ANGSD-wrapper: utilities for analyzing next generation sequencing data

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Abstract

High throughput sequencing has changed many aspects of population genetics, molecular ecology, and related fields, affecting both experimental design and data analysis. The software package ANGSD allows users to perform a number of population genetic analyses on high-throughput sequencing data. The package is specifically designed to produce more accurate results for samples with low sequencing depth and makes use of full genome data while handling a wide array of sampling and experimental designs. Here we present ANGSD-wrapper, a user-friendly interface for running ANGSD and visualizing results. ANGSD-wrapper includes a number of 'wrapper' scripts that facilitate configuration and execution of multi-step analyses and provides interactive graphing of ANGSD results to enhance data exploration. We demonstrate the usefulness of ANGSD-wrapper by analyzing resequencing data from populations of wild and domesticated *Zea*. ANGSD-wrapper is freely available from https://github.com/mojaveazure/angsd-wrapper.

Introduction

High throughput sequencing has revolutionized evolutionary genetics, allowing researchers to quickly assay large numbers of individuals or survey fine-scale patterns of variation along the genome. Application of these methods has led to changes in both experimental design and data analysis [1]. Many of the popular software packages used by researchers [see 2] were not designed to handle these novel data types or efficiently analyze the large volumes of data now being generated. In particular, short read sequencing has brought new challenges, including highly variable coverage, missing data, and high per-nucleotide error rates.

A number of tools have recently been published to handle high throughput sequencing data [3, 4, 5, 6], but the majority of these either make limiting assumptions about the data (e.g., all sites have been sequenced, all genomes are haploid, sequencing is to sufficient depth, all individuals are outcrossing) or are specialized tools offering a narrow set of analysis options. Korneliussen et al. [7] recently published the software package ANGSD, which enables users to flexibly perform a large number of common population genetic analyses, including diversity statistics, Patterson's D-statistic test of admixture [8], site frequency spectrum estimation [9], and neutrality test statistics [10]. One of the most important features of ANGSD is that most analyses are performed directly on genotype likelihoods, freeing users from the requirement of calling variants or genotypes and permitting analysis of low-coverage data or sequences with large amounts of missing data.

Here we present ANGSD-wrapper, a user-friendly interface to ANGSD. ANGSD-wrapper takes the form of a set of configuration files and 'wrapper' scripts (Figure S1) that streamline the execution of

Methods Implemented	Interactive Graphing
ABBA BABA	Yes
Admixture	Yes
Ancestral Sequence	N/A
Genotype Likelihoods	N/A
Inbreeding Coefficients	N/A
PCA	Yes
Site Frequency Spectrum	Yes
Theta Calculation	Yes

Table 1: Table of methods implemented in ANGSD-wrapper

multi-step pipelines inherent in ANGSD as well as pipelines involving related programs such as ngsPopGen, ngsF[11], and ngsAdmix [12]. Because the large volume of data associated with high throughput sequence analysis is often difficult to explore by hand, ANGSD-wrapper also provides a suite of interactive visualization tools to plot results and explore patterns at multiple scales. We demonstrate some of the analyses possible using ANGSD-wrapper using low-coverage whole-genome data from domesticated maize and two related wild teosinte subspecies. ANGSD-wrapper is freely available from https://github.com/mojaveazure/angsd-wrapper.

Methods

ANGSD-wrapper is a set of configuration files and scripts written in the Bash UNIX shell. The scripts can be run either on a standalone computer with a UNIX terminal, or on computing clusters where they can be submitted to a queuing system such as SGE [13], Slurm [14] or TORQUE [15]. An installation of the statistical software R [16] is required to make use of the visualization tools incorporated in ANGSD-wrapper. The visualization portion of ANGSD-wrapper also requires installation of the R packages shiny [17], genomeIntervals [18], and ape [19].

ANGSD-wrapper is divided into scripts associated with analytical approaches implemented in ANGSD and associated software. ANGSD-wrapper provides a common configuration file, common.conf, which holds variables that are likely to remain constant across analyses, including identifiers for chromosomal regions and the paths to project directories. In ANGSD-wrapper, each method is self-contained in a shell script which uses information from the common configuration file and a method-specific configuration file. Each analysis is run using a simple command:

```
$ angsd-wrapper <method> <configuration_file>
```

Analyses supported by ANGSD-wrapper are shown in table 1, and a detailed flowchart of each of these workflows is shown in Figure S1, and additional details, documentation, a tutorial, and a wiki can be found on the GitHub page: https://github.com/mojaveazure/angsd-wrapper/wiki.

The visualization software included with ANGSD-wrapper is contained within it's own directory called shinyGraphing. This application must be started in R and can be accessed locally from a web browser. This software provides a graphical user interface (GUI) to quickly and interactively plot results obtained from ANGSD-wrapper. Each tab in the GUI contains plots for different ANGSD methods.

In order to use the plotting software, the user navigates to the desired tab and uploads the appropriate file of results. The Shiny server automatically parses ANGSD output files and creates the resulting plot(s) (Figure 1), which can be saved using the browser's built in image saving capabilities.

Results

As a demonstration of analyses in ANGSD-wrapper, we explore patterns of diversity in maize and teosinte. We used resequenced samples from the HapMap2 project [20] and calculated summary statistics using a 10 megabase region on chromosome 10.

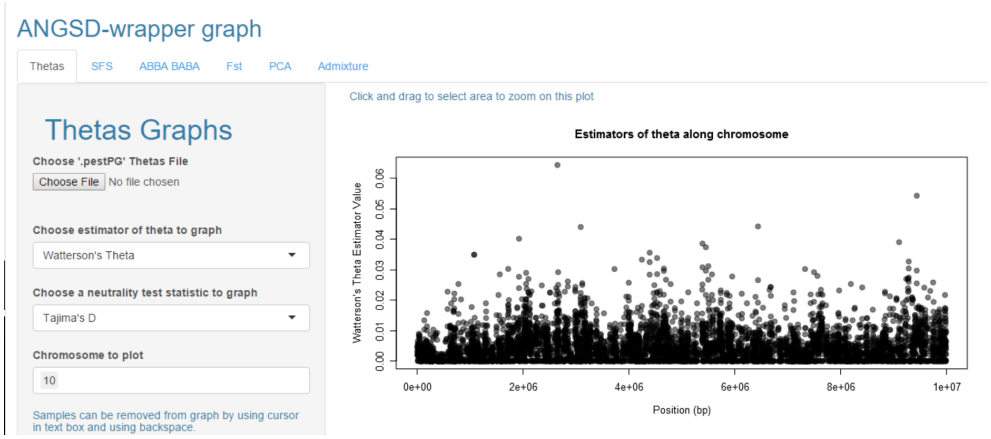

Figure 1: A visualization of Watterson's θ estimated by ANGSD across the first 10 megabases of chromosome 10 in *Zea mays* spp. *mays* using ANGSD-wrapper

Discussion

Conclusions

Our software ANGSD-wrapper provides an intuitive and easy-to-use interface to employ the powerful and flexible suite of population genetic analyses developed in ANGSD [7] and permits the exploration of genome-scale results through interactive visualization. ANGSD-wrapper is under active development to incorporate updates to the ANGSD software package.

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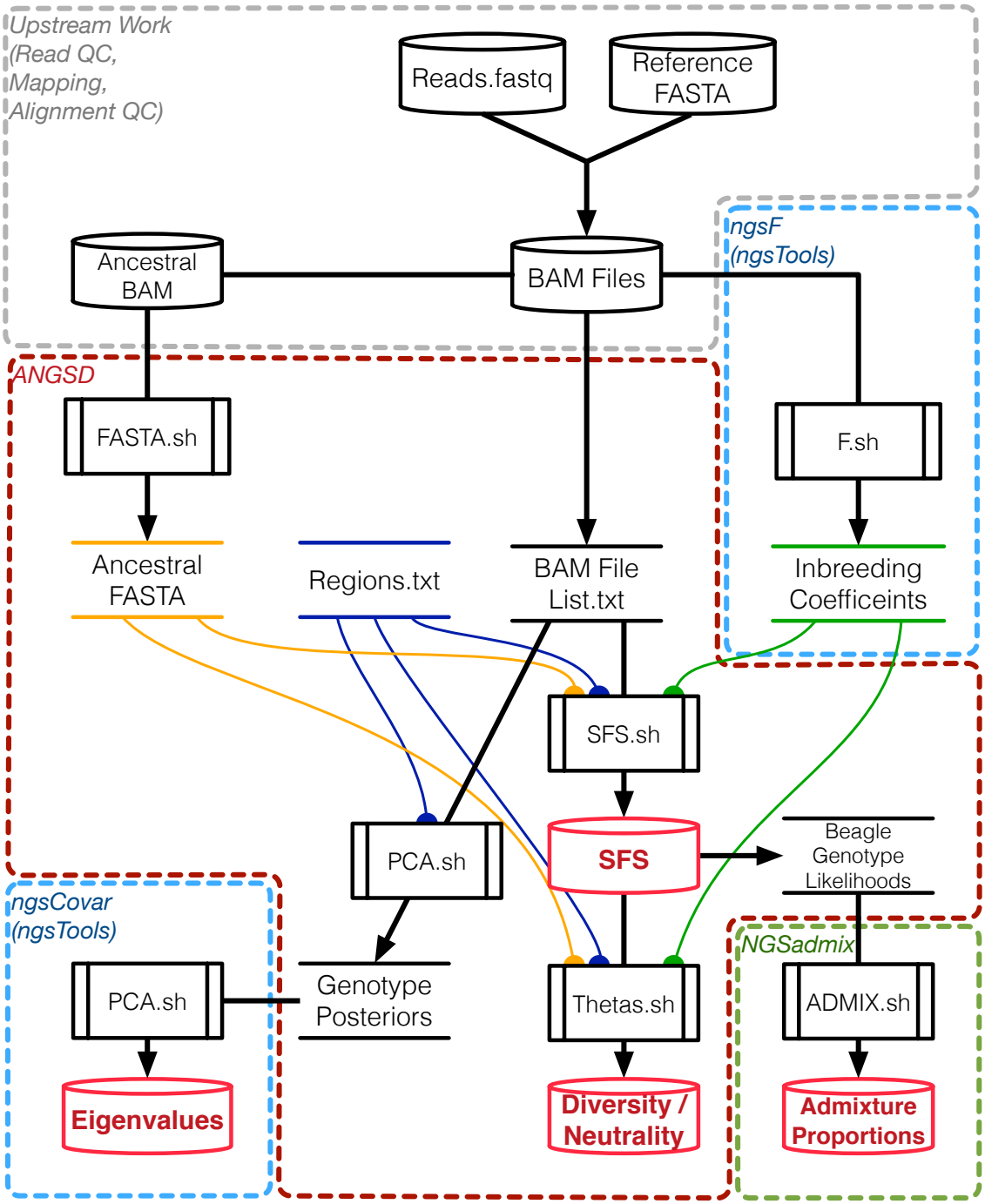

Figure 2: Example analysis workflow diagram.

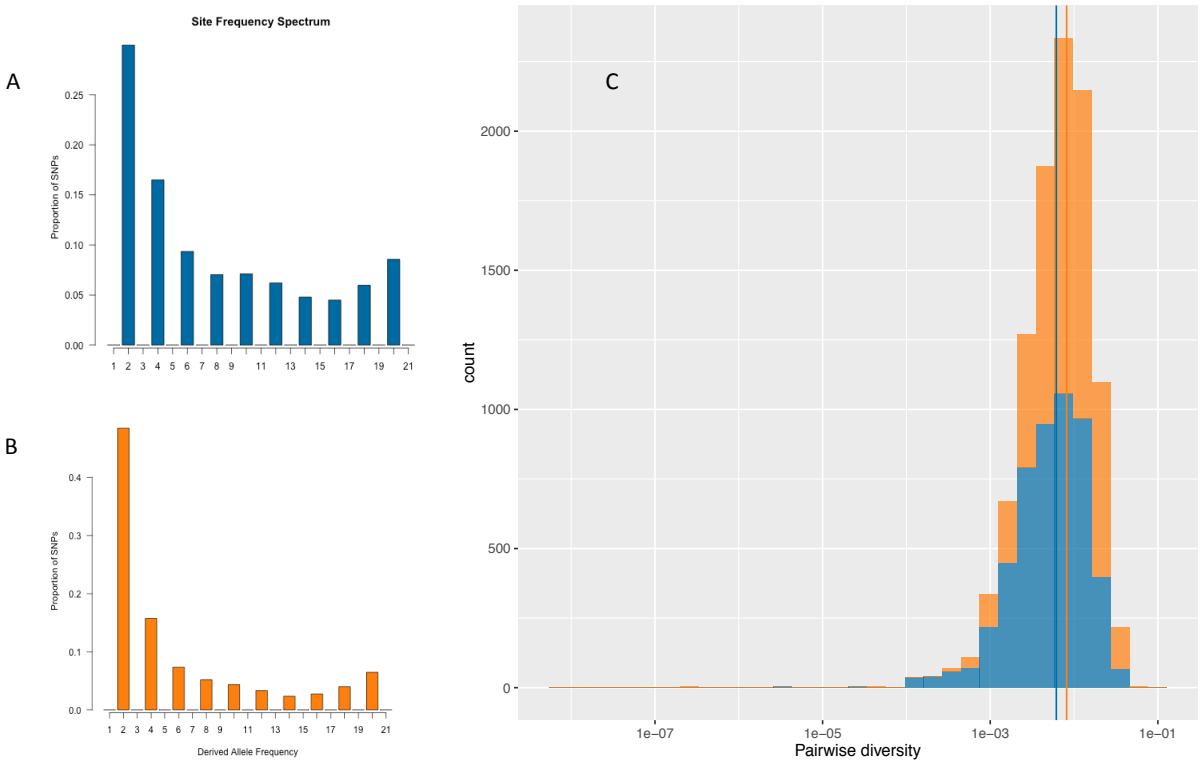

Figure 3: Summary statistics for *Zea mays*. Site frequency spectra for A. maize and B. teosinte. C. distribution of pairwise differences for maize (blue) and teosinte (orange).

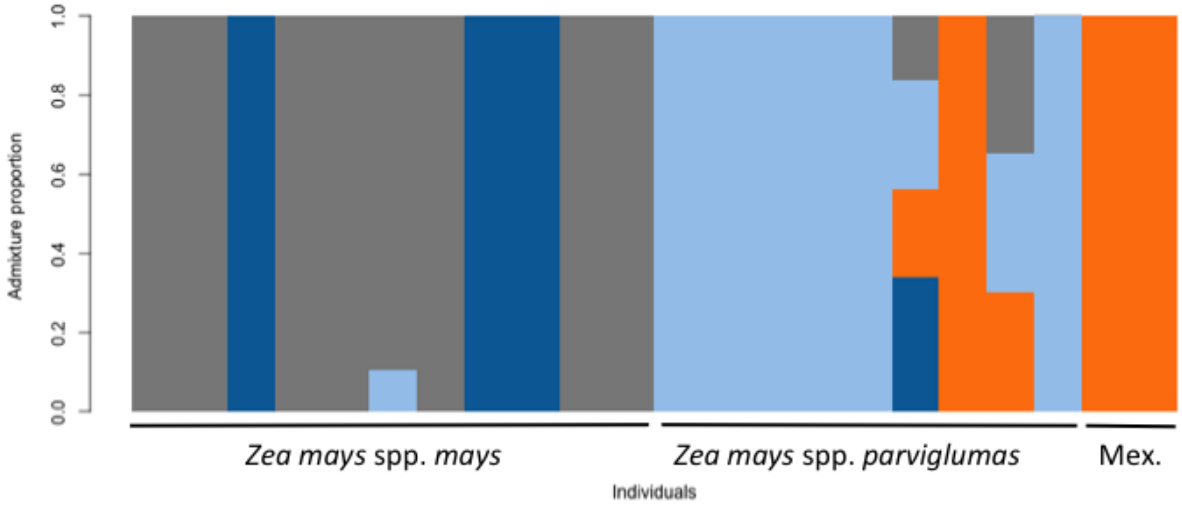

Figure 4: Admixture analysis for *Zea mays* spp. *mays*, *Zea mays* spp. *parviglumas*, and *Zea mays* spp. mexicana (Mex.) with K=4 source populations.

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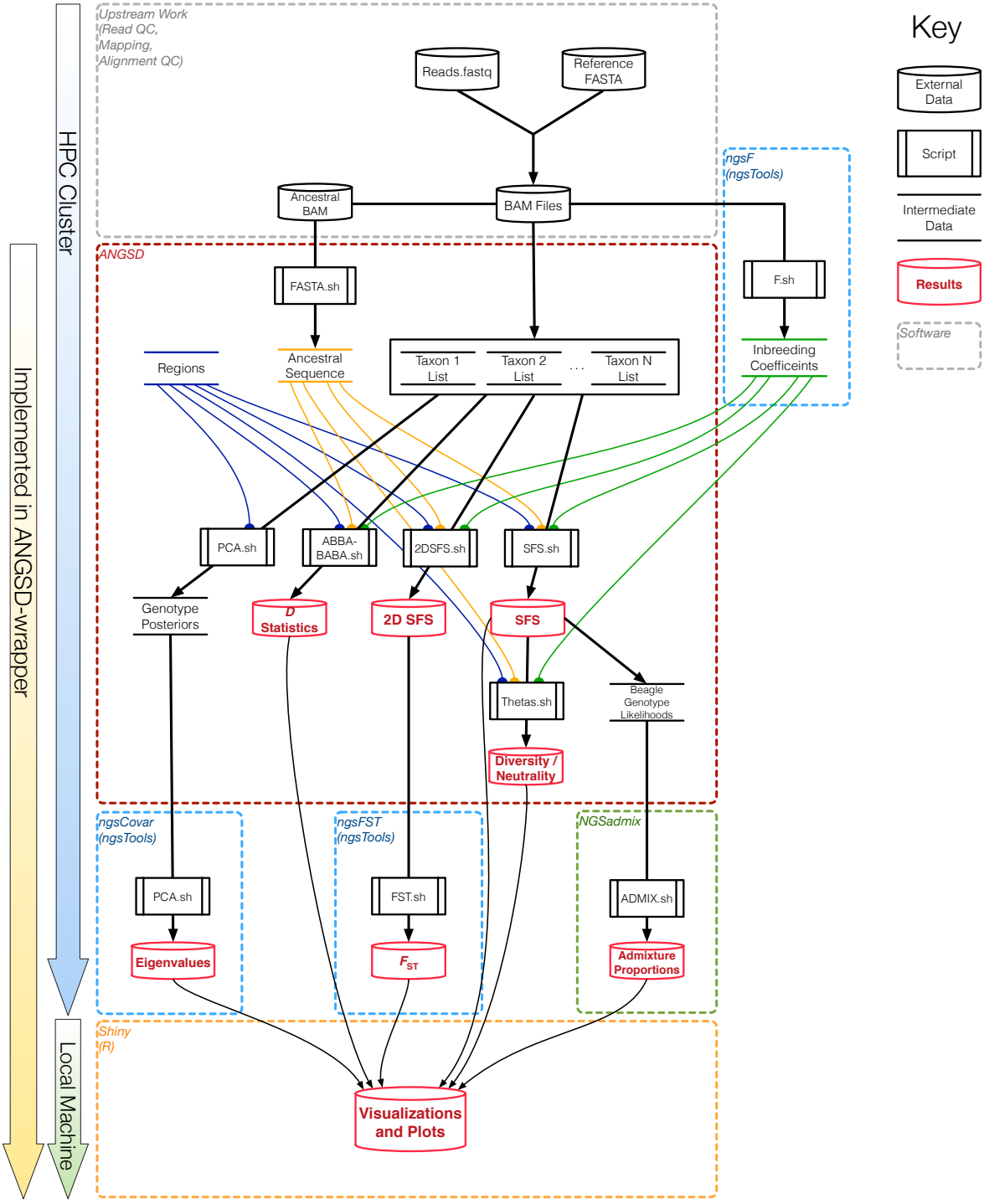

Figure S1: Workflow diagram for all methods available in ANGSD-wrapper.