California Cling Peach Advisory Board
2013 Annual Report

Project Titles: Development of New Cling Peach Varieties

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Objectives:

A. Continue to evaluate field performance and genetic data (including molecular marker/mapping data) from previous seasons to identify the most promising breeding lines (pedigrees) as well as the most promising individuals within selected pedigrees for grower testing as and/or advanced breeding.
B. Continue to develop and implement low cost, high through-put field selection methods targeting high fruit quality and yield.
C. Generate 5,000 new seedling progeny trees through controlled recombinations primarily through cross-hybridization targeting high productivity with reduced grower and processor costs

Summary:

Despite early 2013 field losses of approximately 30% of seedling transplants (from 2012 seed), the target of 5,000+ new seedlings from breeding crosses should be achieved. Based on current numbers of greenhouse seedlings and seeds currently being prepared for germination, field plantings in 2014 should exceed the target of 8,000 new seedling trees. Seedlings are generated by controlled hybridizations to combine desired traits from selected parents, then typically followed by self-pollinations to sort out desired from undesired traits. With the mechanization of most other aspects of the breeding program, the high labor requirements of controlled hybridizations remain an important constraint. Despite these limitations and the continuing cutbacks in University funding, very large breeding populations have been achieved with over 30,000 genotypes evaluated in 2013. Of these, over 400 selections were advanced to caning evaluations at the UCD processing pilot plant. The incorporation of extensive new germplasm in the first phase of this breeding program has resulted in a number of distinct breeding lineages showing promising improvements in crop productivity, quality and disease resistance. In addition, the consequent large size and diversity of the UCD processing peach breeding program has leveraged extensive outside funding for assessing the potential of molecular techniques to accelerate breeding progress. The probable value of these molecular approaches appears mixed and strongly dependent on the specific trait. More traditional methods, however, have identified promising new selections for advancing to grower trials. Advanced UCD selection *Extra-Early#1* is in the process of being patented and released as a *Carson-Dixon* period processing peach variety.

Project Summary: 2013.

The goal of the UCD Processing Peach Breeding program is the development of new cultivars to maintain competitiveness the California industry in the face of increasing international competition. Thus, industry funds are used entirely for generating breeding populations targeting individual genotypes optimized for the multiple fruit production and quality traits required for commercial success. A high priority of the early phase of this breeding program was the identification and incorporation of new germplasm and new traits from outside sources to meet changing grower/processor needs. (This was required since most traditional California processing cultivars represent an inbred genetic population, possessing little new variability for crop improvement). A result of this early genetic improvement phase was the incorporation of new genes and traits from diverse new germplasms (including European, Asian, and South American peaches, the wild peach species *Prunus davidiana* and *Prunus mira* and related almond species *Prunus dulcis, Prunus scoparia, Prunus argentea, and Prunus tangutica*), into breeding lineages selected to be fully adapted to California production and processing requirements. The current phase of the breeding program targets the continued genetic recombination of this material to develop the rare, elite individuals possessing all field and processing characteristics required for long-term commercial success. As discussed in the 2011 and 2012 annual reports, the genetic complexities of this goal will require the generation of much larger breeding populations then occurred in the first phase of the breeding program though at a much reduced costs (necessitated by University and industry cutbacks). By dramatically reducing all phases of our field and greenhouse expenses, the program has achieved and consistently surpassed targeted breeding population goals (Table 1). Based on seedlings currently growing in our greenhouses or being prepared for planting, we expect to surpass our 2013 goal of 5,000 seedlings by at least a thousand seedlings. (Typically about a quarter of our seedlings are rogued-out in greenhouse selection based on disease resistance and plant vigor. Thus, total seedlings generated by the program are about 25% higher than numbers advanced to field planting). Plantings in 2014 will also include approximately 3000 seedlings left over from 2013 seed lots. Unusually warm and dry conditions during a later than usual 2013 planting resulted in an approximately 30% loss in seedling survival so that remaining seed was maintained in the screenhouse for fall, 2013 planting. The early predictions (and subsequent occurrence) of unusual freezing temperatures in the fall, 2013 resulted in our decision to further delay planting to Spring 2014 plantings. The exceptionally high field planting success from 2008 reflects our success at optimizing field management efficiencies. Lower populations in 2012-2013 were the result of simultaneous cutbacks in both university and industry funding at that time. The expected recovery of larger populations in 2014 plantings (2013 generated

Table 1. Targeted size for yearly seedling populations of the UCD breeding program compared to actual field plantings from 2008 to present.

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 (~3,000 in greenhouse)
2014	8,000	~9,000 plus ~3,000 from 2013

seed) reflects further improvement in breeding program efficiencies, including a trend towards direct field planting of pre-germinated seed rather than the traditional process of seed germination in the lab, followed by growth in a greenhouse and screenhouse, followed by transplanting to the field. While this bulk-approach maximizes the potential size of breeding populations, it also inherently increases the risks of large-scale failures in the future. (For this reason several thousand seed are kept in reserve or greenhouse-planted as an insurance against significant early germination/field losses).

New breeding populations continue to be generated by controlled, hand-crossing between carefully selected parents to create hybrids recombining desired characteristics from both parents. This is often followed by cycles of self-pollination of these hybrids to sort out desired from undesired traits. Because peach is naturally self-fruitful, selfed populations are relatively easy to achieve, but hybrids are very difficult because all flowers to be hybridized have to be hand-emasculated, hand-pollinated and subsequently hand harvested/processed. This hybridization phase thus remains an important bottleneck for consistently generating large breeding populations as well as elite individuals, since most successful varieties have a hybrid origin.

Advanced selections expressing introduced traits such as brown rot resistance and the 'long-keeper' trait (capacity for on-tree fruit to maintain good eating and processing quality for 1 to 2 weeks after tree ripe stage) are now undergoing regional testing. In addition, several earlier advanced selections are now progressing to the variety release stage. Advanced UCD selection *Extra-Early #1* is currently in the initial patenting and release step with expected availability to nurseries by the 2014 propagation season. *Extra-Early #1* ripens in the desired *Dixon-Andross* season (Figure 1) and has consistently demonstrated good fruit quality and productivity as well as improved brown rot resistance over the last 12 years of regional testing (summarized in the 2013 Regional Testing annual report). A second advanced selection, *Extra-Late#1* is currently being evaluated for possible patenting and release targeting the *Sullivan#4-Corona* season.

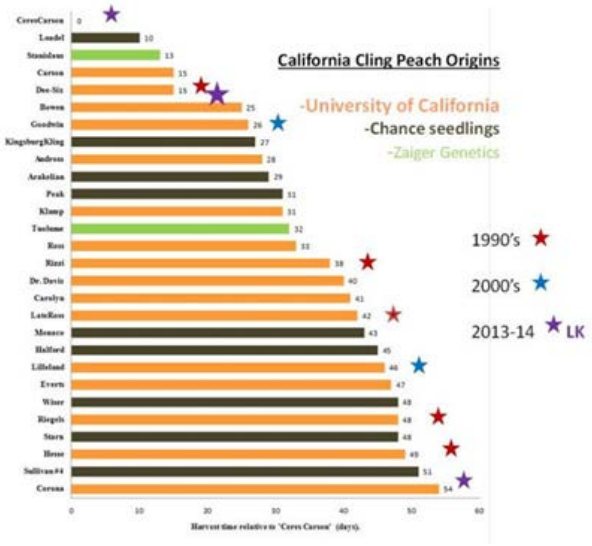

Figure 1. Relative ripening times for recent UCD processing peach selections. *ExtraEarly#1* ripens in the targeted Dee-Six-Bowen period while *ExtraLate#1* ripens with Sullivan and Corona (purple stars).

Although industry funding is directed entirely to applied, field-based genetic-improvement projects, the resulting availability of large segregating populations of diverse, often interspecies origins, has made this material very effective at securing supplemental funding for more basic, often molecular-based studies when combined with the well-established expertise of my collaborators. These studies have allowed large-scale evaluations of the benefits/risks of the new biotechnologies towards applied tree crop breeding such as Marker Assisted Selection (MAS). [Outside funding leveraged by this approach typically matches or exceeds CCPAB funding]. Proposed

advantages of MAS include greater selection efficiency (since the gene is selected directly based on molecular identity rather than field expression, which is often distorted by environmental variability), the ability to make selections at the seedling stage without having to wait until fruit maturity, and the strategic advantage of improved knowledge of the mode of action for genes controlling the trait of interest. An indirect, but important additional advantage, is the opportunity to generate per-reviewed publications which are crucial for advancing basic and applied knowledge as well as university promotions. Three such a long-term federally funded projects funded by RosBreed and USDA-NRI will be summarized in the following sections, with an emphasis towards the potential use of molecular marker data for improving UCD processing peach breeding efficiencies. (A summary of breeding data from these projects and their application to breeding program advancements were summarized in the 2012 annual report).

RosBREED Fruit Quality Study.

An initial assessment of the RosBREED Pedigree-based Analysis (PBA) for mapping quantitative trait loci (QTLs are used as potential markers for quantitative {i.e. not strongly expressed} genes [see more detailed explanations in 2011 and 2012 annual reports]) of the germplasm of the Processing Peach Breeding Program at UC Davis, which includes genetic transfer or introgression from species such as almond [*Prunus dulcis*], *Prunus argentea*, *Prunus davidiana*, and *Prunus mira* has now been completed. Through the application of pedigree correction and subsequently Factor Analysis for Mixed Data (FAMD) to characterize the genetic Structure within the germplasm, we were able to map QTLs for Soluble Solid Content (SSC), Titratable Acidity (TA), pH, Fruit Diameter (FD), Fruit Weight (FW), expression of red color at the pit (RP) and Days to Fruit Maturity (RD) (Figure 2).

Brief methods description. The material contributed to the RosBREED peach germplasm by the UCD Processing Peach Breeding Program integrates a pedigree of 562 individuals, of which 354 form a highly informative pedigree, of which, 258 individuals had whole genotyping information from 215 genome-wide bi-allelic SNPs, and phenotyping information from three consecutive years (2010, 2011 and 2012) for the traits: Soluble Solid Content (SSC) in brix degrees, Titratable Acidity (TA) expressed as malic acid content, pH, Fruit Diameter (FD) in millimeters, Fruit Weight (FW) in grams, presence/absence of red color close to the pit (RP) and Julian Days to Fruit Maturity (RD). The pedigree was previously corrected thought the application of Metropolis Coupled Markov Chain Monte Carlo methods [$(MC)^3$] through the software FRANz 2.0 and the characterization of the Genetic Structure (GS) within that pedigree was determined performing the Factor Analysis for Mixed Data (FAMD) using the library FactoMineR 1.25 implemented in the R package version 2.15.3. The GS was used as a nuisance variable (Group) in the data file entered in FlexQTL®. This nuisance variable was considered as a uniform prior. Taking the 258 individuals included in the FAMD as a base, a pedigree of 354 individuals was set for subsequent entering to FlexQTL® for mapping of QTL for SSC, TA, pH, FD, FW, RP and RD.

The results (Figure 2) show that for these 7 traits, 14 significant QTLs were mapped across the eight chromosomes of peach, indicating that the application of PBA had been successfully applied. Thus, results suggest possible value for Marker-Assisted Breeding (MAB) at the UCD Processing Peach Breeding Program.

Red In Pit (RP):
QTLs= 2
h^2= 0.89-0.93
Chr = 3 & 6
Flaking SNPs:
ss_316025
ss_339562
ss_634798
ss_647983

Soluble Solid Content (SSC):
QTLs= 1
h^2= 0.51-0.71
Chr = 7
Flaking SNPs:
ss_773704
ss_781308

Titratable Acidity (TA):
QTLs= 2?
h^2= 0.61-0.85
Chr = 1 & 7
Flaking SNPs:
ss_112028
ss_122047
ss_129235
ss_767644
ss_781308

Fruit Diameter (FD):
QTLs= 2?
h^2= 0.63-0.81
Chr = 4 & 8
Flaking SNPs:
ss_383699
ss_393509
ss_863993
ss_874345

Fruit Weight (FW):
QTLs= 2?
h^2= 0.69-0.87
Chr = 2 & 6
Flaking SNPs:
ss_194572
ss_197262
ss_605968
ss_629062

Hydrogen Potential (pH):
QTLs= 2?
h^2= 0.54-0.78
Chr = 1 & 5
Flaking SNPs:
ss_3621
ss_37558
ss_553912
ss_583962

Days to fruit ripening (RD):
QTLs= 3?
h^2= 0.84-0.98
Chr = 4
Flaking SNPs:
ss_378668
ss_386692
ss_400613
ss_440662
ss_451993
ss_513211

Fig. 2. Seven pomological traits, Soluble Solid Content (SSC), Titratable Acidity (TA), pH, Fruit Diameter (FD), Fruit Weight (FW), presence/absence of red color close to the pit (RP) and Julian Days to Fruit Maturity (RD), mapped through the application of the PBA on a representative pedigree of 354 individuals from the UCD Processing Peach Breeding Program. The results showed that for those seven traits, 14 QTLs (shown here as concentrated area of data-points and distinct horizontal peaks in the vertical line graph) were mapped across the eight chromosomes of peach. Traits such SSC and TA showed QTL regions in common in chromosome 7. Complex traits such as FD and FW showed at least one QTL with strong evidence for influencing the exhibition of such traits, and which can be pursued through the identification of haplotypes for germplasm screening through SNP chips or development of SSRs, and suggest other promising applications for Marker-Assisted Breeding (MAB). Traits such as RD did not exhibit clear QTLs, which suggest that additional strategies for the identification of genetic components influencing the time to blooming and maturity are needed.

RosBreed results have identified the general locations of genes which appear to contribute to our measured aspects of fruit quality. The SNP markers which identified the sites (as their presence was highly correlated with the presence of the specific trait), while probably not part of the controlling gene, appear located close enough to it to act as a marker for its presence or absence.

	DAF	FD	FW	pH	RD	RP	SSC	TA
DAF	1	0.71	0.87	0.74	0.92	0.31	0.76	0.68
FD	0.71	1	0.94	0.67	0.84	0.41	0.62	0.61
FW	0.87	0.94	1	0.69	0.93	0.47	0.67	0.71
pH	0.74	0.67	0.69	1	0.63	0.52	0.73	0.74
RD	0.92	0.84	0.93	0.63	1	0.27	0.79	0.76
RP	0.31	0.41	0.47	0.52	0.27	1	0.19	0.36
SSC	0.76	0.62	0.67	0.73	0.79	0.19	1	0.87
TA	0.68	0.61	0.71	0.74	0.76	0.36	0.87	1

Figure 6. Correlations between different genes as determined by analysis of the RosBreed data. A high correlation would indicate either that the traits are physiologically related or are genetically linked (located very close to each other on the same chromosome). For example, SNP marker data suggest that at least part of the correlation between titratable acidity (TA) and soluble solids (SSC) may be due to the close proximity on chromosome 7.

A similar chromosome location for different traits, (such as titratable acidity and soluble solids on chromosome 7 in Figure 2) suggest that these traits may be linked. (That is, they are so close together that selection for one inherently selects the other, which would be good if both traits are desirable but a problem if one trait is desirable and the other is undesirable). RosBreed data were used to determine the correlation between different traits (Figure 6). This information is particularly useful when using this germplasm to select for a targeted trait because it informs you on the possible effect on other traits of interest. A more direct application of the immense RosBreed database is the determination of breeding values of different breeding program parent genotypes for the different traits of interest. Table 2 summarizes results of a genetic breeding value analysis of UCD parents for large fruit size. (A high fruit sizing potential is important both to achieve commercial size as well as to achieve more uniform yields, particularly in years with excessive thinning; see 2012 annual report). Results are consistent with established field performance (summarized in 2012 annual report). Potential complications of the RosBreed approach result from

Table 2. Genetic breeding values for large fruit size for selected UCD genotypes as determined by RosBreed analysis. [90_9_116=ExtraEarly#1, D62_193= UltraEarly#1, 96 9 292=Compact#2]

Ranking	Genotype	GBV
1	Klampt	132.66
2	90_9_116	129.15
3	D62_193	120.77
4	96_9_292	117.34
5	Lilliland	115.82
6	2005_10_199	112.01
7	2005_10_247	105.22
8	CarolynG	104.82
9	2005_10_100	101.47
10	18_8_11	101.21
351	2005_17_081	-71.02
352	2005_17_088	-71.14
353	2005_17_119	-71.65
354	2005_17_109	-71.93
355	2005_17_123	-72.17
356	Vilmos	-79.03
357	Jordanolo	-86.61
358	Nonpareil	-99.06
359	F10C_20_51	-99.87
360	persXdavidia	-110.06

the very high diversity of the germplasm tested. For example, many different forms of the genes conferring large-to-small fruit sizing would be expected because both wild and cultivated material was evaluated. However, strong selection has probably already occurred within the cultivated material for large-fruit size genes using traditional breeding methods. Consequently, the genes having major effects on the traits of interest may have already been fixed or concentrated in our advanced breeding lines. Thus, results thus need to be verified within more highly selected breeding lineages. A second problem is that, because of the generalizing nature of the statistical approach used, promising, though relatively isolated and so rare genes (as may have been transferred from a related species) would be less likely to be identified. Yet these novel, previously unexploited genes/traits often have the highest potential for further improving the genotype. A final concern is the ultimate accuracy these markers, since detailed analysis of recombination within individual breeding lines has identified several populations where marker data does not correspond to actual inheritance patterns. [Many of these populations have resulted from interspecific hybridizations and so may reflect higher levels of genetic/genomic complexity then can be measured by these more linear and so limited molecular approaches. (See larger discussion of this potential problem in 2011 and 2012 annual reports].

USDA NRI Fruit Quality Study.

A long term project funded by the USDA-NRI (NIFA) was conducted by the cling peach breeding program of UC Davis over the past several years. From this project a partial whole genome sequence of two specific peach cultivars and an almond by peach introgression breeding line (Freestone, non-melting selection F8,1-42) were obtained, followed by development of 6,657 SNPs and the construction of a high-density consensus SNP map from two mapping populations. To study the relationship between the genome and specific traits, we have completed the first biological peach genome interrogation using bioinformatics tools. We combined classical QTL analysis, genomic annotation resources in peach, and bioinformatics tools that allowed us to obtain an extended list of new candidate genes that can contribute to a more complete understanding of important quality traits in peach (Figures 3, 4 and 5).

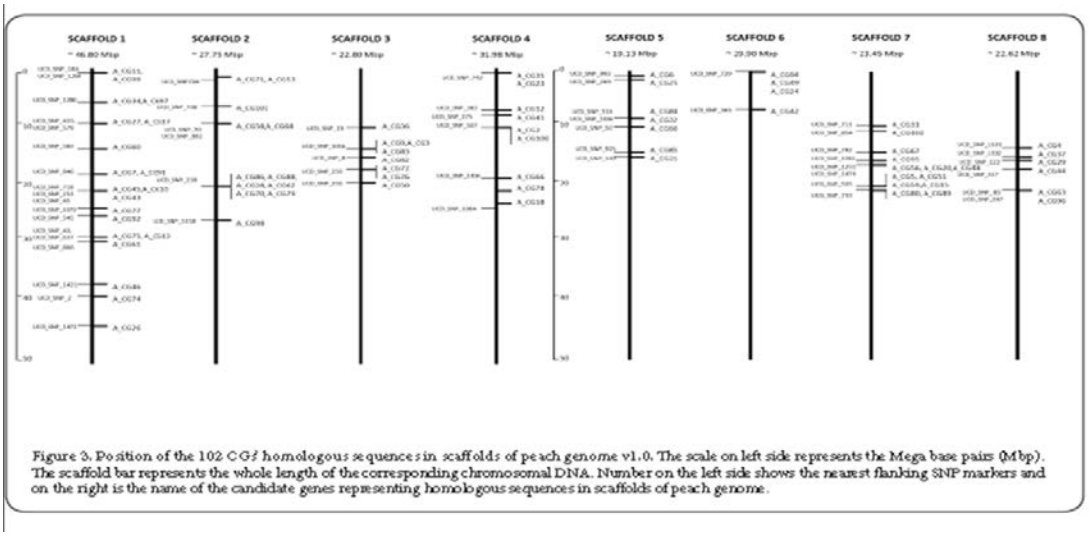

Figure 3. Position of the 102 *CG1* homologous sequences in scaffolds of peach genome v1.0. The scale on left side represents the Mega base pairs (Mbp). The scaffold bar represents the whole length of the corresponding chromosomal DNA. Number on the left side shows the nearest flanking SNP markers and on the right is the name of the candidate genes representing homologous sequences in scaffolds of peach genome.

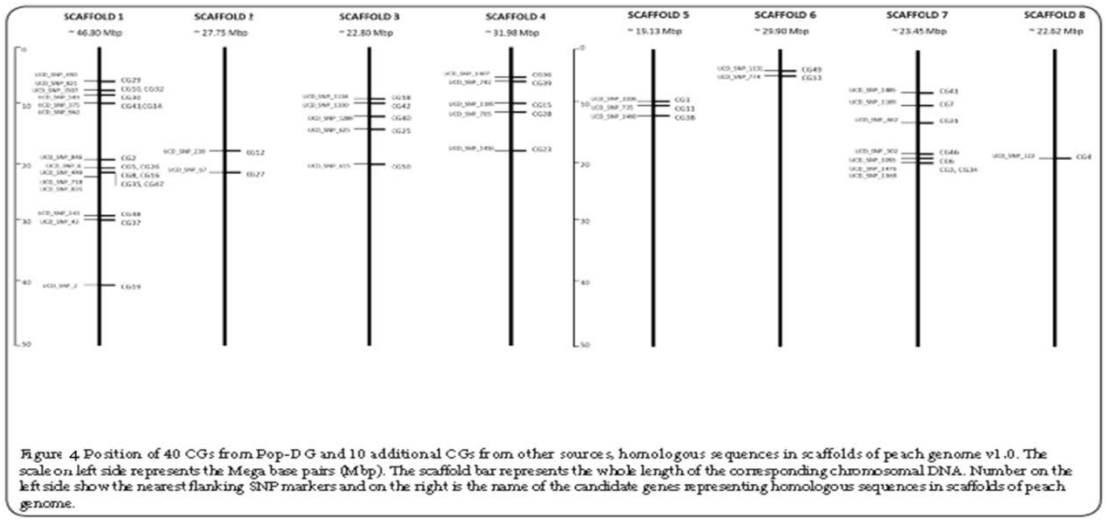

Figure 4. Position of 40 CGs from Pop-DG and 10 additional CGs from other sources, homologous sequences in scaffolds of peach genome v1.0. The scale on left side represents the Mega base pairs (Mbp). The scaffold bar represents the whole length of the corresponding chromosomal DNA. Number on the left side show the nearest flanking SNP markers and on the right is the name of the candidate genes representing homologous sequences in scaffolds of peach genome.

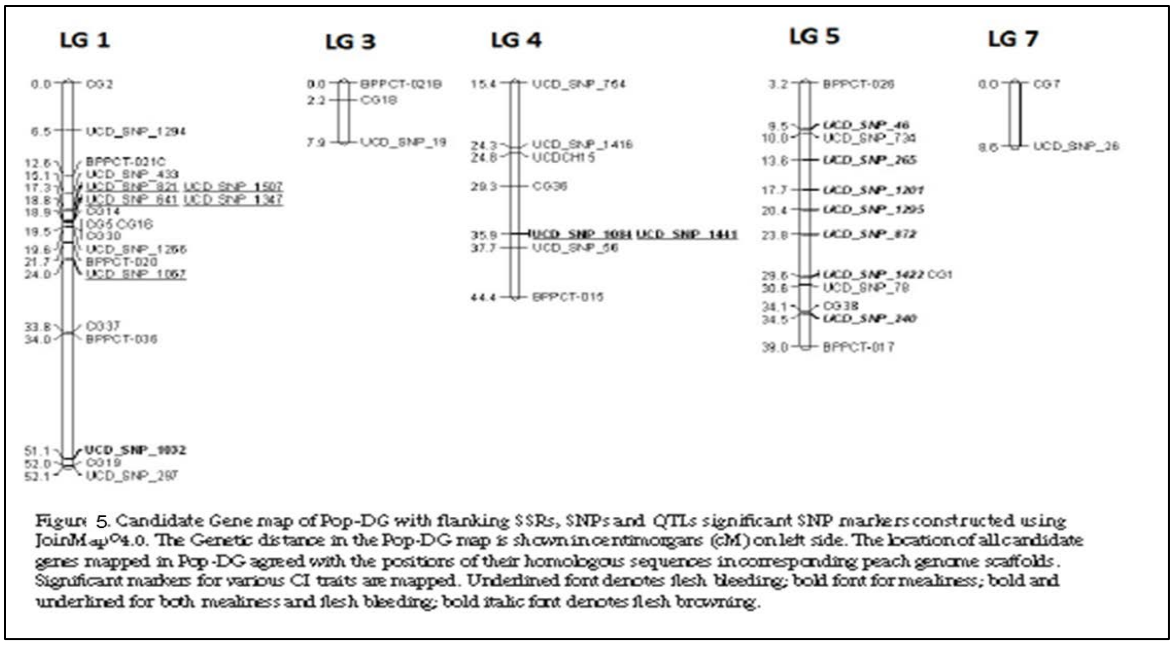

Figure 5. Candidate Gene map of Pop-DG with flanking SSRs, SNPs and QTLs significant SNP markers constructed using JoinMap®4.0. The Genetic distance in the Pop-DG map is shown in centimorgans (cM) on left side. The location of all candidate genes mapped in Pop-DG agreed with the positions of their homologous sequences in corresponding peach genome scaffolds. Significant markers for various CI traits are mapped. Underlined font denotes flesh bleeding; bold font for mealiness; bold and underlined for both mealiness and flesh bleeding; bold italic font denotes flesh browning.

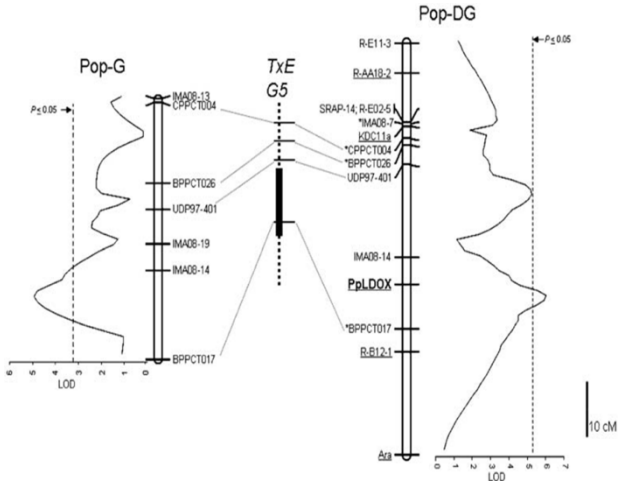

Figure 7. Linkage group G5 of Pop-DG and Pop-G showing the LOD peaks of the peach flesh browning QTL (qP-Brn5.1m) and the location of PpLDOX (in bold). Open vertical bars represent linkage groups. Markers are to the left while LOD chart is to the right of the linkage group in Pop-DG, and vice versa for Pop-G. For Pop-DG integrated map, underlined markers were from 'Dr. Davis', markers with asterisks were heterozygous in both parents, and all other markers were from 'Georgia Belle'. The dashed vertical lines represent LOD thresholds determined for 3 years average data ($P \leq 0.05$) calculated based on 1,000 permutations. A section of G5 of T × E Prunus reference map is represented between Pop-G and Pop-DG showing bin 5:21 (solid bar) and the position of markers. *[Details in Publication #2, 3, 8]*.

Populations studied included the cross of the processing peach cultivar *Dr. Davis* by (a) *Georgia Belle* {an heirloom Freestone variety considered to be an important founder for many modern freestone peaches} and (b) UCD almond to peach introgression line *F8,1-42*, a unique almond by peach hybrid derived non-melting, freestone peach. Fruit quality traits analyzed included flesh browning, flesh bleeding, and mealiness. Preliminary candidate markers associated with these traits are mapped in figures 3 and 4 with a summary of the most promising molecular markers presented in figure 5 for linkage groups (LG) {i.e. chromosomes} 1, 3, 4, 5, and 7. Markers for the candidate gene PpLDOX from *Dr. Davis* appear particularly promising for optimizing resistance to fruit flesh browning (Figure 7). Ongoing research is examining whether this marker can be used as a selection aid for flesh browning resistance and whether it can be used to more fully identify the gene responsible. Flesh browning is an important harvest/post-harvest problem with both processed and fresh-market peaches and is particularly important in developing brown rot resistant fruit since many mechanisms involved increased undesirable fruit flesh browning/ bruising.

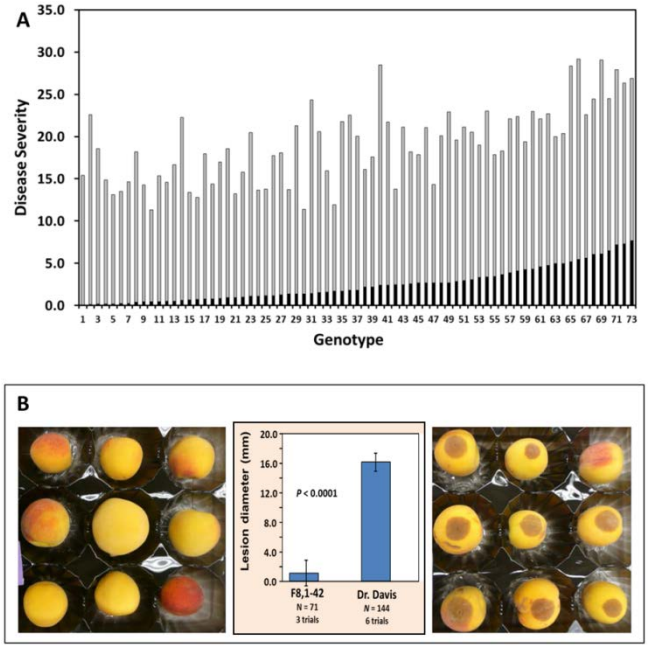

Figure 8. Average disease severity for each of 73 genotypes within the PopDF population over three seasons (2007–2009) in order of increasing values for non-wounded fruit (black column) and the corresponding disease severity in wounded fruit for that genotype (gray column) (A). Representative fruit disease reactions of parents (center), highly resistant (genotype 01,9–38; left panel) and highly susceptible (genotype 01,9–38; right panel) genotypes in PopDF at 72 hours after inoculation with *Monilinia fructicola*. The average disease severity values corresponding to these sets of fruit are 0.0 (left) and 13.7 (right).

USDA-NRI fruit brown rot resistance study.

A companion study evaluated the inheritance patterns of fruit brown rot resistance and related molecular markers in the cross *Dr. Davis* by *F8,1-42* (PopDF). Long-term collaboration with Rick Bostock and our molecular biology collaborators have identified a range of resistances in this material (Figure 8A) as well as the presence of both susceptible and resistant individuals in the parents and their progeny (Figure 8B). Using molecular marker techniques similar to those reported in the previous section, promising candidates for brown rot resistance were identified in linkage group 1 (Figure 9) complementing the markers for associated flesh quality summarized in figure 7. Using the determined location of this brown rot resistance associated SNP marker and an extensive web-based database on gene positions and functions, we have identified several candidate genes located in this region which could theoretically function in disease resistance. From this list of candidates genes, four candidates: zinc finger (ppa007509m, C3HC4-type RING finger) family protein, the AOX1A (ppa014817m,

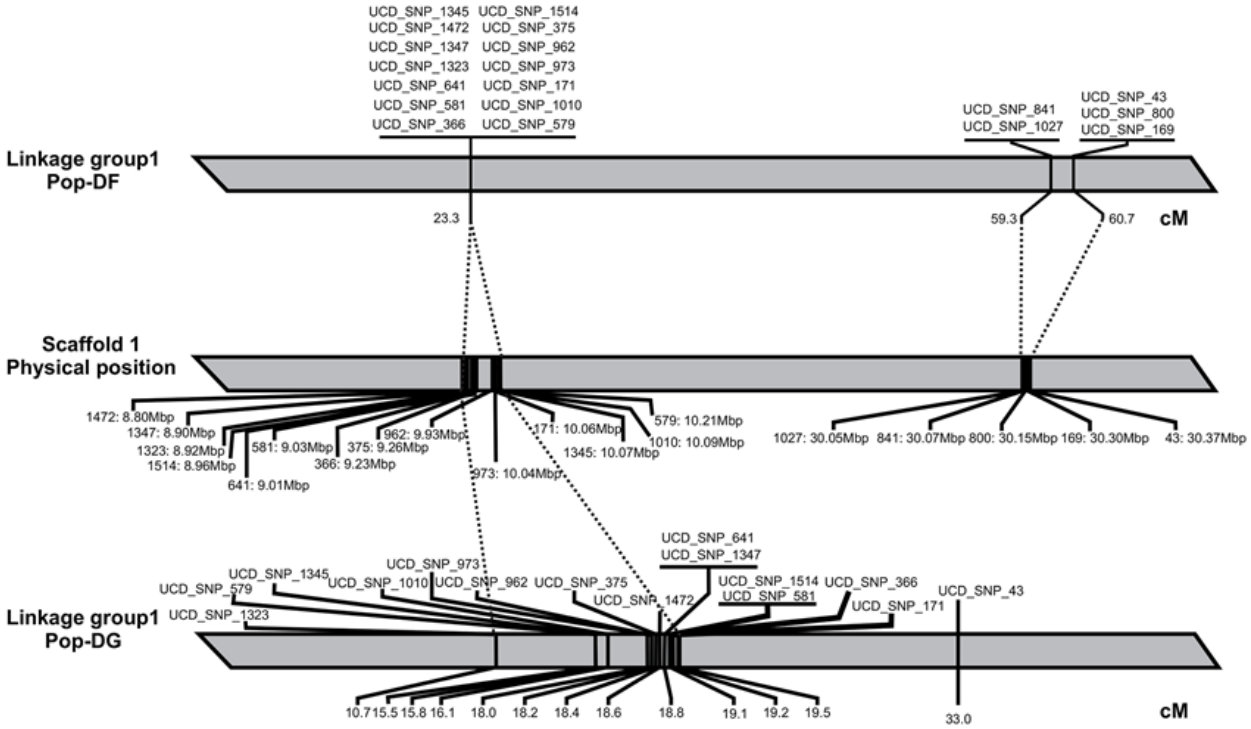

Figure 9. Comparison of genetic map positions of SNPs associated with Brown rot resistance on linkage group 1 of Pop-DF, with their physical map positions on chromosome 1 and their positions in linkage group 1 of Pop-DG. [See Publication 10]

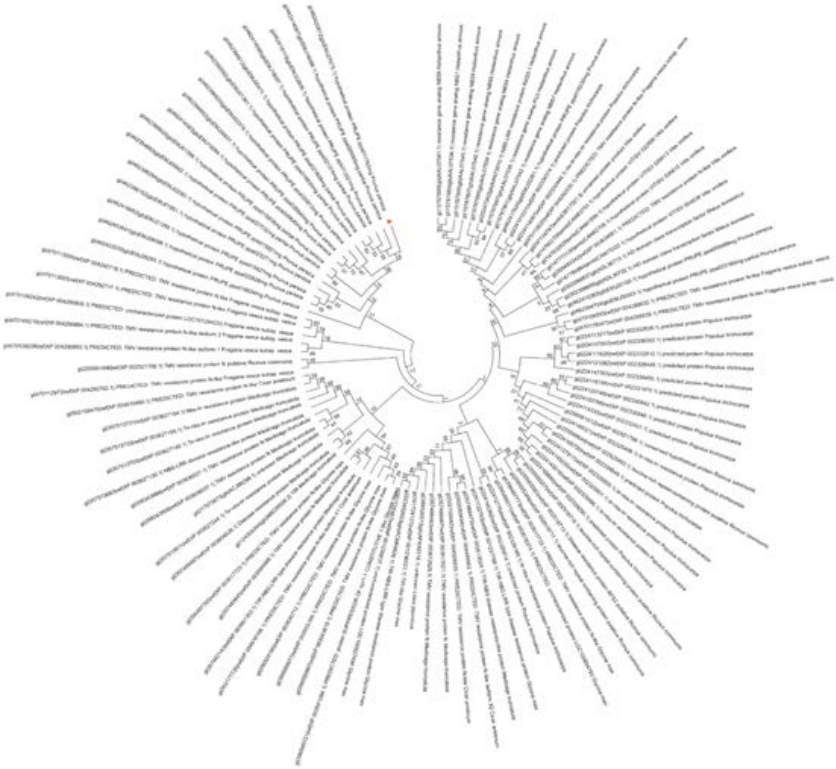

Figure 10. Evolutionary relationships of taxa. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 100 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 85 positions in the final dataset.

alternative oxidase), TMV resistance protein N (ppa011763m) and receptor-like protein

kinase ANXUR2 (ppa026453m), appear to be potential target genes to develop future breeding strategies in peach. Zinc finger (C3HC4-type RING finger) family proteins were involved with cell wall redox, and were associated with freestone-melting flesh, mealiness and flesh bleeding in the companion study. The zinc finger also showed a positive correlation with mealiness at several stages of cold storage and ripening. Similarly, the SNP marker UCD_SNP_1084 could be an important biomarker (a QTL) for fruit quality and chilling injury symptoms such as mealiness and flesh bruising. AOX1A and PPO, also needs to be studied in greater depth to clarify the antioxidant attribute related to this symptom in peach. SNP marker UCD_SNP_641 was associated with a 1293 nucleotides gene with high similarity to the N-gene (ppa011763m,) for Tobacco mosaic virus (TMV) resistance in tobacco. Given the results of this new research, and the absence of NBS and LRR domains in our transcript sequence of ppa011763m, we think that host recognition of brown rot disease-causing organism *M. fructicola* in peach may be mediated by this candidate gene, activating the effector-triggered immunity (ETI) response. To examine this hypothesis, a phylogenetic analysis was conducted using a data set from the alignment (100 sequences) of the amino acid sequences of our ppa011763m gene together with several paralogous proteins and other protein sequences related to our gene but from other species. This analysis (Figure 10) showed that our candidate forms a different clade or grouping from all other paralogous proteins and is supported by a strong (74%) bootstrap support value. The topology observed implies that this gene family has diversified recently (multiple paralogous duplications), perhaps only within the Prunus genus. [This would explain why both almond and peach are affected and also why usable resistance may be transferable from almond.] Absence of more similar sequences in apple or strawberry which are also in the Rosaceae family, implies that gene duplication occurred after divergence between Prunus (stone fruit) and Malus (pome) lineages. Other Prunus transcripts similar to this sequence have the NBS-LRR domains, suggesting that a domain deletion occurred recently. The SNP marker UCD_SNP_1472 was associated with the receptor-like protein kinase ANXUR2 (ppa026453m), a protein that controls pollen tube behavior by directing rupture at proper timing to release the sperm cell. According to the model proposed, our ppa026453m could be a candidate gene for peach to trigger PAMP-triggered immunity (PTI) response for *M. fructicola* in peach. PTI have been observed to be involved in signaling through Ca2+ and H+ influx, early accumulation of reactive oxygen species (ROS), the mitogen-activated protein kinases (MAPKs), etcThis QTL may facilitate the characterization of metabolic pathways and improve protocols for phenotyping by identification of biochemical changes affecting structure or availability of substrates, with subsequent application to peach breeding programs. The marker UCD_SNP_46 was identified by SnpEff as the exon of AOX1A and is a synonomous coding change in the protein. Flesh browning damage expression is related to cell membrane leakage due to cell damage or senescence, which leads to changes in membrane permeability and merging of phenolic compounds (substrate) and the PPO (catalyzer) in the cell, producing a browning reaction. [This biochemical reaction is currently being studied in collaboration with Diane Barrett in Food Science].

In summary, the candidates identified provide both potential markers which may improve breeding selection, and also offer avenues for the identification of the specific gene/resistance mechanism involved (and so provide information on the actual mechanisms and their potential for manipulation). As in previous studies, however, the evaluated populations were very diverse by design (in order to capture for assessment

the highest level trait and genetic variability) and the finding that most of the most desirable genes were inherited from either the UCD processing peach *Dr. Davis* or advanced breeding line *F8,1-42* suggest that these promising traits may have already been fixed within our breeding program. Further and more detailed analysis is thus required. In addition, to improve our ability to identify and transfer new genes to processing peach, information is needed concerning recombination rates within different peach populations as well as the recombination of peach with some of its related species, such as almond. Results of an initial analysis, using both RosBreed and earlier UCD data, show that peach and almond clearly differentiate based on molecular inheritance patterns (Figure 11). Interestingly, the addition of progeny from our crosses to the non-melting, stony-hard Korean peach *Yumyeong'* showed that it could be clearly distinguished from both peach and almond, suggesting that it may represent a unique and isolated peach germplasm. The stony-hard trait from *Yumyeong'* is notable as it has been previously reported to confer both firmer flesh and a *long-keeper* capacity to some of its progeny. However, a multiyear study by our program has found that the increase in fruit firmness and subsequent fruit quality was not sufficient to counteract the undesirable aspects of stony flesh texture (similar to tree-harvested Bartlett pear) on reduced processing flesh texture and color quality.

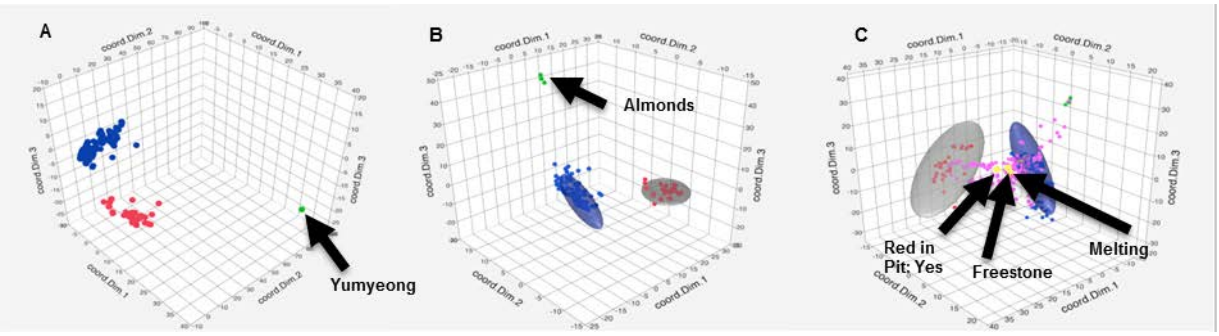

Figure 11. The Genetic Structure identified in a pedigree of 354 individuals, including peach and almond cultivars, as well as introgression breeding lines, from the Processing Peach Breeding Program at UC Davis. (A: Projection of the UC Davis germplasm, in green 'Yumyeong' (male parent), recognized as outlier. B: Projection of the UC Davis germplasm, discarding 'Yumyeong', in green 'F10C,20-51', 'Jordanolo' and 'Nonpareil' (all almonds). C: Projection of the UC Davis germplasm, discarding 'Yumyeong', jointly with 215 bi-allelic SNPS (each pair of alleles is a category) and pomological traits (in yellow)). shows that the clustering of the germplasm is related to "stone-adhesion/flesh-texture", but is not completely driven by these characteristics. This result suggest that genetic phenomena such as the generation of new alleles through the generation of new linkage-blocks (resulting from the recombination of closely related genomes, but each genome having a different evolutionary context {e.g. peach = long LD, almond = short LD}). This recombination may have serious implications for the application of PBA and hence, the mapping of QTL for pomological traits in this breeding germplasm.

The removal of Yumyeong progeny data from our analysis allowed a much clearer separation of the Freestone peach group from the clingstone group as well as a clear separation between the peaches and almonds (Figure 11B). This data is useful in evaluating the potential for transfer of desirable genes/traits between these groups as well as possible risks. For example, the analysis presented in Figure 11C indicates that certain groups are more predisposed to transferring genes for red pigmentation of the pit, which while desirable in many Freestones is not desirable for processing clingstones. A second important conclusion from this study was that linkage

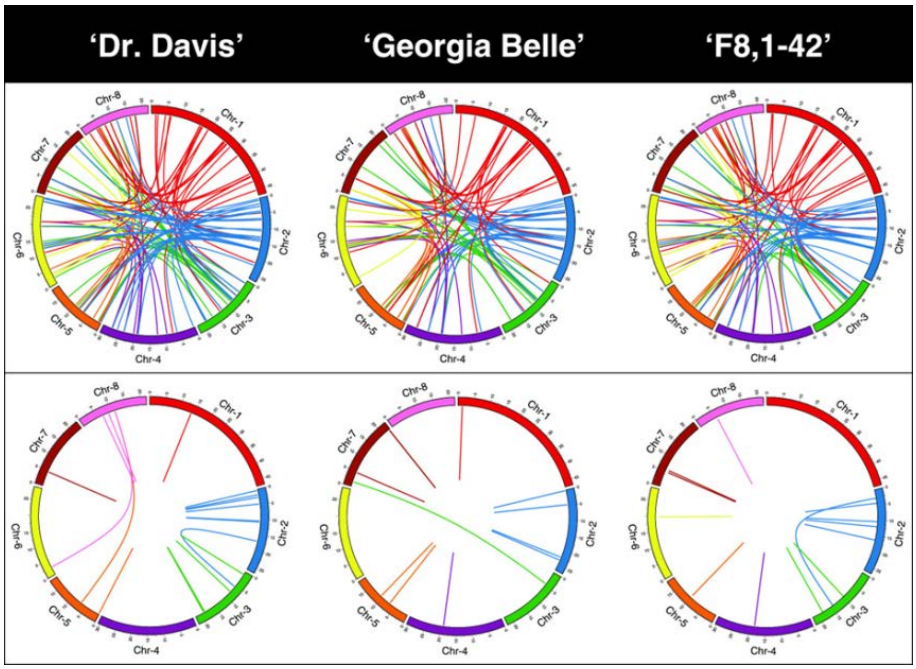

Figure 12.

Visual comparison of the structural variants for three peach cultivars using Circos graphs. The variants were obtained through comparisons with the 'Lovell' Peach Genome Reference Sequence ('Lovell', upper row) and with the exclusive structural variants per genotype (lower row). Non-connected lines correspond to intra-chromosomal variations such as inversions and translocations. [For details, see Publication #9]

disequilibrium (LD) (i.e. the probability for recombination within each linkage group or chromosome section) tends to be lower in peach than an almond. This is important because the transfer of genes from almond to peach require sufficient recombination within the target peach genome to retain the desired almond trait (for example, brown rot resistance) while eliminating by recombination undesired almond traits.

Further molecular analysis of our *Dr. Davis* by *Georgia Belle* (DG) and *Dr. Davis* by *F8,1-42* populations demonstrated that the heirloom cultivar '*Georgia Belle'* and the almond by peach introgression breeding line '*F8,1-42'* are more heterogeneous than the modern processing peach cultivar '*Dr. Davis'* when compared against the '*Lovell'* peach reference genome (Figures 12 and 13). The differences in heterogeneity are reflected in the number of genetic and genomic variants, the types of variants (from DNA insertions and deletions to chromosome inversions and translocations), and the impacts of those variants on the transcribeable and non-transcribeable portions of each genotype analyzed. A pair-wise comparison of consensus genome sequences with 'Lovell' showed that 'F8,1-42' and 'Georgia Belle' are more divergent in compared to 'Dr. Davis in' and 'Lovell'. The results suggest that progenies close to peach founder genotypes conserve more heterogeneity for genetic

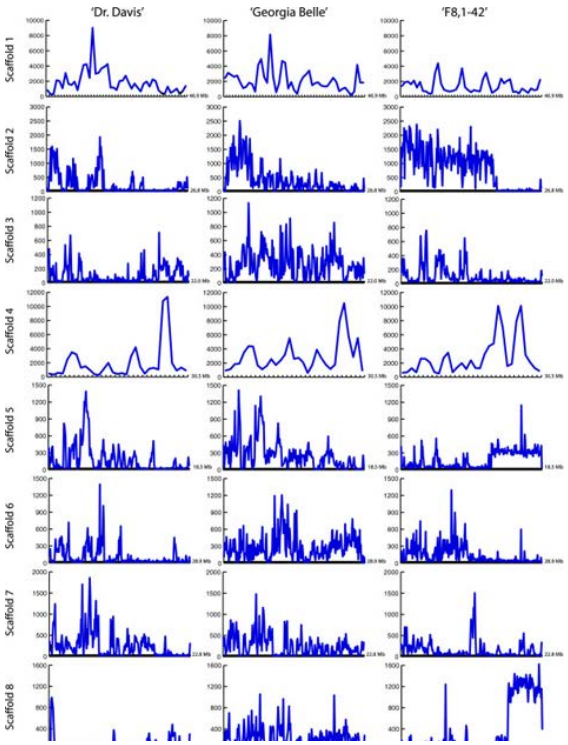

Figure 13. Comparison in the frequency distribution of the variants along each scaffold for 'Dr. Davis', 'Georgia Belle', and 'F8,1-42'. The frequency is given in number of variants per 100 Kb for a particular position in the scaffold.

diversity than modern cultivars do, and that the introgression or transfer of genetic material from related species can promote greater genomic heterogeneity in modern breeding lines. In particular, the research showed that the probability of such variants differed dramatically depending on the specific chromosome or chromosome section (Figure 13). Two implications to applied breeding would be (a) the location of a trait would affect ease of transfer particularly when the transfers is between species (i.e. introgression), and (b) wide crosses including interspecies crosses may be useful for encouraging an increase in recombination which would be desirable, particularly in wide crosses, to sort out desired from undesired genes. [Genetic recombinations resulting from the easement of previously suppressed recombinations, common particularly in central areas of these largely metacentric chromosomes, often results novel phenotypes (i.e. traits not seen in either

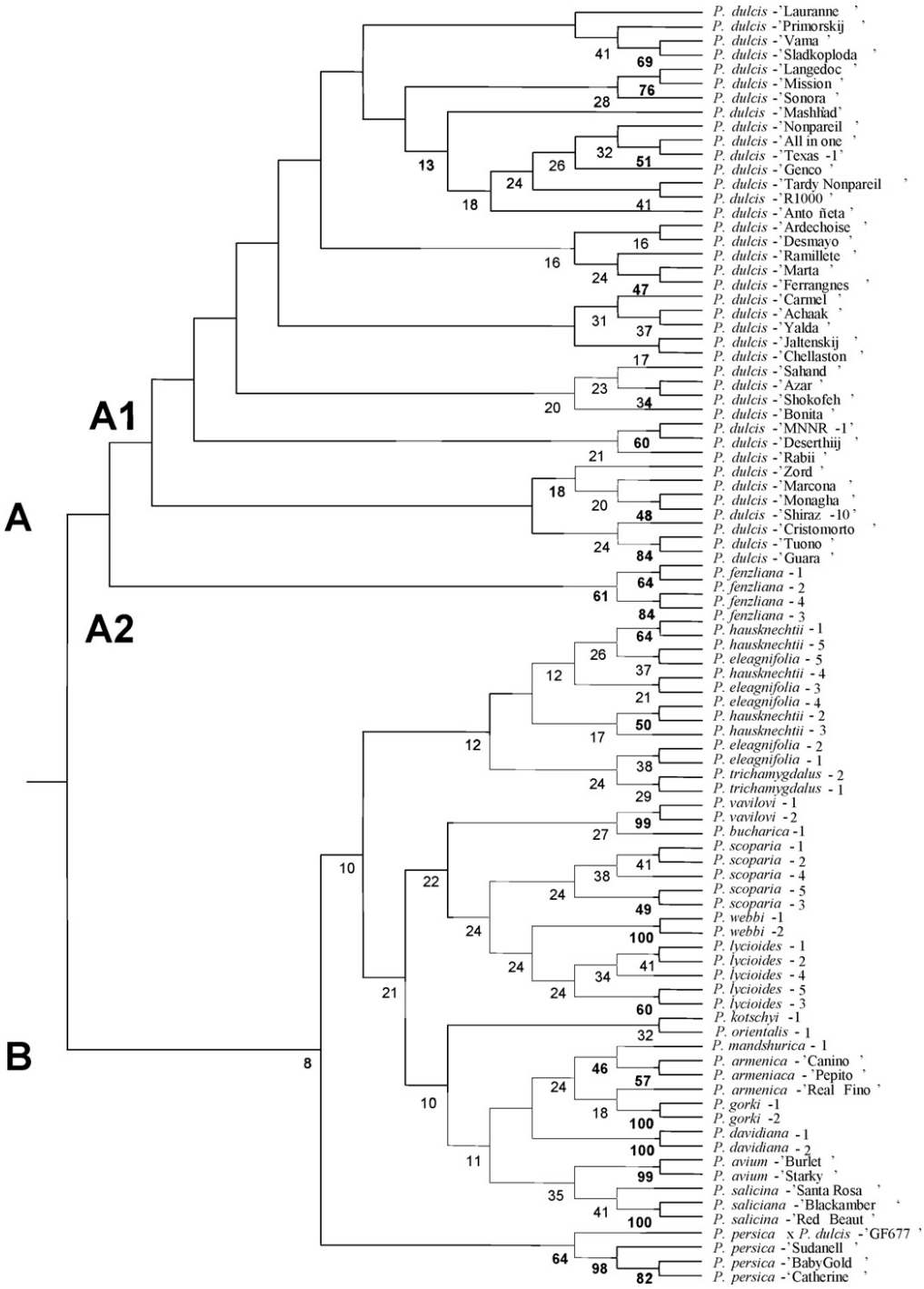

Figure 14. Dendrogram obtained with the similarity Jacard coefficient pair-group method with arithmetic average clustering algorithm (UPGMA) for the 40 almond cultivars and 18 related species. Numbers below branches represent boot strapping values. Most significant bootstrapping values(around50%ormore) were indicated in bold letters. [For details, see Publication #6].

parent, presumably resulting from the novel rearrangement of genes and their chromosome scaffolding). The study of genomic variants is also useful for the determining of genetic control of pomological traits, the characterization of metabolic pathways and the modeling of the inheritance of complex traits, and thus can lead to improved protocols for phenotyping in research and breeding. If verified, interspecific hybrids and subsequent gene introgression to cultivated peach may result in the expression of new phenotypes or traits by two separate methods (a) the transfer specific genes controlling that trait [as in our recent transfer of a mildew resistance gene from almond to peach (see 2011 annual report)], and (b) more complicated genomic restructuring which results in novel trait expression (as in the hybrid vigor resulting from almond by peach hybrid rootstocks (see 2012 annual report). Since the degree of such genomic restructuring appears connected to the degree of relatedness between species, more detailed information is needed in this area of taxonomy and systematics. Results from a recent collaboration with Iranian scientists are presented in Figure 14 which provide a more precise characterization of species relationships and identify a number of potentially useful new species as sources of traits for future peach improvement. [See reference 6 for research details].

In summary, molecular markers have provided promising opportunities for specific genetic improvement as well as new approaches for genomic manipulation to achieve breeding goals. However, many of the most promising traits identified by molecular marker analysis have probably already been incorporated into the UCD breeding program. (We're doing follow-up analysis on more advanced lineages to the test this). Since the complexity of associated genomic interactions is currently poorly understood, we lack the ability to manipulate them efficiently. Because (as demonstrated in the 2012 annual report) optimization of these additional genetic/genomic interactions is essential to optimize cultivar performance, the breeding program needs to proceed in a two-pronged approach: (a) pursue a more detailed understanding of these interactions as a basis for more efficient manipulation, and (b) compensate for the current inefficiencies in our genetic/genomic manipulation by increasing the size of the breeding populations manageable under our current economic constraints.

As documented in Table 1, breeding program efficiency in terms of our ability to generate and effectively do selection on very large populations has increased substantially. This effort has consumed the major portion of CCCAB funding, which, while making possible much of the molecular work highlighted in this report, has as its main product the generation of new breeding lineages and selections. Consequently, this report will conclude with a representative sampling of a few of the over 30,000 genotypes evaluated in 2013 and the 400 selections advanced to evaluation at the UCD processing pilot plant facility. [Results from the 2013 evaluation of advanced selections are summarized in the annual Regional Testing report].

Sample breeding lineages and selections from 2013.

The eight selections summarized in this section were chosen as representative of the diverse breeding lineages currently incorporated into the UCD processing peach breeding program. For more comprehensive overview of the complex pedigrees currently utilized in this program referr to the pedigree summary chart presented in the 2012 annual report.

UCD 2009,25-36. [*Dr. Davis x Compact#2*]. Parents used in this cross have some of the highest genetic breeding values for fruit size (see Table 2). Compact tree size is also transferred to this individual as a major incompletely dominant gene, and so is very predictable in its genetic manipulation. Finally, *Compact#2* represents germplasm brought into the program specifically to bridge the *Dixon-Andross* maturity gap (traditional California genotypes such as *Carson* by *Dr. Davis* would tend to segregate either before or after *Dixon* but rarely ripen at the *Dixon* time. Compact#2 also incorporates resistance to fruit brown rot

UCD 2010,17-170. [*2000,16-22 self*]. *Andross* ripening time. Lineage (UCD 92,14-73) is notable for a uniform yellow-gold flesh color in most to all progeny with absence of any red pigmentation indicative of control by dominant gene(s) and so relatively easy genetic management. Lineage is also a source of *long-keeper* trait. Fruit shown were harvested three weeks after fruit ripe date. Lineage origin is uncertain but appears to be Eastern European. Lineage also contributes resistance to flesh bruising as well as improved tree productivity.

UCD2010,21-450. [*Early#6 x ExtraLate#2*]. *Dixon* season. Early#6 lineage has proven a good source of uniform flesh color without red pigmentation as well as long-keeper trait (as above) but conferring a more luminous or bright gold-yellow color along with good tree productivity but somewhat smaller fruit size. Derivation is from South African germplasm but represents a rare recombinant that avoids the dull orange flesh color and propensity to fruit softness characteristic of this germplasm source. Once isolated, desired traits are readily transferred to progeny from this lineage. Fruit shown were harvested 3 weeks after fruit ripe date.

UCD 2010,1-312. [*Loadel x Early#6*]. *Halford* ripening season. The Early#6lineage again demonstrates uniform, fruit color in progeny as well as long-keeper trait. Fruit shown were harvested 3 weeks after fruit ripe date. Interestingly, although parent Early#6 is *Andross* time, progeny of this cross to Loadel ripens with *Halford*, yet siblings ripen in the 'Early' harvest-season in the previous selection which was crossed with an Extra-Late selection (This demonstrates the failure of progeny ripening times to show a normal distribution between parent dates as is typical in traditional germplasm, but which can deviate significantly when new germplasm is incorporated).

UCD96,1-171. [*R7-1 x Ultra-Early#1*]. *Dixon* season. Ultra-Early#1 lineage is derived from Brazilian (though probably ultimately Portuguese) germplasm and is notable for combining very good productivity and size (note the high genetic breeding value for size in Table 2) in a very early maturing selection. This lineage has also been a good source of brown rot resistance and uniform yellow-gold flesh color. Fruit shown were harvested one week after ripe date. As with Early#6, isolation and concentration of desired genes is the result from multiple targeted backcrosses as well as possible epigenetic manipulations (see 2012 Regional Testing annual report).

UCD2010,5-150. [*Dr. Davis x Crimson Lady*]. *Dixon-Andross* season. *Crimson Lady* was incorporated into the breeding lines shortly after Ultra-Early#1. However, most breeding lines segregated strongly for undesirable red in pit and stronger red blush on skin. Lineage is also a source of the *long-keeper* trait but so far has been less promising because of the occurrence of red in the pit in older fruit as well as some red bleeding into the flesh. Further crosses are being made with the hopes of isolating desirable and undesirable genes as has been achieved in previous breeding lines. Fruit shown were harvested one week after tree ripe date.

UCD2008,33-230. [*2001,9-104 x F8,1-42*]. *Dixon-Andross* season. *F8,1-42* is a unique almond derived peach breeding line which according to RosBreed analysis is genetically non-melting clingstone, yet is distinctly freestone yet still largely non-melting. This lineage also contributes resistance to fruit brown rot and flesh bruising as documented earlier in the report. The non-melting/freestone trait in this lineage is often,

but not always, associated with strong red pigmentation in the pit. This germplasm has been employed towards the development of a consistent non-melting flesh with a more freestone to avoid pit fragmentation problems. While the trait can be strongly pronounced in the parent and some progeny, the inheritance is typically very variable and may involve epigenetic as well as genetic factors. Fruit shown were harvested 3 weeks after tree ripe date.

Recent Publications:

1. Gradziel. T.M. 2012. Classical genetics and traditional breeding. In: A. G. Abbott & C. Kole (eds.). Genetics, Genomics and Breeding of Stone Fruits. Science Publishers,, Plymouth. pg. 22-53.
2. Prabhu Dhanapal, A., Pedro J Martínez-García, Thomas M Gradziel, and Carlos H Crisosto. 2012. First genetic linkage map of chilling injury susceptibility in peach (Prunus persica (L.) Batsch) fruit with SSR and SNP markers. Journal of Plant Science & Molecular Breeding Pg1-12. http://www.hoajonline.com/journals/jpsmb/content/pdf/3.pd
3. Martínez-García P., Peace C., Parfitt D., Ogundiwin E., Fresnedo-Ramírez J., Dandekar A., Gradziel T., Crisosto C. 2012. Influence of year and genetic factors on chilling injury susceptibility in peach (Prunus persica (L.) Batsch). Euphytica: 185:267-280. DOI: 10.1007/s10681-011-0572-1
4. Gradziel, T.M. & Martínez-Gómez, P. 2013, Almond Breeding. Plant Breeding Reviews 37:207-258.
5. 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.
6. Rahemi, A., Fatahi, R., Ebadi, A., Taghavi, T., Hassani, D., Gradziel, T., Folta, K. & Chaparro, J. 2012. Genetic diversity of some wild almonds and related Prunus species revealed by SSR and EST-SSR molecular markers. Plant Systematics and Evolution, 298: 173-192.
7. Gradziel, T., B. Lampinen, F. Niederholzer, and M. Viveros. 2013. 'Sweetheart' Almond: A Fully Cross-compatible Pollenizer for the Early 'Nonpareil' Bloom that Exhibits Very High 'Marcona'-type Kernel Quality. HORTSCIENCE 48:1320–1322.
8. Martínez-García, P.J. D.E. Parfitt, E.A. Ogundiwin, J. Fass, H.M. Chan, R. Ahmad, S. Lurie, A. Dandekar,T.M. Gradziel, and C. H. Crisosto. 2013. High Density SNP Mapping and QTL analysis for fruit quality characteristics in peach (Prunus persica L.) Tree Genetics and Genomes. 9:19-36 DOI 10.1007/s11295-012-0522-7.

9. Fresnedo-Ramírez J., Martínez-García P., Parfitt D., Crisosto C. Gradziel T. 2013. Heterogeneity in the entire genome for three genotypes of Peach [Prunus persica (L.) Batsch] as distinguished from sequence analysis of genomic variants. BMC Genomics. 2013 Nov 1;14(1):750.

10. Martinez Garcia, P.J., Dan E. Parfitt; Richard M. Bostock; Jonathan Fresnedo-Ramirez; Alejandra Vazquez-Lobo; Ebenezer Ogundiwin; Thomas M. Gradziel; Carlos H. Crisosto. (2013). Application of Genomic and Quantitative Genetic Tools to Identify Candidate Resistance Genes for Brown Rot Resistance in Peach. PLOS ONE.

11. Frett, T., K. Kasic, J. Clark, D. Byrne, T. Gradziel and C. Crisosto. 2013. Standardized phenotyping for fruit quality in peach [Prunus persica (L.) Batsch]. J. American Pomological Society. 66:214-219