

# BIO00056I

## Workshop 6: Population Genomics

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### 1 Learning objectives

The aims of this workshop are to:

- Learn more about population genomics data
- View a real-world example of population structure analysis
- To appreciate how population genomics can be applied to eco-epidemiology (the study of the ecology of infectious diseases)

## 2 Introduction

### 2.1 Evolution, populations and genome data

You will be aware now from earlier on in this module that:

- Sometimes hybrids occur between these closely related species.
- Most species contain multiple populations. Genetically, a population is a group of individuals more closely related to each other than to other groups, reflecting shared ancestry.

In practice, it can be hard to tell which population—or even which species—an individual belongs to, especially for microorganisms. In this workshop, we'll use genome data to investigate these questions

In Figure 1 below, each box represents a *population* that contains many individuals. Red and blue boxes represent two species. Some regions have hybrids (mixed colors), and some do not. Some populations show a little mixing; others show a lot.

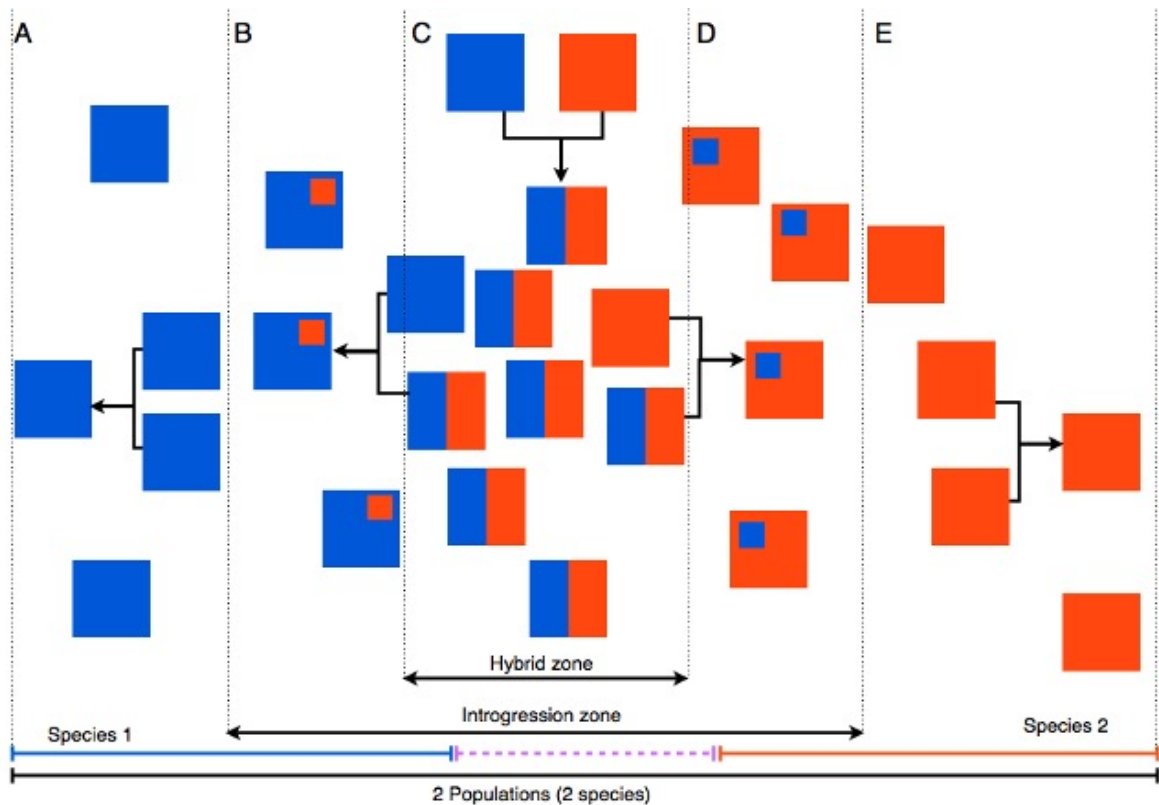


Figure 1: Imagined populations of two species (red and blue), with some hybrids and varying levels of mixing.

## 2.2 The topic for today

In this workshop, we'll look at new genome data from *Leishmania* parasites collected in the Amazon. *Leishmania* are single-celled protozoan parasites that infect mammals, including people. They have two copies of each chromosome (diploid) and reproduce sexually (they undergo meiosis).

We'll focus on *Leishmania guyanensis* and closely related species found in South America. Together they form a species complex called Viannia—closely related species that can sometimes interbreed where their ranges overlap.

These parasites cause cutaneous leishmaniasis (CL), a skin disease that leads to long-lasting sores. CL is zoonotic, meaning it mostly circulates in wild animals; humans are infected but probably play a small role in maintaining the parasite population.

Because Viannia parasites infect many native mammals in South America, they are probably deeply embedded in the region's ecology.

### 2.2.1 Leishmania are spread by sand flies

Three facts about sand flies are important for today:

- Sand flies are mainly found in forests or forest edges (not in sand on the beach)
- They are not strong flyers, so they don't carry *Leishmania* parasites very long distances.
- The different sand flies species, and even populations within species, have their own ecological niches and characteristics.
  - For example, certain populations of sand flies carry some species of *Leishmania*, but not others.

## 2.3 The data we use in this workshop

We're studying the genetics of the Viannia group of *Leishmania* parasites. The analysis is being carried out at the University of York, working with colleagues in Manaus, Brazil—a large city on the Amazon River surrounded by the Amazon Rainforest. Because the Amazon is so biodiverse, several Viannia species may be present there.

Parasites were isolated from patients with cutaneous leishmaniasis (CL) and grown in the lab to extract DNA. We then sequenced the DNA from 70+ parasites using short-read (Illumina) sequencing.

From these genomes, we identified single-nucleotide polymorphisms (SNPs)—tiny differences at single DNA letters. To add context, we also included public data for related Viannia species from the European Nucleotide Archive. A short summary of the computational steps is at the end of this document.

For this workshop, all you need to know is that we have SNP data from:

- 71 *Leishmania* strains collected in Manaus (mostly likely to be *L. guyanensis*)
- 21 *L. braziliensis* strains from various locations in Brazil
- 25 *L. panamensis* strains from Panama
- 34 *L. peruviana* strains from Peru
- one *L. shawi* strain from Brazil

We'll use these data to explore species, populations, and mixing (hybridization) within the Viannia group.

## 3 Exercises

### 3.1 Exploring the data

In this workshop we will explore the results we generated. We will look at three ways to visualise population structure.

#### 3.1.1 Population genomic data files

While genomes contain many types of polymorphisms, population genomic analysis often uses only single nucleotide polymorphisms (SNP) data. This is because SNPs are very abundant and they have some properties that make them easier to model mathematically.

SNPs and other polymorphisms are affected by many processes, such as genetic drift, migration, selection and recombination. However, it can be represented relatively simply as a table.

The standard format for representing SNP data is called VCF (Variant Call Format). Here is a small example of what a VCF file looks like.

```
#CHROM POS REF ALT Lg1 Lg2 Lg3 Lg3
01 2177 A G 0/1 1/1 1/1 0/1
01 2636 G A 0/0 0/1 0/1 0/0
01 9844 G A 0/1 0/0 0/0 0/0
...
35 9999 G A 1/1 0/0 0/1 0/0
```

It is almost like a table that we could load into Excel, where each **row** indicates position in the genome, and **columns** contain information about that position.

It has a header line:

```
#CHROM POS REF ALT
```

This indicates that the first column is the chromosome number (**#CHROM**), the second column is the position on that chromosome (**POS**), the third column is the reference allele (**REF**) and the fourth column is the alternative allele (**ALT**).

After this, we have the genotypes for each sample. In this example, there are four samples:

```
Lg1 Lg2 Lg3 Lg3
```

After this, we have the genotypes for the strains that are in the VCF (four in this case). They are coded as 0 for the reference allele (A), 1 for the second allele (G), so for an A/G polymorphism:

- AA is coded as 0/0
- AG is coded as 0/1
- GG is coded as 1/1

The population genomics community has developed many software tools that process this data to extract some of this information (no tool does it all). In this workshop we will look at a phylogenetic tree, principal components analysis (PCA) and a STRUCTURE plot. These are all used to describe population structure and detect hybrids between populations or between species.

### 3.2 The phylogeny

A phylogenetic tree is a good first look at how similar or different many samples are. But when individuals mix between populations or species (hybridization), a *simple branching* tree can be misleading.

Think of a tree of people grouped by ancestry. Where would you place someone with one parent from Spain and one from Fiji? They don't fit neatly on a single branch with the Spanish and the Fijians. The same data display issue can happen in nature when populations interbreed.

To handle this, we used a network approach (a splits network made with the software *Splitstree*) that can show conflicting signals in the data—like those caused by mixing between populations or species.

Below is our SplitsTree network. There are many samples, so coloured dots cluster tightly. The Manaus samples are dark blue. Most cluster near *Leishmania guyanensis* at the top. We marked the Manaus samples that don't with arrows.

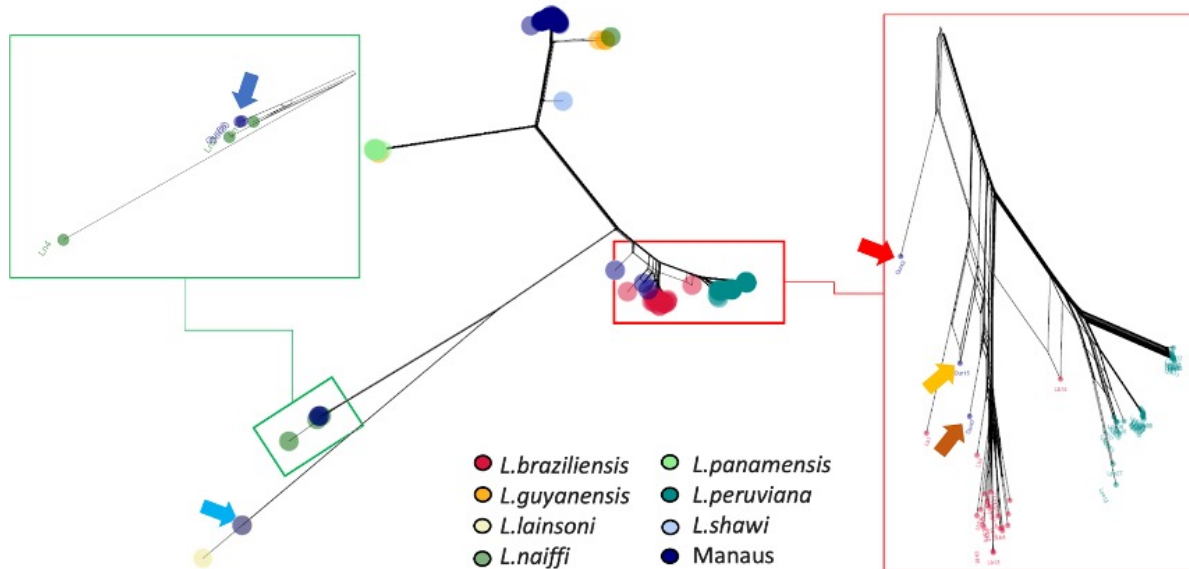


Figure 2: A SplitsTree phylogeny. The overall phylogeny is in the center, and we zoom in to various *clades* (groups of related samples) around the edges. Each dot is a sample, coloured by species. The dark blue dots are samples we collected from Manaus, Brazil.

### 3.2.1 Interpreting phylogeny

Now spend some time discussing the SplitsTree phylogeny, using the using the discussion points below as a guide.

#### 💡 Discussion points: phylogeny

- From this figure, do the *Viannia* species look clearly separated or do some overlap?
- A few Manaus samples don't cluster with *L. guyanensis*. What could explain that?
- What might this suggest about the causes of CL in the Amazon (e.g., multiple species, local ecology, host/vector differences)?
- Look at the *L. peruviana*–*L. braziliensis* area (red box). One strain sits between the two. What could that indicate?
- Examine the data from the single *L. shawi* sample (light blue). Does its branch look unusually long? Remember: longer branches usually mean more mutations (this isn't a dated tree, just a measure of genetic differences).

### 3.3 Principal components clustering

Population structure is an important facet of most (if not all) species. Populations sometimes split into species, and all natural selection begins within a population. So when we start to examine the genetic diversity of a species, it is wise to look at population structure.

Principal components analysis (PCA) is a useful tool for understanding population structure. PCA reduces the variables in our data set (thousands of SNPs in our rows, and many individuals in columns), while preserving a lot of the information about population structure.

A simple way to interpret PCA data is by considering that the distance between two individuals on a two-dimensional PCA plot is a representation of the genetic distance between those samples. So, if they are close on the plot, they are close genetically. And if they are distant in the plot, they are distant genetically. We would expect individuals from within a population, or a species to fall close to each other on a PCA plot.

First look at Figure 3 below. This shows PCA clustering of all the other *Viannia* species, apart from the samples we collected from Manaus. Discuss with the people at your table:

- Do the species cluster genetically?
- Do *L. peruviana* and *L. braziliensis* really look like two different species?

Note: The blue dashed lines are a guide to the eye to help you see the clusters.

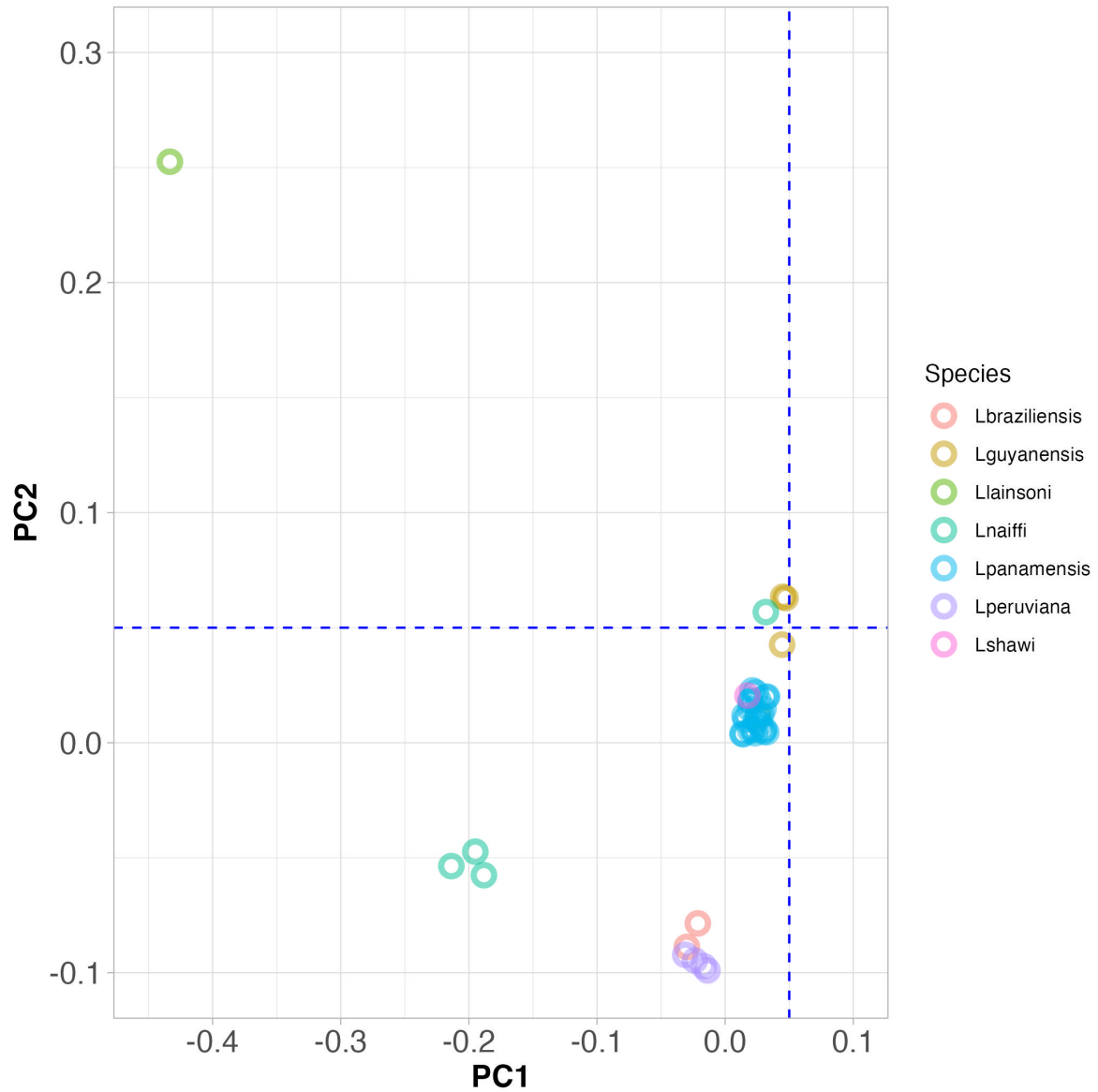


Figure 3. PCA clustering of all the other *Viannia* species from throughout South America

Now look at Figure 4, which shows PCA clustering of all the other *Viannia* species from throughout South America (on the left), and those that were collected from Manaus (on the right). Note that **the PC axes are on the same scale** here.

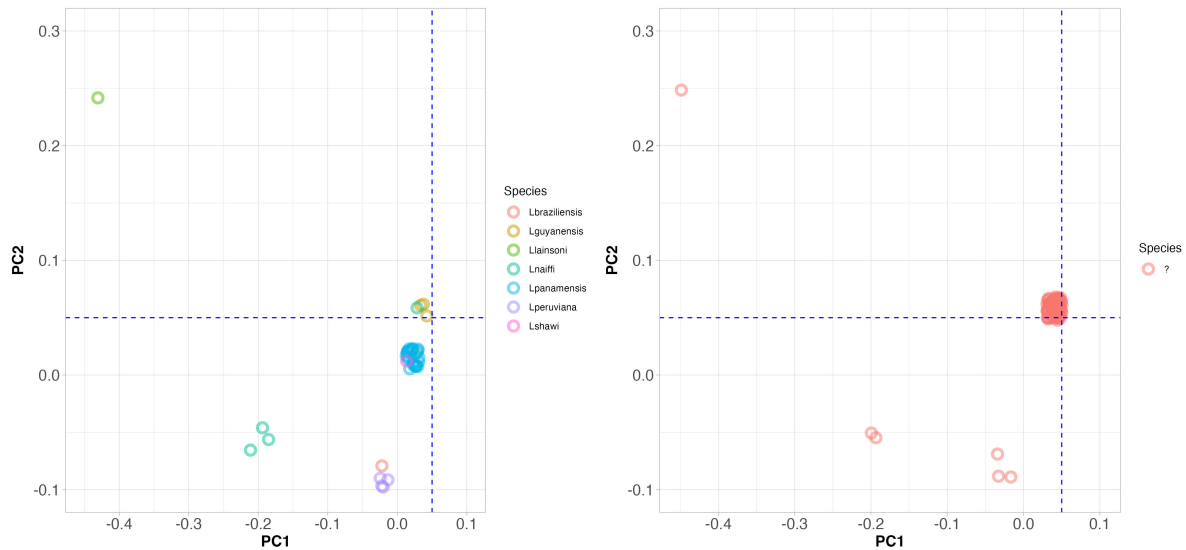


Figure 1: Figure 4. PCA clustering of all the *Leishmania* samples from throughout South America (left), and those that were collected from Manaus (right)

### 3.3.1 Interpreting principal component plots

To learn to interpret principal components plots, using the using the discussion points below for a discuss with the people at your table.

#### 💡 Discussion points: PCA (species)

- Does it look like the samples that came from Manaus are all the same species?
- Since we ensured the positions on the PCA plot identify the species, does it help to identify the species from Manaus?
- What does this tell you about the causes of cutaneous leishmaniasis in the Amazon region?
- Since *Leishmania* are single celled organisms, and they all cause a similar disease, population genomics is the only way to identify which sample belongs to which species. Why would we not merely use PCR to identify the species?

### 3.3.2 Principal components clustering 2: geography and genetics

We can look at the PC plots another way, simply by colouring the samples according to where they came from (geography).

Because sand flies that carry *Leishmania* do not fly very far, it is possible that geographic distance is the main factor that determines the genetic distance between *Leishmania* populations. This concept is called **isolation by distance**.

The *Leishmania* parasite we have genome data from come from Brazil, Peru and Panama which are *very* far apart, so we might expect to see isolation by distance in the PCA plot.

Note that in Figure 4 below: - the *colour* of each point indicates the location where the sample was collected - the *position* on the PCA plot indicated how closely related each sample is. If two strains sit close together on the plot, they are closely related genetically.

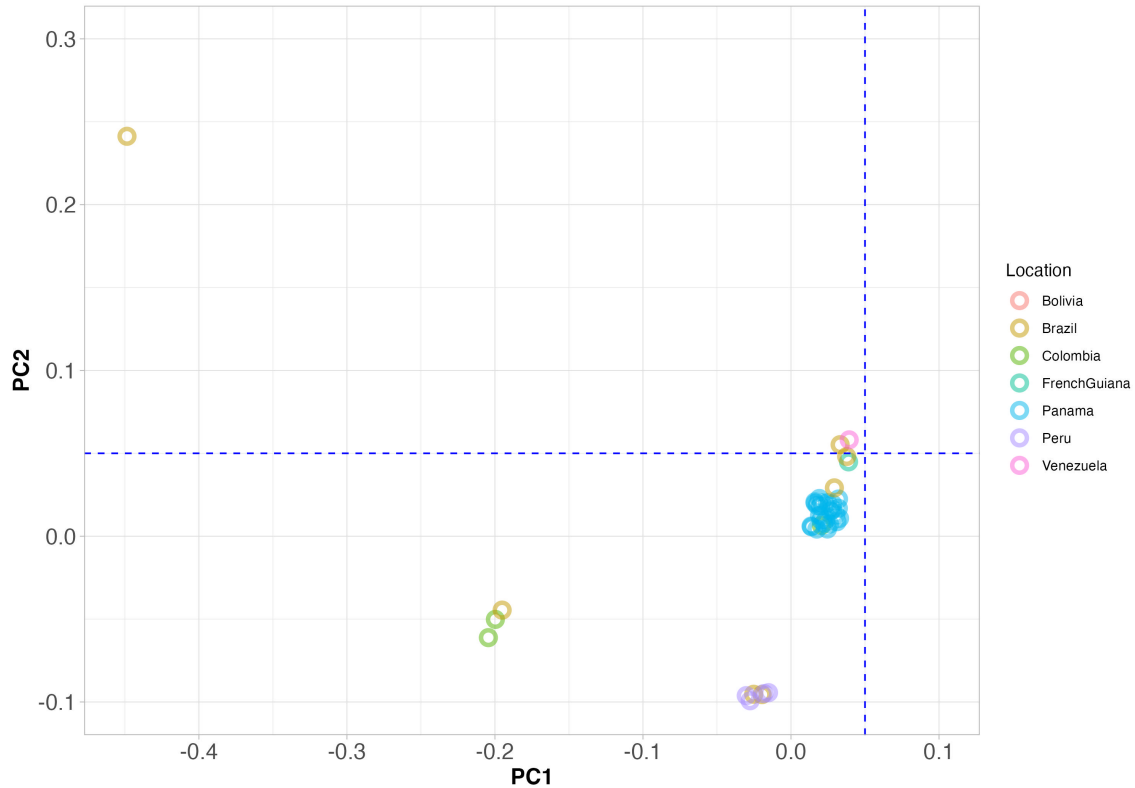


Figure 4. PCA clustering of all the other *Viannia* species from throughout South America. The blue dashed lines are not important here, but will help with interpretation of Figure 5 below.)

#### 💡 Discussion points: PCA and isolation by distance

Note that samples from the *same country* are plotted in the same colour, and samples that are *genetically similar* will be fall close together on the plot.

- Do you see any examples of genetically similar that are all from the same country? These will be one local population of the same species. This is what we expect, from a local population that is one species.
- Do you see any examples of the opposite: genetically similar samples that are close together on the PCA plot, with *different* colours showing that they are from different countries?
- How can we interpret the finding that there are genetically similar individuals living in different countries? It might help to think about this with respect to human populations.

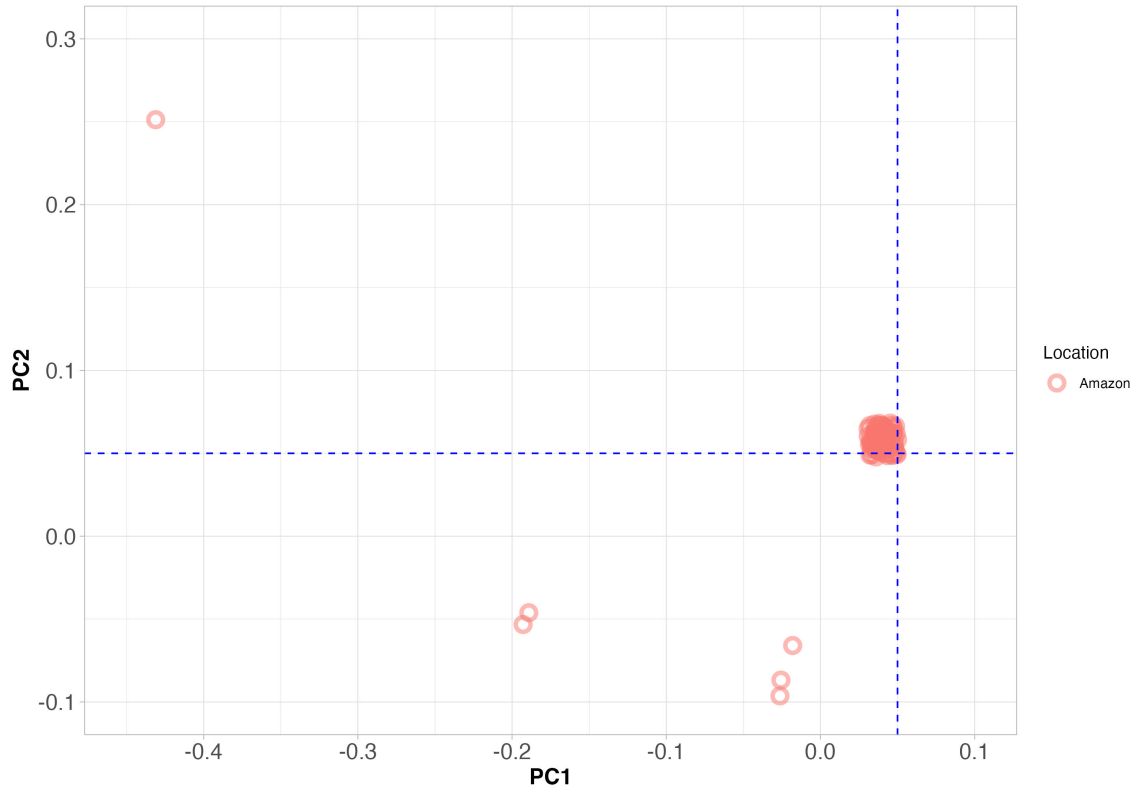


Figure 5. PCA clustering of only the *Leishmania* strain we collected from Manaus. The PCA axes are the same as Figure 4 above. The blue will help with interpretation, compared to Figure 4.

💡 Discussion points: PCA and specis in Manaus

- Since samples that are *genetically similar* will be fall close together on the plot, does it look like all the samples from Manaus are the same species?
- What does this tell us about the causes of genetic differentiation between species?
- In other words, why are all these *Leishmania* samples from Manaus not interbreeding, and becoming genetically related?
- Can you guess what species are present in Manaus, based on the coordinates of species in previous PCA plots?
- Is the idea that there are different *Leishmania* species in Manaus that we obtain from the PCA plots consistent with the phylogeny we looked at earlier?

### 3.4 Admixture/STRUCTURE plots

A common way of showing population genomic data are so-called STRUCTURE plots, generated using a software called STRUCTURE, or a similar one called Admixture, which is what we used. I'll call these STRUCTURE plots for now.

STRUCTURE plots show the proportion of ancestry from different populations (or species) for each individual in the data set. Usually, each column of a STRUCTURE plot represents one individual, and the different colours within each column represent the proportion of ancestry from different populations (or species).

STRUCTURE plots are usually used to describe population structure within a species, but they can also be used to describe structure between closely related species, as we do here.

Our STRUCTURE plot is below. Samples from Manaus are marked in the open rectangle at the bottom of the plot. Again, most look like *L. guyanensis*, and those that do not, are marked with arrows as we did in the phylogeny.

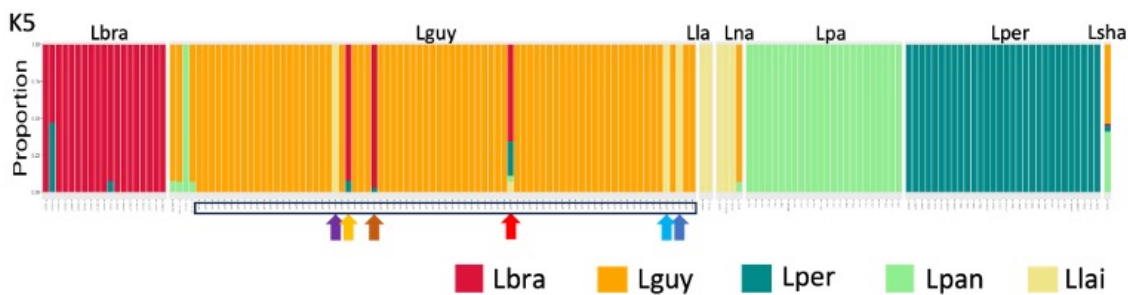


Figure 7. A STRUCTURE plot showing the *Leishmania* species we studied. Samples that were obtained from Manaus are marked in the open rectangle at the bottom of the plot. Most appear to be *Leishmania guyanensis*, but a few do not, and these are marked with arrows as we did in Figure 2, the SplitsTree phylogeny.

#### 💡 Discussion points: the STRUCTURE plot

- Each column is a different strain, and the colours represent the *proportion* of this strain's ancestry that is derived from each species. If there are hybrids between species, we would expect to see columns with multiple colours.
- The most obvious thing about this plot is that the species are different colours.
- Does this visualisation of the data suggest that there is extensive breeding between species of the Viannia?
- Consider the *Leishmania* parasites circulating around Manaus (see Figure 5). Do they all look like one species, or are there multiple species present?

- Look at the one strain of *Leishmania shawi*. This certainly looks like a hybrid between two species.
- Go back to Figure 2 (the phylogeny) and the PCA plots to examine and see how the *Leishmania shawi* sample is placed. Does its placement in those plots make sense, given what we see in the STRUCTURE plot?

## 4 Summary: what we have learned

- Population genomics data can be used to explore population structure and hybridization between populations and species.
- There are different ways of visualising population genomics data, each with their own strengths and weaknesses.
- In this workshop, we have seen examples of:
  - isolation by distance (different species occurring in various counties)
  - multiple species in one location (Manaus) with rare hybrids between species

## 5 After the workshop: exam style questions

### 5.1 Question 1. Populations and migration.

A PCA plot of human populations from Europe is shown in Figure 8 below. Each point is an individual, and they are colored by the country they come from. Small coloured labels represent individuals and large coloured points represent median PC1 and PC2 values for each country.

- Is there evidence for extensive migration of individuals between countries in Europe? Explain your answer.
- What can we infer from this data about the alleles within countries that are close together on the PCA plot (e.g., Spain and Portugal) compared to countries that are far apart on the PCA plot (e.g., Finland and Italy)?
- What can we infer from this data about the interbreeding between countries? Does this look like between country marriages are common, or the exception?

- 
- a**
- PCA plot showing the first two principal components (PC1 and PC2) of genetic variation across 30 European populations. The plot is color-coded by country. An inset map shows the geographic distribution of the populations. The first two principal components explain 12.5% and 10.2% of the genetic variance, respectively.
- b**
- Scatter plot showing the North-south variation in PC1-PC2 space (Y-axis) versus the East-west variation in PC1-PC2 space (X-axis). The plot is color-coded by country: France (green squares), Germany (red diamonds), Italy (blue triangles), and Switzerland (purple circles).
- c**
- Scatter plot showing the Median genetic correlation (Y-axis) versus the Geographic distance between populations (km) (X-axis). The plot is color-coded by country: France (green squares), Germany (red diamonds), Italy (blue triangles), and Switzerland (purple circles). A red curve shows the trend of the median genetic correlation across the geographic distance.

Figure 8. A PCA plot of human populations from Europe. Each point is an individual, and they are colored by the country they come from. Small coloured labels represent individuals and large coloured points represent median PC1 and PC2 values for each country.

## 5.2 Question 2. Understanding populations from DNA

- Describe how population genomic data is collected; starting with the collection of samples from individuals, laboratory work, and the data analysis steps to obtain a VCF file containing SNP data.
- Describe three methods to display population genomic data to show genetic relatedness (population structure) between individuals. For each method, describe one strength and one weakness.
- List three features of the *genetics* of natural populations that we can learn from population genomic data.