# GENETIC INTERRELATIONSHIPS AMONG POPULATIONS OF GILA CYPHA IN THE COLORADO RIVER BASIN

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# *Gila cypha* (Humpback Chub)



Havasu Ck, Grand Canyon, AZ (45 cm TL)

# *Gila cypha* – Life History

- Overall, life history is mostly enigmatic

- Impeded by lack of an historic baseline

- Links between physical and biological parameters largely unknown

- Hampered by the mainstem/LCR continuum

- Little known about interrelationships among populations

- Deficit will continue to hamper attempts at conserving, recovering and managing the species

# *Gila cypha* - Distribution

- Upper Basin:

  - Yampa River
  - Desolation Cn
  - Black Rocks Cn
  - Westwater Cn
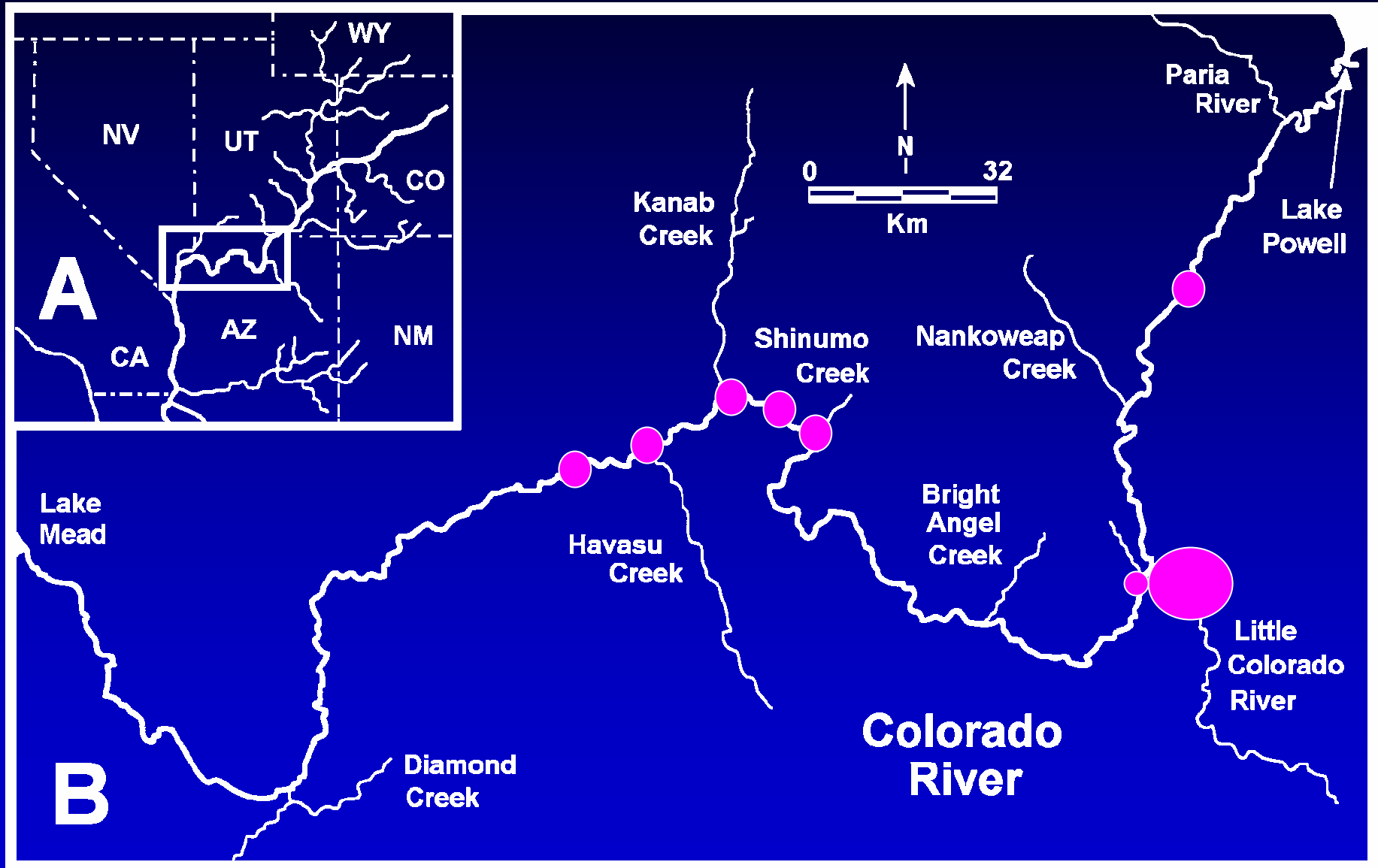  - Cataract Cn
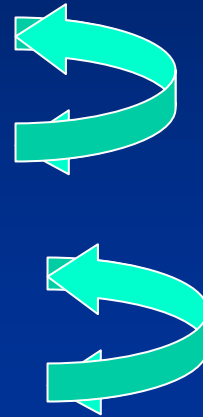
# *Gila cypha* - Distribution

- Lower Basin:

  - 30-Mile Springs
  - Little Colorado River
  - Shinumo Ck
  - Middle Granite Gorge
  - Kanab Ck
  - Havasu Ck
  - Western Grand Cn   (> RM 178)

# Populations – Grand Canyon

# Objectives

- Employ molecular markers to infer genetic interrelationships among populations
  - ❖ genetic diversity within populations
  - ❖ genetic divergence between populations
- If possible, identify genetically distinct units
- Use data to derive a management strategy

# Importance of Genetics

- Population history encoded in genomes
- Historic and contemporary information
  - coalescence
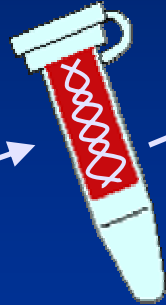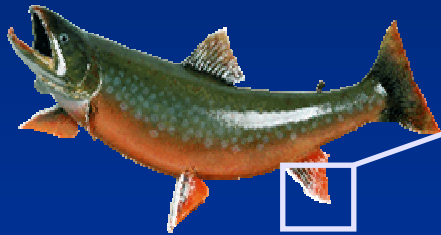  - overall relatedness
  - kinship
  - Ne/N
  - dispersal

# Importance of Genetics

- Information efficiently extracted by genetic methods:
    - advanced molecular techniques
    - computational approaches
    - variety of markers available
        - different temporal & spatial scales

# Molecular Genetic Methods

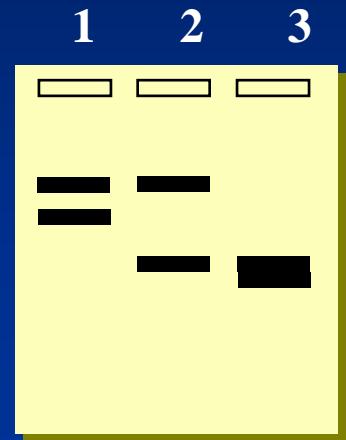**Tissue collection**

**DNA-Extraction**

**DNA Amplification (PCR)**

**Electrophoresis**

1   2   3

# Modern Technology

## Automated Sequencer

✓ **Fluorescent based**
✓ **Automated**
✓ **Sensitive**
✓ **Electronic output**

# DNA Sequence Data
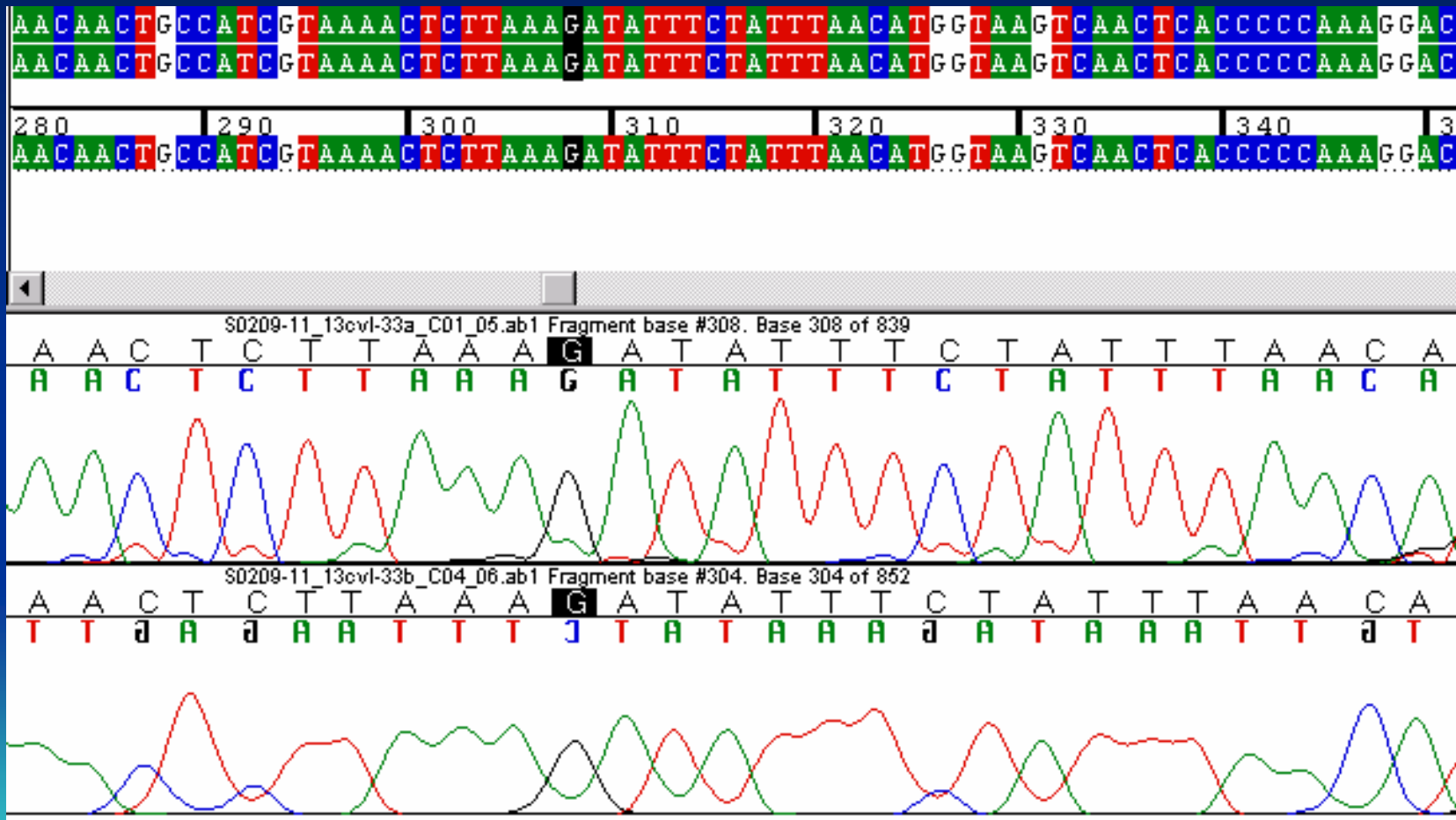
# Microsatellite DNA Analysis

– One of newest approaches used for distinguishing discrete stocks of fishes

– **High** resolution

– **Costly** to develop markers



Multiplex of 5 Microsatellite Loci
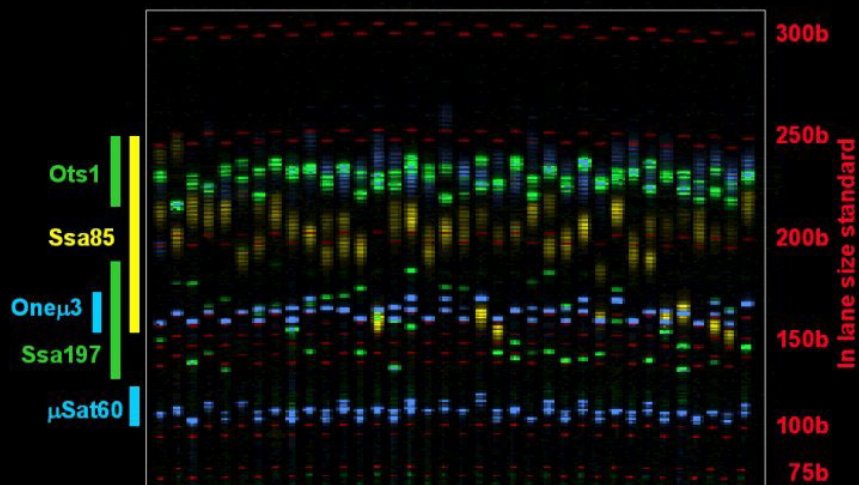


Pink Salmon Microsatellites
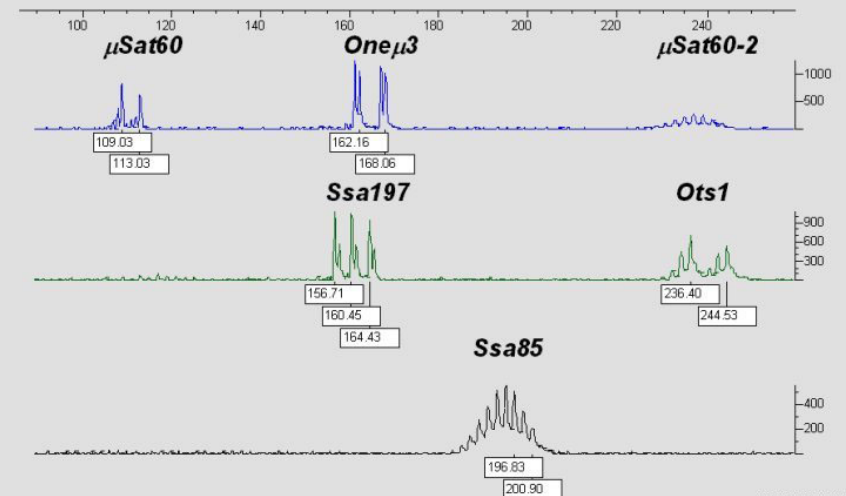
# Why Mitochondrial DNA?

- Inherited maternally with no recombination

- Evolves 5—10x faster than nuclear genes

- Fewer individuals needed to encapsulate its variance within populations

- Some regions conserved, others quite variable

- Phylogenetic analysis possible

# Ancestral Polymorphism (AP)

- Genetic diversity
  - originally present in ancestor population
  - retained in current populations
- AP often link populations even if they are geographically isolated
- "Lineage sorting" changes frequency of AP
  - mutations occur and ascend
  - may eventually eliminates them
- Time is important variable

# Molecular Markers

Today: mtDNA regions = 1,847 bp

- ATP-8 (169 bp)
- ATP-6 (473 bp)
- ND2 (589 bp)
- D-loop (616 bp)

# Sampling: Upper Basin

- 4 locations / 62 individuals

  - Yampa R            5
  - Desolation Cn     21
  - Black Rocks Cn    16
  - West Water Cn     20

# Sampling: Lower Basin

- 9 locations / 152 individuals

  - 30-Mile Springs          9
  - Little Colorado River     54
  - Shinumo Ck            24
  - Middle Granite Gorge    50
  - Kanab Ck             3
  - Havasu Ck            9
  - Western Grand Cn      3

# Genetic Variation: Colorado R Basin

| sites | ATP | ND2 | D-loop |
|---|---|---|---|
| polymorphic | 1% | 2% | 2% |
| Haplotype diversity ($h$) | 0.724 | 0.402 | 0.454 |
| Nucleotide diversity ($\pi$) | 0.00204 | 0.00082 | 0.00085 |

# Haplotypes – ATP86

| | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| YAM | 7 | 7 | 1 | 1 | | | | | | | | 16 |
| DES | 19 | | | 1 | 1 | | | | | | | 21 |
| BKR | 10 | 5 | 5 | 3 | | 1 | | | | | | 20 |
| WWC | 4 | 1 | 1 | | | | | | | | | 5 |
| 30M | 2 | 3 | | | 1 | | 2 | 1 | | | | 9 |
| LCR | 2 | 27 | | | 1 | | 21 | | 1 | | | 54 |
| SHN | | 12 | | | | | 10 | | | 2 | | 24 |
| RAN | 1 | 28 | | | 1 | | 19 | | | | 1 | 50 |
| KAN | | 1 | | | | | 2 | | | | | 3 |
| HAV | | 3 | | | | | 5 | | 1 | | | 9 |
| WGC | 1 | 1 | | | | | 1 | | | | | 3 |
| | 46 | 88 | 2 | 5 | 5 | 1 | 61 | 1 | 2 | 2 | 1 | 214 |

# Basin-Specific Haplotypes

| mtDNA N (shared) | Upper Basin N (unique) | Lower Basin N (unique) |
|---|---|---|
| ATP 11 (3) | 6 (3) | 8 (5) |
| ND2 13 ( 1) | 7 (6) | 7 (6) |
| D-loop 17 ( 2) | 8 (6) | 11 (9) |

# Basin-Specific Haplotypes

| mtDNA<br>N (shared) | Upper Basin<br>N (unique) | Lower Basin<br>N (unique) |
| --- | --- | --- |
| Combined<br>31 (3) | 16 (13) | 18 (15) |
| % | 67% (33%) | 16% (84%) |

# Haplotype Divergence

- Composite Haplotypes

  - p-distance:              1-8

  - Jukes-Cantor:    0.05-0.4%

# Preliminary Conclusions (1)

- Ancestral polymorphism link populations within and across basins

- Upper basin
  - more unique haplotypes, but
  - higher frequency of shared haplotypes

- Lower basin
  - fewer unique haplotypes, but
  - basin-specific haplotypes more frequent

# Preliminary Conclusions (2)

- Haplotype divergence small
  - mtDNA of limited utility in determining relationships among populations

- Apparent regional fixation of haplotypes should be interpreted cautiously as it is sample-size dependent

- Limitations are imposed by evolutionary rate(s) of molecular markers employed

# Preliminary Conclusions (3)

- More to do…….

- …… more to come