

Phylochronology of an Avian Migrant During Autumn Stopover Appears to Show a Time Signal in Genetic Diversity

Kevin Winker¹, Kyle K. Campbell², Chen Wong²,
Joseph F. Fricilone², and Andrew B. Johnson³

Phylochronology, the temporal and spatial dynamics of how population genetic diversity is distributed, can prove useful for the management and conservation of migratory birds. We studied a series of Swainson's Thrushes (*Catharus ustulatus*) taken during autumn migration in southern Belize to determine a) subspecific affinities, b) whether genetic diversity (mtDNA) is randomly distributed through the migratory period, and c) whether this migratory stopover population is a random mtDNA genetic subsample of breeding populations. We found a statistically significant time signal in the genetic data, and in our mtDNA data set the birds passing through southern Belize are apparently a genetically random sample of the eastern North American breeding population (non-significant population structure comparison). Also, a single western bird by phenotype had an eastern genetic haplotype.

Introduction

How breeding populations of migratory birds distribute themselves during the non-breeding season has been a longstanding and difficult problem for ornithologists, wildlife managers, and conservation biologists (Hagan and Johnson 1992, Greenberg and Marra 2005). Outside of species naturally marked with easily diagnosable subspecific characteristics or game species, for which hunters recover banded birds, determining where individuals from particular populations travel and overwinter is difficult. And although tools such as banding, genetics, stable isotopes, transmitters, and geolocators are making headway, the sheer scale of the problem in the large number of nongame migratory populations and species for which we lack data precludes a simple or rapid solution.

As genetic data sets expand for breeding populations, opportunities to survey how genetic variation is distributed during the non-breeding season also expand, and genetics tools are seeing increasing use in studies of bird migration (Haig et al. 1997, Wink 2006). Most efforts thus far have been focused on the spatial distribution of genetic variation between breeding and wintering ranges, often termed migratory connectivity (e.g., Wenink et al. 1993, Webster et al. 2002, Smith et al. 2005, Irwin et al. 2011). In contrast, relatively few studies have examined the temporal component of migrants while they are in migration (e.g., Wenink & Baker 1996, Tiedemann 1999, Wennerberg 2001, Ruegg & Smith 2002, Lopes et al. 2006, Paxton et al. 2013). In this study we emphasize this temporal aspect and suggest that this type of work be expanded under the rubric of *phylochronology*, the temporal and spatial dynamics of the distribution of population genetic diversity (Hadly et al. 2004).

Stopover sites are important for the many migratory species in which individuals cannot fly the full distance between breeding and wintering grounds without refueling. We examine the migration of Swainson's Thrushes (*Catharus ustulatus*) during autumn migration in southern Belize as they migrate south from their breeding grounds across northern North America to wintering grounds in Central and South America (Mack and Wong 2000; Fig. 1). The stopover ecology of this species has been studied at sites where it is a common migrant (e.g., in southern Minnesota; Winker et al. 1992a,b), but the timing of its movements in relation to genetic diversity has not been examined before. Our goals were to determine a) subspecific affinities, b) whether genetic diversity (mtDNA) is randomly distributed

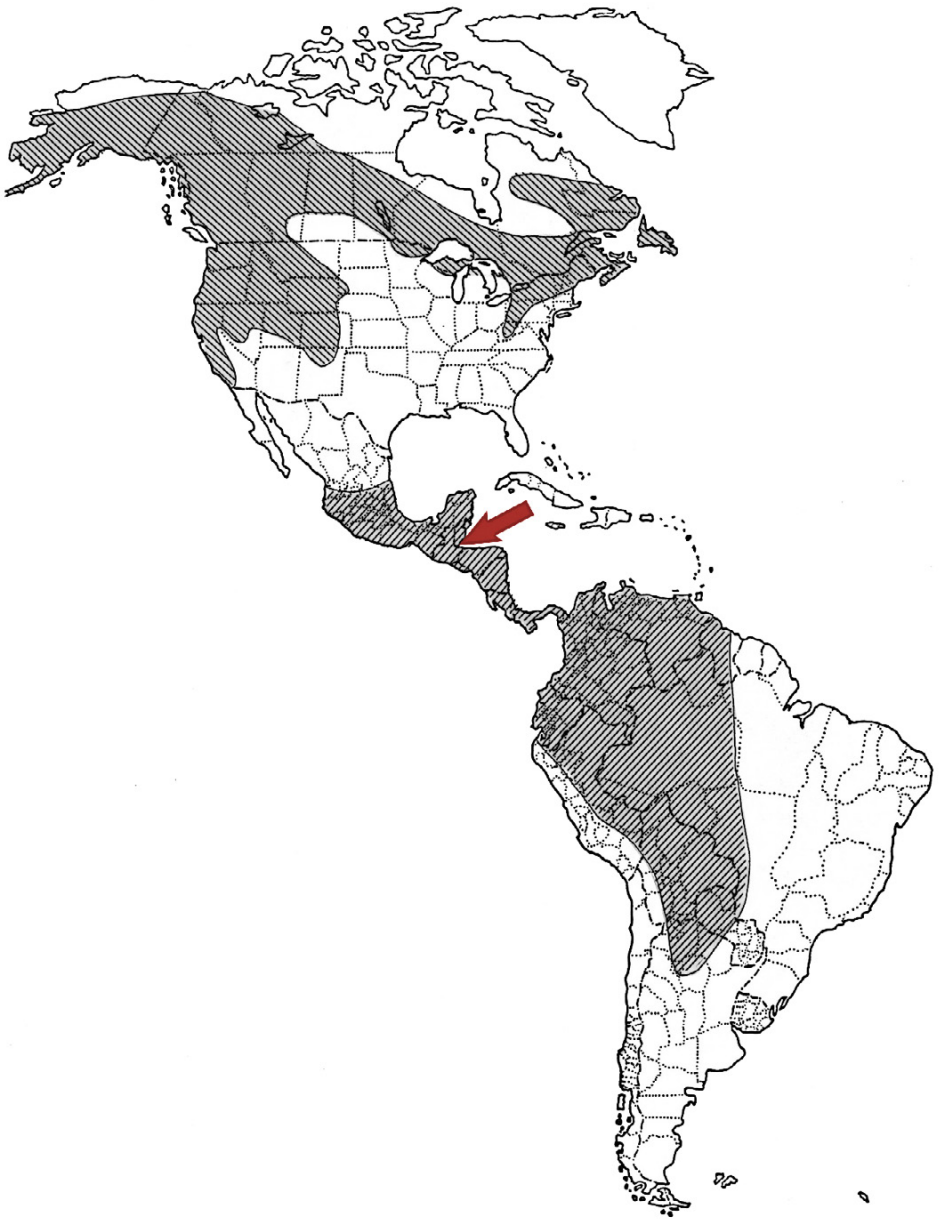


Figure 1. Breeding and wintering distributions of Swainson's Thrush (*Catharus ustulatus*), with an arrow pointing to our Belize study site.

through the migratory period, and c) whether this migratory population is a random genetic subsample of breeding populations.

Methods

During September and October, from 2001 to 2010, we collected 54 Swainson's Thrushes during their autumn migration in Toledo District, southern Belize (Fig. 1). Appropriate permits were obtained for all activities. Differences in the timing of migration between the two subspecies groups are not known, but have not apparently been closely examined (Phillips 1991). Using geolocators on nine birds from near the breeding contact zone in British Columbia, Delmore et al. (2012) found no differences in timing of migration between the two subspecies groups. Large numbers of these thrushes occur in southern Belize as transients during autumn migration, and it is an important stopover refueling site for this species (Johnson & Winker 2008). The dates of our samples for this study spanned 14 Sep to 13 Nov, during which the great majority of individuals of this species occur at this site (unpublished data). Our sampled birds were prepared as museum specimens; skins were examined to identify subspecific group, and tissues were preserved for genetic analyses. Two subspecies groups occur in Swainson's Thrush, the western group of "*ustulatus*" (including *C. u. ustulatus*, *phillipsi*, and *oedicus*; the Russet-backed Thrush of Phillips 1991), and the largely eastern and continental interior group of "*swainsoni*" (including *C. u. swainsoni*, *appalachiensis*, and *incanus* (the Olive-backed Thrush of Phillips 1991, its breeding range spanning from Alaska to Minnesota and Newfoundland). We determined phenotypic affinities to subspecific group (i.e., "*ustulatus*" versus "*swainsoni*") and not to particular subspecies within those groups. Not only are differences within the major groups rather subtle, but Phillips (1991:xliv) cautioned that, due to plumage color changes in museum specimens over time in this genus, "subspecific identifications of *Catharus* spp. (especially if recently taken) are not to be relied on." In short, we can confidently assign specimens to major subspecific group, but not to less distinct subspecies within those two groups. Both subspecies groups occur in Belize (pers. obs., Phillips 1991). We

identified Belize specimens to subspecies group by comparing them to known breeding season specimens.

DNA was extracted from frozen muscle tissue using a DNeasy Tissue Kit (Qiagen, Valencia, CA, USA). Ruegg and Smith (2002) surveyed the genetic diversity of the breeding grounds, using mtDNA control region sequence, and they identified two reciprocally monophyletic haplotype groups corresponding to western coastal and inland (eastern or continental) breeding-ground clades. Using this dataset as our base of comparison for breeding population genetic variation and its distribution, we amplified the mtDNA control region NADH dehydrogenase subunit 1 gene (582 bp) using a species-specific primer (L1TH 5' TGT'TTTCTCATGCTTTACAGGG 3') in combination with primers L437 and H1248 (Tarr 1995, Ruegg & Smith 2002). Amplification was done following standard PCR protocols. Sequencing was performed at the University of Washington, Biochemistry DNA Sequencing Facility (Seattle, Washington). We aligned the sequences by eye without gaps using Sequencher (Gene Codes Corporation Inc., USA). Completed sequences were visualized with a haplotype network (Network 4.6.1.0, Bandelt et al. 1999, Librado and Rozas 2009) that included sequences from breeding populations ($N = 105$, Ruegg and Smith 2002). Julian dates were assigned to each individual taken in migration in Belize. A one-way analysis of variance (Kirkman 1996) was performed for haplotypes that occurred three or more times in the dataset, with the independent variable being haplotype and the dependent variable being Julian date. Finally, we tested whether the Belize birds' mtDNA differed significantly from the eastern breeding population's by calculating F_{ST} between the two populations and determining its significance using Arlequin ver. 3.5 set at 10,000 permutations and using conventional F statistics (Excoffier et al. 2005).

Results and Discussion

All but one of the specimens had plumage characteristics of the eastern subspecies group, *C. u. "swainsoni"* (sensu lato). A higher frequency of "*swainsoni*" was expected in autumn at this site given synthesized accounts of migration in Phillips (1991). The

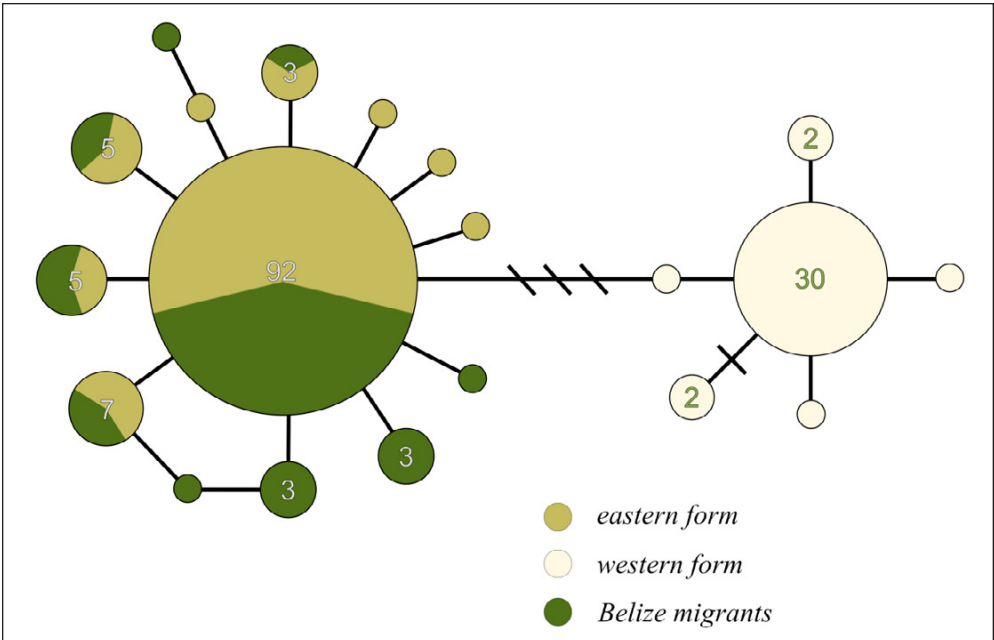


Figure 2. Haplotype network of 582 bp of mtDNA control region sequence. Dark green instances represent birds in our study, whereas the paler colors are from the breeding range data of Ruegg and Smith (2002).

remaining bird (UAM 27813) had a western, “*ustulatus*” subspecies phenotype. MtDNA sequence data revealed nine haplotypes (Appendix, haplotypes A-I), and all were from the eastern (or continental) clade of Ruegg and Smith (2002; Fig. 2). The western bird (by plumage) thus had a phenotype-genotype mismatch; gene flow is known to occur at the contact zone in western North America (Ruegg 2008).

Four haplotypes occurred more than three times in the dataset (Appendix; haplotypes A, B, E, and G). The average Julian dates associated with the passage of these haplotypes differed significantly among them (ANOVA, $F_{3,44} = 3.11, P = 0.031$), indicating a statistically significant time signal in the passage of genetic diversity through this site among years (Fig. 3). Our sample sizes do not allow us to test whether this is a true seasonal phenomenon (i.e., a pattern occurring every year), or whether it is driven by variation in timing of migration among years (e.g., if one haplotype occurred earlier or later one year). Nevertheless, a significant among-year pattern

exists, and among-year variation is an issue with most if not all studies of this type.

Genetic correlations with time have been found in migratory birds before, at stopover sites in Dunlin (*Calidris alpina*) within and between seasons (Tiedemann 1999, Lopes et al. 2006).

Our migratory non-breeding population might represent a distinctive subset of populations from the breeding range of the eastern group. But Belizean birds shared haplotypes with all eight of the eastern/continental breeding populations sampled for sequencing by Ruegg and Smith (2002), and we found no significant difference between these samples ($F_{ST} = 0.00, P = 0.48$). This indicates that the birds passing through southern Belize are effectively a random mtDNA genetic sample of the eastern or continental breeding populations.

What does it mean to have an effectively random mtDNA genetic sampling of populations but with a seemingly nonrandom distribution in time? There are two possibilities, and they are not mutually exclusive: 1) these

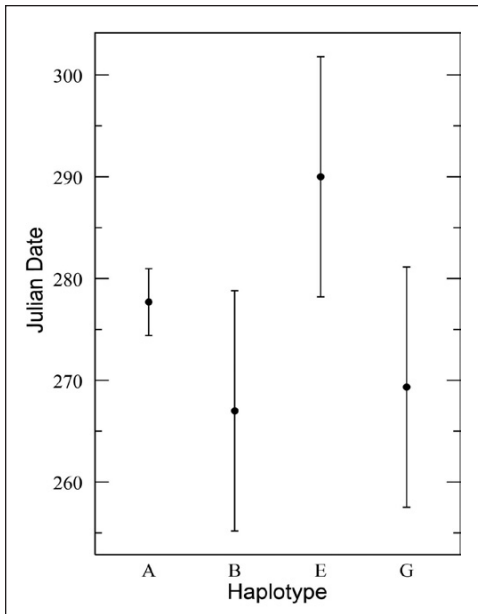


Figure 3. Temporal distribution (mean, 95% CI) by Julian date of the four haplotypes that occurred three or more times in the Belize mtDNA dataset.

may be neutral genetic traits correlated with populations that differ in the timing of their movements through southern Belize; and/or 2) they may be correlated with metabolic efficiencies in flight energetics, as shown by Scott et al. (2011) and Toews et al. (2013) in other bird species. At present, we cannot distinguish between these two possibilities, and clearly much more work will be needed to understand the nonbreeding movements of this species. While we often treat variation in mtDNA as neutral, it is a single, maternally inherited linkage group under strong selection because the mitochondrion is the powerhouse of animal cells. Denser sampling in space, time, and genomics is warranted.

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¹ **Corresponding Author: Kevin Winker, University of Alaska Museum, 907 Yukon Drive, Fairbanks, AK 99775;**

² **University of Alaska Museum, 907 Yukon Drive, Fairbanks, AK 99775;**

³ **Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM 87131.**