# Linking Breeding and Overwintering Areas of Five Nearctic-Neotropical Migratory Passerines Using Molecular Genetic Markers[1]

**B. Milá,[2,3] S. M. Clegg,[2,4] M. Kimura,[2] K. Ruegg,[2] and I. Lovette,[2,5] and T. B. Smith[2,3,6]**

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## Introduction

Demographic studies of Nearctic-Neotropical migrant songbirds have been limited by the difficulty of following them through a complete annual cycle (Webster et al. 2002). As population regulation may conceivably occur on either the breeding area, wintering areas, or on migration routes, determining levels of connectivity of populations between a species' breeding and wintering areas is fundamental to understanding the dynamics of migrant populations. An alternative to marking and tracking individuals (of limited use in small passerines) is to use population-specific genetic markers. A major advantage of this approach is that it relies on the genetic characteristics of the whole population (based on the relatedness among its individuals), and therefore a particular individual does not have to be recaptured or followed.

Molecular genetic markers have been used successfully to examine connectivity in shorebirds (Wenink and Baker 1996, Haig et al. 1997) and more recently in some small passerines (Buerkle 1999, Milot et al. 2000, Kimura et al. 2002, Ruegg and Smith 2002, Lovette et al. in press). In order to apply molecular methodologies effectively to the question of connectivity between populations, genetic variation in these populations needs to be geographically structured (geographic populations need to be genetically differentiated) and the chosen molecular marker must be sensitive enough to detect existing structure. The finer the scale of geographic structure resolved by a particular genetic marker, the more useful it will be in resolving breeding origins.

We have assessed the utility of mitochondrial DNA (mtDNA) markers in determining breeding origins of five long-distance Neotropical migrants: the Yellow-breasted Chat (*Icteria virens*), Nashville Warbler (*Vermivora ruficapilla*), Common Yellowthroat (*Geothlypis trichas*), Wilson's Warbler (*Wilsonia pusilla*), and Swainson's Thrush (*Catharus ustulatus*). We assessed the extent of mtDNA phylogeographic structure and used these data to assign individuals captured on wintering sites in Mexico, Central America, and South America to their respective breeding areas.

## Methods

### *Sampling and Molecular Approaches*

Blood and feather samples were collected from adult birds mist-netted at breeding sites in Canada and the United States, and at overwintering sites in Mexico, Central America, and South America. Blood samples were obtained by sub-brachial venipuncture, and feather samples by plucking the outermost two rectrices. See Kimura et al. (2002) and Ruegg and Smith (2002) for methods of DNA extraction, sequencing, and restriction enzyme digests.

For each species, we first reconstructed a phylogeny based on mtDNA sequence using samples from across the breeding range. We then identified restriction enzymes that were diagnostic of statistically well-supported, geographically defined lineages. Enzyme assays were used to screen samples from individuals captured on overwintering areas to assign them to geographically-defined breeding areas.

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[2]Center for Tropical Research and Department of Biology, San Francisco State University, San Francisco, CA 94132.

[3]Current address: Department of Organismic Biology, Ecology, and Evolution and Center for Tropical Research Institute of the Environment, 621 Charles E. Young Drive South, Room LS 5120, P.O. Box 951606, University of California, Los Angeles, CA 90095-1606.

[4]Current address: Department of Biological Sciences, Imperial College at Silkwood Park, Ascot, Berkshire SL5 7PY, UK.

[5]Current address: Evolutionary Biology Program, Cornell Laboratory of Ornithology, 159 Sapsucker Woods Road, Ithaca, NY 14850.

[6]Corresponding author, e-mail: tbsmith@ucla.edu.

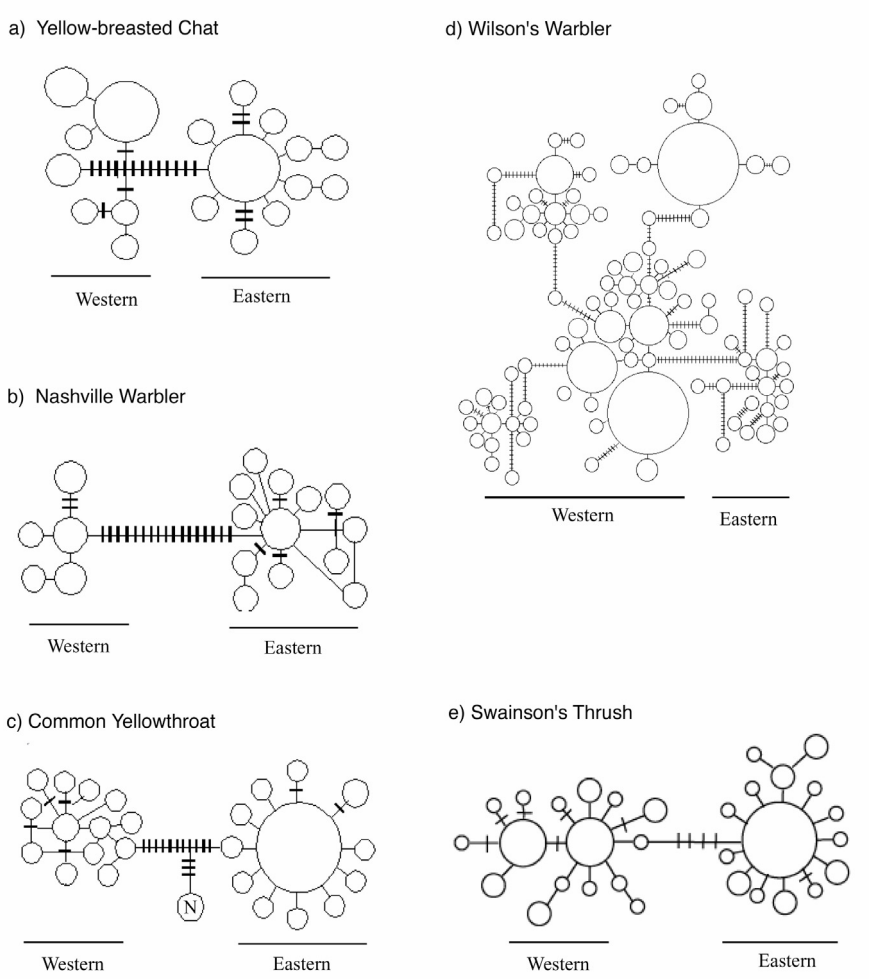


**Figure 1—** Minimum-spanning network with each unique haplotype indicated by a circle and area proportional to the number of individuals sampled. Hatch marks along branches indicate inferred haplotype differences. Eastern and western geographic lineages indicated below each network. (a) Yellow-breasted Chat - mtDNA ATPase sequences were obtained from 34 individuals, including 11 eastern and 7 western individuals. A total of 18 unique haplotypes with 17 nucleotide substitution (1.8 percent sequence divergence) between eastern and western populations (Lovette et al. in press); (b) Nashville Warbler – sequences obtained from 27 individuals, including 18 eastern and 9 western individuals. Eastern and western haplotypes differed by 16 to 22 substitutions, 1.7-2.3 percent sequence divergence (Lovette et al. in press); (c) Common Yellowthroat – sequences from 47 individuals with a maximum of 19 nucleotide substitutions (2 percent) (see Lovette et al. submitted); (d) Wilson's Warbler – mtDNA control region sequences from 200 individuals. 94 unique haplotypes were identified and eastern and western haplotypes differed by a minimum of 22 substitutions (see Kimura et al. 2002). Divergent Nevada haplotype indicated by "N'; (e) Swainson's Thrush - mtDNA control region sequences from 183 individuals showing with a net sequence divergence between lineages of 0.69 percent (Ruegg and Smith 2002).

## Results and Discussion

### *Patterns of Variation on the Breeding Grounds*

A number of common patterns in population genetic structure are evident among all five species (*fig. 1a-e*). The most obvious similarity is that each species is divided into two main haplotype groups associated to varying degrees with eastern and western sampling sites. The level of divergence between these groups was between 0.5 and 2 percent, consistent with a late Pleistocene divergence when using the prevailing molecular clock for mtDNA of 2 percent per million years (Avise and Walker 1998, Kimura et al. 2002, Ruegg and Smith 2002). Another similarity among the five species was the relative lack of geographic structure within eastern and western haplotype groups. These relatively low levels of variation could be due to current or historical gene flow, or past demographic events such as demographic bottlenecks followed by rapid range expansions (e.g. Milá et al. 2000). The high level of homogeneity across broad geographic areas, most evident in the eastern lineage of all sufficiently sampled species, suggests that eastern and western lineages may have had different demographic histories. In general, there was a slightly higher degree of geo-

graphic structure within western groups (*fig. 1*), possibly stemming from less severe effects of glaciation in the west, or the maintenance of higher levels of population subdivision over long periods. In addition, some species showed hints of greater phylogenetic structure that are important to note. In the Common Yellowthroat, there was a divergent haplotype from Nevada separated from the eastern group by 7-9 nucleotide substitutions (point mutations) and from the east by 12-16 substitutions (*Fig. 1c*). This population begs further investigation and may represent a distinct migratory population, or possibly a non-migrant population that may extend southward where we did not sample (Lovette et al. submitted). In Wilson's Warbler, more structure was detected among western populations than we found in the other species. An analysis of molecular variance (AMOVA) revealed both significant within and between-population variation (Kimura et al. 2002). It is possible, however, that similar complexities could be revealed in the other species if sampling were conducted with similar intensity as that for these western Wilson's Warbler populations.

### *Distribution of Genetic Lineages at Overwintering Sites*

The distribution of eastern and western lineages on the wintering grounds differed among species (*fig. 2a-e*). This ranged from complete segregation to some geographic mixing of eastern and western groups at locations on the wintering grounds. In the Yellow-breasted Chat there was no evidence of mixing of eastern and western groups at wintering locations, although samples for any given site were small (*fig. 2a*). Overwintering western groups of the chat were distributed from Southern Baja California to Oaxaca, Mexico. Eastern groups were found from Vera Cruz south through Chiapas, and at sites in Belize and El Salvador. Samples for the Common Yellowthroat were restricted to only three sites, but nevertheless are informative (*fig. 2b*). Only western individuals were found in southern Baja, a mixed population was found in Oaxaca, and only eastern individuals were found in Belize. In contrast, haplotype distributions for the Nashville Warblers revealed only two out of out nine sites with western birds (a site in Sinaloa with 9 individuals and a site in Oaxaca with one individual), while eastern individuals were distributed throughout the wintering range (*fig. 2c*). Limited mixing of breeding lineages at overwintering sites was evident for Wilson's Warbler, mostly in Vera Cruz and Chiapas. Western haplotypes predominated throughout the wintering range (*fig. 2d*). In Swainson's Thrush there was a nearly complete segregation of eastern and western groups on the wintering grounds (*fig. 2e*). Eastern groups were found primarily from Panama to northern South America, while western groups were found in southern Mexico and Central America.

In conclusion, results from the five species examined suggest that connectivity between breeding and wintering areas can be resolved at large geographic scales using mitochondrial DNA variation. The use of other, more variable molecular markers may ultimately increase resolution and the ability to link populations at a finer scale. Also, molecular genetic markers may ultimately be most successful when combined with other types of data such as banding returns, morphologically based subspecific variation, stable isotope markers, radio and satellite telemetry, and disease strain variation.

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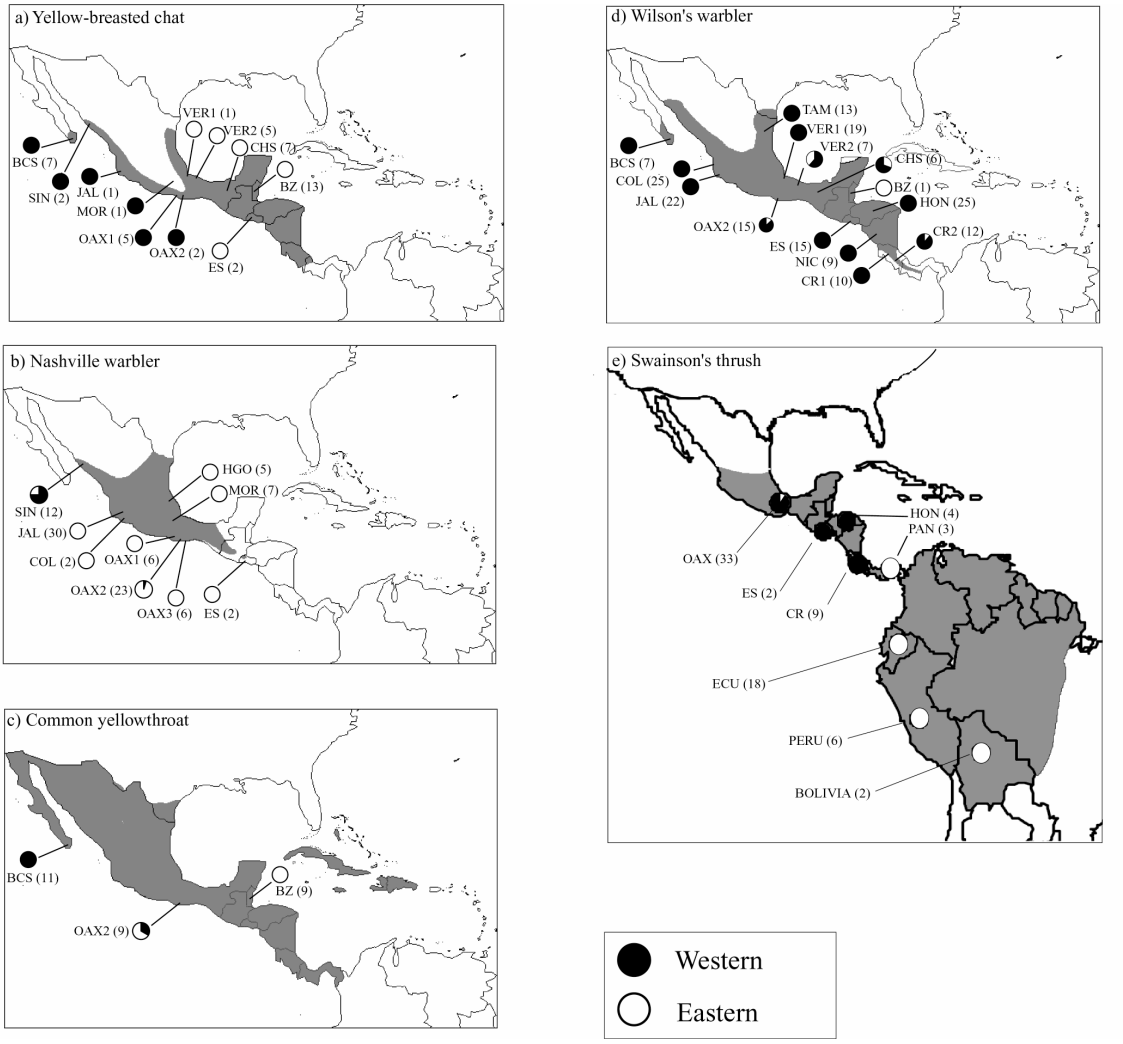


**Figure 2—** Distribution of eastern and western haplotypes in (a) Yellow-breasted Chat, (b) Nashville Warbler, and (c) Common Yellowthroat superimposed on a their overwintering distribution. Western and eastern haplotypes are shown in black and white respectively. Numbers in parentheses indicate sample sizes. Location abbreviations are: BCS, Baja California Sur; BZ, Belize; CHS, Chiapas; COL, Colima; CR (1 &2), Costa Rica; ECU, Ecuador; ES, El Salvador; HGO, Hidalgo; HON, Honduras; JAL, Jalisco; MOR, Morelos; NIC, Nicaragua; OAX (1, 2, and 3), Oaxaca; PAN, Panama; SIN, Sinaloa; TAM, Tamaulipas; VER (1 and 2), Vera Cruz. Modified from Kimura et al. 2002, Ruegg and Smith 2002; and Lovette et al. 2004.