

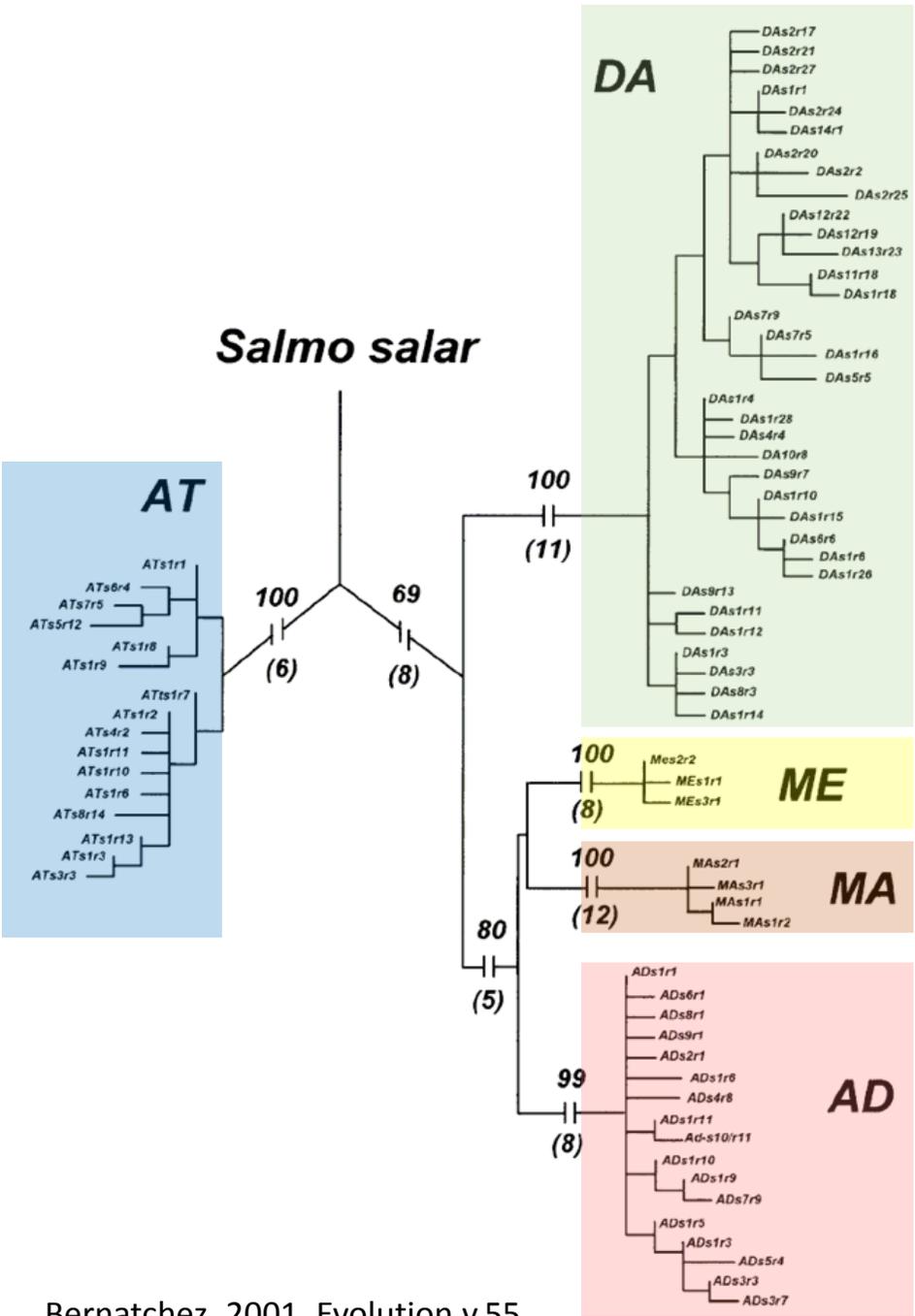
Approche génomique de l'impact des déversements de truites (*Salmo trutta*) domestiques dans les populations sauvages d'origine méditerranéenne



Maëva Leitwein



Salmo salar



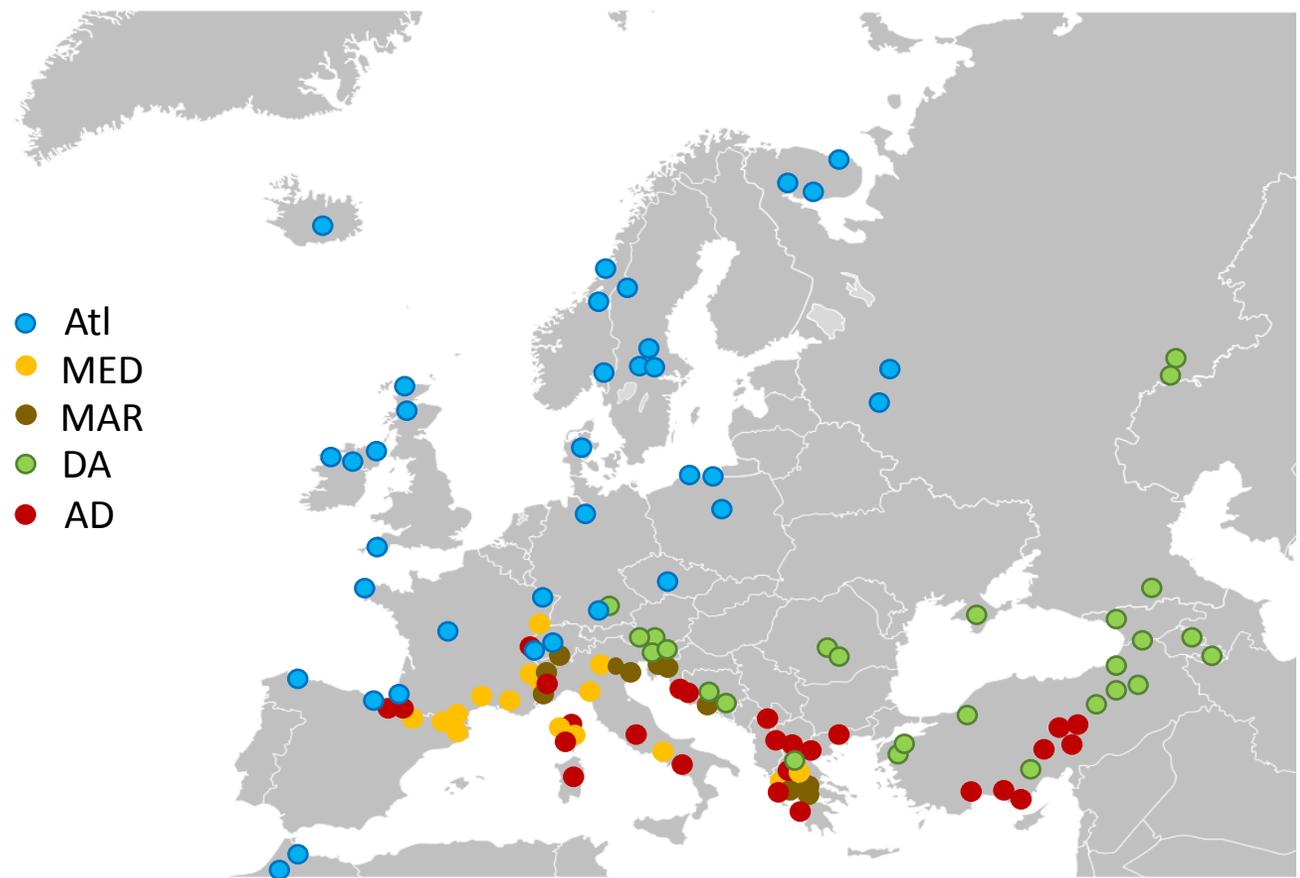
Marbled



Mediterranean



Atlantic





Trout stocking



Enhancement of Atlantic and Mediterranean domestic strains into wild Mediterranean populations

That's a wild one !



Main Questions



What are the impacts of restocking on wild population structure and genetic diversity ?



Main Questions



What are the impacts of restocking on wild population structure and genetic diversity ?

Do domestic alleles introgress into wild populations ?

What is the fitness of the introgressed alleles ?



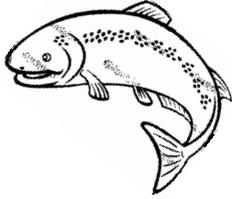
Part 1

→ Development of a high density SNPs array by dd-RAD sequencing



- Wild populations
- Atlantic hatchery strains
- Mediterranean hatchery strains

Wild



3 populations

30 individuals (3×10)

Domestic



2 populations

30 individuals ($1 \times 10 + 2 \times 10$)

Double digest RAD seq

(Paired End, 2×125 bp)



Average number of reads per individual :

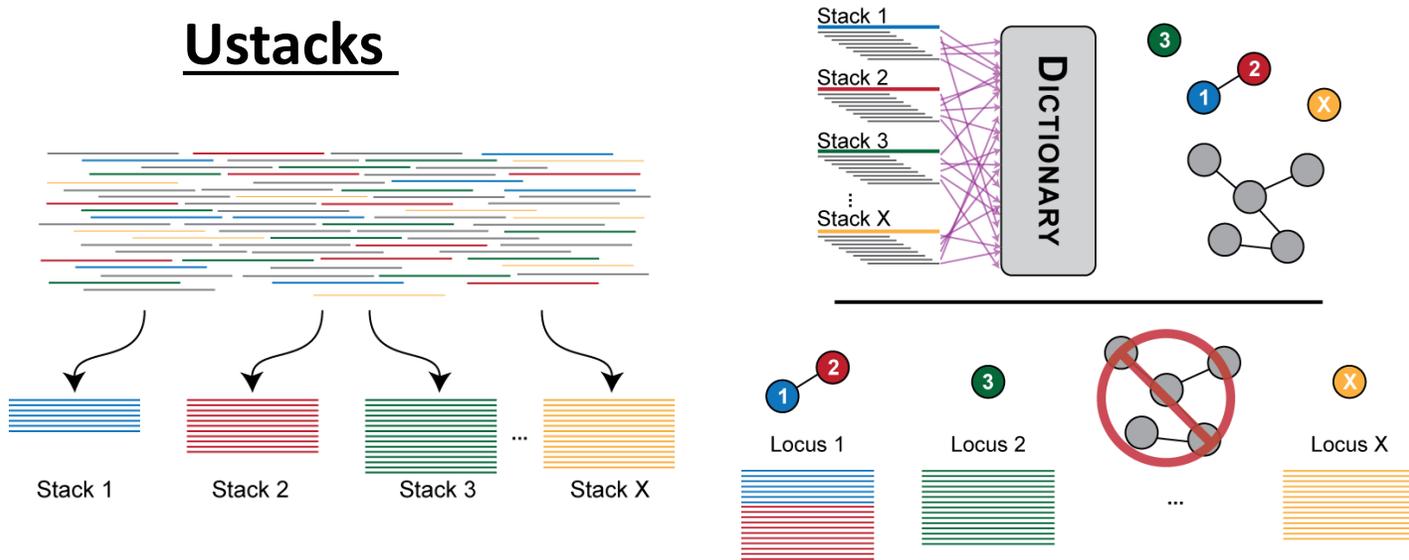
12 millions

Workflow

- *De novo*
- Reference mapping
- Comparison between both methods
- Estimation of nucleotide diversity
- Population structure

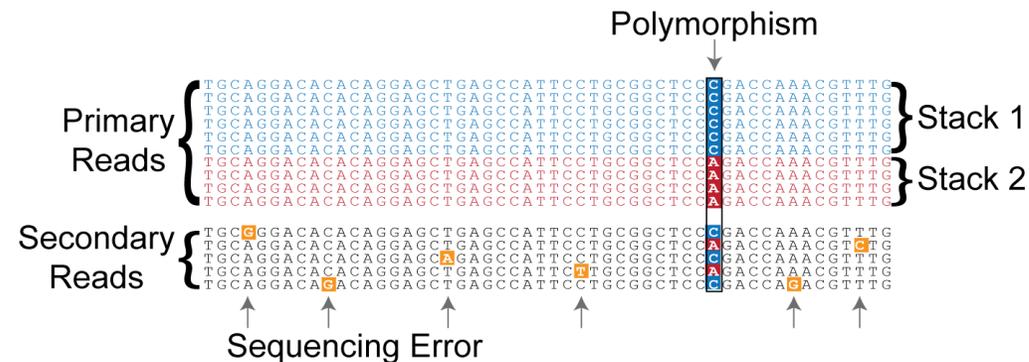
De novo assembly

Ustacks



m= Minimum depth of coverage required to create a stack

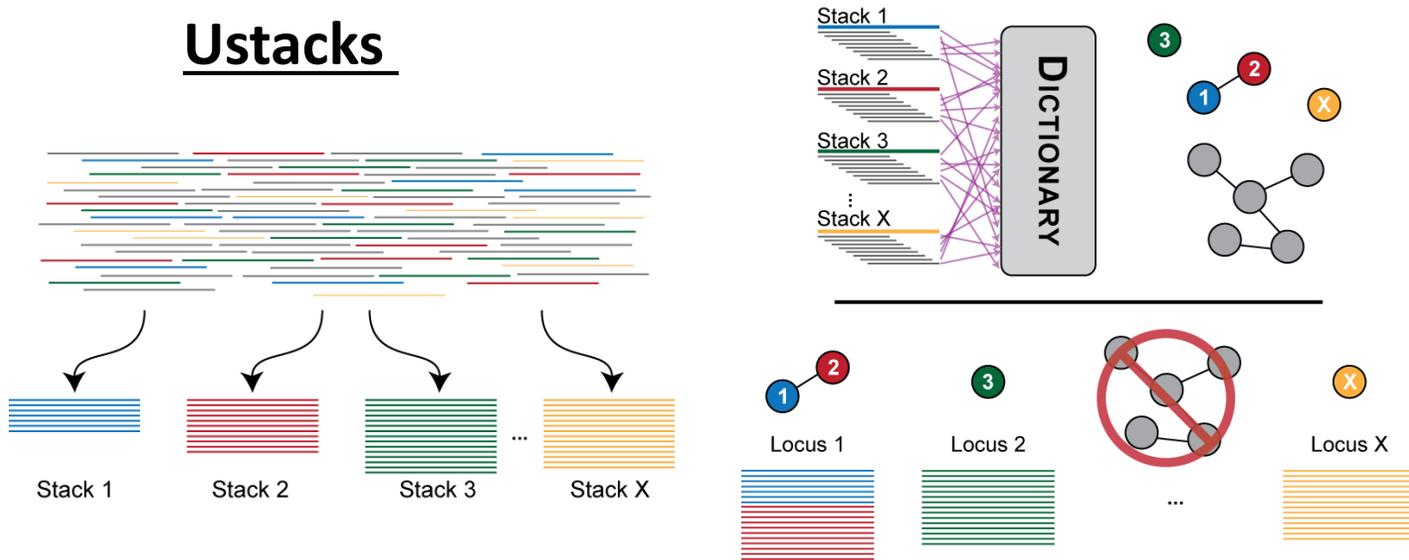
M= Maximum distance allowed between stacks



SNP= Single Nucleotide polymorphism

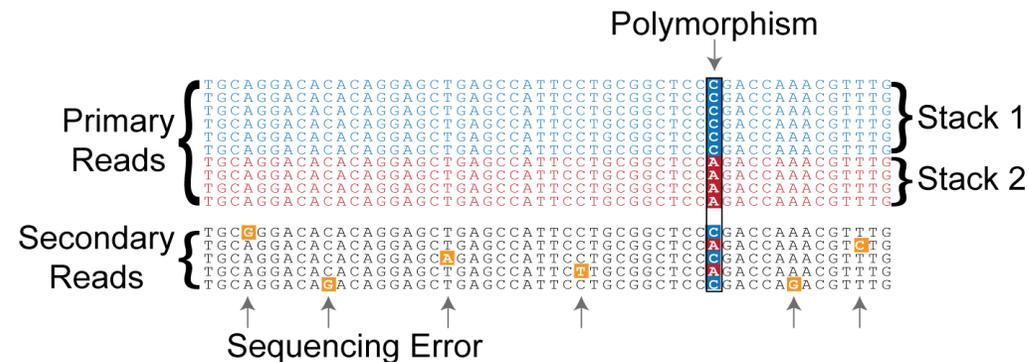
De novo assembly

Ustacks



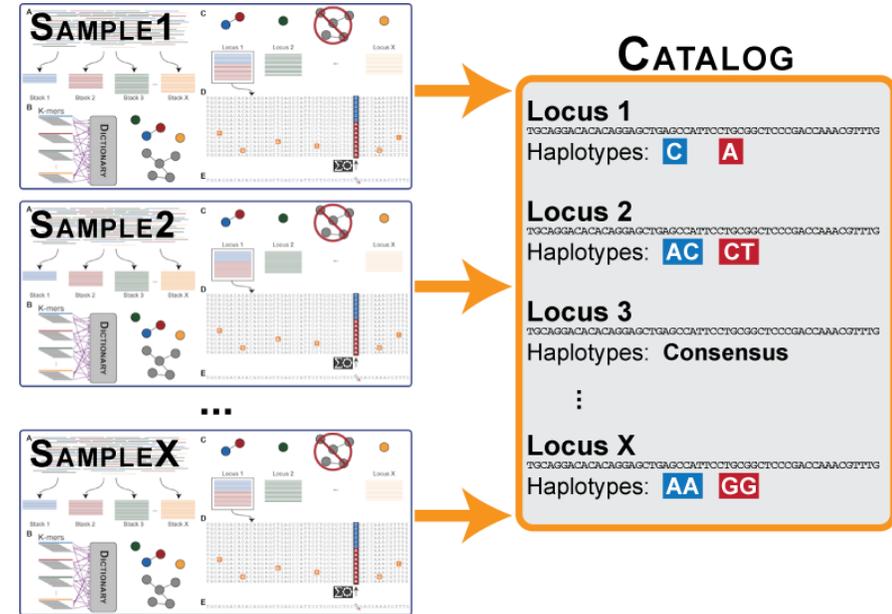
m= Minimum depth of coverage required to create a stack

M= Maximum distance allowed between stacks



SNP= Single Nucleotide polymorphism

Cstacks



n= number of mismatches allowed between loci when generating the catalog

Sstacks

Match to the catalog

Reference mapping

Organism Overview ; [Organelle Annotation Report \[1\]](#)



Salmo salar (Atlantic salmon)

Atlantic salmon; highly valued in fishing and aquaculture

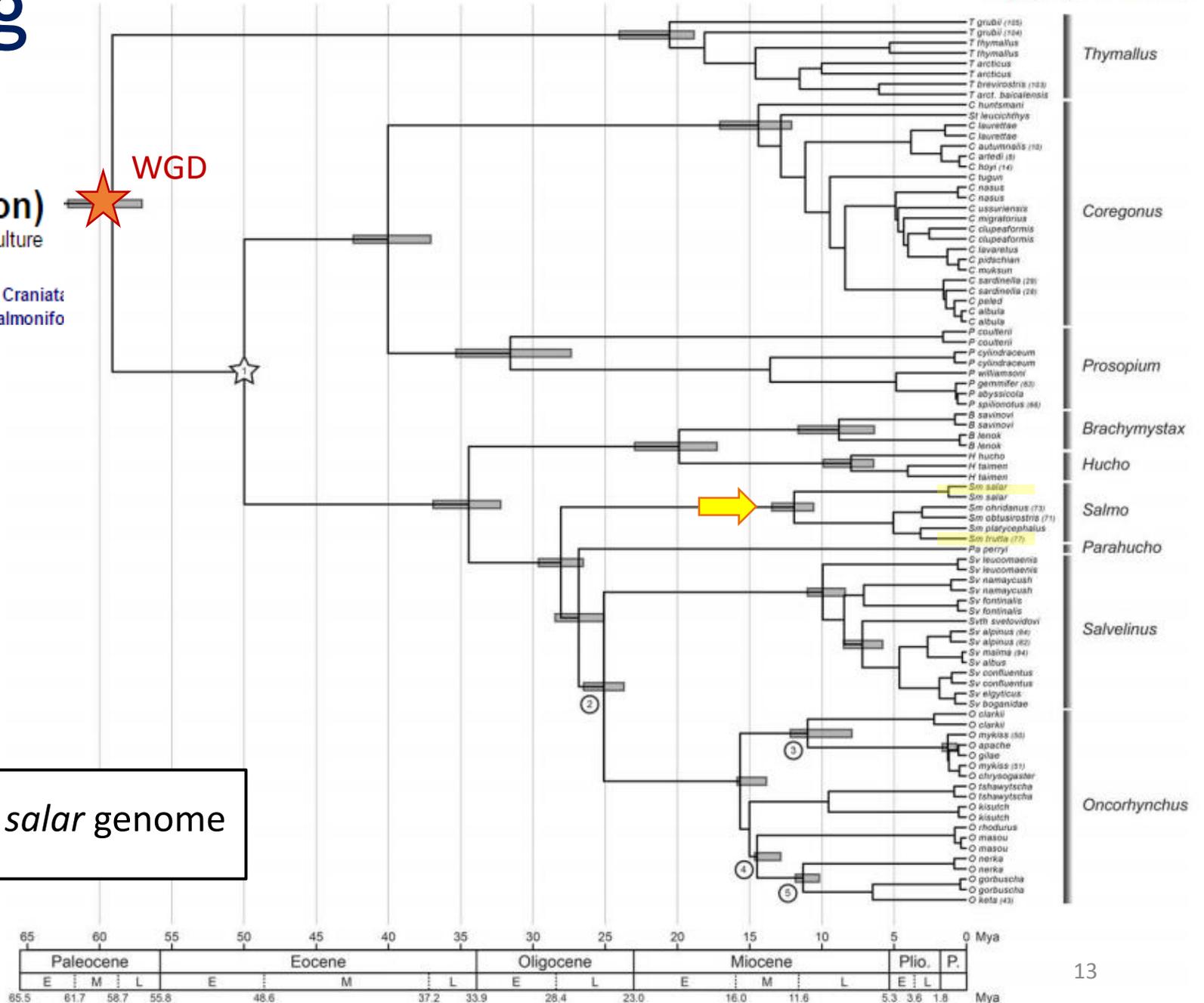
Lineage: Eukaryota[1813]; Metazoa[657]; Chordata[279]; Craniat: Neoptervaiif631; Teleostei[621]; Protacanthoptervaiif21; Salmonifo

Assembly level: Chromosome
 Environment: OxygenReq:Aerobic, Habitat:Aquatic
 Assembly: GCA_000233375.4 ICSASG_v2 scaf
 BioProjects: PRJNA287919, PRJNA72713
 Whole Genome Shotgun (WGS): INSDC: AGKD00000000.4
 Statistics: total length (Mb): 2966.89
 protein count: 13
 GC%: 43.8409
 NCBI Annotation Release: 100

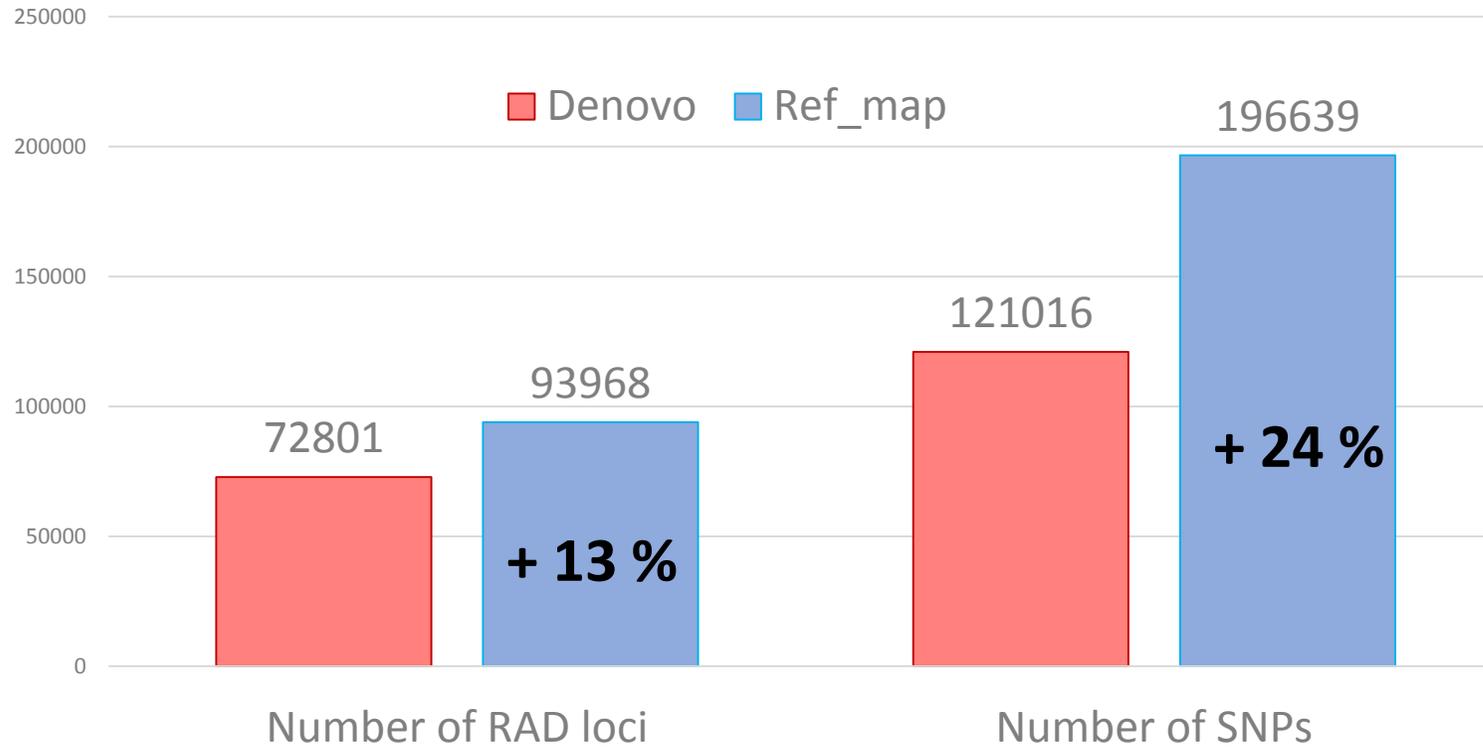
Lien *et al.* 2016

99.6 % of the reads mapped to *Salmo salar* genome

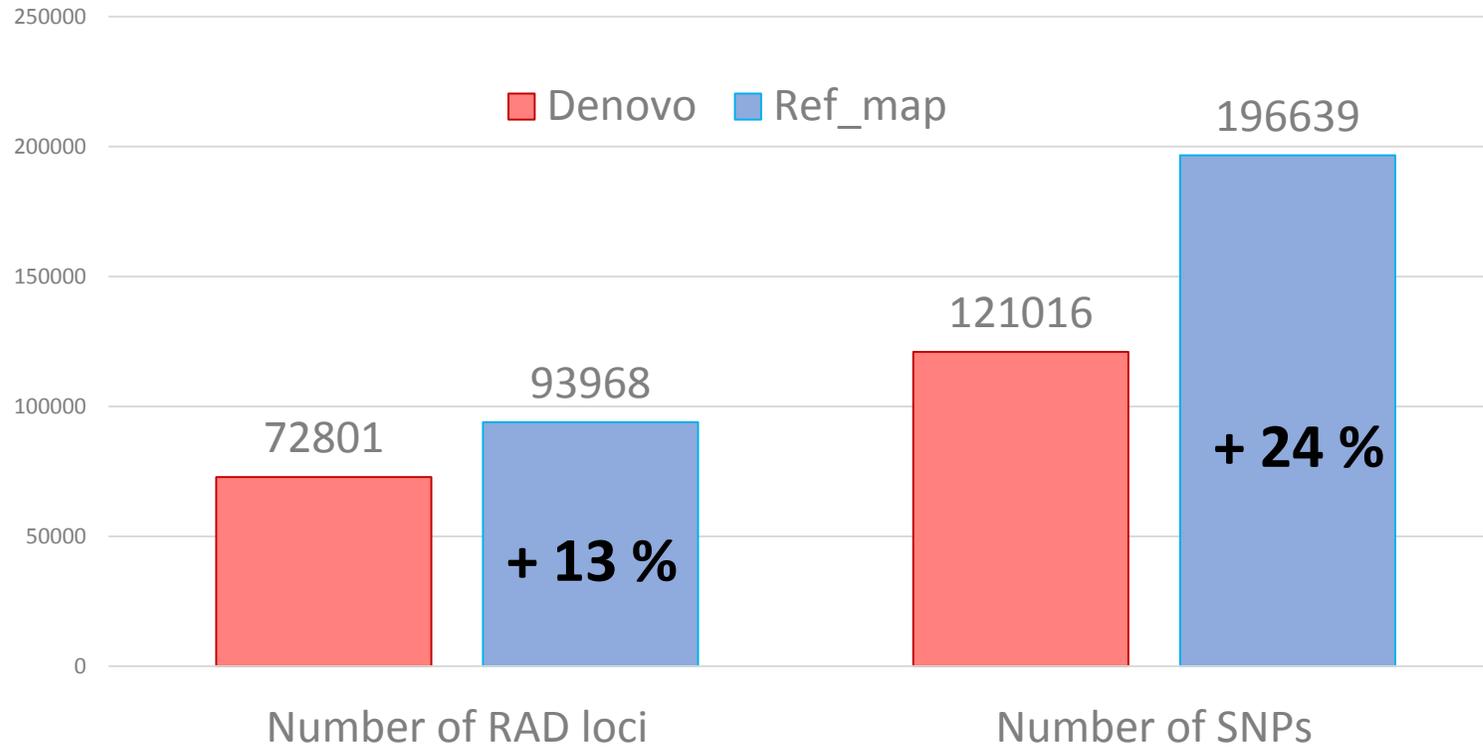
Crete-Lafreniere *et al.* 2012



Denovo VS Reference mapping



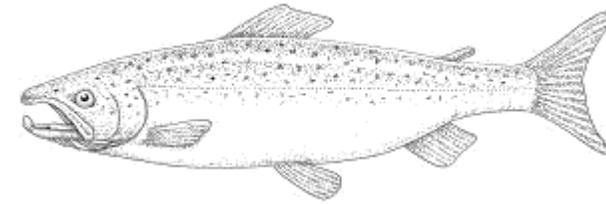
Denovo VS Reference mapping



How many loci are common ?

De novo loci from catalog

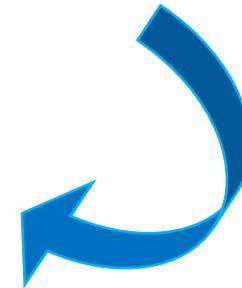
```
>5
CGGCATACAGGCCAGCCAGTGTAAGCAATCTAATATAACATTTTTATCTATGTCAGTTCTAACTGTTTGT
>6
AATTCTTCAGGTAAGGGTTAAGGTTTGGGATAGGCCTAAGACAAAAATCTAAAAACAACCTTTCTACT
>8
CGGAGGACAACAAGATGCAACAAATCAAGTTTTTTTTCTTTCTGTCAATTACATTTTTTCTGTGATGTGA
>11
CGGATCCCCCGATACTGATGCTCGTTCTGGAGGTCTACGATTTCTAGGCTTCACTGAACGGGATTCATTA
>15
AATTCACATATTAATGACATTAGTCAATGGTGCCACCTGTCAATGATTTAGAGGGAGGGATAATAACCAG
>17
CGGTGAAAAATCTGTCCTTTGTTCTGATGAGTCACATTTGAGATTTGGTTCCAACCGCCTTGTTTACAGATG
>19
```



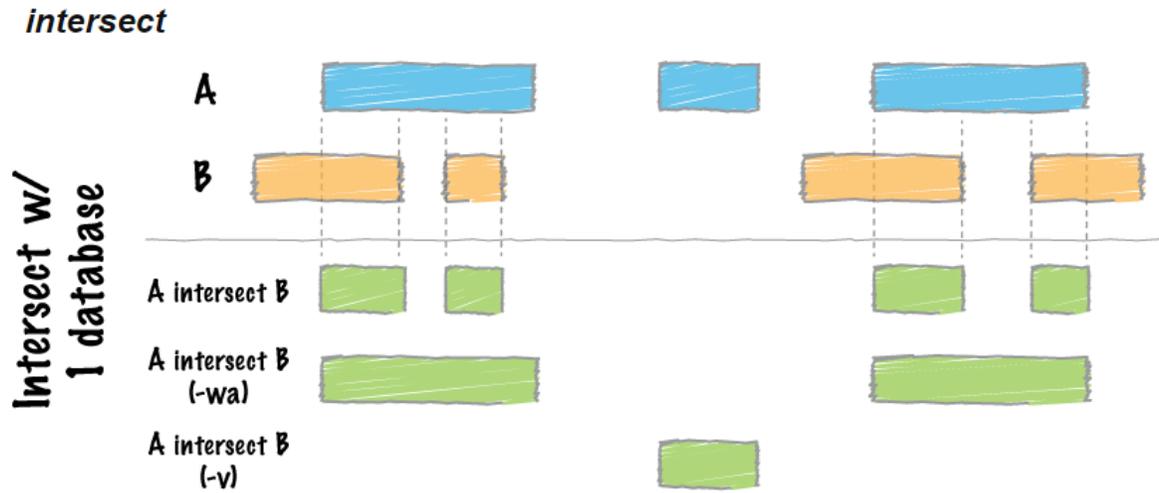
Sam alignment file

```
5      16      CM003298.1      41441601      60      2S118M      *      0      0      CCGAATTCAACACAGG
TAAAAATGTTATATTAGATTGCTTTACTGGCTGGCCTGTATGCCG      *      NM:i:2 MD:Z:35T46C35 AS:i:108 XS:i:21
6      16      CM003291.1      64273448      60      120M      *      0      0      TACGGTGTAACATCT
TTTTGTCTTAGGCCTATCCCAAACCTTAACCCTTACCTGAAGAATT      *      NM:i:2 MD:Z:27C31T60 AS:i:110 XS:i:52
8      16      AGKD04018804.1  7718      60      114M6S      *      0      0      GATGAACTCAGTGTCAAGGGAAGT
AGAAAAAAACTTGATTTGTTGCATCTTGTGTCCTCCG      *      NM:i:2 MD:Z:1G106C5 AS:i:107 XS:i:50
11     0      CM003300.1      46973878      23      24M13D15M7D49M2D32M      *      0      0
AACGGGATTCATTATCATCAACACGGACTGTTGTCTGATTACACACACCTGGTCCCATTC      *      NM:i:23 MD:Z:1A22^ATTCTGGTCACCA1
500120 21M2D50M20C 41
```

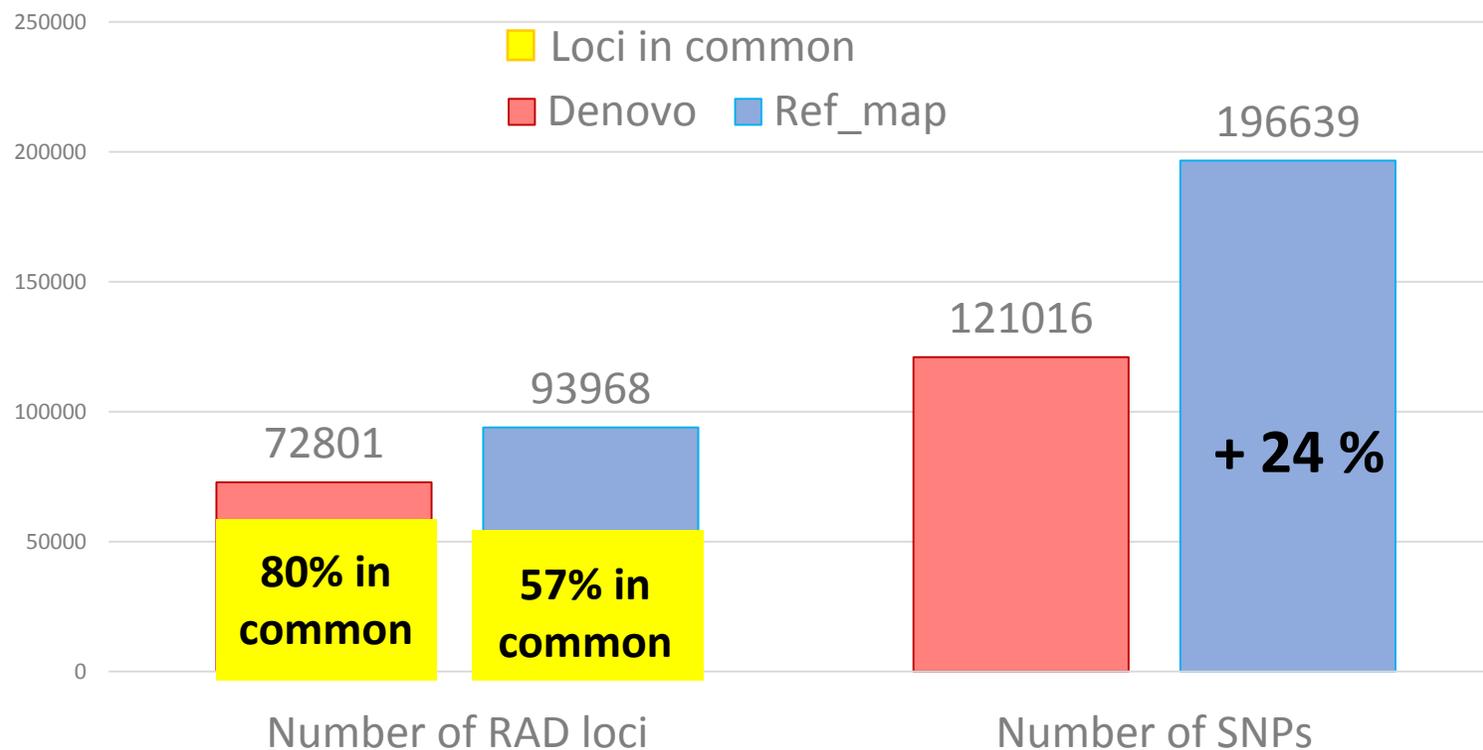
87% (63 164) loci mapped
(104 139 SNPs)



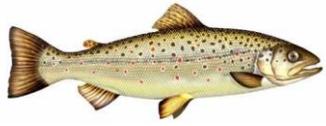
Identifying loci in common



Denovo VS Reference mapping



How are they distributed on chromosomes ?

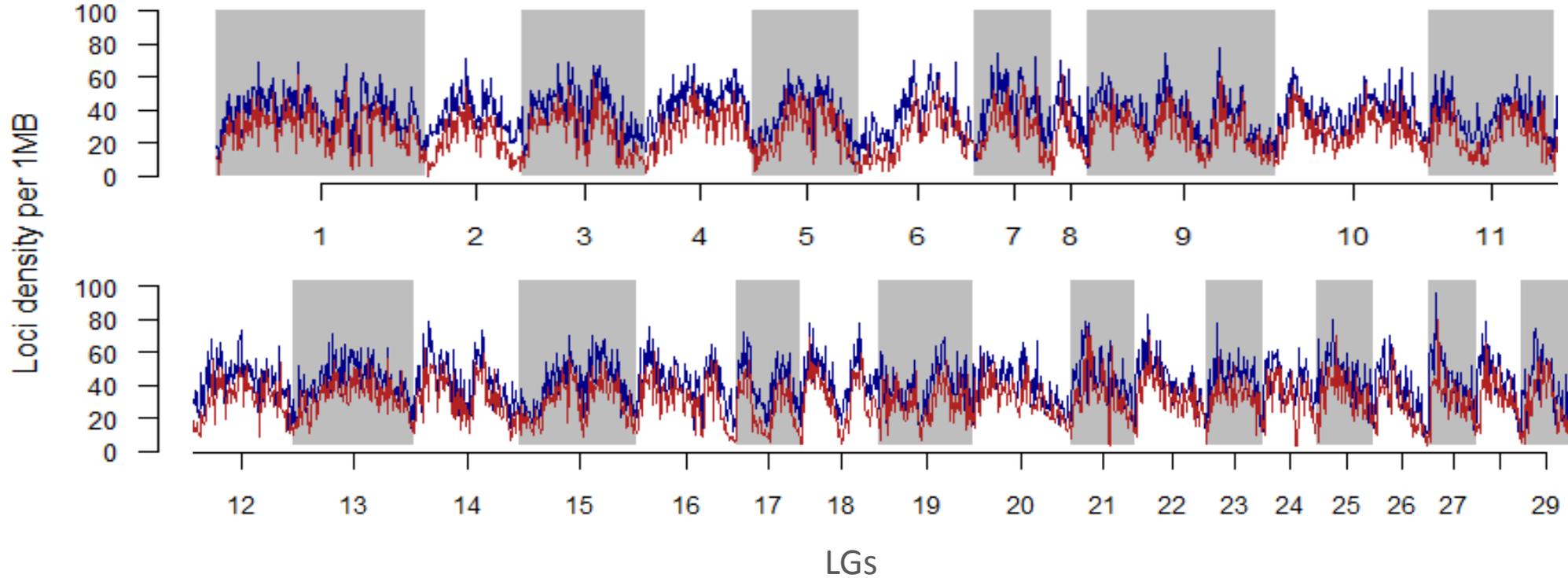


S. trutta

Distribution of brown trout loci along *S. salar* LGs



S. Salar



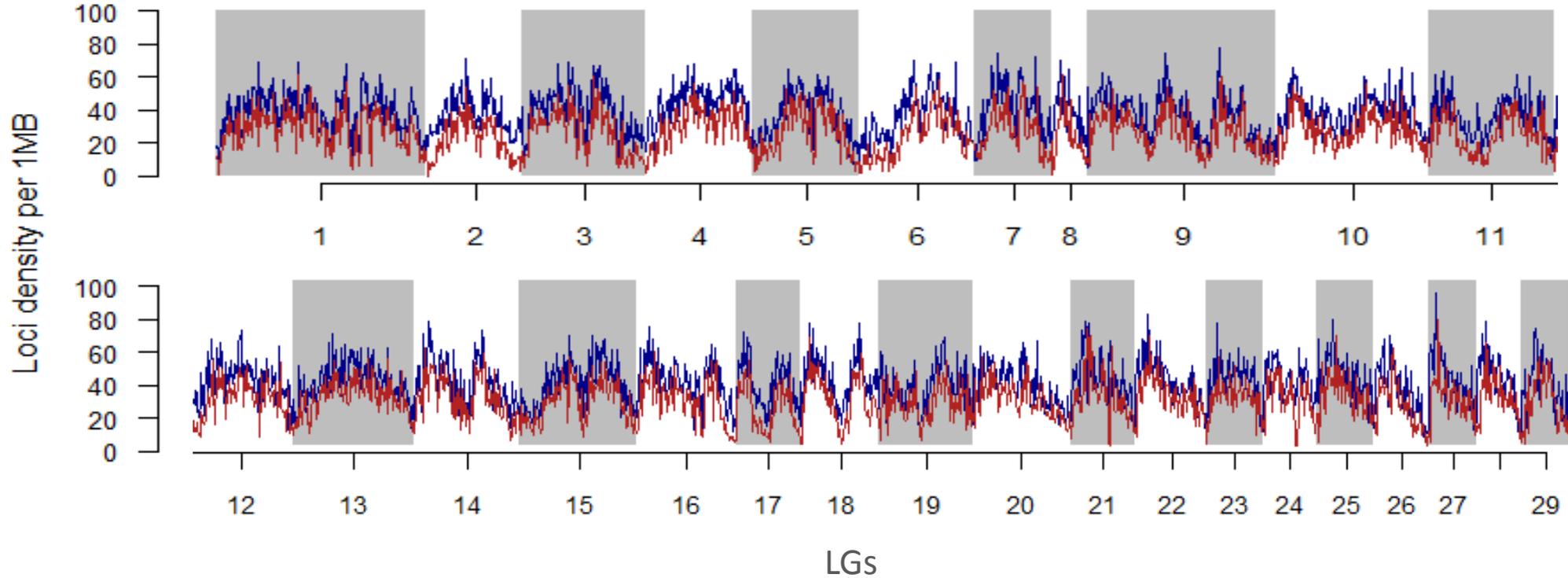
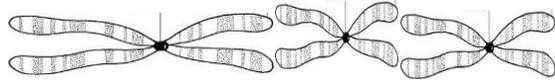


S. trutta

Distribution of Brown trout loci along *S. salar* LGs



S. Salar



■ Ref_map

■ *De novo*

→ Homogeneous distribution

→ Lower density at LGs extremities : paralogous loci ?

Workflow

- *De novo*
- Reference mapping
- Comparison between both methods
- Estimation of nucleotide diversity
- Population structure

 **Stacks** Catchen et al., 2013



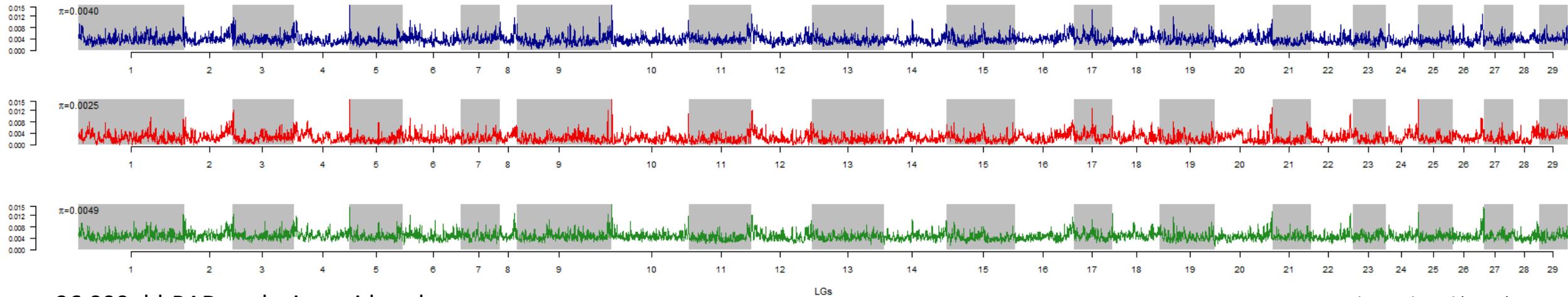
S. trutta

Distribution of brown trout nucleotide diversity along Atlantic salmon (*S. salar*) linkage groups



S. Salar

- Atlantic strain , mean π = 0.0040
- Mediterranean strain, mean π = 0.0025
- Wild Mediterranean trout, mean π = 0.0049



>96,000 dd-RADseq loci considered

Leitwein et al. *J. Fish Biol* (2016)



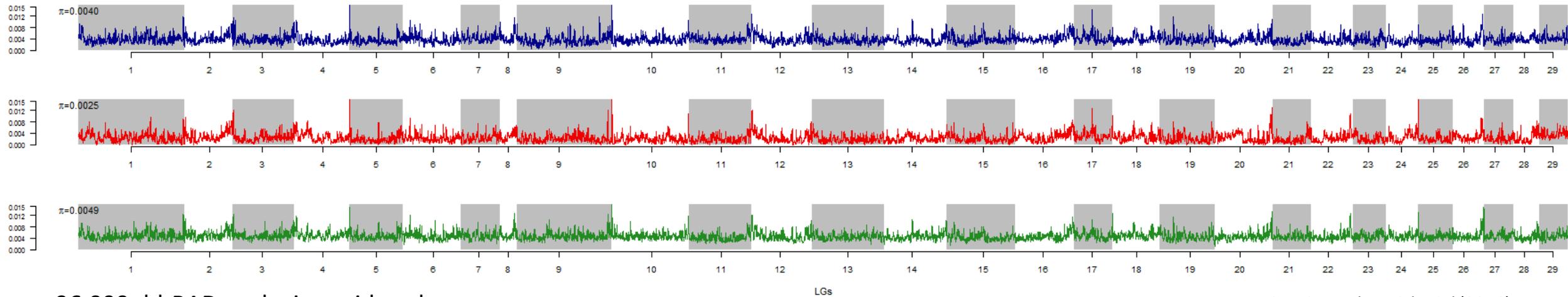
S. trutta

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S. Salar

- Atlantic strain , mean π = 0.0040
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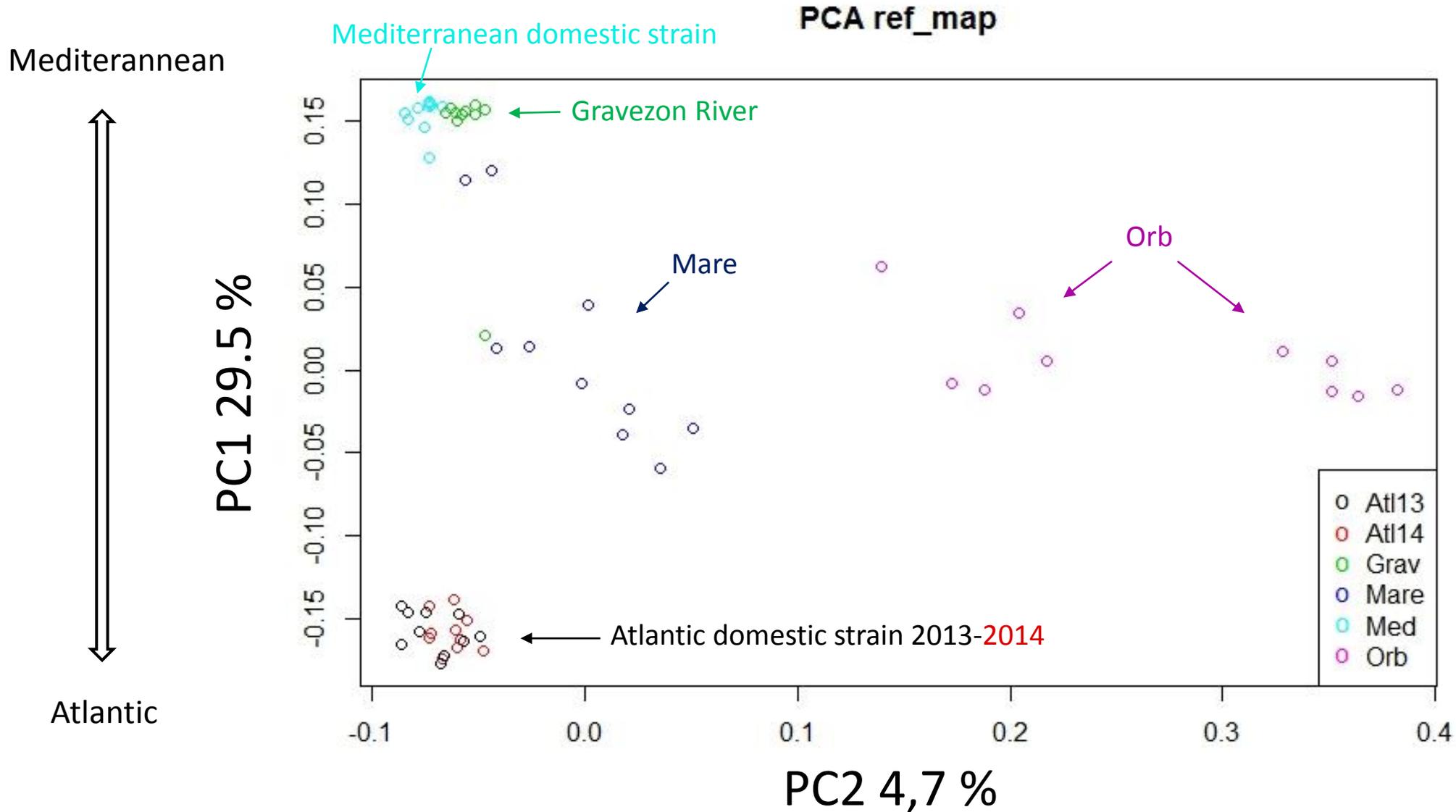


>96,000 dd-RADseq loci considered

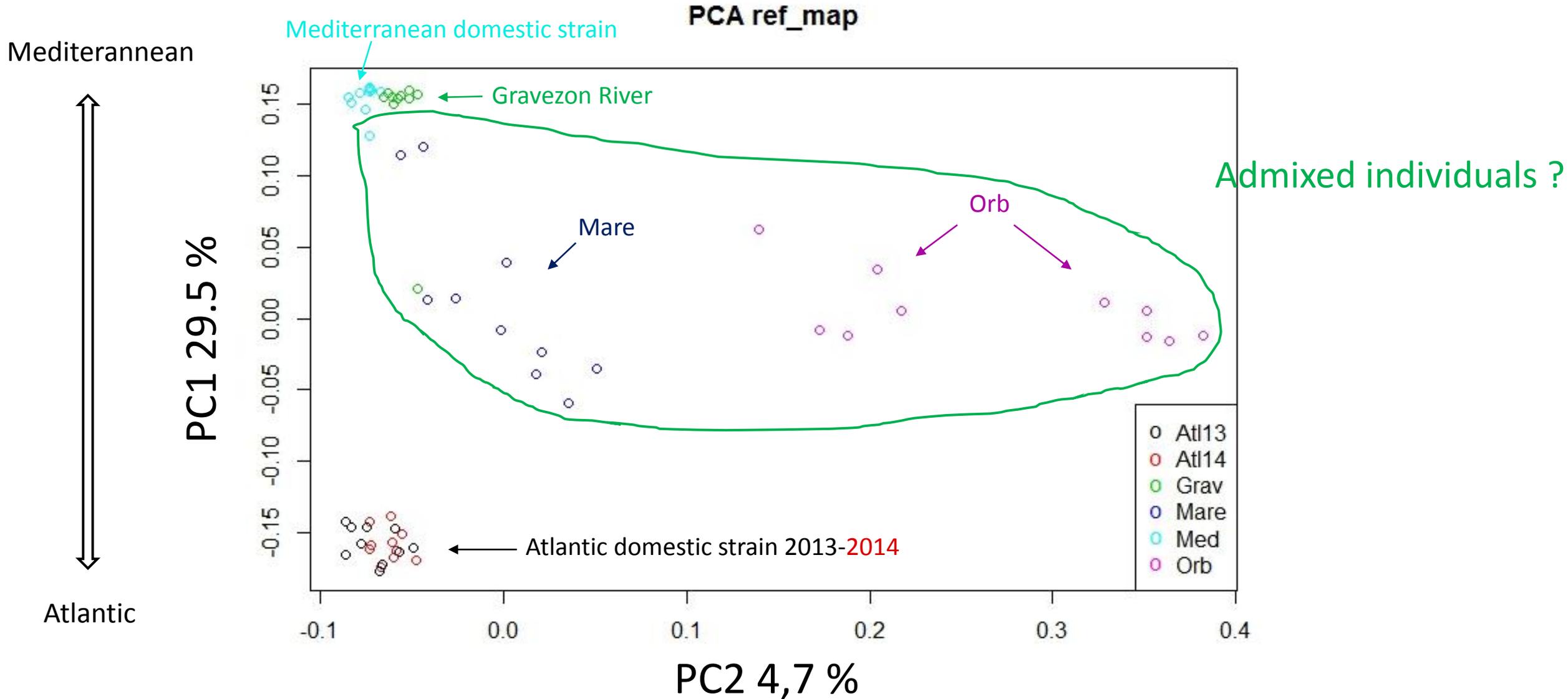
Leitwein et al. *J. Fish Biol* (2016)

- Lower average diversity for the Mediterranean strain
- 'Classical' higher diversity at LGs extremities

Principal Component Analysis (PCA)



Principal Component Analysis (PCA)



Part 1

Concluding remarks

→ **Large number of SNPs** discovered; **similar genome-wide distribution** along LGs using both approaches

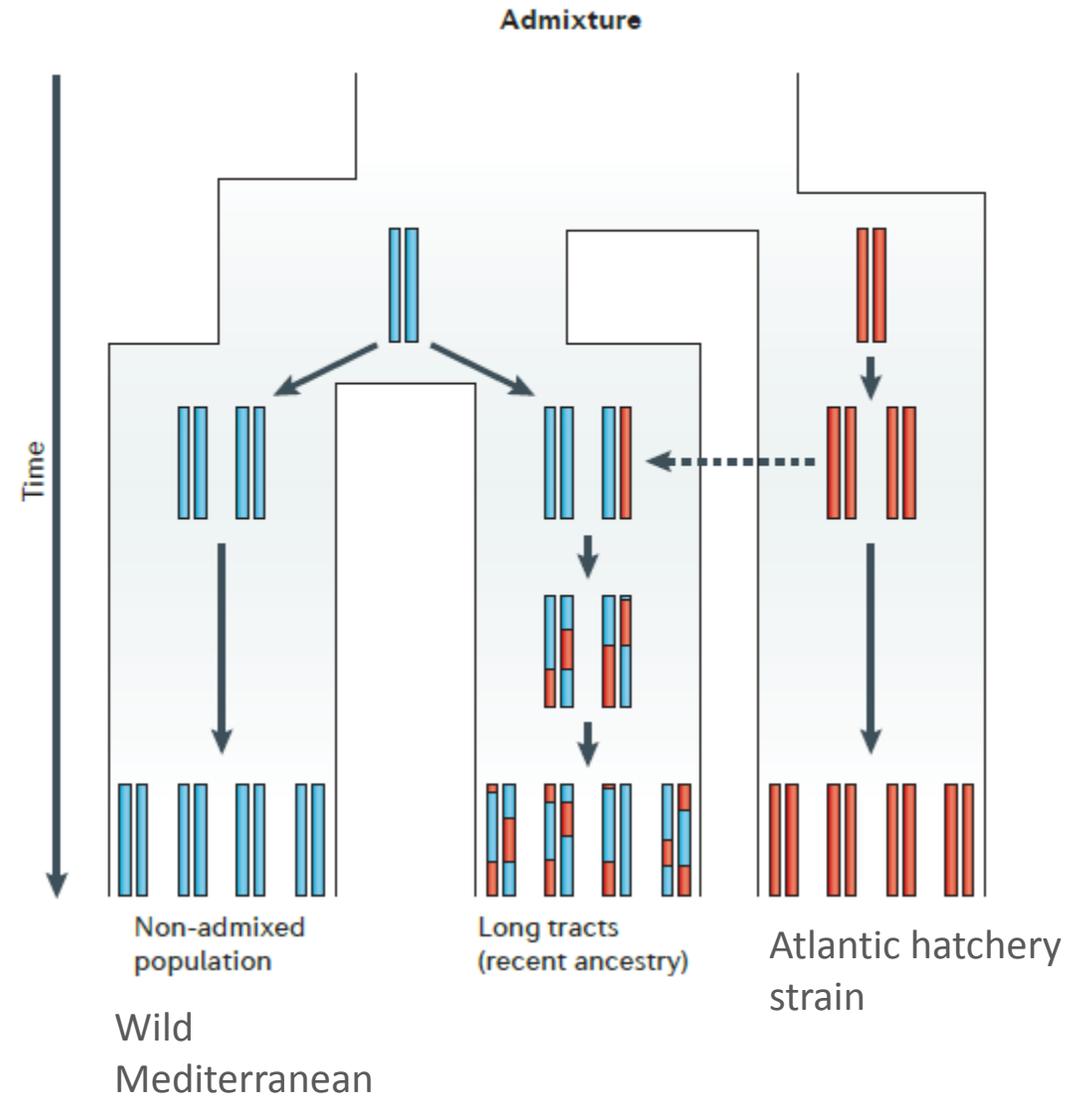
→ **Lower mapping density** and **higher nucleotide diversity** at LGs extremities → footprint of **residual tetrasomy** in these regions ?

→ The **Mediterranean hatchery strain** is twice less diversified than **wild Mediterranean** samples

→ **Presence of admixed individuals in wild populations**

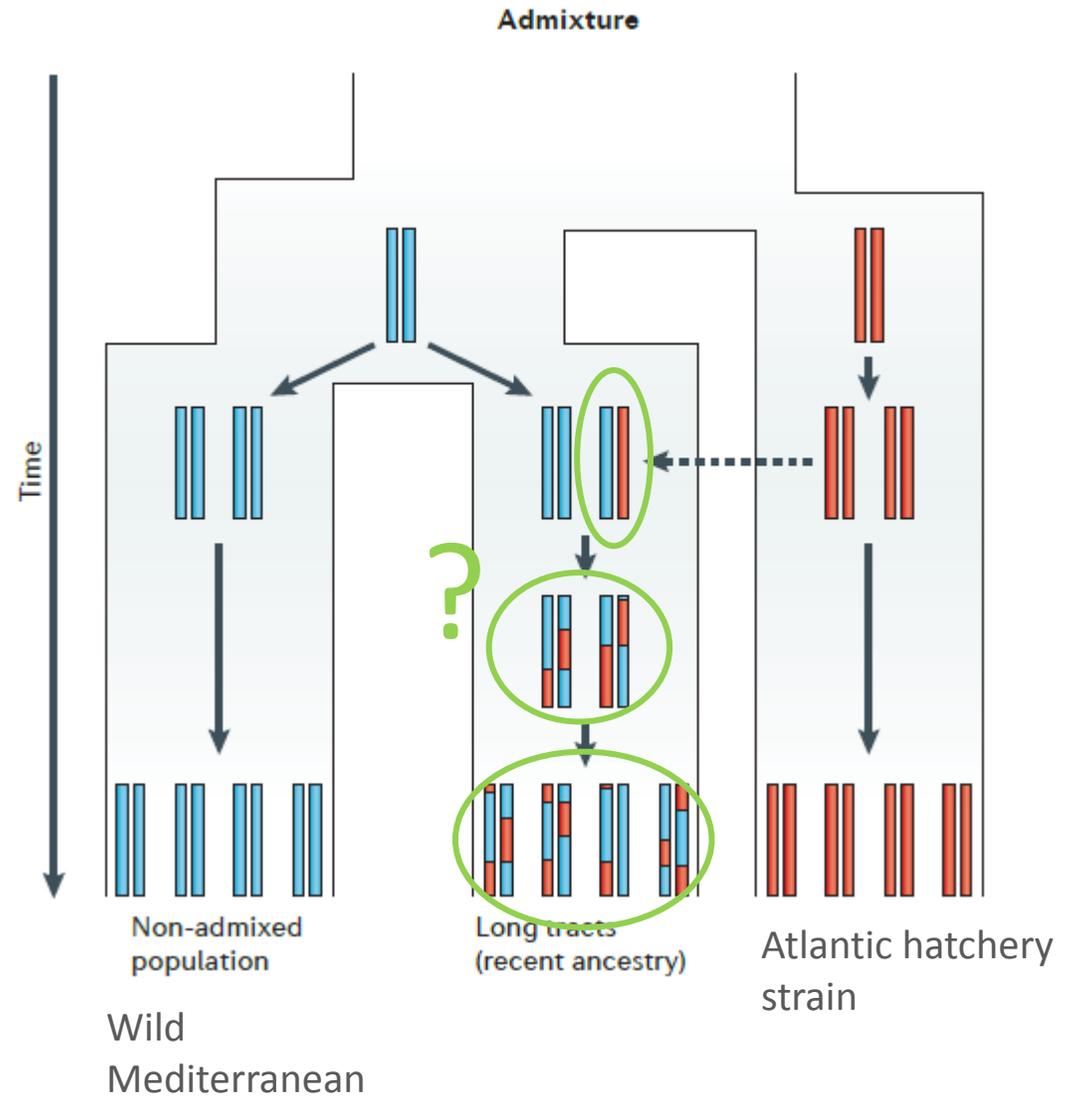
→ Presence of admixed individuals :

What are the distribution patterns of admixture within the genome ?



→ Presence of admixed individuals :

What are the distribution patterns of admixture within the genome ?



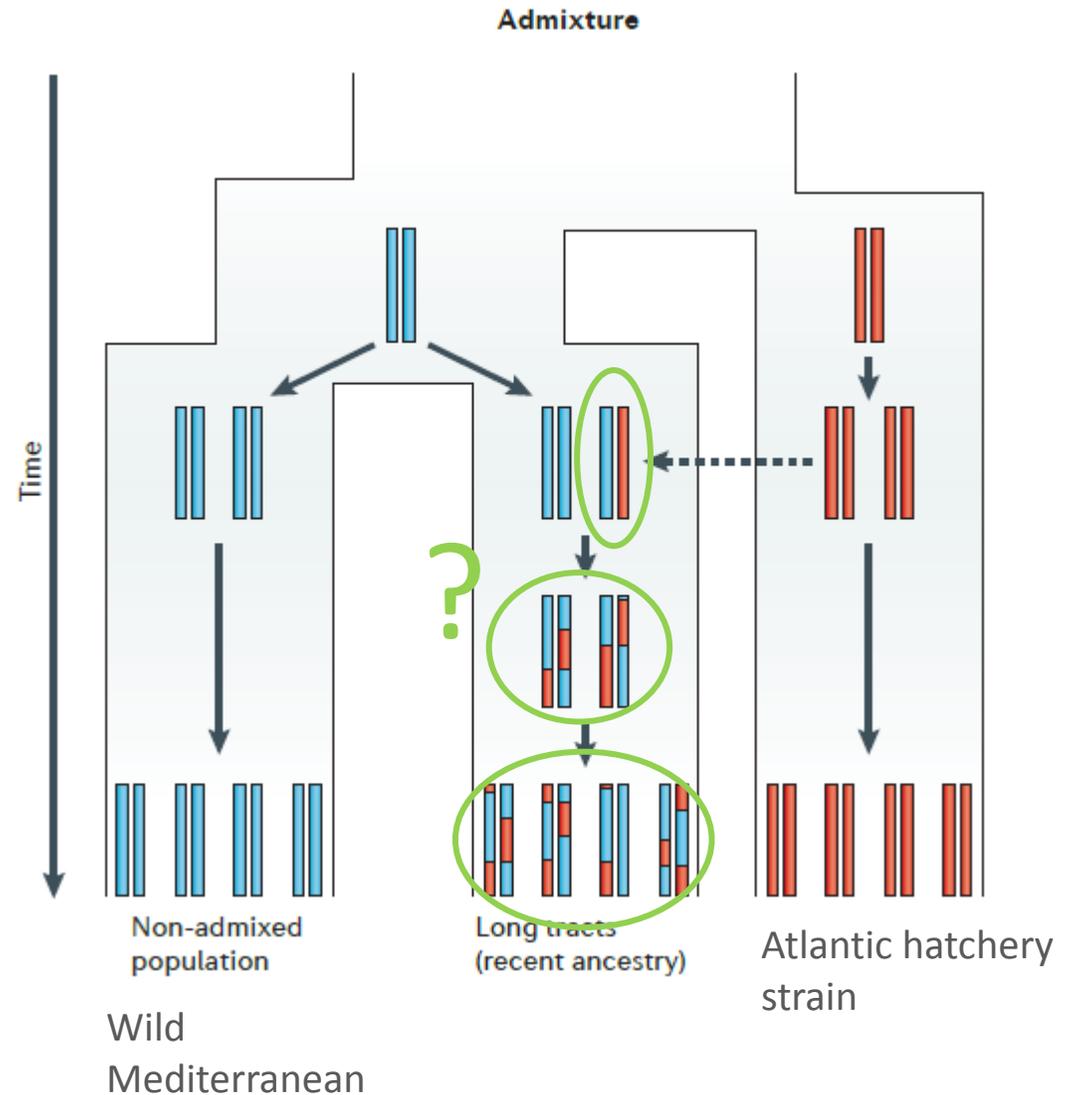
→ Presence of admixed individuals :

What are the distribution patterns of admixture within the genome ?

To estimate the introgression rate along the genome we need :

-> to assess the introgression at haplotype level

-> to infer the recombination rate landscape



→ Presence of admixed individuals :

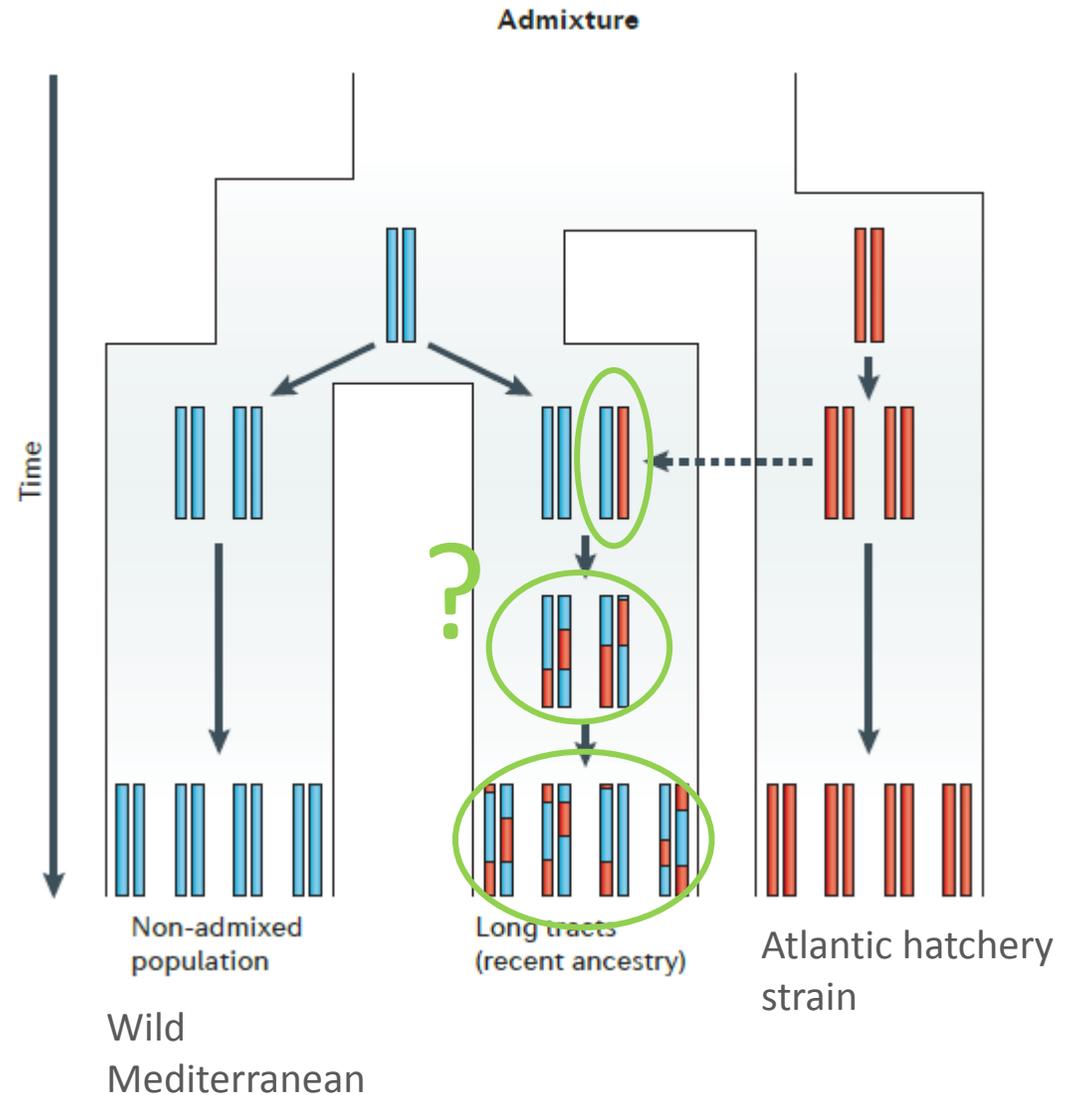
What are the distribution patterns of admixture within the genome ?

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-> to assess the introgression at haplotype level

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Need for *S. trutta* linkage groups



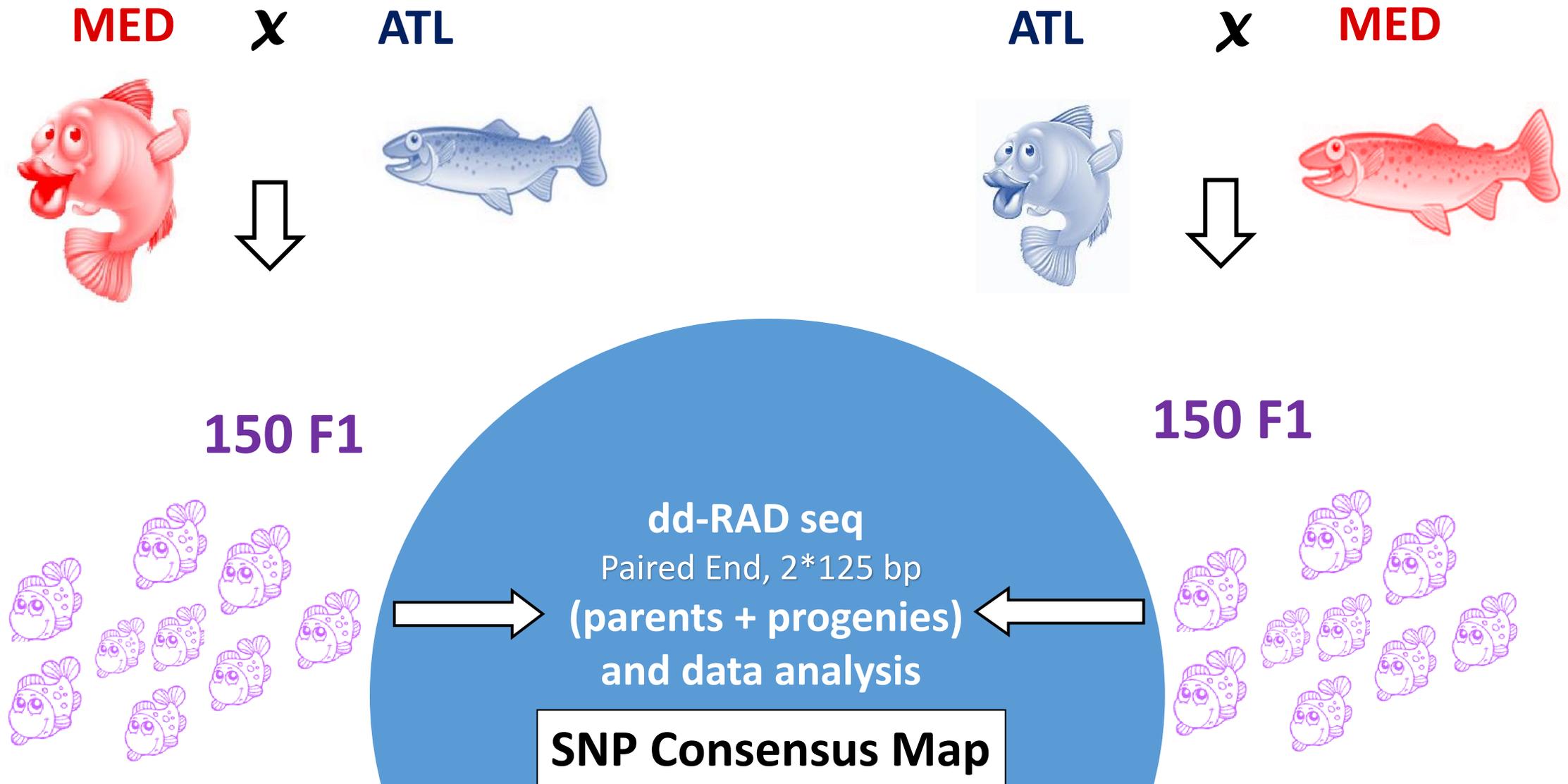
Part 2

High density *S. trutta* linkage map

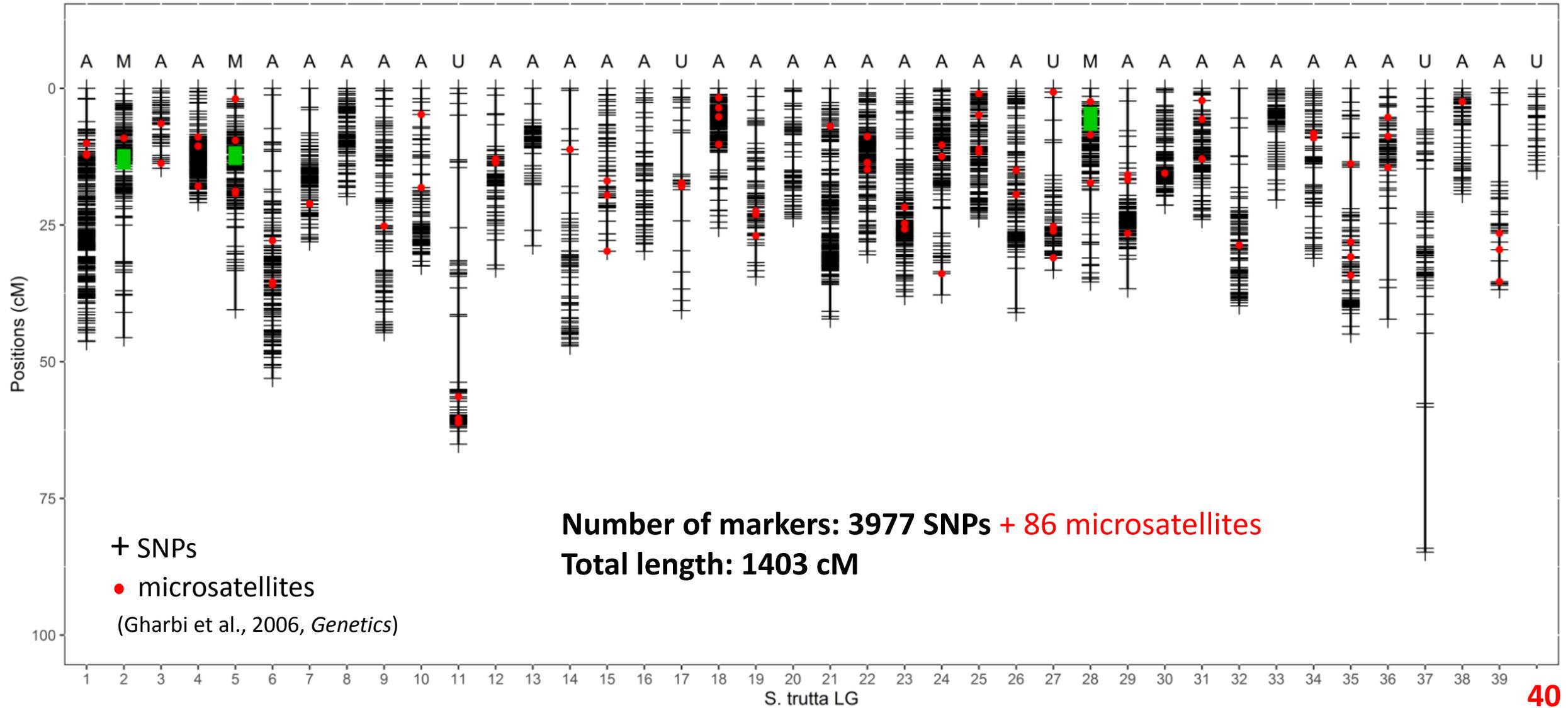
Gharbi et al.'s (2006) brown trout linkage map

- Based on microsatellite (N = 288) + allozyme (N= 13) markers
- **Incomplete: 37** LGs found but **40** LGs expected from karyotypic studies
- Might be improved by high-throughput genomic techniques

The hybrid linkage map design

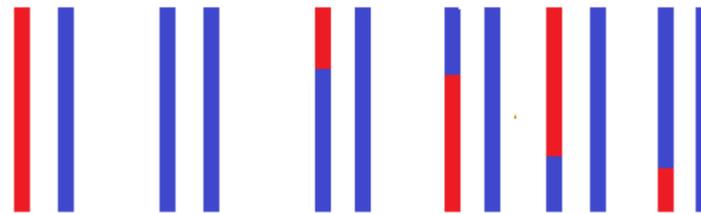
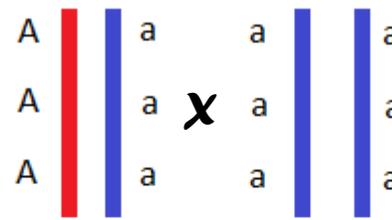
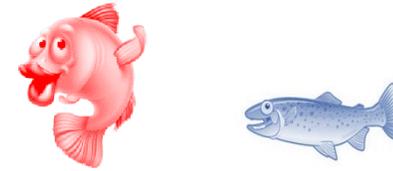
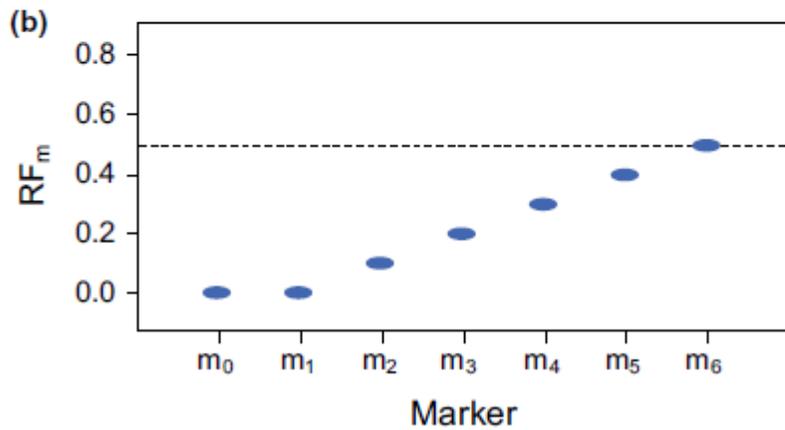
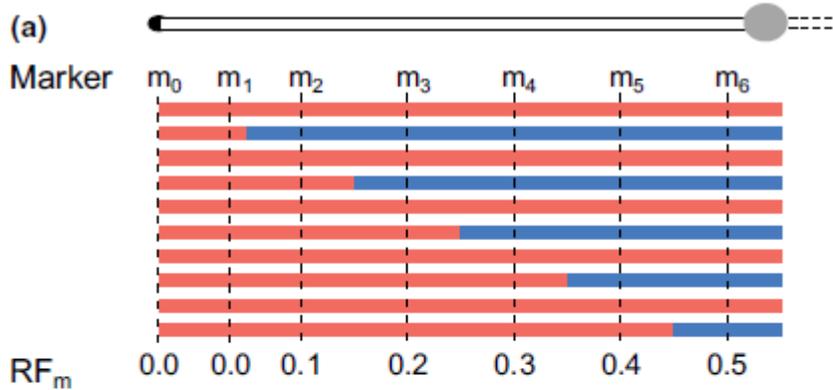


A new *Salmo trutta* linkage map

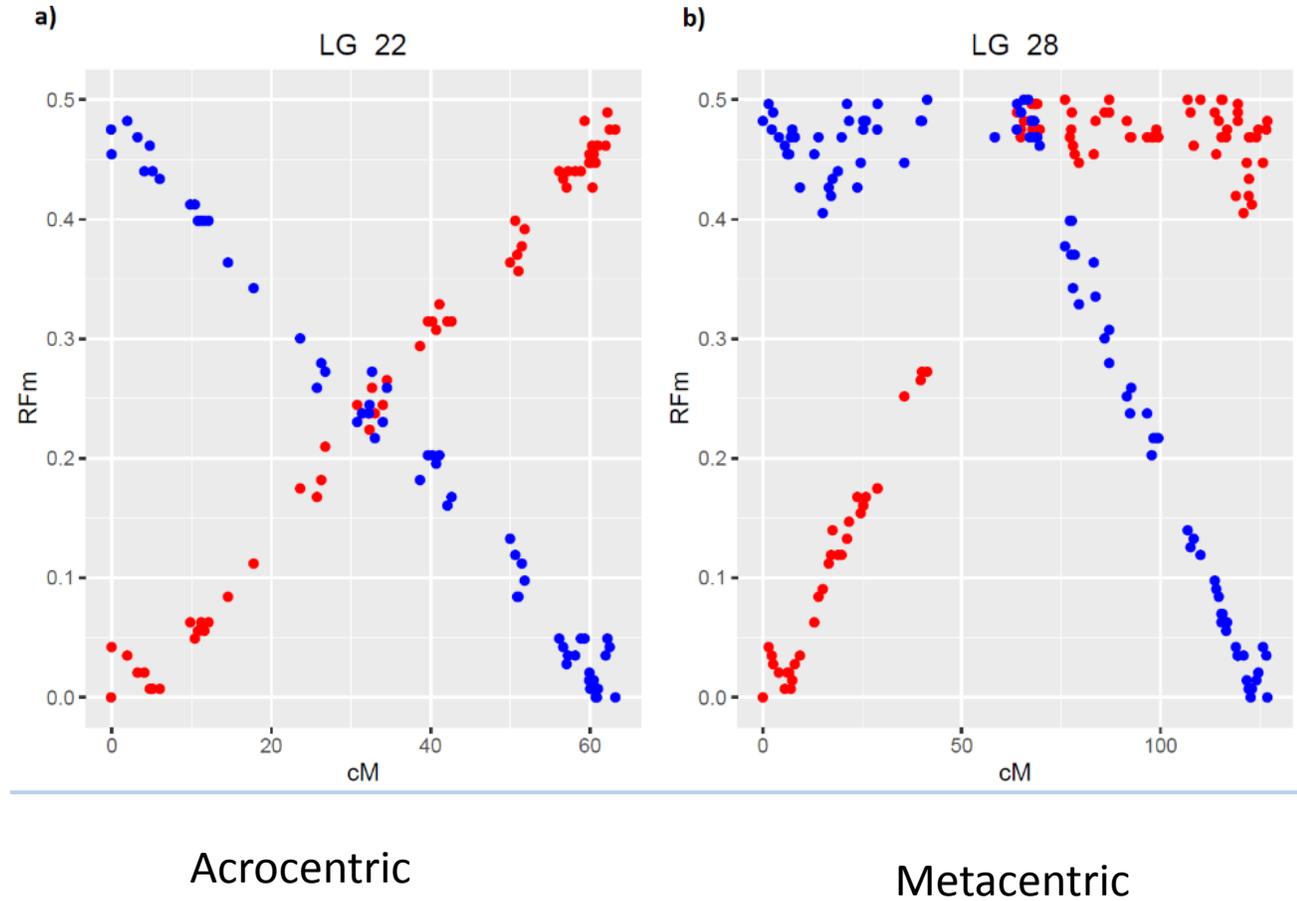
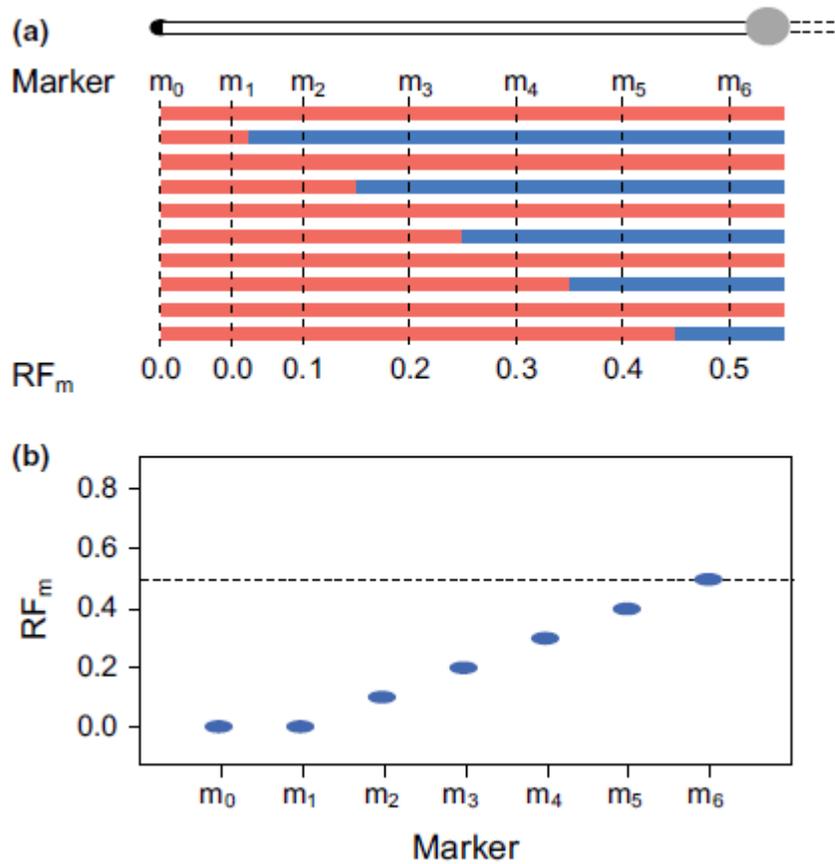


We got
them!

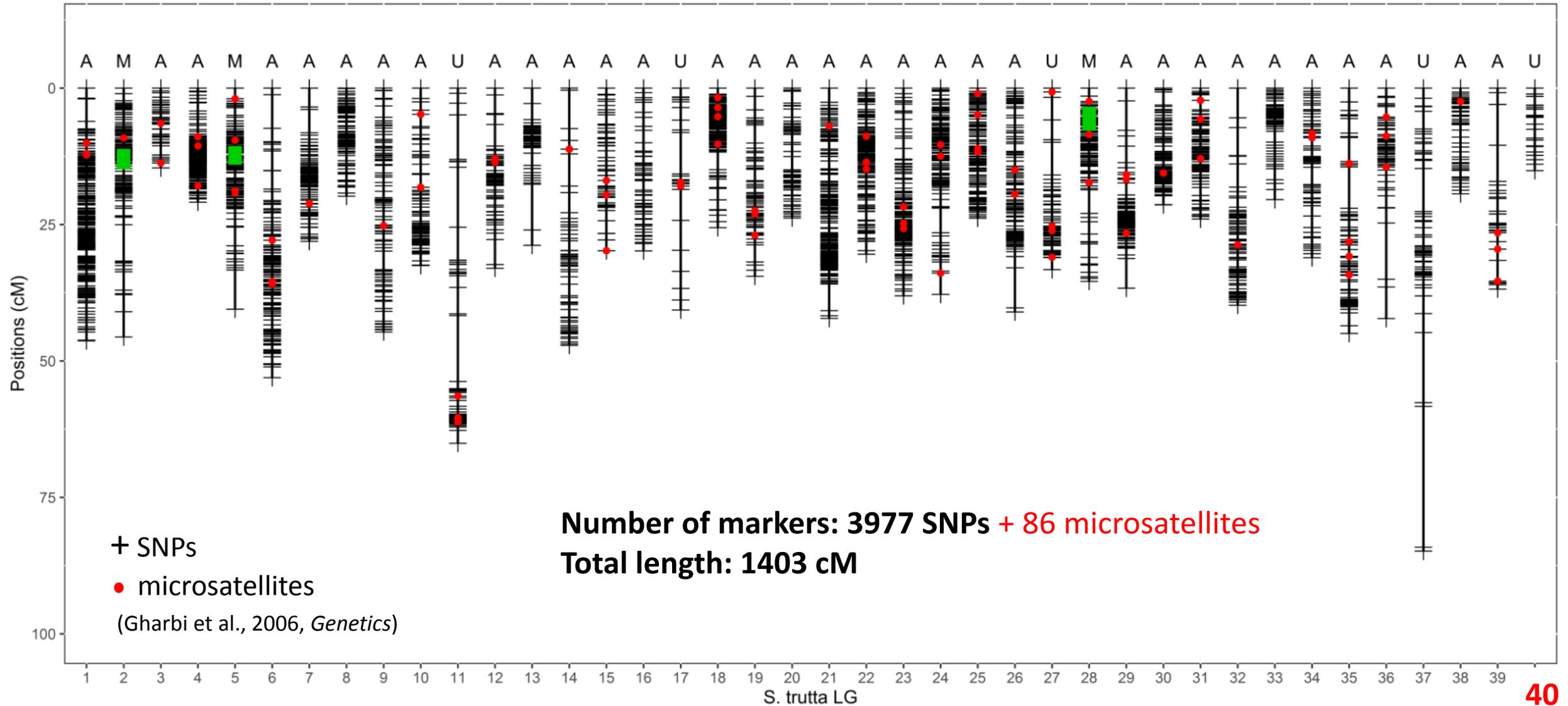
Centromere location and chromosome type



Centromere location and chromosome type



A new *Salmo trutta* linkage map



We got
them!

MapComp

Sutherland et al. (2016)

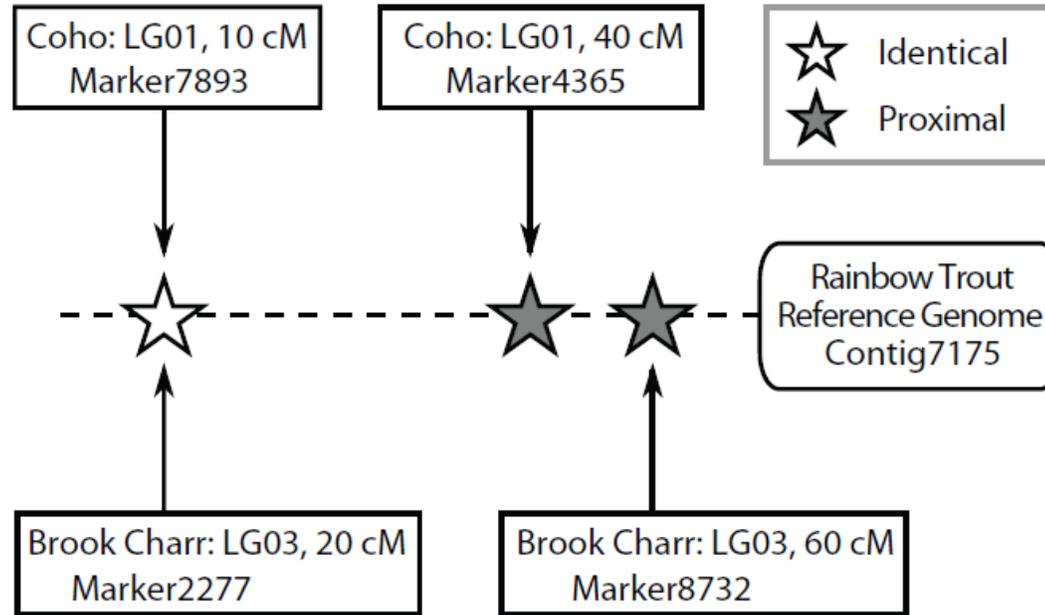


Fig. 1. Schematic of MAPCOMP using a reference genome to pair markers. MAPCOMP compares genetic maps from two different species by mapping marker sequences against a reference genome, then retaining high quality mappings that only hit against one locus in the genome. Markers from each species are paired if they hit against the same contig/scaffold by taking the closest two markers together as each pair. Each marker is paired without replacement, and so any other marker that was second closest to the now-paired marker is discarded. This method captures identical markers (white star in image) and non-identical markers (grey stars). Finally, the linkage group and cM position of each marker is plotted in an Oxford grid. Note that the marker names and contig ID in the schematic are for demonstration purposes only and do not reflect actual pairings.

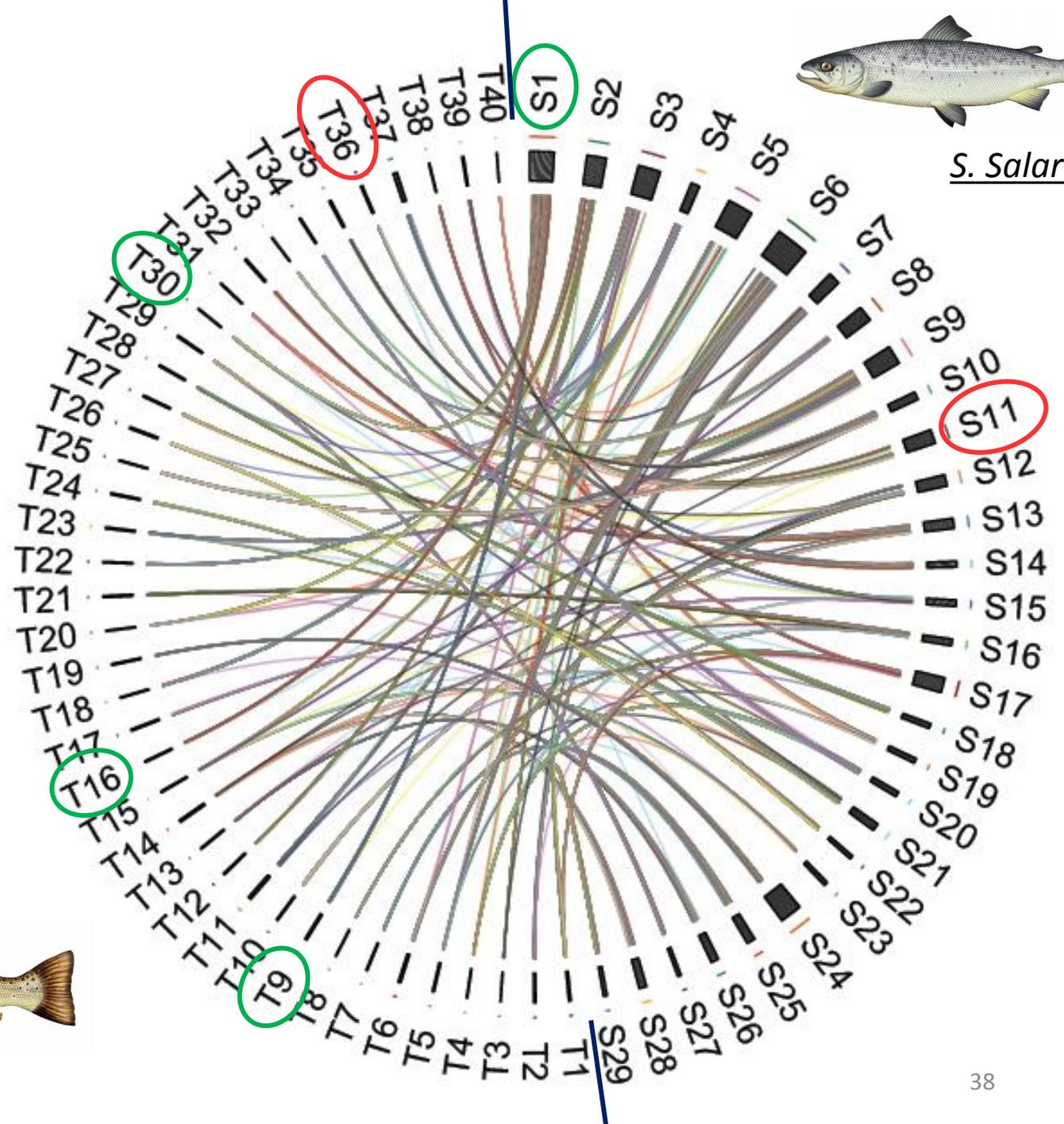
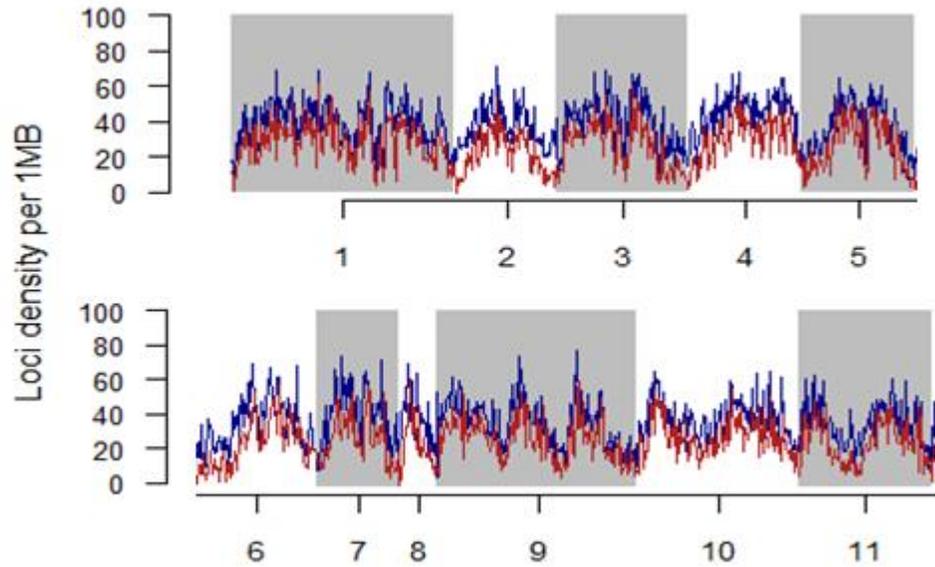
Synteny between *S. salar* and *trutta*



S. salar

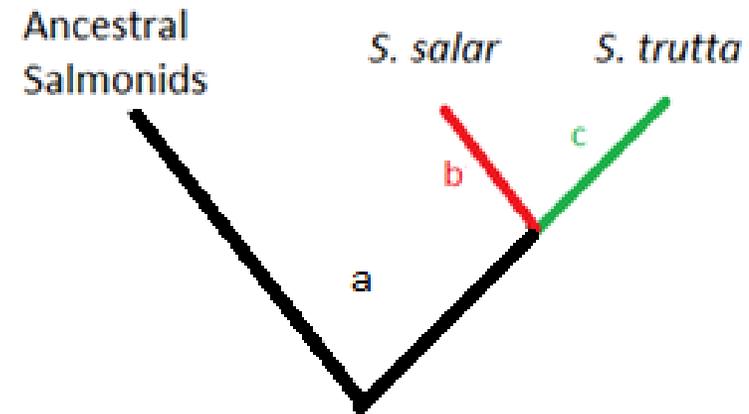


S. trutta



Chromosomal rearrangements

		Fusion	Fission
a	Salmo before speciation	5	3
b	<i>S. salar</i> after speciation	13	2
c	<i>S. trutta</i> after speciation	0	0



Nucleotide divergence between *S. salar* and *trutta*

$$d = \pi_b - \pi_w$$

π_b : nucleotide diversity between *S. salar* and *S. trutta*

π_w : nucleotide diversity within *S. trutta*

Nucleotide divergence between *S. salar* and *trutta*

$$d = \pi_b - \pi_w$$

π_b : nucleotide diversity between *S. salar* and *S. trutta*

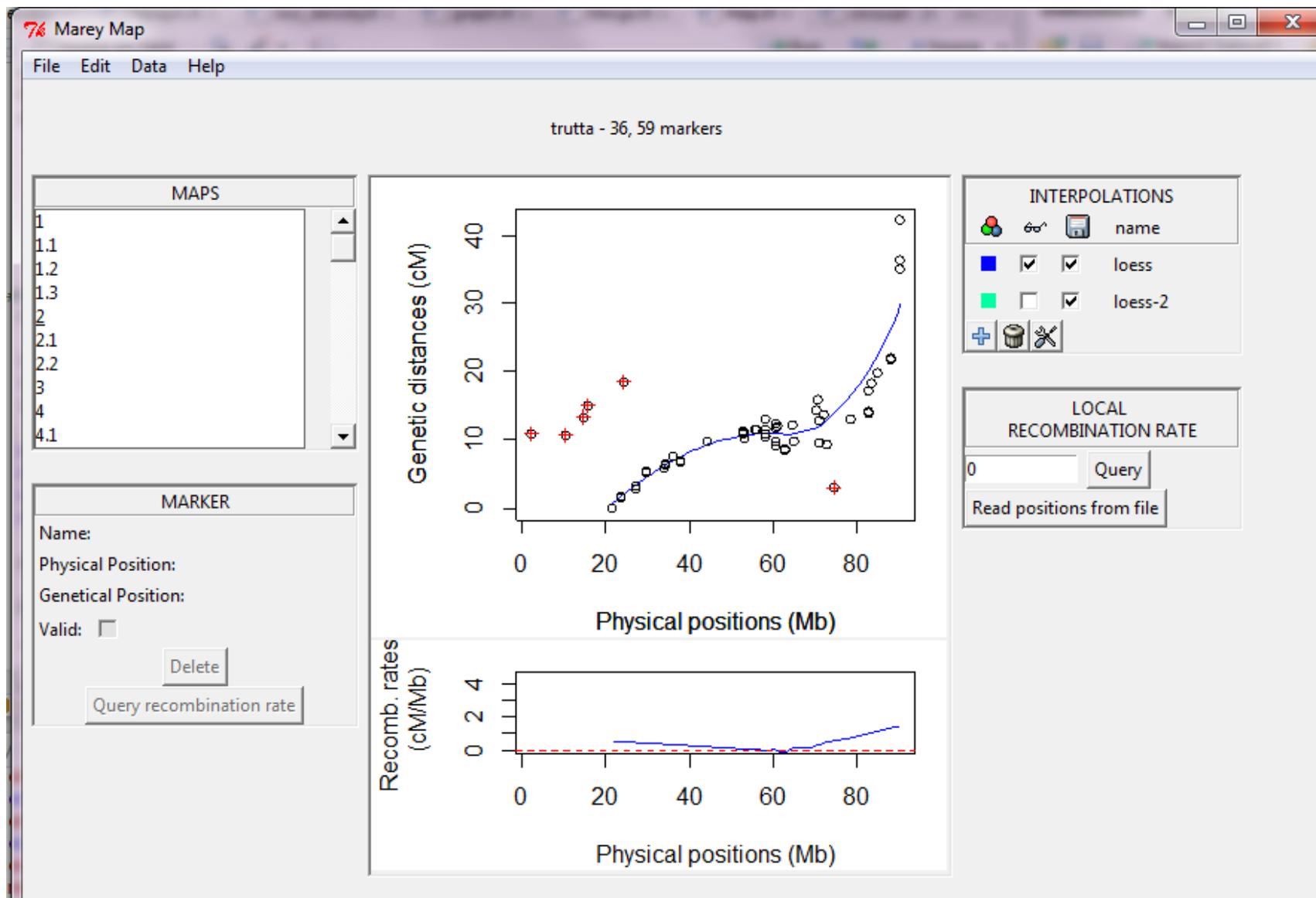
π_w : nucleotide diversity within *S. trutta*

$$d = 0.02285 - 0.0041$$

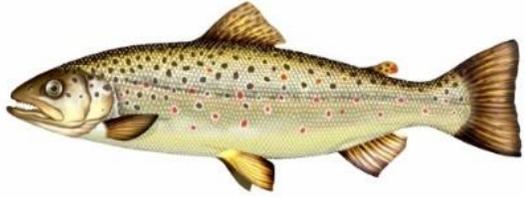
$$d = 1.87\%$$

5.94% (Bernatchez *et al.*, 1992)

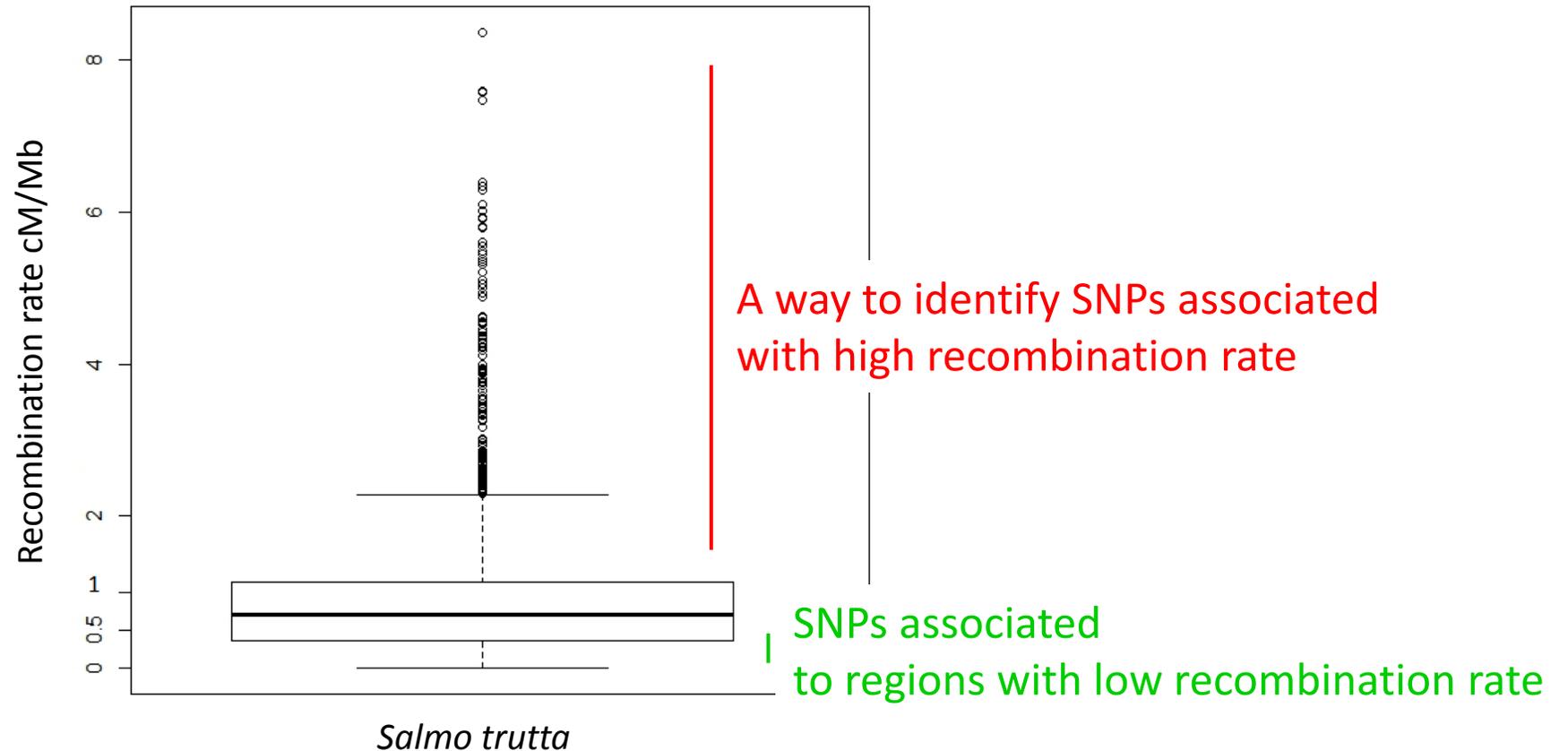
Estimate of recombination rate in the brown trout genome



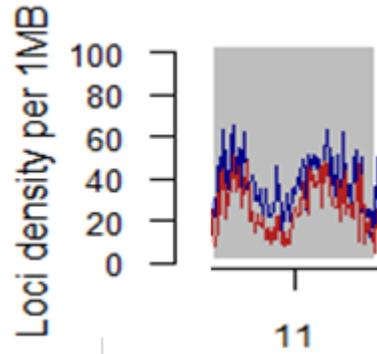
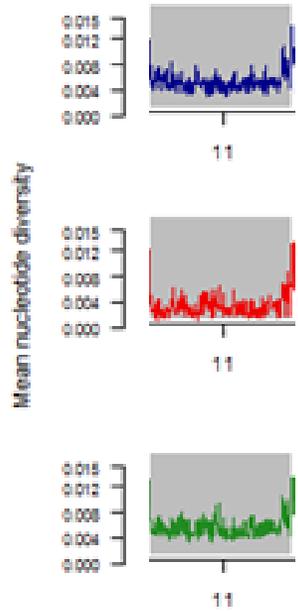
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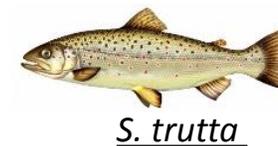
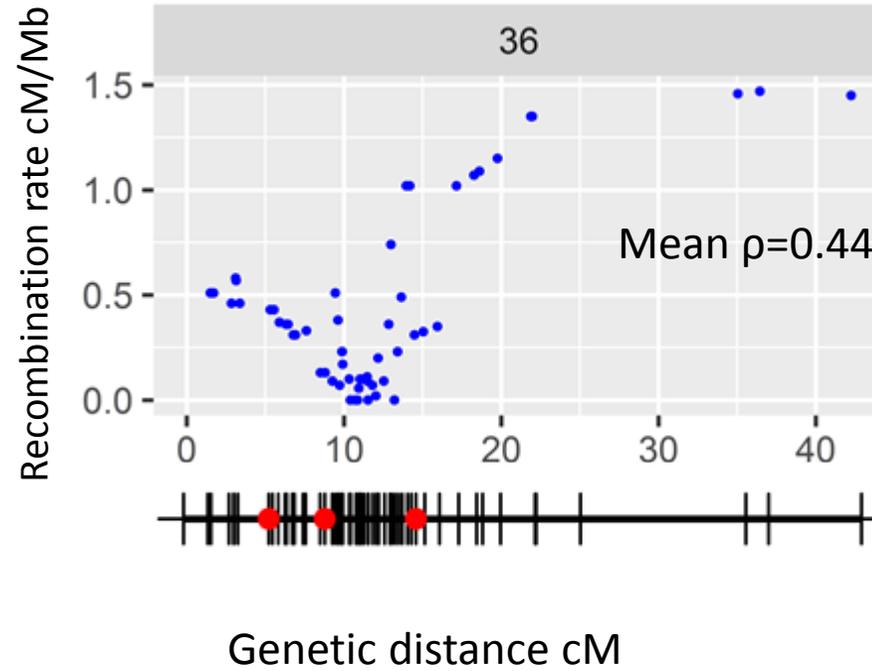
Total mean recombination rate = 0.88 cM/Mb



Recombination rate estimation



LG 11



LG 36

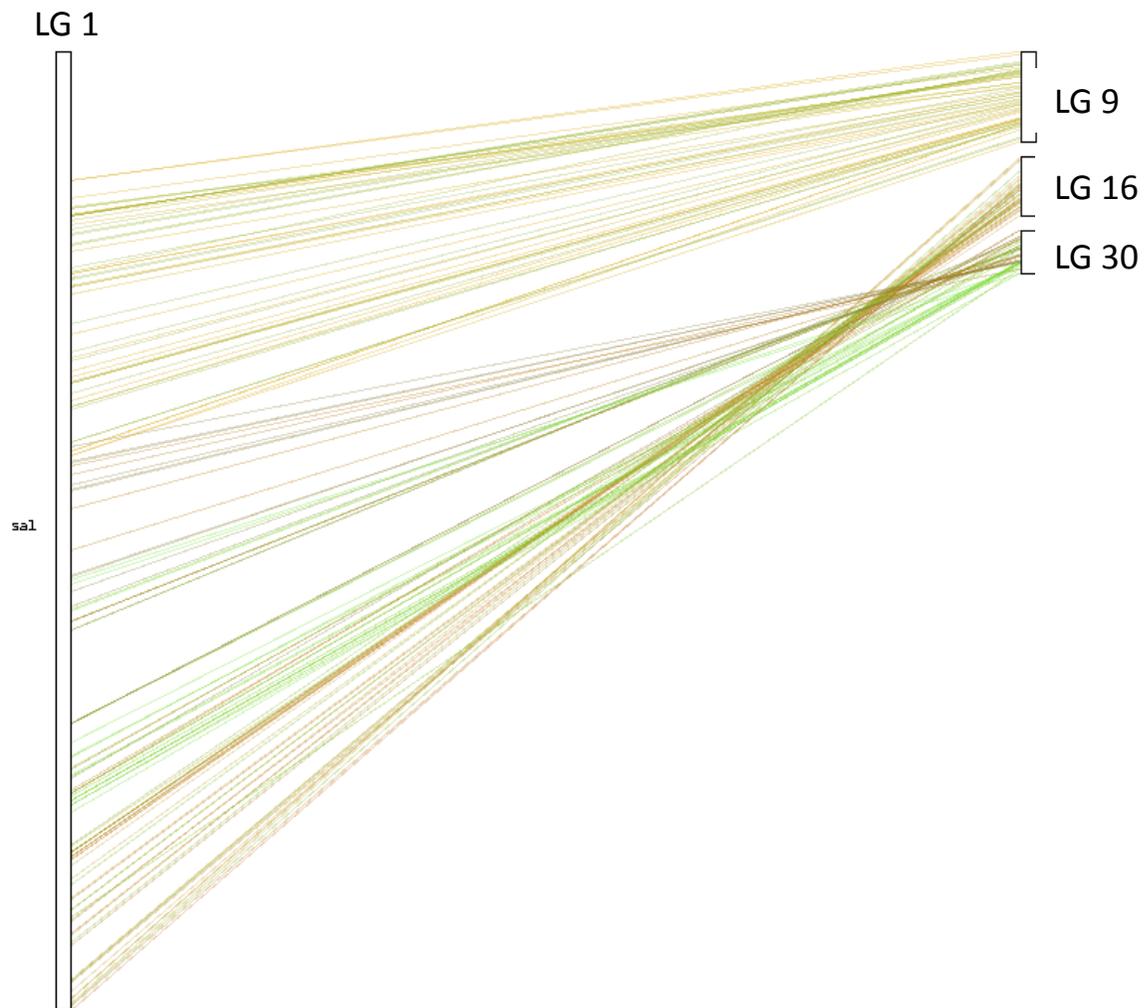
Recombination rate estimation



S. salar



S. trutta



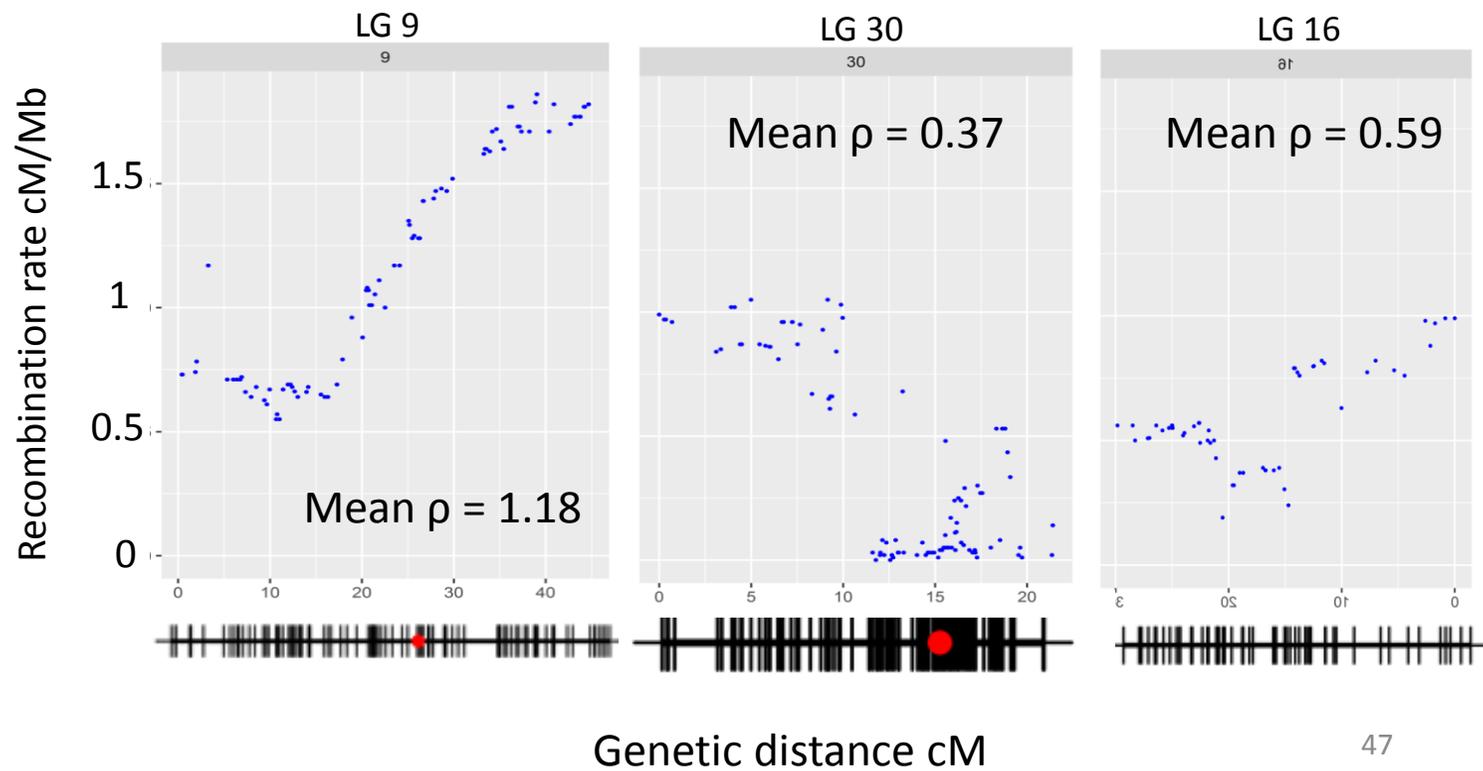
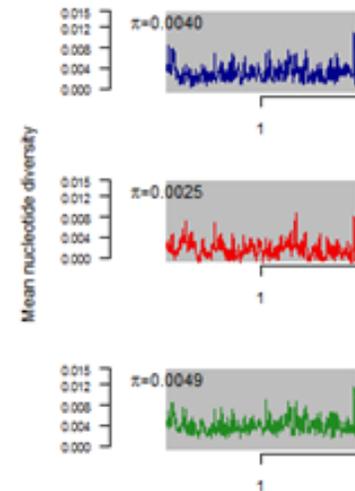
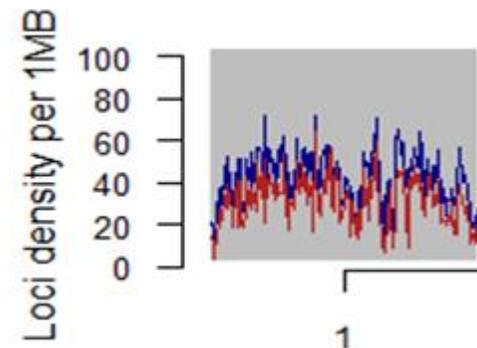
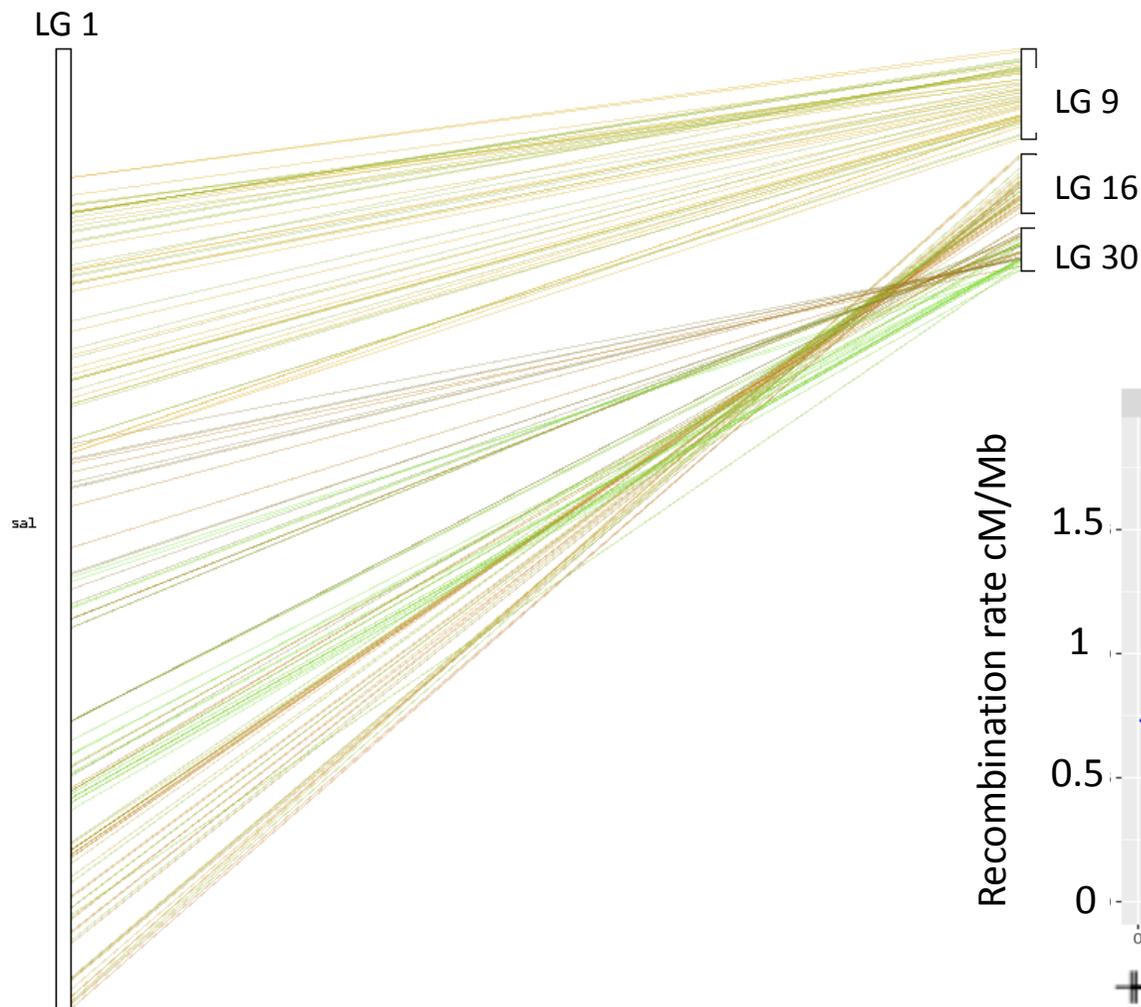
Recombination rate estimation

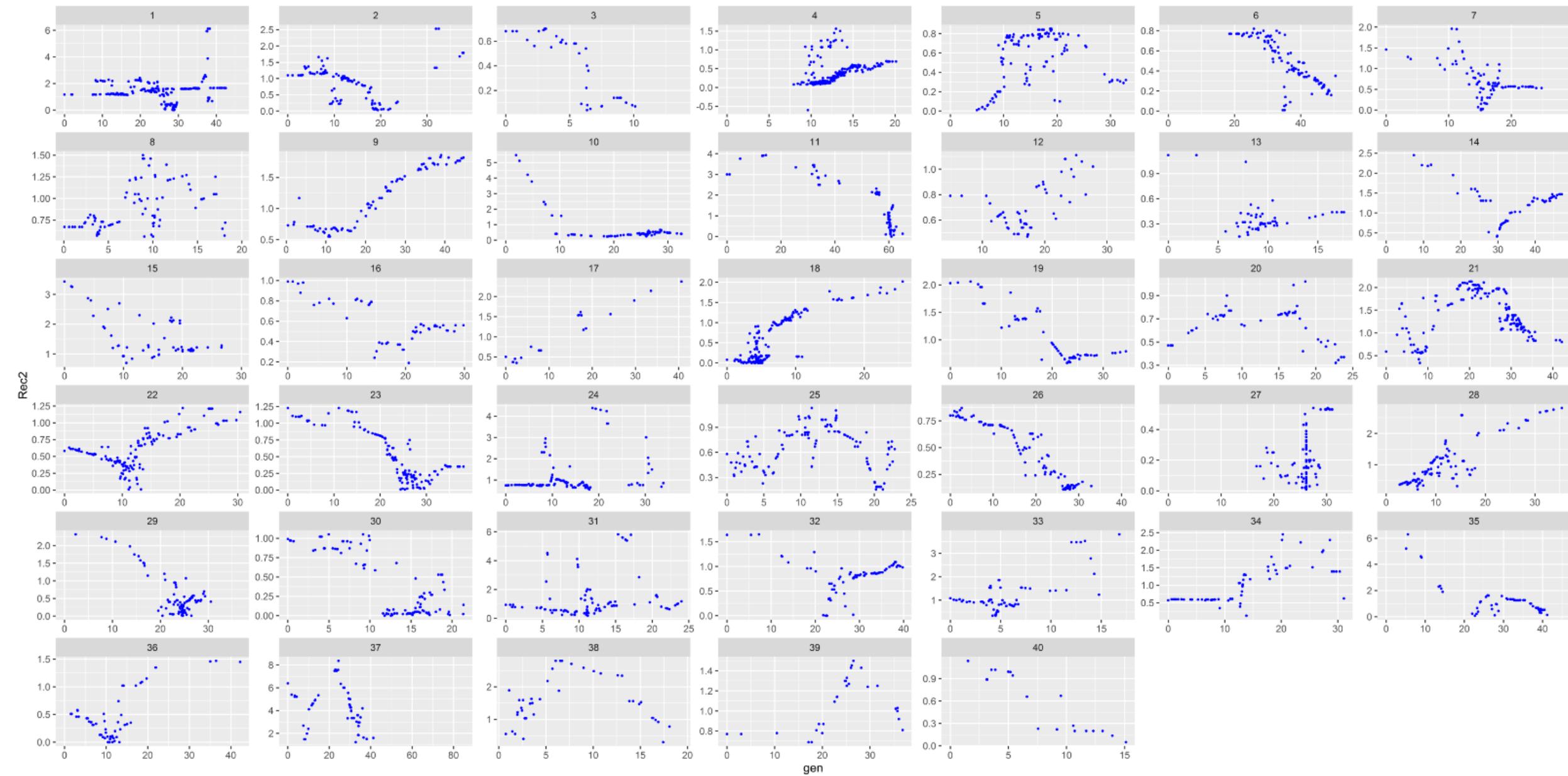


S. Salar

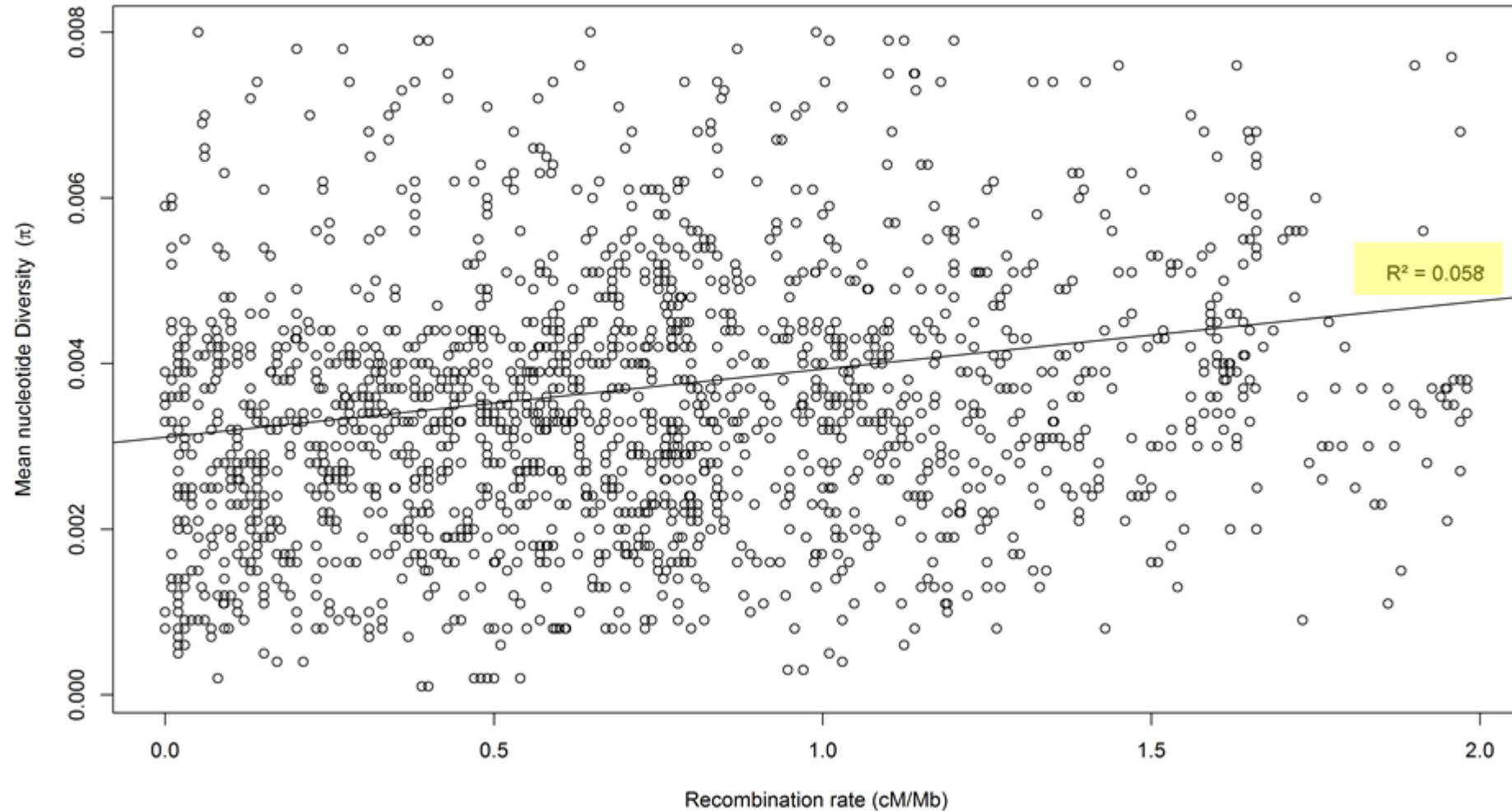


S. trutta





Correlation between the nucleotide diversity and the recombination rate



$P < 0.001$

Part 2

Concluding remarks

- **Strong (and expected...) synteny** between *S. salar* and *S. trutta*
- A improved linkage map is available providing information on **chromosomal rearrangements**:
translocation, fissions, Robertsonian rearrangements between *S. salar* and *S. trutta*
- **Positive correlation** between the **recombination rate** and the **nucleotidic diversity**
- The estimation of the (local) **recombination rate** is accessible in *S. trutta*
- Does the **recombination** landscape **affect local introgression rate** along the genome ?

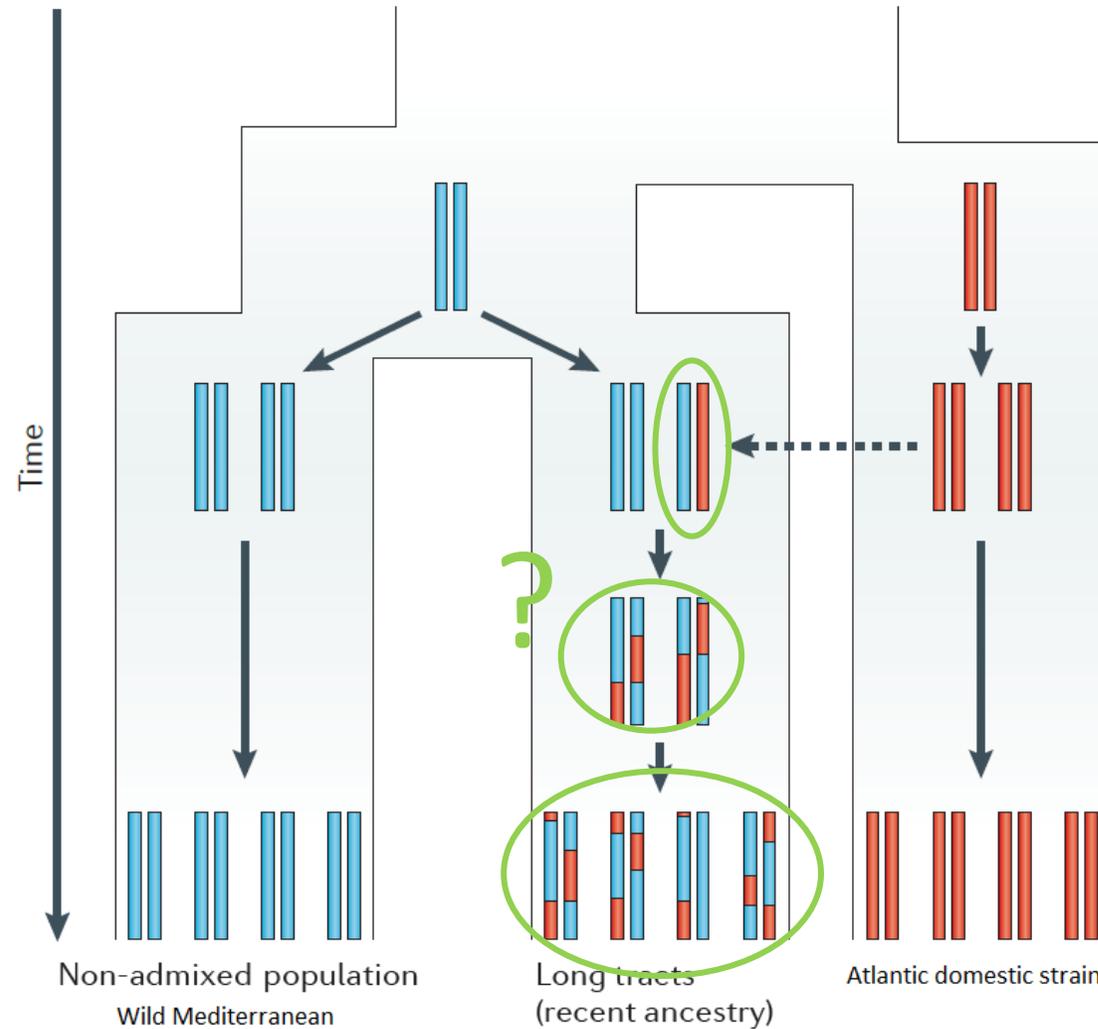
Part 3

→ To identify admixed individuals in the wild:

3 rivers : Mare N= 14
 Orb N= 23
 Gravezon N= 45

} N = 82

(Dom ATL N= 61
 Dom Med N=41)



Part 3

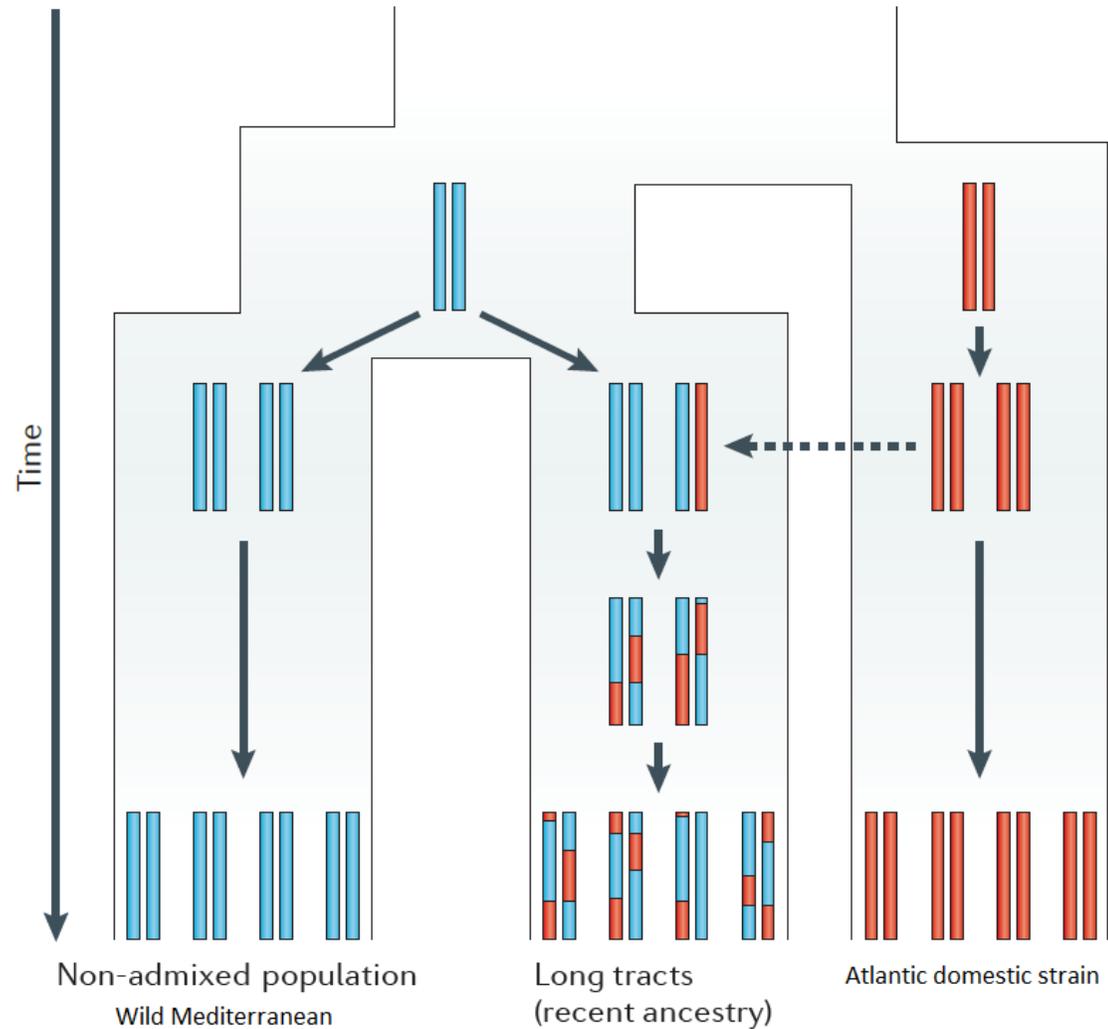
→ To identify introgressed haplotypes

→ To use the **introgressed haplotype**

distribution size as a proxy of the timing of the introgression

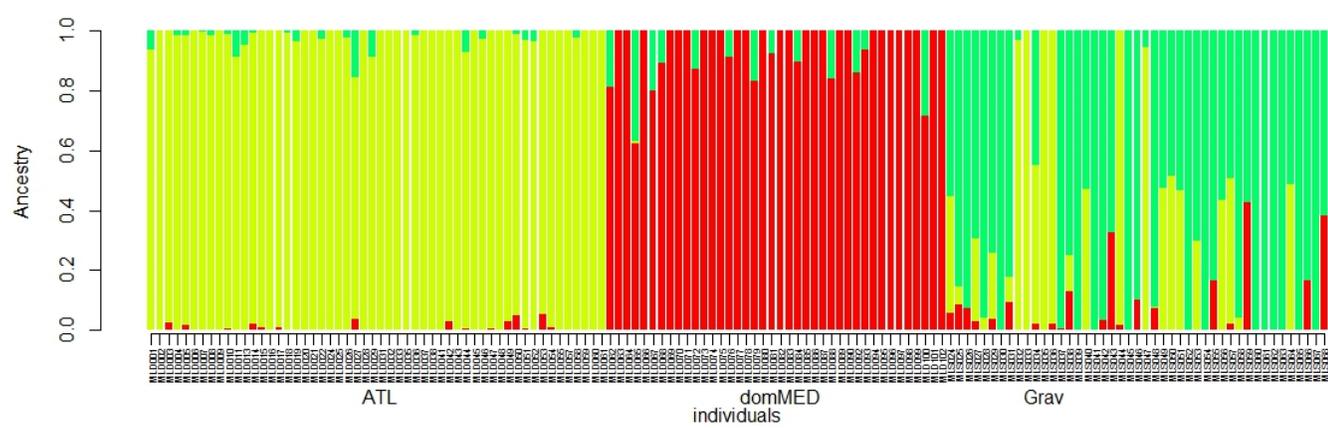
(shorter haplotypes if more

generations/recombination events)

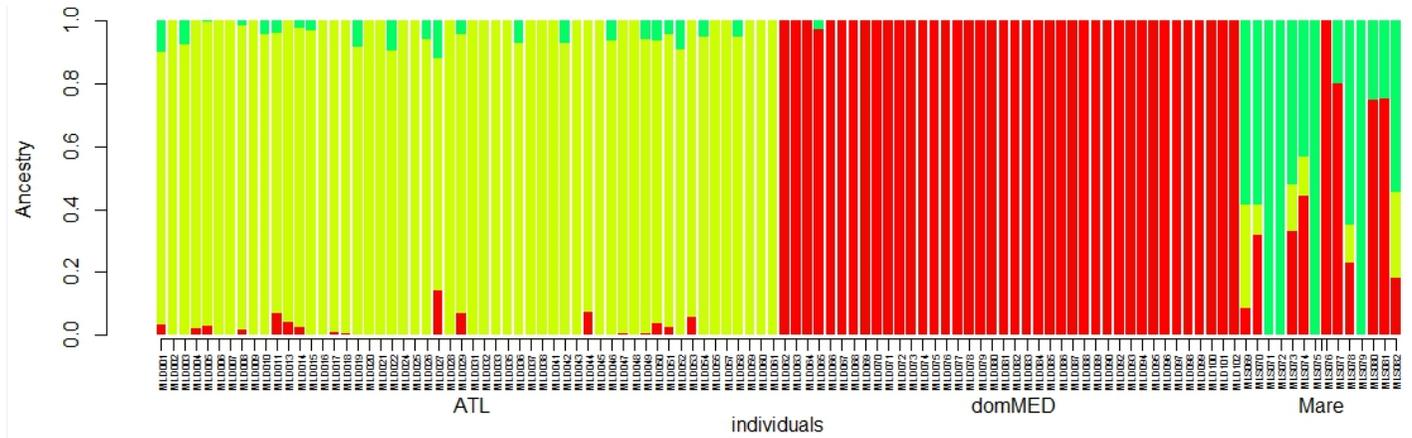


Identification of “pure”
individuals for reference
with Admixture

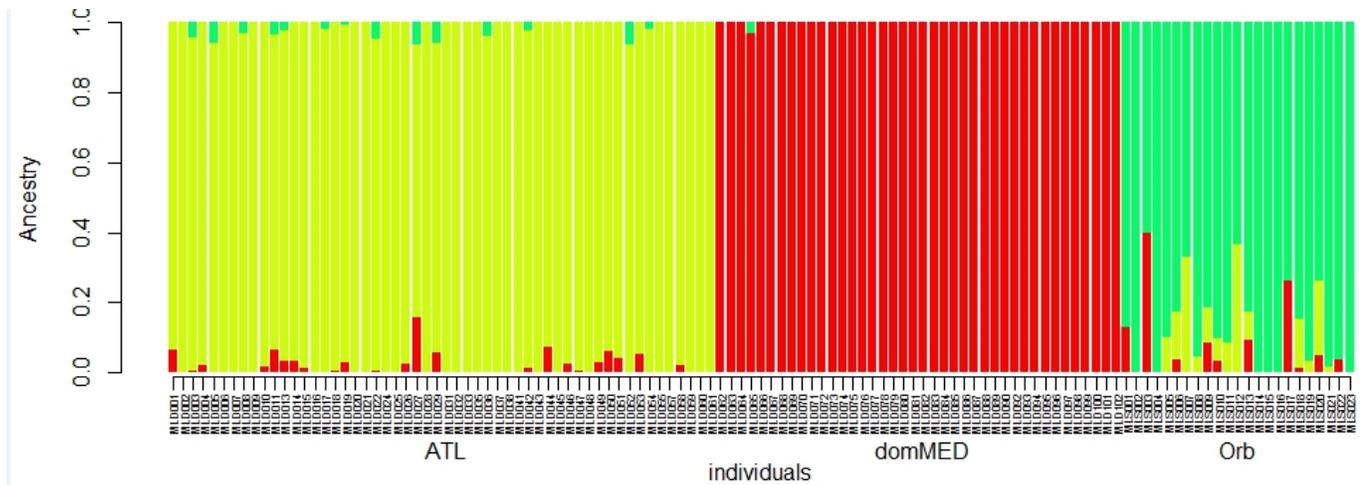
Grav



Mare



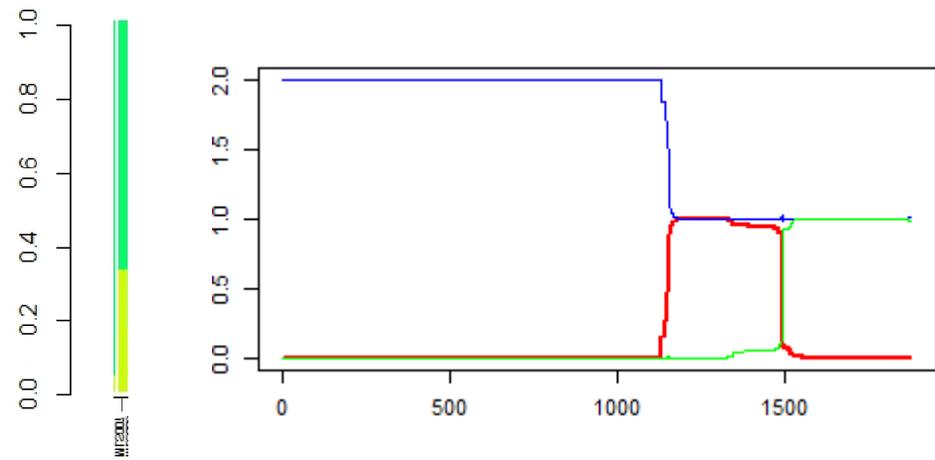
Orb



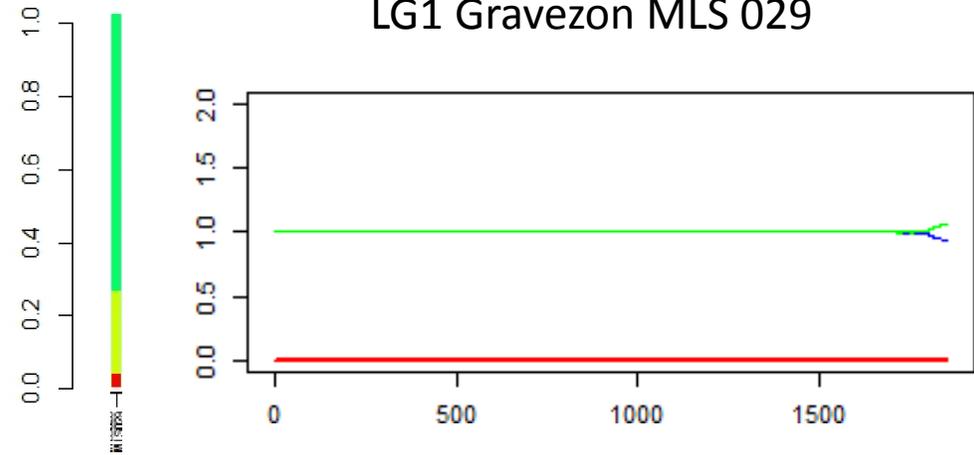
ELAI: Efficient Local Ancestry Inference

Guan Y., 2014

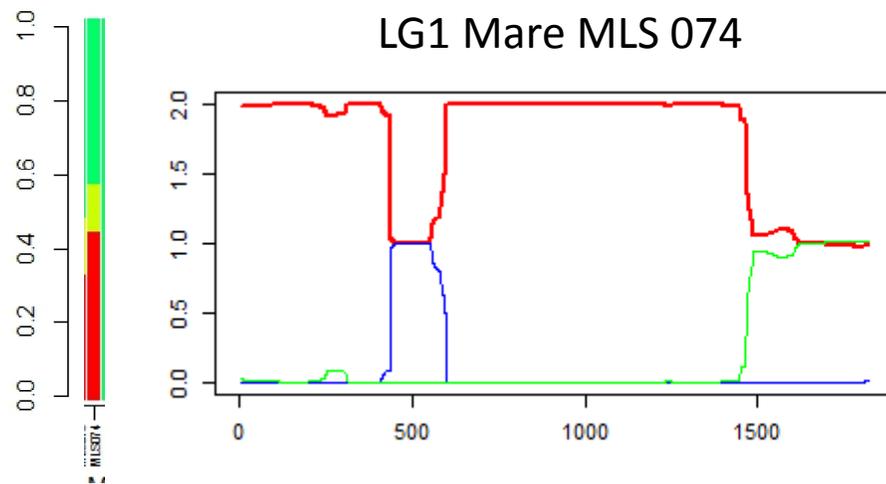
LG1 Orb MLS 007



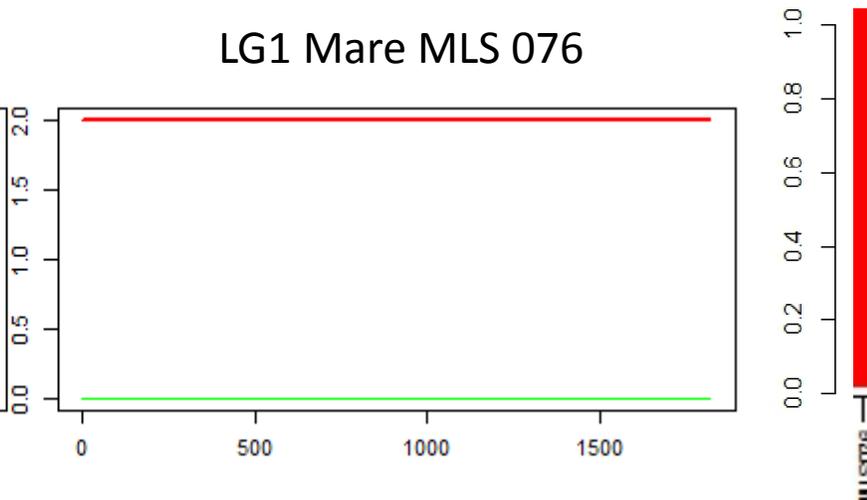
LG1 Gravezon MLS 029



LG1 Mare MLS 074

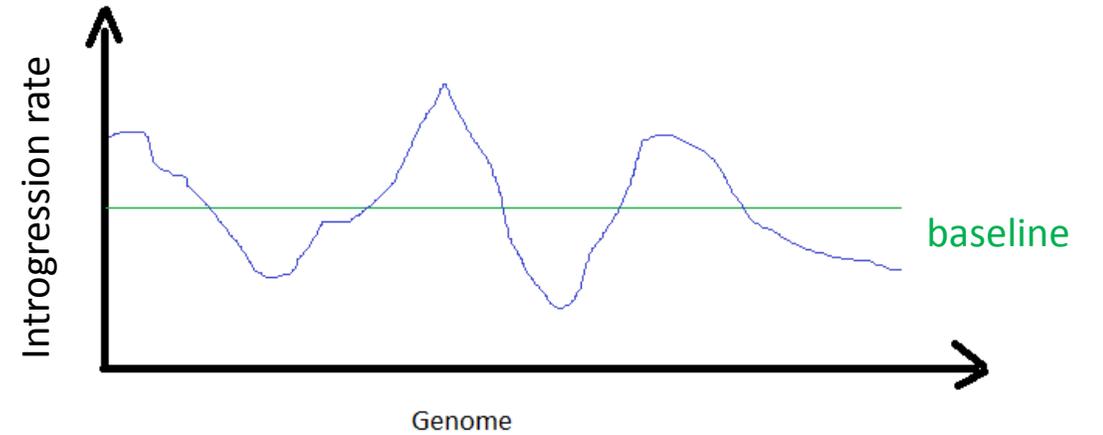
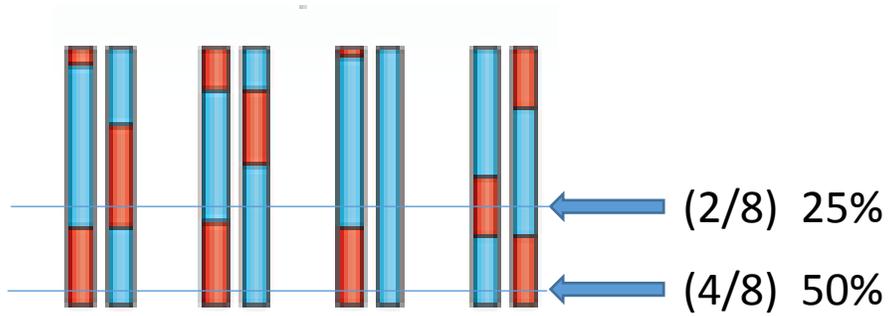


LG1 Mare MLS 076



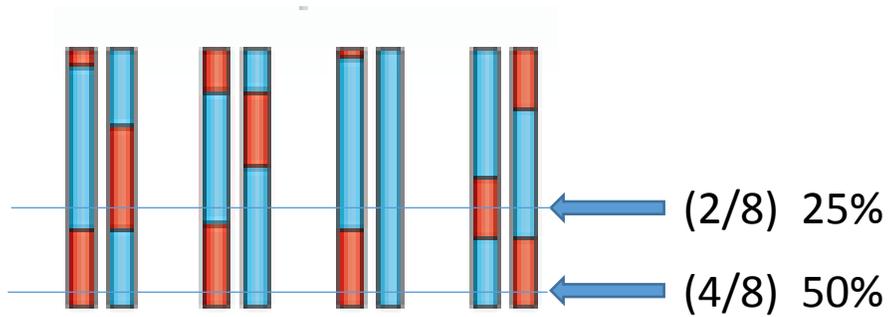
Part 3

→ To determine the introgression rate along the genome

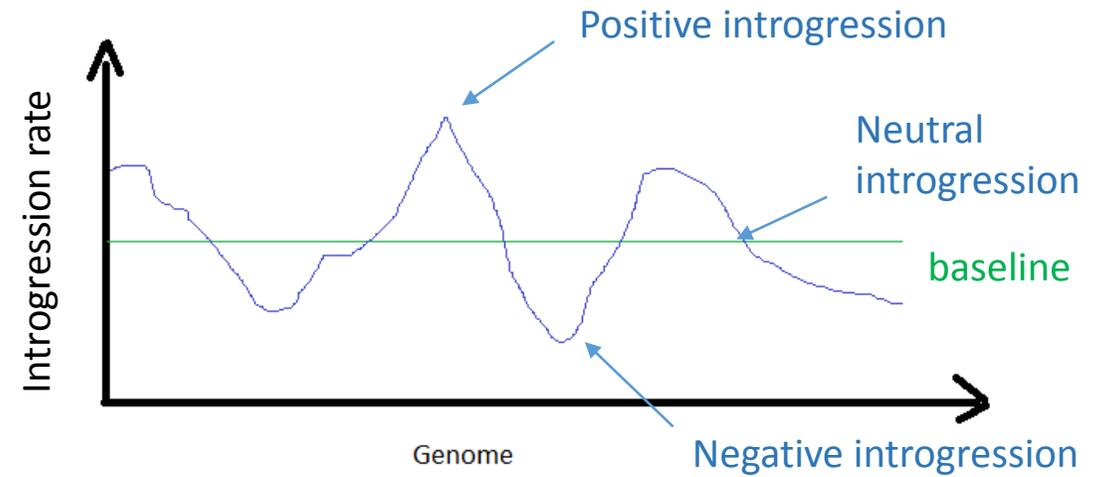


Part 3

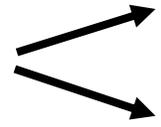
→ To determine the introgression rate along the genome



→ To find signatures of positive or negative introgression



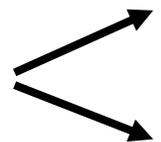
positive introgression



By masking the effect of deleterious alleles

Adaptive introgression

negative introgression

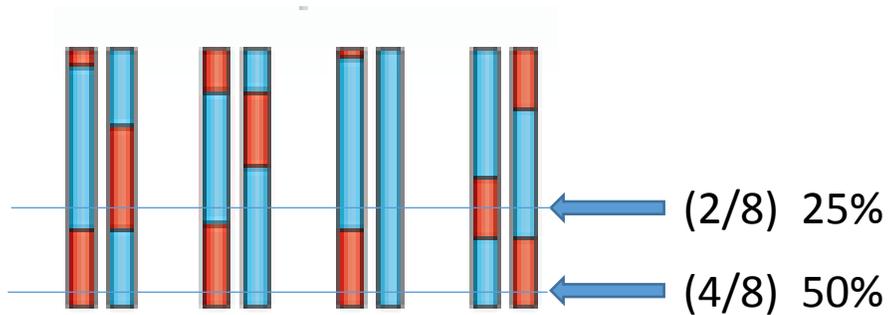


Elimination of non adaptive domestic alleles

Incompatibilities between ATL and MED lineages

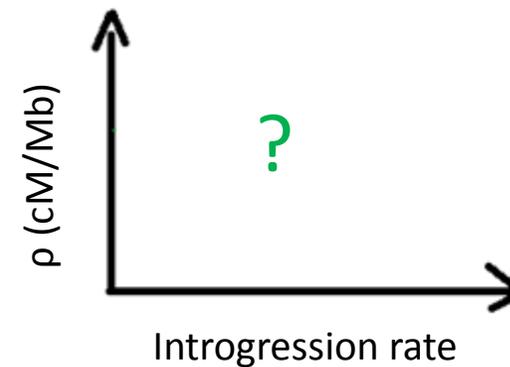
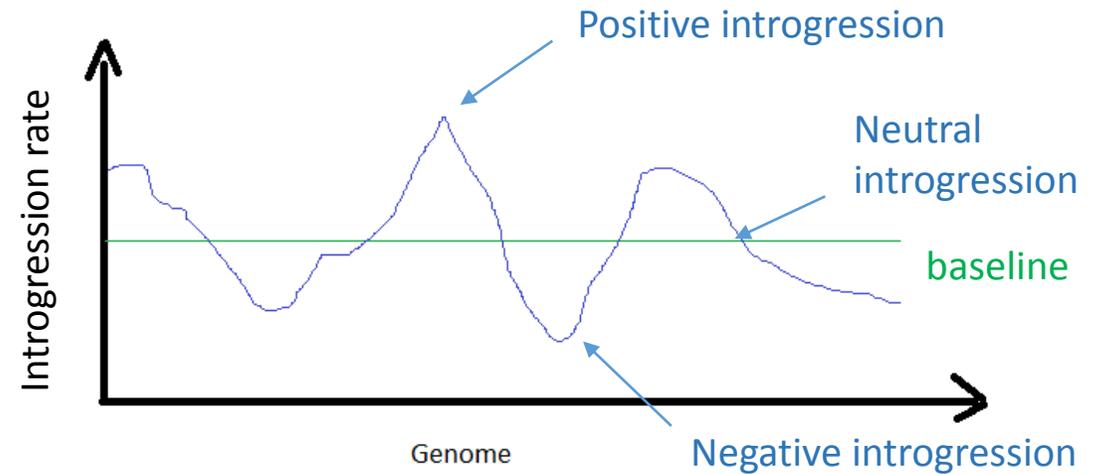
Part 3

→ To determine the introgression rate along the genome



→ To find signatures of positive or negative introgression

→ To associate the recombination landscape to the introgression rate

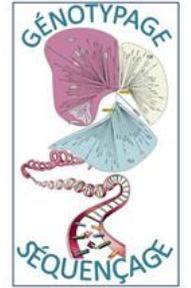


Eric Ravel



Thank you !

Juliette Pouzadoux



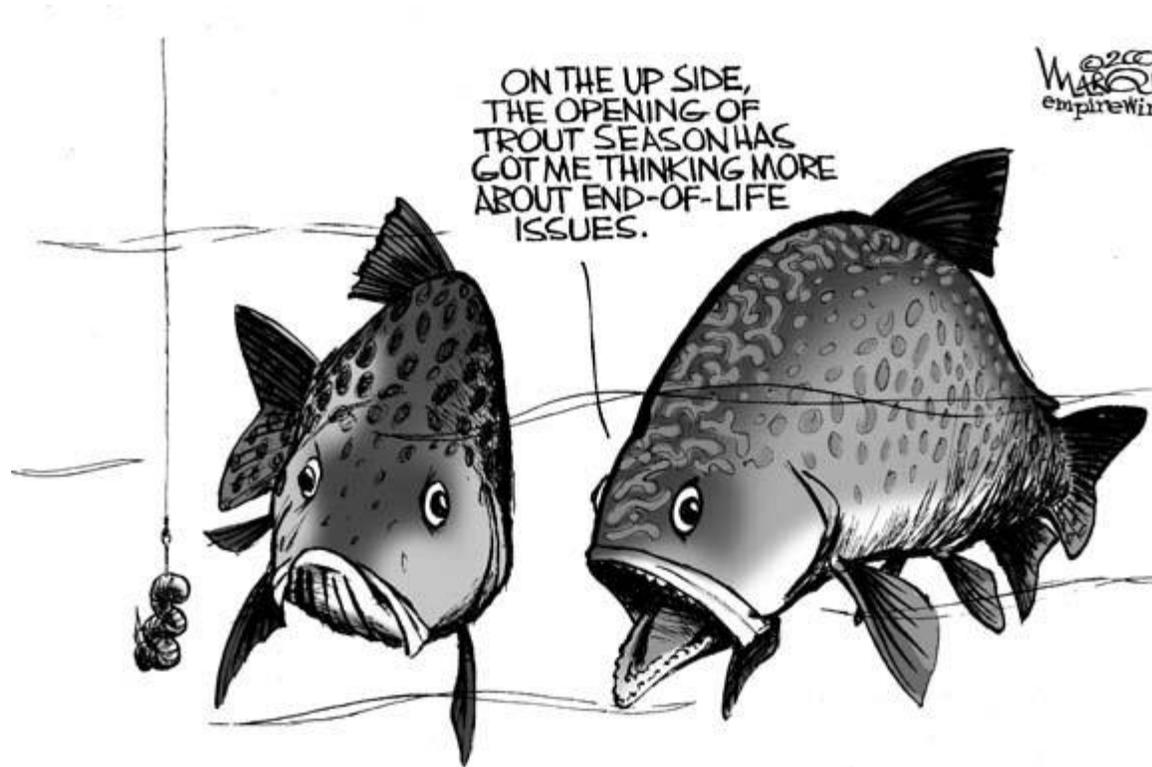
Patrick Berrebi



Bruno Guinand



Pierre-Alexandre Gagnaire



Erick desmarais



Marine Rohmer



Julien veysier, Khalid Belkir, Remy Dernas

→ 3 Metacentrics

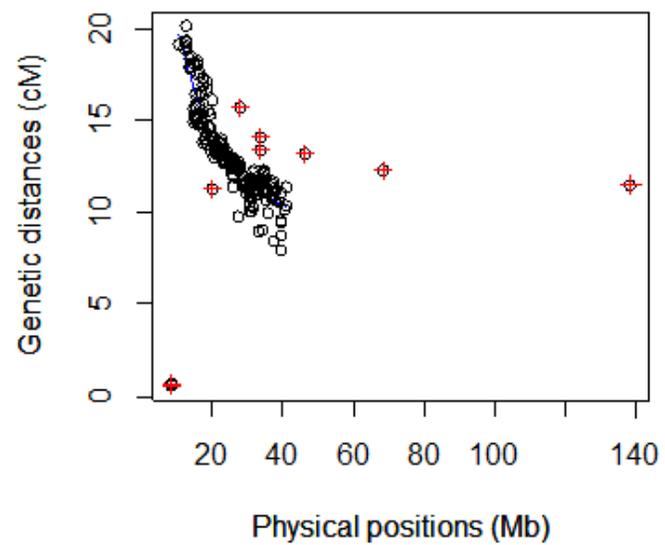
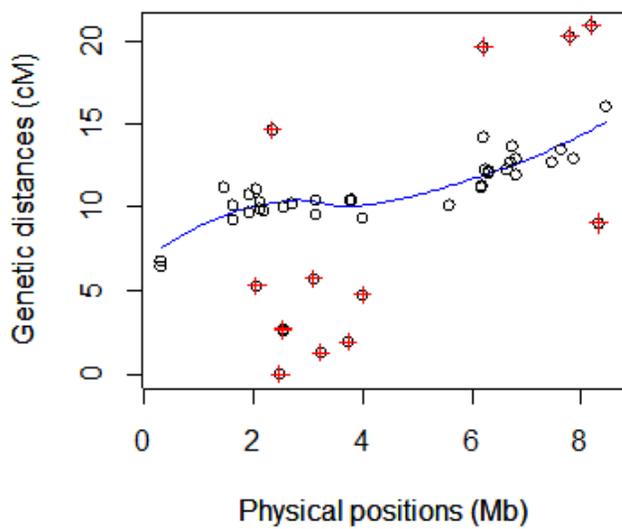
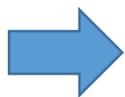
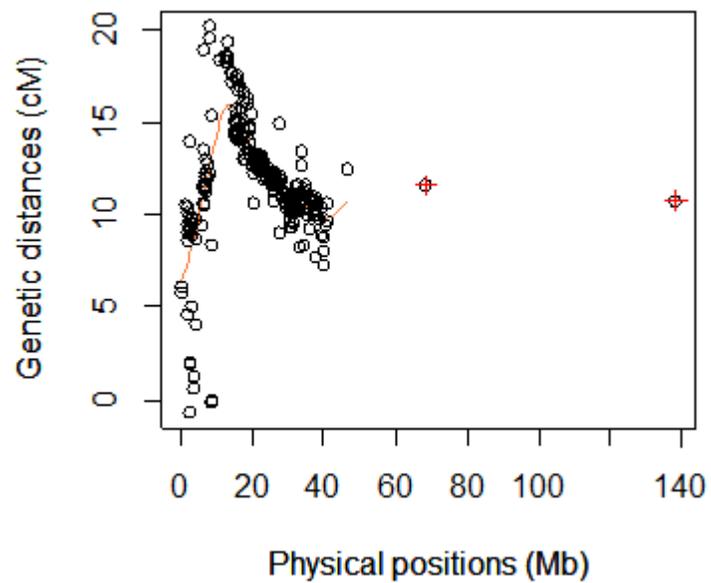
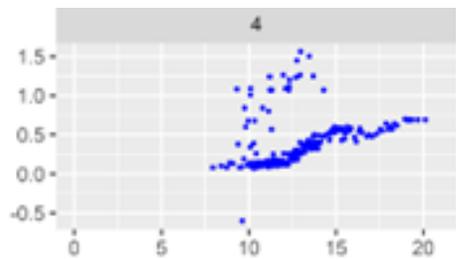
→ 32 Acrocentrics

→ 5 undeterminde



NF = 86 -96

Expected NF (karyotype studies) = 96-104



→ 3 Metacentrics

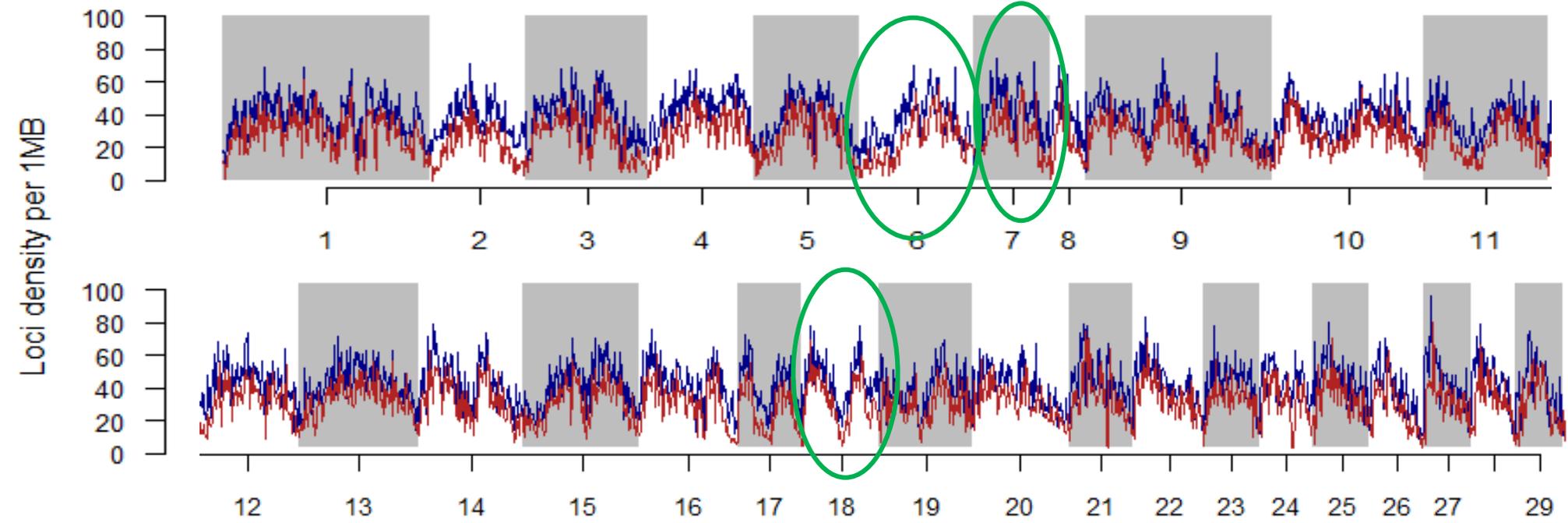
→ 32 Acrocentrics

→ 5 undeterminde



NF = 86 -96

Expected NF (karyotype studies) = 96-104



Part 3

Work to do...

- Detecting hybrid individuals and evaluating the amount of admixture for all individuals samples (N = 184)
- Assess the introgression patterns of individual loci along the genome and identify outliers SNPs
- Use the local recombination landscape to determine linkage disequilibrium between loci
- Assess the introgression at haplotype level then identify introgressed haplotype into wild individuals
- Use the introgressed haplotype size as a proxy of the timing of the introgression
- Determine the introgression rate along the genome
- Find signatures of adaptive introgression or barriers to gene flow