



Breeding sweet cherries at INRA Bordeaux : from conventional techniques to marker-assisted selection

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Breeding sweet cherries at INRA-Bordeaux: from conventional techniques to marker-assisted selection

José Quero García (INRA – Bordeaux)

- ☐ First breeding programmes (1968 – 2005)
(Raymond Saunier-Jacques Claverie)
- ☐ Transition (2000-2005) (Jacques Claverie-
Elisabeth Dirlewanger)
- ☐ Present (2007- today) (UREF-A3C)

Second breeding programme (1980-2005)

New orientations:

- ❖ Introduction of numerous (400) foreign cultivars
- ❖ Establishment of experimental networks (A-B) via a collaboration between INRA and CTIFL

New breeding objectives:

- ❖ Big fruits (= or > 10 g) and firm fruits (Durofel > 60-65)
- ❖ Enlargement of the maturity range
- ❖ Yield precocity (3rd year)
- ❖ High production potential

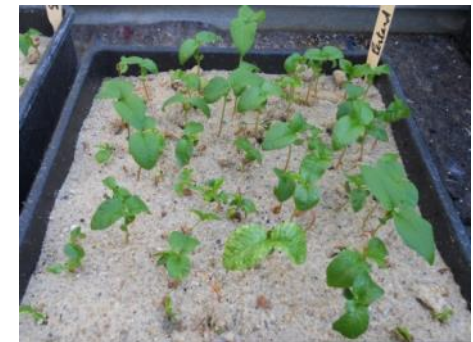
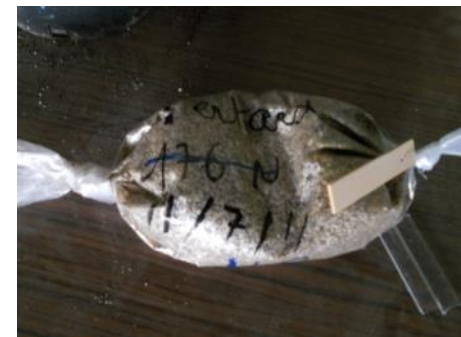


STRUCTURE OF THE PROGRAMME

INRA	Year 0	Hybrid production
	Year 1	Nursery
	Year 2	Field planting on own roots
	Years 5-7	Hybrid evaluation
	Years 8-11	Level 1 evaluation: 3 sites/ 2 cl
	Years 12-15	Level 2 evaluation: 10 sites/ 10 cl

HYBRID PRODUCTION TECHNIQUES

- ❖ Hand-pollination for controlled crosses, open pollinations
- ❖ In-vitro culture for early-maturing hybrids
- ❖ Classical stratification



Second breeding programme (1980-2005)

INRA FERCER



INRA FERPRIME



INRA FOLFER



INRA FERTARD



Second breeding programme (1980-2005)



👉 **Poster** 'The partnership between INRA and CEP INNOVATION'- Quero-Garcia *et al.*

Second breeding programme (1980-2005)

Variety	Maturity period
Primulat®Ferprime	Burlat
Folfer ^(COV)	Between Burlat and Summit
Ferdouce ^(COV)	
Fertille ^(COV)	Summit
Fermina ^(COV)	
V3467	Belge
Ferdiva ^(COV)	Late
Fertard ^(COV)	

Transition (2000-2005)

New research programme on rain-induced fruit cracking:

- ❖ Crosses between **tolerant** (Regina, Fermina, Ferobri) and **susceptible** varieties (Lapins, Garnet, Brooks...)
- ❖ Establishment of a **first genetic map** : Regina x Lapins (125 hybrids) with Prunus SSR markers (Dirlewanger et al., 2004, PNAS 101: 9891-9896)
- ❖ Establishment of an original system to study cracking under **tunnels**



Transition (2000-2005)

Diversity studies conducted on sweet cherry (Tavaud et al, Heredity 2004) with **AFLP and 6 SSR** markers:

- ❖ Large sample of mazzards and sweet cherry varieties
- ❖ Important **genetic differentiation** between wild materials from West Europe, Romania and Georgia
- ❖ Insufficient sample to determine the centers of domestication of sweet cherry. Proximity between wild and cultivated materials due to either **gene flow** or to **domestication** and breeding



Present (2007-today)

- ❖ 2007-2008: new breeding programme and new scientific project: 'Adaptation of sweet cherry to climate change'. Two main targets: **phenology-related traits** and **tolerance** to rain-induced fruit cracking
- ❖ Close relationship with **UEA** (Unité Expérimentale Arboricole): two sites : Toulenné and Bourran
- ❖ **A3C**: 5 researchers, 5 technicians, 1 PhD student, 1 post-doc



Scientific project

GENOMICS

Development of genomic resources
Unigene - RNAseq

Identification of CG
Fonctionnal - Expressionnal

Mapping of CG

Co-localisation CG-QTL

GC Studies
Functional analyses
qRT PCR-epigenetic variation -transformation

PHENOMICS

Search for accurate phenotypic criteria

Phenotyping
Mapping progenies Collections

QTL detection
Multi-site trials
Interaction G x E

**Structure, LD
Association Genetics**
Phenotyping/ **Genotyping**

Climatic data



MAS

- ❖ **Selection criteria:**
 - ☞ **Fruit weight and fruit firmness**
 - ☞ **Phenology-related traits: chilling requirements, flowering and maturity dates**
 - ☞ **Cracking tolerance**
 - ☞ **Self-fertility**
 - ☞ **Organoleptic and nutritional quality**
 - ☞ **Tolerance to diseases (monilia, aphids)**
- ❖ **Marker-assisted selection (MAS) strategy**
- ❖ **Diversification of genetic resources used**



❖ Plant materials for QTL detection studies:

- ➡ Regina x Lapins (**RxL**): 125 inds. (enlarged to 200). Planted in the field on own roots and grafted on Tabel in pots (tunnel system)
- ➡ Regina x Garnet (**RxG**): 120 inds. on own roots (enlarged to 1300)
- ➡ Fercer x Burlat (**FxB**): 115 inds. On own roots

❖ Genetic maps:

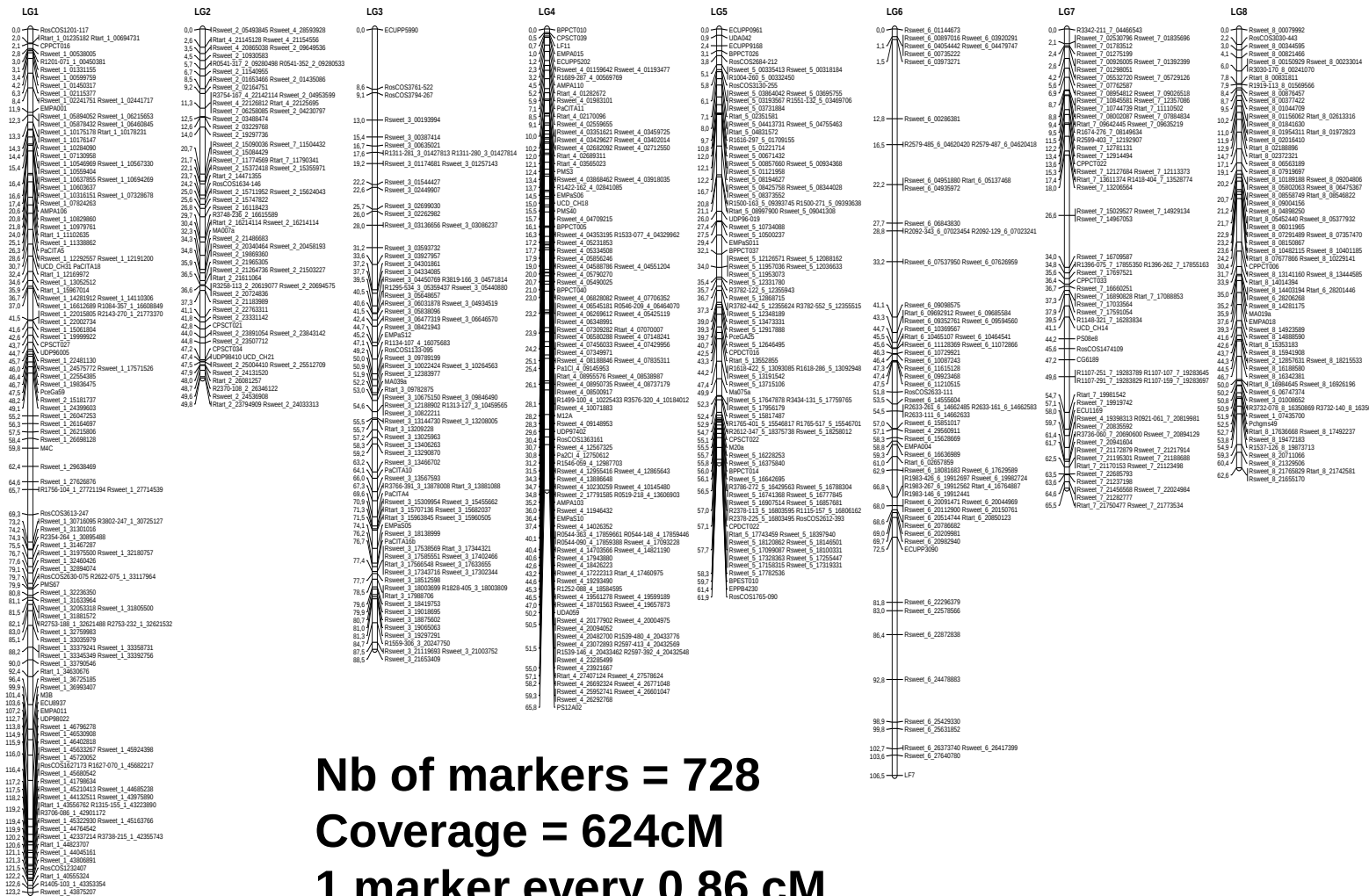
- ➡ RxL and RxG with the **6000 SNP chip** (RosBREED project). FxB in progress

❖ Phenotypic data:

- ➡ Phenology traits: **6-7 years** for flowering and maturity dates (all progenies); **3 years** for CR on RxG
- ➡ Fruit quality traits (weight, firmness, cracking): **5-7 years** for field data (all progenies) and **3 years** for tunnel data (RxL)

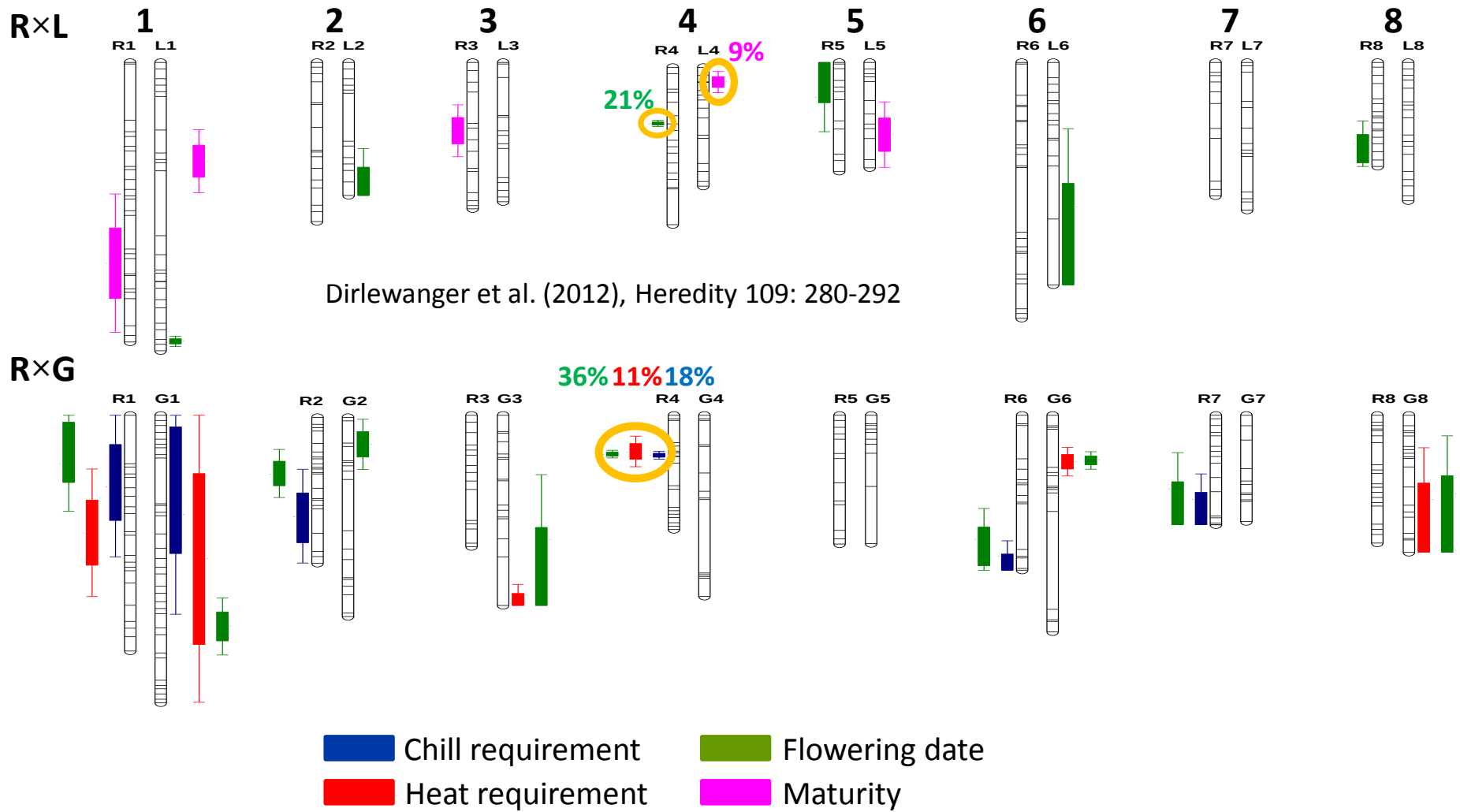


Genetic mapping



Campoy et al. (2013): PlosONE

QTL detection analyses- Phenology traits



Dirlewanger et al. (2012), Heredity 109: 280-292

👉 **Poster P23**– Castède *et al.*

Cracking tolerance evaluation

▪ **Field** : 100 fruits at maturity → percentage

▪ **Tunnel** : 100 fruits, counting during 5 days → percentage

4 types analyzed

Stem end



Pistillar end

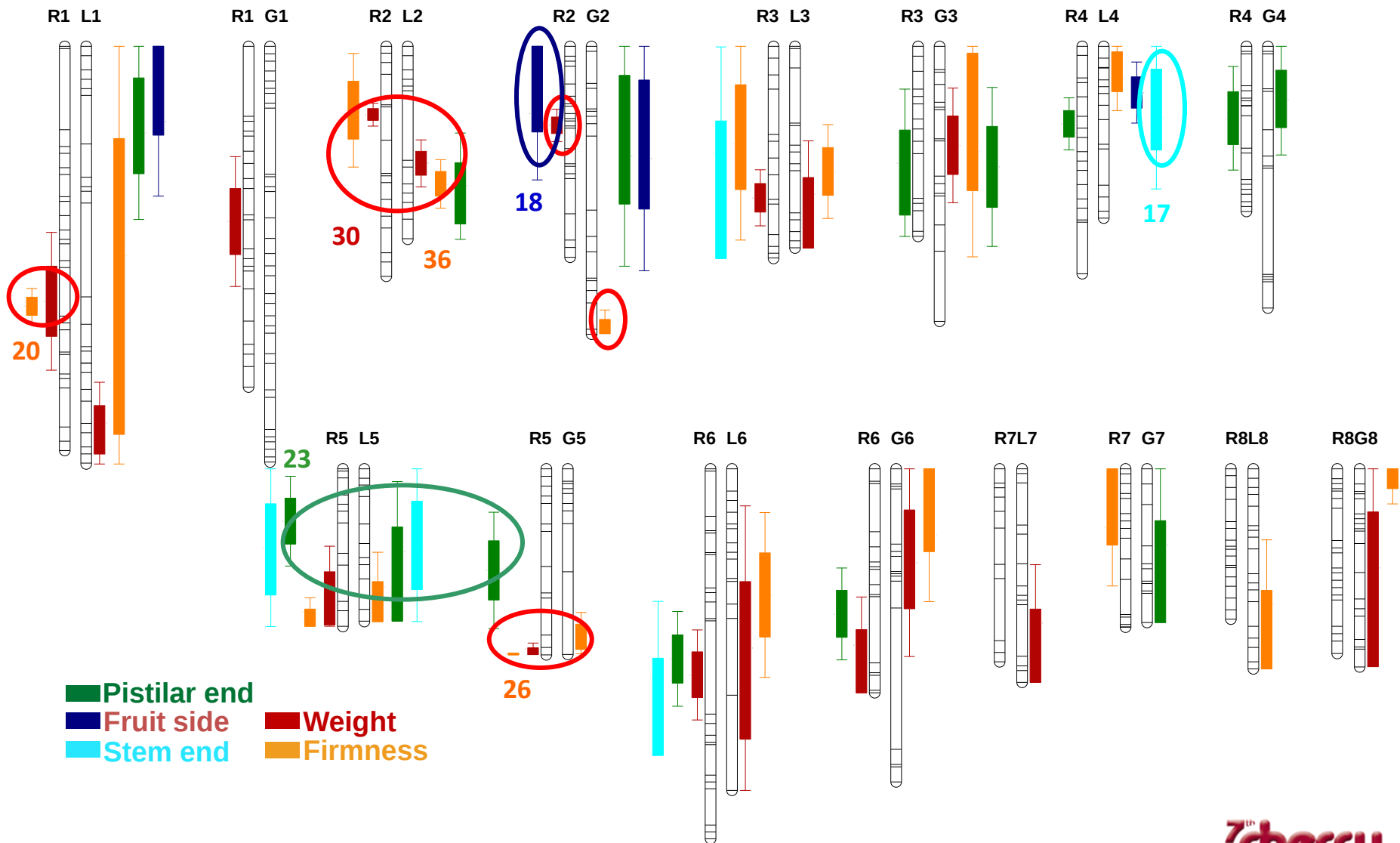


Fruit side



Fruit suture

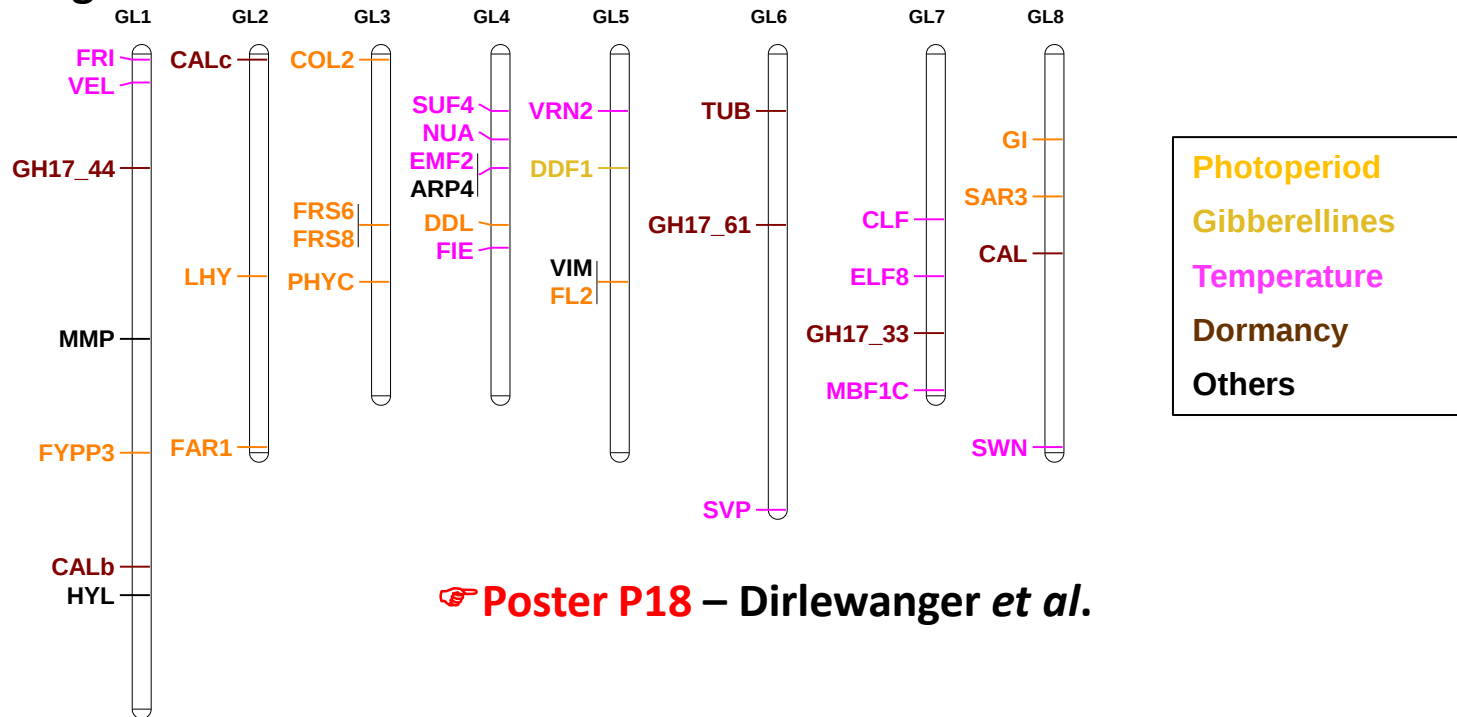
QTL detection analyses- Fruit quality traits



Dirlewanger et al. (2012), RGC6

Candidate Genes (CGs)

❖ Flowering



👉 **Poster P18** – Dirlewanger *et al.*

- ❖ **Fruit weight:** Collaboration with Amy lezzoni's group concerning the **CNR genes** (see next presentation; De Franceschi *et al.*, Molecular Breeding, 2013)
- ❖ **Cracking tolerance:** Collaboration to initiate with Herman Silva's group to map CGs of the biosynthesis pathway of the **cuticle**

- ❖ **Multi-parental** analyses (new QTLs; QTL stability; no need to produce large progenies)
 - ✓ Collaboration with Amy lezzoni's group for fruit weight, use of pedigree-based mapping with FlexQTL software (Rosyara et al., 2013, Mol Breed, accepted with revisions): identification of **6 QTLs** (3 on LG2, one on LGs 1, 3 and 6)
 - ✓ Integration of different crosses, including FxB
- ❖ **Fine mapping**: development of a large RxG population with 1300 hybrids. Planted in the field in 2013. Used for the validation of a MAS strategy
- ❖ Environmental stability (**QTLxE**) studies: multi-site trials implemented within the COST Action: RxL population planted in France, Slovenia, Spain and England. Other populations.

Development of a fine mapping progeny



Establishment of multi-site trials

Bordeaux, France (2001-2002)



Murcia, Spain(2012-2013)



Bordeaux, France (2010-2011)



Maribor, Slovenia (2009-2010 and 2011-2012)

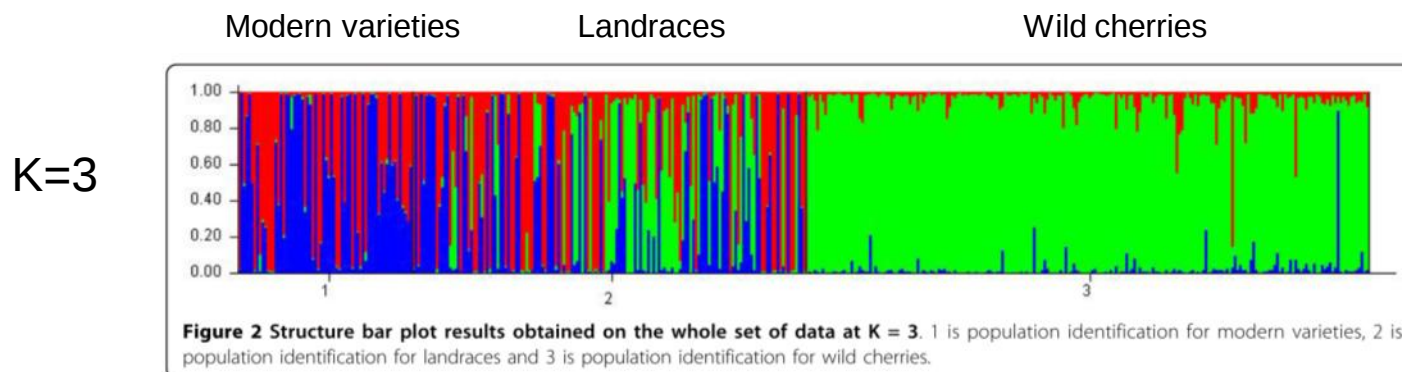
❖ Phenotypic decomposition of complex traits – fruit cracking:

- ✓ New studies under tunnel with RxL progeny grafted on MM14
- ✓ Analyses of **cell wall composition** of the RxL progeny on-going (collaboration with Dr. Marc Lahaye, INRA Nantes)
- ✓ Analyses of the main **metabolites (sugars, acids)** initiated in 2013 in collaboration with Dr. Yves Gibon. Fruits collected from the progenies RxL and FxB. Three replicates per genotype, with three fruits per replicate and three technical replicates.
- ✓ Tri-lateral KBBE project (Spain, Germany and France) submitted in 2012 (waiting for answer). Objective: develop new **protocols for** assessing cracking tolerance taking into account the **composite nature of cracking** (water transport characteristics and mechanical constitution of exocarp) (Prof. Moritz Knoche)

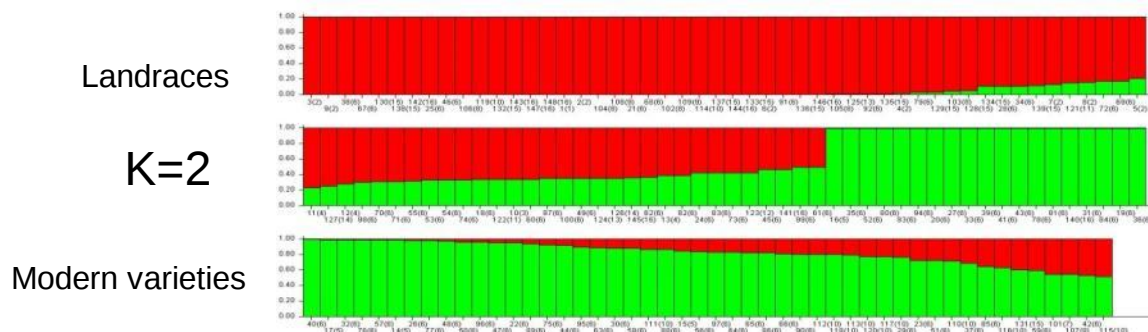


❖ QTL/CG validation by GWA/association genetics analyses :

- ✓ First 'Structure' studies conducted with 26 SSR markers on 207 varieties (141 landraces and 66 modern varieties) (Mariette et al., 2010, BMC Genetics)
- ✓ Study of DL: rapid decay, in particular in wild accessions (Arumyawat et al. 2012, TGG), promising for future association genetics studies



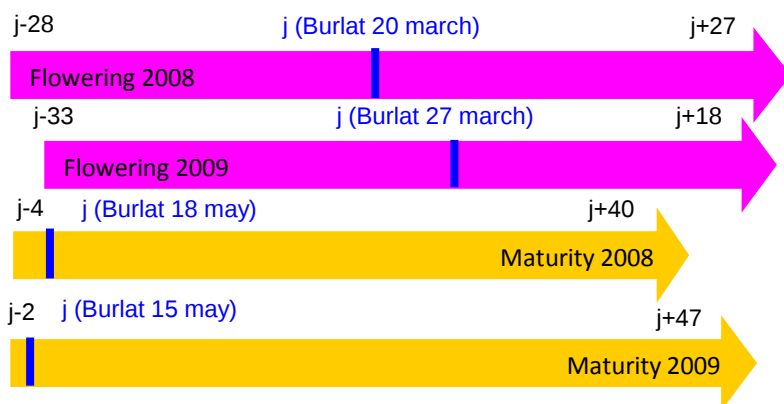
- ✓ Second 'Structure' analyses on-going with the 6K SNP chip on 140 varieties (92 landraces, 55 modern varieties). DL analyses not still initiated (Sandra Robert, MSc).



- ✓ Genetic resources collection genotyped with a subset of 41 CGs SNPs

❖ Phenotypic variability of collections:

- ✓ Based on 20 phenotypic traits (3 years data): flowering date, maturity date, fruit weight, sugar content, etc
- ✓ 380 accessions, among which 160 belong to the French National Collection



Flowering and maturity range of the collection
2 years 380 accessions

Large variability for flowering and maturity dates



❖ Discovery and validation of CGs:

- ✓ Transcriptomics: expressional CGs (**DGE-RNAseq**): on-going for flowering-related genes
 - ☞ **18 cDNA banks**: 2 genotypes (Regina and Garnet), 3 stages (endo-dormancy, dormancy-release, eco-dormancy)
 - ☞ 12-50 millions read/ 100 bases
- ✓ Expressional validation: **qRT-PCR** (PhD S. Castède, 12 flowering CGs)

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Climatic data

Genotype

MODELLING

Phenotype

Ideotypes

MAS

Modelling: from phenology data to MAS

Data

Phenology data

Flowering
Maturity

Climatic data

Temperature

QTLs

Flowering date
Chill requirement
Heat requirement

Molecular data

Candidate genes
Signaling pathways

Models

(1)

Flowering and maturity date
response to temperature
1 cultivar – 1 site

↓ + various sites

(2)

Flowering and maturity date
Wide range of climatic conditions

↓ + cultivars and segregating
populations

(3)

Flowering and maturity date
Model based on mechanisms
Genetic parameters

Outputs

- Analyses of past trends
Temperature / Flowering
- Predictions
→ Flowering and
Maturity dates
- Predictions
→ Effect of genotype
on flowering and
maturity dates
→ G*E interaction

Modelling: from phenology data to MAS

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Phenology data

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Ideotypes construction

Prediction tests

Allele combinations
x
Climatic scenarios
↓
Predicted phenotype



Ideotypes

Ideal alleles combination
for future climatic
conditions



MAS

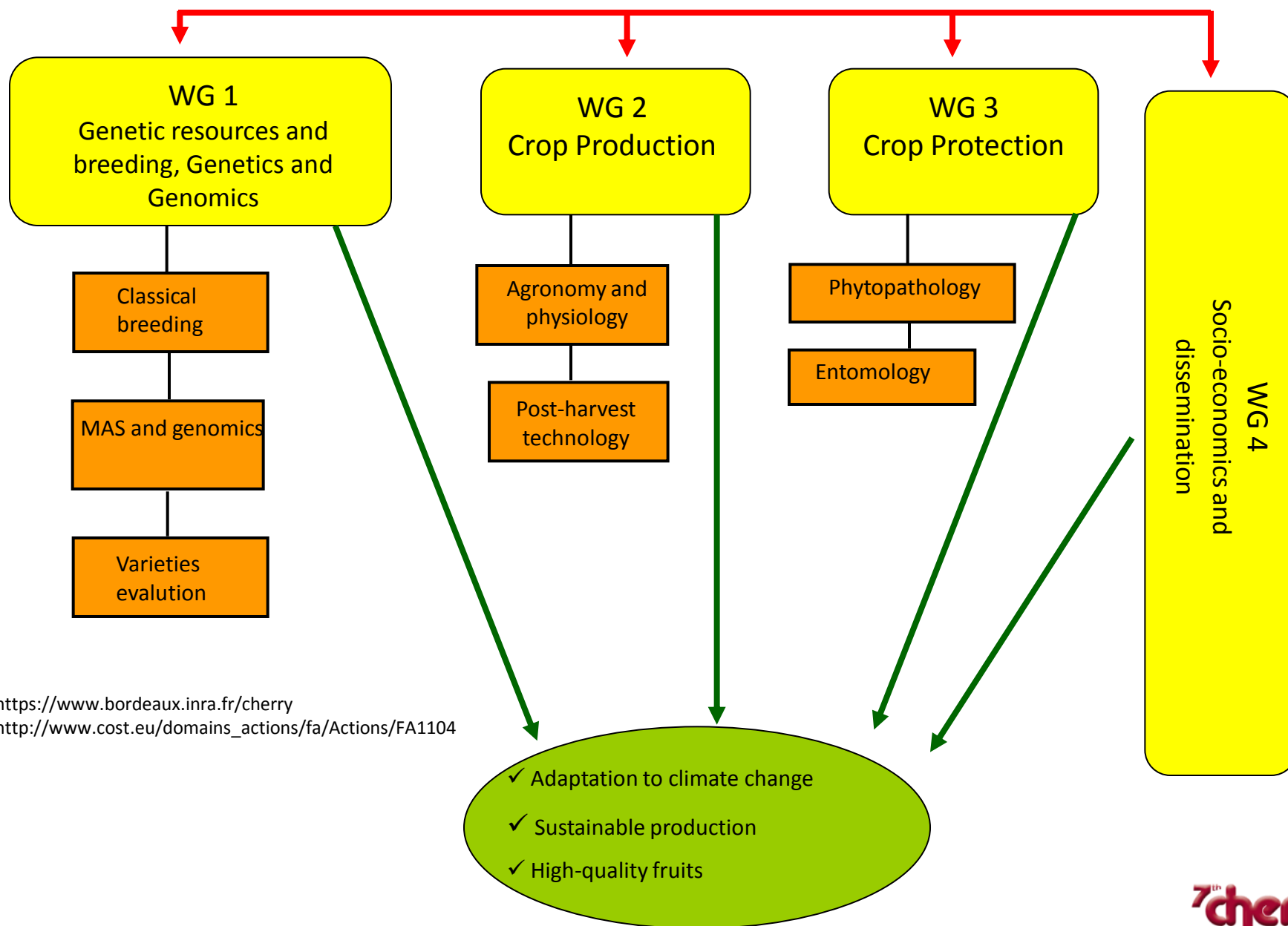
- ❖ Promising for traits with stable and high-effect QTL: flowering and maturity dates, fruit weight and firmness (problem of negative correlations). Still early for very complex traits such as cracking tolerance
- ❖ Trial to validate a multi-trait strategy: select two sets of 150 inds with best and worst allelic combinations and phenotype them (RxG pop)
- ❖ Hybrid production challenge: medium to large sized families, incorporation of genetic diversity
- ❖ Logistic challenge: implement a pipeline. Learn from other experiences (WSU in cherry; other crops, apple, peach...). Which genotyping strategies?

❖ Main objective:

Develop **innovative strategies** to safeguard European cherry production through active networking by:

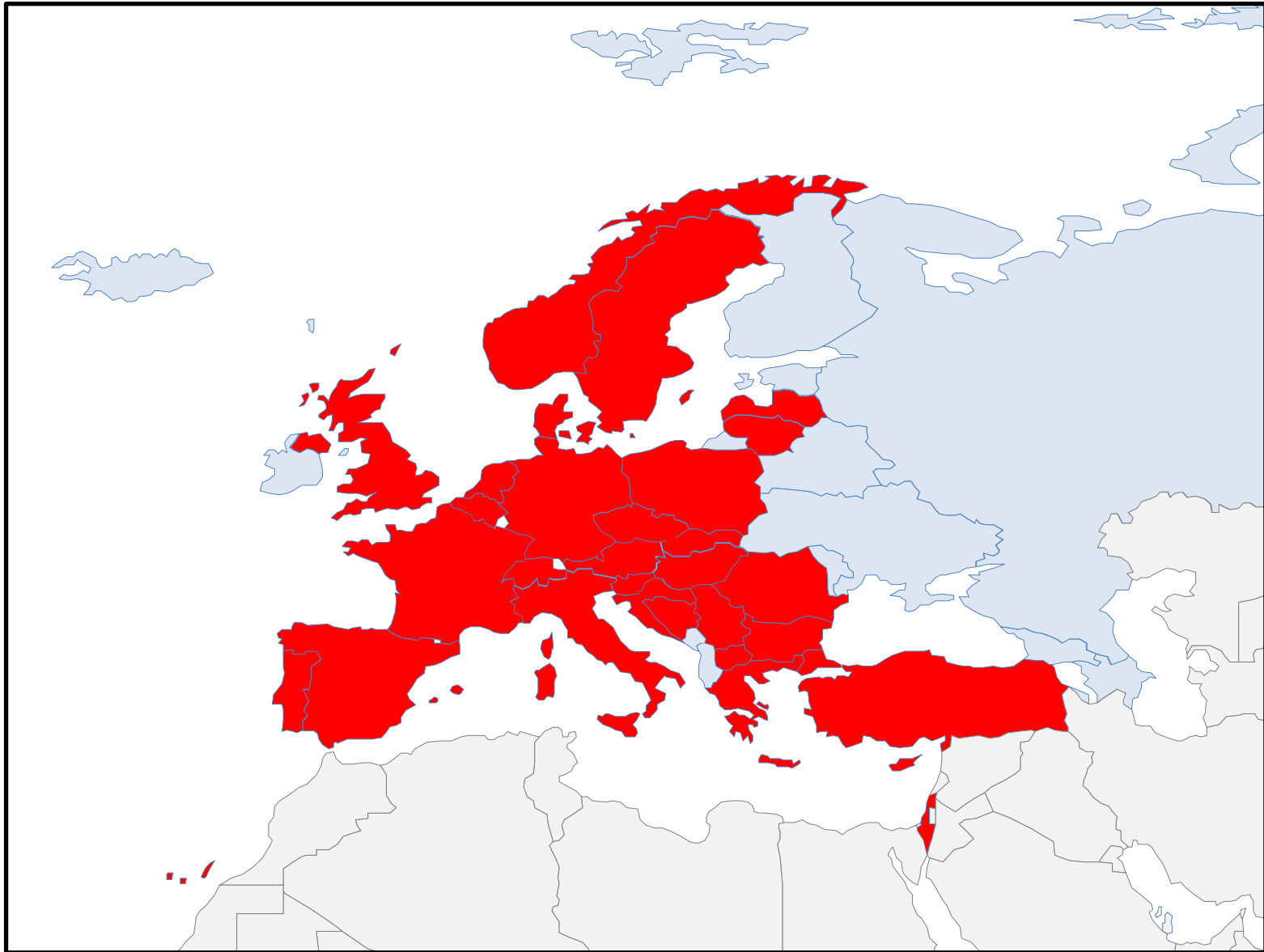
- ✓ The **adaptation** of cherry cultivation to climate change
- ✓ The **implementation** of new cultivation practices aimed at promoting sustainable agriculture
- ✓ The **promotion** of high-quality fruits





<https://www.bordeaux.inra.fr/cherry>

http://www.cost.eu/domains_actions/fa/Actions/FA1104



Acknowledgements



A3C TEAM

Acknowledgements



STUDENTS 2013

**VERY LONG LIST OF NATIONAL
AND INTERNATIONAL
COLLABORATORS!!!!!!**

THANKS FOR YOUR ATTENTION !!!!!

TIME FOR QUESTIONS!!

