Association and Linkage Mapping of Walnut (Juglans regia L.) Phenological Traits
The world in-shell walnut production is increasing.
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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
Background

The world in-shell walnut production is increasing.

1. World production of nuts with shell for 30 last years (FAOSTAT data 2017)

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. Average budbreak date for 'Franquette' and 'Lara'

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

1. The world in-shell walnut production is increasing.

2. China > California > Iran
   France: 7th with 40,000 tons
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   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)
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
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

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
Phenotypic evaluation of phenology and data analysis

1. Budbreak date (leaves and female flowering)
2. Beginning of female flowering date
3. Full female flowering date
4. End of female flowering date
5. Beginning of male flowering date
6. Full male flowering date
7. End of male flowering date

→ 2018 data
→ 2019 data
Phenotypic evaluation of phenology and data analysis

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;

$\mu$ - 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
By calculating the BLUPs, budbreak date within the GWAS panel is considered as normally distributed.
By calculating the BLUPs, budbreak date within the GWAS panel is considered as normally distributed.

Broad-sense heritability: \( H^2 = \frac{\sigma^2_G}{(\sigma^2_G + (\sigma^2_\epsilon / n_{obs/g})]} \)

where \( \sigma^2_G \) - genotypic effect variance;
\( \sigma^2_\epsilon \) - variance of residuals;
and \( n_{obs/g} \) - number of observations by genotype.

\( \rightarrow 0.93 \)
Step 1. To keep SNPs of high resolution from Axiom® Analysis Suite

<table>
<thead>
<tr>
<th>Conversion Type</th>
<th>Number of markers</th>
<th>Percentage of markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>PolyHighResolution</td>
<td>397,921</td>
<td>65.27</td>
</tr>
<tr>
<td>NoMinorHom</td>
<td>75,564</td>
<td>12.39</td>
</tr>
<tr>
<td>MonoHighResolution</td>
<td>36,684</td>
<td>6.02</td>
</tr>
<tr>
<td>CallRateBelowThreshold</td>
<td>27,761</td>
<td>4.55</td>
</tr>
<tr>
<td>OffTargetVariant</td>
<td>4,787</td>
<td>0.79</td>
</tr>
<tr>
<td>Other</td>
<td>66,941</td>
<td>10.98</td>
</tr>
<tr>
<td><strong>Total of retained SNPs</strong></td>
<td><strong>510,169</strong></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>SNPs having no mendelian inheritance</th>
<th>661</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total of retained SNPs</strong></td>
<td><strong>509,508</strong></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>SNPs having genotyping rate &lt;90%</th>
<th>13,993</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total of retained SNPs</strong></td>
<td><strong>495,515</strong></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>SNPs having minor allele frequency &lt;5%</th>
<th>123,751</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total of retained SNPs</strong></td>
<td><strong>371,764</strong></td>
</tr>
</tbody>
</table>

Step 5. To delete duplicated SNPs

<table>
<thead>
<tr>
<th>Duplicated SNPs</th>
<th>7,489</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total of retained SNPs</strong></td>
<td><strong>364,275</strong></td>
</tr>
</tbody>
</table>

609,658 → 364,275 = 59.8%
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.
Confounding effects for GWAS

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)
GWAS results for budbreak date

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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
Female flowering dates

Beginning

Full

End

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Female flowering dates

Beginning

Full

End

Male flowering dates

Beginning

Full

End

Other phenology-related traits
Female flowering dates
Chr 1 – 9,298,520 bp

*Associated SNP

chrosome transmission fidelity protein 8 homolog
LD blocks and candidate genes

1. Female flowering dates
   Chr 1 – 9,298,520 bp
   *Associated SNP

   chromosome transmission fidelity protein 8 homolog

2. Male flowering dates
   Chr 4 – 5,966,307 bp
   Chr 11 – 31,874,617 bp

   trichome birefringence-like 13 protein
   probable trehalose-phosphate phosphatase D
96 unreleased breeding line accessions from WIP, University of California, Davis
KASP marker development and validation for budbreak date

1. 96 unreleased breeding line accessions from WIP, University of California, Davis

2. Kruskal-Wallis test, p-value = $6.88 \times 10^{-13}$

   Budbreak date vs. leafing date

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Thank you for your attention! Any questions?