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COMMENTARY

Genetics, morphology, and ecological niche modeling do not support the subspecies status of the endangered Southwestern Willow Flycatcher (*Empidonax traillii extimus*)

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ABSTRACT

The Willow Flycatcher (*Empidonax traillii*) breeds throughout much of the United States, northwestern Mexico, and southeastern and southwestern Canada. A subspecies found in the southwestern United States and northwestern Mexico, *E. t. extimus* (Southwestern Willow Flycatcher), is listed as endangered under the U.S. Endangered Species Act (ESA). This subspecies was described in 1948, based on comparisons of a few external morphological characters from an unstated number of specimens and localities, although it was excluded from the last official list of subspecies by the American Ornithologists' Union. Like most avian subspecies, its validity has not been tested with modern morphological or genetic methods. Recent assessments of the subspecies have assumed it to be a distinct taxon, and some authors have limited their comparisons to *E. t. extimus* versus *E. t. adastus* (the subspecies immediately to the north of *E. t. extimus*), excluding comparison to western (*E. t. brewsteri*) and eastern (*E. t. traillii*) subspecies. To test subspecies limits, I reanalyzed available quantitative data on plumage coloration, and genetic variation in mitochondrial DNA and nuclear loci, and found no support for the distinctiveness of the Southwestern Willow Flycatcher. A test of niche divergence suggested that the Southwestern Willow Flycatcher does not have a significantly different climatic niche from its nearest geographic neighbor, *E. t. adastus*. I suggest that the Willow Flycatchers of the Southwest represent peripheral populations of an otherwise widespread species that do not merit subspecific recognition, and are therefore inappropriately listed as endangered under the ESA.

Keywords: Endangered Species Act, mitochondrial DNA, niche modeling, plumage coloration, subspecies, Willow Flycatcher

La genética, la morfología y el modelado de nicho ecológico no apoyan el estatus de subespecie en peligro de extinción de *Empidonax traillii extimus*

RESUMEN

Empidonax traillii se reproduce a través de la mayoría de América del Norte al norte del noroeste de México. Una subespecie hallada en el sudoeste de Estados Unidos y noroeste de México, *E. t. extimus*, está listada como en peligro de extinción bajo la Ley de Especies Amenazadas de Estados Unidos. Esta subespecie fue descripta por primera vez en 1948 en base a un número no especificado de especímenes y localidades. Como la mayoría de las subespecies, su validez no ha sido evaluada usando métodos modernos morfológicos o genéticos. En cambio, los estudios publicados han asumido que la subespecie representa un taxón distintivo. El re-análisis de datos cuantitativos disponibles de la coloración del plumaje y la variación genética en el ADN mitocondrial y el loci nuclear no apoyan el carácter distintivo de *E. t. extimus*. Un examen de divergencia de nicho sugiere que *E. t. extimus* no presenta un nicho climático significativamente diferente de su vecino geográfico más cercano, *E. t. adastus*. Por ende, los individuos de *E. traillii* del sudoeste pertenecen a poblaciones periféricas de una especie ampliamente distribuida que no amerita un reconocimiento sub específico, y por lo tanto se encuentra erróneamente listada como en peligro de extinción bajo la Ley de Especies Amenazadas.

Palabras clave: ADN mitocondrial; coloración del plumaje; *Empidonax traillii*; Ley de Especies Amenazadas; modelado de nicho; subespecies

INTRODUCTION

The U.S. Endangered Species Act (ESA) extends protection to species, subspecies, and the distinct population segment (DPS; vertebrates only). Application of the ESA requires that subspecies and DPSs represent evolutionarily significant populations or groups of populations (Moritz 1994). The reason for this expectation is that nearly all species show geographic variation in at least 1 morphological, ecological, physiological, genetic, or behavioral

characteristic, and it is possible to subdivide this variation taxonomically in numerous ways without yielding evolutionarily significant taxa. This situation is often suggested to reflect the subspecies category in general (Wilson and Brown 1953); in ornithology, Remsen (2005:407) noted that "Widespread, long-standing dissatisfaction with subspecies-level designations in bird classification derives from the historical fact that most bird subspecies were described in an era when quantitative methods were unavailable to assess their validity as entities." Barrowclough (1982) noted that authors of comprehensive quantitative surveys of geographic variation often avoided trinomials whereas studies involving qualitative assessments of color or a few study-skin measurements from a few specimens that represented a few widespread sites often included subspecies descriptions. These observations led Barrowclough (1982:601) to remark that "This strongly suggests to me that most subspecies are not to be taken too seriously."

Others find intrinsic value in subspecies (Haig and Winker 2010), and attempts have been made to define subspecies more rigorously (Patten 2010). Patten and Unitt (2002:26) suggested that "A subspecies is a collection of populations within a biological species that are diagnosably distinct from other such collections of populations." The question then is what is meant by "diagnosably." Probably all populations differ in some respect from other populations. For example, an individual in one area might have an allele that is found nowhere else, or there might be an individual that is larger and darker-colored than any other individual in the species. Although both of these unique phenotypes might represent the "raw material" from which the population could adapt to a changing environment, few would consider this sufficient evidence to warrant a subspecies designation if the criterion is that they are diagnosably distinct. Barrowclough (1982) suggested that subspecies should be held to the same standard as all taxa, in that a taxonomic name at whatever rank should be predictive of concordant patterns of character variation. The "75% rule" (Amadon 1949) requires that "75% of a population effectively must lie outside 99% of the range of other populations for a given defining character or set of characters" (Patten and Unitt 2002:27). I suggest that subspecies or other taxa protected under the ESA should be nearly (>95%) if not completely diagnosable (Cracraft et al. 1998).

The lack of a consistent definition of subspecies has resulted in currently described subspecies ranging from evolutionarily distinct taxa to arbitrary divisions of geographic clines in single characters. Thus, some subspecies are not appropriate for certain scientific or conservation uses. If the goal of an investigator is to identify geographic patterns of potentially adaptive variation, all subspecies might be a useful starting point. By contrast, if a subspecies is to be used in an analysis that requires historically significant units, such as listing under the ESA, it should be based on, or evaluated with, modern molecular, morphological, and ecological methods.

Here, I reanalyze published genetic (Paxton et al. 2008) and morphological (Paxton et al. 2010) data to test the validity of the endangered Southwestern Willow Flycatcher (*Empidonax traillii extimus*). I also analyze all available mitochondrial DNA (mtDNA) sequences in GenBank that have not been pooled previously. In addition, I used methods in ecological niche modeling (Warren et al. 2008, 2010) to determine whether the Southwestern Willow Flycatcher is ecologically distinct in its climatic niche. The goal was to analyze variation irrespective of a priori subspecies limits to determine whether there were geographic patterns in genetics, plumage coloration, or ecological niche dimensions that were consistent with described subspecies limits or any other heretofore undiscovered geographically significant divisions.

Review of Taxonomic History and Recent Studies

The Willow Flycatcher has been divided by taxonomists into subspecies, with up to 7 having been recognized at some time in the past (Phillips 1948, Aldrich 1951, Behle 1985). Most (e.g., Unitt 1987) consider the species to include 4 subspecies: *E. t. traillii*, *E. t. adastus*, *E. t. brewsteri*, and *E. t. extimus* (Unitt 1987). Review of the original subspecies descriptions (Brewster 1895, Oberholser 1918, 1932, 1947, Phillips 1948, Aldrich 1951) reveals that few specimens were examined from relatively few areas. For example, Oberholser (1918) described *E. t. brewsteri* from a total of 13 male specimens taken from Oregon, California, Arizona, New Mexico, and Colorado, and a total of 10 females from Oregon, California, Arizona, and Utah. It is difficult to quantify the range of biological variation present within and among populations with such sample sizes; although this may not have been a goal of early taxonomists, it nonetheless obscured whether variation was clinal or discrete. The wide-ranging *E. t. adastus* was described by Oberholser (1932) on the basis of specimens from Hart Mountain in the Warner Valley of south-central Oregon; Miller (1941) was unable to distinguish this subspecies from others, whereas Aldrich (1951) and Phillips (1948) believed it to be valid, although there is disagreement over its distribution. In the description (Phillips 1948) of *E. t. extimus*, no sample sizes were given and no statistical tests performed. Although *E. t. extimus* was described in 1948, it was excluded from the most recent American Ornithologists' Union (AOU) Checklist that included subspecies (AOU 1957). However, taxonomic assessments of *E. traillii* have included *E. t. extimus* after its original description, and it is listed as an endangered subspecies under the ESA (U.S. Fish and Wildlife Service 1995).

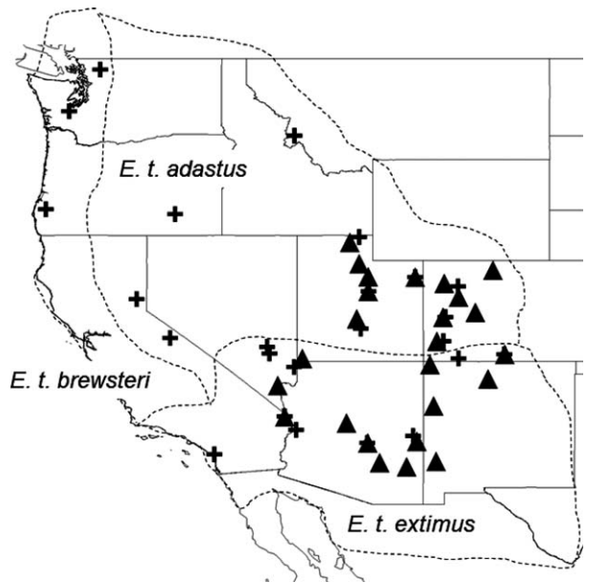

FIGURE 1. Distribution of sample sites for genetics (triangle; Paxton et al. 2008) and coloration (cross, excluding the eastern United States; Paxton et al. 2010), with approximate boundaries of named subspecies.

Several recent studies have compared subspecies of Willow Flycatchers. In a genetic study, Paxton et al. (2008) compared mtDNA sequences and amplified fragment length polymorphisms (AFLPs) from nuclear DNA. Busch et al. (2000) and Paxton (2000) concentrated on AFLP variation within and among subspecies. Paxton et al. (2010) quantified variation in lightness, saturation, and hue of the crown and back. Sample sites (Figure 1) for the 2 larger recent studies (Paxton et al. 2008, 2010) excluded ~75% of the range of *E. t. adastus* and ~50% of the range of *E. t. extimus*. Furthermore, formal comparisons with the subspecies to the west (*E. t. brewsteri*) and east (*E. t. traillii*) were not performed, making it unclear how the study could evaluate the genetic distinctiveness of *E. t. extimus*. Paxton et al. (2011) examined mtDNA sequences from migrant individuals, as well as specimens representing much of the breeding range of *E. traillii* (including all 4 widely recognized subspecies). However, these studies did not provide tests of subspecies limits. For example, Paxton et al. (2010:131) stated that "Because this study was intended to distinguish among established taxonomic units, we grouped breeding sites a priori into one of the four subspecies based on geographic ranges delineated via morphological studies (Unitt 1987, Browning 1993)." However, the samples of *E. t. traillii* were >1,000 km from the nearest sample of *E. t. adastus* or *E. t. extimus*, and the samples of western *E. t. brewsteri* were >900 km from the nearest *E. t. extimus* locality. I contend that the assertion that the subspecies limits are "established" is debatable. Unitt (1987) stated that the most comprehensive study of geographic variation in Willow Flycatchers was by Aldrich (1951). Aldrich's (1951) general review of the subspecies gave no lists of specimens, no values for measurements (except for his description of a new subspecies, *E. t. campestris*, which has subsequently been ignored), and no quantitative character analyses. Unitt (1987) used statistical analyses of character variation, although he too only compared preexisting subspecies limits. Browning (1993) did not perform statistical tests. Therefore, recent studies assumed, rather than tested, whether the data supported described subspecies limits.

A fifth study (Sedgwick 2001) compared vocalizations of Willow Flycatchers from the Southwest, representing localities from within the range of *E. t. adastus* and *E. t. extimus* (again excluding comparison of *E. t. extimus* with *E. t. brewsteri* or *E. t. traillii*). The results suggested that part of the song of *E. t. extimus* differed from that of *E. t. adastus*. Given that *Empidonax* inherit their songs, it might indicate that genetic differentiation exists that was missed by mtDNA and AFLPs. However, Sedgwick's UPGMA phenogram that shows 2 distinct song groups was based on a reduced set of song characters that were chosen because they separated the 2 groups, whereas characters not showing the same (or a different) pattern were excluded. In addition, the 2 clusters did not group samples entirely by subspecies, and Sedwick (2001:366) concluded that his data suggested "moderate introgression of *extimus* genes into the *adastus* gene pool." Lastly, a sampling gap corresponded to the geographic division in song features, reducing the potential to detect gradation in song characteristics between areas. Although the raw data are not available, future studies of vocalizations are warranted.

METHODS

Genetic Differentiation

Paxton et al. (2008) surveyed mtDNA and AFLPs within and among *E. t. adastus* and *E. t. extimus*. The highly variable AFLP data sets (Busch et al. 2000, Paxton 2000, Paxton et al. 2008) were less informative and did not conflict with conclusions drawn from the mtDNA data, and I did not reanalyze the AFLP data. Paxton et al. (2008) documented the occurrence of 33 mtDNA haplotypes at the cytochrome *b* locus in 145 individuals across 25 breeding sites in Arizona (7 sites), New Mexico (4 sites), Colorado (7 sites), and Utah (7 sites). Assuming that table 2 in Paxton et al. (2008) is correct, haplotype C2 in GenBank (AF297261) is incorrect because it has an "A" instead of a "G" at position 708. I reanalyzed the cytochrome *b* data from Paxton et al. (2008) using Arlequin version 3.5.1.3 (Excoffier and Lischer 2010) to compute F_{ST}, a measure of pairwise population differen-

tiation that theoretically ranges from zero (absence of geographic structure) to 1 (fixation), and an analysis of molecular variance (AMOVA), which estimates the proportion of genetic variance attributable to various geographic groupings of populations. Paxton et al. (2011:611) sequenced "316 individuals sampled at 91 sites on the breeding grounds" that represented 62 haplotypes. A search of GenBank (December 13, 2013) found 93 haplotypes for *E. traillii*, but there is no corresponding information on their frequencies or geographic origins, although some are from wintering or migrant individuals (Paxton et al. 2011). Although the geographic localities are not given with the GenBank submissions, Paxton et al. (2011) stated that the sequences represent breeding individuals from *E. t. adastus*, *E. t. extimus*, *E. t. traillii* (New York, Tennessee, Illinois, North Carolina, Virginia, North Dakota, South Dakota, Minnesota, and Maryland), and *E. t. brewsteri* (Washington, Oregon, and California), providing considerable coverage of the breeding range (excluding western Canada and the Maritime provinces). I used Mega6 (Tamura et al. 2013) to construct a neighbor-joining tree (distance = *P* value) with 1,000 bootstrapped replicates, of all 93 haplotypes available in GenBank. Despite uncertainty over the exact geographic origins of haplotypes, if there was significant geographic structure in the species range, it would be recovered by this analysis in the form of significantly supported nodes in the tree.

Morphological Variation

The only available quantitative data (at the level of individuals) that allow testing subspecies limits are from Paxton et al. (2010), who used a colorimeter to measure lightness, saturation, and hue on the back and crown of 374 live-captured individuals from 29 locations. "Lightness" is a measure of how light or dark the color is, whereas saturation and hue reveal color direction in 2-dimensional space. They found that coloration changed over time because of feather wear, and adjusted values for the date measurements were taken by "multiplying the regression coefficient (b) by the date (day of year) starting from the earliest capture date (9 May, day of year_129). These seasonally adjusted color values were used for all subsequent analyses." E. Paxton (personal communication) provided a copy of the adjusted color values (minus the sample from Rush Creek, California). Paxton et al. (2010) divided their samples of breeding individuals into *E. t. adastus* (5 locations, 69 individuals), *E. t. brewsteri* (3, 38), *E. t. extimus* (7, 123), *E. t. traillii* (5, 39), and "boundary" (9, 77). The boundary category included sampling sites between "core" *E. t. adastus* and *E. t. extimus*, although Paxton et al. (2008) previously classified 3 sites (CCCO, DELT, and FICR) as *E. t. adastus* and 1 (MCSP) as *E. t. extimus*. I analyzed the color data using principal component analysis, plotting individuals on orthogonal axes.

Ecological Niche Modeling

The occurrence of Willow Flycatchers in riparian areas of the arid Southwest might be associated with significant ecological distinctiveness from flycatchers breeding elsewhere and representing different subspecies. Thus, in lieu of morphological or genetic distinctiveness of *E. t. extimus*, listing under the ESA could be based on ecological distinctiveness and significance at either the subspecies or DPS level (U.S. Fish and Wildlife Service and National Marine Fisheries Service 1996). I constructed correlative ecological niche models (ENMs; Peterson 2001, Elith et al. 2011) using breeding records from the Breeding Bird Survey (accessed at http://www.pwrc.usgs.gov/bbs) and Ornis2 (http://ornis2.ornisnet.org/) that were input into Maxent version 3.2.2. (Phillips et al. 2006). I divided locality points into those representing *E. t. extimus* ($n = 204$) and *E. t. adastus* ($n = 269$), limiting the latter taxon to localities south of 49°N so as to equalize the areas of the 2 subspecies compared and to avoid confounding variation due to distance with that among putative taxa. There is concern (Unitt 1987) that male *E. t. brewsteri*, at least in California, sing during their migration through the range of *E. t. extimus*, confounding identification of breeding localities from vocalization records (such as the Breeding Bird Survey). Unitt (1987) suggested that only singing male *E. t. extimus* noted after June 20 should be considered breeders, but in the same paper he cited verified breeding dates in May (9, 16, 22, and 26) and early June (5, 12, and 13). Considering data on egg sets, Unitt (1987:158) wrote that "On the basis of 187 dated sets, *extimus* usually begins nesting about 1 June. The sets range in date from 24 May to 30 July; the mean date is 16 June." This suggests that male *E. t. extimus* must sing much earlier when establishing territories. To be conservative, I performed a second niche model in which only localities of singing males noted after June 16 were used (40 *E. t. extimus* and 100 random localities drawn from 184 breeding records of *E. t. adastus*). Climate data (19 layers) were obtained from the Worldclim bioclimatic database (Hijmans et al. 2005).

To determine whether these 2 subspecies differ in niche characteristics, I used the niche identity test and the background test for niche divergence in ENM Tools (Warren et al. 2008, 2010). The niche identity test involved 100 iterations of randomized locality points for the 2 subspecies (maintaining the original number of points for each group), with an ENM constructed for each group in Maxent using the randomized points, followed by calculation of the degree of niche overlap. Schoener's *D* (for details, see Warren et al. 2008, 2010) was used as a measure of niche overlap; it ranges from 0 to 1, with 1 indicating complete overlap. If the observed niche overlap

value is <95% of the randomized niche overlap values (1-tailed test), then the climatic niches of the 2 species are considered significantly different.

The niche identity test assumes that populations "tolerate the exact same set of environmental conditions and have the same suite of environmental conditions available to them" (Warren et al. 2011:17). Thus, it is possible to obtain a significant niche identity test if populations live in differing environments, but this does not mean, necessarily, that the species have diverged ecologically, only that they are able to survive in different climates because of ecological plasticity. Hence, the appropriate test for evaluating whether niches are significantly divergent is the background test, which allows one to assess "whether two species are more or less similar than expected based on the differences in the environmental background in which they occur" (Warren et al. 2011:19). The background test involves multiple iterations in which the niche overlap is calculated between 1 taxon's ENM and an ENM constructed from random points (obtained from Hawth's Tools [Beyer 2004] in ArcGIS9) within the range of the second taxon, where the number of random points is the same as the number of actual points for that taxon (subspecies in the present study). Tests are conducted in both directions. The observed niche overlap between 2 species' ENMs is compared with the distribution of overlap values from the runs in each direction to determine whether species' niches are significantly more or less divergent than expected (2-tailed test). Rejection of the null hypothesis (of niche identity) indicates that observed niche differences are a function of habitat selection and/or availability. If niches overlap more than expected, it is often interpreted as evidence for niche conservatism; if niches overlap less than expected, one can assume niche divergence (Warren et al. 2008, 2010, McCormack et al. 2010). Only in the case of niche divergence would one assume that *E. t. adastus* and *E. t. extimus* had evolved ecological distinctiveness.

RESULTS

Patterns of Genetic Differentiation

Review of prior studies. Paxton et al. (2008) found no fixed mtDNA differences between *E. t. adastus* and *E. t. extimus*. A large percentage of individuals (30%) possessed a common haplotype ("C1"), which was found in 16 localities, although small samples precluded definitive evidence of absence. A second haplotype, D1, was also found in 16 localities although in fewer individuals, and no special significance was attached to it by Paxton et al. (2008). A significant isolation-by-distance (IBD) effect was found using Mantel's (1967) test when considering all samples, which indicates that genetic differences increase with geographic distance between samples. Paxton et al. (2008) did not observe a significant IBD effect within either subspecies, although the geographic distances were much smaller and, hence, do not obviously support their conclusion (Paxton et al. 2008:9) that there is a "rapid change in [haplotype] frequencies at the boundary" between the subspecies. Paxton et al. (2008) also considered how grouping samples into subspecies by moving the boundary north or south affected the amount of genetic variation explained by an AMOVA. This exercise was intended to determine whether a new subspecies boundary would explain more of the genetic variance than the current one. Although there was an effect brought about by changing the boundary, it was not possible to identify a clear and objective boundary. Paxton et al. (2008) constructed a UPGMA dendrogram (their fig. 2) to display the spatial pattern of genetic variation. They found 2 clusters, 1 including populations of *E. t. extimus* and the other a mixture of both subspecies, which does not support the subspecies as described. In addition, the algorithm used to construct UPGMA phenograms results in the "root" being placed at the midpoint of the largest pairwise genetic distance. If the sample of localities is biased geographically, the appearance of 2 clades could recover the pattern of sampling. Even if there were a root to the phenogram, there could be no support for *E. t. extimis*, given the topology.

Paxton et al. (2008) plotted a graph (their fig. 3) of the frequency of the C-group mtDNA haplotypes versus latitude that purports to show a relatively sharp transition between *E. t. extimus* and *E. t. adastus*. Contrary to Paxton et al.'s (2008) figure legend, their figure 3 is not a plot of C-group haplotypes versus latitude but, instead, haplotype frequencies versus a function of latitude and elevation. It is unusual to plot a subspecies boundary in this way, and elevation was not a part of the original subspecies descriptions (reviewed above), which are based solely on latitude and longitude.

New analyses. I plotted (Figure 2) the data from Paxton et al.'s (2008) table 2; these data show a gradual transition between *E. t. extimus* and *E. t. adastus* and, therefore, do not support a sharp genetic transition in C-group haplotypes.

The neighbor-joining tree (not shown) for all 93 unique haplotypes was not consistent with distinct groupings, and the highest bootstrap basal nodal support was 29%. Therefore, although the samples were identified only to U.S. state of origin, there is no phylogenetic structure that could be associated with distinct geographic groupings or named subspecies.

Patterns of Morphological Differentiation

Review of prior studies. Paxton et al. (2010) conducted several analyses, such as a canonical discriminant analysis to "represent the linear relationship of the plumage

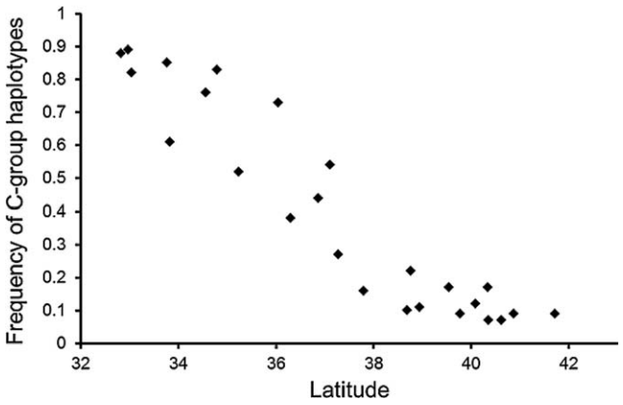

FIGURE 2. Plot showing a smooth, clinal relationship between the latitude of the breeding site and the frequency of the C-group haplotypes for cytochrome *b* data from Paxton et al. 2008 (their table 2).

coloration by subspecies" (Paxton et al. 2010:131). However, this model was built "using only large sample sites within the core area of each subspecies," thereby excluding the boundary sites. Representative conclusions included "While there is overlap in the color values of individual flycatchers from the different subspecies, mean value for the breeding sites tended to cluster together by subspecies" and that the data indicated "intergradation among the subspecies, consistent with a molecular genetic study," that being Paxton et al. (2008).

New analyses. To test the validity of subspecies, I did not group specimens into a priori subspecies, because that process is circular. In Figure 3, I plot the relationship among individuals in *E. t. adastus*, *E. t. brewsteri*, *E. t. extimus*, and individuals from boundary locations on PC1 and PC2. The subspecies are not separated; instead, there is a gradation of variation among them. ~~The same result was obtained when plotting the distribution of individuals in these categories for PC1 and PC2 separately (not shown).~~

Ecological Niche Modeling

The observed niche overlap (Schoener's $D = 0.17$) was much less than the distribution of random values (Figure 4A), which indicates that niche overlap between the subspecies is less than expected at random. However, comparison of the 2 subspecies did not yield a significant background test (Figure 4B), which suggests that the

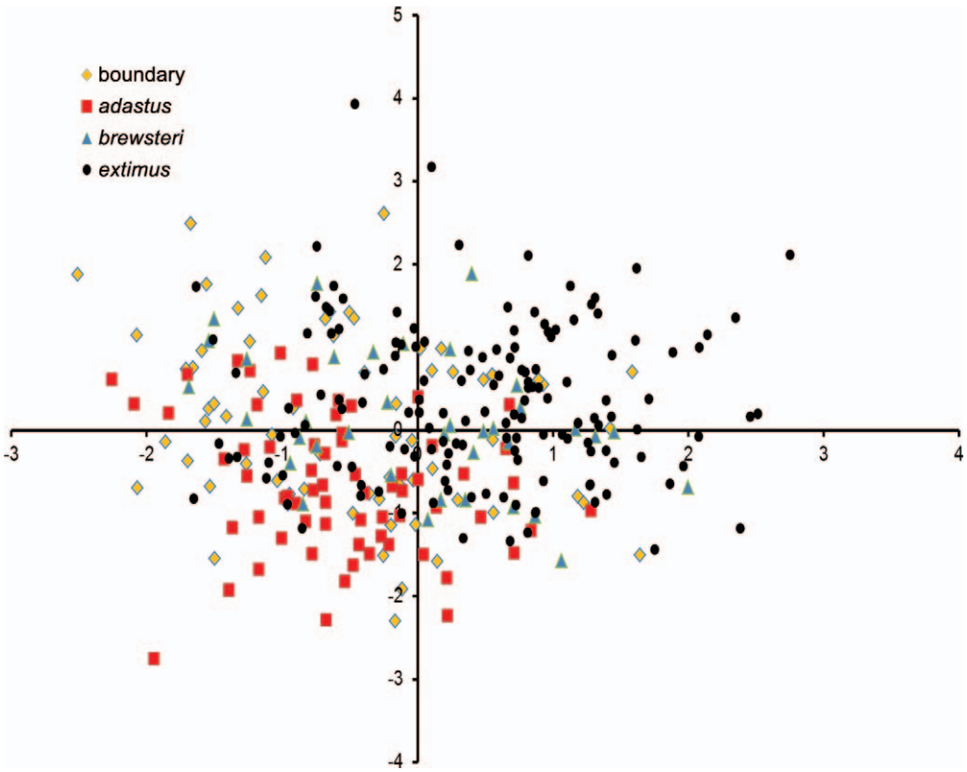

FIGURE 3. Plot of the distribution of 363 individual Willow Flycatchers from 3 subspecies, and boundary localities for the first 2 principal components (PC) based on analysis of 6 color variables (from Paxton et al. 2010). PC1 (horizontal axis) explains 39% of the variation, and PC2 (vertical axis) explains 27%. Note that the subspecies are not distinct and overlap greatly with each other and the boundary locations.

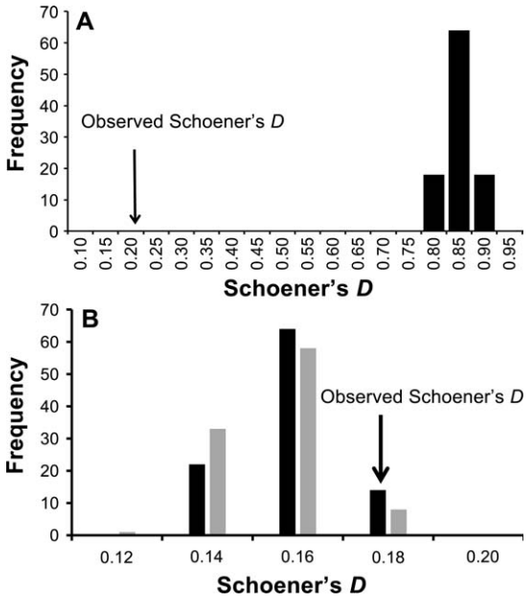

FIGURE 4. (**A**) Results of a niche identity test for *E. t. extimus* versus *E. t. adastus* (100 random repetitions), showing that the observed niche identity (Schoener's *D*; arrow) is much less than expected by chance (black bars) and that the 2 subspecies use different niche characteristics. (**B**) Results of a background test for niche divergence between *E. t. extimus* and *E. t. adastus*, showing that although the subspecies use different niche characteristics, they apparently do not exhibit significant niche divergence. If the subspecies had divergent niches, the observed Schoener's *D* would be less than the random distribution shown by the histogram. ~~Instead the observed value suggests that niches are conserved.~~

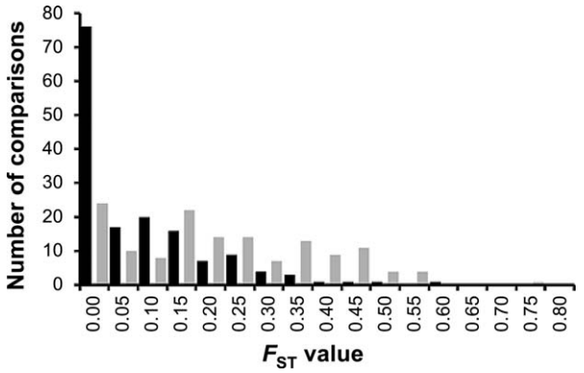

FIGURE 5. Distribution of F_{ST} values between populations within (black bars) and ~~among~~ (gray bars) subspecies in the Willow Flycatcher (data reported by Paxton et al. 2008). The 2 distributions overlap greatly, a result of a gradual transition of genetic variation and a lack of support for distinct subspecies.

observed niche-identity difference is explained by geographic variation in the environmental conditions available to each subspecies within their respective ranges (Warren et al. 2008, 2010, McCormack et al. 2010). That is, the subspecies are using common environmental features as often as one would expect by chance—hence, the species likely has a broad ecological (climatic) tolerance, at least in the area studied, and does not show significant ecological divergence in climate niche dimensions. The same results (not shown) were obtained for the analyses of the samples restricted to a temporal window that was designed to exclude migrant birds (see above).

DISCUSSION

Genetic Differentiation

Empidonax t. extimus is not supported by published genetic (mtDNA or nuclear AFLPs) data as a distinct entity. This result can be illustrated by plotting F_{ST} values within and between subspecies (Figure 5). The 2 distributions overlap because the subspecies boundary is situated arbitrarily on a cline in allele frequencies. The lack of genetic support was noted by Paxton et al. (2008:16): "[T]he evidence for a region of intergradation suggests there is little support biologically for the legal necessity of designating a boundary line. As a result, designating the location of a discrete line separating the 2 subspecies cannot be made unambiguously based on genetic data and thus becomes a value judgment dependent on the philosophy of policy makers." I would argue that if the best available data do not support 2 subspecies, the philosophy of policymakers is irrelevant. Furthermore, the exclusion of comparisons of *E. t. extimus* with *E. t. brewsteri* and *E. t. traillii* is a serious omission, because the validity of *E. t. extimus* cannot be established without testing whether it is distinctive from ~~its other~~ geographically adjacent neighbors. My analysis of all mtDNA sequences in GenBank suggests that none of the subspecies is genetically distinct.

Some authors, as well as the U.S. Fish and Wildlife Service (2011), have concluded that mtDNA alone is insufficient to judge the validity of avian subspecies (Zink et al. 2013). Zink and Barrowclough (2008) pointed out that mtDNA has a greater chance than nuclear loci of detecting genetic differences between recently isolated populations and subspecies, owing to the more rapid coalescence time of mtDNA. Thus, in species without geographically structured mtDNA variation, there is a very low likelihood of differentiation at nuclear loci (Lee and Edwards 2008, Hung et al. 2012). However, because some exceptions exist (Toews and Brelsford 2012), it is important that Paxton (2000) analyzed 197 polymorphic nuclear AFLP markers, although low by modern standards, and found no geographic structure that corresponded to subspecies limits.

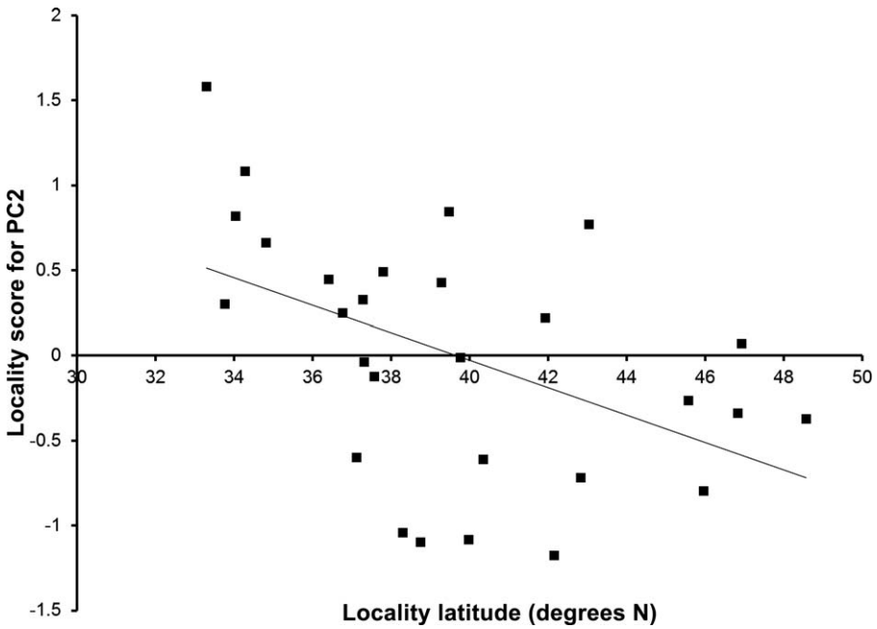

FIGURE 6. Plot of the average score for individuals from 26 sites on principal component 2 (PC2; from analysis of 6 adjusted color variables) versus latitude (from Paxton et al. 2010), showing that if only the geographic extremes were compared, it would give the illusion of statistical significance—when, in fact, there is gradual variation between sites.

Morphological Differentiation

The genus *Empidonax* is known for a high number of sibling species, which are defined as closely related species that lack distinctive morphologies. Hence, one might suggest that analyses of morphology would be uninformative for assessing taxonomic limits below the species level (e.g., subspecies). However, each of the subspecies was described as having differing coloration. For example, *E. t. traillii* is described as having "Entire head, neck and upper parts greenish and relatively uniform" (Phillips 1948:508). *Empidonax t. adastus* was described as "Head (especially sides of neck) grayish, more contrasted with the back than in *traillii* or *alascnesis*; back duller and more grayish green" (Phillips 1948:510). *Empidonax t. extimus* was considered "The palest race of *E. traillii*. Adults most closely resemble *adastus*, but are even paler above" (Phillips 1948:513). Phillips (1948:510) stated that specimens of *E. t. adastus* were "practically identical with those of *E. t. extimus*." Phillips (1948:514) also remarked that "A male in peculiar, retarded plumage from the Gallinas River at Las Vegas, New Mexico, May 23, seems to be typical *extimus*." Presumably, Phillips meant that it was unusual to find a spring male that had not yet molted out of its previous plumage, but he does not describe diagnostic plumage characteristics of the subspecies at different plumage stages, so it is not clear how he ascertained that it was "typical."

The differences in coloration noted in the subspecies descriptions make relevant the quantitative data on head and back coloration of Paxton et al. (2010). Analyses of these color data did not support the subspecies limits, because the coloration grades between them. I predict that a multivariate analysis of standard morphological characters from samples throughout the range (e.g., Zink 1986, Rising 2001, McKay 2008, Skalski et al. 2008) would reinforce the results from the analysis of coloration. The importance of proper sampling is critical. As noted above, Paxton et al. (2010) excluded boundary samples and tested for differences between core areas. The problem with these tests can be appreciated by inspection of Figure 6, which is a plot of PC2 values versus latitude, showing a gradual geographic progression. If only samples from 32–36°N and 45–49°N were compared (putative core areas), there would likely be a statistically significant difference, whereas inclusion of intermediate samples reveals the biased nature of that inference.

It is also a concern that Paxton et al. (2010) obtained data from living specimens that were released, eliminating the possibility of others verifying their measurements. Although analysis of museum specimens is preferable, there are insufficient specimens in collections, and it is not feasible to collect a large series of museum specimens of this endangered bird.

Ecological Distinctiveness

Hatten et al. (2010) provided a perspective on the habitat of *E. t. extimus* but did not make quantitative tests of niche differentiation. I found that niche identities for populations

from different areas differ more than expected by chance (Figure 4A), which is understandable given the large degree of environmental variation that exists across the range. The insignificant background test (Figure 4B) suggests that the species might have a broad ecological tolerance (e.g., plasticity) that allows it to exist across a broad range of environmental conditions without evolving special ecological adaptations, at least in the western portion of the range. Therefore, the available data do not support the Southwestern Willow Flycatcher as being ecologically distinct, although future studies might identify niche dimensions other than climate that could distinguish taxa.

Subspecies Limits and Conservation

The subspecies of Willow Flycatcher, including the Southwestern Willow Flycatcher (*E. t. extimus*), were described using traditional methods that involved few specimens scattered from throughout the range, and few characters that were not analyzed quantitatively or multivariately. Although this was typical for the era when most subspecies were described (Zink and Remsen 1986), use of subspecies for many modern applications requires assessment of their ~~degree of~~ diagnosability. The present study found that molecular, morphological, and ecological data did not support the Southwestern Willow Flycatcher as a distinct subspecies (or DPS). It would seem that the most recent official checklist of subspecies of North American birds (AOU 1957) was prescient in excluding *E. t. extimus*. Hence, the Willow Flycatchers of the Southwest represent peripheral populations of an otherwise widespread species that do not merit subspecific recognition and are therefore inappropriately listed as endangered under the ESA.

The ESA was designed to protect distinct elements of biodiversity. The U.S. Congress directed the Fish and Wildlife Service to use the ESA "sparingly" when listing distinct population segments (Bernhardt 2008), and it would follow that listed taxa should be strongly differentiated in at least 1 genetically based character set. The importance of listing only distinct subspecies is revealed by the cost of endangered species conservation. McCarthy et al. (2012) estimated that $1 billion is needed annually to reduce the threat level of globally threatened bird species, obviously an unrealistic level of support. Perhaps, going forward, the null hypothesis for avian subspecies should be that they are not appropriate for listing without a modern assessment of their validity.

Future Directions

In a sample of 234 North American songbird species, the average number of subspecies is 3.34 (R. M. Zink personal observation). Hence, a primary question: What attributes would qualify a subspecies for recognition as an evolutionarily significant unit, appropriate for listing under the ESA? Barrowclough (1982) suggested that a subspecies name should be predictive of concordant patterns of variation in multiple characters. This criterion would eliminate many named subspecies, including the Southwestern Willow Flycatcher. Zink (2004) and Phillimore and Owens (2006) found that, on average, temperate-breeding bird species possess ~2 evolutionarily significant units per species, based on patterns of genetic variation at neutral loci (e.g., mtDNA). In my opinion, if the Southwestern Willow flycatcher corresponded to ~~1 of these units~~, it would be qualified for ~~listing~~. ~~M~~molecular data indicate that the number of listable avian taxa is twice the number of species but considerably less than the number of subspecies.

I am concerned that a double standard may exist among conservation biologists involving the nature of data required for listing under the ESA. For example, the U.S. Fish and Wildlife Service has relied on results of mtDNA studies in >80 cases since 1989, although they (U.S. Fish and Wildlife Service 2011) did not accept mtDNA evidence that suggested that the California Gnatcatcher (*Polioptila californica californica*) was not distinct (Zink et al. 2013). I suspect that if there were a pattern of reciprocal monophyly in the mtDNA of Southwestern Willow Flycatchers, many would consider it sufficient to establish the evolutionary-independence requisite for ESA listing. However, this was not the case, and some will claim that investigators have just not studied enough characters to disprove a hypothesis of no distinctiveness. Listing under the ESA requires the best available scientific and commercial data—which, in the case of the Southwestern Willow Flycatcher, are not currently supportive of its status. It is important to recognize that sometimes it is necessary to make a listing or delisting decision and not wait for a new technique that might provide a different answer.

It is fair to ponder what new data or analyses might be relevant. I noted above that a thorough study of vocalizations could be useful. I do not think there are enough specimens in museums to conduct a decisive morphological analysis, and although live specimens could be measured and released, it is my opinion that the data cannot be combined with museum specimens, and that all specimens, whether live or in museums, should be measured multiple times by the same observer to minimize and quantify measurement error (Rojas-Soto 2003). The other new horizon in geographic variation research involves the use of "genomics," or surveys of large numbers of loci (Lerner and Fleischer 2010). It remains to be seen whether genomics will reveal patterns of geographic variation that will corroborate the limits of most avian subspecies, or instead will reveal myriad geographic patterns that defy a simple interpretation. It will also be of interest to observe whether genomic studies that fail to support subspecies limits are also considered

inadequate in some way. That is, one might wonder what technique will supplant genomics or whether we have indeed achieved the holy grail of molecular methods.

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