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### ► To cite this version:

Anthony Bernard, Fabrice Lheureux, Elisabeth Dirlewanger. SSR genetic diversity assessment of French walnut (*Juglans* spp.) germplasm collection. XXX. International Horticultural Congress, ISHS, Aug 2018, Istanbul, Turkey. hal-02915374

**HAL Id: hal-02915374**

**<https://hal.inrae.fr/hal-02915374>**

Submitted on 17 Nov 2023

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

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**XXX. INTERNATIONAL  
HORTICULTURAL CONGRESS**

**12 - 16 AUGUST 2018 ISTANBUL - TURKEY**

*Bridging the World through Horticulture*

# INNOV'noyer project

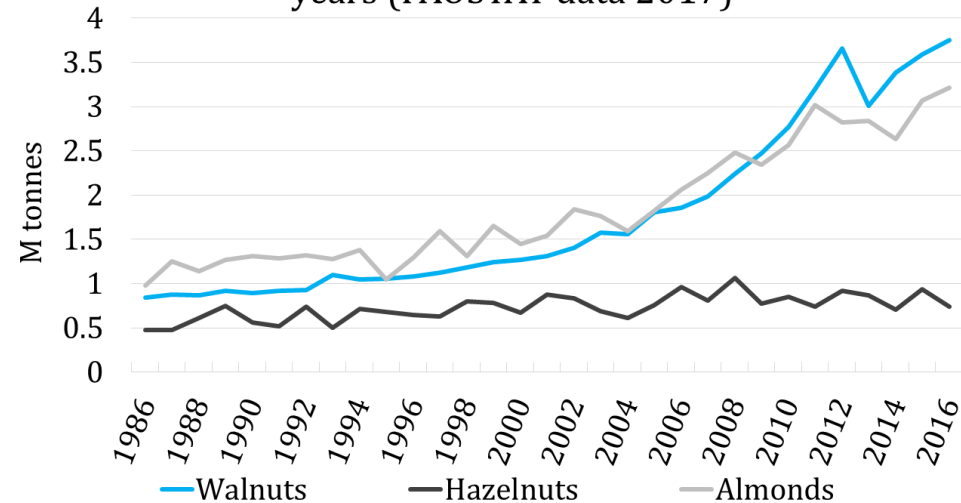


- Study of *Juglans* spp. genetic resources for the implementation of a marker-assisted selection

## 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)



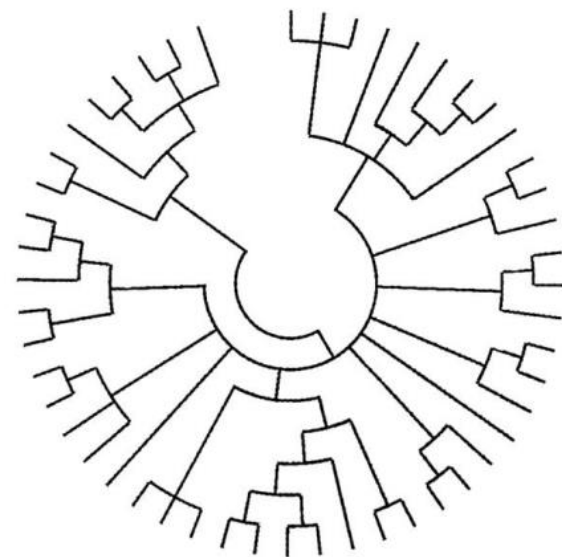
## INNOV'noyer project



- 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



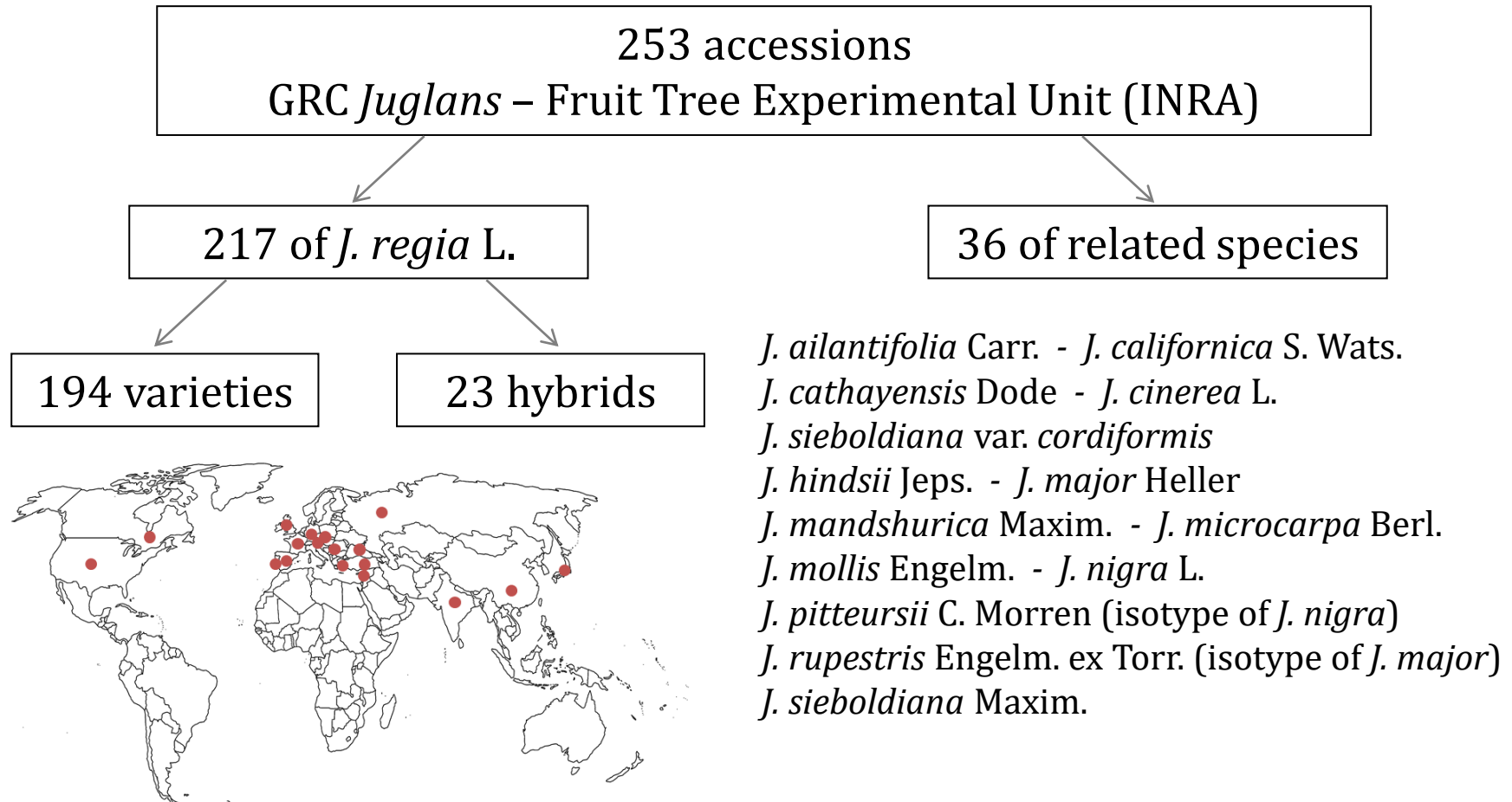
- Study of *Juglans* spp. genetic resources for the implementation of a marker-assisted selection

## 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



# INRA's walnut germplasm



## 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

All		J. <i>regia</i>		Rhysocaryon										Cardiocaryon										
				<i>J. californica</i>	<i>J. hindsii</i>	<i>J. major</i>	<i>J. microcarpa</i>	<i>J. mollis</i>	<i>J. nigra</i>	<i>J. pitteursii</i>	<i>J. rupestris</i>	<i>J. aillantifolia</i>	<i>J. cathayensis</i>	<i>J. cinerea</i>	<i>J. cordiformis</i>	<i>J. mandshurica</i>	<i>J. sieboldiana</i>							
234	234																	234	234					
236	236	236	236	236	236	236	236	236	236	236	236	236	236	236	236	236	236							
238																		238	238	238			238	
240																							240	
242	242	242																					242	242
244																							244	244
246																							246	
248	248	248	248																					
250	250	250	250																					
255	255	255	255																				255	
257	257	257	257																					
259	259	259	259																					
261	261	261	261																					
263	263	263	263																					
265	265	265	265																					
269	269	269	269																					
271	271	271	271																					
273																								



# Some markers: no transferability

## WGA 001

All		<i>J. regia</i>		<i>Rhysocaryon</i>							<i>Cardiocaryon</i>					
				<i>J. californica</i>	<i>J. hindsii</i>	<i>J. major</i>	<i>J. microcarpa</i>	<i>J. mollis</i>	<i>J. nigra</i>	<i>J. pitteursii</i>	<i>J. rupestris</i>	<i>J. ailantifolia</i>	<i>J. cathayensis</i>	<i>J. cinerea</i>	<i>J. cordiformis</i>	<i>J. mandshurica</i>
184	184					184	184									
195		195														
197								197								
198	198	198	198													
199				199	199			199			199					
200	200	200	200													
201	201							201	201		201	201				
202	202	202	202													
203	203	203		203	203	203	203	203	203							
204		204														
205	205							205	205							
206	206	206	206													
207	207					207		207	207							
208	208	208	208													
210	210	210	210													
		213				213		213								
		219							219							

# Genetic diversity of *J. regia* collection

Locus	A	H <sub>o</sub>	H <sub>e</sub>	F <sub>IS</sub>
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<sub>e</sub> > H<sub>o</sub> : deficiency of heterozygotes

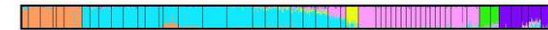
JR 1817 and JR 6439: lower H<sub>o</sub> and H<sub>e</sub>

A: total number of alleles, H<sub>o</sub>: observed heterozygosity,  
H<sub>e</sub>: expected heterozygosity, F<sub>IS</sub>: 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

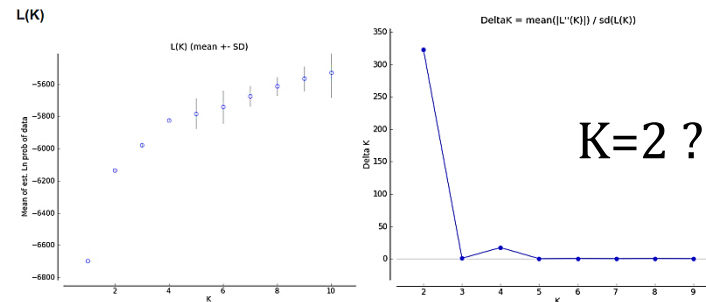
Burn-in period: 5,000

Markov Chain Monte Carlo: 50,000

Admixture model

Correlated allele frequencies

Plateau criterion +  $\Delta K$  method

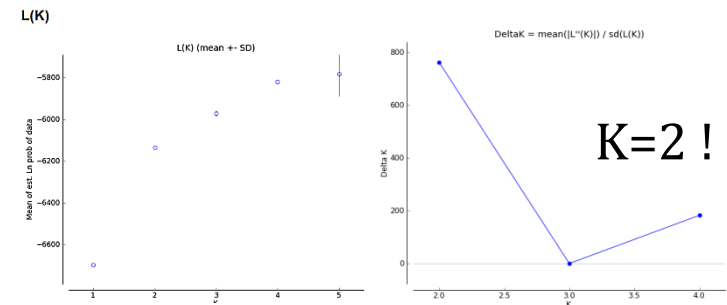


## Step 2

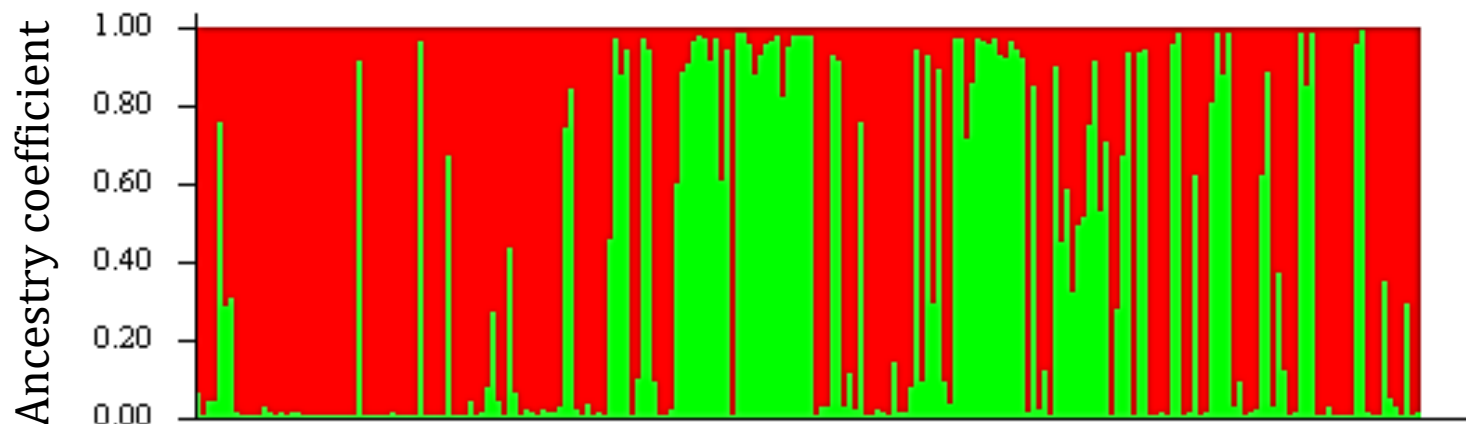
10 runs: K from 1 to 5

Burn-in period: 100,000

Markov Chain Monte Carlo: 750,000



# Bar plot K=2



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

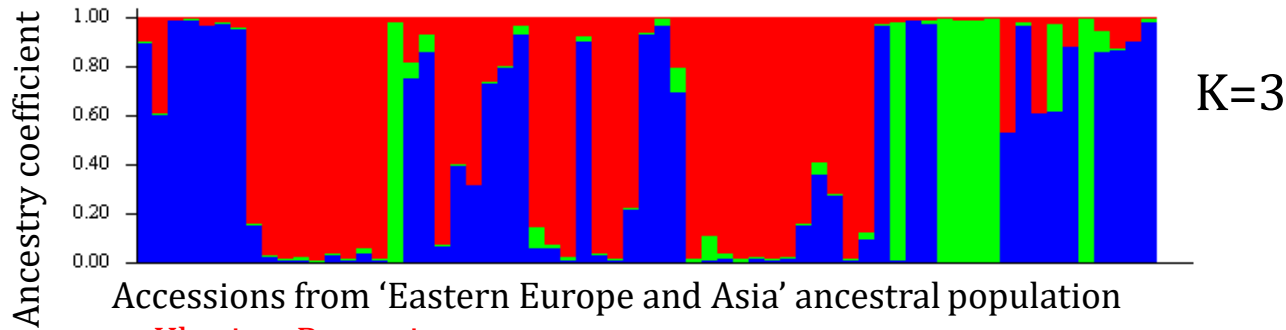
Population 2: Western European and American accessions (x 127)

Pairwise  $F_{ST} = 0.101$

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

Genetic diversity and population structure (6)

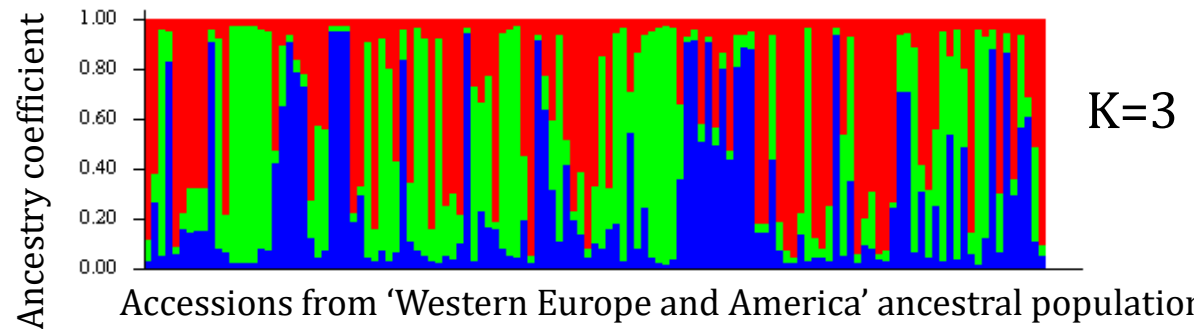
## Subclustering



Ukraine, Romania

China, Japan, Greece

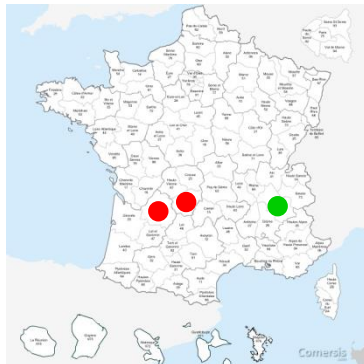
Romania, Bulgaria, Poland, Hungary, Ukraine, Greece, Iran, China, India



France, Germany, Spain, USA

France, USA

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



## 600K SNP genotyping on 170 accessions

GWAS

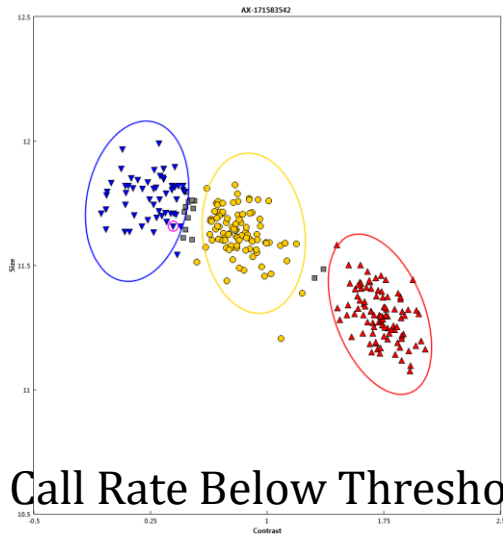
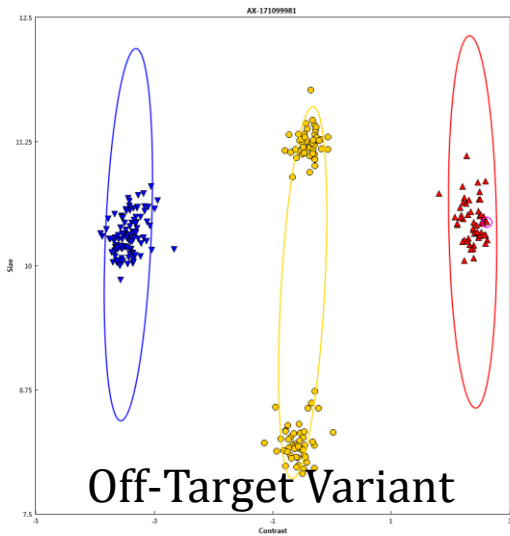
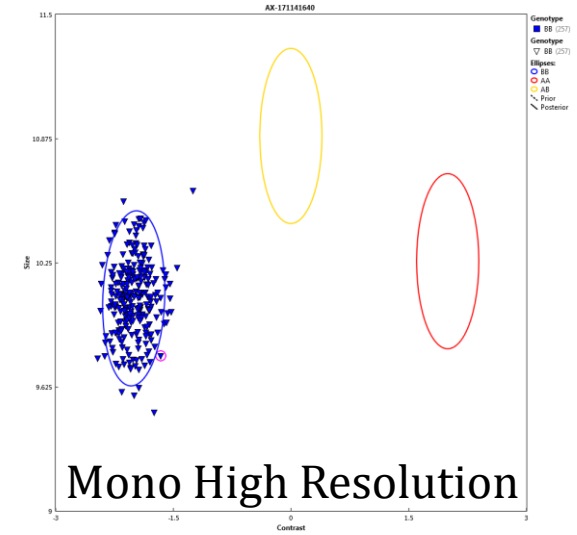
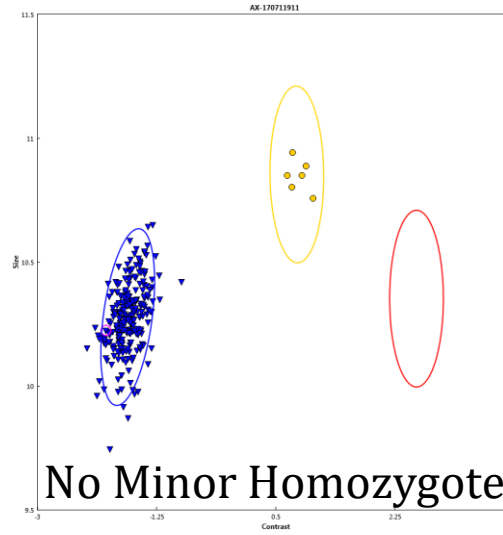
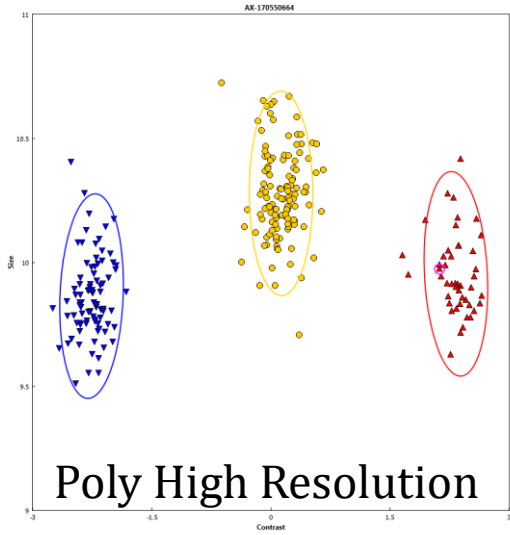
ThermoFisher  
SCIENTIFIC

UC DAVIS  
UNIVERSITY OF CALIFORNIA



Category	Number of markers	% of markers
<b>PolyHighResolution</b>	397,921	<b>65.27</b>
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
<b>Total</b>	<b>609,658</b>	<b>100</b>

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



473,485 SNP –  
77.66%

## Phenotyping on 170 accessions

GWAS

Weight of 100 dry in-shell walnuts



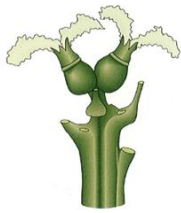
593 g      1,400 g      2,272 g

← Quenouille'      'Del Carrill'      'Carmelo' →

### Phenology



March, 14th      April, 26th



April, 6th      May, 28th



April, 14th      May, 23th

← 'Early Ehrhardt'      'Fertignac' →

← 'Early Ehrhardt'      'Fertignac' →

← 'Lu Guang'      'Saint Jean' →

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   
anrt 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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