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

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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

Filet trout
17 months

Smoked filet trout
24 months

XY male

XX female

- All female production stocks
  - Sexual maturation: ↗ mortality, ↘ flesh quality, ↘ growth rate
  - XX-sex-reversed males (neomales) → hormonal treatment (17αMT, 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)

INRAe

Rainbow trout

Fertilized eggs

Small trout
9-11 months

XY male

Filet trout
17 months

Smoked filet trout
24 months

XX female

Objective:

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

INRAe

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**	

mpf = month post fecundation

Sex recording

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

INRAe
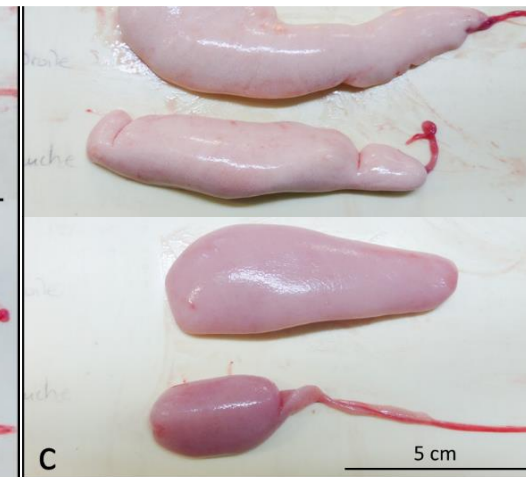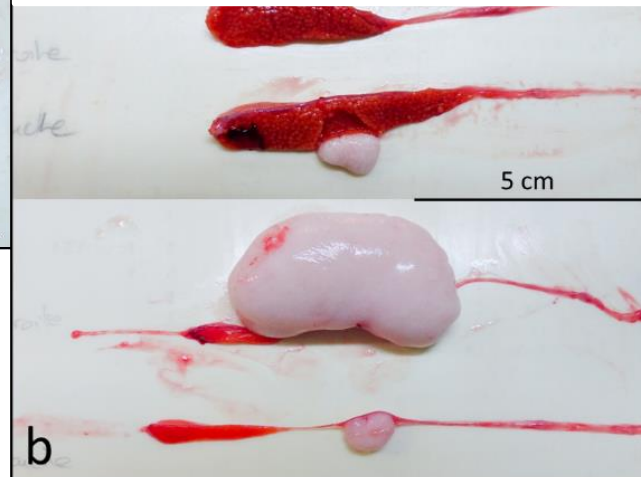
Genotyping and Whole Genome Sequencing
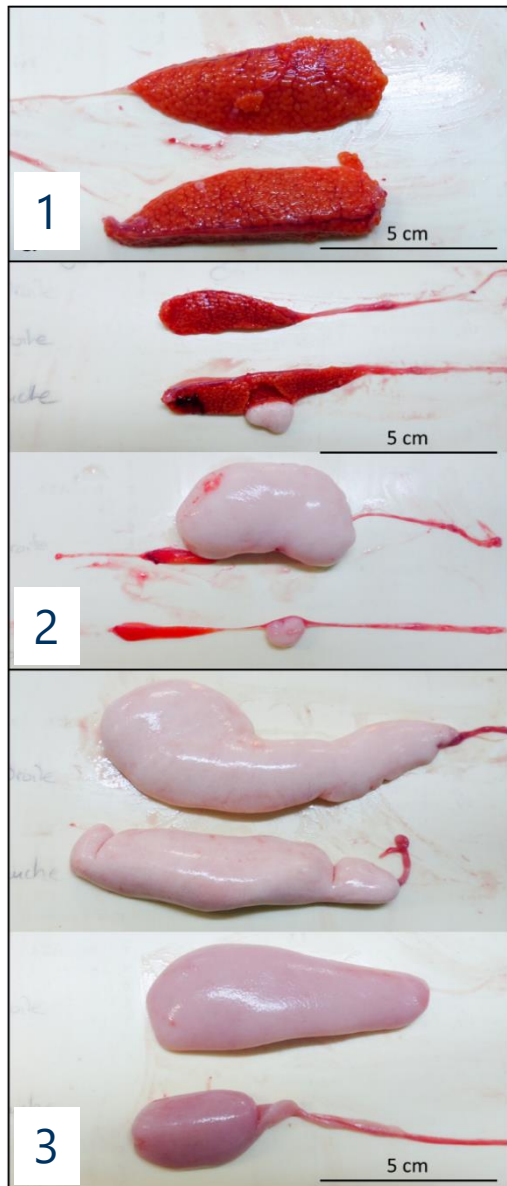
57K SNP: Axiom™ Trout Genotyping Array

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

- **31K SNPs**

Whole genome sequence (TrueSeqNano)
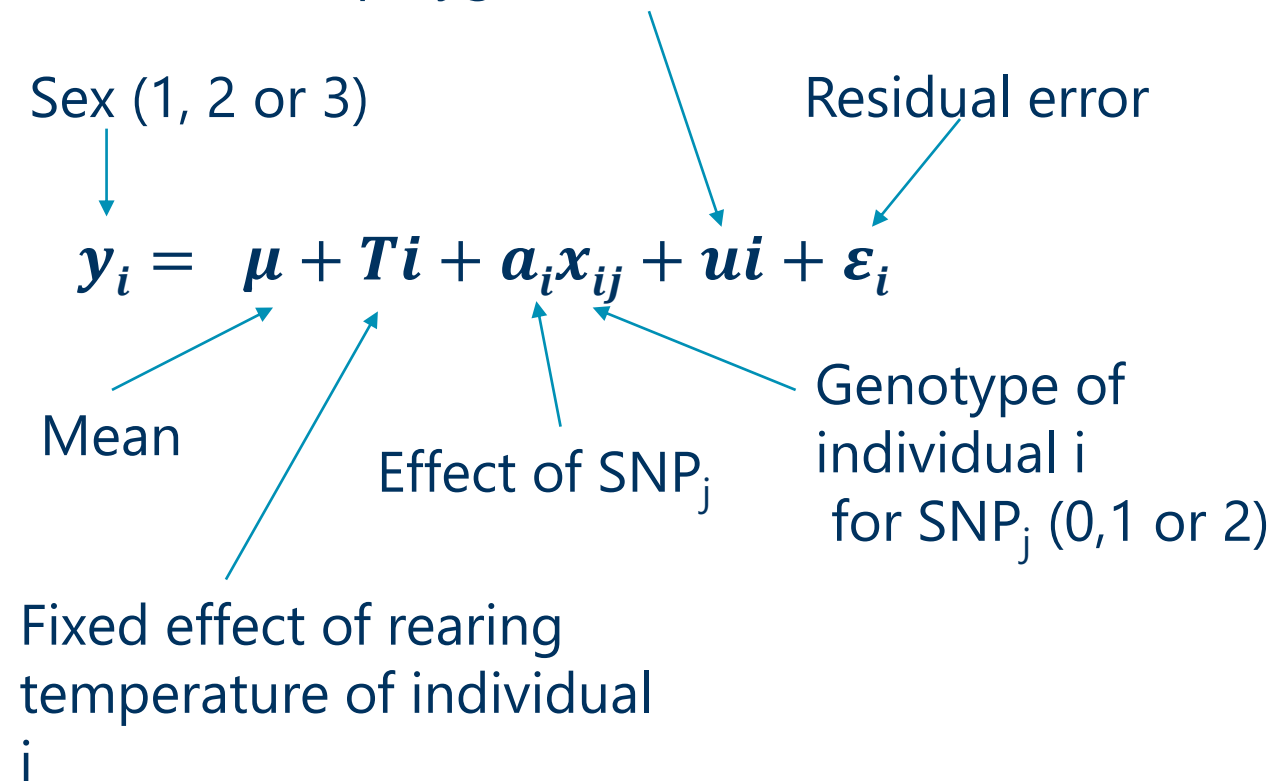
- 50 dams from the mating design
- 8.8 millions SNPs

Imputation

- **8.8 millions SNPs**

Genetic parameters and Genome Wide Association Studies

- Marker-by-Marker (GCTA)

Additive polygenic effect for individual i

Sex (1, 2 or 3)

Residual error

$$y_i = \mu + Ti + a_i x_{ij} + ui + \varepsilon_i$$

Mean

Effect of SNP_j

Genotype of individual i for SNP_j (0,1 or 2)

Fixed effect of rearing temperature of individual i

- Bayes Cπ (BESSiE)

MCMC with 500,000 iterations

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

Indicator variable

δ_{jk} = 1 if SNP_j is selected at a given k iteration
δ_{jk} = 0 otherwise

At every k iteration π = 0.99

INRA𝒆

Genetic parameters and Genome Wide Association Studies
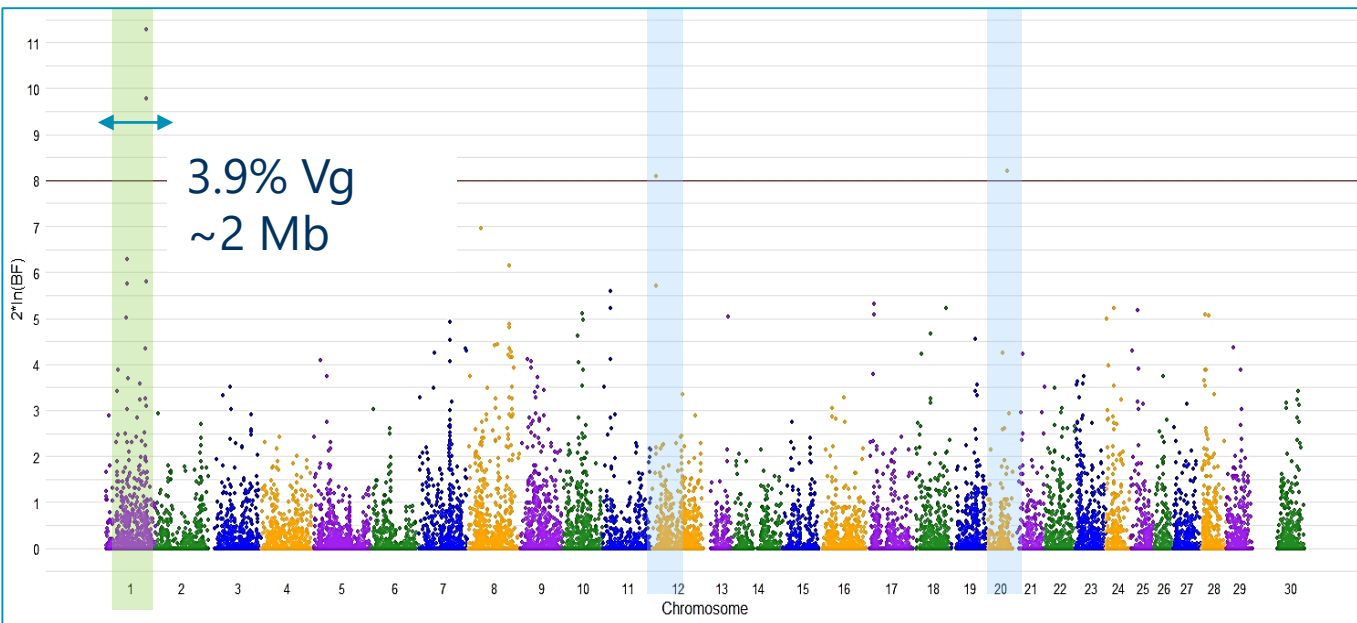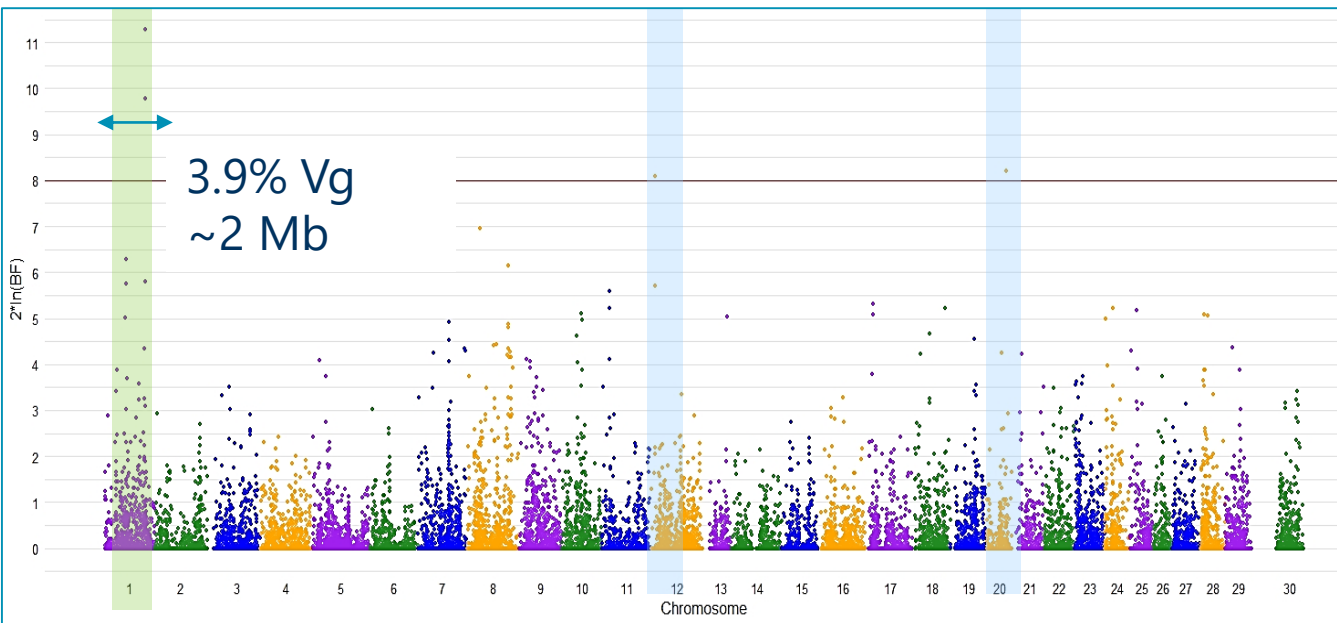
GENOTYPE (31K SNPs)

– **GWAS**

- Bayes Cπ (BESSIE)

Sequence (8.8 millions SNPs)

– $h^2_{seq} = 0.59 / 0.62$

– **GWAS**

- Bayes Cπ (BESSIE) → focus on 4Mb in Omy1 (21K SNPs)

3.9% Vg
~2 Mb

Fraslin et al., 2020

INRAe

Genetic parameters and Genome Wide Association Studies

GENOTYPE (31K SNPs)

- **GWAS**
  - Bayes Cπ (BESSIE)

3.9% Vg
~2 Mb

Sequence (8.8 millions SNPs)

Bayes Cpi run1 analysis

~ 14% Vg
400 – 550 kb

~ 0.5% Vg

Fraslin et al., 2020

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

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[1,10], Florence Phocas[1], Anastasia Bestin[2], Mathieu Charles[1,3], Maria Bernard[1,3], Francine Krieg[1], Nicolas Dechamp[1], Céline Ciobotaru[1], Chris Hozé[1,4], Florent Petitprez[5], Marine Milhes[6], Jérôme Lluch[6], Olivier Bouchez[6], Charles Poncet[7], Philippe Hocdé[8], Pierrick Haffray[2], Yann Guiguen[9] & Edwige Quillet[1]
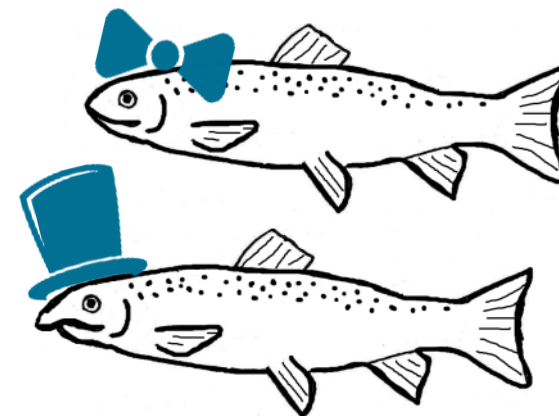
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Thank you for your attention!

INRAe

GABI and GenAqua

LPGP

GENTYANE
PLATE-FORME de
GENOTYPAGE et SÉQUENÇAGE en AUVERGNE

Charles Murgat

SYSAAF

UNION EUROPÉENNE

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.
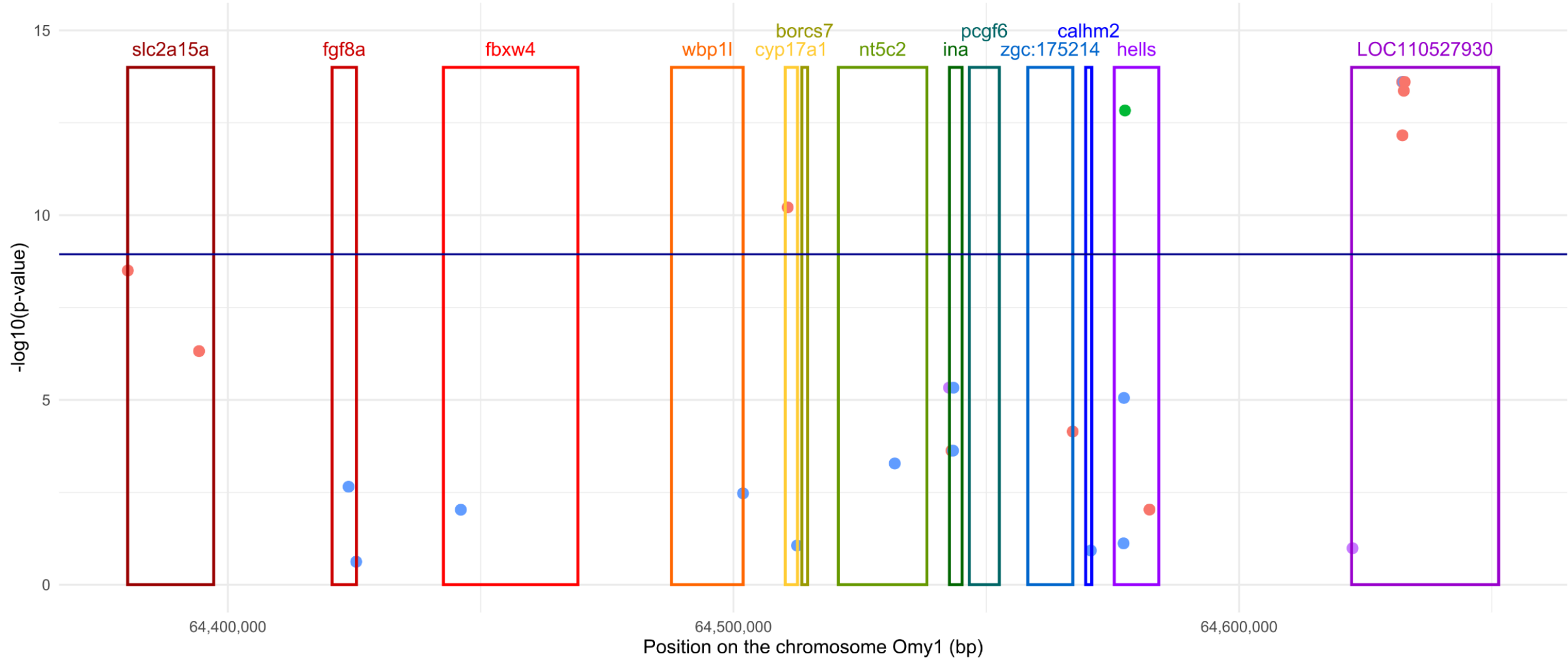
$h^2{}_G$: genomic heritability, calculated as $\sigma a\, 2/\,(\sigma a2+\sigma e2)$. $\sigma a2$: total genetic variance $(=\sigma u2+\sigma g2)$, $\sigma u2$: polygenic variance, $\sigma g2$: genetic variance explained by SNPs, $\sigma e2$: residual variance, $\sigma p2$: phenotypic variance $(=\sigma a2+\sigma e2)$. *value of one MCMC chain among the two used for GWAS, the chain with the closest final π to the 1% or 0.02% was chosen.
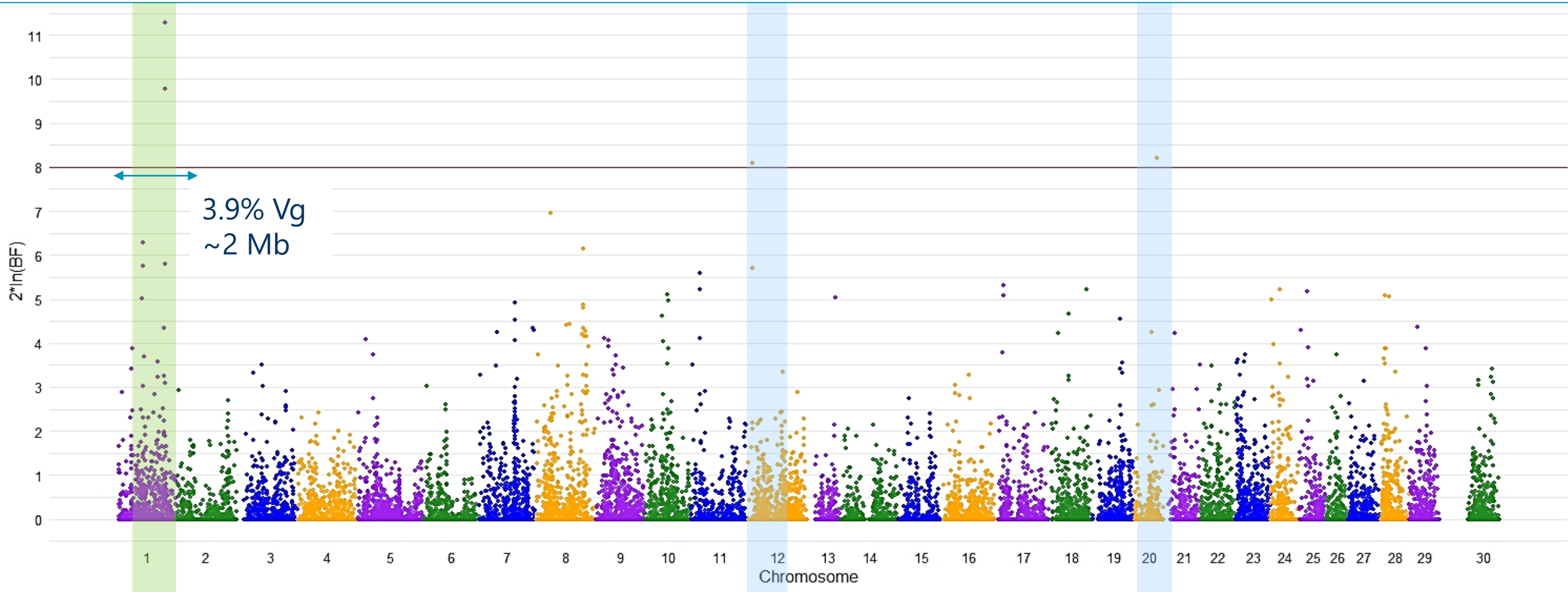
INRAℓ

Bayes Cπ (31K)

3.9% Vg
~2 Mb

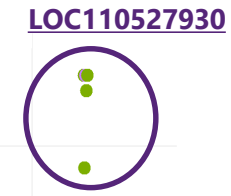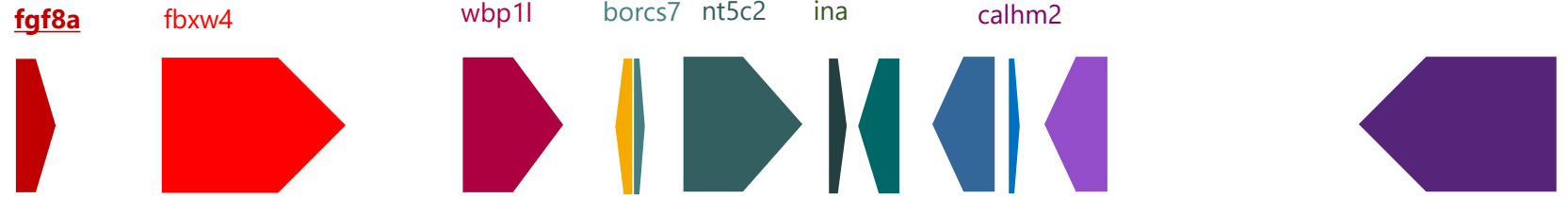Annotated SNPs located within the second QTL region, from 64.360 to 64.633 Mb on Omy1

Fraslin et al., in press

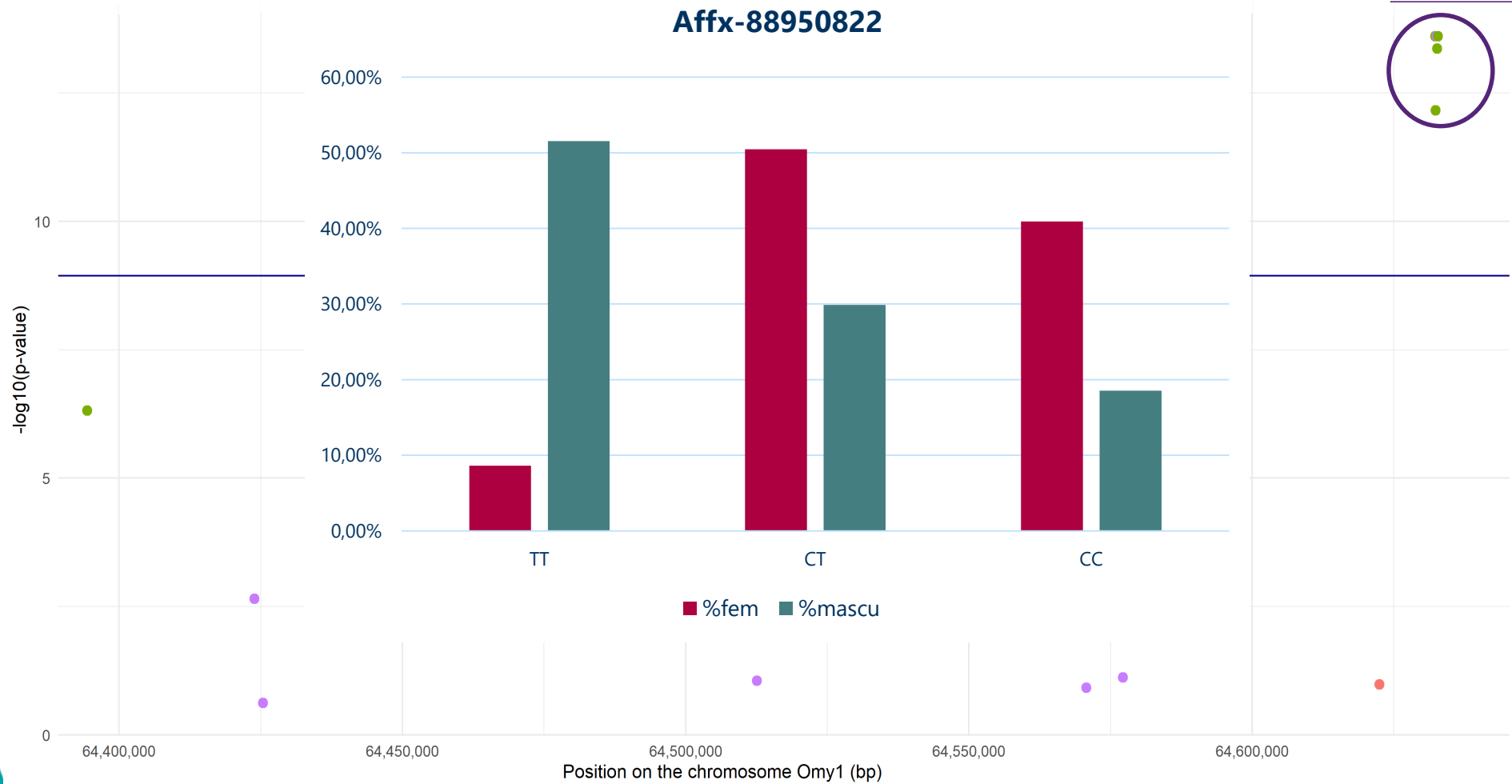# Annotated SNPs located within the second QTL region, from 64.360 to 64.633 Mb on Omy1
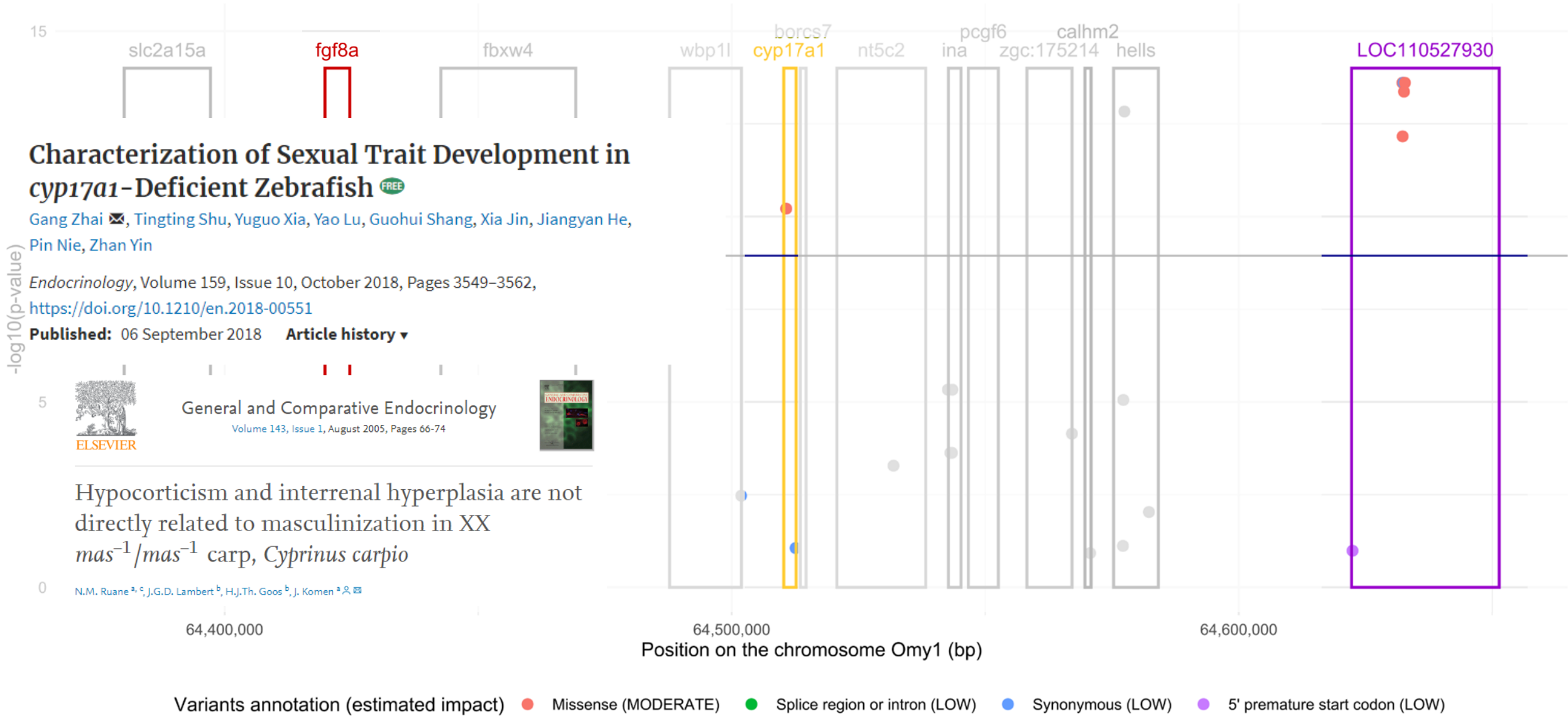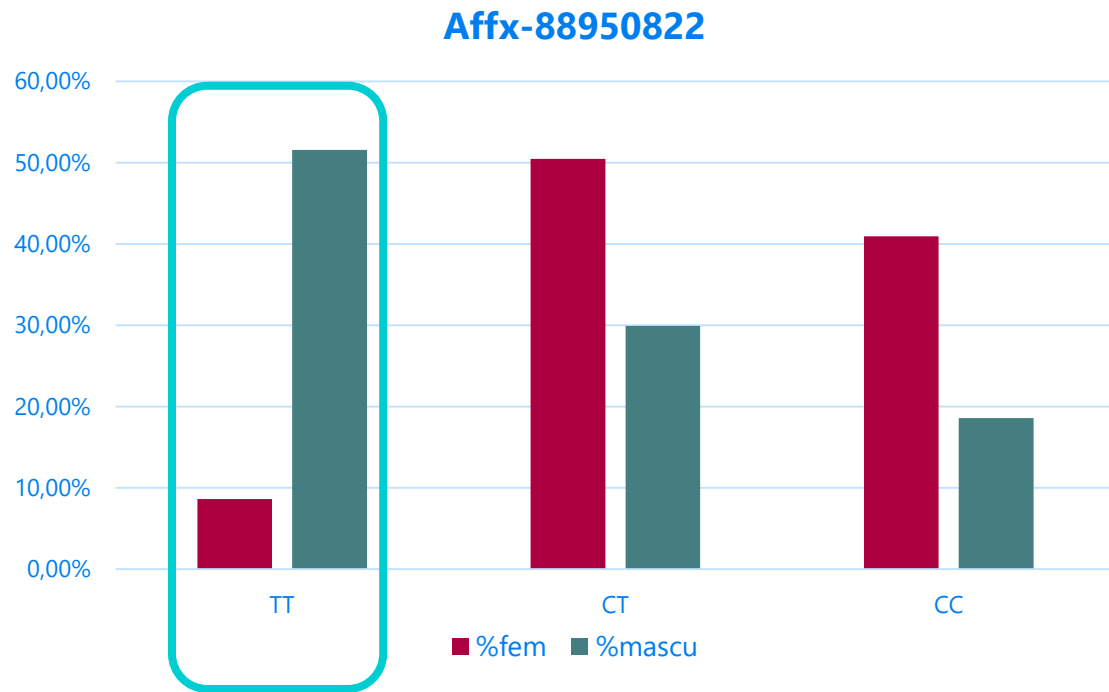

Fraslin et al., in press

Annotated SNPs located within the second QTL region, from 64.360 to 64.633 Mb on Omy1
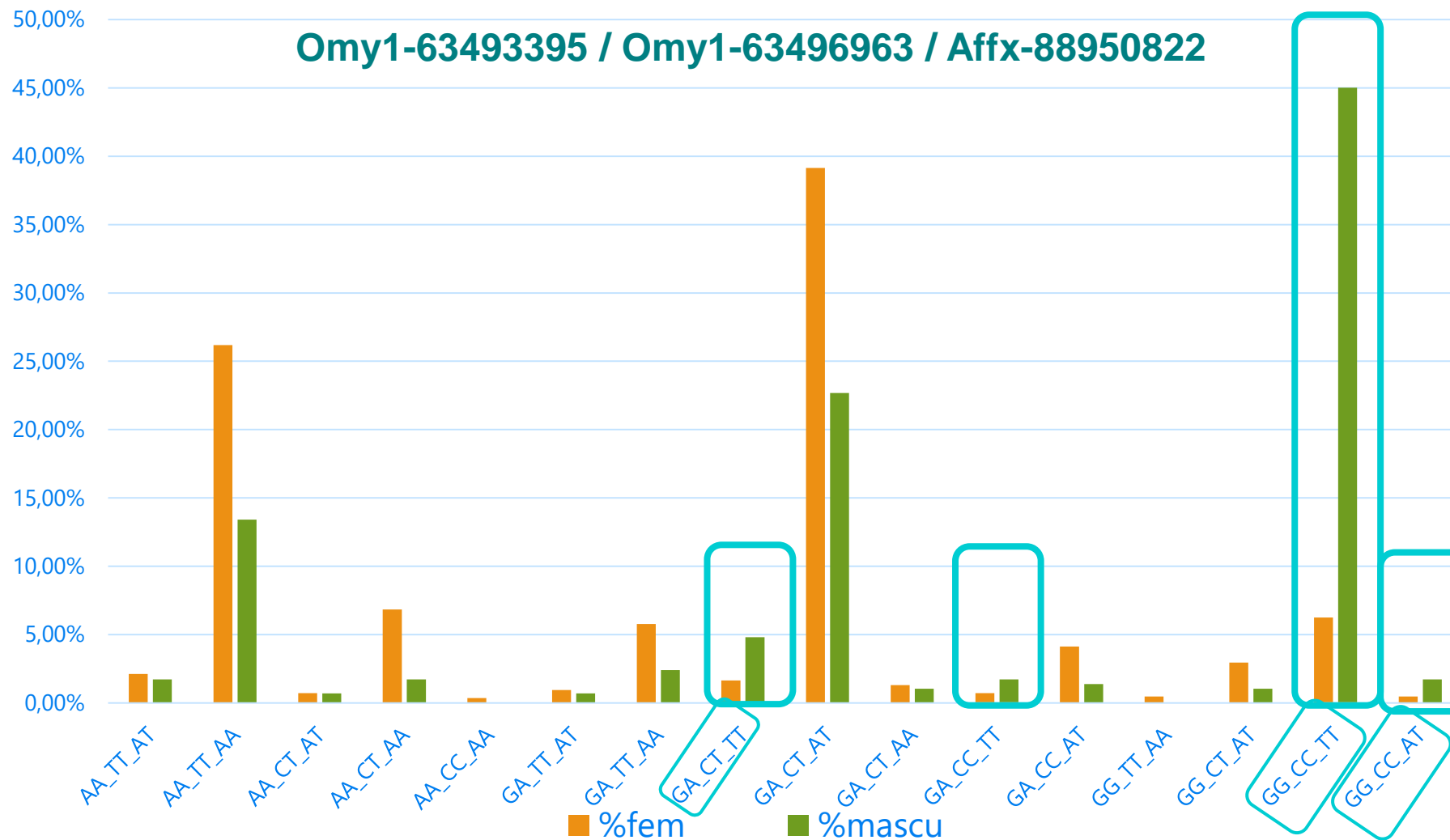
Strategy/tool for the trout producers

- Repress spontaneous maleness
  - A test to detect masculinised fish

Affx-88950822

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

51.6% of masculinised fish are TT (48.4% are CT or CC)
8.6% of true females are TT (91.4% are CT or CC)

INRAe

Omy1-63493395 / Omy1-63496963 / Affx-88950822

53.3% of masculinised fish et **9.1%** of females

INRAe

Hormonal treatment:
17α-Methyltestosterone

*99/22/CE 29 Avril

XX

XX – neomale

X

X
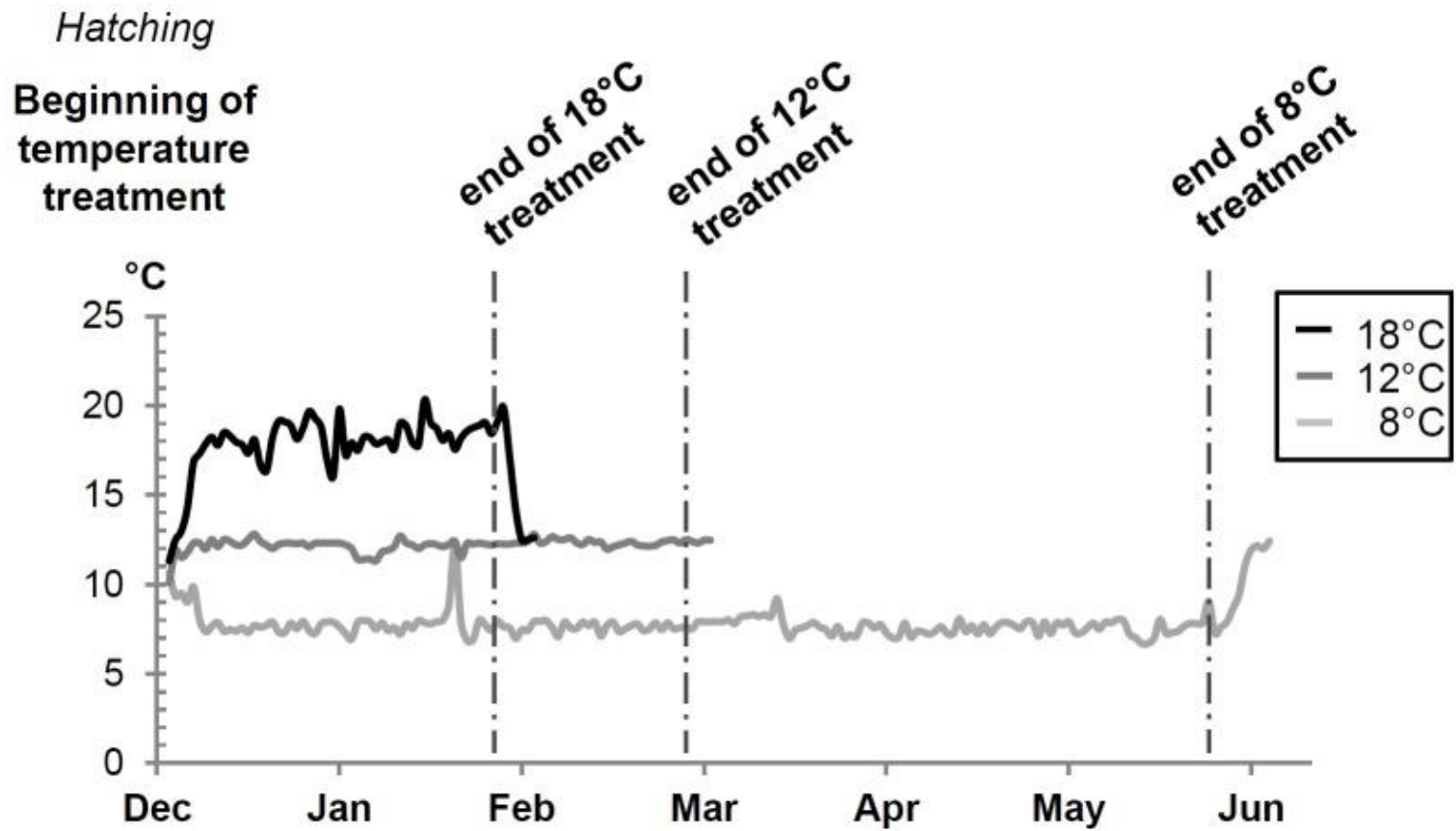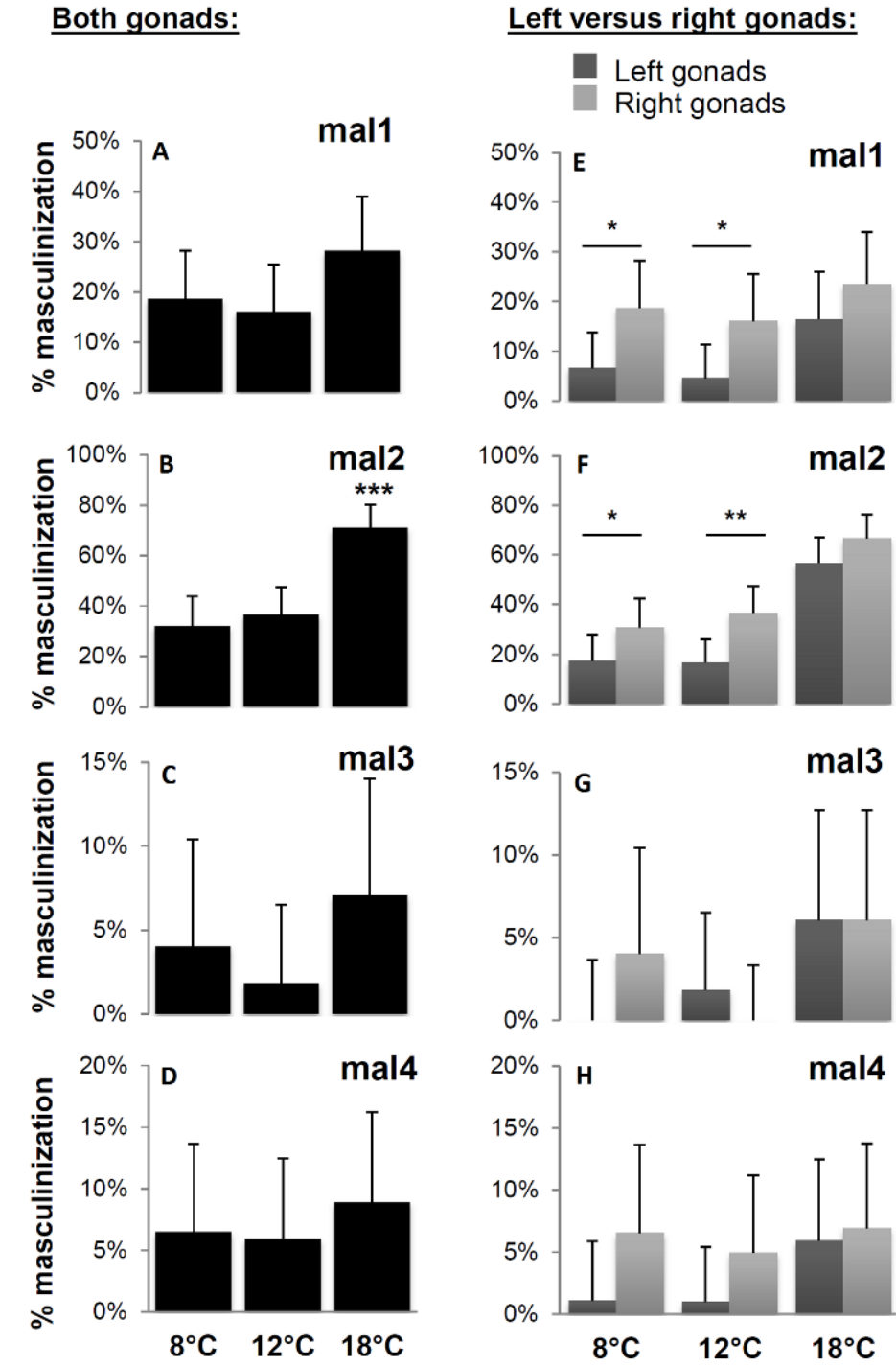
XX - female

XX

XX - female

INRA℮
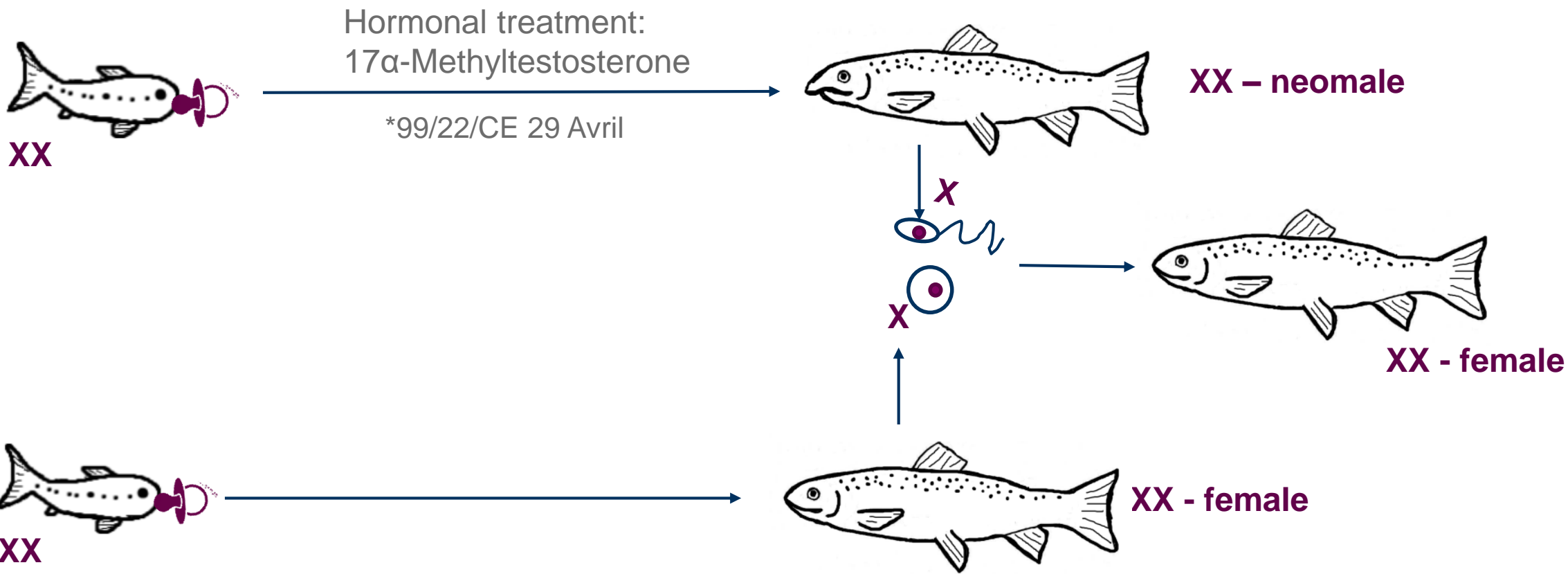
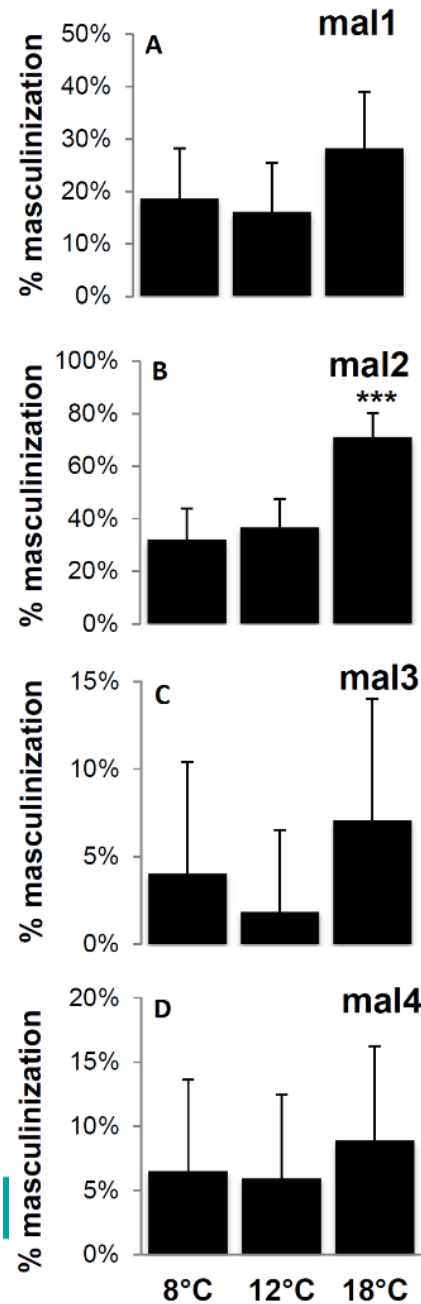**Figure 1. Experimental conditions for the common garden experiment A.**

Valdivia et al., 2014

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

INRAe

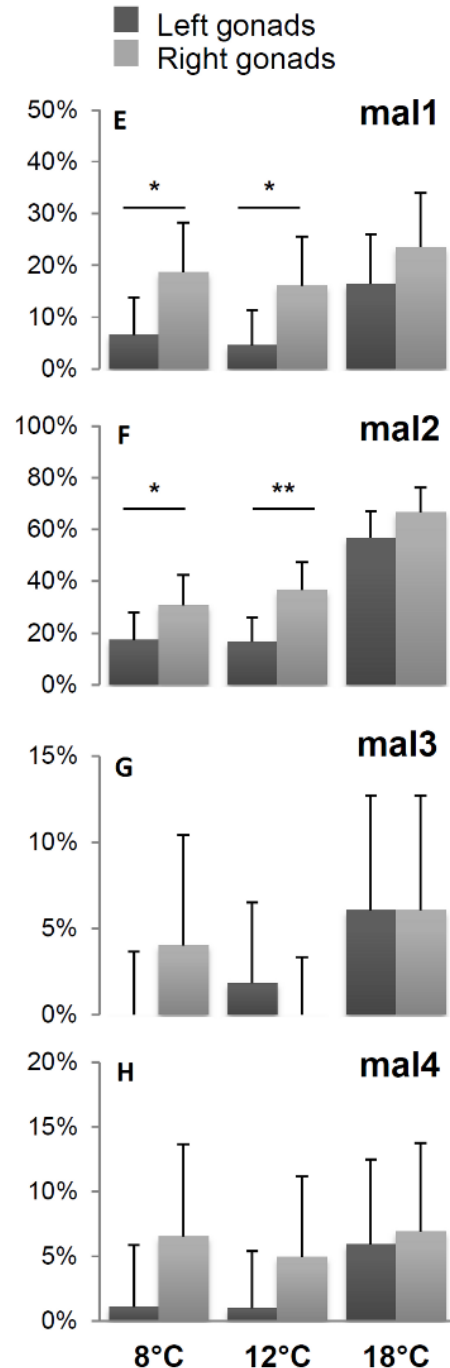**Figure 3. Rates of masculinization of the different *mal-carrying* progeny exposed to different temperatures in experiment A.**

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 ± Confidence Interval at p=0.05%+CI; χ^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
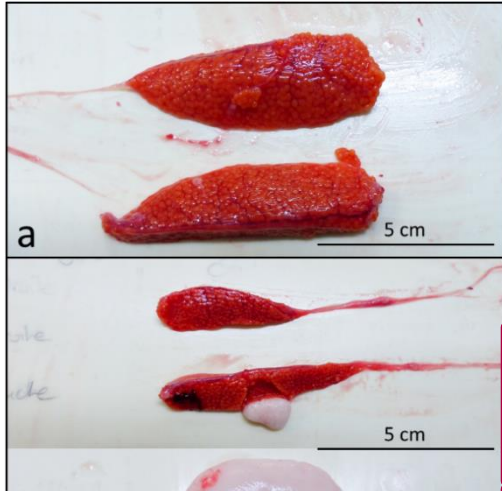17 months

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%