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Characterisation of the pan-genome of *Vitis vinifera* using Next Generation Sequencing

Plant Biology Europe 2018 - June 18-21 - Copenhagen

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Genetic variation in grapevine

North American grapevines

Vitis rupestris



Asian grapevines

Vitis amurensis



Dicot outcrossing perennial species

The pan-genome concept

Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: Implications for the microbial “pan-genome”

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CORE GENOME

- single-copy sequences
- multiple-copy sequences
- transposable elements

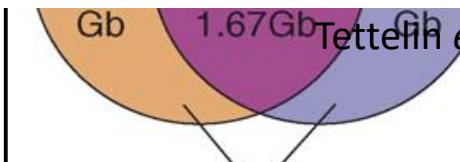
all individuals

Tettelin *et al.* Proc Natl Acad Sci USA, 2005

DISPENSABLE GENOME

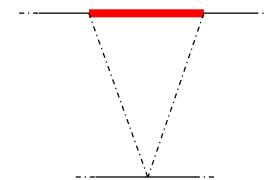
- transposable elements
- multiple-copy sequences
- CNVs + PAVs

only in some individuals



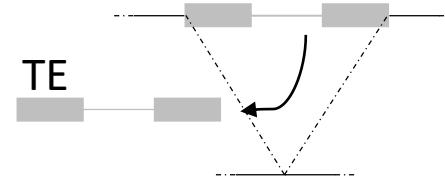
Structural variation calling

reference

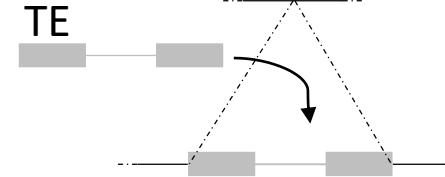


sample

deletion

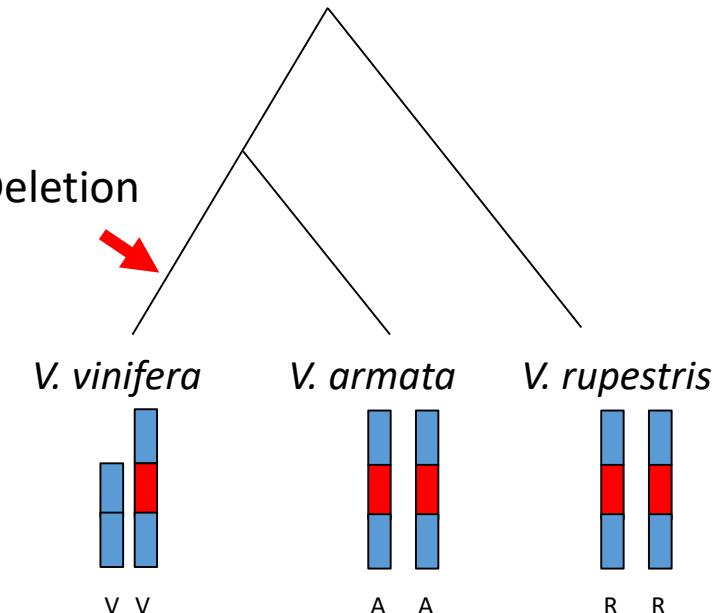


TE excision

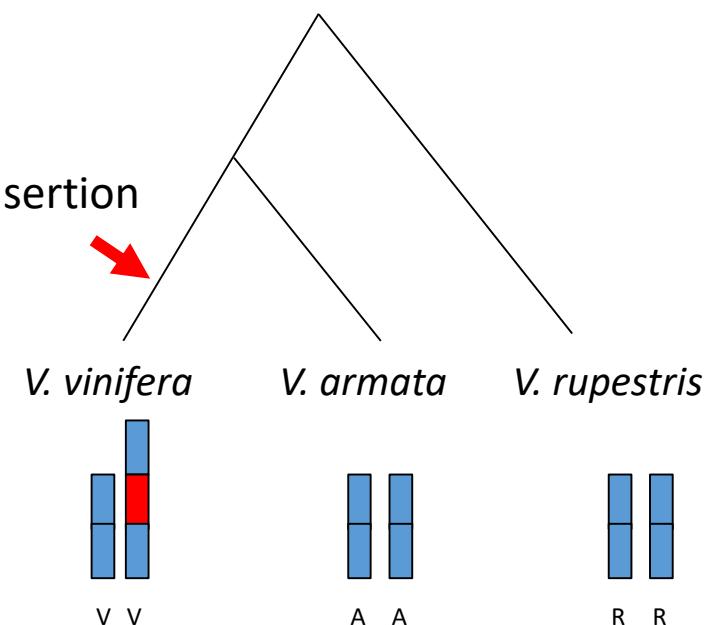


TE insertion

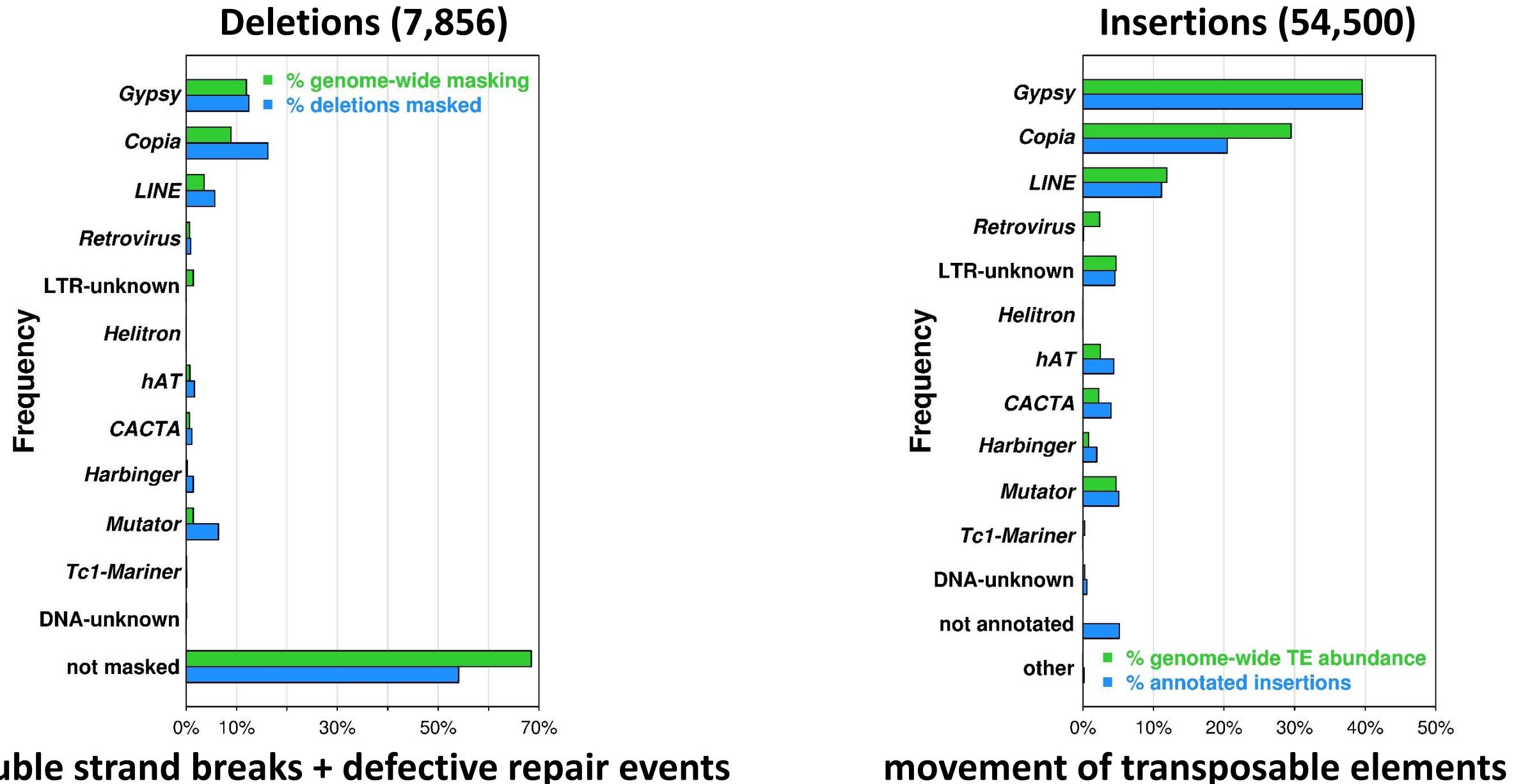
Deletion



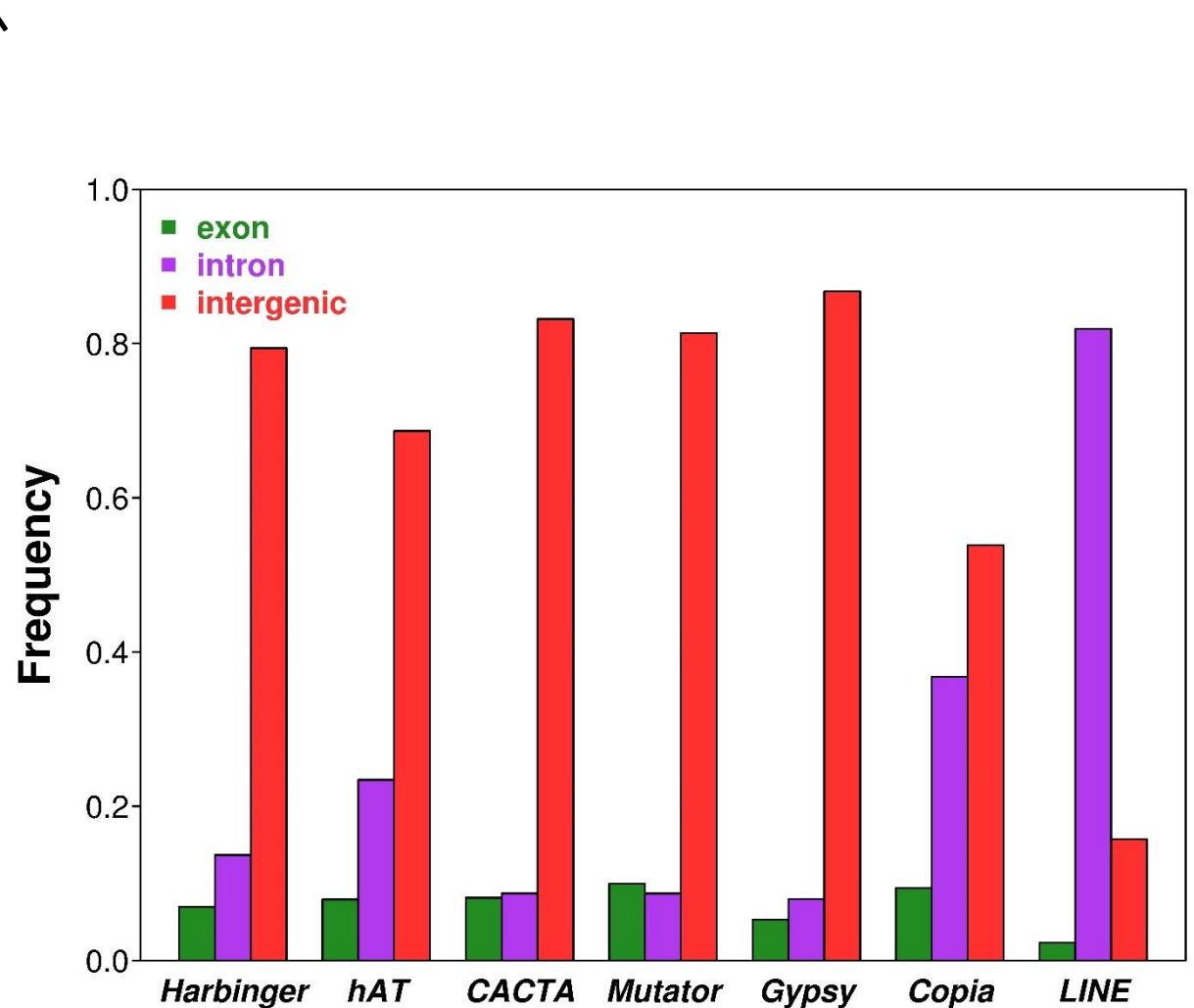
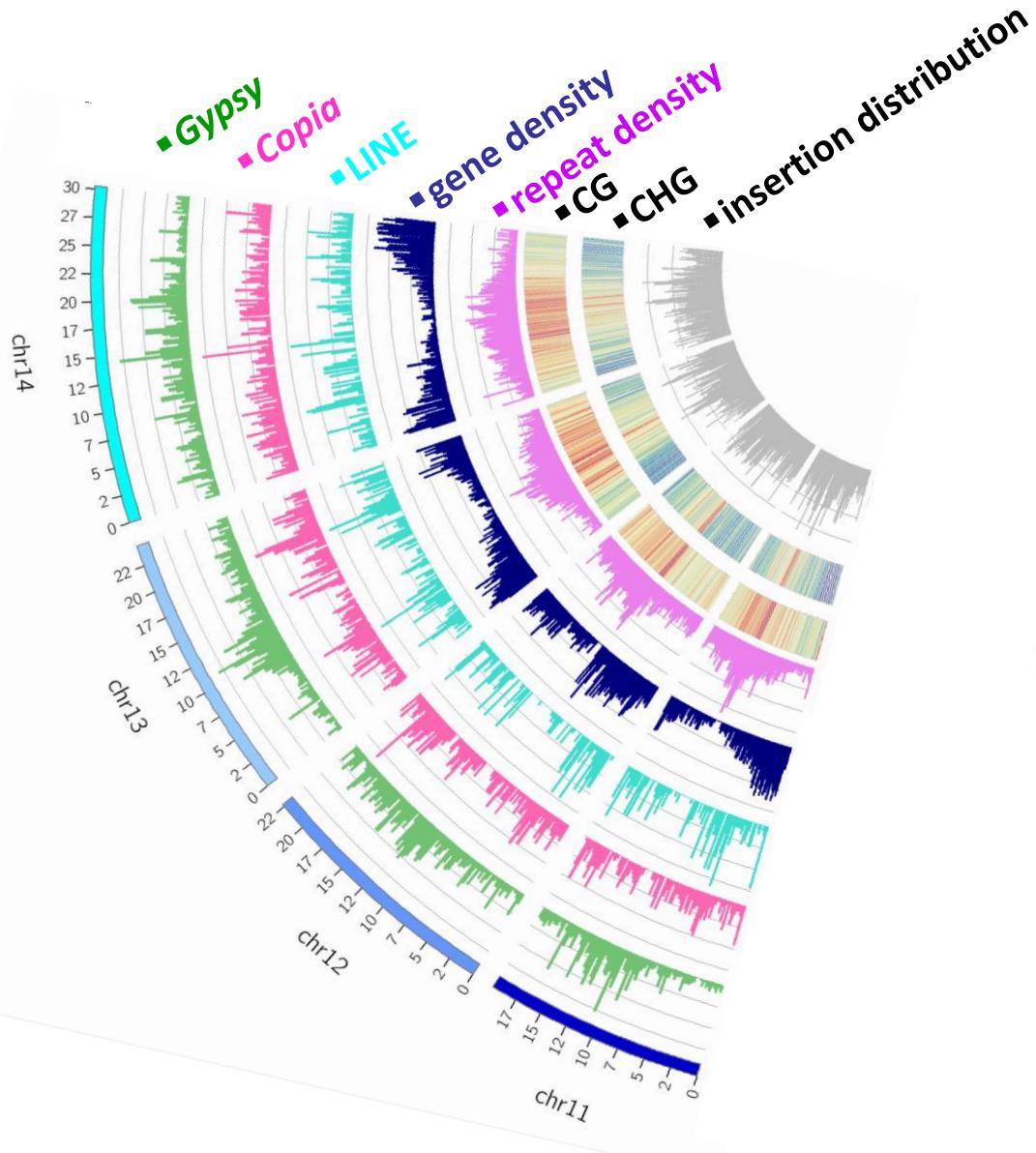
Insertion



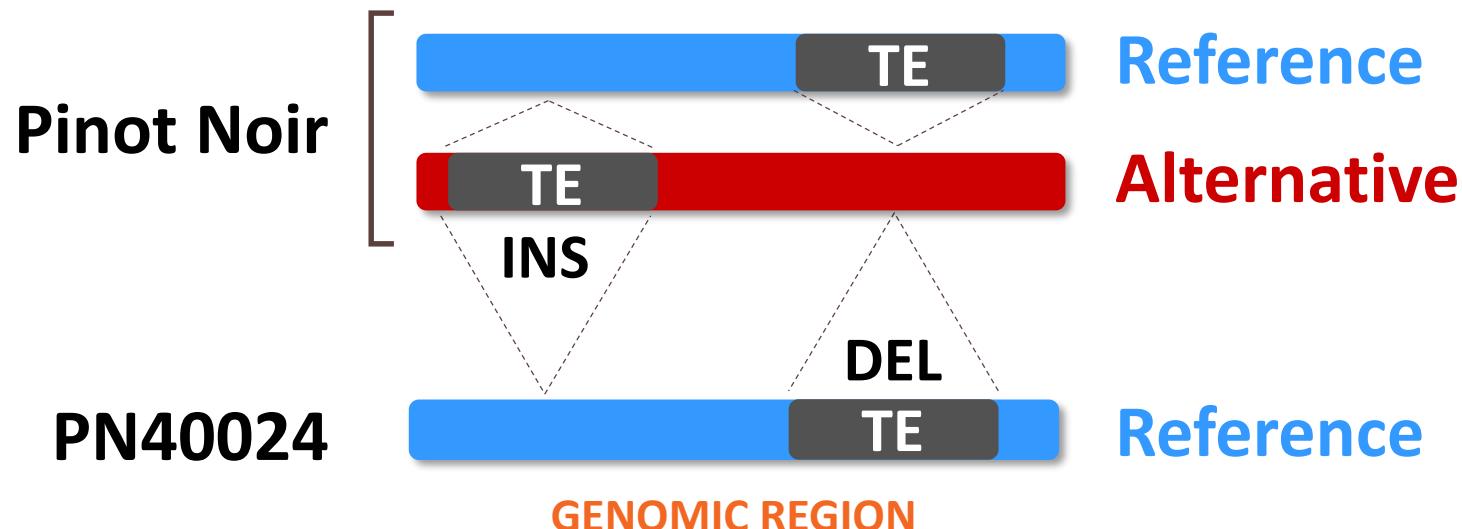
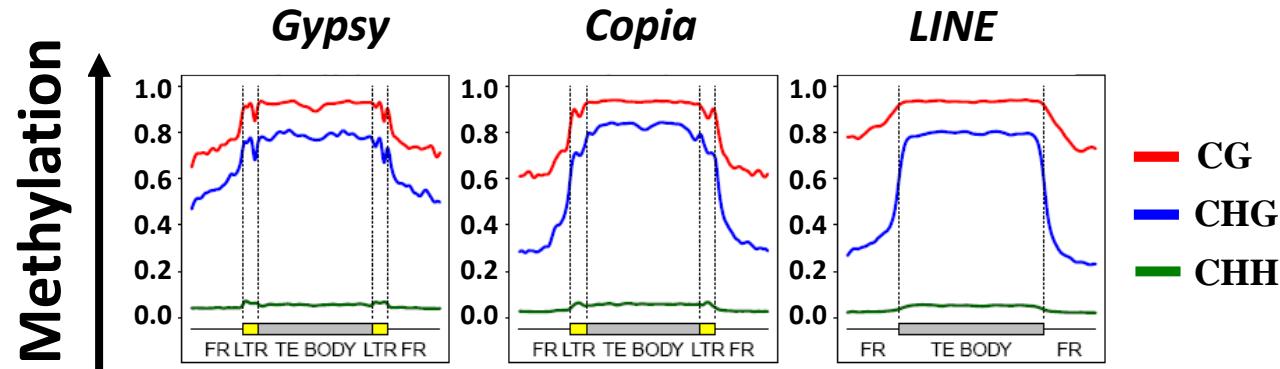
SV composition



SV location

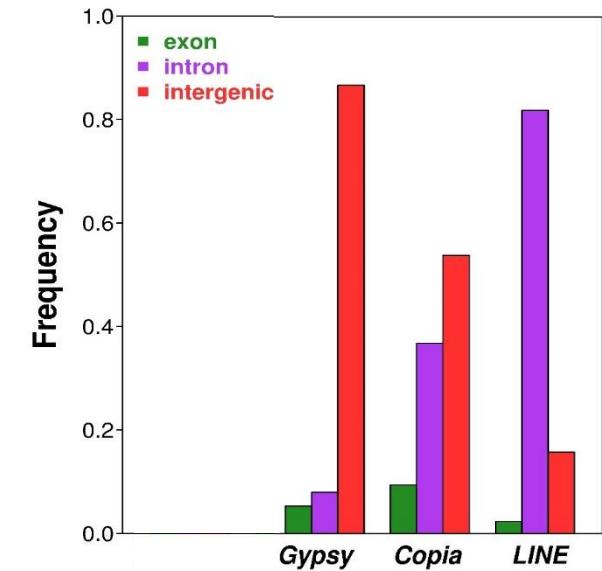
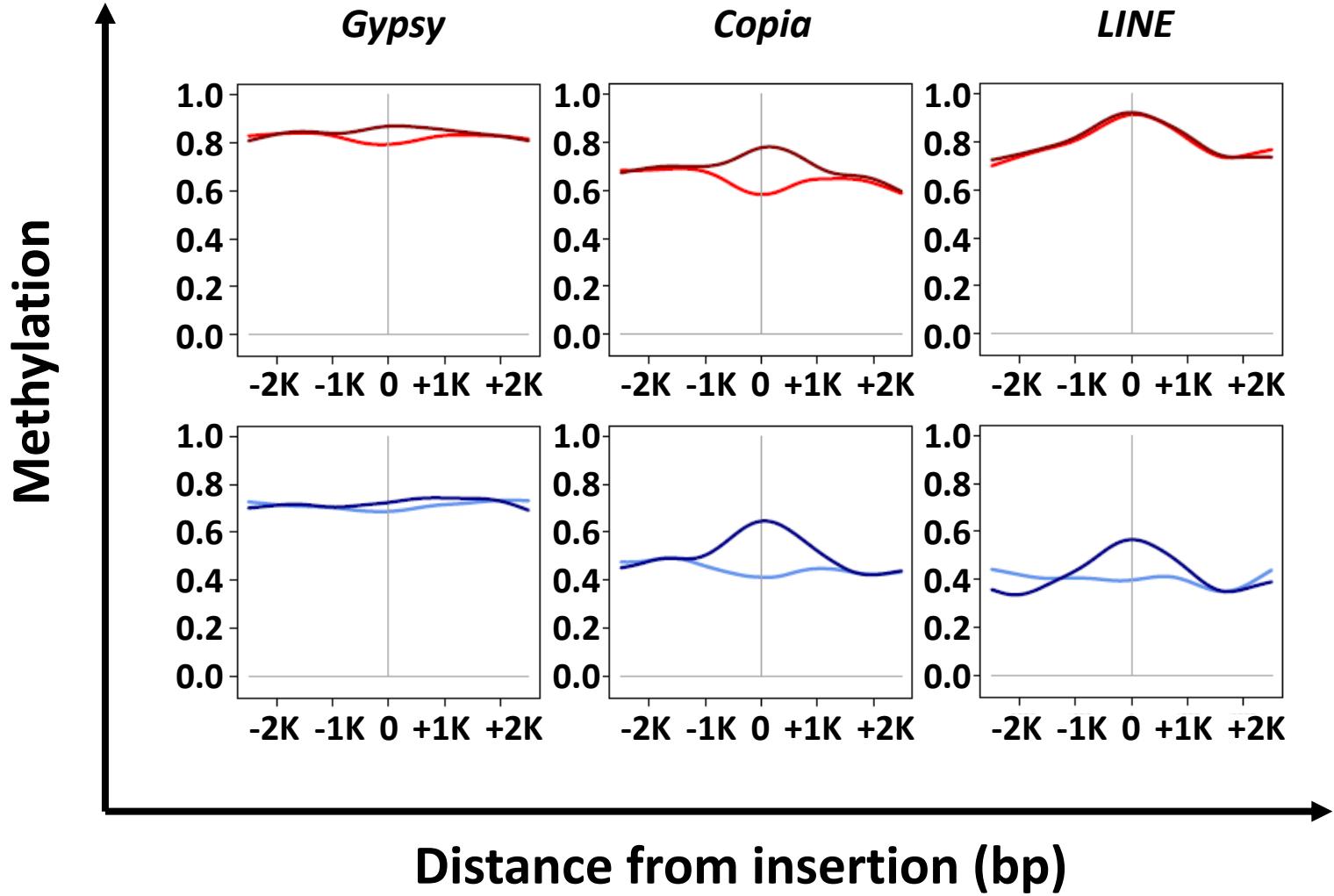


Methylation before and after TE insertion



Pinot noir is one of the two parents of the highly homozygous PN40024 reference

Methylation before and after TE insertion



CG CHG
TE haplotype ———
non-TE haplotype ———

Functional role of the dispensable genome – RNAseq data

Cabernet Franc

Leaves



Tendrils



Berries



Wine



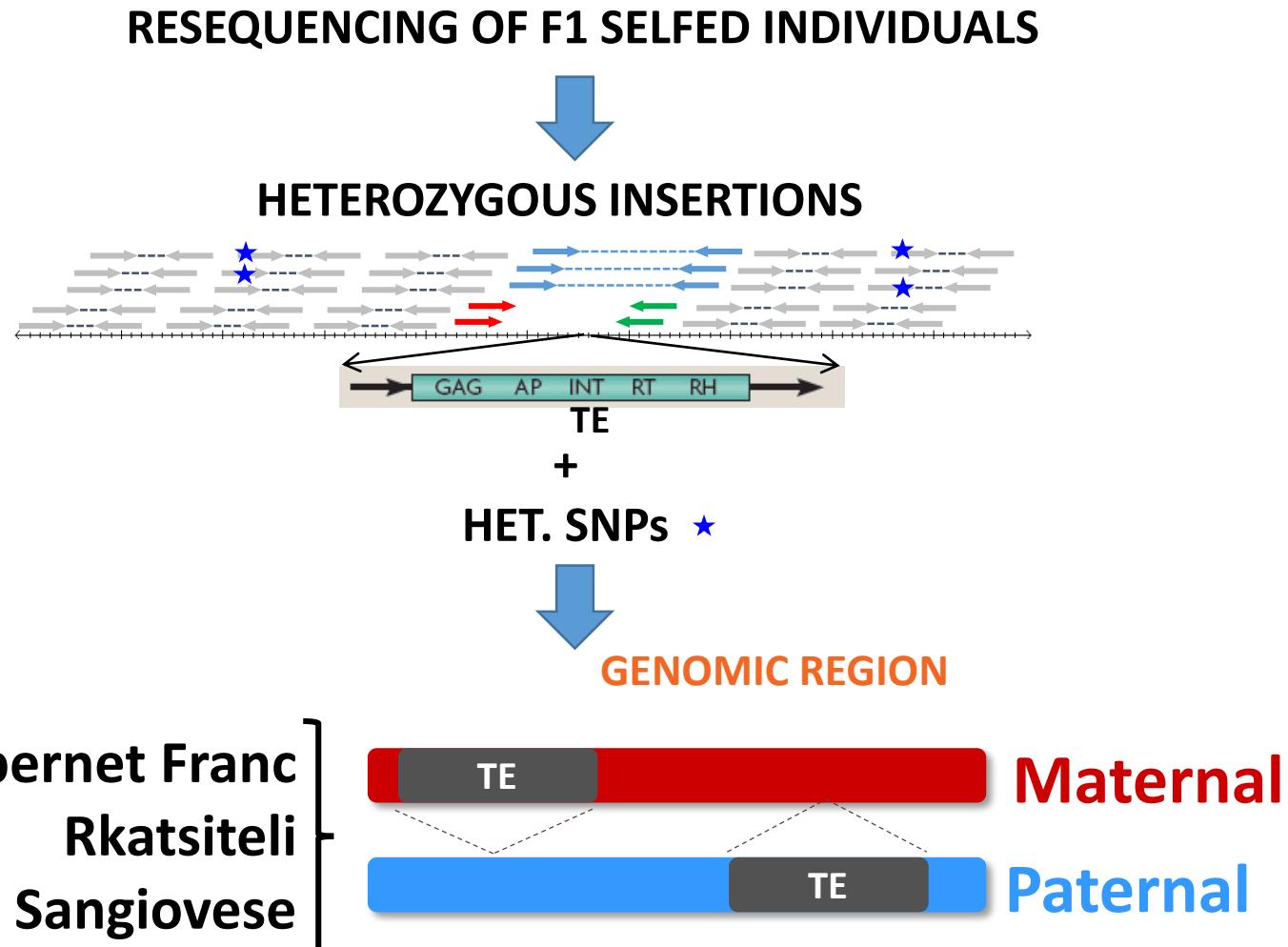
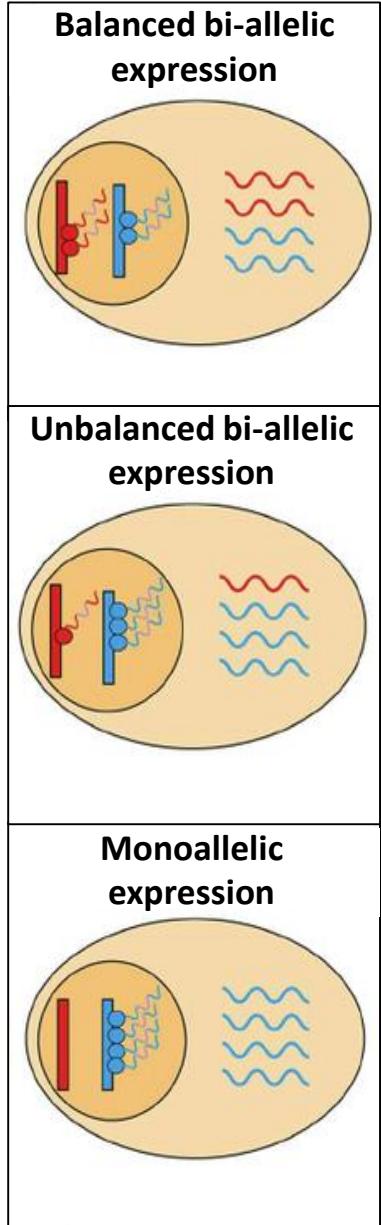
Rkatsiteli



Sangiovese

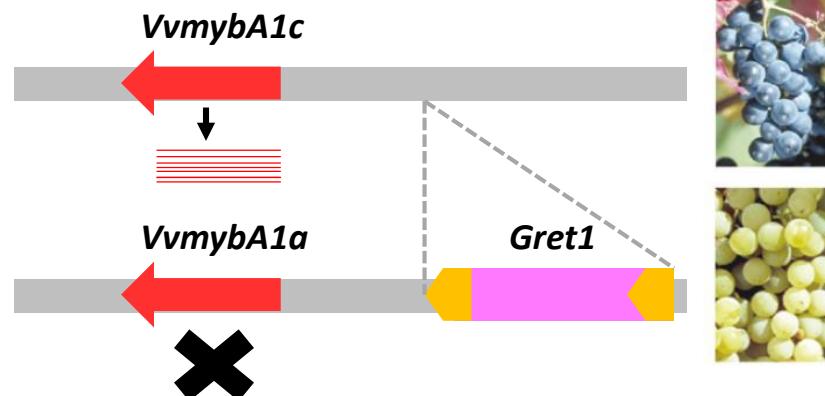
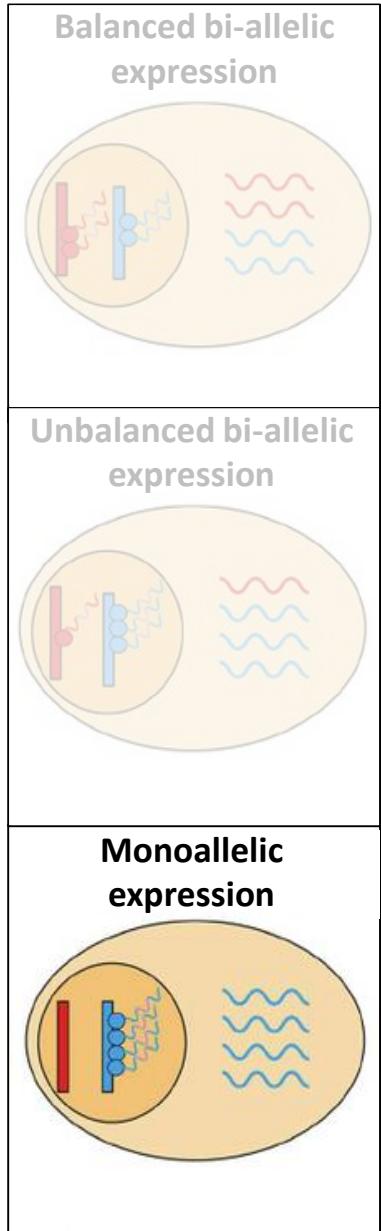


Haplotype phasing & Allele-Specific Expression analysis



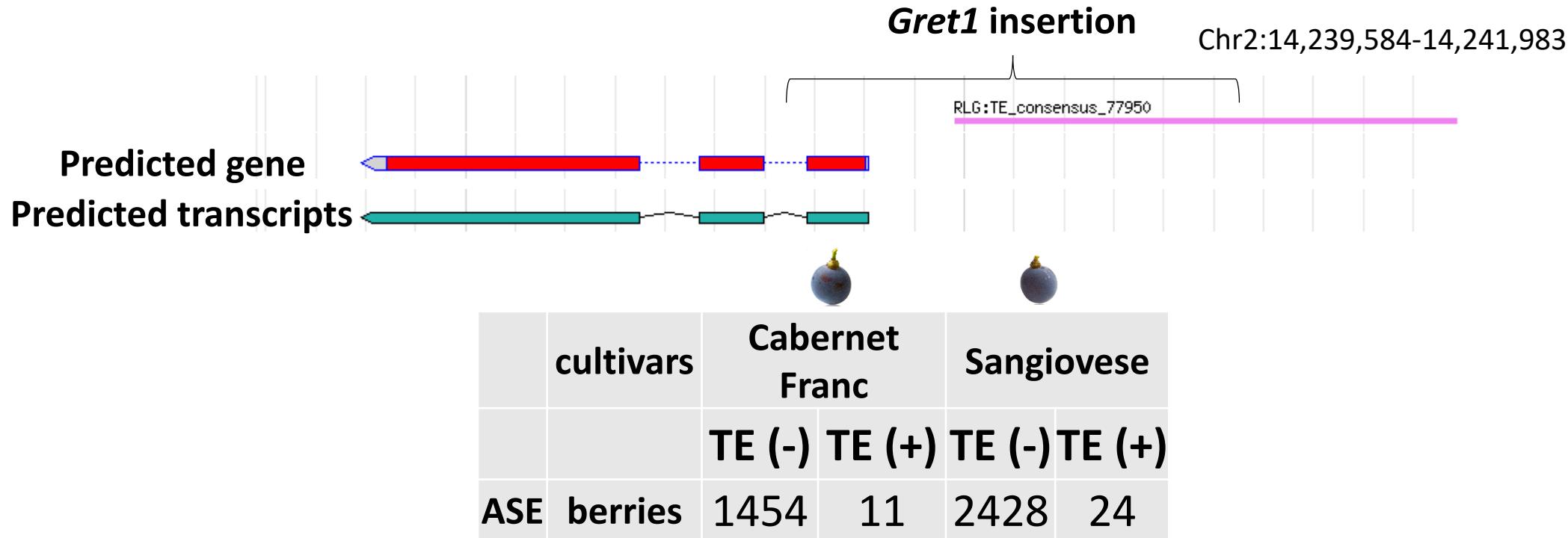
Phase inference for SNPs and SVs allows to perform allele-specific analysis of gene expression in heterozygous individuals

SVs and Monoallelic Expression (MAE): berries colour



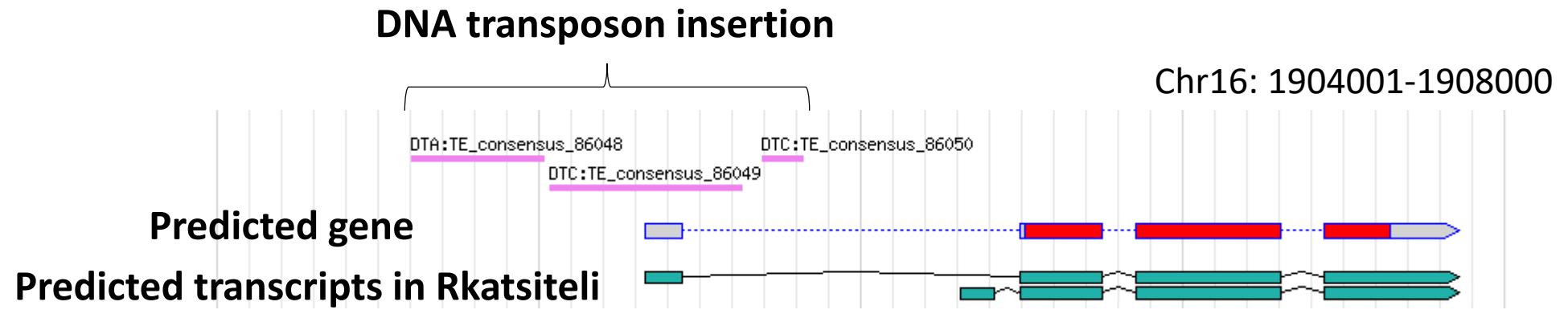
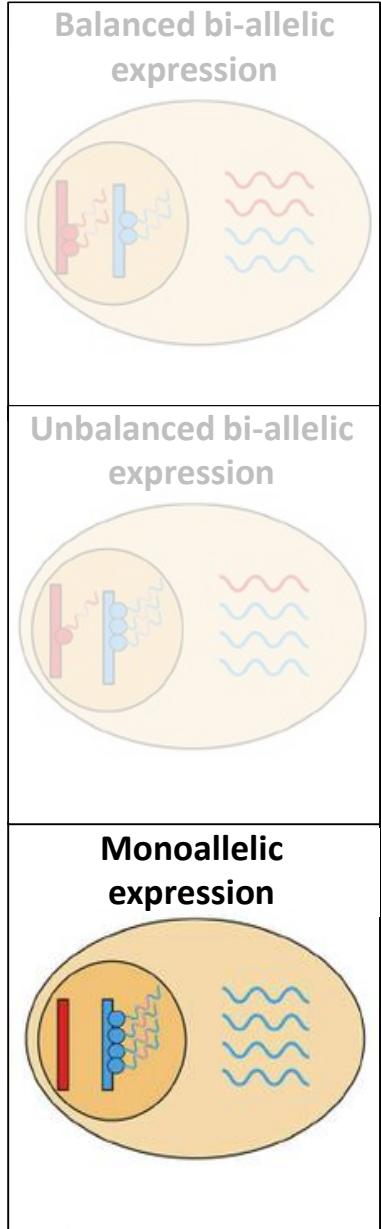
Adapted from Morgante et al. (2007)

The insertion of the retroelement *Gret1* in the promoter region of *VvmybA1* was in strong association with the white-fruited phenotype



Adapted from Kukurba et al. (2014)

SVs and Monoallelic Expression (MAE)



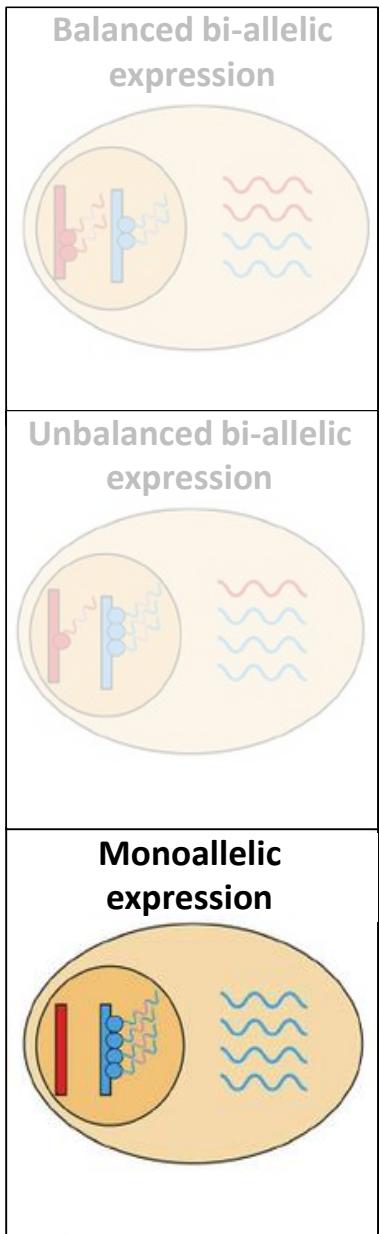
Rkatsiteli allele-specific counts

	TE (-)	TE (+)
berries	0	410
leaves	2	352
tendrils	2	714

	cultivars	Sangiovese	Rkastiteli	Kishmish Vatkana
	TE genotype	-/-	-/+	+/-
FPKM	leaves	0.0	25.4	90.4
	tendrils	0.0	43.3	97.1
	berries	0.0	34.0	80.5

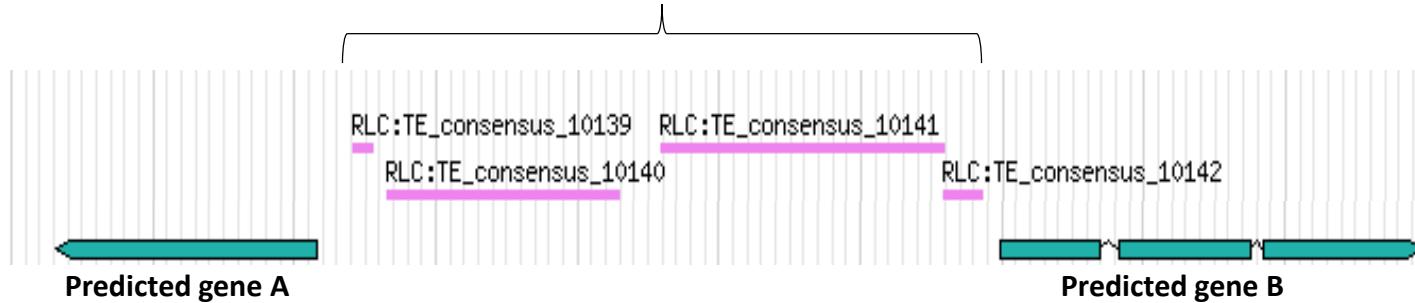
TE insertion increases expression of a *myB* transcription factor and changes gene structure

SVs and Monoallelic Expression (MAE)



Chr5: 23183001-23193000

Copia LTR-retrotransposon insertion



Allele-specific counts

		Cabernet Franc (-/+)		Rkatsiteli (-/+)		Sangiovese (-/-)	
		TE (-)	TE (+)	TE (-)	TE (+)	TE (-)	TE (-)
Gene A (NB-LRR)	berries	106	2	74	2	45	55
	leaves	153	1	211	4	17	57
	tendrils	154	0	220	0	22	27
Gene B (unknown protein)	berries	368	0	663	0	80	302
	leaves	613	1	1157	2	522	1083
	tendrils	662	4	1241	0	588	386

TE insertion shuts down expression of 2 genes (under bidirectional promoter)

Conclusions

- Grapevine has a **pan-genome** composed by a core and a dispensable fraction
 - The dispensable genome originates from deletions and insertions of transposable elements (TE).
 - Cultivated varieties are highly heterozygous / hemizygous
- Transposable elements affect **DNA methylation** in flanking regions
 - Spreading of heterochromatin marks is observed for all classes of TE
 - Effects of different TE on local chromatin are variable and depend on insertion-site methylation status
- Structural variants affect **allelic expression** of flanking genes
 - TE insertions may knock-out genes, contributing to genetic load
 - TE insertions can produce new patterns of gene expression
- Grapevine chromosomes differ **structurally** as well as **epigenetically**

Acknowledgements



European Research Council
Established by the European Commission

Novabreed
ERC-2011-ADG



Fabio Marroni
Sara Pinosio
Michele Vidotto
Alice Fornasiero

Eleonora Paparelli
Mirko Celii
Rachel Schwope
Aldo Tocci

Mara Miculan
Gabriele Di Gaspero
Michele Morgante



Federica Cattonaro
Davide Scaglione
Irena Jurman



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