



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



**XXX. INTERNATIONAL
HORTICULTURAL CONGRESS**

12 - 16 AUGUST 2018 ISTANBUL - TURKEY

Bridging the World through Horticulture

INNOV'noyer project

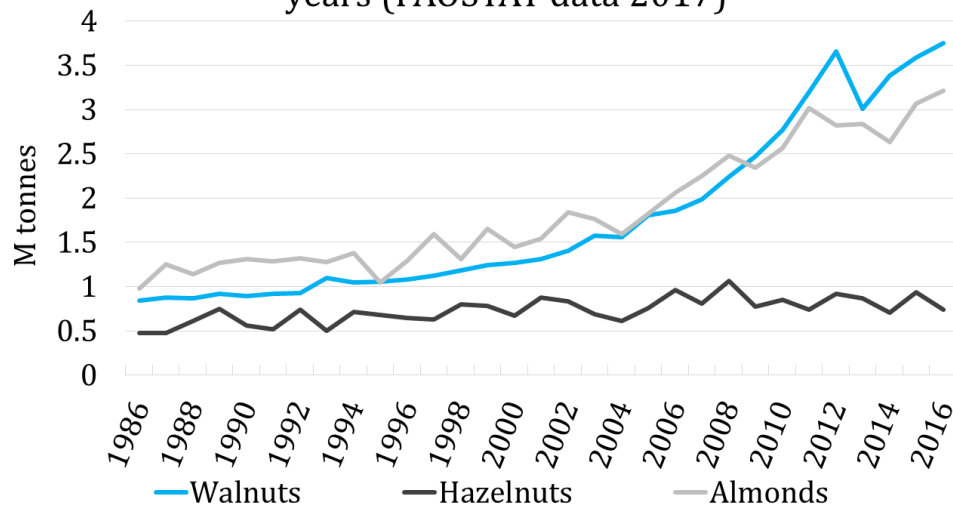


- Study of *Juglans* spp. genetic resources for the implementation of a marker-assisted selection

Context

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

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



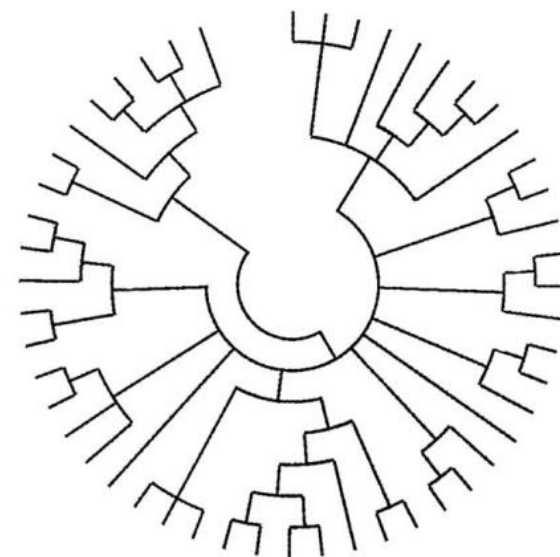
INNOV'noyer project



- 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

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INRA's walnut germplasm

253 accessions

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

217 of *J. regia* L.

36 of related species

194 varieties

23 hybrids



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.

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. aillantifolia		J. cathayensis		J. cinerea		J. cordiformis		J. mandshurica		J. sieboldiana		
234	234																															
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263	263	263	263																													
265	265	265	265																													
269	269	269	269																													
271	271	271	271																													
	273		273																													

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	
184	184									184	184																				
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	219															219															

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

H_e > H_o : deficiency of heterozygotes

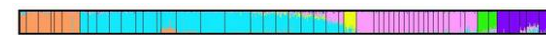
JR 1817 and JR 6439: lower H_o and H_e

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

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

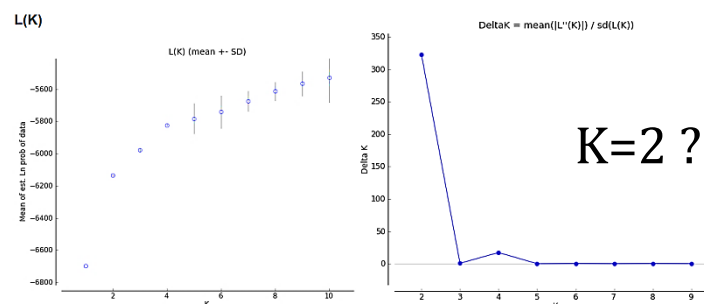
Burn-in period: 5,000

Markov Chain Monte Carlo: 50,000

Admixture model

Correlated allele frequencies

Plateau criterion + ΔK method

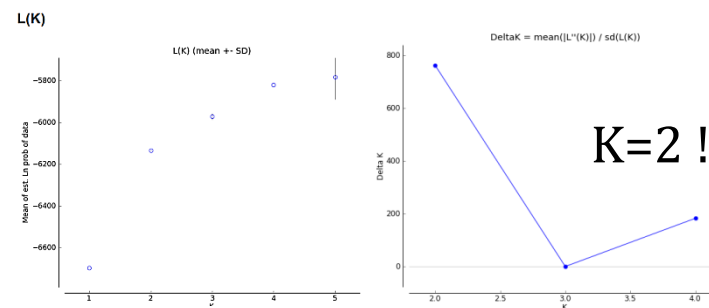


Step 2

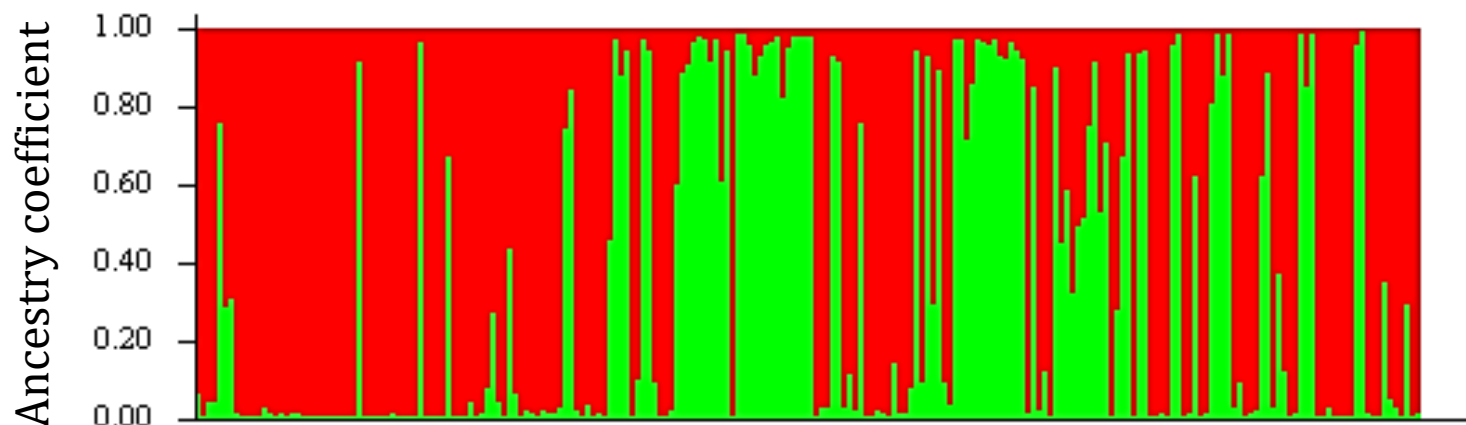
10 runs: K from 1 to 5

Burn-in period: 100,000

Markov Chain Monte Carlo: 750,000



Bar plot K=2



Population 1: Eastern European and Asian accessions (x 63)

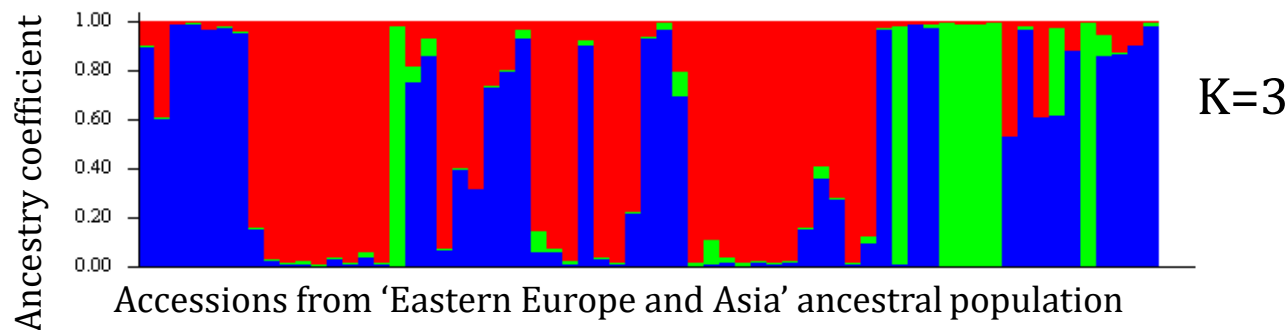
Population 2: Western European and American accessions (x 127)

Pairwise $F_{ST} = 0.101$

H_e , H_o , F_{IS} lower for Population 2

Genetic diversity and population structure (6)

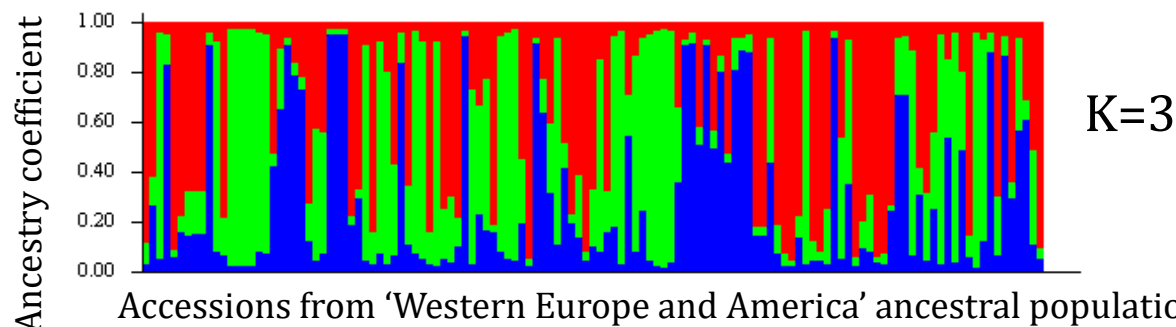
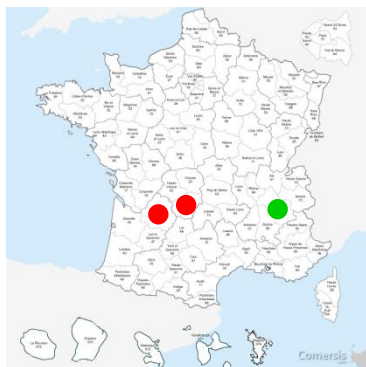
Subclustering



Ukraine, Romania

China, Japan, Greece

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



France, Germany, Spain, USA

France, USA

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

600K SNP genotyping on 170 accessions

GWAS

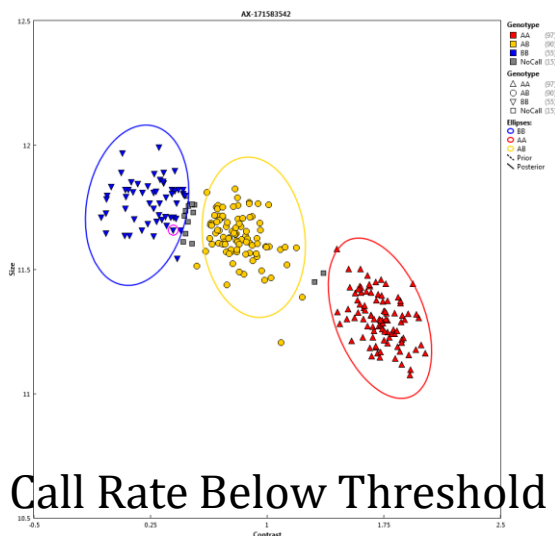
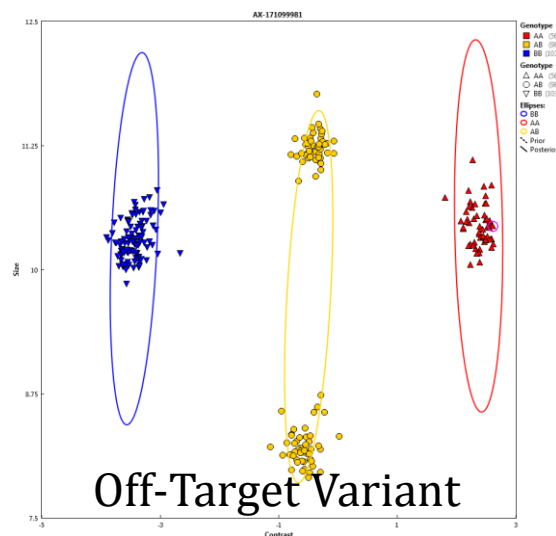
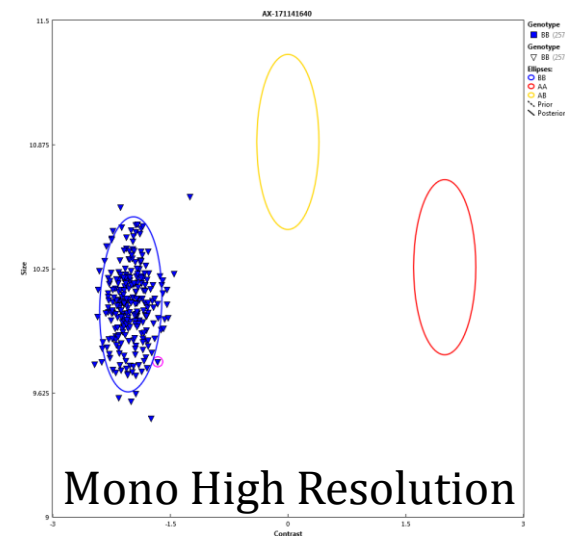
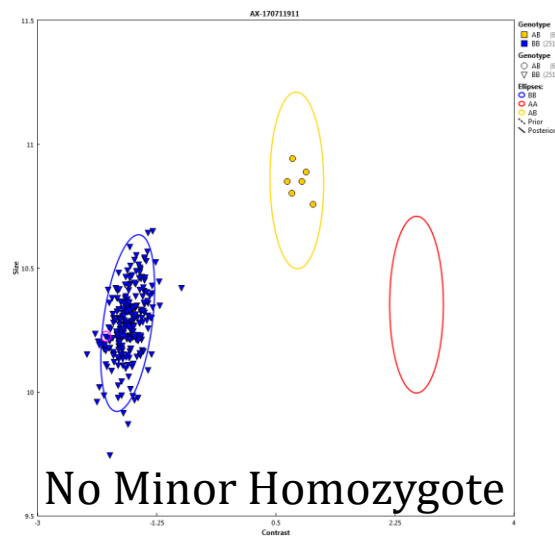
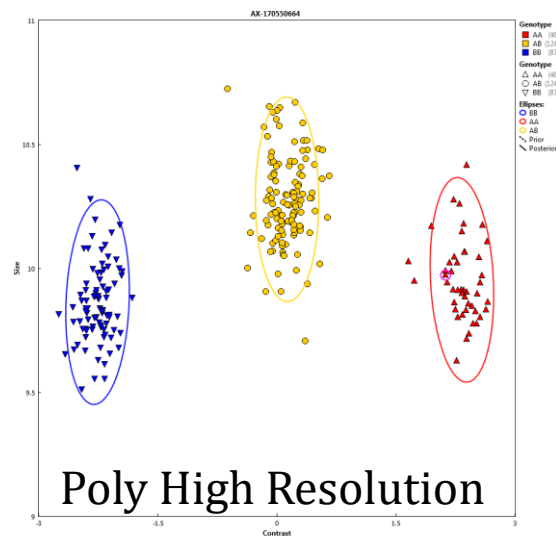
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Category	Number of markers	% of markers
PolyHighResolution	397,921	65.27
NoMinorHom	75,564	12.39
MonoHighResolution	36,684	6.02
CallRateBelowThreshold	27,761	4.55
OffTargetVariant	4,787	0.79
Other	66,941	10.98
Total	609,658	100

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

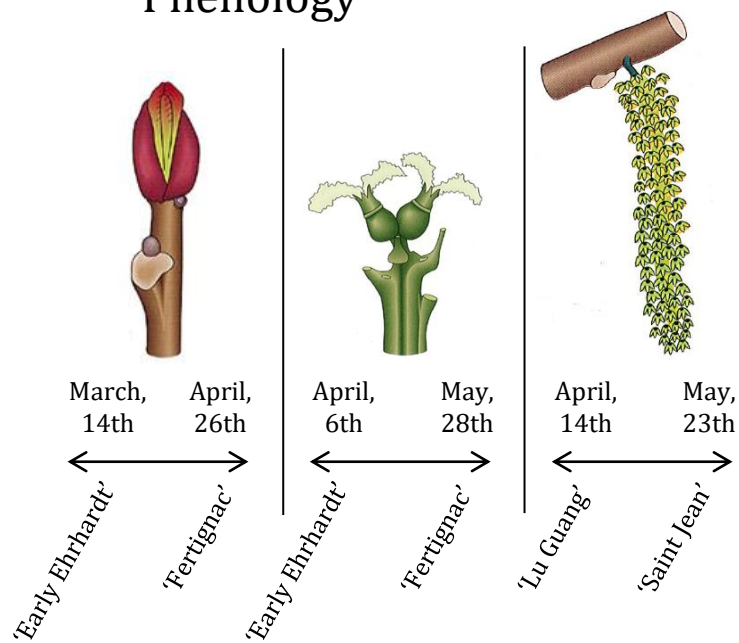


473,485 SNP –
77.66%

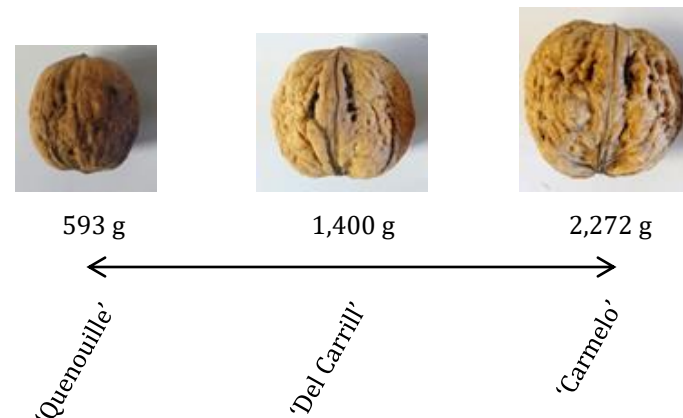
Phenotyping on 170 accessions

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

Acknowledgements

- Eric Germain, former head of INRA's breeding program from 1977 to 2007
- Tree Experimental Unit in Toulence and *Prunus/Juglans* Genetic Resources Center
- « Région Nouvelle-Aquitaine » for funding the project 
- « ANRT » for funding the PhD  
association nationale
recherche technologie
- « BioGEVES » laboratory for SSR amplifications 

İlginiz için teşekkür ederim

Thanks for your attention

Merci de votre attention

Any questions ?

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