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Grape Breeders Search for Reliable DNA Markers

Why the Pinot Noir PN40024 reference genome is not enough

Tim Martinson, Qi Sun, Cheng Zou and Lance Cadle-Davidson

DNA MARKERS ARE REVOLUTIONIZING grape breeding. Inexpensive DNA sequencing has allowed breeders to map the genome and associate observed traits (phenotypes) with DNA markers. Marker-assisted selection (see “Grape breeders no longer flying blind,” Wines & Vines, March 2018) has made it possible to identify which seedlings from conventional crosses carry traits of interest and then discard those that don’t develop early in the process.

The search for markers got a big boost in 2007. Shortly before Christmas, research groups from France and Italy announced they had collaboratively sequenced the grapevine genome—its complete DNA sequence—controlling everything that makes a grapevine a grapevine. A “Science Daily” announcement on December 25 hailed the draft reference genome as “an invaluable tool for creating grape varieties resistant to diseases without altering the quality of the resulting wine.”

This was an enormous effort, involving inbreeding (self-hybridizing) Pinot Noir for five generations to develop a line called “PN40024” (PN40024 is not a commercial Pinot Noir clone and has no commercial value) and “Sanger” shotgun DNA sequencing. Sanger sequencing was state-of-the-art back in 2007, and they needed an inbred line, such as PN40024, to assemble the draft genome with this method. It took several years and cost millions of Euros to put together the PN40024 reference genome and was, at least, a hundred-fold more expensive than current sequencing technology.

In spite of the time and the cost, the PN40024 reference genome turned out to be not good enough.

Breeders link traits to DNA markers by testing mapping populations, which are the “F1” progeny of test crosses. These populations of 100 to 500 progeny produce different combinations of the parental traits, which can then be tested and linked to specific DNA markers. (See “The Phenotyping
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Bottleneck: How grape breeders link desired traits to DNA markers,” Wines & Vines, January 2019)

The problem was that the markers identified using PN40024 genome as the reference didn’t work across the Vitis genus. When applied to North American and Asian Vitis species and hybrids, the markers didn’t transfer. This was a significant problem for breeders, because the disease-resistance traits breeders seek to incorporate into new varieties originate in a broad range of North American and Asian Vitis species.

When VitisGen researchers used a technique called Genotyping by Sequencing to identify 4,000 SNP markers positioned to the PN40024 genome during the first VitisGen project, they found that only 1 to 2 percent of them worked across 17 genetically diverse mapping populations. That’s not good enough for grape breeders.

There are several reasons why the PN40024 genome wasn’t useful to grape breeders.

Twenty million years of evolution separate the sole European species Vitis vinifera from the dozens of North American and Asian wild grape species. While all Vitis species (except muscadine grapes) have 19 chromosomes and are capable of interbreeding, much of the genome has rearranged itself since, through many generations of evolutionary selection and adaptation. The modern human genome (Homo sapiens) has been around for only 200,000 years while the split of North American and European Vitis is 100 times older. A lot can happen in 20 million years.

Markers are developed by comparing DNA sequences from different individuals to the reference genome. This comparative analysis works only if the individuals are closely related to the reference with minimal chromosomal re-arrangement. Otherwise, the markers are not reliable and are error-prone. If you are a breeder seeking to incorporate traits from North American or Asian Vitis species, markers based on the Vitis vinifera reference genome lack those traits would not tell you much about the resistance genes you want.

Another reason is that grapes are highly heterozygous. They inherit two sets of genes, one from each parent, and often have two or more different forms (alleles) of many genes. Unlike many plants (including cultivated grapes), wild grapevines must outcross. Wild grapes are either male or female, with each vine having either stamens (male flowers that release pollen) or pistils (female flowers that produce fruit and seeds). When grapes were domesticated, humans selected vines that were hermaphroditic (both male and female parts on every flower) for obvious reasons. Cultivated grapes can self-polliuate so they don’t need adjacent grapes with male flowers to pollinate the female vines and set fruit. Hermaphroditic flowers freed producers from having to plant and cultivate male vines that produce no fruit and resulted in more predictable and even fruit set.

But heterozygosity is a challenge for DNA sequencing because many genetic loci have two or more different forms (alleles) of each gene. When doing marker-assisted breeding in outcrossing crops, such as grapevines, breeders need to be able to track the two set of genes in each of the two parents separately. In practice, that requires markers to be more accurate and less tolerant of missing data.

Finally, DNA markers are signposts that point to genes but are not the gene itself. Markers in previous genetics platforms were based on one DNA base pair substitution and were referred to by geneticists as Single Nucleotide Polymorphisms (SNPs). Genes from different parents can have a handful of different SNPs and, therefore, more than two alternate forms (alleles) of each gene. Single SNP markers could only distinguish two alternate alleles. The Genotyping by Sequencing markers used in the first VitisGen project were based on one SNP and could only distinguish two alleles (present/absent). They failed to detect a broader range of variable alleles when they were applied to the wider Vitis genus.
Researchers needed a way to identify markers that would work across the entire *Vitis* genus so that they could be confident that markers from wild American and Asian *Vitis*, European *Vitis vinifera* and hybrids would be transferable among species.

**The Core Genome**

The *VitisGen2* genetics team addressed this by targeting the regions of DNA that are evolutionarily conserved (the core genome) across the genus.

To do so, they compared 10 genomes from a broad range of *Vitis* cultivars and species, including wild vines (see **Table 1**). It is notable that seven of these reference genomes were compiled by the *Vitis Gen2* project, a feat that would have been prohibitively expensive just 10 years ago.

By comparing the PN40024 reference genome to the other nine genomes, they found that about 10 percent of the genome was shared by all of the tested accessions and about 70 percent of these are regions that actually code for proteins that make a grapevine a grapevine. Much of the other 90 percent includes what is known as the dispensable genome: “non-coding” DNA that accumulates over evolutionary time, or genes whose presence might be beneficial in some environments but not others.

The Core Genome sequences stayed consistent over 20 million years of evolution; and as a consequence, researchers were able to find and track them. Much like matching the edges of patterned fabric, they were able to align these common gene sequences (the core genome) to each other (see **Figure 1**) and find out what these diverse genomes shared in common. The result is a very detailed map, with a dense network of “mileposts” that provides a solid foundation for identifying common and robust markers.

<table>
<thead>
<tr>
<th>Type</th>
<th>Name of accession</th>
<th>Origin</th>
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<tr>
<td>Wild <em>Vitis</em> species</td>
<td>B9</td>
<td><em>Vitis cinerea</em></td>
</tr>
<tr>
<td></td>
<td>B38</td>
<td><em>Vitis rupestris</em></td>
</tr>
<tr>
<td>Hybrids of wild <em>Vitis</em></td>
<td>Vitis x doaniana</td>
<td><em>V. acerifolia</em> x <em>V. mustangensis</em></td>
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<tr>
<td></td>
<td>Jaeger 70</td>
<td><em>V. aestivalis lincecumii</em> x <em>V. rupestris</em></td>
</tr>
<tr>
<td>Hybrid cultivars</td>
<td>Chambourcin</td>
<td>*V. rupestris, V. riparia, V. cineria, V. vinifera</td>
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<td></td>
<td>Concord</td>
<td><em>V. Labrusca, V. vinifera</em></td>
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<td>Cabernet Sauvignon</td>
<td><em>V. vinifera</em></td>
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<td></td>
<td>Flame Seedless</td>
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<td></td>
<td>Sultanina / Thompson Seedless</td>
<td><em>V. vinifera</em></td>
</tr>
<tr>
<td>Reference genome</td>
<td>Pinot Noir PN40024</td>
<td><em>V. vinifera</em></td>
</tr>
</tbody>
</table>

1 Genome sequence generated by VitisGen project
2 Genome sequence from online database
3 Genome sequence generated by VitisGen project with support from California Table Grape Commission
4 Original reference genome compiled by Jaellon et al (2007)
New, automated sequencing technologies have brought the cost of whole-genome sequencing down from millions of dollars in 2007 to an estimated $3,000 per genotype in the seven grapevines sequenced by the project.

The Resulting Markers
Researchers used the core genome data to develop 2,000 markers, which are physically distributed across the 19 chromosomes and span 99 percent of the grape genome (FIGURE 2). When they tested them against four mapping populations, totaling 1,928 unique vines, they found that 92 percent of the markers worked across the four families, representing a broad range of Vitis breeding parents.

This was a major improvement. Marker transferability went from 1 to 2 percent, using the old Genotyping by Sequencing, to 92 percent with the current methods. Now breeders can be confident that their markers will be transferable to a broad range of Vitis germplasm used in their programs.
The Cost of Analysis

A new technique called rhAmpSeq, which precisely controls how and when the cut-up DNA snippets from samples are amplified during the Polymerase Chain Reaction (PCR), is what makes this rich source of information possible and affordable. This technique allows researchers to use a single reaction to amplify and sequence 2,000 rhAmpSeq markers. In each sequencing experiment, grape breeders are using rhAmpSeq to obtain these markers for 4,608 vines. For a large project like VitisGen2, the cost of getting information on 2,000 markers could now be less than $10 per sample.

New, automated sequencing technologies have brought the cost of whole-genome sequencing down from millions of dollars in 2007 to an estimated $3,000 per genotype in the seven grapevines sequenced by the project. This made it possible to compare the Core Genome across the diverse species in the genus Vitis.

Cornell University grape breeder Bruce Reisch said that at the start of his career, in the early 1980s, making crosses for the grape breeding program was largely a trial and error process, with little genetic information available to guide the effort. There were no mapping populations because extracting genetic information from field populations was a tedious and costly process. Now grape breeders have access to the Core Genome and 2,000 markers to inform their grape-breeding choices. They can use this information to identify new marker-trait associations and use it to screen seedlings produced from their programs, regardless of the parentage used in their crosses. This is a remarkable achievement that is already proving a solid foundation for accelerating progress for grape breeders worldwide.

Marker-assisted selection has brought us Pierce’s Disease-resistant grapes that are about to be released from Andy Walker’s program at UC Davis. French National Institute for Agricultural Research (INRA) researchers have used marker-assisted selection to develop and release four varieties with two powdery mildew and two downy mildew resistance genes—vines that thrive on two fungicide sprays per season. This is only the start.

As more informative markers are identified, the quality of new accessions tested against these markers will also increase and will offer the wine, table, raisin and juice grape industries solutions to thorny problems such as disease susceptibility and the challenge of climate change. WBM

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References:

