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

José Quero-Garcia, José Antonio Campoy, Sophie Castede, Teresa Barreneche, Loick Le Dantec, Bénédicte B. Wenden, Jacques Joly, Lydie Fouilhaux, Elisabeth Dirlewanger

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

José Quero García (INRA – Bordeaux)

VII International Cherry Symposium – 23-27 June 2013, Plasencia, Spain

Plan

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

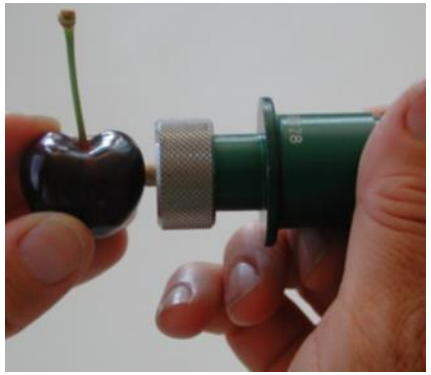
Second breeding programme (1980-2005)

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



Second breeding programme (1980-2005)

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

Second breeding programme (1980-2005)

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

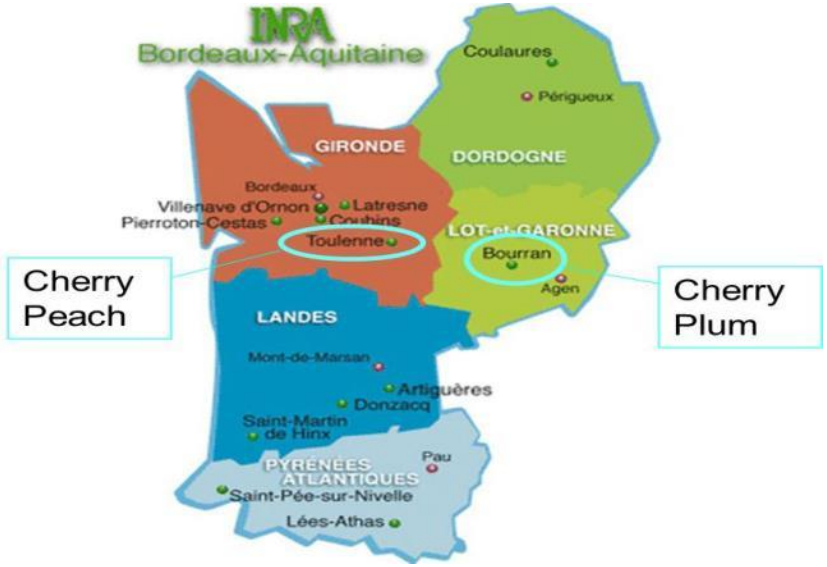
Transition (2000-2005)

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

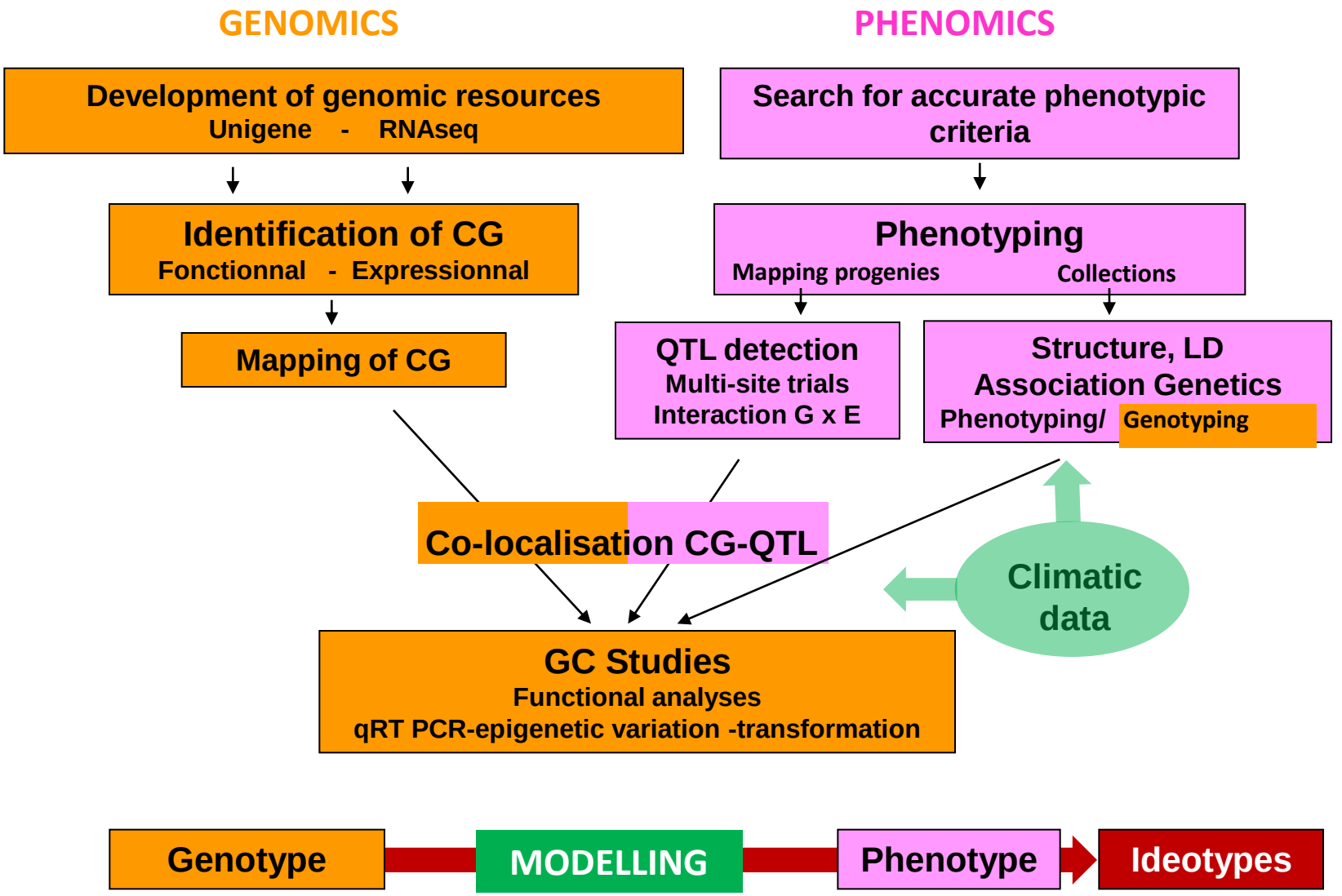
- Large sample of mazzards and sweet cherry varieties
- Important genetic differentiation between wild materials from West Europe, Romania and Georgia
- Insufficient sample to determine the centers of domestication of sweet cherry. Proximity between wild and cultivated materials due to either gene flow 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 : Toulenne and Bourran
- A3C: 5 researchers, 5 technicians, 1 PhD student, 1 post-doc



Scientific project



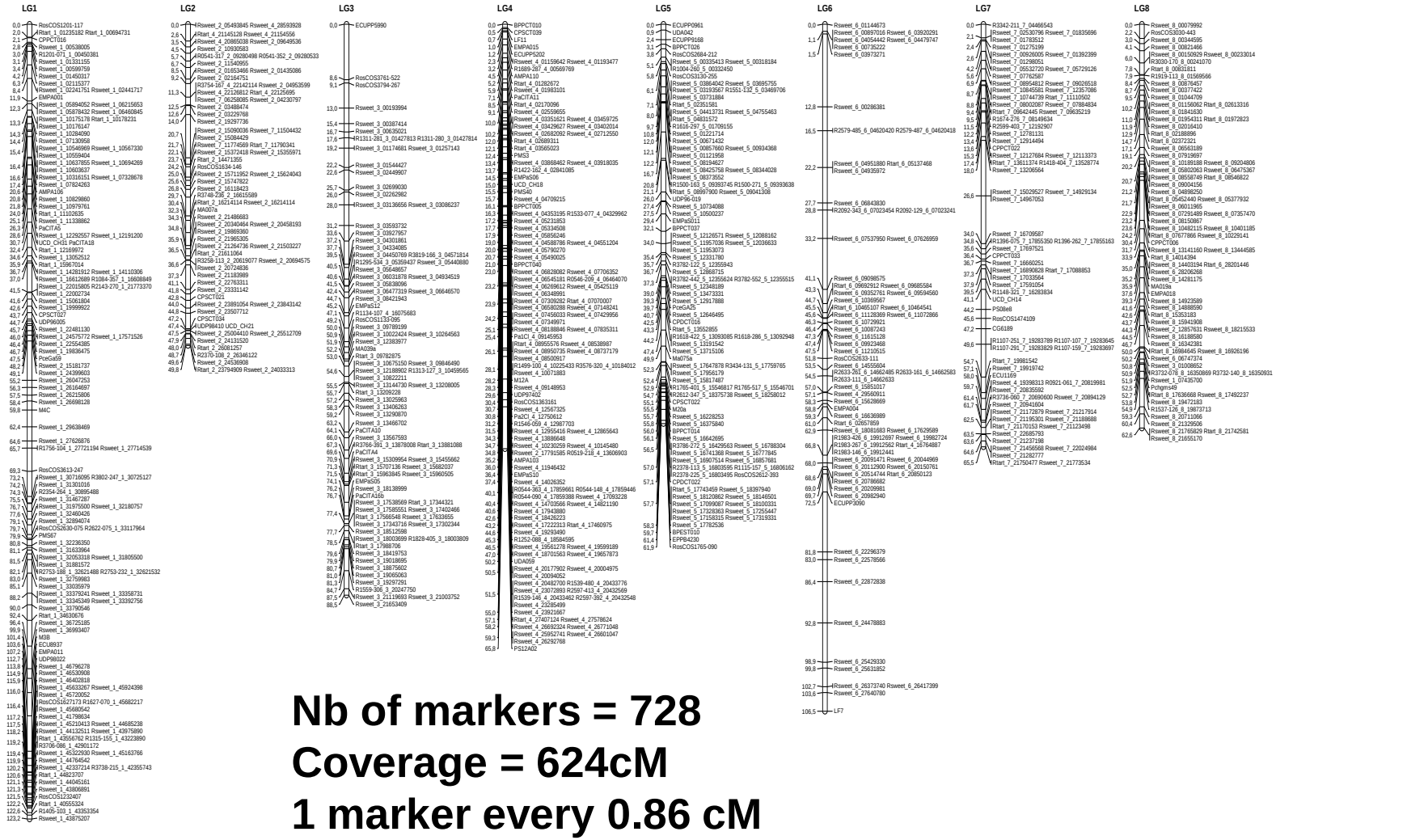
Breeding programme

- **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**

Marker-assisted selection

- **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)

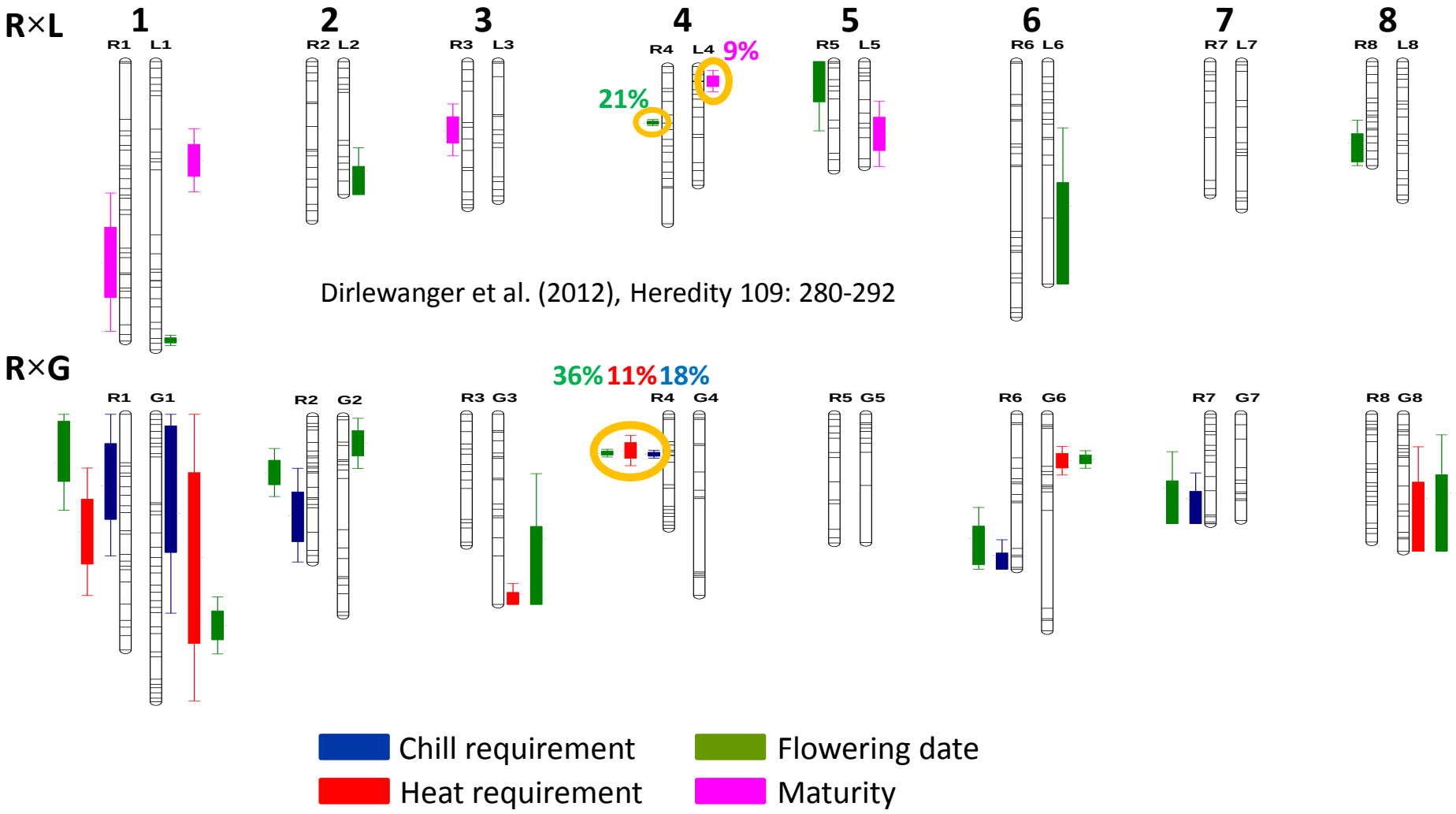
Genetic mapping



Nb of markers = 728
Coverage = 624cM
1 marker every 0.86 cM

Campoy et al. (2013): PlosONE

QTL detection analyses- Phenology traits

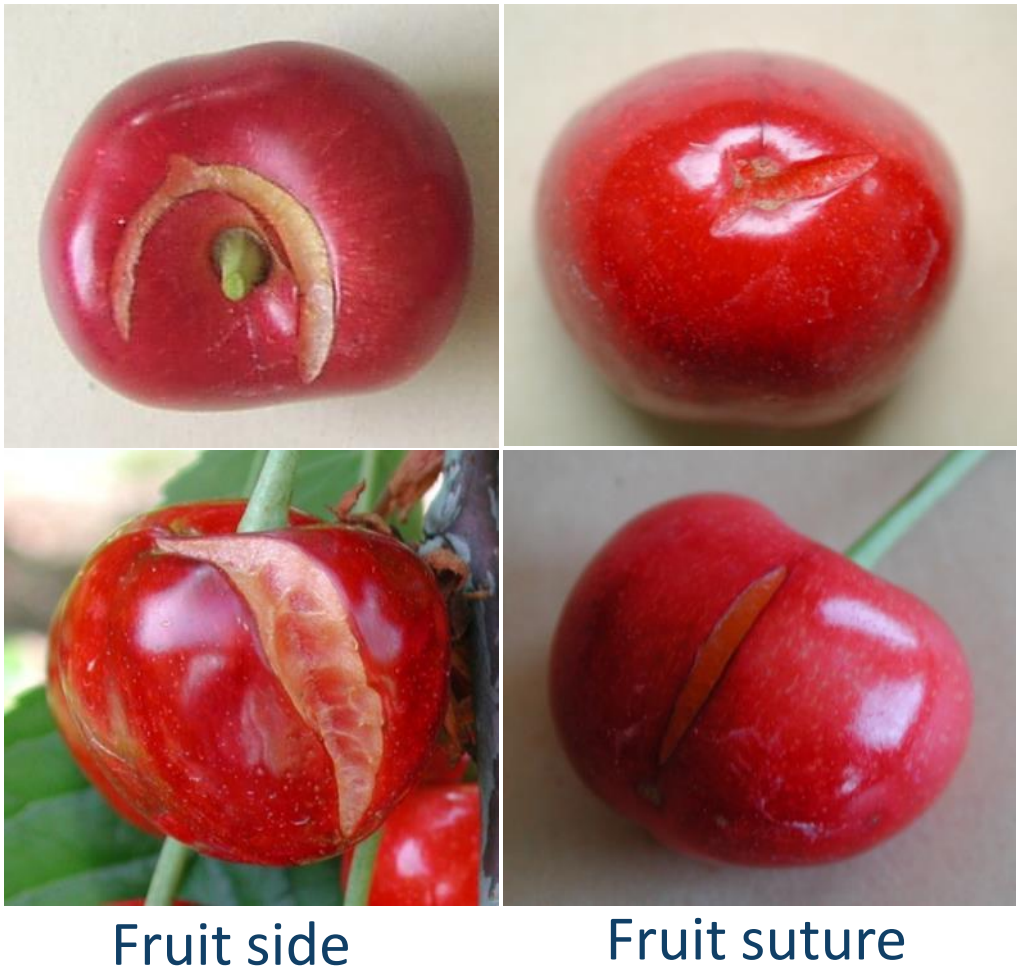


☞ **Poster P23– Castède *et al*.**

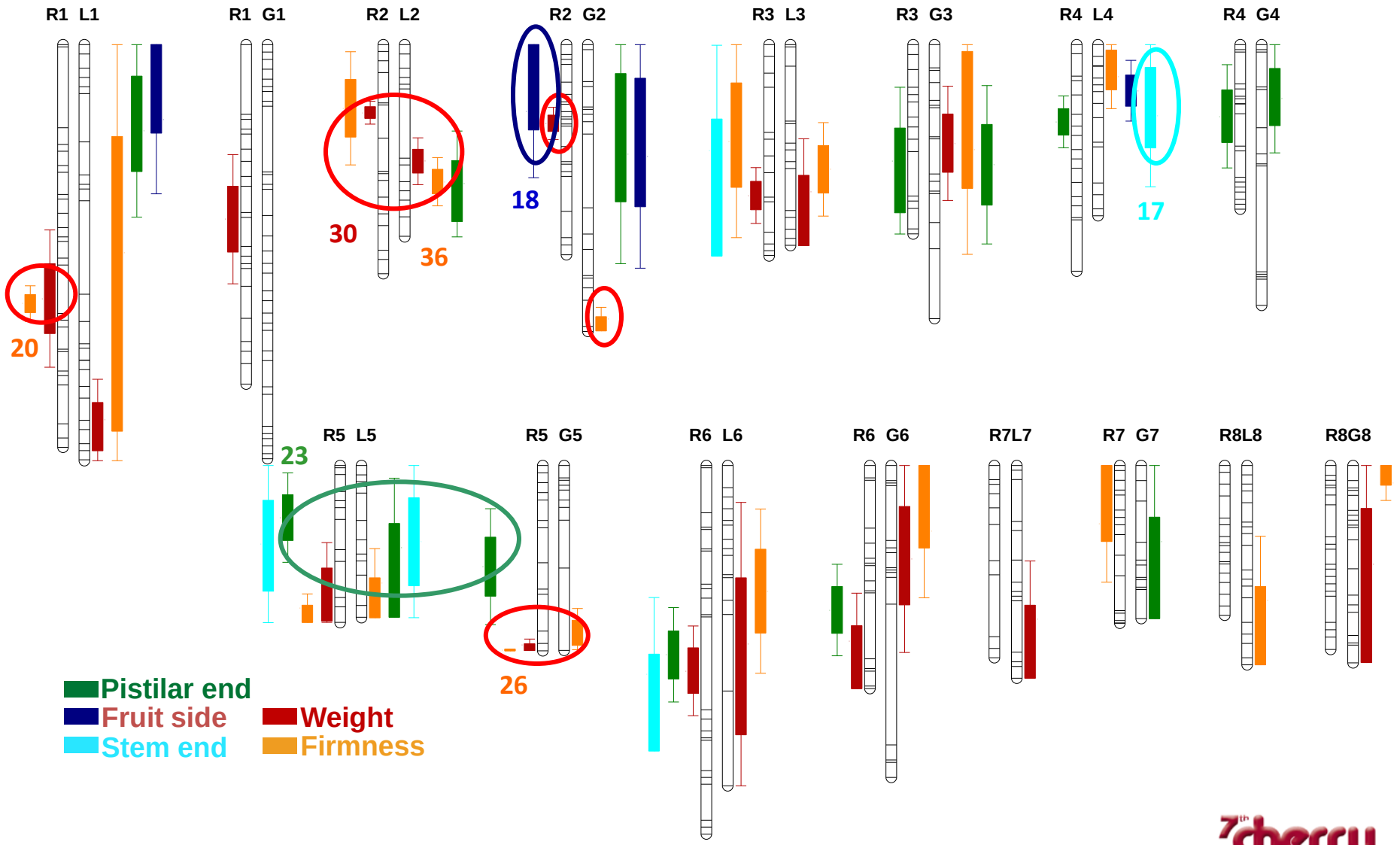
Cracking tolerance evaluation

- **Field** : 100 fruits at maturity → percentage
- **Tunnel** : 100 fruits, counting during 5 days → percentage

4 types analyzed



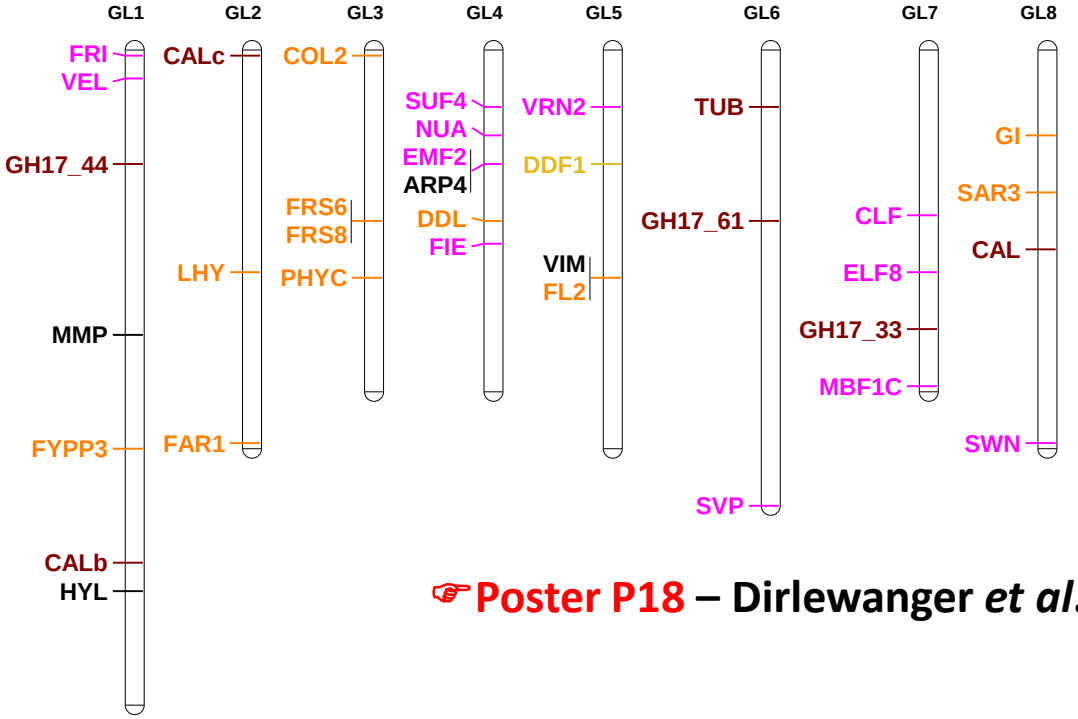
QTL detection analyses- Fruit quality traits



Dirlewanger et al. (2012), RGC6

Candidate Genes (CGs)

❖ **Flowering**



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

❖ **Fruit weight: Collaboration with Amy Iezzoni'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**

QTL detection analyses - Perspectives

- **Multi-parental analyses (new QTLs; QTL stability; no need to produce large progenies)**
  - ✓ **Collaboration with Amy Iezzoni'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)

QTL detection analyses - Perspectives

❖ **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 analyses - Perspectives

❖ 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

K=3

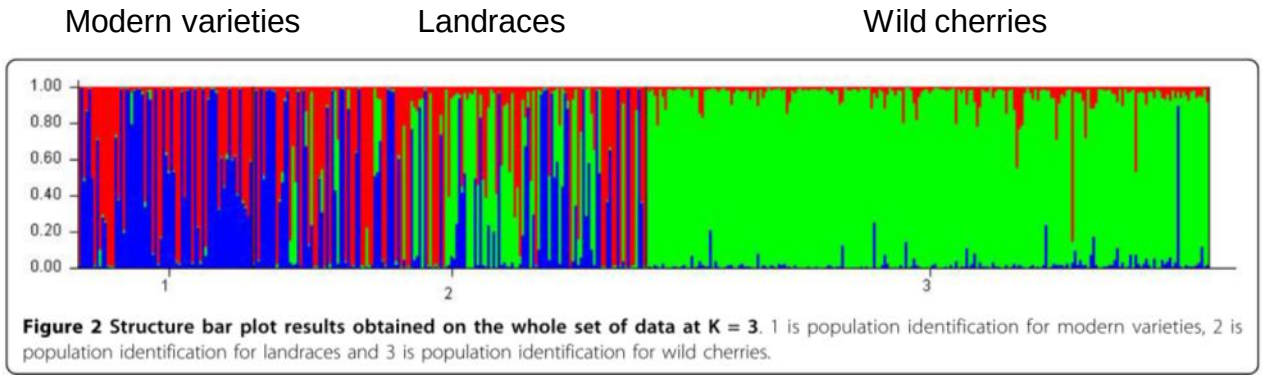
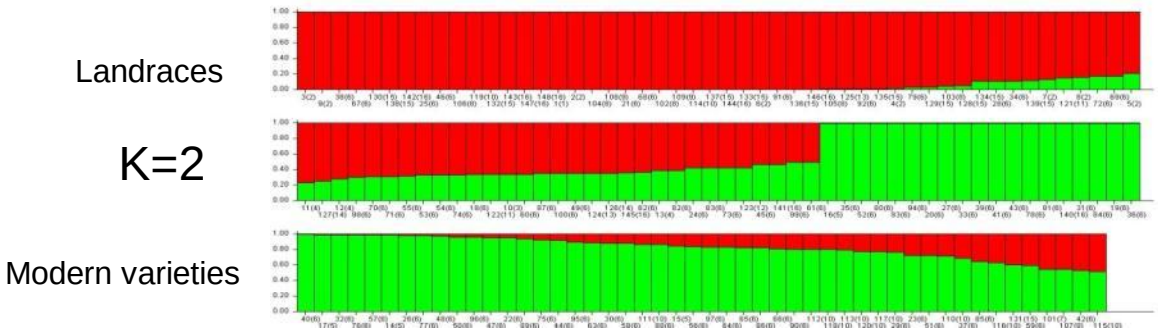


Figure 2 Structure bar plot results obtained on the whole set of data at K = 3. 1 is population identification for modern varieties, 2 is population identification for landraces and 3 is population identification for wild cherries.

✓ 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).

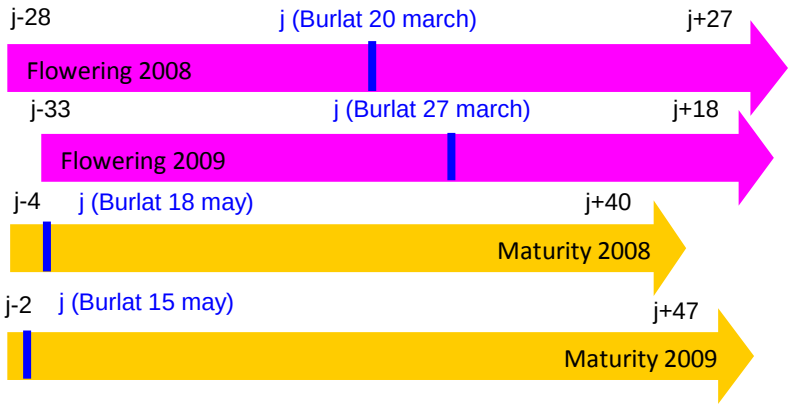


✓ Genetic resources collection genotyped with a subset of 41 CGs SNPs

QTL/CG analyses - Perspectives

❖ 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



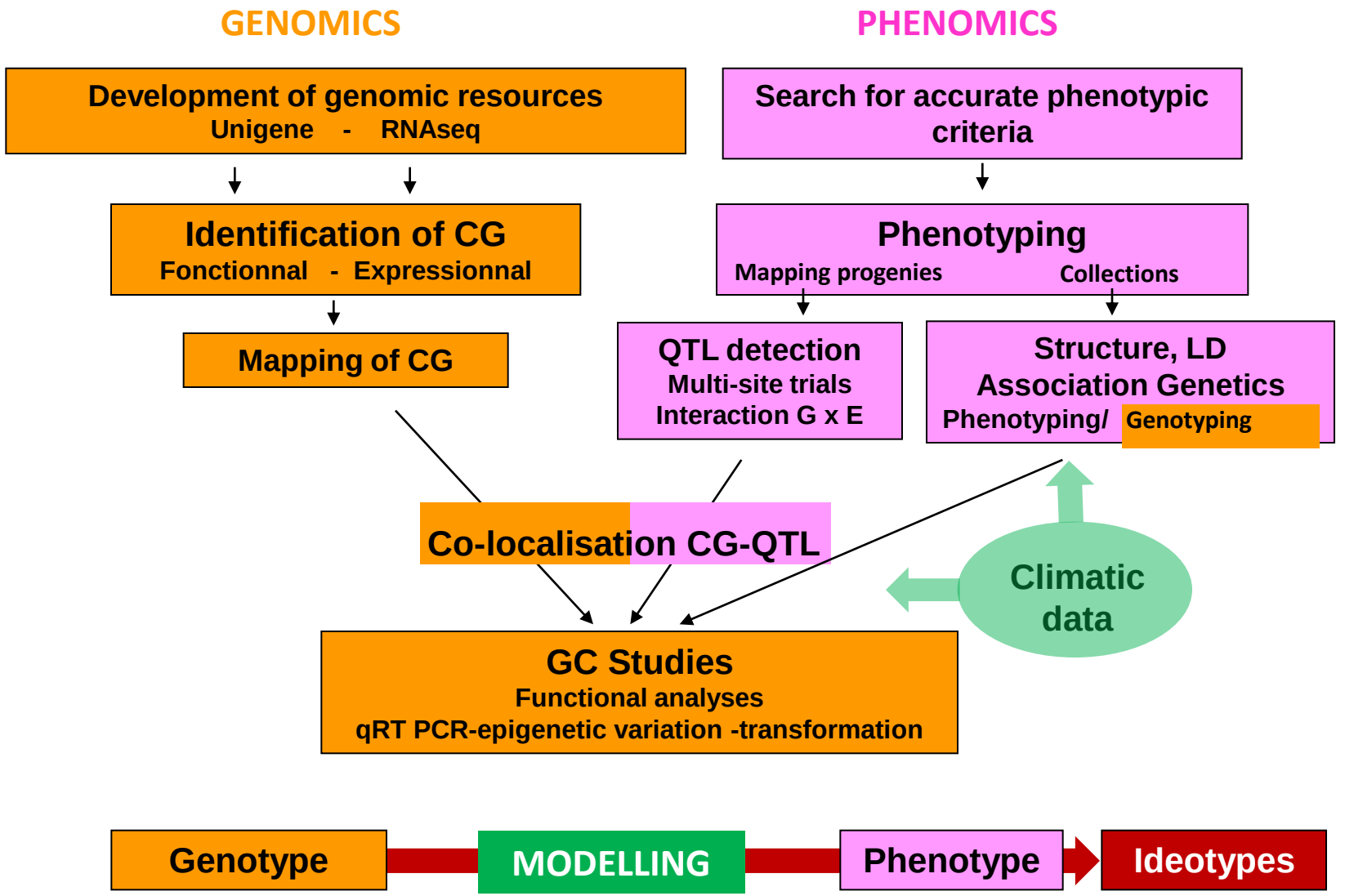
Flowering and maturity range of the collection
2 years 380 accessions

Large variability for flowering and maturity dates

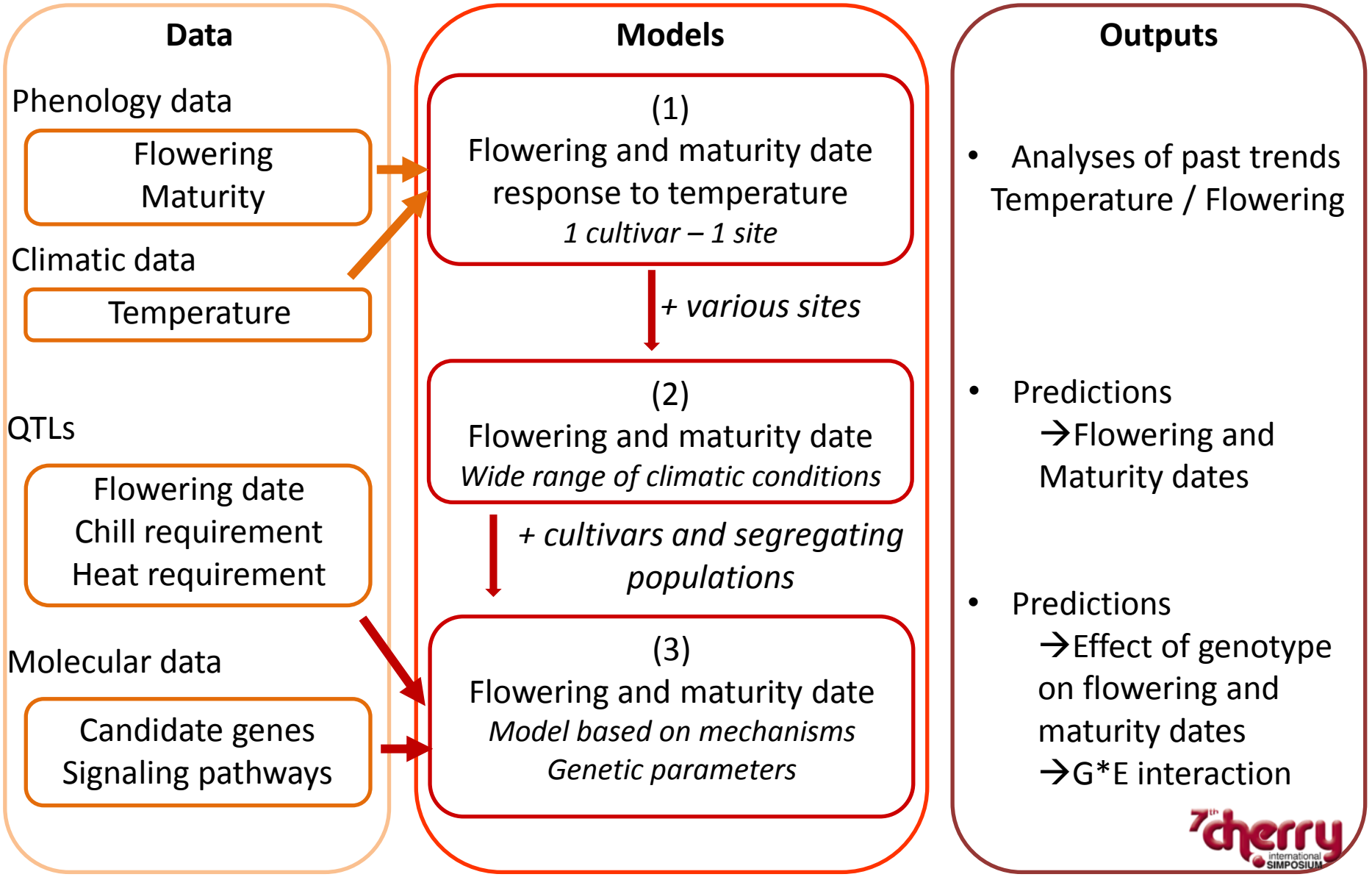
CG analyses - Perspectives

- **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)**

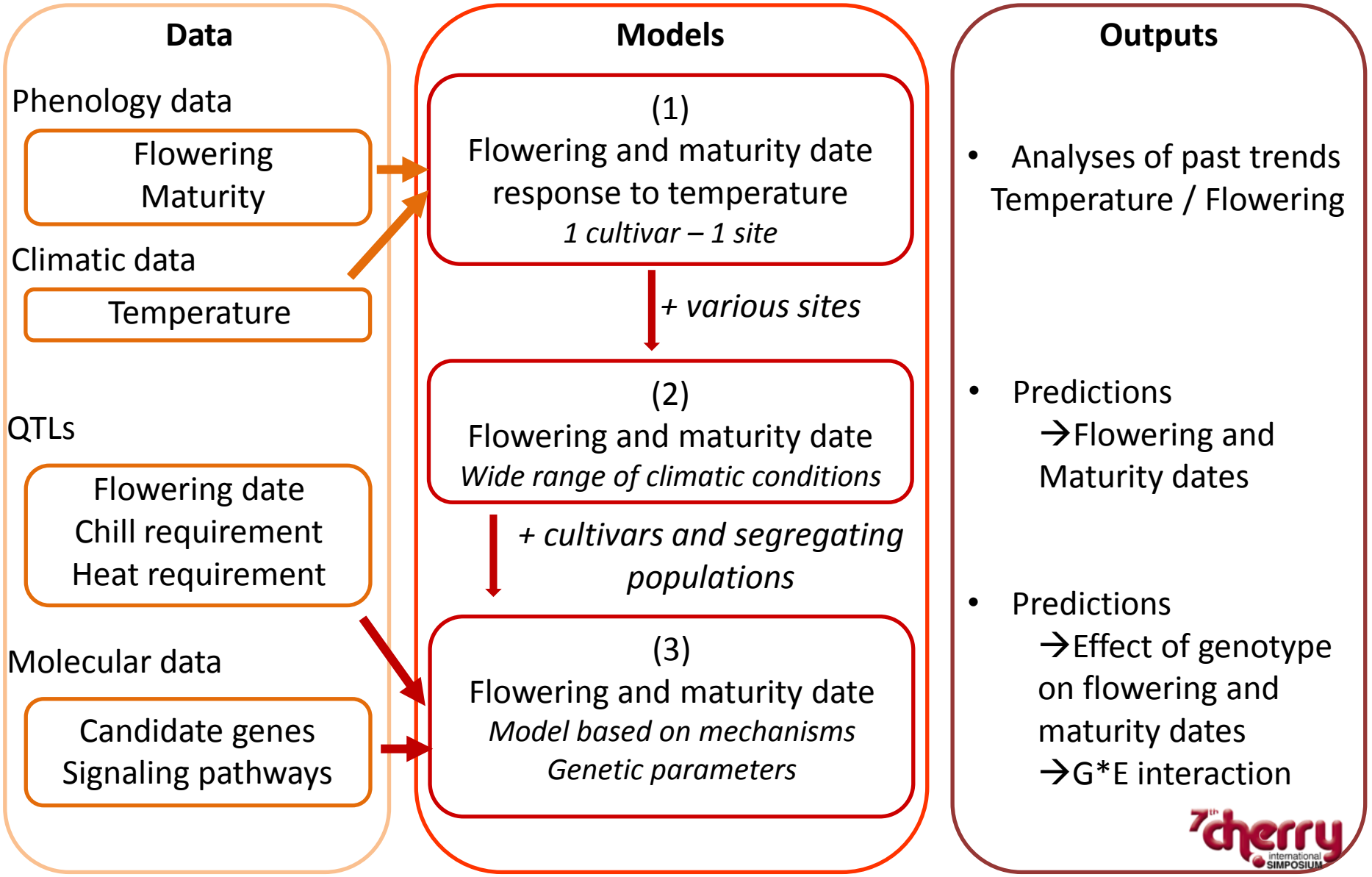
Scientific project



Modelling: from phenology data to MAS



Modelling: from phenology data to MAS



Modelling: from phenology data to MAS

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

Ideotypes construction

Prediction tests

Allele combinations
x
Climatic scenarios

Predicted phenotype

Ideotypes
Ideal alleles combination for future climatic conditions

MAS

MAS strategy - Conclusions

- 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?

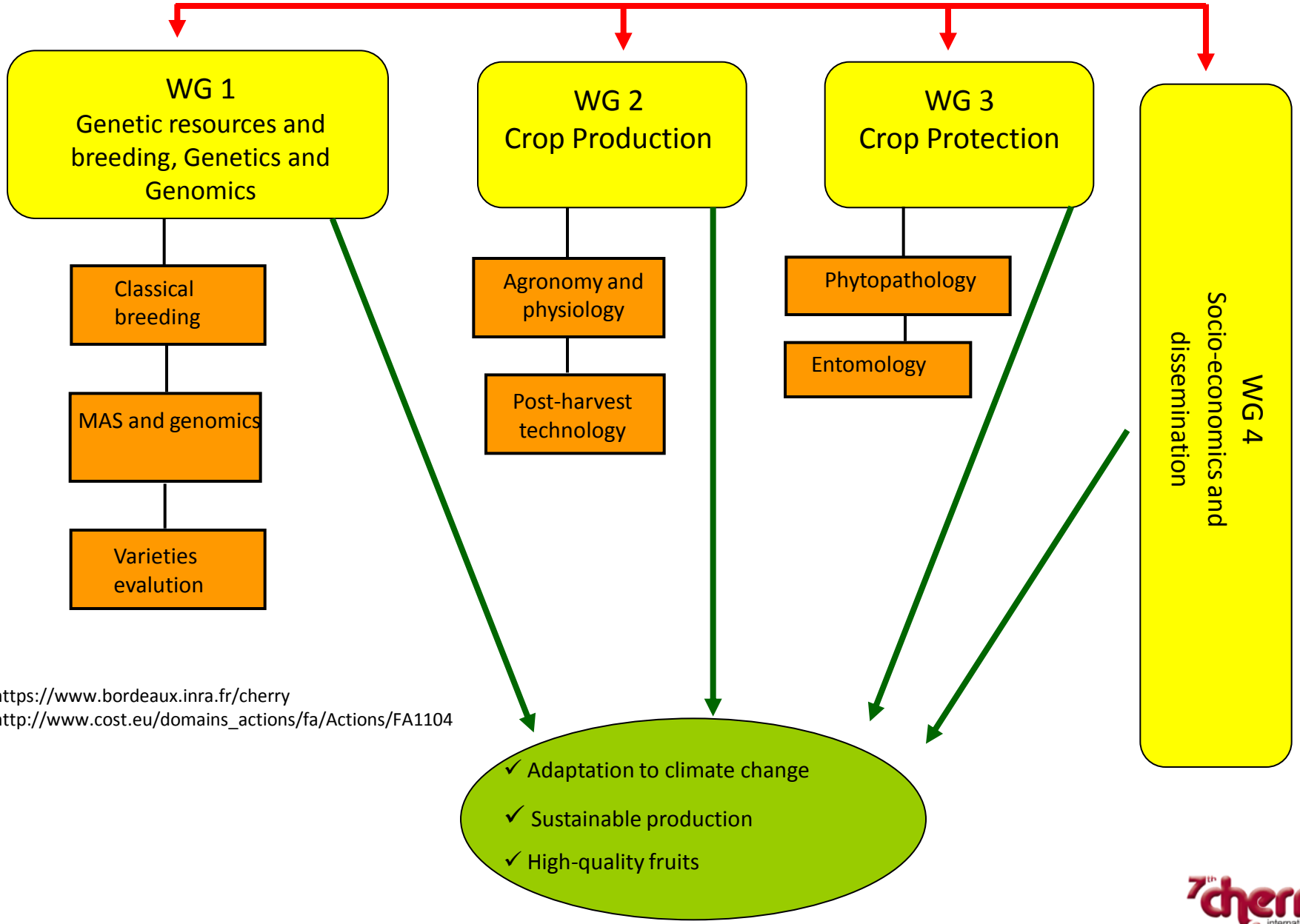
COST Action FA1104 'Sustainable production of high-quality cherries for the European market'

❖ **Main objective:**

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

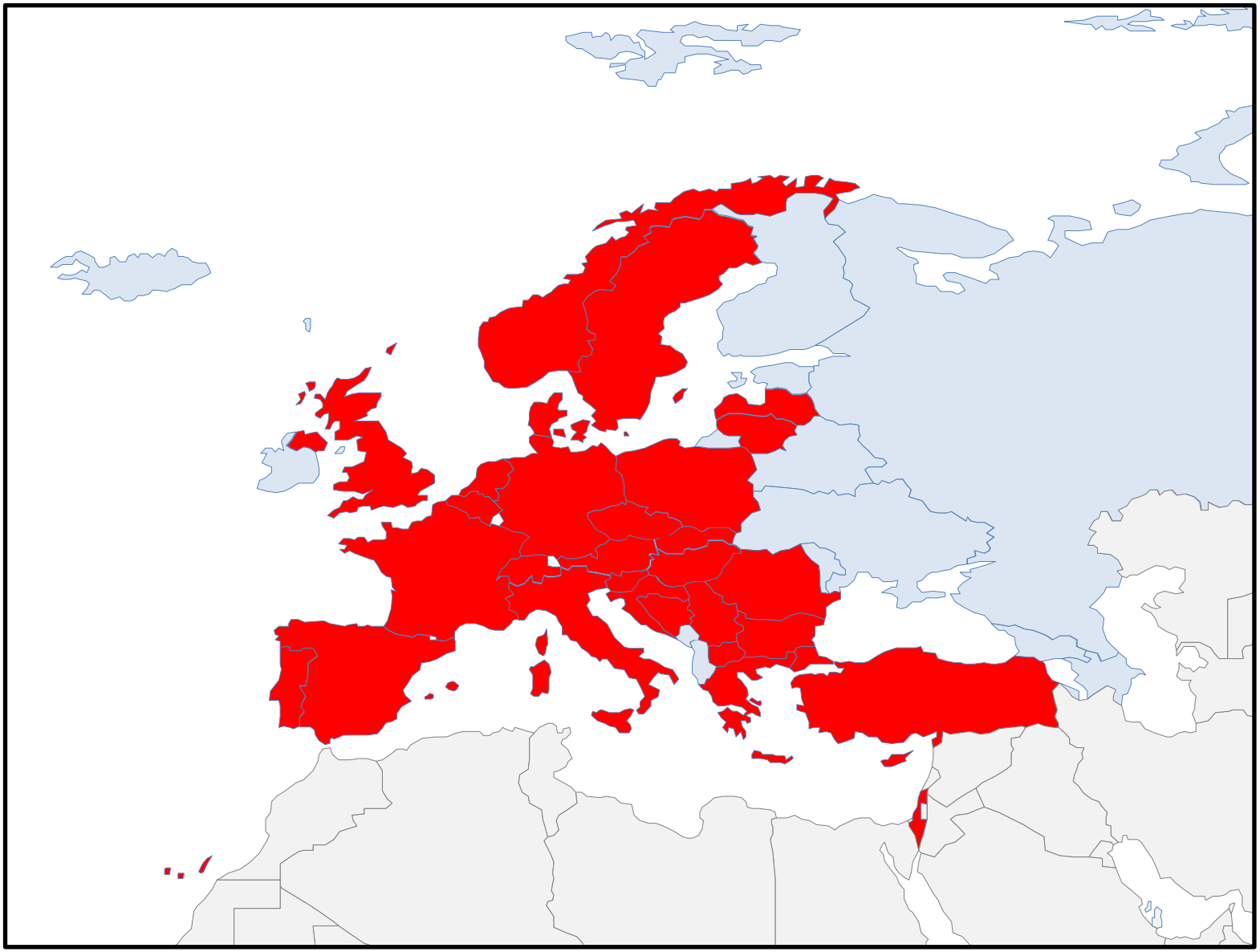
- ✓**The adaptation of cherry cultivation to climate change**
- ✓**The implementation of new cultivation practices aimed at promoting sustainable agriculture**
- ✓**The promotion of high-quality fruits**

COST FA1104



https://www.bordeaux.inra.fr/cherry
http://www.cost.eu/domains_actions/fa/Actions/FA1104

COST FA1104



Acknowledgements

A3C TEAM

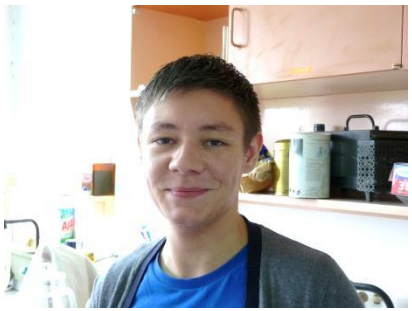
Acknowledgements









STUDENTS 2013

Acknowledgements

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

Acknowledgements

THANKS FOR YOUR ATTENTION !!!!!

TIME FOR QUESTIONS!!