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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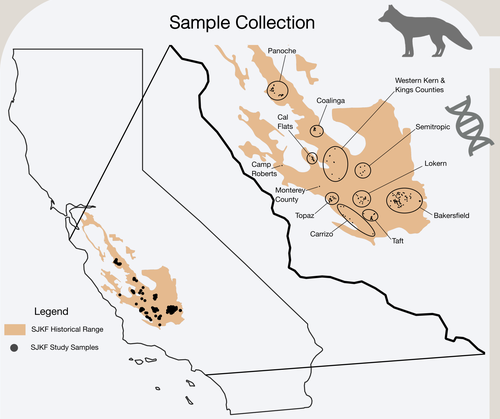
Introduction

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 became extirpated (date?), presumably 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.[7] There has been minimal evidence of mange occurring in exurban kit fox populations outside of the Bakersfield region, which may indicate that dispersal between urban and exurban regions is low.[8] 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[9]
- 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[10] as well as maximum likelihood population assignment (K = 2–10) in the program ADMIXTURE.[11] Additionally, we compared the genetic dissimilarity across the SJKF range using a pairwise F_{ST} analysis in hierfstat.[12]

Characterizing Heterozygosity and Internal Inbreeding

To calculate regional differences in H_e we first calculated individual H_e from the site frequency spectrum [easySFS][13] using genotype likelihoods [angsd][14]. We calculated internal relatedness (IR) which serves as a proxy for inbreeding using GENHET[15]

Preliminary Results

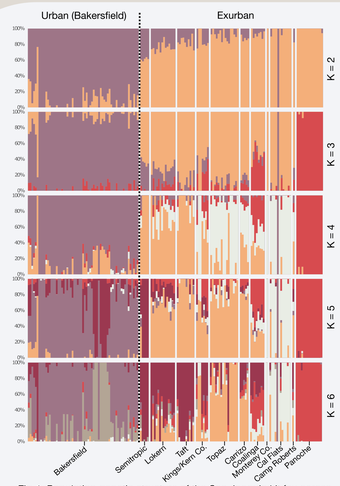


Fig 1. Population genetic structure of the San Joaquin kit fox across its contemporary range identified by the maximum likelihood clustering algorithm implemented in the program ADMIXTURE[CITE] Admixture proportions for each individual are shown as bar plots. We tested K = 2–10 genetic clusters using 11,155 nuclear loci and identified K = 6 had the highest model support (K = 1–6 shown here), and structure appeared hierarchically within the urban and exurban populations after their split at K = 2. .

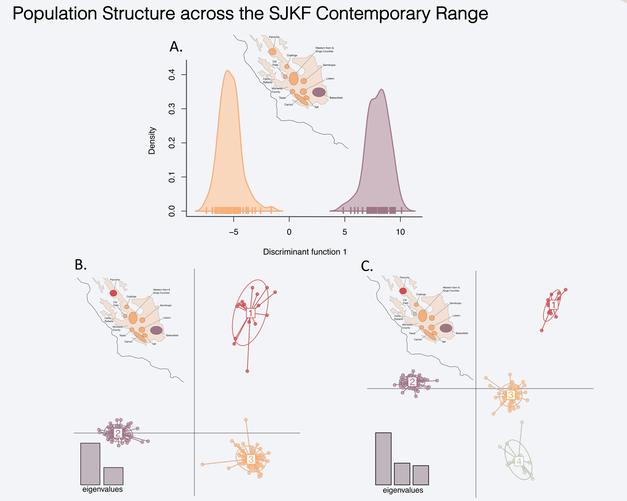


Fig 2. Discriminant analysis of principal components (DAPC) of San Joaquin kit fox individuals based on 11,115 nuclear loci obtained through a reduced representation genotyping-by-sequencing approach. The DAPC analysis had the highest support for 2 genetic clusters (Fig 2A. urban vs. exurban). Increasing the number of genetic clusters resulted in the hierarchical genetic separation of distinct geographic regions within the exurban population, including the Panoche region (Fig 2B.; 3 genetic clusters) and the Cal Flats region (Fig 2C.; 4 genetic clusters).

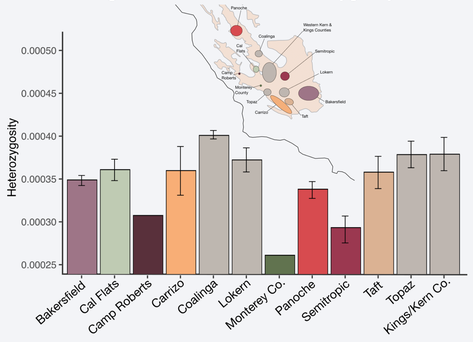


Fig 4. Preliminary results identified variable levels of heterozygosity across distinct regions of the SJKF range. We identified reduced heterozygosity in peripheral regions, when compared to regions found within the center of the range (see map inset). The lowest levels of H_e were identified in Camp Roberts (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.

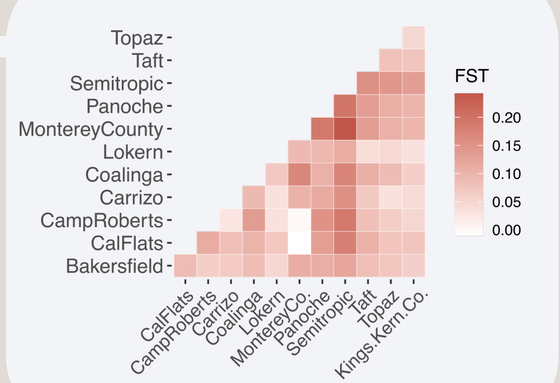


Fig 3. Pairwise matrix of F_{ST} between regions within the contemporary San Joaquin kit fox range highlighting elevated genetic differentiation between

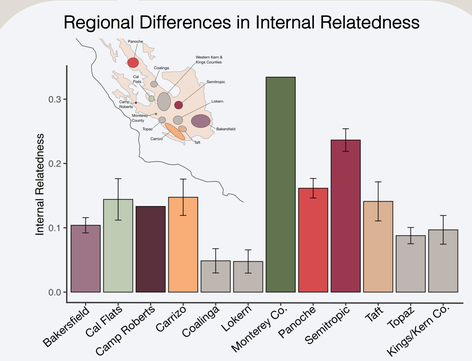


Fig 5. Preliminary results identified variable levels of internal relatedness (IR) across distinct regions of the SJKF range. We identified elevated IR in peripheral regions, when compared to regions found within the center of the range (see map inset). The highest levels of IR were identified in Monterey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

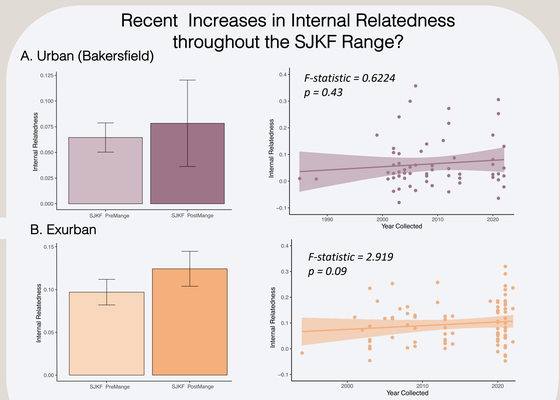
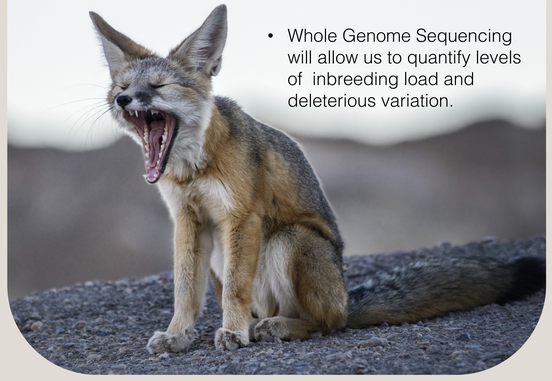


Fig 6.

Conclusions and Next Steps

- Preliminary analyses indicate minimal gene flow between Bakersfield and adjacent exurban populations. While reduced movement between disjunct regions of the SJKF range may effectively limit the transmission of mange from the urban population into exurban regions, it may also result in increased inbreeding and population differentiation which can have negative impacts on the long-term viability of the SJKF population.
- H_e is reduced and internal relatedness (IR) is elevated in peripheral exurban populations, which may indicate these geographic regions are more isolated, and therefore more susceptible to genetic drift and inbreeding.
- We detected an increase in IR over time across both the urban and exurban populations, but these results were only significant in the exurban group. Additional sampling of both historical and contemporary kit foxes could elucidate whether there is in fact a significant increase in inbreeding in the modern SJKF population.
- We plan to conduct isolation by environment analyses to determine whether observed population structure in the SJKF is a result of genetic drift or whether local adaptation contributes to differentiation.
- We will also compare genomic diversity of SJKF to related populations of Desert kit fox (*Vulpes macrotis ssp.*).
- Whole Genome Sequencing will allow us to quantify levels of inbreeding load and deleterious variation.



References

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Acknowledgements

Funding: Central Valley Project Conservation Project (Grant # ????)

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 Work: Maryiam Sadyrova