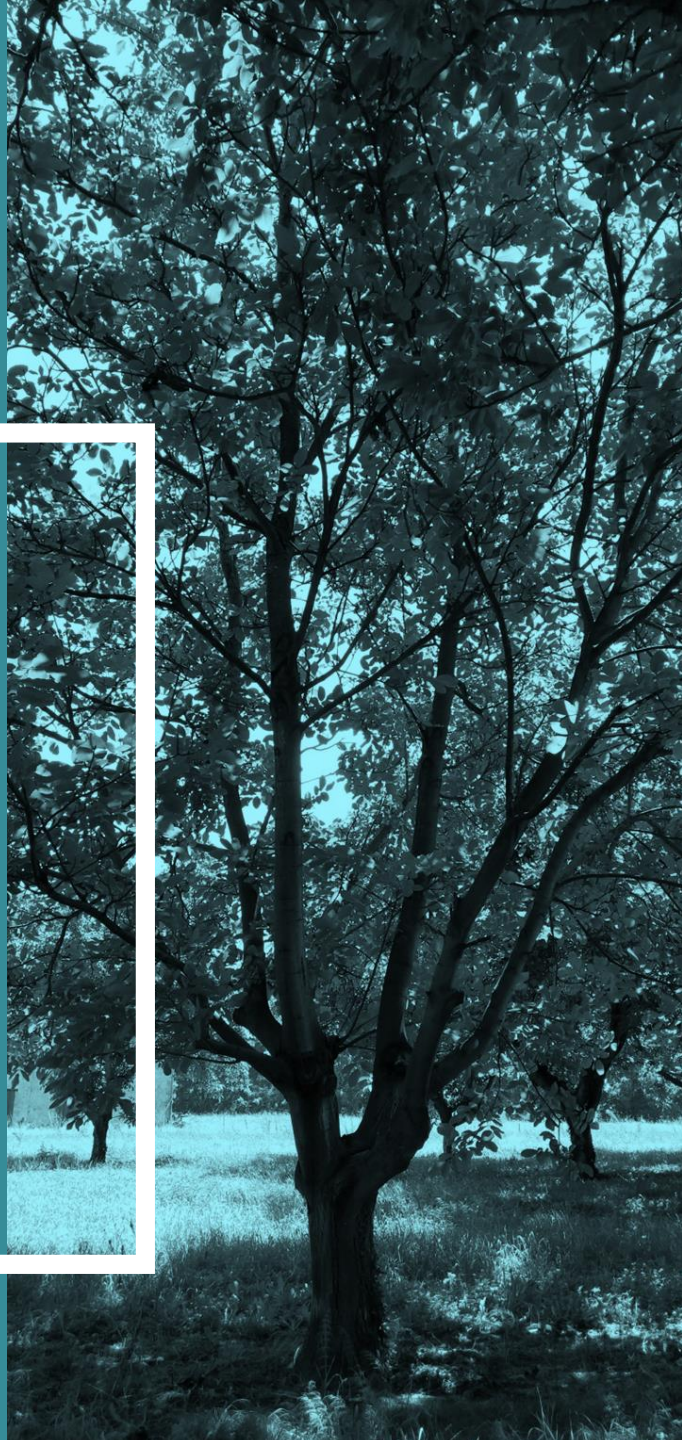
Association and Linkage Mapping of Walnut (Juglans regia L.) Phenological Traits

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

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

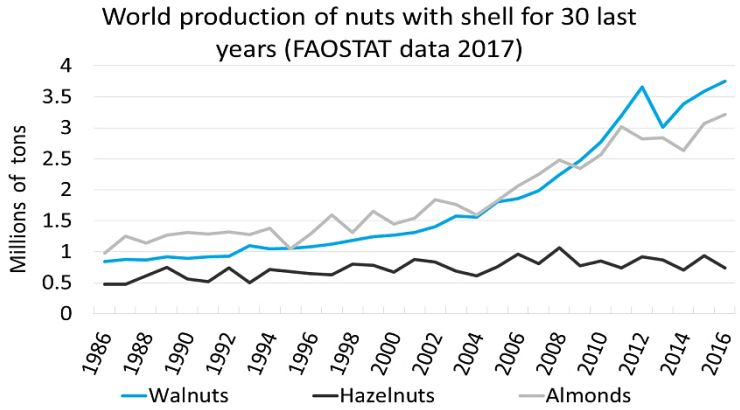
Anthony Bernard
Annarita Marrano
Armel Donkpegan
Patrick J. Brown
Charles A. Leslie
David B. Neale
Fabrice Lheureux
Elisabeth Dirlewanger

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



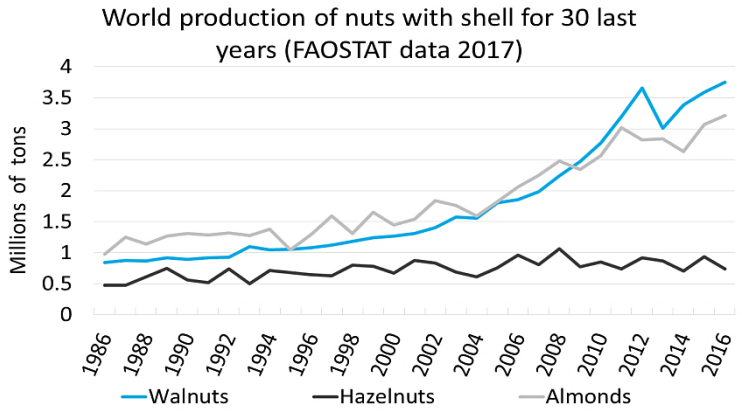
1

The world in-shell walnut production is increasing



1

The world in-shell walnut production is increasing



2

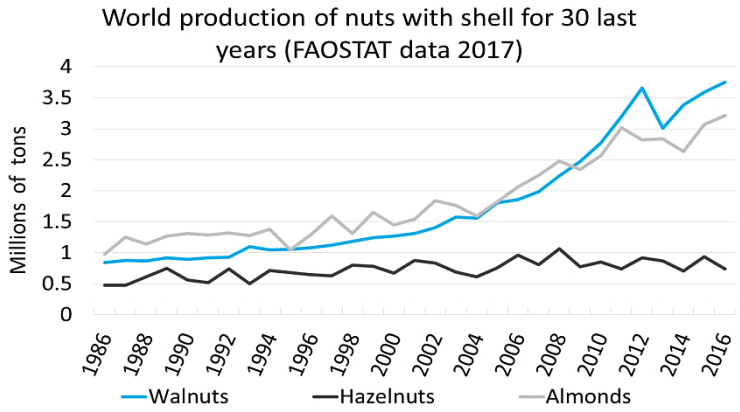
China > California > Iran

France: 7th with 40,000 tons

Area of orchards: 2nd French fruit crop

1

The world in-shell walnut production is increasing



2

China > California > Iran

France: 7th with 40,000 tons

Area of orchards: 2nd French fruit crop

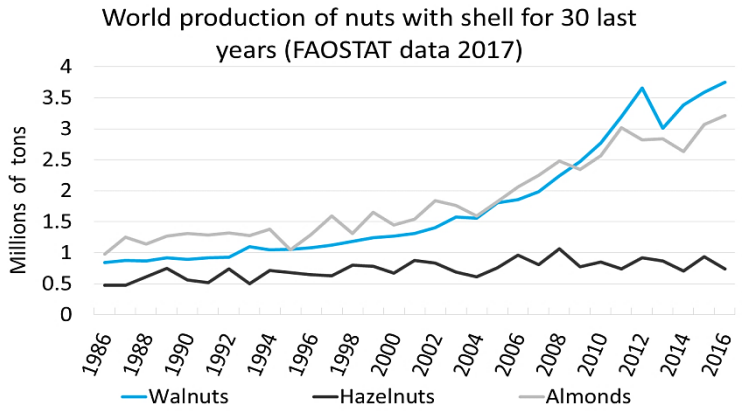
3

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



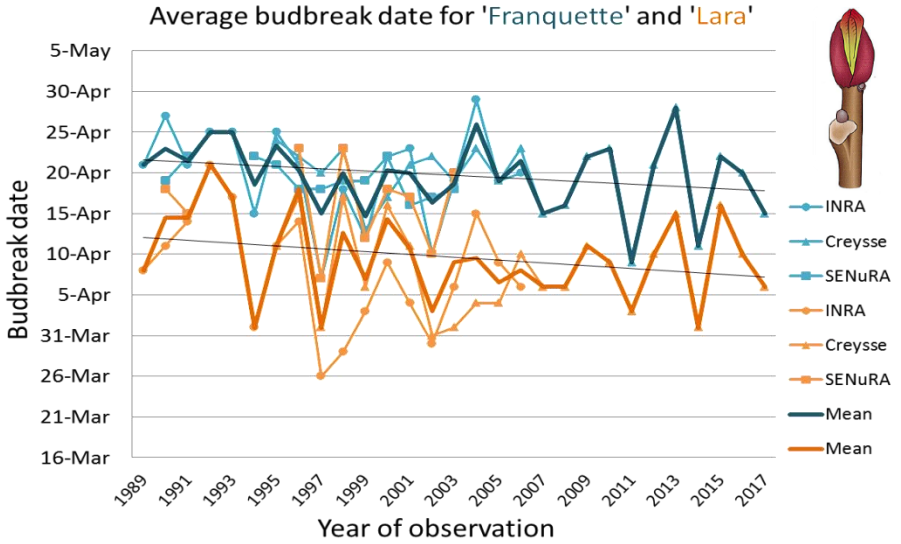
2

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

3

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

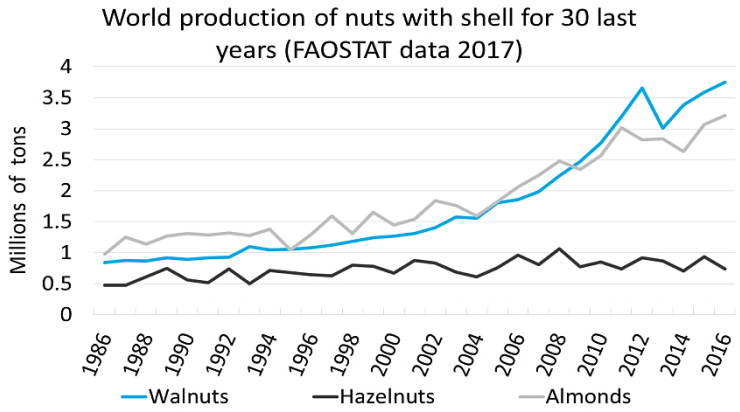
4



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



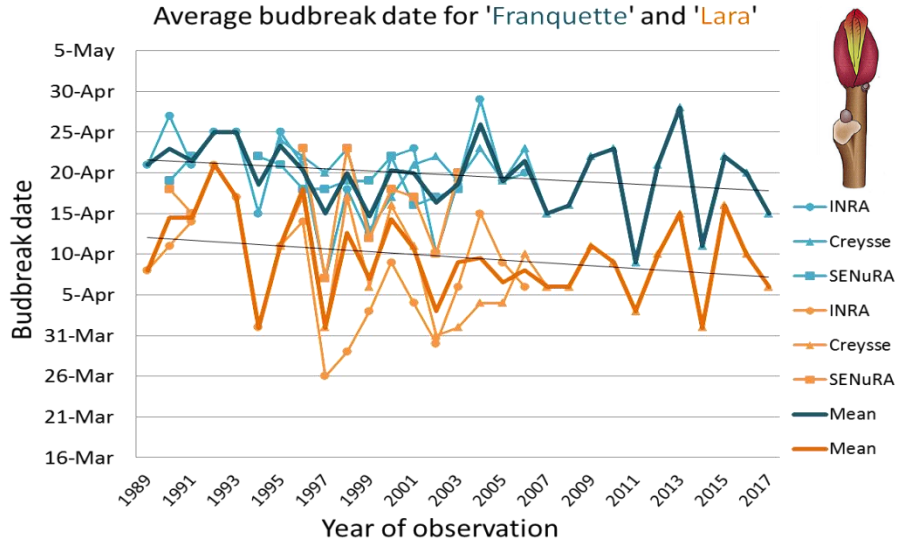
2

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

3

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

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

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: F_1 progeny (78 individuals) segregating for phenology + unique genetic resources core-collection (170 accessions)

→ GWAS, combined with QTLs detection for phenology

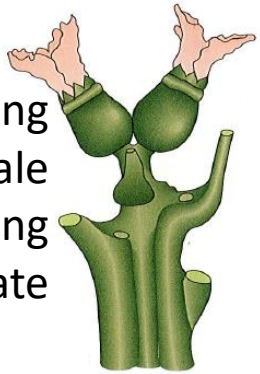
→ marker validation on other plant material

→ choice of genitors

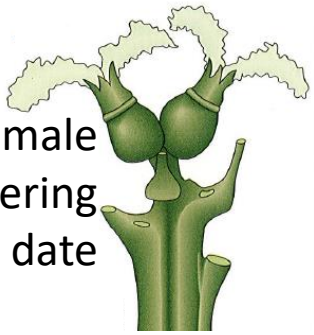
1

Budbreak date (leaves and female flowering)

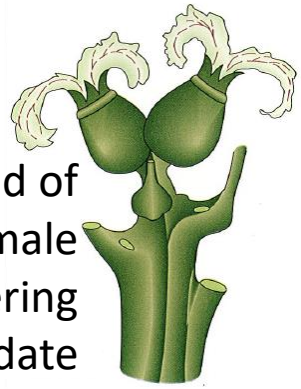
Beginning of female flowering date



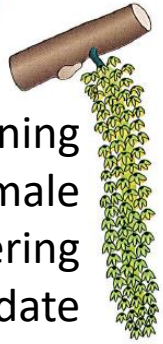
Full female flowering date

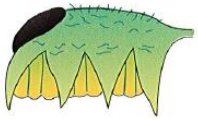


End of female flowering date

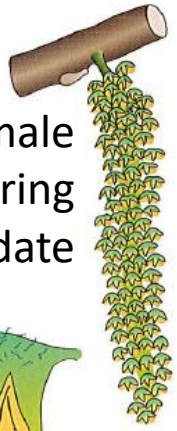


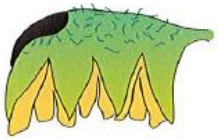
Beginning of male flowering date





Full male flowering date





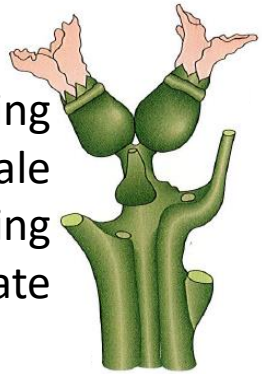
End of male flowering date

→ 2018 data
→ 2019 data

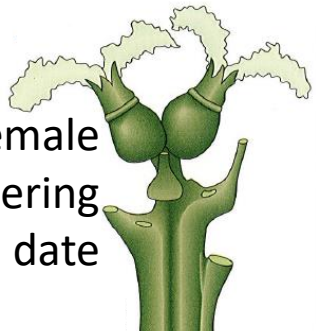
1

Budbreak date (leaves and female flowering)

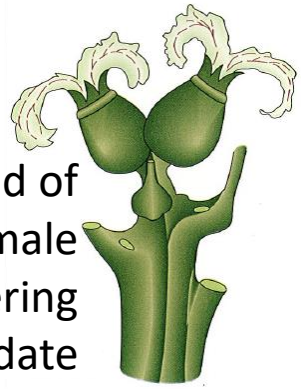
Beginning of female flowering date



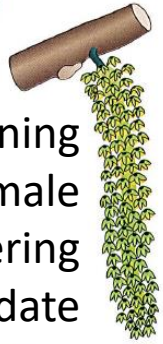
Full female flowering date

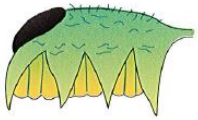


End of female flowering date

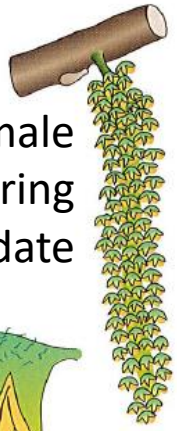


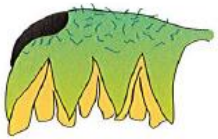
Beginning of male flowering date





Full male flowering 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;

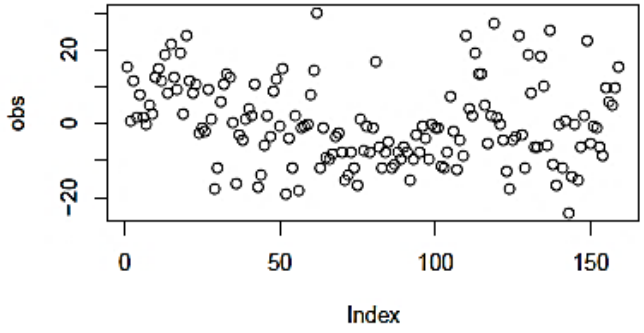
μ - mean value of the trait; Y_i - fixed effect of the i^{th} year;

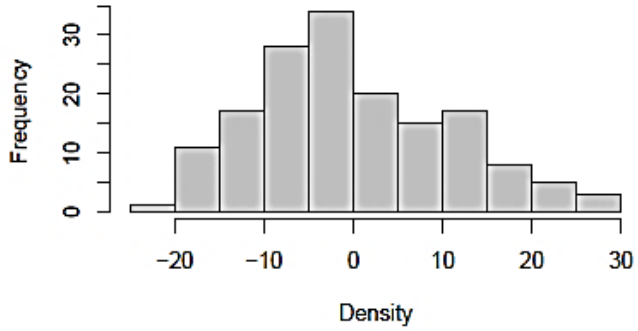
g_k -random effect of the k genotype; and $e_{i(j)k}$ - residuals of the model

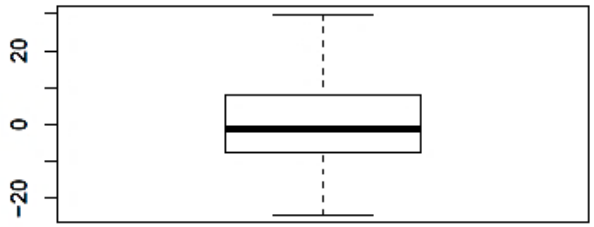
→ lme4

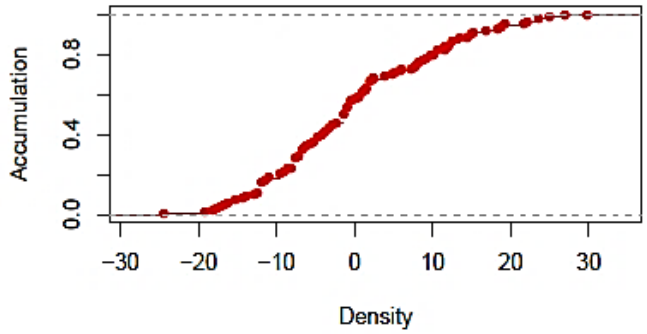
1

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

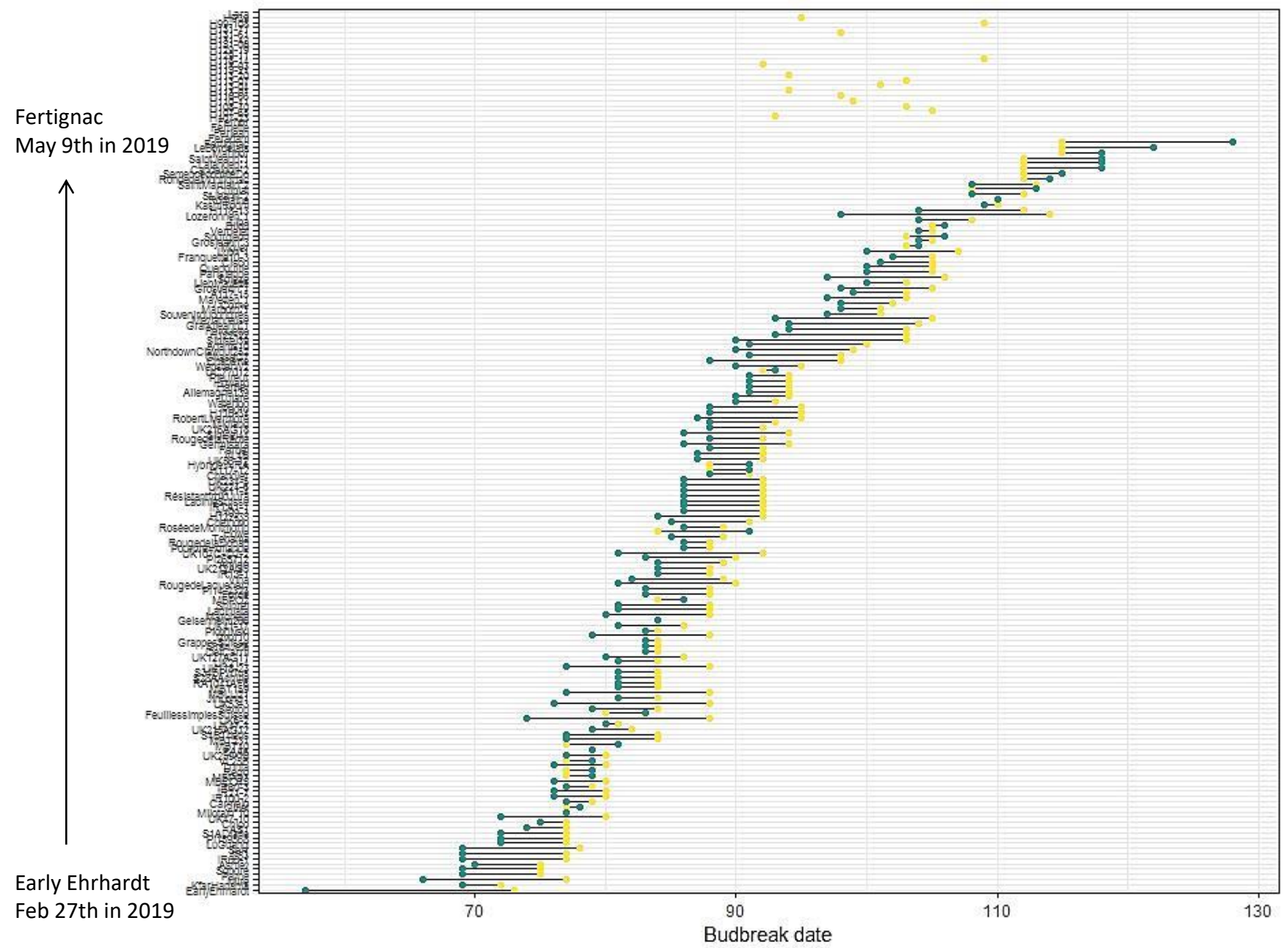








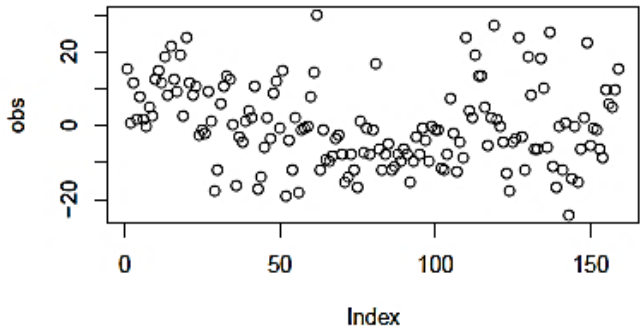
Fertignac
May 9th in 2019

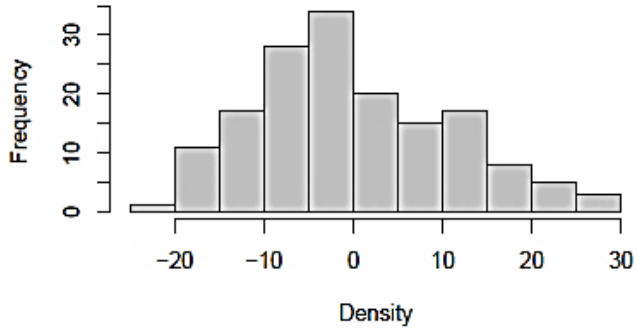


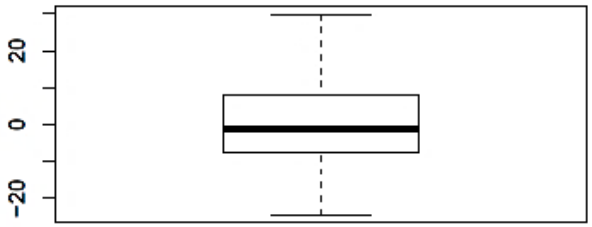
Early Ehrhardt
Feb 27th in 2019

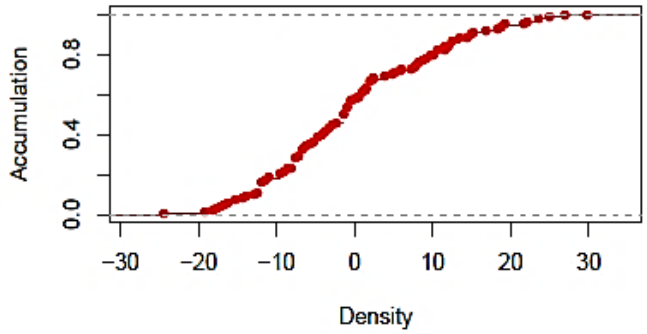
1

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









2

Broad-sense heritability → $H^2 = \sigma^2_G / [(\sigma^2_G + (\sigma^2_\varepsilon / n_{obs/g})]$

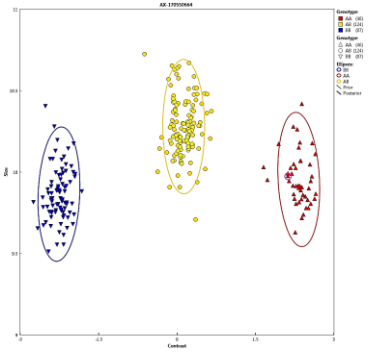
where σ^2_G - genotypic effect variance;

σ^2_ε - variance of residuals;

and $n_{obs/g}$ - number of observations by genotype

→ 0.93

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



Conversion Type	*Number of markers*	*Percentage of markers*
PolyHighResolution	397,921[a]	65,27
NoMinorHom	75,564[a]	12,39
MonoHighResolution	36,684[a]	6,02
CallRateBelowThreshold	27,761	4,55
OffTargetVariant	4,787	0,79
Other	66,941	10,98
Total of retained SNPs	*510,169*	

Step 2. To keep SNPs with mendelian inheritance using F_1 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

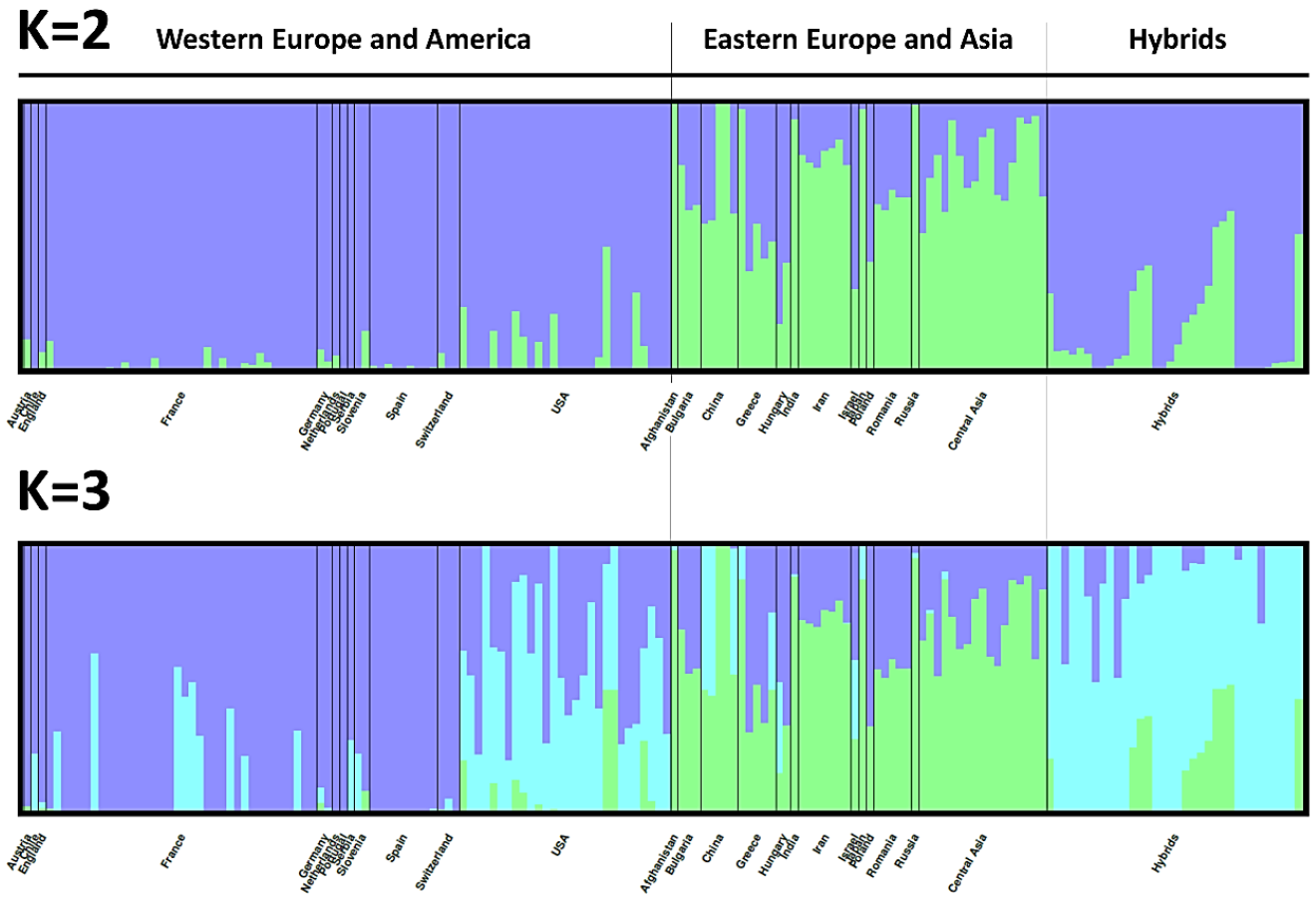
609,658 → 364,275 = 59.8%

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

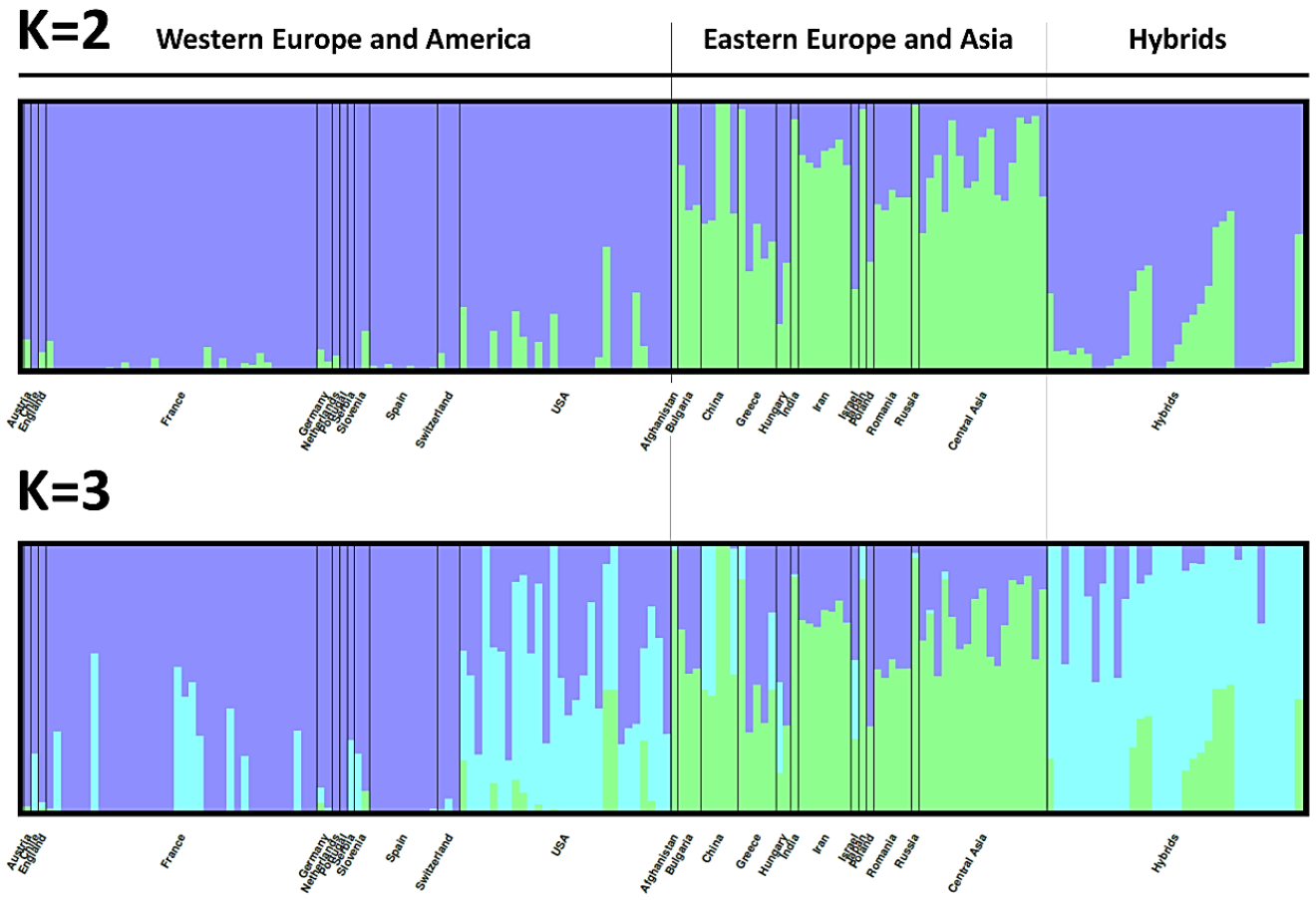


1

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

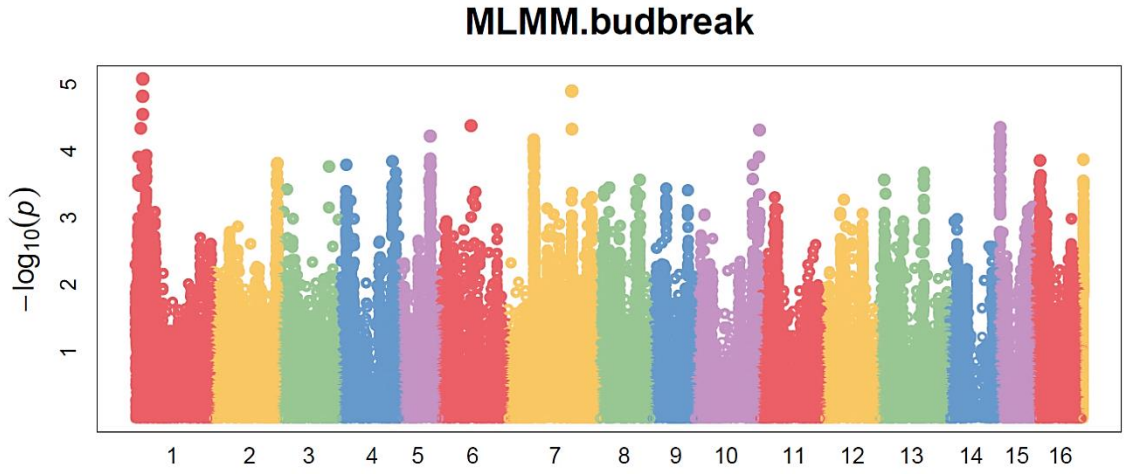


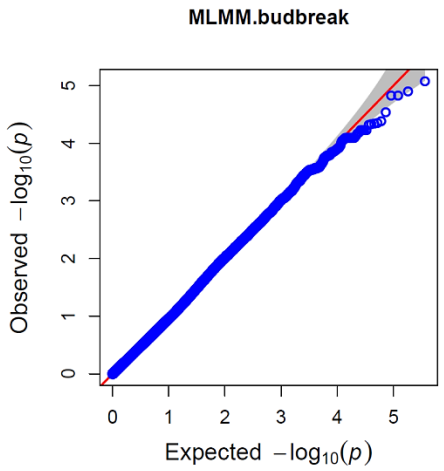
2

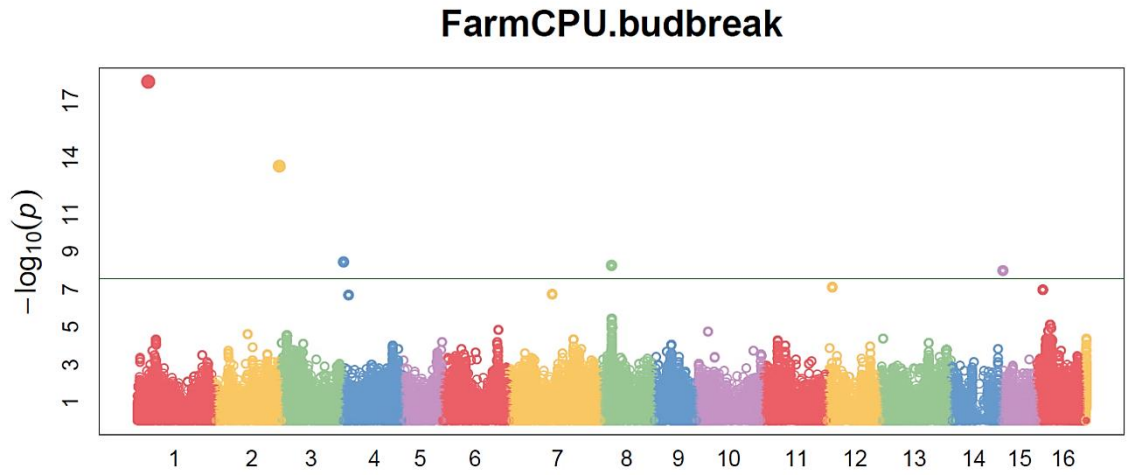
Structure also investigated with PCA

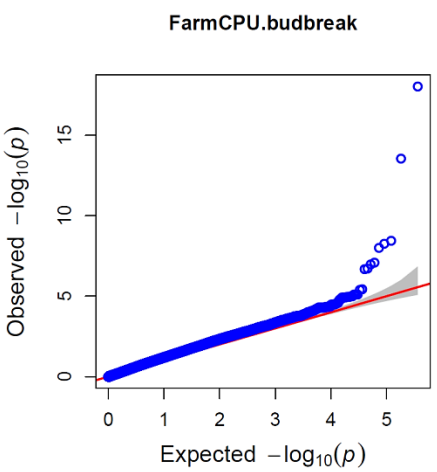
Cryptic relatedness calculated using kinship matrix may account for structure

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









Chr 1 – 6,514,832 bp

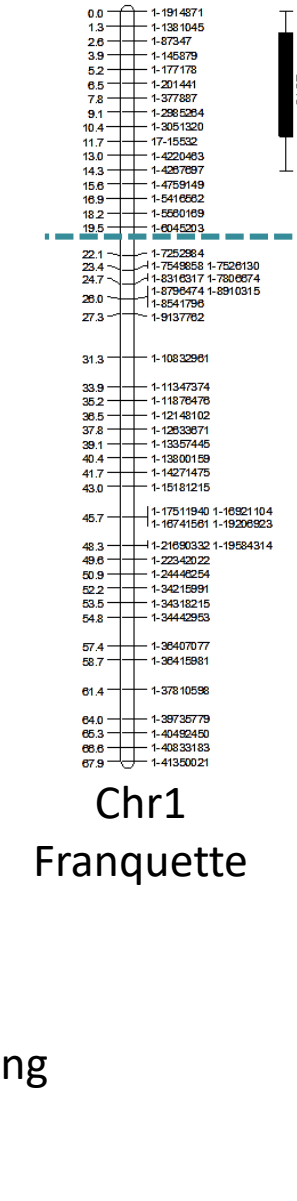
Other work on leafing date: 3 SNPs on chr 1, between 3,187,214 and 4,805,396 bp (Marrano *et al.*, 2019)

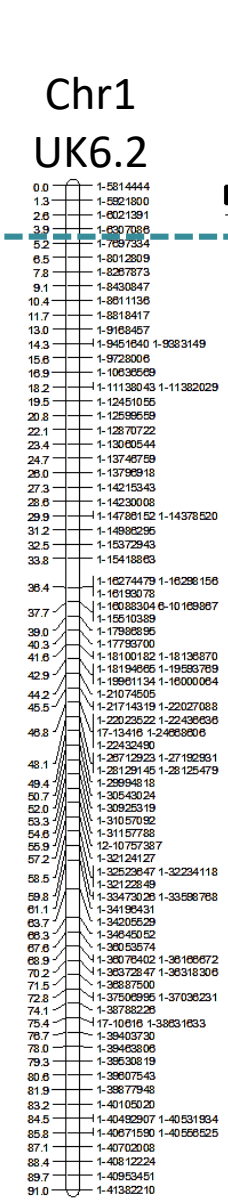
Intraspecific F_1 mapping progeny of 78 individuals

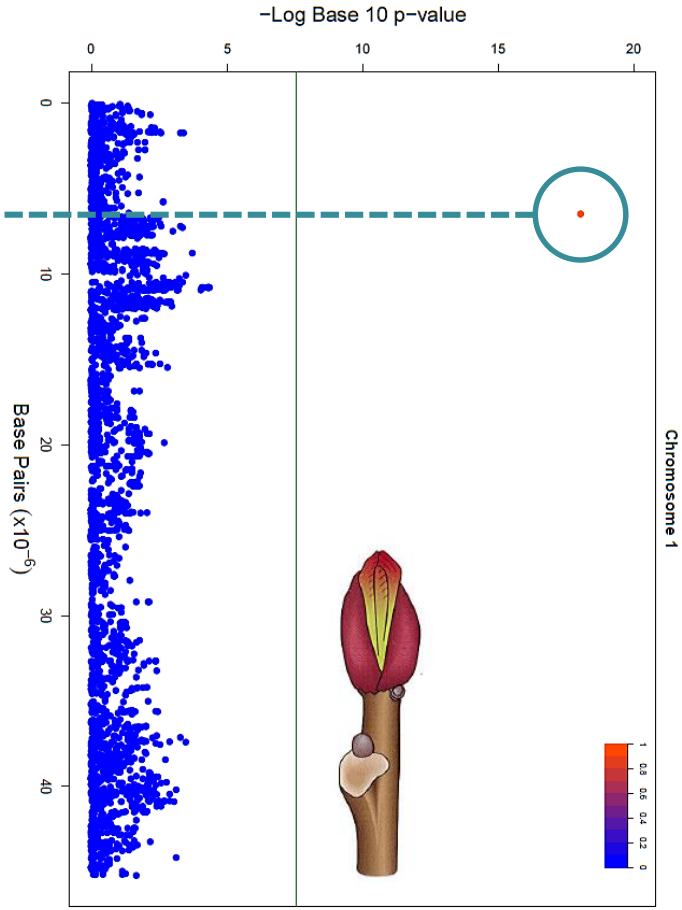
Female: 'Franquette ' (late budbreak date) 849 SNPs

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

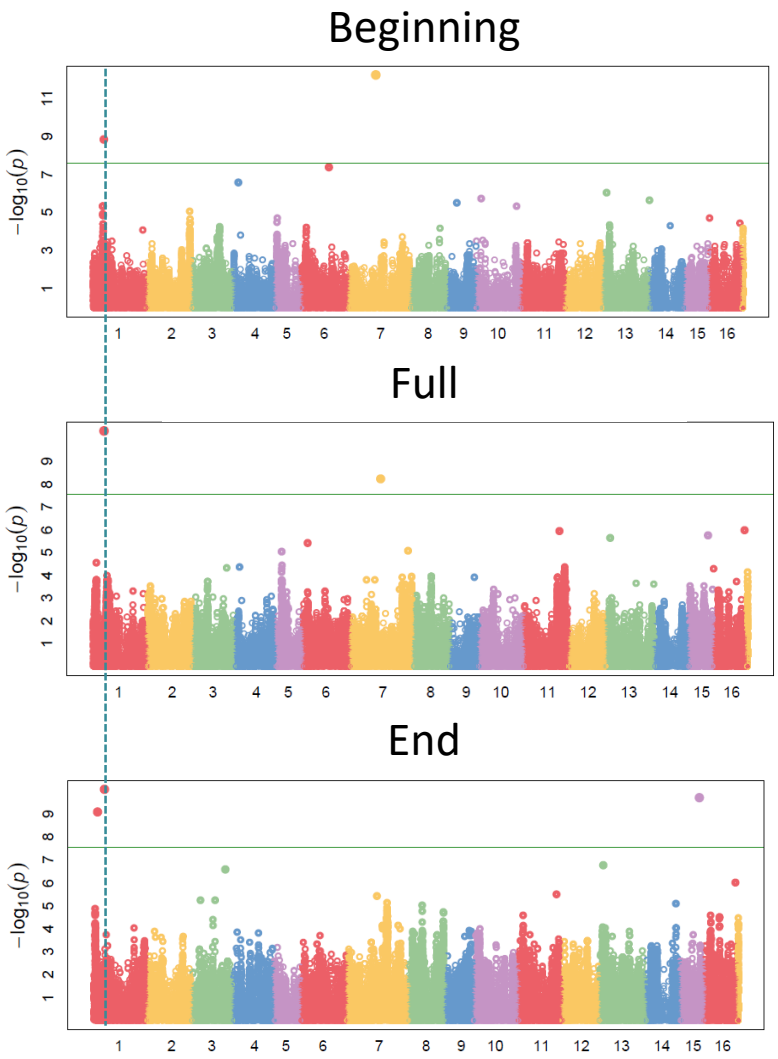






1

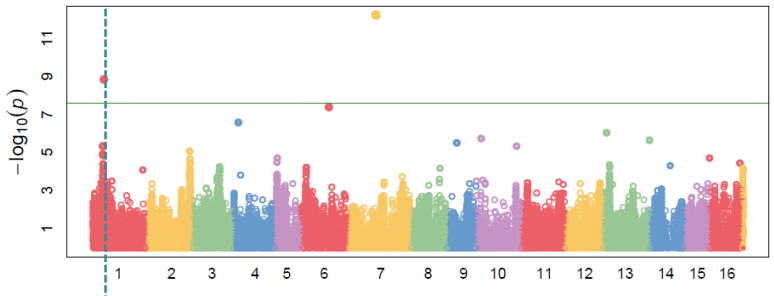
Female flowering dates



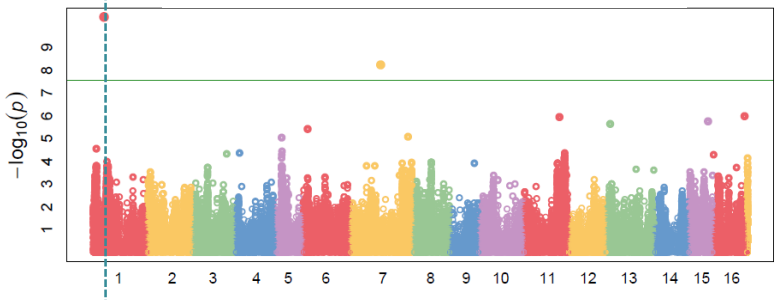
1

Female flowering dates

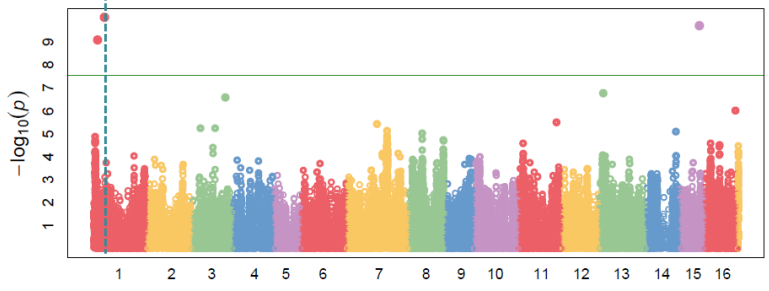
Beginning



Full



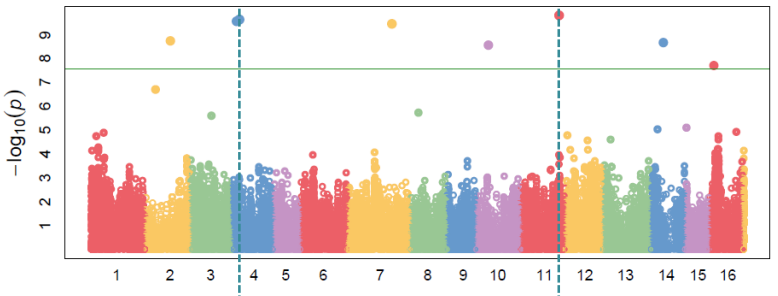
End



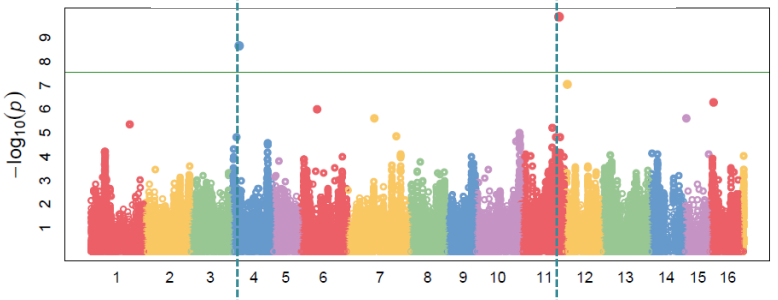
2

Male flowering dates

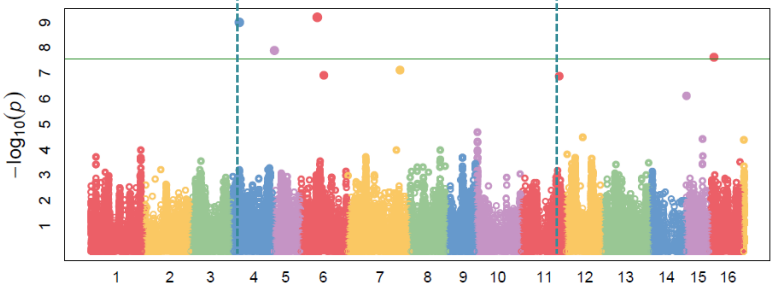
Beginning



Full

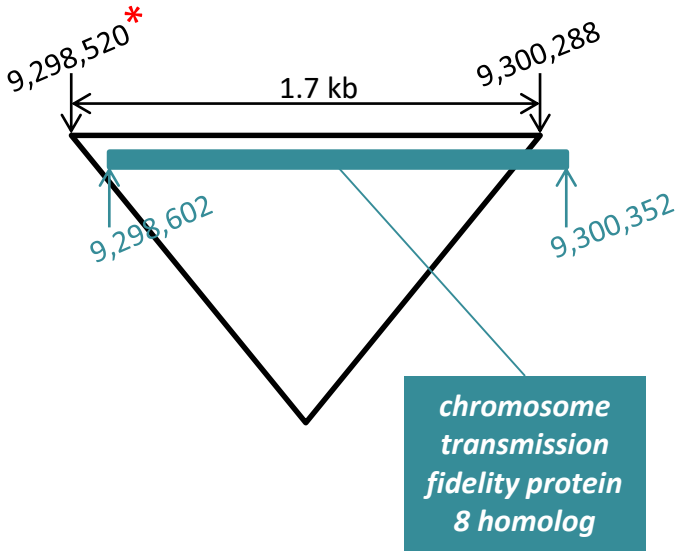


End



1

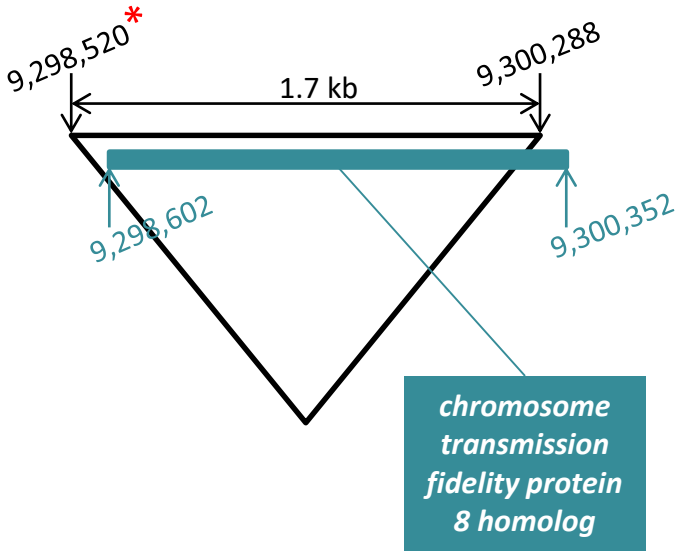
Female flowering dates
Chr 1 – 9,298,520 bp



*Associated SNP

1

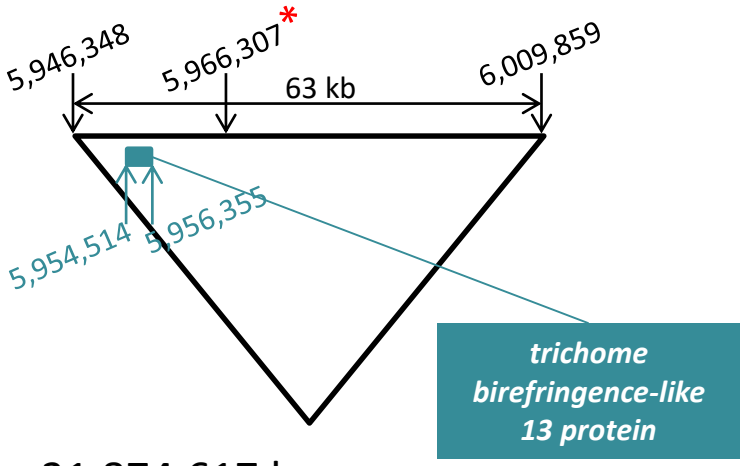
Female flowering dates
Chr 1 – 9,298,520 bp



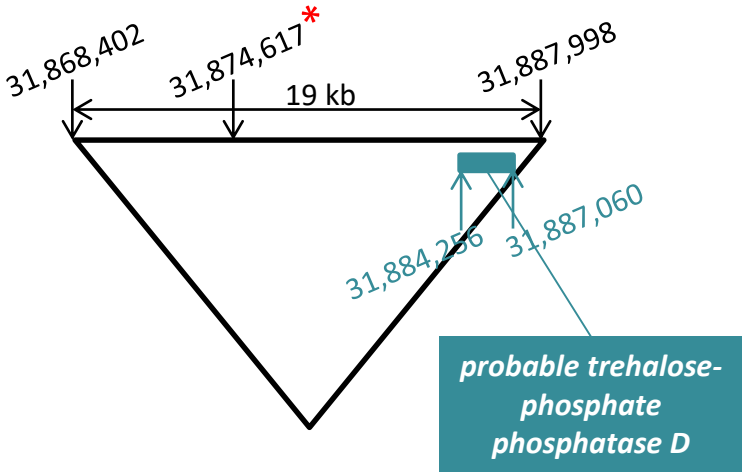
*Associated SNP

2

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

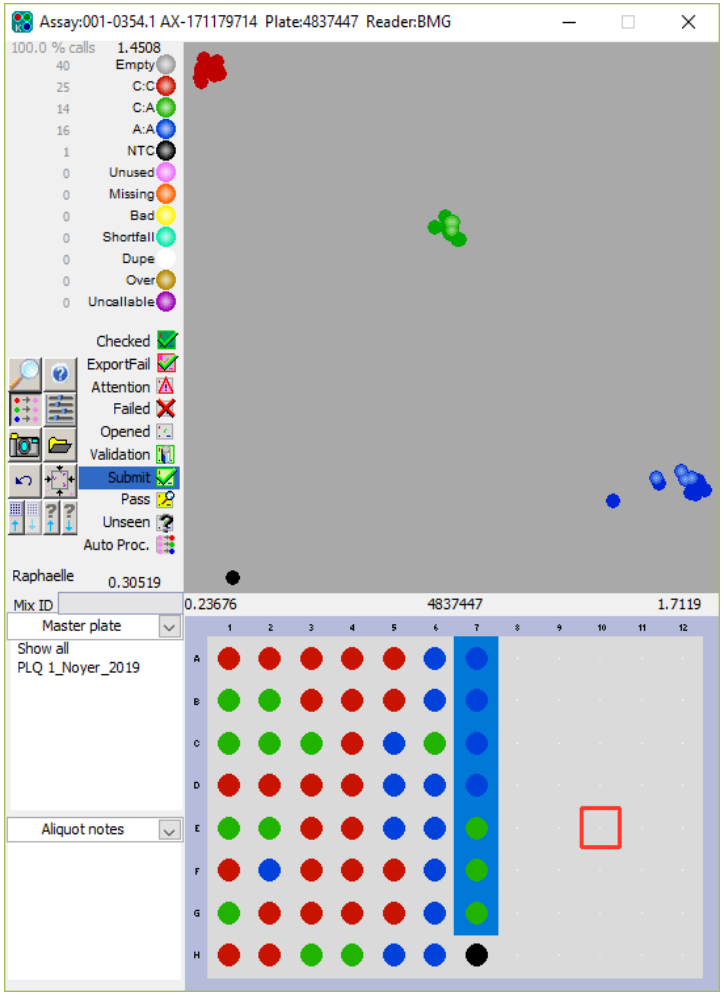


Chr 11 – 31,874,617 bp



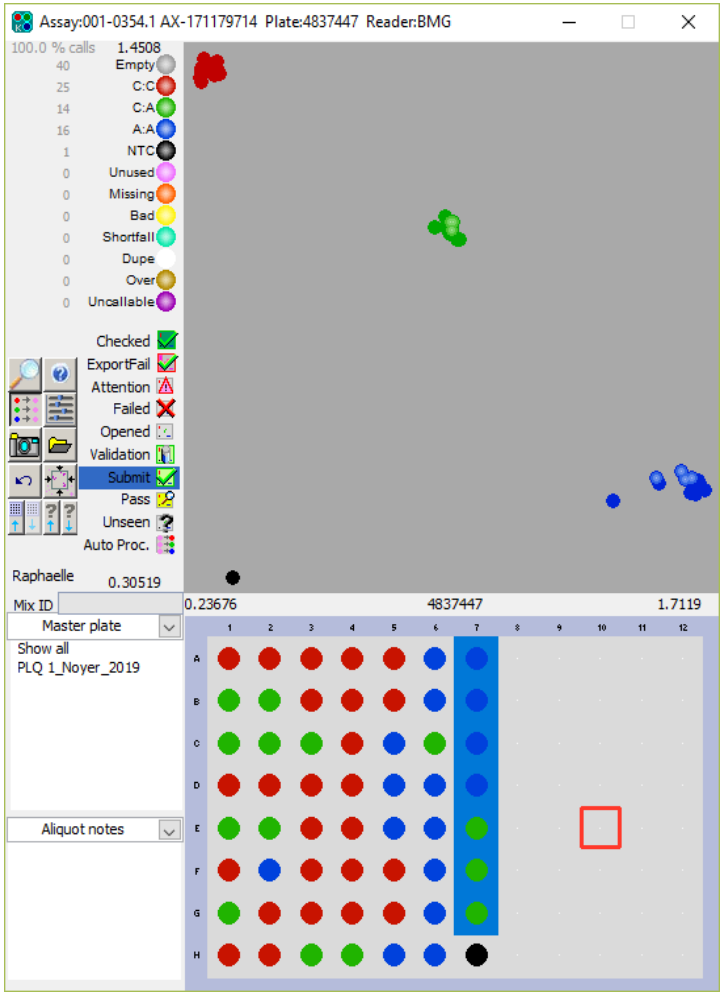
1

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



1

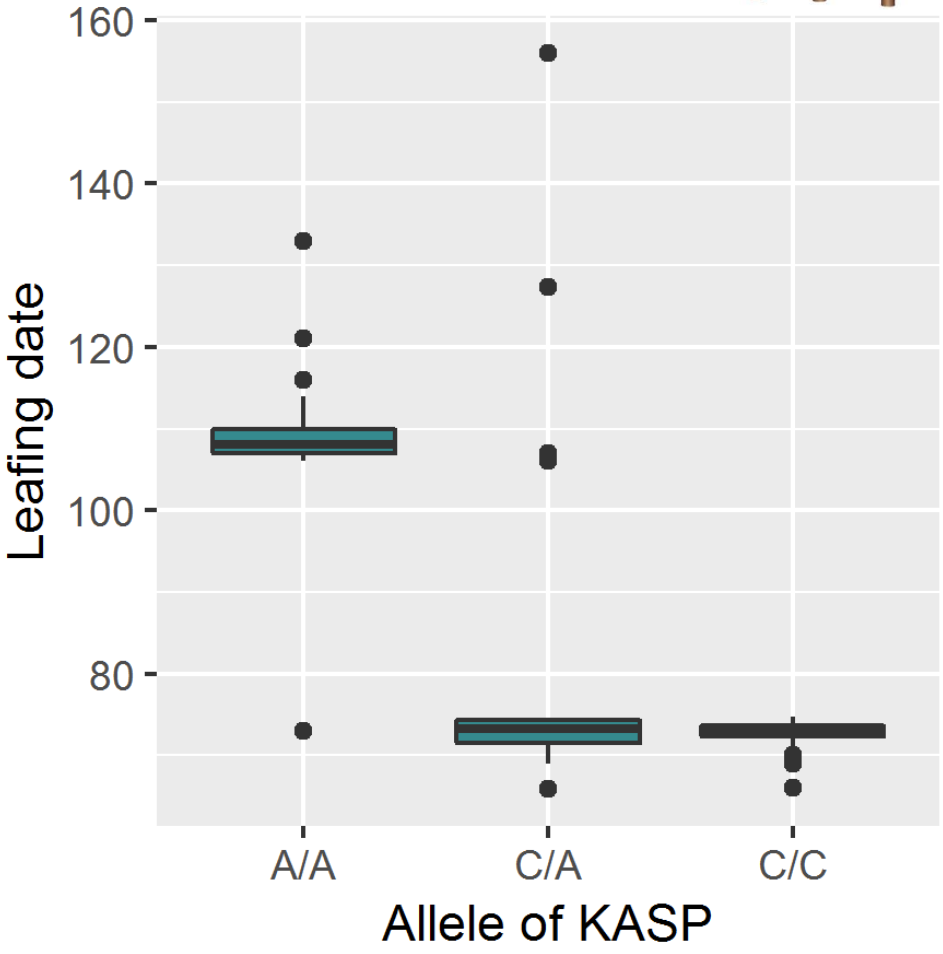
96 unreleased breeding line accessions from WIP, University of California, Davis



2

Kruskal-Wallis test, p-value = 6.88^{e-13}

Budbreak date vs. leafing date



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

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

Thank you for your attention ! Any questions?