“A Genome-wide Association Study for Sex Determination in Atlantic Salmon”

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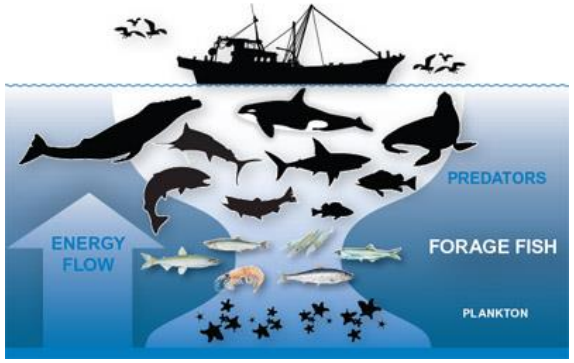
Atlantic salmon (*Salmo salar*)

Commercial and recreational fisheries

Aquaculture

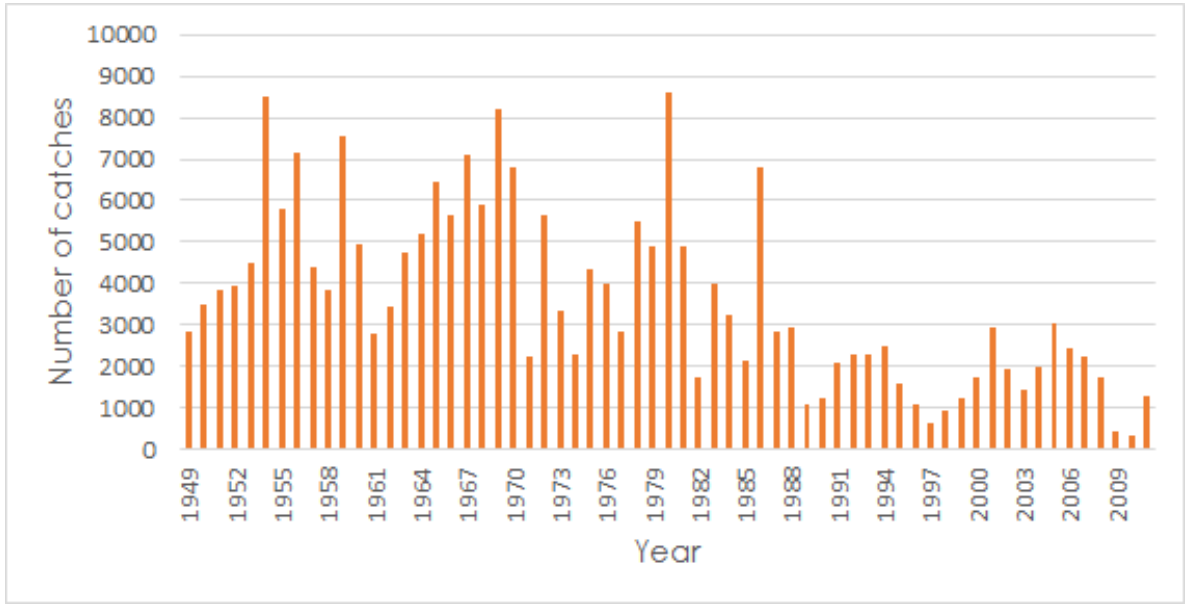


Ecosystem dynamics

Biodiversity

Atlantic salmon (*Salmo salar*)

Decline in salmon Iberian populations



Number of captures accumulated among the main Spanish rivers per year (1949 – 2011)

- Important decrease in number of catches (diminution of 85% approx.)

Causes

- **Dams**
- Overfishing
- Pollution

Conservation programs to recover the populations

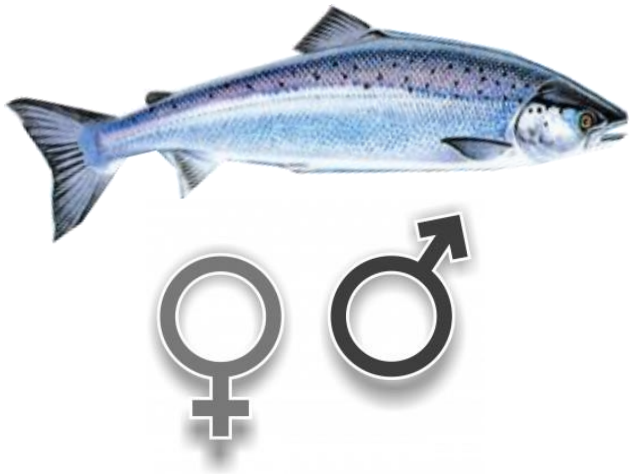
Sex Determination (SD)

- Sex determination is important for **conservation programs** and **aquaculture**.
  - Sex associated genetic markers →Early sex identification
  - Balanced sex ratio or mono-sex production (salmon ♀)

Sex-determining master genes are the main genetic switches controlling the gonadal sex differentiation cascade leading to the development of ovaries or testes

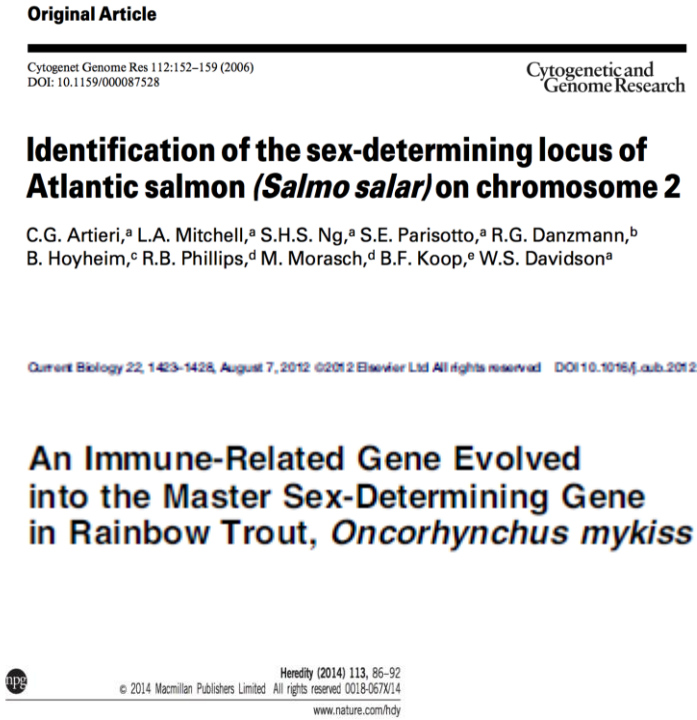
- Fish species: **high genetic variation** for SD

Sex Determination: Salmonids



- Phenotypic sex determined by a genetic **male heterogametic** system.
- **Atlantic salmon** lacks morphologically different sexual chromosomes.
- **Previous studies:** identified sex chromosomes by **genetic mapping** and **FISH** (probes with sex-linked markers)

Sex Determining Loci in salmonids: previous studies



Original Article

Cytogenet Genome Res 112:152–159 (2006)
DOI: 10.1159/000087528

Cytogenetic and Genome Research

Identification of the sex-determining locus of Atlantic salmon *(Salmo salar)* on chromosome 2

C.G. Artieri,[a] L.A. Mitchell,[a] S.H.S. Ng,[a] S.E. Parisotto,[a] R.G. Danzmann,[b] B. Hoyheim,[c] R.B. Phillips,[d] M. Morasch,[d] B.F. Koop,[e] W.S. Davidson[a]

Current Biology 22, 1423–1428, August 7, 2012 ©2012 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2012.05.045

An Immune-Related Gene Evolved into the Master Sex-Determining Gene in Rainbow Trout, *Oncorhynchus mykiss*

Heredity (2014) 113, 86–92
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www.nature.com/hdy

OPEN

ORIGINAL ARTICLE

Evidence for multiple sex-determining loci in Tasmanian Atlantic salmon (*Salmo salar*)

WD Eisbrenner[1], N Botwright[2], M Cook[2], EA Davidson[1], S Dominik[2], NG Elliott[2], J Henshall[2], SL Jones[1], PD Kube[2], KP Lubieniecki[1], S Peng[1] and WS Davidson[1]

Artieri et al 2006

- European Atlantic salmon (Norway): SD locus on the long arm of chromosome 2 (Ssa02)

Yano et al 2012

- ***Sex*** master gene in Rainbow trout, ***sdY***
- *sdY* linked to the SD locus in most salmonids: in SD locus of Atlantic salmon.

Eisbrenner et al. 2013

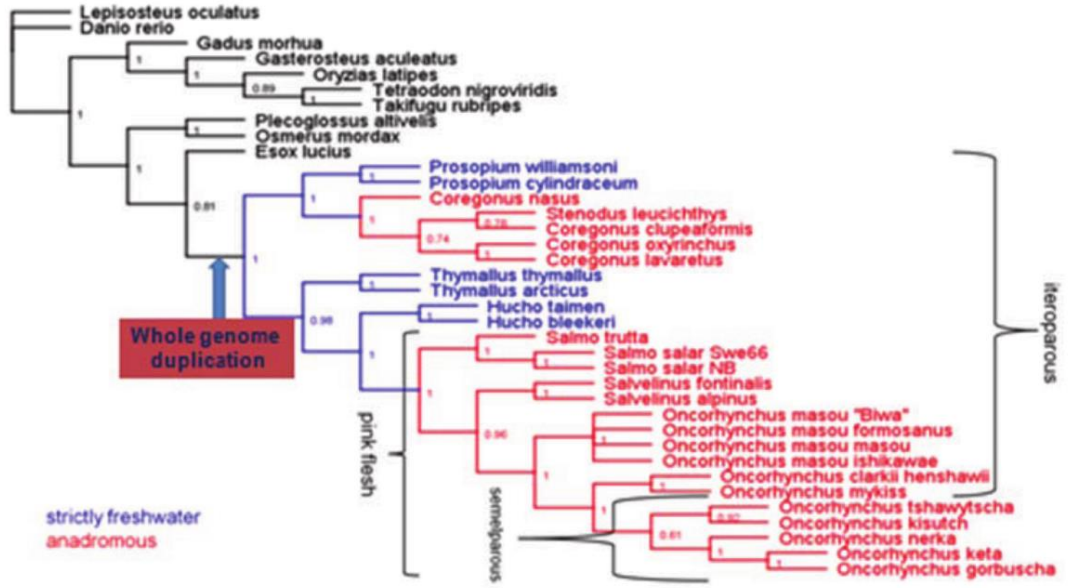
- Tasmanian Atlantic salmon (Canadian origins)
- Three SD loci on chromosomes: Ssa02, Ssa03 and Ssa06.
- All males have *sdY* gene within the 3 SD loci

Background

- Atlantic salmon genome is complex and highly repetitive
- Stemming regions from salmon-specific whole-genome duplication

Davidson 2013

Fig. 1. Phylogenetic relationships within the Salmonidae and other fish species based on the amino acid sequences of mitochondrially encoded proteins.



- Spain is the South distribution limit of the species in Europe
- Small populations. High genetic pressures.

Genotyping array: Atlantic salmon

CIGENE Centre for Integrative Genetics

affymetrix Biology for a better world

96 samples / array

220K SNPs/sample

GWAS analysis

Genome Wide Association Studies

Evaluates the association between each genetic SNP marker with a phenotype in a pool of many individuals.

Objective

Investigate potential association between SNPs and sex in Atlantic salmon Spanish population using genome-wide association approaches (GWAS)

Genotyping Atlantic salmon (220K SNPs array)

Samples

- 16 individuals/river → **96 individuals in total**
- 6 rivers

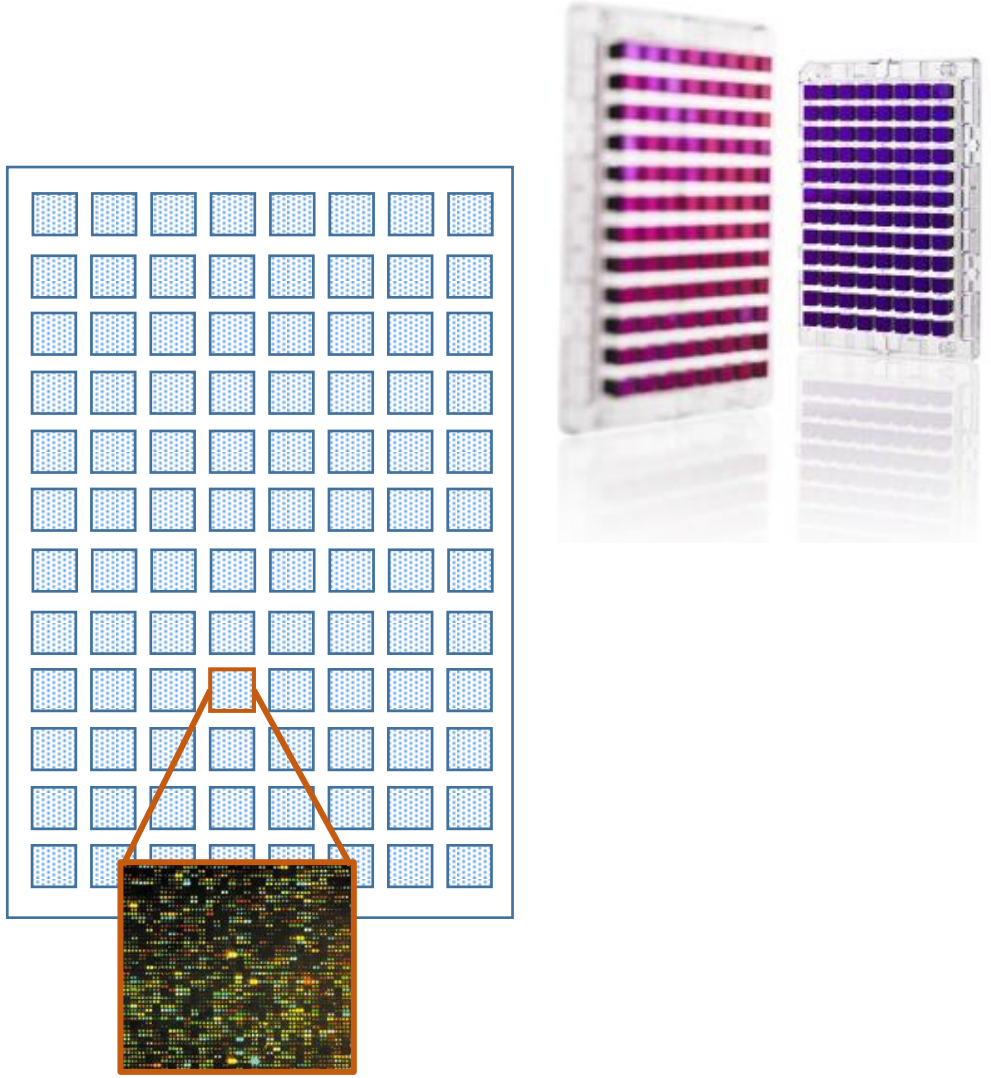
Samples QC

- **DQC** (dish quality control)
  measure of the interference between foreground and background signal distribution

DQC ≥ 0.82

- **Call rate**
  % of DNA samples whose genotype is successfully measured

Call rate ≥ 0.98



MAF

Minor allele frecuency

Excluding those SNP with a very low allele frequence

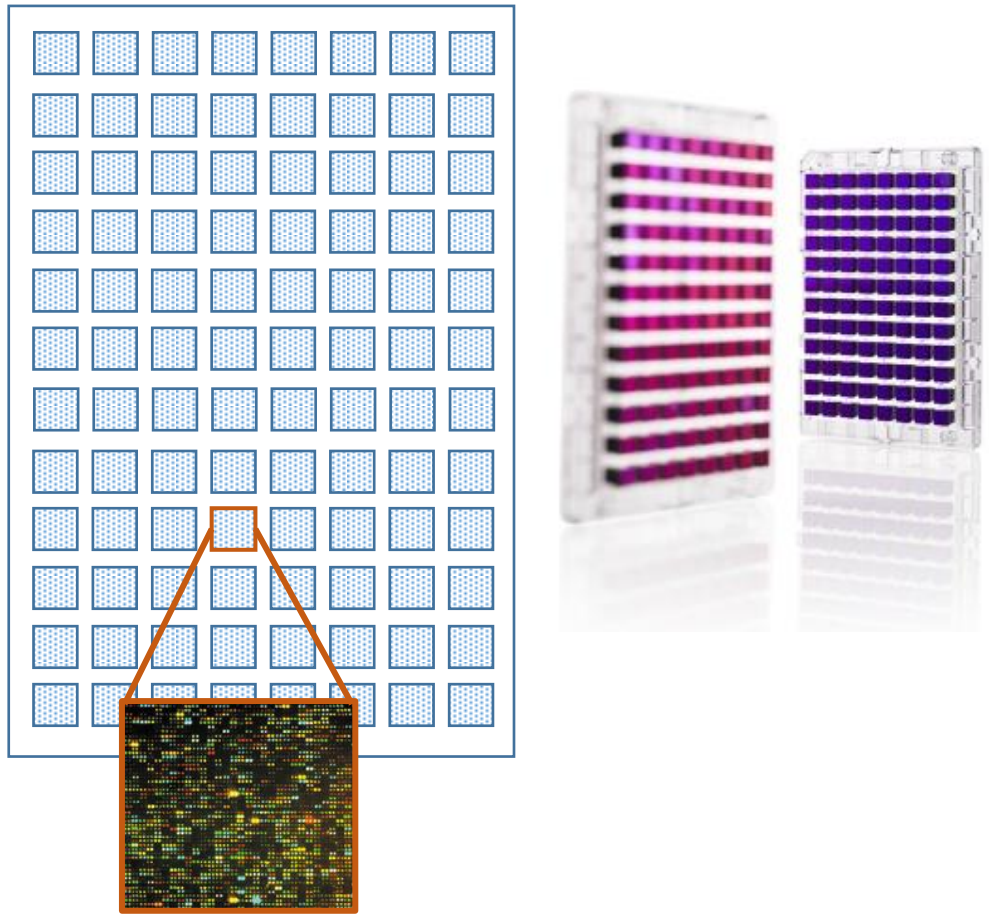
MAF < 0.05

HWE

Hardy-Weinberg Equilibrium

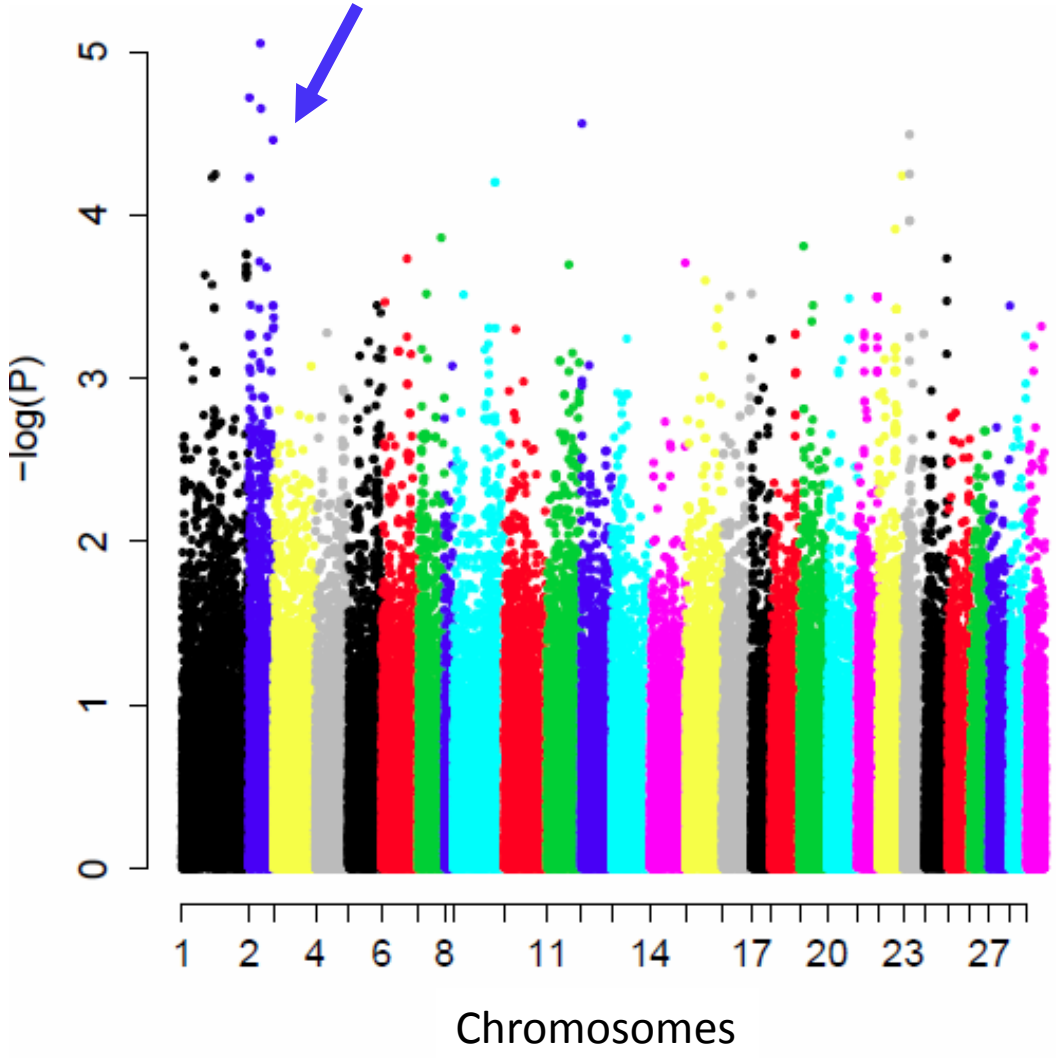
Deviation from HWE can indicate genotyping errors
Small p-values indicate some HW disequilibrium

HWE < $1 \cdot 10^{-5}$



Number of SNPs after filtering: 161486

Analysis results



Package GenABEL

Logistic regression approach (sex=binary trait)

- Interesting SNP p-values trend at chromosome 2
- Making corrections analysis each chromosome indepently:
  reveled **8 significant SNPs** at **chromosome 2**
- SNP positions chromosome 2 (5 different regions):

3545430 4728514 29247973 30835350 66004200

3568542 4775226 29258867

- Spanish Atlantic salmon population seems to have **SD locus** located on the **chromosome 2**, as previously detected in other populations

Next step

- Determinate the exact location of the regions found:
  - Do they overlap previous described regions?
  - Do they contain the *sdY* gene?

- Improve statistical power:
  - Increasing the sample size
  - Pruning by linkage disequilibrium: decrease the number of SNPs: lower threshold for multitest correction

Thank you!!!

Paloma Morán and María Saura

Matthew P. Kent

Pablo Caballero

Jerónimo de la Hoz

Pedro Leunda

Iñaki Bañares

XB2 group

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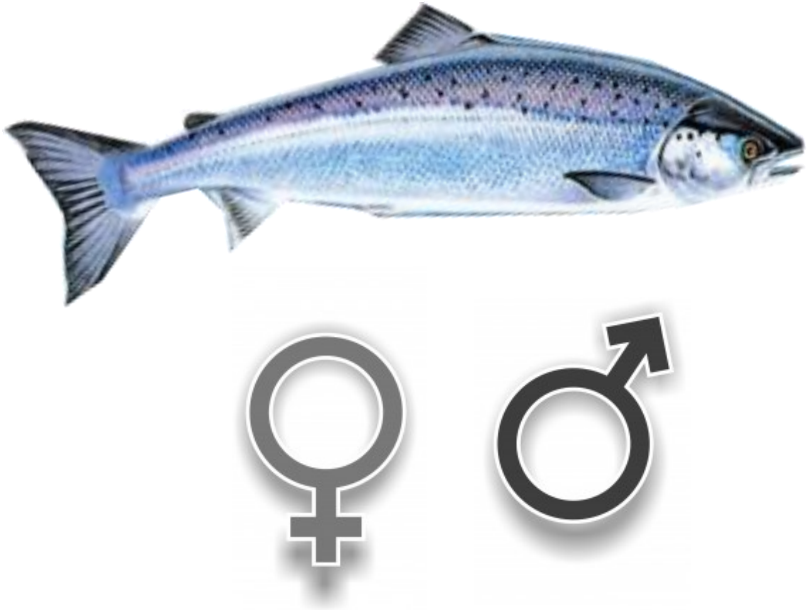
GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

XUNTA DE GALICIA

Sex Ratio: Experiment samples



River	Number of Females	Number of Males
Miño	10	6
Ulla	11	5
Eo	15	1
Sella	13	3
Bidasoa	13	3
Urumea	7	9
TOTAL	69 (72%)	27 (28%)