Developing Microsatellite Primers for Cave Amphipod *Gammarus minus*

Andrew Frank
American University Department of Biology
Just who is *Gammarus minus*?

- Small, shrimp-like crustacean
- Freshwater dweller
- A *detritivore*
- Present in cool springs
- Present in cave streams and subterranean freshwater
Elongated Body
Reduced Pigment
Reduced Number of Ommatidia
Elongated Limbs
Elongated Body
How are cave populations related to spring populations?
Alternatively...
"Although these populations are clearly separated physically, ecologically, and genetically, they have not been separated long enough to accrue a high level of COI divergence."

- Carlini et. al., 2009
Can we characterize the population genetics of *G. minus* in a level of detail that reveals relations between the cave and surface population in finer detail?
Moreover, can we investigate the discrepancy between morphological and genetic characterizations of *G. minus*?
Yes, we can apply microsatellite genetic markers.
Microsatellites are...
Microsatellites are...

Collections of Repeated DNA Sequence Units
Microsatellites are...

Variable by Number of Units, NOT by Number of Nucleotides
Microsatellites are...

Motifs can be between 2 and 6 nucleotides in length
Where are microsatellites in the genome?
This means microsatellites...

1. Evolve *neutrally*
2. Have very variable length
3. Can be abundant in NCRs
The most challenging aspect of using microsatellite data is isolating them *de novo* from newly examined species.
Varies per species, and can vary per population.
Therefore, we have to sample from both cave and spring populations to obtain generalized NCRs.
To isolate microsatellites, you must screen genomic libraries with appropriate DNA probes, and then isolate the regions which positively match the probe’s tandem repeats.
(CATA)$_6$ - CATACATACATACATACATACATA

(CAT)$_8$ - CATCATCATCATCATCATCATCAT

(ACT)$_8$ - ACTACTACTACTACTACTACT

(GT)$_{12}$ - GTGTGTGTGTGTGTGTGTGTGTGT
Size
Selected DNA Fragments
Successful Sequences?
About a 1-2% Success Rate!
Overview & Conclusions

Fragmenting Genomic DNA and Size Selection → Isolating the Microsatellite Containing DNA Strands → Cloning into Vectors and Scanning for Insert

Limiting Steps
Future Directions?

• Complete the library of microsatellite sequences
• Design primers for a number of microsatellite loci
• Sequence these loci using the designed primers
• Analyze sequences for population genetics thus revealing structure
Thank you for coming today!

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References


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