Department of War
Legacy Resource Management Program

Genomics Imperiled Species-23

Using Genomic Resources to Proactively Monitor Imperiled Species on Department of War Lands

Alexander R. Krohn, PhD
JJ Apodaca, PhD

April 2026

Using Genomic Resources to Proactively Monitor Imperiled Species on Department of War Lands

Table of Contents

Abstract

Department of Defense (DoW) installations support high levels of biodiversity, including species of conservation concern that are important to monitor to prevent encumbrances on the mission. By monitoring and proactively managing species that are not yet threatened or endangered, DoW can potentially avoid listing decisions that would encumber the mission on installations where those species occur. This report demonstrates an additional monitoring tool that requires little field effort, but yields important insights into the status of such species. In a single calendar year, Tangled Bank Conservation (TBC) collected or amassed tissue samples from three species (alligator snapping turtle [*Macrochelys sp.*], northern red-bellied cooter [*Pseudemys rubriventris*], and gopher frog [*Rana capito*]) across 15 DoW installations in seven states. Using genomic sequencing methods, TBC sequenced 616 total samples to derive levels of genomic variation, inbreeding, effective population size, and gene flow for each installation. From these analyses, TBC created base-specific monitoring and management plans to ensure species persistence and thus prevent encumbrances. The data assess the current genetic status of each species on an installation, place DoW installations in a landscape and species-specific context, and create a baseline against which future change can be measured. To that end, TBC trained managers in genomic sample collection and storage at eight installations (Avon Park Air Force Range, Fort Belvoir, Fort Chaffee, Fort Indiantown Gap, Joint Base McGuire-Dix-Lakehurst, Moody Air Force Base, Naval Air Station Pensacola, and Naval Weapons Station Yorktown) so that these monitoring programs can continue at relevant intervals. This work represents the first year of monitoring, targeting three of nine total target species planned over the subsequent two years. In this first year, TBC showed that incorporating genomic monitoring can be a cost-effective way to efficiently census populations of target species. By adding genomic monitoring into the toolkit of installation managers, DoW can proactively manage for a variety of species, and more efficiently prevent those species from encumbering the mission.

Introduction

Department of Defense (DoW) lands contain the highest density of threatened or endangered species on all federal lands (Stein et al. 2008; Petersen et al. 2018; Schultz et al. 2024). Every year, DoW deploys extensive resources to carry out transect surveys and capture-mark-recapture studies for imperiled species, which aid in the estimation of important population parameters (e.g. population sizes, population health, and gene flow levels/patterns). These activities are costly, labor intensive, and often ineffective at providing robust demographic estimates for hard-to-detect species or those with small body sizes. Additionally, despite capturing cryptic individuals, these methods do not create data for novel analyses in the future; they only create data based on present recapture methods.

The purpose of the first year of this work is to 1) conduct field surveys on military sites for three of DoW's mission-sensitive amphibian and reptile species, 2) use genomics to answer critical population monitoring questions, and 3) create lasting genomic resources to monitor species with greater efficiency into the future.

In Year 1, target species are alligator snapping turtle (*Macrochelys sp.*), northern red-bellied cooter (*Pseudemys rubriventris*), and gopher frog (*Rana capito*). Deliverables for Year 1 are listed by task below and will include: genetic datasets of the three target species to more easily re-sequence populations for future monitoring efforts; analysis and report summarizing levels of genetic variation, gene flow, effective population size for each species for each installation sampled; and a standard operating procedure for managers outlining how to collect genome-scale tissue samples in the field. These resources will optimize monitoring efforts on military lands and, with training, prepare the military for the future of monitoring while saving money and preventing recovery efforts from jeopardizing the mission.

This work directly supports the goals of the *Memorandum of Agreement between Naval Facilities Engineering Systems Command and the Office of the Deputy Assistant Secretary of War, Environment and Energy Resilience for Contracting Services in Support of the Department of Defense Partners in Amphibian and Reptile Conservation Network*. This project has the support of the Office of the Assistant Secretary of War (Energy, Installations, and Environment) and is being funded by the DoW Legacy Resource Management Program in support of the DoW Partners in Amphibian and Reptile Conservation Network.

Project Description

Objectives

1. Evaluate the current genomic health of target species populations on DoW sites by estimating recent population trajectories, levels of genetic variation among populations, effective population sizes, and gene flow levels among populations.

2. Create an installation-specific genomic assessment of the species present, assessing specifically the species' resiliency, redundancy, and representation (the three R's) based on the US Fish and Wildlife Service's (USFWS) guide for assessing a species' status.

3. Create lasting genomic resources (genetic datasets of the target species; an analysis and report summarizing levels of genetic variation, gene flow, and effective population size for each species for each installation sampled; and a standard operating procedure for managers outlining how to collect genome-scale tissue samples in the field) that will form the baseline for future studies.

4. Create an ongoing monitoring framework based on these results to assess how the genetic health of these species changes over time due to stressors such as land use changes.

5. Create a targeted, installation-specific future genomic monitoring protocol.

6. Train installation natural resource managers to collect samples and design future genetic monitoring protocols and programs.

Methodology

For each of the three target species for Year 1, the goal was to collect at least five samples on each target installation and five reference samples from lands adjacent to each installation. Tangled Bank Conservation (TBC) spent up to seven days at each installation, surveying both on the installation and on adjacent areas.

TBC surveyed for and captured alligator snapping turtles using two 4 ft diameter hoop nets connected by a single fyke net. Nets were placed in suitable habitat, baited with fish or cat food, secured to living vegetation or to an implanted PVC pipe, and kept partially submerged using jerry cans filled with air inside each net. Nets were checked daily. TBC followed DoW best practices for monitoring alligator snapping turtles whenever possible (Department of Defense Partners in Amphibian and Reptile Conservation 2021).

TBC surveyed for and captured northern red-bellied cooters using either unbaited 2 ft diameter hoop nets, modified basking traps, or hand capture. All traps were checked daily. TBC

followed DoW best practices for monitoring northern red-bellied cooters whenever possible (Department of Defense Partners in Amphibian and Reptile Conservation 2020).

TBC surveyed for and captured gopher frogs using dip nets and visual surveys for tadpoles, eggs or adults. TBC followed DoW best practices for gopher frog monitoring whenever possible (Department of Defense Partners in Amphibian and Reptile Conservation 2019).

Objective 1: Evaluate Current Genomic Health

To evaluate the current genomic health of target species populations, TBC conducted fieldwork at each installation in order to gather tissue samples from target species, then sequenced thousands of loci per sampled individual. TBC used 3RAD, a cost-effective, genome-scale sequencing method that allows precise population genetic estimates at a low per-individual cost (Bayona-Vásquez et al. 2019; Apodaca et al. 2023; Krohn et al. 2024).

First, TBC gathered tissue samples from captured individuals of each target species at each location. Tissue samples from turtles consisted of 0.1 - 0.3 µL of blood smeared on a Flinders Technology Associates (FTA) card (Qiagen, Germantown, Maryland). Tissue samples from gopher frogs included whole eggs, pierced in ethanol; toe clips in ethanol; or tadpole tail clips in ethanol.

TBC extracted blood samples using Quick-DNA Miniprep Kits (Zymo Research, San Diego, CA) following the manufacturer's protocols. TBC verified extraction quality by gel electrophoresis and quantified each sample using a Qubit 3.0 Fluorometer (Life Technologies, Carlsbad, California). TBC prepared the restriction-associated digestion sequencing (RADseq) library following a modified 3RAD protocol (Bayona-Vásquez et al. 2019). The library preparation steps were 1) double enzyme digest, 2) adapter ligation, 3) limited cycle PCR, and 4) a 1.2X concentration Serapure SpeedBead cleanup (Rohland and Reich 2012). In the double enzyme digest mixture, TBC increased the amount of genomic DNA to 10 µL and decreased dH_2O to 0.5 µL. TBC used the restriction enzymes ClaI, BamHI, and MspI (New England Biolabs, Ipswich, Massachusetts) for the digestion. To multiplex each sample, TBC used i5 and i7 iTru adapters and primers as dual internal indexes (Glenn et al. 2019). Following the SpeedBead cleanup, TBC visualized these libraries on a gel and quantified their concentrations using a Qubit. TBC pooled libraries to 100 ng/µL in pools of 48 individual libraries, cleaned them again using SpeedBeads, and eluted the pools to 32 µL. TBC then size-selected the pools to the range of 400–600 bp using a Pippin Prep (Sage Science Inc., Beverly, Massachusetts), and performed a final 0.8X SpeedBead cleanup. TBC quantified the final DNA concentration using a Qubit and then sent samples to Genewiz (Azenta Life Sciences, South Plainfield, New Jersey) for sequencing on an Illumina NovaSeq lane with 150 bp paired-end reads.

TBC used ipyrad (Eaton and Overcast 2020) for RAD sequencing data filtering and assembly. For all analyses except GONE (Santiago et al. 2020), TBC assembled the individuals *de novo*. TBC demultiplexed all individuals by their cut site and internal adapters, and used default parameters except for the minimum depth required to call a base (increased to 10X) and

minimum final length of paired reads (increased to 100). For GONE analyses, TBC aligned the alligator snapping turtle samples to the Suwannee alligator snapping turtle reference genome (NCBI GenBank assembly GCA_033296515.1), the gopher frog samples to the American bullfrog reference genome (NCBI GenBank assembly GCA_042186555.1), and the northern red-bellied cooter samples to the red-eared slider reference genome (NCBI GenBank assembly GCF_013100865.1). All used the same parameters as above. For downstream analyses, TBC removed individuals with fewer than 100,000 raw reads, or greater than 90% overall missing data.

Objective 2: Installation-Specific Genomic Assessment

For each installation, TBC assessed the resilience, redundancy, and representation of each of the three target species by analyzing the sequencing data generated by TBC (Objective 1). TBC used a variety of methods to assess each of the 3R's. To quantify levels of population structure for each species between DoW installations and adjacent areas (redundancy of DoW lands, representation), TBC used a principal component analysis (PCA) and TESS (Caye et al. 2016; Chambers et al. 2023). TBC defined resilience as high when the population has high genetic variation, low inbreeding, and/or high effective population sizes, especially relative to other locations of the same species. TBC defined representation as high if there were many nearby localities of the same genetic population. Finally, TBC defined redundancy as high if those populations were also well connected genetically and showed high resilience.

TBC quantified levels of genetic variation, effective population size, and population size changes, all indicators of resilience, over time using SNPRelate (Zheng et al. 2012), Estimated Effective Migration Surfaces (EEMS; Petkova et al. 2016), and GONE (Santiago et al. 2020). See below for specific methods and filtering parameters.

Given that RADseq shears the genome randomly, but can only use SNPs shared across multiple individuals, missing data is a normal and common part of RADseq datasets (Eaton et al. 2017). Each species varies in the amount of missing data it returned from the sequencer (see Results), and each analysis varies in its sensitivity to missing data (Hodel et al. 2017; Eaton et al. 2017; O'Leary et al. 2018). Because the PCA imputes for missing data using the built-in ipyrad analysis tools (Eaton and Overcast 2020), TBC used the least stringent method to filter the dataset, keeping one biallelic single nucleotide polymorphism (SNP) per RAD locus present in at least 50% of individuals. For all other analyses, TBC filtered to one biallelic SNP per RAD locus present in at least 70% of individuals. Given the high number of gopher frogs, and their larger genome size compared to the turtles, they showed variable, but high, rates of missing data (see Results). As such, TBC ran all gopher frog analyses with one biallelic SNP per RAD locus present in 50%, 60% and 70% of individuals. TBC then compared the results (PCA, TESS), or took the mean and range of those estimates (genetic variation, effective population size, population trajectories) so that the uncertainty due to missing data could be quantified.

To calculate levels of genetic variation, TBC calculated the observed heterozygosity (H_O; Nei 1987) and within-population gene variation (i.e., expected heterozygosity, H_S; Nei 1987;

Goudet 2005) using the package 'hierfstat' (Goudet 2005) in R. Both of these values are the proportion of sites that are heterozygous and range from 0 to 1, with higher values indicating more heterozygosity and thus more genetic variation. To quantify levels of inbreeding, TBC calculated within-population subdivision (F_{IS}; Nei 1987) using the package 'hierfstat' (Goudet 2005). F_{IS}, also known as the inbreeding coefficient, ranges from -1 to 1, and expresses the excess or paucity of heterozygotes. Populations with $F_{IS} > 0$, especially $F_{IS} > 0.1$, have fewer heterozygotes than expected and may be experiencing inbreeding.

For alligator snapping turtles and northern red-bellied cooters, TBC also calculated whether F_{IS} was significantly different than expected with a random distribution of individuals (i.e. no population structure) using a permutation test. TBC created 100 random permutations of the same samples used in each analysis, randomly assigning each individual to a population. TBC calculated F_{IS} for those 100 random distributions to get a null expectation of F_{IS}. If the true value was higher or lower than the 95% confidence interval of the random distributions (a two-tailed test), TBC considered it to be significantly different at $\alpha = 0.05$. Because TBC used three different datasets to calculate a range of F_{IS} values for gopher frogs, TBC did not assess significance for gopher frogs, but instead interpreted the magnitude.

To measure population differentiation, which can be explained by levels of gene flow, TBC calculated pairwise F_{ST} (Weir and Goudet 2017), again using 'hierfstat' (Goudet 2005). F_{ST} varies from zero to one, with zero being a panmictic population and one being two populations that never interbreed. Generally, F_{ST} can drop below 0.2 with one migrant per generation (Nielsen and Slatkin 2013). For example, humans from different continents have a mean F_{ST} below 0.15 (Elhaik 2012).

To further assess levels of gene flow, TBC calculated estimated effective gene flow surfaces using the program EEMS (Petkova et al. 2016). EEMS uses a Markov Chain Monte Carlo simulation to interpolate gene flow rates across a geographic area using a gene flow lattice. TBC ran EEMS for four million iterations, sampling every 999 iterations, and discarding the first million iterations as burn-in. TBC used a number of demes that roughly approximated the distances between the sampling locations. TBC ran EEMS for all samples (i.e. across the species) for red-bellied cooters and alligator snapping turtles. Because of the levels of missing data, the genetic distance matrix was not positive definite for an analysis of all gopher frogs (Petkova et al. 2016), so TBC used F_{ST} as above to estimate general levels of gene flow. Given the strong sample sizes and high-quality samples, TBC ran GONE for the Camp Blanding gopher frog samples as well.

Finally, to assess effective population sizes over time, TBC used the program GONE (Santiago et al. 2020). GONE uses the decay of Linkage Disequilibrium (LD) between SNPs to measure how effective population size, both currently and in the recent past (2-600 generations prior), has changed over time. TBC used the reference-aligned datasets for GONE analyses (see above). Given that GONE depends on LD to make its calculations, TBC kept all SNPs present in more than 70% of individuals. GONE assumes no population structure among samples, so TBC ran each installation and its reference population to determine whether they had different

population trajectories. TBC used a genome-wide recombination rate of 1.45 for gopher frogs based on that of the common frog (*Rana temporaria*) (Palomar et al. 2017), and one for the two turtles based on data from snakes, turtles and chickens (Matsuda et al. 2005; Groenen et al. 2009; Hoge et al. 2024). For each installation or reference population where calculations were possible, contemporary effective population sizes (N_e) are shown from the past 1-4 generations, and historic effective population sizes from the past 400-600 generations.

Objective 3: Lasting Genomic Resources

As part of the sequencing and analysis in Objectives 1 and 2, TBC generated genome-scale datasets that can be accessed and compared in subsequent monitoring years. The data are stored and publicly available at the NCBI's Short Read Archive (Project Number PRJNA1262440). This report represents a summary of the findings for the first instance of monitoring. To create lasting standard operating procedures for installation managers, TBC created tissue sampling procedures for distribution to installation managers.

Objective 4: Ongoing Monitoring Framework and Protocol

For each installation and each species, TBC created a tailored ongoing monitoring protocol based on the results presented in this report. The monitoring protocol reflects the ability to detect recent versus historic trends in genetic variation or population sizes, and the overall genetic health of the DoW population compared to neighboring sites.

Objective 5: Genomic Monitoring Protocol

In the conclusion section of this report, TBC outlines monitoring intervals necessary to determine changes to genetic diversity over time. Moreover, after training and receiving protocols (Objectives 4 and 6), installation managers will be capable of collecting sufficient tissue samples for future monitoring efforts.

Objective 6: Train Installation Natural Resource Managers

At each installation that TBC visited, TBC distributed genetic sampling protocols, worked with managers to tailor sampling to specific management questions and positioned managers to successfully take samples by providing training on genetic sampling techniques and by sending and leaving genetic sampling kits ahead of time.

Results and Discussion

Sample Collection Results

Over the course of 2024, TBC sampled at 12 DoW installations across six states, spending between five and seven days at each installation. Through collaborations and with existing samples at TBC, TBC added three more DoW installations to the analyses. This brought the sampling effort to a total of 15 installations across seven states (Table 1). Overall, TBC sequenced 616 samples from the three target species. TBC does not include Moody Air Force Base or Fort Indiantown Gap in Table 1 as TBC did not collect any samples, despite conducting fieldwork there.

When a species was present on a given installation, TBC met or exceeded the sampling goals. These tallies include newly collected samples, samples from collaborators or natural resource managers, or samples already held by TBC.

Despite surveying, TBC did not detect alligator snapping turtles at Moody Air Force Base. While there have been previous positive eDNA detections for this species, the base generally lacks the deep riverine or wetland areas that this species prefers.

Additionally, TBC did not detect any northern red-bellied cooters at Fort Indiantown Gap. Managers explained that they only rarely see one or two individuals. Based on the range of the species in Pennsylvania, state biologist Kathy Gipe believes this may represent an introduced population (K. Gipe, pers. comm.).

Species-Specific Results

Alligator snapping turtle

TBC sequenced 49 samples from target installations and nearby off-installation reference populations that passed all quality filters (Table 1). In addition to those 49 samples, TBC added 46 additional samples from collaborators or from TBC's previous sequencing efforts. Knowing that alligator snapping turtles show strong genetic divergence among river drainages that exit to the ocean (Apodaca et al. 2023; Gordon et al. 2023), TBC chose samples from the installations' river drainages and from adjacent river drainages. The final alligator snapping turtle dataset thus included 95 high-quality samples.

Installation	Sample Size	Off Installation Reference	Sample Size
Alligator snapping turtle			
Fort Chaffee	5	Dale Bumpers National Wildlife Refuge	2
Fort Polk	8	Calcasieu River	14

Fort Benning	2	Uchee Creek and Pine Knot Creek	7
Naval Air Station Pensacola	5	Escambia and Perdido Rivers	6
Gopher frog			
Avon Park Air Force Range	6	Archbold Biological Station	9
Camp Blanding Joint Training Center	59	Jennings State Forest	23
Eglin Air Force Base	18	Apalachicola National Forest	28
Fort Benning	7	Fall Line Sandhills Wildlife Management Area	8
Fort Bragg	2	Sandhills Game Land	15
Fort Stewart	11	Alligator Creek Wildlife Management Area	7
Marine Corps Base Camp Lejeune	10	Holly Shelter	9
Northern red-bellied cooter			
Fort Belvoir	5	Fountainhead Regional Park	10
Joint Base McGuire-Dix-Lakehurst	7	Whitesbog	5
Naval Weapons Station Yorktown	10	Newport News Park	10

Table 1: Samples included in the final datasets from on and off-installation sites that passed all quality filters. These do not include additional reference samples from sites outside DoW installations and reference sites that were included in the total 616 sequenced samples.

Alligator snapping turtles for this project showed strong population structuring around bays that exit to the ocean. To highlight the population structure, TBC plotted the results of the PCA on a map (Figure 1). Samples are plotted on the map as dots where they were collected. The dots are colored by the results of the PCA: red is mapped to higher values of principal components axis one (PC1), green is mapped to higher values of PC2, and blue is mapped to higher values of PC3. Thus, dots that are similar in color are more genetically similar, and dots that are drastically different in color, especially along the R, G and B axes, are genetically

different from each other. The PCA of all 95 individuals used 2,088 SNPs. Overall, alligator snapping turtle population structures are not substantially different on DoW installations than the range-wide pattern of genetic variation partitioned by the bay that meets the ocean.

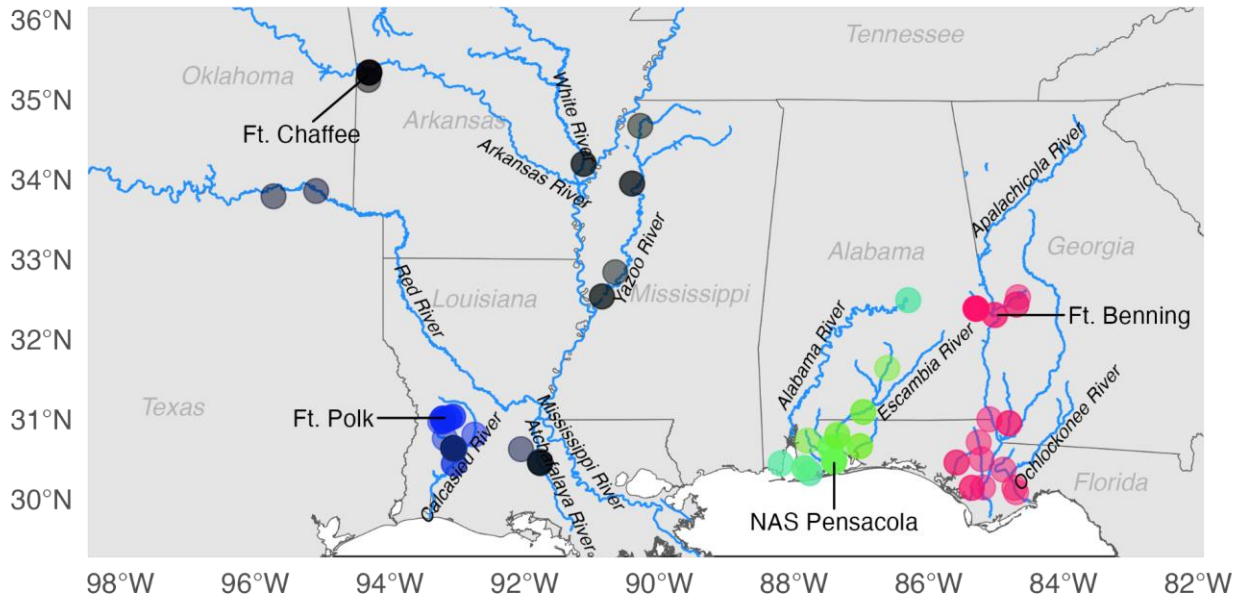


Figure 1: Alligator snapping turtle samples (n = 95) plotted on a map, colored by their Principal Component scores. Samples that are more different in color are more genetically different, with red representing high scores on PC1, green representing high scores on PC2, and blue representing high scores on PC3. Genetic variation is structured strongly by the point where river drainages meet the ocean, with DoW installations following that overall drainage pattern.

TESS analyses corroborated the results of the PCA. TESS analyses used 14,333 polymorphic SNPs. Cross-validation methods showed that three populations (K = 3) best explained the data, and most other values of K showed similar patterns to K = 3 (Figure 2). See Supplementary File 1 for plots of all values of K. TESS analyses showed the strongest differentiation among the river drainages that exited to different bays, with some admixture among them. The only exception to this was the Calcasieu River, which clustered strongly with the Mississippi River drainages. TESS showed strong clustering of the Mississippi River and all drainages west in other analyses (Apodaca et al. 2023), which revealed subtler patterns of bay-based clustering when more samples were added (Gordon et al. 2023).

In conclusion, alligator snapping turtles show overall patterns of genetic variation based on the bay that meets the ocean. DoW installations are not different from this overall pattern, which is likely species-specific and historic in nature (Echelle et al. 2010; Apodaca et al. 2023)

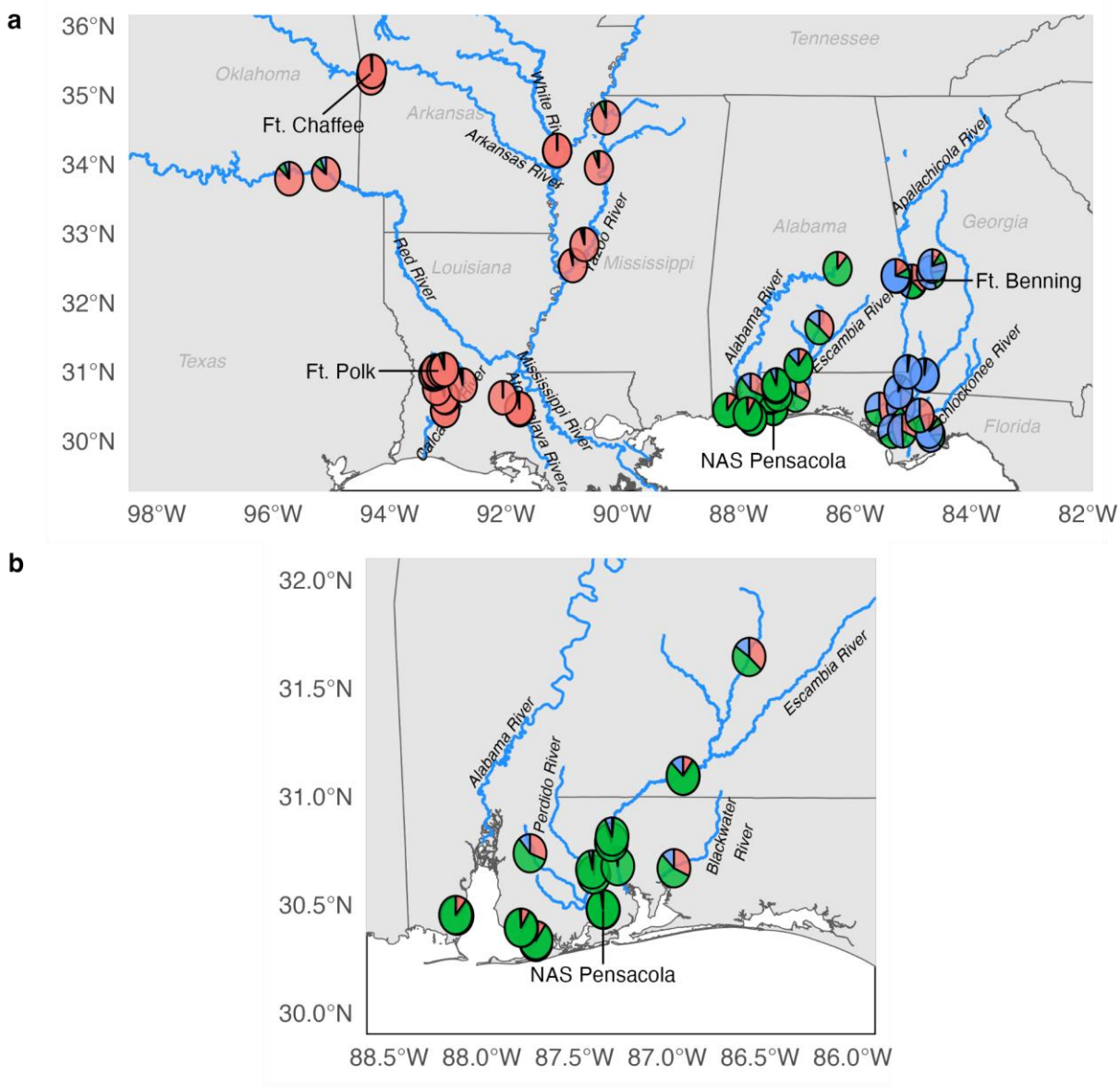


Figure 2: TESS population structuring results for three populations (K = 3) of alligator snapping turtles. All individuals (n = 95) shown (a), and zoomed into the areas around NAS Pensacola (b) to avoid overplotting. Each pie represents one individual, colored by the proportion of genetic variation that individual draws from each of the modeled populations. As in the PCA, there are strong differences among the major river drainages where they meet the ocean. However, here the Calcasieu River and Fort Polk cluster most strongly with the Mississippi River, despite not sharing a bay. There is some admixture, or genetic mixing, among all of the groups shown here.

Gopher frog

TBC sequenced 212 gopher frogs from the target installations and from reference off-installation populations nearby (Table 1). Working with partners from the North Carolina Wildlife Resources Commission (NCWRC), TBC added two DoW installations that had not originally been proposed for inclusion: Marine Corps Base Camp Lejeune and Fort Bragg. The

samples from Fort Bragg represent the first gopher frogs that NCWRC has been able to collect samples from, as the breeding pond is located within Fort Bragg's impact zone. To those 212 samples, TBC added an additional 308 samples from other partners and TBC's previous sequencing efforts for a total of 520 gopher frog samples from across the species' range.

The PCA of all 520 gopher frog samples using 605 SNPs showed a strong break between peninsular Florida and other Coastal Plain samples. TBC plotted the PCA with samples in PC-space, such that samples that are closer in PC-space are also more genetically similar. In general, samples that were geographically closer together were also genetically similar. This likely means that geographic distance plays a strong role in the genetic structure of these gopher frog populations, a pattern known as Isolation by Distance (Wright 1943). In general, DoW installations followed this range-wide pattern and did not cluster together among themselves, or apart from their reference populations.

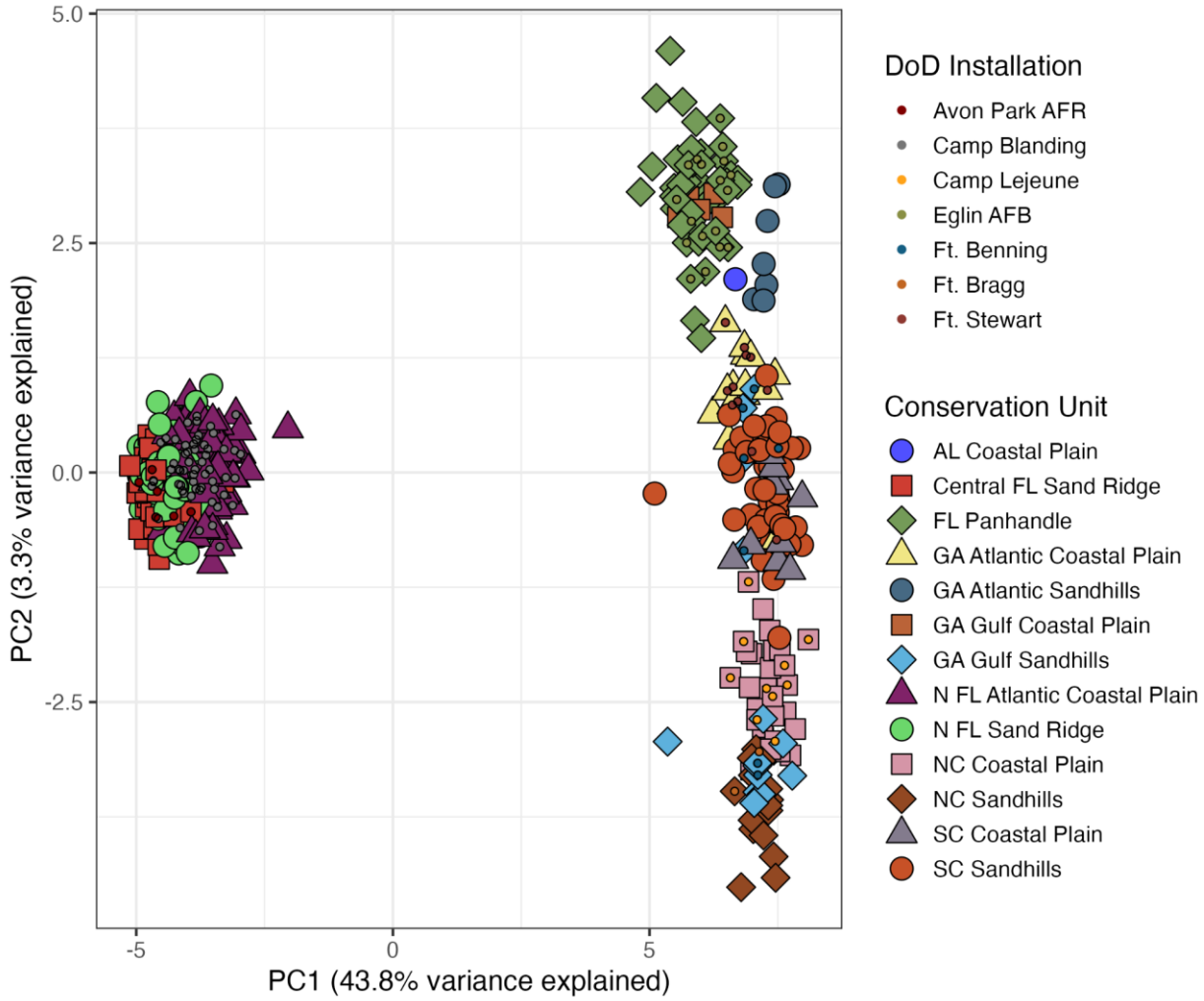


Figure 3: PCA plot of the genetic variation among 520 gopher frogs. Individual colors and shapes represent Conservation Units (Crawford and Maerz 2021), while small dots indicate sampled DoW installations. There is a stark difference between peninsular Florida (N FL Sand Ridge, N FL Atlantic Coastal Plain, Central FL Sand Ridge) and the Coastal Plain samples, with a much smaller difference between the Florida Panhandle and the rest of the Coastal Plain. DoW samples cluster as expected with the non-DoW samples.

TESS analyses corroborated the strong break in gopher frog population structure between peninsular Florida and the rest of the range. TESS analyses used a mean of 2,268 SNPs. All three datasets agreed that the most likely K, among K = 1 through K = 9, was K = 3, generally separating peninsular Florida from the Coastal Plain and Florida Panhandle (Figure 3). TESS analyses show admixture among all of these groups (Figure 4). TESS plots for all other values of K at all missing data levels are included in Supplementary File 2.

These results are in agreement with other work on the population structure of gopher frogs. Previous work had documented a strong break at the Aucilla River (Richter et al. 2014; Devitt et al. 2023), separating the Florida Panhandle from the Coastal Plain (Devitt et al. 2023).

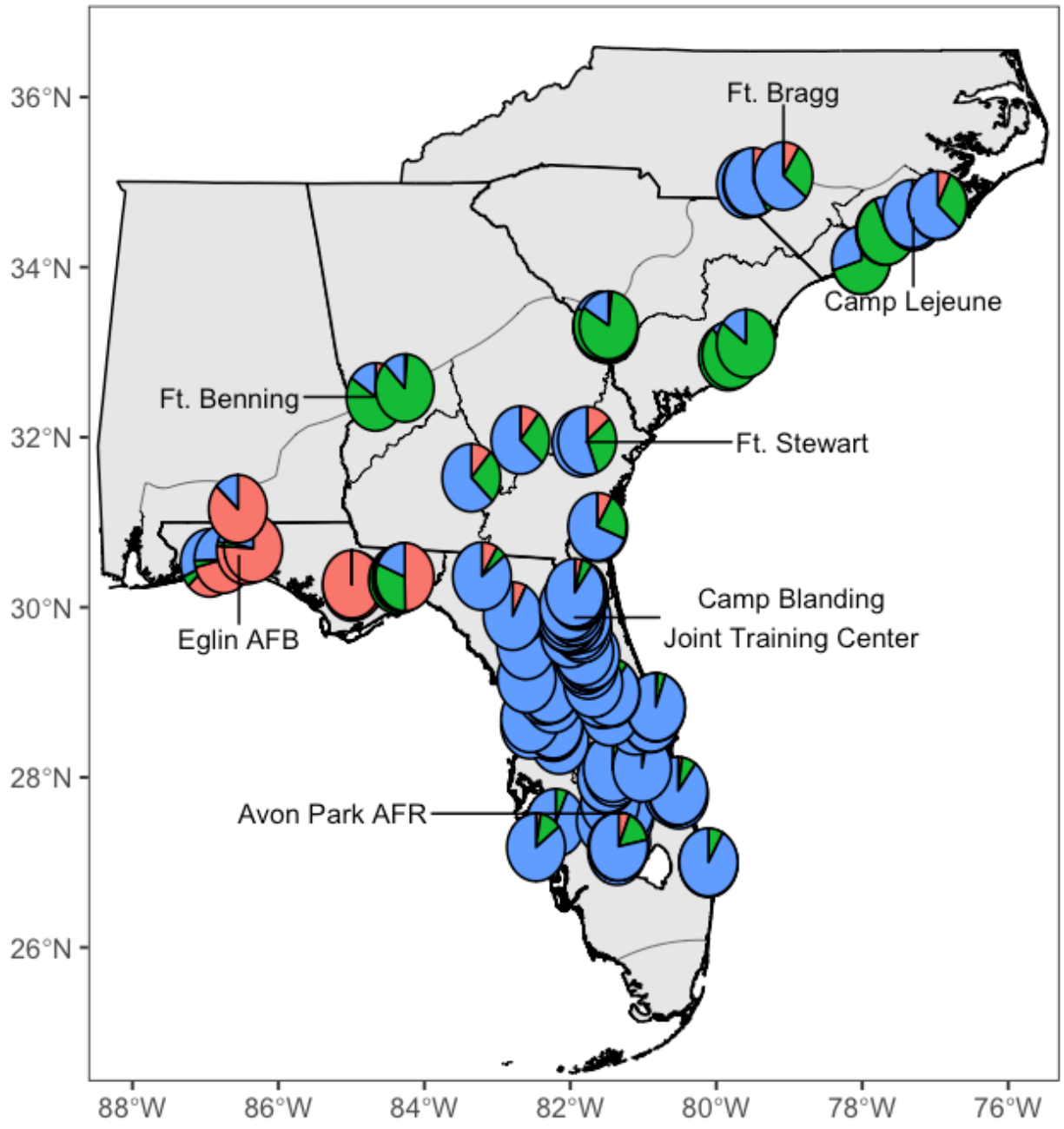


Figure 4: TESS population clustering results for 520 gopher frogs. K = 3 best explains the data, with populations generally centered in the Florida Panhandle, peninsular Florida, and the Coastal Plain. Conservation Unit (Crawford and Maerz 2021) boundaries are shown, in addition to state boundaries. There is admixture among all of these populations.

Northern red-bellied cooter

TBC sequenced 47 northern red-bellied cooters from both on-installation and off-installation reference sites (Table 1). The PCA using all 47 individuals and 16,999 SNPs revealed some structure among DoW installations. While the three installations were genetically

different from each other in PC-space, each installation did not differ from its reference population. This indicates that the differences among installations may be due to their geographic distance, or their origins in different river drainages.

TESS findings are similar, using 7,839 SNPs, with K = 3 best explaining the data. K = 3 shows the three DoW installations as distinct, with some admixture among them (Figure 5). All other TESS plots (K = 2 through K = 10) show similar distinctions, although with increasing amounts of admixture (Supplementary File 3).

Overall, northern red-bellied cooter populations on each of the three installations are distinct from each other genetically. This may be due to geographic distance or their origins in different riversheds, but one cannot accurately distinguish without samples from intermediate locations. Regardless, DoW installations are not distinct from their neighboring reference population, indicating that while there is little genetic connectivity among distant DoW installations, there is substantial genetic connectivity among neighboring sites.

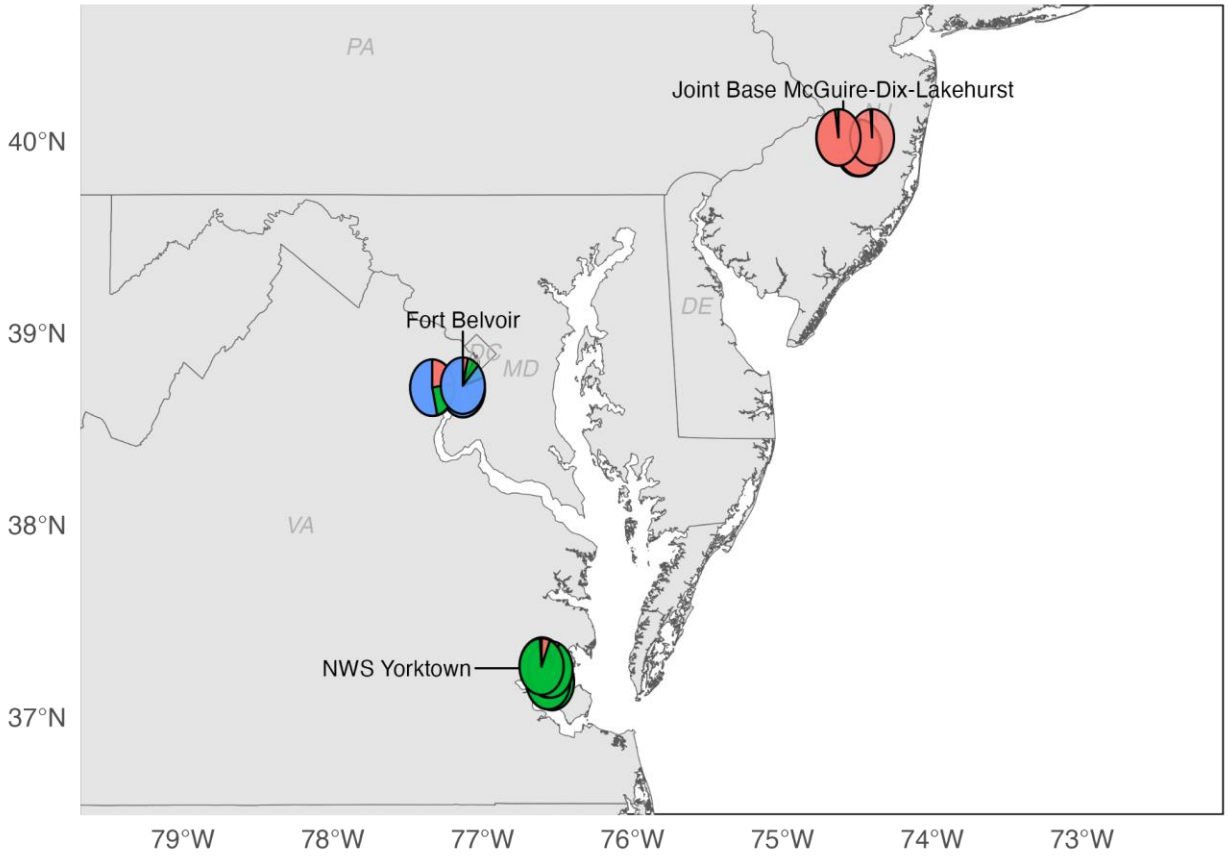


Figure 5: TESS population clustering results for 47 northern red-bellied turtles. K = 3 best explains the data, showing genetic differences among the three sampled DoW installations, but not between a given DoW installation and its nearby reference population.

Installation-Specific Results

The installation-specific measures of genomic health – levels of genetic variation, levels of gene flow, and population trajectories – are outlined below.

Avon Park Air Force Range

Gopher frog

Compared to other populations in the Peninsular Florida population, Avon Park Air Force Range (Avon Park) gopher frogs have similar levels of genetic variation (Table 2). However, other measures of genetic variation are hard to distinguish from sampling error (Waples 2022). For example, despite being relatively close by, and likely from similar ancestral populations, Avon Park appears to have had an ancestral effective population size an order of magnitude larger than its reference population. Moreover, the contemporary effective population cannot be distinguished from sampling error, indicating that there are either too few SNPs, too little genetic variation, or too few individuals in the sampling scheme to accurately calculate N_e. This is also corroborated by F_{IS} being less than -1. This indicates that the expected heterozygosity of this population is erroneously lower than the observed heterozygosity, indicating problems with low genetic variation, high missing data, and low numbers of individuals. Given that five to ten individuals and hundreds to thousands of SNPs are often sufficient to calculate measures of genetic variation and effective population sizes (Krohn et al. 2018; Felsenstein 2006; Prunier et al. 2013; Barley et al. 2015), this may indicate that drift is occurring in this population. Drift may increase genetic differences among individuals such that one would need many more individuals to capture the total variation in the population without violating assumptions of "ideal" populations or random sampling.

In terms of gopher frog gene flow and connectivity, Avon Park is not genetically distinct from its surrounding areas (F_{ST} = 0.15 between Avon Park and Archbold). This indicates at least historic connectivity between these areas. Combined with evidence from larger population structure showing a single population across Peninsular Florida, it does not appear that installation management strategies are isolating the Avon Park gopher frogs.

Overall, Avon Park thus has low resilience due to low genetic variation and low effective population sizes. Despite low genetic variation and effective population sizes, there are many gopher frog ponds in the region, which appear to be well connected. This may be from a founder effect associated with gopher frogs colonizing the paleo islands that made up Avon Park and the surrounding Lake Wales Ridge, however TBC did not evaluate why most nearby ponds have low resilience. Still, with many nearby ponds and seemingly high connectivity among them, redundancy and representation are high.

Installation-specific management recommendations:

As is the case across much of peninsular Florida, genetic variation in gopher frogs appears similarly low across the peninsular range. While census sizes may be high in certain areas, low genetic variation indicates low resilience to future threats. TBC encourages Avon Park

to participate in potential translocation or assisted gene flow efforts for gopher frogs to boost genetic variation, ideally keeping translocations within the historic population of peninsular Florida.

While there do not appear to be substantial genetic differences separating Avon Park gopher frogs from neighboring populations, genetic variation could be improved by increasing connectivity with surrounding areas, and increasing census sizes. Following the best management practices for gopher frogs (Department of Defense Partners in Amphibian and Reptile Conservation 2019), this would involve improving pine flatwoods and savannah habitat, increasing the number of vernal wetlands and burning those areas regularly. Given the presence of suitable gopher frog habitat surrounding Avon Park, this habitat management could be done anywhere on the installation to help facilitate more gopher frog gene flow. Moreover, given connectivity between Avon Park and nearby areas, plus a single population across Peninsular Florida, it does not appear that installation management strategies are impacting gopher frogs relative to nearby populations.

Camp Blanding Joint Training Center

Gopher frog

In terms of overall gopher frog genetic variation, Camp Blanding Joint Training Center (Camp Blanding) has similar levels of genetic variation and slightly lower inbreeding levels than nearby Jennings State Forest (Table 2). Both Camp Blanding and Jennings State Forest had much higher effective population sizes in the recent past, and have much smaller effective population sizes contemporarily. Camp Blanding does maintain higher levels of effective population size and lower levels of inbreeding compared to Jennings State Forest, indicating that it likely has higher resilience as well.

In terms of connectivity, because of large sample sizes at both locations, TBC ran EEMS for just Camp Blanding. EEMS showed average or higher than average gene flow among Camp Blanding Joint Training Center, Camp Blanding Wildlife Management Area, and Jennings State Forest (Figure 6). There is lower than average effective gene flow between those sites and Etoniah Creek State Forest. Although all of the locations drain to the St. Johns River, they are in different conservation units (Crawford and Maerz 2021) that represent the break between peninsular Florida and the North Florida Coastal Plain units. Thus, the conservation unit break here does correspond to gopher frog genetic differences among the populations. Corroborating the connection between Camp Blanding and Jennings State Forest, F_{ST} values between the two locations are low ($F_{ST} = 0.03$). Thus, from both EEMS analysis and F_{ST}, it does not appear that installation activities are isolating gopher frogs or preventing gene flow onto or off of Camp Blanding. The breaks in gene flow observed by EEMS are likely due to phylogeographic or physiographic differences that are reflected in the conservation units (Crawford and Maerz 2021), rather than installation-specific actions.

Installation	Species	n	Ho	Hs	Fis	Historical N_e	Contemporary N_e
Avon Park	Gopher frog	6	0.015 (0.013-0.017)	0.007 (0.005-0.01)	-1.144 (-1.944 - -0.744)	682283	Infinity
Archbold Biological Station	Gopher frog	9	0.01 (0.007-0.013)	0.013 (0.012-0.014)	0.208 (0.087 - 0.403)	65339	Infinity
Camp Blanding Joint Training Center	Gopher frog	58	0.015 (0.014-0.017)	0.017 (0.015-0.019)	0.077 (0.032 - 0.103)	863344	90
Jennings State Forest	Gopher frog	23	0.015 (0.013-0.017)	0.017 (0.014-0.02)	0.105 (0.07 - 0.139)	443958	33
Camp Lejeune	Gopher frog	10	0.014 (0.01-0.019)	0.016 (0.006-0.025)	-0.228 (-1.283 - 0.353)	4995938	40
Holly Shelter	Gopher frog	8	0.009 (0.003-0.012)	0.008 (0.003-0.01)	-0.066 (-0.164 - 0)	NA	NA
Eglin Air Force Base	Gopher frog	18	0.031 (0.023-0.039)	0.036 (0.026-0.046)	0.128 (0.1 - 0.148)	13	53
Apalachicola National Forest	Gopher frog	28	0.022 (0.009-0.035)	0.026 (0.009-0.044)	0.121 (-0.005 - 0.215)	4996990	254
Fort Bragg	Gopher frog	2	0.009 (0-0.015)	0.005 (0-0.015)	-Inf (-Inf - -0.026)	NA	NA
Sandhills Game Land	Gopher frog	15	0.019 (0.014-0.022)	0.042 (0.02-0.056)	0.505 (0.294 - 0.648)	18	29
Fort Benning	Gopher frog	7	0.009 (0-0.018)	0.013 (0-0.025)	0.281 (0.258 - 0.303)	3	74

Fall Line Sandhills Wildlife Management Area	Gopher frog	8	0.005 (0-0.012)	0.006 (0-0.012)	0.058 (0.022 - 0.094)	307	20
Fort Stewart	Gopher frog	11	0.017 (0.006-0.024)	0.016 (0.006-0.022)	-0.065 (-0.098 - 0)	4185	2551
Alligator Creek Wildlife Management Area	Gopher frog	7	0.01 (0.003-0.016)	0.008 (0.003-0.013)	-0.287 (-0.699 - 0.044)	3190111	197
Fort Belvoir	Northern red-bellied turtle	5	0.164	0.176	0.071*	82262	114
Fountainhead Regional Park	Northern red-bellied turtle	10	0.091	0.111	0.182	516	18959
Joint Base McGuire-Dix-Lakehurst	Northern red-bellied turtle	7	0.094	0.105	0.107*	5063	59
Whitesbog	Northern red-bellied turtle	5	0.088	0.1	0.117*	386	104
Naval Weapons Station Yorktown	Northern red-bellied turtle	10	0.117	0.132	0.113*	183733	41
Newport News Park	Northern red-bellied turtle	10	0.123	0.138	0.11*	1052	102
Fort Chaffee	Alligator snapping turtle	5	0.075	0.081	0.071*	302	44
Dale Bumpers White River NWR	Alligator snapping turtle	2	0.107	0.104	-0.029*	NA	NA
Fort Polk	Alligator snapping turtle	8	0.1	0.112	0.109*	350	44
Other Calcasieu	Alligator snapping	17	0.117	0.142	0.175*	2888	28

	turtle						
Fort Benning	Alligator snapping turtle	2	0.105	0.112	0.061*	NA	NA
Uchee Creek	Alligator Snapping turtle	7	0.112	0.108	-0.035*	75	13
Naval Air Station Pensacola	Alligator Snapping turtle	6	0.135	0.136	0.002*	1437	25
Perdido River	Alligator snapping turtle	3	0.105	0.106	0.011*	6	26

Table 2: Installation-specific population genetic statistics: sample size (n), observed heterozygosity (H_O), expected heterozygosity without gene flow, selection or inbreeding (H_S), inbreeding coefficient (F_{IS}), mean historical effective population size (N_e) from 400-600 generations ago, and mean contemporary effective population size from one to four generations ago. Inbreeding levels are significantly different than expected when marked by an asterisk (*). Significance was not measured for gopher frogs, due to varying levels of missing data. For gopher frogs, the mean H_O, H_S and F_{IS} values are reported for each missing data threshold, with the range in parentheses. Rows are ordered by installation (highlighted in gray), with the reference population below it (not highlighted). See Methodology section for more information on each population genetic statistic.

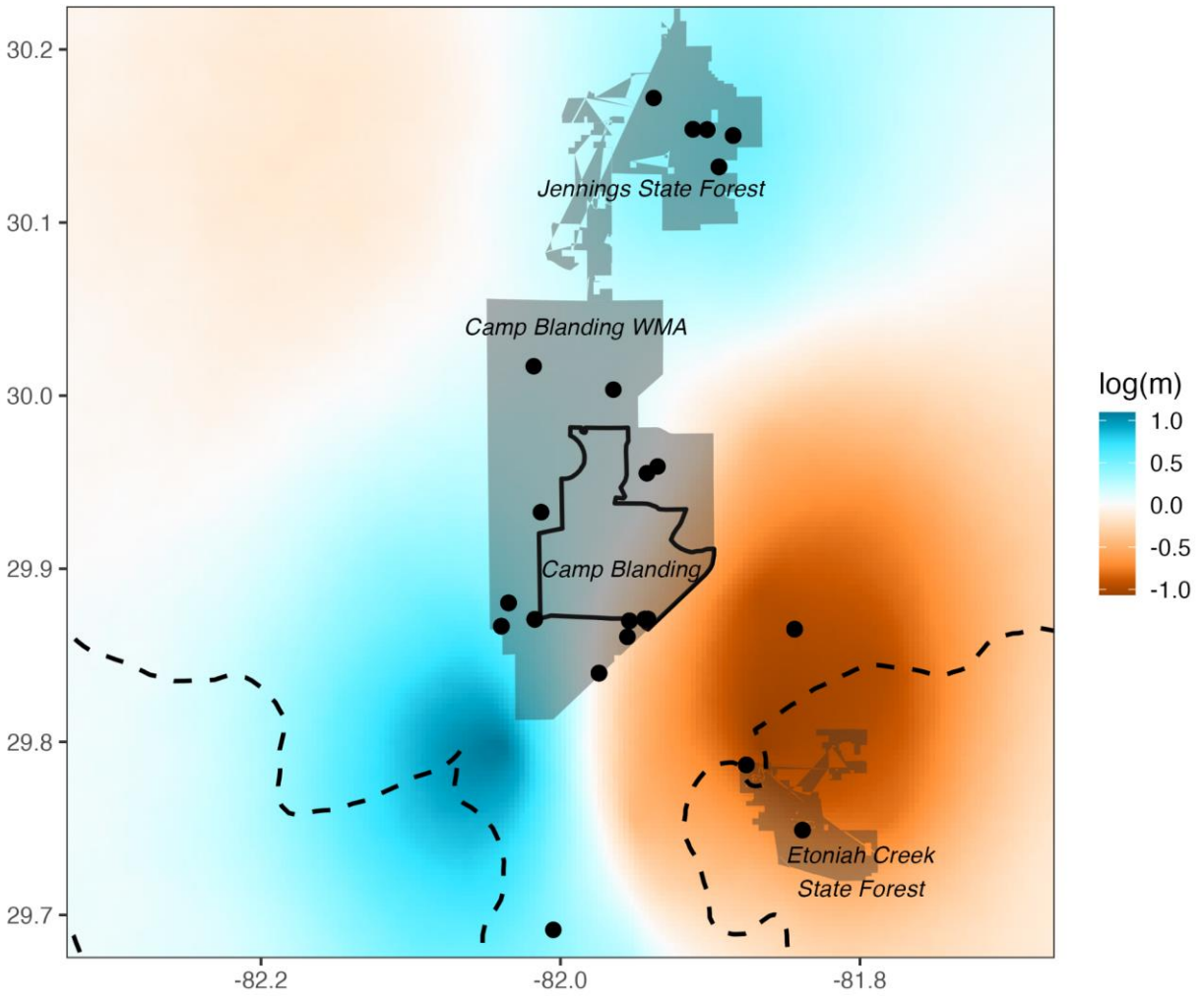


Figure 6: Estimated Effective Migration Surface (EEMS) plot for gopher frogs (n = 93 samples) on and around Camp Blanding Joint Training Center (Camp Blanding). EEMS shows that the logarithmic effective gene flow levels (m) are highest among Camp Blanding Joint Training Center (black outline), Camp Blanding Wildlife Management Area and Jennings State Forest (gray polygons). This extends to the southernmost samples from Ordway-Swisher Biological Station. There is lower than average effective gene flow between Etoniah Creek State Forest (gray polygons) and the other sites, which corresponds to the break in conservation units (Crawford and Maerz 2021), depicted by the dashed line.

Although gopher frog genetic variation is low range-wide, Camp Blanding has a higher effective population size than neighboring areas. Moreover, with many ponds, high census sizes, and ongoing effective gene flow among neighboring populations, Camp Blanding offers an area of high representation, redundancy, and resiliency for the species. Finally, Camp Blanding is

interesting from a genetic perspective, likely representing a healthy population on the edge of a conservation unit.

Installation-specific management recommendations

Overall, gopher frogs at Camp Blanding appear relatively healthy, scoring well in aspects of resiliency, redundancy, and representation. This excellent status of gopher frogs at Camp Blanding may be directly a result of management actions. Management should continue apace to maintain their management actions in the future. Following best management practices, like maintaining pine flatwoods and savannah habitat, increasing the number of vernal wetlands, and burning those areas regularly would certainly help maintain gopher frog populations (Department of Defense Partners in Amphibian and Reptile Conservation 2019).

One of the strengths of the Camp Blanding analysis is the high sample sizes, which give a much more nuanced picture of genetic variation and gene flow across a variety of ponds. Given the high census size and variety of ponds on the installation, TBC suggests regular genetic monitoring on the installation. By sampling every three to five years, managers can easily track changes in genetic variation over time, understand fine-scale movement patterns, and compare their estimates to TBC's baseline data.

Eglin Air Force Base

Gopher frog

Compared to Apalachicola National Forest, Eglin Air Force Base (Eglin) has higher gopher frog genetic variation and similarly high levels of inbreeding. Overall, effective population sizes for both areas are currently very low; however, Eglin also shows low historic effective population sizes. It is possible that populations at Eglin have persisted despite low effective population sizes for many generations. There is some evidence of this occurring in other species, but it seems likely that occasional gene flow would be necessary to maintain this scenario (Ralls et al. 2020). Accordingly, despite low effective population sizes apparently persisting for many generations, the overall risk of extirpation remains high (Frankham 2015; 2005), and this population likely does not have high resilience or redundancy.

Eglin shows high shared genetic variation (Figure 3) and connectivity between it and neighboring areas (F_{ST} between Apalachicola National Forest = 0.02). Knowing the small dispersal ranges of gopher frogs (Arbogast et al. 2022) and the amount of unsuitable habitat separating these areas, this connectivity likely represents shared historical connections rather than ongoing gene flow (see also Figure 3). Still, if the populations are not presently connected, the persistence of these similarities indicates that the populations have not diverged due to drift. Given the apparent genetic connection between Apalachicola National Forest and Eglin, it appears that installation-specific management techniques are not presently affecting levels of genetic connectivity. There is higher than expected redundancy in between Eglin and Apalachicola National Forest.

Low genetic variation and effective population size decreases the resiliency of gopher frogs at Eglin Air Force Base. With nearby populations at Blackwater River State Forest and farther away in Apalachicola, representation is medium. Redundancy is high, as there appears to be shared genetic connectivity even along larger distances between Apalachicola and Eglin.

Installation-specific management recommendations

Given the lower than desired resilience and redundancy, it is most important to bolster genetic variation at Eglin. TBC suggests that Eglin readily participates in any translocation efforts that occur, either among Panhandle populations or across the Coastal Plain population.

Given the maintained connectivity between Eglin and Apalachicola, it appears that Eglin is doing a good job at maintaining population sizes high enough to prevent genetic drift. However, the high inbreeding levels ($F_{IS} > 0.1$) may indicate that vernal pools are few and far between and that gopher frogs are not often moving to new vernal ponds. Thus, TBC recommends increasing the number of vernal pools on the landscape, especially > 1.5 km away from existing ponds (Arbogast et al. 2022) that are managed with the best management practices (Department of Defense Partners in Amphibian and Reptile Conservation 2019).

Fort Belvoir

Northern red-bellied cooters

Northern red-bellied cooters on Fort Belvoir have more genetic variation than neighboring Fountainhead Regional Park. However, both Fort Belvoir and Fountainhead Regional Park have lower than expected genetic variation (H_S) under neutral conditions and thus have elevated inbreeding levels (F_{IS} significantly greater than expected at Fort Belvoir; $F_{IS} > 0.1$ at Fountainhead Regional Park; Table 1). Surprisingly, Fountainhead Regional Park maintains a high effective population size, even increasing from historic levels. Fort Belvoir does not, indicating a reduction in genetic variation and effective population size over time.

In terms of gene flow, Fort Belvoir and Fountainhead Regional Park are connected ($F_{ST} = 0.08$), potentially with more than one migrant per generation (Nielsen and Slatkin 2013). EEMS analysis shows approximately average gene flow levels between Fort Belvoir and Fountainhead Regional Park, with this average level of gene flow extending to the Norfolk area (Figure 7). There is lower than average gene flow between these Virginia populations and populations in New Jersey. This lower gene flow may reflect historical separations between the Delaware and Chesapeake/Potomac Bays, or may reflect low sampling levels around the Delmarva Peninsula. Regardless, northern red-bellied cooters on Fort Belvoir are well connected to local and even regional populations, indicating that the base is not inhibiting gene flow.

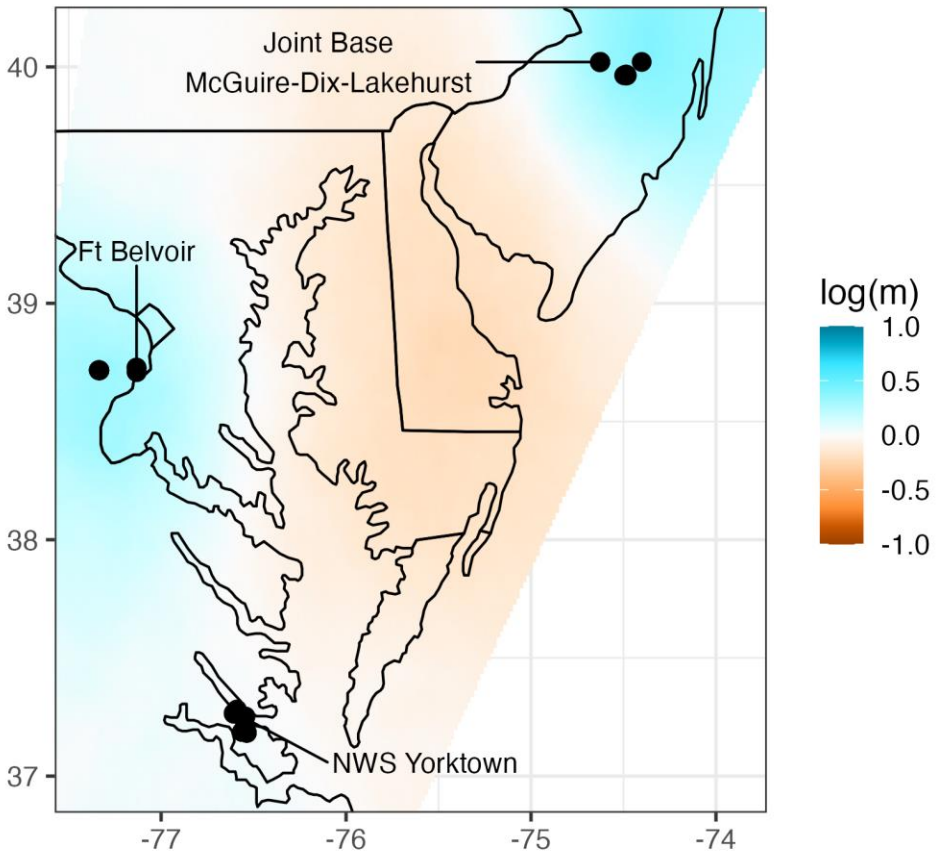


Figure 7: Estimated Effective Migration Surface (EEMS) plot for northern red-bellied cooters (n = 49). EEMS shows that the logarithmic effective gene flow levels (m) are average or slightly above average among Virginia locations and separately among New Jersey populations, with an area of low gene flow on the Delmarva Peninsula. This may reflect historic separations between the Delaware and Potomac/Chesapeake Bays, a lack of sampling on the Delmarva Peninsula, or a true population-level difference. It is not possible to distinguish without further sampling. Regardless, there are no effects to gene flow between DoW installations and their neighboring reference populations.

Thus, with high genetic variation, some evidence of inbreeding, and low effective population size, the population of northern red-bellied cooters shows medium levels of representation and resilience. With strong gene flow both locally and regionally, the representation of this population is high.

Installation-specific management recommendations

Given the high genetic variation and high connectivity to neighboring areas, it does not appear that management actions are impacting the genetic health of northern red-bellied cooters at Fort Belvoir. Still, numerous actions can be taken to boost the population size of northern red-bellied cooters at Fort Belvoir, which will likely help decrease inbreeding levels and potentially increase effective population sizes. First, following best management practices of identifying and

protecting nesting habitat and increasing basking habitat for monitoring purposes will help make monitoring easier and boost population size (Department of Defense Partners in Amphibian and Reptile Conservation 2020). Second, given strong connectivity throughout the Potomac and Chesapeake regions, even as far south as Norfolk, translocations may be considered among these areas to both increase genetic variation, and increase census population sizes.

Fort Benning

Alligator snapping turtle

Alligator snapping turtles on Fort Benning have similarly low levels of genetic variation and high inbreeding to other localities, both in the Apalachicola drainage and elsewhere. While many of the samples collected were contaminated with lymph and did not sequence well, given the numerous comparison populations, it is likely that Fort Benning does not have a high effective population size.

Gene flow levels are lower than expected, both between Fort Benning and reference populations, and among populations in the Apalachicola drainage. EEMS shows lower than average gene flow among all populations in the Apalachicola drainage, even though there was higher than average gene flow between Fort Benning and neighboring Pine Knot Creek (Figure 8). F_{ST}, on the other hand, is unexpectedly low given the EEMS finding between Fort Benning and Uchee Creek ($F_{ST} = 0.03$). Given that F_{ST} represents factors other than gene flow, such as shared population history, population subdivision, etc., this may be an artefact of these samples being a single population rather than strong gene flow between them. Alternatively, the apparent lower than average gene flow shown in EEMS may be an artefact of plotting and deme numbers, given the likely connectivity over such short geographic areas compared to those in other measured alligator snapping turtle populations.

Overall, Fort Benning shows similar levels of resilience, redundancy and representation as other alligator snapping turtles. Levels of resilience are low due to low population size, low genetic variation, and higher than expected inbreeding levels. While representation may be high with numerous populations of alligator snapping turtles in the Apalachicola drainage, redundancy is lower given the lower than average gene flow levels observed among them. Overall, as one of the northernmost populations of a potentially unique alligator snapping turtle species (*M. apalachicolae sensu* Thomas et al. 2014), the population on Fort Benning appears to be faring similarly to other populations in the drainage.

Gopher frog

Gopher frogs on Fort Benning show lower than expected levels of genetic variation, high levels of inbreeding, and low effective population sizes (Table 2). The levels of genetic variation are lower and inbreeding higher than those at Fall Line Sandhills Wildlife Management Area (WMA), which is very nearby. While Fall Line Sandhills WMA has a slightly lower effective population size, both are worryingly low. Interestingly, both populations have had low effective population sizes for a long period of time, similar to Eglin Air Force Base. Again, while some

species can persist at such low levels of genetic variation, their resilience is reduced (Frankham 2015; 2005; Ralls et al. 2020). Moreover, high levels of inbreeding further reduce resilience, and may lead to a population crash in the near future (Frankham 2005).

In terms of gene flow, Fort Benning is well connected to Fall Line Sandhills WMA (F_{ST} = 0.08). While it is unlikely that this low F_{ST} represents present-day gene flow, it is evidence of historical connectivity and shared genetic variation.

Overall, Fort Benning is similar to many other gopher frog sites in having low resilience due to low levels of genetic variation, high levels of inbreeding, and low effective population size. While there is some redundancy with neighboring Fall Line Sandhills WMA, these two populations were the only ones sampled in the Georgia Sandhills, with the next Sandhills population being Savannah River Site in South Carolina (Devitt et al. 2023; Richter et al. 2014). Thus, representation and redundancy are low.

Installation-specific management recommendations

Alligator snapping turtles are most commonly encountered in the Chattahoochee River along the border of Fort Benning. They are also present in Upatoi Creek, Pine Knot Creek, Uchee Creek, and potentially in other smaller drainages, especially as they get wider and deeper with their union with the Chattahoochee. TBC encourages further monitoring of alligator snapping turtles throughout the base, as the detections at the upper reaches of Upatoi and Pine Knot Creek indicate they may be present in wetlands throughout the base. Best management practices, including avoiding disturbance of large wetlands, will promote the continued health of alligator snapping turtles on base (Department of Defense Partners in Amphibian and Reptile Conservation 2021). The data show conflicting patterns of migration between Fort Benning and neighboring areas, with low F_{ST} values indicating strong migration, but surprisingly low migration estimated by EEMS. As such, installation actions may be affecting alligator snapping turtle migration onto and off of the base. In addition to the best management practices mentioned above (Department of Defense Partners in Amphibian and Reptile Conservation 2021), managers can encourage migration across and along the Chattahoochee by avoiding dredging, and removing abandoned fishing gear in the areas of the river adjacent to Fort Benning.

Gopher frogs on Fort Benning are in more dire straits than alligator snapping turtles. With low resilience, redundancy, and representation, considering both Fort Benning and across the Georgia Sandhills, TBC encourages management to bolster this population to prevent it from becoming an encumbrance to the mission. As it stands, there is little evidence that management activities themselves are responsible for these declines, as similar declines have been noted across the region, and there is still strong genetic connectivity between Fort Benning and neighboring areas. However, there are a number of management activities that can boost resilience and redundancy. TBC suggests increasing the number of vernal ponds within 1.5 km of each other, regularly burning upland and wetland habitats (especially in the summer/fall when breeding is not occurring), and otherwise maintaining more gopher frog habitat according to best management practices (Department of Defense Partners in Amphibian and Reptile Conservation

2019; Arbogast et al. 2022). Secondarily, TBC suggests that Fort Benning receive translocated gopher frogs from healthier populations in order to bolster genetic variation, census sizes, and resilience of the population.

Fort Bragg

Gopher frog

Most known gopher frog ponds at Fort Bragg occur in the impact zone or have not had breeding in the past many years. Thus, it is important to include Fort Bragg in this analysis, despite only having two samples.

In terms of genetic variation, Fort Bragg has roughly half the amount of genetic variation as Sandhills Game Land (Table 2). While there are not adequate samples to derive population-level metrics like effective population size and inbreeding statistics, TBC can compare Fort Bragg to Sandhills Game Land. The two areas are geographically proximate, and likely were once connected. Despite high census sizes at Sandhills Game Lands, there appears to be very high inbreeding levels and low historic and contemporary effective population sizes (Table 2). This may reflect the paucity of breeding ponds at Sandhills such that, when frogs breed, they always return to their natal breeding pond and may breed with related individuals (Arbogast et al. 2022). Given that census sizes are likely lower for Fort Bragg and that their genetic variation is half of Sandhills, the picture may be more grim at Fort Bragg.

While one should be cautious interpreting population-level statistics like F_{ST} from two samples, the F_{ST} values between Sandhills Game Land and Fort Bragg support the assumptions above. Despite being very close together and likely a part of a historically connected population, Fort Bragg and Sandhills have a high F_{ST} ($F_{ST} = 0.23$). This may reflect very little contemporary gene flow or, more likely given the F_{ST} values found elsewhere (e.g. Eglin Air Force Base), may reflect divergence via drift for these small, inbred populations.

Given the low genetic variation at Sandhills Game Land, high levels of inbreeding, and levels of effective population sizes, it may be that both Fort Bragg and Sandhills Game Land are undergoing similar levels of genetic drift from an ancestrally connected population. With genetic drift likely occurring at both populations, both populations likely have low representation, resilience, and redundancy, despite higher census sizes at Sandhills Game Land.

Installation-specific management recommendations

As on all bases where there is low redundancy, resilience, and representation, TBC suggests increasing representation and redundancy on base by increasing useable habitat, increasing habitat connectivity, and increasing the number of breeding ponds following best practices for DoW installations (Department of Defense Partners in Amphibian and Reptile Conservation 2019). This involves maintaining pine savannahs and flatwoods, plus numerous well-connected vernal ponds, all with regular burning.

In addition to increasing the number of usable breeding ponds within dispersal distance and improving upland habitat, it is important to facilitate gene flow among populations to

increase genetic variation through genetic rescue (Frankham 2015; Ralls et al. 2020). TBC suggests Fort Bragg participate in translocation efforts to bolster genetic variation. This could include individuals from Sandhills Game Land, but should likely involve less inbred, larger populations, even from the Coastal Plain (Ralls et al. 2020). With only two samples on base, it is hard to draw conclusions about how DoW management actions are affecting genetic health. However, the patterns observed at Fort Bragg are similar to those across the range of gopher frogs.

Fort Chaffee

Alligator snapping turtle

As with most alligator snapping turtle sites measured, alligator snapping turtles at Fort Chaffee have low levels of genetic variation, low effective population size, and significantly higher than expected levels of inbreeding (Table 2). Effective population size may have been historically low in this species, given low historic effective population sizes across the species' range.

However, genetic connectivity across large spatial scales is evident in turtles from Fort Chaffee. According to EEMS, turtles from Fort Chaffee show higher than average gene flow levels from the Arkansas River to the Mississippi River Delta (Figure 8). F_{ST} values corroborate this finding, with low/medium F_{ST} between Fort Chaffee and Dale Bumpers White River National Wildlife Refuge ($F_{ST} = 0.10$), despite being separated by more than 500 river kilometers.

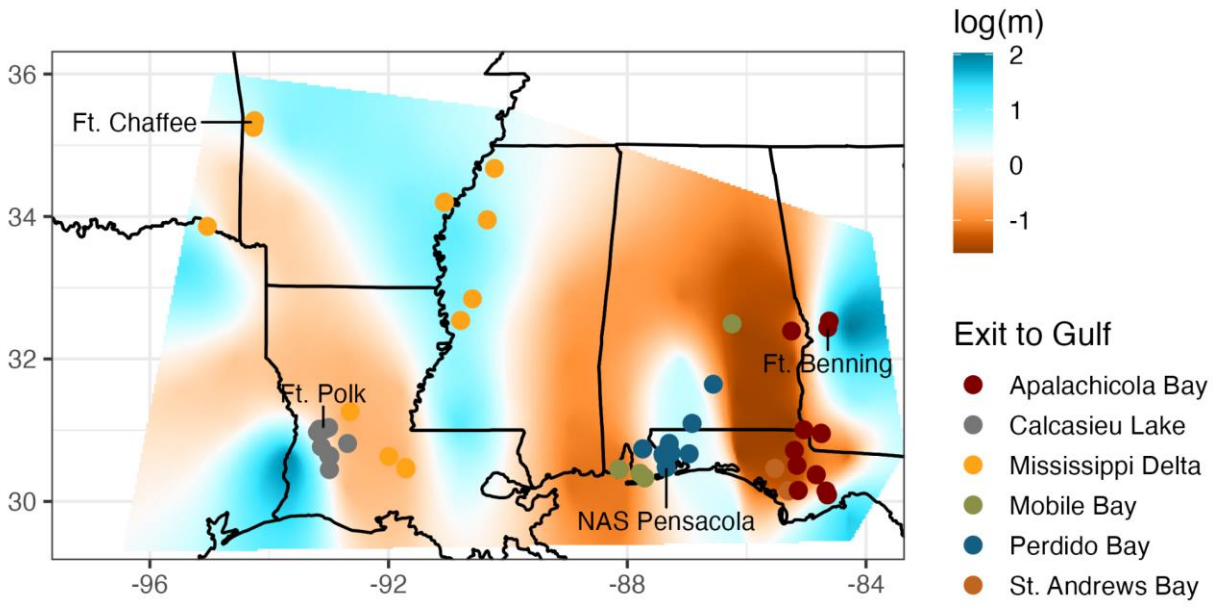


Figure 8: Effective migration (EEMS) plot for alligator snapping turtles (n = 94). EEMS shows that the logarithmic effective gene flow levels (m) are average or slightly above average between the DoW installations and other areas within their drainage for Naval Air Station Pensacola, Fort Chaffee, and Fort Polk. While Fort Benning is in an area of higher migration, there appears to be less than average migration among the Apalachicola Bay populations.

Overall, resilience based on levels of genetic variation is likely low in alligator snapping turtles, both at Fort Chaffee and elsewhere. With many populations of alligator snapping turtles in the Mississippi River drainage that are apparently well connected genetically, representation and redundancy are still apparently high. However, as genetic differences take many generations to accumulate, and there are many dams that likely prevent gene flow between New Orleans and Fort Chaffee, redundancy may be lower than measured (Gordon et al. 2023; Marsack and Swanson 2009; Baggio et al. 2018; Ruzich et al. 2019). Finally, while other species also seem to persist with low genetic variation and effective population sizes for many generations (Ralls et al. 2020), overall extirpation risk remains high (Frankham 2005; 2015), and thus, resilience is also low. While representation is high due to numerous populations along the Arkansas River (Wagner et al. 1996), redundancy may be medium given the known impediments to gene flow. These factors are not specific to Fort Chaffee, which appears similar to reference populations in all aspects. Thus, it does not appear that installation operations are affecting alligator snapping turtle populations.

Installation-specific management recommendations

Deep blackwater swamps or slow-moving rivers, the ideal habitat for alligator snapping turtles, are limited on Fort Chaffee to the areas immediately around Vache Creek. While alligator snapping turtles appear to be abundant in these areas (Trauth et al. 2005), there are actions that

can be taken to maintain alligator snapping turtle population sizes. For example, limiting wetland diversions and disturbances, protecting nesting habitat, and controlling subsidized nest predators (Department of Defense Partners in Amphibian and Reptile Conservation 2021) can all help maintain the population of alligator snapping turtles on Fort Chaffee.

Fort Indiantown Gap

Northern red-bellied cooter

Despite four days of scouting on base, TBC failed to detect any northern red-bellied cooters at Fort Indiantown Gap. Installation managers reported that they are rare, with only one or a handful actually present (J. Shinskie, personal communication). Moreover, state herpetologist Kathy Gipe suspected that they may represent an extralimital introduction of captive turtles (K. Gipe, personal communication). Without samples in hand, TBC could not perform any genetic analyses.

Installation-specific management recommendations

TBC suggests managers continue to monitor for northern red-bellied cooters. Given the managers' experience trapping other turtles, if northern red-bellied cooters are observed, they could be trapped and a blood sample taken. With a single blood sample, TBC could compare the Fort Indiantown Gap samples to other samples in New Jersey and Virginia to better estimate whether the turtles are likely introduced or native. With multiple samples, TBC could determine whether they are related, or have similar levels of genetic variation to other wild populations.

Fort Polk

Alligator snapping turtle

Fort Polk has similar levels of genetic variation and inbreeding as other populations on the Calcasieu River (Table 2). Reference individuals for Fort Polk come from a variety of areas in the Calcasieu River drainage: from areas south of Leblanc, Bundick Creek, Bundick Lake and higher-order streams closer to Fort Polk (Birds, West Fork Sixmile). Thus, it is unsurprising that the historic effective population size of this reference population is higher than that of Fort Polk. However, it is remarkable that this higher sample size and higher geographic coverage results in a lower current effective population size and higher levels of inbreeding. It is likely that the Calcasieu River drainage, including Fort Polk, had a higher effective population size in the past that has shrunk to today's low levels.

Levels of gene flow among the individuals sampled in the Calcasieu River drainage are average according to EEMS (Figure 8). F_{ST} calculations corroborate this finding, with low FST values between Fort Polk and other Calcasieu locations ($F_{ST} = 0.07$). As is true across alligator snapping turtles, individuals cluster strongly within a drainage, such that there is strong gene flow from the Gulf to Fort Polk along the Calcasieu River (more than 100 river km), but very little between Fort Polk and neighboring Red River/Mississippi River turtles just 40 air

kilometers away. Base operations are not impacting gene flow patterns of alligator snapping turtles onto or off of Fort Polk.

Overall, levels of representation, redundancy, and resilience are similar between Fort Polk and other alligator snapping turtle populations. With low genetic variation and effective population sizes, Fort Polk has low resiliency, as do most alligator snapping turtle populations. With a large number of individuals along the Calcasieu drainage, there is high representation. With few structures impeding flow above Lake Charles, it is possible that the gene flow levels observed may still be present today, indicating a high level of redundancy, including at Fort Polk.

Installation-specific management recommendations

Fort Polk lies on the highest reaches of the Calcasieu, harboring a potential source of alligator snapping turtles that can move downstream. Alligator snapping turtles and their habitat are common on Fort Polk and neighboring Kisatchie National Forest, especially along streams and floodplains of the numerous creeks. Overall Fort Polk alligator snapping turtles appear similar to surrounding areas, indicating that Fort Polk is managing the species similarly to nearby populations. While there are no specific management issues with alligator snapping turtles noted on Fort Polk, following best management practices for alligator snapping turtles (Department of Defense Partners in Amphibian and Reptile Conservation 2021), especially avoiding impacts in wetland areas, will ensure that alligator snapping turtles do not impact the mission in the future.

Fort Stewart

Gopher frog

Compared to Alligator Creek Wildlife Management Area (WMA), Fort Stewart gopher frogs have similar levels of genetic variation and similarly low levels of inbreeding. While effective population sizes at both Fort Stewart and Alligator Creek WMA have decreased over time, effective population sizes at Fort Stewart are the highest yet observed (Table 2), boding extremely well for the resilience of this population compared to other gopher frog populations.

In terms of gene flow and connectivity, Fort Stewart shows strong connectivity with nearby Coastal Plain populations (F_{ST} between Fort Stewart and Alligator Creek = 0; F_{ST} between Fort Stewart and Ceylon WMA = 0.006). Given there are few occupied gopher frog areas in the region, there is low intervening habitat suitability and few observations of gopher frogs moving between sites. Therefore, it is doubtful that these represent current-day connectivity. Still, the results show strong shared genetic variation among Georgia Coastal Plain populations. Given that strong shared genetic variation, it is unlikely that installation management actions on Fort Stewart are negatively impacting gopher frogs more than elsewhere in their range.

Overall, Fort Stewart appears to be one of the healthiest gopher frog populations that TBC has measured. Compared to other gopher frog populations, it has high resiliency in terms of low inbreeding, high effective population size, and high genetic variation. While representation

of gopher frogs is reduced in the Coastal Plain of Georgia, there are strong historical connections between Fort Stewart and the other Coastal Plain sites, indicating some remaining redundancy.

Installation-specific management recommendations

With one of the most resilient populations of gopher frogs in Georgia, Fort Stewart stands to benefit in a number of ways. First, it is unlikely that gopher frogs on Fort Stewart will encumber the mission. Second, gopher frogs at Fort Stewart could potentially act as a source population for increasing genetic variation elsewhere. This could be at other DoW sites (e.g. Fort Benning) or at other sites in the Coastal Plain. TBC encourages participation in any translocation efforts that managers are comfortable with. Finally, it is clear that management activities on base are working well to promote a variety of breeding ponds in addition to open longleaf savannah and flatwoods habitat. Any additional best management practices (Department of Defense Partners in Amphibian and Reptile Conservation 2019) that can be implemented to expand and improve habitat will help ensure gopher frogs' security and thus the security of the mission at Fort Stewart.

Joint Base McGuire-Dix-Lakehurst

Northern red-bellied cooters

Genetic variation of northern red-bellied cooters is slightly higher on base than at neighboring Whitesbog, although both had significantly less genetic variation than expected and had high inbreeding levels (Table 2). Effective population sizes for both areas are low and have decreased substantially from historical levels, although Whitesbog may have had historically low effective population size (Ralls et al. 2020). Thus, resilience of northern red-bellied cooters, both on base and off, is overall low.

Gene flow levels and connectivity are strong between Joint Base McGuire-Dix-Lakehurst and Whitesbog (Figure 7). There may be some genetic separation between the New Jersey and Virginia sites sampled, but that might also reflect the lack of sampling on the Delmarva Peninsula. F_{ST} is low ($F_{ST} = 0.03$) and also indicates strong connections between Joint Base McGuire-Dix-Lakehurst and Whitesbog. That strong connection between reference populations and similar levels of genetic diversity, compared to other populations across the range, indicate that management activities are not likely impacting the genetic health of northern red-bellied cooters on Joint Base McGuire-Dix-Lakehurst.

Overall, Joint Base McGuire-Dix-Lakehurst shows low resilience in terms of genetic variation but is similar to surrounding areas. Given the commonness of this species in New Jersey and the apparent strong gene flow among New Jersey populations sampled, representation and redundancy may be high in this area.

Installation-specific management recommendations

To increase resilience, TBC suggests following best management practices of bolstering habitat, decreasing road mortality, and protecting nesting sites (Department of Defense Partners

in Amphibian and Reptile Conservation 2020). Moreover, with a better understanding of the relatedness of Joint Base McGuire-Dix-Lakehurst to other New Jersey populations, receiving translocated northern red-bellied cooters would also bolster genetic variation on base.

Marine Corps Base Camp Lejeune

Gopher frog

In terms of genetic variation, Camp Lejeune has similar levels of genetic variation as Holly Shelter, and appears to have slightly less inbreeding (Table 2). However, levels of inbreeding varied depending on thresholds of missing data, ranging from very outbred to inbred (F_{IS} range = -1.283 - 0.353). Thus it is unclear how inbred or outbred Camp Lejeune truly is. This may be an indication of genetic drift (as in Avon Park), or potentially a data quality issue that could be resolved with better quality samples taken in the field. As with most gopher frog populations examined, Camp Lejeune had a historically very high effective population size that has crashed recently. While comparisons between Camp Lejeune and Holly Shelter are not possible because Holly Shelter did not have enough SNPs in the GONE analysis remaining after filtering, it appears that Camp Lejeune has similar levels of low effective population size to other Coastal Plain locations (e.g. Fort Benning, Fort Stewart).

In terms of gene flow, Camp Lejeune shows surprisingly high divergences from nearby populations (F_{ST} between Holly Shelter = 0.365), and lower divergence between geographically distant ones (F_{ST} between Sandhills Game Land = 0.09). Given the low genetic variation at Sandhills Game Land, high levels of inbreeding and levels of effective population sizes, it may be that both Camp Lejeune and Sandhills Game Land are undergoing similar levels of genetic drift from an ancestrally connected population. Overall, Camp Lejeune fares similarly to other Coastal Plain populations in terms of genetic variation. Given the low effective population size, it likely has low resilience to stochastic events. With the poor connectivity to other populations, and other potential indications of genetic drift, it is likely low on scales of redundancy and resilience as well.

Despite this low genetic diversity, low connectivity, and potentially high inbreeding, it is unclear that management activities on Camp Lejeune are directly to blame for these factors. These factors are present in all North Carolina populations, and most other gopher frog populations that have been measured (Arbogast et al. 2022; Devitt et al. 2023). It is likely that improving management techniques (see below) can improve their circumstances, but it isnot clear that current operations are the direct cause of this low genetic health.

Base specific management recommendations

As on all bases where there is low redundancy, resilience, and representation, TBC suggests increasing representation and redundancy on base by increasing usable habitat, increasing habitat connectivity, and increasing the number of breeding ponds following best practices for DoW installations (Department of Defense Partners in Amphibian and Reptile Conservation 2019). Similar to other gopher frog populations, it is crucial to increase the number

of vernal pools within 1.5 km of each other, and regularly maintain upland pine savannah, pine flatwoods and wetlands with burning, especially in the growing season once the vernal pools are empty (Department of Defense Partners in Amphibian and Reptile Conservation 2019; Arbogast et al. 2022).

To increase connectivity and genetic variation at Camp Lejeune, it is important to expand suitable habitat outside Camp Lejeune's boundaries. In the absence of direct connections to nearby existing populations (Holly Shelter and Croatan), TBC suggests Camp Lejeune participate in translocation efforts to bolster genetic variation, both at Camp Lejeune and in other populations. Depending on census sizes each year, Camp Lejeune can act as a source during boom years or as a recipient population for translocated frogs when other populations have boom years (Ralls et al. 2020; Frankham 2015). Increasing resilience by increasing census sizes, genetic variation, and effective population size will ensure that gopher frog populations are healthy at Camp Lejeune and do not encumber the mission.

Moody Air Force Base

Alligator snapping turtle

Despite a week of trapping in Beatty Branch, where managers had detected alligator snapping turtles using eDNA, TBC did not detect any alligator snapping turtles on Moody Air Force Base. Given the mostly isolated nature of the wetlands and a lack of deep, vegetated water, there is limited suitable habitat for alligator snapping turtles. TBC trapped the neighboring Grand Bay Wildlife Management Area but also failed to detect any alligator snapping turtles. Given that other surveys have resulted in only one eDNA-based detection, it is likely that alligator snapping turtles are present rarely, if ever, on Moody Air Force Base.

Installation-specific management recommendations

TBC recommends managers continue to survey for alligator snapping turtles. The population at Moody Air Force Base would be near the limit of the Suwanee alligator snapping turtle range (Thomas et al. 2014; Apodaca et al. 2023) and would represent an important record for that part of Georgia (Jensen and Birkhead 2003). However, given their rarity, it may be most effective to monitor for the species using eDNA (Sternhagen et al. 2024) rather than expending extra time and expense trapping for a species that is rarely present.

Naval Air Station Pensacola

Alligator snapping turtle

The main area of Naval Air Station Pensacola (Forest Sherman Field) is surrounded by the Pensacola Bay, and has little suitable freshwater habitat for alligator snapping turtles. Turtles are more common near Saufley Field, specifically in Eight Mile Creek. At Saufley Field, alligator snapping turtles have higher genetic variation and lower inbreeding compared to neighboring Perdido River samples. Both Perdido River and Naval Air Station Pensacola alligator snapping turtles have a low effective population size, with Naval Air Station

Pensacola's effective population size decreasing markedly from a historical high. Similar to other alligator snapping turtle populations measured, this may be due to long-term low genetic variation (Ralls et al. 2020). Regardless, the resilience of these populations is low.

In terms of connectivity, Naval Air Station Pensacola is well connected to neighboring Perdido River turtles (F_{ST} = 0.09). It is likely they are more closely related to the Perdido River than the Escambia River (Figure 2) despite the proximity to both. Overall gene flow levels are higher than average across the Perdido and Pensacola Bay river drainages, with a strong break between Perdido/Pensacola Bay and Mobile Bay, as well as between Perdido/Pensacola Bay and Apalachicola/St. Andrews Bay (Figure 8).

Similar to other alligator snapping turtle populations, Naval Air Station Pensacola has low resilience due to low effective population sizes and low genetic variation. It fares slightly better in that it does not have strong inbreeding levels observed. In terms of representation and redundancy, there are many populations of alligator snapping turtles in the rivers that drain to the Perdido and Pensacola Bays, which maintain high levels of connectivity. Thus, representation and redundancy are relatively high. Given the similar levels of genetic variation to neighboring populations and high gene flow onto and off of the installation, it is unlikely that installation activities are impacting alligator snapping turtles.

Installation-specific management recommendations

Given the scarcity of alligator snapping turtles at Forest Sherman Field, managers should monitor for the presence of these turtles. Survey methods could use eDNA (Sternhagen et al. 2024) as an alternative to intensive trapping, given the likely rarity at Forest Sherman Field. Tissue samples should be taken from any turtles trapped in this area as it may be closer aligned with the Escambia River drainage rather than the Perdido. As with other alligator snapping turtle populations measured, it is likely that larger-scale species-wide patterns will explain patterns of genetic variation rather than installation-specific ones.

At Saufley Field, best management practices (Department of Defense Partners in Amphibian and Reptile Conservation 2021), especially avoiding impacts in wetland areas, can help maintain strong connectivity onto and off Naval Air Station Pensacola and prevent alligator snapping turtles from impacting the mission.

Naval Weapons Station Yorktown

Northern red-bellied cooters are common and well studied at Naval Weapons Station Yorktown. Overall, they have slightly less genetic variation and lower effective population sizes than neighboring Newport News cooters. However, both populations have elevated inbreeding levels and have experienced declines in contemporary effective population size compared to historical times, indicating that both populations may be facing similar threats.

Gene flow levels and connectivity are strong between Naval Weapons Station Yorktown and Newport News. EEMS analyses (Figure 7) and population clustering (Figure 5) show average to higher than average gene flow levels all along the coast of Virginia up to Fort Belvoir.

F_{ST} values are also low between Naval Weapons Station Yorktown and Newport News (F_{ST} = 0.03), corroborating strong gene flow onto and off the installation.

Overall, similar to other northern red-bellied cooter populations, resilience is low due to low effective population sizes, little genetic variation, and elevated inbreeding. Representation and redundancy appear high, as cooters are common along the coast of Virginia and show strong connections both locally and regionally. Given the strong connectivity between other populations, it does not appear that installation activities have impacted northern red-bellied cooters genetically.

Installation-specific management recommendations

Populations are currently well monitored on the installation. Managers should continue their monitoring efforts and continue to encourage research on these turtles. Addressing nesting success and road mortality may also help to bolster populations and avoid inbreeding (Department of Defense Partners in Amphibian and Reptile Conservation 2020). Furthermore, if relocations or translocations occur for this species, TBC encourages Naval Weapons Station Yorktown to participate in order to bolster genetic variation and further decrease inbreeding in their population.

Manager Training and Lasting Resources

As part of Objectives three and six, TBC trained installation managers and created lasting resources to improve monitoring into the future. First, TBC created a standard genomic sampling protocol to ensure high-quality genomic samples can be taken in perpetuity (Supplementary File 4). The protocol maximizes sample quality and ease of use, while minimizing cost per sample and harm to the animals. TBC distributed this protocol to seven installations: Avon Park Air Force Range, Camp Blanding, Camp Grayling, Fort McCoy, Joint Base McGuire-Dix-Lakehurst, Naval Air Station Pensacola, and Marine Corps Base Camp Lejeune. (Note: some target installations from Year 2 are included here because the protocol was distributed to them in Year 1, and because it is relevant to training installation managers in genetic sampling techniques.) From those installations, TBC received usable samples from five installations that used our protocol.

Secondly, TBC helped train installation managers directly in the field at eight installations: Avon Park Air Force Range, Fort Belvoir, Fort Chaffee, Fort Indiantown Gap, Joint Base McGuire-Dix-Lakehurst, Moody Air Force Base, Naval Air Station Pensacola, and Naval Weapons Station Yorktown. This training included hands-on fieldwork and/or demonstrations of the technique.

Finally, the genomic data generated from this project represents a lasting resource against which future changes can be measured. The raw genetic data generated for this study is publicly available at NCBI's Short Read Archive (Project Number PRJNA1262440).

Conclusions

In conclusion, with one season of fieldwork and many collaborators, TBC assessed the genetic health of populations of three species of conservation concern at and adjacent to 15 installations in seven states. TBC evaluated the genomic health of these populations and assessed the health for each installation in terms of US Fish and Wildlife Services' three R's, thus completing Objectives one and two. The main findings are summarized in Table 3.

Those genomic results are presented above and represent a baseline against which future population monitoring can occur. Moreover, the raw genetic data is publicly archived at NCBI's Short Read Archive (Project Number PRJNA1262440) so that direct comparisons can be made with the raw data. TBC trained installation managers at eight installations (see above for specific installations), created a standard genomic sampling protocol, and distributed that protocol widely. Installations across the Eastern US are now ready to continue taking high-quality genomic samples to continue this monitoring into the future, thus completing Objectives three and six.

Finally, based on those results, TBC created installation-specific management recommendations for each installation above as part of Objectives four and five. More details on continued genomic monitoring are below.

Military Mission Benefits

With this test case on three species, it is possible for DoW to integrate these monitoring practices into their routine monitoring for any species of conservation concern. TBC has demonstrated the cost-effective nature of this monitoring. Assessing just population size change or migration levels in a single species at a single installation would require at least two employees working for at least two field seasons using traditional mark-recapture methods. Two employees with TBC, with help from five installation partners, collected adequate samples to assess those same metrics in three species on 15 installations across the eastern United States in a single field season. And yet the insights from the datasets are profound, shedding light on population connectivity and population sizes, in addition to population size change over time and gene flow.

Importantly, this work makes proactive management easier for DoW. While none of these species are officially federally listed species (alligator snapping turtles are proposed as threatened as of April 2021), future listing decisions could encumber the mission at these DoW installations. Based on these results, there are some conclusions and proactive management steps that can help prevent these species from encumbering the mission:

DoW installations fare better, or at least similarly to reference populations

TBC found no evidence DoW management actions caused a species to fare worse on DoW land than its reference population or across the species' range. Instead, DoW installations are subject to the population trends of surrounding areas and often fare similarly to nearby areas.

Thus, overall, management is working at least as well on DoW land as it is in surrounding areas. It is also an indication that any ongoing active management should continue.

Species with low resilience and redundancy will benefit from larger populations, better connectivity, and translocation programs

Many installations suffer from low resilience in terms of low genetic variation and low effective population sizes (Table 3). While these were not unique to DoW installations, these trends can be abated by decreasing mortality, increasing breeding opportunities, and connecting populations. For gopher frogs, that means increasing the number of vernal pools within 1.5 km of each other (Arbogast et al. 2022) to create metapopulations of breeding pools and prevent inbreeding opportunities where individuals return to their natal pond. This also means active habitat management, including burning upland pine flatwoods and pine savannahs, reducing basal tree area, and burning vernal pool wetlands once they dry down (Department of Defense Partners in Amphibian and Reptile Conservation 2019). For alligator snapping turtles and northern red-bellied cooters, that includes protecting wetlands from impacts, protecting nesting areas, reducing overabundant nest predators, and reducing road mortalities (Department of Defense Partners in Amphibian and Reptile Conservation 2020; 2021). Finally, extending these habitat alterations to the edge of installations may help promote connectivity with nearby populations, thereby increasing redundancy and representation.

Supporting translocation programs is another way to boost resilience. Translocating animals to boost genetic variation and effective population size can help prevent inbreeding depression. It is known as genetic rescue (Bell et al. 2019; Frankham 2015; Ralls et al. 2020). Based on these results, there is a better understanding of which DoW installations can act as sources and which are better served as recipients. For gopher frogs, individuals could be sourced from Camp Blanding or Fort Stewart to help bolster localities in nearby populations (Figures 2 and 3). Any population could receive and would likely benefit from translocated animals. For alligator snapping turtles and northern red-bellied cooters, animals could be translocated wherever possible while maintaining historic populations (Figures 2 and 4). Overall, the benefit of gene flow generally outweighs the caution around outbreeding. Good source populations are those with robust census sizes (reviewed in Ralls et al. 2020), so boom years from any of the species might indicate potential for translocations.

TBC strongly encourages installations to work with state agencies and with this genetic data to plan translocations wherever possible.

Monitoring should include genomic sampling at intervals relevant to the species

As stated previously, the large payoff in information from genomics is well worth the relatively low effort needed to collect five to ten samples. As such, genetic sampling of new individuals should occur at regular intervals, and be a standard part of DoW monitoring protocols.

Monitoring intervals will vary depending on the generation time of the species. With long-lived turtles, genetic differences might take dozens or hundreds of years to accumulate (Gordon et al. 2023; Marsack and Swanson 2009; Baggio et al. 2018; Ruzich et al. 2019). However, if managers only took genetic samples from hatchlings or unmarked juveniles, they could ensure they are always sampling the newest generation. Alternatively, managers could sample juveniles after 10-20 years to maximize the likelihood of sampling a new generation. For species with shorter generation times, like gopher frogs and most snakes, genomic data can help track changes in effective population size and genetic variation over time (Arbogast et al. 2022; Adams and Edmands 2023). For those species, sampling every two to three generations would be sufficient.

Installation	Species	Resilience	Redundancy	Representation
Avon Park Air Force Range	Gopher frog	Low	High	High
Camp Blanding Joint Training Center	Gopher frog	High	High	High
Eglin Air Force Base	Gopher frog	Low	High	Medium
Fort Belvoir	Northern red-bellied cooter	Medium	Medium	High
Fort Benning	Gopher frog	Low	Low	Low
Fort Benning	Alligator snapping turtle	Low	Medium	High
Fort Bragg	Gopher frog	Low	Low	Low
Fort Chaffee	Alligator snapping turtle	Low	Medium	High
Fort Polk	Alligator snapping turtle	Low	High	High
Fort Stewart	Gopher frog	High	Medium	Low
Joint Base McGuire-Dix-Lakehurst	Northern red-bellied cooter	Low	High	High
Marine Corps Base Camp Lejeune	Gopher frog	Low	Low	Low
Naval Air Station Pensacola	Alligator snapping turtle	Low	High	High
Naval Weapons Station Yorktown	Northern red-bellied cooter	Low	High	High

Table 3: Assessments of resilience, redundancy, and representation for the three focal species on the 13 installations where TBC collected samples (i.e. not including Moody Air Force Base or Fort Indiantown Gap, which were lacking the target species). TBC defined high resilience as

having high genetic variation, low inbreeding, and/or high effective population sizes, especially relative to other locations of that species. TBC defined representation as high if there were many nearby localities of the same genetic population and defined redundancy as high if those populations were also well connected genetically and/or showed high resilience.

Final Conclusions

In summary, this report demonstrates the effectiveness of genomic monitoring for assessing the genetic health of three species across the southeastern US. TBC trained installation managers in how to properly take samples, and created a baseline dataset from which future monitoring can occur. While the status and needs of installations vary, it is clear that populations on DoW installations are subject to similar population trends as their neighboring populations. Monitoring of these species of conservation concern should continue, and genomics represents an efficient way to get population-scale data from relatively little field effort.

Acknowledgements

TBC is grateful to a number of installation managers, researchers, state biologists, and TBC staff members who sent samples, collected samples in the field, facilitated installation access, and did the lab work for this project. This project would not have been possible without their help and collaborative spirit. TBC is especially grateful to Jessica Baker, Rian Burris, Lawrence Carlile, Louisa Collins, Riley Dunn, Kevin Enge, Magenta Fougerat, Jim Godwin, Jeff Hall, Eva Kerr, Stacey Lance, Alexandria Long, John Maerz, Hannah Nelson, Jonathan Neufeldt, Thomas Olexa, Sarah Pearce, Christopher Petersen, John Pilcicki, Vivian Porter, Jamie Shinskie, Matthew Smith, Mark Stevenson, Vanessa Terrel, Kameron Voves, and Abigail Wetzel.

Literature Cited

Adams, Nicole E., and Suzanne Edmands. 2023. "Genomic Recovery Lags behind Demographic Recovery in Bottlenecked Populations of the Channel Island Fox, *Urocyon Littoralis*." *Molecular Ecology* 32 (15): 4151–64. https://doi.org/10.1111/mec.17025.

Apodaca, Joseph J., Alexander R. Krohn, Louisa Collins, James C. Godwin, Luke Pearson, and Andrew D. Walde. 2023. "Population Structure and Genetic Differentiation in Extant Alligator Snapping Turtles (Genus Macrochelys) with Implications for Taxonomy and Conservation." *Southeastern Naturalist* 22 (12): 1–24.

Arbogast, Brian S., Stephanie J. Kamel, Nathaniel T. Akers, and Jeffrey G. Hall. 2022. "Kinship and Breeding Site Philopatry Drive Fine-Scale Genetic Structure in Fragmented Populations of the Gopher Frog (Rana Capito) in North Carolina." *Journal of Herpetology* 56 (2): 249–57. https://doi.org/10.1670/20-140.

Baggio, Rafael A., Sabrina B. L. Araujo, Daniel Ayllón, and Walter A. Boeger. 2018. "Dams Cause Genetic Homogenization in Populations of Fish That Present Homing Behavior: Evidence from a Demogenetic Individual-Based Model." *Ecological Modelling* 384 (September): 209–20. https://doi.org/10.1016/j.ecolmodel.2018.06.019.

Barley, Anthony J., Patrick J. Monnahan, Robert C. Thomson, L. Lee Grismer, and Rafe M. Brown. 2015. "Sun Skink Landscape Genomics: Assessing the Roles of Micro-Evolutionary Processes in Shaping Genetic and Phenotypic Diversity across a Heterogeneous and Fragmented Landscape." *Molecular Ecology* 24 (8): 8. https://doi.org/10.1111/mec.13151.

Bayona-Vásquez, Natalia J., Travis C. Glenn, Troy J. Kieran, et al. 2019. "Adapterama III: Quadruple-Indexed, Double/Triple-Enzyme RADseq Libraries (2RAD/3RAD)." *PeerJ* 7 (October): e7724. https://doi.org/10.7717/peerj.7724.

Bell, Donovan A., Zachary L. Robinson, W. Chris Funk, et al. 2019. "The Exciting Potential and Remaining Uncertainties of Genetic Rescue." *Trends in Ecology & Evolution* 34 (12): 12. https://doi.org/10.1016/j.tree.2019.06.006.

Caye, Kevin, Timo M. Deist, Helena Martins, Olivier Michel, and Olivier François. 2016. "TESS3: Fast Inference of Spatial Population Structure and Genome Scans for Selection." *Molecular Ecology Resources* 16 (2): 540–48. https://doi.org/10.1111/1755-0998.12471.

Chambers, E. Anne, Anusha P. Bishop, and Ian J. Wang. 2023. "Individual-based Landscape Genomics for Conservation: An Analysis Pipeline." *Molecular Ecology Resources*, October 26, 1755-0998.13884. https://doi.org/10.1111/1755-0998.13884.

Crawford, Brian A., and John C. Maerz. 2021. *Strategic Conservation Management for Gopher Frogs at Site-Specific to Range-Wide Scales*. United States Fish and Wildlife Service.

Department of Defense Partners in Amphibian and Reptile Conservation. 2019. "Recommended Best Management Practices for the Gopher Frog on Department of Defense Installations." *Department of Defense Legacy Resource Management Program*.

Department of Defense Partners in Amphibian and Reptile Conservation. 2020. "Recommended Best Management Practices for the Northern Red-Bellied Cooter on Department of Defense Installations." *Department of Defense Legacy Resource Management Program*. https://parcplace.org/wp-content/uploads/2021/02/Red-bellied-Cooter_BMP_Final.pdf.

Department of Defense Partners in Amphibian and Reptile Conservation. 2021. *Recommended Best Management Practices For the Alligator Snapping Turtle on Department of Defense*

Installations.
Devitt, Thomas J., Kevin M. Enge, Anna L. Farmer, et al. 2023. "Population Subdivision in the Gopher Frog (Rana Capito) across the Fragmented Longleaf Pine-Wiregrass Savanna of the Southeastern USA." *Diversity* 15 (1): 1. https://doi.org/10.3390/d15010093.
Eaton, Deren A R, and Isaac Overcast. 2020. "Ipyrad: Interactive Assembly and Analysis of RADseq Datasets." *Bioinformatics* 36 (8): 2592–94. https://doi.org/10.1093/bioinformatics/btz966.
Eaton, Deren A. R., Elizabeth L. Spriggs, Brian Park, and Michael J. Donoghue. 2017. "Misconceptions on Missing Data in RAD-Seq Phylogenetics with a Deep-Scale Example from Flowering Plants." *Systematic Biology* 66 (3): 3. https://doi.org/10.1093/sysbio/syw092.
Echelle, A. A., J. C. Hackler, J. B. Lack, et al. 2010. "Conservation Genetics of the Alligator Snapping Turtle: Cytonuclear Evidence of Range-Wide Bottleneck Effects and Unusually Pronounced Geographic Structure." *Conservation Genetics* 11 (4): 4. https://doi.org/10.1007/s10592-009-9966-1.
Elhaik, Eran. 2012. "Empirical Distributions of FST from Large-Scale Human Polymorphism Data." *PLOS ONE* 7 (11): e49837. https://doi.org/10.1371/journal.pone.0049837.
Felsenstein, Joseph. 2006. "Accuracy of Coalescent Likelihood Estimates: Do We Need More Sites, More Sequences, or More Loci?" *Molecular Biology and Evolution* 23 (3): 3. https://doi.org/10.1093/molbev/msj079.
Frankham, Richard. 2005. "Genetics and Extinction." *Biological Conservation* 126 (2): 131–40. https://doi.org/10.1016/j.biocon.2005.05.002.
Frankham, Richard. 2015. "Genetic Rescue of Small Inbred Populations: Meta-Analysis Reveals Large and Consistent Benefits of Gene Flow." *Molecular Ecology* 24 (11): 11. https://doi.org/10.1111/mec.13139.
Glenn, Travis C., Todd W. Pierson, Natalia J. Bayona-Vásquez, et al. 2019. "Adapterama II: Universal Amplicon Sequencing on Illumina Platforms (TaggiMatrix)." *PeerJ* 7 (October): e7786. https://doi.org/10.7717/peerj.7786.
Gordon, M, B Giles, K Garcia, et al. 2023. *Baseline Study of Alligator Snapping Turtle (Macrochelys Temminckii) Population Viability in Texas Watersheds, Final Report. (Report No. EIH23-002)*. Nos. EIH23-002. Prepared for the Texas Comptroller of Public Accounts (Contract 20-6997BG).
Goudet, Jerome. 2005. "Hierfstat, a Package for r to Compute and Test Hierarchical F-Statistics." *Molecular Ecology Notes* 5 (1): 1. https://doi.org/10.1111/j.1471-8286.2004.00828.x.
Groenen, Martien A.M., Per Wahlberg, Mario Foglio, et al. 2009. "A High-Density SNP-Based Linkage Map of the Chicken Genome Reveals Sequence Features Correlated with Recombination Rate." *Genome Research* 19 (3): 510–19. https://doi.org/10.1101/gr.086538.108.
Hodel, Richard G. J., Shichao Chen, Adam C. Payton, Stuart F. McDaniel, Pamela Soltis, and Douglas E. Soltis. 2017. "Adding Loci Improves Phylogeographic Resolution in Red Mangroves despite Increased Missing Data: Comparing Microsatellites and RAD-Seq and Investigating Loci Filtering." *Scientific Reports* 7 (1): 1. https://doi.org/10.1038/s41598-017-16810-7.
Hoge, Carla, Marc de Manuel, Mohamed Mahgoub, et al. 2024. "Patterns of Recombination in Snakes Reveal a Tug-of-War between PRDM9 and Promoter-like Features." *Science* 383

(6685): eadj7026. https://doi.org/10.1126/science.adj7026.
Jensen, John B., and William S. Birkhead. 2003. “Distribution and Status of the Alligator Snapping Turtle (Macrochelys Temminckii) in Georgia.” *Southeastern Naturalist* 2 (1): 25–34.
Krohn, Alexander R., Chris J. Conroy, Risa Pesapane, Ke Bi, Janet E. Foley, and Erica Bree Rosenblum. 2018. “Conservation Genomics of Desert Dwelling California Voles (Microtus Californicus) and Implications for Management of Endangered Amargosa Voles (Microtus Californicus Scirpensis).” *Conservation Genetics* 19 (2): 2. https://doi.org/10.1007/s10592-017-1010-2.
Krohn, Alexander R., Brian Folt, Joseph J. Apodaca, Craig Guyer, and Jeffrey M. Goessling. 2024. “Using Genomic Data to Estimate Population Structure of Gopher Tortoise (Gopherus Polyphemus) Populations in Southern Alabama.” *Conservation Genetics* 25 (3): 755–70. https://doi.org/10.1007/s10592-024-01601-1.
Marsack, Kelly, and Bradley J. Swanson. 2009. “A Genetic Analysis of the Impact of Generation Time and Road-Based Habitat Fragmentation on Eastern Box Turtles (Terrapene c. Carolina).” *Copeia* 2009 (4): 4. https://doi.org/10.1643/CE-08-233.
Matsuda, Yoichi, Chizuko Nishida-Umehara, Hiroshi Tarui, et al. 2005. “Highly Conserved Linkage Homology between Birds and Turtles: Bird and Turtle Chromosomes Are Precise Counterparts of Each Other.” *Chromosome Research* 13 (6): 601–15. https://doi.org/10.1007/s10577-005-0986-5.
Nei, Masatoshi. 1987. *Molecular Evolutionary Genetics*. Columbia University Press. https://doi.org/10.7312/nei-92038.
Nielsen, Rasmus, and Montgomery Slatkin. 2013. *An Introduction to Population Genetics: Theory and Applications*. Sinauer Associates.
O’Leary, Shannon J., Jonathan B. Puritz, Stuart C. Willis, Christopher M. Hollenbeck, and David S. Portnoy. 2018. “These Aren’t the Loci You’e Looking for: Principles of Effective SNP Filtering for Molecular Ecologists.” *Molecular Ecology* 27 (16): 16. https://doi.org/10.1111/mec.14792.
Palomar, Gemma, Freed Ahmad, Anti Vasemägi, Chikako Matsuba, Alfredo G Nicieza, and José Manuel Cano. 2017. “Comparative High-Density Linkage Mapping Reveals Conserved Genome Structure but Variation in Levels of Heterochiasmy and Location of Recombination Cold Spots in the Common Frog.” *G3 Genes|Genomes|Genetics* 7 (2): 637–45. https://doi.org/10.1534/g3.116.036459.
Petersen, Chris, Rob Lovich, and Sarah Stallings. 2018. “Amphibians and Reptiles of United States Department of Defense Installations.” *Herpetological Conservation and Biology* 13 (3): 652–61.
Petkova, Desislava, John Novembre, and Matthew Stephens. 2016. “Visualizing Spatial Population Structure with Estimated Effective Migration Surfaces.” *Nature Genetics* 48 (1): 1. https://doi.org/10.1038/ng.3464.
Prunier, J. G., B. Kaufmann, S. Fenet, et al. 2013. “Optimizing the Trade-off between Spatial and Genetic Sampling Efforts in Patchy Populations: Towards a Better Assessment of Functional Connectivity Using an Individual-Based Sampling Scheme.” *Molecular Ecology* 22 (22): 22. https://doi.org/10.1111/mec.12499.
Ralls, Katherine, Paul Sunnucks, Robert C. Lacy, and Richard Frankham. 2020. “Genetic Rescue: A Critique of the Evidence Supports Maximizing Genetic Diversity Rather than Minimizing the Introduction of Putatively Harmful Genetic Variation.” *Biological*

Conservation 251 (November): 108784. https://doi.org/10.1016/j.biocon.2020.108784.
Richter, Stephen C., Eric M. O'Neill, Schyler O. Nunziata, et al. 2014. "Cryptic Diversity and Conservation of Gopher Frogs across the Southeastern United States." *Copeia* 2014 (2): 2. https://doi.org/10.1643/CG-13-040.
Rohland, Nadin, and David Reich. 2012. "Cost-Effective, High-Throughput DNA Sequencing Libraries for Multiplexed Target Capture." *Genome Research* 22 (5): 5. https://doi.org/10.1101/gr.128124.111.
Ruzich, Jenna, Keith Turnquist, Nathan Nye, David Rowe, and Wesley A. Larson. 2019. "Isolation by a Hydroelectric Dam Induces Minimal Impacts on Genetic Diversity and Population Structure in Six Fish Species." *Conservation Genetics* 20 (6): 6. https://doi.org/10.1007/s10592-019-01220-1.
Santiago, Enrique, Irene Novo, Antonio F Pardiñas, María Saura, Jinliang Wang, and Armando Caballero. 2020. "Recent Demographic History Inferred by High-Resolution Analysis of Linkage Disequilibrium." *Molecular Biology and Evolution* 37 (12): 3642–53. https://doi.org/10.1093/molbev/msaa169.
Schultz, Alan D., Fischer ,Richard A., Lovich ,Robert E., et al. 2024. "Successful Conservation of United States Department of Defense Lands: Response to Emily Rabung & Eric Toman (2022), Soldiers in the Garden: Managing the US Military Training Landscape." *Landscape Research* 49 (2): 301–7. https://doi.org/10.1080/01426397.2023.2252291.
Stein, Bruce A., Cameron Scott, and Nancy Benton. 2008. "Federal Lands and Endangered Species: The Role of Military and Other Federal Lands in Sustaining Biodiversity." *BioScience* 58 (4): 339–47. https://doi.org/10.1641/B580409.
Sternhagen, Elizabeth C., Mark A. Davis, Eric R. Larson, et al. 2024. "Comparing Cost, Effort, and Performance of Environmental DNA Sampling and Trapping for Detecting an Elusive Freshwater Turtle." *Environmental DNA* 6 (2): e525. https://doi.org/10.1002/edn3.525.
Thomas, Travis M., Michael C. Granatosky, Jason R. Bourque, et al. 2014. "Taxonomic Assessment of Alligator Snapping Turtles (Chelydridae: Macrochelys), with the Description of Two New Species from the Southeastern United States." *Zootaxa* 3786 (2): 2. https://doi.org/10.11646/zootaxa.3786.2.4.
Trauth, Stanley E., Robert G. Neal, and Malcolm L. McCallum. 2005. *Herpetofaunal Inventory of Fort Chaffee Maneuver Training Center*. Fort Chaffee Maneuver Training Center Environmental Branch.
Wagner, Brian K, Dave Urbston, and Dennis Leek. 1996. "Status and Distribution of Alligator Snapping Turtles in Arkansas." *Proceedings of the Annual Conference of Fish and Wildlife Agencies* 50: 264–70.
Waples, Robin S. 2022. "What Is Ne, Anyway?" *Journal of Heredity* 113 (4): 371–79. https://doi.org/10.1093/jhered/esac023.
Weir, Bruce S, and Jérôme Goudet. 2017. "A Unified Characterization of Population Structure and Relatedness." *Genetics* 206 (4): 4. https://doi.org/10.1534/genetics.116.198424.
Wright, Sewall. 1943. "Isolation by Distance." *Genetics* 28 (2): 2. https://doi.org/10.1093/genetics/28.2.114.
Zheng, X., D. Levine, J. Shen, S. M. Gogarten, C. Laurie, and B. S. Weir. 2012. "A High-Performance Computing Toolset for Relatedness and Principal Component Analysis of SNP Data." *Bioinformatics* 28 (24): 24. https://doi.org/10.1093/bioinformatics/bts606.

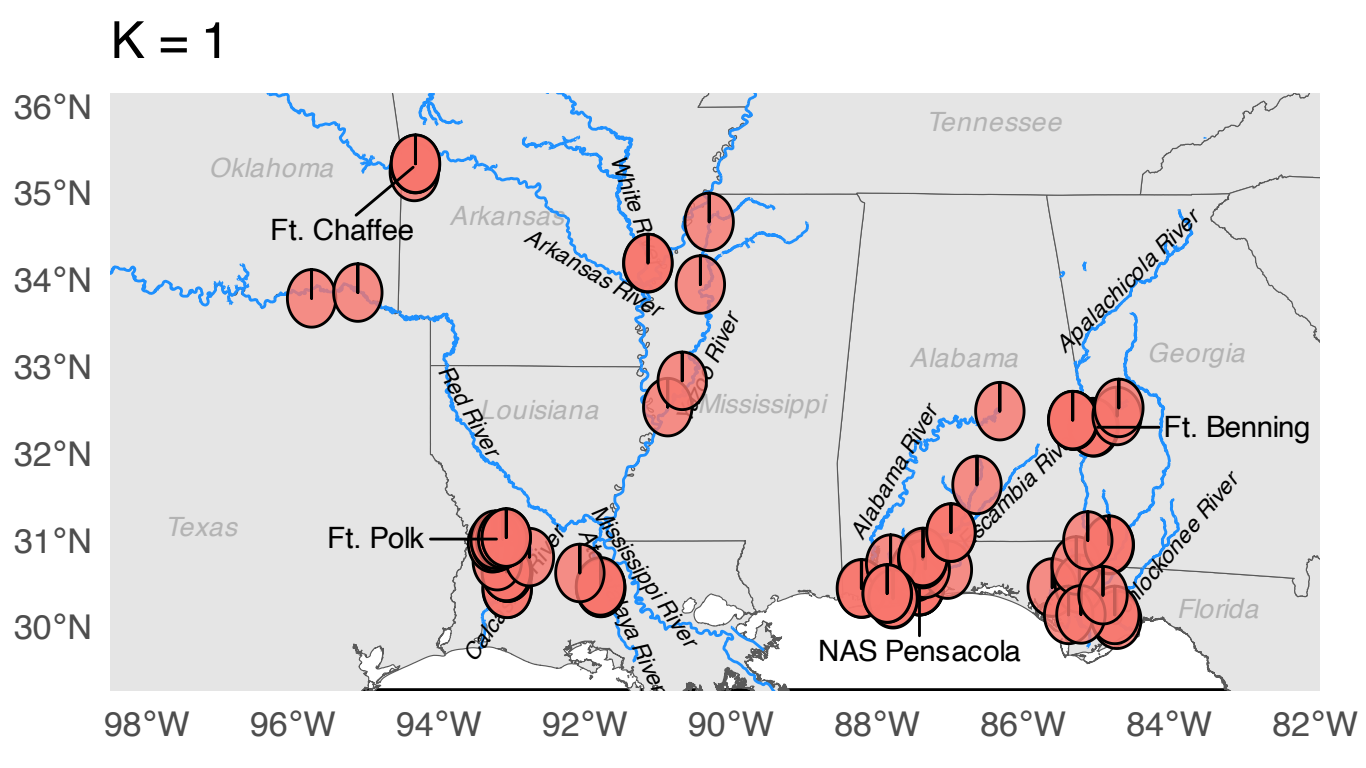
Appendices

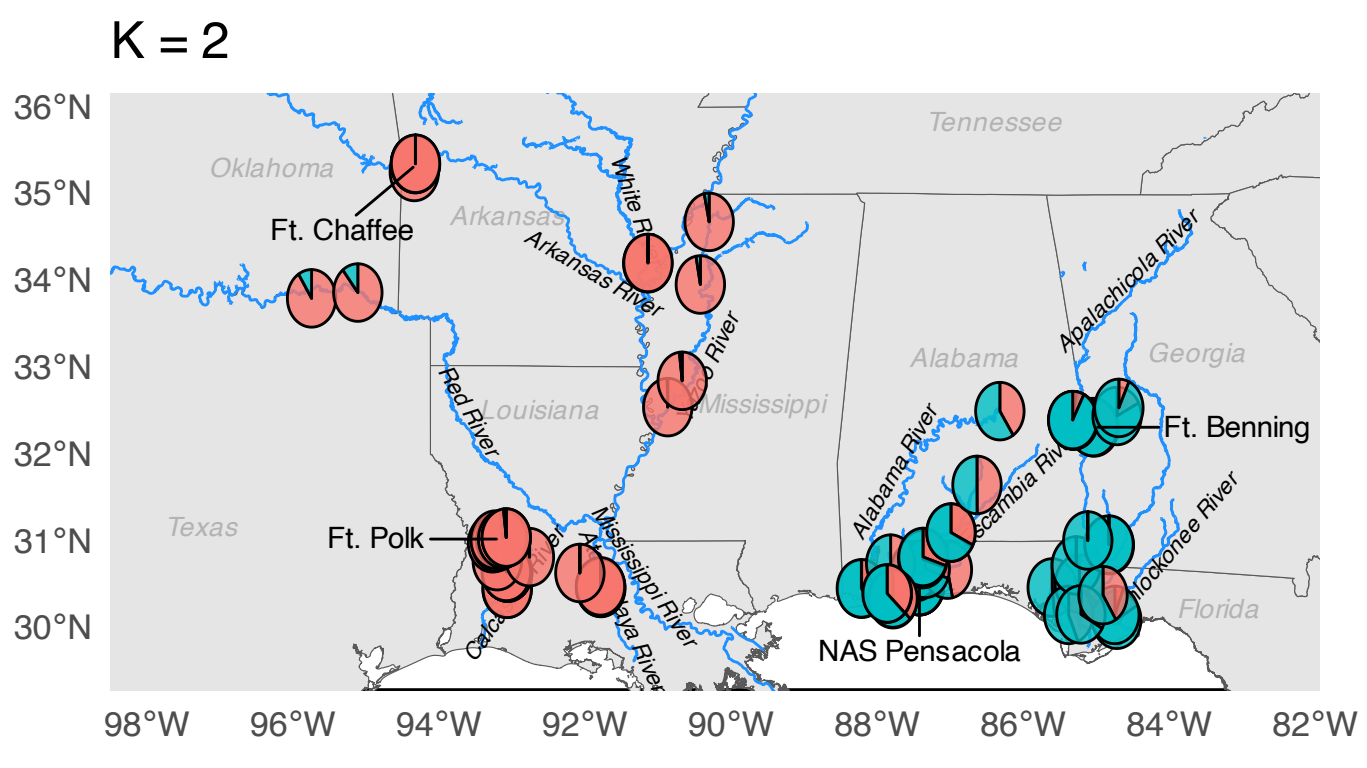
Supplementary File 1: Maps of TESS outputs for each number of populations (K) tested (K = 2 through K = 10 total) for all alligator snapping turtles. Each plot is titled with the number of populations tested. Three populations best described the dataset. Each pie represents one individual, colored by the proportion of genetic variation that individual draws from each of the modelled populations. Each color represents admixture from a different population

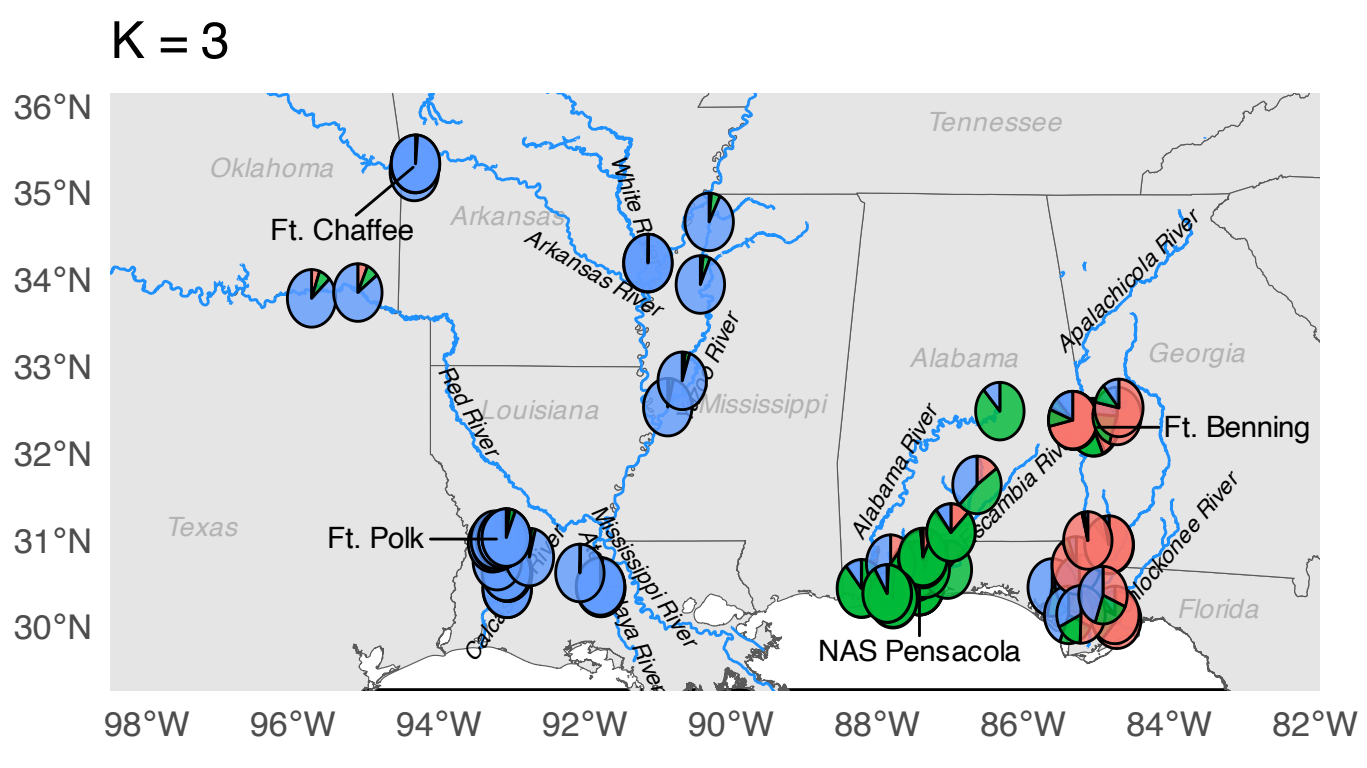
Supplementary File 2: TESS barplots for all gopher frog individuals at all missing data levels. TESS was run for two through nine populations (K = 2 through K = 9), at three different missing data levels: up to 50%, 40%, or 30% of sites allowed with missing data per individual. For simplicity, individuals are plotted as bars on a bar graph, with each bar colored by the proportion of that individual's genome derived from each of the tested populations (K). Each color represents admixture from a different population. Conservation units are separated by dashed white lines and labelled. Individuals are in the same order in every plot. Each page shows a different value of K, at all three missing data levels.

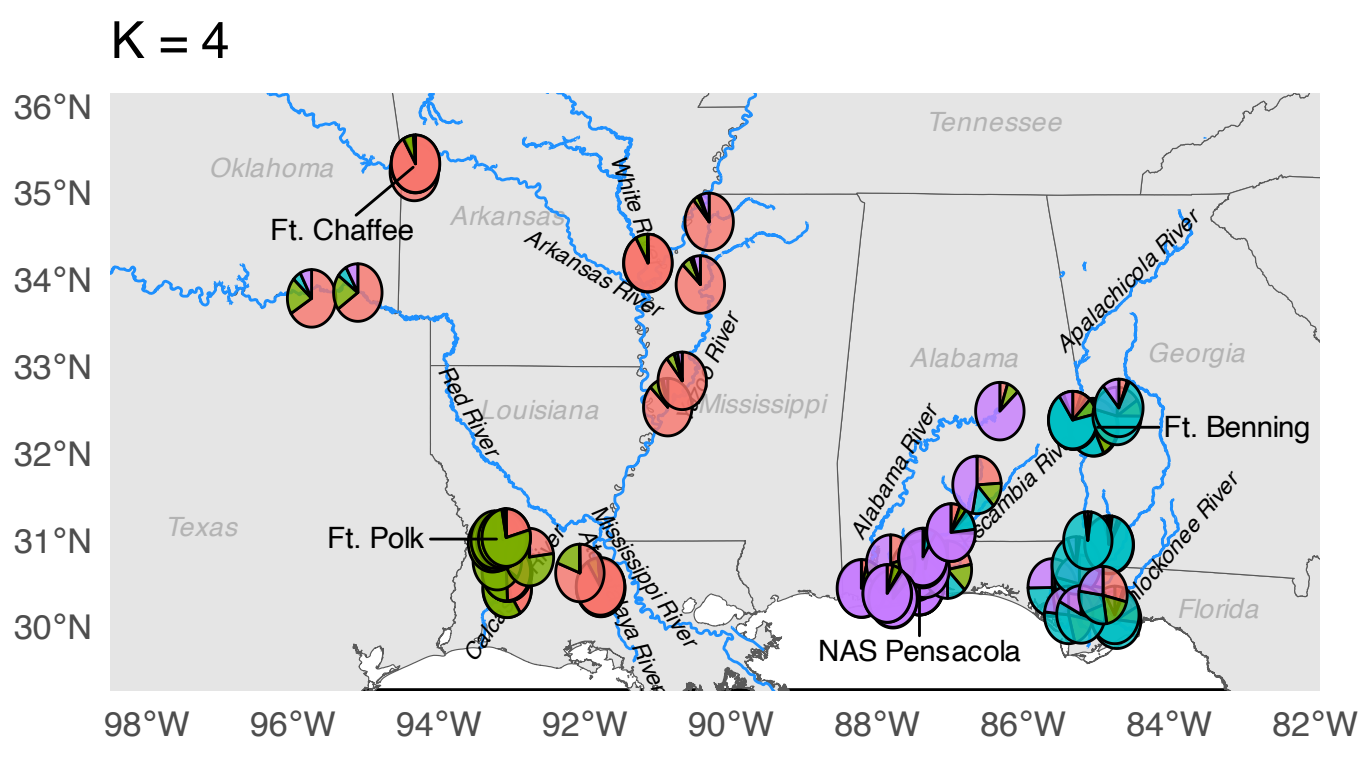
Supplementary File 3: Maps of TESS outputs for each number of populations (K) tested (K = 2 through K = 10 total) for all northern red-bellied turtles. Each plot is titled with the number of populations tested. Three populations best described the dataset. Each pie represents one individual, colored by the proportion of genetic variation that individual draws from each of the modeled populations. Each color represents admixture from a different population

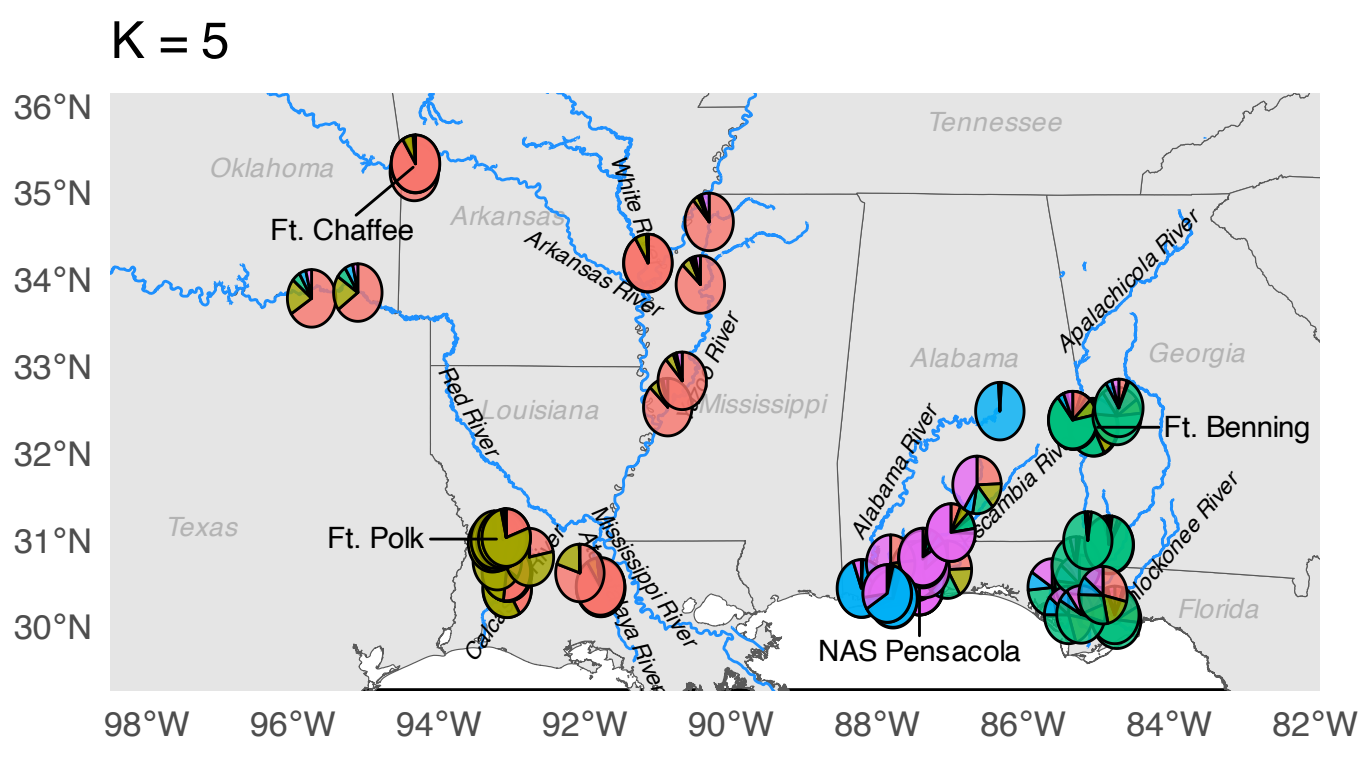
Supplementary File 4: Genetic sampling protocol for various reptiles and amphibians developed as part of this project, and distributed to installation managers.

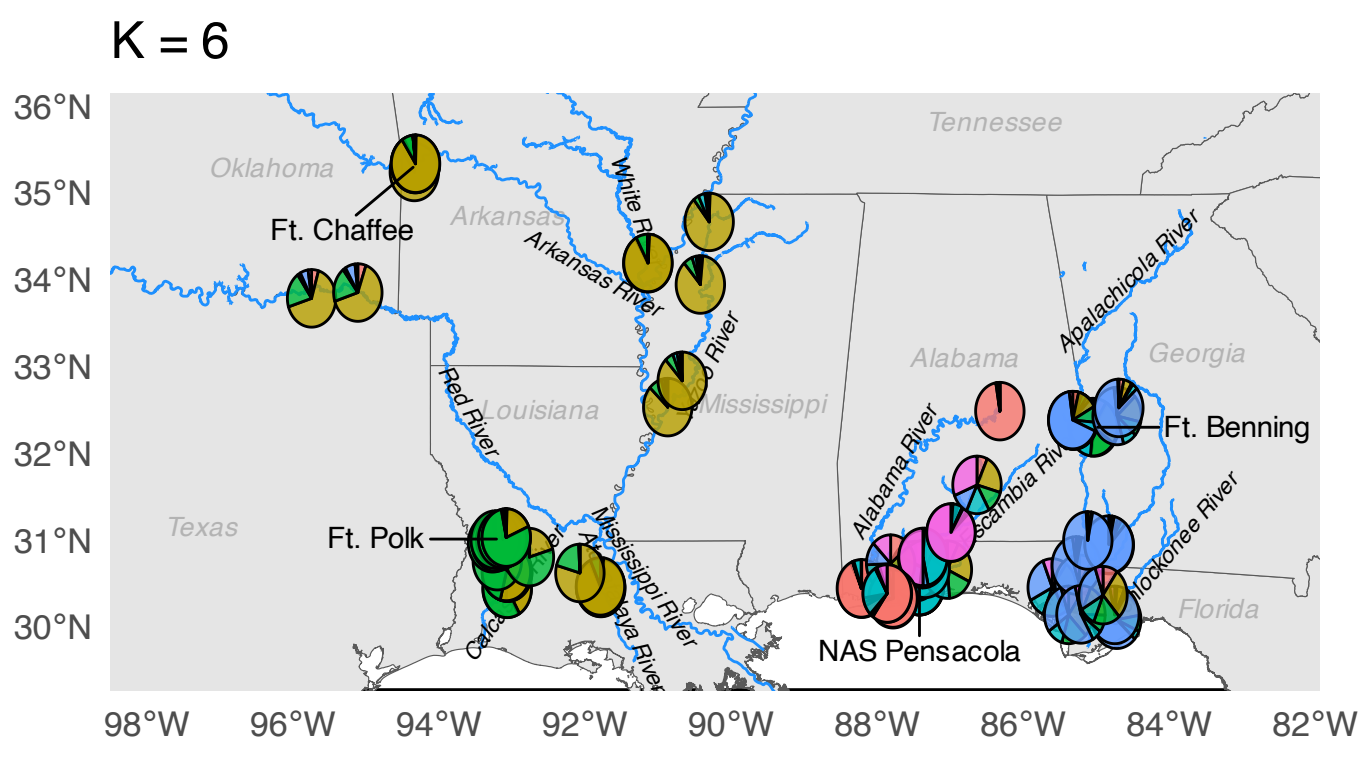

K = 1
36°N
35°N
34°N
33°N
32°N
31°N
30°N
98°W
96°W
94°W
92°W
90°W
88°W
86°W
84°W
82°W
Oklahoma
Arkansas
Louisiana
Texas
Tennessee
Mississippi
Alabama
Georgia
Florida
Ft. Chaffee
Ft. Polk
Ft. Benning
NAS Pensacola
Arkansas River
White R.
Red River
Mississippi River
Alabama River
Apalachicola River
Ochlockonee River

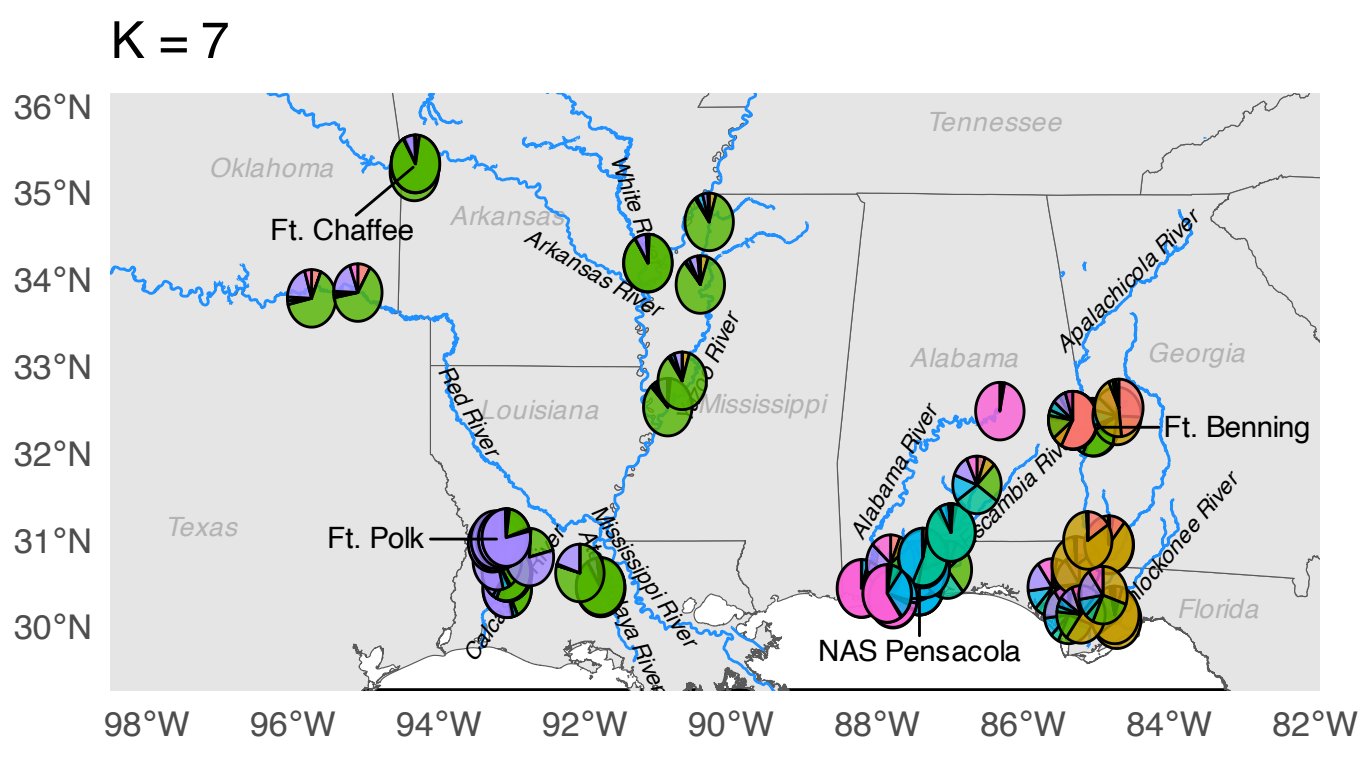

K = 2
36°N
35°N
34°N
33°N
32°N
31°N
30°N
98°W
96°W
94°W
92°W
90°W
88°W
86°W
84°W
82°W
Oklahoma
Arkansas
Louisiana
Texas
Tennessee
Mississippi
Alabama
Georgia
Florida
Ft. Chaffee
Ft. Polk
Ft. Benning
NAS Pensacola
Arkansas River
White R
Red River
Mississippi River
Alabama River
Apalachicola River
Blackwater River

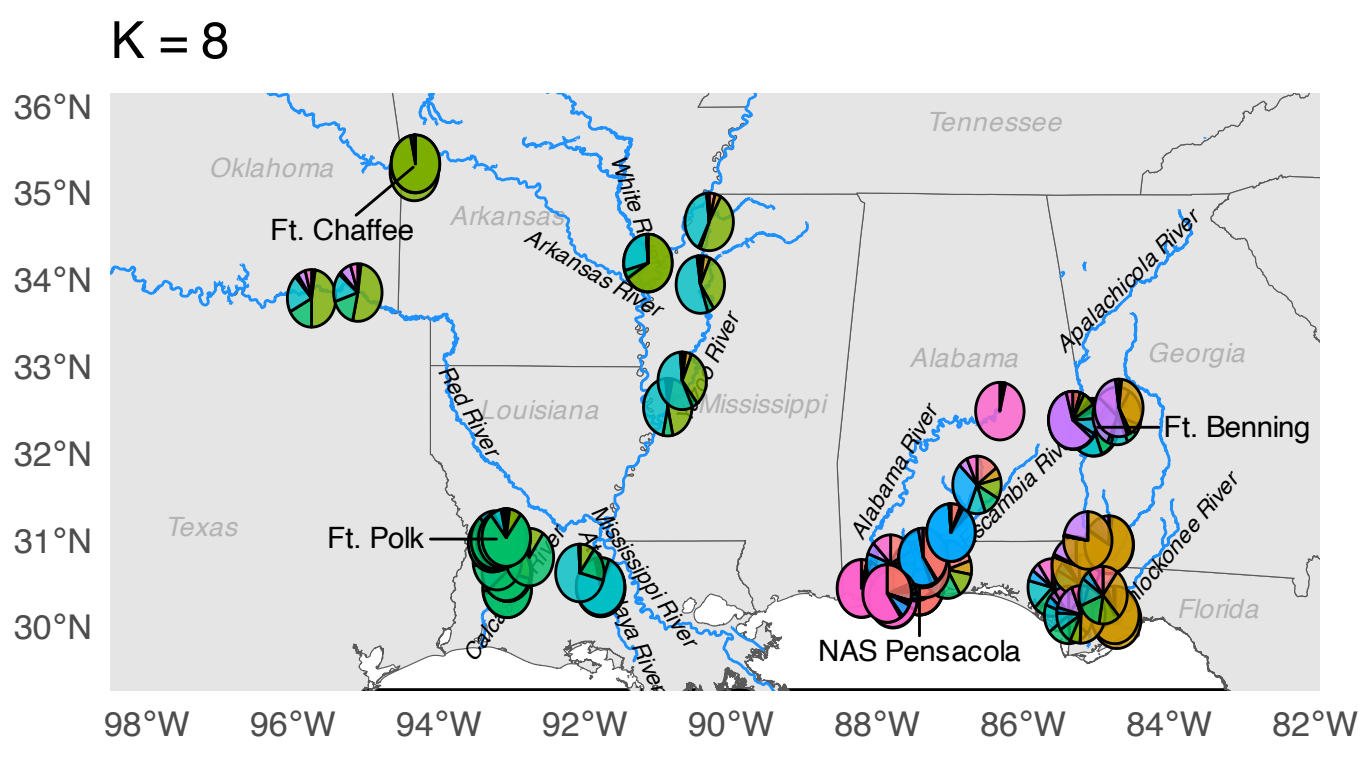

K = 3
36°N
35°N
34°N
33°N
32°N
31°N
30°N
98°W
96°W
94°W
92°W
90°W
88°W
86°W
84°W
82°W
Oklahoma
Arkansas
Louisiana
Texas
Tennessee
Mississippi
Alabama
Georgia
Florida
Ft. Chaffee
Ft. Polk
Ft. Benning
NAS Pensacola
Arkansas River
White River
Red River
Mississippi River
Alabama River
Apalachicola River

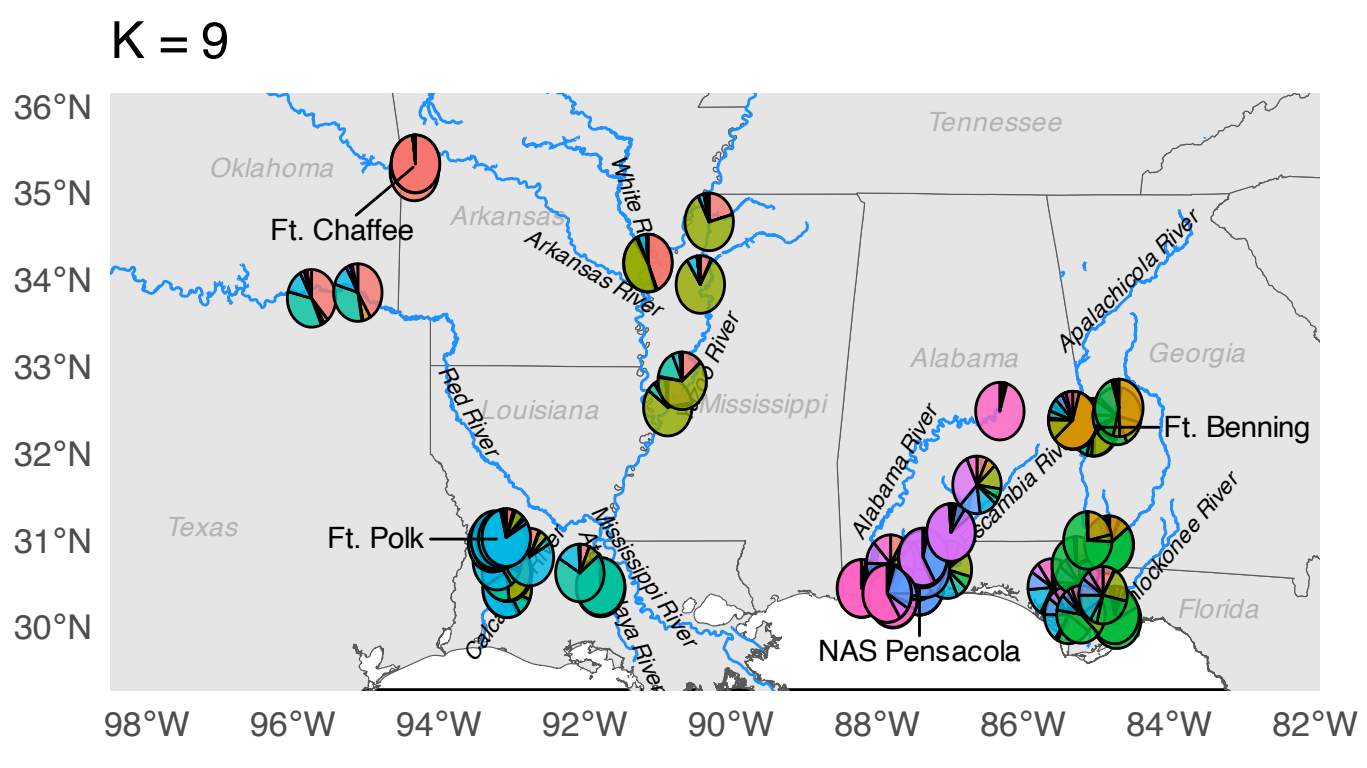

K = 4
36°N
35°N
34°N
33°N
32°N
31°N
30°N
98°W
96°W
94°W
92°W
90°W
88°W
86°W
84°W
82°W
Oklahoma
Arkansas
Louisiana
Texas
Mississippi
Alabama
Georgia
Florida
Tennessee
Ft. Chaffee
Ft. Polk
Ft. Benning
NAS Pensacola
Arkansas River
White R
Red River
Mississippi River
Alabama River
Apalachicola River
Florida

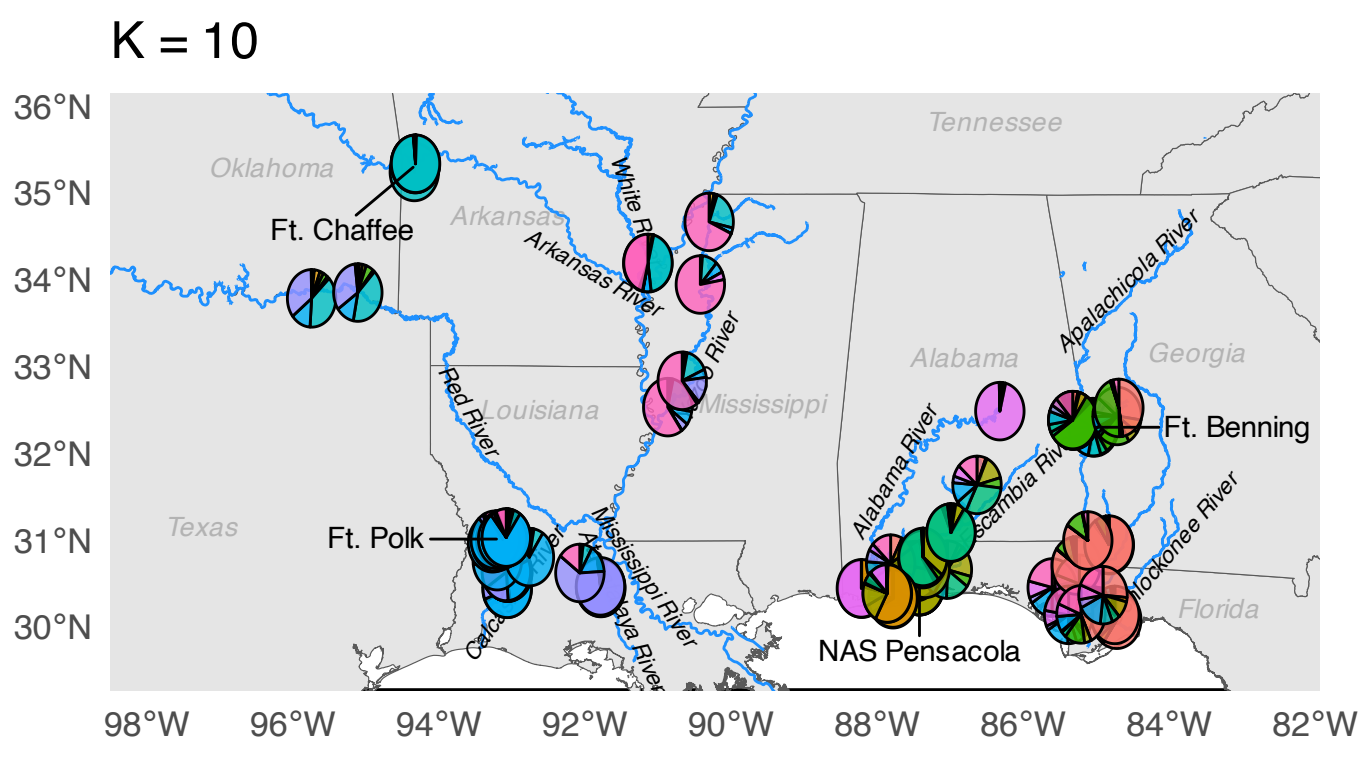

K = 5
36°N
35°N
34°N
33°N
32°N
31°N
30°N
98°W
96°W
94°W
92°W
90°W
88°W
86°W
84°W
82°W
Oklahoma
Arkansas
Tennessee
Louisiana
Mississippi
Alabama
Georgia
Florida
Texas
Ft. Chaffee
Ft. Polk
Ft. Benning
NAS Pensacola
Arkansas River
White River
Red River
Mississippi River
Alabama River
Apalachicola River
Ochlockonee River

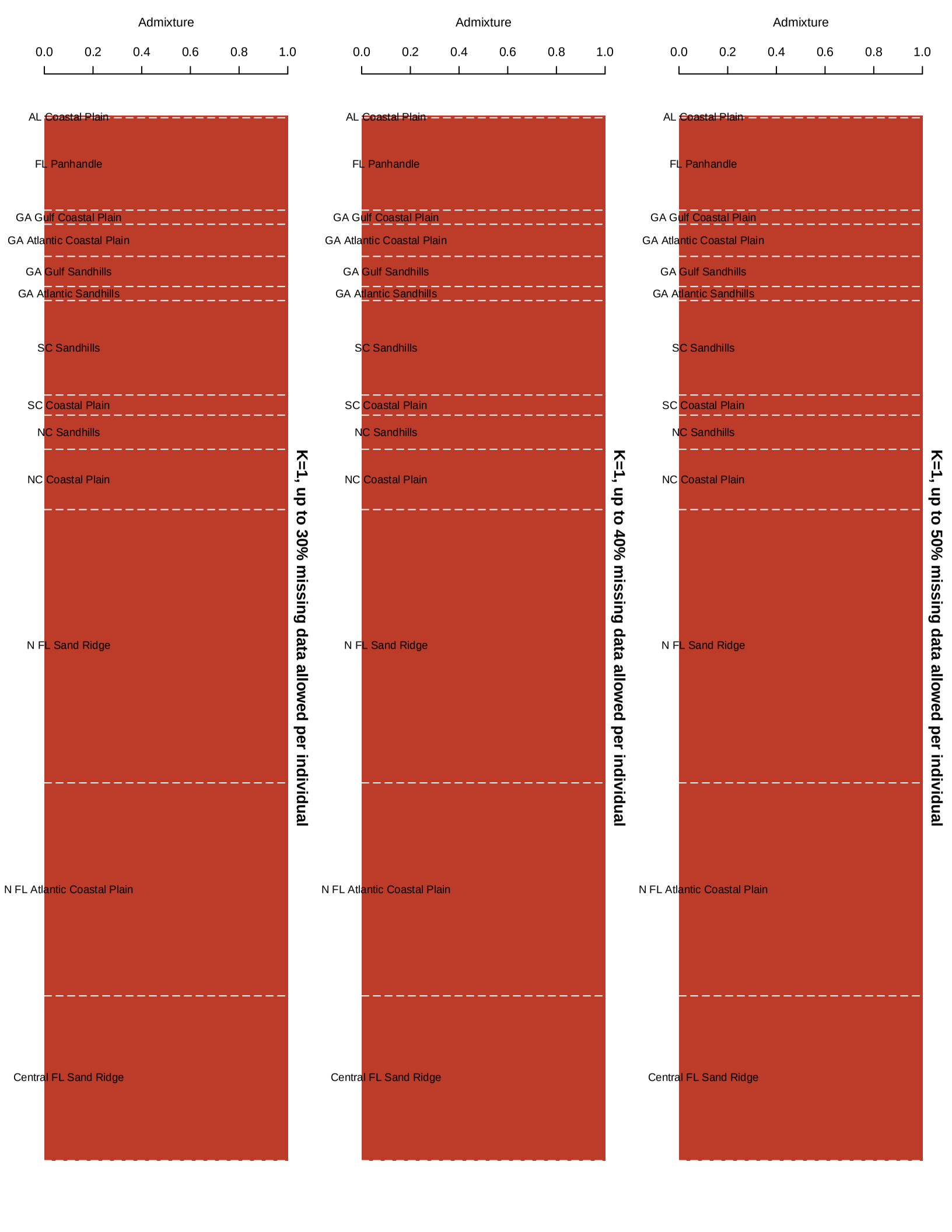

K = 6
36°N
35°N
34°N
33°N
32°N
31°N
30°N
98°W
96°W
94°W
92°W
90°W
88°W
86°W
84°W
82°W
Oklahoma
Arkansas
Louisiana
Texas
Mississippi
Alabama
Georgia
Florida
Tennessee
Ft. Chaffee
Ft. Polk
Ft. Benning
NAS Pensacola
Arkansas River
White R
Red River
Mississippi River
Alabama River
Apalachicola River
Florida

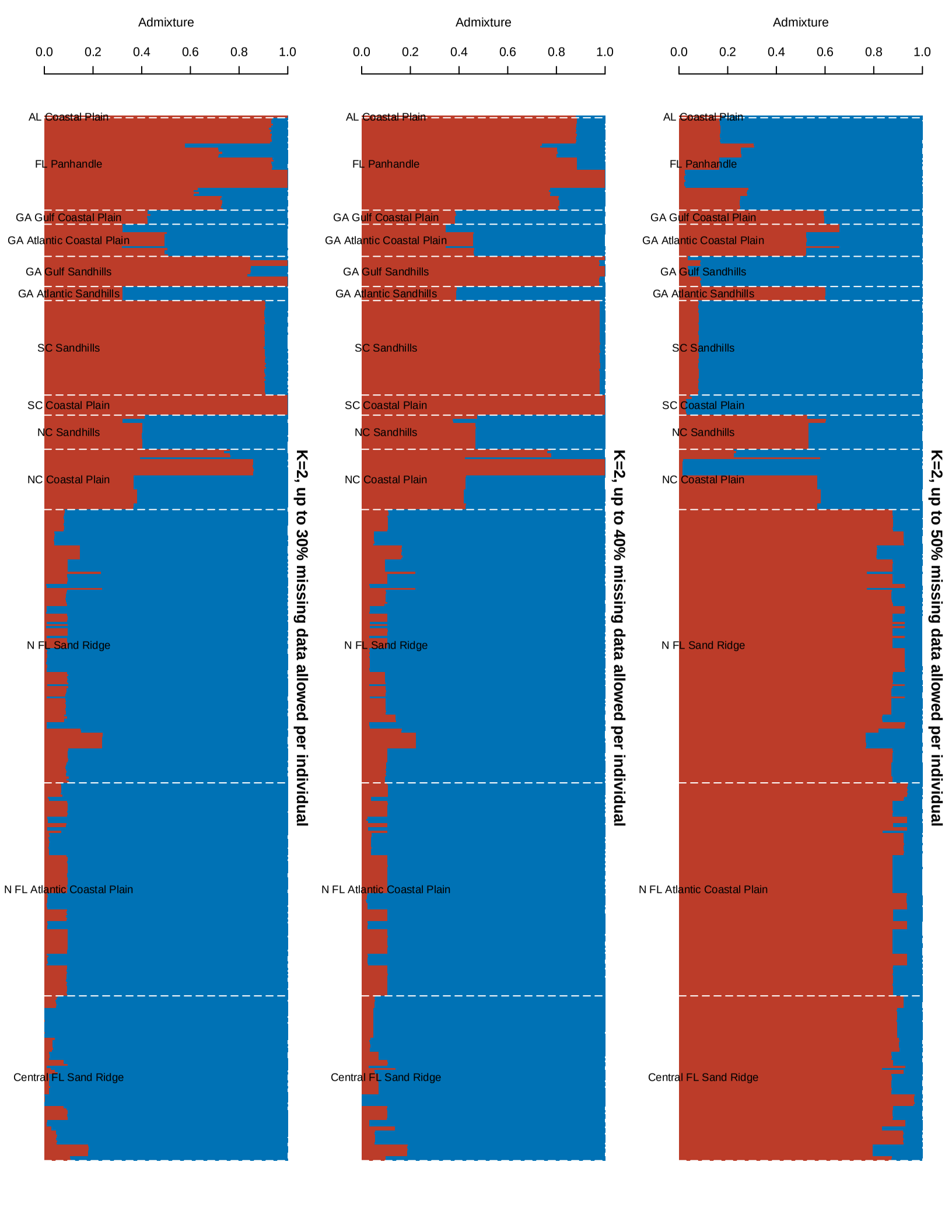

K = 7
36°N
35°N
34°N
33°N
32°N
31°N
30°N
98°W
96°W
94°W
92°W
90°W
88°W
86°W
84°W
82°W
Oklahoma
Arkansas
Louisiana
Texas
Mississippi
Alabama
Georgia
Florida
Tennessee
Ft. Chaffee
Ft. Polk
Ft. Benning
NAS Pensacola
Arkansas River
White River
Red River
Mississippi River
Alabama River
Apalachicola River

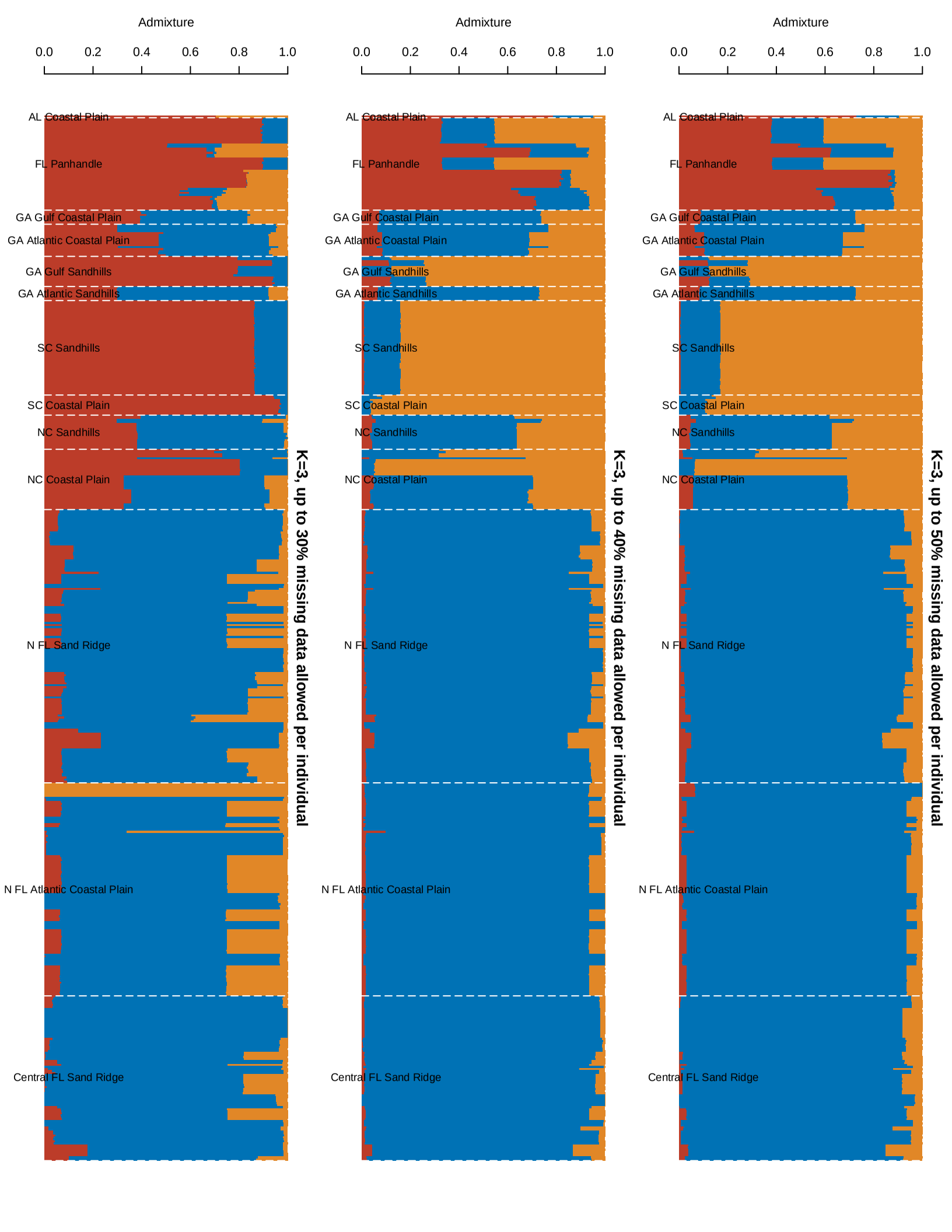

K = 8
36°N
35°N
34°N
33°N
32°N
31°N
30°N
98°W
96°W
94°W
92°W
90°W
88°W
86°W
84°W
82°W
Oklahoma
Arkansas
Tennessee
Louisiana
Mississippi
Alabama
Georgia
Florida
Texas
Ft. Chaffee
Ft. Polk
Ft. Benning
NAS Pensacola
Arkansas River
White R
Red River
Mississippi River
Alabama River
Apalachicola River
Ochlockonee River

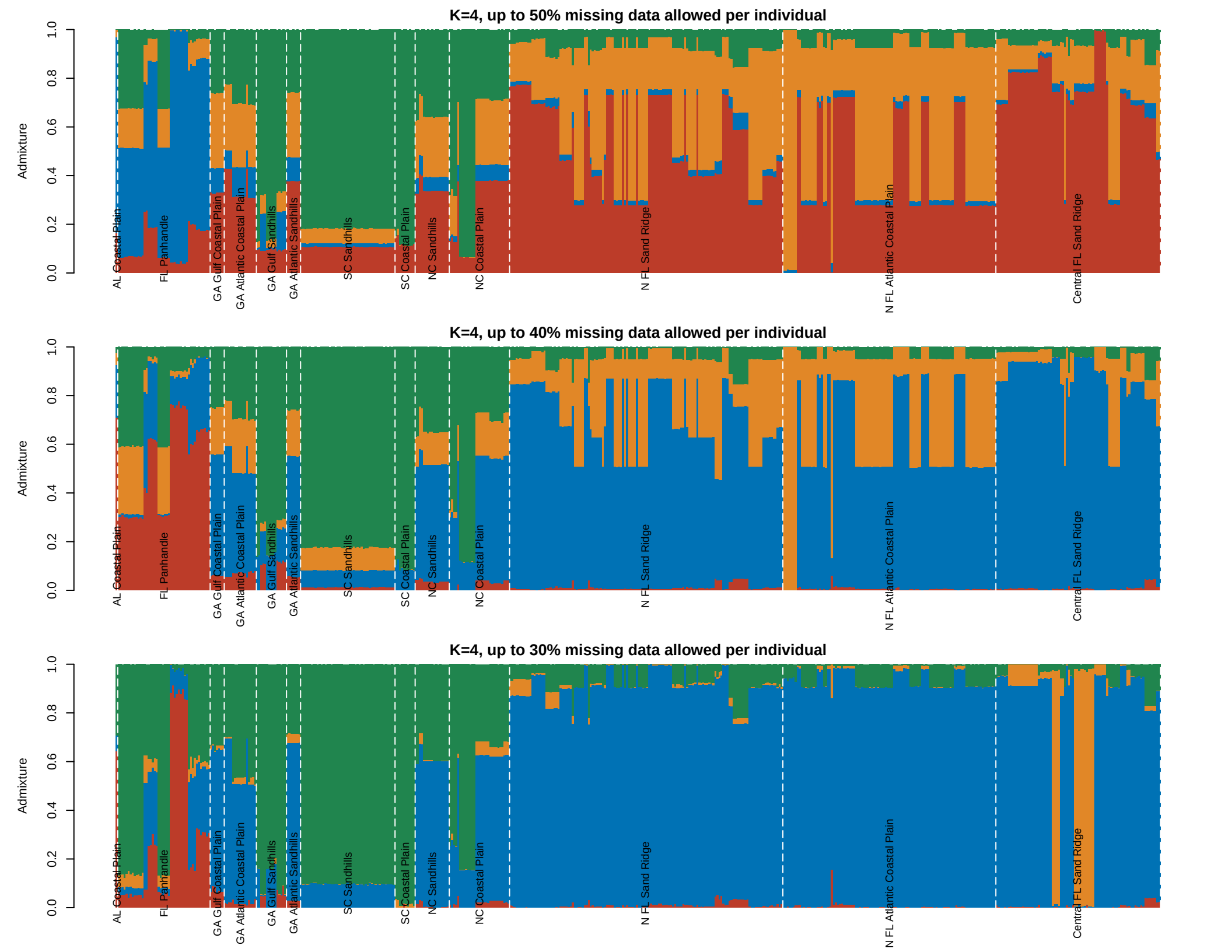

K = 9
36°N
35°N
34°N
33°N
32°N
31°N
30°N
98°W
96°W
94°W
92°W
90°W
88°W
86°W
84°W
82°W
Oklahoma
Arkansas
Tennessee
Louisiana
Mississippi
Alabama
Georgia
Florida
Texas
Ft. Chaffee
Ft. Polk
Ft. Benning
NAS Pensacola
Arkansas River
White R
Red River
Mississippi River
Alabama River
Apalachicola River
Florida

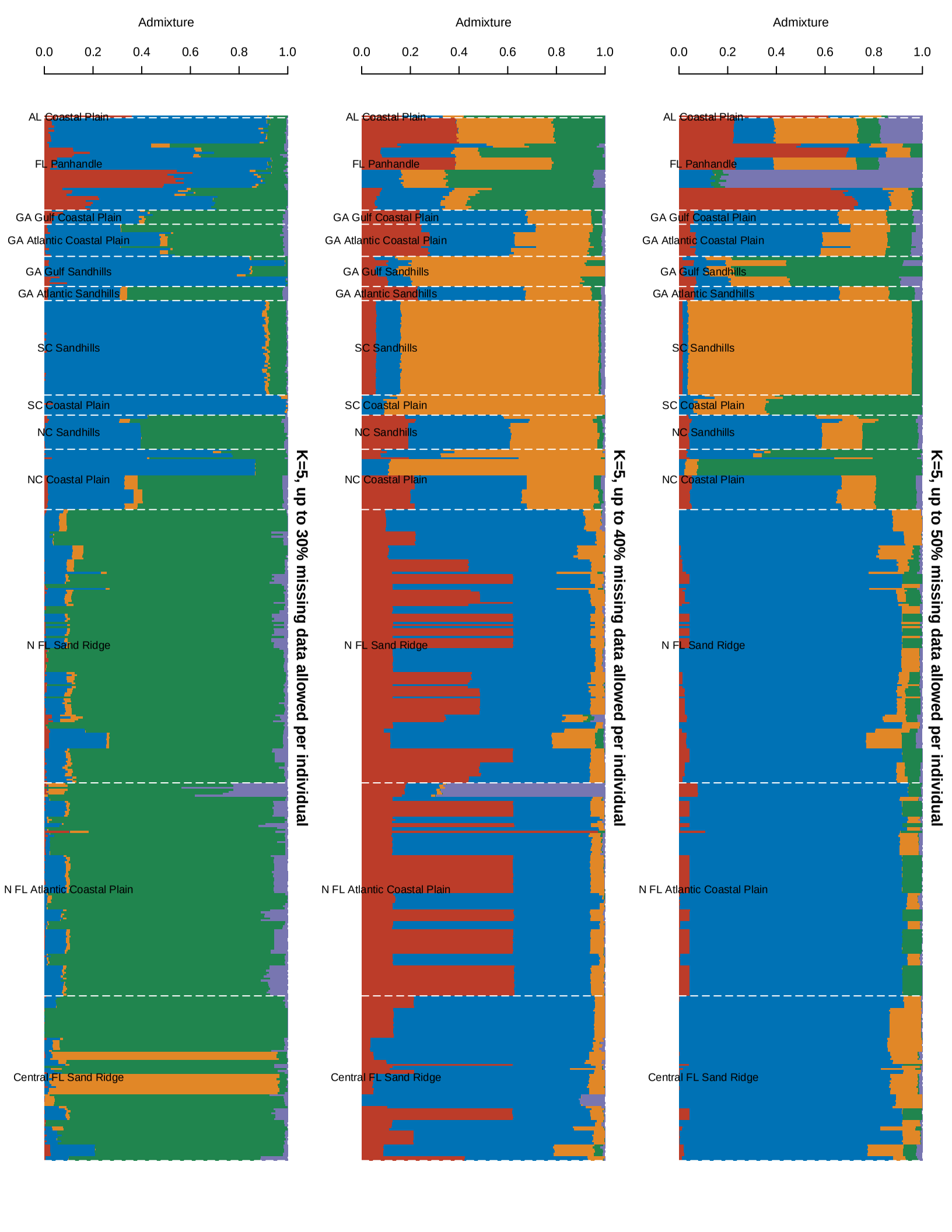

K = 10
36°N
35°N
34°N
33°N
32°N
31°N
30°N
98°W
96°W
94°W
92°W
90°W
88°W
86°W
84°W
82°W
Oklahoma
Arkansas
Louisiana
Texas
Tennessee
Mississippi
Alabama
Georgia
Florida
Ft. Chaffee
Ft. Polk
Ft. Benning
NAS Pensacola
Arkansas River
White R
Red River
Mississippi River
Alabama River
Apalachicola River
Florida

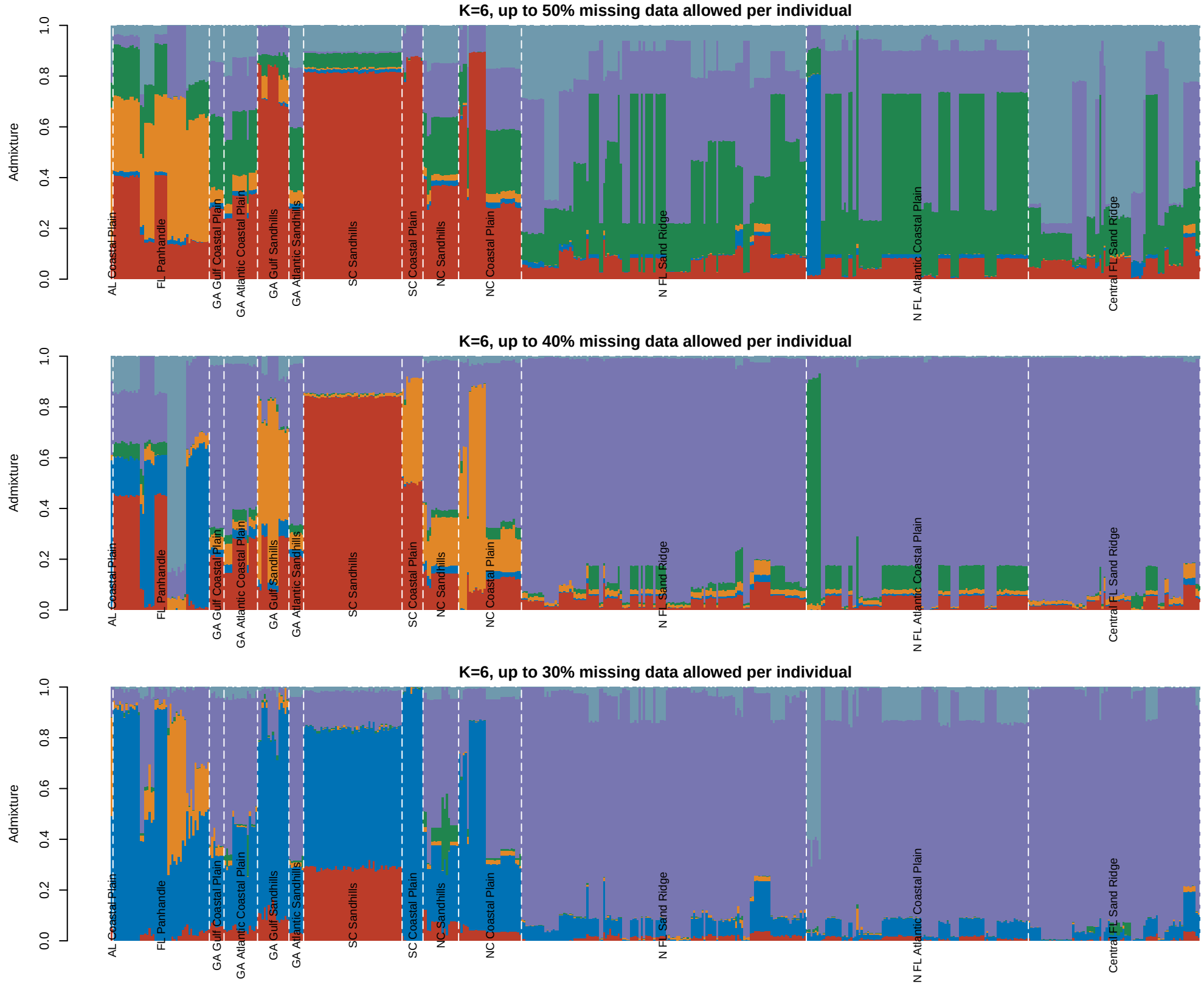

Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge
K=1, up to 30% missing data allowed per individual
Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge
K=1, up to 40% missing data allowed per individual
Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge
K=1, up to 50% missing data allowed per individual

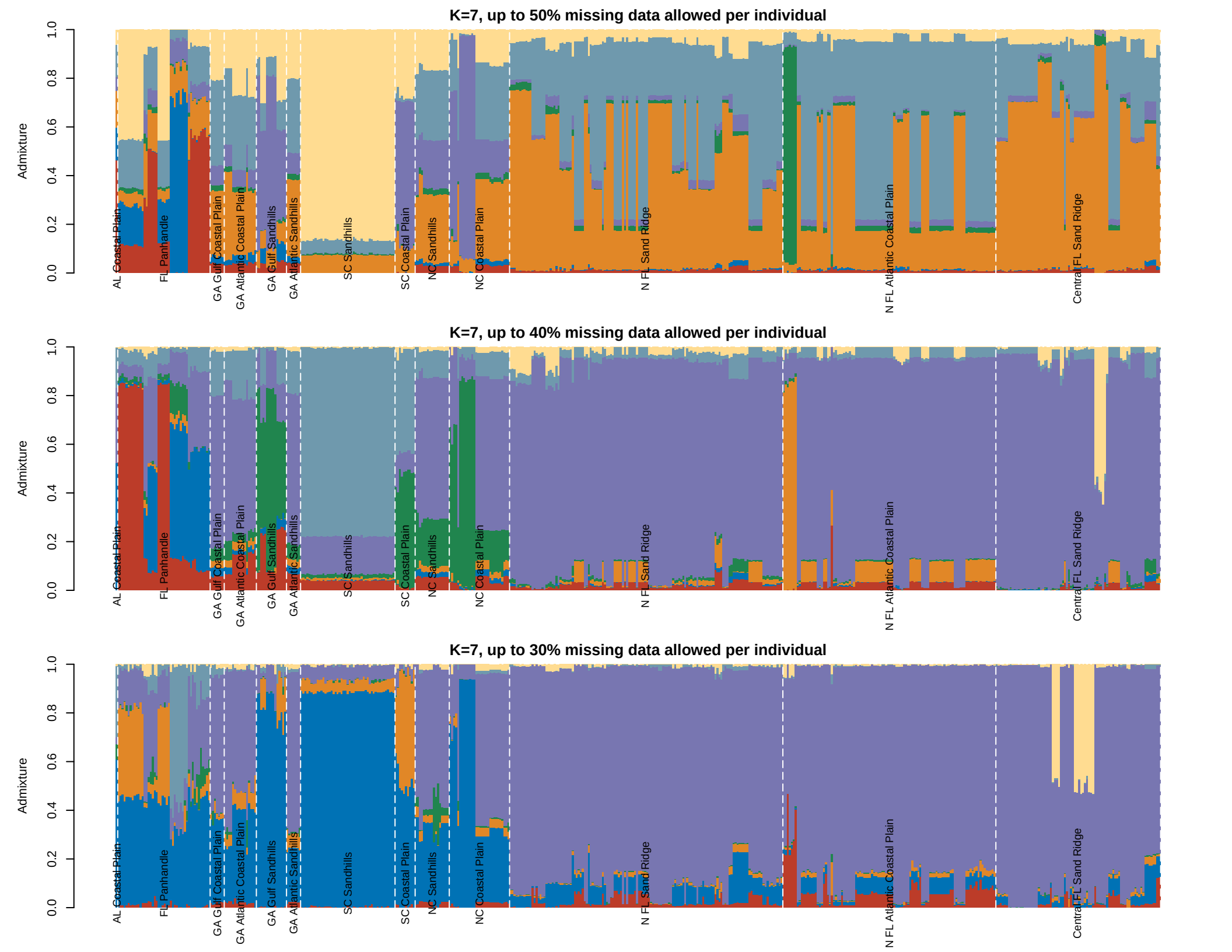

Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge
K=2, up to 30% missing data allowed per individual
K=2, up to 40% missing data allowed per individual
K=2, up to 50% missing data allowed per individual

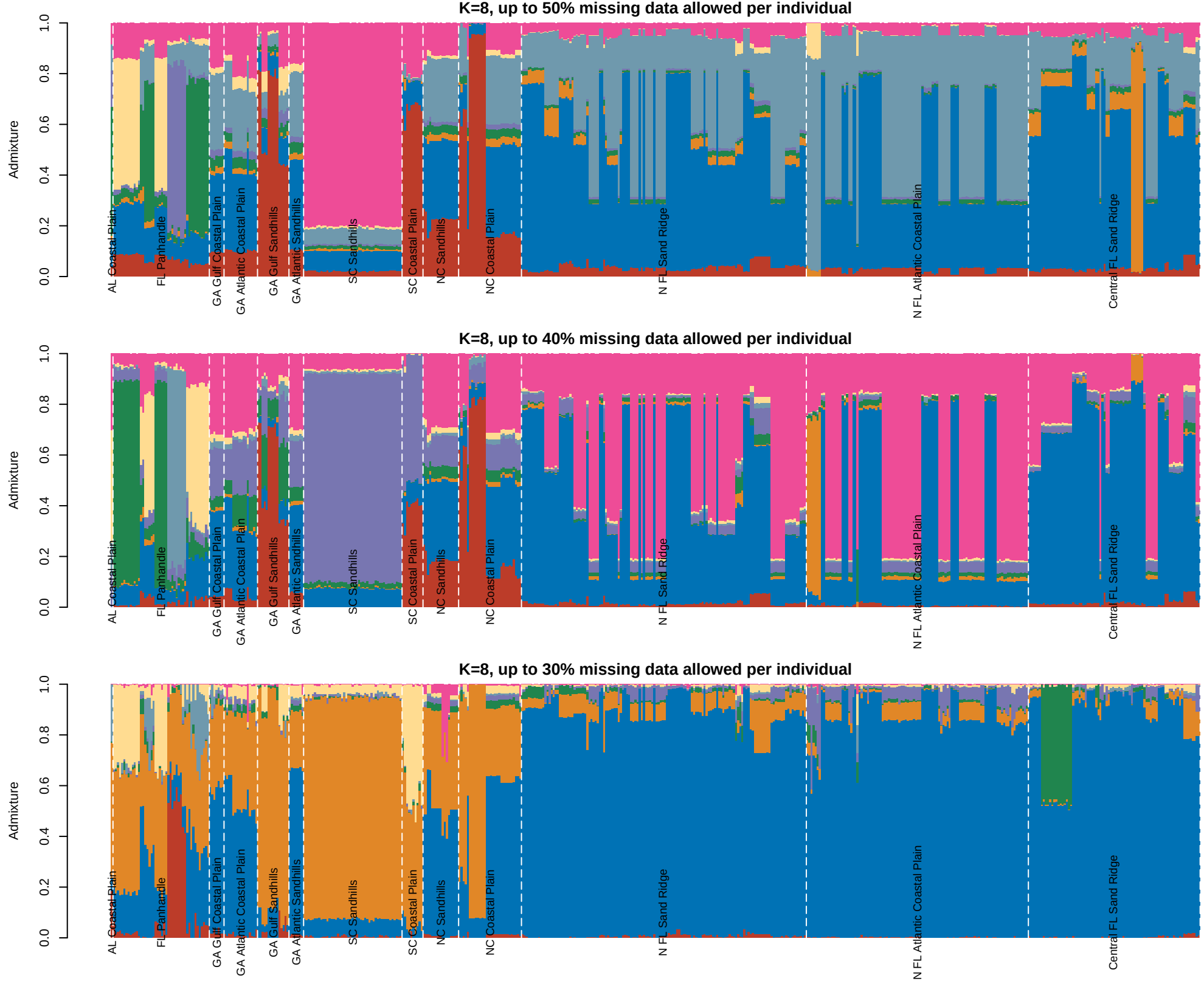

Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge
K=3, up to 30% missing data allowed per individual
K=3, up to 40% missing data allowed per individual
K=3, up to 50% missing data allowed per individual

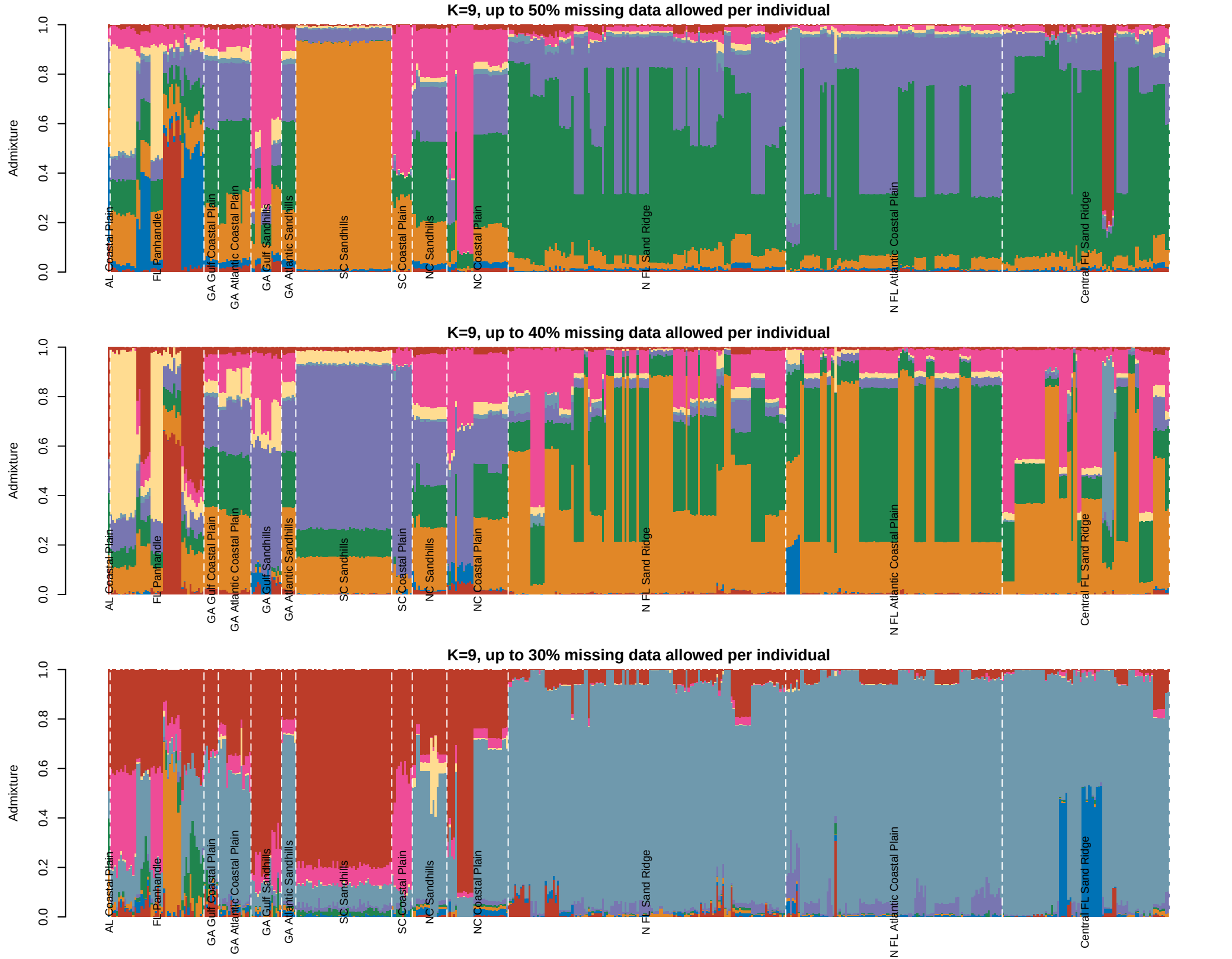

K=4, up to 50% missing data allowed per individual
K=4, up to 40% missing data allowed per individual
K=4, up to 30% missing data allowed per individual
Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge

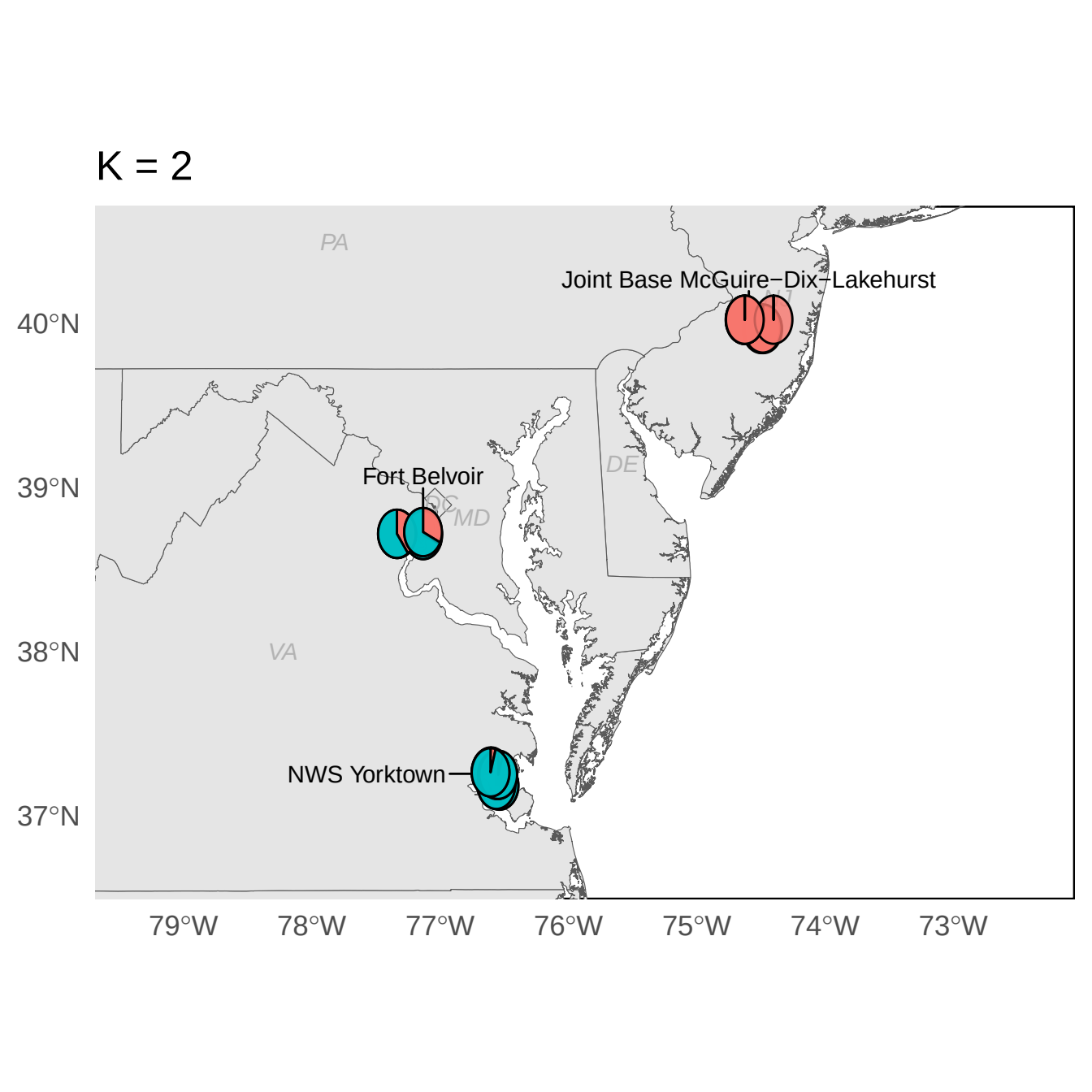

Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge
K=5, up to 30% missing data allowed per individual
Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge
K=5, up to 40% missing data allowed per individual
Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge
K=5, up to 50% missing data allowed per individual

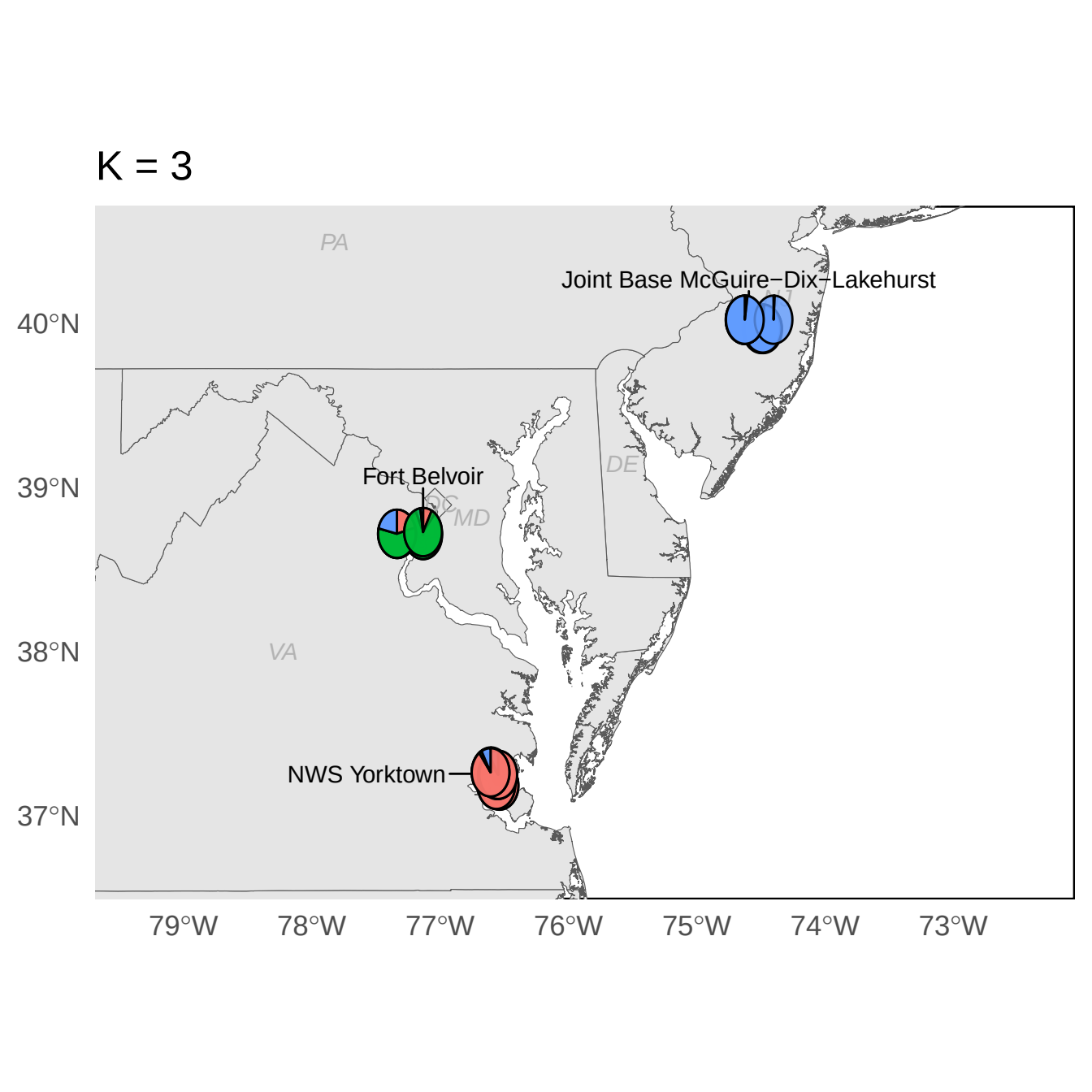

K=6, up to 50% missing data allowed per individual
K=6, up to 40% missing data allowed per individual
K=6, up to 30% missing data allowed per individual
Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge

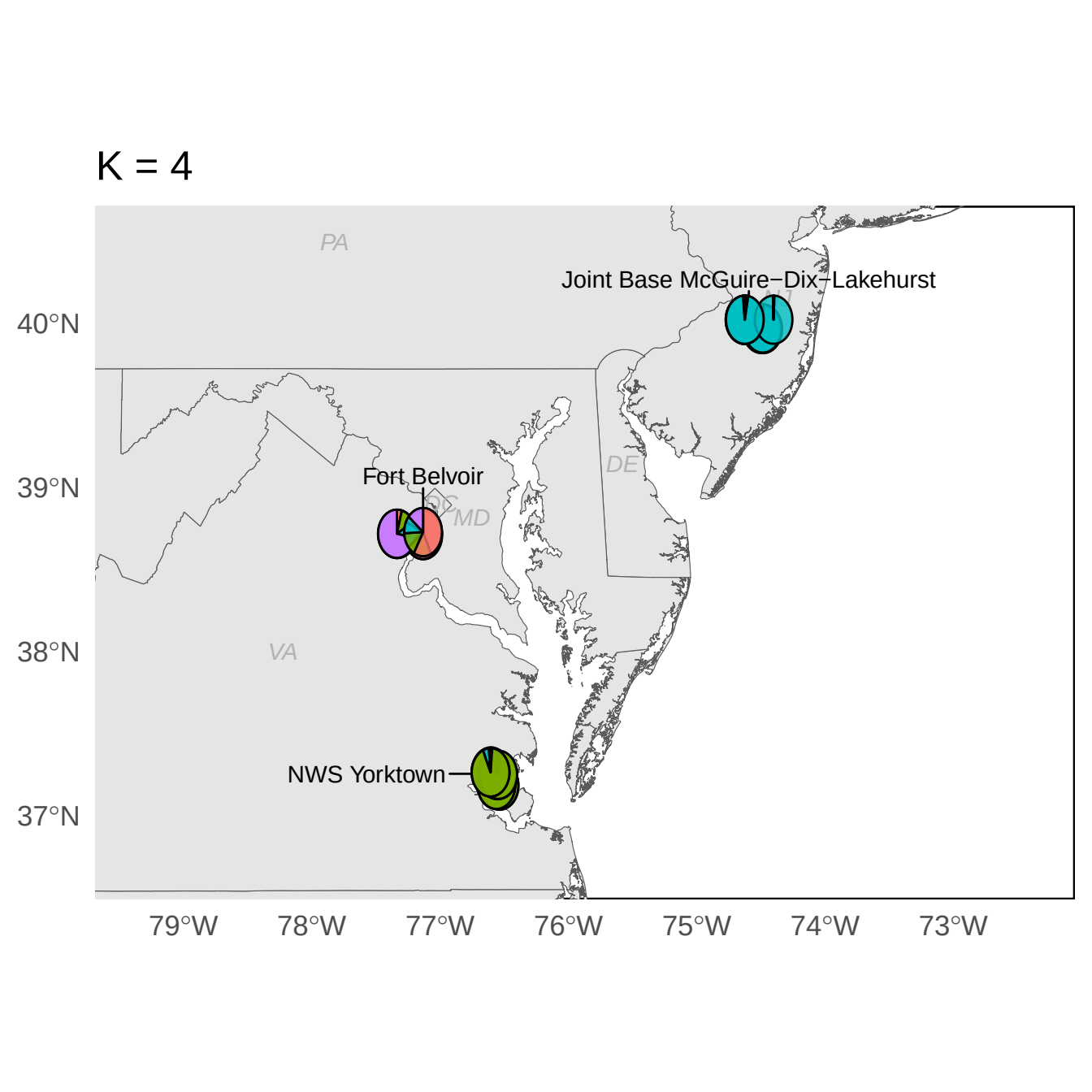

K=7, up to 50% missing data allowed per individual
K=7, up to 40% missing data allowed per individual
K=7, up to 30% missing data allowed per individual
Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge

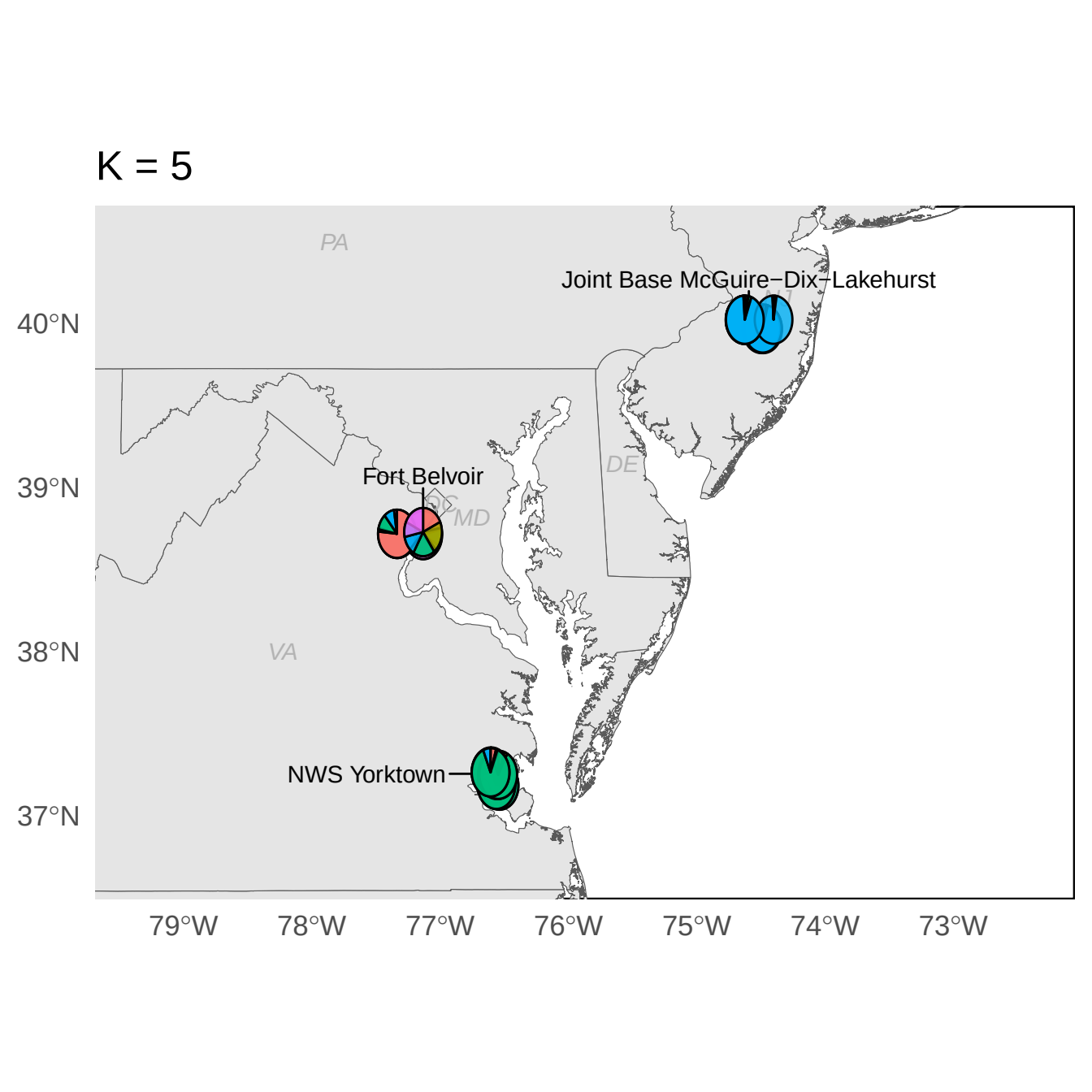

K=8, up to 50% missing data allowed per individual
K=8, up to 40% missing data allowed per individual
K=8, up to 30% missing data allowed per individual
Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge

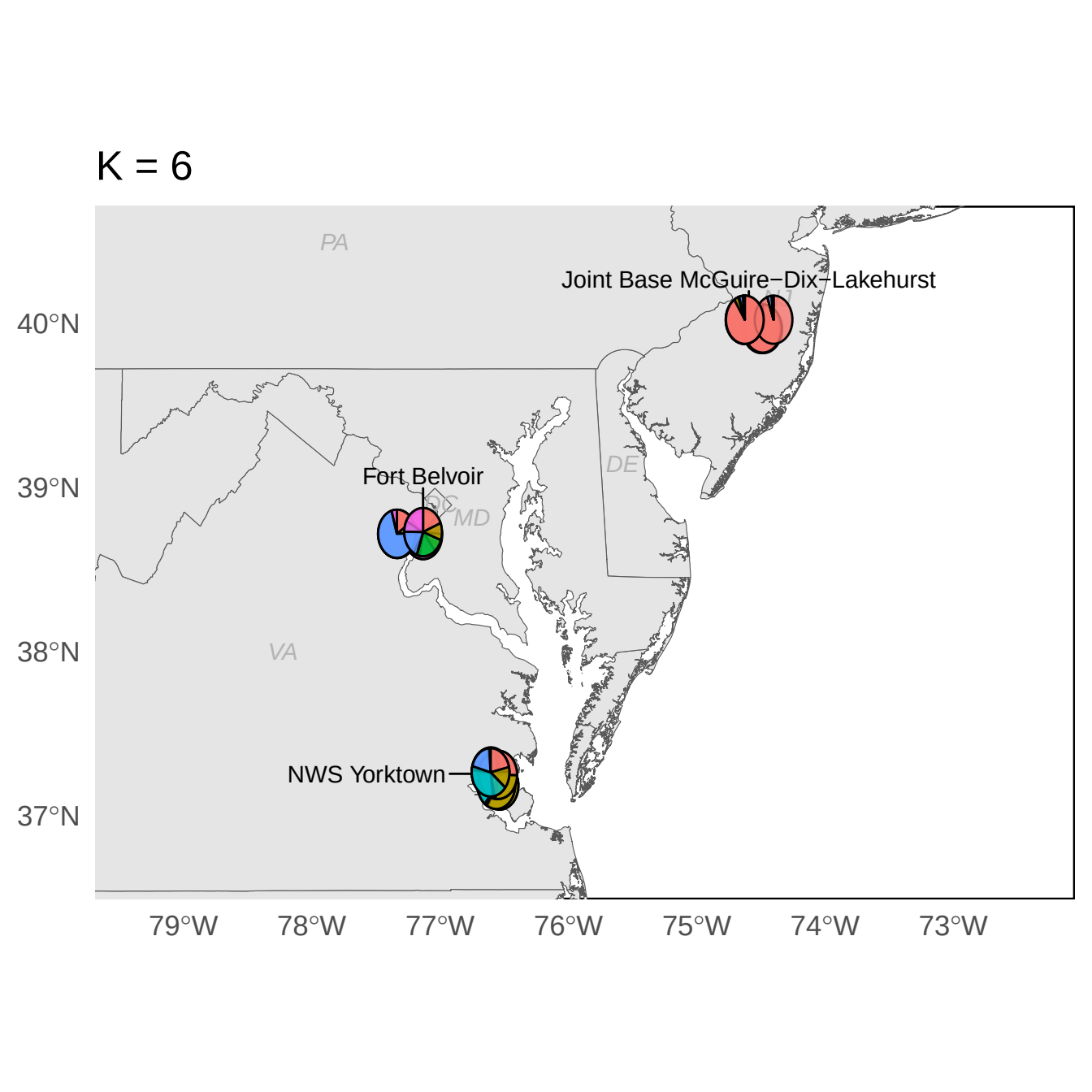

K=9, up to 50% missing data allowed per individual
K=9, up to 40% missing data allowed per individual
K=9, up to 30% missing data allowed per individual
Admixture
0.0
0.2
0.4
0.6
0.8
1.0
AL Coastal Plain
FL Panhandle
GA Gulf Coastal Plain
GA Atlantic Coastal Plain
GA Gulf Sandhills
GA Atlantic Sandhills
SC Sandhills
SC Coastal Plain
NC Sandhills
NC Coastal Plain
N FL Sand Ridge
N FL Atlantic Coastal Plain
Central FL Sand Ridge

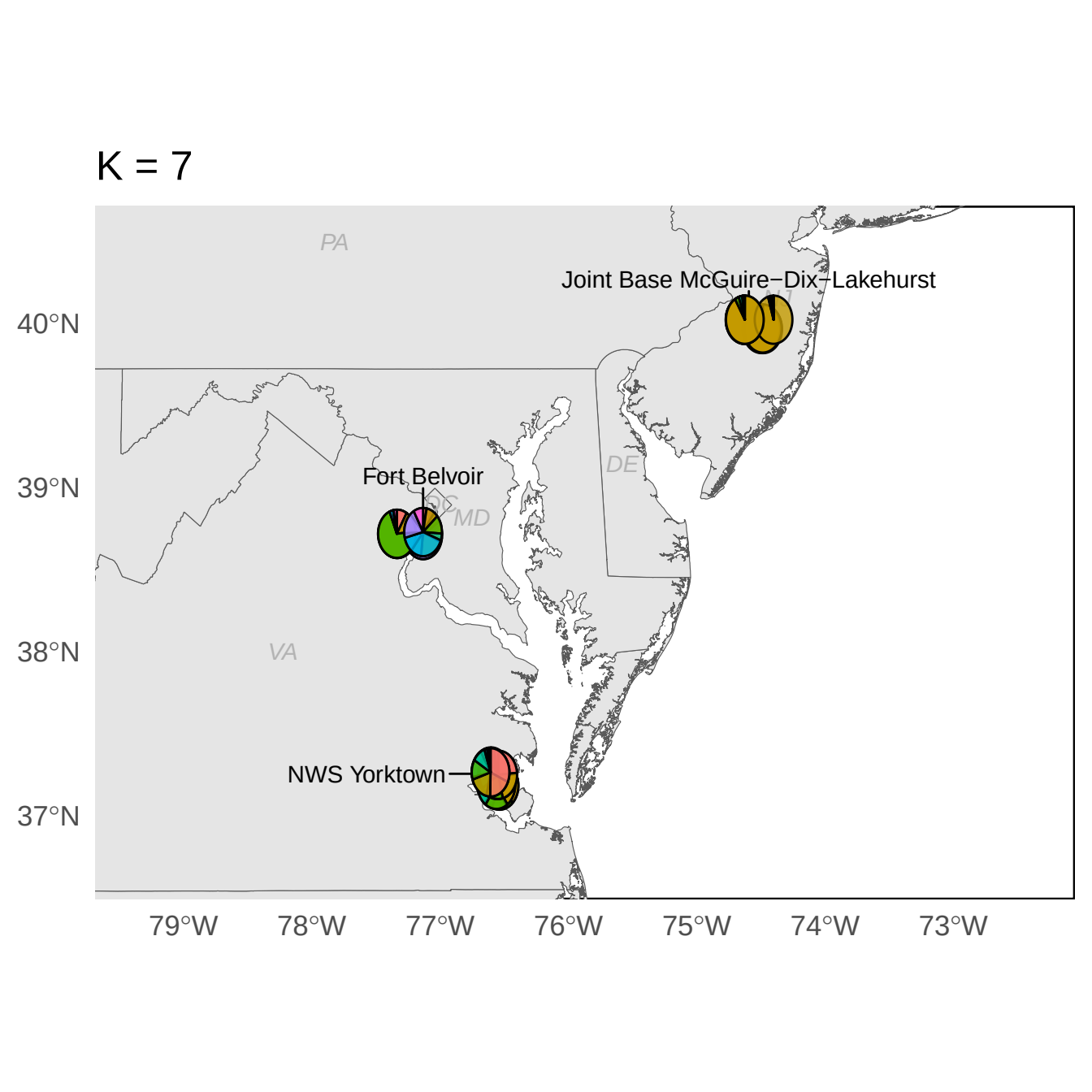

K = 2
PA
Joint Base McGuire−Dix−Lakehurst
NJ
40°N
DE
Fort Belvoir
DC
MD
39°N
VA
38°N
NWS Yorktown
37°N
79°W
78°W
77°W
76°W
75°W
74°W
73°W

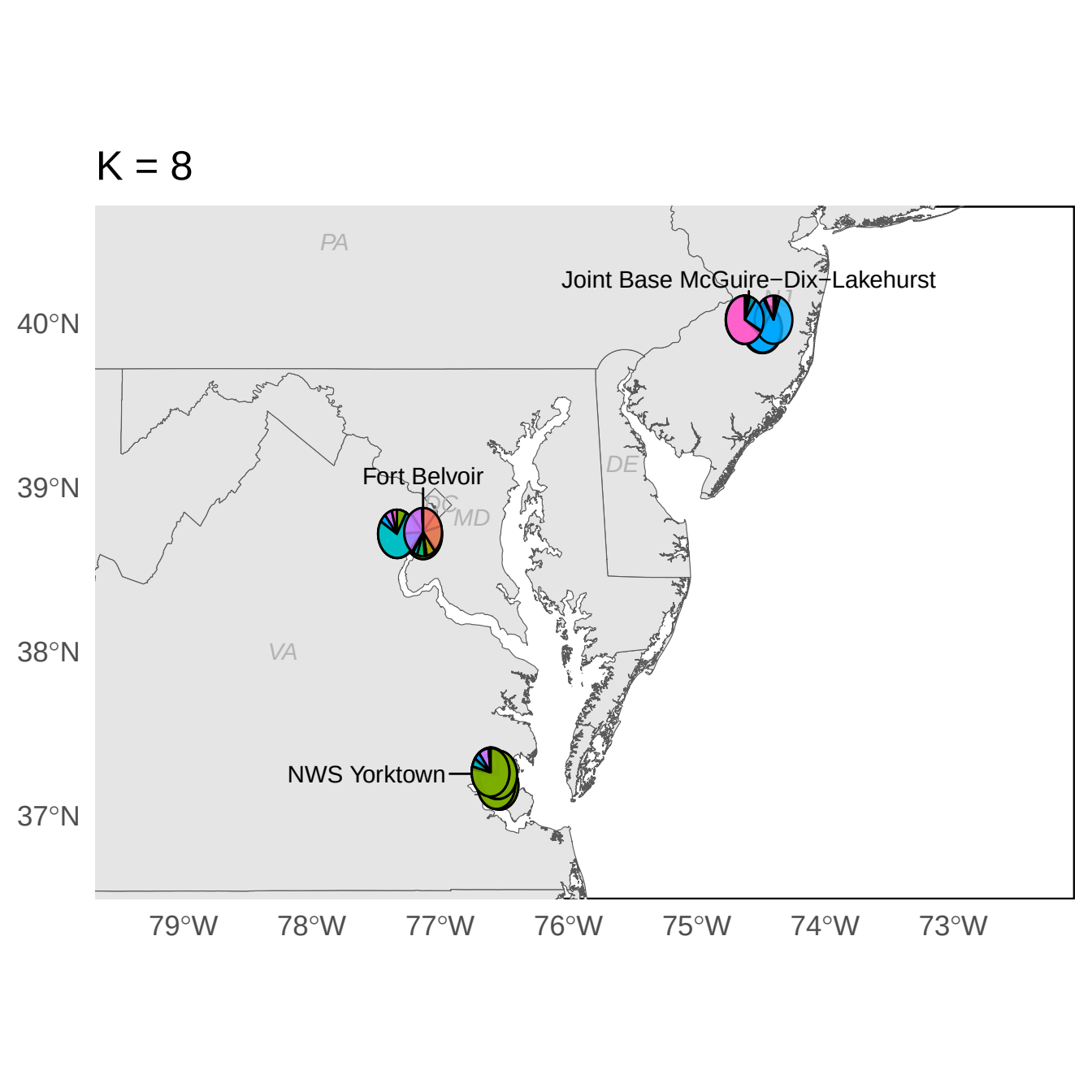

K = 3
PA
Joint Base McGuire−Dix−Lakehurst
NJ
40°N
DE
Fort Belvoir
DC
MD
39°N
VA
38°N
NWS Yorktown
37°N
79°W
78°W
77°W
76°W
75°W
74°W
73°W

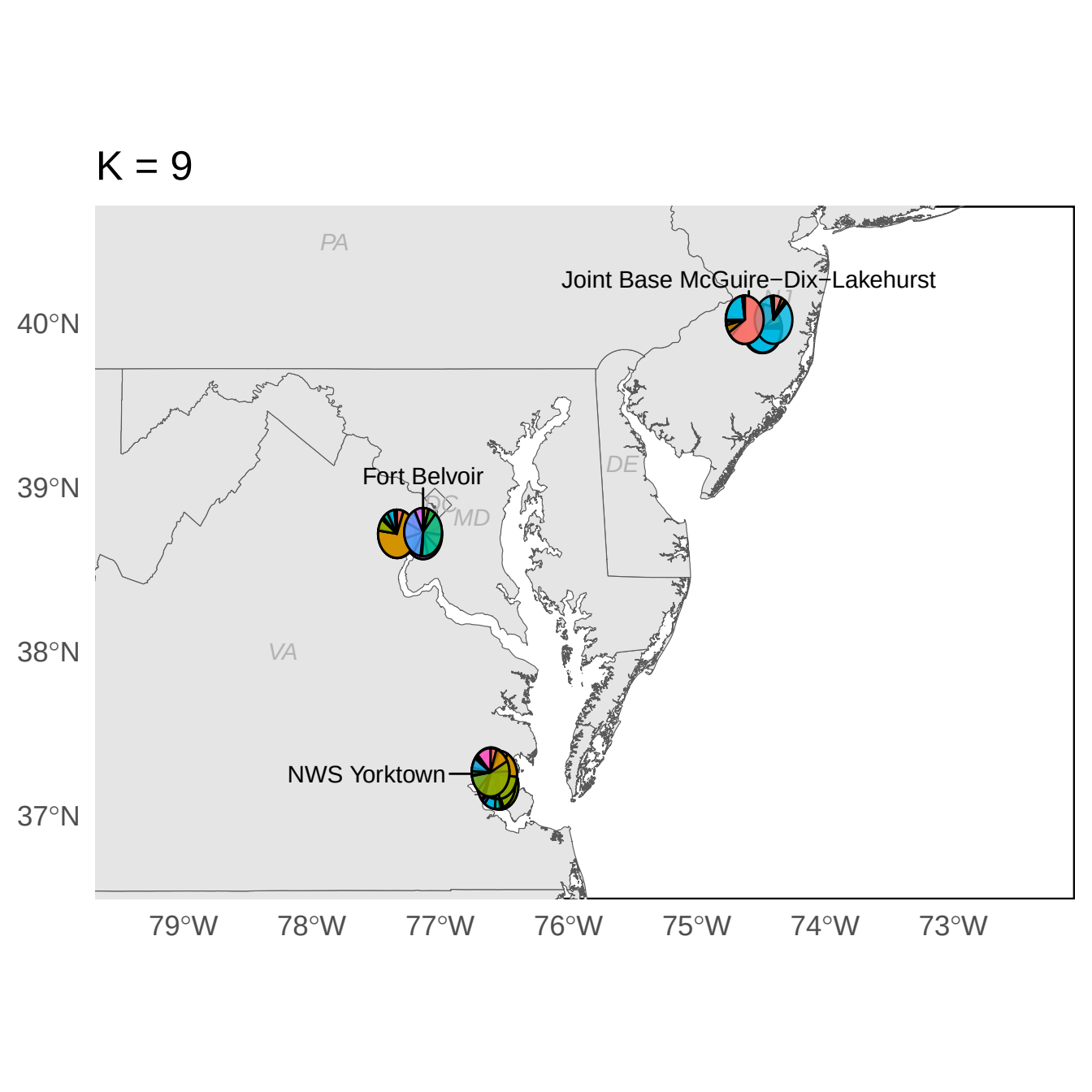

K = 4
PA
Joint Base McGuire−Dix−Lakehurst
NJ
40°N
DE
Fort Belvoir
DC
MD
39°N
VA
38°N
NWS Yorktown
37°N
79°W
78°W
77°W
76°W
75°W
74°W
73°W

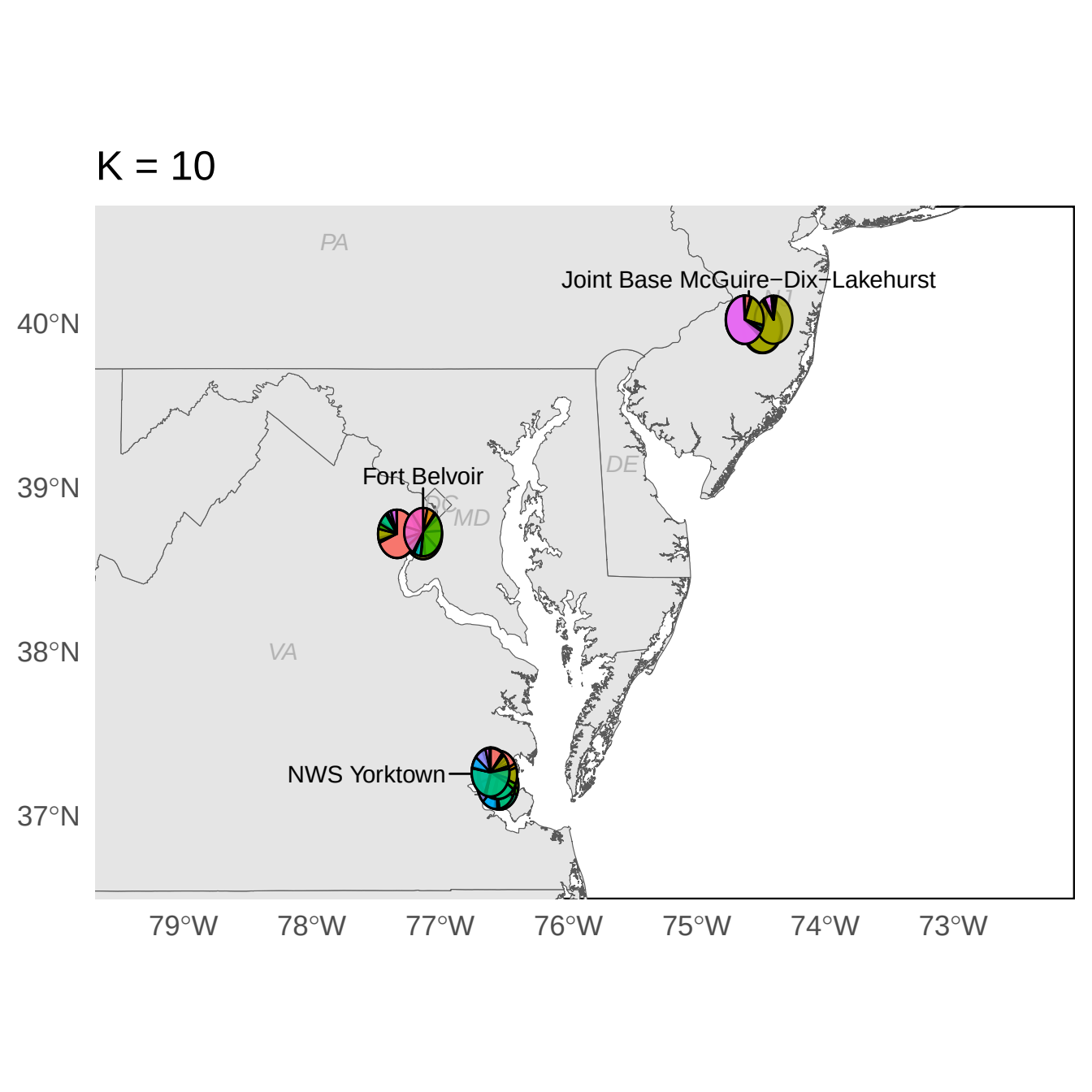

K = 5
PA
Joint Base McGuire−Dix−Lakehurst
NJ
40°N
DE
Fort Belvoir
DC
MD
39°N
VA
38°N
NWS Yorktown
37°N
79°W
78°W
77°W
76°W
75°W
74°W
73°W


K = 6
PA
Joint Base McGuire−Dix−Lakehurst
NJ
40°N
DE
Fort Belvoir
DC
MD
39°N
VA
38°N
NWS Yorktown
37°N
79°W
78°W
77°W
76°W
75°W
74°W
73°W


K = 7
PA
NJ
DE
MD
VA
Joint Base McGuire−Dix−Lakehurst
Fort Belvoir
NWS Yorktown
40°N
39°N
38°N
37°N
79°W
78°W
77°W
76°W
75°W
74°W
73°W


K = 8
PA
Joint Base McGuire−Dix−Lakehurst
NJ
40°N
DE
Fort Belvoir
DC
MD
39°N
VA
38°N
NWS Yorktown
37°N
79°W
78°W
77°W
76°W
75°W
74°W
73°W


K = 9
PA
Joint Base McGuire−Dix−Lakehurst
NJ
40°N
DE
Fort Belvoir
MD
39°N
VA
38°N
NWS Yorktown
37°N
79°W
78°W
77°W
76°W
75°W
74°W
73°W


K = 10
PA
Joint Base McGuire−Dix−Lakehurst
NJ
40°N
DE
Fort Belvoir
MD
39°N
VA
38°N
NWS Yorktown
37°N
79°W
78°W
77°W
76°W
75°W
74°W
73°W

TANGLED BANK
CONSERVATION™

Blood and Tissue Sampling Protocols

Table of Contents

Turtle Blood Draw

Preferred DNA Collection Method for Turtles

Protocol Usage Chart

	Protocol to Use Based on Size/Weight		
	Protocol A	*Protocol B*	*Protocol C*
Small Turtles (< 150 g)	*X*	*X (maybe)*	
Medium Turtles (> 150 g)		*X*	
Large Turtles (> 4 kg)			*X*

Notes before starting:

- Based on our research and experience, we find these blood draws to be the least risky to the turtle, most efficient in the field, and yield the highest quality DNA. If you are permitted in doing so, you may use another method.
- All blood draws here can be done with a 1” 25 gauge needle. The toenail clip can be done with a pair of dissecting scissors or a large nail clipper.
- As a general rule, do not draw more than 4mL/kg of blood from any animal. That equates to 0.4mL from a 100g turtle. Do not draw blood from turtles that are under 100 grams.
- For all turtles, we recommend drawing 0.1 mL (100 µL) and no more than 0.5 mL of blood, being mindful to take less blood from smaller animals. If you draw lymph (clear/yellow fluid) along with blood, discard the sample. Lymph is a contaminant for DNA analyses. You can try again with a new needle, or take a tissue sample instead.
- Do not attempt more than 3 needle sticks per turtle. If you are not successful after 3 sticks, try a tissue sample.
- Use a new pair of gloves for each turtle that is handled.
- Each FTA card and ziploc bag will be labeled with a numeric TBC lab ID. Please also label each FTA card (and ziploc) with a unique sample ID of your choosing.
- The FTA cards and paper barriers are cut down from larger FTA cards, so there may be printed text on some and not others- please disregard this text. Each card and ziploc will have a TBC ID printed on a label sticker, which serves as the unique identifier for all FTA cards. Please use this ID for the sample intake form.
- Be sure to record the precise location for each sampled individual and enter latitude and longitude coordinates into the sample intake form.
- Store used needles and syringes in a hard-sided, sealable, sharps container (i.e an empty laundry detergent bottle). *Please dispose of all sharps containers in accordance with your local safe disposal guidelines.*

Turtle Blood Draw - Protocol A

Toenail Clip and FTA Swab

Materials:

1 small ziploc containing an FTA card, folded paper barrier, and paper clip

- If purchasing separately, FTA cards can be cut into four corresponding to the circles on the card

1 tube with 75 or 95% ethanol

1 pair of dissecting scissors or nail clippers

1 alcohol wipe

Vial containing styptic powder, or styptic powder stick

Equipment for sterilizing dissection tools (hydrogen peroxide, lighter, etc.)

Pair of nitrile/latex gloves

Instructions:

1. Using a gloved finger, or a gloved finger and gauze, extend one of the turtle's hind limbs and, using two fingers, gently massage the foot in a circular motion for about 1-2 minutes. This helps encourage blood flow.
2. Hold the same hind limb extended in order to give clear access to a toe. Wipe the chosen nail/toe area with the alcohol wipe and allow the alcohol to evaporate.
3. Position the turtle's foot so that the target toenail is above the ethanol tube. Clip the toenail down to the quick, being sure not to cut any skin, and allow the toenail to fall into the ethanol tube. You want the nail/toe to produce a few drops of blood. If need be, gently massage the foot to help squeeze a bit of blood out. *If you are not getting any blood to come out after about 30-60 seconds of massaging, either try to clip the nail down further (ONLY if you can do this without cutting skin) or try with another toenail. If you do clip the nail a second time or clip a second toenail, make sure to place the toenail in the ethanol tube, as well.*
4. Dab the drops of blood onto the center FTA card. Avoid pooling the blood. Instead, dab in a polka dot pattern.
5. If the nail/toe continues to bleed, apply styptic powder.
6. Allow blood to dry, then fold the paper barrier over the FTA card. Paperclip the paper and FTA card together so the FTA card doesn't fall out.
7. Place the paper clipped packet into the ziploc and seal it shut.
8. Clean any scissors or nail clippers with hydrogen peroxide, or flame, before continuing to the next turtle.

The FTA card should be stored in its ziploc bag at room temperature in a low-humidity environment. The ethanol vial can be frozen. Samples can be stored in these conditions and shipped back on ice in one batch at the end of the season.

Turtle Blood Draw - Protocol B

Subcarapacial Sinus Blood Draw

Materials:

1 small ziploc containing an FTA card, folded paper barrier, and paper clip

- If purchasing separately, FTA cards can be cut into four corresponding to the circles on the card

1 25 gauge 1 inch needle with attached syringe

1 alcohol wipe

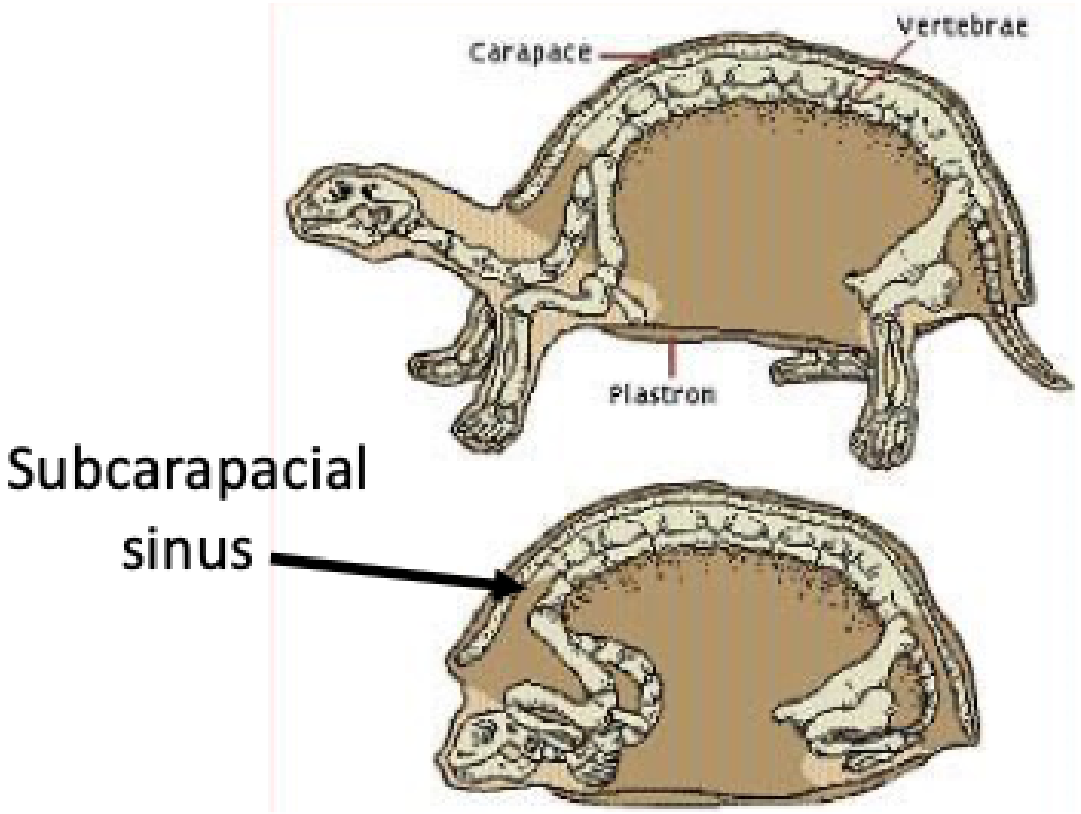
Styptic powder

Pair of nitrile/latex gloves

Instructions:

1. Using a gloved finger, or a gloved finger and gauze, push the turtle's head back in its shell and hold gently but firmly. Be sure to leave the area above its head free from gauze and fingers.
2. Insert the needle above the turtle's head, aiming upwards along the midline to between the first and second vertebral scute.
3. Once the needle is below the skin, raise the plunger slightly to create and maintain negative pressure as the needle moves in. Gently move the needle posteriorly and dorsally until blood flows into the syringe. Stop moving once blood starts flowing into the syringe. Slowly retract the needle once you have drawn the recommended amount.
4. If you draw lymph (yellow-ish or clear liquid), remove the needle, change syringes and retry.
5. If you hit bone or anything hard, retract the needle anteriorly and redirect.
6. If the turtle bleeds once the needle is removed, the person holding the turtle's head should apply pressure to the draw site with gauze or a gloved hand for 30-60 seconds. If the blood does not clot, apply styptic powder.
7. The person with the syringe should dispense the blood in a polka dot pattern on the FTA card, allowing the blood to absorb with minimal pooling. *Note: Dispense the blood as soon as you are able, or it may clot within the syringe and make it difficult to dispense through the needle.*
8. Allow blood to dry, then fold the paper barrier over the FTA card and paperclip the paper and FTA card together so the FTA card doesn't fall out.
9. Place the paper clipped packet into the ziploc and seal it shut.
10. Dispose of the needle and syringe in a hard-sided, sealable, sharps container.

The FTA card should be stored in its ziplock bag at room temperature in a low-humidity environment. Samples can be stored in these conditions and shipped back in one batch at the end of the season.



Subcarapacial sinus location (ventral to the carapace)

Turtle Blood Draw - Protocol C

Dorsal coccygeal blood draw

Materials:

1 small ziploc containing an FTA card, folded paper barrier, and paper clip

- If purchasing separately, FTA cards can be cut into four corresponding to the circles on the card

1 25 gauge 1 inch needle with attached syringe

1 alcohol wipe

Vial containing styptic powder

Pair of nitrile/latex gloves

---

1. This protocol will require two people: one to restrain the turtle, and the second to draw the blood.
2. Hang the turtle's tail off of the edge of the surface where the turtle is being held. Expose the dorsal surface of the tail by either bending the tail or letting it fall.
3. Find scales about midway down the ventral surface of the tail. This is usually between the second and third bony plate of an Alligator Snapping Turtle. Swab the areal with the alcohol swab.
4. Insert the needle at a low angle (0-45 degrees) relative to the tail, in the fleshy area between the two scales. Once the needle is below the skin, reangle the syringe to a higher angle (45-90 degrees).
5. Raise the plunger slightly to create negative pressure. Move the needle ventrally until you feel it pierce the vein. This will often be right below the skin. Stop when blood starts to fill the vacuum in the syringe, or readjust to find the vein. If you fail to find the vein, reorient, retract, and attempt entry again.
6. Once you have the desired volume, remove the needle from the turtle.
7. If the turtle bleeds from the injection site, apply pressure to the draw site with gauze or a gloved hand for 30-60 seconds. If the blood does not clot, apply styptic powder.
8. The person with the syringe should dispense the blood in a polka dot pattern on the FTA card, allowing the blood to absorb with minimal pooling. *Note: Dispense the blood as soon as you are able, or it may clot within the syringe and make it difficult to dispense through the needle.*
9. Allow blood to dry, then fold the paper barrier over the FTA card and paperclip the paper and FTA card together so the FTA card doesn't fall out.
10. Place the paper clipped packet into the ziploc and seal it shut.
11. Dispose of the needle and syringe in a hard-sided, sealable, sharps container.

The FTA card should be stored in its ziplock bag at room temperature in a

low-humidity environment. Samples can be stored in these conditions and shipped back in one batch at the end of the season.

Turtle Cloacal Swab

Materials:

1 small ziploc containing an FTA card, folded paper barrier, and paper clip

- If purchasing separately, FTA cards can be cut into four corresponding to the circles on the card

Cotton or Mawi swab (example cotton swab, but see note below)

Pen

Sharpie

Pair of nitrile/latex gloves

Notes:

The size and material of provided swabs will depend on the species of turtle being sampled. Smaller species (ex. bog turtle, spotted turtle) require narrower swabs than larger species (ex. box turtle). If you have requested sampling supplies for multiple species, please be sure to use only the supplies from the sampling kit designated for the species you are sampling.

Instructions:

1. Put on latex gloves. A new pair of gloves should be used for each turtle that is handled.
2. Remove FTA card packet from the ziploc, then remove paper clip and folded paper barrier. The FTA card and ziploc will already be labeled with a TBC lab ID, but please add an additional label on both the FTA card and ziploc with a unique sample ID of your choosing.
    a. Note: *Sample ID can be any unique identifier (For example, "[Turtle notch ID], [Site Code]" or "[Your initials][Site name][sample#]"). The most important thing is that the sample ID is the same on the FTA card and the intake form, and that all information is included on the intake form.*
3. Collect GPS coordinates for each sample. This does not need to be labeled on the FTA cards or ziploc, but ***will need to be included on the sample intake form***.
4. Use one swab to collect the first cloacal sample. Gently insert the cotton tip of the swab into the turtle's cloaca and rotate the swab 1-3 times.
5. Firmly rub and twist the swab onto one half of the circle on the FTA card.
6. Discard the first swab, then repeat step 1 using the second swab.
7. Firmly rub and twist the swab onto the second half of the circle on the FTA card.
8. Fold the paper barrier over the FTA card, then paper clip them together so the FTA card doesn't fall out.
9. Place the paper clipped packet into the ziploc and seal it shut.

The FTA card should be stored in its ziplock bag at room temperature in a low-humidity environment. The swab can be stored in its vial, in the bag with the FTA card. Samples can be stored in these conditions and shipped back in one batch at the end of the season.

Snake Blood Sample

Preferred DNA Collection Method for Snakes

Materials:

1 small ziploc containing an FTA card, folded paper barrier, and paper clip

- If purchasing separately, FTA cards can be cut into four corresponding to the circles on the card

1 25 gauge 1 inch needle with attached syringe

1 alcohol wipe

Vial containing styptic powder

Pair of nitrile/latex gloves

Notes:

- *As a general rule, do not draw more than 4mL/kg of blood from any animal. That equates to 0.4mL from a 100g snake.*

Instructions:

1. Put on nitrile gloves. New gloves should be used for each snake handled.
2. Restrain the snake so that the body is supported and the person drawing blood has easy access to the ventral part of the tail. Generally it is best to have one person supporting the snake, and a second person drawing blood. If the snake is small enough and it can be done safely, position the head above the tail so that blood will "pool" in the tail.
3. Remove FTA card packet from the ziploc, then remove paper clip and folded paper barrier. The FTA card and ziploc will already be labeled with a TBC lab ID, but please add an additional label on both the FTA card and ziploc with a unique sample ID of your choosing.
    a. Note: *Sample ID can be any unique identifier (For example, "[Ventral Scale ID], [Site Code]" or "[Your initials][Site name][sample#]"). The most important thing is that the sample ID is the same on the FTA card and the intake form, and that all information is included on the intake form.*
4. Collect GPS coordinates for each sample. This does not need to be labeled on the FTA cards or ziploc, but ***will need to be included on the sample intake form***.
5. If the needle and syringe are separately packaged, attach the needle to the syringe by screwing in the base of the needle into the top of the syringe.
6. Sanitize the ventral side of the tail of the snake approximately halfway between the cloaca and the tip of the tail with the alcohol swab. This will be the blood draw site. We are aiming for the ventral coccygeal vein. Keep in mind that male snakes have

hemipenes and musk glands distal to their cloaca that we do not want to puncture. The person holding the snake should put pressure on the cloaca to avoid musking/bowel movements during the procedure.

7. Insert the needle at a low angle (0-45 degrees) between two ventral scales.
8. Once the needle is below the skin, reangle the syringe to a higher angle (45-90 degrees). Raise the plunger slightly to create negative pressure. Move the needle down (dorsally) until you feel vertebrae. Back the needle out slowly until blood fills the vacuum in the syringe. Stop there until you've drawn up at least 0.1 mL (100 µL) but no more than 0.5 mL of blood from the vein, being mindful to take less blood from smaller animals. If you draw anything except red blood (e.g. clear/yellow lymph), discard the sample and try another location 1-2 ventral scales towards the cloaca.
9. Dispense the blood in the middle of an FTA card.
10. If the snake is bleeding at the injection site, sprinkle the wound area with styptic powder.
11. Fold the paper barrier over the FTA card, then paperclip the paper and FTA card together so the FTA card doesn't fall out.
12. Place the paper clipped packet into the ziploc and seal it shut.
13. Dispose of the needle and syringe in a hard-sided, sealable, sharps container. A new needle and syringe should be used for each snake. *Note: Please dispose of all sharps containers in accordance with your local safe disposal guidelines.*

The FTA card should be stored in its ziplock bag at room temperature in a low-humidity environment. Samples can be stored in these conditions and shipped back in one batch at the end of the season.

Tissue Samples

E.g. amphibian eggs, frog toes, snake ventral scales, tissue from a Dead on Road (DOR) sample

Materials:

1 tube with 75% or 95% ethanol
Dissecting scissors
Dissecting tweezers
Equipment for sterilizing dissection tools (hydrogen peroxide, lighter, etc.)
Pair of nitrile/latex gloves

Note:

Tissue samples from DORs should only be taken from very freshly killed animals. If there are signs of putrefaction – e.g. discolored organs, rotten smells, etc. – or if the DOR is highly desiccated, the DNA will likely already be too degraded to use.

Instructions:

1. Put on latex gloves. A new pair of gloves should be used for each animal/specimen that is handled.
2. Position the tube so it stands upright. The tube may already be labeled with a TBC lab ID. Regardless of whether it is already labeled with a TBC lab ID or not, please add an additional label to the tube with a unique sample ID of your choosing.
    a. Note: *Sample ID can be any unique identifier (For example, "[Site Code]" or "[Your initials][Site name][sample#]"). The most important thing is that the sample ID is the same on the tube and the intake form, and that all information is included on the intake form.*
3. Collect GPS coordinates for each sample. This does not need to be labeled on the tube, but ***will need to be included on the sample intake form***.
4. Unscrew the cap of the tube. Add approximately 50 mg of tissue to the ethanol vial. This should be the size of 3 grains of rice, and should have a volume no greater than 50% the volume of ethanol in the tube. In other words, there should be at least as much ethanol as tissue in the tube.
    a. For DORs, use your dissecting scissors to locate and sample (in descending order of preference) the brain, the liver or muscle from the DOR.
    b. For amphibian eggs, add as many eggs as possible to meet the volume requirement. Pierce the gel capsule of each egg. Take the most developed eggs possible. (Unfertilized eggs only have half the total genome and are not helpful.)
    c. For salamanders, use a blunt object (like the dull side of the scissor blade) to fracture the tip of the salamander's tail.
    d. For turtles, cut a piece of nail with a small piece of cuticle. If the cuticle bleeds while taking the sample, blot the blood on an FTA card following the Turtle Blood Draw instructions. Taking the cuticle with the nail gives greater DNA yield than just the keratinized nail. You may also take a small piece of clean foot webbing

or a tip of the tail. Ensure that you cut the webbing or tail so that ethanol can penetrate the entire tissue.

e. For frogs, use the scissors to cut the tip of a toe.
f. For snakes, use the scissors to cut pieces of the ventral scales. Getting skin with the scale is OK.

5. **Close the tube and store at room temperature if shipping immediately. If shipping more than one week after collection, freeze the samples. See shipping instructions (provided after intake form is completed) for more information.**
6. Clean any tools used with hydrogen peroxide, or flame, before continuing.

Sample Submission Instructions

When you are ready to submit your samples, email info@tbconservation.org for more information on shipping and submitting your samples' metadata.