10/31/2017
Joseph Goergen
Coordinator - Science Based Conservation Programs and Research
SCIF Conservation Department
501 2nd St NE
Washington, DC 20002

RE: Safari Conservation International Foundation grant submission

Dear Mr. Goergen,

We at the U.S. Geological Survey, Smithsonian Institution, Maine Department of Inland Fisheries and Wildlife, U.S. Forest Service, and the University of Massachusetts look forward to the opportunity of partnering with you to improve monitoring and management of Canada lynx and bobcat populations in North America. Using the very latest population genetic approaches, we will provide more reliable methods for understanding past demographic events, estimating effective population sizes, and assessing regional patterns and associated landscape dynamics. Along with our partners, we will use these data and tools to provide more detailed and better informed regional and range-wide conservation strategies for managing, protecting, and recovering wildlife populations. The framework and methods employed in this proposal will place public trust resource management agencies in the vanguard for application of population estimation approaches that meet standards of peer review and other external scrutiny. Longer-term, the objective of this project is to provide conservation practitioners, particularly State wildlife agencies, with powerful tools to sustain our natural resources.

Since 2016, we have been working to sequence, assemble, and annotate a reference genome for Canada lynx with a multi-disciplinary group of collaborators in field biology, genetics, bioinformatics, and conservation planning. At this juncture, we are organizing to expand the utility and impact of our research tools and findings to inform management and conservation of both Canada lynx and bobcat. The two species, although closely related, have different needs and face unique natural and regulatory challenges that require diverse strategies. Both species would benefit from a better understanding of the health, connectivity, and long-term viability of their populations. Without the support of the Safari Club International Foundation, we would not be able to achieve these additional goals.

Our proposal requests $148,813 USD in funding over three years to leverage ongoing research in development of more-efficient and effective tools for monitoring and managing Canada lynx and bobcat populations. Importantly, we have samples in hand, agreements with partners, and access to reference samples and other resources from the Smithsonian Institution. We appreciate the SCIF taking an interest in our approach and in possibly supporting conservation through the expansion of this work. Please contact me at (413) 687-5789 if you require any further information or have any questions concerning this proposal.

Thank you,
John F. Organ, Ph.D.
Chief, USGS Cooperative Fish & Wildlife Research Unit Program
& Adjunct Associate Professor, Department of Environmental Conservation
160 Holdsworth Way, University of Massachusetts
Amherst, MA 010

A genomic toolbox to enhance management of extant Canada lynx and bobcat populations

Authors: John Organ[1,6], Warren Johnson[2], Stephen DeStefano[3,6], Michael Schwartz[4], Jennifer Vashon[5], Tanya Lama[3,6]

[1] U.S. Geological Survey Cooperative Fish & Wildlife Research Units Program
[2] Smithsonian Institution
[3] Massachusetts Cooperative Fish & Wildlife Research Unit
[4] U.S. Forest Service National Genomics Center for Wildlife & Fish Conservation
[5] Maine Department of Inland Fisheries & Wildlife
[6] University of Massachusetts Amherst

Abstract/Project Summary

Advancements in genome science have the potential to dramatically impact how we make decisions about managing, conserving, and recovering wildlife populations. Management of wildlife populations is often made in the absence of reliable population estimates or metrics of population structure, connectivity, and viability. To address this challenge, we propose the development of an analysis toolkit that can be used by the broader conservation community to address fundamental genetic questions about wild populations of Canada lynx and bobcat. Management strategies for the two species differ significantly, as the former has a distinct population segment in the lower-48 states currently protected under the Endangered Species Act and a harvested population in Alaska and Canada, while the latter is a widely-distributed generalist that is harvested during hunting and trapping seasons in states across its distribution. We will demonstrate the utility of this toolkit to inform management strategies for both species by translating our findings into actionable recommendations for managers. Population monitoring and management have generally been based on harvest records, labor intensive radio-telemetry studies, and a small number of genetic markers. However, the complexities of managing contemporary wildlife populations demand more efficient tools and high-resolution data. Our approach takes advantage of important advances in genome science. Genetic variation can now be characterized across thousands of genes distributed throughout the genome. Assays based on these markers are robust, easy to standardize and can be linked with important adaptive traits and other indicators of population health.

Introduction

Carnivores in general are inherently difficult to survey because of their large home ranges, low population densities, and nocturnal activity. Due to their elusive nature, wild cats are especially difficult to monitor and study at a meaningful scale, and thus, demographic data are often unavailable to guide appropriate conservation action or test hypotheses regarding population viability (Gompper et al. 2006). The bobcat *(Lynx rufus)* is widespread across North America but exists at low densities at the edges of its distribution in the northern United States (Anderson and Lovallo 2003) where it appears to be repopulating historically occupied regions (Roberts and Crimmins 2010, Linde et al. 2012). Bobcats in the United States are taken by both trapping and hunting. State agencies manage populations by adjusting harvest length, timing, intensity, and method, often relying on data from mandatory or voluntary surveys and submission of biological samples (tissue, tooth, or carcass) by hunters and

trappers. Although baseline information on population size, structure, and connectivity and detailed modeling of population sustainability and adaptive potential are largely unknown across most of the bobcat range, many states are well positioned to help address these knowledge gaps as they already collect the necessary samples to estimate bobcat population abundance and reconstruct long-term trends in demography and structure.

Similarly, basic metrics including regional abundance estimates are largely unknown among U.S. populations of Canada lynx *(Lynx canadensis)*, which exist non-contiguously at the southern extent of the species distribution and present a different set of conservation challenges. Listed as threatened under the Endangered Species Act (Federal Register, 2000), State agencies are tasked with protecting lynx from "take" and participating in all aspects of developing and implementing recovery and monitoring programs. Significant gaps in our knowledge (e.g., population estimates, connectivity, genetic adaptability) make it difficult to design effective management strategies and accurately quantify species recovery. States such as Maine, in which bobcat and lynx co-occur, have an extensive collection of lynx handled during the annual fur-trapping season. Trappers comply with State guidelines to mitigate "take" by reporting incidental capture of lynx, allowing biologists on call to document the animal's condition and collect biological samples such as blood, tissue, and hair prior to release.

The reliance of managers on limited data, generally based on hunter success (e.g., take per unit effort) as an index to population size and trend (e.g., increasing, stable, decreasing) ultimately makes it harder to defend policy decisions and communicate effectively with the broader community. For example, animal rights advocates continue to challenge the scientific defensibility of furbearer management, often through the popular referendum process that exists in 24 states. These referendums, such as "Oregon Trapping Ban Initiative" (2014, 2016, denied), "Massachusetts Ban on Leghold Traps Initiative, Question 1" (1996, approved) and "Colorado Prohibited Methods of Taking Wildlife, Initiative 14" (1996, approved) jeopardize sustainable harvest of fur as a natural resource, vilify the hunting and trapping community, and compromise public trust in State wildlife agencies. For example, a coalition of anti-hunting groups recently took the first steps toward a 2018 ballot initiative that would outlaw the hunting and trapping of both bobcat and mountain lion *(Puma concolor)* in the state of Arizona.

Given these needs and challenges, a reliable set of tools, baseline metrics and efficient models are needed to make informed management decisions. Metrics based on robust monitoring of bobcat and Canada lynx populations in near real-time, and at a meaningful scale would include robust estimates of demography, connectivity, and adaptive potential (genetic variation). We propose to design and implement these relatively inexpensive and vastly more-informative tools using new molecular approaches being pioneered in the field of conservation genomics. Designing tools applicable to both species will increase their utility for conservation management and provide additional context for conservation planning, especially in areas where they both occur. Additionally, our proposed methods will provide valuable information as to the separation and speciation of lynx and bobcat and the factors that influenced it (Johnson et al. 2006). Finally, we will document at a fine scale the extent and patterns of bobcat/lynx hybridization beyond the F1 generation, between a pure lynx and a pure bobcat, and assess the genetic consequences of interspecies hybridization on fitness and local adaptation (Schwartz et al. 2004).

Recent scientific studies (polar bear, Malenfant et al. 2015; river otter, Stetz et al. 2016) have demonstrated the potential of these genomic methods to inform conservation strategies to 1) maintain meta-populations on a mosaic of private and public lands; 2) monitor population dynamics through

shifting environmental conditions and land-use patterns; 3) manage isolated and endemic populations; 4) model population structure and local adaptation for better design and implementation of translocation, reintroduction, and recovery strategies; 5) provide fast and accurate genetic testing for law enforcement; 6) document past and ongoing patterns of hybridization and resultant impacts on fitness and local adaptation; 7) directly assess physiological changes in gene expression related with changes in habitat, disease, pollution, disturbance, and other perturbations; 8) directly measure effects of habitat fragmentation and urbanization on isolated populations, including changes in connectivity, local adaptation, and inbreeding; and 9) provide tools for modeling future change based on management decisions and other external impacts.

To date, a primary impediment to the effective use of genomics in conservation is the need to assemble a coordinated group of experts capable of integrating field biology, conservation planning, genomics, molecular biology, and computational and analytical methods. 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 (e.g., the critically endangered Iberian lynx). Our approach focuses on similar multidisciplinary collaborations and engagement among academic researchers, State and Federal agencies, and non-profit partners. We will interact and partner with conservation practitioners and user groups to help develop relatively inexpensive and versatile tools that can be adapted as baseline knowledge. Our project will provide the tools and data to obtain recurring, reliable metrics for conservation applications. Coupled with public outreach, this approach will help foster broader societal interest in science and trust in science-based management decisions enacted by managers at state agencies. Our proposed consortium and the tools it produces will make lynx and bobcat an exemplary model of scientifically robust wildlife management. The framework of the project and its resultant products will serve as a roadmap for the management of other widely-distributed fur-bearing species (e.g., beaver) and species of special conservation importance.

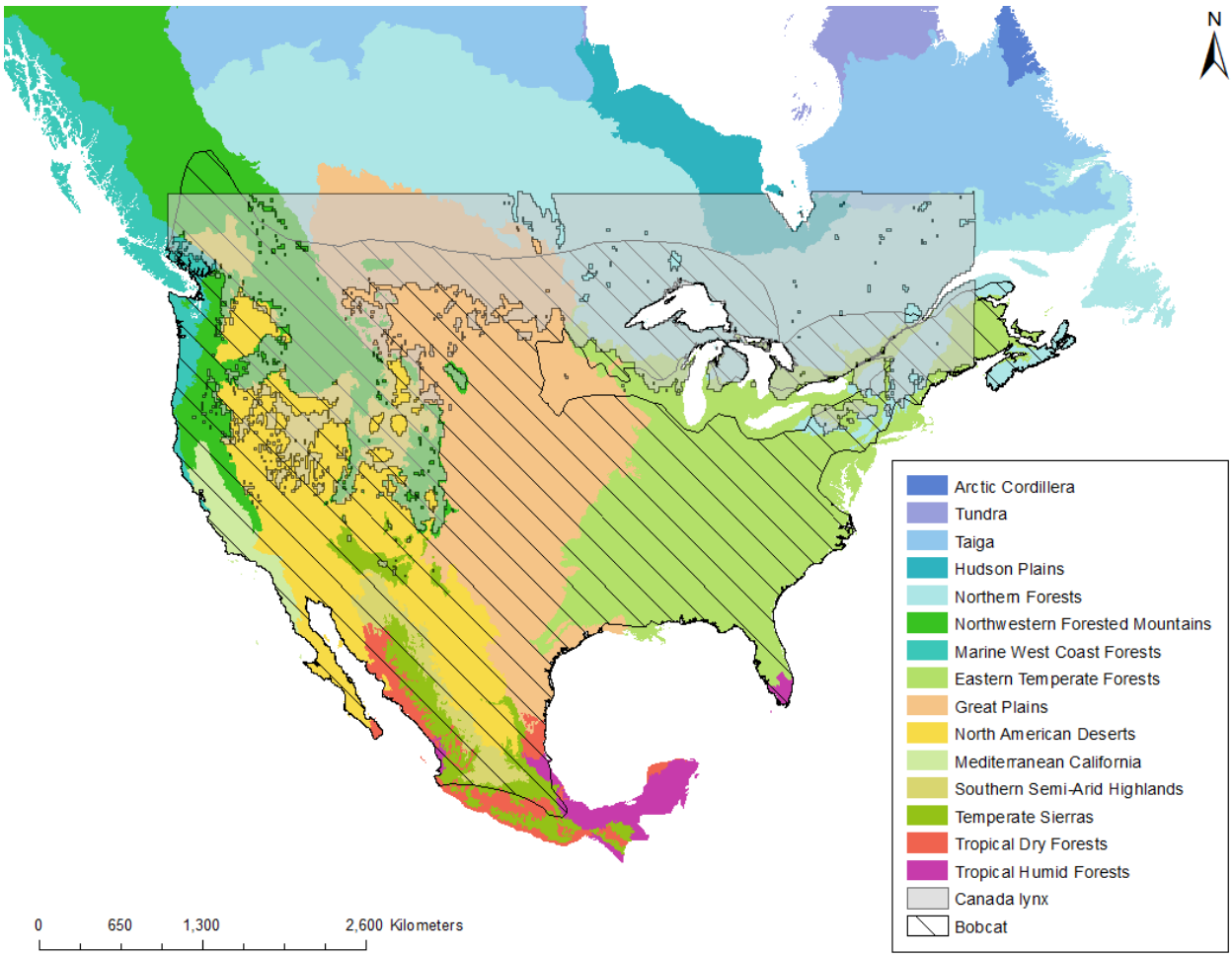

Figure 1. Historic ranges of bobcat *(Lynx rufus)* and Canada lynx *(Lynx canadensis)* populations, delineation of level 1 ecological regions of North America (Commission for Environmental Cooperation 1997).

Goals

Through this project we will 1) perform innovative conservation research; 2) provide conservation practitioners with powerful tools and novel insights on contemporary and historic bobcat and lynx populations; 3) design and implement scientifically defensible monitoring systems and models to better inform management decisions; and 4) increase familiarity with and use of new genomic approaches for monitoring and managing natural resources.

Objectives

Our objectives are centered on a series of challenging but fundamental questions relative to bobcat and Canada lynx species conservation. We will produce a new generation of tools for science-based management and monitoring, facilitate cross-disciplinary collaboration, and translate science into actionable conservation strategies. We will:

1. Develop a suite of genetic tools, models, and metrics to produce baseline estimates of population size, structure, and viability for reliable long-term monitoring.

2. Assess how population structure and demography among populations are affected by disturbance (e.g., urbanization, habitat conversion, fragmentation, and climate change).
3. Determine the adaptive capacity of populations under varying conditions including habitat availability, quality and connectivity, population size, and disturbance.
4. Provide genome-scale platform and data for more-sophisticated and robust studies on how individual animals respond to environmental changes and disturbances (e.g., pollution, disease, toxicity, habitat)

Methodology

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. The samples needed are currently held at the Smithsonian Frozen Collection, Maine Department of Inland Fisheries and Wildlife, and National Genomics Center for Wildlife & Fish Conservation. 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, with available 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.

Developing reference genomes for Canada lynx and bobcat

A reference genome represents the complete nucleotide sequence for all chromosomes in the species of interest – essentially serving as a physical map of its contents (genes). We are currently constructing a high-quality genome of a male Canada lynx with support from Pittman-Robertson Wildlife Restoration Program monies granted to the State of Maine Department of Inland Fisheries and Wildlife. The overarching goal of that project is to inform management and recovery strategies for lynx using genomic data. Here, we are asking for funds to complement and expand the lynx research by designing a genomic toolbox with utility for *both* bobcat and lynx management. Crucial to that goal, we propose the construction of a high-quality genome specific to bobcat and the development of tools (assays) that will be relevant to the management of both species across their respective population distributions.

Our approach is robust and has been successfully applied to other model organisms (Abascal et al. 2016). DNA will be extracted from high quality tissue samples using standard extraction kits, fragmented, and then sequenced using a combination of next-generation platforms (e.g., Illumina and 10X; Illumina and PacBio). We propose a whole genome shotgun sequence (WGS) strategy designed to yield a long-range, high-quality assembly with near complete coverage of the bobcat genome. We propose collecting additional paired-end reads generated from various insert-size libraries (e.g., 200bp, 500bp, 5kb). By using different library sizes, we hope to minimize bias and use a hierarchical approach in the assembly process.

Computer algorithms will be used to analyze the genome using multiple approaches. We will initially piece the sequence reads together into a continuous stretch of sequence (*de novo)*. Using our knowledge of the Canada lynx genome as a reference for aligning the reads, we will proceed with an assisted assembly. Gaps in this patchwork of sequence reads will be closed using longer reads that span the length of the sequence to help fill in any missing base-pairs. To harness the full potential of our genome, we will annotate using genome-mapping techniques. Genome annotation is the

computationally complex process of attaching biologically relevant information to genome sequence data. This can range from gene models, functional information, pathways, or epigenetic modifications using lines of evidence from genomes of other well-studied species, such as the domestic cat *(Felis catus)*, dog (*Canis familiaris*), and human.

Characterizing genome-wide patterns of variation

Once the initial sequence and assembly of the genome is available, we will enrich the assembly by characterizing genome-wide population-level patterns of variation, and identifying polymorphisms, mutations, and structural variation. We will conduct low-coverage (20X) whole genome re-sequencing from a panel of individual lynx and bobcat sampled across their respective distributions. For bobcat, we aim to incorporate animals from each of the 13 proposed subspecies and a representative variety of occupied habitats (Figure 1). For lynx, we aim to incorporate samples from each of the 14 states in the contiguous US range with historically or currently resident lynx populations and from representative Canadian provinces. Specifically, we will propose generation of ~100,000 reads from each population. These reads will be aligned to our draft genome assembly, forming an ascertainment panel from which over a million SNPs (single nucleotide polymorphisms or base-pairs that differ among individuals) will be discovered throughout the genome. A subset of ~20,000 SNPs 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 compromising fitness; 4) map regional patterns in structure.

Designing optimized SNP panels for conservation

A major advantage of using genomic methods is that these approaches allow us to more reliably estimate effective population size, describe population structure, detect migration, and assign local ancestry at contemporary timescales of one to 20 generations. To optimize marker-assisted monitoring and management, we will select a panel of the most informative SNPs from which we can designate subsets optimized for the following tasks typical in conservation applications: population size, structure, and adaptive potential. This will be an iterative process requiring input from academics and State agency biologists to ensure maximum utility of these tools. The first iteration of the array will be used in Year 1 to provide coarse, regional scale estimates of the size, structure and viability of a regional bobcat population (e.g. southwest including Arizona). Additional testing and refinement of the array will take place in Years 2 and 3. Each marker will be genotyped and an assay will be designed to test large volumes of samples at that specific SNP, insertion, or deletion of interest. Ultimately, we will leverage findings to infer future population change under various environmental scenarios, particularly relative to climate change and urbanization.

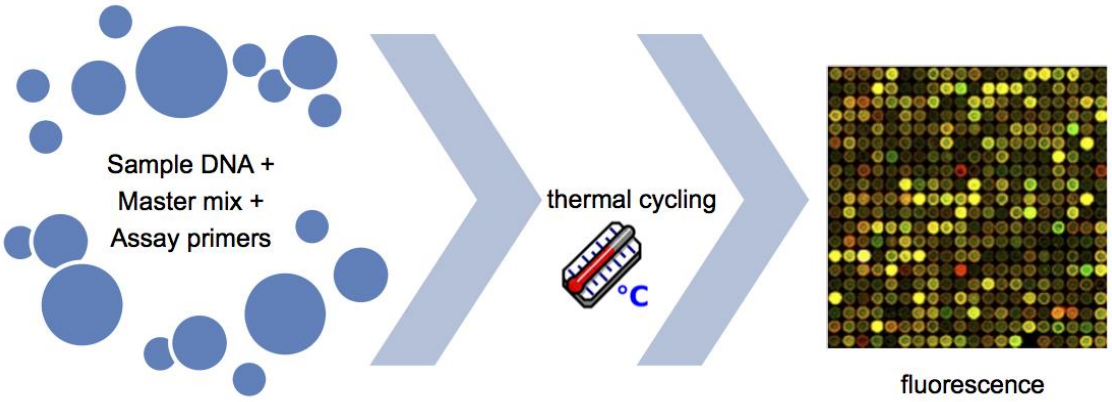

Figure 2: Genotyping assays use three components: sample DNA, allele-specific primers, and buffer solution. The three components are combined on a 96-well PCR plate, and undergo thermal cycling followed by an end-point fluorescent read on a qPCR instrument. The allele-specific primers each correspond with a fluorescent signal.

1) Population size

Effective population size (Ne) is a key population genetic parameter that describes the amount of genetic drift in a population. We will use SneP (a software toold) to estimate baseline metrics for monitoring population status and trend and gauging the scope and rate of declines in viability. This method is reported to perform well in non-ideal populations (e.g., skewed sex ratio, non-random mating, or small sample size) and will provide a reliable framework for management decisions relative to harvest season length, timing, and tag limits. This component of our research design will specifically catalyze engagement with state and federal partners. It is our goal for this information to improve state implementation of bobcat management and protect/maintain/enhance public opportunities for use of the resource.

2) Population structure

Our analyses will assess the impact of fragmentation, small population size, and isolation to identify populations most at-risk, and direct conservation actions that mitigate inbreeding, isolation, or local extinction. We will ultimately use geographic information system (GIS) techniques to generate a map of connectivity and to better understand the specific biotic and abiotic factors influencing gene flow between subpopulations. Further, bobcats from distinct subspecies are currently managed for harvest as separate populations, primarily because of substantial differences in regional pelt values. We will analyze population structure to determine whether bobcat populations are subdivided into genetically discernible populations that support current management regulations at a regional scale.

3) Adaptive potential

Preserving adaptive genetic variation is a principle concern for conservation as it maximizes the potential for populations to respond to changing environmental conditions (e.g., climate change) and endure disturbances (e.g., disease). Adaptive variation can be identified through analysis of low-coverage (20X) genomic sequences. Genes influencing local adaptation will tend to have low genetic diversity in flanking regions and will be more highly differentiated between populations due

to divergent natural selection (Korneliussen 2013). We predict that small isolated populations will have high levels of deleterious variants – mutations which reduce fitness (Keller & Waller 2002; Reed 2005) and populations occupying unique habitats (e.g., desert) will show genetic evidence of local adaptation, highlighting the need for their conservation. Using full genome sequences, we can test these predictions. We can also identify genes that are likely under selection. We will study SNPs within functional genes to identify those which may be compromising fitness and predict the consequences of these mutations. This will provide a "map" of adaptive and deleterious variation within a given population – allowing us to identify populations with unique or critical local adaptations, and those which are most under threat of genetic decline and extinction. We will use population genetic models to assess the overall impact of deleterious variation on fitness and predict the future viability of populations in response to changes in population size and connectivity. It is our goal to translate these findings into recommendations for specific management practices that foster the conservation of each population's adaptive potential.

Observing responses to environmental change and disturbance

In order to more efficiently identify genes within the genome and compare patterns in gene expression across individuals, we will continue to collect blood samples for RNA sequencing (RNA-seq). Observing changes in gene expression can help us describe how individual animals are responding to environmental change in real time. Recently, RNA-seq has shown that short-term environmental challenges (e.g., extreme high or low temperatures characteristic of severe weather/climate change) can be mediated by changes in gene expression. RNA-seq involves the sequencing of RNA transcripts with the goal of estimating expression of all genes in a given tissue (e.g., brain, ovary, lung). Gene expression represents the first-line response to environmental challenges and is poorly researched in non-model organisms. We will perform RNA-seq analysis of individuals in targeted populations through blood sampling and tissue-specific collection. The transcriptomes will then be directly mapped onto our reference genome using a combination of currently available software (e.g., TopHat, Bowtie, BWA). Our goal is to determine if there are population-specific patterns in gene expression that vary across (and allow occupancy of) habitats unique in size, composition, quality, or disturbance (e.g. pollutants, toxicants, stressors). We will use ~5 replicates of each condition (e.g., small isolated population vs. connected core habitat). We predict that environmental stressors will affect gene expression and survival.

Project timeline & itemized budget

This project requires three years of support. We request the following support ($135,285) from Safari Club International Foundation to cover direct costs for supplies, sequencing and analyses, assay development and validation, and communicating findings. The multi-disciplinary nature of this project will be actively sustained by quarterly team conference calls organized and led by co-PI Johnson. These meetings will keep project leaders up to date on research progress and coordinate future directions. A critical deficit in conservation science is the translation of results into recommendations for management and policy. We will convey management recommendations specifically through annual meetings for resource managers and agency personnel as well as webinar communications aimed at conservation practitioners. These efforts will enhance direct communication between all parties.

The requested budget, supplementary to current resources from the Maine Pittman Robertson Wildlife Restoration Fund, will be sufficient for the activities described. However, we expect that there

will be immediate interest from existing projects to help evaluate our tools and gain early access to applying them in current studies. In these cases, we will develop cost sharing and collaborative agreements to ensure early access to the community. Upon the expiration of this funding, we envision developing an MOU with individual State agencies interested in continued population monitoring and expect that there will be opportunity and need to continue the development and refinement of these or similar tools for use in additional species of interest (e.g., mountain lion).

Year 1 ($44,855)

In the Year 1, we will focus on collecting samples, generating data, conducting genome-wide analyses and testing the first iteration of the array in a pilot study. We will collect samples for target bobcat and lynx populations across their respective distributions. Given that all or the majority of the samples are already in place, and the persistence of long-term field programs (e.g., Maine), we are confident that we can meet or exceed this deadline. We will quickly select an individual bobcat (adult, male) for high-coverage whole genome sequencing. Our computational and analytical partners at the Vertebrate Genome Laboratory will generate the sequencing data and complete the first draft assembly and annotation. This will be an iterative process. Every draft assembly and annotation is the result of a series of analytical processes and should accordingly be treated as a working hypothesis. While the bioinformatics team focuses on these computationally complex tasks, we will select 30 individual bobcat and Canada lynx for additional low-coverage genome re-sequencing. These reads will then be aligned to our draft genome assembly. We will proceed by characterizing genome-wide patterns of population-level variation (SNPs, insertions, deletions). Approximately 20,000 markers will meet minimum standards for inclusion in genome-wide analyses of demography, evolution, and population genetics. We will conclude Year 1 by designing 24 custom genotyping assays as the first iteration of our array. The array will be employed in a pilot study to provide estimates of population size, structure and viability of a regional bobcat population (e.g. southwest).

Supplies

Sample collection, storage, permitting, dry ice, shipping	**$1,200**
Genomic DNA extraction kit	**$1,500**
DNA quality assay	**$270**
Library preparation (multiple insert sizes) and quality control	**$9,200**
subtotal	**$12,170**

Sequencing and analyses

10X library preparation and sequencing	**$10,000**
Illumina HiSeq4000 sequencing $2850 per lane x 1	**$2850**
Standard analyses: read alignment, de-duplication, base quality recalibration, local realignment, SNP and insertion/deletion calling	**$400**
Genome assembly DISCOVAR *de novo*	**$1,000**

Genome annotation: gene prediction, repetitive elements, functional annotation	**$600**
Genome re-sequencing Illumina HiSeq4000	**$7,800**
Population-level variant discovery GATK & compute time	**$1000**
subtotal	**$23,650**

Assay development and validation (Year 1 pilot)

KASP by Design assay development	**$7,200**
KASP assay reagents	**$185**
Supplies, testing and validating assays	**$1650**
subtotal	**$9,035**

Year 2 ($45,530)

A final version of the draft genome will be used in Year 2 to assist with the analyses, low-coverage re-sequencing, assay development, and data interpretation. Our formal affiliation with the Genome10K initiative and Vertebrate Genome Laboratory provide access to the necessary computational expertise to develop the proposed products, and we expect other partners will be interested in other aspects of the analyses (e.g. detailed analyses of immune system genes, partners working on Iberian and Eurasian lynx conservation). We will focus on designing optimized SNP panels for conservation applications by selecting ~150 markers based on location and function. We will then genotype each marker (SNP, insertion or deletion) of interest and develop individual custom assays for each. This collection of individual assays will be arranged into a “panel” on a 96-well PCR plate (Figure 2). We will optimize subsets of assays into three “panels” designed for conservation applications -- assessment of population size, structure, and adaptive potential. We will then employ these optimized panels to assay high volumes of DNA samples from individual bobcat and Canada lynx at a fine, subpopulation scale. These results will be compiled into a map of connectivity, including biotic and abiotic factors influencing gene flow. Second, we will incorporate these findings into predictive modeling of population change under various environmental scenarios (e.g., climate change, habitat fragmentation).

Assay development and validation

KASP by Design assay development	**$43,000**
KASP assay reagents	**$930**
Supplies, testing and validation	**$1,600**
subtotal	**$45,530**

Year 3 ($44,900)

In Year 3, we will enrich our draft genomes with additional sequence data from RNA transcripts. We will sequence RNA transcripts from blood samples and organ tissues (e.g. brain and ovary/testes) from individual bobcat. RNA transcripts will be mapped onto the annotated genome assembly. We will then estimate the expression of genes in each given tissue and compare patterns in gene expression across individuals and populations. Population-specific patterns in gene expression will be described under various conditions (e.g. population size, connectivity, disturbance). We will interpret the impact of environmental stress on observed changes in gene expression and consequently, survival.

In Year 3 we will focus on communicating recommendations directly to State agency personnel at workshops and webinars, culminating in a joint meeting for the purposes of sharing results and defining future direction. These efforts will foster necessary communication between researchers and conservation practitioners. Review articles will outline general project achievements, key findings and novel insight from our research. Minimally, we can expect one high-profile submission, and in addition, a number of publications reporting regional findings and conservation applications, co-authored and led by various partners.

Sequencing and analyses

RNA sequencing	**$19,400**
Standard bioinformatics and exploratory analyses: FASTQ format output; read mapping and alignment; estimation of gene expression; differential gene expression analysis; pairwise comparison(s) for MRNA	**$400**
Functional annotation	**$600**
subtotal	**$20,400**

Travel and coordination

Joint meeting National Conservation Training Center, Shepherdstown, WV	**$13,500**
Conference travel	**$8,000**
Publishing	**$3,000**
subtotal	**$24,500**

Overhead ($13,528)

Matching funds in the amount of $66,966 will be applied to the project as waived overhead by the University of Massachusetts (i.e., Standard Overhead Rate of 59.5% - 10% = 49.5% waived overhead). We request $13,538 in additional support to cover the remaining 10% of the Standard Overhead Rate.

Additional support ($270,556)

$270,556 in funding have been awarded by the State of Maine (Pittman-Robertson Wildlife Restoration Fund) to cover direct costs apportioned as follows: $115,485 for a 3-year stipend and 20-hour graduate student appointment; $5,817 for Co-PI effort; $58,310 for genome sequencing and analyses; $37,674 for travel expenses; $2,121 for conference travel, and $1,500 for publications. Matching funds in the amount of $146,250 are applied through donated services. $72,000 in donated services have been awarded from the Smithsonian Institution (W. Johnson, 90 days @ $800/day) for technical direction. $63,972 have been awarded in waived overhead from the University of Massachusetts.

FY2016: $127,676
FY2017: $71,440
FY2018: $71,440

Intellectual contributions

The success of this project relies on strong collaborations and innovative application of molecular, computational, and analytical techniques principally used in studying the human genome. Our formal affiliation with the Genome 10k Initiative provides access to expert collaborators who have considerable experience with developing these products; co-PI Johnson serves as chair of the G10k mammal subcommittee. We will translate our findings into recommendations for management of a threatened species and monitoring of bobcat, both critical concerns given rising rates of habitat fragmentation and threat of climate change. Achieving the ambitious goals set forth in this proposal will require the assembly and coordination of experts across multiple disciplines and interests.

(PI) John Organ

Dr. Organ is the Chief of the USGS Cooperative Fish and Wildlife Research Units and previously served as Chief of Wildlife and Sport Fish Restoration for the Northeast Region of the US Fish & Wildlife Service. John is a certified wildlife biologist and past president and fellow of The Wildlife Society. He is also a professional member of the Boone and Crockett Club and Senior Specialist in the Fulbright Scholar Program. He is a member of the IUCN Sustainable Use and Livelihoods Specialist Group and the Sustainable Use of Wildlife Committee of the Association of Fish and Wildlife Agencies. John serves on the Advisor Board of the Conservation Leaders for Tomorrow Program. John initiated the Maine Canada lynx study in 1997 and served as Co-PI.

(Co-PI) Stephen DeStefano

Stephen DeStefano is Leader of the Massachusetts Cooperative Fish & Wildlife Research Unit (USGS) and Research Professor in the Department of Environmental Conservation, University of Massachusetts-Amherst. Dr. DeStefano directs research, education, and outreach applied to conservation biology and management, primarily for large mammals (deer, moose, bears) in cooperation with several agencies and organizations. As a member of the Cooperative Research Unit Program, DeStefano works closely with state and federal cooperating agencies to address issues of concern and provide research findings and consultation that can be applied to management and conservation of wildlife populations, habitat, and human-wildlife interactions. He serves as a Co-

Principal Investigator for the lynx project and handles issues related to UMass, federal, and state logistics and bureaucracy as necessary.

(Co-PI) Warren Johnson (technical direction)
PI-Johnson will be charged with monitoring progress and compliance with the timeline above. He is a conservation geneticist and evolutionary biologist at the Smithsonian Conservation Biology Institute and is the coordinator of the Smithsonian Institute for Biodiversity Genomics. His research spans multiple disciplines, including applied wildlife ecology, comparative genomics and molecular ecology and often addresses evolutionary questions in model organisms or natural populations with a strong biomedical or veterinary component. He has been involved in numerous multi-disciplinary projects, many focusing of felids species, and has published over 150 scholarly and popular articles with hundreds of collaborators.

(Co-PI) Michael Schwartz
Michael Schwartz is the Director of the US Forest Service's National Genomics Center for Wildlife and Fish Conservation, located in Missoula, Montana. He is also a Research Professor of Wildlife Biology in the College of Forestry and Conservation at the University of Montana. He received his Master's Degree in 1996 from American University, where he worked with the Smithsonian Institution's Center for Conservation and Evolutionary Genetics. He subsequently received his Ph.D. in Wildlife Biology in 2001 from the University of Montana. Dr. Schwartz's team at the National Genomics Center currently works on over 60 species ranging from bull trout and sage grouse to moose and wolverines. They are dedicated to using genetics to find broad scale conservation solutions.

(Collaborators) Jennifer Vashon & Cory Mosby
Jennifer Vashon serves as the Maine Department of Inland Fisheries and Wildlife species specialist for Canada lynx and Black bear. She joined the Maine Canada lynx Study in 1999 as project lead under John Organ and Craig McLaughlin. Cory Mosby serves as the species specialist for small mammals and bobcat for the state of Maine. Maine Inland Fisheries and Wildlife recently instituted new protocols to assess hunter effort and require mandatory tooth and tissue submission for genetic analyses of bobcat. Jennifer and Cory maintain working relationships with hunters and trappers throughout the state.

(Collaborator) Tanya Lama
Tanya is a PhD student at University of Massachusetts Amherst, advised by Drs. Organ, DeStefano and Johnson. She is a current member of the Massachusetts Cooperative Fish & Wildlife Research Unit with a MS (2016) and five years of professional experience with the US Fish & Wildlife Service and US Geological Survey as a Pathways Biological Career Intern. Tanya is a professional member of the Boone & Crockett Club, Conservation Leaders for Tomorrow graduate, and certified waterfowl identification and hunter safety course instructor.

Expected Outcomes (benefit to conservation & sustainable use. Tangible products, who benefits?

The key deliverables of the project are: 1) Generation of reference-quality, nearly gap-less, chromosomal level genome assemblies for bobcat and Canada lynx; 2) Publicly available annotated genomes for bobcat and Canada lynx; 3) A framework, sampling design, and analytical workflow to address testable hypotheses relative to bobcat and Canada lynx conservation; 4) Optimized SNP panels for conservation applications; 5) Range-wide map of connectivity and biotic/abiotic impacts on gene flow; 6) Population viability analyses; 7) Recommendations for management and policy conveyed to appropriate State, Federal, and non-profit entities; 8) Publications co-authored and led by members of our consortium.

State benefits

Significantly, the active involvement of state fish and wildlife agencies in this endeavor further reinforces their status as a trustee of wildlife resources. States have legal ownership of most wildlife under the Public Trust Doctrine (except for federal ownership rights reserved under the constitution for the federal government) and science is considered the proper tool for discharging wildlife policy (Leopold 1933:18; Organ et al. 2012). The application of highly advanced science tools to harvest (bobcat) and at-risk species (lynx) management will greatly assist states against challenges to their management programs by those purely opposed to harvest (e.g. by public referendum). Furthermore, greater precision in population estimation and variation will help ensure the sustainability of harvest programs for future generations. Additionally, the findings of this study should lead to development of conservation strategies for lynx that utilize landscape genetics to ensure connectivity of the contiguous United States populations to those in Canada. Such strategies could conceivably bolster scientific support for de-listing and return of lynx to state management authority. The application of science to actionable management that leads to recovery of an at-risk species and sustainable use of a related species will demonstrate to the public the value of science and the robustness of North American wildlife conservation.

Conservation genomics is a powerful tool with the potential to change the way we manage wildlife and fisheries populations, relevant to both State and Federal agencies. We anticipate developing an MOU with those interested in long-term population monitoring and/or continued refinement of tools or development of new ones for additional applications that would allow for long-term funding. There are many examples of this kind of arrangement, often accomplished through Cooperative Research Units (of which PI-Organ is Chief and PI-DeStefano is Unit Leader). PI Organ has already approached State leadership from (Maine, Massachusetts, Wisconsin) about their interest in such a program, and has written statements of enthusiastic support (attached; Supplementary Material). We anticipate working across agencies to develop long-term support for this work. Additional avenues for funding include NSF, USDA (Wildlife Services), EPA, NOAA, USFWS and the National Park Service. Additionally, a long list of NGOs (e.g., Panthera) will be interested in our findings at the conclusion of this three year period and may be interested in supporting future endeavors.

Academic benefits

Our project will provide the research community with a broad and powerful platform for basic and applied research. Our annotated, high-quality genome assembly will attract researchers generally focused more broadly on other species or on specific biological topics such as infectious disease. These genomic links will increase our ability apply research and methods from other species, including

humans, agricultural species, and species with similar management needs. By comparing the genomic sequences of species at different evolutionary distances, we can identify functional sequences and non-coding sequences with regulatory functions and determine which are unique to each species.

A reference genome helps efficiently identify new informative polymorphisms (SNPs) and address genome-wide analyses of demography, evolution, and fitness through intraspecific and interspecific comparisons. This project will provide a very strong catalyst for collaboration among the international community, including those working on similar species (e.g. the Endangered Iberian lynx and the heavily managed European lynx) or similar biological questions (e.g. hybridization, infectious disease, and predator/prey dynamics).

Public engagement

Conservation of both species addressed in this proposal are of general societal interest. The geographic range and diversity of ecosystems occupied (Figure 1) across the two species distributions are considerable, offering the general conservation community a wide diversity of examples to educate and motivate interest in conservation. Charismatic species such as bobcat and Canada lynx provide additional opportunities for engagement with stakeholders and the general public. To reach a broad public audience, our results will be featured in public webinars, published in scientific journals, social media, and in classes taught by co-PIs. The consortium of investigators and institutions collaborating on this project (two federal agencies, one state agency, one university, one government laboratory) are well situated to communicate and engage with the general public regarding the approaches and results of our research.

Letters of Support:

Maine Department of Inland Fisheries & Wildlife, Canada lynx and bobcat species specialists Jennifer Vashon and Cory Mosby (Appendix)
(207) 941-4238
jennifer.vashon@maine.gov

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PAUL R. LEPAGE
GOVERNOR

STATE OF MAINE
DEPARTMENT OF INLAND FISHERIES & WILDLIFE
WILDLIFE RESOURCE ASSESSMENT SECTION
650 STATE STREET
BANGOR ME 04401

CHANDLER E. WOODCOCK
COMMISSIONER

October 30, 2017

Joseph Goergen, Coordinator
Science Based Conservation Programs and Research
SCIF Conservation Department
501 2nd St NE
Washington, DC 20002

Re: A genomic toolbox to enhance management of extant Canada lynx and bobcat populations

Dear Mr. Goergen,

We are writing to show our support for PI Steven DeStefano's proposed project, "*A genomic toolbox to enhance management of extant Canada lynx and bobcat populations*." We believe Dr. DeStefano's proposed project will greatly benefit our agency and further the efforts of those working to conserve and manage Canada lynx and bobcat populations. We have worked closely with Dr. DeStefano, co-PI Organ, co-PI Johnson, and their PhD student, Ms. Lama, over the last several years.

We have been biologists for the State of Maine Department of Inland Fisheries and Wildlife for a combined 17 years with the responsibility of managing and conserving Canada lynx and bobcats. Our mission, to protect and enhance the state's wildlife populations while providing sustainable use of resources, has been supported by Dr. John Organ through multiple research projects, such as a 12-year telemetry study of Canada lynx in northern Maine. Through this project, we were introduced to Dr. Warren Johnson, who developed collection protocols for long-term DNA storage from captured lynx.

The status of Canada lynx in Maine had been a matter of speculation, resultant in lynx being listed as a single DPS under the Endangered Species Act in 2000. As a result, several research projects were initiated to assess historic and current status of lynx in Maine. However, these studies have not addressed uncertainty over historic population levels, or future persistence, and population viability. Conservation genetics could help tackle these knowledge gaps but, to date, have largely been inconclusive due to the limited number of available lynx-specific markers. The genomic approaches detailed in Dr. DeStefano's proposal will produce powerful tools for estimating key parameters in wildlife management, such as effective population size and the ability to test hypotheses regarding demography, connectivity, and hybridization.

Bobcats in Maine are taken by both trapping and hunting, and the State has historically managed the population by adjusting harvest length, timing, intensity, and defining legal methods of take. We

recently instituted mandatory tooth and tissue submissions from bobcat taken in the 2016 hunting season, resulting in a collection of 33 tissue samples available for analysis. A reliable estimate of population abundance would be the most useful metric resultant of this proposal for the purposes of long-term monitoring. Additional insights into the status of our bobcat population would facilitate effective communication about management decisions, particularly relative to bobcat harvest with stakeholders and the general public.

At the most fundamental level, this research will provide resources in support of science-based management for bobcat and lynx in Maine. We eagerly anticipate continued collaboration on this project.

Sincerely,

Jennifer Vashon
Lynx Biologist
Maine Department of Inland Fisheries and Wildlife

Cory Mosby
Furbearer Biologist
Maine Department of Inland Fisheries and Wildlife