Molecular Ecology (2002) **11**, 1605–1616

Phylogeographical approaches to assessing demographic connectivity between breeding and overwintering regions in a Nearctic–Neotropical warbler (*Wilsonia pusilla*)

M. KIMURA,* S. M. CLEGG,* I. J. LOVETTE,*† K. R. HOLDER,* D. J. GIRMAN,*‡ B. MILÁ,*§ P. WADE* and T. B. SMITH*¶

**Center for Tropical Research and Department of Biology, San Francisco State University, San Francisco, USA; †Cornell Laboratory of Ornithology, Ithaca, USA; ‡Department of Biology, Sonoma State University, Rohnert Park, USA; §Department of Organismic Biology, Ecology, and Evolution, University of California, Los Angeles, USA*

Abstract

We characterized the pattern and magnitude of phylogeographical variation among breeding populations of a long-distance migratory bird, the Wilson's warbler (*Wilsonia pusilla*), and used this information to assess the utility of mtDNA markers for assaying demographic connectivity between breeding and overwintering regions. We found a complex pattern of population differentiation in mitochondrial DNA (mtDNA) variation among populations across the breeding range. Individuals from eastern North America were differentiated from western individuals and the eastern haplotypes formed a distinct, well-supported cluster. The more diverse western group contained haplotype clusters with significant geographical structuring, but there was also broad mixing of haplotype groups such that no haplotype groups were population specific and the predominance of rare haplotypes limited the utility of frequency-based assignment techniques. Nonetheless, the existence of geographically diagnosable eastern vs. western haplotypes enabled us to characterize the distribution of these two groups across 14 overwintering locations. Western haplotypes were present at much higher frequencies than eastern haplotypes at most overwintering sites. Application of this mtDNA-based method of linking breeding and overwintering populations on a finer geographical scale was precluded by the absence of population-specific markers and by insufficient haplotype sorting among western breeding populations. Our results suggest that because migratory species such as the Wilson's warbler likely experienced extensive gene flow among regional breeding populations, molecular markers will have the greatest utility for characterizing breeding–overwintering connectivity at a broad geographical scale.

Keywords: connectivity, migration, mtDNA, phylogeography, *Wilsonia pusilla*

Received 3 September 2001; revision received 25 April 2002; accepted 25 April 2002

Introduction

Migratory New World songbirds that move between temperate breeding and tropical overwintering locations have received relatively little attention from an intra-specific phylogeographical perspective. Concomitantly, little is known about the pattern and magnitude of genetic variation among breeding populations of most Neotropical migrants, or about the extent of breeding population mixing during the nonbreeding season. In the few migratory or irruptive species for which genetic information is available, levels of molecular variation are generally low and these species often exhibit low or negligible geographical population structure (Ball & Avise 1992; Seutin *et al*. 1995; Buerkle 1999; Arguedas & Parker 2000; Gibbs *et al*. 2000; Milá *et al*. 2000; Winker *et al*. 2000; Lovette & Bermingham 2001). Migration itself may

Correspondence: Thomas B. Smith. ¶Present address: Department of Organismic Biology, Ecology, and Evolution and Center for Tropical Research Institute of the Environment, 621 Charles E. Young Drive South, Room LS5120, PO Box 951606, University of California, Los Angeles, CA 90095–1606 USA. Fax: +1 310 825 5446; E-mail: tbsmith@ucla.edu

inhibit phylogeographical differentiation. Studies of marked individuals show that migratory Neotropical songbird species typically exhibit very low levels (< 10% return rates) of natal philopatry (Weatherhead & Forbes 1994). This high magnitude of natal dispersal suggests that long-distance gene flow may occur over short ecological time scales, either via the long-distance dispersal of individuals or incrementally via shorter distance dispersal over several generations. High levels of gene flow would tend to limit population-level genetic differentiation and render any demographic structure difficult to reconstruct using molecular techniques. The weak phylogeographical structure in these vagile species contrasts with trends seen in resident songbirds in North America and elsewhere, many of which exhibit high levels of phylogeographical variation (e.g. Gill *et al*. 1993; Klein & Brown 1994; Zink 1997; Lovette *et al*. 1998).

Although many Neotropical migratory songbird species may exhibit a high degree of present-day gene flow, the presence of intraspecific geographical variation in morphology, behaviour and life history traits in some species suggests that historical biogeographical processes may have contributed to intraspecific diversification. Pleistocene vicariance leading to differentiation in North American songbirds was first suggested by Mengel (1964), who hypothesized that Pleistocene glacial cycles drove species-level diversification. However, more recent genetic evidence has shown that many North American songbird species originated prior to the Pleistocene (Bermingham *et al*. 1992; Klicka & Zink 1997; Lovette & Bermingham 1999). Thus, glacial cycles and resulting changes in habitats seem to have played a larger role in causing differentiation at the intraspecific level (Avise & Walker 1998). In particular, migratory songbird species that extended their breeding range as forested habitats expanded during the present interglacial are likely to have experienced demographic bottlenecks in one or more forest refugia during previous glacial maxima.

The potential for phylogeographical structure in any particular migratory species therefore depends both upon the magnitude of present-day gene flow and upon the degree of population structure caused by historical events earlier in the species' history. Documenting the pattern (or lack) of phylogeographical variation is valuable both from a comparative evolutionary perspective and because of the potential applications to conservation. In an applied context, the presence or absence of breeding-range phylogeographical structure in Neotropical migrants has important implications for the use of molecular markers to link breeding and overwintering populations of these species. The inability to accurately define and follow population units throughout the annual cycle has been a major hindrance to the study of demographic changes in Neotropical migrant species (Webster *et al*. 2002), some of which are undergoing severe population declines of indeterminate cause. Traditional nonmolecular methods of tracking individuals through their annual cycle have proved highly inefficient. Typically, few individuals banded on breeding grounds are recovered from overwintering areas and alternative methods such as radio telemetry of individuals remain technologically unfeasible for small songbirds that migrate long distances. One promising technique is the use of geographically specific stable isotopes in feathers to assign nonbreeding birds to the breeding regions where the feathers were grown; at present, however, this approach is only informative over broad latitudinal or longitudinal zones (Chamberlain *et al*. 1997, 2000; Hobson 1999; Cherel *et al*. 2000; Hobson *et al*. 2001; Rubenstein *et al*. 2002).

Population-specific molecular markers seem to have the potential to greatly augment the limited information on breeding–wintering connectivity available from these nonmolecular sources (Webster *et al*. 2002). To apply molecular techniques to the question of breeding–wintering connectivity, it is necessary to first assess genetic variation among breeding populations and thereby identify markers that can be used to assign an overwintering individual to its region of breeding origin (e.g. Wenink & Baker 1996; Haig *et al*. 1997). Despite the strong interest in passerine Neotropical migrants engendered by their recent population declines (e.g. Robbins *et al*. 1989; DeGraaf & Rappole 1995), there have been no studies of their breeding–wintering population connectivity based on analyses of phylogeographical variation in molecular markers. The utility of such molecular methods for assaying population-level links will depend largely on the geographical scale of the breeding season population structure in these migratory species. Strong genetic structure on a fine scale would allow tight links between breeding and overwintering regions to be documented. This would potentially allow demographic trends to be associated with land use changes, thereby informing conservation decisions. Conversely, breeding season panmixis would preclude the use of molecular markers to link breeding and overwintering regions.

We reconstructed phylogeographical variation among Wilson's warbler (*Wilsonia pusilla*) breeding populations using mitochondrial DNA (mtDNA) sequences and used this information to explore the utility of molecular markers to link breeding and overwintering populations. Wilson's warbler is locally common and widespread across western and northern North America, with a breeding range that extends from California and New Mexico north to Alaska and across Canada to Newfoundland (see map portion of Fig. 1). The existence of three geographical subspecies, *W. p. chryseola, W. p. pileolata* and *W. p. pusilla* (Lowery & Monroe 1968), distinguished by subtle plumage and behavioural differences (Ammon & Gilbert 1999) suggested that this polytypic species might also exhibit useful

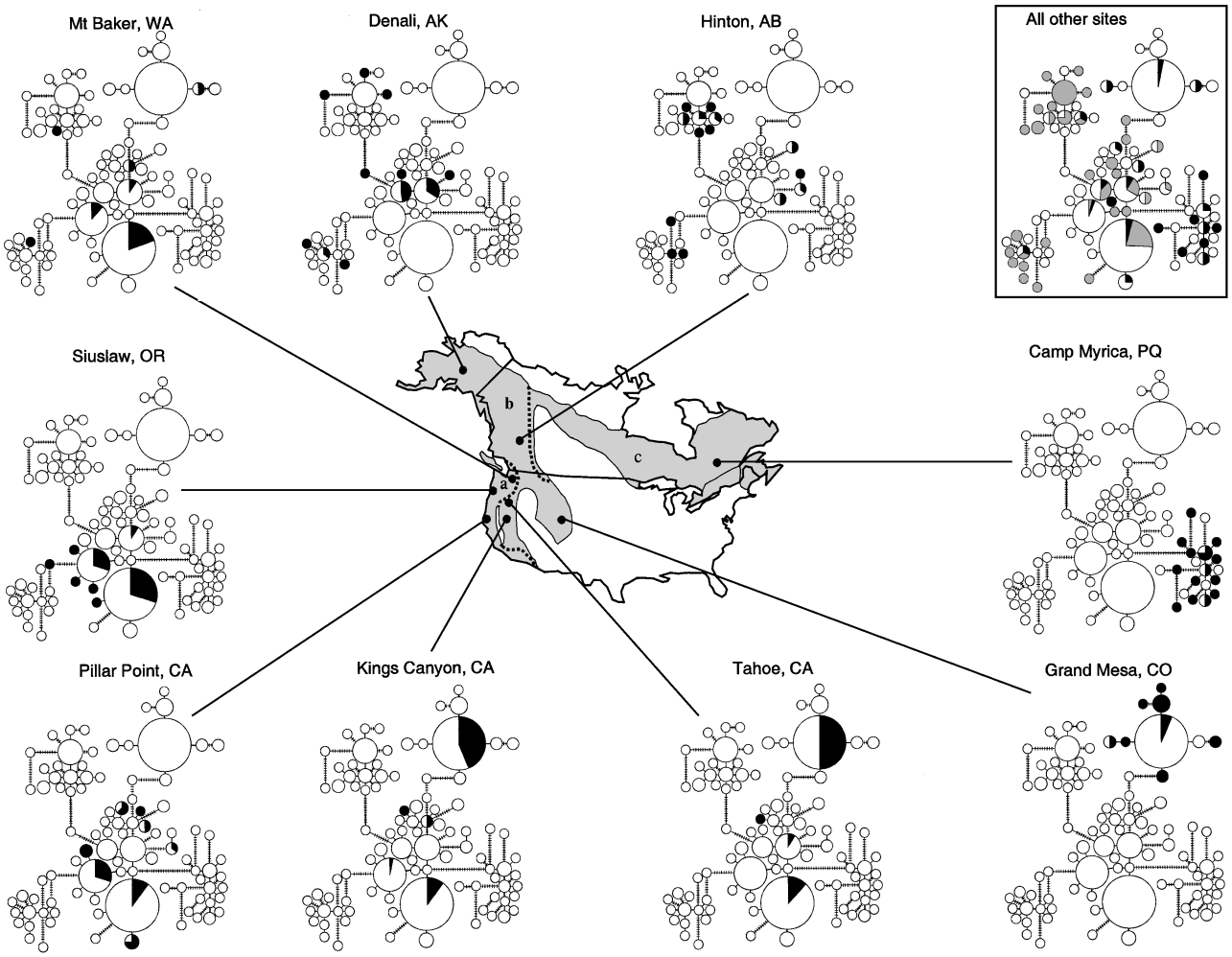

Fig. 1 Distribution of control region haplotypes at the nine most intensively sampled sites ($n \geq 12$) across the breeding distribution of Wilson's warbler. The map at centre depicts the location of these nine sites superimposed on the geographical ranges (dashed lines) of the three currently recognized morphological Wilson's warbler subspecies: (a) *Wilsonia pusilla chryseola* in Pacific coast ranges, (b) *W. p. pileolata* in the centre of the species range, and (c) *W. p. pusilla* in the north-east. The presence and relative frequencies of haplotypes found at each site are indicated by dark shading in the minimum spanning network associated with each site (see Fig. 2 for additional details on this network). The boxed network at upper right shows the frequencies of haplotypes recovered from breeding sites where $n \leq 12$ (black shading) and the frequencies of haplotypes recovered only from individuals sampled from overwintering locations (grey shading).

mtDNA population structure. Furthermore, some regional breeding populations have undergone significant population declines in recent years, raising conservation concerns, whereas others have increased, suggesting that different breeding populations are demographically distinct (Breeding Bird Survey data; Sauer *et al*. 2000). The overwintering range of Wilson's warbler extends from northern Mexico south throughout Central America to western Panama, but very little is known about where particular breeding populations overwinter. For example, although over 140 000 Wilson's warblers (*W. pusilla*) have been banded in the USA and Canada, only 3 of those individuals (0.002%) have been recovered on their overwintering areas in Mexico and Central America (Bird Banding Laboratory, Laurel, MD, USA).

Our aims here are threefold: (i) to reconstruct the recent evolutionary history of Wilson's warbler in North American breeding regions to evaluate the role of late Pleistocene glacial vicariance in promoting intraspecific differentiation in this species; (ii) to assess the degree of population genetic structure across the breeding range; and (iii) to use this information to infer patterns of connectivity between breeding and overwintering areas.

Materials and methods

Sample collection

We sampled a total of 338 individual Wilson's warblers (*Wilsonia pusilla*) for this study: 152 individuals from 13

Table 1 Population locations and sample size (n) for breeding and overwintering sites. For breeding sites with $n \geq 12$, haplotype diversity (h) and nucleotide diversity (π) are indicated

Sampling site	Abbr.	Latitude–Longitude	n	h ± SD	π ± SD
Breeding					
A. Denali Natl Park, AK	DE	63°35′ N–149°36′ W	15	0.94 ± 0.05	0.022 ± 0.012
B. Hinton, AB	AB	52°40′ N–111°18′ W	14	1.00 ± 0.03	0.023 ± 0.013
C. Mt Baker Natl Forest, WA	MB	48°09′ N–121°27′ W	12	0.86 ± 0.08	0.015 ± 0.009
D. Umatilla Natl Forest, OR	UM	45°48′ N–117°51′ W	5	1.00 ± 0.13	0.020 ± 0.013
E. Siuslaw Natl Forest, OR	SIU	44°16′ N–123°51′ W	16	0.83 ± 0.08	0.005 ± 0.003
F. Tahoe Natl Forest, CA	TA	39°37′ N–120°31′ W	15	0.54 ± 0.13	0.011 ± 0.007
G. Kings Canyon Natl Park, CA	KC	36°44′ N–118°58′ W	12	0.67 ± 0.14	0.013 ± 0.008
H. Pillar Point, CA	PILL	37°30′ N–122°29′ W	17	0.90 ± 0.04	0.007 ± 0.004
I. Big Sur, CA	BS	36°16′ N–121°48′ W	8	1.00 ± 0.06	0.013 ± 0.008
J. Grand Mesa, CO	CO	39°02′ N–107°57′ W	14	0.90 ± 0.06	0.006 ± 0.004
K. Camp Myrica, PQ	QU	49°43′ N–73°20′ W	16	0.98 ± 0.03	0.012 ± 0.007
L. Fredericton, NB	NB	45°48′ N–66°39′ W	4	1.00 ± 0.18	0.008 ± 0.006
M. Hilliardton, ON	ON	47°30′ N–79°40′W	4	1.00 ± 0.18	0.014 ± 0.010
Wintering					
N. Baja California Sur, Mex.	BCS	22°53′ N–109°54′ W	7	—	—
O. La Maria, Colima, Mex.	COL	19°14′ N–103°43′ W	25	—	—
P. Las Joyas Biol. Station, Jalisco, Mex.	JAL	19°46′ N–104°22′ W	22	—	—
Q. El Cielo Bios. Reserve, Tamaulipas, Mex.	ELC	23°00′ N–99°08′ W	13	—	—
R. Coatepec, Veracruz, Mex.	COA	19°27′ N–96°58′ W	19	—	—
S. Catemaco, Veracruz, Mex.	CAT	18°25′ N–95°07′ W	7	—	—
T. Oaxaca, Mex.	OAX	17°03′ N–96°43′ W	15	—	—
U. El Ocote Reserve, Chiapas, Mex.	ELO	16°45′ N–93°07′ W	6	—	—
V. Cockscomb Basin, Belize	BZ	16°47′ N–89°00′ W	1	—	—
W. Tegucigalpa, Honduras	HON	14°06′ N–87°13′ W	25	—	—
X. San Salvador, El Salvador	ES	13°42′ N–89°12′ W	15	—	—
Y. Estelí, Nicaragua	NIC	13°05′ N–86°23′ W	9	—	—
Z1. Santa Elena, Costa Rica	SE	10°56′ N–85°41′ W	10	—	—
Z2. San Vito, Costa Rica	SV	08°50′ N–82°58′ W	12	—	—

breeding sites and 186 individuals from 14 overwintering sites (Table 1). To increase the likelihood that birds sampled from breeding locations were local breeders rather than passage migrants, we included only samples from individuals that met the following criteria: (i) they were captured during the core of the breeding season between 1 June and 31 July, and (ii) they possessed physiological indicators of breeding condition (for males a cloacal protuberance and for females a well-defined brood patch; see Pyle 1997). Feather samples from individuals on the breeding grounds were either collected by us, contributed by bird banders participating in the Monitoring Avian Productivity and Survivorship (MAPS) programme, or contributed by independent banders. Samples from overwintering individuals were obtained between 1 December and 28 February. Most of these overwintering individuals are likely to have been captured on their final winter destination, because the available evidence from colour banding and resighting studies suggests that Wilson's warblers occupy stable winter territories from November to April (Rappole *et al*. 1992).

We obtained DNA from blood and/or feather tips from all individuals captured. Blood was sampled via brachial venepuncture and stored in lysis buffer (Seutin *et al*. 1991). Feathers were obtained by collecting the two outer rectrices from each individual. Blood and feather samples were kept at ambient temperatures until they were returned to the laboratory, when they were archived at –20 °C.

DNA amplification and sequencing of mitochondrial control region

Genomic DNA was extracted from blood following standard salt or phenol/chloroform procedures (Hillis *et al*. 1996) or, alternatively, from the quill tips of feathers using a QIAAMP® kit (QIAGEN Inc., Valencia, CA, USA) following the manufacturer's instructions. To explore the relatedness of intraspecific lineages, the degree of population structure and levels of intrapopulation diversity, we amplified and sequenced 343 bp of control region I using primers LGL2 and H417 (Tarr 1995). Amplification volumes totalled 25 μL and included 1 μL purified DNA

solution (concentration variable), 2.5 μL 1× PCR Buffer II (Perkin-Elmer), 2.0 mM $MgCl_2$, 0.8 mM dNTPs (Sigma), 0.4 μM each primer and 0.5 U of Amplitaq polymerase (Perkin-Elmer). Reactions were denatured for 3 min at 94 °C, followed by 30 cycles of 94 °C denaturing for 30 s, 50 °C annealing for 30 s and 72 °C extension for 45 s and terminated with a 5-min extension at 72 °C. Sequencing reactions were conducted with ABI dye terminator chemistry (Applied Biosystems, Foster City, CA, USA) following the standard ABI cycle sequencing protocol and were electrophoresed on an ABI 377 automated sequencer. The resulting sequences were aligned and assembled using SEQUENCHER Version 3.0 (GeneCodes Corp., Ann Arbor, MI, USA).

As discussed in more detail below, we encountered difficulties in amplifying a portion of the cytochrome *b* gene from some *W. pusilla* samples owing to the co-amplification of an apparent nuclear-encoded copy (numt) of that gene. Several lines of evidence, however, suggest that the control region I sequences used for the phylogenetic analyses presented here are mitochondrially encoded. First, the extremely high levels of variation among individuals are consistent with mitochondrial rather than nuclear origin (Kidd & Friesen 1998). Second, we generated replicated sequences from several individuals from DNA extracted from blood (relatively nuclear genome enriched), feathers (unknown mtDNA vs. nuclear copy number) and pectoral muscle (relatively mtDNA genome enriched), and in all cases the sequences from a given individual were identical and unambiguous. Finally, we encountered none of the anomalies in our control region chromatograms such as 'double peaks' that were indicative of numt contamination in the cytochrome *b* amplifications.

Analyses of phylogeographical variation

We conducted analyses of genetic variation using the 343 bp of control region I sequence obtained from 200 individuals comprising 153 individuals from 13 breeding sites and 47 individuals of unknown breeding origin sampled from two overwintering sites. Sequences for the control region have been deposited in GenBank (Accession nos AF499469–AF499562). Overwintering individuals were sequenced to increase the probability of detecting lineages that might have gone undetected because of geographical gaps in the breeding-season sampling.

Because the number of haplotypes vastly exceeded the number of potentially parsimony informative sites, we generated a preliminary phylogenetic reconstruction via a distance-based method (neighbour-joining) on a Kimura 2-parameter distance matrix (Kimura 1980) in PAUP* (Swofford 1999). This reconstruction was midpoint rooted because large genetic distances separated *W. pusilla* from its two congeners, *W. citrina* and *W. canadensis*. The large number of very similar control region I haplotypes we recovered suggests, however, that tree-based reconstructions are unlikely to depict reliably the actual relationship among haplotypes, because many haplotypes in our sample are likely to be derived from other sampled haplotypes. We therefore generated a minimum spanning network in the program ARLEQUIN Version 2.000 (Schneider *et al*. 2000).

To test for geographical structure in control region haplotype variation, we conducted analyses of molecular variance (AMOVA, Excoffier *et al*. 1992) using ARLEQUIN 2.000. AMOVAS were based on Kimura 2-parameter distances, and included the nine sampling sites for which we obtained large numbers of control region sequences. Each of these sampling sites was treated as a separate population in the AMOVA to explore the extent of population subdivision across the breeding range. In addition, we used ARLEQUIN to generate population pairwise F_{ST} values for all breeding sampling sites.

To compare levels of genetic divergence among populations, we calculated haplotype diversity (*h*; *sensu* Nei 1987 equation 8.5) and nucleotide diversity (π; *sensu* Nei 1987 equation 10.5) indices for all populations where $n \geq 12$. The *h* index reflects the probability that two randomly chosen haplotypes in a population will differ, whereas the π index reflects the probability that two randomly chosen homologous nucleotides in a population will differ.

To estimate net sequence divergence among genetically differentiated haplotype groups identified in the minimum spanning network, we calculated Nei's genetic distance δ, corrected for within-population polymorphism, as follows:

$$\delta = \pi_{xy} - 0.5(\pi_x + \pi_y)$$

where π_{xy} is the mean pairwise sequence divergence between individuals from population x compared with those from population y, and π_x and π_y are the mean pairwise sequence divergences of individuals in populations x and y, respectively (Nei & Li 1979). This approach assumes that present-day levels of intralineage polymorphism are indicative of the variation present in the common ancestral lineage. We therefore also calculated the minimum, maximum and average pairwise sequence divergence between eastern and western haplotypes without employing Nei's correction factor. These latter values assume all between-lineage divergence has arisen since the lineages became evolutionarily independent.

RFLP-based genotyping of overwintering individuals

After characterizing phylogeographical patterns among breeding-season Wilson's warbler populations, we used restriction digests to assign overwintering birds to genetically differentiated (see Results) eastern vs. western breeding regions. Although a number of control region I variable nucleotides were diagnostic of either the eastern

or western group, no commercially available restriction enzymes cleaved these diagnostic control region sites. We therefore conducted restriction enzyme digests on amplified cytochrome *b* products for which enzymes were available. Although our efforts to sequence this cytochrome *b* fragment were somewhat confounded by the presence of an apparent nuclear-encoded copy that resulted in ambiguities at some nucleotide sites in some individuals, we obtained a substantial number of 'clean' cytochrome *b* sequences that had a typical cytochrome *b* pattern of nucleotide variation and which clustered into eastern vs. western groups in a pattern completely congruent with the corresponding control region I-based reconstructions for the same individuals. We therefore used restriction enzymes that cleaved reciprocally diagnostic cytochrome *b* sites to distinguish overwintering birds from eastern vs. western breeding sites.

Cytochrome *b* amplifications targeted a 316-bp product and were conducted using primers MVZ 03′ and MVZ 04′ as modified by Johnson & Cicero (1991) from L14841 and H15149 (Kocher *et al.* 1989). Sequences for the cytochrome *b* region have been deposited in GenBank (Accession nos AF499563–AF499595). Reaction conditions included an initial denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 45 s, and a final extension at 72 °C for 5 min. Eastern birds possessed a cleavage site for the restriction enzyme *Nsi*I (New England Biolabs, Inc.) absent in western birds, which yielded two bands of 69 and 247 bp. Western birds possessed a cleavage site for *Hin*cII (New England Biolabs, Inc.) absent in eastern birds, which yielded two bands of 137 and 179 bp. We performed restriction digests using 5 μL of polymerase chain reaction (PCR) product and 1 U of enzyme per reaction. We treated each unknown overwintering individual with both enzymes and included known eastern and western breeding birds as positive controls. Products were electrophoresed on 6% acrylamide gels and bands were visualized under UV light following ethidium bromide staining. The size differences of the resulting fragments enabled individuals to be readily scored by eye as eastern or western, and there were no cases in which both enzymes simultaneously cut or failed to cut.

Results

Phylogeographical variation in control region

A total of 94 unique control region haplotypes were defined by 61 variable nucleotide sites among the 200 individuals sequenced. No insertions or deletions were present and the resulting nucleotide alignment was therefore unambiguous. Transition differences occurred at 47 sites, transversions at 4 sites, and both transitions and transversions at 10 sites. Overall sequence divergence among all control region haplotypes based on Kimura 2-parameter distances ranged from 0.3 to 6.4% (mean 2.9%). Most of the haplotypes (65/94) were unique to individuals, 29 haplotypes were shared by more than 1 individual and 4 common haplotypes were shared by 10–21 individuals. Sixty-one haplotypes were identified from breeding individuals only, 22 from overwintering individuals only, and 11 were identified in both. All 47 overwintering individuals sequenced from 2 sites in western Mexico fell within 1 of the haplotype clusters identified in the sample of breeding individuals, suggesting that no additional, well-differentiated haplotype groups were missed in our breeding ground sampling.

Phylogenetic structure among samples from across the breeding range of Wilson's warbler was revealed by patterns of haplotype clustering in a minimum spanning network (Fig. 2) with four haplotype clusters emanating from a central cluster. These peripheral clusters differed from the central group by 8–22 nucleotide substitutions. With Nei's δ correction factor, each peripheral haplotype group differed from the central group by between 0.7 and 1.3%. One of the peripheral clusters comprised individuals from Ontario, Quebec and New Brunswick, representing a monophyletic group with strong support (94% bootstrap value in a neighbour-joining tree; results not shown). The central and remaining three peripheral clusters contained western breeding birds only, but these western groups collapsed when forced into a neighbour-joining tree. Of all western sampling locations, only Pillar Point, California, comprised members of a single haplotype group (Fig. 1). Current subspecies designations are only partially concordant with patterns of control region phylogeographical variation: eastern populations of *Wilsonia pusilla pusilla* can be genetically distinguished from populations falling in the range of *W. p. chryseola* and *W. p. pileolata*, but we found no concordance between control region haplotype variation and the boundaries of these latter two subspecies (Fig. 1). Although there is a trend towards increased genetic diversity in Wilson's warbler populations at higher latitudes (Fig. 3), this trend is not statistically supported for either haplotype diversity (*h* index; Spearman rank correlation, $r_s = 0.64$, $P = 0.07$), or nucleotide diversity (π index; $r_s = 0.47$, $P = 0.20$), although it is near significance for haplotype diversity.

When all breeding ground sampling sites were considered separate populations, an AMOVA showed that the majority of the total genetic variance was due to among-population variation (df = 8; $\phi_{ST} = 55\%$; $P < 0.001$), however, a significant amount of variation was also attributed to variation within populations (df = 120 $\phi_{SC} = 45\%$; $P < 0.001$). When the analysis was restricted to western populations only, most variation occurred within populations (df = 105; $\phi_{SC} = 60\%$; $P < 0.001$), although the among-population variance component remained significant (df = 7; $\phi_{ST} = 40\%$; $P < 0.001$). In addition, there was some evidence for geographical structure in the west, based on the frequency

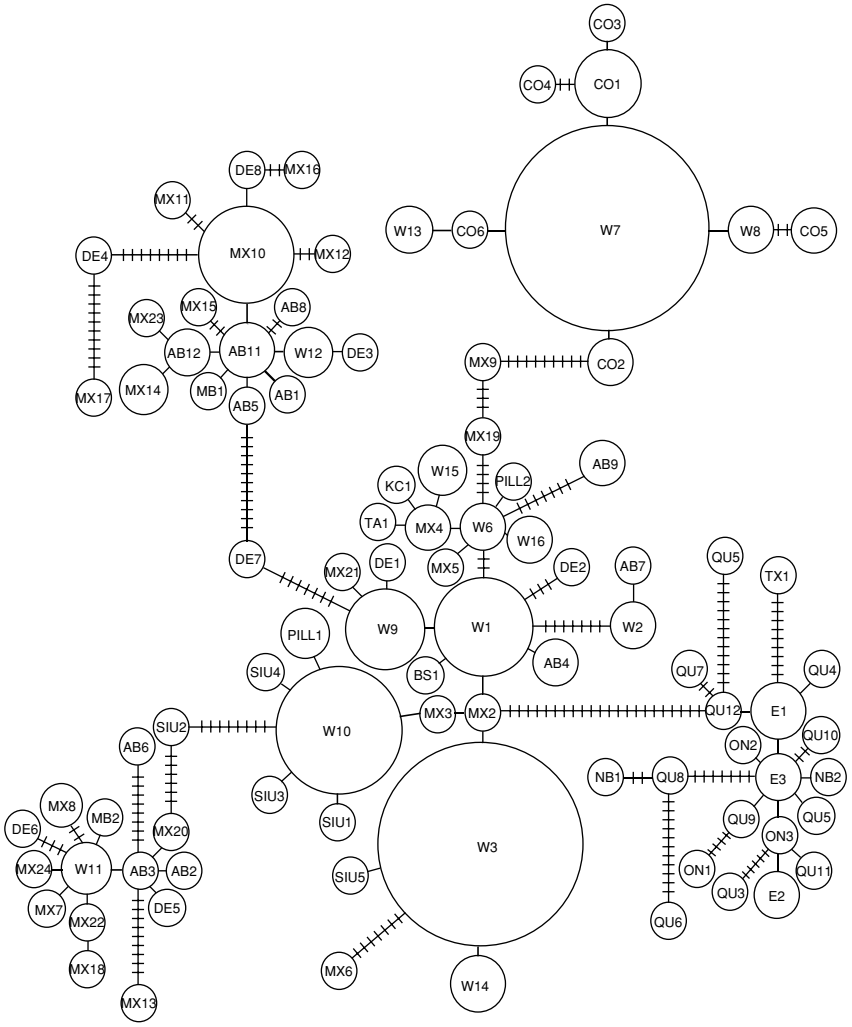

Fig. 2 Minimum spanning network for the 94 Wilson's warbler control region haplotypes. The size of the circle denoting each haplotype is proportional to the number of sequenced individuals with that haplotype. Hatch marks along branches indicate the inferred number of nucleotide substitutions separating haplotypes. Haplotype designations indicate location of origin (see Table 1 for site-specific abbreviations; W = haplotypes recovered from ≥ 2 western breeding locations; E = haplotypes recovered from ≥ 2 eastern breeding locations; MX = haplotypes recovered only from overwintering locations). The group of haplotypes at bottom right is the 'eastern group' referred to in the text; all other haplotypes fall in the western group.

and distribution of haplotypes in the minimum spanning tree in which shared haplotypes tend to occur in geographically adjacent or nearby sites (Fig. 1).

Pairwise F_{ST} values ranged from –0.08 (Umatilla/Tahoe) to 0.89 (Siuslaw/New Brunswick) (Table 2). Alberta yielded a significant F_{ST} compared with every other population. The three eastern sampling sites had nonsignificant F_{ST}s among themselves, and significant F_{ST}s compared with all other western populations. Among western populations, the nontransitive nature of gene flow between certain pairs of populations made it difficult to assign these populations to larger regional groups. For example, although Big Sur had a nonsignificant F_{ST} value compared with both Denali and Mt. Baker, Mt. Baker compared with Denali yielded a significant F_{ST}.

Distribution of lineages among overwintering region

Of 186 overwintering individuals genotyped via sequencing or restriction digests, only 10 belonged to the 'eastern' breeding group. The remaining 173 were of western origin, but could not be assigned with confidence to a particular subregion of the western breeding range. A total of 5 of 14 overwintering sampling sites contained individuals with the eastern mtDNA lineage, and of these, four contained a mixture of eastern and western lineages (Fig. 4). All other overwintering sampling sites contained only western breeding individuals. The sites that contained eastern birds were clustered in the centre of the overwintering range with the exception of the southern Costa Rican overwintering site, which contained one eastern individual (Fig. 4). The western birds thus appear to be more abundant at most sites sampled and occupy a broader latitudinal overwintering range than do eastern birds.

Discussion

The existence of phylogeographical structure in mtDNA indicates that historical biogeographical processes have influenced patterns of intraspecific molecular variation in Wilson's warbler. This variation is only partially concordant

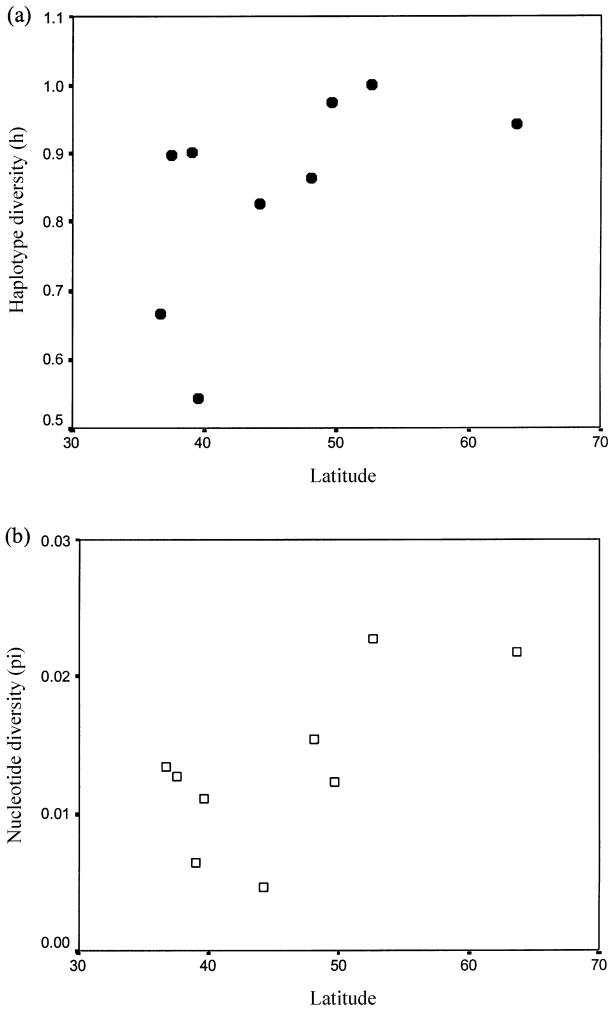

Fig. 3 (a) Haplotype diversity (h), and (b) nucleotide diversity (π) indices as a function of latitude for breeding sites from which $n \geq 12$ control region I sequences were obtained.

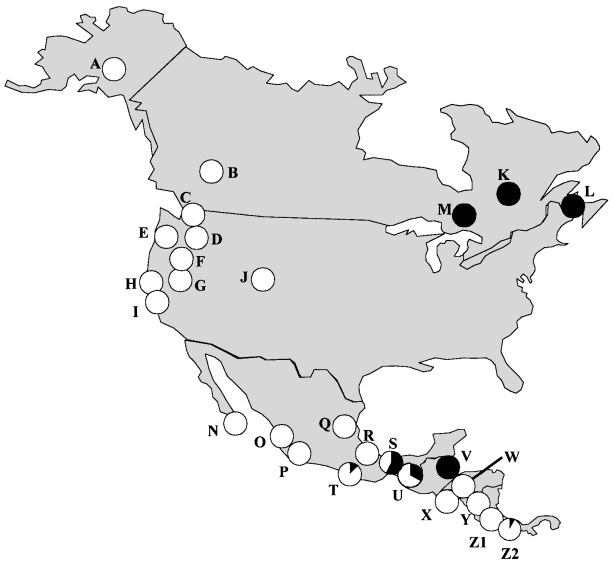

Fig. 4 Frequencies of eastern (black shading) and western (white shading) Wilson's warbler haplotypes at breeding and overwintering sites. Letters correspond to sites described in Table 1.

with current subspecies designations, with eastern populations of one widespread race (*Wisonia pusilla pusilla*) forming a well-supported genetic lineage readily distinguished from the two western races (*W. p. chryseola* and *W. p. pileolata*). Our sampling regime does not allow us to determine whether central and western populations of *W. p. pusilla* are most closely related to eastern populations of *W. p. pusilla* or to populations of the neighbouring western subspecies. Irrespective of the genetic affinities of central and western *W. p. pusilla* populations, *W. p. chryseola* and *W. p. pileolata* are not phylogenetically distinguishable from each other using this mtDNA marker.

Table 2 Pairwise F_{ST} values for all breeding populations. In the lower diagonal, significant values ($P < 0.05$) are indicated by a plus; nonsignificant values are indicated by a minus

	DE	AB	MB	UMA	SIU	TA	KC	PILL	BS	CO	QU	NB	ON
DE		+	+	+	+	+	+	+	−	+	+	+	+
AB	0.076		+	+	+	+	+	+	+	+	+	+	+
MB	0.123	0.168		+	+	+	−	+	−	+	+	+	+
UMA	0.202	0.212	0.195		+	−	−	+	+	−	+	+	+
SIU	0.382	0.438	0.082	0.562		+	+	−	+	+	+	+	+
TA	0.308	0.325	0.262	−0.078	0.537		−	+	+	+	+	+	+
KC	0.249	0.267	0.150	−0.071	0.430	−0.056		+	+	+	+	+	+
PILL	0.349	0.414	0.104	0.548	0.056	0.540	0.440		+	+	+	+	+
BS	0.052	0.149	0.003	0.347	0.247	0.414	0.309	0.132		+	+	+	+
CO	0.507	0.461	0.503	0.098	0.749	0.132	0.171	0.732	0.650		+	+	+
QU	0.692	0.685	0.723	0.684	0.822	0.701	0.692	0.814	0.753	0.765		−	−
NB	0.670	0.667	0.737	0.685	0.892	0.739	0.719	0.868	0.785	0.840	−0.064		−
ON	0.657	0.643	0.704	0.651	0.865	0.726	0.698	0.843	0.749	0.818	−0.031	−0.037	

The only geographically monophyletic group among our nine intensively sampled sites was the cluster of eastern haplotypes found in Quebec and other locations in eastern North America that were sampled less intensively. As suggested previously for North American birds (Avise & Walker 1998), Pleistocene vicariant events are the most likely explanation for the divergence between the eastern and western haplotype groups. It is apparent, however, that the separation of the eastern group does not represent the species' only historical population subdivision. In western North America, we found one central haplotype group from which the eastern group and three western haplotype groups are derived in the minimum spanning network. Although the three peripheral western groups are nearly as highly differentiated as is the eastern group, each intensively sampled location in western North America contained individuals representing two or more such groups. The pattern of sharing of divergent haplotypes among these western locations is consistent with a scenario of allopatric divergence, probably during late Pleistocene glacial maxima, followed by postglacial population admixture.

Under the application of the most widely used calibration for rates of avian control region I sequence divergence (20.8% per million year; e.g. Quinn 1992) the magnitude of nucleotide divergence between the central group and the four peripheral groups corresponds to estimates of divergence times ranging from 33 654 to 62 500 years. These estimates must be interpreted with caution for several reasons, including the absence of a rate calibration from a closely allied taxon, assumptions about historical population sizes, and errors associated with the stochastic nature of single locus nucleotide differentiation (e.g. Edwards & Beerli 2000). Nonetheless, even allowing for a 10-fold error in our estimate, the divergence of Wilson's warbler populations clearly falls within the late Pleistocene. Our results add to a growing body of evidence implying that the late Pleistocene, in particular, was an important period for intraspecific differentiation (e.g. Avise & Walker 1998). Most of the current breeding range of Wilson's warbler was glaciated for at least part of the late Pleistocene, suggesting that the species' current broad distribution has resulted from a northward expansion from multiple refugia following the last glacial maximum. The molecular signature of this type of geographical and demographic expansion depends upon the size and number of refugia, and on the rate of demographic increase. Species restricted to a single small refugium during some period of the Pleistocene would be expected to have a low level of genetic diversity over their present-day breeding range as a result of population bottlenecking followed by rapid postglacial expansion (e.g. Gill *et al*. 1993; Milá *et al*. 2000). Northward colonization from genetically depauperate southern refugia has been inferred for several Northern Hemisphere vertebrates, based on a recurring pattern of decreasing molecular diversity at higher latitudes (Merila *et al*. 1997; Hewitt 2000).

Although an association between latitude and level of genetic diversity was suggested in Wilson's warblers, it was in the opposite direction to this general trend: the highest levels of genetic diversity were found in northern Wilson's warbler populations (Alaska and Alberta) in areas that were probably colonized most recently. One explanation for the trend for increased molecular diversity in northern latitudes is that multiple colonizations of the north from more than one southern source may have occurred as ice sheets receded and new habitats became available. For example, high genetic diversity in Aleutian Islands Rock Ptarmigan (*Lagopus mutus*) is attributable to multiple colonizations of that archipelago from three different refugial sources (Holder *et al*. 2000). Alternatively, Wilson's warbler populations at northerly locations may have retained more of the mtDNA polymorphisms that characterized a diverse, ancestral colonizing front than did southern populations. However, under this latter scenario it is unclear what mechanism would result in such differential retention of mitochondrial haplotypes.

Some population-level mtDNA differentiation was evident among western sampling sites. A qualitative assessment of the geographical distribution of western haplotypes (Fig. 1) reveals that adjacent geographical regions tend to have similar complements of haplotypes. For example, as one moves from the southern coast inland towards Kings Canyon and Tahoe in the Sierra Nevada, and then to the Colorado Rocky Mountains, there is a gradual replacement in the representation of several common, shared haplotypes (e.g. haplotypes designated W3, W7, and W10 in Fig. 2). This pattern is consistent with an isolation-by-distance model, but it could also result from allopatric divergence (e.g. in the coastal vs. Rocky Mountain populations) followed by secondary contact and haplotype mixing in geographically intermediate locations (such as in the Sierra Nevada).

This complicated pattern of gradual haplotype replacement among western locations is difficult to describe statistically for several complementary reasons. First, most haplotypes (67%) were found only in single individuals and therefore contribute no information on geographical structure to analyses that do not take into account the phylogenetic relationships among haplotypes. Second, haplotypes that were present in higher frequencies tended to be shared among multiple sampling locations, but sample sizes for frequency-based analyses of only these shared haplotypes were low. Finally, owing to the gradual replacement of haplotype groups across E–W and N–S transects (Fig. 1), there are no clear breakpoints in the geographical distribution of haplotypes that help us to identify historically allied local populations that can be

grouped for statistical analyses of geographical variation. Nevertheless, the significant among-site variation identified in the AMOVA analysis when all western sampling sites were treated as separate populations suggests that there is statistical support for the presence of substantial genetic differentiation among western sites. However, no single western population is readily distinguishable from other such populations.

Conservation implications: linking breeding and overwintering populations

A primary motivation behind our investigation of phylogeographical variation in Wilson's warbler was to explore the utility of using mtDNA markers to link breeding and overwintering populations of this and other Neotropical migrant birds. This application of molecular genetic data is potentially of great conservation relevance because the inability to follow individual migratory birds throughout the year has been a major barrier to integrating the events and processes that occur over their annual cycle. Such integration is important because it is difficult to identify how migratory species are demographically regulated without information on the level of connectivity between particular breeding and overwintering populations (Latta & Baltz 1997; Webster *et al*. 2002). Furthermore, the demographic processes occurring in the two seasons may not be independent; for example, recent studies of other migratory wood warblers suggest that events during the nonbreeding season influence reproductive success in the subsequent breeding season (Marra *et al*. 1998; Sillett *et al*. 2000).

We were successful in identifying mtDNA markers that characterized the two principal haplotype groups within the breeding distribution of Wilson's warbler, and we used these markers to survey for the presence of the corresponding eastern and western lineages in birds sampled at 14 overwintering sites in Mexico and Central America (Fig. 4). Most (93%) of these overwintering individuals had the western mtDNA lineage, which was present at high frequencies at sites spanning the species' entire north–south overwintering range. Individuals with the eastern lineage were present at only five overwintering sites, including four clustered sites in southern Mexico and Belize and one site in Costa Rica (Fig. 4). These patterns suggest that the eastern breeding lineage has a considerably more restricted overwintering distribution than the western breeding lineages. Furthermore, under the assumption that the overwintering individuals were sampled randomly with respect to these eastern vs. western haplotype groups, the breeding population with the eastern mtDNA lineage has a considerably smaller total population size than the western breeding population. An alternative explanation for the numerical discrepancy between eastern and western overwintering birds is that individuals with the eastern lineage may be more common at overwintering locations that were not well-represented in our sample, particularly at interior, highland sites in southern Mexico and Central America.

Molecular markers have been used with mixed success to detect population genetic structure in other taxa with high dispersal abilities (e.g. Wirgin *et al*. 1993; Bowen *et al*. 1995; Larsen *et al*. 1996; Wenink & Baker 1996; Haig *et al*. 1997; McParland *et al*. 1999; Petit & Mayer 2000). The results presented here suggest that mitochondrial markers will be most useful at assessing large-scale patterns of connectivity of breeding and overwintering populations of Neotropical passerines. Mitochondrial markers will likely be less useful in assessing local-scale connectivity. Other highly vagile species may have patterns similar to the Wilson's warbler, in which the high haplotype diversities and extensive sharing of haplotypes across the western breeding region precluded the identification of fine-scale, population-specific haplotypes, even though qualitative examination of the geographical distribution of some haplotype groups suggest that these western populations are not completely panmictic. This pattern may be due to a combination of factors such as the intrinsically high potential for past and ongoing gene flow, and cycles of population bottlenecks during the late Pleistocene glacial maxima and demographic expansions during interglacial periods. A molecular marker that could identify distinct local breeding populations of these birds would need to evolve very rapidly to produce population-specific genotypes on a very short time scale. At present, microsatellite loci would appear to be the most likely such markers, but preliminary analyses of microsatellite variation in Wilson's warblers suggest that even these highly variable markers will not provide increased geographical resolution among the breeding sites discussed here (Clegg *et al*. manuscript submitted).

Considered in concert, the results presented here and previous studies (e.g. Buerkle 1999; Milot *et al*. 2000) suggest that a molecular phylogenetic approach is best utilized to determine connectivity at a large geographical scale. We have shown that determining where eastern vs. western birds overwinter may be accomplished easily using lineage-specific restriction enzymes. Breeding–wintering connectivity on a finer geographical scale, however, would be better explored using other approaches. Molecular genetic data may be most useful when combined with information from other sources, such as morphology and feather isotope ratios (Chamberlain *et al*. 1997), that provide additional information about the breeding-region origin of overwintering migrants. One promising avenue of inquiry involves assaying individual overwintering birds using both molecular and nonmolecular techniques and combining the breeding-region probability assessments generated by these complementary techniques.

Acknowledgements

We thank D. DeSante and the Institute for Bird Populations, the Monitoring Avian Productivity and Survivorship banders, J. Booker, R. Brito, R. Colwell, T. Doyle, P. Marra, J. Steele, and Point Reyes Bird Observatory and the Colorado Bird Observatory for contributing genetic samples. We would also like to thank the many generous individuals who collected and sent us feather samples that were not analysed in this study. For sharing their study sites and/or facilitating fieldwork, we thank Y. Aubry, P. Bichier, R. Carlson, A. Cruz, O. Cruz, R. Dickson, O. Figueroa, T. Gavin, K. Holl, L. Imbeau, O. Komar, B. Murphy, E. Rodriguez, E. Ruelas, E. Santana, W. Schaldach, C. Spytz, P. Thorn and the Belize Audubon Society. For research permits we thank the Canadian Wildlife Service and Environment Canada, Provinces of Quebec, New Brunswick, Ontario and Alberta; US Fish & Wildlife Service; National Park Service; F. Villaseñor for help obtaining permits in Mexico; Instituto Nacional de Ecologia, Secretaria de Medio Ambiente y Recursos Naturales in Mexico; Forestry Department of the Ministry of Natural Resources in Belize; Organization for Tropical Studies and Ministerio del Ambiente y Energia in Costa Rica; and Government of El Salvador. For assistance in the field and/or laboratory we thank T. Erwin, H. Gates, J. Gearlds, L. Gentle, J. Larson, M. Macabeo-Ong, L. Nguyen, K. Ruegg, C. Saux and B. Wang. We thank H.L. Gibbs, M. Stephens, R.K. Wayne and two anonymous reviewers for comments on an earlier version of the manuscript. This work was supported by grants from San Francisco State University, NIH Office of Research on Minority Health (grant #5P20RR11805), a Graduate Assistance in Areas of National Need fellowship to MK Environmental Protection Agency (grant # R827109-01-0) and National Science Foundation (grant # DEB-9726425 and IRCEB-9977072) to TBS.

References

Ammon EM, Gilbert WM (1999) Wilson's warbler (*Wilsonia pusilla*). In: *The Birds of North America, no. 478* (Poole A, Gill F, eds), pp. 1–27. The Birds of North America, Inc, Philadelphia.

Arguedas N, Parker PG (2000) Seasonal migration and genetic population structure in house wrens. *Condor*, **102**, 517–528.

Avise JC, Walker D (1998) Pleistocene phylogeographic effects on avian populations and the speciation process. *Proceedings of the Royal Society of London, Series B*, **265**, 457–463.

Ball RM, Avise JC (1992) Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk*, **109**, 626–636.

Bermingham E, Rohwer S, Freeman S, Wood C (1992) Vicariance biogeography in the Pleistocene and speciation in North American wood warblers: a test of Mengel's model. *Proceedings of the National Academy of Sciences of the USA*, **89**, 6624–6628.

Bowen BW, Kamezaki N, Limpus CJ, Hughes GR, Meylan AB, Avise JC (1995) Trans-Pacific migrations of the loggerhead turtle (*Caretta caretta*) demonstrated with mitochondrial DNA markers. *Proceedings of the National Academy of Sciences of the USA*, **92**, 3731–3734.

Buerkle CA (1999) The historical pattern of gene flow among migratory and non-migratory populations of prairie warblers (Aves: Parulinae). *Evolution*, **53**, 1915–1924.

Chamberlain CP, Bensch S, Feng X, Akesson S, Andersson T (2000) Stable isotopes examined across a migratory divide in Scandinavian willow warblers (*Phylloscopus trochilus trochilus* and *Phylloscopus trochilus acredula*) reflect their African winter quarters. *Proceedings of the Royal Society of London, Series B*, **267**, 43–48.

Chamberlain CP, Blum JD, Holmes RT, Feng X, Sherry TW, Graves GR (1997) The use of isotope tracers for identifying populations of migratory birds. *Oecologia*, **109**, 132–141.

Cherel Y, Hobson KA, Weimerskirch H (2000) Using stable-isotope analysis of feathers to distinguish molting and breeding origins of seabirds. *Oecologia*, **122**, 155–162.

DeGraaf RM, Rappole JH (1995) *Neotropical Migratory Birds: Natural History, Distribution, and Population Change*. Cornell University Press, Ithaca, NY.

Edwards SV, Beerli P (2000) Perspective: gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. *Evolution*, **54**, 1839–1854.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: applications to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Gibbs HL, Dawson RJG, Hobson KA (2000) Limited differentiation in microsatellite DNA variation among northern populations of the yellow warbler: evidence for male-biased gene flow? *Molecular Ecology*, **9**, 2137–2147.

Gill FB, Mostrom AM, Mack AL (1993) Speciation in North American chickadees. I. Patterns of mtDNA genetic divergence. *Evolution*, **47**, 195–212.

Haig SM, Gratto-Trevor CL, Mullins TD, Colwell MA (1997) Population identification of western hemisphere shorebirds throughout the annual cycle. *Molecular Ecology*, **6**, 413–427.

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Hillis DM, Moritz C, Mable BK (1996) *Molecular Systematics*. Sinauer Associates, Sunderland, MA.

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia*, **120**, 314–326.

Hobson KA, McFarland KP, Wassenaar LI, Rimmer CC, Goetz JE (2001) Linking breeding and wintering grounds of Bicknell's thrushes using stable isotope analyses of feathers. *Auk*, **118**, 16–23.

Holder K, Montgomerie R, Friesen VL (2000) Glacial vicariance and historical biogeography of rock ptarmigan (*Lagopus mutus*) in the Bering region. *Molecular Ecology*, **9**, 1265–1278.

Johnson NK, Cicero C (1991) Mitochondrial DNA sequence variability in two species of sparrows of the genus *Amphispiza*. *Acta XX Congressus Internationalis Ornithologici*, 600–610.

Kidd MG, Friesen VL (1998) Sequence variation in the guillemot (Alcidae: Cepphus) mitochondrial control region and its nuclear homolog. *Molecular Biology and Evolution*, **15**, 61–70.

Kimura M (1980) A simple method for estimating the evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 111–120.

Klein N, Brown WM (1994) Intraspecific molecular phylogeny in the yellow warbler (*Dendroica petechia*), and implications for avian biogeography in the West Indies. *Evolution*, **48**, 1914–1932.

Klicka J, Zink RM (1997) The importance of recent ice ages in speciation: a failed paradigm. *Science*, **277**, 1666–1669.

Kocher TD, Thomas WK, Meyer SA *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and

sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the USA*, **86**, 6196–6200.

Larsen AH, Sigurjonsson J, Oien N, Vikingsson G, Palsboll P (1996) Populations genetic analysis of nuclear and mitochondrial loci in skin biopsies collected from central northeastern North Atlantic humpback whales (*Megaptera novaeangliae*): population identity and migratory destinations. *Proceedings of the Royal Society of London, Series B*, **263**, 1611–1618.

Latta SC, Baltz ME (1997) Population limitation in neotropical migratory birds: Comments on Rappole & McDonald (1994). *Auk*, **114**, 754–762.

Lovette IJ, Bermingham E (1999) Explosive speciation in the New World *Dendroica* warblers. *Proceedings of the Royal Society of London, Series B*, **266**, 1629–1636.

Lovette IJ, Bermingham E (2001) Mitochondrial perspective on the phylogenetic relationships of the Parula wood-warblers. *Auk*, **118**, 211–215.

Lovette IJ, Bermingham E, Seutin G, Ricklefs RE (1998) Evolutionary differentiation in three endemic West Indian warblers. *Auk*, **115**, 890–903.

Lowery GH, Monroe BL (1968) Family Parulidae. In: *Checklist of Birds of the World*, Vol. 14 (ed. Paynter RA), pp. 5–93. Museum of Comparative Zoology, Cambridge, MA.

Marra PP, Hobson KA, Holmes RT (1998) Linking winter and summer events in a migratory bird by using stable-carbon isotopes. *Science*, **282**, 1884–1886.

McParland TL, Ferguson MM, Liskauskas AP (1999) Genetic population structure and mixed-stock analysis of walleyes in the Lake Erie–Lake Huron corridor using allozyme and mitochondrial DNA markers. *Transactions of the American Fisheries Society*, **128**, 1055–1067.

Mengel RM (1964) The probable history of species formation in some northern wood warblers (Parulidae). *Living Bird*, **3**, 9–43.

Merila J, Bjorklund M, Baker AJ (1997) Historical demography and present day population structure of the greenfinch, *Carduelis chloris*: an analysis of mtDNA control-region sequences. *Evolution*, **51**, 946–956.

Milá B, Girman DJ, Kimura M, Smith TB (2000) Genetic evidence for the effect of a postglacial population expansion on the phylogeography of a North American songbird. *Proceedings of the Royal Society of London, Series B*, **267**, 1033–1040.

Milot E, Gibbs HL, Hobson KA (2000) Phylogeography and genetic structure of northern populations of the yellow warbler (*Dendroica petechia*). *Molecular Ecology*, **9**, 667–681.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Nei M, Li W-H (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the USA*, **76**, 5269–5273.

Petit E, Mayer F (2000) A population genetic analysis of migration: the case of the noctule bat (*Nyctalus noctula*). *Molecular Ecology*, **9**, 683–690.

Pyle P (1997) *Identification Guide to North American Birds*. Slate Creek Press, Bolinas, CA.

Quinn TW (1992) The genetic legacy of *Mother Goose*: phylogeographic patterns of lesser snow goose *Chen caerulescens caerulescens* maternal lineages. *Molecular Ecology*, **1**, 105–117.

Rappole JH, Morton ES, Ramos MA (1992) Density, philopatry, and population estimates for songbird migrants wintering in Veracruz. In: *Ecology and Conservation of Neotropical Migrant Landbirds* (eds Hagan JM, Johnston DW), pp. 337–344. Smithsonian Institution Press, Washington, DC.

Robbins CS, Sauer JR, Greenberg RS, Droege S (1989) Population declines in North American birds that migrate to the neotropics. *Proceedings of the National Academy of Sciences of the USA*, **86**, 7658–7662.

Rubenstein DR, Chamberlain CP, Holmes RT *et al.* (2002) Linking breeding and wintering ranges of a migratory songbird using stable isotopes. *Science*, **295**, 1062–1065.

Sauer JR, Hines JE, Thomas I, Fallon J, Gough G (2000) *The North American Breeding Bird Survey, Results and Analysis 1966–99*, Version 98.1. USGS Patuxent Wildlife Research Center, Laurel, MD.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN, Version 2.000: a Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Seutin G, Ratcliffe LM, Boag PT (1995) Mitochondrial DNA homogeneity in the phenotypically diverse redpoll finch complex (Aves: Carduelis: Carduelinae: flammea-hornemanni). *Evolution*, **49**, 962–973.

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, **69**, 82–90.

Sillett TS, Holmes RT, Sherry TW (2000) Impacts of a global climate cycle on population dynamics of a migratory songbird. *Science*, **288**, 2040–2042.

Swofford DL (1999) *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, MA.

Tarr CL (1995) Primers for amplification and determination of mitochondrial control-region sequences in oscine passerines. *Molecular Ecology*, **4**, 527–529.

Weatherhead PJ, Forbes MRL (1994) Natal philopatry in passerine birds: genetic or ecological influences? *Behavioral Ecology*, **5**, 426–433.

Webster MS, Marra PP, Haig SM, Bensch S, Holmes RT (2002) Links between worlds: unraveling migratory connectivity. *Trends in Ecology and Evolution*, **17**, 76–83.

Wenink PW, Baker AJ (1996) Mitochondrial DNA lineages in composite flocks of migratory and wintering dunlins (*Calidris alpina*). *Auk*, **113**, 744–756.

Winker K, Graves GR, Braun MJ (2000) Genetic differentiation among populations of a migratory songbird: *Limnothlypis swainsonii*. *Journal of Avian Biology*, **31**, 319–328.

Wirgin I, Maceda L, Waldman JR, Crittenden RN (1993) Use of mitochondrial DNA polymorphisms to estimate the relative contributions of the Hudson River and Chesapeake Bay striped bass stocks to the mixed fishery on the Atlantic coast. *Transactions of the American Fisheries Society*, **122**, 669–684.

Zink RM (1997) Phylogeographic studies of North American birds. In: *Avian Molecular Evolution and Systematics* (ed. Mindell DP), pp. 301–324. Academic Press, San Diego.

All authors are current or former members of the Center for Tropical Research (CTR) at SFSU (CTR has since moved to UCLA). Since 1994, CTR has collaborated with the Monitoring Avian Productivity and Survivorship (MAPS) programme, with the Point Reyes Bird Observatory, and with individual bird banders from across North America to obtain nondestructively sampled genetic material from a variety of Neotropical migrant species. This project will continue to generate comparative data on the pattern and magnitude of phylogeographical variation in migrant songbirds with the ultimate goal of using that information to help assess demographic connectivity between breeding and overwintering areas in migrant species of special concern.
