

**Analysis of candidate gene polymorphisms of rosebushes
from 18th and 19th centuries by a combination of
Access-Array Fluidigm PCR and Illumina MiSeq sequencing**

Jérémy CLOTAULT, Mathilde LIORZOU, Nathalie JACQUIER, Douae
BEN HDECH, and the members of the project FloRHiGe

Context: project FloRHiGe

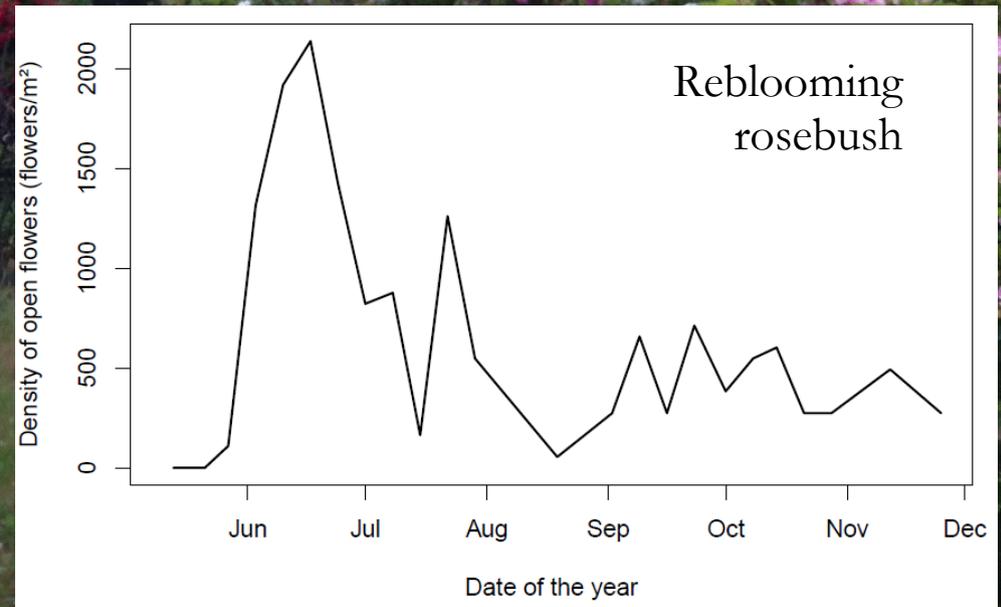
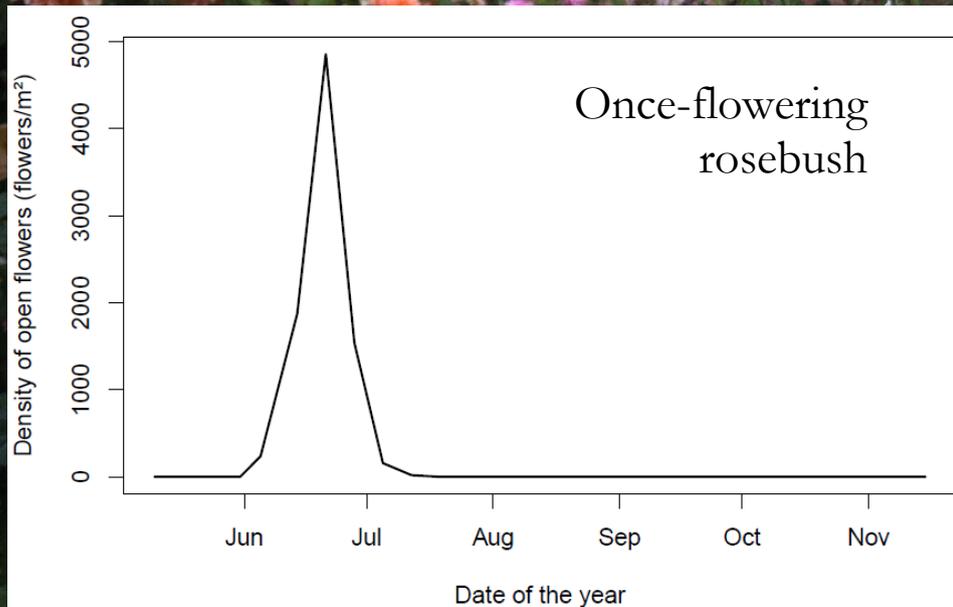


- Main issue: studying the historical construction of the diversity of rosebushes during the 18th and 19th centuries
- Interdisciplinary project between biologists (IRHS, EPGV) and historians (CERHIO, CFV)
- Funding: Region 'Pays de la Loire'

Context: rose breeding in 19th century

The 19th century was the Golden Age for rose breeding

Among the selected traits, recurrent blooming, introduced from China

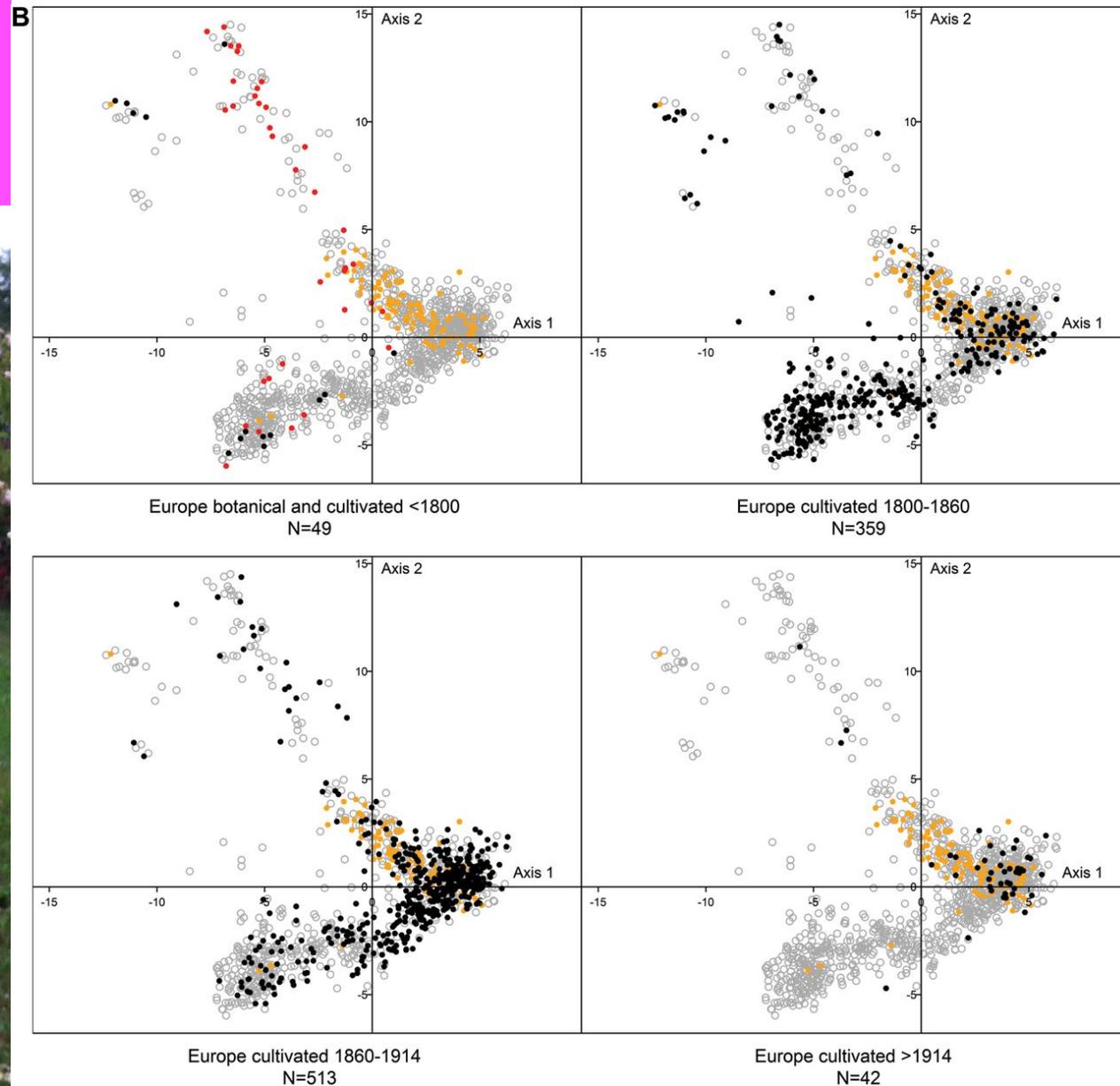


And several other traits, like perfume, colour, etc.

Context: some results for genetic background

Methods: 1228 rosebushes genotyped by 32 SSR markers

Results: Genetic background of European hybrids shifted towards Chinese background during the 19th century



Liorzou et al. 2016 – J. Exp. Bot. 67 (15) 4711-4725

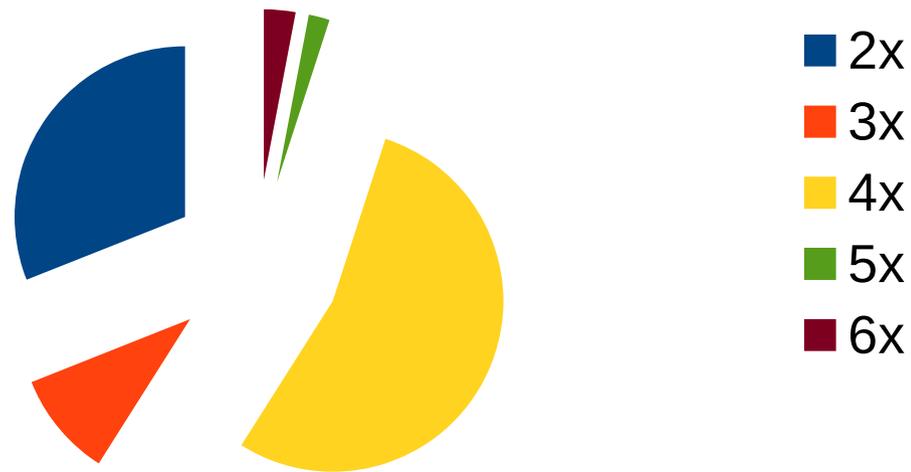
Objectives

- Detection of polymorphisms of candidate genes
 - SNPs, but also InDels, frequent in interspecific hybrids
 - Allele dosage in a sample with mixed ploidy
- Study of the impact of the breeding history on the gene diversity
 - Link with phenotype?
 - Signatures of selection?

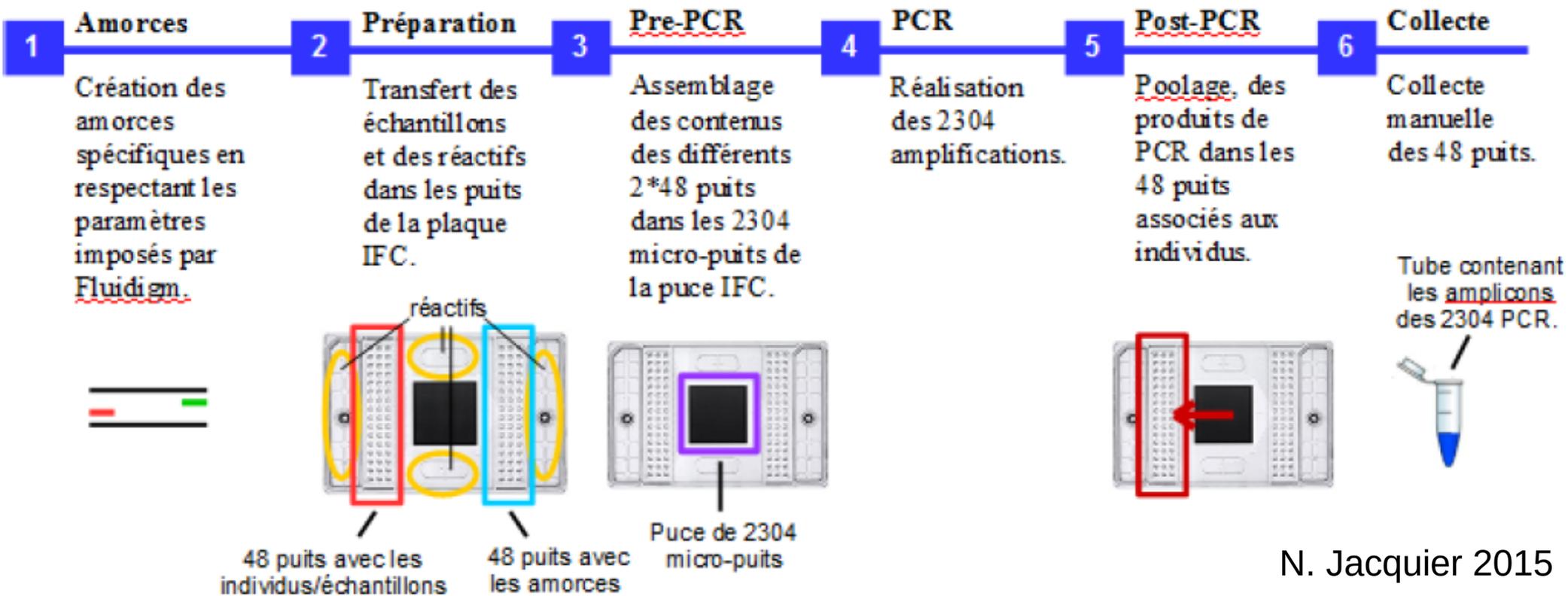
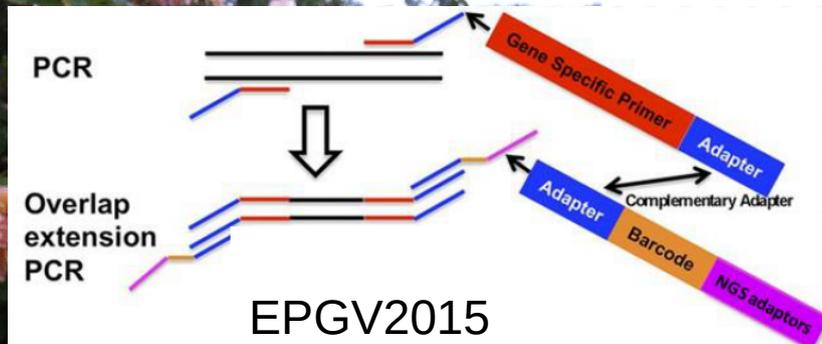
Materials and methods: the sample

365 rosebushes representative from the diversity of Liorzou et al. (2016)

Ploidy level



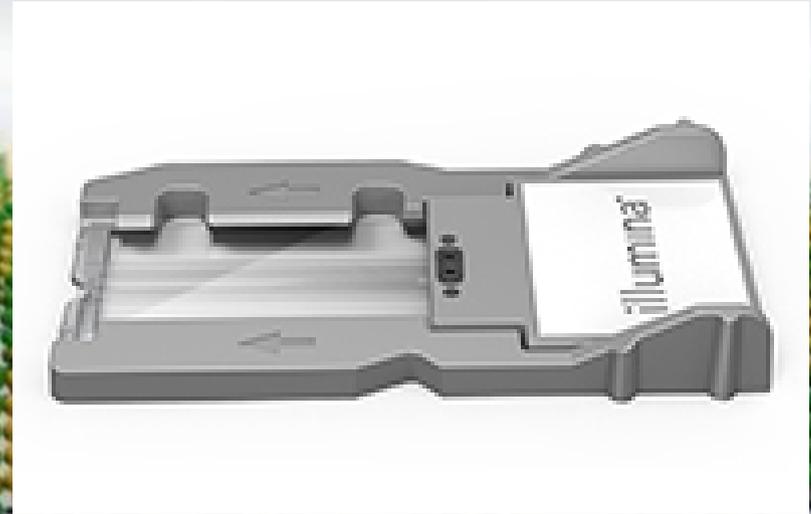
Materials and Methods: Access Array Fluidigm PCR



Materials and Methods: Illumina MiSeq sequencing



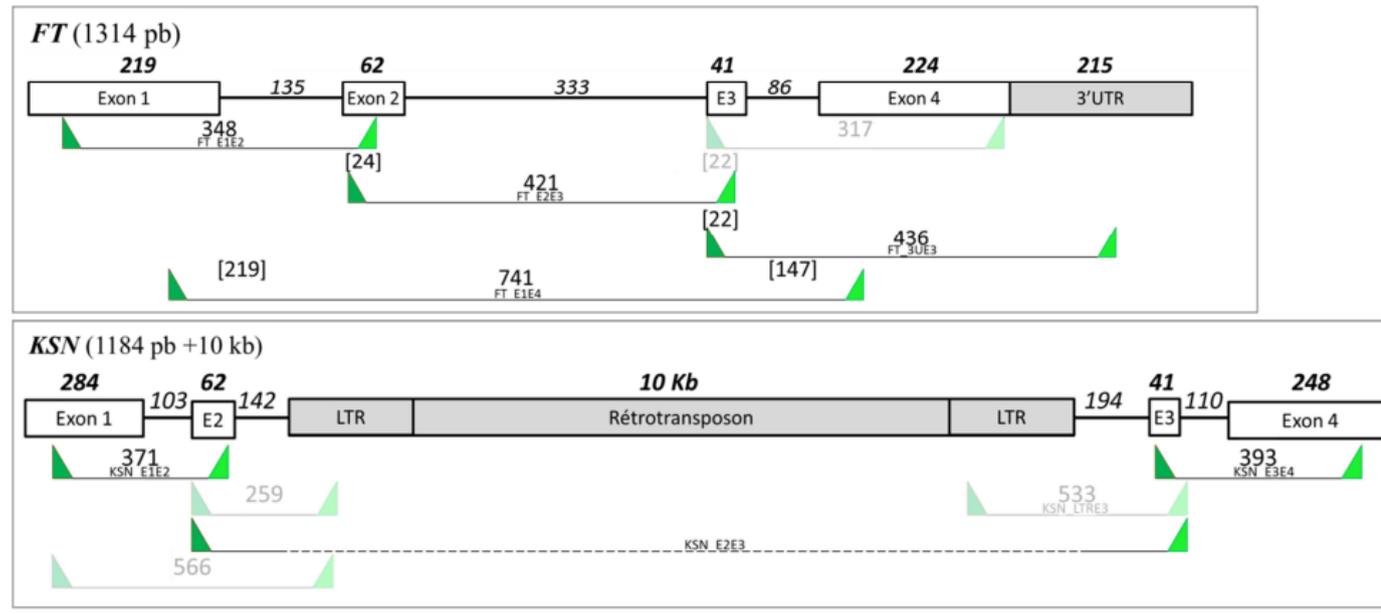
MiSeq sequencer



MiSeq flow cell

Materials and Methods: genes

Floraison

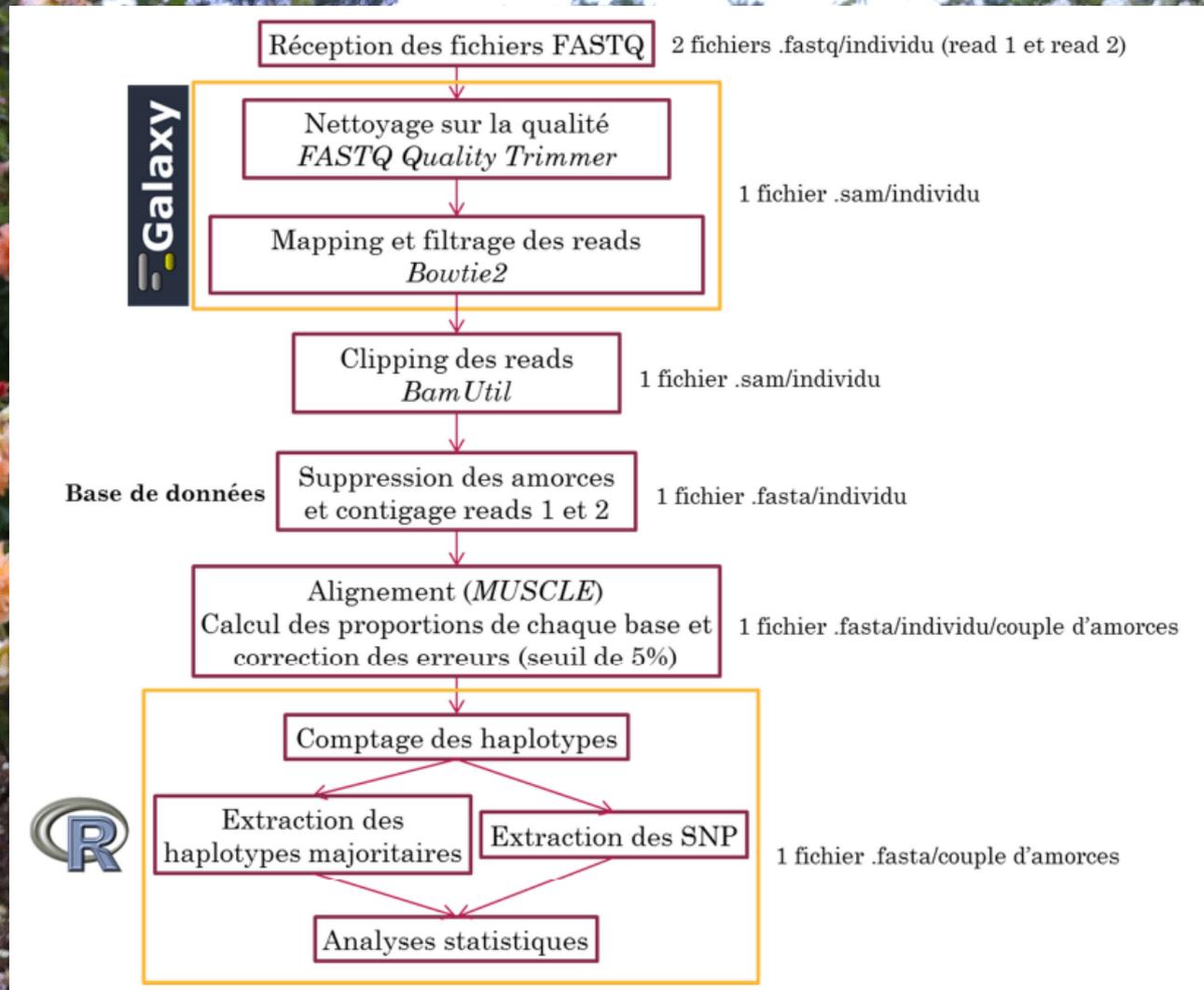


+ 9 other candidate genes
Double flower (1)
Colour (5)
Perfume (2)
Plant shape (1)

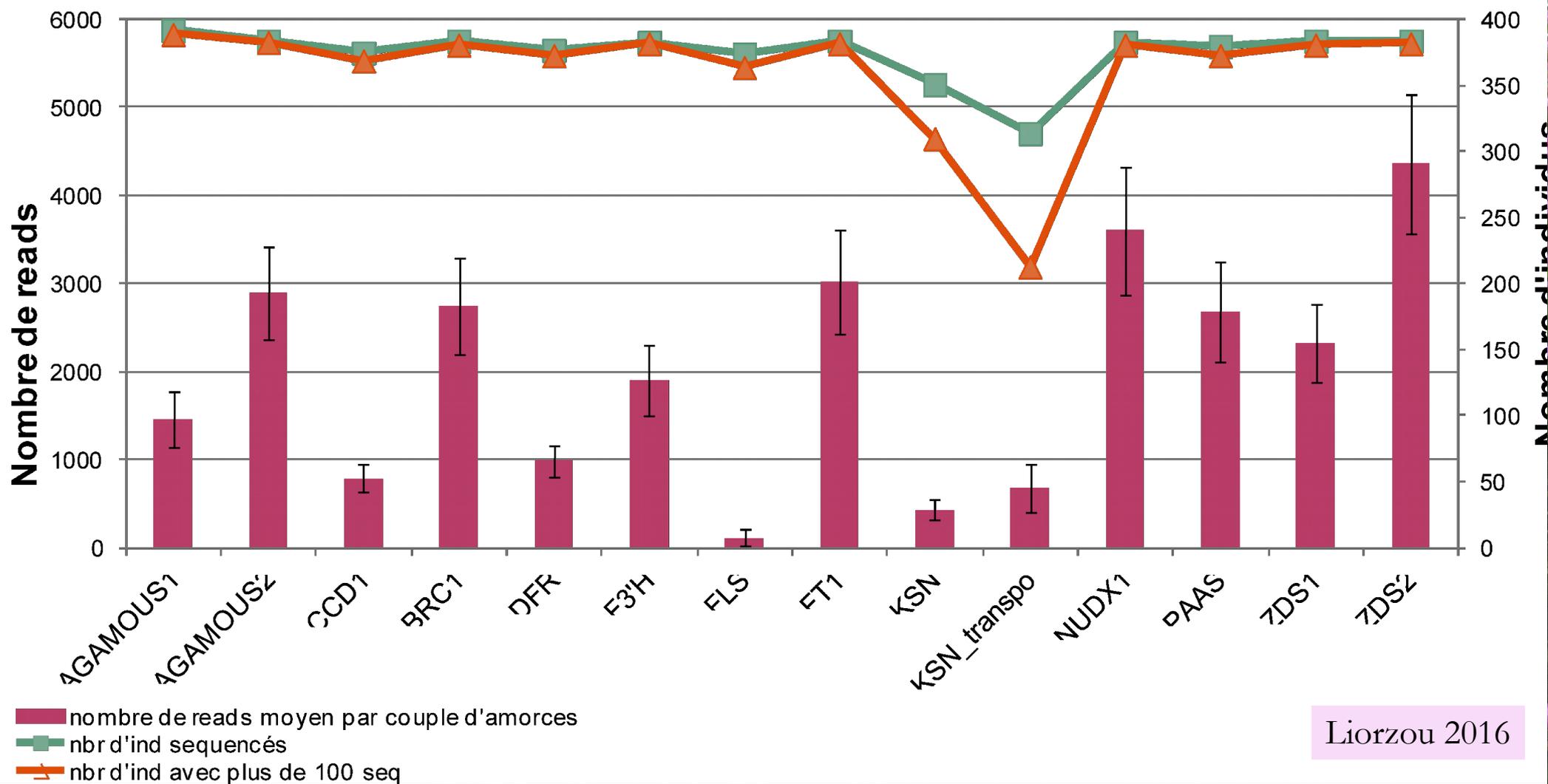
+ 50 portions of randomly chosen genes
(amplified in duplex)

Liorzou 2016

Materials and Methods: bioinformatics

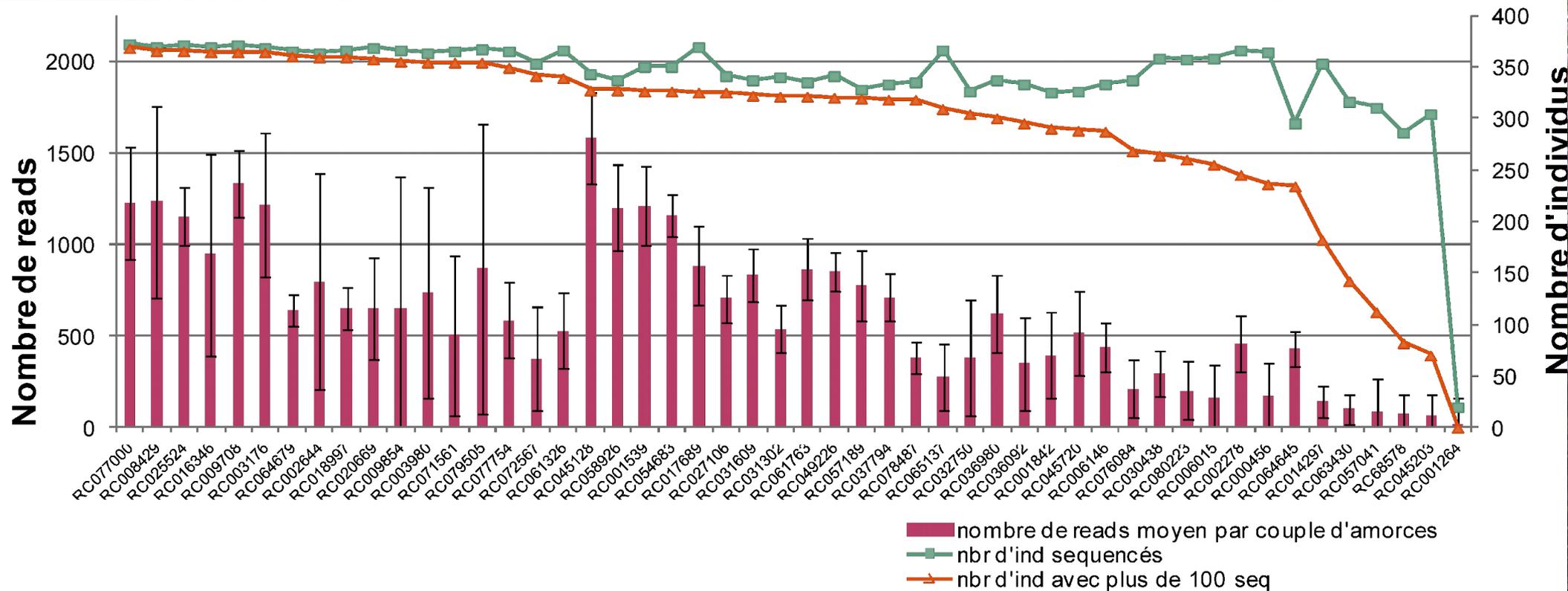


Results / success rates: candidate genes



Liorzou 2016

Results / success rates: randomly chosen genes



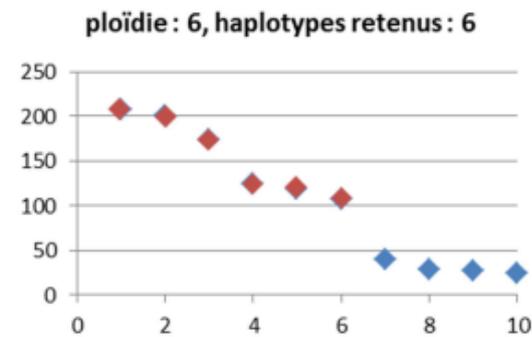
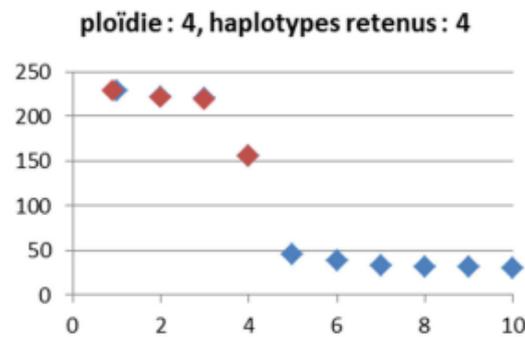
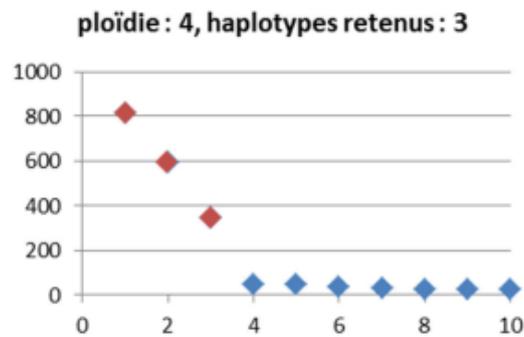
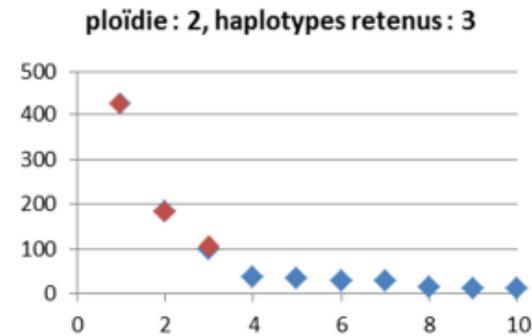
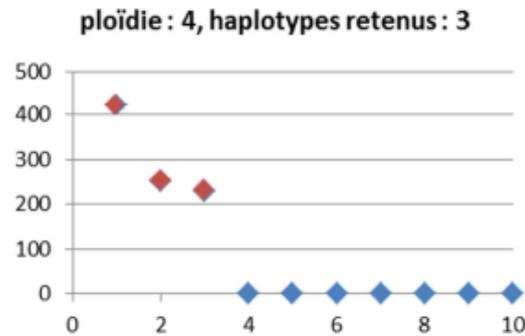
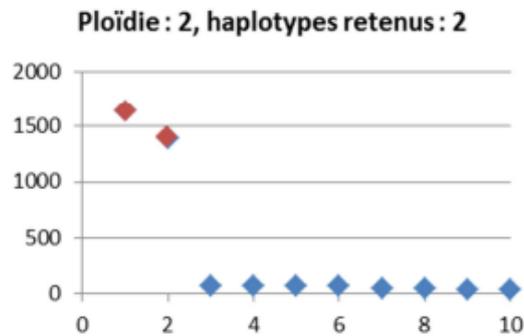
Liorzou 2016

Results / success rates: conclusions

- Of the 141 primer pairs, 86 worked well (gene portions having more than 100x depth per individual and having worked for more than half of individuals) : success rate of 61 %
- Main reasons:
 - Primer sites
 - Lower conservation for primers in introns or UTRs
 - High polymorphism rate in the sample (interspecific hybrids)
 - Amplicon itself
 - No amplification if large unpredictable indels
 - Elimination of small amplicons (<300 bp) during purification
 - PCR competition for duplexes of randomly chosen genes ?

Results / haplotype identification & allele dosage

- Haplotype identification - Were kept:
 - Only the n haplotypes above the frequency plateau, if $n \leq x$ (ploidy level)
 - Only the x most frequent haplotypes, if $n > x$



Results / haplotype identification & allele dosage

- Comparison of observed haplotype frequencies with expected haplotype frequencies according ploidy level

Expected haplotype frequency boundaries

2x	0-0.5		0.5-1			
3x	0-0.33	0.33-0.67	0.67-1			
4x	0-0.25	0.25-0.5	0.5-0.75	0.75-1		
5x	0-0.2	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1	
6x	0-0.17	0.17-0.33	0.33-0.5	0.5-0.67	0.67-0.83	0.83-1

$$\left| \frac{f_h - V}{L} \right|$$

- Allele dosage according the proximity of a boundary
- Use of a confidence index:

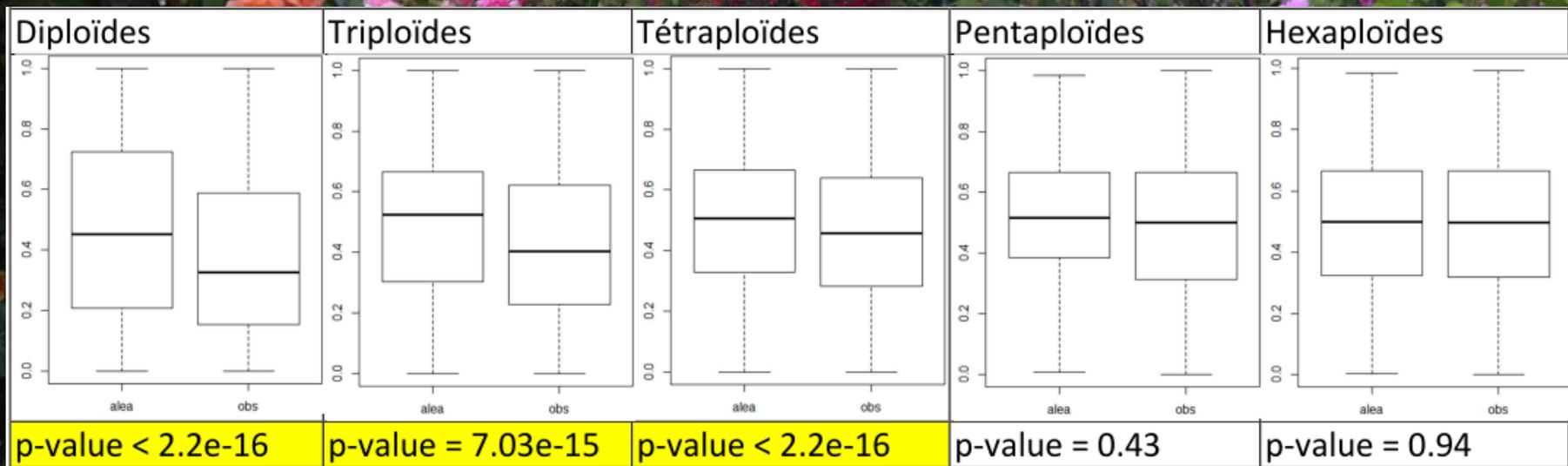
f_h : observed frequency

V: nearest expected boundary

L: inter-mid-boundary width

Results / haplotype identification & allele dosage

- Comparison between observed dataset and simulated datasets:



- Even if dosage is better for 2x-4x than random haplotype frequency, there is a huge variance

Conclusions: technical aspects

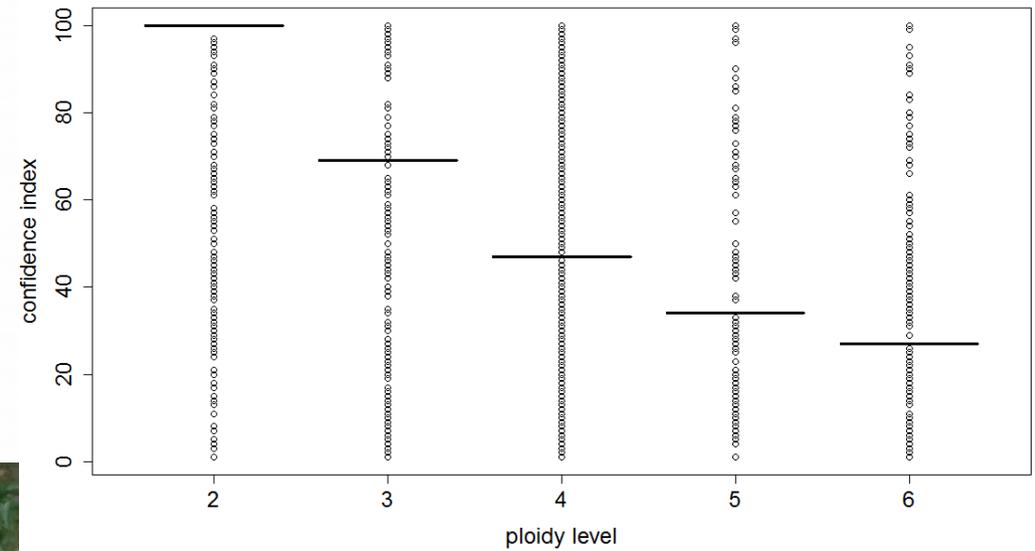
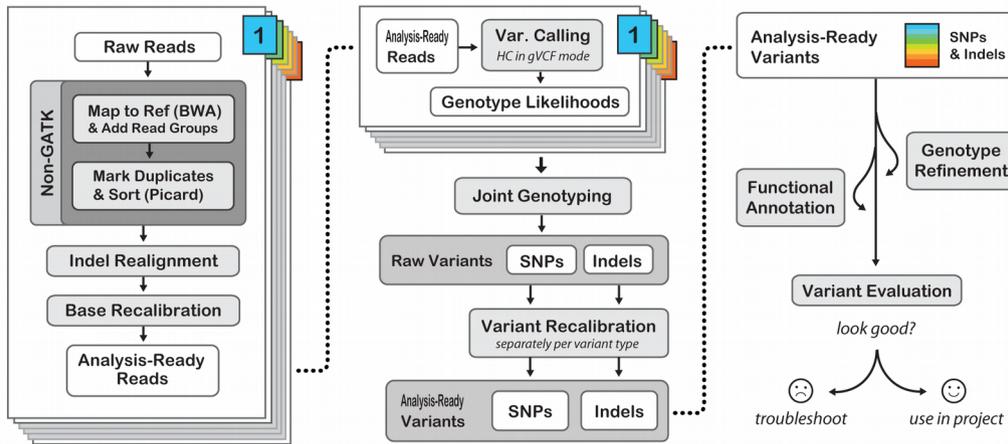
- Combination of Access-Array Fluidigm PCR and Illumina MiSeq sequencing is efficient to detect polymorphisms for candidate genes
- The possibility of allele dosage in polyploids by this approach requires further examination

Conclusions: technical aspects

Pipeline for variant genotyping



Data Pre-processing >> Variant Discovery >> Preliminary Analyses

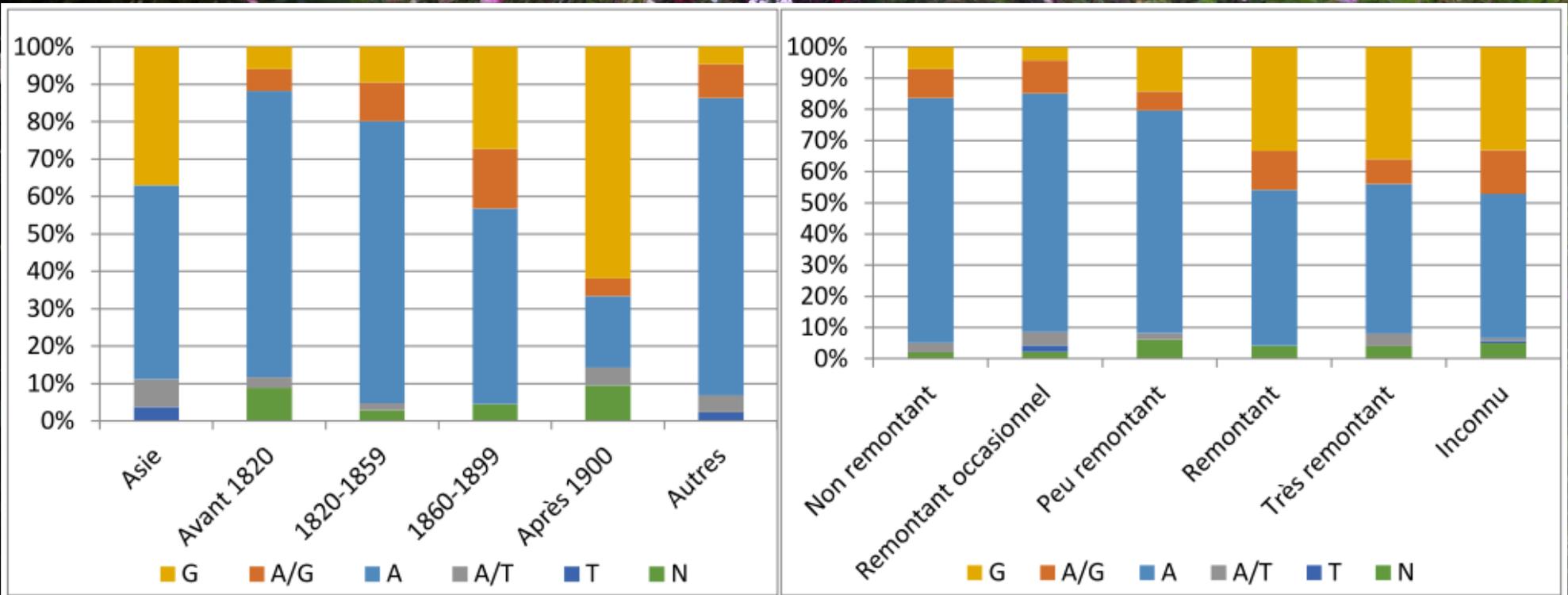


Pipeline description

Confidence index according ploidy level

Conclusions: some biological results

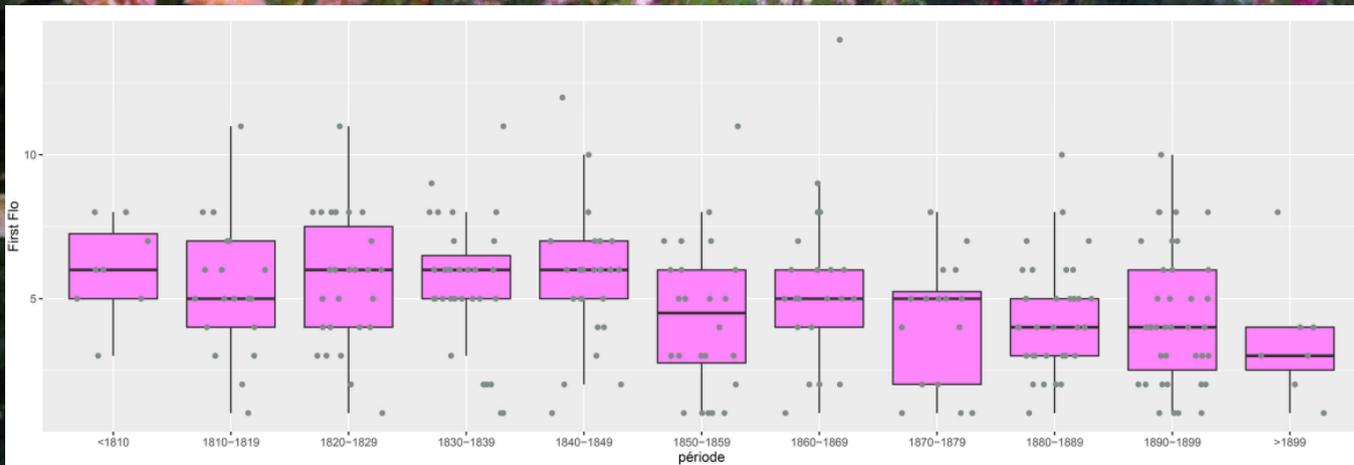
- *RoKSN*: selection for the copia allele, inactivating the floral repressor → increase of recurrent blooming during the 19th century



Frequency of presence of alleles at SNP A292G, linked to copia

Conclusions: some biological results

- Earlier flowering along the 19th century



Date of first flowering according breeding periods

- *RoFT*: Some allele variants are associated to date of flowering (Ben Hdech 2016)

First Flo	Sites	258	289	358	515
10 rosiers les plus précoces	<i>Rosa reversa</i>	0,33	0,66	1	1
	Stanwell Perpetual	0,5	0,25	1	1
	Aristide (Repartee)	0,66	1	1	1
	<i>Rosa holikensis</i>	0,25	1	1	1
	Dawson	0,5	1	1	1
	<i>Rosa albertii</i>	0	1	1	1
	Meg Merrilies	0	1	1	1
	Cibles	0	1	1	1
	Petite Ecossoise	0	0,75	1	1
	Souv De Philemon Cochet	0	1	1	1
10 rosiers les plus tardifs	<i>Rosa wichurana</i>	0	1	1	1
	Felicite Bohain	1	0,25	1	0,5
	Horace Vernet	0,75	0,75	1	0,75
	<i>Rosa subgallicoïdes</i>	1	0	1	1
	Belle Virginie	1	0,5	1	0,75
	Delille	0,75	0,25	0,75	1
	Belvedere	1	0,5	0,5	1
	Blanc De Vibert	1	0,5	1	0,75
	<i>Rosa phoenicia</i>	0,5	0	1	1
	<i>Rosa bracteata</i>	1	1	1	0

Comparison of variant frequency for early- and late-flowering roses

Acknowledgements

EPGV

Marie-Christine Le Paslier, Aurélie Bérard, Aurélie Chauveau, Elodie Marquand, etc.

Rose gardens

Parc Floral de la Beaujoire, Roseraie de Commer, La roseraie du Désert, Le jardin de Jumaju, Roses Loubert, Grande roseraie de Lyon, SCRADH, Roseraie du parc botanique de la Tête d'Or, La roseraie du Val-de-Marne

Historians

Annie Antoine, Céline Briée, Cristiana Oghina-Pavie, Stéphane Tirard

Platforms

Plateau ANAN, Plateforme Gentyane

Members of the GDO team

Mathilde Liorzou, Annie Chastellier, Fabrice Foucher, Agnès Grapin, Laurence Hibrand-Saint Oyant, Gilles Michel, Alix Pernet, Vanessa Soufflet-Freslon, Tatiana Thouroude

Ancient members of the GDO team

Nathalie Jacquier, Douae Ben Hdech, Vivien Bauchat, Shubin Li

