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



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Trout stocking



That's a wild one!





Enhancement of Atlantic and Mediterranean domestic strains into wild Mediterranean populations







Main Questions

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







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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 ?







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



- Wild populations
- Atlantic hatchery strains
- Mediterranean hatchery strains









3 populations

2 populations

30 individuals (3*10)

30 individuals (1*10 + 2*10)

Double digest RAD seq

(Paired End, 2*125 bp)

<u>Average number of reads per individual :</u>

12 millions

Workflow

\rightarrow De novo

- \rightarrow Reference mapping
- \rightarrow Comparison between both methods
- \rightarrow Estimation of nucleotide diversity
- \rightarrow Population structure



De novo assembly





m= Minimum depth of coverage required to create a stack

M= Maximum distance allowed between stacks



SNP= Single Nucleotide polymorphism

De novo assembly





m= Minimum depth of coverage required to create a stack

Stack 1

M= Maximum distance allowed between stacks





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

Cstacks

De novo assembly





m= Minimum depth of coverage required to create a stack



M= Maximum distance allowed between stacks





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

Sstacks

Match to the catalog



33.9

23.0

11.6

85.5

Denovo VS Reference mapping



Denovo VS Reference mapping



How many loci are common ?

De novo loci from catalog

>5
CGGCATACAGGCCAGCCAGTGTAAAGCAATCTAATATAACATTTTTATCTATGTCAGTTCTAACTGTTTGT
>6
AATTCTTCAGGTAAGGGTTAAGGTTTGGGATAGGCCTAAGACAAAAATCTCAAAAACAACTTTCTATCACT
>8
CGGAGGACAACAAGATGCAACAAATCAAGTTTTTTTTTT
>11
CGGATCCCCCCGATACTGATGCTCGGTCTGGAGGTCTACGATTTCTAGGCTTCACTGAACGGGATTCATTA
>15
AATTCACATATTAATGACATTAGTCAATGGTGCCACCTGTCAATGATTTTAGAGGGAGG
>17
CGGTGAAAATCTGTCCTTTGTTCTGATGAGTCACATTTGAGATTTGGTTCCAACCGCCTTGTTTACAGATG



Sam alignement file

5 16	CM003	298.1	41441601	60	2S118M	*	Θ	Θ	CCGAATTCAACACAGG
ΤΑΑΑΑΑΤGTT	ATATTAGATTG	CTTTACACTGG	CTGGCCTGTATGCCG	*	NM:i:2	MD:Z:351	F46C35	AS:i:108	XS:i:21
6 16	CM003	291.1	64273448	60	120M	*	Θ	Θ	TACGGTGTAAACATCT
TTTTTGTCTT#	AGGCCTATCCC	AAACCTTAACC	CTTACCTGAAGAATT	*	NM:i:2	MD:Z:270	C31T60	AS:i:110	XS:i:52
8 16	AGKD0	4018804.1	7718 60	114M6S	*	Θ	Θ	GATGAACT	CAGTGTCAAGGGAAGT
AGAAAAAAAAA	CTTGATTTGTT	GCATCTTGTTG	TCCTCCG *	NM:i:2	MD:Z:161	106C5	AS:i:107	7	XS:i:50
11 0	CM003	300.1	46973878	23	24M13D15	5M7D49M20)32M	*	ΘΘ
AACGGGATTC	ATTATCATCAA	ACACGGACTGT	TGTCTGATTACACACA	CCTGGTTC	CCATTTC	*	NM:i:23	MD:Z:1A2	2^ATTCTGGTCACCA1
500100 01M0	DEOMOOC 4.								

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



Identifying loci in common





Denovo VS Reference mapping 250000 Loci in common Denovo Ref_map 196639 200000 150000 121016 93968 100000 72801 + 24 % 50000 80% in 57% in common common 0 Number of RAD loci Number of SNPs How are they distributed on chromosomes?

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



Ref_map

De novo



LGs

Distribution of Brown trout loci along S. salar LGs





 \rightarrow Homogeneous distribution

 \rightarrow Lower density at LGs extremities : paralogous loci ?

Workflow

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



Atlantic strain , mean $\pi = 0.0040$

Mediterranean strain, mean π = 0.0025

Wild Mediterranean trout, mean $\pi = 0.0049$





S. trutta

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



Atlantic strain , mean $\pi = 0.0040$

Mediterranean strain, mean π = 0.0025

Wild Mediterranean trout, mean $\pi = 0.0049$



 \rightarrow Lower average diversity for the Mediterranean strain

 \rightarrow 'Classical' higher diversity at LGs extremities

Principal Component Analysis (PCA)



Principal Component Analysis (PCA)





→ 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

 \rightarrow Presence of admixed individuals in wild populations

What are the distribution patterns of admixture within the genome ?



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To estimate the introgression rate along the genome we need :

- -> to assess the introgression at haplotype level
- -> to infer the recombination rate landscape



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Admixture Non-admixed Long tracts Atlantic hatchery population (recent ancestry) strain Wild Mediterranean

Time



High density S. trutta linkage map

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

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

The hybrid linkage map design



A new Salmo trutta linkage map



them!

Centromere location and chromosome type





Limborg et al., 2015

Centromere location and chromosome type





Acrocentric

Metacentric

Limborg et al., 2015

A new Salmo trutta linkage map



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.

Syntenies between *S. salar* and *trutta*



T38 T40

<u>S. Salar</u>

58

59

S





<u>S. trutta</u>

Tsai et al., 2016 Lien et al., 2016

<u>S. Salar</u>

39

Chromosomal rearrangements

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



Nucleotide divergence between S. salar and trutta



 $\pi_{\rm b}$: nucleotide diversity between *S. salar* and *S. trutta* $\pi_{\rm w}$: nucleotide diversity within *S. trutta*

Nucleotide divergence between S. salar and trutta

$$d = \pi_{\rm b} - \pi_{\rm w}$$

 $\pi_{\rm b}$: nucleotide diversity between *S. salar* and *S. trutta* $\pi_{\rm w}$: nucleotide diversity within *S. trutta*

d = 0.02285 - 0.0041

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

Estimate of recombination rate in the brown trout genome



Rezvoy et al. 2007

Estimate of recombination rate in the brown trout genome



Total mean recombination rate = 0.88 cM/Mb



Recombination rate estimation





Recombination rate estimation



Genetic distance cM



Correlation between the nucleotide diversity and the recombination rate



Recombination rate (cM/Mb)

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
- \rightarrow The estimation of the (local) **recombination rate** is accessible in *S. trutta*

→ Does the **recombination** landscape **affect local introgression rate** along the genome ?

 \rightarrow To identify admixed individuals in the wild:

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







Racimo et al. (2015)

 \rightarrow 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



ELAI: Efficient Local Ancestry Inference





54

Guan Y., 2014

 \rightarrow To determine the introgression rate along the genome





 \rightarrow To determine the introgression rate along the genome





 \rightarrow To find signatures of positive or negative introgression



 \rightarrow To determine the introgression rate along the genome



 \rightarrow To find signatures of positive or negative introgression

 \rightarrow To associate the recombination landscape to the introgression rate



Eríc Ravel





Juliette Pouzadoux



Eríck desmaraís



Maríne Rohmer



Julíen Veysíer, Khalíd Belkír, Remy Dernat 59



Patrick Berrebi



Bruno Guínand





Pierre-Alexandre Gagnaire



NF = 86 -96 Expected NF (karyotype studies) = 96-104



😠 JoinMap 4 - Map2familles	_	-	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_				_ 0
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	279 3	84893 <hkxhk></hkxhk>	{01} (hh,hk,kk)	55.901 hk	hk kk kk	hh kk	hk hk h	h hh h	h hk h	k hk k	ik kk h	k hh hi	h hk kk	kk hk	kk kk	hk hk h	k hk hh	k hh kk	kk kk	hk hh hh	kk hk hl	hh kk hh hk
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Group 10	1021 2	76023 <efxeg></efxeg>	{01} (ee,ef,eg,fg)	47.639 ef	ef fg fg	ee	- eg e	eg ee e	e ef e	ef eg -	- fg e	f ee ee	e eg fg	ef	ef fg	ee fg e	g eg ee	g ee	ef fg	ee ee eg	fg fg er	ee ee ef
	2738 3	84977 <efxeq></efxeq>	(01) (ee ef eq fq)	57 740 ef	ef ef fa	ee fa	ea e	e ee e	e ef e	f ea f	a fa e	f ee ef	f ea fa	fa ea	fa fa	eg ef e	a ea ee	a ee fa	fa fa	eg ee ee	fa ef er	ee fa ee ef









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