

Fall 2008 ORCA Mentoring Grant Application

Meloidogyne Phylogeny: A Supertree Approach

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Abstract

Phylogenetic trees allow us to see how closely related different species are by comparing a few genes from many species. While many phylogenies of the genus have been created in the past, none of them summarize relationships across the entire *Meloidogyne* nematode genus because of incompatibility between taxa and genetic datasets. The objective of my research is to merge several phylogenies for different genes in the *Meloidogyne* genus and make a complete phylogenetic tree using a supertree approach. Knowing the phylogenetic relationship will help us to control *Meloidogyne*, an agricultural pest.

Goal and Purpose

Meloidogyne nematodes are agricultural pests that cause about 5% of global crop loss each year (Sasser et al., 1985). *Meloidogyne* enter plant roots using the same path that nitrogen-fixing bacteria use; however, unlike nitrogen-fixing bacteria, they kill the plant once inside. In order to better understand the evolution of pathogenesis and develop more effective control strategies, a clearer image of how the many species of *Meloidogyne* are related is needed. A phylogenetic tree is a hypothesis of the relationship between different species. Phylogenetic trees allow us to see how closely related different species are by comparing a few genes from many species. Although numerous phylogenies of the genus have been constructed in the past, none of them summarize relationships across the entire genus because of incompatibility (incomplete congruence) between taxa and genetic datasets. The objective of my research is to merge several phylogenies for different genes in the *Meloidogyne* genus and make a comprehensive phylogenetic tree using a supertree approach.

Importance of the Project

Numerous phylogenetic hypotheses of the genus *Meloidogyne* have been created; however, each of them sampled different taxa and was constructed using different genes, therefore a single, multigene analysis is not feasible. The tree I am making will be a better representation than existing hypotheses because it will reflect the relationships between all species already sequenced. It will be useful for all elements of the comparative method—coevolution, cospeciation, comparative genomics, phylogeography, biogeography, and character evolution, all of which are important research tools for exploring the evolution of plant parasitism in this genus and devising novel control strategies.

Main Body

Meloidogyne spp. sequence data was obtained from Genbank for the 28S, 18S, and ITS ribosomal DNA gene regions; mitochondrial DNA; and four nuclear genes (RNA polymerase II, dystrophin, EF1 α , and major sperm protein). Since each of these datasets contains different species, it will not be possible to do a single, multigene analysis. Instead, I am going to generate individual phylogenies using each of these datasets and then summarize them with a supertree.

To ensure that all possible sequences available in Genbank are obtained, I have conducted blast searches as well as keyword searches for sequences under several names. Having collected all the sequences, I am now aligning them using Clustal X (Larkin et al., 2007). Occasionally, sequences don't align. In these I will align the reverse-complement of the problem sequences, which will resolve the majority of these difficulties. Sequences that are very short compared to the average sequence are removed, because very short sequences may not align well and may introduce problems into the phylogenetic analysis. I am using PUZZLE v. 4.0.2 (Strimmer and Von Haeseler 1996) to remove identical sequences.

After I'm sure the sequences align properly, I will make parsimony and maximum likelihood phylogenetic trees for each gene. Then I will merge the phylogenies to make a phylogenetic supertree using RadCon (Thorley & Page 2000). This program generates a matrix from each of the individual phylogenies that can be used to create a most parsimonious summary of relationships. If the parsimony and likelihood trees differ, I will perform separate supertree analyses for each method. With the supertree method, I can combine trees that have at least a few overlapping taxa to generate a tree that shows relationships between all taxa.

Null Hypothesis: All of the genes, and each of the treebuilding methods, will produce the same phylogeny.

Alternative Hypothesis: Some of the genes and some treebuilding methods will produce discordant phylogenies, which will likely decrease resolution of the supertree. I will explore the reasons for these differences, but focus on combining them to make a super-tree that will produce a best estimate of their relationships

Anticipated Academic Outcome

This work will appear in a book, Root-knot Nematodes, published by CABI Publishing as a chapter that I will coauthor with my mentor. I will also present this work at the annual meetings of the Society of Nematologists in July 2009.

Qualifications

I have taken classes such as Genomics, Molecular Biology, and Genetics, which allow me to efficiently carry out my project. I have already started the work and have finished collecting the sequences. I have started aligning in Clustal X; the sequences are similar, which is encouraging.

Project Timetable

October 2008—Finish gathering sequences, make sure they align, remove identical sequences

November 2008—Complete making phylogenetic trees; construct supertree

December 2008-April 2009—Work on manuscript and presentation

Fits with BYU's Mission

This project will expand the existing knowledge about meloidogyne relationships. This knowledge will help us to control an agricultural pest.

Sources

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947-2948.

Sasser JN, Carter CC. (1985). Overview of the International Meloidogyne Project 1975–1984. In *An Advanced Treatise on Meloidogyne*. Edited by: Sasser JN, Carter CC. Raleigh: North Carolina State University Graphics; 1985:19-24.

Strimmer, K. Von Haeseler, A. (1996). Quartet puzzling: a Quartet Maximum-Likelihood Method for reconstructing tree topologies. *Mol. Biol. Evol.*, 13: 964–969.

Thorley, J. L., and R. D. M. Page. (2000). RadCon: phylogenetic tree comparison and consensus. *Bioinformatics* 16:486-487.

Mentor's Qualifications

Dr. Adams researches comparative phylogeography, host-parasite relationships, coevolution, molecular systematics, phylogenetic theory, speciation and species concepts, and biodiversity. Last year he published "Phylogeny and evolution," which is an article in the book Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbionts. The project I am working on now is similar this and other research he has done.