# Detecting Selection

with EAA and Fst outlier tests

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***Workshop on Population and Speciation Genomics 2020***

# Practical learning goals:

- Become familiar with running differentiation-based and environmental association analyses (EAA)
- Apply different parameter sets and evaluate how different settings impact outlier detection.
- Plot and interpret results of different tests

<u>Data for the practical come from:</u>

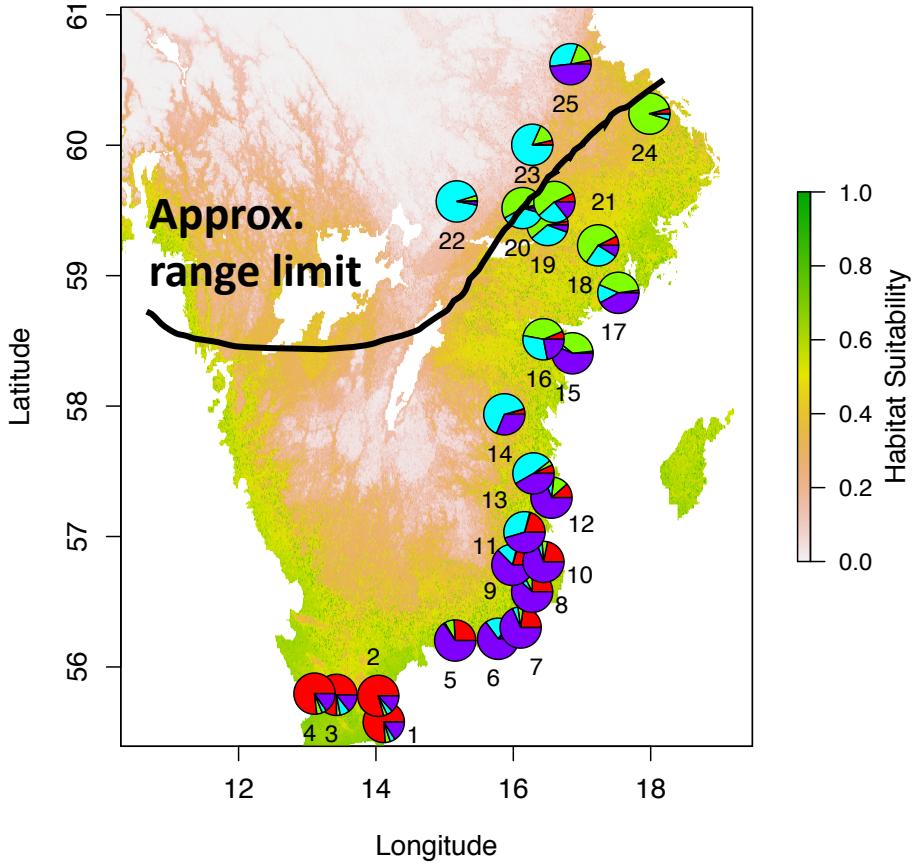
Dudaniec RY, Yong CJ, Lancaster LT, Svensson EI, Hansson B (2018) Signatures of local adaptation along environmental gradients in a range-expanding damselfly (*Ischnura elegans*). *Molecular Ecology*. 27(11): 2576-2593

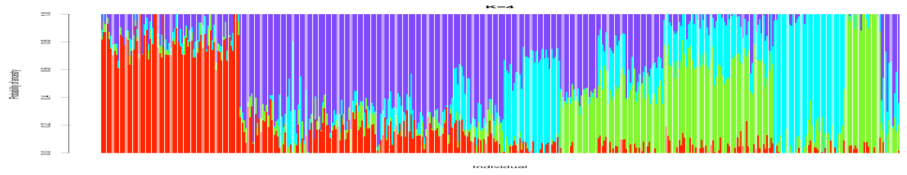
# THE GENETIC DATA

*Ischnura elegans* –
Blue tailed damselfly

- 426 *I. elegans* (2013)
- RADseq/13,612 SNPs
- 500km gradient

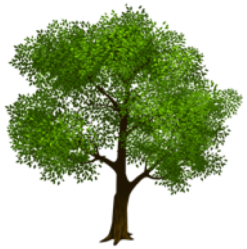
4 admixed clusters



*Dudaniec et al. 2018, Mol Ecol*

# THE ENVIRONMENTAL DATA

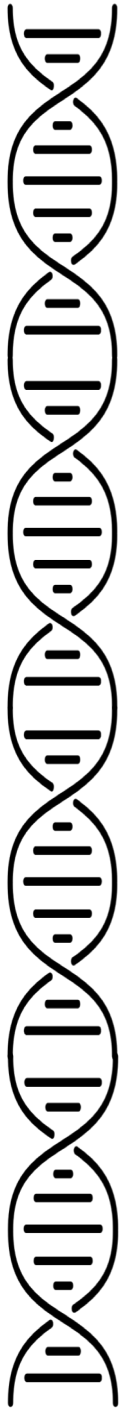


Percentage tree cover

Mean Annual Temperature
Mean Maximum Temp Warmest Quarter (Summer)

Wind Speed

Mean Annual Precipitation



# What we'll cover

**Each Part has a separate R script file:**

Part 1.0 The Data:

Load and examine genetic data, plot and correlate environmental variables

Part 2.0 Outliers:

Differentiation-based (Fst) outlier detection with *pcadapt* and *OutFLANK*

Part 3.0 Multivariate EAA:

Ordination approach using Redundancy Analysis (*RDA*)

Part 4.0 Univariate EAA:

Single locus tests with Latent Factor Mixed Models (*LFMM*)

# PART 1.0 The Data

1) Visualise and examine the dataset
2) Plot and examine environmental variables
3) Check for correlations in environmental variables

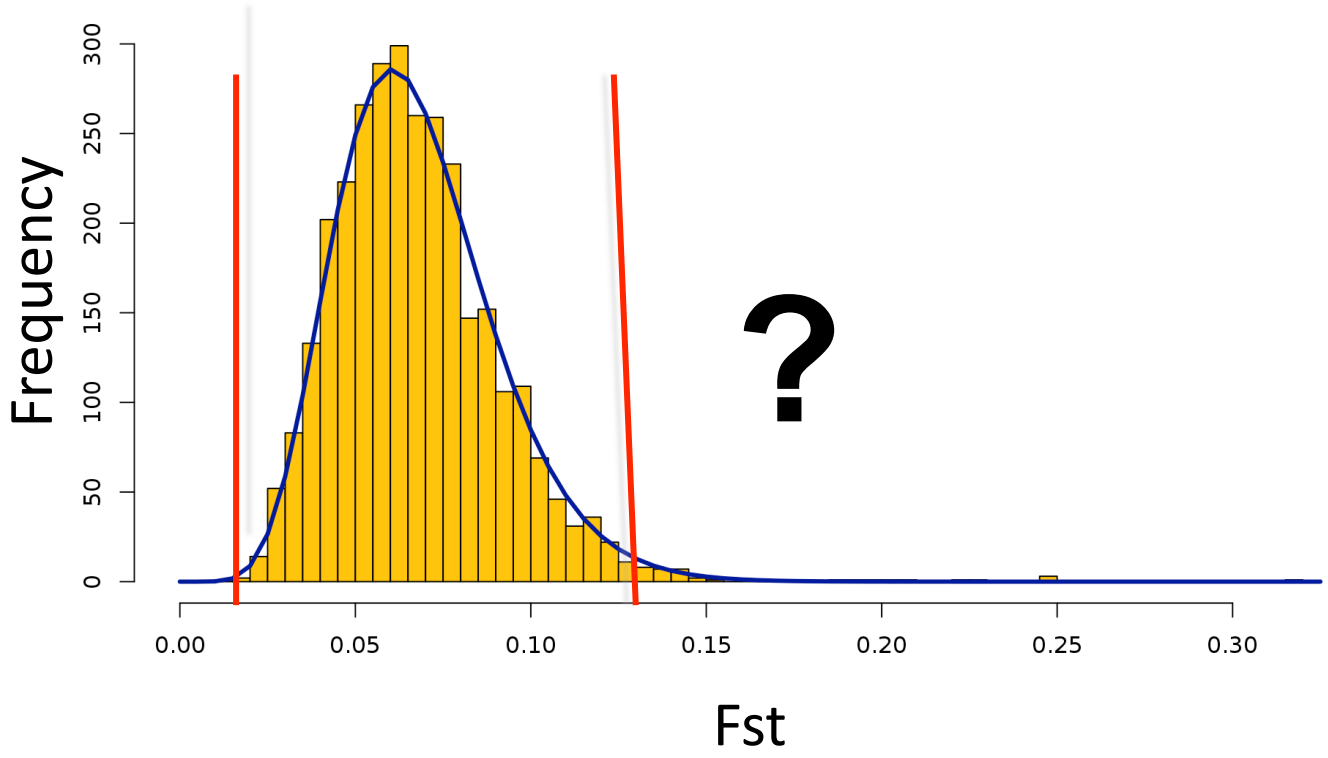
# PART 2.0. Outliers

1) Run *OutFLANK* (Whitlock and Lotterhos 2015)
2) Modify parameters, fill in Table 1 – compare N outliers with different parameters:
    - Left and Right Trim Fraction
    - q- threshold (FDR rate)

# Left and Right Trim Fraction

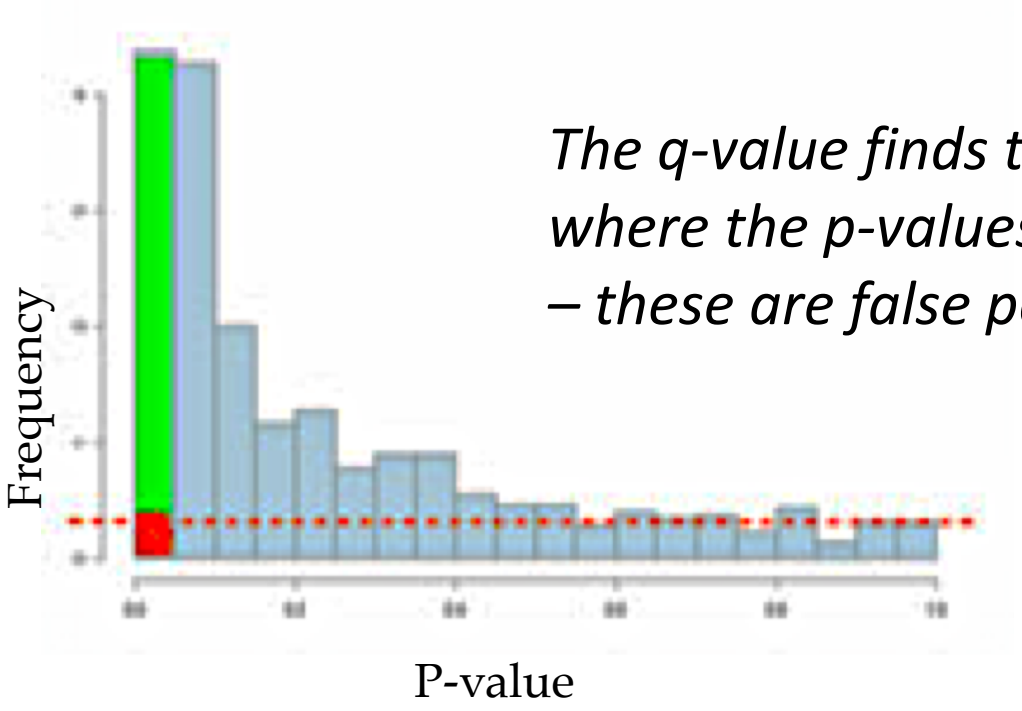
Tells OutFLANK what fraction of extreme Fst values to remove from the left and right tails before estimating the **null Fst distribution**

If you trim too few off the tails, it will result in a much wider chi-square distribution (smaller df) and fewer outliers called.



# False discovery rate:‘q threshold’

- *q* sets the % threshold for determining an ‘outlier’ = a measure of the false discovery rate
- The *q-value* is calculated based on the right tail (+) *p-values* for each locus
- If a locus is below the threshold then it may be included as a candidate




*The q-value finds the height where the p-values ‘flatten out’ – these are false positives*

q-threshold

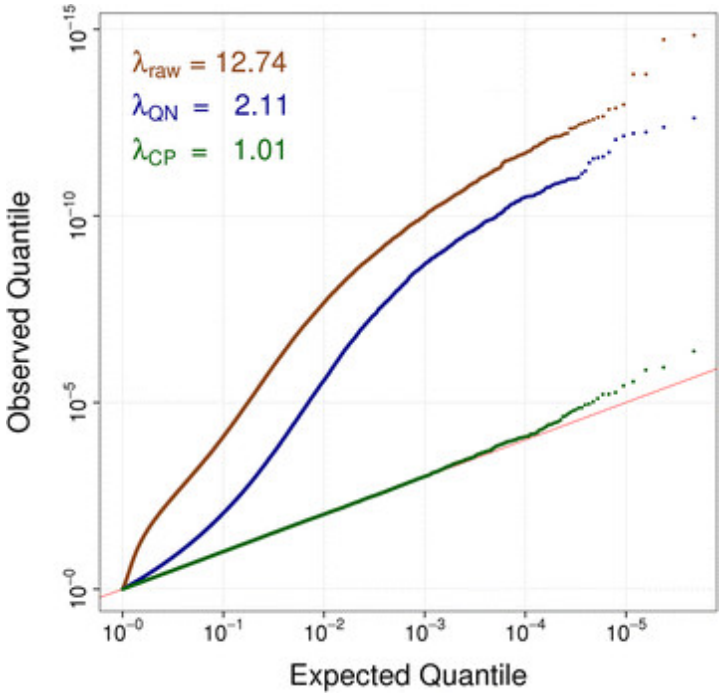
# PART 2.0. Outliers

1) Run *OutFLANK* (Whitlock and Lotterhos 2015)
2) Modify parameters, fill in Table 1 – compare N outliers with different parameters:
    - Left and Right Trim Fraction
    - q- threshold (FDR rate)
3) Run *pcadapt*
    -Modify GIF – compare to original GIF
    -Define FDR (q-value cut off)
    - Examine N outliers

# Genomic Inflation Factor (GIF)

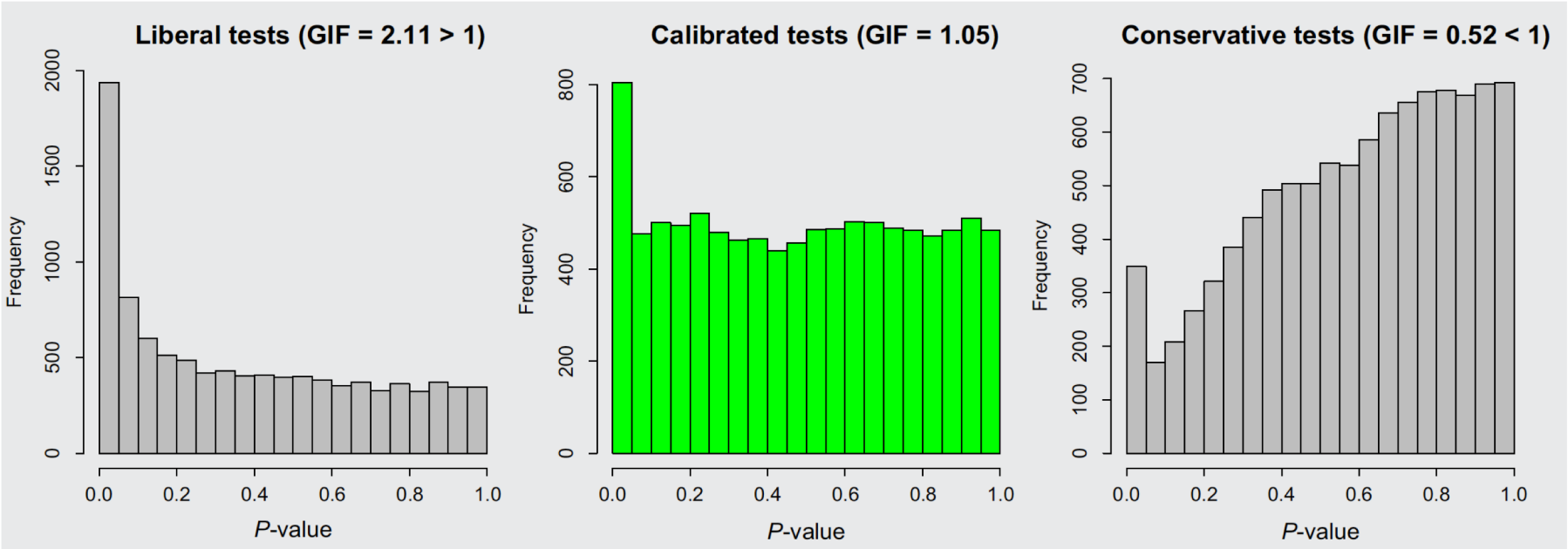
Used to recalibrate test scores (p-values) to control FDR

Expresses the **deviation** of the distribution of the <u>observed</u> test statistic from the distribution of the <u>expected</u> test statistic, **i.e. inflation of scores**




*GIF can vary depending on sample size, relatedness, LD, population substructure, and N causal variants.*

# Calibrating P-values with GIF




**Box Fig. 1.** Histograms of test significance values (*P*-values) prior to the application of FDR control algorithms (artificial data). GIF is the genomic inflation factor for each data set.

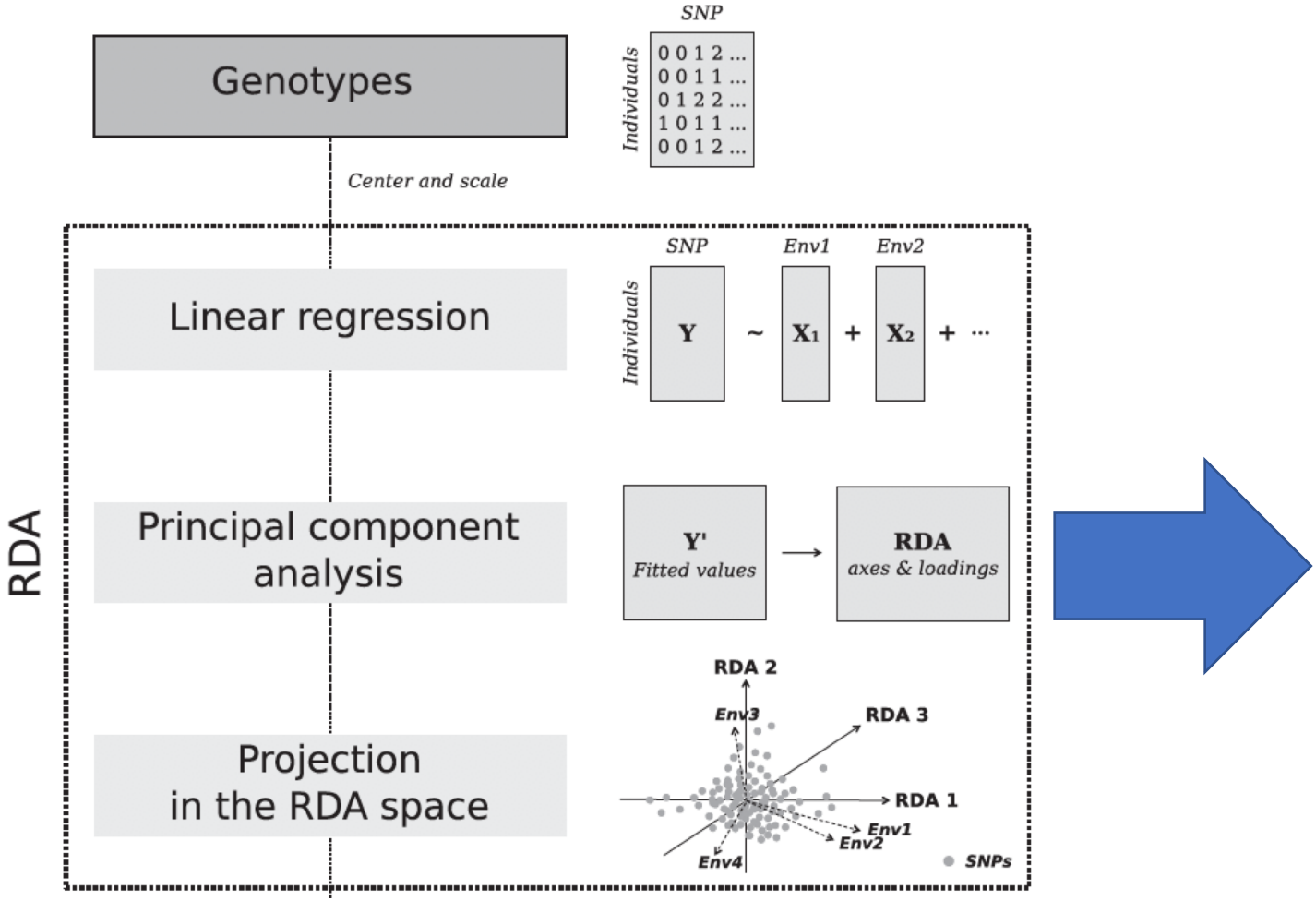
GIF ~ 1= well calibrated

GIF > 1= liberal (too many small p-values)

GIF < 1= conservative (too many large p-values)

# PART 3.0. Multivariate EAA

## RDA – Redundancy Analysis (R package **vegan**)

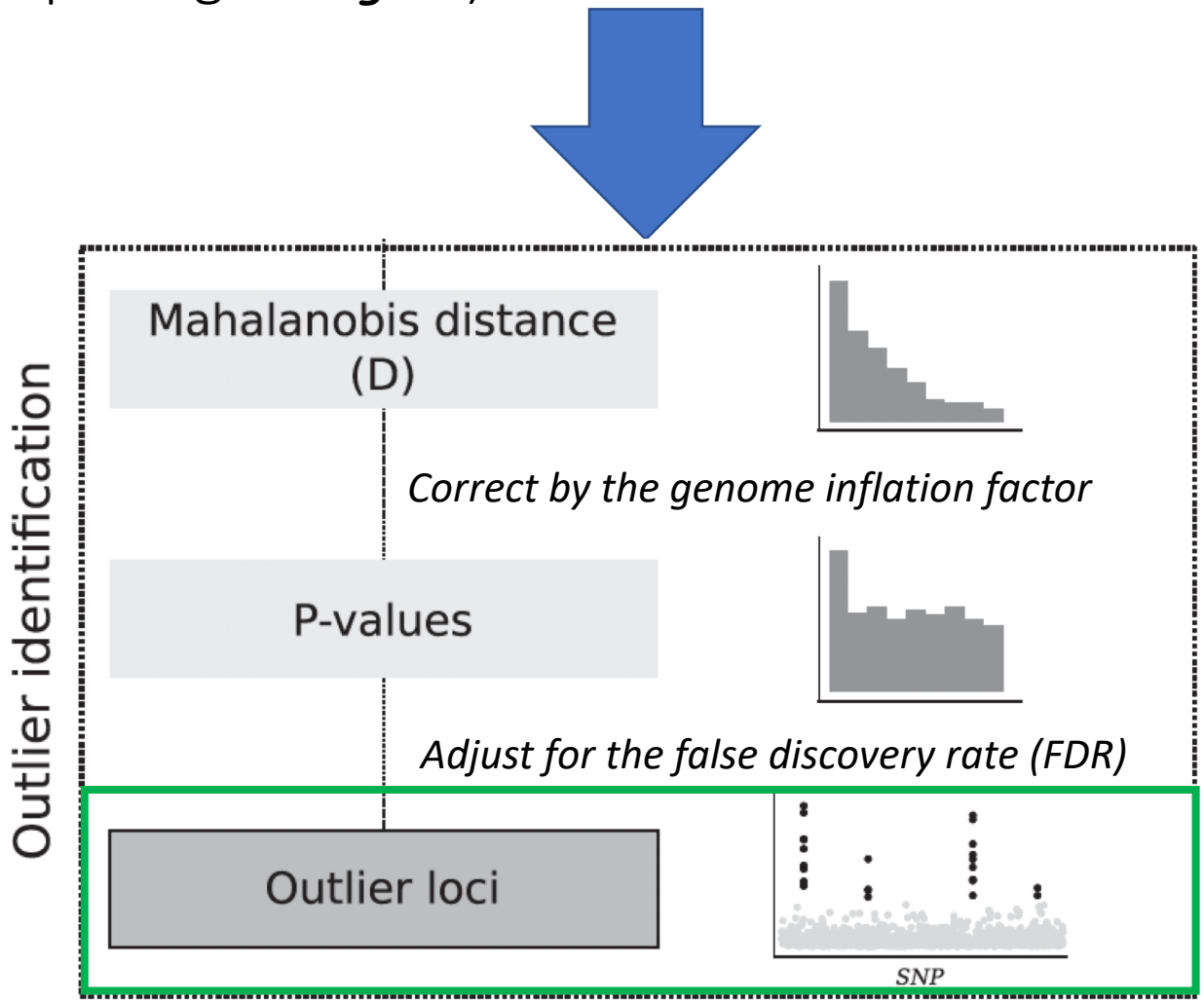



*Recover loci loadings on K number of axes*

*Capblancq et al. 2018*

# PART 3.0. Multivariate EAA

RDA – Redundancy Analysis
(R package **vegan**)




*= a multi-dimensional measure of how many SDs each locus is from the mean distribution*

*Capblancq et al. 2018*

# PART 3.0. Multivariate EAA

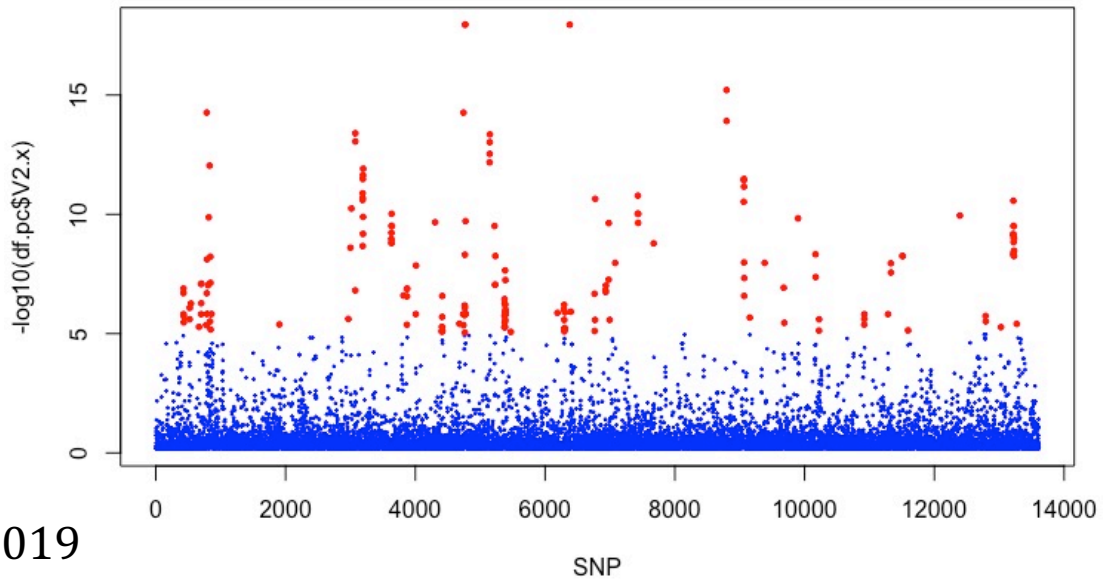
RDA – Redundancy Analysis
(R package **vegan**)

1) Identify outliers using *Mahalanobis distance* approach (Capblancq et al. 2018)
2) Compare default and modified GIFs
3) Identify outliers using a *P-value (SD) cut-off* approach
4) Correlate SD outliers with environmental predictors and plot

# PART 4.0. Univariate EAA

Latent factor mixed modelling **LFMM2**

- Linear mixed model that uses ‘K’ genetic groups as latent factors (representing random effects)
- The environmental variables are the fixed effects
- Each SNP x ENV association is tested separately




See Caye et al. 2019

# PART 4.0. Univariate EAA

Latent factor mixed modelling **LFMM**

1. Run LFMM
2. Examine GIF for each environmental predictor
3. Outlier detection – 1 variable at a time
4. Modify GIF and apply FDR cut-offs
5. *Optional:* Run a PCA on environmental predictors and re-run LFMM - identify PC outliers

# What we'll cover

**Each Part has a separate R script file:**

Part 1.0 The Data:

**Feel free to work with others. Parts 2-4 are independent so can be done in any order. Feel free to pick and choose, or do it all ☺**

Ordination approach using Redundancy analysis (*RDA*)

Part 4.0 Univariate EAA:

Single locus tests with Latent Factor Mixed Models (*LFMM*)