Preliminary Comparison of Genetic Diversity in the Endangered San Joaquin Kit Fox (*Vulpes macrotis mutica*) Before and After a Mange Outbreak

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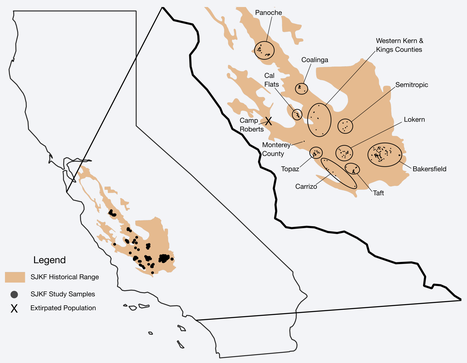
Introduction

The San Joaquin kit fox (SJKF; *Vulpes macrotis mutica*) is a federally endangered species.[1] Today, fewer than 5,000 individuals are thought to occur across their range, and the contemporary metapopulation is distributed across three core regions and several smaller satellite populations.[2,3] While anthropogenic habitat loss is believed to be the fundamental cause of the historical decline of the SJKF, localized disease outbreaks today proximately threaten remaining populations both demographically and genetically[4,5]. Two known satellite populations (Camp Roberts, Fort Hunter Liggett) became extirpated, possibly as a result of inbreeding depression or disease.[6] Additionally, in the last decade, sarcoptic mange has caused significant demographic declines in a formerly abundant urban SJKF population in Bakersfield.[5] There has been minimal evidence of mange occurring in exurban kit fox populations outside of the Bakersfield region (small scale outbreak identified in neighboring Taft in 2019), which may indicate that dispersal between urban and exurban regions is low.[7] We aimed to assess whether there is population structure between kit foxes within Bakersfield and those found in other portions of their range. We then compared differences in genetic diversity and internal relatedness (a proxy metric for quantifying inbreeding) over both geographic space and across time to see whether the mange outbreaks have had a significant impact on genetic diversity and inbreeding. We used reduced-representation genomic sequencing approach to compare population structure, landscape connectivity, genetic diversity, and internal relatedness of historical SJKF individuals sampled prior to the mange outbreak (n = 89) as well as of SJKF individuals sampled from the contemporary population (n = 109), focusing on both urban (n = 82) and exurban (n = 116) regions.

Methods

Sample Collection



We obtained 198 kit fox tissue samples that were collected across California from 1985–2022. These included samples from the urban population in Bakersfield (n = 82) as well as from the exurban regions (n = 116)

Lab Work: Extraction + GBS

- DNA extraction using Qiagen DNEasy Blood and Tissue Kit
- Genotyping-by-Sequencing[8]
- We retained 157 SJKF samples that were sequenced at ~33x coverage across 11,155 nuclear loci

Population Structure

We conducted a discriminant analysis of principal components (DAPC) in adegenet[9] as well as maximum likelihood population assignment (K = 2–10) in the program ADMIXTURE.[10] Additionally, we compared the genetic dissimilarity across the SJKF range using a pairwise F_{ST} analysis in hierfstat.[11]

Characterizing Heterozygosity and Internal Inbreeding

To calculate regional differences in H_e we calculated individual H_e from the site frequency spectrum [easySFS][12] using genotype likelihoods [angsd][13]. We calculated internal relatedness (IR), which serves as a proxy for inbreeding, using the program GENHET[14]

Preliminary Results

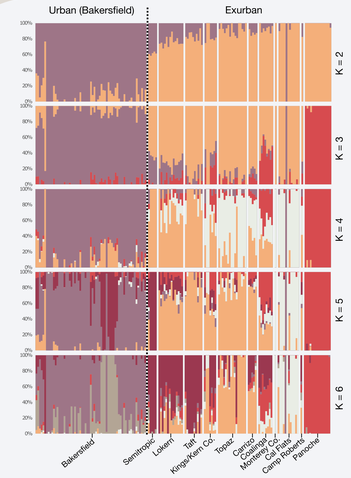


Fig 1. Population genetic structure of the San Joaquin kit fox across its contemporary range identified by the maximum likelihood clustering algorithm implemented in the program ADMIXTURE[10] Admixture proportions for each individual are shown as bar plots. Structure appeared hierarchically within the urban and exurban populations after their split at K = 2 with the highest model support at K = 6 (Tested K = 2–10 clusters).

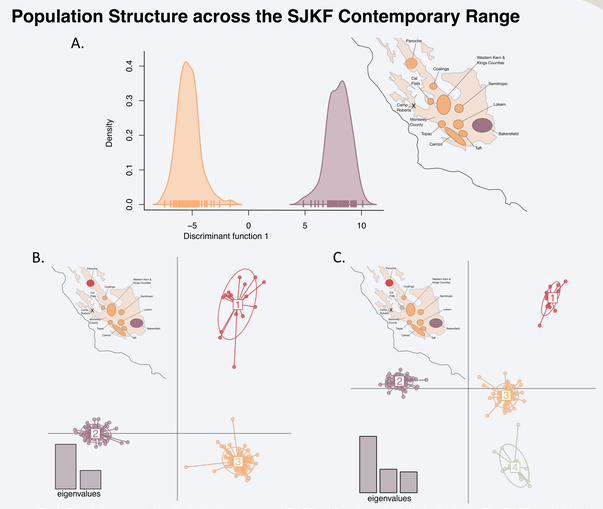


Fig 2. Discriminant analysis of principal components (DAPC) of San Joaquin kit fox individuals.. The DAPC analysis had the highest support for two genetic clusters (A), differentiating the urban (purple) and exurban (yellow) populations. Increasing the number of genetic clusters in the DAPC analysis resulted in the hierarchical genetic separation of distinct geographic regions within the exurban population, including the Panoche region (B; 3 genetic clusters) and the Cal Flats region (C; 4 genetic clusters).

Regional Differences in Heterozygosity

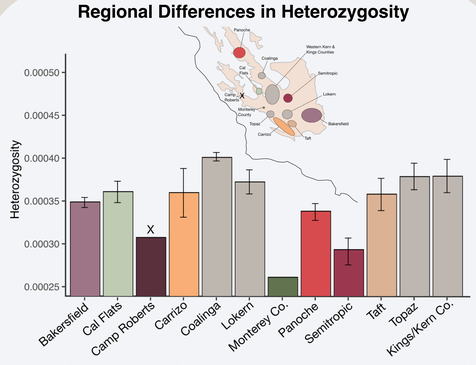


Fig 4. Preliminary results identified variable levels of heterozygosity across distinct regions of the SJKF range. We identified reduced heterozygosity in peripheral regions, when compared to regions found within the center of the range (gray; see map inset). The lowest levels of H_e were identified in Camp Roberts (X; currently extirpated), Monterey County, Semitropic, and Panoche, followed by Cal Flats, the Carrizo Plains, and Taft. The urban population in Bakersfield had moderate levels of H_e when compared to exurban populations.

Genetic Differentiation Among SJKF Regions

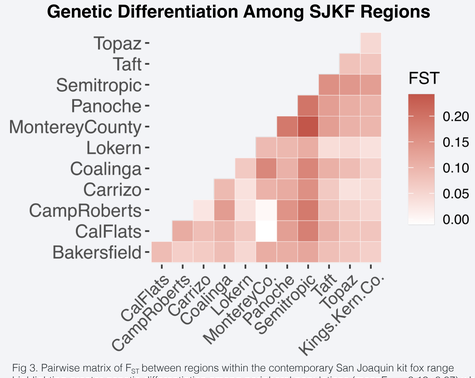


Fig 3. Pairwise matrix of F_{ST} between regions within the contemporary San Joaquin kit fox range highlighting greater genetic differentiation among peripheral populations (meanF_{ST} = 0.12±0.07) when compared to differentiation among both central populations (meanF_{ST} = 0.06±0.02) and the exurban population as a whole (meanF_{ST} = 0.09±0.05). Additionally, peripheral populations showed greater genetic differentiation from Bakersfield (meanF_{ST} = 0.09±0.2) when compared to central populations (meanF_{ST} = 0.06±0.02), despite some of them (Semitropic) being geographically proximate)

Regional Differences in Internal Relatedness

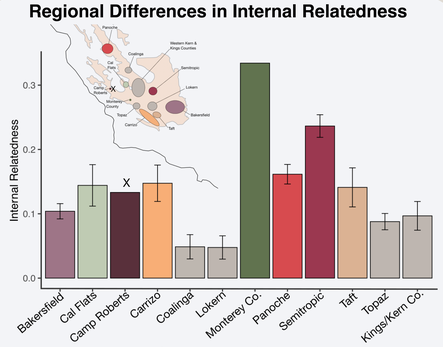


Fig 5. Preliminary results identified variable levels of internal relatedness (IR) across distinct regions of the SJKF range. We identified elevated IR in peripheral regions, when compared to regions found within the center of the range (gray; see map inset). The highest levels of IR were identified in Monterey County, Semitropic, and Panoche, followed by Cal Flats, the Carrizo Plains, and Camp Roberts (X; currently extirpated). The urban population in Bakersfield had moderate levels of IR when compared to exurban populations.

Recent Increases in Internal Relatedness throughout the SJKF Range?

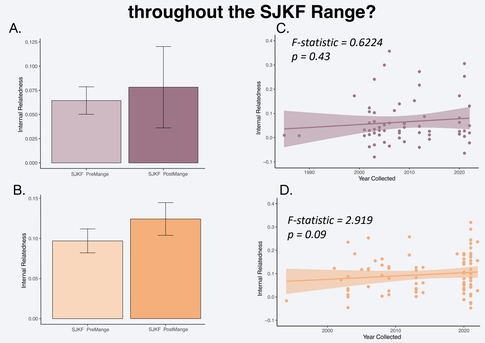


Fig 6. Changes in mean internal relatedness (IR) in individuals sampled prior to (<2012) and after the major (>2020) mange outbreak from both urban (A; purple) and exurban (B; yellow) regions. A linear model comparing individual level IR across time additionally supported increases in IR in more recent time periods for urban (C; purple) and exurban (D; yellow) regions, however this trend was only marginally significant (e.g., at alpha = 0.1) in the exurban population (F-statistic = 2.919, p = 0.09).

Conclusions and Next Steps

- Preliminary analyses indicate minimal gene flow between Bakersfield and adjacent exurban populations.
- Future assignment analyses will investigate exploratory movements that do not result in gene flow but could transmit mange and other pathogens.
- Reduced gene flow may result in increased inbreeding and population differentiation which can have negative impacts on the long-term viability of the SJKF population.
- H_e was lower and internal relatedness (IR) higher in peripheral than central exurban populations, which suggests these geographic regions are more isolated and more susceptible to genetic drift and inbreeding.
- Next, whole genome sequencing will allow us to quantify levels of inbreeding load and deleterious variation.
- We detected potential increases in IR over time across both the urban and exurban populations. Additional sampling of historical and contemporary kit foxes is needed to clarify these apparent trends.
- We plan to conduct isolation by environment analyses to investigate whether local adaptation contributes to differentiation.
- We will also compare genomic diversity of SJKF to related populations of Desert kit fox (*Vulpes macrotis ssp.*).

References

Scan for digital poster & references

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Acknowledgements

Funding: Central Valley Project Conservation Project (Bureau of Reclamation Agreement No. R22AP00305)

Sample Acquisition: Chris Conroy, Museum of Vertebrate Zoology at University of California Berkeley; California Department of Fish and Wildlife; Endangered Species Recovery Program at Cal State University Stanislaus.

Lab Assistance: Mariyam Sadyrova

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