Using flow cytometry and low-copy nuclear DNA sequences to identify new polyploid taxa in *Isoëtes* (Lycopodiophyta)

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Highlights

- *Isoëtes* (quillworts) can be tentatively assigned to species by screening with flow cytometry and low-copy nuclear DNA sequence data
- Strong correlation between genome size and homeologue copy number (r^2 = 0.78, p < 2e-16)
- DNA sequences clearly indicate diploid progenitors
- Combination of genome size, homeologue copy number, and parentage suggests more polyploid species in e. North America than currently recognized
- DNA sequences from polyploids suggest interactions with unknown diploid species

Methods

Flow Cytometry

Fresh *Isoetes* leaf and two standards (*Raphanus sativus* 'saxa' and *Glycine max* 'Polanka') were chopped in LB01 buffer and stained with propidium iodide. A BD Accuri C6 flow cytometer was used to measure nuclei fluorescence. Fluorescence signals were screened with a FL-2, 580/20 nm-bandpass filter and a FL-3, 670-nm longpass filter. Analysis of unfiltered homogenate was based on light-scatter (SSC-A) vs. fluorescence signals (FSC-A). Genome size was calculated using the equation:

Sample 2C DNA content =
(Sample G1 peak mean / Standard G1 peak mean) X Standard 2C DNA content (pg DNA)

Low-Copy DNA Sequencing

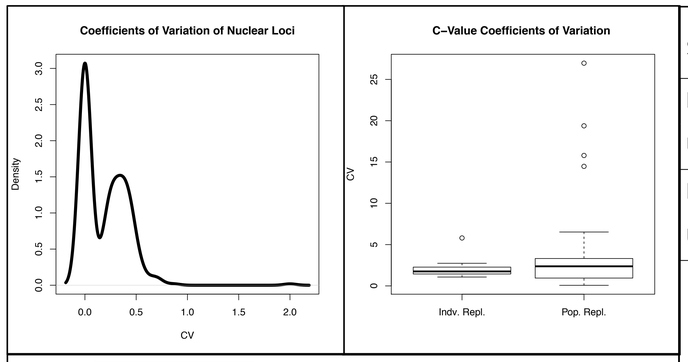
Amplicons were generated by PCR using barcoded primers for *LEAFY* intron 2, *pgiC* spanning exons 12-17, and *IBR3* spanning exons 4-6 and 11-16. Sequences were generated on the Pacific Biosciences Sequel platform and processed using the Pipeline for Unraveling Reticulate Complexes (PURC; Rothfels et al. 2017). MrBayes and RAxML were used for phylogenetic inference.

Hypothetical Species Assumptions

- Unique combinations of diploid genomes are intersterile and arise from independent hybridizations
- Hybrids between different ploidy levels are sterile
- Fixed heterozygosity at homeologue loci
- Each unique combination of diploid genomes and ploidy level represents a species

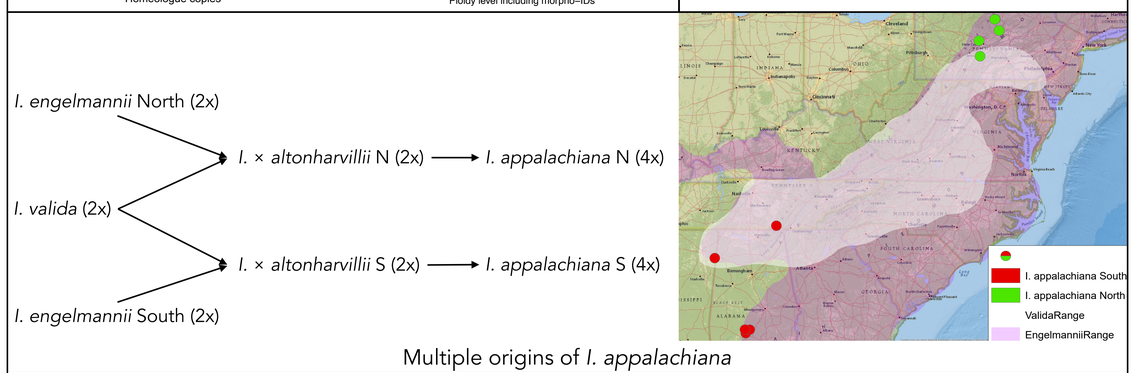
Results

- Individual and population replicates of 2C-values show no significant difference (p = 0.999)
- Within species, most variation in 2C-values is between populations (p = 0.0002, F = 12.9)
- 2C-values and inferred ploidy level showed a strong positive correlation (r^2 = 0.78, p < 2e-16)
- Coefficients of variation (CVs) of gene copies across loci mostly 0
- 21 unique genotype+ploidy level combinations representing hypothetical new species
- New parentage scenarios of polyploids were identified

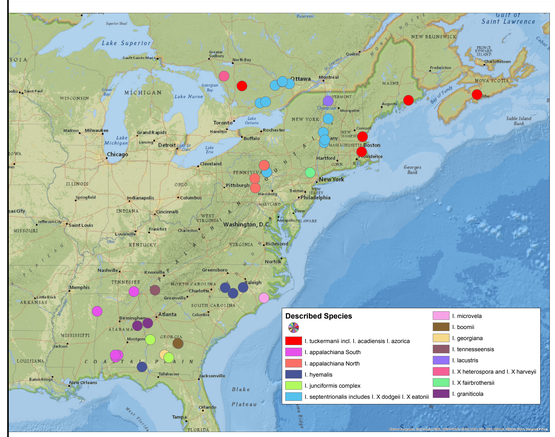


Summary of species in e. North America	
Described Diploids	15
Undescribed Diploids	12
Described Polyploid Species	14
Undescribed Polyploid Species	21

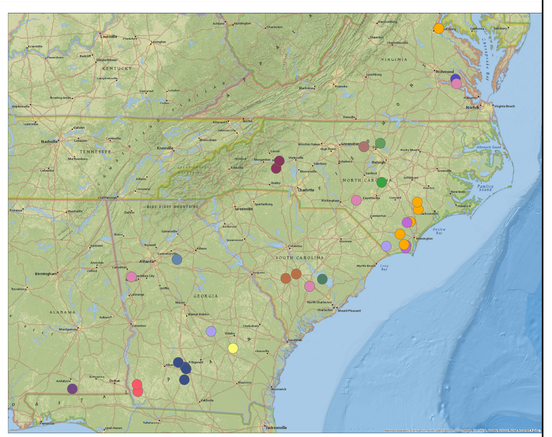
Isoëtes valida (L) and *I. appalachiana* (R)



Multiple origins of *I. appalachiana*



Described polyploid *Isoëtes* in e. North American



Undescribed polyploid *Isoëtes* in e. North America

Conclusions

Broadscale screening using flow cytometry and low-copy DNA sequencing provides a useful method for quickly assessing hundreds of individuals involved in polyploid complexes. In *Isoëtes*, we easily identified mixed-ploidy populations and individuals fitting taxonomic species concepts. This allowed us to focus resources (chromosome counts, NGS sequencing) on samples with ambiguous results.

Acknowledgements

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Rothfels et al. 2017. Next generation polyploid phylogenetics… *New Phytologist*.