REQUIRED Cover Page

APPLICATION FOR PROFESSIONAL DEVELOPMENT GRANT

**All applicants please complete this cover page.

Choose one:	
[ ] Creative activity [X] Research activity [ ] Professional Enhancement activity	**Date of Last PDG Award** (Semester and Year awarded): May 2006 **Date of ATU Faculty Appointment** (Semester and Year): Fall 1998

1. Project Title: Population genetics of the striped scorpion (*Centruroides vittatus*): Expanding previous studies and identifying new genetic markers

2. Name of Principal Investigator/Project Director: Tsunemi Yamashita

3. School (abbrev): PLS **4. Department:** BIOLOGY

5. Campus Mail Address: McE 10 **6. PI/PD Campus Phone:** 968-0327

7. Amount Requested: $ 7050.00 **8. Total Cost of Project: $** 7050.00

9. Does this project involve: **10. Duration of Project:** four months

Yes	No	
[ ]	[X]	human subjects?
[ ]	[X]	animals/animal care facility?
[ ]	[X]	radioactive materials?
[ ]	[X]	hazardous materials?
[ ]	[X]	biological agents or toxins restricted by the USA Patriot Act?
[ ]	[X]	copyright or patent potential?
[ ]	[X]	utilization of space **not** currently available to the PI/PD?
[ ]	[X]	the purchase of equipment/instrumentation/software currently **available** to the PI/PD?

NOTE: If the answer is "yes" to any of the above questions, the investigator must attach appropriate documentation of approval or justification for use/purchase.

SIGNATURES

Department Head Date 4/26/07

Dean Date 4-26-07

REQUIRED COVER PAGE

This Section to be completed by the Office of Academic Affairs

PDC Committee Award Recommendation: Yes____ No____
PDC Committee Proposal Rank: _____ of _____ Total Proposals.
Recommendation of VPAA: Yes____ No _____
Recommendation of President: Yes____ No _____
Award Date: _____

Professional Development Grant

Project Title: Population genetics of the striped scorpion (*Centruroides vittatus*): Expanding previous studies and identifying new genetic markers.

B. Abstract:

In previous research, the population genetic structure of the striped scorpion (*Centruroides vittatus)* was investigated, and it was determined that these scorpions expanded their geographic range into the Ozarks and Ouachitas of Arkansas within the past 4,000 to 8,000 years. In addition, analysis of toxin genes in this scorpion revealed distinct genetic changes have occurred in the toxin proteins when compared to other, more toxic members of the same genus. I plan to further my investigations with an expanded analysis of scorpion populations as well as identifying new genetic markers to better discriminate among similar populations of this scorpion.

C. Purpose/Objectives

This project is focused upon conducting further analyses of striped scorpion populations and identifying better genetic markers for population level analyses that may be fruitful in later investigations. These additional studies are primarily planned because reviewers of a submitted manuscript to the journal Molecular Ecology suggested gathering further samples from populations as this scorpion encompasses a large geographic range (see attached manuscript review). This journal is regarded as one of the best journals in ecology and evolutionary studies, with these rankings- ISI Journal Citation Reports® Ranking: 2005: 60/261 (Biochemistry & Molecular Biology); 10/112 (Ecology); 7/33 (Evolutionary Biology) Impact Factor: 4.301. I plan to complete these objectives to complete the project goals.

A. Collect ten to 15 individuals from approximately 15 new populations across its large US geographic range (TX, NM, CO, KS, OK, AR, MO, and LA)

B. Conduct a screening of scorpion DNA to identify specific regions that house genetic markers (microsatellites) for population analyses.

C. Expand the data base of nucleotides employed in the current DNA sequence analysis from 600 nucleotide to 1400 nucleotides.

D. Significance/Need

The reviewer's comments indicated several populations in the manuscript dataset were of low sample number (n=2). Several of these populations are in areas of the scorpion's geographic range in the Trans-Pecos region of west Texas where the scorpion exhibits very large genetic separation among populations. I plan to revisit these areas and other sites in the Trans-Pecos and Big Bend region of Texas to increase the sample size for a more robust analysis. This additional work will also allow a better analysis to determine if the Big Bend populations are as genetically isolated from other Texas populations as the original investigations indicated. Moreover, as this scorpion is widespread across its geographic range, it can be used to

investigate patterns of genetic diversity that may be representative of historic and evolutionary patterns in other Chihuahuan desert organisms.

Another aspect of the research described in this proposal will extend a project that was initially funded through the AR NIH BRIN Award in 2004. The goal of the BRIN award was to identify microsatellite genetic markers for toxin gene research. In this award, we were not able to identify these genetic markers, but did identify a gene region that was employed in the current population genetics study with the striped scorpion. This study revealed the recent expansion of this scorpion from the west into the Ozarks and Ouachitas. After the time period of the award, better techniques were developed and disseminated to identify microsatellite markers from organisms that had shown difficulty in previous attempts (Hamilton et al. 1999, DeWoody 2002). The identification of these genetic markers would be significant as few genetic microsatellite markers have been identified for arachnids and none have been identified for scorpions. These genetic markers can help reveal patterns of genetic differentiation among scorpion populations that are geographically close to each other or have recently separated from each other. These markers are also important in that they represent nuclear markers from "normal" genes, whereas the majority of the genetic markers employed in our research are mitochondrial gene markers that may not discriminate among geographically close populations as well. Moreover, the development of these markers will assist with further research with population level analyses in other scorpions.

E. Process for attainment of objectives/Goals

1. Scorpion collections:
The collecting trip will target sites along the scorpion's south Texas populations, along the Rio Grande River in Texas and New Mexico, and in populations within the Trans-Pecos region of Texas. Collection permits have been obtained from state wildlife and parks agencies and will be updated prior to field work. We plan to collect approximately 150 new individuals to increase the sample size necessary for a robust analysis. Of the individuals collected, 75 individuals will be sampled for DNA analyses for 300 DNA sequencing reactions.

2. Expansion of the DNA database to encompass a larger number of nucleotides:
We plan to conduct further analysis of samples employed in the original research with additional PCR primers to expand the gene regions utilized in subsequent analyses. These DNA regions will be sequenced at the UAMS sequencing center. We plan to conduct 200 DNA sequencing reactions to update the original samples.

3. Develop microsatellite markers for additional genetic analysis:
Here we plan to use the updated protocols for enriching DNA samples for microsatellite markers outlined in new protocols (DeWoody 2002, Roed et al. 2006). These protocols involve the use of DNA binding beads that allow selective collection of specific DNA regions and greatly improve the isolation of microsatellite regions.

4. Analysis of DNA sequence data:
After DNA sequencing results are obtained, we will proofread the sequences, align the critical regions to each other with a DNA alignment program, and then input the aligned DNA sequences into several phylogenetic analysis software program for tree building and

final analysis. First, we plan to create a larger, more inclusive phylogenetic tree with expanded DNA sequences that includes more populations from across the scorpion's geographic range. This tree should allow us to determine if southern populations are more genetically separated than populations in the north.

F. Dissemination of Results

These results will update existing data for publication in a leading ecology and evolutionary biology journal, Molecular Ecology. The data and analyses conducted with this expanded study will also be presented at the Arkansas Academy of Sciences and the American Arachnological Society annual meetings.

G. Repeated Requests

This project is a second request for this project. It differs from the original project as it is an expansion of the data collected from the first project. The results from the previous project indicate scorpion populations in the Chihuahuan desert show greater genetic structuring than those outside this region. Further analysis of scorpion populations in this region will allow better resolution of the patterns of genetic diversity in this organism.

H. Budget Justification

Supplies $4750.00

These funds will purchase reagents necessary for DNA extraction, quantification, and PCR (approximately $1000.00). In addition, these funds will defray the costs of DNA sequencing at UAMS. We plan to submit several individuals from all populations with forward and reverse sequencing to reduce error. Each DNA sequencing run costs $7.50, thus approximately $3,750.00 is budgeted for sequencing costs alone.

Travel $2300.00

These funds will be used for travel to field sites over a 14 day collecting excursion. We plan to travel to west Texas and New Mexico to collect from populations approximately 60 – 80 miles apart. We expect to travel 4,500 miles in our field collection trip. Most of the sites will be in the Trans-Pecos region of Texas as scorpion populations appear to exhibit the greatest genetic diversity in this area.

Budget Total: $7050.00

I. Bibliography

MB Hamilton, EL. Pincus, A Di Fiore, and RC. Fleischer. 1999. Universal Linker and Ligation Procedures for Construction of Genomic DNA Libraries Enriched for Microsatellites. BioTechniques 27: 500-507.

JA DeWoody 2002. DeWoody's Microsatellite Cloning Protocol-Spring 2002

Molecular Ecology

Editorial Office: Division of Plant Sciences
University of Nottingham, Sutton Bonington Campus
Loughborough, LE12 5RD, UK
Email: mecol@ejournals.co.uk

MS#: 07/196

Ref#: 1

This form will be sent to the authors

Title: Striped scorpion, Centruroides vittatus, (Scorpiones: Buthidae) phylogeography indicates rapid and recent expansion into Ozark and Ouachita Interior Highlands

Authors: Tsunemi Yamashita and Douglas Rhoads

Please indicate your assessment by checking the appropriate boxes:

Quality of Science		Importance of Science*		Quality of Presentation	
Experimentally and/or theoretically excellent reliable data, no fatal flaws	☐	Research addresses a consequential question in ecology, evolution, behaviour, or conservation	☐	Writing is clear, methods and data analyses are transparent, ideas make sense, proper grammar and spelling is employed, redundancy is avoided	☐
Mostly competent, but suffering from flaws of a technical or analytical nature	☐	Research primarily descriptive and/or only relevant to the taxon being studied	☒	Ideas and methods are mostly clear, but grammar and/or spelling is poor, format does not follow guidelines, and/or there is redundancy between sections	☐
Weak, major flaws or inconsistencies	☒	Research outside the scope of *Molecular Ecology*, or of little interest to readers	☐	Writing lacks clarity, methods and ideas are hard to follow	☒

Note: Molecular Ecology editorial policy requires that papers address consequential questions of general relevance to the fields of ecology, evolution, behaviour, or conservation

Please provide an overall assessment by checking one box only:

Accept for publication as is, or after minor editorial changes	☐
Accept after minor revision not requiring further referee assessment	☐
Reject in present form, but encourage submission of new manuscript	☒
Reject without prospect of resubmission	☐

	Yes	No
Does this manuscript require significant reduction in length?	☐	☒

If "Yes", please indicate where shortening is required in your specific comments, or by annotations to the manuscript

Molecular Ecology

Editorial Decision

Subject Editor: Emerson

MS# 07-196

Decision: Reject

Decision Statement:

This manuscript has been seen by two referees, both of whom are very critical of aspects of the data and analyses. Both referees highlight problems with relaxing connection limits for the construction of a network. As pointed out by referee 1, relaxing connection limits involves relaxing the confidence that can be placed upon these connections being correct. Increasing the connection limit to the extent that the authors have done raises serious concerns about the validity of their networks. Referee 1 points out a number of other serious concerns related to the network analysis, as does referee 2. Perhaps of greater concern is the rather unusual patterns of variation observed within the nuclear marker, which questions to what extent it really can be considered a single copy gene. Both referees offer suggestions for improvement of the paper, but these are not trivial. In the absence of convincing evidence that the nuclear gene is single copy referee 1 suggests removal of this data entirely. Referee 2 points out that sample sizes are not really appropriate for a population level study over such a broad geography. I concur with this, and for me it is not immediately clear how many COI haplotypes there are? There are 100 individuals sequenced, with from 1 to 4 at each locality (which is low), and the GenBank accessions in the appendix would imply there are 100 unique sequences. But that is clearly not the case when one looks at the figures. It would be useful to have some mention in the results section as to the number of unique haplotypes, and include this information for each locality in appendix 1.

Given the substantial concerns raised by both of the referees, I am afraid my recommendation for this manuscript is rejection. The authors have an interesting and useful study system, and the referees offer a number of useful suggestions to strengthen and broaden the appeal of this paper, however, this will require a substantial amount of work.

CURRICULUM VITAE

PERSONAL INFORMATION

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PROFESSIONAL INFORMATION

Academic Degrees:

1993 Ph.D.	Department of Biology, Vanderbilt University, Nashville, TN 37235
1985 B.A.	Hendrix College, Conway, AR 72032

Professional experience:

2003-Present	Associate Professor Department of Biological Sciences Arkansas Tech University
1998-2003	Assistant Professor Department of Biological Sciences Arkansas Tech University
1998	Adjunct Assistant Professor Department of Biology Northeast Louisiana University
1994-1998	Assistant Professor (Graduate Faculty) Department of Biology Northeast Louisiana University
1992-1994	Post-doctorate, Division of Hematology Vanderbilt University Medical School
1986-1991	Teaching Assistant Vanderbilt University

Grant Awards:

2006 (T Yamashita, K McArthur, and R Snead) The Population Genetics of Stargazing Darter in Arkansas. ATU Undergraduate Research Office. $2,700.00

2006 (J Stoeckel and T Yamashita) Status and genetics of the stargazing darter in Arkansas. Arkansas Game and Fish Commission. $25,000.00

2005 (T Yamashita) Molecular biology of the striped scorpion (*Centruroides vittatus*): toxin gene analysis. ATU Sponsored Programs. $3,300.00

2004 (T Yamashita) Molecular biology of the striped scorpion (*Centruroides vittatus*): population genetic structure and toxin gene analysis. ATU Sponsored Programs. $1,600.00

2004 (T Yamashita) Molecular biology of the striped scorpion (*Centruroides vittatus*) : population genetic structure and toxin gene analysis. ATU Undergraduate Research Office. $2,800.00

2004 (T Yamashita and DR Rhoads) Identification and characterization of microsatellite loci, SNPs, and toxir genes for genetic analysis of a medically important scorpion species. Arkansas BRIN. $16,985.00

2003 (T Yamashita) BRIN Equipment Supplement to Summer BRIN award. Arkansas BRIN. $25,350.00

2003 (T Yamashita and DR Rhoads) Identification and characterization of microsatellite loci for genetic analysis of a medically important scorpion species. Arkansas BRIN. $15,590.00

2001 (T Yamashita and Joe Stoeckel) Employing genetic markers to reveal taxa in a morphologically similar mussel complex.
Arkansas Tech University Undergraduate Research Office $3750.00

1999 (T Yamashita) An evaluation for investigation of genetic markers for population analysis.
Center for Energy, Natural resources and Environmental Studies. $7,732.00

1998 (R Buchholz and T Yamashita) Investigation of the parasitological and genetic mechanisms shaping sexually selected characters in the wild turkey in Louisiana. Louisiana Education Quality Support Fund (LeQSF). $70,000.00

1998 (A Findley, S Hecht, T Yamashita, and M Taylor) Teaching Molecular Biology in the Laboratory. NLU developmental Grants Program. $10,000.00

1996 (T Yamashita) Improving the undergraduate genetics laboratory. NLU Teaching and Learning Resour Center Grant Award. $2,855.00

1995 (T Yamashita and K Tolson) PCR detection of Mycobacterium leprae in armadillo lymphatic tissue. NLU Faculty Research Award. $3,510.00

1994 (T Yamashita) Population genetics analysis of cottonmouth populations in the upper Saline River. NLU Faculty Research Award. $3,000.00

1988 California Academy of Sciences: Exline-Frizzell Fund for Arachnological Research. $500.00

1987 Sigma Xi, The Scientific Research Society $300.00

1987-1990 Vanderbilt University: Graduate Travel and Research Grants $2,000.00

Publications:

Yamashita,T and D Rhoads. Striped scorpion, *Centruroides vittatus*, (Scorpiones: Buthidae) phylogeography indicates rapid and recent expansion into Ozark and Ouachita Interior Highlands. Submitted for publication in Molecular Ecology.

Yamashita, T. 2004. Surface activity, biomass, and phenology of the striped scorpion, *Centruroides vittatus* in Arkansas. Euscorpius -Occasional Publications in Scorpiology 17: 25-33.

J Lewter, T Yamashita, and AL Szalanski. 2003. DNA sequence analysis of the freshwater mussel, *Lampsilis hydiana* (Bivalvia: Unionidae), in select Ozark and Ouachita mountain streams of Arkansas. J. of the Arkansas Academy of Science 57: 216-220.

Yamashita, T. and V. Fet 2000 Molecular approaches to Biogeography. In G. Polis and P. Brownell (Eds) Scorpion Biology. Oxford University Press, NY.

Bondurant, M.C., T. Yamashita, K. Muta, S.B. Krantz, and M.J. Koury. 1996. c-myc expression affects proliferation but not terminal differentiation or survival of explanted erythoid progenitor cells. Journal of Cellula Physiology 168: 255-263.

Yamashita, T and G.A. Polis. 1995. A Geographic Analysis of Scorpion Populations on Habitat Islands. Heredit 75 (5): 495-505.

Yamashita, T. and G.A. Polis. 1995. A test of the central-marginal model using sand scorpion populations (Paruroctonus mesaensis, Vaejovidae). Journal of Arachnology 23: 60-64.

Polis, G.A. and T. Yamashita 1990. The ecological importance of predaceous arthropods in desert communities. In G. Polis (Ed) The Ecology of Desert Communities. Univ. of Arizona Press, Tucson, AZ

Abstracts:

Tsunemi Yamashita, Katherine McArthur, and Ryan Snead. A survey of Population Genetic Markers to Discriminate population differentiation in the Stargazing darter, *Percina uranidea.* Arkansas Academy of Sciences, April 2007.

Tsunemi Yamashita, Bryan Stobaugh, and Phillip Choi. Sodium channel peptide toxin genomics in the striped scorpion. American Arachnological Society Meeting, June 2006.

T Yamashita, Maria Longing, and Nick Pridgin, Phylogeography of the striped scorpion, *Centruroides vittatus* in the southwestern United States. American Arachnological Society Meeting, June 2005.
T Yamashita, Maria Longing, and Nick Pridgin, Phylogeography of the striped scorpion, *Centruroides vittatus* in the southwestern United States. Arkansas Academy of Sciences, April 2005.

T Yamashita and DR Rhoads. Identification and characterization of microsatellite loci , SNP's, and toxin genes for genetic analysis of a medically important scorpion species, *Centruroides vittatus*. Arkansas BRIN Research Mentoring Program-Research Day. July 2004

T Yamashita. Surface activity, biomass, and phenology of the striped scorpion, *Centruroides vittatus* in Arkansas. American Arachnological Society Meeting, June 2004.

T Yamashita. Life history characteristics, biomass, and phenology of the striped scorpion, *Centruroides vittatus* in Arkansas. Arkansas Academy of Sciences. April 2004

T Yamashita and DR Rhoads. Microsatellite identification in a medically important scorpion species, *Centruroides vittatus*. Arkansas BRIN Research Mentoring Program-Research Day. July 2003

T Yamashita. Phylogeography of the striped scorpion, *Centruroides vittatus*. Arkansas Academy of Science, April 2003

J Lewter, T Yamashita, and AL Szalanski. DNA sequence analysis of the freshwater mussel, *Lampsilis hydiana* (Bivalvia: Unionidae), in select Ozark and Ouachita mountain streams of Arkansas. Arkansas Academy of Science, April 2003

Yamashita, T and D. Hodgson. A screening of two scorpion populations, Centruroides vittatus (Say) for random amplified polymorphic DNA markers. American Arachnological Society Meeting, July 2000.

Yamashita, T and J. Qin PCR detection of Mycobacterium leprae in the armadillo, Dasypus novemcinctus. Association of Southeastern Biologists meeting, April 1998.

Williams, R and T Yamashita. Population genetics of an introduced ladybird beetle (Harmonia axyridis) Louisiana Academy of Sciences meeting, February 1998.

Bondurant, M., K. Muta, T. Yamashita, L. Kelley, S.B. Krantz, and M. Koury. Erythropoietin stimulates expression of c-myc without stimulating cell division in explanted mouse and human erythroblasts. Abstract submitted for the American Society of Hematology meeting, December 1993.

Invited Lectures:

Striped scorpion, *Centruroides vittatus*, (Scorpiones: Buthidae) phylogeography indicates rapid and recent expansion into Ozark and Ouachita Interior Highlands. Graduate seminar, Entomology Department, UA-F, March 2007.

Ecology and population biology of the Striped Scorpion, *Centruroides vittatus*. Thirty-seventh Arkansas Junior Science and Humanities Symposium, April 2003

PROPOSED BUDGET
FACULTY RESEARCH GRANT
(include budget categories as appropriate)

1.	Graduate assistant stipend	$_____
	Fringe benefits @ .4% (4/10 percent) of graduate assistant stipend	_____
2.	Non-work study stipend	_____
	Fringe benefits @ .4% (4/10 percent) of non-work study stipend	_____
3.	*Supplies (please list items to be purchased and estimated price per item including taxes and shipping, if appropriate):	
	Item No. 1 (Microsatellite markers) Estimated Price	1000.00
	Item No. 2 (DNA Sequencing) Estimated Price	3750.00
	Item No. 3 () Estimated Price	_____
	(additional lines as needed)	
	Total estimated supplies	4750.00
4.	Travel (please list travel expenditures by date and estimated costs):	
	Travel No. 1 5/17/2007 – 5/30/2007 Estimated Price	2300.00
	Travel No. 2 Estimated Price	_____
	Travel No. 3 Estimated Price	_____
	(additional lines as needed)	
	Total estimated travel	2300.00
5.	*Capital Outlay (please list items to be purchased and estimated price per item including taxes and shipping, if appropriate):	
	Item No. 1 Estimated Price	_____
	Item No. 2 Estimated Price	_____
	Item No. 3 Estimated Price	_____
	(additional lines as needed)	
	Total estimated capital outlay	_____
	TOTAL PROPOSED BUDGET	$ 7050.00

*Items purchased under $2,500 (including taxes and shipping) are considered supply items. Capital Outlay items are those which cost $2,500 or more (including taxes and shipping). Please contact the Purchasing Office for questionable items.