Determine Phytoene Desaturase Gene Copy Number in *Hydrilla verticillata*

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Introduction

- *Hydrilla verticillata* is an invasive aquatic species that has infested water bodies in 21 states in the U.S.[1] (Fig. 1).
- U.S. hydrilla is called "the perfect aquatic weed" for its ability to reproduce asexually and grow rapidly in a wide range of environment, causing millions of dollars worth of economic loss and damage[2] (Fig. 2).
- Fluridone herbicide targets the enzyme phytoene desaturase (PDS), which is essential for photosynthesis. Fluridone treatment causes bleaching and halts growth in hydrilla[3].
- Repeated and extensive use of the herbicide fluridone has lead to fluridone resistance in Florida hydrilla[4].
- Resistant hydrilla exist as diploid, triploid, and tetraploid plants[5].
- Puri et al. 2007 reported that fluridone resistance in hydrilla exists along a continuum from low to high[5].
- We hypothesized that amplification of the PDS gene, either through genome duplication (polyploidy) or PDS duplication into other regions of the genome, can explain this continuous range of fluridone resistance.
- We asked two questions:
  1. Does PDS copy number correspond to the ploidy level?
  2. Is there evidence for PDS gene amplification in resistant hydrilla?

Methods

1. Karyotyping for determining ploidy level of standard sample from Texas
   - Hydrilla root tips were collected during a period of high cell proliferation activity, pressed to spread out the chromosomes, and stained (Fig. 4).
   - Chromosome number of each cell were counted under the microscope.
2. Genome walking method for determining PDS copy number
   - DNA was extracted from leaf tissue.
   - PDS primers annealing to a region 60-70 bp away from each end of the PDS cDNA sequence were designed via Primer3.
   - Genome walking was performed as shown in Fig. 3.
3. Quantitative real-time PCR (qPCR) for determining PDS gene copy number
   - Microsatellite genotyping was used to tentatively predict ploidy levels.
   - 18S ribosomal RNA was chosen as the housekeeping gene.
   - Primers for 18S rRNA and PDS were designed via Primer3.
   - qPCR reactions were run in triplicate.
   - $2^{-\Delta\Delta Ct}$ method was used to determine the relative PDS gene copy number
   - One-way ANOVA with a Tukey post-hoc test was used to compare PDS copy number between hydrilla populations.

Results

- The ploidy level of the standard Texas sample was confirmed as triploidy.
- Products larger than 400 bp from the genome walking method were sequenced but there was no match with the ends of the PDS gene.
- PDS copy number was significantly higher in diploid hydrilla than triploid hydrilla (Fig. 5).
- There was no significant difference between Texas (susceptible) 3n and Florida (resistant) 3n hydrilla (Fig. 5).

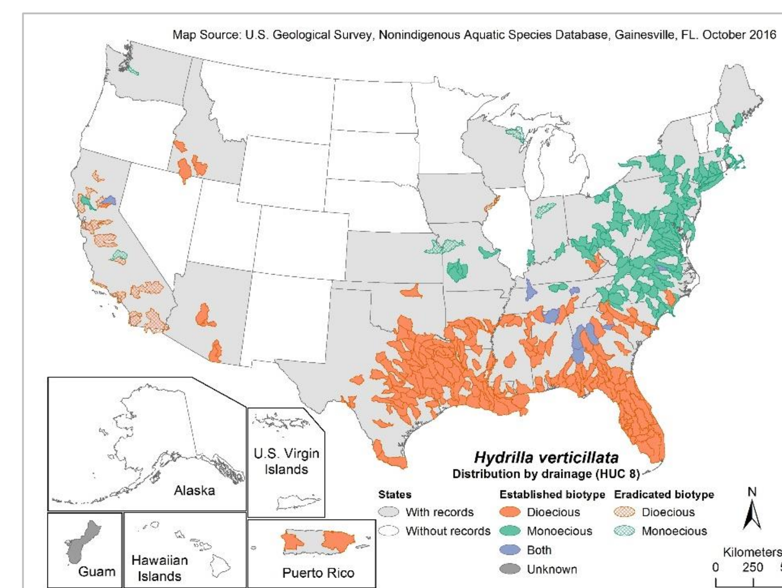

Figure 1. Hydrilla distribution map (2016). Two different biotypes are found in the U.S.: female dioecious (plants with female flowers only) & monoecious (plants with both male and female flowers).

Figure 2. Hydrilla outcompetes native species, blocks irrigation canals, disrupts fishery communities, increases sedimentation and flooding, and impairs water supplies.

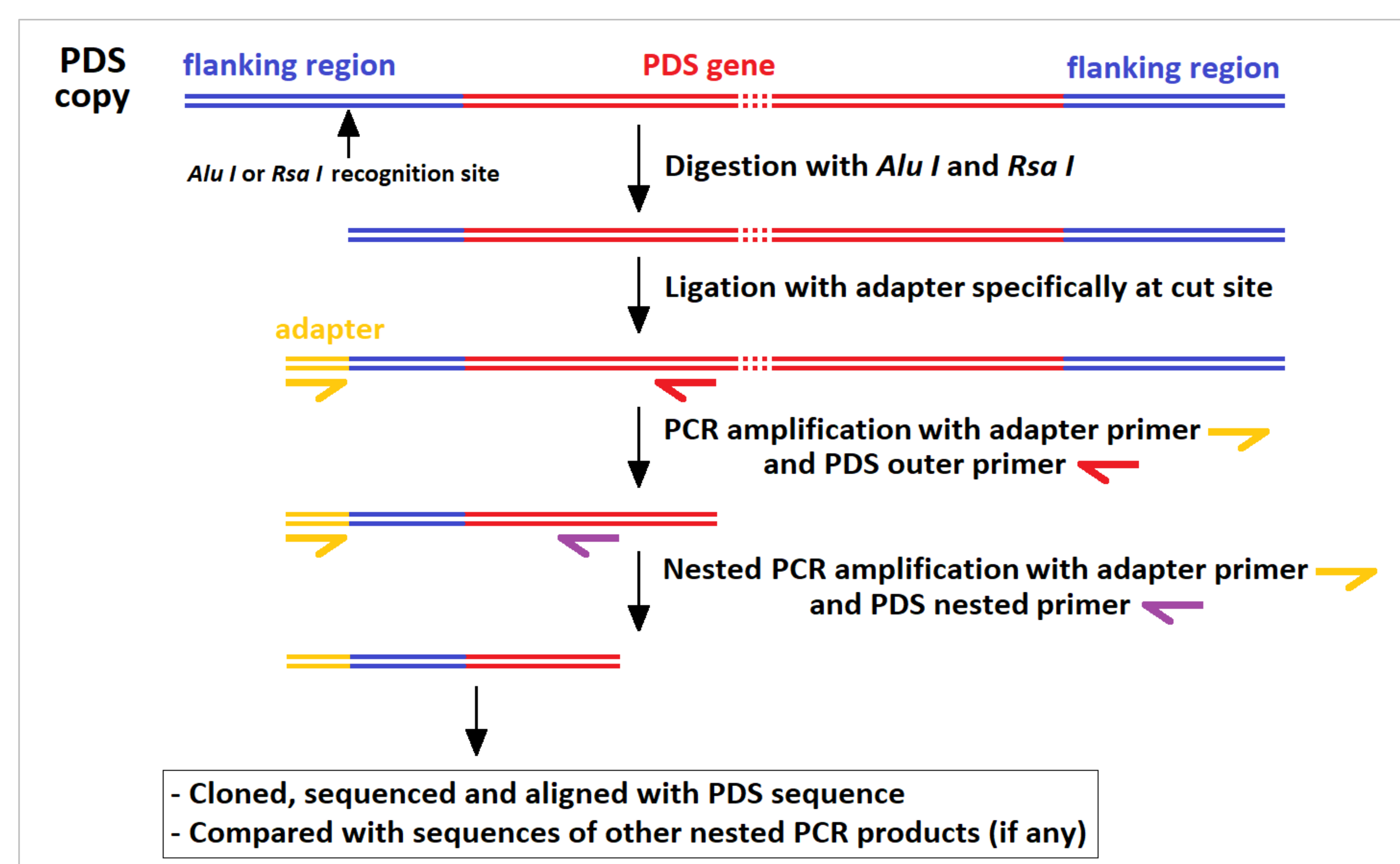

Figure 3. Step-by-step genome walking. PCR products were retrieved through gel electrophoresis. Diagram is not to scale.

Figure 4. Chromosome spreading.

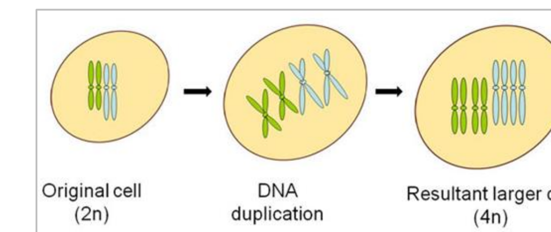

Figure 6. Endopolyploidy or endoreduplication.

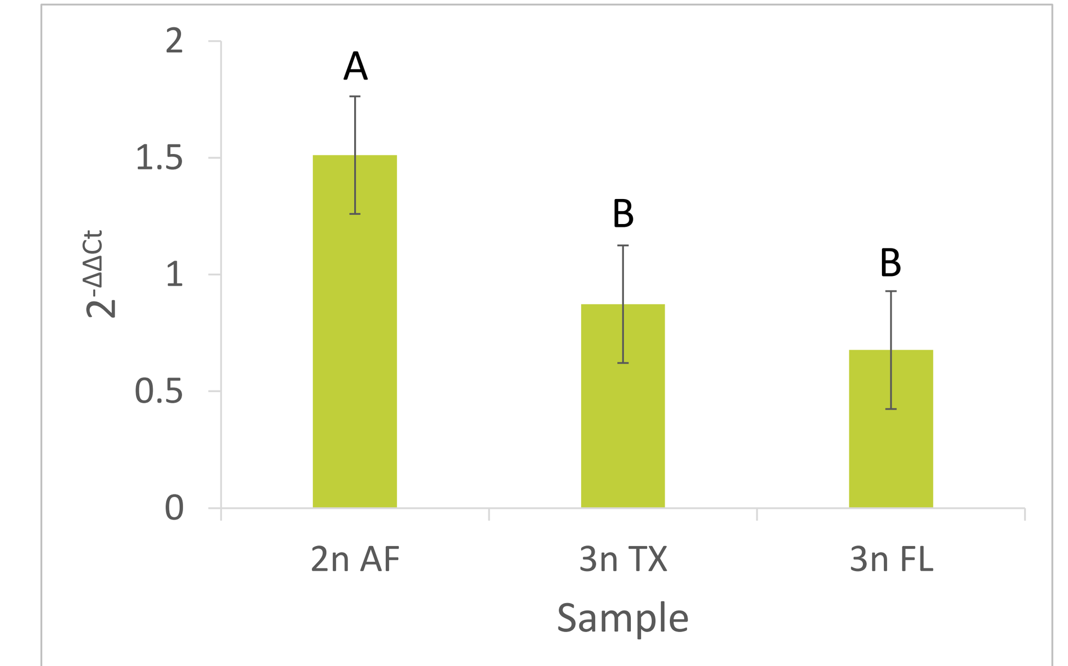

Figure 5. Mean $2^{-\Delta\Delta Ct}$ values with standard error of the populations: Africa diploid (2n AF), Texas triploid (3n TX, susceptible) and Florida triploid (3n FL, resistant).

Discussion

- The genome walking method is based on the fact that if the PDS gene gets duplicated and inserted back into the genome elsewhere, then the DNA regions lying before the promoter area may differ from one duplicate copy to another and between the duplicate copy and the parental copy.
- Genome walking did not yield valuable results because it relied on many indeterminable factors due in part to our lack of the entire PDS genomic DNA sequence.
- Endopolyploidy (elevated nuclear DNA quantities resulting from consecutive doubling of the original unreplicated diploid cell[7]) was observed only in diploid hydrilla before and can possibly explain the higher PDS copy number in 2n compared to 3n hydrilla[8] (Fig. 6).
- No significant difference in PDS number between FL and TX 3n hydrilla suggested PDS amplification was unlikely to be the mechanism behind fluridone resistance. Other possible mechanisms include epigenetic modifications, undescribed PDS mutations, or non-target site resistance factors[5,9,10].
- Future studies should validate the qPCR results via Southern blot analysis or the use of other housekeeping genes and the PDS region for qPCR[11].

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