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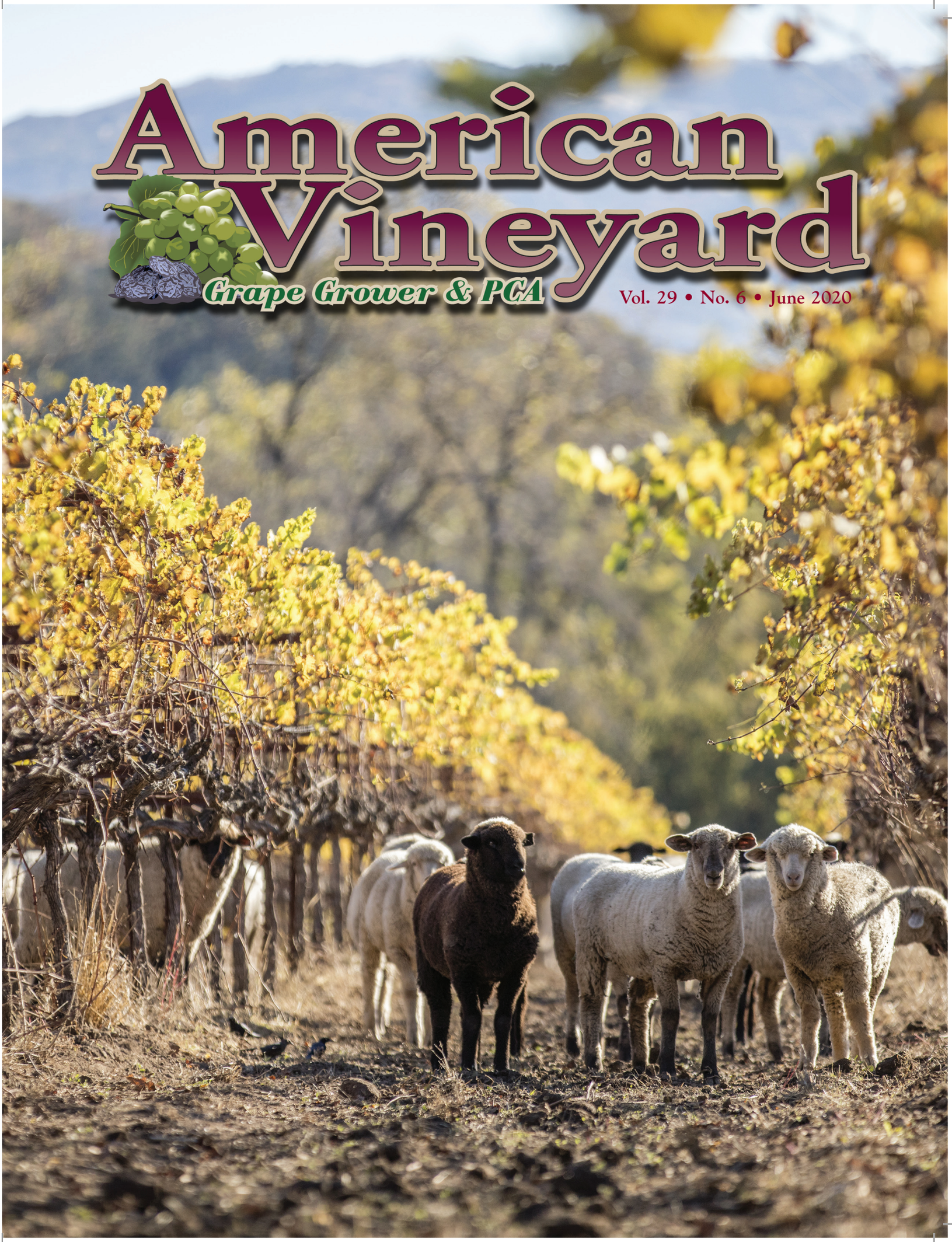

Getting the Perfect Cluster Shape

Defining Table & Wine Grape Traits with DNA Markers

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What defines good architecture in grape clusters and how do plant breeders select for it? Is it the long loose clusters of 'Autumn King' or the smaller, more compact, clusters of 'Chardonnay'? Evaluating cluster architecture, the overall shape of a cluster or bunch, and what defines "good" architecture is important to both table and wine grape breeders and growers.

Until recently, grape breeders had to rely on cumbersome, time-consuming manual observations to evaluate cluster shape in breeding lines. But now USDA and University of Minnesota scientists are using automated image analysis, along with the so-called 'rhAmpSeq' markers developed by the VitisGen2 project, to speed up the process and link traits observed in breeding lines and mapping populations to DNA markers.

What makes a good cluster shape varies greatly between the table and wine grape industries.

For table grapes, large and loose conical clusters with definitive shoulders and large berries that catch the eye of grocery shoppers are key. In wine grape varieties, reducing cluster compactness and small berry size are the priorities. Highly compacted clusters are more likely to develop sour rot or Botrytis bunch rot, and small berries ensure a high skin to flesh ratio – desirable particularly for extraction of flavors and color in red wine varieties.

Grape clusters vary in size, color, berry shape, berry number and size, and rachis branching patterns. Table and wine grape breeders at the USDA Agricultural Research unit in Parlier and the University of Minnesota are replacing time-consuming manual evaluations of cluster architecture with image analysis – allowing more objective and faster 'scoring' of breeding lines and mapping populations.

Defining Cluster Architecture

Cluster architecture and compactness can be affected by a number of traits including berry number and size, rachis branch number, length and even rachis angle. Evaluating each of these traits in mapping populations to develop DNA tests is time consuming and a challenge for grape breeding programs.

Manually evaluating all of these traits can take 10-15 minutes per cluster – and when you multiply that by 1000 clusters in a typical 'mapping population', it quickly becomes impractical. Fortunately, advancements in image-based analyses are making it possible to dramatically reduce the time it takes to evaluate grape cluster characteristics.

These image-based analyses can be used with traditional genomic tools as well as a new genomic tool called 'rhAmpSeq' to locate and identify DNA markers associated with grape cluster architecture traits. Once discovered, these DNA tests can be used by grape breeders to predict and select promising lines at the seedling stage instead of waiting until vines are 2-3 years old.

Image Analysis for Evaluating Cluster Architecture

Over the past four years, we have collected more than 10,000 2-D images of grape clusters, rachises and berries across two segregating grape breeding populations at the USDA-ARS San Joaquin Valley Agricultural Science Center in Parlier, CA. Using a flatbed scanner or a standard SLR camera and a scale bar, we collected images from clusters of roughly 350 grapevines with vastly different shapes and sizes (**Figure 1 page 24**).

We are analyzing these images to determine heritable traits such as berry shape and size, cluster shape, size and compactness.

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Cluster Shape

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Through collaborative efforts with USDA-ARS engineer Dr. Amy Tabb in Kearneysville, WV, we are evaluating each trait for its effect on overall cluster shape.

We classify each cluster into different types: conical (short, shouldered or long) or cylindrical (single, winged or double winged) and also rate cluster compactness (e.g. the number of berries per unit of rachis). Using image-based analysis tools allows us to evaluate more clusters, and provide more detailed data on the traits contributing to cluster architecture.

At the University of Minnesota, we are using image-based analyses to evaluate two key wine grape populations. Our key "mapping population" of about 125 vines resulted from a cross between two parents that differed in cluster compactness, berry color, and powdery mildew resistance. MN1264 has compact clusters, dark berries, powdery mildew resistance and pistillate (female only) flowers. MN1246 has loose clusters with pink berries (**Figure 3**). A second population has been imaged, but the berries are all green-yellow in this family, which has created a problem with separating the rachis, which is a similar color.

Our setup involves a backlighted backdrop that provides contrast to the clusters being imaged (**Figure 4**). For two years, these mapping populations have been the subject of image analysis for cluster compactness, berry color, and other traits with approximately 6,000 2-D images collected.

Figure 1. Cluster architecture varies. We capture that variability through 2-dimensional scanning and image analysis. Grape clusters with and without berries are scanned with a surrounding "aruco pattern" (pattern with known metrics to replace a ruler or more conventional scale bar) that provides precise measurements for image analysis.

Linking Image Analysis to Genetic Markers.

Knowing what traits determine cluster shape is only the first step. Moving from image-based data to DNA marker development is crucial for breeding the perfect cluster shape into new grape varieties.

Thanks to the VitisGen2 project, we now have a new tool called "rhAmpSeq" that provides ~2,000 DNA markers covering all 19 chromosomes of the grapevine genome – at a cost of about $10 per sample. These markers provide the backbone or supplement existing markers of a genetic map, e.g. the roadmap, for each segregating mapping population.

Figure 2: Scanned images of grapes (left) and computer image-analysis of berry shape and circumference (right).

We are using this rhAmpSeq tool, along with imaging data, to pinpoint the exact location of the genes associated with the desirable traits – and map them to specific chromosomes. The resulting 'Quantitative Trait Loci' (called QTLs by breeders) then become DNA markers that are useful to breeding programs.

Figure 3. Two numbered selections at the University of Minnesota with differing cluster architecture were crossed to form a mapping population of 125 siblings. MN1264 (female parent) has tight clusters with dark berries and no 'wings'. MN 1246 has loose clusters with pink berries and extensive branching. Image analysis of the mapping population allows researchers to identify genetic loci associated with different cluster attributes.

To date, the UMN grad student Anna Underhill used image analysis on 6000 images from the mapping population to identify QTLs (DNA marker linkages) associated with berry color and cluster architecture traits. Current UMN student Laise Moreira is using the same tool to identify QTLs associated with cluster architecture traits using

mapping populations at the USDA in Parlier.

At Parlier, Individual vines in our mapping population vary in flowering time, cluster size/shape and weight, berry number, berry shape and color. We will be using the rhAmpSeq tool to identify and validate potential QTLs for many of these traits. The challenge will be to understand which of the 15 different traits are the most important contributors to fruit architecture. Our hope is that a few QTLs will stand out enough to use as DNA markers in selection and breeding programs.

Associating these QTL DNA markers with traits means that breeders can predict the performance offspring well before the plants reach maturity. With populations evaluated in wine grape and table grape programs from different research labs, grape breeders will have access to more information about the inheritance of cluster architecture and other important production traits.

To date, the VitisGen2 project has identified 70 DNA markers that will be useful for marker-assisted selection in both table grapes and wine grapes. This will allow breeders to select the best new seedlings for breeding lines and potential new cultivars earlier in the process, making grape breeding programs more efficient and lowering the cost of developing new varieties.

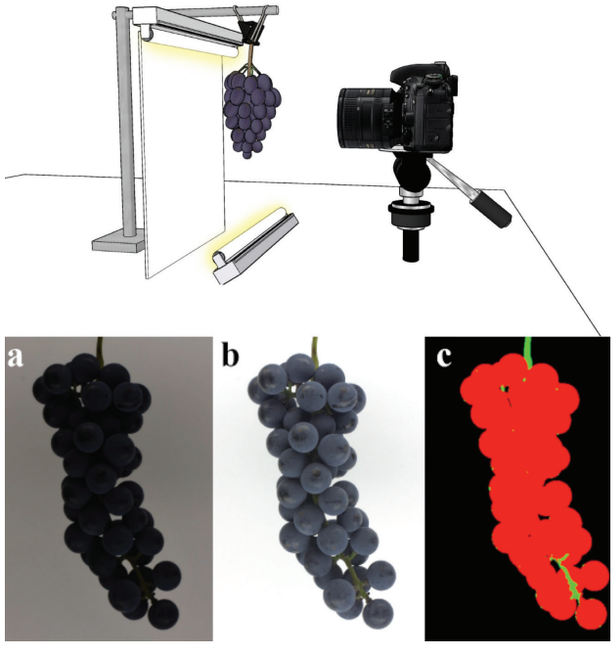

Figure 4. 2-D Cluster imaging setup at the University of Minnesota (Top). Image analysis (Bottom) first includes acquisition of a raw image (a), color correction (b), and an image segmentation pipeline that uses color so identify berries, background, and rachis(c).

Acknowledgement

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