**North Carolina School of Science and Mathematics (NCSSM) Endophyte Diversity Workshop**

**An outreach activity organized by the Lutzoni lab, Duke University, for the NSF funded Dimensions of Biodiversity project entitled "An interdisciplinary study of hyperdiverse endophytic fungi and their function in boreal forests"**

**http://www.endobiodiversity.org**

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*** Students will need to bring with them lab coats for Part I on March 23, because we will be using bleach.***

**Schedule:**
½ day lab at Duke University (March 23, 2013; 12 - 4 PM) – Workshop part I
Full day lab at Duke University (April 27, 2013; 10 AM - 4 PM) – Workshop part II

**Objectives:**
1. Students will be able to describe endophytes and how they are different from other fungal microorganisms.
2. Students will know where endophytes can be found and how to isolate them from plant tissue in a lab setting.
3. Students will be able to explain why it is important to study endophytic fungi and fungi in general.
4. Students will be able to estimate infection frequencies, and determine if they have sampled sufficiently to characterize endophytic communities.
5. Students will use basic online software tools (e.g., BLAST) on the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/) in an attempt to identify unknown endophyte cultures using their DNA sequences.
6. Students will compare endophytic fungal communities in seedlings versus mature loblolly pine trees.
7. Two selected students will experience conducting research in the Lutzoni lab at Duke University for a period of up to two years.

**Key Vocabulary:**
Ascoma
Ascomycota
Axenic culture
Commensalism
DNA
Diversity
Endolichenic fungus
Endophyte
Endophytic fungus
Horizontal transmission
Hyphae
Leotiomyceta
Lichen
Mutualism
Mycelium
Parsimony
Pathogen
Pezizomycotina
Polymerase Chain Reaction (PCR)
Saprophyte
Secondary metabolite
Species
Species richness
Stomata
Spore
Symbiosis
Vertical transmission

**Workshop Part I (Duke University):**

**Pre-course evaluation (10 min)**

**Introduction to Fungi and Fungal Endophytes (1 hr)**

Instructors will introduce students to the world of fungi and fungal endophytes in a classroom setting. We will briefly cover background biology of fungi and of plants to place the endophytes within a familiar context for the students. Topics to be covered:

A. What are endophytes? They are defined functionally as fungi living within plants or lichens without causing detectable symptoms of disease. Endophytes are known as hyperdiverse, and horizontally transmitted. They have been found in every plant (endophytic fungi) and lichen species (endolichenic fungi) examined thus far. They are increasingly recognized as an accessible but under-exploited trove of ecological, genetic, and functional diversity.

B. What are fungi? What are plants? What are lichens? What are the different groups of fungi? Which subgroup of fungi are endophytic?

C. Are endophytes all found inside plants? Are they found on plants? What parts of a plant do they infect? Are they found on mosses? Ferns? Algae? Lichens?

D. What are endophytes doing in the plant? Are they parasites? Are they saprobes? What are some current hypotheses? We will pass around examples of endophytes.

E. What are endophyte communities like in old and young pine needles? Set up a hypothesis (e.g., endophyte communities will be different between young vs. old leaves).

**Field Trip to Duke campus to collect plants (30 minutes)**

We will go collect plants outside and bring them back to the lab. Describe the plant, approximate age of leaves, etc. We will collect from host plants from which we already have available endophyte DNA sequences, e.g. adult loblolly pine, and pine seedlings. Each student will collect one seedling and 10 needles from one adult pine.

**Lab stations at Duke to culture and learn about lab protocols (1 ½ - 2 hours)**

Students will be divided into two groups and sterilize/cut/plate their plant tissues at the laminar flow hoods (gloves and lab coat required). Students will (1) surface sterilize plant tissues with 100% ethanol, 0.5% NaOCl (2 min), 70% ethanol (2 min), (2) cut one 2 mm section from each needle (10 needles from adults and 10 needles from seedlings) for a total of twenty 2 mm sections, and (3) plate them on 2% MEA plates.

Students will receive instructions on how to take care of their plates and how to analyze infection frequencies for the Workshop Part II.

**<u>Workshop Part II (Duke University; in lab with computers):</u>**

**Discussion of findings from Workshop Part I (1 hr)**

Students will share their findings from Workshop Part I. We will discuss infection frequency, contamination rates, morphological types, species richness, Shannon diversity index, and evenness (based on morphotypes).

We will have a short exercise to understand rarefaction curves using morphotypes as "species". We will divide all the endophytes from all the students into morphotypes and label the plates – morph 1, morph 2, etc. We will randomly pick plates from a box to simulate randomization and derive a rarefaction curve. We will do this three times manually and then online once with the same dataset to see how close our manual rarefaction curve is to the curve generated by a computer's randomization.

We will revisit our original hypotheses with regard to endophytic communities in seedlings *versus* adult pine trees based on the morphotype diversity.

**Brief introduction to molecular biology (30 minutes, Ryoko)**

Students will get an overview on how to extract DNA from fungal cultures and how to amplify and analyze DNA sequences. We will go over Sanger sequencing and the types of molecular markers used in fungal community ecology.

**Brief introduction to BLAST and Sequencher (30 minutes, Emilie)**

Students will get an overview on BLAST algorithms and Sequencher software.

**Lunch Break (1 hr)**

**Computer Activity: Learn bioinformatics tools that help researchers classify endophytes with DNA (2 hours)**

A. Students will be given DNA sequences of endophytes collected from the same Workshop Part I plant hosts (e.g., mature loblolly pines, loblolly pine seedlings). Sequences will be provided by Lutzoni lab researchers.

B. Students will try to determine how many OTUs they have with Sequencher.

C. We will make rarefaction curves and calculate diversity indices to compare the endophyte communities of adult and seedling loblolly pine trees.

D. Students will use BLAST, an online bioinformatics tool, to identify endophytes from their DNA sequences.

E. We will have a discussion of why we use OTUs and genotypes to study endophytes.

F. We will compare our diversity indices and conclusions based on morphotypes *vs.* sequence OTUs.

G. We will review the original hypotheses.

**Presentation of Anita Simha's Data (15 minutes Ryoko)**

**Introduction to research on endophytes (30 minutes, Ryoko, Emilie, Ko-Hsuan)**

Instructors will present briefly (10 minutes each) on why and how researchers study endophytes using Powerpoint presentation slides.

**Wrap-up Assessment: (15 minutes)**

Evaluations will be given out to workshop participants to assess learning and to find students who may be interested in working in the Lutzoni lab.