The Condor 113(3):608–618
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WINTER DISTRIBUTION OF WILLOW FLYCATCHER SUBSPECIES

Eben H. Paxton[1,2,6], Philip Unitt[3], Mark K. Sogge[4], Mary Whitfield[5], and Paul Keim[1]

[1]*Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011*
[2]*U.S. Geological Survey, Southwest Biological Science Center, Colorado Plateau Research Station, Box 5614, Northern Arizona University, Flagstaff, AZ 86011*
[3]*San Diego Natural History Museum, P. O. Box 121390, San Diego, CA 92112*
[4]*U.S. Geological Survey, Flagstaff, AZ 86001*
[5]*Southern Sierra Research Station, Weldon, CA 93283*

Abstract. Documenting how different regions across a species' breeding and nonbreeding range are linked via migratory movements is the first step in understanding how events in one region can influence events in others and is critical to identifying conservation threats throughout a migratory animal's annual cycle. We combined two studies that evaluated migratory connectivity in the Willow Flycatcher (*Empidonax traillii*), one using mitochondrial DNA sequences from 172 flycatchers sampled throughout their winter range, and another which examined morphological characteristics of 68 museum specimens collected in the winter range. Our results indicate that the four subspecies occupy distinct but overlapping regions of the winter range. Connectivity between specific breeding and winter grounds appears to be moderate to strong, with distributions that suggest migration patterns of both the chain and leap-frog types connecting the breeding and nonbreeding grounds. The Pacific lowlands of Costa Rica appear to be a key winter location for the endangered Southwestern Willow Flycatcher (*E. t. extimus*), although other countries in Central America may also be important for the subspecies.

Key words: Empidonax traillii extimus, *migratory connectivity, mixed-stock analysis, Southwestern Willow Flycatcher, winter* distribution.

Distribución Invernal de las Subespecies de *Empidonax traillii*

Resumen. Documentar cómo diferentes regiones que hacen parte de la distribución reproductiva y no reproductiva de una especie están ligadas mediante movimientos migratorios es el primer paso para entender cómo los eventos sucedidos en una región pueden afectar eventos en otras. Además, es crítico para identificar amenazas de conservación a lo largo del ciclo anual de los animales migratorios. Combinamos dos estudios que evaluaron la conectividad migratoria en *Empidonax traillii*, uno que empleó secuencias de ADN mitocondrial de 172 individuos muestrados a lo largo de su distribución invernal y otro que examinó rasgos morfológicos de 68 especímenes de museo coleccionados en la distribución invernal. Nuestros resultados indican que las cuatro subespecies ocupan regiones diferentes, pero superpuestas, de la distribución invernal. La conectividad entre áreas de cría y de invernada específicas parece ser moderada a fuerte, y las distribuciones sugieren patrones de migración entre las áreas reproductivas y no reproductivas de tipo cadena y de tipo salto de rana. Las tierras bajas del Pacífico de Costa Rica parecen ser una localidad de invernada clave para la subespecie amenazada *E. t. extimus*, aunque otros países centroamericanos también podrían ser importantes para esta subespecie.

INTRODUCTION

Understanding how different regions are linked via migratory behavior, termed connectivity, is important for understanding a migratory bird's ecology and population dynamics through its annual cycle (Webster et al. 2002). There is growing evidence that the three periods of a migratory bird's annual cycle (breeding, wintering, and migration) are linked to one another in terms of carryover effects in productivity, dispersal, and survivorship (Marra et al. 1998, Smith et al. 2003, Moore et al. 2005, Studds et al. 2008). For example, the quality of winter habitat may have repercussions for a bird's fitness in the subsequent breeding season (Norris et al. 2004), as individuals wintering in high-quality locations may be in better condition and therefore migrate faster and arrive at the breeding grounds earlier than do conspecifics wintering in poor habitat. Where a breeding population winters, and the strength of the connectivity linking specific subsets of the breeding and winter ranges, also influences migration distance, migration routes, and potentially migration strategies (Clegg et al. 2003, Smith et al. 2005, Kelly et al. 2005, Paxton et al. 2007a). For species of conservation concern, knowing where populations

Manuscript received 17 October 2009; accepted 31 January 2011.

[6]Current address: U.S. Geological Survey, Pacific Island Ecosystems Research Center, P. O. Box 44, Hawaii National Park, HI 96718. E-mail: eben_paxton@usgs.gov

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occur throughout the annual cycle is crucial for assessing threats and targeting conservation efforts at those areas critical to the species' long-term viability (Bairlein 2003, Baker et al. 2004).

For most migratory birds, details of the connectivity between the breeding range, winter range, and migratory stopovers sites are unknown. Some of the earliest information on connectivity came from studies of museum specimens describing the distribution of distinct morphological types (e.g., subspecies) on the nonbreeding grounds (e.g., Swarth 1920, Marshall 1988) and studies of waterfowl, for which the relatively large rates of recovery of banded birds provided information linking different geographic regions (Diefenbach et al. 1988, Hepp and Hines 1991). Recently, satellite transmitters have allowed large birds to be tracked in real time, but for most species sample sizes are still small (Kanai et al. 2002, Higuchi and Pierre 2005). Direct tracking of most small birds is not yet possible (Bairlein 2003, Wikelski et al. 2007, but see Stutchbury et al. 2009). However, intrinsic markers such as morphological characteristics, genetic markers, and stable isotopes can be used to link individuals to particular populations and regions, providing a method for establishing connectivity between breeding, wintering, and migration sites (Webster et al. 2002, Hobson 2005, Smith et al. 2005, Paxton et al. 2007a).

Our objectives in this study were to use intrinsic markers to estimate the distribution of the subspecies of the Willow Flycatcher (*Empidonax traillii*) on their winter grounds and gain insight into the strength of migratory connectivity. The Willow Flycatcher is a long-distance migrant breeding across much of the contiguous United States and southern Canada and wintering from the Pacific coast of Sinaloa, Mexico, south to Colombia, Ecuador, and Venezuela (Sedgwick 2000; Fig. 1). Four subspecies of the Willow Flycatcher are recognized (Fig. 1): *E. t. adastus*, breeding in the Great Basin and central Rocky Mountains, *E. t. brewsteri*, breeding in the Pacific coastal region north of southern California, *E. t. extimus*, breeding in the southwest portion of the United States, and *E. t. traillii*, breeding east of the Rocky Mountains (Phillips 1948, Aldrich 1951, Unitt 1987). The Southwestern Willow Flycatcher (*E. t. extimus*) was declared an endangered species in 1995 (USFWS 1995), and identifying locations where it winters is considered a top priority for long-term conservation (USFWS 2002). One study to date has addressed the issue with stable isotopes (carbon, nitrogen, and hydrogen) collected in the feathers of Willow Flycatchers, which molt in their winter range (Kelly et al. 2008). However, a lack of strong geographic patterns in hydrogen-isotope values in Central and South America and high variability in all three isotopes resulted in difficulties in predicting the winter location of breeding flycatchers on the basis of analysis of their feathers, calling for other methods.

We combined two studies, one using molecular genetic markers sampled from wintering populations, 1997–2007, and another evaluating the morphology and plumage coloration of museum specimens collected in the species' winter range from 1882 to 1995. The subspecies are defined on the basis of subtle differences in color, pattern, and proportions (Unitt 1987, Browning 1993), but previous molecular genetic studies of the Willow Flycatcher documented strong differences among the subspecies in the frequency of mitochondrial DNA (mtDNA) haplotypes (average F_{ST} among subspecies = 0.15; Paxton 2000), which can be exploited to link individuals in nonbreeding areas to their breeding grounds. This study is the first step in assessing connectivity between the breeding and winter ranges and ultimately linking different regions for a better understanding of the life history of this species throughout its annual cycle. In addition, identifying locations where the endangered subspecies winters will allow for conservation measures in the winter range to be focused where they will be most effective.

METHODS

MOLECULAR GENETIC STUDY

From 1997 to 2007, Willow Flycatchers were sampled across their winter range as part of a demographic study (Koronkiewicz et al. 2006) and surveys of the winter range from Sinaloa, Mexico, to Ecuador (Lynn et al. 2003, Nishida and Whitfield 2006, Schuetz et al. 2007). On both the breeding and winter grounds Willow Flycatchers respond aggressively to playback of their song (Sogge et al. 2001, 2007), and we used tape-playback methods to survey for flycatchers, ensuring species identification through diagnostic vocalizations to avoid misidentification of similar *Empidonax* spp. When time and resources permitted, attempts to capture (Sogge et al. 2001) and sample DNA were made in those locations where Willow Flycatchers were detected. All wintering flycatchers were sampled from December to February, outside the period of migration.

Upon capture, flycatchers were banded so individuals could be identified, and a drop of blood was collected for DNA analysis. Blood was washed into a microcentrifuge tube containing a buffer solution and stored in a cooler until it could be frozen. DNA was extracted via standard techniques outlined in Busch et al. (2000). This study used 1063 nucleotides of the cytochrome *b* gene that begin 80 bases downstream from the start codon of the gene. All sequences for this region were confirmed on both strands. Using the polymerase chain reaction (PCR), we sequenced DNA extracts directly with primers obtained from Helm-Bychowski and Cracraft (1993; L14827: 5' CCACACTCCACACA GGCCTAATTAA 3', H16065: 5' GGAGTCTTCAGTCTCTGGTTTACAAGAC 3'). PCRs were carried out with 50 ng of DNA, 1× PCR buffer, 3 μM $MgCl_2$,

FIGURE 1. Locations of Willow Flycatchers sampled for molecular genetic information on the breeding and winter grounds (circles) and locations of museum specimens identified to subspecies on the winter range (squares). Gray lines, outlines of subspecies' breeding ranges; shading, the Willow Flycatcher's known winter range.

200 μM deoxynucleotide triphosphates (dNTPs), 1 μM of each primer, and 1 unit of *Taq* DNA polymerase. Conditions of cycling were as follows: 35 cycles of 30 sec at 94 °C, 30 sec at 55 °C, and 2 min at 72 °C. PCR products were concentrated with a QIAquick PCR purification kit (Qiagen), then sequenced on an ABI 377 DNA sequencer. We aligned the sequences manually and edited them with Sequence Navigator version 1.0.1 (Applied Biosystems). All unique cytochrome *b* sequences were deposited in GenBank (accession numbers AF297237–AF297276, GU207885–GU207935).

MUSEUM SPECIMEN STUDY

Specimens of the morphologically similar Willow and Alder (*E. alnorum*) Flycatchers collected across their nonbreeding range in Mexico, Central, and South America were requested from 22 museums (see acknowledgments); the request yielded 670 specimens. Of these, 232 were in molt or defective in some way and missing one or more of the morphological characteristics of interest, and 103 were identified as juveniles by buffy wing bars and were excluded. Finally, we chose to only consider those individuals collected outside the Willow

TABLE 1. Mitochondrial DNA haplotypes of the Willow Flycatcher grouped by subspecies and region of the winter range. For those haplotypes detected on both the breeding and winter grounds, the frequency (and number) of each haplotype is shown, along with column totals for numbers of individuals, sites, and haplotypes analyzed.

Haplotype	*n*	*E. t. adastus*	*E. t. brewsteri*	*E. t. extimus*	*E. t. traillii*	N. Mexico	S. Mexico	El Salvador	Costa Rica	Panama	Ecuador
A1	14	0.36 (4)	0	0.09 (1)	0.55 (6)	0	0	0	0.67 (2)	0	0.33 (1)
A2	12	0.30 (3)	0.10 (1)	0.30 (3)	0.30 (3)	0	0	0	0	0.50 (1)	0.50 (1)
A4	3	0	0	0	1.0 (2)	0	0	0	0	0	1.0 (1)
A5	6	0.33 (1)	0.33 (1)	0.33 (1)	0	0	0	0	1.0 (3)	0	0
A6	2	0	1.0 (1)	0	0	0	1.0 (1)	0	0	0	0
A8	6	0.33 (1)	0	0.33 (1)	0.33 (1)	0	0	0	1.0 (3)	0	0
A11	2	1.0 (1)	0	0	0	0	0	0	1.0 (1)	0	0
B1	72	0.80 (32)	0.18 (7)	0.03 (1)	0	0.31 (10)	0.22 (7)	0.13 (4)	0.25 (8)	0.09 (3)	0
B2	11	0.40 (2)	0	0.60 (3)	0	0.33 (2)	0.33 (2)	0.17 (1)	0.17 (1)	0	0
B3	4	0	1.0 (2)	0	0	0	0	0.50 (1)	0.50 (1)	0	0
B12	2	1.0 (1)	0	0	0	0	0	0	1.0 (1)	0	0
B13	2	1.0 (1)	0	0	0	0	0	0	1.0 (1)	0	0
B17	2	0	1.0 (1)	0	0	0	0	0	1.0 (1)	0	0
C1	72	0.15 (10)	0	0.85 (55)	0	0.14 (1)	0	0	0.86 (6)	0	0
C4	5	0.25 (1)	0	0.75 (3)	0	0	1.0 (1)	0	0	0	0
C6	2	1.0 (1)	0	0	0	0	0	0	1.0 (1)	0	0
D1	150	0.40 (35)	0.23 (20)	0.15 (13)	0.23 (20)	0.13 (8)	0.13 (8)	0.06 (4)	0.48 (30)	0.10 (6)	0.10 (6)
D4	5	0	0	0	1.0 (4)	0	0	0	0	1.0 (1)	0
D6	4	0.67 (2)	0.33 (1)	0	0	0	0	0	1.0 (1)	0	0
D7	5	1.0 (4)	0	0	0	0	0	0	1.0 (1)	0	0
D11	2	0	1.0 (1)	0	0	0	0	0	1.0 (1)	0	0
D15	3	1.0 (1)	0	0	0	0	0	0	1.0 (2)	0	0
D19	4	0	0	0	1.0 (2)	0	0	0	0	0	1.0 (2)
E1	5	1.0 (4)	0	0	0	0	0	0	1.0 (1)	0	0
Number individuals		104	35	81	38	21	19	10	65	11	11
Number sites		39	15	21	16	10	8	5	7	4	9
Number haplotypes		17	9	9	7	4	5	4	18	4	5

Flycatcher's period of probable migration, 15 September–15 April, resulting in a total of 96 Willow and Alder Flycatchers from Middle and South America.

We developed models to predict the winter distribution of the subspecies on the basis of characteristics of museum specimens from the breeding range of the Willow and Alder Flycatchers. A total of 146 individuals from 7 museums (see acknowledgments) collected from 1 May to 1 August were considered, consisting of 17 specimens of *E. t. adastus*, 36 of *E. t. brewsteri*, 21 of *E. t. extimus*, 27 of *E. t. traillii*, and 45 of *E. alnorum*. Using morphological traits found to be valuable in distinguishing among the subspecies (Unitt 1987), we measured bill length from nostril, tail length from insertion of central rectrices, wing chord, wing chord divided by tail length, and four variables expressing the shape of the wing: the difference between the longest primary (9, often equaled by 7 and 8) and primary (p) 10, the difference between p9 and p5, the difference between p9 and p6, and (p9 – p10)/(p9 – p5). The color of the back and crown was quantified with a Minolta CR-300 colorimeter. The colorimeter provides three values for each location, *L* (light to dark), *a* (green to red), and *b* (blue to yellow), resulting in six values per specimen. Each specimen had a total of 24 measurements taken per location, which were averaged into a single number per color value. All measurements were taken by P. Unitt.

STATISTICAL ANALYSIS

MOLECULAR GENETIC STUDY

We grouped wintering Willow Flycatchers into one of six regions: northern Mexico (Sinaloa south to Acapulco, 17° N), southern Mexico, El Salvador, Costa Rica, Panama, and Ecuador (Table 1). The cytochrome *b* sequences from flycatchers sampled on their winter grounds were compared to sequences from 316 individuals sampled at 91 sites on the breeding grounds, 1996 to 2004. We assigned individuals sampled on the breeding grounds a priori to one of the four subspecies on the basis of location of capture and published distributions of the subspecies (see Unitt 1987).

We used a mixed-stock analysis to estimate the winter distribution of the subspecies on the basis of shared mtDNA haplotypes. A mixed-stock analysis estimates the contribution of each subspecies to each subset of the winter range by comparing the distributions of mtDNA haplotypes encountered in different groups (subspecies) on the breeding grounds and in the sections of the winter range (Fournier et al. 1984). Mixed-stock analysis is widely used in fisheries (Okuyama and Bolker 2005) and occasionally with birds (Pearce et al. 2000). Mixed-stock analysis is well suited for evaluating mtDNA patterns because the analysis, unlike assignment tests, does not require multi-locus markers (Manel et al. 2005), providing probability-based estimates of contribution by incorporating information from private alleles, haplotypes that occur in only one subspecies, and frequency differences. The greater the genetic differences among groups, the stronger the inference, but estimates based on mixed-stock analysis are typically associated with large confidence intervals (Reynolds and Templin 2004). In this study, we used a constrained maximum-likelihood approach with the mixed-stock package in program R (www.r-project.org).

MUSEUM SPECIMEN STUDY

We used a canonical discriminant analysis to create a predictive model to assign specimens of unknown origin collected on the winter grounds to subspecies. Prior to constructing the discriminant function, we adjusted most of the variables to account for sexual dimorphism and changes in plumage coloration due to age of the specimen. The Willow Flycatcher is sexually dimorphic, with males on average larger than females. For those morphological characteristics that varied by sex, we subtracted (or added) the average difference between males and females from the males' measurements as follows: wing, –4.27; (p9 – p6), –1.07; (p9 – p5), –1.47; (p9 – p10)/(p9 – p5), 0.22; tail, –2.56. Additionally, the colors of many bird specimens shift from a darker, grayer color to a paler, redder one with years in a museum collection, a change termed foxing (Doucet and Hill 2009). In the Willow Flycatcher, foxing shifts primarily the values of *a*, but also values of *L* and *b* to a lesser extent. We used an analysis of covariance, controlling for differences among the subspecies, to evaluate how each of the color values changed as a function of year (1882 to 1995). We found significant differences in *L* and *a* of both the head and back (the shifts appear linear and constant over the ages of the specimens), and we adjusted these values for each specimen as a function of the year collected (*L*_crown year = –0.0068, *a*_crown year = –0.0083, *L*_back year = –0.011, *a*_back year = –0.0091). While solar radiation and abrasion can cause plumage coloration to change over time (Paxton et al. 2010), we did not address seasonal fading in this study because of uncertainty on how fading progresses through the nonbreeding season. However, seasonal fading is of greatest concern with adults in fall migration, which are excluded from this analysis, as Willow Flycatchers initiate molt when they arrive on their winter grounds.

We used a two-step approach to identify the winter specimens to subspecies. First, we constructed a canonical discriminant function model to distinguish Willow Flycatchers from the morphologically similar Alder Flycatcher. The model was applied to the specimens from the winter range, and those individuals predicted as Willow Flycatchers with ≥90% probability were analyzed further. Then we developed a second discriminant function model that considered just Willow Flycatchers to assign specimens to one of the four subspecies. Although distinguishing between the eastern (*E. t. traillii*) and western subspecies is not possible on the basis of color alone (Paxton et al. 2010), the addition of measurements allows for discrimination between the two groups. All analyses of the museum specimens were run in JMP 8.0 (SAS, Inc.).

RESULTS

For the molecular genetic study, we obtained cytochrome *b* sequences from 172 wintering flycatchers from 43 sites across five countries, detecting a total of 54 haplotypes (Table 1). Of these 54 haplotypes, 24 (44%) matched the 62 haplotypes sequenced from 316 individuals sampled on the breeding grounds. While over half of the haplotypes detected in the breeding range were not detected in the winter range, all common (high-frequency) haplotypes were detected in both regions. For example, 87% of the haplotypes found in the breeding range but not in the winter range were low-frequency haplotypes (≤2 individuals), and all haplotypes found in >4 individuals in the breeding range were also detected in the winter range. Likewise, of the 54 haplotypes detected in the winter range, 30 (56%) did not match haplotypes detected in the breeding range, but each of these 30 occurred in only 3 or fewer individuals. The resulting 24 haplotypes detected in both the breeding and winter ranges provided a dataset of 137 individuals from the winter grounds (80% of individuals sampled) and 274 individuals from the breeding grounds (87% of individuals sampled) that were used for the mixed-stock analysis.

The mixed-stock analysis estimated *E. t. adastus* as the dominant subspecies in northern Mexico, continuing south to Costa Rica in decreasing frequency but with a spike in occurrence at its southernmost estimated winter location, Costa Rica. On the opposite end of the winter range, *E. t. traillii* was the dominant subspecies in Ecuador, extending north into Panama at a lower frequency, and was not estimated to winter in regions farther north. *Empidonax t. brewsteri* had an estimated winter range from southern Mexico to Panama, with its highest frequency in the southern portion of this range, and *E. t. extimus* was estimated to occur primarily in Costa Rica. The width of the confidence intervals around the estimates from the mixed-stock analysis (Table 2) implies considerable uncertainty regarding the exact ratios of subspecies at particular locations, but the geographic location of highly informative haplotypes illustrates the general pattern derived from the mixed-stock analysis. For example, *E. t. traillii* had three private alleles that were detected on the winter grounds only in Ecuador ($n = 2$) and Panama ($n = 1$), while alleles unique to *E. t. brewsteri* were found in southern Mexico ($n = 1$), El Salvador ($n = 1$), and Costa Rica ($n = 3$). All private haplotypes from *E. t. adastus* were found in Costa Rica ($n = 7$), but one haplotype that occurs in high frequency within its breeding range (B1) was found at its highest frequency in northern Mexico, and then in decreasing frequency toward the south (Table 1). No private haplotypes of *E. t. extimus* were detected on the winter grounds; however, one haplotype strongly associated with the endangered subspecies (C1, with 97% of all detections within its range or in its zone of intergradation with *E. t. adastus*, and 56% of all examples of *E. t. extimus* sampled possessing the haplotype; Paxton et al. 2008) was detected in six individuals in Costa Rica and one individual in southern Mexico.

TABLE 2. Results of mixed-stock analysis of the contribution of the four subspecies of the Willow Flycatcher to six regions of the winter range and the number of males and females sampled within each region. Each point estimate is an unconstrained maximum-likelihood estimate of the contribution of each subspecies to each region, with bootstrapped 95% confidence estimates indicating uncertainty around the estimates.

	Subspecies contribution (95% CI)			
Region	*E. t. adastus*	*E. t. brewsteri*	*E. t. extimus*	*E. t. traillii*
N. Mexico	1.0	0.0	0.0	0.0
	(0.00, 0.99)	(0.00, 0.89)	(0.00, 0.27)	(0.00, 0.00)
S. Mexico	0.65	0.35	0.0	0.0
	(0.00, 0.99)	(0.00, 0.94)	(0.00, 0.33)	(0.00, 0.00)
El Salvador	0.46	0.54	0.0	0.0
	(0.00, 0.99)	(0.00, 0.99)	(0.00, 0.26)	(0.00, 0.00)
Costa Rica	0.62	0.32	0.06	0.0
	(0.30, 0.85)	(0.07, 0.54)	(0.00, 0.24)	(0.00, 0.25)
Panama	0.0	0.71	0.0	0.29
	(0.00, 0.87)	(0.00, 0.99)	(0.00, 0.00)	(0.00, 0.85)
Ecuador	0.0	0.0	0.0	1.0
	(0.00, 0.00)	(0.00, 0.35)	(0.00, 0.00)	(0.63, 0.99)

The discriminant function combining 14 measures of plumage coloration and morphology (Table 3) allowed us to distinguish the specimens of the Willow and Alder Flycatchers from the breeding range with an estimated 99% accuracy. Application of this species-level model to the winter specimens ($n = 96$) yielded 69 wintering Willow Flycatchers (those predicted as ≥90% probability of being Willow Flycatchers; 9 specimens with probabilities between 50% and 90% excluded). The second discriminant model for distinguishing the four subspecies of the Willow Flycatcher, based on 92 breeding adults, had a 15% misclassification rate overall but a 2% misclassification rate at the 90% confidence threshold (31 specimens with probabilities between 50% and 90% excluded) (Fig. 2). Of the 69 winter specimens, 34 were predicted with ≥90% confidence: 2 as *E. t. adastus*, 10 as *E. t. brewsteri*, 5 as *E. t. extimus*, and 17 as *E. t. traillii* (Table 4). Of the five specimens identified as *E. t. extimus*, 3 were collected in Costa Rica, 1 in El Salvador, and 1 in Guatemala.

DISCUSSION

The results from both analyses indicate that the four subspecies occupy distinct but overlapping subsets of the winter range (Table 2, Fig. 3). Both agree that the eastern subspecies, *E. t. traillii*, occupies the southernmost portion of the winter range, being the dominant subspecies in South America and

TABLE 3. Mean and 95% CI of the 14 characteristics of the Willow Flycatcher measured from museum specimens from the breeding range and used to build the predictive model for the winter specimens of unknown origin.

Measurement	*E. t. adastus*	*E. t. brewsteri*	*E. t. extimus*	*E. t. traillii*
Sample size	17	36	21	27
Wing chord	69.5 (68.2, 70.8)	66.4 (65.2, 67.7)	65.9 (64.4, 67.5)	70.0 (68.8, 71.2)
Tail	59.4 (58.4, 60.5)	57.3 (56.3, 58.3)	57.0 (55.7, 58.2)	56.6 (55.6, 57.5)
Wing/tail ratio	1.17 (1.16, 1.18)	1.16 (1.15, 1.17)	1.16 (1.14, 1.17)	1.24 (1.23, 1.25)
Bill	9.1 (8.9, 9.3)	9.2 (9.0, 9.4)	9.3 (9.0, 9.5)	9.0 (8.8, 9.2)
p9 – p10	9.1 (8.6, 9.6)	8.6 (8.2, 9.1)	8.6 (8.0, 9.2)	8.1 (7.6, 8.5)
p9 – p6	4.1 (3.7, 4.5)	3.5 (3.1, 3.9)	3.0 (2.5, 3.5)	4.2 (3.8, 4.6)
p9 – p5	8.2 (7.8, 8.7)	7.3 (6.8, 7.7)	6.4 (5.8, 6.9)	8.2 (7.8, 8.6)
(p9 – p10)/(p9 – p5)	1.12 (1.03, 1.21)	1.20 (1.11, 1.29)	1.37 (1.27, 1.48)	0.99 (0.91, 1.08)
L crown	28.1 (27.4, 28.7)	26.9 (26.3, 27.5)	29.9 (29.1, 30.7)	28.9 (28.3, 29.5)
a crown	2.2 (2.0, 2.3)	2.0 (1.9, 2.2)	2.0 (1.8, 2.2)	1.8 (1.7, 2.0)
b crown	9.9 (9.6, 10.3)	10.0 (9.7, 10.4)	10.8 (10.4, 11.3)	10.4 (10.1, 10.8)
L back	33.8 (33.3, 34.4)	32.3 (31.8, 32.8)	35.4 (34.7, 36.0)	34.9 (34.4, 35.4)
a back	1.1 (0.9, 1.3)	1.3 (1.1, 1.5)	1.2 (0.9, 1.4)	0.8 (0.6, 0.9)
b back	13.0 (12.4, 13.5)	13.8 (13.3, 14.4)	14.8 (14.2, 15.5)	13.4 (12.9, 13.9)

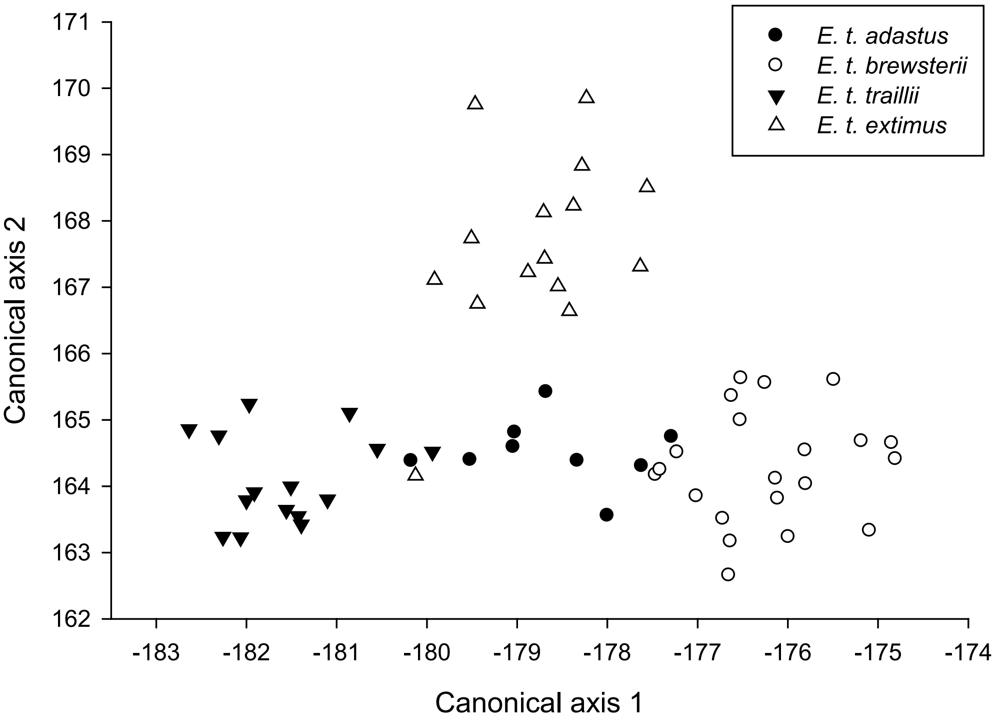

FIGURE 2. Canonical discriminant function analysis of 14 morphological characteristics showing the relative relationship in ordination space of individual Willow Flycatchers collected from across the breeding ranges of the four subspecies. Only those individuals predicted to one of the four subspecies with >90% confidence are shown; in this case, one individual, from the breeding range of *E. t. extimus*, was incorrectly classified.

TABLE 4. Frequency of Willow Flycatcher subspecies (≥90% probability threshold) predicted on the basis of morphological features from museum specimens collected in the winter range.

Region	*n*	*E. t. adastus*	*E. t. brewsteri*	*E. t. extimus*	*E. t. traillii*
Mexico	7	0	7 (100%)	0	0
Northern Central America	6	1 (17%)	3 (50%)	2 (33%)	0
Southern Central America	9	1 (11%)	0	3 (33%)	5 (56%)
South America	12	0	0	0	12 (100%)
Total	34	2	10	5	17

extending northward into Central America in decreasing frequency and not detected in the northernmost portion of the winter range. The winter distributions of the subspecies of the Great Basin and central Rocky Mountains, *E. t. adastus*, and of the northern Pacific slope, *E. t. brewsteri*, estimated by the two approaches differ somewhat, with the molecular genetic study suggesting *E. t. adastus* as the dominant subspecies at the northernmost portion of the winter range, while the evidence from the museum specimens points to *E. t. brewsteri* as the dominant subspecies in Mexico. However, distinguishing between these two subspecies via molecular genetic and morphological characteristics can be difficult (Unitt 1987, Paxton 2000), and both studies agree that the two subspecies combined are dominant in the northern and central parts of the winter range. The Southwestern Willow Flycatcher, *E. t. extimus*, appears to winter primarily in the center of the winter range, with the molecular genetic study estimating Costa Rica as the primary location among those countries sampled and the museum specimens suggesting a broader distribution across Central America but likewise with a focus on Costa Rica (3/5 winter specimens of *E. t. extimus*). Although sample sizes were generally small, variance was high, and the accuracy of the predictive models was unknown, the agreement of the two independent studies strengthens conclusions generalized from both, and the geographic distribution of the subspecies suggests a nonrandom, biologically driven pattern of distribution. Furthermore, given that both studies sampled much of the winter range and the genetic study detected all of the haplotypes common on the breeding grounds, the results suggest that a representative sample of the subspecies on the winter grounds was achieved.

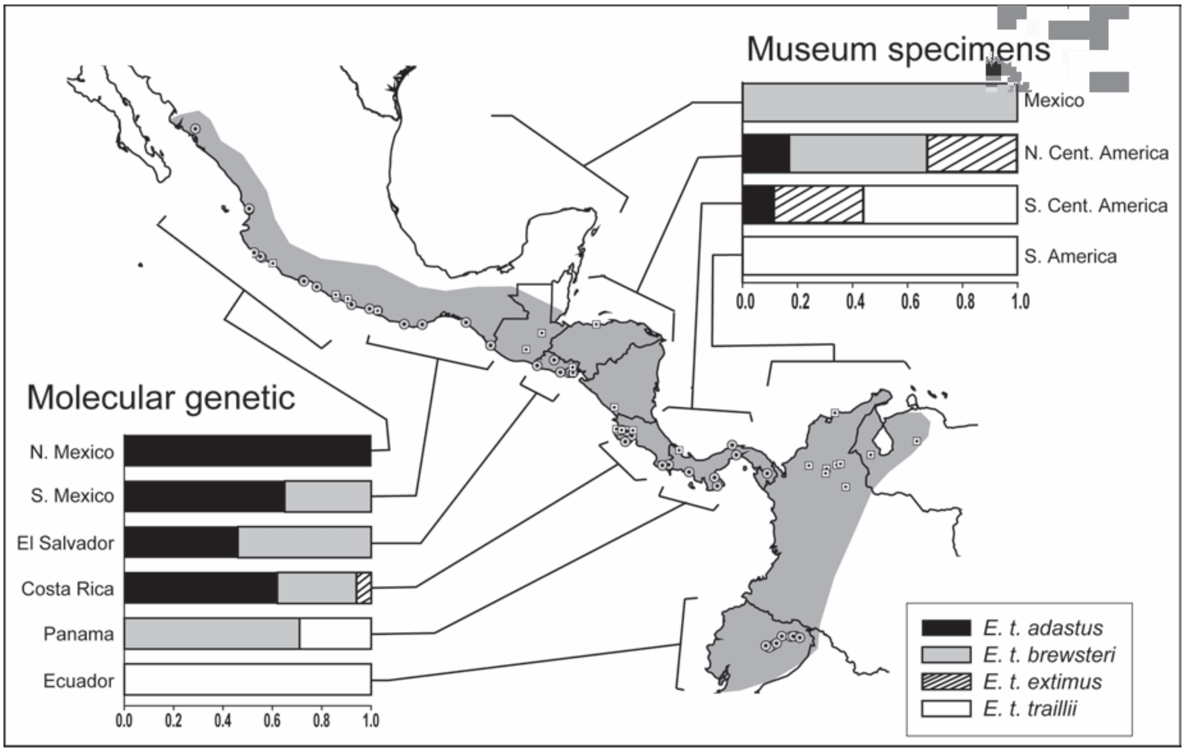

FIGURE 3. Frequency of the four subspecies of the Willow Flycatcher in subsets of the winter range, based on molecular genetic and morphological traits. The molecular genetic study considered six regions of the winter range: northern Mexico, southern Mexico, El Salvador, Costa Rica, Panama, and Ecuador. The museum specimen study considered Mexico, northern Central America (Guatemala, El Salvador, Honduras, and Nicaragua), southern Central America (Costa Rica and Panama), and South America (Colombia, Venezuela, and Ecuador).

The importance of the Pacific lowlands of Costa Rica as a site for the southwestern subspecies' wintering is based on several lines of evidence. First, Costa Rica was chosen for the long-term winter demographic study that began in 1997 (Koronkiewicz et al. 2006) on the basis of early work with museum specimens from the winter range (Unitt, unpubl. data) suggesting that this country was an important location for *E. t. extimus*. Second, this study sampled six individuals from Costa Rica that had the haplotype (C1) strongly associated with the Southwestern Willow Flycatcher, with the same haplotype detected only once in another region, southern Mexico. Third, two individuals initially banded within the southwestern subspecies' breeding range were recaptured in northwestern Costa Rica and, on the basis of resighting of their colored bands, overwintered at the sites of recapture (Koronkiewicz and Sogge 2001). Both individuals were subsequently confirmed as returning to and breeding at the locations where initially banded, including one female that migrated between the same sites of breeding (Roosevelt Lake, Arizona) and of wintering (Bolson, Costa Rica) for at least four consecutive years. Of the currently estimated 3.2 million Willow Flycatchers (Rich et al. 2004), approximately 45% are *E. t. brewsteri*, 33% are *E. t. traillii*, 22% are *E. t. adastus*, and about 0.2% are *E. t. extimus*. If wintering Southwestern Willow Flycatchers were randomly distributed over an area equivalent to that of the other subspecies, the likelihood of more than the occasional *E. t. extimus* being sampled from any one area by random chance alone would be very small. During the period that many of the museum specimens were collected (as early as 1882), *E. t. extimus* may have been more numerous, but it probably has always been rarer than the other subspecies. These results suggests that either Costa Rica is the core of the winter range of *E. t extimus*, with most individuals found in that region, or the subspecies' winter range consists of several clusters of relatively high density, one of which is Costa Rica. However, the rarity of *E. t. extimus* implies that detecting the subspecies can be difficult, and further sampling in Central America will be important for a better understanding of the distribution of the endangered subspecies. For example, three museum specimens from southern Mexico were predicted to be *E. t. extimus* with high, but less than 90% confidence, and may indicate that the winter range of *E. t. extimus* extends north to that region.

Our results suggest that in the Willow Flycatcher connectivity between the breeding and winter grounds is moderate to strong. The distribution of the three western subspecies on their breeding and winter grounds suggests a chain migration (Salomonsen 1955), where the more northern subspecies (*E. t. adastus* and *E. t. brewsteri*) winter in the northernmost portion of the winter range and the southernmost subspecies

(*E. t. extimus*) winters farther south. However, the eastern subspecies appears to migrate in a leap-frog pattern relative to the three western subspecies. The eastern subspecies winters primarily south of the western subspecies and circumnavigates the Gulf of Mexico (Sedgwick 2000), passing through the winter ranges of the three western subspecies to a more southerly winter range. Studies of other migratory passerines also tend to show moderate to strong connectivity between the breeding and winter ranges (Norris et al. 2006). However, the type of migration pattern (e.g., chain, leap-frog) varies by species and may reflect the nuances of demographic histories, responses to Pleistocene glaciation, and other evolutionary pressures (Boulet and Norris 2006). Interestingly, the Willow Flycatcher and its sibling species, the Alder Flycatcher, exemplify a leap-frog pattern. The Alder breeds north of the Willow Flycatcher across Canada and Alaska, and migrates through the eastern United States, circumnavigating the Gulf of Mexico (many specimens of the Alder were collected in Veracruz, Mexico, and along the Caribbean slope of Central America, with no evidence of other migratory routes), to winter primarily south of the Willow Flycatcher in South America along the east flank of the Andes, south to Bolivia (Lowther 1999, this study). This pattern may reflect migration habits that developed before the two species diverged and have persisted to the present.

The strength of migratory connectivity between geographic regions has important implications for the ecology and conservation of populations. Events in one region can influence events in another, via crossover effecs, depending on the strength of migratory connectivity between the regions (Marra et al. 2006). Furthermore, mortality of passerines is estimated to occur primarily in the nonbreeding period (Sillett and Holmes 2002, Paxton et al. 2007b), suggesting that breeding populations could be affected by events far from the breeding grounds. Baker et al. (2004) documented this type of linkage by demonstrating that declines of the breeding population of the Red Knot (*Calidris canutus*) were due to the loss of critical food resources at a stopover site. Surveys of wintering Willow Flycatchers and their habitats, in Costa Rica, Ecuador, Guatemala, Mexico, and Panama (Lynn et al. 2003, Nishida and Whitfield 2006, Schuetz et al. 2007), have found wintering flycatchers occupying a wide range of habitats, generally characterized by trees or woody shrubs bordering standing or moving water. These habitats range from mature trees to young successional regrowth in disturbed habitats, and given the abundance of secondary growth in all of these regions, Willow Flycatcher populations may not be currently limited by the availability of winter habitat. However, natural sites with mature trees are rare, and possibly of higher quality than younger sites (Koronkiewicz et al. 2006), suggesting that continuing anthropogenic changes on the winter grounds may be degrading overall habitat quality. Our results suggest that conservation efforts for the endangered Southwestern Willow Flycatcher focused on Costa Rica may be the best strategy initially, with continued surveys and research warranted to determine the extent of this endangered subspecies' winter range more fully.

Ultimately, more studies are needed to establish exactly how conditions on the breeding and winter grounds, and the stopover habitats in between, influence one another so the challenges faced in the eventual recovery of this endangered species can be understood fully. This study, by establishing some of the linkages between the breeding and winter grounds, is an important first step in understanding such cross-seasonal effects. Additionally, this study demonstrates the value of using multiple intrinsic markers to compare patterns of geographic distribution independently and therefore providing greater confidence in the overall conclusions.

ACKNOWLEDGMENTS

Funding for this study was provided by the U.S. Geological Survey, Southern Sierra Research Station, and the Bureau of Reclamation. We are indebted to the many field biologists who assisted in field efforts, and Jessica Girard helped with DNA sequencing. Thank you to Ben Bolker for assistance in running the mixed-stock analysis and to Karen Messer for assistance with the analysis of the specimens. Kristina Paxton and Tad Theimer provided critical reviews of early drafts. Thank you to the authorities of the museums that lent specimens: American Museum of Natural History, New York; Academy of Natural Sciences, Philadelphia; Carnegie Museum of Natural History, Pittsburgh; California Academy of Sciences, San Francisco; Cornell University, Ithaca, New York; Denver Museum of Nature and Science, Denver; Delaware Museum of Natural History, Wilmington; Field Museum, Chicago; Natural History Museum of Los Angeles County, Los Angeles; Louisiana State University Museum of Natural Science, Baton Rouge; Moore Laboratory of Zoology, Occidental College, Los Angeles; Museum of Vertebrate Zoology, University of California, Berkeley; Peabody Museum of Natural History, Yale University, New Haven, Connecticut; University of Alaska, Fairbanks; University of Arizona, Tucson; Florida Museum of Natural History, University of Florida, Gainesville; Bell Museum of Natural History, University of Minnesota, Minneapolis; Museum of Zoology, University of Michigan, Ann Arbor; National Museum of Natural History, Washington, DC; Burke Museum, University of Washington, Seattle; Western Foundation of Vertebrate Zoology, Camarillo, California. The use of product trade names in this paper does not constitute product endorsement by the U.S. Geological Survey.

LITERATURE CITED

Aldrich, J. W. 1951. A review of the races of the Traill's Flycatcher. Wilson Bulletin 63: 192–197.

Bairlein, F. 2003. The study of bird migrations—some future perspectives. Bird Study 50:243–253.

Baker, A. J., P. M. Gonzalez, T. Piersma, L. J. Niles, I. L. S. Nascimento, P. A. Atkinson, N. A. Clark, C. D. T. Minton, M. K. Peck, and G. Aarts. 2004. Rapid population decline in Red Knots: fitness consequences of decreased refueling rates and late arrival in Delaware Bay. Proceedings of the Royal Society of London B 271:875–882.

Boulet, M., and D. R. Norris. 2006. The past and present of migratory connectivity. Ornithological Monographs 61:1–13.

Browning, M. R. 1993. Comments on the taxonomy of *Empidonax traillii* (Willow Flycatcher). Western Birds 24:241–257.

Busch, J. D., M. P. Miller, E. H. Paxton, M. K. Sogge, and P. Keim. 2000. Genetic variation in the endangered Southwestern Willow Flycatcher. Auk 117:586–595.

Clegg, S. M., J. F. Kelly, M. Kimura, and T. B. Smith. 2003. Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a neotropical migrant, Wilson's Warbler (*Wilsonia pusilla*). Molecular Ecology 12:819–830.

Diefenbach, D. R., J. D. Nichols, and J. E. Hines. 1988. Distribution patterns during winter and fidelity to wintering areas of American Black Ducks. Canadian Journal of Zoology 66:1506–1513.

Doucet, S. M., and G. E. Hill. 2009. Do museum specimens accurately represent wild birds? A case study of carotenoid, melanin, and structural colours in Long-tailed Manakins *Chiroxiphia linearis.* Journal of Avian Biology 40:146–156.

Fournier, D. A., T. D. Beacham, B. E. Riddell, and C. A. Busack. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Canadian Journal of Fisheries and Aquatic Sciences 41:400–408.

Helm-Bychowski, K., and J. Cracraft. 1993. Recovering phylogenetic signal from DNA sequences: relationships within the corvine assemblages (class Aves) as inferred from complete sequences of the mitochondrial DNA cytochrome-*b* gene. Molecular Biology and Evolution 10:1196–1214.

Hepp, G. R., and J. E. Hines. 1991. Factors affecting winter distribution and migration distances of Wood Ducks from southern breeding populations. Condor 93:884–891.

Higuchi, H., and J. P. Pierre. 2005. Satellite tracking and avian conservation in Asia. Landscape and Ecological Engineering 1:33–42.

Hobson, K. A. 2005. Flying fingerprints, p. 235–246 *In* R. Greenberg and P. Marra [eds.], Birds of two worlds: the ecology and evolution of migration. Johns Hopkins University Press, Baltimore.

Kanai, Y., M. Ueta, N. Germogenov, M. Nagendran, N. Mita, and H. Higuchi. 2002. Migration routes and important resting areas of Siberian Cranes (*Grus leucogeranus*) between northeastern Siberia and China as revealed by satellite tracking. Biological Conservation 106:339–346.

Kelly, J. F., K. Ruegg, and T. Smith. 2005. Combining isotopic and genetic markers to identify breeding origins of migrant birds. Ecological Applications 15:1487–1494.

Kelly, J. F., M. J. Johnson, S. Langridge, and M. Whitfield. 2008. Efficiency of stable isotope ratios in assigning endangered migrants to breeding and wintering sites. Ecological Applications 18:568–576.

Koronkiewicz, T. J., and M. K. Sogge. 2001. Southwestern Willow Flycatchers recaptured at wintering sites in Costa Rica. North American Bird Bander 26:161–162.

Koronkiewicz, T. J., M. K. Sogge, C. van Riper III, and E. H. Paxton. 2006. Territoriality, site fidelity, and survivorship of Willow Flycatchers wintering in Costa Rica. Condor 108:558–570.

Lowther, P. E. 1999. Alder Flycatcher (*Empidonax alnorum*), no 446. *In* A. Poole and F. Gill [eds.], The birds of North America. Birds of North America, Inc., Philadelphia.

Lynn, J. C., T. J. Koronkiewicz, M. J. Whitfield, and M. K. Sogge. 2003. Willow Flycatcher winter habitat in El Salvador, Costa Rica, and Panama: characteristics and threats. Studies in Avian Biology 26:41–51.

Manel, S., O. E. Gaggiotti, and R. S. Waples. 2005. Assignment methods: matching biological questions with appropriate techniques. Trends in Ecology & Evolution 20:136–142.

Marra, P. P., K. A. Hobson, and R. T. Holmes. 1998. Linking winter and summer events in a migratory bird by using stable-carbon isotopes. Science 282:1884–1886.

Marra, P. P., and R.T. Holmes. 2001. Consequences of dominance-mediated habitat segregation in American Redstarts during the nonbreeding season. Auk 118:92–104.

Marra, P. P., D. R. Norris, S. M. Haig, M. Webster, and J. A. Royle. 2006. Migratory connectivity, p. 157–183. *In* K. Crooks and M. Sanjayan [eds.], Conectivity conservation. Cambridge University Press, New York.

Marshall, J. T. Jr. 1988. Birds lost from a giant sequoia forest during fifty years. Condor 90:359–372.

Moore, F. R., R. J. Smith, and R. Sandberg. 2005. Stopover ecology of intercontinental migrants; en route problems and consequences for reproductive performance, p. 251–261. *In* R. Greenberg and P. Marra [eds.], Birds of two worlds: the ecology and evolution of migration. Johns Hopkins University Press, Baltimore.

Nishida, C., and M. J. Whitfield. 2006. Winter distribution of the Willow Flycatcher (*Empidonax traillii*) in Ecuador and northern Mexico. Report to U.S. Bureau of Reclamation, Boulder City, AZ.

Norris, D. R., P. P. Marra, G. J. Bowen, L. M. Ratcliffe, J. A. Royle, And T. K. Kyser. 2006. Migratory connectivity of a widely distributed songbird, the American Redstart (*Setophaga ruticilla*). Ornithological Monographs 61:14–28.

Norris, D. R., P. P. Marra, T. K. Kyser, T. W. Sherry, and L. M. Ratcliffe. 2004. Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. Proceedings of the Royal Society of London B 271:59–64.

Okuyama, T., and B. M. Bolker. 2005. Combining genetic and ecological data to estimate sea turtle origins. Ecological Applications 15:315–325.

Paxton, E. H. 2000. Molecular genetic structuring and demographic history of the Willow Flycatcher. M.Sc. thesis. Northern Arizona University, Flagstaff, AZ.

Paxton, K. L., C. van Riper, T. C. Theimer, and E. H. Paxton. 2007a. Spatial and temporal migration patterns of Wilson's Warbler in the southwestern United States revealed by stable isotopes. Auk 124:162–175.

Paxton, E. H., M. K. Sogge, S. L. Durst, T. C. Theimer, and J. R. Hatten. 2007b. The ecology of the Southwestern Willow Flycatcher in central Arizona: a 10-year synthesis report. USGS Open File Report 2007-1381.

Paxton, E. H., M. K. Sogge, T. C. Theimer, J. Girard, and P. Keim. 2008. Using molecular genetic markers to resolve a subspecies boundary: the northern boundary of the Southwestern Willow Flycatcher in the four-corner states. U.S. Geological Survey Open File Report 2008-1117.

Paxton, E. H., T. J. Koronkiewicz, M. K. Sogge, and M. A. McLoud. 2010. Geographic variation in the plumage coloration of Willow Flycatchers. Journal of Avian Biology 41:128–138.

Pearce, J. M., B. J. Pierson, S. L. Talbot, D. V. Derksen, D. Kraege, and K. T. Scribner. 2000. A genetic evaluation of morphology used to identify harvested Canada Geese. Journal of Wildlife Management 64:863–874.

Reynolds, J. H., and W. D. Templin. 2004. Detecting specific populations in mixtures. Environmental Biology of Fishes 69:233–243.

Phillips, A. R. 1948. Geographic variation in *Empidonax traillii*. Auk 65:507–514.

Rich, T. D., C. J. Beardmore, H. Berlanga, P. J. Blancher, M. S. W. Bradstreet, G. S. Butcher, D. W. Demarest, E. H. Dunn, W. C. Hunter, E. E. Iñigo-Elias, J. A. Kennedy, A. M. Martell, A. O. Panjabi, D. N. Pashley, K. V. Rosenberg, C. M.

Rustay, J. S. Wendt, and T. C. Will [online]. 2004. Partners in Flight North American Landbird Conservation Plan, version March 2005. Cornell Lab of Ornithology. Ithaca, NY. <http://www.partnersinflight.org/cont_plan/>.

Salomonsen, F. 1955. The evolutionary significance of bird migration. Biologiske Meddeleser 22:1–62.

Schuetz, J. G., M. J. Whitfield, and V. A. Steen. 2007. Winter distribution of the Willow Flycatcher (*Empidonax traillii*) in Guatemala and Mexico. Report to U.S. Bureau of Reclamation, Boulder City, AZ.

Sedgwick, J. A. 2000. Willow Flycatcher (*Empidonax traillii*), no 533. *In* A. Poole and F. Gill [eds.], The birds of North America. Birds of North America, Inc., Philadelphia.

Sillett, T. S., and R. T. Holmes. 2002. Variation in survivorship of a migratory songbird throughout its annual cycle. Journal of Animal Ecology 71:296–308.

Smith, T. S., S. M. Clegg, M. Kimura, K. C. Ruegg, B. Mila, and I. J. Lovette. 2005. Molecular genetic approaches to linking breeding and overwintering areas in five neotropical migrant passerines, p. 222–234. *In* R. Greenberg and P. P. Marra [eds.], Birds of two worlds: the ecology and evolution of migration. Johns Hopkins University Press, Baltimore.

Smith, R. B., T. D. Meehan, and B. O. Wolf. 2003. Assessing migration patterns of Sharp-shinned Hawks *Accipiter striatus* using stable-isotope and band encounter analysis. Journal of Avian Biology 34:387–392.

Sogge, M. K., T. J. Koronkiewicz, C. van Riper III, and S. L. Durst. 2007. Willow Flycatcher nonbreeding territory defense behavior in Costa Rica. Condor 109:475–480.

Sogge, M. K., J. C. Owen, E. H. Paxton, and S. M. Langridge. 2001. A targeted mist net capture technique for the Willow Flycatcher. Western Birds 32:167–172.

Studds, C. E., T. K. Kyser, and P. P. Marra. 2008. Natal dispersal driven by environmental conditions interacting across the annual cycle of a migratory songbird. Proceedings of the National Academy of Sciences, USA 105:2929–2933.

Stutchbury, B. J. M., S. A. Tarof, T. Done, E. Gow, P. M. Kramer, J. Tautin, J. W. Fox, and V. Afanasyev. 2009. Tracking long-distance songbird migration by using geolocators. Science 323:896.

Swarth, H. S. 1920. Revision of the genus *Passerella*, with special reference to the distribution and migration of the races in California. University of California Publications in Zoology 21:75–224.

Unitt, P. 1987. *Empidonax traillii extimus*: an endangered subspecies. Western Birds 18:137–162.

U.S. Fish and Wildlife Service (USFWS). 1995. Final rule determining endangered status for the Southwestern Willow Flycatcher. Federal Register 60:10694–10715.

U.S. Fish and Wildlife Service (USFWS). 2002. Southwestern Willow Flycatcher (*Empidonax traillii extimus*) final recovery plan. U.S. Fish and Wildlife Service, Albuquerque, NM.

Webster, M. S., P. P. Marra, S. M. Haig, S. Bensch, and R. T. Holmes. 2002. Links between worlds: unraveling migratory connectivity. Trends in Ecology & Evolution 17:76–83.

Wikelski, M., R. W. Kays, N. J. Kasdin, K. Thorup, J. A. Smith, and G. W. Swenson. 2007. Going wild: what a small-animal tracking system could do for experimental biologists. Journal of Experimental Biology 210:181–186.