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Author(s): Scott F. Lovell and M. Ross Lein

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GEOGRAPHICAL VARIATION IN SONGS OF A SUBOSCINE PASSERINE, THE ALDER FLYCATCHER (*EMPIDONAX ALNORUM*)

SCOTT F. LOVELL¹ AND M. ROSS LEIN^{1,2}

ABSTRACT.—Although there is a large body of literature dealing with the nature of geographic variation in the songs of birds, few studies have examined such variation across the entire range of species of suboscine birds. We measured time and frequency characteristics of songs of Alder Flycatchers (*Empidonax alnorum*) from six regions spanning almost the entire range of the species, from Alaska to Maine. Both univariate and multivariate analyses demonstrated significant differences in song characteristics among regions, and discriminant function analysis classified 69% of songs to the correct region. We found no relationship between geographic separation and magnitude of difference in songs among regions—songs of birds from some widely-separated regions were more similar than they were to songs of birds from neighboring regions. We argue that these regional differences have a genetic basis, but the pattern of variation does not appear to be consistent with a simple “isolation by distance” model. The variation may reflect differing adaptation to optimize acoustic transmission in varying habitats across the range. However, more detailed studies, including examination of genetic variation among populations, are required to test such suggestions rigorously. Received 21 May 2012. Accepted 10 November 2012.

Key words: Alder Flycatcher, *Empidonax alnorum*, geographic variation, song, suboscine.

The study of birdsong exploded after the mid-1950s as the result of technological advances in field recording and spectrographic analysis (Baptista and Gaunt 1994). One of the first topics to be explored extensively using these technologies was the nature of geographic variation within species (Thielcke 1969, Mundinger 1982). However, the vast majority of such research has dealt with the songs of oscine passerines (Passeriformes, suborder Passeri). Some form of learning is believed to be involved in the ontogenetic development of song in members of this group (Kroodsma 1982, 1996; Slater 1989), and variation in the precision of the learning process has probably had a major role in producing the diversity of patterns of geographic variation demonstrated by oscines (Podos and Warren 2007).

In contrast, relatively few studies have examined intraspecific geographic variation in the songs of suboscine passerines (suborder Tyranni). This is a consequence, at least in part, of the fact that most suboscines are found in subtropical or tropical regions where very little research has been conducted (until recently). However, learning is believed to play little or no role in the ontogeny of song in most, if not all, suboscines (Kroodsma 1984, 1985, 1989; Kroodsma and Konishi 1991). As a consequence, most suboscines show relatively little intraspecific variation in song, both within

and between populations, in comparison to oscine species. Some studies, based primarily on visual inspection of audiospectrograms, suggested that significant intraspecific geographic variation was absent (e.g., Kellogg and Stein 1953; Stein 1963; Lanyon 1978; Kroodsma 1984, 1993).

Because of this claim of extremely limited variation in the songs of suboscine species, differences between populations that are separated in geographic space are frequently interpreted as an indication of evolutionary divergence between such populations. Isler and others (1998) have argued that the demonstration of a small number of diagnosable differences in vocalizations between pairs of antbird populations warrants recognition of such populations as valid species.

Recently, detailed quantitative analyses of frequency and temporal features have demonstrated that songs of a number of species of suboscine passerines are individually distinctive, even though this is often difficult to detect through visual inspection of audiospectrograms (Lovell and Lein 2004, Ríos Chelén et al. 2005, Wiley 2005, Lein 2008, Fernández-Juricic et al. 2009, Kirschel et al. 2009, Clark and Leung 2011), and that such differences are sufficient for individual recognition (Lovell and Lein 2005). Our understanding of the significance of geographic variation in suboscine songs is hampered by the paucity of similar quantitative analyses of variation across the ranges of single species. Without such knowledge, it is difficult to evaluate the significance of differences between the songs of

¹Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada.

²Corresponding author; e-mail: mrlein@ucalgary.ca

isolated samples separated in geographic space. To address this issue, we analyzed songs of Alder Flycatchers (*Empidonax alnorum*) from localities across North America to determine the possible extent and patterns of geographic variation.

METHODS

Study Species.—The Alder Flycatcher (*Empidonax alnorum*) is considered a monotypic species (Lowther 1999). Phillips (1948) reported that individuals from Alaska, Yukon, and the Northwest Territories were larger than average with slightly paler backs. He named this form *alascensis*, but it was not recognized subsequently (Aldrich 1951, AOU 1957). Geographic variation in morphology and plumage remains poorly known and worthy of further evaluation (Lowther 1999).

Alder Flycatchers breed in damp, brushy thickets and shrubby wetlands across most of northern North America. During the breeding season, Alder Flycatchers can be found from central Alaska to Newfoundland, south across Michigan and Pennsylvania, and along the Appalachian Mountains into Tennessee and North Carolina (AOU 1998, Lowther 1999). The song repertoire of Alder Flycatchers consists of one relatively simple song type (Fig. 1), described verbally as ‘fee-bee-o’ (Stein 1963).

Study Areas and Recordings.—We analyzed tape recordings of songs of male Alder Flycatchers from Alaska, Alberta, Maine, Michigan, New York, and Ontario, covering almost the full width of the range of this species (Fig. 2). Alberta recordings were from our field research in 2000–2001; recordings from other regions were obtained from the Borror Laboratory of Bioacoustics (Ohio State University), and the Macaulay Laboratory of Natural Sounds (Cornell University) (locality information in Appendix 1). All recordings were made during the breeding season for the species in the various regions. Individual songs were acquired as digital sound files using RTSD Version 1.10 and SIGNAL Version 3.1 bioacoustical software (Engineering Design, Belmont, MA, USA), using a sample rate of 25 kHz with 16-bit amplitude resolution. We chose songs for analysis after viewing audiospectrograms with RTSD to ensure they were free of background noise in the frequency range of Alder Flycatcher song. We restricted our analysis to regions from which we had recordings of at least five males. We measured a single song for each individual to

maximize sample sizes for regions other than Alberta (some recordings from archives included as few as one song). This decision is justified, because individual Alder Flycatcher males show almost no variation among successive songs in a recording (Lovell and Lein 2004). Measurements of single songs from 26 individuals were highly correlated (mean $r = 0.90$) with mean values for 10 songs from the same individuals. In addition, the use of single songs is conservative in tests for differences among regions. Inclusion of some songs with extreme deviations from mean values for individuals would reduce the probability of detecting differences among regions. The data set included songs from 57 individual Alder Flycatchers from six geographic regions (Table 1). We used a program in the SIGNAL language to assist in measurement of 12 temporal variables and six frequency variables for each ‘fee-bee-o’ song (Appendix 2; Lovell and Lein [2004] provide a detailed description of these procedures).

Statistical Analyses.—SYSTAT 10.2 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. We conducted univariate one-way ANOVAs to examine which of the original variables differed significantly among geographic regions. Eleven variables did not differ significantly among the regions (Table 1) and were removed from further analysis. Many of the seven remaining variables were correlated with one another. We performed a principal components analysis (PCA) on these seven variables to reduce the number of variables and to eliminate problems caused by collinearity.

We used a Mantel test to examine whether a matrix of geographic distances was correlated with a matrix of song distances. The geographic distances between each pair of recording locations were calculated from latitudes and longitudes and song distances were the Euclidean distances between each pair of songs in principal component space.

We used discriminant function analysis (DFA) of principal component scores of songs to determine whether songs of individuals could be identified reliably to the correct geographic location. Results of jack-knifed classifications, in which each song was classified using discriminant functions computed from all songs in the data set except the case being classified, are reported as percentages of songs classified correctly. This technique is a conservative estimate of the power of the classification technique

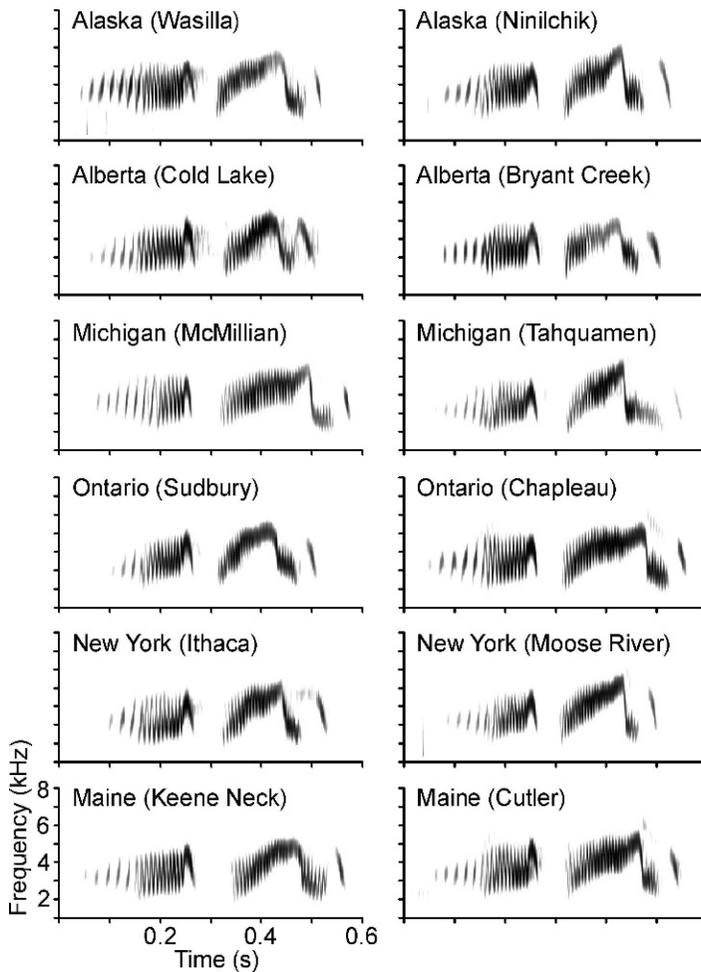


FIG. 1. Representative examples of the ‘fee-bee-o’ songs of Alder Flycatchers from the six geographic regions.

(Manly 1994). We calculated Cohen’s Kappa to measure the improvement of this classification over chance assignment (Titus et al. 1984).

RESULTS

Only seven of the 18 time and frequency variables differed significantly ($P < 0.05$) among the geographic regions (Table 1). T1, T2, T9, and T12 demonstrated the greatest differences among the geographic location ($P < 0.001$) (Table 1). Principal component analysis generated four principal components with eigenvalues >1.0 from the seven variables, explaining $\sim 85\%$ of the variance among the geographic regions (Table 2). PC 1 had high positive loadings for three time variables measuring duration (T1, T2, T8) and a negative loading for a frequency change variable

(F2) (Table 2). This indicates that birds with high PC 1 scores had relatively long songs during which the frequency of the ‘fee’ phrase increased at a slower rate in comparison to songs of birds with low PC 1 scores. PC 2 had high positive loadings for the duration of the first four introductory notes of the fee phrase (T9) and high negative loadings for the duration of the first four FMs of the ‘o’ portion of the ‘bee-o’ phrase (T12) and the maximum frequency of the terminal note (F6) (Table 2). PC 3 had high positive loadings for the duration of the first four introductory notes of the ‘fee’ phrase (T9) and the maximum frequency of the terminal note (F6), and high negative loadings for the duration of the interval between the fee and the bee-o phrases (Table 2). Three of the four PCs differed significantly among the geographic

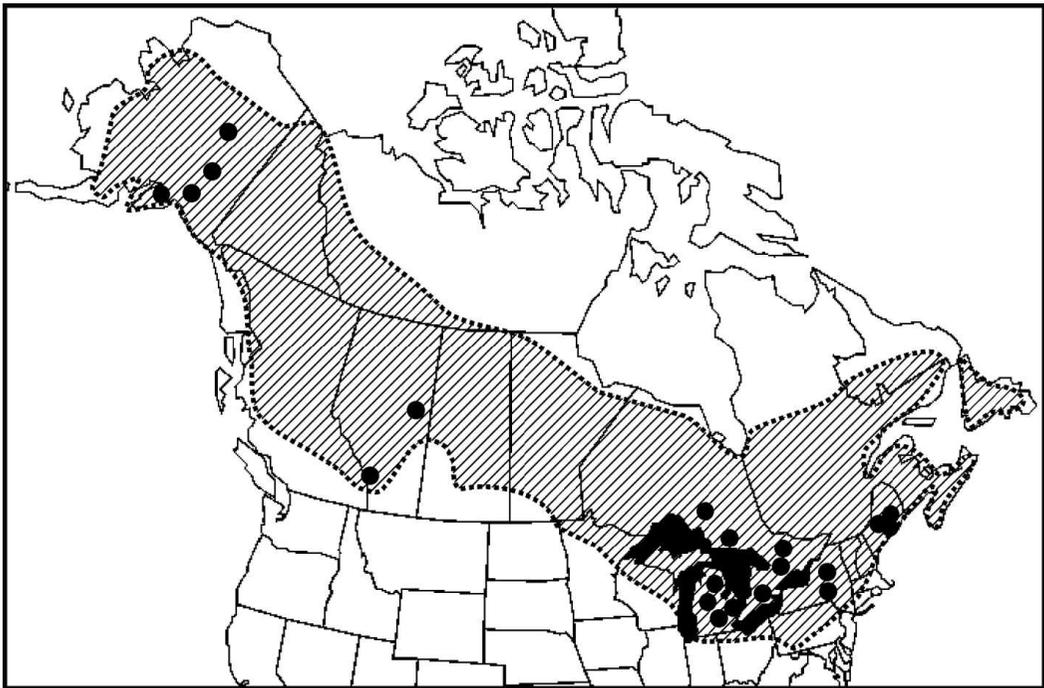


FIG. 2. Breeding range (cross-hatched) of the Alder Flycatcher in North America. Recording localities are indicated by filled circles.

TABLE 1. Means and standard errors for 18 time and frequency variables measured for ‘fee-bee-o’ songs of Alder Flycatchers from six geographic regions, along with results of ANOVAs testing for differences among regions. Variables that differed significantly among regions are indicated by bold face. See Appendix 2 for detailed description of variables.

Variable	Geographic Region (N)						F _{5,51}	P
	Alberta (14)	Alaska (5)	Maine (12)	Michigan (8)	New York (5)	Ontario (13)		
T1	519.9 ± 7.9	480.2 ± 17.1	506.5 ± 8.5	493.6 ± 11.8	455.2 ± 21.0	457.5 ± 12.0	5.62	<0.001
T2	235.9 ± 7.8	216.8 ± 14.9	232.5 ± 4.4	212.4 ± 8.6	189.0 ± 10.0	194.6 ± 7.2	5.80	<0.001
T3	120.4 ± 3.6	116.8 ± 10.3	113.5 ± 3.0	116.6 ± 5.8	106.2 ± 5.6	105.8 ± 3.1	1.94	0.10
T4	187.7 ± 5.5	178.8 ± 5.7	181.2 ± 4.1	194.0 ± 6.6	179.4 ± 13.1	180.1 ± 5.6	0.82	0.54
T5	136.6 ± 5.5	126.2 ± 6.5	130.8 ± 4.5	137.4 ± 7.3	132.0 ± 12.1	126.7 ± 4.5	0.64	0.67
T6	50.9 ± 2.2	52.6 ± 4.6	50.2 ± 3.5	56.5 ± 4.7	47.6 ± 4.0	53.5 ± 2.7	0.63	0.68
T7	40.6 ± 1.4	36.6 ± 4.3	39.1 ± 2.1	34.5 ± 1.6	38.0 ± 3.4	35.9 ± 1.6	1.31	0.27
T8	55.8 ± 2.1	48.4 ± 2.3	53.8 ± 2.7	52.5 ± 3.3	48.0 ± 3.8	46.8 ± 4.4	2.63	0.034
T9	18.9 ± 0.3	26.4 ± 0.9	19.1 ± 0.4	25.6 ± 1.2	25.2 ± 0.7	18.7 ± 0.5	32.15	<0.001
T10	7.0 ± 0.2	7.0 ± 0.5	6.8 ± 0.2	7.0 ± 0.2	7.0 ± 0.3	6.6 ± 0.1	0.69	0.64
T11	6.3 ± 0.1	5.8 ± 0.2	6.1 ± 0.2	6.4 ± 0.2	6.2 ± 0.2	6.0 ± 0.1	1.97	0.09
T12	5.9 ± 0.2	5.9 ± 0.1	7.6 ± 0.4	5.9 ± 0.2	6.0 ± 0.3	7.8 ± 0.1	15.41	<0.001
F1	4,092.3 ± 60.6	4,330.0 ± 157.4	3,949.2 ± 114.7	4,015.6 ± 126.4	4,017.6 ± 157.0	4,202.8 ± 122.5	1.17	0.34
F2	8.0 ± 1.1	8.6 ± 1.1	6.7 ± 1.4	11.9 ± 2.2	14.0 ± 2.7	12.2 ± 1.3	3.09	0.016
F3	4,387.5 ± 65.7	4,592.2 ± 80.5	4,521.6 ± 96.7	4,358.4 ± 89.8	4,355.8 ± 69.2	4,527.2 ± 48.9	1.34	0.26
F4	20.6 ± 1.1	22.6 ± 0.8	24.3 ± 1.9	20.1 ± 2.6	21.6 ± 1.6	24.2 ± 1.9	1.04	0.41
F5	5,081.0 ± 77.5	5,366.4 ± 131.4	5,058.7 ± 73.9	5,077.4 ± 69.3	5,104.5 ± 97.6	5,336.5 ± 83.8	2.37	0.053
F6	4,246.5 ± 91.4	4,376.4 ± 165.9	4,394.4 ± 86.1	3,921.0 ± 83.0	4,262.2 ± 117.6	4,391.2 ± 91.2	2.88	0.023

TABLE 2. Factor loadings of the principal component analysis performed on seven song variables of Alder Flycatchers from six geographic regions. Loadings (> |0.5|) are shown in boldface.

Song Variable	PC1	PC2	PC3	PC4
T1	0.930	0.128	0.177	0.187
T2	0.880	0.002	0.326	-0.092
T8	0.617	0.257	-0.454	0.426
T12	0.039	-0.813	-0.037	0.294
F6	0.116	-0.680	0.507	0.037
T9	-0.222	0.540	0.722	0.065
F2	-0.427	0.115	0.161	0.837
Eigenvalue	2.28	1.51	1.15	1.02
% Variance explained	32.39	21.59	16.42	14.53

regions (one-way ANOVAs; PC 1: $F_{5,51} = 9.764$, PC 2: $F_{5,51} = 25.17$, PC 3: $F_{5,51} = 2.968$, all $P < 0.01$). *Post-hoc* tests showed multiple significant differences among pairs of geographic regions. Songs of Alder Flycatchers from each region differed from those from two or more other regions in scores on one or more principal components, and songs from individuals from Alberta differed from those from all other regions on at least one principal component (Table 3). There was no significant correlation of song distance between pairs of birds with geographic distances between them (Mantel test; $r = 0.055$, $P = 0.92$).

A MANOVA conducted as part of the DFA showed highly significant differences among multivariate means for the different geographic regions ($F_{15,129} = 13.45$; $P < 0.001$). Jack-knifed classification assigned 40 of the 58 songs (69%) to geographic region correctly, significantly better than expected by chance (Cohen’s Kappa = 0.63; $P < 0.001$; 95% CI = 0.48–0.77). The distribution of values for individual songs in discriminant function space shows this separation clearly. There is little or no overlap of songs from different geographic regions on the first two discriminant functions (Fig. 3) with additional separation

of regions on the third discriminant function (not shown).

DISCUSSION

Our findings demonstrate that there are limited, but significant, differences in songs of Alder Flycatchers among the six geographic regions examined. These differences are apparent in both the time and frequency traits of the songs of individuals (Table 1) and in multivariate comparisons (Table 3, Fig. 3). However, the geographic pattern of this variation is not simple. Some comparisons of songs between pairs of regions that are close geographically show significant differences (Table 3, Fig. 3), whereas other comparisons between pairs of regions well-separated in space show much less differentiation. For example, songs from birds from Alberta and Maine showed considerable overlap in discriminant function space (Fig. 3), and DFA assigned almost 50% of Maine birds to Alberta, despite the fact that these samples are separated by ~2,400 km. This complex geographic pattern of variation requires an evolutionary explanation.

Although learning or copying of song models is believed to play no role in the ontogeny of song in most groups of suboscine birds, the Alder

TABLE 3. Results of post-hoc tests comparing pairs of regions following ANOVAs indicating significant variation ($P < 0.05$) in PC 1, PC 2, and PC 3 scores for songs of Alder Flycatcher among six geographic regions.

Location	Differences in PC1	Differences in PC2	Differences in PC3
Alberta (AB)	MI, NY, ON	ME, MI, ON	AK
Alaska (AK)	—	ME, ON	AB, ON
Maine (ME)	NY, ON	MI, NY	—
Minnesota (MN)	AB	ON	—
New York (NY)	AB, ME	ON	—
Ontario (ON)	AB, ME	NY	AK

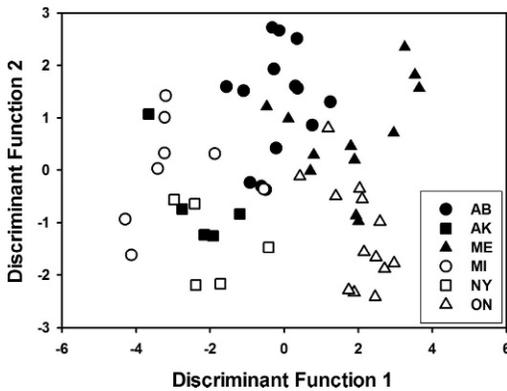


FIG. 3. Plot of values for individual songs from the six geographic regions in the space defined by the first two discriminant functions.

Flycatcher is one of the few species in which this has been verified experimentally (Kroodsmma 1984). However, there is evidence that variation in song structure among individuals functions in individual recognition (Lovell and Lein 2005) and therefore should be favored by natural selection. The type of modest variation among individuals that is found within a local population (Lovell and Lein 2004) possibly could result from non-specific, chance variation in song development processes, resulting in unique songs for each individual. However, such an explanation is inconsistent with the pattern of geographic variation that we observed. Individuals from the same population or geographic region tend to have songs that are similar to one another. This finding suggests that there is some genetic component influencing the variation in song development, and that individuals from a given location are more likely to have similar genetic complements.

If genetic variation is responsible for at least part of the variation that occurs among individuals, and among localities, there are a number of possible explanations. First, the variation could be ‘neutral’ in nature, generating functional variation among the songs of individuals in a population, but without any particular pattern of variation being selectively advantageous. The important factor in this situation would be that songs of individuals are sufficiently different to permit individual recognition. If this were true, we might expect to observe an ‘isolation by distance’ effect when comparing songs of birds from different geographic regions. Assuming that there is no population structuring, or barriers to

gene flow across the range of the species, we would expect to find that differences among songs of birds from different populations would increase with increasing geographic distance separating them. Such variation in song features has been described for several oscine passerines (e.g., Morton 1987, Benedict and Bowie 2009). This is not the case for Alder Flycatchers. There is no correlation between song difference in multivariate space and geographic distance separating pairs of birds, and there is some suggestion that songs of birds from widely-separated populations may be quite similar (e.g., Maine and Alberta).

Alternatively, the pattern of variation that is seen might be adaptive, resulting from selection to optimize sound transmission in the particular structural environment in which songs are used. Many studies have shown that vegetation structure can influence sound transmission and, consequently, favor temporal and frequency characteristics that result in efficient communication (Wiley and Richards 1992, Brumm and Naguib 2009). This type of acoustic adaptation potentially could explain the similarity of songs within populations or regions, the variation across the range of the species, and the similarity of songs between widely-separated regions. Ippi and others (2011) related geographic variation in songs of the suboscine Thorn-tailed Rayadito (*Aphrastura spinicauda*) to structural differences in the forests inhabited by this species across its range. However, they compared some populations that are differentiated at the subspecific level, and historical processes may have contributed to this divergence. Testing whether the geographic variation we describe is related to habitat differences may be difficult. Alder Flycatchers occupy brushy habitats that show limited physiognomic variation across the range of the species (Lowther 1999), reducing the potential importance of this selective force. In addition, most songs are delivered from elevated song perches above the canopy of the vegetation, minimizing the possible influence of sound transmission through vegetation.

Our study is one of the first to examine geographic variation in song across the entire range of a suboscine bird, albeit with small sample sizes and no samples from large portions of the range. It indicates a mosaic pattern of minor but significant variation in temporal and frequency characteristics of songs of Alder Flycatchers across their range. Payne and Budde (1979) and Lein (2008)

failed to find geographic variation in the songs of the congenetic Acadian Flycatcher (*Empidonax vireescens*) and Buff-breasted Flycatcher (*E. fulvifrons*), respectively. However, these studies looked for variation over short geographic distances. Geographic variation in song has been demonstrated in the Willow Flycatcher (*E. traillii*) (Sedgwick 2001), but this occurs between recognized subspecies that are differentiated morphologically and genetically (Paxton et al. 2008), and probably reflects former geographic isolation of the populations involved.

Studies of geographic variation in a number of Neotropical subspecies also have demonstrated pronounced differences among geographically-separated populations, which have been interpreted as evidence of population structuring and reproductive isolation among the populations (Isler et al. 1998). However, one subspecies, the Variable Antshrike (*Thamnophilus caerulescens*) exhibits clinal variation in a song characteristic that was concordant with variation in mitochondrial DNA among populations (Isler et al. 2005). These authors warn that taxonomic judgments based on song differences between widely-separated populations must remain tentative until the pattern of variation in intervening populations can be demonstrated. We require multiple studies that sample song variation intensively across the ranges of subspecies, preferably with corresponding genetic analyses, in order to understand fully the nature and significance of geographic variation in the songs of this important group of birds.

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APPENDIX 1. Recordings used in analysis of geographic variation in song of Alder Flycatchers. Acronyms for recording archives: BLB = Borror Laboratory of Bioacoustics; LNS = Macaulay Library of Natural Sound; MRL = Bioacoustic Collection of M. R. Lein.

Location	Recording Accession Numbers
Alaska	BLB18618; BLB18780; BLB18809; BLB23930; LNS77076
Alberta	MRL04605; MRL04606; MRL04609; MRL06677; MRL06688; MRL06697; MRL06707; MRL06755; MRL06790; MRL06800; MRL06820; MRL06839; MRL06840; MRL07183
Maine	BLB2032; BLB6526; LNS7533; LNS7534; LNS7535; LNS7550; LNS85215; LNS101054; LNS101061; LNS101091; LNS102250; LNS112041
Michigan	BLB2802; LNS7506; LNS7507; LNS7508; LNS7555; LNS7556; LNS7557; LNS7565
New York	LNS7526; LNS7527; LNS7543; LNS7544; LNS100850
Ontario	LNS7509; LNS7538; LNS7539; LNS7558; LNS7559; LNS7561; LNS7562; LNS55520; LNS59741; LNS59743; LNS59746; LNS63985; LNS63987

APPENDIX 2. Description of song variables measured for audiospectrograms of 'fee-bee-o' songs of male Alder Flycatchers. Detailed description of procedures used to measure temporal and frequency variables are presented in Lovell and Lein (2004).

Code	Variable
T1	Duration of the entire song (msec)
T2	Duration of the 'fee' phrase (msec)
T3	Duration of the frequency modulation (FM) portion of the 'fee' phrase (msec)
T4	Duration of the 'bee-o' phrase (msec)
T5	Duration of the 'bee' portion of the 'bee-o' phrase (msec)
T6	Duration of the 'o' portion of the 'bee-o' phrase (msec)
T7	Duration of the interval between 'bee-o' phrase and terminal note (msec)
T8	Duration of the interval between the 'fee' and 'bee-o' phrases (msec)
T9	Duration of the first four introductory notes of the 'fee' phrase (msec)
T10	Duration of last four FMs of the 'fee' phrase (msec)
T11	Duration of first four FMs of the 'bee' portion of the 'bee-o' phrase (msec)
T12	Duration of first four FMs of the 'o' portion of the 'bee-o' phrase (msec)
F1	Maximum frequency of the 'fee' introductory notes (Hz)
F2	Rate of frequency change in the 'fee' introduction (Hz/msec)
F3	Maximum frequency of the 'fee' terminal chevron (Hz)
F4	Rate of frequency change of the 'bee' portion of the 'bee-o' phrase (Hz/msec)
F5	Maximum frequency of the 'bee-o' phrase (Hz)
F6	Maximum frequency of the terminal note of the 'fee-bee-o' song (Hz)