



# IDENTIFICATION AND MAPPING OF LOCI CONTROLLING VIABILITY IN *VITIS VINIFERA* SELF-CROSSES

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# *Vitis vinifera*

- dicot outcrossing perennial species with highly variable genome
- cultivated varieties are highly **heterozygous / hemizygous**
- **vegetatively** propagated for cultivation
  - less recombination events compared to other cultivated species
- **high genetic load** not purged by selection



# Analysis of putatively deleterious variants in grapevine germplasm

128 *V. vinifera* varieties resequenced  
3 Proles: *occidentalis*, *orientalis*, *pontica*

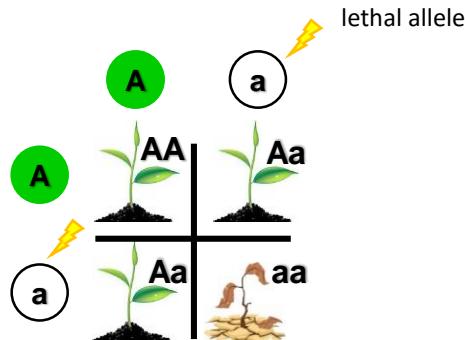
Type	# genes carrying mutations	# mutations in genes
Deletions	5,669	6,451
Insertions	7,142	13,970
Frameshift ins	3,610	4,647
Frameshift del	5,134	7,304
Stopgain SNPs	6,163	9,536
Stoploss SNPs	1,145	1,194
Total	16,732	44,102

V2.1 Gene prediction: 31,845 genes

# Segregation distortion in selfed progenies to uncover deleterious alleles

**Deleterious recessive alleles** found in **homozygosis** in selfed progeny decrease fitness and result in **inbreeding depression**

## DISTORTION IN SEGREGATION



Genotypic ratio  
1 AA : 2 Aa

Phenotypic ratio  
3 viable : 1 dead

# Germination in progenies deriving from self- and out- cross



self cross seedlings (~ 2 weeks)



outcross seedlings (~ 2 weeks)

# Germination in progenies deriving from self- and out- cross



self cross seedlings (~ 2 weeks)



outcross seedlings (~ 2 weeks)



self cross seedlings (~ 4 weeks)



normal phenotype



chlorotic phenotype

# Effects of selfing at germination and after the first vegetative season



~ 4 weeks after germination



overwintered seedlings

# Effects of selfing at germination and after the first vegetative season



~ 4 weeks after germination



overwintered seedlings



2<sup>nd</sup> vegetative season

# Workflow of data collection for GBS analysis

gDNA extraction



Genotyping by  
Sequencing libraries  
(GBS-Seq)



1.  
sequencing

Stacks

Catchen, Cresko et al.,  
Mol. Ecol., 2013.

SNPs and  
genotypes call

Raw data cleaning  
from erroneous calls



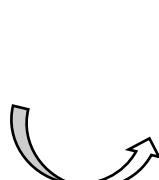
2.  
data calling  
and cleaning

- Single locus distortion analysis
- Pairs of loci distortion to seek epistatic interactions



3.  
distortion analysis

genetic  
mapping



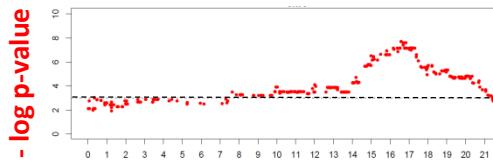
data integration



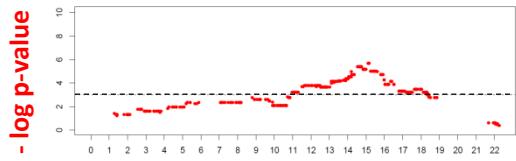
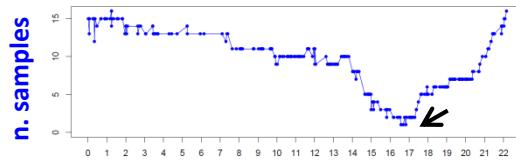
recombination  
along genome

4.  
recombination  
analysis

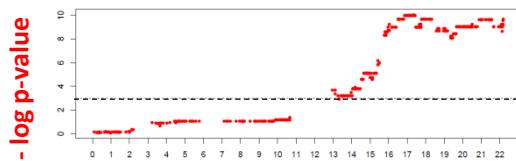
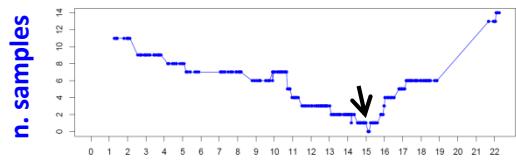
# Single locus SD in self-crosses: hotspot on chromosome 8



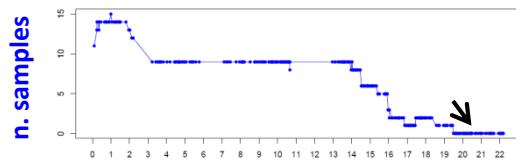
Rkatsiteli



Sangiovese

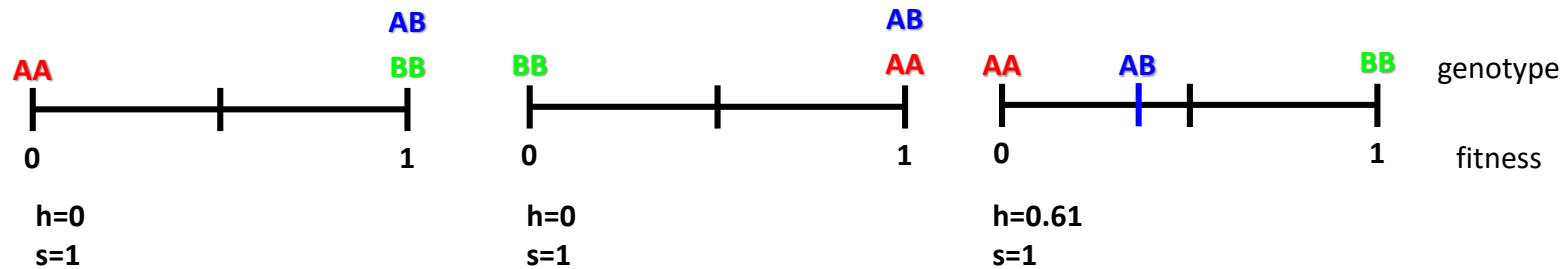
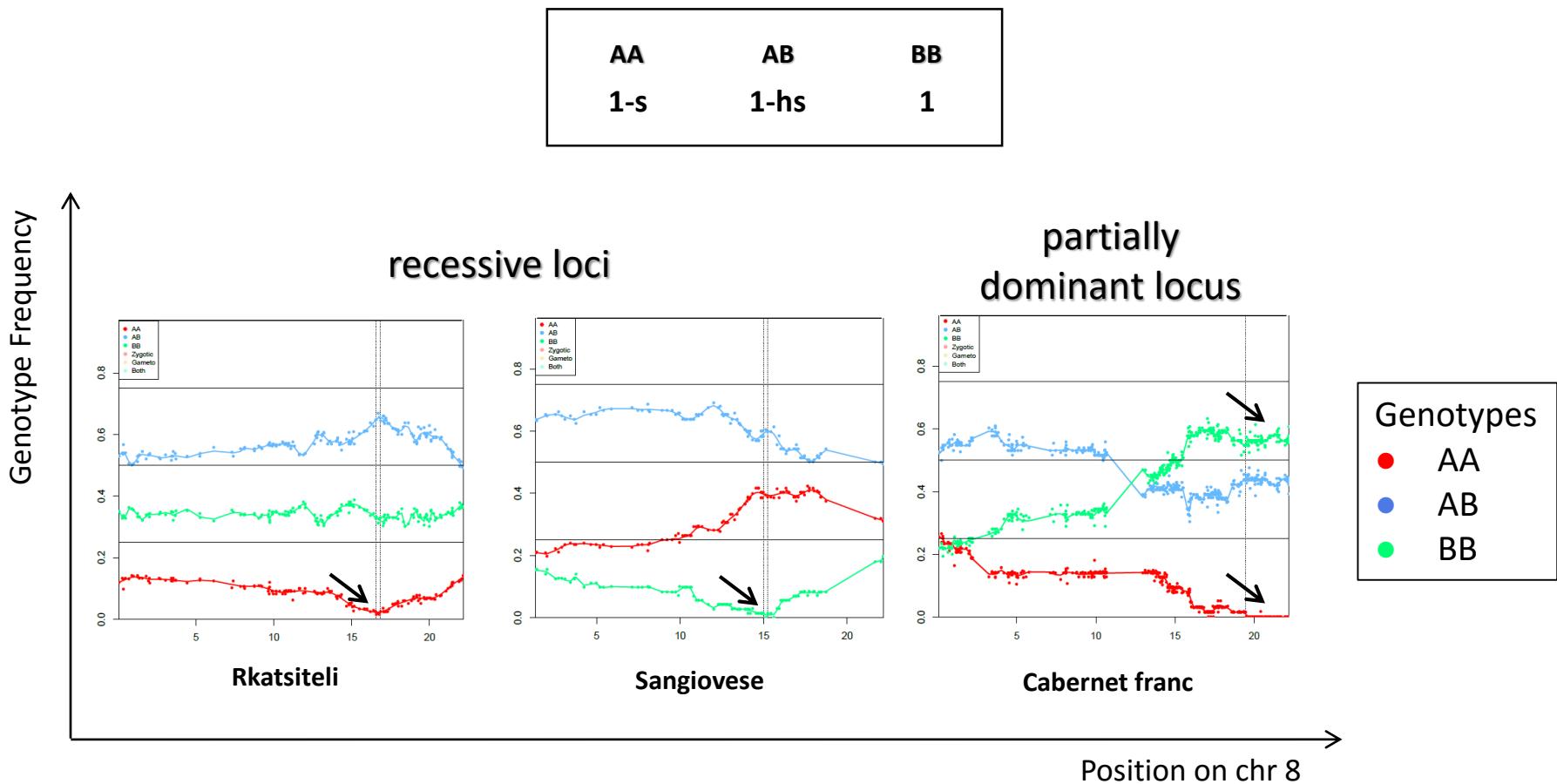


Cabernet franc



- Chi-square test on single loci
- p-value of 0.001
- Bonferroni correction

## Different allelic effects on distortion in chromosome 8



# Single locus SD effects in selfed progenies

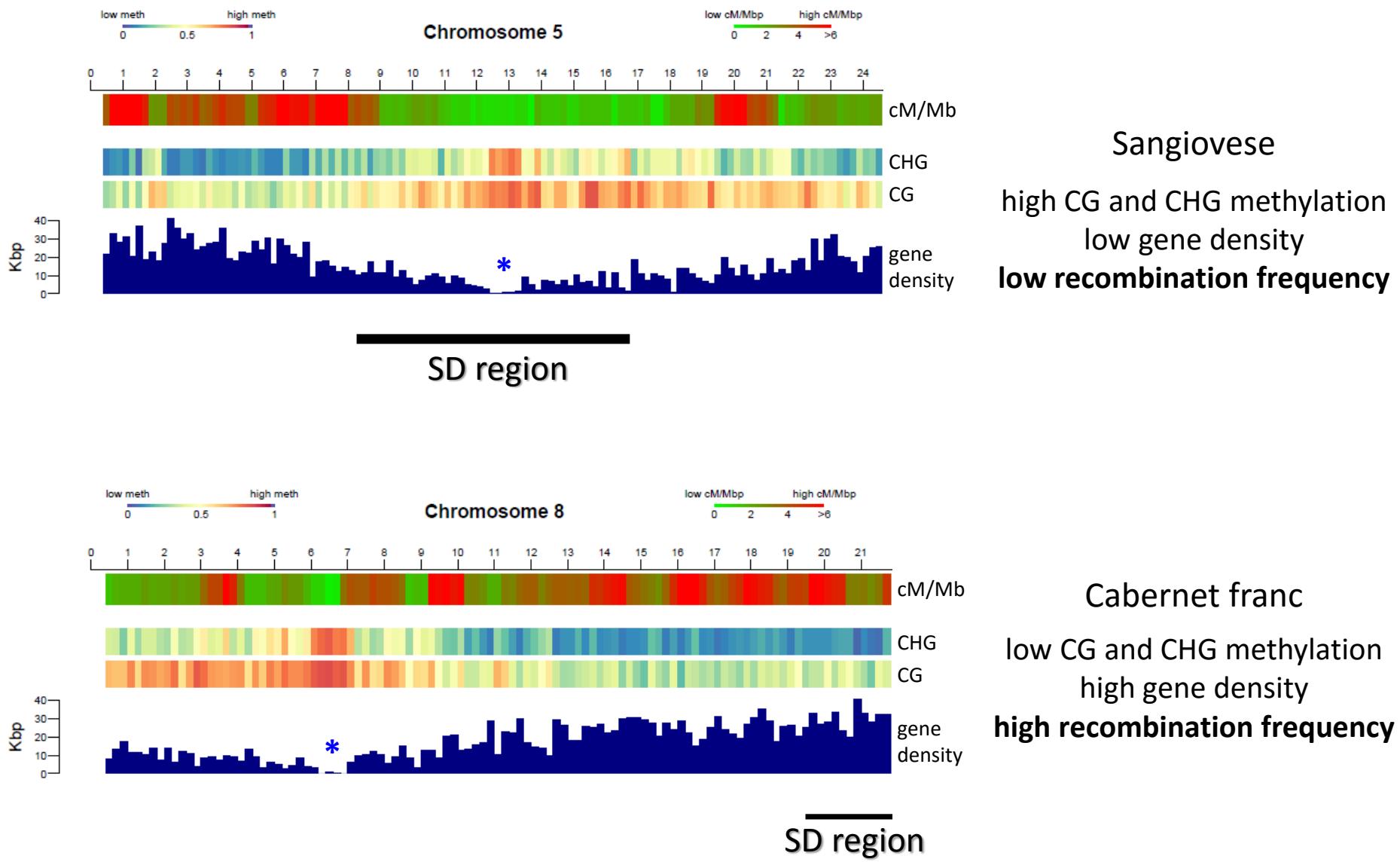
cross	# samples	SD locus	T0 (1 month)	T1 (1 year)	T2 (2 years)	Segregation
Rkatsiteli self	238	chr 8 (250 Kb)	ns	ns	L	recessive
		chr 18 (1.3 Mb)	ns	ns	L	recessive
Primitivo self	43	chr 4 (2.5 Mb)	-	-	L	recessive
		chr 11 (2.8 Mb)	-	-	L	partially dominant
		chr 12 (1.3 Mb)	-	-	L	dominant
Cabernet franc self	68	chr 8 (3 Mb)	L	L	L	partially dominant
Sangiovese self	87	chr 5 (8.9 Mb)	L	-	-	recessive
		chr 8 (260 Kb)	L	-	-	recessive
		chr 9 (5.4 Mb)	L	-	-	recessive
Pinot nero self	85	chr 11 (2.6 Mb)	D	-	-	overdominant
		chr 15 (10 Mb)	D	-	-	dominant
		chr 15 (3.5 Mb)	L	L	L	recessive
Schiava grossa self	91	chr 4 (9.5 Mb)	D	D	D	recessive

L: lethal

D: deleterious

ns: non significative

# Single locus SD regions and genome context



# Fine mapping of distorted regions: population level haplotype analysis

cross	chr	SD Candidate region length (Kbp)	
		Segregation defined	Population defined
Rkatsiteli	8	250	150
Rkatsiteli	18	1300	920
Primitivo	4	2500	1130
Primitivo	11	2800	1480
Primitivo	12	1300	760
Cabernet franc	8	3000	620
Sangiovese	5	8900	2580
Sangiovese	8	260	34
Sangiovese	9	5400	2390
Sangiovese	11	2600	1000

# Pairs-of-loci SD in selfed progenies: epistatic interactions

Fisher's Test:

- to determine if there are interactions between **pairs of loci**

Ongoing:

- test **1:15 ratio** (double recessive homozygotes)

		chr1				
		AB	Ab	aB	ab	
		AB	AABB	AABb	AaBB	AaBb
		Ab	AABb	AAbb	AaBb	Aabb
		aB	AaBB	AaBb	aaBB	aaBb
		ab	AaBb	Aabb	aaBb	aabb

# Conclusions and Perspectives

- Self-fertilization of *V. vinifera* varieties leads to high levels of **lethality** detectable as segregation distortion of markers in the progenies
- Analysis of single locus distortion revealed **10 regions of lethality** and **3 deleterious regions** in the 6 self-crosses assessed
- 6 loci of SD are located in **low-recombination** regions, while 7 loci are located in **high-recombination** regions
- Population level haplotype analysis allowed **restriction** of genetically defined distorted regions
- Future work will be oriented to the identification and characterization of **target genes/SVs as candidates for SD**

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Principal Investigator  
Co-supervisor  
Structural Variation & Population Analysis  
Population Analysis & Crosses  
GBS & Genetic Mapping  
Methylation Analysis  
NGS Sequencing

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