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Association and Linkage Mapping of Walnut (*Juglans regia* L.) Phenological Traits

Anthony Bernard, Annarita Marrano, Armel Donkpegan, Patrick J Brown, Charles A Leslie, David B Neale, Fabrice Lheureux, Elisabeth Dirlewanger

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INRAE

la science pour la vie, l'humain, la terre

université
de **BORDEAUX**

INTERNATIONAL PLANT &
ANIMAL GENOME XXVIII
JANUARY 11-15, 2020
SAN DIEGO, CA, USA

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Armel Donkpegan

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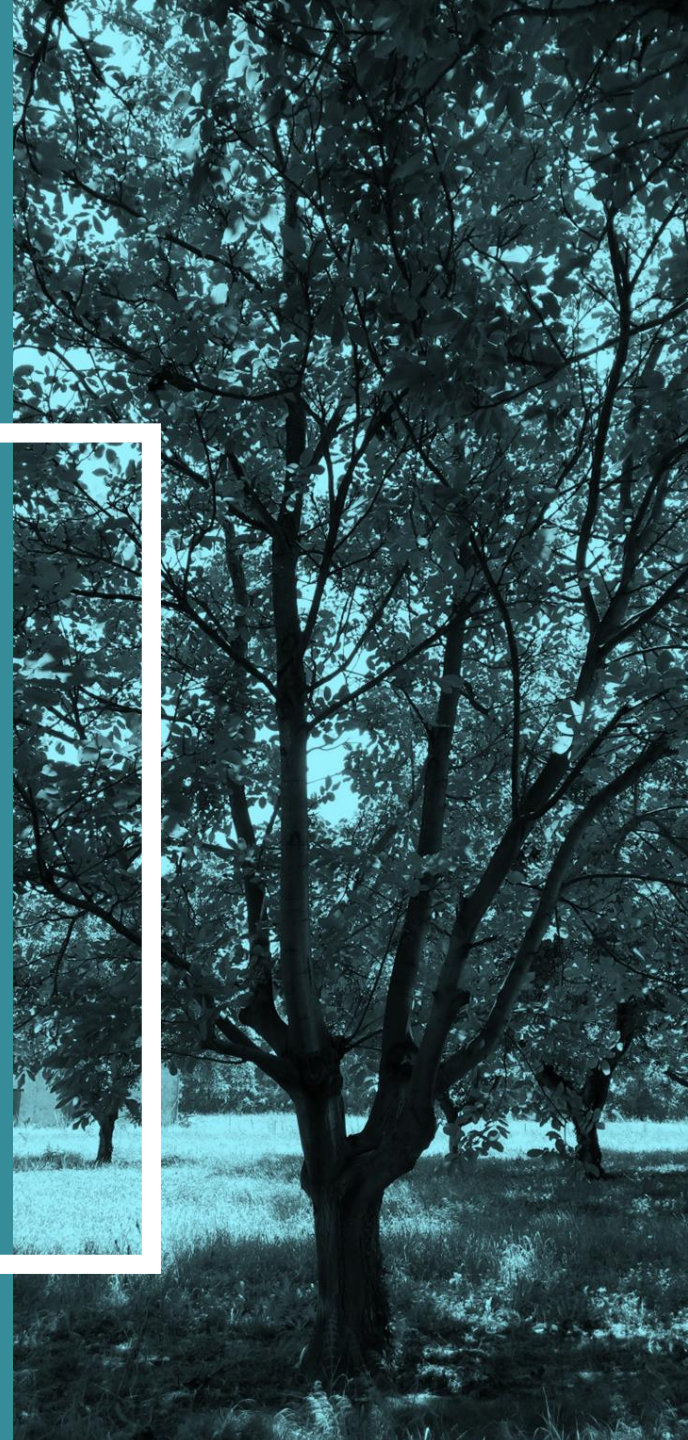
Charles A. Leslie

David B. Neale

Fabrice Lheureux

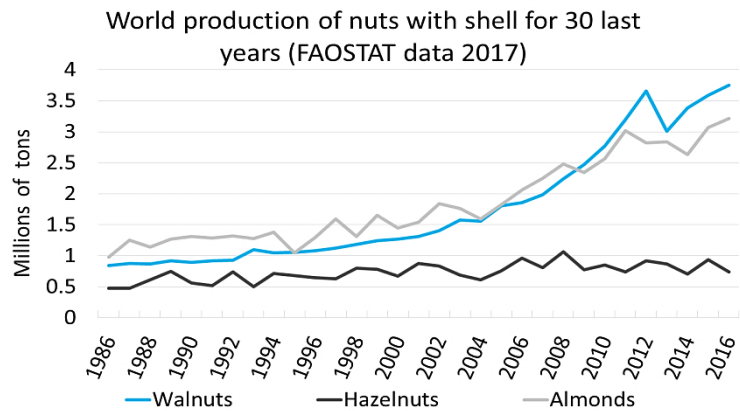
Elisabeth Dirlewanger

Association and Linkage Mapping of Walnut (*Juglans regia* L.) Phenological Traits



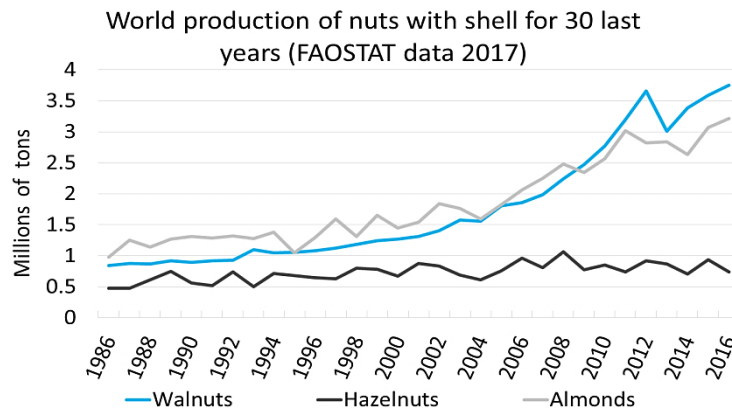
1

The world
in-shell
walnut
production
is increasing



1

The world
in-shell
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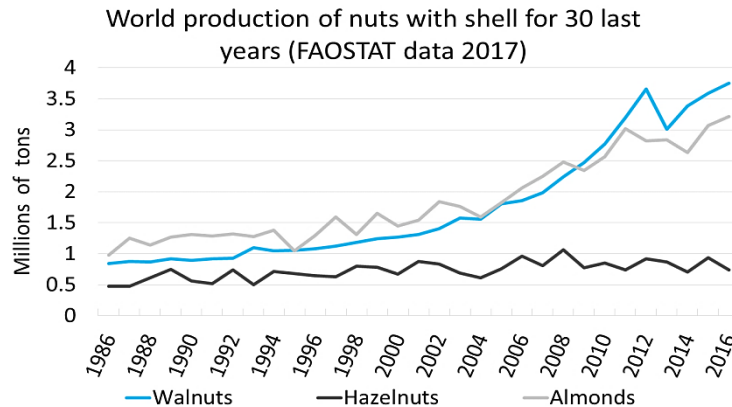


2

China > California > Iran
France: 7th with 40,000 tons
Area of orchards: 2nd French fruit crop

1

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2

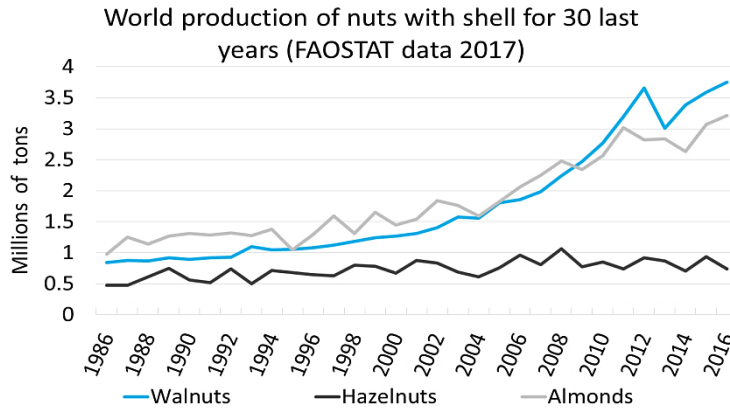
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3

Walnut breeding goals in France:
increased yield, larger nut size, ease of
cracking, adaptation to climatic
conditions (late spring frosts)
→ phenology

1

The world in-shell walnut production is increasing



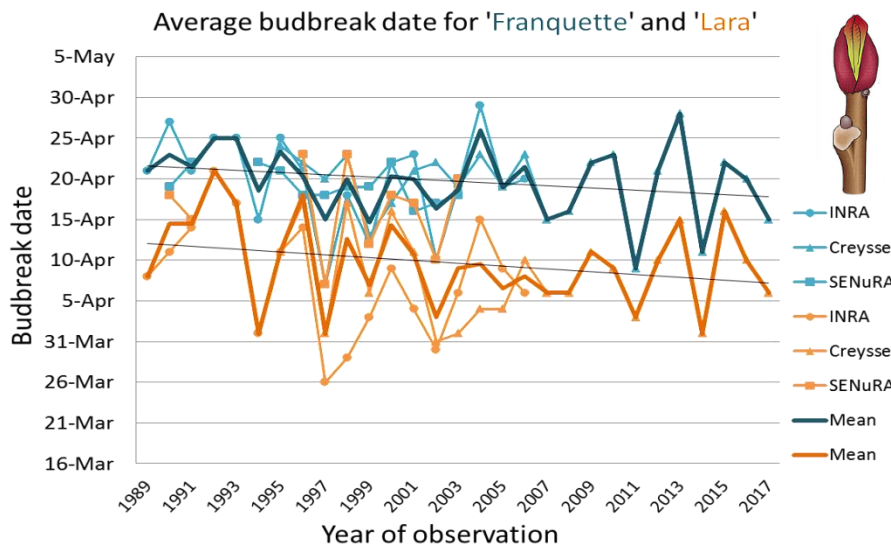
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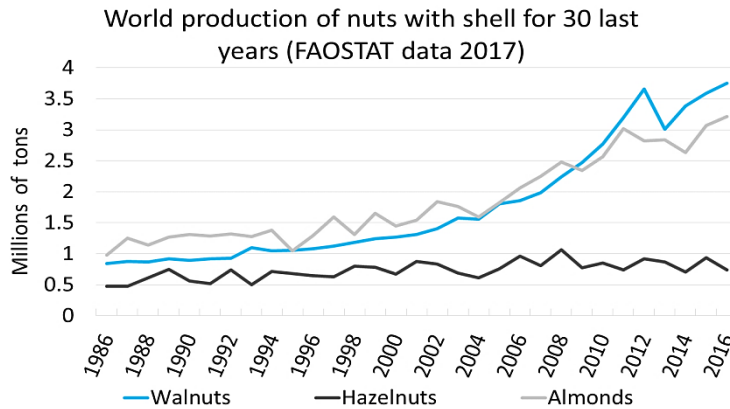
4



Effect of climate change, but breeding possible since phenology-related traits are also controlled by genetic background

1

The world in-shell walnut production is increasing



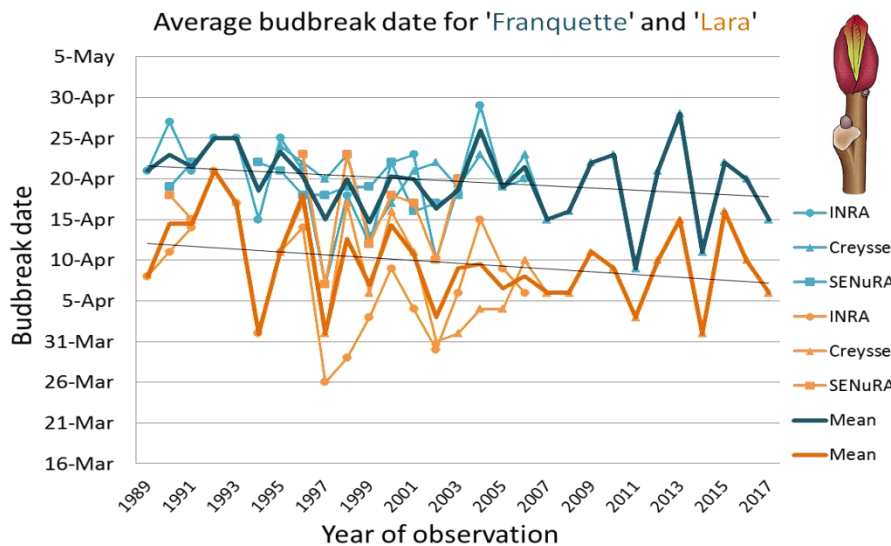
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 → phenology

4



Effect of climate change, but breeding possible since phenology-related traits are also controlled by genetic background

5

Axiom™ *J. regia* 700K SNP array (Marrano *et al.*, 2019)
 GWAS on walnut: in-shell walnut and kernel traits (Arab *et al.*, 2019), water use efficiency (Famula *et al.*, 2019), and yield, lateral bearing, pellicle color, leafing date and harvest date (Marrano *et al.*, 2019)

1

Goals: study of *Juglans regia* genetic resources for the implementation of a marker-assisted selection

- basic research: genetic diversity and structure evaluation of INRA germplasm repository (Bernard *et al.*, 2018), and genetic architecture deciphering of main traits of interest
- applied research: establishment of necessary tools for marker-assisted selection

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2

Action plan:

- phenotyping: 2017, 2018, 2019, 2020 for many traits related to phenology, nut in-shell and kernel
- genotyping: using SSRs and Axiom™ *J. regia* 700K SNP array
- plant material: F₁ progeny (78 individuals) segregating for phenology + unique genetic resources core-collection (170 accessions)
- GWAS, combined with QTLs detection for phenology
- marker validation on other plant material
- choice of genitors



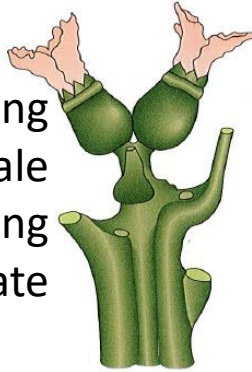
1

Budbreak
date

(leaves and
female
flowering)



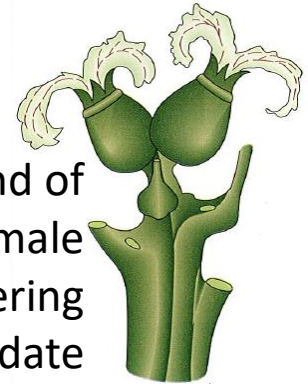
Beginning
of female
flowering
date



Full female
flowering
date



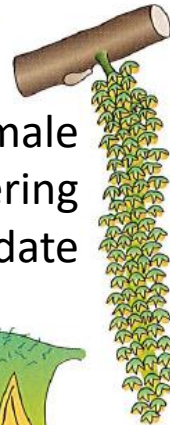
End of
female
flowering
date



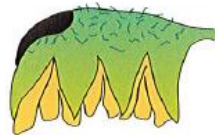
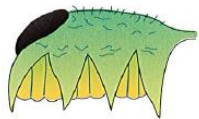
Beginning
of male
flowering
date



Full male
flowering
date



End of
male
flowering
date



→ 2018 data

→ 2019 data

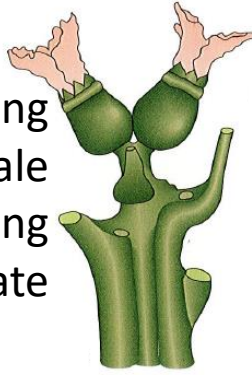
1

Budbreak
date

(leaves and
female
flowering)



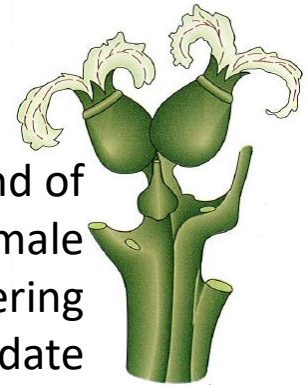
Beginning
of female
flowering
date



Full female
flowering
date



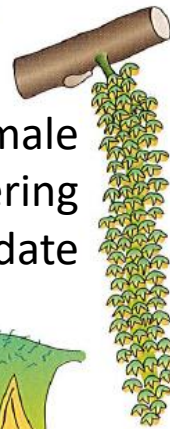
End of
female
flowering
date



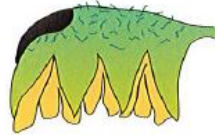
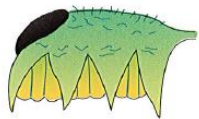
Beginning
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flowering
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Full male
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date



End of
male
flowering
date



→ 2018 data
→ 2019 data

2

Best Linear Unbiased Predictions (BLUPs) → $P_{ik} = \mu + Y_i + g_k + e_{ik}$

P_{ik} - observed phenotype of the k^{th} accession in the i^{th} year;

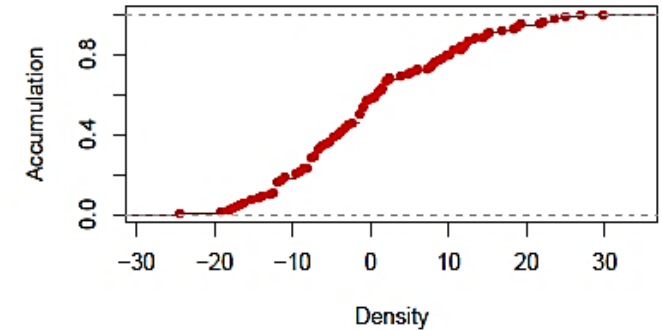
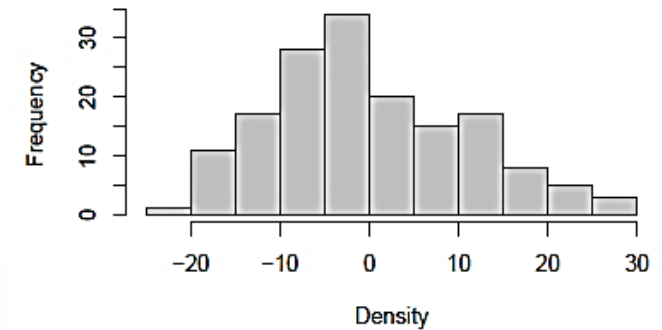
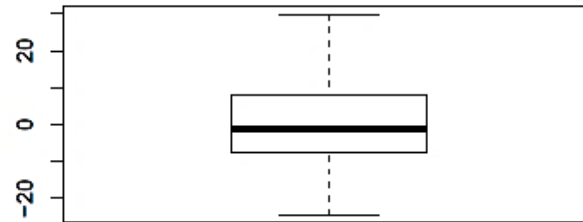
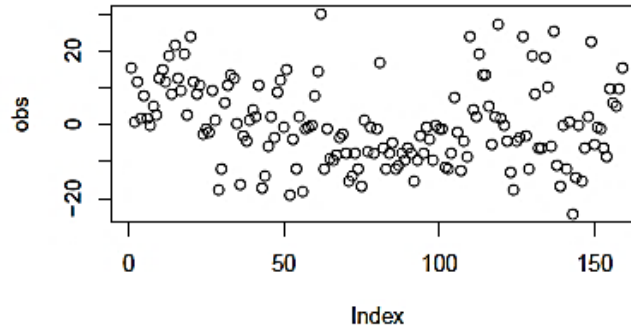
μ - mean value of the trait; Y_i - fixed effect of the i^{th} year;

g_k - random effect of the k genotype; and $e_{i(j)k}$ - residuals of the model

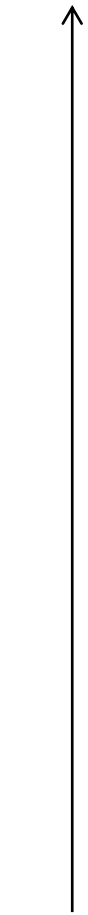
→ lme4

1

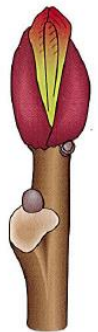
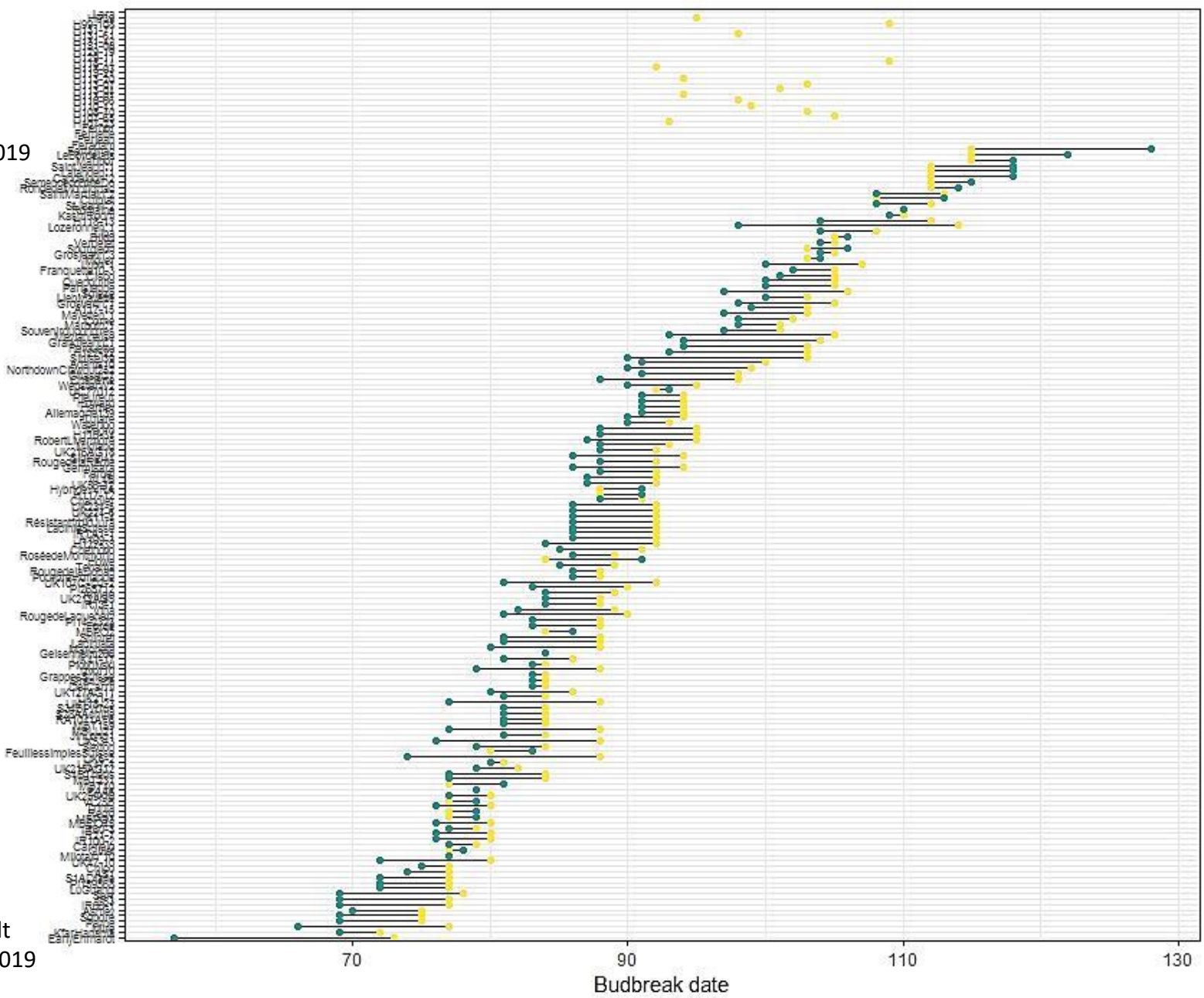
By calculating the BLUPs, budbreak date within the GWAS panel is considered as normally distributed



Fertignac
May 9th in 2019



Early Ehrhardt
Feb 27th in 2019

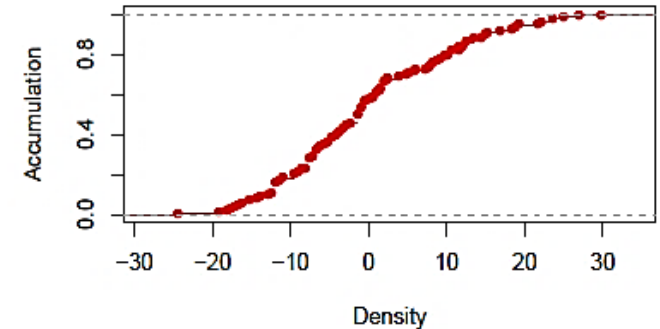
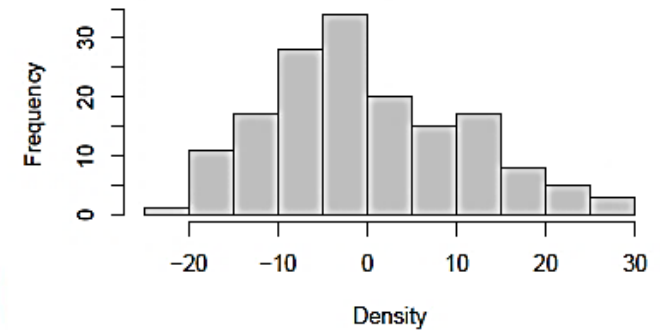
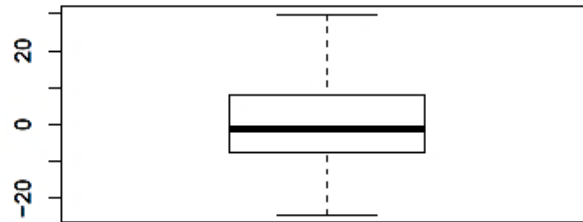
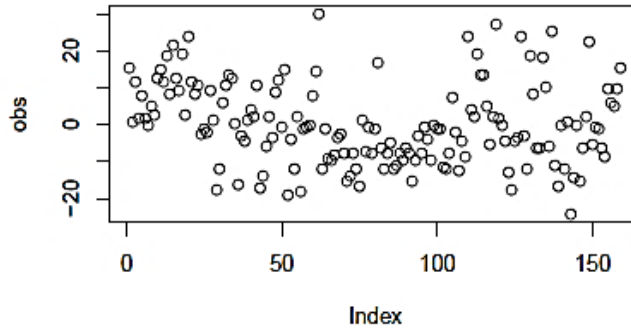


Year

- 2018
- 2019

1

By calculating the BLUPs, budbreak date within the GWAS panel is considered as normally distributed



2

Broad-sense heritability $\rightarrow H^2 = \sigma_G^2 / [(\sigma_G^2 + (\sigma_\varepsilon^2 / n_{\text{obs/g}}))]$
 where σ_G^2 - genotypic effect variance;
 σ_ε^2 - variance of residuals;
 and $n_{\text{obs/g}}$ - number of observations by genotype

$\rightarrow 0.93$

Step 1. To keep SNPs of high resolution from Axiom® Analysis Suite*Conversion Type*

PolyHighResolution

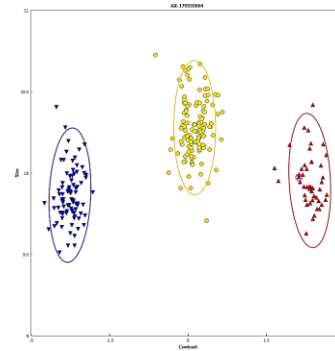
NoMinorHom

MonoHighResolution

CallRateBelowThreshold

OffTargetVariant

Other

Total of retained SNPs*Number of markers*397,921^a75,564^a36,684^a

27,761

4,787

66,941

510,169

Percentage of markers

65,27

12,39

6,02

4,55

0,79

10,98

Step 2. To keep SNPs with mendelian inheritance using F₁ progeny

SNPs having no mendelian inheritance

661

Total of retained SNPs

509,508

Step 3. To keep SNPs having genotyping rate >90%

SNPs having genotyping rate <90%

13,993

Total of retained SNPs

495,515

Step 4. To keep SNPs having minor allele frequency >5%

SNPs having minor allele frequency <5%

123,751

Total of retained SNPs

371,764

Step 5. To delete duplicated SNPs

Duplicated SNPs

7,489

Total of retained SNPs

364,275

^a SNPs of high resolution

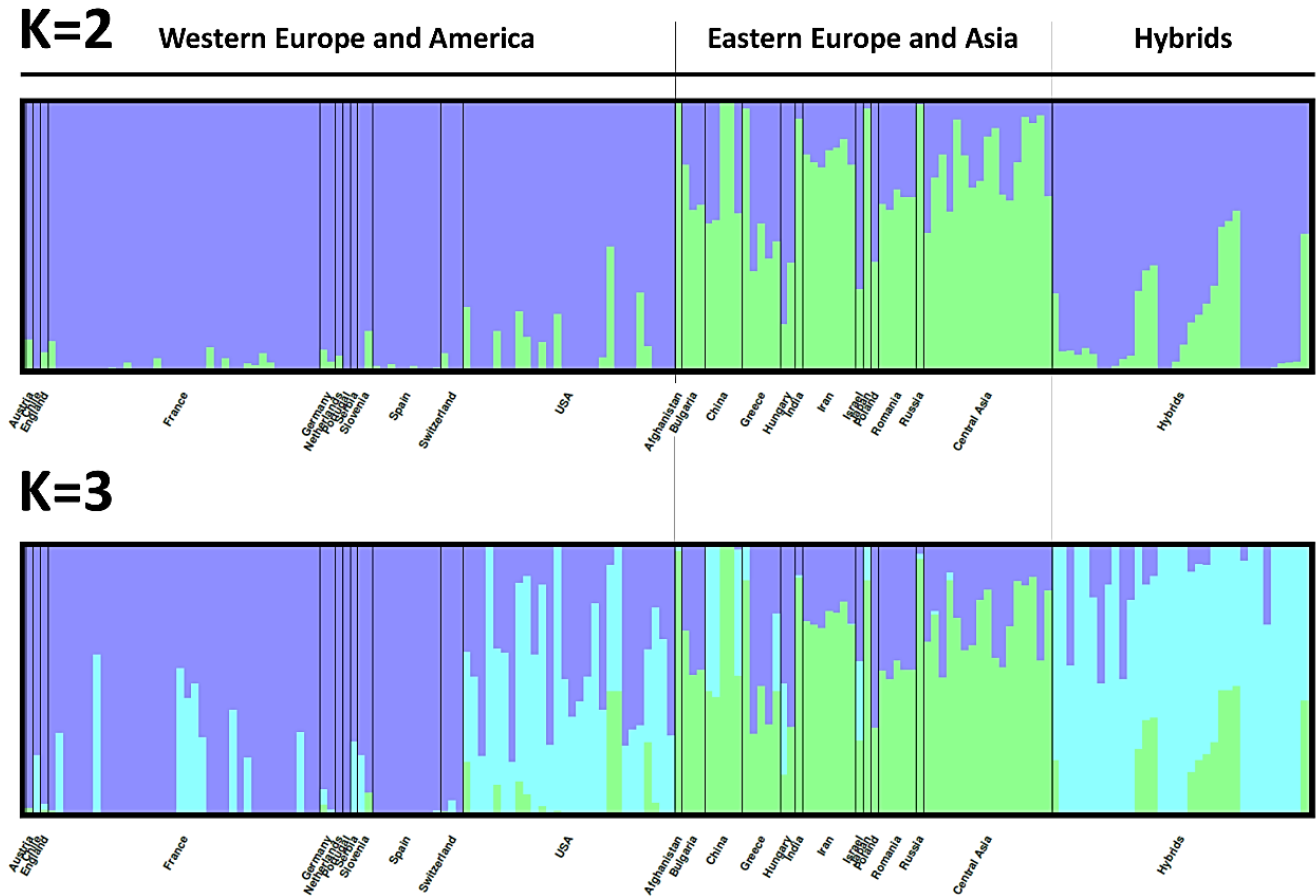
609,658 → 364,275 = 59.8%

1

Structure analysis shows clustering according to geographical origin

K2: one group with accessions from Western Europe and America, other with accessions from Eastern Europe and Asia

K3: highlights hybrids

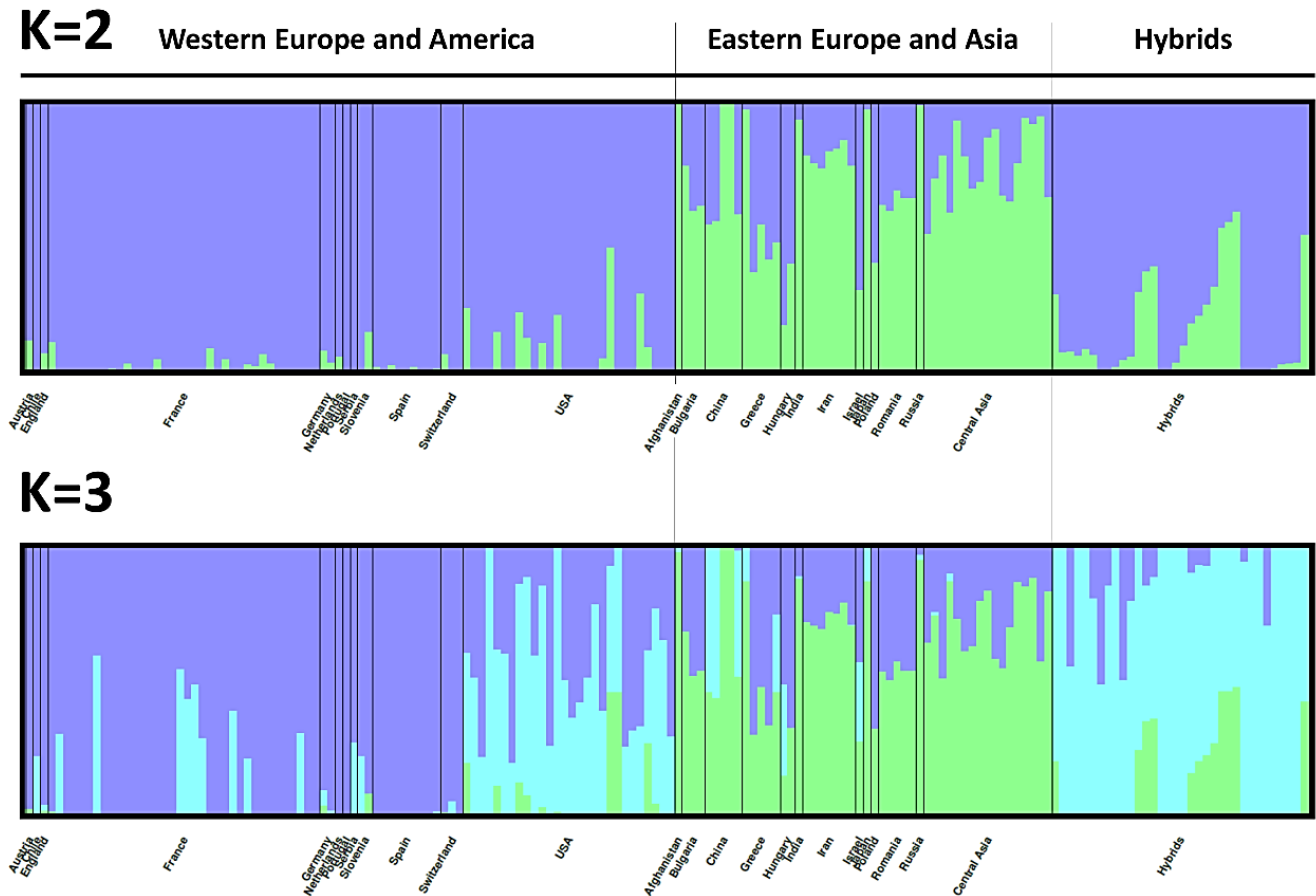


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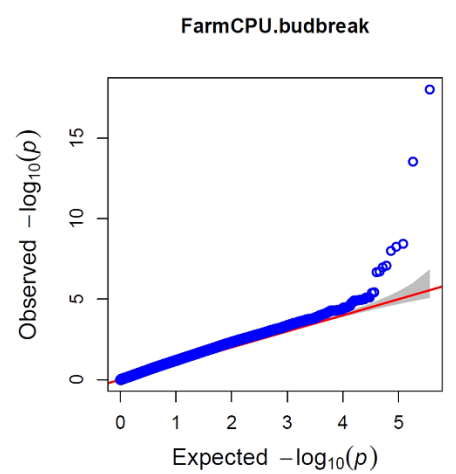
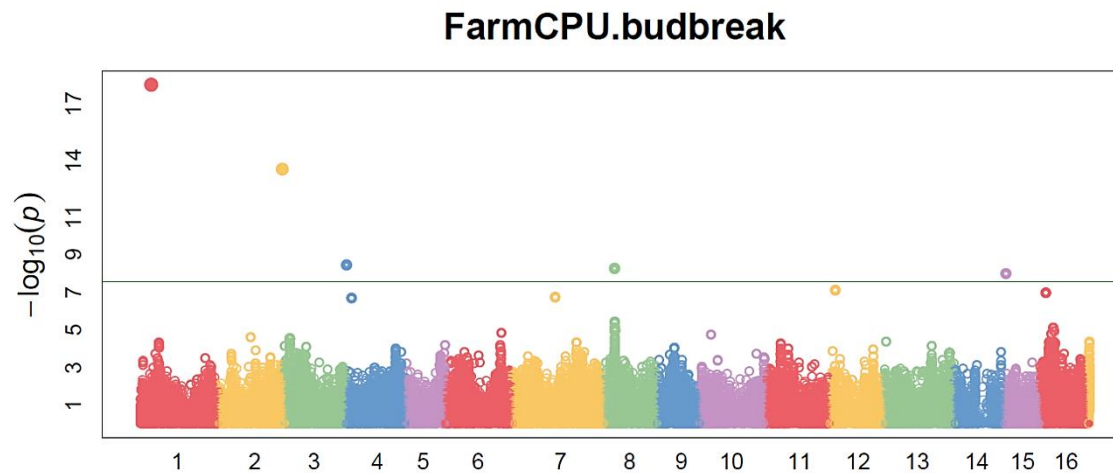
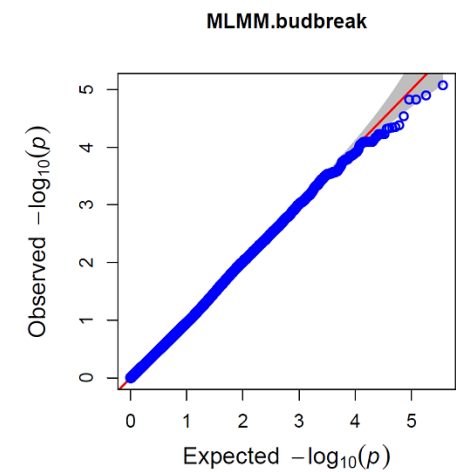
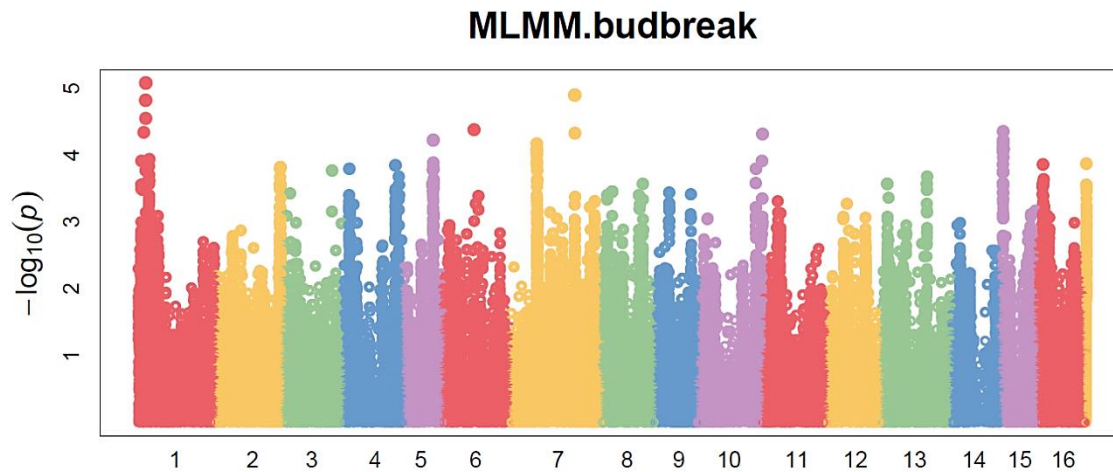


2

Structure also investigated with PCA

Cryptic relatedness calculated using kinship matrix may account for structure

→ Best number of PCs to include = 0 (Bayesian Information Criterion)



Chr 1 – 6,514,832 bp

Other work on leafing date: 3 SNPs on chr 1,
between 3,187,214 and 4,805,396 bp (Marrano *et al.*, 2019)

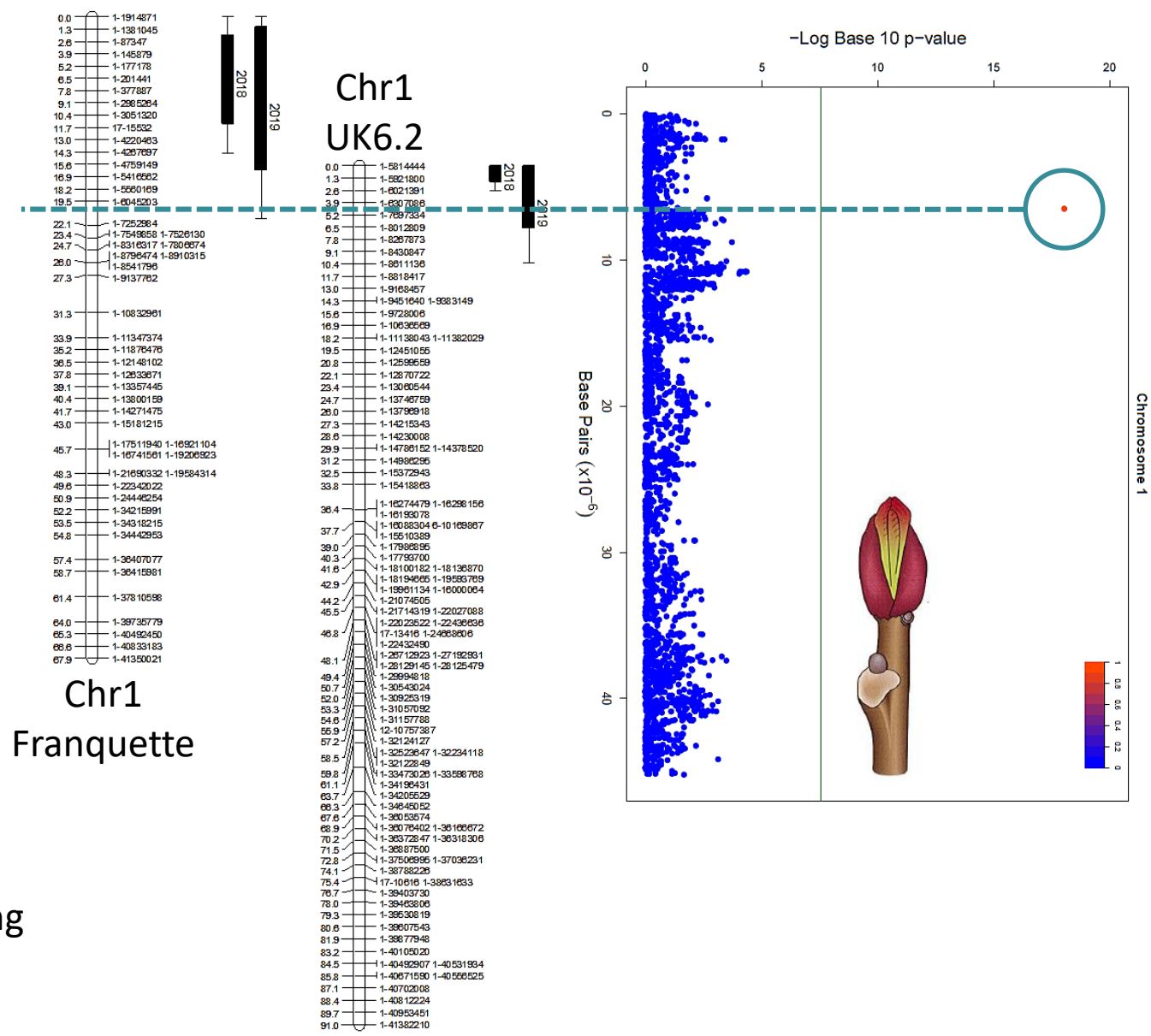


Intraspecific F₁ mapping progeny of 78 individuals

Female: 'Franquette' (late budbreak date) 849 SNPs

Male: 'UK6.2' (intermediate to early) 1,088 SNPs

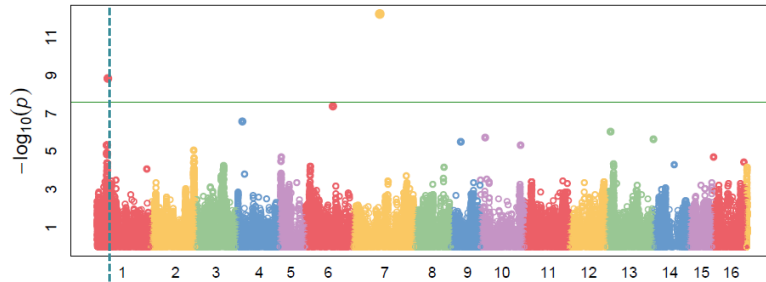
Pseudo-testcross strategy: JoinMap4.0 LOD 16.0 for mapping Kosambi's function MultiQTL 2.6 Multiple Interval Mapping



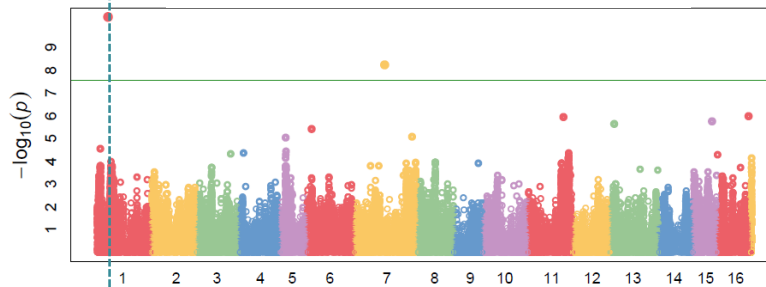
1

Female flowering dates

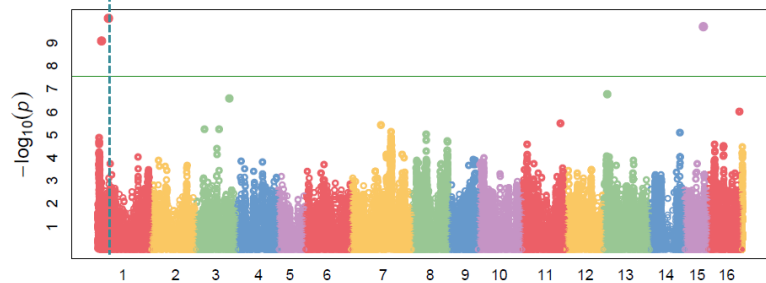
Beginning



Full



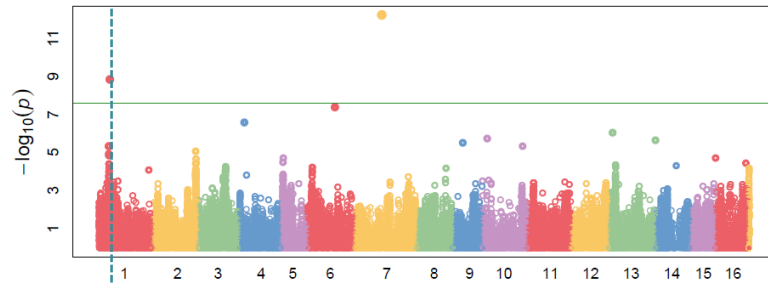
End



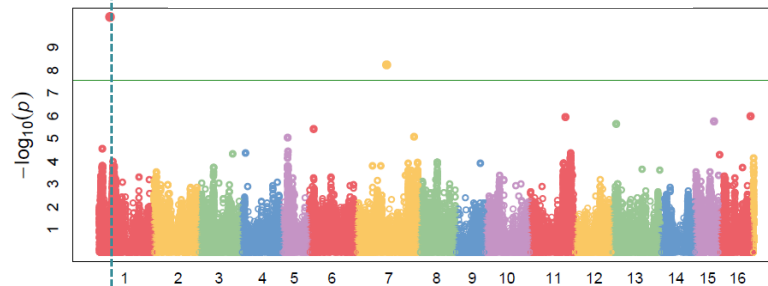
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Female flowering dates

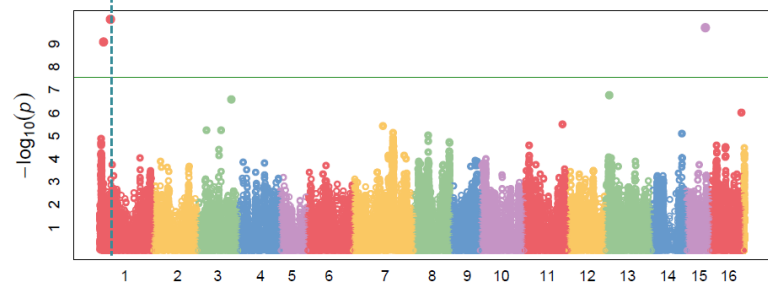
Beginning



Full



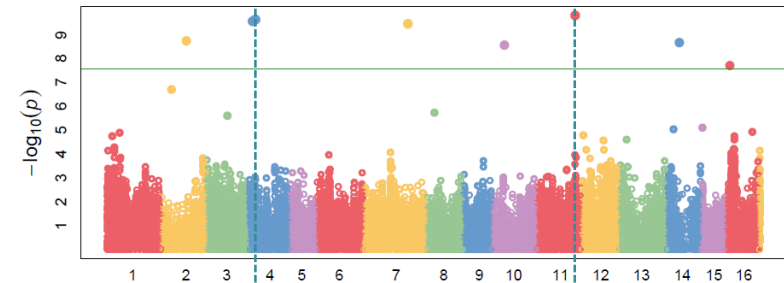
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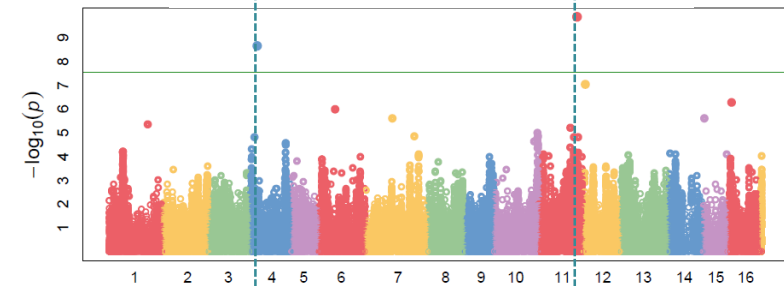
2

Male flowering dates

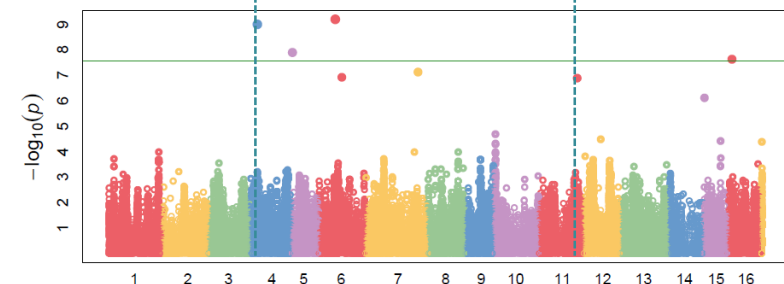
Beginning



Full

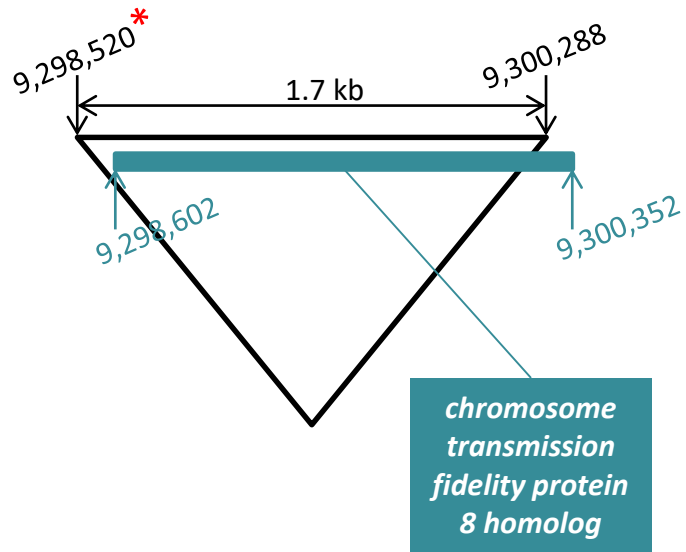


End



1

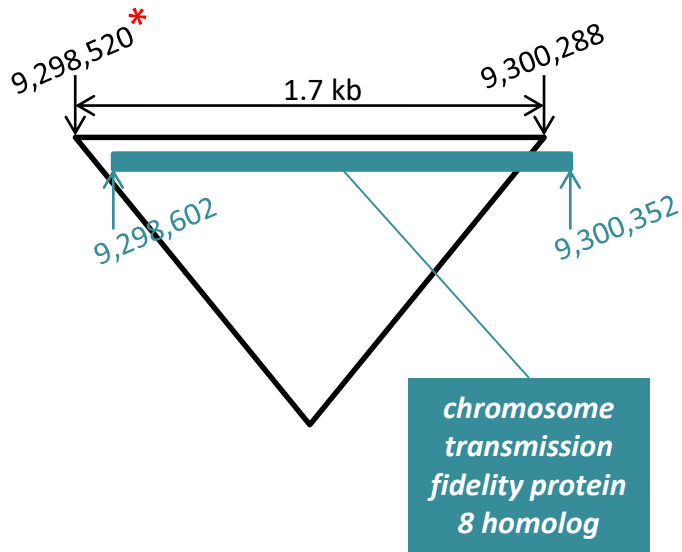
Female flowering dates
Chr 1 – 9,298,520 bp



*Associated SNP

1

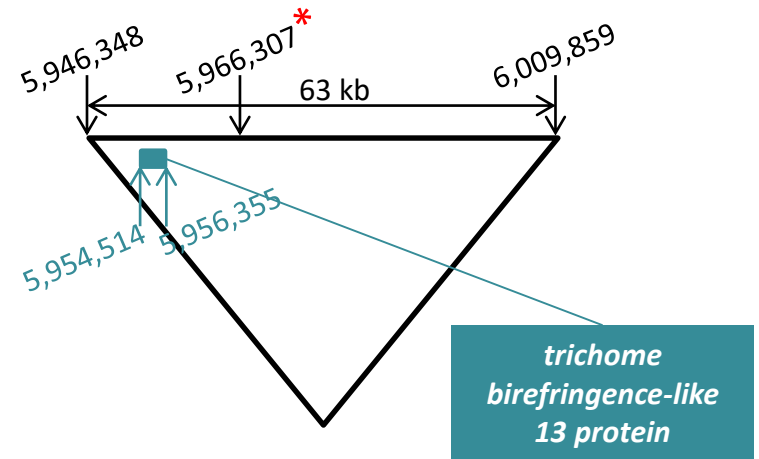
Female flowering dates
Chr 1 – 9,298,520 bp



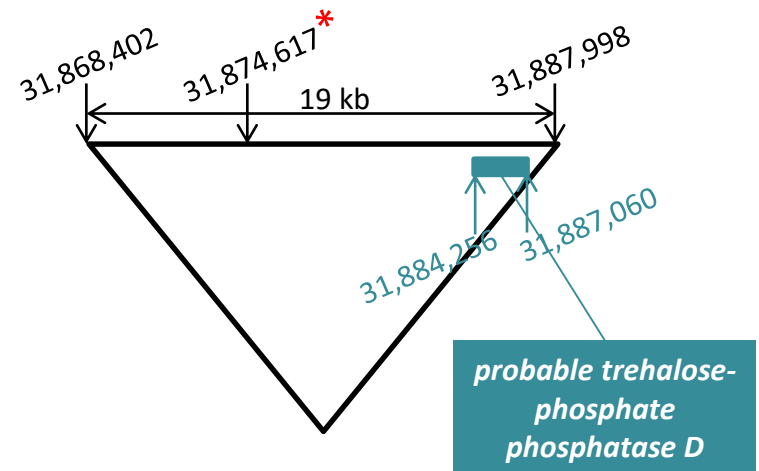
* Associated SNP

2

Male flowering dates
Chr 4 – 5,966,307 bp

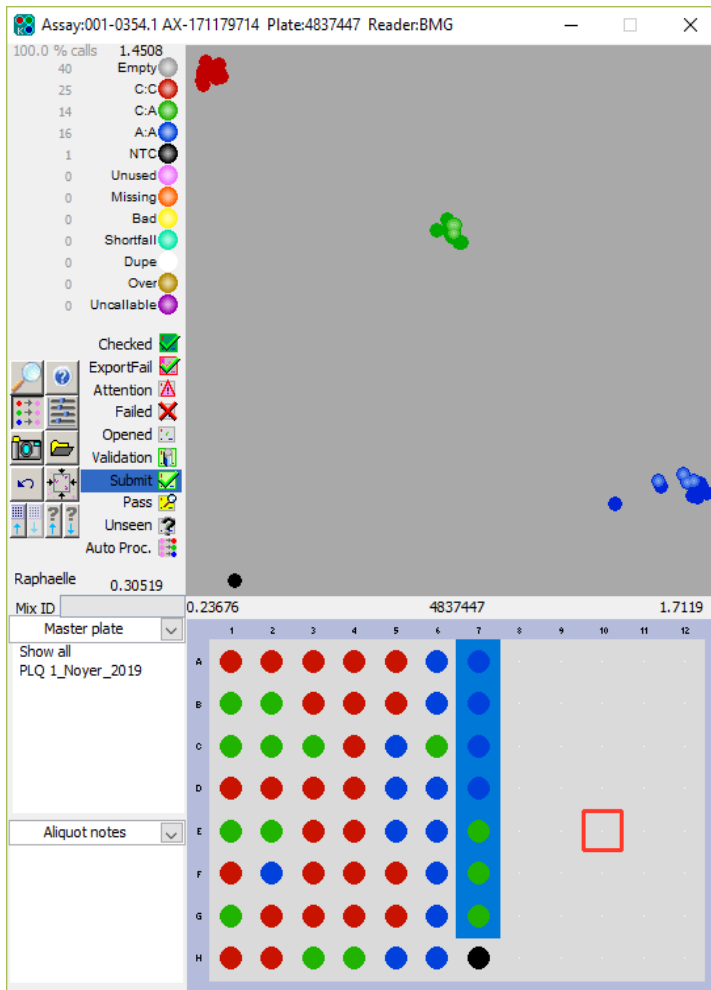


Chr 11 – 31,874,617 bp



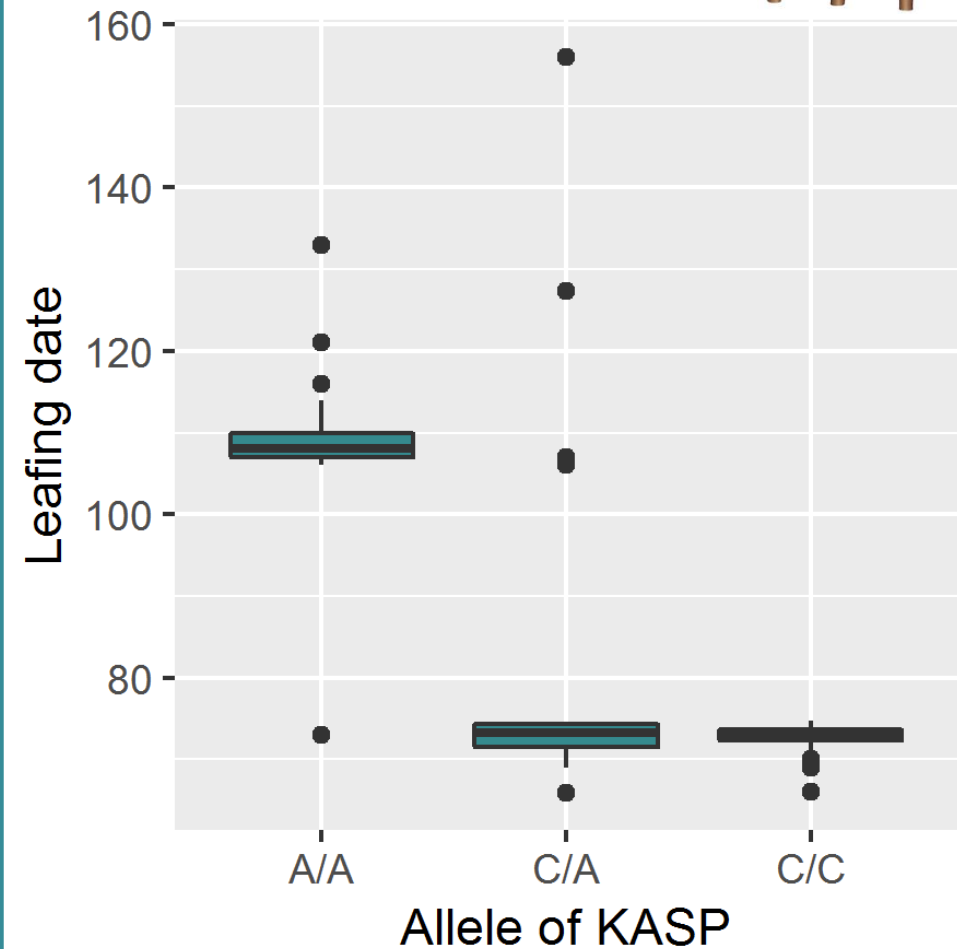
1

96 unreleased breeding line accessions
from WIP, University of California, Davis



2

Kruskal-Wallis test, $p\text{-value} = 6.88 \times 10^{-13}$
Budbreak date vs. leafing date





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**Thank you for
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Any questions?**