Radiation of the grape retroelement (GRET1) in Vitis spp. of the grapevine germplasm repository.

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Mobile genetic elements have been found in nearly all living species and are known to insert into both hetero- and euchromatic regions of the genome. The effects of this movement on the genome can be the expansion of repetitive, noncoding regions, disruption of wildtype gene function leading to pseudogene status, modified gene functioning leading to altered, selectable phenotypes, and chromosomal rearrangements leading to speciation, to name a few (Kidwell and Lisch 2001). In European grapevine, Vitis vinifera, there are only three retroelements known to date, Gret1, Vine-1, and Tvv1 (Kobayashi et al. 2004, Verries et al. 2000). Interestingly, both Gret1 and Vine-1 were first identified by their having inserted into the regulatory sequences upstream of functional genes (Kobayashi et al. 2004, Verries et al. 2000). Currently, only Gret1 has been fully sequenced and is associated with mutations causing most, if not all, white-fruited V. vinifera via its insertion into the promoter of VvMybA1, the transcription factor controlling the final step in anthocyanin biosynthesis during ripening (Kobayashi et al. 2004, This et al. 2007).

Grapes and their wild relatives have a worldwide distribution, with most species being native to North America and Asia. Traditionally, these wild species have not been useful in wine production as European grapes have, but rather may be used for the production of unfermented juices. As a consequence, much less breeding and selection has gone into wild germplasm except for their use in the introgression of disease and insect resistance into V. vinifera hybrids and rootstocks. Interestingly, whereas V. vinifera includes many berry color phenotypes such as black, grey, red, rose and white, these other Vitis species are almost entirely black and rarely red. The only species containing white-fruited genotypes are V. labrusca, V. riparia, V. rotundifolia and V. aestivalis, each of which may claim single or very few white genotypes.

In light of these facts, the question becomes: Do the wild Vitis species contain the retroelement, Gret1, and if so, is it present in similar copy numbers to V. vinifera? In the present study, we test the hypothesis that native American grapevine species do contain Gret1 by screening the 1356 accessions of the USDA-ARS Plant Genetic Resources Unit Grapevine Germplasm collection using realtime PCR (qPCR) specific for Gret1.

Materials and Methods
DNA was isolated from all 1356 accessions of the USDA-ARS Plant Genetic Resources Unit Grapevine germplasm repository in Geneva, NY using a modification of Lin and Walker (1997). Significant changes to this protocol were the scaling of all reagents to facilitate tissue collection and DNA extraction in a 96-well plate format, and the addition of 200 mM Na$_2$B$_4$O$_7$•10H$_2$O to the extraction buffer for removal of phenolic compounds. DNA was quantified using A$_{260}$ and diluted to 25 ng/ul prior to amplification.

Primers and a labeled probe specific to the Vitis Gret1 element were designed using PrimerExpress (Biorad; www.bio-rad.com; Hercules, CA, USA) and synthesized by MWG Biotech (http://www.mwg-biotech.com/html/all/index.php; High Point, NC, USA) as follows:

Forward 5' - GCAGGAATGACGACTGGATCA - 3'
Reverse 5' - GTTGTTACCTCGCGTCTTTGG - 3'
Probe 5' – 6-FAM – CGTCCATCCATCTGTTACTACGTGGACC-Black Hole Quencher 1 – 3'

The PCR reactions were set up in 25 µl of 1x Biorad iQsupermix, 0.5 µM each primer, 0.5 µM labeled probe, 25 ng genomic DNA, 40 ng/µl sheared salmon sperm DNA (Sigma). qPCR was carried out using an iCycler (Biorad) for 60 cycles of 30 seconds at 95°C, 20 seconds at 60°C, 10 seconds at 72°C.

Three amplification replicates of each accession were conducted. In order to be able to compare threshold cycles between 96-well plates, a dilution series from 0 ng to 100 ng of genomic DNA of either V. vinifera cv. ‘Pinot Blanc’ or ‘Pinot Gris’ was included on each plate. It was determined by southern blot analysis that these two cultivars have similar copy numbers of Gret1 and no statistical difference was found between dilution series of the two genotypes. Three replicates per plate were also performed for the dilution series. ANOVA and Regression were used to analyze the dilution series. It was found that there was no statistical difference between qPCR plates (“plate”) and that dilution was a significant variable (“dil”). Based on these results, the qPCR threshold cycle values from a single 96-well plate were...
normalized based on the 10 ng dilution series data point from that plate and analyzed with the entire data set. All statistical calculations were performed using SAS statistical software (SAS Institute, Cary, NC).

**Results and Discussion**

The data presented here indicate that *Gret1* is present and that there is variation for copy number across all *Vitis* species tested. Variation was also found within species as demonstrated by the error bars shown in Figure 5 with *V. thunbergii* and *V. cognetiae* representing the extremes. This suggests an ancient appearance for *Gret1* but recent, possibly domestication-related, movement (Docking et al. 2006).

In screening 14 genotypes representing diverse species and berry colors using primers specific to the *Gret1* insertion in *VvMybA1*, it was found that the retroelement had not inserted into the promoter of this gene as it has in *V. vinifera* white berry genotypes.


Figure 1. Graphical display of ANOVA Least Significant Difference analysis for Grapevine collection qPCR data (threshold cycle) normalized to the 10 ng dilution series datapoint (see text). Low values indicate a low threshold cycle and therefore higher *Gret1* copy number. Analysis was conducted with respect to species, which is listed on the x-axis. Bars labeled with the same letters are not statistically different using α=0.05. Error bars represent one standard deviation and for those species without error bars only one accession was tested.