Almond Variety Development

Project No.: **10-HORT1-Gradziel**

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Objectives:

A. Utilize expanded grower trials to evaluate regional performance of advanced breeding lines to identify the most promising genes/selections for resistance and market quality for inclusion in new Regional Variety Trials.
B. Improve selection efficiency for required traits (productivity, resistance to disease/pest/environmental stress, marketability, sustainability). Prioritize required traits in partnership with growers, handlers and processors.
C. Accelerate the variety development cycle through expanded controlled hybridizations followed by more efficient screening of progeny trees for self-compatibility, tree productivity, kernel quality and resistance to key pests, diseases and environmental stresses.

Interpretive Summary:

Commercial success of a new variety is determined not only by improved performance in a specific area, but also a consistently superior performance for the wide range of required traits. This is particularly relevant for almond were orchards are expected to be productive for 20 or more years and where failed varieties cannot be readily plowed under and replanted. Almonds also differ from most field crops in that they are not seed propagated but clonally propagated. At UCD, vegetative propagation combined with clone based selection strategies is proving to be one of the most effective methods for capturing the fullest range of breeding potential, including additive, dominance, epistatic, epigenetic and genomic interactions for almond improvement. The common practice of clonal propagation of a small number of elite varieties, however, inherently decreases the genetic variability for that crop and so increases its genetic vulnerability to diseases and cultural changes. The majority of California cultivars are derived from only 2 parental cultivars: *Nonpareil* and *Mission*. To incorporate new traits such as self-compatibility and improved disease resistance, the UCD almond breeding program has

brought in a wide range of new germplasm, including material from related species. The ongoing challenge is to employ the most efficient traditional and molecular breeding strategies to transfer required new genes from this diverse parental material into a genetic background that is well-adapted the Central Valley production and market systems. UCD selection *2-19E*, combining late flowering and high productivity with a *Nonpareil* type kernel is currently being prepared for patenting and release.

Materials and Methods:

The University of California at Davis (UCD) Almond Variety Development Program will be presented as a general overview of the breeding approach being developed with more detailed information presented for key components (breeding strategy, development and assessment of current parents, and regional evaluation of advanced selections). Detailed results are presented as figures and tables and will be more thoroughly discussed in the associated captions to allow a more expedient summary of program status, while the main text will pursue a more general discussion of the importance and interconnectedness of the different components. Although the use of technical language has been minimize, the inclusion of some standard genetic terms is inevitable, though definitions are available in standard references such as Wikipedia.

Crop breeding strategies.

In the century since the genetic basis of inheritance was rediscovered and exploited for crop improvement, a large number of diverse breeding strategies have evolved. Most, however, are based on four fundamental approaches: *Inbreeding, Hybridization, Synthetics* and *Cloning* (**Figure 1**). *Inbreeding* and *Hybridization* are commonly used for crops which are self-pollinating and so tolerant of inbreeding. *Synthetics* and *Clones* are more often used in cross-pollinating crop species such as almond where self-pollination may result in reduced fitness, including inbreeding depression.

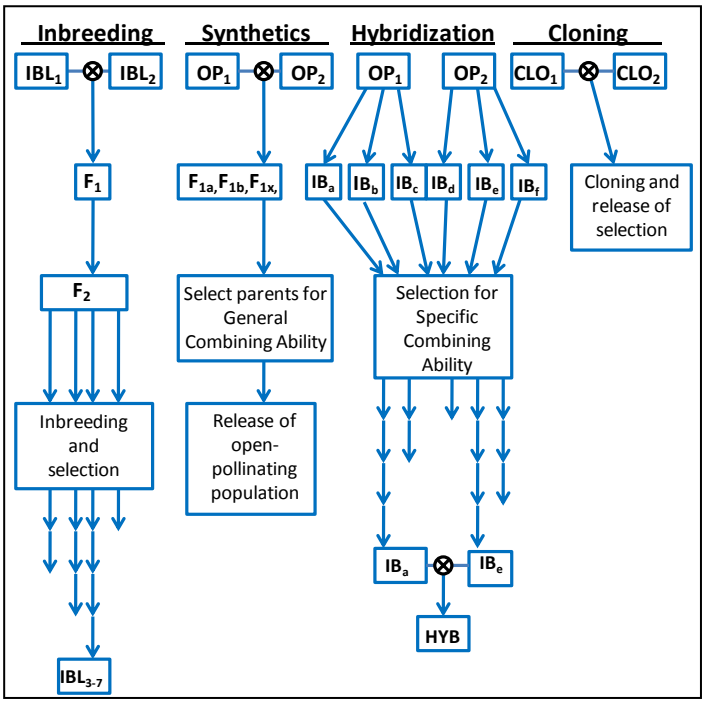


Figure 1. The 4 basic breeding strategies for cultivar development. (The horizontal length of individual boxes roughly reflects genetic variability while the number of tiers of vertical arrows approximate number of breeding cycles.

Inbreeding.

Inbreeding typically involves the recurrent inbreeding of populations which are thus more genetically homogeneous than would occur with random mating. Ranging from recurrent selection to the development of inbred pure lines, this strategy is characterized by the selection of transgressive phenotypes

in the F_2 to F_7 generations. Inbreeding drives individual loci towards homozygosity and so primarily targets additive genetic effects. The increasing level of homozygosity with each inbred generation is a distinct advantage in seed-propagated crops as individuals in advanced inbred lines will be more homogenous and so consequently more true-breeding in seed provided to growers.

Hybrid seed.
Hybridization involves the development of hybrids between inbred parental lines which have been carefully selected for their specific combining ability (typically heterotic vigor or heterosis). Resulting hybrid progeny are genetically uniform (homogenous) yet can be highly heterozygous and so capable of exploiting additive, dominance and epistasis interactions. However, the full exploitation of these genetic effects is limited by the tedious parental combining-ability testing required for each desired inbred line combination.

Synthetics.
While *Hybridization* involves the selection of inbred parent pairs based on their specific combining ability (as determined by previous assessment of progeny performance), Synthetics involve the selection of a number of genotypes for good general combining ability (i.e. moderate to good heterosis recovered in all possible crossing combinations). While capable of exploiting additive, dominance and epistatic genetic effects, synthetics are generally less efficient in accumulating additive genetic effects than inbreeding with recurrent selection, and less efficient than hybridization at capturing dominance and epistasis effects since the realized genetic gain is the average of the many potential hybrids and so difficult to optimize. Because heterosis can be partially maintained in growers' fields through continued natural outcrossing, synthetics have proven particularly useful in perennial forage crops such as alfalfa where naturally occurring annual re-seeding is required.

Cloning.
Cloning depends upon the capability for asexual or vegetative propagation of the cultivar from the breeding program to the grower's field, and is thus common in perennial, woody crops such as almond. It usually involves an initial hybridization between two distinct parents, but may also involve self-pollination of genotypes where inbreeding depression is not a problem (**Figure 2**). Unlike *Inbreeding* and *Hybridization*, there is typically no pre-breeding requirement (i.e. no development of early generation inbred lines, etc.) in cloning and, because selected genotypes can be asexually propagated, all genetic potential is essentially captured for the grower without the risk of the often regressive, genetic recombination associated with foundation seed increase for seed propagated crops. Consequently, *Cloning* can fully capture additive, dominance and epistasis effects in cultivars which then remain true-to-type in subsequent vegetative propagations [1]. The level of genetic gain is limited only by the quality and diversity of the breeding parents and the size of the progeny population. Cloning of interspecies hybrids has also been shown to be very effective for the breeding of vigorous and often disease resistant rootstocks for almond such as the *Hansen* and *Nickels* peach by almond hybrids [1]. Vegetative growth vigor in

interspecies hybrids which is sometimes termed 'luxuriance' to distinguish it from intra-species hybrid vigor or heterosis can often transgress well beyond that of even a highly-vigorous parent, and appears to involve both gene-gene and even genome-genome interaction.

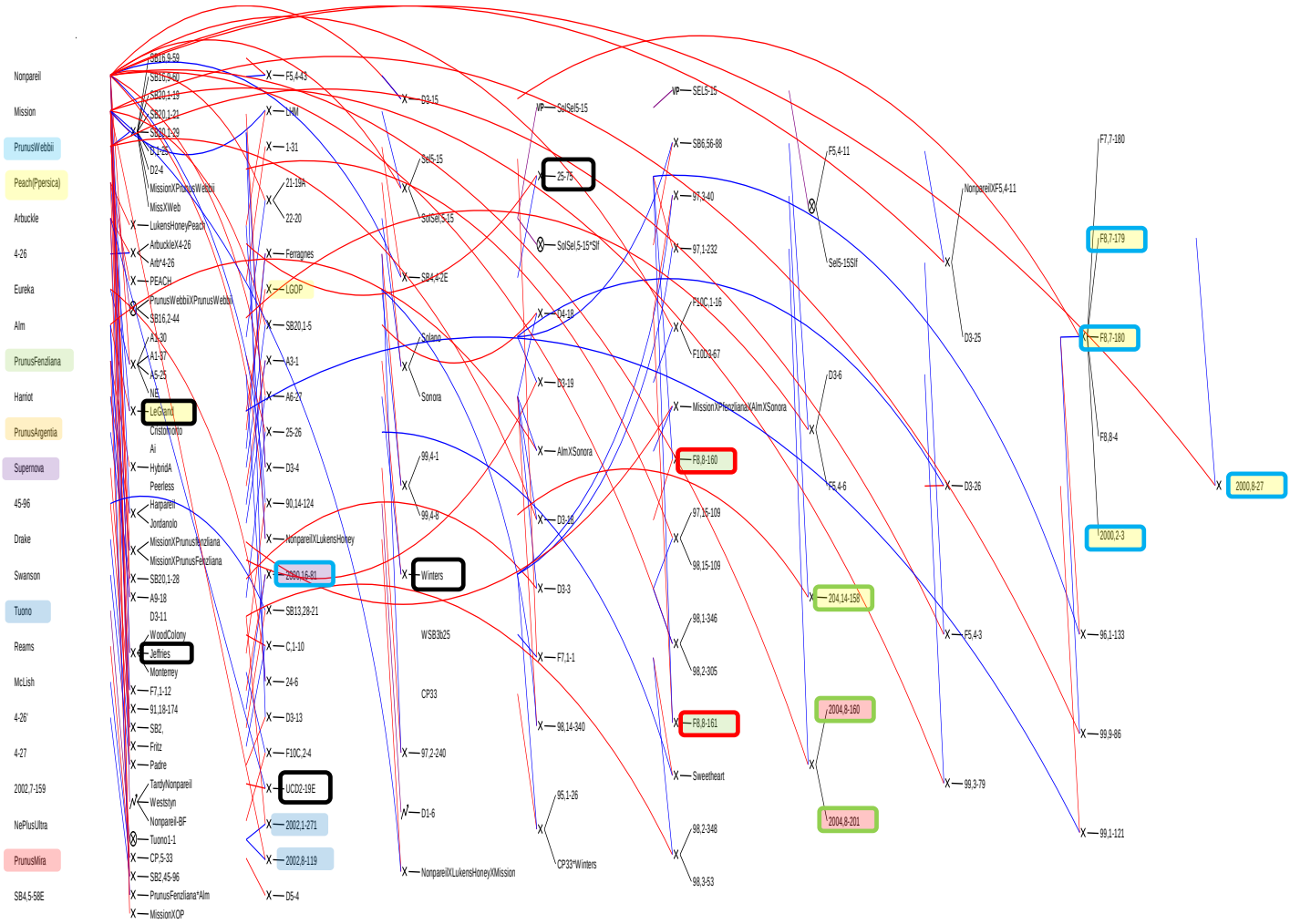


Figure 2. Flowchart showing the lineages of UCD advanced selections and parents currently used in breeding for self compatibility and improved productivity, including disease and pest resistance. Sources of self-compatibility have been independently transferred from the almond variety *LeGrand*, the Italian variety *Tuono*, the induced-mutation *Supernova*, and related species including *Prunus webbii, P. mira* and *P. persica* (peach). Additional sources of disease resistance and improved kernel and tree quality have been transferred from heirloom almond varieties, *P. fenzliana, P. persica, a*nd *P. argentia*. Advanced breeding lines are color-coded relative parent source. Several advanced selections have Incorporated traits such as self-compatibility from multiple sources as breeding experience has shown improved performance and improved stability over years and locations when multiple, diverse sources were combined. Similar results have been found for disease and pest resistance. Other important breeding lines discussed in the report are boxed in black. (Red lines identify the seed parent while blue lines identify pollen parent). {Image can be enlarged for easier visualization. Detailed descriptions and recent field data for these advanced selections are provided in the appendices.}

Results and Discussion:

UCD Almond breeding strategies

Historically, the term 'breed' referred to a type of domesticated animal such as the Australian Shepherd dog which has been selected for specific phenotypes or well-defined traits. The term 'breeding', in turn, refers to the selection of parent combination to achieve the desired phenotype in subsequent offspring. Just as natural selection can result in the gradual evolution of individuals and populations towards greater fitness within the selecting environment, human selection of parent combination and resultant progeny can result in pronounced phenotype changes in individuals and populations which can occur relatively rapidly depending upon the intensity of selection. A primary objective of most breeding approaches is to maximize the desired response to selection or genetic gain. In plant systems, the goal of breeding is also the development of an improved phenotype which is often referred to as a 'variety', or more specifically a 'cultivar' (derived from 'cultivated variety') to distinguish it from the more broadly defined 'botanical variety'. Because most perennial, woody plants such as almond can be asexually propagated, a typical cultivar is usually a single genotype which may be the result of selection over a very large number of years and/or from a very large population of progeny [2]. For example, virtually all commercial sweet orange (*Citrus sinensis*) plantings are essentially asexual propagations of a single ancestral genotype . Chance mutations leading to improved phenotypes (improved flavor, sweetness, color, later maturity, etc.) among the millions of otherwise genetically identical clonal trees cultivated over the past several hundred years have been discovered and, if found to be true-to-type following asexual propagation, are often propagated and distributed as new orange cultivars such as the Washington Navel and Valencia cultivars. Similarly, recent evidence indicates that tree crops such as fig (*Ficus carica*) have been cultivated for over 11,000 years, supporting a very early domestication of fruit and nut crops and so an extended time for the selection of truly exceptional individual clones or cultivars. Many modern almond cultivars have been cultivated continuously for hundreds to thousands of years since their initial selection [2,6], presumably derived from the leading cultivars of their day. The capacity of asexual propagation to essentially capture these very rare, horticulturally-elite genotypes and, in addition, allow their continued improvement through the accumulation of desirable sports or mutations, offered considerable advantages over early breeding efforts with cereals and other seed-propagated crops. This is because propagation by seed inevitably results in a risk reshuffling of desirable genes leading to genetically and so phenotypically variable progeny.

The reduced genetic reshuffling, however, can also act to reduce genetic options as environmental and cultural conditions change. For example, the California and Florida orange industries are under a real threat of extinction from the *citrus-greening* disease since, because of the genetic uniformity of the crop worldwide, no durable genetic resistance is readily available through traditional breeding. More recently, almond production in California has been put in jeopardy by an unpredictable supply of required honeybee pollinators, owing to economic and disease problems. Although many cultivars are currently planted in California, the almond industry remains highly inbred

since most commercially important cultivars are derived from only two parental cultivars: *Nonpareil* and *Mission*, which also appear to be related (**Figure 3**) probably having been derived from common germplasm in the Languedoc region of France [3,9] {unique for its soft-shell varieties in a continent otherwise dominated by hard-shell almonds}.

Variety	B004Fa	B004Fb	B039Ha	B039Hb	B040Na	B040Nb	B002Fa	B002Fb	U003Ha	U003Hb	M040Na	M040Nb	M024Fa	M024Fb	T004Ha	T004Hb	T012Na	T012Nb
Nonpareil	182	194	130	148	142	146	211	233	99	110	212	259	224	236	155	155	148	158
Mission	196	216	122	146	130	136	199	203	108	114	227	227	234	236	129	147	136	148
TardyNonpareil	182	194	130	148	142	146	211	233	99	110	212	259	224	236	155	155	148	158
Jeffries	182	194	130	148	142	146	211	233	99	110	212	259	224	236	155	155	158	158
Booth	182	194	130	148	142	146	211	233	99	110	212	227	224	236	129	155	148	158
Carmel	182	196	122	148	130	142	199	233	99	114	212	227	234	236	147	155	136	158
Thompson	194	216	122	130	130	142	203	211	99	114	212	227	224	236	147	155	136	148
Monterey	182	216	146	148	130	142	203	233	99	114	227	259	224	234	129	155	148	158
Ruby	194	196	122	142	136	146	199	211	108	110	227	227	224	236	147	155	148	156
Livingston	194	196	122	130	136	146	199	233	108	110	229	259	224	236	147	155	136	158
Fritz	194	196	142	146	130	146	199	211	110	114	221	227	224	236	129	155	148	158
Norman	194	196	122	130	130	146	199	211	110	114	212	227	224	236	147	155	148	158
Kochi	182	194	142	148	142	146	211	233	99	110	225	225	224	224	155	155	148	158
Butte	182	196	122	130	136	146	199	233	99	114	227	259	224	236	147	155	148	158
Price	194	196	146	148	130	148	199	211	110	114	212	227	224	236	129	155	148	148
Aldrich	182	196	122	130	130	142	199	233	110	114	212	227	236	236	147	155	148	148
LeGrand	182	194	142	148	130	136	211	233	108	114	227	259	224	236	155	155	148	158
LGOP	194	194	148	148	130	136	211	211	108	114	227	259	224	224	155	155	148	158
Sonora	182	194	148	148	130	142	211	233	99	99	255	259	224	236	145	155	138	158
Padre	182	196	122	180	136	142	199	209	99	108	227	227	236	244	129	147	148	148
Winters	182	200	130	136	132	132	233	233	116	116	227	229	236	242	155	155	146	158
2004,8-201	182	194	136	148	142	142	211	233	99	99	212	229	236	236	155	155	146	148
2000,16-81	194	196	130	184	130	142	199	211	99	120	213	259	236	236	147	155	146	158
2000,2-3	182	194	122	130	136	142	211	233	99	108	227	259	224	236	147	155	150	158
2000,8-27	182	194	130	148	136	146	211	233	108	110	212	227	224	236	155	155	148	150
2002,1-271*	196	200	144	184	138	142	199	199	95	99	225	259	236	250	143	155	152	158
2002,8-119	196	200	144	146	136	136	199	199	95	108	225	227	236	250	129	155	136	152
2004,14-158	182	194	148	184	130	130	211	233	108	120	229	259	224	224	155	155	146	158
2004,8-160	182	194	140	148	142	142	211	233	99	99	212	229	224	224	134	155	146	148
F7,1-1	182	194	130	184	142	146	211	233	99	110	227	229	224	236	155	161	148	156
F8,7-179	194	205	122	130	136	142	211	235	99	108	227	259	224	250	147	155	150	158
F8,8-160	194	196	184	184	130	130	199	211	99	120	213	229	224	236	135	155	146	158
F8,8-161	196	205	140	168	130	142	199	235	120	120	213	255	236	250	147	155	138	146

Figure 3. Results from the DNA fingerprinting (using SSR markers [5]) for traditional California almond cultivars as well as advanced UCD breeding selections identified in **Figure 2**. Note that virtually all California almond cultivars share markers with Nonpareil and Mission, supporting their derivation from these early California varieties. In addition, the similarity of markers between Mission and Nonpareil support a common origin for both which is in agreement with historical records suggesting the area of Languedoc, France is the origin for both [2,3]. Exceptions are the UCD developed varieties *Sonora, Padre* and *Winters*, in which outside germplasm was intentionally incorporated to increase breeding options and to decrease the genetic vulnerability of California almond. The prevalence of novel markers (indicated in red text with a blue background) in UCD cultivars and advanced selections supports a further increase in novel genetic opportunities as well as a decrease in general genetic vulnerability in these breeding lines despite intensive selection for kernel and tree types adapted the Central Valley production and markets (see **Figure 13** and appendices).

Genetic analysis as a basis for applied breeding.

Early almond breeders were generally aware that the characteristics or phenotypes of progeny from a specific set of parents were determined by the environmental conditions during their development as well as by genetic factors inherited from parents. The only way to determine a given individual's genetic or breeding potential, however, was through experience; that is by keeping track of the general breeding value for each individual parent as well as the specific value of each individual parental combination. Such trial and error approaches required both extensive experience as well as a good understanding of various environment effects on the final phenotype since the final heritability of the trait was determined by the proportion of the total phenotypic or observable variability that was due to parent (genetic) contribution relative to the total variability from genetic and environmental causes. Breeding was largely reactive since the heritability of a specific trait from a specific parent combination had to first be developed empirically and then, if desired, reproduced on a larger scale. More proactive and analytical approaches to cultivar breeding resulted from the discovery in the early to mid-1900s, that genes coded by unique DNA sequences were the factors controlling heredity, and the rediscovery of Mendel's research showing that genes can be inherited in predictable patterns. An example of the proactive breeding potential of Mendelian analysis is apparent in the classical single gene (1:2:1) ratio expected in heterozygous diploid crosses (**Figure 4**). With sufficient knowledge of the inheritance for the trait of interest and the genetic composition of the parents, the breeder could accurately predict the proportion of progeny expected to inherit the traits (and thus determine the minimum number of progeny required to obtain at least a few individuals possessing the desired traits). Similarly, by analyzing segregation ratios of progeny from known crosses, the breeder could sometimes deduce both the genetic control for the trait as well as the genetic composition of the parents. For example, selfing Nonpareil or Mission (or crossing Nonpareil by Mission) would give a progeny population which segregated roughly 3:1 for sweet: bitter kernel indicating that both parents were heterozygous (Aa) for this trait (**Figure 4**). However, selfing the Winters or Butte varieties would result in all seedlings having sweet kernels, indicating that these varieties were homozygous dominant (AA) for the trait [7]. (This also explains why these varieties are particularly susceptible to deer-feeding). The major Mendelian or qualitative genes identified through this process for peach, (the stone fruit with the most extensive genetic database), and then verified in our UCD breeding lines for almond are summarized in **Table 1**.

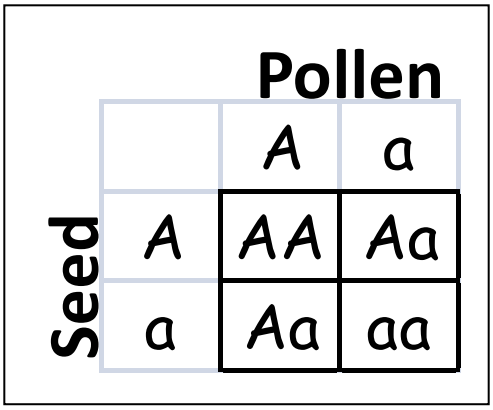


Figure 4. Punnett square diagram showing predicted gamete (1/2A:1/2a) as well as progeny genotypes (1AA:2Aa:1aa) and their probabilities from a cross between two diploid plants heterozygous at locus Aa where A- dominates in expression. This segregation pattern is common for bitterness (aa) in almond kernels where sweetness (AA or Aa) dominates.

Table 1. Qualitative traits identified in almond. Of these, only kernel sweetness vs. bitterness and self-incompatibility/compatibility have significant commercial importance.

Phenotype and symbol	Genotype	Note
Leaf		
Willow-leaf (*Wa2*)	*wa2*/*wa2*	
Crinkle leaf (*CL*)	*cl*/*cl*	
Flower		
Pink petal (*P*)	*P*/–	
Self-incompatibility(S*x*)	*Sa/Sb*	
Self-compatibility *(Sf)*	*Sf*-	
Fruit		
Sweet kernel *(A)*	*A*-	Multiple modifier genes
Tree		
Canker susceptibility *(CK)*	*ck/ck*	High susceptibility to bark canker
Dwarf *(Dw)*	*dw/dw*	Dwarf tree with very short internodes

Table 2. Estimates of heritabilities (proportion of trait due to genetic control) for almond traits evaluated at UCD [1,4,10]. Note, for example, that kernel bitterness is determined entirely by genetic factors rather than growth/storage environment.

Trait	Heritability	Standard Deviation
hull dehiscence	0.02	0.32
shell type	0.55	0.17
shell seal	0.14	0.20
retention of outer shell	0.34	0.20
width of shell opening	0.21	0.20
in-shell weight	0.81	0.17
kernel length	0.77	0.17
kernel thickness	0.71	0.20
kernel mass	0.64	0.17
kernel width	0.62	0.17
double kernels	0.51	0.30
kernel color	0.42	0.22
shell color	0.05	0.22
kernel bitterness	1.00	0.36
kernel crease	0.79	0.20
worm damage	0.30	0.17
hull pubescence	0.28	0.22

The reductionist approach made possible through Mendelian analysis remains the foundation for the genetic manipulation of most readily observable segregating or *qualitative* genes. Similarly, the recognition that genetic contributions could be isolated and then recombined in a largely additive manner forms the basis for most molecular marker approaches, including both marker assisted selection (MAS) and marker assisted breeding (MAB). From **Table 1**, however, it can be seen that traits controlled by single segregating genes are rare in almond and even where important examples exist, such as self-compatibility, become complicated by specific genetic background (as discussed in following sections). For most important horticultural traits, segregation ratios become increasingly complex, and, the ability to discriminate the diminishing individual genetic effect from environmental effect becomes limiting so that for traits controlled by three or more genes, an analysis based on statistical probabilities is usually required to help discriminate genetic from environmental background effects (**Figures 8 and 10**). In such *quantitative* genetic analysis, the variation in traits or phenotypic expression is partitioned into environmental and genetic components where genes are generally assumed to be independent in action and alleles contribute equal and additive effects to final phenotype. Heritability (H) in this narrow sense can then be defined by the ratio of additive genetic variance [V_G] to total variance (genetic [V_G] + environmental [V_E] + genetic by environment interaction [V_{GxE}]) resulting in the formula: Heritability (H) = V_G /(V_G + V_E +V_{GxE}).

Heritability estimates for almond calculated by the UCD program (including extensive early work by Dale Kester) is presented in **Table 2**. Traditional breeding methods by necessity targeted those alleles whose heritability (extent of genetic control) is large enough to be differentiated from background environmental variance. As new germplasm is incorporated into the breeding program, however, new genes and genetic relationships are introduced which can change final heritability values. An extensive new germplasm has been Incorporated to the almond breeding program over the past two decades in efforts to identify the best sources of self-compatibility and disease and pest resistance (**Figures 2, 3 & 13**). [The most promising parents, possessing both the desired trait as well as a good adaptedness to Central Valley conditions, have also been made available to public breeding programs in California]. Because these elite breeding lines have resulted from recurrent backcrossing to California-adapted material (see **Figure 13**) the majority of their genes are derived from Californian germplasm with the inclusion of a relatively few new genes selected for their desired traits (see **Figure 3**). However, because novel and often exotic traits (such as self-compatibility) have been transferred to cultivated almond backgrounds, previously established heritability values may no longer be accurate and need to be reestablished on a case-by-case basis.

Effective molecular markers (such as shown in **Figure 3**), combined with advanced statistical analysis techniques offer the opportunity for more accurate discrimination between exotic and more traditional genes, as well as between genetic and environmental effects, resulting in the opportunity for more efficient, incremental genetic improvement. Thus MAS has been particularly successful in the genetic improvement of self-pollinating crops such as most cereals and vegetables, since most important genes act in an additive manner, and most advanced selections have been inbred to

near homozygosity. In out-crossed crops such as almond, however, high levels of heterozygosity exist [8], with additional and often exploitable genetic contributions resulting from interactions within individual loci (dominance), among different loci (epistasis and other genetic interactions) and even between genomes (as in the interspecies hybrid vigor of hybrid rootstocks [1,7]). The relative importance of these different genetic components for almond and many other tree crops can be better appreciated by comparing the breeding strategies which has been shown to be most effective in their genetic improvement.

Genetic components of almond fitness.

Because breeding strategies differentially exploit the different genetic components contributing to final cultivar fitness, the approach ultimately converged upon by crop breeders can often be informative concerning the genetic components critical to that crop. While recurrent mass selection and synthetics have been utilized in European breeding programs in the early to mid-1900s for low-input, low output almond production [2], virtually all modern almond as well as all modern *Prunus* stone fruit breeding programs employ versions of the *Hybrid-Clone* strategy.

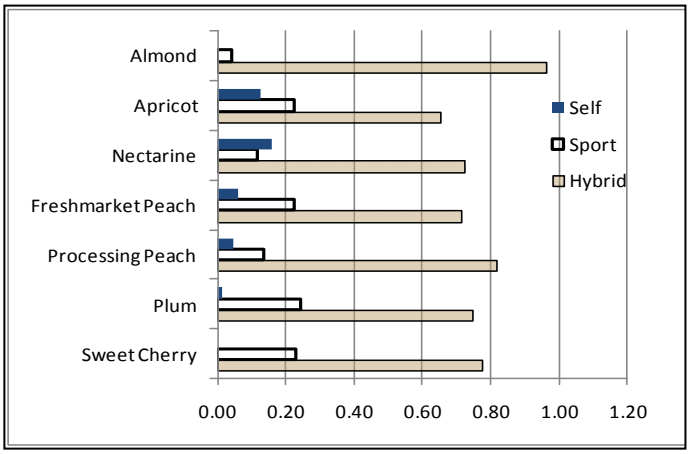


Figure 5. Results from a survey on cultivar origins (hybridization, selfing or sport mutation) for the different stone fruit cultivars having parentage reported in the 1997 Brooks and Olmo Register of Fruit and Nut Varieties showing predominance of hybrids [11].

The observed fitness of hybrids relative to self-pollinations is consistent with the out-breeding nature of almonds and many stone fruit where deleterious recessive alleles would be expected to accumulate due to inbreeding with selfing [11]. Hybridization would encourage greater heterozygosity at these vulnerable loci, where a dominant allele would mask expression of deleterious recessive alleles. For example, when we recently obtained self seed from Nonpareil almond using forced inbreeding techniques, approximately 1/4 of the seedling progeny show severe canker disease suggesting a segregation at a single allele with high canker susceptibility (indicating susceptibility is a homozygous recessive trait as for 'aa' in **Figure 4** and that Nonpareil is a heterozygous (and so relatively resistant) carrier. At certain loci, the heterozygote may also show a fitness advantage over either homozygote, presumably because the greater allelic diversity confers greater overall fitness in differing environments. This situation, sometimes called heterozygote advantage would further encourage hybridization over selfing. An example is the sweet:bitter heterozygote (see **Figure 4**) in Nonpareil, Mission and all California almond cultivars except Butte and Winters, since the kernels will be sweet (and so marketable) while the leaves and bark retain enough bitterness (and cyanide) to repel herbivores (such as browsing deer and some foliar pests).

Such improved hybrid fitness, which may involve beneficial interactions at the intra-locus (heterosis), inter-locus (epistasis) and even inter-genomic level (luxuriance, as in interspecies hybrid rootstocks and introgression lines), would confer significant crop performance advantages particularly in the extensive year by site replicated trials common in almond Regional Variety Trials (RVT) evaluations. Improved vegetative vigor may be involved, but improved fitness or productivity could also result from the accumulation of such beneficial genetic, inter-locus and genomic interactions. In addition, the chromosomes in almond are primarily meta-centric, meaning that the centromere (point of attachment for the strands which align the chromosomes to its proper orientation within the cell) are located in the middle of the chromosomes [5]. Because of the physical nature of the centromeres, there appears to be suppression of genetic recombination on large sections of the adjacent chromosome DNA in almond and peach (**Figure 6**). The consequence would be significant suppression of genetic recombination for a large proportion of the genes. Selection, particularly for groups of genes that interact well together, could still occur at those largely centromere-fixed genes but would have to have occurred over long time periods (as is common for many clonally propagated crops). While commonly associated with greater vegetative vigor, such fixed heterozygotes often also show improved harvest index, which in the predominantly spur-bearing almond crops might be expected to confer increased final yields.

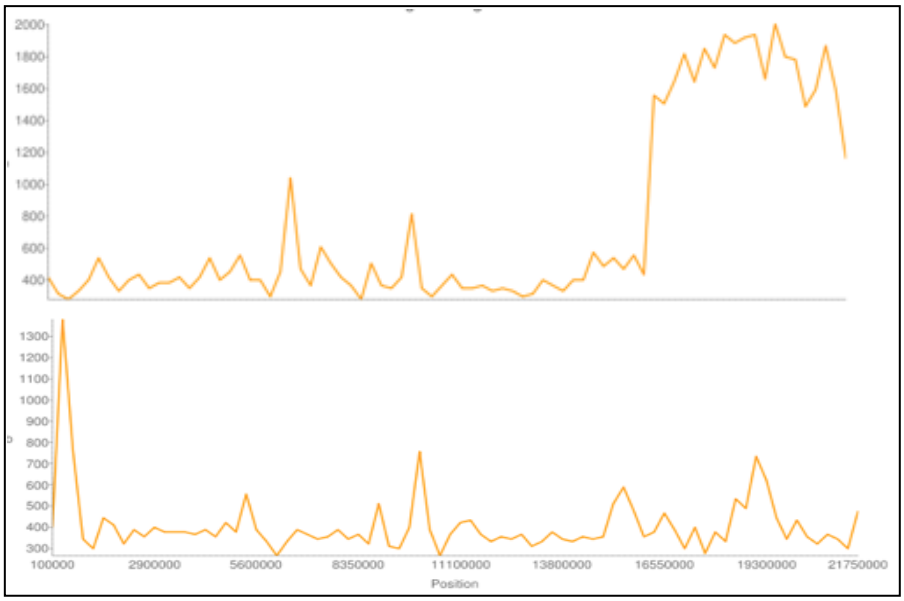


Figure 6. Levels of genetic variation typically observed along the physical length of peach and almond chromosomes (bottom; = chromosome 8 of the peach cultivar 'Dr. Davis'). Dramatic increase in the level of genetic variability in UCD breeding line 'F8,1-42' (top) which is a 'Nonpareil' almond by 'Dr. Davis' peach introgression line, suggesting that such interspecific hybridization may allow greater genetic recombination and so greater access to novel gene combinations for use in breeding.

Taken together, these findings indicate that, unlike many seed-propagated crops, genetic control of important almond traits is not determined by genes acting in a largely additive manner, but supports a much greater importance of the interactions within gene locus (dominance based heterosis) and among genes (epistasis and other desirable

inter-locus interactions) and even among chromosomes and genomes (epigenetics, etc.) [11]. If verified, this finding would have important consequences almond breeding approaches since the promised improved breeding efficiency of marker assisted selection (MAS) and similar molecular-based approaches assumes genetic control is almost entirely additive.

ALMOND BREEDING APPROACHES

Genetic improvement vs. cultivar development.

Breeding goals can be divided into two major categories: genetic improvement and cultivar development. Genetic improvement typically has a well-defined, focused goal such as improved disease resistance within locally adapted genetic background. In contrast, success at cultivar development is indicated by sizable commercial plantings over the long production time required for commercially profitability. For example, a successful almond cultivar is expected to have an average annual kernel production of over 3000 pounds per acre and an orchard-life expectancy of at least 20 years in order to be commercially viable. Cultivar success, then, is rarely determined by superior performance in one or a few traits, but rather is determined by the absence of deficiencies for the large number of fruit and tree characteristics required for commercial viability [11]. The need in almond crops to simultaneously optimize a large number of essential traits remains the greatest challenge to breeding strategies including the use of MAS and other molecular-based techniques.

In genetic improvement, the specific strategy utilized for trait manipulation will depend on the nature of genetic control. Genetic control is traditionally classified into three groups: monogenic, oligogenetic, and polygenic, each of which has unique opportunities and limitations.

Monogenic traits.

In a monogenic trait, the controlling gene will segregate in a classic single gene Mendelian ratio (**Figure 4**) which can be readily manipulated. Since almond is diploid (that is, having 2 complete sets of genes), progeny will inherit one complete set each from the seed and pollen parents. Thus, not only are the progeny genotypes predictable but unknown parental genotypes can be readily deduced once the progeny genotypes are determined. An example is the fingerprinting patterns shown in **Figure 3** for standard California almond cultivars, where all the molecular markers observed can be traced back either Nonpareil or Mission, thus identifying them as likely parents. Self-incompatibility/self-compatibility is another important monogenic trait in almond (**Figure 7**). Each diploid almond cultivar would have 2 possible forms (alleles) of the self-incompatibility gene and any haploid pollen which has genetic identity with either of the seed parent forms will prove incompatible. Pollen carrying the cross-compatible form, however, will be compatible on all crosses. [Dihybrid (2 genes) ratios are also simple enough to also be considered within this group].

A unique advantage of *Clone*-based breeding methods is the ability to accumulate desirable monogenic or single gene mutations (sometimes referred to as point

mutations). Naturally occurring mutations are often identified as bud-sports (novel phenotypes originating from a single bud) which, while typically rare, become increasingly likely with larger planting size and time periods. Desirable mutations in an established cultivar have the advantage of providing a discrete improvement in an otherwise well-established genotype, (i.e. a cultivar whose cultural management and marketing has already been well worked out), making them very desirable. The commercial value of cultivars originating from bud-sports is well documented by their large numbers in **Figure 5**.

An example of a beneficial bud-sport is the *Tardy-Nonpareil* cultivar which flowers approximately 10 days after standard *Nonpareil* and so has greater frost/disease avoidance. The bud-sport origin of *Tardy Nonpareil* can be verified by the identical DNA fingerprinting with Nonpareil, as shown in **Figure 3**. Induced mutations, while rarer, can be also be valuable, as in the induction of self-compatibility in the Italian almond cultivar *Supernova*. While bud sports and induced mutations are typically limited and discrete genetic changes, the risk of negative associated or pleiotropic effects still requires careful field evaluations of these altered genotypes before commercial release as cultivars. Sometimes apparent pleiotropic effects are the result of closely linked genes rather than a secondary effect of the primary gene mutation. An example is the association of a lower yield potential with a later flowering in the *Tardy-Nonpareil* budsport. Evidence that lower yield potential is linked to the later flowering *of Tardy-Nonpareil* and not an associated effect of the same mutation has been shown by our

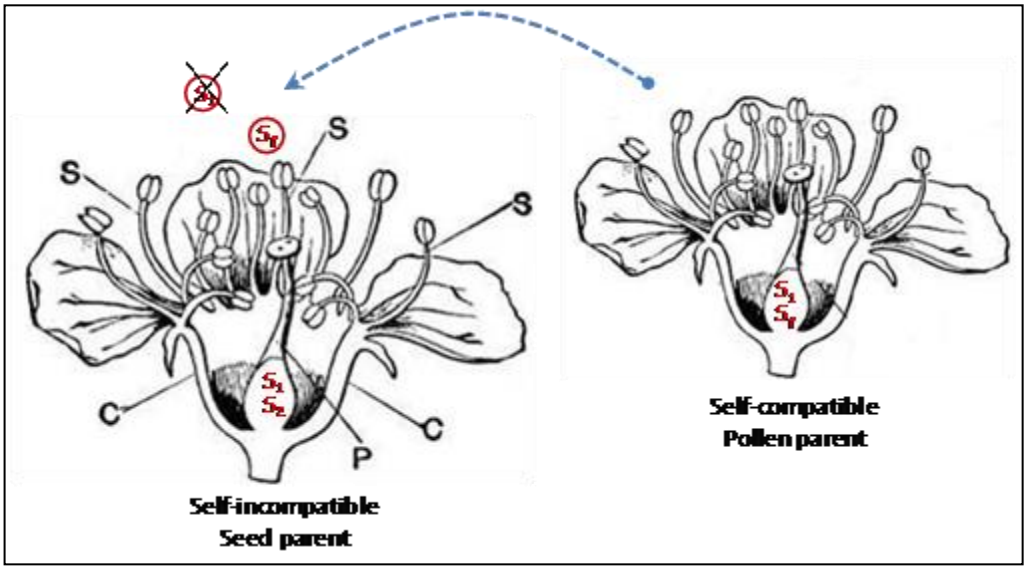


Figure 7. The cross-incompatibility mechanism in almond demonstrating a marker assisted breeding (MAB) crossing strategy which ensures that all progeny will inherit the S_f allele and so be self-compatible since all donor pollen not possessing the S_f allele will possess the S_1 pollen which is cross-incompatible on the S_1S_2 seed parent. (S-stamen, P- pistil, C-floral cup. Note that for a controlled cross onto a self-compatible flower as seed parent, the seed parent anthers are removed before pollen dehiscence by pinching and removing the top-half of the floral cup –and so all attached anthers and petals).

ability to break the linkage with proper genetic hybridizations. UCD advanced selection 2-19E which is currently being prepared for patent and release, is a progeny between *Tardy-Nonpareil* and *Arbuckle* and a rare example of the successful recombination a late-flowering with high yielding Nonpareil-type almond (**Appendix A, B, C, D & E**).

For several reported almond budsports, however, molecular analysis has shown the actual origin was from sexual crosses [3]. An example is the *Jefferies* almond cultivar which has been reported to be a budsport of Nonpareil. Jefferies' DNA fingerprint, however, does not match that of Nonpareil (**Figure 3**) but is consistent with it being a progeny between Nonpareil and Mission. In the early to mid- 1900s, almond scion cultivars were often grafted onto the lower market value and greater disease resistant Mission seedling rootstocks (which were usually pollinated by Nonpareil as it initiated flowering approximately a week earlier). In a small number of propagations, the scion bud failed and a rootstock bud grew instead. Where the rootstock phenotype was clearly different, it was recognized as an 'escape' and rebudded. But where it is similar enough, it was frequently mistaken as a budsport, which, if of sufficiently good quality was propagated as a new cultivar. Molecular analysis of monogenic markers can, thus, be very effective in determining genetic origin.

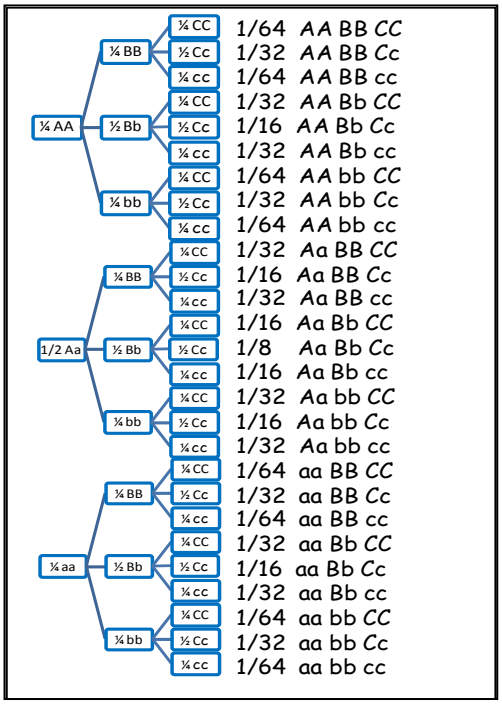


Figure 8. Tree diagram showing genotypes and their probabilities predicted from a cross between two diploid plants heterozygous at unlinked loci A/a, B/b and C/c.

Traits controlled by 1 to 2 genes can be readily transferred to locally adapted genetic backgrounds through recurrent selection, as has been achieved in our transfer of self-compatibility from peach to cultivated almond (**Figure 13**). Because of the longer generation time and smaller progeny population sizes typical of almond crops, recurrent backcrossing is often utilized as it allows more efficient concurrent improvement in both recurrent population and targeted traits. Approximately 60% of the UCD almond breeding crosses on 2010 and 2011 involved recurrent backcrosses of the most promising self-compatible and or disease resistant individuals to a California adapted parent such as Nonpareil (as with early crosses in **Figure 13**). The remaining crosses involved hybridization among advanced lineages to maximize genetic recombination, with subsequent selecting for 'Nonpareil' kernel and tree types (as with more recent crosses in **Figure 13**). Over 7,000 seedlings from approximately 40,000 crosses among 14 parents were recovered from 2010 crosses. Although similarly large numbers of crosses were made in 2011, final seed set is expected to be much lower as a result of poor weather conditions at bloom (rain and frost) and related disease loss, particularly from *Monilinia* blight and bacterial blast. [These difficult weather and disease

conditions, however, are providing valuable opportunities for field evaluation of disease resistance in advanced breeding lines].

Oligogenic traits.

For oligogenic traits, which are controlled by a relatively few genes, the expected Mendelian segregation ratios become increasingly complex and so increasingly difficult to distinguish from background environmental variance (**Figure 8**). MAS and associated molecular marker strategies should be particularly effective for oligogenic manipulation provided the number of genes remains relatively low. Although, as previously discussed, self-compatibility is typically considered a monogenic trait (i.e. single gene control), it has recently been shown that the level of self compatibility can vary depending on environment and a relatively small numbers of modifier genes (**Figure 9**) [1]. Thus, while self-compatibility can be recovered with the relatively simple single-gene transfer (typically through recurrent selection as in **Figure 13**), to achieve consistently high levels of self-compatibility over different years and environments, the appropriate modifier genes need to be concurrently selected. As the number of controlling and/or modifier genes increases, the additive value of individual genes diminishes as does its final breeding value. More significantly, as the number of genes contributing additive affect to traits such as crop yield increases, the population size required to ensure that an individual will be present that possess all or even most of the desired genes becomes prohibitively large (**Figure 10**) even if effective molecular markers were identified for all targeted genes. In these situations, molecular markers can be employed to identify parents homozygous for some of the desired alleles, which could then be fixed in subsequent progeny populations. By such sequential and recurrent selection/fixation, additional targeted loci can be 'pyramided' in the progeny populations though many of the multitude of other genes required for commercial success are often lost from the recurrent breeding population in the process. In addition the improved understanding of the genetic control of targeted traits made possible by molecular analysis may be of considerable value to the breeder and may lead to novel breeding strategies. For example, some of the earliest application of marker assisted breeding (MAB) in tree crop improvement was the development of molecular markers which could allow the identification/selection of UCD almond breeding lines possessing desired cross-incompatibility genotype (S-alleles, including S_f, the self-compatibility allele) at the seedling stage and so eliminate timely process of tree growth and field screenings [1,7]. However, because self-compatibility/incompatibility is a gametophytic trait in the

Year	2003	2004	2005	2007	2008	2009	2010	2011
Winters	15	6	22	33	3	5	4	2
LeGrand	15	3		25	6	3		
LGOP	1	27		5	8	0		
F8,8-161	29	12	20	34	7	35		
F8,8-160	23	26	30	35	30	32		
F8,7-179	20	8	35	7	1	12		
F7,1-1	31	20		33	10	15		
2004,8-160					38	35	30	32
2004,8-201					32	25	25	26
2000,16-81		5	4	37	3			
2000,2-3				28	33	30		
2000,8-27	10	1	2	34	31	41		

Figure 9. Levels of natural (i.e. no hand or honeybee pollinations) self-sets in advanced breeding lines from Fig. 2 showing typical year-to-year variability resulting from genetic and environmental interactions with the self-compatibility gene.

targeted crops, knowledge of parental genotypes was sufficient to devise crossing combinations (**Figure 7**) which essentially ensured all progeny would be self-compatible without the need for extensive molecular analysis of progeny.

Polygenic traits

As genetic control for a given trait becomes more complex, Mendelian segregation ratios becomes less discernible against the environmental background variability and the trait is analyzed instead in terms of the probabilities of its expression using appropriate statistical analysis. This can occur with genetic control by as few as 3 genes for low heritability traits, and for 4 or more genes even for traits showing moderate heritabilities. The statistical or quantitative methods employed are typically reactive in their analysis, (i.e. previously established, segregating populations are prerequisite to predicting future progeny performance). With recurrent selection strategies such quantitative analysis becomes increasingly accurate as each new generation informs and improves upon the overall genetic model. Although quantitative methods are being developed to distinguish additive from dominance effects, the unwieldy statistical approaches currently used largely precludes a reliable characterization of dominance or other intra- or even inter-locus interactions in breeding programs.

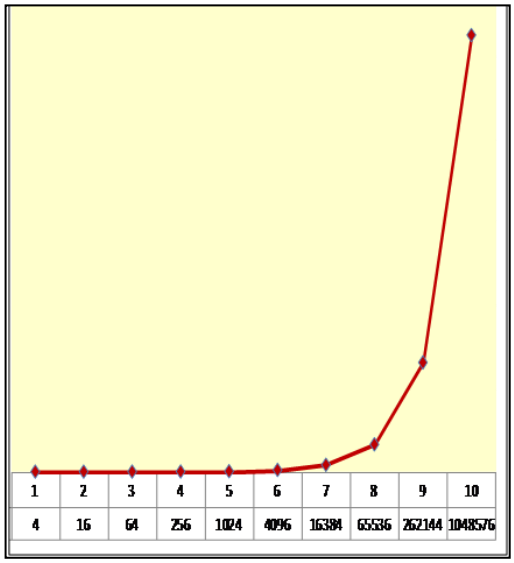


Figure 10. Plot showing the minimum population size (Y-axis and bottom row) predicted by Mendelian analysis for obtaining a desired homozygous genotype at increasing numbers of independent almond loci (X-axis and top row).

Efficient quantitative methods are similarly not available for manipulating genome-genome and associated epigenetic interactions. Part of the reason is that these interactions remain poorly understood and also are not readily captured and manipulated by traditional breeding methods developed for seed propagated crops. Cloning, however, can capture even highly complex and poorly understood genetic interactions making it arguably the most efficient breeding technique for combining, in true-breeding cultivars, the fullest range of desirable genetic, epistatic, epigenetic and genomic interactions [11]. This capacity also makes cloning particularly promising for the characterization and eventual manipulation of these largely underutilized interactions. Towards this goal, however, molecular-based approaches may have to move beyond the current emphasis on DNA-based markers. Clone analysis also offers unique opportunities for the study of epigenetic interactions since different and often heritable phenotypes (juvenility, imprinting, gene-silencing, etc.) of the same clone (genotype) in the same environment would be the expression of epigenetic rather than genetic or environmental factors. For example, Noninfectious Bud-failure in almond appears to be an epigenetic-like clonal aging condition where the genetic (DNA) composition of affected cultivars

remains unchanged but where gene activity is altered in a heritable manner. Although it is a major production problem in almond, it appears a poor candidate for MAS since the DNA sequence appears identical in both affected and unaffected genotypes (see [3]). Similarly, genome-genome interactions which appear to play important roles in enhancing vegetative vigor, as characterized by interspecies hybrid rootstocks, appear be the result of both genetic as well as genomic differences between the parents, possibly including differences in chromosome orientations, scaffold structure, histone composition, methylation patterns, synteny differences, etc. Although providing valuable tools for a more thorough dissection/characterization of these crop improvement opportunities, molecular-genetic analysis, as currently employed, may ultimately hinder breeder utilization of these germplasm resources because of its very specialized and so inherently reductionistic, additive gene focus.

Cultivar Development.

The definitive aim of plant breeding is the development of successful cultivars. A successful cultivar can be conveniently defined as providing a net improvement over the cultivar to be replaced. That is, it must be at least as good as the cultivar it is to replace in the areas of horticultural, quality, disease/pest resistance, market, etc., yet possess improvements valuable enough to result in sizable commercial plantings. Powerful genetic strategies are becoming available for genetic improvement. The major barrier to successful cultivar development, however, is not the process of genetic improvement but rather the process of simultaneously maintaining commercial quality for the wide range of other essential traits. This is the reason bud-sport mutations have been an valuable source of new cultivars (**Figure 5**) since they can confer a distinct improvement to an otherwise genetically unreshuffled, commercially proven cultivar. A well established dogma of tree fruit breeding is that the success of a new cultivar is determined not by its exceptional performance in specific areas but rather a uniformly superior performance across a broad range of characteristics or traits. For example, one of the major goals the UCD almond breeding program is the development of self-compatible varieties to facilitate cultural management as well as the greater year-to-year production consistency. However, Regional Variety Trial results have shown that self-compatibility is in no way a guarantee for commercial success. **Figure 11** shows the performance of the early self-compatible variety, *LeGrand* in Delta RVT trials. Although possessing the self-

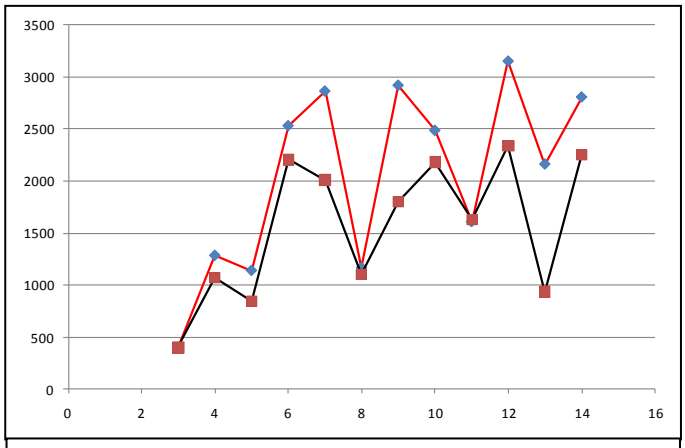


Figure 11. Production (lbs/A) for the self-compatible almond cultivar *LeGrand* (red) and self-incompatible *Thompson* (black) in adjacent rows at the Delta College plot showing increased production but no year-to-year stability in this multiyear test.

compatibility gene, *LeGrand* failed commercially because of a number of crucial flaws, including relatively low and inconsistent levels of self-compatibility (because of the lack of proper modifier genes), poor flower structure to encourage self-pollination, and a nut stick-tight problem which significantly reduced actual yields.

Similarly, UCD25-75 is a highly self-compatible and highly self-pollinating selection tested in the 1994-2006 RVT. (Branches bagged at flowering to exclude honeybee pollinators) set as heavily as adjacent open-pollinated branches). Despite a high and consistent productivity and very good kernel quality in the first 10 bearing years (**Figure 12**), UCD25-75 eventually failed because it's tree architecture resulted in excessive internal shading and poor shake-ability and so inevitably lower yields, particularly after 14th leaf.

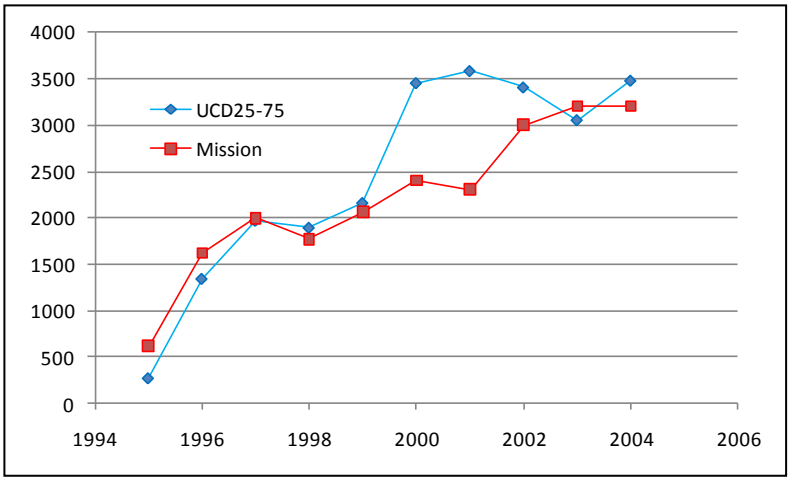


Figure 12. Production (lbs./A y axis) of highly self-compatible and highly self-pollinating selection *UCD25-75* and adjacent *Mission* row at Kern RVT. Despite good productivity and kernel quality, UCD25-75 ultimately failed due to problems with tree architecture.

Consequently, it is the absence of serious deficiencies which will ultimately determine commercial success of a new variety. This is particularly relevant in tree crops were orchards are expected to be productive for 20 years or more in order to be commercially viable, and where failed cultivars cannot be readily plowed under and replanted as with cereal and vegetable crops. The ecologist and author Jared Diamond (1998) has termed this decisive vulnerability to a broad spectrum of potential deficiencies the *Anna Karenina* effect based on Leo Tolstoy's classic opening sentence in his novel of that name: *"All happy families are alike; each unhappy family is unhappy in its own way."* In addition to kernel quality, good performance is required for numerous traits in a broad range of essential categories, including tree structure, productivity and longevity, disease and insect resistances, harvest time, uniformity and ease-of-harvest, precocity, freedom from alternate bearing, post-harvest performance, rootstock compatibility, market type, consumer preference, etc. Thus, while genetic improvement may benefit from a focused, reductionist approach to trait improvement, successful cultivar development requires the simultaneous, holistic manipulation of a large number of essential traits. As demonstrated in **Figure 10**, a traditional additive-gene based MAS approach would quickly become overwhelmed by the number of required markers. This incongruity, while complicating cultivar development may also be undermining future breeding progress. Genetic improvement strategies, including MAS, are becoming increasingly efficient at the partitioning and so manipulating the

principal additive genetic interactions affecting the target trait, but because they are resource intensive, these inherently reductionist approaches may lead to reduced effectiveness of successful tree cultivar development if not fully complemented with the equally essential holistic cultivar development approaches.

Tree crops are also unique in that successful cultivars need to demonstrate this superior fitness over a much broader range of environmental variation (position on tree, planting site, age of tree, age of clone, varying disease and insect pressures, changes in climate and weather pattern, etc.). Superior fitness over a range of environments is ultimately more important to final cultivar productivity than exceptional performance within a narrow environmental niche [11]. Tree crop cultivars thus resemble the clonal colony or 'genet' of ecology such as the "Pando" clone of Quaking Aspen (*Populus tremuloides*) in the Wasatch Mountains of Utah, USA, which cover 43 hectares and so is often considered the world's largest organism by mass. Since the estimated 47,000 individual clonal trees ('ramets' in ecological terms) which constitute the *Pando* clone have developed over a wide range of differing ecological niches, its competitive advantage appears to result from a broad adaptability rather than being highly adapted to a specific niche. In comparison, the *Nonpareil* almond clonal cultivar is planted on over 116,000 hectares (at over 48 trees/ha) in California alone, with additional plantings in Europe, Asia, North Africa, South America and Australia. Although there are other cultivars which out-yield or have higher market value than Nonpareil in certain production areas and periods [12], Nonpareil continues to dominate this crop because of a superior overall fitness (i.e. economic returns over the typical orchard life of 20+ years) with over 5.6 million trees currently planted over an ecologically diverse 700 km stretch ranging from Redding to Bakersfield.

Improved environmental buffering has been shown to be associated with the higher genetic heterozygosity typical of most stone fruit cultivars (**Figure 5**). Even with predominantly inbreeding species such as peach recombination from hybridizations would increase the opportunity for beneficial intra (dominance) and inter-locus rearrangements. Though relatively rare, such desirable rearrangements once selected would be largely fixed by linkage disequilibrium leading to the equivalence of heterosis over extended selection periods. Extended periods of selection for broad environmental adaptability would occur (for many almond varieties, particularly in Europe and Asia, selection has been occurring for hundreds to thousands of years) and would thus identify rare, elite selections where the maximum potential of additive, dominance, epistatic, genomic and epigenetic interactions was combined. Clonal propagation allows the capture of these rare elite genotypes for future plantings as well as future genetic improvements through bud-sport mutation or further, albeit rare, favorable recombinations. Inbreeding would be deleterious to such buffered fecundity, which could help explain the preponderance of hybrids versus self-pollinations in successful stone fruit cultivars (**Figure 5**). MAS when applied to multiple traits is inherently targeting additive genes and so ineffective in selecting other beneficial gene interactions.

Bearing in almond is primarily on spurs [10]. Spur production, with its highly efficient fruit-to-vegetative ratio (harvest index) often dominates crop production in mature, highly productive orchards. In almond, these spur-based production-units are also considerably autonomous and competitive, that is carbohydrate flow is primarily from nearby leaves to the more competitive local sink, whether individual developing nut or vegetative apices (i.e. DeJong model). While the clone (genet) or individual tree (ramet) may be the target of natural selection of wild clonal colonies, the fruiting-spur (compet) may prove as important or more important in the more synthetic and often more intensive crop breeding selection. Recent research in almond has shown that the most productive orchards are those where both the quantity (number) and quality (fecundity) of spurs are optimized during the multi-year, peak production phase of the planting [13].

Productivity remains the most important attribute in new almond cultivars but because of its complexity and all-inclusive nature is often managed as a nebulous quantitative trait which frustrates a more thorough analysis and manipulation by both traditional as well as molecular approaches. Molecular-approaches such as association mapping, offer unprecedented opportunities to more fully characterize important components of yield as a basis for future genetic and cultural manipulation but require a more detailed understanding of the biological basis [10]. It is informative how biotech progress over the last 3 decades has advanced to the point where sequencing individual almond breeding lines can now be readily achieved, yet our understanding of the physiological and developmental components of a trait as critical as yield has made only rudimentary progress over the same time period. This precarious biological knowledge-base, along with the traditionally insular nature of molecular genetic analysis remains a major impediment to more efficient cultivar breeding in tree nut crops. The inherent capacity of clone-based cultivars to capture the fullest range of beneficial genetic, epigenetic and genomic interactions for applied crop improvement provides both a prerequisite and unique opportunity for researchers to evolve beyond the current reductionistic additive-gene approach, but would require (perhaps stimulate) significant parallel progress in our understanding of the basic underlying developmental and inheritance mechanisms at the epigenetic and genomic as well as genetic level [14]. An even greater challenge/opportunity would be the progression from the present focus on single trait genetic improvement to an emphasis on the concurrent management/advancement of the multitude of traits required for commercial success, i.e. cultivar breeding.

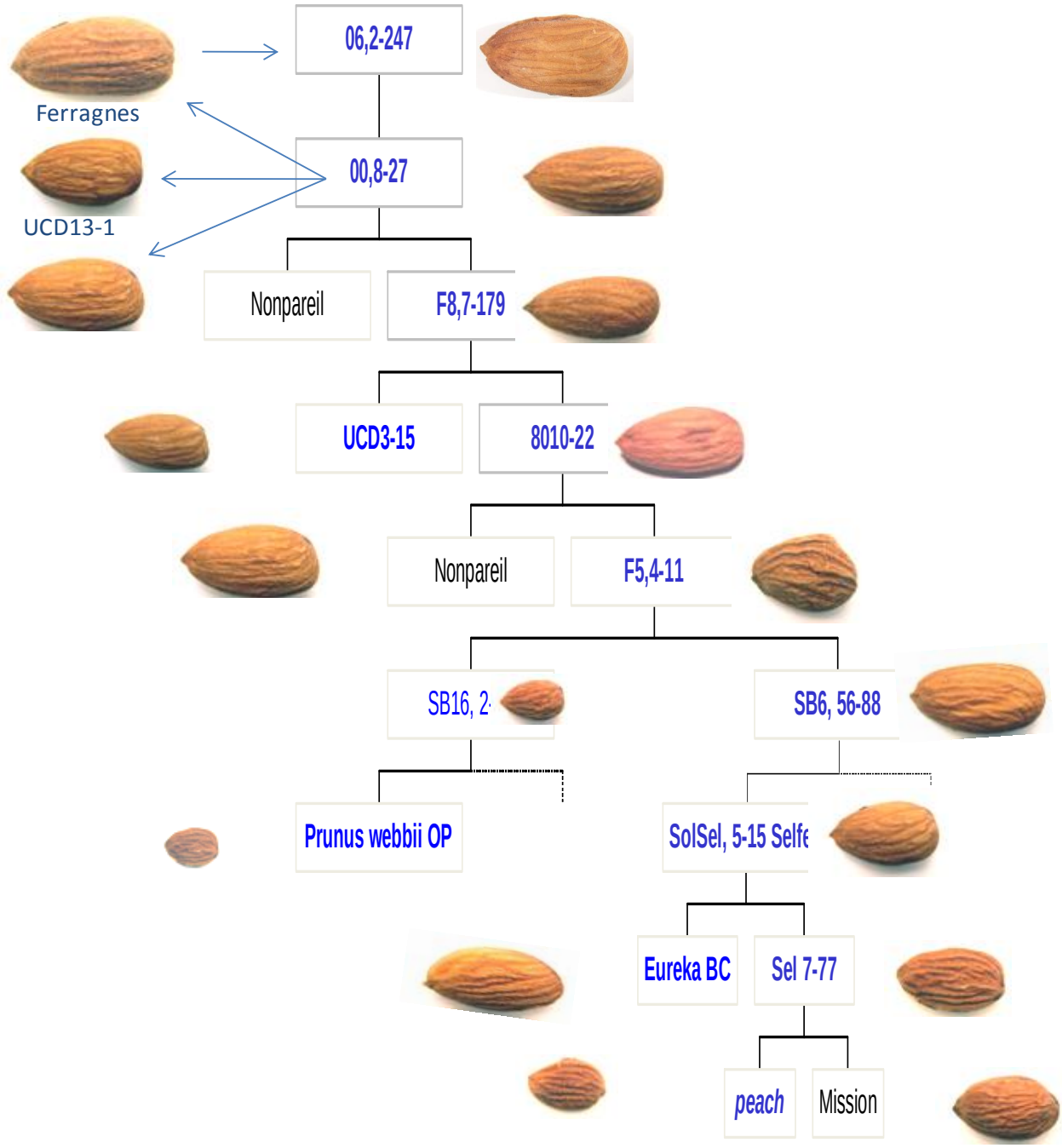
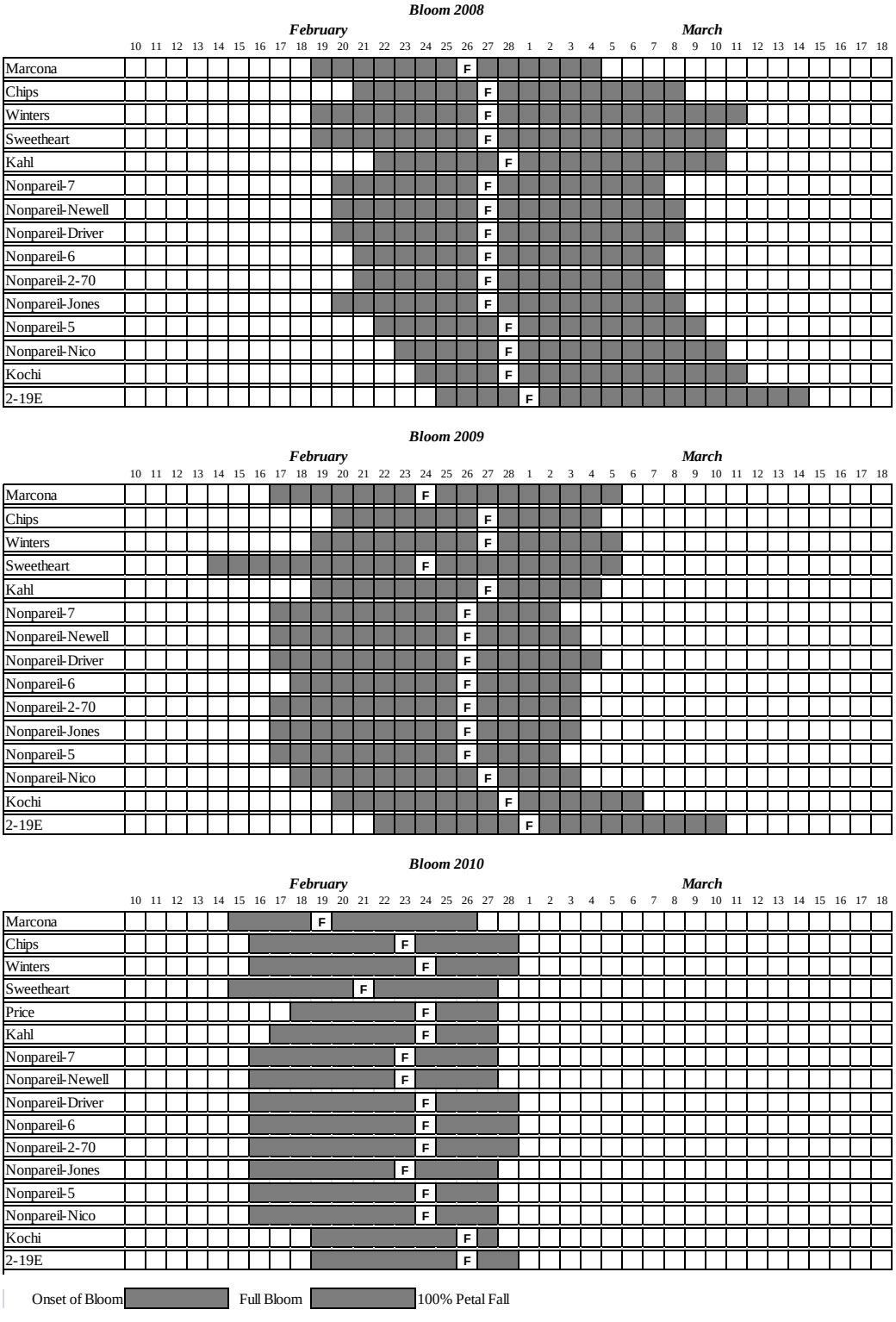


Figure 13. Lineage (pollen parent to right; seed parent to left) showing transfer of self-compatibility from peach and the wild almond Prunus *webbii* to advanced UCD selections. [Levels of self-compatibility are reported in **Figure 9** with kernel and tree traits provided in Appendices A-E.] Early breeding efforts involved a complex series of crosses to transfer traits (self-compatibility and hull rot resistance) from wild relatives to cultivated almonds. A series of recurrent backcrosses to Nonpareil-type almonds then transferred desired traits to a Central valley adapted background. More recent crosses target high commercial quality with maximum productivity (and so maximum genetic variability). A major challenge has been small kernel size in donor material resulting in only a small proportion of progeny having good commercial kernel size. {Table 2 shows a relatively high heritability estimate of 0.77 for kernel size}. Recent advanced selections have achieved kernel sizes larger than Nonpareil which when used as parents will result in a much larger proportion of seedling progeny having commercially desirable sizes.

Appendix A. Selected breeding lines used as parents for self-compatibility and disease resistance. (Bloom: days after *Nonpareil*; Origin refers to the germplasm source of the major trait of interest (i.e. self-compatibility, disease resistance, etc.); Self-set refers to average self-set (bagged) compared to adjacent set on unbagged, insect pollinated branch). [Averaged from last 5 years of production}.

Selection	Bloom vs. Nonp.	Kernel (g)	Shell-out (%)	Doubles (%)	Origin	Length (mm)	Width (mm)	Thick (mm)	Self-set (%)
UCD2-19E	4	0.98	0.61	2	Californa almond	21.6	11.6	8.1	8
LG-0P	7	1.07	0.62	6	Peach	22.2	12.1	9.6	62
F8,8-161	5	1.17	0.53	13	Prunus mira	25.0	11.8	8.1	81
F8,8-160	5	1.22	0.62	0	Prunus mira	27.3	12.2	8.4	85
F8,7-180	2	1.24	0.61	0	Prunus webbii	29.6	13.2	7.7	11
F8,7-179	2	1.09	0.61	2	Peach	27.1	12.4	8.7	77
95,1-26	1	1.78	0.54	2	European almond	29.3	14.3	9.8	48
2000,2-3	1	1.17	0.57	3	Peach	24.5	12.2	9.3	93
2000,16-81	4	0.95	0.55	5	Irradiated almond	21.3	11.5	9.5	84
2000,8-27	2	1.04	0.52	9	Prunus webbii	24.4	11.8	8.3	91
204,14-158	1	1.46	0.44	0	Prunus fenzliana	25.5	13.8	8.4	54
2002, 7-159	3	1.12	.62	4	Tuono almond	26.3	11.1	8.5	63
2004,8-160	2	1.87	0.62	0	Prunus mira	30.5	15.6	8.6	96
Nonpareil	0	1.11	0.63	1	Standard	23.3	13.2	8.2	3

Appendix B. Bloom patterns for UCD Almond Advanced Selections and Standards for the 2006 through 2010 seasons. (Compiled by P. Schrader and B. Lampinen as part of Kern RVT study). [Note to good bloom overlap Of *Winters* and *Sweetheart* with the early *Nonpareil* bloom, and a good overlap of *2-19E* with later *Nonpareil* bloom.]



Chilling hours from November 1, 2009 to February 28, 2010 **1039**

Appendix C. Yield, number of nuts, average kernel weight, shelling percentage and kernel pound per acre yield for the 2006 through 2010 seasons. Data for each year is sorted by cumulative yield. Note the dominance of 2-19E until 2010 when insufficient 2009 nitrogen and water inputs resulted in reductions in 2010 flower and so crop development. (Compiled by Lampinen & Gradziel labs as part of Kern RVT study).

2006				Kernel pounds per		Cumulative kernel yield
Variety	No. of nuts/tree	Average kernel wt (g)	Shelling percentage	Tree	Acre	(lbs/acre)
2-19e	6852 a	0.94 g	53.0 d	14.2 a	1718 a	1718 a
Winters	6648 a	0.87 h	53.4 d	12.7 a	1540 a	1540 a
Marcona	3611 bcd	1.31 a	30.7 f	10.4 b	1258 b	1258 b
Nonpareil-Ni	4246 b	1.09 cde	67.2 a	10.2 b	1232 bc	1232 bc
Nonpareil-5	3713 bcd	1.12 bcd	67.9 a	9.1 bcd	1110 bcd	1110 bcd
Nonpareil-D	3867 bc	1.07 def	63.4 abc	9.1 bcd	1103 bcd	1103 bcd
Nonpareil-3-8-2-70	3848 bc	1.07 cde	64.6 ab	9.1 bcd	1101 bcd	1101 bcd
Nonpareil-Ne	3815 bc	1.07 cde	67.7 a	9.0 bcd	1086 bcd	1086 bcd
Nonpareil-6	3886 bcd	1.12 bc	67.0 a	8.9 bcd	1075 bcd	1075 bcd
Nonpareil-J	3717 bcd	1.08 cde	64.0 abc	8.8 bcd	1066 bcd	1066 bcd
Chips	3623 bcd	1.02 f	53.8 d	8.1 bcde	985 bcde	985 bcde
Kochi	3134 cd	1.16 b	59.9 c	8.0 cdef	965 cdef	965 cdef
Nonpareil-7	3288 bcd	1.08 cde	65.1 a	7.8 def	940 def	940 def
Kahl	3139 cd	1.06 ef	47.8 e	7.3 def	889 def	889 def
Sweetheart	2777 d	0.95 g	67.8 a	5.8 f	588 f	588 f

2007				Kernel pounds per		Cumulative kernel yield
Variety	No. of nuts/tree	Average kernel wt (g)	Shelling percentage	Tree	Acre	(lbs/acre)
2-19e	13149 a	0.78 e	54.3 d	22.8 a	2756 a	4474 a
Winters	11972 ab	0.83 de	60.2 b	21.8 ab	2634 ab	4173 a
Nonpareil-Newell	10659 bc	0.90 bc	67.3 a	20.9 abc	2536 abc	3626 b
Nonpareil-Nico	9260 cde	0.92 bc	66.0 a	18.8 abcde	2279 abcde	3511 b
Nonpareil-Driver	9793 cd	0.91 bc	65.6 a	19.6 abcd	2370 abcd	3474 b
Nonpareil-3-8-2-70	9340 cde	0.92 bc	66.3 a	18.9 abcde	2291 abcde	3393 b
Nonpareil-5	8905 cdef	0.95 b	67.0 a	18.6 abcde	2251 bcde	3323 bc
Marcona	6938 fg	1.08 a	29.8 f	16.5 defg	1995 defg	3252 bcd
Kahl	9594 cd	0.91 bc	47.6 e	19.3 abcd	2332 abcd	3222 bcd
Nonpareil-J	9137 cde	0.89 bcd	65.5 a	17.8 bcde	2152 bcdef	3218 bcd
Nonpareil-6	8396 def	0.94 b	67.1 a	17.4 def	2103 def	3178 bcd
Nonpareil-7	9517 cd	0.92 bc	67.9 a	19.3 abcd	2332 abcd	3140 bcd
Chips	7681 defg	0.87 cd	54.4 d	14.7 efg	1780 efg	2766 bcd
Kochi	6006 g	1.08 a	59.4 bc	14.3 fg	1729 fg	2694 de
Sweetheart	6767 fg	0.89 bcd	66.6 a	13.1 g	1588 g	2165 e

2008				Kernel pounds per		Cumulative kernel yield
Variety	No. of nuts/tree	Average kernel wt (g)	Shelling percentage	Tree	Acre	(lbs/acre)
2-19e	13472 a	0.93 g	54.3 d	27.5 cd	3321 cd	7795 a
Nonpareil-Nico	13879 a	1.10 cd	66.0 a	33.5 a	4056 a	7567 ab
Nonpareil-Newell	11916 bcd	1.09 de	67.3 a	28.6 cd	3456 cd	7110 bc
Nonpareil-3-8-2-70	12506 bcd	1.17 cd	66.3 a	30.7 b	3714 b	7106 bc
Nonpareil-Driver	12729 abc	1.07 de	65.6 a	29.8 bc	3611 bc	7085 bc
Nonpareil-5	12883 ab	1.08 de	67.0 a	30.5 b	3692 b	7001 bc
Winters	9872 e	1.02	60.2 b	22.1 fg	2670 fg	6843 c
Nonpareil-7	13250 ab	1.06 de	67.9 a	31.1 ab	3763 ab	6802 c
Nonpareil-6	10707 de	1.16 c	67.1 a	27.3 cd	3300 cd	6478 cd
Nonpareil-J	11071 d	1.09 cde	65.5 a	26.6 de	3224 de	6442 cd
Kahl	10720 de	0.96 fg	47.6 e	22.6 fg	2733 fg	5954 de
Chips	11465 cd	0.97 fg	54.4 d	24.4 ef	2956 ef	5722 e
Sweetheart	13149 ab	0.82 g	66.6 a	23.9 ef	2893 ef	5059 f
Marcona	4721 f	1.39 a	29.8 f	14.4 h	1748 h	5001 f
Kochi	5882 f	1.28 b	59.5 bc	16.5 h	2002 h	4996 f

Appendix C (continued)

2009				Kernel pounds per		Cumulative kernel yield
Variety	No. of nuts/tree	Average kernel wt (g)	Shelling percentage	Tree	Acre	(lbs/acre)
Nonpareil-Nico	13773 ab	1.05 bcd	74.7 ab	32.9 a	3977 a	11417 a
Nonpareil-Newell	14513 a	1.03 bcd	74.8 ab	33.1 a	4004 a	11145 ab
2-19e	14706 a	0.84 f	65.6 f	27.1 c	3285 c	11080 ab
Nonpareil-Driver	13856 ab	1.08 ab	75.8 a	32.9 a	3977 a	11062 ab
Nonpareil-3-8-2-70	13756 ab	1.04 bcd	74.6 ab	31.4 ab	3798 ab	10905 abc
Nonpareil-5	12070 bcd	1.08 ab	74.2 ab	28.7 bc	3476 bc	10494 bcd
Nonpareil-7	13051 ab	1.03 bcd	72.6 abc	29.5 bc	3571 bc	10393 bcd
Nonpareil-6	13505 ab	1.02 bcd	71.2 cd	30.3 abc	3661 abc	10139 cd
Nonpareil-J	12803 abc	1.04 bcd	71.6 bcd	29.0 bc	3513 bc	9955 de
Winters	9434 ef	0.96 bcde	61.6 g	20.0 e	2415 e	9258 ef
Kahl	11035 cde	0.87 ef	59.1 g	21.1 de	2559 de	8513 fg
Chips	9771 ef	0.93 def	58.6 g	20.0 e	2422 e	8144 gh
Sweetheart	12798 abc	0.85 ef	73.3 abc	24.0 d	2906 d	7965 gh
Marcona	8977 fg	1.07 abc	32.5	21.2 de	2562 de	7563 hi
Kochi	7252 g	1.17 a	68.9 de	18.7 e	2259 e	6955 i

2010			Shelling	Kernel pounds per			Cumulative kernel
Variety	No. of nuts/tree	Average kernel wt (g)	percentage	unit PAR int.	Tree	Acre	yield (lbs/acre)
Nonpareil-Nico	9521.8 abc	1.24 abcdef	72.5 ab	49.7 ab	25.9 ab	3141 ab	14558 a
Nonpareil-Newell	8429.4 cde	1.31 ab	73.6 a	45.2 abc	24.2 ab	2931 ab	14099 ab
Nonpareil-3-8-2-70	8823.4 bcd	1.28 abcd	72.3 ab	47.0 ab	24.8 ab	3011 ab	13915 abc
Nonpareil-Driver	8368.2 cde	1.28 abcd	71.0 ab	46.2 abc	23.5 ab	2849 ab	13910 abc
Nonpareil-5	9410.2 abc	1.24 abcde	72.3 ab	50.8 a	25.8 ab	3130 ab	13579 abc
Nonpareil-7	10611.8 ab	1.16 bcdef	69.8 ab	49.4 ab	27.1 a	3282 a	13510 abc
Nonpareil-6	9498.9 abc	1.21 abcdef	71.8 ab	48.7 ab	25.4 ab	3081 ab	13219 bc
2-19e	6832.8 efg	1.10 bcdef	56.1 e	33.7 e	16.6 cd	2020 cd	13100 bc
Nonpareil-Jones	8314.7 cde	1.23 abcdef	70.9 ab	43.8 abcd	22.6 b	2737 b	12691 c
Winters	6601.3 efg	1.11 bcdef	60.7 cde	38.5 cde	16.0 de	1945 cd	11203 d
Chips	9089.0 abc	1.15 bcdef	65.9 abc	48.4 ab	23.0 b	2789 b	10933 d
Sweetheart	10915.5 a	0.80 g	71.8 ab	42.1 bcd	19.3 ab	2803 ab	10768 de
Kahl	7587.0 cde	1.01 f	56.5 de	43.4 abcd	16.9 c	2048 c	10561 de
Marcona	5072.7 gh	1.28 abc	26.2	36.7 de	14.4 cde	1745 cde	9307 fg
Kochi	3902.2 h	1.40 a	64.4 bcd	23.5 f	12.1 e	1466 e	8421 g

Appendix D. Hullrot strikes per tree for the 2010 season at the McFarland trial. Letters indicate significant difference at the 5% level of significance. (Compiled by P. Schrader and B. Lampinen as part of RVT study).

Variety	*2010 Hull Rot Strikes/tree*	
Kahl	8.33	a
Sweetheart	11.00	a
Marcona	13.33	a
2-19E	18.83	a
Price	23.01	a
Chips	24.00	a
Nonpareil-Nico	30.67	a
Nonpareil 3-8-2-70	61.33	a
Nonpareil-J	62.67	a
Nonpareil-5	65.17	a
Nonpareil-7	72.67	a
Nonpareil-6	82.83	a
Nonpareil-Newell	83.67	a
Nonpareil-DR	98.17	a
Kochi	262.00	b
Winters	539.67	c

Appendix E. Description of current UCD Self-compatible breeding parents from Regional Variety Trials. Breeding selections represent a very wide genetic variability due to their interspecific origins. In addition to self-compatibility, novel genetic options for disease and insect resistance have been incorporated into much of the material represented below. Establishing evaluation plots in different areas of the Sacramento and San Joaquin Valleys, allows a more thoroughly evaluation of their value as parents for further self-compatibility and resistance breeding, as well as their potential as possible cultivar releases.

UCD2-19E. Lineage: Tardy Nonpareil X Arbuckle. This selection was one of the highest producing varieties at the Kern RVT plot with an accumulated (1996-2005) yield of 26,112 pounds per acre following an exceptionally high crop of 4890 pounds per acre in 2003. plots. UCD2-19E can show a strong alternate bearing habit where years of high crop yield are followed by low crops. In years of very high crop, insufficient nutrients are available to the overloaded fruiting spurs to initiate the number of flowers needed to maintain the crop, and in some cases to maintain the very viability of the spur into the next season. In the current Kern County RVT, we have been successful in maximizing year-to-year production by closely monitoring current season crop yield and providing increases in both irrigation water and fertilizer nutrients as needed. Under these conditions, UCD2-19E has been the highest producer in 2006, 2007, and 2008 and amongst the highest in 2009 (see Appendix B.). In addition to its very high crop, 2-19E shows good kernel quality, a late bloom ~7d after Nonpareil, and resistance to flower blight. Low hull rot and Alternaria blight disease levels have also been observed in all plots to 2009.

LG-OP. Lineage: *LeGrand*-Open-Pollinated. Kernels have good quality and a Padre-type shape, though are somewhat larger. Shells are soft, moderate in thickness with good seals. Kernels show moderate levels of doubles (~8%) and creasing. The tree is more compact, like Carmel, with upright scaffolds but allowing good productivity because of a shorter internode distance between leaves and spurs. Most production in the mature trees is on spurs which are well distributed throughout the canopy. The level of self-compatibility has been inconsistent like the *LeGrand* parent. Trees have shown good productivity both at the Winters and southern San Joaquin evaluation plots. Bud-failure has been observed in progeny of LG-0P indicating an increased BF-potential of the parent but no symptoms have been observed on 15th leaf parent trees; some yield loss from flower blight in Kern County in 2009-10. Bloom starts ~8 d after Nonpareil.

F8,7-179. Lineage: D3-15 (Nonpareil X F5,4-43 {P.webbii X P.webbii}{SEL5-15Selfed})) X D3-25 [(Nonpareil X F5,4-11{P.webbii X P.webbii}{SEL5-15Selfed})]. Combining multiple and distinct sources of self-compatibility (from both peach and P. webbii), this selection has shown good levels of self compatibility even in seasons were spring storms have suppressed cross-pollination. Improved levels of both foliar (including Alternaria leafspot) and blossom disease resistance have also been observed though susceptibility to hull rot has also been observed. Kernels show good quality and are of uniform size and shape with some doubles though with a darker seed coat color. The shells are paper, though only 50% sealed. Early productivity in regional trials has been moderate to high. Regional trials are being watched closely for disease susceptibility and bearing wood renewal. Bloom starts ~3 d after Nonpareil.

F8,8-160. Lineage: D4-18 [(Mission X {P.mira X Alm}) X Sonora] x 25-75. This and F8,8-161 have incorporated genes from the wild almond species *P. mira* into a cultivated almond background. F8,8-160 was selected for its consistent level of self-compatibility and its good-quality kernel. Seed coat color is like Mission or darker. Shells are paper, and moderately (70%) sealed. Trees have shown good productivity both at the Arbuckle and southern San Joaquin evaluation plots. Kernels are uniformly elliptical and relatively thick resulting in good kernel weights and so improved yield potential. In regional test plantings, trees are upright-spreading to bushy with moderate to good crop distribution primarily on spur bearing wood resulting in a tree size similar to Plateau or Carmel. Bloom occurs approximately 5 d after Nonpareil and can be profuse. Harvest occurs approximately 5 weeks after Nonpareil. Average kernel length/width/thickness is 2.2/1.2/0.9 cm. Ave. kernel weight is 1.0 g; kernel/kernel + shell crackout is 0.57.

F8,8-161. Lineage: as F8,8-160. F8,8-161 was selected for its consistent level of self-compatibility and its good-quality kernel. Shells are comparable to, to slightly thicker than Carmel, having good (98%) seals. Trees have shown good productivity both at the Arbuckle and southern San Joaquin evaluation plots. Doubled nuts (two nuts developing on a unique T-shape spur) are often observed and may contribute to the higher yield potential this selection. Pollen is fully cross compatible with Nonpareil and most major commercial almond varieties. Tree is upright and similar in size and vigor to Fritz. Bloom occurs approximately 6 d after Nonpareil and is profuse. Harvest occurs approximately 26 d after Nonpareil. Average kernel length/width/thickness is 2.3/1.2/0.8 cm. Ave. kernel weight is 1.2 g; kernel/kernel + shell crackout is 0.63. Doubles (~14%) may be a problem and Monilinia flower blight and Alternaria leaf spot has been observed in San Joaquin valley plantings.

2000,2-3. Lineage: D3-15 (Nonpareil X F5,4-43{P.webbii X P.webbii}{SEL5-15Selfed})) X D3-25 [(Nonpareil X F5,4-11{P.webbii X P.webbii}[{SEL5-15Selfed})]. A relatively recent selection, 2000,2-3 represents an advancement of the D3-25 selection by incorporating improved tree structure disease resistance and productivity. Self-compatibility and a Nonpareil-type kernel were derived from the D3-25 parent. The D3-15 parent contributed a more upright-spreading tree structure, a more uniform, spur based productivity, and a more durable and well-sealed shell. Tree structure is upright to upright-spreading with a very high productivity resulting from a uniform and high nut distribution. The original tree also shows evidence of improved foliar disease resistance. The tree is semi-upright with radial branching , being 10% narrower than Nonpareil but similar in height. Bloom is approximately 5 d after Nonpareil with harvest approx. 21d after Nonpareil. Kernel quality is good. High yielder in 2009 Fresno County test plots. Average kernel length/width/thickness is 2.4/1.2/0.9 cm. Ave. kernel weight is 1.2 g; kernel/kernel + shell crackout is 0.55. Shell-seal is moderate with approximately 70% of the nuts showing complete seals. This selection resulted from a complex series of crosses involving *Prunus persica* (peach) and *Prunus webbii* in its lineage. Bud Failure like symptoms observed in 2009 in 10 year old seedling tree.

2000,8-27. Lineage: Nonpareil X F8,7-179. As with selection 2000,2-3, (above), this selection represents the next breeding generation derived from selection F8,7-179 (described above). The backcross to Nonpareil has resulted in an improved Nonpareil-type kernel quality and improved shell seal. High levels of self-compatibility have also been recovered as have good tree architecture and uniform crop distribution, primarily on spur bearing wood. The tree also exhibits improved levels of foliar disease resistance when compared to both parents. Kernel uniformity is very high with low levels of doubled or damaged kernels. The tree is upright-spreading and approx. 20% smaller than Nonpareil. The bearing-habit is similar in terms of the ratio of spur to shoot flower buds. The selection blooms approximately 3 d after Nonpareil and harvest approx. 15 d after Nonpareil. Average kernel length/width/thickness is 2.2/1.2/0.9 cm. Ave. kernel weight is 1.2 g; kernel/kernel + shell crackout is 0.64. The paper shells give good crack out but have poor 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.

2004,14-158. Lineage: 99,4-8 (Ferragnes * LGOP) * 97, 3-40 (P. webbii * Winters). Tree his upright-spreading to spreading. Bloom occurs approximately 2 days before Nonpareil. Harvest is approximately 3 weeks after Nonpareil. Flowers are self-compatible but not consistently so. Kernels are large and of uniform, with good quality and with moderately thin but well sealed shells. Branches are very productive leading to some breakage of seedling trees. Currently used primarily as a parent for improved tree architecture.

UC95,1-26. Lineage: USDA Selection CP33 * Winters. Tree is upright-spreading and productive with large, attractive nuts. Shell-seal is good as is the shell integrity. Tree shows good levels of self-compatibility in some years, but is more erratic in others. Flowering time is approx 8 d after Nonpareil. No disease problems observed to 2008-10. Because of its very good kernel quality and diverse lineage, this selection is being used in crosses to optimize self-compatibility and kernel quality and yield.

2004,8-160. Lineage: NP * 97,1-232[25-75 [Arb * 4-26]*[SB4, 4-2E] * Winters /97,3-40[D4-18 (Mis * [P.fenzliana *Alm])** Winters]. Tree is upright-spreading to spreading. Production of large attractive nuts on high density spurs resulting in very consistent and high production. Good shell seal and kernel quality though some kernel creasing is common due to the larger size. Seed coat size is darker with a somewhat dusty appearance. Tree is highly self-compatible and highly self-fruitful (self-pollinating). Flowers approx 4 d after Nonpareil. Crossing studies to Nonpareil, however, have shown lower than expected Nonpareil seed sets, requiring further studies in 2011-12. The exceptional size and quality of the kernel make this a particularly promising parent for future crosses.

2004,8-201. Lineage: NP * 97,1-232[25-75 [Arb * 4-26]*[SB4, 4-2E] * Winters /97,3-40[D4-18 (Mis * [P.fenzliana *Alm])** Winters]. [Sister line to 2004,8-160]. Tree is upright and very productive. Bloom time is approx. 7 d after Nonpareil. Nuts are of good quality and well-sealed. Kernels are medium to large and somewhat flat. Branches show high density of spur production and show no disease despite the consistently high crops. No kernel defects observed to 2008 and 2009. The high kernel quality and high levels of self-compatibility and yield make this parent particularly promising for developing late flowering Nonpareil-like cultivars.

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