

RosBREED's Community Breeders' Page

A success story in Rosaceae marker-assisted breeding: larger fruit for sweet cherry.

What can we learn?

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Quantitative trait loci – they're a dime a dozen, right? For the last decade every DNA lab has been churning out QTLs, and along with each is the promise of great utility in breeding. Yet the cherry fruit size QTL on *Prunus* chromosome 2, May 2010's "Jewel in the Genome", *is* actually influencing decisions about crossing, seedling selection, and elite selection advancement. So what makes this QTL different, and how soon can we polish up more jewels like this??

The jewel

Many features of the cherry fruit size QTL are the same as for most QTLs. A large, statistically significant effect was detected among its segregating alleles in the experimental population dedicated to the study (in the PhD project of Jim Olmstead at MSU, supervised by Amy Iezzoni). Physiological dissection of the trait strongly indicated that the locus' effect was through cell number, a highly heritable component of fruit size. Yet the experimental population (n=190) was from a cross between a medium-sized old founder cultivar and a tiny-fruited wild forest accession, where the largest-fruited offspring maxed out at less than half the weight of fruit from top cultivars on the market today. This begs the question (the first question that in the RosBREED approach we pose to every QTL with dreams of grandeur): Does the QTL have commercial and breeding relevance?

A precious jewel

Amy Iezzoni chooses the targets of her efforts carefully. Size matters: cherry fruit size is directly tied to industry profitability and consumer preference. Size (as measured by weight or diameter, or the industry's box row size) is not only a factor of yield but is itself a fruit quality trait where larger sizes return a premium to growers. A worthy breeding target. Yet breeding populations are typically skewed to small fruit compared to the parental average, and so crosses based only on phenotypic information produce few larger-fruited seedlings. This trait is therefore highest priority in the Pacific Northwest sweet cherry breeding program (PNWSCBP). Back to the QTL mapping population, the medium-fruited parent cultivar was heterozygous for the detected QTL. Surprisingly, the wild forest cherry parent was also heterozygous, and carried the same QTL allele associated with large fruit. So those seedlings homozygous for this allele

had significantly larger fruit than the rest. The proportion was even less than the expected one-quarter, due to segregation distortion in this genomic region. Hopefully starting a trend among QTL reporters, Dr. Iezzoni and colleagues reported the functional haplotypes at this QTL, as delimited by alleles of the two flanking SSRs. What I'll call the "good" haplotype was "190-255", denoting that trees carrying the 190 bp allele of CPSCT038 and the 255 bp allele of BPPCT034 together on one of their two copies of chromosome 2 are extremely likely to also hold an allele for a gene involved in providing fruit with more cells à larger fruit on average (Figure 1).

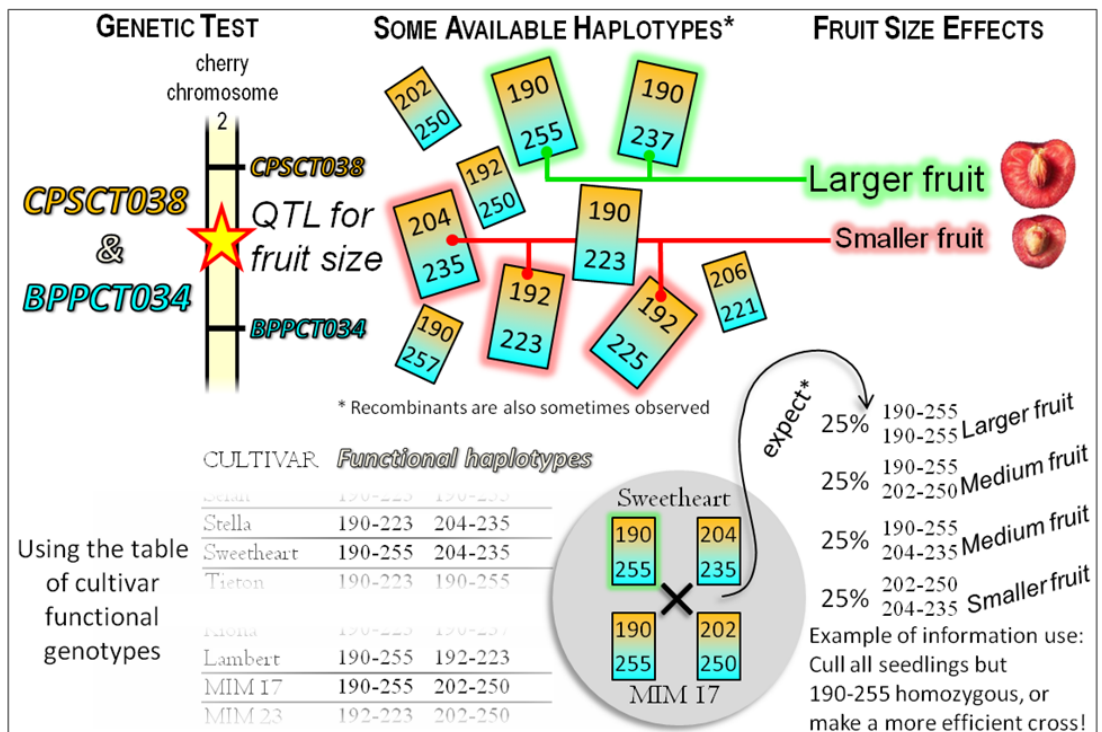


Figure 1. Use of a valuable jewel in the sweet cherry genome, involving SSR markers flanking a fruit size QTL on chromosome 2. A table of functional genotypes for various sweet cultivars can be found at www.rosbreed.org/breeding/jewel-use

Breeding utility

Without translation to breeding utility, a QTL is merely a squiggle on a map in a journal archive. Dr. lezzoni followed up the QTL discovery with federal funding (in landmark NRI-funded projects that also first introduced Pedigree-Based Analysis, SNP-based genome scans, and genome-wide comparative “RosCOS” markers to U.S. rosaceous crops). I became involved too, combining MSU and WSU data to catalog available haplotypes and assess their association with fruit size and other important traits in breeding germplasm of the PNWSCBP.

A key piece of knowledge for breeding utility is the distribution of functional alleles in parent breeding germplasm. As it turns out, the “190-255” haplotype is very common in the parent pool of the PNWSCBP, representing about 50% of all haplotypes, and its homozygotes are abundant. Of more than ten other haplotypes observed, no other comes close – even the next most frequent is a recombination of the first. Therefore, Dr. lezzoni’s “good” QTL allele is found beyond just her original experimental population – it is breeding-relevant!

Associations of haplotypes with fruit size were ascertained in two independent studies. The first, at MSU, included 41 pedigree-linked cultivars and selections and two mapping populations (n= 124 and 103). FlexQTL™ analyses *validated* the existence of the QTL across this wider germplasm set. *Utility* of the markers was addressed in 2009 in the second study, by calculating effects of alleles and haplotypes in the first fruiting seedlings of the new PNWSCBP – 21 populations derived from 17 parents (total n = 217) – as well as several further parents and selections. This effort distinguished several new alleles, defined the breeding haplotype pool, and even indicated that further traits (firmness and sweetness) may be associated with the locus. For example, the good “190-255” may bring along undesirable softness. But the rare “190-237” haplotype was associated with large *and* firm fruit. Several haplotypes were connected to small fruit, which are no good commercially even if associated with firmness and good flavor – unless linked trait loci rather than pleiotropy is the underlying mechanism.

Validated. Utility. Sound familiar? Validation and Utility are core stages (#4 and 5) in the MAB Pipeline. In essence, these efforts in 2005-2009 described above were ad hoc. Were the parent pools wide enough to represent the breadth of breeding germplasm? Were the seedling numbers sufficient to confirm statistical significance? Were confounding effects of relatedness accounted for? RosBREED is bringing a systematic approach to the conversion of promising jewels into valuable components of new cultivars. For this cherry jewel, and others throughout Rosaceae crops, the coordinated approach to validating genetic tests crop-wide and assessing their utility within individual breeding programs began in the 2010 season. With our comprehensive and statistically rigorous Pipelining approach, the answer to all such questions of analytical robustness from now on will be “Yes!”

Breeding use

With steadfast attention, consideration in the context of breeding reality, and persevering beyond mere academic interest, the chromosome 2 sweet cherry fruit size jewel is now used routinely in breeding.

The fruit size genetic test is used to *inform crossing decisions* (marker-assisted parent selection, MAPS) in Nnadozie Oraguzie’s breeding program. With the “190-255” haplotype being so common, Dr. Oraguzie is pleasantly surprised with the number of homozygous “good” seedlings that appear in open-pollinated progenies – knowing that open pollination can be a reliable fallback is reassuring! But controlled crossing can achieve greater enrichment for favorable fruit QTL alleles, even to fixation (that avoids the need for marker-assisted seedling selection), especially for improving the proportion of other large-fruit haplotypes or avoiding small-fruit ones (Figure 1). Unlike the common “190-255”, the rarity of the large-fruited “190-237” haplotype necessitates deliberate and even multi-generational planning to truly exploit – plans that Dr. Oraguzie has indeed already hatched. A certain haplotype combination associated with small fruit, “192-223” and “204-235”, arises from many combinations of important breeding parents. This observation reflects a common realization at first use of predictive genetic tests that many previous crosses are inefficient for obtaining target genotypes. Since spring 2010, Dr. Oraguzie has conducted MAPS to enrich his germplasm for large-fruit alleles.

The genetic test is used to *inform seedling selection decisions* (marker-assisted seedling selection, MASS) in Dr. Oraguzie’s program. A second genetic test, for self-fertility, is also used. Following calculations and considerations of *MASS Cost Efficiency and Logistics* (Pipeline stage 7), a *Trial* run (Pipeline stage 8) was conducted in the fall of 2010, which culled 500 seedlings, from 800, predicted to be small-fruited and self-infertile. Cost-wise, this 2010 MASS was estimated to save ~\$25K in projected costs of maintaining and evaluating genetically inferior seedlings. Logistics-wise, these efforts highlighted the need for careful labeling of individual plants and that the simplest window would be when seedlings are in the lath house rather than earlier in the greenhouse. Also, the breeding crew was relieved to have less a strenuous task for out-of-sync fall planting. The successful trial run paved the way for routine MASS the next year. In 2011, almost 2000 seedlings were genetically screened and more than half culled.

The genetic test is used to *evaluate cultivar candidates*. Available advanced selections that had previously been elevated on phenotype alone, were screened for the chromosome 2 fruit size markers and the self-fertility test. The

genotyping results factored into decisions about whether to advance the selections further, and several selections were dropped before further resources were expended on them. In the future, genetic screening for these available tests will be conducted and considered earlier in selection advancement decisions. However, it is never too late within a breeding program to consider all available information on the prediction of genetic potential. Results from new markers (whether for other traits or further QTLs for fruit size) and up-to-date information on existing markers (such as refinements of predicted phenotype, interactions with other loci, or linkage involving other traits) will continue to be part of Dr. Oraguzie's decisions in the PNWSCBP.

A jewel for any sweet cherry breeder

The table of functional haplotypes for the chromosome 2 sweet cherry fruit size jewel can be found at: www.rosbreed.org/breeding/jewel-use

Breeder's intuition

This jewel's discovery by a breeder was no coincidence. Dr. lezzoni's breeder's intuition from years of experience with cherry germplasm told her that there must be major trait loci segregating in her cherry populations. Community Breeders, your intuition of the complexity of trait inheritance is very valuable! Are there important traits for which you believe major controlling loci are segregating in breeding germplasm? Tell your local molecular genetics support team! Tell RosBREED! These could be relatively easy high value MAB targets.

Many lessons

Discovery to breeding application of the chromosome 2 sweet cherry fruit size QTL has taught many MAB lessons. With our Demonstration Breeding Programs representing so many breeding situations, traits, genetic architectures, and breeder preferences, we expect to learn and describe many more!



Figure 2. Dr. Oraguzie with the fruits of MAB labors.