California Cling Peach Advisory Board
2014 Annual Report

Project Titles: Development of New Cling Peach Varieties

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Objectives:

1. Continue to evaluate multi-year field performance and genetic data (including molecular marker/mapping data) to identify the most promising breeding lines (pedigrees) as well as the most promising individuals within selected pedigrees for grower testing as and/or further crossing.
2. Continue to develop and implement low cost, high through-put field selection methods targeting high fruit quality and yield with greater emphasis in 2014 on plant developmental indices.
3. Generate 9,000 new seedling progeny trees through controlled recombinations primarily through cross-hybridization targeting high productivity with reduced grower and processor costs.

Introduction:

The needs for processing peach varieties differ from fresh market fruit since the lower returns for processed fruit require greater production efficiency and crop consistency over an expected orchard life of 20 years or more. Achieving these needs requires the identification and incorporation of new germplasm for increasing productivity and processing quality while reducing grower inputs, as well as the long-term regional testing to identify deficiencies prior to large-scale industry plantings. The UCD variety development program has become an international leader in the identification, testing, and incorporation of new germplasm into commercial varieties in order to meet rapidly changing industry needs. We have also been able to leverage the resulting large and diverse breeding populations to successfully obtain complementary funding (USDA, SCRI, etc.) for the more costly and technically demanding analysis required to develop molecular markers for important traits. (Molecular markers for a trait such as fruit brown rot resistance offer improved breeding efficiency as one could select the resistance gene directly rather than the difficult and costly indirect field screenings). While many tree crop breeding programs have solicited industry funding for molecular marker research, our policy has been to apply all industry funds to develop large-scale, field-based variety development programs and to then leverage these established and genetically diverse breeding populations to solicit outside funding for the more demanding molecular studies. We are currently starting the 2nd phase of the breeding project with a strong applied focus on developing new processing peach cultivars with the required new traits to meet changing grower and processor needs and reinforced at the molecular marker level by expanded support from the new SCRI RosBreed project for developing molecular markers and informatics assessment tools .

Progress Summary- 2014

Continued breeding progress in 2014 has resulted in two exceptional achievements. The first was the release to the California industry of the cultivar *Kader* which provides the industry with a *Carson-Bowen* season processing peach with high orchard as well as case yields. Additional advanced selections for the *Andross* and pre-*Loadel* harvest season are moving through the breeding pipeline with anticipated releases in the next few years. Concurrently, a new generation of experimental peaches which promise continued high yields with lower grower inputs, is being prepared for a new round of regional testing. This progress was possible because of a singular focus of breeding program resources towards identification of higher-quality germplasm, its incorporation through genetic recombination into California adapted breeding lines, and a subsequent rigorous, long-term testing to rogue out deficient material and identify the most promising new genotypes for evaluation and release as new varieties. The second exceptional achievement was the selection for a second round of SCRI funding for the new RosBreed project in which the number of UCD peach selections for which molecular markers would be developed was increased to 1,000 individuals, representing a threefold increase from the first RosBreed project in which UCD cling peach breeding material also represented the largest proportion of peaches analyzed. This was achieved largely because we had large breeding populations already established with the required genetic diversity for effective molecular analysis. The current RosBreed project is multi-crop in scope (apples, peaches, cherries, strawberries, etc.) and multistate and international in its execution, representing an impressive logistical achievement directed by Prof. Amy Iezzoni in Michigan State University. We are now in the process of identifying the most promising individuals for molecular analysis, targeting established populations from 2010 and 2011 plantings since 2013-14 field data show these populations to be segregating for crucial commercial traits including disease resistance, fruit quality and harvest efficiency. However, continued maintenance of 2010 and 2011 plantings (originally scheduled for removal in 2015 and 2016) will require reductions in newer field plantings in order to stay within breeding budget. Over 10,000 breeding seed were recovered in both 2013 and 2014, exceeding program objectives (Figure 1). Half of the 2013 seed has been germinated and grown in greenhouses with over 4,000 selected seedlings being field-planted in 2014 with an additional 3,000 seedlings being stored for winter stratification and possible direct seeding in early 2015. We have elected to delay the full 2014 field plantings and to reduce 2013 field plantings since participation in the five-year RosBreed project would require the postponement of the scheduled removal of approximately 10,000 bearing trees from earlier 2010 plantings. The development of molecular-based techniques to improve assessment of breeding value for selections is particularly valuable for UCD material since the majority of our advanced breeding lines possess new and so otherwise poorly characterized germplasm introduced to achieve previously unattainable goals [primarily fruit brown rot resistance, fruit integrity allowing delayed and once-over harvest, and certain

Year	Target	Field planting
2008	8,000	9,061
2009	8,000	12,038
2010	6,000	11,637
2011	5,000	8,211
2012	5,000	5,141
2013	5,000	2,073
2014	9,000	~4,000

Figure 1. Targeted versus actual field plantings by the UCD processing peach breeding program.

maturity times (*Dixon/Andross* and season extension)]. Once the final list of 2010-Block and 2011-Block individuals for RosBreed testing has been completed in the spring of and summer of 2015, a large proportion of those blocks will be removed freeing up resources for later field plantings. Considerable resources, however, will have to be diverted to the multiyear data collection for the 1, 000 test trees from these blocks, representing a limit to future breeding program size. Consequently, the focus of this report will be to summarize and further analyze progress in our ability to develop molecular markers to facilitate future peach breeding efforts, including both novel opportunities and inherent limitations to their utilization. Field-related updates of 2014 progress for the Cling Peach Variety Development Project are included in the 2014 Regional Evaluation Report since a goal of the this year's activity is the identification of the most promising advanced selections for a new round of regional testing.

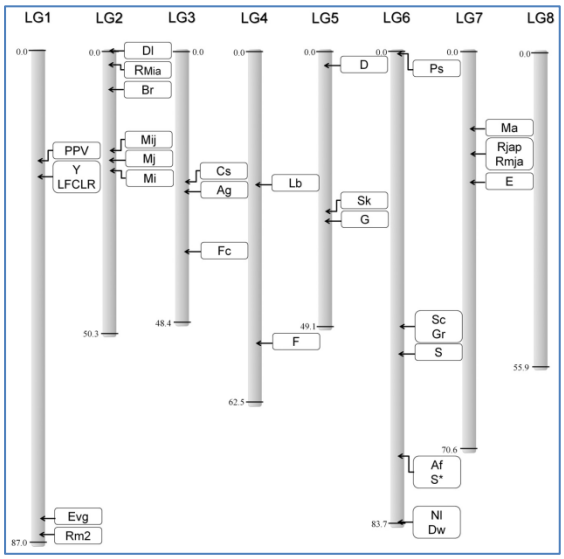

Figure 2. Map position of simply inherited traits in peach is shown according to the 2012 Prunus CMap available at http: //www.rosaceae.org. PPV: Plum Pox Virus Resistance; Y: Fruit Flesh Colour; LFCLR: Senesced Leaf Colour; Evg: Evergrowing; Rm2: Green Peach Aphid Resistance; Dl: Double Flowers; RMia, Mij, Mj and Mi: Root-knot nematode resistance; Br: Branched Growth habit; Cs: Flesh color around stone; Ag: Anther Color; Fc: Flower Color; Lb: Blooming time; F: Flesh adhesion D: Non AcidFruit; Sk: Kernel Taste; G: Skin Hairiness; Ps: Pollen Sterility; Sc: Fruit Skin Colour; Gr: LeafColour; S: Self-Incompatibility; Af: AbortingFruit; S*: Fruit Shape; Nl: LeafShape;; Ma, Rjap, Rmja: Root-Knot Nematode Resistances.

The opportunities and limitations of molecular markers to facilitate UCD peach breeding.

A plant appearance, or phenotype, is determined by its chemical makeup, which, in turn, is determined by the specific genes that it contains. Genes are coded by short DNA segments on a much longer DNA strand. Different species have different numbers of DNA strands which when highly condensed or coiled are recognized as chromosomes. Because each chromosome or strand contains thousands of genes for different plant processes, genes on the same strand or chromosome are said to be linked-together or simply linked. In plant growth and development, normal mitosis ensures that each daughter cell receives an exact copy of each chromosome so that the plant appearance or phenotype on one shoot is identical to another shoot. (Similarly, the Ross variety, because it is propagated as a vegetative clone, is identical for all trees propagated). In sexual recombination, however, chromosomes of the same type but from different parents can recombine genetic material by reshuffling whole chromosomes and, within chromosomes, exchanging equivalent segments of DNA (but containing different alleles or forms of a given gene such as white or yellow flesh color). Because this is inherently a physical process, genes very close together on the same DNA linkage group or chromosome are less likely to be separated (and are said to be tightly linked) than those at distant ends of the linkage group. For many traits, such as flesh color, characterization (or phenotyping) requires a mature producing tree and so a delay of several years before characterization can take place. In addition, because there are different degrees of yellow flesh color, and because the level of color will vary with the

environment (temperature, sunlight, etc.) as well as specific allele type (genotype), it is often impossible to know the specific genotype from its general phenotype or appearance. Molecular markers offer the opportunity to identify the specificity of a gene by reading it directly or indirectly on the DNA. A direct reading would be possible by determining the DNA code at the location or locus for that gene once it is known and then scanning for that specific code using appropriate technologies. Even when the location is not known, if nearby DNA or molecular markers were identified, they could be used as indicators of the presence of that gene because they are physically linked. Molecular markers thus offer the opportunity to know the specific genotype precisely even at the seedling stage and even under differing environments. One could thus select seedlings having the desired traits (disease resistance, etc.) obviating the need for large the field land requirements and multiple years needed through conventional selection methods. An additional advantage of molecular markers which is particularly useful when new germplasm is being introduced (as in the UCD program) is that it can also be used to characterize genes nearby and so linked to the desired target gene. Having undesirable genes such as small fruit size linked to desirable genes such as disease resistance would make that targeted gene less valuable unless the linkage could be broken. The probability that linkage can be broken is directly related to the distance between the genes. By knowing the linkage distance, one could predict how large a population would be needed to effectively break the linkage and separate the undesired from the desired gene. Therefore, the development of good linkage maps for crop species offers powerful opportunities to improve the breeding efficiency. Because each chromosome or linkage group contains thousands of genes interspersed with even more spacer DNA, a good linkage map would have to have thousands of molecular markers saturating the entire length of the DNA strand or linkage group. As few as 10 years ago, the best linkage map for peach was that shown in Figure 2 and was developed by multimillion dollar European consortium. The map was of scientific interest but of limited breeding value because there were very few markers per linkage group (LG) which made their exact location and even verification difficult to determine. In addition, those few mapped traits were of little value to most advanced breeding programs since those traits (for example, yellow versus white flesh) had already been strongly selected for or against by the programs. Because of its strong applied focus, the UCD processing peach breeding program has developed among the largest and most genetically diverse peach populations in the world. Because large established and segregating populations are a prerequisite for molecular marker discovery, we have been very successful in securing outside funding for marker research on different applied topics as part of a research collaboration with the DNA analysis lab of Dr. Carlos Crisosto (Figure 3). Our continued focus on generating and evaluating large and genetically diverse breeding populations has continued to provide ideal populations for molecular analysis (and successful acquisition of new molecular funding), and the UCD peach molecular analysis consortium (Crisosto, Dandekar, Parfitt, Martinez-Garcia and Gradziel) have become an internationally recognized center for tree fruit

Title:	Candidate genes for fruit softening in Prunus	Survey and molecular genetic analysis of resistance/tolerance to the brown rot pathogen (Monilinia fructicola)	Peach, almond and related species as mutual sources of useful genetic variation for fruit and nut quality	SNP and QTL discovery of peach fruit quality genes	Integration of genomic tools for next generation peach and almond cultivar development	Combining Disease Resistance With Horticultural Quality In New Rosaceous Cultivars
Agency:	USDA	UCANR	UC Discovery Grant	NRI-CSREES	RosBREED SCRI	RosBREED SCRI
Amount:	$445,000	$67,232	$528,600	$378,000	$191,144	~$200,000
Start	9/1/2005	9/1/2007	9/1/2006	9/1/2008	9/1/2009	9/1/2014
End	09/01/2008	09/01/2008	09/01/2009	09/01/2011	09/01/2012	9/1/2019

Figure 3. Recent processing peach molecular marker development projects achieved by leveraging applied breeding program resources.

molecular marker development. [Just as breeding funds are directed entirely to processing peach cultivar development activities, molecular funds are directed entirely to molecular marker development (though this does cover some field data collection). Molecular funds are also used for project management including report and sub- grant writing which are largely under the direction of Dr. Crisosto and for UCD projects under the direction of Dr. Iezzoni for RosBreed].

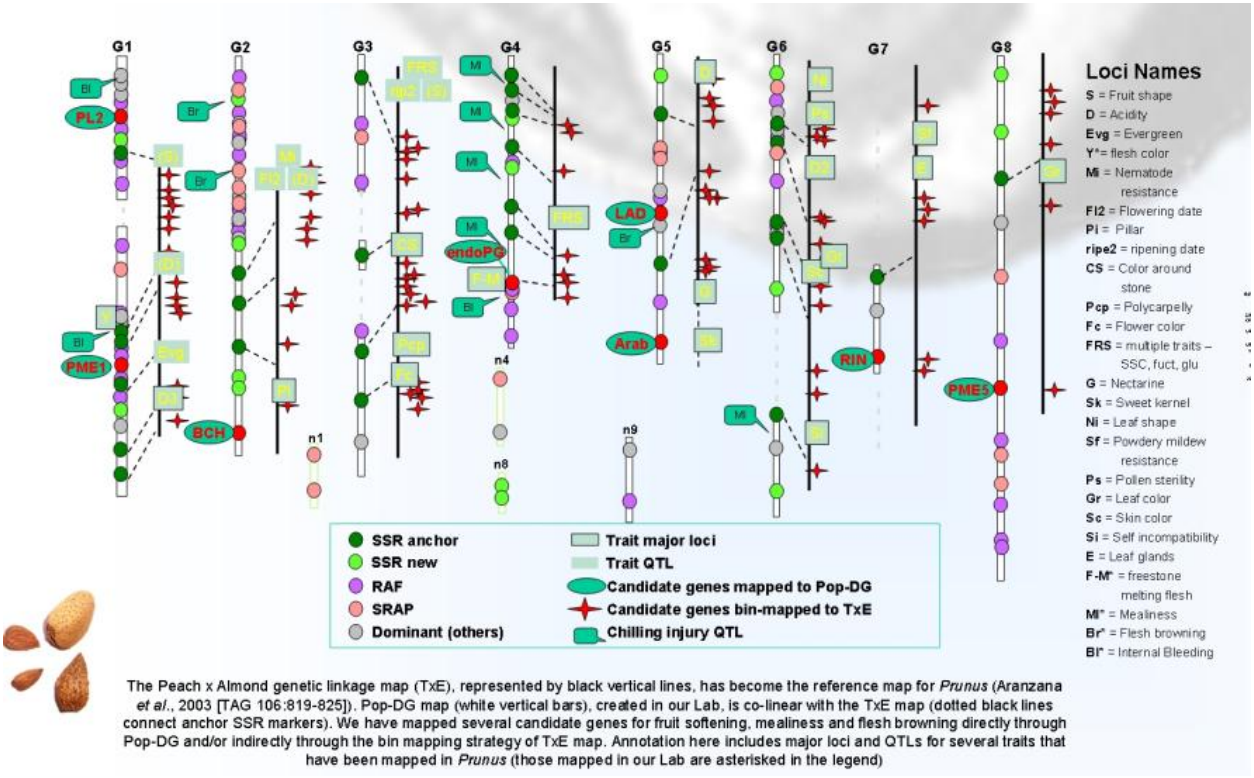

Figure 4. Early UCD molecular map.

UCD development of peach molecular maps.

Early UCD molecular maps utilized diverse processing peach breeding populations including almond by peach derived breeding lines. While peach and almond are closely related and easily hybridized, peach, because it is self-fruitful and derived from a very small and relatively uniform initial germplasm, is highly inbred, so that there is not a lot of variability at each genetic loci. This is undesirable from a breeding point because genetic variability is the source for trait improvement. Similarly, it is undesirable for developing molecular maps because, again, progeny segregating for a large number of traits are required to distinguish the relationship (possible linkage) of genes controlling those traits. An early UCD developed peach genetic linkage map is presented in Figure 4, and represents an improvement over the earlier map in Figure 2 both for the number of points mapped as well as the increased accuracy for mapping the location or loci of different traits. (For example note that the FM (flesh adhesion or clingstone/freestone trait) locus in Figure 2 is more accurately placed in the lower portion of chromosome 4 (G4) in Figure 4). Note also that FM locus is very close to endoPG or the endopolygalacuronase gene location (or loci) providing the first solid evidence that endoPG may be determining the freestone/clingstone trait. [Subsequent ‘candidate gene’ analysis, summarized in earlier annual

reports and described in the later brown rot resistance sections of this report, allowed us to identify different forms of endoPG and to effectively use them as predictors of flesh adhesion type without the need to analyze actual fruit. Similar analysis identified LAD (Leucoanthocyanidin dioxygenase) in the middle of G5 to be very closely associated with undesirable flesh browning in peach]. Because genetic mapping is a statistically-based method, the location of the targeted gene is given not as a precise map point but as a probability over certain sections of the chromosome or linkage group. With increasing markers and/or increased replications of the data, the predicted gene location becomes more precise. Figure 5 shows such a probability map for traits identified in Figure 4 but now showing the zones which most likely contains the locus based on two years of consecutive data. To develop even this general map, approximately 1500 molecular markers (mostly SSRs but also RAP and RAFs) were generated and mapped for two separate processing peach breeding lines (Figure 6). Genetic variability was developed in the first breeding line by incorporating genes from almond (as a great grandparent) in a cross to *Dr. Davis* (Figure 6-top), and in the second breeding line by crossing *Dr. Davis* to the old heirloom variety *Georgia Belle* (Figure 6-bottom). To determine the final gene order, including the proximity or linkage between traits, all maps were aligned against a standard almond by peach consensus map known as the TxE map. Even these general maps provide considerable information. Loci that are close together (such as mealiness and bleeding on G4 in Figure 5) may represent closely linked genes or different expressions of the same gene. The freestone/clingstone (FM) loci is also at this location and it is known that stone adhesion has a strong effect both on flesh mealiness and color bleeding in the pit, suggesting that these represent distinct or pleiotropic effects of the same gene.

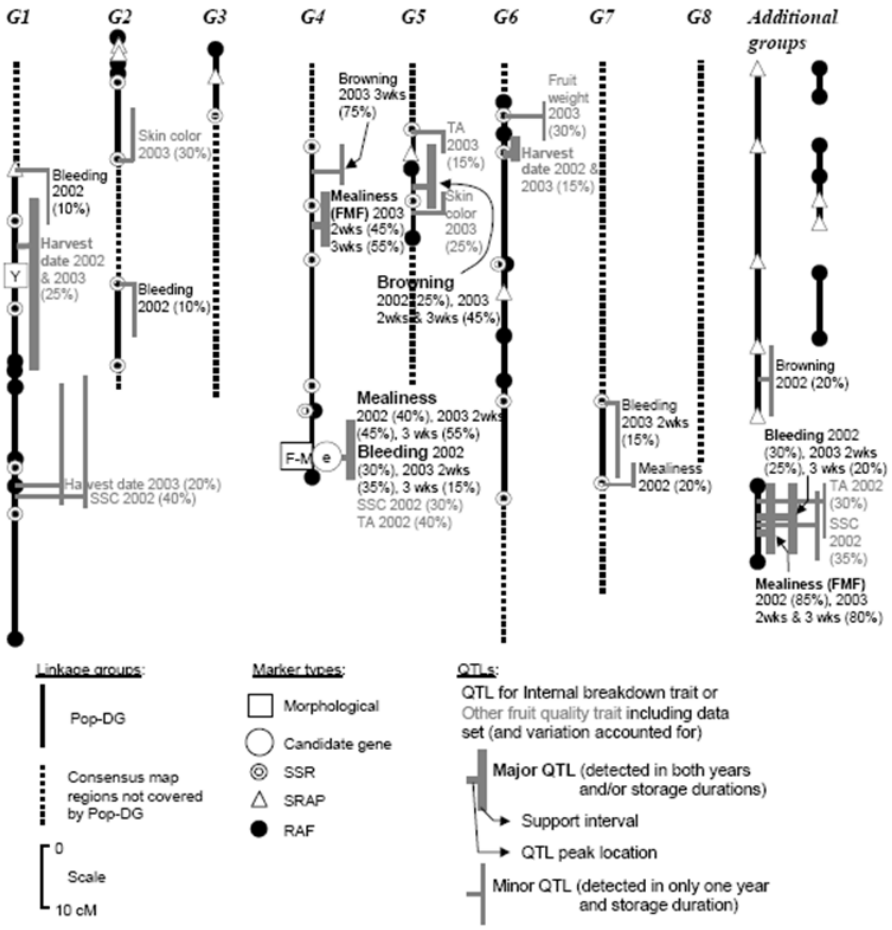

Figure 5. A partial genetic map of processing peach with fruit quality QTLs. The linkage groups are shown overlaid on the eight consensus Prunus linkage groups.

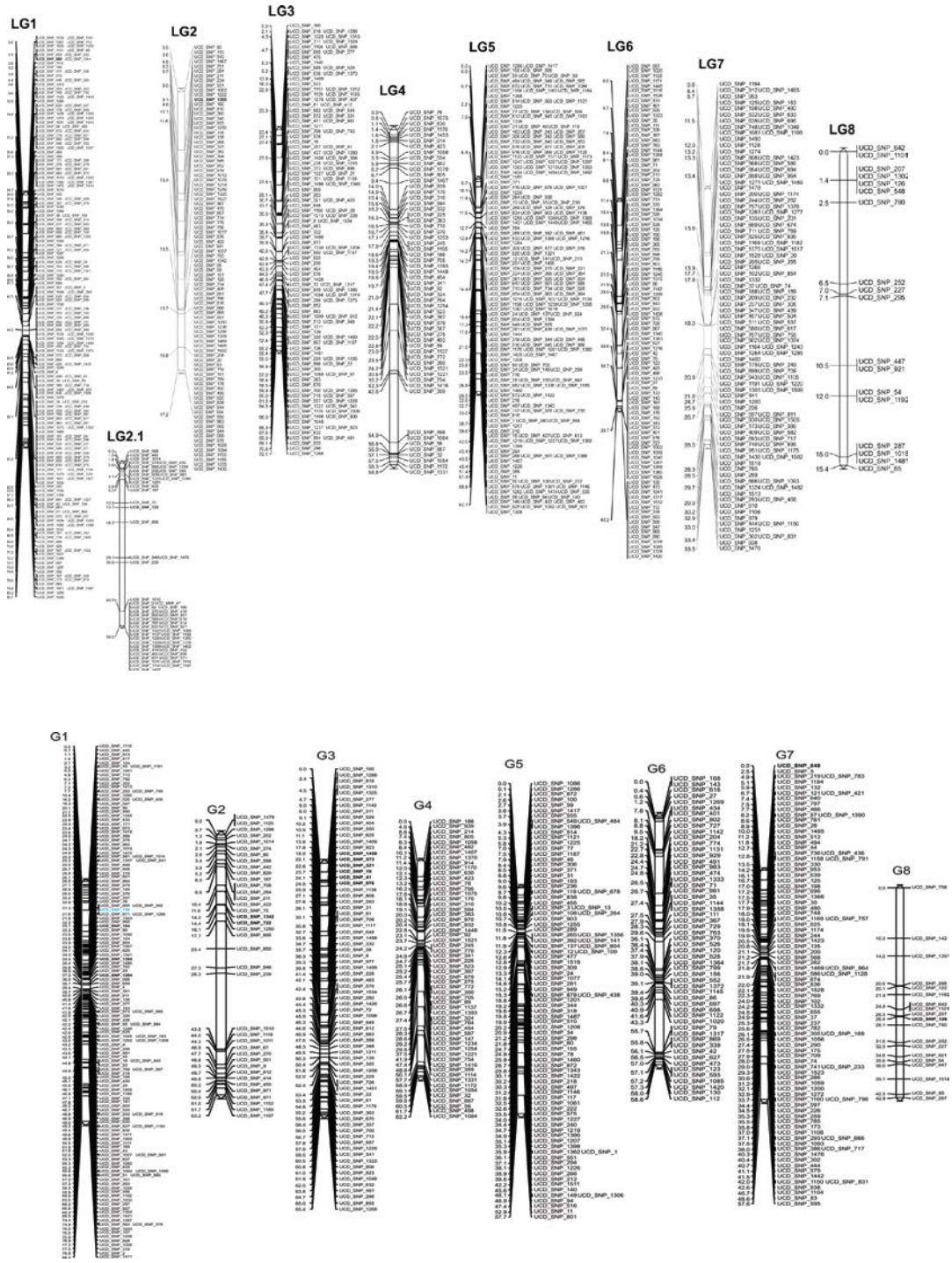

Figure 6. Medium-high density UCD molecular marker maps for peach by almond (top) and peach by heirloom peach (bottom) processing peach breeding lines.

RosBreed marker development.

The first RosBreed project was a multi-state, multi-crop project which by consolidating marker development for a range of closely related tree crops in the Rosaceae family could develop large numbers of markers much more cost-effectively. The statistical methods for marker development in RosBreed were also more powerful for detecting effective markers because they used the known pedigrees of the different breeding lines to more accurately predict markers associated with the desired traits and lineages. In addition, RosBreed developed more intuitive molecular tools to allow the crop breeder improved capacity for evaluating his breeding material. This is demonstrated in Figure 7 where the inheritance of markers Q and q [representing, in this case, two different forms (or alleles) for a gene controlling fruit size] are plotted based on known breeding pedigrees while also displaying color-coded data for fruit size (light violet for small fruit to dark violet for large fruit) as well as critical marker contribution (orange versus blue colors and bar charts). This approach not only offers more statistical power compared to traditional methods (which looked primarily at the degree of correlation or association between different molecular markers and the targeted trait in segregating populations), but also provide the breeder with improved understanding of his breeding material. For example, Figure 7 shows how the incorporation of almond germplasm (*Nonpareil*) contributed small fruit size to its progeny but also a diversity of unique molecular markers as well as modifier genes which facilitated the detection and mapping of an allele for large fruit size inherited from Dr. Davis through Goodwin. This allows better breeding decisions to be made since marker value can be determined lineage by lineage. This more precise analytical tool has also clearly demonstrated that, despite the general 'one molecular map fits all' approached to marker assisted breeding, marker value depends upon lineage. (For example in Figure 7,

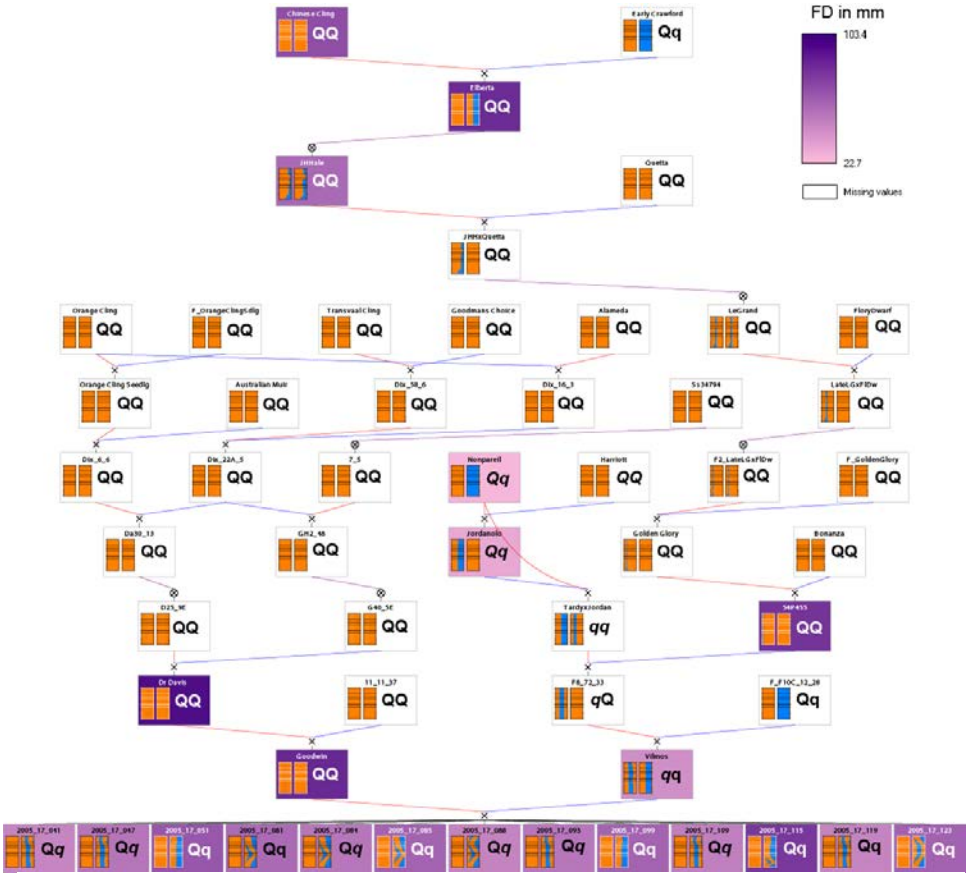

Figure 7. Using breeding pedigrees to better determine trait inheritance from different lineages.

components for improved fruit size may have existed within traditional peach germplasm but cannot be detected and/or enhanced until the inclusion of more exotic almond germplasm). The importance of pedigree to the final value of different markers for assisting breeding selection is demonstrated in Figure 8. In this analysis of fruit size using the pooled total RosBreed data (including data from the University of Arkansas, California (UCD), Clemson-South Carolina, and Texas A&M) a positive breeding value for a fruit size loci identified on linkage group/chromosome 6 could only be identified for UCD pedigrees.

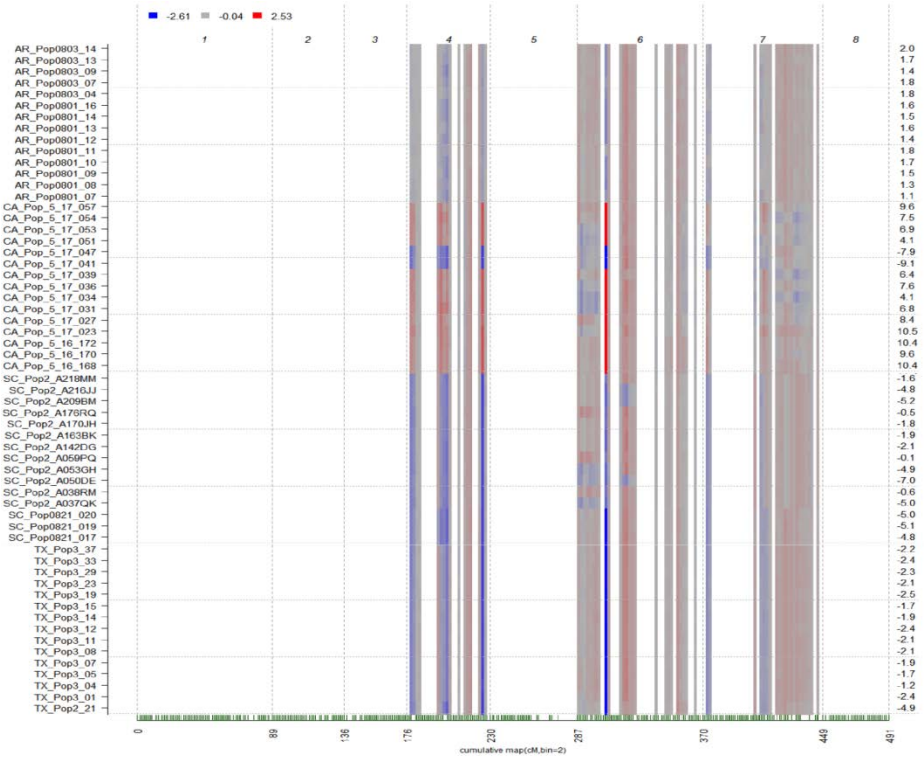

Figure 8. Calculated Genomic Estimated Breeding Values (GEBVs) for fruit size in 2011 data from representative progenies for the four peach breeding programs involved in RosBreed. Gradual coloring intensity in blue, gray and red colors indicate negative, intermediate and positive breeding values for the chromosomal segment showing positive evidence for effective molecular markers. Number on the right represents the accumulated genomic breeding values per accession.

This particular pedigree was unique because the great grandparent was almond (as charted in Figure 7). Note also that while the total accumulated breeding value (in the right-hand column of Figure 8) was uniformly low for most breeding lines, the UCD material showed exceptional variability ranging from almost -10 to over +10. Results support a stronger contribution to fruit size in this particular lineage by the specific allele tagged by the marker. [The specific allele (Q or q) could be determined either by general pedigree analysis (as shown in Figure 7) or even more precisely by identifying the specific allelic genotype and so protein for that locus (as shown in Figure 12).

Differences in marker effectiveness among different pedigrees appear to be the result of different genetic backgrounds (i.e. other genes present can either enhance or suppress the final effectiveness) as well as differences in genomic or structural conditions (for example, differences in chromosome structure can enhance or suppress final effectiveness). The importance of the specific genetic background to final gene expression has been well documented for affecting genes and are known as modifier genes. The importance of genomic or structural differences is less well understood but appears to be particularly important in germplasm derived from interspecies crosses. When specific marker locations for the two UCD processing peach breeding populations (DF and DG) shown in Figure 6 are compared (Figure 9), most markers lined up at similar (though not necessarily exact) positions on the reference chromosome (blue bar). Most

linkage groups (LG or chromosomes), however, often also displayed lines going distinctly against the pattern indicating that for these sites chromosome rearrangement (such as inversions or translocations) had occurred (as for A versus 'a' in LG 2). Such rearrangements both change the location (and so interactions) of the mapped gene as well as change the general capacity for gene expression at those sites were structural changes occurred. [In some cases dormant genes can be activated or normally active genes are suppressed]. Although such large-scale genomic or chromosomal rearrangements can be used to enhance or suppress desired gene expression, they

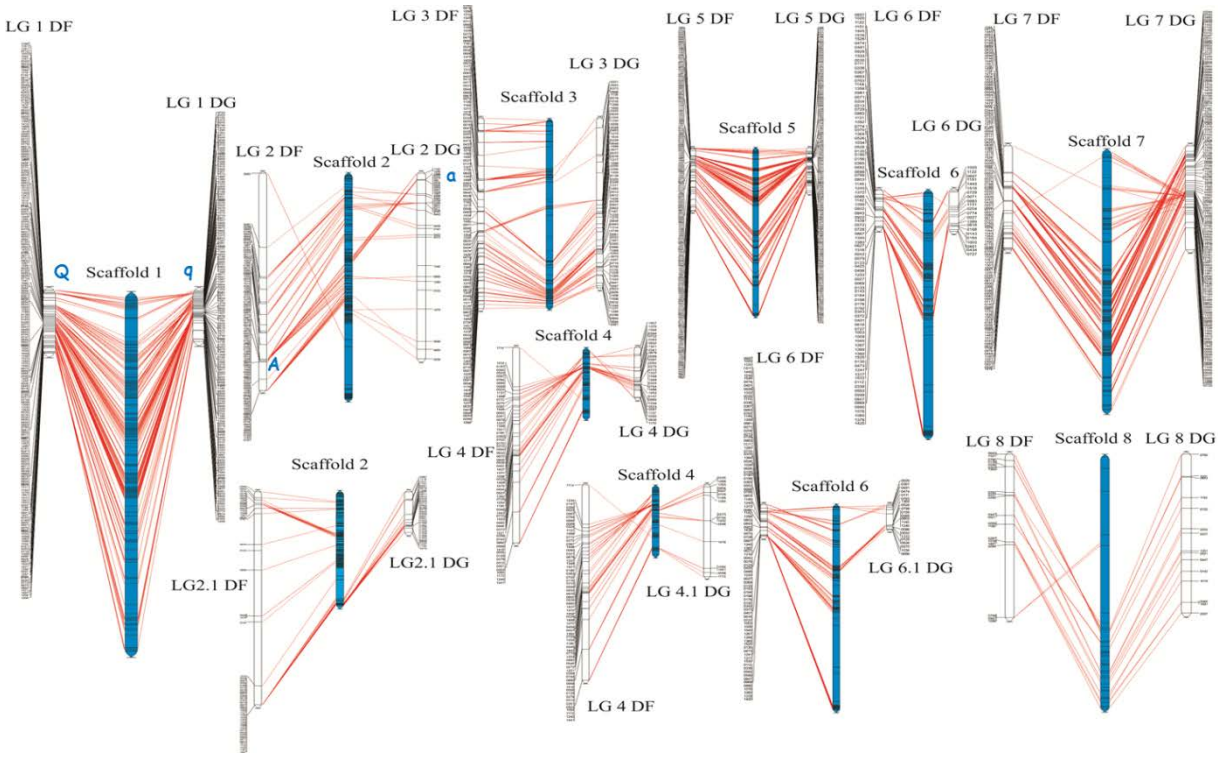

Figure 9. Alignment of markers from UCD processing peach breeding populations DF and DG against the TxE reference (blue bar). While most markers (for example, Q and q on LG 1) show similar alignment, other markers (such as A and a on LG2) showed distinctly different locations demonstrating the widespread occurrence of chromosome rearrangements particularly in interspecies derived breeding lines.

have rarely been used in most (primarily seed propagated) crops because of their infrequent occurrence and unpredictable transfer through seed propagation. In clonally propagated crop such as peach, their stability can be maintained through asexual propagation and the frequency of occurrence can be increased through interspecies hybridizations as occurred with breeding population DF (as plotted in Figure 7).

Genomic changes associated with interspecific hybridization can sometimes also have more dramatic changes. A unique marker segregation in in advanced UCD processing peach breeding line derived from an initial almond by peach interspecies hybridization is shown in Figure 10 where colored dots in the top row represent different markers at each chromosome (colored bars at top and numbers at bottom) for the interspecies derived parent. As expected, there is a high variability in marker (color) type. Each of the 50 rows below the top row plots the markers for selfed progeny. Although standard Mendelian segregation would dictate that markers should segregate randomly in each self-progeny row (much as with reshuffling and re-dealing a deck of cards) very little recombination is actually detected with the few instances identified by

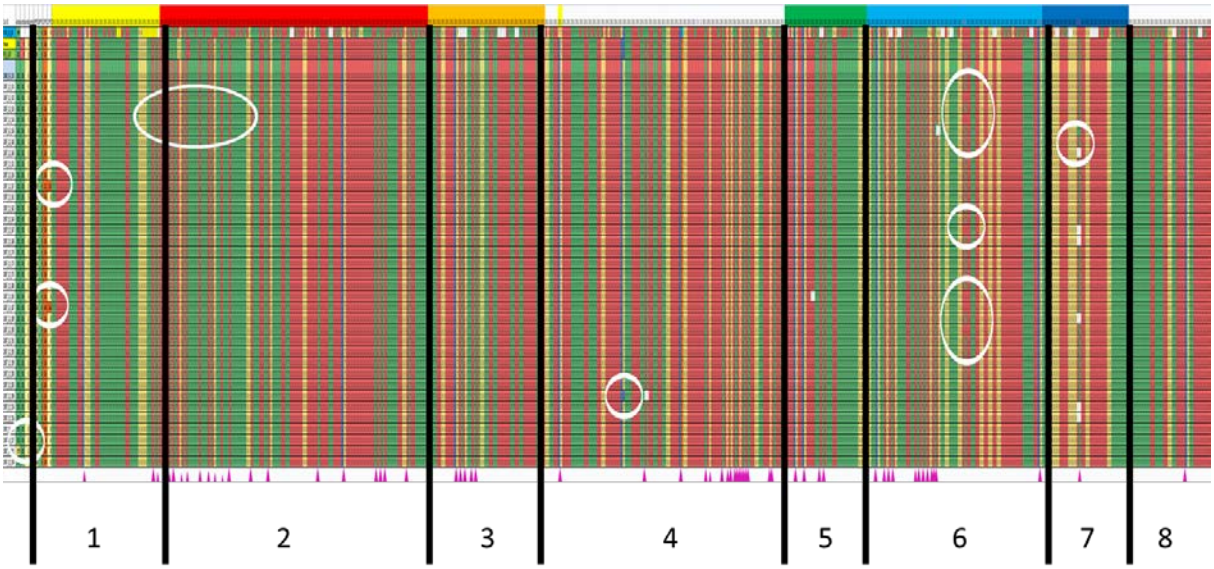

Figure 10. Failure of marker segregation among the eight linkage groups or chromosomes of peach (top color bar and bottom numbers) from a peach parent derived from an almond by peach interspecific hybrid [top row of colored dots: each dot representing a specific marker]. Each row below the top row represents markers for an individual selfed progeny. Extremely few recombinants were detected (white circles) demonstrating large-scale suppression of marker expression through unknown mechanisms.

encircling in white. While the mechanism involved is still poorly understood, it suggests an opportunity for large-scale chromosome manipulation such as the general suppression of all chromosomes from one breeding parent (which has been demonstrated in some highly studied plants such as Arabidopsis and maize). The chromosome structure is actually very complex with many regions known to possess few active genes yet which nonetheless appear very important in some trait expression. It is much more difficult to disassemble and reassemble components of chromosome structure then it is to segregate out, and recombine genes within the DNA it contains and for this reason we know much more about DNA structure and behavior than of the chromosome within which it resides. Observations like this also cautioning against routine interpretation of marker data when interspecies crosses are involved. Unfortunately, because of the very large numbers of complex data analyzed, most mapping is done by powerful computer software and results printed out often without a clear understanding of what was actually analyzed. For example, since this population of over 40 individuals represents a large proportion of the 350 UCD individuals analyzed in the first RosBreed project and even the approx.. 1000 total individuals evaluated by RosBreed for peach, it's totally aberrant segregation pattern should have raised concerns of a possible problem. The statisticians involved in the final RosBreed mapping were not even aware of its presence, when they were made aware, did not seem concerned. Part of the reason is that the more important research product for many of them (i.e. basis of promotions, etc.) is publications rather than valid markers.

Molecular marker map utilization in UCD Processing Peach Variety Development.

The most current iteration of the UCD peach molecular marker map is presented in Figure 11, showing proposed location for traits considered important for processing peach. Improved knowledge concerning the location of targeted traits (usually molecular markers strongly associated with those traits) allows their more effective manipulation in breeding programs. An

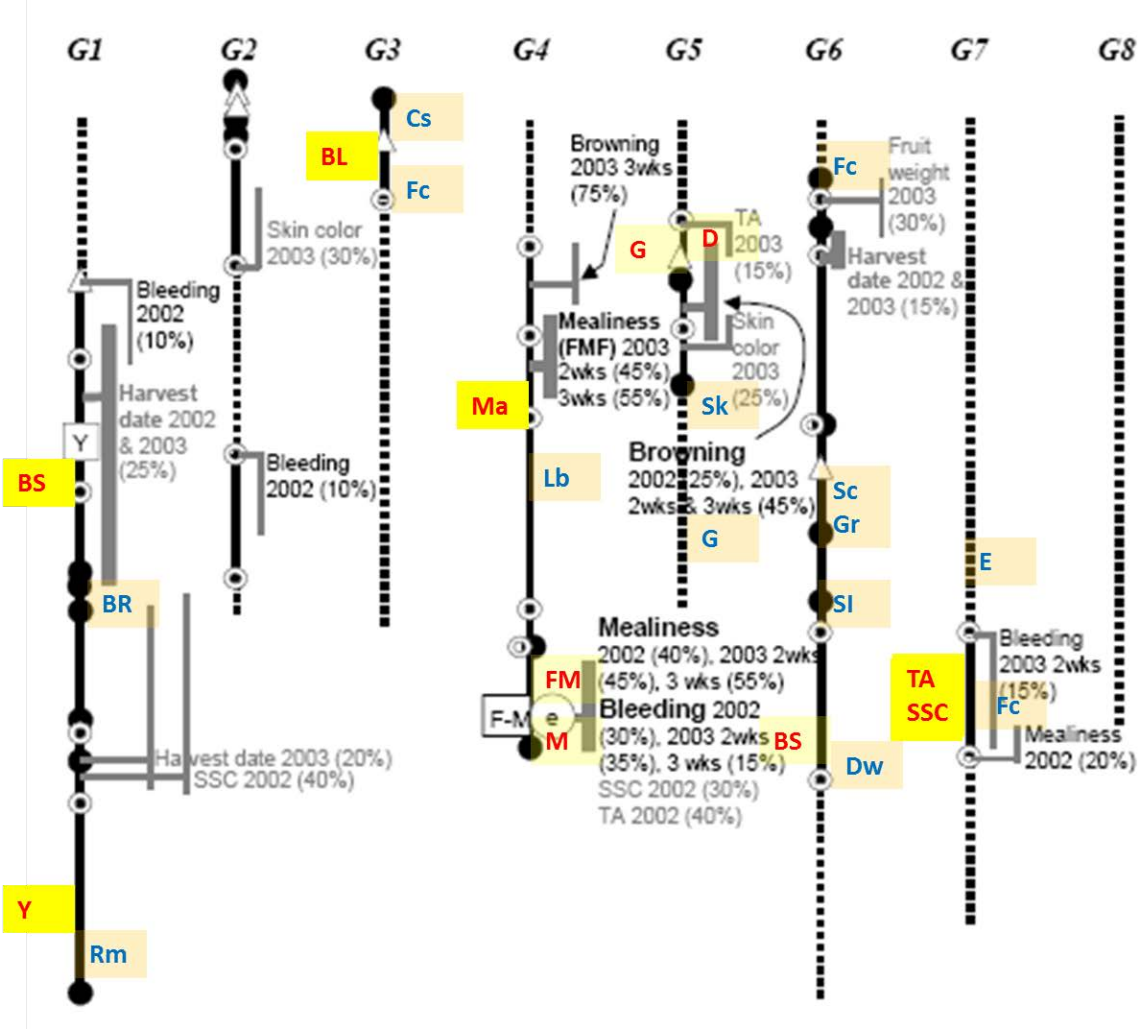

Figure 11. Current iteration of the UCD peach molecular map. Yellow boxes show previously established markers, tan boxes show more recently established markers while black text identify proposed location for new traits.

advantage frequently touted is the ability to do selection at the seedling stage. However, as demonstrated by the previous examples, the final level of expression still depends on the genetic/genomic background and thus can only be determined by assessing field performance. In addition, unique interactions involved with the diverse germplasm utilized, can sometimes result in unique or novel expression of traits which may be commercially useful. Again, the only way to ascertain this would be through actual field evaluations. If the breeding program was highly focused for a trait such as disease resistance considered essential for commercial sustainability, then the focused selection for just one or a few traits at the seedling stage has advantages. However, when cultivar success is dependent on high performance for a large number of traits (productivity, quality, resistance, etc.), the breeding program does not have the luxury for such focus but instead has to pursue the rare individuals containing high-level performance for all required traits. Having effective markers for critical traits still has advantages as it allows a better understanding of the inheritance of the targeted trait as well it's interactions. Interactions could

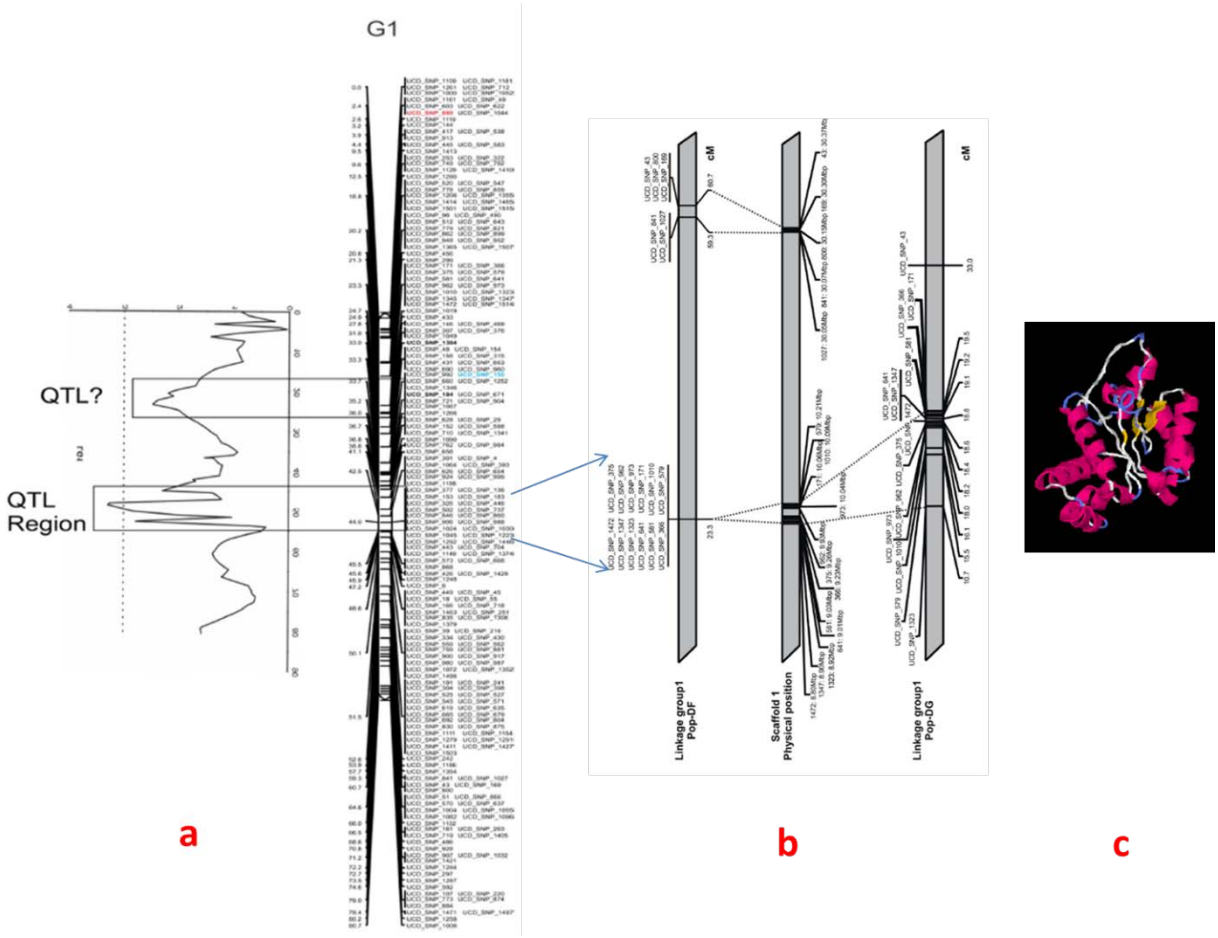

Figure 12. Fruit brown rot resistance marker identification and candidate gene characterization. Statistical procedures identify high correlations between individuals displaying high brown rot resistance and possessing specific molecular markers (a) [this marker can now be used as a general indicator of the presence of the desired form of the gene in marker assisted selection]. The DNA strand at the section identified by the markers is sequenced and this unique sequence is then compared against established databases for known plant genes to identify possible functional genes. Genes within the targeted site which have been reported to have similar resistance function are then selected as candidate genes. Information available from extensive genetic databases can provide a range of data from putative gene function to protein structure (c) further informing the breeding process.

include different gene loci affecting the same trait, such as combining or pyramiding genes for low flesh browning from both G4 and G5 in Figure 11. The exploitation of such interactions could also facilitate desired trait combinations such as improved acidity (TA) and soluble solids (SSC) located at the basal portion of G7. In some cases the interactions identified may be conflicting as with the association of the freestone melting (FM) trait at the basal portion of G4 with both mealiness and flesh color bleeding (and to a lesser extent with SSC and TA levels). This association may be the result of a close linkage of separate controlling genes or it may indicate *pleiotropic* or multiple effects of the same gene. For example, the expression of both mealiness and flesh color bleeding is highly dependent on the texture of the flesh (for example mealiness is much more common in melting flesh than clingstone non-melting flesh). Here the association may be due less to different genes than to the presence of the FM freestone-melting allele which promotes the condition. Larger numbers and higher resolution of markers promised by the second RosBreed project would go a long way to clarify many of these issues. In addition,

knowing the general location for a trait allows much more focused research into identifying the specific genes controlling that trait and even its mechanism of action. This is demonstrated in some of our recent research with brown rot resistance in processing peach as diagrammed in Figure 12. This research identified sections in the central region of chromosome 1 (G1) which were associated with higher levels of fruit brown rot resistance (that is breeding progeny showing high levels of resistance also had a significantly higher occurrence of these markers). The molecular markers which were particularly highly correlated with this high resistance can now be used as markers for the presence of the still unknown gene. In addition, the cost of sequencing DNA has now decreased to the point that the same markers can be used to isolate DNA from this region which is then sequenced for its specific DNA code. That code is then compared against huge databases established from other plant and even animal and human research to identify similar DNA sequences as well as the specific proteins that they code for. Proteins identified in this manner, which have been shown in other biological systems to have the type of function or activity associated with disease resistance (in this case) can then be tagged as likely candidate genes responsible for the observed resistance in peach. Once identified in this manner, the particular candidate can be tested since the researcher now knows rather precisely what to look for. [The ideal test would be to use genetic engineering to knock-out the candidate gene and verify that the trait expression is also knocked-out, though this powerful tool is not available to peach genetics because of our inability to genetically engineer this or related species].

In summary, molecular-based marker assisted selection (MAS) is often touted as having the capacity to identify genes that will have a major impact on breeding success. (For example the advertised objective of the RosBreed projects is to find “the gems in the genome”). For most applied breeding programs, genes with such a pronounced desirable effect have already been selected owing to their major effect and so relative ease of detection. MAS is particularly valuable for traits which have a smaller but still sizable contribution. Traits controlled by 1 to 2 major genes are relatively easy to detect and select by traditional methods. Traits controlled by 3 or more major genes are much more difficult. MAS is particularly valuable for traits controlled by 3 to 5 major genes (for example, if 4 genes combined would give good field resistance). Currently, many of our most challenging traits in the processing peach breeding program (including fruit brown rot resistance, the once-over harvest ability, delayed fruit deterioration following the full ripe stage, as well as certain environmental stress tolerance) appeared to be in this 3-5 controlling gene range. These are also the type of traits for which the next round of RosBreed analysis will be particularly powerful at characterizing. This improved capacity for molecular analysis comes at a particularly opportune time for the processing peach breeding because we are now entering a final phase of new germplasm-to-new cultivar development program and so we have large populations of relatively well-adapted peach genotypes which will greatly facilitate the identification of those genes with the most promising real-world commercial value. While effective markers for such genes appear to have the potential to significantly increase breeding success on their own, they also open the door to discovery of extensive and detailed information concerning the genetic control of that trait, including its potential value in different genetic and environmental backgrounds. However, despite the opportunities of MAS to identify, tag, and recover useful breeding traits, our experience has clearly shown that be truly valuable these markers and their underlying data have to be thoroughly validated by careful genetic and field performance testing. Five years from now, we should be much more knowledgeable concerning the genetic basis (as well as our capacity to select) desired traits, but successful fruit cultivar breeding will still be a largely field-based endeavor.

Recent Relevant Publications

Martínez-García P. Fresnedo-Ramírez J. Parfitt D. Gradziel T. Crisosto C. 2013. Effect prediction of identified SNPs linked to fruit quality and chilling injury in peach [Prunus persica (L.) Batsch]. Plant Molecular Biology: 81:161–174. DOI 10.1007/s11103-012-9989-8.

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