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SSR genetic diversity assessment of French walnut (Juglans spp.) germplasm collection

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Genetic diversity and population structure assessment of INRA's walnut (*Juglans* spp.) germplasm collection using SSR markers

Anthony Bernard,
Teresa Barreneche, Fabrice Lheureux & Elisabeth Dirlewanger

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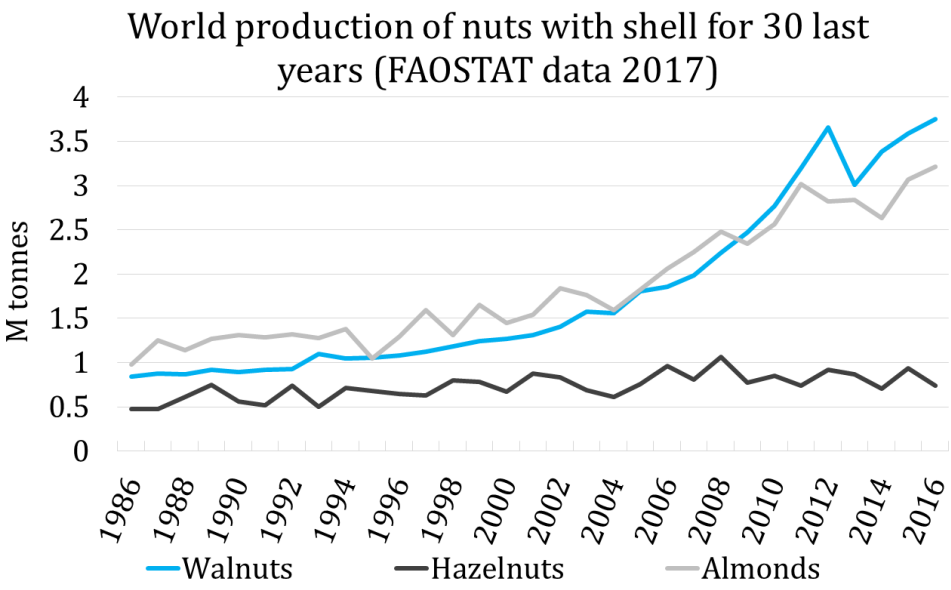
INNOV'noyer project

- Study of *Juglans* spp. genetic resources for the implementation of a marker-assisted selection

Context

- Important economic development of walnuts
- France: insufficient choice of cultivars
- Global competition, climate change, European plan Ecophyto II
- Genetic improvement: important lever



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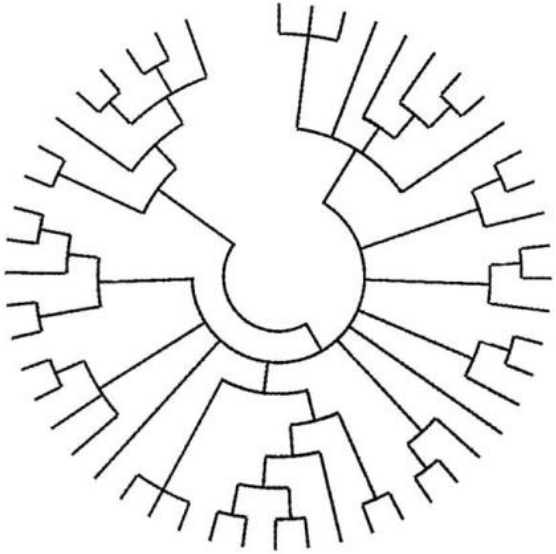
INNOV'noyer project

- Study of *Juglans* spp. genetic resources for the implementation of a marker-assisted selection

Goals

- Initiation of a new breeding program
- Basic research:
  - Genetic diversity evaluation of germplasm repository
  - Genetic determinism identification of traits of interest
- Applied research: establishment of necessary tools for marker-assisted selection achievement



INNOV'noyer project

- Study of *Juglans* spp. genetic resources for the implementation of a marker-assisted selection

Action plan

- Characterization of the 253 accessions from Genetic Resources Center *Juglans*:
  - Available phenotyping data (from 1970 to 2006) and new phenotyping in 2017, 2018, 2019
  - Traits: tree, phenology, fruit and diseases
- Genotyping: SSRs and 600K SNP array Axiom®
- Genome-Wide Association Study

INRA's walnut germplasm

253 accessions
GRC *Juglans* – Fruit Tree Experimental Unit (INRA)

- 217 of *J. regia* L.
  - 194 varieties
  - 23 hybrids
- 36 of related species

J. ailantifolia Carr. - *J. californica* S. Wats.
J. cathayensis Dode - *J. cinerea* L.
J. sieboldiana var. *cordiformis*
J. hindsii Jeps. - *J. major* Heller
J. mandshurica Maxim. - *J. microcarpa* Berl.
J. mollis Engelm. - *J. nigra* L.
J. pitteursii C. Morren (isotype of *J. nigra*)
J. rupestris Engelm. ex Torr. (isotype of *J. major*)
J. sieboldiana Maxim.

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Genetic diversity

253 accessions + 15 SSR markers

WGA 001
WGA 004
WGA 009
WGA 027
WGA 069
WGA 072
WGA 202
WGA 276
WGA 349
WGA 376

Genomic SSRs from ***J. nigra***
- Dangl *et al.*, 2005
- Woeste *et al.*, 2002

~~JR 0160~~
~~JR 1739~~
JR 1817
JR 6160
JR 6439

EST-SSR from ***J. regia***
- Dang *et al.*, 2016

Some alleles: specific of some species

WGA 376

		J. regia		*Rhysocaryon*												*Cardiocaryon*											
All				*J. californica*		*J. hindsii*		*J. major*		*J. microcarpa*		*J. mollis*		*J. nigra*		*J. pitteursii*		*J. rupestris*		*J. ailantifolia*		*J. cathayensis*		*J. cinerea*		*J. cordiformis*		*J. mandshurica*		*J. sieboldiana*	
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269	269	269	269																												
271	271	271	271																												
	273		273																												

Genetic diversity and population structure (2)

Some markers: no transferability

WGA 001

		J. regia		*Rhysocaryon*																*Cardiocaryon*											
All				*J. californica*		*J. hindsii*		*J. major*		*J. microcarpa*		*J. mollis*		*J. nigra*		*J. pitteursii*		*J. rupestris*		*J. ailantifolia*		*J. cathayensis*		*J. cinerea*		*J. cordiformis*		*J. mandshurica*		*J. sieboldiana*	
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Genetic diversity of *J. regia* collection

Locus	A	H_o	H_e	F_{IS}
WGA 001	8	0.65	0.71	0.08
WGA 004	7	0.46	0.50	0.08
WGA 009	7	0.59	0.66	0.11
WGA 027	2	0.43	0.46	0.07
WGA 069	9	0.57	0.76	0.25
WGA 072	6	0.32	0.42	0.24
WGA 202	17	0.68	0.78	0.13
WGA 276	16	0.63	0.76	0.17
WGA 349	11	0.49	0.78	0.37
WGA 376	13	0.61	0.66	0.08
JR 1817	5	0.10	0.15	0.33
JR 6160	10	0.43	0.49	0.12
JR 6439	5	0.14	0.17	0.18
Min	2	0.10	0.15	0.07
Mean	8.92	0.47	0.56	0.17
Max	17	0.68	0.78	0.37

A: total number of alleles, H_o: observed heterozygosity, H_e: expected heterozigosity, F_{IS}: inbreeding coefficient

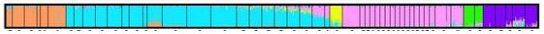
$H_e > H_o$: deficiency of heterozygotes

JR 1817 and JR 6439: lower H_o and H_e

Pop. structure of *J. regia* collection

➢ Method used to determine the « best » K ?

***Structure* Software**

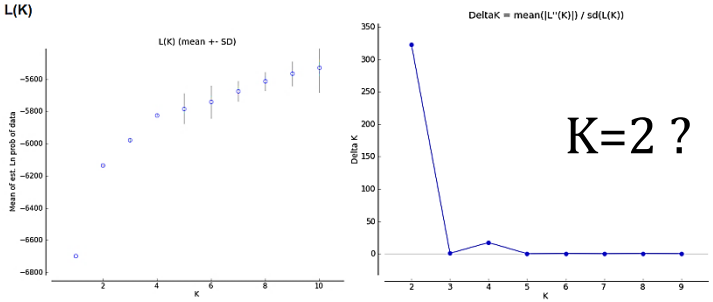


Structure Harvester

Web v0.6.94 July 2014, Plot vA.1 November 2012, Core vA.2 July 2014

Step 1

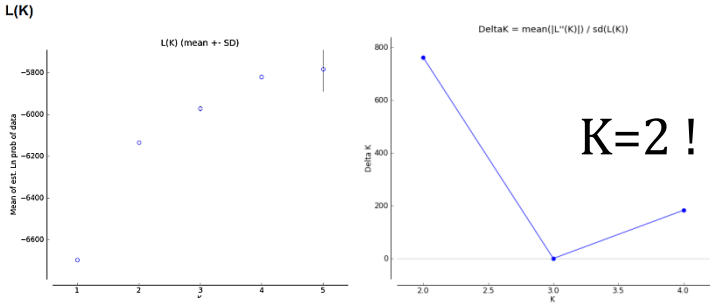
20 runs: K from 1 to 10
Burn-in period: 5,000
Markov Chain Monte Carlo: 50,000
Admixture model
Correlated allele frequencies
Plateau criterion + ΔK method



K=2 ?

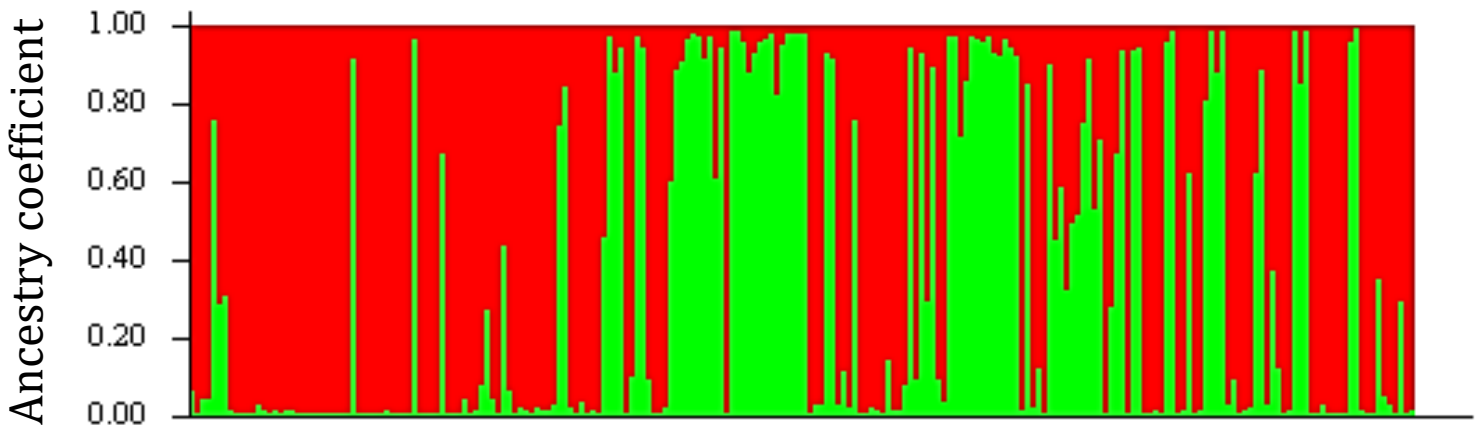
Step 2

10 runs: K from 1 to 5
Burn-in period: 100,000
Markov Chain Monte Carlo: 750,000



K=2 !

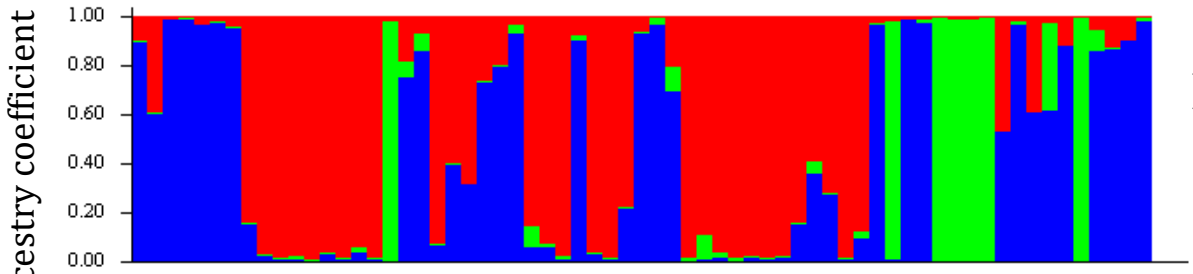
Bar plot K=2



Population 1: Eastern European and Asian accessions (x 63)
Population 2: Western European and American accessions (x 127)

Pairwise F_{ST} = 0.101
H_e, H_o, F_{IS} lower for Population 2

Subclustering



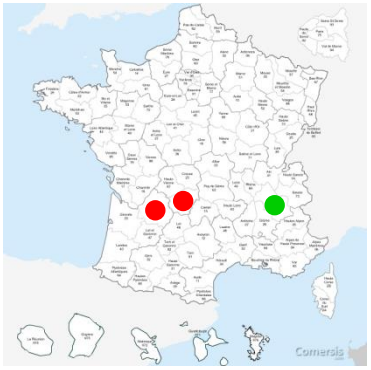
K=3

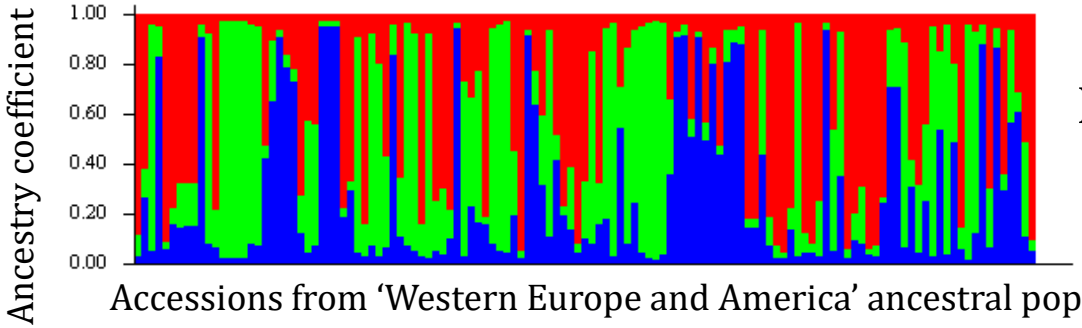
Accessions from 'Eastern Europe and Asia' ancestral population

Ukraine, Romania

China, Japan, Greece

Romania, Bulgaria, Poland, Hungary, Ukraine, Greece, Iran, China, India





K=3

Accessions from 'Western Europe and America' ancestral population

France, Germany, Spain, USA

France, USA

USA, Chile, France, England, Portugal, Hungary, Greece

Genetic diversity and population structure (7)

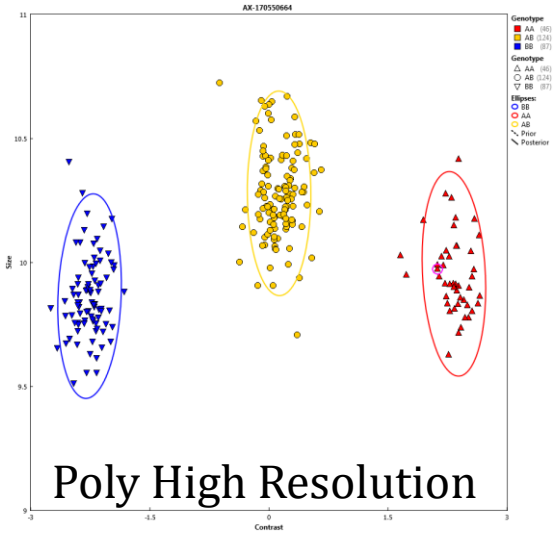
600K SNP genotyping on 170 accessions

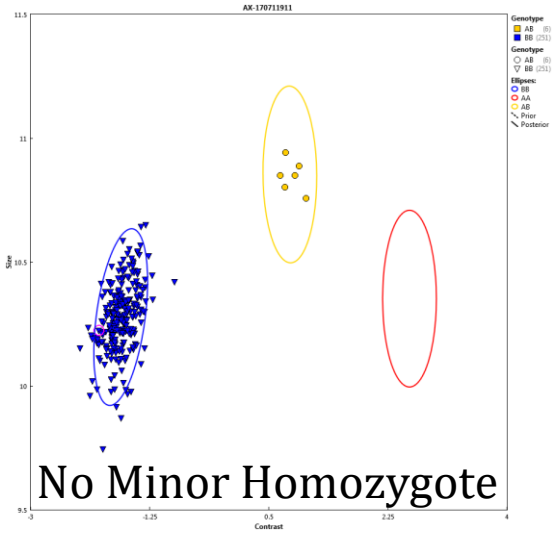
GWAS

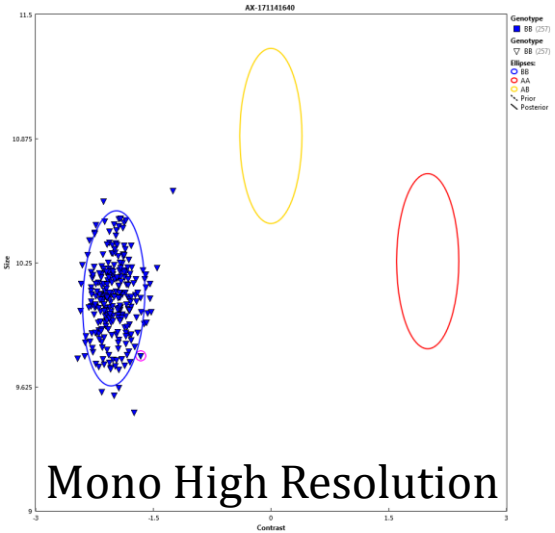
Category	Number of markers	% of markers
PolyHighResolution	397,921	65.27
NoMinorHom	75,564	12.39
MonoHighResolution	36,684	6.02
CallRateBelowThreshold	27,761	4.55
OffTargetVariant	4,787	0.79
Other	66,941	10.98
Total	609,658	100

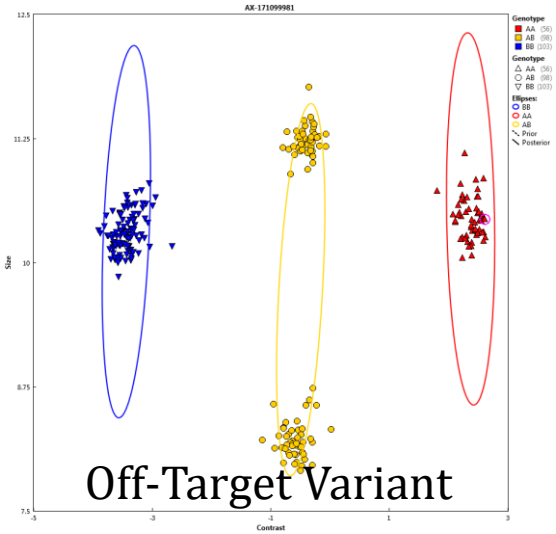
Genome-wide SNP genotyping (1)

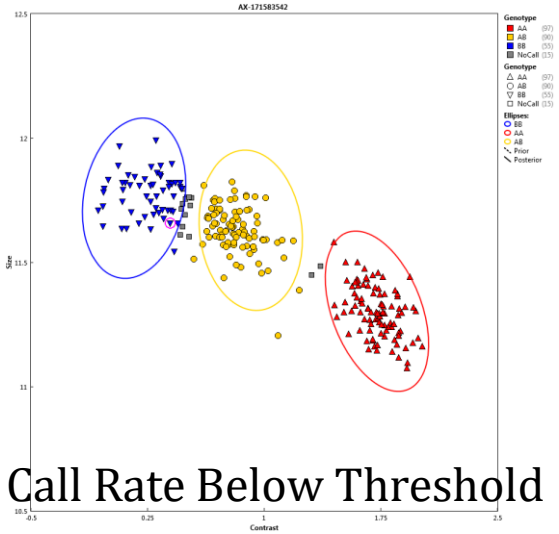
SSR Genetic Diversity Assessment of the INRA's Walnut Germplasm Collection











473,485 SNP – 77.66%

Genome-wide SNP genotyping (2)

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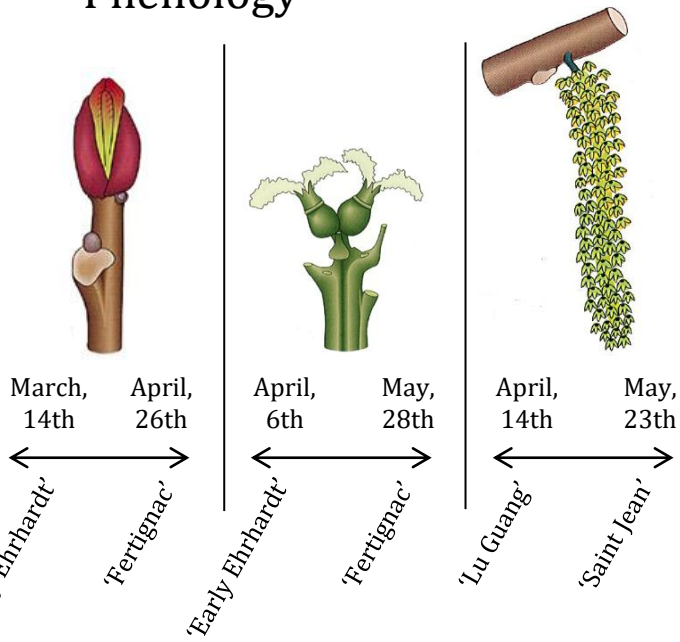
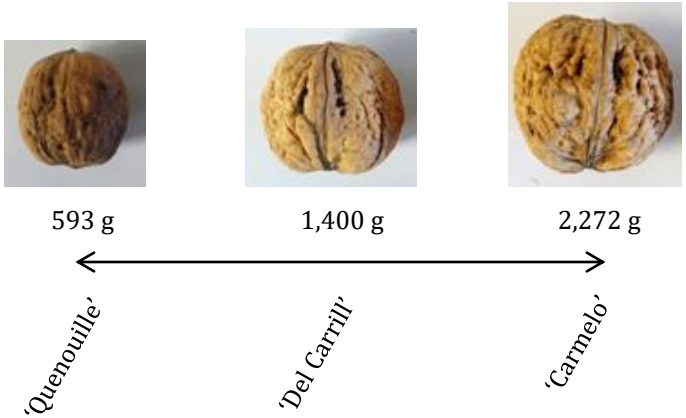
Phenotyping on 170 accessions

GWAS

Phenology



Weight of 100 dry in-shell walnuts



Fruit bearing habit

Walnut size

Walnut shape

Suture strength

Maturity fruit date

Shell thickness

Growth habit

Kernel weight

Kernel color

Diseases

Prospects

- GWAS on a wide range of traits observed over many years
- To provide tools for marker-assisted selection

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- Eric Germain, former head of INRA's breeding program from 1977 to 2007
- Tree Experimental Unit in Toulenne and *Prunus/Juglans* Genetic Resources Center
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- « BioGEVES » laboratory for SSR amplifications

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İlginiz için teşekkür ederim

Thanks for your attention

Merci de votre attention

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Any questions ?

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