FSF FORENSIC SCIENCES FOUNDATION
EMERGING FORENSIC SCIENTIST AWARD

Lucilia mexicana and its Population Structure in Texas, USA

Department of Biological Sciences

Department of Forensic Sciences

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Forensic Entomology

Application of insects to civil and criminal investigations

Urban

Stored Products

Medico-legal

Insects in Medico-Legal Investigations

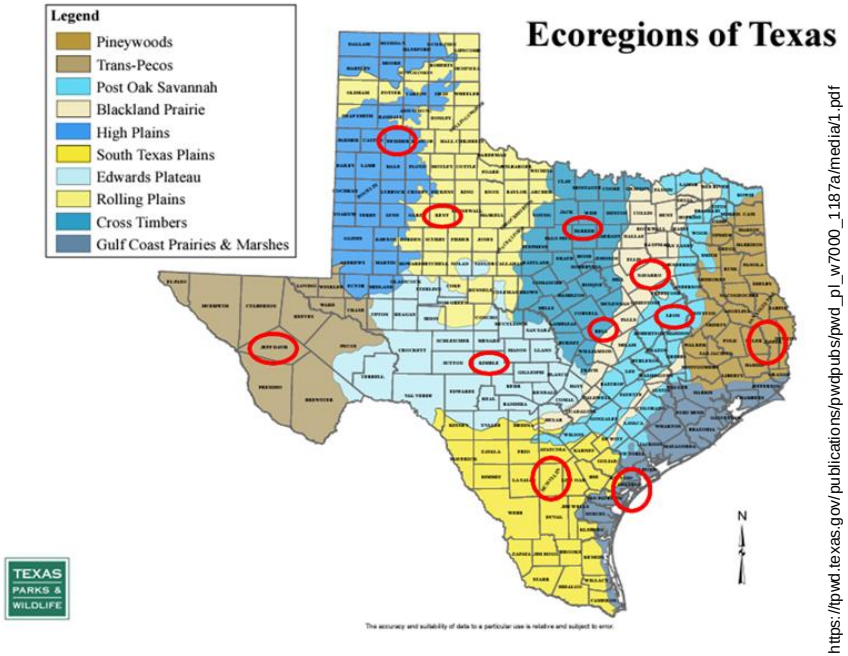
- Insects are the first to arrive at a body
  - Most common flies and beetles
- Insects are an important tool[1]
  - Post-mortem Interval (PMI) Estimation
  - **DNA Analysis**
- Entomological evidence
  - Geographical region
  - Season

Forensic Entomology (Student Magazine Illustration), Oguchi Anyaele. (n.d.). https://oguchiart.artstation.com/projects/3oLm22 (accessed February 5, 2021).

1. Amendt J, Richards CS, Campobasso CP, et al (2011) Forensic entomology: Applications and limitations. Forensic Science, Medicine, and Pathology 7:379–392. https://doi.org/10.1007/s12024-010-9209-2

Lucilia mexicana

- Southwestern United States distribution[2,3]
- First colonizer of mammalian carrion in Texas[4]
  - Myiasis-infestation of living tissue by maggots
- Population Studies
  - Differences in development[5-7]
- Texas ecoregions[8,9]
  - Differences in soil, vegetation, and climate



https://tpwd.texas.gov/publications/pwdpubs/pwd_pl_w7000_1187a/media/1.pdf

2. Hall DG (1948) The blowflies of North America. Thomas Say Foundation
3. Jones N, Whitworth T, Marshall SA (2019) Blow flies of North America: Keys to the subfamilies and genera of Calliphoridae, and to the species of the subfamilies Calliphorinae, Luciliinae and Chrysomyinae. Can J Arthropod Identif 1–191. https://doi.org/10.3752/cjai.2019.39
4. Archambeault AD (2012) Population structure of Lucilia Mexicana Macquart 1843 (Diptera: Calliphoridae) in Texas with a discussion of colonization and genetics. Thesis, Sam Houston State University
5. Gallagher MB, Sandhu S, Kimsey R (2010) Variation in Developmental Time for Geographically Distinct Populations of the Common Green Bottle Fly, *Lucilia sericata* (Meigen)*. J Forensic Sci 55:438–442. https://doi.org/10.1111/j.1556-4029.2009.01285.x
6. Tarone AM, Picard CJ, Spiegelman C, Foran DR (2011) Population and Temperature Effects on *Lucilia sericata* (Diptera: Calliphoridae) Body Size and Minimum Development Time. J Med Entomol 48:1062–1068. https://doi.org/10.1603/ME11004
7 Owings C, Spiegelman C, Tarone A, Tomberlin J (2014) Developmental variation among *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) populations from three ecoregions of Texas, USA. Int J Legal Med 128:709
8. Gould FW, Hoffman GO, Rechenthin CA (1960) Vegetational Areas of Texas.
9. Griffith G, Bryce S, Omernik J, Anne Rogers (2007) Ecoregions of Texas

Objectives

- Objective 1: Design microsatellite multiplex for *L. mexicana*

- Objective 2: Assess the population structure of *L. mexicana* across the Texas ecoregions

Methods

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Cat
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DNA

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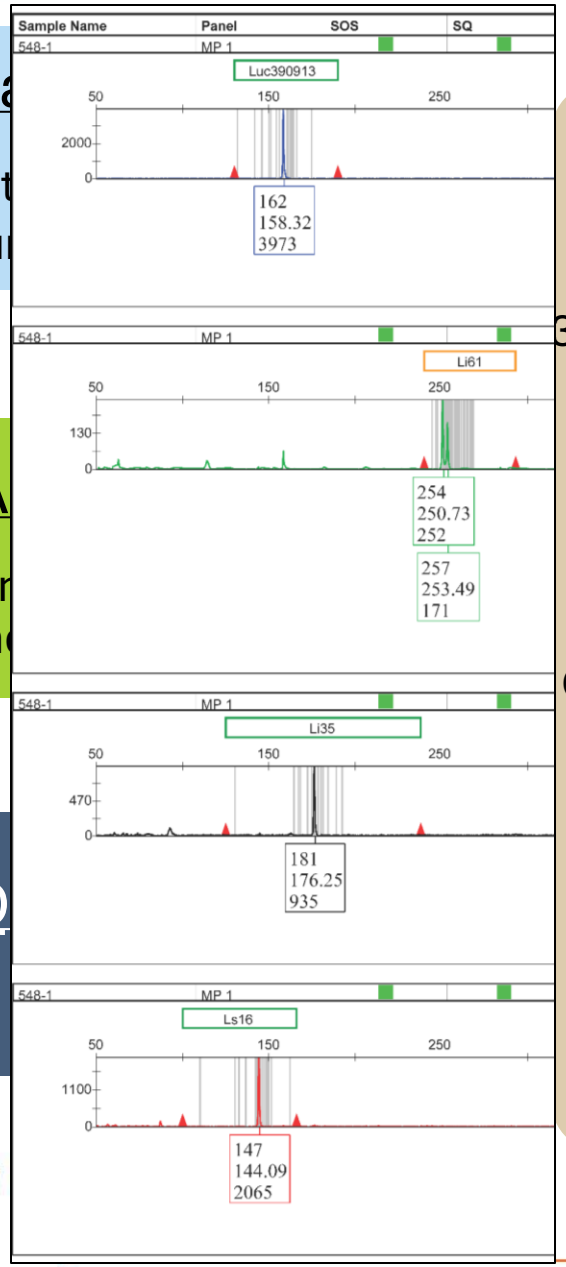
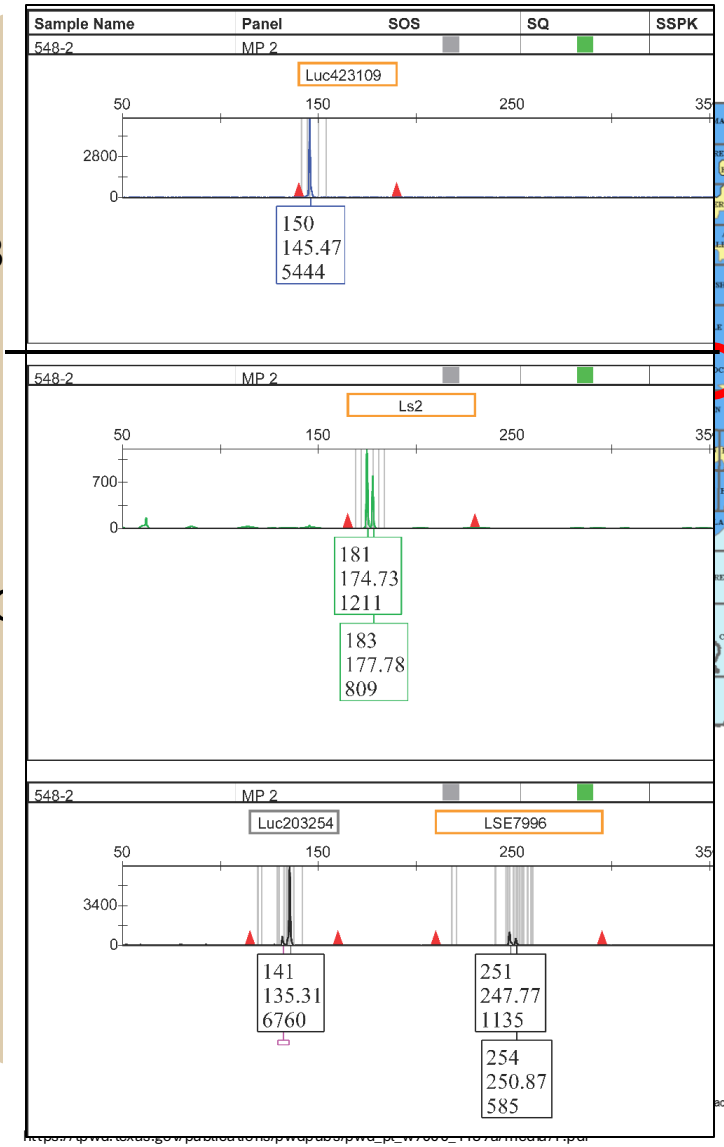
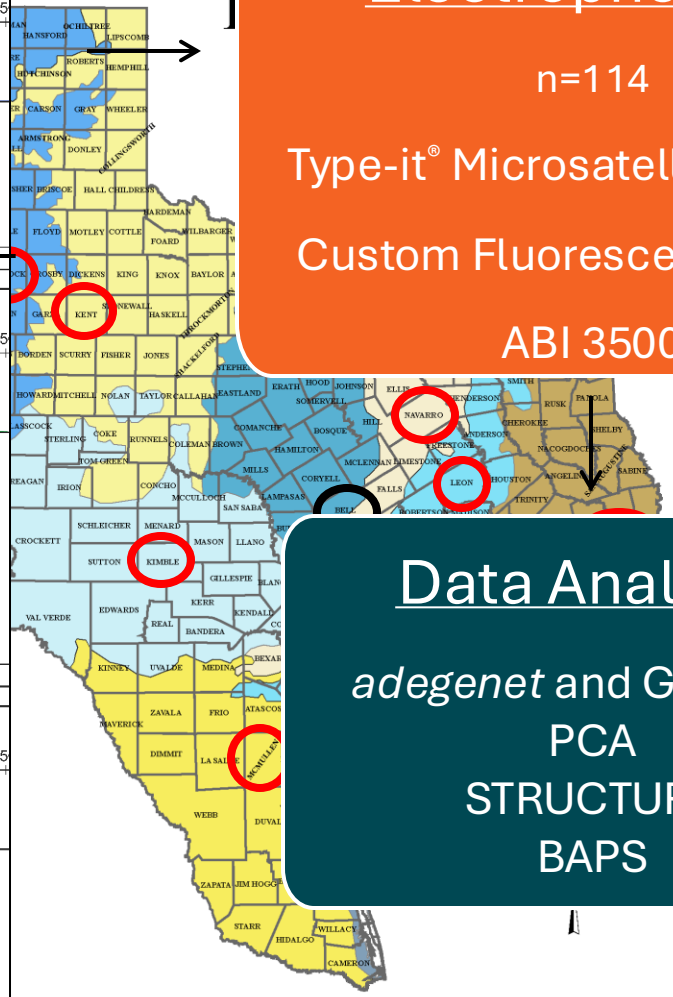
DNA Q







PCR and Capillary Electrophoresis

n=114

Type-it® Microsatellite PCR Kit

Custom Fluorescent Primers

ABI 3500

Data Analysis

adegenet and Genepop
PCA
STRUCTURE
BAPS

10. Florin A-B, Gyllenstrand N (2002) Isolation and characterization of polymorphic microsatellite markers in the blowflies *Lucilia illustris* and *Lucilia sericata*. Molecular Ecology Notes 2:113–116. https://doi.org/10.1046/j.1471-8286.2002.00165.x
11. An H-E, Do DT, Lee, et al (2021) Development of the new microsatellite markers of *Lucilia sericata* (Diptera: Calliphoridae) from Korea. Mol Biol Rep 48:8245–8248. https://doi.org/10.1007/s11033-021-06750-x
12 Meglécz E, Pech N, Gilles A, et al (2014) QDD version 3.1: a user-friendly computer program for microsatellite selection and primer design revisited: experimental validation of variables determining genotyping success rate. Molecular Ecology Resources 14:1302–1313. https://doi.org/10.1111/1755-0998.12271

Markers

Name	Forward and Reverse Primers 5'-3' Sequence	Final Reaction Concentration (µM)	Size (bp)*	Alleles	H_o/H_e	Motif^	Panel and Dye
Li35[10]	TGTCTTGTTCAATTTGATGCTTG TTCATACTTCTCTATTATTATATTTT	0.4	130-200	12	0.32/0.67	$TA_2(CATA)_2TA_2(CA)TA_3$	1-NED
Li61[10]	CCAATTACAAACTTACATCC GGAATTGTTATAATTTAATT	0.1	245-275	19	0.67/0.89	-	1-VIC
Ls2[10]	TCCCACGTCAACAACAAAAA CTTGTCGAACCCTGAAGGAA	0.075	170-190	6	0.45/0.70	$(CAA)_2TAA(CCA)CAA_3$	2-VIC
Ls16[+10]	TGTAAATCATTTTAGAGAAATTCA TTTGTTGTTTTCCTCTGACCAA	0.2	130-170	14	0.30/0.64	-	1-PET
LSE-7996[+11]	GTCTCTTATTGTGGCATCAT GGTGAATGCCGGTTATAA	0.075	220-270	14	0.24/0.80	-	2-NED
Luc203254	TTGTAAAAACTCGACCACAC TTTGATTAGCGTGAGGAAATGA	0.1	120-150	8	0.21/0.53	$AC_2TA(TGT)_2$	2-NED
Luc390913	CAAGAGTTGATTGGAATACCG GGCCACTAGCTTGATGTACG	0.05	130-180	14	0.52/0.59	$GGAC_3$	1-6-FAM
Luc423109	AATGATTGTCAACAACCACTGG GAACGAAGGTTTGGCATCAGC	0.05	140-160	5	0.15/0.14	$(CGTC)_3$	1-6-FAM

*size is based on sequencing results
^motif from Positive Control (Huntsville), (-) mononucleotide repeat (s)
+ Departure from Hardy-Weinberg
No linkage detected between the loci

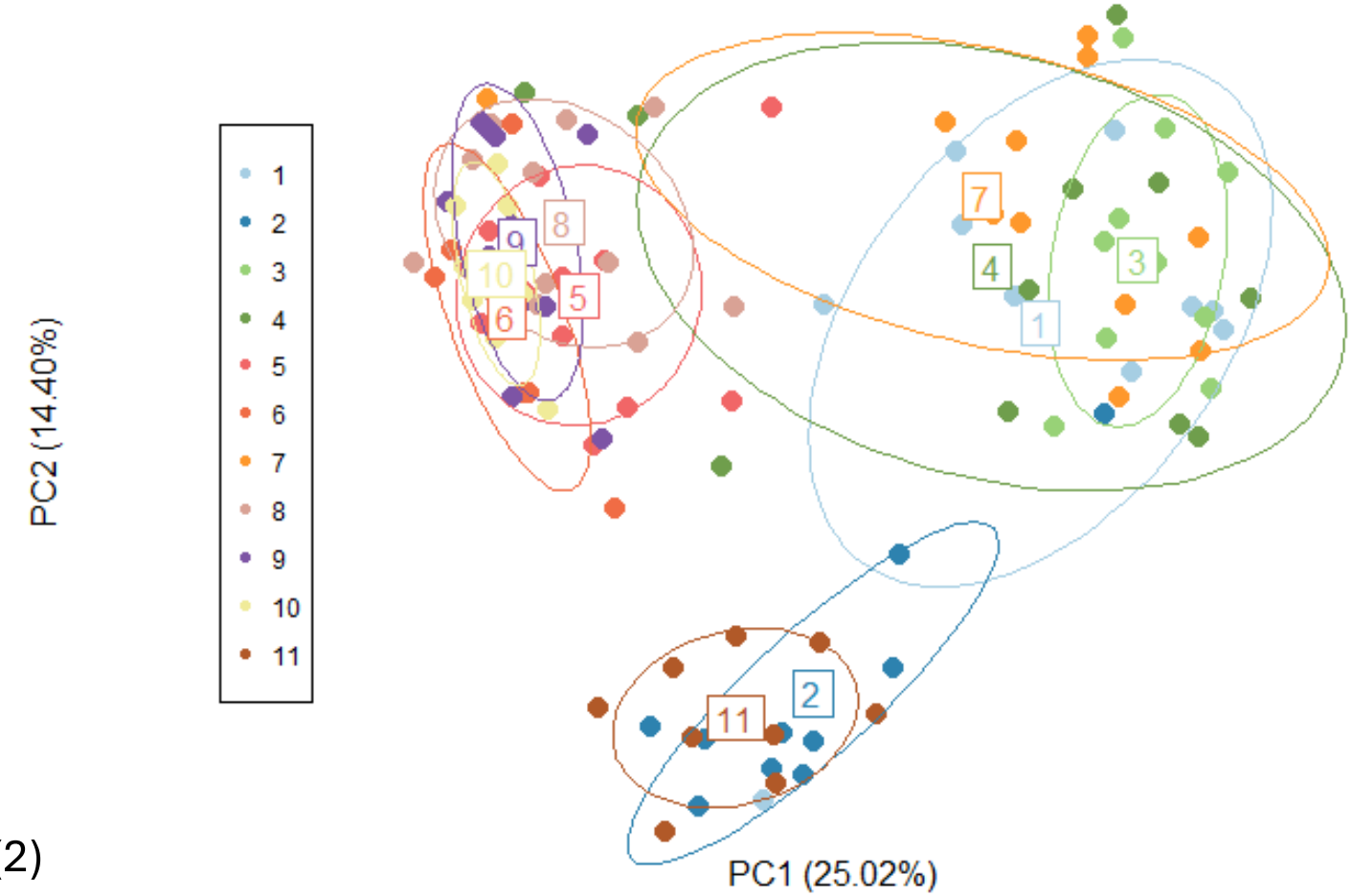
Pairwise F_{st}

	Blackland Prairies	Gulf Coast Prairies and Marshes	Pineywoods	Cross Timbers North	Trans-Pecos	Cross Timbers South	Post Oak Savannah	High Plains	Rolling Plains	Edwards Plateau
Gulf Coast	0.1903									
Pineywoods	0.0005	0.3103								
Cross Timbers N	-0.0233	0.2167	-0.0038							
Trans-Pecos	0.2791	0.2845	0.3777	0.2395						
Cross Timbers S	0.2816	0.2507	0.4058	0.2298	0.0912					
Post Oak Savannah	-0.0026	0.266	0.0424	-0.0045	0.2566	0.2669				
High Plains	0.2599	0.2966	0.3616	0.2154	0.1148	0.007	0.2175			
Rolling Plains	0.2869	0.2956	0.3953	0.2387	0.0942	-0.0253	0.2464	-0.0046		
Edwards Plateau	0.3003	0.3024	0.4318	0.2593	0.1097	-0.015	0.278	0.0233	0.0041	
South Texas Plains	0.2538	0.0033	0.3749	0.268	0.2913	0.2355	0.323	0.3026	0.2922	0.297

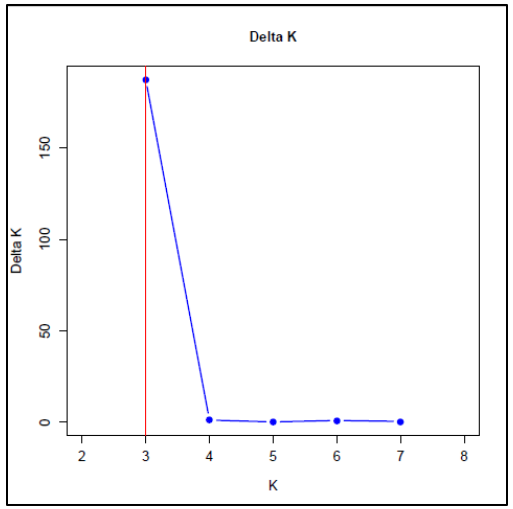
0 to 0.05: Low to no genetic differentiation
0.05 to 0.15: Moderate differentiation
0.15 to 0.25: High differentiation
Above 0.25: Very high differentiation

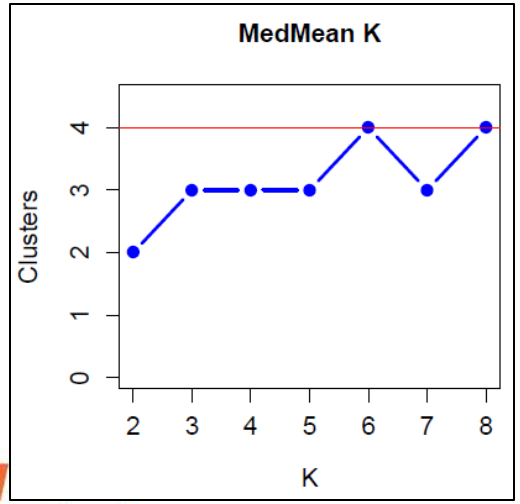
PCA

- Cluster 1
  - Blackland Prairies (1)
  - Pineywoods (3)
  - Cross Timbers North (4)
  - Post Oak Savannah (7)
- Cluster 2
  - Trans-Pecos (5)
  - Cross Timbers South (6)
  - High Plains (8)
  - Rolling Plains (9)
  - Edwards Plateau (10)
- Cluster 3
  - Gulf Coast Prairies and Marshes (2)
  - South Texas Plains (11)



STRUCTURE

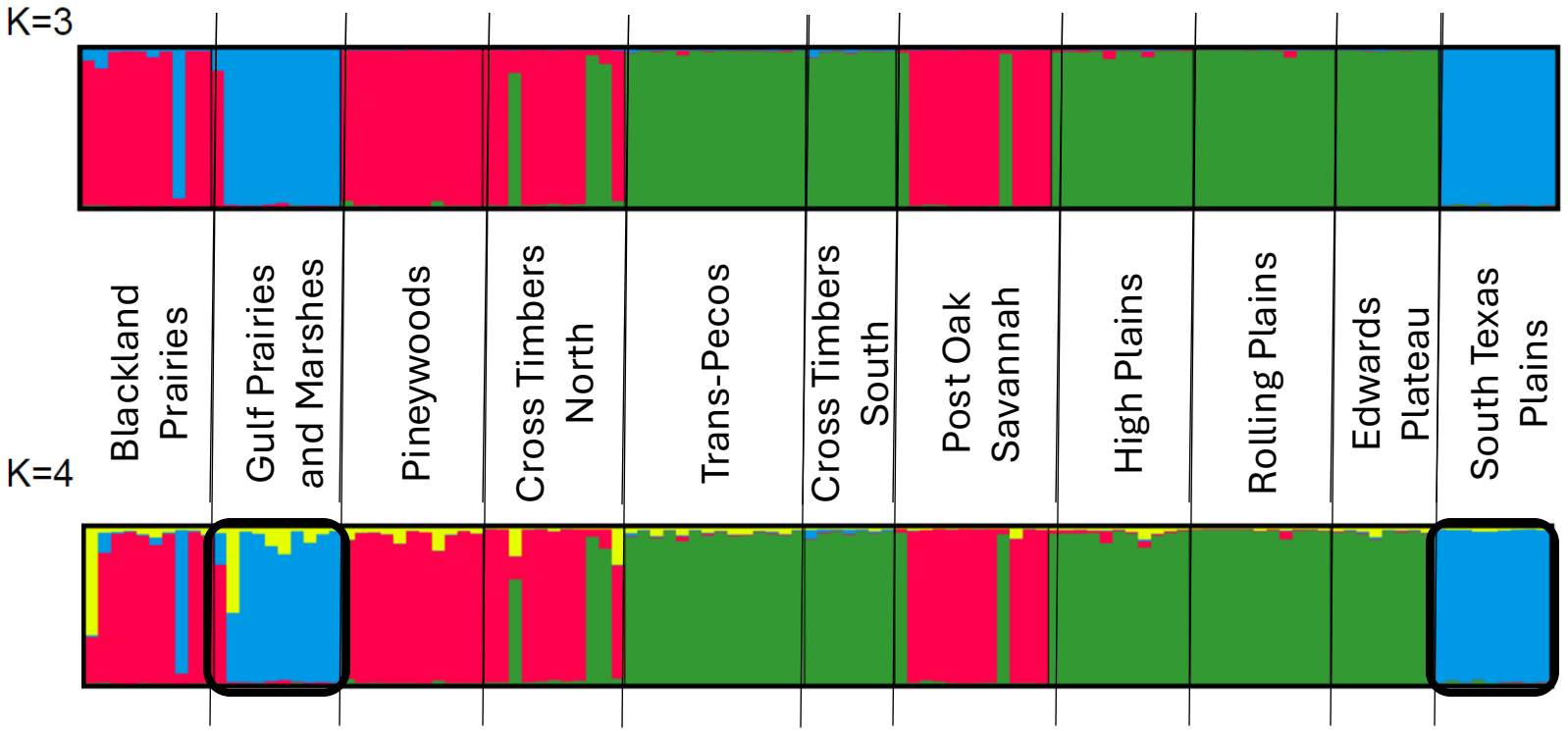




Various methods to determine best K

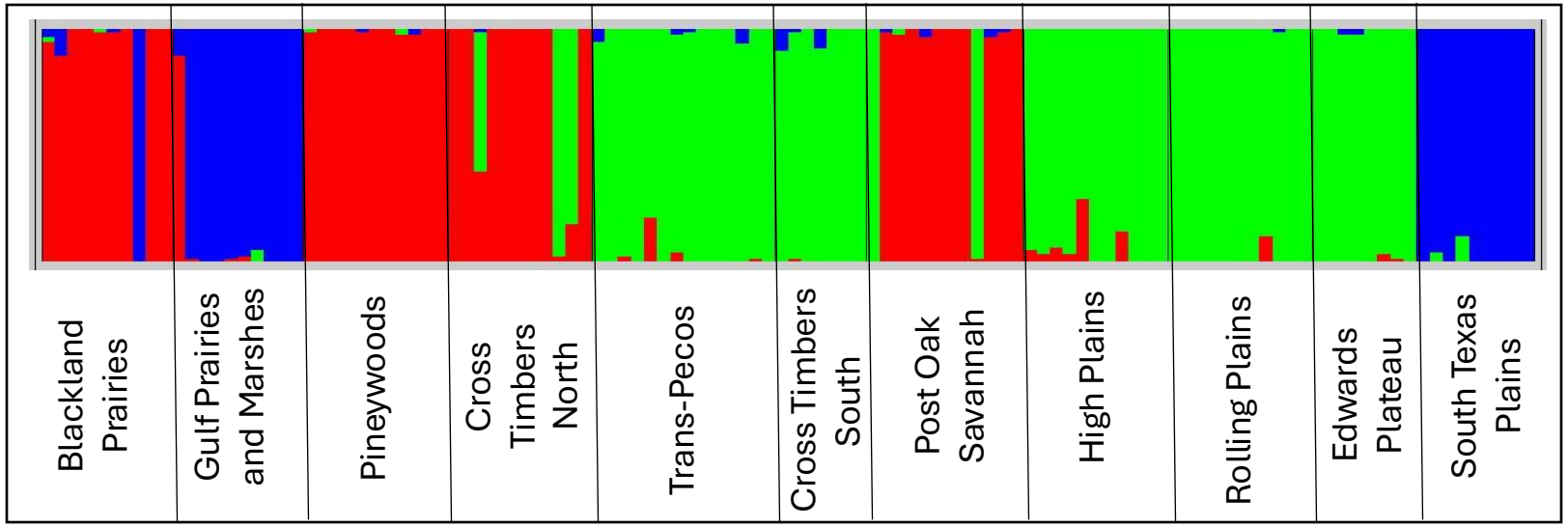
K=genetic clusters

3 or 4 clusters

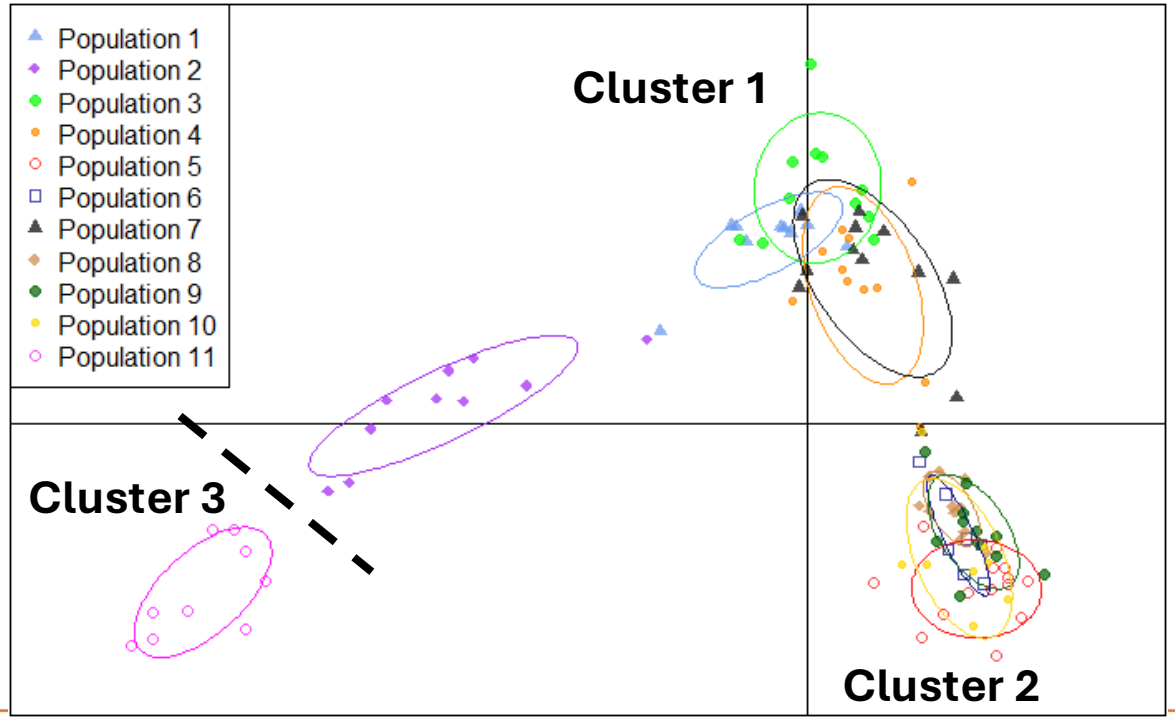


BAPS and DAPC

- BAPS
  - 3 clusters identified



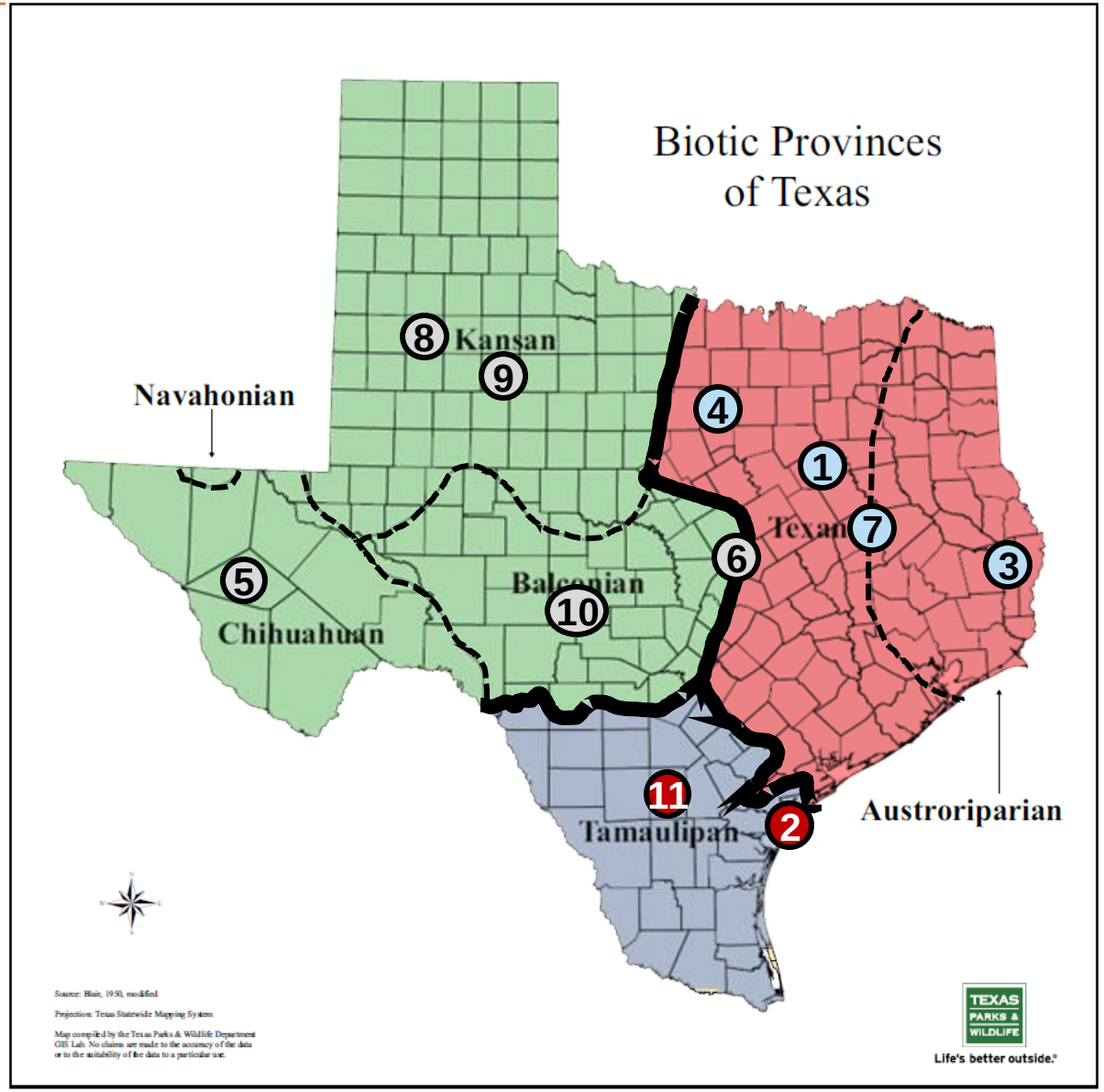
- DAPC
  - p<0.001
  - Separated out Cluster 3



Correlation to Location

- Populations not correlated to ecoregion

- Three genetic clusters correlating with the three major biota of Texas[13]
  - Austroriparian
  - Sonoran
  - Neotropical
- Experience different temperature and precipitation

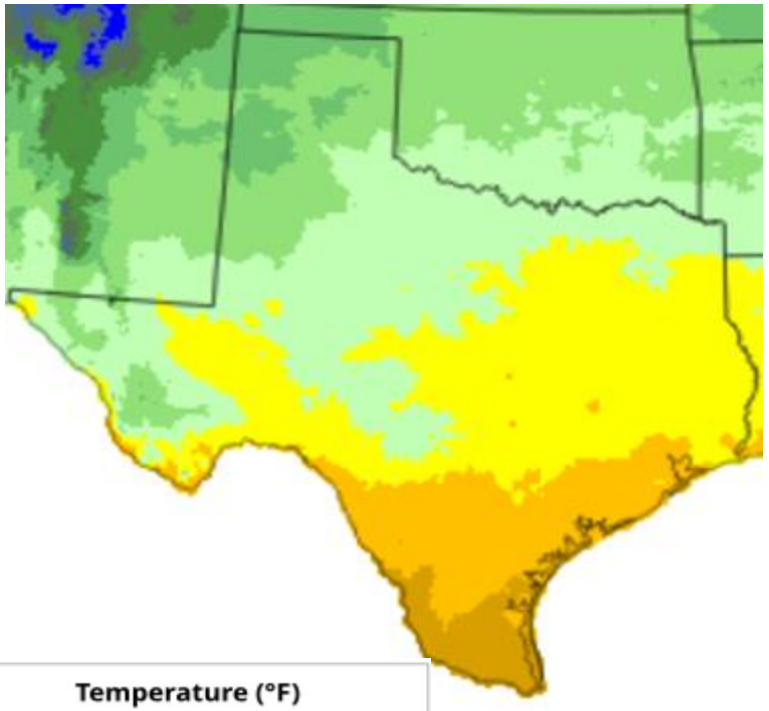


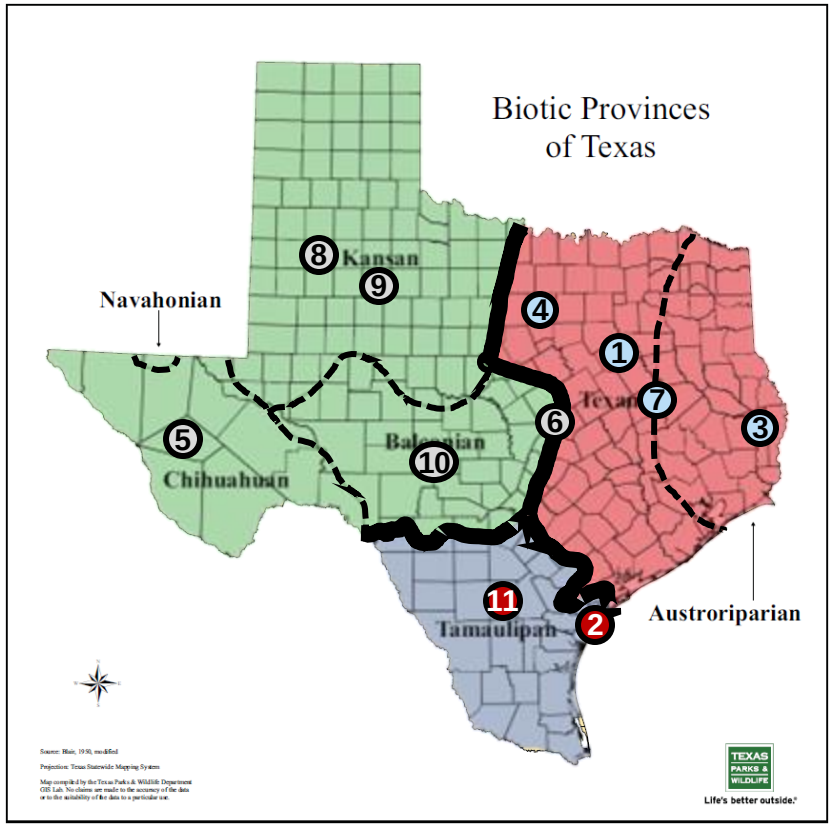
https://tpwd.texas.gov/publications/pwdpubs/media/pwd_mp_e0100_1070ae_08.pdf

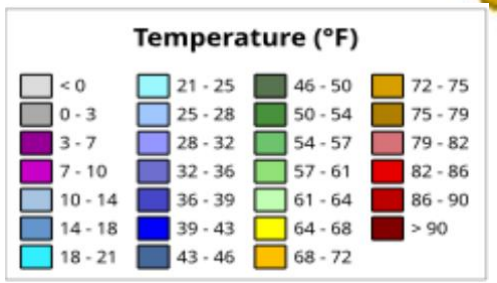
13. Cope ED (1880) On the zoological position of Texas

Temperature and Precipitation

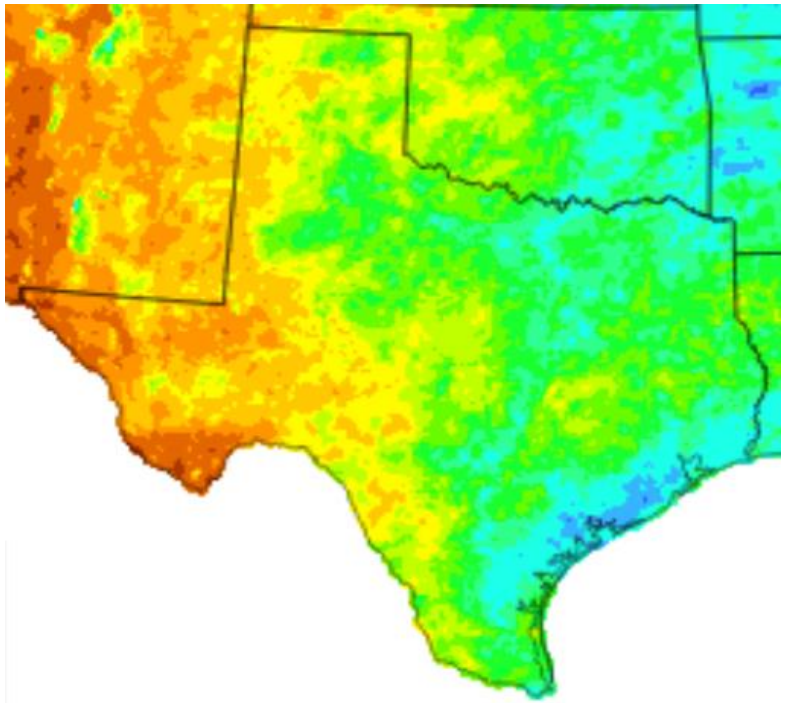
Mean Temperature for 2010

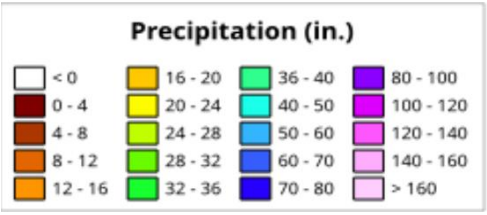






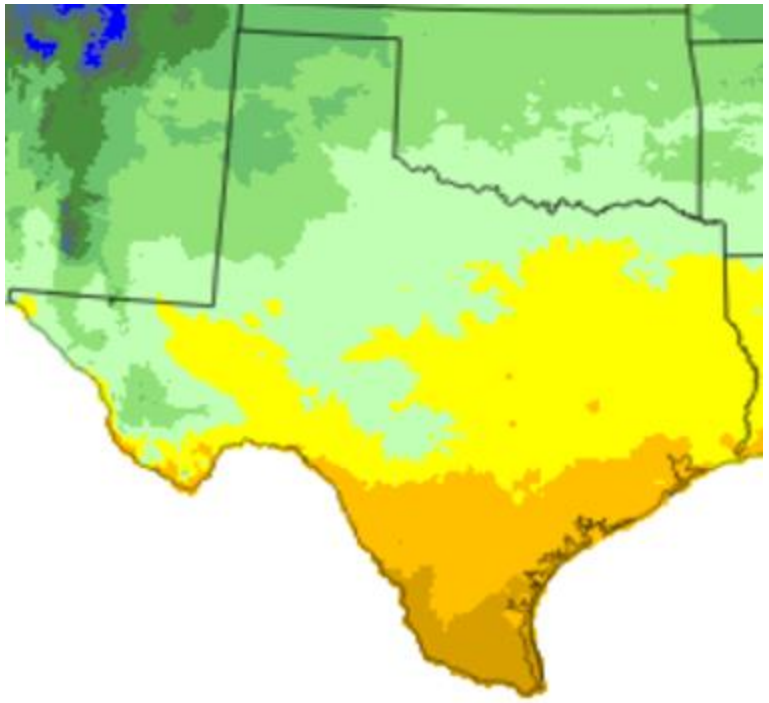
Total Precipitation for 2010

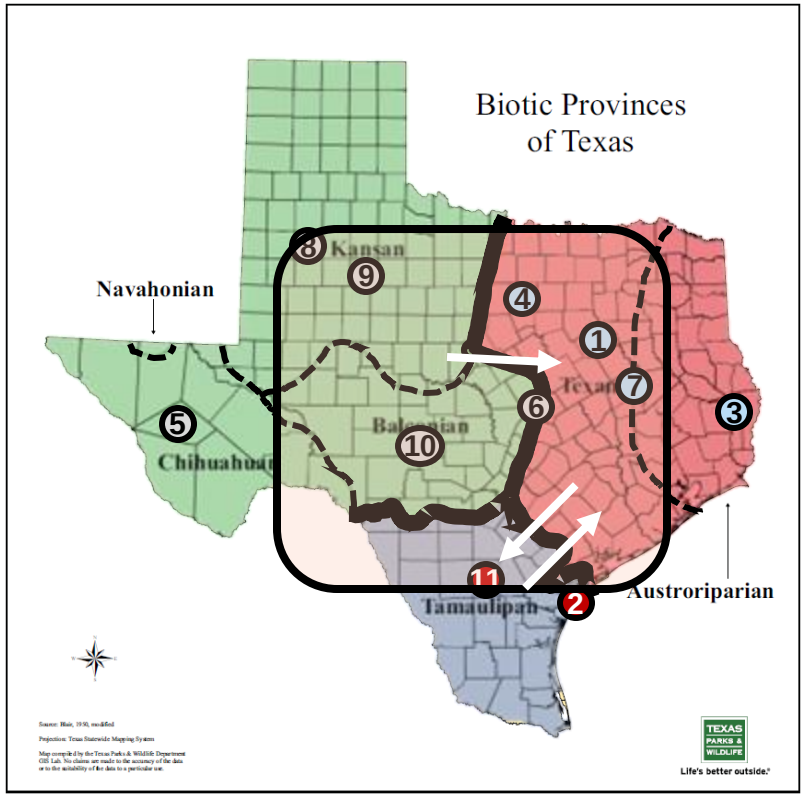




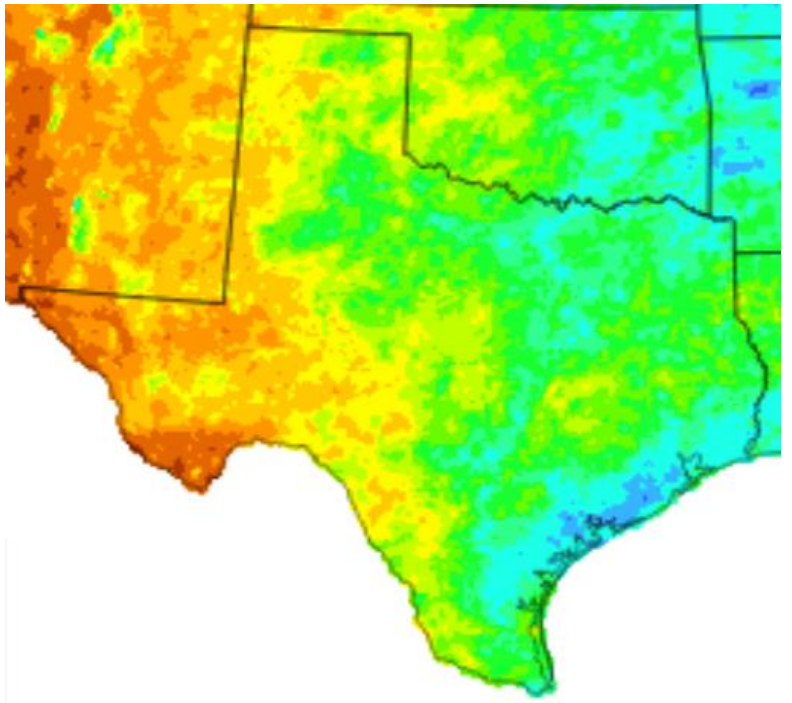
Transition Area and Migrants

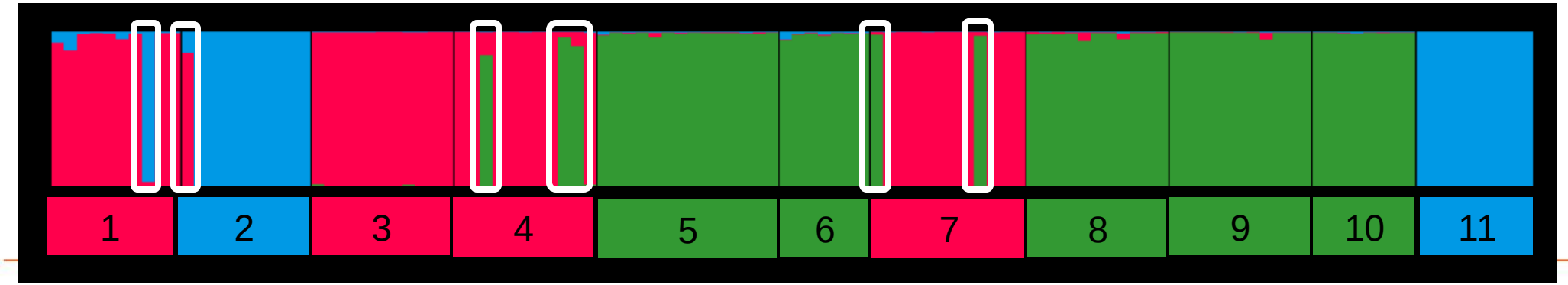
Mean Temperature for 2010

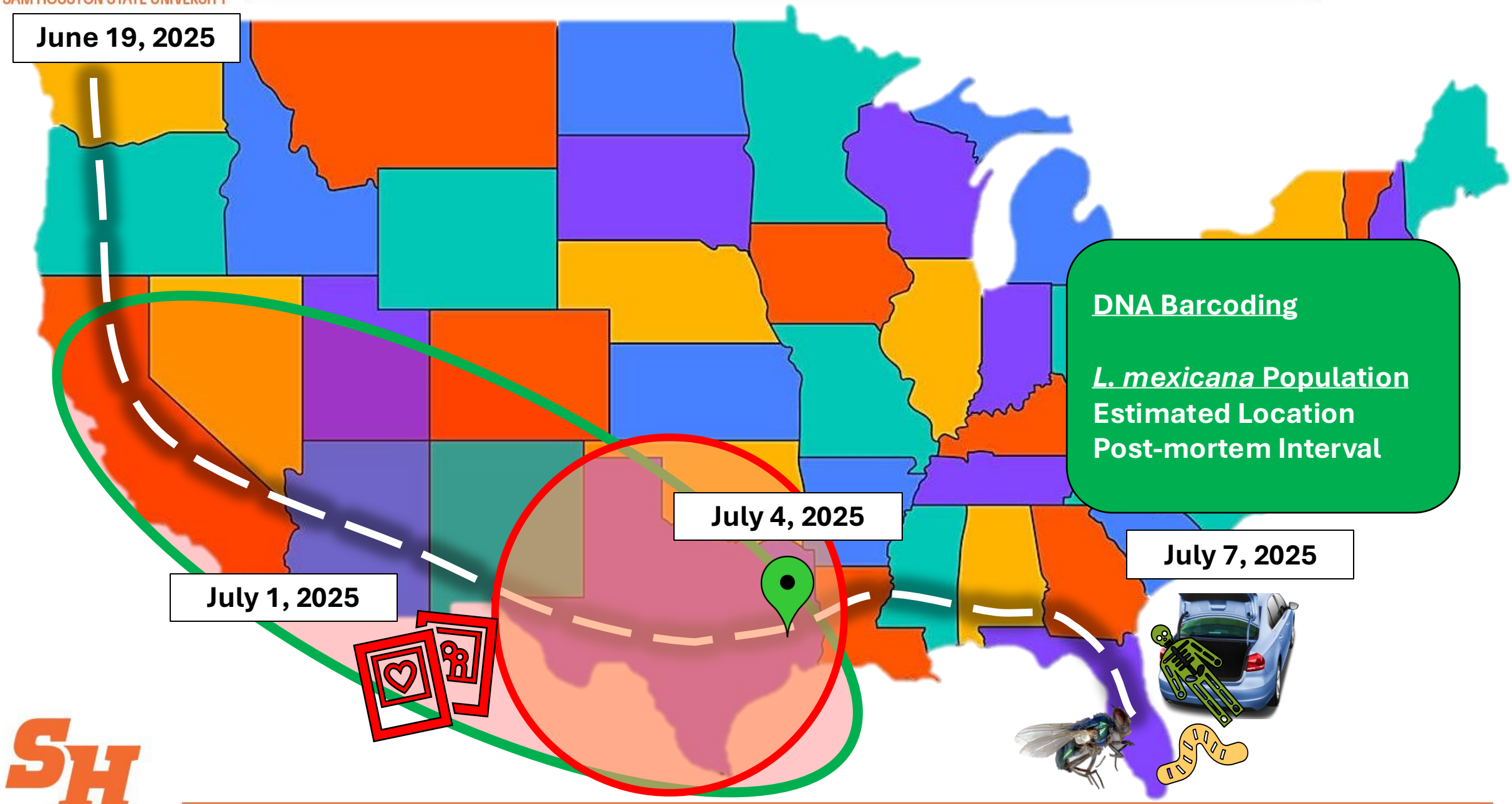




Total Precipitation for 2010






June 19, 2025
July 1, 2025
July 4, 2025
July 7, 2025
DNA Barcoding
L. mexicana Population
Estimated Location
Post-mortem Interval

Conclusion

- Populations not differentiated based on ecoregions
- Clusters correlated with the three major biota of Texas
  - Temperature and precipitation gradients
- Different temperatures could lead to different development requirements and thresholds
- Future studies include the optimization of the panel
  - Additional informative markers
- Examine more recently collected *Lucilia mexicana*
  - Effects of climate change in the past 10+ years

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Sam Houston State University
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Biological Sciences

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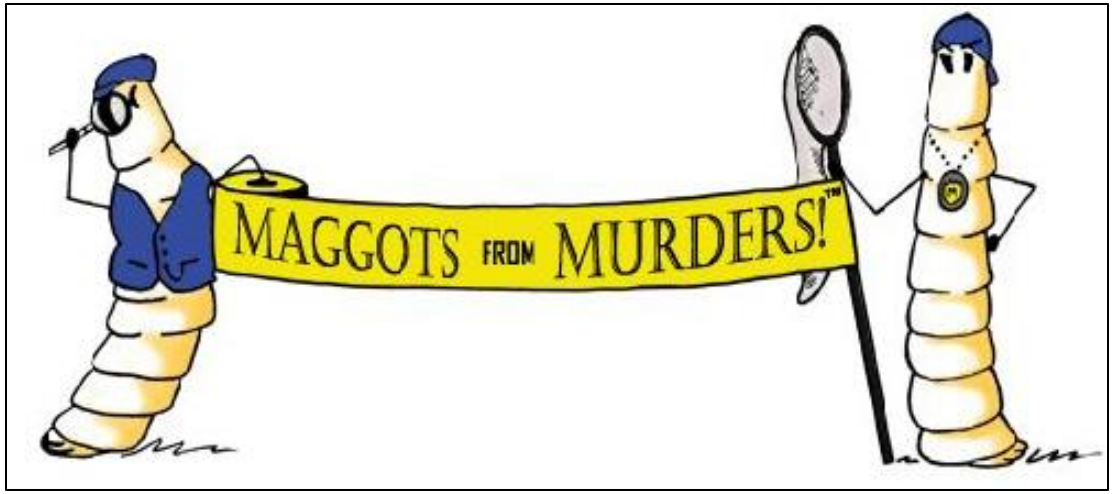

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