Wild cherry triploids: a chance for forest breeders ?

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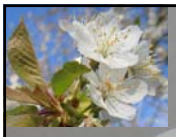
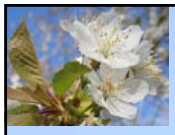
VALBRO – Growing Valuable Broadleaved Tree species
2008, October 6-8 Freiburg - Germany

ALIMENTATION
AGRICULTURE
ENVIRONNEMENT

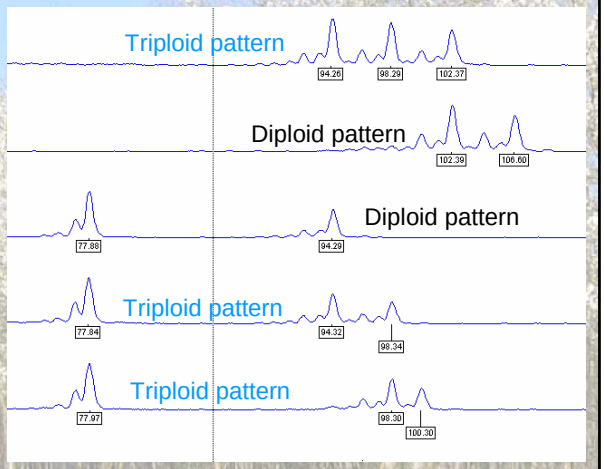
INRA

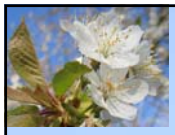


Detection of triploids in the INRA collection



12 SSRs
S incompatibility locus marker
On 5 chromosomes out of 8
→ Obvious triploid patterns for 3 to 8 markers
→ Flow cytometry confirmed triploidy

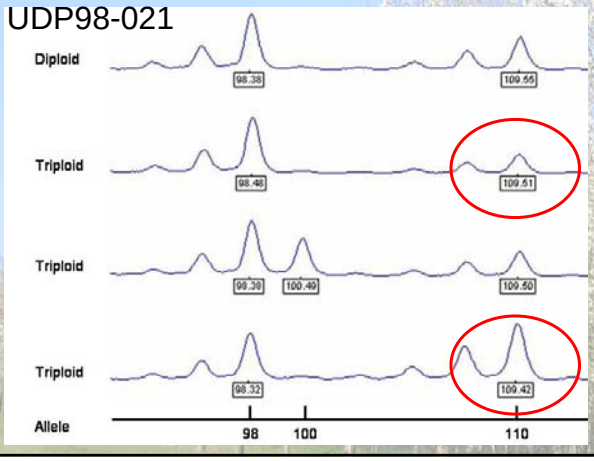


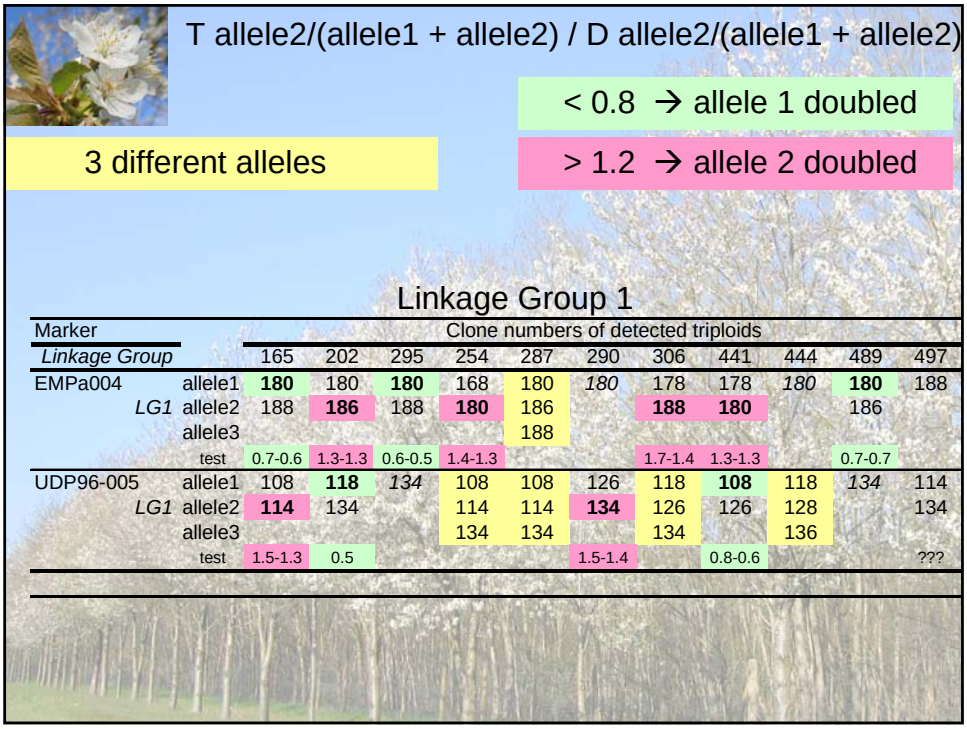


Which allele is doubled when the genotype presents 2 alleles ? Diploids sharing the same two alleles as a triploid were used for comparison:

$$\text{Test} = \frac{\text{T allele2/(allele1 + allele2)}}{\text{D allele2/(allele1 + allele2)}}$$

UDP98-021





T allele2/(allele1 + allele2) / D allele2/(allele1 + allele2)

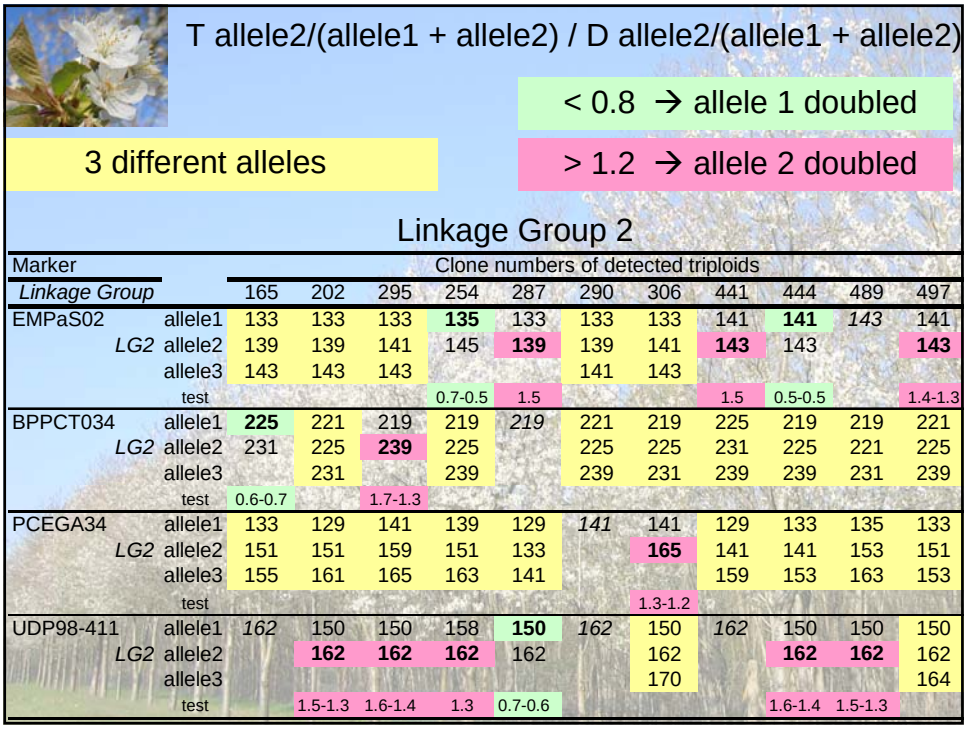
< 0.8 → allele 1 doubled

3 different alleles

> 1.2 → allele 2 doubled

Linkage Group 1

Marker		Clone numbers of detected triploids										
Linkage Group		165	202	295	254	287	290	306	441	444	489	497
EMPa004	allele1	**180**	180	**180**	168	180	*180*	178	178	*180*	**180**	188
LG1	allele2	188	**186**	188	**180**	186		**188**	**180**		186	
	allele3					188						
	test	0.7-0.6	1.3-1.3	0.6-0.5	1.4-1.3			1.7-1.4	1.3-1.3		0.7-0.7	
UDP96-005	allele1	108	**118**	*134*	108	108	126	118	**108**	118	*134*	114
LG1	allele2	**114**	134		114	114	**134**	126	126	128		134
	allele3				134	134		134		136		
	test	1.5-1.3	0.5				1.5-1.4		0.8-0.6			???



T allele2/(allele1 + allele2) / D allele2/(allele1 + allele2)

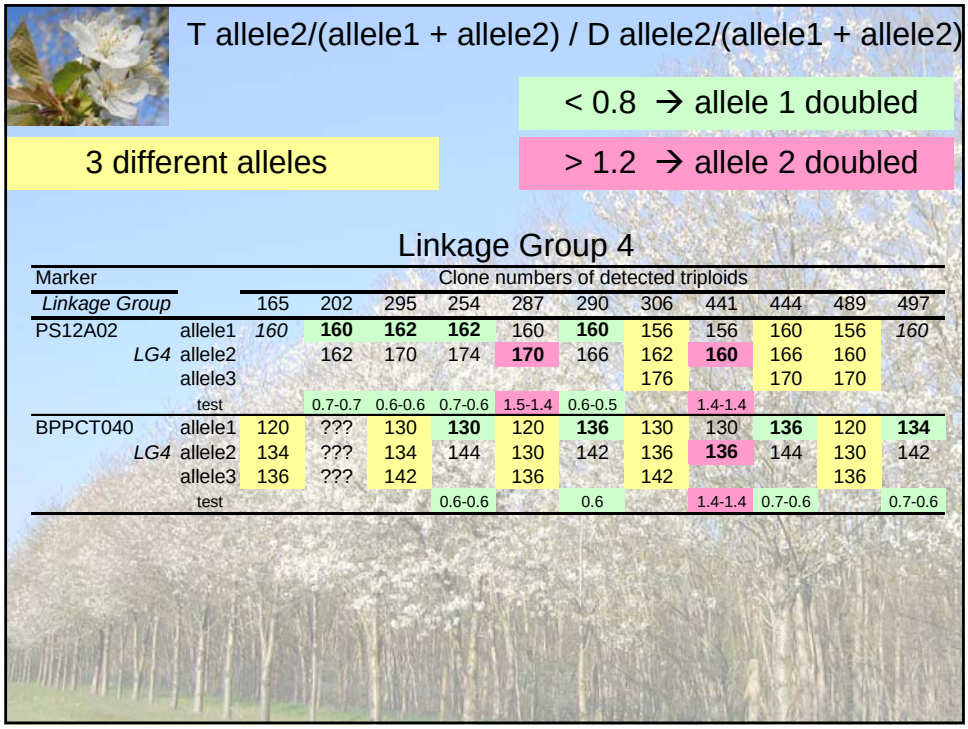
< 0.8 → allele 1 doubled

3 different alleles

> 1.2 → allele 2 doubled

Linkage Group 2

Marker		Clone numbers of detected triploids										
Linkage Group		165	202	295	254	287	290	306	441	444	489	497
EMPaS02	allele1	133	133	133	**135**	133	133	133	141	**141**	*143*	141
LG2	allele2	139	139	141	145	**139**	139	141	**143**	143		**143**
	allele3	143	143	143			141	143				
	test				0.7-0.5	1.5			1.5	0.5-0.5		1.4-1.3
BPPCT034	allele1	**225**	221	219	219	*219*	221	219	225	219	219	221
LG2	allele2	231	225	**239**	225		225	225	231	225	221	225
	allele3		231		239		239	231	239	239	231	239
	test	0.6-0.7		1.7-1.3								
PCEGA34	allele1	133	129	141	139	129	*141*	141	129	133	135	133
LG2	allele2	151	151	159	151	133		**165**	141	141	153	151
	allele3	155	161	165	163	141			159	153	163	153
	test							1.3-1.2				
UDP98-411	allele1	*162*	150	150	158	**150**	*162*	150	*162*	150	150	150
LG2	allele2		**162**	**162**	**162**	162		162		**162**	**162**	162
	allele3							170				164
	test		1.5-1.3	1.6-1.4	1.3	0.7-0.6				1.6-1.4	1.5-1.3	



T allele2/(allele1 + allele2) / D allele2/(allele1 + allele2)

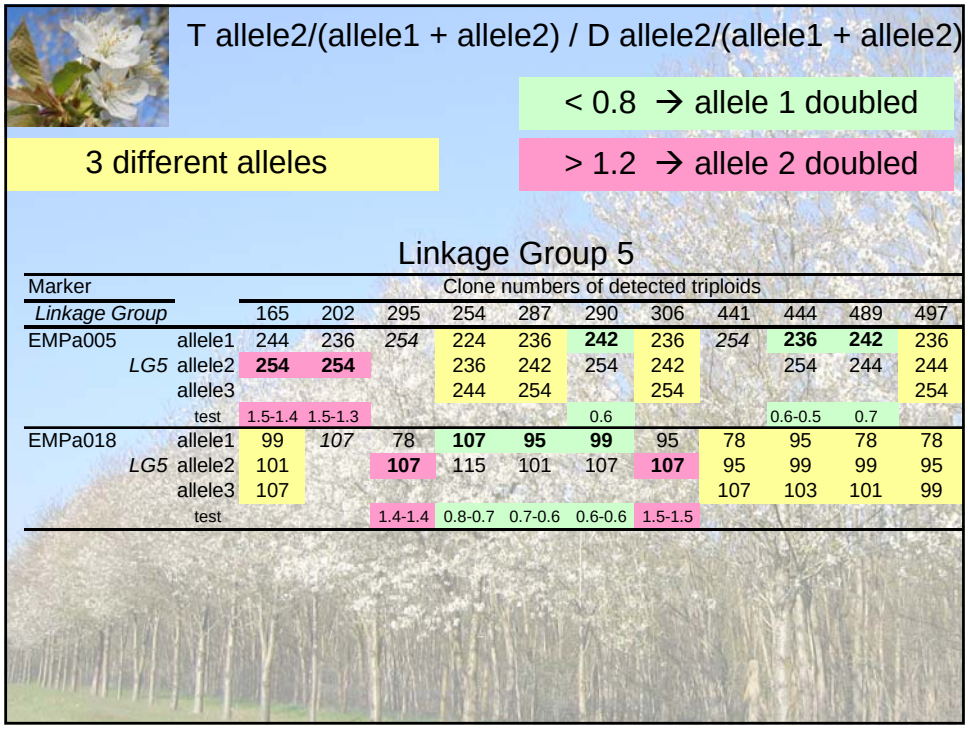
< 0.8 → allele 1 doubled

3 different alleles

> 1.2 → allele 2 doubled

Linkage Group 4

Marker		Clone numbers of detected triploids										
Linkage Group		165	202	295	254	287	290	306	441	444	489	497
PS12A02	allele1	*160*	**160**	**162**	**162**	160	**160**	156	156	160	156	*160*
LG4	allele2		162	170	174	**170**	166	162	**160**	166	160	
	allele3							176		170	170	
	test		0.7-0.7	0.6-0.6	0.7-0.6	1.5-1.4	0.6-0.5		1.4-1.4			
BPPCT040	allele1	120	???	130	**130**	120	**136**	130	130	**136**	120	**134**
LG4	allele2	134	???	134	144	130	142	136	**136**	144	130	142
	allele3	136	???	142		136		142			136	
	test				0.6-0.6		0.6		1.4-1.4	0.7-0.6		0.7-0.6



T allele2/(allele1 + allele2) / D allele2/(allele1 + allele2)

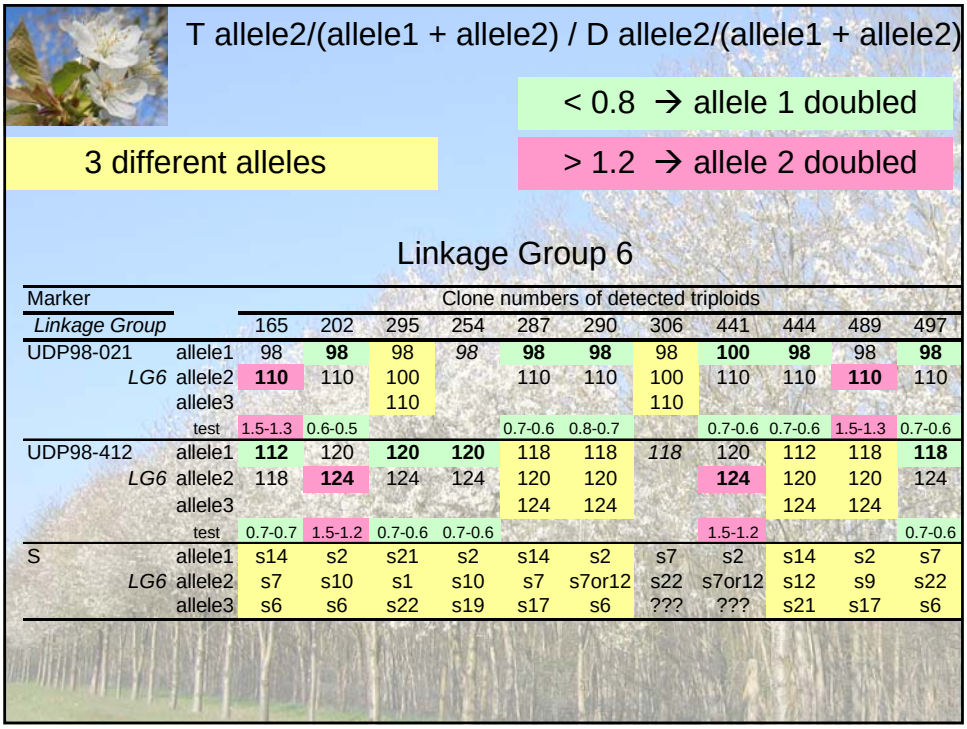
< 0.8 → allele 1 doubled

3 different alleles

> 1.2 → allele 2 doubled

Linkage Group 5

Marker		Clone numbers of detected triploids										
Linkage Group		165	202	295	254	287	290	306	441	444	489	497
EMPa005	allele1	244	236	*254*	224	236	**242**	236	*254*	**236**	**242**	236
LG5	allele2	**254**	**254**		236	242	254	242		254	244	244
	allele3				244	254		254				254
	test	1.5-1.4	1.5-1.3				0.6			0.6-0.5	0.7	
EMPa018	allele1	99	*107*	78	**107**	**95**	**99**	95	78	95	78	78
LG5	allele2	101		**107**	115	101	107	**107**	95	99	99	95
	allele3	107							107	103	101	99
	test			1.4-1.4	0.8-0.7	0.7-0.6	0.6-0.6	1.5-1.5				



T allele2/(allele1 + allele2) / D allele2/(allele1 + allele2)

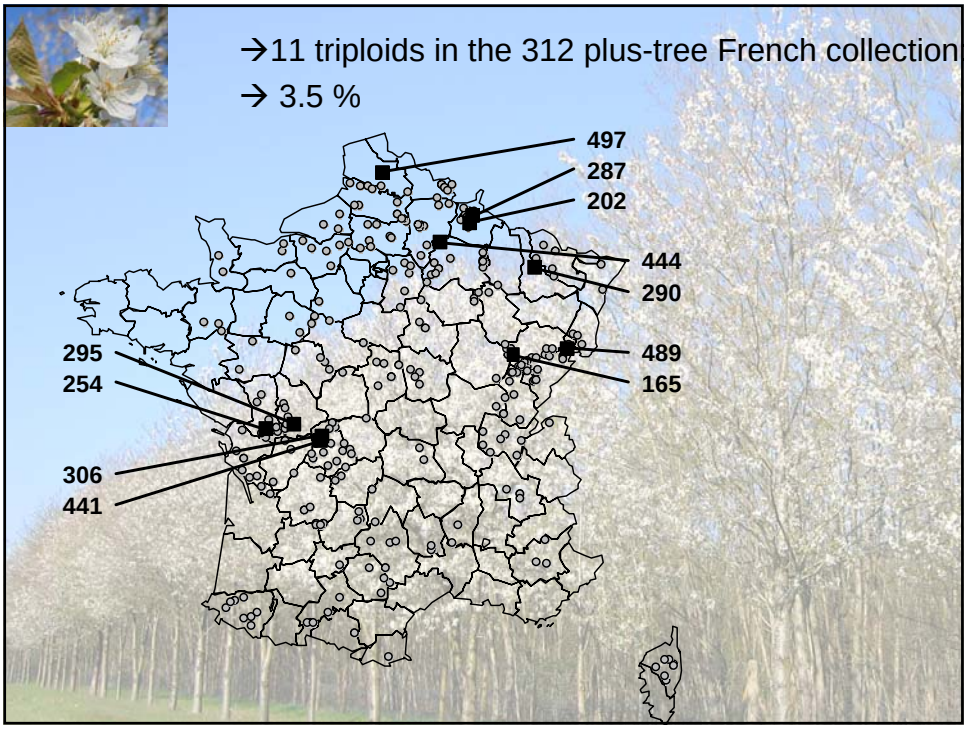
< 0.8 → allele 1 doubled

3 different alleles

> 1.2 → allele 2 doubled

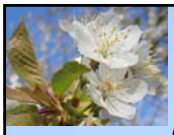
Linkage Group 6

Marker		Clone numbers of detected triploids										
Linkage Group		165	202	295	254	287	290	306	441	444	489	497
UDP98-021	allele1	98	**98**	98	*98*	**98**	**98**	98	**100**	**98**	98	**98**
LG6	allele2	**110**	110	100		110	110	100	110	110	**110**	110
	allele3			110				110				
	test	1.5-1.3	0.6-0.5			0.7-0.6	0.8-0.7		0.7-0.6	0.7-0.6	1.5-1.3	0.7-0.6
UDP98-412	allele1	**112**	120	**120**	**120**	118	118	*118*	120	112	118	**118**
LG6	allele2	118	**124**	124	124	120	120		**124**	120	120	124
	allele3					124	124			124	124	
	test	0.7-0.7	1.5-1.2	0.7-0.6	0.7-0.6				1.5-1.2			0.7-0.6
S	allele1	s14	s2	s21	s2	s14	s2	s7	s2	s14	s2	s7
LG6	allele2	s7	s10	s1	s10	s7	s7or12	s22	s7or12	s12	s9	s22
	allele3	s6	s6	s22	s19	s17	s6	???	???	s21	s17	s6

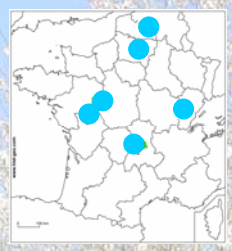


→11 triploids in the 312 plus-tree French collection

→ 3.5 %

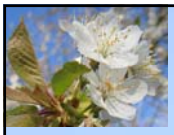


… none in 1489 trees, genotyped with the same markers, in 6 French wild populations (❶, unpublished data)



Elsewhere ?
One (confirmed) in a 26-tree Belgian plus-tree collection ❷
One (confirmed) in a German forest ❸
Two samples with tri- or tetraploid banding pattern in Germany in a 166 tree population ❹
None in other published data (551 ❺ and 134 ❻ trees)

❶ Stoeckel S., Castric V., Mariette S., Vekemans X. J. Evol. Biol. 21: 889-899 (2008)
❷ De Cuyper B., Sonneveld T., Tobutt K.R. Molecular Ecology 14: 945-955 (2005)
❸ Von Schelhorn M. TAG, 17 (6-8): 232-235 (1947)
❹ Schueler S., Tusch A., Sholz F. Mol. Ecol. 15: 3231-3243 (2006)
❺ Vaughan SP., Cottrell JE., Moodley DJ., Connolly T., Russell K. For. Ecol. Manag. 242: 419-430 (2007)
❻ Holtken AM., Gregorius HR. BMC Ecology 6:13 (2006)



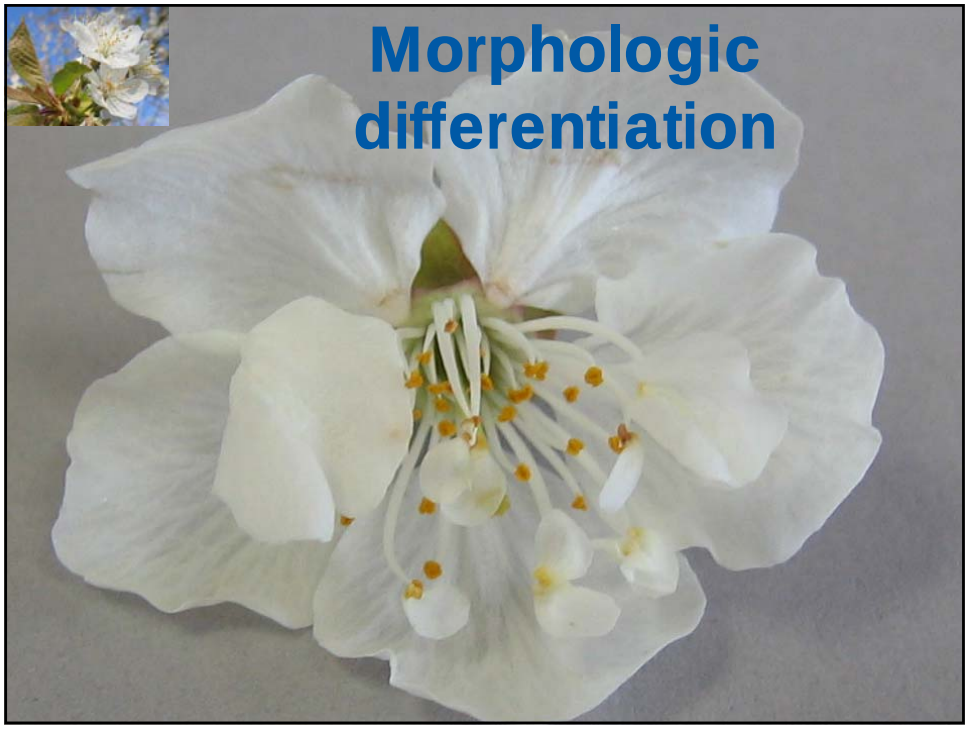
Are triploids *Prunus avium* x other *Prunus* hybrids ?
Or produced thanks to diploid *Prunus avium* gametes ?

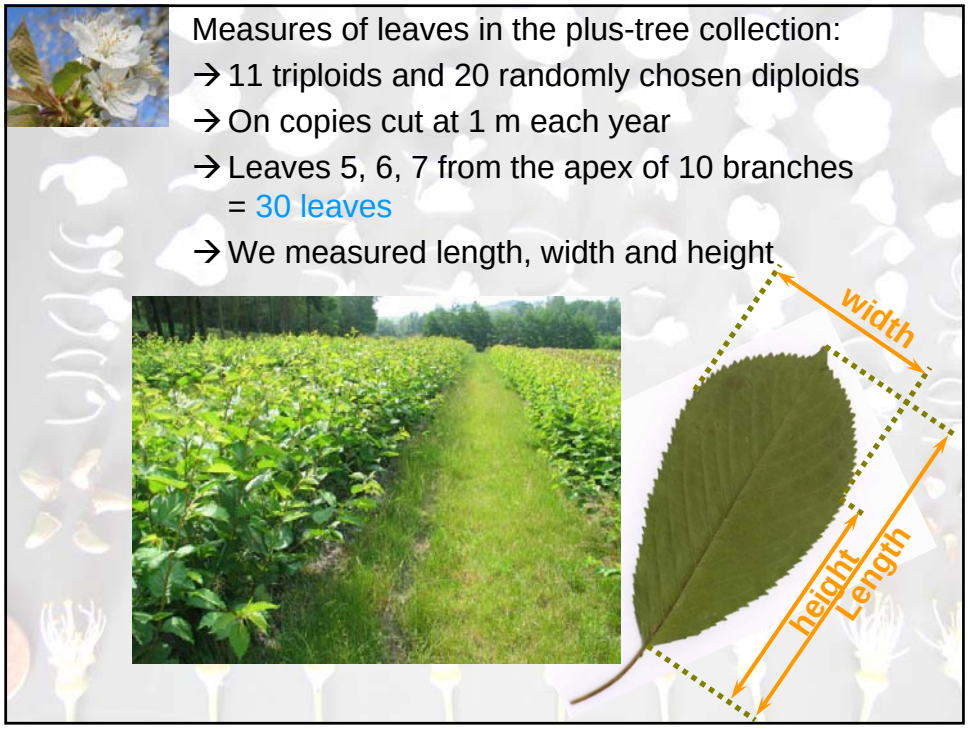
→ Triploids or tetraploids have been already observed in sweet cherry breeding programmes.

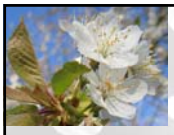
→ Alleles of triploids are all present in the diploid collection

→ Triploids are most likely *P. avium* trees

	Numbers of alleles		Allelic frequencies	
Marker	T	D	min	max
PCEGA34	*12*	22	*0.02*	*0.2*
BPPCT034	*5*	19	*0.11*	*0.24*
S	*12*	18	*0.01*	*0.12*
PS12A02	*7*	16	*0.02*	*0.55*
UDP98-411	*5*	14	*0.02*	*0.5*
EMPa005	*5*	13	*0.01*	*0.52*
BPPCT040	*6*	13	*0.07*	*0.27*
EMPa018	*7*	12	*0.01*	*0.26*
UDP96-005	*7*	12	*0.01*	*0.34*
UDP98-412	*4*	11	*0.03*	*0.35*
EMPaS02	*6*	9	*0.01*	*0.5*
UDP98-021	*3*	7	*0.19*	*0.46*
EMPa004	*5*	4	*0.01*	*0.35*


Morphologic differentiation


Measures of leaves in the plus-tree collection:
→ 11 triploids and 20 randomly chosen diploids
→ On copies cut at 1 m each year
→ Leaves 5, 6, 7 from the apex of 10 branches = 30 leaves
→ We measured length, width and height
width
height
Length

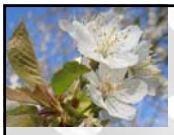


Measures on petals in the plus-tree collection:

→ 9 triploids and 20 randomly chosen diploids

→ 30 petals from several flowers

→ We measured length and width



Leave size is significantly different between D and T:

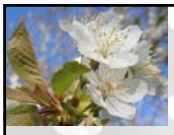
→ Triploids' leaves are longer + 3%

→ And moreover, larger + 17%

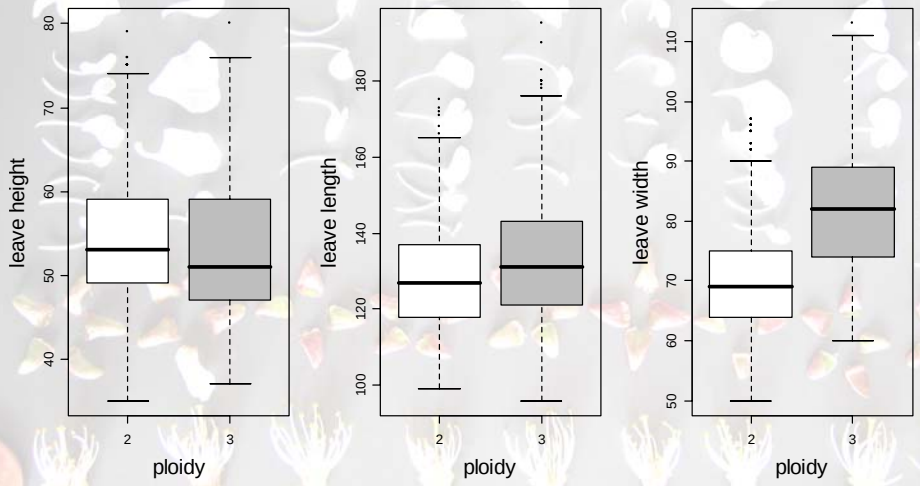
		mean		ANOVA	
		diploids	triploids	ploidy	clone in ploidy
leaves (mm)	length	128.4	132.4	23.3***	19.1***
	width	69.8	81.6	599.5***	25.7***
	height	53.9	53.1	2.9 °	17.0***

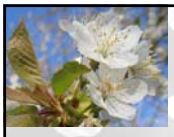
*** P-value < 0.001

° P-value < 0.1



Variability inside diploid and triploid leave sizes prevents to discriminate them clearly





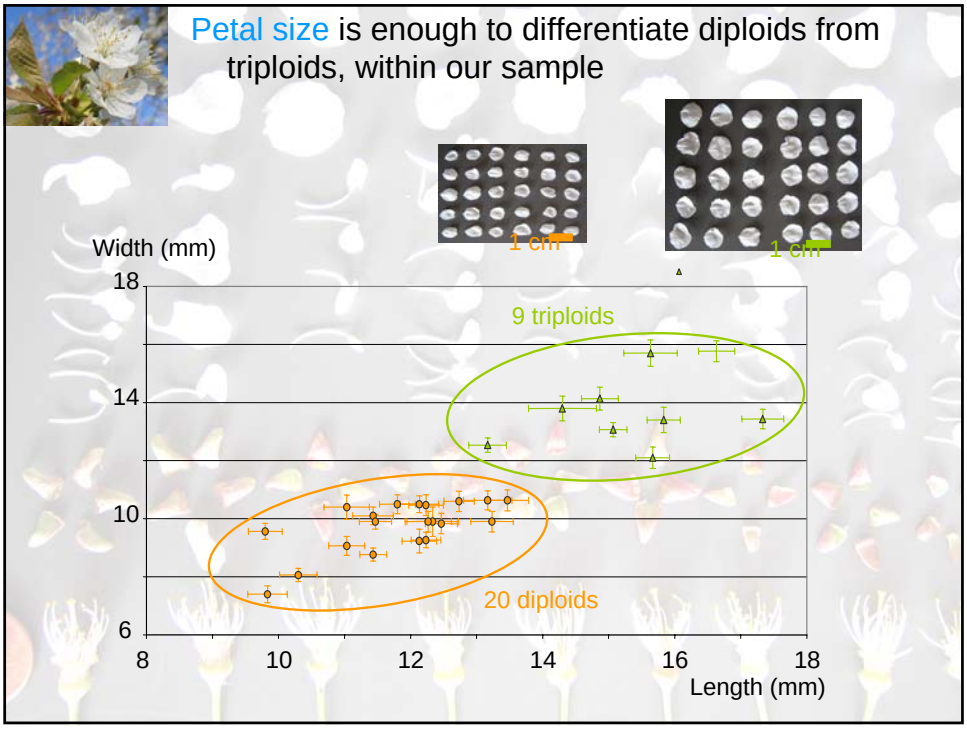
Petal size is highly significantly different between D and T:

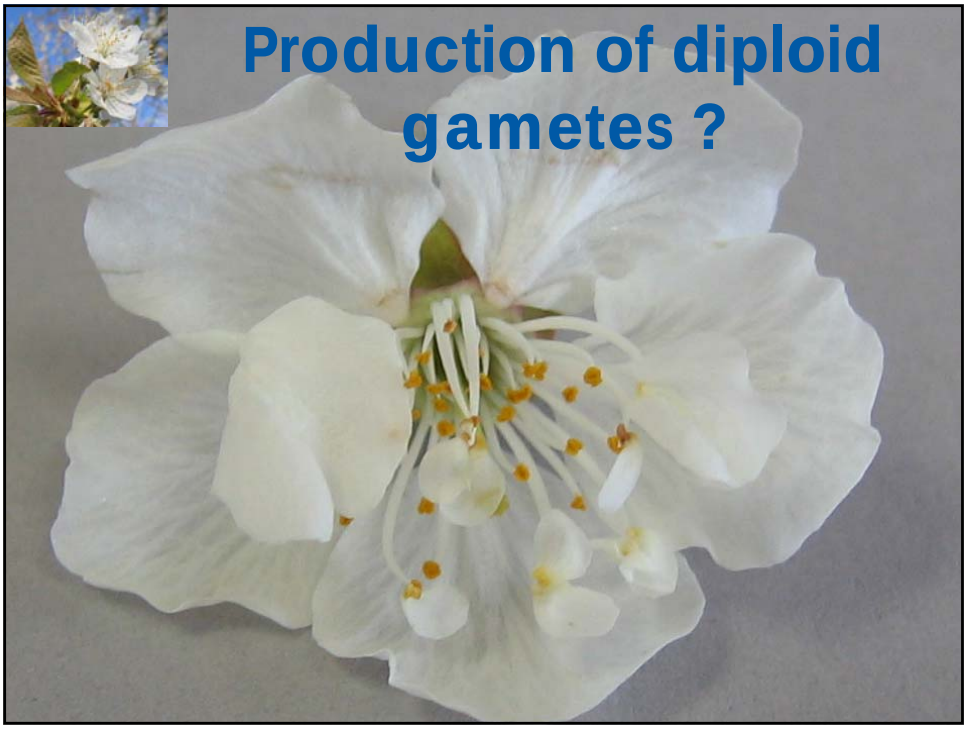
→ Triploids' petals are longer + 30%

→ And moreover, larger + 42%

		mean		ANOVA	
		diploids	triploids	ploidy	clone in ploidy
petals (mm)	length	11.8	15.4	3334.4***	51.6***
	width	9.7	13.8	3241.9***	33.0***

*** P-value < 0.001

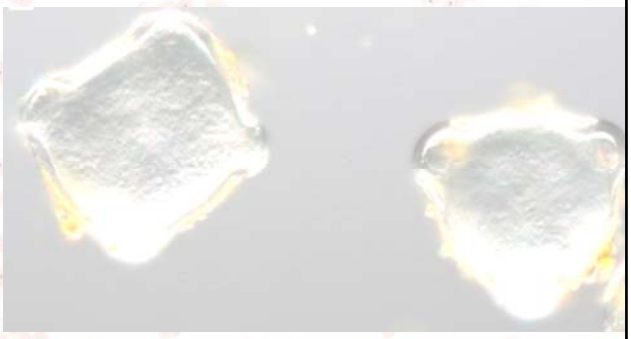

Petal size is enough to differentiate diploids from triploids, within our sample
1 cm
1 cm
Width (mm)
18
14
10
6
9 triploids
20 diploids
8
10
12
14
16
18
Length (mm)


Production of diploid gametes ?

Counting of 1000 pollen grains / tree

→ 9 triploids and 20 diploids

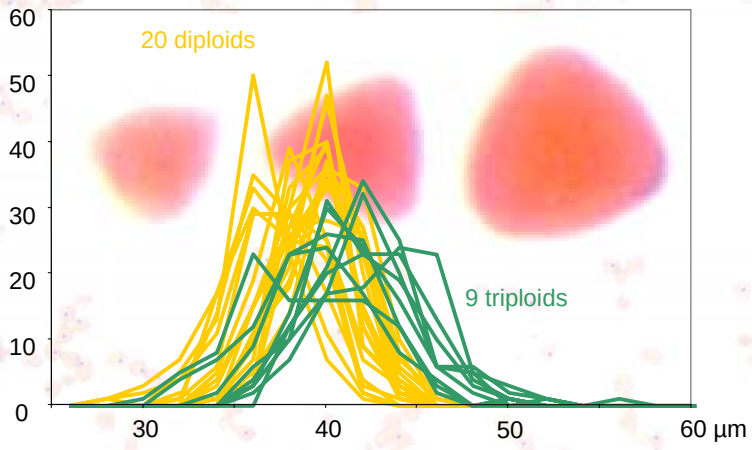
→ % of quadrangular grains larger for triploids



Triangular pollen grains:

→ We observed 100 pollen grains per tree

→ Triploid pollen grains are more variable, some have a bigger size



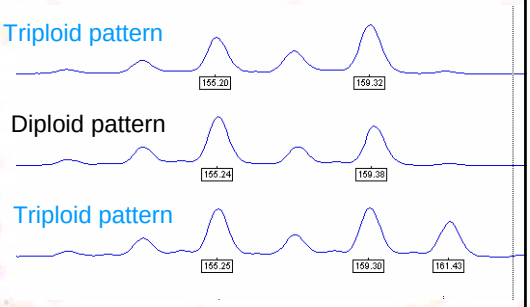
Open pollinisation on a triploid:

→ We collected 12 ungerminated seeds,

→ genotyped with 10 SSRs:

→ 5 seem diploids, 3 seem triploids,

4 seem aneuploids



ps12A02

Open pollinated crosses on 6 triploids:

→ 274 seedlings

→ cytometry

→ 169 triploids

→ 1 tetraploid

parents		progeny				
mother	fathers	total	triploids	diploids	aneuploids	% triploids
165	?	15	11	4	0	73
202	?	17	13	4	0	76
287	?	13	12	1	0	92
306	?	18	15	3	0	83
295	?	149	118	30	0	79
295	?	43	0	43	0	0
290	?	19	0	19	0	0

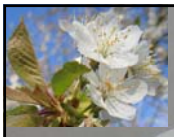
Controlled crosses with triploids as males:

→ 33 4-years-old trees in INRA nursery

→ cytometry

→ 2 triploids

parents		progeny			
mother	father	total	triploids	diploids	aneuploids
141	165	5	1	4	0
172	165	1	0	1	0
182	165	11	1	10	0
184	165	10	0	10	0
154	254	1	0	1	0
253	254	5	0	5	0



Agronomic interest of triploids

Multiclonal field tests:

	years of growth	nb clones
→ Gan	6	71
→ Bergerac	7	85
→ Fontenoy	10	75
→ Sarrazac	14	38

Growth measures:

→ Increase in height

→ Increase in circumference

→ Leaf spot susceptibility

Multiclonal field tests:

→ Increase in circumference different between triploids and diploids: +34%

→ Increase in height different between triploids and diploids: +21%

→ Triploids are more resistant to leaf spot

	F-value	Bergerac	Fontenoy	Gan	Sarrazac
increase in height	ploidy	1.6	49.4***	272.0***	111.8***
	clone in ploidy	8.3***	11.3***	4.4***	28.3***
increase in circumference	ploidy	5.7*	70.3***	50.9***	452.1***
	clone in ploidy	9.7***	14.1***	4.0***	31.2***
leaf spot	ploidy	98.6***	89.7***	27.4***	715.7***
	clone in ploidy	25.6***	13.8***	8.3***	63.8***

*** P-value < 0.001 * P-value < 0.05

Multiclonal field tests:

→ Negative correlation between growth and susceptibility to leaf spot

→ Comparisons should imply clones with similar level of resistance

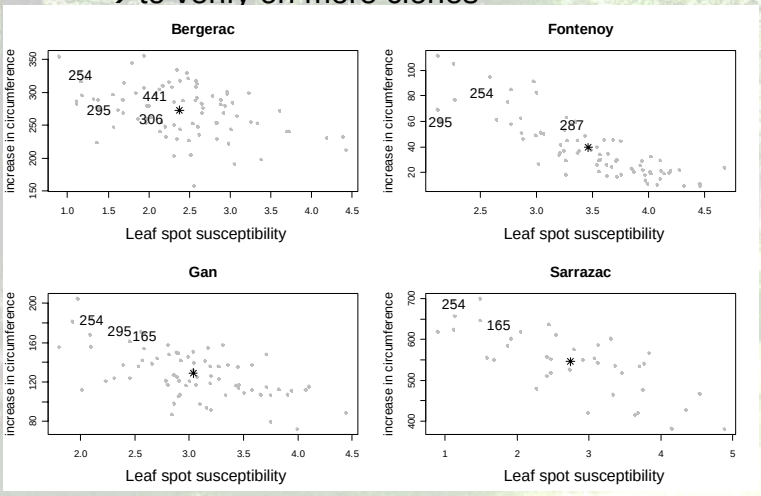
trials		Bergerac	Fontenoy	Gan	Sarrazac
correlation	height / circumference	0.45	0.59	0.74	0.64
	height / leaf spot	-0.12	-0.6	-0.22	-0.57
	circumference / leaf spot	-0.28	-0.67	-0.24	-0.62

Multiclonal field tests, triploids compared to diploids with similar leaf spot resistance:

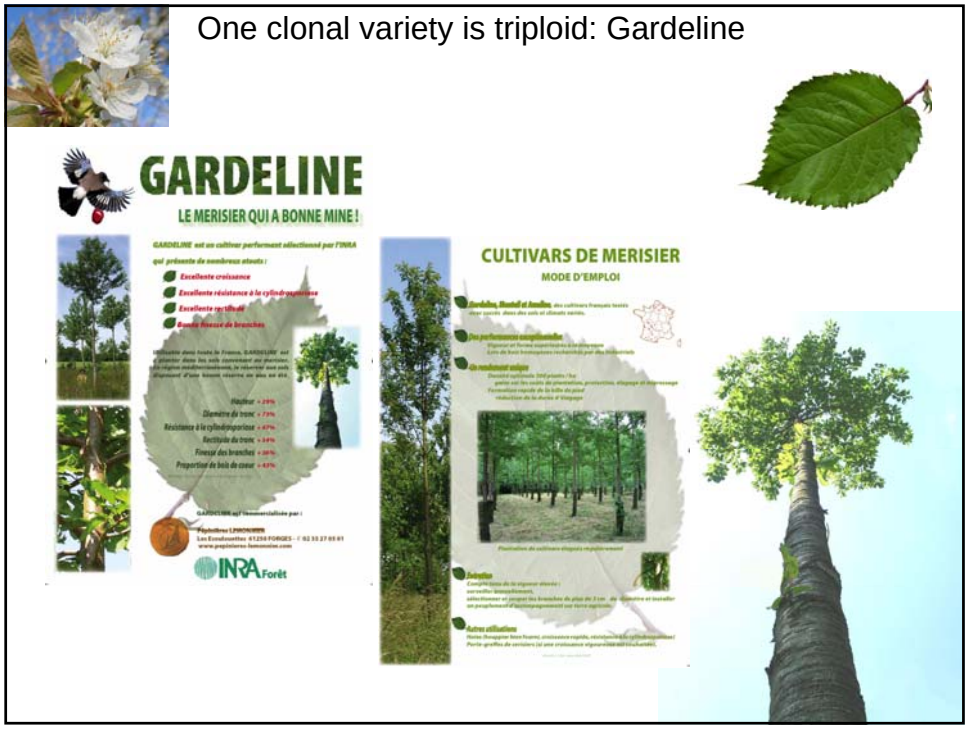
→ 254, 441, 165, 287: good growth

→ 295: variable growth, 306: average growth

→ to verify on more clones




Which place for triploids in the wild cherry breeding programme in France ?


One clonal variety is triploid: Gardeline
GARDELINE
LE MERISIER QUI A BONNE MINE !
CULTIVARS DE MERISIER
MODE D'EMPLOI
INRA Forêt

Two triploids included in a seed orchard

parents		progeny				
mother	fathers	total	triploids	diploids	aneuploids	% triploids
287	?	13	12	1	0	92
295	?	149	118	30	0	79
295	?	43	0	43	0	0

Breeding programme for wild cherry

Phenotypic selection → collection

Plus-trees

Clonal field tests

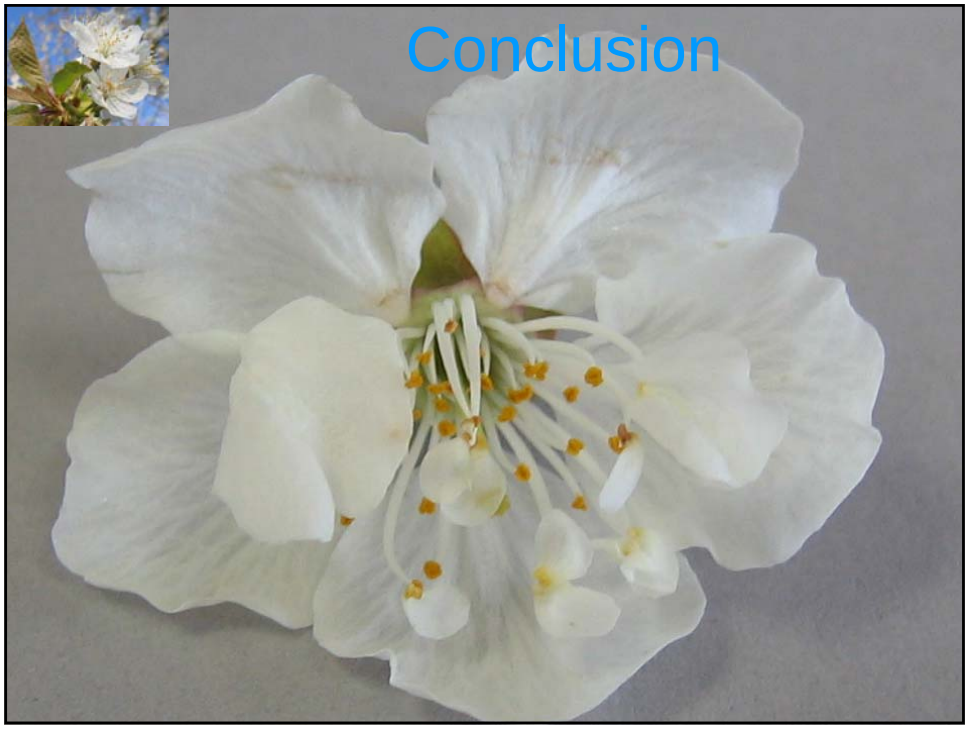
10 clonal Varieties

Seed orchards Cabrerets Avessac

On the best:
1) Full-sib families
2) Open pollinated families

Nursery tests, Clonal field tests

… New varieties



Conclusion

SSRs, cytometry, pollen, leave and petal morphology in the INRA wild cherry collection:

→ excess of triploids compared to natural populations

→ triploids are produced by diploid gametes of cherry

→ SSRs leave and moreover flower morphology characterize triploids, but cytometry is the most efficient and cheaper method to find them

Triploids as males:

→ 4-years old trees are triploid and diploid

Triploid as females:

→ seeds are triploid, diploid or aneuploid

→ but young plants are either triploid or diploid

Growth and resistance to leaf spot better for triploids
→ Phenotypic plus tree selection has been efficient
→ After further selection on form, 3 triploids already included in varieties

Is there any problem to include triploids in seed orchards ?
→ If triploidy is an intrinsic advantage for leaf spot resistance and growth, diploids produced by a triploid may be of average rather than superior quality
→ Control of the comparative quantity and quality of diploids and triploids obtained from a triploid

Is there an interest to produce more triploid clonal varieties?
→ Triploid clonal patches are natural: example of clone 497, sampled in a very homogeneous 0.5ha stand, proposed as seed stand but producing almost no seed
→ Clonal varieties have nevertheless a limited impact on natural populations, as there are produced only for the most active foresters

Thank you !