Theor Appl Genet (2008) 116:1079–1094
DOI 10.1007/s00122-008-0737-z

ORIGINAL PAPER

Genomic amplification of the *Gret1* retroelement in white-fruited accessions of wild *Vitis* and interspecific hybrids

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Received: 17 September 2007 / Accepted: 18 February 2008 / Published online: 12 March 2008
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Abstract Retrotransposons are retrovirus-related mobile sequences that have the potential to replicate via RNA intermediates and increase the genome size by insertion into new sites. The retroelement, *Gret1*, has been identified as playing a key role in generating fruit color variation in cultivated grape (*Vitis vinifera* L.) due to its insertion into the promoter of *VvMybA1*. Fruit color variation is an important distinguishing feature of cultivated grapes and virtually no fruit color variation is observed in wild grape species. The presence and relative copy number of *Gret1* was assessed using quantitative PCR on 22 different *Vitis* species, only four of which (plus interspecific hybrids) are known to contain white accessions. *Gret1* copy number was observed to vary by species as well as by color within species and was significantly higher in white-fruited accessions across all taxa tested. Additionally, genomic regions surrounding *Gret1* insertion were sequenced in white *V. vinifera*, hybrid, *V. labrusca*, *V. aestivalis*, and *V. riparia* accessions.

Introduction

Mobile genetic elements have been found in nearly all species of living organisms and are known to insert into both hetero- and euchromatic regions of the genome. The effects of this movement on the genome can lead to the expansion of repetitive, noncoding regions, disruption of wildtype gene function leading to pseudogene status, modified gene function resulting in altered, selectable phenotypes, and chromosomal rearrangements (Kidwell and Lisch 2001). At the population level, these changes can lead to species evolution as well as crop evolution and domestication (Kidwell and Lisch 2001; Naito et al. 2006).

Only three retroelements have been described in the European grapevine *Vitis vinifera*: *Gret1*, *Vine-1*, and *Tvv1* (Kobayashi et al. 2004; Verries et al. 2000; Pelsy and Merdinoglu 2002). Interestingly, both *Gret1* and *Vine-1* were first identified by their insertion into the regulatory sequences upstream of functional genes (Kobayashi et al. 2004; Verries et al. 2000). In fact, work by Pereira et al. (2005) suggests that *Gret1* inserts primarily in the euchromatin in low–moderate copy numbers within the *V. vinifera* genome. *Gret1*, a *Ty3-gypsy*-type element, is believed to be responsible for the mutation causing nearly 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; Lijavetzky et al. 2006; This et al. 2007). This insertional event results in the complete loss of expression of *VvMybA1* and can be complemented by transformation with the wildtype allele (Kobayashi et al. 2002). Recent evidence has shown that another similar gene, *VvMybA2*, which is physically linked to *VvMybA1*, also contains mutations that can lead to loss of color, and the presence of these mutations is perfectly correlated with the presence of *Gret1* (Walker et al. 2007).

V. vinifera is the most widely cultivated species of grape worldwide and includes many berry color phenotypes such as black, grey, red, rose, and white. Compared to *V. vinifera*, there is notably little fruit color diversity in

Communicated by A. Schulman.

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wild grape species. Of the approximately 30 *Vitis* species native to North America, vines are almost exclusively black-fruited, with rare reports of red or white types for a few species. In the non-*vinifera* species represented in the US *Vitis* germplasm collections, the only species containing white-fruited genotypes are *V. labrusca*, *V. riparia* and *V. aestivalis*, each of which may claim only a single reported white genotype. The grape berry color locus has been suggested to be a domestication gene and it may be that differences in crop evolution and early selection, or the lack thereof, between the wild and domesticated species of *Vitis* could explain this variation (This et al. 2007). That is, domestication of *V. vinifera* in Europe and Asia is thought to be the result of selection of favorable genotypes close to the origin of diversity in the Near East along with interbreeding with wild European *V. vinifera* genotypes as humans, and possibly a wine culture, migrated west (Arroyo-Garcia et al. 2006). The additional meioses associated with these domestication events could account for the amplification of berry color in *V. vinifera* as opposed to the lack of crossing and human selection in non-domesticated species.

Recently, our lab and others have demonstrated that sequence variation at the *VvMybA1* locus is strongly associated with all known *V. vinifera* berry color phenotypes (Lijavetzky et al. 2006; This et al. 2007). Whereas black and white berries represent full and null expression of this gene, respectively, many red and rose varieties possess alleles with two additional mutations in the footprint of *Gret1* or one of two SNPs in the promoter downstream from the *Gret1* insertion site (This et al. 2007). It is possible that this sequence variation in the promoter of red and rose varieties leads to reduced *VvMybA1* expression levels, thus leading to reduced anthocyanin production, although this has not yet been tested. Additional work has shown that the grey-fruited variety "Pinot gris" is a chimera, possessing a genotypically white L2 cell layer and a genotypically black L1, resulting in berries that are intermediate between white and black (Hocquigny et al. 2004). Such chimerism also appears to be a reasonable explanation for the appearance of rose-colored berries (Walker et al. 2006). These findings suggest that the genetic variation underlying grape berry color evolution is, indeed, quite restricted.

In contrast to the lack of genetic diversity in *Vitis* berry color, other species such as *Petunia hybrida*, *Zea mays*, *Antirrhinum majus*, and *Lycopersicon esculentum*, all contain multiple natural color mutants (Table 1) (De Jong et al. 2004; Ludwig et al. 1989; Martin et al. 1991; Mol et al. 1983; Paz-Ares et al. 1987; Quattrocchio et al. 1993; Schiefelbein et al. 1988). Nearly every structural and regulatory gene in the well-conserved anthocyanin biosynthetic pathway has been identified as causal or cosegregating with loss-of-color mutation. These mutations affect color in flowers, seed coat, aleurone, pericarp and pubescence owing to the tissue-specific control conferred by the action of *myb*- and *myc*-like transcription factors (Ludwig et al. 1989; Paz-Ares et al. 1987). Color variants derived from induced mutagenesis (e.g., transposon tagging, radiation or chemical) in these species as well as the laboratory model, *Arabidopsis thaliana*, consistently uncover additional alleles at both the biosynthetic pathway genes and *myb* and *myc* regulatory genes (van Houwelingen et al. 1998). Further, a surprisingly large proportion of the natural loss-of-color mutations are apparently caused by the presence of mobile genetic elements such as *Gret1* in *V. vinifera*, *Tgm* in *Glycine max*, *Ac*/*Ds* in *Z. mays* and *dTph* in *P. hybrida* (Kobayashi et al. 2004; Kroon et al. 1994; Matsumara et al. 2005; Schiefelbein et al. 1988; Xu and Palmer 2005; Zabala and Vodkin 2005).

Considering the remarkable conservation between species within the anthocyanin biosynthetic pathway, the identification of only a single anthocyanin mutation locus in *V. vinifera* is noteworthy. The phenotypic data from the North American species also suggest that few white alleles exist in nature. The role of *Gret1* in producing white berries in *V. vinifera* is well documented. In the present study, we test the null hypothesis that this retroelement is limited to European grapevine by a quantitative PCR (qPCR) assay of a large collection of North American grape germplasm. We have further assayed this population for mutant alleles at the *VvMybA1* locus and other loci related to flavonoid biosynthesis. Our data demonstrate that there are species-level differences in *Gret1* copy number within *Vitis* species and that the white phenotype is associated with higher copy number. Additionally, we have identified several additional loci containing *Gret1* insertions in the genome of N. American *Vitis* species.

Materials and methods

Plant material

Grapevine accessions maintained by the USDA–ARS Plant Genetic Resources Unit (PGRU) Grapevine germplasm repository in Geneva, NY, were used for these experiments (Table 2). Most members of this collection are indigenous to North America with a few accessions originating from Asia. Additionally, *V. aestivalis* cv. "Pixiola" from the National Clonal Germplasm Repository in Davis, CA, was included in these analyses as the only known example of a white-fruited *V. aestivalis*. Data on berry color was downloaded from the Germplasm Resources Information Network (GRIN) database (http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl) and confirmed visually upon

Table 1 Selected natural color mutations in diverse species

Genotype	Gene product	Tissue specificity	Mutation	Reference
Antirrhinum				
candica (*candi*)	ANS/LDOX	Flower color	TE insertion	Martin et al. 1991
nivea (*niv*)	CHS	Petal epidermis	TE insertion	Comba et al. 2000
incolorata	F3H	Flower color	Likely TE	Forkmann and Stotz 1981; Martin et al. 1991
eosinea (*eos*)	F3′H	Flower color	Likely TE	Holton and Cornish 1995
delila (*del*)	Myc (bHLH)	Flower color	Likely TE	Docking et al. 2006; Goodrich et al. 1992
eluta (*el*)	Transcription factor	Flower color	Likely TE	Martin et al. 1991
Capsicum annuum				
a	Myb	Fruit	?	Daskalov and Poulos 1994
Dianthus caryophyllus				
flavonoid3 (fl3, fl3-m)	GST	Flower color	TE	Larsen et al. 2003
Glycine max				
I	CHS	Seed coat	CHS tandem repeats	Matsumara et al. 2005; Nicholas et al. 1993
w3	DFR	Flower color	Likely TE	Fasoula et al. 1995
wp	F3H	Flower color	TE (Tgm-Express 1)	Zabala and Vodkin 2005
T	F3′H	Pubescence; seed coat	Deletion, protein truncation	Nicholas et al. 1993; Toda et al. 2002
Im	?	Seed coat	Likely TE	Nicholas et al. 1993
w4	?	Flower color	TE	Xu and Palmer 2005
Petunia hybrida				
po	CHI	Anther color	Regulatory sequence mutation	van Tunen et al. 1989; Vantunen et al. 1991
'red star'	CHS	Flower color	siRNAs	Koseki et al. 2005; Mol et al. 1983
hf1, hf2	F3′5′H	Flower color	TE (dTph9, rTph1)	Matsumara et al. 2005; Wiering 1974
ht1, ht2	F3′H	Flower color	TE (dTph1)	van Houwelingen et al. 1998; Wiering 1974
ph4	Myb	Flower color	TE (dTph1)	Quattrocchio et al. 2006
rt	RT (UFGT-like)	Flower color	Defective TE (dTph1, dTph3)	Kroon et al. 1994
Pisum sativum				
A	Myc (bHLH)	Flower color	?	Uimari and Strommer 1998
A2	Myc (bHLH)	Flower color	?	Uimari and Strommer 1998
Sorghum bicolor				
yellow seed 1 (y1)	Myb	Pericarp; glume	Deletion	Boddu et al. 2005
Lycopersicon esculentum				
anthocyanin without (*aw*)	DFR	Whole plant	?	Goldsbrough et al. 1994
anthocyanin less (*a*)	F3′5′H	Seed coat, stem, leaf	?	De Jong et al. 2004
anthocyanin gainer (*ag*)	Myb	Whole plant	?	De Jong et al. 2004
anthocyanin absent (aa)	?	Whole plant	?	http://tgrc.ucdavis.edu/
Vitis vinifera				
VvMybA1	Myb	Berry skin	Gret1 insertion (TE)	Kobayashi et al. 2002, 2004
Zea maize				
pr	F3′H	Aleurone	?	Coe et al. 1988
bronze 2 (*bz2*)	GST	Aleurone	DS (TE)	Marrs et al. 1995; Takahashi et al. 1994
c1	Myb	Aleurone	likely TE	Paz-Ares et al. 1987
r1	Myc (bHLH)	Aleurone	TE	Bhardwaj and Wilkinson 2005; Ludwig et al. 1989
bronze 1 (*bz1*)	UFGT	Aleurone	Ac/Ds (TE)	Schiefelbein et al. 1988

berry maturation. Twenty-two species, including interspecific hybrids, were represented, five of which contain both white and nonwhite berries (Table 2). × *andersonii* is the designation for the natural hybrid of *V. coignetiae* × *V. riparia*; × *champinii* for *V. mustangensis* × *V. rupestris*; × *doaniana* for *V. mustangensis* × *V. acerifolia*; and × *novae-angliae* for *V. labrusca* × *V. riparia*.

DNA isolation and PCR

DNA was isolated from young leaf tissue of all 1,356 accessions using a modification of Lin and Walker (1997). Significant changes to this protocol included the scaling of all reagents to facilitate tissue collection and DNA extraction in a 96-deepwell plate format, and the addition of 200 mM $Na_2B_4O_7{\cdot}10H_2O$ to the extraction buffer for removal of phenolic compounds. DNA was quantified by measuring the absorbance (A_{260}) and the quality checked by agarose gel visualization. All DNAs were normalized to 25 ng/μl prior to amplification.

Table 2 List of *Vitis* and related species in the North American *Vitis* collection in Geneva, NY, USA

	# Accessions	
	White	Nonwhite
Vitis acerifolia	0	15
Vitis aestivalis	1	10
Ampelopsis brevipedunculata	0	1
Vitis amurensis	0	22
Vitis cinerea	0	48
Vitis coignetiae	0	3
Vitis hybrid	123	503
Vitis labrusca[a]	4	38
Vitis monticola[b]	0	12
Vitis palmata	0	4
Vitis piasezkii	0	2
Vitis riparia	1	186
Vitis rupestris	0	26
Vitis sp. (unknown)	0	60
Vitis thunbergii	0	5
Vitis vinifera	5	15
Vitis vulpine	0	32
× *andersonii*	0	1
× *champinii*	0	2
× *doaniana*	0	1
× *novae-angliae*	0	1
Vitis yenshanensis	0	33
Total	134	1,020
Grand total	1,154	

[a] Three of the four white *V. labrusca* are probably hybrids with *V. vinifera*

[b] *V. monticola* is probably mislabeled in the Geneva, NY, germplasm collection as this species would not survive the climate. A more likely classification is *V. riparia*

Sixteen representative samples from each 96-well plate were amplified using primers to 18S rDNA to confirm that the DNAs were amplification-competent. The 18S rDNA primers were as follows: forward 5′-CTGGCGACGCAT CATTC-3′ and reverse 5′-GAATTACCGCGGCTGCT-3′ and amplification of 18S rDNA was performed for 30 cycles of 95° for 1 min, 54° for 45 s, and 72° for 1 min. A 10-fold dilution series (100, 10, 1, 0.1, 0.01, 0 ng) of DNA from each species included in this study was amplified using the same 18S primers and amplification conditions in a Sybr green-based qPCR assay. Dilution series were analyzed using PROC REG of the SAS version 9.1 statistical software (SAS Institute, Cary, NC). No statistical difference was detected between species ($P = 0.6613$), thus confirming that 18S is a suitable control and that all species can be amplified with equal efficiency.

qPCR primers and a labeled probe specific to the *Vitis Gret1* element were designed using PrimerExpress (Applied Biosystems, Foster City, CA) and synthesized by MWG Biotech (High Point, NC, USA) as follows: forward 5′-GCAGGAATGACGACTGGATCA-3′; reverse 5′-GTT GTTACCTCGCGTCTTTGG-3′; probe 5′-6-FAM-CGTCC ATCCATCTGGTTACTACGTGGACC-BHQ1-3′. The qPCR reactions were set up in 25 μl of 1× Biorad IQSupermix, 0.5 μM each forward and reverse primer, 0.5 μM labeled probe, 50 ng genomic DNA, and 40 ng/μl sheared salmon sperm DNA (Sigma, St Louis, MO). qPCR was carried out using an ICycler (Biorad, Hercules, CA) for 60 cycles of 95° for 30 s, 60° for 20 s, and 72° for 10 s. Representatives from each species in the collection were amplified and visualized by gel electrophoresis, all of which resulted in a single band of the expected size (data not shown).

Detection of the *Gret1–VvMybA1* and wildtype *VvMybA1* alleles was performed according to Kobayashi et al. (2004) for all white accessions in the PGRU collection and Pixiola, a white-fruited *V. aestivalis*. This method entailed the use of forward primers based either in the 3′ LTR of *Gret1* (A) or immediately 5′ to *Gret1* (B) in combination with a reverse primer within the *VvMybA1* coding region (C). The AC primer combination is a marker for the presence of *Gret1* and BC is a marker for the absence.

Primers to detect the presence of *Gret1* inserted into other anthocyanin biosynthetic or other candidate genes were designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and are listed in Table 3. Candidate genes selected for this analysis were either known structural genes in the anthocyanin biosynthetic pathway or genes identified in other plant systems as

Table 3 Primers used for the amplification of genomic sequence surrounding *Gret1* and anthocyanin biosynthesis candidate genes

Primer name	Genbank accession[a]	Direction	Length	T_m (°)	Sequence (5′ → 3′)	Annealing temperature in combination with universal forward primer
COMT		Reverse	20	54.8	ATGCCAGGTTCTTCGAGATG	51°
CHIchimera	X75963	Reverse	20	55	CAGCTTCAATTTTCTCATCC	NA[b]
CHSchimera	X75969	Reverse	20	55	GTGGGAGTTCAATTTTGGTA	57°
DFRchimera	Y11749	Reverse	20	55	AATAATGAGCTTCGTTTCCA	NA
F3Hchimera	X75965	Reverse	20	54.8	ATGAGCTTCTCGCTGTACTC	NA
F3′5′H		Reverse	21	54.2	GGTTTTAAGGAAAGCCTGAGC	52°
Gret1 universal	AB242301	Forward	22	51	TTTTTTAACCCGCCCATTTTT	–
GSTchimera	AY971515	Reverse	20	54.9	GCACTCACACTCTTCCTCTC	NA
MRPchimera	AF488698	Reverse	20	55	AGTCAACTTCCAACTCGAAA	NA
PHYA		Reverse	20	54.7	CCGTCATTGACAACAACTGC	52°
UFGTchimera	DQ513314	Reverse	20	55	GAGGTGGGTTTTCTTTCTTT	55°

[a] Accession number of sequences used for primer design

[b] NA indicates no amplification in *V. vinifera* or any wild species

being involved in anthocyanin processing or transport. Candidate gene insertional mutants were detected by PCR with 32 cycles of 95° for 1 min, 45 s at annealing temperature (see Table 3), and 72° for 2 min.

Gret1 quantification experimental design and analysis

Three amplification replicates of each accession in the germplasm collection were conducted. In order to be able to compare threshold cycles between 96-well plates, a 10-fold dilution series from 100 to 0 ng (100, 10, 1, 0.1, 0.01, 0 ng) of genomic DNA of either *V. vinifera* cv. 'Pinot Blanc' or 'Pinot Gris' was included on each plate. Pinot Gris is phenotypically grey due to its chimeric nature: Pinot Noir L1 layer and Pinot Blanc L2 layer. Thus, while the fruit of the variety differs phenotypically from Pinot Blanc, the vast majority of its cells are genetically identical. The two different genotypes were used as plate controls due to limitations in DNA quantities; however, no statistical difference in *Gret1* copy number was found at $\alpha = 0.05$ between dilution series of the two genotypes ($P = 0.067$). Three replicates per plate were also performed for the dilution series to ensure statistical rigor. Experimental raw data were expressed as threshhold cycle (Ct) and data from each plate were normalized as a proportion of the Ct of the 10 ng Pinot Blanc or Pinot Gris control sample to the Ct of the sample (Ct_{norm}):

$$Ct_{norm} = \frac{Ct_{pinot}}{Ct_{experimental}}$$

This normalization results in higher Ct_{norm} values representing higher *Gret1* copy number. Normalized data were analyzed using PROC MIXED of the SAS Version 9.1 statistical software (SAS Institute, Cary, NC) comparing species means as well as color within species.

Amplification of genomic sequences flanking *Gret1*

Inverse PCR (IPCR) was performed as in Ochman et al. (1988). Genomic DNA (5 µg) from cvs. Pinot Blanc (*V. vinifera*), Pinot Noir (*V. vinifera*), Pixiola (*V. aestivalis*), Bougher (*V. riparia*), Alba (*V. labrusca*), and the interspecific hybrid Siebel 8229 (pedigree includes: *V. riparia* Michx, *V. labrusca* L., *V. vinifera* L., *V. rupestris* Scheele, *V. lincecumii* Buckl., *V. cinerea* Engelm) were digested with *Hin*dIII and column purified (Qiagen, Valencia, CA). Circularization of *Hind*III fragments was achieved by ligation at 12°C for 16 h using T4 DNA ligase (Promega, Madison, WI) according to the manufacturer's instructions. DNA was purified by passing over a second column and then used as a template in PCR using primers designed with Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) based on the *Gret1* sequence (GenBank accession AB111101) near the LTR. The primer sequences used for IPCR were: forward 5′-CATCTTAC TAGCACCCTTCACTG-3′ and reverse 5′-ATGGTGCCA AAGACTGTCG-3′. Amplification was carried out in 1× Promega colorless PCR buffer (Promega), 0.2 mM dNTPs (Promega), 0.4 µM of each primer, 1 U GoTaq DNA polymerase (Promega) with 15 ng template DNA for 35 cycles of 94° for 1 min, 45° for 45 s, and 72° for 3 min. If no distinct bands were present following the initial round of IPCR, a second PCR was carried out using the same conditions using 1 µl of the initial PCR reaction as

template. All resulting bands were gel-extracted and purified, taking a portion of the gel surrounding the bands as well in order to capture similarly sized yet underrepresented products. The purified products were cloned using the TOPO cloning kit for sequencing (Invitrogen, Carlsbad, CA) and sequenced using the vector T3 and T7 primer sites. Sequencing was performed at the Cornell University Biotechnology Resource Center on an Applied Biosystems Automated 3730 DNA Analyzer.

Sequence analysis

Sequence analysis, vector trimming, and alignment were carried out using Sequencher 4.1.4 (Gene Codes Corperation, Ann Arbor, MI). Consensus sequences from IPCR clones were BLASTed against the draft *V. vinifera* cv. 'PN40024' genome sequence available from Genoscope Centre National de Sequencage (http://www.genoscope.cns.fr/externe/English/corps_anglais.html) (The French–Italian Public Consortium for Grapevine Genome Characterization 2007). Genomic contigs resulting from these searches were used to query BLAST against the EST database for all organisms in Genbank to identify putative expressed sequences within the genomic *Vitis* sequence. Any identified ESTs were then submitted to the BLASTx search to identify possible protein candidates. The sequences identified in the present study were submitted to Genbank and assigned the accession numbers EF119791–EF119830).

Results

The Taqman assay developed for these experiments targets the LTR region of *Gret1* that may be more susceptible to mutation than other portions of this element (Bennetzen 2000). Our primers are specific to *Gret1* only and amplified the predicted 150 bp fragment in all species tested (data not shown). It is entirely possible that this assay detects fragmented *Gret1* where only a portion of the element or the LTR remains. Because our aim is to detect all *Gret1* elements resulting from both ancient and recent insertions, the detection of these truncated forms is well within the scope of this study.

Species-level differences in *Gret1* copy number exist within N. American *Vitis* germplasm. Ct_{norm} values ranged from 0.5 to 1.3 where a Ct_{norm} value of 1 indicates equivalent *Gret1* copy numbers to those of the control genotypes *V. vinifera* Pinot Blanc and Pinot Gris. Of the 1,356 accessions in the grapevine collection, 187 did not exceed the plate threshold value (i.e., did not amplify) and were set at 70 cycles in order to create a "did not amplify" datapoint for the purpose of statistical analysis. Statistical analysis using the SAS mixed model Ct_{norm} = species color(species) revealed statistical differences in *Gret1* copy number between species (Fig. 1). Notably, *V. vinifera* and *V.* hybrid, a synthetic taxonomic grouping mostly containing *vinifera* interspecific hybrids, had the highest mean copy number and were not significantly different from each other. *V. vinifera* and *V.* hybrid also had significantly different *Gret1* copy number compared to the other species in the analysis, except *V. aestivalis* and *V. riparia*, as well as *Ampelopsis brevipedunculata* and × *doaniana*, each being represented by single accessions. *V. piasezkii*, *V. coignetiae*, × *andersonii*, *V. amurensis*, and *V. monticola*, as a group contain significantly fewer copies than *V. vinifera*, *V.* hybrid, × *doaniana*, *V. palmata*, *V. aestivalis*, *V. vulpina*, *V. labrusca*, *V. rupestris*, *V. acerifolia*, *V. riparia*, *V. cinerea*, and *V. yenshanensis*. The grouping *Vitis* sp. (unknown) likely consists of a mix of species that are as yet, unclassified, and shows the greatest statistical similarity to moderately low copy number species × *andersonii*, *V. amurensis*, *V. monticola*, × *champinii*, × *novae-angliae*, *A. brevipedunculata*, and *V. thunbergii*. Recently, Pelsy (2007) compared many of these same species using both microsatellite and *Tvv1* untranslated leader region markers. Phylogenies generated in that study do not seem to correlate with the Ct_{norm} values reported here. That is, as genetic distance from *V. vinifera* increases, Ct_{norm} values do not decrease but rather appear randomly associated. Thus, decreasing Ct_{norm} values are likely not associated with sequence divergence at the primer or probe sites but rather with fewer *Gret1* copies.

White accessions have statistically significant higher *Gret1* copy number than colored accessions

Significant differences in *Gret1* copy number were observed between color classes within several species (Fig. 2). Mean copy number for white accession within a species is consistently higher than colored accessions for that same species. This difference is significant for *V. riparia* ($P = 0.01$, $n = 186$ colored, 1 white), *V. labrusca* ($P < 0.001$, $n = 38$, 4), and hybrid ($P = 0.03$, $n = 503$, 123). *V. aestivalis* ($P = 0.06$, $n = 10$, 1) is only significant at $\alpha = 0.1$ and *V. vinifera* ($P = 0.11$, $n = 15$, 5) is non-significant. All but one of the 123 *V.* hybrid white accessions tested are known to have at least one *V. vinifera* parent in their pedigrees or to have an unclear pedigree but displaying *V. vinifera* characteristics (e.g., perfect flowers). In addition to the differences between color within a species, Fig. 2 also highlights the significantly higher *Gret1* copy number in *V. vinifera* and *V.* hybrid colored accessions than colored accessions of *V. riparia*, *V. aestivalis*, and *V. labrusca* ($\alpha = 0.01$). In contrast, there are no statistical differences among white accessions regardless of species.

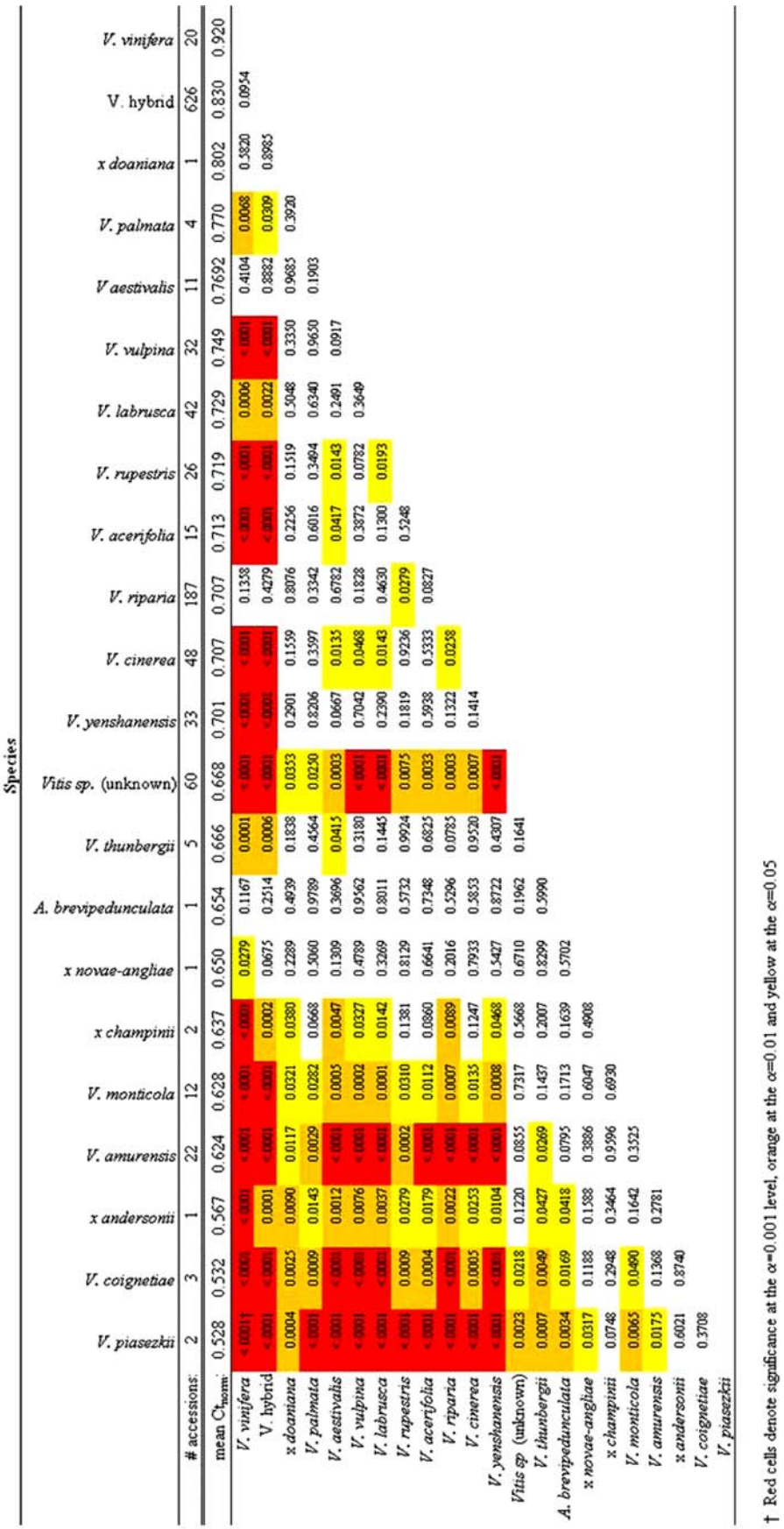

Fig. 1 Statistical output from proc mixed analysis of qPCR threshhold cycle for *Gret1* copy number amplification with respect to species. *Dark grey (Red) shading* indicates significance at the $\alpha = 0.001$ level, *medium grey (orange) shading* at the $\alpha = 0.01$ level, and *light grey (yellow) shading* at the $\alpha = 0.05$ level. $Ct_{norm} = Ct_{pinot}/Ct_{experimental}$

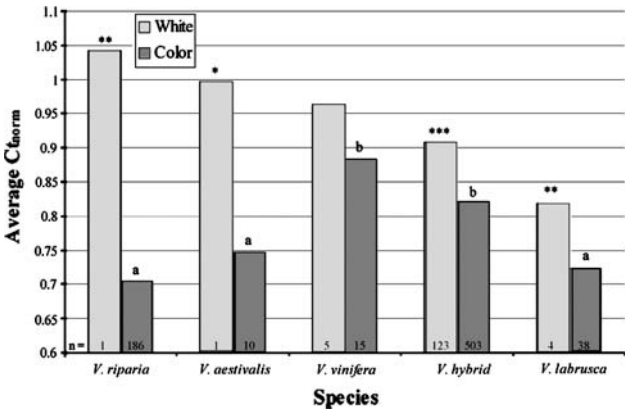

Fig. 2 Statistical output from proc mixed analysis of qPCR threshhold cycle for *Gret1* copy number amplification with respect to color within species. *Light bars* indicate white accessions; *dark bars* indicate colored accessions. *Asterisks* denote statistically significant differences between white and colored accessions for that species (* $\alpha = 0.1$, ** $\alpha = 0.05$, *** $\alpha = 0.01$). Letters (*a* and *b*) *above dark bars* denote statistically significant differences when comparing colored accessions between species ($\alpha = 0.01$). There are no significant differences between white accessions between species. $Ct_{norm} = Ct_{pinot}/Ct_{experimental}$

Greater variation in *VvMybA1* allelic diversity in white-fruited North American *Vitis* germplasm compared to *V. vinifera*

All white accessions ($n = 134$) were screened using AC and BC primer combinations designed by Kobayashi et al. (2004) for amplification of *VvMybA1*. The AC primer set is designed to amplify the *Gret1*-containing *MybA1* allele whereas BC amplifies the wildtype allele. Each of these primer sets amplified across the five species tested; however, AC was not present in 33 of 145 (22.8%) white accessions and BC was present in 98 of 145 (67.6%) white accessions (representative genotypes shown in Fig. 3). This is in contrast to white-fruited *V. vinifera* in which AC is present in 98% and BC is present in less than 2% of the white-fruited samples (Lijavetzky et al. 2006; This et al. 2007).

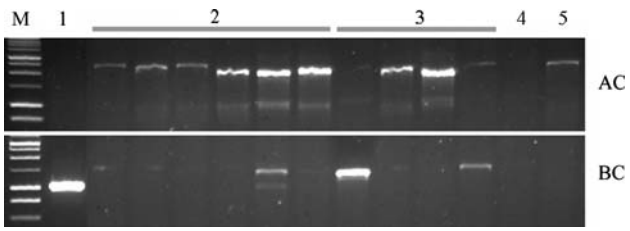

Fig. 3 PCR amplification of *VvMybA1* wildtype and *Gret1* alleles in representative white-fruited species. *Lane 1 V. aestivalis*, *2* six different *Vitis* hybrid accessions, *3* four different *V. labrusca* accessions, *4 V. riparia*, *5 V. vinifera*. *AC* denotes amplification from the 3′ end of *Gret1* to the 3′ end of *VvMybA1*. *BC* denotes amplification from the *VvMybA1* promoter, upstream of the *Gret1* insertion, to the 3′ end of *VvMybA1*. Thus, a band is expected in AC if *Gret1* is present and a band in BC if no *Gret1* is present. Both bands indicate a heterozygote. *M* 1 kb molecular weight ladder (Promega, Madison, WI)

Amplification of candidate genes reveals fragmented insertion of *Gret1* upstream of fragmented UFGT

Primers designed to amplify between the 3′ end of *Gret1* and the coding sequence of anthocyanin biosynthetic candidate genes, chalcone isomerase (CHI), chalcone synthase (CHS), dihydroflavonone reductase (DFR), flavonone 3-hydroxylase (F3H), glutathione *S*-transferase (GST), multidrug resistance protein (MRP), and UDP glucose–flavonoid glucosyl transferase (UFGT) were designed and tested on white and colored accessions from the grape collection and white and colored *V. vinifera* (Table 3). CHI, DFR, F3H, GST, and MRP did not amplify in any genotype in either *V. vinifera* or any of the species represented in the grape collection. Faint bands were visualized in CHS in *V. vinifera* (data not shown), which were cloned and sequenced. Strong bands were amplified using UFGT primers in both *V. vinifera* and the North American hybrid species (data not shown) and these were also cloned and sequenced. Analysis of the resulting sequence revealed that while both UFGT and *Gret1* sequence were present, both were fragmented, and it is likely that this UFGT locus is not functional. Other clones contained short fragments of UFGT and putative retroelement sequences. In no case were either UFGT or *Gret1* intact. CHS sequence analysis revealed clones of entirely CHS sequence or putative retroelements, but not *Gret1*.

Sequences flanking *Gret1* insertion sites

To better understand the genomic sites of insertion of *Gret1*, IPCR was conducted to identify genomic sequence flanking *Gret1*. More than 200 sequences were generated from IPCR representing 26 unique sequences and deposited in Genbank (accession nos. EF119791–EF119830). These are listed, along with their Genoscope assembled contig hits, Genbank protein hits, and genotypes from which they were amplified, in Table 4. *Gret1* sequence is present in 19.2% of contigs, and retroelement-related sequences are present in an additional 27%. It is reasonable that *Gret1* does not appear in all cases because we have compared North American species sequence to that of *V. vinifera*. Interestingly, both Pixiola (*V. aestivalis*) and Bougher (*V. riparia*) resulted in a large proportion of sequences not identified in the other species tested. Of the sequences identified through this assay, about half are clearly related to retroelements and about 8% are putatively housekeeping. A few sequences, however, stand out as having potential roles in secondary metabolism and anthocyanin development: PhytochromeA (PHYA; ABA46868), found in Alba (*V. labrusca*) and Pinot Blanc;

Table 4 Genomic sequence surrounding Gret1 in representative white-fruited genotypes and Pinot Noir

Genbank accession	Genoscope contig			Genotypes (# clones sequenced)					
	Designation	# Bases	ORFs	Alba (24)	Bougher (34)	Pinot blanc (48)	Pinot noir (51)	Pixiola (40)	Siebel (28)
EF119811, EF119801, EF119814	232698	6164	AB111101 *Vitis vinifera* gypsy-type retrotransposon Gret1	×		×	×		×
			ABE86426 (*Medicago truncatula*) IMP dehydrogenase/GMP reductase						
			NP_683491 (*Arabidopsis thaliana*) unknown protein; mRNA deadenylase, exonuclease subunit and related nucleases						
			Q7M443 (*Tulipa bakeri*) Chitinase 2 (Tulip bulb chitinase-2) (TBC-2)						
EF119830, EF119802, EF119803, EF119810	287992/290539	21085/2685	AB111101 *Vitis vinifera* gypsy-type retrotransposon Gret1		×	×	×		×
			AAQ98059 (*Vitis cinerea* × *Vitis rupestris*) unknown protein						
			AAO72990 (*Populus alba*) cyclin D						
EF119804, EF119805, EF119812	308051/249661	3753/1106	AB111101 *Vitis vinifera* gypsy-type retrotransposon Gret1			×	×		×
			NP_181056 (*Arabidopsis thaliana*) GRL/NAP1/NAPP; transcriptional activator						
			NP_192202 (*Arabidopsis thaliana*) GTP binding/translation elongation factor						
EF119822, EF119809	321511/322228	5463/1545	ABA99600 [*Oryza sativa* (*japonica* cultivar-group)] retrotransposon protein, putative, unclassified			×	×		
EF119818, EF119808	210041	16583	BAA33460 (*Panax ginseng*) cycloartenol synthase		×		×		
			BAB01046 (*Arabidopsis thaliana*) phosphate/phosphoenolpyruvate translocator protein-like						
			ABE81450 (*Medicago truncatula*) integrase, catalytic region						
			ABE90049 (*Medicago truncatula*) hypothetical protein MtrDRAFT_AC147501g18v1						
EF119829	264486	2484	YP_398414 (*Triticum aestivum*) respiratory-chain NADH dehydrogenase				×		
EF119792	261316	2252	DQ424856 (chloroplast *Vitis vinifera*) photosystem II protein D1	×					
			YP_398414 (*Triticum aestivum*) respiratory-chain NADH dehydrogenase						

Table 4 continued

Genbank accession	Genoscope contig			Genotypes (# clones sequenced)					
	Designation	# Bases	ORFs	Alba (24)	Bougher (34)	Pinot blanc (48)	Pinot noir (51)	Pixiola (40)	Siebel (28)
EF119795, EF119823	224615/205636	8618/9661	BAD93174 (*Ginkgo biloba*) MADS-box transcription factor GbMADS10		×	×			
			ABE81450 (*Medicago truncatula*) integrase, catalytic region						
EF119798, EF119821	213431	8589	ABA46868 (*Solanum tuberosum*) phytochrome A	×		×			
	201607/289983	5214/5709	CAB78602 (*Arabidopsis thaliana*) hypothetical protein, similar to retrotransposon Ta11-1	×					
			ABE93177 (*Medicago truncatula*) RNA-directed DNA polymerase (reverse transcriptase)						
EF119824	326330	44404	ABI34371 (*Solanum demissum*) Gag/pol polyprotein, 3′-partial, putative			×			
			NP_085590 (*Arabidopsis thaliana*) hypothetical protein ArthMp112; Mitovirus RNA-dependent RNA polymerase						
EF119813,	200492	12909	AB111101 *Vitis vinifera* gypsy-type retrotransposon Gret1	×					×
EF119817			AAB67741 (*Arabidopsis thaliana*) isopentenyl pyrophosphate isomerase						
EF119791	304456	6678	NP_177535 (*Arabidopsis thaliana*) protein binding/ ubiquitin-protein ligase/zinc ion binding	×					
EF119799	202286	13172	ABE78617 (*Medicago truncatula*) reverse transcriptase (RNA-dependent DNA polymerase), putative	×					
			AAM14182 (*Arabidopsis thaliana*) unknown protein						
EF119820	216998	22977	ABD33028 (*Medicago truncatula*) UBA/TS-N domain, putative		×				
			ABD33027 (*Medicago truncatula*) dual specificity protein phosphatase						
			NP_191234 (*Arabidopsis thaliana*) unknown protein						
EF119797	215602	1576	AAX95662 [*Oryza sativa* (*japonica* cultivar-group)] protein kinase domain, putative		×				
EF119819	204249/233023	24503/1693	ABA93715 [*Oryza sativa* (japonica cultivar-group)] plant protein family protein		×				
			AY327514 (*Vitis vinifera*) SCAR marker linked to grape seedlessness gene						

Table 4 continued

Genbank accession	Genoscope contig			Genotypes (# clones sequenced)					
	Designation	# Bases	ORFs	Alba (24)	Bougher (34)	Pinot blanc (48)	Pinot noir (51)	Pixiola (40)	Siebel (28)
EF119800	222580	1976	NP_001056985 [*Oryza sativa* (*japonica* cultivar-group)] ubiquitin interacting motif domain containing protein		×				
			NP_180916 (*Arabidopsis thaliana*) serine/threonine protein kinase						
EF119796	223088	2251	ABE66133 (*Arabidopsis thaliana*) pentatricopeptide repeat-containing protein		×				
			NP_173314 (*Arabidopsis thaliana*) RWP-RK domain transcription factor						
EF119826	255928/319469	1000/12277	ABC86840 (*Vitis vinifera*) flavonoid 3′,5′-hydroxylase	×				×	
EF119825	227722	28671	NP_182034 (*Arabidopsis thaliana*) cyclin-dependent protein kinase					×	
			AAM10933 (*Arabidopsis thaliana*) putative bHLH transcription factor						
EF119794, EF119806	231470	5288	AB111101 *Vitis vinifera* gypsy-type retrotransposon Gret1	×	×			×	
EF119827	289982	10835	ABE81882 (*Medicago truncatula*) RNA-directed DNA polymerase (reverse transcriptase)					×	
EF119828	290606/289827/300401	38631/2629/1726	BAD25386 [*Oryza sativa* (*japonica* cultivar-group)] *S*-ribonuclease binding protein-like					×	
			AAT38786 (*Solanum demissum*) Gag-pol polyprotein, putative						
			AAC09388 (*Actinidia deliciosa*) xyloglucan endotransglycosylase precursor						
EF119816	285375/287167/320425	10534/4413/20212	BAD89562 NTMTP1A (*Nicotiana tabacum*), Co/Zn/Cd efflux system component					×	
			Q43047 (*Populus kitakamiensis*) caffeic acid 3-*O*-methyltransferase						
			ABA95820 (*Oryza sativa*) retrotransposon protein, putative, unclassified						
			NP_195338 (*Arabidopsis thaliana*) ATP binding/transmembrane receptor						
EF119815	297919	17582	AB111101 *Vitis vinifera* gypsy-type retrotransposon Gret1					×	
EF119807	300401/290829	1715/2431	AAU90288 (*Solanum demissum*) Polyprotein, putative					×	

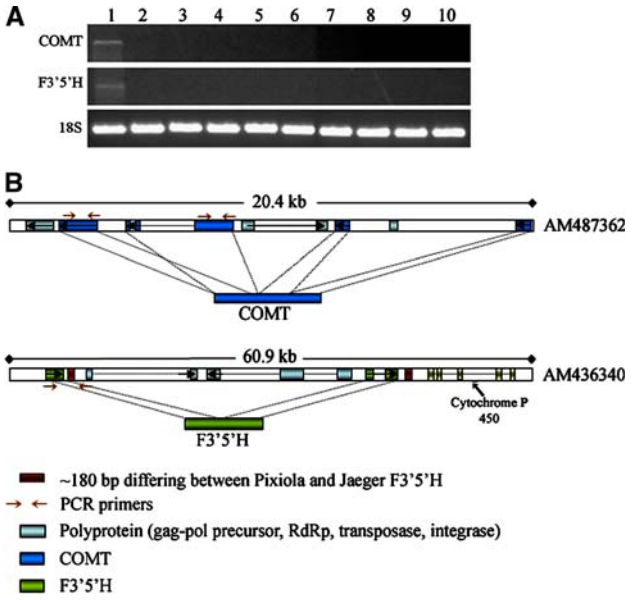

Fig. 4 Analysis of candidate genes in *V. aestivalis* accessions. **a** Caffeic acid 3-*O*-methyltransferase (COMT) and flavonoid 3′,5′-hydroxylase (F3′5′H) were identified as being in the genomic region near a *Gret1* insertion by IPCR. *Gret1*-containing alleles for these candidates were amplified using one *Gret1*-specific primer and one gene-specific primer. *Lane 1* is the white-fruited *V. aestivalis*, cv. 'Pixiola'. *Lanes 2–10* are nonwhite *V. aestivalis*. 18s rDNA was amplified in each accession to verify amplification competence. **b** Structures of genomic regions surrounding *Gret1* insertion into F3′5′H and COMT in *V. vinifera*. No genomic sequence is available for *V. aestivalis*, so comparisons were made in *V. vinifera* to detect potential locus structures. *Jaeger* is a black-fruited *V. aestivalis* accession

flavonoid 3′5′-hydroxylase (F3′5′H; ABC86840), found in Pixiola and Bougher; and caffeic acid 3-*O*-methyltransferase (COMT; Q43047), identified only in Pixiola. *Gret1* insertion in or near these genes was confirmed via PCR amplification from *Gret1* to the candidate gene and subsequent sequence analysis suggests the presence of retroelement insertions at these loci even in *V. vinifera* (Table 3, Fig. 4). In the case of *V. aestivalis*, *Gret1* insertions into F3′5′H and COMT were only present in the white-fruited accession (Fig. 4a). Insertion of *Gret1* in PHYA was not limited to white accessions in either *V. labrusca* or *V. vinifera* (data not shown).

Discussion

V. vinifera is the main cultivated species of grape in the world. The retroelement *Gret1* was found to be significantly responsible for the creation of white-fruited accessions of *V. vinifera* and was likely an important source of genetic variation during the early selection and domestication of grape. Our analysis has revealed that *Gret1* is also present in additional *Vitis* species and that significant differences in *Gret1* copy number exist between species. While it is to be expected that different species might have differing predispositions for transposition, it is unexpected that amplification of a single element is associated with a particular trait. The work described herein demonstrates this phenomenon in grapes, their wild North American relatives, and a few Asian species, and explores the nature of the insertion sites in some of the more interesting genotypes.

Gret1 is widespread within *Vitis* species as well as within the *V. vinifera* genome (Lijavetzky et al. 2006; Pereira et al. 2005; This et al. 2007). While the taxonomic relationships between the species within *Vitis* are still being debated and incomplete (Di Gaspero et al. 2000; Soejima and Wen 2006), it is known that *V. vinifera* originated at a location geographically isolated from the North American species (Galet 1988). This being the case, there was some basis for the hypothesis that *Gret1* is a retroelement limited to or primarily active in *V. vinifera* and *V.* hybrid, owing to the vast majority of this synthetic species' *V. vinifera* parentage. We have found that *Gret1* is present in all species assayed, although there are significantly more copies in *V. vinifera*, *V.* hybrid, and × *doaniana* than in the other species tested (Fig. 1). Interestingly, when species are separated into white and non-white groups, there are differences in *Gret1* copy number within species (Fig. 2). This is true for *V. riparia*, *V. labrusca*, *V. aestivalis*, and *V.* hybrid, but not for *V. vinifera*. Further, there is no statistical difference among white-fruited genotypes between species while colored *V. vinifera* and *V.* hybrid contain significantly higher *Gret1* copy numbers than *V. riparia*, *V. aestivalis*, and *V. labrusca*. One possible explanation for these results is that a flurry of *Gret1* transpositional activity occurred coincidentally with or preceded the appearance of white-fruited individuals.

Previous work on anthocyanin regulation in grapes has shown that the *Gret1* insertion upstream of *VvMybA1* is responsible for white berry color in nearly all *V. vinifera* varieties (Kobayashi et al. 2004; Lijavetzky et al. 2006; This et al. 2007). Attempts to detect the *Gret1–VvMybA1* allele in the species represented by the PGRU *Vitis* collection provided anomalous results with white genotypes often containing the wildtype or novel mutant *VvMybA1* alleles (Fig. 3), suggesting that additional mutant alleles have led to the white-fruited phenotype in these accessions.

It is well documented that transposons, retroelements, and retroviruses insert into animal genomes in a non-random pattern, with 5′ ends or transcriptional starts of expressed genes being preferred (Kidwell and Lisch 2001; Liao et al. 2000; Wu et al. 2003; Yant et al. 2005). In plants, there is often an insertion site bias; however, this may be for either heterochromatic or euchromatic regions (Lonnig and Saedler 2002). Shankar et al. (2001)

demonstrated the random distribution of the non-autonomous transposon *sTag1* in *Arabidopsis*. In contrast, the *Ac* transposon in maize preferentially inserts in genomic hypomethylated, albeit repetitive, regions of the genome (Kolkman et al. 2005). Using FISH, the insertion of *Gret1* in *V. vinifera* has been shown to insert primarily in euchromatic regions, although single insertion events are difficult to detect using this method and may not have been accounted for (Pereira et al. 2005). Differences in insertion site preference have been explained as "selfish," as in the case of heterochromatic insertion where nontranscribed and inactive regions of the genome are available for the uninhibited amplification of a transposable element (Bennetzen 2000). In the case of transposition into actively transcribed regions, it is thought that the genomic DNA is less tightly associated with the histones and is, therefore, more accessible to integration (Becker et al. 2002; Lonnig and Saedler 2002). Both by candidate gene analysis and by sequence analysis of random flanking sequences, we have demonstrated that *Gret1* is inserted both in transposon- and gene-rich areas of the genome (Table 4). Further, we have identified putative alleles of functional importance within *Vitis* by virtue of a *Gret1* insertion site proximal to the following candidate genes: phytochrome A, flavonoid 3′,5′-hydroxylase and caffeic acid 3-*O*-methyltransferase (Ageorges et al. 2006; Bogs et al. 2006; Castellarin et al. 2006; Duek and Fankhauser 2003).

The most striking feature of the present research is the finding that *Gret1* copy number is associated with color which raises interesting questions concerning the role of transposons in providing the genetic and phenotypic diversity required for crop domestication and the diversifying selection observed in *V. vinifera*. Although rare in nature, our analysis shows nonrandom association of *Gret1* specifically with white berry color of all known examples of white-fruited wild species of the 38-chromosome *Vitis* species (section *Euvitis*) (Fig. 2). In the case presented here, the colored, wild accessions show a low *Gret1* copy number in comparison with colored, domesticated varieties, while the same high *Gret1* copy number is present in white accessions of all species, regardless of domestication level. A model that may explain these data could be that *Gret1* amplification in *Vitis* genomes greatly increases the probability of the emergence of novel phenotypes that provided important variation for selection by humans during the domestication of *V. vinifera*. This model may also explain why there was no statistical difference between white and colored fruit within *V. vinifera*. If transpositional activity played a role in the domestication of this species, it can be expected that high *Gret1* copy number be uniformly high and not associated with specific traits.

Alternatively, the amplification of *Gret1* in the grape genome may be due to genomic instability following interspecific hybridization, particularly in the case of *V.* hybrid. This group of accessions represents the largest group in the present analysis with 123 white and 503 colored genotypes. The pedigrees of most of these accessions contain multiple *Vitis* species including, but not limited to, all of the species in the present study. Since white-fruited wild grapes are rare in nature, it is probable that many white-fruited, interspecific hybrids derive white alleles from *V. vinifera*. Consequently, the high *Gret1* copy number observed in white-fruited hybrids may be a result of hybridization with *V. vinifera*, which has the highest *Gret1* copy number observed, or possibly as a result of the genomic stress applied by the wide cross (Fontdevila 2005; McClintock 1984). Of the 123 white *V.* hybrid accessions, only one does not apparently contain *V. vinifera* in its pedigree.

Why have more color loci not been identified in *V. vinifera*? Recent studies have shown that in up to 95% of vinifera genotypes assayed, white genotypes are homozygous for the *Gret1–VvMybA1* allele (Lijavetzky et al. 2006; This et al. 2007). One reason for this may be the highly heterozygous and clonal nature of *V. vinifera* as a crop and that many grape cultivars have been clonally propagated for hundreds of years. Breeding programs exist, as with other crops, in which cross hybridization between different genotypes is utilized; however, during the course of breeding a new line, far fewer meioses occur in grape than in breeding programs of annual species. Instead, a small number of generations are generally employed with selection and clonal propagation of desirable individuals. Random mutation and transposition events (whether random or nonrandom) generally result in hemizygous and recessive genome alterations and will only become phenotypically apparent through segregation. Furthermore, as in many domesticated species, a genetic bottleneck exists in the grape gene pool as a result of a small number of genotypes giving rise to the majority of the existing genotypes (Arroyo-Garcia et al. 2006; Bowers et al. 1999). Thus, whether through spontaneous mutation or by descent, mutations of phenotypic consequence are rare.

This is not to say that visible spontaneous mutations never arise in grape. Rather, this crop is notable for the number of bud sports that have been propagated by breeders and viticulturalists. A classic example of this is the ancient variety 'Pinot Noir' which may be considered the wildtype version of color variants 'Pinot Blanc', 'Pinot Meunier', and 'Pinot Gris' (Regner et al. 2000). Each of these genotypes has been shown to be derived from Pinot Noir in the absence of meiotic recombination. What this mutability translates to in wild populations is spontaneous appearance of domestication-related traits that are, without selection, normally expunged from the population due to lack of selection by humans or other seed dispersal agents

or lack of transmission to progeny owing to inbreeding depression and dioecy. Grape is a highly heterozygous crop (Arroyo-Garcia et al. 2006), and as a result, there are likely loci in the genome for which a single mutation will reveal a phenotypic change. The fact that so many bud sports occur in grape in the absence of recombination is indicative of a mutagenic force at work, perhaps in the form of active transposons. While the fact that so many color mutations (e.g., Blanc, Gris from Noir) being propagated could be due to their high visibility, their frequency along with the data presented here suggests a mutational bias.

The discovery of *Gret1* in all species of *Vitis* so far tested has a great potential benefit towards the development of both a transposon-based marker system as well as mutagenesis system. Marker systems using retroelements or other transposons have been shown to be useful in assessing diversity or gene identification in a number of species including maize, petunia, barley, and pea (De Keukeleire et al. 2001; Ellis et al. 1998; Lee et al. 2005; Lin et al. 2006). The potential utility of *Gret1* for retroelement microsatellite amplified polymorphism (REMAP) and inter-retroelement amplified polymorphism (IRAP) markers, has been demonstrated previously (Pereira et al. 2005). To date, little attention has been paid to grape as a genetic model, despite it being a perennial, fruited, non-climacteric species with a small genome, hundreds of years of pedigree records, and a history of viable bud sports. Adding efficient markers that can function in all *Vitis* species as well as a transposon mutagenesis system will help to highlight this genus as being genetically tractable.

The work presented here suggests many more avenues for future basic and applied research. As has been suggested previously, the *Gret1* element may be highly useful in IRAP or REMAP marker systems (Pereira et al. 2005), not only in *V. vinifera*, but also in its wild relatives. Further, we have identified several genes into which *Gret1* has transposed in domesticated and wild grape accessions, both with and without an association with white berry color. The identification of insertions associated with berry color leads us to the hypothesis that *Gret1* may have played a critical role as a mutagenic force during crop evolution and domestication. The resolution of the issues raised here may lead us not only to the elucidation of berry color genetics in this non-model, high value species but also to a clearer picture of the role of mobile element transposition in all plants.

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