Almond Variety Development

Project No.: **15-HORT1-Gradziel**

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Objectives:

Develop (1) improved pollenizers for Nonpareil, and ultimately, (2) varieties that possess self-fertility and improved market value and resistance to disease, insects and environmental stress. Specific objectives for 2015-2016 include:

1. Generate 16,000 new seedling progeny with subsequent field plantings of ~10,000 new trees. Evaluate and reduce by an additional 20% the ~22,000 progeny trees currently in breeding trials through continued development and implementation of low-input/high-throughput breeding efficiency strategies.
2. Identify effective predictors of yield potential (annual and cumulative) to assess opportunities/limitations of traditional as well as evolving biotech approaches including molecular marker-assisted-selection (MAS).
3. Assess opportunities and limitations of advance breeding germplasm currently being tested in Regional Variety Trials (RVTs). Expand smaller regional grower new trials to evaluate next generation selections.

Summary Abstract:

The goal of the UC Davis almond variety development program is the development of new varieties possessing genetic solutions to meet the changing demands of California almond production. Improved genetics, providing advantages ranging from self-fruitfulness to resistance to diseases, pests and environmental stresses, has been achieved through a long-term effort of transferring desired genes from wild and cultivated relatives. A concurrent goal of this genetic improvement program is the development of California adapted germplasm possessing a broad diversity of potential genetic solutions to both immediate challenges as well as unanticipated challenges such as resistance to new pests/diseases and compatibility with new cropping systems, and make this germplasm generally available to both public and private California breeders. Tree crop improvement is particularly challenging because of the extensive requirements of land and time; thus high breeding efficiency is essential. Breeding efficiency has been achieved through the development of effective strategies for tree and data management to allow the large population sizes and rapid population cycling required for breeding progress. Breeding crosses in 2016 have resulted in over 16,000 seed, with approximately 10,000 seed targeted for field planting after initial greenhouse culling. Molecular marker methods are being utilized to improve our understanding of individual gene as well as larger-scale chromosome or haplotype inheritance. Ten UCD selections have been advanced to the Regional Variety Trials following extensive initial grower testing. An additional 22 UCD selections are currently being propagated for new grower testing in the different almond production areas. The variety Kester has been released providing the industry with a high quality, productive and late-flowering pollenizer producing pollen that is fully cross compatible with Nonpareil as well as all other major California cultivars.

Interpretive Summary

Commercial production in California is dependent almost entirely on the variety *Nonpareil* and a relatively few closely-related pollenizers, most of which have *Nonpareil* and *Mission* as direct parents. A long-term emphasis of the UCD almond breeding program has been the identification and incorporation of new and diverse germplasm. Genetic solutions to emerging production challenges are now becoming available from this improved germplasm, including regionally-adapted selections expressing high productivity, self-fruitfulness, and increased insect, disease and environmental stress resistance. Improved breeding lines also offer opportunities to expand market demand by optimizing phytonutrients in new cultivars, such as the high heart-friendly oleic acid content in the recently released Sweetheart variety (see Reference 1), while minimizing potential health and marketing risks including aflatoxins, allergens and salmonella.

A concurrent goal of this genetic improvement program is the development of California adapted germplasm possessing a broad diversity of potential genetic solutions to both immediate challenges as well as unanticipated challenges such as resistance to new pests/diseases and compatibility with new cropping systems, and make this germplasm generally available to both public and private California breeders. Tree crop improvement is particularly challenging because of the extensive requirements of land and time; thus high breeding efficiency is essential. Breeding efficiency has been achieved through the development of effective strategies for tree and data management to allow the large population

sizes and rapid population cycling required for breeding progress. Breeding crosses in 2016 have resulted in over 16,000 seed, with approximately 10,000 seed targeted for field planting after initial greenhouse culling. Molecular marker methods are being utilized to improve our understanding of individual gene as well as larger-scale chromosome or haplotype inheritance. Ten UCD selections have been advanced to the Regional Variety Trials following extensive initial grower testing. An additional 22 UCD selections are currently being propagated for new grower testing in the different almond production areas. The variety *Kester* has been released providing the industry with a high quality, productive and late-flowering pollenizer producing pollen that is fully cross compatible with *Nonpareil* as well as all other major California cultivars.

Following long-term RVT and grower testing in all major California production regions, the UCD breeding program has released the *Kester* almond variety. This variety is the result of a cross between Tardy-Nonpareil (a late-flowering mutation of Nonpareil) and *Arbuckle*. Kester is fully cross-compatible with *Nonpareil* as well as all other commercial pollenizers and blooms approximately 4 days after *Nonpareil* and so is less vulnerable to damage by early spring frosts. *Kester* kernels are similar to *Nonpareil* but with well-sealed, worm resistant shells. The variety produces low frequencies of double kernels and twin embryos. Harvest is 4 to 7 days after Nonpareil. Trees are upright to spreading and moderately vigorous, being about 80% of *Nonpareil* size at maturity. The *Kester* variety has consistently been among the most productive of all evaluated selections and varieties in over 16 years of Regional Variety Trials. Long-term regional testing also showed no Noninfectious Bud-failure or pronounced susceptibility to commercially important diseases and pests.

1. Generate new seedling progeny.

The target for the 2015 crossing season was the generation of 16,000 new seedling progeny with subsequent field plantings of ~10,000 new trees. To make room for this expanded planting we had also targeted a reduction of approximately 20% the ~22,000 progeny trees currently in breeding trials including the removal of remaining Wolfskill (WEO) blocks 4 through 7. Because of the wide genetic diversity present in these breeding evaluation blocks (as demonstrated by the diverse lineages in **Figure 1**), it was subsequently decided that, rather than bulldozing the blocks, they would be retained through 2016 but without irrigation in order to assess differences in breeding lineages to drought stress.

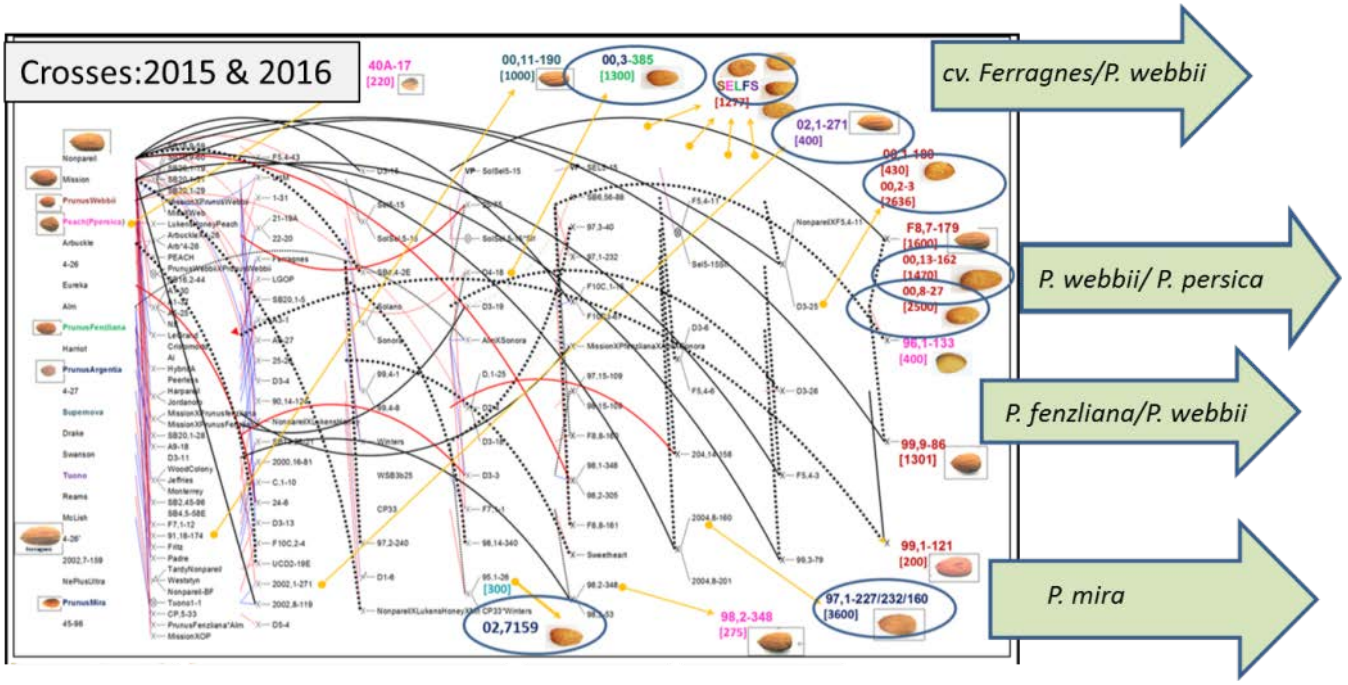

Figure 1. Summary of the UCD breeding germplasm pedigrees from diverse and often wild almond and related species parents (left column) for advanced selections currently being tested in regional variety trials (circled items). Lines connect progeny to parents; solid lines identify seed parent while dotted lines identify the pollen parent. Broad green arrows identify UCD lineages targeted for further, focused improvement in 2015 and 2016 breeding crosses, with the initial germplasm donor being identified within the arrows. Greater emphasis in 2016 targets increased introgression of germplasm containing *P. fenzliana* and *P. webbii* genes.

A summary of crossing results for 2015 and 2016 (preliminary) is presented in **Table 1**. Directed crosses were achieved through the isolation of branches or entire trees through mesh enclosures followed by hand and or bumblebee-mediated pollination as well as through cross pollination in previously established isolation blocks. **Figure 1** is modified from the 2014 Annual Report to show our increased emphasis in 2016 on the inclusion of lineages containing *P. webbii* and *P. fenzliana* in advanced introgression lines in order to better capture the potential disease and drought resistance demonstrated by current introgression lines. (Introgression refers to the hybridization with the related donor species followed by a series of backcrosses to cultivated almond types so that the trait of interest [for example self-compatibility] can be sequentially transferred to a cultivated almond genetic background. This introgression can be visualized in **Figure 1** through the lines depicting the seed and pollen

parents for the multiple generations between the initial wild species cross to the final advanced selection (for example, the wild peach *P mira* with small and poor kernel quality in the left-hand column and the advanced selection 97, 1-227 with commercial almond kernel quality as well as self-fruitfulness in the lower right-hand corner of the box.

The delayed removal of the WEO blocks has also delayed new plantings though the number of seed recovered in 2015 is on target to achieve our goal of approximately 10,000 field planted seedlings. New seedling evaluation blocks have been developed at Davis with over 1,000 seedlings field-planted planted and over 3,000 seedlings in greenhouses with an additional 6000 seed either undergoing stratification or in storage. The Davis plots provide better cultural conditions for early almond growth but extensive crow damage is anticipated when trees come into production, requiring more tedious early-summer evaluation/selection. Additional transplanting at both (WEO) as well as Davis blocks is planned for this fall.

Table1. Summary of breeding crosses and seed/seedling development from 2015 and 2016.

	2015	**2016**
Total crosses	~30,000	~20,000
Recovered seed	~12,000	~2000++
Transplanted seedlings	1327	
Greenhouse seedlings	3568	
Seed in stratification	~3000	
Seed in storage	~3000	

[++ Data for recovered seed in 2016 is preliminary as the harvest is just beginning]

Since the goal of the UCD Almond Genetic Improvement Program is both the development of new cultivars as well as the improvement of germplasm for future cultivar development by both public and private breeders, we are attempting to maintain as broad a genetic diversity as possible while still improving commercial quality in advance lines. While some of the more promising germplasm has been transferred to USDA Prunus Germplasm Repository, current reductions in both USDA land and funding has limited the number transferred though increases are anticipated in the near future.

2. Identify effective predictors of yield including molecular marker-assisted-breeding.
We continue to work closely with Dr. Bruce Lampinen on field-based determinants of cultivar yield, utilizing data from light-interception, tree architectures, and spur-dynamics, at these have proven the most effective predictors of potential crop yield to date (as summarized in previous reports). A goal for 2015-16 was a more detailed assessment of the value of molecular-markers for improved almond breeding efficiency. A specific goal was to assess the accuracy of previous RosBreed (see *https://www.**rosbreed**.org/*) developed markers for genetic analysis in almond and its close relatives. In the UC Davis component of the RosBreed project, over 500 markers uniformly spread across the 8 Prunus chromosomes were analyzed for over 400 genotypes of diverse interspecies lineages. [While the targeted crop was peach, our extensive utilization of almond and its wild relatives for peach genetic improvement, allowed the inclusion of a large number of almond and related species as well as their introgression lineages for RosBreed analysis. Because of the enormous amount of information generated by this project (over 1000 different molecular markers for each of over 1000 peach genotypes as well as associated phenotypic data on fruit and tree characteristics), assessment of individual marker

value required complex statistical analysis using advanced informatics software. Early results suggest value of molecular markers for identifying important components involved in the genetic control of fruit size, crop maturity **Table 2**) and various disease resistances in peach (references 2, 3, 4, 7 and 14). However, the value of many markers has been found to be largely restricted to the particular genetic lineage in which it was identified, which significantly limited its general utility in a breeding program such as ours that utilizes an extended germplasm base. Frequently, markers developed for peach failed to distinguish different genetic alleles and even closely related species such *as P mira* and *P davidiana* as well as almond, *P dulcis*. More detailed results and discussion are presented in that 2015 annual report for Project No. 2015.15-HORT10-Gradziel (Rootstock Breeding Germplasm). Although RosBreed molecular-markers developed for peach lack the precision for precise genotyping in almond and even closely related peach relatives (that is, a specific marker does not identify a specific genotype but rather may give the same result for several related genotypes), a more generalized evaluation of marker distribution does provide valuable information on the larger scale inheritance of common chromosome segments (haplotypes) from different parental sources (pedigrees). [To facilitate visualization of these inheritance patterns, RosBreed marker data has been color-coded in **Figure 2** and other summary charts provided in this report]. Because these plants are diploid, each marker can have 2 possible alleles which are plotted as different colors. Each paired-allele (diploid locus) column shows results for a specific set of marker alleles at that locus or position on the chromosome. The marker position on the chromosome advances left-to-right showing its relative position on the chromosome. The different seedling genotypes within breeding lineages are shown on different rows of the plot. This allows a rapid visual assessment of the similarities/differences among different progeny genotypes (rows) for the different molecular marker alleles (columns) contained in the different chromosomes. [Note that each diploid locus column-pair is genetically distinct and color differences indicate genetic differences only within that column-pair. Thus, similar colors (red, green, yellow) in different columns are only used for visual separation and do not indicate genetic similarities between different loci]. In **Figure 2** the top color bar indicates the identity of short segments of the end of chromosome 4 (gold color) and the beginning of chromosome 5 (blue color). The first data row shows the marker pattern for a genetic standard with the 2nd row showing the *O'Henry* peach parent. Mid-chart, the *Nonpareil* almond parent pattern is shown with the pattern for the almond-peach introgression line *F8, 1-42* in the row above. The rows below *Nonpareil* show patterns for individual progeny from the cross *O'Henry* by *F8, 1-42*. What is striking is the near-total genetic uniformity of all progeny at the section of chromosome 4 (gold) shown with a greater, though still limited variability shown for the beginning segment of chromosome 5 (blue). Because genes (including molecular marker genes) from 2 distinct parents should randomly recombine in progeny (much like to reshuffling of a deck of cards), these more fixed patterns indicate that recombination and so inheritance is being restricted. Similarly, the fourth row of the chart shows the marker pattern for *05,16-152*, a specific progeny from the O'Henry by *F8, 1-42* cross with the 9 rows below it showing 9 progeny from a self-pollination of this individual. The first 5 rows show evidence consistent with a normal, random genetic reshuffling or recombination for those marker alleles which were heterozygous (i.e., possessed 2 distinct alleles, rather than homozygous where both parent alleles are the same and so all progeny will be the same) in the *05,16-152* parent. However, even here, the subsequent progeny showed a distinct suppression of recombination. The data show that even in these advanced introgression lineages, the reshuffling at certain chromosome locations appears to occur in larger blocks rather than by individual allele as would be expected. This

has important breeding implications as it indicates that larger components of the donor genome can be transferred, which can sometimes be beneficial. If undesirable genes are embedded in these larger chromosome blocks, however, it can be detrimental. Because the molecular markers allow the identification of these larger inheritance blocks or haplotypes, this type of evaluation can help identify breeding lineages which are more effective in transferring the desired trait to cultivated backgrounds.

Name	Parent1	Parent2
03,1_329	DrDavis	F_03,1_329
OHenry	Merrill2on	El2erta
05,16_101	F8_1_42	F8_1_42
05,16_152	OHenry	F8_1_42
05,16_152_01	05,16_152	05,16_152
05,16_152_02	05,16_152	05,16_152
05,16_152_03	05,16_152	05,16_152
05,16_152_04	05,16_152	05,16_152
05,16_152_05	05,16_152	05,16_152
05,16_152_06	05,16_152	05,16_152
05,16_152_07	05,16_152	05,16_152
05,16_152_08	05,16_152	05,16_152
05,16_152_09	05,16_152	05,16_152
F8_1_42	90_1_4	90_1_4
Nonpareil	-	-
05,16_153	OHenry	F8_1_42
05,16_156	OHenry	F8_1_42
05,16_157	OHenry	F8_1_42
05,16_158	OHenry	F8_1_42
05,16_159	OHenry	F8_1_42
05,16_160	OHenry	F8_1_42
05,16_161	OHenry	F8_1_42
05,16_162	OHenry	F8_1_42
05,16_146	OHenry	F8_1_42
05,16_164	OHenry	F8_1_42
05,16_166	OHenry	F8_1_42
05,16_167	OHenry	F8_1_42
05,16_168	OHenry	F8_1_42
05,16_170	OHenry	F8_1_42
05,16_147	OHenry	F8_1_42
05,16_149	OHenry	F8_1_42
05,16_150	OHenry	RioOsoGem

Figure 2. The visualization of the inheritance patterns of different sets of molecular markers at different positions of chromosome 4 (top bar-gold color) and 5 (blue color) for *Nonpareil* almond and *O'Henry* peach as well as advanced breeding introgression of almond to peach.

A possible reason for these distorted inheritance patterns is shown in **Figure 3** where a full genomic analysis (500 to 1,000 markers distributed over all 8 chromosomes) of the *Dr. Davis* peach *by F8,1-42* almond-peach introgression line (DF) is compared to the peach standard *Georgia Belle* (DG). In this graph the position of individual *F8,1-42* markers on each of the 8 chromosomes (scaffolds or LG (linkage groups)) is compared *with Georgia Belle*. Most markers occupy similar positions on the homologous chromosomes although often the spacing between markers varies (indicating that the genetic density may vary among the different chromosomes). Occasionally a marker is located on a totally different section of the homologous chromosome (indicated by a diagonal line). This indicates that during the separate evolution of almond and peach that these markers were moved to a different position on the chromosome either through translocation or through inversions (that is, segment of the chromosome inverted causing an inversion of the embedded genetic sequences). For unrestricted genetic recombination to occur, the DNA in chromosomes needs to be precisely aligned with its homolog. Because inversions and translocations change the chromosome and

so DNA positions, alignment and so normal recombination is often restricted, though because the genes remain on the same chromosome, recombination is eventually possible but more tedious. This can have advantages if all the genes on these stable haplotype blocks are beneficial (many disease resistances often behave in this manner) but can be a disadvantage of one or more of the embedded genes is an undesirable trait such as kernel bitterness).

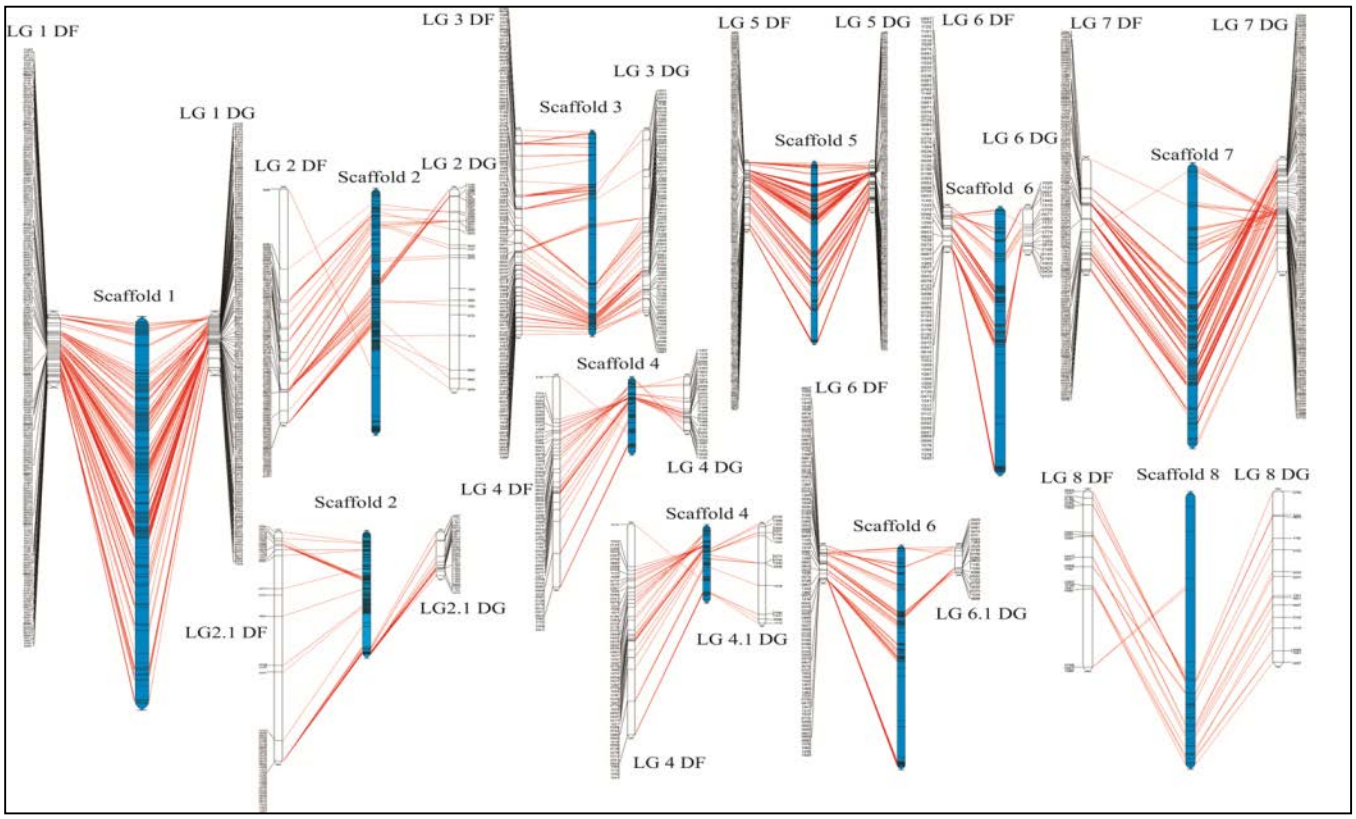

Figure 3. The relative position of individual Dr. Davis by F8, 1-42 markers (DF) on each of the 8 chromosomes (scaffolds or LG (linkage groups)) as compared with Georgia Belle peach standard (DG).

A more complex breeding progeny inheritance pattern is shown in **Figure 4** which shows an advanced almond-peach introgression line which has been inbred to the point of near homozygosity (all alleles are identical within each locus, and all progeny possess the same allelic combinations or genotype). Genetic uniformity or homozygosity is indicated by the solid green and red bars in the upper portion of the plot which indicate that all introgression line progeny (highlighted blue) are uniform at those loci. In this introgression lineage, this homozygosity or genetic uniformity extends to all 8 chromosomes except for the limited section of chromosome 6 shown here. The marker distribution patterns in this example indicate chromosomal rearrangements not within the same chromosome as previously described, but between 2 different chromosomes. In **Figure 5**, the results of a comprehensive genomic evaluation of structural variants among different chromosomes in this population are

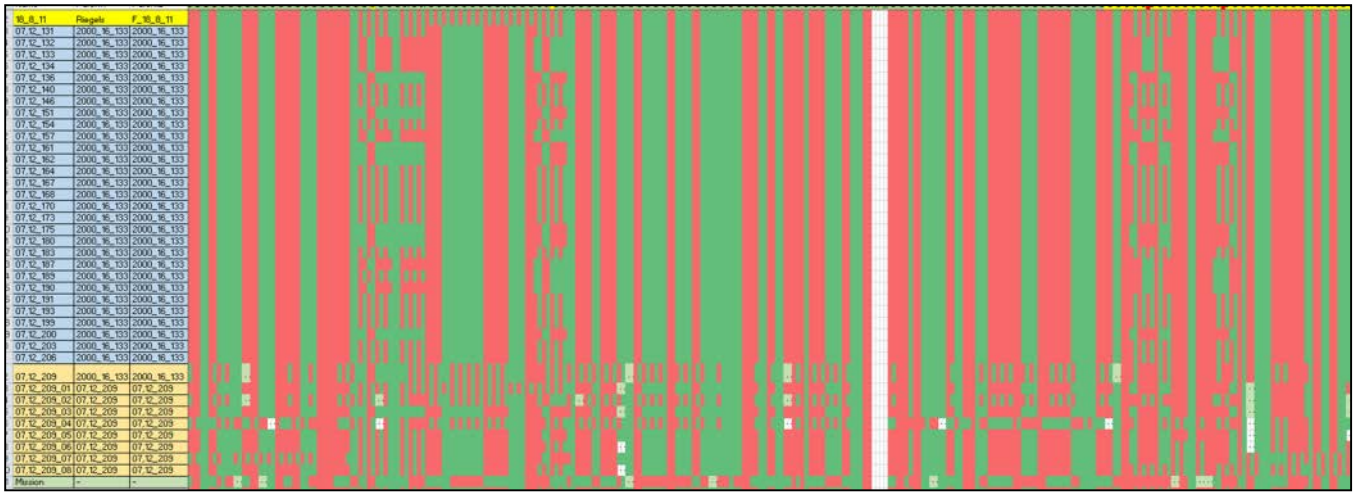

Figure 4. The visualization of the inheritance patterns of different sets of molecular markers at a small portion of chromosome 6 for an almond by Reigels peach advanced breeding introgression population.

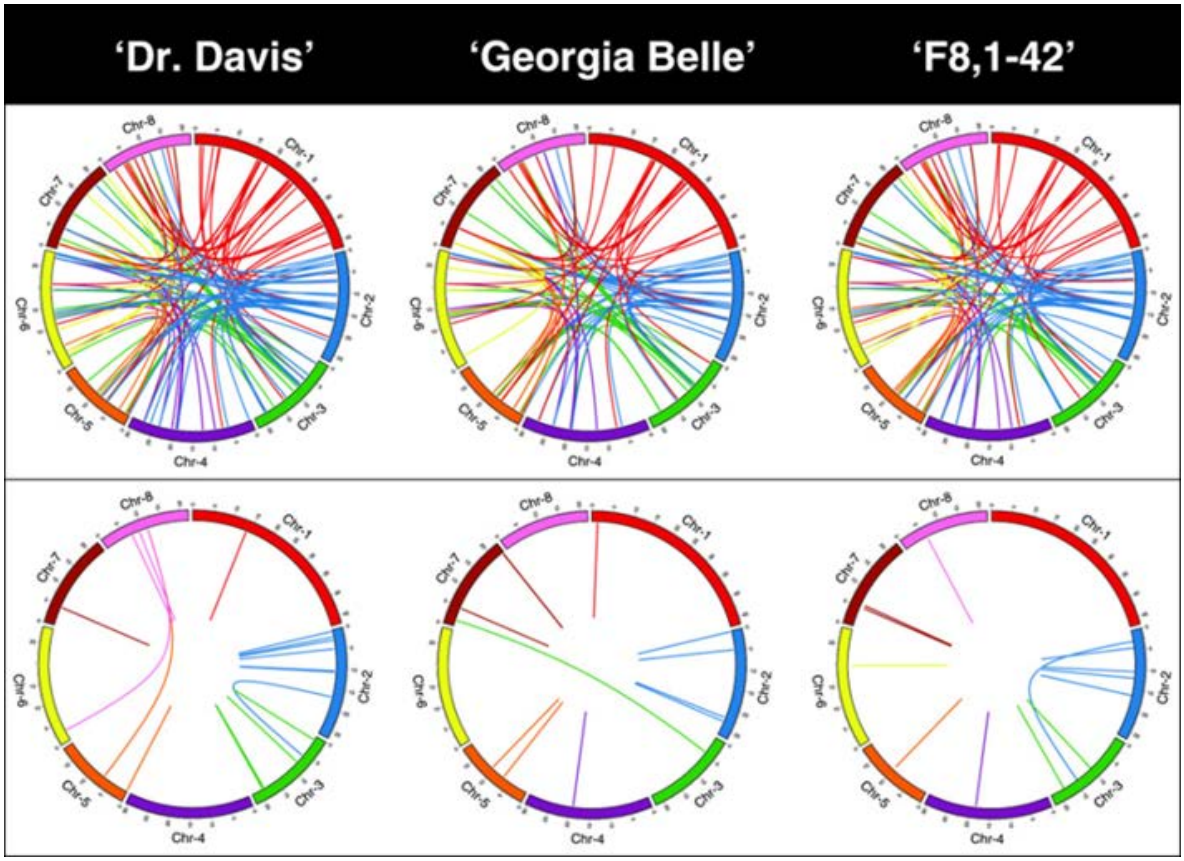

Figure 5. Visual comparison of the structural variants for three peach cultivars using Circos graphs. The variants were obtained through comparisons with the 'Lovell' Peach Genome Reference Sequence ('Lovell', upper row) and with the exclusive structural variants per genotype (lower row). Non-connected lines correspond to intra-chromosomal variations such as inversions and translocations. [For details, see Reference #4]

presented. Results show that intra-chromosomal rearrangements are present in Georgia Belle which is an important founder for much of the US peach germplasm. Further rearrangements within and between chromosomes are encouraged when more diverse germplasm is incorporated into the breeding lineages, as exemplified by the results for Dr. Davis and F8, 1-42. The translocation of DNA to different chromosome (which has no homologous segment of DNA for pairing and subsequent recombination) can result in the entire chromosome segment behaving as if it were a single gene (i.e., no recombination occurs within it). These segments can be very stable and so readily transferred to progeny. If undesirable genes are embedded in the segment, however, they are very difficult to rogue out since internal recombination (i.e., reshuffling) is rare.

Figure 6. Unusual 'primal 'selections infrequently found in advanced almond to peach introgression lines which combine high vigor and disease resistance with good kernel quality including kernel sweetness and often self-fruitfulness.

Occasionally, in advanced almond-peach introgression lines, rare progeny will be found which are distinctly almond-type in appearance (despite appearing in peach-like populations) but are more genetically homozygous than either almond or similar almond-peach hybrids. Since extensive genetic differences separate peach from almond, the spontaneous transition from a peach to an almond type would be expected to be rare. However, since a relatively small number of genes appear to be involved in the domestication of the fleshy peach (see reference 13), a more discreet genetic change might cause a genetic reversion to a more primitive peach type (which, based on wild peach relatives might be expected to look fairly almond-like). For this reason, we collectively referred to these types as 'primals' and because they can often

retain some of the vigor and disease resistance associated with almond-peach hybrids, we have made selections for individuals with commercial almond potential. In **Figure 6**, several such individuals are shown which while retaining a thick European type shell as well as improved vigor and disease resistance, also display kernel qualities (including sweetness) which may have commercial potential. We are also utilizing molecular marker analysis to help identify the origin of these primal types. An example of the molecular marker patterning of 07, 12-209, a primal-type found in the highly inbred population shown in **Figure 4** is shown highlighted in yellow in **Figure 4** along with several self-progeny derived from it. Marker inheritance is erratic, appearing normal in some cases and highly restricted in others, and we are currently analyzing a more extensive population of primals and their selfed progeny using more advanced markers based on an Ilumina array (for further details see Project No. 2015-15-HORT10-Gradziel--Rootstock Breeding Germplasm report).

Figure 7. A primal-type selection derived from a *P mira* to almond advanced introgression line which combine high vigor and disease resistance with good kernel quality including kernel sweetness and self-fruitfulness.

Similar primal types are also occasionally found in advanced breeding lines being introgressed to almond types. Because of the stronger almond background in these lineages, primals retain more of the commercial almond traits while demonstrating exceptional tree vigor and associated stress tolerance. Breeding selection *07,2-292* shown in **Figure 7**, while distinctly almond-like, demonstrates improved vigor, disease resistance, as well as self-fruitfulness.

Molecular markers have thus proven useful for genetic analysis at both the individual loci and so trait level, as well as the larger levels of chromosome recombination and inheritance. Preliminary results with Prunus fruit types including peach, have demonstrated that markers can be identified which have a higher association and so predictive value for traits of interest including fruit size, maturity date and disease resistance. Because we used SNP markers, the association and so predictability is not the result of the marker being part of the gene controlling the trait but rather a result of the marker being located on the DNA close enough to the trait to be consistently inherited with it. Because this SNP marker-trait loci association will differ depending upon the breeding lineage, particularly when other species are used as a source of the trait, the association and so commercial trait predictability is often lost. Even in these situations, sufficient marker variability is present to provide information on the larger patterns of chromosomal rearrangements and so general inheritance. Tools developed within the RosBreed program to rapidly characterize the inheritance of these larger chromosome blocks or haplotypes further facilitate this strategy for visualizing inheritance patterns to allow more efficient breeding decisions. An example of the early application of this haplotyping tool developed in peach but applied to almond is shown in **Table 2**. This tool is most useful in the

Table 2. Haplotype mapping for the central section of chromosome 4 which contains several genes known to control crop maturity. The full haplotype complement can then be used to predict maturity in the individual genotypes without specific knowledge of the controlling genes.

Individual	Parent1	Parent2			1	2	3	4
2006_1_107	18_8_11	P_Tang_Mix			h	B	B	h
2000_2_8	Loadel	*P.argentea*		4	B	B	B	B
2000_2_9	Loadel	*P.argentea*		4	B	B	B	B
2001_7_180	Andross	*P.argentea*		4	B	B	B	B
2005_20_143	2000_3_205	2000_3_205			B	B	B	B
2007_10_214	2000_2_9	dummy026		4	B	B	B	B
2008_53_47	Riegels	2000_12_110		4	B	B	B	B
2009_18_187	Andross	96_9_292		4	B	B	B	h
2009_19_18	Andross	96_9_292		4	B	B	B	h
2009_28_152	18_8_11	dummy027		4	B	B	B	B
Carmel	*Nonpareil*	*Mission*	4	4	B	B	B	B
F10C_20_51	F8_76_45	F_F10C_20_51	4	4	B	B	B	B
F5C_6_8	*Nonpareil*	Mission	4	4	B	B	B	B
F5C_6_9BF	Nonpareil	Mission	4	4	B	B	B	B
F8_7_179	Nonpareil	A80_10_22	4	4	B	B	B	B
Jordanolo	Nonpareil	Harriott	4	4	B	B	B	B
Mission	*	*	4	4	B	B	B	B
Mission_BF	Mission	*MUT	4	4	B	B	B	B
Nickels	CP_5_33	Nemaguard			h	h	h	B
Nonpareil	*	*	4	4	B	B	B	B
persXdavidiana	P. persica	P. davidiana		4	B	B	B	B
Sonora	Nonpareil	F_Sonora	4	4	B	B	B	B
Stukey_6_27H	Nonpareil	Mission	4	4	B	B	B	B
Stukey_6_9BF	Nonpareil	Mission	4	4	B	B	B	B
TardyNonpareil	Nonpareil	*MUT	4	4	B	B	B	B
2005_18_191	2000_2_8	2000_2_8	4	4	B	B	B	B
TX_Pop9_05	TXW1490_1	CAF2		4	B	B	B	h
Hansen1	Almond	1_8_2peach		4	A	B	B	B
2005_20_192	91_16_154	F10C_20_51			A	B	B	A
18_8_11	Riegels	F_18_8_11		4	B	B	B	A
Andross	Fortuna	Dix_5A_1		4	B	B	B	A
F8_5_171	90_10_91	90_10_91		4	B	B	B	A
40A_17	*	*	4	4	A	A	A	B

more advanced introgression lines to peach but shows decreasing ability for haplotype discrimination when more substantial portions of the genomic drive from almond or even related peach species. Greater predictive precision for these types of haplotyping tools will become available as molecular markers with greater specificity to the donor species are developed. However, specificity may be limited to just that donor species or even to specific lineages from which the initial markers were developed.

3. Assess advanced breeding germplasm.

Because commercial success of a new almond variety will require dependable production of quality crop over a broader range of physical, cultural, and temporal environments, long-term and regional testing is critical to ensuring that any deficiencies are identified prior to large-scale grower and industry investment. Because a deficiency in any of a large number of requirements for commercial profitability can be the undoing of a new variety, there is a high likelihood that new selections advanced to regional testing will be found to be wanting in some crucial characteristic within the first 5 to 10 years. This is particularly true when novel traits such as self-fruitfulness are being incorporated since, as demonstrated by the previous genetic data, the introduction of new improved germplasm runs a significant risk of introducing new undesirable traits as well. A large number of UCD advanced experimental almonds have been selected to be included in the new regional variety trials based on their previous positive performance in smaller scale grower trials as well as the diverse germplasm that they contribute. While this expanded diversity increases the likelihood that undesirable characteristics may be present, it is seen as essential in maintaining the genetic and so production flexibility required if California production is to remain sustainable in these changing and increasingly erratic climates.

Table 3. Summary of UCD experimentals advanced Regional Variety Trial testing. Bloom and harvest times are given in days relative to *Nonpareil*

Selection	Bloom	Harv.	Self-fruitfulness	Origin
UCD3-40	-5	11	Partially Self-fruitful	*P.fenzliana*
Winters	-3	20	Self-incompatible	Harriot
Sweetheart	-2	18	Partially Self-fruitful	*P. persica*
UCD18-20	1	20	Partially Self-fruitful	*P. persica*
UCD1-16	3	12	Partially Self-fruitful	*P.fenzliana*
UCD8-27	4	12	Self-fruitful	*P. webbii*
UCD8-160	4	15	Self-fruitful	*P. mira*
UCD97,1-232	5	13	Self-fruitful	*P. mira*
UCD1-271	5	14	Self-fruitful	Tuono
UCD7-159	5	16	Self-fruitful	*P. webbii*
UCD2-19E *Kester*	6	10	Self-incompatible	TardyNonp. * Arb.
UCD8-201	7	18	Self-fruitful	*P. mira*

A summary of UCD selections included in the new regional variety trials is presented in **Table 3** with a more detailed description provided in Appendix A. While some of the UCD selections had sufficient crop to warrant harvest 2015, the lower crop on the maturity of other selections was deemed too small for the effort, with the first general harvest scheduled for the fall 2016. Differences in tree performance within plots are already becoming apparent despite the young age of the orchards. Of particular interest is differences in performance among plots since the selection may be perceived as doing very well at one site and yet fairly poorly at another, with the difference primarily due to differences in tree architectures. Differences in disease resistance/susceptibility are also becoming apparent despite good spray regimes practiced at all sites. An example is shown in **Figure 8** which plots the intensity of the leaf rust outbreak this summer at the Chico RVT. While most varieties at the site of the outbreak showed some susceptibility, UCD 1-232 showed very little damage despite being at the epicenter of the outbreak. Interestingly, both UCD 8-160 and UCD 8-201 are progeny of UCD 1-232 indicating that the resistance (if actually present) may have been lost with continued introgression to more a traditional almond genetic background. This is because each generation of backcrossing to traditional almond will dilute the new germplasm by 50%. While focused selection on the main traits of interest (in this case self-fruitfulness) will ensure its retention in the progeny, the opportunity to retain other desirable traits (such as resistance to new or established diseases/pests) can be greatly reduced with each generation of backcross introgression.

24	Supareil	24	Y117-86-03	24	Winters	24	Sterling
25	Nonpareil	25	Nonpareil	25	Nonpareil	25	Nonpareil
26	Durango	26	2-19E Kester	26	Jenette	26	Wood Colony
27	Nonpareil	27	Nonpareil	27	Nonpareil	27	Nonpareil
28	Wood Colony	28	UCD7-159	28	Capitola	28	Durango
29	Nonpareil	29	Nonpareil	29	Nonpareil	29	Nonpareil
30	Marcona	30	self-fruitful P16.013	30	Bennett--12 short	30	UCD1-271
			Irrigation in one or two blocks split here.				
ow	Rep 2	Row	Rep 2	Row	Rep 4	Row	Rep 4
31	Nonpareil	31	Nonpareil	31	Nonpareil	31	Nonpareil
32	Jenette	32	UCD8-27	32	UCD18-20	32	Sweetheart--12 short
33	Nonpareil	33	Nonpareil	33	Nonpareil	33	Nonpareil
34	UCD7-159	34	UCD18-20	34	Booth	34	UCD8-27
35	Nonpareil	35	Nonpareil	35	Nonpareil	35	Nonpareil
36	Y117-86-03	36	UCD1-271	36	Bennett--4 short	36	Eddie--9 short
37	Nonpareil	37	Nonpareil	37	Nonpareil	37	Nonpareil
38	Marcona	38	2-19E Kester/Hansen	38	UCD8-160	38	UCD1-271
39	Nonpareil	39	Nonpareil	39	Nonpareil	39	Nonpareil
40	Y116-161-99	40	UCD1-16	40	UCD1-16	40	Y117-91-03
41	Nonpareil	41	Nonpareil	41	Nonpareil	41	Nonpareil
42	Capitola	42	Folsom	42	UCD1-232	42	Jenette
43	Nonpareil	43	Nonpareil	43	Nonpareil	43	Nonpareil
44	UCD8-201	44	Sweetheart	44	2-19E Kester/Hansen	44	Y117-86-03--6 short
45	Nonpareil	45	Nonpareil	45	Nonpareil	45	Nonpareil
46	self-fruitful P16.013	46	UCD3-40	46	UCD8-201	46	Marcona
47	Nonpareil	47	Nonpareil	47	Nonpareil	47	Nonpareil
48	Self-fruitful P13.019	48	Y117-91-03	48	self-fruitful P16.013	48	Aldrich
49	Nonpareil	49	Nonpareil	49	Nonpareil	49	Nonpareil
50	2-19E Kester	50	Sterling	50	Supareil	50	Durango
51	Nonpareil	51	Nonpareil	51	Nonpareil	51	Nonpareil
52	UCD1-232	52	Aldrich	52	Winters	52	Wood Colony
53	Nonpareil	53	Nonpareil	53	Nonpareil	53	Nonpareil
54	Eddie	54	Winters	54	UCD7-159	54	Self-fruitful P13.019
55	Nonpareil	55	Nonpareil	55	Nonpareil	55	Nonpareil
56	UCD8-160	56	Durango	56	UCD3-40	56	2-19E Kester
57	Nonpareil	57	Nonpareil	57	Nonpareil	57	Nonpareil
58	Bennett	58	Supareil	58	Capitola	58	Folsom
59	Nonpareil	59	Nonpareil	59	Nonpareil	59	Nonpareil
60	Wood Colony	60	Booth	60	Y116-161-99	60	Sterling
61	Nonpareil	61	Nonpareil	61	Nonpareil	61	Nonpareil

Figure 8. Color mapping of a leaf rust outbreak at the Chico RVT in June-July, 2016 where a more intense shading indicates greater disease damage.

Over 20 additional genotypes, selected for self-fruitfulness and improved productivity, are currently being propagated for regional grower testing supplementing the previous 24 UCD advanced selections currently under grower testing. A summary of propagated items is presented in **Table 4** with samples showing kernel and nut characteristics presented in **Figure 9**.

Table 4. Summary of UCD experimentals currently being propagated for regional grower trials. (Origin: *M -Prunus mira, F -P. fenzliana, P -P. persica, W -P. webbii.*

UCD Advanced Selection	Seed Parent	Pollen Lineage	Origin
UCD4-8-149	Nonpareil	25-75 * Winters	*MF*
UCD4-16-266	Nonpareil	25-75 * Winters	*MF*
UCD4-17-20	Nonpareil	25-75 * Winters	*MF*
UCD5-3-47	Ferragnes	D3-4(Miss*WebF2) * Ferrag	*F*
UCD5-4-60	UCD13-1	25-75 [Arb * 4-26]*[SB4, 4-2E] * F10D103-9wnt	*M*
UCD5-4-170	UCD13-1	25-75 [Arb * 4-26]*[SB4, 4-2E] * F10D103-9wnt	*M*
UCD5-5-3	UCD13-1	25-75 [Arb * 4-26]*[SB4, 4-2E] * F10D103-9wnt	*M*
UCD5-5-17	UCD13-1	25-75 [Arb * 4-26]*[SB4, 4-2E] * F10D103-9wnt	*M*
UCD5-5-80	UCD13-1	25-75 [Arb * 4-26]*[SB4, 4-2E] * F10D103-9wnt	*M*
UCD 5-5-367	UCD13-1	25-75 [Arb * 4-26]*[SB4, 4-2E] * F10D103-9wnt	*M*
UCD 5-6-331	UCD13-1	25-75 [Arb * 4-26]*[SB4, 4-2E] * F10D103-9wnt	*M*
UCD5-6-340	UCD13-1	25-75 [Arb * 4-26]*[SB4, 4-2E] * F10D103-9wnt	*M*
UCD5-6-369	UCD13-1	25-75 [Arb * 4-26]*[SB4, 4-2E] * F10D103-9wnt	*M*
UCD5-6-390	UCD13-1	25-75 [Arb * 4-26]*[SB4, 4-2E] * F10D103-9wnt	*M*
UCD5-7-30	UCD13-1	25-75 [Arb * 4-26]*[SB4, 4-2E] * F10D103-9wnt	*M*
UCD5-9-100	UCD13-1	D3-15 * D3-25	*WP*
UCD5-9-358	UCD13-1	D3-15 * D3-25	*WP*
UCD5-9-396	UCD13-1	D3-15 * D3-25	*WP*
UCD6-1-254	Nonpareil	D4-18'(P. webbii)* Sonora	*W*
UCD6-3-91	Nonpareil	D3-25 * 25-75	*WM*
UCD6-3-319	Nonpareil	D3-25 * 25-75	*WM*
UCD6-3-105	Nonpareil	D3-25 * 25-75	*WM*

Figure 9. Shell and kernel characteristics from representative UCD selections propagated for grower testing and summarized in **Table 4** with Nonpareil and advanced selection UCD97,1-232 provided for reference.

Seed Parent	Kester Pollen	Self Pollen	S-genotype
Fritz	+++++	-	S1S6
Carmel	+++++	-	S5S8
Mission	+++++	-	S1S5
Butte	+++++	-	S1S8
Padre	+++++	-	S1S18
Price	+++++	-	S1S7
Thompson	+++++	-	S5S7
Sonora	+++++	-	S8S13**
Monterey	+++++	-	S1S8
Winters	+++++	-	S1S14
Nonpareil*	+++++	-	S7S8
Kester	-		S8S23**

*-reciprocal cross also positive

** S-genotype not yet verified

Figure 10. Cross-compatibility of the new UCD variety *Kester* with other commercial almond varieties representing major cross-incompatibility groups.

Figure 11. Shell and kernel characteristics of the new UCD variety *Kester* compared to adjacently planted *Nonpareil* from the 2014 McFarland RVT.

Advanced UCD breeding selection 2-19E has been fully released to the California industry as the later Nonpareil-bloom pollinizer '*Kester'*. Virus-tested and certified true-to-type foundation stock has been made available to all interested nurseries. Field test-crosses completed in 2016 have verified previous molecular characterizations of the S-allele controlling cross-incompatibility, that the variety Kester is compatible with other commercial almond varieties (**Figure 10**). Since these varieties also represent the major cross-incompatibility groups, results support the cross-incompatibility of Kester with most if not all remaining commercial almond varieties since the *Kester* S23 allele inherited from the cv. *Arbuckle* is unique and may act as a universal donor. The *Kester* variety results from a long-term effort to develop a later-flowering pollenizer for *Nonpareil* which is comparable to *Nonpareil* in nut quality and yield (**Figure 11**) while having a flowering time approximately 3-5 days after Nonpareil and developing a smaller, more compact tree than Nonpareil. The later bloom would allow Kester to cover the economically critical peak and later *Nonpareil* bloom as well as the more straggling late bloom becoming common with warming winter temperatures. A key goal of the later bloom, however, was its greater protection frost damage. The smaller tree size yet high productivity of *Kester* allows high yields but even higher yields for interplanted *Nonpareil* rows, as more sunlight and so more photosynthesis can occur on the premium priced *Nonpareil* trees.

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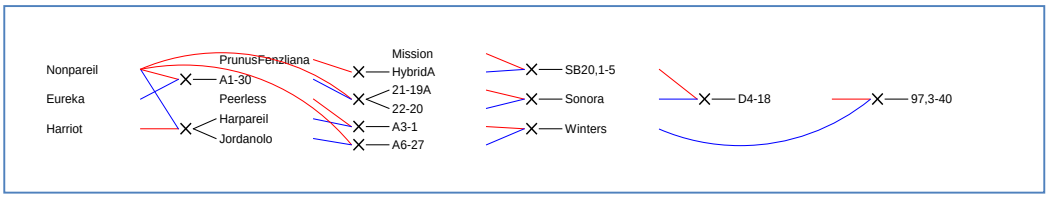
Appendix A. Characteristics of UCD selections included in the new RVT trials.

UCD3-40



UCD3-40 combines a large, high-quality kernel with good tree form and productivity. Regional testing in the Sacramento and lower San Joaquin valleys has also demonstrated good disease and pest resistance. The pedigree includes a complex parentage with a sizable contribution from *P. fenzliana,* which is often considered the species from which cultivated almond was derived. Parentage also includes *Nonpareil* and *Jordanolo* which have a history of noninfectious bud failure. Extensive and long-term testing of this selection has thus far been free from any indication of noninfectious bud failure risk. Bloom has consistently been just before *Nonpareil*, even in low chill years. The large, attractive kernels may also facilitate the development of new premium quality (*Sonora*, etc.) markets.

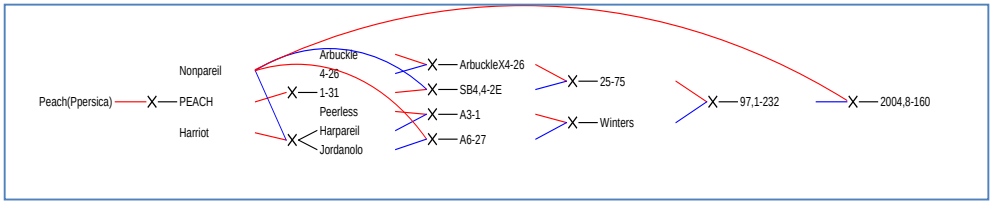
UCD18-20

The seed parent of this selection is F10D5-11, a USDA item from the early Professor Kester/Dr. Jones UCD/USDA breeding collaboration which appeared to have high levels of self-compatibility. The pollen parent is the UC cultivar *Winters* which, while genetically self-incompatible, demonstrates relatively high background levels of self-compatibility (which, unfortunately, has not been consistent from year to year). This selection has similarly shown moderate levels of self-compatibility in some years, but is more erratic in others and so is considered only partially self-compatible. Long-term trials at WEO in the Davis pathology block have demonstrated good productivity as well as good general disease and pest resistance.

UCD1-16

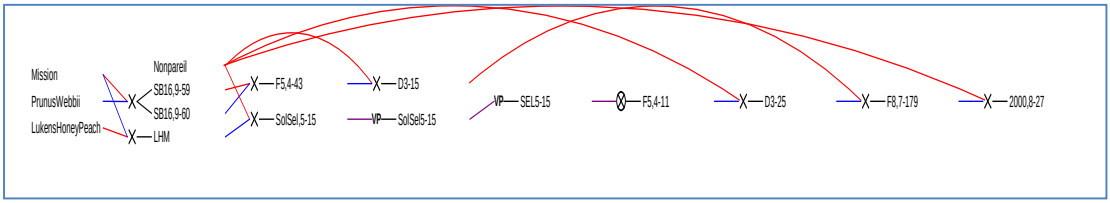
This selection was developed from a separate *P. fenzliana* lineage [Nonpareil X D3-19 {(Mission X *P*. *fenzliana*) X Solano}]. The selection is considered self-incompatible (*P. fenzliana* has not been a useful breeding source for self-compatibility). Both shell and kernel quality have been very good in long-term WEO and Nickels Soils Lab testing. Selections have also showed good general disease resistance in grower regional trials. Kernels pellicles also show a very desirable blonde-yellow color, comparable to Sonora. Trees are medium in size and productive.

UCD8-160



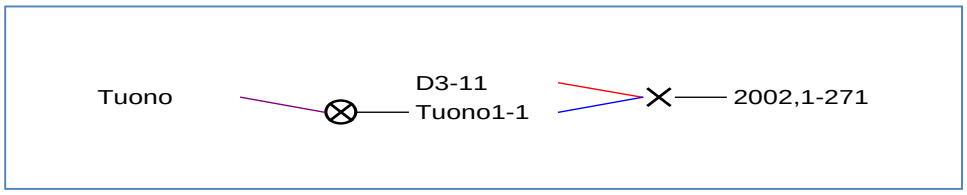
UCD8-160 has become one of the most promising of the new UCD breeding parents because of its combination of good kernel size and quality and consistently high self-compatibility. It is a progeny of UCD2-232 which is also included in the regional trials. Trees, while more compact than *Nonpareil*, are very productive and with production primarily on spurs. Multiyear testing at Nickels, WEO, and McFarland have demonstrated good general disease resistance despite the more compact structure of the trees.

UCD8-27



This selection represents a complex pedigree combining traits from both peach and *Prunus webbii*. High levels of self-compatibility have been recovered as have good tree architecture and uniform crop distribution, primarily on spur bearing wood. [Earlier research has shown that the control of self-compatibility from *P webbii* is in the pistil while control from peach is in the pollen. By combining different genes and so different mechanisms for self-compatibility, we are attempting to improve both maximum performance and year-two-year consistency. The tree is upright-spreading and approx. 20% smaller than Nonpareil The paper shells give good crack out but have poorer seals (60%) though the worm infestation has not been a problem to date. Kernels show good-quality though double kernels (~10%) may be a concern.

UCD1-271

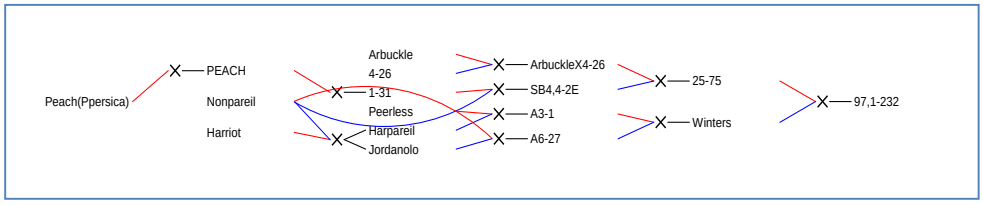


UCD1-271 utilizes the Italian cultivar *Tuono* as the source of self-compatibility, which has been heavily utilized by the earlier USDA Fresno breeding program as well as most Spanish and Italian breeding programs. While the *Tuono* almond is a very hard shelled, irregularly shaped kernel cultivar, its commercial almond background allows self-compatibility to be readily transferred to advanced breeding lines. Our experience suggests it also contributes poorer kernel quality, in particular significant kernel creasing in progeny. Most of the undesirable traits have been bred out in this selection while retaining good kernel quality and high self-compatibility. This selection is the result of targeting increased year-to-year production as well as self-compatibility consistency through combining more traditional breeding sources.

UCD7-159

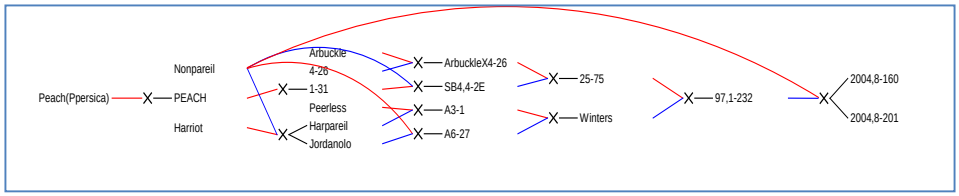
This selection resulted from the cross of *Nonpareil* by 95,1-26 (USDA selection CP33 crossed with *Winters*) based on previous USDA data which indicated that 95,1-26 had a novel source of self-compatibility. Recent test crossing at UCD has shown only moderate levels of self-compatibility in the 95,1-26 parent. Relatively high levels of self-compatibility have been identified in UCD7-159 suggesting that the 95,1-26 may still be a useful and unique source of self-compatibility but that expression is masked in the parent by its particular genetic background. Both tree and kernel show promising quality with good yields and low disease when trees have only been evaluated at WEO and Nickels plots.

UCD1-232



UCD1-232 has been one of our most effective parents for the transfer of self-compatibility as well as good disease resistance. It combines desirable traits from peach as well as a range of heirloom California cultivars. Kernel qualities are not as good as the other advanced RVT selections yet within the range of commercially important current California cultivars. Long-term testing has shown consistent levels of productivity, self-compatibility and disease resistance in this selection as well as in many breeding progeny using this selection is a parent. [In addition to assessing commercial value of these accessions, the new multisite RVTs will allow more detailed evaluations of individual diseases in differing environments. This more extensive information will allow better assessment of these items both as potential cultivar releases as well as parents for future crosses].

UCD8-201



UCD8-201 is a sister line to UCD,8-160, described previously as one of our most promising sources of self-compatibility and kernel quality. Although kernels of this selection do not show the uniform high quality of UCD,8-160 (kernels are medium to large and somewhat flat), the tree is particularly productive with a desirable upright spreading structure. Nuts are well-sealed. Branches show high density of spur production and have shown no significant disease despite the consistently high crops.