

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

UCDAVIS

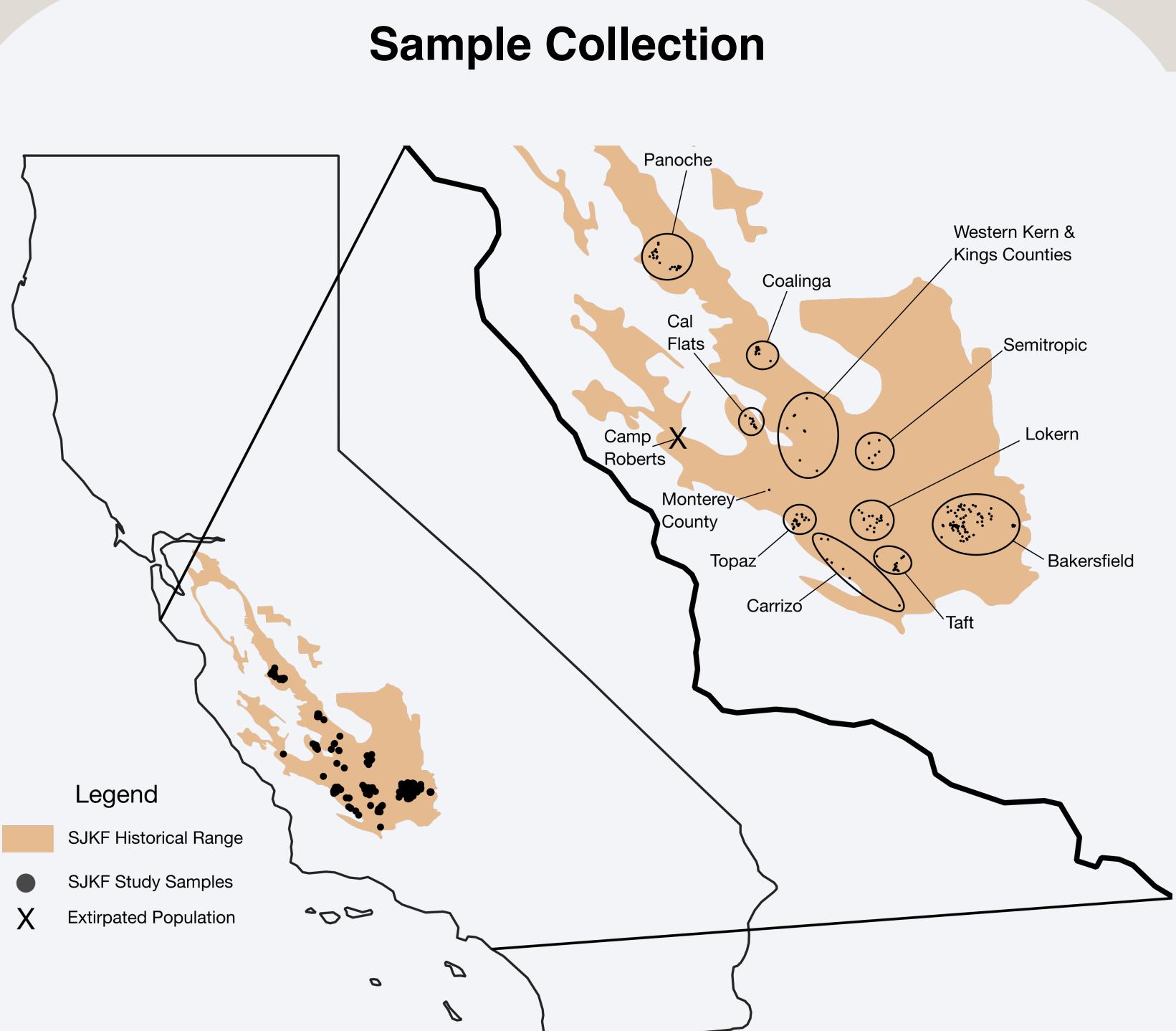
Sophie Preckler-Quisquater¹, Katelyn Sanchez^{2,3}, Brian Cypher⁴, Jaime Rudd⁵, Deanna Clifford^{5,6}, Stevi Vanderzwan¹, and Ben Sacks^{1,7}

(1) Mammalian Ecology and Conservation Unit, Veterinary Genetics Laboratory, School of Veterinary Medicine, University of California, Davis, CA (2) Texas A&M University, College Station, Texas (3) Ecological and Evolutionary Response to Rapid Environmental Change NSF Research Experience for Undergraduates, University of California, Davis, Davis, Ca (4) Endangered Species Recovery Program, California State University-Stanislaus, Turlock, CA 95382, USA (5) Wildlife Health Laboratory, California Department of Fish and Wildlife, Rancho Cordova, CA 95670, USA (6) Department of Medicine and Epidemiology & Karen C. Drayer Wildlife Health Center, School of Veterinary Medicine, University of California, Davis, Davis CA 95616 USA (7) Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA.

Introduction

The San Joaquin kit fox (SJJKF; *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 SJJKF, 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, 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 SJJKF 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 SJJKF individuals sampled prior to the mange outbreak ($n = 89$) as well as of SJJKF individuals sampled from the contemporary population ($n = 109$), focusing on both urban ($n = 82$) and exurban ($n = 116$) regions.

Methods



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 ($n = 116$).



Sample Collection

- DNA extraction using Qiagen DNEasy Blood and Tissue Kit
- Genotyping-by-Sequencing⁸
- We retained 157 SJJKF samples that were sequenced at ~33x coverage across 11,155 nuclear loci

Population Structure

We conducted a discriminant analysis of principal components (DAPC) in adegenet⁹ as well as maximum likelihood population assignment ($K = 2–10$) in the program ADMIXTURE.¹⁰ Additionally, we compared the genetic dissimilarity across the SJJKF range using a pairwise F_{ST} analysis in hierfststat.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 GENHET¹⁴.

Preliminary Results

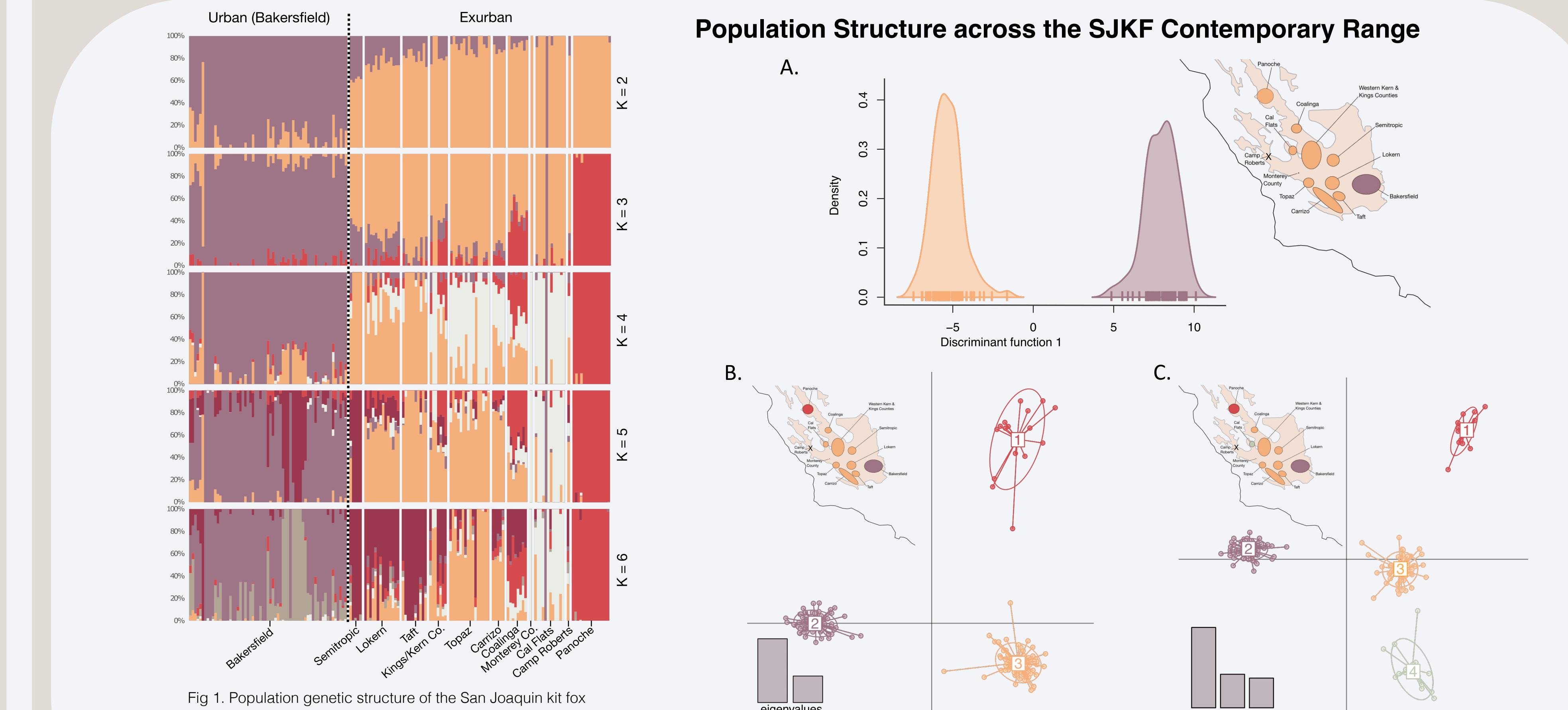


Fig 2. Discriminant analysis of principal components (DAPC) of San Joaquin kit fox individuals. The DAPC analysis had the highest support for two genetic clusters (A), differentiating the urban (purple) and exurban (yellow) populations. Increasing the number of genetic clusters in the DAPC analysis resulted 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

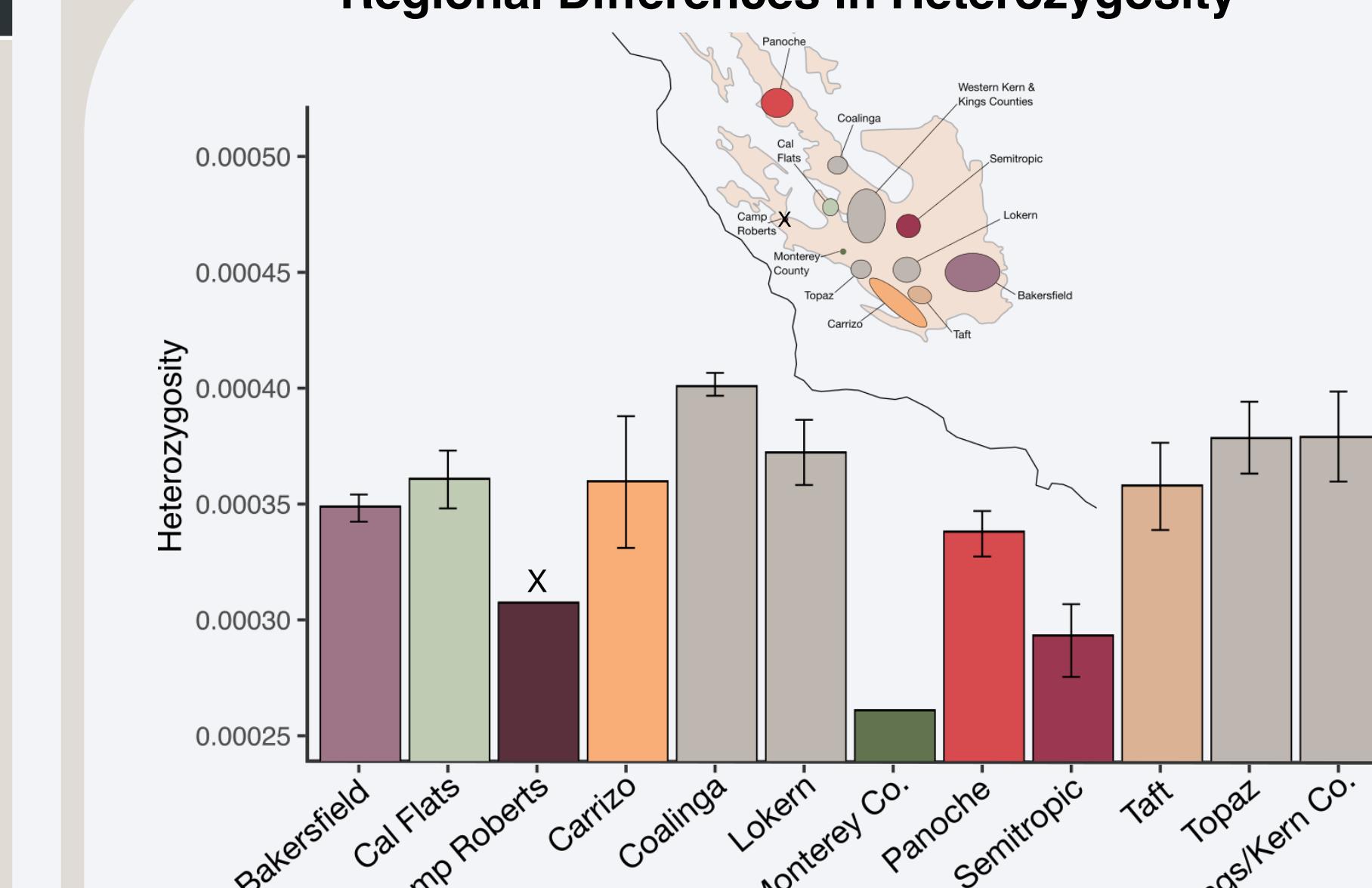


Fig 4. Preliminary results identified variable levels of heterozygosity across distinct regions of the SJJKF range. We identified reduced heterozygosity in peripheral regions, when compared to regions found within the center of the range (gray; see map inset). The lowest levels of H_e were identified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

Regional Differences in Internal Relatedness

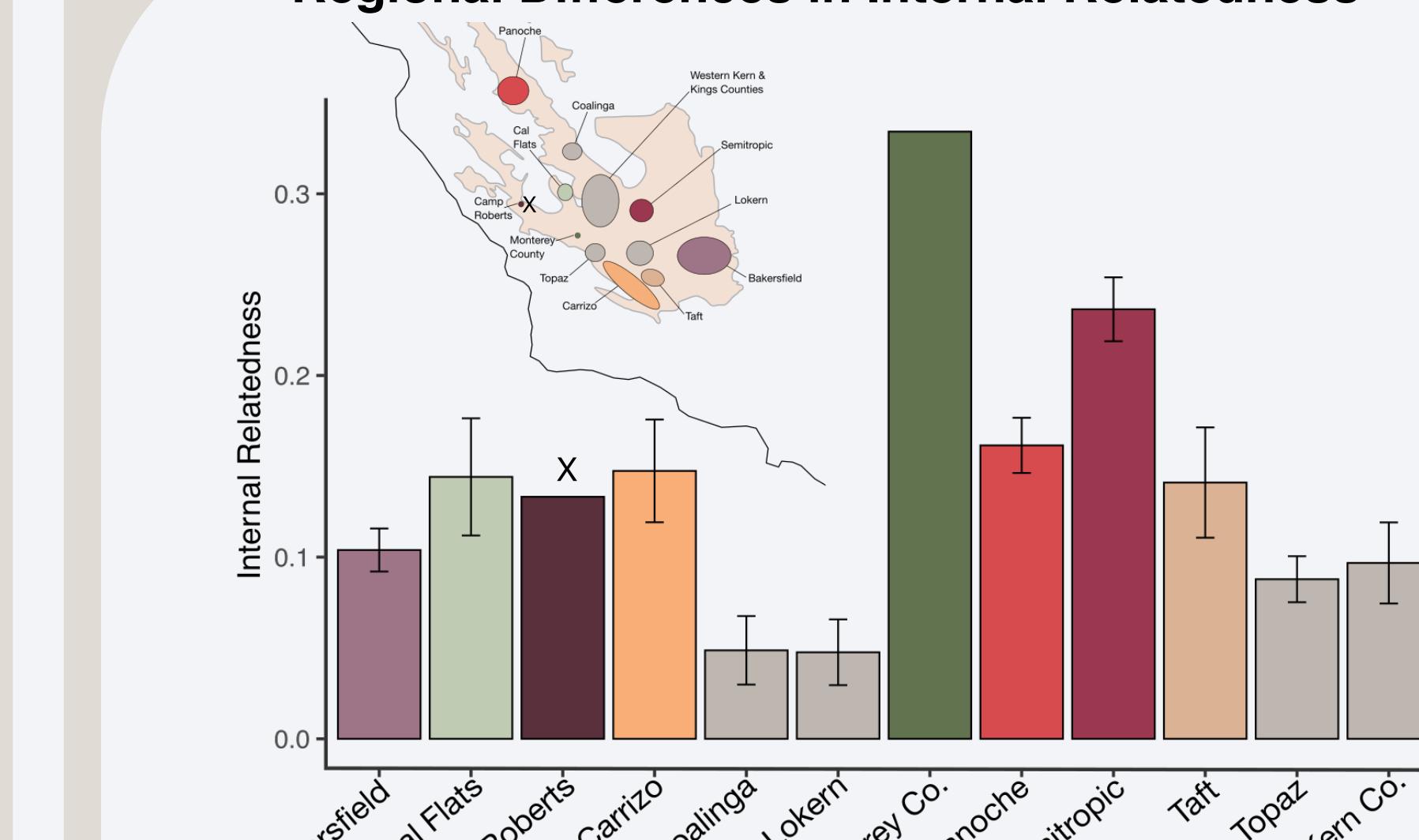


Fig 5. Preliminary results identified variable levels of internal relatedness (IR) across distinct regions of the SJJKF range. We identified elevated IR in peripheral regions, when compared to regions found within the center of the range (gray; see map inset). The highest levels of IR were identified 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.

Genetic Differentiation Among SJJKF Regions

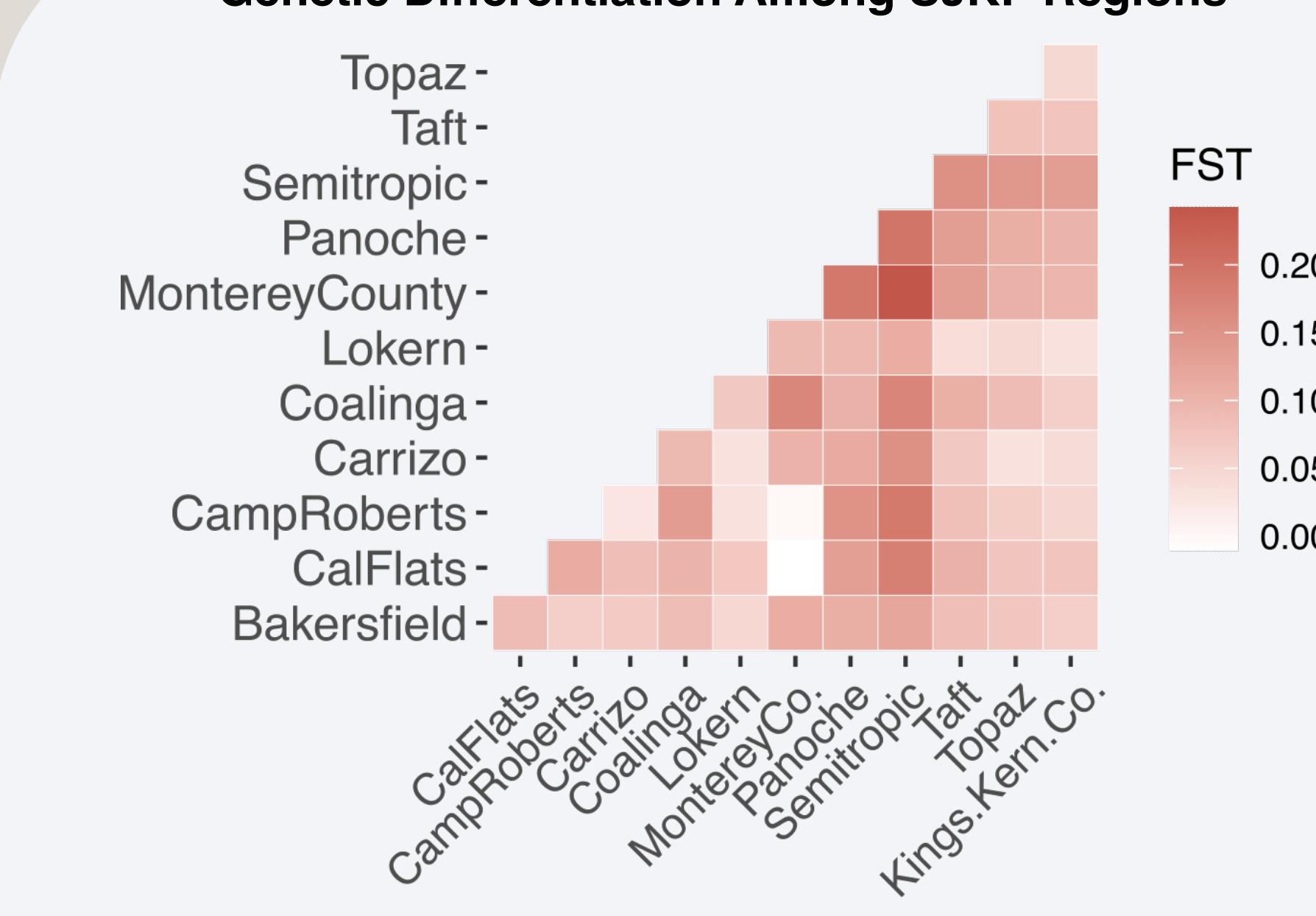


Fig 3. Pairwise matrix of F_{ST} between regions within the contemporary San Joaquin kit fox range highlighting greater genetic differentiation among peripheral populations ($\text{mean } F_{ST} = 0.12 \pm 0.07$) when compared to differentiation among both central populations ($\text{mean } F_{ST} =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

Recent Increases in Internal Relatedness throughout the SJJKF Range?

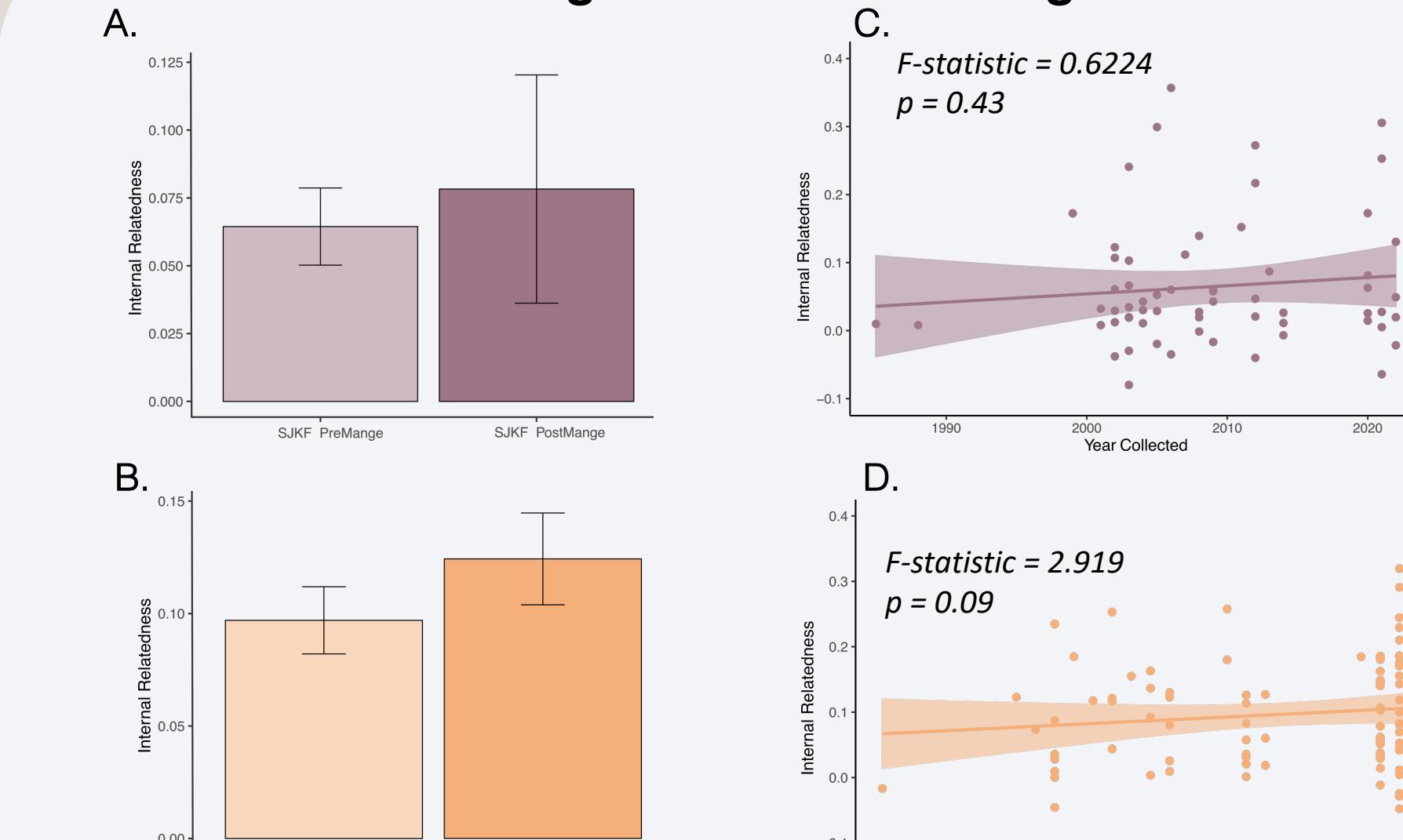


Fig 6. Changes in mean internal relatedness (IR) in individuals sampled prior to (<2012) and after the major (>2020) mange outbreak from both urban (A; purple) and exurban (B; yellow) regions. A generalized linear model comparing individual level IR across time additionally supported increases in IR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

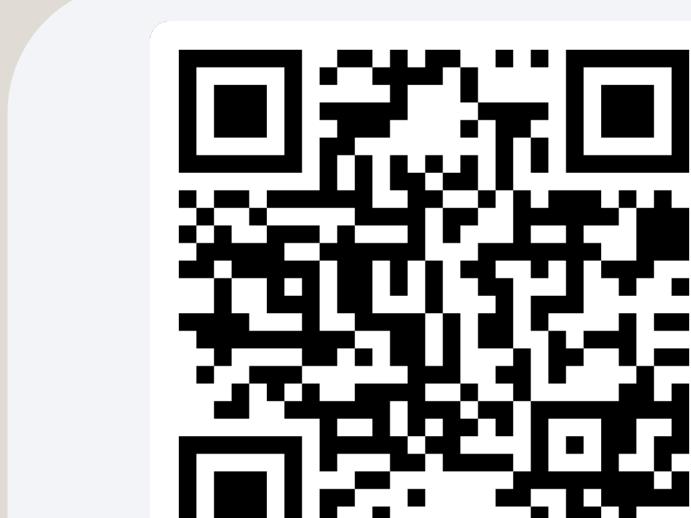
Conclusions and Next Steps

- Preliminary analyses indicate minimal gene flow between Bakersfield and adjacent exurban populations. While reduced movement between disjunct regions of the SJJKF 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 SJJKF 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. Whole Genome Sequencing will allow us to quantify levels of inbreeding load and deleterious variation.
- 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 SJJKF population.
- We plan to conduct isolation by environment analyses to determine whether observed population structure in the SJJKF is a result of genetic drift or whether local adaptation contributes to differentiation.



- We will also compare genomic diversity of SJJKF to related populations of Desert kit fox (*Vulpes macrotis* ssp.).

References



Scan for digital poster & references

Contact Info



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 Work:** Mariyam Sadyrova

References

1. Federal Register. (1967) Department of Interior, Office of the Secretary, Native Fish and Wildlife, Endangered Species. *Federal Register* 32:4001
2. U.S. Fish and Wildlife Service. (2010) San Joaquin kit fox (*Vulpes macrotis mutica*) 5-year review: summary and evaluation. United States Fish and Wildlife Service, Sacramento, CA.
3. Cypher, B. L., S. E. Phillips, and P. A. Kelly. (2013) Quantity and distribution of suitable habitat for endangered San Joaquin kit foxes: conservation implications. *Canid Biology and Conservation* 16:25-31.
4. Cypher, B. L., S. C. McMillin, T. L. Westall, C. Van Horn Job, R. C. Hosea, B. J. Finlayson, and E. C. Kelly. (2014). Rodenticide exposure among endangered kit foxes relative to habitat use in an urban landscape. *Cities and the Environment* 7(1): Article 8.
5. Cypher, B. L., J. L. Rudd, T. L. Westall, L. W. Woods, N. Stephenson, J. E. Foley, D. Richardson, and D. L. Clifford. (2017). Sarcoptic mange in endangered kit foxes: case histories, diagnoses, and implications for conservation. *Journal of Wildlife Diseases* 53:46-53.
6. White PJ, Berry WH, Eliason JJ, Hanson MT (2000) Catastrophic decrease in an isolated population of kit foxes. *The Southwestern Naturalist* 45: 204–211.
7. Rudd, J.L.; Clifford, D.L.; Cypher, B.L.; Hull, J.M.; Jane Riner, A.; Foley, J.E. (2020) Molecular epidemiology of a fatal sarcoptic mange epidemic in endangered San Joaquin kit foxes (*Vulpes macrotis mutica*). *Parasites Vectors*, 13, 456.
8. Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., Mitchell, S.E., (2011) A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for a High Diversity Species. *PLoS ONE* 6, e19379.
9. Jombart T (2008). “adegenet: a R package for the multivariate analysis of genetic markers.” *Bioinformatics*, 24, 1403-1405.
10. D.H. Alexander, J. Novembre, and K. Lange. (2009) Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, 19:1655–1664
11. Goudet, J. (2005) Hierfstat, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Notes*. 5: 184-186
12. RN Gutenkunst, RD Hernandez, SH Williamson, CD Bustamante (2009) Inferring the joint demographic history of multiple populations from multidimensional SNP data *PLoS Genetics* 5:e1000695
13. Korneliussen et al. (2014)ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinformatics*, 15:356.
14. Coulon A. (2010). GENHET: an easy-to-use R function to estimate individual heterozygosity. *Molecular Ecology Resources* 10:167–169.