SEEDS "YOU CHOOSE" AWARD APPLICATION

A NEXT-GENERATION APPROACH TO MEASURING THE RATE OF MOLECULAR EVOLUTION

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Abstract

Measuring the rate of DNA mutations, the molecular basis of evolution, is a fundamental question in evolutionary biology. While mutation rates for mitochondrial DNA have been well documented, the rate of mutation of the nuclear DNA that makes up more than 99% of the genome is not well understood. Here we propose to utilize Next-Generation Sequencing technology to estimate this critical measure. This project will further the careers of three female scientists at different points in their research careers. Funding for this project will provide these women with the experience and publications they need help them reach their career goals.

Project Goals

The goal of the proposed research is to calibrate the rate at which DNA mutations accumulate across the nuclear genome. This critical measure has applications across biological disciplines, allowing investigators to estimate the timing of evolutionary events. Obtaining this measure is challenging because mistakes in DNA replication are extremely rare events that cannot be measured in the course of human lifetimes. It is possible, however, to indirectly estimate this rate if an independent calibration point is available. Ideally, fossil records provide this reference point. However, in the absence of fossil evidence, a well-dated vicariant event (i.e. an event that has separated once continuous populations) can be used. Once this reference is established, mutation rates are estimated by comparing DNA sequences from both descendent populations and dividing their genetic difference by separation time. This project will use populations separated by the Isthmus of Panama to estimate the rate and variance of DNA mutations across the genome.

The formation of the Isthmus of Panama is an ideal calibration point for estimating DNA mutation rates as: 1) the timing of its completion has been well documented at ~3 MYA 2) it serves as a virtually impenetrable barrier to movement of marine species between the Caribbean and Pacific Ocean and 3) hundreds of species were split simultaneously by this event, creating the replication needed to estimate the variance of mutation rates across lineages. Among these lineages, the snapping shrimp genus Alpheus contains the most transisthmian species-pairs of any taxonomic group studied; at least nineteen transisthmian species-pairs have been identified. For this reason Alpheus has been identified as a model for examining rates of molecular evolution.

We propose to use Alpheus sister-species to calibrate mutation rates across the nuclear genome. Previous studies have used Alpheus for estimating mutation rates in the mitochondrial genome, but measuring rates of nuclear DNA mutation has been prohibitively time intensive and costly. Within the last five years, advances in DNA sequencing technologies have made it possible to sequence entire genomes quickly and affordably. We will selectively sequence the nuclear genomes of three different transisthmian Alpheus species-pairs. Using newly acquired Illumina Systems available at UM’s Human Genomics Core and a novel protocol for reducing genome complexity we will be able to generate 25 Gb of sequence data for each species-pair. Using CLC-bio software, we will identify orthologous regions of DNA for each species-pair and mutation rates will be estimated using the Isthmus’ closure as the calibration point. Rate comparisons across these three pairs will be used to establish the variance in mutation rates across lineages. More than 200 published studies have incorporated Isthmus-calibrated mutation rates for the mitochondrial genome, however, a nuclear-based mutation rate is now needed to interpret the genomic
datasets that are quickly becoming standard for phylogenetic and phylogeographic studies. This study will not only provide this fundamental measure of evolutionary change, but will also demonstrate the applicability of new sequencing technologies to a wider range of biological disciplines.

Career Goals

This award will serve to further the career goals of three female scientists early in their careers:

Dr. Carla Hurt is research faculty in the Department of Biology and will oversee all aspects of the proposed research. This project will help her to establish herself in the scientific community in three ways.

1.) First, publication and recognition for her research are critical for advancement. Publications from this project will likely have a large impact in molecular ecology. Here, we propose to examine one of the most critical measures of evolution, the tempo and variance of DNA mutations in the nuclear genome. This project is given added appeal as it combines A) *Alpheus* — a model system in molecular evolution B) the Isthmus of Panama — what has been called “the greatest natural experiment in evolution” and C) the latest advances in Next Generation Sequencing (NGS). As evidence of this potential, the first paper that used *Alpheus* to measure rates of mitochondrial DNA mutation was published in Science and has received nearly 300 citations. Resulting publications will not only improve Dr. Hurt’s opportunities for advancement, but will increase recognition for her work.

2.) Secondly, as director of the Molecular Core Lab, it is essential that Dr. Hurt is familiar with the latest technologies. The techniques utilized in this project have only been available since early 2010. Termed Next-Generation Sequencing (NGS), these advances will allow evolutionary biologists to address longstanding questions that were previously inaccessible due to technological or financial limitations. This project will provide the experience Dr. Hurt needs to utilize these techniques effectively in her own research and to advise other UM faculty how NGS methods can be used to meet their research goals.

3.) Finally, this project will provide a foundation for developing hypotheses related to Dr. Hurt’s research program in speciation and the genetics of biodiversity. Specifically, the resulting information about the *Alpheus* genome will be used to identify regions of the genome under differential selection in sister-species from the Caribbean and Pacific that warrant further study.

Dr. Jane Indorf, a recent Ph.D. graduate from UM’s Department of Biology, will be responsible for all data generation and analysis. Dr. Indorf is currently seeking a postdoctoral position where she can further develop her own research program in molecular ecology. Many advertised positions are now requiring that applicants have some experience in NGS. The experience and publications she receives from this project will greatly enhance her competitiveness for all future job prospects.

Katie Silliman is an undergraduate Singer scholar in the Marine Sciences Department and intends on pursuing a career in research specializing in ecological and conservation genetics. She has been a research assistant with Dr. Hurt since the beginning of her freshman year. Katie will participate in all aspects of this project from generating sequence data to analysis and writing. The valuable experience Katie will
gain from participating in this project and the chance to co-author resulting publications will give her a distinct advantage as she applies for graduate programs.

**Budget**

The internal cost for a single lane run on the Illumina Hi-Seq 2000 at UM's Human Genomics Core is $2,000 for a paired-end read and the cost for generating a standard library is $500.00. This will generate the sequence data for a single species-pair comparison. The remaining $5,000.00 needed to sequence the other two species pairs and complete this project will come from a General Research Support Award (GRSA).

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CURRICULUM VITAE

EDUCATION

1999 – 2005  Ph.D. Arizona State University - Genetics

EMPLOYMENT HISTORY

2007 – 2007  Research Assistant Professor– Department of Biology, University of Miami
2005 - 2007  Postdoctoral Researcher – Smithsonian Tropical Research Institute
1998 – 1999  Research Assistant in Developmental Genetics Laboratory– Arizona State University

PUBLICATIONS


GRANTS

2010 Smithsonian Scholars Fund – Smithsonian Institute - $32,000.00

2008 General Research Support Fund – University of Miami - $6,800.00

2003 Center for Bioinformatics – Graduate Fellowship – $3,936.00

2003 AZ Game and Fish – Genetic Variation in AZ Springsnails-Heritage Grant – $36,000

2001 AZ Game and Fish - Genetic Variation in AZ Springsnails-Heritage Grant – $47,000

PRESENTATIONS AND WORKSHOPS


2009 Workshop in Statistical Phylogeography. Portal, Arizona


2006 Summer Institute in Statistical Genetics. Seattle, WA.


2004 Hurt, C. R. Oral presentation. Genetic and Biological Consequences of Geographic Isolation. School of Natural Resources Seminar Series, University of Arizona, Tuscon, AZ.


ASSISTANTSHIPS

2004  Research assistant, Department of Biology, Arizona State University

2003  Teaching assistant, Department of Biology, Arizona State University. Organic Evolution

2001 – 2002  Research assistant, Department of Biology, Arizona State University

1999 – 2000  Teaching assistant, Department of Biology, Arizona State University. Natural History of Arizona, Human Anatomy and Physiology

COURSES

2007 Fall  Graduate Reading Seminar – Basics of Coalescent Theory

2008 Fall  Molecular Ecology Laboratory

2009 Fall  Molecular Core Techniques
2009 Spring  Population Genetics

2010 Spring  Molecular Core Techniques

SYNERGISTIC ACTIVITIES

2007 – 2008  Journal Club – I organized a weekly journal club meeting MIMEE (Methods In Molecular Evolution and Ecology) to discuss the applications of coalescent theory in population genetics.

2008 – 2010  Mentoring Undergraduate Research – Since beginning my career at UM I have trained three female undergraduate research assistants; two of these students will co-author peer-reviewed journal articles.

2008  Reviewer – Graduate Research Support Awards, Department of Biology UM

2007 – 2010  Invited Editorial Reviewer
Proceedings of the Smithsonian Marine Science Network Symposium
BMC Evolutionary Biology
Evolutionary Ecology

2009 – 2010  Graduate Student Committees – I am currently serving on the graduate committees of five Ph.D. students in the Department of Biology
BIOGRAPHICAL SKETCH

Jane L. Indorf

PROFESSIONAL PREPARATION
Boston University  Biology (Ecology/Evolution)  B.A.  2003
University of Miami  Biology (Evolution)  Ph.D.  2010

APPOINTMENTS
National Science Foundation GK-12 Graduate Fellow  2007 – 2009

PUBLICATIONS


SYNERGISTIC ACTIVITIES
Resident Scientist in Miami-Dade County Public Schools, 7th and 8th grade science classrooms, 2007 – 2009

ADVISORS AND COLLABORATORS
Dr. Tom Kunz, Boston University, undergraduate advisor
Dr. Michael Sorenson, Boston University, undergraduate research advisor
Dr. Michael Gaines, University of Miami, graduate advisor
Dr. Carla Hurt, collaborator

EDUCATION, OUTREACH, AND TRAINING
Candice Guevara, undergraduate
Teaching assistant for undergraduate Genetics Laboratory course (BIL 251), University of Miami, 2009 – 2010, 2005 – 2007
Howard Hughes Medical Institute and University of Miami Department of Biology Research in Ecology Program, Graduate Mentor, summers 2010, 2009, 2004
Subject: SEEDS You Choose Award
From: Kathryn Tosney <ktosney@miami.edu>
Date: Thu, 20 Jan 2011 16:42:24 -0500
To: <hurte@bio.miami.edu>
CC: Natasha Jobbagy Schiller <natasha@bio.miami.edu>

Dear Carla,

Congratulations, your SEEDS You Choose application to help support a research project that will engage three women scientists has been selected and will be fully funded! Cost share was provided by Biology.

You and the other winners will soon be profiled on the SEEDS home page and on awards page, http://www.as.miami.edu/seeds/. As described in the application, your proposal will be uploaded to the SEEDS site later this term, to form a model for others.

Attached is a SEEDS Quick Guide which will help you when you begin to put your proposal into action. Our SEEDS Program Manager, Natasha Jobbagy Schiller, cc’d here, will help you to process any payments and assist you as needed. Please consult with her prior to making any purchases to ensure that your purchase is within budget and within sponsored programs regulations. All funding should be spent and payments processed by 08/31/12 when our SEEDS grant is scheduled to end.

Please note that you are required to submit a quarterly report with updates on your progress towards achieving your goals outlined in this proposal. A concluding report is also needed.

SEEDS is excited to support your research and we look forward to hearing about your progress.

Best,
Kathryn

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Professor Kathryn Tosney
Chair of Biology
Director of SEEDS (Scientists and Engineers Expanding Diversity and Success)
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view my calendar at http://penguin.bio.miami.edu/calendar/week.php?user_ktosney

SEEDS Quick Guide.pdf
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