

**1. DETERMINATION OF POPULATION STRUCTURE IN MOUNTAIN QUAIL TO FACILITATE MANAGEMENT**

**2. Amount Requested – \$142,335**

**3. Applicant Contact Information:**

1. Applicant: Pheasants Forever Inc. -- 501(c) (3)tax id number:41-1429149
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**4. Project Priority:** Research: Mountain Quail Management

**5. Introduction:**

The secretive nature of the Mountain Quail (*Oreortyx pictus*) makes it a difficult species to study. Consequently, many aspects of Mountain Quail biology remain poorly known, including population dynamics and vital rates, dispersal and migration behavior, and the degree of isolation among populations. Addressing the latter gap in our knowledge is of particular importance for understanding the distinct evolutionary histories of populations, delineating management units, and guiding potential translocation efforts (Pope & Crawford 2004).

Much of the current data on patterns of population differentiation come from qualitative analyses of plumage variation. Five subspecies of Mountain Quail have been described based on these plumage analyses, four of which occur in California (van Rossem 1937; Gutiérrez & Delehanty 1999). *O. p. palmeri* is found in mesic coast ranges from Washington state south to San Luis Obispo Co., California with a gap around the bay area. *O. p. pictus* is found in the interior coast ranges eastward across northern California and south through the central sierras. *O. p. eremophilus* occurs in the more arid mountains of southern California and ranges northward through both the southern Sierras and southern coast ranges. Finally, *O. p. russelli* is confined to the Little San Bernardino Mountains of southern California. The validity of these subspecies has been questioned and the geographic boundaries among subspecies is fairly arbitrary in certain cases (Grinnell & Miller 1944). Genetic data will be important for testing subspecies designations, refining management units for Mountain Quail, and determining whether local adaptation to different climatic regimes exist within the species.

Genetic data have long played a critical role in documenting patterns of population structure that can inform conservation units (Allendorf et al. 2022). The power to resolve even fine-scale patterns of genetic structure has improved dramatically with increasing affordability of genome-scale datasets. Genomic datasets also provide novel opportunities to understand the genetic health of populations through quantification of genetic diversity and to explore local adaptation to distinct environments that may be important to consider in population management. For example, genomic analyses of sage-grouse (*Centrocercus* spp.) revealed fine-scale patterns of population structure and documented signatures of selection at several genes that may be associated with their dietary specialization on sagebrush (*Artemisia* spp.), plants rich in toxic secondary metabolites (Oh et al. 2019). Little genetic data of any kind exist for Mountain Quail.

Whole genome sequences from 29 California samples of Mountain Quail were recently obtained as part of the California Conservation Genomics Project (CCGP; Shaffer et al. 2022). Preliminary analyses of this dataset indicate that structure does exist among California populations of the Mountain Quail (**FIGURE 1**). The principal divide is between populations in the mountains of southern California and more northern populations. Evidence for additional structure also exists within the Sierra Nevada, and between northern coastal ranges and the Sierra Nevada (Fig. 1). However, major geographic gaps exist among the samples included in preliminary analyses. These gaps make it difficult to exclude the possibility of isolation by distance and hamper population delimitation

efforts. Filling these gaps will be critical for establishing accurate management units for Mountain Quail in California. To this end, we are partnering with CDFW personnel to obtain an additional 100 Mountain Quail samples from these sample gaps. We plan to generate whole genome sequences from these additional quail samples to address the following objectives:

**Objectives:**

- (1) Resolve patterns of population structure among Mountain Quail populations in California.
- (2) Quantify genetic diversity across different populations of Mountain Quail.
- (3) Explore patterns of adaptive differentiation among populations to further establish the importance of certain populations as management units.

**6. Project Description:** *Provide a detailed description of work to be performed, including the following:*

1. *Location of the project:* California, statewide
2. *Describe all personnel in the budget, their roles in the project, and their qualifications. Include titles and responsibilities of each.*

**Al Eiden:** Director of Field Operations. He will manage the overall budget, coordinate with CDFW, and outcomes of this proposal. Al will coordinate with the University and ensure all aspects of this project meet the goals and objectives of any approved grant activities, and work with PF accounting staff to ensure fiscal activities meet all legal requirements for work in California.

**Casey Waterman,** Senior Accountant. Casey will provide all accounting activities including invoicing to CDFW, submitting payment to the University for work accomplished, and will provide all financial reporting needed for this project.

**Sarah Marquart.** Agreement Specialist. Sarah will provide assistance with creating agreements and/or contract needed to accomplish the goals and objectives between CDFW, US Berkely, and any other potential partners.

**Dr. Phred Benham** will serve as a postdoctoral scholar working with Professor Rauri Bowie at UC Berkeley to analyze the resulting data. A laboratory technician will be hired to collect the whole genome sequence data from the 100 Mountain Quail. Dr. Benham is presently working on the California Conservation Genomics Project and has performed the analyses of whole-genomes from 29 Mountain Quail reported here as preliminary data. Professor Bowie is an expert with 20 years experience in wildlife genetics.

3. *Describe contractors and subcontractors (if any) and their responsibilities*

Professor Rauri Bowie, Museum of Vertebrate Zoology, University of California, Berkeley. His lab will be responsible for generating and analyzing the genetic data to be collected from 100 Mountain Quail individuals.

4. *Implementation plan, approach, methodology;*

We plan to partner with CDFW to obtain 1-3 individuals per locality from multiple sites throughout the state of California for a total of 100 samples. Major priorities for sampling will be: (1) mountain ranges in southern California (San Bernardino Mountains, San Gabriel Mountains, Transverse Ranges, etc.), (2) the central Sierra Nevada between Calaveras and Madera counties, and (3) northern California on either

side of the Sacramento River headwaters (**TABLE 1 & FIGURE 2**). Samples will be obtained through targeted scientific collection and by cooperating with hunters in the different CDFW quail management units. Tissue samples will be vouchered when possible (either by a study skin, skeleton, or wing), and samples will be archived either at the CDFW or the Museum of Vertebrate Zoology, Berkeley.

We plan to sequence whole genomes of the 100 sampled Mountain Quail to an average of 10x coverage. Preliminary subsetting of the CCGP genomic dataset suggested that datasets with >11,000 single nucleotide polymorphisms (SNPs) would be necessary to uncover finer-scale patterns of genetic diversity. Reduced representation sequencing methods, such as target capture or RADseq, are more cost effective but based on our preliminary analyses of 29 Mountain Quail may not provide sufficient power to identify population genetic clusters in the state. The greater resolution and increased capacity to perform sophisticated population structure, genetic diversity, and selection scan analyses points to whole genome sequencing (WGS) as the method which will provide the most benefits to management of the species.

5. *Materials/equipment necessary to implement the project and by whom the materials will be provided;*

DNA extractions from quail tissue and sample library preparations will be performed in the Evolutionary Genetics Lab (EGL) at the University of California, Berkeley. The EGL is fully equipped with the necessary instruments to perform this work. EGL staff and personnel on this grant proposal have extensive experience working with avian DNA and preparing libraries for whole genome sequencing. For example, we have recently completed sequencing of over 1,800 bird and mammal genomes for the CCGP to similar levels of coverage we are proposing here. This includes the 29 Mountain Quail presented as preliminary data in this proposal. The lab protocols developed by the EGL for CCGP sequencing will be followed for the proposed work. Sequencing of prepared libraries will be performed on an Illumina Novaseq S4 platform at the QB3 sequencing core at UC Berkeley.

6. *Explanation of how this work addresses the selected priority(ies):*

Raw sequencing data will be cleaned, mapped, and variants called using standard pipelines that have been extensively used in the Bowie lab. Cleaned sequencing reads will be mapped to a chromosome-level Mountain Quail reference genome currently being sequenced by the CCGP using BWA (Li & Durbin 2009). Single nucleotide polymorphisms (SNPs) in mapped reads will be identified using bcftools mpileup software (Li et al. 2011). These SNPs will be used for all downstream analyses.

**Objective 1.** To determine population structure, several methods will be used to assess patterns of population structure across the state. These will include principal components analyses and model-based clustering methods (e.g. ADMIXTURE; Alexander et al. 2009). We will additionally construct Estimated Effective Migration Surfaces (EEMS; Petkova et al. 2016) to identify regions of the state where deviations from isolation by distance exist. This will help establish both potential barriers to gene flow among populations and identify corridors with high connectivity among populations.

**Objective 2.** Once distinct population clusters have been identified, we will quantify a number of population genetic parameters (e.g. nucleotide diversity, heterozygosity) in the scikit-allel package (Miles et al. 2020) to determine spatial patterns of genetic diversity. Populations with increased levels of inbreeding exhibit longer tracts of homozygosity relative to outbred populations. To determine whether any quail populations exhibit signatures of inbreeding, we plan to measure runs-of-homozygosity using the program ROHan (Renaud et al. 2019).

**Objective 3.** To explore patterns of local adaptation among populations, we will collect remote-sensing data from sampled populations on vegetative characters (e.g. [NDVI dataset](#)) and 19 bioclimatic variables (<https://worldclim.org>). These data will be used to quantify the ecological distinctiveness of genetically differentiated clusters using a species distribution modeling approach (e.g. Elith & Leathwick 2009). We will couple this analysis with a variety of methods to detect signatures of selection in the genomes of Mountain Quail. First, we will estimate a variety of population genetic statistics in sliding windows

between populations in distinct environments. Regions of the genome that show high  $F_{st}$  and  $D_{xy}$  divergence and low nucleotide diversity will be considered outliers potentially under divergent selection between populations. We will then use a combination of redundancy analysis (RDA; Capblancq & Forrester 2021) and linear factor mixed models (LFMM; Caye et al. 2019) to look for statistical associations between genotypes and specific environmental variables (e.g. precipitation). All of these approaches for inferring the genetic basis of local adaptation have limitations; however, genes identified as outliers across all methods will provide the strongest evidence for playing a role in local adaptation to different climate regimes.

We have access to the computational resources to perform all filtering, mapping, variant calling, and data analysis steps proposed above. This includes servers at the Museum of Vertebrate Zoology, UC Berkeley dedicated to the analysis of genomic data of this scale and with all software necessary for this project already installed. Project personnel (Benham) have extensive experience analyzing these kinds of datasets and have worked with all proposed methods.

7. *Proof of environmental permitting compliance (if necessary).* N/A

## **7. Expected Management Benefits**

We anticipate the results of this study will provide a number of management benefits for Mountain Quail in California. First, this study will be the first to establish patterns of population genetic structure in Mountain Quail. Our preliminary data indicate that this species likely exhibits meaningful levels of population structure in the state that are not necessarily associated with subspecies limits or current quail management units. The proposed work will refine patterns of population structure and provide more robust evidence for the demarcation of quail management units. Second, the proposed whole genome sequencing approach will allow for sophisticated analyses of the genetic health of different population genetic clusters of Mountain Quail. These analyses will help identify populations with lower diversity and higher inbreeding levels that may require additional monitoring and management interventions. The final component of the proposed work will also help identify the ecological distinctiveness of population genetic clusters and help further delineate management units that preserve adaptive diversity in the species. If translocations among populations are ever needed to mitigate inbreeding depression or population declines, data from these final analyses will help inform which populations would lead to the greatest success of the translocation program. For example, translocations from ecologically divergent populations may be more likely to fail due to outbreeding depression.

## **8. Schedule and List of Deliverables**

CDFW collection of Mountain Quail samples – October 2022 to April 2023

Whole Genome Data Collection – May to July 2023

Analyses and writing of report for CDFW and Scientific Publications – August 2023 to July 2024

**9. Budget Narrative:** *Describe anticipated project costs by activity, task and/or potential phases of the project with appropriate implementation cost for each.*

Whole genome sequencing will be subcontracted to the Professor Rauri Bowie at UC Berkeley. His lab will be responsible for generating the data from samples provided by CDFW and for analyses of these data. To accomplish these goals, Bowie will hire a lab technician (480 hours) and a postdoctoral researcher for 1 year.

## **10. Itemized Budget:**

Include separate line items for the following budget categories:

1. Personnel. List titles corresponding with Project Description (list benefits on separate line item).

***Pheasants Forever and Quail Forever Inc.***

Project Coordinator (Al Eiden): 63 hours/ year @ \$60/hour= \$3,800  
Accountant (Casey Waterman): 30 hours/year @ \$37.50/hour=\$1,125  
Agreement Specialist (Sarah Marquart): 20 hours/year @ \$45.50/hour= \$910

***UC Berkeley Subcontract***

Laboratory Technician for Whole Genome data generation = 480 hours @ \$26/hour	= \$12,480
Postdoc for DNA data analyses – 12 months Salary	= \$6350/Month = \$76,200
Benefits	= \$900/month = \$10,800

2. Agreement Administration (include overhead % rate if applicable).

***UC Berkeley Subcontract Overhead:*** @ 11% = \$14,520

3. Operating Expenses. List materials and cost on a per unit basis. Provide separate line items for travel and subcontractors etc.

***UC Berkeley Subcontract***

DNA reagents and sequencing of 100 genomes = \$225/genome = \$22,500

See table below for the same information in the example table provided in the application.

**Line-Item Budget Detail for < DETERMINATION OF POPULATION STRUCTURE IN MOUNTAIN QUAIL TO FACILITATE MANAGEMENT >**

**A. PERSONNEL SERVICES**

<u>Project Coordinator (Al Eiden)</u> : 63 hours/ year @ \$60/hou	\$3,800
<u>Accountant (Casey Waterman)</u> : 30 hours/year @ \$37.50/hour	\$1,125
<u>Agreement Specialist (Sarah Marquart)</u> : 20 hours/year @ \$45.50/hour	\$910
Laboratory Technician for Whole Genome data generation = 480 hours @ \$26/hour	\$12,480
Postdoc for DNA data analyses – 12 months	\$76,200
<b>Subtotal Personnel Services</b>	<b>\$94,515</b>
Staff Benefits	\$10,800
<b>Total Personnel Services</b>	<b>\$105,315</b>

**B. OPERATING EXPENSES: GENERAL**

<i>Field Supplies</i>	\$ xxx
<i>Travel</i>	\$ xxx
<b>Subtotal Operating Expenses: General</b>	<b>\$ xxx</b>

**C. OPERATING EXPENSES: SUBCONTRACTORS**

DNA reagents and sequencing of 100 genomes = \$225/genome	\$22,500
<i>Subcontractor 2 – description of services</i>	\$ xxx
<i>Subcontractor 3 – description of services</i>	\$ xxx
<b>Subtotal Operating Expenses: Subcontractors</b>	<b>\$22,500</b>

**D. OPERATING EXPENSES: EQUIPMENT**

<i>Equipment item</i>	\$ xxx
<b>Subtotal Operating Expenses: Equipment</b>	\$ xxx
<b>E. SUBTOTALS &amp; INDIRECT COSTS</b>	
SUBTOTAL A + B (Personnel Services + Operating Expenses: General)	\$ xxx
SUBTOTAL C (Operating Expenses: Subcontractors)	\$ xxx
SUBTOTAL D (Operating Expenses: Equipment)	\$ xxx
Indirect Charge Rate 11% <i>UC Berkeley Overhead</i> (Applies to Sections A + B only)	\$14,520
<b>F. GRAND TOTAL</b>	<b>\$142,335</b>

## APPENDICES

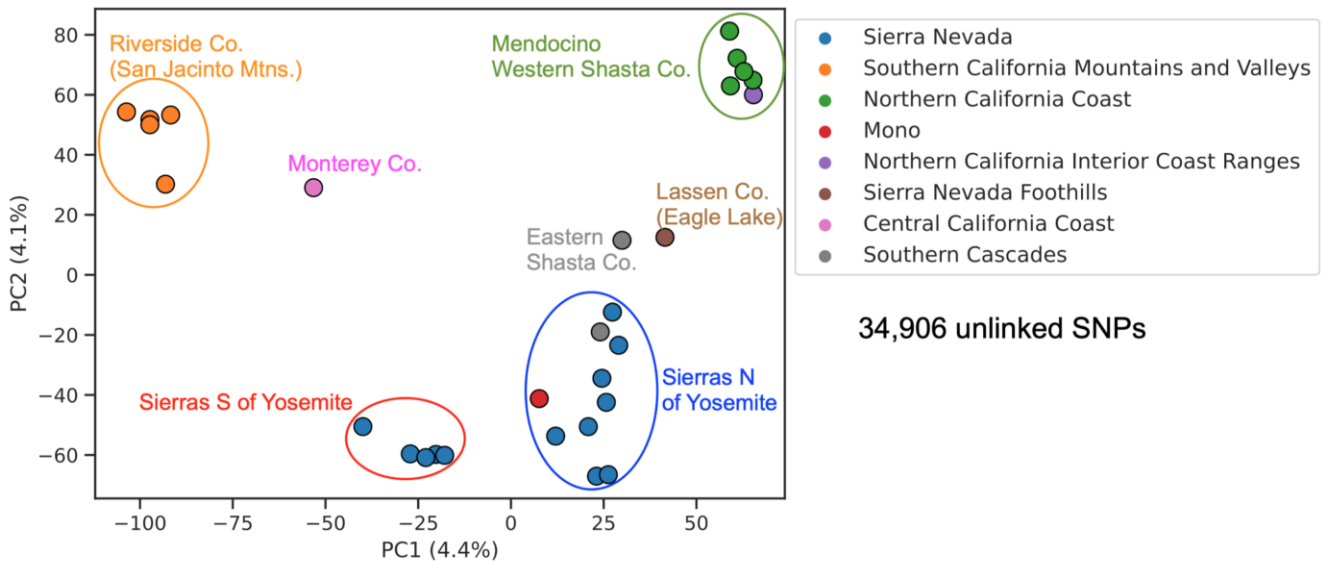
**FIGURE 1.** (see below)

**FIGURE 2.** (see below)

**TABLE 1.** (see below)

**Literature cited.** (see below)

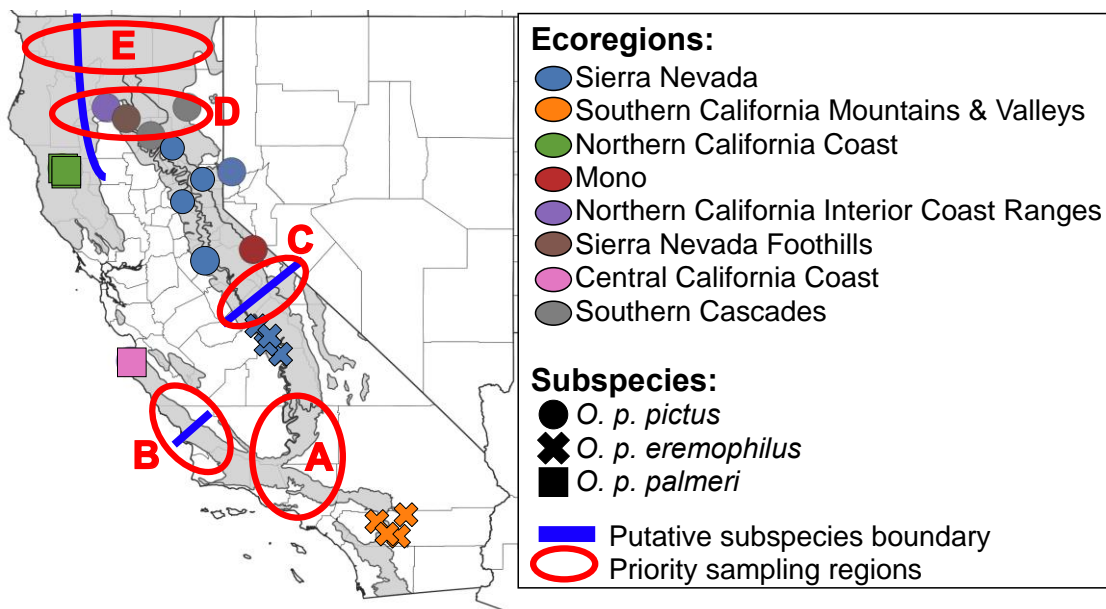
# APPENDICES



**Figure 1.** Analyses of 29 Mountain Quail Genomes obtained through the California Conservation Genetics Project suggest that Mountain Quail populations distributed across California may comprise several management groups.

**Table 1.** Proposed sampling localities: see corresponding sampling map (Figure 2) for outline of major sampling areas.

Subspecies	Mountain range	County
<b>Priority sampling area A</b>		
<i>eremophilus</i>	San Bernardino Mountains	San Bernardino
<i>eremophilus</i>	NW San Gabriel Mountains	Los Angeles
<i>eremophilus</i>	SE San Gabriel Mountains	Los Angeles
<i>eremophilus</i>	Santa Monica Mountains	Los Angeles
<i>eremophilus</i>	Mt. Pinos	Kern
<i>eremophilus</i>	Tehachapi Mtns	Kern
<i>eremophilus</i>	Greenhorn Mtns	Kern
<i>eremophilus</i>	S. Sierra Nevada	Kern
<i>eremophilus</i>	S. Sierra Nevada	Southern Tulare
<b>Priority sampling area B</b>		
<i>eremophilus</i>	Santa Ynez Mountains	Santa Barbara
<i>eremophilus</i>	Sierra Madre Mountains	Santa Barbara
<i>eremophilus/palmeri</i>	Sierra Madre Mountains	San Luis Obispo
<i>eremophilus/palmeri</i>	S Santa Lucia Range	San Luis Obispo
<i>palmeri</i>	N Santa Lucia Range	Monterey
<b>Priority sampling area C</b>		
<i>eremophilus/pictus</i>	Central Sierras	Mariposa
<i>eremophilus/pictus</i>	Central Sierras	Tuolumne
<i>eremophilus/pictus</i>	Central Sierras	Mono
<b>Priority sampling area D</b>		
<i>eremophilus</i>	Lassen National Forest	Lassen
<i>eremophilus</i>	Shasta lake area	Shasta
<i>eremophilus</i>		Shasta
<i>palmeri/eremophilus</i>		Trinity
<b>Priority sampling area E</b>		
<i>pictus</i>	Warner Mtns	Modoc
<i>pictus</i>	anywhere...	Siskiyou
<i>pictus/palmeri</i>	Klamath Mtns.	
<i>palmeri</i>	anywhere...	Humboldt/Del Norte



**Figure 2.** Proposed sampling areas for Mountain Quail.



## Literature cited

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