Molecular Marker Validation on Interspecific Breeding Germplasm for Rootstock Development

Project No.: **15-HORT10-Gradziel**

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Objectives:

A. Evaluate efficacy of marker expression in different interspecies hybridization/introgression lines.
B. Assess the marker inheritance patterns in segregating populations from objective (A).
C. Determine whether inheritance patterns from (B) may be sufficient to determine probable parentage of unknown or ambiguous rootstock clones or selections.

Summary Abstract:

The adaptability of cultivated almond to different growing environments, both in terms of changing production regions as well as changing climates, is strongly dependent on the availability of suitable genetic rootstocks. New rootstocks are currently being developed to meet these changing demands. Rootstock improvement has benefited from the increasing understanding of both the extensive genetic as well as phenotypic (disease/stress resistance, etc.) variability available in almond and its related species. However, rootstock breeding is traditionally very tedious and time-consuming. The use of molecular markers is increasingly being used to improve breeding efficiency by allowing geneticist to identify genotypes and so traits of interest at much earlier stages in the breeding program. These molecular markers have been shown to be frequently species-specific, which confounds their utility when wide species crosses or divergent species introgression's are attempted. (A common introgression program would involve the transfer of the desired trait such as self-fruitfulness or nematode resistance from the donor species to the cultivated crop through a series of recurrent backcrosses). In much of this diverse germplasm, molecular markers developed for peach showed varying levels of efficacy when applied to species hybrids and their introgression lines. Despite a frequent failure of molecular markers developed in one species to discriminate the genetic alleles of the different species, the use of marker-assisted-breeding proved valuable for understanding general inheritance trends as well as identifying possible barriers as well as potential novel opportunities for exotic gene transfer for rootstock improvement.

Interpretive Summary:

Changes in planting practices, including the quantity and quality of land and irrigation water, have led to the need for a new generation of almond rootstocks and a number of public and private efforts have been initiated to develop and test new candidates. Germplasm derived from other species, either directly or through species hybridization, is often pursued to attain the greatest range of vigor, stress and disease tolerance and desirable horticultural traits. However, the acquisition of such exotic germplasm is often difficult and time-consuming. At UC Davis, a diverse germplasm has already been developed combining almond, peach as well as related species *including P. argentea, P. bucharica, P. davidiana, P. fenzliana, P. domestica, P. mira, P. orthosepala, P. scoparia, P. tangutica* and *P. webbii*. In this project, selected germplasm from the UCD almond and peach breeding programs with value for rootstock improvement have been characterized both phenotypically and at the molecular marker level. Detailed pedigree relationships as well as trait expression data have been transferred to the RosBreed web-site (http://www.rosbreed.org/) and published to allow end-user analysis. This germplasm demonstrates extensive diversity both genetically and in the range of traits useful to rootstock improvement programs. Field evaluations have identified unique and potentially useful characteristics in parents and progeny, including modification of tree architecture from *P. scoparia* hybrid rootstocks, drought tolerance in a peach by *P. argentea* hybrid, and a pronounced invigoration of scion growth in several advanced interspecies introgression lines.

The genetic characterization of parent species, species-hybrids and subsequent breeding progeny has been completed using over 500 molecular markers showing a fairly uniform distribution over all eight of the Prunus chromosomes. While rootstock improvement has benefited from the increased understanding of both the extensive genetic as well as phenotypic (disease/stress resistance, etc.) variability available in almond and its related species, traditionally breeding remains very tedious and time-consuming. The use of molecular markers is increasingly being used to improve breeding efficiency by allowing geneticist to identify genotypes and so traits of interest at much earlier stages in the breeding program. These molecular markers have been shown to be frequently species-specific, which confounds their utility when wide species crosses or divergent species introgression is attempted. (A common introgression activity would involve the transfer of the desired trait such as self-fruitfulness or nematode resistance from the donor species to the cultivated crop through a series of recurrent backcrosses). In much of this diverse germplasm, molecular markers developed for peach showed varying levels of efficacy when applied to species hybrids and their introgression lines. Despite a frequent failure of molecular markers developed in one species to discriminate the genetic alleles of different species, the use of marker-assisted-breeding proved valuable for understanding general inheritance trends as well as identifying possible barriers as well as potential novel opportunities for exotic gene transfer for rootstock improvement.

Materials and Methods (as developed in year one of the proposal):

Materials analyzed in this report were developed and characterized in the first year of the study as summarized below and in the 2014 Annual Report for this project (14-HORT10-Gradziel). In addition, promising germplasm was propagated for evaluation by other researchers including USDA Germplasm Repository. Research materials were developed to represent the broad range of germplasm presently available for almond rootstock improvement, including *P. persica, P. mira, P. webbii I P. tangutica, P. scoparia, P. davidiana* and *P. dulcis species,* interspecies F2's and backcrosses (**Figure 1**).

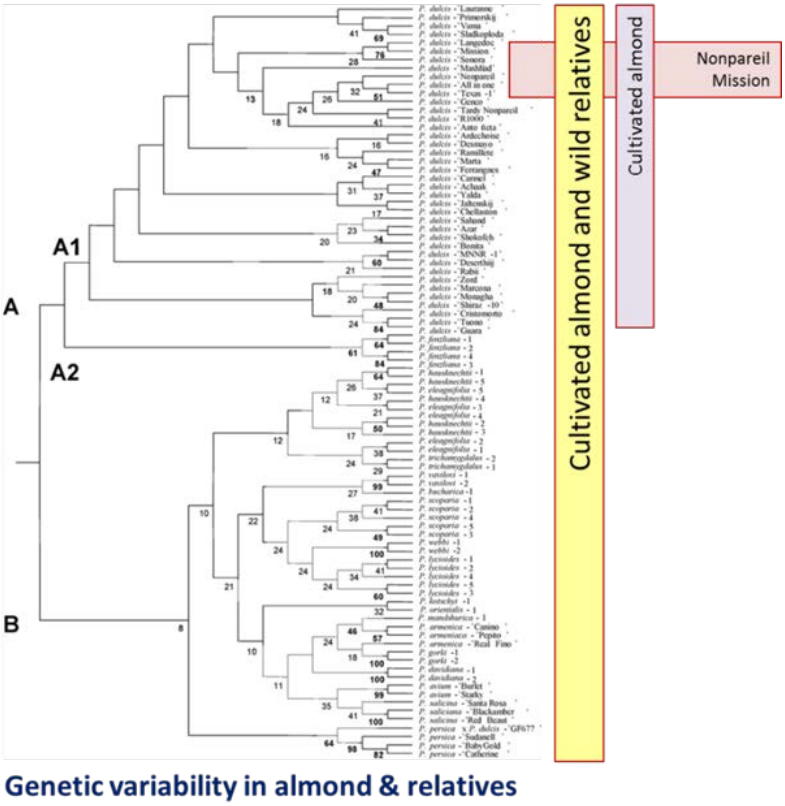


Figure 1. Plot showing the relationship of different peach and almond species the cultivated almond. Length of the horizontal lines connecting to species indicates the relative genetic distance between them. Highlighted red box indicates the extent of germplasm currently utilized in California, the violet shaded box delineates the extent of germplasm available within cultivated almonds worldwide while the yellow shaded box indicates the extent of germplasm available when related almond and peach species are exploited.

Methods include the use of RosBreed developed SSR and SNP molecular markers (see https://www.rosbreed.org/) to test whether these markers assort normally from parent to progeny in interspecies crosses or whether aberrant segregation patterns (due to chromosomal rearrangements such as translocations/inversions and associated gene methylation) are evident as detailed in references 4, 5, 6, 12, & 13). Distortions from expected patterns are identified using standard genomic analysis software such as Pedimap and Rosebreed haplotyping tools as well as also by direct examination of the data. Where discrepancies are identified, inheritance patterns of individual molecular markers have been characterized using standard qualitative methods.

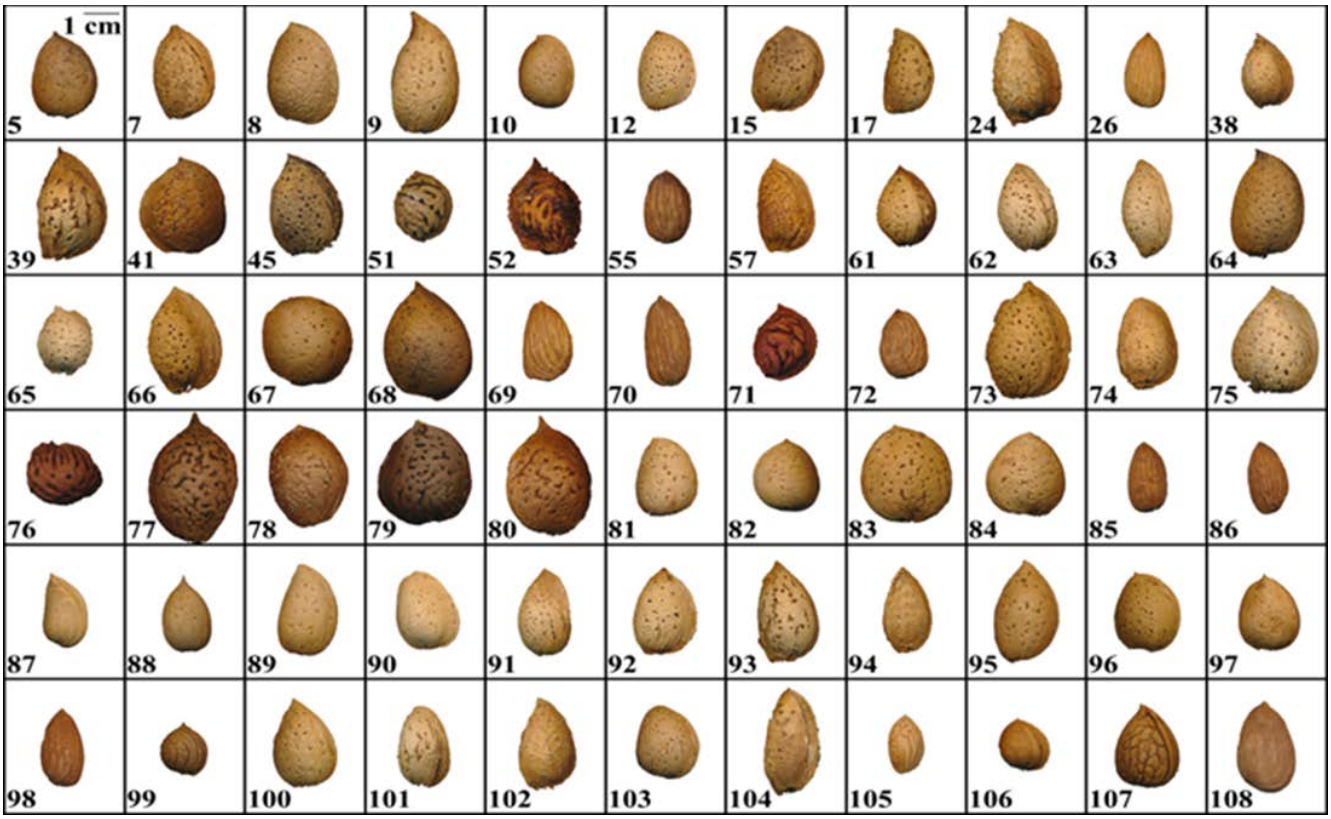


Figure 2. Representative sample showing the diversity of almond types present in this germplasm. The numbering of the seeds is the same as in **Table 1**. All seeds are in-shells except # 26 (Nonpareil), 55, 69, 70, 72, 85, 86, 98, and 108. [Results are from a published (ref. #14) collaborative study with Dr. Sathe's group at the University of Florida].

Species represented within the UCD almond germplasm *include Prunus tangutica, P persica, P davidiana, P mira, the argentea, P scoparia, P dulcis,* and *P webbii* which taken together represent a much broader range of genetic breeding options than present in the traditional almond breeding germplasm and in particular the California germplasm which is largely derived from 2 cultivars: Nonpareil and Mission (**Figure 1**). An extensive diversity is also captured for each species within introgression lineages. Considerable variability has been captured and demonstrated for both fruit and nut type (**Figure 2** and **Table 1**) and tree types, including germplasm previously shown to have disease resistance value when utilized as a rootstock (**Figure 3**). Genotypes range from interspecies hybrids with a well-established rootstock value owing to their typical high vigor and so related disease/stress resistance/ avoidance (**Figure 4**), to genotypes demonstrating the ability to modify scion architecture and physiology (**Figure 5**). The species donor in **Figure 5** is *Prunus scoparia*, which also possesses tremendous adaptability to arid environments. (The inset shows native *P. scoparia* growing at a 3000m mountain pass in the Zagros Mountains of Iran were conditions are so extreme that no other plants survive).

Table 1. UCD breeding germplasm analyzed for seed characteristics in a collaborative study with University of Florida. The numbering of the individual genotypes is the same as in **Figures 7** and **8**. Color shading laws visualization of the differences within each trait (column) with red being low to green high.

No.	Genotype	Origin	(%) Almond genome	Nut Length (mm)	Kernel Length (mm)	Nut Width (mm)	Kernel Width (mm)	Nut Thickness (mm)	Kernel Thickness (mm)	Nut Mass (g)	Kernel Mass (g)	Soluble protein (g/100 g)	R-ELISA	R-WB	R-DB
99	A7-23	*P. argentea* (bitter seed)	0	19.03	13.38	15.25	9.73	12.05	6.04	1.47	0.37	17.28	0.61	0.64	0.61
107	A7-25	*P. webbii* (bitter seed)	0	28.97	20.39	18.28	11.75	13.66	7.26	2.93	0.82	19.09	0.51	0.75	0.57
102	F10D,2-14	*P. fenzliana* (F2)	0	30.55	22.3	16.48	11.38	11.33	8.43	4.54	1.03	19.21	1.66	0.78	1.1
52	Andross	Peach (*P. persica*) (bitter seed)	0	35.31	17.77	26.12	11.43	19.5	3.89	6.21	0.36	20.65	0.39	0.53	1.01
105	A10-4	*P. bucharica* (bitter seed)	0	19.14	14.33	10.33	6.62	7.35	4.66	0.58	0.21	20.94	0.59	1.01	0.82
87	A7-28	*P. webbii* (bitter seed)	0	25.72	18.43	14.13	9.1	10.2	6.3	1.39	0.49	21.04	0.88	1.11	1.03
101	F10D,2-12	*P. fenzliana* (F2)	0	26.49	20.61	16.11	10.78	11.51	7.04	1.41	0.77	21.38	1.53	1.06	1.44
71	P11-58	*P. mira* (bitter seed)	0	26.55	14.48	17.75	9.86	12.82	4.33	2.48	0.29	23.39	0.53	0.79	0.83
76	A13-1	*P. persica* × *P. davidiana* (bitter seed)	0	21.47	13.77	20.69	11.41	17.83	6.1	3.83	0.46	23.41	0.45	0.5	0.9
51	40A-17	Peach (*P. persica*) (bitter seed)	0	24.25	13.35	16.83	7.18	12.45	3.35	1.81	0.11	23.74	0.51	0.52	1.02
106	A2-11	*P. tangutica* (bitter seed)	0	16.54	13.36	15.23	10.28	12.4	8.28	1.34	0.49	25.44	0.7	0.94	0.87
88	F5,4-42	*P. webbii* (F2)	0	26.82	18.54	14.98	9.46	10.83	6.7	1.96	0.55	25.8	0.64	1.1	1.06
5	**F5,4-10**	*P. webbii* × (Nonpareil × *P. persica*) BC1	38	27.5	19.69	18.32	11.94	12.78	7.22	2.69	0.78	22.12	0.53	1.02	0.96
77	Hansen2168	Almond × *P. persica*	50	44.06	27.95	28.46	15.71	18.29	7.34	9.07	1.44	12.35	1.57	0.81	1.31
97	F10D,3-24	*P. webbii* (BC1)	50	25.71	19.33	19.52	13.23	13.29	6.13	2.66	0.71	13.39	1.27	0.95	1.16
79	Nickels	Almond × *P. persica*	50	36.88	23.87	28.7	16.37	20.85	8.75	9.18	1.53	13.79	0.75	0.09	0.85
104	F10D,3-50	*P. fenzliana* (BC1)	50	36.2	27.32	19.3	13.93	13.31	8.75	2.37	1.59	15.37	2.18	0.73	0.91
96	F10D,3-13	*P. webbii* (BC1)	50	25.39	19.4	19.08	12.02	13.66	8.03	1.85	0.83	17.07	0.47	0.57	0.87
100	F10D,3-3	*P. argentea* (BC1)	50	29.57	23.42	18.62	12.41	13.8	7.01	1.88	0.96	17.47	0.26	0.4	0.65
93	F10D,3-2	*P. webbii* (BC1)	50	30.57	19.71	17.83	11.09	13.64	6.99	1.53	0.77	17.84	0.66	0.51	1.09
94	F10D,2-5	*P. webbii* (BC1)	50	28.66	20.83	14.57	9.81	11.31	8.07	1.23	0.76	17.99	0.47	0.75	0.86
89	F10D,3-15	*P. webbii* (F2BC1)	50	33.32	24.03	20.99	12.86	14.64	7.18	4.1	0.96	18.58	0.33	0.91	0.82
41	F10C,12-28	(Nonpareil × *P. persica*) F2	50	35.09	20.24	23.93	13.04	17.99	9.02	4.96	1.08	19.32	1.76	0.71	1.19
92	F10D,1-2	*P. webbii* (BC1)	50	29.97	20.76	19.8	12.21	14.21	7.15	1.59	0.84	20.4	0.68	1.11	1.14
91	F10D,1-4	*P. webbii* (BC1)	50	30.79	23.09	18.07	11.93	13.31	7.57	1.94	0.95	20.5	1.32	0.62	1.1
90	F10D,1-22	*P. webbii* (F2BC1)	50	28.91	21.59	21.35	12.65	15.24	7.72	2.45	0.97	21.05	1.78	1.01	1.11
78	Hansen536	Almond × *P. persica*	50	34.51	23.82	24.61	13.93	18.9	7.46	7.44	1.12	21.06	0.66	1	0.77
95	F10D,3-26	*P. webbii* (BC1)	50	33.55	24.05	20.27	11.4	14.4	7.45	3.23	0.93	21.17	1.06	1.02	0.89
45	F10C,20-51	(Nonpareil × *P. persica*) F2 (bitter seed)	50	35.12	25.14	21.25	12.63	14.98	7.31	2.43	1.1	23.87	0.56	0.7	0.69
57	F5,16-60	(Mission × *P. argentea*) F2	50	32.85	23.77	17.06	11.1	11.9	7.34	1.56	0.87	24.08	0.44	0.57	0.99
84	F10D,3-23	Padre × F5,4-4	69	27.45	20.37	19.82	11.85	13.38	7.71	2.32	0.84	14.48	1.49	0.76	1.11
12	F5,20-42	Padre × F5,4-10	69	26.76	21.42	17.85	12.07	14.03	8.18	1.87	1	16.72	0.65	0.99	0.66
83	F10D,1-26	Nonpareil × F5,4-4	69	30.84	23.05	24.82	14.16	15.84	6.87	3.88	1.11	17.64	1.61	1.27	0.99
17	F8N,7-4	F5,4-10 × Sonora	69	31.96	22.74	16.12	10.66	10.66	6.21	1.17	0.76	19.52	0.65	1.14	0.81
39	8010-22	Nonpareil × F5,4-10	69	37.57	24.6	19.31	12.5	14.1	7.07	1.9	1.05	21.06	2.09	1.43	0.89
55	SB13,25-75	Nonpareil × F5,4-10	69	NA	23.08	NA	12.54	NA	7.76	NA	1.17	22.18	1.78	0.94	1.49
82	F10D,2-18	Nonpareil × F5,4-4 (see No. 4)	69	24.86	19.04	17.53	10.8	13.13	8.47	1.95	0.8	22.4	0.76	0.65	1.2
15	F8N,6-68	F5,4-10 × Solano	69	30.72	21.57	19.93	12.47	14.38	7.19	1.89	0.96	23.47	0.88	1.02	0.91
81	F10D,3-7	Almond × *P. webbii* × *P. persica* (BC2)	84	26.25	20.45	16.6	10.62	12.64	6.74	1.41	0.69	15.35	0.42	0.55	0.77
72	97,1-232	SB13,25-75 × Winters (see No. 55)	85	NA	23.62	NA	13.42	NA	8.16	NA	1.29	20.61	2.06	0.99	0.9
9	F5,13-54	(Mission × *P. fenzliana*) BC1 × Sonora	88	37.19	23.69	19.52	11.92	16.68	8.31	2.94	1.05	16.28	0.7	0.54	0.68
10	F5,10-9	(Mission × *P. fenzliana*) BC1 × Sonora	88	27.28	21.12	18.82	12.24	14.15	7.04	3.08	0.82	18.11	0.61	1.08	0.99
103	F10D,2-3	(Mission × *P. fenzliana*) BC1 × Sonora	88	27.63	21.82	20.09	13.17	16.3	8.92	3.24	1.13	20.71	1.56	0.89	1.15
7	F5,6-13	(Mission × *P. fenzliana*) BC1 × Sonora	88	32.03	22.06	17.25	10.83	10.51	6.71	1.66	0.84	25.6	0.95	0.83	0.84
8	F5,6-1	(Mission × *P. fenzliana*) BC1 × Sonora	88	33.78	23.01	23.68	14.64	16.75	7.38	5.08	1.33	25.88	0.92	1.13	0.94
108	97,3-40	Almond × *P. webbii* × *P. persica* (BC3)	92	NA	33.26	NA	15.14	NA	8.7	NA	2.08	25.31	0.9	0.9	0.87
75	2004,9-1	Nonpareil × 97,1-232	93	34.28	24.96	23.78	13.46	18.07	7.54	3.15	1.24	14.54	1.89	1.1	0.86
74	2004,8-201	Nonpareil × 97,1-232	93	32.14	24.05	21.45	12.97	13.97	8.13	2.06	1.26	15.81	1.67	1.09	1.22
73	2004,8-160	Nonpareil × 97,1-232 (see No. 72)	93	38.45	28.58	22.52	14.18	15.41	8.64	2.96	1.77	19.84	2	1.15	0.85
38	SB13,54-39E	(Nonpareil × *P. persica*) BC3	94	26.19	16.93	15.83	10.23	12.33	8.18	1.05	0.7	21.51	1.96	0.72	1.16
98	97,2-240	*P. webbii* (BC4)	94	NA	23.76	NA	12.61	NA	9.45	NA	1.28	22.22	0.4	0.61	1
80	2005,20-192	(*P. persica*) BC4	94	37.09	20.55	26.46	14.58	19.27	7.37	7.31	0.99	23.91	0.63	0.88	1
85	2000,2-3	Almond × *P. webbii* × *P. persica* (BC4)	96	NA	23.91	NA	11.62	NA	8.97	NA	1.17	19.89	1.93	0.96	0.75
86	2000,8-27	Almond × *P. webbii* × *P. persica* (BC4)	96	NA	24.26	NA	12.13	NA	8.62	NA	1.2	23.92	0.55	0.87	0.9
68	Tuono	Almond variety	100	38.43	26.35	27.67	16.27	18.3	8.22	5.45	1.58	17.14	0.32	0.96	0.8
69	2004,18-20	Almond variety	100	NA	26.34	NA	13.12	NA	8.7	NA	1.54	18.72	0.68	0.85	0.99
61	Mission	Almond variety	100	27.89	20.76	19.79	12.36	15.77	8.87	2.55	1.04	19.17	0.46	0.84	0.93
64	Ferragnes	Almond variety	100	36.35	26.8	23.06	14.18	17.04	8.29	4.09	1.48	19.37	1.56	1.02	1.21
70	95,1-26	Almond variety	100	NA	29.66	NA	14.25	NA	9.47	NA	1.98	20.94	1.1	0.66	0.74
24	Sonora	Almond variety	100	37.02	27.7	18.89	13.08	12.69	7.8	2.25	1.52	22.07	0.74	1.08	1.13
67	Marcona	Almond variety	100	29.38	21.96	25.83	17.26	19.62	8.75	5.55	1.55	22.22	0.88	1.02	1
66	**Winters**	Almond variety	100	36.41	26.33	19.25	11.87	14.08	8.13	2.09	1.21	22.37	1.05	0.73	1.3
26	**Nonpareil**	Almond variety	100	NAc	24.74	NA	13.49	NA	7.86	NA	1.31	23.07	1.02	1	1
65	Sweetheart	Almond variety	100	22.47	19.1	18.98	12.51	14.33	8.84	1.54	0.98	25.52	1.73	1.12	1.16
63	Kahl	Almond variety	100	34.27	25.95	17.03	12.11	14.99	8.04	2.2	1.2	26.29	1.22	1.12	0.9
62	Chips	Almond variety	100	28.66	21.51	19.45	12.68	14.71	8.18	2.02	1.06	26.46	1.68	0.91	1.17

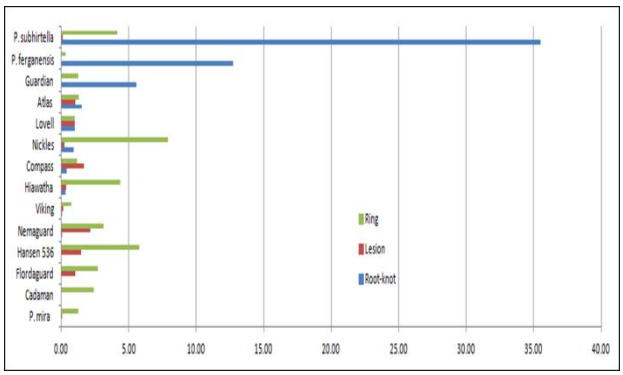


Figure 3. Differences in interspecies germplasm resistance to various nematodes as documented by previous studies by Bliss et al. at UCD.

Figure 4. Increased tree size and vigor typical of an interspecies hybrid (left) versus standard peach rootstock seedling (right).

A major challenge to rootstock breeding is the maskings effect that interspecies hybrid vigor has on any specific disease resistance genes within the germplasm. This is because the rootstock may be essentially able to outgrow the disease even though it is susceptible. Since the individual can be clonally propagated, this genotype can still be used directly as a rootstock, but often multiple improved traits are desired in the next generation of California rootstocks (nematode resistance, soilborne disease resistance, graft compatibility, scion architecture, etc.) which would require additional intercrossing to recombine all desired traits into one genotype. Because hybrid vigor is usually lost in subsequent selfings and inter-crosses, most species- hybrid-vigor advantage would be lost in rootstock breeding progeny which would confound both selection progress as well as the use of molecular markers to identify traits of interest. The only way to accurately determine the breeding value of such material would be to make and analyze the appropriate crosses. For example, **Figure 6** shows an advanced backcross to peach of the *P scoparia* interspecies F1 hybrid shown in **Figure 5)** which was left to grow under dryland conditions after 4 seasons of irrigated culture. Tolerances to non-irrigated conditions are evident in the continued tree growth and development as well as by the setting and cropping of a heavy fruit set. Consequently, rootstock breeding is typically

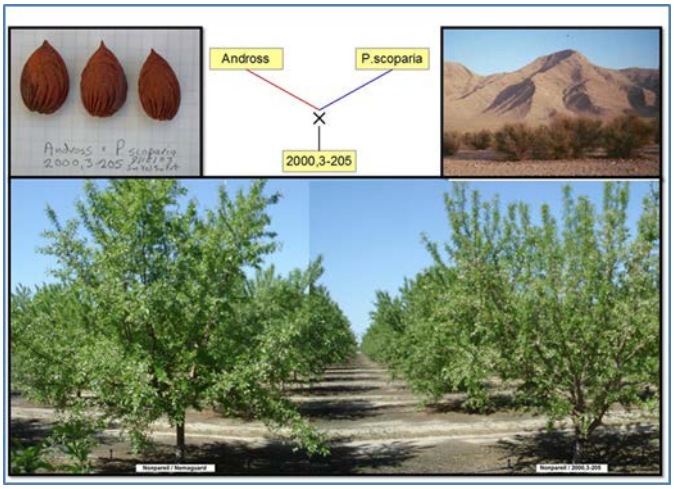


Figure 5. Derivation of the peach by *P. scoparia* hybrid UCD2000,3-205 and its effect on Nonpareil tree architecture when used as a rootstock (Nonpareil on Nemaguard at left and on 200,3-205 at right, in a collaborative evaluation by UCD, Fowler Nurseries and Paramount Farms. (Image courtesy of C. Fleck).

Figure 6. Seedling at right is an advanced backcross to peach of the *P scoparia* interspecies hybrid shown in **Figure 6** demonstrating heritability of some aspects of drought tolerance from the wild species.

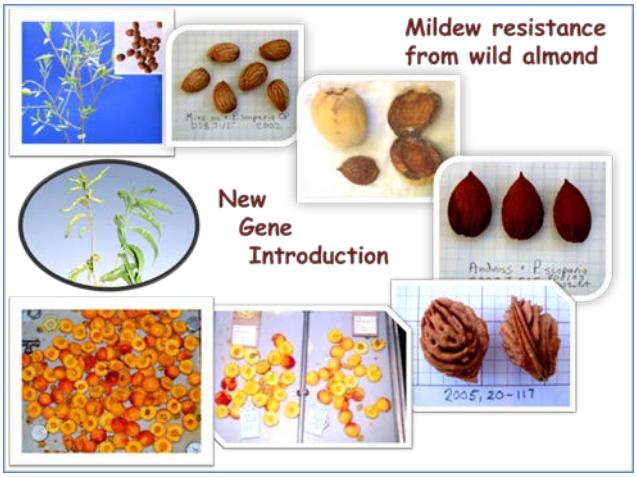


Figure 7. Introgression of mildew resistance from wild P scoparia to cultivated peach. Images denote different parents and the multi-generation backcross to peach.

pursued by two separate strategies: 1) generate interspecies rootstocks which combine desired traits (such as nematode resistance and graft compatibility) with vigorous root and tree growth derived from the nature of the interspecies hybrid, and 2) recombine multiple desired traits from multiple parents using traditional crossing and backcrossing methods. The development of interspecies hybrids can be rapidly obtained and resultant vigor captured through clonal propagation, though the number of desirable traits combined in a single rootstock is limited. Traditional breeding allows the recombination of the greater number of traits but involves a considerable amount of time for rootstock development. While molecular markers would facilitate traditional breeding, the multigenerational breeding pedigrees required to validate these markers would need to be developed and so require multiple years to develop and characterize. An example of such wild species trait introgression's demonstrated in **Figure 7**. In this example interspecies hybrid was backcross to California adapted peach for four consecutive generations while at the same time selecting for mildew resistance and good commercial peach quality. Several such introgressions have been included in the UCD germplasm providing both immediate opportunities for new trait introduction as well as required populations for developing useful molecular markers.

In our breeding program we have also identified a rare but intriguing introgression-type which combines the vigor and growth habit typically found in interspecies hybrids with greater genetic uniformity (i.e., greater heritability in progeny) which we have characterized as a primal-type since the tree characteristics almost always shows the more primal species phenotypes.

Often found in advanced introgression lines (**Figure 8**) at very low frequencies (1/500-1/1000), these rare individuals appear to be reversions to a more primal, undomesticated phenotype which almost always displays the almond-like leaf, tree and fruit traits characteristic of wild Prunus (**Figure 9**). This reversion-type also typically shows augmentation of tree vigor and tree architecture commonly associated with interspecies hybrids (**Figure 8**). Although rare, the size and genetic diversity of UCD breeding program material has allowed the collection of over 30 primal-type genotypes, most often in peach-almond and in peach-mira introgression lines though less frequently in crosses involving other species. In some advanced introgression material, primals have been selected which display a high quality, sweet kernel in an otherwise hybrid vigor background and so may have unique opportunities for commercial almond production under high disease/environmental stress conditions (**Figure 9**). Because of their rarity, there is virtually no information concerning such genetic throwbacks in the literature. Based on the UCD experience, such interspecies derived germplasm may be relatively common in domesticated almond (see references 5 & 6) and some of the domesticated plums. Peach-almond reversion lines are particularly useful because they are easy to identify given the distinctive tree/leave/fruit characteristics, and because that specific species hybrid group has proven particularly valuable for rootstock development. Over 10 of these unique genotypes have been propagated for the rootstock trials with cooperating nurseries and growers.

Figure 8. The appearance of a primal -type in an almond times *P mira* backcross introgression breeding line. While rare, primal types are readily identified by an increase in vigor and associated disease resistance as well as leaf and fruit traits more characteristic of the wild type.

Figure 9. Characteristic leaf and fruit traits typical for the primal-group of interspecies introgression genotypes. This tree was derived from an initial almond by peach hybrid and has medium-large sweet kernels yet good foliar disease resistance.

Almond by peach interspecies hybrid breeding lines, however, represent only a small proportion of the potential variability in tree growth and performance available with hybridization to other species. Examples of the range of tree architectures and growth habits are shown in **Figures 5** and **10** while the range in

performance (potential drought tolerance as indicated by the critical leave temperature) of advanced breeding lines is shown in **Figure 11**.

Figure 10. Range in tree growth habits of selected UCD interspecies breeding lines which are among the over 200 individuals which have been transferred to USDA/ARS germplasm repository for public access by rootstock researchers and breeders.

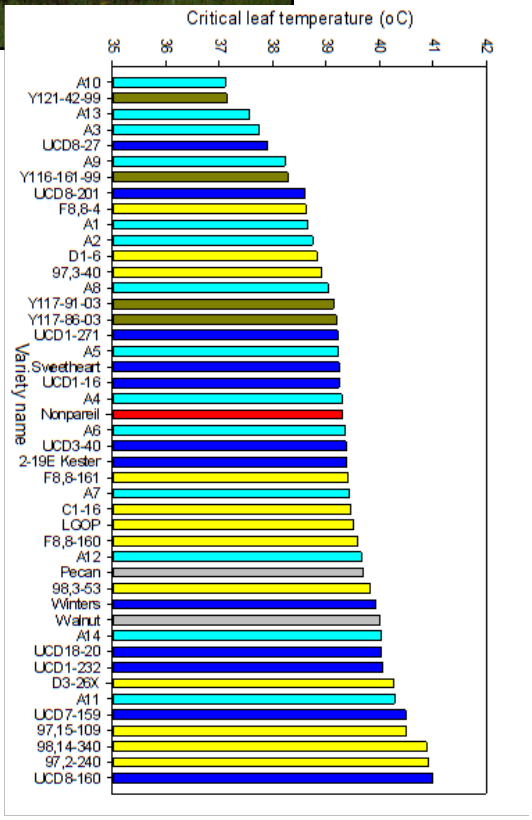


Figure 11. (Right) Range in potential drought tolerance as indicated by the critical leave temperature for UCD and USDA/ARS almond and interspecies germplasm planted at the Chico and Arbuckle RVT and analyzed in 2015 by Matthew Gilbert, UCD.

Results:

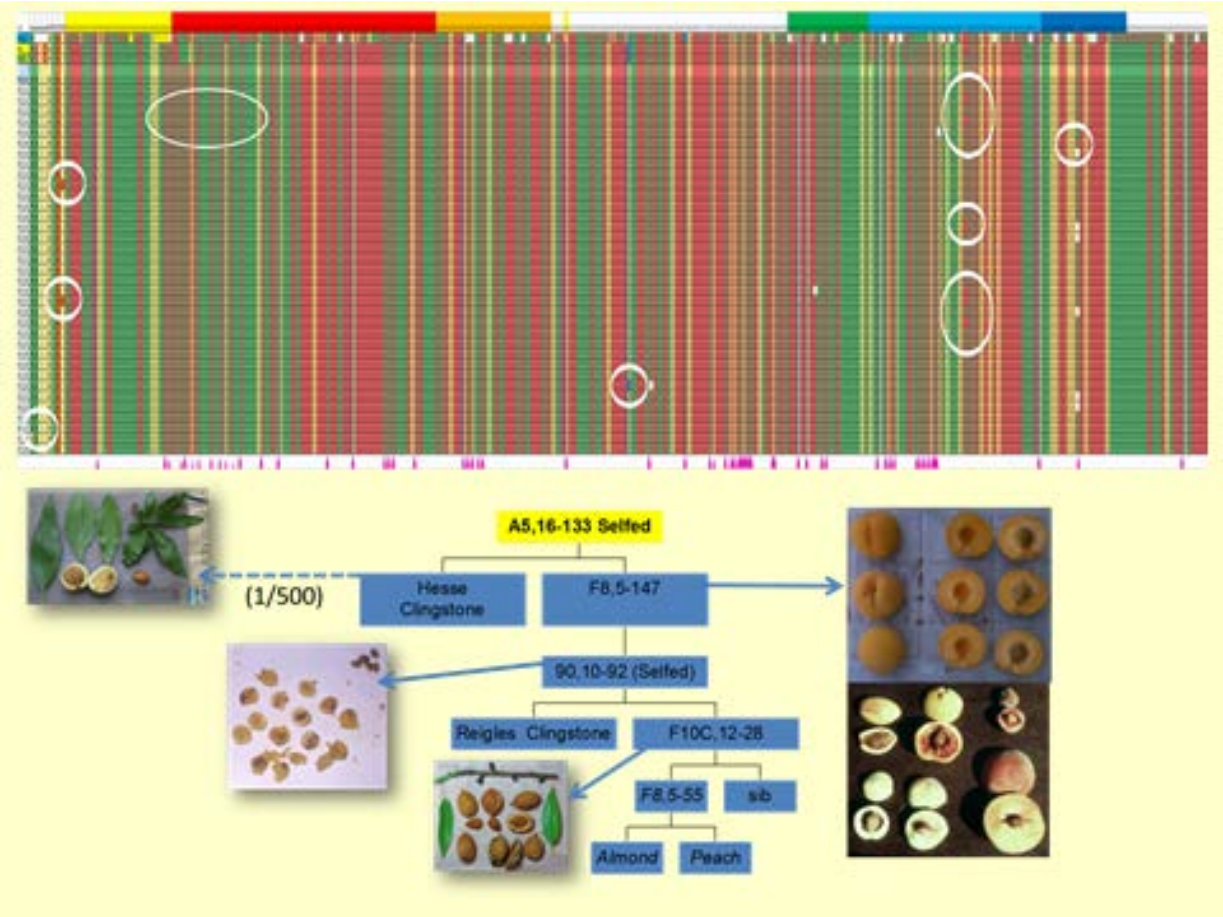


Figure 12. Representative RosBreed marker patterns for each of the eight Prunus chromosomes analyzed (colored bars in the top row). [While only about 30 markers are shown for each chromosome, they are representative of the over 500 markers analyzed. (Pink triangles at the base of the chart identify loci located throughout the chromosome regions that remain heterozygous despite the apparent high level of overall inbreeding).

Figure 12 shows results from the early analysis of interspecies populations using the RosBreed data and tools (see https://www.**rosbreed**.org/). In the UC Davis component of the RosBreed project, over 500 markers spread across 8 chromosomes were analyzed for over 400 genotypes of diverse interspecies lineages. To facilitate visualization, RosBreed marker data has been color-coded in the summary charts used in this report. Figure 12 shows a representative sample. The top color bar indicates the identity of each chromosome in the order of 1 through 8. The 1st data row shows the marker pattern for a standard genotype, in this case the primal peach 07,12-209 with the next row showing results for the A5, 16-133 parent and all subsequent rows showing results for the 40 non-primal progeny. Because these plants are diploid, each marker (colored column) can have 2 possible alleles which are shown in this and subsequent plots as different colors. Each paired-color column shows results for a specific set of marker

alleles at that locus or section of the chromosome. (The chromosome number advances 1 to 8 left to right, on the broad color bar at top, while the marker position on the chromosome also advances left-to-right showing its relative position on the chromosome. The different genotypes within breeding lineages are shown on different rows of the plot. This allows a rapid 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 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 on the chromosomes are only used for visual separation and do not indicate genetic similarities].

The breeding population evaluated in **Figure 12** is an advanced almond to peach introgression line as shown in the lineage beneath the plot. The rows in the plot are include the A5, 16-133 parent in the 2nd row with the remaining row showing self progenies of this parent. Surprisingly, all but one of the 40 progeny gave virtually identical like killer fingerprints despite differing phenotypically in important commercial traits in the field. In fact, the only genetic differences in all 40 of the non-primal progeny as well as the A5, 16-133 parent are circled in the chart with the remaining 490 markers being identical throughout. The one exception is the 07, 12-209 primal seedling shown in the top row which displays a tremendous amount of genetic variability. The breeding lineage involves an almond to peach hybridization followed by 6 generations of backcrossing and selfing. While each generation would reduce the almond molecular component by 50%, even for this many generations of backcrossing should retain a large number of almond genes. (The uniform retention of heterozygosity for a large number of loci positioned fairly uniformly across the different chromosomes is also highly inconsistent with traditional genetic inbreeding. In addition, this lineage has also been the source of several novel traits previously unknown in peach including the primal -type shown in figure 12 as well as resistance to plum pox virus which is present in almond but absent in peach). These highly anomalous marker segregation patterns indicated that either a typical genetic recombination mechanisms were at work, the lineage had somehow been selected for near total homozygosity for the peach genome, and/or the markers developed for peach were not consistent in their ability to discriminate alleles from almond and other related species.

To further test efficacy of standard RosBreed molecular markers for genetic discrimination when interspecific material is analyzed, additional molecular data was generated in 2016 through a 9K SNP mini-array based on RosBreed markers but developed with a private service provider (Ilumina). A large number of additional primal's were included in this analysis. The Ilumina array was originally pursued to see if this platform could successfully translate the trait predictions developed from the larger RosBreed data but from a more specialized array of markers. As with the earlier RosBreed analysis, simple translation from marker presence to trait presence proved not possible and it still requires molecular and breeding expertise to identify the most promising markers, interpret results, and troubleshoot system glitches. Nonetheless, large haplotypic diversity in UCD almond and peach breeding germplasm was documented. In general, this DNA test was simply predictive (i.e., it was easy to

interpret results) for approximately 60% of germplasm but require a more careful analysis for the remainder (which usually involved interspecific crosses). In particular, a large number of trait prediction failures were common to the primal material (as

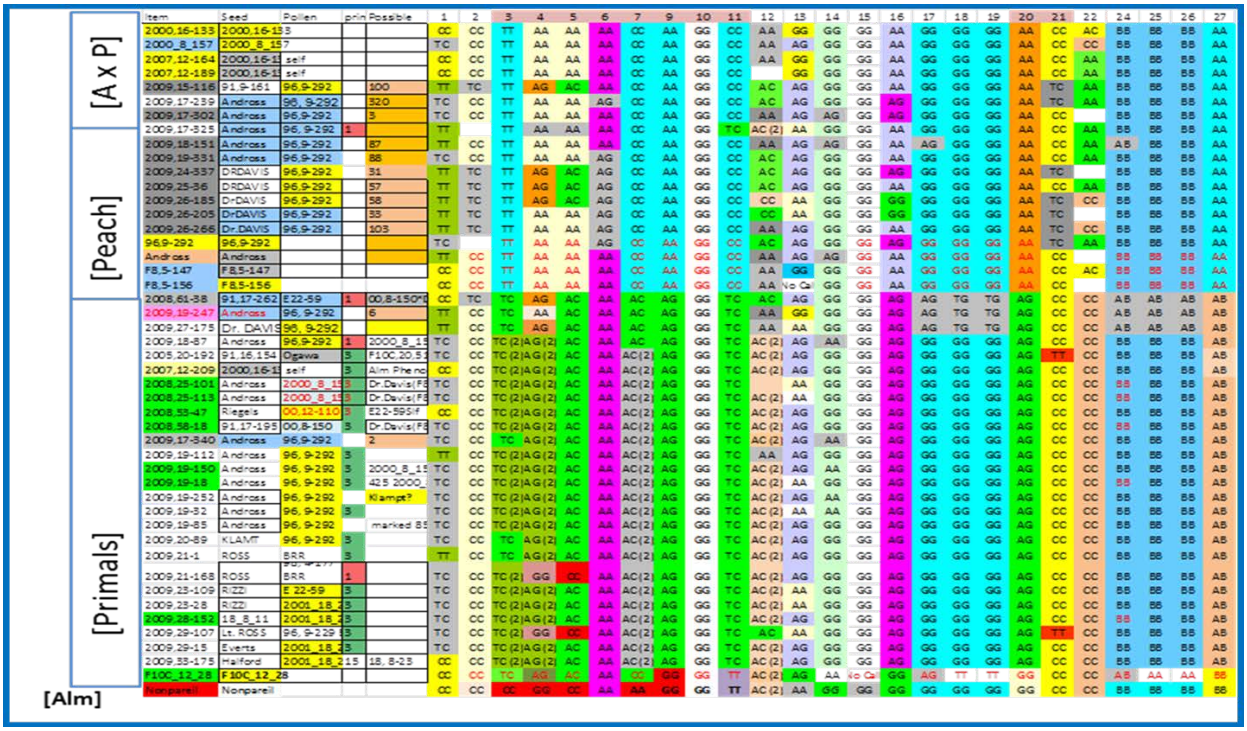
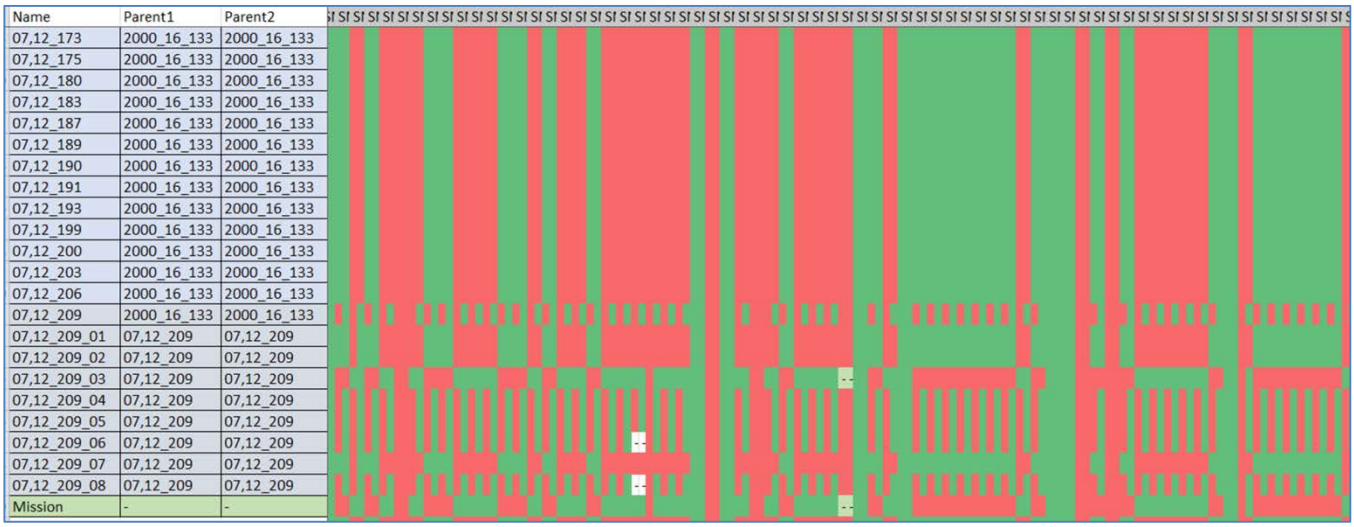


Figure 13. Analysis of molecular markers developed using the 9K SNP mini-array showing distinctive patterns between peach, almond, and almond by peach hybrids, as well as between almond by peach hybrids and the primal genotypes, despite their being phenotypically similar.

previously observed in **Figure 12**). For example, markers scored with two heterozygous groups (e.g., "AC (2)") are always from interspecies introgression populations, since they detect the presence of a third allele coming from almond or another non-peach species but could not uniquely identify that allele. When the 9K SNP mini-array patterns with over 1000 markers which used to evaluate our interspecies introgression lines as well as the primals, the markers were able to clearly distinguish primals from the general peach population as well as the peach almond hybrids lineages [AxP] and almond (**Figure 13**).

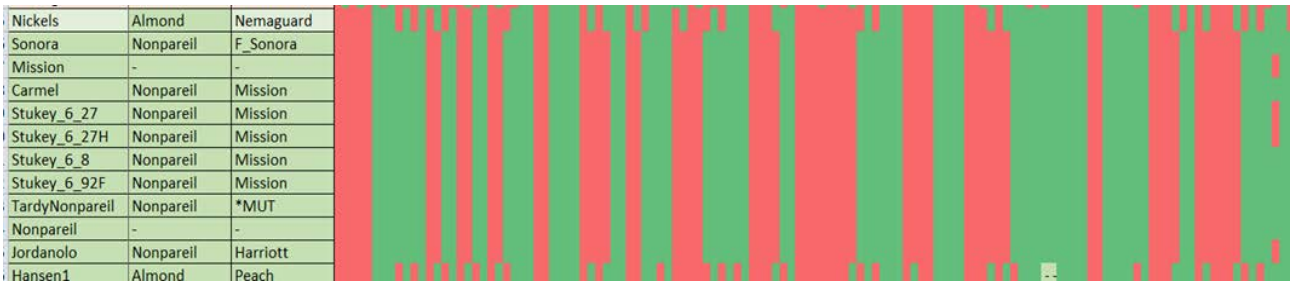
Despite the improved genetic discrimination of this approach, reanalysis of the A5, 16-133 selfed population shown in **Figure 12**, using the 9K SNP mini-array again showed near-total uniformity between the parent and all progeny except the primal 07, 12-209. In **Figure 14**, rows below the primal show representative marker patterns for progeny of 07, 12-209 demonstrating in many cases expected genetic recombination in progeny though in other cases inconsistent inheritance patterns.



Name	Parent1	Parent2
07,12_173	2000_16_133	2000_16_133
07,12_175	2000_16_133	2000_16_133
07,12_180	2000_16_133	2000_16_133
07,12_183	2000_16_133	2000_16_133
07,12_187	2000_16_133	2000_16_133
07,12_189	2000_16_133	2000_16_133
07,12_190	2000_16_133	2000_16_133
07,12_191	2000_16_133	2000_16_133
07,12_193	2000_16_133	2000_16_133
07,12_199	2000_16_133	2000_16_133
07,12_200	2000_16_133	2000_16_133
07,12_203	2000_16_133	2000_16_133
07,12_206	2000_16_133	2000_16_133
07,12_209	2000_16_133	2000_16_133
07,12_209_01	07,12_209	07,12_209
07,12_209_02	07,12_209	07,12_209
07,12_209_03	07,12_209	07,12_209
07,12_209_04	07,12_209	07,12_209
07,12_209_05	07,12_209	07,12_209
07,12_209_06	07,12_209	07,12_209
07,12_209_07	07,12_209	07,12_209
07,12_209_08	07,12_209	07,12_209
Mission	-	-

Figure 14. Reanalysis of the A5, 16-133 selfed population shown in figure 12, using the 9K SNP mini-array again showed near-total uniformity between the parent and all progeny except the primal 07, 12-209. Rows below the primal show representative marker patterns for progeny of 07, 12 209 showing in many cases expected genetic recombination in progeny though in other cases inconsistent inheritance patterns.

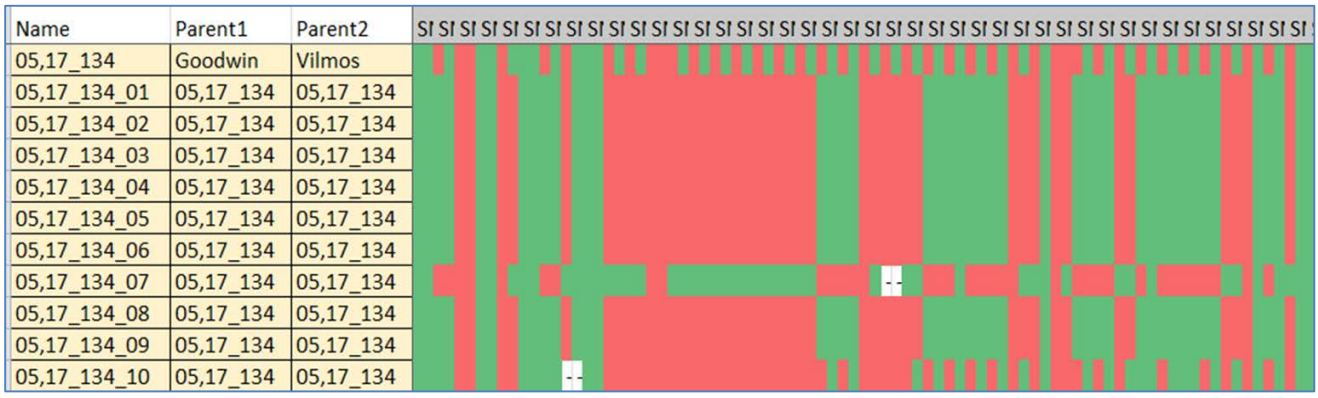
A key test of the RosBreed molecular marker ability to discriminate almond and other non-peach alleles involved the comparison of several different almond genotypes, as shown in **Figure 15**. Because almond is self-sterile, it requires constant outcrossing and consequently is genetically very highly heterozygous (i.e., the 2 possible alleles at each genetic locus are rarely identical because they have been inherited from different parents). For the most part, the high genetic variability in almond was not detectable using the RosBreed markers. Because these markers were specifically developed for peach, they apparently cannot discriminate among genotypes in almond, a closely related species, and thus characterize all non-peach genotypes as indistinguishable.



Nickels	Almond	Nemaguard
Sonora	Nonpareil	F_Sonora
Mission	-	-
Carmel	Nonpareil	Mission
Stukey_6_27	Nonpareil	Mission
Stukey_6_27H	Nonpareil	Mission
Stukey_6_8	Nonpareil	Mission
Stukey_6_92F	Nonpareil	Mission
TardyNonpareil	Nonpareil	*MUT
Nonpareil	-	-
Jordanolo	Nonpareil	Harriott
Hansen1	Almond	Peach

Figure 15. Representative molecular marker plots for different almond cultivars and genotypes as compared to the peach by almond hybrids Nicells and Hansen showing failure of the markers to discriminate among the known heterozygotes in almond. (Note that some markers on the right side of the plot were able to be discriminated, but these were rare).

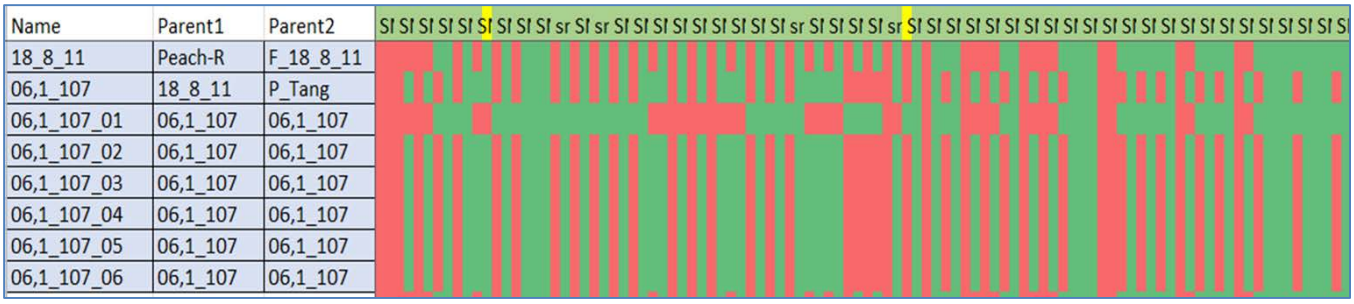
To test whether inheritance distortions may also be involved, (in addition to the failed perception of inheritance because of the inability of peach markers to distinguish almond alleles), we have resynthesized the 1st backcross to peach shown in **Figure 12** with results shown in **Figure 16** where the Goodwin peach has been crossed to the peach almond F2 F10C12-28 (identified here as Vilmos). While the progeny (represented by 05, 17-134 in **Figure 16**) are highly heterozygous and would be expected early in the almond introgression to peach, the self-pollination of 0517-134, again show a very distorted inheritance, despite the ability of RosBreed markers to distinguish between the peach and almond alleles in this section of chromosome. [Since the progeny should be composed of a reshuffling of the 05, 17-134 parent alleles, they should show a random recombination of markers in the top row rather than the solid blocks shown. The distortions may be due to chromosomal rearrangements such as inversions or translocations and or sterility barriers. While pollen or ovule sterility was not apparent in any of the progeny, functional levels of sterility could encourage outcrossing rather than the intended selfing, which would also lead to distortions from expected inheritance patterns. We are currently trying to develop more consistent markers for discriminating such interspecies material to further study these possibilities.



Name	Parent1	Parent2
05,17_134	Goodwin	Vilmos
05,17_134_01	05,17_134	05,17_134
05,17_134_02	05,17_134	05,17_134
05,17_134_03	05,17_134	05,17_134
05,17_134_04	05,17_134	05,17_134
05,17_134_05	05,17_134	05,17_134
05,17_134_06	05,17_134	05,17_134
05,17_134_07	05,17_134	05,17_134
05,17_134_08	05,17_134	05,17_134
05,17_134_09	05,17_134	05,17_134
05,17_134_10	05,17_134	05,17_134

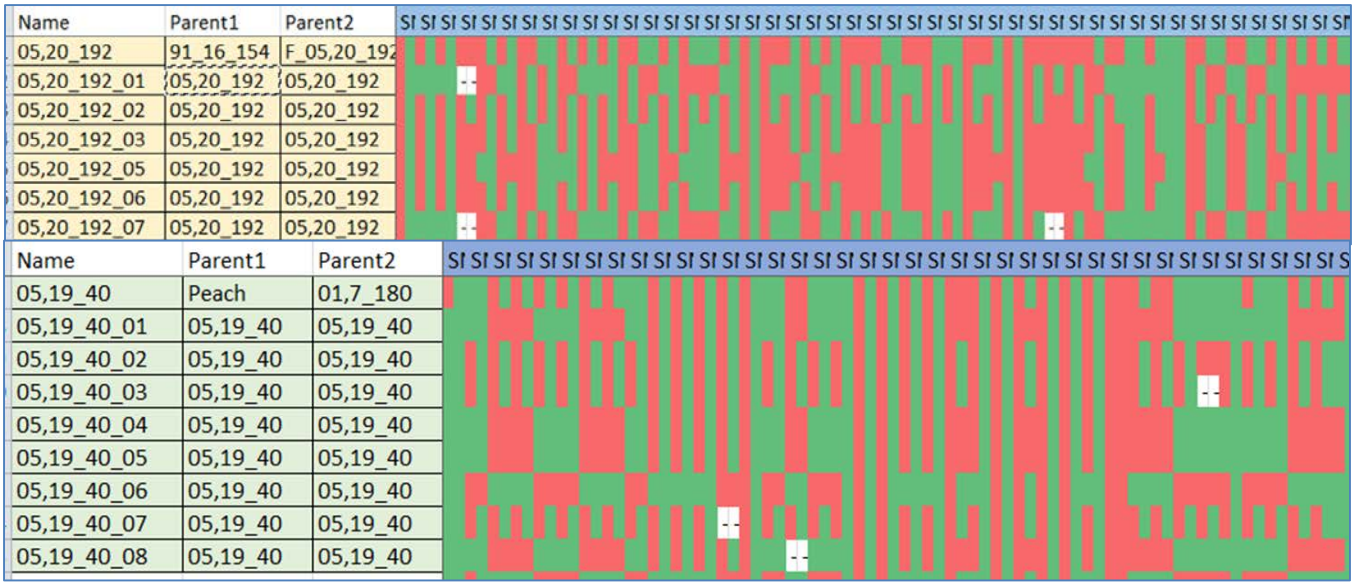
Figure 16. Results from the resynthesis the 1st backcross to peach shown in figure 12 where progeny (represented by 05, 17-134 in figure 16) are highly heterozygous but subsequent self-pollinations, again show a very distorted inheritance.

The degree of distortion from expected inheritance patterns differs with different species introgression lines which may be an indication of differences in barriers to recombination (i.e., different chromosome rearrangements and associated sterility) or differences in the peach markers ability to among the different species. In the interspecies cross between peach and *Prunus tangutica*, the inheritance pattern is more consistent with standard expectations (**Figure 17**). [That is, for the most part, each allele identity (color) for each selfed progeny can be explained with inheritance of one or 2 possible forms present in the original 06, 1-107 parent]. However, the genetic variability or heterozygosity of the species hybrid is much less than the peach parent which is contrary to expectation since peach is normally self-pollinated and so highly inbred. In addition, while more markers in the progeny are consistent with the given selfed parent, markers interspecies hybrid often failed to show the contribution from peach, again indicating aberrations in expected pattern of inheritance.



Name	Parent1	Parent2
18_8_11	Peach-R	F_18_8_11
06,1_107	18_8_11	P_Tang
06,1_107_01	06,1_107	06,1_107
06,1_107_02	06,1_107	06,1_107
06,1_107_03	06,1_107	06,1_107
06,1_107_04	06,1_107	06,1_107
06,1_107_05	06,1_107	06,1_107
06,1_107_06	06,1_107	06,1_107

Figure 17. Molecular marker inheritance pattern of peach (top row), a peach by Prunus tangutica interspecies hybrid (2nd row) with lower row showing patterns for 6 F2 progeny (selfs of the species hybrid) showing that distortion from expected inheritance patterns differs with different species introgression lines.



Name	Parent1	Parent2
05,20_192	91_16_154	F_05,20_192
05,20_192_01	05,20_192	05,20_192
05,20_192_02	05,20_192	05,20_192
05,20_192_03	05,20_192	05,20_192
05,20_192_05	05,20_192	05,20_192
05,20_192_06	05,20_192	05,20_192
05,20_192_07	05,20_192	05,20_192

Name	Parent1	Parent2
05,19_40	Peach	01,7_180
05,19_40_01	05,19_40	05,19_40
05,19_40_02	05,19_40	05,19_40
05,19_40_03	05,19_40	05,19_40
05,19_40_04	05,19_40	05,19_40
05,19_40_05	05,19_40	05,19_40
05,19_40_06	05,19_40	05,19_40
05,19_40_07	05,19_40	05,19_40
05,19_40_08	05,19_40	05,19_40

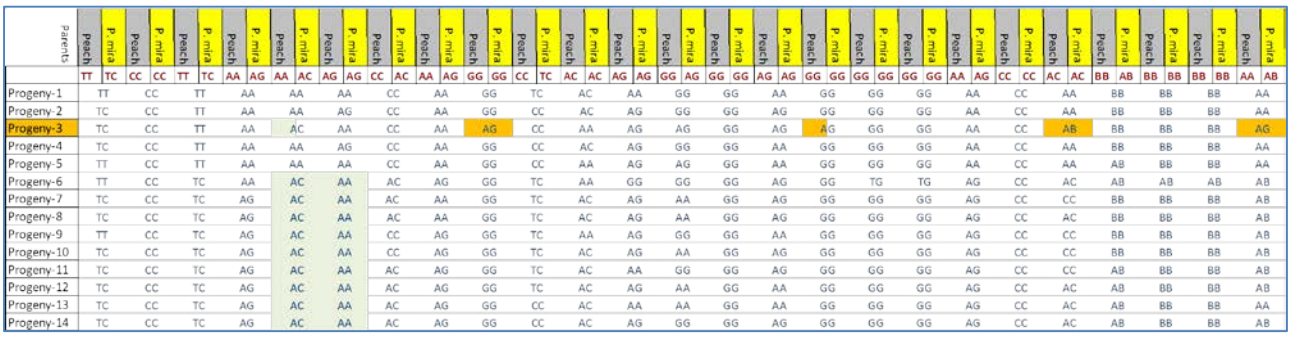
Figure 18. Molecular marker inheritance patterns of primal genotypes derived from advanced almond to peach introgression lines (05, 20-192 at top) in Prunus argentea to peach introgression lines (05, 19-40 at bottom) as well as patterns for 7 and 8 selfed progeny, respectively, showing largely normal inheritance patterns.

In contrast, the inheritance patterns for most interspecies derived primal genotypes show relatively normal inheritance across all chromosomes **(Figure 18).** Because of the advanced state of introgression, this might be expected as the populations should be composed largely of peach alleles. However, a predominance of peach alleles in these primal individuals makes it more difficult to explain 3rd distinct peach by almond type phenotype since the expectation was these would be more similar genetically to species hybrids. We are currently trying to develop improved markers to further study this inconsistency. The more traditional inheritance patterns, however, may facilitate the development of such primal types either as highly stress tolerant cultivars (see 2015 Almond Variety Development Annual Report, 15-HORT1-Gradziel) or as rootstocks.

Name	Parent1	Parent2
P.mira19	-	-
Riegels	Jungerman	Everts
Lovell	StJohn	F_Lovell
persXdavidiana	Peach	P. davidiana
Nemaguard	Peach	P. davidiana
Nickels	Almond	Nemaguard
Sonora	Nonpareil	F_Sonora
Mission	-	-
Carmel	Nonpareil	Mission

Figure 19. Representative molecular marker patterns on chromosome 1 for different species and species hybrids showing the value of general trends in identifying possible pedigrees. The pattern for Nemaguard shows a much greater similarity between an established (peach x *P davidiana*) interspecies hybrid then either almond or Prunus mira, supporting the general perception that P davidiana was a parent of Nemaguard.

General molecular marker patterns can also provide insight concerning possible species contributions to current rootstocks. In **Figure 19**, the pattern for Nemaguard shows a much greater similarity between a known (peach x *P. davidiana*) interspecies hybrid then either almond or *Prunus mira*, supporting the general perception that *P davidiana* was a parent of Nemaguard and probable contributor of the genes for nematode resistance. The power of this approach, however, may be limited to species closely related peach, including *P davidiana and P mira*.



Parents	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira	Peach	P. mira
	TT	TC	CC	CC	TT	TC	AA	AG	AA	AC	AG	AG	CC	AC	AA	AG	GG	GG	CC	TC	AC	AC	AG	AG	GG	AG	GG	GG	AG	AG	GG	GG	GG	GG	AA	AG	CC	CC	AC	AC	BB	AB	BB	BB	BB	BB	BB	AA	AB
Progeny-1	TT		CC		TT		AA		AA		AA		CC		AA		GG		TC		AC		AA		GG		GG		AA		GG		GG		GG		AA		CC		AA		BB		BB		BB		BB		AA	
Progeny-2	TC		CC		TT		AA		AA		AG		CC		AA		GG		CC		AC		AG		GG		GG		AG		GG		GG		GG		AA		CC		AA		BB		BB		BB		BB		AA	
Progeny-3	TC		CC		TT		AA		AC		AA		CC		AA		AG		CC		AA		AG		AG		GG		AG		GG		GG		GG		AA		CC		AB		BB		BB		BB		BB		AG	
Progeny-4	TC		CC		TT		AA		AA		AG		CC		AA		GG		CC		AC		AG		GG		GG		AA		GG		GG		GG		AA		CC		AA		BB		BB		BB		BB		AA	
Progeny-5	TT		CC		TT		AA		AA		AA		CC		AA		GG		CC		AA		AG		AG		GG		AA		GG		GG		GG		AA		CC		AA		AB		BB		BB		BB		AA	
Progeny-6	TT		CC		TC		AA		AC		AA		AC		AG		GG		TC		AA		GG		GG		GG		AG		GG		TG		TG		AG		CC		AC		AB		AB		AB		AB		AB	
Progeny-7	TC		CC		TC		AG		AC		AA		AC		AA		GG		TC		AC		AG		AA		GG		AG		GG		GG		GG		AG		CC		CC		BB		BB		BB		BB		AB	
Progeny-8	TC		CC		TC		AG		AC		AA		AC		AA		GG		TC		AC		AG		AA		GG		AG		GG		GG		GG		AG		CC		AC		BB		BB		BB		BB		AB	
Progeny-9	TT		CC		TC		AG		AC		AA		CC		AG		GG		TC		AA		AG		GG		GG		AA		GG		GG		GG		AG		CC		CC		BB		BB		BB		BB		AB	
Progeny-10	TC		CC		TC		AG		AC		AA		CC		AG		GG		TC		AC		AG		AA		GG		AG		GG		GG		GG		AG		CC		CC		BB		BB		BB		BB		AB	
Progeny-11	TC		CC		TC		AG		AC		AA		AC		AG		GG		TC		AC		AA		GG		GG		AG		GG		GG		GG		AG		CC		CC		AB		BB		BB		BB		AB	
Progeny-12	TC		CC		TC		AG		AC		AA		AC		AG		GG		TC		AC		AG		AA		GG		AA		GG		GG		GG		AG		CC		AC		AB		BB		BB		BB		AB	
Progeny-13	TC		CC		TC		AG		AC		AA		AC		AG		GG		CC		AC		AA		AA		GG		AA		GG		GG		GG		AG		CC		AC		AB		BB		BB		BB		AA	
Progeny-14	TC		CC		TC		AG		AC		AA		AC		AG		GG		CC		AC		AG		GG		GG		AG		GG		GG		GG		AG		CC		AC		AB		BB		BB		BB		AB	

Figure 20. Analysis of introgression progeny from a cross between *P, persica* (peach) and *P. mira* showing molecular markers (9K SNP mini-array) as inherited by progeny. Hi-lighted (yellow) markers identify a probable contamination. Light shading identifies suspicious inheritance patterns between 2 linked loci.

As introgression lines between these more closely related species become more advanced (that is, become more peach-like genetically and phenotypically) inheritance patterns becomes much more normalized so that consistent testing becomes feasible. An analysis of introgression progeny from a cross between *P, persica* (peach) and *P. mira* showing generally expected inheritance patterns (**Figure 20**). Hi-lighted (yellow) markers identify a probable contamination. Light shading, however, identifies suspicious inheritance patterns can still be detected between 2 linked loci.

Results thus demonstrate reliable genetic analysis is possible when markers are available which distinguish the targeted species. With interspecies hybridization and subsequent introgression, many markers will prove efficient in discriminating alleles from the more divergent species. This distortion appears to be minimized when species are closely related to becoming more significant as species become more divergent.

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