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A PHYLOGEOGRAPHIC AND POPULATION GENETIC ANALYSIS OF A WIDESPREAD, SEDENTARY NORTH AMERICAN BIRD: THE HAIRY WOODPECKER (*PICOIDES VILLOSUS*)

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Abstract.—The Hairy Woodpecker (*Picoides villosus*) has one of the broadest breeding distributions of any North American bird and is also one of the most morphologically variable with as many as 21 described subspecies. This wide distribution and high degree of phenotypic diversity suggests the presence of underlying genetic structure. We used ND2 sequence from 296 individuals from 89 localities throughout the Hairy Woodpecker distribution to address this question and to explore this species' evolutionary history. Phylogenetic analyses identified three main Hairy Woodpecker clades, each ~1.5% divergent from one another. One clade was comprised of birds from boreal and eastern zones of North America (N&E); the second, of birds from western and southwestern North America (S&W), and the third included only birds from a disjunct population in Costa Rica and Panama. Population genetic analyses and climatic niche models indicated that the N&E and S&W clades have very different recent evolutionary histories. Populations in the N&E are characterized by a lack of genetic structure and a genetic signature of recent population expansion. In contrast, S&W populations are highly structured and relative population stability was inferred. The S&W clade is further structured into three additional geographically and genetically isolated groups: Pacific Coast ranges, interior ranges, and southern Mexico. The continental-scale patterns of genetic variation observed suggest that the complex topography of the montane west has probably been more important than latitude in generating phylogenetic diversity within this species. *Received 17 November 2010, accepted 24 January 2011.*

Key words: Hairy Woodpecker, mitochondrial DNA, molecular systematics, phylogeography, *Picoides villosus*, population genetics.

Análisis Filogeográfico y de Genética Poblacional de un Ave Norteamericana, Sedentaria y de Amplia Distribución: *Picoides villosus*

Resumen.—El carpintero *Picoides villosus* presenta uno de los ámbitos de distribución reproductiva más amplios entre las aves norteamericanas. Además, es una de las especies más variables morfológicamente, pues comprende hasta 21 subespecies descritas. La amplia distribución y el alto grado de diversidad fenotípica sugieren la existencia de estructura genética subyacente. Utilizamos secuencias del gen ND2 obtenidas de 296 individuos de 89 localidades ubicadas a través del ámbito de distribución de *P. villosus* para abordar esta pregunta y para explorar la historia evolutiva de la especie. Los análisis filogenéticos identificaron tres clados principales de *P. villosus*, divergentes entre sí en un ~1.5%. Un clado incluyó a las aves de zonas boreales y del este de Norte América (N&E), el segundo a las aves del oeste y suroeste de Norte América (S&W) y el tercero sólo a las aves de una población disyunta de Costa Rica y Panamá. Los análisis de genética poblacional y los modelos de nicho basados en variables climáticas indicaron que los clados del N&E y del S&W han tenido historias evolutivas recientes muy diferentes. Las poblaciones del N&E se caracterizan por la ausencia de estructura genética y por una señal genética de expansión poblacional reciente. En contraste, las poblaciones del S&W están altamente estructuradas y se infiere que su tamaño ha sido estable. Además, el clado del S&W está estructurado en tres grupos adicionales que están aislados geográfica y genéticamente: las montañas de la costa del Pacífico, las montañas del interior y el sur de México. Los patrones de variación genética observados a escala continental sugieren que la topografía compleja de las zonas montañosas del oeste ha tenido una importancia probablemente mayor que la latitud como factor generador de diversidad filogenética dentro de esta especie.

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Evolutionary biologists have long been interested in the relationship between earth history and biotic diversification (biogeography). Of particular interest has been the effect of Pleistocene glacial cycles, which are known to have fragmented species and reconfigured their distributions on a continental scale. Early work on the effect of Pleistocene climates on North American birds (Mengel 1964, Hubbard 1973, Rising 1983) focused on explaining the evolution of distributions among species pairs, or groups of closely related species. More recently, modern molecular and coalescent methods have made it possible to study historical biogeography at the intraspecific level (phylogeography, Avise et al. 1987), the level at which the recent Pleistocene events have had the most profound effect (Klicka and Zink 1997, Avise and Walker 1998). Although single species studies (e.g., Zink et al. 2000, Barrowclough et al. 2004, Alexander and Burns 2006, Mila et al. 2007a, Spellman et al. 2007) remain of great interest to avian systematists and evolutionary biologists, a better understanding of the evolution of species distributions and the processes that may have shaped them comes from comparative molecular studies of co-distributed species (Zink 1997, Avise 1998). By comparing phylogeographies across a suite of North American taxa, we can test specific hypotheses regarding the locations and roles of refugia in shaping community structure (Hewitt 1996). Differential success of colonization from these refugia into higher latitudes reveals organismal responses to changing environments. Comparative studies make it possible to detect whether regional diversity reflects common biogeographical histories.

In North America, regional comparative phylogeographical studies have been conducted for the Pacific Northwest (e.g., Brunsfield et al. 2001), southern California (Calsbeek et al. 2003), and the southeastern U. S. (Avise 2000, Soltis et al. 2006). Although some birds are mentioned, they do not figure prominently in these works and in general, few comparative phylogeographic studies exist for North American birds. To fill this void, we have begun to assemble the framework from which to study the comparative phylogeography of western North American montane birds. We have identified about a dozen co-distributed avian species that co-occur in pine (*Pinus*) and pine-oak (*Quercus*) habitats throughout North and Middle America. Comparative phylogeographic study of these taxa is the ultimate goal, and common patterns are beginning to emerge. Thus far, studies on White-breasted Nuthatch (*Sitta carolinensis*; Spellman and Klicka 2007), Mountain Chickadee (*Poecile gambeli*; Spellman et al. 2007) and Brown Creeper (*Certhia americana*; Manthey et al. 2011) have found significant genetic structure between Rocky Mountain and Sierra Nevada populations of these birds, much like that discerned earlier for Fox Sparrow (*Passerella iliaca*; Zink 1994) and Blue Grouse (*Dendragapus obscurus*; Barrowclough et al. 2004). Our preliminary results also suggest that the Peninsular and Transverse ranges of southern California have acted as a refugium for at least some western montane bird species. This pattern is identified for both Mountain Chickadee (Spellman et al. 2007) and White-breasted Nuthatch (Spellman and Klicka 2007) and is consistent with recent findings for White-headed Woodpecker (*Picoides albolarvatus*; Alexander and Burns 2006). In contrast, different evolutionary histories are suggested for the Pygmy Nuthatch (*S. pygmaea*; Spellman and Klicka 2006) and Red-breasted Nuthatch (*S. canadensis*, G. M. Spellman, unpubl. data), two species that exhibit little genetic variation across their entire distributions. In this paper, we add to this growing body of knowledge by using mitochondrial DNA (mtDNA) sequences to investigate the phylogeographic history of the Hairy Woodpecker (*P. villosus*).

Few non-migratory North American birds are as widely distributed as the Hairy Woodpecker. It occurs across all of North America, and from central Alaska south to the Isthmus of Panama. A relatively common bird throughout most of this distribution, it is considered a resident in forest and woodland regions, preferring, but not restricted to spruce (*Picea*), pine and pine-oak habitats (Jackson et al. 2002). The Hairy Woodpecker is also among the most geographically variable of North American birds, displaying extensive variation in size and plumage coloration across its range (Oberholser 1911, Ridgway 1914, Jackson 1970, Ouellet 1977). This variation has been partitioned into as many as 21 subspecies (Peters 1948). Two main groups of subspecies are recognized on the basis of underpart coloration (Ouellet 1977) and these correspond with two broad geographic regions, western North America plus Middle America, and boreal and eastern North America. The general pattern of variation observed for continental birds is a north to south diminution in size and a darkening of plumage west and south of the Rocky Mountains (Jackson et al. 2002). Unfortunately, most of the variation in the Hairy Woodpecker is clinal such that many putative subspecies intergrade broadly, making identification to race not always possible (Short 1982, Jackson et al. 2002). The distinctive regional differences in plumage and size suggest the presence of accompanying regional genetic differences. On the other hand, the clinal nature of much of this variation suggests substantial gene flow may be occurring between many of these geographically distinctive forms. These alternatives can be assessed with rapidly evolving mtDNA markers. In this study, we use such a marker to assess the phylogeographic and population-genetic structure of the Hairy Woodpecker. Discerning patterns of genetic variation will allow us to assess the degree of correspondence between phenotypic and genotypic variation in this species. Quantifying this genetic variation will allow us to explore the evolutionary history of this species, particularly its response to climate change during the last ~21,000 years. With its continent-wide distribution, results of this phylogeographic and population-genetic analysis of Hairy Woodpecker will not only inform directly questions concerning the evolution of western montane birds, but it will also represent one of the few data sets available to explore evolutionary patterns on a continental scale.

Methods

Sampling strategy and generation of sequence data.—Hairy Woodpecker tissue samples were obtained for 296 individuals from 89 localities distributed throughout species' range (Fig. 1 and online Appendix 1 [see Acknowledgments]; locality and voucher data for all specimens used are available from the senior author). An effort was made to maximize the sampling of morphological diversity. To that end, 15 of 17 widely recognized subspecies (following Ouellet 1977) are represented in this study. The congeners *P. mixtus* and *P. pubescens* were used as outgroup taxa. Total genomic DNA was extracted using a DNeasy tissue extraction kit (Qiagen, Valencia, CA) following the manufacturer's protocol. We amplified all 1,041 base pairs (bp) of the mtDNA NADH dehydrogenase

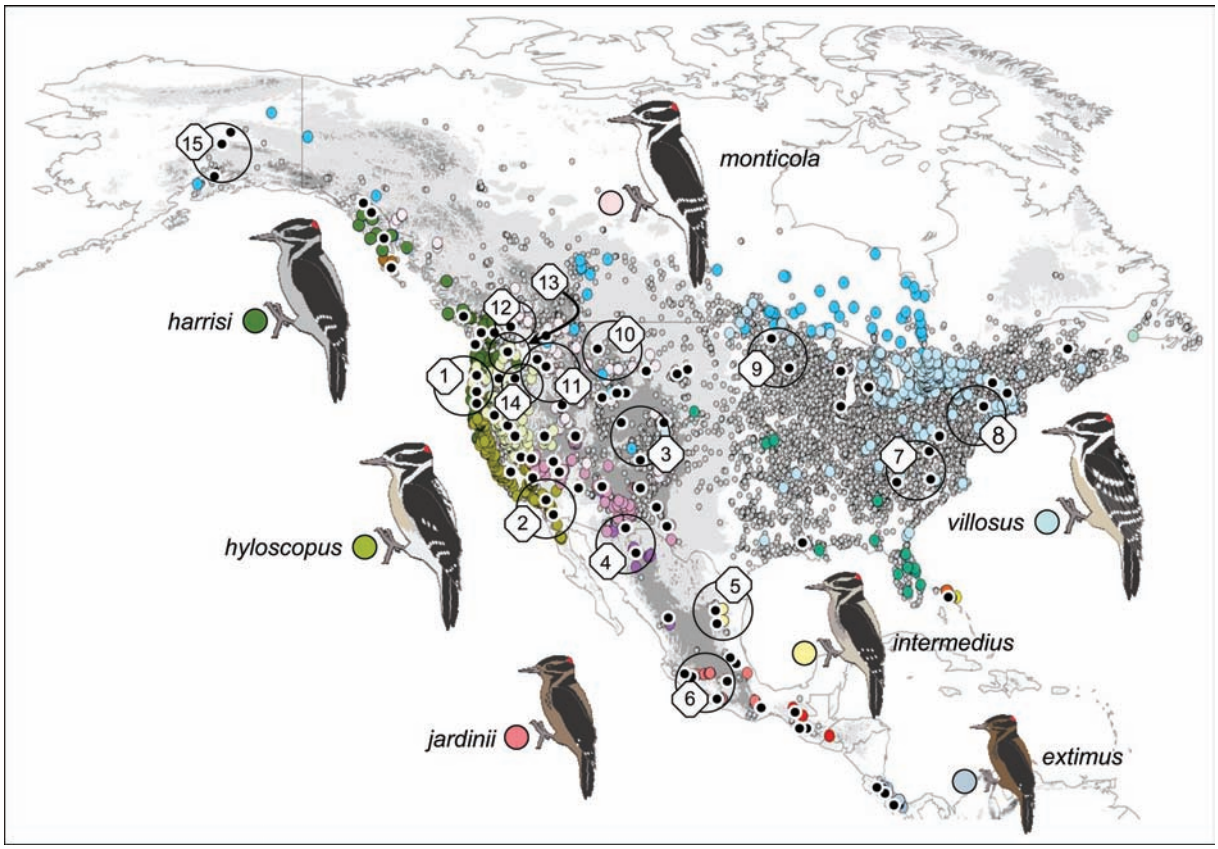

FIG. 1. Hairy Woodpecker (*Picoides villosus*) distribution map. Small gray dots represent 17,620 unique, recent observation records (www.avianknowledge.net) and indicate approximate limits of the Hairy Woodpecker distribution. Colored circles indicate approximate subspecies distributions as defined by 869 specimen records (ORNIS data portal, www.ornisnet.org, search date of 27 July 2010; see Acknowledgments for complete list of data providers). Black circles identify all sampling localities; large open, numbered circles identify those samples pooled (if necessary) for population genetic analyses. Population genetic samples: 1 = Oregon Coast (OR_W); 2 = southern California (CA_S); 3 = southern Rocky Mountains (CO-UT); 4 = southeastern Arizona and the Sierra Madre Occidental (SM Occ); 5 = Sierra Madre Oriental (SM Ori); 6 = southern Mexico (MX_S); 7 = the Appalachian Mountains (NC-VA); 8 = northeastern U.S. (NY); 9 = Minnesota (MN); 10 = western Montana (MT_W); 11 = northeast Oregon (OR_NE); 12 = northeast Washington (WA_NE); 13 = central Washington (WA_C); 14 = central Oregon (OR_C); 15 = Alaska (AK). Selected subspecies images depict general direction and degree of changes in plumage and size. Additional subspecies not shown include: *picoideus* (Queen Charlotte Is., brown); *orius* (light green); *leucothorectis* (mauve); *icastus* (purple); *sanctorum* (red); *piger* (Bahamas, Grand Bahama, Abaco, bright yellow); *maynardi* (Bahamas., New Providence, Andros, orange); *audubonii* (blue-green); *terranovae* (Newfoundland, light green-blue) and *septentrionalis* (dark blue).

subunit 2 (ND2) gene using primers L5215 (Hackett 1996) and TrC (Miller et al. 2007). Amplifications were done in 12.5 µL reactions under the following conditions: denaturation at 94°C, followed by 40 cycles of 94°C for 30 s, 54°C for 45 s, and 72°C for 1 min. This was followed by a 10 min extension at 72°C and a 4°C soak. PCR products were sent to High-Throughput Genomics Unit (University of Washington) for all subsequent sequencing steps. There, products were purified using ExoSAP-IT (USB Corporation, Cambridge, MA) and these purified products were cycle-sequenced using BigDye (Applied Biosystems, Foster City, CA) on a high-throughput capillary sequencer. Complementary strands of each gene were unambiguously aligned using SEQUENCHER, version 4.9 (Gene Codes Corporation, Ann Arbor, MI). Data quality was verified by sequencing both light and heavy DNA strands. No gaps, insertions, or deletions were apparent in the aligned sequences, and all sequences translated correctly into amino acid form (using MEGA version 4, Kumar et al. 2008).

Phylogenetic analyses.— Phylogenetic relationships among haplotypes were examined using maximum likelihood (ML) and Bayesian analyses. For most analyses, the data were divided into one of two codon partitions (first and second position sites combined and third positions; the "CP" model of Shapiro et al. 2006). MR-MODELTEST (Nylander 2004) and the Akaike Information Criterion (AIC) method (see Posada and Buckley 2004) were used to identify the best-fit model for each. A ML tree was constructed with the Program TREEFINDER (Jobb 2006) using all unique ingroup haplotypes and the GTR + I model of sequence evolution for both partitions. The nature of the data precluded performing standard ML bootstrapping analyses, so nodal support was assessed via Bayesian inference using the Program MRBAYES,

version 3.1.2 (Huelsenbeck and Ronquist 2001). For this analysis, the same partitioned data were used and the appropriate models assumed, although specific model parameters were left undefined and estimated by the program. Four Markov chains were run for 3 million generations and sampled every 100, yielding 30,000 trees, 10,000 of which were discarded to ensure stationarity. The procedure was repeated to ensure thorough sampling. Both runs converged on similar distributions, so the trees from each analysis were combined to yield 40,000 topologies from which a 50% majority rule consensus tree was constructed. Nodes having posterior probabilities ≥ 95% were deemed significantly supported (Huelsenbeck and Ronquist 2001).

We also used the Program BEAST, version 1.5.4 (Drummond and Rambaut 2007), to provide a phylogeny estimate and to approximate among-clade divergence times. BEAST uses Bayesian Markov chain Monte Carlo (MCMC) methods and samples over many trees, each weighted according to their posterior probabilities. As a consequence, uncertainty in the phylogeny is accounted for, yielding parameter estimates (such as divergence times) with 95% credibility intervals (CI). A BEAST input file was generated using Program BEAUTI, version 1.5.4. A likelihood ratio test for a molecular clock indicated that the data were sufficiently clocklike ($-2 \log \Delta = 77.75$, df = 90, $0.9 > P > 0.5$) enabling a strict clock model to be used in the BEAST analyses. Thus, the divergence time credibility intervals measure the variance in divergence times among the posterior distribution of gene trees, and not variance in rate heterogeneity along branches. Because ND2 appears to evolve in birds at a slightly faster rate than cytochrome-*b* (Smith and Klicka 2010), we used a prior substitution rate of 0.015 (2.3% divergence my^{-1}) rather than the commonly used cytochrome-*b* rate of 0.01 (2% my^{-1}). The data were partitioned as described above, the GTR + I model was implemented for both partitions, and the tree prior was set for constant population size. To achieve sufficiently high (>200) effective sample size (ESS) values we used log normal parameter prior distributions. Analyses were run for 80 million generations and trees were sampled every 1,000. Program TRACER, version 1.5, was used to assess convergence, and TREEANNOTATOR, version 1.5.4, was used to generate summary trees. We also used the Program AWTY (Nylander et al. 2008) to confirm that available tree space had been sufficiently sampled. Final topologies, with divergence times and 95% credibility intervals were visualized with FIGTREE, version 1.3.1. Most of these programs are available as part of the BEAST package (see Acknowledgments).

The analyses above indicated clear but shallow genetic structuring within Hairy Woodpecker, a common result in intraspecific studies where genetic divergences are low and many clades have not yet achieved monophyly. Networks are useful for visualizing such data, so we used the Program NETWORK, version 4.516 (Bandelt et al. 1999), to construct a median-joining network using all 296 available sequences. Subsequently, networks were independently constructed for individual clades that were identified in our phylogenetic analyses.

Population genetic analyses.—To reach acceptable sizes for population genetic analyses ($n \approx 10$, see Harding 1996), we pooled samples from geographically proximate locations for some sites. The result was 15 geographic populations (Fig. 1 and online Appendix 1 [see Acknowledgments]) with sample of 8 to 21 individuals (total $n = 185$). Because we were interested in the underlying genetic structure in each population, individuals presumed to represent recent introgression events (based on mtDNA "mismatches") were omitted from these analyses. Genetic diversity indices were generated using DnaSP, version 5.0 (Librado and Rozas 2009), for each of the 15 populations. These included number of haplotypes, haplotype and nucleotide diversity, and number of private haplotypes (those occurring in only one population). Private haplotype frequencies were calculated by hand. We also used RAREFAC (Petit et al. 1998) to calculate a corrected measure of haplotype diversity (haplotype richness) that accounts for differences in population sample sizes. To obtain a measure of genetic differentiation among populations, we tabulated pairwise-F_{ST} values with ARLEQUIN, version 3.11 (Excoffier et al. 2005). Significance was assessed after a Bonferroni correction for multiple comparisons. Population differentiation was also tested with an exact test (Raymond and Rousset 1995). Based on haplotype frequencies, this test was run in ARLEQUIN using 100,000 Markov chain steps. Population structure was further evaluated with an analysis of molecular variance (AMOVA) using ARLEQUIN (Excoffier et al. 2005). AMOVA uses the frequencies of haplotypes and the number of mutations among them to test the significance of variance components within populations, among populations within groups, and among groups.

Historical demography.—We tested for population demographic changes in several ways. For each of the 15 populations, we performed a mismatch distribution (Slatkin and Hudson 1991) on the basis of 1,000 test replicates to test a model of sudden population expansion. Distributions were plotted using DnaSP (Librado and Rozas 2009) and the mismatch statistics SSD (significant sum of squared deviation) and *r* (Harpending's raggedness index) were calculated with ARLEQUIN (Excoffier et al. 2005). If significant ($P < 0.05$), the null hypothesis of sudden population expansion can be rejected. ARLEQUIN mismatch analyses also generate estimates of the time since expansion began (τ) and relative population sizes for before (Θ_0) and after (Θ_1) population expansion. The statistic τ was used to calculate the time (*t*, in years) at which individual populations began to expand with $\tau = 2ut$, and $u = 2\mu k$, with μ being the mutation rate (0.015 mutations / lineage my^{-1}) and *k* the sequence length (Rogers and Harpending 1992).

Because mismatch tests are known to be conservative (Ramos-Onsins and Rozas 2002), we also computed the more powerful Fu's F_S (Fu's test of selective neutrality; Fu 1997) and R_2 (Ramos-Onsins and Rozas 2002) statistics to assess population demography. Fu's F_S (1997) values were generated via 5,000 coalescent simulations using ARLEQUIN. Negative F_S values result from an excess of rare alleles, suggesting population expansion (or background selection), whereas positive values indicate a stable population. Simulation analyses have indicated that for evaluating Fu's F_S statistics a significance level of $\alpha = 0.02$ is appropriate (Fu 1997, Excoffier et al. 2005). We also calculated Fu and Li's (1993) D^* and F^* statistics in DnaSP (Librado and Rozas 2009). When compared to Fu's F_S values, these statistics can help distinguish background selection from population growth. If an F_S value is significant and D^* and F^* statistics are not, population growth is indicated. Conversely, significant D^* and F^* statistics suggest that background selection is the likely cause of the observed variation (Fu 1997). R_2 values were generated with DnaSP (Librado and Rozas 2009). Significance ($\alpha = 0.02$) was assessed by comparing the observed value with a null distribution simulated using empirical sample sizes and numbers

of segregating sites. Simulations suggest that R_2 test works better on smaller sample sizes (like those in our study) and F_S works better on larger ones (Ramos-Onsins and Rozas 2002).

In general, the proportion of private haplotypes in a population should increase as a function of population size and isolated populations should retain more private alleles than populations with high emigration rates. To explore these relationships among our populations, we regressed the proportion of private haplotypes against an estimate of effective population size for each population. As a proxy for population size, we used Θ_k estimates obtained using ARLEQUIN. Under conditions of neutrality, constant population size, constant mutation rate (across populations), and the infinite-alleles model, differences in Θ_k should reflect differences in effective population size for females (N_{fe}, Ewens 1972). Assuming that departures from these assumptions are approximately equivalent for all populations, Θ_k can be considered an effective relative indicator of N_{fe} (see Helgason et al. 2001). In the regression plot, a greater distance above the regression line suggests increasing isolation while the greater the distance below the line indicates an increasing degree of connectedness among populations.

Genetic structure and geography.—Geographic patterns of genetic variation were examined in several ways. An hypothesis of Late Pleistocene postglacial geographic expansion (e.g., Hewitt 1996, 2000) was tested by plotting nucleotide diversity by latitude, first for all populations combined and again with the data partitioned into the two major clades. In theory, populations now occupying recently glaciated regions, should exhibit lower genetic diversity. On the same data sets, we performed Mantel tests (Mantel 1967) in ARLEQUIN using 1,000 random permutations to evaluate whether the observed genetic patterns were due, at least in part, to isolation by distance (IBD), that is, significant correlations between genetic ($F_{ST}/[1 - F_{ST}]$) and geographic (km) distances. To assess the potential role of elevation on genetic structuring we plotted F_{ST} values against average elevation for each pair of population samples.

Two additional procedures were used to characterize patterns of genetic divergence across the species' distribution. First, we used our 15 population samples and the Program SAMOVA ("spatial analysis of molecular variance"; Dupanloup et al. 2002) to identify partitions of geographically adjacent sampling areas that were maximally differentiated genetically. This approach operates under a simulated annealing procedure to maximize the statistic F_{CT}, which is an indicator of the proportion of total genetic variance due to differences between groups of populations. The optimal number of genetic "groups" (k) in the data set was determined by the largest, still significant F_{CT} value obtained. Second, a genetic landscape shape analysis was done using Alleles in Space (AIS; Miller 2005) using the full, ungrouped data set divided into two runs, one for each of the geographically distinct major clades. Pairwise genetic distances were calculated and assigned to midpoints between sampling sites using the Delauney triangulation-based connectivity network (Miller et al. 2006). Next, a simple interpolation procedure was used to infer genetic distances at locations on a uniformly spaced grid that overlaid the entire sample netscape. We used residual genetic distances derived from a linear regression of geographic and genetic distances to account for potential correlation between these two measures (Manni et al. 2004, Miller et al. 2006). A three-dimensional surface plot of the interpolated genetic distances was produced, where X and Y coordinates correspond to geographical locations within the network and genetic distance is depicted in the Z dimension. Peaks on these plots correspond to areas where genetic differences are unusually high after IBD is accounted for; valleys identify areas where distances are unexpectedly low. For both SAMOVA and AIS analyses, the identified groupings of populations are presumed to be separated by genetic barriers.

To gain insights into the geographic distribution of individual clades over time, we constructed climatic niche models (CNM) using 1,190 unique geographic occurrence records (690 N&E points, 500 S&W points). These included our own sampling localities and a subset of the observation records shown in Figure 1. Our bioclimatic variables were from the WorldClim dataset (version 1.4), with a resolution of 2.5 min (Hijmans et al. 2005). Seven variables were correlated with others ($r > 0.90$); therefore, only 12 of 19 temperature and precipitation variables were used (i.e., BIO1, BIO2, BIO3, BIO5, BIO6, BIO8, BIO9, BIO12, BIO14, BIO15, BIO18, and BIO19). Individual CNMs were generated using the maximum entropy algorithm in MAXENT, version 3.3 (Phillips et al. 2006), for each of the two main clades (S&W, N&E) identified in our phylogenetic analyses. We used default settings (regularization = 1, convergence threshold = 0.00001, iterations = 500), 10 replicates, and the random test percentage was set to 0.25 for model evaluation. The models were then applied to the Model for Interdisciplinary Research on Climate (MIROC; Hasumi and Emori 2004) layers to estimate a suitable climatic envelope for Hairy Woodpeckers during the last glacial maximum (LGM). We visualized models in ARCGIS, version 9.3 (ESRI Redlands, CA). Because the distribution of the Hairy Woodpecker is well known, we used a digital range map (Ridgely et al. 2007) to set the logistic climate suitability threshold values for the S&W and N&E clades and applied these same threshold values to the paleo-CNMs. For a more detailed overview of CNM methodology, see Elith et al. (2006).

RESULTS

Phylogenetic analyses.—Sequences of 296 Hairy Woodpecker individuals for the complete ND2 gene (1,041 bp; GenBank accession numbers HQ889319–HQ889613) yielded 90 variable nucleotide sites, 40 of which occurred in only single individuals. Overall, 92 unique haplotypes were identified. The most common haplotype occurred in 28.4% ($n = 84$) of all individuals (Fig. 2, haplotype 5) with the next most common occurring in 7.4% ($n = 22$, Fig. 2, haplotype 33). The ML and Bayesian topologies produced were similar (Figs. 2 and 3) with respect to internal nodes. Each identified three major clades (Fig. 3, column B). One clade contained most individuals from boreal and eastern regions of North America (hereafter referred to as N&E clade). A second contained most individuals from montane regions of western and southwestern North America along with all individuals from Mexico and Guatemala (hereafter S&W clade). A third clade was comprised of only birds from the southernmost extent of the species range, Costa Rica and Panama. Limited introgression between the N&E and S&W clades was evident in the northwestern United States, whereas the third clade was monophyletic. Genetically, each of these three clades was approximately equidistant from one another genetically at ~1.5% uncorrected sequence divergence. A standard mtDNA rate of 2% sequence divergence my^{-1} (see Lovette 2004) would indicate

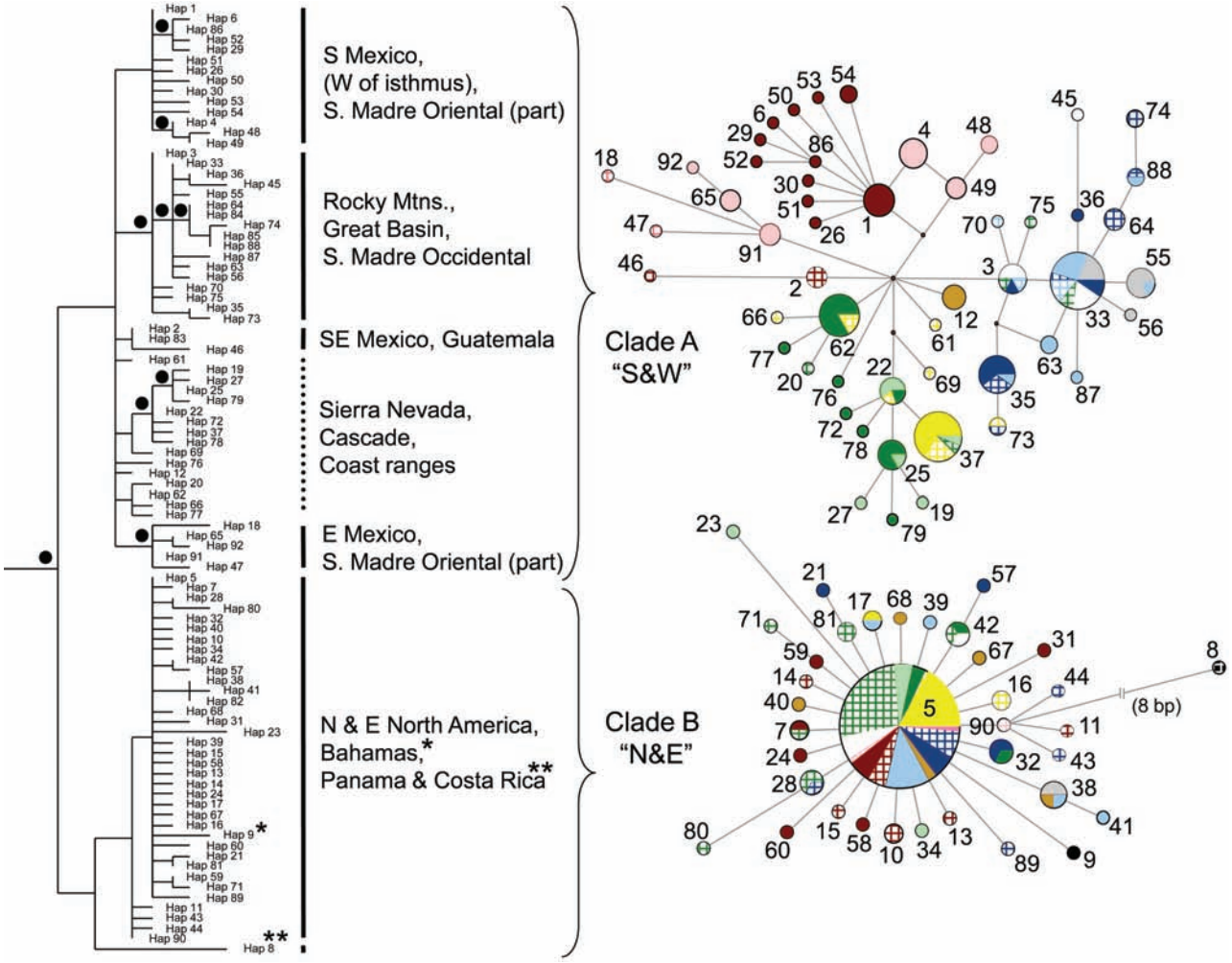

Fig. 2. Phylogenetic and network relationships based on 92 Hairy Woodpecker haplotypes. On left, a ML tree derived using partitioned data (see Methods) and a GTR + I model of sequence evolution. Those nodes receiving significant Bayesian posterior support (≥95%) are indicated by black circles. On right, median-joining networks of the two major clades. The branch (not shown) connecting the two main clades contains a minimum of eight mutational steps. In the following description N, S, W and E refer to north, south, west and east, respectively, and their intermediate (e.g., NW = northwest). Standard abbreviations are used for U.S. states. Color code for S&W clade: dark (dk) green = OR coast; light (lt) green = WA coast, Vancouver Island; dk green cross hatch (ch) = Cascade Range, E OR, E WA; yellow = S CA (Transverse and Peninsular Ranges); yellow ch = Sierra Nevada Range; orange = Queen Charlotte Islands.; dk blue = S Rocky Mountains.; dk blue ch = NV; lt blue = AZ; lt blue ch = NM, white = central Rocky Mountains. (ID, MT, WY, SD); gray = Sierra Madre Occidental; red = S Mexico (Transvolcanic Belt, S. Madre del Sur); red ch = SE Mexico (Chiapas, Guatemala); pink = S Madre Oriental; pink ch = E Mexico (Veracruz, Hidalgo. For N&E clade: yellow = AK, yellow ch = SE AK, dk green = OR coast; dk green ch = Cascade Range, E WA, E OR; lt green = WA coast, Vancouver Island; white = central Rocky Mountains. (ID, WY, MT, SD); pink = NE CA; pink ch = New Brunswick; dk blue = NE WA; dk blue ch = N Rocky Mountains (MT, ID); red = Appalachian Mountains (WV, NC, VA); red ch = Northeast (NY, NH); lt blue = MN; brown = Midwest (MI, WI, IL); gray = Southeast (LA); black = Bahamas (Abaco); and black ch = Panama, Costa Rica.

an initial divergence within this lineage at roughly 750,000 years before present (ybp). This timing is concordant with an estimate of 690,000 ybp (95% credibility interval [CI]: 470,000–925,000) obtained from a Bayesian analysis using coalescent priors and a slightly faster rate of 2.3% my^{-1} for the ND2 gene (see Methods).

Relationships within each major clade were less clear. The ML and Bayesian topologies differed considerably nearer the terminal nodes (Fig. 2 vs. Fig. 3). A lack of power in the data set (only 50 phylogenetically informative characters) was revealed by shallow genetic structure and low nodal support. Median-joining networks (Fig. 2) provided a better perspective on within-clade relationships. They indicated very different evolutionary histories for the S&W and N&E clades. The N&E clade was comprised of 137 sequences that were defined by 37 haplotypes. In this clade over 61% of individuals were represented by a single dominant haplotype (hap 5), whereas most of the remaining haplotypes differed from this one by a single nucleotide change. Such "star-shaped" networks are consistent with a recent population expansion from a single source (Slatkin and Hudson 1991). In contrast, the S&W clade (159 individuals, 55 haplotypes) showed a high

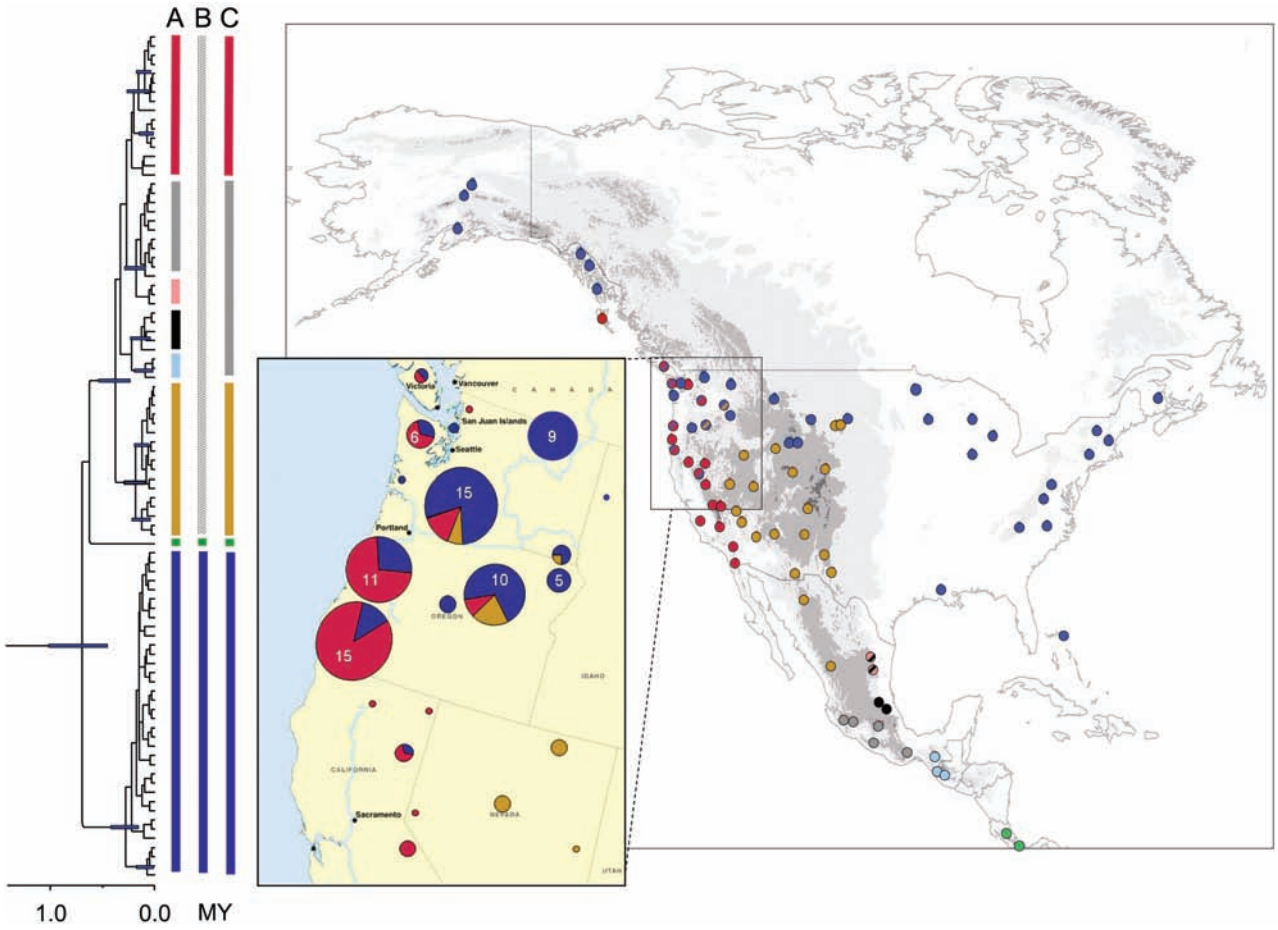

FIG. 3. Phylogeographic results. The tree on the left is a phylogenetic hypothesis of clade relationships and divergence times obtained from Bayesian methods (using BEAST; see Methods). The distributions of the geographically structured, color-coded terminal clades (A) are indicated on the map on the right. The inset map highlights an apparent zone of introgression in the Pacific Northwest involving individuals of three otherwise distinct clades. Column B identifies the three main clades identified in the text. Column C identifies five evolutionary groupings identified via the combined SAMOVA and AIS analyses (see text).

degree of genetic structuring into multiple discrete geographic units (discussed below). This pattern may indicate longer periods of isolation with reduced gene flow among geographically segregated populations.

Population genetic analyses.—F_{ST} statistics were able to quantify the results suggested by median-joining networks for each major clade (Fig. 3, column B). All six populations in the S&W clade (Figs. 1 and 2) differed significantly from one another both in F_{ST} comparisons (Table 1) and in exact tests of population differentiation (Raymond and Rousset 1995). By contrast, little genetic structure was evident among any of the nine N&E clade populations. Among the N&E populations, a single significant F_{ST} value was obtained (northeast Washington vs. Alaska), whereas exact tests of differentiation suggested that only Alaska differed significantly ($\alpha = 0.05$, results not shown) from the northeast Washington, northeastern U.S., Appalachian Mountain, and western Montana populations. AMOVA analyses (Table 2) confirmed that a high degree of genetic structure occurred in the S&W clade but not in the N&E clade. In the former, 35.0% of the variation occurred among populations ($F_{ST} = 0.3498$, $P < 0.0001$), whereas, in the latter, only 3.4% of the total variation occurred among populations ($F_{ST} = 0.03369$, $P = 0.055$).

Curiously, the differences in population structure within the S&W and N&E clades did not appear to be reflected by most of the genetic diversity indices. Haplotype diversity was relatively high in populations from both clades, ranging from 0 in southern California, where all 11 individuals shared the same haplotype, to 4.364 and 4.029 (corrected values) for the Appalachian Mountains and southern Mexico respectively (Table 3). The frequency of private haplotypes in each population varied from 0 (Alaska, central Washington) to 0.53 (southern Mexico) and averaged 0.23 for N&E populations combined and 0.29 for the combined S&W samples. Nucleotide diversity estimates were also comparable across clades (Table 3). The highest values in the S&W occurred along the Oregon coast (0.0025) and in the Nuevo Leon (0.0030) population (Table 3). For the N&E clade, the highest values obtained were for the Appalachian Mountains in the southeastern US (0.0014) and western Montana (0.0014).

Table 1. Pairwise F_{ST} values (below the diagonal) for all 15 Hairy Woodpecker population samples, by clade. Asterisks indicate statistical significance after a Bonferroni correction. The average number of pairwise nucleotide differences within a population are shown on the diagonals (in italics), while the average number of pairwise differences between populations are given above the diagonal.

Population[a]	A	B	C	D	E	F	G	H	I
S&W Clade									
(A) CA_S	*0.0000*	4.5000	3.2381	6.4167	3.8824	6.4444			
(B) SM Ori	0.5887*	*3.1699*	4.6746	6.1389	3.7157	6.1667			
(C) OR_W	0.5282*	0.3904*	*2.5524*	5.3691	4.8291	5.3545			
(D) SM Occ	0.9568*	0.6659*	0.6738*	*0.5303*	6.2990	1.9722			
(E) MX_S	0.7473*	0.3472*	0.5575*	0.8121*	*1.6618*	6.3007			
(F) CO-UT	0.8680*	0.5661*	0.5684*	0.4152*	0.7229*	*1.8890*			
N&E Clade									
(A) AK	*0.1333*	0.4417	0.6222	0.5571	0.7333	0.7939	0.7939	0.6917	0.3167
(B) OR_NE	0.0527	*0.7500*	0.9306	0.8750	1.0139	1.0796	1.0796	1.0000	0.6250
(C) NY	0.0950	0.0272	*1.0556*	1.0556	1.2222	1.2828	1.2828	1.1806	0.8056
(D) MN	0.0266	0.0180	0.0496	*0.9560*	1.1508	1.2273	1.2273	1.1250	0.7500
(E) WA_NE	0.0900*	–0.0038	0.0455	0.0363	*1.2778*	1.3939	1.3737	1.2361	0.9167
(F) NC-VA	0.2381	–0.0314	0.0181	0.0225	0.0185	*1.4546*	1.4546	1.3523	0.9621
(G) MT_W	0.0489	–0.0147	0.0324	0.0373	0.0176	0.0125	*1.4182*	1.3296	0.9470
(H) OR_C	0.1231	0.0357	0.0547	0.0583	0.0058	0.0224	0.0199	*1.7857*	0.8333
(I) WA_C	0.0603	0.0355	0.0671	0.0445	0.0633	0.0042	0.0073	0.0322	*0.4697*

[a]See legend to Figure 1 for full names of populations represented by the abbreviations.

Table 2. AMOVA results for each of the two major geographic divisions (Fig. 2) within the Hairy Woodpecker.

Clade	Source of variation	Percentage of variation	F_{ST}	*P*
Northern and Eastern (N&E) North America	Among populations	3.37%	0.0337	0.05474
	Within populations	96.63%		
Southern and Western (S&W) North America & Central America	Among populations	34.95%	0.3495	0.00000
	Within populations	65.05%		

Table 3. Genetic diversity indices and mismatch statistics for 15 Hairy Woodpecker populations. Sample size (*n*), number of haplotypes (*H*), number of private haplotypes (Priv. *H*), haplotype diversity (H_d), haplotype richness, (H_r, see Methods), nucleotide diversity (π), Fu's F_S, R_2 test for population expansion, raggedness index (*R* index), mismatch sum of squared deviation (SSD), and time elapsed (in years) since population expansion began (*t*[yrs]). Significance for F_S and R_2 tests set at $\alpha = 0.02$. The null model for mismatch statistics (*R* index, SSD) is that the population is growing exponentially ($\alpha = 0.05$).

Locality[a]	*n*	*H*	Priv. *H*	H_d	H_r	π	F_S	R_2	*R* index	SSD	*t* (yrs)
Clade A—South & West											
CA south (CA_S)	11	1	1	0.0000	0.0000	0.0000	0.0000	na	na	na	na
S Madre Oriental (SM Ori)	18	6	6	0.8366	3.6980	0.0030	0.8372	0.1933	0.0814	0.0479	104,787
Oregon coast (OR_W)	21	8	8	0.7381	3.2220	0.0025	–1.2473	0.1132	0.1537	0.0732	74,384
S Madre Occidental (SM Occ)	12	2	1	0.5303	1.0000	0.0005	1.1521	0.2652	0.2849	0.0295	13,160
Mexico South (MX_S)	17	9	9	0.7868	4.0290	0.0013	–1.9543	0.0946	0.3357	0.0912	39,689
S Rockies (CO-UT)	9	4	3	0.7500	2.7780	0.0018	0.2703	0.2227	0.1073	0.0352	53,090
Clade B—North & East											
Alaska (AK)	15	2	0	0.1333	0.5330	0.0001	–0.6490	0.2494	0.5555	0.0379	47,643
Oregon northeast (OR_NE)	8	3	1	0.4643	2.0000	0.0007	–0.3050	0.2320	0.1365	0.0104	35,431
New York (NY)	9	5	4	0.8056	3.6670	0.0010	–2.3600*	0.1275*	0.2862	0.0467	21,524
Minnesota (MN)	14	5	3	0.5055	2.2860	0.0009	–1.7480	0.0976*	0.0943	0.0427	24,768
Washington northeast (WA_NE)	9	4	3	0.6944	2.7780	0.0012	–0.5365	0.1708	0.0363	0.0046	27,586
North Carolina - Virginia (NC-VA)	11	7	5	0.8182	4.3640	0.0014	–3.9620*	0.0937*	0.0760	0.0045	25,456
Montana west (MT_W)	11	6	3	0.7273	3.6360	0.0014	–2.5080*	0.0965*	0.0955	0.0239	30,515
Oregon central (OR_C)	8	3	2	0.6071	2.0000	0.0011	0.5063	0.2538	0.1773	0.2332*	3,602
Washington central (WA_C)	12	3	0	0.4394	1.5760	0.0005	–0.7246	0.1576	0.1673	0.0112	9,446

[a]See legend to Figure 1 for full names of populations represented by the abbreviations.

Historical demography and landscape genetics.—Under the presumption that populations were pressed southward and reduced in size during late Pleistocene glacial advances, a genetic signature of recent population expansion was expected for birds currently occupying north-temperate and boreal regions. This scenario appeared to be supported in most cases by mismatch distributions. The more conservative mismatch statistics, raggedness index and SSD (Table 3), rejected the null hypothesis of recent range expansion for only a single population in central Oregon (OR_C, N&E clade). However, the more powerful Fu's F_S and R_2 tests indicated that only four N&E populations (northeastern U.S., Minnesota, Appalachian Mountains, and western Montana) were likely to have undergone a rapid expansion while all S&W populations were either at demographic equilibrium or possessed genetic structure themselves (Table 3). Fu and Li's (1993) D^* and F^* statistics were significant for one population (Appalachian Mountains; $D^* = -2.320$, $P < 0.02$; $F^* = -2.511$, $P < 0.02$; other results not shown), indicating that for most populations, significant F_S values were likely due to population growth rather than background selection. Estimates of the elapsed time since expansion began for those populations deemed rapidly expanding (northeast U.S., Minnesota, Appalachian Mountains, Montana) averaged 25,566 ybp. This value is consistent with population expansions that began around the end of the LGM.

Isolation by distance was evident among populations in the N&E clade (Mantel test, $R^2 = 0.464$, $P = 0.03$) but not within the S&W ($R^2 = -0.384$, $P = 0.917$; plots not shown). A similar result was obtained when we examined relationships between nucleotide diversity and latitude (Fig. 4A). In the N&E, the expected significant relationship was observed ($R^2 = -0.546$, $P = 0.023$); however, no relationship existed for the S&W populations ($R^2 = 0.003$, $P = 0.918$). Plotting pairwise estimates of population differentiation (F_{ST}) against pairwise average elevation also indicated a distinction between populations in the N&E versus S&W clades (Fig. 4B). It seems unlikely that higher elevations by themselves have led to greater population structuring among western populations, but rather, lower elevations allow greater population connectivity in the more continuously forested east whereas higher elevation western habitats are frequently separated by vast expanses of unsuitable habitat. An approximation of "population connectivity" was obtained by plotting frequency of private haplotypes versus relative population size (as estimated by Θ_k) for each population (Fig. 4B). All six S&W populations were located on or above the regression line, with three outside the 95% CI interval. Of the nine N&E populations, six were located below the line with four of these outside the 95% CI. This result suggests an overall higher degree of isolation for S&W populations and greater connectivity among those in the N&E.

Our SAMOVA analysis indicated that the 15 North American Hairy Woodpecker populations in our study were best divided into five genetically and geographically discrete assemblages (Table 4). The N&E clade was maintained as one group and the S&W clade was subdivided into a Southern California group, a Pacific Coast group (exclusive of Southern California), a Rocky Mountain plus Sierra Madre Occidental group, and a group containing all remaining Mexican populations. Not a part of the SAMOVA analysis, the Costa Rica–Panama clade formed a sixth partition, yielding a total of six maximally differentiated sampling areas. Slightly different results were obtained from our genetic landscape shape interpolation analysis (AIS). These differences

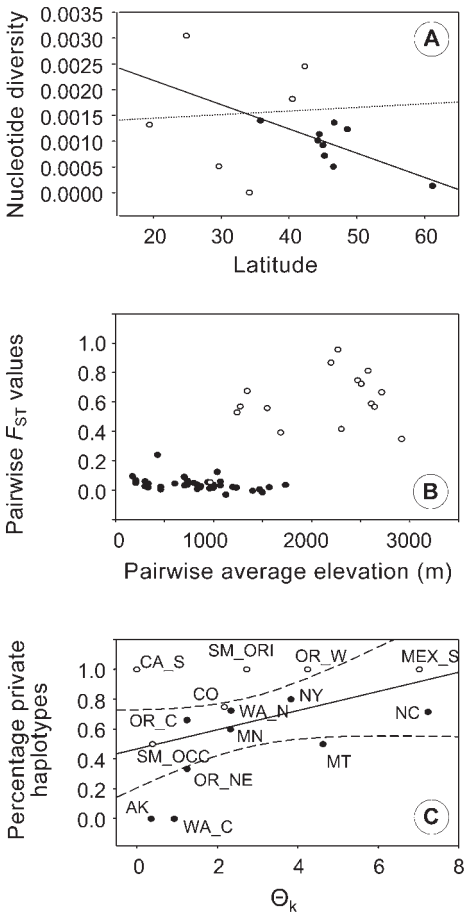

FIG. 4. Comparisons of population characteristic for 15 Hairy Woodpecker population-genetic samples. S&W populations are designated by open circles (with dashed regression line) and for N&E populations they are closed (solid regression line). (A) The relationship between latitude and nucleotide diversity: a strong negative slope for N&E populations supports the post-glacial expansion hypothesis. (B) The relationship between population pairwise elevation and pairwise genetic differentiation (F_{ST}): demonstrates the importance of forest discontinuity in the genetic structuring of populations in the montane region occupied by the western clade (open circles). (C) The relationship between effective population size (Θ_k) and haplotype diversity: a greater distance above the regression line suggests increasing isolation while the greater the distance below the line indicates an increasing degree of connectedness among populations.

were presumably due to the use of all North American samples (S&W, $n = 159$; N&E, $n = 131$) for AIS whereas only a subset of these were used for SAMOVA. For the N&E clade, some genetic discontinuity was evident between western Montana and the combined Alaska, northeastern Washington, and central Oregon samples (Fig. 5), suggesting that even with the relative lack of genetic structure, the Northern Rocky Mountains were an apparent barrier to gene flow. Three groupings were defined for the S&W clade. A Rocky Mountains–Sierra Madre grouping was again recognized, but due to more complete population sampling the southern California and Oregon Coast groups were combined into a larger Sierra Nevada-Cascades-Coast Range group (Fig. 5). The AIS analysis also revealed a Mexico grouping (exclusive of

Table 4. Population structure inferred by SAMOVA (spatial analysis of molecular variance). Percentages of molecular variation explained by "within and among" groupings are indicated. Accompanying fixation indices for values shown were all highly significant ($P < 0.00001$). The highest F_{CT} value (82.72) was obtained when $k = 5$, the optimal number of genetic and geographic groupings. Because of its smaller sample size ($n = 6$), the Costa-Rican and Panamanian birds were not a part of this analysis, although they are considered an additional "group". By contrast, the OR_W (coastal Oregon) and CA_S (southern California) groups suggested here to be groups, were not when additional data were examined (see Fig. 5 and text).

k	Grouping[a]	Among groups (F_{CT})	Among populations within groups	Within populations
2	[all S&W] [all N&E]	74.51	13.06	12.43
3	[OR_W, CA_S, SM Ori, S_MEX] [SM Occ, CO-UT] [all N&E]	78.70	7.92	13.38
4	[OR_W, CA_S] [SM Ori, S_MEX] [SM Occ, CO-UT] [all N&E]	80.89	4.79	14.31
5	[OR_W] [CA_S] [SM Ori, S_MEX] [SM Occ, CO-UT] [all N&E]	82.72	2.84	14.44
6	[OR_W] [CA_S] [SM Ori, S_MEX] [SM Occ, CO-UT] [OR_C] [remaining N&E]	81.60	3.21	15.91
7	[OR_W] [CA_S] [SM Ori, S_MEX] [SM Occ, CO-UT] [OR_C] [OR_NE] [remaining N&E]	80.41	3.73	15.97

[a]See legend to Figure 1 for full names of populations represented by the abbreviations

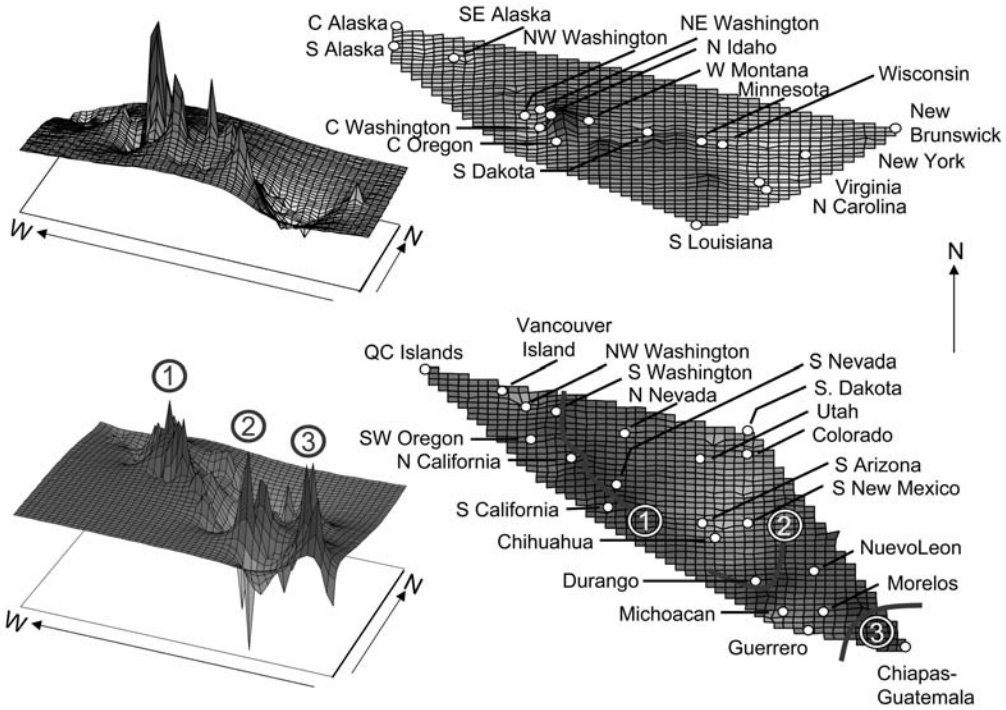

Fig. 5. Results of genetic landscape shape interpolation analyses for N&E (top) and S&W (bottom) clades. Both were constructed using a 50 x 50 grid and a distance weighting parameter of one. The *X* and *Y* axes correspond to geographic locations within the landscape and surface plot heights (*Z* axis) reflect genetic distances. Peaks denote above-average genetic distances in relation to scaled geographic distance and likely indicate barriers to gene flow. A subset of sampling localities are indicated by white dots to provide geographic context in the two-dimensional plot depictions (right side of figure). Barriers identified in the lower panels include: (1) Sierra Nevada–Cascade Range rain shadow (western Great Basin); (2) Mexican Plateau; and (3) the Isthmus of Tehuantepec. Scale differs between top and bottom figures; in a combined analysis (not shown), the magnitude of peaks in the N&E interpolation are much reduced relative to those in the S&E.

Sierra Madre Occidental) but separated birds from Chiapas and Guatemala (not included in the SAMOVA analysis) as a distinct group. Three putative geographic barriers were identified within the range of the S&W clade. A "ridge" (indicating above-average genetic distance, see Fig. 5) running in a northwesterly direction approximated the divide between the Great Basin and the Sierra Nevada and Cascade ranges in the western U.S. A set of peaks near the SE corner of the three-dimensional plot (Fig. 5) identified a second barrier, the Mexican Plateau. Covered mostly by deserts and xeric shrublands, this plateau isolates Sierra Madre Occidental and Rocky Mountain populations from those in the mountain ranges of eastern and southern Mexico. The Isthmus of Tehuantepec formed a third well established barrier that isolated our samples from Chiapas and Guatemala from those throughout the rest of Mexico. Our CNM reconstructions performed better than random predictions. The area under the receiver operating characteristic (ROC) curve was close to one (AUC > 0.96) for the models of each clade. The models predicted relatively severe habitat reductions during the LGM for populations in the N&E clade, and no net loss of habitat for those in the S&W clade (Fig. 6).

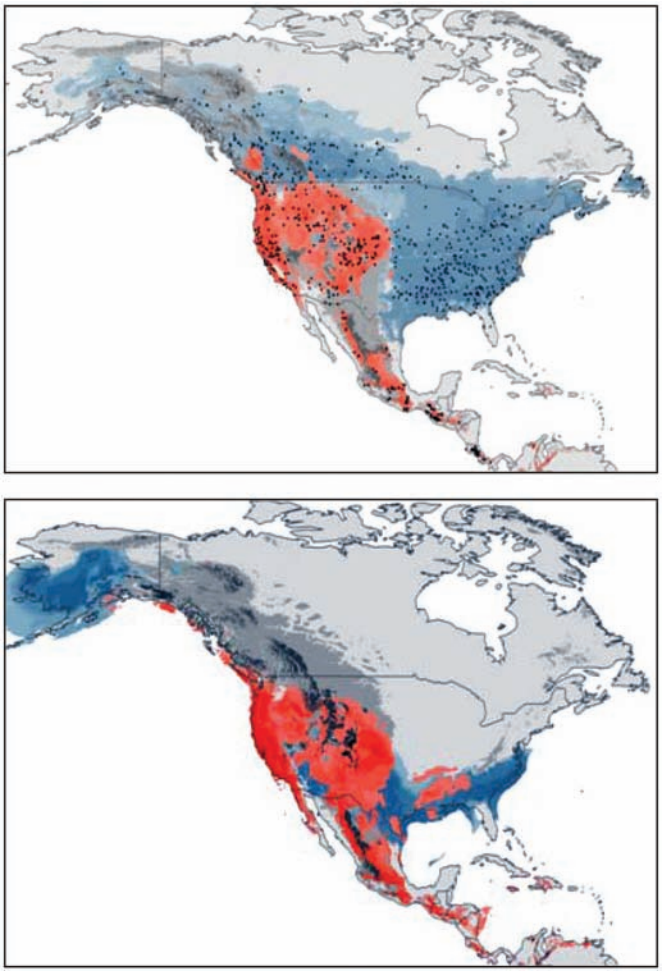

FIG. 6. Averaged climatic niche models for N&E (blue) and S&W (red) clades of the Hairy Woodpecker. The top panel depicts predicted current range based on climate data associated with occurrence records (black dots). The lower panel depicts the predicted distribution of each clade during the LGM (21,000 ybp). Darker red and blue shadings indicate higher logistic probabilities of the species' occurrence. Gray shadings indicate topographic relief.

DISCUSSION

Evolutionary patterns.—Hairy Woodpeckers are partitioned genetically into three distinct mitochondrial clades (Fig. 3, column B): southern Central America (Costa Rica, Panama), boreal and eastern North America (N&E), and southwestern and western North America (S&W). The latter clade is highly structured genetically and geographically with three main additional groups that are identifiable (Fig. 3, column C): Pacific Coast, Rocky Mountain–Sierra Madre Occidental, and Mexico (excluding Sierra Madre Occidental). Despite a relatively extensive zone of introgression among three of these five groups (Fig. 3, map inset) in the Pacific Northwest, gene flow among clades (as measured via mtDNA) appears otherwise to be limited or nonexistent across much of these groups' distributions. A more precise gene flow estimate will require the examination of nuclear genetic markers. We were surprised by this finding, given the clinal nature of size and plumage variation in this species and the presumption that ongoing gene flow was responsible for the general lack of discrete character boundaries (e.g., Mayr 1963; but see Endler 1977). Each of the five identified "groups" (Fig. 3C) appears to be on its own evolutionary trajectory and each could arguably be described as an incipient phylogenetic species or phylogroup; however, both reproductive isolation and reciprocal monophyly appear to be lacking for most.

The breeding distribution of the Hairy Woodpecker extends from central Alaska, south to the Isthmus of Panama. Few non-migratory avian taxa share this broad geographic distribution; however, several widely distributed North American species show a similar pattern of being genetically divided into broad eastern and/or boreal, and western montane assemblages. Examples can be found within several "species complexes" in which members are clearly close relatives but morphologically distinctive enough to have easily been discerned by taxonomists. These include the *Carpodacus* finches (*cassini, purpureus*), sapsuckers (*Sphyrapicus ruber, S. nuchalis,* and *S. varius*), vireos (*Vireo cassini, V. plumbeus,* and *V. solitarius*), Fox Sparrow (thick-billed [*megarhyncha*], sooty [*unalaschensis*], slate-colored [*schistacea*], and red [*iliaca*] forms), Yellow-rumped Warbler (*Dendroica auduboni* and *D. coronata*), bluebirds (*Sialia mexicana, S. currucoides*, and *S. sialis*) and Northern Flicker (red [*cafer*] and yellow-shafted [*auratus*] forms). Less distinct and more cryptic taxa sharing this pattern have been identified using molecular methods, including Swainson's Thrush (*Catharus ustulatus*; Ruegg and Smith 2002), Winter Wren (Drovetski et al. 2004; since split into *Troglodytes pacificus* (W) and *T. hiemalis* (E) [AOU 2010]), and White-breasted Nuthatch (Spellman and Klicka 2007). In the Fox Sparrow, the western montane grouping is further divided into distinctive Rocky Mountain and Sierra Nevada–Cascade range forms (Zink 1994), a pattern also observed in the Blue Grouse (Barrowclough et al. 2004) and the Hairy Woodpecker S&W clade. Our ongoing comparative study of western montane birds suggests that this pattern may not be uncommon. Thus far, we have recovered significant genetic structuring within White-breasted

Nuthatch (Spellman and Klicka 2007), Mountain Chickadee (Spellman et al. 2007), and Brown Creeper (Manthey et al. 2011) along the Great Basin–Sierra Nevada interface.

For those species (or species complexes) whose western distribution extends southward into Mexico, recent molecular studies have consistently identified a genetic discontinuity that separates populations associated with montane regions of Mexico from the major mountain ranges in the U.S. Examples include Yellow-eyed and Dark-eyed Junco (*Junco phaeonotus*, *J. hyemalis*; Milá et al. 2007a) and Yellow-rumped Warbler (Milá et al. 2007b). The observed lack of structure across this region in Chipping Sparrow (*Spizella passerina*; Milá et al. 2006) and White-breasted Nuthatch (Spellman and Klicka 2007) has been interpreted as evidence of recent range expansion out of southern refugia. A novel pattern was observed in Hairy Woodpecker, in which the populations in the southern Rockies and Sierra Madre Occidental formed a group, with the genetic discontinuity pushed farther south, possibly to Central Nayarit where suitable highland habitat is fragmented by several low river valleys (Rio San Pedro Mezquital, Rio Grande de Santiago, and Rio Huaynamota).

Evolutionary history.—Given an evolutionary tree, it is tempting to begin imagining biogeographic evolutionary scenarios that would explain the apparent relationships among clades. In our trees (Figs. 2 and 3), shallow divergences and a general lack of support for many clades suggest that such an exercise would be problematic. The distinctive Costa Rica–Panama form *extimus* provides an example. Plumage and morphometric characters, along with geographic position, strongly suggest affinities with morphologically similar and geographically proximate S&W forms. Bayesian analyses yielded that very result (Fig. 3); however, an alternative placement within the N&E clade is supported by our ML analysis (Fig. 2). Such discrepancies are due in part to a paucity of phylogenetically informative characters but also likely reflect the high degree of coalescent stochasticity associated with mtDNA (single locus) parameter estimates (Edwards and Beerli 2000). This source of error is probably most acute in the S&W clades where less continuously distributed habitats may lead to relatively smaller population sizes. Such small populations are more prone to a loss of diversity due to genetic drift, subsequently leading to a reduction in coalescence times. A more detailed discussion of biogeography and population-specific demographic parameters (such as divergence time estimates, relative population sizes, gene flow estimates, etc.) must await a multilocus data set.

Even when viewed cautiously and under this light, it is clear that the N&E and S&W Hairy Woodpecker clades have had very different recent evolutionary histories. In the S&W, populations are highly structured both geographically and genetically (Table 1) whereas little structure is detected in the widespread (Gulf Coast to central Alaska) N&E clade. Given the northern extent of its distribution, at least some Hairy Woodpecker populations were necessarily displaced southward during cycles of glacial advance. Our patterns of variation (e.g., see Fig. 2, networks) suggest that the N&E clade was compressed into a single, southern refugial population. In contrast, the degree of genetic sorting observed within the S&W clade suggests that each of the three main groups identified (Pacific Coast; Rocky Mountain–Great Basin–Sierra Madre Occidental;, and, eastern and southern Mexico) occupied their own geographically discrete refugium during the LGM.

The climatic niche model of putative Hairy woodpecker distributions during the LGM supports these predictions (Fig. 6). The model predicts that suitable habitat during the LGM for N&E birds was restricted to the southern one-third of the U.S. and widely across southern Alaska and Beringia. The Alaskan population is the most genetically depauperate of all sampled N&E populations (Table 3), indicating that although perhaps inhabitable, Alaska was not occupied by Hairy Woodpeckers during the LGM. This genetic signature is consistent with that of a "leading edge" expansion (*sensu* Hewitt 1996) from southern latitudes. Genetic indices (haplotype diversity, private alleles) for N&E populations in the Pacific Northwest (central Washington, northeast Oregon) were also characteristic of a population bottleneck, suggesting recent colonization into this region from elsewhere. However, all remaining N&E populations exhibited relatively high levels of haplotype diversity and multiple private alleles, unexpected characteristics of populations comprised of recent colonists. We interpret this to mean that either the true refugium was much less restricted than predicted, or it was large enough to retain much of the preexisting genetic diversity, with expansion out of this region at the end of LGM occurring across a broad latitudinal front.

The paleo-model (Fig. 6) for the S&W clade differs considerably, suggesting no net loss of habitat for western Hairy Woodpecker populations during the LGM. Highest probabilities of occurrence are discontinuously distributed and are indicated for the entire Pacific Coast and associated mountain ranges, the southern tip of the northern Rockies (southern Idaho), the central and southern Rockies through the Sierra Madre Occidental, and the Sierra Madre Oriental through the Trans-volcanic ranges in Mexico. These regions correspond well with those defined by the genetic data, suggesting that populations in each region passed the LGM in relative isolation. The relatively high genetic distances, high frequencies of private haplotypes, and geographic structuring of most sampled S&W populations are consistent with a model of long-term population persistence and limited gene flow (Kerdelhue et al. 2009) as suggested by the CNM. The paleo-model also suggests that during the LGM members of both the S&W and N&E clades were more broadly distributed across the southern U.S., with suitable habitat for the N&E clade predicted as far west as the Sierra Nevada range. It is not clear whether the model has overpredicted LGM distributions, or if perhaps competition played a role in maintaining separate eastern and western forms. Nevertheless, our data provide no indication that introgression occurred in the southern U.S. during the LGM.

The western montane groups.—Significant genetic structure was found within each of the three western groups (Pacific Coast, Rockies and Sierra Madre Occidental, Mexico). The geographic and genetic isolate (ssp. *picoideus*) on the Queen Charlotte Islands (haplotype 12, Fig. 2; see also Topp and Winker 2008) is morphologically distinct from the mainland form (Ouellet 1977). Our data show that this is probably because these birds belong to different phylogenetic groups; *picoideus* is part of the S&W clade while British Columbia mainland birds are members of the N&E clade (*contra* Ouellet 1977). Our data suggest that the Queen Charlotte population may represent a geographic holdover from a time when the S&W clade was more widely distributed along the northern Pacific Coast (Fig. 6). Distinctive northern and southern components occur in the Pacific Coast group, where the southern

California ($n = 11$) and Oregon coast ($n = 21$) populations are reciprocally monophyletic. Only when additional intervening sampling localities were added was it apparent that the single haplotype (37, Fig. 2) possessed by all southern California birds also occurs in low frequencies throughout much of California and Oregon. The single southern California haplotype is derived from within a larger overall west coast assemblage (Fig. 2). This fixation of variation in southern California birds could be explained by two evolutionary phenomena: (1) the population is of recent origin from a smaller founder population and has had no subsequent gene flow; or (2) the population has persisted as a small, peripheral population through the LGM (as suggested by the CNM) and as a consequence has lost genetic diversity (Barton 2001, Eckert et al. 2008, Miller et al. 2010). Regardless, the result is surprising because this region has a high probability of Hairy Woodpecker occurrence during the LGM (Fig. 6) and has been identified as a Late Pleistocene refugia for a number of other montane birds (e.g., Spellman and Klicka 2007, Spellman et al. 2007, Alexander and Burns 2006).

The Mexican distribution (excluding Sierra Madre Occidental) of Hairy Woodpecker has three lineages, two of which sort geographically. Although small ($n = 4$), our sample from east of the Isthmus of Tehuantepec (ssp. *sanctorum*) is monophyletic, corroborating the importance of the Isthmus as a common point of diversification for many avian taxa (e.g., Barber and Klicka 2010). Birds in eastern Mexico (ssp. *intermedius,* Nuevo León, Hidalgo, Veracruz) are genetically structured with respect to the more widespread southern form (ssp. *jardinii*) that occupies the Sierra Madre del Sur and the Transvolcanic range. The haplotype exclusivity (Fig. 2, haplotypes 41, 44, 45) exhibited by these two forms suggests an historic separation of populations in eastern and southern Mexico. Determining whether the lack of monophyly is due to recent gene flow or retained ancestral mtDNA polymorphism will require additional sampling.

More subtle structuring is evident within the Rocky Mountain–Sierra Madre Occidental assemblage. Only three haplotypes were identified within the latter (ssp. *icastus*), and only one of these (Fig. 2, haplotype 7) occurred widely throughout the southern Rocky Mountains (ssp. *leucothrectis, monticola*). The Sierra Madre Occidental birds also harbor relatively low levels of genetic diversity (Table 3). From a biogeographical perspective, this lack of diversity is puzzling. The CNM model suggests that Hairy Woodpecker habitat likely expanded in this region during the LGM, and paleoecological data confirm that forests in this region have contracted since then (see Ramamoorthy et al. 1993). Because of the central position of this Sierra within the species' distribution, its relatively immense area, and the likelihood that it has harbored suitable habitat throughout the Pleistocene, it seems unlikely that the observed pattern is due to peripheral or isolation effects (*sensu* Eckert et al. 2008). Instead, perhaps some form of Hairy Woodpecker was present historically, but it was replaced recently by the lineages occurring there today.

Taxonomic considerations.—Below we briefly discuss our findings regarding the evolutionary history of this lineage within the context of current views on subspecies taxonomy. Our goal is to provide, where possible, some insight and clarification; it is not to provide a complete taxonomic revision of the group. The value of subspecies as evolutionary entities has been hotly contested (e.g., Zink 2004, Phillimore and Owens 2006) and it is not our goal to enter into this debate. Nevertheless, few avian taxonomists working today would argue that all recognized avian subspecies are valid (Haig and Winker 2010). Although Hairy Woodpeckers are highly variable morphologically, the clinal nature of the observed variation makes it difficult to satisfactorily define geographically and morphologically discrete entities (Ridgway 1914, Jackson 1970). The problem was succinctly summarized by Lester Short in the most recent complete taxonomic revision of Hairy Woodpecker: "As is often the case in American birds, many races have been described, some of them very weakly characterized and of little or no significance" (Short 1982:326).

Much of the mtDNA variation occurring within Hairy Woodpecker does occur among subspecies. An AMOVA analysis with all samples assigned to putative subspecies (based on distribution, not morphology) indicated that 52.2% of variation was captured by subspecies while 47.8% remained unaccounted for (within subspecies). For comparison, an AMOVA with all individuals assigned to one of the five main geographic groupings (Fig. 3, column C) indicated that 78.6% of the variation occurred among groups and only 21.4% within them.

Distinctions between major eastern and western Hairy Woodpecker groups have long been recognized by avian taxonomists and these roughly correspond to our N&E and S&W clades. This division was based primarily on underpart color (Ouellet 1977, Jackson et al. 2002), which separates the eastern forms *septentrionalis*, *villosus*, *audubonii*, *terraenovae* (Newfoundland, unsampled), *maynardi* (Bahmas, N. Providence, Andros, unsampled), and *piger* (Grand Bahama, Abaco; distributions shown in Fig. 1) from all others. Our results suggest that the British Columbian (B.C.) coastal elements currently recognized as northern forms of *harrisi* are also members of this eastern assemblage. In some taxonomies (e.g., AOU 1957, Short 1982) these B.C. populations were recognized as *sitkensis*, distinct from *harrisi.* Ouellet (1977) recognized some differences in the B.C. birds, but considered these to represent intergradation between *harrisi, septentrionalis*, and *monticola*. Upon corroboration with other characters, either *sitkensis* should be resurrected, or it should be subsumed by the widespread boreal form *septentrionalis*. Our data also suggest that neither *harrisi* nor *monticola* extend north into Canada as subspecies distributions would predict. Rather, in the Pacific Northwest, the northern form *septentrionalis* (of the N&E clade) extends west to the Pacific Coast and south into the northern tier of the United States. The apparent "mismatch" between morphological and genetic groups in this region may be caused by high levels of mtDNA introgression. In hybridizing lineages, rates of mtDNA and nuclear DNA introgression can be discordant (Coyne and Orr 2004). An analysis of nuclear genetic markers will be required for clarification.

Farther east, we found no genetic evidence of differentiation among the three main eastern subspecies (*septentrionalis, villosus, audubonii*). Morphological variation in these is clinal in all respects, with lighter-colored (whiter) and larger birds in the north grading towards slightly darker and much smaller birds along the coast of the Gulf of Mexico (Jackson et al. 2002). We were unable to adequately assess the insular Bahamian forms, *maynardi* and *piger.* Our single sample of the latter was nested within the N&E clade (Fig. 2, haplotype 9) and differed by 3 nucleotide changes from the most common haplotype. The modest genetic differentiation observed is

not matched by the relatively dramatic morphological differentiation in this form. These two forms also differ considerably from one another and additional work on both of them is warranted.

Our Pacific Coast group (excluding *sitkensis*) is comprised of three additional subspecies *picoideus, harrisi* (excluding those north of Vancouver Island), and *hyloscopus*. Genetic structuring along the coast is roughly concordant with the proposed distributions of these subspecies. The Rocky Mountain–Great Basin–Sierra Madre Occidental group identified with genetic data includes four subspecies (Fig. 1), *orius, leucothrectis, icastus*, and *monticola*. Morphology suggests that the dominant form in the Sierra Nevada range is the Great Basin (mostly) subspecies *orius*. We found that most of the Sierra Nevada birds sampled instead possessed Pacific Coast haplotypes. Some introgression along the eastern foothills is likely, although it was not detected by our sampling (Fig. 3). We also found no genetic support for *leucothrectis* in the U.S. southwest. Apparently, strong morphological support is also lacking for this taxon, because neither Short (1982) nor Phillips et al. (1964) could distinguish it from its northern neighbor, *orius*. However, Short (1982) also could not find any consistent differences between the Rocky Mountain form *monticola* (Fig. 1) and the more northerly *septentrionalis* whereas our genetic data place them in alternate S&W and N&E clades. These two forms are similar in size and both typically have snowy white underparts, a feature found in most eastern subspecies. We note that *monticola* (and all other S&W forms) can be distinguished consistently from eastern birds by having significantly less white dorsally, especially on the wing coverts, a character apparently not given much weight by some taxonomists.

The Mexican forms *jardinii, intermedius* and *sanctorum* (Fig. 1) fall out within another of our genetic groups. The geographic and genetic data are concordant with the recognition of these three subspecies. Of these southern forms, Short (1982) failed to recognize (rejected) *intermedius*, and he lumped the Costa Rica–Panama endemic *extimus* in with the morphologically similar but disjunct form *sanctorum*. Despite the morphological similarity, our study indicates that *extimus* and *sanctorum* are genetically distinct and likely not even sister taxa.

The Hairy Woodpecker and continental-scale patterns of diversity.—When a single species occupies a very wide geographic range, we are able to consider how broad-scale phenomena affect the generation and partitioning of diversity within that lineage. There is a well-recognized latitudinal gradient in biodiversity, which is most evident among taxa at the species level and higher (Hillebrand 2004). Although there is some evidence from comparative studies that these among-species latitudinal patterns begin within species, it is not clear whether shorter term, within-species evolutionary phenomena drive the patterns that we see at higher taxonomic levels (Mittelbach et al. 2007, Martin and Tewksbury 2008). In this respect, the insights that we can gain from a single widespread lineage are important. Within the Hairy Woodpecker, latitude is associated with the accumulation of population genetic variation (Fig. 4). Indeed, the N&E clade might be considered a classic example of glacial effects on within-species biodiversity, in that its genetic diversity decreases with increasing latitude and its populations lack structure, especially across regions from which it was excluded during the LGM. However, the S&W clade appears to have been largely decoupled from this same glacial phenomenon and as a consequence harbors more incipient phylogenetic species than its sister clade to the east and north. This strongly suggests that the heterogeneous topography of western North America has been more important than latitude in generating phylogenetic diversity in this lineage.

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