No Evidence of Heteroplasmy found in Mitochondrial DNA of *Fundulus heteroclitus* and *Fundulus grandis* hybrids

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Introduction

- In most organisms, egg-sperm mechanisms target the mitochondrial DNA (mtDNA) in the sperm, destroying it so only the mtDNA of the mother remains, resulting in a pattern of maternal inheritance.[1]
- Directionality of a hybrid can be detected via mtDNA, as it is typically maternally inherited.[2]
- In very rare instances of hybridization, some egg-sperm recognition mechanisms fail to recognize the gametes of another species, resulting in inheritance of the father's mtDNA and paternal leakage.[3]

Hypothesis

Previous data suggests that a small number of hybrids of *Fundulus heteroclitus* and *Fundulus grandis* inherit mitochondrial DNA from both parents, resulting in potentially heteroplasmic offspring. This investigation seeks to answer two questions

- Can evidence of heteroplasmy in hybrid offspring be confirmed?
- Does heteroplasmy occur equally in both directions, or is more common when a certain species is the mother?

Methods

Cytochrome-b Sequencing

11 samples were amplified via PCR and purified, and were sent to GENEWIZ for sequencing. Upon return of the results, sequencies were aligned, visually inspected, and analyzed using Sequencher, DNAsp, and Fabox haplotype collapser.

Restriction Digest

87 samples left over from the Barbas and Gilg (2018) study that were known to be hybrids with known mothers were amplified via PCR and underwent a restriction digest using Nsi-I, which cuts the allele characteristic of one species and not the other.

Directionality

A G test of independence was used to determine significance in directionality.

Results

Sequencing of Cytochrome-b Gene Shows No Evidence of Heteroplasmy

A

Total Base Pairs	468
Polymorphic Sites	30
Halotypes	3
Haplotype Diveristy	0.345
Nucleotide Diversity	0.01166

B

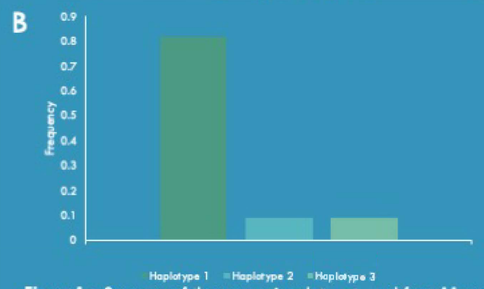

Figure 1 – Summary of the sequencing data returned from 11 successful samples. Panel A shows values obtained from sequences, including the haplotype and nucleotide diversity. One haplotype is more common than the others found, which is reflected in the value and illustrated in Panel B, where the frequencies of the three haplotypes are plotted.

A

B

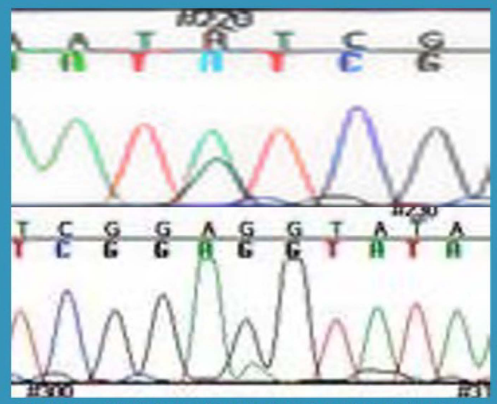

Figure 2 – Comparison of heterozygosity and observed results. Panel A is a sample of a chromatograh from lactate dehydrogenase B, a nuclear locus. It displays a clear example of heterozygosity, showing a clear "double peak" pattern characteristic of overlapping nucleotide bases. Panel B shows an example of observed results from the mitochondrial locus cytochrome b, with no cleanly overlapping peaks. All returned sequences were reviewed in search of heteroplasmy, but of 22 forward and reverse sequences combined together to make 11 complete sequences, no overlapping peaks indicative of heteroplasmy were found.

Restriction Digest of Cytochrome-b with Nsi-I Shows a Strict Pattern of Maternal Inheritance of mtDNA in Hybrids

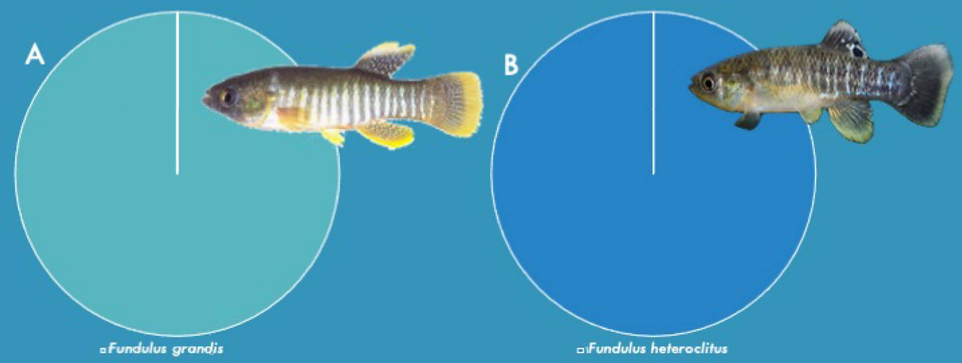

Figure 3 – Restriction digest data shows that in all cases examined, the genotype of the mtDNA of the offspring was the same as the mother. (A) The tanks containing hybrids created from a cross with a *Fundulus grandis* mother and a *F. heteroclitus* father created offspring with mtDNA 100% characteristic of *F. grandis* (n = 38). (B) The same trend holds true for hybrids yielding from tanks with a *F. heteroclitus* mother and a *F. grandis* father, as they all contained mtDNA characteristic of their mother (n=44). This data indicates that these hybrids show a strict pattern of maternal inheritance of mtDNA, meaning that the directionality of a hybrid can be reliably determined via a restriction digest of their cytochrome-b gene. A G-test of independence was performed on these results and verified significance, returning a G value of 0, less than the P value of 1.

Conclusions

Question 1: Can evidence of heteroplasmy in hybrid offspring be confirmed?

- No heteroplasmy was detected in any individuals.
- The restriction digest data showed uniparental inheritance, and the sequencing data showed no heteroplasmy, supporting each other.
- The previous results suggesting heteroplasmy was taking place and prompting this investigation seem likely to have been caused by faulty enzymes or an incomplete digest.

Question 2: Does heteroplasmy occur equally in both directions, or is it more common when a certain species is the mother?

- No heteroplasmy was found during this investigation, so it is impossible to answer this question fully.
- However, *Fundulus grandis* and *Fundulus heteroclitus* hybrids' had genotypes matching that of their mother, suggesting a strict pattern of maternal inheritance of mtDNA.
- Therefore, the directionality of crosses can be determined reliably by examining the mtDNA, as offspring will match the mother.

References

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