The basis of variation in the size and composition of Shiraz berries

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Introduction
Variation is an inherent part of all biological systems and vineyards are no exception. Variation in the vineyard is multilevelled and it occurs between berries, bunches and vines. This discussion will focus explicitly on variation between berries which derives from asynchronous berry development within the bunch. In extreme cases, this is referred to as 'hen and chicken' or 'millerandage'. Two components of berry-to-berry variation are significant in grape and wine production: one pertains to berry size and the other to berry composition. Variation in berry size affects vineyard yield and wine quality. Variation in berry composition affects fruit flavour and wine quality. Comparative studies have tended to assess relative variation using the coefficient of variation, a unitless measure that expresses the sample variability relative to the sample mean. In this study, the coefficient of variation was used to identify fluctuations in the level of variation in berry size and berry composition throughout the post-flowering period of berry development. High levels of variation in the early post-flowering period suggest that variation originated prior to berry set, most likely as a result of asynchronous cell division in the floral primordium at budburst. Decreasing levels of variation indicate points of resynchronisation in the berry growth cycle: the more synchronised the event, the lower the variation. The lowest variation in berry size occurred at harvest, indicating one such point of resynchronisation in berry development. By contrast, berry flavonoid variation was lowest at softening, indicating a separate point of resynchronisation exists for flavonoid metabolism. The wine industry presumes that all variation has a negative impact on crop level, fruit composition and wine quality. Although this presumption may prove true, it has not yet been scientifically substantiated.

Defining variation
Variation can be defined as ‘an instance of varying or changing; an alteration or change in something, esp. with frequent or ready change or difference within certain limits’ (Simpson and Weiner 1989). Other words that are frequently encountered in this context include:

- Variable (n) – ‘Something which is liable to vary or change; a changeable factor, feature, or element’;
- Variability (n) – ‘The fact or quality of being variable in some respect; tendency towards, capacity for, variation or change’; and
- Vary (n) – ‘Of things: To undergo change or alteration; to pass from one condition, state, etc., to another, esp. with frequent or ready change or difference within certain limits’.

All of these words have the same Latin origin (variare) that revolves around a central theme of ‘change’. Variation is about change and change can occur on many levels.

Levels of variation
The levels of variation form an inter-related sequence (Table 1). Variation occurs between nations within the world, between vineyards within the nation, between vines within the vineyard, between bunches within the vine, between berries within the bunch, and between cells within the berry (Amerine and Roessler 1958). The variation at each successive level is dependent on the variation in the preceding level. The statistical method of residual maximum likelihood (REML) allows us to calculate variance components for each level by equating the residual mean squares to their expectations (Rankine et al. 1962, Robinson 1987, Genstar 5 Committee 1987). The sources of variation, degrees of freedom, and estimated mean square for variance components at all levels are listed in Table 1. The discussion that follows will focus on variation that occurs at the vineyard level, the vine level, the bunch level, and the berry level.

Vineyard level
Variation between vineyards contributes to the imbalance between supply and demand that is a threat to the stability of the Australian wine industry. The area of vines planted in Australia between 1902 and 200 is shown in Figure 1.

Historically, three major planting events dominate this graph:

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Table 1. The levels of variation form an inter-related sequence from the national down to the cellular level. The variation at each successive level is dependent on the variation in the preceding level. Variance components at each of these levels can be calculated from estimated mean squares using the method of residual mean likelihood (REML).

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
<th>No.</th>
<th>Variance</th>
<th>Degrees of Freedom</th>
<th>Estimated Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>national</td>
<td>nations within world</td>
<td>a</td>
<td>$\sigma^2_a$</td>
<td>a-1</td>
<td>$\sigma^2_a$</td>
</tr>
<tr>
<td>vineyard</td>
<td>vineyards within nation</td>
<td>b</td>
<td>$\sigma^2_b$</td>
<td>ab(1)</td>
<td>$\sigma^2_a+\sigma^2_b$</td>
</tr>
<tr>
<td>vine</td>
<td>vines within vineyard</td>
<td>c</td>
<td>$\sigma^2_c$</td>
<td>abc(1)</td>
<td>$\sigma^2_a+\sigma^2_b+\sigma^2_c$</td>
</tr>
<tr>
<td>bunch</td>
<td>bunches within vine</td>
<td>d</td>
<td>$\sigma^2_d$</td>
<td>abcd(d-1)</td>
<td>$\sigma^2_a+\sigma^2_b+\sigma^2_c+\sigma^2_d$</td>
</tr>
<tr>
<td>berry</td>
<td>berries within bunch</td>
<td>e</td>
<td>$\sigma^2_e$</td>
<td>abcde(e-1)</td>
<td>$\sigma^2_b+\sigma^2_c+\sigma^2_d+\sigma^2_e$</td>
</tr>
<tr>
<td>cellular</td>
<td>cells within berry</td>
<td>f</td>
<td>$\sigma^2_f$</td>
<td>abcdef(f-1)</td>
<td>$\sigma^2_f$</td>
</tr>
</tbody>
</table>
• between 1916 and 1926, planting area increased by 92%;
• between 1970 and 1979, planting area increased by 26%; and
• between 1989 and 2005, planting area increased by 185%.

This last planting event is the source of much concern to grapegrowers and winemakers alike.

Grape production in Australia between 1902 and 2005 is also shown in Figure 1. Production increased steadily from 1902 until 1996 at the rate of 9,500 t/yr. However, in the period 1997 to 2005, production increased by 132,000 t/yr (115%). Much of this production is surplus to current winemaking requirements.

Planting area and grape production data from Figure 1 can be combined to plot the course of grapevine yield in Australia for the period 1902 to 2005 (Figure 2). Despite some fluctuations, grapevine yield has increased steadily from 2.9 t/ha in 1902 to 13.2 t/ha in 2005. Three peak yields are apparent from the data:

• in 1938, yield reached 11.0 t/ha;
• in 1970, yield reached 14.6 t/ha; and
• in 1992, yield reached 17.5 t/ha.

Although yield per hectare may seem relatively consistent, annual yield variation in Australia for the same time frame is quite the opposite (Figure 2). Variation between one year and the next can be as high as 86%. The imbalance between supply and demand is a direct consequence of this variation.

Yield components
Many components contribute to grapevine yield (Figure 3: May 1972). Variation in any one of these components will contribute to variation in the final crop. The yield prediction models grapegrowers currently use tend to oversimplify this complex relationship between the grapevine and its environment (Clingeleffer et al. 2001). In addition, the grapevine itself is capable of self-regulation (Clingeleffer 1983) and yield component compensation (Freeman et al. 1979, Smart et al. 1982). While many of these components cannot be controlled directly, vineyard managers do have the capacity to manipulate some parameters in the vineyard. Pruning regulates node number per vine and budburst. Carbohydrate reserves can be modified to influence bud fruitfulness and fruit set (Smith and Holzapfel 2003). Grapevine canopies can be managed to enhance budburst, bud fruitfulness and berry growth (Baldwin 1964, Buttrose 1974, Smart and Robinson 1991, Smart 1992).

Vine level
The next level to consider is variation between vines. The inherent variation among individual vines can have a more significant impact on yield than external influences such as soil gradients and drainage or fertility irregularities (Strickland et al. 1932). Variation in sugar levels, titratable acidity and bunch weight between vines can be much greater than within vines (Rankine et al. 1962). Recent developments in spatial analysis techniques have made this area of research much more accessible to the industry. Yield maps produced by a mechanical harvester fitted with an on-board yield monitor and global positioning system (GPS) allow the grapegrower to visualise areas of different productivity in the vineyard (Bramley and Hamilton 2004). Another approach is to collect aerial images of the vineyard using satellite or aircraft mounted sensors and calculate the normalised difference vegetation index (NDVI) for each vine. These maps can be used to visualise differences in vine vigour or relative biomass on a vineyard scale (Hall et al. 2002). Many variables can be measured at the vine level, including: soil characteristics; carbohydrate reserves; bud fruitfulness; percent budburst; inflorescence primordia number; node number; shoot number; bunch number; and vine position.

Bunch level
The next level down is the variation between bunches. Differences in bunch size are commonplace in most vineyards (Figure 4). Since

![Figure 1. Grape production in Australia from 1902 to 2005 has witnessed annual fluctuations of ±40%, despite the relative stability of the planting area.](Image)

![Figure 2. Grapevine yield in Australia has increased steadily with time from 2.9 t/ha in 1902 to 13.2 t/ha in 2005.](Image)

![Figure 3. Grapevine yield is determined by a series of complex interactions between the grapevine and its environment.](Image)
yield forecasting and maturity testing procedures at wineries often rely on bunch sampling, differences in bunch size can be a major source of error in these protocols. Stratified bunch and berry sampling programs have been devised to overcome some of these problems (Roessler and Amerine 1963) but seasonal, varietal and site-specific considerations still confound any general sampling protocol (Wolpert and Howell 1984, Kasimatis and Vilas 1985). The variables that contribute to variation between bunches include: inflorescence primordia size; flower number; fruit set; berry number; bunch weight; and bunch position.

Berry level

Although variation between berries is poorly understood, it potentially has the greatest impact on grape and wine quality. A typical berry follows a double sigmoid growth curve during its post-flowering development, but two berries on the same bunch may follow quite different paths (Figure 5: Matthews et al. 1987). The divergence of the growth curves becomes apparent shortly after flowering and the timing of this divergence is responsible for the extent of the difference between the two berries at harvest. This uneven berry development is called asynchronous development, meaning ‘out-of-phase’. The extent and impact of asynchronous development at the berry level is largely undocumented. Stout (1936) noted that mixtures of berry types within a bunch, in respect of seededness and size, were a frequent occurrence. He named the phenomenon ‘partial variation’. Coombe (1984) pointed out that asynchrony between berries at the onset of Stage III would be missed in samples from mixed populations. To ensure synchronous populations he advocated the use of individual berries in developmental studies (Coombe and Iland 1987). Two recent studies from New Zealand (Trought 1996, Trought and Tannock 1996) have determined the extent of variation in the size (weight and volume) and composition (°Brix) of berries within bunches of the varieties Chardonnay, Cabernet Sauvignon and Pinot Noir. Seed weight, berry volume and vascular development of the pedicel were all inter-correlated. The variables that contribute to variation between berries include: berry size; berry composition; seed number; seed size; and berry position.

Impact of variation (theoretical)

Coombe and Iland (2004) proposed the following model to explain the theoretical impact of variation between berries on grape and wine quality (Figure 6). If ten individual berries each develop at a different rate (asynchronously), then each berry will reach its optimum quality potential at a slightly different time. Thereafter, the quality rating of each berry will decline. Since overall quality of the juice is simply the average quality of all ten berries, asynchronous berry development will result in a reduction in overall quality (modified after Coombe and Iland 2004).
optimum quality potential at a slightly different time. Thereafter, the quality rating of each berry will decline. Since overall quality of the juice is simply the average quality of all ten berries, asynchronous berry development will result in a reduction in overall quality. By the same logic, if it were possible to resynchronize berry development, this would lead to an improvement in overall quality (Figure 7).

Measuring variation
Statistical science allows the analysis of natural variation. Several techniques are used to quantify the level of dispersion around a population mean. These included range, mean deviation, sum of squares, variance, standard deviation, and the coefficient of variation. Additionally, standard error and probability tests employ the central limit theorem to measure departures from normality (Zar 1974). Comparative studies have frequently assessed relative variation using the coefficient of variation:

\[
\text{coefficient of variation (CV)} = \frac{\text{standard deviation (s)}}{\text{mean (x)}} \times \frac{100}{1}
\]

This is a unitless measure of the sample variability relative to the sample mean expressed as a percentage.

The value of comparing coefficients of variation is illustrated in Figure 8. The following function was used to generate theoretical frequency distributions for samples with different relationships between the mean and the standard deviation (Zar 1974):

\[
y = \frac{1}{\sqrt{2\pi}} \cdot e^{-(x-x)^2/2s^2}
\]

Three possible outcomes are indicated in the diagram:

- increasing population variation between sample A and sample B (Figure 8(a));
- decreasing population variation between sample A and sample B (Figure 8(b)); and
- no difference in population variation between the two samples (Figure 8(c)).

Source of variation (timing)
CVs were used to compare Shiraz berry samples taken from a vineyard in McLaren Vale. Whole bunches were sampled at seven developmental stages throughout the post-flowering period: setting; pea-sized; softening; coloured; pre-harvest; harvest; and post-harvest (Table 2). Individual berries were removed from their bunch, weighed and measured. Callipers were used to measure berry diameters in three dimensions (x, y, z) to estimate berry surface area and volume.

Seeds were removed, counted and weighed and the remaining berry flesh and skin was homogenised with a mortar and pestle. Subsamples of the homogenate were extracted with acidified ethanol and centrifuged (Iland et al. 1993). Spectrophotometric techniques were employed to determine the amount and concentration of anthocyanins (Niketić-Aleksić and Hrazdina 1972), flavonols (Price et al. 1995), and other phenolics (Singleton 1966, Somers and Vérette 1988) in the centrifugate.

The relationships of mean berry fresh weight (mg), volume (mm³), and surface area (mm²) with berry age (days after flowering) all followed double-sigmoid patterns (Figure 9). Maximum berry weight, volume and surface area occurred at 91 days after flowering. Thereafter, berry size declined by approximately 20% during the final three developmental stages (from pre-harvest to post-harvest). This phenomenon is referred to as berry shrinkage.

Berry flavonoid composition followed a different trend to berry size. Figure 10 shows changes in anthocyanin concentration during berry development. Anthocyanin concentration declined with increasing berry size at pea-sized and softening stages. By contrast, anthocyanin concentration increased with increasing berry size at pre-harvest stage. This contradicts the prevailing theory that smaller berries exhibit higher anthocyanin concentrations as a result of their greater surface area to volume ratio (Freeman 1983, Matthews and Anderson 1988).

Flavonol concentration during berry development declined with increasing berry size at all three stages: pea-sized; softening; and pre-harvest (Figure 10). The same trend was seen in the graph of phenolic concentration during berry development (Figure 10).

Table 2. Shiraz berry samples were taken from a vineyard in McLaren Vale. Whole bunches were sampled at seven developmental stages throughout the post-flowering period: setting; pea-sized; softening; coloured; pre-harvest; harvest; and post-harvest. The corresponding E-L growth stage, berry age (days after flowering), and estimated juice TSS (°Brix) are recorded for comparison.

<table>
<thead>
<tr>
<th>Developmental Stage</th>
<th>E-L Stage</th>
<th>Berry Age (days after flowering)</th>
<th>Estimated Juice TSS (°Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>flowering</td>
<td>23</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>setting (S)</td>
<td>27</td>
<td>23</td>
<td>–</td>
</tr>
<tr>
<td>pea-sized (P)</td>
<td>31</td>
<td>33</td>
<td>4</td>
</tr>
<tr>
<td>softening (SO)</td>
<td>34</td>
<td>72</td>
<td>7</td>
</tr>
<tr>
<td>coloured (C)</td>
<td>36</td>
<td>91</td>
<td>15</td>
</tr>
<tr>
<td>pre-harvest (PR)</td>
<td>37</td>
<td>120</td>
<td>20</td>
</tr>
<tr>
<td>harvest (H)</td>
<td>38</td>
<td>135</td>
<td>24</td>
</tr>
<tr>
<td>post-harvest (PO)</td>
<td>39</td>
<td>149</td>
<td>27</td>
</tr>
</tbody>
</table>

![Figure 8](https://example.com/figure8.png)

**Figure 8.** Theoretical frequency distributions are plotted for samples A (–) and B (—). Modifying the relationship between the mean (x) and the standard deviation (s) results in different levels of variation, indicated by the coefficient of variation (CV). In (a), where \( s_x = s \) and \( s_y = s/2 \), there is a doubling in variation between samples A and B (CV₂ = 2CV₁). In (b), where \( s_x = 2s \) and \( s_y = s/2 \), there is a halving in variation between samples A and B (CV₂ = CV₁/2). However, in (c), where \( s_x = 2s \) and \( s_y = 2s \), there is no change in variation between samples A and B (CV₂ = CV₁).

![Figure 9](https://example.com/figure9.png)

**Figure 9.** The relationships between mean berry fresh weight (mg) (–), mean berry volume (mm³) (—), mean berry surface area (mm²) (—), and berry age (days after flowering) all follow truncated double-sigmoid curves. Developmental stages are abbreviated across the top of the graph. Berry shrinkage is indicated by a decline in weight, volume and surface area during the last three growth stages.
Variation in berry size
A sequential comparison of CVs can reveal both the source of variation and the points of resynchronisation in the berry’s developmental cycle. CVs for each berry parameter at each developmental stage are listed in Table 3. Taking the parameters of berry size as a group (weight, volume, surface area), the CVs were already high at setting. This implies that variation in berry size originated at an earlier stage in the developmental cycle. Asynchronous cell division during floral differentiation at budburst is the most likely source. Since the CVs declined at harvest, this stage represents a point of resynchronisation in the developmental cycle.

Variation in berry composition
When the same principles are applied to the analysis of variation in berry composition (anthocyanins, flavonols, other phenolics), it is clear that the CVs for berry flavonoids were already high at the pea-sized stage (Table 3). Once again this implies that variation in berry composition originated at an earlier developmental stage. Since the CVs declined at softening, this stage represents another point of resynchronisation in the developmental cycle.

Relevance to grape and wine quality
If winemakers concede that variation in berry size and berry composition has a negative impact on quality (albeit not empirically substantiated), then floral differentiation, berry softening, and berry harvest are all critically important phenomena in the production of high quality grapes and wine.

Managing variation
The options for managing variation are level-dependent. The three sub-sections below outline some of the approaches that might be used to manage variation at the level of the vine, the bunch, and the berry.

Vine level
Zonal management and zonal harvest are appropriate techniques where the grapegrower has ready access to the necessary technology. Alternative approaches include irrigation management and reserve management, particularly in the warm irrigated regions. Recent research has demonstrated that the post-harvest period is important in replenishing carbohydrate reserves that have been depleted during the previous growing season (Howell 2001). Nevertheless, the best approach is site selection. Variation can be avoided by choosing a site free from variation in soil, topography, and extreme weather events.

Bunch level
Variation at the bunch level is best managed by applying viticultural best-practice to promote uniform budburst, shoot growth, inflorescence development, flowering, bunch exposure, and berry development (Coome and Iland 2004).

Berry level
Our knowledge of managing variation at the berry level is still very much in the research phase. Areas of promising research activity include:

<table>
<thead>
<tr>
<th>Berry Parameter</th>
<th>Coefficients of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>weight</td>
<td>setting 53</td>
</tr>
<tr>
<td></td>
<td>volume 55</td>
</tr>
<tr>
<td></td>
<td>surface area 40</td>
</tr>
<tr>
<td></td>
<td>anthocyanins -</td>
</tr>
<tr>
<td></td>
<td>flavonols -</td>
</tr>
<tr>
<td></td>
<td>phenolics -</td>
</tr>
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</table>

Table 3. A sequential comparison of CVs can reveal both the source of variation and the points of resynchronisation in the berry’s developmental cycle. Variation in berry size (weight, volume, surface area) originates prior to setting and is resynchronised at harvest. Variation in berry composition (anthocyanins, flavonols, other phenolics) originates prior to pea-sized and is resynchronised at softening.
• the role of vascular function in berry growth and development;
• the relationship between seed development and berry development;
• the significance of bunch architecture to berry size and composition;
• the influence of extreme weather events on grapevine reproductive biology; and
• the relative importance of cell division and cell expansion throughout the entire developmental cycle.

As researchers discover more about these basic physiological processes, our capacity to manage variation at the berry level will be significantly enhanced.

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