EEB 5350. Molecular Systematics. 2 Credits- half-semester module, 24 March-30 April 2014

Lectures: M & W 12:30-1:45 Bio-Pharm 3rd floor conference room
Labs: M & W 2:00-4:00 (first half-hour in conference room, remainder in BioPharm 325).

Instructor: Chris Simon, Biopharm 305D, 6-4640, <chris.simon@uconn.edu>
Graduate Assistant: Russ Meister, Biopharm 325A, <Russell.Meister@uconn.edu>; 6-3947

Readings: will be posted as PDF's.

Handy reference books: 1) Molecular Systematics, 2nd ed. (Hillis, Moritz & Mable, eds. 1996, Sinauer) especially Chapter 11 by Swofford et al. on Phylogenetic Inference; 2) Molecular Evolution: A phylogenetic Approach (Page & Holmes 1998, Blackwell); 3) Inferring Phylogenies (Felsenstein 2004, Sinauer); The Phylogenetic Handbook (eds. Philippe Lemey, Marco Salemi, and Anne-Mieke Vandamme, 2010).

Lecture Goals: The course will focus on the basics of molecular systematics theory and practice **from the point of view of the data**. We will explore the ways in which an understanding of processes of evolution of molecular data can help in the construction of evolutionary trees. Lectures will examine some of the most serious problems in evolutionary tree construction: nucleotide bias, alignment, homoplasy, among-site rate variation, taxon sampling, long branches, big trees, heterogeneous rates of evolution among branches, covarion shifts.

Laboratory Goals: Labs will cover basic techniques in molecular systematics from DNA extraction to sequencing, alignment and cloning. This lab will be of interest to both experienced and novice molecular systematists because we will try newly developed kits/techniques and compare them to older ones.

Short Assignments:
1) For each topic a bibliography will be provided including one focal paper for which the PDF will be posted. Each student will need to turn in a one-page summary of the importance of each focal paper (1 or occasionally 2 papers per week).

2) The week prior to the start of classes you will be given a checklist discussing practical considerations, organization and data checks for molecular systematics. In certain sections you are asked to answer questions and explain how these procedures are modified in your lab.

3) There will be a short "secondary structure alignment assignment" during the semester.

4) Each student will keep a laboratory notebook and hand-in data collected during the course in the form of an alignment and a nexus data file. Various exercises will be performed in laboratory and some will be finished outside of class. These are detailed in the laboratory syllabus.

5) For each Lab, one student will present a 10-15 minute Powerpoint presentation relating to techniques used in that day's lab. Russ will be available to advise you, but use web searches and try to do as much as possible on your own. These Powerpoint presentations will be posted on the class website so that in the future when you teach a molecular systematics class, they can be used as a starting point to revise and develop lectures of your own.

Final Exam: The final exam will be a take home test in which each student critiques the first draft of a paper submitted to Systematic Biology (submitted in the past but making comments as if it were submitted today). Each student will also compare the submitted version to the published version. The answer key will be the actual review containing reviewers, associate editors, and editor's comments (with permission of authors, reviewers and editors) and a list of critical points that need to be considered by the authors.

Lecture Topics by Date (Lab on next pages):

M 24 March
Lecture 1. An introduction to looking at your data: How molecules evolve.
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W 26 Mar
Lecture 2. Homoplasy, The history of molecular systematics, models of evolution, among site rate variation

M 31 Mar
Lecture 3. Problems associated combining data, multiple gene histories for single taxa (Species trees and gene trees)
|
W 3 April
Lecture 4. Choosing partitions, comparing trees

Prior to Class on April 7th there will be a guest lecture/informal seminar in the Bamford Conference Room at 11AM by Prof. **Karl Kjer** of Rutgers University entitled: "A new Insect phylogeny from the 1KITE initiative" **(**The 1000 Insect Transcriptomes Project) Abstract. Despite extensive efforts over the past 50 years, the sequence and timing of many insect relationships remain controversial. We addressed this problem by reconstructing the phylogeny of insects based on phylogenomic data from 1,478 protein-coding genes for 144 taxa and estimated dates of the appearance of all major extant insect groups, using a rigorously validated set of fossil calibrations. Separate phylogenetic analyses using either site-specific rate models or novel domain-specific amino acid substitution models, provide robust and congruent results. Polyneoptera (e.g., stoneflies, earwings, grasshoppers, termites, mantids, roaches) was recovered as a natural group that includes the enigmatic Zoraptera. Lice (Psocodea) were more closely related to Holometabola (beetles, flies, wasps, bees, etc.) than to true bugs (Hemiptera) and thrips (Thysanoptera). Temporal analyses show that insects colonized land simultaneously with plants in the Silurian, rapidly evolved the ability to fly (~ 404-395 Ma), and had already begun to diversify into modern lineages in the Late Carboniferous. However, the extraordinary taxonomic diversity of modern insects appears to have mostly Cretaceous origins. Our phylogenomic analysis provides a uniquely robust temporal and phylogenetic framework for studying the causes and consequences of morphological and physiological innovations in insects and for comparative analyses in the prospering field of insect genomics.

M 7 Apr
Lecture 5. Guest Speaker. **Paul Frandsen**. Kjer Lab, Rutgers University. "Automatic selection of optimal partitioning schemes in phylogenetics" Abstract: Accounting for variations in the rate and pattern of the evolutionary process is an important part of modeling in phylogenetics. One way to improve our ability to do this is to partition a sequence alignment into subsets that share similar evolutionary characteristics and apply independent models to each alignment subset. However, determining the optimal number of subsets and where to assign each site can be difficult. Some of the most commonly used methods rely on *a priori* assumptions about the data

and often fail to account for a substantial amount of evolutionary variation. I will introduce a new algorithm that automatically partitions sequence alignments into subsets of similar sites based on site-wise parameter estimates. I find that this method generates partitioning schemes with drastic improvements in model fit over other commonly used approaches."

Paul's talk will last about 30 min and there will be time for discussion. In the remaining time we will start on the next topic: Secondary structure, structural evolution, & alignment

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W 9 Apr

Lecture 6. Secondary structure & alignment (cont.); Molecular clocks

M 14 Apr

Lecture 7. Long branches, taxon sampling, Felsenstein-zone & anti-felsenstein zone; long branch pruning strategy

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W 16 Apr

Lecture 8. Big Trees, Long Branches, & Simulations

M 21 Apr

Lecture 9: Among Lineage rate variation: nucleotide bias among taxa

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W 23 Apr

Lecture 10: Among Lineage rate variation: Covarion evolution: codon models

M 28 Apr

Lecture 11: ALRV: heterotachy, covarion models; long branch problems, taxon sampling, meaning of "basal taxon"

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W 30 Apr

Lecture 12: Tests of topology and problems associated with nodal support

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Sunday 4th May: Lab project and notebook due. Take Home FINAL EXAM handed out.
Sunday 11th May: Take home final due

Lab Topics by Date:

1- M 24 Mar-

First hour: Data checks at every step. Chris
LAB: Start Nucleospin kit extractions-goes overnight- 30 min
Second hour: Mechanics of Lab; Explanation of class *Tettigades* project. Russ

2- W 26 Mar-

Mini-presentation: DNA extraction- ultrapure to ultradirty, (phenol-chloroform/CsCl gradients to filters to salting out to chelex, etc.) Russ
LAB: Chelex extraction. Finish Nucleospin extractions

3- M 31 Mar-

Before lab, read the introduction to the primer compilation, study the primer comparisons among animals for the COI and COII genes in Simon et al. 1994. And Simon et al. 2006. Mini-presentation: Primer Design- Primer exercise introduction; the beginning of Genious. Russ
LAB: Run extractions on gels. Demonstrate DNA & RNA extraction quantification and the use of

the nanodrop. Homework: Troubleshoot and improve "universal" primers for COI and COII in comparison to four complete *Tettigades* sequences

4- W 3 Apr--
Mini-presentation: The Polymerase Chain Reaction- how it works & optimizing reactions.

LAB: Set-up PCR reaction (mtDNA of *Tettigades* species, COI barcode, two directions), run gel

5- M 7 Apr-
Minipresentation: Different methods for cleaning PCR products for sequencing reactions

LAB: Purify PCR products and set-up sequencing reactions

6- W 9 Apr-
Minipresentation: How Big Dye works, chromatograms, and troubleshooting

LAB: Sephadex and put samples on the ABI; Looking at sequences using Sequencher/Geneious, making contigs, blasting sequences in Genbank

7- M 14 Apr-
Minipresentation: - Cloning DNA

LAB: Cloning- Long Lab.

8- W 16 Apr-
Minipresentation: Depositing sequences in GenBank

LAB: PCR clones/Set up sequencing reactions- Long Lab

9- M 21 Apr-
Minipresentation: Ancient DNA & Museum DNA protocols

LAB: Sephadex and put clone samples on ABI:

10-W 23 Apr-
Minipresentation: Numts

LAB: Compare products with those from PCR with DNA vs cloning template and complete mtDNA sequences.

11-M 28 Apr-
Minipresentation: RNA: extraction and what it can be used for
LAB: RNA isolation- Nucleospin RNA Kit

12-W 30 Apr-
Guest Lecture: **Beth Wade**, Next Gen sequencing applications, Transcriptomics, Rad Tags, Class Discussion on the implications for modeling data for phylogenetic analysis.