

UNIVERSITY OF MASSACHUSETTS AMHERST
GRADUATE SCHOOL DISSERTATION FIELDWORK GRANT APPLICATION

A completed Dissertation Fieldwork Grant Application includes this form and a letter of recommendation from your faculty advisor. Send application materials or questions to researchgrant@grad.umass.edu.

Name (Last, First, Middle; as it appears in SPIRE): Lama, Tanya, Marjorie

Student ID #: 28295140

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College or School:

- | | |
|---|--|
| <input type="checkbox"/> College of Education | <input type="checkbox"/> College of Nursing |
| <input type="checkbox"/> College of Engineering | <input type="checkbox"/> College of Social & Behavioral Sciences |
| <input type="checkbox"/> College of Humanities and Fine Arts | <input type="checkbox"/> Isenberg School of Management |
| <input type="checkbox"/> College of Information & Computer Sciences | <input type="checkbox"/> School of Public Health & Health Services |
| X College of Natural Sciences | <input type="checkbox"/> Other (specify) Click here to enter text. |

Department/Degree Program: Environmental Conservation/ PhD

In what semester did you begin the PhD program? Fall 2015

Name of Graduate Program Director: Timothy Randhir

Email for Graduate Program Director: randhir@eco.umass.edu

Name of Faculty Supervisor/Advisor: Stephen DeStefano

Email for Faculty Supervisor/Advisor: sdestef@eco.umass.edu

Name of departmental bookkeeper/financial administrator: Linda Fortin

Email for departmental bookkeeper/financial administrator: lsfortin@eco.umass.edu

Amount Requested (between \$1,000 and \$5,000; round to nearest \$): \$4,976

Date (month, year) you expect to begin the proposed research: June, 2018

Date (month, year) you expect to complete the proposed research: December, 2018

Where will your proposed dissertation research take place? Include all locations:

Maine Department of Inland Fisheries & Wildlife Field Station (Bangor, Maine)

Does your research involve any of the following? Please check all that apply.

- ☐ Research with human subjects
- ☐ Research with vertebrate animals
- ☐ Travel outside the United States

By initialing below I verify that I have not previously received a UMass Graduate School Dissertation Fieldwork Grant. I further agree that if a grant is provided for this project I will (1) use the funds for the purposes outlined in my budget; (2) submit proof of IRB approval if the research involves Human Subjects before funds will be released; (3) provide the graduate school with a written report indicating how funds were spent and return unused funds; and (4) acknowledge this grant in all documents, papers, and publications associated with the research supported by the grant.

Applicant's Initials: TL

Date:02/28/2018

Project Abstract

The Canada lynx (*Lynx canadensis*) is a widely-distributed, native North American felid that exists at low-densities in boreal forests capable of supporting its primary prey source, the snowshoe hare. As a habitat and prey specialist listed as threatened under the Endangered Species Act, Canada lynx are vulnerable to adverse impacts of habitat fragmentation, climate change, and competition from generalist carnivores like bobcat. Management of lynx populations has traditionally been informed by genetic analyses using a small number of neutral markers. However, the complexities of managing this species demand more efficient tools and high-resolution data. We have produced the first reference genome for Canada lynx – an advancement in conservation science that allows genetic variation to be characterized across thousands of genes. At this juncture, we are seeking support for genetic analyses that will provide reliable estimates of population size, structure, connectivity, and viability for wildlife managers.

Project Budget

1. Amount Requested (between \$1,000 and \$5,000; round to nearest \$): \$4,976

2. What is the estimated cost for ALL dissertation research? \$270,000

3. If this amount is greater than the amount requested in this Dissertation Fieldwork Grant please explain how the balance will be covered. (e.g. external funding, out-of-pocket, advisor start-up funds):

This research is extramurally funded by Maine Inland Fisheries & Wildlife with in-kind matching contributions from the U.S. Geological Survey, Smithsonian Institution, and Rockefeller University's Vertebrate Genome Laboratory. Funds have been allocated for the sequencing, assembly, and annotation of a reference genome for Canada lynx. The support requested here would maximize the utility of the reference genome by further characterizing genomic variation in lynx across their geographic distribution and would contribute greatly to the conservation and management of wild populations.

4. Do you have other funding applications under review to cover the research costs outlined in this grant? No

5. Since beginning your graduate education at UMass Amherst, what forms of financial support (e.g. TA/RA position, departmental or university fellowship, external grant or fellowship) have you received?

I have received full-time (20h) research assistantships and anticipate continued support through my graduation (Spring 2020). I have also been awarded the Silvio O. Conte Memorial Scholarship and gained professional experience and financial compensation through externships with the US Fish & Wildlife Service and US Geological Survey. I have not yet fulfilled my required one-semester teaching assistantship.

<u>Budget Category</u>	<u>Amount (round to nearest \$)</u>
Travel as itemized and explained in Budget Justification below (including each destination and associated expenses [e.g. airfare, ground transportation, excess baggage fees, visa fees])	\$407
Living Expenses as itemized and explained in Budget Justification below (including each destination and associated expenses [e.g. food, lodging, other expenses])	0
Supplies and Equipment as itemized and explained in Budget Justification below	\$1,209
Communication (e.g. local cell phone at field site(s), printing; duplication; postage) as itemized and explained in Budget Justification below	0
Services/Personnel (e.g. transcription; gifts or payment to research subjects) as itemized and explained in Budget Justification below	\$3,360
Other Costs as itemized and explained in Budget Justification below	0
TOTAL REQUESTED (this should match your response to Question #1 above)	\$4,976

Budget Justification

Travel (\$406.56)			
<i>Month(year)</i>	<i>Description</i>	<i>Mileage</i>	<i>Cost*</i>
June-18	Travel to/from Amherst, MA and Bangor, Maine	614	\$331.56
June-18	Fuel		\$50
June-18	Tolls		\$25
Total Cost	\$ 406.56		

*Cost of mileage was calculated using a rate of \$0.54/mile.

Supplies and Equipment (\$1,209)			
<i>Product</i>	<i>Description</i>	<i>n</i>	<i>Cost per unit</i>
QIAamp DNA Mini Kit (50)	50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	3	\$165.00
QIAamp DNA Mini Kit (250)	250 DNA preps: 250 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	1	\$714.00
Total Cost	\$1,209		

Services/ Personnel (\$3,360)			
<i>Service</i>	<i>Description</i>	<i>n</i>	<i>Cost per unit</i>
Nextera DNA Library Preparation	Preparation of an organism's total genomic DNA for next generation sequencing, with a low DNA input requirement.	6	\$110.00
Whole genome sequencing	Sequencing on an Illumina HiSeq2000 machine	6	\$450.00
Total Cost	\$3,360		

Project Proposal

Due to their elusive nature and low population densities, wild cats are especially difficult to study at meaningful scales, and thus, demographic data are often unavailable to guide appropriate conservation action or test hypotheses regarding population viability (Gompper et al. 2006). Listed as threatened under the Endangered Species Act, the Canada lynx (*Lynx canadensis*) is a widely-distributed North American felid that exists at low-densities in boreal forests capable of supporting its primary prey source, the snowshoe hare. As habitat and prey specialists, lynx populations are broadly exposed and highly vulnerable to adverse impacts of habitat loss and fragmentation, climate change, and competition from generalist carnivores like bobcat (Peers et al. 2013). Species of concern often have small population sizes with low genetic diversity and are vulnerable to local extinction without management action. Traditionally, management of lynx populations has been informed by genetic analyses that rely on a small number of neutral markers (e.g. microsatellites). However, the poor resolution offered by these markers has failed to produce reliable information for wildlife managers. Given the complexities of managing lynx and mitigating the threat of local extinction, more efficient tools and high-resolution data are needed to inform management decisions. This project uses whole genome data to enhance the resolution and inferential power of traditional conservation genetic assessments for informed management of six lynx conservation units in the United States (Northern Maine, Northeastern Minnesota, Northwestern Montana/Northeastern Idaho, North-central Washington, the Greater Yellowstone Area, Western Colorado). We seek to test the hypothesis that these six units are acting as a single population (panmixia) connected by long-distance dispersers and genetic interchange with the core lynx population in central Canada. Second, we aim to inform conservation strategies by describing the genetic diversity, genetic differentiation, and population health of each unit. Ultimately, our goal is to provide a scientific basis for the management of Canada lynx populations and translate our findings into actionable conservation strategies for managers.

Recent technological advances in genetics have enabled the investigation of thousands of molecular markers. This "big-data" revolution is collectively termed *genomics* and has been made possible by two key technologies being widely adopted among conservation geneticists: 1) next-generation sequencing (NGS) and 2) high-throughput genotyping of single nucleotide polymorphisms (SNPs). When applied to conservation, genomics enhances the power of genetic assessments and dramatically impacts how we manage, conserve, and recover populations of threatened and endangered species. The drastic reduction in NGS costs has made it possible for conservation geneticists to sequence the entire genome or several genomes of a non-model species (e.g. the giant panda; Li et al. 2010; Zhao et al. 2013). Complete genomes allow biologists to measure (rather than estimate) genetic variation, which can be monitored and managed closely in small populations. Genomic approaches significantly enhance the resolution of traditional genetic assessments including describing population structure and function, estimating gene flow and dispersal rates, effective population size, number of breeders, and

testing hypotheses on the impacts of fragmentation and disturbance on population viability (Allendorf et al. 2010). Results guide species management actions such as habitat restoration, translocations, re-establishment programs, and long-term genetic monitoring plans.

A primary impediment to the effective use of genomics in conservation is the need to assemble a coordinated group of experts capable of integrating expertise from field biology, conservation planning, genetics and bioinformatics. Consortia such as the Genome 10K Initiative (Koepfli et al. 2015) have been critical in advancing non-model genome studies by fostering multi-disciplinary collaborations and translating findings into recommendations for managers and actionable conservation strategies. We have successfully engaged academic researchers, State and Federal agency biologists, and non-profit partners in the production of a complete genome sequence from a Canada lynx in northern Maine. Here, we are asking for funds to complement and expand the lynx research by characterizing genetic variation from at least one lynx in each of the remaining five conservation units (Minnesota, Montana/Idaho, Washington, Greater Yellowstone Area, Colorado). Without these additional data, we cannot conduct any comparative measures among the six lynx conservation units and our assessments of genetic diversity, differentiation, and population health will be limited to lynx in Northern Maine. Consistent evaluation of the six units will enable transboundary research and management strategies critical to conservation of broadly-distributed, low-density, long-distance dispersing carnivores. Further, comparative measures may aid in the prioritization of management for conservation units that are most at-risk of local extinction.

Methods

The field of genomics and bioinformatics is rapidly evolving and consequently the methods and state-of-the-art sequencing technologies and analytical software continue to evolve. Methodology at the time of data analyses may change accordingly but will be very similar to what we propose here. All proposed work will take place at the Maine Department of Inland Fisheries and Wildlife Field Office in Bangor, Maine. Data analyses and workflows will leverage an established partnership with the Genome 10K Initiative (www.genome10k.soe.ucsc.edu) and the Vertebrate Genome Laboratory at Rockefeller University, including bioinformatics support, training, and analytical pipelines for data management, genome assembly, annotation, and visualization. The resources available among our consortium of partners will enable efficient data analysis and large-scale knowledge discovery.

Our approach is robust and builds on conservation genomic approaches successfully applied to other non-model organisms (e.g. polar bear, Malenfant et al. 2015; river otter, Stetz et al. 2016). DNA will be extracted from tissue samples using standard extraction kits, fragmented, and then sequenced on a next-generation sequencing platform (e.g. Illumina). We will conduct low-coverage (10X) whole genome re-sequencing of at least one lynx from each of the remaining conservation management units. These data will be aligned to our draft genome assembly, forming an ascertainment panel from which we will characterize genome-wide

patterns of variation including polymorphisms, mutations, and structural variants. A subset of ~20,000 loci distributed across all chromosomes will be selected and validated based on parameters and location within functional genes and gene pathways. This comprehensive set of SNP markers will be used to carry out genome-wide analyses of demography, evolution, and population viability. We will 1) identify historically significant population bottlenecks, founder events, and migrations; 2) describe genomic diversity relative to short- and long-term viability and adaptive potential; 3) assess deleterious variants and substitutions as signatures of genetic erosion (inbreeding, introgression) compromising fitness; 4) map regional patterns in structure.

Statement of Need

Obtaining funds to pay for travel, genomic DNA extraction, and genome sequencing is critical to meeting the research objectives of my project. I have secured funds and successfully generated a high-quality reference genome for Canada lynx based on a sample from Northern Maine. Furthermore, I have engaged a multidisciplinary team that is successfully bridging the gap between research and conservation practice. Without genome data from each of the remaining five conservation units (Minnesota, Montana/Idaho, Washington, Greater Yellowstone Area, Colorado) we cannot conduct any comparative measures among the six units, and our assessments of genetic diversity, differentiation and population health will be limited in scope. A consistent approach to evaluating the status of the six units will help prioritize conservation action for populations most at-risk of local extinction, and second, will enable a common and shared approach to monitoring these populations going forward.

Literature Cited

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- Murray DL, Steury TD & Roth JD (2008) Assessment of Canada Lynx Research and Conservation Needs in the Southern Range: Another Kick at the Cat. *Journal of Wildlife Management*. 72(7), 1463–1472.
- Peers MJL, Thornton DH & Murray DL (2013) Evidence for large-scale effects of competition: niche displacement in Canada lynx and bobcat. *Proceedings of the Royal Society*. 280 (1773), 2013–2495.
- Zhao S, Zheng P, Dong S, et al. (2013) Whole-genome sequencing of giant pandas provides insights into demographic history and local adaptation. *Nature Genetics*. 45(1): 67-71.