

COMPARATIVE PHYLOGEOGRAPHY OF SOUTHERN INDOCHINA

Introduction

The emergence of Statistical Phylogeography represents a mandate to explicitly test hypotheses about population-level processes that shape genetic variation across geographic landscapes (1). Elucidating these processes is integral to understanding speciation and how biodiversity is generated and maintained. Southeast Asia represents an ideal model system for testing hypotheses about the historical processes that have shaped the genetic structure of natural populations and the assembly of rich communities. In particular, Southern Indochina is a biodiversity hotspot with a complex and well-understood climatical and geological history, allowing the genetic patterns of contemporary populations to be used to test hypotheses about which historical events were the primary drivers of biodiversity.

The Southern portion of the Indochinese peninsula is primarily a large flood basin that receives drainage from surrounding plateaus to the north. Isolated within this low-lying basin are the Cardamom Mountains of Southwestern Cambodia. Seasonal fluctuations in water levels are more extreme in Southern Indochina than anywhere else in the world (2). The area covered by Tonle Sap Lake in Central Cambodia changes more than six-fold between the dry and wet season (2); much of the interior of the country is inundated during the latter. Fluctuations were even more extreme throughout the Pleistocene. During periods of high sea levels, much of Southern Indochina was likely inundated, whereas during low sea levels, the region was completely landlocked as a central part of the Sunda Shelf (3-5). Furthermore, recent analyses of pollen data from lake sediments in Cambodia suggest that cool, dry glacial conditions had a prolonged hold on Southern Indochina, and the warm, monsoon climate did not begin to develop until approximately 8400 years before present (6).

Hypotheses

Given the dramatic fluctuations in water levels and the recent glacial climate in Southern Indochina, the rich, monsoon-adapted biota currently inhabiting Southern Indochina can be explained in two ways: 1) There were refugia within the isolated Cardamom Mountains that allowed the biota to persist through the Pleistocene (the refugial hypothesis), or 2) the biota of Southern Indochina colonized the region from other areas of Southeast Asia following the onset of the monsoon climate (the dispersal hypothesis).

Objectives

I propose to use molecular sequence data from multiple, co-distributed taxa to statistically test the refugial and dispersal hypotheses, and infer the major historical biogeographic processes that shaped genetic and species diversity within Southern Indochina.

Methods

Target Taxa— I will sample four species that are co-distributed across Southern Indochina from five different regions within the Cardamom Mountains, and five additional regions surrounding the Cardamoms. The target species include two lizards (*Calotes versicolor* and *Sphenomorphus maculatus*) and two frogs (*Polypedates leucomystax* and *Microhyla pulchra*). The species were selected because they are distantly related, easily

collected, broadly distributed across Indochina, and exhibit disparate life history traits and dispersal abilities. Because shared population genetic patterns among these species cannot be attributed to common ancestry, but rather large-scale biogeographic processes, the four species will be used in concert to test the refugial and dispersal hypotheses.

Data Collection and Analyses—Ten individuals per species will be collected from each locality. For all individuals, I will sequence *Cytochrome b* and four anonymous, single-copy nuclear loci. All heterozygous nuclear loci will be phased using a combination of cloning and the program SITES (7) until all alleles are determined with greater than 95% certainty. To determine the number of, and relationships among, populations represented by the samples from each species, I will use genetic clustering analyses (8) and standard and coalescent-based (9) phylogenetic analyses. For populations within the Cardamom Mountains, maximum likelihood (10) and Bayesian (11) analyses will be used to test for past refugia (i.e. recent population expansion). I will use the programs IMA (12), BEST, and LaGrange (13) to test for phylogeographic patterns consistent with dispersal from other areas, and whether such patterns are congruent among species. I will develop a novel parametric bootstrapping method and use MsBayes (14) to statistically test whether population expansions (i.e. refugia) and/or dispersals were contemporaneous among species and with the onset of the monsoon climate. For each species, I will compare the observed sequence data to null distributions generated via coalescent simulations under refugial and dispersal scenarios to determine if either can be statistically rejected over a broad range of parameter settings.

Significance and Broader Impacts

This will be the first study to statistically test explicit hypotheses about the large-scale biogeographic processes that shaped genetic diversity and community assembly across the landscape of Indochina. If the refugial hypothesis is correct, the Cardamom Mountains may be an important area of endemism, explaining the recent surge of new species being described from this region (15-18). If the colonization hypothesis is correct, Southern Indochina may be an important biogeographical link among surrounding areas of Southeast Asia, explaining why species boundaries tend to fall out in this region (19-21). Furthermore, the four broadly distributed taxa targeted by this study likely comprise complexes of multiple species. This study will reveal the true species-level diversity represented by these complexes. All of these results will have important conservation implications.

The Cardamom Mountains represent one of the largest tracts of undisturbed forest in mainland Southeast Asia, harboring nearly all of Cambodia's biodiversity and many species that are extinct throughout the rest of their native ranges (22). The results of this study will help the Cardamom Mountain Wildlife Sanctuaries Project make informed policy decisions to better safeguard this region from increasing pressure from logging and slash-and-burn agriculture.

This work will be a collaborative effort with Cambodia's Ministry of Environment and Fauna and Flora International, as they are currently in the process of founding a national museum in Phnom Penh. As part of this project, I will be training students from the Royal University of Phnom Penh and Ministry of Environment staff in fieldwork, specimen preparation and preservation, and curatorial techniques. This training will be vital as they begin to assemble their own zoological collections.

Timeline

I am a second year PhD student in the Department of Ecology and Evolutionary Biology at the University of Kansas. From previous fieldwork in Cambodia, I already have the necessary samples for all four species from two areas within the Cardamom Mountains. I will spend June–August of 2009 in Cambodia collecting samples from three additional areas within the Cardamoms and from five areas surrounding the mountains. During September and October of 2009, I will sequence *Cytochrome b* for all collected samples and conduct preliminary analyses on these data. I will use these preliminary results to apply for additional funding, including an NSF Doctoral Dissertation Improvement Grant, in order to develop and sequence four nuclear loci for each species for my dissertation.

Literature Cited

1. Knowles LL & Maddison WP (2002) Statistical phylogeography. *Molecular Ecology* 11:2623-2635.
2. Bonheur N (2006) Tonle Sap Biosphere Reserve Environmental Information Database. (Tonle Sap Biosphere Reserve Secretariat, Phnom Penh, Cambodia).
3. Hanebuth T, Statterger K, & Grootes PM (2000) Rapid Flooding of the Sunda Shelf: A Late-Glacial Sea-Level Record. *Science* 288(5468):1033-1035.
4. Sathiamurthy E & Voris HK (2006) Maps of Holocene Sea Level Transgression and Submerged Lakes on the Sunda Shelf. *The Natural History Journal of Chulalongkorn University Supplement* 2:1-43.
5. Voris HK (2000) Maps of the Pleistocene Sea Levels in South East Asia: Shorelines, River Systems, Time Durations. *Journal of Biogeography* 27:1153-1167.
6. Maxwell AL (2001) Holocene Monsoon Changes Inferred from Lake Sediment Pollen and Carbonate Records, Northeastern Cambodia. *Quaternary Research* 56:390-400.
7. Hey J & Wakely J (1997) A Coalescent Estimator of the Population Recombination Rate. *Genetics* 145:833-846.
8. Pritchard JK, Stephens M, & Donnelly P (2000) Inference of Population Structure Using Multilocus Genotype Data. *Genetics* 155:945-959.
9. Liu L & Pearl DK (2007) Species Trees from Gene Trees: Reconstructing Bayesian Posterior Distributions of a Species Phylogeny Using Estimated Gene Tree Distributions. *Systematic Biology* 56(3):504-514.
10. Kuhner MK, Yamato J, & Felsenstein J (1995) Estimating Effective Population Size and Mutation Rate From Sequence Data Using Metropolis-Hastings Sampling. *Genetics* 140:1421-1430.
11. Drummond AJ, Rambaut A, Shapiro B, & Pybus OG (2005) Bayesian Coalescent Inference of Past Population Dynamics from Molecular Sequences. *Molecular Biology and Evolution* 22(5):1185-1192.
12. Hey J & Nielsen R (2007) Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. *Proceedings of the National Academy of Science* 104(8):2785-2790.
13. Ree RH & Smith SA (2008) Maximum Likelihood Inference of Geographic Range Evolution by Dispersal, Local Extinction, and Cladogenesis. *Systematic Biology* 57(1):4-14.
14. Hickerson MJ, Stahl EA, & Lessios HA (2006) Test for Simultaneous Divergence using Approximate Bayesian Computation. *Evolution* 60(12):2435-2453.
15. Ohler A, Swan SR, & Daltry JC (2002) A Recent Survey of the Amphibian Fauna of the Cardamom Mountains, Southwest Cambodia with Descriptions of Three New Species. *The Raffles Bulletin of Zoology* 50(2):465-481.
16. Daltry JC & Wuster W (2002) A New Species of Wolf Snake (Serpentes: Colubridae: *Lycodon*) from the Cardamom Mountains, Southwestern Cambodia. *Herpetologica* 58(4):498-504.
17. Grismer LL, *et al.* (2008) Additional Amphibians and Reptiles from the Phnom Samkos Wildlife Sanctuary in Northwestern Cardamom Mountains, Cambodia, with Comments on their Taxonomy and the Discovery of Three New Species. *The Raffles Bulletin of Zoology* 56(1):161-175.
18. Stuart BL & Emmett DA (2006) A Collection of Amphibians and Reptiles from the Cardamom Mountains, Southwestern Cambodia. *Fieldiana: Zoology New Series No.* 109:1-27.
19. Bain RH, Lathrop A, Murphy RW, Orlov NL, & Cuc HT (2003) Cryptic Species of a Cascade Frog from Southeast Asia: Taxonomic Revisions and Descriptions of Six New Species. *American Museum Novitates* 3417:1-60.
20. Matsui M, Shimada T, Ota H, & Tanaka-Ueno T (2005) Multiple invasions of the Ryukyu Archipelago by Oriental frogs of the subgenus *Odorrana* with phylogenetic reassessment of the related subgenera of the genus *Rana*. *Molecular Phylogenetics and Evolution* 37:733-742.
21. Wuster W, Thorpe RS, Cox MJ, Jintakune P, & Nabhitabhata J (1995) Population systematics of the snake genus *Naja* (Reptilia: Serpentes: Elapidae) in Indochina: Multivariate morphometrics and comparative mitochondrial DNA sequencing (cytochrome oxidase I). *Journal of Evolutionary Biology* 8:493-510.
22. Fauna & Flora International (2000) Biological Survey of the Cardamom Mountains, Southwestern Cambodia: Interim Report, April 2000. Cambridge, UK.

Table 1. Summary of proposed budget.

400 Guanidine thiocyanate DNA extractions at \$0.06 each	\$ 18
400 PCR reactions at \$0.16 each	\$ 64
1,600 cycle sequencing reactions at \$1.10 each	\$ 1760
Total	\$ 1848
Total requested from SSB	\$ 1850

Budget Justification

For the proposed project, I am requesting \$1850 for sequencing costs associated with collecting one mitochondrial locus for 400 individuals (four species; 10 localities per species; 10 individuals per locality per species). This will require 400 DNA extractions and PCR reactions. For all the species, sequencing the entire reading frame of *Cytochrome b* requires four cycle sequencing reactions (i.e. four primers) for each PCR reaction. Thus, I will perform 1,600 cycle sequencing reactions. In Table 1, the cost per PCR reaction includes reagents and buffers, and clean-up of the reaction products with dilute ExoSAP-IT. The cost per cycle sequencing reaction includes ABI Big Dye and buffer, Sephadex medium, and the lane charge associated with the visualization of the sequence on an ABI 3730 DNA Analyzer. All laboratory work will be performed at the University of Kansas Biodiversity Research Center Molecular Sequencing Lab. The cost of fieldwork is not included in the budget, because it will be covered by the NSF Biological Surveys and Inventories Grant (DEB 0344430) awarded to Town Peterson and Rafe Brown.

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Curriculum Vitae

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EDUCATION

Ph.D. in Ecology & Evolutionary Biology, University of Kansas (KU), 2007-present (anticipated May 2012)
M.S. in Biology, Louisiana State University (LSU), August 2007, GPA: 4.0
B.S. in Biology, University of Wisconsin Oshkosh, *Summa Cum Laude*, May 2004, GPA: 3.92

PROFESSIONAL EXPERIENCE

2008-present Research Assistant for the NSF EPSCoR grant "Understanding and Forecasting the Ecological Consequences of Environmental Changes" (EPS-0553722)
2007-present Curatorial Assistant for the KU Natural History Museum Herpetology Collection
2006-2007 Coordinator of the LSU Museum of Natural Science Seminar Series
2006-2007 Curatorial Assistant for the LSU Museum of Natural Science Herpetology Collection
2006 Teaching Assistant for Herpetology, LSU
2004-2006 Research Assistant in the LSU Museum of Natural Science
2003-2004 Tutor for the University of Wisconsin Oshkosh Student Support Services

GRANTS & AWARDS

2009 Estimating Species Trees Workshop Travel Grant, University of Michigan Museum of Zoology (\$500)
2008 The KU Biodiversity Research Center Panorama Small Grant Program (\$900)
2004-2007 Graduate Student Enhancement by the LSU Biology Dept. (\$30,000)
2007, '06 & '05 LSU Department of Biological Sciences BioGrads Award (\$300 each)
2007 & '06 LSU Museum of Natural Science Graduate Student Research Grant (\$600 each)
2005 Sigma Xi Grant-in-Aid of Research (\$1000)
2004 University of Wisconsin Oshkosh Outstanding Biology Major Award (\$500)
2003 & '02 Leslie-Allen Fellowship in Ecology and Field Biology (\$500 each)
2001 University of Wisconsin Oshkosh Undergraduate Research Grant (\$2500)

PRESENTATIONS

- **Oaks, J.R.** Accommodating Among-Site Rate Variation in Phylogenetic Inference: Data Partitioning as a Random Variable and the Objective Choice of Partition Strategy. KU Natural History Museum Graduate Student Organization Retreat, Lawrence, Kansas, December 2008. Oral contribution.
- **Oaks, J.R.** Evolution of the True Crocodiles (*Crocodylus*). Sigma Xi Research Paper Competition, University of Kansas, Lawrence, Kansas, April 2008. Oral contribution (Awarded 2nd place).
- **Oaks, J.R.** Phylogenetic Systematics and Biogeography of the True Crocodiles (*Crocodylus*). KU Natural History Museum Seminar Series, Lawrence, Kansas, November 2007. Oral contribution.
- Carling, M., Z. Cheviron, J. Grismer, A. Jennings, and **J.R. Oaks**. Fieldwork at the LSU Museum of Natural Science: Where in the World are the Museum Students? LSU Museum of Natural Science Seminar Series, Baton Rouge, Louisiana, April 2007. Oral contribution.

PUBLICATIONS

- **Oaks, J.R.**, J.M. Daul, and G.H. Adler. 2008. Life span of a tropical forest rodent, *Proechimys semispinosus*. *Journal of Mammology*, 89(4):904-908.
- Grismer, L.L., T. Neang, T. Chav, P.L. Wood, **J.R. Oaks**, J. Holden, J.L. Grismer, T.R. Szutz, and T.M. Youmans. 2008. Additional amphibians and reptiles from the Phnom Samkos Wildlife Sanctuary in Northwestern Cardamom Mountains, Cambodia, with comments on their taxonomy and the discovery of four new species. *Raffles Bulletin of Zoology* 56(1):161-175.