CONTINENT-SCALE GENETIC STRUCTURE IN A BOREAL FOREST MIGRANT, THE BLACKPOLL WARBLER (*SETOPHAGA STRIATA*)

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ABSTRACT.—We describe the range-wide phylogeography of Blackpoll Warblers (*Setophaga striata*), a migratory passerine with a broad breeding range in North America's boreal forest that encompasses several possible biogeographic barriers but shows no phenotypic geographic variation. We used mitochondrial control-region sequences from 304 individuals in combination with ecological niche models and coalescent simulations to test alternative historical hypotheses about the number of Pleistocene refugial populations and divergences among modern populations. Population pairwise F_{ST} and spatial analyses of molecular variance suggested significant genetic structure among western, eastern, and Newfoundland populations, but no structure among sky-island populations at the southeastern periphery of the breeding range. Inferred gene flow fits a model of isolation-by-distance. Coalescent simulations rejected all multiple-refugia hypotheses in favor of a single refugium. Paleodistribution models and modern migratory pathways suggested that the refugium was located in southeastern North America. In contrast to previous studies that have invoked multiple Pleistocene refugia as the cause of genetic structure in North American bird species, our analyses suggest that geographic structure in Blackpoll Warblers results from isolation-by-distance rather than a history of sundered populations. *Received 23 November 2011, accepted 20 March 2012.*

Key words: Blackpoll Warbler, boreal forest, coalescent analysis, migratory birds, mitochondrial DNA, Pleistocene refugia, population genetics, *Setophaga striata*, statistical phylogeography.

Structure génétique à l'échelle du continent chez un migrateur de la forêt boréale, *Setophaga striata*

RÉSUMÉ.—Nous décrivons la phylogéographie de *Setophaga striata* à travers son aire de répartition. Ce passereau migrateur a une vaste aire de reproduction dans la forêt boréale de l'Amérique du Nord qui englobe plusieurs barrières biogéographiques possibles mais qui ne présente aucune variation géographique phénotypique. Nous avons utilisé des séquences de la région mitochondriale de contrôle de 304 individus en combinaison avec des modèles de niche écologique et des simulations coalescentes pour tester les hypothèses historiques alternatives portant sur le nombre de populations refuges du Pléistocène et les divergences entre les populations modernes. Les FST par paire des populations et les analyses spatiales de la variance moléculaire ont suggéré une structure génétique importante parmi les populations de l'ouest, de l'est et de Terre-Neuve, mais aucune structure parmi les populations des îles biogéographiques à la périphérie sud-est de l'aire de reproduction. Le flux génétique inféré correspond à un modèle d'isolement par la distance. Les simulations coalescentes rejettent toutes les hypothèses de refuges multiples en faveur d'un seul refuge. Les modèles de paléodistribution et les voies migratoires modernes suggèrent que le refuge était situé dans le sud-est de l'Amérique du Nord. Contrairement aux études antérieures qui ont évoqué les refuges multiples du Pléistocène comme la cause de la structure génétique chez les espèces aviaires d'Amérique du Nord, nos analyses suggèrent que la structure géographique chez *Setophaga striata* résulte de l'isolement par la distance plutôt que de populations scindées historiquement.

GENETIC DIVERSITY WITHIN and among populations of North American plants and animals is structured, in part, by the distributional changes associated with climate cycles of the past 2 million years (Avise 2000, Hewitt 2000). As glacial ice inundated northern habitats, populations were repeatedly pushed into one or more ice-free refugia (Pielou 1991). Modern gene pools may hold the genetic legacy of historical population bottlenecks and expansions or isolation in refugia, and patterns of genetic variation are commonly used to infer population histories and the numbers and locations of glacial refugia (Hewitt 2000). Phylogeographic studies of North American birds have revealed genetically distinct groups within the contiguous ranges of species and some common patterns of divergence among codistributed North American species (Zink 1996, Klicka and Zink 1999, Soltis et al. 2006). Many studies have revealed population structure east and west of the Rocky Mountains or Midwestern plains (Milot et al. 2000, Ruegg and Smith 2002, Clegg et al. 2003, Johnson and Cicero 2004, Lovette et al. 2004, Weir and Schluter 2004, Lovette 2005, Peters et al. 2005, Spellman and

Klicka 2007), a pattern often interpreted as the result of a history of isolation in two or more Pleistocene refugia.

Most phylogeographic studies of North American birds have focused on temperate-zone species, presumably because species that breed at higher latitudes tend to have fewer named subspecies (Martin and Tewksbury 2008, Phillimore 2010). But subspecific taxonomy and plumage variation appear to be poor indicators of genetic structure (Zink 2004), and studies of monotypic species often reveal cryptic geographic structure attributed to multiple Pleistocene refugia (Peters et al. 2005, Omland et al. 2006, Colbeck et al. 2008, Barrowclough et al. 2011). In fact, the effect of Pleistocene glaciation on genetic divergence may be disproportionately high for bird species that breed at high latitudes and in the boreal forest, compared with birds distributed at lower latitudes and in other habitats (Weir and Schluter 2004, Drovetski et al. 2010). Proposed boreal-forest-type refugia include unglaciated regions south of ice sheets, coastal refugia along the northern Pacific and Atlantic coasts, and ice-free Beringia. Pollen, fossil, and genetic data suggest that Beringia and the Queen Charlotte Islands (also known as Haida Gwaii) off the west coast of British Columbia also supported boreal tree species (McTaggart 1989, Brubaker et al. 2005, Anderson et al. 2006, Zazula et al. 2006) and may have provided a refuge for birds (Burg et al. 2005, 2006; Saitoh et al. 2010). Boreal-forest tree species also persisted through the Pleistocene in now-submerged areas along the Atlantic shelf from Newfoundland to New England, which may have been important refugia for forest birds (Gill et al. 1993, Zink and Dittmann 1993, Holder et al. 1999, Schauffler and Jacobson 2002, Colbeck et al. 2008). In addition to these northern refugia, boreal forest birds likely survived in unglaciated areas south of the ice sheets in western and eastern North America (Pielou 1991; Zink 1996; Milá et al. 2007a, b; Colbeck et al. 2008).

Here, we describe the range-wide phylogeography of the Blackpoll Warbler (*Setophaga striata*), a Neotropical–Nearctic migrant that breeds in the boreal forest of North America. Blackpoll Warblers are phenotypically uniform across their range (Hunt and Eliason 1999), but their current distribution covers many proposed refugia discussed above and spans possible barriers to gene flow, including the Rocky Mountains. As with other boreal-forest species, the breeding range of Blackpoll Warblers includes several geographically isolated populations on continental-shelf islands in the Canadian Maritimes and on mountaintop "sky islands" at the southern periphery of their range in northeastern North America. We investigated geographic patterns of genetic variation in Blackpoll Warblers in relation to both their current boreal distribution and their inferred distribution during the Last Glacial Maximum of the Pleistocene (LGM) and, thereby, examined the relative roles of historical and contemporary barriers to gene flow in this species.

Our approach combined population samples of genetic data and model-based simulations that account for coalescent stochasticity and uncertainties in parameter estimations, providing a statistical framework to choose among alternative hypotheses of population history (Edwards and Beerli 2000; Knowles 2001, 2009; Carstens et al. 2005; Beaumont et al. 2010; Lim et al. 2011). This statistical phylogeography approach gains rigor if hypotheses are generated *a priori* from independent data, such as fossil evidence or ecological niche modeling (Spellman and Klicka 2006, Carstens and Richards 2007, Knowles et al. 2007, Richards et al. 2007). We developed *a priori* hypotheses regarding the number of Pleistocene

refugia based on the number of suitable areas identified in a paleo-distribution model for the Blackpoll Warbler. We tested 10 alternative historical population models spanning hypotheses of one, two, and three refugia and four possible divergence times using coalescent simulations and mitochondrial control-region sequences from Blackpoll Warblers sampled throughout their range.

Methods

Tissue collection and laboratory methods.—Blackpoll Warbler tissues were obtained from breeding individuals captured or collected in June and July throughout the species' breeding range (Fig. 1). Our sampling strategy was to focus on geographically disjunct populations in eastern North America where Blackpoll Warblers are isolated on high-elevation boreal-forest "sky islands" and continental islands. When possible, we sampled several isolated mountains within isolated mountain ranges in order to establish the geographic scale of genetic structure. Eastern North American populations were sampled via blood or feathers taken from live banded birds or tissues from museum specimens collected between 2007 and 2010. Blackpoll Warblers from central and western Canada and Alaska were sampled entirely from museum specimens (toe pads and frozen tissues).

Whole genomic DNA was extracted from all samples using phenol-chloroform extractions or a DNeasy Animal Blood and Tissue Extraction Kit (Qiagen, Valencia, California). Mitochondrial control-region sequence variation has proved useful in revealing the recent histories of North American bird species (Zink and Barrowclough 2008), including several parulid warblers (Milot et al. 2000; Davis et al. 2006; Milá et al. 2007a, b; Colbeck et al. 2008; Grus et al. 2009). A 355-bp segment of Domain I of the control region, homologous to that obtained in the above-cited studies, was amplified using polymerase chain reaction (PCR) primers Dpdl-L5 and Dpdl-H4 (Milot et al. 2000). Extractions and PCR setups from toe pads were carried out in a designated ancient-DNA laboratory at the New York State Museum to prevent contamination from exogenous DNA sources. Internal primers DsJR-L1 (5′–CACATTCTTGTTTCAGGTAC–3′) and DsJR-H1 (5′–TTAGCTGTAGGTAGGAGCAT–3′) were designed from aligned Blackpoll Warbler control-region sequences so that toe-pad samples could be amplified in two overlapping segments. The PCR cycles consisted of 3 min denaturation at 94°C, followed by 50 cycles of 15 s at 94°C, 15 s primer annealing at 55°C, and 60 s extension at 65°C. The PCR products were visualized on an agarose gel, cleaned using ExoSAP-IT (Affymetrix, Santa Clara, California), and sequenced in both directions using Big Dye chemistry and an ABI 3730XL automated sequencer (Applied Biosystems, Foster City, California). Sequences were aligned using SEQUENCHER (Gene Codes, Ann Arbor, Michigan), and all variable sites were double checked against original chromatograms for accuracy.

Genetic analyses.—Gene genealogies were estimated for all sampled birds using maximum likelihood (ML) and Bayesian likelihood methods under a model of sequence evolution selected in MODELTEST (Posada and Crandall 1998) according to Akaike's information criterion (AIC). Trees were searched in MRBAYES (Ronquist and Huelsenbeck 2003) along four Markov chains for 10^7 generations, sampling trees every 10^3 steps. The first 2,000 trees were discarded as burn-in, and a consensus topology following the
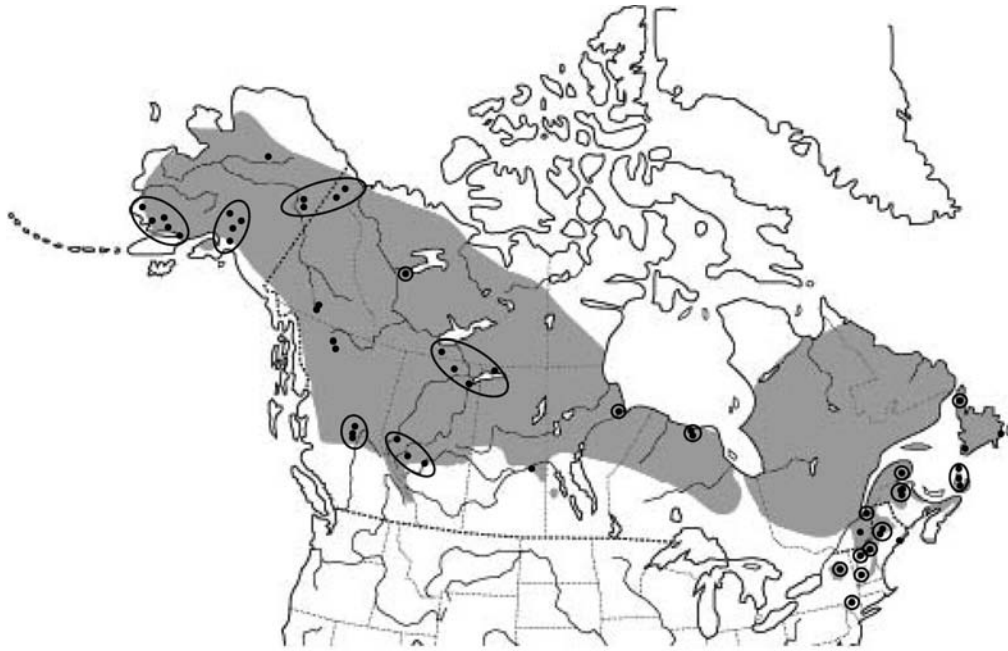
FIG. 1. Breeding distribution of Blackpoll Warbler (shaded gray; from Hunt and Eliason 1999) showing all sampling localities as black dots. Circles and ellipses denote groupings of localities into populations for data analyses.

50% majority rule was constructed using the final 8,000 trees. The program TRACER and the standard deviation of split frequencies were used to ensure convergence. The ML analyses and 1,000 bootstrap replicates were performed in RaxML according to the GTRMIX model (Stamatakis 2006). Four unique Yellow Warbler (*S. petechia*) sequences were obtained from GenBank (accession nos. AF205953–AF205955, AF205959) and used as an outgroup in all phylogenetic analyses. Relationship among haplotypes was further explored with a median-joining parsimony network built in the program NETWORK (Bandelt et al. 1999).

We used Google Earth to plot and measure the distance between localities, and samples were grouped into populations comprising at least five individuals, using a nearest-neighbor approach. Groupings represent disjunct localities from eastern North America (i.e., mountain ranges or islands) and, for central and western North America, pooled samples collected within 250 km of each other but separated from other groups by ≥350 km, resulting in 20 populations (Fig. 1). We also pooled samples into second-order groupings, representing localities within 550 km of each other but separated from other groups by ≥650 km. Results from second-order groupings did not differ from our first-order groups and are not further discussed. Measures of genetic diversity, including the number of haplotypes and nucleotide and haplotype diversity, were calculated for each population in DNASP (Rozas et al. 2003). Fu's F_S (Fu 1997) and mismatch distributions (Rogers 1995) were calculated to test for historical demographic expansion under the assumption of selective neutrality. Harpending's (1994) raggedness index was calculated for each mismatch distribution, and its significance was determined using a parametric bootstrap (100 replicates) in ARLEQUIN (Excoffier et al. 2005). The parameter τ and 95% confidence intervals were also calculated in ARLEQUIN and used to estimate the timing of demographic expansion with the equation τ = 2μ*kt*,

where μ is the mutation rate, *k* is the number of sites in each sequence, and *t* is the time to expansion. In all analyses, we used a range of substitution rates (μ = 0.075, 0.15, and 0.30 substitutions site^{-1} Ma^{-1}) spanning those previously used in studies of control-region variation in *Setophaga* (Milot et al. 2000, Davis et al. 2006, Milá et al. 2007b, Colbeck et al. 2008) and other birds, and used a generation time of 2 years. Generation times of 1.8 years (Yellow-rumped Warbler [*S. coronata*]; Milá et al. 2007b) and 2.8 years (Pygmy Nuthatch [*Sitta pygmaea*]; Spellman and Klicka 2006) were estimated for similarly sized passerine birds on the basis of adult survival rate and age of maturity (following Lande et al. 2003).

Geographic structure among populations was examined using pairwise F_{ST} implemented in ARLEQUIN with 100 permutations to assess significance, and spatial analyses of molecular variance (SAMOVA), which can be used to determine the most likely number of groups (*K*) and assign membership of populations to these groups. To test for a pattern of isolation-by-distance, we compared the relationship between geographic distance and population pairwise F_{ST}. Mean latitude and longitude were used for populations with samples pooled from multiple localities.

Paleodistribution modeling and hypothesis testing.—Occurrence data from 74 georeferenced July specimen records of Blackpoll Warblers were downloaded from the ORNIS database (see Acknowledgments) in November 2010 and visually inspected for accuracy. No records were excluded on the basis of collection year. The Blackpoll Warbler climate niche was estimated in MAXENT (Phillips et al. 2006) by combining occurrence data with 19 biologically relevant climate variables describing temperature, precipitation, seasonality, and climatic extremes at a resolution of 2.5 arc-minutes (WorldClim data [see Acknowledgments], Hijmans et al. 2005). Eighty percent of occurrences were used to train the model, and 20% were withheld to test model accuracy.
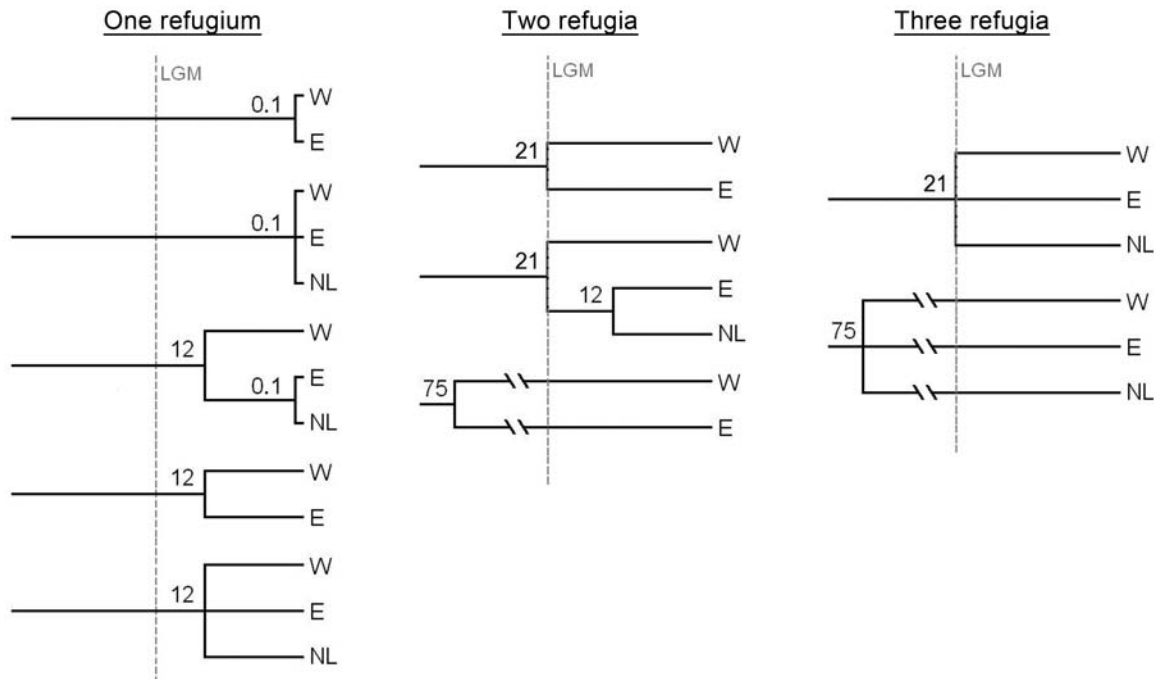
Fig. 2. Ten population models tested with coalescent simulations. Two-population models assume an east–west division and three-population models assume west–east–Newfoundland divisions for Blackpoll Warblers as defined in the text. Nodes are labeled with time in units of thousands of years before present. The gray dashed line represents the LGM (~21 kya). The number of branches crossing the dashed line at LGM represents the proposed number of refugia. Populations diverging after LGM are considered to have arisen in one refugium. Each population model was tested with and without demographic growth.

The modeled niche was extrapolated onto the MIROC climate model for LGM (21,000 years BP) to project the paleodistribution of Blackpoll Warblers. Model performance was measured as the area under the receiver operating curve (AUC), which can be interpreted as the probability of correctly classifying a presence and an absence (Phillips et al. 2006).

We developed alternative hypotheses regarding the number of Pleistocene refugia based on the number of suitable areas identified qualitatively from the paleodistribution model and on results from SAMOVA analyses and pairwise F_{ST}. Ten alternative historical population models were tested that span hypotheses of one, two, or three refugia and four possible divergence times (T) (Fig. 2). Our method is tree-based, requiring branching models that describe divergence among populations at dates selected *a priori* on the basis of geological events that may have divided populations. To test single-refugium models, we used divergence times of $T = 0.1$ kya to represent a hypothesis of little or no divergence between modern populations, and $T = 12$ kya corresponding to a time of expected population movements following glacial retreat from areas now dominated by boreal forest (Dyke et al. 2003). Under the 12-kya scenario, modern populations that expanded from a single refugium may have become genetically structured because of current barriers to gene flow. Models of $T = 21$ kya and 75 kya are multiple-refugia hypotheses with divergence times that correspond to the LGM and the end of the Sangamonian interglacial, respectively (Duk-Rodkin et al. 2004). These models explicitly test whether populations of Blackpoll Warblers were isolated by vicariance as ice sheets invaded

northern habitats. Three-refugia models are either tritomies, assuming contemporaneous splitting of three populations, or models in which two eastern populations split from one another more recently than a third, western population (Fig. 2). All models were tested both presuming constant population size and allowing for demographic growth.

The 10 population models were tested in two ways: with simulated coalescent genealogies and with simulated DNA sequences. First, Slatkin and Maddison's (1989) S statistic, a measure of discord between population and gene trees, was calculated from a Bayesian reconstruction of all sampled Blackpoll Warblers and compared with the distribution of S values from 1,000 coalescent genealogies simulated under each model using the program MESQUITE (see Acknowledgments). The distribution of S for each model is used to calculate the probability (p) of the observed S, and offers a two-tailed statistical framework for choosing among alternative hypotheses. If the observed S was <5% or >95% of simulated S values under a given model, that model was rejected. Second, 500 sequence data sets that matched the sample size and model of sequence evolution of the empirical data set were simulated under each population model in the program SIMCOAL (see Acknowledgments). The level of nucleotide divergence (π_{net}) between western and eastern groups, and between the eastern and Newfoundland groups (when applicable), was calculated for the empirical data set and compared with the distribution of π_{net} from simulated data sets. Population models were rejected if observed π_{net} fell outside of 95% of simulated values (Hickerson et al. 2006).

Coalescent simulations require a user-defined populations size (N_e), which we estimated in two ways: (1) by multiplying published estimates of breeding population density (number of nests ha^{-1}, assuming that number of nests ≈ number of females ≈ N_e) at sites throughout the breeding range (data from Theberge 1976, Erskine 1977, Eliason 1986, Kessel 1998, Hunt and Eliason 1999) by the area of current breeding distribution as modeled in MAX-ENT; and (2) from sequence data, using the equation $\Theta = 2N_e\mu$ for mitochondrial DNA. Estimates of Θ were averaged over three runs in MIGRATE-N (see Acknowledgments), sampling 10 short chains every 100 steps for 10^5 steps and sampling 2 long chains every 100 steps for 20 million generations, following burn-ins of 20,000 steps. The coalescent process is sensitive to small changes in mutation rate and N_e; using a range of mutation rates ($\mu = 0.075$, 0.15, and 0.30 substitutions site^{-1} Ma^{-1}) in coalescent simulations and to convert Θ to N_e allowed us to test the robustness of our analyses against a range of effective population sizes.

RESULTS

Genetic diversity and demographic expansion.—We detected 81 haplotypes among 304 Blackpoll Warbler sequences sampled throughout the breeding range (Fig. 3). All DNA sequences are archived in GenBank (accession nos. JX020986–JX021289). Fu's F_S

was significant, indicating historical rapid demographic expansion throughout the range of Blackpoll Warblers. Mismatch distributions for the complete sample and for samples from individual populations were smooth and unimodal, and Harpending's raggedness indices were low and nonsignificant (all $P > 0.05$, except QUm $P = 0.04$), supporting a model of rapid historical population expansion. Estimated mean times of demographic expansion for the complete sample were 24 kya (range: 0.9–37 kya), 48 kya (2–74 kya), and 97 kya (4–148 kya) using mutation rates of 0.30, 0.15, and 0.075 substitutions site^{-1} Ma^{-1}, respectively.

Geographic structure.—Bayesian and ML gene-tree reconstructions were highly congruent and showed no geographic structure (not shown). Collapsing branches with <0.50 Bayesian posterior probabilities or <50% bootstrap support in ML analysis resulted in one large polytomy. Relationships within a haplotype parsimony network were highly reticulate and showed no major genetic breaks (Fig. 3).

After excluding from population-level analyses 17 samples that did not meet our criteria for population groupings, we detected 77 haplotypes in the remaining 287 samples (Table 1). Haplotype diversity was high, averaging 0.859 across all populations. Pairwise F_{ST} and SAMOVA revealed evidence of significant geographic structure between populations east and west of the Rocky Mountains. SAMOVA identified $K = 2$ as the most likely number of groups and included western Alaska, central Alaska, and British Columbia in
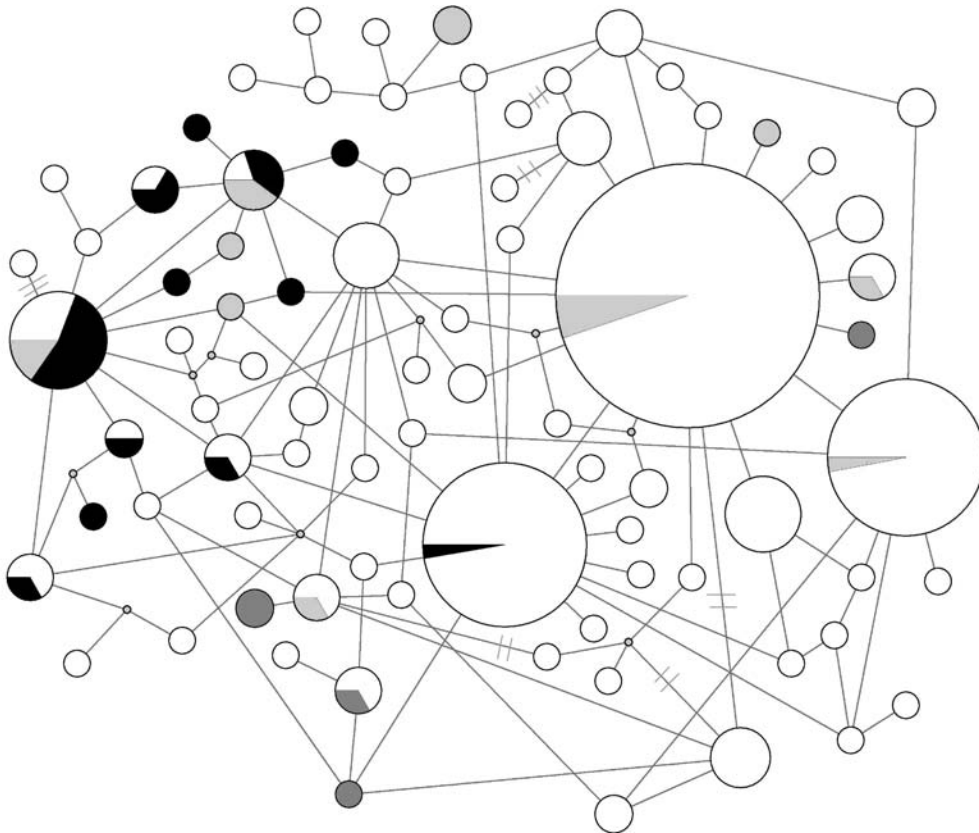

FIG. 3. Parsimony haplotype network. Haplotypes (circles) are sized proportional to observed frequency and shaded depending on whether the Blackpoll Warbler they were found in was from western (black), eastern (white), or Newfoundland (dark gray) groups as defined by SAMOVA and F_{ST} results. Light gray haplotypes represent those not included in population-level analyses because they did not meet inclusion criteria for population grouping. Hash marks denote haplotypes separated by two mutations; all others are separated by one mutation.

Table 1. Population sample sizes (*n*) and the number of detected haplotypes (*h*), haplotype diversity (*H*), nucleotide diversity (π), and the expansion parameter τ for each Blackpoll Warbler population included in the present study.

Population	Code	*n*	*h*	*H*	π	τ
Western Alaska	wAK	8	6	0.893	0.005	1.87
Central Alaska	cAK	7	7	1.000	0.006	2.34
Eastern Alaska and Northern Yukon Territory	eAK	6	6	1.000	0.006	2.20
British Columbia	BC	5	3	0.700	0.005	2.33
Northwest Territories	NT	5	4	0.900	0.010	4.80
Alberta	AB	6	4	0.867	0.008	5.21
Central Canada	cCAN	6	5	0.933	0.011	5.53
Manitoba	MB	20	11	0.868	0.007	3.33
Ontario	ON	9	7	0.917	0.008	3.05
Adirondacks, New York	NYa	23	7	0.731	0.003	1.13
Catskills, New York	NYc	29	8	0.714	0.004	1.43
Mount Mansfield, Vermont	VTm	19	8	0.865	0.004	1.75
Stratton Mountain, Vermont	VTs	10	7	0.867	0.006	1.90
New Hampshire	NH	29	10	0.835	0.004	1.55
Maine	ME	17	5	0.728	0.003	1.16
Mont Gosford, Quebec	QUg	25	10	0.857	0.002	1.56
Mine Madeline, Quebec	QUm	18	13	0.954	0.009	3.31
New Brunswick	NB	29	12	0.842	0.005	1.70
Nova Scotia	NS	11	7	0.818	0.004	1.62
Newfoundland	NL	5	4	0.900	0.008	4.10
Total		287	77	0.859	0.006	2.59

one group separate from eastern Alaska and all other populations. We use these as our definitions of "western" (western Alaska, central Alaska, and British Columbia) and "eastern" (all other sampled populations) groups for further analyses. SAMOVA estimated that 39.72% of genetic variation in Blackpoll Warblers is due to the difference between these western and eastern groups. Pairwise F_{ST} values between western and eastern populations averaged 0.378 and were higher than those within western (−0.051) and eastern (0.118) groups (Table 2). Populations from eastern Alaska, Manitoba, central Canada, and Alberta had significant pairwise F_{ST} when compared with eastern, but not western, populations (Table 2), which suggests an alternative grouping that would place these populations in the western group. We used both the grouping identified by SAMOVA and this alternative grouping in coalescent simulations and refugium hypothesis testing, but found that it made no difference. Pairwise F_{ST} values were significant between Newfoundland and 16 of the other 19 populations (mean = 0.269), including its closest geographic neighbor, Nova Scotia (F_{ST} = 0.307).

Pairwise F_{ST} values between high-elevation populations isolated at the southeastern periphery were not significant (mean = −0.003). Aside from several haplotypes recovered from single individuals (singletons), we found no haplotypes that were confined to any of the high-elevation populations isolated in the Catskill Mountains (New York), Adirondack Mountains (New York), Green Mountains (Vermont), and White Mountains (New Hampshire). Analyses with IBDWS revealed significant isolation-by-distance across all populations (*r* = 0.733, *P* < 0.0001; Fig. 4). Only 7 of 81 haplotypes were found in both eastern and western groups. These results suggest some restriction of gene flow among Blackpoll Warbler populations at a continental scale and that geographic distance is a more important structuring mechanism than physical isolation in high elevations.

Paleodistribution modeling and hypothesis testing.—Current and LGM distribution models performed well (mean AUC = 0.949, *n* = 7 replicates), and the projected current distribution matched published distribution maps (Fig. 5). Modeled distribution during LGM identified three areas with high occupancy probability (Fig. 5), all of which have previously been suggested as Pleistocene refugia for birds. The largest area was located in ice-free Beringia; a second was located in the Grand Banks, a now submerged area off the east coast of Newfoundland. The third area, located in eastern North America near modern-day Chesapeake Bay, had the highest probability of presence (i.e., best fit to modern Blackpoll Warbler climate niche). To test alternative refugia hypotheses, samples were assigned to western and eastern groups (as identified by F_{ST} and SAMOVA) or, in the case of three-refugia models, Newfoundland (NL) was removed from the eastern group and used as the third population.

From values for Θ calculated by MIGRATE-N, we estimated the effective population sizes for these three putative populations as $N_{e(east)}$ = 70,000–280,000 females, $N_{e(west)}$ = 8,000–34,000, and $N_{e(NL)}$ = 6,000–25,000, with the range of values reflecting the range of mutation rates. Our estimates using published breeding-pair density data and current breeding-range area (from MAXENT) resulted in values of $N_{e(east)}$ = 126,000, $N_{e(west)}$ = 26,000, and $N_{e(NL)}$ = 10,000, which fall within our estimates from single-locus genetic data.

Blackpoll Warbler samples required *S* = 20 and *S* = 24 sorting events to reconcile the observed tree with hypothetical east–west and east–west–Newfoundland population trees, respectively. The distribution of *S* from 1,000 genealogies simulated in MESQUITE under each population model rejected all multiple-refugia models at all mutation rates, failing to reject models with *T* = 0.1 kya (*P* < 0.001 for all models of *T* = 12, 21, and 75 kya). These results were consistent across simulations encompassing 95% confidence intervals of Θ, and models

TABLE 2. Pairwise F_{ST} values between all possible pairs of populations. Significance at the α = 0.05 level from 100 permutations in ARLEQUIN are denoted by gray shading. The Blackpoll Warbler populations are arranged from west to east.

|	wAK	cAK	eAK	BC	NT	AB	cCAN	MB	ON	NYa	NYc	VTm	VTs	NH	ME	QUg	QUm	NB	NS	NL
wAK	X																			
cAK	−0.037	X																		
eAK	0.13	0.12	X																	
BC	−0.109	−0.007	0.122	X																
NT	0.317	0.33	0.182	0.273	X															
AB	0.153	0.244	0.1	0.07	0.013	X														
cCAN	0.104	0.087	0.018	0.008	0.006	−0.013	X													
MB	0.092	0.155	0.01	0.039	0.101	−0.016	0.001	X												
ON	0.265	0.299	0.104	0.215	−0.043	−0.017	0.016	0.025	X											
NYa	0.574	0.602	0.457	0.562	0.062	0.211	0.283	0.268	0.091	X										
NYc	0.55	0.582	0.417	0.528	0.087	0.183	0.25	0.244	0.082	−0.006	X									
VTm	0.482	0.53	0.364	0.451	0.035	0.089	0.195	0.185	0.057	0.004	0.002	X								
VTs	0.453	0.49	0.335	0.431	−0.031	0.084	0.143	0.167	0.009	−0.023	−0.005	−0.031	X							
NH	0.544	0.566	0.415	0.531	0.049	0.216	0.261	0.265	0.087	−0.01	0.011	0.032	−0.001	X						
ME	0.553	0.613	0.468	0.541	0.113	0.116	0.256	0.209	0.076	0.057	0.036	−0.027	0.003	0.085	X					
QUg	0.516	0.541	0.38	0.497	0.064	0.174	0.223	0.232	0.074	−0.014	0.003	0.016	−0.023	−0.009	0.071	X				
QUm	0.346	0.387	0.265	0.299	−0.043	0.055	0.105	0.161	0.015	0.058	0.057	0.039	0.003	0.069	0.028	0.071	X			
NB	0.451	0.493	0.3	0.413	0.029	0.085	0.171	0.152	0.019	0.009	−0.006	−0.023	−0.013	0.027	0.001	0.044	0.034	X		
NS	0.51	0.538	0.375	0.496	0.045	0.126	0.188	0.216	0.046	−0.02	−0.015	−0.007	−0.019	−0.025	0.036	0.01	0.034	−0.002	X	
NL	0.381	0.407	0.27	0.382	0.101	0.161	0.163	0.165	0.066	0.364	0.38	0.3	0.169	0.335	0.39	0.312	0.183	0.277	0.307	X
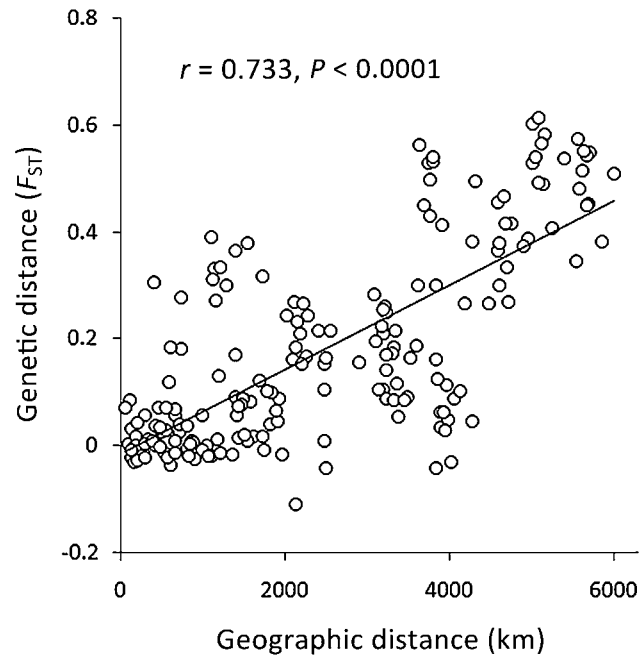
FIG. 4. Correlation of geographic distance (km) and genetic distance (pairwise F_{ST}) among all populations of Blackpoll Warblers as calculated in the program IBDWS.

accounting for demographic growth did not affect results. Results of hypothesis tests based on SIMCOAL were somewhat less conclusive. The observed mean nucleotide divergences (π_{net}) were 1.391 between east and west and 0.573 between east and Newfoundland. Using a mutation rate of 0.30 substitutions site^{-1} Ma^{-1}, these empirical values of π_{net} fell below 95% of π_{net} simulated using T = 12, 21, and 75 kya, which means that our data are less divergent than expected under these models. However, when slower mutation rates of 0.15 and 0.075 substitutions site^{-1} Ma^{-1} were used, divergence times of T = 0.1, 12, and 21 kya could not be rejected. Therefore, simulation results from SIMCOAL reject all multiple-refugia hypotheses when a fast mutation rate is used, but fail to distinguish between single and multiple refugia hypotheses at slower rates. Results did not differ when an alternative scenario including eastern Alaska, Alberta, central Canada, and Manitoba into the western group was used.

DISCUSSION

Molecular phylogenies indicate that species-level divergences are more recent in boreal-forest birds than in temperate and tropical birds (Weir and Schluter 2004), which suggests that Pleistocene glaciation had a disproportionately large effect on species whose current breeding ranges were completely inundated by ice as recently as 12,000 years ago. Few studies have examined patterns of genetic variation within boreal-forest bird species, and the extent to which their ranges were fragmented by glacial cycles remains unclear. Our finding of high pairwise F_{ST} and significant SAMOVA values reveals genetic structure on a continental scale between eastern and western populations, and between Newfoundland and eastern populations of
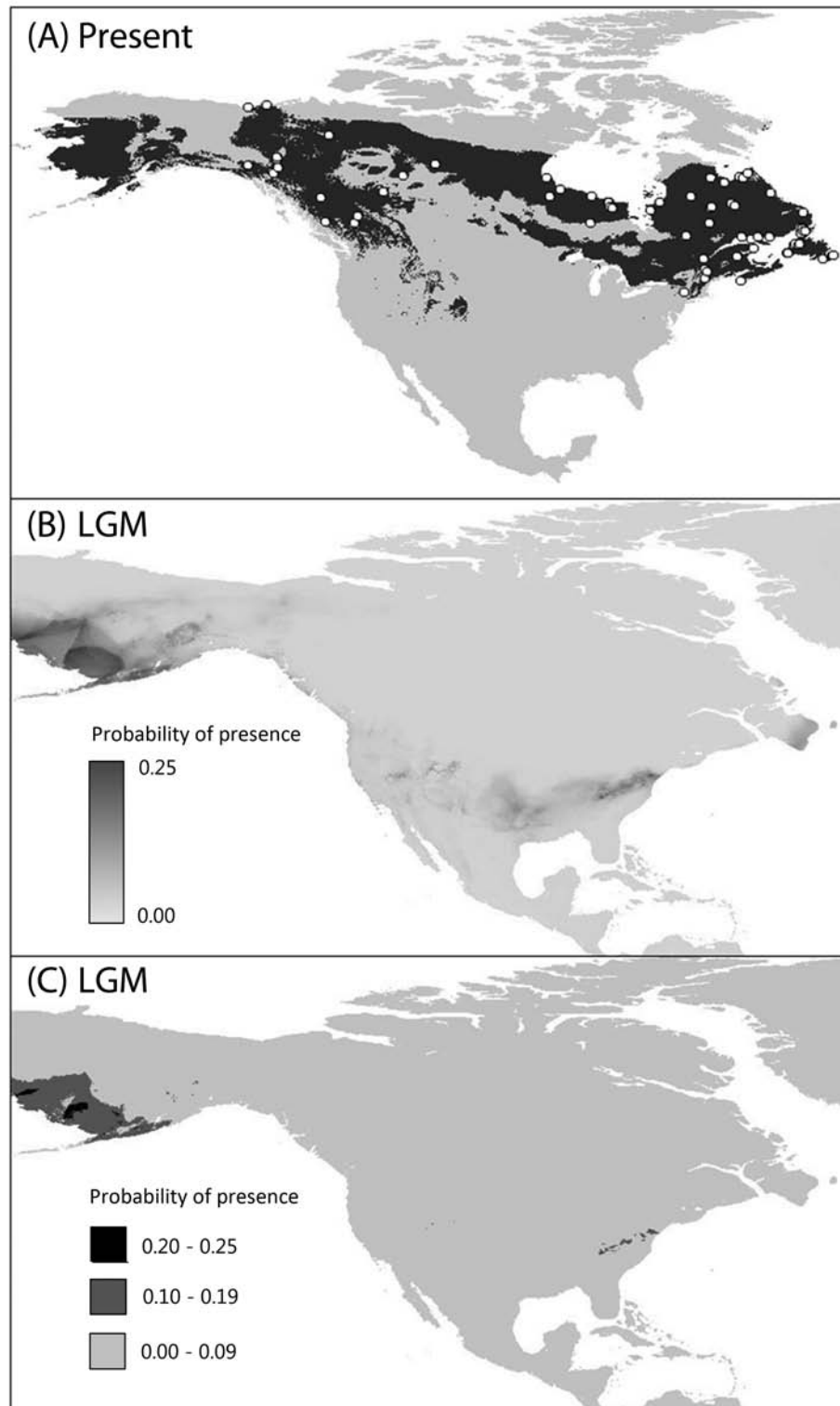
Fig. 5. Models of Blackpoll Warbler distribution under (A) current and (B and C) LGM conditions as modeled by the MIROC climate model using 76 July occurrences (white circles in A). Current modeled presence (black in A) was mapped from probability of presence using a threshold of 10th percentile of training data (Phillips et al. 2006). (B) Paleodistribution suggests three distinct suitable regions that may have held refugia. (C) When probability thresholds of 0.10 and 0.20 are applied, only two potential refugia remain.

Blackpoll Warblers. However, nucleotide divergence is low; we did not find evidence of genetic breaks (divergence) in our haplotype network, and there are no clades of haplotypes in our phylogenetic trees, all of which suggests a lack of historical population fragmentation. We also did not find significant genetic structure among currently isolated populations at the southeast periphery of the species' breeding range. Instead, we found a pattern of continental-scale geographic genetic structure that is consistent with a model of isolation-by-distance. A scenario of isolation-by-distance is also supported by the fact that both western and eastern populations share haplotypes with central populations (Alberta and Manitoba) but not with each other, which indicates that gene flow is limited at large geographic scales, even for this long-distance migrant.

It is surprising that we did not observe significant geographic structure among isolated sky-island populations at the southern periphery of the species' breeding range. McCormack et al. (2008) reported an interesting case of genetic structure evolving among sky-island populations of Transvolcanic Jays (*Aphelocoma ultramarina*) that became isolated in only the last 12,000 years. Microsatellite data obtained from the eastern, disjunct populations of Blackpoll Warblers also indicate a lack of structure among sky-island populations (J. Ralston and J. J. Kirchman unpubl. data). Direct estimates of dispersal using mark–recapture methods are needed to clarify rates of gene flow among high-elevation boreal-forest isolates in eastern North America.

Our results suggest that the genetic structure in Blackpoll Warblers is not due to biogeographic breaks or a history of fragmentation into multiple refugia, but rather results from limited gene flow across the species' huge breeding range. Paleodistribution models indicate that Blackpoll Warblers might have persisted in three refugial areas of North America during the LGM. Our approach was to regard these areas as potential refugia, and to test them as alternative hypotheses against our genetic data. These tests rely on several assumptions and parameter estimates, including selective neutrality of the mitochondrial control region and estimates of effective population size, generation times, and the substitution rate of the control region. Despite model sensitivity to alternative mutation rates, we find general support for recent expansion out of a single refugium. Our fastest mutation rate of 0.30 substitutions site^{-1} Ma^{-1}, which has been applied in many previous studies using control-region domain I in passerines (Milot et al. 2000, Davis et al. 2006, Kirchman and Franklin 2007, Colbeck et al. 2008), is faster than phylogenetically determined rates calibrated with fossils or biogeographic events older than 1 million years (Weir and Schluter 2008) but may be more appropriate for short-term divergences such as those we investigated here (Ho et al. 2005). Peterson and Masel (2009) have shown that the time dependency of mutation rates is real and that the faster rates that pertain to intraspecific genetic divergences are due to the persistence of ancestral polymorphism. Faster rates provide stronger support for a single refugium in Blackpoll Warblers, though this conclusion is not contingent on fast mutation rates. A single-refugium hypothesis could not be rejected even at the slowest rate.

Our discovery that Blackpoll Warblers from Newfoundland are genetically distinct (mean pairwise F_{ST} = 0.155) mirrors the finding of Colbeck et al. (2008) that Newfoundland populations of American Redstarts (*S. ruticilla*) are distinct from all other continental populations of that species. Although American Redstarts are monotypic across their extensive range and have low phylogeographic structure, they are thought to have survived the Pleistocene in two refugia, one in southeastern North America and one on the now-submerged Atlantic coastal shelf near Newfoundland (Colbeck et al. 2008). The importance of northeastern Atlantic coastal refugia in the genetic structuring of modern breeding populations of North American birds should be further investigated, with particular attention paid to populations from Newfoundland. To date, very few phylogeographic studies have included samples from Newfoundland (Zink and Dittmann 1993, Milot et al. 2000, Colbeck et al. 2008).

The location of the Blackpoll Warbler refugium is difficult to infer from our genetic data alone. Levels of neutral genetic diversity should be highest near centers of origin (i.e., refugia) and lowest at the leading front of a population expansion, but this signature of directional expansion is difficult to detect in our data set because of uneven sampling across the east–west axis. Migratory pathway offers an alternative means of inferring the location of refugia. Ruegg and Smith (2002) proposed that autumnal migration pathways in birds trace historical routes of postglacial expansion and used this to explain the circuitous migration in Swainson's Thrush (*Catharus ustulatus*). Blackpoll Warblers have a unique migration route among North American passerines. Individuals breeding throughout Canada and as far west as Alaska migrate east in the fall and collect along the Atlantic coast before migrating south over the Atlantic to their wintering grounds in South America. This path brings all Blackpoll Warblers near the modeled refugium south of the ice sheets in eastern North America, regardless of their breeding latitude (Kirchman et al. 2011). Migratory behavior may also explain observed patterns of genetic structure among Blackpoll Warbler populations in central Canada. Several authors have proposed that dispersal (and gene flow) is facilitated along the migratory route (Helbig 2003, Boulet and Gibbs 2006). We found that unlike populations from Alberta, central Canada, and Manitoba, the Northwest Territories population groups strongly with the east and shares no haplotypes with western populations. This pattern may be the result of birds migrating east from Alaska, Yukon, and British Columbia through Alberta, Saskatchewan, and Manitoba, facilitating dispersal into these populations, but passing to the south of Northwest Territories. The most parsimonious explanation of both the modern migratory pathway and observed genetic structure is expansion from a single refugium in southeastern North America. As ice sheets retreated and breeding populations expanded north and northwest, autumn migration continued to track ancestral routes to the coast and over the Atlantic to tropical wintering grounds. This interpretation is congruent with inferred histories of other broadly codistributed migratory passerines, including Swainson's Thrush (Ruegg and Smith 2002, Ruegg et al. 2006) and eastern Yellow-rumped Warblers (*S. c. coronata*; Milá et al. 2007b) that also underwent postglacial expansion out of an eastern North American refugium. Thus, in concordance with studies of other North American bird species, we have found significant population genetic structuring on a continental scale in Blackpoll Warblers. Unlike in these other species, however, patterns in Blackpoll Warblers seem to reflect very recent or ongoing processes rather than historical fragmentation into Pleistocene refugia.

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