



Model-based phylogenetic methods and hypothesis testing, ENTOM 4610

Elizabeth Murray

Spring 2016, 3 credits

I. Course Information

Instructor: Dr. Elizabeth Murray

Office: Comstock 3128

255-2096, em573@cornell.edu

Office hours: Wednesdays 2:30 – 4:00 and by appointment

TA: Peter Lannoo. Peter will be available during part of the lab period and will also be monitoring Piazza. He has experience dealing with big data and computer programming. He'll be available Fridays by appointment to help with student questions, so email him if needed!

Lectures: Tuesdays and Thursdays, 12:20 - 1:10, Comstock B106

Labs: Thursdays, 1:25 - 4:25, Comstock 2123 (Conference Room)

Prerequisite: BIOEE 1780 or BIOMG 2800 or equivalent, or permission of instructor.

Course description:

A variety of disciplines in biological research address questions that rely on a phylogenetic framework for hypothesis testing, including the fields of ecology, epidemiology, behavior, physiology, evolution, and genomics. This course will provide an advanced undergraduate/graduate level introduction to model-based methods of phylogenetic analysis including maximum likelihood and Bayesian methods. The emphasis will be on DNA sequence data and issues associated with reconstructing phylogenetic trees from multiple gene loci. In addition, the course will cover how phylogenies can be used in the context of evolutionary hypothesis testing (including fossil-calibrated phylogenies, character mapping, ancestral state estimation, and historical biogeography) using rigorous statistical methods. The course will include a computer laboratory for performing analyses using real data sets. Beginning skills in R programming will be introduced, and students will build an independent dataset to analyze using the techniques introduced in class.

II. Course materials

Required:

- The Phylogenetic Handbook (Lemey *et al.*, 2009)
Download free electronic editions of these through Cornell libraries, will be utilized during course:
- Analysis of Phylogenetics and Evolution with R (Paradis, 2012)
- Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology (Garamszegi, 2014)

Programs required by first day of class:

- R [<http://cran.r-project.org/>]
- text editor, for example:
 - o TextWrangler for macs [<http://www.barebones.com/products/textwrangler/download.html>]
 - o Notepad++ for PCs [<http://notepad-plus-plus.org/>]

Download for lab work by Jan 28th:

- Analysis of Phylogenetics and Evolution with R (Paradis, 2012)

III. Course Aims and Outcomes

This course is designed to prepare you to assemble a dataset and make the best hypothesis of phylogenetic relationships from the data at hand. Yet there is more to phylogenetics than just estimating the tree. After obtaining the phylogenetic results, you'll learn tools to explore evolutionary patterns and answer macroevolutionary questions. The class is focused on practical knowledge of the analytical methods, with a foundation in the theoretical aspects and background needed to run a chosen analytical package successfully and correctly.

Phylogenetic relationships and evolutionary relatedness are important for many aspects of biological research, so this course material is applicable for all types of careers (not just those who want to enter the field of systematics or phylogenetics). You will be able to apply your skills to your own research and / or to an examination of others' research methods and conclusions. You'll develop skills in scientific presentation and writing. We'll also be exercising valuable skills of critical thinking, problem solving, and communication.

Specific Learning Outcomes:

Outcome 1:

- 1.1 Be able to describe relationships among gene sequences/ individuals/ species based on a phylogeny.
- 1.2 Understand the difference between gene trees and species trees.

Outcome 2:

- 2.1 Learn how to use a variety of programs to analyze data in a phylogenetic framework.
- 2.2 Build an aligned sequence data set and infer phylogenies using various programs.
- 2.3 Assess and discuss issues with evaluating statistical support for relationships.

Outcome 3:

- 3.1 Implement methods for macroevolutionary analyses, including comparative analyses and ancestral estimation.
- 3.2 Determine which tests are appropriate for a specific evolutionary question.
- 3.3 Develop alternative hypotheses of evolution and rigorously examine statistical support for these alternatives.

Outcome 4:

- 4.1 Write questions and hypotheses and be able to evaluate others' research.
- 4.2 Discuss scientific literature with peers and peer-review other classmates' scientific writing.

Outcome 5:

- 5.1 Develop an independent project based on [the student's] own interests.
- 5.2 Build a dataset and run appropriate analyses for the individual project.
- 5.3 Interpret the results of these analyses and communicate findings through scientific writing.
- 5.4 Present results of independent research orally.

Piazza through Blackboard will be available as an online discussion forum. Students are encouraged to answer others' inquiries. You may post anonymously, but it's preferred that you identify yourself. The TA, Peter Lannoo, will also be monitoring the discussion and answering questions. If you think your question will be applicable to the rest of the class, please post it here! We will potentially have you post a comment occasionally for class discussion.

IV. Course format

ATTENDANCE: Please contact me if you foresee any problems in attending class. I will be taking attendance for lectures and labs, and I'll be checking in with you if there are over three absences. This course comprises a lot of interactive learning, so it's beneficial for you to attend classes.

1) TUESDAY LECTURE:

Typically will consist of a powerpoint presentation or interactive work.

2) THURSDAY LECTURE:

Typically will consist of a short lecture, group work, or a discussion on a paper topic.

3) COMPUTER LAB:

Will start with a short lecture or interactive introduction to topic. Each lab will have a homework assignment associated. If you complete the homework in class, you are encouraged to then use any remaining time to implement the techniques learned onto your personal dataset.

3) ANALYTICAL RESEARCH PROJECT:

Ongoing project with components compiled using the programs and skills we learn each week through computer lab and lectures. Individual datasets will be chosen and research activities will culminate at end of semester with a class presentation and a final paper.

- **Presentation:** Oral presentation of 10 min (depending on class size), to take place during the last full week of class. Modeled after a scientific meeting presentation, the details of which will be discussed in class.
- **Paper:** Final paper modeled after a manuscript submitted to a scientific journal, following formatting of 'Molecular Phylogenetics and Evolution'. This includes a title, abstract, introduction, materials and methods, results, discussion, conclusion (optional), and references cited. The use of figures and tables is expected. Additional detail will be given in class.

V. Assignments / Deliverables

DEADLINES: I expect all work to be on time. If you know of something in advance that will cause tardiness for a deadline, contact me. Otherwise, 10% a day will be deducted for late work.

Submitting assignments:

Much of the homework will involve electronic submissions. These assignments should be turned in via Blackboard. Grades will also be available on Blackboard. If assignments need to be emailed to me, make sure to include your last name in the name of the file.

- 1) Lecture quizzes:
There will be 6-7 short quizzes given. They will be open-book and completed over Blackboard. You are not to consult with anyone else or get assistance with answers!
[[These quizzes are not meant to 'trip you up', but are to keep you up to date on reading, focused on important information, and also show me if there are areas needed for review.]]
- 2) Lab homework:
Each week, there will be homework to be done *individually* and turned in electronically by the following Wednesday.
[[This is meant to be an extension of lab-time learning and will give you a chance to review the lesson and potentially to be more creative with your data / graphics manipulations.]]
- 3) Groupwork (sometime involving preparing a draft or other materials prior to class):
For project development, as we go through the semester there will be group consultation and group feedback / editing of written components of the paper. Some of these will involve turning in summaries or other paperwork.
[[Receiving feedback from colleagues is a useful process for improving your work and clarifying your thoughts or your writing. Also, you will practice providing constructive comments to others.]]
- 4) Final project:
Delivered to class as a short presentation. Students will present on May 5th (May 3rd also if needed)
[[Here is a chance to practice speaking skills as modeled after a scientific conference or perhaps a segment of a job talk. Also, it's exciting to show your research progress to your peers.]]
- 5) Final Paper:
Due on or before **May 16th**, 5:00 pm.
[[Scientific writing is the primary method to disseminate your research and so this paper is modeled on a journal article format. This is a chance to work on a skill useful in future employment!]]

EXTRA CREDIT:

Attend a phylogenetics or systematics seminar on campus and submit a summary of the talk, including your review (educated opinion) of the research and presentation – must be at least 250 words.

Summary is due no more than two weeks after presentation.

Worth 5 points [2 points for summary, 2 points for your review, 1 point for grammar, flow, etc.]

VI. Grading:

Lab Homework:	25%
Project Assignments/Group work:	15%
Quizzes* (6-7 total):	10%
Attendance/Participation/Discussion:	15%
Final Oral Presentation:	10%
Final Paper:	25%

*Quizzes will be **Feb** 4, 11, 18, 25; **Mar** 3, 10, 17, 24. These dates are Thursdays, but the online Blackboard quiz will not be due until Monday morning. Blackboard-based 'quizzes' are open-book. Late quizzes will be accepted, at a reduction of 20% per day.

Explanation of grades

S/U: 70% and above will be given a satisfactory

Letter grades:

A+	>100	C+	77-79
A	93-100	C	73-76
A-	90-92	C-	70-72
B+	87-89	D+	67-69
B	83-86	D	63-66
B-	80-82	D-	60-62

VII. Academic Integrity

Each student in this course is expected to abide by the Cornell University Code of Academic Integrity. Any work submitted by a student in this course for academic credit will be the student's own work. For this course, collaboration is allowed in the in-class computer tutorials. During weekly online quizzes, you must do your own work. Discussion is not permitted, nor may you compare or collaborate in any way.

You are encouraged to discuss information and concepts covered in lecture and the sections with other students. You can give or receive consulting help from such students. However, this permissible cooperation should never involve one student having possession of a copy of all or part of work done by someone else, in the form of an electronic version or a hard copy.

Should copying occur, both the student who copied work from another student and the student who gave material to be copied will both receive a zero for the assignment. Penalty for violation of this Code can also be extended to include failure of the course and University disciplinary action.

Plagiarism [*Latin* plagiarius: kidnapper] will not be tolerated.

VIII. Accommodations for students with disabilities

In compliance with the Cornell University policy and equal access laws, I am available to discuss appropriate academic accommodations that may be required for student with disabilities. Requests for academic accommodations are to be made during the first three weeks of the semester, except for unusual circumstances, so arrangements can be made. Students are encouraged to register with Student Disability Services to verify their eligibility for appropriate accommodations.

IX. Expectations

The instructors will provide -- and expect from students -- an atmosphere for learning that respects diversity. Respect and integrity are paramount. Respect in the classroom includes: being on time for class, directing attention to the speaker, refraining from browsing the internet during class, and other social courtesies appropriate in a community setting.

If you have concerns, questions, or need some guidance, please feel free to contact Elizabeth or Peter. I prefer face-to-face or phone for more detailed questions that require discussion or explanations.

2016 Course Schedule:

May change slightly to accommodate student needs

Please read by first day:
The Tree-Thinking Challenge
(Baum *et al.*, 2005)

1 INTRODUCTION TO SYSTEMATICS AND PHYLOGENETICS

January 28

Lecture:

- Welcome and syllabus
- What are phylogenetic trees? Brief history of tree-thinking, phylogenetic hypotheses
- how to read and write trees, tree structure, systematics

Lab:

- Introduction to R – installing packages, entering data, working with matrices, importing trees and nexus files, plotting phylogenies

Readings for the week:

- The Tree-Thinking Challenge (Baum *et al.*, 2005)

2 ASSEMBLING A DATASET

February 2 & 4

Lecture:

- Traditional gene selection, primer design, and sequencing
- Assembling a dataset –which genes, how many genes, different data types used to build trees

Lab:

- BLAST searches; GenBank, accessing GenBank sequences through R
- Dryad, TreeBase; nexus, newick, XML, Phylip, FASTA formats
- Assembling and annotating dataset, nexus command block; SequenceMatrix, Mesquite

Readings for the week:

- Chapters 1- 3, The Phylogenetic Handbook (Lemey *et al.*, 2009); (focus on 1 & 3);for Ch 2-3, only the “Theory” section is required and not the “Practice” section at the end of each chapter
- [Skim for lab] Ch. 1-2, Analysis of Phylogenetics and Evolution with R (Paradis, 2012)

Programs to download for lab:

- Mesquite v3.04 [<http://mesquiteproject.wikispaces.com/installation>]
- SequenceMatrix v1.8 [<http://gaurav.github.io/taxondna/>]
- FigTree v1.4.2 [<http://tree.bio.ed.ac.uk/software/figtree/>]
- Make sure you have java installed! v8 [<http://java.com/en/download/>]

3 HISTORY OF PHYLOGENETIC METHODS, PARSIMONY, AND THE TREE-BUILDING PROCESS

February 9 & 11

Lecture:

- Philosophical framework of systematics; History of parsimony and distance methods
- Optimality criteria, branch swapping
- Measures of branch support – bootstrap, jackknife; Types of consensus trees

Lab:

- calculating sequence distances, parsimony tree-building
- Intro to PAUP

- Introduction of Final project: scientific writing, journal format, generating topic ideas, timelines

Readings for the week:

- Chapter 2, Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology (Garamszegi, 2014)
- Chapters 5 [skim] & 8 [read] “Theory” sections, The Phylogenetic Handbook (Lemey *et al.*, 2009)

Programs to download for lab:

- PAUP (GUI test version) [http://people.sc.fsu.edu/~dswofford/paup_test/]

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NUCLEOTIDE SUBSTITUTION MODELS & CRITERIA

February 18

Lecture:

- Models of sequence evolution, nucleotide substitution model selection
- Criteria for choosing best model fit

Lab:

- jModelTest2
- Introduction to likelihood framework

Readings for the week:

- Chapters 4 and 10 “Theory” section, The Phylogenetic Handbook (Lemey *et al.*, 2009)
- jModelTest: phylogenetic model averaging (Posada, 2008) [skim]
- PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses (Lanfear *et al.*, 2012) [skim]

Programs to download for lab:

- jModelTest v2.1.7 [<https://code.google.com/p/jmodeltest2/wiki/Download>]
- PartitionFinder v1.1.1 [<http://www.robertlanfear.com/partitionfinder/>]

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USING ML METHODS

February 23 & 25

Lecture:

- Maximum likelihood

Lab:

- how to calculate ML on tree
- Likelihood-based tests and phylogenetic reconstruction

Readings for this week:

- Chapter 6 “Theory” section, The Phylogenetic Handbook (Lemey *et al.*, 2009)
- Phylogeny estimation and hypothesis testing using maximum likelihood (Huelsenbeck & Crandall, 1997)
- Model Use in Phylogenetics: Nine Key Questions (Kelchner & Thomas, 2007)

Programs to prepare for lab:

- set up account to access CIPRES website [<https://www.phylo.org/portal2/login!input.action>]

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BAYESIAN INFERENCE

March 1 & 3

Lecture:

- Everything Bayesian, from prior/posterior probability distributions to MCMC
- Demonstrations using Paul Lewis' MCRobot [<http://phylogeny.uconn.edu/software/>]

Lab:

- MrBayes
- Tracer for visualizing results

Readings for the week:

- Chapter 7 "Theory" section, The Phylogenetic Handbook (Lemey *et al.*, 2009)
- Phylogeny Estimation (Holder & Lewis, 2003)
- **Recommended** as an entertaining overview: The State of Bayesian Phylogenetics: Bayes for the Uninitiated (Brown, 2003)

Programs to download for lab:

- MrBayes 3.2.6 [<http://mrbayes.sourceforge.net/download.php>]
- mac users must also download BEAGLE for MrBayes to work—see help information for link
- Tracer v1.6 [<http://tree.bio.ed.ac.uk/software/tracer/>]

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TESTING TREE TOPOLOGY, TROUBLESHOOTING A PHYLOGENETIC TREE

March 8 & 10

Lecture:

- Extending MrBayes - reversible jump MCMC and stepping stone analyses
- Statistical measures to assessing trees – alternative trees, harmonic means – BF (constraint tree)
- Supermatrices and issues with large datasets, impacts of **missing data**
- Discussion of "Ribosomal protein genes of holometabolan insects reject the Halteria, instead revealing a close affinity of Strepsiptera with Coleoptera" (Longhorn *et al.*, 2010)

Lab:

- Gblocks – removing noise in alignments

Readings for this week:

- Chapters 9&12 "Theory" section, The Phylogenetic Handbook (Lemey *et al.*, 2009) [skim]
- Phylogenomics resolves the timing and pattern of insect evolution (Misof *et al.*, 2014) [look through SI for many different tests / analyses they ran] [skim]

Programs to download for lab:

- None, all are available through web interfaces

TIMETREES

March 15 & 17

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Lecture:

- From substitutions/site to absolute age – calibrating a tree
- Fossils, issues with dating, types of calibrations, practical considerations, rate heterogeneity

Lab:

- BEAST 2

Readings for the week:

- Chapters 11 & 18 “Theory” sections, The Phylogenetic Handbook (Lemey *et al.*, 2009)
- Relaxed molecular clocks for dating historical plant dispersal events (Renner, 2005)
- Beyond fossil calibrations: Realities of molecular clock practices in evolutionary biology (Hipsley & Muller, 2014)
- **Recommended:** BEAST: Bayesian evolutionary analysis by sampling trees (Drummond & Rambaut, 2007)

Programs to download for lab:

- BEAST2 [<http://www.beast2.org/>]
 - o BEAST download includes the programs: BEAUTi, LogCombiner, TreeCombiner, and DensiTree
 - o There is also a (previously-free) draft of the book BEAST2 – get that from Blackboard! It covers theory and tutorials, and is available now as a published book for a fee.
- **Optional** – BEAGLE [<https://code.google.com/p/beagle-lib/>]
 - o used to speed up the BEAST analysis, but not necessary to implement for our lab

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CHARACTERS – MAPPING, ANCESTRAL STATE ESTIMATION

March 22 & 24

Lecture:

- Analyses and programs for ancestral state reconstruction, overview of current methods
- Character evolution
- cophylogenetic analysis, if interest?

Lab:

- Mesquite and BayesTraits for ancestral state reconstruction

Readings for this week:

- Chapter 1&10, Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology (Garamszegi, 2014)
- Effects of phylogenetic signal on ancestral state reconstruction (Litsios & Salamin, 2012)

Programs to download for lab:

- BayesTraits v2 [<http://www.evolution.rdg.ac.uk/BayesTraits.html>]
-

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SPRING BREAK WEEK

March 29 & 31

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HISTORICAL BIOGEOGRAPHY

April 5 & 7

Lecture:

- Guest speaker April 5: **Christian Rabeling**, University of Rochester
- History of biogeography, ancestral area reconstruction methods

Lab:

- BioGeoBEARS

Readings for this week:

- "Biogeography" Chapter 47, Sanmartín; from the textbook 'The Tree of Life',
- Discussion paper TBA

Programs to download for lab:

- Install BioGeoBEARS in R

Dataset summary due on the 8th

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DIVERSIFICATION ANALYSES, NEXT-GENERATION SEQUENCING

April 12 & 14

Lecture 1:

- Yule, birth-death models, speciation and extinction rates
- Analysis of diversification patterns in a phylogenetic context

Lecture 2 and Lab:

- **Guest speaker – Jacob Berv, EEB**; bird tree, next-gen sequencing methods, BEAST dating
- BAMM & BAMMtools, other R-based methods: laser, DiversiTree, apTreeShape, etc.

Readings for the week:

- Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates (Alfaro *et al.*, 2009)
- Skim through intro to BAMM [<http://bamm-project.org/documentation.html>]

Programs to download for lab:

- BAMM [<http://bamm-project.org/index.html#>]

Nexus file and outline of paper due on 15th

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TRAIT SIMULATION AND PHYLOGENETIC COMPARATIVE ANALYSES

April 19 & 21

Lecture:

- Character evolution continued, tests of correlated characters
- simulating trees and characters in R, use of simulations for hypothesis testing

Lab:

- R implementation of various methods to test for phylogenetic signal in a character
- PIC and PGLS

Readings for the week:

- Chapter 13, Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology (Garamszegi, 2014) – simulating data
- chapters 4-6 would be beneficial to anyone interested in using comparative methods
- Phylogenies and the Comparative Method (Felsenstein, 1985)

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WORK ON RESEARCH PROJECT

April 26 & 28

Lecture 1:

PEER REVIEW: exchange materials and methods

Lecture 2:

- Let's Talk: components of a research presentation
- Discussion of readings

Lab:

- Work on project

Readings for the week:

- Seeing the forest for the trees: the limitations of phylogenies in comparative biology (Losos, 2011)

Materials and Methods peer review in class on the 26th

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RESEARCH PRESENTATIONS

May 3 & 5

Lecture:

- group discussion of presenting results as a powerpoint

Lab:

- presentations of final projects

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CONCLUSIONS

May 10

Lecture:

- future directions of model-based phylogenetic methodology, finding the big picture
- Post-molecular systematics and the future of phylogenetics (Pyron, 2015)
- The Tree of Life (Maddison, 2012)
- FIELD TRIP to the genomics sequencing facility (meet in the classroom)

2016 Course Schedule:

week	Tues	Thurs	Topic	Programs for Lab	Assignments that are due
1		28-Jan	Intro to Systematics and Phylogenetics	R	
2	2-Feb	4-Feb	Assembling a Dataset	Mesquite, SequenceMatrix, FigTree	lab HW 1 from 28th
3	9-Feb	11-Feb	Phylo Methods, Parsimony, Tree-Building	PAUP	lab HW 2
4	x	18-Feb	Nucleotide Substitution Models & Choice	jModelTest, PartitionFinder	lab HW 3
5	23-Feb	25-Feb	ML	CIPRES site - RAxML	lab HW 4
6	1-Mar	3-Mar	Bayesian Inference	MrBayes, Tracer	lab HW 5
7	8-Mar	10-Mar	Topology Tests, Troubleshooting	Gblocks, RogueNaRok	lab HW 6
8	15-Mar	17-Mar	Timetrees	BEAST	lab HW 7
9	22-Mar	24-Mar	Discrete/Continuous Characters; ACE	BayesTraits, Mesquite	lab HW 8, bibliography (25 th)
10	29-Mar	31-Mar	spring break		
11	5-Apr	7-Apr	Historical Biogeography	BioGeoBears (in R)	dataset summary
12	12-Apr	14-Apr	Diversification Analyses, Simulating Trees	R packages (MEDUSA?), BMM JAKE BERV GUEST LECTURE – 14 TH	nexus file/outline
13	19-Apr	21-Apr	Phylogenetic Comparative Analyses	R packages	work on project
14	26-Apr	28-Apr	work on research project		materials & methods peer review
15	3-May	5-May	class projects and presentations		prepare for final paper
16	10-May	x	last day – Field Trip		

Readings:

- Alfaro, M.E., Santini, F., Brock, C., Alamillo, H., Dornburg, A., Rabosky, D.L., Carnevale, G. & Harmon, L.J. (2009) Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 13410-4.
- Baum, D.A., Smith, S.D. & Donovan, S.S.S. (2005) The Tree-Thinking Challenge. *Science*, 979-980.
- Brown, J.W. (2003) The State of Bayesian Phylogenetics: Bayes for the Uninitiated. In, p. 71. Queen's University
- Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.
- Felsenstein, J. (1985) Phylogenies and the Comparative Method. *The American Naturalist*, **125**, 1-15.
- Garamszegi, L.Z. (2014) *Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology*. Springer-Verlag, Berlin.
- Hipsley, C.A. & Muller, J. (2014) Beyond fossil calibrations: realities of molecular clock practices in evolutionary biology. *Front Genet*, **5**, 138.
- Holder, M. & Lewis, P.O. (2003) Phylogeny estimation: traditional and Bayesian approaches. *Nature Reviews Genetics*, **4**, 275-84.

- Huelsenbeck, J.P. & Crandall, K.A. (1997) Phylogeny Estimation and Hypothesis Testing Using Maximum Likelihood. *Annual Review of Ecology and Systematics*, **28**, 437-466.
- Kelchner, S.A. & Thomas, M.A. (2007) Model use in phylogenetics: nine key questions. *Trends in Ecology and Evolution*, **22**, 87-94.
- Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. (2012) PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, **29**, 1695-701.
- Lemey, P., Salemi, M. & Vandamme, A.-M. (2009) *The Phylogenetic Handbook*, Second edn. Cambridge University Press, New York.
- Litsios, G. & Salamin, N. (2012) Effects of phylogenetic signal on ancestral state reconstruction. *Syst Biol*, **61**, 533-8.
- Longhorn, S.J., Pohl, H.W. & Vogler, A.P. (2010) Ribosomal protein genes of holometabolan insects reject the Halteria, instead revealing a close affinity of Strepsiptera with Coleoptera. *Molecular Phylogenetics and Evolution*, **55**, 846-59.
- Losos, J.B. (2011) Seeing the forest for the trees: the limitations of phylogenies in comparative biology. (American Society of Naturalists Address). *The American Naturalist*, **177**, 709-27.
- Maddison, D.R. (2012) The Tree of Life. *Systematic Biology*, **62**, 179-179.
- Misof, B., Liu, S., Meusemann, K., Peters, R.S., Donath, A., Mayer, C., Frandsen, P.B., Ware, J., Flouri, T., Beutel, R.G., et al. (2014) Phylogenomics resolves the timing and pattern of insect evolution. *Science*, **346**, 763-767.
- Paradis, E. (2012) *Analysis of Phylogenetics and Evolution with R*, Second edn. Springer.
- Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253-6.
- Pyron, R.A. (2015) Post-molecular systematics and the future of phylogenetics. *Trends Ecol Evol*, **30**, 384-9.
- Renner, S.S. (2005) Relaxed molecular clocks for dating historical plant dispersal events. *Trends in Plant Science*, **10**, 550-8.