Hunting for nuclear markers in green algal lineages: Molecular evolution of glucose-6-phosphate isomerase

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Introduction

- The Chlorophyta exhibit a remarkable cytological diversity ➔Fig 1
- Unravelling the evolutionary history of this diverse group is a difficult task, due to:
  - antiquity of the major defining lineages
  - considerable rate variation among lineages
  - scarcity of useful molecular phylogenetic markers

➔ examination of several independent nuclear markers is needed, therefore:

- different genes with known function and sequence data available are tested
- a cDNA library is screened to search for new useful genes

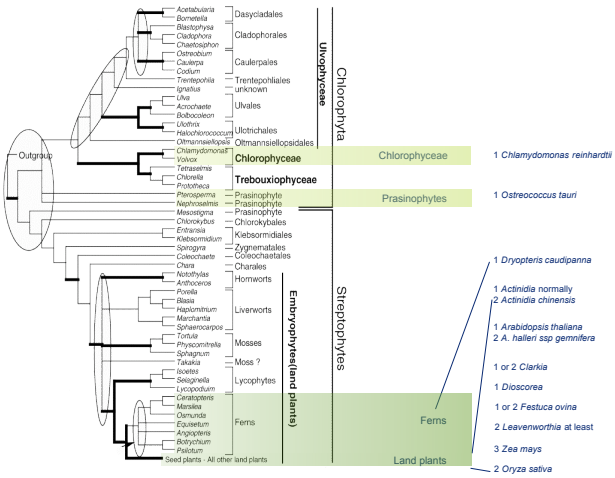


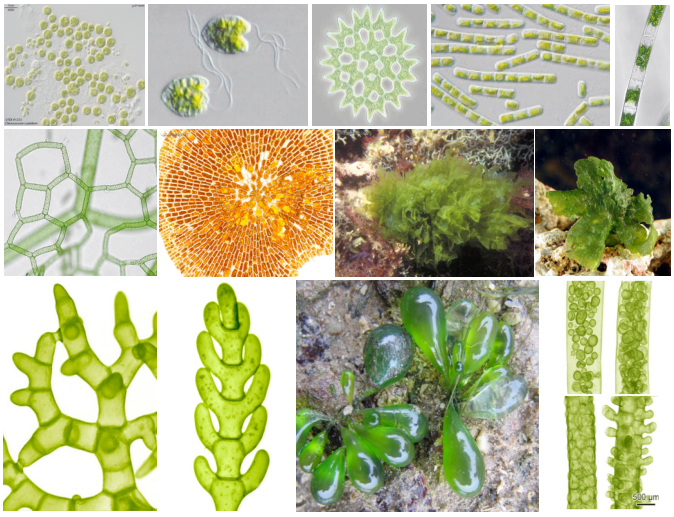
Fig 2: Evolution and copy number of G6PI in the Chloroplastida Tree after D. Mandoli *et al.* Toward resolution of the "Fuzzy Nodes" in green plant phylogeny (http://faculty.washington.edu/mandoli/fuzzy.ph)

Aims of the study

- Identification of nuclear genes useful for phylogenetic reconstruction in the Chlorophyta
- Investigation of the G6PI gene(s) useful as a phylogenetic marker in the Chlorophyta

Methods

- Primer design based on the G6PI genome sequences of *Arabidopsis thaliana, Oryza sativa, Chlamydomonas reinhardtii* and *Ostreococcus tauri*
- DNA extraction➔ PCR➔ Cloning ➔ Sequencing
- RNA extraction➔ RT-PCR➔ PCR➔ Sequencing



Chlorococcum acaberium[1]	Haloshpaera cf. minor[2]	Pediastrum duplex[2]	Klebsormidium flaccidum[3]	Ulothrix sp.[2]
Hydrodictyon reticulatum[2]	Phycopeltis arundinacea[3]	Ulva sp.	Anadyomene wrightii[4]	
Phyllodictyon anastomosans[4]	Boodlea composita[4]	Boergesenia forbesii	Siphonocladus tropicus[4]	

Photographers: 1. www.bio.utexas.edu/research/utex/ 2. Lewis and McCourt 2004 3. www.Algaebase.org 4. Frederik Leliaert

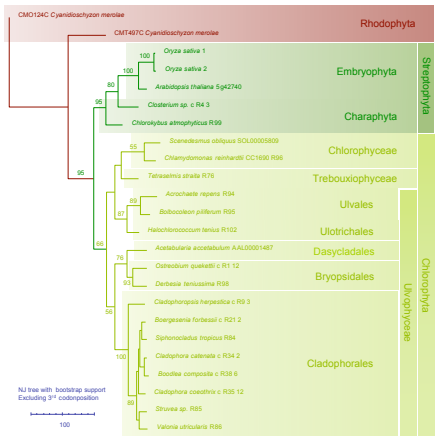
Fig 1: Remarkable cytological diversity in the Chlorophyta ranging from unicellular microscopic algae with a single nucleus, over multicellular filaments and foliose blades, to coenocytic and siphonous life forms that are essentially composed of a giant cell containing thousands of nuclei.

Glucose-6-phosphate isomerase (G6PI)

- Enzyme of the carbohydrate biosynthesis pathway, after the Calvin cycle, in photosynthetic organisms and of the glycolysis in all living organism
- Genome sequences of *C. reinhardtii* and *O. tauri* revealed that both have a single copy of G6PI ➔ Fig 2

Results

- G6PI phylogenetic tree of several green algae



Conclusions

- Epiphytic or endophytic bacteria interfere with direct DNA amplification of nuclear genes
- Information content of G6PI depends on taxonomic level
  - ➔deep phylogenies require exclusion of 3rd codon pos
  - ➔ species level relationships are generally well-supported
  - ➔ introns at fixed positions may offer opportunities towards studies at the intraspecific level
- Most likely only one (functional) copy of G6PI gene is present in the taxa tested

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