Applying genomics to grapevine clones

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Recent breakthroughs in grapevine clonal genetics at the AWRI have enabled the identity of grapevine clones and the relationships between them to be accurately determined. This work has expanded knowledge of Chardonnay and unravelled the heritage of the Gingin clone, whose origins have long been shrouded in mystery.

IN BRIEF

- For the first time, genomics has been applied to define the differences between clones of Chardonnay grapevines.
- Mapping the mutations found in different clones has allowed their propagation history to be understood.
- The origins of the Gingin Chardonnay clone have been determined, after many years of confusion.
- There are potential applications of this work in authenticating or certifying grapevine clonal material.

INTRODUCTION

Chardonnay is one of the most widely grown grapevine varieties worldwide and produces some of the world's best table and sparkling wines. Chardonnay is Australia's second-largest grapevine variety and the top white variety, accounting for approximately 20% of Australia's total winegrape crush in 2020 (Wine Australia 2020). Australian Chardonnay plantings increased from the late '70s and early '80s onwards, and this was accelerated in large part by the availability of new clonal selections from UC Davis and Foundation Plant Services (FPS) in the USA.

Early clonal selections aimed to provide grapevine material that was virus free and had maximum productivity - the characteristics desired by industry at the time. Today, however, there are many clones of Chardonnay available, offering a more diverse selection for sensory characteristics, performance and fruit quality, driven by winemakers who are seeking to achieve a specific Chardonnay style. Interestingly, one of Australia's more sought-after Chardonnay clones (Gingin) is also one of the oldest.

The Chardonnay clone Gingin was first imported into Western Australia in 1957 (Fennessy 2018). It was shared throughout Western Australia and has since become one of the state's most coveted clones. Records describing the source material for this importation no longer exist (or never existed at all), but it was suspected by interested viticulturists to be the same as the Old Foundation Chardonnay (hereafter OF Chard) clone (Robinson 2018). In addition to OF Chard, Gingin has also been considered to be synonymous with a third clone called Mendoza, a Chardonnay selection from Argentina which was imported into Australia in 1968 from FPS at the same time as OF Chard (Fennessy 2018, Sweet 2018). While there is no historical reason that these two clones should be related, the similar vine characteristics and importation time for OF Chard and Mendoza have been a source of confusion throughout the Australian wine industry (Witt 2012, Hayward 2017).

The AWRI Biosciences team applied genomics to better understand the genetic characteristics and heritage of Chardonnay and its clones. The world of genomics has exploded in the last five years following breakthroughs in sequencing technology, with innovations in a wide range of applications. In grapevine development, understanding the genomic changes associated with key differences in the vineyard will be crucial to future breeding and selection efforts. Where a genetic mutation is known to cause a desired characteristic, molecular methods could greatly accelerate the process of selecting for this characteristic in new cultivars or clones. Furthermore, identifying the genetic differences among clones is the first step towards being able to identify and certify clonal vine material.

Genomes are windows to the past and future

Genomics can help unravel the heritage of and relationships between grapevine cultivars. The completion of a Chardonnay genome assembly in 2018 (a digital representation of the genetic material that encodes the Chardonnay vine), combined with sequencing of DNA from Chardonnay's parents (Pinot Noir and Gouais Blanc), uncovered clear evidence that Gouais Blanc and Pinot Noir are also very closely related to each other, with Chardonnay representing the fortuitous result of inbreeding (Roach *et al.* 2018). In some parts of the Chardonnay genome, both chromosome copies matched both parents, which is only possible where a close relationship occurs between the parents (Figure 1, see page 40). The relationship between these two ancient winegrape cultivars was previously unknown but was easily identifiable using the new generation of genome sequencing technology.

High-quality digital representations of grapevine genomes, known as genome assemblies, will improve future grapevine selections by accelerating research aimed at understanding how genetic differences translate into changes in the vineyard. Since the Chardonnay genome assembly was published, this resource has been used by several other groups to study genes involved in grapevine domestication (Badouin *et al.* 2020), disease resistance (Andolfo *et al.* 2019) and specific sensory attributes (Smit *et al.* 2019, Yang *et al.* 2020). With climate change now affecting the global wine industry, there is a very real need to identify and select new varieties and clones that can better cope with issues such as severe weather events, increased disease pressure, compressed vintages and smoke taint.

Unravelling clonal propagation histories

New clones arise when a plant, or part

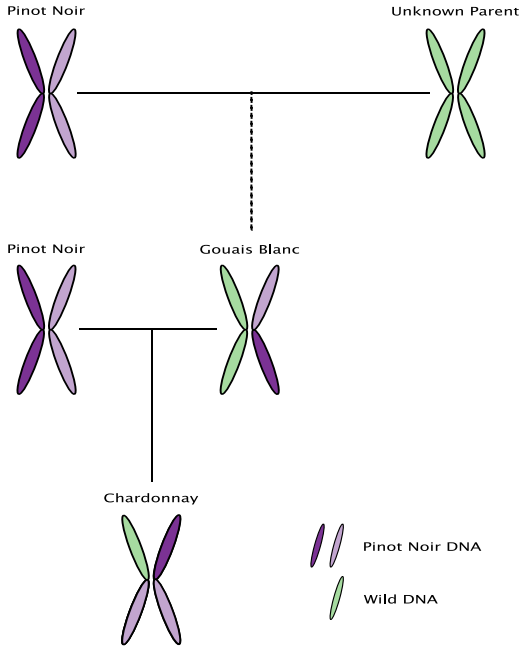

Figure 1. Evidence of inbreeding between Gouais Blanc and Pinot Noir in the Chardonnay genome. A cross somewhere in Gouais Blanc's history introduced Pinot Noir DNA into its genome. A second cross between Pinot Noir and Gouais Blanc created regions of double Pinot Noir DNA in the Chardonnay genome.

of a plant, showing a favourable trait (often referred to as a 'sport') is used as a mother vine for taking cuttings for new plantings, enabling that favourable trait to be fixed in the subsequent generations. Bud sports arise due to the fact that random genetic mutations can occur when cells divide during plant growth. When cuttings are taken from this mutated growth, these mutations are passed onto the new plants that are established from these cuttings. Over time, these new plants can accumulate further mutations. By using genomics to identify the shared and unique mutations present in each specific clone (or sub-clone), it is possible to unravel the unique propagation history of grapevine clonal material (Figure 2).

In 2018, 15 clones of Chardonnay were sequenced to identify clonal mutations present in some popular clones, and this was expanded to 26 samples for 18 clones in 2020 to include the historically significant Gingin and OF Chard clones (Roach *et al.* 2018, Roach *et al*. 2020). The unique pattern of mutations present in each sample was used to generate an evolutionary tree for Chardonnay, which then defined the relationships between each sample (Figure 3). A close genetic relationship was seen between I10V1 and its bud sports — CR Red and Waite Star. As both CR Red and Waite Star are derived from I10V1 it is expected that these plants contain all I10V1's mutations, as well as each containing some of their own mutations. Similarly, duplicate samples from different vineyards for clones 76, 95 and 96 were also closely related.

Samples of Mendoza, Gingin and OF Chard were included to determine if these clones were actually related, and to ascertain if any relationship(s) with other clones existed. As expected, both Gingin and OF Chard were very closely related. Unexpectedly, Mendoza also was found to have a shared heritage with these two clones, although with a higher level of genetic variation. While the origin of Gingin is therefore largely resolved, the real mystery now is how grapevines from Argentina (Mendoza) and the USA (Gingin and OF Chard) are related, becoming reunited at FPS before making their way to Australia.

Using clonal mutations in authentication?

With the cataloguing of clonal mutations comes the possibility of using these unique mutational signatures to authenticate or certify grapevine clonal material. Currently, the accurate identification and distribution of grapevine clones is entirely dependent on maintaining thorough records and using stringent viticultural practices to avoid mislabelling of material. Identifying an unknown clone via ampelography is rarely possible and mistakes in foundational nursery material have the potential to go unnoticed throughout the entire distribution chain. Establishing a new vineyard is a large investment and using the right clone (or wrong clone by mistake) can affect the vineyard's output and performance for years to come. For these reasons it is desirable to establish robust methods to identify and certify clonal material.

After identifying clonal mutations for the original panel of 15 Chardonnay clones, further work was conducted to validate their suitability for Chardonnay clonal identification. Independently sourced duplicate samples of clones 76, 95, and 96 were included in the data set and as expected, the duplicates shared most of their marker mutations (Roach *et al*. 2018). From this it was inferred that most mutations identified should be suitable for use in clonal identification. To push the boundaries of what was possible, sequencing data was obtained for eight independently sourced Chardonnay clones, sequenced at low coverage (Roach *et al*. 2018). Low coverage sequencing has the advantage of sequencing more samples at lower cost, with the trade-off being less data for each sample. These low coverage datasets were screened to look for previously identified clonal mutations. While most mutations were missed due to the limited data, it was still possible to identify a large enough proportion of the total mutations to unambiguously identify the clone for each

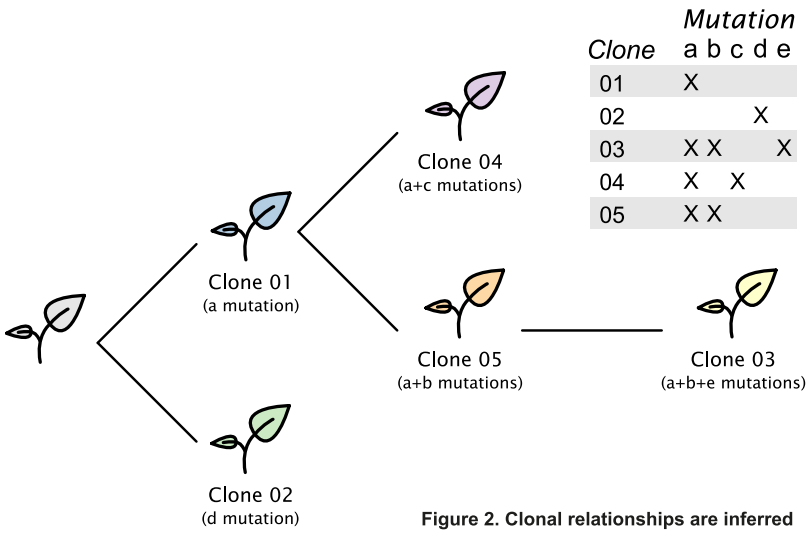

Figure 2. Clonal relationships are inferred from shared and unique mutations.

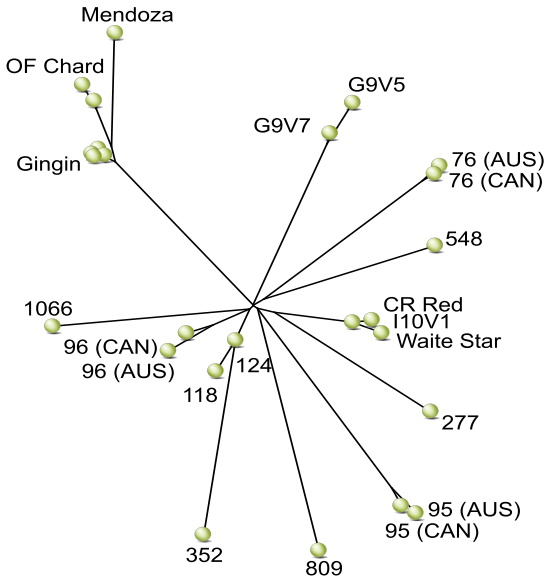

Figure 3. A phylogenetic tree of Chardonnay clones. Figure adapted from Roach et al. (2020) with permission from the original publisher.

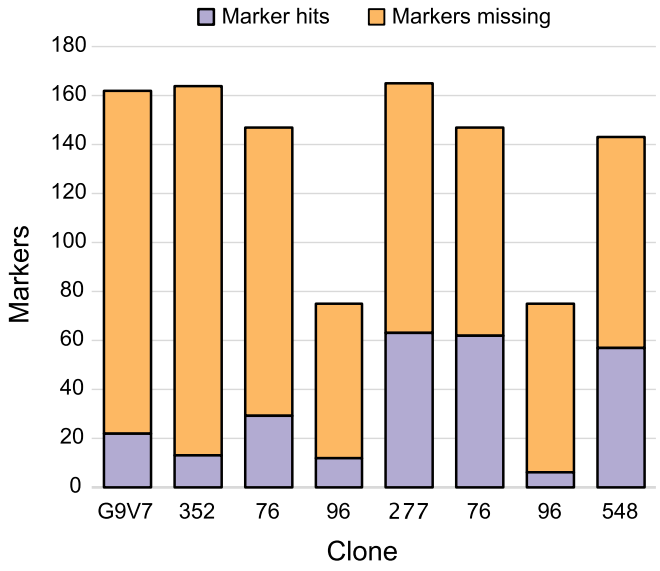

Figure 4. Low-coverage sequencing was found to recover sufficient mutations for positive identification of different Chardonnay clones.

sample (Figure 4). Additional research is now needed to reduce the cost of these tests (while maintaining robustness) to be able to transition towards widely applicable diagnostic testing.

WHAT'S NEXT?

This work in Chardonnay has demonstrated wide-ranging benefits and opportunities from applying genomics to grapevines. New understanding has been gained of the heritage of the Chardonnay variety, and a clonal mystery that has been occupying viticulturists' minds for decades has been solved. Most excitingly, there is potential for clonal mutations to be used as markers for definitive clonal identification, something that is not possible with non-genetic methods. The techniques used are of course also applicable to other grapevine varieties, with work on Pinot Noir clones recently commencing at the AWRI, in partnership with Adelaide Hills Vine Improvement Inc., the South Australian Government and Wine Australia.

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