Molecular Ecology (2007) **16**, 159–173 doi: 10.1111/j.1365-294X.2006.03119.x

Speciation and rapid phenotypic differentiation in the yellow-rumped warbler *Dendroica coronata* complex

BORJA MILÁ, THOMAS B. SMITH and ROBERT K. WAYNE
Department of Ecology and Evolutionary Biology, University of California Los Angeles, 621 Charles E. Young Dr, Los Angeles, CA 90095, USA; and Center for Tropical Research, Institute of the Environment, University of California Los Angeles, 619 Charles E. Young Dr, Los Angeles, CA 90095, USA

Abstract

The relative importance of the Pleistocene glacial cycles in driving avian speciation remains controversial, partly because species limits in many groups remain poorly understood, and because current taxonomic designations are often based on phenotypic characteristics of uncertain phylogenetic significance. We use mtDNA sequence data to examine patterns of genetic variation, sequence divergence and phylogenetic relationships between phenotypically distinct groups of the yellow-rumped warbler complex. Currently classified as a single species, the complex is composed of two North American migratory forms (myrtle warbler *Dendroica coronata coronata* and Audubon's warbler *Dendroica coronata auduboni*), and two largely sedentary forms: *Dendroica coronata nigrifrons* of Mexico, and *Dendroica coronata goldmani* of Guatemala. The latter are typically considered to be races of the Audubon's warbler based on plumage characteristics. However, mtDNA sequence data reveal that sedentary Mesoamerican forms are reciprocally monophyletic to each other and to migratory forms, from which they show a long history of isolation. In contrast, migratory myrtle and Audubon's warblers form a single cluster due to high levels of shared ancestral polymorphism as evidenced by widespread sharing of mtDNA haplotypes despite marked phenotypic differentiation. Sedentary and migratory forms diverged in the early Pleistocene, whereas phenotypic differentiation between the two migratory forms has occurred in the Holocene and is likely the result of geographical isolation and subsequent range expansion since the last glaciation. Our results underscore the importance of Quaternary climatic events in driving songbird speciation and indicate that plumage traits can evolve remarkably fast, thus rendering them potentially misleading for inferring systematic relationships.

Keywords: Holocene, phylogeography, Pleistocene, plumage evolution, postglacial expansion, speciation

Received 22 February 2006; revision received 30 May 2006; accepted 7 August 2006

Introduction

Climatic cycles during the Pleistocene had a dramatic impact on the distribution and phylogeographical pattern of species (Avise 2000; Hewitt 2000), yet the relative role of climatic change during this epoch in driving bird speciation remains controversial (Lovette 2004). Using molecular markers to time the splits between sister species has led some to propose that most speciation events predate the Pleistocene (Klicka & Zink 1997; Zink *et al*. 2004), while other studies have concluded that the late Pleistocene was indeed an important time for speciation (Avise & Walker 1998; Johnson & Cicero 2004; Weir & Schluter 2004). This ongoing debate underscores the need for detailed phylogeographical studies that document patterns of lineage splitting and establish species limits in poorly studied groups. Furthermore, the revision of species limits using molecular data is important as recent studies show that phylogenetic inference based on certain phenotypic traits can lead to erroneous conclusions regarding phylogenetic relationships and evolutionary history (Crochet *et al*. 2000;

Correspondence: Borja Milá, Fax: (310) 825 5446;
E-mail: bmila@ucla.edu.

Table 1 Main morphological characteristics of yellow-rumped warbler groups (males only)

Group	Throat colour	Auriculars	White postocular stripe	White supraloral spot	Posterior white patch on crown	Breast and flanks	Upperparts	Outer rectrices with white spots	Mass (mean ± SD)*
coronata	White	Gray	Yes	Yes	No	Streaked	Gray	3	11.57 ± 0.597
auduboni	Yellow	Gray	No	No	No	Streaked	Gray	5	12.15 ± 0.671
nigrifrons	Yellow	Black	No	No	No	Blackish	Blackish	5	13.56 ± 0.850
goldmani	Yellow with white posterior patches	Black	No	No	Yes	Black	Black	5	13.57 ± 0.666

* based on field measurements taken with an electronic scale on the following number of males per group: *coronata* (n = 24), *auduboni* (n = 28), *nigrifrons* (n = 24), *goldmani* (n = 33).

Omland & Lanyon 2000; Weckstein 2005; Moore *et al*. 2006). For example, recent molecular phylogenetic studies have revealed striking patterns of homoplasy in plumage traits caused by sexual selection (Prum 1997; Omland & Lanyon 2000) or natural selection (Crochet *et al*. 2000; Warren *et al*. 2005; Moore *et al*. 2006), leading to substantial systematic revisions and a better understanding of evolutionary process in general and phenotypic trait evolution in particular.

Here, we use mitochondrial DNA (mtDNA) variation to investigate the evolutionary history of the yellow-rumped warbler *Dendroica coronata* complex of North and Central America. Given its broad latitudinal range from Alaska to Guatemala, and marked phenotypic differentiation among subspecific groups, the complex provides a good model to test the role of Pleistocene climate cycles in driving differentiation and to study the evolution of plumage traits. The current taxonomic classification of the yellow-rumped warbler complex consists of a 'myrtle warbler *Dendroica coronata coronata* group' of eastern and northern North America, and an 'Audubon's warbler group', composed of three subspecies: *Dendroica coronata auduboni* of western North America, *Dendroica coronata nigrifrons* of the Sierra Madre Occidental in Mexico, and *Dendroica coronata goldmani* of the Guatemalan highlands (Hubbard 1970; Hunt & Flaspohler 1998) (Table 1, Fig. 1). The two North American forms are short- to long-distance migrants, whereas *D. c. nigrifrons* and *D. c. goldmani* are largely sedentary. Despite marked phenotypic differentiation between the main four groups (hereafter referred to as *coronata*, *auduboni*, *nigrifrons* and *goldmani*), the presence of a narrow hybrid zone between *coronata* and *auduboni* in British Columbia and Alberta (Hubbard 1969) (Fig. 1), resulted in the grouping of all forms into a single species (A.O.U. 1973). Previously, *D. coronata* and *D. auduboni* were considered separate species, with *nigrifrons* and *goldmani* regarded as subspecies of *D. auduboni*, although *goldmani* has been considered a separate species by some authors based on plumage characteristics (Davis 1972; Navarro-Sigüenza & Peterson 2004).

To date, the systematics of the group have been based on plumage characteristics only (Hunt & Flaspohler 1998). Here we investigate the evolutionary history of the four yellow-rumped warbler groups using mtDNA markers, and examine patterns of current and historical gene flow, the geographical distribution of lineages, the phylogenetic relationships among groups, and the demographic history of populations. In addition, we generate estimates of divergence time between taxa to infer the role of climatic patterns in driving lineage differentiation in the complex. We also use the phylogenetic relationships and divergence time estimates generated from molecular data to infer the evolutionary history of plumage patterns in the complex. Specifically, we test the validity of the current split into myrtle and Audubon's warbler 'groups'. If this hypothesis is correct, both groups should form reciprocally monophyletic groups. The alternative 'paraphyly' hypothesis (migratory *coronata* and *auduboni* are more closely related to each other than either is to the Mesoamerican groups), would mean that the markedly distinct plumage traits of *coronata* are autapomorphic and have evolved unusually fast. Finally, we compare patterns of variation in three different mtDNA regions to investigate the usefulness of each to infer evolutionary history and demarcation of species limits in this system. Specifically, we compare variation in the cytochrome *c* oxidase *I* gene (COI), the standard marker proposed for 'barcoding' studies (Hebert 2004) to that of two other mtDNA markers, ATPase 6 & 8 genes, and the control region.

Materials and methods

Field sampling

Populations of all four yellow-rumped warbler groups were sampled in the field using mist nets and playback of song recordings to attract individuals to the nets when necessary. Sampling localities and sample sizes for each group were as follows: *Dendroica coronata coronata*: Juneau

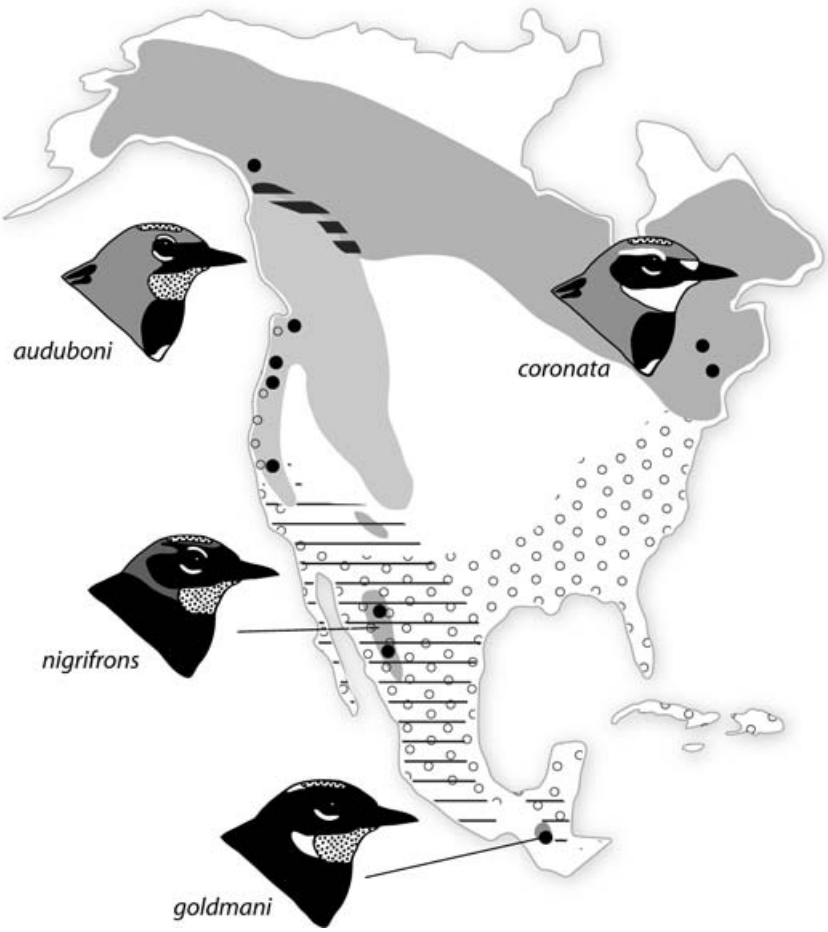

Fig. 1 Sampling localities (black dots), breeding and wintering ranges, and basic plumage characteristics of males of the four main yellow-rumped warbler groups: *Dendroica coronata coronata* ('myrtle warbler'); *Dendroica coronata auduboni* ('Audubon's warbler'); *Dendroica coronata nigrifrons* ('black-fronted warbler'); and *Dendroica coronata goldmani* ('Goldman's warbler'). Stippled areas on bird schematics represent yellow plumage. For a more detailed description of phenotypic characteristics of the complex, see Table 1 and Hunt & Flaspohler (1998). Hatched area corresponds to wintering range of *D. c. auduboni*, and area of empty circles represents wintering range of *D. c. coronata*. Thick-hatched area in the northwest corresponds approximately to the hybrid zone between *coronata* and *auduboni*.

(58.4166°N, 134.55°W) $n = 8$, Alaska; Rangeley (44.98°N, 70.66°W) $n = 16$, and New Brunswick (43.8888°N, 69.9204°W) $n = 8$, Maine, USA; *Dendroica coronata auduboni*, El Portal (37.7555°N, 119.7528°W) $n = 10$, California; Agness (42.6083°N, 123.8528°W) $n = 9$, Elgin (45.8139°N, 117.8611°W) $n = 11$, and Paisley (42.5917°N, 120.8139°W) $n = 10$, Oregon; and Naches (46.75°N, 120.9°W) $n = 20$, Washington, USA; *Dendroica coronata nigrifrons*, San Diego Tenaenz (24.9466°N, 105.9203°W) $n = 15$, Durango, and El Vergel (26.4671°N, 106.3881°W) $n = 17$, Chihuahua, Mexico; and *Dendroica coronata goldmani*, Culchemal (15.5333°N, 91.4883°W) $n = 12$, and Chichim (15.5365°N, 91.5536°W) $n = 14$, Huehuetenango, and Ixchiguan (15.1493°N, 91.9124°W) $n = 4$, San Marcos, Guatemala. Captured individuals were aged, sexed, weighed and marked with a uniquely numbered aluminium band. For genetic analysis we collected a blood sample (by venipuncture of the sub-brachial vein), and the two outermost tail feathers. Blood was stored in lysis buffer (Seutin 1991) and kept at ambient temperature until storage at –80 °C in the laboratory. Feathers were placed in clean, dry paper envelopes and stored at –20 °C.

Laboratory methods

We extracted genomic DNA using a QIAGEN extraction kit and followed the manufacturer's protocol. We used the polymerase chain reaction (PCR) to amplify the following three regions of the mtDNA genome: 300 base pairs (bp) of the hypervariable region I of the control region using primers H417 (H2607, 5′-AGTAGCTCGGTTCTCGTGAG-3′) and LGL2 (L2263, 5′-GGCCACATCAGACAGTCCAT-3′) (Tarr 1995); 655 bp of the cytochrome *c* oxidase I (COI) gene using primers 'BirdF1' (5′-TTCTCCAACCACAA AGACATT-3′) and 'BirdR1' (5′-ACGTGGGAGATAAT-TCCAAATCCTG-3′) (Hebert 2004); and a 648-bp fragment that spanned 358 bp of the ATP-synthase 6 gene and the entire ATP-synthase 8 gene (hereafter referred to as ATPase), using primers L8929 (5′-GGACAATGCTCAGAAATCT-GCGG-3′) and H9855 (5′-ACGTAGGCTTGGATTATK-GCTACWGC-3′) (Sorenson *et al*. 1999). PCR cycles were the following: 3-min denaturation at 94 °C followed by 36 cycles of 94 °C for 30 s, 54 °C (48 °C for the control region fragment) for 45 s, and 72 °C for 45 s, with a final extension of 5 min at 72 °C. Products were purified with an Ultra-Clean

(Mo Bio Laboratories) PCR purification kit and run in a dydeoxy-terminator cycle-sequencing reaction using a CEQ cycle sequencing kit by Beckman-Coulter. Products of this reaction were purified with an ethanol precipitation and sequenced in a Beckman-Coulter CEQ 2000 automated sequencer. Sequences were automatically aligned using SEQUENCHER 4.1. (GeneCodes) and variable sites were checked visually for accuracy. Coding gene sequences (COI and ATPase) were unambiguously translated into their amino acid sequence. Some of the control region sequences obtained from blood samples [which generally have a high nuclear-to-mitochondrial DNA ratio (Sorenson & Quinn 1998)] produced double peaks on the sequence chromatographs, suggesting the amplification of double product and the possibility of nuclear copies of the control region. A single product was produced in most cases when genomic DNA was re-extracted from the epithelial cells found in feather samples, which are richer in mitochondria. Eleven CR and seven ATPase sequences in which double peaks persisted were excluded from the analyses. All sequences used in this study have been deposited in GenBank under accession nos DQ855136–DQ855241.

Phylogenetic analysis

We combined the three mtDNA segment data sets (COI and ATPase 6 and 8 genes and control region) for a total of 1602 bp. Each of the three data sets was analysed with MRMODELTEST (Nylander 2004) to determine the model of sequence evolution that best fit the data. Bayesian analysis of the sequence data was then conducted using MRBAYES version 3.1.2 (http://mrbayes.csit.fsu.edu/) (Huelsenbeck *et al*. 2001) using the model of sequence evolution obtained from MRMODELTEST for each of the mtDNA regions. Two Markov chains were run simultaneously for 1 million generations and sampled every 100 generations. Trees generated prior to stationarity were excluded, and a consensus topology was obtained from the last 195 000 trees in the chain. We also constructed phylogenies of mtDNA haplotypes with the neighbour-joining (NJ) algorithm in PAUP* (Swofford 1999) using uncorrected distances. Node support in the NJ tree was tested with a bootstrap analysis (Felsenstein 1985) using 10 000 replicates. In both Bayesian and NJ analyses we rooted trees with *Dendroica palmarum* as the outgroup due to its phylogenetic proximity to *D. coronata* (Lovette & Bermingham 1999).

Traditional bifurcating trees may not adequately represent intraspecific phylogenies, where ancestral and derived haplotypes can co-exist in a given sample (Posada & Crandall 2001). In order to maximize inference power from haplotype relationships and frequencies, we constructed minimum-spanning networks of absolute distances between haplotypes for each mtDNA segment using the molecular variance parsimony algorithm (Excoffier & Smouse 1994) as implemented in ARLEQUIN 2.0 (Schneider *et al*. 2000). In these networks, haplotypes are represented as circles at the nodes of a tree instead of at the tips, with the size of the circle being proportional to the frequency of the haplotype in the population.

Genetic differentiation and population structure

We used the number of nucleotide changes between haplotypes to calculate corrected genetic distances between groups by taking into account intragroup polymorphism (Nei 1987), so that $D_{xy} = d_i xy - 0.5(d_i x + d_i y)$, where x and y are the groups being compared and d_i is uncorrected average genetic distance (Wilson *et al*. 1985).

Population structure was deduced with an analysis of molecular variance (AMOVA) using ARLEQUIN 2.0. AMOVA uses the frequencies of haplotypes and the number of mutations among 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 nonparametric permutation methods (Excoffier *et al*. 1992). In order to identify groups of populations based on genetic differences, we grouped sampling localities in a way that maximized the among-group variance component (Φ_{ct}). Differentiation between groups was tested with an exact test of population differentiation (Raymond & Rousset 1995) using 10 000 Markov chain steps in ARLEQUIN 2.0.

Demographic history

To test for past sudden changes in effective population size we used Fu's test of neutrality (Fu 1997), which detects departures from neutrality in scenarios characterized by an excess of rare alleles and young mutations in nonrecombining sequences. We used ARLEQUIN 2.0 to generate values of F_s for each of the four yellow-rumped warbler groups and for each mtDNA segment (COI and ATPase and control region). Significant large negative values of F_s indicate an excess of recent mutations and reject population stasis (Fu 1997). In addition, we compared distributions of pairwise nucleotide differences among mtDNA haplotypes (mismatch distributions) with expectations of a sudden-expansion model (Rogers 1995). Populations that have undergone a sudden demographic expansion are expected to show a Poisson-shaped mismatch distribution, whereas populations that have been in equilibrium for a relatively long period of time are expected to show a bimodal or 'ragged' distribution (Slatkin & Hudson 1991). We generated mismatch distributions for the different groups using ARLEQUIN 2.0. The model also generates an estimate of the time (t) elapsed between the effective population size before the expansion (N_0), and the present population size (N_1) (Rogers & Harpending 1992).

Estimation of divergence times

We calculated divergence times among groups with a nonequilibrium coalescence model that uses the variance in pairwise differences between DNA sequences to generate estimates of divergence time independent of gene-migration rates between pairs of populations (Nielsen & Wakeley 2001). We used Markov chain Monte Carlo simulations as implemented in the program MDIV (Nielsen & Wakeley 2001) to generate maximum likelihood estimates of θ, twice the effective female population size (N_{fe}) times the mutation rate (μ); *T*, the divergence time between two populations scaled by population size; and *M*, the gene migration rate between the two populations, also scaled by population size. We assumed uniform prior distributions and set maximum values for *T* and *M* of 10 and 30, respectively. To allow for the possibility of multiple mutations at the same nucleotide site, we used the Hasegawa–Kishino–Yano (HKY) model (Hasegawa *et al*. 1985) instead of an infinite sites model, which did not apply to our data. We used Markov chains of 4 000 000 cycles preceded by a 'burn-in' period of 500 000 cycles for each pairwise population comparison, and run the analysis three times for each population comparison using different random seeds. We run MDIV on a data set that combined the two coding regions (COI and ATPase) for a total of 1302 bp, and used a standard mutation rate of 0.02 substitutions/site/million years ago (Ma).

In addition, we estimated divergence times by applying a molecular clock to genetic distances corrected for intragroup polymorphism. Previous work on mtDNA variation in small landbirds has shown that the 2% divergence-per-Ma rule is reasonably consistent for coding regions of the mtDNA across species (Baker & Marshall 1997; Lovette 2004). However, in order to account for possible departures from this rate (García-Moreno 2004), we calculated divergence times using three rates for coding region data (0.01, 0.02 and 0.04 substitutions/site/Ma). Mutation rates for the control region are more variable than those of coding genes, and published rates span an order of magnitude, from 20% in penguins (Lambert *et al*. 2002) and 15% in dunlin (Wenink *et al*. 1994) to about 2% in sparrows (Zink & Weckstein 2003). We therefore used a range of mutation rates (0.02, 0.04, 0.1 and 0.15 subst./site/Ma) to estimate divergence times from control region data. Finally, we assessed levels of gene flow between populations from the degree of divergence estimated by F_{ST}, given that $F_{ST} \approx 1/1 + 2N_{fe}m$, where $N_{fe}m$ denotes the effective number of females exchanged between populations per generation in an island model (Wright 1969).

Because most passerine bird species reach sexual maturity at 1 year of age, most previous population genetic studies of passerines have used a generation time (*T*) of 1 year. However, theoretical work has shown that the generation time of a population, defined as the average age of mothers of newborn individuals in a population with a stable age distribution is $T = \alpha + s/(\lambda - s)$, where α is the age in years at first breeding, λ is the annual geometric growth rate of the population, and *s* is the annual probability of survival (Lande *et al*. 2002). Assuming the population is in demographic equilibrium (λ = 1) and using an estimated value of *s* of 0.445 (Stewart 1988), we used a generation time *T* of 1.8 years.

Results

Phylogenetic relationships

Sequencing of 126 individuals for COI, 93 for ATPase and 109 for the control region (CR) produced 32, 18 and 56 unique haplotypes, respectively (Table 2). For the phylogenetic analysis, we concatenated the sequence from all three markers into a single data set of 1602 bp. Only individuals for which sequence from all three segments was available were used, thus the final number of haplotypes in the concatenated data set was 41 (Fig. 2). The concatenated haplotypes produced 130 variable sites, of which 80 were parsimony informative. Models of sequence evolution for the three mtDNA segments obtained from MRMODELTEST were HKY + I + G for COI and CR, and GTR + G for ATPase.

Table 2 mtDNA haplotypes per sequenced region found at each sampling site. Numbers in parentheses indicate frequency when greater than one. See text for exact localities

	Population	COI (*n* = 126)	ATPase (*n* = 93)	CR (*n* = 109)
coronata	Alaska	J, P2, W3	H(3), N, O, P, Q, R	M, N, O, P, Q(4)
	Maine	J(17), K, L(2), M	H(7), J, L, M	O2, O3(3), Q(4), T, V4(3), Z1, Z2, Z4, Z5, Z6, Z7
auduboni	Washington	J(8), J2, O, T(4), V, W, W2, X, Y, Z	H(4), I(4)	T2, T3, V3, V4, Z
	Oregon	J(9), J4, O, P, Q, R(4), S, T(4), T2(2), U, X	H(8), I(5), K	O3, R(2), R1, R2, S, S1, T1, T6(2), T7, U, U1, V3, V5, V6(3), W, W2, Y(2), Z8, Z9
	California	J(2), J3, M2, T(3), X	H(5), I(3)	Q(2), T, V1, V2, V3(3), W1, X
nigrifrons	Mexico	A(3), C(7), E, F, G(2), H(2), I(4)	E(10), F(5), G	C, D(3), E, E1, F, G, H, H1, I, J(4), K, L
goldmani	Guatemala	A(23), B(4)	A(20), B(3), C, D(5)	A(25), B(2)

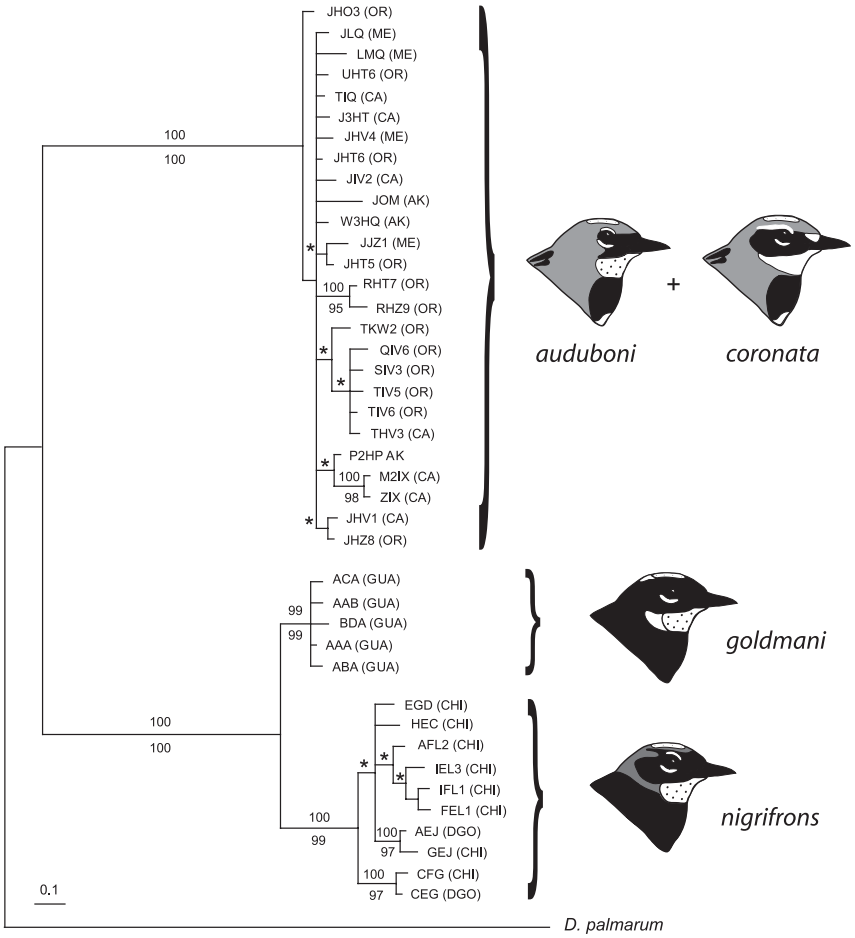

Fig. 2 Bayesian analysis of 1602 bp of mtDNA sequence (combined COI, ATPase and CR data sets), showing phylogenetic relationships between yellow-rumped warbler morphotypes. Consensus topology of 195 002 trees obtained after one million generations based on the HKY + I + G model for COI and CR, and GTR + G for ATPase. Figures along branches represent node support from Bayesian analysis (above) and neighbour joining (below). Asterisks correspond to nodes that obtained support values between 50% and 94% in both analyses. Haplotype designations (concatenated from COI, ATPase and CR haplotypes on Table 1) are shown at branch tips, followed by the geographical location of the haplotype (CA, California; OR, Oregon; AK, Alaska; ME, Maine; DGO, Durango, Mexico; CHI, Chihuahua, Mexico; GUA, Guatemala). Stippled areas on bird schematics represent yellow plumage.

Bayesian analysis and the neighbour-joining algorithm produced identical phylogenies with respect to nodes that obtained > 95% branch support in the Bayesian analysis (Fig. 2). Phylogenetic analysis revealed that *nigrifrons* and *goldmani* form well-differentiated, reciprocally monophyletic sister groups, whereas *coronata* and *auduboni* cluster together into an unresolved sister clade (Fig. 2). Minimum-spanning networks constructed for each of the three mtDNA markers examined show a consistent pattern of haplotype sharing between *coronata* and *auduboni* groups (Fig. 3). In these groups, the pattern for COI and ATPase data is characterized by the presence of a single haplotype of high frequency (J and H, respectively), accompanied by several other closely related haplotypes of lower frequency. The control region was considerably more variable than the coding regions, and although this pattern is also apparent (see haplotype Q in Fig. 3c), there was a much larger number of low-frequency haplotypes, probably due to the relatively higher mutation rate of this marker. The ATPase and control region data sets were able to resolve the separation of *nigrifrons* and *goldmani* groups (Fig. 3b, c). In contrast, the COI data set revealed sharing of haplotype A between individuals of Mexico and Guatemala (Fig. 3a). Given the central position of haplotype A in the network and its relative high frequency, this pattern in COI appears to represent shared ancestral polymorphism due to the relatively slower mutation rate of this gene compared to that of ATPase and CR.

Genetic structure

Significant F_{ST} values were found between all population pairs, including the *coronata* and *auduboni* groups (COI: $F_{ST} = 0.068$, $P = 0.003$; ATPase: $F_{ST} = 0.133$, $P = 0.002$; CR: $F_{ST} = 0.118$, $P < 0.001$). Exact tests of population differentiation based on haplotype frequencies (Raymond & Rousset 1995) were also significant for all population pairs, including the *coronata*/*auduboni* pair (COI: $P = 0.029$; ATPase: $P < 0.001$; CR: $P = 0.012$). The grouping of yellow-rumped warbler individuals into the three main clades revealed by the phylogenetic analysis (Fig. 2) explained close to 92% of the genetic variance among groups and yielded the highest values of Φ_{ct} in an AMOVA (Table 3). Grouping individuals into four groups according to phenotype (*coronata*, *auduboni*,

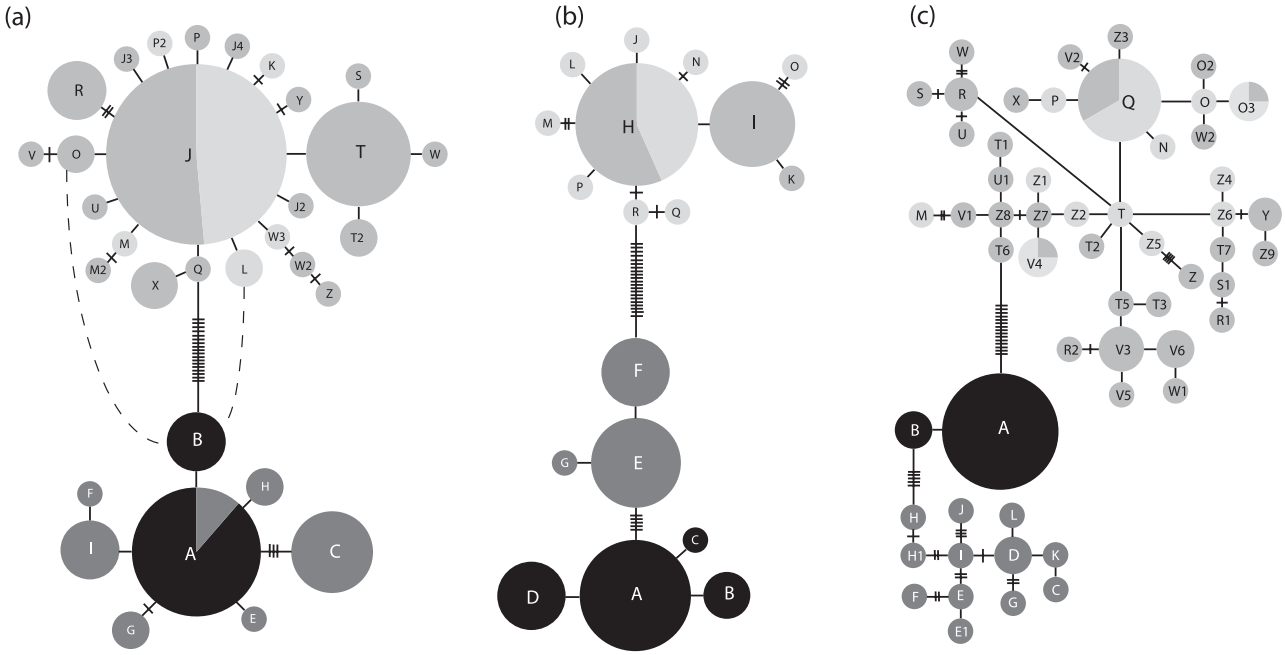

Fig. 3 Minimum-spanning networks of absolute distances between mitochondrial DNA haplotypes of (a) COI gene (b) ATPase 8 gene, and (c) control region. Each circle represents a haplotype, with size proportional to the haplotype's frequency in the population. Colors represent the four main yellow-rumped warbler morphotypes: *coronata* group (light grey), *auduboni* group (medium grey), *nigrifrons* group (dark grey) and *goldmani* group (black). Unhatched network branches represent a single nucleotide substitution, and hatch marks along branches represent additional substitutions. Broken lines represent alternative pathways of equal likelihood (additional alternative pathways for the CR network, not shown for clarity, are as follows: G-D, J-D, F-E, H-E, O-T2, Z4-O2, M-O, Z7-Z6, V4-Q, W1-W2, S-T5, R1-Y, Y-R, Z-Z3).

nigrifrons and *goldmani*) decreased Φ_{ct} to a nonsignificant value below 90%, emphasizing the genetic similarity between the two migratory groups despite their marked phenotypic divergence.

Genetic distance and divergence times

Average percent divergence (corrected for intrapopulation polymorphism) between North American forms (*coronata* + *auduboni*) and Mesoamerican forms (*goldmani* + *nigrifrons*) was 3.07% for COI, 3.48% for ATPase, and 4.86% for control region data (uncorrected distance values were 3.33%, 3.84%, and 6.38%, respectively). Distances between *coronata* and *auduboni* and between *nigrifrons* and *goldmani*, were markedly smaller (see Table 4).

The standard clock calibration of 2% per Ma for coding data yields an estimated time to the common ancestor between migratory and sedentary forms of about 1.7 Ma (for COI) to 1.9 Ma (for ATPase), corresponding approximately to the early Pleistocene. Using the control region data, and given that in this species variation in this marker is about twice that found in coding regions, divergence time between migratory and sedentary forms would be 1.2 Ma using a clock calibration of 4% per Ma, and 0.49 Ma using a faster calibration of 10% per Ma. Estimated divergence times between *coronata* and *auduboni* groups using the coding region calibration yields about 10 000 years BP with the COI data set (0.13% divergence), and 12 000 years BP with the ATPase data set (0.15% divergence). The control region data yields times between 41 000 and 16 400 years BP using the same calibrations as above. These results are generally consistent with a Holocene separation of *coronata* and *auduboni* following the last glacial maximum about 18 000 years BP.

Maximum-likelihood values of θ and T estimated from the nonequilibrium coalescence model for the *coronata*/*auduboni* group comparison using MDIV were 5.60 and 0.30, respectively (Table 6). These values correspond to a divergence time of about 16 129 years BP using a mutation rate of 0.02 subst/site/Ma. The divergence time estimate between *nigrifrons* and *goldmani* ($\theta = 3.39$, $T = 1.93$) was about 62 814 years, and between North American and Mesoamerican groups ($\theta = 8.72$, $T = 4.82$) was about 403 517 years.

Genetic diversity and demographic history

Populations of North American groups *coronata* and *auduboni* showed evidence of a recent population expansion. The pattern of genetic diversity in both migratory groups is

Table 3 Analysis of molecular variance (AMOVA) of the main seven yellow-rumped warbler populations (Maine, Alaska, Washington, Oregon, California, Mexico and Guatemala). Significance values associated with the variance components were obtained from 1000 random permutations of the DNA sequences using ARLEQUIN 2.0

Grouping tested	Marker	d.f.	SS	Variance component	% of variance
(*coronata*) (*auduboni*)	COI				
(*nigrifrons*) (*goldmani*)	Among groups (ϕ_{ct})	3	602.72	6.732	89.74
	Among populations (ϕ_{sc})	3	3.27	0.027	0.37***
	Within populations (ϕ_{st})	119	88.34	0.742	9.90***
	ATPase				
	Among groups (ϕ_{ct})	3	591.11	8.665	95.37*
	Among populations (ϕ_{sc})	3	1.39	0.005	0.06***
	Within populations (ϕ_{st})	86	35.77	0.416	4.58***
	Control region				
	Among groups (ϕ_{ct})	3	473.60	5.802	79.39*
	Among populations (ϕ_{sc})	3	8.68	0.143	1.96***
	Within populations (ϕ_{st})	102	139.02	1.363	18.65***
(*coronata*) (*auduboni*)	COI				
(*nigrifrons* + *goldmani*)	Among groups (ϕ_{ct})	2	595.72	7.330	89.48*
	Among populations (ϕ_{sc})	4	10.27	0.119	1.46***
	Within populations (ϕ_{st})	119	88.34	0.742	9.06***
	ATPase				
	Among groups (ϕ_{ct})	2	527.77	8.467	83.19*
	Among populations (ϕ_{sc})	4	64.74	1.295	12.72***
	Within populations (ϕ_{st})	86	35.78	0.416	4.09***
	Control region				
	Among groups (ϕ_{ct})	2	394.05	4.698	61.62
	Among populations (ϕ_{sc})	4	88.23	1.563	20.50***
	Within populations (ϕ_{st})	102	139.02	1.363	17.88***
(*coronata* + *auduboni*)	COI				
(*nigrifrons*) (*goldmani*)	Among groups (ϕ_{ct})	2	600.06	8.832	91.76*
	Among populations (ϕ_{sc})	4	5.94	0.051	0.53***
	Within populations (ϕ_{st})	119	88.34	0.742	7.71***
	ATPase				
	Among groups (ϕ_{ct})	2	588.69	10.379	95.64*
	Among populations (ϕ_{sc})	4	3.81	0.057	0.52***
	Within populations (ϕ_{st})	86	35.77	0.416	3.83***
	Control region				
	Among groups (ϕ_{ct})	2	464.31	7.403	82.02*
	Among populations (ϕ_{sc})	4	17.97	0.259	2.87***
	Within populations (ϕ_{st})	102	139.02	1.363	15.10***

*, $P < 0.05$; ***, $P < 0.001$.

characterized by the presence of one or two high-frequency haplotypes accompanied by several other closely related haplotypes in lower frequencies, a pattern that is particularly apparent in the COI and ATPase data sets (Fig. 3a, b). Further evidence for a population expansion in the north is provided by the results of Fu's neutrality test, which show large negative and significant values in both *coronata* and *auduboni* groups (Table 5). Finally, the mismatch distributions for the *coronata* and *auduboni* groups show a good fit to the expansion model curve and appear as a Poisson-shaped wave, especially in the control region data set (Fig. 4), as expected for a population that has undergone a sudden expansion in effective population size (Slatkin & Hudson 1991; Rogers & Harpending 1992). The estimated time to the expansion based on parameter τ ranged between 48 310 and 12 077 years BP for coding region data, and between 176 916 and 35 383 years BP for control region data.

In contrast to migratory groups, the pattern of the Mexican *nigrifrons* group suggests a history of long-term population stasis and a lack of a clear expansion. Levels of genetic diversity, especially nucleotide diversity, are relatively high across markers (Table 5) and values of F_s are generally

Table 4 Corrected and uncorrected genetic distances among and within yellow-rumped warbler groups. For each mtDNA segment, above diagonal: corrected average percent pairwise distances; below diagonal: uncorrected average percent pairwise distances, and along diagonal: average percent divergence within group

	coronata	*auduboni*	*nigrifrons*	*goldmani*
COI				
coronata	*0.097*	0.019	3.048	3.112
auduboni	0.216	*0.296*	3.053	3.136
nigrifrons	3.332	3.437	*0.471*	0.081
goldmani	3.188	3.304	0.336	*0.040*
ATPase				
coronata	*0.303*	0.023	3.657	3.609
auduboni	0.219	*0.089*	3.802	3.736
nigrifrons	3.854	3.891	*0.090*	0.944
goldmani	3.803	3.824	1.032	*0.086*
Control Region				
coronata	*1.450*	0.164	5.998	5.073
auduboni	1.296	*0.813*	6.284	5.197
nigrifrons	7.404	7.372	*1.363*	2.501
goldmani	5.821	5.627	3.206	*0.047*

nonsignificant (Table 5). Furthermore, the mistmatch distribution is more ragged (less smooth) than that of the *coronata*/*auduboni* group and shows a worse fit to the expansion model frequencies (Fig. 4). The Guatemalan *goldmani* group appears to have undergone either long-term small population size or a severe and recent genetic bottleneck, as evidenced by extremely low levels of genetic diversity across markers (Table 5, Fig. 3).

Table 6 Maximum likelihood values of parameters θ, *M* (2*Nm*), and *T* (*t*/2 N) estimated with a nonequilibrium coalescence model using MDIV

Groups	θ (SD)	*M* (SD)	*T* (SD)
(*coronata*) (*auduboni*)	5.60 (0.02)	1.20 (0.13)	0.30 (0.04)
(*nigrifrons*) (*goldmani*)	3.39 (0.02)	0.02 (0.02)	1.93 (0.02)
(*cor* + *aud*) (*nigr* + *gold*)	8.72 (0.12)	0.02 (0.01)	4.82 (0.05)

Discussion

Speciation in the yellow-rumped warbler complex

Lineage divergence between sedentary forms (*nigrifrons* and *goldmani*) and migratory forms (*coronata* and *auduboni*) of the yellow-rumped warbler using a standard molecular clock appears to have occurred in the early Pleistocene, a pattern that is broadly consistent with the radiation of several other *Dendroica* taxa as previously inferred from a genus-wide phylogenetic study (Lovette & Bermingham 1999). In turn, sedentary forms *nigrifrons* and *goldmani* have attained reciprocal monophyly in mtDNA markers (except in the COI gene), and the genetic distance between the two forms suggests they split from a common ancestor within the last million years, or the middle Pleistocene. Reproductive isolation between *goldmani* and other groups cannot be ascertained in the wild because its distribution does not overlap with that of other groups. Reproductive isolation between *nigrifrons* and *auduboni*, however, seems likely. Large numbers of *auduboni* individuals visit the highlands of western Mexico during the nonbreeding season, where they live sympatrically with *nigrifrons* individuals (both groups can be captured in the same mist nets), and

Table 5 Genetic diversity indices. Sample size (*n*), number of haplotypes (#hap.), haplotype diversity (*h*), nucleotide diversity (π), and Fu's F_s per mtDNA segment and population

mtDNA marker	*n*	#hap.	*h*	π	F_s
COI					
coronata	25	7	0.8344 ± 0.0394	0.00296 ± 0.00189	−12.955***
auduboni	54	19	0.4867 ± 0.1211	0.00097 ± 0.00087	−4.901***
nigrifrons	20	7	0.8316 ± 0.0568	0.00471 ± 0.00285	0.088
goldmani	27	2	0.2621 ± 0.0972	0.00040 ± 0.00051	0.479
ATPase					
coronata	18	9	0.7059 ± 0.1198	0.00302 ± 0.00201	−3.783**
auduboni	30	3	0.5356 ± 0.0480	0.00089 ± 0.00082	0.343
nigrifrons	16	3	0.5417 ± 0.0985	0.00090 ± 0.00085	−0.094
goldmani	29	4	0.5000 ± 0.0985	0.00086 ± 0.00081	−0.994
Control region					
coronata	26	15	0.8954 ± 0.0487	0.00813 ± 0.00507	−9.51012***
auduboni	29	23	0.9771 ± 0.0134	0.01449 ± 0.00814	−24.50843***
nigrifrons	17	12	0.9338 ± 0.0456	0.01363 ± 0.00799	−4.69191*
goldmani	27	2	0.1425 ± 0.0862	0.00048 ± 0.00079	−0.34896

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

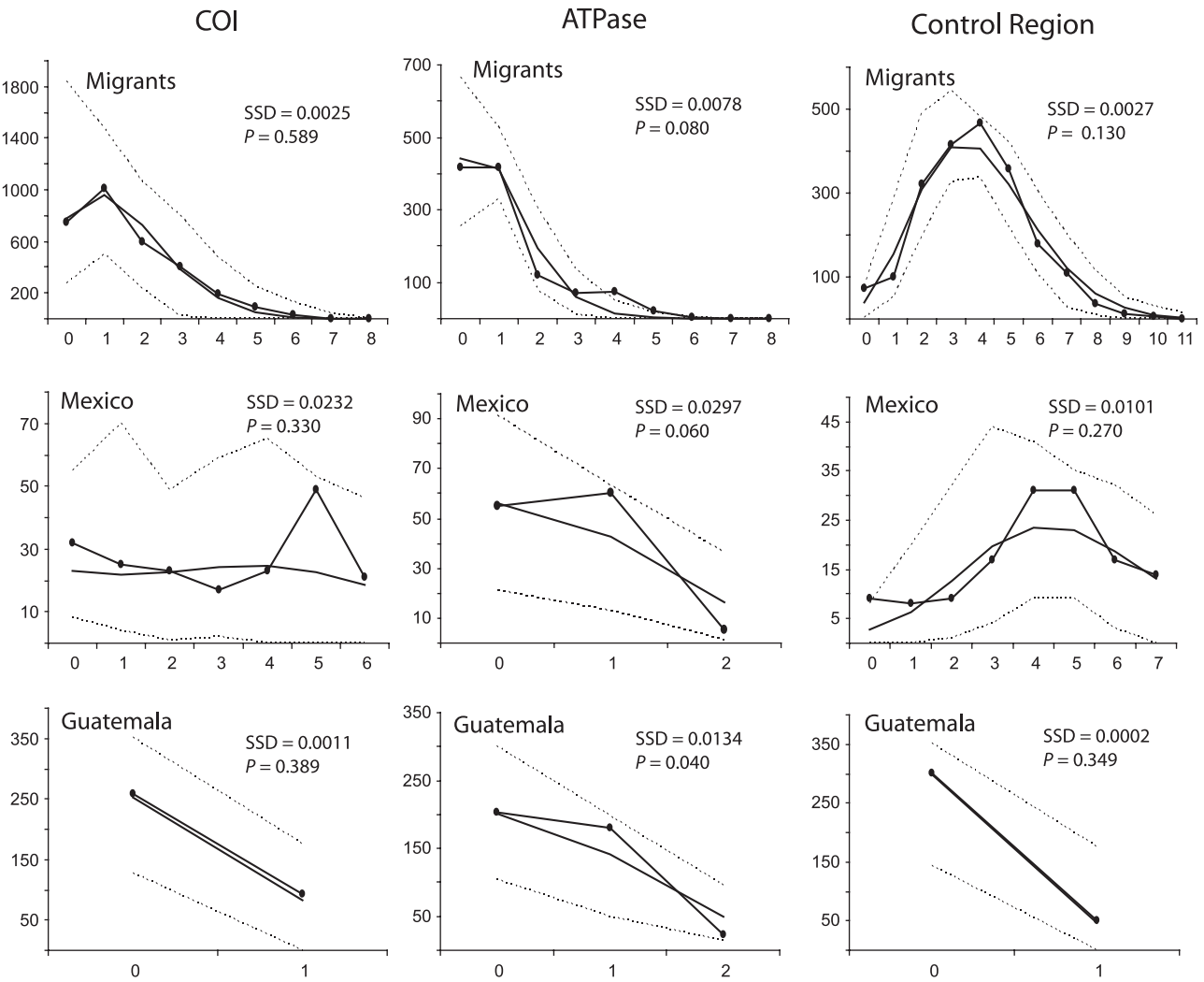

Fig. 4 Mismatch distributions from migratory *coronata* and *auduboni*, Mexican *nigrifrons* and Guatemalan *goldmani* yellow-warbler populations based on COI (first column), ATPase (second column) and control region (third column) data. Black circles correspond to observed frequencies of pairwise nucleotide differences, broken lines represent upper and lower bounds for the 95% confidence intervals around the observed distribution ($\alpha = 0.05$), and solid lines represent the expected frequencies under a sudden expansion model.

they do not vacate these areas until *nigrifrons* is well into its breeding activity, including territory establishment and nest building (late April; B. Milá, personal observation). Furthermore, the similarity in the breeding habitat of *auduboni* and *nigrifrons* suggests that the two groups likely lived sympatrically (or in close parapatry) during at least the last glacial maximum, when temperate coniferous forests were restricted to the southern US and northwestern Mexico (Markgraf 1993; Wright *et al*. 1993), yet no intermixing appears to have occurred over the last several hundred thousand years as demonstrated by the complete differentiation of their mitochondrial genomes. However, because mtDNA is strictly matrilineal, analysis of nuclear markers will be needed to confirm the patterns of gene flow among groups suggested by our results.

In contrast to sedentary forms, genetic differentiation between *coronata* and *auduboni* is considerably lower (Table 4), haplotypes of the two taxa cluster into a single unresolved clade (Fig. 2), and high-frequency haplotypes are shared between the two groups. Unexpectedly, our results indicate that *auduboni* is more closely related to *coronata* than to the other members of the so-called 'Audubon's warbler group' (*nigrifrons* and *goldmani*), with which *auduboni* shares such prominent plumage characters as a yellow throat and lack of white on lores and supercilium (Fig. 1, Table 1). Three different estimates of divergence

time between the two forms consistently point to their separation during the Holocene.

The sharing of haplotypes between *coronata* and *auduboni* forms can be attributed to one of three main processes, including incomplete lineage sorting due to recent divergence, recent gene flow, or ancient introgression through hybridization. Haplotypes shared by the two groups are haplotype J in COI, H in ATPase, and Q in the control region sample (Table 2, Fig. 3). The relatively high frequency of these haplotypes and their central position in star-like minimum-spanning networks indicate that they are ancestral to other sampled haplotypes (Templeton *et al*. 1992). Because only ancestral haplotypes are shared between the two populations, and because the hybrid zone where the two groups meet in western Canada is narrow (~56 km) and stationary (Hubbard 1969; Barrowclough 1980), recent gene flow seems an unlikely explanation. Incomplete lineage sorting is expected in the early stages of speciation between recently diverged populations. Coalescence theory predicts that populations between which gene flow has been interrupted will eventually reach reciprocal monophyly at neutrally evolving markers (Slatkin & Maddison 1989), and for mtDNA markers that should take place in about $4N_{fe}$ generations, where N_{fe} is the female effective population size. However, for expanding populations of large effective size such as those of *coronata* and *auduboni*, achievement of reciprocal monophyly might take much longer (Neigel & Avise 1986; Nichols 2001).

Ancient introgression following hybridization (Lehman *et al*. 1991; Weckstein *et al*. 2001) early in cladogenesis is expected to generate a similar pattern to incomplete lineage sorting, and thus distinguishing the two processes can be difficult (Moore 1995; Funk & Omland 2003). Because mtDNA is inherited as a single unit, hybridization cannot be detected using mtDNA alone and nuclear loci should be examined. However, because coalescence times in nuclear genes may be four times longer than in mtDNA (Nichols 2001), a large number of autosomal loci will be required to attain the necessary resolving power. A study by Barrowclough (1980) revealed that genetic distance between the two taxa inferred from eight allozyme loci was only 0.006 (SE = 0.0022) and F_{ST} values were low and nonsignificant (global $F_{ST} = 0.005$, $P > 0.05$), suggesting that genetic divergence in nuclear markers is low and consistent with mtDNA variation. Moreover, recent genetic divergence accompanied by fast plumage evolution despite incomplete lineage sorting has been recently documented in a number of species (see below), suggesting that fast plumage differentiation in the absence of lineage sorting might not be rare in nature.

In light of the above evidence, we believe hybridization to be a less parsimonious explanation than incomplete lineage sorting, but further assays of nuclear genes are necessary to confirm this pattern. In either case, given the marked phenotypic differentiation, significant F_{ST} values, and the absence of current gene flow (except at a narrow hybrid zone), the *coronata* and *auduboni* groups appear to be in the early stages of speciation and given their largely allopatric distributions can be considered to be on separate evolutionary trajectories.

Pleistocene climate and evolutionary history

The Pleistocene appears to have been a crucial period for differentiation in the yellow-rumped warbler complex. Changes in habitat distribution caused by cyclical glaciations over the last 2 million years (Wright *et al*. 1993) are likely to have played a role in driving the divergence of the different groups. Specifically, restriction of forest patches during glacial maxima may have confined populations to small isolated refugia. On these habitat patches, warbler populations probably suffered severe reductions in effective population size, which catalysed allopatric differentiation and speciation (Mengel 1964; Hubbard 1970; Weir & Schluter 2004). The pattern of genetic diversity and haplotype frequencies in the *auduboni* and *coronata* groups is consistent with an expansion from a population of small effective size, a pattern that seems to support the forest refuge hypothesis. This pattern of low genetic diversity and lack of genetic structure has been reported for a number of other temperate passerines (Ball & Avise 1992; Zink 1997; Milá *et al*. 2000; Milot *et al*. 2000; Veit *et al*. 2005) and suggests that genetic bottlenecks during glacial maxima followed by postglacial expansions may be of common occurrence. Based on current distributions and observed patterns of genetic diversity, we hypothesize that the *auduboni* group expanded northward along the western half of the continent, whereas *coronata* expanded up the eastern half, then across the boreal zone to reach Alaska and western Canada, giving rise to a distributional pattern that is typical of many boreal passerines (Morse 1989; Sibley 2000; Weir & Schluter 2004). The evolution of this distributional pattern was inferred from molecular data in Swainson's thrush (*Catharus ustulatus*) and it was shown to be concordant with the advance of boreal forest since the last glacial maximum (Ruegg & Smith 2002).

In his influential study on the role of Quaternary glacial cycles in driving speciation in wood warblers, Mengel (1964) postulated that glacial remnants of eastern deciduous forest in southeastern North America hosted the ancestral forms from which western species evolved following postglacial expansions northward and westward across the boreal zone. However, Mengel proposed the yellow-rumped warbler as one of the few exceptions to his overarching speciation model, hypothesizing that given the myrtle warbler's affinity for coniferous forest habitats, this species is 'a recent (Wisconsin) differentiate resulting from

eastward re-expansion of (a) pro-*Dendroica auduboni*' (Mengel 1964; p. 31). Although our results generally favour this hypothesis, we believe that differentiation in southern forest refugia during the last glacial maximum followed by a northward expansion to their current ranges as explained above, appear to be a more plausible scenario based on our data.

Whether sedentary forms are ancestral to migratory forms or vice versa cannot be established from our molecular data alone, as the two clades are separated by a single node (Fig. 2). According to the available genus-wide phylogeny of *Dendroica* wood warblers (Lovette & Bermingham 1999), all potential outgroups for the yellow-rumped warbler tree are fully migratory species, which means that inference of ancestry based on character evolution using migratory behaviour would favour the hypothesis of migratory ancestry and thus a loss of migration in *nigrifrons* and *goldmani*. However, the possibility that extant sister species had sedentary populations that went extinct at some point in their histories cannot be ruled out (Cox 1985), and thus migratory behaviour might not be an appropriate trait to infer the evolution of the group. A more informative set of traits might be the plumage characteristics within the ingroup. These suggest that a yellow throat and lack of white supercilium, shared by *auduboni*, *nigrifrons* and *goldmani*, constitute the ancestral state, with the white throat and supercilium found in *coronata* representing derived characters that evolved rapidly in the Holocene. In light of our genetic results, we believe this assessment to be the most parsimonious.

Rapid plumage divergence in the coronata *group*

Our results indicate that the marked plumage differentiation of the *coronata* group with respect to other members of the complex (Fig. 1, Table 1) has evolved in just a few thousand years. Recent divergence with fast plumage evolution has been recently documented in *Icterus* orioles (Baker *et al*. 2003; Kondo *et al*. 2004), redpolls (Seutin *et al*. 1995), bluethroats (Questiau *et al*. 1998; Zink *et al*. 2003), yellow wagtails (Ödeen & Björklund 2003), and swamp sparrows (Greenberg *et al*. 1998). Sexual selection is typically invoked as the most likely factor in driving the rapid evolution of plumage divergence in the above studies, as it is known to be capable of driving the rapid evolution of striking plumage characteristics (Prum 1997; Omland & Lanyon 2000), and its role in speciation has received considerable theoretical and empirical attention (Lande 1981; Takimoto *et al*. 2000; Panhuis *et al*. 2001; Price 2002). The role of natural selection cannot be discounted and has been proposed as a factor in plumage evolution (Crochet *et al*. 2000; Greenberg *et al*. 1998; Warren *et al*. 2005), although it is thought to operate at slower rates than sexual selection. Given the fact that the main differences in plumage found in *coronata* are restricted to patterning of the head area, a role for natural selection appears unlikely and we favour the hypothesis that sexual selection may be involved in the rapid evolution of these plumage traits.

Relative utility of different mtDNA markers

Patterns of mtDNA variation in our study revealed significant differences in the degree of phylogenetic resolution afforded by the three different markers used. The control region appeared to be the most variable marker, followed in order by the ATPase 6 and 8 genes and COI. The most significant difference in terms of phylogenetic signal was the pattern of incomplete lineage sorting found in the COI data set with respect to two of the groups (*nigrifrons* and *goldmani*), which the other two markers grouped into reciprocally monophyletic clades. This is likely due to the relatively lower mutation rate of this marker and suggests that the use of COI as a gene for 'barcoding' avian species (Hebert 2004) may not detect reciprocally monophyletic groups in all situations. Despite the greater variation found in the control region, this marker was not found to be more informative than the ATPase genes in terms of phylogenetic signal. However, due to its higher mutation rate, this marker was the most effective in detecting a signature of expansion in the migratory populations as evidence by a Poisson-shaped mismatch distribution (Fig. 4).

Implications for the systematics of the complex

Our study reveals that *Dendroica coronata coronata* is more closely related to *Dendroica coronata auduboni* than either one is to *Dendroica coronata nigrifrons* or *Dendroica coronata goldmani* (Fig. 2), which makes the 'Audubon's warbler group' paraphyletic. Based on plumage traits and the molecular phylogenetic data presented here, we believe that the assignation of species rank to *D. c. nigrifrons* and *D. c. goldmani* is likely to be uncontroversial. However, whether *D. c. auduboni* and *D. c. coronata* should be treated as separate species is less clear given the existence of a hybrid zone between them (Hubbard 1969) and the pattern of incomplete lineage sorting shown here. Because the evolution of reciprocal monophyly is due to the stochastic process of gene sorting and may or may not be correlated with the evolution of phenotypic characters, reciprocal monophyly may not always be a useful criterion for the establishment of species limits (O'Hara 1994; Doyle 1995; de Queiroz 1998; Baker *et al*. 2003). However, the presence of hybrid zones between putative species poses a special problem, and factors such as its width, degree of introgression and back-crossing, and projected permanency of the hybrid zone should be further investigated and taken into account (Helbig *et al*. 2002).

Historical demography of Mesoamerican forms: implications for conservation

The exceptionally low levels of genetic diversity found across all mtDNA markers in the *goldmani* group suggests that this relatively small and isolated population in the western highlands of Guatemala has either suffered a severe and recent bottleneck or has remained small for a long period of time. At the high elevations preferred by *goldmani* [typically above 2500 m (Howell & Webb (1995); B. Milá, personal observation], relatively small changes in the altitudinal distribution of high-elevation pine and juniper species may have had a dramatic effect on the area of its suitable habitat, leading to reductions in effective population size. Present-day anthropogenic disturbance of natural habitats for agriculture and grazing could be exerting significant pressure on this geographically restricted and poorly studied group. Similar concerns apply to the *nigrifrons* group in the Sierra Madre Occidental of Mexico, where its preferred habitat (dry pine and pine-oak forest primarily above 2000 m) is being rapidly depleted for timber production. Detailed analysis of the current geographical distribution of *goldmani* and *nigrifrons*, as well as data on population density and trends, are urgently needed to ensure the protection of these unique taxa.

Acknowledgements

Martin Cody, Fritz Hertel, John McCormack and two anonymous reviewers provided helpful comments on previous versions of the manuscript. Allison Alvarado, Ingrid Arias, Omar Espinosa, Adrián Gutiérrez, Mari Kimura, Sergio Larios, Allison Lee, John McCormack, Adán Oliveras, Miguel Ramírez and Vicente Rodríguez provided outstanding help in the field. The Institute for Bird Populations, Gwen Baluss and Dennis Vroman kindly contributed feather samples. A. Navarro-Sigüenza at the Museo de Zoología Alfonso Herrera at UNAM facilitated Mexican samples. For financial support, we thank the UC MEXUS Program, the UCLA Latin American Center, an American Ornithologists' Union's Wetmore Award, UCLA's Department of Ecology and Evolutionary Biology, the Turner Foundation, the Environmental Protection Agency and the National Science Foundation.

References

A.O.U. (1973) Thirty-second supplement to the American Ornithologists' Union checklist of North American Birds. *Auk*, **90**, 411–419.

Avise JC (2000) *Phylogeography*. Harvard University Press, Cambridge, MA.

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

Baker A, Marshall H (1997) Mitochondrial control region sequences as tools for understanding evolution. In: *Avian Molecular Evolution and Systematics* (ed. Mindell DP), pp. 49–80. Academic Press, San Diego.

Baker JM, López-Medrano E, Navarro-Sigüenza A, Rojas-Soto OR, Omland KE (2003) Recent speciation in the orchard oriole group: divergence of *Icterus spurius spurius* and *icterus spurius fuertesi*. *Auk*, **120**, 848–859.

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

Barrowclough GF (1980) Genetic and phenotypic differentiation in a wood warbler (genus *Dendroica*) hybrid zone. *Auk*, **97**, 655–668.

Cox GW (1985) The evolution of avian migration systems between temperate and tropical regions of the New World. *American Naturalist*, **126**, 451–474.

Crochet P-A, Bonhomme F, Lebreton JD (2000) Molecular phylogeny and plumage evolution in gulls (Larini). *Journal of Evolutionary Biology*, **13**, 47–57.

Davis LI (1972) *A Field Guide to the Birds of Mexico and Central America*. University of Texas Press, Austin, Texas.

Doyle JJ (1995) The irrelevance of allele tree topologies for species delimitation, and a non-topological alternative. *Systematic Botany*, **20**, 574–588.

Excoffier L, Smouse PE (1994) Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: molecular variance parsimony. *Genetics*, **136**, 343–359.

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

Felsenstein J (1985) Confidence intervals on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.

Fu YX (1997) Statistical neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Funk DJ, Omland KE (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology and Systematics*, **34**, 397–423.

García-Moreno J (2004) Is there a universal mtDNA clock for birds? *Journal of Avian Biology*, **35**, 465–468.

Greenberg R, Cordero P, Droege S, Fleischer RC (1998) Morphological adaptation with no mitochondrial DNA differentiation in the coastal plain swamp sparrow. *Auk*, **115**, 706–712.

Hasegawa M, Kishino H, Yano T (1985) Dating the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, **22**, 160–174.

Helbig AJ, Knox AG, Parkin DT, Sangster G, Collinson M (2002) Guidelines for assigning species rank. *Ibis*, **144**, 518–525.

Hebert PDN (2004) Identification of birds through DNA barcodes. *PLoS Biology*, **2**, 1657–1663.

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

Howell SNG, Webb S (1995) *A Guide to the Birds of Mexico and Northern Central America*. Oxford University Press, New York.

Hubbard JP (1969) The relationships and evolution of the *Dendroica coronata* complex. *Auk*, **86**, 393–432.

Hubbard JP (1970) Geographic variation in the *Dendroica coronata* complex. *Wilson Bulletin*, **82**, 355–369.

Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, **294**, 2310–2314.

Hunt PD, Flaspohler DJ (1998) Yellow-rumped warbler (*Dendroica coronata*). In: *The Birds of North America* (eds Poole A, Gill F). The Birds of North America, Inc, Philadelphia, Pennsylvania.

Johnson NK, Cicero C (2004) New mitochondrial DNA data affirm the importance of Pleistocene speciation in North American birds. *Evolution*, **58**, 1122–1130.

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

Kondo B, Baker JM, Omland KE (2004) Recent speciation between the Baltimore oriole and the black-backed oriole. *Condor*, **106**, 674–680.

Lambert DM, Ritchie PA, Millar CD *et al*. (2002) Rates of evolution in ancient DNA from Adélie penguins. *Science*, **295**, 2270–2273.

Lande R (1981) Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Sciences, USA*, **78**, 3721–3725.

Lande R, Engen S, Saether B-E *et al*. (2002) Estimating density dependence from population time series using demographic theory and life-history data. *American Naturalist*, **159**, 321–337.

Lehman N, Eisenhawer A, Hansen K *et al*. (1991) Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. *Evolution*, **45**, 104–119.

Lovette IJ (2004) Mitochondrial dating and mixed support for the '2% rule' in birds. *Auk*, **121**, 1–6.

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

Markgraf V (1993) Climatic history of Central and South America since 18 000 yr BP: comparison of pollen records and model simulations. In: *Global Climates Since the Last Glacial Maximum* (eds Wright HE, Kutzbach JE, Webb T III *et al*.). University of Minnesota Press, Minneapolis.

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

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, Biological Sciences*, **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.

Moore WS (1995) Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. *Evolution*, **49**, 718–726.

Moore WS, Weibel AC, Agius A (2006) Mitochondrial DNA phylogeny of the woodpecker genus *Veniliornis* (Picidae, Picinae) and related genera implies convergent evolution of plumage patterns. *Biological Journal of the Linnean Society*, **87**, 611–624.

Morse DH (1989) *American Warblers, an Ecological and Behavioral Perspective*. Harvard University Press, Cambridge.

Navarro-Sigüenza A, Peterson AT (2004) An alternative species taxonomy of the birds of Mexico. *Biota Neotropica*, **4**, 1–32.

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

Neigel J, Avise JC (1986) Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: *Evolutionary Processes and Theory* (eds Nevo E, Karlin S), pp. 515–534. Academic Press, New York.

Nichols R (2001) Gene trees and species trees are not the same. *Trends in Ecology & Evolution*, **16**, 358–364.

Nielsen R, Wakeley J (2001) Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics*, **158**, 885–896.

Nylander JAA (2004) *MRMODELTEST (version 2). Program distributed by the author at* http://www.csit.fsu.edu/~nylander/, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.

O'Hara RJ (1994) Evolutionary history and the species problem. *American Zoologist*, **34**, 12–22.

Ödeen A, Björklund M (2003) Dynamics in the evolution of sexual traits: losses and gains, radiation and convergence in yellow wagtails (*Motacilla flava*). *Molecular Ecology*, **12**, 2113–2130.

Omland KE, Lanyon SM (2000) Reconstructing plumage evolution in orioles (*Icterus*): repeated convergence and reversal in patterns. *Evolution*, **54**, 2119–2133.

Panhuis TM, Butlin R, Zuk M, Tregenza T (2001) Sexual selection and speciation. *Trends in Ecology & Evolution*, **16**, 325–413.

Posada D, Crandall KA (2001) Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology & Evolution*, **16**, 37–45.

Price TD (2002) Domesticated birds as a model for the genetics of speciation by sexual selection. *Genetica*, **116**, 311–327.

Prum RO (1997) Phylogenetic tests of alternative intersexual selection mechanisms: trait macroevolution in a polygynous clade (Aves: Pipridae). *American Naturalist*, **149**, 668–692.

de Queiroz K (1998) The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. In: *Endless Forms: Species and Speciation* (eds Howard DJ, Berlocher SH). Oxford University Press, New York.

Questiau S, Eybert M-C, Gaginskaya AR, Gielly L, Taberlet P (1998) Recent divergence between two morphologically differentiated subspecies of bluethroat (Aves: Muscicapidae: *Luscinia svecica*) inferred from mitochondrial sequence variation. *Molecular Ecology*, **1998**, 239–245.

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution*, **49**, 1280–1283.

Rogers AR (1995) Genetic evidence for a Pleistocene population explosion. *Evolution*, **49**, 608–615.

Rogers AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, **9**, 552–569.

Ruegg KC, Smith TB (2002) Not as the crow flies: a historical explanation for circuitous migration in Swainson's thrush (*Catharus ustulatus*). *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **269**, 1375–1381.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN 2.0: A software for population genetics data analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

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

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

Sibley DA (2000) *National Audubon Society the Sibley Guide to Birds*. Alfred A. Knopf, New York.

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.

Slatkin M, Maddison WP (1989) A cladistic measure of gene flow inferred from the phylogenies of alleles. *Genetics*, **123**, 603–613.

Sorenson MD, Quinn TW (1998) Numts: a challenge for avian systematics and population biology. *Auk*, **115**, 214–221.

Sorenson MD, Ast JC, Dimcheff DE, Yuri T, Mindell DP (1999) Primers for a PCR-based approach to mitochondrial genome

sequencing in birds and other vertebrates. *Molecular Phylogenetics and Evolution*, **12**, 105–114.

Stewart PA (1988) Annual survival rate of yellow-rumped warblers. *North American Bird Bander*, **13**, 106.

Swofford DL (1999) PAUP*: *Phylogenetic Analysis Using Parsimony (*and Other Methods)*, version 4.0b8. Sinauer Associates, Sunderland, Massachusetts.

Takimoto G, Masahiko H, Yamamura N (2000) A deterministic genetic model for sympatric speciation by sexual selection. *Evolution*, **54**, 1870–1881.

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

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics*, **132**, 619–633.

Veit ML, Robertson RJ, Hamel PB, Friesen VL (2005) Population genetic structure and dispersal across a fragmented landscape in cerulean warblers (*Dendroica cerulea*). *Conservation Genetics*, **6**, 159–174.

Warren BH, Bermingham E, Prys-Jones RP, Thebaud C (2005) Tracking island colonization history and phenotypic shifts in Indian Ocean bulbuls (*Hypsipetes*: Pycnonotidae). *Biological Journal of the Linnean Society*, **85**, 271–287.

Weckstein JD (2005) Molecular phylogenetics of the *Ramphastos* toucans: implications for the evolution of morphology, vocalizations, and coloration. *Auk*, **122**, 1191–1209.

Weckstein JD, Zink RM, Blackwell-Rago RC, Nelson DA (2001) Anomalous variation in mitochondrial genomes of White-crowned (*Zonotrichia leucophrys*) and Golden-crowned (*Z. atricapilla*) sparrows: Pseudogenes, hybridization, or incomplete lineage sorting? *Auk*, **118**, 231–236.

Weir JT, Schluter D (2004) Ice sheets promote speciation in boreal birds. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **271**, 1881–1887.

Wenink PW, Baker AJ, Tilanus MGJ (1994) Mitochondrial control-region sequences in two shorebird species, the turnstone and the dunlin, and their utility in population genetic studies. *Molecular Biology and Evolution*, **11**, 22–31.

Wilson AC, Cann RL, Carr SM *et al.* (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society*, **26**, 375–400.

Wright S (1969) *Evolution and the Genetics of Populations. Vol. 2. The Theory of Gene Frequencies*. Chicago University Press, Chicago, Illinois.

Wright HE, Kutzbach JE, Webb T III *et al.* (1993) *Global Climates Since the Last Glacial Maximum*. University of Minnesota Press, Minneapolis.

Zink RM (1997) Phylogeographic studies of North American birds. In: *Avian Molecular Evolution and Systematics*, pp. 301–324. Academic Press, New York.

Zink RM, Weckstein JD (2003) Recent evolutionary history of the fox sparrows (genus: *Passerella*). *Auk*, **120**, 522–527.

Zink RM, Drovetski SV, Questiau S *et al.* (2003) Recent evolutionary history of the bluethroat (*Luscinia svecica*) across Eurasia. *Molecular Ecology*, **12**, 3069–3075.

Zink RM, Klicka J, Barber BR (2004) The tempo of avian diversification during the Quaternary. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **359**, 215–220.

Borja Milá is a postdoctoral fellow interested in using phylogeographic and genomic approaches to study speciation and related evolutionary processes. Other interests include the evolution of migration, migratory connectivity, bird-parasite interactions and conservation genetics. This work was part of his dissertation on the role of Quaternary climate in shaping phylogeographic patterns and driving speciation in songbird species of North and Central America. Thomas B. Smith is a professor at the Department of Ecology and Evolutionary Biology and the director of the Center for Tropical Research at UCLA. He is interested in evolutionary genetics and ecology, and his research focuses on the mechanisms of speciation, migratory connectivity, bird-parasite interactions and the conservation of tropical vertebrates. Robert K. Wayne is a professor at UCLA's Department of Ecology and Evolutionary Biology. He is interested in applying molecular genetic techniques to address questions in population genetics, systematics, genomics and conservation genetics of vertebrates.
