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Genetic evidence for the effect of a postglacial population expansion on the phylogeography of a North American songbird

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Phylogeographical studies of Nearctic songbirds conducted to date have yielded unexpectedly low levels of genetic differentiation and weak phylogeographical structure in mitochondrial DNA lineages as compared with species studied in Neotropical areas. Factors leading to this pattern may include (i) gene flow, (ii) population expansions from bottlenecked populations, and (iii) selective sweeps. Here we provide evidence for the role played by Pleistocene postglacial population expansions on the phylogeography of MacGillivray's warbler (*Oporornis tolmiei*), a long-distance migratory bird. Samples from 12 breeding localities in the temperate USA were compared with those from two localities in north-eastern Mexico. The former showed evidence of a Late Pleistocene population expansion as indicated by low haplotype and nucleotide diversity, a star-like phylogeny of alleles, and a mismatch distribution indicating a sudden increase in effective population size. By contrast, the Mexican population showed high levels of genetic diversity and a mismatch distribution as expected for a population unaffected by sudden demographic change. Haplotypes from the two regions formed two distinct phylogroups which separated roughly one million years ago according to a conventional molecular clock for songbirds. This study provides support for the Pleistocene expansion hypothesis in MacGillivray's warbler and suggests that postglacial expansion of bottlenecked populations is responsible for the lack of variation and structure reported for most North American songbird species.

Keywords: genetic diversity; MacGillivray's warbler; phylogeography; Pleistocene; songbirds; speciation

1. INTRODUCTION

Patterns of geographical differentiation at the molecular level may reveal valuable information on underlying evolutionary processes and past demographic events. With few notable exceptions (Avise & Nelson 1989; Girman *et al*. 2000; Zink 1994), studies of intraspecific phylogeographical patterns in Nearctic songbirds have yielded markedly low levels of genetic differentiation and a weak phylogeographical structure in mitochondrial DNA (mtDNA) lineages (Avise *et al*. 1988; Ball & Avise 1992; Buerkle 1999; Zink 1997). Low levels of genetic differentiation have been found even in species showing relatively high levels of phenotypic variation (Ball *et al*. 1988; Greenberg *et al*. 1998; Seutin *et al*. 1995; Zink & Dittman 1993*a*,*b*). These results are in contrast to patterns found in Neotropical bird species, where relatively high levels of mitochondrial genetic differentiation and a strong phylogeographical pattern have been documented (Bates *et al*. 1999; Caparella 1988; Escalante-Pliego 1991; Klein & Brown 1994; Peterson *et al*. 1992; Seutin *et al*. 1994).

Several factors have been proposed to explain the relative lack of structure and phylogeographical signal in songbirds of temperate latitudes. Assuming similar mtDNA mutation rates across songbird taxa (Klicka & Zink 1997), the absence of genetic structure in the mitochondrial genome across a species' range could be due to (i) gene flow among geographically distant populations (Edwards 1993; Zink 1994), (ii) historical demographic events such as range expansions from bottlenecked populations (Rogers 1995), or (iii) range-wide selective sweeps, wherein a given haplotype favoured by selection spreads across the species range (Maruyama & Birky 1991). Because all three factors may produce a similar pattern of haplotype variation (namely the presence of a single or a few widely distributed shared alleles accompanied by other, lower-frequency alleles), distinguishing among alternative hypotheses has proven difficult, and the relative importance of each factor in shaping current phylogeographical patterns remains a topic of debate (Zink 1997).

Recently, habitat contractions caused by Pleistocene glacial cycles across temperate regions have been proposed as a major process in reducing genetic diversity in northern latitudes (Hewitt 1996; Merilä *et al*. 1997; Rising & Avise 1993). According to this hypothesis, loss of allelic diversity due to genetic drift in bottlenecked refugial populations would have been followed by rapid range expansions northward as ice sheets receded. If such expansions occurred too recently for mtDNA to accumulate point mutations, low genetic diversity and weak phylogeographical patterns would be observed today. A testable prediction of this Pleistocene expansion hypothesis is that populations that were not affected by glacial habitat contractions—such as those in tropical or subtropical latitudes—should show higher levels of genetic

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diversity and subdivision than those in northern populations, and similar to those found in tropical species. However, most studies have focused on species or populations with current distributions restricted to temperate North America and have not included populations to the south. Because no intraspecific north–south comparison has been attempted, the relative importance of the Pleistocene expansion hypothesis to the phylogeography of North American birds has been difficult to assess.

Temperate species whose ranges include breeding populations in southern (tropical to subtropical) latitudes provide an excellent opportunity to test the Pleistocene expansion hypothesis. Here we examine mtDNA variation across the range of MacGillivray's warbler (*Oporornis tolmiei*), a long-distance migratory songbird whose breeding distribution comprises most of western North America (from the Pacific to the Rocky Mountains, and from south-eastern Alaska to southern California and central New Mexico), including a small and isolated but thriving breeding population in north-eastern Mexico (figure 1*a*) (Pitocchelli 1995). This population is also migratory and lies just north of the species' wintering grounds, which span northern Mexico south through Central America (Howell & Webb 1995), and is separated from the rest of the breeding range by over 1000 km.

We predict that populations that experienced genetic bottlenecks as a result of Pleistocene habitat contractions should show evidence of low historical effective population size as indicated by low haplotype and nucleotide diversity (Birky *et al*. 1983). Subsequent postglacial expansion over the present range would result in (i) a star-like phylogeny of haplotypes with few high-frequency ancestral haplotypes and numerous low-frequency alleles separated from the ancestral ones by a few mutational steps (Slatkin & Hudson 1991), (ii) low levels of genetic subdivision within and among populations, and (iii) a Poisson distribution of pairwise nucleotide differences among haplotypes indicating a sudden increase in effective population size (Rogers & Harpending 1992). By contrast, refugial populations in the south unaffected by Pleistocene habitat contractions should show higher levels of nucleotide diversity, stronger phylogeographical structure, and a multimodal distribution of pairwise nucleotide differences indicating long-term demographic stability.

2. MATERIAL AND METHODS

We examined mtDNA sequence variation in the cytochrome *b* gene and the control region from a total of 177 individual MacGillivray's warblers from 14 locations throughout the species' breeding range (figure 1*a*). Individual birds were captured with mist-nets during the breeding season, and blood and/or feather samples were collected in the field in addition to information on age, sex and reproductive condition.

Whole genomic DNA was extracted from blood or feather samples using a commercially available Qiagen^{TM} (Valencia, CA, USA) kit. The polymerase chain reaction was used to isolate and amplify a 316 base pair fragment of the cytochrome *b* gene and 339 base pairs of the control region in the mitochondrial genome (Saiki *et al*. 1988), with primer pairs MVZ03′ and MVZ04′ (modified by C. Cicero and N. Johnson, University of California at Berkeley, USA, from Kocher *et al*. (1989)), and H417 and LGL2 (Tarr 1995), respectively. All samples were sequenced in both directions in an ABI Prism 377 automated sequencer (Applied Biosystems, Inc., Foster City, CA, USA). Cytochrome *b* data were obtained from 159 birds and control region sequences were obtained from 122 birds, with 104 individuals having both fragments sequenced. To ensure that sequences were of mitochondrial origin and not nuclear inserts, two individuals for which both blood and feather samples were available were sequenced using both sources of DNA and identical results were obtained. Translation of cytochrome *b* sequences yielded a single amino-acid change in one haplotype and no stop codons.

Estimates of nucleotide diversity (π) and haplotype diversity (h) were obtained with the program Arlequin (Schneider *et al*. 1996). Phylogenetic relationships among haplotypes were reconstructed with the neighbour-joining algorithm in the program MEGA v. 1.02 (Kumar *et al*. 1993) and with maximum parsimony using PAUP* (Swofford 1999). A Kimura two-parameter model (Kimura 1980) was used to estimate genetic distances among cytochrome *b* haplotypes, and a gamma distribution ($a = 0.5$) of Tamura–Nei distances was assumed for the control region sequences (Kumar *et al*. 1993; Tamura & Nei 1993). Branch support was tested by performing 500 bootstrap replicates. Minimum-spanning trees, in which haplotypes are the nodes of a network rather than the terminal tips of a tree (Stanley *et al*. 1996), were constructed from absolute distance values using the program NTSYS (Rohlf 1993). Estimates of sequence divergence between populations were corrected for polymorphism within populations using Nei's genetic distance (Nei 1987; Wilson *et al*. 1985).

To estimate population structure we conducted an analysis of molecular variance (AMOVA) using the program Arlequin. AMOVA uses the frequencies of haplotypes and the number of mutations between them to test the significance of the variance components associated with various hierarchical levels of genetic structure (within populations, among populations within groups, and among groups) by means of non-parametric permutation methods (Excoffier *et al*. 1992). Sampling sites were first treated as individual 'populations' to test for overall genetic subdivision. In order to identify larger-scale genetic populations, sites were then grouped to maximize among-group variance (i.e. ϕ_{ct} -values). Those groupings that maximized values of ϕ_{ct} and were significantly different from distributions of individuals generated from 1000 random permutations of the DNA sequences, were assumed to reflect the most probable geographical subdivisions (Excoffier *et al*. 1992).

To infer the demographic history of populations, we compared mismatch distributions of pairwise nucleotide differences among control region haplotypes with expectations of a sudden-expansion model (Rogers 1995) using the programs Arlequin (Schneider *et al*. 1996) and MISMATCH (v. 4–3, provided by A.* Rogers, University of Utah, USA). Variation in cytochrome *b* was too low for the model to be applied. The model estimates three parameters of interest: the effective population size before the expansion (N_0), the present population size (N_1), and the time elapsed between the two (t). N_0 was estimated as $\theta_0 = 2N_0u$, and N_1 as $\theta_1 = 2N_1u$, where $u = 2\mu k$, where μ is the mutation rate and k is the length of the sequence. θ_0 was estimated as $\sqrt{(v - m)}$, where m and v are, respectively, the observed mean and variance of the pairwise sequence differences. To estimate the time from N_0 to the present, we used $\theta = 2ut$, where t is the time elapsed between the initial population and the current population, and θ was estimated as $m - \theta_0$ (Rogers & Harpending 1992). Statistical significance of a population expansion was tested using Fu's F_s-test of

Table 1. *Sampling sites, mtDNA haplotypes and sample sizes*

sampling sites	cytochrome *b* haplotypes[a]	no. of individuals	no. of haplotypes	control-region haplotypes[a]	no. of individuals	no. of haplotypes
USA sites	—	116	7	—	101	33
Alaska	A(6), G	7	2	b, d, f, j, m, w, x	7	7
North California	A(11), G, H, I	14	4	b(4), bb, c, cc, d, h	9	6
East California	A(12), H(2)	14	2	b(4), c, d, ff, gg(2), u	10	6
South California	A(7)	7	1	a, b(5), d, n(3)	10	4
Colorado	A(7)	7	1	d, f, g(5), t	8	4
East Oregon	A(4)	4	1	b, d, g(2)	4	3
South Oregon	A(8), B, H, I, J	12	5	a, aa, b(5), bb, c(2), dd, l, z(2)	14	8
Idaho	A(9)	9	1	c, d(2), g, hh, ii, jj, kk, r	9	8
Montana	A(11), B(3)	14	2	b(3), d(4), g(2), l(3), v	13	5
Utah	A(6), K	7	2	c, g(3), l, w	6	4
Washington	A(14), G	15	2	b(2), e, f, g(2)	6	4
Wyoming	A(6)	6	1	g, s, o, d, y	5	5
Mexican sites	—	43	11	—	21	7
Coahuila	A(2), C, D(11), E(2), L(3), Q(3)	22	6	ee, nn, p(4), q(2)	8	4
Nuevo León	C(5), D(7), E(2), F, L, M(2), N, O, P	21	9	k, ll, mm, p(4), q(6)	13	5
totals	—	159	17	—	122	40

[a] Figures in parentheses indicate the number of individuals carrying haplotype if more than one.

neutrality (Fu 1997), which detects departures from neutrality in scenarios characterized by an excess of rare alleles and young mutations in non-recombining sequences. Large negative values of F_s indicate an excess of recent mutations and reject population stasis (Fu 1997).

3. RESULTS

A total of 17 cytochrome *b* and 40 control region haplotypes were found in the breeding range of MacGillivray's warbler (table 1). The cytochrome *b* haplotype *A* predominated in USA populations, with other haplotypes appearing in lower frequencies. A similar pattern was seen for control region data, where haplotypes *b*, *d*, and *g* were found in higher frequencies than the rest and were shared by many populations. Furthermore, low-frequency haplotypes differed from the high-frequency ones by one or two base pairs, giving rise to a star-like phylogeny of haplotypes (figure 1*b*,*c*). None of the haplotypes detected in north-eastern Mexico were found in the USA populations except for cytochrome *b* haplotype *A*, which was found in two individuals from the Coahuila sample (one of which carried control-region haplotype *ee*).

Haplotypes found in the northern (USA) and the southern (north-eastern Mexico) populations formed two highly divergent phylogroups according to a neighbour-joining reconstruction (figure 2). Trees produced with maximum parsimony had similar topologies, and bootstrap values for the node separating the USA and Mexican clades were 81 and 88 for cytochrome *b* and control region, respectively. Given the marked divergence between haplotype *A* and the other haplotypes found in Mexico, the two individuals detected in Coahuila carrying haplotype *A* were considered to represent recent introgression from the north. For cytochrome *b* data, average divergence between northern (*A*, *B*, *G*, *H*, *I*, *J* and *K*) and southern (*C*, *D*, *E*, *F* and *L*, *M*, *N*, *O*, *P*) haplotypes was 0.0241 (s.d. = 0.00462, range: 0.0128–0.0327). When divergence between the two phylogroups was corrected for intrapopulation polymorphism, the divergence value among the two phylogroups was 0.018. When the two individuals carrying haplotype *A* in the Mexican group were excluded, the divergence estimate was 0.019. Conventional avian cytochrome *b* molecular clock calibrations (2% per million years) (Quinn 1992; Tarr & Fleischer 1993) suggest that the two phylogroups separated roughly one million years ago.

Analysis of population subdivision revealed significant levels of genetic subdivision in the breeding range of MacGillivray's warbler ($\phi_{st} = 0.74$, $p < 0.001$). When populations were divided into two main groups (USA and Mexico), over 89% of the overall variance was explained by among-group variation (table 2). Variation among populations within those groups and among individuals within populations was low as indicated by low ϕ_{sc}- and ϕ_{st}-values, respectively.

Marked differences in genetic diversity were found between USA and Mexican populations. For cytochrome *b* data, average haplotype diversity (h) for the Mexican populations was over twice that found in the USA populations (table 3). Average nucleotide diversity (π) was higher in the Mexican population than in the USA populations by an order of magnitude. These differences were maintained when the two individuals carrying haplotype *A* in the Coahuila (Mexico) population were excluded from the analysis. Without these individuals, the values for h and π were 0.776 ± 0.056 and 0.005 ± 0.003, respectively.

The mismatch distribution from the USA population showed the smooth wave predicted for a population that has undergone a sudden demographic expansion (Rogers & Harpending 1992), while that of the Mexican population was rough and resembled the distribution expected from a non-growing population (Slatkin & Hudson 1991)

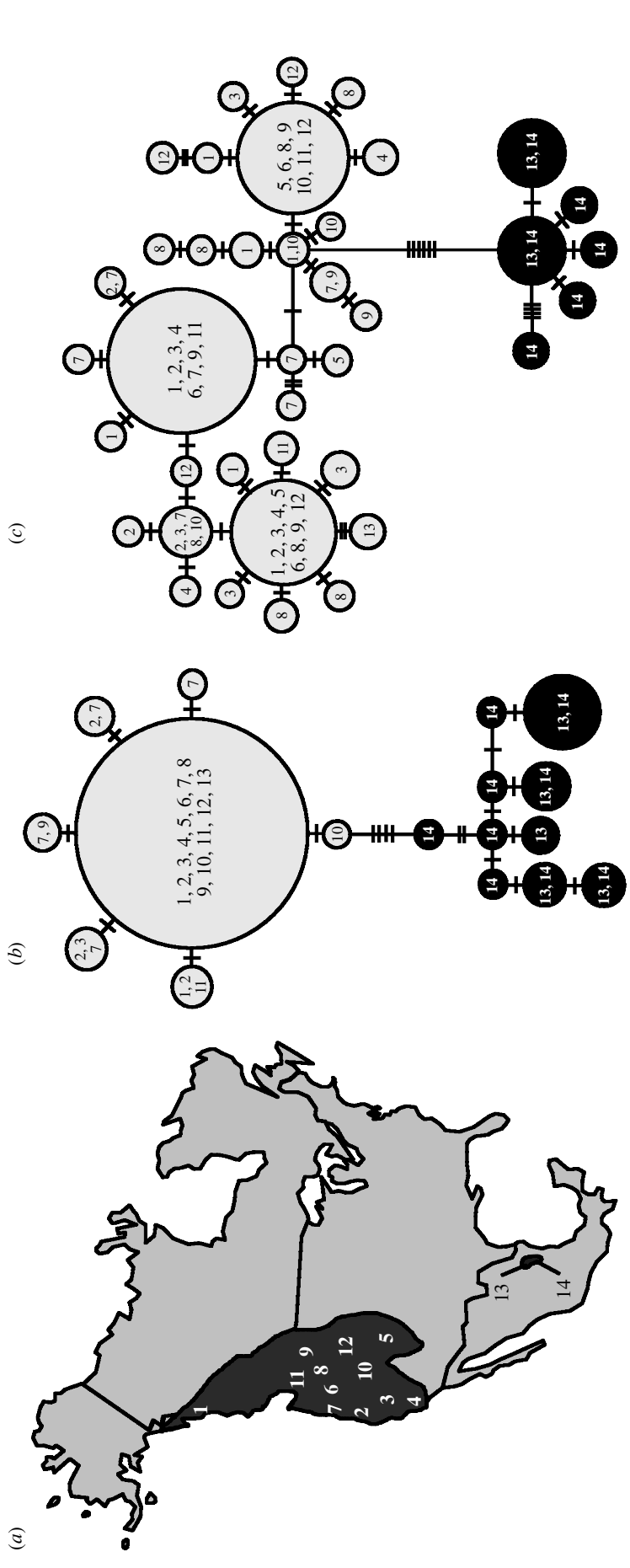

Figure 1. (*a*) Sampling localities across MacGillivray's warbler breeding range included in the study: (1) Juneau, Alaska; (2) Mount Shasta, northern California; (3) Sierra Nevada, California; (4) Big Bear Lake, southern California; (5) Brighton, Colorado; (6) Willamette National Forest, Oregon; (7) Grants Pass, Oregon; (8) Yellowstone National Park, Wyoming and Targhee National Forest area, Idaho; (9) Flathead, Montana; (10) Mount Timpanogos, Utah; (11) Wenatchee National Forest, Washington; (12) Lander, Wyoming, in the USA; and (13) San Antonio de las Alazanas, Coahuila; (14) Cerro Potosi, Galeana, Nuevo León, in Mexico. The shaded area indicates MacGillivray's warbler breeding range (wintering range not shown). See table 1 for sample sizes and haplotype designations. (*b*) Minimum-spanning tree of cytochrome *b* haplotypes and (*c*) control region haplotypes. Light-grey shaded circles represent haplotypes found in the USA sites and black circles represent haplotypes found in the Mexican sites. Bars across branches indicate single nucleotide changes (branches not drawn to scale). The size of each circle is proportional to the frequency of the haplotype it represents, and numbers within circles indicate the sampling localities where the haplotypes were detected.

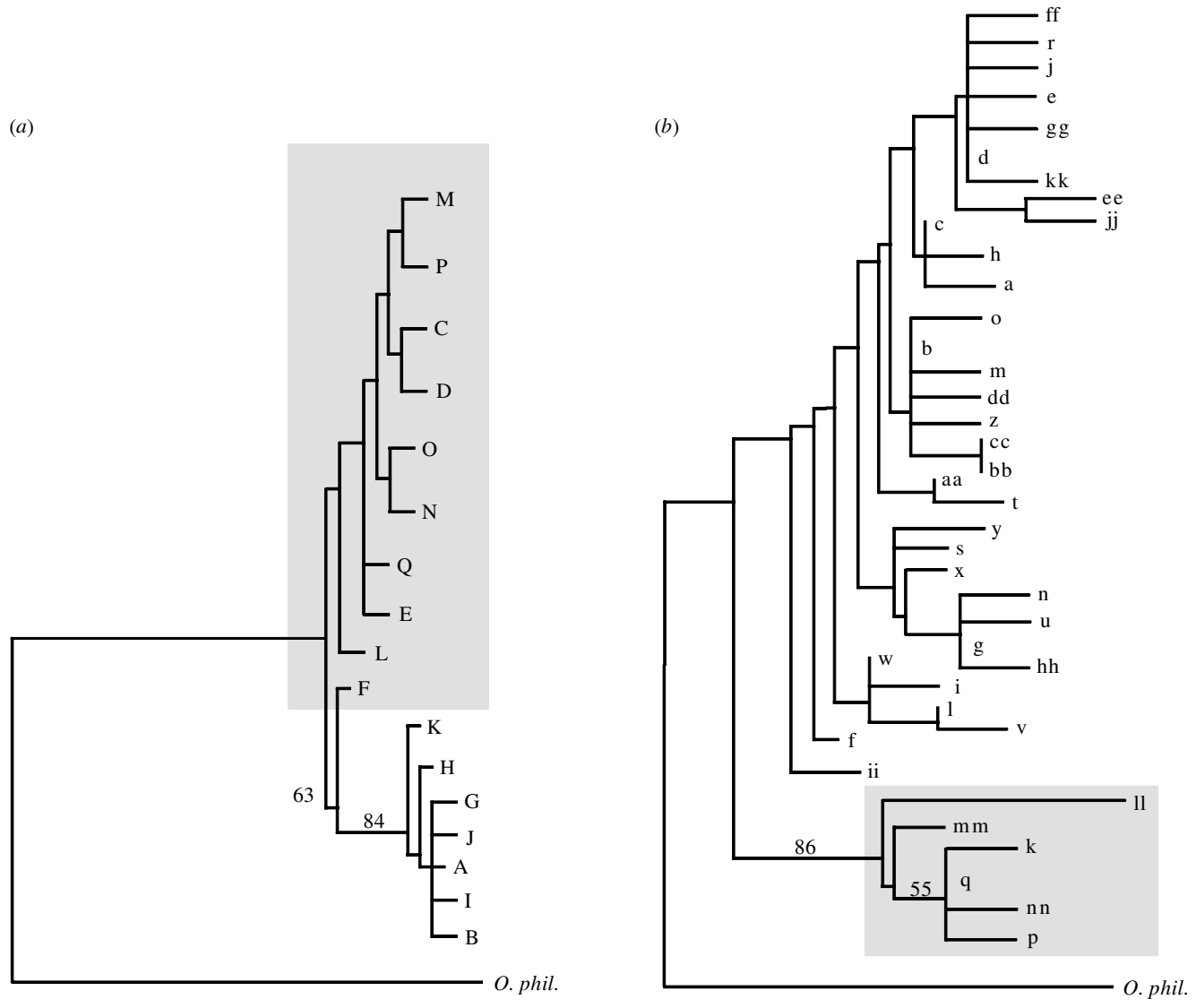

Figure 2. Neighbour-joining trees of (*a*) cytochrome *b* and (*b*) control region haplotypes from USA and Mexican populations of MacGillivray's warbler with mourning warbler (*O. philadelphia*) as an outgroup. Haplotypes found in the Mexican sites only are shaded. Bootstrap values greater than 50% are indicated on tree branches.

(figure 3). Parameter estimates obtained using the method described by Rogers (1995) were $\theta_0 = 0.3$, $\theta_1 = 8.17$, $\tau = 2.454$, implying that the expansion was of the order of a 27-fold increase in female effective population size (from $N_0 = 1495$ to $N_1 = 40710$) and took place approximately 12 500 years before present. It is notable that the estimate of the time to the expansion coincides with the recession of the ice sheets following the last glacial maximum, 18 000 years ago (Webb & Bartlein 1992). Fu's test supported the demographic expansion of the USA population rejecting stasis at the 0.05 significance level with an F_s-value of -20.457.

4. DISCUSSION

(a) *Pleistocene effects on songbird speciation*

The relative importance of Pleistocene glacial cycles in promoting divergence and speciation in North American songbird taxa is currently under debate (Arbogast & Slowinski 1998; Avise & Walker 1998; Klicka & Zink 1997, 1999). Our data contribute to a body of evidence suggesting that Pleistocene events played an important role in the differentiation and divergence of songbird populations. According to prevailing molecular clock calibrations, divergence values reported here between the two main phylogroups in MacGillivray's warbler indicate that cladogenesis in this species took place roughly one million years before present (for caveats of molecular clocks, see Klicka & Zink (1997)). These divergence values are significantly higher than those found among some pairs of congeners (e.g. Bermingham *et al.* 1992; Rising & Avise 1993; Zink & Avise 1990; Zink & Dittman 1993*b*), yet whether the USA and Mexican phylogroups have achieved species status depends on taxonomic definitions that will not be addressed here. However, the timing of the divergence between the two groups, coupled with the dramatic effect of a single glacial cycle on the phylogeographical structure of the species, points to the Pleistocene as an important period for differentiation and divergence in this taxon.

Table 2. *AMOVA of MacGillivray's warbler populations divided into USA and Mexican groups*

source of variation	degrees of freedom	variance components	p	percentage of variation
among groups	1	$\phi_{ct} = 2.9781$	< 0.02	89.37
among populations within groups	12	$\phi_{sc} = -0.00718$	< 0.001	-0.22
within populations	145	$\phi_{st} = 0.2801$	< 0.001	10.85

Table 3. *Haplotype* (h) *and nucleotide* (π) *diversity indices calculated from cytochrome* b *data*

sampling sites	h	π
USA sites	0.2405 ± 0.0526	0.000806 ± 0.001006
Alaska	0.2857 ± 0.1964	0.000907 ± 0.001238
North California	0.3956 ± 0.1588	0.001361 ± 0.001462
East California	0.2637 ± 0.1360	0.000837 ± 0.001096
South California	0	0
Colorado	0	0
East Oregon	0	0
South Oregon	0.5758 ± 0.1634	0.002117 ± 0.001960
Idaho	0	0
Montana	0.3626 ± 0.1302	0.001150 ± 0.001321
Utah	0.2857 ± 0.1964	0.000907 ± 0.001238
Washington	0.1333 ± 0.1123	0.000423 ± 0.000746
Wyoming	0	0
Mexican sites	0.7951 ± 0.0526	0.006299 ± 0.004026
Coahuila	0.7273 ± 0.0868	0.006976 ± 0.004469
Nuevo León	0.8429 ± 0.0572	0.005257 ± 0.003591

Nevertheless, our results are in agreement with the main conclusions of Klicka & Zink (1997) and contribute to the mounting evidence against the Late Pleistocene origin model (Mengel 1964, 1970), which suggests that the last 250 000 years have been a crucial time for songbird speciation.

(b) *Effect of postglacial expansions on MacGillivray's warbler phylogeography*

Population expansions over large continental areas following Pleistocene glacial maxima have been proposed as causes for the lack of variation and phylogeographical structure in several avian species in temperate North America (see the review in Zink 1997) and Europe (Bensch *et al*. 1999; Merilä *et al*. 1997). Patterns of mtDNA variation within the USA populations of MacGillivray's warbler are consistent with expectations of a Pleistocene population expansion over the northern part of the species' range following a period of low female effective population size. The presence of one or a few common, widespread ancestral haplotypes (*A* for cytochrome *b*, and *b*, *d* and *g* for the control region), low haplotype and nucleotide diversity, a star-like phylogeny of alleles, and a Poisson-shaped mismatch distribution of control region haplotypes, are consistent with a demographic expansion from a population of small effective size. By contrast, the mtDNA patterns in the north-eastern Mexican population are consistent with expectations of a population unaffected by sudden demographic change as predicted for a Pleistocene refugium. Haplotype and nucleotide diversity values are higher than in the north, and the mismatch distribution fits expectations of a population in long-term demographic stability. Data from the north-eastern Mexican populations reveal what are probably pre-expansion patterns of variation and diversity, consistent with those found in Neotropical species examined to date (Bermingham *et al*. 1997).

While we cannot definitively reject the possibility of a selective sweep to explain the observed patterns of genetic variation, we believe selection is unlikely. A generalized pattern of decreasing haplotype diversity with increasing latitude has been reported across a wide range of taxa (Hayes & Harrison 1996; Hewitt 1996; Merilä *et al*. 1997). Because selective pressures are unlikely to be the same across those taxa, we consider the occurrence of selective sweeps to be a less parsimonious explanation than a postglacial expansion.

The role of gene flow in shaping the pattern of variation found in the northern populations of MacGillivray's warbler and other avian species remains unclear. A postglacial population expansion would probably mask any geographical genetic structure for a long time, making it difficult to detect current and past levels of gene flow among populations. The presence of several unique haplotypes at each of the two Mexican sites (*F*, *M*, *N*, *O* and *P* in Nuevo León, and *Q* in Coahuila), and the fact that the two sampling sites are 30 km from each other (a very short distance for a migratory bird), suggest that current levels of gene flow, at least for these two populations, might be low. However, larger samples will be necessary to fully address this question. In any case, the present study shows that caution should be used when

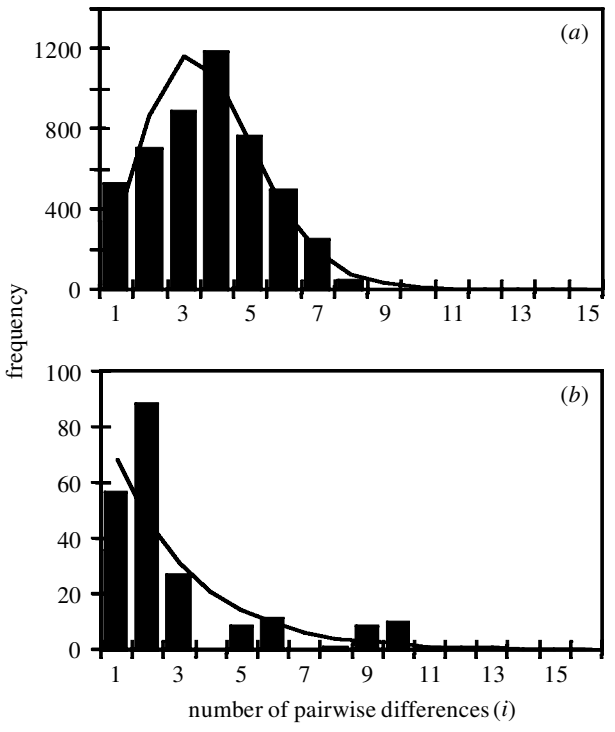

Figure 3. Distribution of pairwise nucleotide differences among members of (*a*) USA populations and (*b*) north-eastern Mexican populations of MacGillivray's warbler. Black histograms represent the observed differences, while thin lines represent the ideal distributions predicted by the model.

interpreting patterns characterized by extensive sharing of haplotypes among populations (especially in temperate regions), so as to avoid mistaking the ubiquity of ancestral post-expansion haplotypes for evidence of gene flow.

The intraspecific test of the Pleistocene expansion hypothesis presented here provides strong evidence for the importance of Pleistocene events in shaping current patterns of genetic diversity and structure in MacGillivray's warbler. Our study also suggests that postglacial expansion of bottlenecked populations might be responsible for the lack of variation and structure reported for most North American species studied to date. Nearctic species with tropical or subtropical populations have the potential to reveal historical patterns of genetic variation and provide crucial insights into general phylogeographical patterns of North American songbird species. Furthermore, when refugial populations harbour large proportions of a species' total genetic diversity and show high levels of differentiation, they represent evolutionarily significant units (Moritz 1994) and may be important targets for conservation efforts.

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