

# Phylogenetic analysis of *Heterobasidion annosum* P ISG from regions in North America



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## INTRODUCTION

*Heterobasidion annosum* is an ecologically and economically important basidiomycete pathogen that infects temperate, coniferous forests worldwide. Previously thought to be genetically and pathogenetically uniform, three separate *H. annosum* "intersterility groups" (ISGs) were identified in 1978. Designated P, S and F, these groups are characterized, somewhat loosely, by lack of interfertility, host affinity and morphology. Hybrid S/P ISG *H. annosum* have also been identified and represent one of few fungal pathogen hybrids. Phylogenetic and isozyme studies have shown the three ISGs to be distinct, and in some cases, further separated according to continent. However, the relationships at a finer scale of geographic resolution are less studied.

### Specific Aims

- Use a phylogenetic approach to examine genetic relatedness and biogeographical patterns *within* an *H. annosum* ISG at the continental level; focus is on the North American P ISG.
- Compare trees constructed from nuclear regions with those from mitochondrial regions. Incongruence between organelle and nuclear sequence trees can indicate possible reticulation.

## METHODS

### Isolates:

71 isolates from 17 locations:

- 57 P ISG isolates: 56 from North America, 1 from Europe.
- 14 European F and S and North American S ISG isolates (included for purposes of analysis).
- All European isolates from Aymaville (Valle d'Aosta), Italy.

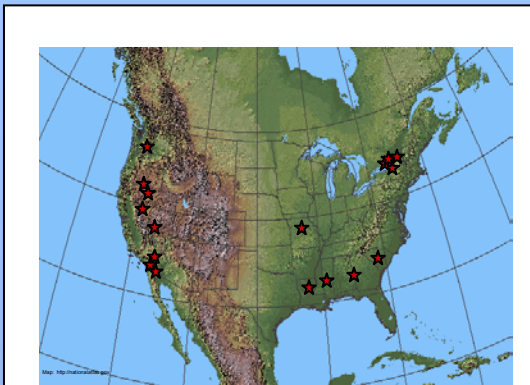


Fig. 1: Distribution of North American P ISG isolates used in this study.

### Regions amplified:

- 1) Elongation Factor 1- $\alpha$ 
  - Nuclear coding region (with non-coding inserts): primers (1).
- 2) ATPase subunit 6 (*atp6*)
  - Mitochondrial coding region: primers (2).
- 3) DNA insertion in the mitochondrial large rRNA gene
  - Mitochondrial non-coding: primers and protocols available upon request.
  - Some isolates have a 1.8 kb insert, some have a 2.0 kb insert and European P isolates have no insert. 1.8 and 2.0 kb inserts do not align.

### Data Analysis:

- Sequences edited and aligned in Sequencher, with manual refinements to alignments performed using Se-AL.
- Trees constructed in PAUP using maximum parsimony methods.
- Gaps coded as missing data, and sequence insertions present in some isolates but not others coded as discrete characters: 0 (insertion absent) and 1 (insertion present).
- As sequences from an appropriate outgroup for all regions have not yet been obtained, all trees are midpoint rooted.
- Consensus trees calculated using "strict consensus" algorithm in PAUP.

## RESULTS

- Multiple, equally parsimonious trees for each analysis.
- Strict consensus trees for each region shown below.
- As 1.8 and 2.0 kb inserts into the mitochondrial large rRNA gene do not align, two trees shown for this region.

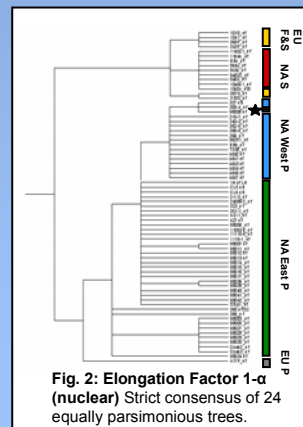


Fig. 2: Elongation Factor 1- $\alpha$  (nuclear) Strict consensus of 24 equally parsimonious trees.

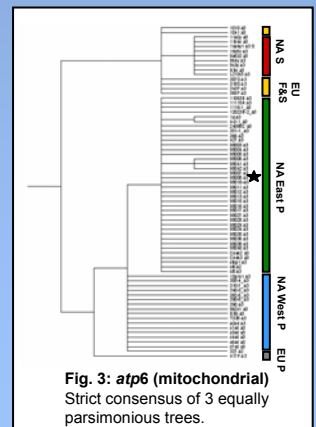


Fig. 3: *atp6* (mitochondrial) Strict consensus of 3 equally parsimonious trees.

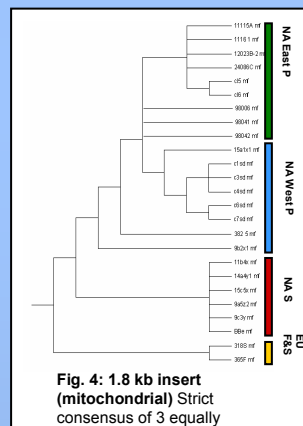


Fig. 4: 1.8 kb insert (mitochondrial) Strict consensus of 3 equally parsimonious trees.

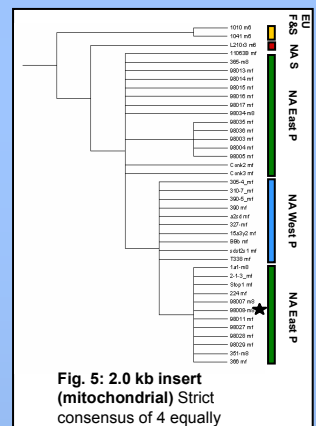


Fig. 5: 2.0 kb insert (mitochondrial) Strict consensus of 4 equally parsimonious trees.

## CONCLUSIONS

- There is evidence of geographic structure within the North American P ISG isolates; isolates divide into "East" and "West" North American clades. No consistent evidence of further geographic structure is indicated by these analyses.
- United States Midwest isolates (Missouri) fall into East North American clades.
- A single Canadian isolate (★) shows evidence of incongruent nuclear and mitochondrial gene trees. It falls into a clade with the East North America isolates for both mitochondrial regions, but into a clade with the West North America isolates in the nuclear region. This isolate will be investigated further for signs of reticulation.

## ACKNOWLEDGMENTS

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## REFERENCES

- <sup>1</sup> Johannesson, H. and J. Stenlid. 2003. Molecular markers reveal genetic isolation and phylogeography of the S and F intersterility groups of the wood-decay fungus *Heterobasidion annosum*. Mol. Phy. and Evol. In Press. Available online at [www.sciencedirect.com](http://www.sciencedirect.com) from May 2003.
- <sup>2</sup> Kretzer, A. M. and T.D. Bruns. 1999. Use of *atp6* in fungal phylogenetics: An example from the Boletales. Mol. Phy. and Evol. 13(3):483-492.