

# Genetic Assessment of Wild and Refugia Populations of Texas Wild Rice

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US Fish and Wildlife Service

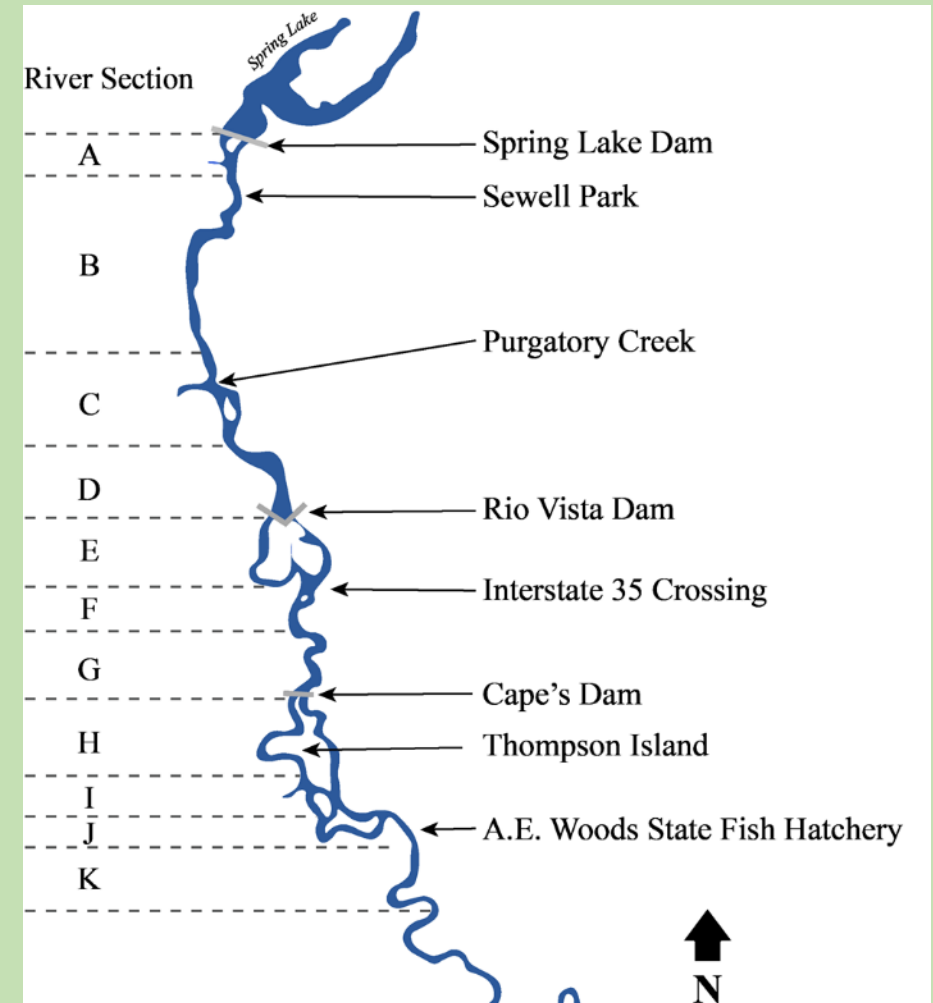
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# Texas Wild Rice

- Population restricted to 2 miles of the upper San Marcos river
- River sections designated by structures like bridges
- Grows in gravelly substrates in swift moving water, no more that 2 meters?



# Two modes of propagation

## Sexual Reproduction



Photo Credit: Jackie Poole – Texas Parks & Wildlife Dept.

## Clonal production of rhizomes



Zottoli, World Press, <https://zottoli2.wordpress.com/zone-3/>

# Coverage Has Changed Over Time

Year	Coverage
1998/1999	~ 1,650 m <sup>2</sup>
2012	4,996 m <sup>2</sup>
2020	16973 m <sup>2</sup>



# Refugia Needs



Collection



Husbandry



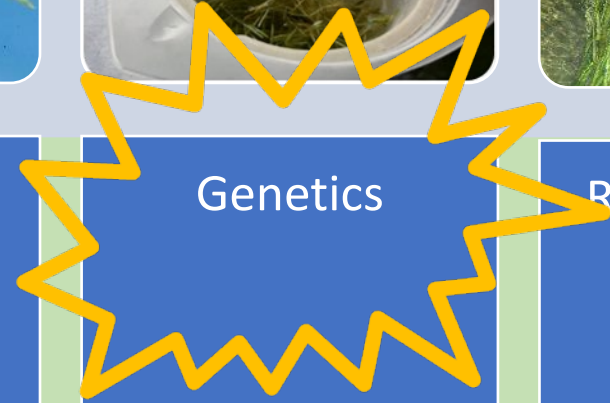
Propagation



Genetics



Reintroduction



# Importance of Genetics

Reflect wild population

- Preserve genetic variation

See change through time

- Abiotic
- Anthropogenic
- Conservation efforts

Improvements to Refugia

Increased coverage in the wild





# Objectives

1. Determine if the TWR populations at the SMARC and UNFH reflect the genetic diversity of the wild population
2. Compare the current genetic diversity of wild TWR to historical genetic diversity
3. Determine if any changes should be made to our current protocols
  - Target a river section
  - Modify number of plants in refugia
  - Remove duplicate plants



# Methods – Sample Collection

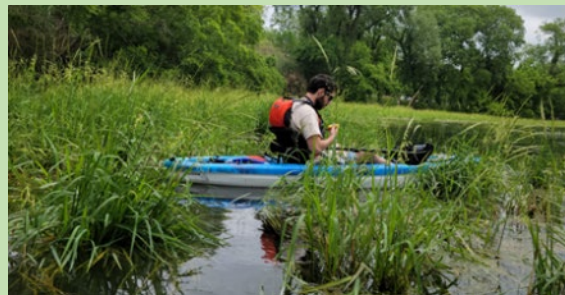
10-cm leaf clip

- Labeled with river section, stand number, and sample number
- Frozen

Randomly selected wild stands – by river section

Sample from middle of small stands

Sample every 2 m in larger stands





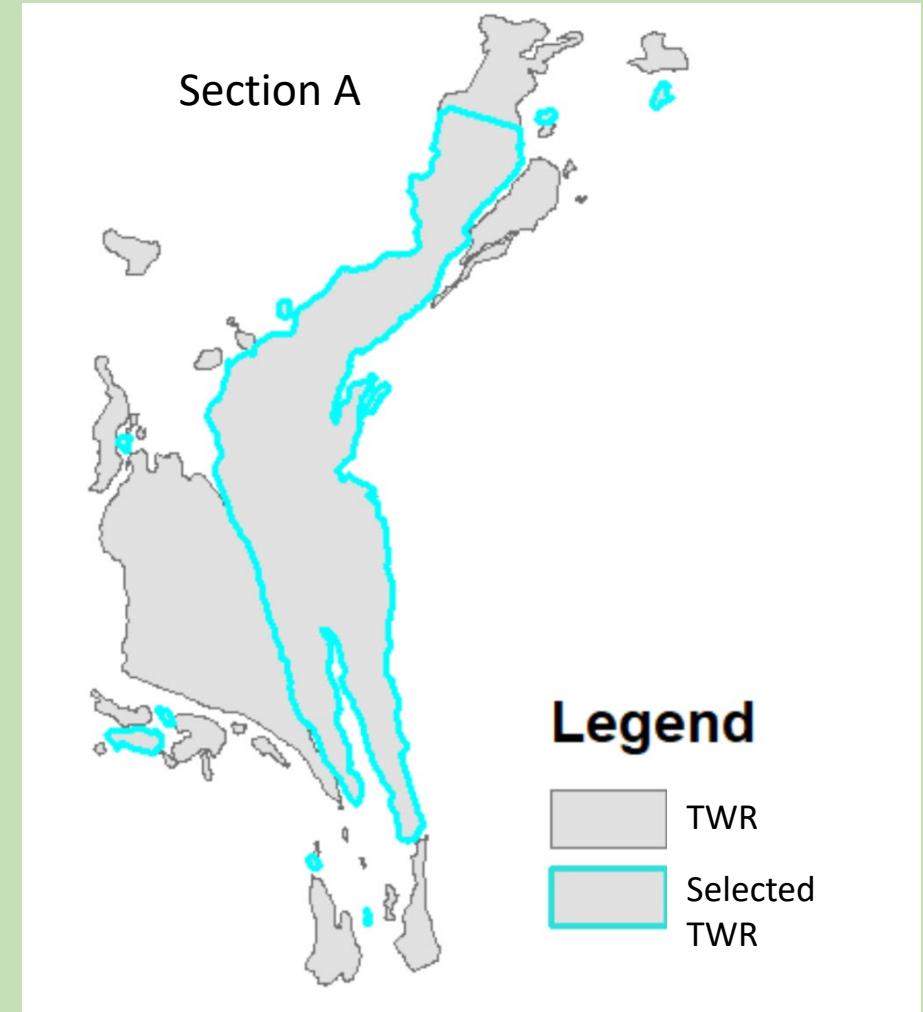
# Methods – Sample Collection

Coverage estimation by river section

- BIO-WEST survey maps
- Aerial imagery from Meadows Center
- ArcMap

Scaled number of samples by density

Minimum – 5-10 samples per section



# Methods – Sample Collection



# Methods – Genetic Sampling

## Microsatellite analysis

- Ten amplified, seven used

## Composite genotypes – GeneMapper





# Methods – Data Analysis

- Genetic metrics calculated:
  - Heterozygosity ( $H_E$ )
  - Heterozygosity per locus ( $H_O$ )
  - Number of alleles per locus ( $N_A$ )
  - Average inbreeding coefficients ( $F_{IS}$ )
  - Allelic richness ( $A_R$ )
  - Number of genetic clusters ( $K$ )



# Metrics of Genetic Diversity

- Allelic Diversity
  - Number of alleles per locus ( $N_A$ )
  - Allelic richness ( $A_R$ )
    - Private alleles; show diversity specific to a site
- Heterozygosity
  - Expected Heterozygosity ( $H_E$ ) vs Observed heterozygosity ( $H_O$ )
    - Deviations from Hardy-Weinberg Equilibrium
- $F_{is}$  and  $F_{st}$  (AMOVA)
- STRUCTURE

# Metrics of Genetic Diversity: Heterozygosity

- Two Alleles at locus A
  - A and a

- Three genotypes at Locus A
  - AA, Aa, aa

- Heterozygosity measures deviations from Hardy-Weinberg Equilibrium (Null)
  - Equal allele frequency,  $A = 0.5$  and  $a = 0.5$ , heterozygosity (Aa) = 0.5
  - Unequal allele frequency,  $A = 0.33$  and  $a = 0.66$ , heterozygosity (Aa) = 0.55
  - Unequal allele frequency,  $A = 0.66$  and  $a = 0.33$ , heterozygosity (Aa) = 0.45

- Deviations from Null suggest one or more of the following assumptions are not true
  - Random mating
  - Infinite population size
  - No selection
  - No migration/gene flow
  - No mutation

	A	a
A	AA	Aa
a	Aa	aa

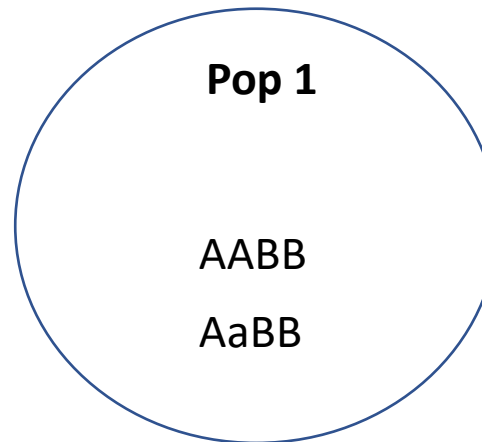


# Metrics of Genetic Diversity

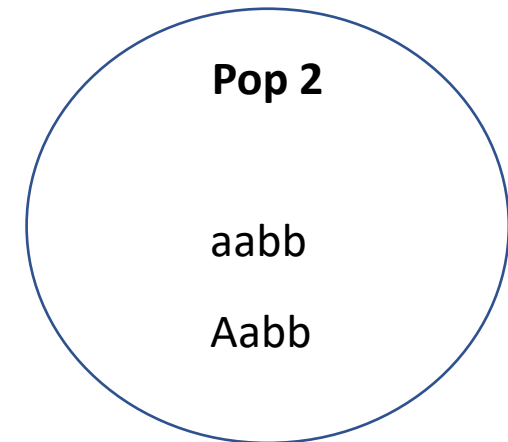
	AB	AB	ab	ab
AB	AABB	AABB	AaBb	AaBb
AB	AABB	AABB	AaBb	AaBb
ab	AaBb	AaBb	aabb	aabb
ab	AaBb	AaBb	aabb	aabb

## At this Locus

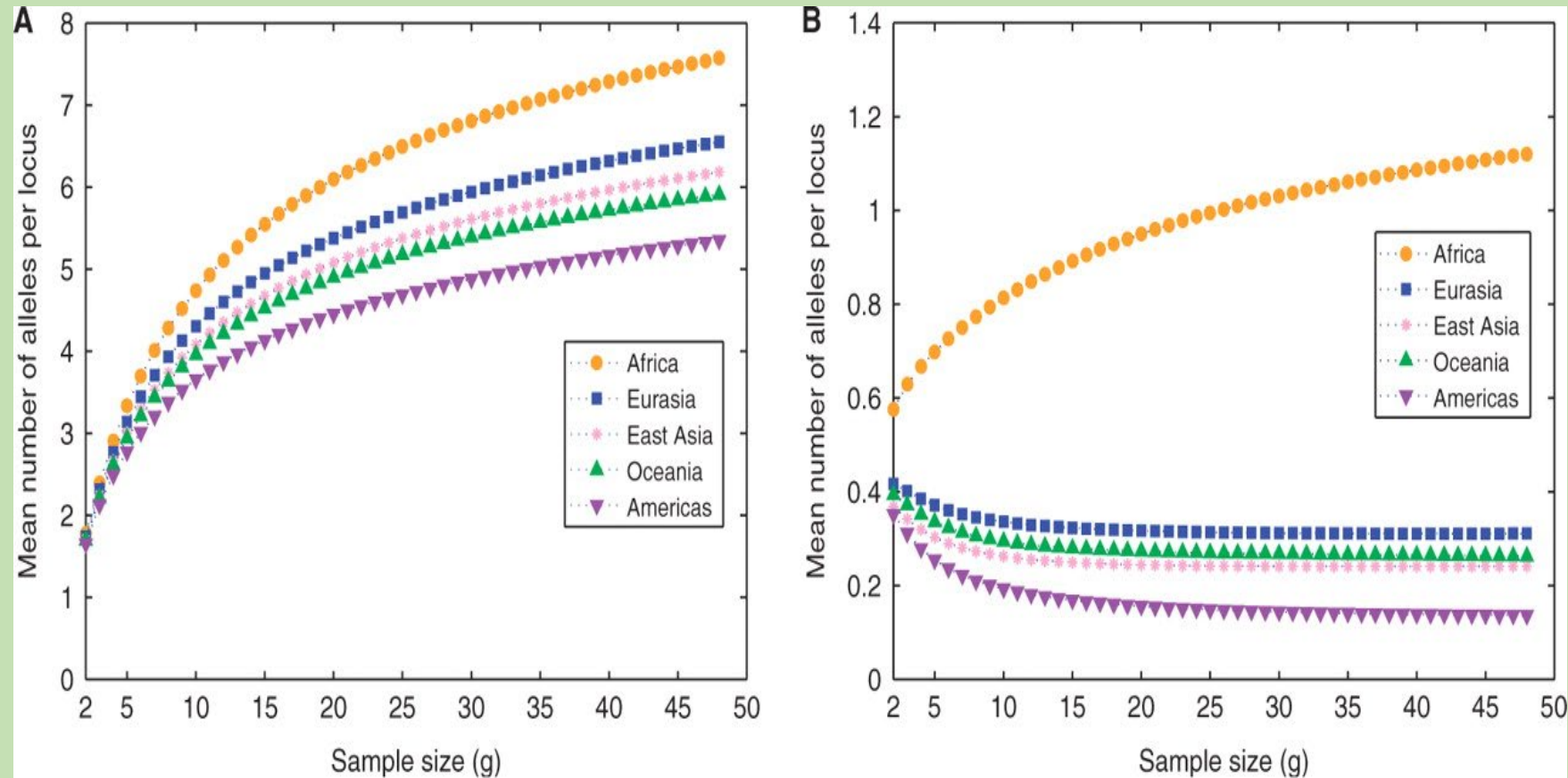
- Four alleles
  - A, a, B, b
- Three genotypes
  - AABB, aabb, AaBb
- Heterozygosity = 0.5
- Private alleles or **Allelic richness ( $A_R$ )** is increased



Missing b allele



Missing B allele



**Fig. 4.** The mean number of (A) distinct alleles per locus and (B) private alleles per locus, as functions of standardized sample size for five major geographic regions

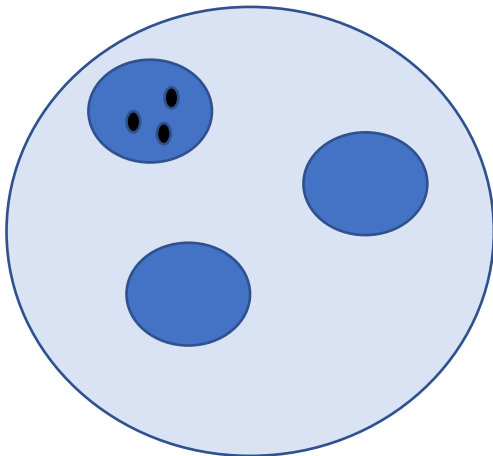
# Analysis of Molecular Variance (AMOVA)

- Basically, a one-way ANOVA
  - Determine if there are significant differences in the means of three or more independent groups

$F_{IS}$  = individual relative to subpopulation

$F_{ST}$  = subpopulation relative to total

$F_{IT}$  = individual relative to total



## ANOVA: Analysis of Variance is a *variability ratio*

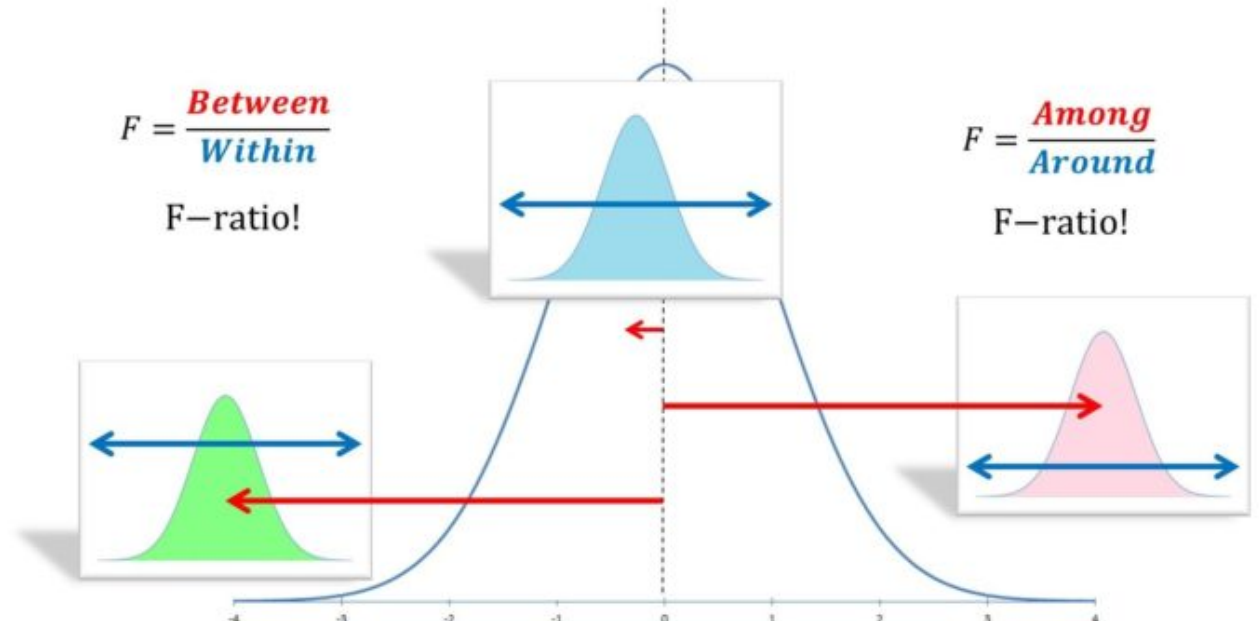
*Variance Between + Variance Within = Total Variance*

$$F = \frac{\text{Between}}{\text{Within}}$$

F-ratio!

$$F = \frac{\text{Among}}{\text{Around}}$$

F-ratio!





# STRUCTURE

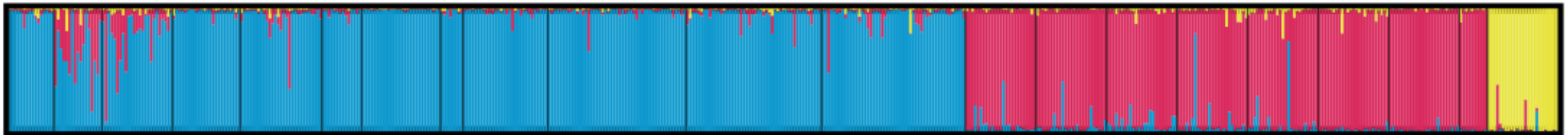
- Bayesian clustering approach using Markov Chain Monte Carlo (MCMC) estimation
- Randomly assign individuals to populations, allele frequencies are estimated and the probability of observing these estimated frequencies is calculated based on observed data
- Individuals are reassigned based on the estimated allele frequencies
- The process is repeated thousands of times until it converges on the highest probability of observing the estimated allele frequency estimates and probability of an individual's membership to a population
- STRUCTRE does this under an assumed number of populations or genetic groups (K)
- Assume No Admixture, Admixture, Gene Linkage

# STRUCTURE Plot

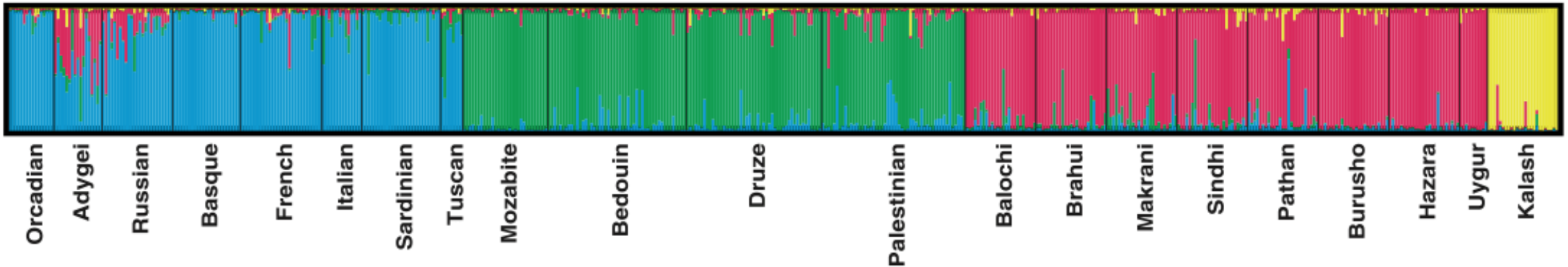
- K = the number of genetic lineages
- Different colors represent each K
- Vertical lines represent a single individual
- Individuals are grouped by population or sampling site

## Eurasia

K=3



K=4



# Sample Collections

## Refugia Population

- 212 plants from SMARC
- 180 plants from UNFH

## Wild Population

- 379 total plants sampled

## Post Data Analysis

- Total of 771 individuals analyzed
  - 652 after within river segment duplicates removed
  - 600 Unique genotypes



# No Significant Genetic Loss

<b>Locus</b>	<b>Saltzgiver et al 2021 (in situ only) n = 331</b>	<b>Wilson et al 2017 (in situ only) n = 156</b>	<b>Richards et al 2007 (in situ only) n = 298-346</b>
<b>Zt-1</b>	2	3	4
<b>Zt-13</b>	15	9	20
<b>Zt-16</b>	5	4	-
<b>Zt-21</b>	14	13	15
<b>Zt-22</b>	7	5	7
<b>Zt-23</b>	14	9	13
<b>Zt-26</b>	2	2	-



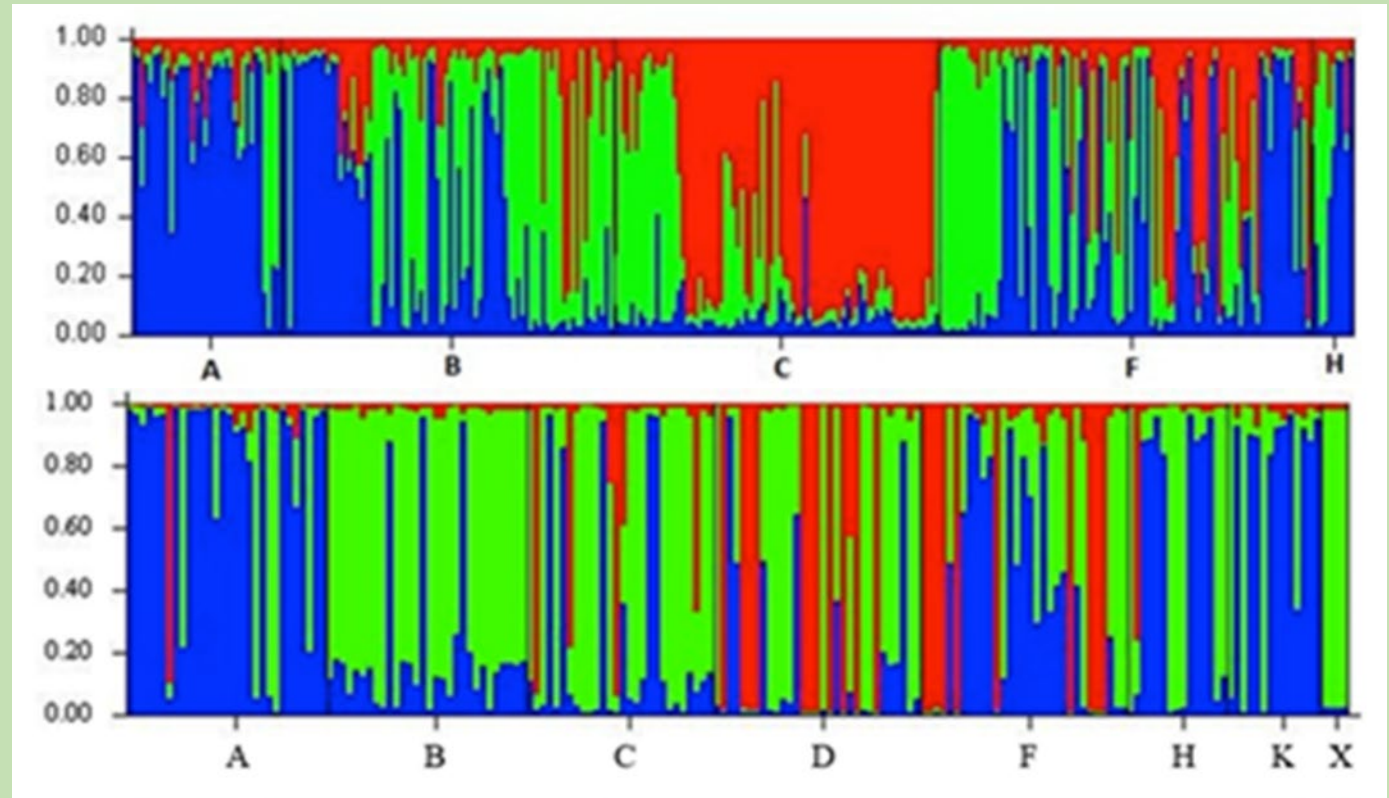
# Previous Studies

Richards et al. 2007

- Collected in 1998, 1999, 2002

Wilson et al. 2017

- Collected in 2012

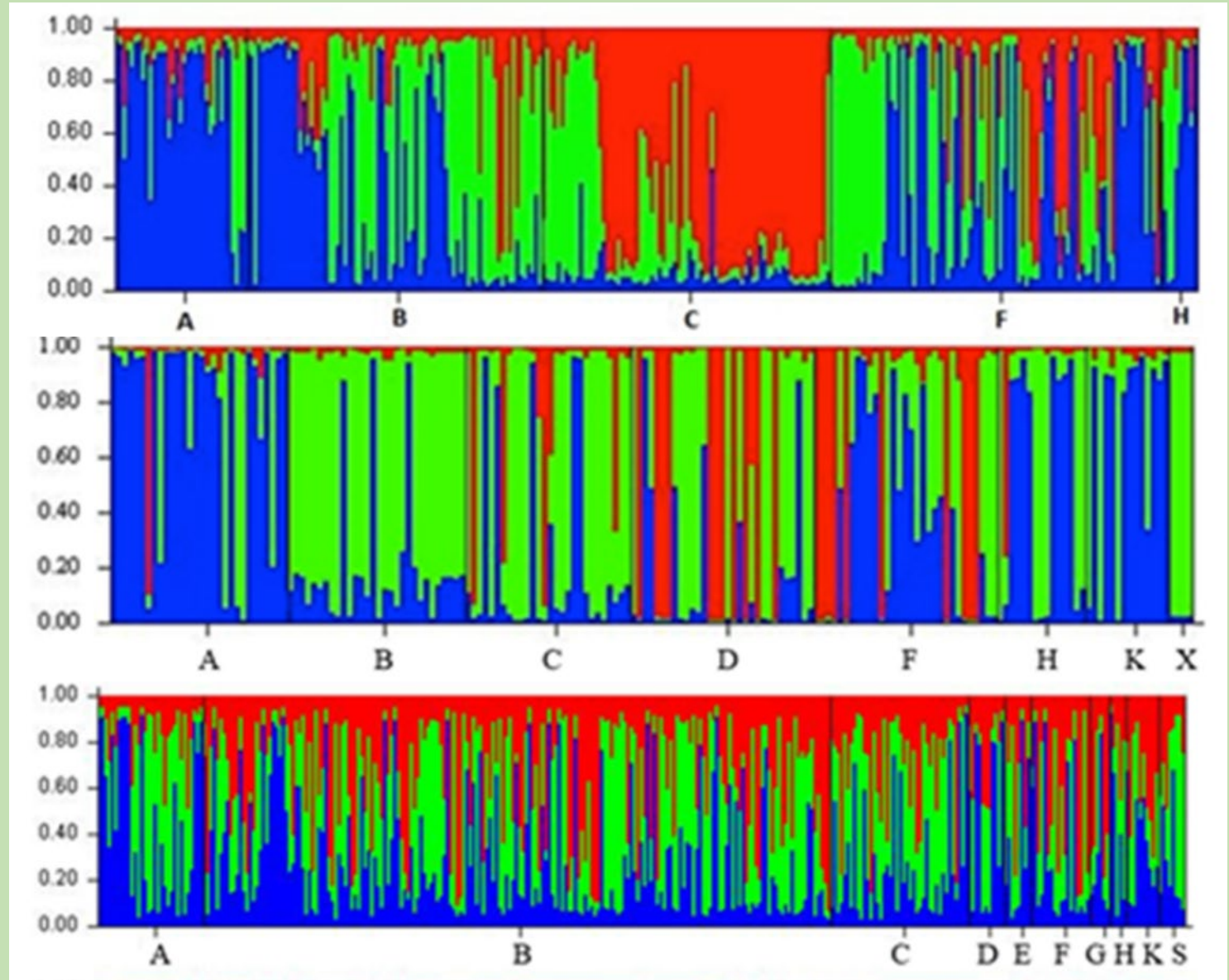


Richards, C.M., M.F. Antolin, A. Reille, J. Pool, and C. Walters. 2007. Capturing genetic diversity of wild populations for ex situ conservation: Texas wild rice (*Zizania texana*) as a model. *Genetic Resources and Crop Evolution* 54: 837–848.

Wilson, W.D., J.T. Hutchinson, and K.G. Ostrand. 2015. Genetic diversity assessment of in situ and ex situ Texas wild rice (*Zizania texana*) populations, and endangered plant. *Aquatic Botany* 136:212-219.

# Results – Wild

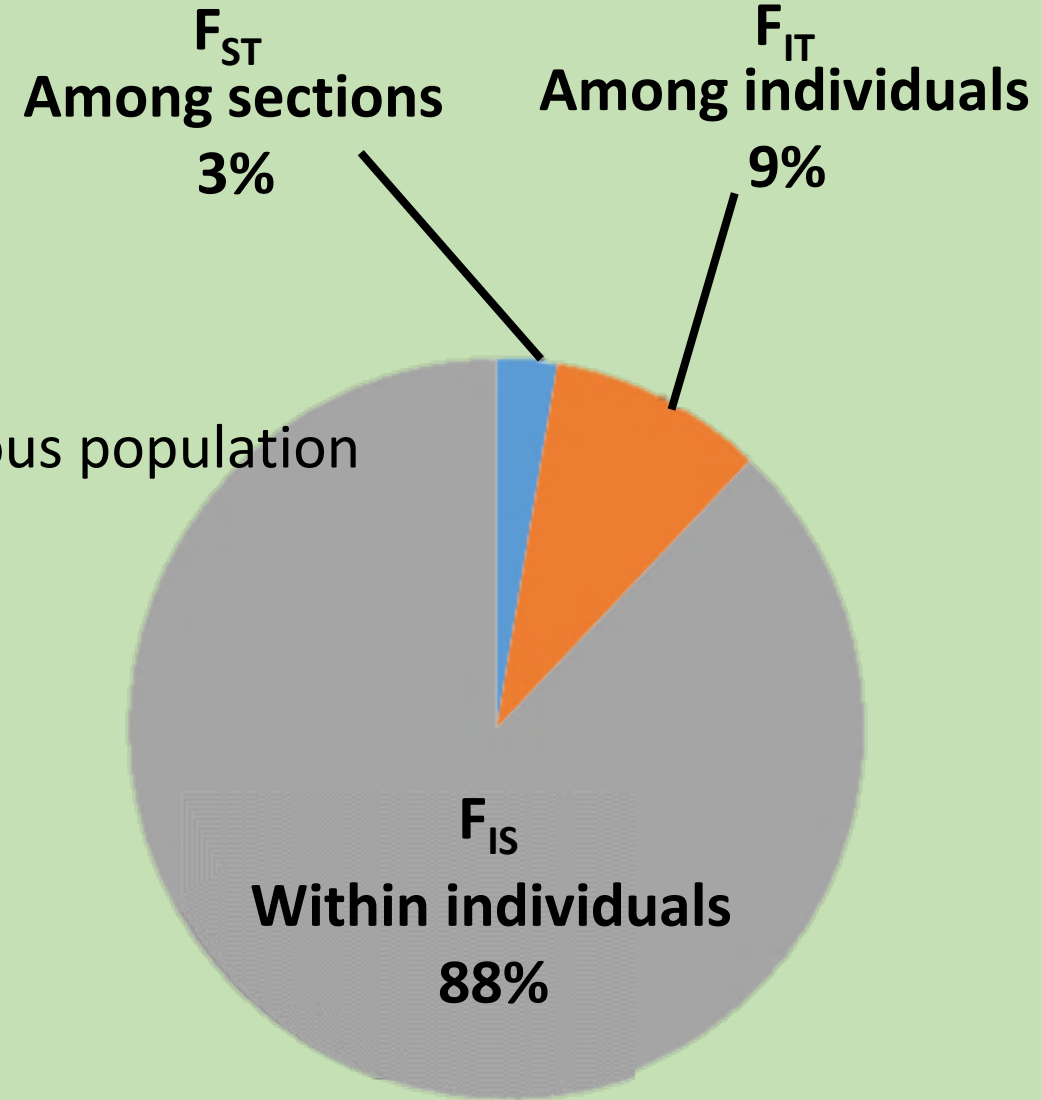
- Wild STRUCTURE across time
  - 1998/99 – top
  - 2012 – middle
  - 2021 – bottom
- Colors do not represent the same clusters across charts



# Wild Genetic Diversity

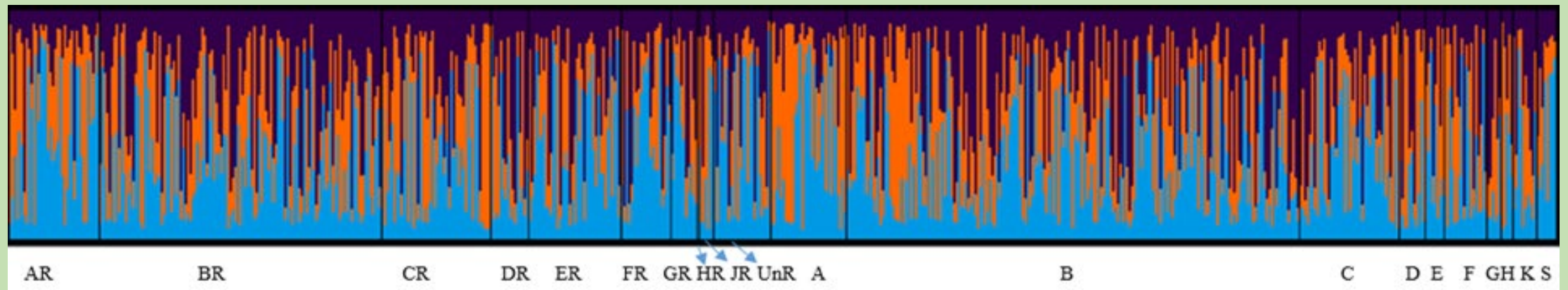
## Analysis of Molecular Variance

- Most variance within individuals
  - Small  $F_{st}$  Indicates a panmictic or homogeneous population



# Refugia Reflects the Wild

- Refugia is as homogenous as the wild
- Diversity in Refugia reflects the wild
- Minimum number of plants needed in the refugia is 200 unique individuals
  - Wild population randomly sampled four times (25%, 40%, 50%, 75% of genotypes)
  - One-sided t-test vs all genotypes to determine loss of allelic richness at each sampling size
  - Determined minimum # of plants to sample to not have a significant loss in representation





# Refugia Population Improved

- Number of alleles in refugia between Wilson 2017 and new study
- Refugia population has improved since last assessment
- The SMARC and UNFH populations are not mirrored

<b>Locus</b>	<b>Saltzgiver et al 2021 (ex situ only) n = 321</b>	<b>Wilson et al 2017 (ex situ only) n = 48</b>
<b>Zt-1</b>	2	2
<b>Zt-13</b>	18	8
<b>Zt-16</b>	5	3
<b>Zt-21</b>	18	14
<b>Zt-22</b>	6	4
<b>Zt-23</b>	14	7
<b>Zt-26</b>	3	2

# Conclusions

- The wild population has become more homogenous across river sections, but not less diverse overall
  - Replanting efforts move genetics
  - Monitor genetics moving forward to assess genetic loss
- The refugia population has improved from incorporating recommendations from Wilson et al. (2017)
- The SMARC and UNFH do not mirror each other well – room for improvement
- Refugia needs at least 200 genetically unique individuals for captive assurance



# Conclusions – Refugia

- Caveats
  - samples collected before recreation areas reopened
- More to come
  - Which plants are duplicates
  - Which plants are unique





# Thank You!

- Edwards Aquifer Authority
- Husbandry team
- SMARC staff
- SNARRC staff





The background of the image is a photograph of a pond or stream. The water is dark and still, reflecting the surrounding greenery. Numerous long, thin, green blades of grass or algae are submerged in the water, some standing upright and others leaning over. The overall color palette is dominated by various shades of green, from bright lime to deep forest green, with some brownish tones from submerged plant matter.

# Questions?