Connectivity of Leopard Frog Populations

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Clark County MSHCP Project Progress Report
Symposium, 12 August 2009

SCHOOL OF LIFE SCIENCES
UNLV
UNIVERSITY OF NEVADA LAS VEGAS

Conservation Agreement and Strategy (CAS)

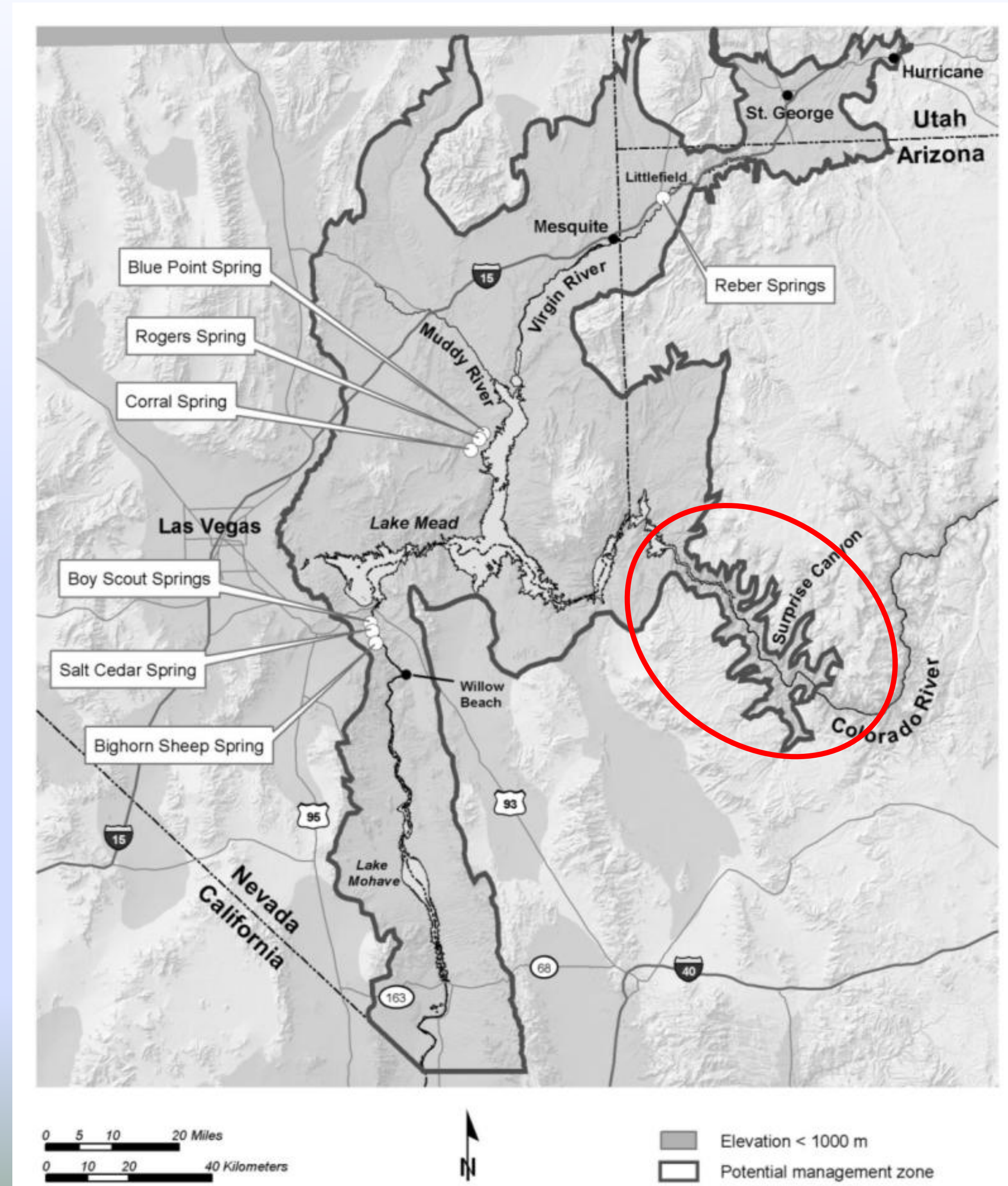
A Major CAS Objective:

“Establish additional populations of relict leopard frogs.”

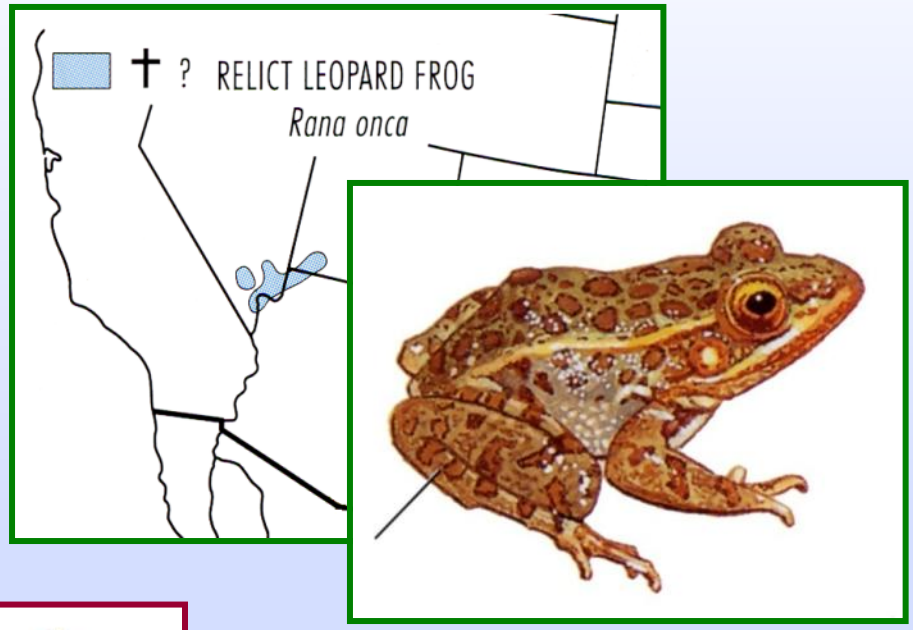
Potential Management Zone

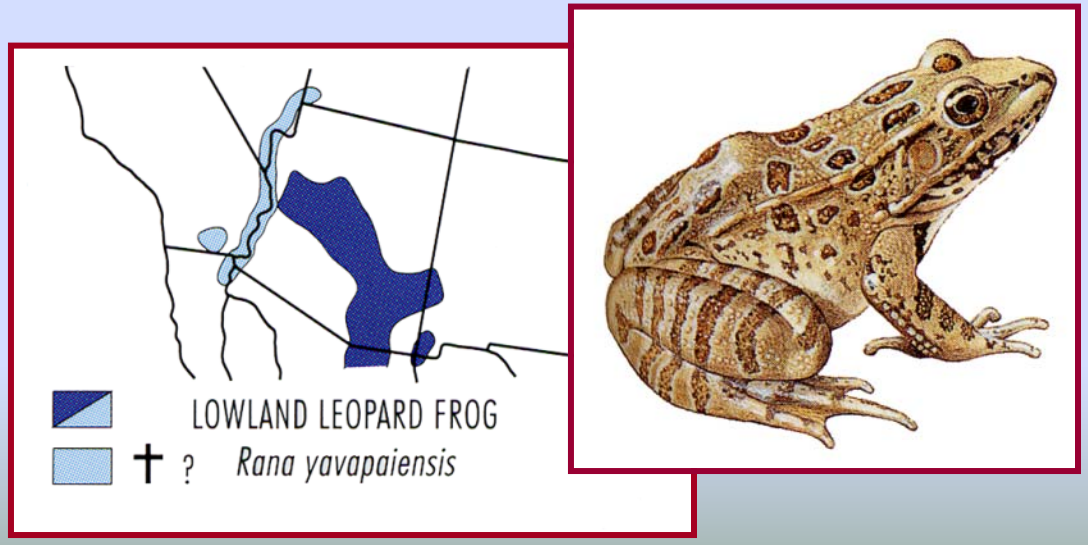
Represents a feasible approximation of where Relict Leopard Frogs may have once existed…

...and that contains sufficient potential habitat for conservation actions



Frog from Surprise Canyon





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Colorado River from mouth of Spencer Canyon

Photo: Jef Jaeger, Oct 2009

Photo: Jef Jaeger, Oct 2008

Pearce Ferry Rapid, Oct 2008


Photo: Jef Jaeger, Oct 2008

Separation Canyon

Project Goals

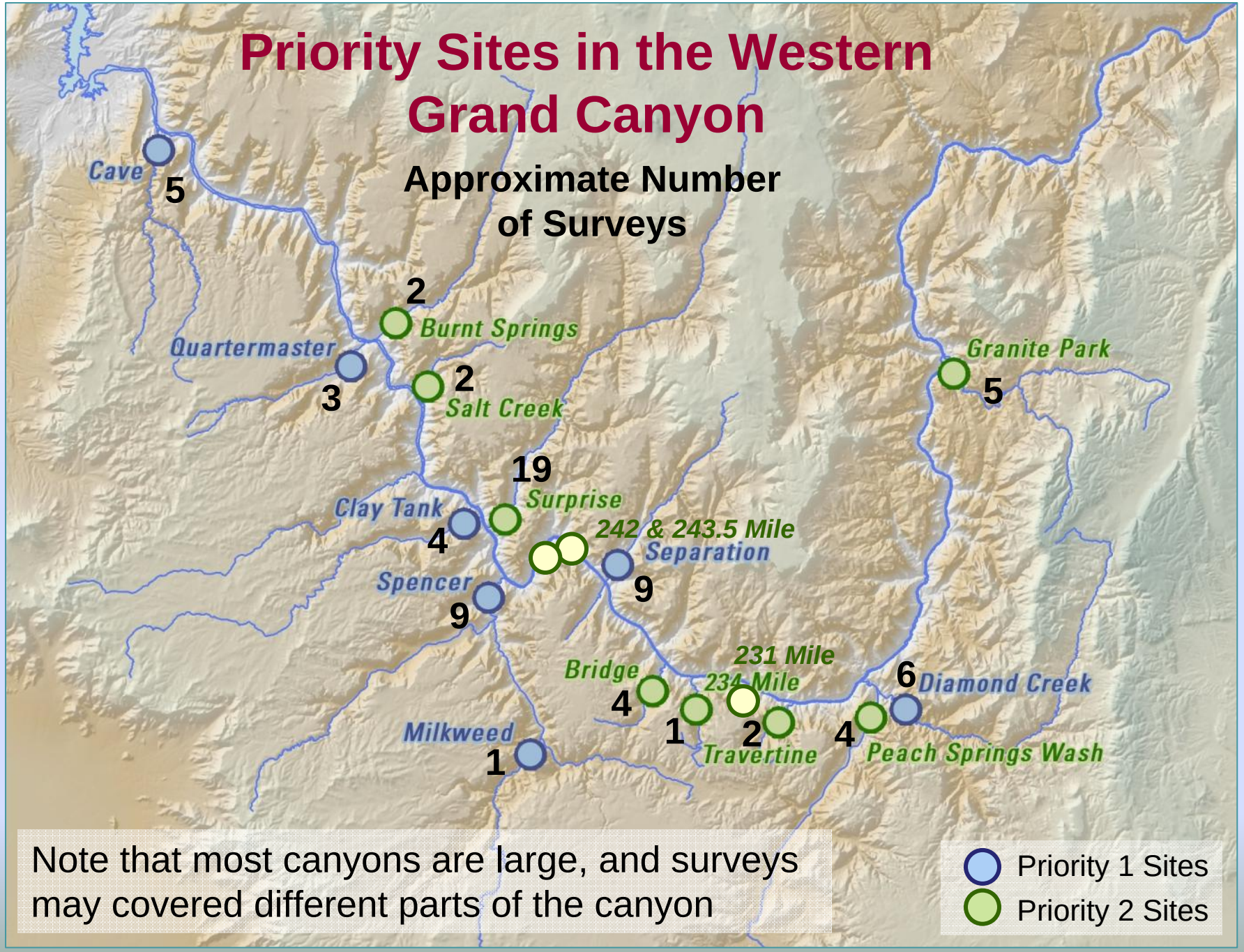
Assess the distribution of leopard frogs within the Grand Canyon portion of the PMZ, and determine the taxonomic identity of the populations within

Identify genetic structure among leopard frog populations throughout the PMZ

Include samples from:
- Relict Leopard Frog populations
- Regional populations of Lowland Leopard Frogs
- Any new leopard frog populations encountered

Field Surveys

- Identified priority areas within the Western Grand Canyon
- Conduct visual encounter surveys
  - methods specified in field protocol and data management documents
- Survey trips along the river hiking up canyon, or from the rim hiking down canyon
- Mostly multiple day journeys by raft and/or backpacking
- Logistics of survey trips often required targeting only portions of a site/canyon, thus requiring multiple site visits


Priority Sites in the Western Grand Canyon
Approximate Number of Surveys
Cave
5
2
Burnt Springs
Quartermaster
3
2
Salt Creek
19
Surprise
Clay Tank
4
242 & 243.5 Mile Separation
9
Spencer
9
Granite Park
5
231 Mile
Bridge
4
234 Mile
1
2
Travertine
6
Diamond Creek
4
Peach Springs Wash
Milkweed
1
Note that most canyons are large, and surveys may covered different parts of the canyon
Priority 1 Sites
Priority 2 Sites

Areas where we may still conduct additional surveys this coming fall:

- Upper areas of Peach Springs Wash
- Upper Tributaries of Spencer Canyon, including areas of Milkweed and West Water Canyons

These areas are considered sensitive by the Hualapai Tribe...

Columbine Fall and Cave Canyon

MtDNA Analysis

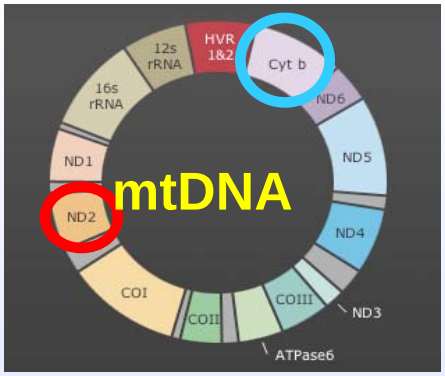
“Completed”

Manuscript submitted for review (July 2009):

“Phylogeography of declining relict and lowland leopard frogs in the desert Southwest of North America”

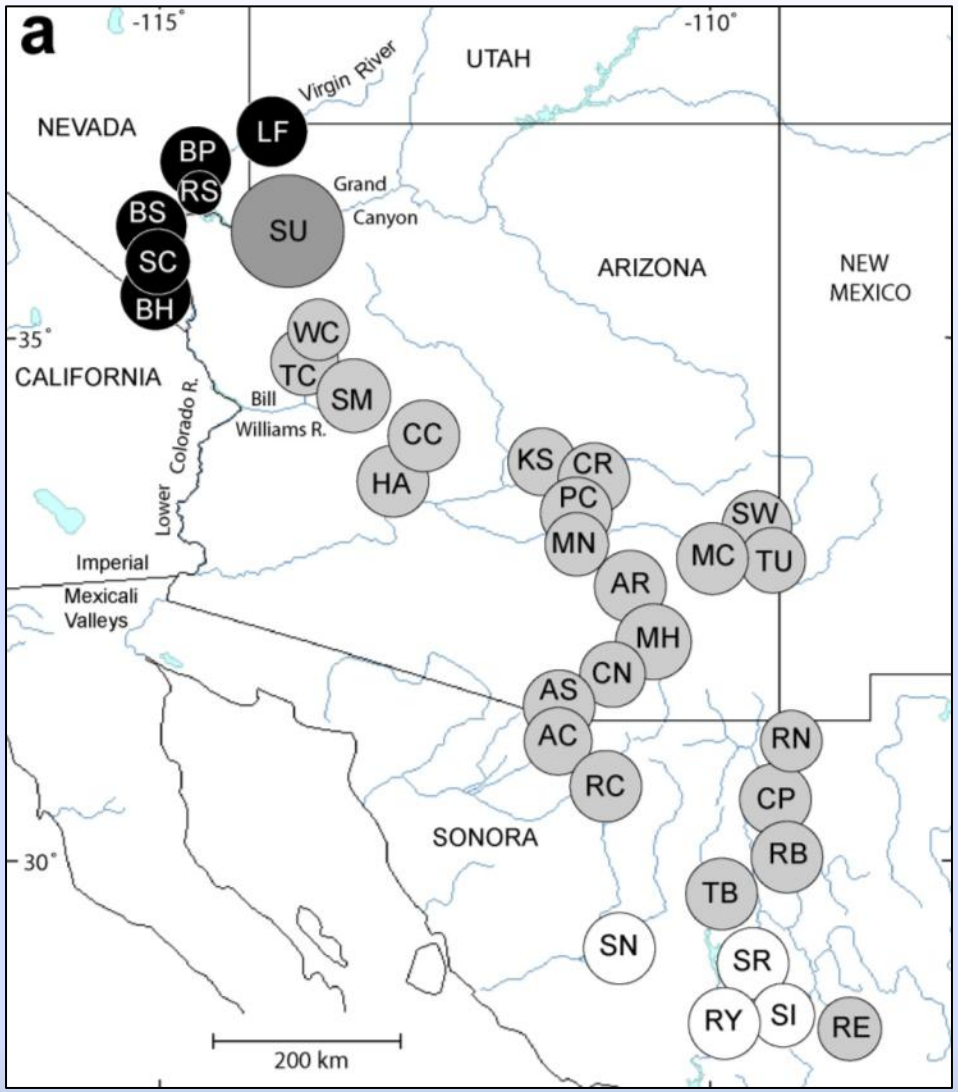
Viktória Oláh-Hemmings, Jef Jaeger, Michael Sredl, Martin Schlaepfer, Randy Jennings, Charles Drost, David Bradford, and Brett Riddle.

MtDNA Analysis

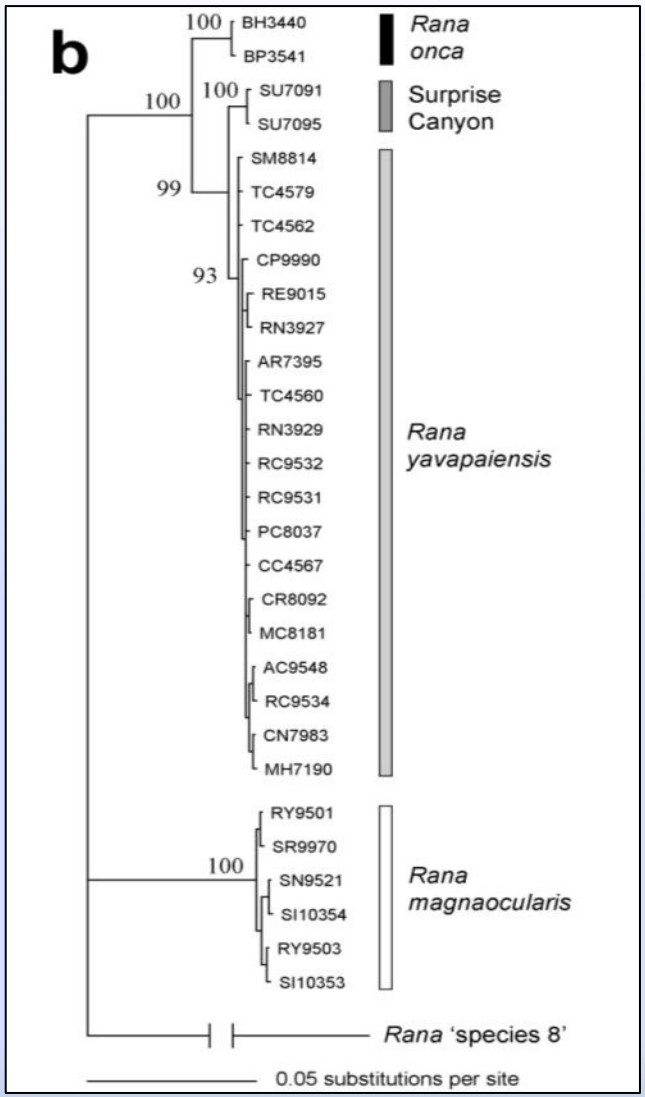


- Assessed sequences data from 1035 bp of ND2 and confirmed phylogenetic patterns with 962 bp of Cyt b
- Range-wide sampling of extant populations of *R. onca* and *R. yavapaiensis*
  - 51 samples of *R. onca* from 6 sites
  - 23 samples from the population in Surprise Canyon
  - 202 samples of *R. yavapaiensis* from 23 sites in Arizona and Mexico
  - 37 outgroup samples

MtDNA Analysis



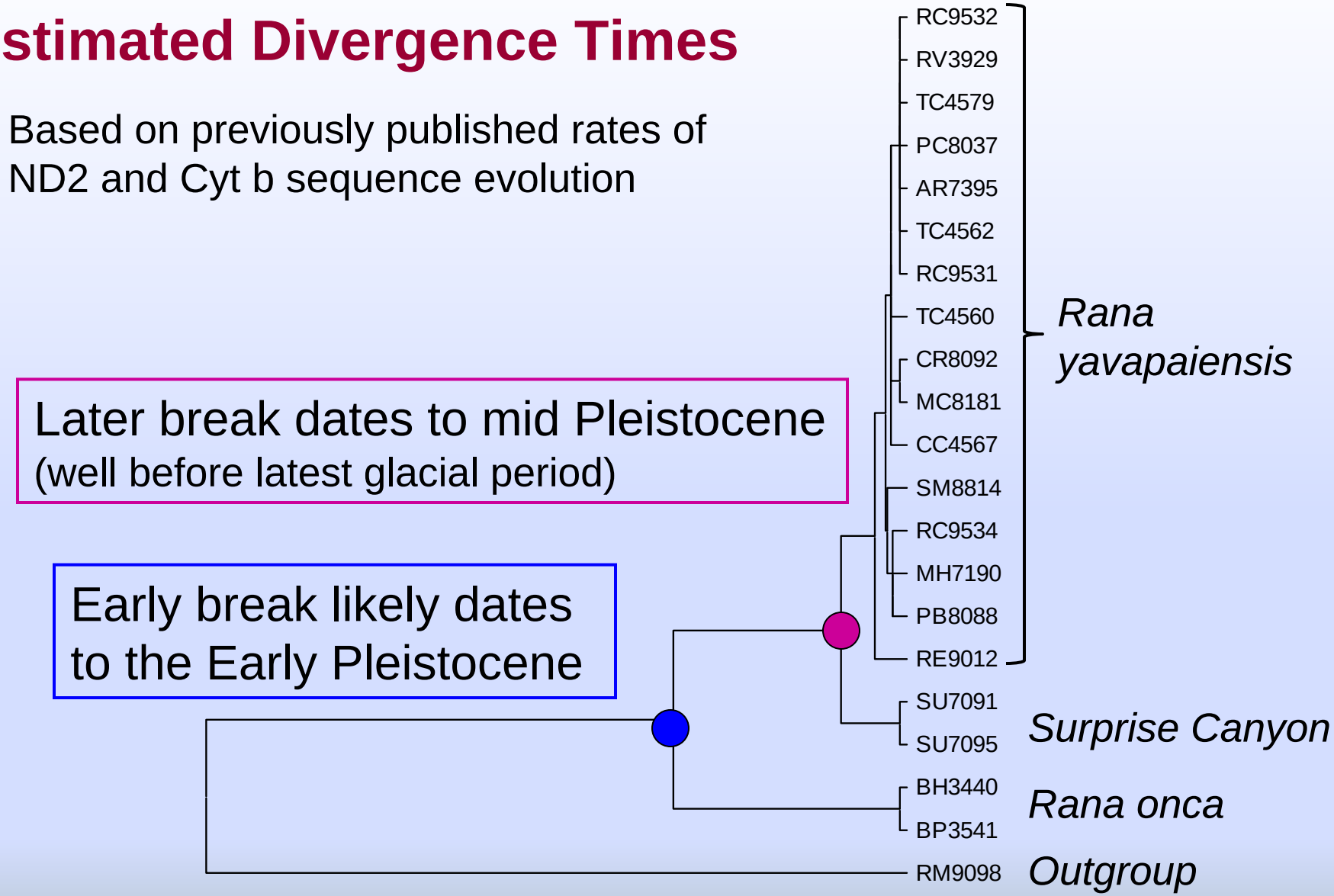
Sample sites and relative sample sizes (circle size)



Bayesian consensus tree from ND2 and Cyt b haplotype data

Estimated Divergence Times

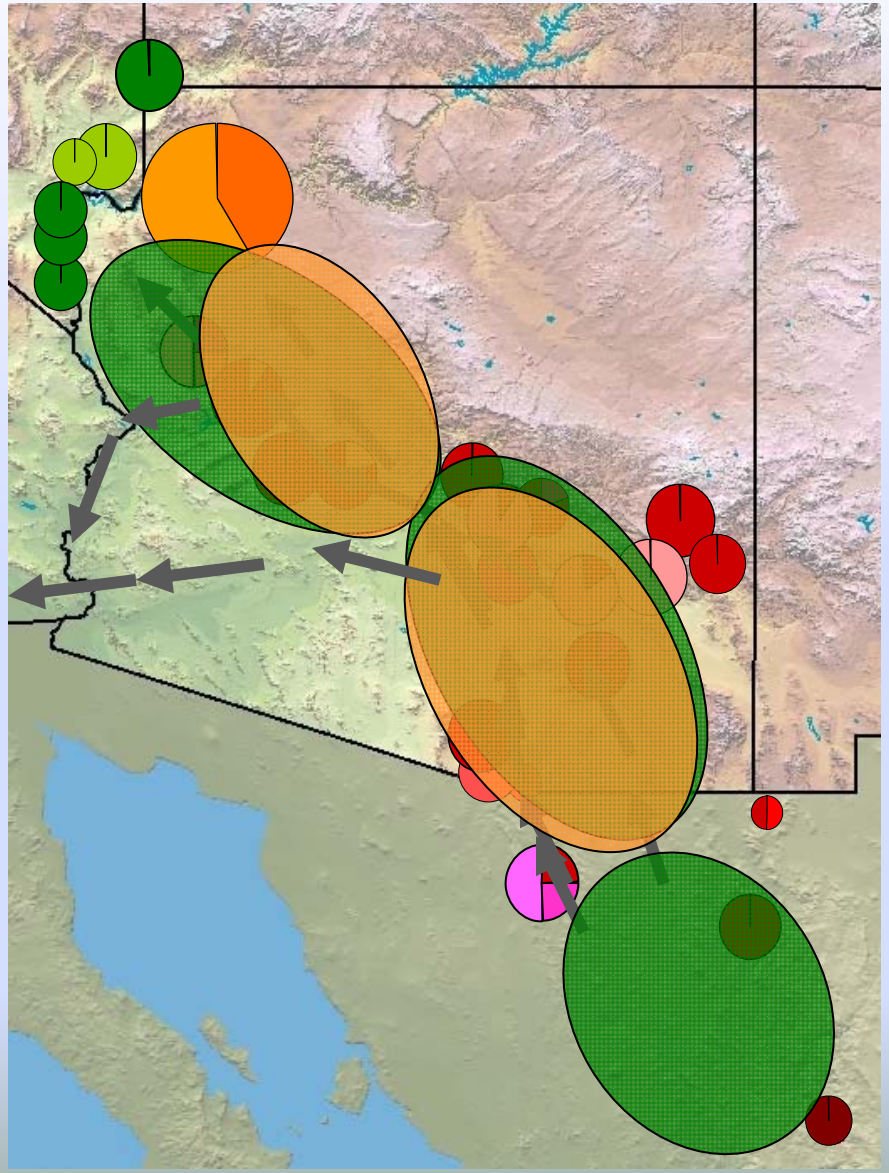
Based on previously published rates of ND2 and Cyt b sequence evolution



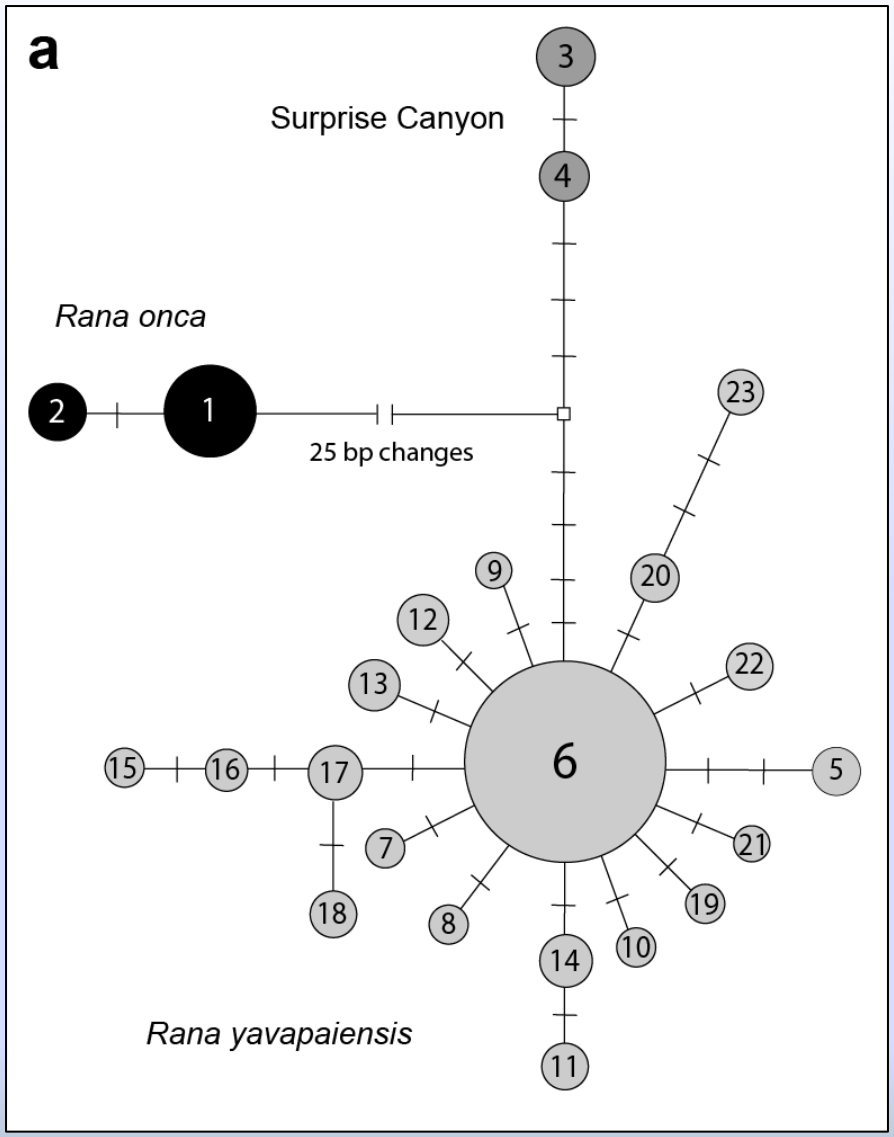
Preliminary ND2 Neighbor-Joining haplotype tree, clock enforced

Phylogeography based on MtDNA analysis

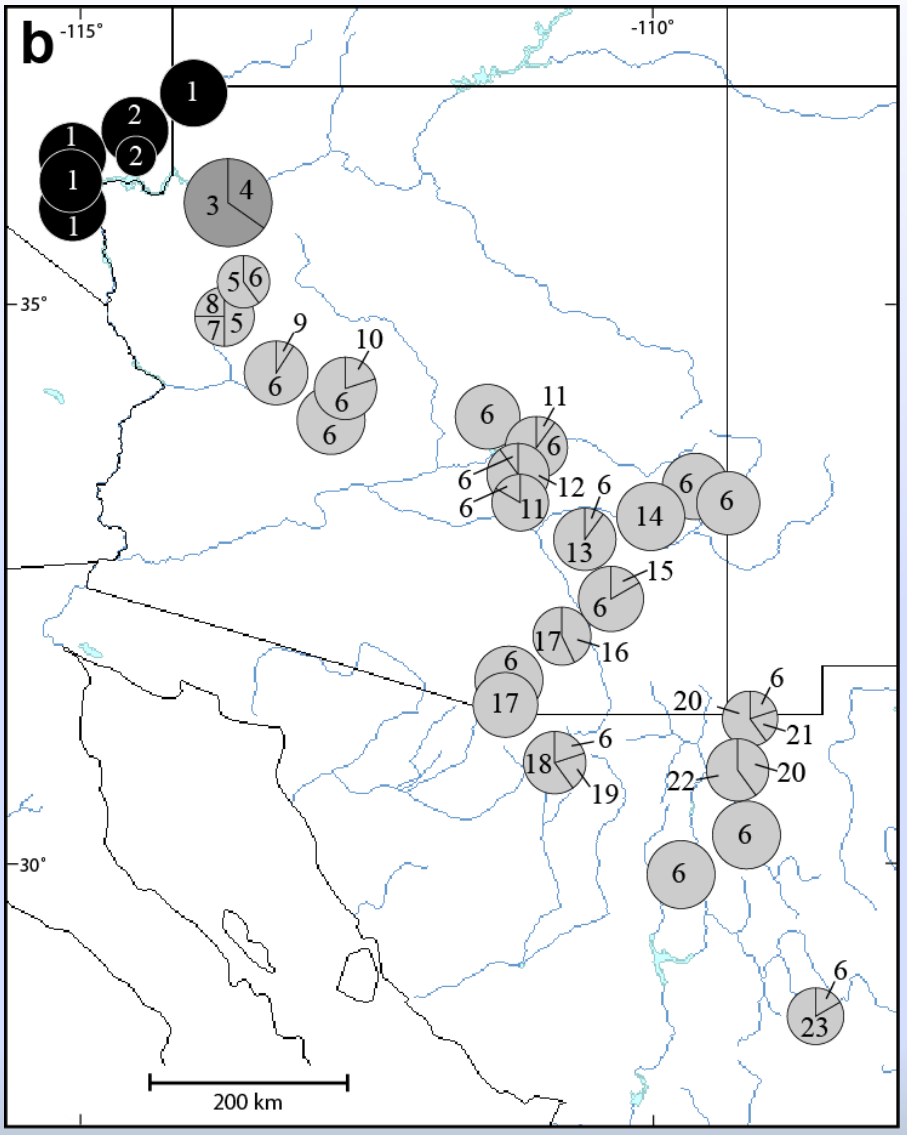
Complex history of range expansion, contraction, and isolation



MtDNA Analysis – ND2 Haplotype Assessment



ND2 Haplotype Network

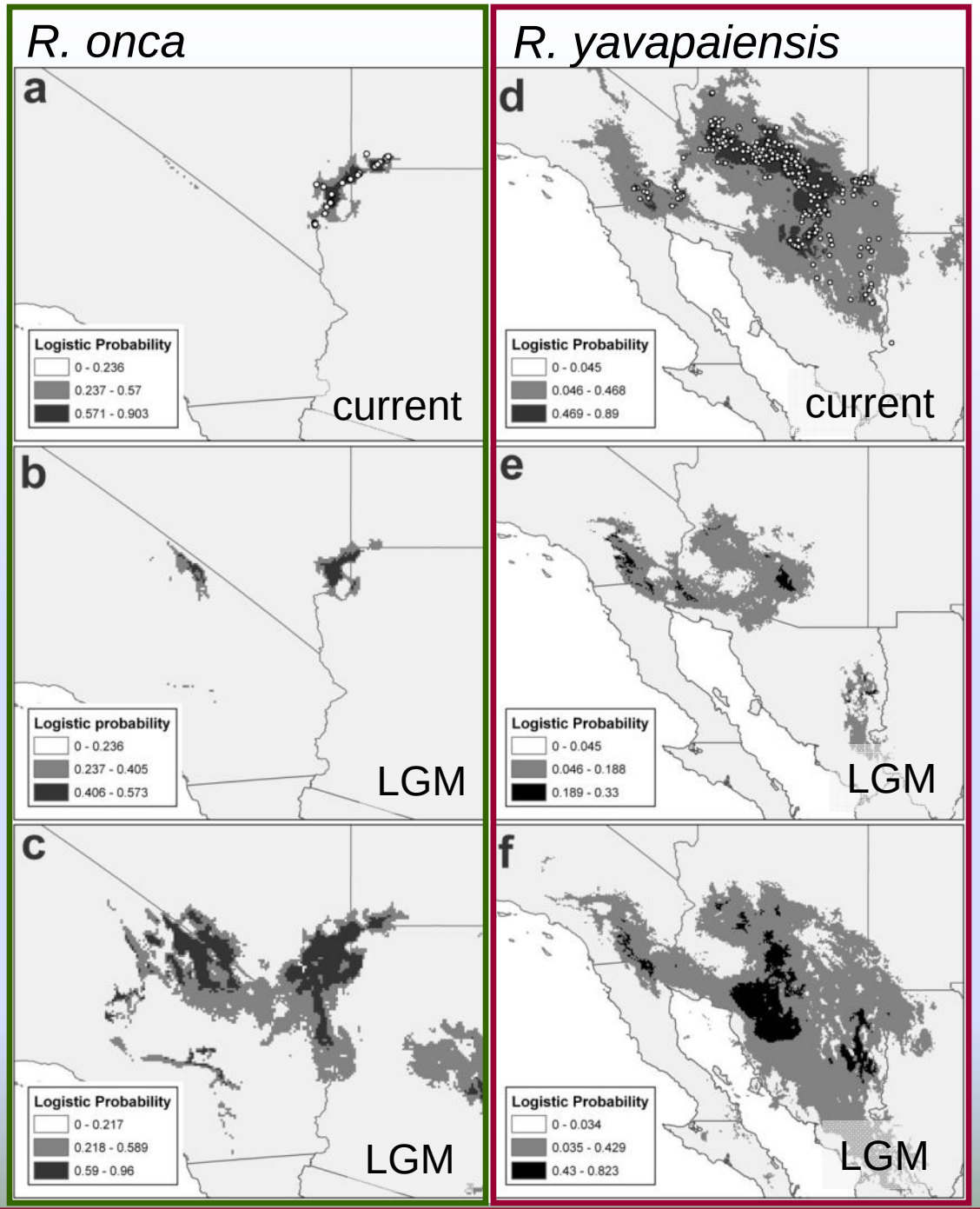


Distribution of ND2 Haplotypes

Climatic Niche Models

Base on temperature and precipitation variables

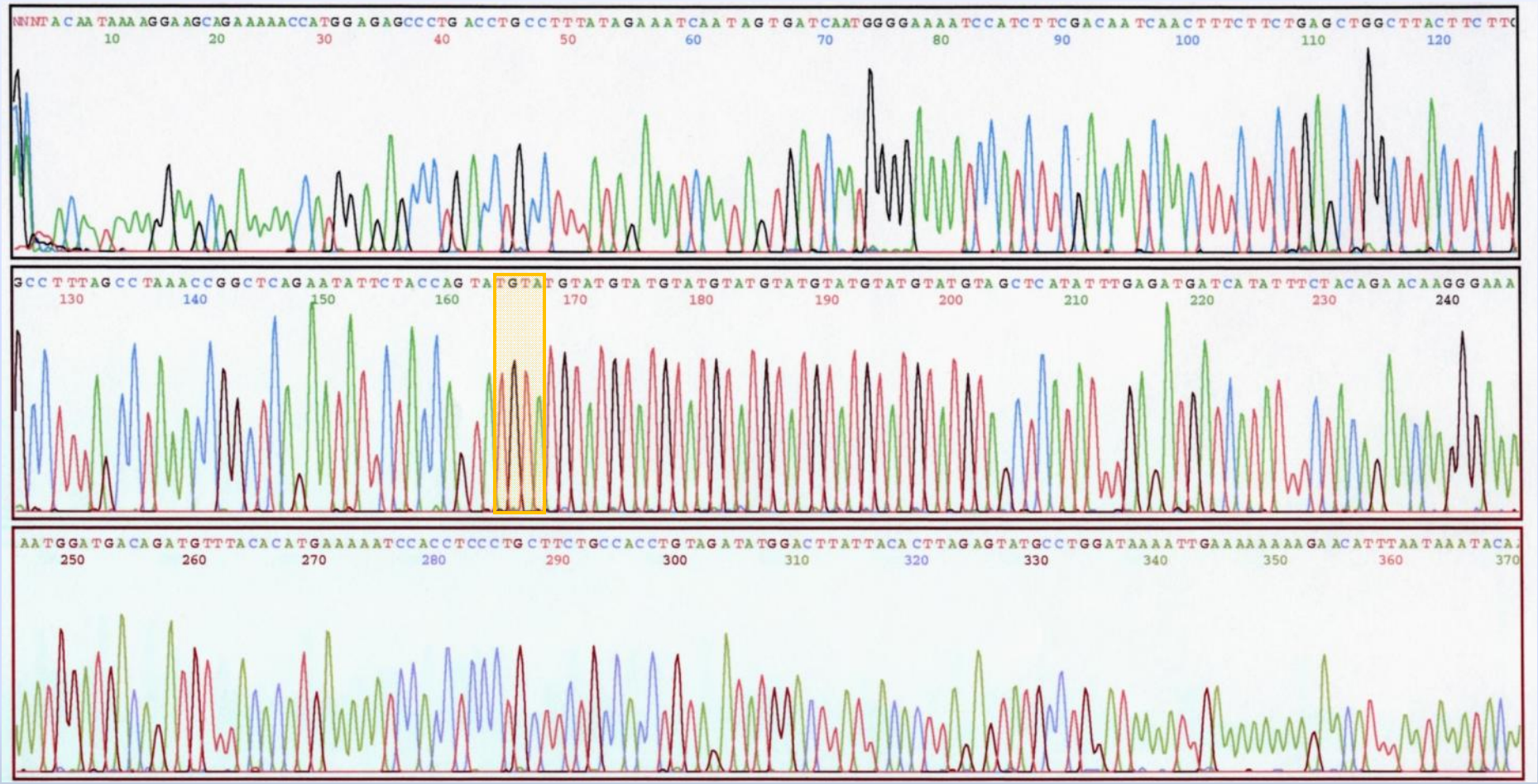
Two different climatic reconstructions of the Latest Glacial Maximum



Oláh-Hemmings et al. (*in review*)

Microsatellite Analysis

Quickly evolving, tandem repeating regions of nuclear DNA



Microsatellite containing sequence from R. onca, $(TGTA)_{10}$ repeat region

Development Microsatellite Markers/Primers

Savage A.E., and J.R. Jaeger (2009)

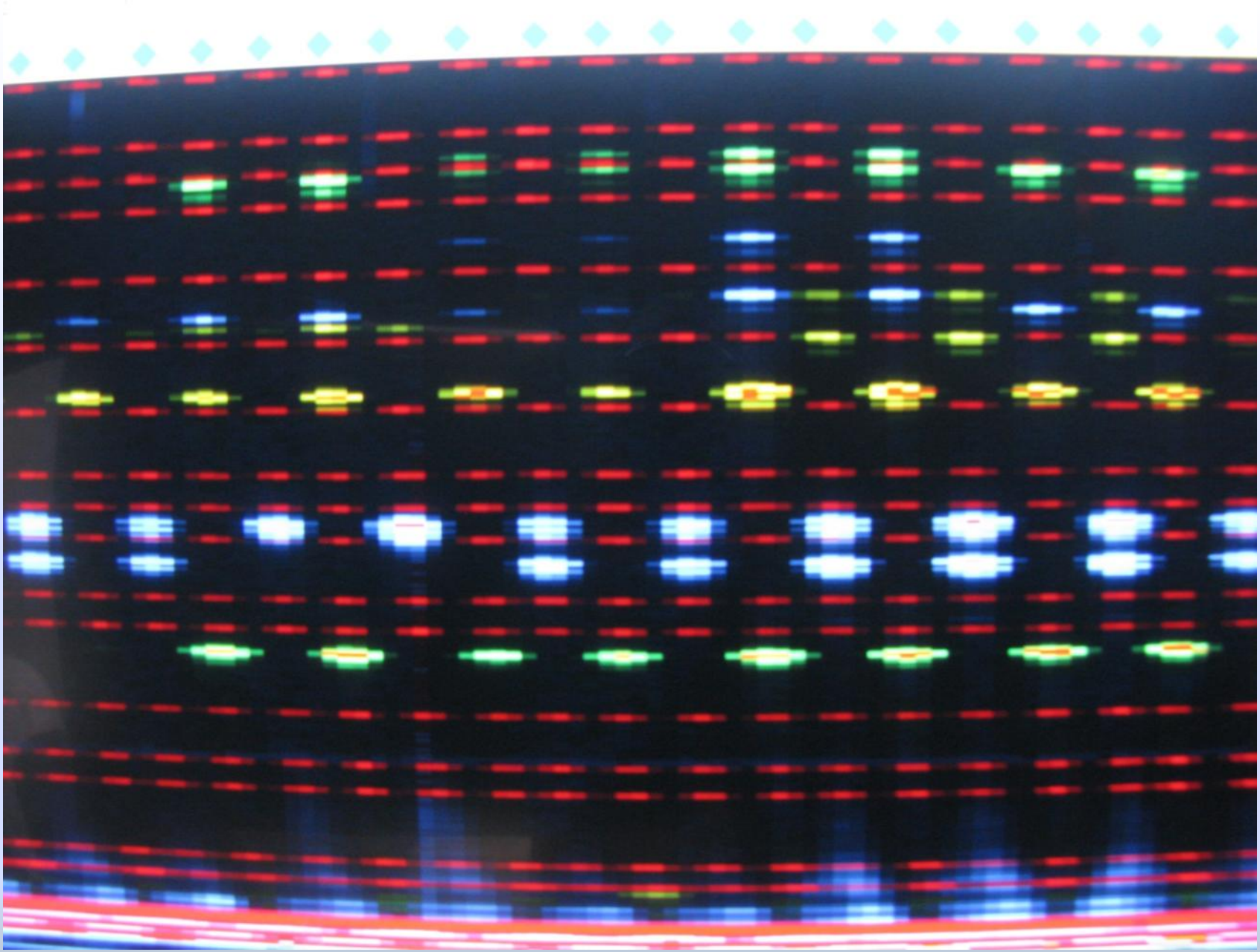
Isolation and characterization of microsatellite markers in the lowland leopard frog (*Rana yavapaiensis*) and the relict leopard frog (*R. onca*), two declining frogs of the North American desert southwest.

Molecular Ecology Resources 9, 199–202

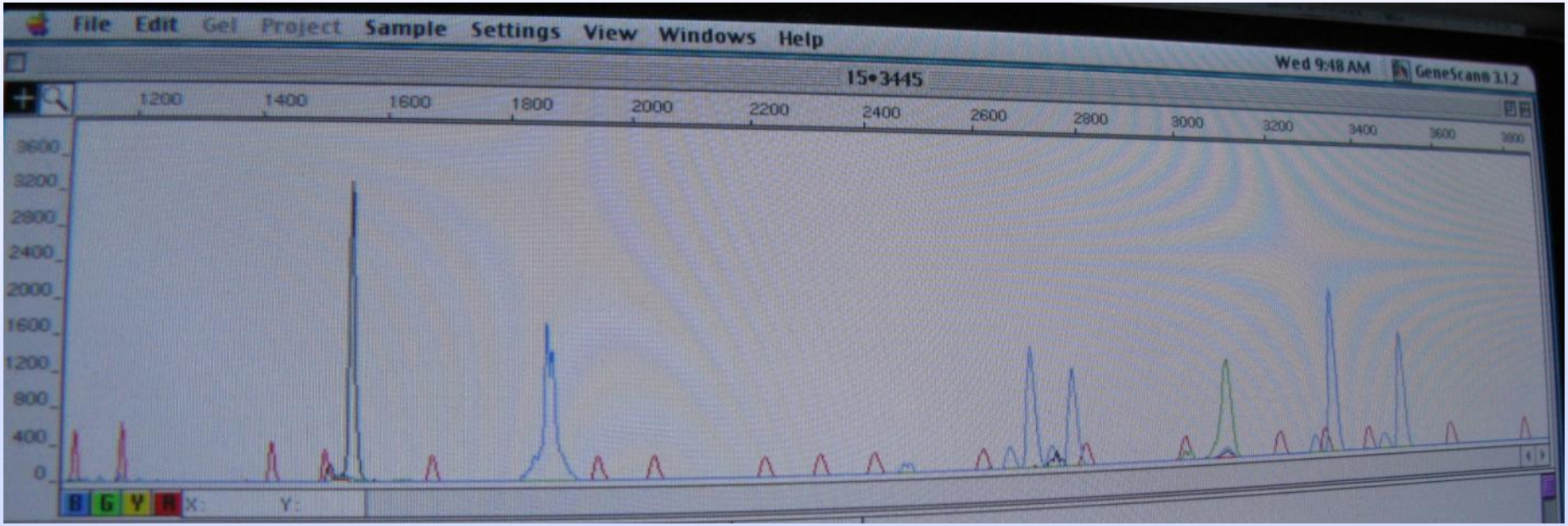
Microsatellite Markers/Primers

Locus	Repeat motif	Primer sequence (5'-3')	Size range
*Ro*C4	$(ATAG)_{19}(ATAC)_{12}$	F: **(6-FAM)**TTACCAAGGTAGCACTCTTTG R: ACTGCAAACCGGAAATGT	195-243
*Ro*C123	$(TGTA)_{10}$	F: **(PET)**GGCTTACTTCTTGCCTTTAGC R: CATCCATTTTTCCCTTGTTC	124-156
*Ro*D102	$(TATC)_{19}$	F: **(NED)**ATGGAGATTTAAGTGCAAGAGT R: CTTCCCAATATGGCATAGATAT	195-251
*Ro*D125	$(AGAT)_{15}$	F: **(6-FAM)**TCAATGGTGGTGTGTCAC R: GCTCTGAAGTCAACTGGTC	282-298
*Ro*C9	$(AT)_5(AGAT)_{10}(ACAT)_8$	F: **(NED)**CCAGCTCTAAACACATTAGCTC R: CAGCATAGGTTGGAATACAAAT	184-239
*Ro*D124	$(TCTA)_{19}(TCTCTA)_6(TC)_3$	F: **(VIC)**ACCCCTCCAAGTCAAAATC R: AAAGGTGGGCAAACTCAA	232-292
*Ro*D120	$(ATCT)_{11}$	F: **(NED)**TATCCGAGGCTTAAAATCCTTC R: ATATCGGTGCAACCCTAATACA	100-124
*Ro*D122	$(TATC)_{19}(TA)_1(TATC)_2$	F: **(NED)**CTCTGAGTCTGTCTGTCTGTCT R: TAGTGGCTTAGTCCATTCTATG	190-260
*Ro*C110	$(ATAC)_7(AC)_6$	F: **(VIC)**GGACCTGTCATACACAATGTC R: AAATGCTCTCAAACCAAGTAAC	124-148
*Ry*DI-7	$(GT)_{14}$	F: **(VIC)**AATACTCTGTTCATCTTTGTCATCTGTTCTG R: AAATCCTTAGCACTCCTTCTGGTCACT	232-280
*Ry*TET-G	$(ATCT)_{13}$	F: **PET)**GGTGGTGTACAGAGCCAAAAGGATTAGAATTGTGTTGAT R: AACCCCTAGACGCGCACGAGGTGAAGAAG	124-224
*Ry*TET-f	$(CTAT)_{16}$	F: **(6-FAM)**CTTTACCCTTAAGTTGTTTAGTGGATAA R: ACTTACAAACACAATAATATAACAGGACCGAGTA	219-289
*Ry*TET-e	$(CAAA)_6$	F: **(PET)**ATAGTTCAGCAATTTTGTATATTTTTGTGCAAGATGTATG R: ACTTCTCCGAGGGGGTCAGCAGATGT	219-271
*Ry*DI-2r	$(AC)_{11}$	F: **(NED)**TTCAACGTCCCATTAAGAGGAAACT R: GACTATTTGGGCGATATCAGAAAA	182-194
*Ry*2	$(CA)_6CC(AC)_4ACC(AC)_6$	F: **(PET)**GTGTGCGGCAGAGCCATGTGC R: GGCATATCCATTTTGATGGG	166-186

Savage & Jaeger (2009)



Screen-photo of a portion of a gel run of *R. onca* microsatellites



Screen-photo of 4 *R. onca* microsatellite markers for a signal individual as seen on analysis software

Project Status

- ✓ All milestones and deliverables have been met to date...
- ✓ Field surveys completed, all primary targeted sites within western Grand Canyon region have been surveyed
  - *No new populations found*
  - *Final report and data transfer pending*
- ✓ Phylogeographic analysis based on mtDNA "completed"
  - *Manuscript submitted to scientific journal for peer-review*
  - *Final report pending*
- ✓ Available microsatellite markers/primers assessed and polymorphic loci identified
  - *Information provided in a peer-reviewed publication*
- ✓ Microsatellite data currently being generated
  - *Analysis and final report, pending*

Acknowledgements

- Funding: Clark County, Support also from UNLV, NSF, and USGS

- Additional assistance from: Lisa Gelczis, Randy Jennings, Brett Riddle, Jim Rorabaugh, Mike Sredl, Martin Schlaepfer, and Kelly Zamudio

- Special thanks to the field crews and volunteers that helped conduct surveys...