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## Genetic determinism of spontaneous masculinisation in XX-female rainbow trout (*O. mykiss*)

Florence Phocas, Edwige Quillet, Yann Guiguen, Anastasia Bestin, Clémence Fraslin

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Submitted on 10 Jan 2024

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# Genetic determinism of spontaneous masculinisation in XX-female rainbow trout (*O. mykiss*)



Clémence Fraslin

F. Phocas, A. Bestin, P. Haffray, P. Hocdé, Y. Guiguen, E. Quillet, et al.,

# Rainbow trout

Fertilized  
eggs



Small trout  
9-11 months



XY male

Filet trout  
17 months






Smoked filet trout  
24 months



XX female



- All female production stocks

- Sexual maturation:  mortality,  flesh quality,  growth rate
- XX-sex-reversed males (neomales) → hormonal treatment (17 $\alpha$ MT, 99/22/CE 29 April)
  - XX-female + XX-neomale = XX-offspring

- Spontaneous maleness

- ~1% of fish in most production stocks
- Minor QTL detected in a XX-trout line (INRAE)

# Rainbow trout



## Objective:

**Characterise the genetic architecture of spontaneous maleness in an all-female commercial trout population**



# Fish production

Yolk resorption

Fish rearing

Sex recording

12°C

12-14.5°C

10/15 mpf

18°C  
(1134 °- days)

12-14.5°C

RESEARCH ARTICLE

## High Temperature Increases the Masculinization Rate of the All-Female (XX) Rainbow Trout ‘Mal’ Population

Karina Valdivia<sup>1</sup>, Elodie Jouanno<sup>1</sup>, Jean-Nicolas Volff<sup>2</sup>, Delphine Galiana-Arnoux<sup>2</sup>, René Guyomard<sup>3</sup>, Louise Helary<sup>1</sup>, Brigitte Mourot<sup>1</sup>, Alexis Fostier<sup>1</sup>, Edwige Quillet<sup>3</sup>, Yann Guiguen<sup>1\*</sup>

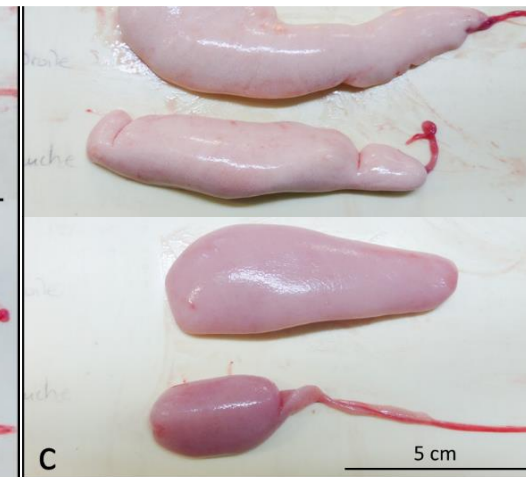
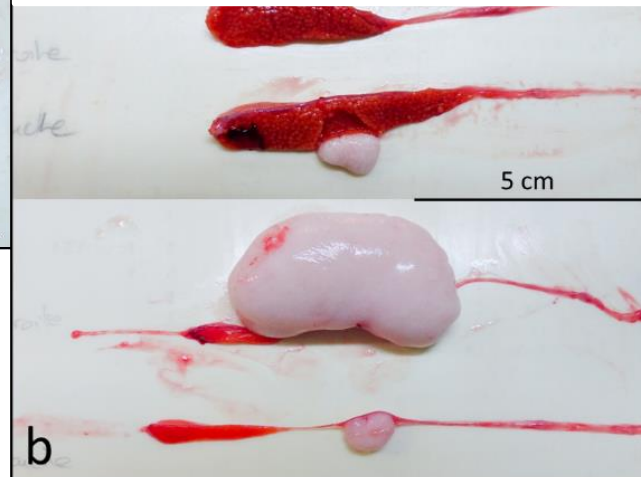
1. INRA, UR1037 LPGP Fish Physiology and Genomics, F-35000, Rennes, France, 2. IGFL, UMR5242 CNRS/INRA/Université Claude Bernard Lyon I/ENS, Lyon, Cedex 07, France, 3. INRA, UMR1313 GABI Génétique Animale et Biologie Intégrative, Domaine de Vilvert, 78352, Jouy-en-Josas Cedex, France

\*yann.guiguen@rennes.inra.fr



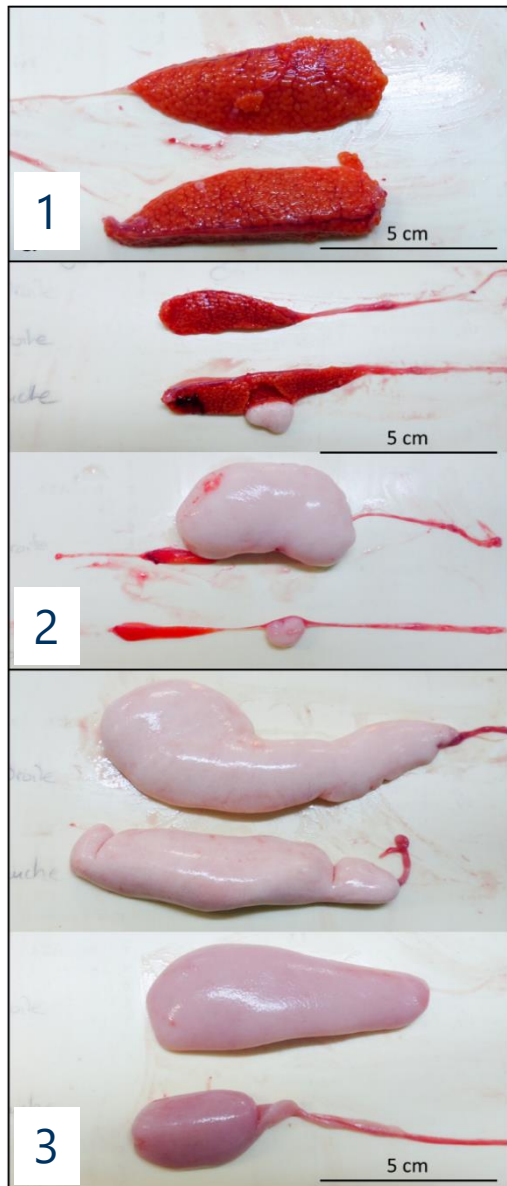
# Sex recording

	Females
12°C	9,771
18°C	10,061
Total	19,832





# Genotyping and Whole Genome Sequencing



## 57K SNP: Axiom™ Trout Genotyping Array

- 1,139 fish
  - 1 = females (848)
  - 2 = intersex (131)
  - 3 = males (160)

– **31K SNPs**

## Whole genome sequence (TrueSeqNano)

- 50 dams from the mating design
- 8.8 millions SNPs

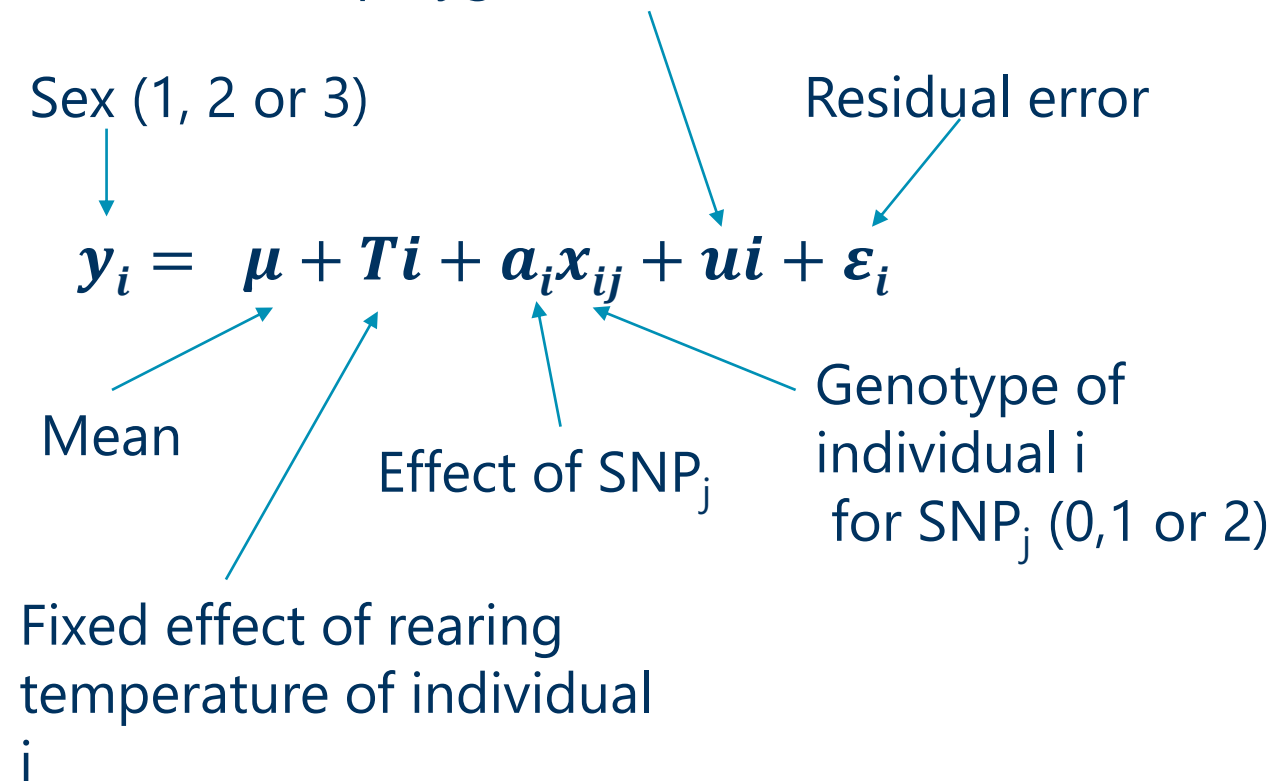
## Imputation

- 8.8 millions SNPs

# Genetic parameters and Genome Wide Association Studies

- Marker-by-Marker (GCTA)

Additive polygenic effect for individual  $i$



- Bayes Cp (BESSiE)

MCMC with 500,000 iterations

$$y_i = \mu + Ti + u_i + \sum_{j=1}^{n \text{ SNP}} \delta_{jk} a_j g_{ij} + \varepsilon_{ik}$$

Indicator variable

$\delta_{jk} = 1$  if SNP $_j$  is selected at a given  $k$  iteration  
 $\delta_{jk} = 0$  otherwise

At every  $k$  iteration  $\pi = 0.99$



# Genetic parameters and Genome Wide Association Studies

## GENOTYPE (31K SNPs)

### – GWAS

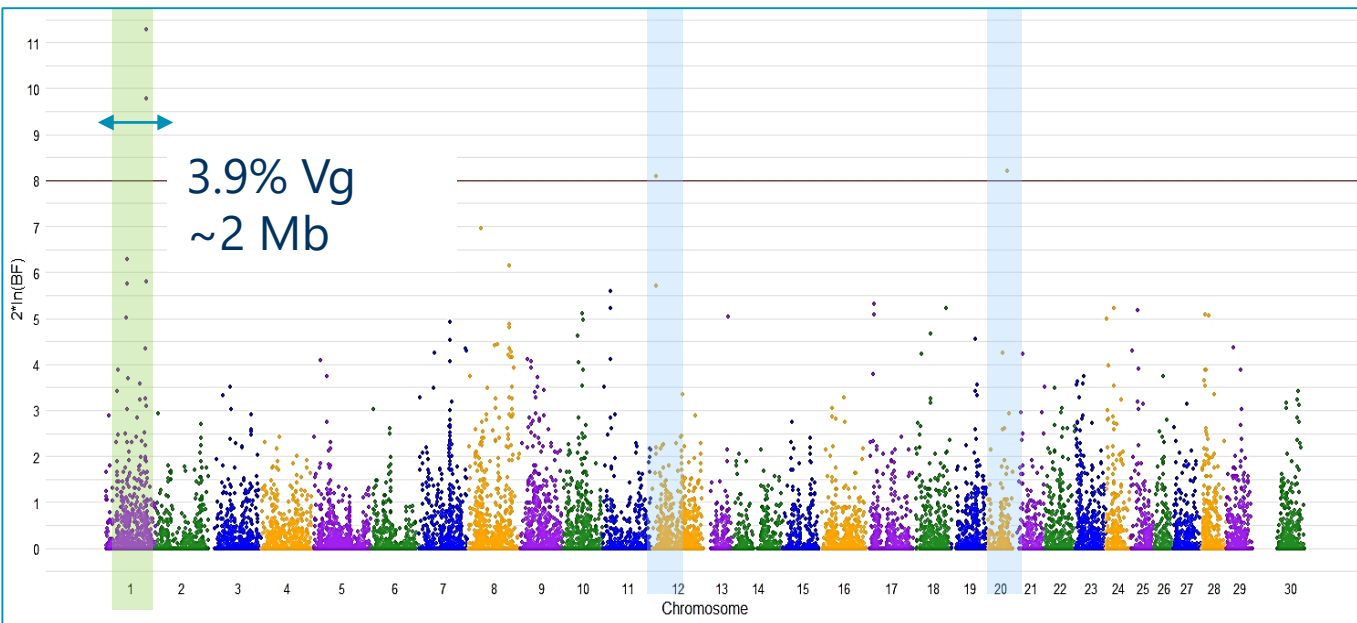
- Bayes C $\pi$  (BESSIE)

## Sequence (8.8 millions SNPs)

–  $h^2_{seq} = 0.59 / 0.62$

### – GWAS

- Bayes C $\pi$  (BESSIE) → focus on 4Mb in Omy1 (21K SNPs)

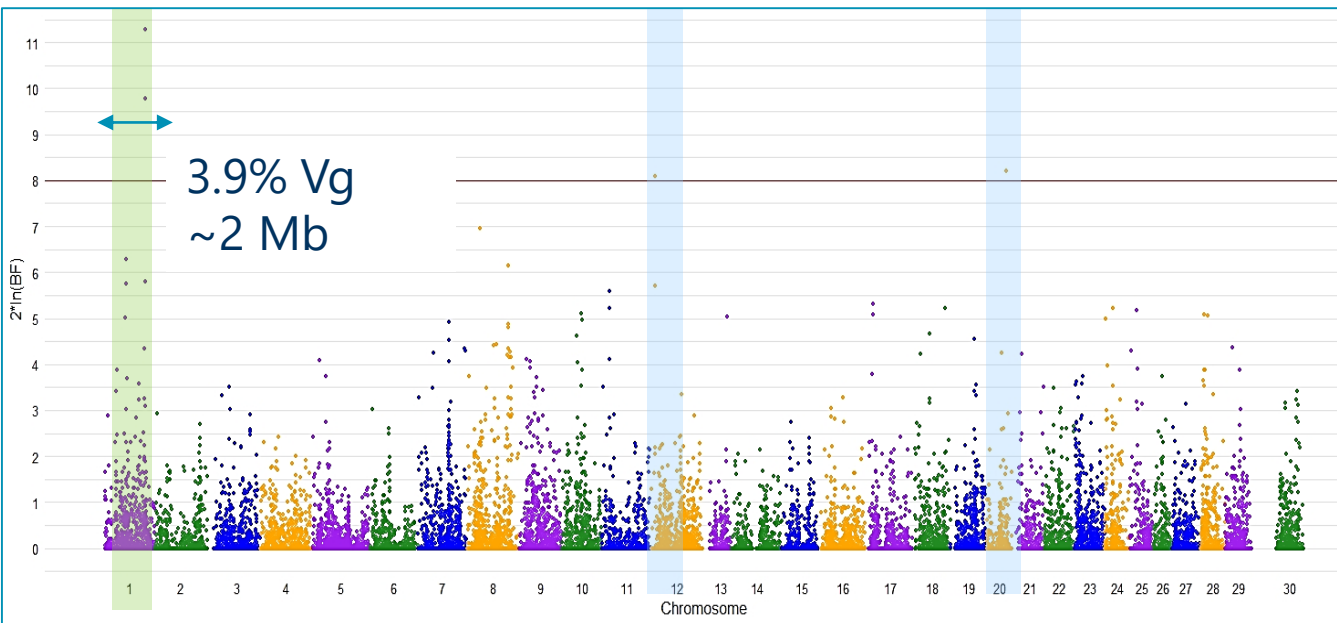


# Genetic parameters and Genome Wide Association Studies

## GENOTYPE (31K SNPs)

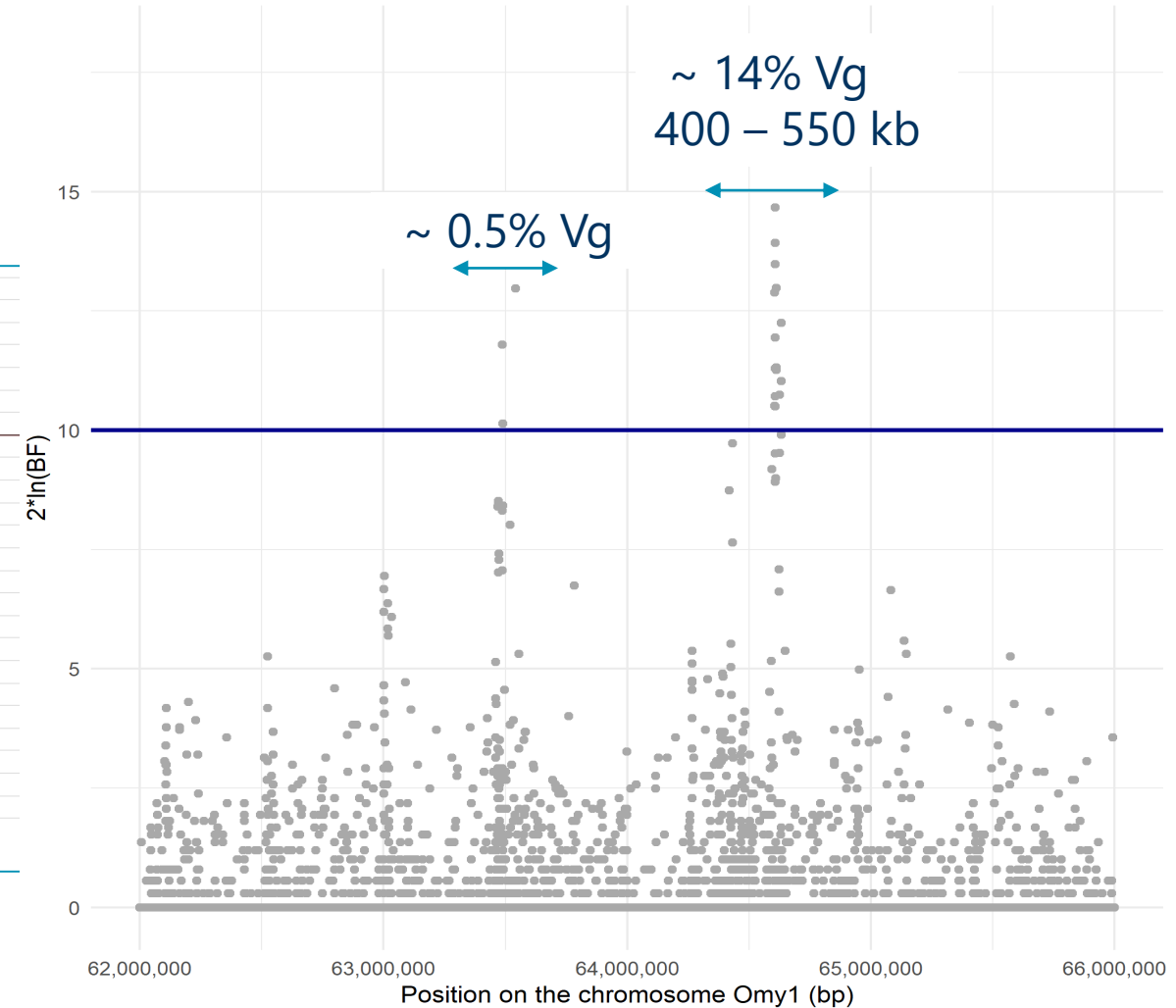
### – GWAS

- Bayes C $\pi$  (BESSIE)

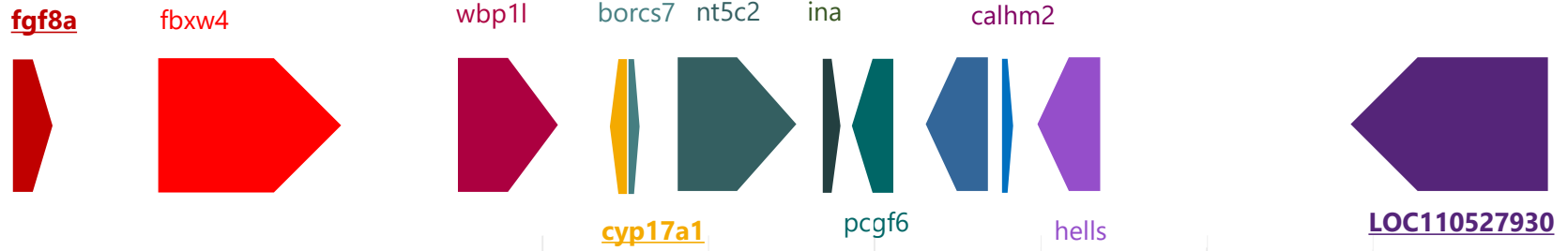


## Sequence (8.8 millions SNPs)

Bayes Cpi run1 analysis



# Annotated SNPs located within the second QTL region, from 64.360 to 64.633 Mb on Omy1



## Characterization of Sexual Trait Development in *cyp17a1*-Deficient Zebrafish FREE

Gang Zhai ✉, Tingting Shu, Yuguo Xia, Yao Lu, Guohui Shang, Xia Jin, Jiangyan He, Pin Nie, Zhan Yin

*Endocrinology*, Volume 159, Issue 10, October 2018, Pages 3549–3562,

<https://doi.org/10.1210/en.2018-00551>

Published: 06 September 2018 [Article history](#) ▾



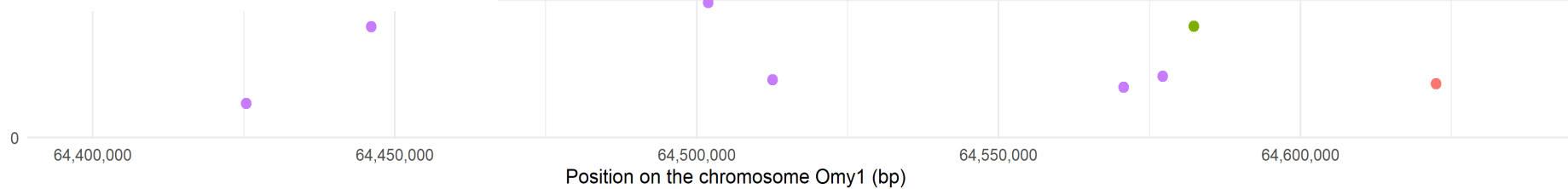
General and Comparative Endocrinology

Volume 143, Issue 1, August 2005, Pages 66-74



Hypocorticism and interrenal hyperplasia are not directly related to masculinization in XX *mas*<sup>-1</sup>/*mas*<sup>-1</sup> carp, *Cyprinus carpio*

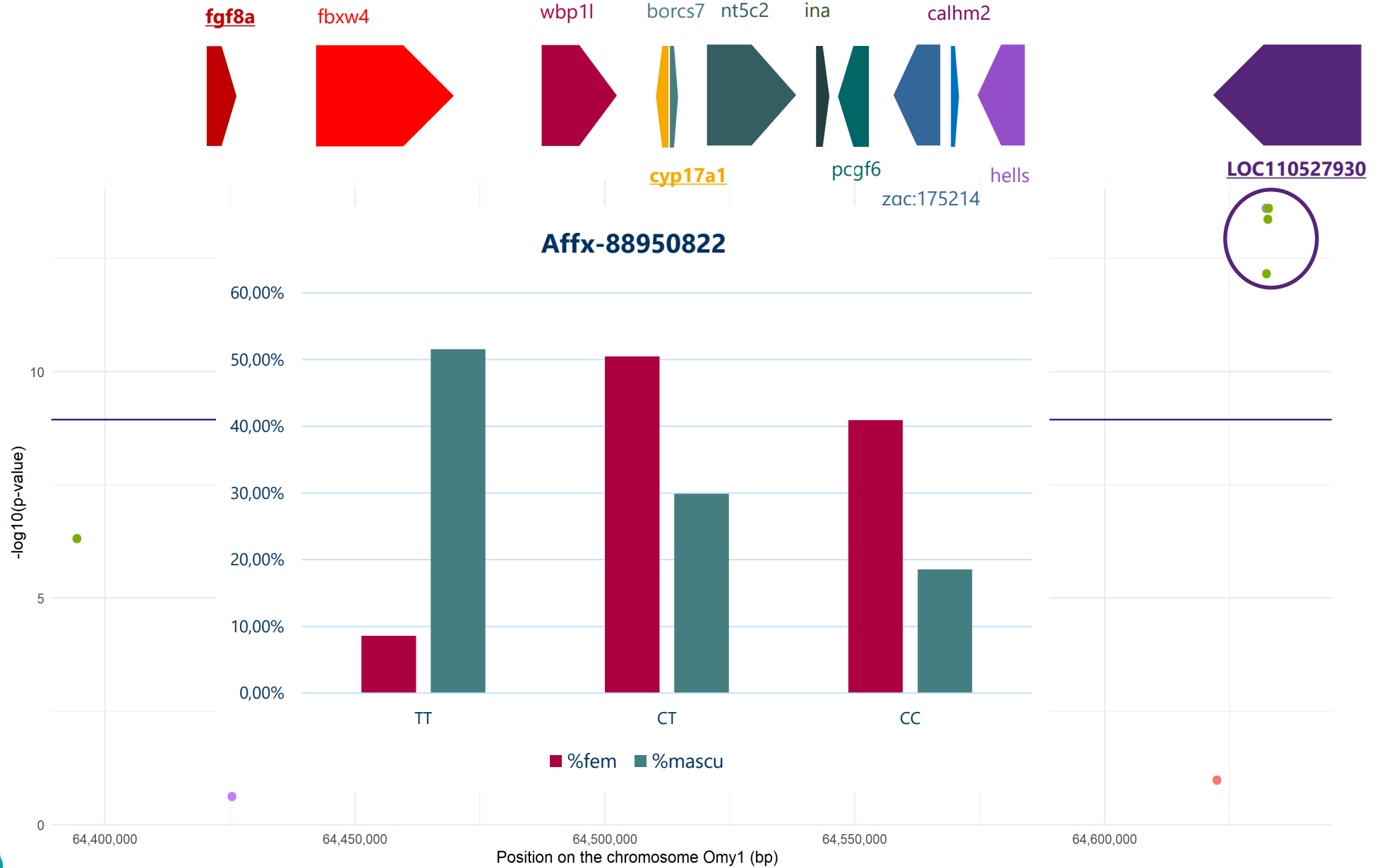
N.M. Ruane<sup>a, c</sup>, J.G.D. Lambert<sup>b</sup>, H.J.Th. Goos<sup>b, j</sup>, J. Komen<sup>a, k</sup> ✉



Variants annotation (estimated impact) ● 5' premature start codon (LOW) ● Missense (MODERATE) ● Splice region or intron (LOW) ● Synonymous (LOW)



# Annotated SNPs located within the second QTL region, from 64.360 to 64.633 Mb on Omy1



# Minor genetics factors involved in spontaneous masculinisation of XX-female rainbow trout

- High heritability 0.59 / 0.62
- 1 major QTL on Omy1
- 3 smaller QTLs on Omy1, 12 and 20
- Candidate genes
  - *fgf8a*, *cyp17a1*, uncharacterised protein

OPEN

**Genetic determinism of spontaneous masculinisation in XX female rainbow trout: new insights using medium throughput genotyping and whole-genome sequencing**

Clémence Fraslin<sup>1,10</sup>, Florence Phocas<sup>1</sup>, Anastasia Bestin<sup>2</sup>, Mathieu Charles<sup>1,3</sup>, Maria Bernard<sup>1,3</sup>, Francine Krieg<sup>1</sup>, Nicolas Dechamp<sup>1</sup>, Céline Ciobotaru<sup>1</sup>, Chris Hozé<sup>1,4</sup>, Florent Petitprez<sup>5</sup>, Marine Milhes<sup>6</sup>, Jérôme Lluch<sup>6</sup>, Olivier Bouchez<sup>6</sup>, Charles Poncet<sup>7</sup>, Philippe Hocdé<sup>8</sup>, Pierrick Haffray<sup>2</sup>, Yann Guiguen<sup>9</sup> & Edwige Quillet<sup>1</sup>

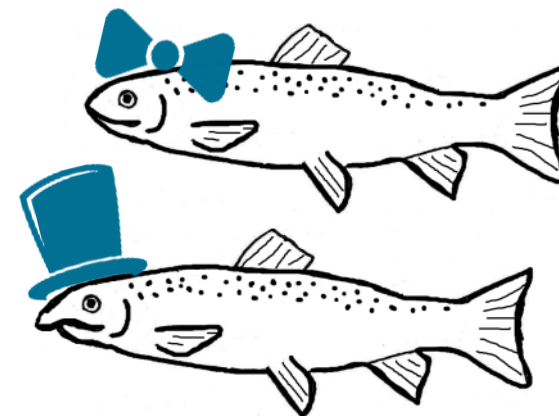
# Thank you for your attention!

## INRAE

GABI and GenAqua

LPGP

**GENTYANE**  
PLATE-FORME de  
GENOTYPAGE et SÉQUENÇAGE en AUVERGNE



Charles Murgat



SYSAAF



Funded by the UE (FEAMP & FranceAgriMer)  
n°R FEA470016FA1000008

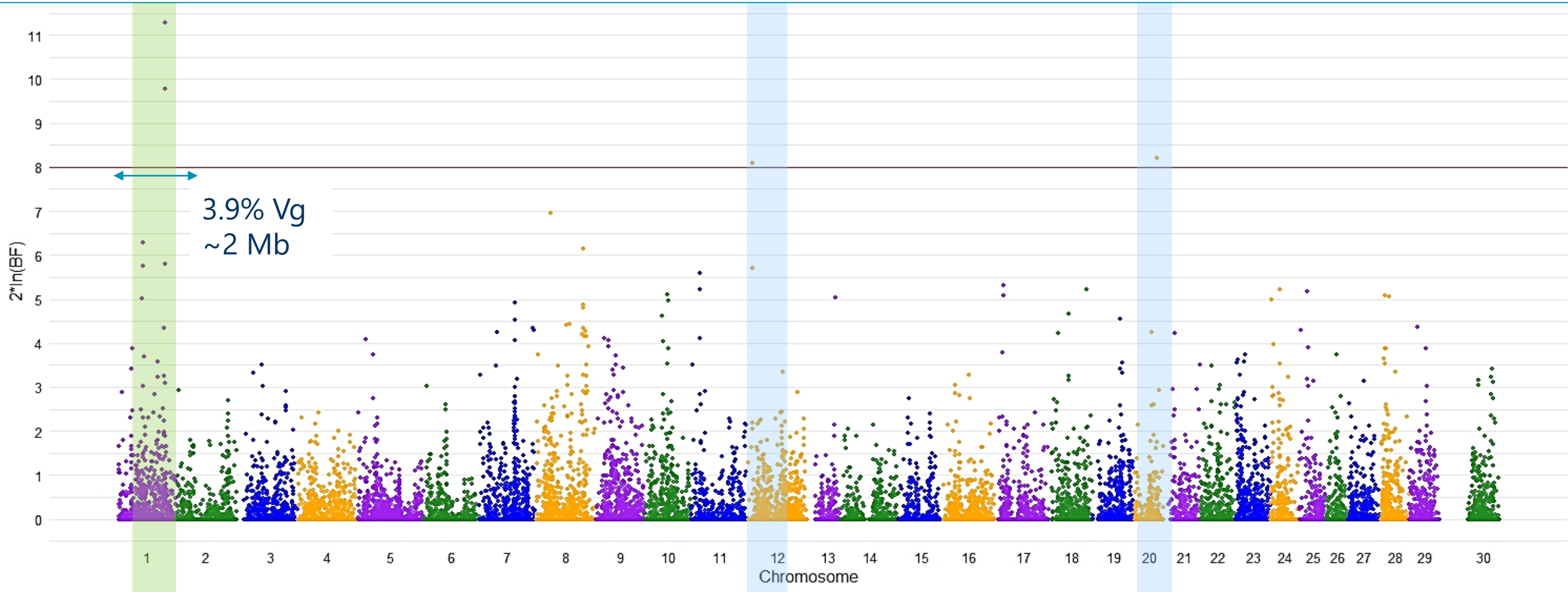
Analysis	$h_g^2 \pm SE$	$\sigma_p^2 \pm SE$	$\sigma_e^2 \pm SE$	$\sigma_a^2 \pm SE$	$\sigma_u^2 \pm SE$	$\sigma_g^2 \pm SE$
GCTA-chip	0.48 ± 0.04	0.43 ± 0.02	0.22 ± 0.01	0.21 ± 0.03	-	-
BCπ-chip*	0.56 ± 0.05	0.43 ± 0.02	0.19 ± 0.02	0.24 ± 0.03	0.10 ± 0.03	0.14 ± 0.03
GCTA-seq	0.62 ± 0.06	0.42 ± 0.02	0.16 ± 0.02	0.26 ± 0.03	-	-
BCπ-seq*	0.59 ± 0.05	0.44 ± 0.02	0.17 ± 0.02	0.25 ± 0.03	0.21 ± 0.03	0.04 ± 0.01

**Table 3. Estimates of genetic and genomic parameters for spontaneous maleness under the different statistical models.**

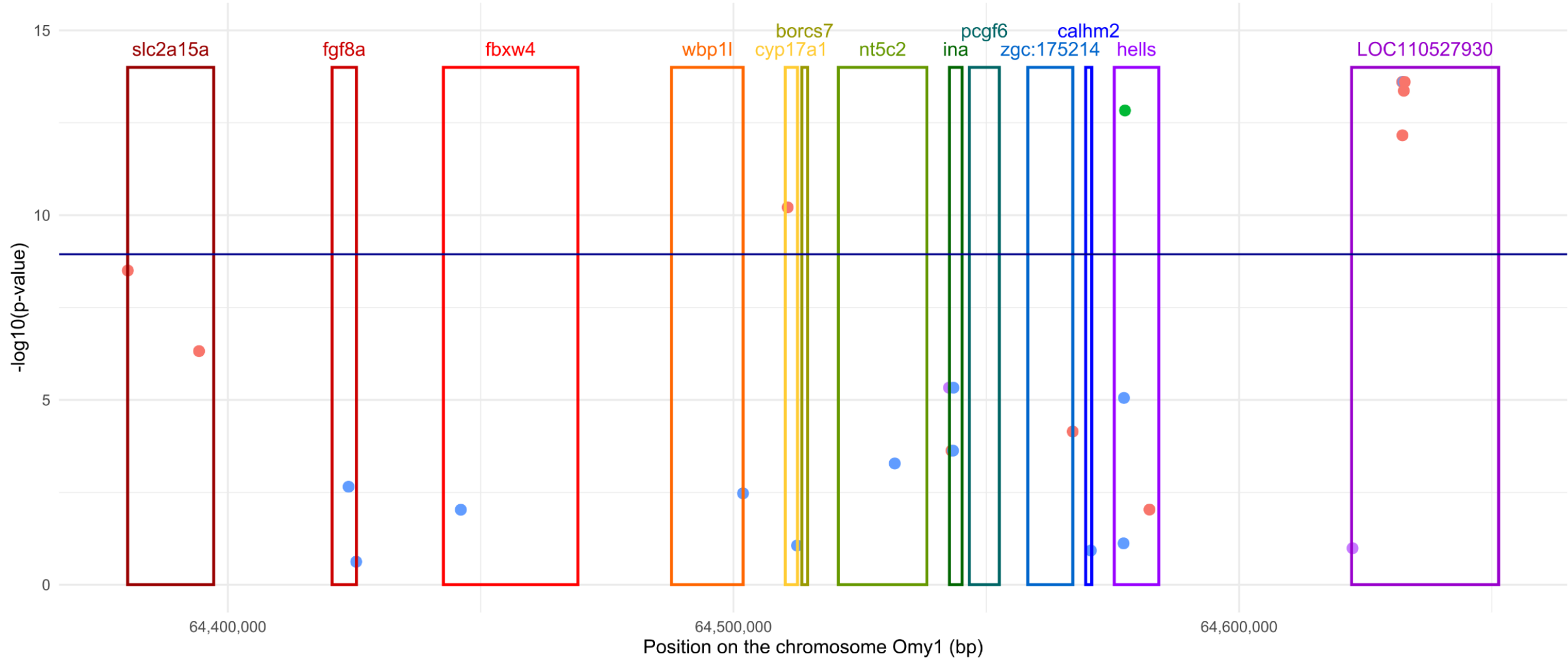
$h_g^2$ : genomic heritability, calculated as  $\sigma_a^2 / (\sigma_a^2 + \sigma_e^2)$ .  $\sigma_a^2$ : total genetic variance ( $= \sigma_u^2 + \sigma_g^2$ ),  $\sigma_u^2$ : polygenic variance,  $\sigma_g^2$ : genetic variance explained by SNPs,  $\sigma_e^2$ : residual variance,  $\sigma_p^2$ : phenotypic variance ( $= \sigma_a^2 + \sigma_e^2$ ). \*value of one MCMC chain among the two used for GWAS, the chain with the closest final  $\pi$  to the 1% or 0.02% was chosen.



# Bayes Cp (31K)



# Annotated SNPs located within the second QTL region, from 64.360 to 64.633 Mb on Omy1

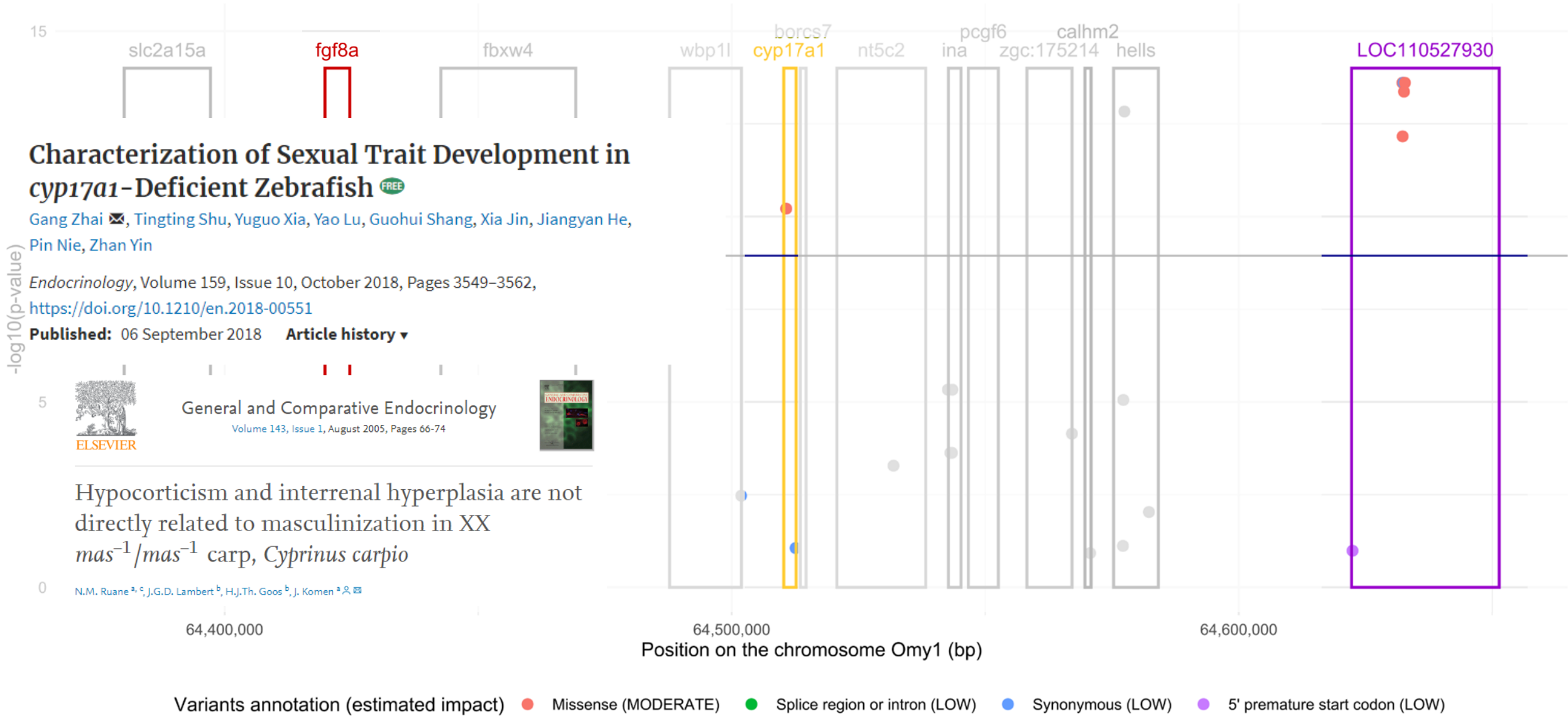


Variants annotation (estimated impact) ● Missense (MODERATE) ● Splice region or intron (LOW) ● Synonymous (LOW) ● 5' premature start codon (LOW)

Fraslin et al., in press

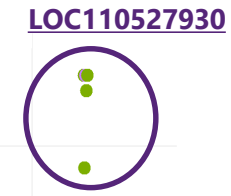
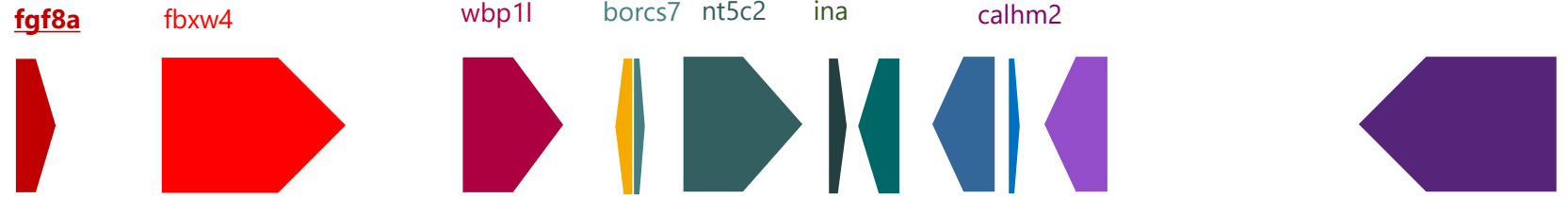


# Annotated SNPs located within the second QTL region, from 64.360 to 64.633 Mb on Omy1

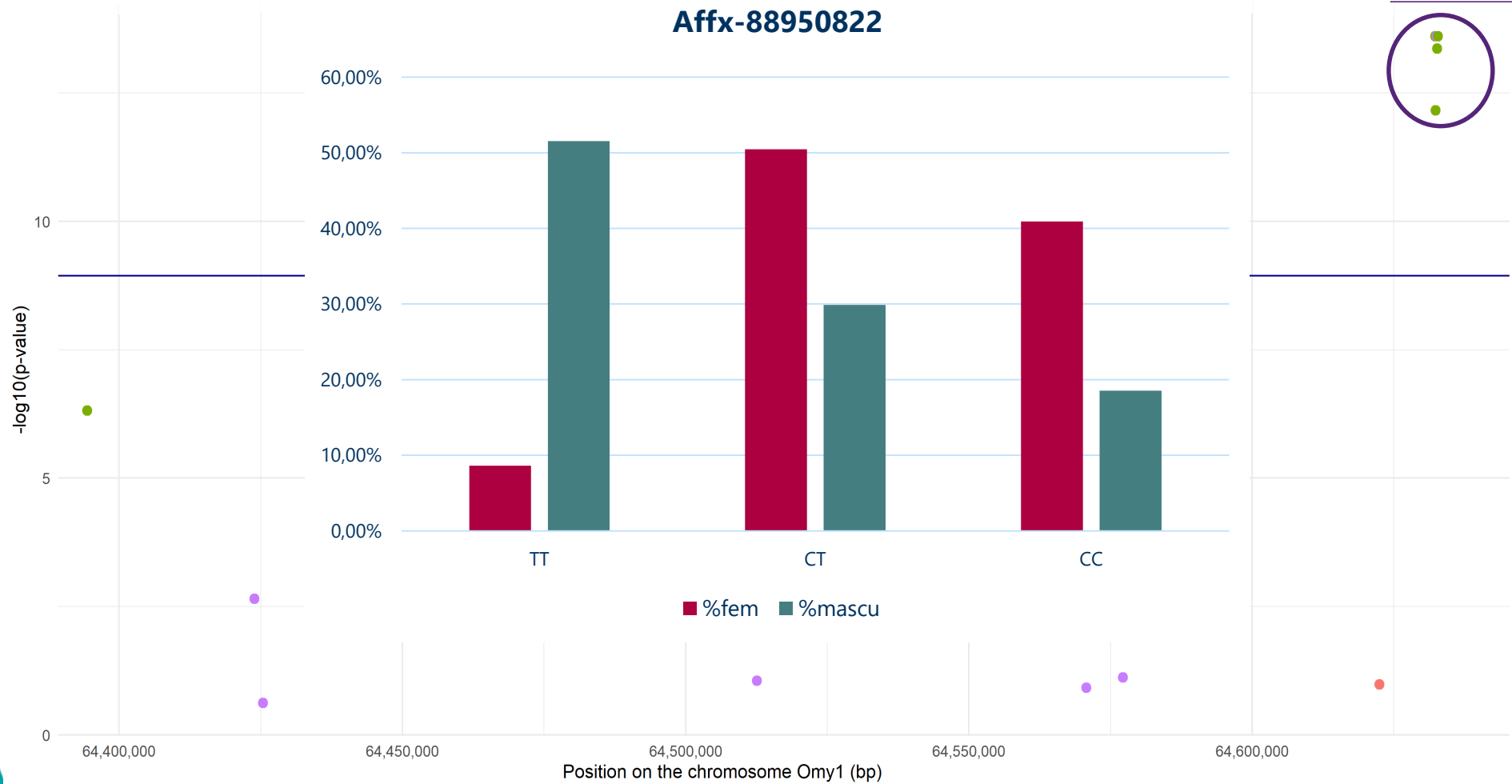


Fraslin et al., in press

# Annotated SNPs located within the second QTL region, from 64.360 to 64.633 Mb on Omy1

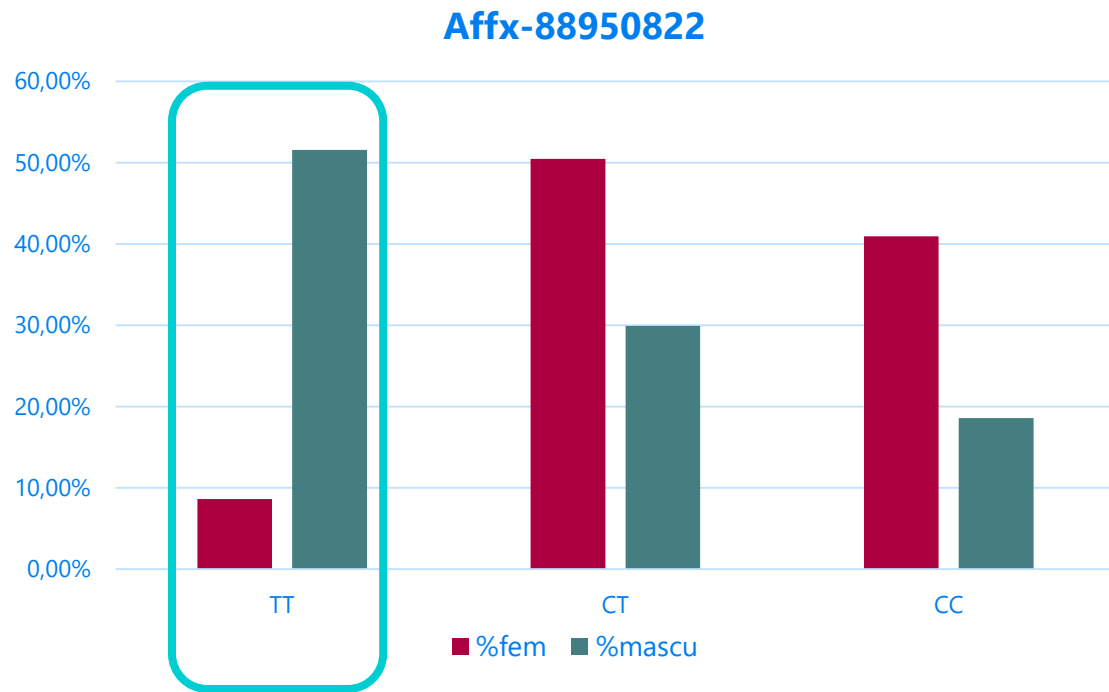


**Affx-88950822**



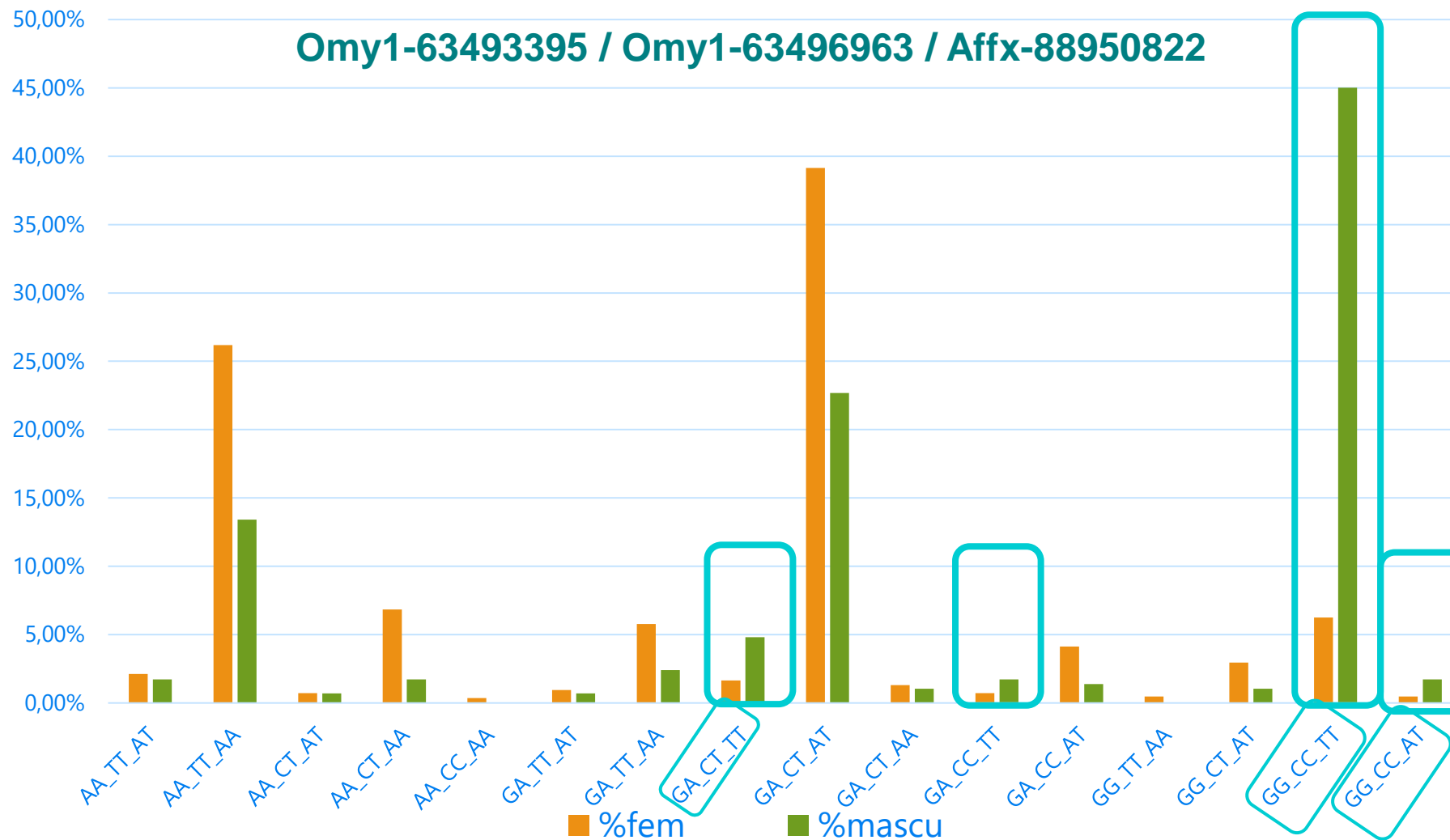
# Strategy/tool for the trout producers

- Repress spontaneous maleness
  - A test to detect masculinised fish

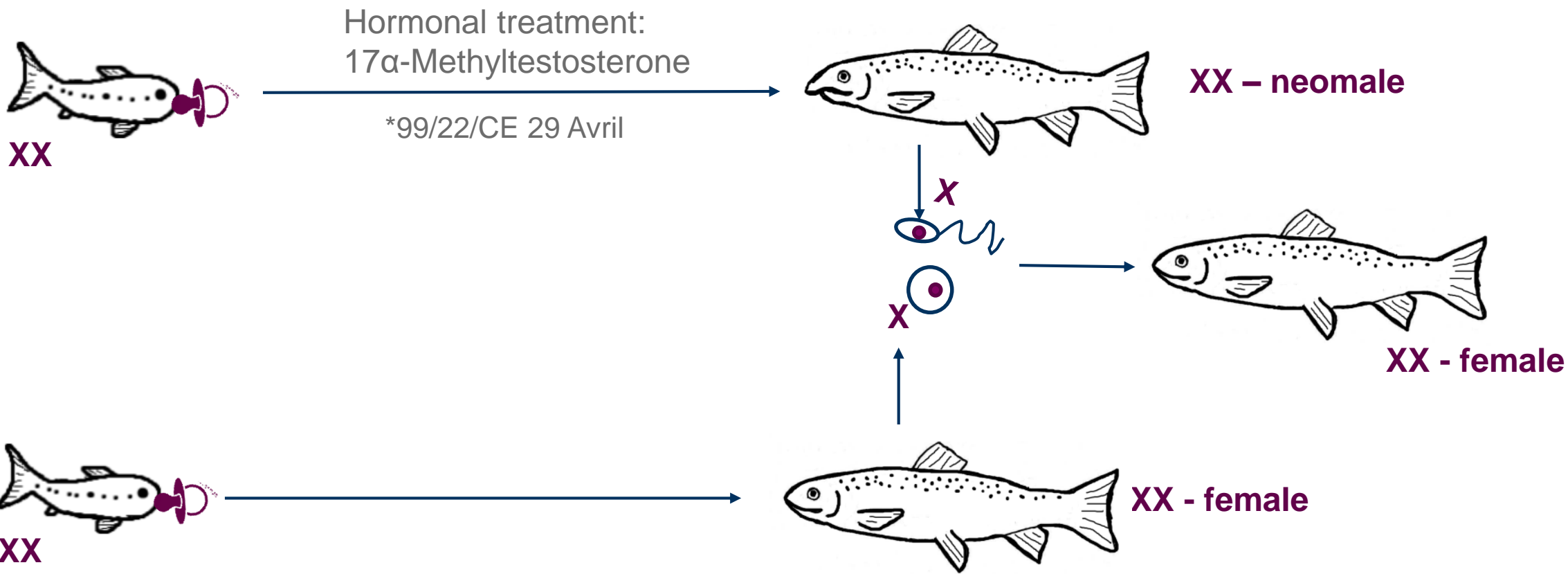


- Enhance spontaneous maleness
  - MAS
  - High heritability ~ 0.60
  - GxE ?

Omy1-63493395 / Omy1-63496963 / Afx-88950822



53.3% of masculinised fish et 9.1% of females





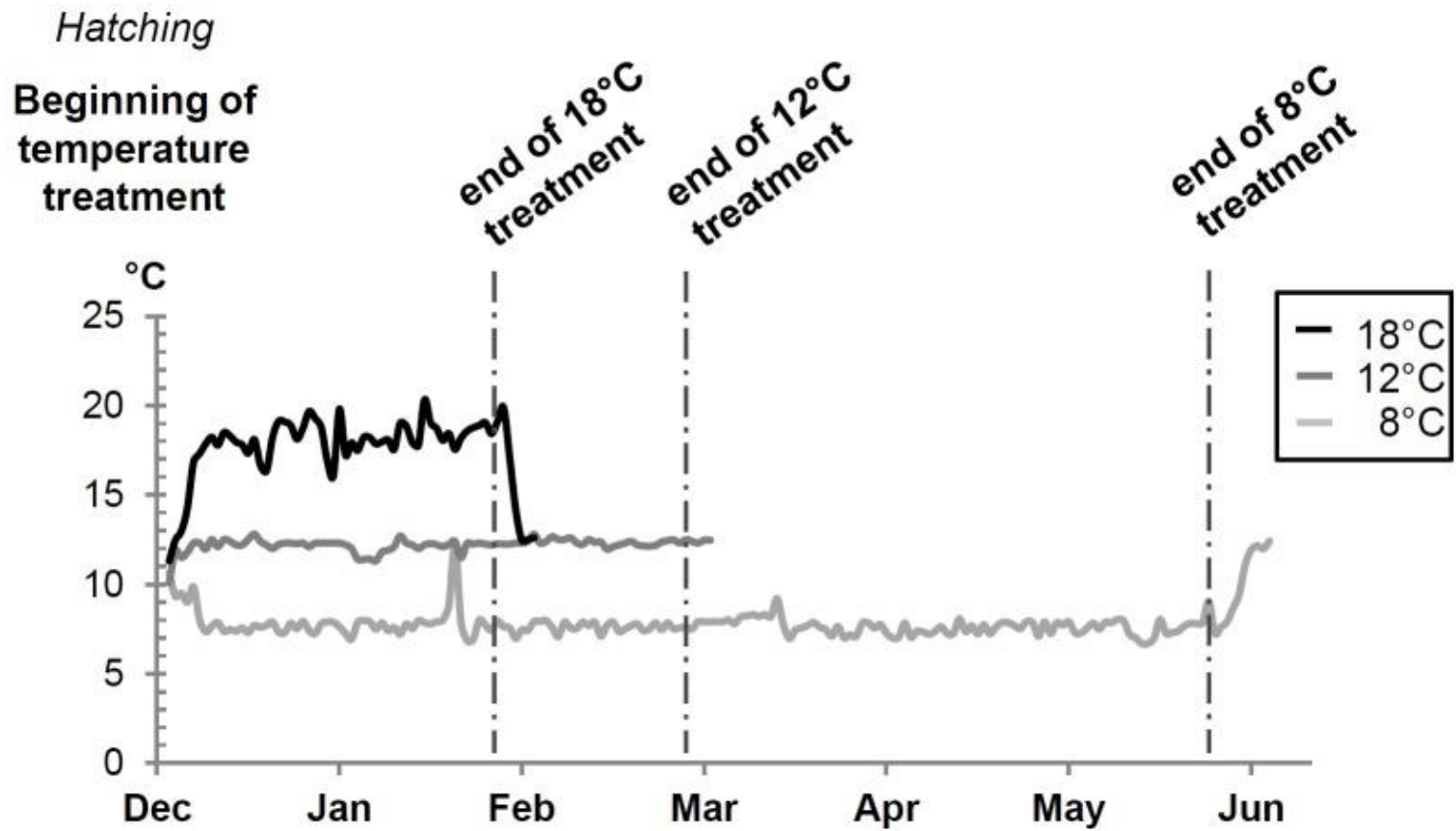
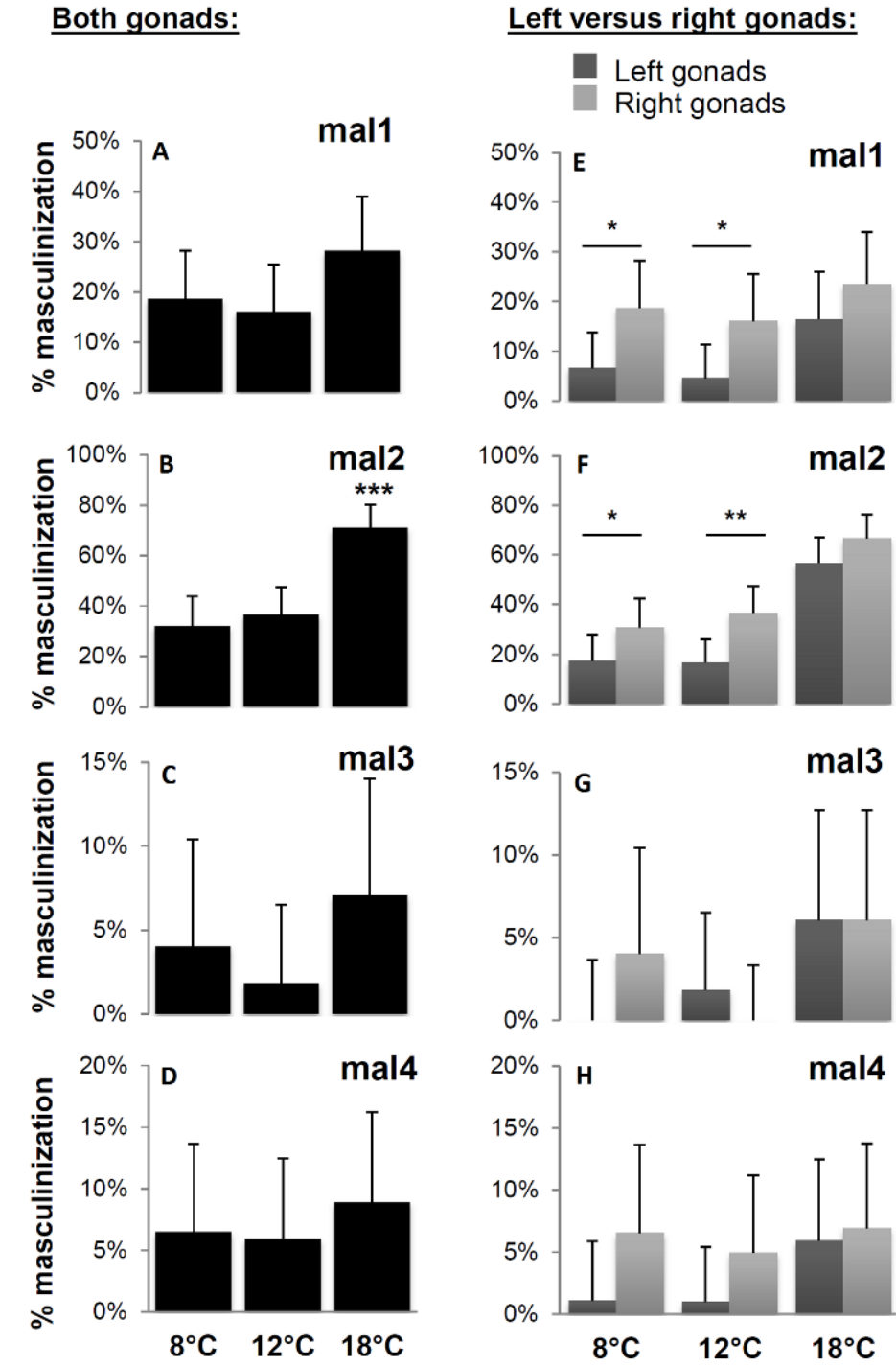


Figure 1. Experimental conditions for the common garden experiment A.

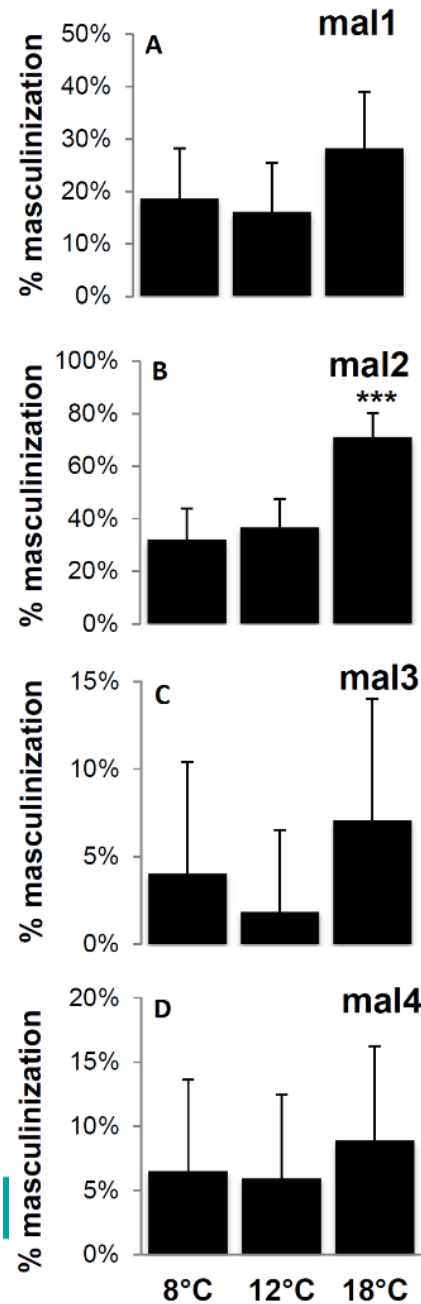
Valdivia et al., 2014



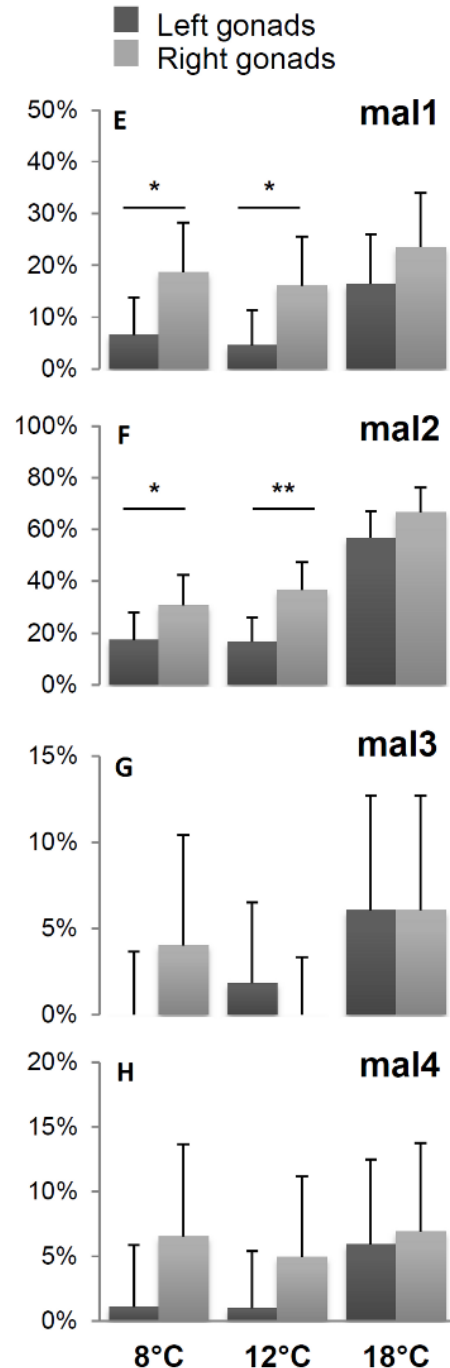
Figure 3. Rates of masculinization of the different *mal*-carrying progeny exposed to different temperatures in experiment A.



### Both gonads:



### Left versus right gonads:



**Figure 3. Rates of masculinization of the different *mal*-carrying progeny exposed to different temperatures in experiment A.**

Panels A to D: Individual masculinization rates (each individual is considered masculinized when at least one gonad is masculinized) following different temperature treatments (8, 12 and 18°C).

Panels E to H: Masculinization rates of left versus right gonads following different temperature treatments (8, 12 and 18°C) (in percentage  $\pm$  Confidence Interval at  $p=0.05\%+CI$ ;  $\chi^2$ ; \*  $p<0.05$ , \*\*  $p<0.01$ , \*\*\*  $p<0.001$ ).

Numbers of animals analyzed at 8, 12 and 18°C, respectively: mal1 = 99, 108 and 99; mal2 = 92, 101 and 101; mal3 = 91, 87 and 85; mal4 = 75, 90 and 90.

# Rainbow trout in France

Fertilized  
eggs



Small trout  
9-11 months



Filet trout  
17 months



Smoked filet trout  
24 months



XY male

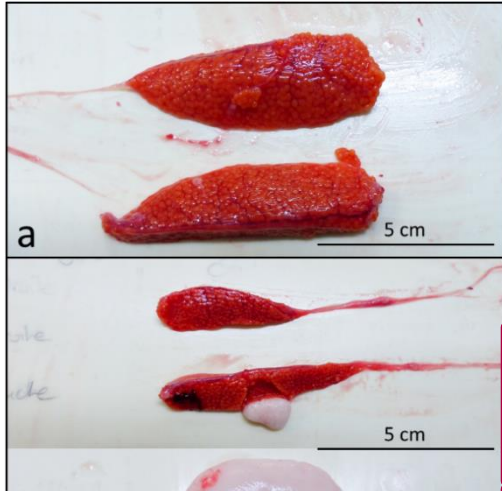


XX female

**Objective:**

**Characterise the genetic architecture of spontaneous maleness in an all-female commercial trout population**

# Sex recording



	12°C	18°C	Total
Total number of fish	10,010	10,194	20,204

Females			19,832
			132
			161
			1.5%

