Genomic Sequencing across a Zone of Secondary Contact Uncovers Complex Demographic History and Admixture between Cryptic Gray Fox Lineages

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Introduction

Past climatic fluctuations have heavily influenced current species distributions, generating complex evolutionary histories through periods of isolation in refugia as well as secondary contact and gene flow post-expansion. The gray fox (Urocyon cinereoargenteus) represents the most basal extant canid lineage and occurs only in the Americas. Previous mitochondrial analysis identified deeply divergent (up to 1 Mya) eastern and western lineages, and a major phylogeographic break between lineages along the Great Plains Suture Zone, indicating that gray foxes have likely been isolated for numerous glacial-interglacial cycles.[1,2] However, it is still unclear whether these lineages maintained complete reproductive isolation during this time, or whether there were periods of secondary contact and admixture post-divergence. Using a combination of reduced-representation (n = 259) and whole-genome (n = 42) sequencing of gray foxes across their US range, we generated estimates of nuclear split times and assessed concordance with previously published mitochondrial estimates.[1,2] We additionally quantified genome-wide ancestry proportions, to identify whether there was any evidence of nuclear admixture between eastern and western lineages at the previously described contact zone. Using a local ancestry inference approach, we then tested whether gene flow was recent (potentially due to human induced landscape changes in the last 100 years) or whether it occurred during an older, post-Pleistocene expansion event. We also explored whether selective introgression of beneficial genetic variation may have played a role in the evolutionary history of these two lineages. Understanding the complexities surrounding divergence and secondary contact between these gray fox lineages will allow us to better understand the role past and future climate shifts may play in the overall diversity of species.

Methods

Sample Collection

We obtained 376 gray fox samples during 2013–2019 from three different sources across North America including 211 DNA extracts from Texas, 26 DNA extracts from California, and 139 tissue samples from fur-trapped foxes across several eastern and western states

Lab Work Part 1: Extraction + GBS

- DNA extraction using Qiagen DNEasy Blood and Tissue Kit
- Genotyping-by Sequencing[3]

Global Ancestry Inference

We conducted a principal components analysis as well as Bayesian population assignment to assess admixture (K = 2) using 44,931 GBS loci in fastStructure.[4]

Estimating Divergence Time

To assess the phylogenetic relationship of eastern and western gray foxes and estimate nuclear divergence time, we incorporated GBS data from other members of the Canidae family, and first generated a maximum likelihood phylogram.[5] We then converted this tree to a semi-parametric time calibrated tree with a root age estimated between 9 and 11.9 mya.[6]

Lab Work Part 2 : WGS

We conducted whole genome sequencing on a subset of our samples that included unadmixed Western (n = 12) and Eastern (n = 11) reference gray foxes as well as gray foxes from each of the putatively admixed eastern (n = 9) and Western (n = 9) populations.

Local Ancestry Inference and Estimating Admixture Timing

In order to identify the distribution of eastern vs western gray fox ancestry throughout the hybrid genomes, we first identified a set of ~500k Ancestry Informative SNPs, and then used Ancestry-HMM, a hidden Markov model that can classify each chromosomal region (eastern H_o, western H_o, H_e) using information derived from non-admixed Western and Eastern reference populations.[7] This approach simultaneously infers local ancestry and estimates the most likely number and timing of admixture pulses that generated the observed ancestry patterns.

Identifying Selectively Introgressed Regions

To identify putative genomic regions that have undergone selective introgression across the zone of secondary contact, we averaged the relative introgressed ancestry proportions at each site across the genome within the western and eastern admixed populations.

Preliminary Results

Global Ancestry Inference

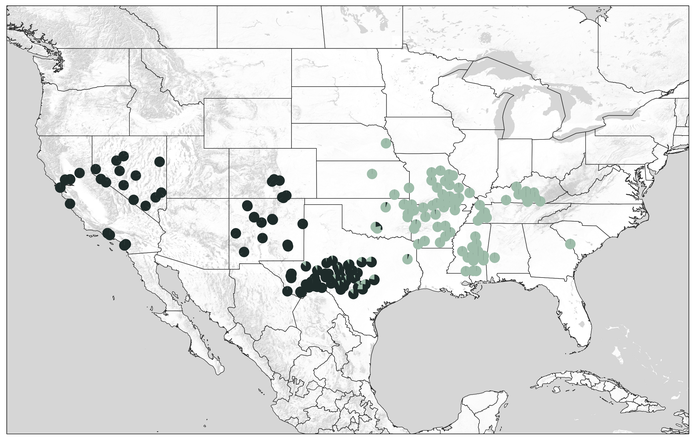


Fig 1A. Population assignments of North American gray fox samples identified using the program fastStructure (K = 2) based on 44,931 nuclear SNPs obtained through a reduced representation genotyping-by-sequencing approach. The primary division between the eastern (light green) and western (dark green) lineages was identified in the southern Great Plains region. We also identified low levels of introgression (1 – 28%) across the zone of secondary contact as indicated by the variable proportions of ancestry shown in each individual pie chart.

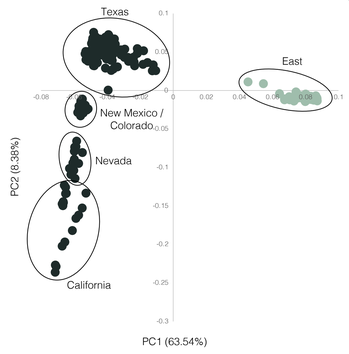


Fig 1B. Principal components analysis (PCA) of gray fox individuals based on 44,931 nuclear SNPs obtained through a reduced representation genotyping-by-sequencing approach. The first principal component axis accounted for most of the variance (63.5%) and discriminated between the eastern (light green) and western (dark green) lineages. The second principal component axis explained 8.4% of the variance and revealed some population structure within the western lineage. Each circle represents an individual fox colored by its geographic location with broader labeled circles indicating more fine-scale geographic distribution.

Local Ancestry Inference

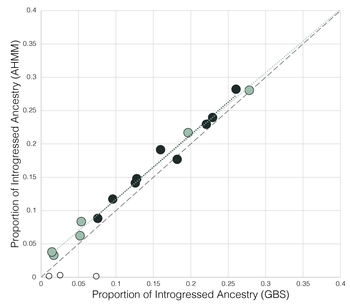


Fig 3. Relative proportions of introgressed ancestry were highly concordant for both western (dark green) and eastern (light green) admixed individuals when comparing our global ancestry approach (44,931 GBS loci + Bayesian clustering approach), and our local ancestry inference (500k AIM loci + Hidden Markov Model). Open circles indicate three samples that were identified as admixed in our global ancestry approach but had <1% introgression in our local ancestry inference and were therefore removed from downstream analyses.

Divergence Time Estimate

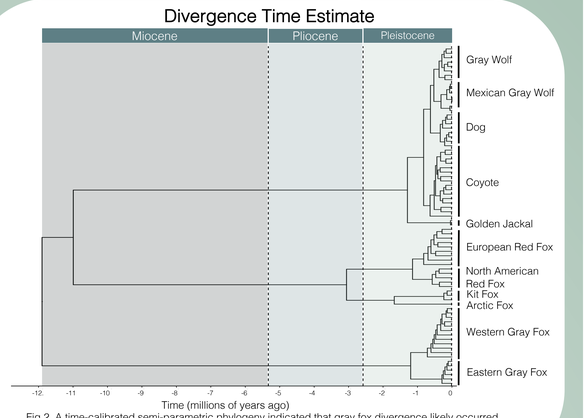


Fig 2. A time-calibrated semi-parametric phylogeny indicated that gray fox divergence likely occurred sometime between the divergence times of Eurasian/North America red fox, and arctic/kit fox. This supports a mid-Pleistocene split, in line with previously published mitochondrial estimates, and is much older than splits typically used to classify intraspecific divisions in North American carnivores

Estimating Timing of Admixture Pulses

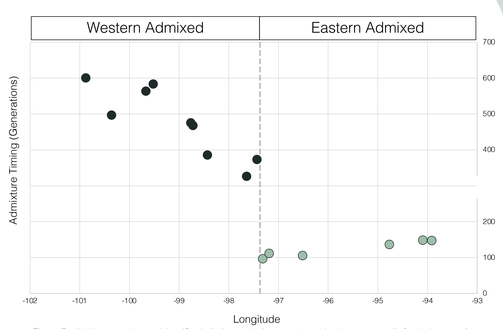


Fig 4. Preliminary analyses identified distinct admixture pulses in the western (left; dark green) and eastern (right; light green) populations. Inferred timing of the migration pulse was estimated separately for each western (n = 9) and eastern (n = 6) admixed genome. The western admixed population appears to have experienced an older pulse of eastern introgression (300 – 600 generations ago), while the eastern admixed population experienced a more recent pulse of western introgression (100 –150 generation ago).

Identifying Selectively Introgressed Regions

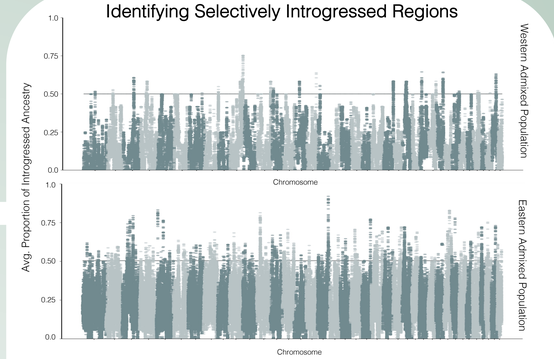
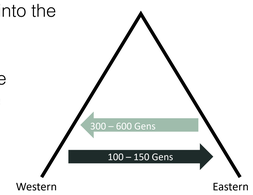


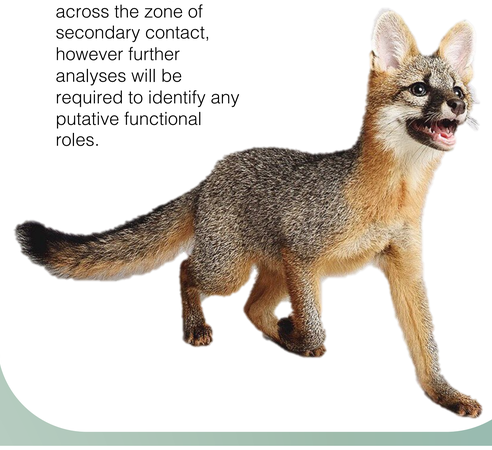
Fig 5. Proportion of eastern and western introgressed ancestry at each SNP averaged across all individuals within the western (left panel; n=9) and eastern (right panel; n=6) admixed populations, respectively. We identified the top 1% outliers, denoted by the gray horizontal line, for the western (>0.504) and eastern (>0.499) admixed populations based upon the distribution of the average introgression proportions for all loci across the genome.

Conclusions

- A zone of secondary contact with nuclear gene flow between eastern and western gray fox lineages was identified in the southern Great Plains region.
- Divergence estimates correspond to the Early-Middle Pleistocene Transition (1.4 – 0.4 mya) in the Irvingtonian land mammal age, which is substantially older than those typically characterizing intraspecific divisions for most North American carnivores.[8,9]
- We identified an older pulse of eastern ancestry into the western gray fox population approximately 300-600 generations ago. We also identified a more recent pulse of western ancestry into the eastern gray fox lineage approximately 100-150 generations ago. This more recent pulse may coincide with human-induced landscape changes facilitating migration.



- Several regions may have undergone selective introgression across the zone of secondary contact, however further analyses will be required to identify any putative functional roles.



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