

# ANGSD-wrapper: utilities for analyzing next generation sequencing data

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Running head: ANGSD-wrapper

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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. ANGSD uses probabilistic approaches which can directly make use of genotype likelihoods, and SNP calling is thus not required for comparative analyses. This takes advantage of all of the sequencing data and produces more accurate results for samples with low sequencing depth. Here we present ANGSD-wrapper, a set of wrapper scripts that provide a user-friendly interface for running ANGSD and visualizing results. ANGSD-wrapper supports multiple types of analyses including estimates of nucleotide sequence diversity and performs neutrality tests, principal component analysis, estimation of admixture proportions for individuals samples, and calculation of statistics that quantify recent introgression. ANGSD-wrapper also 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 and examine genome-wide variation. Application of these approaches has led to changes in both experimental design and data analysis (Ekblom and Galindo, 2011). Many popular software packages used by researchers for analysis of comparative resequencing data (see Excoffier and Heckel, 2006) were not designed to handle these novel data types or efficiently analyze the large volumes of data now being generated. Despite the decreasing cost of sequencing, researchers must nonetheless allocate finite resources and balance the depth of sequencing and the breadth of a sample. This poses a challenge for population genetics analysis, which generally requires accurate polymorphism calls in a broad sample (Felsenstein, 2006; Pluzhnikov and Donnelly, 1996). While these experimental design challenges in molecular population genetic studies have existed for at least two decades, high throughput sequencing brings the added technical challenges of highly variable coverage, missing data, and high per-nucleotide error rates (Johnson and Slatkin, 2007; Lynch, 2008).

A number of tools have recently been published to estimate population genetic descriptive statistics using high throughput sequencing data (Danecek et al., 2011; Garrigan, 2013; Hutter et al., 2006; Purcell et al., 2007). The software package ANGSD Korneliussen et al. (2014), is noteworthy because it enables users to perform a large number of common population genetic analyses, including estimation of diversity statistics, admixture analysis including Patterson’s D statistic (Durand et al., 2011), site frequency spectrum estimation (Nielsen et al., 2012), and calculation of neutrality test statistics (Ko-

rneelissen et al., 2013). ANGSD works directly with alignment formats produced from standard high throughput sequence analysis pipelines, which removes the need for the user to transform the data into a software-specific format. One of the most important features of ANGSD is that analyses can be integrated over all sites, rather than on predetermined polymorphic sites *a priori*. This permits ANGSD to calculate common population genetic descriptive statistics on low-coverage sequencing data, and handle missingness due to variation in coverage. Further, this probabilistic framework is appealing to researchers studying non-model organisms where the lack of existing genomic resources or cost considerations prevent the ability to obtain high quality data. Also, many existing tools do not implement statistical models that account for biological issues such as non-random mating. Recent use of ANGSD highlights these benefits, allowing population genetic analyses in non-model organisms such as the blue-eyed black lemur (Meyer et al., 2015) and *Ficedula* flycatchers (Burri et al., 2015) and inbred lines of wild and cultivated maize (Beissinger et al., 2016).

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 multi-step pipelines required for data analysis in ANGSD. ANGSD-wrapper also assists with configuration of ANGSD-related programs such as ngsTools (Fumagalli et al., 2014), ngsF (Vieira et al., 2013), ngsAdmix (Skotte et al., 2013), and PCA (Fumagalli et al., 2013). As in ANGSD, ANGSD-wrapper allows users to perform whole genome analysis or analyze a set of user-defined windows across the genome. The wrapper scripts are written against a frozen versions of ANGSD (v0.902-48-g8b89ba4) and supporting tools for consistency of analysis. Because the large volume of data associated with high throughput sequence analysis is often difficult to visualize, 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 when applied to low-coverage resequencing using 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 primarily in the Bash scripting language. 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 (Gentzsch, 2001), Slurm (Jette et al., 2002) or TORQUE (Staples, 2006). A Python installation (van Rossum, 2016) is required for some light, dynamic pre-processing of the data, and the statistical software R (R Core Team, 2014) 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** (Chang et al., 2015), **APE** (Paradis

Table 1: Table of methods implemented in ANGSD-wrapper

Method	Calculations	Interactive Graphing
SFS	Site frequency spectrum	Yes
2DSFS	Joint site frequency spectrum, $F_{ST}$	Yes
ABBA/BABA	Patterson’s D statistic	Yes
Ancestral	Extract ancestral sequence from BAM file	No
Genotypes	Genotype likelihood estimations	No
PCA	Principal component analysis	Yes
Thetas	Diversity statistics ( $\theta_w$ , $\theta_\pi$ , Fu and Li’s $\theta$ , Fay’s $\theta$ ) and neutrality tests (Tajima’s D, Fu and Li’s D, Fu and Li’s F, Fay and Wu’s H, Zeng’s E )	Yes
Inbreeding	Calculate per-individual inbreeding coefficients with ngsF	No
Admixture	Perform admixture analysis	Yes

et al., 2004), **Lattice** (Sarkar, 2008), **Hmisc** (Jr et al., 2015), **data.table** (Dowle et al., 2015), **DT** (Xie, 2015), and **shinythemes** (Chang, 2015), as well as **genomeIntervals** (Gagneur et al., 2015) from Bioconductor (Huber et al., 2015); these are installed automatically upon first run of the visualization interface.

ANGSD-wrapper is divided into scripts associated with analytical approaches implemented in ANGSD and associated software. It provides a common configuration file, **Common.Config**, 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:

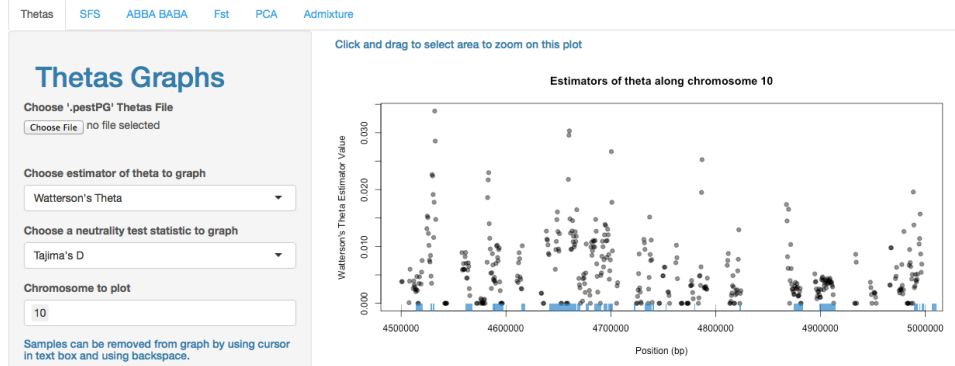
```
$ angsd-wrapper <method> <configuration_file>
```

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

The visualization software included with ANGSD-wrapper is contained in a directory called ‘shiny-Graphing.’ This application is started from within ANGSD-wrapper and launched locally from a standard 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 results file. The Shiny server automatically parses standard ANGSD output files and creates the resulting

Figure 1: Visualization of Watterson’s  $\theta$  estimated by ANGSD across a 1.5Mb region of chromosome 10 in *Zea mays* ssp. *mays* using ANGSD-wrapper. Darker colors indicate a higher density of points. Blue boxes indicate gene annotations provided by a GFF file.

#### ANGSD-wrapper graph



plot(s) (Figure 1), which can be saved using the browser’s built-in image export options.

## Results and Discussion

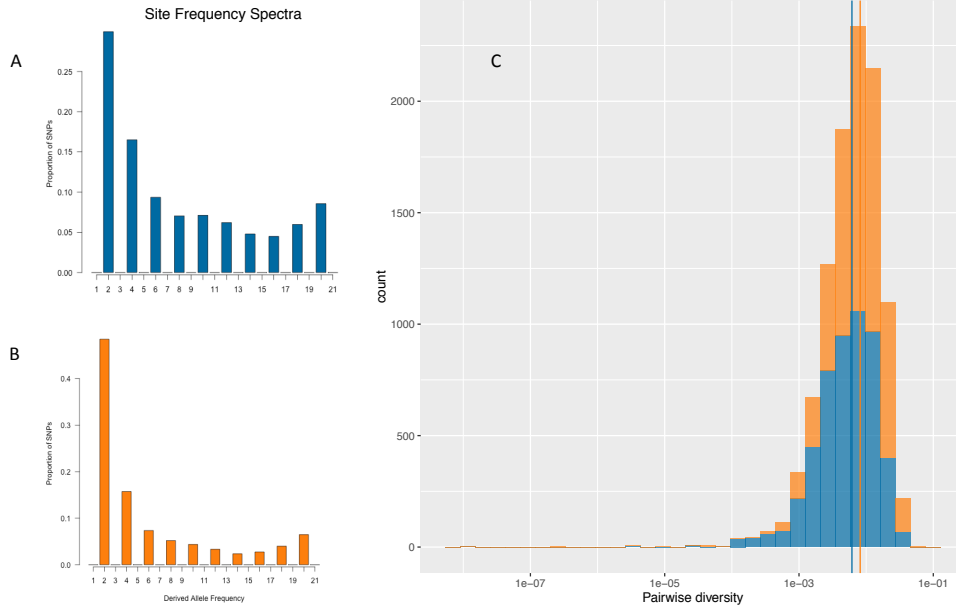
As a demonstration of analyses in ANGSD-wrapper, we explore patterns of pairwise nucleotide diversity in a single genomic region of domesticated maize and wild teosinte. We used a subset of the resequenced samples from the Maize HapMap2 project (Chia et al., 2012) and calculated summary statistics using a 10Mb region on chromosome 10 (Table S1). The data are available at [https://figshare.com/articles/Example\\_Data\\_tar\\_bz2/2063442](https://figshare.com/articles/Example_Data_tar_bz2/2063442) and can be downloaded from within ANGSD-wrapper using the command

```
$ angsd-wrapper setup data
```

In the following we refer to methods listed in Table 1 when describing analyses.

We first use the “SFS” method to estimate the site frequency spectrum (SFS) of both maize and its wild progenitor *Zea mays* ssp. *parviglumis*, assuming an inbreeding coefficient of  $F = 1$  for these highly inbred samples. The SFS and diversity statistics were calculated using ancestral states inferred by the “Ancestral Sequence” method from a single resequenced genome of *Tripsacum dactyloides*. We show that the maize SFS is skewed toward intermediate-frequency variants (Figure 2A-B, Tajima’s D of 0.2 and 0.0085 in maize and teosinte, respectively), likely a result of the bottleneck associated with maize domestication (Beissinger et al., 2016; Eyre-Walker et al., 1998). Using the “Thetas” method we find further evidence of the domestication bottleneck, with mean levels of pairwise nucleotide diversity in this region in maize  $\approx 25\%$  lower than in teosinte (0.0061 and 0.0082, respectively; Figure 2C). Using the “2DSFS method,” which includes an  $F_{ST}$  calculation, we find a mean  $F_{ST}$  in this region of 0.116, nearly identical to the genome-wide value of 0.11 reported in Hufford et al. (2012). None of the genes in this

Figure 2: Summary statistics for *Zea mays*. Derived site frequency spectra for A. maize and B. teosinte. C. distribution of pairwise nucleotide diversity for maize (blue) and teosinte (orange). Mean values for each taxon are represented by corresponding vertical lines. Pairwise nucleotide diversity results are visualized separately from the interactive graphics and colors were added to A and B using a custom script.



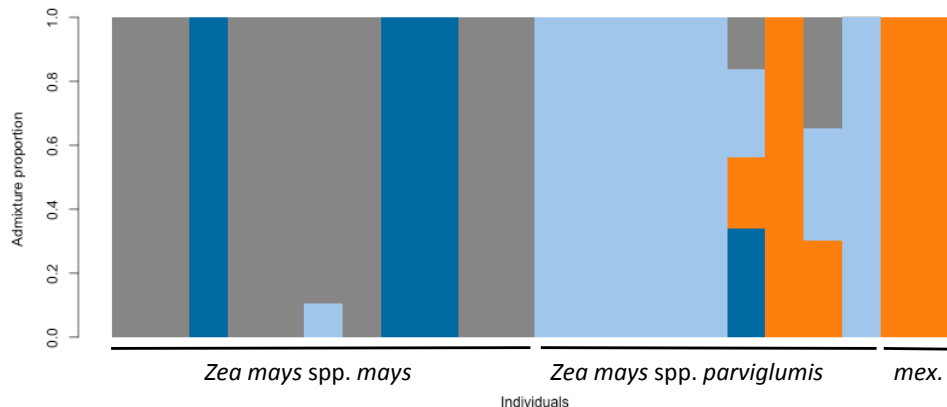
region have been identified as potential domestication candidates Hufford et al. (2012), consistent with the lack of extended regions of high  $F_{ST}$  in our analysis (Figure S3).

Finally, we include two samples of the related wild teosinte *Zea mays* ssp. *mexicana* to assess evidence for admixture. We ran the “Admixture” method, which implements an estimate of admixture proportion from genotype likelihoods (Skotte et al., 2013). We identify structure within domesticated maize separating three high-latitude temperate landraces from the other tropical accessions (Figure 3). *Zea mays* ssp. *mexicana* clusters into its own group (orange), along with a single accession of ssp. *parviglumis* collected from a region in which many teosinte populations appear to be the result of admixture between the two subspecies (Fang et al., 2012). Consistent with an independent analysis from SNP genotyping (Hufford et al., 2013), the lowland maize samples included here show no evidence of admixture with ssp. *mexicana*. Most ssp. *parviglumis* accessions fall primarily into a single (light blue) cluster, but two accessions show assignment to multiple clusters, perhaps due to the limited resolution resulting from analysis of a single genomic region and restricted geographic sampling.

## Conclusions

ANGSD-wrapper provides an easy-to-use interface that simplifies many population genetic analyses implemented in ANGSD (Korneliussen et al., 2014) and permits the exploration of genome-scale results through interactive visualization. ANGSD-wrapper is under active development to incorporate new anal-

Figure 3: Admixture analysis for *Zea mays* ssp. *mays*, *Zea mays* ssp. *parviglumis*, and *Zea mays* ssp. *mexicana* (*mex*). Colors represent the proportion of each individual’s genome assigned to one of the  $K=4$  source populations. Individuals are shown in the same order as Table S1. For results using other values of  $K$  see Figure S2.



yses and updates to the ANGSD software package.

## Acknowledgments

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Table S1: Table of samples used in analysis with mean depth over the region 15000000-25000000 on chromosome 10.

Sample	Mean Depth	Species
BKN009	7.00194	<i>Zea mays</i> spp. <i>mays</i>
BKN011	6.9777	<i>Zea mays</i> spp. <i>mays</i>
BKN014	6.78829	<i>Zea mays</i> spp. <i>mays</i>
BKN015	3.739	<i>Zea mays</i> spp. <i>mays</i>
BKN019	3.71944	<i>Zea mays</i> spp. <i>mays</i>
BKN022	3.71268	<i>Zea mays</i> spp. <i>mays</i>
BKN025	3.53799	<i>Zea mays</i> spp. <i>mays</i>
BKN026	3.91798	<i>Zea mays</i> spp. <i>mays</i>
BKN027	7.05576	<i>Zea mays</i> spp. <i>mays</i>
BKN033	3.85336	<i>Zea mays</i> spp. <i>mays</i>
BKN035	3.74839	<i>Zea mays</i> spp. <i>mays</i>
TIL01	3.60133	<i>Zea mays</i> spp. <i>parviglumis</i>
TIL03	4.07518	<i>Zea mays</i> spp. <i>parviglumis</i>
TIL04	6.09163	<i>Zea mays</i> spp. <i>parviglumis</i>
TIL07	5.11419	<i>Zea mays</i> spp. <i>parviglumis</i>
TIL09	5.29004	<i>Zea mays</i> spp. <i>parviglumis</i>
TIL11	3.15477	<i>Zea mays</i> spp. <i>parviglumis</i>
TIL15	6.87873	<i>Zea mays</i> spp. <i>parviglumis</i>
TIL16	2.67186	<i>Zea mays</i> spp. <i>parviglumis</i>
TIL17	2.61892	<i>Zea mays</i> spp. <i>parviglumis</i>
TIL08	6.09453	<i>Zea mays</i> ssp. <i>mexicana</i>
TIL25	13.1566	<i>Zea mays</i> ssp. <i>mexicana</i>
TDD39103	8.62	<i>Tripsacum dactyloides</i>

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Xie, Y. (2015). *DT: A Wrapper of the JavaScript Library 'DataTables'*. R package version 0.1.

Figure S1: Workflow diagram for all methods available in ANGSD-wrapper. Some workflows are supported by ANGSD, but not incorporated here. Please see the ANGSD paper for more details (Korneliussen et al., 2014).

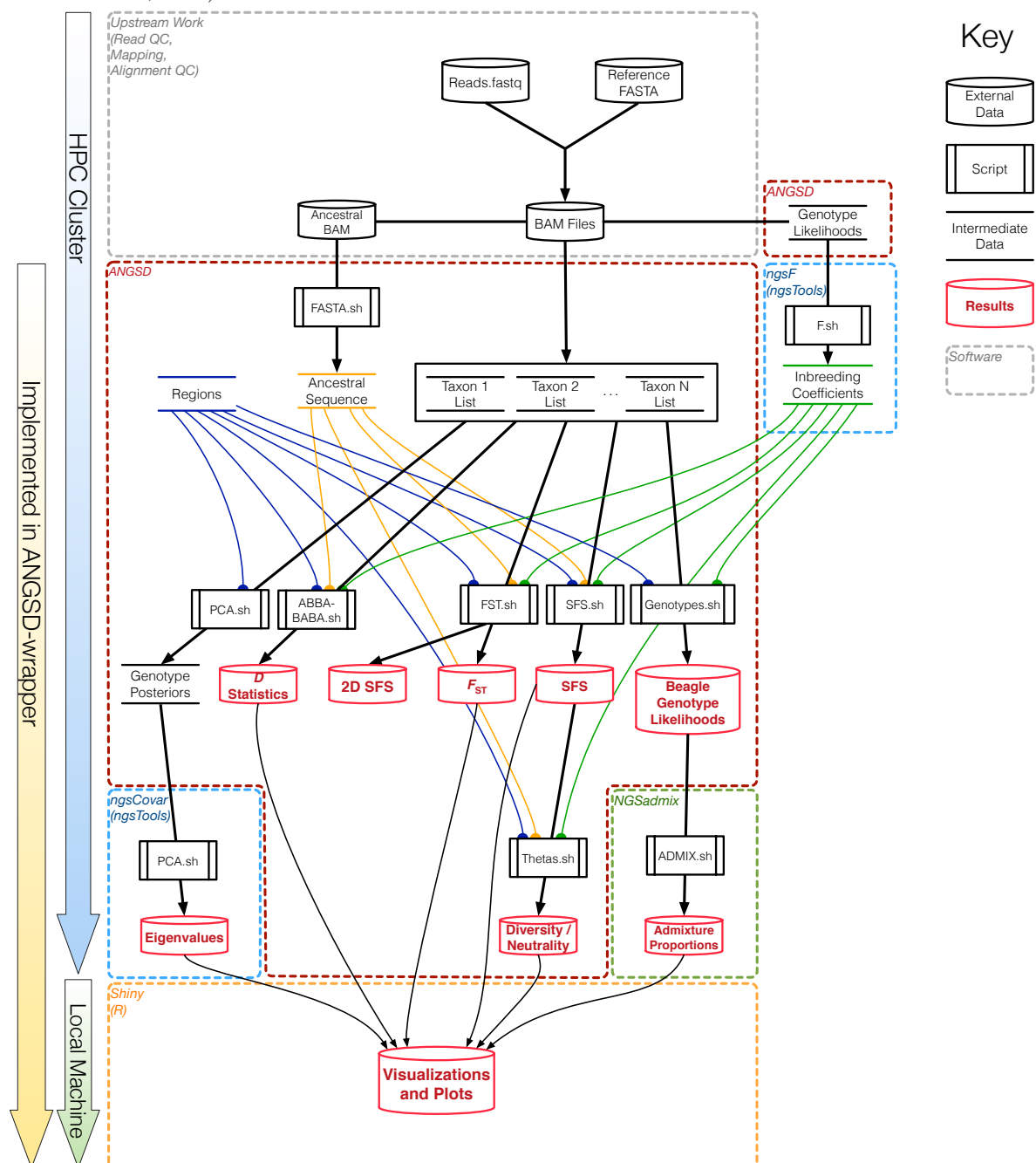


Figure S2: Admixture analysis for K=2 (top), K=3 (middle), and K=4 (bottom).

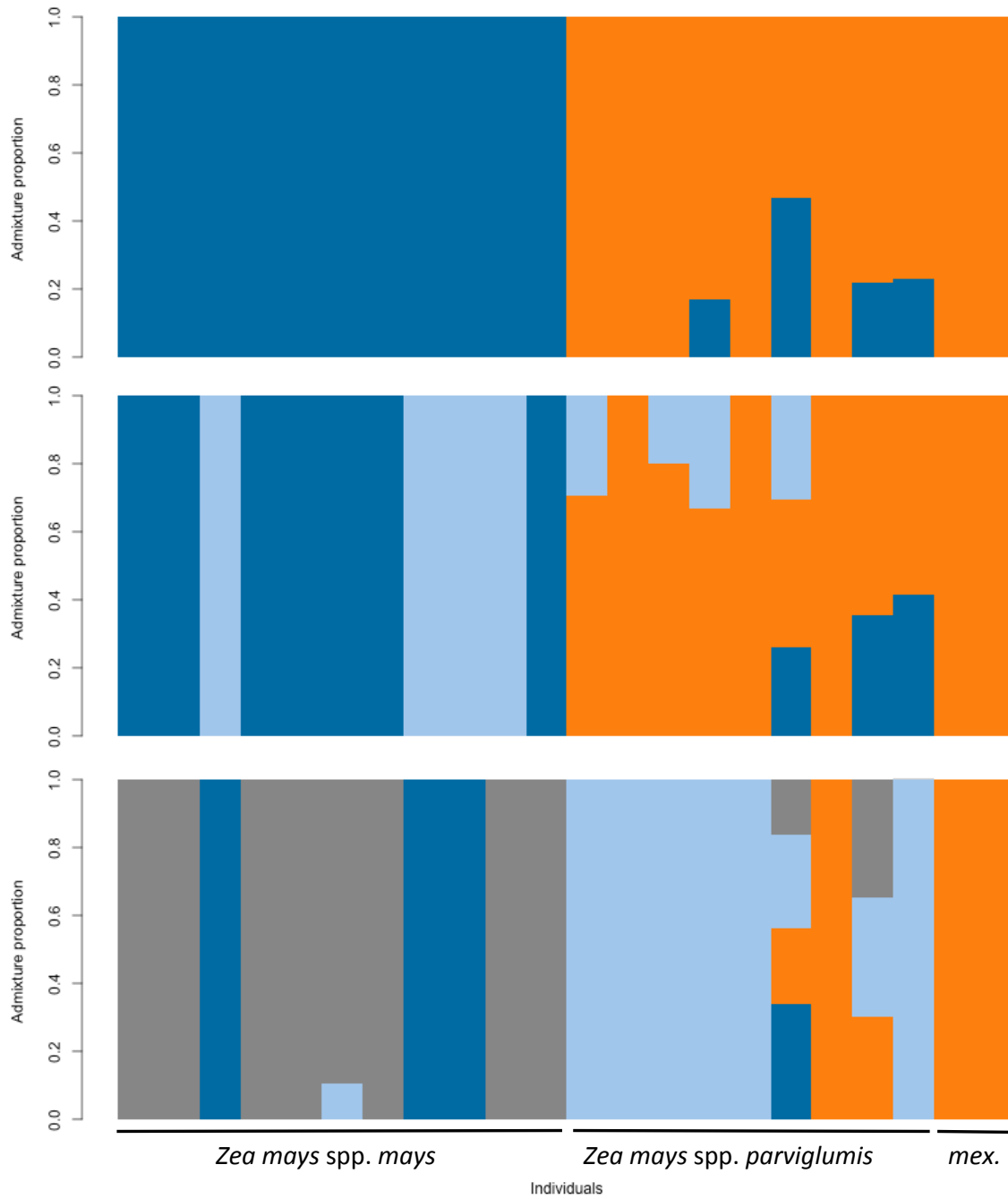


Figure S3:  $F_{ST}$  values plotted against base pair position on chromosome 10 of maize.  $F_{ST}$  is calculated between the maize and teosinte samples.

