

GENETIC VARIATION IN THE ENDANGERED SOUTHWESTERN WILLOW FLYCATCHER

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ABSTRACT.—The Southwestern Willow Flycatcher (*Empidonax traillii extimus*) is an endangered Neotropical migrant that breeds in isolated remnants of dense riparian habitat in the southwestern United States. We estimated genetic variation at 20 breeding sites of the Southwestern Willow Flycatcher (290 individuals) using 38 amplified fragment length polymorphisms (AFLPs). Our results suggest that considerable genetic diversity exists within the subspecies and within local breeding sites. Statistical analyses of genetic variation revealed only slight, although significant, differentiation among breeding sites (Mantel's $r = 0.0705$, $P < 0.0005$; $\theta = 0.0816$, 95% CI = 0.0608 to 0.1034; $\Phi_{ST} = 0.0458$, $P < 0.001$). UPGMA cluster analysis of the AFLP markers indicates that extensive gene flow has occurred among breeding sites. No one site stood out as being genetically unique or isolated. Therefore, the small level of genetic structure that we detected may not be biologically significant. Ongoing field studies are consistent with this conclusion. Of the banded birds that were resighted or recaptured in Arizona during the 1996 to 1998 breeding seasons, one-third moved between breeding sites and two-thirds were philopatric. Low differentiation may be the result of historically high rangewide diversity followed by recent geographic isolation of breeding sites, although observational data indicate that gene flow is a current phenomenon. Our data suggest that breeding groups of *E. t. extimus* act as a metapopulation. Received 4 March 1999, accepted 18 October 1999.

CHARACTERIZING GENETIC variation in fragmented populations is an important part of endangered species conservation. Decline and extinction of wild populations have been linked to inbreeding (Keller et al. 1994, Saccheri et al. 1998) and loss of genetic diversity (Westemeier et al. 1998). Although demographic and environmental factors also are important in population declines (Lande 1988, Caro and Laurenson 1994), genetic factors such as gene flow, diversity, and population differentiation can be useful for evaluating population viability (Frankham 1995, Haig 1998). Identifying genetic issues enhances the effectiveness of management, especially in early stages when options may be the most flexible (Haig 1998). Genetic surveys also advance our knowledge of the microevolutionary processes of genetic differentiation and diversification.

We report the results of a rangewide genetic survey of the Southwestern Willow Flycatcher (*Empidonax traillii extimus*), a federally endan-

gered subspecies of *E. traillii*. Southwestern Willow Flycatchers breed in the scattered and isolated remnants of dense riparian habitat in the southwestern United States. This subspecies has declined in numbers and range through the 20th century (Unitt 1987, Browning 1993). The current population is estimated to be approximately 700 breeding pairs, with more than half of the known breeding sites consisting of five or fewer adult pairs (United States Geological Survey unpubl. data). The United States Fish and Wildlife Service identified reduced gene flow among remaining breeding sites as a potential threat to the subspecies (USFWS 1993, 1997). Previous genetic studies of Willow Flycatchers (Zink and Johnson 1984, Seutin and Simon 1988, Winker 1994) did not include *E. t. extimus*, making our study the first in-depth genetic assessment of this endangered subspecies.

We used the recently developed amplified fragment length polymorphism (AFLP) technique (Vos et al. 1995) to generate markers for our analysis. This PCR-based method produces a DNA fingerprint for each individual that may

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be used to estimate genetic variation in populations. AFLPs are similar to restriction fragment length polymorphisms (RFLPs) in their ability to detect changes at restriction sites. However, because they also are sensitive to nucleotide substitutions at any of six selective positions (three on the forward primer and three on the reverse), their mutation rate is more comparable to that of randomly amplified polymorphic DNAs (RAPDs) than to RFLPs. Like RAPDs, AFLPs generate dominant markers that cannot be used to directly measure allele frequencies. Nonetheless, they possess a high degree of resolving power that is effective for paternity analysis (Questiau et al. 1999) and the detection of population structure that would be predicted *a priori* (Travis et al. 1996, Yan et al. 1999). Several qualities make this technique valuable in genetic surveys of birds. First, generating AFLPs does not require previous knowledge of the genome of interest or a large investment of development time. Second, studies of endangered species are often complicated by small sample sizes. One way to approach this problem is to analyze a larger number of markers, which can be generated quickly using the AFLP method. Third, AFLP is a PCR-based approach, and only small amounts of tissue or blood are needed as a DNA template source (thereby minimizing disturbance to the study organism).

Our goals were to estimate the level of genetic diversity in *E. t. extimus*, assess the degree of genetic structuring in *E. t. extimus*, and determine whether any breeding groups were genetically unique. One criticism of genetic surveys is that they typically lack observational data (Bossart and Prowell 1998, Haig 1998). We compared observational data from ongoing field studies (Paxton et al. 1997, Netter et al. 1998) against the results of our genetic analyses. We found slight genetic structuring among breeding groups, which may be explained by the philopatry that was observed in the majority of banded birds. Importantly, we also documented extensive gene flow among breeding groups and an absence of any genetically unique and/or isolated groups, which probably resulted from among-site movements that we documented in the field.

METHODS

Field collections.—We collected blood samples from Southwestern Willow Flycatchers at breeding sites in

Arizona, California, Colorado, New Mexico, and Nevada (Fig. 1, Table 1). All of these sites are considered biologically (Unitt 1987, Browning 1993) or administratively (USFWS unpubl. data) to contain *E. t. extimus*. To avoid including migrants in the samples, we used only adults that were confirmed to be reproductive or territorial at a single breeding site during the nonmigratory period of the breeding season (15 June to 20 July 20; Unitt 1987). This resulted in samples from 290 adults in 1996 and 1997 (Table 1). We played recorded vocalizations to attract adults into mist nets. Each captured bird was fitted with a unique combination of color bands and a numbered aluminum band before release.

We obtained blood by clipping a toenail and rinsing the drop of blood into a 1.5-mL tube with about 40 μ L of collection buffer ($1 \times$ SSC, 50 mM EDTA). Samples were stored on ice and then frozen. Between each use, nail clippers were thoroughly cleaned with sterile alcohol swabs as a safety measure for the birds and to prevent transfer of blood between samples. Recapture studies have not identified discernible negative effects from toenail clipping (Paxton and Sogge 1996, Netter et al. 1998). This method is quick and does not require venipuncture (therefore reducing trauma) and yields enough DNA (200 ng to 20 μ g) for multiple uses in PCR-based protocols.

DNA analysis.—DNA was isolated from blood following the procedure described by Mullenbach et al. (1989). Blood was digested overnight at 55°C in lysis buffer (10 mM Tris, 1 mM EDTA, 1% SDS, 100 mM NaCl, pH 8) with 200 μ g/mL proteinase K and 2M DTT (final concentration for both). This was extracted with chloroform and followed by isopropanol precipitation. An aliquot of each DNA extraction was electrophoresed on 0.7% agarose gels to assess template quality and quantity.

Genetic markers were generated using the AFLP procedure as described by Vos et al. (1995) and Travis et al. (1996) with the following modifications. Restriction-ligation reactions (RLs) were conducted using 50 to 200 ng of DNA in $1 \times$ RL buffer (10 mM Tris-base, 10 mM K-Ac, 10 mM Mg-Ac, 5 mM DTT, pH 7.5) with 5 U of both *EcoRI* (Gibco BRL) and *MseI* (NEB) restriction enzymes. RLs were incubated for 1 h at 37°C, at which time the following ligation reagents were added in a 10- μ L aliquot: 5 pmol each of two *EcoRI* adapters (Vos et al. 1995), 50 pmol each of two *MseI* adapters (Vos et al. 1995), 1 U T4 DNA ligase, $1 \times$ RL buffer, and 0.2 mM ATP (final concentration for the latter two reagents). RLs were incubated for an additional 3 h at 37°C and then stored at -20°C. An aliquot of each sample was diluted 1:10 in TE (10 mM Tris, 0.1 mM EDTA, pH 8.0) for use as template in the first selective amplification.

The first and second selective amplifications and polyacrylamide gel electrophoresis took place according to Vos et al. (1995) with modifications described in Travis et al. (1996). Adenine was used as

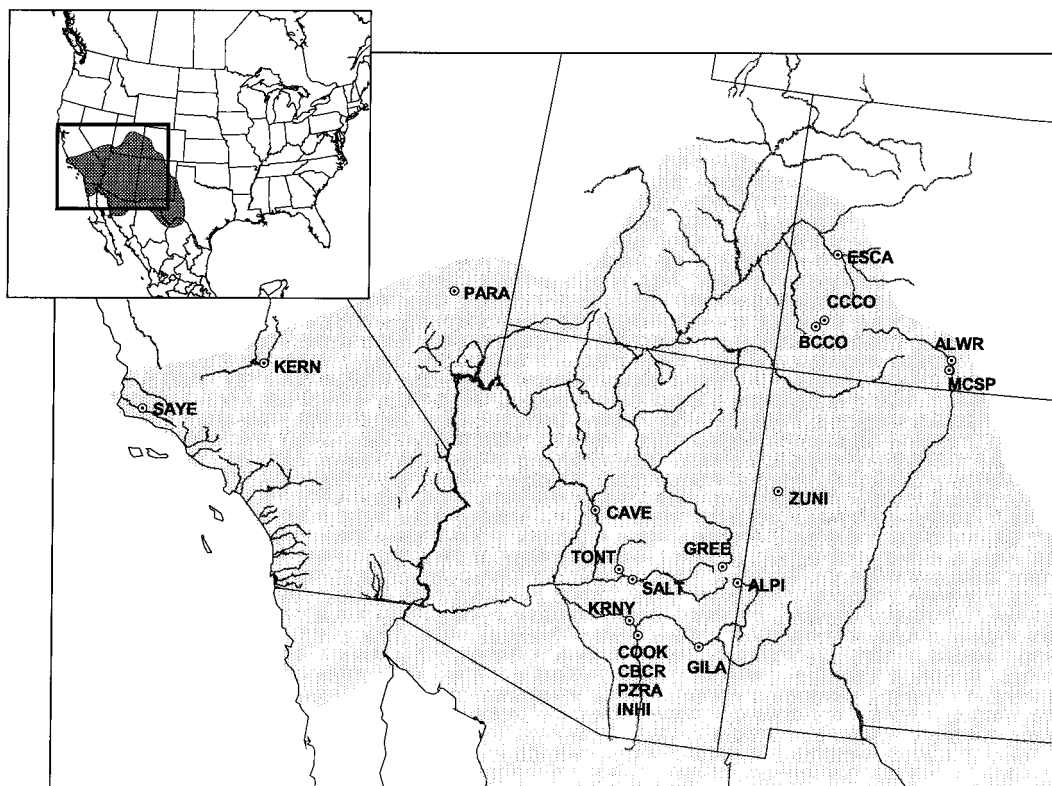


FIG. 1. Breeding range of the Southwestern Willow Flycatcher and collection locales (circles). Shading indicates the approximate boundary (Unitt 1987:figure 1, Browning 1993). Site abbreviations are in Table 1. COOK, CBCR, PZRA, and INHI are represented by a single circle but are distinct breeding sites in close proximity to each other.

the selective nucleotide in the first selective amplification. The selective nucleotides used in the second selective amplifications were AGC/ACA, AGC/AGC, AGC/ACT, AGC/AGC, AGC/AGG, and AGC/ATC (*EcoRI/MseI* primers, respectively).

AFLP data set.—We scored polymorphic AFLP markers manually. To avoid scoring ambiguous markers, we took the conservative approach advised by Haig et al. (1994) and scored only those markers that were distinct, intense, and highly reproducible. An important criterion for marker choice was the complete absence of any background noise at null allele positions. From 197 total polymorphic markers, we chose a set of 38 to construct a data set pooled over all fragments and primer combinations from 290 birds collected in 1996 and 1997.

To detect within-year errors caused by data entry and/or AFLP discrepancies, we replicated 15 birds collected in 1996 and 43 birds collected in 1997 as blind controls. We observed an error rate of 0.77% in 1996 and 0.72% in 1997, demonstrating a high within-year reproducibility for these 38 AFLP markers. However, we also discovered a 6.24% error rate that

arose from between-year inconsistencies. This between-year error rate was measured empirically by replicating 20 birds collected in 1996. Products from the first selective PCR were prepared in 1996 and frozen for one year. DNA templates from these same 20 birds were also frozen for one year and then taken through the entire AFLP procedure in 1997. The 1996 and 1997 preparations were placed in neighboring lanes on polyacrylamide gels to negate errors from gel artifacts. The overwhelming majority of the between-year inconsistencies (5.60 of 6.24%) occurred in only 3 of the 20 birds. Therefore, it is possible that a minority of birds accounts for most of the error in the combined data set. To ascertain between-year effects, we separated all 1996 and 1997 birds by year (110 and 180, respectively) and ran the same statistical tests as were run on the combined data set. Results from each year were similar to those of the combined analysis, providing strong evidence that between-year errors did not impair our genetic study.

Data analysis.—Genetic diversity at each breeding site was estimated using average heterozygosity (H) and proportion of polymorphic loci (P) as described

TABLE 1. Collection locales, sample sizes, and estimates of genetic diversity for Southwestern Willow Flycatchers.

Site	Location	State	<i>n</i>	<i>H</i> ^b	<i>P</i> ^c	Territories ^a	
						1996	1997
ALPI	Alpine, San Francisco River	AZ	4	0.221	52.63	3	—
GILA	Gila River near Safford	AZ	6	0.286	73.68	4	—
GREE	Greer, Little Colorado River	AZ	14	0.289	81.58	11	7
INHI	Indian Hills, San Pedro River	AZ	17	0.343	86.84	—	15
CAVE	Camp Verde, Verde River	AZ	21	0.312	86.84	6	10
CBCR	CB Crossing, San Pedro River	AZ	5	0.265	71.05	—	5
COOK	Cook's Lake and Cook's Seep	AZ	19	0.348	89.47	17	13
KRNY	Kearny, Gila River	AZ	8	0.320	81.58	—	8
PZRA	PZ Ranch, San Pedro River	AZ	26	0.322	89.47	13	5
SALT	Salt River inflow, Roosevelt Lake	AZ	40	0.323	84.21	22	18
TONT	Tonto Creek inflow, Roosevelt Lake	AZ	26	0.299	84.21	17	21
KERN	Kern River near Isabella Reservoir	CA	36	0.325	81.58	34	30
SAYE	Santa Ynez River	CA	9	0.278	76.32	—	10
ALWR	Alamosa NWR, Rio Grande	CO	16	0.306	84.21	30	30
BCCO	Beaver Creek	CO	6	0.235	65.79	—	10
CCCO	Clear Creek	CO	8	0.288	76.32	—	9
ESCA	Escalante State Wildlife Area	CO	12	0.316	81.58	10	8
MCSP	McIntire Springs	CO	6	0.251	71.05	—	8
ZUNI	Zuni Pueblo	NM	5	0.256	63.16	—	5
PARA	Pahranagat NWR	NV	6	0.273	68.42	—	5

^a Estimated number of territories (based on Langridge and Sogge 1997, Owen and Sogge 1997, Sferra et al. 1997, Whitfield et al. 1997, USGS unpubl. data).

^b Estimated heterozygosity (Nei 1978).

^c Percent polymorphic loci.

in Hartl and Clark (1989). These descriptive statistics were calculated using the Tools for Population Genetic Analyses (TFPGA) software package (Miller 1997). Each AFLP marker was assumed to be dominant and to correspond to an independently segregating Mendelian locus whose genotype frequencies conformed to Hardy-Weinberg equilibrium. TFPGA estimated allele frequencies using the Taylor expansion approach of Lynch and Milligan (1994). *H* was estimated using Nei's unbiased heterozygosity (Nei 1978), and *P* was calculated as the proportion of loci at which the most common allele had an estimated frequency of less than 0.95. Because *H* and *P* can vary in relation to population size (Stangel et al. 1992, Travis et al. 1996), we performed a linear regression analysis of the estimated number of territories at each site (*N*) with *H* and *P*. At breeding sites where *N* changed between 1996 and 1997, we used the average from the two years. We also regressed sample size against *H* and *P* to determine if it was related to these two measurements.

Because AFLP markers have not been used widely in genetic surveys of natural populations, we ran three different statistics that are capable of detecting population structure: Mantel's *r* (Sokal and Rohlf 1995, Miller 1999), θ (Weir 1996, Weir and Cockerham 1984), and Φ_{ST} (Excoffier et al. 1992). The hypothetical null population against which we tested our data is considered to be an unfragmented population where within-site genetic similarities are equivalent

to between-site genetic similarities, allele frequencies are the same among sites, and barriers to gene flow are absent. Essentially, Mantel's *r*, θ , and Φ_{ST} test whether birds from a particular breeding site are more similar to each other than to birds from any other site. If they are, then some degree of population structure exists.

We used the program MANTEL-STRUCT (Miller 1999) to calculate average within- and between-site similarities of individual AFLP marker profiles as quantified by the Jaccard coefficient. MANTEL-STRUCT was then used to perform a variation of a Mantel test (Sokal and Rohlf 1995, Miller 1999) to test the null hypothesis that no differences existed in within- and between-site similarities of individuals, a condition that would suggest a lack of population structure. This analysis was conducted on individuals from all sites simultaneously by calculating the correlation between the interindividual similarity coefficient matrix and a congruent binary matrix containing 1s in the positions of within-site similarities and 0s in the positions of between-site similarities. In such an analysis, larger correlation coefficients indicate greater genetic differentiation of sites. The significance of the correlation was obtained using the asymptotic approximation provided by Mantel (1967).

Values of θ and Φ_{ST} are commonly used indicators of genetic differentiation that estimate Wright's F_{ST} . If all subpopulations (breeding sites) exhibit similar

allele frequencies, then F_{ST} will equal 0 (Hartl and Clark 1989). Therefore, departures from 0 in θ and Φ_{ST} would indicate spatial genetic heterogeneity among *E. t. extimus* breeding sites. TFPGA calculated θ using the same assumptions and allele frequencies for estimating H and P (above). We tested the significance of θ by generating 95% confidence intervals around the statistic through the use of a bootstrapping procedure (5,000 replicates). Confidence limits around θ that did not overlap with 0 were taken as evidence of significant genetic differentiation of breeding sites. Because the assumption of Hardy-Weinberg equilibrium might be invalid in *E. t. extimus* breeding groups and some of the sample sizes were small, we calculated Φ_{ST} with an analysis of molecular variance (AMOVA) using Arlequin 1.1 (Schneider et al. 1996). This procedure generated an F_{ST} analog based solely on AFLP marker phenotypes as opposed to the estimated allele frequencies used in the calculation of θ . We calculated interindividual genetic distances (D) by subtracting the Jaccard similarity coefficients (S) from one ($D = 1 - S$). The significance of Φ_{ST} was evaluated with a Monte-Carlo procedure consisting of 9,999 replicates.

Finally, we obtained a graphical representation of average individual between-site similarities (computed from MANTEL-STRUCT) using the UPGMA cluster analysis feature of NTSYS-pc (Rohlf 1993). The UPGMA dendrogram was used to visually demonstrate the level of relatedness between breeding groups and identify those that may be genetically unique.

RESULTS

AFLP patterns.—We generated a total of 708 AFLP markers (511 monomorphic and 197 polymorphic) using six primer combinations. Numbers of total markers produced by each primer combination were: AGC/ACA = 143, AGC/ACG = 33, AGC/ACT = 144, AGC/AGC = 153, AGC/AGG = 109, and AGC/ATC = 126 (*EcoRI/MseI* primers, respectively). No two flycatchers had identical AFLP profiles for the 38 polymorphic markers we used, nor did we find site-specific diagnostic markers from 20 breeding groups that were sampled across the subspecies' range.

Genetic diversity and differentiation.—Among the 20 breeding sites, H ranged from 0.221 to 0.348 and P from 52.63 to 89.47% (Table 1). Both H and P increased significantly with N and n (Figs. 2A–D), which means that predicting H and P with estimated numbers of territories may be confounded by sample size.

All three statistics that we used to assess

population structure indicated significant genetic differentiation among breeding sites. Of the two F_{ST} estimates, θ was highest ($\theta = 0.0816$, 95% CI = 0.0608 to 0.1034) and Φ_{ST} was lowest ($\Phi_{ST} = 0.04578$, $P < 0.001$). Confidence limits for θ did not overlap with zero, which provides evidence that genetic differentiation among sites was statistically significant. Although not an F_{ST} analog, Mantel's r yielded a comparable level of population differentiation ($r = 0.0705$, $P < 0.0005$). In these analyses, larger values indicate greater genetic differentiation of sites. The low values generated by each method imply that breeding site differentiation is slight. We ran these same statistical tests on the 1996 and 1997 birds separately to factor out between-year errors. Results for each separate year were similar to those reported above and were significant at the same levels (data not shown).

The UPGMA results were consistent with the above patterns and also suggested low genetic differentiation. The dendrogram displayed long branch lengths between breeding sites but short branch lengths separating interior nodes, and no one site stood out as uniquely separated from the others (Fig. 3). The 20 breeding sites formed several weak geographic clusters that would be predicted *a priori*. For example, the two California sites (KERN and SAYE), the three western Colorado sites (ESCA, CCCO, and BCCO), the two high-elevation Arizona sites (ALPI and GREE), and three of the southern Arizona sites (CBCR, KRNY, and PZRA) were in clusters that contained close geographic neighbors. However, some features of the dendrogram could not be explained by geography, such as California sites clustering with Colorado sites and Arizona sites (INHI, COOK) that did not cluster with their nearest neighbors.

DISCUSSION

Utility of AFLPs.—The AFLP technique was very useful in this survey of genetic variation in Southwestern Willow Flycatchers. We found its main advantages to be (1) fast development time, (2) high within-year reproducibility, (3) the ability to be based on small quantities of DNA, and (4) the ability to generate large numbers of polymorphic markers from just six primer combinations. This marker system was

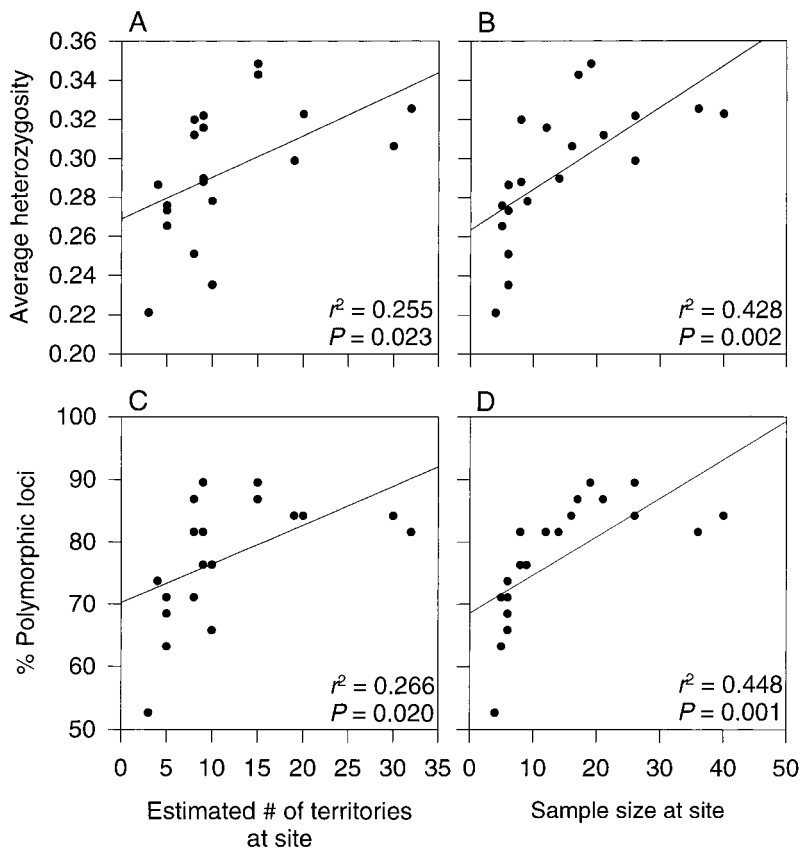


FIG. 2. Linear regression of genetic diversity with estimated number of territories per site (A, C) and sample size per site (B, D).

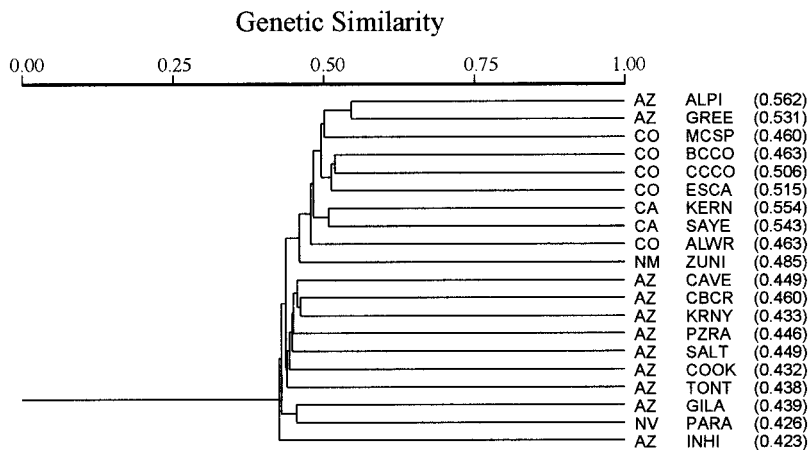


FIG. 3. UPGMA dendrogram of average between-site genetic similarities of Southwestern Willow Flycatcher breeding sites. Scale indicates genetic similarity (Jaccard coefficient). Numbers in parentheses are average within-site similarities of individuals.

overlooked in a recent review of molecular methods used for conservation (Haig 1998). This is unfortunate, because the AFLP technique can be applied to species for which little or no genomic information is available, as is often the case in conservation studies.

The six primer pairs we used yielded very complex marker patterns, with the exception of AGC/ACG. This primer pair produced simpler patterns and the highest ratio of scorable markers (data not shown). We have discovered in subsequent AFLP studies on Southwestern Willow Flycatchers and Wild Turkeys (*Meleagris gallopavo*) that most primer pairs with three selective nucleotides produce highly complex patterns when used against bird templates. However, *EcoRI* primers with the selective nucleotides ACG, ACC, and AGG produce simple, easily resolved marker patterns (20 to 30 loci), especially when combined with *MseI* primers that are GC-rich.

The main limitations of this technique were (1) production of dominant markers, and (2) problems with between-year reproducibility. When using dominant markers such as AFLPs and RAPDs, allele frequencies must be estimated from Hardy-Weinberg assumptions. This makes AFLP markers more susceptible to bias than codominant markers such as RFLPs, microsatellites, and allozymes (Yan et al. 1999, Zhivotovsky 1999). The cause of our between-year error is not known, although Arens et al. (1998) reported that AFLPs are sensitive to methods of DNA extraction. Factors affecting our between-year results would include fresh versus frozen DNA, differences in the efficiency of the restriction-ligation reaction, and minute changes in the first and second selective PCRs. Therefore, when multiyear studies are planned, we recommend preparing templates collected in different years at the same time, even if that means re-running a subset of templates. This will demand more DNA from older samples, but the AFLP technique does not require a large amount of template. At the very least, a set of between-year replicates needs to be included, something that is not routinely exercised in conservation genetics analyses.

In an attempt to see how between-year errors affected our results, we split our data set by year and ran the entire genetic analysis on the 1996 and 1997 birds separately. The results from these analyses were similar to those from

the combined analysis and were significant at the same levels. One possible explanation for this is that most of the between-year inconsistencies occurred in only 3 of the 20 birds, and in the combined data set a similar minority accounted for most of the error and was offset by the large overall sample size. We conclude that between-year inconsistencies did not impair our survey of genetic variation in *E. t. extimus*.

Genetic diversity and differentiation.—In the southwestern United States, modification and loss of riparian habitat have contributed to the decline and fragmentation of the rangewide distribution of *E. t. extimus* (Unitt 1987). Our estimates of H and P suggest that substantial genetic diversity remains in the breeding groups we sampled. This is encouraging, because most of the sites were small and geographically disjunct, which leaves them more susceptible to drift and fixation than large breeding sites. Although estimates of diversity were higher in larger breeding groups, increases in H and P may not have been due solely to increases in the number of territories at a breeding site, because sample size also plays a role in estimating H and P .

Each of the three indicators of genetic differentiation that we used suggested slight but significant genetic differentiation among breeding groups of *E. t. extimus*. The statistically significant structure detected by these tests does not appear to translate into biologically significant structuring of breeding groups. Multiple lines of genetic evidence suggest that these breeding groups function as a metapopulation and regularly exchange genetic material. First, we found no site-specific diagnostic markers from 20 breeding groups sampled rangewide, nor were any two AFLP profiles identical for the 290 birds sampled. This indicates that substantial genetic variation exists rangewide. Second, values of r , θ , and Φ_{ST} each indicated that a low proportion of the total genetic variation in these birds resulted from partitioning individuals by breeding site. In our study, more than 90% of the total genetic variation occurred among individuals. Finally, the UPGMA dendrogram exhibited a weak clustering of breeding sites according to geographic location but did not identify any breeding groups that were uniquely separated from the others.

Observational data from field studies are consistent with genetic results and demon-

strate a high degree of movement between breeding groups. Of the adults banded at Arizona breeding sites from 1996 to 1998 (Arizona being the focus of the banding study), 47% have been recaptured or resighted (Paxton et al. 1997, Netter et al. 1998). One-third of these (15% of total) changed breeding sites between years, usually in the same river drainage and less frequently between drainages up to 190 km distant (Netter et al. 1998). In addition, none of the nine banded juveniles from Arizona resighted between 1996 and 1999 was at its natal site. These observations indicate a high rate of dispersal in juveniles and adults. The remaining two-thirds of resighted or recaptured adults in Arizona (32% of total) displayed breeding-site philopatry. If a similar level of fidelity occurs rangewide, the slight but statistically significant structure we found may be due to small population sizes and philopatry rather than geographic or genetic isolation of breeding groups. As such, it would have only transient effects on differentiation. Thus, genetic and field data suggest that the level of movement in these birds is sufficient to provide for widespread gene flow, maintenance of high genetic variation, and lack of biologically important structure.

In addition to current dispersal of individuals among breeding sites, genetic diversity in Southwestern Willow Flycatchers may be a function of historic levels of rangewide diversity. A high level of diversity may have been facilitated by a greater number of individuals, less geographic fragmentation of riparian habitat, and dispersal among breeding sites. Indirect estimates of gene flow given by molecular marker methods often cannot distinguish between contemporary and historic patterns (Bossart and Prowell 1998). The movement of reproductive individuals observed by Paxton et al. (1997) and Netter et al. (1998) indicates that current levels of gene flow suggested by AFLP markers are not due solely to historic patterns of gene flow.

Management implications.—Our AFLP patterns and related observational data indicate that genetic options for Southwestern Willow Flycatcher recovery are flexible. Our analysis did not reveal any highly differentiated breeding groups of special management concern. However, certain breeding sites may be significant management units owing to their high-

quality habitat and reproductive productivity. Zink (1997) reviewed mitochondrial studies that have documented the influence of geography on genetic differentiation in birds and concluded that geographic distance alone is not a strong factor in genetic divergence of populations, whereas barriers such as oceans, mountains, and deserts often are associated with population structuring. Likewise, our data suggest that geographic separation of breeding sites is not a genetic barrier for *E. t. extimus*, which is capable of long-distance dispersal.

The genetic strategy for managing this endangered subspecies must be rangewide. Priorities should include protection of habitat at productive breeding sites and an increase in numbers of flycatchers, especially in smaller breeding groups. The preservation and expansion of existing breeding habitat would help maintain the low level of differentiation that we observed. Because movement occurs more often between adjacent sites than between distant sites, gene flow might depend on a network of breeding sites along the same drainage, with such networks distributed throughout the range of Southwestern Willow Flycatchers to facilitate long-distance dispersal.

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