

INTRODUCTION



Figure 1. Perched Willow Flycatcher (*Empidonax traillii brewsteri*) (Photo: Vickie J Anderson)

- Four subspecies of the Willow Flycatcher (*Empidonax traillii*) (Unitt, 1987; Figure 2):
 - Empidonax traillii adastus* breeds in the Great Basin and central Rocky Mountains
 - E. t. brewsteri* breeds in the Pacific coastal region above southern California (Figure 1).
 - E. t. extimus* breeds in the southwest portion of the United States
 - E. t. traillii* breeds east of the Rocky Mountains

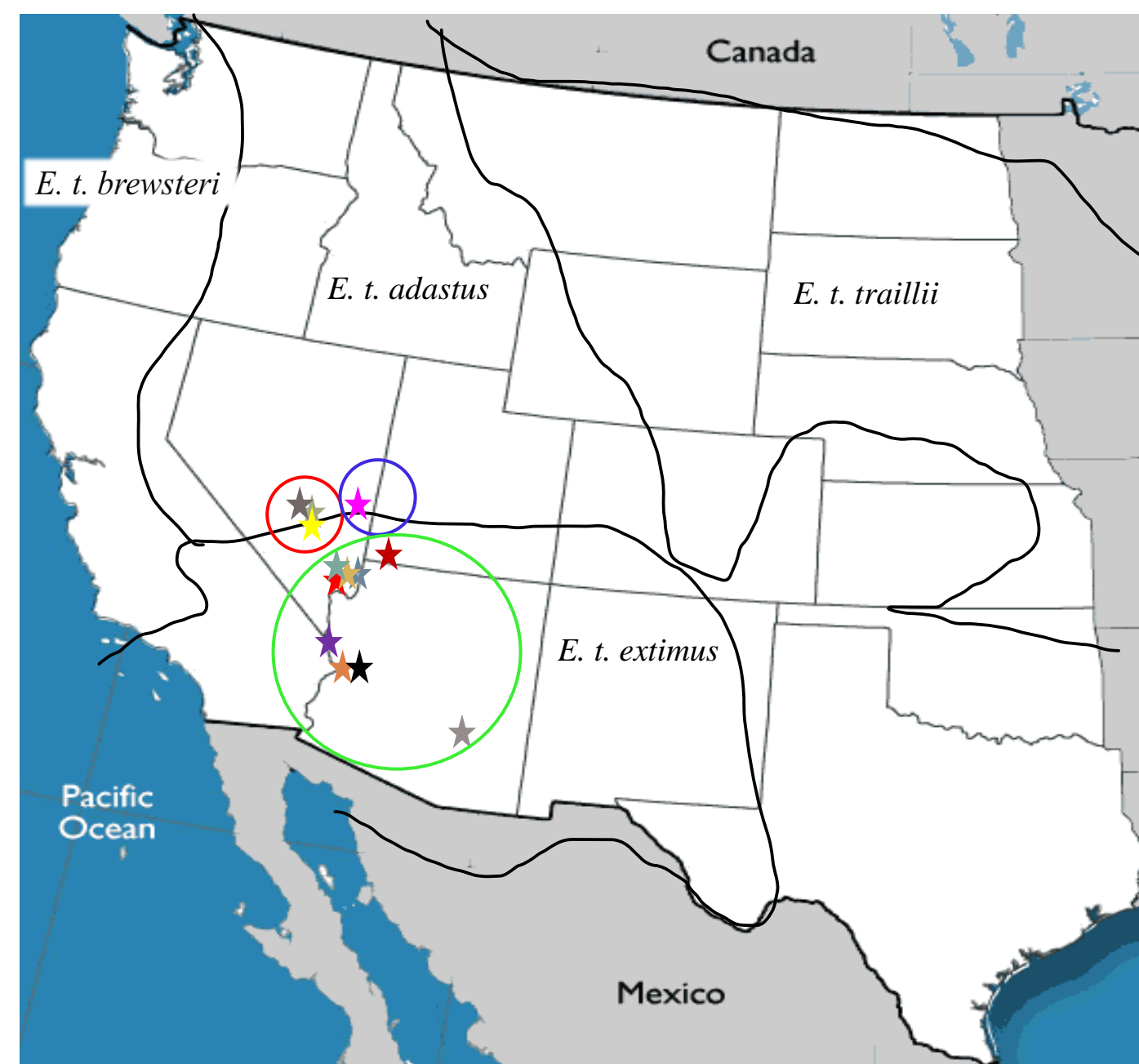


Figure 1. Theoretical boundaries for subspecies of Willow Flycatchers (Paxton et. al, 2005)

- The southwestern willow flycatcher (SWFL) has been classified as endangered since 1995; one reason for the decline is habitat loss (USFWS, 2014).
- SWFLs breed strictly in riparian habitat. Riparian zones are scarce in the Southwestern US, contributing to only 1% of the land area but are an important oasis for wildlife (Hatten, 2016).
- At the SWFL and *E. t. adastus* boundary line, there are no physical barriers and breeding populations are scattered (Figure 1).
- The area between two subspecies where they can interact and interbreed is called an introgression zone.
- Geographical separation and ecological factors such as varying elevation, temperatures, water sources, and different kinds of vegetation drive adaptations and eventually speciation. (Orr, 2005)

RESEARCH OBJECTIVES

- Identify introgression zones along the *E. t. adastus* and *E. t. extimus* boundary
- Examine how ecological factors like varying elevations, temperatures, and vegetation composition are related to this boundary

METHODS

- 13 Study Sites in Nevada, Arizona, and Utah (Figure 1, Figure 2)
 - 4 northern sites range from 1,000-1,200 m in elevation
 - 9 southern sites range from 140-1000 m in elevation

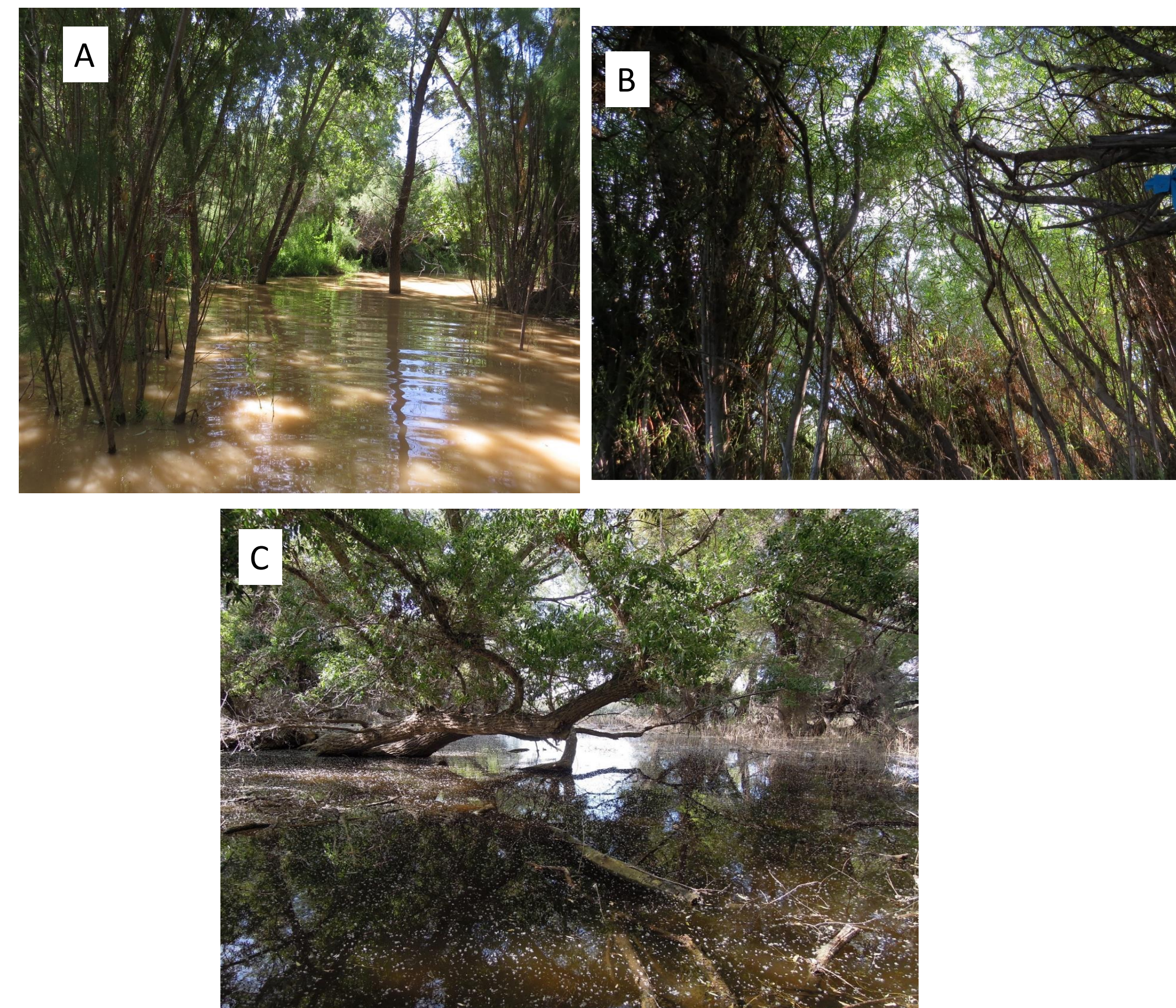


Figure 2. Examples of vegetation in riparian habitats at A) MVWA, mix of native/nonnative vegetation, with patches of open areas and patches of denser understory areas; B) KEPI, mostly nonnative tamarisk habitat with a very dense understory; and C) PAHR, native cottonwood/willow with an open understory (Photos: Mary Anne McLeod)

- Between 2012 and 2017, southwestern willow flycatchers were captured using target mist netting and blood was extracted from each individual by clipping the toenail.
- We extracted DNA and sent samples to the UCLA genomics lab to genotype 96 single nucleotide polymorphisms (SNPs).
- We analyzed the SNP results using STRUCTURE (Version 2.3.3) which determines the probability of each individual belonging to a different genetic population
- We assigned all individuals with 90% of successful SNPs to a specific genetic cluster based on its genetic code using Rubias (performed by the UCLA genomics lab).
 - Southwest genetic cluster and Southern California Coast genetic cluster both correspond to *E. t. extimus*
 - Pacific northwest cluster corresponds to the northern portion of the *E. t. brewsteri* range
 - Interior west genetic cluster corresponds to *E. t. adastus*
- We compared the STRUCTURE results to Rubias assignment test to assess whether changing ecological factors such as temperature, habitat availability, and elevation have impacted the genome of breeding populations along the boundary line, and to see if zones of introgression may be present.

RESULTS

- We sent 400 samples for genotyping.

STRUCTURE

- 374 individuals were assigned to 1 of 3 genetic populations with STRUCTURE represented by 3 colors (Figure 3)

 - Individuals from the 9 sites within the SWFL range (Figure 2) were mainly assigned to green population
 - Individuals from 3 sites in the northern part of the southwestern subspecies range (Figure 2), were assigned to the red population
 - Individuals from MVWA (Figure 2), were assigned to a third population depicted in blue (Figure 3).

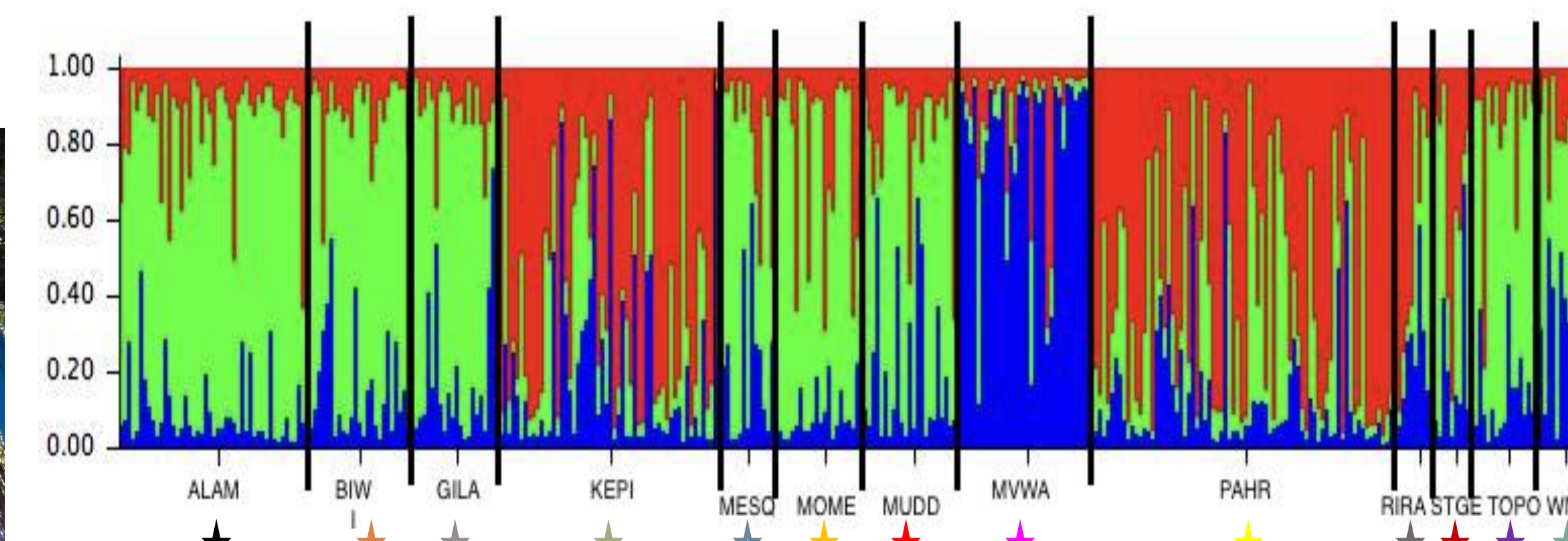


Figure 3. STRUCTURE assignments of individual willow flycatchers to one of three populations corresponding to the three different colors.

RUBIAS

- 185 samples that were 90% successful were assigned to 1 of 4 genetic clusters (Table 1)
- Excluding MVWA, 174 out of 176 individuals came from the Southwestern genetic cluster
- In MVWA, Rubias assigned at least 1 individual to each genetic cluster (Table 1).

Table 1. Number of individuals from each site assigned to the genetic clusters identified by Rubias assignment testing

	Southwest	Pacific Northwest	Southern California Coast	Interior West	Total Individuals
Alamo Lake State Park	29	0	0	0	29
Bill Williams	13	0	0	0	13
GILA river	12	0	0	0	12
Key Pittman WMA	27	0	0	1	28
Mesquite	9	0	0	0	9
Moapa Valley	14	0	0	0	14
Muddy River	13	0	0	0	13
Meadow valley wash	3	1	1	4	9
Pahrnagat NWR	24	0	0	1	25
Rio Rancho	4	0	0	0	4
St. George, UT	8	0	0	0	8
Topock Marsh NWR	10	0	0	0	10
Warm springs NA	10	0	0	0	10



Figure 4. Isolated riparian habitat at Pahrnaghat (Photo: Mary Anne McLeod)

CONCLUSIONS

- Inferences from STRUCTURE alone and inferences from considering both STRUCTURE and Rubias were contradictory

STRUCTURE

- Identified 3 genetic populations. We expected to find 2 genetic populations corresponding to the subspecies *E. t. extimus* and *E. t. adastus*
- KEPI, RIRA, and PAHR appear to be in a zone of introgression based on the relative proportions of the 3 colors (Figure 3) and on their location near the boundary line between *E. t. adastus* and *E. t. extimus* (Figure 1)
- The 9 southern populations could correspond to individuals of *E. t. extimus*
- The blue population confined to MVWA are likely *E. t. adastus*

STRUCTURE and Rubias

- 98.9% of Individuals from the red and green populations from STRUCTURE were assigned to the Southwest genetic cluster indicating 2 distinct populations within the subspecies which could indicate future speciation of the SWFL
- MVWA had breeding individuals assigned to all 4 genetic clusters indicating its location in a zone of introgression between *E. t. adastus* and *E. t. extimus*

Table 2. Ecological factors of the study sites impacting adaptations of the Willow Flycatcher during the breeding season (McLeod & Pellegrini, 2019)

	Avg. Elevation	Avg. temp (°F) May-July	Habitat
ALAM	341 m	96	Shrubland with wetland habitat near Alamo Lake
BIWI	154 m	103	Mix of native forest & tamarisk, beaver ponds and cattail marsh
GILA	532 m	100	Mixed native vegetation, confluence of Gila and Colorado river
KEPI	1,165m	90	Dense understory of nonnative tamarisk, declining water levels throughout breeding season
MESQ	466 m	100	Mainly tamarisk vegetation, wetland habitat
MOME	577 m	102	Wetland habitat with nonnative vegetation
MUDD	375 m	102	Wetland with fluctuating water levels, tamarisk vegetation
MVWA	1,213 m	86	mix of native/nonnative vegetation, perennial stream and beaver pond
PAHR	1,023 m	92	native cottonwood willow, open understory, open water
RIRA	1,165m	90	Cultivated native vegetation near perennial stream
STGE	822m	95	Nonnative riparian habitat in developed area
TOPO	140m	105	Tamarisk with patches of Gooding's willows, open water
WMSP	1,000m	97	Cultivated shrubland with mainly nonnative vegetation

Driving factors for future speciation

- Small differences in environmental conditions (Table 2) across riparian habitats (Figure 2; Figure 4) may contribute to the differentiation between breeding populations at each site across Willow Flycatcher subspecies
- Key ecological factors possibly responsible for genetic differentiation within the subspecies (Orr, 2005; Table 2):
 - Elevation of study sites
 - Summer ambient temperature
 - Habitat composition

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