Assessing the Genetic Determination of Valuable Avocado Traits Using Microsatellite (SSR) Markers and Quantitative Trait Locus (QTL) Analysis

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I. Goals

Avocado breeding is challenged by the inability to perform controlled pollination, the long juvenility period of the trees (4–7 years) and their considerable size. Because of these difficulties, varietal improvement has long relied on multi-year field trials during which large numbers of seedlings are grown to maturity and screened for desirable (phenotypic) characteristics. Inferior trees are culled as their deficiencies become apparent, leaving only the most promising genotypes. Clearly, the time, land resource, and labor costs associated with growing trees to the appropriate stage of development are huge. This financial burden is further compounded by conventional breeding approaches that use open-pollination, which results in a complete re-shuffling of genes—good and bad—in the hope of coming across a superior seedling. This approach happened to work well for Rudolph Hass who discovered such a chance seedling in his back yard, but Mr. Hass was a very lucky man indeed.

The use of molecular markers as surrogates for tangible traits reduces the screening task by between 1–2 orders of magnitude: Markers can be detected in DNA from young seedlings, eliminating the need to grow large numbers of trees to maturity, thus reducing land and labor expenditures. Marker-assisted selection (MAS) also permits much greater intensities of selection because large numbers of seedlings can be assessed for marker transmission shortly after germination. Only seedlings with a desirable marker variant are retained, insuring a pool of breeding material enriched for the 'good' gene(s) despite the inevitable gene reshuffle.

In preparation for MAS, our project has advanced on three fronts:

(1) Development of genetic populations to assess the heritability of major traits
(2) Development of genetic markers
(3) Evaluation of levels of genetic variation in avocado accessions to assess the potential for genetic improvement

II. Developing Genetic Populations

In 1999 we initiated the establishment of a replicated experimental avocado population of over 800 trees. The 204 distinct avocado genotypes that represent our experimental population consist of open-pollinated progeny of cultivar Gwen. Each of the 204 trees has been clonally replicated four-fold, with two clonal replicates of each genotype growing at each of two locations (coastal and inland Southern California). This population fulfills a very important function in that the experimental replication—coupled with classical quantitative genetic analyses—provides a means of eliminating the non-genetic component of a trait measurement to reveal its genetic (heritable) portion. In other words, "what you see is not what you get" until after these analyses have removed the non-genetic noise.

In the spring of 2002, we started to collect data on growth rate, flowering, and—since 2004—fruit yield and quality. For each data set, we calculated heritabilities and related parameters including genotype x environment interaction effects and trait correlations (all data from Chen et al. 2007): broad-sense heritability for tree height, canopy diameter and stem girth was 34.3, 29.7, and 28.5%, respectively, and corresponding values for flowering abundance and fruit yield per tree accounted for 32.3 and 23.4%, respectively. These values are not as high as anticipated but sufficient to permit breeding advance. Genotype x environment interaction was weak for flowering and fruit yield per tree. Growth rates did not correlate with flowering abundance, and only a moderate correlation was found between growth rate and fruit yield per tree.

Since 2006, in collaboration with Dr. Arpaia and the UC Riverside Scion Breeding Program, we have gathered data on fruit quality parameters (fruit size, shape, skin and flesh attributes; Fig. 1). These fruit quality data and the previously collected flowering and growth-related measurements will be fed into a quantitative trait locus (QTL) analysis that detects associations between a measurement and a molecular marker. The same quantitative genetic analyses mentioned above will be applied in a study of markers associated with the nutritional composition of avocado fruit. The proposal for this project was submitted to the University of California Industry-University Cooperative Research Program (IUCRP) in October 2007.

Figure 1. Fruit collected from a subset of the 'Gwen' progeny population, showing a considerable range in fruit size, shape, skin color and skin texture.

III. Developing Molecular Markers

During 1999–2003, we developed a set of 127 microsatellite markers from a genomic library of 'Hass' screened for dinucleotide and trinucleotide repeat motifs (Ashworth et al. 2004). Since then, we have been genotyping each tree in the experimental population using these markers. Figure 2 shows marker alleles for 8 avocado genotypes (each row corresponds to one genotype) at one locus. To date, we have gathered marker data in 204 individuals at 23 microsatellite loci. The marker data have indicated that approximately a quarter each of the 204 'Gwen' progeny genotypes originated from pollination by 'Bacon', 'Fuerte', and 'Zutano' pollen (together 75% of the 204 genotypes), with the remaining 25% originating from pollination by unidentified pollen sources or miscellaneous identified varieties. Knowing the pollen source enables us to analyze the data according to both maternal and paternal contributions: for example, we have detected significant differences due to the paternal parent for mean tree height, with 'Gwen' × 'Zutano' progeny being significantly taller and 'Gwen' × 'Fuerte' progeny significantly shorter (Chen et al. 2007).

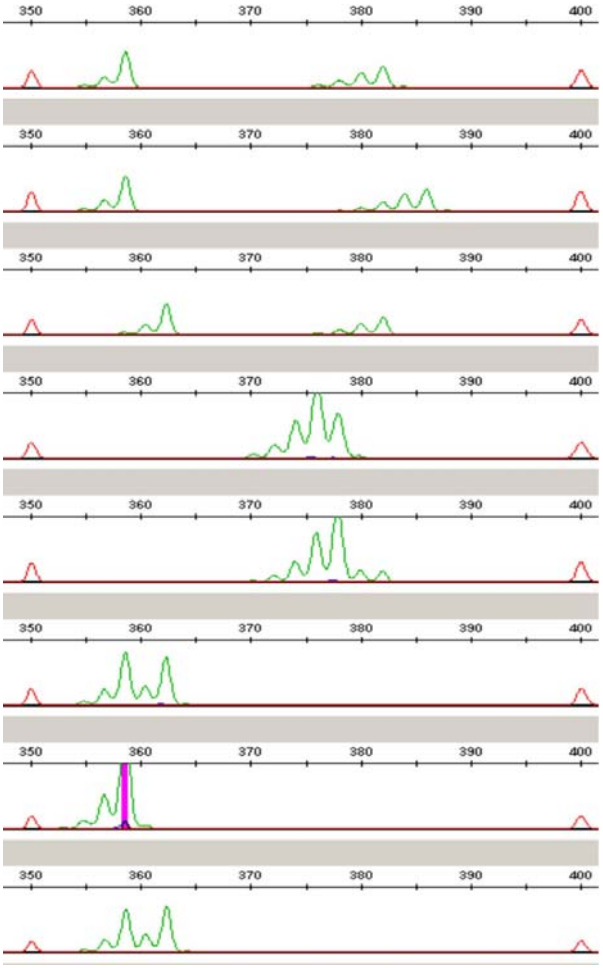

Figure 2.
Electropherogram illustrating micro-satellite marker variants in eight avocado geno-types at one marker locus (AUCR 418)

IV. Fresh Perspectives on Genetic Improvement

New insights are being gained from a new class of molecular markers called SNPs (Single Nucleotide Polymorphic DNA) developed for avocado in our lab (Chen et al., in press). This work revealed 213 SNPs in four genes (5690 base pairs of DNA) sequenced in 33 cultivars and 21 wild avocado accessions. The SNP markers were used to reassess cultivar origins and the relative contributions of the three horticultural races of avocado (Chen et al., in prep.). In several cases, prevailing assignments in terms of Guatemalan and/or Mexican race ancestry were challenged. Moreover, Mexican race ancestry emerged as being further subdivided in relation to elevation and latitude (data not shown). A histogram (Fig. 3) depicts the racial composition of a panel of avocado cultivars. By improving our knowledge of the ancestry of existing cultivars, breeders can refine their choice of suitable source material. Work proposed under the IUCRP is designed to search for additional SNP markers that are specifically associated with genes controlling the nutritional composition of avocado fruit.

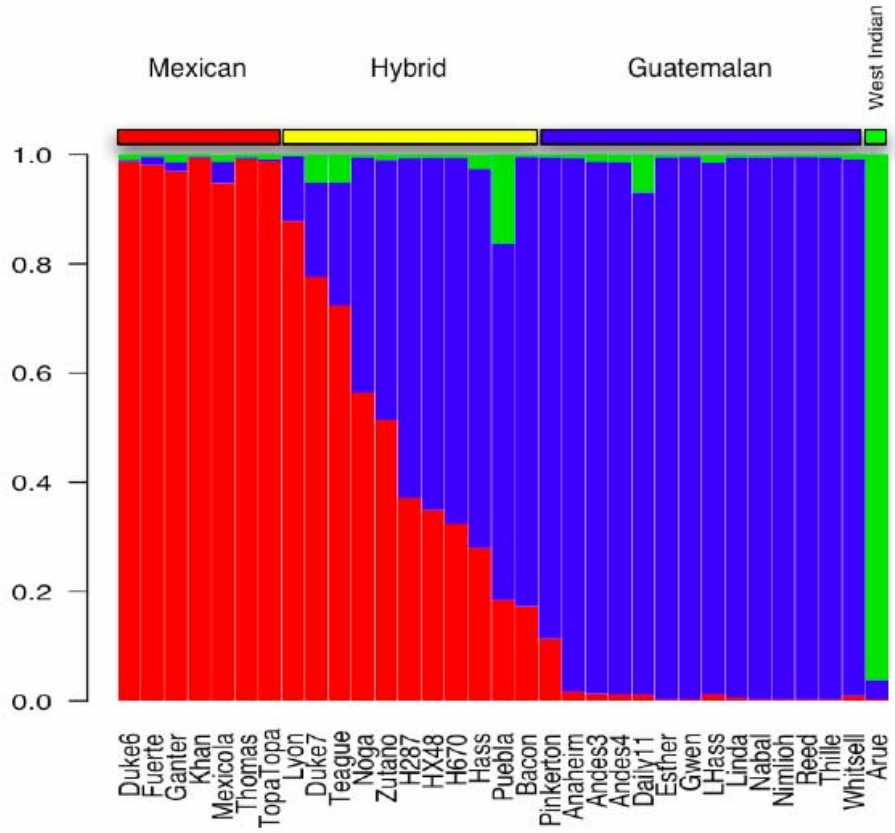

Figure 3. Histogram depicting the relative contribution of the Mexican (red) and Guatemalan (blue) horticultural race in the ancestry of 33 avocado cultivars. The West Indian contribution (green) is expected to be small in California-grown cultivars.

V. The Future

As soon as we reach a threshold number of ca. 50 microsatellite markers we will be able to perform a QTL analysis on our accumulated data to look for markers that track traits of interest. Our goal is to re-run this analysis with 100 microsatellite markers to maximize our ability to detect associations with commercially and biologically relevant traits. Initial implementation of MAS would focus on seedlings derived from a subset of trees in our 'Gwen' progeny population.

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