California Cling Peach Advisory Board
2012 Annual Report

Project Titles: Development of New Cling Peach Varieties

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Location: Dept. of Plant Sciences, Univ. of California at Davis

Objectives:

A. Generate 5,000 new seedling progeny trees through controlled recombinations primarily through cross-hybridization targeting high productivity with reduced grower and processor costs.

B. Evaluate breeding populations from 2008-2011 plantings for desired traits with greater development and use of lower-cost, higher-throughput field evaluation techniques.

C. Critically evaluate the potential and current limitations of molecular based marker assisted selection/breeding using standard as well as novel approaches with a greater emphasis on crop yield potential.

Summary:

Major cutbacks in University funding during the last several years have driven dramatic changes in field practices to minimize breeding costs. Recently implemented changes, which include high density, high fertilizer-water management plantings and greater mechanization of transplanting, pruning (by mechanical hedging), weed control (through precision mowing and herbicide application), thinning (Darwin mechanical thinner), propagation (by winter budding onto potted rootstock), and tree removal (herbicide and bulldozing), target a more consistent, and so efficient evaluation of seedling tree breeding value while minimizing hand labor costs. The effectiveness of these changes has allowed the processing peach breeding program to meet and usually significantly exceed targeted breeding population sizes with over 40,000 seedling trees currently in various stages of evaluation (Table 1). [In fact, current average breeding size is larger than the pre-budget-cut era. The dependence on hand labor not only was limiting budget-wise but also time-wise, putting a lower ceiling on manageable population size.] The 2012 targeted breeding population of 5,000 seedling progeny has been met and exceeded, though because of industry funding cutbacks and our already tenuous UCD budget, extra seed was not planted but carried over to the 2013 season. The greater selection efficiencies required to accurately evaluate these larger populations is being achieved by targeting only the clearly elite seedling trees for further data collection and UCD pilot plant cannery test-processing. This was not feasible in earlier stages of the breeding program because the emphasis on incorporating new germplasm possessing

desirable traits (though usually poorly adapted to California growing conditions), required compromising final field and processing quality with desired trait value (such as improved resistance to fruit brown rot). Because the resulting diverse and relatively large peach breeding populations also represent ideal test material for molecular marker-based genetic analysis, many of our advanced breeding lines were included in the multimillion dollar SCRI funded RosBreed genetic mapping project at no cost to the program. Initial analysis from the last three years of detailed RosBreed data indicate that molecular-based trait markers presently have important but limited applications for the development of new commercially viable processing peach cultivars. [See 2011 Annual Report for a comparison of breeding approaches]. Ironically, a major limitation may result from the incorporation of novel germplasm, as its novelty is often missed by the RosBreed pedigree analysis approach which depends on commonality among many breeding lineages for effective detection of horticultural with molecular marker associations. These novel traits, (which include improved fruit firmness, texture and tree architecture, as well as disease, pest and environmental stress resistance), however, often appear to offer the most promising opportunities for cultivar improvement. Breeding strategies will need to combine proven traditional population performance-based approaches combined with largely predictive molecular-based methodologies to maximize future selection efficiency. Early strategic decisions by the breeding program decisions to expand the genetic base and so genetic options has identified novel options that may satisfying traditional field and processing needs while allowing significant reductions in production costs. These improvements include improved resistance to fruit brown rot, reduced pit fragments/color defects and so higher case yields, the capacity for efficient once-over (hand or mechanical) harvest, and opportunities in the *Compact*-cultivar series to reduce thinning, pruning, harvest and maintenance costs. Three advanced UCD selections (*Ultra-Early#1 {pre-Loadel}, Extra-Early#1 {Dixon-Andross} and Extra-Late#1 {Starn-Corona}* are currently being prepared for patenting and release to the industry and represent important advances in these key breeding objectives. The next

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	6,000+
2014	8,000	

Table 1. Proposed vs. actual UCD processing peach breeding populations.

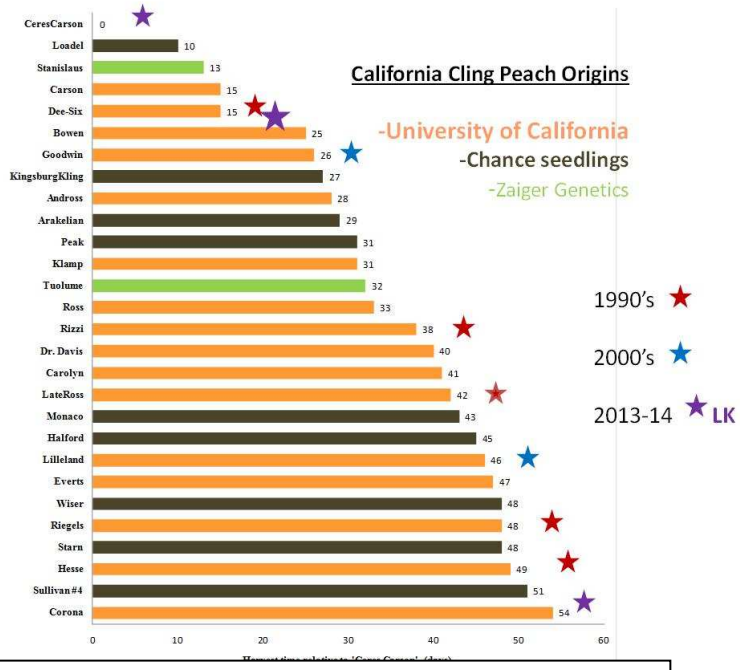

Fig. 1. California processing peach harvest times and origins. (Stars identify recent UCD releases).

generation of cultivars, including the *Compact-tree* series will offer greater opportunities for production efficiency but only if coupled with novel field practices.

Processing Peach Breeding Program Summary-2012.

The initial goal of the breeding program has been cultivar replacement, primarily within the *Dixon-Andross* and *Halford-Corona* maturity season (**Figure 1**). In addition, opportunities for season extension are also being pursued, particularly the Ultra-Early season before *Loadel*, and to a lesser extent, extending the season beyond *Corona*. More recent goals include the breeding of processing peach cultivars requiring lower inputs of labor and agrochemicals, including fungicides. Towards this goal, advanced, California adapted breeding selections have been developed with improved fruit brown rot resistance as well as more compact tree size with more manageable tree architecture (as detailed in previous reports). Efforts have also been directed towards developing breeding lines maintaining good fruit quality and low fruit drop both at the full-ripe stage and up to 3 weeks thereafter. This on-tree maintenance of fruit quality allows additional time for under- ripe, undersized fruit to continue to full-ripe stage, contributing to greater yields, as well as allowing a once-over harvest (manually or mechanically) of all fruit.

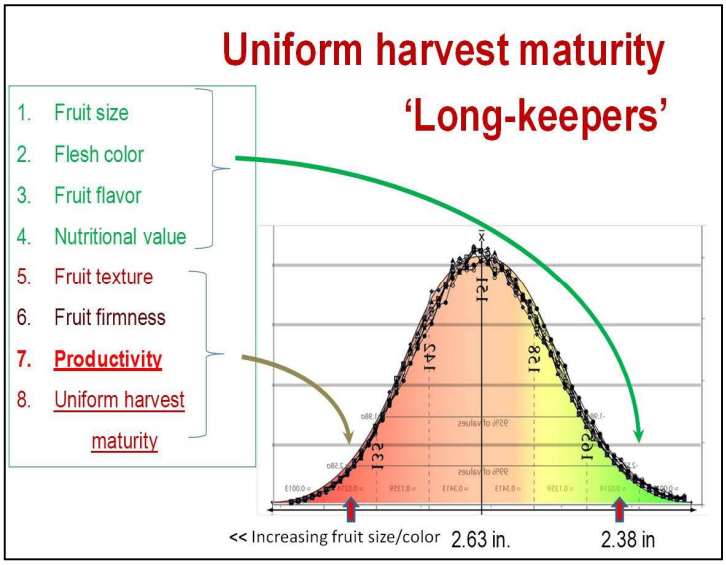

Fig. 2. Fruit ripening plot identifying advantages of later harvest times.

*This objective is demonstrated in **Figure 2** where the proportion of fruit of different sizes and maturities are plotted for a typical tree at harvest. Because fruit in different sections of the tree will develop/ripen at differing times depending on different exposures to heat, light, etc., the fruit size and maturity at any one time which approach a normal or Bell-shaped distribution. To maximize yield, the grower harvests when the majority of fruit are ripe and before many of the first-to-ripen fruit become overripe (left Bell-edge on chart) even though many fruit have not yet achieved the 2.38 inch minimum and so are too small, too green, and too hard for processing. In 'Long-Keeper' (**LK**) varieties the natural development to the over-ripe stage is delayed for a week or more allowing immature fruit to develop to maturity. Besides allowing a single harvest, such varieties would lead to overall improvements in fruit size, color, texture and so productivity as well as a general improvement in nutritional value.*

Advanced California adapted selections expressing this LK trait and, in many cases, improved fruit brown rot resistance, have been bred from numerous, diverse sources including germplasm from Brazil, South Africa, China, as well as related species including almond (*Prunus dulcis*) and many of its wild relatives (including *P. webbii, P.*

scoparia, P. argentea, and the wild peaches *P. davidiana* and *P. mira.* (**Figures 3 and 4**).

Figure 3 *shows advanced processing peach selections derived from these diverse sources as part of the early breeding program objective to incorporate new germplasm. Improved levels of fruit brown rot resistance have also been incorporated in UltraEarly, Extra-Early, and Extra-Late selections. Selections in red font, including Ultra-Early#1, Extra-Early#1 and Extra-Late#1 are currently being prepared for patenting and release to the California industry. Release decisions on the remaining items are expected in the next four years. Compact selections resulted from the selection of a bud-sport mutation within more traditional California breeding material. The compact trait is expressed as an approximately 60 percent reduction in tree size while retaining near-normal leaf and flower bud densities. Improved fruit texture and a limited 7-10 day long-keeping ability is also expressed in these lines. While having the potential to dramatically reduce orchard thinning, pruning and harvest costs, commercialization of this trait will require novel cultural management techniques. Early results from regional trials are described in the 2012 Regional Testing report. Almond has also proven a valuable source of genes for long-keeping ability; fruit brown rot and possible late season extension (see Figures 4 and 16 for breeding lineages).*

Fig. 3. Advanced processing peach selections derived from diverse sources and possessing novel traits.

Flowchart in ***Figure 4*** *provides both an overview as well as detailed relationships among important UCD lineages at the start of the RosBreed project in 2009. [Many additional lineages are not included as most generally represent exploration of yet unproven germplasm sources.] Sources of post-ripe fruit integrity (long-keeper [LK]) have been independently transferred from Brazilian and South African varieties and related species including Prunus argentea, P. mira and P. dulcis (almond). Additional sources of disease resistance and improved fruit and tree quality have been transferred from European and Brazilian peach varieties, P. davidiana, P. scoparia, and P. dulcis. Several of our most advanced breeding lines (not yet added to flowchart) have incorporated traits such as fruit LK from multiple sources, as breeding experience has shown improved performance and improved stability over years and locations when multiple, diverse sources were combined. Similar results have been found for disease and pest resistance. This extensive and diverse lineage has proven particularly useful for the RosBreed project as it attempts find consistent associations between specific traits and specific molecular (DNA-based) markers through pedigree-analysis (i.e. analyzing both progeny as well as parents, grandparents, etc. for common trait-DNA associations). The association is a consequence of that particular trait being coded for either by that particular DNA, or, more commonly, by DNA physically close enough to the controlling DNA to be used as a marker of its presence and identity. RosBreed also leverages the common genetic origins the rosaceous crops (peach, cherry, apple and strawberry) by searching for common trait-DNA associations among these related species. See website for additional information.*

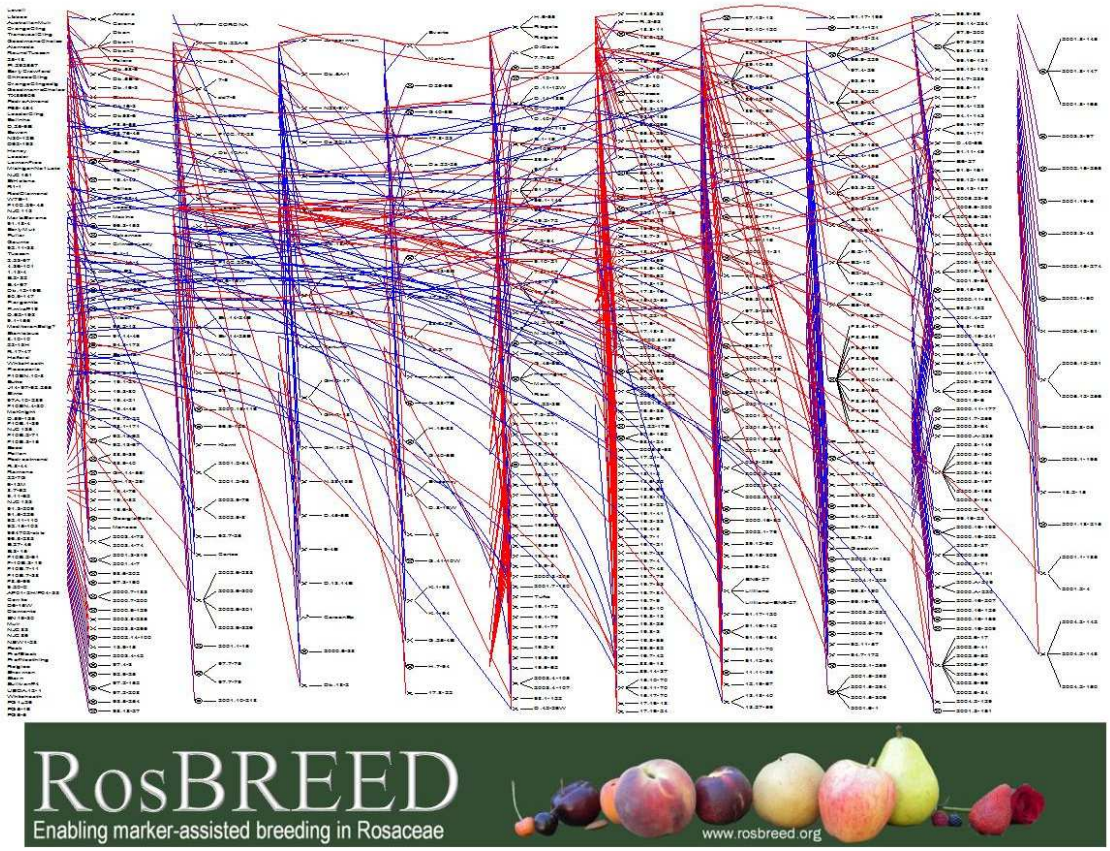

Fig. 4. Important UCD breeding lineages and relationships at the start of the RosBreed project.

Because virtually all current domestic peach cultivars, both fresh market and processing, are derived from as few as 4-6 original Chinese selections, the genetic variability in the early 1990's was very limiting for both breeding improvement as well as molecular-marker-based analysis (i.e. because of the high level of genetic uniformity there were relatively few genetic differences to map). Consequently the genetically very diverse material developed by the UCD processing peach breeding program was included as a major component of the RosBreed peach molecular marker development project. Over the last three years, detailed characterization of fruit and tree quality for almost 400 UCD processing peach breeding selections have been collected and analyzed for specific associations with specific DNA-based molecular markers which were simultaneously developed for these individuals as well as peach breeding lines from Texas, Arkansas and South Carolina (see RosBreed.com website). This combined data is currently being analyzed using sophisticated software and powerful computer processors to identify possible trait-marker trends. The direct selection of these markers (using still expensive but increasingly less expensive DNA-based diagnostics) may offer a more efficient method to select for traits expressed late in plant development (i.e. selecting for final fruit color at the seedling stage), traits controlled by a relatively small and so otherwise hard to distinguish genetic effects, and traits where the genetic expression is variable depending upon the environment in which it is grown. At UCD,

we are also analyzing our data at a more detailed level in order to better identify important breeding traits as well as unique DNA-based molecular markers providing information/understanding for the expression and inheritance those traits. (An additional though frequently overlooked value of the RosBreed exercise is a more intimate understanding of the genetic opportunities and limitations of our different germplasm sources, as well as a better understanding of the importance of different traits to final cultivar value). As examples, the inheritance patterns of several important processing peach traits are summarized in the following plots to better understand the limitations and opportunities of the extended UCD germplasm.

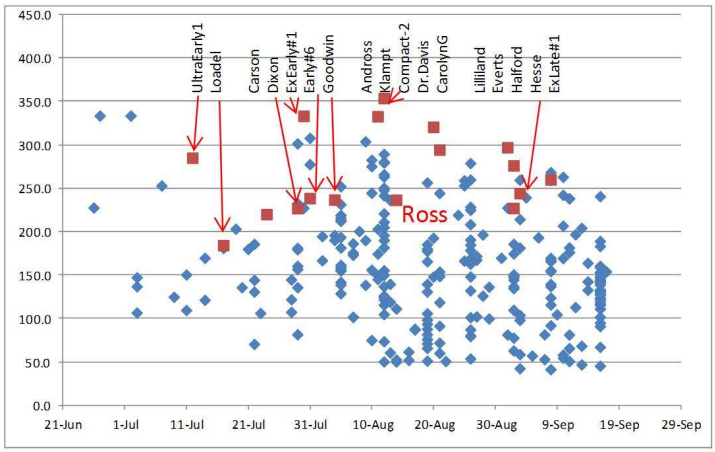

Fig. 5. Maximum potential fruit weight (g) relative to fruit ripe date for different UCD RosBreed selections.

Maximum potential fruit weight (in grams, achieved by flower thinning to 1 fruit or less per meter of branch) for different UCD RosBreed project selections is plotted in **Figure 5** relative to fruit ripe date. Although a wide distribution of fruit size is evident, all commercially established cultivars analyzed are consistently located at the higher fruit masses above 200 grams even though the minimum acceptable fruit size for canning peach is 60 millimeters or approximately 80 grams. [Typical diameters for 200 gram fruit are 80 to 85 millimeters while those for the 350 gram fruits of *Klampt* and *Compact-2* is a respectable 10 centimeters]. Although fruit diameters of 10 centimeters would be generally undesirable for processing peach, the ability to aggressively increase fruit size when resources are available (as would happen with over- thinning) is desirable to consistently approach maximum tree yield potential. This capacity for compensatory sizing appears particularly important for *Extra-Early* and *Early* cultivars, becoming somewhat less important for *Late* and *Extra-Late* cultivars (*Ross* and later) where the extended season may allow more opportunities for crop compensation. Most UCD advanced selections show very high fruit sizing potential. *Ultra-Early-1*, particularly distinguishes itself in this area despite its very early maturity. (All other large fruit accessions in this very early maturity season are freestones where varying degrees of pollen sterility contribute to low fruit set and so large fruit sizes). The unique and exceptional performance of *Ultra-Early-1,* which is derived from Brazilian and Eastern European germplasm, highlights the value of incorporating new genetic material to expand breeding opportunities. *Extra-Early-1,* which is also being prepared for patenting and release, also shows exceptional fruit sizing potential under these conditions. The largest sizes of just over 350 grams (or 10 centimeters fruit diameter) were achieved by the high yielding variety *Klampt* and the early season compact tree selection *Compact-2*. A potential downside of high fruit sizing potential may be a loss of flesh firmness with increasing size. Average fruit firmness for tested accessions is plotted against average fruit diameter In **Figure 6**. No strong correlation is evident in the diverse materials

tested and while large-fruit cultivars such as *Dr. Davis* and *Klampt* show moderate flesh firmness, all cultivars are above the 6 lb. standard pressure test level. Two UCD selections, Extra-Late-7 and 92,14-73 show exceptional firmness scores with moderate fruit sizes. Both have been important parents in breeding for good harvest/post-harvest fruit integrity including the LK trait though they need to be matched with a larger fruit size potential parent such as *Dr. Davis*. [The small but firm 'primal peach' shown in Figure 6 is an almond-like wild peach that is generated at very low frequencies within some advanced interspecific breeding lines; see Figure 18].

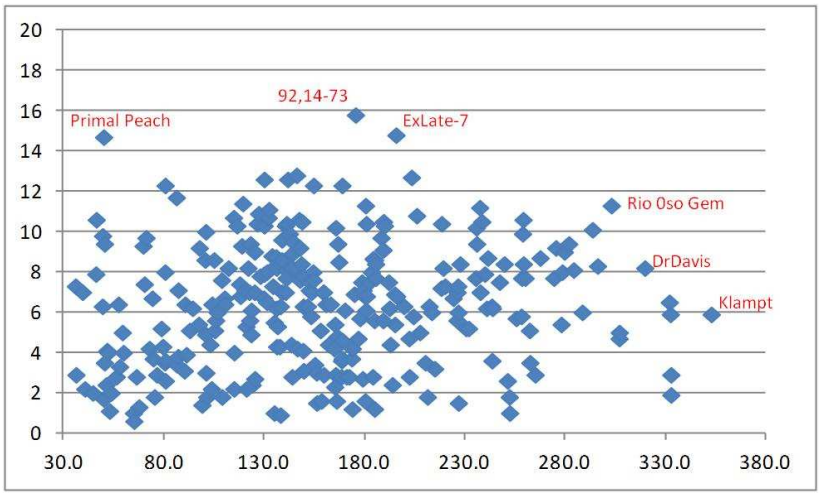

Fig. 6. Average fruit firmness (lbs.) plotted against average fruit diameter (mm).

These multiyear results support one of our accelerated breeding strategies of selecting for aggressive fruit sizing potential within the first two years of seedling tree growth. This is achieved by mechanically hedging high density seedling progeny trees to approximately 7 feet and mechanically thinning until only a few flowers remain on each branch. Subsequent fruit growth will thus not be limited by crop load and will show maximum genetic potential for size as well as quality. Minimum to no hedging/thinning occurs in the second and final field fruiting season, which allows selection for high crop loads combined with good fruit size and quality. For both selection years, selection is made only for those trees which lack any production or processing deficiency (i.e. no irregular or soft fruit, red pits or flesh, splitting, poor branch architecture, etc., etc.). These trees are marked with color coded flagging to identify the ripe date. After 1 week, only fruit still having good horticultural/processing quality are given a second color-coded flag. Finally, only those flagged selections maintaining horticultural/processing quality to the third week are tagged for more detailed sample evaluation and possible test processing.

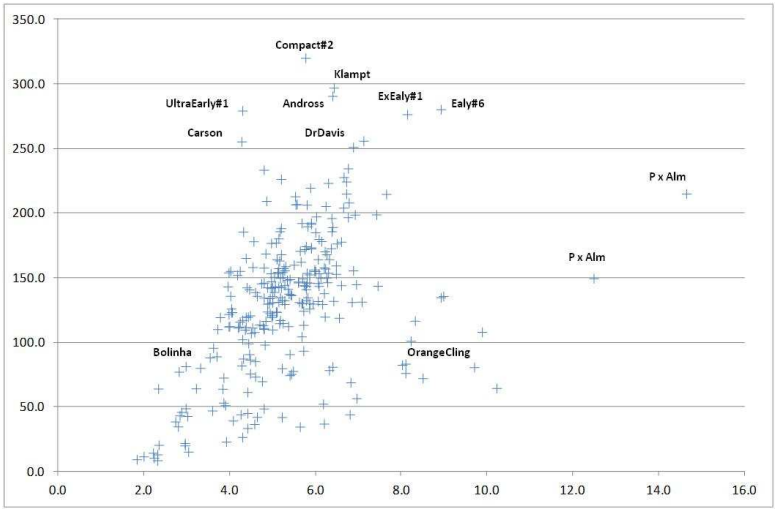

Fig. 7. The relation between fruit and pit weight (g).

The relation between fruit weight and endocarp or pit weight is plotted in **Figure 7**. While the traits are generally correlated, as would be expected, both *Ultra-Early-1* and *Compact-2* show relatively smaller pit size for the given fruit size. This would be desirable as it would result in greater case yields (provided that the less-massive pits do not show a greater predisposition to split-pits or cracking). *Bolinha*, the Brazilian cultivar which has been an important source of fruit brown rot resistance, shows the relatively low fruit and pit size characteristic of unadapted material as does the old founder variety, *Orange Cling* with its relatively large pit and small fruit size. Several outliers with pit sizes larger than *Orange Cling* are progeny from a peach by wild almond interspecific cross. The large variability in pit and fruit morphology in this material has made them useful for studying the role of pit development to subsequent fruit development. Initial findings suggest that fruit mesocarp thickness (and so final fruit size) are closely associated with and perhaps determined by the volume and architecture of vascular bundles embedded within the lignified endocarp (pit) which 'feed' the mesocarp.

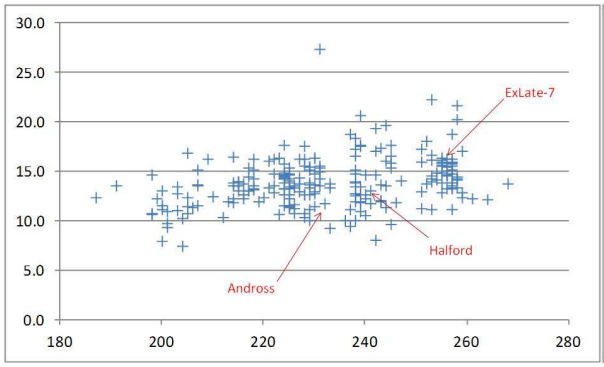

Fig. 8. Fruit Brix relative to harvest time.

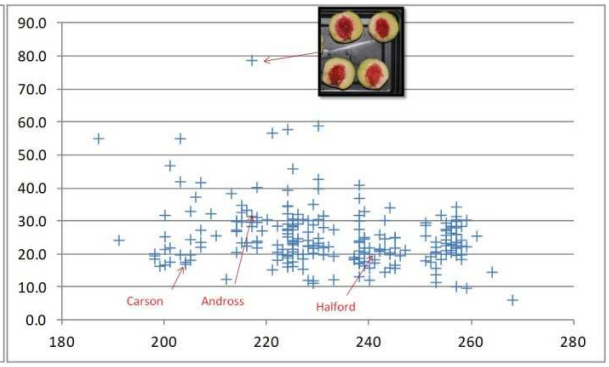

Fig. 9. Brix/TA relative to harvest time.

The final two data plots from this large-scale analysis show a relatively wide range in fruit Brix potential (**Figure 8)** which shows a general increase with increasing (Julian date) time to harvest (as would be expected because of the greater opportunities for energy accumulation), as well as fruit Brix /titratable acidity values (**Figure 9).** Very high levels for both traits (25 % Brix and 78 Brix/TA ratio) were observed in some of our interspecies derived breeding lines (the white fleshed, red pitted fruit shown in inset), demonstrating opportunities for fairly dramatic trait improvement if the germplasm can be fully incorporated. (Sugar content is so high in some of these selections that it protects the fruit from microbial infection during ripening and after-ripening so that the fruit dry on the tree much like prunes).

RosBreed data analysis can provide important information on genetic control independent of molecular marker analysis. The distinct segregation for non-red and red pits (anthocyanin production in the fruit flesh adjacent to the pit or endocarp) apparent in the cross between the firm-fleshed ('stony-hard') cultivar *Yumyeong* and *Loadel* demonstrates genetic control by relatively few number of genes **(Figure 10).** In contrast, the consistent and uniform expression of red pits in all progeny from the cross between the almond-derived non-melting freestone selection *F8,1-42* (no anthocyanin production, see Figure 12) by *Elberta* (anthocyanin production) suggest that the genetic

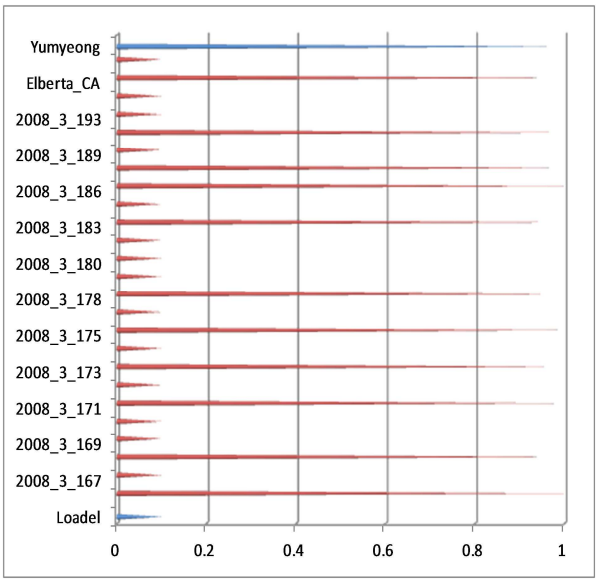

Fig. 10. Segregation for non-red (short bar) and red pits (long bar) in progeny from the cross *Yumyeong* by *Loadel.*

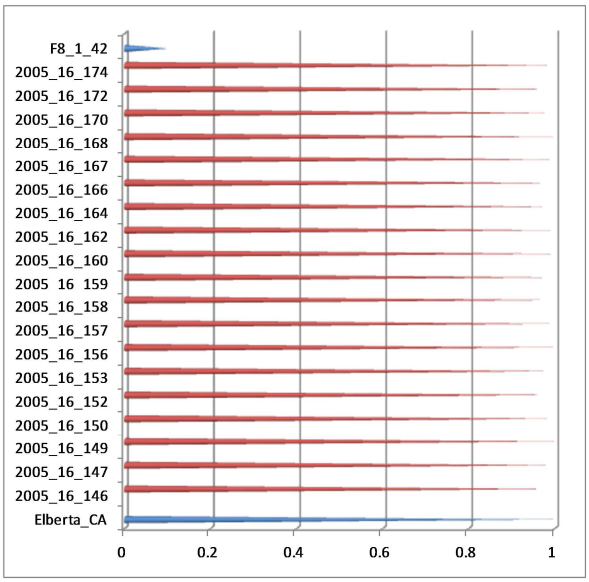

Fig. 11. All progeny showing red pits indicating dominance of the *Elberta* controlling genes.

factors in *Elberta* dominate in the progeny (**Figure 11)** making this genotype less desirable as a parent in a processing peach breeding program cross.

> *Interestingly, some non-melting/low-melting freestone fruit types were also identified in these progeny however the red pigment degrades with cooking making it unsuitable for processing peach. [A freestone, nonmelting processing flesh peach could otherwise be useful as pit-fragmentation could be avoided and so case-yields increased. In addition, nonmelting/processing freestone's would require only industrial freestone pitters which may be less costly and easier to maintain than the traditional clingstone pitting machines now used].*

While the UCD almond derived breeding line *F8,1-42* is the only non-melting freestone peach genotype known of this potentially useful combination, the full genetic control is very complex and so a prime candidate for RosBreed-type molecular marker analysis. A long-term collaborative molecular-marker analysis project between our lab and that of Carlos Crisosto has developed a fairly detailed molecular marker map for these populations (**Figure 12,** see also figures 13 and 18). An important early finding of this project was that the traditional freestone/clingstone and melting/nonmelting traits are controlled by two closely linked endopolygalacuronase (endoPG) genes on linkage group (chromosome) 4. [The RosBreed project was in many ways patterned on this earlier fruit brown rot mapping project directed by Dr. Crisosto and largely implemented by the visiting scientists Dr. Peace, Dr. Ogundiwin', and Dr. Martinez-Garcia (see publication lists)]. UCD selection F8,1-42, however, defies this otherwise universal association, since it's endoPG molecular marker genotype is clingstone despite its obvious freestone phenotype (inset, lower center). However, because these markers allow us to rule out the standard genetic mechanisms for F8,1-42, they provide useful information and insights concerning the actual genetic control. Similar

information/insights may ultimately prove as valuable as the more tenuous and lineage-specific molecular markers developed by the larger-scale RosBreed analysisl.

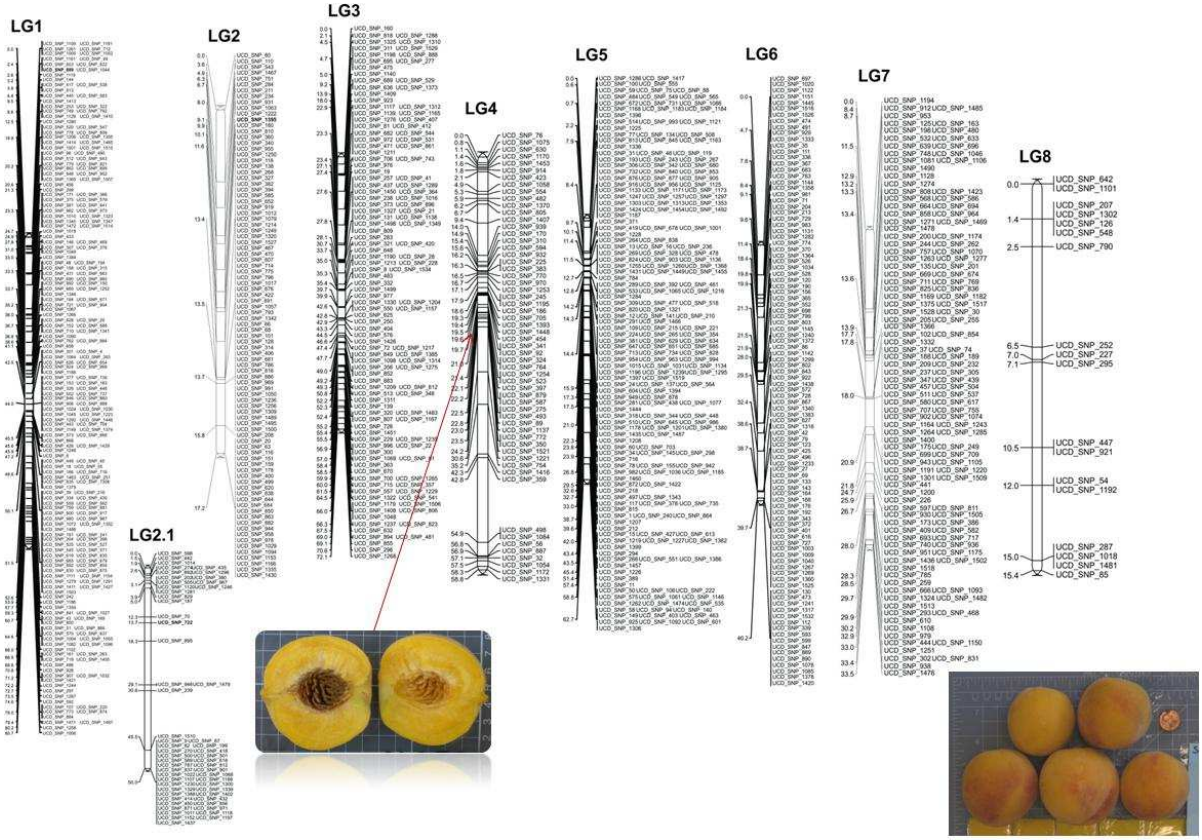

Fig.12. Molecular marker map for brown rot resistance genes developed with Crisosto lab showing the location of the endoPG genes discriminating melting flesh and freestone from non-melting/clingstone.[Center inset: *UCD-F8,1-42* nonmelting freestone. Right inset: example of fruit size variability in RosBreed *Loadel* sample.

[One of the more serious challenges to RosBreed accuracy is the need for a very precise phenotyping (trait measurement) if associations with certain markers are to be identified. For example, even if an individual in the population possesses a putative gene for a large fruit size (phenotype), any number of field variables (shading, over cropping, disease, etc.) could act to suppress full expression of that trait resulting in an incorrect phenotyping and consequently a failure to accurately correlate specific phenotypes with specific molecular markers. This type of problem is demonstrated in the variable sized Loadel fruit shown in the lower right-hand corner of Figure 11. Despite our extensive efforts to obtain uniform results, this type of environmentally induced variation was relatively common. Because the full RosBreed data set will combine data from California, Texas, Arkansas and North Carolina research plots (with different environments at each site), this type of environmental variation will almost certainly be magnified. Other traits being analyzed such as soluble solids, acidity, blush, red-in-pit, ripe date, etc. are probably even more profoundly influenced by variable environments.]

A more recent finding of our Fruit Brown Rot mapping project with Dr. Crisosto's lab indicates that major genes controlling brown rot resistance are located on linkage groups (chromosomes) 1 and 4 (**Figure 13**). This mapping population was also segregating for the clingstone-freestone trait which, as discussed above, is controlled by the endoPG gene complex located in the central region of chromosome 4; a region also identified as having a putative resistance gene. Because nonmelting, clingstone types are generally more resistant to fungal lesion development then melting-freestone types, the resistance in chromosome 4 may, in fact, turn out to be the endoPG gene. If confirmed, this QTL molecular marker would have no value for processing peach breeding since the nonmelting clingstone types have already been fixed in these breeding populations. Two markers for possible resistance genes were identified on chromosome 1 (see figure 13 on the left, where only those phenotype-marker correlation peaks higher than the dotted line are considered legitimate {i.e. not spurious}). Again, environmental influences can mask results if not carefully controlled. High correlations were found between markers and disease resistance for years 1 and 2 (top and middle left plots). In year 3, however, environmental variation (field climates, insect damage, decreased inoculum potency, incipient infections, etc.) effectively masked the genetic control components when the full three-year summary (vs. year by year) data is analyzed (plots at right) [similar to what is planned for the RosBreed data].

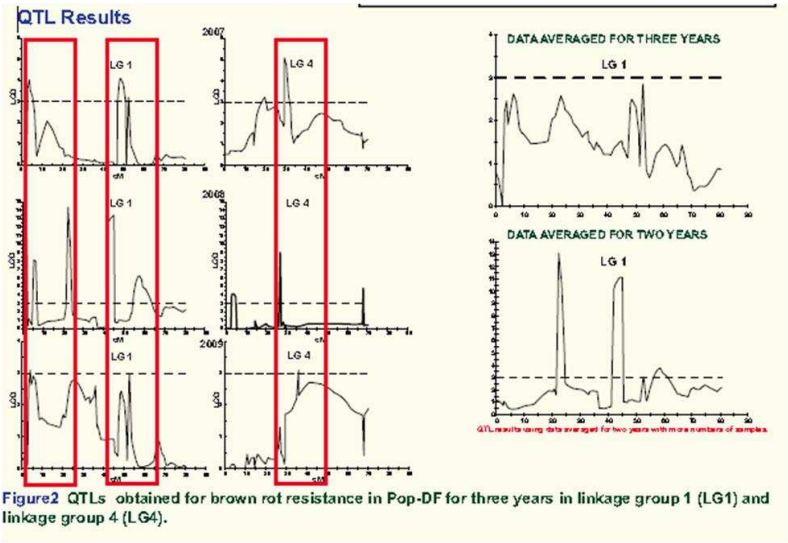

Figure2 QTLs obtained for brown rot resistance in Pop-DF for three years in linkage group 1 (LG1) and linkage group 4 (LG4).

Fig. 13. Markers associated with brown rot resistance in linkage group 1 and linkage group 4 based on three years evaluation with Crisosto and Bostock labs of a cross between *Dr.Davis* by *F8,1-42*

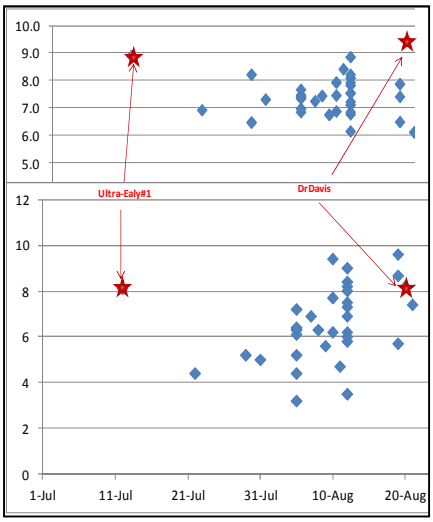

Fig. 14. Progeny performance for fruit size and firmness for the cross Dr. Davis by Ultra-Early-1

Equally important in selecting parents for target traits such as fruit brown rot resistance, is the capacity of the parents to confer the range of other traits (such as size, color, firmness ripe date, etc.) required for commercial success. Because most of these traits are quantitative (i.e. controlled by many genes with small affect) individual progeny performance is often normally distributed between the values of parents. Thus, it is

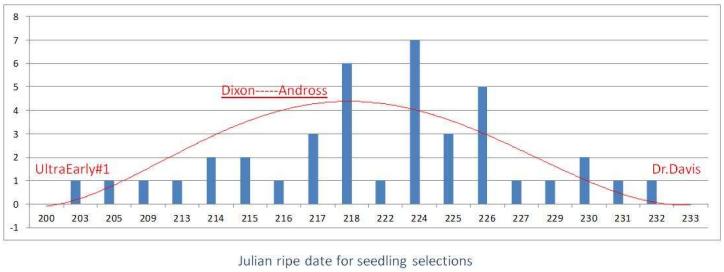

Fig. 15. Expected (red curve) and observed distribution of ripe dates for progeny from the cross *Dr. Davis* by *Ultra-Early-1.*

important to select parents having a high probability of conferring all of the required commercial traits to progeny. Progeny performance for fruit size and fruit firmness are plotted out in **Figure 14** for the cross *Dr. Davis* by *Ultra-Early-1.* Previous testing has shown both parents to possess moderate to high levels of fruit brown rot resistance, fruit size, firmness and color. In addition, average ripening time between the two parents would be concentrated in the area of the desired *Dixon-Andross* target maturity season if the trait was distributed in the expected normal distribution. Even with the heavy RosBreed-project thinning, fruit size of all progeny was below that of the parents (Figure 14, top plot) yet typically well above the 6 millimeter minimum size. This suggests that both *Dr. Davis* and *Ultra-Early-1* had near optimum genetic composition for fruit size so that any genetic reshuffling in the progeny resulted in sub optimal genetic compositions and so smaller fruit. To maximize the previously discussed compensatory-sizing potential (compensate for over thinning by producing larger sizes in the remaining fruit), only the larger fruit would be selected from these progeny for further testing. For fruit firmness, many progeny performed as well or better than the parents (Figure 14, lower plot) with many individuals showing very good fruit firmness (with several of these also showing good size). This indicates that these particular parents complement each other for this trait. [Earlier breeding studies have identified *Dr. Davis* is a good parent for conferring higher levels of fruit firmness in many progeny, despite this variety often appearing only moderately firm at maturity. This indicates that while *Dr. Davis* has some superior genes for firmness it also has sufficient inferior genes to moderate overall affect. Proper crossing combinations, however, can concentrate the superior genes in progeny while concurrently purging out the inferior ones. Molecular markers associated with these superior firmness genes would be particularly informative. However, since the progeny populations will also be segregating for a range of other required traits, having a good molecular marker for only one of them would not justify the still excessive laboratory costs since trees would still have to be grown and evaluated for remaining traits in the field.

As shown in Figure 14, harvest dates for the progeny were more congregated towards the *Dr. Davis* parent. Because time to harvest is typically controlled by a large number of genes with small effects, progeny usually tend to be distributed in a normal distribution between the two parents with the largest number of progeny tending to be at the midpoint between the parents. A principal reason for making the *Dr. Davis* by *Ultra-Early-1* cross was made was to generate most progeny ripening at the desired *Dixon-Andross* target time.

[Previous experience with traditional California germplasm was that for crosses in which the parents bracketed the Dixon-Andross maturity time the progeny would congregate both before and after the Dixon-Andross gap, with only a few individuals within. This is believed to be a stage 2 development/ transition problem resulting from the high inbreeding of

California germplasm and is probably the reason for the earlier failures to develop a commercially successful processing peach cultivar for the season. It was also one of the incentives for bringing in outside germplasm such as UltraEarly-1 with its more exotic (Brazil and Eastern Europe) derivation.

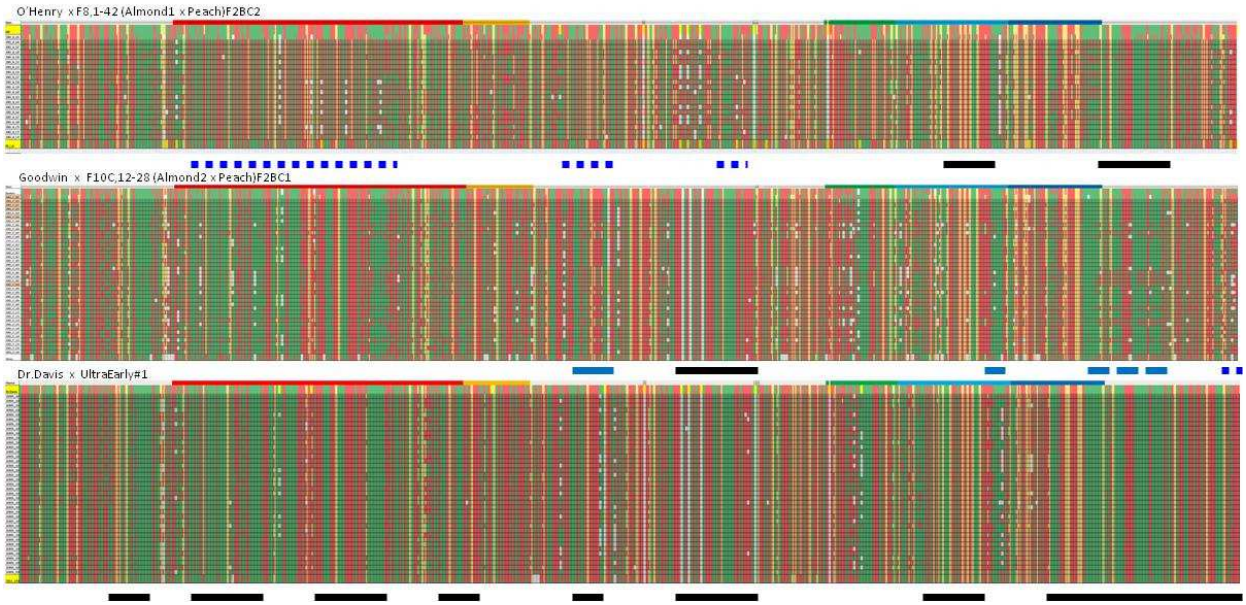

Fig. 16. Color-coded RosBreed markers for the *Dr. Davis* by *Ultra-Early-1* cross (bottom) and two UCD processing peach breeding lineages derived from almond by peach interspecific crosses.

Figure 14 presents average data from the 3 RosBreed years (2010-12). Thus, some of the later maturity times may be the consequent of the generally delayed ripening (because of cooler spring temperatures) in 2011-12. Progeny ripening times in 2010 are plotted in **Figure 15** in Julian days (along with the 2010 ripe dates for both parents). While the standard expectation would be a normal or Bell-curve distribution (red line), results continue to show a distinct bias towards later ripe dates with a distinct depression during the targeted *Dixon-Andross* time. However, while the number of progeny in the targeted *Dixon-Andross* season is reduced, backcrossing and intercrossing the low number recovered has allowed some progress to be made in this objective. Even with the incorporation of peach cultivars from divergent regions, the amount of new genetic variation (and so new trait variation) is limited since most of these foreign cultivars also can be traced back to a relatively few numbers of founder peaches initially brought from China. This pronounce genetic uniformity is demonstrated in **Figure 16** which charts the genetic variability of the *Dr. Davis* by *Ultra-Early-1* cross in comparison to two other UCD processing peach breeding lineages which were derived from initial almond by peach interspecific crosses.

Each cross has from 20 to 40 progeny. Approximately 500 molecular markers covering all of the 8 chromosomes (indicated by horizontal colored bands at the top of each chart) were determined for each progeny and parent. The top row (just beneath the color-coded chromosome bands) shows marker results from the first parent while the bottom row shows

marker results for the second parent. Each row in between represent the marker results for an individual progeny of that cross. Since peach is diploid, each marker has two possible alleles or forms. For this chart, markers alleles were color-coded so that the thinnest vertical bands represent variation for all parents and progeny at one given marker. While this allows a fairly detailed analysis of genetic differences among progeny and between crosses (image may be enlarged so that individual progeny/markers can also be analyzed). It also allows a convenient big-picture 'snap-shot' of the amount and areas of variation for each cross.

A general overview of the marker-charts for the three populations shows a pronounced uniformity of vertical colored bands (i.e. less 'noise' caused by multiple different colored points within each vertical band) for the *Dr. Davis* by *Ultra-Early-1* cross. Many vertical color bars show no change from parent-1 (in the top row) through each individual progeny in the intervening rows to parent-2 in the bottom row. [These highly conserved areas are identified by solid black horizontal bars beneath each conserved section]. No marker variation (and so probably no genetic variation for different traits) is present in these sections because both parents are identical at these locations (loci). These highly uniform regions predominate in the peach by peach cross and are a visual indication of the limited genetic variation even in this relatively wide (Brazilian by Californian) cross within peach. Considerably more variation is apparent in the almond derived crosses, though a few (black banded) uniform sections are present, indicating genetic inbreeding resulting from the multiple backcrosses to peach from the initial exotic interspecific cross (see lineage in Figure 18). [In both cases of almond by peach lineages, 3 to 4 generations of backcrossing or selfing towards peach types have occurred so that the progeny are distinctly peach-like]. Present in the almond derived crosses and absent in the peach by peach crosses are areas where the parents differ yet most progeny retained the genotype of only one parent (rather than being uniformly distributed (identified by shorter horizontal blue bars under the chart). These may represent areas where the chromosomes have different arrangements between peach and almond (translocations, inversions, deletions) which result in poor alignment during meiosis and consequently reduced recombination in the progeny. Genes in these sections would generally be inherited as a block (almond type or peach type) with little reshuffling or recombination of genes within the block. Thus, if a desired resistance gene was located within an almond-block, it could be very difficult to transfer the individual resistance gene to a more pure peach background (called *linkage drag* because the undesired almond genes would be dragged along with the

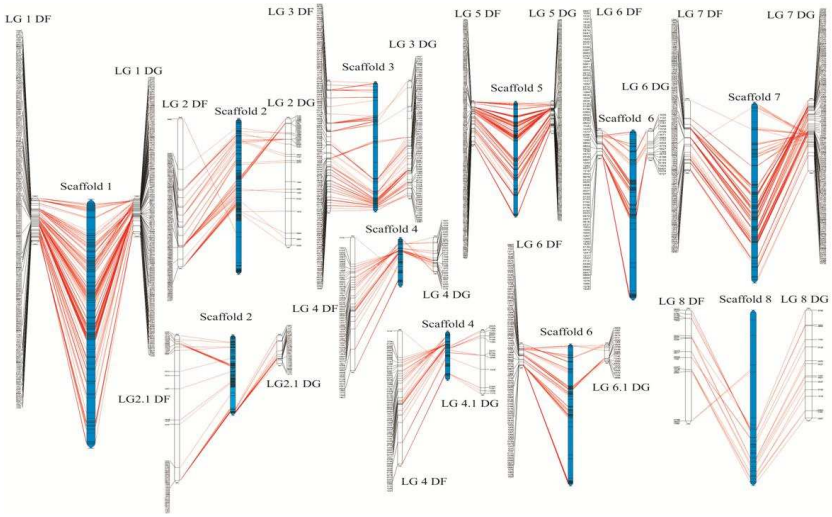

Fig. 17. Evidence for chromosomal rearrangements in markers for *Dr. Davis* by *F8,1-42* progeny compared against maps of almond (left) vs. peach (right).

desired resistance gene because they are closely linked or connected in the desired DNA). Particularly intriguing are sections in the *O'Henry* by *F8,1-42* cross (top chart) where more marker variation and so apparently more genetic recombination is occurring then would be expected based solely on parental differences (horizontal blue dots). These types of super-variable regions, while rarer, sometimes occur in breeding lines derived from interspecies crosses and may represent an opening-up to recombination of chromosome sections that are normally repressed.

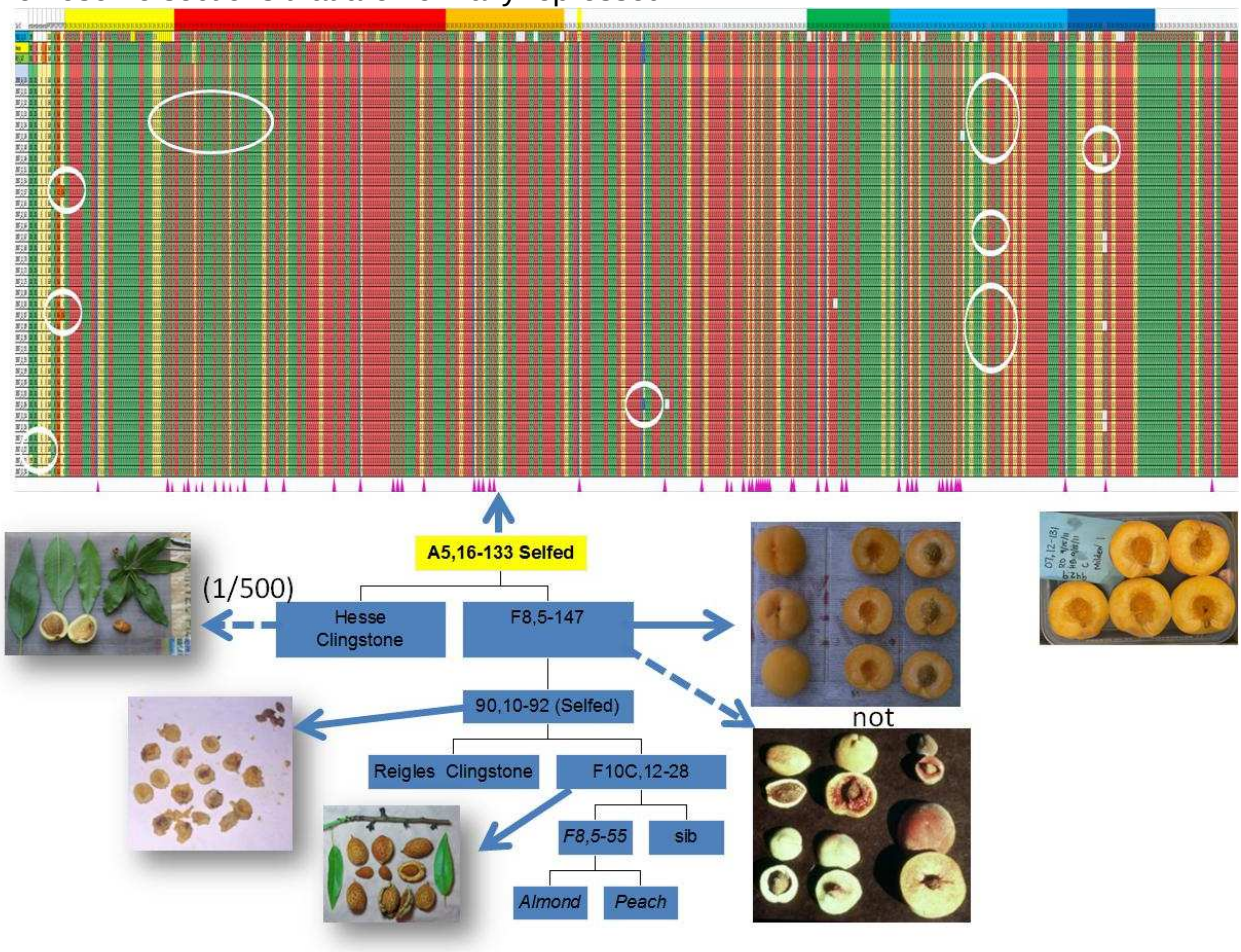

Fig. 18. Expected (red curve) and observed distribution of ripe dates for progeny from the cross *Dr. Davis* by *Ultra-Early-1.*

[Despite the high variability observed in these almond derived lines, progeny were distinctly peach (i.e. indistinguishable from commercial peach) with a few almond-like exceptions in the *Goodwin* by *F10C,12-28* cross (see figure 18)]. Additional support for the occurrence of chromosomal rearrangements in these populations comes from our earlier work with the Crisosto and Bostock labs in mapping brown rot resistance genes in the *Dr. Davis* by *F8,1-42* population (**Figure 17**). The higher mapping resolution in this project identified several regions were homologous sections of peach chromosomes were inverted or translocated relative to the corresponding almond chromosome. Because recombination requires close DNA/chromosome alignment at meiosis, these

altered chromosome architectures would act to restrict or suppress recombination in these areas. Similarly, recombination in areas near the mostly central centromere of each chromosome is normally repressed (multiple markers in these regions would appear as a single marker because they are rarely reshuffled). Chromosomal rearrangements within these areas could transfer some of these markers away from the repressed centromeric region resulting in the types of super-variability observed in some of the interspecies RosBreed data. In this scenario, interspecies derived germplasm would have greater opportunities for genetic recombination (at certain typically exotic locations) than would be achievable in standard peach by peach crosses. [Further information supporting such increase genetic recombination to chromosome reshuffling was presented in the 2011 annual report].

The most intriguing RosBreed result, however, came from a more advanced population from the *Goodwin* by *F10C,12-28* lineage shown in Figure 16 and summarized in **Figure 17**. The bottom-center flow-chart shows lineage of the 'Long-Keeper' (LK) peach lineage (*F10C,12-28 , F8,5-147* and sister lines, while A5,16-133 is a self of F8,5-147 selected for its LK ability plus fruit quality. [Sample fruit for key parents are shown as insets]. *F8,5-147* was initially intriguing since it and all of its sibs had distinct peach phenotypes (inset) rather than segregating for peach or almond types as would be normal for this generation (dotted line inset). *F8,5-147* is also interesting since in rare instances, further backcrosses to peach will generate mostly peach progeny and occasional distinct almond-type or primal-peach type fruit and trees (left image) which has never occurred in more traditional crosses. The top colored marker-chart, however, is most intriguing as it shows RosBreed markers for UCD RosBreed population 2007,12- seedlings 131 to 200 which is an self of A5,16-133 (also shown at top rows are the A5,16-133 parent and F8,5-147 grandparent. While a relatively high rates recombination should be apparent in this lineage (comparable to previous charts) virtually no recombination is apparent. (Circles show the very few recombinations identified in ~500 markers for over 40 progeny). This lack of (apparent) recombinations occurs even for heterozygous loci {pink wedges at bottom} in F8,5-147 and all of its sibs lines as well as A5,16-133 and all 40 progeny. [Heterozygous loci possess on allele or gene form from each parent (Aa) and when self-pollinated as in this case should randomly segregate the three types (1AA: 2Aa: 1aa)]. This segregation is not occurring in this population. Field trees however, are segregating for leaf gland, flower type etc., and for RosBreed fruit quality data. [Fruit sample from progeny tree 2007,12- 131 shown in inset on right]. The aberrant to highly aberrant segregation patterns found in figures 16 and 18, respectively significantly undermines confidence of marker assisted selection to this breeding program. One possible explanation is that the molecular methods used were inappropriate for this material resulting in many loci being misidentified. However, as proposed in the 2011 annual report, such anomalies might be expected and may even represent unique breeding opportunities in interspecies-derived and clonally propagated crops. This point of view is summarized in **Figure 19** where the linear strand of DNA is shown expanding out from one of the eight chromosomes found in peach and almond. The core premise of marker-assisted-selection is that the DNA, being linear, represents information much as in a book chapter or data table. (In this analogy, different chromosomes would represent different

chapters or tables). To increase the expression of a trait such as resistance or yield, you increase the number positive genetic loci contributing to it, much like playing poker. While this area of research is generally called ‘Genomics’ in reference to the genome, the genome actually refers to the full complement of all hereditary material within the cell nucleus.

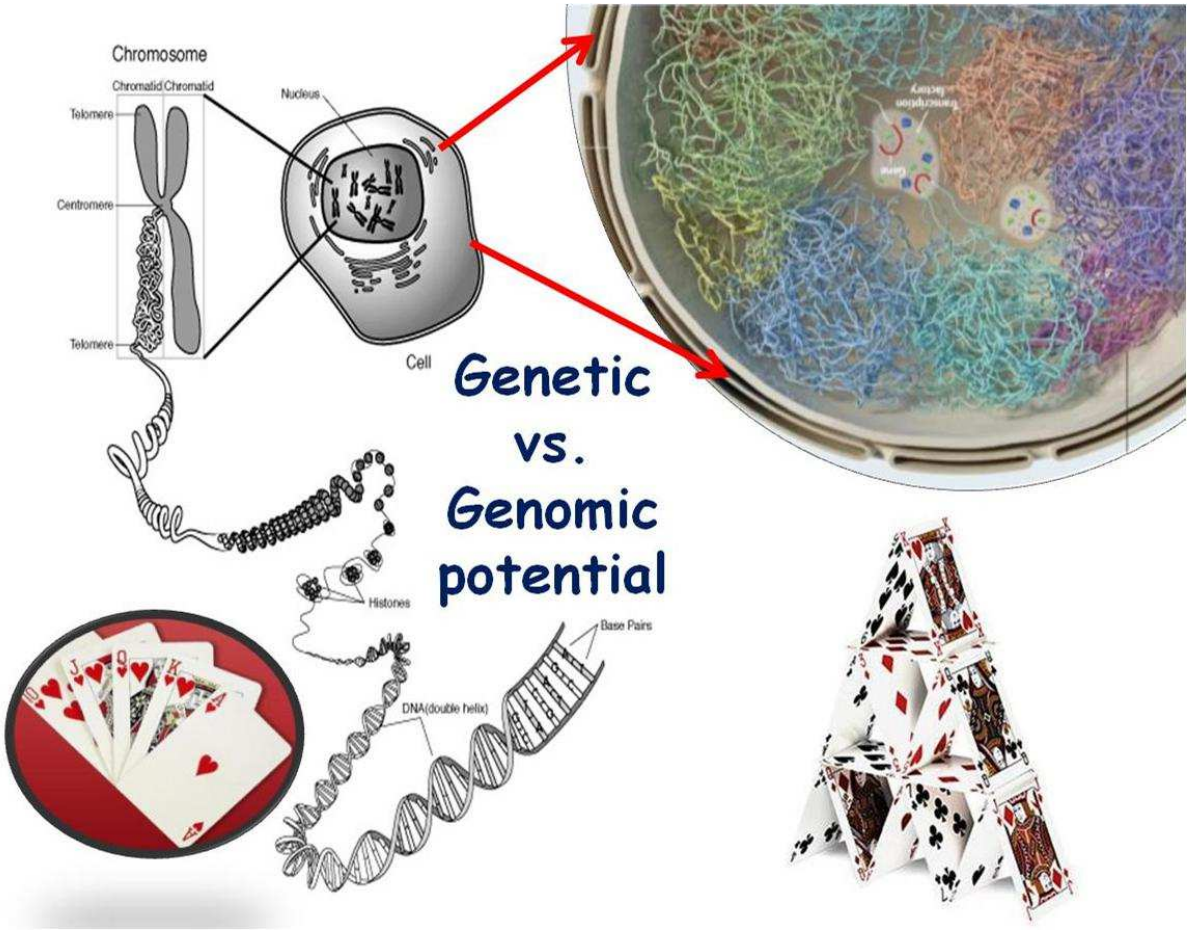

Fig. 19. Genomic perspective emphasizing the full complement of all hereditary material within the cell nucleus and their accessibility for positive breeding selection.

Ongoing research, mostly in human genetics, now indicates that trait expression can be much more complex and that a specific gene interacts with its surroundings (i.e. the adjacent chromosome chemistry can turn genes on or off, though molecular markers would always show them as present and so presumably on), other genes (epistasis), and even other chromosomes (top right image in figure 18). These interactions are even more complex in interspecies derived breeding lines because novel interactions resulting in novel and sometimes valuable phenotypes are more prevalent. [It has also been shown in interspecific material that whole chromosomes can be turned on or off depending on parentage, position, etc.].

An alternative ‘Genomic ‘model being developed by our program is that any genomic variation available to natural selection will be acted on by natural selection, including

interactions within genes (allelic), between individual genes, between genes and their surrounding (including different levels of chromosome chemistry/architecture) as well as among chromosomes. Since all are functional in determining the final level of trait expression particularly for complex traits such as disease resistance and yield, all need to be considered in breeding selection as well if maximum performance is to be achieved. Most of these complex interactions, however, are altered/reshuffled and so not 'inherited' along with the DNA (genes) during meiosis and the subsequent seed development process essential for seed propagated agronomic and vegetable crops. Because we do not yet have the sophistication to characterize and predict these very complex interactions, they are generally considered non-genetic (non-DNA) and non-heritable and lumped into the Environmental (i.e. not amenable to breeding manipulation) component of trait expression. Because fruit tree crops such as peach are clonally propagated, superior forms of these complex but poorly understood interactions can be (and most likely have been) captured and propagated. [This would explain why it's very difficult to improve on elite long-established clonal of long-lived crops such as citrus, apple, pear, grape, pistachio, prune, walnut, etc. using marker assisted selection and why most successful genetic improvements come from bud-sports (i.e. specific genetic change while genome interactions are maintained). This genomic analogy would be better represented by a house of cards were both the individual value of the card (gene) as well as its association with all other cards is important in determining final trait value. The challenge is how to breed for whole genomic value.

A major attraction of marker assisted selection is that the breeder might predict the outcome based solely on the DNA content (proactive), thus eliminating the need for preliminary test crossing to identify elite parental combinations (reactive) typical in traditional breeding methods. Traditional breeding methods, with their emphasis on proof-of-value before large-scale crossing, are inherently well structured to identify these more complex genomic interactions even when they are not well understood. Such traditional quantitative breeding methods (summarized in the 2011 annual report) have typically considered only heritable (i.e. passed from seed to seed) components to be genetic/genomic and would have ruled out the type of genomic interactions discussed above as being unstable and so undesirable in seed propagated crops. Breeding programs for clonally propagated crops may need to be less strict with this concept of heritability, and focus more on what can be captured/maintained through vegetative propagation. An sole emphasis on molecular marker assisted selection in this scenario would be counterproductive to applied breeding. However, both current and emerging molecular analysis methods offer the opportunities to identify, if not precisely characterize, genomic variants. For example, in our breeding data the patterns of trait segregation (heritability) suggested a suppression of recombination consistent with chromosome rearrangement. The precision of molecular marker analysis appears to confirm these rearrangements, and so identified possible genomic variants which could be manipulated towards applied breeding goals. Because of the complexity and so rarity of elite genomic interactions, successful breeding program would have to be largely reactive (i.e. capable of generating very large seedling populations and effectively selecting the rare elite genetic/genomic individuals). This perspective has driven the following modifications of the current processing peach breeding program:

1. *Varieties succeed because they lack deficiencies for any of a large number of traits needed for commercial success (not because they have superior performance for any single trait).*
    a. *Rapidly rogue out all progeny which show any deficiencies.*
    b. *Identify accurate predictors of complex traits such as yield.*
        i. *Fruit compensatory sizing ability on heavily thinedn trees.*
        ii. *Cropping ability on unthinned trees.*
        iii. *Minimum fruit size on heavily cropped unthinned trees.*
    c. *Evaluate advanced selections over multiple years and multiple environments before release.*
2. *Elite commercial varieties are the result of optimizations at both the genetic and genomic level.*
    a. *Incorporate interspecific germplasm into the breeding program to increase genetic and genomic variability accessible to breeding selection.*
    b. *Select for both traditional breeding priorities as well as novel phenotypes which could advance reading goals (for example, see figure 2).*
    c. *Generate very large population sizes to adjust for the very low probability of truly elite individuals within the population.*
        i. *Mechanize all aspects of the breeding program to maximize breeding population size and optimize uniformity of seedling production environments.*
        ii. *Rapidly identify superior selections so that the breeding populations can be rapidly removed to advance next cycle.*
    d. *Identify superior genetic/genomic performance based on clonal performance as well as segregating seed progeny performance.*
        i. *Utilize traditional quantitative as well as evolving molecular methods to both differentiate and select for useful (genetic, genomic) components versus spurious environmental effects.*
        ii. *Utilize emerging metabolomic analysis to more accurately select genetic/genomic factors with the greatest contribution to breeding goals.*

While seemingly complex, the manipulation of complexity is an inherent and so essential component of applied breeding programs. Progress can be measured in the improvement of targeted traits such as disease resistance and performance of advanced selections in grower trials (see Regional Testing annual report). Ultimately, it is the performance of the advanced breeding selections upon which the breeding program efficiency needs to be evaluated. **Figure 17** shows several 2012 selections in the targeted *Dixon-Andross* season which appear to possess good commercial quality and the LK capacity to hold fruit on the tree with minimum deterioration (note that all samples were harvested three weeks after initial tree ripe date) and in several cases improve brown rot resistance and/or compact tree structure.

Fig. 20. Examples of 2012 selections in the targeted *Dixon-Andross* season which demonstrate the LK capacity to hold fruit on the tree with minimum deterioration (all samples were harvested three weeks after initial tree ripe date). *UCD09,40-35* (left: LK derived from Eastern germplasm); *UCD09,39-41* (center: somatic mutation combining LK with compact tree structure); *UCD08,25-115* (right: LK with brown rot resistance derived from almond by peach interspecies hybrid).

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5. Riaz Ahmad, Dan E. Parfitt, Joseph Fass, Ebenezer Ogundiwin, Amit Dhingra, Thomas M. Gradziel, Dawei Lin, Nikhil A. Joshi, Pedro J. Martinez-Garcia, Carlos H. Crisosto. 2012. Whole genome sequencing of peach (Prunus persica L.) for SNP identification and selection. BMC Genomics 2011, 12:569 doi:10.1186/1471-2164-12-569
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7. Frett, T., K. Kasic, J. Clark, D. Byrne, T. Gradziel and C. Crisosto. In-press. Standardized phenotyping for fruit quality in peach [Prunus persica (L.) Batsch]. HortScience.
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