Population Genetic Diversity and Structuring in Yellow-billed Cuckoos

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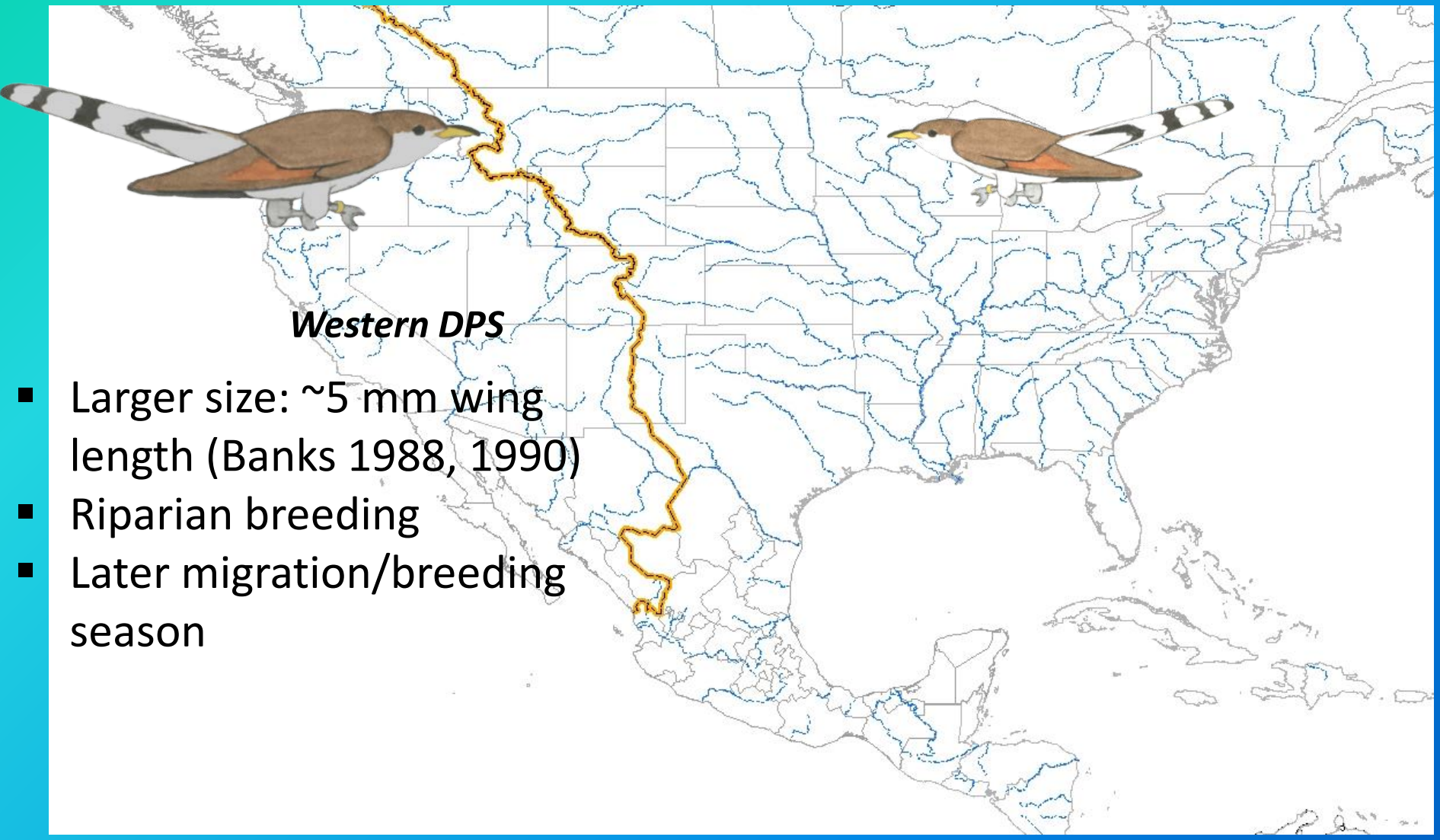
Western versus Eastern Yellow-billed Cuckoo

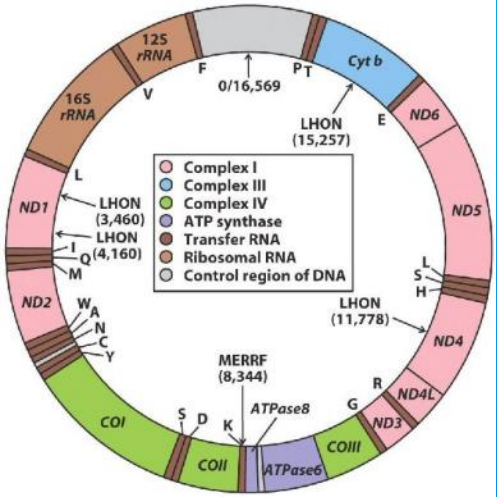
- Two subspecies - Ridgeway 1887



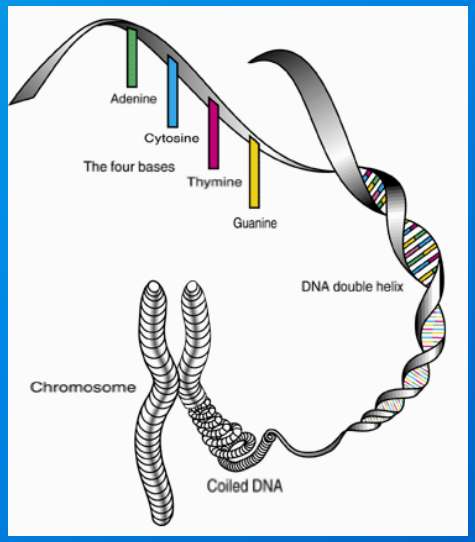
- Larger size: ~5 mm wing length (Banks 1988, 1990)
- Riparian breeding
- Later migration/breeding season

Previous Genetic Research

Fleischer (2001), Farrell (2006, 2013):

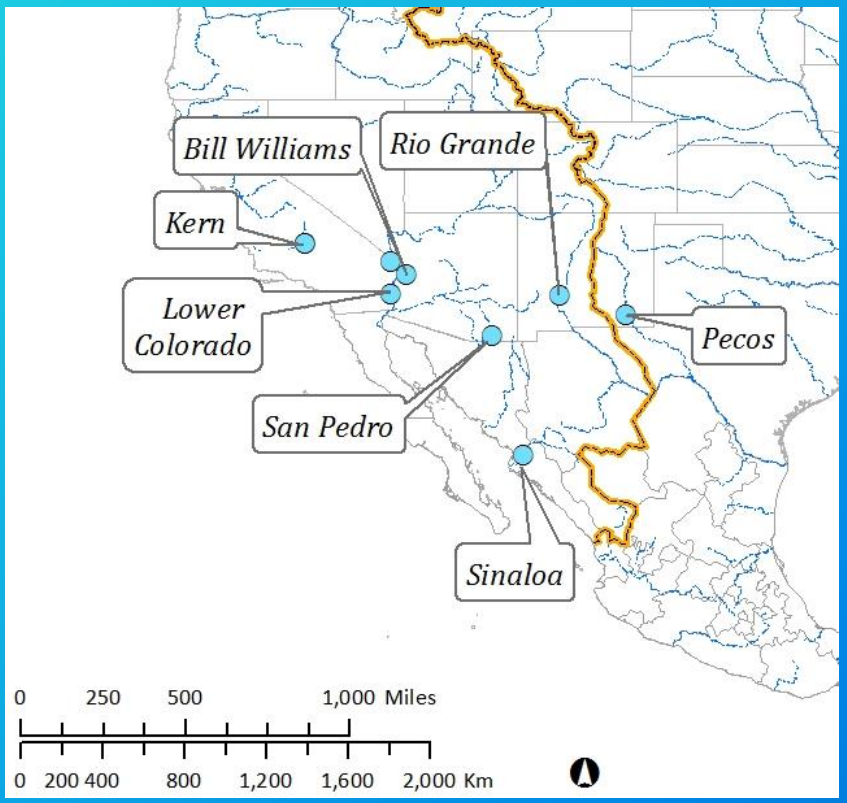


- Cyt-*b* gene (mtDNA), n = 66, n = 31
- Insignificant genetic differences
- Substantial variation within western samples (Farrell 2013) - potential structuring due to dispersal barriers?
- More informative markers, e.g. microsatellites, recommended



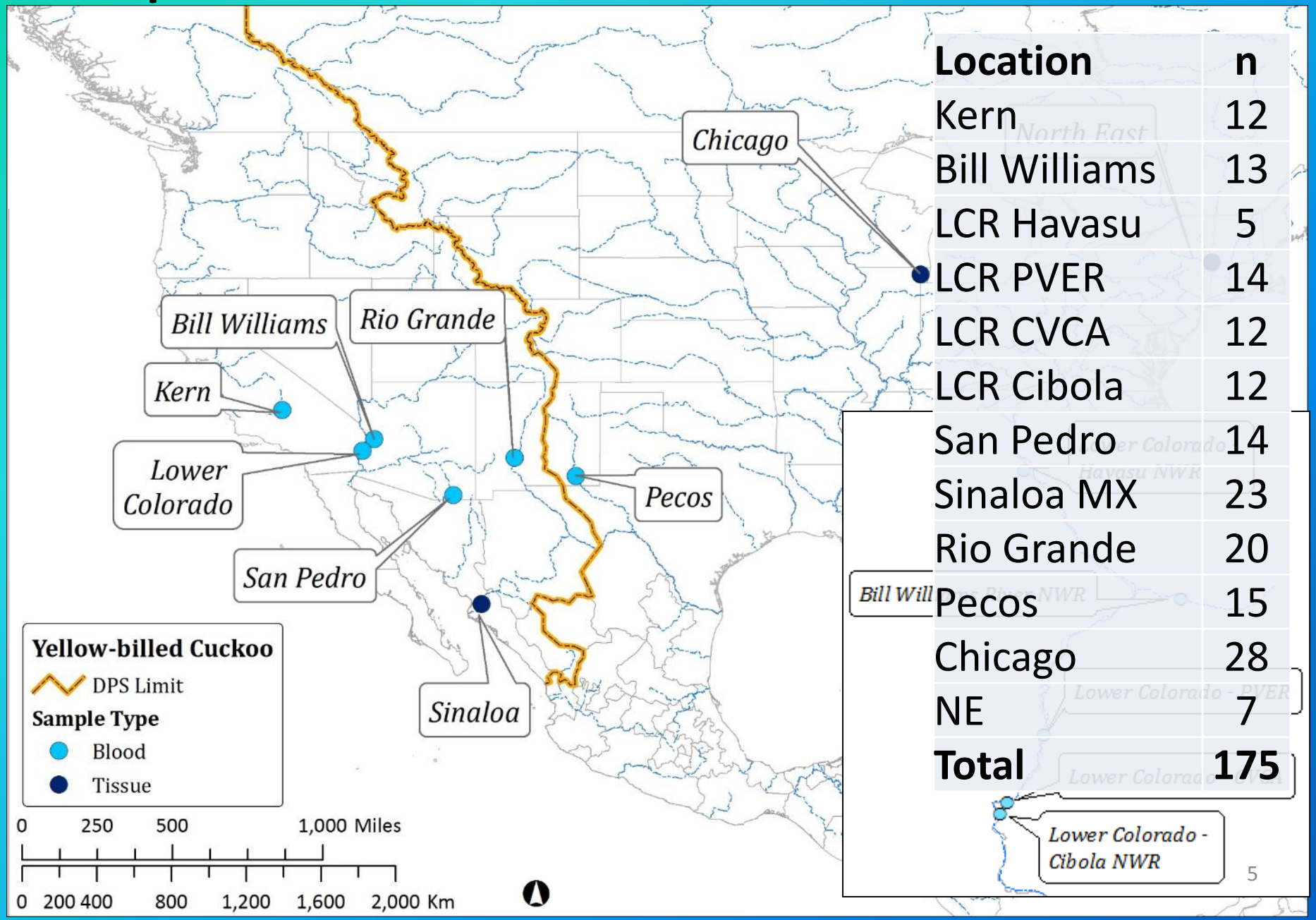
Objectives

- Dispersal barriers: among isolated **western** sites, measure
  - Genetic diversity
  - Population structuring
  - Inbreeding



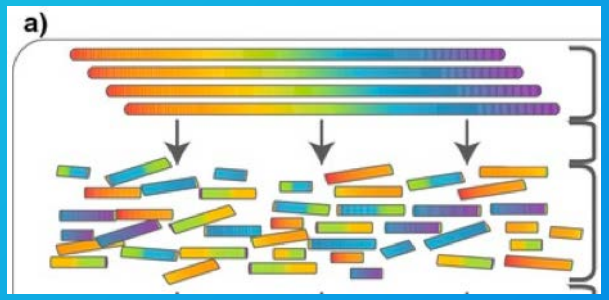
- Assess placement of western DPS limit: Include Pecos River NM?
- Measure differentiation between **western** and **eastern** yellow-billed cuckoos

Sample locations: 7 western* 2 eastern

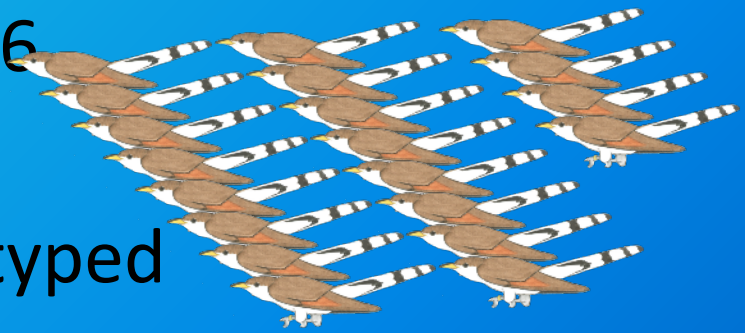


Location	n
Kern	12
Bill Williams	13
LCR Havasu	5
LCR PVER	14
LCR CVCA	12
LCR Cibola	12
San Pedro	14
Sinaloa MX	23
Rio Grande	20
Pecos	15
Chicago	28
NE	7
Total	**175**

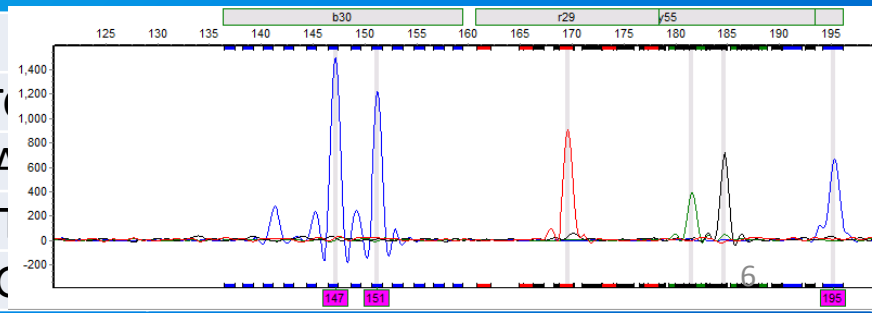
Novel Microsatellite Development



- 1 YBCU pyrosequenced (R Fleischer / Smithsonian Institution)
- 75 'PALs' identified/primers designed
- Tested on 1 bird for PCR amplification, 34 successfully amplified
- Tested these on 20 birds, found 16 polymorphic
- Designed 2 multiplex PCRs, genotyped 175 samples at 14 loci, 3 replicates



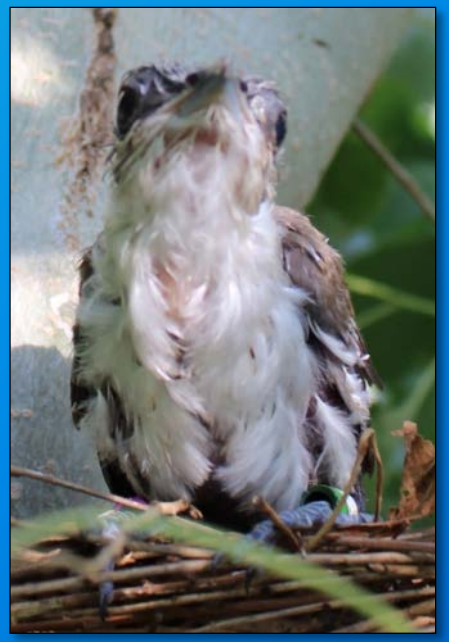
Locus	Type	Repeat Motif	Forward sequence
y01	Di	TG	AACAGCAATGTGTGTGT
y02	Di	GT	AGGTGTGGTGTGCAGCA
y03	Di	TG	AGTCTCAGCTCTGTGTGT
y04	Tetra	GACA	TTTCCACCCTGGCAACTC



DNA Sampling

- Target-netting (adults), blood sampling (**unrelated** adults + chicks) : n = 119
  - **Contemporary** museum tissue samples - UWBM, FMNH, AMNH : n = 73
  - DNA extraction (Qiagen kits)

Cibola Valley Conservation Area

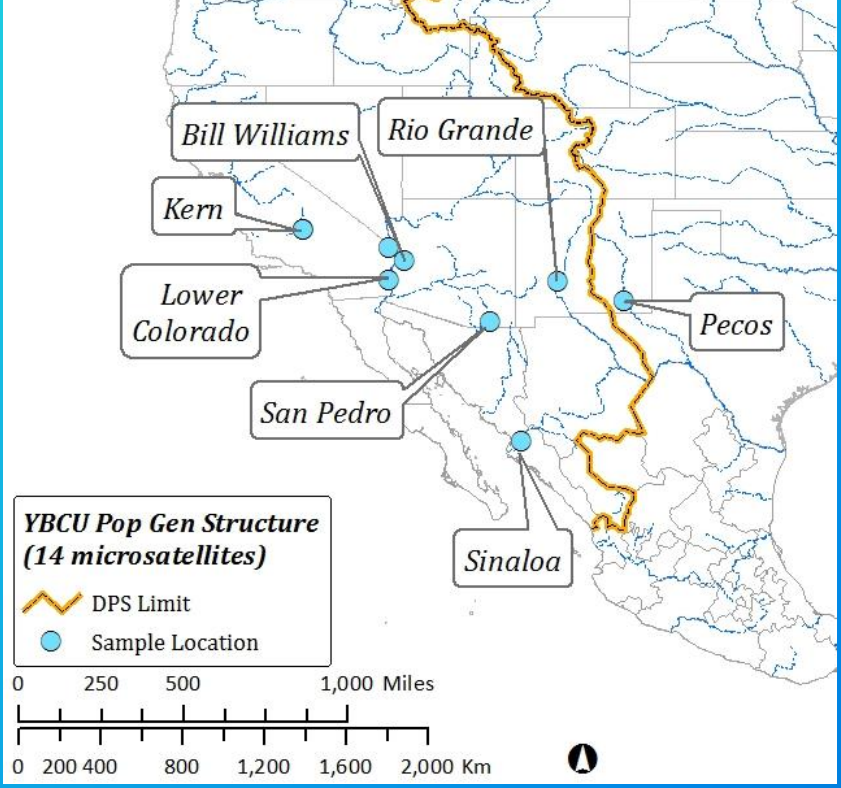


Palo Verde Ecological Reserve

Analysis

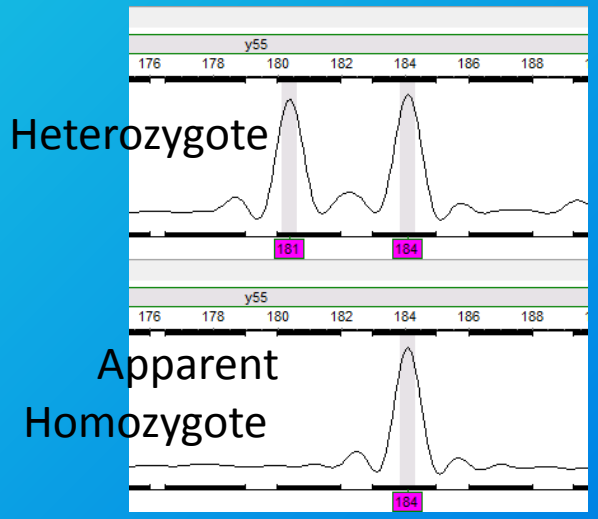
- Among isolated **western** sites: measure levels of
  - Genetic diversity – **H**
  - Structuring – **F_{ST} / G''$_{ST}$**
  - Inbreeding – **F_{IS}**
- Assess placement of western DPS limit – **F_{ST} / G''$_{ST}$ between Pecos and other sites**
- Measure differentiation between **western** and **eastern** yellow-billed cuckoos – **F_{ST} / G''$_{ST}$ between eastern/western sites**

  – **compare variation in size (wing length) by longitude**



Analysis

- Genetic Diversity:
  - Heterozygosity (H): expected (H_E) and observed (H_O)
  - Test HWE, heterozygote deficiency
  - Test for null alleles



Heterozygote

Apparent Homozygote

Microsatellite Locus y56

Results: Ho, He, F by Locus (n = 14 loci)

Locus	# Alleles	Ho	He	P (HWE)	Freq. NA
b38	6	0.29	0.32	0.1782	0
b46	5	0.63	0.62	0.8294	0
b25	8	0.58	0.70	0.0001	0.07
g57	5	0.53	0.69	0	0.09
y56	2	0.39	0.47	0.0171	0
y34	5	0.15	0.16	0.1323	0
r23	5	0.23	0.51	0	0.29*
r24	5	0.50	0.57	0.0054	0
r26	4	0.48	0.52	0.0308	0*
b30	14	0.66	0.82	0	0.09*
b68	3	0.07	0.12	0.0002	0.05*
g43	4	0.48	0.58	0.004	0
y55	5	0.09	0.14	0.0006	0.41
r29	3	0.05	0.05	1	0
Mean	5.29	0.37	0.45	0	0.07

Population Statistics : 9 populations, 14 loci

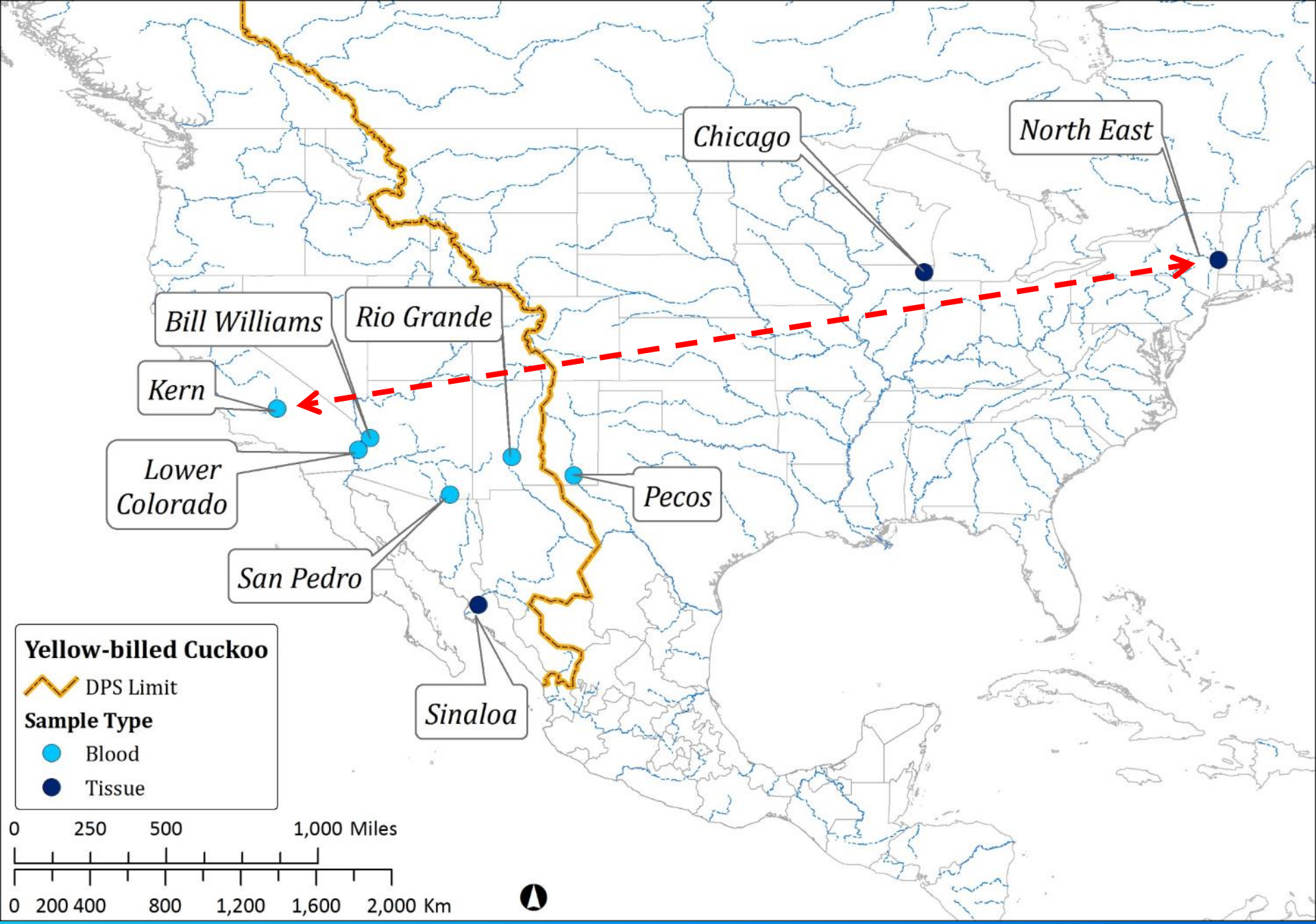
Population	N	Av. # Alleles	H_O	H_E	P (HWE)	F_{IS}
Kern	12	2.86	0.346	0.391	0.0664	0.119
Bill Williams	13	3.57	0.377	0.438	0.0096	0.144
Colorado	43	4.07	0.378	0.440	0.0000*	0.142
San Pedro	14	3.43	0.364	0.454	0.0001*	0.205*
Sinaloa	23	3.57	0.364	0.444	0.0000*	0.184*
Rio Grande	20	3.71	0.339	0.451	0.0000*	0.253*
Pecos	15	3.43	0.372	0.464	0.0022	0.184
Chicago	28	3.71	0.372	0.452	0.0000*	0.181*
NE	7	2.50	0.383	0.414	0.2344	0.083
Global	175	5.29	0.367	0.446	0.0000*	0.162

* P-value: significant after Bonferroni correction * FIS: higher than expected by chance

Pairwise G''_{ST} Scores

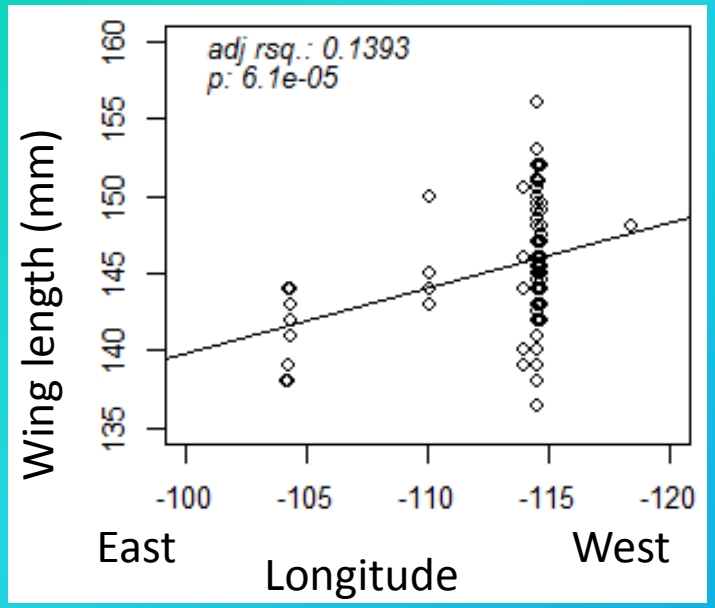
	Kern	Bill Williams	Colorado	San Pedro	Sinaloa	Rio Grande	Pecos	Chicago	NE
Kern		0.100	0.003	0.573	0.024	0.008	0.388	0.092	0.002
Bill Williams	0.035		0.304	0.296	0.654	0.390	0.302	0.289	0.050
Colorado	0.065	0.006		0.164	0.647	0.430	0.126	0.387	0.036
San Pedro	-0.010	0.008	0.014		0.488	0.658	0.843	0.883	0.094
Sinaloa	0.047	-0.010	-0.005	-0.002		0.800	0.141	0.344	0.167
Rio Grande	0.060	0.002	0.001	-0.012	-0.014		0.169	0.310	0.231
Pecos	0.004	0.010	0.017	-0.024	0.020	0.020		0.647	0.016
Chicago	0.028	0.009	0.002	-0.020	0.004	0.005	-0.010		0.093
NE	0.133	0.063	0.054	0.052	0.029	0.021	0.091	0.042	
	Kern	**Bill Williams**	**Colorado**	**San Pedro**	**Sinaloa**	**Rio Grande**	**Pecos**	**Chicago**	**NE**

Exact G test for genotypic differentiation between Kern and NE samples: p-value significant after Bonferroni correction ($\alpha = 0.05$)

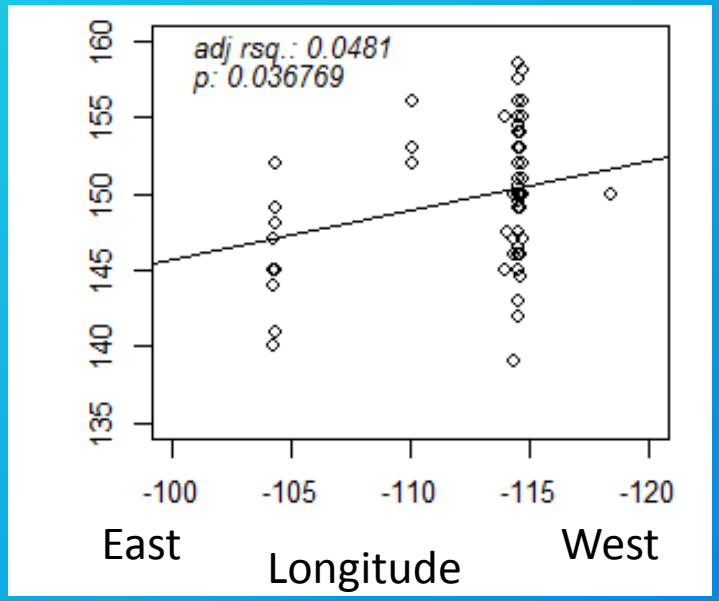

Chicago
North East
Bill Williams
Rio Grande
Kern
Lower Colorado
Pecos
San Pedro
Sinaloa
Yellow-billed Cuckoo
DPS Limit
Sample Type
Blood
Tissue
0 250 500 1,000 Miles
0 200 400 800 1,200 1,600 2,000 Km

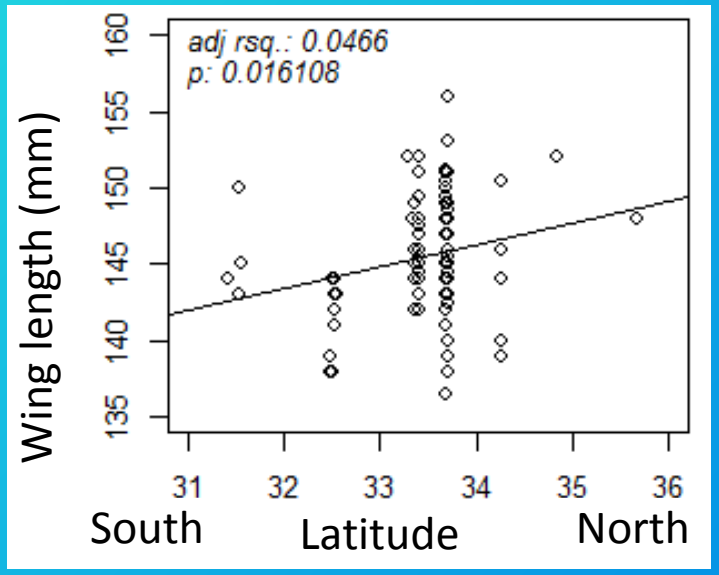
Wing length east -> west, south -> north: clinal increase

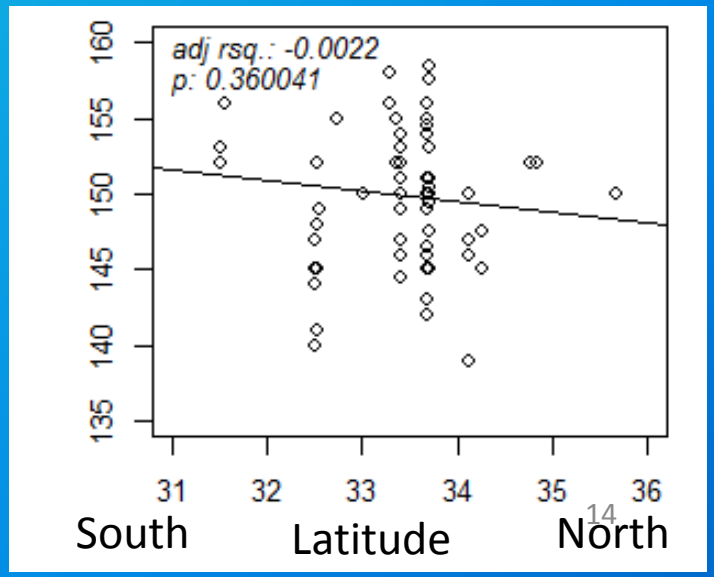
Males



Females

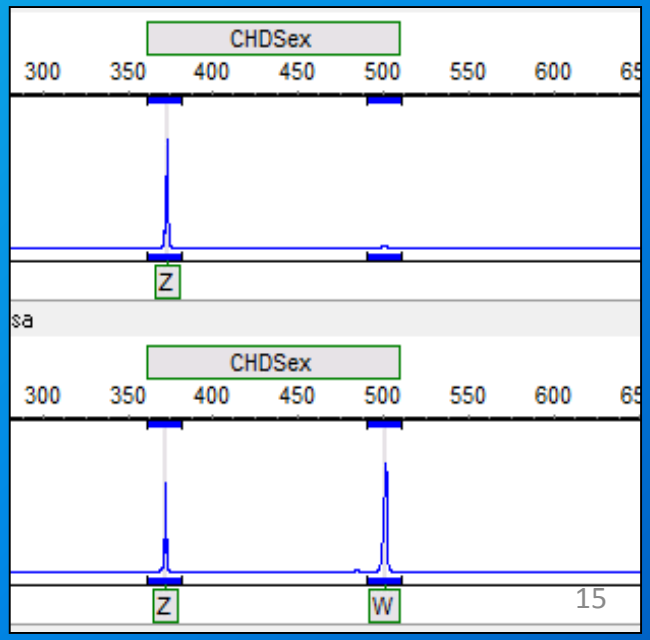


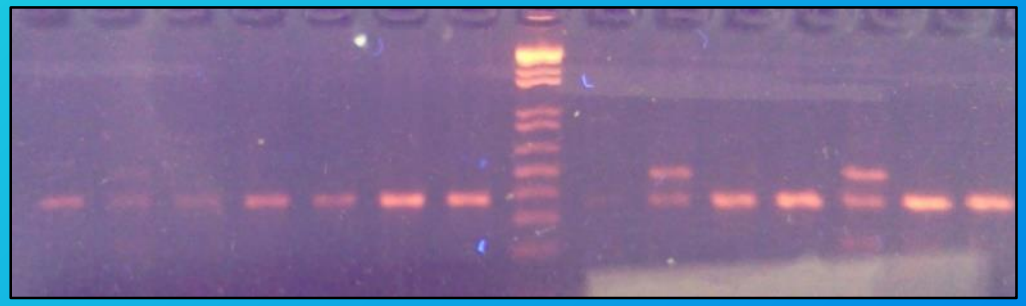




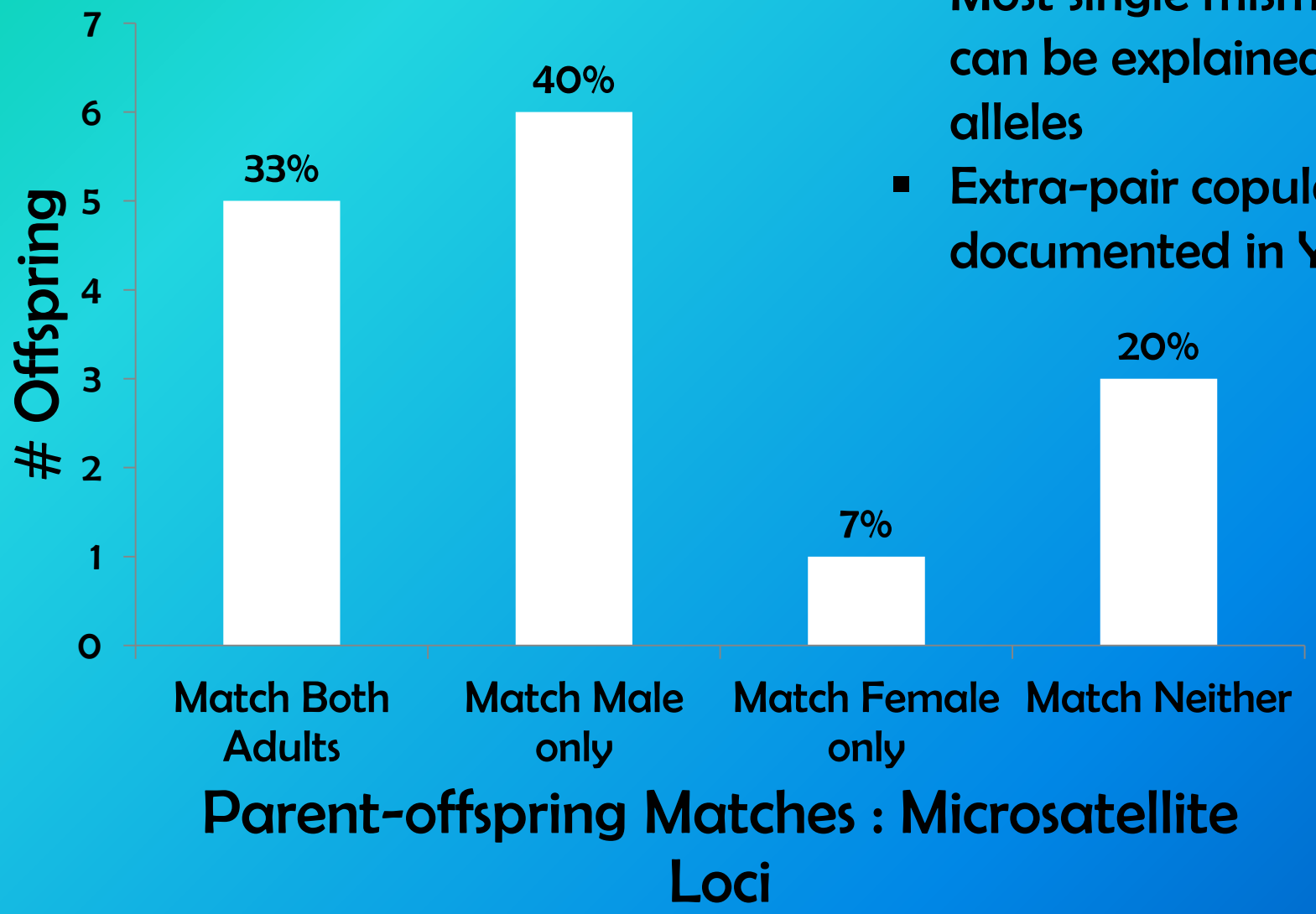
Error Testing

- Genotyped six family groups : nesting male + female + 1-4 chicks
- DNA-sexing (based on Han et al. 2009)
- Cytochrome-b sequencing of females and chicks





Results: microsatellite loci parent-offspring mismatches (6 nests, 15 chicks, 14 loci)



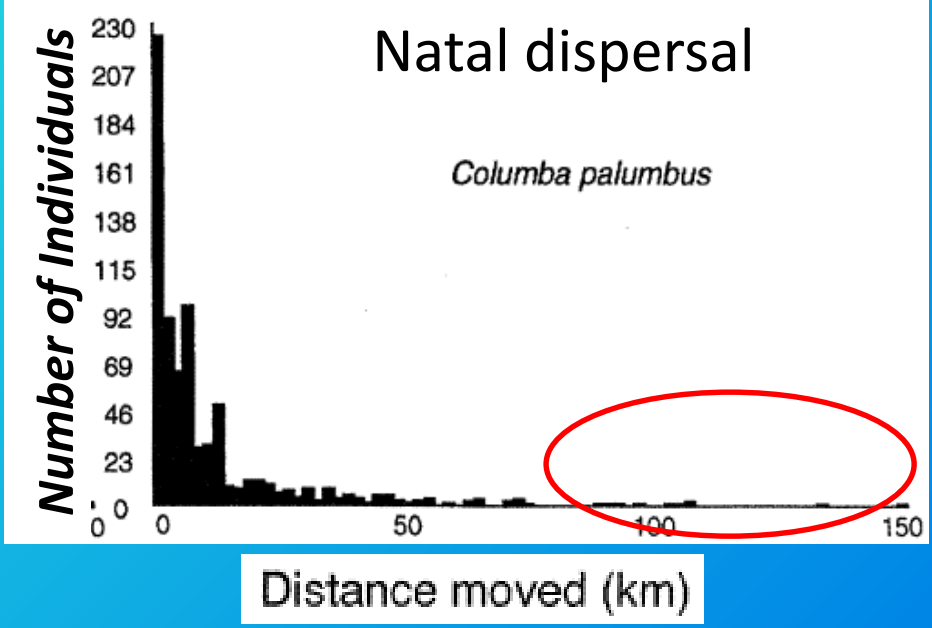
- Most single mismatches can be explained by null alleles
- Extra-pair copulations documented in YBCU

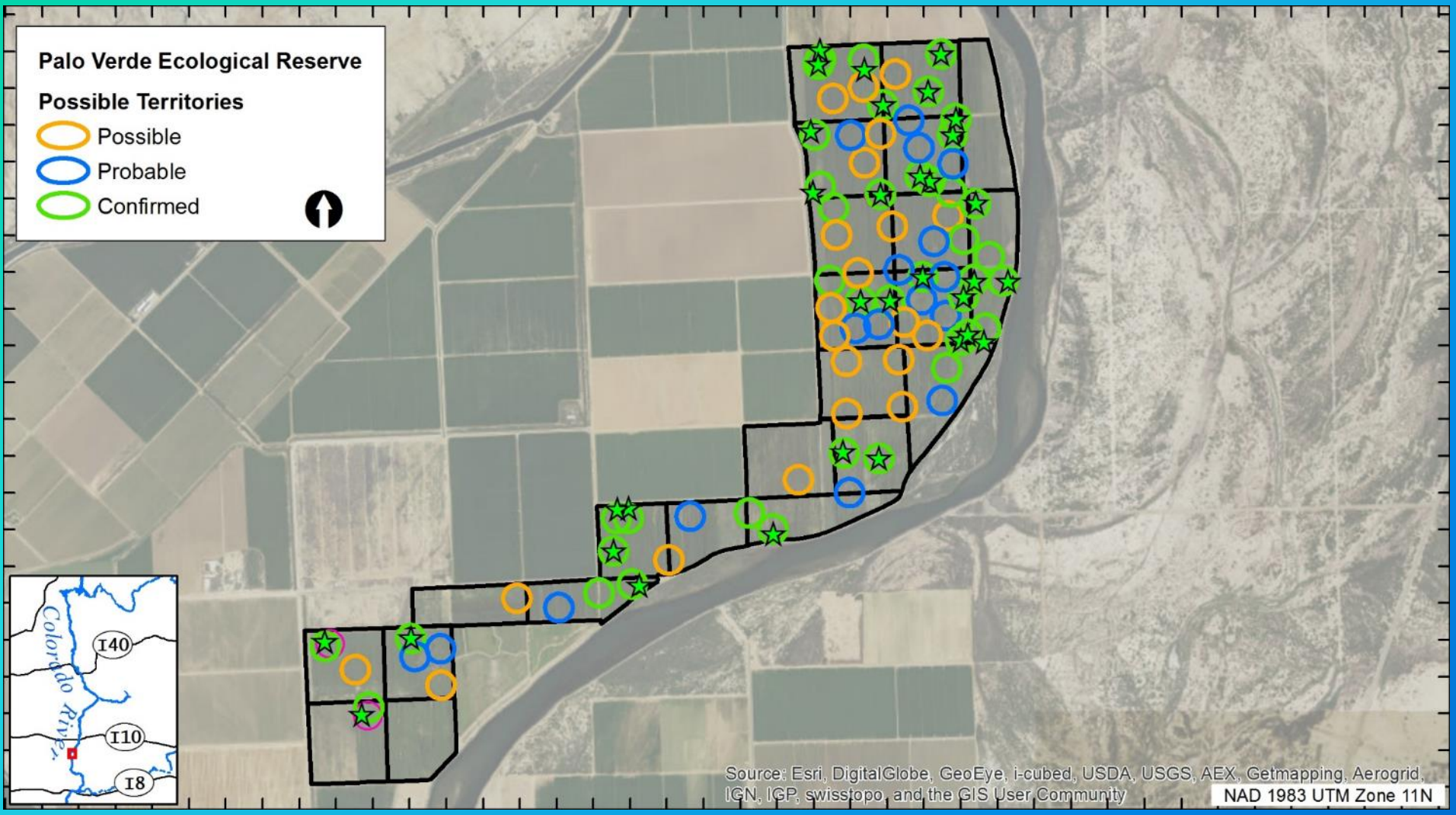
Nest #	Chick #	Microsatellite Allele Mismatches			Cyt-b Match
		Total Mismatches	Mismatches With Male	Mismatches with Female	
1	1	0	0	0	✓
	2	**5 CP**	**1 E**	**4 CP**	X
	3	0	0	0	✓
2	1	1 N	1 N	0	✓
	2	1 N	1 N	0	✓
	3	**4 CP**	0	**4 CP**	X
	4	**7 CP**	**1 E**	**6 CP**	X
3	1	1 N	0	1 N	✓
	2	0	0	0	✓
4	**1**	**5 P***	0	**5 P***	✓
5	1	1 E	0	1 E	✓
	2	**7**	**1-5**	**0-4 P***	✓
6	1	0	0	0	✓
	2	0	0	0	✓
	3	1 N	1 N	0	✓

N Possible null allele **CP confirmed parasitism (cty-b mismatch)**, **P* probable parasitism, ♀♀ same haplotype.** E either scoring error or different parent.

Summary

- No evidence of restricted gene flow
- Lower H, higher F_{IS} due to increased inbreeding
- High rate of conspecific nest parasitism
- Restoring large patches of riparian habitat may help increase the size of the western population




Palo Verde Ecological Reserve
Possible Territories
Possible
Probable
Confirmed
Colorado River
I40
I10
I8
Source: Esri, DigitalGlobe, GeoEye, i-cubed, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community
NAD 1983 UTM Zone 11N

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