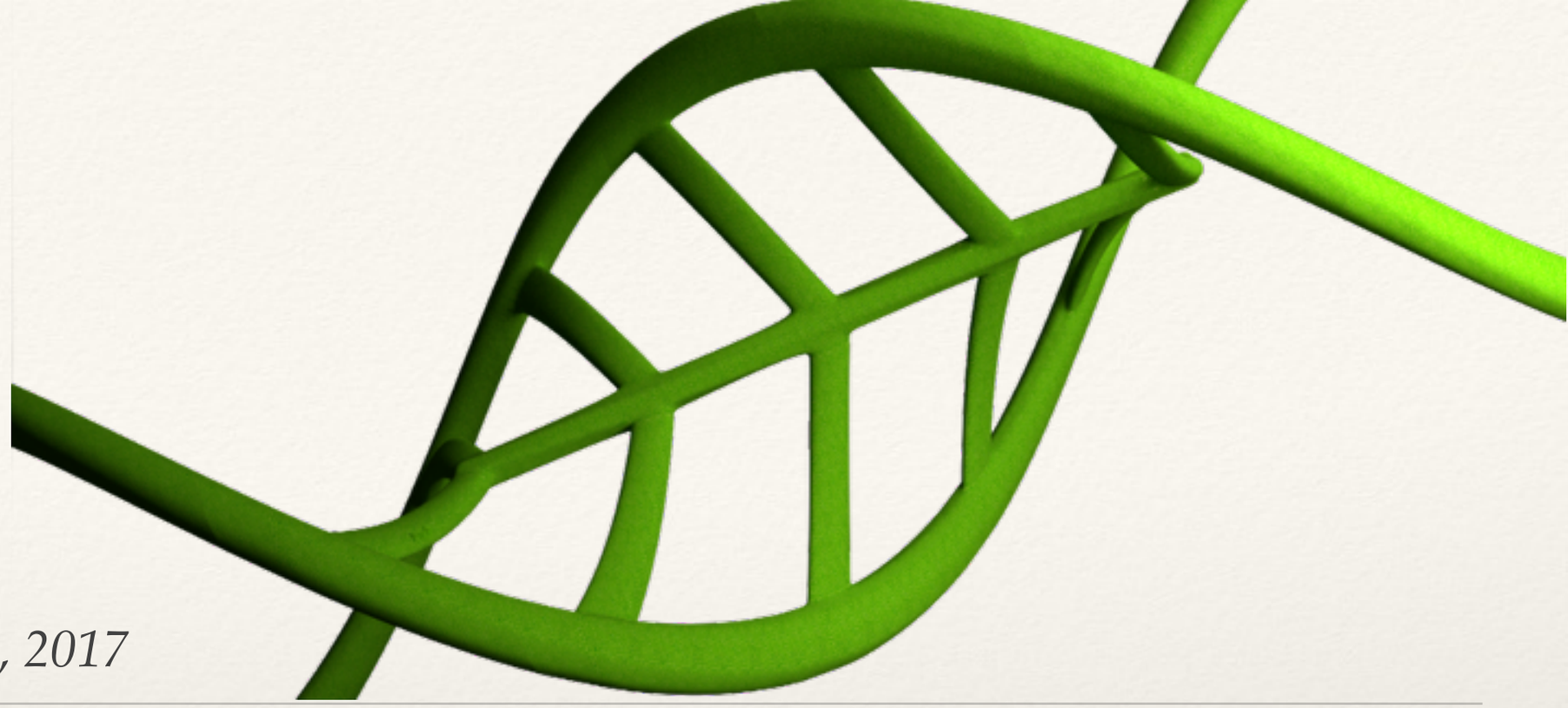
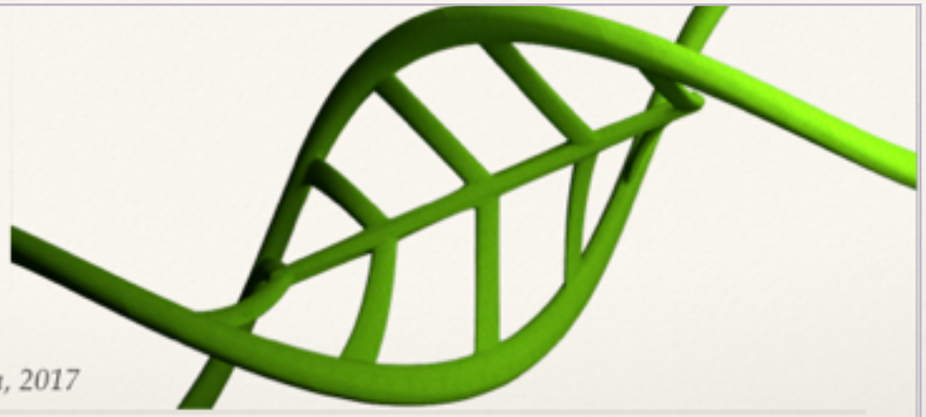


Montpellier, November 7th, 2017

Bioinformatic and analytical tools for the analysis of whole-genome sequence polymorphism data

Sebastián E. Ramos-Onsins

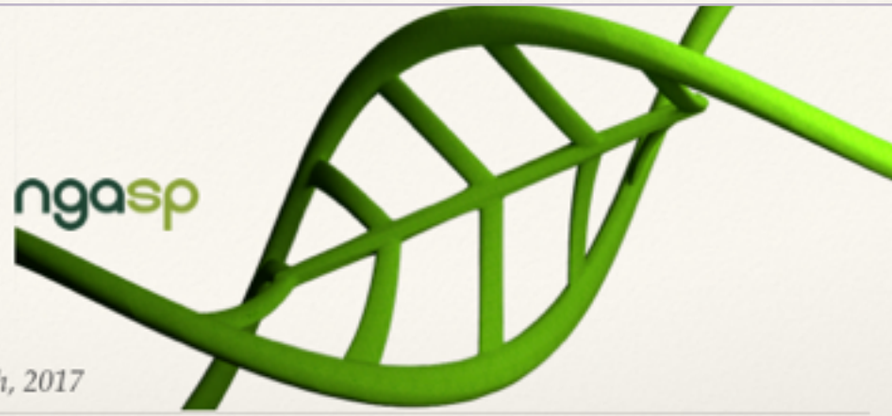
CRAG CENTRE DE RECERCA EN AGRIGENÒMICA
CSIC IRTA UAB



Montpellier, November 7th, 2017

DIGUP: Detection of Incompatible Genealogies Using Unphased Data

Mireia Vidal Villarejo
Luca Ferretti
Sebastián E. Ramos-Onsins



Montpellier, November 7th, 2017

ngasp : A Computational Tool for Population Genomic Analyses of NGS Datasets

Sebastián E. Ramos-Onsins
Gonzalo Vera

Montpellier, November 7th, 2017

ngasp : A Computational Tool for Population Genomic Analyses of NGS Datasets

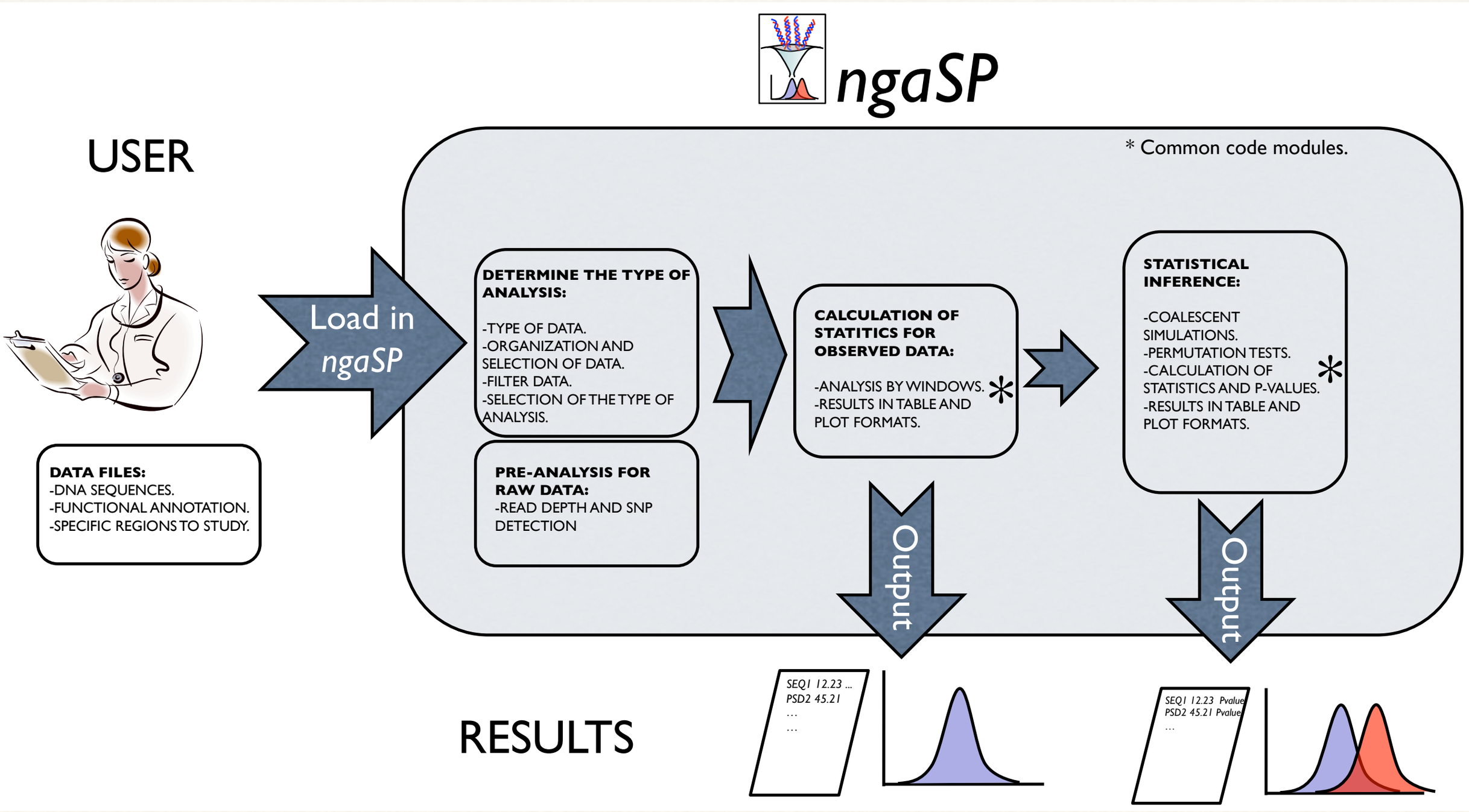
Sebastián E. Ramos-Onsins
Gonzalo Vera

CRAG CENTRE DE RECERCA EN AGRIGENÒMICA
CSIC IRTA UAB

ngasp

- The ***ngasp*** (**n**ext **g**eneration **a**nalyses of **s**equence **p**olymorphisms) starts from the necessity of having a user-friendly tool to perform the analysis of sequence variability dealing with NGS data.

ngasp


ngaSP
USER
Load in ngaSP
DATA FILES:
-DNA SEQUENCES.
-FUNCTIONAL ANNOTATION.
-SPECIFIC REGIONS TO STUDY.
* Common code modules.
DETERMINE THE TYPE OF ANALYSIS:
-TYPE OF DATA.
-ORGANIZATION AND SELECTION OF DATA.
-FILTER DATA.
-SELECTION OF THE TYPE OF ANALYSIS.
PRE-ANALYSIS FOR RAW DATA:
-READ DEPTH AND SNP DETECTION
CALCULATION OF STATITICS FOR OBSERVED DATA:
-ANALYSIS BY WINDOWS. *
-RESULTS IN TABLE AND PLOT FORMATS.
STATISTICAL INFERENCE:
-COALESCENT SIMULATIONS.
-PERMUTATION TESTS.
-CALCULATION OF STATISTICS AND P-VALUES. *
-RESULTS IN TABLE AND PLOT FORMATS.
Output
Output
SEQ1 12.23 ...
PSD2 45.21
...
...
SEQ1 12.23 Pvalue
PSD2 45.21 Pvalue
...
RESULTS

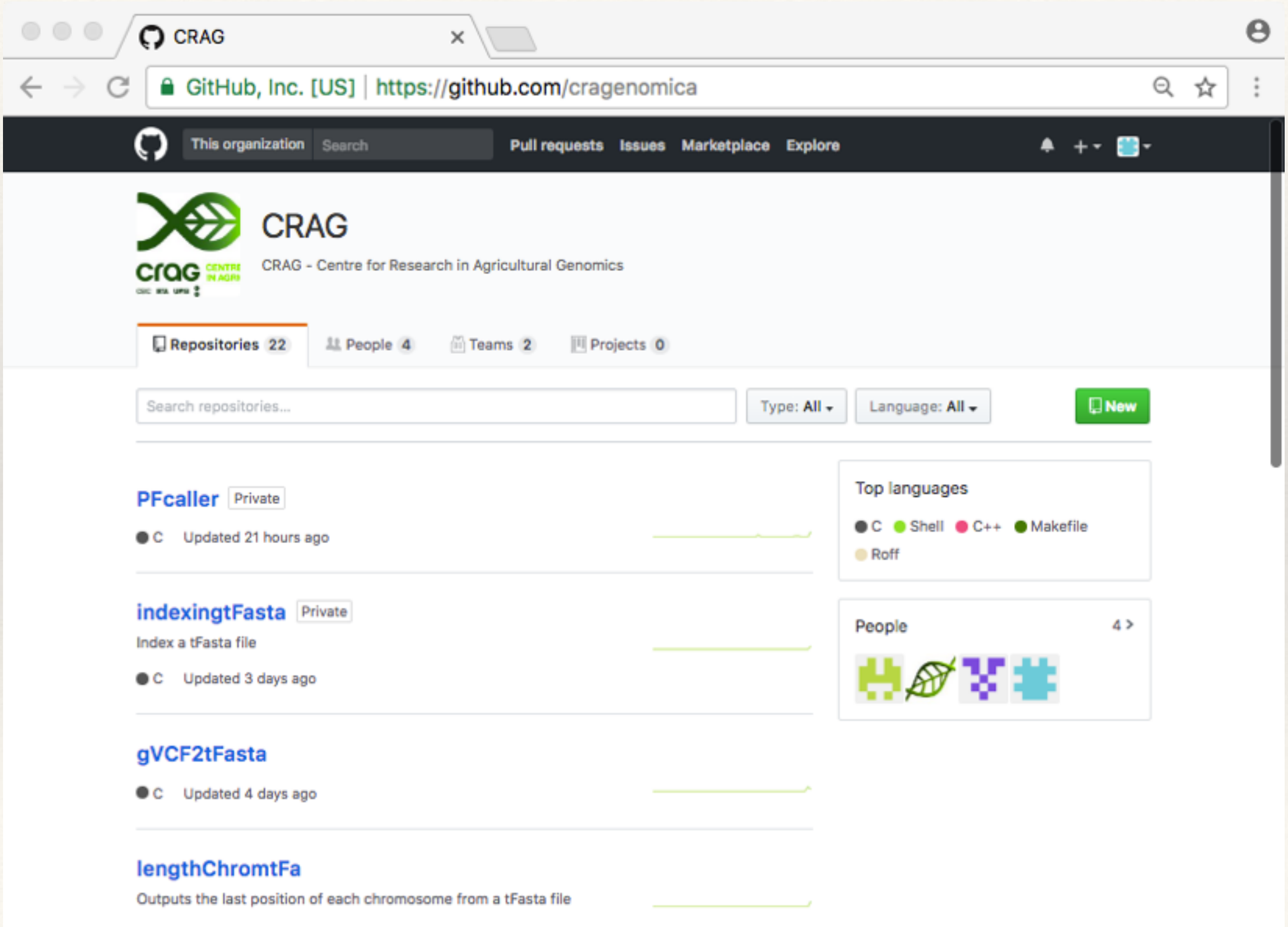
ngasp

- Project in collaboration with computer engineers (in CRAG and in the school of Engineers at the UAB)
- Includes multiple software tools for population genetic analysis (own-in-house and external):
  - manage BAM and gVCF and fasta files. Defined tfasta format.
  - SNP callers (pooled data, polyploid, diploid, haploid data).
  - Format converter tools.
  - Filter tools (BED files, GFF annotation).
  - Tools for sequence analyses with missing data.
  - Whole data or Sliding windows analysis..
  - Outputs: plots and/or tables.
- A web and graphical interface to manage project analysis as well as command line (JavaScript).
- Different kinds of users:
  - Experimental designer (final user)
  - Pipeline designer
  - Calculation designer
- Incorporating computational optimizations using distributed architectures.

Software for Analysis of Variability of NGS data

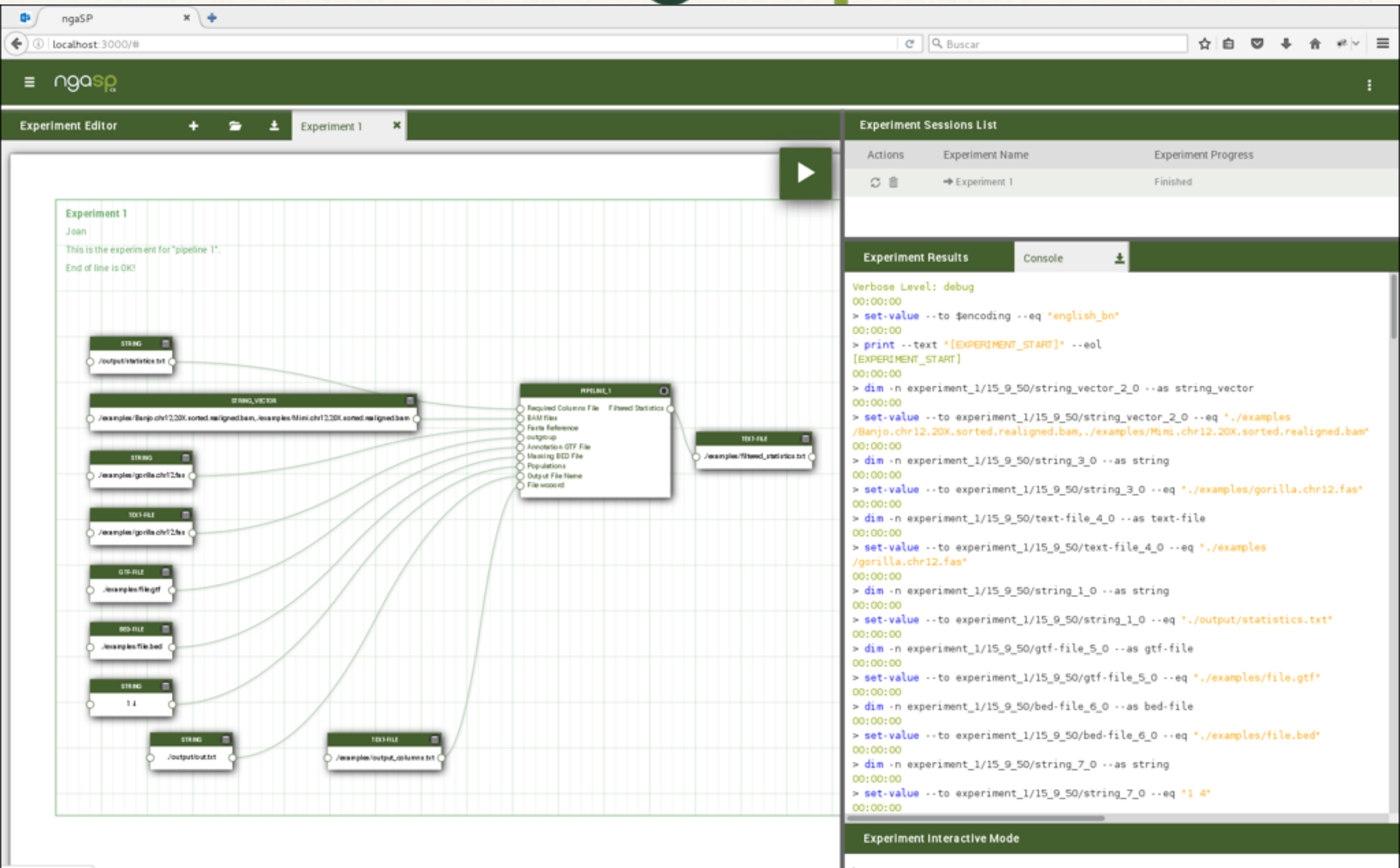
https://bioinformatics.cragenomica.es/projects/ngaSP

https://github.com/cragenomica

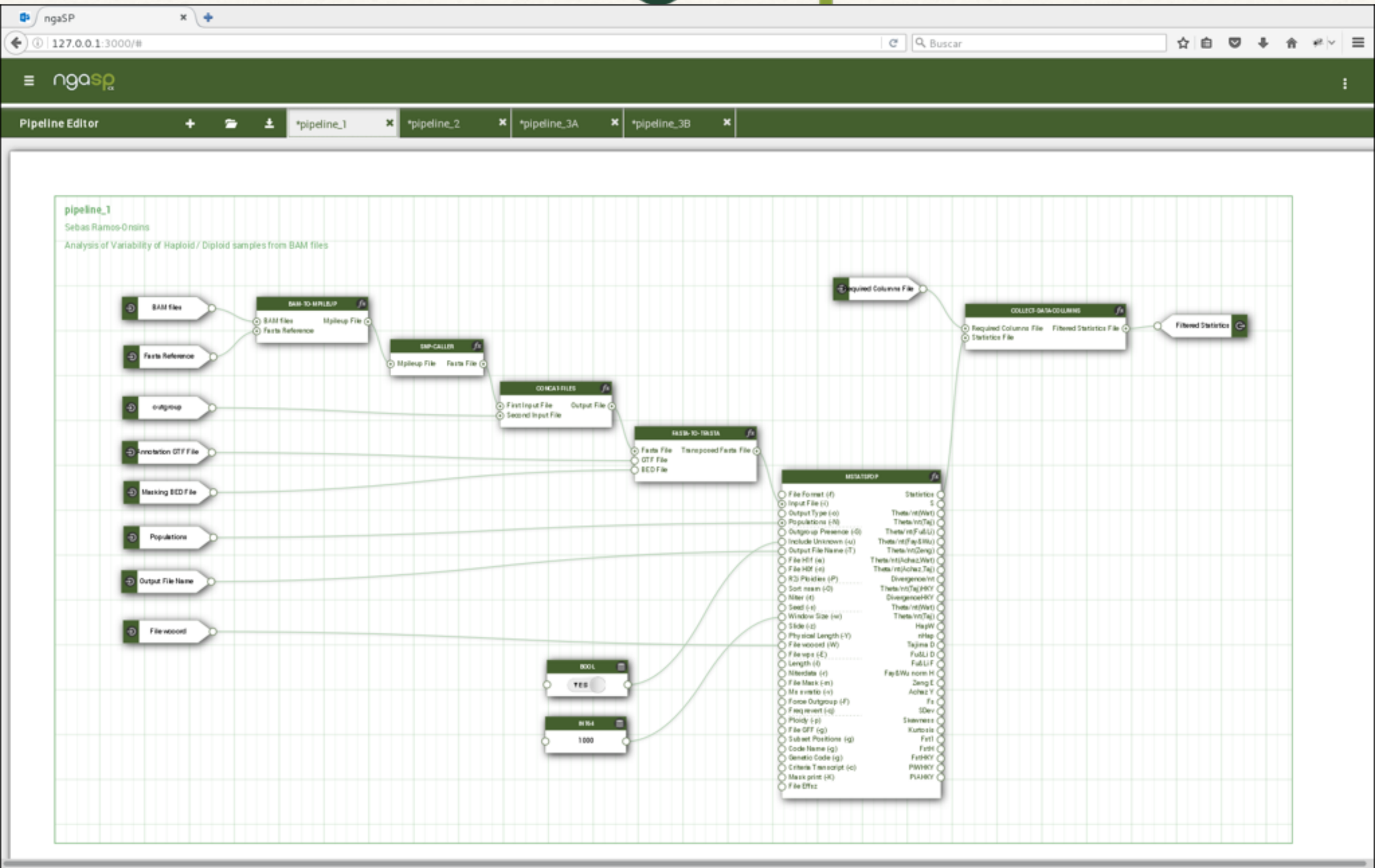


https://bioinformatics.cragenomica.es/projects/ngaSP

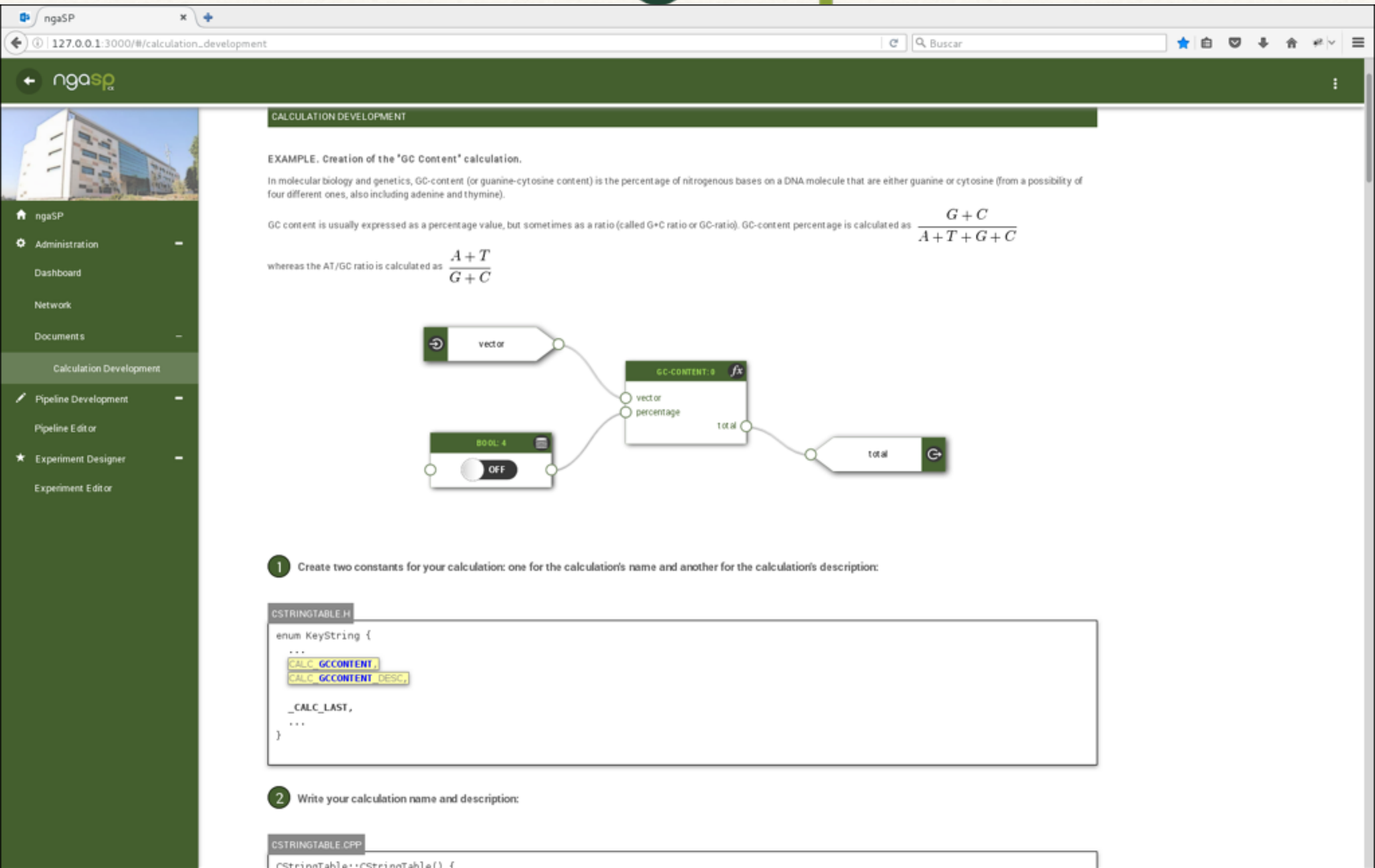
Experimental designer



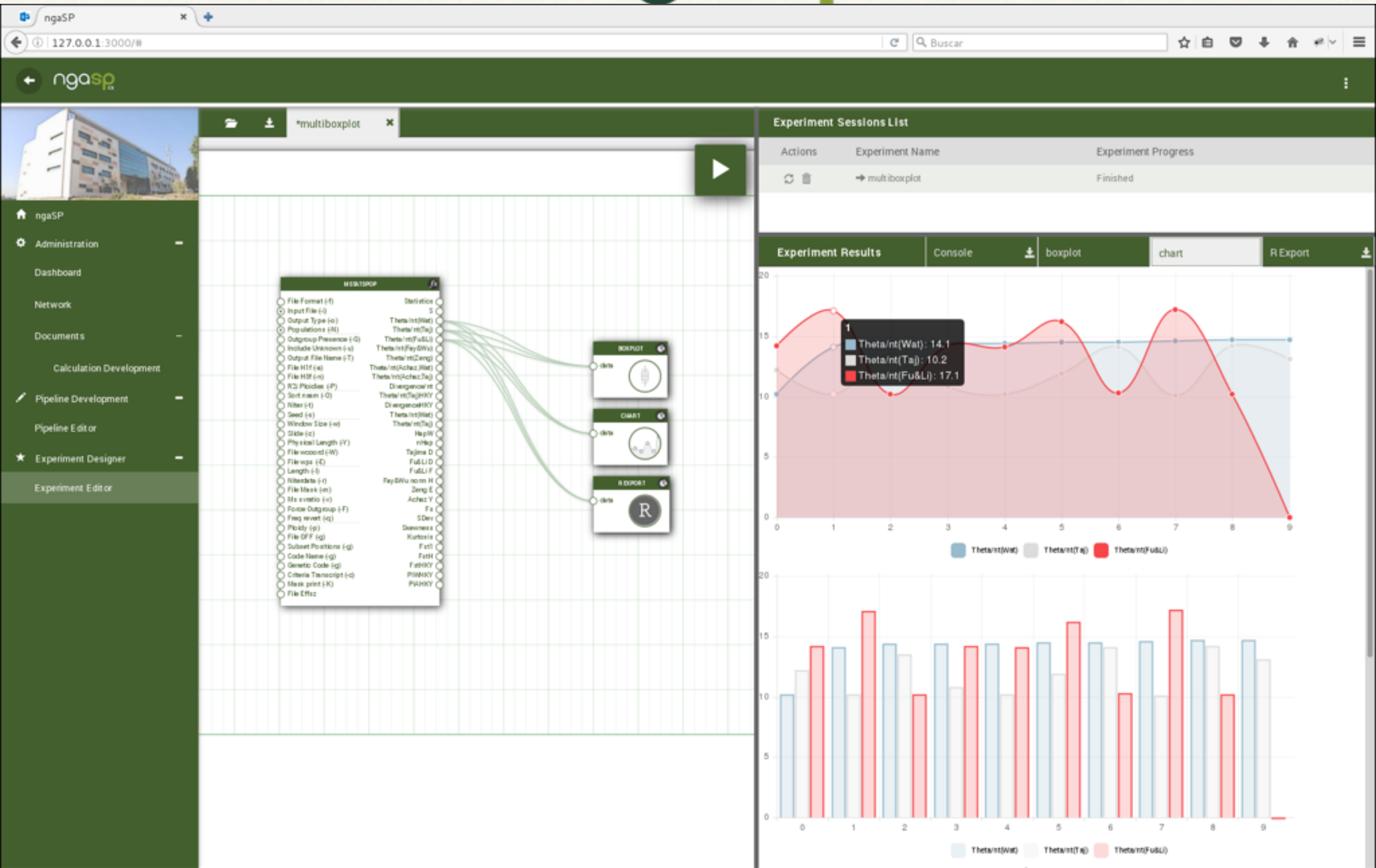
Pipeline developer



Developer of Calculation boxes

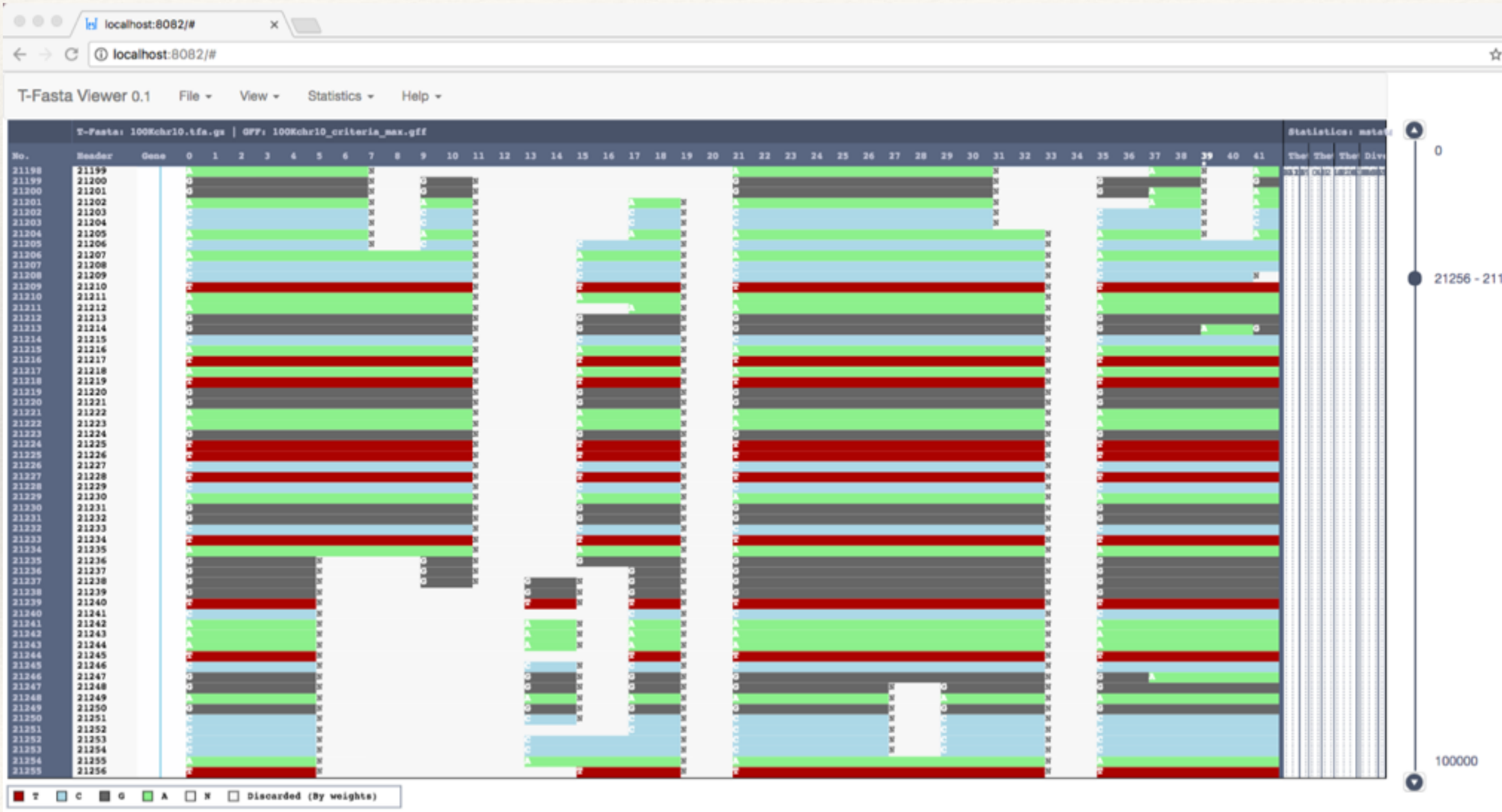


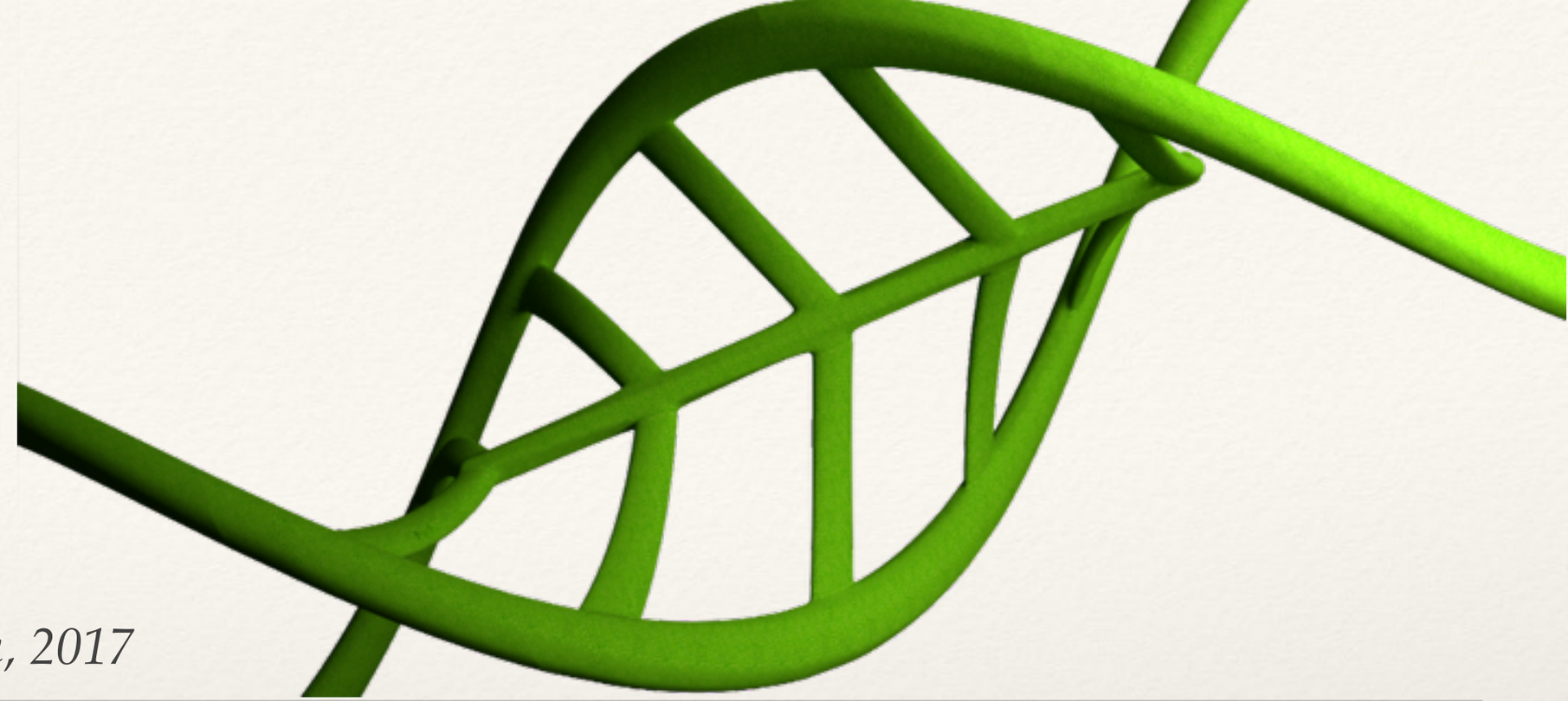
Output results



Output results

Output results





Montpellier, November 7th, 2017

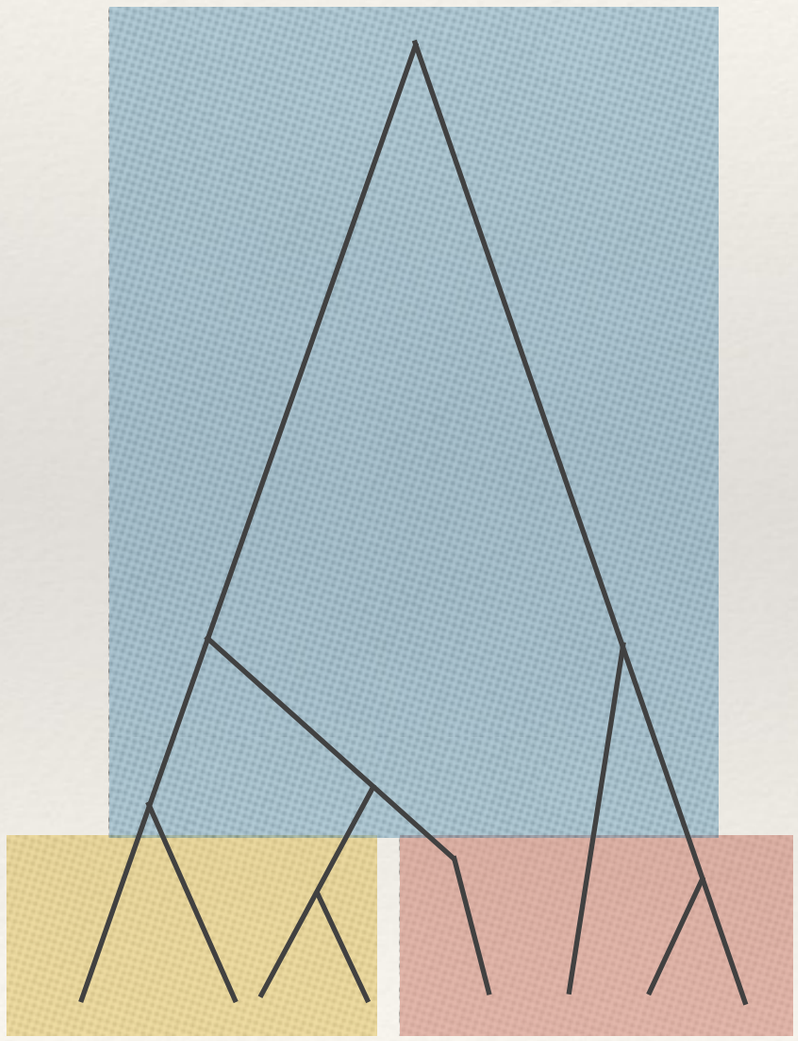
DIGUP: Detection of Incompatible Genealogies Using Unphased Data

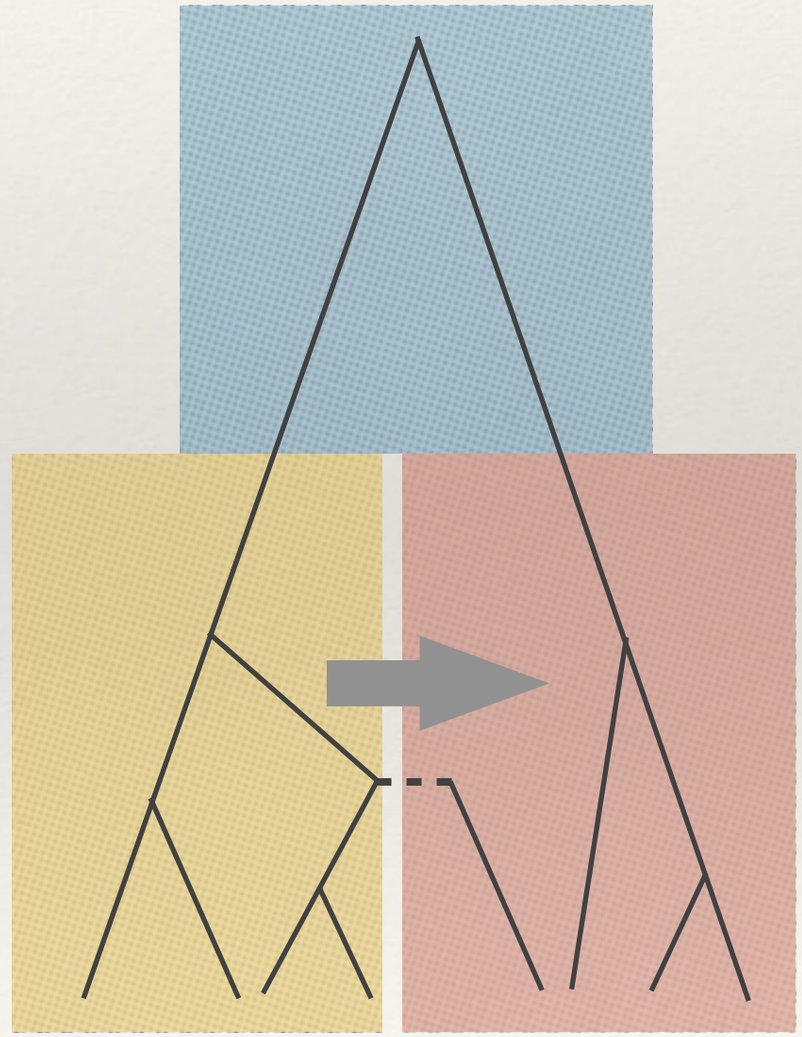
Mireia Vidal Villarejo
Luca Ferretti
Sebastián E. Ramos-Onsins

CRAG CENTRE DE RECERCA EN AGRIGENÒMICA
CSIC IRTA UAB

Ancestral Polymorphism vs Migration, Recombination and Incompatible Genealogies

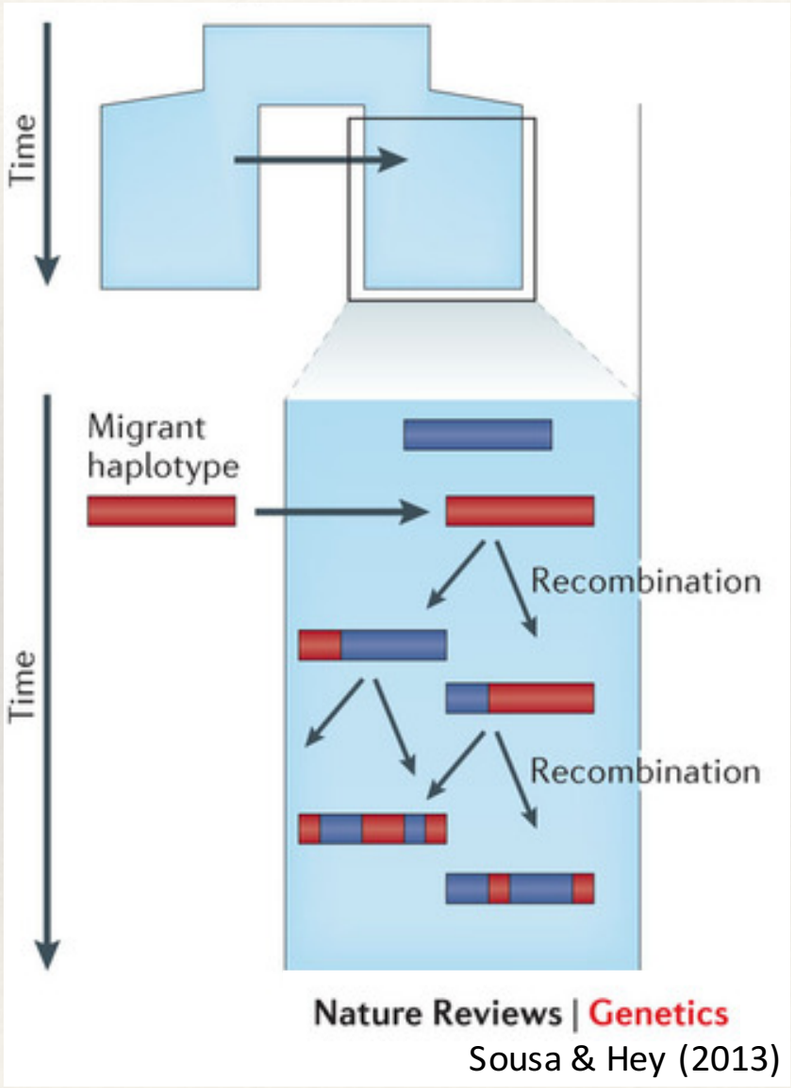
- Ancestral Polymorphism and Migration can be confounded:





Ancestral Polymorphism vs Migration, Recombination and Incompatible Genealogies

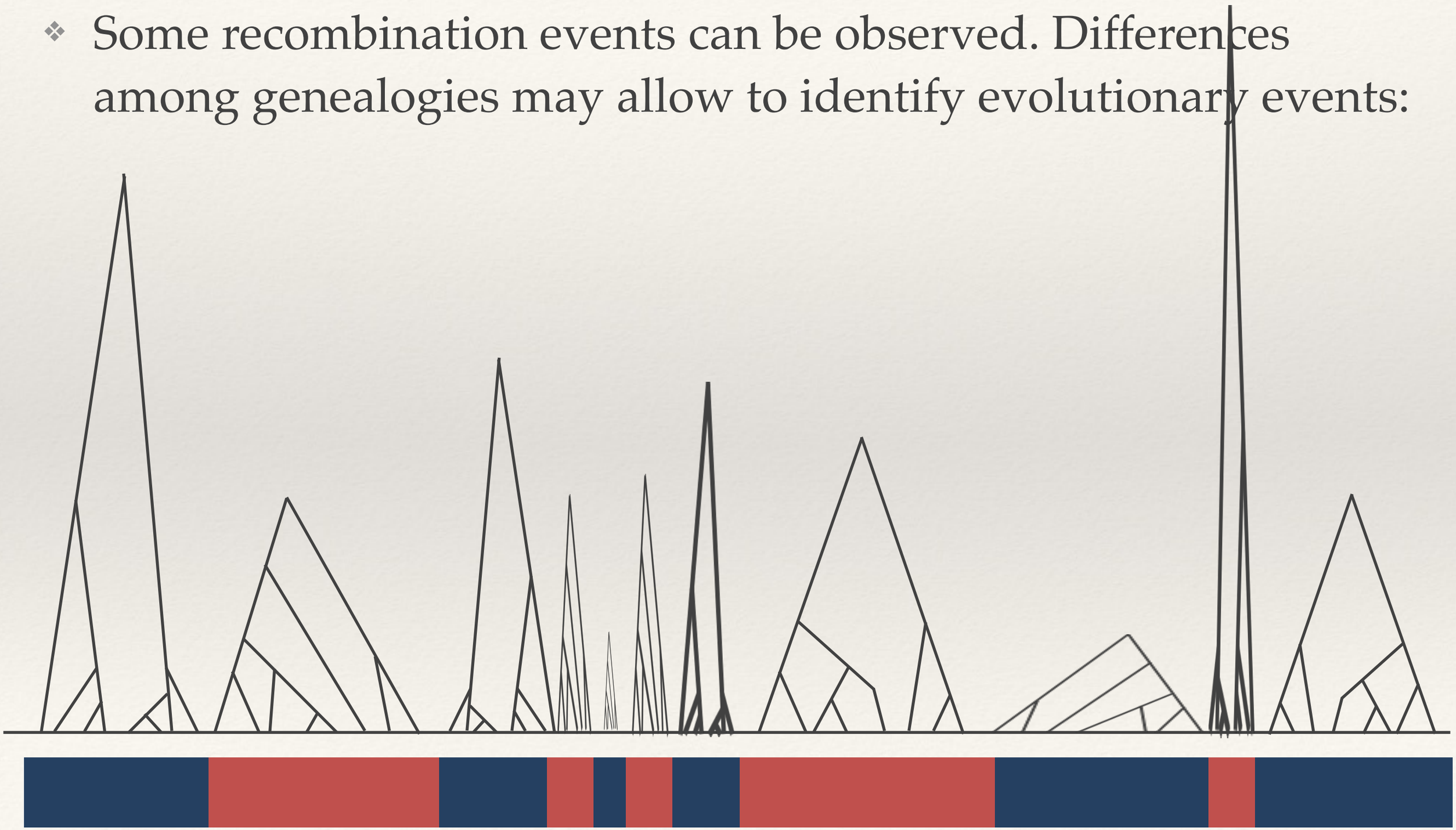
- Recombination cut and join different genealogies:



Sousa & Hey (2013)

Ancestral Polymorphism vs Migration, Recombination and Incompatible Genealogies

- Some recombination events can be observed. Differences among genealogies may allow to identify evolutionary events:



Ancestral Polymorphism vs Migration, Recombination and Incompatible Genealogies

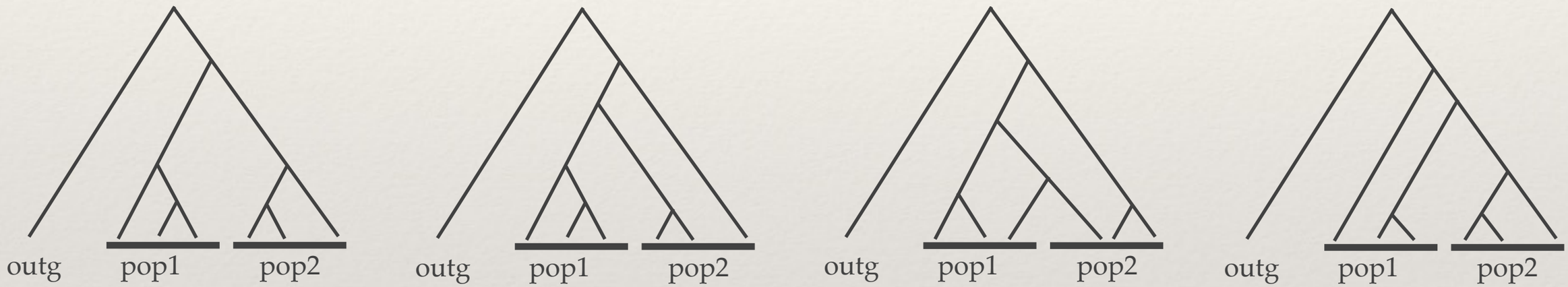
- Pooled data adds complexity to the study:
  - For each position, different individuals are considered, and also different sample sizes can be used.
    - The genealogy of a region (or a position) can not be directly compared because the samples are different.
- Missing data can be considered as a similar problem, as we can have information from different individuals of the populations with different sample sizes per position.

Study of the Variability in Populations

- We aim to:
  - Design simple statistics and algorithms that describe the variability among populations involved in the genome.
  - Detect incompatible genealogies and their lengths across the genome, using unphased data.
  - Study the expected patterns of these statistics (or algorithms) under different conditions.

Methodology

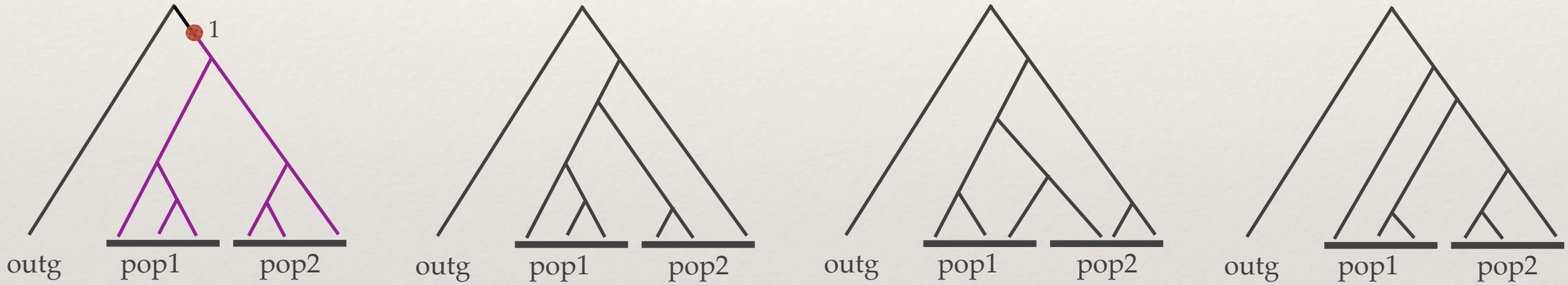
- Find incompatible genealogies along the genome considering TWO populations and one ancestral outgroup population (4 rooted genealogies):



- We define three possible states for each population:
  - **A**: Ancestral (all samples equal to the outgroup)
  - **D**: Derived (all samples different to the outgroup)
  - **P**: Polymorphic

Methodology

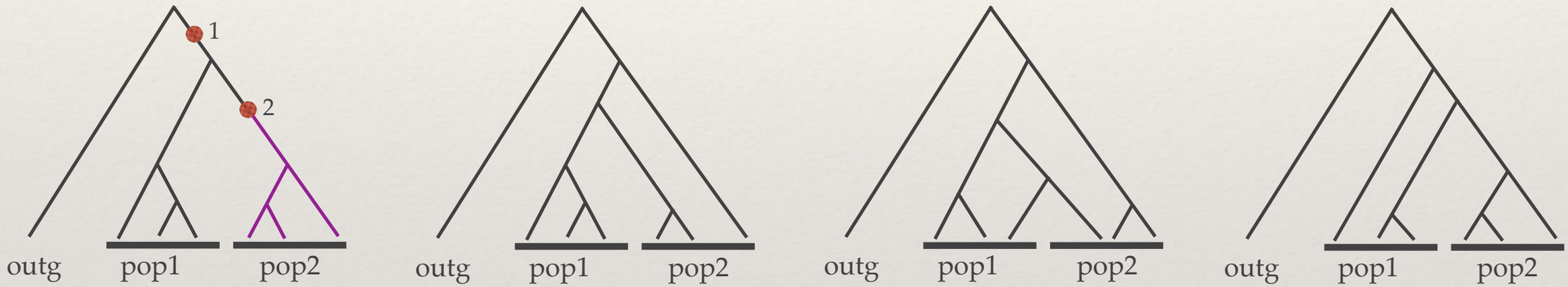
- Find incompatible genealogies along the genome considering TWO populations and one ancestral outgroup population:



1	D	D

Methodology

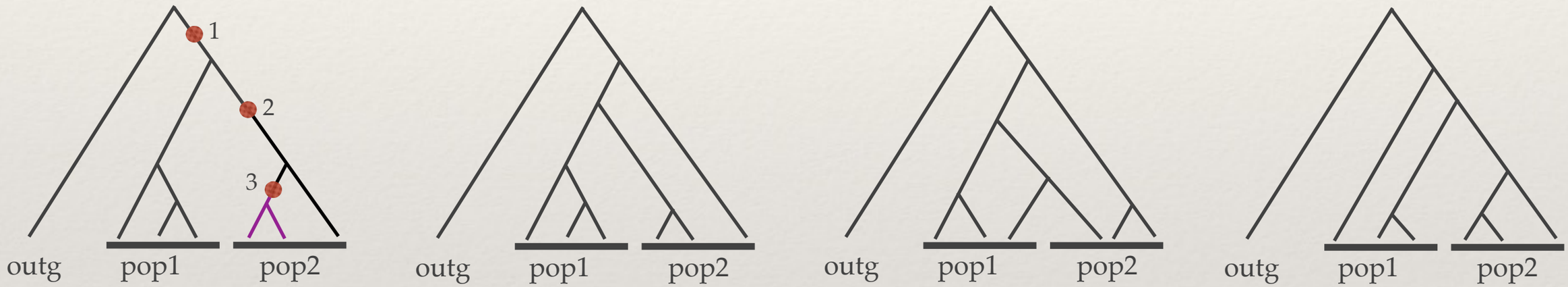
- Find incompatible genealogies along the genome considering TWO populations and one ancestral outgroup population:



1	D	D
2	A	D

Methodology

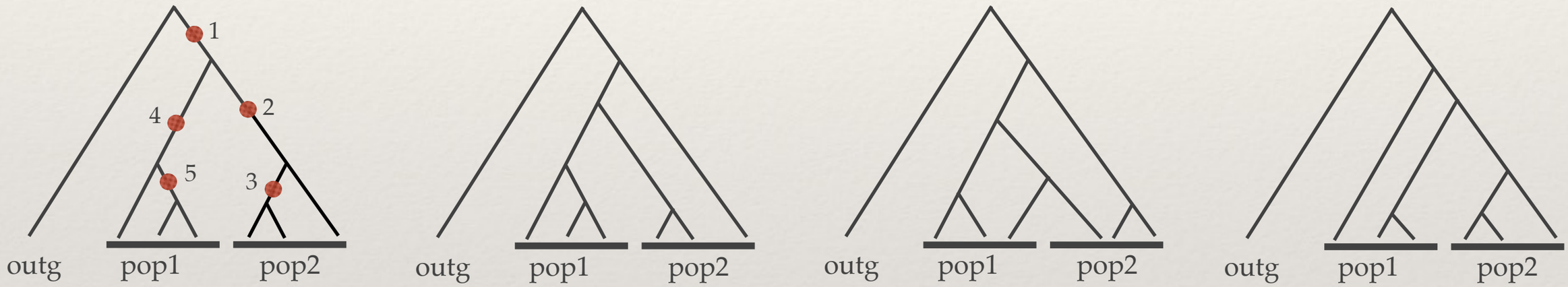
- Find incompatible genealogies along the genome considering TWO populations and one ancestral outgroup population:



1	D	D
2	A	D
3	A	P

Methodology

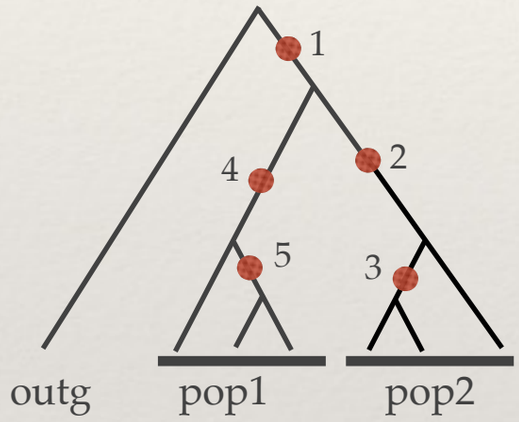
- Find incompatible genealogies along the genome considering TWO populations and one ancestral outgroup population:



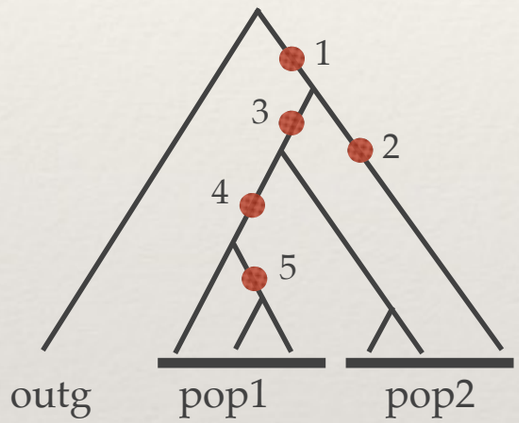
1	D	D
2	A	D
3	A	P
4	D	A
5	P	A

Methodology

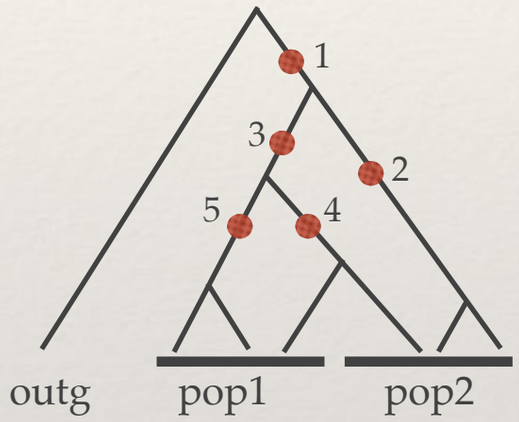
- Find incompatible genealogies along the genome considering TWO populations and one ancestral outgroup population:



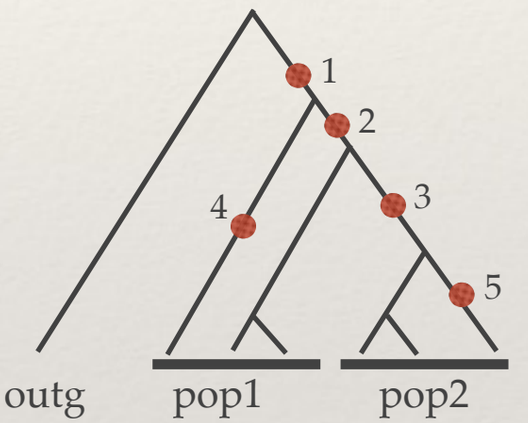
1	D	D
2	A	D
3	A	P
4	D	A
5	P	A



1	D	D
2	A	P
3	D	P
4	D	A
5	P	A



1	D	D
2	A	P
3	D	P
4	P	P
5	P	A

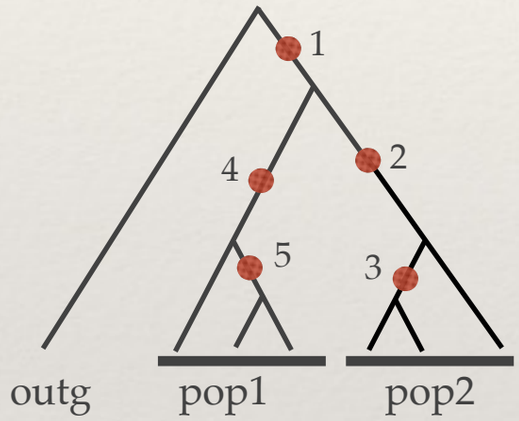


1	D	D
2	P	D
3	A	D
4	P	A
5	A	P

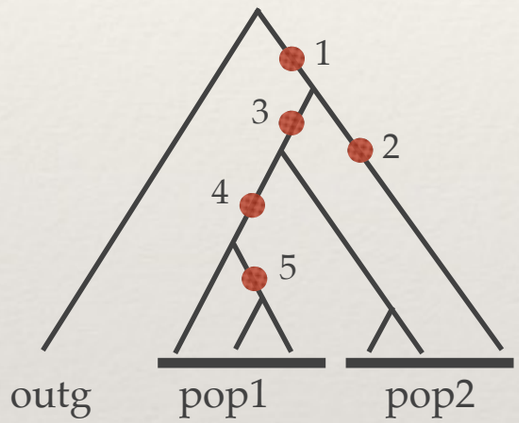
Methodology

- Find incompatible genealogies along the genome considering TWO populations and one ancestral outgroup population:

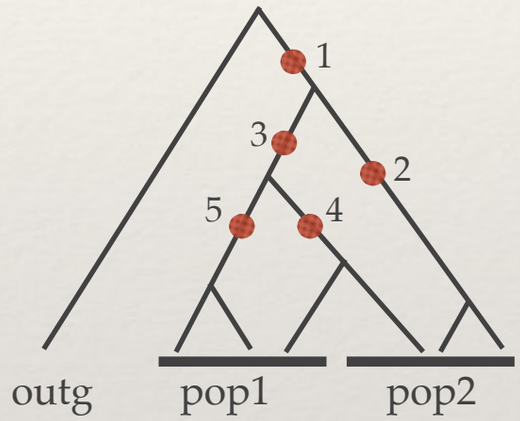
COMPATIBLE COMBINATIONS?



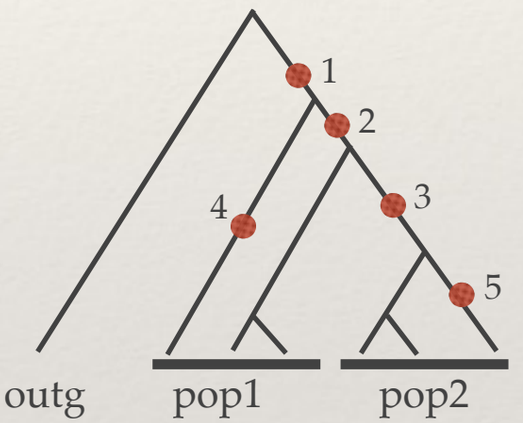
1	D	D
2	A	D
3	A	P
4	D	A
5	P	A



1	D	D
2	A	P
3	D	P
4	D	A
5	P	A



1	D	D
2	A	P
3	D	P
4	P	P
5	P	A

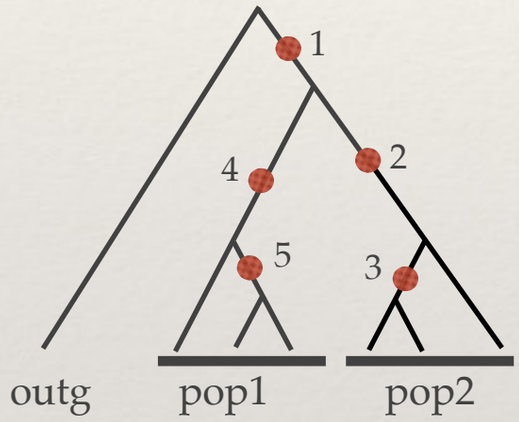


1	D	D
2	P	D
3	A	D
4	P	A
5	A	P

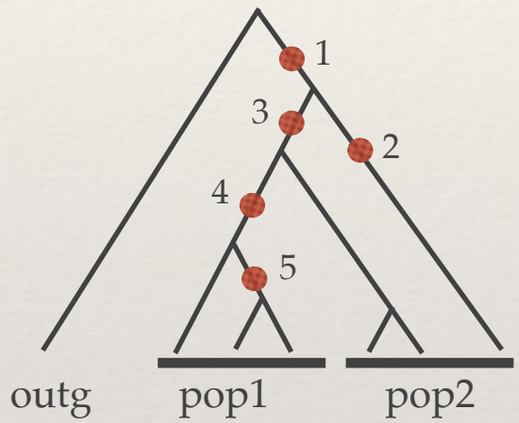
Methodology

- Find incompatible genealogies along the genome considering TWO populations and one ancestral outgroup population:

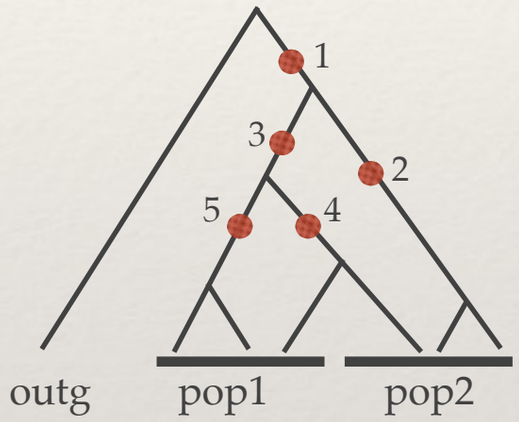
INCOMPATIBLE COMBINATIONS?



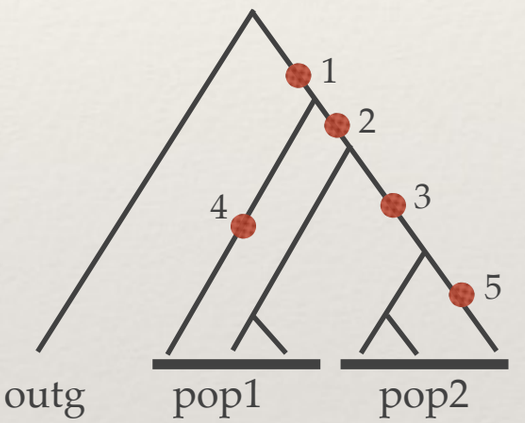
1	D	D
2	A	D
3	A	P
4	D	A
5	P	A



1	D	D
2	A	P
3	D	P
4	D	A
5	P	A



1	D	D
2	A	P
3	D	P
4	P	P
5	P	A



1	D	D
2	P	D
3	A	D
4	P	A
5	A	P

Methodology

- Find incompatible genealogies along the genome considering TWO populations and one ancestral outgroup population:

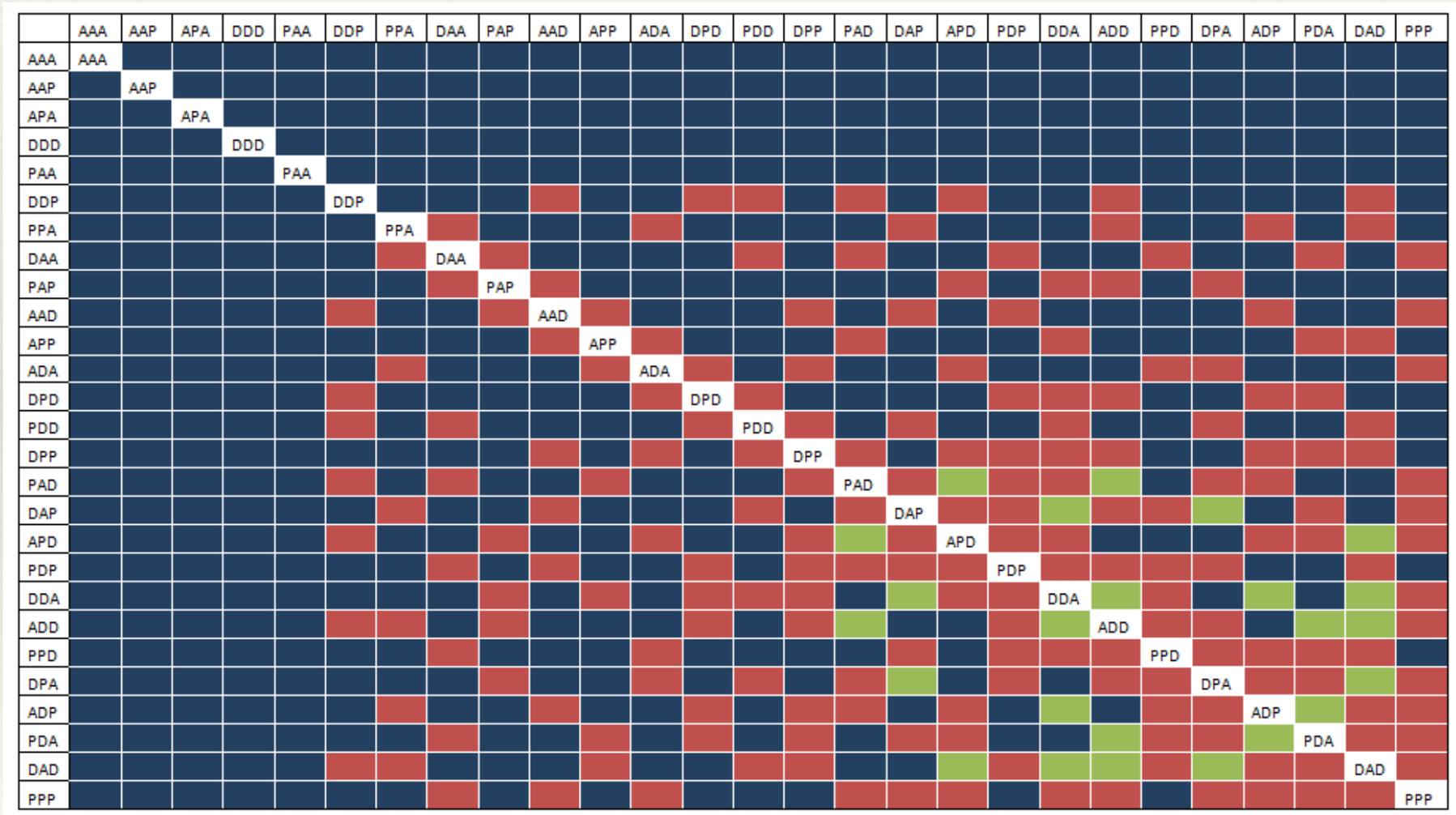
INCOMPATIBLE COMBINATIONS

	AA	PA	AP	DD	PP	DA	AD	DP	PD
AA	AA								
PA		PA							
AP			AP						
DD				DD					
PP					PP				
DA						DA			
AD							AD		
DP								DP	
PD									PD

RED: incompatible combinations

Methodology

- Find incompatible genealogies along the genome considering THREE populations and one ancestral outgroup population (105 rooted bifurcating genealogies):



RED: incompatible combinations in two populations.

GREEN: incompatible combinations in three populations.

Methodology

- From these two simple examples we infer two main rules of incompatibility:

  - Incompatibility between two pops:
    AD vs DP
    AD vs PP
    PD vs DP

  - Incompatibility between three pops:
    DAX vs DXA (X can be D or P)

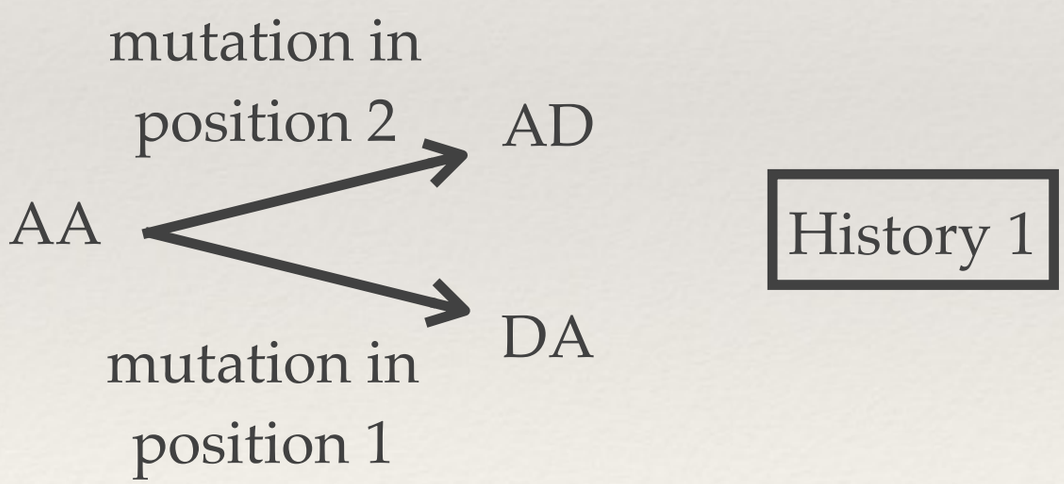
Methodology

- Find incompatible genealogies along the genome considering N populations and one ancestral outgroup:

  All incompatibilities between states are obtained by combinations of two or three populations.

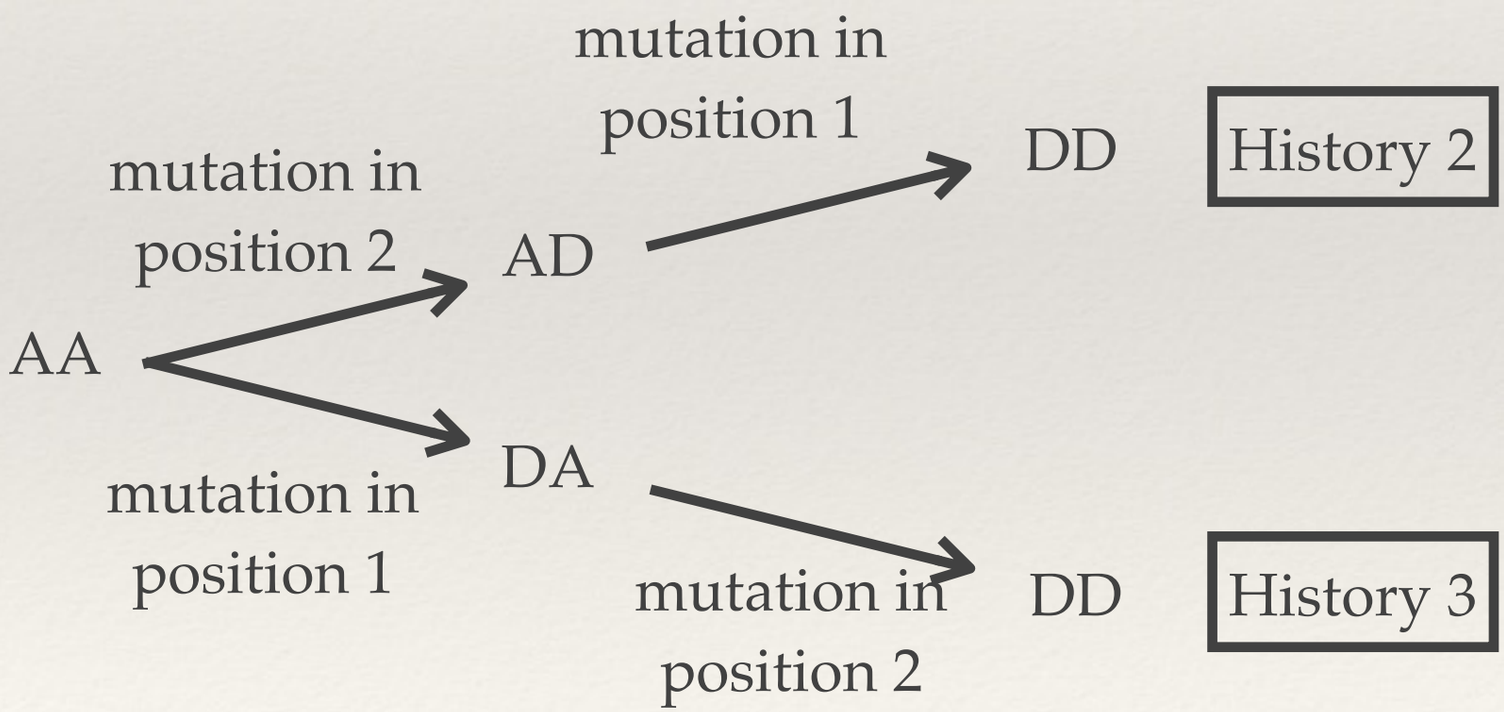
Methodology

- **The rule of the four haplotypes for two positions:** Assuming no recurrent mutation and having no recombination (same genealogy), no more than three different haplotypes can be formed.



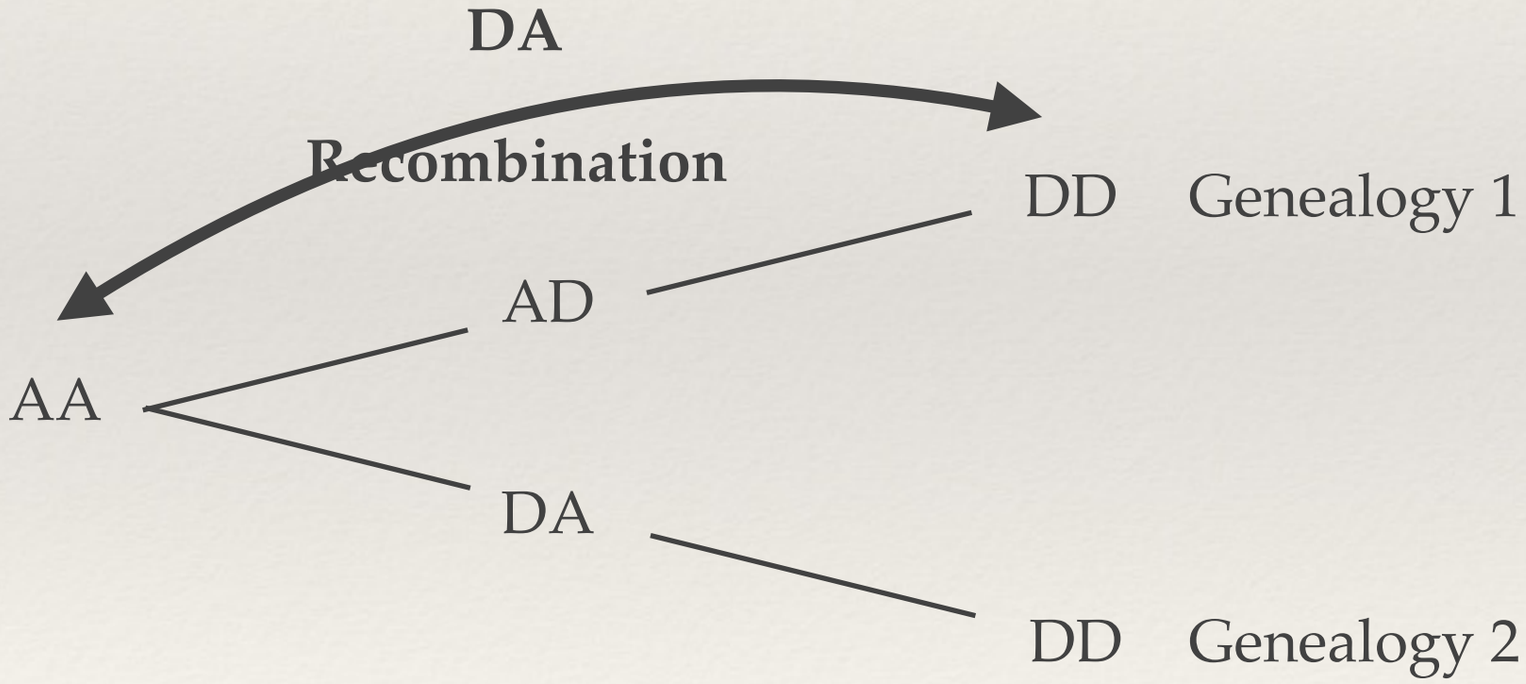
Methodology

- **The rule of the four haplotypes for two positions:** Assuming no recurrent mutation and having no recombination (same genealogy), no more than three different haplotypes can be formed.



Methodology

- **The rule of the four haplotypes for two positions:** Assuming no recurrent mutation and having no recombination (same genealogy), no more than three different haplotypes can be formed.



Methodology

- **The rule of the four haplotypes for two positions:** Assuming no recurrent mutation and having no recombination (same genealogy), no more than three different haplotypes can be formed.

- As expected, all combinations producing incompatibilities between genealogies have the four possible haplotypes.

- No more than three populations (plus the outgroup) are necessary to observe the four haplotypes (that is, one haplotype per population).

Methodology

- Selecting the incompatible fragments:

```
+-----------------+
| GENERAL SUMMARY |
+-----------------+
Type    #positions

AAP     383
DDD     1129
PAP     33
DPP     10
DDP     91
APA     214
PAA     204
PPP     40
APP     8
DAP     3
PDP     8
PPA     24
AAD     18
DDA     14
DPD     1
PPD     2
DPA     6
APD     5
```

1. Look for all types of variants.
2. Find the incompatible combinations.
3. Sort each state by its position.
4. Assign the fragments that are incompatible with the contiguous.

Methodology

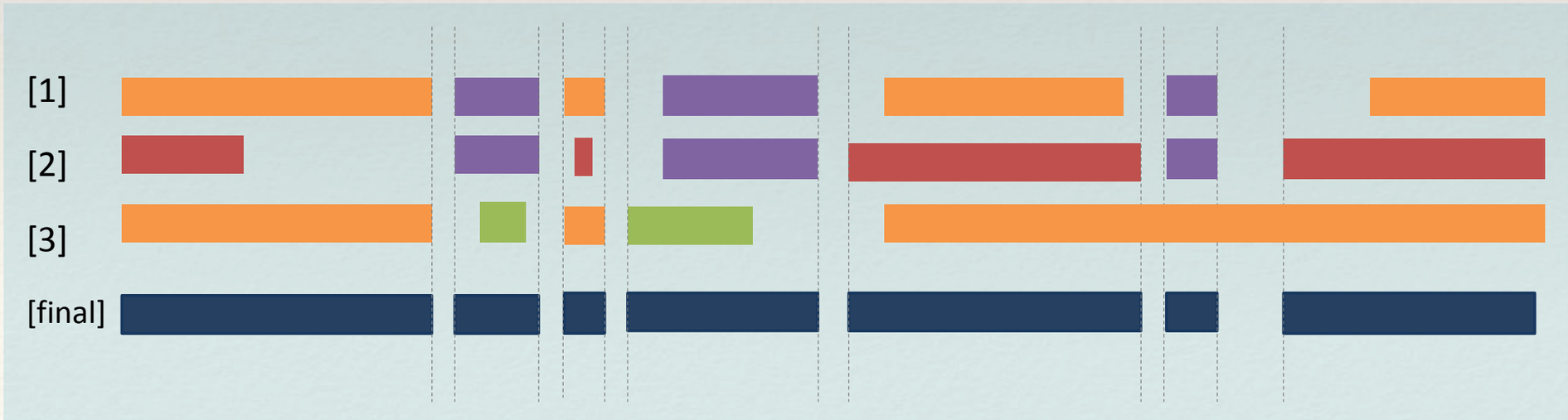
- Selecting the incompatible fragments:

[9a] DPP 12,15,16,22,25,35,45,47,64
[9b] APD 3,4,5,8,19,20,29,32,33,36,54,58,72,90

3,4,5,8, 12,15,16,19,20, 22,25, 29,32,33, 35,36,45,47,54,58,64,72,90

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

Using all incompatibility combinations, we can have some overlapping:



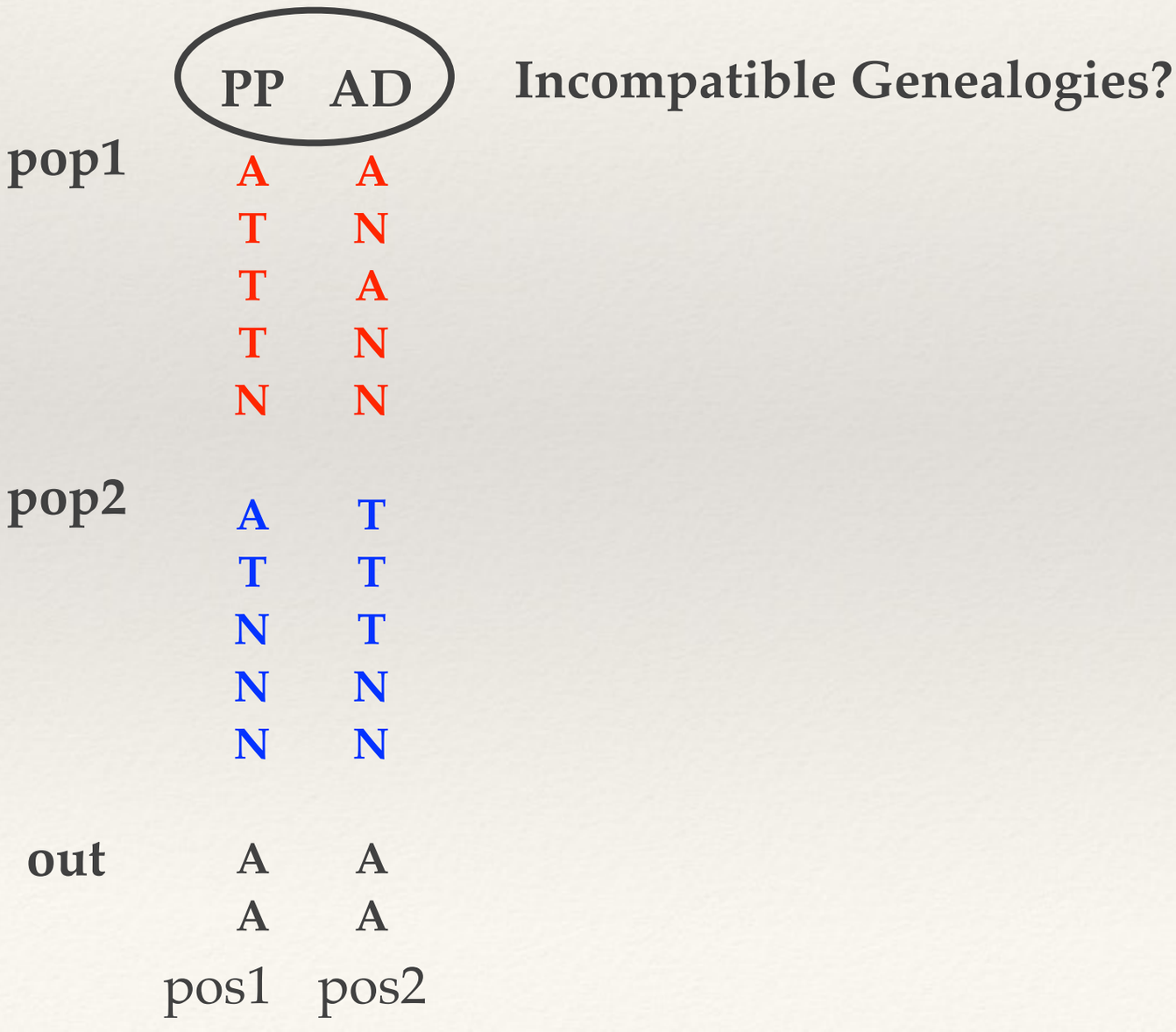
Methodology: missing data

- Considering a weight factor for positions having missing data:

	PP	AD
pop1	A	A
	T	A
	T	A
	T	N
	N	N
pop2	A	T
	T	T
	N	T
	N	N
	N	N
out	A	A
	A	A
	pos1	pos2

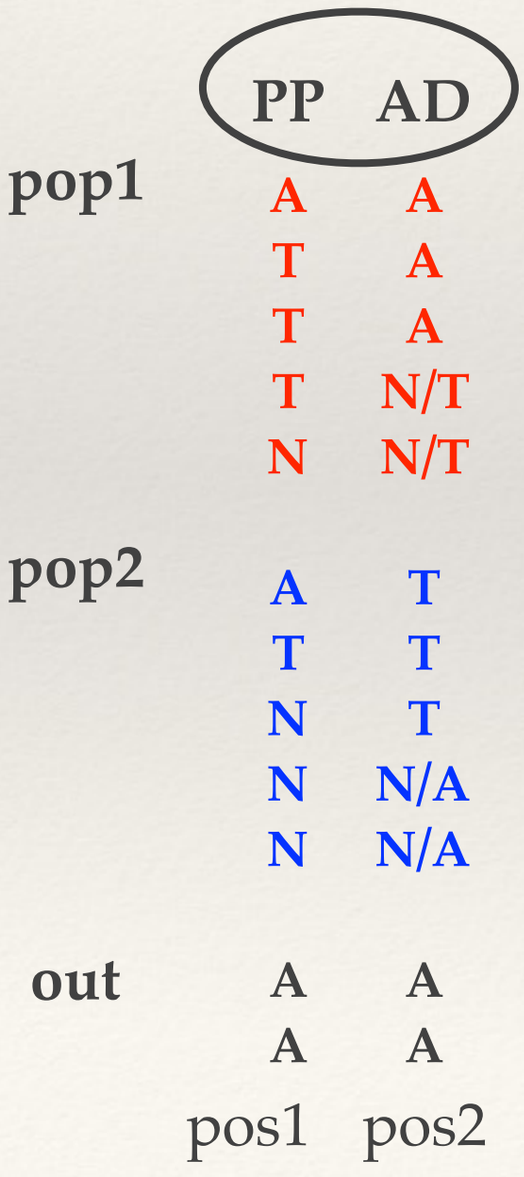
Methodology: missing data

- Consider a weight factor for positions having missing data:



Methodology: missing data

- Consider a weight factor for positions having missing data:



Incompatible Genealogies?

Given the missing data, AD may be PD, or AP, or PP, and the order of the individuals within population is unknown, the genealogies would be compatible!

Methodology: missing data

- Similarity of missing data versus pooled data:

INCOMPATIBLE FRAGMENTS				
[...end]	[start...]	comb1	comb2	w
1847	4428	DDPP	PADD	1.0
4428	5521	PADD	PDPP	1.0
5521	6786	PDPP	PADP	0.99
6786	8164	PADP	PPPP	0.99
8164	9163	PPPP	PDAA	1.0
10245	11552	PAPP	AAPD	0.96
11552	12316	AAPD	PAPP	0.99
13993	14080	PDPP	PAPD	0.9
14080	14527	PAPD	PDPP	0.99
14533	14861	PDPP	AAPD	0.9
14861	15104	AAPD	PAPP	0.99
15581	15701	PPPP	PDAP	1.0
16938	17290	PAPP	PADA	0.99
17290	17767	PADA	PDPD	0.99
18409	18681	PDPP	PADP	0.99
18681	22726	PADP	PDPP	1.0
22726	22961	PDPP	DAPA	0.56
22961	23511	DAPA	PPAA	1.0
24441	24799	AADP	AAPD	0.99
25946	27251	AAPD	PDPP	0.99
28820	29028	DDPP	AAPD	0.96
29292	29548	AAPD	DDPP	0.99
29548	29599	DDPP	AAPD	0.97
29742	30004	PDPD	PPDD	1.0
30004	30085	PPDD	DDPP	1.0
30552	30715	DDPP	AAPD	0.97

- The probability that we have in the entire sample a state (A or D) given the observation with missing data can be calculated:

$$P(\text{Ant} \mid \text{Ans}) + P(\text{Pnt} \mid \text{Ans}) = 1$$
$$P(\text{Dnt} \mid \text{Dns}) + P(\text{Pnt} \mid \text{Dns}) = 1$$

- Assuming a simple model of polymorphism versus divergence for each population, these probabilities are easily obtained using conditional probabilities and coalescent theory.

Results: Coalescent simulations

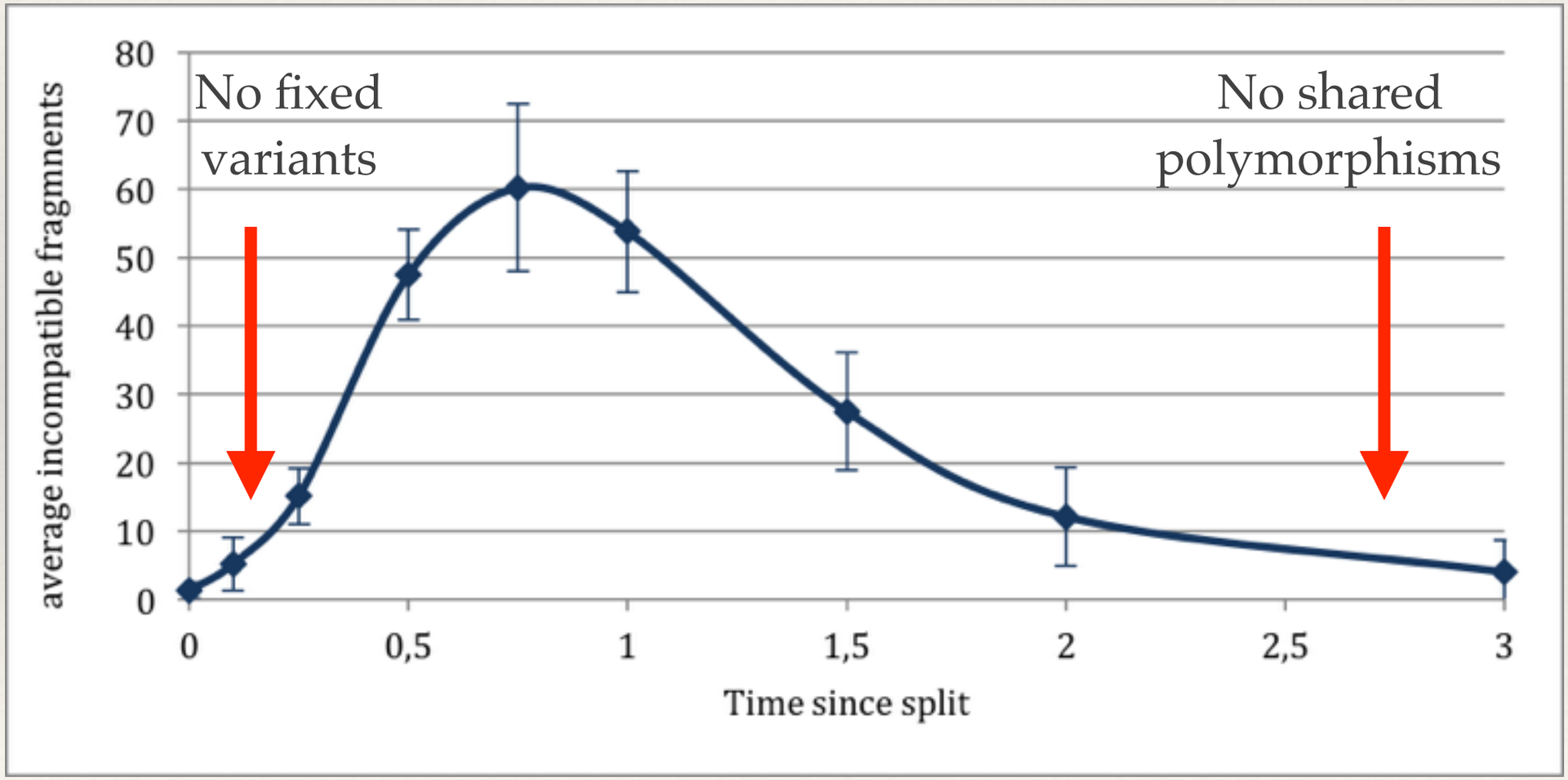
- Detection of recombinant events. True and false positive detection of Incompatible Genealogies:

VALIDATION

- No incompatible fragments were observed in simulations with no recombinations.
- In case using R>0, we never find incompatible fragments in the same real tree (the real tree was obtained using he *check tree* function in *ms* software, which show all trees).

Results: Coalescent simulations

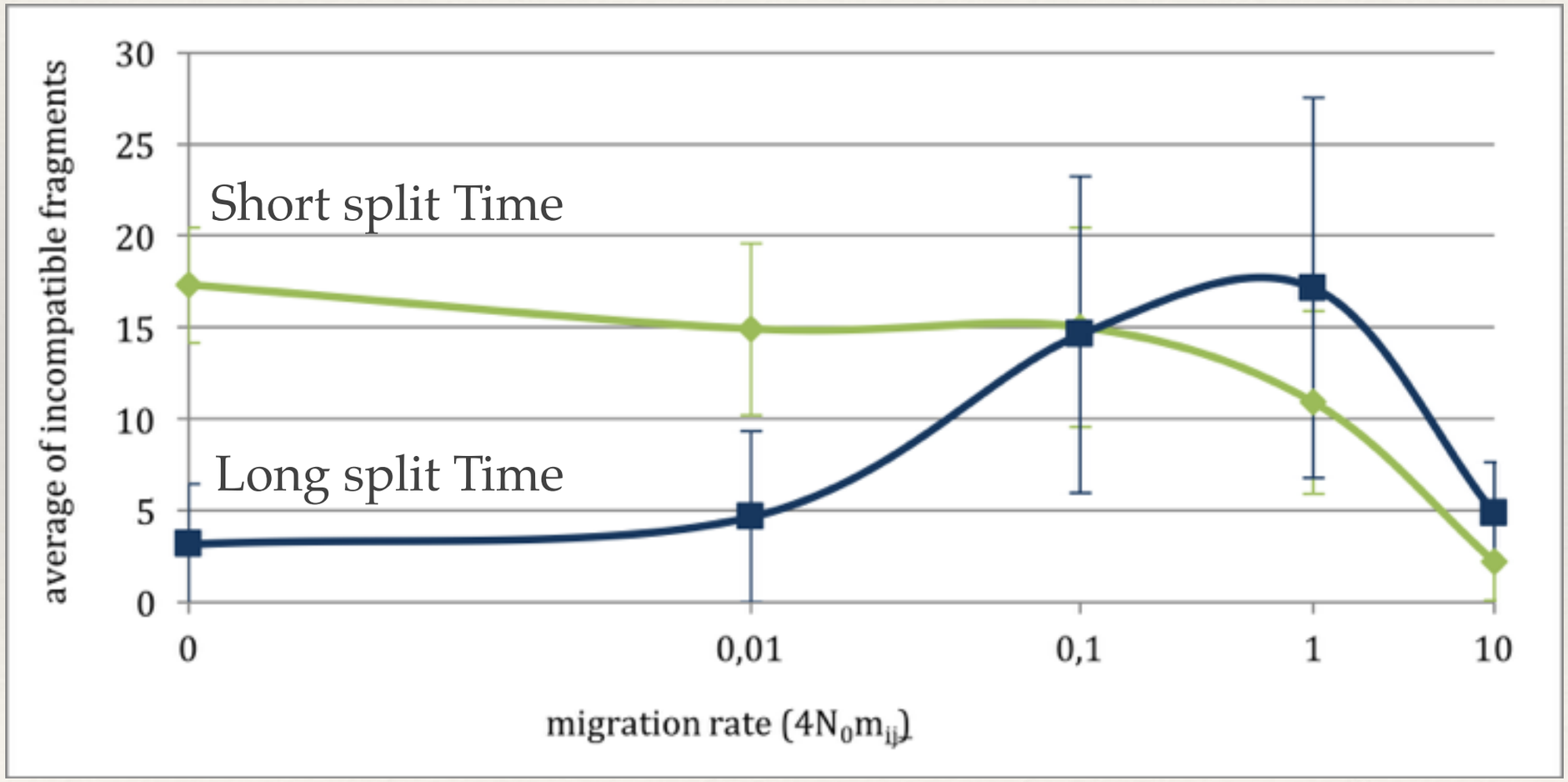
- The Time of Split among populations and the Detection of Incompatible Genealogies:



Relation between time since split (relative to 4N generations) between two populations and incompatible fragments found. No migration among populations.

Results: Coalescent simulations

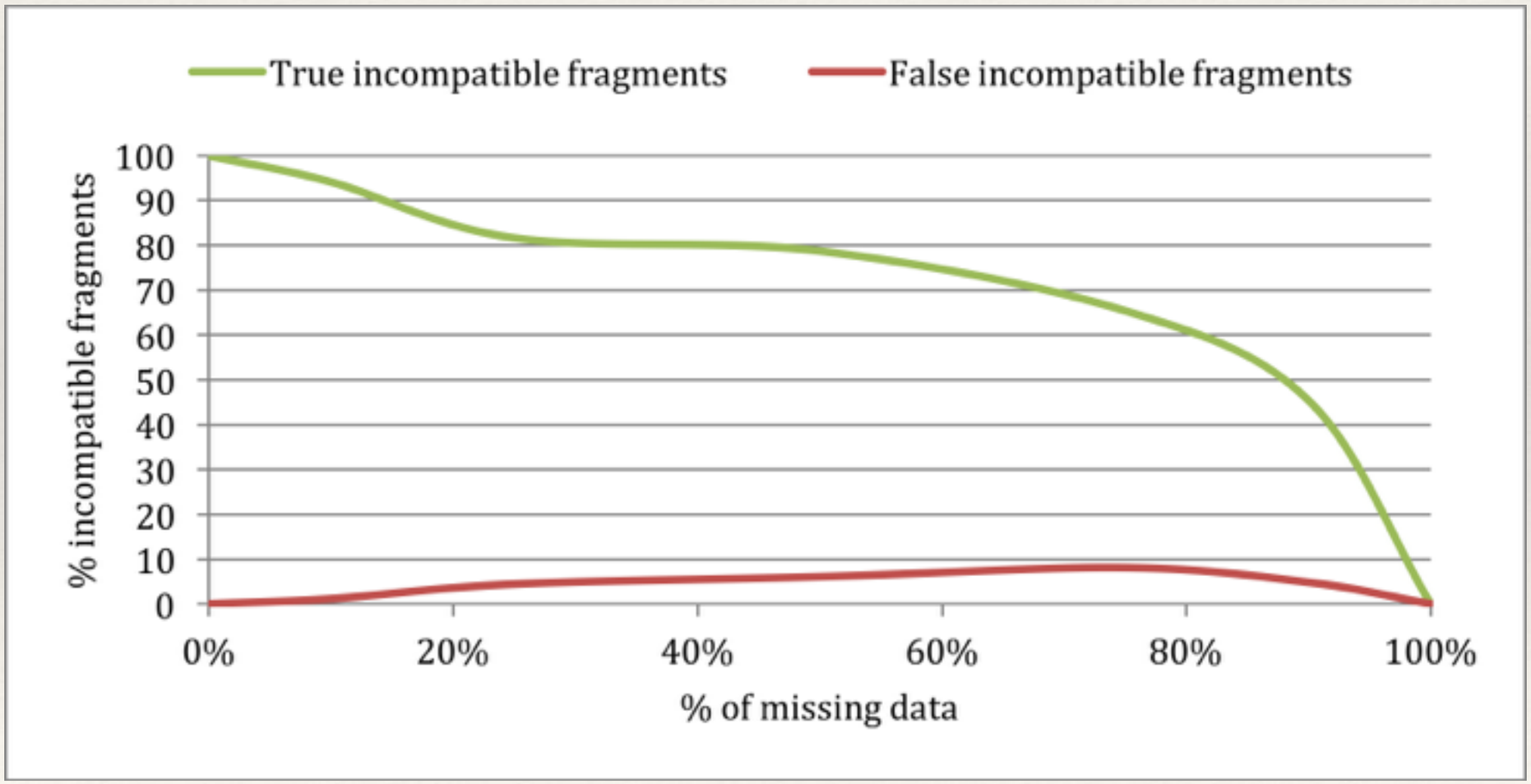
- The Migration parameter and the Detection of Incompatible Genealogies:



Relation between different migration rates ($4N_0m_{ij}$) and average number of incompatible fragments. Analysis done in two populations, with unidirectional migration. Green: short time (0.25 · 4N generations) since populations' split. Blue: long) time (3 · 4N generations) since populations' split.

Results: Coalescent simulations

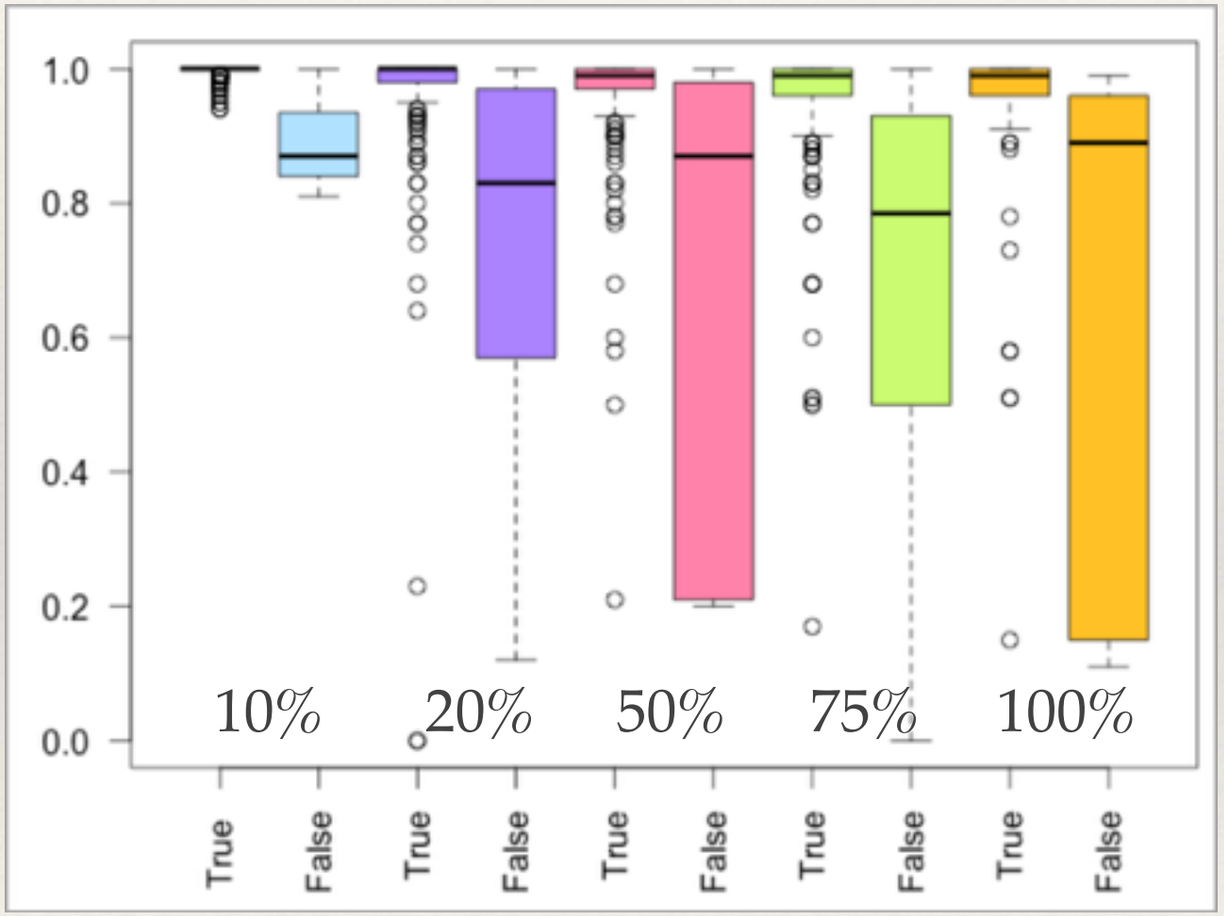
- The Missing data and the Detection of Incompatible Genealogies. True and False Positives:



Percentage of true and false incompatible fragments in different masks of missing data in relation to a sample with no missing data.

Results: Coalescent simulations

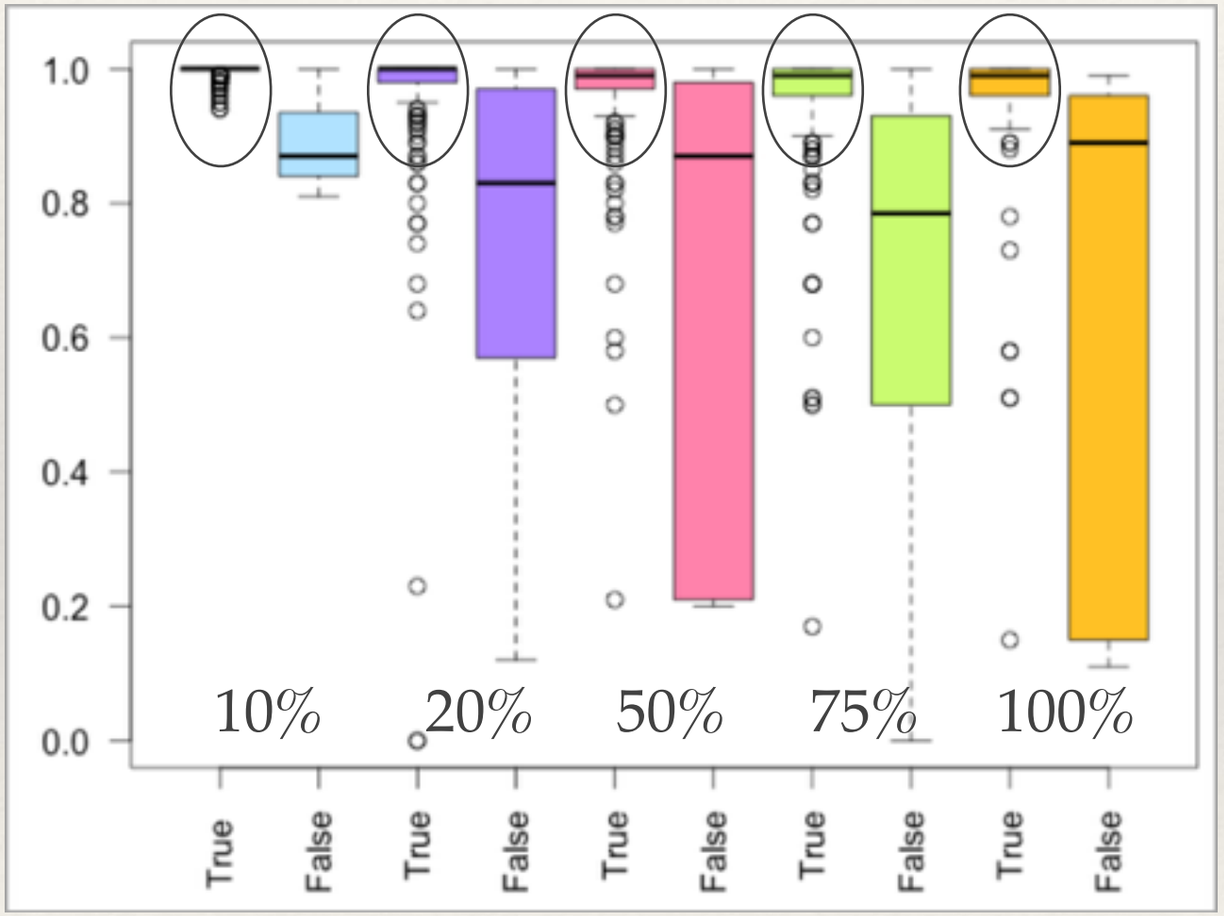
- The Missing data and the Detection of Incompatible Genealogies. The weight factor:



Boxplot of the normalised weight of reliability in true and false incompatible fragments for each mask with different percentage of missing data simulated**.** Percentage of missing in order: 10%, 25%, 50%, 75%, 90%.

Results: Coalescent simulations

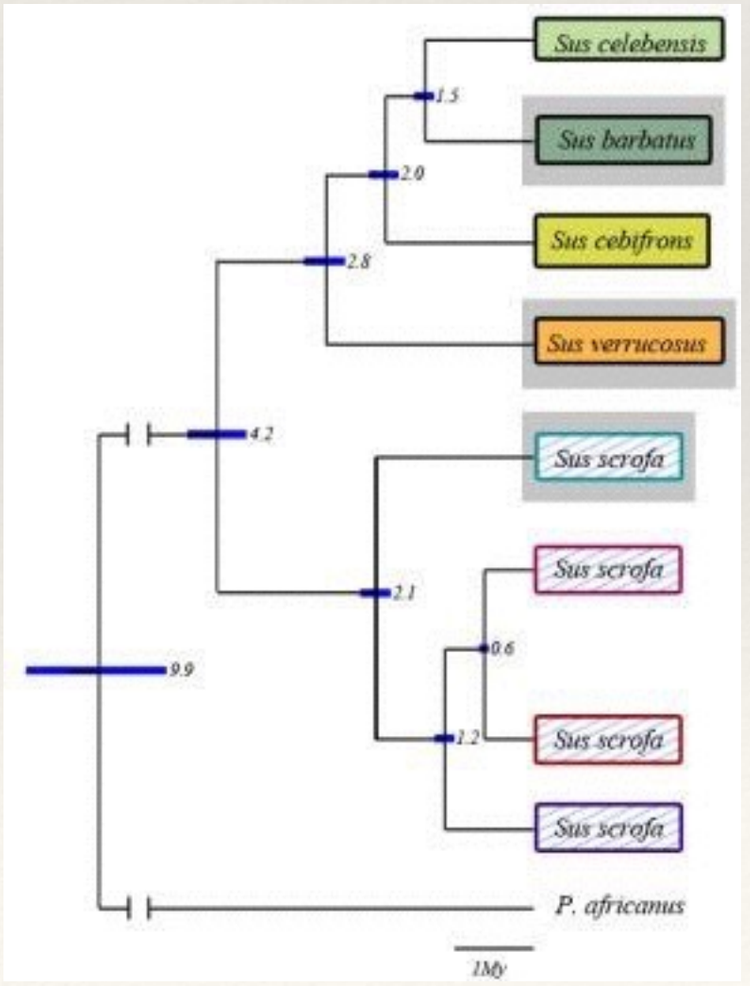
- The Missing data and the Detection of Incompatible Genealogies. The weight factor:



Boxplot of the normalised weight of reliability in true and false incompatible fragments for each mask with different percentage of missing data simulated**.** Percentage of missing in order: 10%, 25%, 50%, 75%, 90%.

Results: Real data

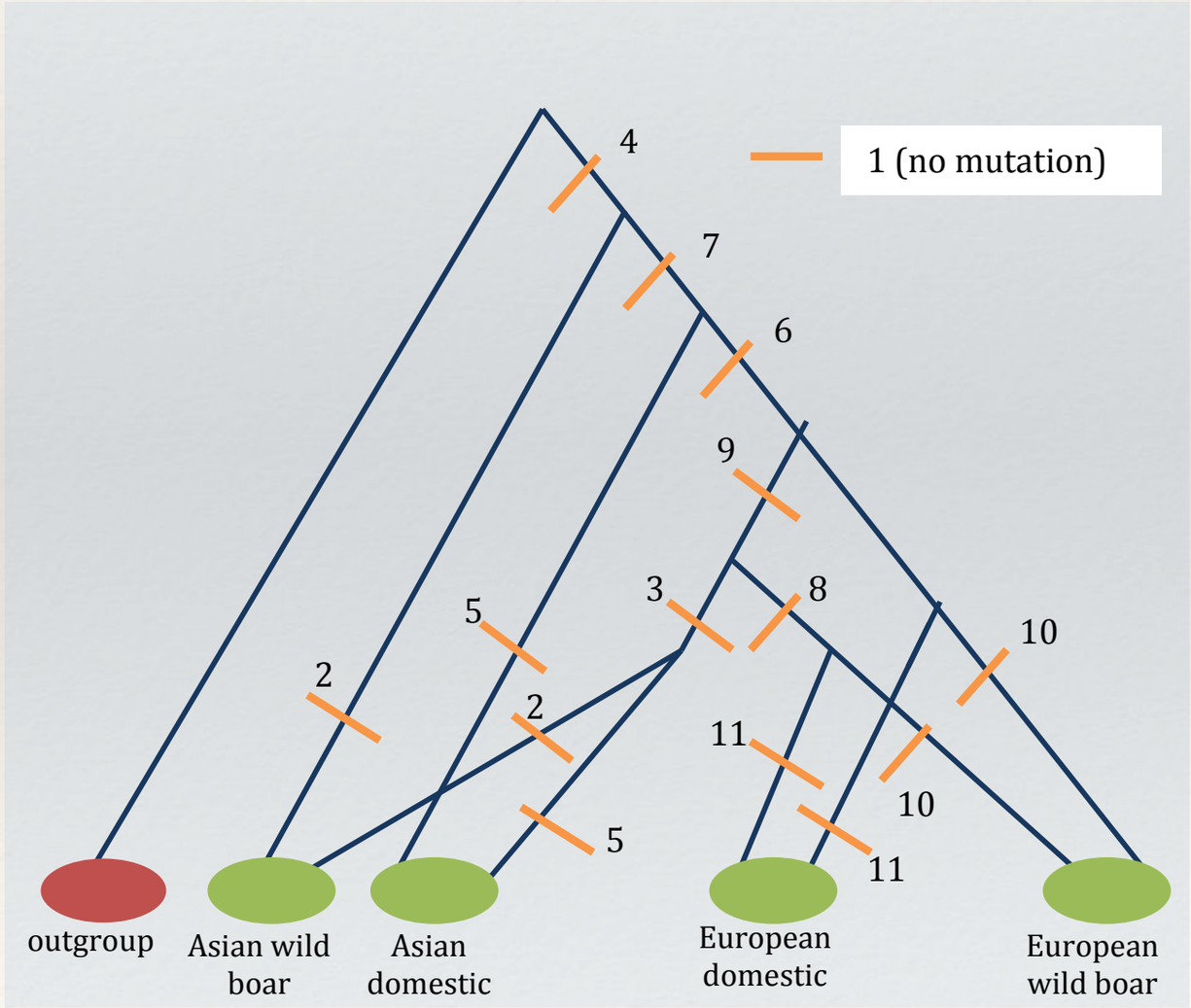
- Study of the variant sites along the chromosome 10 in four populations (around 10 samples each) of the species *Sus scrofa* (pig).
  - The More General Tree and other frequent Tree Genealogies.
  - The recombination rate and the length size of incompatible genealogies.
  - The Distribution of Tree length genealogies.



Results: Real data

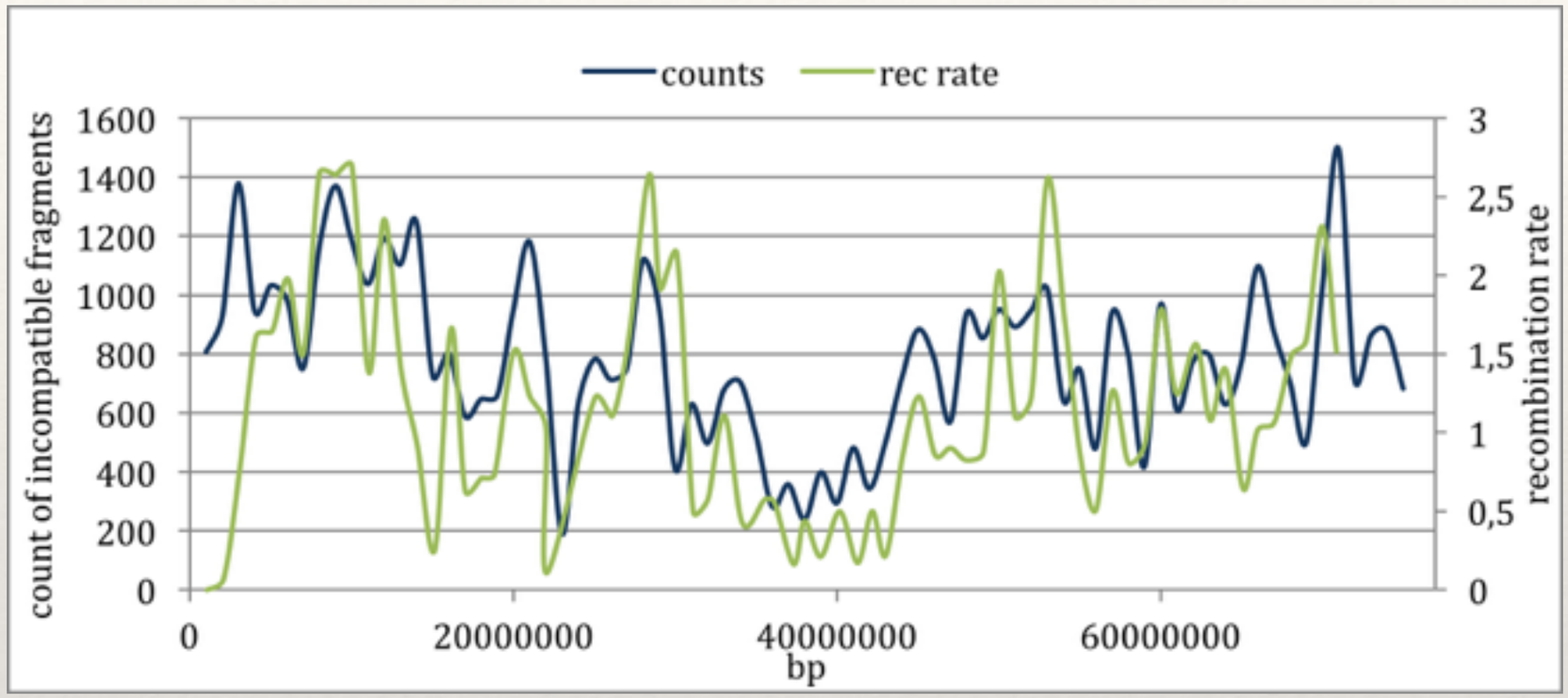
- The 11 more frequent type of combinations (85% variants) and their genealogical reconstruction.

#	Combination type	Counts
1	AAAA	51403963
2	PAAA	414110
3	PPAA	163531
4	DDDD	127040
5	APAA	71559
6	PPDD	64476
7	PDDD	38528
8	AAPP	31307
9	PPPP	29767
10	AAAP	28245
11	AAPA	24377



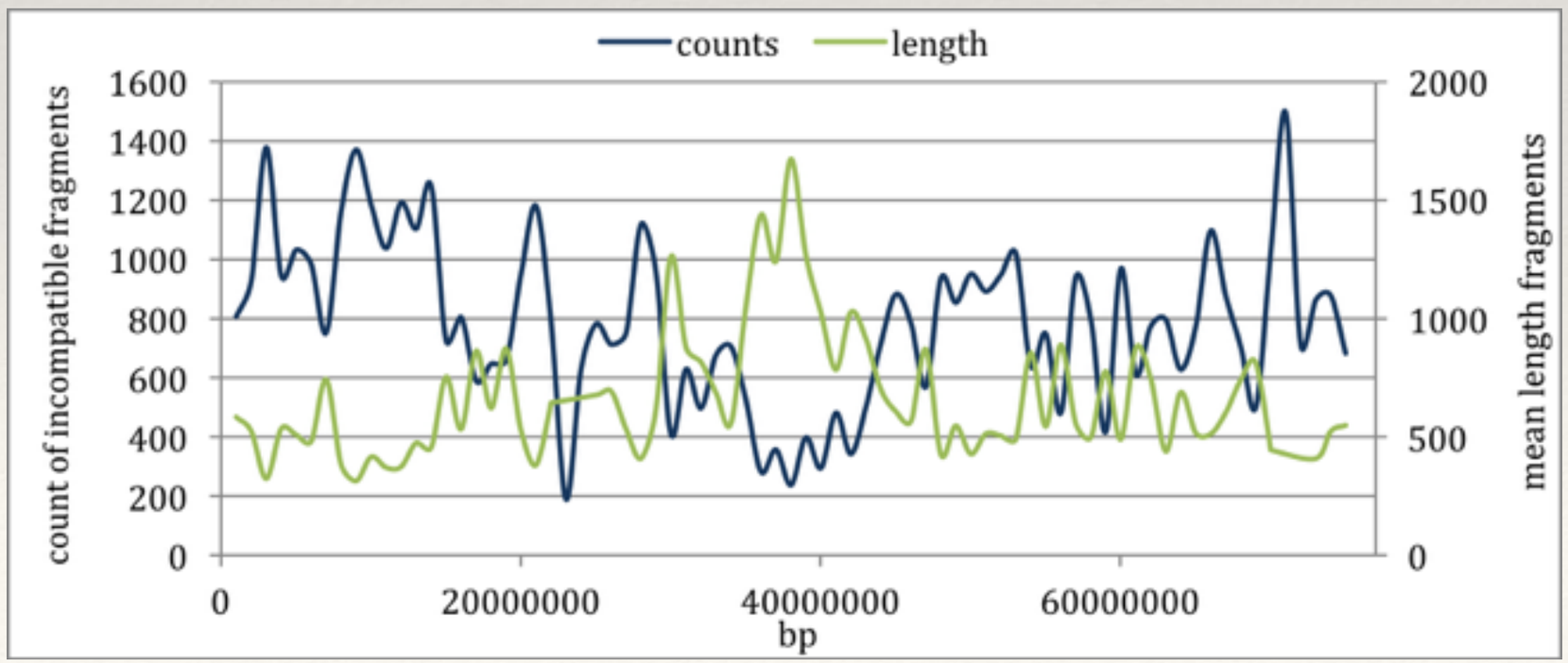
Results: Real data

Number of Incompatible fragments



Recombination rate

Number of Incompatible fragments

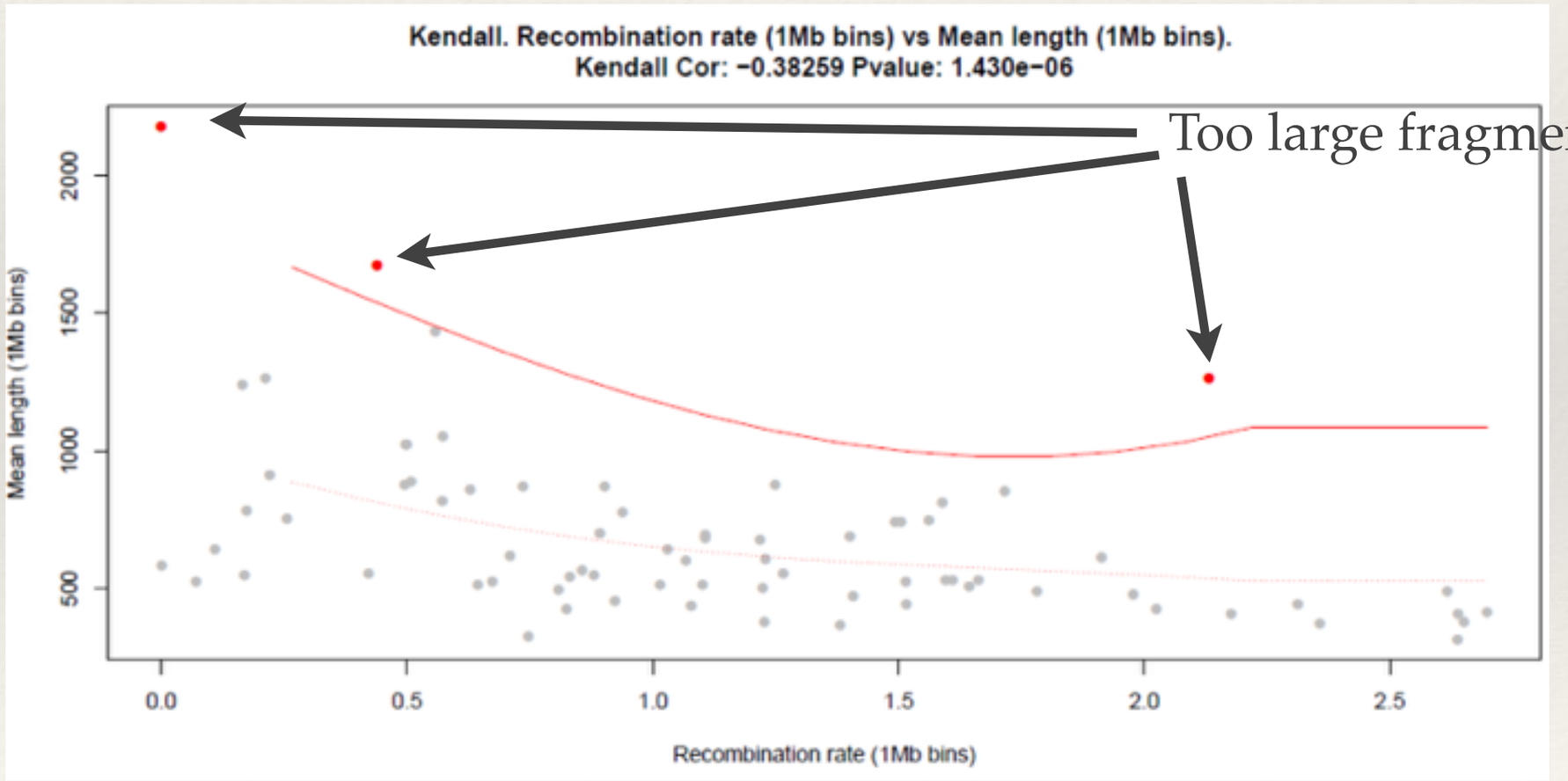


Mean length of Incompatible fragments

Results: Real data

- Comparison between lengths of incompatible fragments and recombination rate. Empirically, we find few outliers.

Mean length of Incompatible fragments



Recombination rate

Perspectives

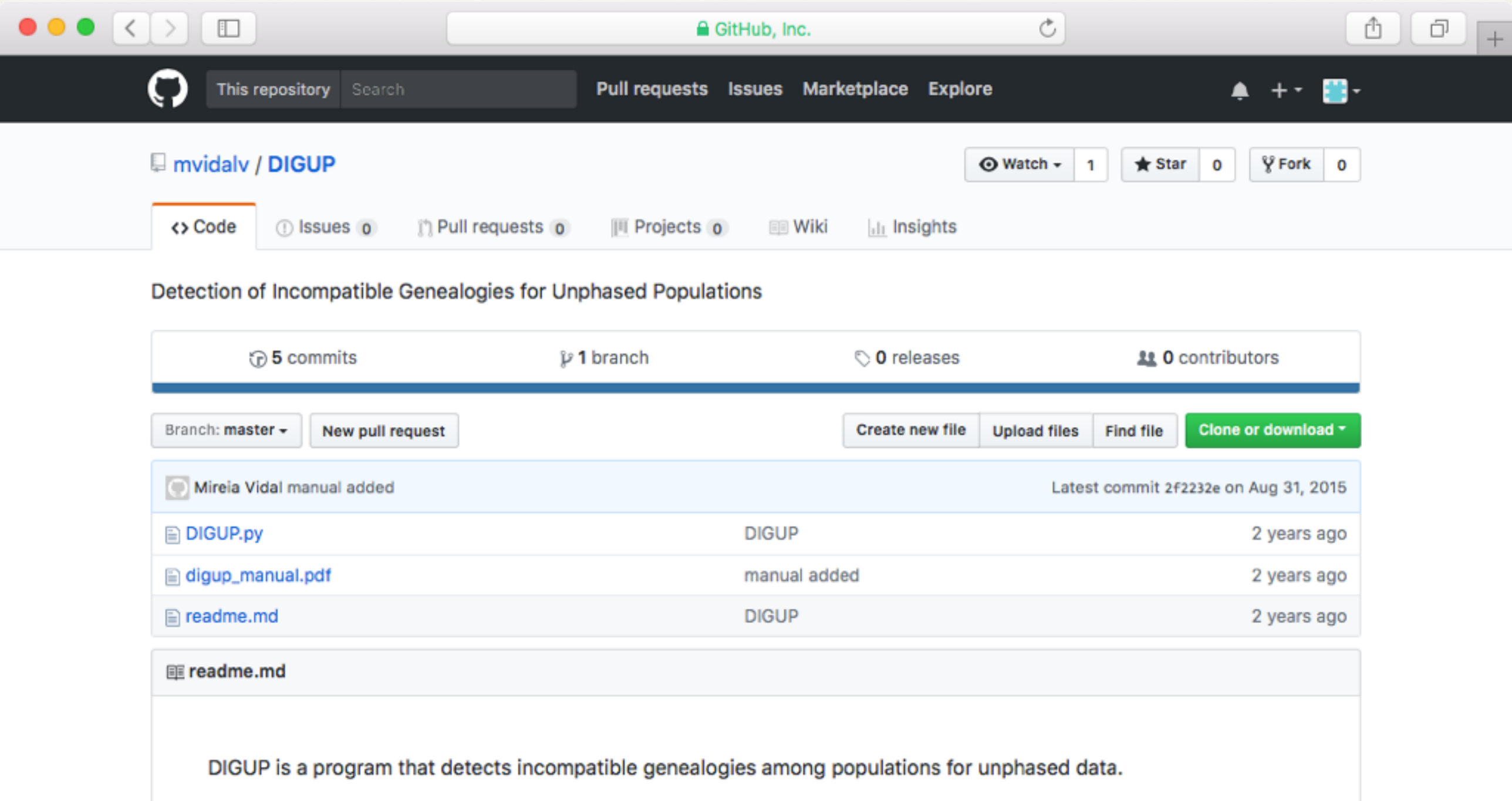
- Useful for discretising the genome into non-incompatible windows when using a sliding windows analysis.
- Useful for counting all different branches appearing in the sample and reconstructing the history of the species for the whole and at each genomic region.
- Factors used for weighting missing data: consider other weights. For example use the number of incompatible variants versus contiguous fragments as a factor for the reliability of the incompatibility.

Perspectives

- Detection of local evolutionary events:
  - Relationship between recombination rate and number and length of incompatible genealogies. The NO fit of recombination map versus patterns of incompatible genealogies observation can be caused by additional evolutionary processes.
  - An excess of a given type of a variant (a mutation in a specific branch) in some regions may be unexpected under the general genealogical pattern, which may indicate a rare evolutionary process. Study the distribution of variant types and the distribution of incompatible fragment lengths versus different evolutionary models.
  - Combination with other methodologies (for example D-statistic).
  - A HMM may be constructed for differentiating regions having migration from each popA to each popB, or no migration, considering the incompatible genealogical regions.

Software: DIGUP

https://github.com/mvidalv/DIGUP



Software: DIGUP

https://github.com/mvidalv/DIGUP

DIGUP usage

DIGUP is able to read both *fasta* and *ms* format and includes several arguments, ones are optional and others are required. Below, DIGUP usage and detailed explanation of each argument.

```
Usage: DIGUP.py input_file -n n -i i1 i2.. ip [-o {1,2,12}] [-ms]
[-l length] [-nt nt1 nt2.. ntp] [-G]
```

-n total number of sequences (including the outgroup).

-i total number of individuals in each population (in the same order as in the input file). Last population is considered to be the outgroup.

-ms if input file is in *ms* format (default reading is for *fasta* format)

-o output type (1, 2 or both as 12) for the classification of variant positions. Default output type is 1, which includes classification, of each population, of all variant positions. Output type 2 includes same

DIGUP project

Mireia Vidal-Villarejo (Hohenheim U., Germany)
Luca Ferretti (Pirbright I., UK)
Sebastian E. Ramos-Onsins (PI)

ngasp core team

Jordi Leno-Colorado (co-directed PhD in Genetics)
Joan Jené (Computer Scientist Engineer)
Gonzalo Vera (Head Engineer)
Sebastian E. Ramos-Onsins (PI)

Luca Ferretti (Pirbright I., UK)
Javier Navarro (Comp. Sc., PCB)
Carlos Montemuiño (co-directed PhD in Comp. Sc.)
Sara Guirao-Rico (CRAG)
Miguel Pérez-Enciso (ICREA-CRAG)

Julio Rozas (UB-Barcelona)
Alejandro Sánchez-Gracia (UB-Barcelona)
Porfidio Hernández-Budé (UAB-Barcelona)
Emanuele Raineri (CNAG-Barcelona)

https://bioinformatics.cragenomica.es/numgenomics/people/sebas/