REQUIRED COVER PAGE

APPLICATION FOR FACULTY RESEARCH GRANT

**All questions must be completed to be considered for grant award.

Choose one:	
[ ] Creative P [X] Research	**Date of Last FRG Award** (Semester and Year awarded): NA **Date of ATU Faculty Appointment** (Semester and Year): Fall 1998

1. Project Title: Molecular biology of the striped scorpion - Population Genetic structure & toxin Gene Analysis

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

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

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

7. Amount Requested: $ 1600.00 **8. Total Cost of Project: $** 1600.00

9. Does this project involve: **10. Duration of Project:** June 30, 2005

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 Contribution (if applicable): **$**_____

Account Number: _____ **Chairperson** **Date**

School Contribution (if applicable): **$**_____

Account Number: _____ **Dean** **Date**

This Section to be completed by the Office of Academic Affairs

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

Faculty Research Grant

Project Title: Molecular biology of the striped scorpion (*Centruroides vittatus*): population genetic structure and toxin gene analysis.

B. Abstract:
Population genetics is a subdiscipline of genetics that examines genetic variability within populations and genetic differentiation among them. This project investigates the genetic structure of a widespread scorpion species, *Centruroides vittatus*. Employing molecular DNA techniques, we plan to create a robust evolutionary tree that shows the genetic separation among scorpion populations. In addition, we plan to investigate the differentiation within toxin genes among scorpion populations. The impetus for the genetic analysis of population structure stems from preliminary data that suggests this scorpion species expanded from a glacial refuge in the south. The toxin gene analysis breaks new ground as no systematic investigation of toxin gene variability among populations within a species has been completed. In addition to the research aspects, this project will also improve the preliminary data needed to attract larger grant awards from outside sources. Lastly, this project will support the implementation of new equipment provided through a UAMS BRIN equipment award into the biology curriculum.

C. Purpose/Objectives
This project will determine the genetic structure of striped scorpion populations across their wide geographic range. In addition, this project will investigate genetic diversity in toxin genes among these populations. There are several objectives to be completed that will assist in the completion of the major goals.

A. Produce DNA sequence data from several gene areas located throughout the scorpion's DNA for genetic variability comparison among populations. DNA sequence data is the actual chemical make up of the genes. Investigating these data allows investigators to quantify genetic differences among genes, individuals, and populations.

B. Produce DNA sequence data from several toxin genes to allow comparison among scorpion populations for genetic divergence in these specific genes.

D. Significance/Need
This project is focused on the genetic analysis of striped scorpion populations across its geographic range. This scorpion encompasses a large geographic area that includes Texas, eastern New Mexico, southern Colorado, Kansas, southern Nebraska, southern Missouri, a small portion of western Illinois, western Arkansas, western Louisiana, and Oklahoma (Shelly and Sissom 1995). In several states, *Centruroides vittatus* is the only scorpion reported: Kansas, Nebraska, Missouri, Illinois, Arkansas, and Oklahoma. This study will determine how these scorpion populations are structured across their geographic range. Knowledge of population structure is a key component in population genetics and this knowledge can identify those populations that are genetically unique and may be targeted for conservation efforts. Specifically, this avenue of research answers questions such as "Does this population show a unique genetic signature that indicates evolutionary separation from other populations?"

In addition, this project is an expansion of work conducted by undergraduates funded through the 2003 Undergraduate Research Award. One of the intriguing findings from the 2003 project was that scorpion populations in Texas exhibited greater genetic separation than those sampled further north in Oklahoma, Arkansas, Kansas, and Missouri (Figure 1). These findings suggest northern populations expanded from glacial refugia possibly during the Hypsithermal Interval approximately 8,000 to 4,000 years ago with other western prairie species such as the collared lizard (*Crotaphytus collaris*) (Delcourt & Delcourt 1991, Hutchison et al. 1999). These findings lead Dr. Yamashita to conduct a more through sampling of the scorpion across its geographic range. In the 2004 summer, a collecting trip yielded samples from 18 additional populations. Individuals were obtained from populations in LA, TX, NM, CO, KS, and OK. Researchers in TX and NE added to the collection effort to result in a total of 30 new populations for analysis. The hypothesis we plan to test is that populations in southern regions (LA, TX, NM) will exhibit greater genetic separation than those in northern areas. We predict our phylogenetic (evolutionary) tree that illustrates population separation will reveal a bushy region interconnected with short lines, representing northern populations, connected to regions interconnected with longer lines representing the greater genetic separation in southern populations (see Figure 1).

The undergraduates engaged in this Faculty Research Grant will examine population genetic structure through PCR (Polymerase Chain Reaction) and DNA sequencing analysis. The PCR process will allow us to select and amplify select gene regions from extracted scorpion DNA. We plan to then clean the PCR products and send them to a DNA sequencing facility at UALR to determine the nucleotide composition of these genes. After the sequencing results are returned, we plan to analyze the sequence data and create a phylogenetic tree that will illustrate the genetic separation among populations. We also plan to apply additional analytical techniques to better investigate our data to test if current population structure is primarily due to historical population structure. Contemporary population structure could result from several processes, some are current; other processes are due to the history of the population in a geographic area.

Another facet of this work is the continued examination of diversity in scorpion toxin genes. A summer 2003 and 2004 UAMS-BRIN award allowed Dr. Yamashita to investigate genetic markers appropriate to determine population structure and toxin gene variability in this scorpion. From the 2004 summer, we were able to target specific gene regions and produced three markers suitable for further analysis. In addition, we surveyed seven toxin genes and identified three genes that produced DNA products useful to determine toxin gene variability in this scorpion. Employing similar techniques as those outlined above, we plan to investigate the toxin gene DNA sequence variability among populations in this scorpion. This research is unique as it examines if toxin genes vary among populations. The characterization of the genes involved in toxin production is limited with the majority of scorpion toxin studies examining genes among different species. Moreover, with regard to humans, this scorpion species exhibits mild venom when compared to the more medically important sister species, *Centruroides exilicauda*. This research could provide details that may allow investigators to understand better the genetic aspects of venom toxicity.

This proposal will also help to improve instructional materials in the biology program as the undergraduates funded from the proposal will utilize new equipment purchased from a 2003 UAMS - BRIN equipment grant. These equipment items include a wide band fluorometer, DNA isolation equipment, gel electrophoresis analysis, and water purification equipment (fall

2004). The undergraduates will utilize these items and determine how they can best fit into the laboratory exercises of existing courses such as genetics, physiology, evolutionary biology, and molecular genetics.

This project will have benefits to promote Dr. Yamashita's research and teaching activities in several avenues. First, progress will be made on a previous undergraduate project from the 2003/2004 academic year. Results and activities from this proposal will be an important contribution to a BRIN grant renewal application in the spring of 2005 to show continued activity on the summer research. These research awards will also provide preliminary data for potential proposals to federal granting agencies (NSF or NIH). Moreover, this proposal will fund two undergraduate students engaging in research activities. Lastly, this project will fund the continued usage of new equipment and its incorporation into the laboratory teaching curriculum.

Lastly, the funds from this proposal will supplement an ATU Undergraduate Research award that primarily funded undergraduate students who will conduct this project. The funds generated through this proposal will pay for additional supplies and fees (see below in budget justification).

E. Process for attainment of objectives/Goals

The students employed in this project will complete several activities to analyze genetic differentiation among scorpion populations and toxin genes diversification.

1. DNA isolation and purification- The students will isolate genomic DNA from alcohol preserved scorpions. The procedure they will use is a standard DNA extraction protocol. The students will also purify the extracted DNA to remove any unwanted chemicals and contaminants.

2. DNA amplification via PCR- The PCR process allows a targeted amplification (copying) of a specific portion of the scorpion's DNA with nucleotide primers specific for scorpion toxin genes. With this procedure, the students will create large amounts of DNA from mitochondrial DNA regions and three toxin genes that can then be sequenced for further analysis. Once the DNA has been PCR amplified, the students will clean the products to remove contaminants before packaging them for shipment to UALR for DNA sequencing.

3. 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 a phylogenetic analysis software program for tree building and final analysis. First, we plan to create a larger, more inclusive phylogenetic tree that includes 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. Second, we plan to compare our toxin gene results to published toxin gene regions to determine if specific DNA regions of toxin genes differ among striped scorpion populations and also between the striped scorpion and the more toxic sister species, *C. exilicauda*.

F. Dissemination of Results

Each student will produce a final report and a seminar that summarizes their findings from the research. In addition, the students will participate in the Undergraduate Honor's Research Colloquium in the 2005 spring semester. After the larger population genetics project is completed, the work will be submitted for publication in Molecular Ecology. In addition, faculty seminars from this work will be presented at the 2005 Arkansas Academy of Sciences and the American Arachnological Society conference.

G. Repeated Requests

This project is a first request.

H. Budget Justification

Supplies $1300.00

These funds will purchase reagents necessary for DNA extraction, quantification, and PCR (approximately $600.00). In addition, these funds will defray the costs of DNA sequencing at UALR. We plan to submit two individuals from each of 30 populations with forward and reverse sequencing to reduce error. Each DNA sequencing run costs $10.00, thus approximately $1,200.00 for sequencing costs alone. With the addition of second runs for un-readable DNA sequences, we expect a total sequencing cost of about $1,400.00 for the population study. When the cost of toxin gene sequencing is included (approximately $600.00), we can expect an overall total cost of $2,000.00. We were able to obtain approximately 60% of the sequencing costs from the Undergraduate Research Award.

Travel $300.00

These funds will be used for travel to field sites for additional scorpion collecting as needed as well as travel to local meetings (AR Academy of Sciences).

The PI will spend approximately 20-30% of the week during the semester on the project and approximately 40% of the week during non-academic time.

I. Bibliography

Delcourt, H.R. and PA Delcourt. 1991. Late-quaternary vegetation history of the interior highlands of Missouri, Arkansas, and Oklahoma. In D Henderson & LD Hedrick (eds) *Restoration of Old Growth Forests in the Interior Highlands of Arkansas and Louisiana.* Proceedings of the Conference, Winrock International, Morrilton, AR

Hutchison, D. W., S. T. Malcomber, and L. S. Pletscher. 1999. A multidisciplinary investigation of the applicability of the Pleistocene herpetofaunal stability model to collared lizard (*Crotaphytus collaris*). Herpetological Monographs 13: 81-141.

Shelley, R.M. and W. D. Sissom. 1995. Distributions of the scorpions *Centruroides vittatus* (Say) and *Centruroides hentzi* (Banks) in the United States and Mexico (Scorpiones, Buthidae). J. of Arach. 23: 100-110

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 (e.g., software)	Estimated Price		
Item No. 2 (e.g., copying costs)	Estimated Price	DNA Isolation Reagents	400.00
Item No. 3	Estimated Price	DNA Sequencing Costs	900.00
(additional lines as needed)			

Total estimated supplies 1300.00

4. Travel (please list travel expenditures by date and estimated costs):

Travel No. 1	Estimated Price		
Travel No. 2	Estimated Price	local	100.00
Travel No. 3	Estimated Price	Ar. Acad. Sci	200.00
(additional lines as needed)			

Total estimated travel 300.00

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 $ 1600.00

*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.

CURRICULUM VITAE

PERSONAL INFORMATION

Tsunemi Yamashita
SS#: 360-52-6433
Department of Biological Sciences
Arkansas Tech University
Russellville, AR 72801

(501) 968-0327 office
(501) 968-4964 home
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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

Grant Awards:

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

Publications:

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:

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 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:

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

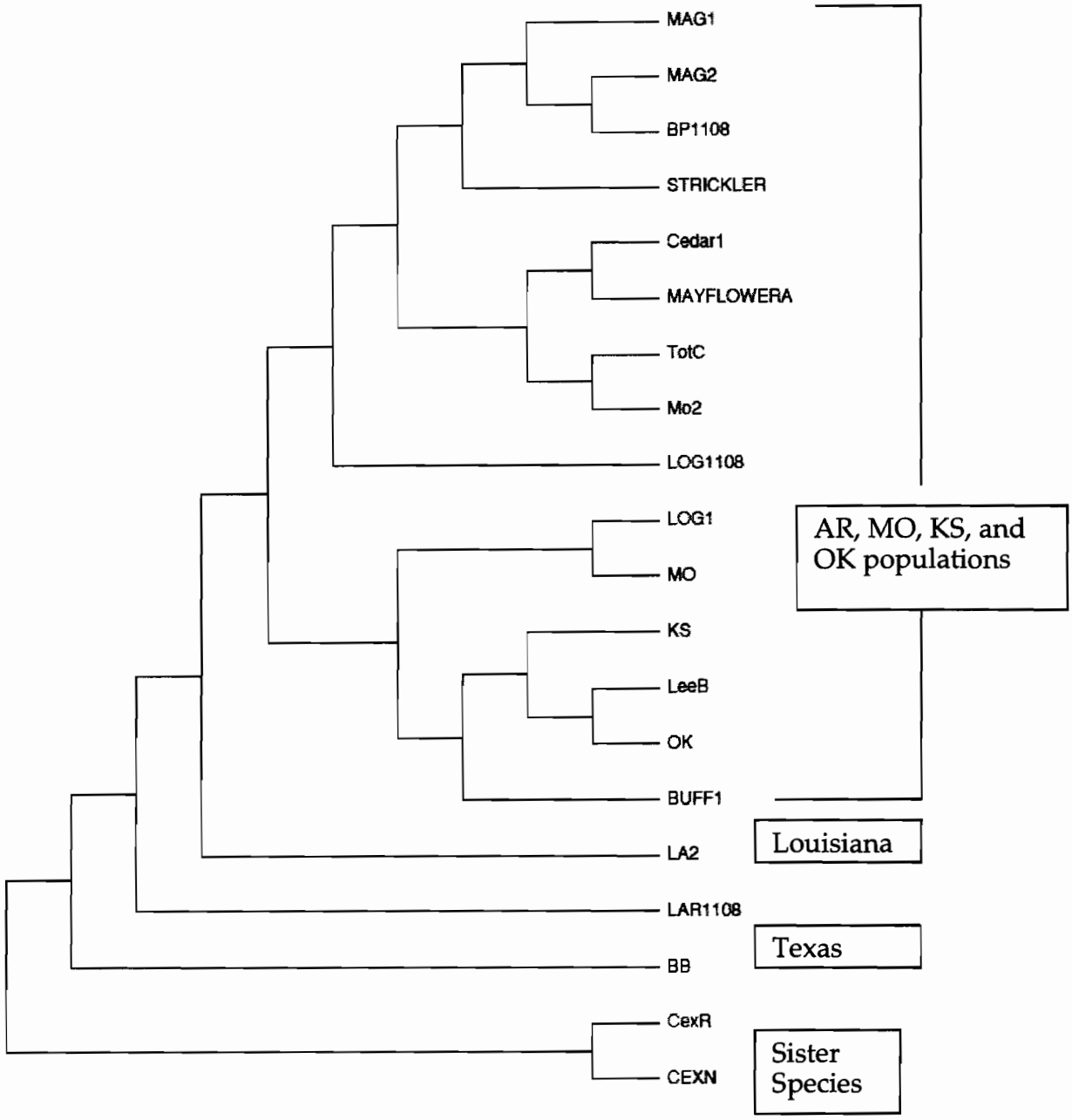

Figure 1. A phylogenetic tree created from DNA data. This tree suggests *C. vittatus* populations in the north are more genetically alike than those in the south.

OFFICE OF ACADEMIC AFFAIRS

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1509 North Boulder Avenue
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November 5, 2004

Dr. Tsunemi Yamashita
Associate ~~Assistant~~ Professor of Biology
Arkansas Tech University
McEver, Room 10
Russellville, AR 72801

Dear Dr. Yamashita:

Congratulations! Academic Affairs is pleased to announce your application for the Spring, 2005 Faculty Research Grant has been recommended by the Faculty Salary, Benefits, and Awards Committee. Based on this recommendation, Academic Affairs has approved the $1,600 budget for your research of molecular biology of the striped scorpion-population genetic structure & toxin gene analysis. Requisitions regarding the grant will be processed through your Dean's office and should be expended by June 30, 2004.

Your research on this project is sure to not only benefit your department, but Arkansas Tech University as a whole. We wish you success with this endeavor.

Sincerely,

Jack Hamm
Vice President for Academic Affairs

Copy: Dr. Charlie Gagen
Dr. Richard Cohoon
File

Jennifer Fleming

From: Richard Cohoon [Richard.Cohoon@mail.atu.edu]
Sent: Monday, October 18, 2004 10:22 AM
To: jennifer.fleming@mail.atu.edu
Subject: Yamashita's research grant propopsal

Ms. Fleming:
I fully support and recommend Dr. T. Yamashita's research proposal. It is a continuation of the research project he has been working on for the last two years with support from UAMS-BRIN.

R.R. Cohoon
Dean, P&LS
