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Postglacial population genetic differentiation potentially facilitated by a flexible migratory strategy in Golden-crowned Kinglets (*Regulus satrapa*)

T.M. Burg, S.A. Taylor, K.D. Lemmen, A.J. Gaston, and V.L. Friesen

Abstract: Relatively recently, temperate regions in North America were covered by extensive ice sheets, making them inhospitable to contemporary flora and fauna. Since the retreat of the ice sheets, these regions have been recolonized by a diversity of taxa, some of which have undergone rapid postglacial divergence. Evidence supports the hypothesis that some taxa persisted in unglaciated refugia during the Last Glacial Maximum, such as on Haida Gwaii (formerly the Queen Charlotte Islands). Many taxa on Haida Gwaii are genetically distinct from mainland populations at neutral molecular markers possibly as the result of isolation in refugia or postglacial colonization. The Golden-crowned Kinglet (*Regulus satrapa* Lichtenstein, 1823) is a continentally distributed, short-distance migratory passerine inhabiting mature conifer forests including those on Haida Gwaii. We used five microsatellite markers and a 568 base-pair fragment of the mitochondrial control region to determine the likelihood that Haida Gwaii region acted as a refugium for this species during the last ice age. We report significant gene flow between Haida Gwaii and the western North American mainland from mitochondrial markers, but significant population genetic differentiation at nuclear markers. We also report genetic divergence between eastern and western Golden-crowned Kinglets, as well as higher genetic diversity and population substructuring within the western population than within the eastern population. The east-west differentiation probably arose due to isolation in separate Pleistocene refugia south of the ice sheets. However, population differences within the west are likely caused by more recent processes; contemporary differentiation of Haida Gwaii Golden-crowned Kinglets most likely occurred postglacially.

Key words: Haida Gwaii, gene flow, population differentiation, Queen Charlotte Islands, refugia, Golden-crowned Kinglet, *Regulus satrapa*.

Résumé : Il n'y a pas si longtemps, les régions tempérées de l'Amérique du Nord étaient recouvertes de vastes calottes glaciaires les rendant non accueillantes pour la flore et la faune contemporaines. Depuis le retrait des calottes, ces régions ont été recolonisées par divers taxons, dont certains ont connu une divergence postglaciaire rapide. Des données appuient l'hypothèse voulant que certains taxons aient persisté dans des refuges épargnés par la glaciation durant le dernier pléniglaciaire, comme l'archipel Haida Gwaii (anciennement les îles de la Reine-Charlotte). De nombreux taxons de Haida Gwaii sont génétiquement différents des populations du continent au niveau de marqueurs moléculaires neutres, possiblement en raison de leur isolement dans les refuges ou de la colonisation postglaciaire. Le roitelet à couronne dorée (*Regulus satrapa* Lichtenstein, 1823) est un passereau de répartition continentale migrant sur de courtes distances, qui vit dans des forêts de conifères matures, y compris celles de Haida Gwaii. Nous avons utilisé cinq marqueurs microsatellites et un fragment de 568 paires de bases de la région de contrôle de l'ADN mitochondrial pour déterminer la probabilité que la région de Haida Gwaii ait servi de refuge pour cette espèce durant la dernière glaciation. Nous faisons état d'un important flux génétique entre Haida Gwaii et la partie occidentale du continent nord-américain indiqué par des marqueurs mitochondriaux, mais également une importante différenciation génétique des populations au niveau de marqueurs nucléaires. Nous faisons également état d'une divergence génétique entre les roitelets à couronne dorée de l'Est et de l'Ouest, ainsi que d'une plus grande diversité génétique et une sous-structuration plus importante de la population de l'Ouest que de la population de l'Est. La différenciation est-ouest découle probablement de l'isolement dans des refuges pléistocènes distincts au sud des calottes glaciaires. Cependant, les différences entre populations dans l'Ouest découlent probablement de processus plus récents; la différenciation contemporaine des roitelets à couronne dorée de Haida Gwaii est vraisemblablement postglaciaire. [Traduit par la Rédaction]

Mots-clés : Haida Gwaii, flux génétique, différenciation des populations, îles de la Reine-Charlotte, refuge, roitelet à couronne dorée, *Regulus satrapa*.

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Introduction

Ice sheets covered the majority of North America until approximately 21 000 years ago (Pielou 1991). Consequently, the distributions of most North American taxa shifted since the last glacial maximum (LGM), and some are still shifting northwards at a relatively rapid rate (Chen et al. 2011). During the LGM, North American flora and fauna persisted both south of the Laurentide and Cordilleran ice sheets in unglaciated areas, as well as in unglaciated refugia bordering, and sometimes within, the ice sheets (e.g., on nunataks and island archipelagos) (Soltis et al. 1997; Jaramillo-Correa et al. 2009; Shafer et al. 2010). Refugial populations of a diverse array of taxa including vertebrates and plants (reviewed in Soltis et al. 1997; Jaramillo-Correa et al. 2009; Shafer et al. 2010) underwent varying levels of population differentiation at neutral genetic markers while isolated. Contemporary population genetic differentiation in many North American taxa can be attributed to genetic drift and (or) selection that occurred in refugia, often combined with postglacial differentiation following range expansion (Burg et al. 2005, 2006). Documenting and attempting to understand the influence of past climate change on population differentiation of North American plant and animal taxa is important towards understanding contemporary geographic variation and the potential future impacts of climate change on biodiversity.

Significant evidence exists that Haida Gwaii or a bordering region (e.g., Hecate Strait) remained unglaciated during the LGM and acted as a refugium for numerous taxa (Clague 1989; Pielou 1991; Byun et al. 1999). A number of taxa are endemic to the archipelago, and Haida Gwaii populations of several other species are morphologically and genetically distinct from their mainland counterparts (Heusser 1989; Burg et al. 2005; Deagle et al. 2013). Interestingly, patterns of neutral genetic variation indicate that at least some of the differentiation of Haida Gwaii taxa occurred postglacially, following range expansion and colonization of the archipelago (Byun et al. 1997, 2002; Burg et al. 2005; Bull et al. 2010). The cause of this rapid postglacial differentiation is unknown, but could be related to the geographic isolation of the archipelago, which sits 80 km from the coastal mainland.

Avian taxa inhabiting Haida Gwaii are differentiated from their mainland counterparts to varying degrees, and nine endemic subspecies have been described (see Table 1 in Bull et al. 2010). Some show evidence of having diverged in refugial populations (e.g., Steller's Jay, *Cyanocitta stelleri* (Gmelin, 1788); Northern Saw-whet Owl, *Aegolius acadicus* (Gmelin, 1788); Hairy Woodpecker, *Picoides villosus* (L., 1766); Pine Grosbeak, *Pinicola enucleator* (L., 1758); Topp and Winker 2008), while others appear to have undergone postglacial divergence (e.g., Orange-crowned Warbler, *Vermivora celata* (Say, 1823); Bull et al. 2010). The best predictors for this pattern appear to be habitat requirement and migratory status: avian taxa that are habitat generalists and do not undergo migration are more likely to have persisted in a Haida Gwaii refugium than are migratory species that require mature forests.

The Golden-crowned Kinglet (*Regulus satrapa* Lichtenstein, 1823) is a short-distance migratory passerine that inhabits most of the contiguous United States and all Canadian provinces (Fig. 1). As inhabitants of mature conifer forest, Golden-crowned Kinglets were most likely confined to glacial refugia, either south of or within the ice sheets, during the LGM and have only occupied the bulk of their current breeding distribution for approximately 12 000 years. Interestingly, the breeding distribution of this species is still changing, with recent documentation of expansions south into spruce and pine plantations (Ingold and Galati 1997). Widespread movement of this nature will erode population differentiation that may have developed in glacial refugia; however, this southern expansion has primarily taken place within the widely distributed eastern subspecies, *Regulus satrapa satrapa* Lichtenstein, 1823 (Ingold and Galati 1997).

In the present study, we analyzed variation in nuclear and mitochondrial DNA in Golden-crowned Kinglets to investigate the likelihood that isolation in a glacial refugium on or near Haida Gwaii during the LGM lead to genetic differentiation of this primarily migratory, mature-forest specialist. Specifically, we genotyped 260 Golden-crowned Kinglets at five microsatellite markers and sequenced a 568 base-pair (bp) fragment of the mitochondrial control region to test the following hypotheses: (i) Populations of Golden-crowned Kinglets on Haida Gwaii are genetically isolated and distinct from mainland populations and from nearby island populations (i.e., Vancouver Island), and if so, (ii) the divergence of Haida Gwaii Golden-crowned Kinglets took place postglacially rather than in a refugium on Haida Gwaii.

Materials and methods

Sample collection

Four to six subspecies of Golden-crowned Kinglets are recognized (Jenks 1936; Ingold and Galati 1997), three of which inhabit regions where samples were collected for the present study (Fig. 1): the Western Golden-crowned Kinglet (*Regulus satrapa olivaceus* Baird, 1864) breeds along the Pacific Coast from Sitka, Alaska, to southern Oregon west of the Cascade and Coast Mountains. The Arizona Golden-crowned Kinglet (*Regulus satrapa apache* Jenks, 1936) inhabits southeastern British Columbia between the Rocky Mountains and the Coast and Cascade Mountain ranges. *Regulus satrapa satrapa* is widespread throughout eastern North America. The two other subspecies recognized by the American Ornithologists' Union, *Regulus satrapa aztecus* Lawrence, 1887 (Mexico) and *Regulus satrapa clarus* Dearborn, 1907 (Mexico and Guatemala), are at the extreme southern edge of the range and were not sampled as part of this study. Differences between these three subspecies consist of subtle variation in plumage and size (Chui and Doucet 2009). All three subspecies are migratory. Blood and tissue samples were obtained from 260 individual Golden-crowned Kinglets from 11 populations in Alaska, British Columbia, Alberta, Washington, Oregon, Ontario, Nova Scotia, and Newfoundland (Fig. 1). Local breeding adults were caught during the summer months using mist nets and song playback, and blood was taken from the brachial vein, dried on filter paper, and stored in individual bags. Muscle samples from Ontario were obtained from the Royal Ontario Museum. To the best of our knowledge no migrating birds were sampled. DNA was extracted using standard proteinase K – phenol – chloroform extraction followed by ethanol precipitation (Sambrook and Russell 2001).

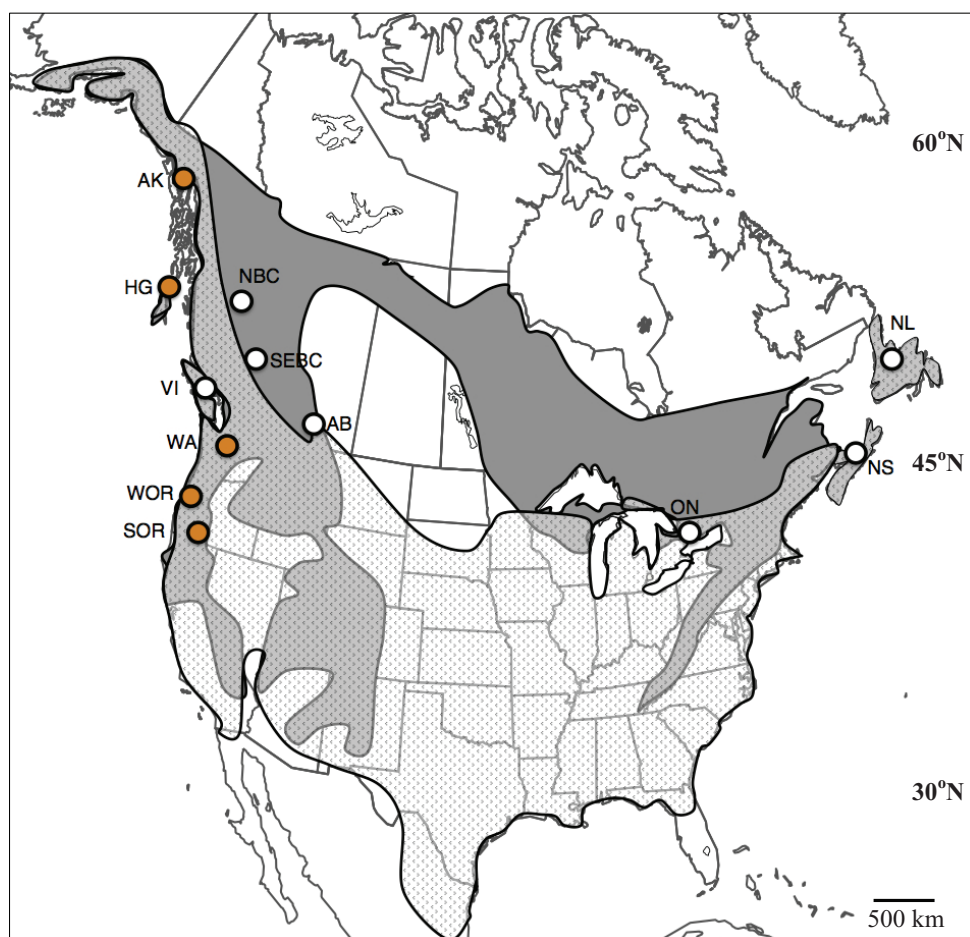
Microsatellite genotyping

Samples from Alberta, Nova Scotia, and Newfoundland were collected at a later date and were not included in microsatellite analyses because of the small sample size ($n < 5$) and changes in sequencing platforms. Primers for five microsatellite markers isolated from other avian species were used to genotype Golden-crowned Kinglets: *Ase18*, *Ase56*, *Pat43*, *Pdo5*, *Poc66* (Bensch et al. 1997; Otter et al. 1998; Griffith et al. 1999; Richardson et al. 2000). All loci were amplified using a two-step annealing procedure: 1 cycle for 2 min at 94 °C, 45 s at T_{A1} , 60 s at 72 °C; 7 cycles of 60 s at 94 °C, 30 s at T_{A1} , 45 s at 72 °C; 30 cycles of 30 s at 89 °C, 30 s at T_{A2} , 45 s at 72 °C; and 1 final cycle of 5 min at 72 °C. For loci *Ase56* and *Poc66*, $T_{A1} = 48$ °C and $T_{A2} = 50$ °C; for locus *Pdo5*, $T_{A1} = 50$ °C and $T_{A2} = 52$ °C; for loci *Ase18* and *Pat43*, $T_{A1} = 55$ °C and $T_{A2} = 57$ °C. Polymerase chain reaction (PCR) products were run on a 6% acrylamide gel on a Licor 4200 IR2. Known allele standards were run on each gel to ensure that alleles were sized consistently between gels. Alleles were scored using GeneImagIR (Licor), and sizing was confirmed by visual inspection.

Microsatellite statistical analyses

All populations and loci were tested for departures from Hardy-Weinberg and linkage equilibrium using GENEPOP (Raymond and

Fig. 1. Breeding (grey), winter (white with chevrons), and year-round (grey with chevrons) distributions of Golden-crowned Kinglets (*Regulus satrapa*) in North America. In “year-round” regions (Ingold and Galati 1997; Swanson et al. 2012), different individuals are present in summer and winter. Previously glaciated sampling sites indicated by white circles, unglaciated sampling sites indicated by orange circles on the Web (grey circles in print): Alaska (AK), British Columbia (northern BC, NBC; southeast BC, SEBC; Queen Charlotte Islands/Haida Gwaii, HG; and Vancouver Island, VI), Washington (WA), Oregon (western OR, WOR; southern OR, SOR), Alberta (AB), Ontario (ON), Nova Scotia (NS) and Newfoundland (NL).



Rousset 1995) and for large allele dropout and null alleles using MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2004). As the number of detected alleles is highly dependent on the number of individuals sampled, allelic richness was calculated for each locus and each population based on the minimum sample size of 24 using FSTAT version 2.9.3.2 (Goudet 2001). For microsatellites, 25–30 individuals per sampling site should represent population-level variation (Hale et al. 2012). FSTAT version 2.9.3.2 (Goudet 2001) was also used to estimate pairwise F_{ST} and R_{ST} values; significance was tested with 50 000 permutations. Tests for homogeneity of allele frequencies were performed using TFGPA version 1.3 (20 batches, 1000 dememorization steps, and 20 000 permutations/batch; Miller 1997).

The “admixture” model with sampling location as prior information and “correlated allele frequencies” was used to determine the number of genetic clusters in the data set using the program STRUCTURE version 2.3.3 (Pritchard et al. 2000). Hubisz et al. (2009) recommends using sampling locations as prior information when the number of loci is low or structure is weak. Five independent runs of 10^5 Markov chain Monte-Carlo (MCMC) iterations were performed with a 50 000 burn-in to estimate the number of populations (K) for $K = 1$ –7. Results from runs at each value

of K were averaged and $\Pr(K = X)$ was determined using Bayes’ factor and STRUCTURE HARVESTER (Pritchard et al. 2000; Evanno et al. 2005; Earl and vonHoldt 2012).

Mitochondrial DNA sequencing

A 568 bp fragment of the control region, containing Domain I and a section of Domain II, was amplified using the primers *RegulusMCR-60L* (5′-AAG GGT ATG TAT TAC TTC GC-3′) and *RegulusMCR-760H* (5′-GTC AAT CGA CGG TCA TGT G-3′) designed from previously published *R. satrapa* sequence (Päckert et al. 2006). This gene region was targeted, as it is typically the most variable in passerines and therefore the most useful for detecting recent population subdivision. For details on the control region amplification, sequencing, and alignment see the supplementary material.¹

Population genetic analyses of the mitochondrial control region

Ewens–Watterson (Ewens 1972; Watterson 1978) and Chakraborty’s (Chakraborty 1990) tests of selective neutrality were conducted for each sampling site using ARLEQUIN version 3.11 (Excoffier et al. 2005). FLUCTUATE version 1.4 (Kuhner 1998) was used to address

¹Supplementary material and Table S1 are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2013-0217>.

the null hypothesis that population growth rate was zero. FLUCTUATE was run using an initial population growth rate of one and a Watterson estimate of θ (Watterson 1975; Kuhner 1998). Population size was allowed to vary and each run consisted of 20 short chains of 1000 steps, followed by 3 long chains of 10^5 steps. Chains were sampled every 20 steps and runs were performed three times with different random seeds. Statistical significance was determined by testing whether g (the growth parameter) was significantly different from zero using log-likelihood ratio tests (Kuhner 1998). Based on results from estimates of population differentiation (see below), the data set was divided into eastern and western geographic regions for FLUCTUATE analyses.

To index population genetic structure, pairwise population differentiation (Φ_{ST}) and net sequence divergence (δ) were calculated from mitochondrial control region sequences using ARLEQUIN, which was also used to evaluate the significance of geographic subdivisions among sampling sites using a hierarchical analysis of molecular variation (AMOVA). To test for hierarchical structure, sampling locations were combined into larger geographic groups (i) based on results from pairwise population differentiation, (ii) based on the geographic separation of sampling sites, and (iii) to test the hypothesis that Haida Gwaii acted as a glacial refugium for this species. Larger geographic regions were created in an attempt to maximize global between-region population differentiation (Φ_{CT}), assuming that the most likely geographic subdivisions of populations were those that maximized global Φ_{CT} (Stanley et al. 1996). All analyses were conducted using Kimura's two-parameter substitution model (Kimura 1980) with a rate parameter (α) of 0.45 determined using MrModelTest version 2.3 (Nylander 2004). Significance was determined by comparing the results to 10 000 random permutations of the data at a significance level of 0.05 using the Benjamini-Yekutieli correction for multiple tests (Narum 2006).

A three-population IMA2 analysis was used to estimate gene flow between the three genetic groups detected with STRUCTURE (see Results; Hey and Nielsen 2007; Hey 2010). The Bayesian IM model implemented in IMA2 assumes constant population sizes, selective neutrality, random mating, an absence of linkage disequilibrium between loci, and no recombination within loci (Hey and Nielsen 2007). Limitations to the IM approach have been evaluated by recent simulation studies, which report that the model is robust to the presence of population structure and recombination within loci, but not to ancestral population structure, variation in gene flow through time, or deviations from the assigned mutation model (Sousa et al. 2011). Short preliminary runs were used to determine appropriate demographic priors. Multiple chain runs with geometric heating were then conducted, using a burn-in of at least 200 000 steps and results were recorded every 30 min. Runs were allowed to continue until effective sample size (ESS) values reached a minimum of 250. To assess convergence, the demographic parameters from each population tree were evaluated three times using different random number seeds. Convergence was also evaluated by assessing mixing properties of the MCMC (ensuring the absence of long-term trends in parameter estimate plots) during each run. Separate analyses were conducted for all three possible population tree topologies—1: ((Haida Gwaii, western North America) eastern North America); 2: ((Haida Gwaii, eastern North America) western North America); 3: ((eastern North America, western North America), Haida Gwaii). All population trees resulted in similar parameter estimates, so estimates are reported for the most likely population tree based on the glacial history of North America and on geography—(Haida Gwaii, western North America) eastern North America). Model parameters are scaled to the mutation rate and are reported as such because control region mutation rate estimates for passerine birds vary by an order of magnitude (e.g., Ho et al. 2005; Emerson 2007). The migration parameter estimate was considered significantly different from zero if the lowest bin of the associated 95% highest

posterior density interval (HPD) was greater than zero: HPD intervals represent the minimum length confidence intervals for a Bayesian posterior distribution (Hey and Nielsen 2004).

Results

Microsatellites

Populations contained 8–44 alleles per locus with an overall total of 19–68 alleles at each locus (supplementary Table S1).¹ No deviations from Hardy-Weinberg or linkage equilibrium (supplementary Table S1),¹ or evidence of null alleles or allelic drop out were found. Overall allelic richness based on the smallest sample size ($n = 24$) ranged from 13 to 17.5 (Table 1). Ontario showed the lowest allelic diversity, even when compared with populations with similar sample sizes. Pairwise F_{ST} estimates ranged from 0.001 to 0.041 and R_{ST} from 0.000 to 0.256 (Table 2). Ontario was significantly different from all western populations, and within the west, Haida Gwaii and Alaska were significantly different from most of the other western populations. Alaska was different from both northern British Columbia and Washington-Oregon, and northern British Columbia was significantly different from Washington-Oregon. Results of tests for allelic homogeneity were similar to results of comparisons of F_{ST} for Ontario and Haida Gwaii, except Haida Gwaii was significantly different from south-east British Columbia.

Bayesian structure analysis showed similar $\ln P(D)$ at $K = 1$ –3, with the highest level at $K = 2$ (−6927), Bayes' rule ($\Pr(K = 2) = 1$), with western samples having a high probability of belonging to one cluster (mean $Q = 0.944$) and samples from Ontario having a high probability of belonging to a second (mean $Q = 0.739$). The $K = 3$ ($\ln P(K = 2) = -6984$) was supported by Evanno's method with samples from Haida Gwaii having a high probability of belonging to a group separate from the other western samples (mean Q values for each group are 0.853, 0.878, and 0.760 for Haida Gwaii, remaining western populations, and Ontario, respectively) (Fig. 2). Two of the loci (*Pdo5* and *Ase18*) were hypervariable containing 68 and 59 alleles, respectively. To determine if these loci were biasing our results, three sets of additional analyses were done excluding each locus and both loci. Excluding *Pdo5* did not affect the results; removing *Ase18* or both *Pdo5* and *Ase18* resulted in lower Q values, however, individuals from Haida Gwaii separated from other western populations. Pritchard et al. (2000) urged caution when estimating K , particularly when differences in likelihood estimates are small; however, three clusters agree with both F_{ST} and goodness-of-fit tests.

Mitochondrial control region

Among 245 Golden-crowned Kinglets, 42 mitochondrial control region haplotypes were defined by 28 variable sites (GenBank accession Nos. KC514474–KC514516). Haplotype diversity ranged from 0.50 ± 0.27 in Newfoundland to 1.00 ± 0.18 in Nova Scotia (Table 1). Nucleotide diversity was highest in Washington and lowest in Newfoundland (Table 1). Significant growth was detected using FLUCTUATE for both the eastern (growth parameter, $g = 1818$, $\chi^2 = 11.1$, $P < 0.005$) and the western ($g = 721$, $\chi^2 = 152.8$, $P < 0.005$) geographic groups. No Ewens-Watterson test statistics were significantly different from expected values; however, Chakraborty's tests indicated that the observed number of haplotypes was significantly higher than the expected number for four western sampling sites (Table 1).

Twenty-one estimates each of pairwise population differentiation (Φ_{ST}) and net sequence divergence (δ) were significantly greater than zero after Benjamini-Yekutieli correction: all of the comparisons were between eastern and western sampling site locations (Table 2). No comparisons within eastern North America or within western North America were significant; however, small sample sizes for the two most eastern sampling sites may contribute to this result as estimates of both Φ_{ST} and δ were high. AMOVA

Table 1. Number of individual Golden-crowned Kinglets (*Regulus satrapa*) genotyped for microsatellites (N_{msat}) or sequenced for mitochondrial control regions (N_{mt}), allelic richness averaged across loci (A_r), mean number of alleles/locus (A), haplotype diversity (h ; mean \pm SD), and nucleotide diversity (π ; mean \pm SD).

Site	N_{msat}	A_r	A	N_{mt}	h	π ($\times 10^{-3}$)	Ewens–Watterson F	
							Observed	Expected
AK	32	317.5	21.0	32	0.84 \pm 0.05	3.1 \pm 2.1	0.19	0.15
NBC	50	17.2	26.8	49	0.83 \pm 0.05	3.8 \pm 2.4	0.19	0.12
SEBC	26	16.9	19.4	24	0.74 \pm 0.09	2.8 \pm 1.9	0.29	0.17
HG	49	16.7	24.2	49	0.73 \pm 0.07	3.8 \pm 2.4	0.28	0.14
VI	34	17.5	23.4	33	0.73 \pm 0.07	4.5 \pm 2.8	0.29	0.19
WA	28	17.5	21.4	26	0.74 \pm 0.09	5.0 \pm 3.0	0.28	0.18
AB	0			6	0.80 \pm 0.17	2.3 \pm 1.9	0.33	0.30
ON	24	13.0	13.0	13	0.78 \pm 0.10	2.1 \pm 1.6	0.28	0.25
NS	0			4	1.00 \pm 0.18	2.6 \pm 2.4	NA	NA
NL	0			4	0.50 \pm 0.27	0.9 \pm 1.1	0.63	0.59

Note: Locations of sampling sites and site abbreviations are listed in Fig. 1. NA, not available.

Table 2. Pairwise Φ_{ST} / net sequence divergence (δ) estimates (from control region variation; lower matrix) and F_{ST} / R_{ST} estimates (from micro-satellite variation; upper matrix) between the sampling locations of Golden-crowned Kinglets (*Regulus satrapa*).

	AK	NBC	SEBC	HG	VI	WA	AB	ON	NS	NL
AK		0.003 / 0.174	0.004 / 0.231	0.006 / 0.150	0.002 / 0.099	0.010 / 0.201		0.041 / 0.165		
NBC	0.00 / 0.002		0.002	0.008 / 0.017	0.001 / 0.016	0.007 / 0.005		0.029 / 0.215		
SEBC	−0.02 / −0.03	−0.01 / −0.02		0.006 / 0.017	0.002 / 0.054	0.002 / 0.010		0.029 / 0.231		
HG	0.00 / 0.01	−0.01 / −0.01	0.00 / 0.00		0.009 / 0.057	0.009 / 0.042		0.029 / 0.121		
VI	0.01 / 0.03	−0.01 / −0.03	0.00 / −0.005	0.01 / 0.017		0.003 / 0.026		0.037 / 0.208		
WA	0.01 / 0.01	−0.01 / −0.03	0.00 / −0.006	0.00 / 0.00	−0.02 / −0.06			0.037 / 0.256		
AB	−0.01 / 0.01	0.02 / 0.01	−0.03 / −0.02	−0.02 / 0.02	0.00 / 0.09	0.01 / 0.11				
ON	0.78 / 5.64	0.72 / 5.15	0.80 / 5.66	0.73 / 5.20	0.69 / 4.77	0.66 / 4.44	0.84 / 6.41			
NS	0.77 / 5.76	0.71 / 5.21	0.78 / 5.76	0.71 / 5.32	0.66 / 4.79	0.62 / 4.51	0.82 / 6.50	−0.02 / −0.05		
NL	0.77 / 5.76	0.71 / 5.23	0.79 / 5.78	0.71 / 5.32	0.66 / 4.85	0.62 / 4.53	0.86 / 6.50	−0.08 / −0.01	−0.14 / −0.12	

Note: Estimates in boldface type were significantly different from zero after a Benjamini–Yekutieli correction. Population abbreviations are given in Fig. 1.

detected significant global population structure ($\Phi_{\text{ST}} = 0.32$, $P < 0.0001$), and the maximum estimate of between group population structure (Φ_{CT}) was obtained by combining all western samples and all eastern populations into separate groups ($\Phi_{\text{CT}} = 0.71$, $P = 0.002$).

The mitochondrial haplotype network generated in TCS showed two distinct clades with strong, although not perfect, geographic concordance between the eastern clade and the western clade (Fig. 2). No western haplotypes were found at any eastern sampling site, and only small proportions of eastern haplotypes were found at the majority of western sampling locations (Fig. 3).

IMa2 analyses revealed significant gene flow between Haida Gwaii and western North America ($m = 5.4$, 95% HPD = 1.88–35.4), and significant but limited gene flow between western North America and Ontario ($m = 0.53$, 95% HPD = 0.18–21.8). Gene flow between Haida Gwaii and Ontario did not differ significantly from zero ($m = 0.075$, 95% HPD = 0.025–22.1). The estimated divergence date was significantly greater than zero between the eastern and the western populations ($t = 3.26$, 95% HPD = 0.39–5.88) but not for Haida Gwaii versus western populations ($t = 0.003$, 95% HPD = 0.000–1.083).

Discussion

Both nuclear and mitochondrial markers showed significant population differentiation between Golden-crowned Kinglets sampled from eastern versus western breeding sites in the northern part of their range (Figs. 3, 4). Furthermore, Golden-crowned Kinglets inhabiting Haida Gwaii are genetically differentiated at nuclear loci, but not mitochondrial loci, from their mainland counterparts in western North America (Table 2). Genetic differences did not correspond to current subspecies designations for the three northern subspecies.

Continent-wide patterns of differentiation

Both mitochondrial and nuclear data show the presence of at least two genetically distinct Golden-crowned Kinglet clades: one in western North America and the other mainly in eastern North America (Figs. 3, 4). Reductions in gene flow also are evident across the range, particularly between eastern and western populations. Sampling in the east was not as extensive and a gap in sampling exists in the Prairie provinces; additional samples from the central portion of the range are required to determine where the split occurs and if it is gradual (e.g., Ralston and Kirchman 2012; Lait and Burg 2013) or more abrupt (Boulet and Gibbs 2006). Whereas the winter ranges of eastern and western populations of Golden-crowned Kinglets are connected, the summer distributions are distinct and the narrowest gap between eastern and western populations is <450 km (breeding bird survey and Christmas bird count; Ingold and Galati 1997). Both summer and winter distributions show highest densities on the east and west coasts, which may further reduce dispersal opportunities. Genetic diversity in Golden-crowned Kinglets is higher in western North America than in the east. One explanation could be the uneven distribution of sampling in the two areas. However, gene flow may also account for some of the patterns. Estimates from IMa2 show significant, but low, gene flow between western and eastern populations. A regular influx of even a small number of individuals from eastern populations could introduce genetic variation to western populations. An additional explanation is the possibility that Golden-crowned Kinglets persisted in multiple glacial refugia, or a larger refugium, in western North America during the last ice age.

The historical biogeographic patterns of many forest inhabitants are closely tied to tree species, and Golden-crowned Kinglets are dependent on spruce (genus *Picea* A. Dietr.) and fir (genus *Abies* Mill.) during the breeding season. Studies on both black

Fig. 2. Statistical parsimony network for mitochondrial control region haplotypes in North American Golden-crowned Kinglets (*Regulus satrapa*). Inset shows birds sampled in eastern (white) and western (black) North America. Sampling site abbreviations as in Fig. 1.

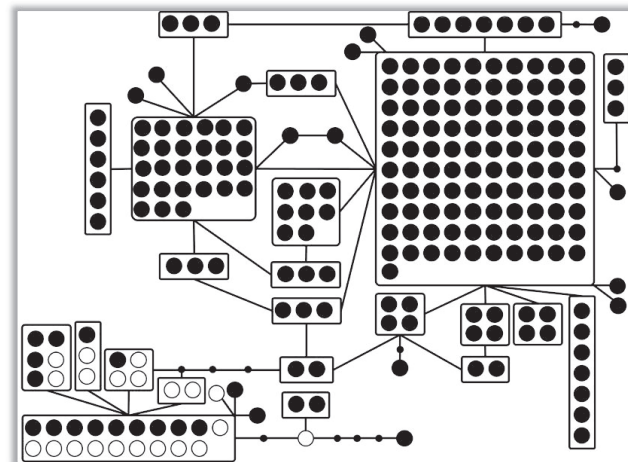
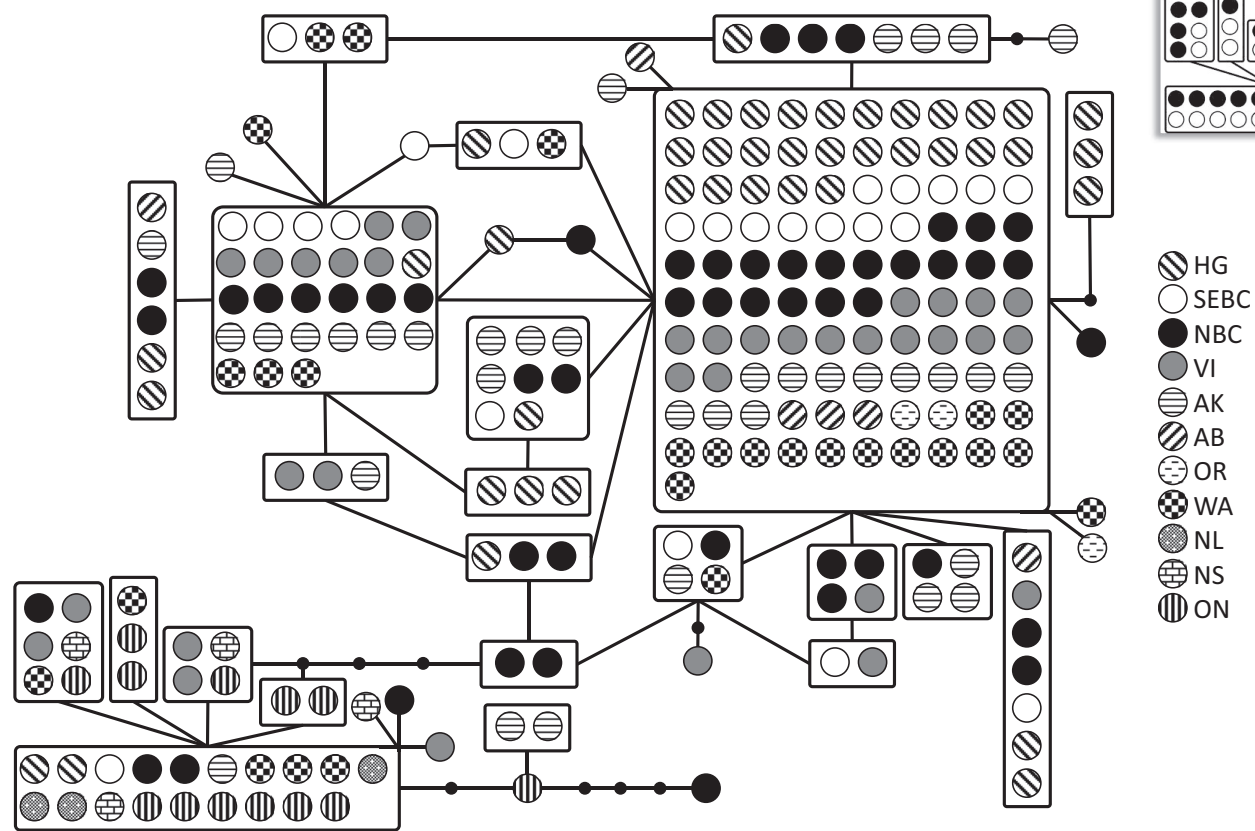


Fig. 3. Distribution of eastern (white) and western (black) Golden-crowned Kinglet (*Regulus satrapa*) mitochondrial control region haplotypes across North America. Pie size is not proportional to sample size from each region.

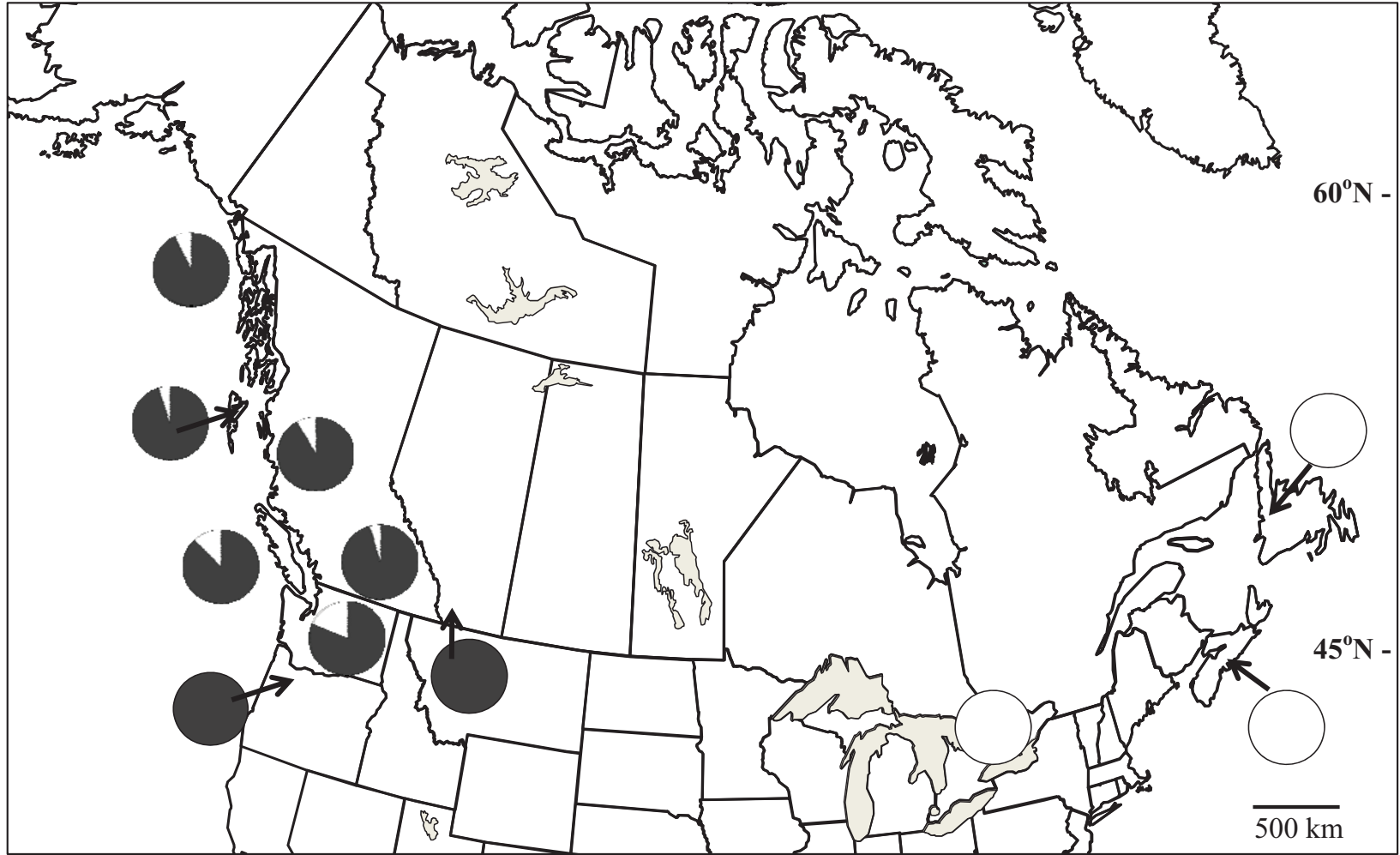
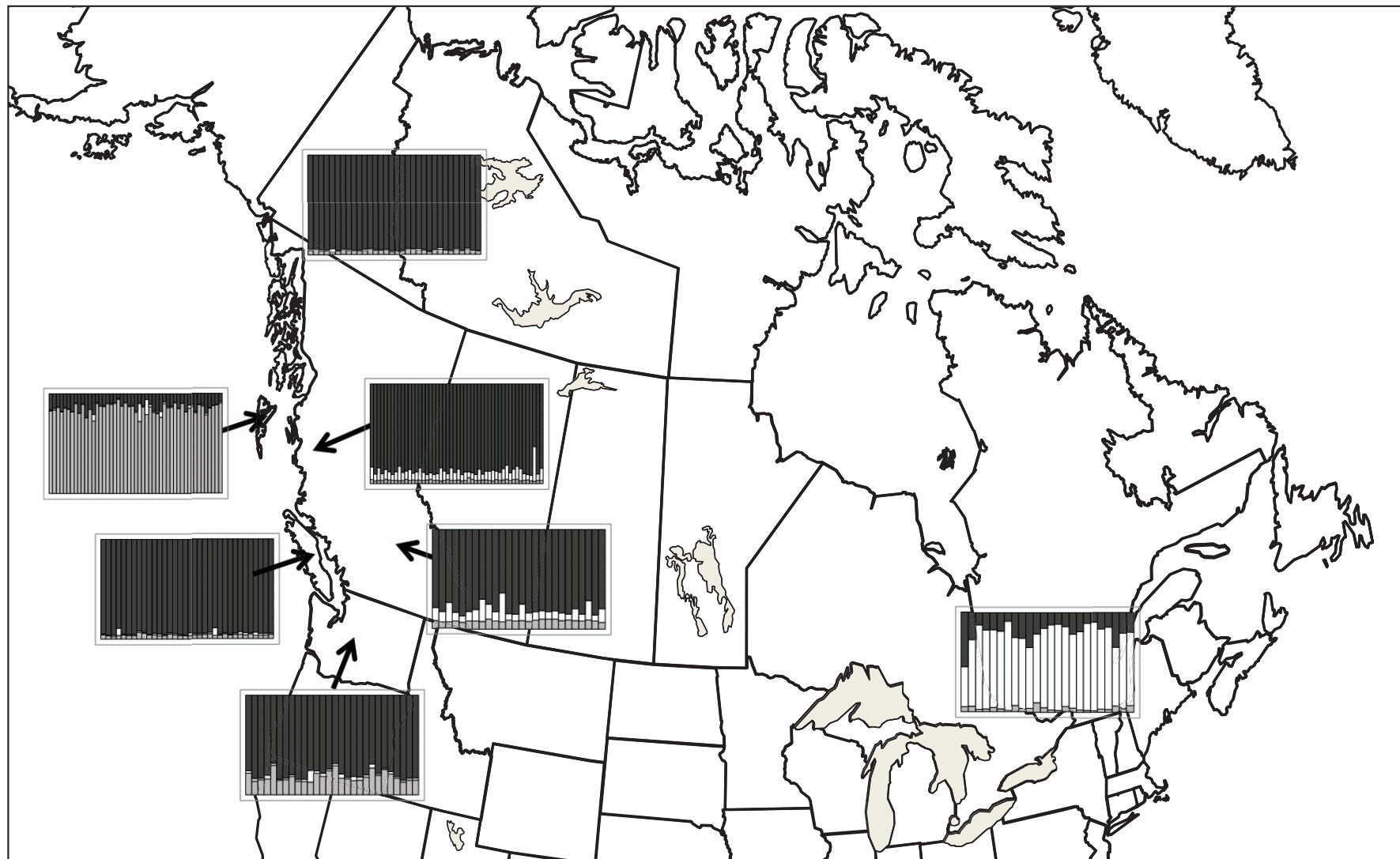


Fig. 4. Probability of assignment to each of three genetic populations for individual Golden-crowned Kinglets (*Regulus satrapa*) inferred by STRUCTURE. Membership to each cluster: eastern (white), western (black), and Haida Gwaii (light grey) are indicated for each individual.



spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) and white spruce (*Picea glauca* (Moench) Voss) found evidence of multiple refugia with at least one in the east and one in the west (Jaramillo-Correa et al. 2004; de Lafontaine et al. 2010), which could help explain the east–west differentiation that we documented in Golden-crowned Kinglets. A number of other continentally distributed avian taxa show an east–west split (Lovette et al. 2004; Bull et al. 2010; Manthey et al. 2011a; Graham and Burg 2012). Thus, it seems likely that the two Golden-crowned Kinglet mitochondrial lineages persisted in isolated areas during the LGM with limited gene flow.

Rapid post-Pleistocene differentiation on Haida Gwaii

Microsatellite data show that individuals from Haida Gwaii are genetically distinct from other western populations (Table 2, Fig. 2). However, our results are inconsistent with a separate refugial population of Golden-crowned Kinglets on, or near, Haida Gwaii during the LGM. There is no evidence of deep divergence in the control region haplotype network associated with Haida Gwaii or any neighbouring region; Haida Gwaii does not differ from the coastal mainland in haplotype frequencies; and IMA2 results indicate significant maternal gene flow exists between Haida Gwaii and mainland populations. Instead, it appears that, as hypothesized, Golden-crowned Kinglets underwent rapid postglacial differentiation on Haida Gwaii. Similar patterns have been reported in other taxa with comparable geographic distributions (Burg et al. 2005; Bull et al. 2010; Manthey et al. 2011a, 2011b; Graham and Burg 2012; Walstrom et al. 2012), suggesting an important role for postglacial divergence in generating contemporary biodiversity of North American taxa.

An alternative possibility is that Haida Gwaii was part of a larger western refugium within which weak population genetic structure existed. However, at least four lines of evidence argue against this possibility. First, it would require a large expanse of mature forest, and there is little evidence of large trees in the Haida Gwaii area prior to approximately 11 000 years ago (Mandryk et al. 2001). Second, the fact that population genetic structure was detected using microsatellite data and not using mitochondrial data suggests that the restrictions in gene flow are very recent. Third, the divergence between Haida Gwaii and mainland populations of kinglets, estimated from control region variation using IMA2, did not differ significantly from zero. Finally, no deep branches suggestive of even a large refugial population were present in the mitochondrial gene tree.

The migratory patterns of Golden-crowned Kinglets are not well characterized, and the species is present year-round in many parts of its range (Fig. 1; Swanson et al. 2012). Whether some individuals are true residents or some individuals at a particular location are migrants warrants further investigation. Current data support the hypothesis that Haida Gwaii Golden-crowned Kinglets are resident year-round and do not migrate seasonally like many other kinglet populations in the north (Ingold and Galati 1997). Sedentary species (McDonald et al. 1999; Coulon et al. 2008; McCormack et al. 2008; Manthey et al. 2011a; Graham and Burg 2012) tend to show higher levels of population structure than migratory species (Ruegg and Smith 2002; Lovette et al. 2004; Boulet and Gibbs 2006), and resident populations of species with mixed migration strategies tend to show increased genetic isolation compared with migratory populations (Boulet and Gibbs 2006; Seki et al. 2007).

Across their range, winter migration of Golden-crowned Kinglets involves only short-distance southward movements (Swanson et al. 2012). The extent of their movements appears to be flexible and is related to the severity of winter weather and food availability. Additionally, the species has recently undergone expansion of its southern range, probably in response to reforestation of these areas with spruce (Hall 1984). This apparent migratory flexibility may have allowed Golden-crowned Kinglets on Haida Gwaii to become resident more rapidly than could a long-distance migrant

such as the Orange-crowned Warbler, facilitating rapid postglacial divergence. Combined with the physical isolation of Haida Gwaii, located 80 km from the coastal mainland, these factors may help explain the contemporary genetic isolation that we detected.

Summary

Golden-crowned Kinglets appear to have experienced rapid postglacial differentiation on Haida Gwaii, potentially the result of their flexible short-distance migration strategy. Recent southward expansions of this species in numerous states, apparently tied to regeneration of spruce forests, highlight their relative flexibility and opportunistic behaviour. Future research should aim to better characterize the flexibility of the short-distance migration strategy employed by this species. If flexible, this species may be well equipped to respond to habitat change associated with climate change in the future.

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