DoD Environmental Planning and Conservation Webinar Series

Assessment of the population genetics of Golden-cheeked Warblers: updates from genome-enabled studies

June 21, 2023

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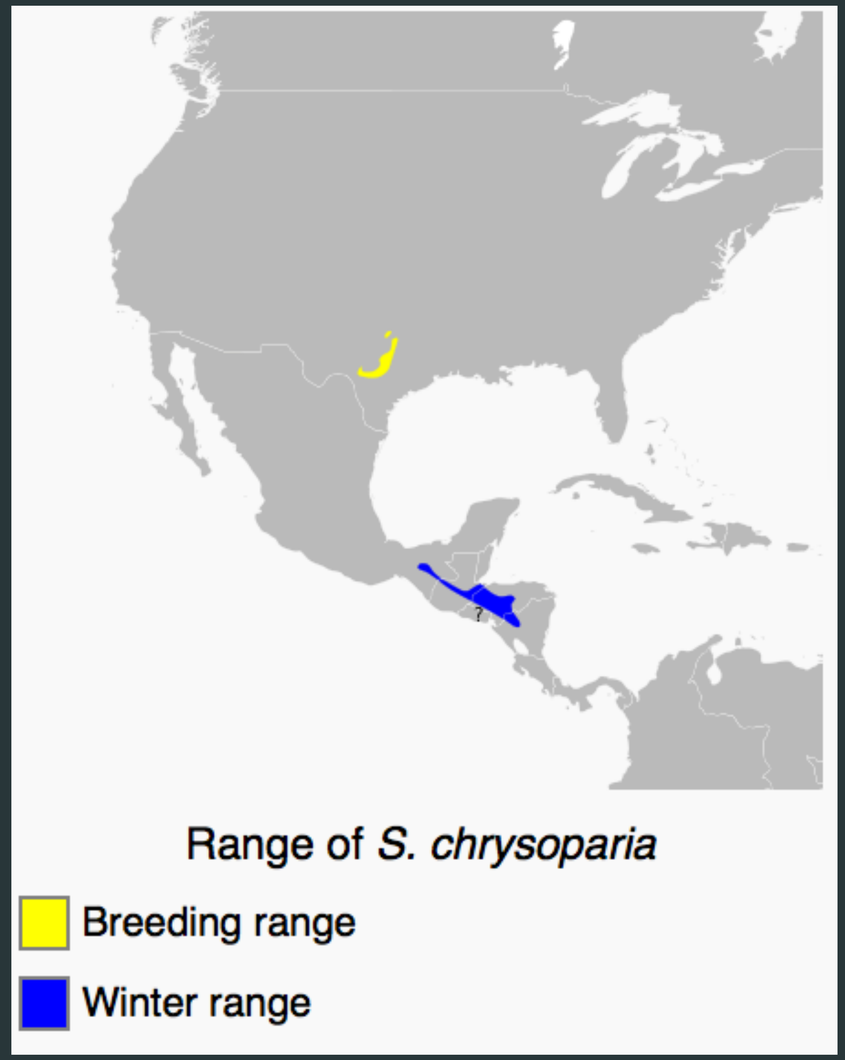
Assessment of Population Genetics of Golden-cheeked Warblers

Giri Athrey, Ph.D, Texas A&M University
Laurel Moulton, Ph.D, City of Austin
Lisa O'Donnell, M.S, City of Austin

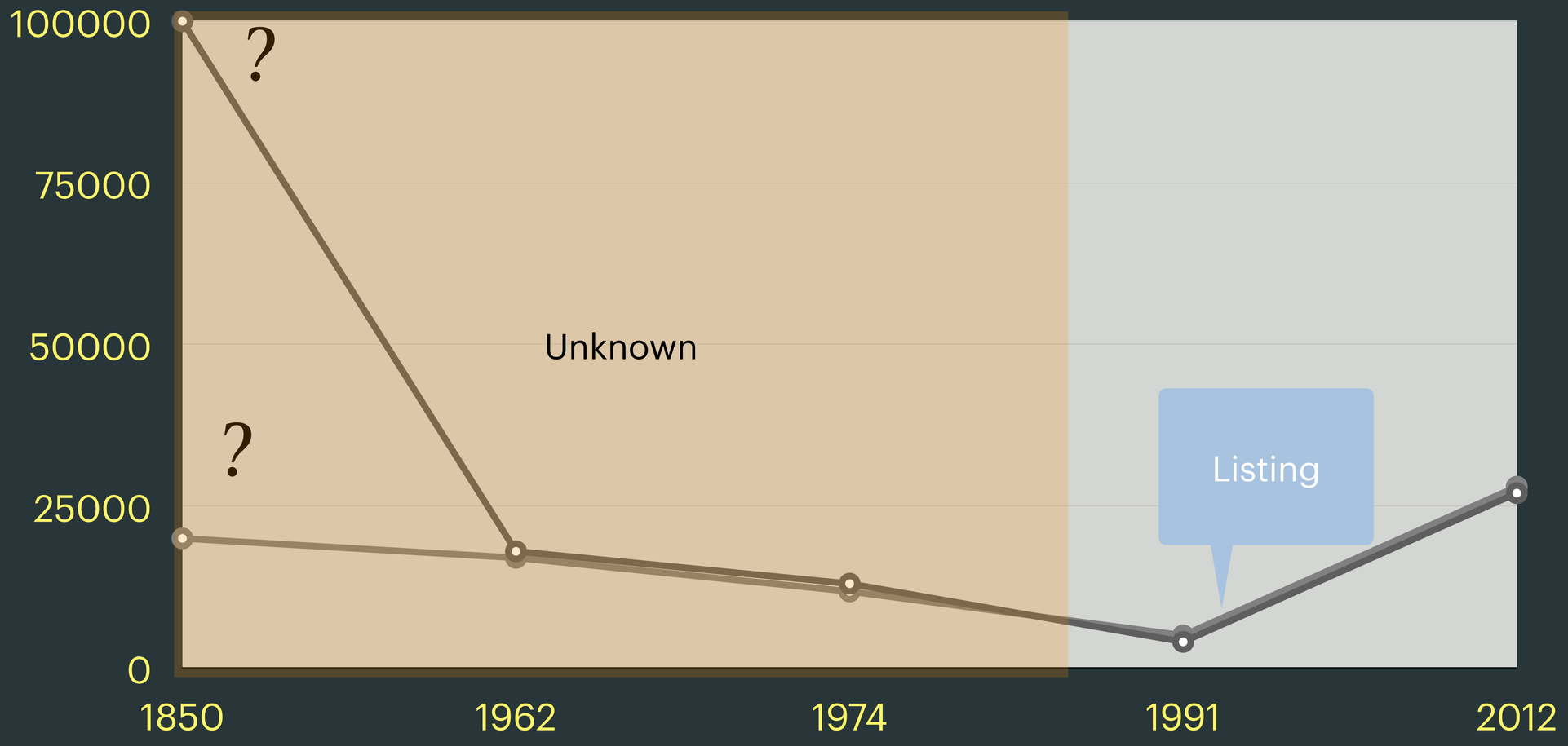
Funded through USACE W9126G- 19-2-0014

TEXAS A&M UNIVERSITY

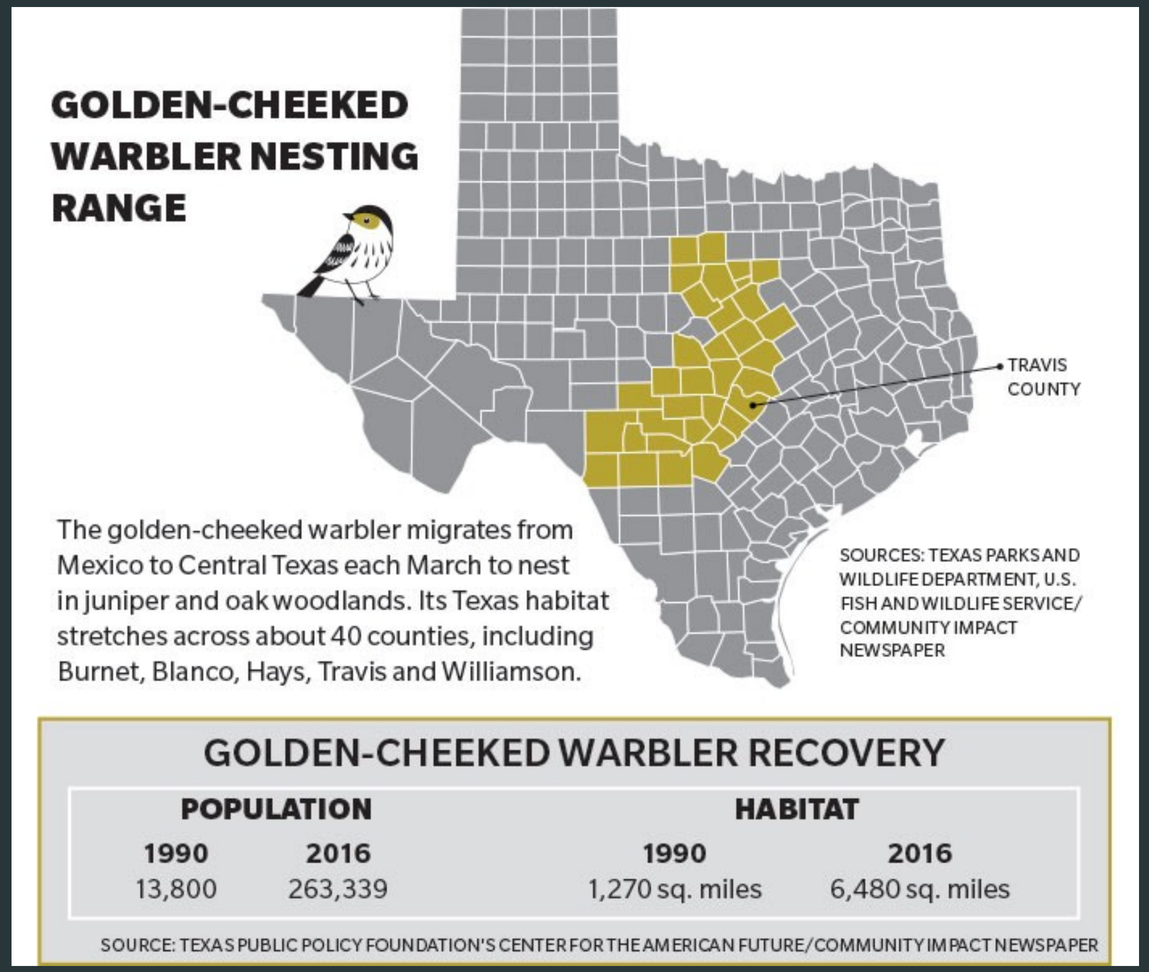
Setophaga chrysoparia
The Golden-cheeked Warbler

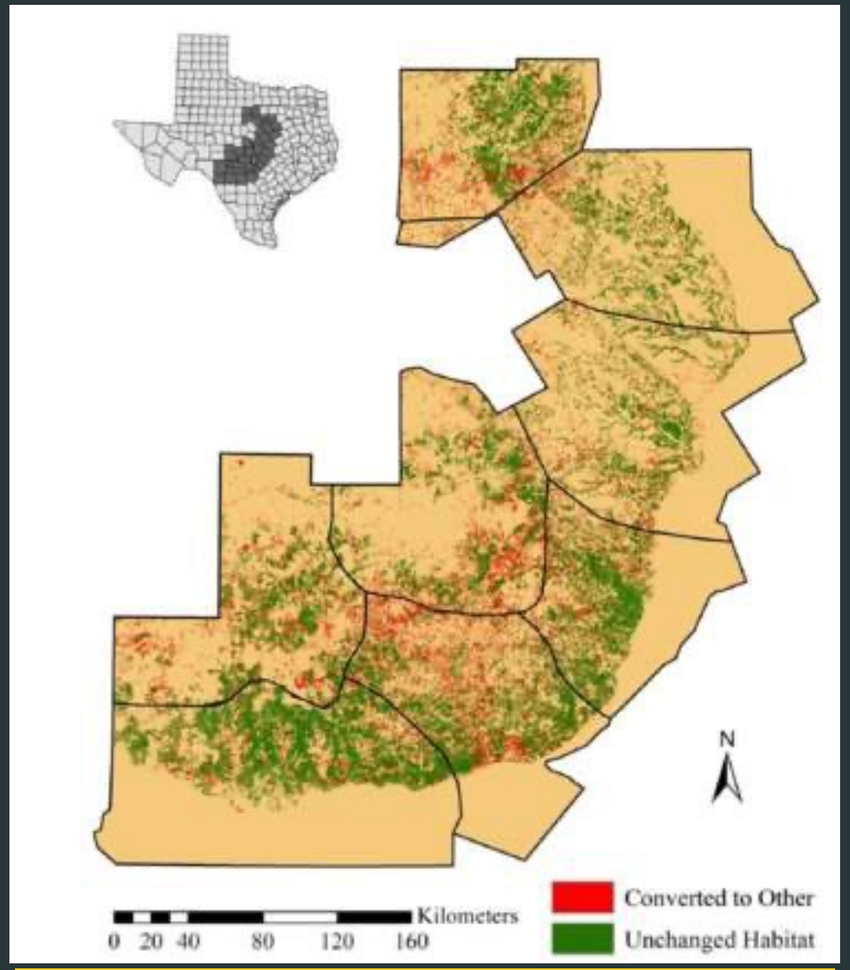


Population estimates of GCWA



Management and Recovery Status





Estimated ~30% reduction in warbler breeding habitat between 2001-2011.

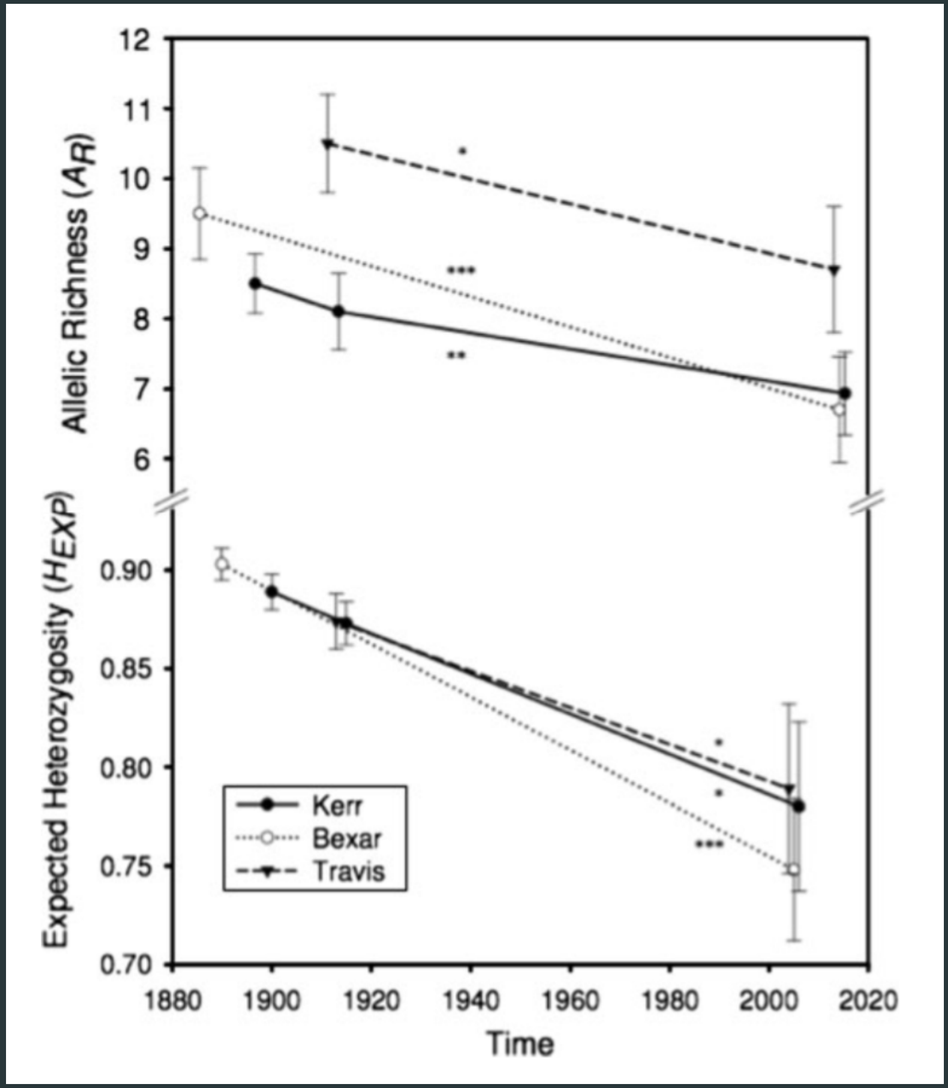
Source: Land Change Science @ TX State

Previous genetic study showed decline in genetic diversity

How did habitat changes and population size changes impact genetic diversity?

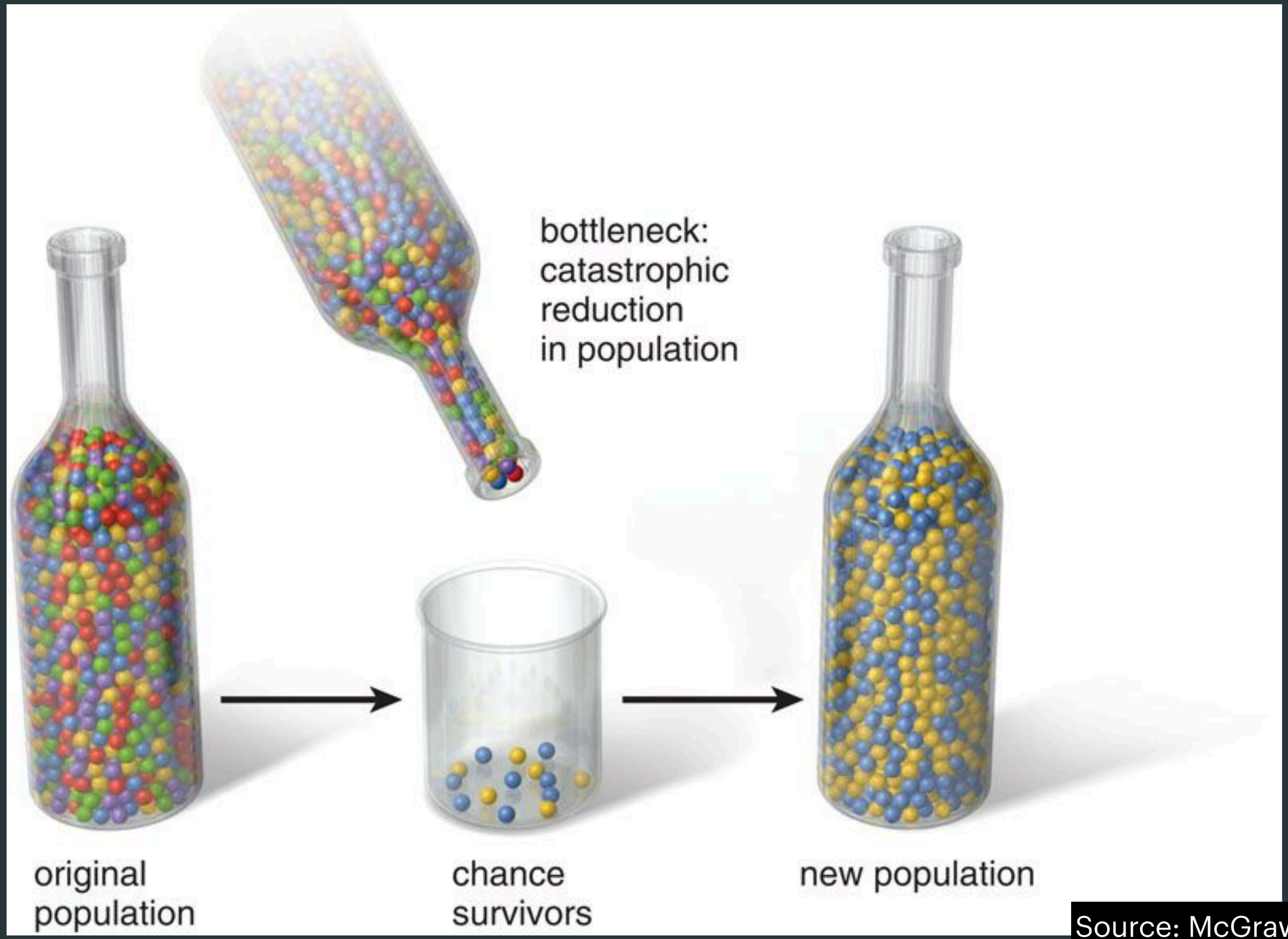
Historical context needed to answer this question

How steep was the decline?

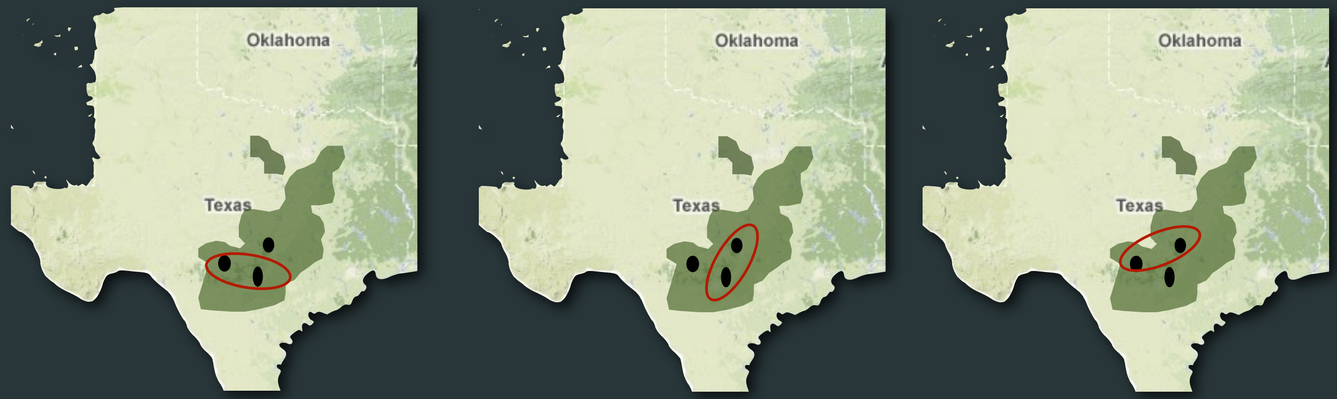


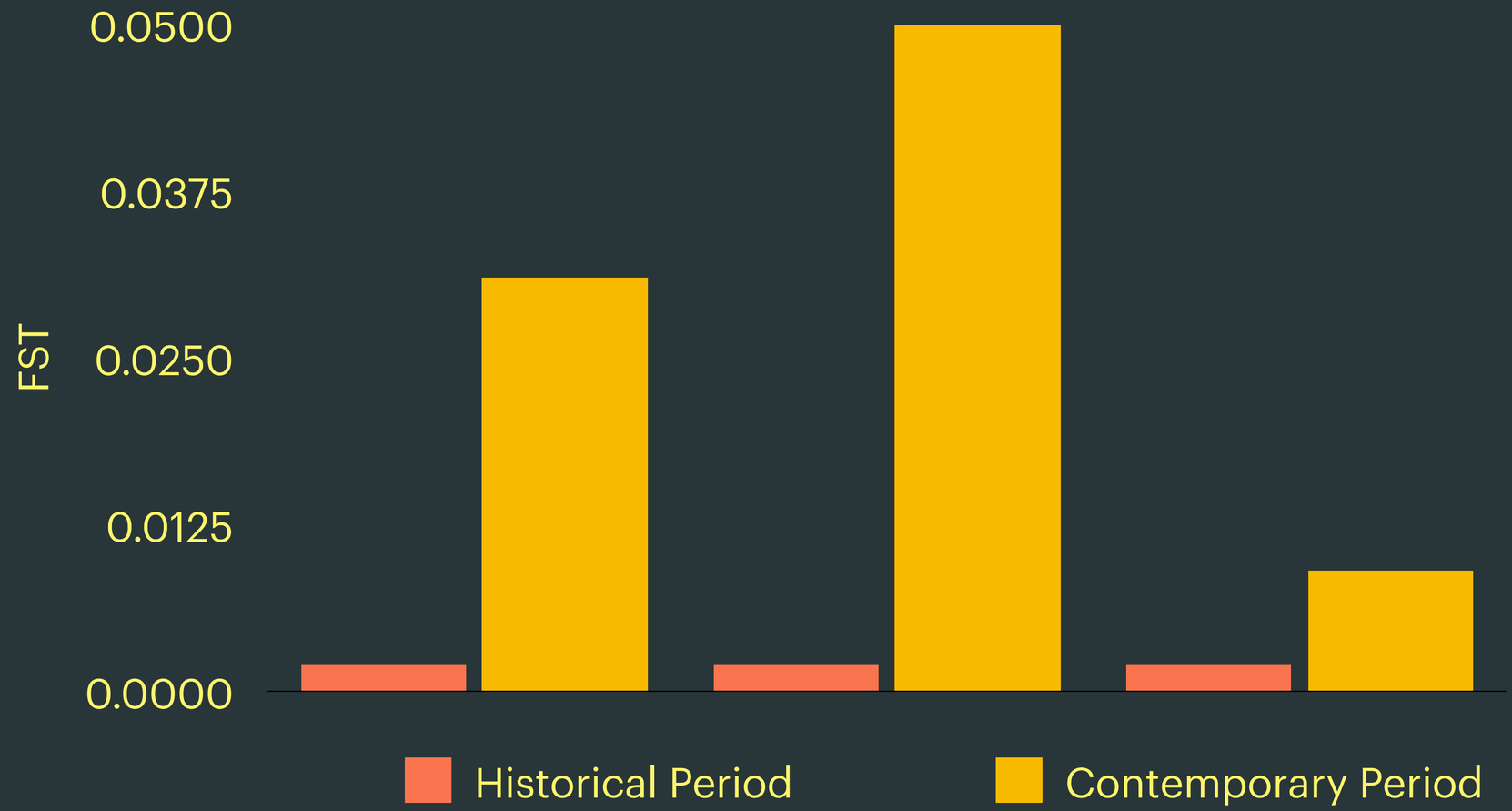
20-30% decline in measures of genetic diversity (on average)

Athrey *et al* 2012



Source: McGraw Hill Education





Athrey *et al* 2012

Objective 1

To generate a *de novo* assembly of the GCWA genome using a combination of short-read and long-read sequencing technologies to be made freely to the public for future studies of GCWA and closely related species.

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What is a genome reference?

- A reference genome is a **haploid representation** of a species' genome
- Can be constructed from a single individual (more common now), or from a pool (common earlier)
- Alternate alleles (when known from population data) are assembled alongside reference locus
- Organized into chromosomes (best), or scaffolds (better), or contigs/supercontigs (ok).

Copies of genomic DNA

Sequence reads

"All the News That's Fit to Print"

The New York Times.

LATE CITY EDITION

VOL. XCIV ..No. 31,980. NEW YORK, WEDNESDAY, AUGUST 15, 1945. THREE CENTS

JAPAN SURRENDERS, END OF WAR! EMPEROR ACCEPTS ALLIED RULE; M'ARTHUR SUPREME COMMANDER; OUR MANPOWER CURBS VOIDED

HIRING MADE LOCAL

Communities, Labor and Management Will Unite Efforts

6,000,000 AFFECTED

Draft Quotas Cut, Services to Drop 5,500,000 in 18 Months

By LEWIS WOOD

Third Fleet Fells 5 Planes Since End

SECRETS OF RADAR GIVEN TO WORLD

Its Role in War and Uses for Peacetime Revealed in Washington and London

By WILLIAM S. WHITE

ALL CITY 'LETS GO'

Hundreds of Thousands Roar Joy After Victory Flash Is Received

TIMES SQ. IS JAMMED

Police Estimate Crowd in Area at 2,000,000—Din Overwhelming

By ALEXANDER FEINBERG

PRESIDENT ANNOUNCING SURRENDER OF JAPAN

Mr. Truman reading the message in the White House. Seated are Admiral William D. Leahy, Secretary of State James F. Byrnes and former Secretary of State Cordell Hull. Standing (left to right) are Maj. Gen. Philip Fleming, head of the Federal Works Administration; William H. Davis, Economic Stabilizer; John W. Snyder, Reconversion Director; James Forrestal, Secretary of the Navy; Fred Vinson, Secretary of the Treasury; Tom Clark, Attorney General, and Lewis Schoenbrunch, Secretary of Labor.

PETAIN CONVICTED, SENTENCED TO DIE

Jurors Recommend Clemency Because of His Age—Long Indictment Upheld

By G. H. ARCHAMBAULT

Terms Will Reduce Japan To Kingdom Perry Visited

By JAMES B. RESTON

TREATY WITH CHINA SIGNED IN MOSCOW

Complete Agreement Reached With Chungking on All Points at Issue, Russians Say

YIELDING UNQUALIFIED, TRUMAN SAYS

Japan Is Told to Order End of Hostilities, Notify Allied Supreme Commander and Send Emissaries to Him

MAC ARTHUR TO RECEIVE SURRENDER

Formal Proclamation of V-J Day Awaits Signing of Those Articles—Cease-Fire Order Given to the Allied Forces

By ARTHUR KROCK

WASHINGTON, Aug. 14—Japan today unconditionally surrendered the hemispheric empire taken by force and held almost intact for more than two years against the rising power of the United States and its Allies in the Pacific war.

The bloody dream of the Japanese military caste vanished in the text of a note to the Four Powers accepting the terms of the Potsdam Declaration of July 26, 1945, which amplified the Cairo Declaration of 1943.

Like the previous items in the surrender correspondence, today's Japanese document was forwarded through the Swiss Foreign Office at Berne and the Swiss Legation in Washington. The note of total capitulation was delivered to the State Department by the Legation Charge d'Affaires at 6:10 P. M., after the third and most anxious day of waiting on Tokyo, the anxiety intensified by several premature or false reports of the finale of World War II.

Orders Given to the Japanese

President Truman summoned a special press conference in the Executive offices at 7 P. M. He handed to the reporters three texts.

The first—the only one he read aloud—was that he had received the Japanese note and deemed it full acceptance of the Potsdam Declaration, containing no qualification whatsoever; that arrangements for the formal signing of the peace would be made for the "earliest possible moment;" that the Japanese surrender would be made to General MacArthur in his capacity as Supreme Allied Commander in Chief; that Allied military commanders had been instructed to cease hostilities, but that the formal proclamation of V-J Day must await the formal signing.

The text ended with the Japanese note, in which the Four Powers (the United States, Great Britain, China and Russia) were officially informed that the Emperor of Japan had issued an imperial rescript of surrender, was prepared to guarantee the necessary signatures to the terms as prescribed by the Allies, and had instructed his commanders to cease active operations, to surrender all

Continued on Page 2, Column 3

Hirohito on Radio; Minister Ends Life

Two-Day Holiday Is Proclaimed; Stores, Banks Close Here Today

World News Summarized

WEDNESDAY, AUGUST 15, 1945

Cruiser Sunk, 1,196 Casualties; Took Atom Bomb Cargo to Guam

MacArthur Begins Orders to Hirohito

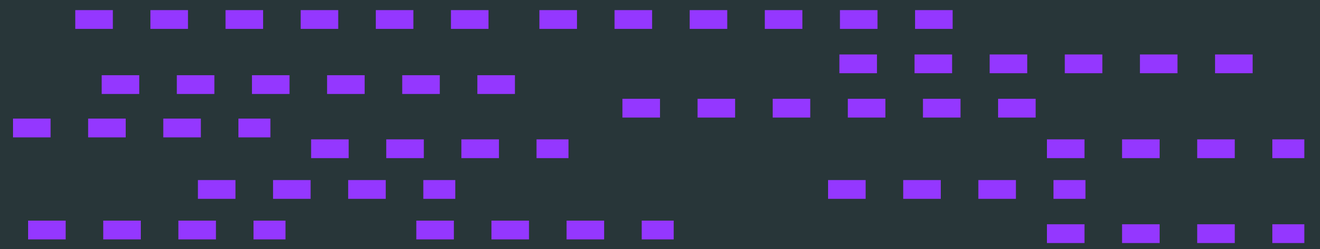
Assembled Genome

Assembly from WGS

Actual Genome sequence

*** Unknown and perhaps unknowable!**

sequenced READS



Perfect Assembly

Typical Assembly

Repetitive regions and or poor sequencing leave gaps

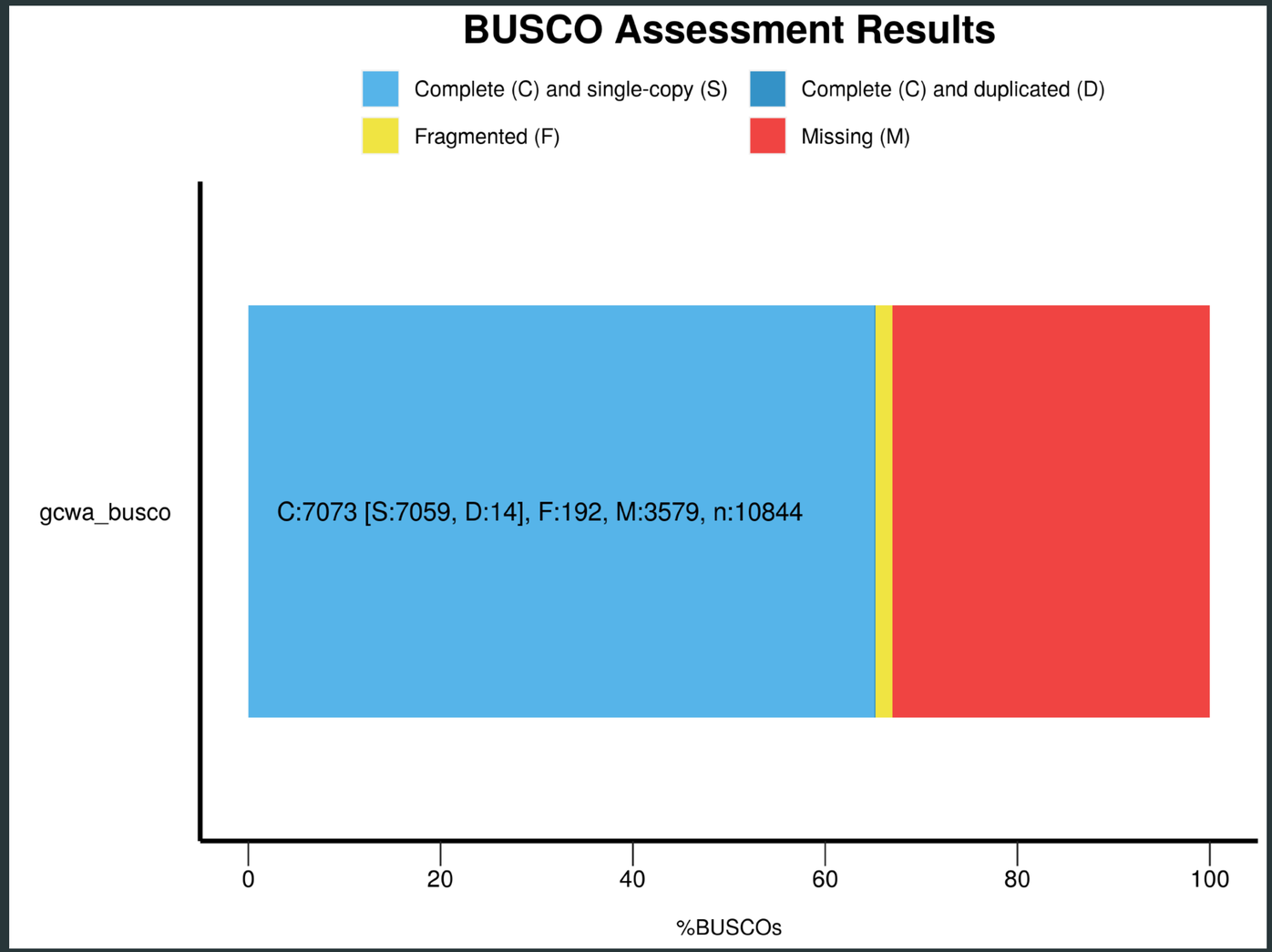
Repeats or un-sequenced

GCWA Assembly quality

Ref Free metric	Haslr Hybrid Assembly	Scaffolded	Gapped Scaffolds
N. Contigs	4719	3862	2450
Longest Contig	4.95 Mbp	6.283 Mbp	12.6 Mbp
N50	703 Kbp	957 Kbp	1.518 Mbp
N/100kpb	0	0.61	414
Total Length	982.98 Mbp	982.57 Mbp	817.252 Mbp

Assembly Completeness

Benchmarking Universal Single Copy Orthologs



Objective 2

Perform a genetic assessment of GCWA population genetic diversity, structure, and population size history across the breeding range to inform species action plan and upcoming USFWS status review.

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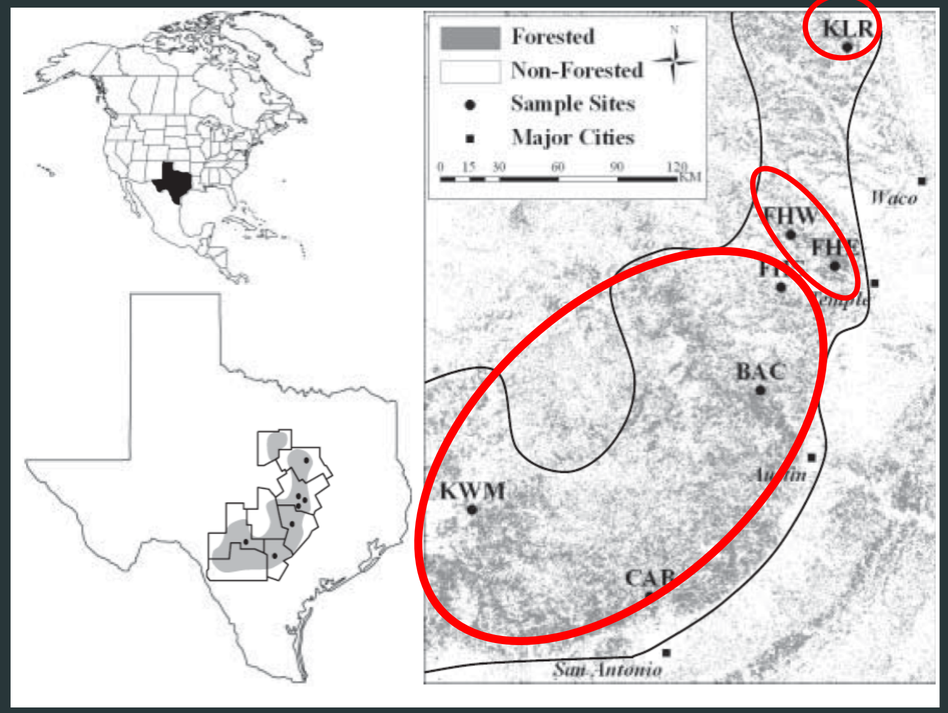
Rationale

Update work done by Lindsay et al. 2008 and Athrey et al. 2011 and to inform species action plan and upcoming USFWS status review.



Lindsay et al. 2008:

From Athrey et al. 2011:

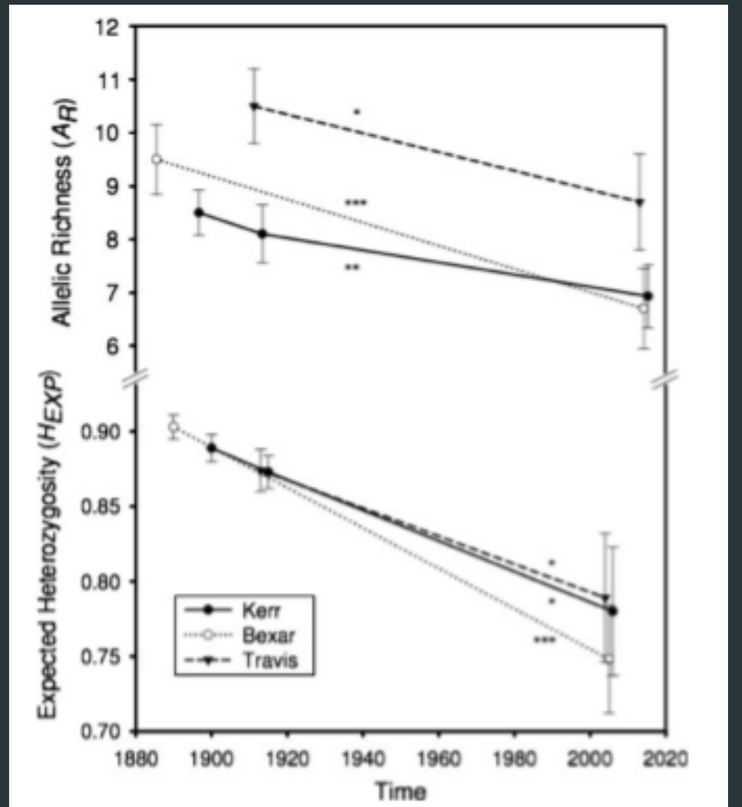
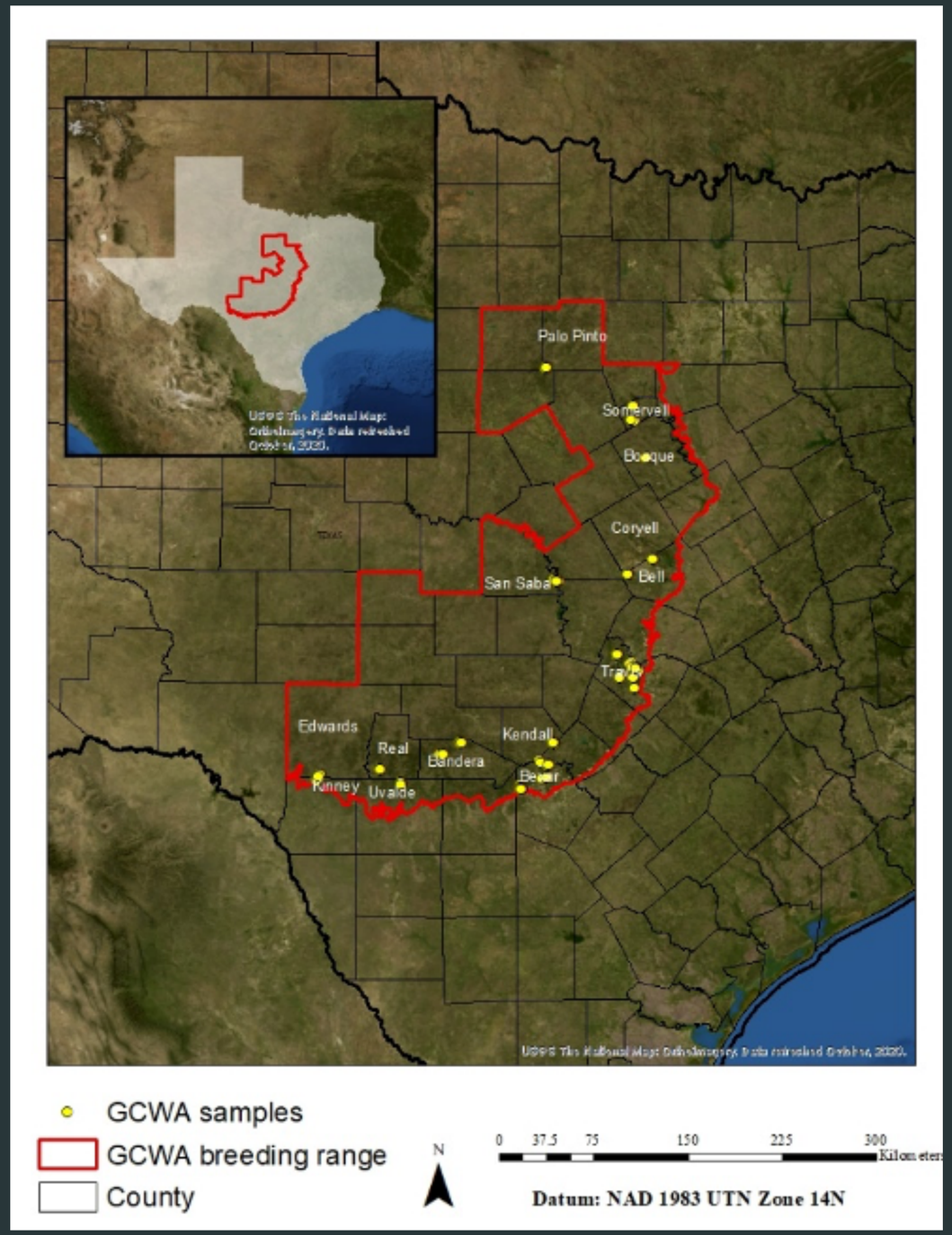


Fig. 2 Estimates of mean genetic diversity (with SEs) for historical and contemporary populations of *D. chrysoparia*. The hypotheses of no difference in estimates of allelic richness (A_R) and expected heterozygosity (H_{EXP}) were evaluated with a *T*-test ($\alpha = 0.05$). *Symbols above each bar* indicate significant pairwise differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)



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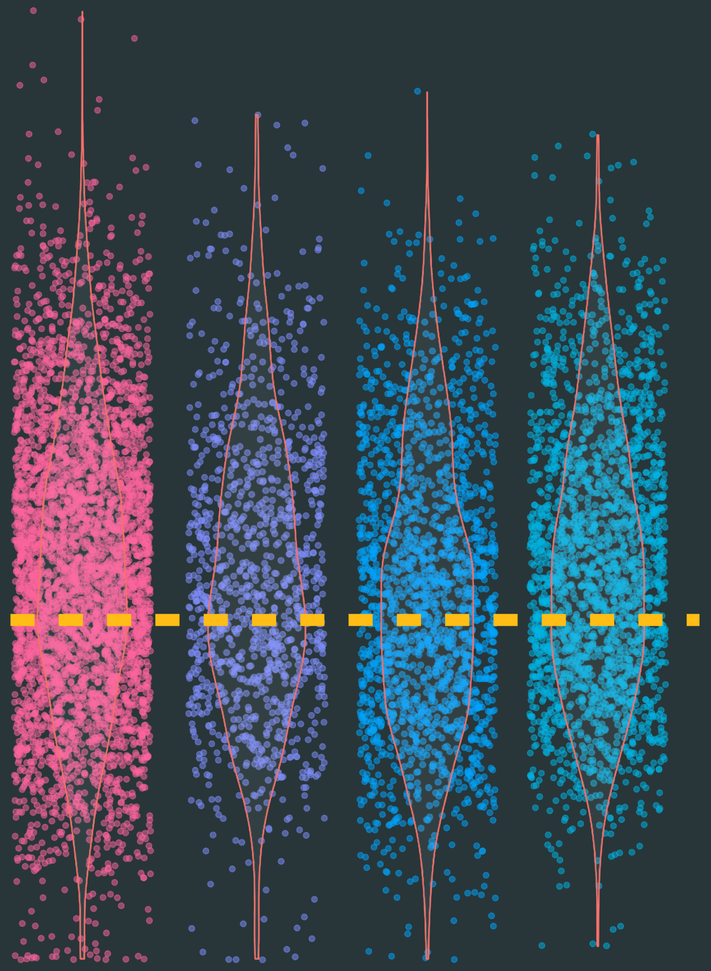
Row Labels	F	M	Grand Total
Bandera	**2**	**25**	**27**
Hatfield Ranch	1	11	12
Love Creek Preserve	1	14	15
Bexar	**1**	**44**	**45**
Camp Bullis		22	22
Government Canyon SNA	1	22	23
Bosque	**1**	**14**	**15**
Meridian State Park	1	14	15
Coryell/Bell	**2**	**21**	**23**
Ft Hood	2	21	23
Kendall		**27**	**27**
Guadalupe River State Park		27	27
Kinney/Edwards		**15**	**15**
Dobbs Run Ranch		3	3
Kickapoo Caverns State Park		12	12
Palo Pinto		**16**	**16**
Palo Pinto State Park		16	16
San Saba		**20**	**20**
Colorado Bend State Park		20	20
Somervell	**2**	**17**	**19**
Cahopo Ranch	2	5	7
Fossil Rim Wildlife Center		5	5
Marsh Ranch		7	7
Travis	**7**	**43**	**50**
BCNWR (Victoria)		17	17
BCP - Barton Creek		2	2
BCP - Collins		2	2
BCP - Emma Bike	1	6	7
BCP - Emma Expansion		3	3
BCP - Emma Long	1	4	5
BCP - Forest Ridge	2	3	5
BCP - Hamilton	2		2
BCP - Kent Butler	1	2	3
BCP - Reicher Ranch		2	2
BCP - Vista Point		2	2
Uvalde	**5**	**20**	**25**
Garner State Park	2	13	15
Shield Ranch	3	7	10
Grand Total	**20**	**262**	**282**

- Sequenced DNA from all samples.
  - 43 samples had lower template quality/ library quality, and were not sequenced.
- Short PE libraries (150bp).
- Generated 12-15x genome coverage
  - Coverage is sufficient for calling heterozygotes and numerous population genetics analyses
  - Analysis of genetic diversity and structure with open source tools.

Genome wide nucleotide diversity

π=0.002

π=0.001



π=0.0014

π=0.0039

π=0.0011

π=0.005

π=0.0032

Contemporary Genetic Diversity

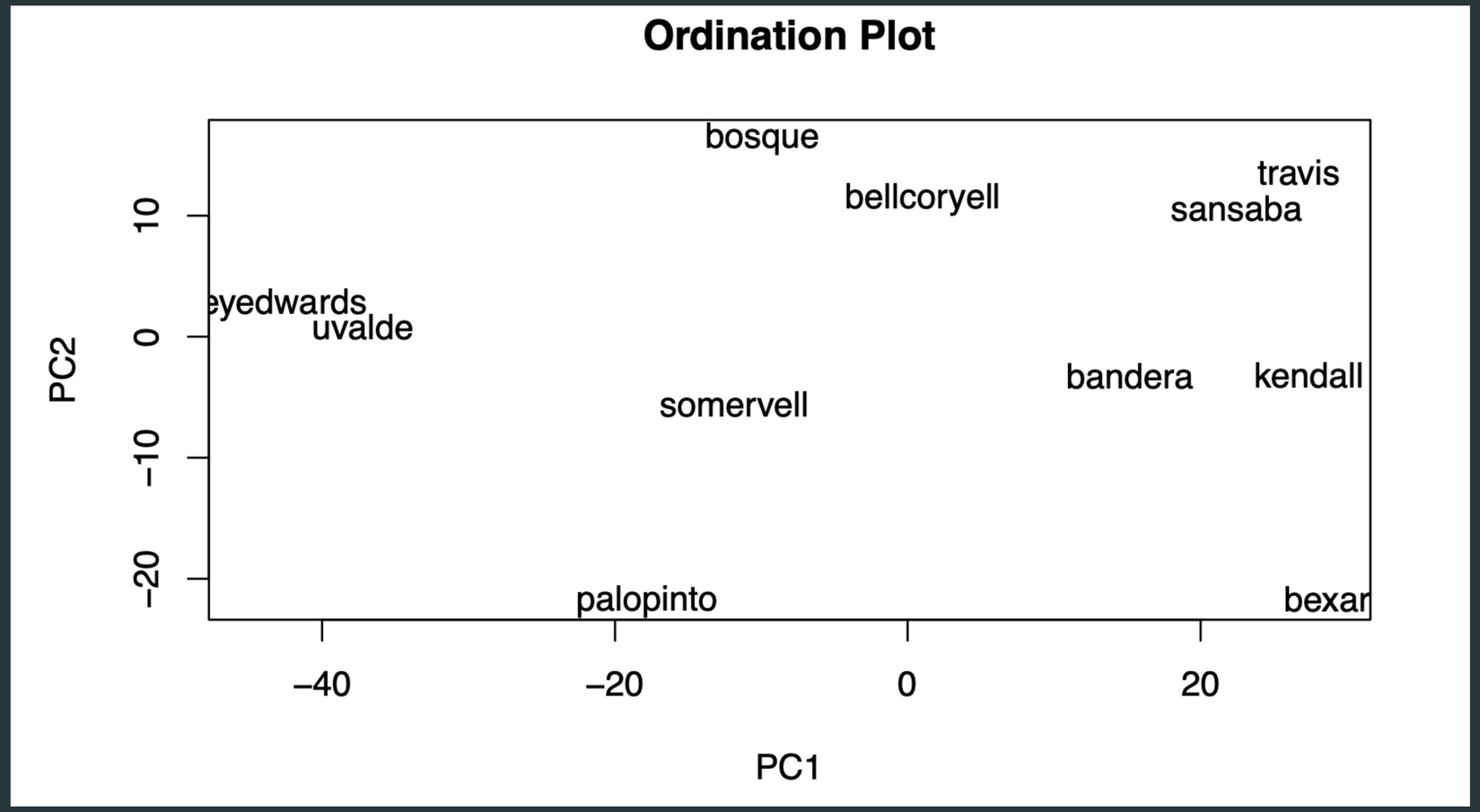
Table 2: Summary of the sample sizes per population, the population-wise inbreeding coefficients (Fi), and the observed heterozygosity (Ho). Genome-wide averages are presented by population, and a final species average is presented.

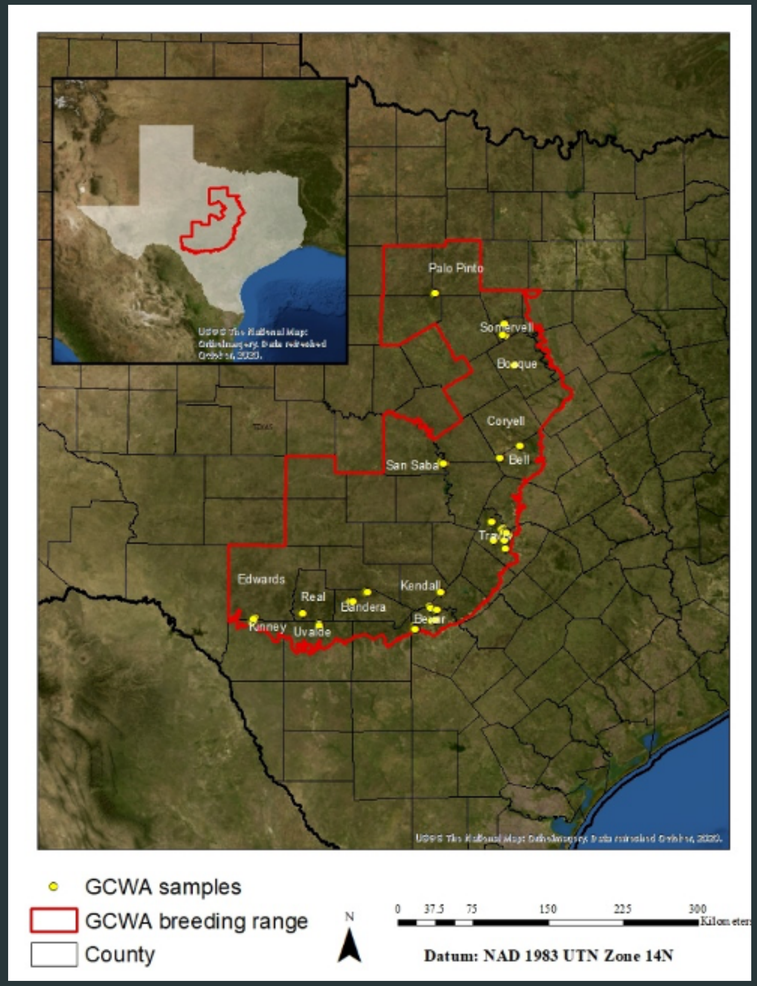
Population	Sample Size (N)	Avg. F_{IS}	Avg. Ho
Bandera	25	0.097	0.025
Bell/Coryell	23	0.095	0.033
Bexar	42	0.075	0.022
Bosque	15	0.114	0.040
Kendall	25	0.070	0.024
Kinney Edwards	15	0.112	0.041
Palo Pinto	16	0.119	0.034
San Saba	19	0.101	0.035
Somervell	18	0.084	0.030
Travis	15	0.124	0.035
Uvalde/Real	25	0.096	0.025
Total	239	0.099 (Avg.)	0.031 (Avg.)

Population Genetic Structure

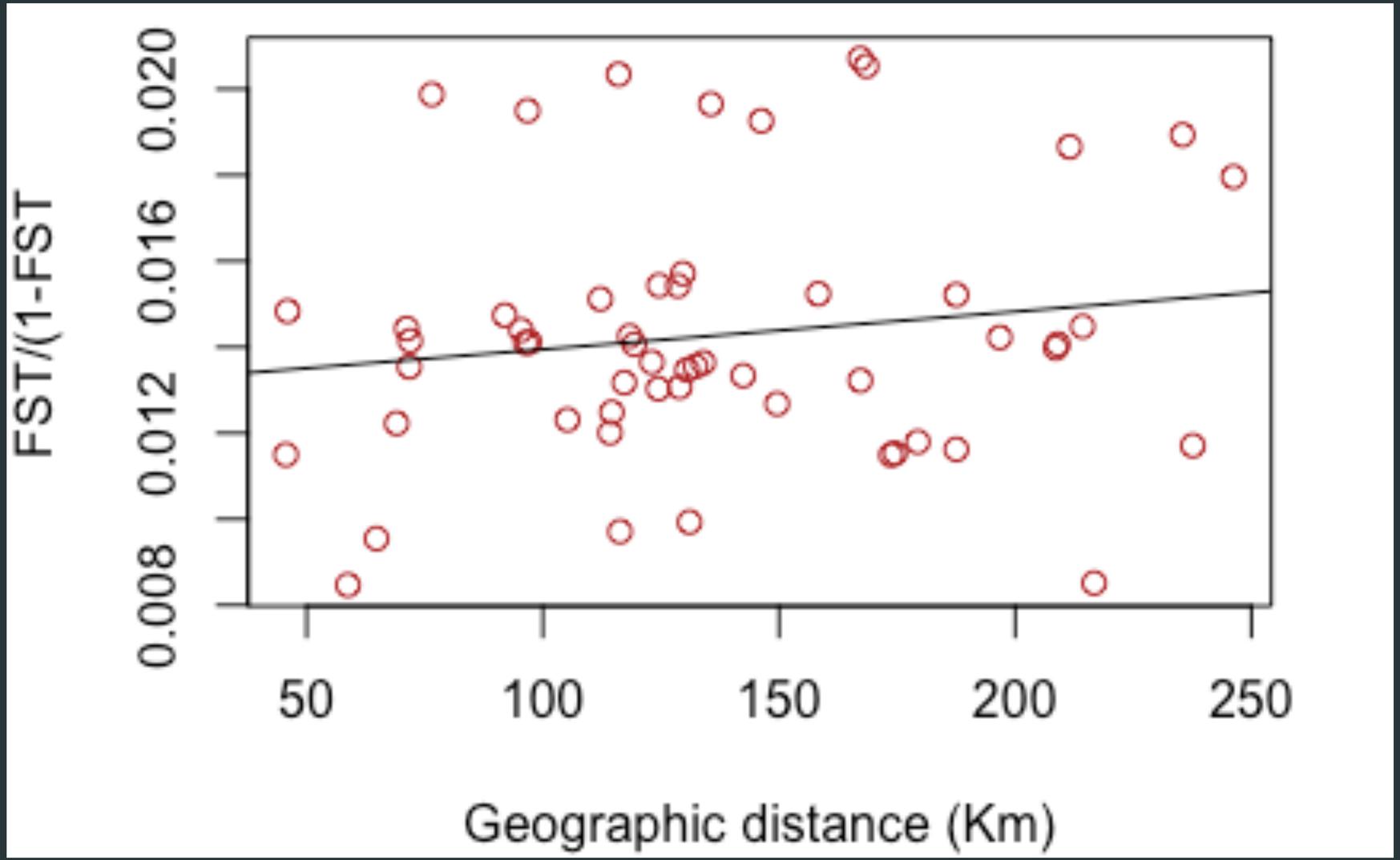
	bellcoryell	bexar	bosque	kendall	kinneyedwa	palopinto	sansaba	somervell	travis	uvalde
bandera	0.011533	0.008467	0.01347	0.011498	0.012311	0.018657	0.013071	0.014068	0.013174	0.009711
bellcoryell		0.009931	0.013541	0.012485	0.014223	0.019261	0.013647	0.014064	0.014116	0.011708
bexar			0.012681	0.009546	0.011795	0.017957	0.011622	0.013032	0.012009	0.008513
bosque				0.014256	0.015239	0.019498	0.01474	0.014396	0.015427	0.01398
kendall					0.013639	0.019651	0.013336	0.014099	0.014147	0.011492
kinneyedwards						0.020535	0.015435	0.015709	0.015215	0.012226
palopinto							0.019875	0.020342	0.020711	0.018936
sansaba								0.01443	0.015119	0.013225
somervell									0.014837	0.014478
travis										0.013552
uvalde										

Population Genetic Structure



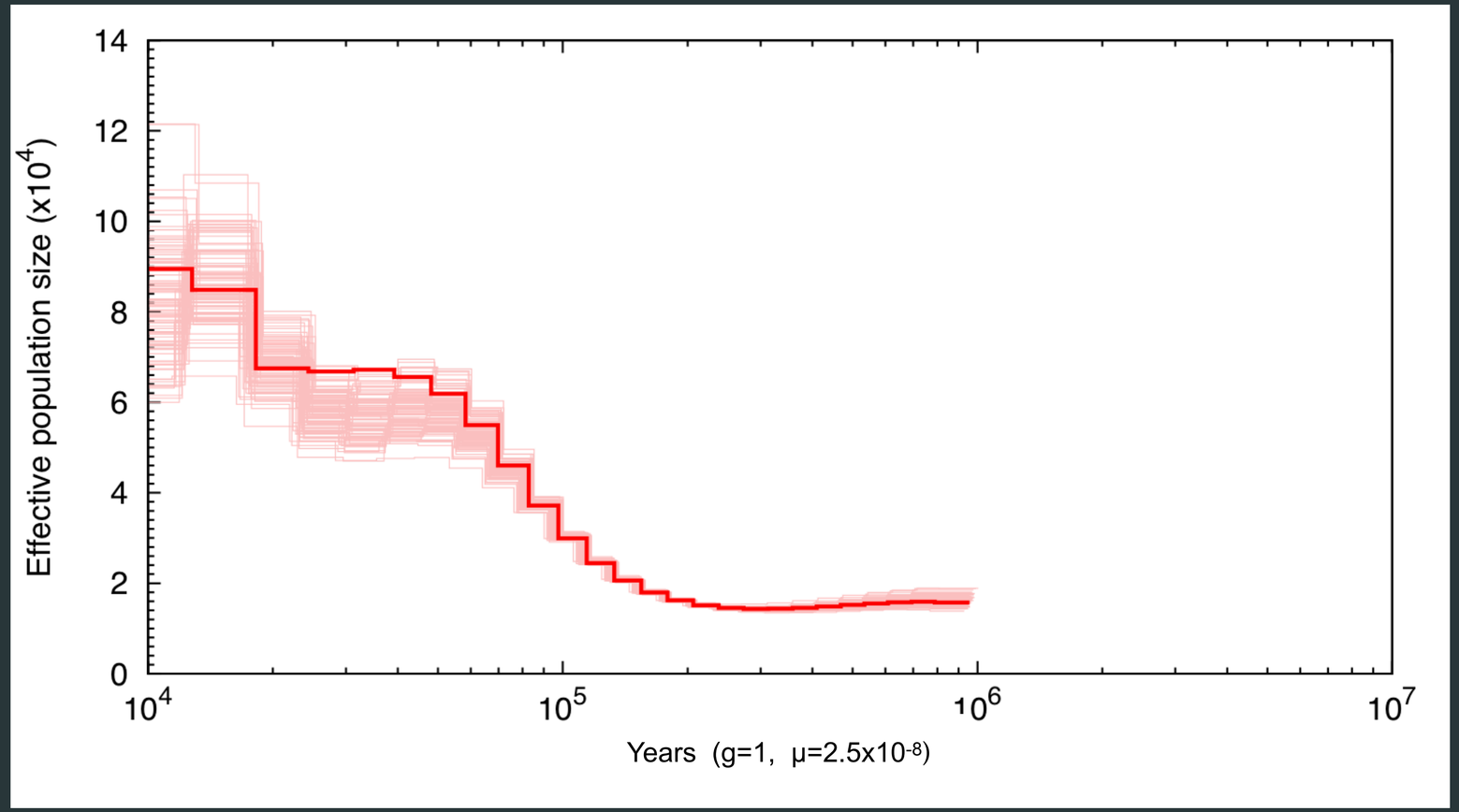


Population Genetic Structure



Historical Demography

Historical demographic reconstruction (with PSMC) based on heterozygous loci indicates that GCWA were relatively abundant during the Pleistocene and entering the Holocene (last 10k years).

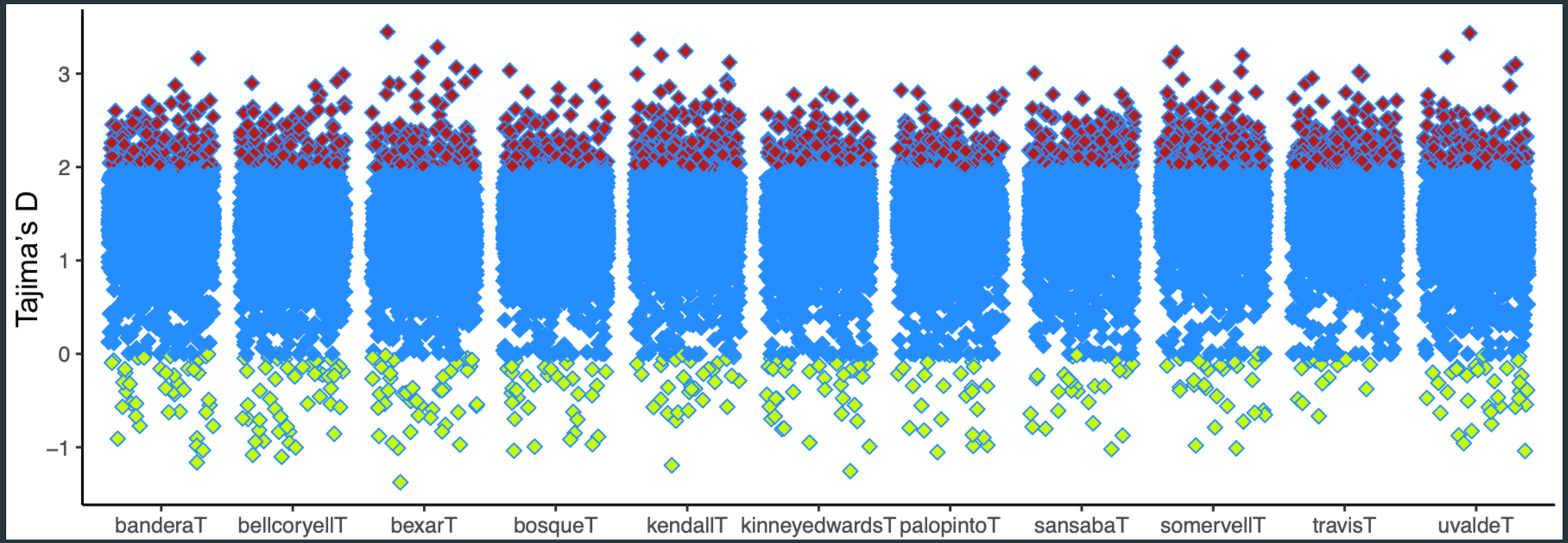


Current Effective Population Sizes

Table 3: Summary of the estimates of effective population sizes using three different methods in this study. When available, the numbers from the temporal estimates (MLNe) generated in the 2011 study are reported alongside. 95% confidence intervals are shown in parentheses when available.

Population	2011 study	N_e (Coancestry)	N_e (LD)	N_e (Het)
Bandera		152 (108-191)	78 (29-103)	5.9
Bell Coryell		140 (73-178)	72 (34-97)	4.8
Bexar	49 (24-212)	254 (159-361)	130 (73-161)	6.1
Bosque		82 (41-104)	46 (31-53)	4.3
Kendall		156 (110-181)	80 (54-201)	3.9
Kinney Edwards		92 (76-123)	47 (24-77)	2.17
Palo Pinto		98 (66-134)	51 (19-81)	7.75
San Saba		116 (91-133)	59 (41-73)	5.17
Somervell		110 (101-145)	52 (38-89)	4.7
Travis	273 (46-621)	89 (53-99)	49 (23-108)	3.47
Uvalde/Real		110 (93-128)	70 (49-103)	4.8

Genome wide Neutrality Tests



Conclusions

- GCWA shows a genome-wide average nucleotide diversity (π) of 0.0014
- Low levels of heterozygosity in every population surveyed, with a species average of 0.31
- There is a high level of inbreeding within each population, with medium to high levels of inbreeding coefficients
- Bexar and Palo Pinto population show high degree of isolation
  - Bell Coryell is not evenly connected with all populations
- Lack of significant Isolation-by-distance, which can be suppressed by low heterozygosity
- Effective population size estimates show clear evidence for small population sizes and recent bottlenecks
- **Overall, lack of genomic evidence supporting a genetic recovery, and increasing isolation of some populations**

Funded by USACE W9126G- 19-2-0014

Thanks to these people who help support this work in various ways
Dr. Elizabeth Galli-Noble
Mr. Chris Harper
Ms. Lisa O'Donnell
Ms. Zia Burns
Mr. Greg Bonnell
Mr. Ryan Orndorff
TAMU NRI and Texas A&M AgriLife Research
Dr. Byron Stone
Travis Audubon and TOS
Land owners across the state

Thank you to John Chenowith, Cristina Campbell, Jonny Scalise, Colin Strickland, Cindy Sperry, and Josie Kerrigan for help with collecting samples!