Chloroplast DNA reveals uniparental plastid inheritance from *Isoetes engelmannii* in two allotetraploid speciation events

Gaurav S. Kandlikar[α,β], Forrest D. Freund[γ], Gabriel P. Johnson[α], W. Carl Taylor[α], Elizabeth A. Zimmer[α]

Introduction

Green plants are uniquely fundamental as primary producers in all terrestrial ecosystems. Therefore, understanding the origin and persistence of plant species is crucial for biodiversity conservation. Unlike in animals, speciation in plants is commonly driven by hybridization and subsequent chromosome doubling to yield novel allopolyploid species[1].

Isoetes L. is a genus of heterosporous lycopsids comprised of >200 species with a cosmopolitan distribution. Lycopsids with isoetalean characteristics are first found in the fossil record during the Late Devonian, and fossils similar to extant *Isoetes* appear by the Jurassic[2]. Almost 50% of the *Isoetes* species found in North America are polyploids, which suggests that allopolyploidy may be a major cause of speciation. This is especially true in aquatic *Isoetes*, where individuals of various species can cohabitate one body of water[1].

Isoetes spp. growing in braided streams in Manassas, Virginia.

Two North American allotetraploid species of *Isoetes* are *I. riparia* Engelm. ex A. Braun and *I. appalachiana* D.F. Brunt. & D.M. Britton. The putative hybridization pathway, based on distribution, morphology, spore ornamentation, and molecular analyses of these allotetraploid species is shown below[1,3,4].

I. echinospora (2n=22) **x** ***I. engelmannii (2n=22)***

I. x. eatonii (2n=22)

↓ Chromosome doubling

I. riparia (2n=44)

I. valida (2n=22) **x** ***I. engelmannii (2n=22)***

I. x. alton-harvillii (2n=22)

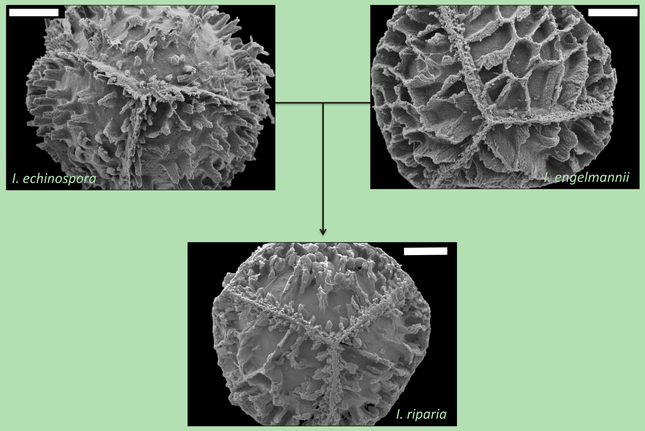
↓ Chromosome doubling

I. appalachiana (2n=44)

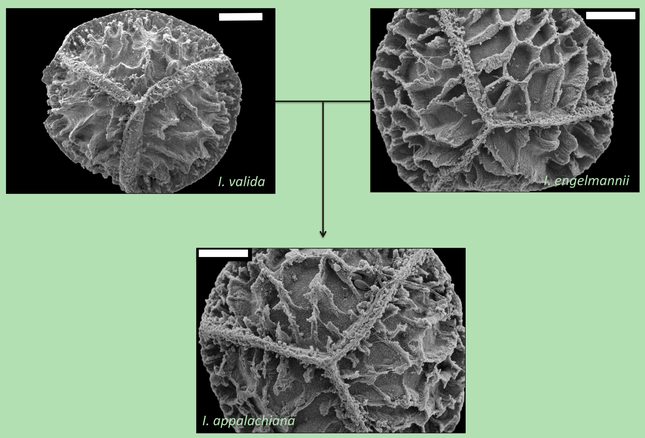
In the present study, DNA sequences were generated for various chloroplast markers to determine which of the parental species was the chloroplast donor (likely maternal parent) to *I. riparia* and *I. appalachiana*.

Spore Morphology

The megaspore ornamentation of *Isoetes* often contains the most diagnostic characters used in species discrimination. The SEM images below show the mature megaspores of the diploid parental and allotetraploid species considered in this study.



Mature megaspores of *Isoetes echinospora, I. engelmannii,* and their putative allotetraploid, *I. riparia*. The spore ornamentations of the hybrid shows characteristics of both parental spores. Bar = 100µM.



Mature megaspores of *Isoetes valida, I. engelmannii,* and their putative allotetraploid, *I. appalachiana*. The spore ornamentations of the hybrid shows characteristics of both parental spores. Bar = 100µM

References, Affiliations, and Acknowledgements

[1]Hoot, S.B., Napier, N.S & Taylor, W.C.. 2004. Am. J. Bot. 91(6): 899-904.
[2]Pigg, K. B. 2001. American Fern Journal 91(3): 99-114.
[3]Brunton, D.F., and Britton, D.M. 1997. Rhodora 99(898): 118-133.
[4]Taylor, W.C., and Hickey, R.J. Ann. Missouri Bot. Gard. 79: 613-622
[5]Geneious version 6.1.6 created by Biomatters. Available from www.geneious.com/

[α]Dept. of Botany, Smithsonian National Museum of Natural History
[β]Contact: gaurav.kandlikar@gmail.com
[γ]Rancho Santa Ana Botanic Gardens

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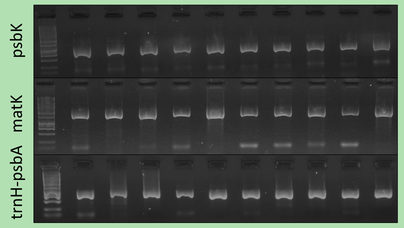
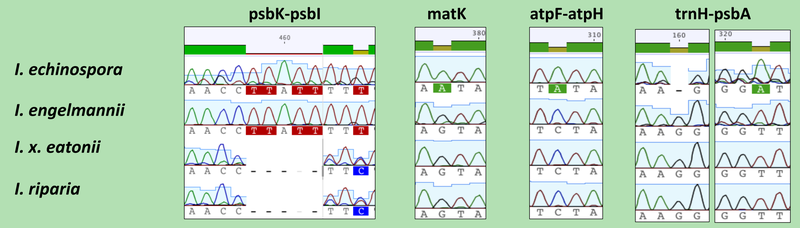
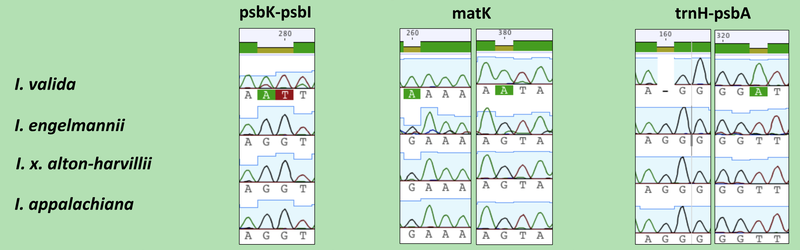
DNA Sequencing Results

DNA was extracted from *Isoetes* leaf tissue using a Qiagen® Plant DNeasy Mini Kit and amplified according to standard PCR protocols. Amplicons were treated with ExoSAP-IT and directly Sanger sequenced using Big Dye. Chromatograms were visualized and edited in Geneious®[5].

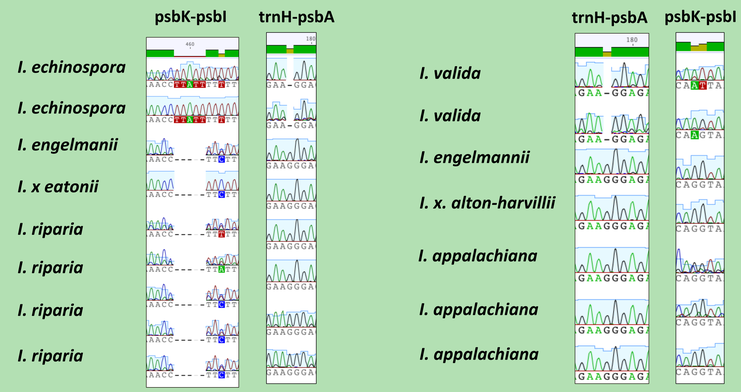




Two indels, two transversions and one transition across four markers separate *I. echinospora* chloroplast DNA from *I. engelmannii*, *I. x eatonii* and *I. riparia*



One indel, two transversions and three transitions across four markers separate *I. valida* chloroplast DNA from *I. engelmannii, I. x alton harvillii* and *I. appalachiana*



Sequencing of additional individuals from other geographically distinct populations support the status of *I. engelmannii* as the chloroplast donor in the allotetraploids

Conclusions

Sequences from four chloroplast regions indicate that ***I. engelmannii* is the chloroplast donor** to the primary hybrids *I. x eatonii and I. x alton-harvillii* and allotetraploids *I. appalachiana* and *I. riparia*. Increased sampling of primary hybrids will determine the frequency of plastid inheritance from *I. engelmannii*. Complete plastome sequencing of basic diploids, putative hybrids, and allotetraploids will confirm unidirectional plastid inheritance.