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GENETIC PATTERNS OF DIFFERENTIATION AMONG FIVE LANDBIRD SPECIES FROM THE QUEEN CHARLOTTE ISLANDS, BRITISH COLUMBIA

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Abstract.—The Queen Charlotte Islands (QCI), British Columbia, have many putative endemic avian subspecies. We evaluated four species—Northern Saw-whet Owl (*Aegolius acadicus*), Hairy Woodpecker (*Picoides villosus*), Steller's Jay (*Cyanocitta stelleri*), and Pine Grosbeak (*Pinicola enucleator*), each with a phenotypically described endemic subspecies from QCI—for uniqueness, conservation concern, and management. The Chestnut-backed Chickadee (*Poecile rufescens*), with no endemic subspecies from QCI, was included for comparison. We hypothesized that the four endemics would have similar phylogeographic patterns of genetic divergence and coalescence between QCI and possible source populations, because they may share a glacial-refugium history. Cytochrome *b* was sequenced for all species from Alaska, Washington, and QCI. The four species with endemic phenotypes from QCI had significant genetic divergence from nearby conspecific populations, though variation in divergence times indicated varying colonization histories. Given the corroboration between morphological and genetic evidence for derived populations from QCI, the four endemic subspecies exhibit hallmarks of being evolutionarily significant units (ESUs) and, at the least, should be considered separate management units (MUs), distinct population segments (DPSs), or designatable units (DUs). This is reflected in existing subspecific nomenclature, which our genetic results support. Chestnut-backed Chickadees had genetic differentiation in southeast Alaska as a separate MU but no significant differentiation in QCI. Our results indicate that QCI has been an important area for the generation of avian diversity below the species level and that it is an important area for the conservation and management of birds in northwestern North America. *Received 30 November 2006, accepted 22 August 2007.*

Key words: conservation, endemism, Haida Gwaii, mtDNA, phylogeography, Queen Charlotte Islands, subspecies.

Patrons génétiques de différentiation chez cinq espèces d'oiseaux terrestres des îles de la Reine-Charlotte, en Colombie-Britannique

Résumé.—Les îles de la Reine-Charlotte (IRC), en Colombie-Britannique, ont plusieurs sous-espèces aviaires endémiques putatives. Nous avons évalué quatre espèces—*Aegolius acadicus*, *Picoides villosus*, *Cyanocitta stelleri* et *Pinicola enucleator*, lesquelles ont une sous-espèce endémique aux IRC décrite phénotypiquement—pour leur unicité, leur problématique de conservation et leur gestion. *Poecile rufescens*, qui n'a pas de sous-espèce endémique aux IRC, a été incluse pour fins de comparaison. Nous avons émis l'hypothèse que les quatre sous-espèces endémiques auraient des patrons phylogéographiques de divergence génétique similaires et une coalescence entre les IRC et les populations sources possibles, car elles peuvent partager une histoire de refuge glaciaire. Le cytochrome *b* a été séquencé pour toutes les espèces venant d'Alaska, de l'état de Washington et des IRC. Les quatre espèces avec des phénotypes endémiques aux IRC présentaient une divergence génétique significative avec les populations de même espèce avoisinantes, même si la variation de la divergence dans le temps indiquait des histoires de colonisation variables. Étant donné la corroboration entre les évidences morphologiques et génétiques des populations dérivées des IRC, les quatre sous-espèces endémiques présentent des marques montrant qu'elles sont des unités évolutionellement significatives (UES) et, à tout le moins, qu'elles devraient être considérées comme des unités de gestion séparées (GE), des segments de populations distincts (SPD) ou des unités désignables (UD). Ceci se reflète dans la nomenclature subspécifique existante, laquelle est supportée par nos résultats génétiques. *P. rufescens* présentait une différenciation génétique dans le sud-est de l'Alaska comme une UG séparée mais sans différenciation significative aux IRC. Nos résultats indiquent que les IRC ont été une zone importante pour générer une diversité avienne sous le niveau des espèces et qu'il s'agit d'une zone importante pour la conservation et la gestion des oiseaux dans le nord-ouest de l'Amérique du Nord.

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A high percentage of extinctions occurs on islands, so the taxonomic validity of island endemics, their distributions, and their histories should be a priority both for an understanding of lineage history and for regional management and conservation (BirdLife International 2000, Mayr and Diamond 2001, Cook et al. 2006). Phylogeographic structure may be particularly pronounced in northern areas affected by past glaciations that caused the separation of populations over evolutionary time (Hewitt 1996, Avise 2000, Griswold and Baker 2002). Genetic studies of isolated populations and taxonomic subspecies can be useful for understanding the process of speciation and for determining which populations are evolutionarily significant units (ESUs) and, thus, important for the conservation of biodiversity (Moritz 1994, Avise 2000, Cook and MacDonald 2001). Comparative phylogeography is often used to seek genetic patterns across taxa with similar geographic ranges and can provide insight into the processes and areas generating biodiversity and the regional importance of such areas for conservation (Avise 1994, 2000; Bermingham and Moritz 1998; Moritz and Faith 1998; Cook et al. 2001; Calsbeek et al. 2003).

An important area of within-species biodiversity on the northwest Pacific coast of North America is the Queen Charlotte Islands (QCI, or Haida Gwaii), located ~80 km from the coast of mainland British Columbia and about 50–70 km from the two closest islands of the Alexander Archipelago in southeast Alaska. British Columbia and southeast Alaska were mostly covered with ice during the Wisconsin glaciation, but it has been suggested that there was either a refugium or multiple refugia near QCI that isolated populations and caused differentiation from mainland populations in many taxa (e.g., Warner et al. 1982, Heusser 1989, Pielou 1991, Hetherington et al. 2004, Lacourse et al. 2005, Cook et al. 2006). Endemic species and subspecies described from QCI include plants (Ogilvie 1989), insects (Kavanaugh 1989, Clarke et al. 2001), fish (Moodie and Reimchen 1976, O'Reilly et al. 1993), birds (American Ornithologists' Union [AOU] 1957, Cowan 1989, Sealy 1998), and mammals (Cowan 1989). There are also regional patterns of species with genetically distinct clades or named endemic subspecies with ranges that include QCI and southeast Alaska or coastal Canada; examples include Ermine (*Mustela erminea*; Fleming and Cook 2002) and Northern Goshawks (*Accipiter gentilis*; Sonsthagen et al. 2004). Described phenotypic endemism, coupled with the glacial history of the region, make QCI and southeast Alaska a potentially important area for the generation of biodiversity at high latitudes. Given the importance of this region for forestry and the often dramatic effects that timber harvest has on habitat availability for endemic, forest-dependent lineages, these areas are also important from a regional management and conservation perspective (Cook et al. 2006).

Here, we examine the phylogeography of five regionally codistributed, forest-dependent avian species from QCI to determine whether there is a genetic pattern of differentiation as suggested by subspecific (i.e., phenotypic) endemism and to bring genetic data to bear on regional issues of management and conservation. We asked three main questions: (1) Do avian populations of endemic sedentary subspecies from QCI show genetic differentiation from other regional populations? (2) Are patterns of phylogeographic differentiation and coalescence similar among species? And (3) do genetic data indicate that these areas are important for conservation and management? Specifically, we examined populations of Northern Saw-whet Owls (*Aegolius acadicus*), Hairy Woodpeckers (*Picoides villosus*), Steller's Jays (*Cyanocitta stelleri*), and Pine Grosbeaks (*Pinicola enucleator*). We chose these species because they are forest-dependent and have putative subspecific (i.e., phenotypic) endemism in the region of QCI, and because the endemic populations are nonmigratory (AOU 1957, Cowan 1989, Sealy 1998). Furthermore, we corroborated the phenotypic characters on which the attribution of subspecific endemism was based by reference to the same specimens used to study the population genetics. We also included Chestnut-backed Chickadees (*Poecile rufescens*) for comparative purposes, because they have no described phenotypic endemism in the study region (AOU 1957).

We sequenced the mitochondrial gene cytochrome *b* (cyt *b*) and compared patterns of genetic differentiation within and among these five species. We hypothesized that the four species with phenotypically described endemism from QCI would have similar phylogeographic structure, patterns of genetic divergence, and coalescent properties. Conversely, the Chestnut-backed Chickadee, with no described endemism in the region, is a natural control and would probably not share patterns of differentiation with endemics or have much phylogeographic structure.

Mitochondrial sequence data have little bearing on the validity of phenotypically described subspecies, because the predominantly silent substitutions of mitochondrial DNA (mtDNA) variation at the intraspecific level are expected to be decoupled from differentiation resulting from selection on phenotype (e.g., Bulgin et al. 2003, Mumme et al. 2006). However, subspecific variation does suggest underlying genetic differentiation, and mitochondrial genetic data can help us understand some of the deeper evolutionary history of intraspecific variation. It can also be valuable for genetic diagnoses of populations and regions that warrant special management or conservation attention (Moritz 1994; Avise 2000; Cook et al. 2001, 2006; Phillimore and Owens 2006).

Methods

Sampling.—We included four landbird species with phenotypic endemism from QCI in the study: Northern Saw-whet Owl and its QCI endemic, *A. a. brooksi*; Hairy Woodpecker and its QCI endemic, *P. v. picoideus*; Steller's Jay and its QCI endemic, *C. s. carlottae*; and Pine Grosbeak and its QCI endemic, *P. e. carlottae* (AOU 1957, Cowan 1989, Sealy 1998). The Chestnut-backed Chickadee was used for comparison. All these species are nonmigratory except the nominate Northern Saw-whet Owl, and *A. a. brooksi* is nonmigratory (Sealy 1998). Cytochrome *b* was used because it is a well-studied gene with a fairly constant rate of evolution and has proved useful in many intraspecific population-level studies (Moore and DeFilippis 1997, Avise 2000). Voucher specimens are listed in the Appendix.

For each species comparison, we used three main sample regions: Alaska, Washington–Oregon, and QCI (Fig. 1). Although these are political units, they make biological sense in the context of the northwest Pacific coastal distributions of these species. The Washington–Oregon region covers coastal mainland populations south of QCI. Alaska does not, at first, seem to be a single region, given its large size; however, most Alaska specimens were from the continuous southern coastal area between mountain ranges and

the ocean that shares a glacial history and matches the Alaska distributions for all these species except Pine Grosbeaks and Hairy Woodpeckers, which are also found in the interior (Pielou 1991, Cannings 1993, Greene et al. 1998, Adkisson 1999, Dahlsten et al. 2002, Jackson et al. 2002). We included a single Hairy Woodpecker from Minnesota and several from interior Alaska to increase sample size in this species; the Minnesota sample was not used in statistical population comparisons.

Mitochondrial DNA.—Total genomic DNA was extracted from muscle tissue following Glenn (1997) or DNeasy DNA purification kit protocols (Qiagen, Valencia, California). DNA was amplified for most or all of cytochrome *b* using the following forward and reverse primers: L0-25 (5'-ATGGCCCCAAACATCCGAAAGTCTC-3') and H1117 (5'-GGGTGCTTGCTATTGGGAGTAGGACGAGG-3') for Northern Saw-whet Owls (971 base pairs [bp]); L14841 (Helm-Bychowski and Cracraft 1993) and H16065 (Kocher et al. 1989) for Hairy Woodpeckers, Steller's Jays, and Chestnut-backed Chickadees (1,045 bp); and L14851 (Kornegay et al. 1993) and H16064 (Harshman 1996) for Pine Grosbeaks (1,143 bp). Primer numbers correspond to cyt-*b* nucleotide positions in the chicken, *Gallus gallus domesticus* (Desjardins and Morais 1990). All amplifications were performed using *Taq* DNA Polymerase with buffer B (Promega Corporation, Madison, Wisconsin) and standard polymerase chain reaction (PCR) protocols (Hillis et al. 1996). Samples were purified with PEG precipitation and cycle-sequenced using Big Dye Terminator 3.1 (Applied Biosystems, Foster City, California). The amplified cycle-sequenced product was cleaned using sephadex purification columns and sequenced in both directions using standard protocols on an ABI 373 or 3100 automated sequencer (Applied Biosystems).

Mitochondrial sequence data were edited, aligned, and checked for stop codons indicative of nonfunctional nuclear copies using SEQUENCHER, version 4.1 (Gene Codes, Ann Arbor, Michigan). We then blasted sequence data on NCBI GenBank to ensure

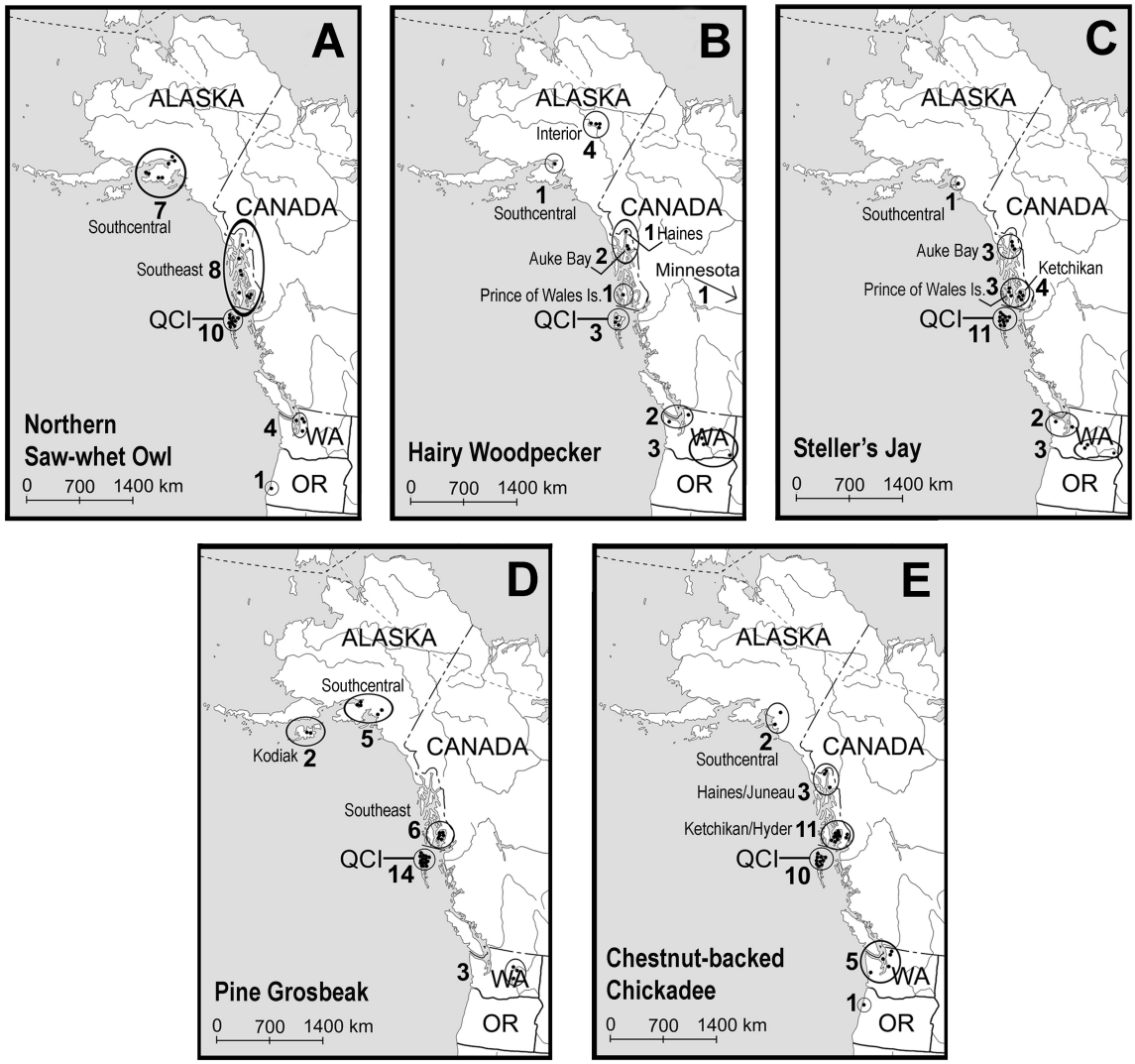

Fig. 1. General sample locations are shown with large circles. Numbers equal sample size within each circle. Black dots indicate approximate locations of individuals and help show sample density in each area.

that the closest matches were avian cyt *b*. Using DNASP, version 3.99.5 (Rozas and Rozas 1999), sequences were examined for haplotype variation, variable base pairs, fixed differences in populations, and number of segregating sites (S). Unrooted median-joining networks were constructed for each species in NETWORK, version 4.2.0.2 (Bandelt et al. 1999; see Acknowledgments). For comparison, statistical-parsimony networks were made with TCS 1.21 (Clement et al. 2000). We also imported sequences into PAUP*, version 4.0b10 (Swofford 2002), and made unrooted parsimony networks for each species and mapped mutations and ambiguity circles onto them by hand. These three methods were used to find the most parsimonious haplotype networks.

Phylogenetic analyses.—The best-fit maximum-likelihood (ML) model of molecular evolution for each species was selected using ML scores from PAUP* and Akaike's information criterion (AIC) for model selection as implemented in MODELTEST, version 3.06 (Posada and Crandall 1998, Posada and Buckley 2004). Maximum-likelihood analyses with heuristic search algorithm, 100 random additions, and TBR branch-swapping were used to reconstruct phylogenetic relationships among individuals in PAUP*, using the selected best-fit models of evolution. Bootstrap support was evaluated by resampling each data matrix 1,000 times (Felsenstein 1985). Trees were rooted with outgroup taxa thought to be closely related. The outgroup sequences were acquired from GenBank or from University of Alaska Museum specimens (GenBank accession numbers available on request).

Bayesian analyses using the same MODELTEST parameters for each species were conducted using MRBAYES, version 3.1 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003, Altekar et al. 2004). Four independent runs starting from random trees were used for each species to ensure that the Markov chain converged on the optimal likelihood value. Trees were sampled every 10,000 generations, and the analyses were run for 8 million generations. All trees sampled before the Markov chain plateaued were discarded (the "burn-in"), and remaining trees were used to approximate posterior probabilities for each phylogeny (Huelsenbeck and Ronquist 2001). Trees were then imported into PAUP*, where majority-rule consensus trees were made with the posterior probabilities of each clade recorded as the percentage of that clade occurring among all the sampled trees (Huelsenbeck and Ronquist 2001).

Population structure and differentiation.—Population differentiation was investigated with population pairwise F_{st} estimates from haplotype frequencies using ARLEQUIN, version 2.0 (Schneider et al. 2000). Significance of *P* values was determined after sequential Bonferroni corrections. To examine population structure, we used Nei's average population pairwise comparisons to test for differentiation between sample regions and to compare QCI and all other samples, again in ARLEQUIN. Homogeneity of mtDNA haplotype distributions within and among populations was assessed using analysis of molecular variance (AMOVA), implemented in ARLEQUIN.

Divergence levels.—An estimate of QCI population-divergence time was calculated for each species using 1.6% (Fleischer et al. 1998) to 2% (Shields and Wilson 1987) sequence divergence per million years. This calculation used net number of nucleotide substitutions per site between QCI and all other populations as calculated in DNASP.

To test the hypothesis of simultaneous divergence or colonization times among species from QCI, we used MSBAYES (Hickerson et al. 2006). This program uses an approximate Bayesian computational (ABC) framework that tests for simultaneous divergence across multiple codistributed taxon pairs using a hierarchical model that incorporates intrinsic variation such as ancestral coalescence and among-taxon demographic histories (Hickerson et al. 2006). This method allows for the simultaneous estimation of three hyper-parameters that characterize the mean ($E[\tau]$), variability (Ω), and number of separate divergence events (Ψ) across multiple population pairs (Hickerson et al. 2006). The ABC method obtains these estimates by simulating data and their summary statistics from the joint prior distribution under a model and then sampling from the resulting joint posterior distribution using probabilities based on the similarity between the summary statistic vector for observed versus simulated data (Hickerson et al. 2006).

We ran 2 million simulations in MSBAYES using the following starting parameters for the upper and lower bounds of prior distributions: θ lower = 0.5 (default), θ upper = 10.0 (based on the highest π_W from observed summary statistics as recommended by Hickerson et al. [2006]), τ upper = 10.0 (based on relatively recent divergence in the past 1 million years), migration rate upper = 10.0 (some migration is possible), recombination rate upper = 0.0 (mtDNA has no recombination), and ancestral population size upper = 0.5 (default). We report joint posterior estimates based on the summary statistic vector **D** that includes the 20 summary statistics (π_{net}, π, θ_W, $\mathrm{Var}[\pi - \theta_W]$ per taxon pair) and a tolerance of 0.001, which yielded estimates based on 2,000 draws from the joint posterior, given that there were 2 million simulated draws from the joint prior.

Results

Haplotype variation and networks.—The five species exhibited varying degrees of intraspecific genetic diversity, with a range of 3–22 haplotypes within each species (Fig. 2). Segregating sites (S) for each species varied accordingly: 2 in Northern Saw-whet Owls, 20 in Hairy Woodpeckers, 20 in Steller's Jays, 37 in Pine Grosbeaks, and 8 in Chestnut-backed Chickadees. Most nucleotide mutations for all species were third-position synonymous changes. The four regionally polytypic species had one to seven haplotypes that were found only from QCI (not shared with other locations), though the haplotype networks showed phylogeographic patterns that were different for each species (Fig. 2).

Haplotype networks made with TCS and NETWORK were almost identical, except for one ambiguity loop in the Steller's Jay network that was not seen with TCS (not shown). However, neither program produced the shortest possible networks across all five species when compared with nucleotide mutations. Northern Saw-whet Owl and Chestnut-backed Chickadee relationships were identical under all methods. Steller's Jays were also very similar. Pine Grosbeaks and Hairy Woodpeckers had larger haplotype divergences that may have interfered with the programs' abilities to find the shortest networks, adding two or three more steps than necessary (not shown). To visualize the most parsimonious networks with relationship ambiguities for all species, we mapped mutations and ambiguity loops onto networks by hand (Fig. 2).

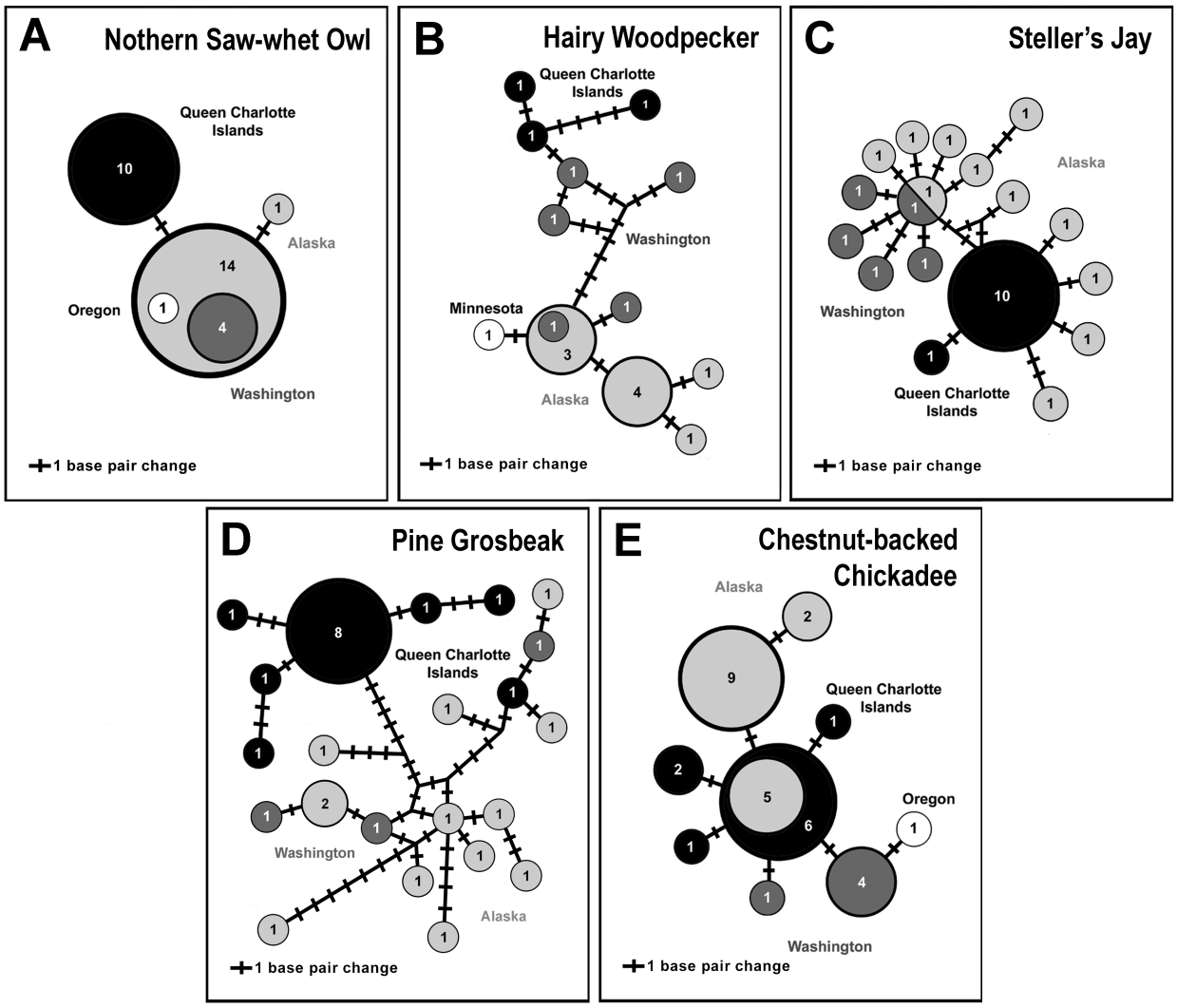

Fig. 2. Haplotype networks showing the relationships of haplotypes and the number of individuals with each haplotype. Colors indicate sample locations: black = Queen Charlotte Islands, light gray = Alaska, dark gray = Washington, and white = Oregon or Minnesota. The size of each circle is proportional to the number of individuals with each haplotype. The length of connecting lines is proportional to the number of base-pair differences between haplotypes.

The endemic population of *A. a. brooksi* from QCI had a single haplotype that differed by one fixed base pair from all other haplotypes (Fig. 2A). Similarly, Hairy Woodpeckers of the endemic population *P. v. picoideus* from QCI had one fixed base pair that differed from all the other specimens (Fig. 2B). The woodpecker network had two distinct groups that were separated by at least six mutations (Fig. 2B). Each of these groups included individuals from Washington. The QCI Steller's Jay population (*C. s. carlottae*) did not share haplotypes with any other population (Fig. 2C). However, five birds from Alaska shared one base-pair difference with QCI that differentiated this group from Washington specimens and the other six Alaska birds (Figs. 2C and 3C).

Six of seven Pine Grosbeak haplotypes from QCI (all birds phenotypically identified as *P. e. carlottae*) shared three fixed mutations and four more almost-exclusive mutations that were each shared with one of three Alaska individuals (not apparent in the network; some mutations are mapped multiple times because haplotypes are very divergent; Fig. 2). The seventh haplotype from QCI occurred in one bird (also identified as *P. e. carlottae*) that was in a separate group that included one Washington and three Alaska birds; this group was separate from the QCI and Washington–Alaska groups by three exclusive base-pair changes (Fig. 2D). All QCI Pine Grosbeak haplotypes had another mutation that was shared with four Alaska and one Washington individual (Fig. 2D).

In contrast to the other species, 6 of 10 Chestnut-backed Chickadees from QCI shared a common haplotype with 5 individuals from Alaska (Fig. 2E). Chickadees also had one fixed difference in a group of 11 southeast-Alaska individuals from Ketchikan and Hyder (Figs. 1E and 2E).

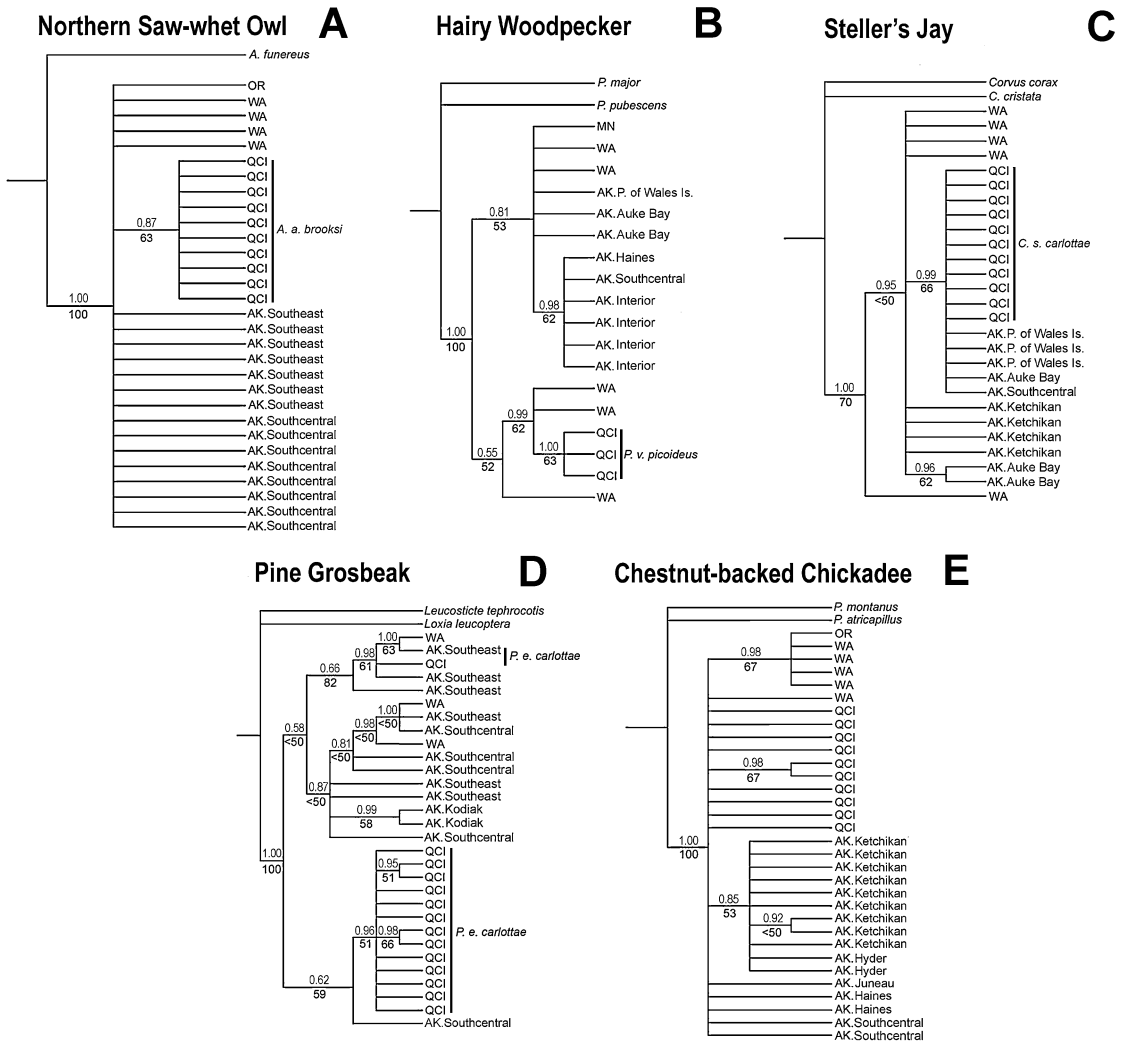

Fig. 3. Fifty-percent majority-rule consensus trees for the five species in the study. Bayesian and maximum-likelihood (ML) trees had the same topologies. Numbers above branches are Bayesian posterior probabilities. Numbers below branches are ML bootstrap support from 1,000 replications. Sample locations: AK = Alaska, QCI = Queen Charlotte Islands, WA = Washington, OR = Oregon, and MN = Minnesota. Subspecies are indicated only where they are important to the discussion.

Phylogenetic patterns.—The best-fit ML models inferred with MODELTEST were used in reconstructing phylogenetic trees for each species: F81 for Northern Saw-whet Owls, K81uf for Hairy Woodpeckers, TrN+I for Steller's Jays and Pine Grosbeaks, and HKY for Chestnut-backed Chickadees (Posada and Crandall 1998). Maximum-likelihood trees reconstructed using cyt-*b* sequence data with 1,000 bootstrap replicates showed the same topology as the Bayesian majority-rule consensus trees (Fig. 3). For all species, a 100,000-generation burn-in was sufficient to sample the Markov chain after it reached a plateau. Therefore, 791 trees were used from across 8 million generations to build majority-rule consensus trees for each species.

The Northern Saw-whet Owl phylogeny had one clade that exclusively included individuals of *A. a. brooksi* from QCI and was supported by a posterior probability of 0.87; the other owls were not significantly differentiated (Fig. 3A).

Hairy Woodpeckers had geographically distinct clades with posterior probabilities of 0.98 and higher (Fig. 3B). One of these clades included the QCI birds, and another was dominated by interior-Alaska birds (Fig. 3B). All the southeast-Alaska individuals, two Washington individuals, and the one Minnesota individual were related to the interior-Alaska-dominated clade (Fig. 3B). Three Washington individuals were more closely associated with the QCI clade (Fig. 3B).

Steller's Jays also showed geographic structure. Five Alaska individuals (three Prince of Wales Island, one Auke Bay, and one south-central) occurred in a strongly supported clade with all the QCI birds (Fig. 3C). Another well-supported clade consisted of

Table 1. Population pairwise F_{st} values (above) and P values (± SD, below). Values in bold are significant P values for F_{st} with experimentwise $\alpha = 0.05$ after Bonferroni corrections.

Species	WA: AK	QCI: WA	QCI: AK	QCI: not QCI
Northern Saw-whet Owl	–0.099	1.000	0.920	0.933
	0.991 ± 0.003	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**
Hairy Woodpecker	0.078	0.000	0.163	0.075
	0.099 ± 0.025	0.991 ± 0.003	0.144 ± 0.031	0.324 ± 0.051
Steller's Jay	–0.019	0.498	0.389	0.359
	0.991 ± 0.003	**0.000 ± 0.000**	**0.000 ± 0.000**	**0.000 ± 0.000**
Pine Grosbeak	0.008	0.212	0.008	0.155
	0.671 ± 0.005	0.122 ± 0.004	**0.001 ± 0.000**	**0.001 ± 0.000**
Chestnut-backed Chickadee	0.395	0.374	0.231	0.172
	0.000 ± 0.000	**0.000 ± 0.000**	0.018 ± 0.018	**0.009 ± 0.009**

Abbreviations: WA = Washington, AK = Alaska, and QCI = Queen Charlotte Islands.

two Auke Bay specimens, but the third Auke Bay specimen was in the QCI clade (Fig. 3C). Southeast-Alaska specimens from Prince of Wales Island and Ketchikan were different; the Prince of Wales Island birds clustered with QCI, and all the Ketchikan birds grouped with Washington (Fig. 3C).

Pine Grosbeaks had the most structure and complexity. The QCI individuals were paraphyletic, but most were in a single clade with a posterior probability of 0.96 (Fig. 3D). One QCI individual occurred in another well-supported clade that included three southeast-Alaska specimens and one from Washington (Fig. 3D). One of the latter was phenotypically identified by plumage and measurements as *P. e. carlottae*, the putative endemic QCI subspecies (UAM 6758), as was the specimen in this clade from QCI (UAM 9265). This result was unexpected but was verified by recutting, re-extracting, and resequencing these samples. Bayesian and ML trees also had a south-central-Alaska specimen (UAM 13086) as sister to the QCI population of *P. e. carlottae* (Fig. 3D). This specimen has been identified by phenotype as an Alaska subspecies, *P. e. leucura*.

Chestnut-backed Chickadees showed less geographic structure than the other species. One of the two most strongly supported clades, each with a posterior probability of 0.98, included four Washington individuals and the single specimen from Oregon (another Washington bird was not in this clade; Fig. 3E). The other clade included two QCI individuals. The 11 southeast-Alaska individuals from Ketchikan and Hyder formed a clade with a posterior probability of 0.85 (Figs. 1E and 3E).

Genetic differentiation.—Population pairwise F_{st} values were used to estimate differentiation between populations (Table 1). Northern Saw-whet Owls showed significant differentiation between QCI and all other populations, with F_{st} values of 0.9–1.0. The Washington and Alaska owl populations were not significantly differentiated (Table 1). Hairy Woodpeckers exhibited nonsignificant differentiation between QCI and other populations, despite substantial phylogeographic structure, likely because our small sample size reduced the power to detect differentiation (Table 1 and Fig. 3B). Steller's Jays had significant F_{st} values between all population pairs except Washington and Alaska (Table 1). Pine Grosbeaks showed significant differentiation between QCI and Alaska and between QCI and all other specimens combined (Table 1). The lack of significant differentiation between QCI and Washington is probably attributable to our small sample size from Washington, given that there was pronounced phylogenetic differentiation (Table 1 and Fig. 3D). Chestnut-backed Chickadees had similar F_{st} values across all population pairs and significant P values after Bonferroni corrections, except for the Alaska-versus-QCI comparison (Table 1). Nei's population pairwise differences using haplotype frequencies (not shown) had similar results.

Analysis of molecular variance within and between populations was conducted to determine how total genetic variation was partitioned among populations within each of the five species (Table 2). Northern Saw-whet Owl was the only species that showed a higher percentage of variation among populations than within populations; in the other species, >68% of total genetic variation occurred within populations (Table 2).

Divergence levels.—Rough estimates of divergence time for each species between QCI and other populations were as follows: 51,500–64,375 years before present (ybp) for Northern Saw-whet Owls, 281,500–351,875 ybp for Hairy Woodpeckers, 46,500–58,125 ybp for Steller's Jays, 243,500–304,375 ybp for Pine Grosbeaks, and 14,500–18,125 ybp for Chestnut-backed Chickadees.

Estimates calculated in MSBAYES for the ratio of variance to mean divergence times ($\Omega = 0.521$, 95% quantiles: 0.045–5.711) and the number of divergence times across taxon pairs ($\Psi = 4.68$, 95% quantiles: 1.126–5.000) did not support a history of simultaneous

Table 2. Analysis of molecular variance among and within populations for all five species in the study. These numbers are the percentage of total genetic variation explained by among-population versus within-population variation.

Species	Among populations (%)	Within populations (%)
Northern Saw-whet Owl	90.96	9.04
Hairy Woodpecker	9.06	90.94
Steller's Jay	30.47	69.53
Pine Grosbeak	14.56	85.44
Chestnut-backed Chickadee	31.64	68.36

divergence or colonization of QCI populations of the five species in the study. Whereas $\Omega = 0$ is expected for a set of species pairs with one divergence event, the probable number of divergence events across all species in the present study was very close to five, or one for each species.

Discussion

The four species with putative phenotypic endemism in QCI populations, Northern Saw-whet Owl, Hairy Woodpecker, Steller's Jay, and Pine Grosbeak, all showed genetic differentiation when QCI was compared with other nearby populations using cyt-*b* sequence data (Figs. 2 and 3). These genetic data support the endemic subspecies originally described from phenotypic differences. However, these species do not share the same phylogeographic pattern in the QCI region, which suggests different divergence times and colonization histories and, possibly, different levels of conservation concern for taxa with QCI endemism (Figs. 2 and 3).

Differentiation.—Results indicate that the putative endemic QCI subspecies *A. a. brooksi* has significant genetic differentiation in addition to phenotypic and behavioral differentiation restricted to QCI (Sealy 1998, Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2006). Phylogenetic analyses indicate substantial separation of QCI Hairy Woodpeckers, but F_{st} values were not significant (Figs. 2 and 3; Table 1). This observed variation in support of QCI differentiation between analytical methods was probably a result of low sample size, though coastal versus interior differentiation (Figs. 1B, 2B, and 3B) may also have contributed. This species proved difficult to obtain in numbers from any of our study areas and warrants more work with increased sampling (Fig. 1B). Despite our small sample size from QCI, it seems that this population is genetically different from other populations.

The relationships found in Steller's Jays may indicate (1) some level of relatively recent gene flow from QCI into southeast Alaska after a history of separation or (2) a recently separated population in QCI that shares ancestral haplotypes with Prince of Wales Island. Either recent movement or incomplete lineage sorting might explain why strong phylogeographic differentiation was not observed in the Bayesian or ML trees outside of the clade containing all QCI individuals (Fig. 3C). At the present time, QCI Steller's Jays appear to represent a genetically and phenotypically distinct population. Supporting this conclusion, a recent study with larger sample sizes and more loci found high levels of differentiation in QCI populations of this species (Burg et al. 2005).

The unexpected clade found in Pine Grosbeaks (Fig. 3D), with two phenotypically identified *P. e. carlottae* having divergent ("non-QCI") haplotypes, suggests that gene flow, incomplete lineage sorting, or both are occurring between QCI and other populations. The fact that one of these birds was from mainland Alaska suggests that gene flow is occurring from QCI to the mainland (AOU 1957). The relationship between the south-central-Alaska individual and the main QCI clade (Fig. 3D) also suggests gene flow or incomplete lineage sorting between Alaska and QCI populations. Uneven sampling may have affected results, because we were able to obtain only three samples from Washington. The complex genetic relationships of Pine Grosbeaks in our study region warrant further research with larger samples sizes, more extensive sampling, and additional loci to disentangle gene flow from lineage sorting as factors affecting the genetic isolation of the QCI population. However, our results indicate that Pine Grosbeaks from QCI are significantly divergent in phenotype and genotype from other conspecific populations.

The observed differentiation among populations of Chestnut-backed Chickadees may be attributable to isolation by distance; this species does not migrate and has very limited movements. Our results support significant genetic differentiation in lower southeast Alaska and between Washington and both QCI and Alaska populations. The most central, and possibly ancestral, haplotype included individuals from south-central Alaska, QCI, and northern southeast Alaska (Fig. 2E), which suggests possible colonization into southeast Alaska from a larger source population. This is tentative, because we have few samples from Washington and Oregon and none from mainland British Columbia.

A recent microsatellite study of Chestnut-backed Chickadees in the same region found high levels of allelic variation in all populations (Burg et al. 2006), which is consistent with our AMOVA analysis and significant F_{st} results found using cyt *b*. Burg et al. (2006) also found distinct genetic differentiation in northern southeast Alaska and QCI, which is not congruent with our mitochondrial results showing differentiation in southern southeast Alaska. Differences in mutation rates and lineage sorting between the mitochondrial and nuclear marker systems used in Burg et al. (2006) and the present study probably contributed to these observed differences.

Patterns across species.—Genetic patterns of these codistributed species in the QCI region seem to differ in several details, while also—among those with phenotypically-based QCI endemics—sharing QCI-related genetic differentiation. The haplotype networks and phylogenetic trees showed that genetic haplotype diversity varies greatly among these species (Figs. 2 and 3). The Northern Saw-whet Owl had low genetic diversity, three haplotypes separated by single base-pair changes, but clear differentiation of the QCI population (Figs. 2A and 3A). In comparison, Hairy Woodpeckers and Pine Grosbeaks had many more haplotypes (12 and 22, respectively) within and among populations than Northern Saw-whet Owls, and they had more complex genetic structure and relationships, with more base-pair differences between haplotypes (Figs. 2 and 3). Steller's Jays also had high genetic diversity (17 haplotypes), but the genetic relationships showed less structure and distance than, for example, Hairy Woodpeckers, and the phylogenetic tree did not have complete genetic separation of the QCI subspecies *C. s. carlottae*, even though haplotypes were not shared between QCI and other populations (Figs. 2C and 3C). Chestnut-backed Chickadees had more genetic variation than Saw-whet Owls but had very few mutations between haplotypes when compared with Hairy Woodpeckers and Pine Grosbeaks.

In agreement with variation in levels of genetic divergence, rough estimates of divergence times based on an assumed avian cyt-*b* clock (Shields and Wilson 1987, Fleischer et al. 1998) suggested multiple different divergence events among these species. On the basis of these date ranges, all the QCI populations of these species appear to have diverged before the end of the last glacial maximum at ~13,000 ybp, except perhaps the Chestnut-backed Chickadee (Sutherland Brown 1968, Pielou 1991, Hetherington et al. 2004). These findings provide some support for divergence in a

glacial refugium in or near QCI for the endemic populations, as opposed to divergence following postglacial colonization; however, these divergence estimates are far from exact (Lovette 2004).

The test of simultaneous divergence of QCI populations using MSBAYES agreed with our other findings that among these species the QCI populations most likely did not diverge simultaneously but at as many as five different times. Observed differences in divergence patterns and haplotype variation among these species are probably attributable to different colonization histories, gene flow, and lineage sorting, but the overlying outcome of the present study is that the species with phenotypically described subspecific endemism from QCI also have significant genetic differentiation of those populations (Maddison and Knowles 2006).

These results are given with the caveat that sampling effects may play some role in the among-species variance in the genetic differentiation observed from QCI. We were unable to sample mainland British Columbia, and these species have wider ranges across North America that we did not sample. Resolution of refugial locations, contemporary levels of gene flow, vicariant histories, and directions of colonization will require increased sampling: numerically, geographically, and with more genetic markers.

Conservation and management.—For conservation and management efforts in southeast Alaska and QCI, the present study provides genetic evidence for population-level divergence among four avian species with endemic subspecies based on phenotypic differentiation ("QCI endemics") and also for one species without such recognized phenotypic endemism (Chestnut-backed Chickadees in southeast Alaska). Patterns of genetic endemism in this region are correlated with phenotypic (subspecific) endemism and are concentrated in QCI. The QCI endemics seem to be on independent evolutionary trajectories compared with mainland populations and are consistent with the high levels of endemism seen among other taxa from QCI (AOU 1957, Moodie and Reimchen 1976, Cowan 1989, Kavanaugh 1989, Ogilvie 1989, O'Reilly et al. 1993, Sealy 1998, Clarke et al. 2001). Isolation in QCI has generated avian diversity below the species level that has been recognized phenotypically and that has genetic corroboration; this diversity should be managed and conserved.

Since scientists first determined the need for defining intraspecific units for effective conservation management, the concepts and unit criteria have been highly debated, and there is still no consensus (Moritz 2002, Green 2005). Four units are often used to define populations for conservation management and evolutionary importance. (1) "Evolutionarily significant unit" (ESU) has been given several proposed definitions, but they all agree that an ESU includes populations that are significantly distinct from other populations on the basis of correlation between more than one type of data, usually including genetic data or distinct adaptive variation (Ryder 1986, Waples 1991, Moritz 1994). (2) "Management unit" (MU) is a population with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles (Moritz 1994). (3) "Distinct population segment" (DPS) is a population that is morphologically or genetically distinct (U.S. Department of the Interior and U.S. Department of Commerce 1996). And (4) "designatable units" (DUs) below the species level may be defined with any of the following criteria: named subspecies or variety, genetically distinct unit, major range disjunction with no gene flow, or biogeographically distinct units that inhabit different ecogeographic regions (COSEWIC 2005, Green 2005). Evolutionarily significant units focus on historical population structure, mtDNA phylogenies, and long-term conservation (Moritz 2002). Management units are concerned with current population structure, allele frequencies, and short-term management (Moritz 1994). The DPS is a politically engendered biological unit used for making conservation or management policy in the United States of America and may or may not be the same as the other categories. Similarly, DUs are politically engendered biological units used by COSEWIC for status assessment (COSEWIC 2005, Green 2005). We used these general descriptions to describe distinct intraspecific units for the five species in the present study.

Given the corroboration of morphological and genetic evidence for derived populations from QCI, the four endemic subspecies of Northern Saw-whet Owls, Hairy Woodpeckers, Pine Grosbeaks, and Steller's Jays seem to exhibit hallmarks of being ESUs, are clearly all DPSs and DUs, and should be considered separate MUs (Ryder 1986, Moritz 1994, U.S. Department of the Interior and U.S. Department of Commerce 1996, COSEWIC 2005, Green 2005). This is reflected in existing subspecific nomenclature, with which our genetic results are concordant. On the basis of genetic data, Chestnut-backed Chickadees in lower southeast Alaska also represent a DPS and a separate MU. These results indicate that QCI has been an important area for the generation of avian diversity below the species level and that it is an important area for bird conservation and management in northwestern North America.

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Appendix. Voucher numbers and GenBank accessions.

Species	Museum	Catalogue numbers	GenBank accession
Aegolius acadicus	UAM	5488, 5851, 6500, 6501, 6901, 6904, 8989–8990, 9180–9181, 13949, 13996, 14940, 17882–17883, 17957, 19042, 19474, 19479, 19481–19485, 10153–10154.	EU075383–EU075412
	UWBM	C. D. Spaw 5022, 67021, 67190, 68205.	
Picoides villosus	UAM	6994, 7579, 9378, 11035, 11540–11541, 13077, 14017, 14096–14097, 15188–15189, 17705.	EU075502–EU075519
	UWBM	50088, 59091, 62606, 62629, 62643.	
Cyanocitta stelleri	UAM	6046–6048, 8507–8509, 8878, 8986, 8961, 9292–9293, 10099, 10138, 10173, 10199, 11199, 11711, 12435, 12444, 13138, 13498, 13929.	EU075413–EU075439
	UWBM	43187, 58632, 59042, 62608, 63692.	
Pinicola enucleator	UAM	6479, 6483, 6649, 6737, 6745, 6758, 8501–8504, 8532, 8794, 9264–9266, 10157, 10174–10175, 11040, 11285–11286, 11287, 11653, 13086, 15182, 17958–17959.	EU075440–EU075469
	UWBM	D. R. Froehlich 176, 60576, 60608.	
Poecile rufescens	UAM	6494, 8365–8369, 11162, 11203, 11315–11316, 11537, 13158–13159, 15109–15110, 15396, 15421, 17563, 17576, 17706–17707, 17746, 17884, 17961–17964.	EU075470–EU075501
	UWBM	47924, 62616, 62642, 63698, 71208.	

Abbreviations: UAM = University of Alaska Museum, and UWBM = University of Washington Burke Museum.