Assessing the impact of components of the pan-genome on segregation in Vitis vinifera crosses

Supervisor: Prof. Michele Morgante

PhD student: Alice Fornasiero

Co-supervisor: Dott. Fabio Marroni

UNIVERSITĂ







Vitis vinifera

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



lethal recessive alleles show their effects when varieties are self-crossed and cause **segregation distortion** because **homozygous individuals** cannot survive



Deviation of segregation frequencies from the expected Mendelian ratios in a pedigree



SEGREGATION WITH DISTORTION



Genotypic ratio 1 AA : 2 Aa

Phenotypic ratio 3 viable : 1 dead

Pan-genome

Core genome genomic features common to all individuals Dispensable genome partially shared and/or non shared DNA sequence elements

INTRA-SPECIFIC VARIATION

Structural Variants (SVs):

- Insertions, deletions, duplications (CNVs and PAVs)
- Inversions and translocations (balanced)

Structural variants can generate SD

SVs can generate unbalanced gametes that may themselves die or cause zygotes to die if inherited in homozygosis



Conditional dispensable genome

The portion of dispensable genome that comprises genes belonging to gene families, a given proportion of which (but not necessarily all) are needed for survival. Marroni, Pinosio, Morgante, *Curr Opin Plant Biol.*, 2014



Goals of the project

1) Assess segregation distortion (SD) in Vitis vinifera crosses

2) Investigate the role of SVs in SD

3) Define and characterize translocation in Rkatsiteli

Germination in Cabernet franc self progeny vs. outcross progeny





chlorotic phenotype

Germination in Sangiovese self progeny



Sangiovese self progeny ~ 4 weeks



Sangiovese self progeny: normal vs. chlorotic phenotype Effects of Rkatsiteli selfing at germination and after the first vegetative season



Germination of Rkatsiteli self progeny ~ 4 weeks



Rkatsiteli self progeny after the first vegetative season

Effects of Rkatsiteli selfing on phenotype after the first vegetative season









stem Ø: > 3 mm many buds sprout

stem Ø: ~ 3 mm few buds sprout stem Ø: ~ 3 mm no bud sprouts stem Ø: < 1 mm no bud sprouts

Stems and buds phenotypes

Methods

1) Assess **segregation distortion** (SD) in *Vitis vinifera* self-crosses

Genotyping of progenies from selfed varieties:
ddRAD-Seq libraries construction and analysis

(internal protocol modified from Peterson at al., PlosOne, 2012.)

One-locus distortion:

Chi-squared Test to measure lacking of genotypic classes and to look at reproductive barriers (Harushima et al., *Genetics*, 2001.)

Two-loci distortion:

Fisher's Test for distortion due to **two-loci epistatic interactions** (Alheit et al., *BMC Genomics*, 2011.)

Chi-squared Test for testing 1:15 model

double digest RAD-Seq workflow



Methods: self-crosses genotyping

	Before o	After cleaning	
	Samples	ddRAD loci	ddRAD loci
Rkatsiteli self progeny1	86	12,015	6,607
Rkatsiteli self progeny 1+2	(+152) 238	9,264	5,152
Primitivo self	62	15,648	8,498
Cabernet self	68	16,303	8,628
Sangiovese self	87	17,690	7,293
Pinot nero self	85	in progress	

Monomorphic markers SNPs in homozygous regions SNPs in repetitive regions Manual curation

Single locus SD in self-crosses





- Chi-square Test on single loci
- p-value of 0.05
- p-values adjusted for *fdr control*

Position (Mb)

Single locus SD in self-crosses





Single-locus SD and recombination



- Sangiovese self progeny Rkatsiteli self progeny
 - Cabernet franc self progeny

single locus SD in self-crosses: dominant lethal mutations

Primitivo self progeny - chr 12



Pairs-of-loci SD in self-crosses: epistatic interactions

Fisher's Test:

 to determine if there are interactions between pairs of loci in a dihybrid cross

Chi-square Test:

to test 1:15 model



Goals of the project

1) Assess segregation distortion (SD) in Vitis vinifera crosses

2) Investigate the role of SVs in SD

3) Define and characterize translocation in Rkatsiteli

Haplotype showing distortion



Haplotype showing distortion



If haplotype is lethal, shouldn't be found in **homozygous state in the population** short haplotype blocks





Haplotype showing distortion



If haplotype is lethal, shouldn't be found in **homozygous state in the population** short haplotype blocks



Position[Mb]

Main goal: to find lethal SVs, non-sense SNPs and indels in expressed genes

		one locus SD regions length (Kbp)		n. het SVs/SNPs in genes	
	Chr	Segregation defined	Population defined	Segregation defined	Population defined
Cabernet franc	8	3000	618	55	15
Rkatsiteli	8	250	149	1	0
Rkatsiteli	18	1300	919	13	4
Primitivo	4	2500	478	24	7
Primitivo	11	2800	1490	29	3
Primitivo	12	1400	na	18	6
Sangiovese	5	8900	2580	78	18
Sangiovese	8	260	34	5	2
Sangiovese	9	5400	2390	79	3
Sangiovese	11	2600	1790	50	14

Goals of the project

1) Assess segregation distortion (SD) in Vitis vinifera crosses

2) Investigate the **role of SVs in SD**

3) Define and characterize translocation in Rkatsiteli

Pairs-of-loci SD in Rkatsiteli self



- excess of some double-homozygous (AABB, aabb) and double-heterozygous (AaBb)
- lack of some double-homozygous (aaBB, AAbb) and single-heterozygous (Aabb, AaBB, AABb, aaBb)

PCR confirms translocation of chr 1 on chr 11 in Rkatsiteli



PCR confirms translocation also in Alexandrouli, Mtsvane kachuri and Gorula over about 250 varieties tested

Perspectives

DISTORTION ANALYSIS

- Define haplotypes of all varieties
- Find **lethal mutations in expressed genes** that correlate to the haplotypes implied in segregation distortion → FIND TARGETS
- Perform distortion analysis on pollen to improve distinction between pre- and post-zygotic selection in one-locus SD

RKATSITELI

Find the reciprocal translocation breakpoint

Perform ASE and specific allele methylation analysis to uncover whether chromosome re-location affects chromatin structure and gene expression

Acknowledgements

Michele Morgante

Fabio Marroni

Gabriele Di Gaspero

Gabriele Magris

Irena Jurman

Mirko Celii

Aldo Tocci

Ettore Zapparoli







European Research Council

Established by the European Commission

Supporting top researchers from anywhere in the world

Supported by the ERC project NOVABREED - Novel variation in plant breeding and the plant pangenomes (Grant agreement no.: 294780).

Bibliography

- Alheit KV, Reif JC, Maurer HP, Hahn V, Weissmann EA, Miedaner T and Würschum T, 2011. Detection of segregation distortion loci in triticale (*xTriticosecale* Wittmack) based on a high-density DArT marker consensus genetic linkage map. *BMC Genomics*, **12**:380.
- Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA, 2013. Stacks: an analysis tool set for population genomics, *Mol Ecol*. 22(11):3124-40.
- Gonen S, Lowe NR, Cezard T, Gharbi K, Bishop C, and Houston RD, 2014. Linkage maps of the Atlantic salmon (*Salmo salar*) genome derived from RAD sequencing. *BMC Genomics*, 15:166.
- Harushima, Nakagahra, Yano, Sasaki, Kurata, 2001. A Genome-Wide Survey of Reproductive Barriers in an Intraspecific Hybrid. *Genetics*, 159(2 883-892)
- Marroni F, Pinosio S, Morgante M, 2014. Structural variation and genome complexity: is dispensable really dispensable? *Curr Opin Plant Biol.*, 31:6
- Muñoz-Amatriaín M, Eichten SR, Wicker T, Richmond TA, Mascher M, Steuernagel B, Scholz U, Ariyadasa R, Spannagl M, Nussbaumer T, Mayer KF, Taudien S, Platzer M, Jeddeloh JA, Springer NM, Muehlbauer GJ, Stein N, 2013. Distribution, functional impact, and origin mechanisms of copy number variation in the barley genome. *Genome Biol.* 14(6).
- Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE, 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One.* 7(5).