Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Genetic characterization of *Macrotus californicus* along the lower Colorado River

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Outline

- Life History/Distribution
- Conservation Status
- Objectives
- Methods
- Results

Life History

- Medium sized bat (45-58mm forearm)
- Long ears (>25mm)
- Nose leaf

Life History

- Family: Phyllostomidae
- Non-migratory
- Colonial, utilize geothermally heated roosts ~29°C
- Described 1858
  - Subspecies of *M. waterhousii* 1969
  - Species status 1974

Life History

- Nightly outflights at dusk
  - Glean insects off desert scrub
  - Active year-round
- Maternity colonies form in summer
  - Breeding in fall
  - 1-2 young born in June

Conservation Status

- Not listed as threatened or endangered by USFWS
- IUCN ranking: least concern
- Species of special concern by BLM in Arizona, California, Nevada
- California: Vulnerable
- Arizona: Vulnerable
- Primary threats: Roost disturbance, loss of foraging habitat

Conservation Status

- One of five evaluation Species under LCR-MSCP
  - Lowering of groundwater elevations could reduce production and abundance of insect prey
- CLNB1: Conduct surveys to locate CLNB
- CLNB2: Create covered species habitat near roost sites

Distribution

Objectives

- Document genetic structuring of roosts along the LCR
- Provide demographic estimates, including current and historic effective population size of the LCR populations

Methods

Capture/Tissue collection

- Netting
  - Restoration areas
  - Roosts
- Wing punch

- 20 localities
  - 9 from the LCR-MSCP boundary
  - 6 from south of the border

- DNA Extraction
- PCR amplification
- Sequencing
  - Mitochondrial
    - Non-recombinant
    - First pass for phylogenetic studies
  - Genomic
    - Microsatellite
    - Next-gen sequencing

Results

Analysis

Mitochondrial DNA

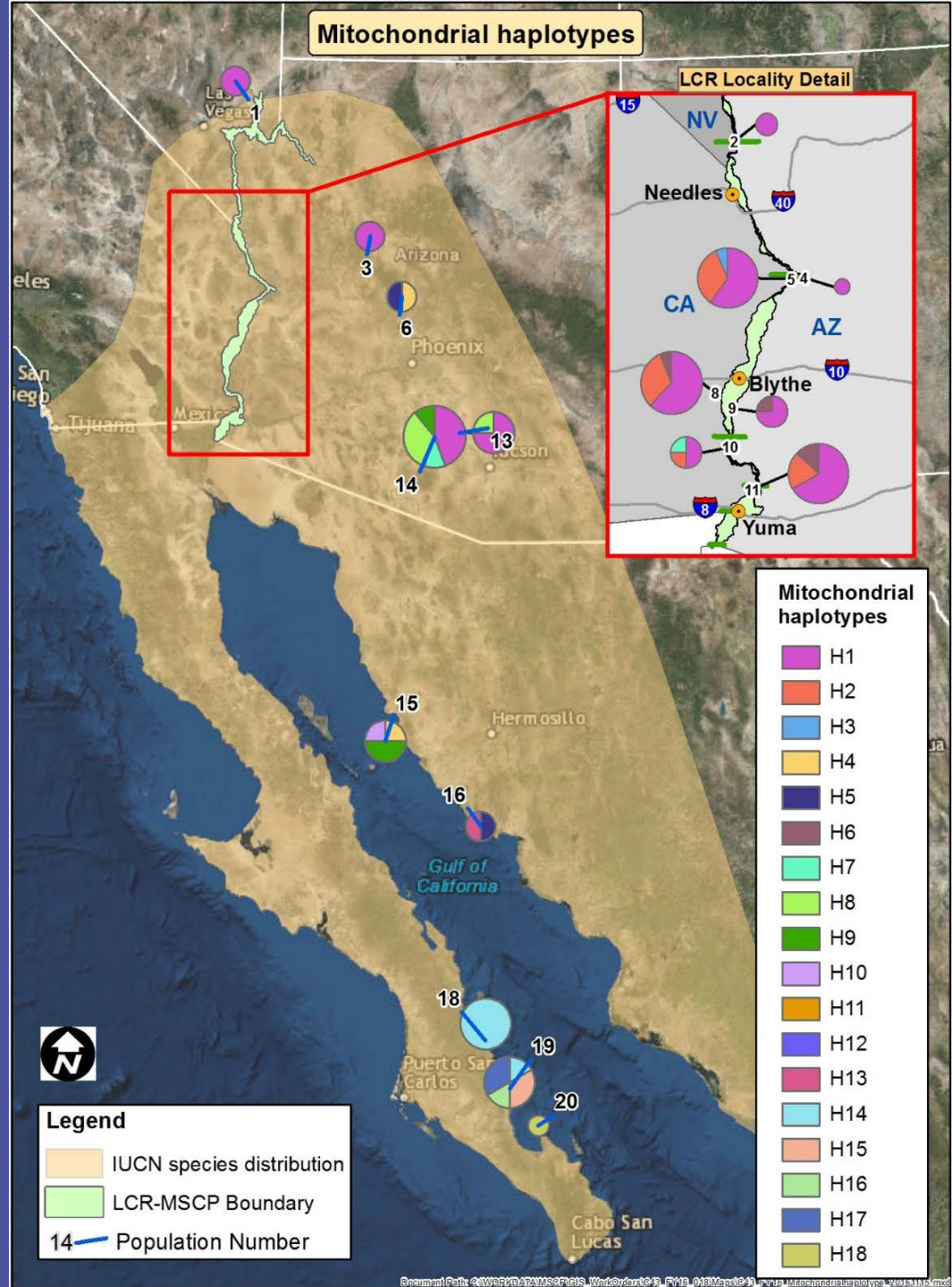
- Haplotype map
- Pairwise Fst
- AMOVA
- Tajima's D
- Mismatch Distribution

Microsatellite DNA

- Pairwise Fst
- AMOVA
- Program STRUCTURE

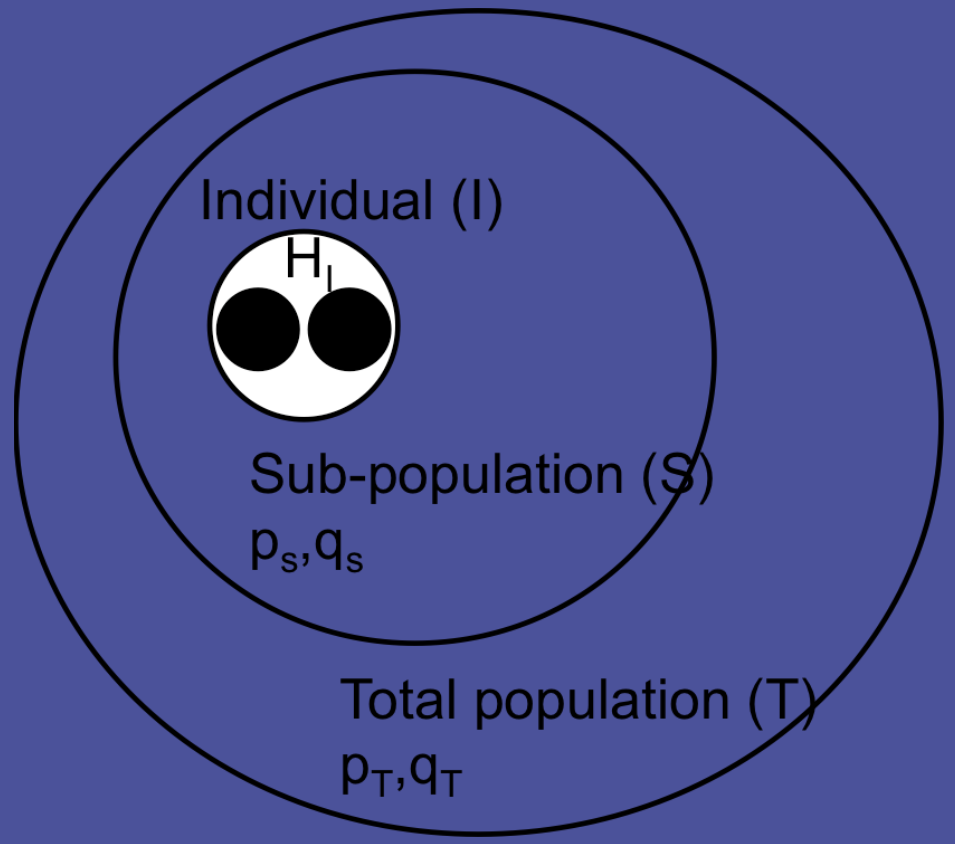
MtDNA

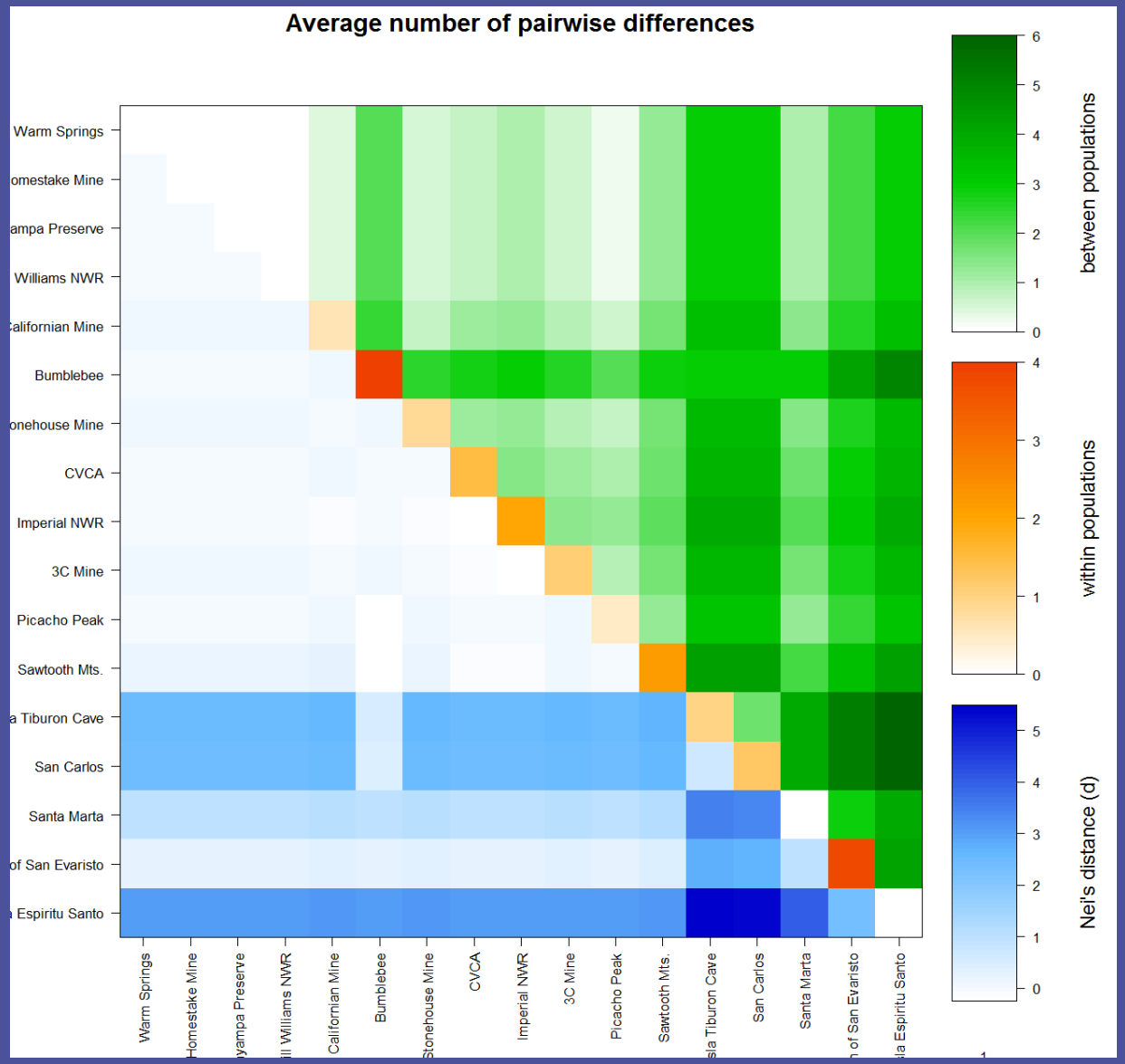
- 916 base pairs mtDNA cytochrome *b* gene
  - 99 individuals
  - 17 localities
- 18 haplotypes
  - 5 present in LCR
    - 3 unique to LCR



F_{ST}(Fixation index)

- Measure of population differentiation due to genetic structure
- π = average pairwise difference
  - Fst = (πbetween – πwithin) / πbetween
- Range 0-1
  - 0 = no differentiation
  - 1 = complete isolation




Average number of pairwise differences
Warm Springs
omestake Mine
ampa Preserve
Williams NWR
alifornian Mine
Bumblebee
onehouse Mine
CVCA
Imperial NWR
3C Mine
Picacho Peak
Sawtooth Mts.
a Tiburon Cave
San Carlos
Santa Marta
of San Evaristo
Espiritu Santo
Warm Springs
Homestake Mine
yampa Preserve
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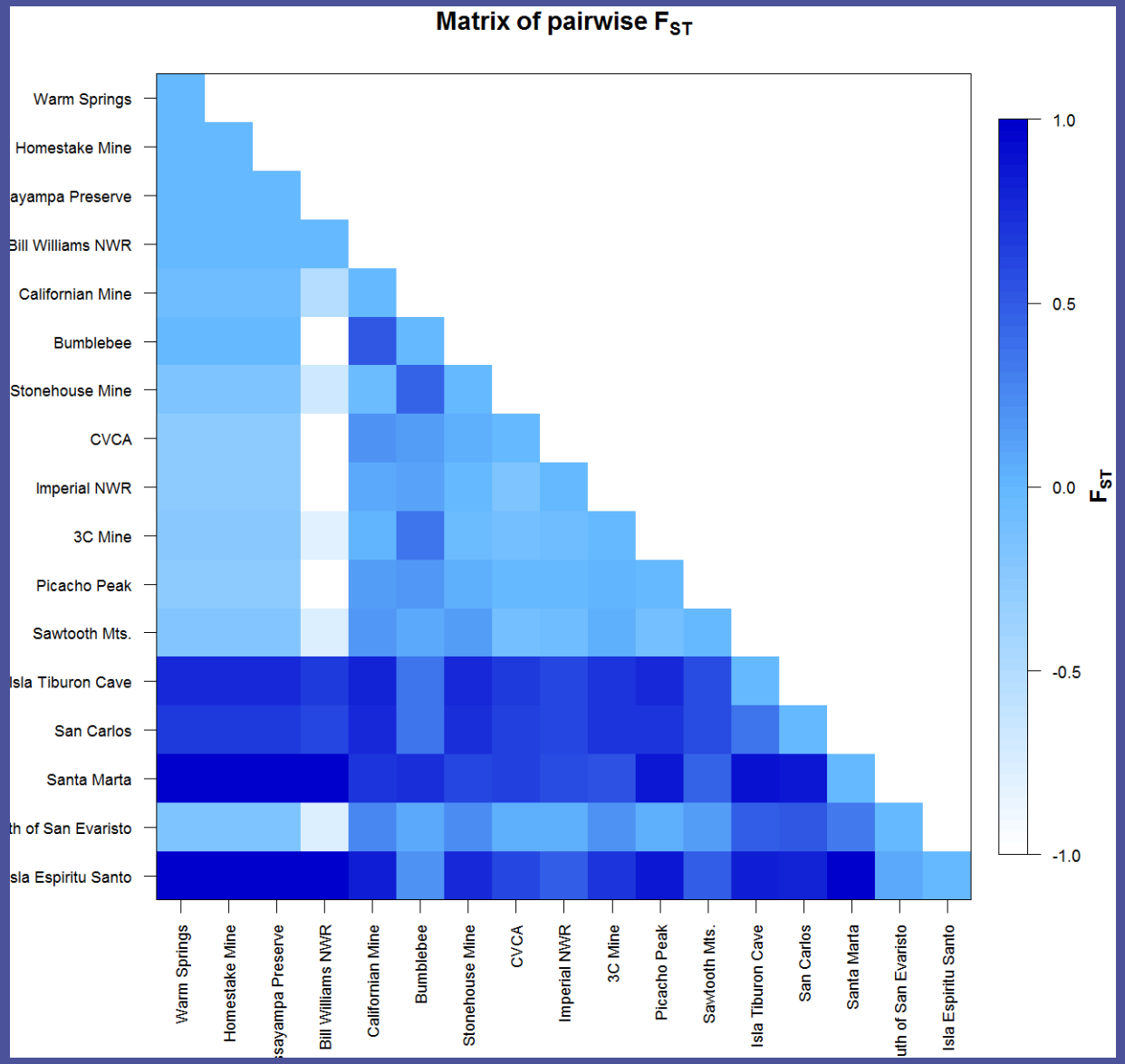
between populations
within populations
Nei's distance (d)


Matrix of pairwise F_{ST}
Warm Springs
Homestake Mine
ayampa Preserve
Bill Williams NWR
Californian Mine
Bumblebee
Stonehouse Mine
CVCA
Imperial NWR
3C Mine
Picacho Peak
Sawtooth Mts.
Isla Tiburon Cave
San Carlos
Santa Marta
th of San Evaristo
sla Espiritu Santo
F_{ST}

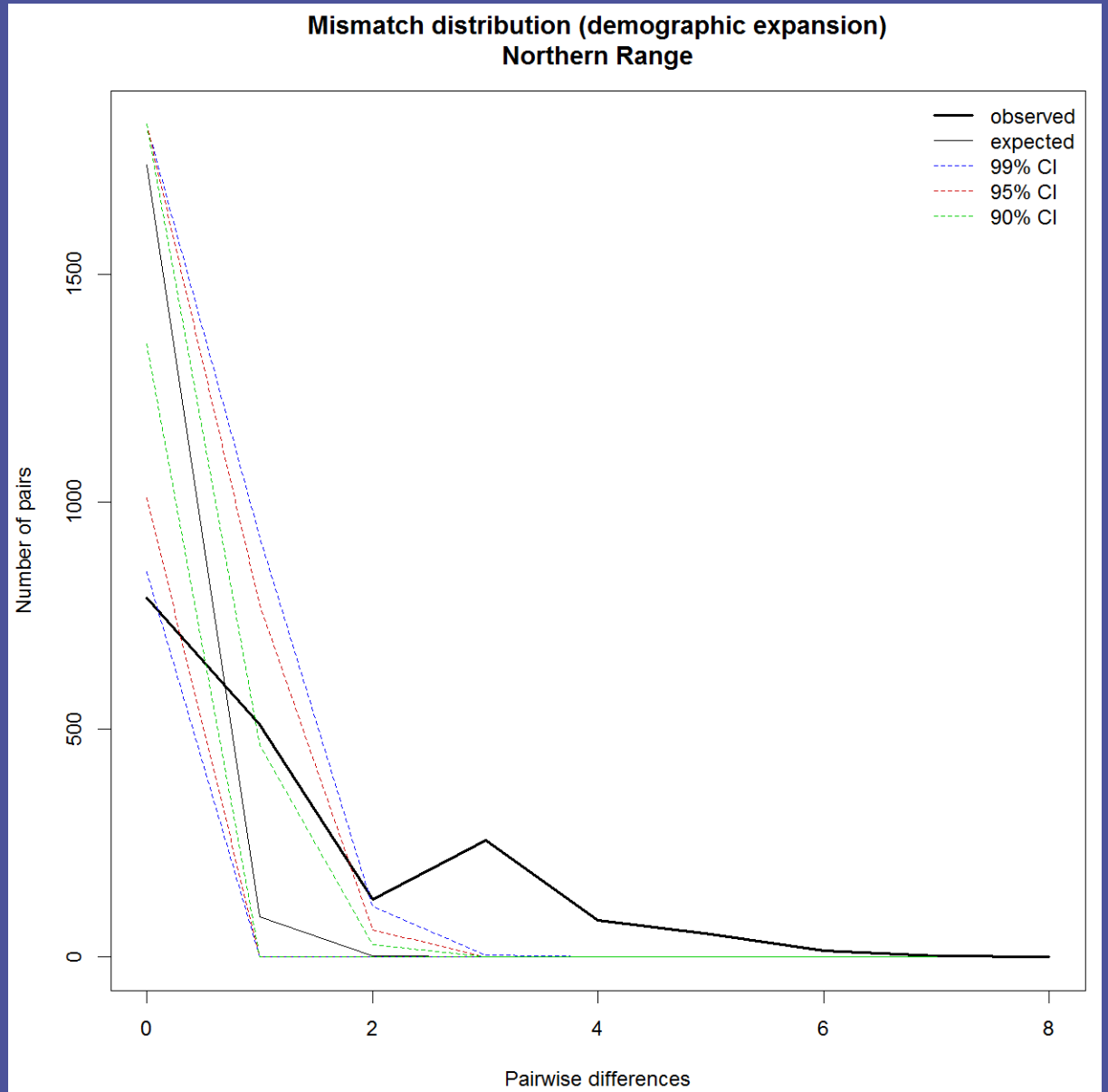
mtDNA AMOVA

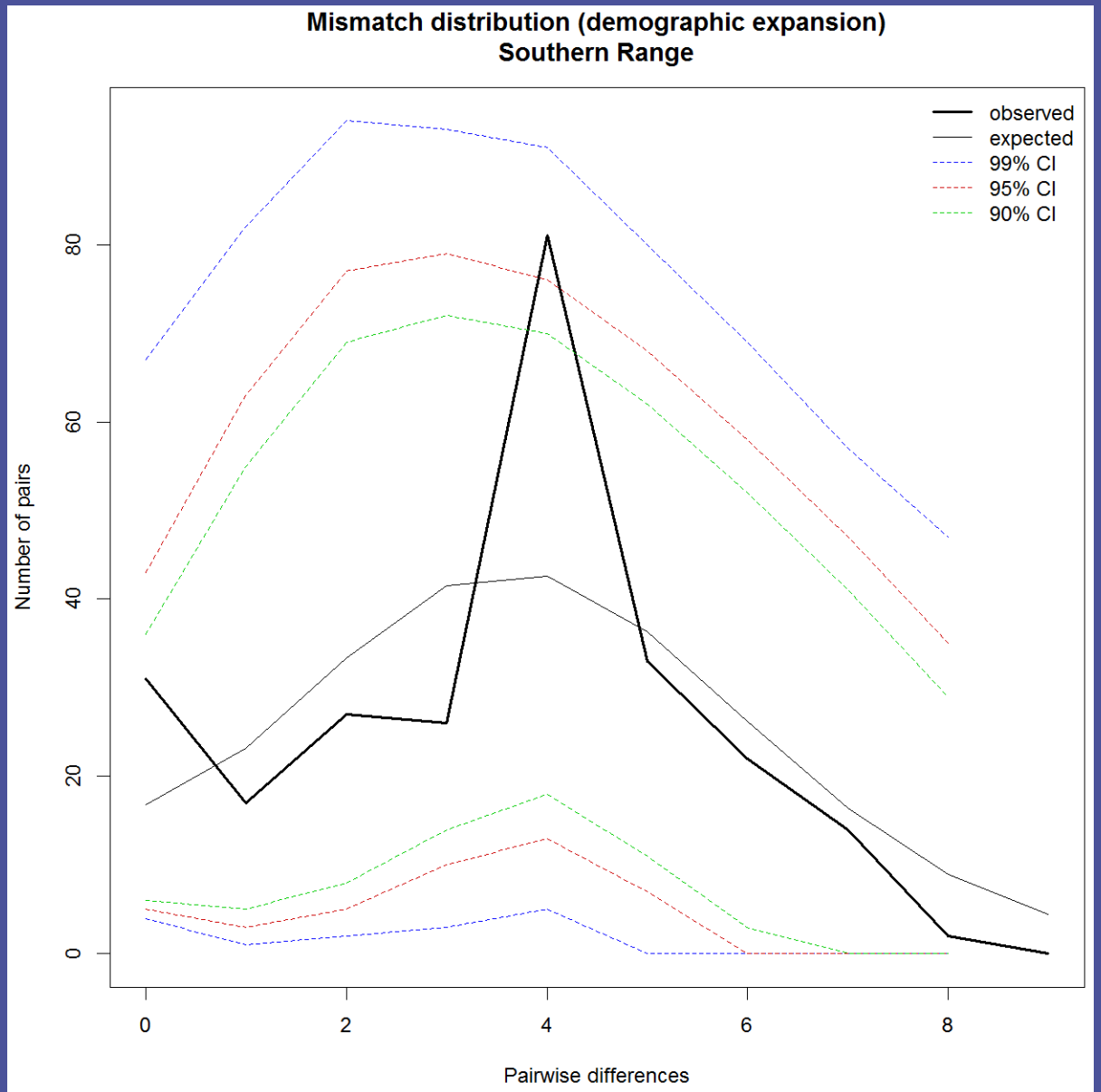
- Variation explained by
  - Groups: 48.74%
  - Populations within groups: 6.65%
  - Within populations: 44.61%

Tajima's D results

- π = Average pairwise difference
- Θ = Expected pairwise difference
- Tajima's D = Normalized version of π - Θ
- Northern range samples (n=76): -1.52
- Southern range samples (n=23): -0.50

Mismatch distribution





Genomic results

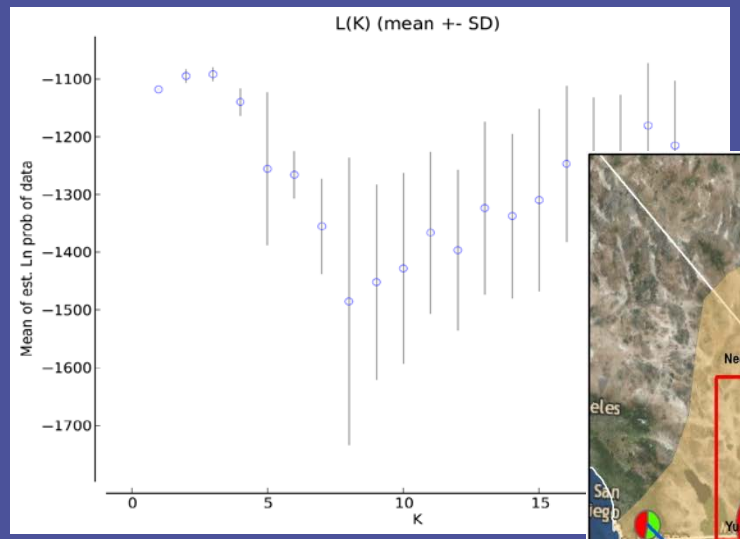
- 2000-5000 loci isolated per individual sent for next-gen sequencing
  - ➢ Filter for variability
- 6 microsatellite loci
  - – 87 individuals
  - – 19 localities

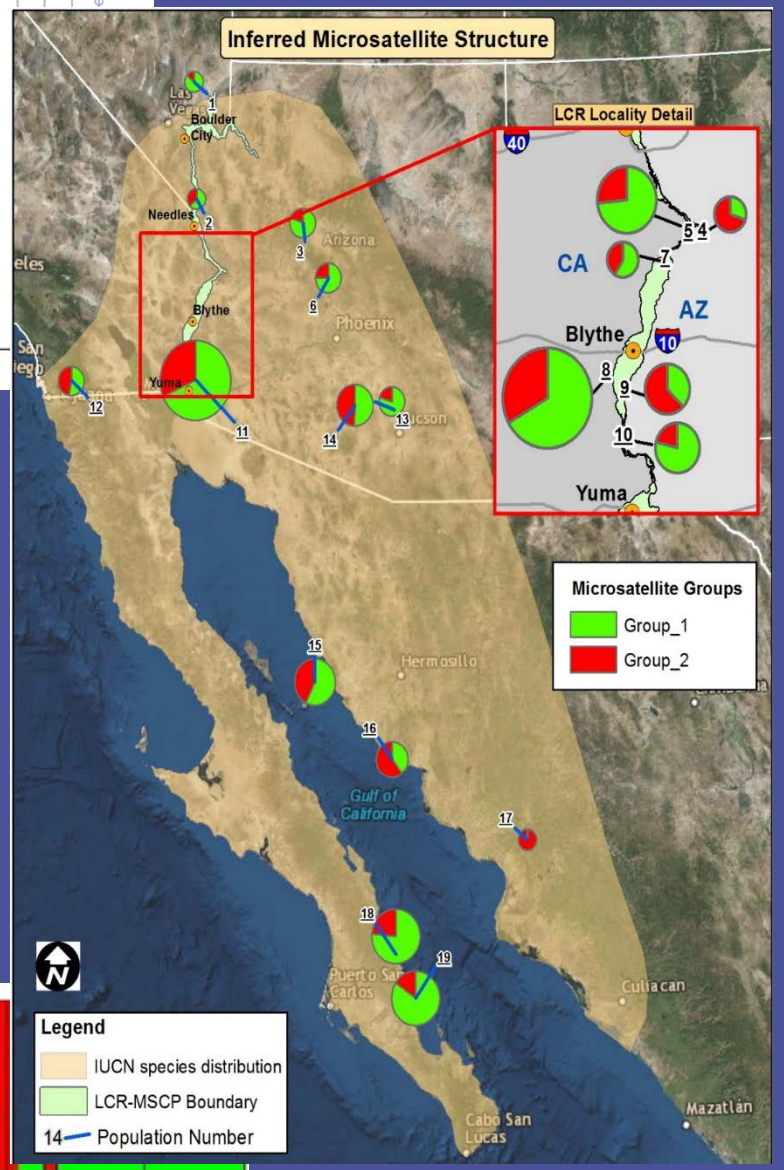
Microsatellite AMOVA

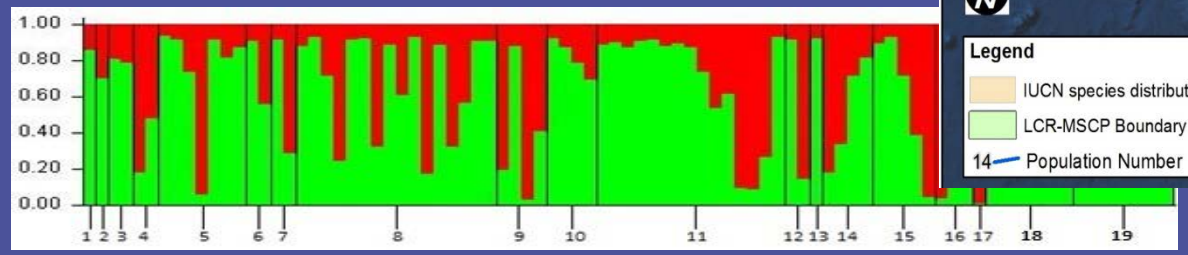
- Among groups: 0.05%
- Among populations within groups: 0.35%
- Within populations: 99.60%

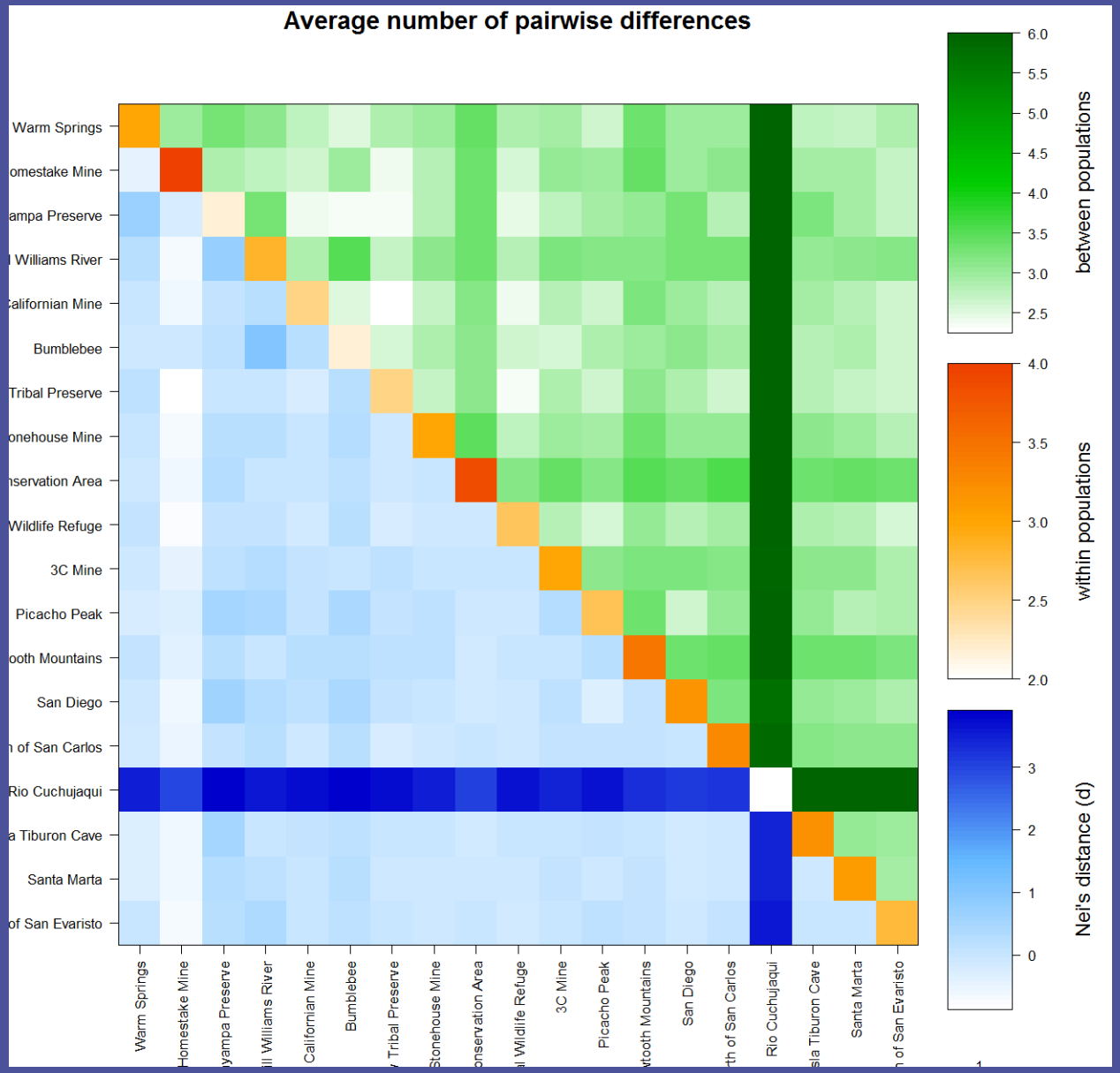
Program STRUCTURE

- Bayesian clustering algorithms Estimate number of groups (K) given allele frequencies
- Supports multilocus datasets
- Useful in detecting population structure where it may be weak
- Ran with 50,000 step burn-in, 50,000 MCMC reps, 20 independent runs
- K=2 most supported





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between populations
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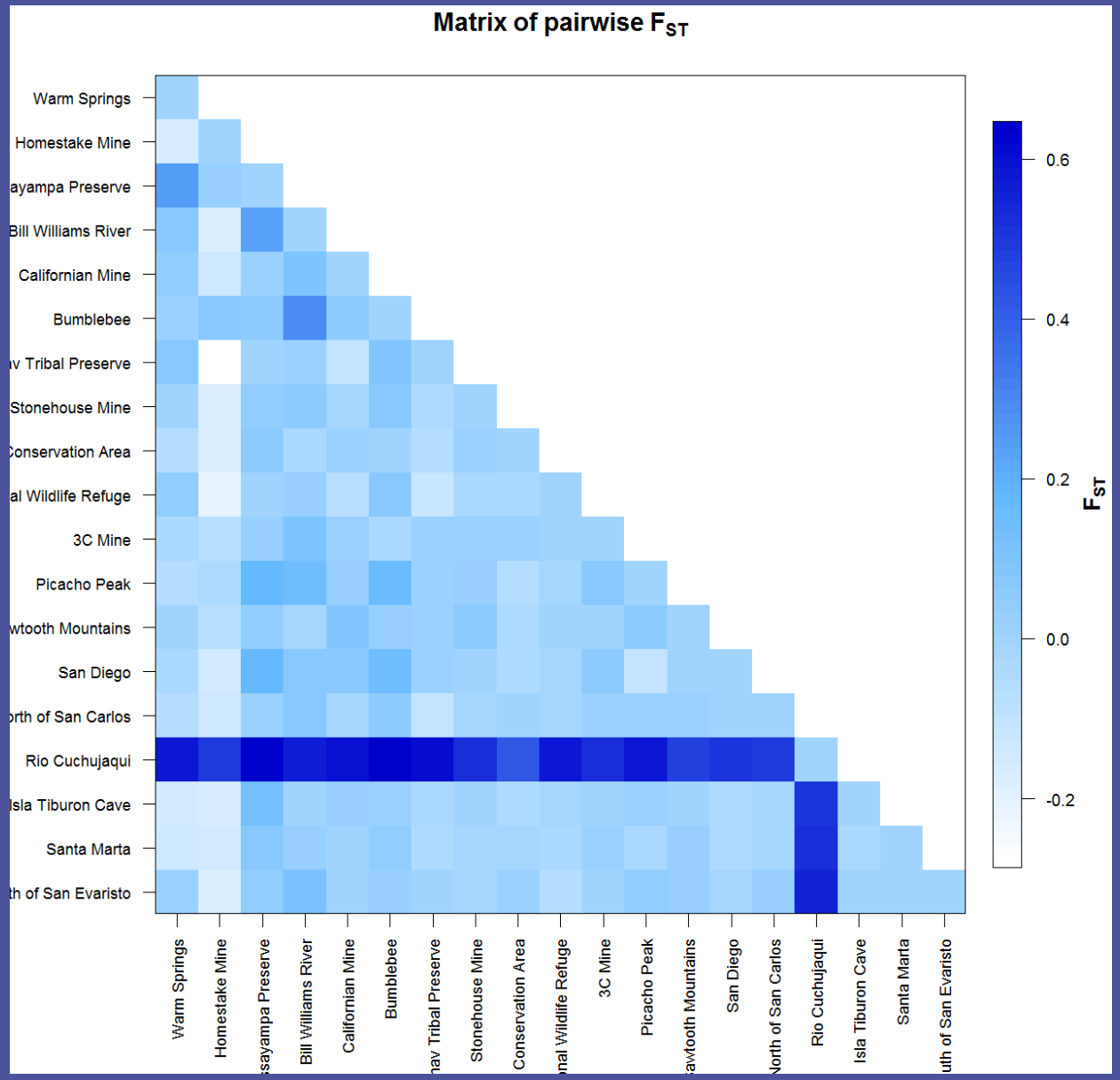
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F_{ST}

Takeaways

- Higher genetic structuring than would be expected in panmictic population
- Demographic parameters suggest northern range may be recent expansion
- Microsatellite dataset not structured enough to be informative for roost fingerprinting
- Exercise caution when applying new techniques!

Acknowledgements

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