Genoscape and Migratory Connectivity of the Loggerhead Shrike

Holden Fox, MS Student, Colorado State University
1717 W Drake Rd, Fort Collins, CO 80526 | (425)-466-9678 | Holden.Fox@colostate.edu
Co-investor: Kristen Ruegg, Colorado State University

Objective: Grassland birds are experiencing persistent and widespread declines, despite existing conservation efforts. I propose to conduct a comprehensive genetic analysis of migratory patterns in the loggerhead shrike, a grassland species of conservation concern. I will use whole genome resequencing of shrikes across their full annual cycle to delineate genetically distinct breeding populations and quantify the extent of population connectivity. By creating a map of range-wide genetic variation, my study can be used to inform targeted, region-specific management and conservation strategies, addressing the critical need for a holistic approach to conserving this species.

Background: Grassland birds are in peril. Recent estimates show that 75% percent of grassland bird species are declining, with more than 50% of individuals lost since 1970.[7] Among these species is the loggerhead shrike. Once abundant in grassland, shrubland, and shrub-steppe habitats across North America, loggerhead shrikes have experienced significant range-wide population declines.[10] These declines surpass projections based on habitat loss alone.[6] Additionally, migrant populations face more severe and persistent declines compared to resident populations,[10] which suggests that populations are limited along migratory routes and in the wintering grounds. However, the challenge of distinguishing between resident, partial migrant, and long-distance migrant shrikes across the annual cycle has hindered past conservation efforts. Shrikes display intricate migration and residency patterns: those breeding above 40N are typically migrants, while those at or below 40N are generally partial migrants or year-round residents. Furthermore, the wintering range of migratory shrikes significantly overlaps with that of residents.[6] To advance conservation efforts, there is a crucial need for tools capable of distinguishing between different migratory forms and accurately mapping the intricate migratory patterns of the loggerhead shrike.

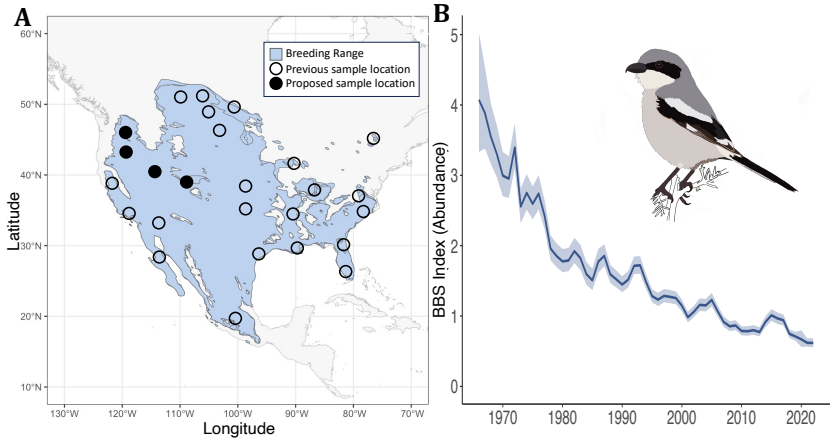

Figure 1. (A) Map of loggerhead shrike sampling areas. (B) Breeding Bird Survey index of loggerhead shrike abundance in North America (1970-2022).

Previous work to understand the migratory connectivity of loggerhead shrikes combined nuclear genetic microsatellite markers and stable hydrogen isotope methods to assign wintering birds to their breeding grounds.[1] These methods successfully identified general regions of origin for several primary shrike wintering areas; however, they lacked

precision in assigning individual shrikes to specific locations. Higher resolution population assignment is needed to manage shrike population declines at a finer scale. Recent advances in whole genome sequencing have made it possible to identify population structure at a finer scale than possible using other methods, allowing for highly accurate population assignment.[8,9] My proposed work will use whole genome resequencing of loggerhead shrikes across their full annual cycle to assess range-wide genetic variation and quantify the strength of migratory connections. Specifically, I will address the following questions: 1) How is genetic variation distributed between populations across the breeding range of loggerhead shrikes? 2) Where do genetically distinct populations of loggerhead shrikes over-winter? 3) What are the strengths of migratory connections between genetically distinct groups across the annual cycle?

Relevance to DFO grant program mission:
Colorado Loggerhead shrikes are steadily declining. Despite intensive research and conservation effort, the factors responsible for this decline remain poorly understood. Research on the genetic variation and connectivity of breeding populations of shrikes will further our understanding of Colorado shrike migratory patterns, and has the potential to inform targeted, region-specific management strategies for Colorado shrikes.

Locations: Sampling will take place at four sites in Colorado (Mesa County), Nevada (Elko and White Pine County), Oregon (Harney County), and Washington (Benton, Grant, Adams, and Franklin Counties). Initial sampling locations were chosen using Ebird hotspot data and through communication with local experts. Lab work and data analysis will take place at Colorado State University in Fort Collins, CO.

Timeline: Fieldwork for this project will be completed by July 2024, and the entire project (lab work and data analysis) will be completed by the end of spring 2025. I am planning on graduating with my Masters in the Fall of 2025 and will also be working to publish my results at this time.

Methods: 201 genomic DNA samples from loggerhead shrike breeding populations in the eastern portion of the range have been sequenced. An additional 500 DNA samples from across the breeding range including Mexico and the Southwestern United States have been contributed by collaborators within the loggerhead shrike working group and are currently housed at Colorado State University. Despite extensive sampling to date, gaps remain in key areas in the western United States. To fill these sampling gaps and identify range-wide genetic variation for loggerhead shrikes, I will collect a target of 10 or more feather and blood samples per site during the breeding season of 2024. I will use a modified potter trap to catch the shrikes. Individuals will be banded, and genetic sample taken before immediate release.

I will extract and purify genomic DNA from these genetic samples (Qiagen DNeasy Blood & Tissue Kit). I will use a modified version of Illumina's NextEra Library Preparation protocol to prepare whole-genome sequencing libraries and pool the libraries by equal mass prior to sequencing. Pooled libraries will be sequenced on an individual Illumina NovoSeq 6000 lane with a target coverage of 9x per individual. I will use a pipeline adapted from the Genome Analysis Toolkit (GATK) Best Practices Guide to process raw reads. GATK

haplotype caller and samtools[4] will be used to call raw genotypes and filter for missingness and quality scores (minimum base quality Phred score of 20), and the intersection of these high-quality variants as input for recalibration with BaseRecalibrator.[11] To identify distinct genetic clusters on the breeding range, I will generate a PCA using GATK.[11] I will assess how genetic variation is distributed across by space by creating a genetic admixture plot using STRUCTURE.[5] Population assignment will be done using WGSassign[3] and the strength of migratory connections will be quantified using the network modeling approach described in the Mignette R-package.[2]

Literature Cited: [1]Chabot A, Hobson K, Wilgenburg S, Pérez G, & Lougheed S (2018) Migratory connectivity in the loggerhead shrike (Lanius ludovicianus). *Nat Ecol Evol* **8**: 10662-10672. [2]Desaix M (2023) Mignette. *GitHub*. [3]DeSaix M, Rodriguez M, Ruegg K, Anderson E (2023) Population assignment from genotype likelihoods for low-coverage whole-genome sequencing data. *Authorea.* [4]Li H & Durbin R (2009) Fast and accurate short read alignment with burrows–wheeler transform. *J Bioinform* **25**: 1754–1760. [5]Pritchard J, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**: 945-959. [6]Pruitt L (2000) Loggerhead shrike status assessment. Fort Snelling, Minnesota: U.S. Fish and Wildlife Service. [7]Rosenberg K, Dokter A, Blancher P, Sauer J, Smith A, Smith P, *et al.* (2019) Decline of North American Avifauna. *J Sci* **366**: 120–4. [8]Rueda-Hernández R, Bossu CM, Smith T B, Contina A, Canales del Castillo R, Ruegg K *et al.* (2023) Winter connectivity and leapfrog migration in a migratory passerine. *Nat Ecol Evol* **13**: e9768. [9]Ruegg K, Brinkmeyer M, Bossu C, Bay R, Anderson E, Boal C, Dawson R *et al.* (2021) The American Kestrel (Falco sparverius) genoscape: implications for monitoring, management, and subspecies boundaries. *J Ornithol* **138**: ukaa051. [10]Sauer J, Hines J.E, Fallon J, Pardieck K, Ziolkowski D Jr, & Link W (2017) The North American breeding bird survey, results and analysis 1966–2016. Laurel, MD: USGS Patuxent Wildlife Research Center. [11]Van der Auwera G, Carneiro M, Hartl C, Poplin R, Angel G, Levy-Moonshine A et al. (2013) From FastQ data to high-confidence variant calls: The genome analysis toolkit best practices pipeline. *Curr Protoc Bioinform* **43**: 10-11.

Budget:

Item	Amount	Cost	Source
Sequencing	1 Lane	$2,000	Requested from DFO
Blood sampling supplies	Up to 300 samples	$500	Pending
Gas	$100/10 fills	$1,000	Pending
Hotel	$175/14 nights	$2,450	Pending
Per Diem	$69/15 days	$1,035	Pending
Total		$6,985	
Total requested from DFO		$2,000	

Budget Justification: The funding requested from DFO will be critical as it will support the cost of NGS sequencing, allowing me to fill current sampling gaps for this project.

Previous Publications:

Cordes M, Sundman A, Fox H, Binford G (2023) Protein salvage and repurposing in evolution: Phospholipase D toxins are stabilized by a remodeled scrap of a membrane association domain. *Protein Sci* **32**: e4701.

Colorado State University

DEPARTMENT OF BIOLOGY
1878 Campus Delivery
Fort Collins, Colorado 80523
www.biology.colostate.edu

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To whom it may concern,

I am writing in support of the application from Holden Fox, entitled, "Genoscape and Migratory Connectivity of the Loggerhead Shrike." Holden is a first year Master student in my lab and, if funded, I can confirm that he is well qualified to carry out the proposed research. I selected him to join my lab from a large pool of applicants based on his previous research experience and his excellent letters of recommendation. Since joining my group in the Fall of 2023, it has been a pleasure to watch Holden engage in his research and teaching with the skills of a highly competent student. He is organized, curious, independent, methodical, and able to seek out answers to questions as they arise.

As his Master thesis supervisor, I can also confirm that Holden will be provided the logistical and financial support necessary to complete the other aspects of his work. His proposed research builds off an NSF-funded project in my lab aimed at connecting populations of migratory birds across their full annual cycle using genomics. As part of this project, Holden is supported by a close nit group of staff research scientists and other graduate students who are all available to assist Holden in his graduate training. Further, through the existing NSF grant Holden has access to funding for laboratory work, but the funding from this proposal will allow him to fill in critical sampling gaps.

Please don't hesitate to reach out if you have any additional questions.

Kind regards,

Assistant Professor
Colorado State University
Phone: 510-292-5099
Email: Kristen.Ruegg@colostate.edu