EVIDENCE FROM THE GENETICS OF LANDBIRDS FOR A FORESTED PLEISTOCENE GLACIAL REFUGIUM IN THE HAIDA GWAII AREA

Christin L. Pruett[1,2,6], Carrie M. Topp[2], James M. Maley[2,3], Kevin G. McCracken[2],
Sievert Rohwer[4], Sharon Birks[4], Spencer G. Sealy[5], and Kevin Winker[2]

[1]*Department of Biological Sciences, Florida Institute of Technology, Melbourne, FL 32901*
[2]*University of Alaska Museum and Institute of Arctic Biology, 907 Yukon Dr., Fairbanks, AK 99775*
[3]*Biodiversity Institute, Haub School of Environment and Natural Resources, 1000 E. University Ave.,
Dept. 4304, Laramie, WY 82071*
[4]*University of Washington, Department of Biology and Burke Museum, Box 351800, Seattle, WA 98195*
[5]*Department of Biological Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada*

Abstract. Pleistocene refugia likely contributed to the modern biodiversity of northern areas. Using the mitochondrial DNA cytochrome-*b* gene, we compared 11 forest-dwelling bird species from Haida Gwaii (formerly the Queen Charlotte Islands) with populations from Alaska, Washington, and other locations in the United States. If Haida Gwaii was an unglaciated refugium, its modern populations should feature a high number of endemic lineages and divergence times that predate the end of the last glacial maximum, ca. 13,000–19,000 years before present (ybp). Furthermore, the genetic diversity of these populations should be higher than that in areas colonized after the glacial retreat. Four of the species examined from Haida Gwaii showed old divergences and a high percentage of endemic lineages: the Northern Saw-whet Owl (*Aegolius acadicus*), Hairy Woodpecker (*Picoides villosus*), Steller's Jay (*Cyanocitta stelleri*), and Pine Grosbeak (*Pinicola enucleator*); all four have endemic subspecies on these islands. The Pacific Wren (*Troglodytes pacificus*) and Song Sparrow (*Melospiza melodia*) showed genetic trends associated with populations in refugia, including high genetic diversity on Haida Gwaii. Estimated divergence dates of these six species were fairly uniform (~20,000–30,000 ybp), being greatest for the Hairy Woodpecker (>70,000 ybp) and Pine Grosbeak (>120,000 ybp). There was an association between apparent occurrence in a refugium and a sedentary life-history strategy and a trend for endemic subspecies (4 of 6) also to show this association. Our findings suggest that the Haida Gwaii area hosted a forested refugium during the cycles of climatic change in the late Pleistocene.

Key words: *community genetics, population genetics, seasonal migration, endemism, Queen Charlotte Islands.*

Genética de las Aves Continentales como Evidencia de una Refugio Glaciar Pleistocénico de Bosque en el Área de Haida Gwaii

Resumen. Los refugios pleistocénicos probablemente contribuyeron a la biodiversidad moderna de las áreas del norte. Usando el gen citocromo-*b* del ADN mitocondrial, comparamos 11 especies de aves del bosque de Haida Gwaii (anteriormente conocidas como las Islas de la Reina Charlotte) con poblaciones de Alaska, Washington y otros lugares de los Estados Unidos. Si Haida Gwaii fue un refugio glaciar, sus poblaciones modernas deberían presentar un alto número de linajes endémicos y tiempos de divergencia anteriores al final del último máximo glaciar, ca. 13,000–19,000 años antes del presente (AP). Más aún, la diversidad genética de estas poblaciones debería ser mayor que la de áreas colonizadas luego del retroceso glaciar. Cuatro de las especies examinadas de Haida Gwaii mostraron divergencias viejas y un alto porcentaje de linajes endémicos: *Aegolius acadicus*, *Picoides villosus*, *Cyanocitta stelleri* y *Pinicola enucleator*; las cuatro tienen subespecies endémicas en estas islas. *Troglodytes pacificus* y *Melospiza melodia* mostraron tendencias genéticas asociadas con poblaciones en refugios, incluyendo una alta diversidad genética en Haida Gwaii. Las fechas estimadas de divergencia de estas seis especies fueron bastante uniformes (~20,000–30,000 años AP), siendo mayores para *P. villosus* (>70,000 años AP) y *P. enucleator* (>120,000 años AP). Hubo una asociación entre la presencia aparente en un refugio y una estrategia de historia de vida sedentaria, y una tendencia de las subespecies endémicas (4 de 6) también a mostrar esta asociación. Nuestros resultados sugieren que el área de Haida Gwaii hospedó un refugio de bosque durante los ciclos de cambio climático de fines del Pleistoceno.

INTRODUCTION

Genetic, palynological, and paleontological evidence shows that refugia from glaciation have played an important role in shaping the biodiversity of northern areas (Hewitt 2000, Weir and Schluter 2004, Provan and Bennett 2008). Identifying these refugia and the species they harbored through Pleistocene glacial cycles can inform us about previously unknown genetic diversity among high-latitude lineages (Taberlet et al. 1998, Taberlet and Cheddadi 2002). Furthermore, the habitat requirements of species that persisted in these refugia should inform us about the ecology of refugia during glaciation (Waltari et al. 2007, Godbout et al. 2008, Shafer et al. 2010).

The presence of a refugium during the last glacial maximum (LGM) on Haida Gwaii (formerly the Queen Charlotte Islands), British Columbia, has been debated for over 50 years (Foster 1965, Cowan 1989). Biologists argued for the existence of a refugium on the basis of the numerous taxa endemic to Haida Gwaii (Foster 1965, Heusser 1989) and the discovery of apparently unique genetic lineages (O'Reilly et al. 1993, Soltis et al. 1997, Byun et al. 1997, 1999, Clarke et al. 2001, Smith et al. 2001, Fleming and Cook 2002, Topp and Winker 2008). Even the Haida language suggests the Haida people have long lived in this isolated place: linguists classify Haida as an isolate, unrelated to the language of any other west-coast tribe (Vaillant 2005). However, early geological studies suggested that during the Pleistocene Haida Gwaii and neighboring British Columbia (Fig. 1) were completely glaciated (except for exposed mountaintops, or nunataks), becoming completely ice-free approximately 13,000 years before present (ybp) (Sutherland Brown and Nasmith 1962). In addition, genetic lineages that were once considered unique to Haida Gwaii have been found elsewhere, casting doubt on whether these lineages evolved in a Haida Gwaii refugium (Demboski et al. 1999, Johnson and Taylor 2004). Studies have identified areas on Haida Gwaii that might have been ice-free as early as 16,000 ybp (Warner et al. 1982, Ramsey et al. 2004); Clark et al. (2009), however, suggested that the end of the LGM in the northern hemisphere began approximately 19,000 ybp. Thus being ice-free 16,000 ybp does not necessarily support the idea that a refugium existed on Haida Gwaii.

Here we analyze the population genetics of a suite of forest-dwelling landbirds to shed further light on whether there was a Pleistocene refugium on or near Haida Gwaii. Birds are useful for this comparison because several endemic subspecies breed only on Haida Gwaii, and for the Song Sparrow (*Melospiza melodia*), Steller's Jay (*Cyanocitta stelleri*), and Townsend's Warbler (*Setophaga townsendi*) genetic evidence has suggested a refugium in the Haida Gwaii area (Zink and Dittmann 1993, Fry and Zink 1998, Burg et al. 2005, Krosby and Rohwer 2009, Lait et al. 2012). We sampled populations of 11 forest bird species. Six of these species have subspecies endemic to Haida Gwaii (Table 1). These 11 species represent a large portion of the forest-dwelling birds currently breeding on Haida Gwaii (Cowan 1989) and almost all of its endemic subspecies. We contrasted Haida Gwaii populations of each of these species with populations from Washington and Oregon (a refugium south of the ice during the LGM; Pielou 1991), from southeast Alaska, from more northerly locations in Alaska if the species breeds that far north, and from several other locations in the United States that might correspond to other refugia (Weir and Schluter 2004, Waltari et al. 2007; Fig. 1).

Isolation in refugia should leave a variety of genetic signatures in populations that persisted in them (Fig. 2). For example, if Haida Gwaii populations were isolated during glacial cycles and are still isolated on the islands, they should include divergent lineages in which lineage sorting is complete or nearly complete. Furthermore, the estimates of these lineages' dates of divergence should predate the end of the last ice age, which occurred between 13,000 (Sutherland Brown and Naismith 1962) and 19,000 ybp (Clark et al. 2009), as found for one of two haplotype lineages of Townsend's Warbler from the Haida Gwaii area (Krosby and Rohwer 2009). Where populations did not persist in a refugium, rapid post-glacial population expansion tends to leave a pattern of reduced genetic diversity in populations founded from ancestors isolated in a refugium (Ibrahim et al. 1996, Hewitt 1996, 2000), a pattern typical of many northern populations (Hewitt 2001). When a refugial population serves as a source for other populations, we expect higher genetic diversity in the refugial source population than in the population founded after glaciation (Hewitt 1996). However, if a refugial population was small and remains isolated from immigrants (as might be expected for island taxa), then lower genetic diversity is expected (Nei et al. 1975).

The 11 species we sampled vary in seasonal status, from being sedentary (seven species) to partial migrants (two species) to obligate seasonal migrants (two species; Table 1). Seasonal migration might facilitate dispersal and thus increase
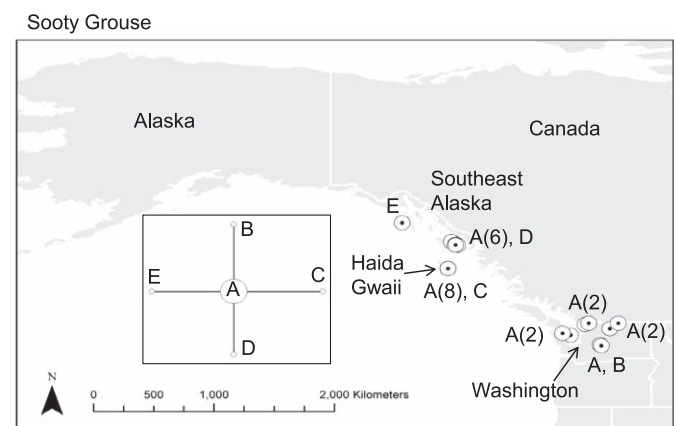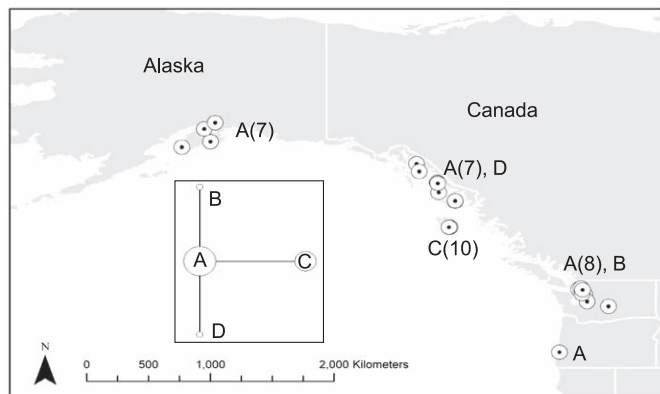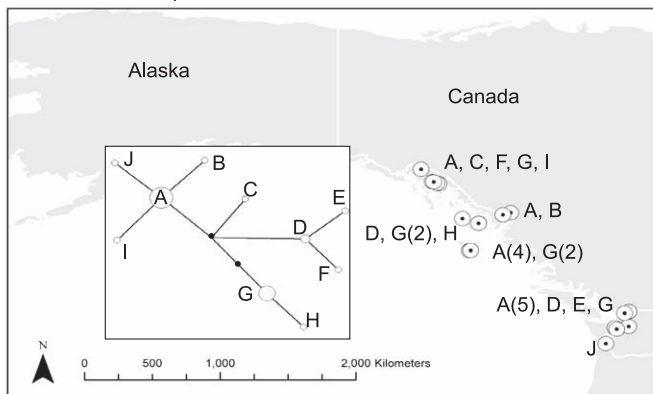

FIGURE 1.   Locations of sampling (labeled on map for the Sooty Grouse) and mtDNA haplotype networks for the 11 species addressed in this study. Haplotypes are uniquely identified, and the number of individuals possessing the haplotype is in parentheses.
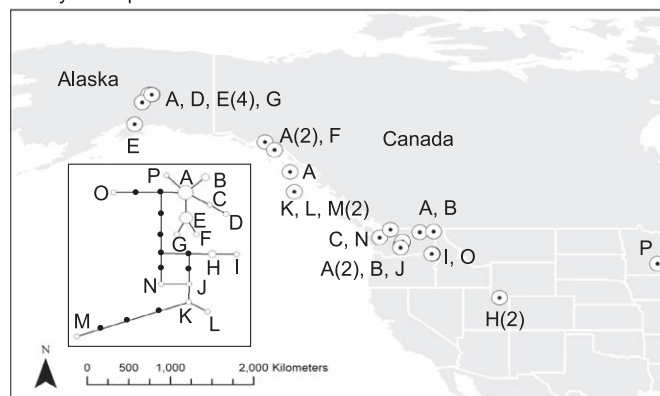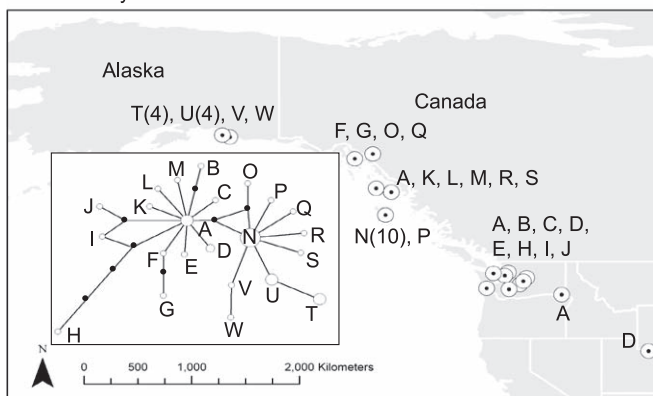
FIGURE 1.   *Continued*

Pacific Wren

Chestnut-backed Chickadee

Song Sparrow

Pine Grosbeak
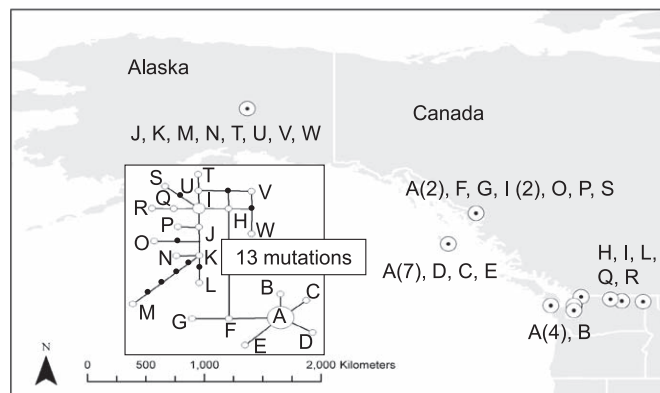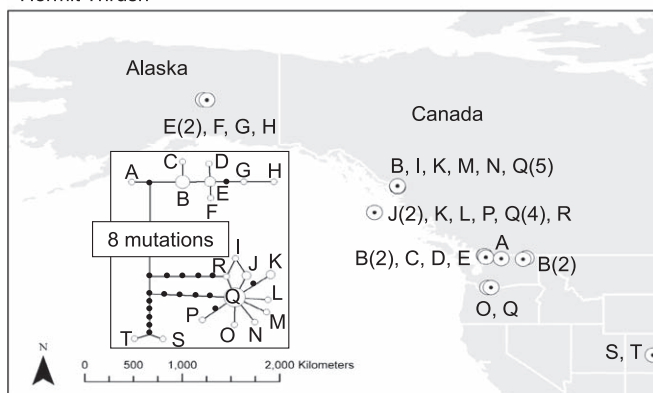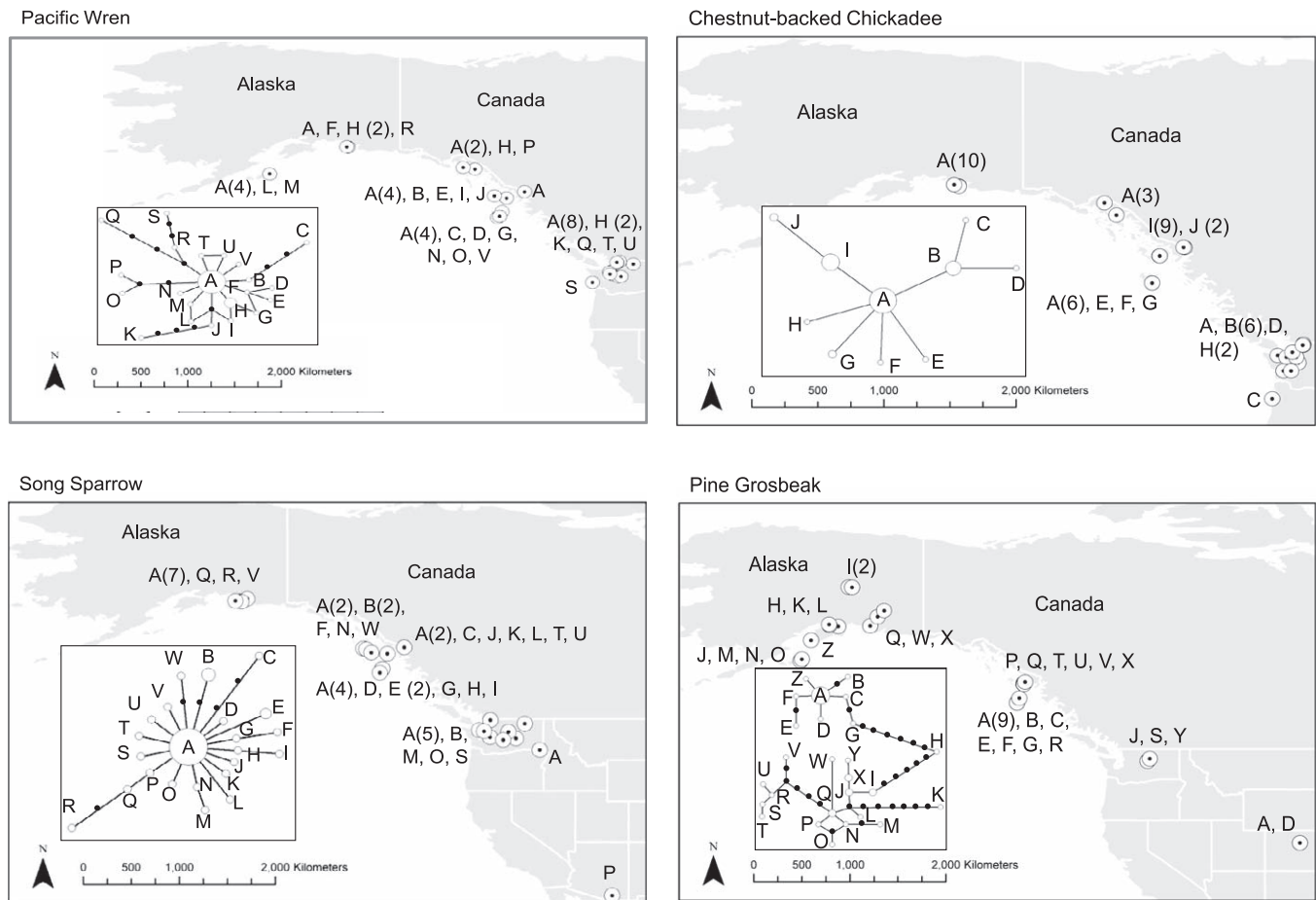
FIGURE 1.   *Continued*

gene flow among migratory populations (Paradis et al. 1998, Arguedas and Parker 2000, Pruett et al. 2008). If the rate of gene flow among seasonally migratory populations is currently high, this could swamp the genetic signal of a refugium by lowering the number of endemic lineages or by increasing the genetic diversity of a population that colonized after the LGM.

We examined genetic data in relation to possible trends that isolation in a refugium might cause in affected populations (Fig. 2). This allowed us to address the following questions: (1) Is there evidence of a forested refugium during the LGM among Haida Gwaii landbirds? (2) Do migratory and resident populations show different genetic signals of isolation? (3) Do taxa with recognized subspecies show genetic signals of isolation? And (4) what general inferences can be made about the historical ecology of this region?

METHODS

We sequenced the mitochondrial (mt) DNA cytochrome-*b* gene. This is a well-characterized marker that has a fairly uniform evolutionary rate across a variety of taxa, demonstrated utility

in the identification of populations that have been isolated, and has proven useful in dating population divergences and in comparative studies among species (Moore and DeFilippis 1997, Avise 2000). Specimens of migratory populations were collected during the breeding season, except for a few individuals from Washington (Appendix, available at http://dx.doi.org/10.1525/cond.2013.120123).

We extracted whole-genomic DNA from muscle tissue from each bird with a DNeasy tissue kit (Qiagen, Valencia, CA), and we amplified and cycle-sequenced most of the mitochondrial cytochrome-*b* gene (943–1143 bp) with a variety of primer sets. Primers used for each species were as follows: Sooty Grouse (*Dendragapus fuliginosus*; 1, 2), Northern Saw-whet Owl (*Aegolius acadicus*; 3, 4), Red-breasted Sapsucker (*Sphyrapicus ruber*), Hairy Woodpecker (*Picoides villosus*; 1, 2), Pacific Wren (5, 6), Steller's Jay (1, 2), Swainson's Thrush (*Catharus ustulatus*; 5, 7), Hermit Thrush (*C. guttatus*; 7, 8), Chestnut-backed Chickadee (*Poecile rufescens*; 1, 2), Song Sparrow, and Pine Grosbeak (*Pinicola enucleator*; 7, 9), with 1 representing L14841 (Helm-Bychowski and Cracraft 1993), 2 H16065 (Kocher et al. 1989), 3 owlF, 4 owlR (Topp and Winker

TABLE 1.   Taxa sampled from Haida Gwaii with their status as endemics and migrants.

Species	Subspecies[a]	Migration	References
Sooty Grouse (*Dendragapus obscurus*)	*munroi*	Sedentary	Griscom 1923, Zwickel and Bendell 2005
Saw-whet Owl (*Aegolius acadicus*)	*brooksi*	Sedentary	Rasmussen et al. 2008, Sealy 1998
Red-breasted Sapsucker (*Sphyrapicus ruber*)		Sedentary	Walters et al. 2002
Hairy Woodpecker (*Picoides villosus*)	*picoideus*	Sedentary	Jackson et al. 2002
Steller's Jay (*Cyanocitta stelleri*)	*carlottae*	Sedentary	Greene et al. 1998
Pacific Wren (*Troglodytes pacificus*)		Partial migrant	Hejl et al. 2002
Swainson's Thrush (*Catharus ustulatus*)	*phillipsi*	Obligate migrant	Mack and Yong 2000
Hermit Thrush (*Catharus guttatus*)		Obligate migrant	Jones and Donovan 1996
Song Sparrow (*Melospiza melodia*)		Partial migrant	Arcese et al. 2002
Pine Grosbeak (*Pinicola enucleator*)	*carlottae*	Sedentary	Adkisson 1999
Chestnut-backed Chickadee (*Poecile rufescens*)		Sedentary	Dahlsten et al. 2002

[a]If endemic to Haida Gwaii.

2008), 5 L1650 (5′-ATTGCCTCCCACCTAATCGACC-3′), 6 H1150 (5′-GGAGGTTGGCAACTAGGGTTC-3′), 7 H16064 (Harshman 1996), 8 L14703 (C. Huddleston, pers. comm.), and 9 L14851 (Kornegay et al. 1993). We sequenced the amplified products in both directions with an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA) and aligned the sequences and verified protein coding with Sequencher 3.1 (Gene Codes, Ann Arbor, MI). All sequences were deposited in GenBank (accession numbers KF208899–209128; see Pruett 2002, Topp and Winker 2008, Pruett and Winker 2010).

STATISTICAL ANALYSES

To assess levels of genetic diversity, we calculated haplotype diversity (*H*; a haploid equivalent of heterozygosity; Nei 1987) and nucleotide diversity (π) in the program DnaSP (Rozas and Rozas 1999). We compared estimates of haplotype diversity in Haida Gwaii with those of other sampled populations by comparing 95% confidence intervals based on standard deviations from DnaSP. We illustrated haplotype networks with the software NETWORK version 4.5.10 (Bandelt et al. 1999). We tested the neutrality of mtDNA sequence variants via Tajima's (1989) *D* and Fu and Li's (1993) *D** and *F** statistics, as implemented in DnaSP. Significance of neutrality tests were assessed by the total number of mutations in each sample.

We used the software IM (Nielsen and Wakeley 2001, Hey and Nielsen 2004) to simultaneously estimate the population-size parameter θ, migration rate (*m*), and time since divergence in years (*t*) between two populations scaled to the neutral mutation rate. Pairwise comparisons were between Haida Gwaii and all other locations that, on the basis of haplotype networks, possessed a haplotype of the same haplotype grouping as Haida Gwaii. For example, the Pine Grosbeak has two clades of haplotypes (Fig. 1), one of which is made up primarily of individuals from Haida Gwaii, except for two individuals from
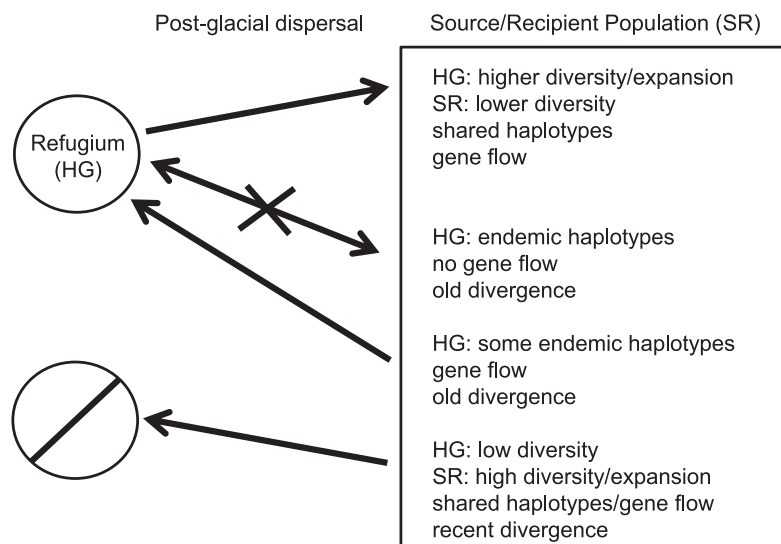

FIGURE 2.   Diagram of genetic signatures expected in mitochondrial DNA if Haida Gwaii was or was not an unglaciated refugium for birds during the last glacial maximum (LGM).

Colorado (haplotypes A and D), and haplotype Z, which is found in Alaska. In IM analyses, specimens from Haida Gwaii ($n =$ 15) were compared with a sample ($n = 15$) from Colorado and Alaska combined. Specimens from southeast Alaska and Washington were not included in the analyses because they are clearly divergent and do not share haplotypes. We used this approach to examine the most likely cause (e.g., incomplete lineage sorting, recent gene flow, recent divergence) of haplotypes in Haida Gwaii and other sampled locations being shared or similar. Mutation rates (substitutions per locus per year) were based on 2% sequence divergence per million years (MY) from molecular clocks developed for cytochrome-*b* across a variety of bird taxa (Weir and Schluter 2008). There is debate about the utility of molecular clocks for dating recent population divergences (e.g., Ho et al. 2005, Penny 2005). Therefore, we also used a range of mutation rates (1–5 % per MY) in our analyses to set prior values for scalars of mutation rates. More complex models that incorporated differences in effective population size or migration rates among populations were unable to converge, probably because of the limited size of the datasets and the single-locus approach. Therefore, we used a simple model in IM that assumes equal migration between pairs of populations and equal effective population sizes. We were examining populations that might have expanded their distribution recently and thus violate an assumption of the IM model. However, recent simulation studies have found that IM is robust to violations of the assumption of stable population size (Strasburg and Rieseberg 2010).

We used the HKY model of molecular evolution (Hasegawa et al. 1985), which takes into account multiple nucleotide substitutions at the same position, nucleotide-frequency differences, and any transition/transversion bias, to generate Bayesian posterior distributions for each estimated parameter. Following the approach of Peters et al. (2007), we first ran IM with large, flat priors and then defined priors that encompassed the posterior distribution of each parameter. To ensure that the Markov chain Monte Carlo algorithm was converging correctly, we ran multiple independent chains (at least three per analysis pair) with different starting values and ran chains of varying lengths (500,000–1,000,000 burn-in; 1,000,000–10,000,000 steps) and assessed effective samples size for each parameter for each run. We accepted only runs with effective samples sizes >150 for each parameter (Peters et al. 2007). We derived parameter estimates from the modes of each distribution and determined Bayesian credibility intervals by taking the shortest intervals containing 95% of the posterior distributions. IM analyses of the Pacific Wren failed to converge, so we excluded that species from the set of those analyses.

RESULTS

GENETIC DIVERSITY AND ENDEMISM

Values of haplotype (H) and nucleotide (π) diversity in Haida Gwaii populations varied widely among the 11 species studied (Table 2). In some species diversity was very low (e.g., Sooty Grouse, Northern Saw-whet Owl, Red-breasted Sapsucker, and Steller's Jay); in others it was higher (e.g., Hermit Thrush, Pacific Wren, and Song Sparrow). Diversity was also high in the Hairy Woodpecker, but because only four individuals from Haida Gwaii were available this result should be viewed with caution. In some species genetic diversity in Haida Gwaii was significantly lower than in Washington populations; these include the Sooty Grouse, Red-breasted Sapsucker, Steller's Jay, and Swainson's Thrush (Table 2). Genetic diversity in the Pacific Wren and Song Sparrow was greater on Haida Gwaii than in the other areas sampled (Table 2). The Northern Saw-whet Owl had low diversity across all locations examined, with only a single haplotype found on Haida Gwaii. Tajima's D and Fu and Li's $D*$ and $F*$ statistics differed significantly from zero for some populations, including, in Haida Gwaii, the Pine Grosbeak ($D = –2.13$, $P < 0.01$, $D* = –2.62$, $P < 0.02$, $F* = –2.85$, $P < 0.02$), Swainson's Thrush ($D = –1.74$, $P < 0.05$, $D* = –2.01$, $0.10 > P > 0.05$, $F* = –2.179$, $0.10 > P > 0.05$), and Pacific Wren ($D = –1.80$, $P < 0.05$, $D* = –1.98$, $0.10 > P > 0.05$, $F* = –2.18$, $0.10 > P > 0.05$); in southeast Alaska, the Hermit Thrush ($D* = –1.96$, $P < 0.05$, $D* = –2.23$, $P < 0.02$, $F* = –2.44$, $P < 0.02$) and Song Sparrow ($D = –2.09$, $P < 0.05$, $D* = –2.52$, $P < 0.05$, $F* = –2.76$, $P < 0.05$); in Washington, Steller's Jay ($D = –1.32$, $P > 0.10$, $D* = –2.94$, $P < 0.05$, $F* = –3.06$, $P < 0.05$), Song Sparrow ($D = –1.80$, $P < 0.05$, $D* = –2.08$, $P < 0.05$, $F* = –2.25$, $P < 0.05$), and Pacific Wren ($D = –2.10$, $P < 0.05$, $D* = –2.60$, $P < 0.05$, $F* = –2.83$, $P < 0.02$). These results are consistent with either a departure from neutrality or a population expansion.

Ten species had some proportion of endemic haplotypes on Haida Gwaii (Fig. 1). For three species on Haida Gwaii lineage sorting was complete, with no haplotypes shared with any other population: the Northern Saw-whet Owl (1 haplotype), Hairy Woodpecker (3 haplotypes), and Steller's Jay (2 haplotypes). Swainson's Thrushes from Haida Gwaii, Washington, and southeast Alaska shared haplotypes that were not found in interior Alaska, and Hermit Thrushes from Haida Gwaii shared haplotypes with populations in southeast Alaska and south-central Washington on the east side of the Cascade Range along the Columbia River (Fig. 1). In both thrushes divergences between Pacific coastal and interior locations were wide (Fig. 1). The Sooty Grouse, Red-breasted Sapsucker, Chestnut-backed Chickadee, Pacific Wren, and Song Sparrow had haplotypes found commonly at all locations we sampled (Fig. 1). The most unusual pattern occurred in the Pine Grosbeak, in which the most common haplotype on Haida Gwaii (haplotype A; Fig. 1) was also found in Colorado but not at locations nearer Haida Gwaii.

Genetic migration rates. For the Northern Saw-whet Owl, Hairy Woodpecker, Steller's Jay, Swainson's Thrush, Chestnut-backed Chickadee, Song Sparrow, and Pine Grosbeak, rates of effective movement (m) between Haida Gwaii populations and other closely related populations were less than 1.0 and had marginal posterior distributions that overlapped zero (Table 3, Fig. 3), implying little or no gene flow between Haida

Gwaii and other populations. For Swainson's Thrush and the Song Sparrow, however, the upper bound of these distributions became flat (did not return to zero probability of occurrence), so zero and very high levels of gene flow are indistinguishable (Fig. 3). This same pattern (but with a higher mode for the migration parameter) also occurred in the Sooty Grouse and Hermit Thrush (Table 3, Fig. 3), suggesting that information in these species' datasets was insufficient for rates of gene flow to be estimated. For the Red-breasted Sapsucker and Pacific Wren marginal posterior densities did not overlap zero but had flat tails that indicated high gene flow.

DIVERGENCE ESTIMATES

For some species, estimates of the time (t) parameter in years were relatively shallow, suggesting post-glacial colonization of Haida Gwaii; these included the Sooty Grouse, Red-breasted Sapsucker, and Chestnut-backed Chickadee (Table 3). For others, estimates of divergence time predated the LGM (>13,000 ybp; based on the earliest estimate) but the 95% credibility interval overlapped this estimate; these included the Swainson's Thrush, Hermit Thrush, Pacific Wren, and Song Sparrow. The credibility interval for Steller's Jay overlapped the end of the LGM if it occurred at 19,000 ybp (Table 3, Fig. 3), and three species, the Northern Saw-whet Owl, Hairy Woodpecker, and Pine Grosbeak, had credibility intervals that did not overlap the end of the LGM even if it occurred 19,000 ybp (Table 3; Fig. 3). For the Sooty Grouse, Red-breasted Sapsucker, Swainson's Thrush, and Hermit Thrush the time parameters overlapped a divergence estimate of zero and became flat, so t could not be estimated for these species. The estimated divergence of the Pine Grosbeak was the oldest by a large margin (Table 3, Fig. 3).

EFFECTS OF SEASONAL MIGRATION

There was a trend for species sedentary on Haida Gwaii, including the Northern Saw-whet Owl, Hairy Woodpecker, Steller's Jay, and Pine Grosbeak, to show evidence of differentiation in a refugium. Also, four of six species (Table 1) with described endemic subspecies show evidence of isolation in a refugium, although subspecies *munroi* of the Sooty Grouse was not recognized by the AOU (1957). We found no signal of a refugium in one of the two obligate seasonal migrants (Swainson's Thrush). For all four seasonal migrants estimates of gene flow were high (Pacific Wren) or indeterminate (Swainson's Thrush, Hermit Thrush, and Song Sparrow) (Table 3). The Red-breasted Sapsucker, in which the Haida Gwaii population is considered sedentary but neighboring populations are migratory (Walters et al. 2002), did not show a signal of refugial isolation.

DISCUSSION

REFUGIAL SIGNALS

If birds colonized newly available habitats from Haida Gwaii after the LGM, we should find higher genetic diversity on Haida Gwaii than in colonized areas, signals of a population expansion from Haida Gwaii, haplotypes shared between Haida Gwaii and colonized areas, and gene flow (Fig. 2; Ibrahim et al. 1996, Hewitt 1996, 2000). The Song Sparrow and Pacific Wren fit some of these expected patterns; in both species haplotypic diversity was significantly greater on Haida Gwaii than elsewhere (Table 2), Haida Gwaii and other areas shared haplotypes, and the rate of gene flow was high. The Pacific Wren also shows a signal of population expansion on

TABLE 2.   Sample size (n), haplotype diversity (H), and nucleotide diversity (π) of 11 species of forest birds from the four primary sampling locations in this study.

Species	Haida Gwaii			Washington			Southeast Alaska			Other Alaska[a]		
	n	H	π	n	H	π	n	H	π	n	H	π
Sooty Grouse	9	0.22	0.00036	8	0.51[b]	0.00054	8	0.46[b]	0.00075	—	—	—
Saw-whet Owl	10	0.00	0.00000	10	0.20	0.00021	8	0.25	0.00026	7	0.00	0.00000
Red-breasted Sapsucker	6	0.53	0.00154	9	0.78[b]	0.00354	11	0.93[b]	0.00265	—	—	—
Hairy Woodpecker	4	0.83	0.00318	10	0.91	0.00417	4	0.50	0.00096	8	0.64	0.00113
Steller's Jay	11	0.18	0.00017	9	0.92[b]	0.00238	10	1.00[b]	0.00315	10	0.73[b]	0.00138
Swainson's Thrush	10	0.53	0.00094	10	0.87[b]	0.00950	9	0.94[b]	0.00954	8	1.00[b]	0.00475
Hermit Thrush	10	0.84	0.00154	10	0.87	0.00688	10	0.78	0.00429	5	0.90	0.00198
Pacific Wren	10	0.87	0.00269	15	0.72[c]	0.00180	13	0.86	0.00203	11	0.80	0.00146
Chestnut-backed Chickadee	10	0.64	0.00073	11	0.60	0.00067	14	0.56	0.00061	10	0.00	0.00000
Song Sparrow	10	0.84	0.00147	10	0.67[c]	0.00106	15	0.93	0.00208	10	0.53[c]	0.00115
Pine Grosbeak	15	0.66	0.00230	3	1.00	0.00554	6	1.00[b]	0.00519	13	0.99[b]	0.00562

[a]See Fig. 1 for other Alaska sampling locations for each species. The ranges of the Sooty Grouse and Red-breasted Sapsucker do not extend north of southeast Alaska.

[b]Significantly higher haplotype diversity in comparisons with the Haida Gwaii population, assessed on the basis of 95% confidence intervals not overlapping.

[c]Significantly lower haplotype diversity in comparisons with the Haida Gwaii population, assessed on the basis of 95% confidence intervals not overlapping.

TABLE 3.   Number of base pairs (bp) sequenced and parameter estimates of divergence time (t) in years before present and migration (m) for Haida Gwaii versus a grouping of all other locations at which were represented haplotypes of the same clade as Haida Gwaii haplotypes.

Species	bp	t[a]	m
Sooty Grouse	1142	5.5×10^3 (2.3×10^3–2.3×10^5)[b]	>10.0[c,d]
Northern Saw-whet Owl	971	3.3×10^4 (2.6×10^4–1.0×10^6)	0.25[c]
Red-breasted Sapsucker	1042	4.3×10^3 (5.3×10^3–9.3×10^5)[b]	>10.0[d]
Hairy Woodpecker	1045	7.2×10^3 (2.8×10^4–9.2×10^5)	0.12[c]
Steller's Jay	1045	3.3×10^4 (1.6×10^4–3.9×10^5)	0.13[c]
Swainson's Thrush	1060	1.9×10^4 (3.2×10^3–1.3×10^5)[b]	0.15[c,d]
Hermit Thrush	1011	2.0×10^4 (3.6×10^3–2.4×10^5)[b]	>10[c,d]
Pacific Wren	949	—	—
Chestnut-backed Chickadee	1045	1.8×10^4 (1.2×10^4–9.4×10^5)	0.43[c]
Song Sparrow	1137	2.2×10^4 (8.5×10^3–1.2×10^5)	0.81[c,d]
Pine Grosbeak	1143	$.3 \times 10^5$ (3.7×10^4–8.3×10^5)	0.09[c]

[a] 95% credibility intervals in parentheses.
[b] Parameter overlaps zero and has no upper bound.
[c] Parameter's distribution overlaps zero.
[d] No upper bound to the distribution.

Haida Gwaii. On the basis of divergence estimates, three species that we studied likely persisted through the last glacial maximum in a refugium on or near Haida Gwaii but did not colonize adjacent areas after the end of the LGM (Fig. 2): the Northern Saw-whet Owl, Hairy Woodpecker, and Steller's Jay. When we considered the full range of 95% credibility intervals as extremes for our estimates from IM, divergence dates for the Northern Saw-whet Owl and Hairy Woodpecker preceded the LGM. These three species possess haplotypes only found on Haida Gwaii (Fig. 1), and there is limited evidence for gene flow to or from populations elsewhere.

If Haida Gwaii was a refugium but also colonized after the LGM, we might expect to observe a large number of endemic haplotypes on Haida Gwaii but shared haplotypes with some other locations, early estimates of divergence for Haida Gwaii, and evidence for gene flow (Fig. 2). We observed this pattern to some extent in the Pine Grosbeak, which showed an old divergence (the oldest observed in the study), many endemic haplotypes, and a low frequency of haplotypes shared with other locations. We were able to obtain two samples of the Pine Grosbeak from Colorado and discovered that one bird's haplotype was identical to the primary haplotype on Haida Gwaii; the other's differed by only a single mutation (Fig. 1). This was surprising because we did not find the common Haida Gwaii haplotype near Haida Gwaii in southeast Alaska or in Washington (Fig. 1). Possible explanations for these shared haplotypes include incomplete lineage sorting (although the deep divergences between Haida Gwaii and other locations make this idea seem unlikely; Fig. 1), rare long-distance movements into or out of the refugium, or Haida Gwaii receiving colonists from the Rocky Mountains or vice versa (Table 3, Fig. 2). Haida Gwaii and Rocky Mountain populations of the Pine Grosbeak do share similar patterns of vocalization (Adkisson 1999). There might be a historical association between populations of Haida Gwaii and the southwestern U.S. The Hairy Woodpecker shows such a pattern, with the population of Haida Gwaii representing a clade common to the Rocky Mountain region but still possessing haplotypes only found on Haida Gwaii (Klicka et al. 2011). However, other species of which we examined samples from the southwestern U.S., including the Hermit Thrush, Steller's Jay, and Song Sparrow, do not share haplotypes with Haida Gwaii (Fig. 1).

If Haida Gwaii was colonized after the LGM, we would expect genetic diversity there to be lower than in source areas, many shared haplotypes, and a high rate of gene flow. We observed significantly lower diversity in Haida Gwaii populations of the Sooty Grouse, Red-breasted Sapsucker, and Swainson's Thrush. We found a large number of shared haplotypes in these three species and in the Hermit Thrush and Chestnut-backed Chickadee. A high rate of gene flow was estimated for all of these species except the Chestnut-backed Chickadee. Thus post-glacial colonization is likely for these species (Ibrahim et al. 1996, Hewitt 1996, 2000).

ISOLATION AND LIFE HISTORY

Among the sedentary Haida Gwaii populations examined (Sooty Grouse, Northern Saw-whet Owl, Red-breasted Sapsucker, Hairy Woodpecker, Steller's Jay, Pine Grosbeak, and Chestnut-backed Chickadee), there was a trend for evidence of limited gene flow. Four of the seven species (the owl, woodpecker, jay, and grosbeak) showed many of the genetic trends expected from isolation in a refugium (Fig. 2). The Hairy Woodpecker had lineages endemic to Haida Gwaii, divergence estimated to have preceded the LGM, and high diversity. The Northern Saw-whet Owl, Steller's Jay, and Pine Grosbeak had
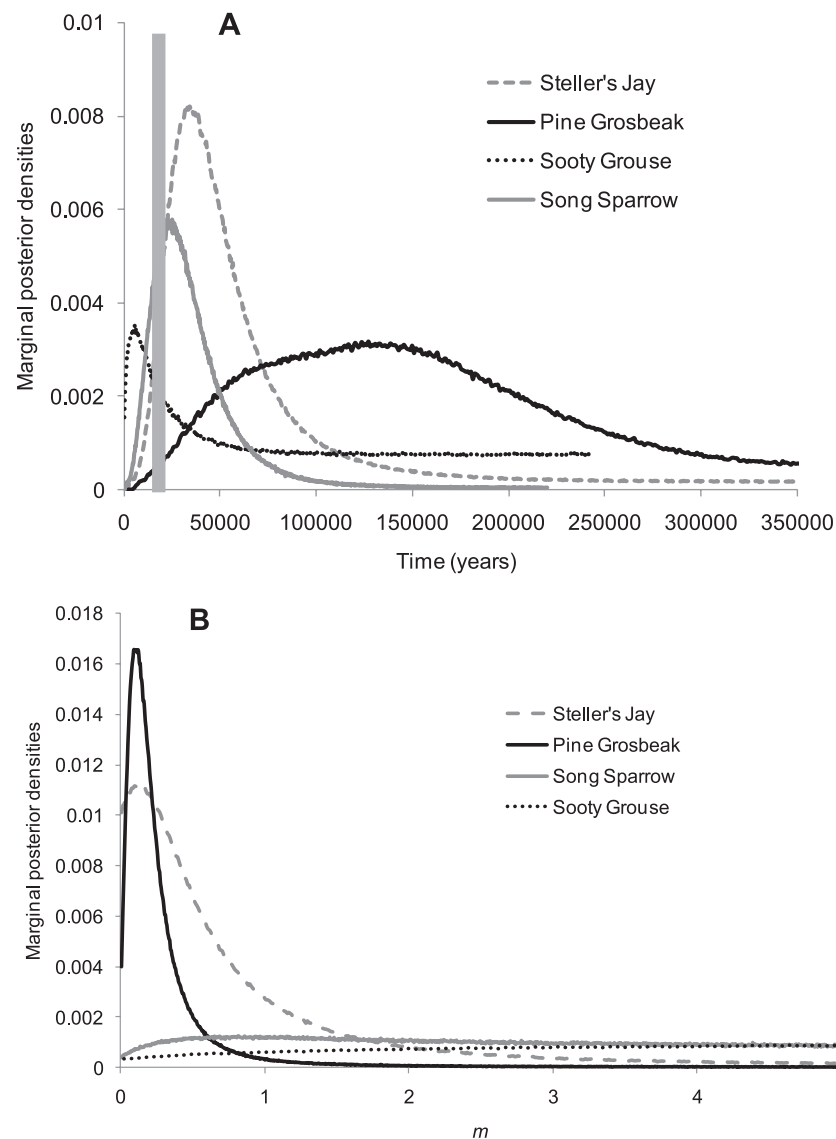
FIGURE 3.   Marginal posterior densities for (A) time (*t*) and (B) migration (*m*) parameters from IM analyses of a representative group of species. The approximate time of the end of the LGM is shown as a gray bar on the time panel.

lineages endemic to Haida Gwaii and divergence times older than 13,000 ybp but their haplotype and nucleotide diversity were lower on Haida Gwaii than in many of the other areas examined. These include Alaska locations that were likely to have been at the leading edge of post-glacial expansion (Table 2). A possible explanation is that a population locked in a refugium might lose genetic diversity when its size is reduced (Frankham et al. 2010). If these populations remained on Haida Gwaii and gene flow was nonexistent (as it appears to have been in the Northern Saw-whet Owl, Steller's Jay, and Pine Grosbeak), then genetic diversity would not be replenished except through new mutations. In addition, long-term isolation during both glacial and interglacial cycles (when

populations of sedentary species would have been isolated because of their inability to disperse across a water barrier) could have increased the divergence between Haida Gwaii and mainland populations.

In the Chestnut-backed Chickadee, genetic diversity on Haida Gwaii is high, divergence is recent, and the estimated rate of gene flow is low, but haplotypes are shared with other populations (Table 3). This suggests that lineage sorting is incomplete, most likely because isolation is recent rather than because of gene flow. This finding matches well with that of Burg et al. (2006, but see Lait et al. 2012), who postulated a post-glacial colonization and subsequent lack of gene flow for this species. The two remaining sedentary species, the Sooty

Grouse and Red-breasted Sapsucker, likely colonized Haida Gwaii after the LGM. These species have low genetic diversity and a low percentage of endemic haplotypes. Unfortunately, information in the datasets was inadequate for reliable estimates of divergence times or gene flow in these latter species.

A life-history strategy of partial migration is one in which some members of a population undertake long-distance seasonal movements, possibly driving higher dispersal, whereas other individuals remain in an area throughout the year. The populations of the Song Sparrow and Pacific Wren in the Haida Gwaii area are thought to be partially migratory (Arcese et al. 2002, Hejl et al. 2002; Table 1). These species showed high genetic diversity, high levels of gene flow, and haplotypes shared with other locations. Two scenarios may explain this pattern: (1) isolation in a Haida Gwaii refugium and a subsequent high rate of gene flow among populations that swamped the genetic signal, or (2) post-glacial colonization. Although credibility intervals are large (because of the use of a single locus), the results of the IM analyses do provide some insight into which pattern is more likely. For both the wren and the sparrow divergence dates were similar at ~22,000–25,000 ybp, and rates of gene flow were high. The IM analyses suggest that the founding of these populations through post-glacial colonization is less likely than a scenario in which lineage sorting is incomplete because recent gene flow (i.e., new colonists coming into an older population) is obscuring a signal of refugial isolation.

The Hermit and Swainson's Thrushes are obligate seasonal migrants to Haida Gwaii (Table 1), so if migration facilitates dispersal, gene flow to and from other locations might be expected to be greater in them than in sedentary species. Some of the trends possibly due to increased gene flow (Table 3) are found in these species, including moderate-to-high levels of genetic diversity and low-to-moderate percentages of haplotypes endemic to Haida Gwaii (Table 2, Fig. 1). For neither species, however, in comparison with populations that share haplotypes with Haida Gwaii, did we have enough information to estimate divergence or gene-flow parameters. On the basis of shared haplotypes, a likely scenario is that Haida Gwaii was colonized from a coastal area to the south. This pattern has been found among a variety of organisms on Haida Gwaii (Demboski et al. 1999, Johnson and Taylor 2004) and matches the results of Ruegg and Smith (2002), who sequenced the mtDNA control region of Swainson's Thrushes. Another interesting pattern is that Hermit Thrushes from Haida Gwaii and south-central Washington

TABLE 4.   Summary of molecular genetic evidence for an unglaciated refugium in the Haida Gwaii area.

Species	Molecular marker	Endemic lineages	Pre-LGM	High diversity	References
Plants					
Sword fern (*Polystichum munitum*)	allozymes, cpDNA			X	Soltis et al. 1997
Lodgepole pine (*Pinus contorta*)	mtDNA	X			Godbout et al. 2008
Gastropods					
Nebria gregaria	mtDNA	X	X		Clarke et al. 2001
Mammals					
Ermine (*Mustela erminea*)	mtDNA	X			Fleming and Cook 2002
Black bear (*Ursus americanus*)	mtDNA	X			Byun et al. 1997[a]
Reptiles					
Garter snake (*Thamnophis sirtalis*)	mtDNA		X		Janzen et al. 2002
Fish					
Stickleback (*Gasterosteus aculeatus*)	mtDNA	X	X		O'Reilly et al. 1993[b]
Silver salmon (*Oncorhynchus kisutch*)	microsatellites, mtDNA	X		X	Smith et al. 2001
Birds					
Northern Saw-whet Owl (*Aegolius acadicus*)	mtDNA	X	X		Topp and Winker 2008; this study
Townsend's Warbler (*Setophaga townsendi*)	mtDNA		X	X	Krosby and Rohwer 2009
Hairy Woodpecker (*Picoides villosus*)	mtDNA	X	X		Topp and Winker 2008; this study
Steller's Jay (*Cyanocitta stelleri*)	microsatellites, mtDNA	X	X		Topp and Winker 2008; Burg et al. 2005; this study
Pacific Wren (*Troglodytes pacificus*)	mtDNA		X	X	this study
Song Sparrow (*Melospiza melodia*)	mtDNA		X	X	Zink and Dittmann 1993; Fry and Zink 1998; this study
Pine Grosbeak (*Pinicola enucleator*)	mtDNA	X	X		Topp and Winker 2008; this study

[a]But see Demboski et al. (1999), Byun et al. (1999).
[b]But see Johnson and Taylor (2004).

share haplotypes but neither location shares haplotypes with samples from across northern Washington (Fig 1). This may be a result of the pattern of leapfrog migration evident in this species (Jones and Donovan 1996).

HISTORICAL ECOLOGY OF THE HAIDA
GWAII REFUGIUM

On the basis of the population genetics of the forest birds of Haida Gwaii that we studied, we can make inferences about the habitat, climate, and species composition that existed in this refugium during the LGM. Warner et al. (1982) found palynological evidence for a late Pleistocene climate on Haida Gwaii that "was moderately oceanic and cooler than the present but was not so severe as to preclude the survival of biota" such as coniferous trees. The birds of Haida Gwaii in which we found a genetic signature of a refugium are species found in areas with temperate or subarctic climates. Most of the species we examined are found in mixed coniferous forests through much of their ranges (Jones and Donovan 1996, Greene et al. 1998, Adkisson 1999, Mack and Yong 2000, Arcese et al. 2002, Dahlsten et al. 2002, Hejl et al. 2002, Jackson et al. 2002, Walters et al. 2002, Zwickel and Bendell 2005, Rasmussen et al. 2008), and all are currently found in this habitat on Haida Gwaii (Martin et al. 1995; pers. obs.). Our genetic evidence of a refugium in this region suggests that the moderating effects of the Pacific Ocean on the region's climate enabled Haida Gwaii to remain hospitable through the LGM for many of the species still there. Thus, the habitats that the Northern Saw-whet Owl, Hairy Woodpecker, Steller's Jay, and Pine Grosbeak (and possibly the Pacific Wren and Song Sparrow) need for nesting and feeding must have been present on the islands. The same holds true for Townsend's Warbler, which also shows strong evidence of inhabiting an unglaciated refugium and which currently breeds abundantly in the Sitka spruce forests that dominated this region during the Quaternary (Krosby and Rohwer 2009).

Our study adds to the growing molecular genetic evidence that a late-Pleistocene refugium existed in the Haida Gwaii area (Table 4). This refugium likely harbored a diverse set of organisms, including plants, invertebrates, and vertebrates. During the last glacial maximum the Haida Gwaii refugium was probably forested and very similar in community composition and ecology to what the islands support today.

ACKNOWLEDGMENTS

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