ASSEMBLING THE FUNGAL TREE OF LIFE
National Science Foundation
Assembling the Tree of Life
AToL

PROPOSAL:

*1500 species of fungi for 8 loci ($\approx 10 \text{ kb}$) in 4 years!

6 nuclear loci: nucSSU rDNA, nucLSU rDNA, ITS rDNA, RPB1, RPB2, EF-1$\alpha$

2 mitochondrial loci: mitSSU rDNA, ATP6

*Ultrastructural features common to all fungi
New data and compilation of all existing data

Starting date: January 1, 2003

See the AFToL website for more information
One proposal from the entire mycological community

113 collaborators from 23 countries

Strong bioinformatic & computational biology component

Preceded by an NSF Network Coordination grant “DEEP HYPHA”
“ASSEMBLING THE FUNGAL TREE OF LIFE” (AFToL)

David Hibbett (Clark University): 400 Basidiomycota
François Lutzoni (Duke University): 400 Lichen-forming Ascomycota et al. & Bioinformatics
David McLaughlin (University of Minnesota): Subcellular characters
Joseph Spatafora (Oregon State University): 400 Ascomycota
Rytas Vilgalys (Duke University): 300 Chytridiomycota, Glomeromycota & Zygomycota
AFTOL

- RPB1 - RPB2
- EF1α - ATP6 - mitSSU rDNA
- nucLSU - SSU - ITS rDNA

Clark

morphological data
(SPB, nuclear division, ...)

Duke

Oregon State

U of Minnesota

1500 species

1500 species (SPB, nuclear division, ...)
TWO MAIN OPERATIONS NEEDED FOR LARGE-SCALE PHYLOGENETIC ANALYSES
Essence of AFToL in solving data acquisition issues

- STANDARDIZATION
- COMMUNICATION
- AUTOMATION
Source Information of DNA Samples

DNA SAMPLE

Morpho & Ultrastructural info

Expected phylum

Frozen material info

Voucher info

Culture info

Providers of material/collaborators

Team:
Clark U
Duke L Lab
Duke V lab
OSU
UofM
Entry in DB of source information of DNA + (voucher, cultures, frozen sample, DNA, etc...) + team providing the material + morphological/ultrastructural info + expected phylum

Automated sequencer

Raw sequences output

Blast check point

Phred & Phrap assembler

When sequencing Completed

Locus 1 align  Locus 2 align  Locus 3 align

Initiate NJ bootstrap analyses for set of taxa common to more than one locus

GenBank Weekly

NJ analysis of gene 1 For taxa in common between DS2 and DS3
NJ analysis of gene 1 For taxa in common between DS2 and DS4
NJ analysis of gene 2 For taxa in common between DS3 and DS2
NJ analysis of gene 2 For taxa in common between DS3 and DS4
NJ analysis of gene 3 For taxa in common between DS4 and DS2
NJ analysis of gene 3 For taxa in common between DS4 and DS2

Congruence test

Server #1

Server #2 BLAST (in house)
Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits

That concludes my list of the co-authors of this paper. And now, I see that my time is up.
One goal of this study

Provide a phylogenetic synthesis for the Fungi and a framework for future phylogenetic studies based on previously unpublished data and all sequences available in GenBank
1) What are the loci that have been sequenced for the highest number of fungal species and appropriate for resolving deep relationships within the Fungi? *nucSSU and nucLSU rDNA*

2) A total of 13,467 GenBank sequences were considered

3) 1010 unique taxa had both sequences available
   Taxonomic name associated with the sequence

4) Taxa excluded:
   - < 600 bp
   - Many errors in GenBank “definition line”

3) Unpublished sequences added from various projects and AFTOL
   Total of 573 taxa
1) What is the next locus that has been sequenced for the highest number of the 573 species selected and appropriate for resolving deep relationships within the Fungi?  

*mitSSU rDNA, RPB2*

2) nucSSU+nucLSU+mitSSU = 253 taxa (but only Asco- and Basidiomycota)

3) nucSSU+nucLSU+RPB2 = 161 taxa (only Asco- and Basidiomycota)

4) nucSSU+nucLSU+mitSSU+RPB2 = 103 taxa (only Asco- and Basidiomycota)
Two-locus Bayesian MCMC tree
nucSSU+nucLSU
558 species, 430 genera, 68 orders, 5 phyla

Ascomycota

Basidiomycota

Glomeromycota

Zygomycota

Chytridiomycota
Three-locus Bayesian MCMC tree
nucSSU+nucLSU+RPB2
157 species, 134 genera, 34 orders, 2 phyla
Four-locus Bayesian MCMC tree
nucSSU+nucLSU+mitSSU+RPB2
103 species, 94 genera, 30 orders, 2 phylia
Second AFToL publication?

150 species X 8 loci (10%; 40 lichen species)
October 30, 2004 deadline for new sequences to be included

Data sets are being assembled
WWW based interface to AFTOL database

Secure access for registered users only

Possibility to enter, retrieve and change data

Each author views and modifies only his/her own data

Dynamic web page construction using Zope
<table>
<thead>
<tr>
<th>Taxon Binomial</th>
<th>AFTOL ID</th>
<th>nucSSU</th>
<th>nucLSU</th>
<th>RPB1</th>
<th>RPB2</th>
<th>ATP6</th>
<th>EF1A</th>
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WHERE ARE WE IN ASSEMBLING THE FUNGAL TREE OF LIFE?

560 publications reporting 595 fungal phylogenetic trees: # loci, # species, # orders (Martin Grube, Elizabeth Baloch, Betsy Arnold)

489 (82.2%) trees based on single locus data set
Summary of survey

- 489 (82.2%) trees based on single locus data set
- 83.9% of fungal phylogenies are based exclusively on sequences from the ribosomal RNA tandem repeats.
- Although the number of species included in published trees has generally increased over time, most studies have included fewer than 100 species, with an overall mean of $34.2 \pm 2.3$ species/study (range: 3–1155 species)
- Few studies have focused on resolving relationships among orders of Fungi
- 354 of 595 trees examined (59.5%) conveyed relationships within single orders
Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits

Focus on progress in assembling the fungal tree of life

How can I be an author on the next AFTOL publication?
Inclusion of co-authors in AFTOL publications

- Samples (dry material, cultures, DNA) were provided and DNA sequences were obtained in time to be included in phylogenetic analyses.
- Provided unpublished DNA sequences that could be included in phylogenetic analyses of multi-locus data sets.
- Contributed to the article (e.g., conducting phylogenetic analyses, writing part of ms, ect.)
Graduate students or postdocs can visit Duke University as part of this project to generate molecular data and learn phylogenetic methods.

- Generate data that are essential to the visitor’s and AFTOL respective project (i.e., placement of genera within the Ascomycota)
- Visits are for a minimum of three months
- Visitors to date:
  - Damien Ertz (P. Diederich & E. Sérusiaux)
  - Anja Amtoft (R. Harris)
  - Alexandra Bachran (B. Büdel)
  - Mónica García Otálora (I. Martinez)
Questions

What is AFTOL?