Biological Boundaries and Conservation of the Kanab Ambersnail

- Mitochondrial DNA sequence
- Nuclear DNA sequence (gene and SNP)
- Anatomical analyses

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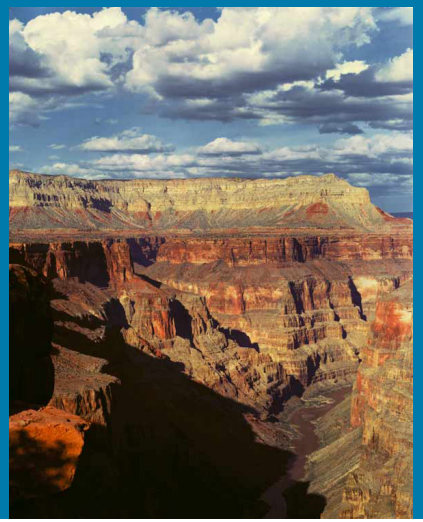
Molecular Taxonomy: Kanab ambersnail

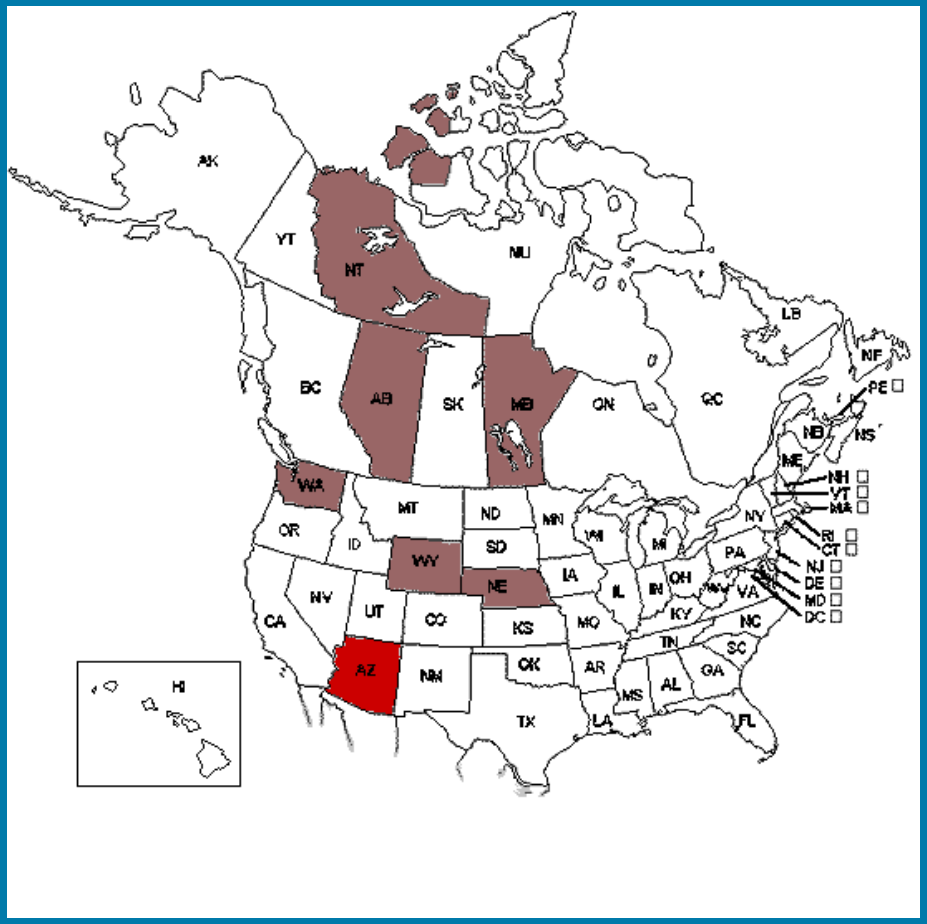
- Genetic and anatomical variation in Kanab ambersnail - USGS (GCMRC)
  - Hans-Werner Herrmann, Postdoc (UA)
  - Anna Carlson, Postdoc (UA)
  - Collaborators: Mark Miller (USU), Barry Roth
  - Sampling: Jeff Sorenson (AGFD)
    1. Resolve Kanab ambersnail taxonomy in *Oxyloma* AZ/ UT populations (AZ, UT) and Canada individuals
    2. Explain prior discordance of morphological and genetic results



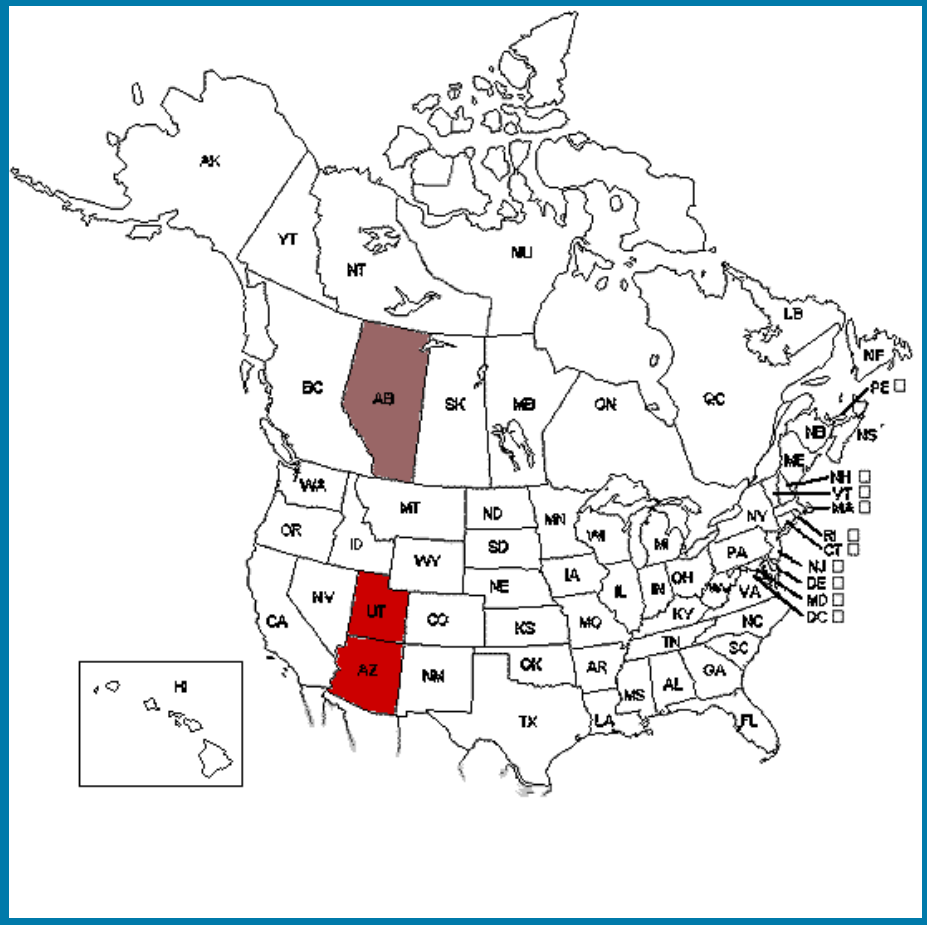
Background

- *Oxyloma* genus, terrestrial snail
  - >12 species in North America
  - Also occurs in Europe and Africa
- *Oxyloma haydeni* occurs in western US and Canada
  - Taxa not well defined
  - Morphology and anatomy have limitations
- Kanab Ambersnail, *Oxyloma haydeni kanabense*
  - Type specimen in Utah, occurs into Canada
  - ESA listed as endangered subspecies
- Family SUCCINEIDAE, Beck, 1837
  - Shells offer little indication if genus or species identity
  - Genera are identified by anatomical traits (reproductive)
  - Species-level resolution poorly understood

Oxyloma haydeni haydeni
(Niobrara ambersnail)



Oxyloma haydeni kanabense
(Kanab ambersnail)



- Distribution pattern are disjunct for both subspecies
- Sympatric distribution in Arizona and Alberta

Niobrara

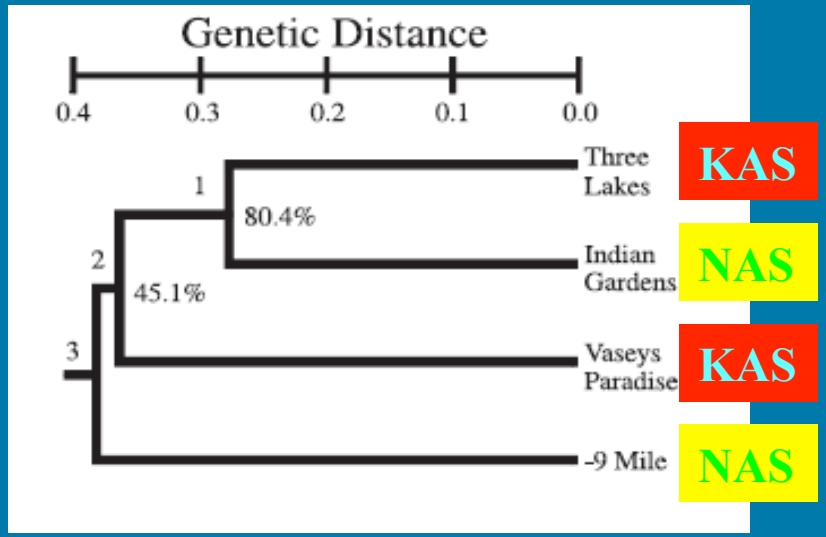
Kanab

Utah/Arizona, Distribution NOT sympatric btwn subspecies

Previous Molecular Studies

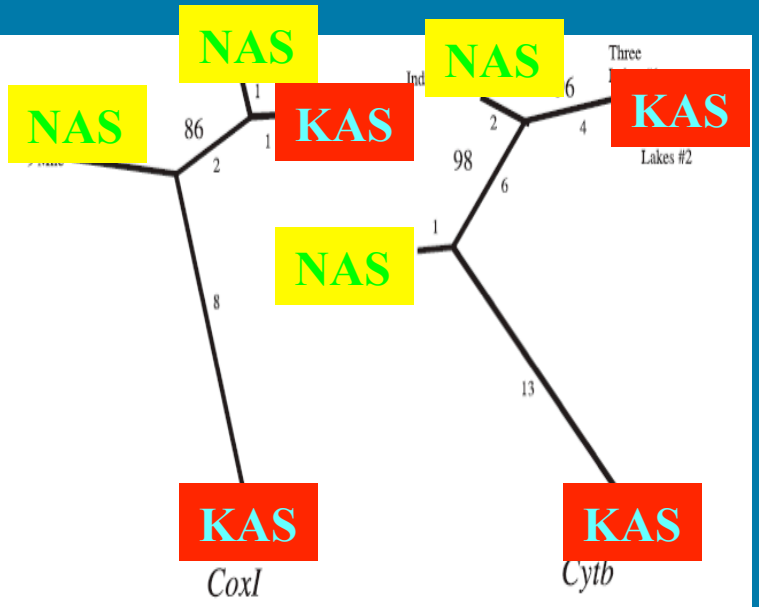
(Miller et al.)

- AFLP
  - 3 Lakes, Indian Garden
    - High diversity
  - Vasey's, -9 mile
    - Low diversity



- mtDNA CoxI and Cytb
  - All equally different taxa?
  - What taxonomic level?

More research is needed!



Current Study

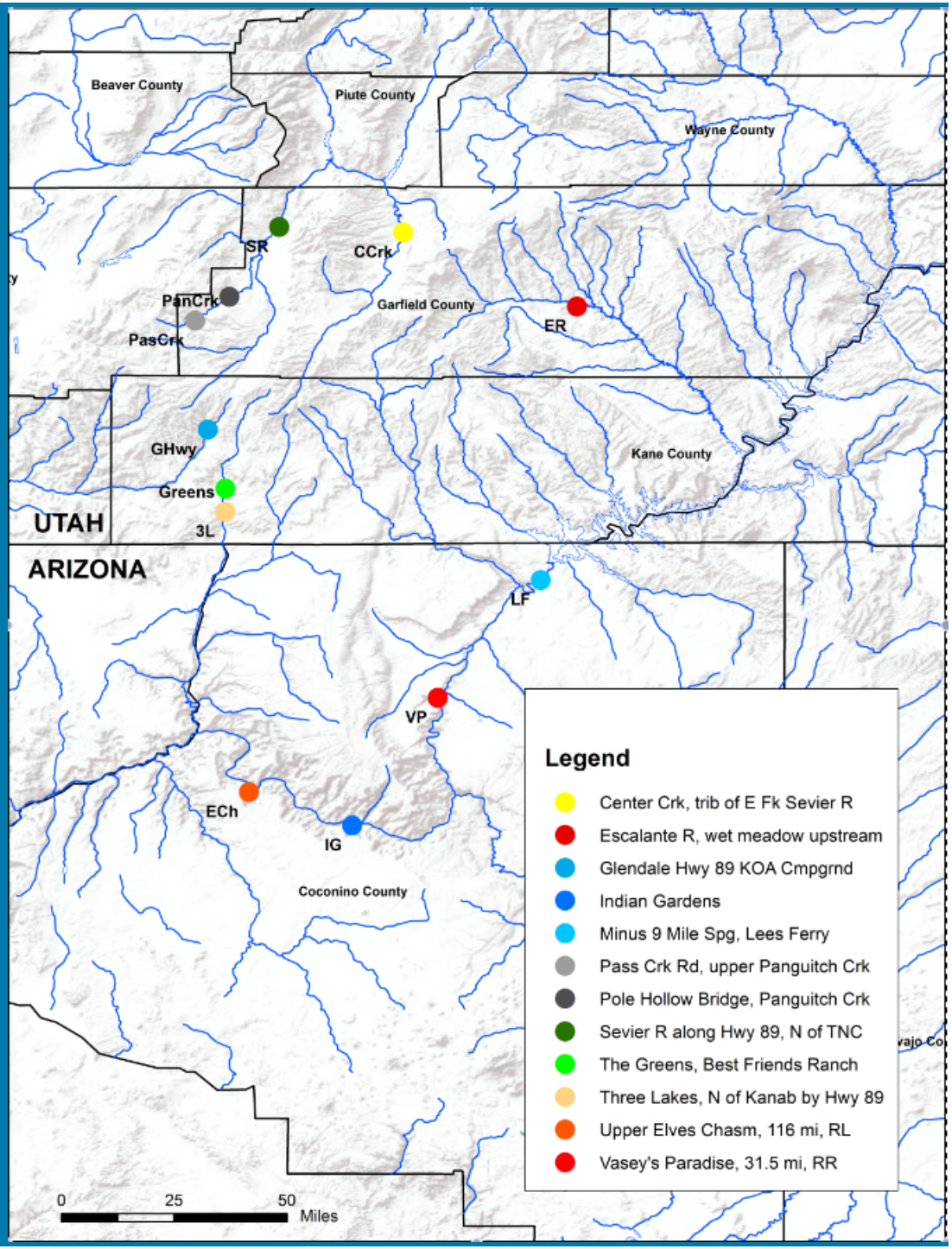
- Molecular Methods
  - mtDNA sequencing - CoxI, Cytb (Mark Miller, USU)
  - Nuclear DNA - SNPs, genes, STR (Culver, Herrmann)
- Morphological and Anatomical Methods (Barry Roth)
  - Shell characteristics
  - Reproductive characteristics
- Management Implications
  - River hydro dynamics?
  - What taxa occur in the Grand Canyon?

Sampling

(Jeff Sorenson and Dan Cox, AGFD)

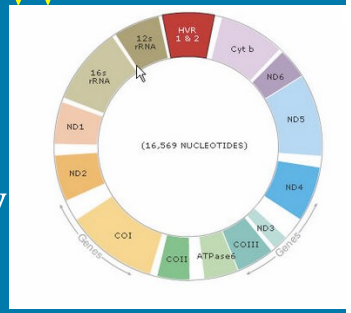
- AZ and Utah sampled
- 11 sampling locations
- 15-25 individs./pop.

- KAS type locality; Greens
- "Named" KAS at 4 locations

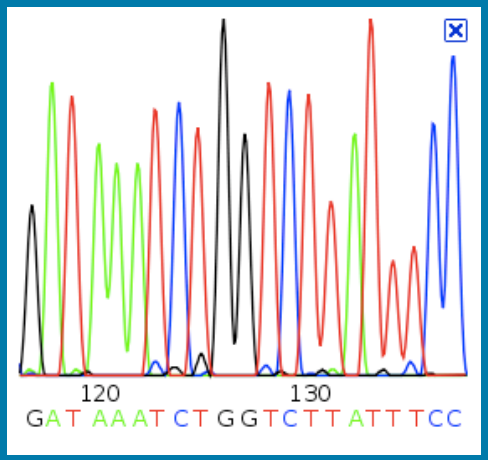


Molecular Markers Overview

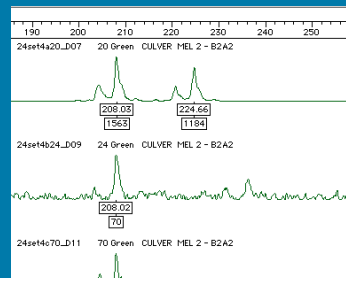
- Mitochondrial DNA (extra- nuclear)
  - Medium to high resolution marker (one single marker)
  - Used to resolve species, subspecies, populations, gene flow
  - Genes and non-coding d-loop (control region)



- Autosomal single copy DNA (nuclear)
  - Low resolution markers (many independent)
  - Used to resolve species or higher order
  - Genes, non-coding (DNA sequence or SNP)



- Autosomal microsatellite STR DNA (nuclear)
  - High resolution markers (many independent)
  - Highly polymorphic (repetitive) motifs [ATATA]….[GCGCG]
  - Used for individual ID, relatedness, gene flow, populations subdivision, subspecies-level resolution
  - Non coding (Fragment length polymorphism)



Nuclear DNA Marker Development

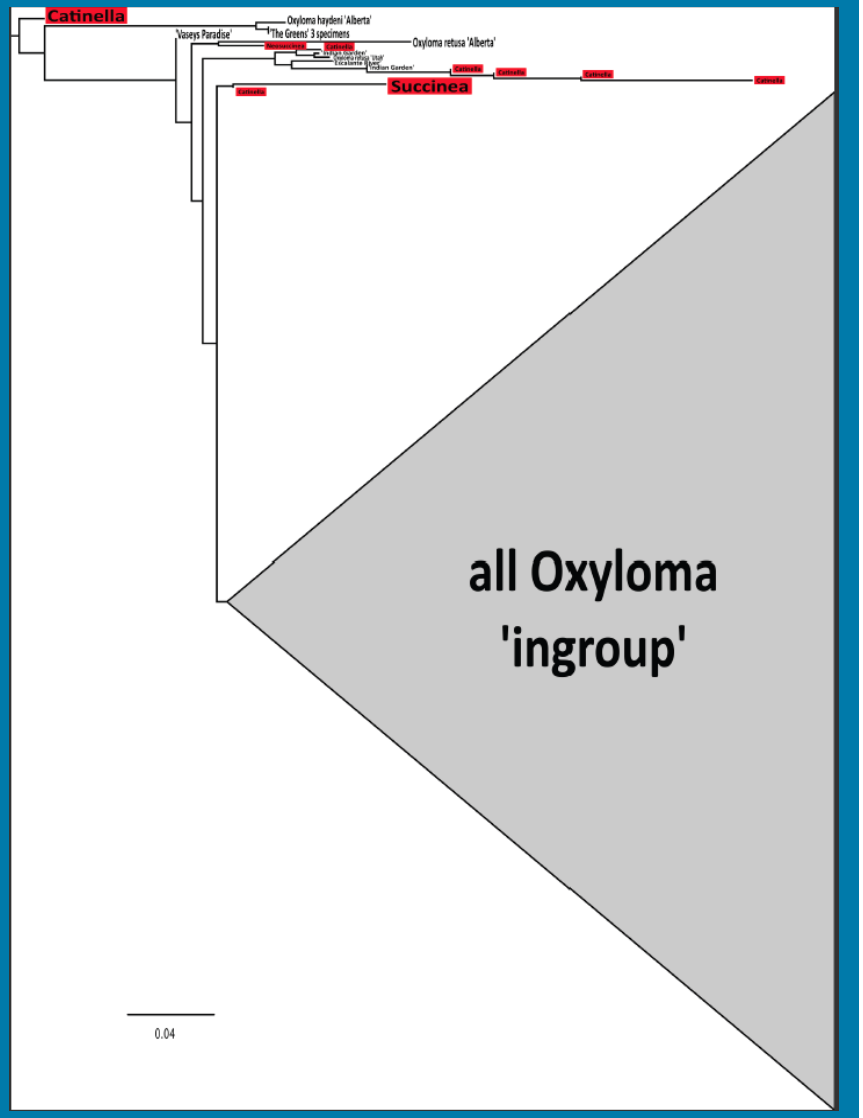
- Nuclear genes
  - Tested 6 genes from mollusc literature
  - 2 produced PCR product
- SNP development in species with no genome information
  - Genomic library
  - Sequenced 150 random clones (65kb), design primers
    - Most primers did not amplify across populations (species/subspecies)
    - Those that did amplify (conserved region), most showed no variability
  - 2 regions found with polymorphic sites
    - 356 - 25 variable (15 parsimony informative) sites
    - 458 - 36 variable (32 parsimony informative) sites, and 5 indels
- STR development
  - Genomic library enriched for simple tandem repeat elements
  - Sequenced 100 random clones, design primers
    - Most primers did not amplify across populations
    - 1 region, KS6511, amplified with 23 polymorphic alleles

DNA Methods Summary

- Mitochondrial DNA
  - DNA sequence for Cox1 (366 bp) and CytB (510 bp)
- Nuclear Genes
  - 2 polymorphic genes
    - S7 (486 bp)
    - ITS (625 bp)
- Nuclear SNPs
  - 2 polymorphic regions out of 150 clones
    - 356 - 25 variable sites
    - 458 - 36 variable sites, and 5 indels
- Nuclear STR
  - 1 polymorphic regions out of 100 clones
    - KS6511 - 23 polymorphic alleles

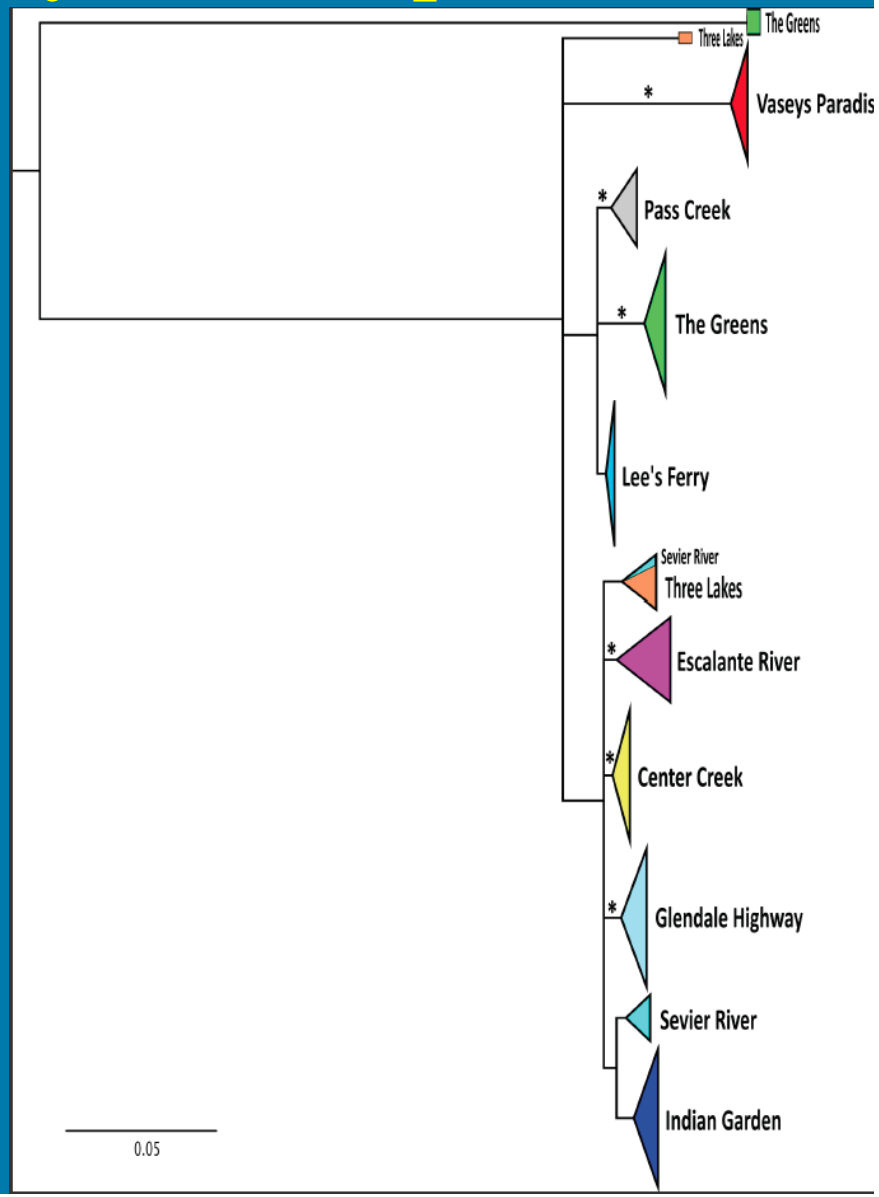
Results - *Oxyloma* ingroup

Outgroups of Catinella, Succinea
And Neosuccinea



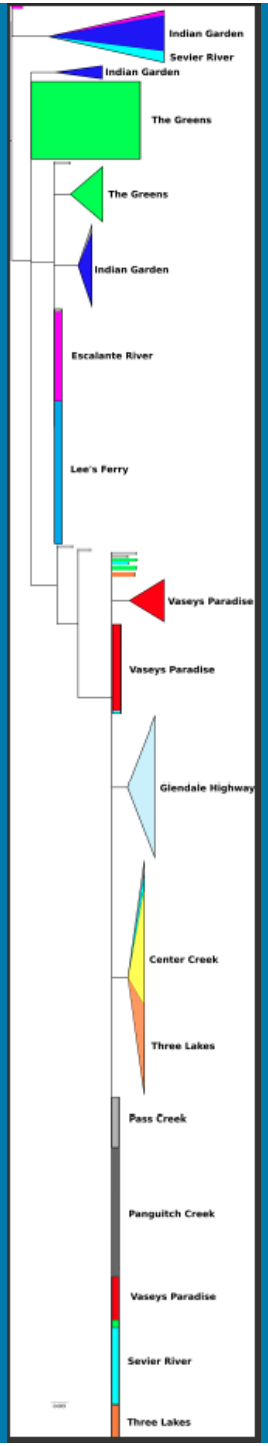
Mitochondrial DNA phylogeny
CoxI (366bp) & Cytb (510bp)

- KAS not distinct taxa
- One Vasey's indiv. has LF haplotype in Cytb gene
  - Short distance dispersal?
- Shallow clades
  - Bottlenecks?
  - Genetic Drift?
  - Gene flow?
- Greens individuals with very long branch length



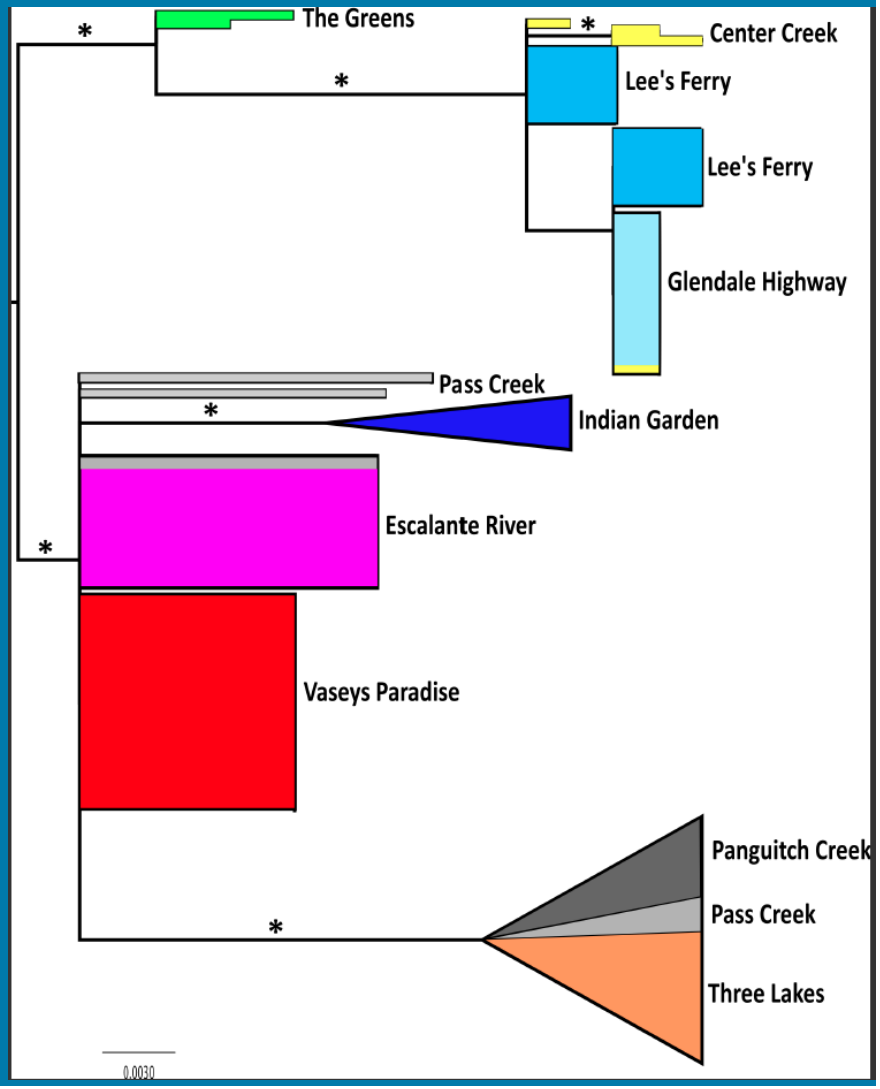
Nuclear DNA S7 gene phylogeny S7(486bp)

- KAS not distinct taxa
- "Mixed lineages" many lineages in most geographic locations



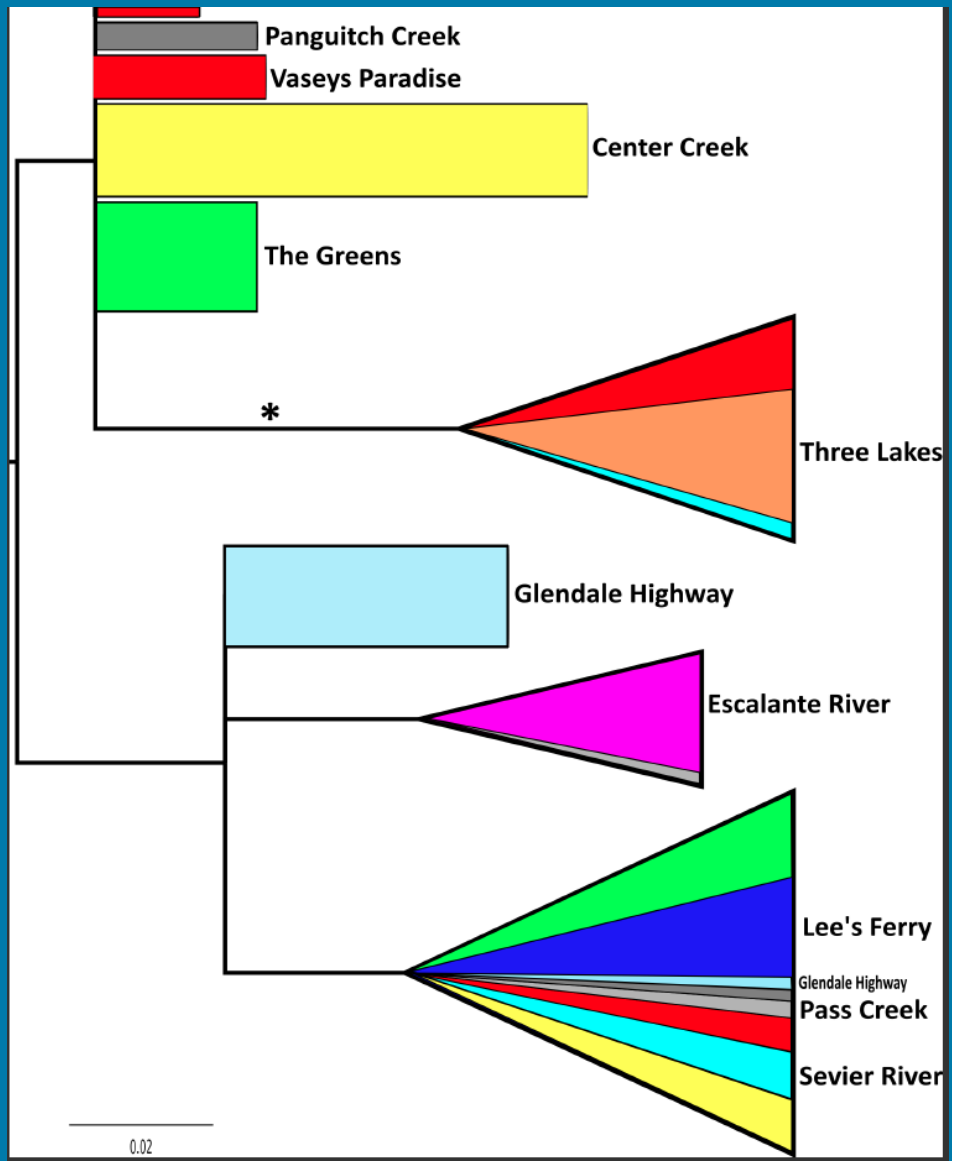
Nuclear DNA gene phylogeny ITS (625bp)

- KAS not distinct taxa
- "Mixed lineages" many lineages in most geographic locations



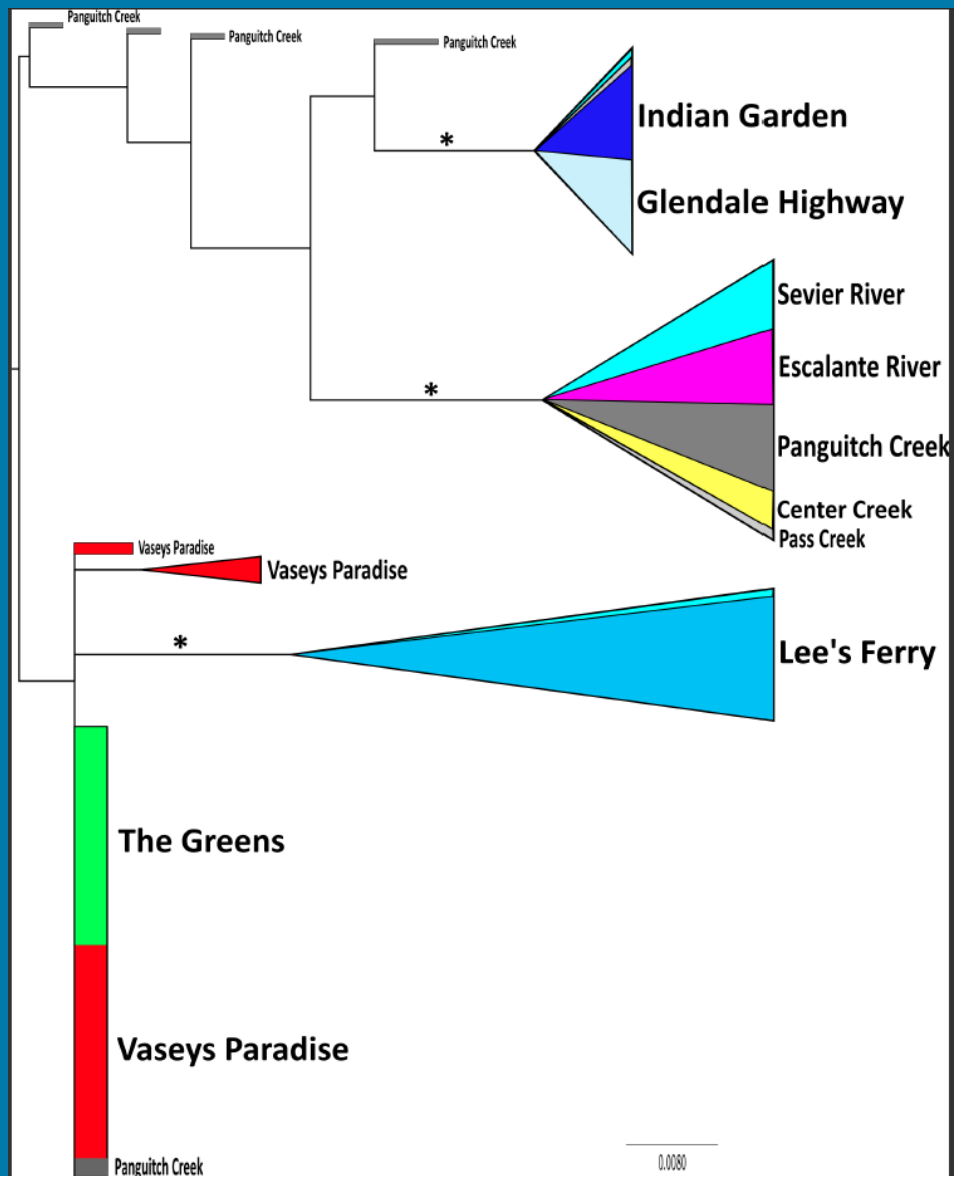
Nuclear DNA clone phylogeny 356(199bp)

- KAS not distinct
- Mixed lineages



Nuclear DNA clone phylogeny 458(528bp)

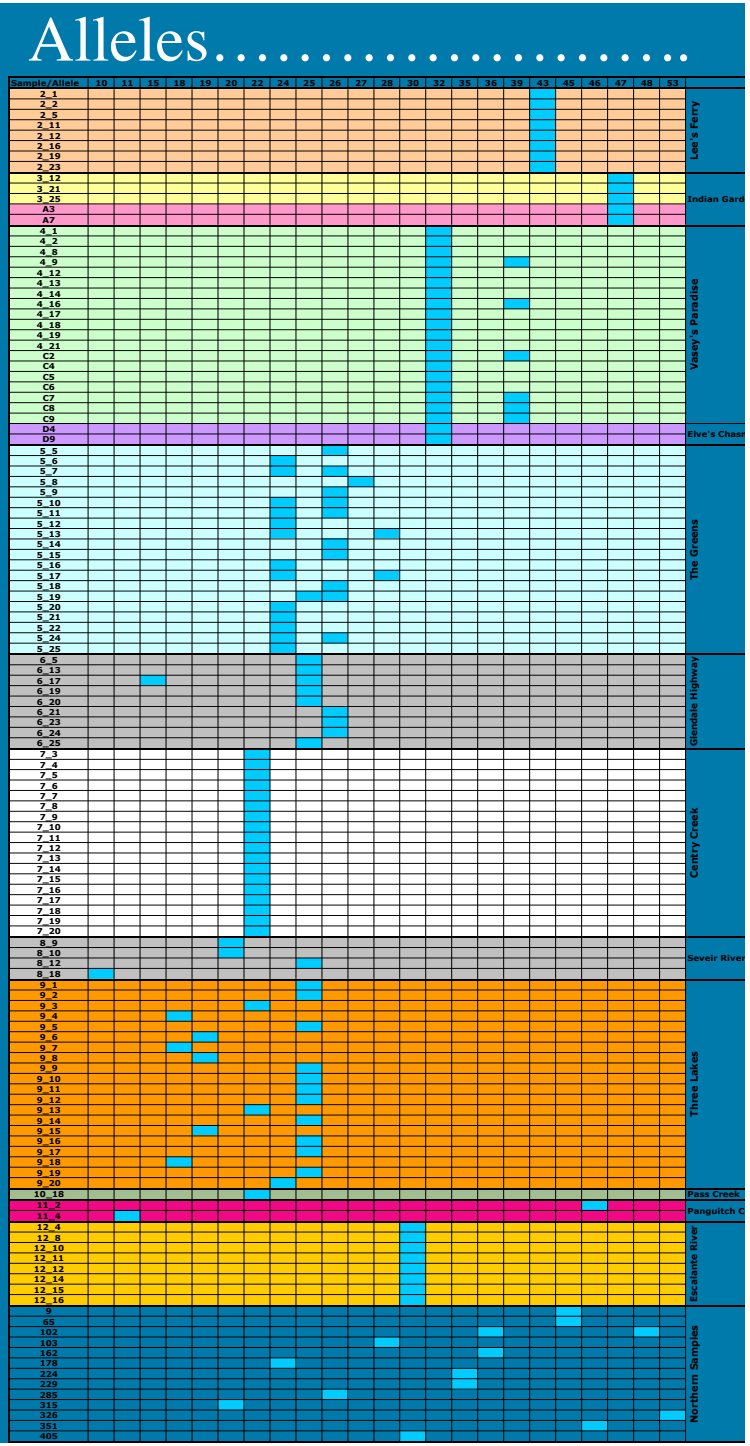
- KAS not distinct
- Mixed lineages



STR Alleles in *Oxyloma*

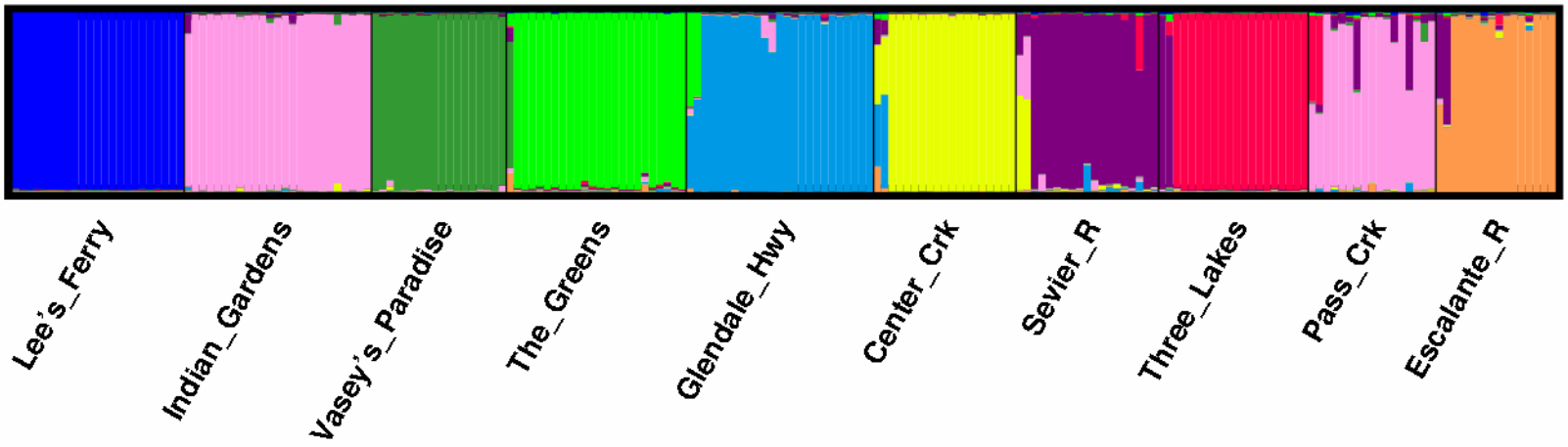
-Canada has full range of alleles
-Arizona and Utah populations mirror alleles found in Canada

In phylogeny, the Greens outliers clustered with *Oxyloma* samples from Canada



AFLP Bayesian STRUCTURE analysis supplement for STR data

K = 9 genetic clusters (Pass Crk and Indian gardens are not distinguishable)

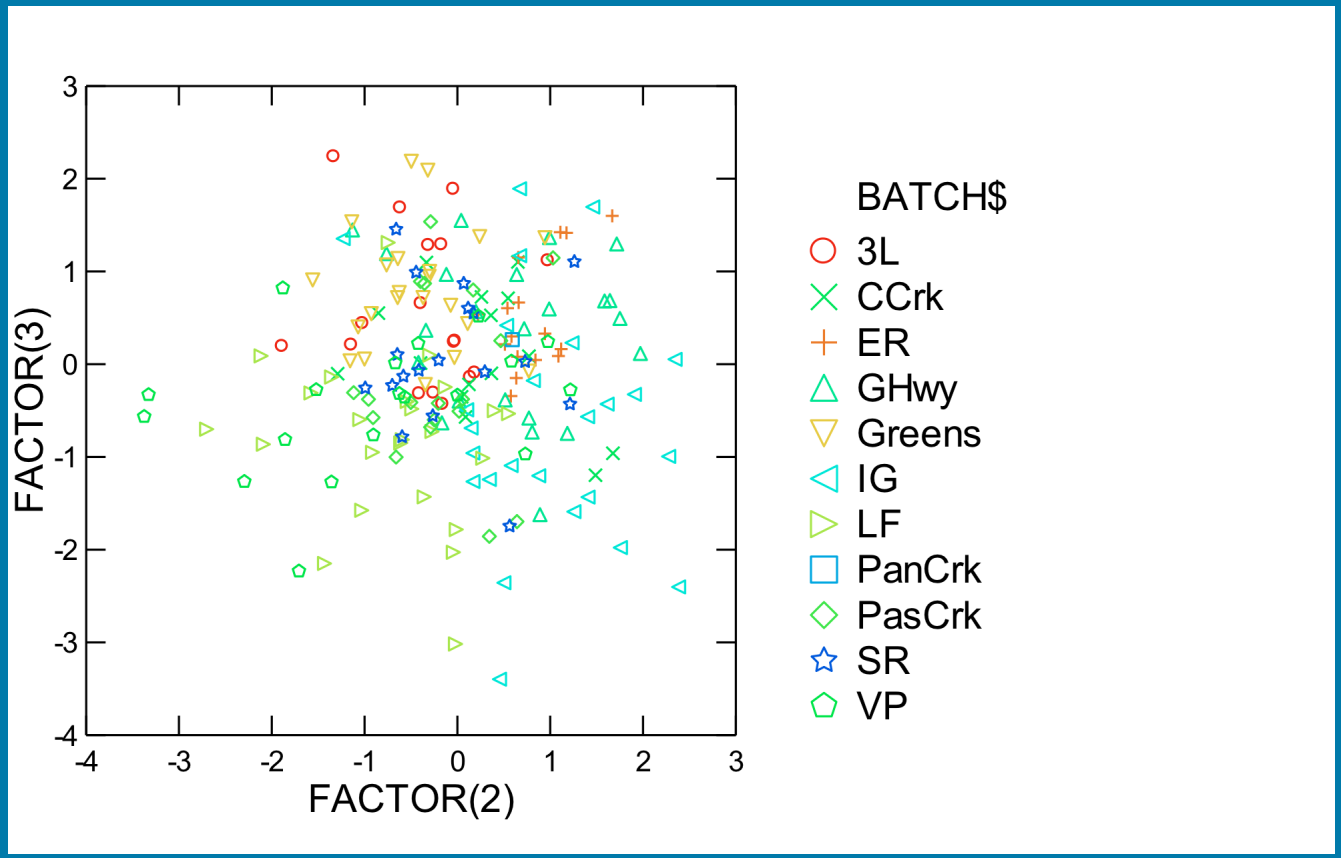


Morphology and Anatomy

- Morphology
  - Used mantle digital images (more reliable method than measurements from the small and fragile shells themselves)
  - Estimated extent and pattern of black pigment – an apparently significant character and possibly a useful field mark
  - Devised set of standard measurements to be taken from shell images
  - Large number of shell characters examined
- Anatomy
  - Used reproductive characters less susceptible to the effects of age, preservation, and individual variation
  - Large number of anatomical characters examined

- No significant differences among populations

PCA for *Oxyloma* samples for second and third components

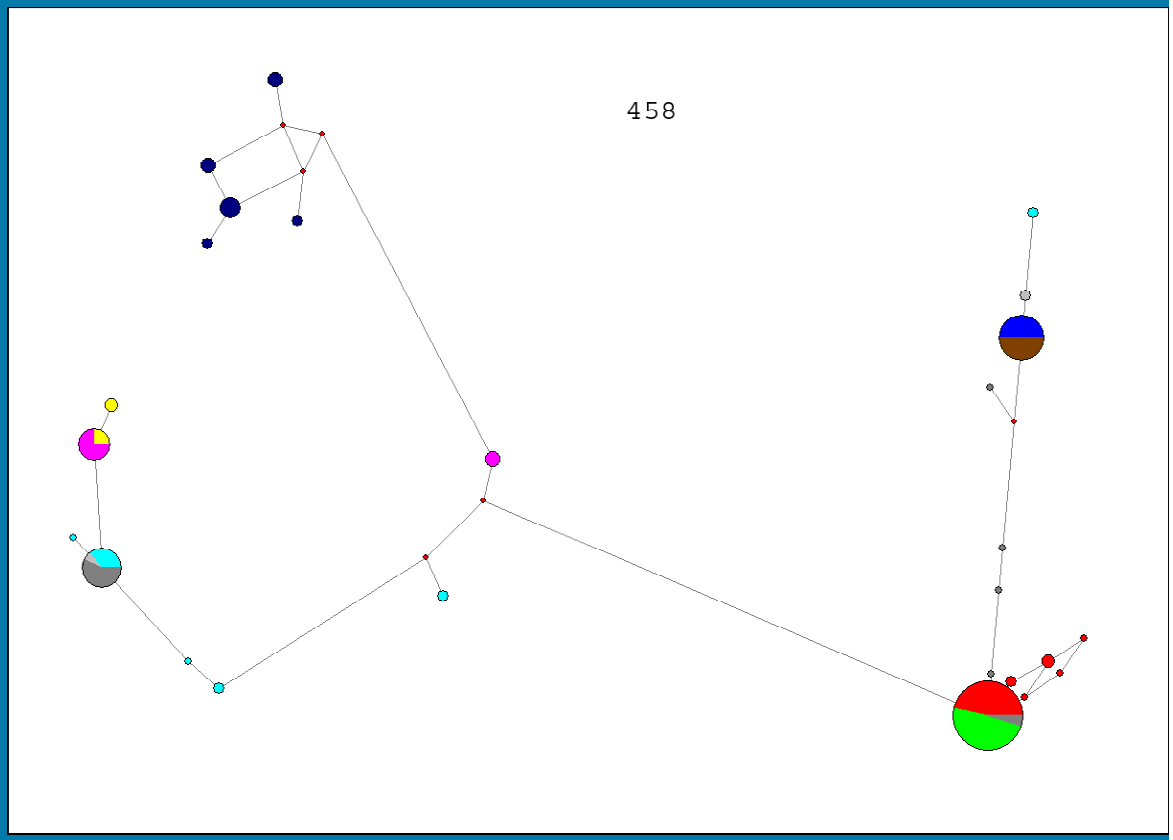


Conclusions

- Extensive gene flow observed among *Oxyloma* populations in AZ and UT
- One taxonomic group (species-level) indicated
- Evidence for short and long distance dispersal
- How to explain dispersal?
  - Short distance may be river flow mediated (Lee's - Vasey's)
  - Long distance may be bird transport (highly divergent lineages found in same geographic location)
- Unique dispersal strategy
  - Oxyloma are hermaphrodites
  - Oxyloma are capable of self fertilization
  - One immigrant can found a population
  - Genetic bottlenecks (and drift) are probably a common occurence

Additional support - 458 Network

- 3 Greens "outliers"
  - clustered with Canada
- 2 - 4 "hubs"
  - Where more lineage mixing occurred



Conservation Implications

- Populations should continue to be monitored for stability/decline in numbers
- Important to protect habitat to allow dynamic process of colonizations to continue
- Managed as one species group
  - Metapop. with historical extinctions/ recolonizations?
  - Ongoing colonizations?

Implications for ESA

- Genetics Policy states:
  - Genetic differences must be addressed during the listing process to determine the taxa being listed
  - No further revision of taxa is allowable, due to genetic data

- Under this policy of no revisions - only delisting or re-listing as different taxa is allowable.

Questions?

Museum samples from Canada

- *Oxyloma haydeni* (long distance dispersal)
- *Oxyloma retusa* (outgroup)
- *Oxyloma nuttalina* (outgroup)

Mitochondrial DNA, all Canada samples added to current samples

- Supports theory of bird transport
  - Canada *haydeni*
    - Greens
- Outgroup species are ingroups
  - *Oxyloma retusa*
    - Greens, Escalante
  - *Oxyloma nuttalina*
    - Greens, 3 Lakes