Estimating the effect of habitat quality on gene flow, genetic diversity, and fitness in Flammulated Owls (Psiloscops flammeolus)

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Objective Inferring rates of gene flow and estimating genetic diversity is critical for managing populations of conservation concern. Here, we aim to advance research in Colorado populations of the Flammulated Owl (Psiloscops flammeolus), a species susceptible to rapid climate change 1 and an indicator species in old growth forests 2 , by proposing to apply genomic sequencing to estimate structure among populations and genetic diversity and individual fitness within populations in relation to habitat quality. We hypothesize that gene flow among populations will be driven by female-biased dispersal, given the high site fidelity exhibited among male Flammulated Owls 3 . Furthermore, we hypothesize that males with high fitness are centrally-located in patches of suitable habitat, suggesting a positive relationship between fitness and habitat quality. Consequently, we expect high genetic diversity at the center of aggregate nesting areas driven by high rates of extra-pair paternity (EPP) in males occupying high quality territories.

Background The climate crisis continues to impact all aspects of ecology, and while species-specific responses frequently vary in impact and severity 4 , avian taxa demonstrate a significant and extremely concerning negative trend 5,6 . An alarming loss of over 2.9 billion birds from North America since 1970 7 suggests many ecological communities and ecosystems may become unstable given rapid environmental change, such as habitat conversion and fragmentation 8 . Theory suggests habitat fragmentation restricts gene flow and, consequently, reduces genetic diversity, thereby rendering local populations vulnerable to extirpation 9,10 . Consequently, it is critically important to quantify genetic diversity within and among populations, particularly of indicator species, to better predict ecosystem health. Indicator species with migratory life history strategies provide a lifeline to holistically understand fragmented ecosystems, as such species are important for spreading biomass and fecal matter and for providing and consuming resources 11 . However, many migratory species are severely threatened as migration routes become increasingly hazardous, and wintering and breeding grounds are lost to anthropogenic expansion 12 . Thus, migratory birds can serve as important indicators of ecosystem health, and can inform managers of the health of mega-systems, connections between fragments and larger ecosystems by migratory species 13 . The Flammulated Owl is a small insectivorous raptor, native to much of the Rocky Mountain West, as well as Washington State, Oregon, California, and parts of California and Mexico 14 . This owl species is highly unique given its annual seasonal migration between central Mexico (wintering grounds) and the western United States (breeding grounds) 15 . The Flammulated Owl thrives in old growth ponderosa pine (Pinus ponderosa) forests, where, as a secondary cavity nester, breeding pairs use abandoned woodpecker cavities to raise young 14 . Despite the curious nature of this owl species, little is known about dispersal patterns, partial migratory tendencies, and size of breeding populations in Canada, Washington State, Oregon, and California. The Linkhart Ornithology Lab (Colorado College) has collected demographic and habitat data in Flammulated Owl populations in the Pike National Forest in Colorado since 1981. Long-term field research has also yielded individual breeding data and blood samples since 2002. The Colorado study spearheaded by Linkart and colleagues represents the longest ongoing study of Flammulated Owls and has produced much of what is currently understood about the species. As part of an ongoing collaboration with the Gamboa Animal Evolutionary Ecology Lab (Colorado College), students have been working to determine DNA sex and relatedness among Flammulated Owls. This collaboration is ongoing and building towards long-term analyses of gene flow and population differentiation of this species across western North America.

Significance Understanding population dynamics and genomics of vulnerable species is crucial for ensuring population persistence and maintaining community stability. While GPS locators, radiotelemetry, and civilian birding programs have been effective in tracking and understanding the pattern of larger and diurnal birds 17 , the Flammulated Owl is small, elusive, quite, and nocturnal. Thus, the Flammulated Owl is well-suited for inferring gene flow and population dynamics across a relatively small geographic extent using modern, non-invasive, molecular techniques, such as next-generation sequencing 18 . Genomic sequencing data will supplement existing demographic data and provide researchers with reliable individual sex and fitness information, as well as population metrics, thereby enabling the identification of more vulnerable populations. By establishing an understanding of how habitat and climate affect Flammulated owl population dynamics, we can generate informed hypotheses about how forest ecosystems may respond to future environmental change. Ultimately, these findings may enable researchers, land managers, and other stakeholders to develop a synergistic, proactive conservation framework for maintaining biodiversity in old growth forests.

Methods During the 2015-2022 summer breeding season, owls were captured and blood samples taken as a part of a long-term owl demographic study (Linkhart Lab, Colorado College). We will extract genomic DNA from blood samples using Qiagen DNeasy Blood & Tissue Extraction Kits and modified extraction protocols (Ruegg Lab, Colorado State University). Approximately 350 individuals spanning the breeding seasons will be included in subsequent genomic analyses. Specifically, we will quantify DNA concentrations and develop genomic libraries following protocols established by the Ruegg Lab (Colorado State University). Final normalized libraries will be sent to Novagene for low-coverage whole genome sequencing (lcWGS) on an Illumina NovaSeq 6000 platform with an estimated 3-5X target depth of coverage. Sequencing data will be used to impute genotypes in Beagle 19 , estimate individual relatedness in NgsRelate 20 , and estimate genetic diversity and population structure and differentiation in ngsTools 21 . To determine whether gene flow is sex-biased, we will assign individual sex using PCR amplification of known sex markers 22 and compare individual sex with estimates of admixture. Population genetic statistics (e.g., heterozygosity, nucleotide diversity) will be correlated with habitat metrics to test the hypothesis that males with high quality territories at the center of aggregate nesting sites sire more offspring through EPP and, as a result, contribute to the inequality of genetic diversity across the landscape.