

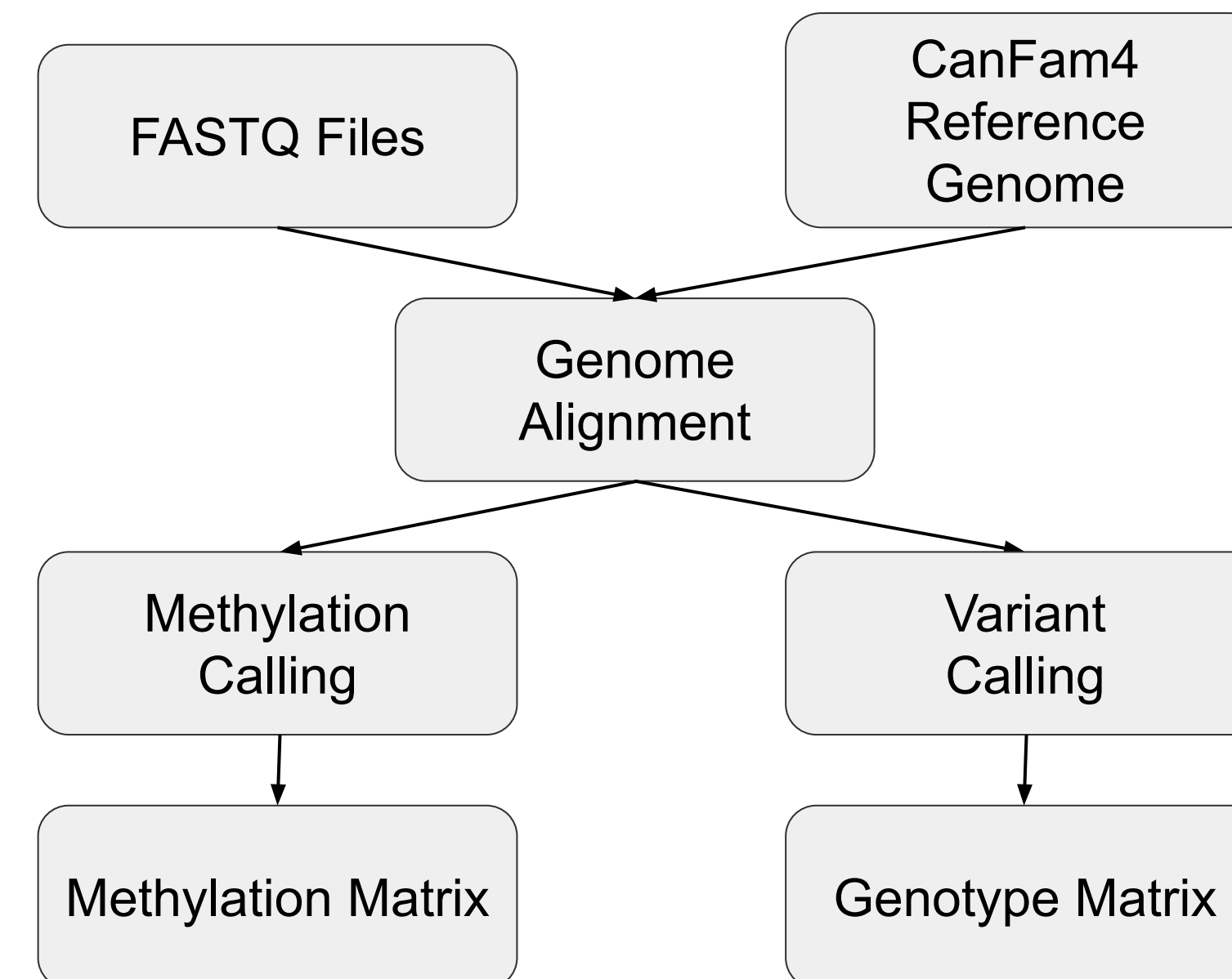
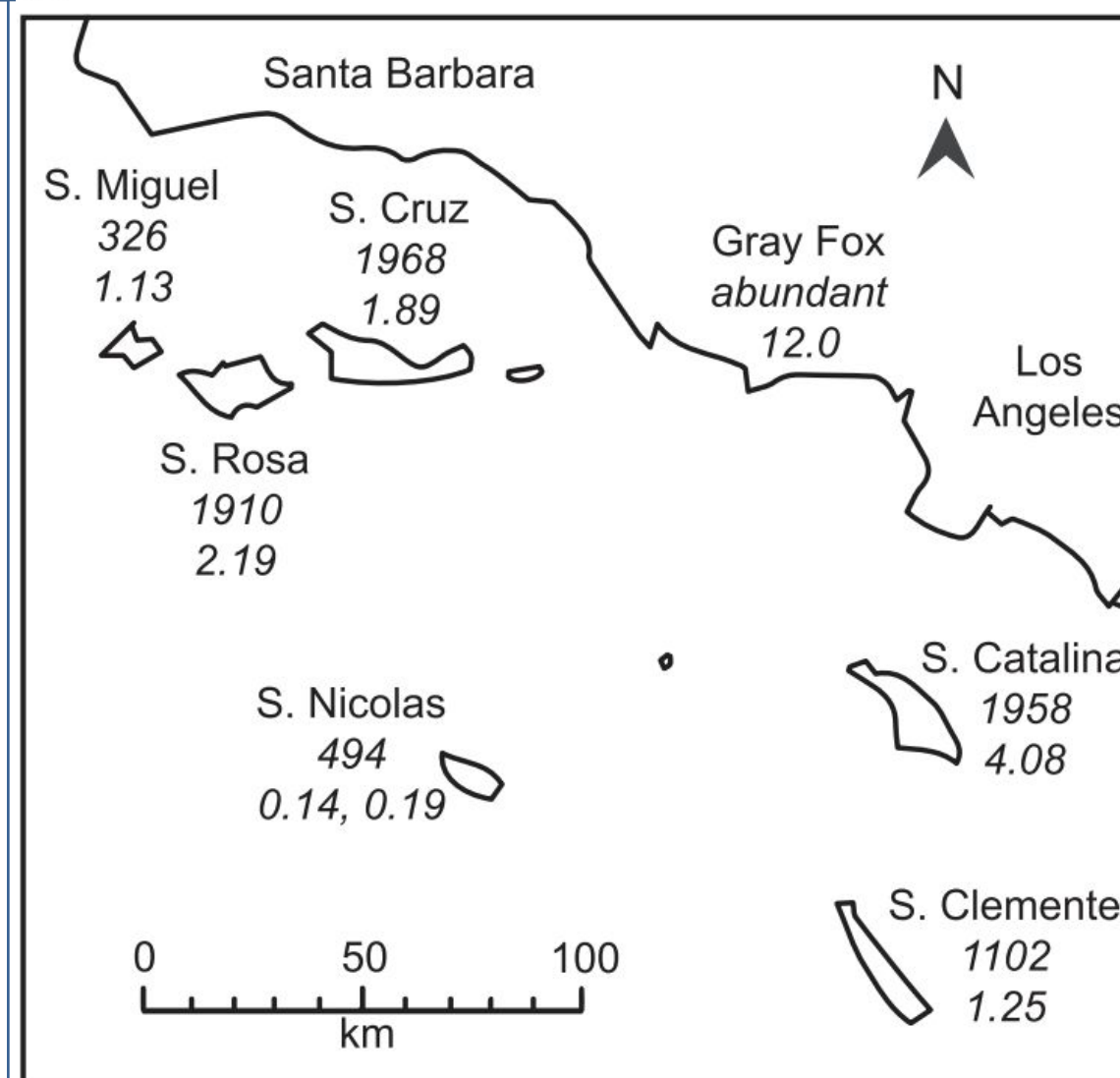
Island Foxes Endure Genomic Flatlining with the Help of Epigenetics

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Introduction

- Island Fox populations are very small
- Island Foxes suffer from extreme lack of genome wide-variation known as genomic flatlining [2]
- It is widely accepted that genomic flatlining is dangerous to population survival [1], but island fox populations have continued to grow considerably in recent years [4]
- Previously, these populations have been studied purely through a genetic lens
- No literature centered on epigenetics, and in particular, DNA methylation
- Methylation has been established to affect gene expression



Results

- ANOVA testing revealed that between gray, San Nicolas, and Santa Catalina fox genomes there exists ($p < 0.05$):
 - 43 distinct methylation sites
 - 104 genetically distinct sites
- Between these three groups, these sites correspond to:
 - 19 genes with differential methylation
 - 73 genes with genetic variation
 - 8 genes with both types of variation

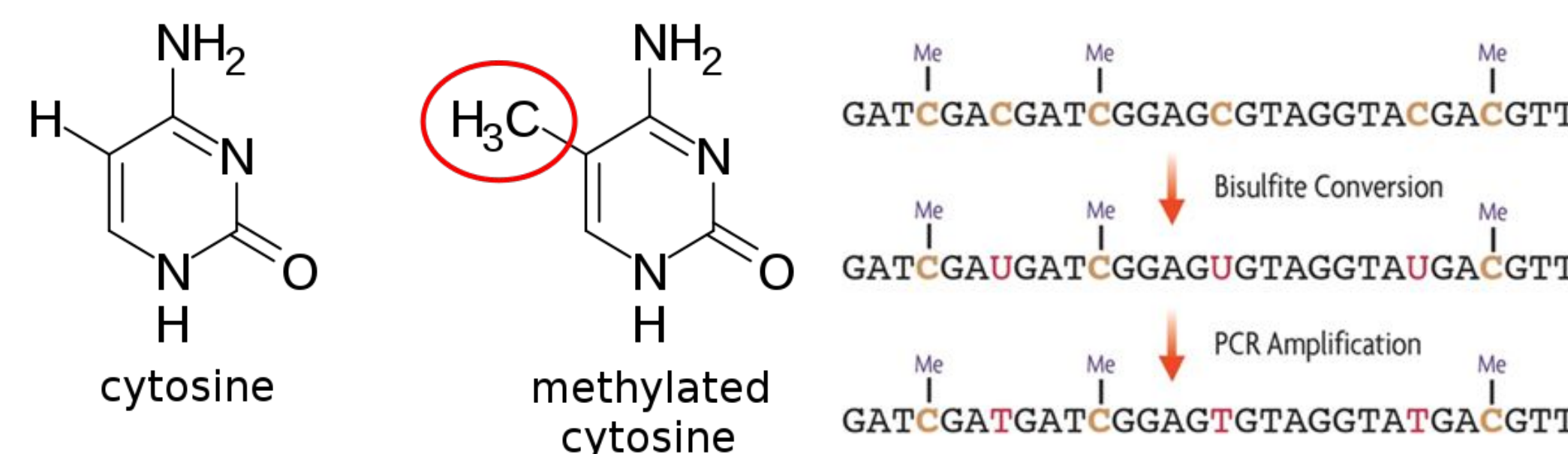
Results

- After controlling methylation values for age and sex, intraindividual methylation variances were calculated for each fox at ~100 sites with the most differential methylation
- Combined Island Fox groups exhibit greater intraindividual methylation variance than the gray mainland foxes ($p < 0.05$)

Group	% genome heterozygous	Mean Intraindividual Methylation Variance
Gray:	$9.7 \times 10^{-4} \%$	0.0058
Santa Catalina:	$7.0 \times 10^{-4} \%$	0.0166
San Nicolas:	$5.6 \times 10^{-4} \%$	0.0195

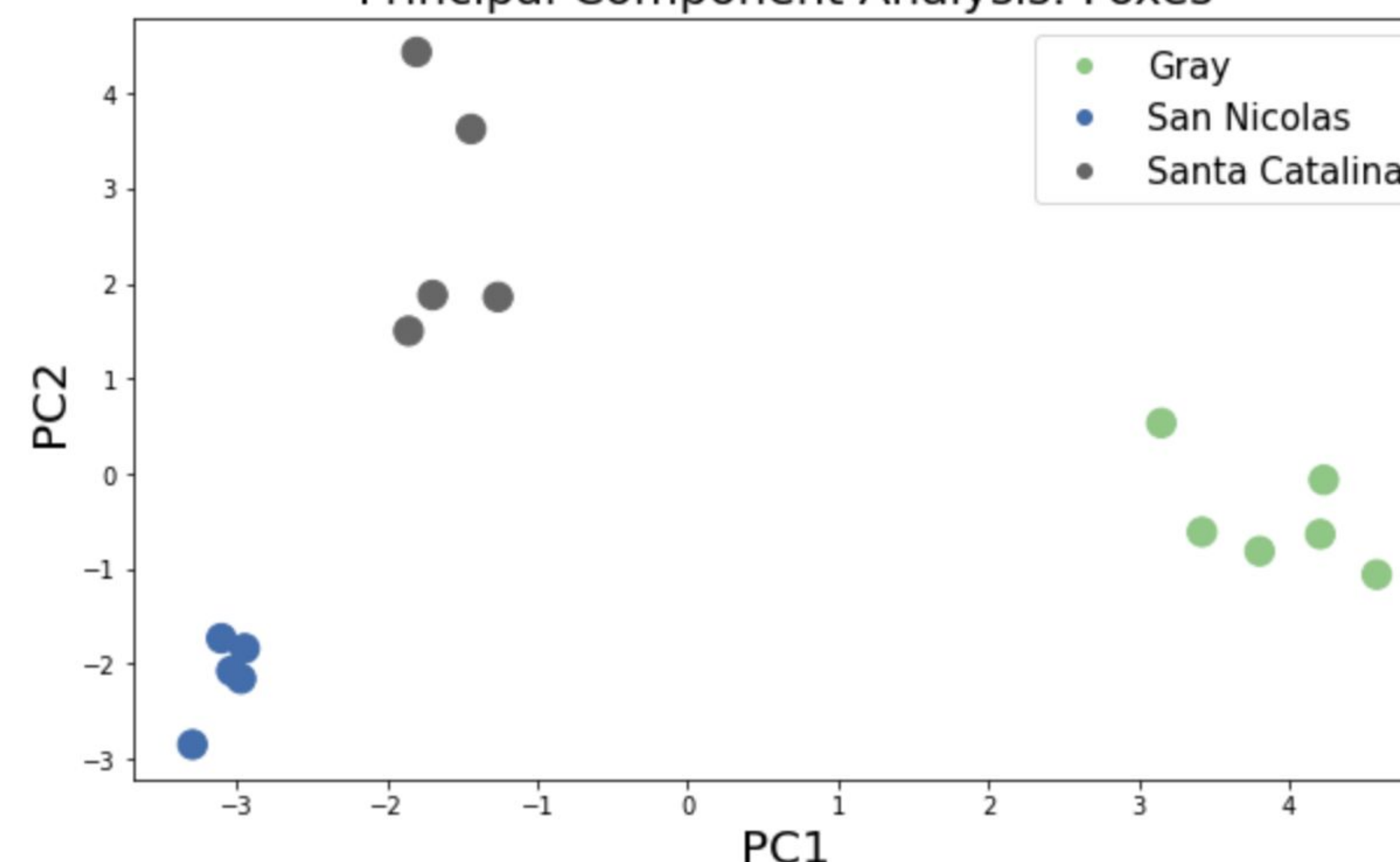
- Between the three groups of foxes, genes that have differential methylation are involved in:
 - growth and differentiation
 - neuronal development and synaptic functions
 - cell and hormone signalling
 - osteoblast differentiation and bone formation
 - embryonic development

Materials and Methods



- Targeted Bisulfite Sequencing was used to identify over 80,000 methylation sites in 16 different foxes (island and gray)
- Output matrix entries have a fraction of cytosines methylated at a particular site in a particular individual

Principal Component Analysis: Foxes



Conclusions

- Island Foxes differentially express genes important for **cell signaling** and **embryonic development**
- Island Fox methylation patterns are themselves **distinct between islands**
- Island Foxes exhibit much **greater epigenetic variation** than gray foxes, suggesting a possible “epigenetic diversity” in their populations

References

- [1] Frankham, R. (2005). Genetics and extinction. *Biol. Conserv.* 126, 131–140.
- [2] Robinson JA, Ortega-Del Vecchyo D, Fan Z, Kim BY, vonHoldt BM, Marsden CD, Lohmueller KE, Wayne RK. Genomic Flatlining in the Endangered Island Fox. *Curr Biol.* 2016 May 9;26(9):1183-9. doi: 10.1016/j.cub.2016.02.062. Epub 2016 Apr 21. PMID: 27112291.
- [3] Robinson JA, Brown C, Kim BY, Lohmueller KE, Wayne RK. Purging of Strongly Deleterious Mutations Explains Long-Term Persistence and Absence of Inbreeding Depression in Island Foxes. *Curr Biol.* 2018 Nov 5;28(21):3487-3494.e4. doi: 10.1016/j.cub.2018.08.066. Epub 2018 Oct 25. PMID: 30415705; PMCID: PMC6462144.
- [4] US Fish and Wildlife Service (2016). Removing the San Miguel Island fox, Santa Rosa Island fox, and Santa Cruz Island fox from the federal list of Endangered and Threatened wildlife, and reclassifying the Santa Catalina Island fox from Endangered to Threatened; final rule. *Fed. Regist.* 81, 53315–53333