Classification system of ectomychorrhizae fungi

Introduction

Cladistics is a method of phylogenetic systematics which aims to determine an organism's classification, by decent, in a taxonomic system. Cladistics uses synapomorphies, which are shared derived characteristics, between groups of organisms to make decisions about how the group fits into the classification system. For this cladistic study the group of organisms known as ectomycorrhizae fungi has been selected.

Ectomychorrhizal (EM) fungi plays a key role in the growth and development of vascular plants (Allen, 1991; Kendrick, 1992). Much work has been done on classification of the types of associations between the fungi and the host plants (Brundrett, 2004) but much work remains on differentiation of the organisms beyond this level of classification (Wilkinson, 2001). Ectomycorrhizae form symbiotic relationships with woody plants and mychorrhizae abundance and diversity have been shown to have effects on plant growth and survival (Hart and Klironomos, 2002). Recently work has been done on identifying the evolutionary history of ectomycorrhizae common in basidiomycetes using DNA samples of 46 species which yielded 10, 000 equally parsimonious trees (Hibbett *et al* , 2000). A key problem with construction of evolutionary histories of ectomycorrhizae is that most is DNA based rather than morphological or behavioural (Bruns and Shefferson, 2004). This data is needed to confirm that the DNA-based cladograms are correct.

The study will focus on ectomycorrhizal associations with woody plants as these are easy to sample and readily available. Field work will be undertaken

in the summer of 2007 when the ground is soft enough for soil samples. The purpose of the study is to be able to identify EM fungi using non-sexual state morphological characteristics which is a cheaper technique than doing a DNA analysis. Identifying which species associate with which woody plants may be helpful in preparing sites for reforestation and could influence how forest sites are prepared for replanting.

Aims & Objectives

The aim of the study is to further develop the classification system of ectomychorrhizae fungi and to complement other studies done in the field. The focus is to confim that current techniques to construct ectomycorrhizal cladograms using DNA are in agreement with ectomycorrhizal cladograms based on morphological and behavioural characteristics. If there is disagreement between the methods than either more development must be done on distinguishing physical characteristics or the DNA identification methods must be reconsidered.

Context/literature review/background

Much work has been done on identifying EM communities but most of this has been done on either sexual states (early literature) or rDNA internal transcribed spacer (ITS) comparison (Horton and Bruns, 2002). Since early work concentrated on fruiting and spore forms of EM, this covered very little of the total EM which occurred mostly below ground (Horton, 2002). However, advances in molecular technology have given mycologists a new way to determine species diversity and taxonomy of these morphologically

difficult to determine EM. Work is being rapidly done on identifying EM using rDNA sequences and building databases for public use (Kõljalg *et al* , 2005).

Major papers reviewed include:

Wilkinson, D. M. (2001). Mycorrhizal evolution. *Trends in Ecology & Evolution* Vol16(2), p. 64-65.

Bruns, T. D. and Shefferson, R. P. (2004). Evolutionary studies of ectomycorrhizal fungi: recent advances and future directions. *Canadian Journal of Botany* . Vol. 82 (8) p. 1122 – 1133.

Horton, T. R. and Bruns, T. D. (2002). The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* 10, p. 1855–1871

Horton, T. R. (2002). Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale . *Plant and Soil* 244: 29–39.

Taylor AFS. (2002). Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. *Plant and Soil* 244: 19-28.

Methodology

Samples will be taken of root material of 4 different types of trees that are commercially important and readily available. To get a wide sampling of EM 2 softwoods and 2 hardwoods were chosen. It would have been possible to sample simply 1 species repeatedly but a wider range of samples is desirable to build more detailed cladograms. Samples will be taken in spring, mid-summer and fall to try and obtain samples of different morphs of the EM.

Abies balsamea , *Pinus resinosa* and *Picea rubens* were the chosen softwoods for their relative abundance and ease of identification in the field. *Fraxinus excelsior* , *Populus tremuloides* and *Betula papyrifera* were the hardwoods chosen for the same reasons.

Root samples will be collected from a tree of each species growing in close proximity. This will lead to duplicate species samples but will give an overall picture of the species diversity of the area. Typically some EM species will be dominant but some other species should be present. The samples will then be stored in cool temperatures to slow sample degradation while awaiting analysis.

Samples will then be analyzed by locating EM, taking a cutting and sending it to a professional molecular lab for rDNA analysis of the ITS region. The same sample will then be fully morphologically characterized. Using the UNITE database (Kõljalg, U *et al* , 2005) identification of the species based on molecular characteristics will be made. This will be compared to the morphological-based cladistic identification to check if the morphological classification system is valid.

Outline of data collected

None to date.

Further analysis

Further analysis depends on results from the comparison of the morphological based cladogram and the molecular analysis. If the two match then work can be continued to included more sample sites and more species

could be identified. If the comparison is not a match, and this is the expected result from the trial, then a resampling should take place and more work should be done on developing a classification system.

Timesheet

—-This section is not possible for me to complete as I have no idea of the time frame of the project. ——–

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a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytologist. Vol 166(3)p. 1063

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