California Cling Peach Advisory Board
2011 Annual Report

Project Titles: Development of New Cling Peach Varieties

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Objectives:

A. Accelerate the breeding of varieties contributing to reduced grower production costs, including once-over harvest, fruit resistance to mechanical bruising, improved processing quality, and reduced pitting problems such a split-pits, fragments and red-staining of pit cavities.
B. Continue breeding for Ultra-Early, Extra-Early, Early and Extra-Late maturity periods, as well as resistance to brown rot and mildew. Develop and test strategies for identifying the most promising seeding candidates within the first years of growth.
C. Generate 5,000 new seedling trees through the controlled crosses within advanced 2nd and 3rd generation breeding lines and locally adapted California selections.
D.

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 processing peach were orchards are expected to be productive for 20 or more years and where failed varieties cannot be readily plowed under and replanted. Peaches 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 peach 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 a small number founding parents. To incorporate new traits such as improved post-ripe fruit integrity improved disease resistance, the UCD peach 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. The breeding efficiencies promoted by the RosBreed project, including molecular-based marker assisted selection as well as greater emphasis on self-pollination as a crossing strategy to combine desirable commercial traits, represent an attractive breeding strategy to allow continued aggressive breeding progress at lower cost. As an example, in 2011, over 20,000 pollination were made among selected parents. Killing frost in the Winters, CA crossing blocks, which occurred in March again in April destroyed many of the hybrid seed. However, by utilizing RosBREED developed markers to verify self-pollination, we were able to harvest, prepare and plant over 4,000 seedlings from selected parents to complement the 14,000 seedlings from previous 2010 controlled crosses (which were planted in 2011). While highly attractive however, the 2011 self-pollination or inbreeding-strategy ultimately targets the consolidation of a relatively few number of desirable genes and, as such, appears to conflict with prior breeding experience which indicate that high levels of genetic recombination, achieved only through complex cross-hybridization, is the only proven path to high yields. The goal of this program review/annual report, therefore, is a comprehensive overview current breeding strategies and their potential for processing peach variety improvement. The University of California at Davis (UCD) Peach 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). More detailed results are presented as figures and tables in this and the Regional Testing Annual Report, 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. Results suggest a serious limitations in the inherently reductionist, RosBREED approach and indicate that to fully exploit molecular-marker-based selection, techniques need to be developed to (a) simultaneously manipulate the large number of genes determining orchard productivity and much of fruit quality as well as (b) to more fully integrate biotech with proven traditional breeding approaches.

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* (Fig. 1). *Inbreeding* and *Hybridization* are commonly used for vegetable and field 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.

Inbreeding

Inbreeding typically involves the recurrent inbreeding (usually by repeated self-pollinations) 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.

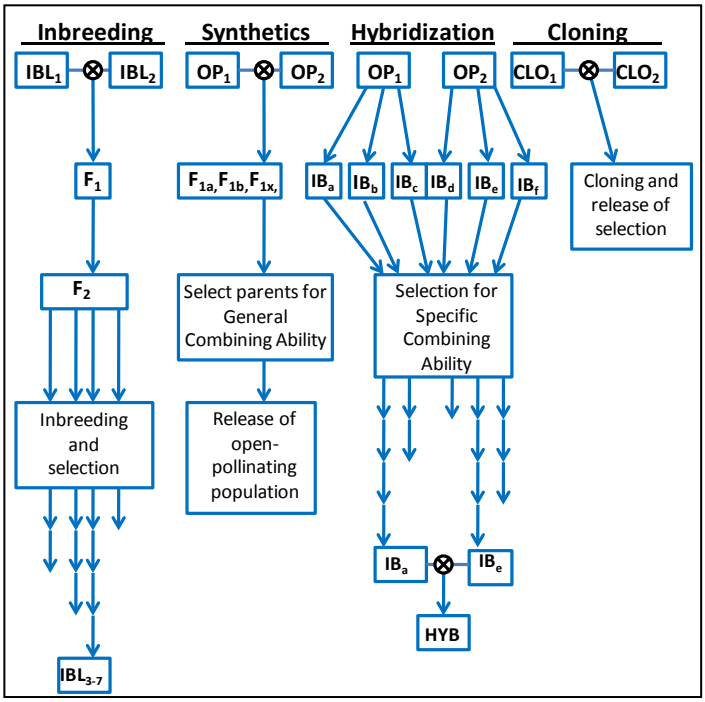

Fig. 1. The 4 basic breeding strategies for cultivar development. (The horizontal length of individual boxes roughly reflects genetic variability while the number of tiers of vertical arrows approximate number of breeding cycles.

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 peach. 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. Unlike *Inbreeding* and *Hybridization*, there is typically no pre-breeding requirement (i.e. no prerequisite development of 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 stone fruit such as the *Hansen* and *Nickels* peach by almond hybrids [2]. 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.

UCD Peach breeding strategies

Historically, the term 'breed' referred to a type of domesticated animal such as the Clydesdale horse that 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-directed 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 peach, 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

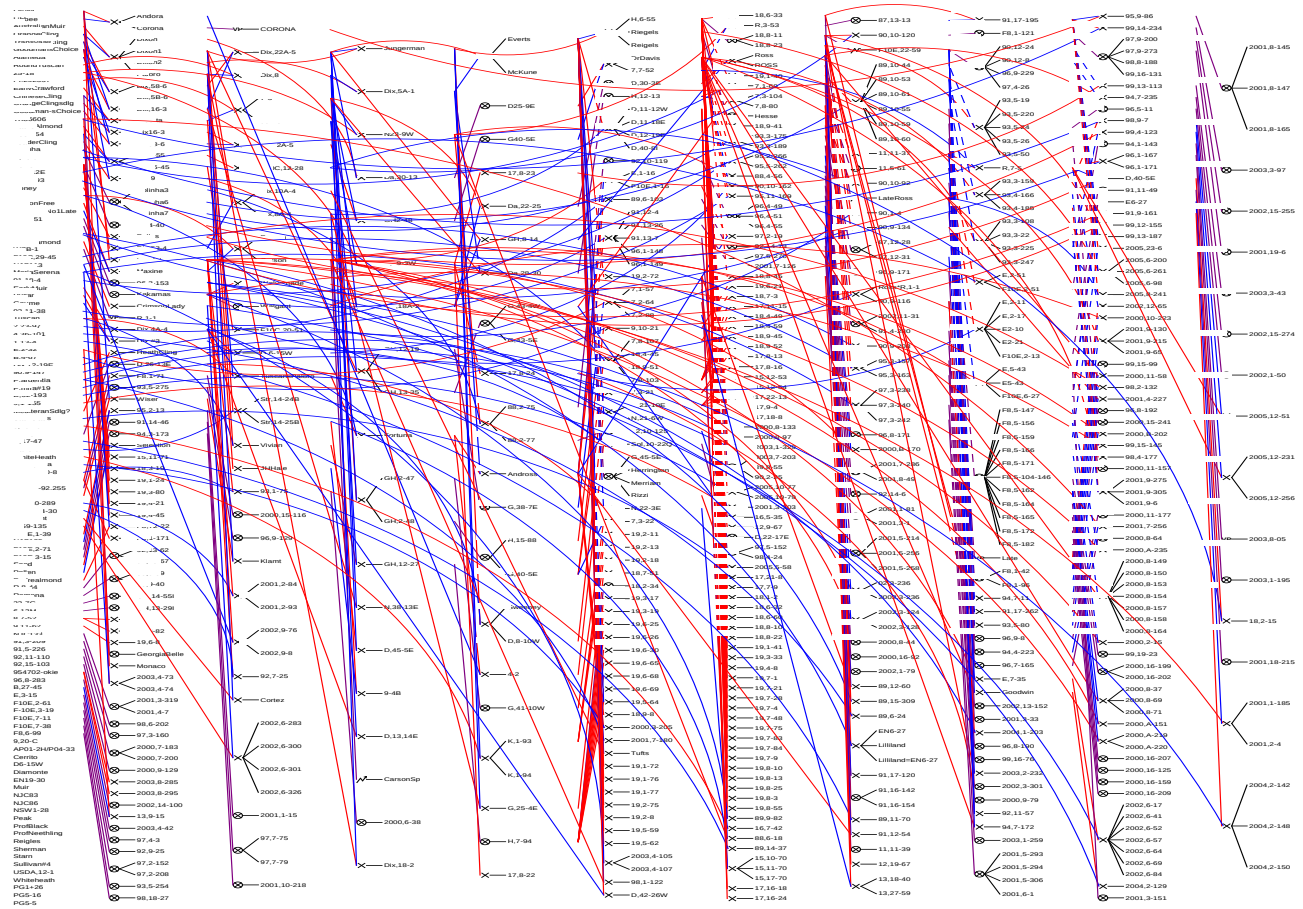

Fig. 2. Flowchart showing updated and corrected lineages of UCD advanced selections and parents currently used in breeding for once-over harvest and improved productivity, including disease and pest resistance. Sources of post-ripe fruit integrity have been independently transferred from South African varieties and related species including *Prunus argentea, P. mira* and *P. dulcis* (almond). Additional sources of disease resistance and improved kernel and tree quality have been transferred from European and Brazilian peach varieties, *P. davidiana, P. scoparia, a*nd *P. dulcis*. Several advanced selections have incorporated traits such as fruit flesh integrity 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. (Red lines identify the seed parent while blue lines identify pollen parent). {Image can be enlarged for visualization of details}.

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 stone fruit cultivars have been cultivated continuously for hundreds to

Genotype	B00	B00	B03	B03	B04	B04	B00	B00	U00	U00	M04	M04	M02	M02	T004	T004	T012	T012
Hesse	198	198	144	144	136	136	229	229	137	137	231	231	240	240	133	133	158	158
18,8-11	198	198	144	144	136	136	229	229	123	137	231	231	240	240	133	133	158	160
FPS-20-13	198	200	144	144	136	136	225	229	137	137	231	231	240	240	133	133	158	158
NSW2-36	198	200	144	144	136	136	225	229	137	137	231	231	240	240	133	133	158	158
NSW2-37	198	200	144	144	136	136	225	229	137	137	231	231	240	240	133	133	158	158
EL4	198	198	144	144	136	136	*229*	229	137	137	231	231	*240*	240	133	133	158	160
EL5	198	198	144	144	136	136	*229*	229	137	137	231	231	*240*	240	133	133	158	160
EL6	198	198	144	144	136	136	*229*	229	137	137	231	231	*240*	240	133	133	158	160
EL7	198	198	144	144	136	136	*229*	229	137	137	231	231	*240*	240	133	133	160	160
2000,2-18	198	198	144	144	136	136	*229*	229	137	137	231	231	*240*	240	133	133	160	160
2005,17-185	198	198	144	144	136	136	229	229	123	137	231	231	240	242	133	133	158	160
2003,1-329	198	198	144	144	136	136	225	225	137	137	231	231	240	240	133	133	158	158
F8,1-42	194	198	144	144	136	136	211	229	137	137	231	231	224	230	133	133	158	158
2005,13-135	198	198	154	154	136	136	225	225	137	137	231	231	240	240	133	133	158	158
2005,17-136	198	200	154	154	136	136	225	225	137	137	231	231	240	242	133	133	158	158
2003,6-171	194	200	144	144	136	136	211	225	137	137	231	231	224	240	133	133	*158*	*158*
2000,3-205	194	198	144	148	136	144	197	229	131	137	231	231	240	242	133	159	144	160
2000,3-205	194	198	144	148	136	144	197	229	131	137	231	231	240	242	133	159	144	160
2005,18-118	198	198	144	154	136	136	209	229	137	139	231	231	240	240	133	133	158	160
2005,20-100	198	198	144	154	136	136	229	229	119	139	231	231	240	240	133	133	158	160
2005,16-147	198	198	144	154	136	136	229	229	119	137	233	251	240	240	133	133	162	162
F10C, 12-128	194	200	148	148	118	146?8	229	233	123	123	212	233	224	240	155	155	148	158
2007,16-53	178	178	144	144	136	136	203	203	137	137	231	233	238	238	133	133	158	158
F10C, 20-51P	194	196	140	150	142	142	199	211	99	99	212	227	236	250	147	155	146	148
2005,20-192	196	198	130	144	136	136	199	229	114	123	231	259	236	240	133	155	158	158
95, 12-130	198	204	144	144	118	118	229	231	123	123	251	255	230	232	133	133	158	158

Fig. 3. Results from the DNA fingerprinting (using SSR markers [5]) for traditional California processing peach cultivars (yellow), advanced UCD breeding selections (pink), early breeding and introgression lines (orange) and interspecies hybrids (green) [many identified in Fig. 2]. Note that virtually all California processing peach cultivars share most markers, supporting their derivation from a relatively few early California varieties. The prevalence of novel markers in UCD cultivars and advanced selections supports increased genetic opportunities as well as a decreased genetic vulnerability (to pests, diseases, climate change, etc.) in these breeding lines despite intensive selection for fruit and tree types for adaptation to Central Valley production and markets (see Fig. 12).

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.

This 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, peach production in Pennsylvania, Eastern Canada and Chile has been put in jeopardy by the introduction of the plum pox virus which has already devastated stone fruit production in parts of Europe. Although many cultivars are currently planted in California, this germplasm remains highly inbred since most commercial cultivars are derived from only a few parental cultivars, which also appear to be related (Fig. 3) [3,10].

Genetic analysis as a basis for applied breeding.

Early peach 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 specific 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 is determined by the proportion of the total phenotypic or observable variability that was due to parent (genetic) 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.

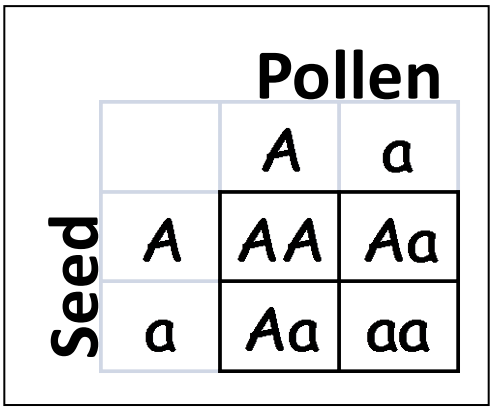

Fig. 4. Punnett square diagram showing predicted gamete (1/2A:1/2a) as well as progeny genotypes (1AA:2Aa:1aa) and their probabilities from a cross between two diploid plants heterozygous at locus Aa where A- dominates in expression. This segregation pattern is common for bitterness (aa) in peach kernels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

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 (Figs. 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

Table 1. Qualitative traits in peach (modified from Monet and Bassi 2008).

Phenotype and symbol	Genotype[a]	Note	Reference
Tree			
Broomy (columnar, pillar) (*Br*)	*br*/*br*	Incomplete dominance; phenotype is upright when *Br* is heterozygous with the alleles for the standard, dwarf, compact or weeping growth habits	Scorza et al. (1989, 2002); Yamazaki et al. (1987); Chaparro et al. (1994)
Upright (*Up*)	*Br*/*br* *br*/*pl* *br*/*dw* *br*/*Ct*	See: Columnar	Scorza et al. (1989)
Arching (*Ar*)	*Brbr*/*plpl*	Upright weeping; similar to the *Up*, but with a distinct curvature of the 1-year-old shoots; from F2 or backcross progenies of columnar (*Br*) × weeping (*Pl*) crosses; *Br* is epistatic to *Pl*	Werner and Chaparro (2005)
Bushy (*Bu*)	*bu*1/*bu*1 *bu*2/*bu*2	*Bu*1 and *Bu*2 are independent.	Lammerts (1945)
Compact (*Ct*)	*Ct*/–		Mehlenbacher and Scorza (1986)
Dwarf (*Dw*)	*dw*/*dw*	Short internode (<10 mm)	Lammerts (1945)
	*dw*2/*dw*2	Very dwarf	Hansche (1988)
	*dw*3/*dw*3	Extremely dwarf, thin stem	Chaparro *et al*. (1994)
Semi-dwarf (*N*)	*n*/*n*	Incomplete dominance	Monet and Salesses (1975)
Weeping (*Pl*)	*pl*/*pl*	Incomplete dominance, featuring open, intermediate canopy when heterozygous (Pl/pl) (Bassi and Rizzo, 2000). Pl from pleureur (to weep, in French)	Monet et al. (1988)
Standard	*Dw*/– *Br*/*Br* *Pl*/*Pl* *ct*/*ct*	This growth habit results from the allelic status of any of these known genotypes	
Graft incompatibility with Damas 1869 plum (*I*)	*I1*/– *I2*/–	Two dominant genes; incompatibility found only in some nectarines	Salesses and Al-Kai (1985)
Corky triangle (*T*)	*t*/*t*	Epidermic suberification at bud base in 1-year-old shoot	Monet and Bastard (1982)
Evergreen (*Evg*)	*evg*/*evg*	Also called evergrowing (Bielenberg *et al*., 2004) terminal buds do not go dormant.	Lammerts (1945); Rodriguez *et al*. (1994)
Anthocyanin deficiency (*An*)	*an*/*an*	Pale pink flowers	Monet (1967)
Anthocyaninless (*W*)	*w*/*w*	White flowers; no red anywhere	Lammerts (1945)
	w^v/ w^v	w^v is unstable producing variegated flowers (peppermint)	Lammerts (1945); Chaparro et al. (1995)
Resistance to root-knot nematode, *Meloidogyne javanica* (*Mj*)	*Mj*1/– *Mj*2/–	*Mj*1 and *Mj*2 are independent	Lownsberry and Thomson (1959) Sharpe et al. (1970); Lu et al. (2000)
Resistance to root-knot nematode, *Meloidogyne incognita* (*Mi*)	*Mi*/–		Weinberger et al. (1943); ; Lu *et al*. (2000)
Resistant to both species	*Mij*/		
Green aphid resistance (*Rm*)	*Rm*1/–		Monet and Massonié (1994)

Phenotype and symbol	Genotype	Note	Reference
Leaf			
Foliar glands (*E*)			
Reniform	*E*/*E*		Connors (1921)
Globose	*E*/*e*	Incomplete dominance	
Eglandular	*e*/*e*	High susceptibility to powdery mildew; serrate leaf margin	
Redleaf (*Gr*)	*Gr*/–	Red incompletely dominant over green in leaves and fruit skin ground colour.	Blake (1937)
Albinism (*C*)	*c*/*c*	Plant does not survive	Bailey and French (1932)
Wavy-leaf (*Wa*)	*wa*/*wa*		Scott and Cullinan (1942)
Willow-leaf (*Wa2*)	*wa2*/*wa2*		Chaparro et al. (1994)
Crinkle leaf (*CL*)	*cl*/*cl*	Associated with very oblate fruit shape	Ledbetter (1996)
Flower			
Non-showy (Sh)	*Sh*/–		*Connors (1920); Bailey and French (1942);* Lammerts (1945)
Large size (*L*)	*L*/-		Connors (1920); Lammerts (1945);
Double (D_1)	d_1/d_1	More than five petals; often incompletely dominant; number of extra petals controlled by one or two recessive ger	Bailey and French (1949); Lammerts (1945)
Fewer extra petals (Dm_1, *Dr*	dm_1/dm_1 dm_2/dm_2	Dm_1 and Dm_2 are independent and additive	Lammerts (1945); Yamazaki al. (1987)
Dark pink petal (*P*)	*P*/–		Lammerts (1945)
Red petal (*R*)	*r*/*r*		Lammerts (1945); Chaparro al. (1995)
Male sterility (*Ps*)	*ps*/*ps*	Sometimes has some viable pollen (from 'J.H. Hale')	Connors (1926); Blake and Connors (1936) ; Scott and Weinberger (1944)
Male sterility (Ps_2)	ps_2/ps_2	(from 'White Glory')	Blake (1932); Werner and Creller (1997)

Phenotype and symbol	Genotype	Note	Reference
Fruit			
Slow ripening (*Sr*)	*sr*/*sr*		Ramming (1991)
Saucer (flat) shape (*S*)	*S*/–	*S*/*S* is lethal (Guo *et al*., 2002)	Lesley (1940)
Aborting fruit (*Af*)[b]	*af*/*af*		Dirlewanger et al. (2006); Blake (1932); Werner et al. (1998)
Blood red flesh (*Bf*)	*Bf*/–	Pigment appears in immature fruit and main leaf vein; often smaller trees	Blake (1932); Werner et al. (1998)
Rough skin (*Rs*)	*rs*/*rs*	Matte skin surface; glabrous flower buds	Okie and Prince (1982) ; Okie (1988b);
Glabrous skin (nectarine) (*G*)	*g*/*g*	Fuzzless	Blake (1932) ; Blake and Connors (1936)
Full red skin (*Fr*)	*fr*/*fr*	Only on fruit	Beckman and Sherman (2003)
Highlighter (*H*)	*h*/*h*	Red colour suppression on fruit skin	Beckman et al. (2005)
White flesh (*Y*)	*Y*/–	Also affects calyx cup and leaf colour	Connors (1920)
Flesh texture /pit adherence *(F)c*	*Fl*		Bailey and French (1932; 1949); Monet (1989); Peace et al. (2005)
Melting freestone			
Melting clingstone	*f/f* *f/f1* *f/fn*		Peace et al. (2005)
Nonmelting clingstone	*f1/f1* *f1/n* *n/n*		Peace et al. (2005)
Stony Hard flesh *(Hd)d*	*hd/hd*		Yoshida (1976); Scorza and Sherman (1996)
	hdhd/F	Stony hard, melting	Haji et al. (2005)
	hdhd/f1f1	Stony hard, nonmelting	Haji et al. (2005)
Low-acid flesh *(D)*	*D/-*	*D* for *douce* (sweet in French)	Monet (1979)
Sweet kernel (Sk)	*Sk/sk*		Werner and Creller (1997)

[a] Traits determined by traditional Mendelian analysis prior to advent of molecular analysis.
[b]The 'aborting fruit' has been reported as a recessive trait causing the abortion of all fruits within 2 months after full bloom; it is still not clear whether the 'aborting fruit' phenotype is regulated from the same locus or from a novel gene (*Af*).
[c]Four alleles at the same locus controlling both flesh texture (endopolygalacturonase enzyme expression) and pit adherence; the fourth, null allele (*n*), has the same effect as the f_1 allele (non-melting clingstone) (Peace *et al.*, 2005) both traditional Mendelian as well as molecular markers used in determination.
[d]The independent inheritance of this trait was demonstrated, also suggesting an epistatic influence on the *F* locus, since the stony-hard, melting (*hdhd*/*f*–) phenotype is induced to soften when exogenous ethylene is applied (Haji et al., 2005).

Table 2. Reported heritability estimates for quantitative traits in peach (from Monet and Bassi, 2008).

Trait	Heritability	Reference
Full bloom	0.39	Hansche et al. (1972)
Amount of ripening	0.38	Hansche et al. (1972)
Ripening date	0.84	Hansche et al. (1972)
Crop	0.08	Hansche et al. (1972)
Fruit length	0.31	Hansche et al. (1972)
Fruit cheek	0.26	Hansche et al. (1972)
Fruit suture	0.29	Hansche et al. (1972)
Fruit firmness	0.13	Hansche et al. (1972)
Fruit acidity	0.19	Hansche et al. (1972)
Soluble solids	0.01	Hansche et al. (1972)
Juvenility (flower number)	0.16	Hansche and Boyton (1986a)
Intensity of browning	0.35	Hansche and Boyton (1986b)

Table 3. Breeding strategy origins for California peach and plum cultivars which have achieved a high commercial success as indicated by a harvest of over 500,000 cases in 2010 (CDFA 2011). ('?' -indicates origin implied but not clearly stated in patent description).

Peach	**Origin**
August Fire	budsport
August Red	OP?
Autumn Flame	hybrid
Brittney Lane	hybrid
Crimson Lady	hybrid
Diamond Bright	hybrid
Elegant Lady	hybrid
Honey Blaze	F_2
O'Henry	hybrid
Rich Lady	Amparo OP?
Ruby Diamond	hybrid
Spring Bright	hybrid
Spring Snow	F_3?
Summer Bright	F_2?
Summer Fire	hybrid
Super Rich	hybrid

Plum	**Origin**
Blackamber	Friar hybrid
Fortune	hybrid
Friar	hybrid
Angeleno	Queen Ann OP?

Table 4. Selected stone fruit traits as prioritized by applied cultivar breeding programs in the United States as part of a RosBREED study to identify promising targets for MAB where, in effect, all traits were rated as essential. (0-nonessential; 5-essential). (Yue et al 2011).

Trait Name	**Sweet Cherry**	**Tart Cherry**	**Peach**	**Apricot**	**Plum**
Fruit firmness	5	5	4.88	5	4.67
Skin color	5	5			
Fruit size	5		4.63		4.67
Flavor	4		4.6	5	4.67
Fruit shape		5	4.71		
Flesh color		5			
Sweetness			4.63	5	5
Soluble solids(Brix)			4.5	5	5
Productivity			4.57		5
Production consistency		5	4.63		4.67
Extended harvest season	5			5	4.67
Fruit uniformity		5	4.75		
Pre-harvest dropping				5	4.5
Fruit juiciness	4				
Pit shape and size		5			
Pit splitting and fragments		5			
Machine harvest ability		5			
Titratable acidity				5	
pH				5	
Aromatics/volatiles				5	
Heat tolerance				5	
Storage disorders					4.5
Heat tolerance			4.57		
Resistance to frost injury	5				
Powdery mildew	5				
Other disease-viral	5				
Self fertility	5				
Graft compatibility		5			

control for the trait as well as the genetic composition of the parents. For example, selfing the variety *Rizzi* would give a progeny population which segregated roughly 3:1 for sweet: bitter kernel indicating that this parent was heterozygous (Aa) for this trait (Fig. 4). However, selfing the and Ross or Halford varieties would result in all seedlings having bitter kernels, indicating that these varieties were homozygous recessive (aa) for the trait [7]. The major Mendelian or qualitative genes identified through this process for peach, (the stone fruit with the most extensive genetic database), are summarized in Table 1.

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 commercially valuable traits controlled by single segregating genes are rare in peach and even where important examples exist, such as flesh color, 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 effects becomes limiting so that for traits controlled by three or more genes, an analysis based on statistical probabilities is usually required (Figs. 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) = $V_G /(V_G + V_E + V_{GxE})$.

Currently published heritability estimates for peach are 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 into the peach breeding program over the past two decades in efforts to identify the best sources of productivity and disease and pest resistance (Figs. 2,3 & 12). [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 Fig. 12) 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 Fig. 3). However, because novel and often exotic traits (such as the *long-keeper* trait) have been transferred to cultivated peach 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 Fig. 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 most stone fruit, 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 peach and many other tree crops can be better appreciated by comparing the breeding strategies which have historically been shown to be most effective in their genetic improvement.

Genetic components of peach 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 apricot and almond production [2], virtually all modern peach as well as all modern stone fruit breeding programs employ versions of the *Hybrid-Clone* strategy.

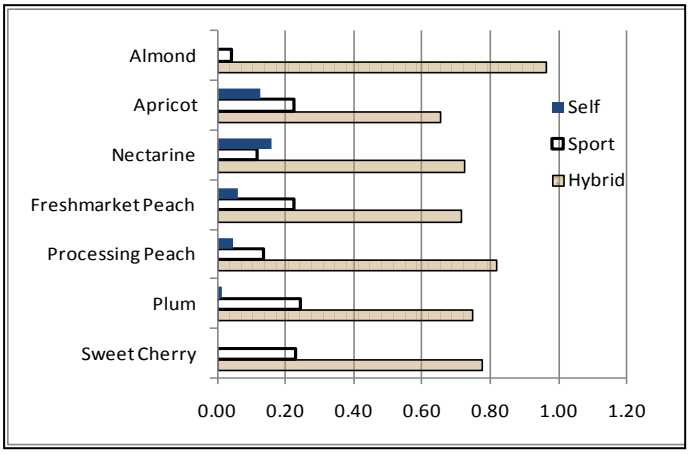

Fig. 5. Results from a survey on cultivar origins (hybridization, selfing or sport mutation) for the different stone fruit cultivars having parentage reported in the 1997 Brooks and Olmo Register of Fruit and Nut Varieties showing predominance of hybrids [1].

Although early 1990s studies had suggested the possibility that hybrid vigor or heterosis was exploitable in peach if inbred parent lines could be properly developed, later results of a long-term peach *Inbreeding* and *Hybridization* study by researchers in France were considered unsuccessful. Most commercially successful stone fruit cultivars appear to result from an initial hybridization between two distinct parents rather than the more convenient self-pollination, even in crops such as peach and sour cherry which are naturally self-pollinated and may show no discernible inbreeding depression. Results from a survey on cultivar origins (hybridization, selfing or sport mutation) for the different stone fruit cultivars having parentage reported in the Brooks and Olmo Register of Fruit and Nut Varieties (Anon 1997) are presented in Fig.

5. Even for nectarine, fresh-market peach, and processing peach, where self-pollination is the natural mating system, a hybrid origin clearly dominates despite the relatively large numbers of cultivars evaluated (162, 540, and 22 respectively). Similar trends are seen for apricot, where selfing is readily achieved for a number of major cultivars. No cultivars originating from self-pollination were observed in plums despite self-pollination being common in several plum species including prominent cultivars, but this may partly due to a prevalence of a more exotic, even interspecies hybrid origins of some plum cultivars. Similarly, no cultivars originating from self-pollination were observed for sweet cherry as might be expected in this mostly outcrossing species (Fig. 5). Results from a recent survey of origins for the major (annual production exceeding 500,000 cases) peach and plum cultivars currently grown in California (CDFA 2011) also shows a clear predominance of a hybrid over self-pollination origin (Table 3) despite the common breeding practice of selfing which is much less tedious. Hybridization in stone fruits often requires careful emasculation of the seed parent flower followed by hand-pollination using previously collected and processed pollen. Self-pollination, in contrast involves simply bagging the flowering branch to exclude outside pollen transfer by visiting insects, or merely allowing the flowers to open-pollinate and then using molecular markers to rogue-out the occasional out-cross. [Under field conditions, insect cross-pollination has been shown to be relatively common for plum and peach with occasional out-crossing proportions of 30% or higher reported.

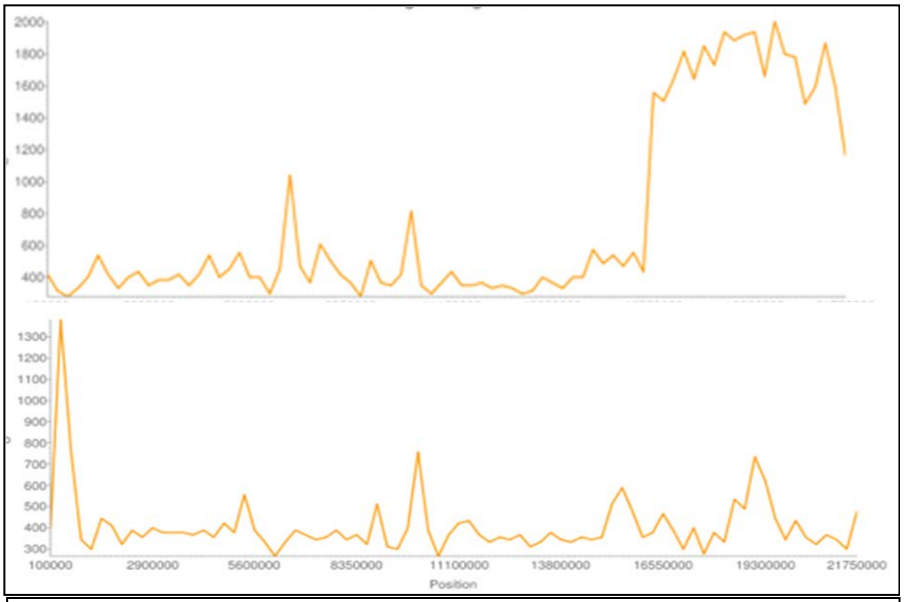

Fig. 6. Levels of genetic variation typically observed along the physical length of peach and almond chromosomes (bottom; = chromosome 8 of the peach cultivar 'Dr. Davis'). Dramatic increase in the level of genetic variability in UCD breeding line 'F8,1-42' (top) which is a 'Nonpareil' almond by 'Dr. 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.

The observed fitness of hybrids relative to self-pollinations is consistent with the out-breeding nature of most stone fruits where deleterious recessive alleles would be expected to accumulate. Hybridization would encourage greater heterozygosity at these vulnerable loci, where a dominant allele would mask expression of deleterious recessive alleles. An example in peach would be the homozygous recessive eglandular

genotype (ee in Table 1) which is associated with high susceptibility to powdery mildew (*Sphaerotheca pannosa* (Wallr:Fr.) disease. Both the homozygous dominant genotype (EE, reniform leaf gland) as well as the heterozygous genotype (Ee, globose leaf gland) show resistance. 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. A possible example of heterozygote advantage may be the previously described leaf gland loci in peach where the homozygous dominant (EE) phenotype has been associated with greater susceptibility to leaf-curl (*Taphrina deformans* (Burk.)) disease but the heterozygote (Ee) shows relatively greater resistance to both leaf-curl and powdery mildew.

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

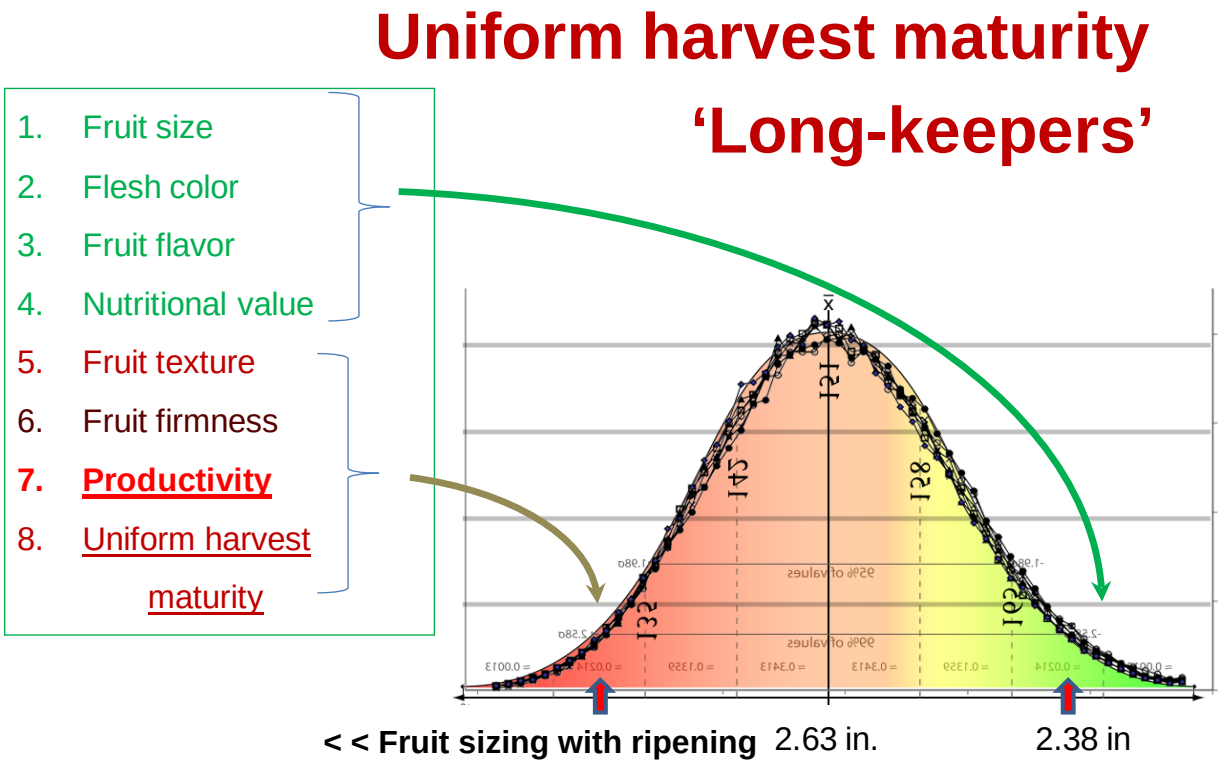

Fig. 7. Breeding strategy targeting the suppression of fruit deterioration after normal tree-ripening (*long-keeper* trait) as a means to improve yields as well as a number of associated fruit traits. *Long-keeper* cultivars would allow a delayed harvest of one week or more, thus allowing green and undersized fruit to continue to develop to full commercial quality. In preliminary tests of such once-over harvests, yields have increased 5-10% with a ~90% decrease in culls.[Traditionally, a grower may harvest when average fruit size is 2.63 in. in order to maximize the number of fruit above the 2.38 payment threshold but minimize loss to overripe fruit (red arrows). The *long-keeper* trait would eliminate these restrictions].

crop performance advantages particularly in the extensive year by site replicated trials common in Cling Peach Regional Testing. 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 peach 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 peach and almond (Fig. 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).

Taken together, these findings indicate that, unlike many seed-propagated crops, genetic control of important peach 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. [1, 11]. If verified, this finding would have important consequences peach 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.

PEACH 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 fruit brown rot 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 processing peach cultivar is expected to have an average annual production of ~20 tons 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 [1, 11]. The need in peach 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 (Fig. 4) which can be readily manipulated. Since peach is diploid (that is, having 2 complete sets of genes), progeny will inherit one complete set from the seed and one from the pollen parent. Thus, not only are the progeny genotypes predictable, but unknown parental genotypes can be readily deduced once the progeny genotypes are determined. [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 Fig. 5.

Examples of a beneficial bud-sports is the *Late Ross* variety, which as a mutation of *Ross* retains many of the good fruit and tree qualities of *Ross* while ripening later. Induced mutations, while rarer, can be also be valuable, as in the induction of self-compatibility and compact, spur-type bearing habit in sweet cherry. While bud sports and induced mutations typically are limited and discrete genetic changes, the risk of negative pleiotropic effects 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 common association of the nectarine trait with reduced fruit size which plagued early breeding efforts. Large sized nectarine genotypes were eventually recovered after extensive breeding efforts to break the relatively tight linkage of nectarine with small fruit size.

Genetically modified organisms (GMOs) are in many ways similar to induced mutations as they provide the opportunity to introduce a discrete new trait to an otherwise well established genetic background, but also run the risk of introducing undesirable characteristics (either through pleiotropy or multiple gene insertion events) and so also require extensive field testing before release as an improved cultivar.

Traits controlled by 1 to 2 gene(s) can also be readily transferred to locally adapted genetic backgrounds through recurrent selection, as might occur with mass-selection, selfing/inbreeding, and backcrossing. Because of the longer generation time and smaller progeny population sizes typical of stone fruit crops, recurrent backcrossing is often utilized as it allows a more efficient concurrent improvement in both recurrent population and targeted traits. An example of our recurrent backcrossing program to transfer *Monilinia* fruit rot resistance from the Brazilian cultivar *Bolinha* to California processing peach breeding lines is presented in Fig. 12. Hybridization is also a very efficient strategy for recombining multiple monogenic traits when controlled by dominant genes. In this approach, which is used extensively in vegetable crop breeding but only

rarely and fruit crop improvement, the two parents are selected for the both presence of complementary dominant genes as well as their specific combining ability.

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 (Fig. 8). MAS and associated molecular marker strategies should be particularly effective for oligogenic manipulation provided the number of genes remains relatively low. Although, fruit flesh color (White versus yellow) is typically considered a monogenic trait (i.e. single gene control), it has recently been shown that the level of flesh color can vary depending on environment and a relatively small numbers of modifier genes [1]. Thus, while flesh color can be recovered with the relatively simple single-gene transfer (typically through recurrent selection as in Fig. 12), to achieve consistently high levels of flesh color over different years and environments, the appropriate modifier genes need to be concurrently selected. {Similar complex relationships are apparent in our breeding for fruit brown rot resistance Fig. 9 and fruit flesh integrity Fig. 11}. 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 (Fig. 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

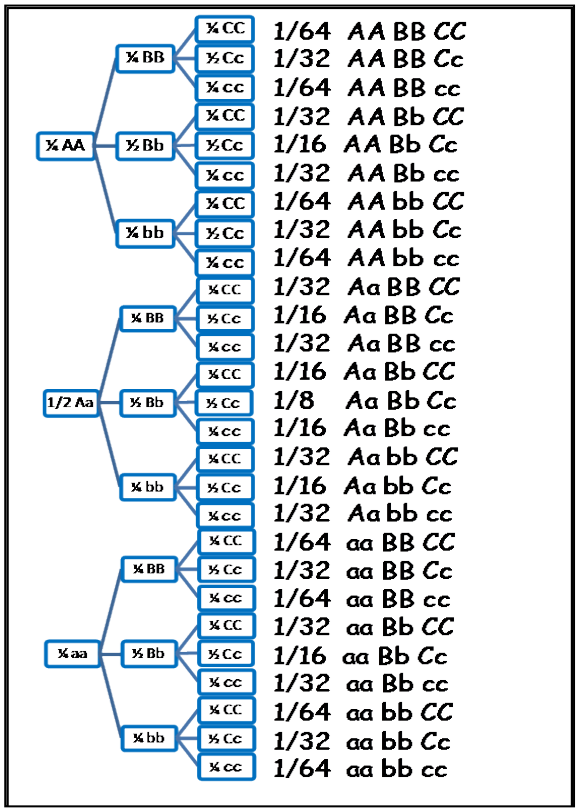

Fig. 8. Tree diagram showing genotypes and their predicted probabilities from a cross between two diploid plants heterozygous at unlinked loci A/a, B/b and C/c.

of targeted traits made possible by molecular analysis may be of considerable value to the breeder and may lead to novel breeding strategies as presented in Fig. 6.

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.

	2009	2010	2011
UltraEarly	2.9	0.3	0.4
Carson	21.8	11.9	12.0
Early#4	1.6	5.6	5.2
Ross	20.1	23.2	21.4

Fig. 9. Year-to-year variability in fruit brown rot resistance as shown as disease severity score (lesion size by incidence) after controlled inoculation of selected UCD peach breeding lines.

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 [1]. 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 and scaffold structure (Fig. 6), 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 such as *Late-Ross* or *Kingsburg Cling* have been a valuable source of new cultivars (Fig. 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 (See Table 4).

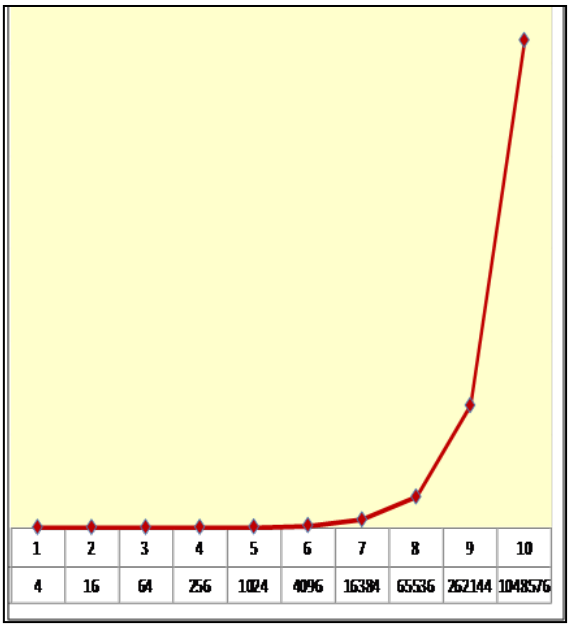

Fig. 10. Plot showing the minimum population size (Y-axis and bottom row) predicted by Mendelian analysis for obtaining a desired homozygous genotype at increasing numbers of independent peach loci (X-axis and top row).

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 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 fruit 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, red-pit staining, 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 Fig. 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.

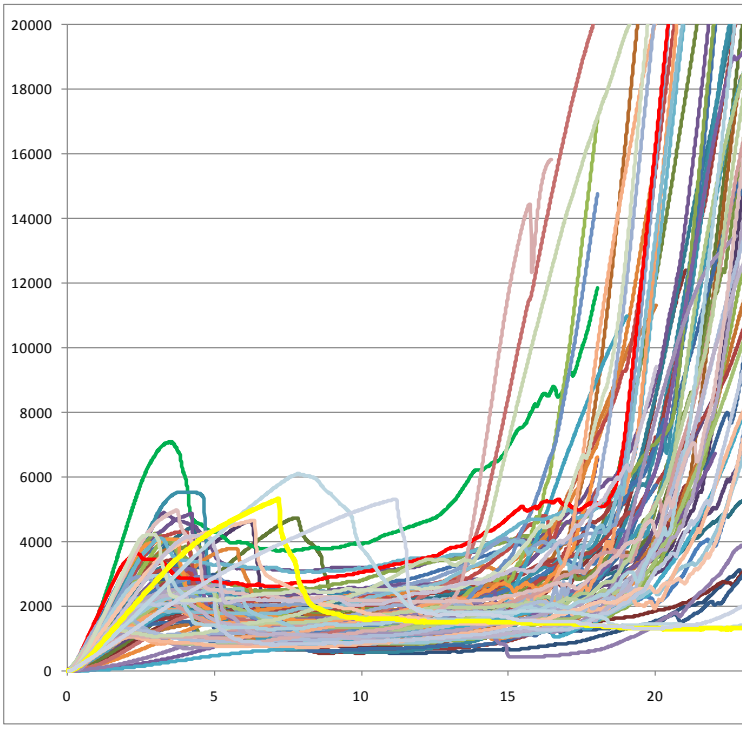

Fig. 11. Fruit integrity as characterized by resistance force (vertical axis) for 3 mm dia. probe at increasing distance (mm) into fruit flesh from skin surface (horizontal axis) for standard processing peach (yellow), Extra-Late#6 *long-keeper* parent (green) and progeny including individual inheriting *long-keeper* trait (red).

Improved environmental buffering has also been shown to be a associated with the higher genetic heterozygosity typical of most stone fruit cultivars (Fig. 5). Even with predominantly inbreeding species such as peach, recombination from hybridizations would increase the opportunity for beneficial intra-allelic (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 have occurred for many stone fruit varieties, particularly in

Europe and Asia, where selection has been occurring for hundreds to thousands of years. This extended selection 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 (Fig. 5). MAS when applied to multiple traits is inherently targeting additive genes and so ineffective in selecting other beneficial gene interactions.

Productivity remains the most important attribute in new peach 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]. An example of successful targeting a critical limiting component of yield to allow rapid improvement with relatively straightforward genetic manipulation is seen in our breeding efforts to suppress post-ripe fruit deterioration (*long-keeper* trait) to effectively increase commercial yield as well as fruit quality (Fig. 7). It is informative, though, how biotech progress over the last 3 decades has advanced to the point where sequencing individual peach breeding lines can now be readily achieved, yet our understanding of the basic 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 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 is 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 successful commercial cultivar breeding.

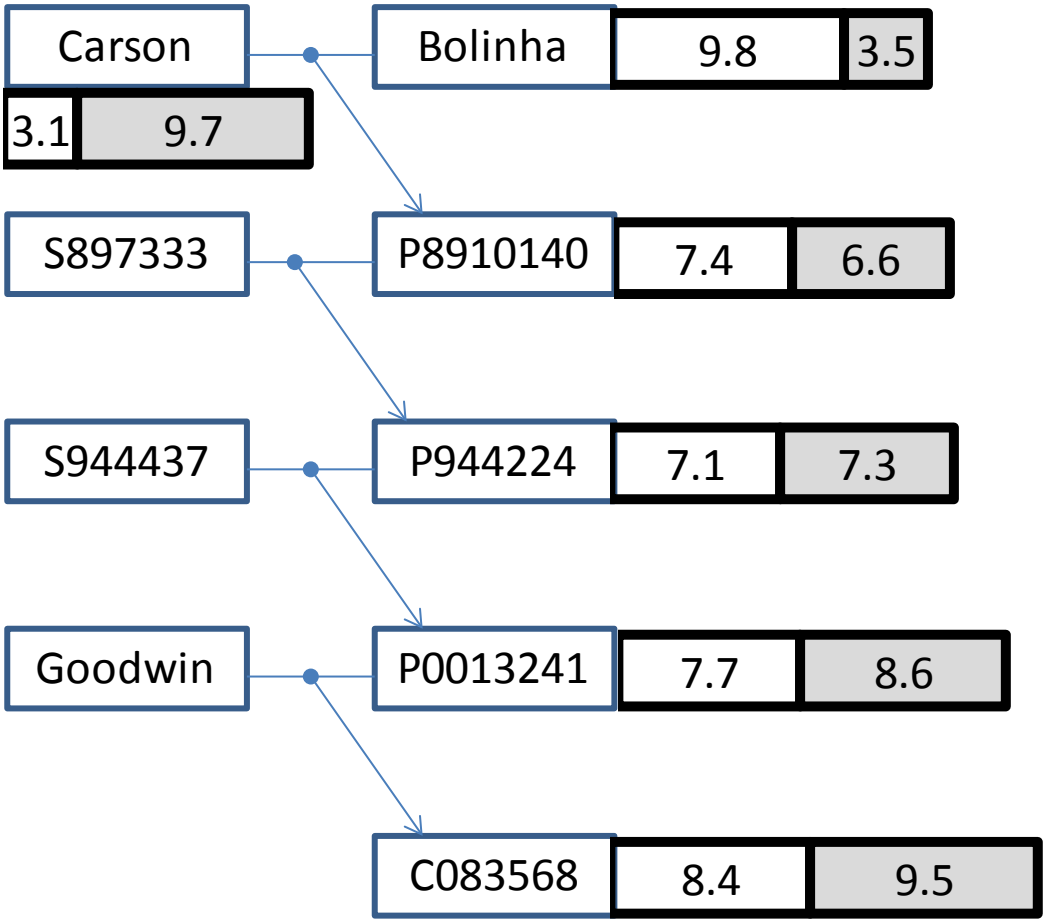

Fig. 12. Lineage (pollen parent to right; seed parent to left) showing transfer of brown rot resistance from the resistant Brazilian variety *Bolinha* to advanced UCD selections with concurrent selection for good processing quality. [Levels of resistance (0-susceptible; 10-resistant) are shown in unshaded central box while processing quality (0-poor; 10-very good) shown in shaded box]

[References Cited]:

1. Gradziel, T.M. (in press) Traditional genetics and breeding. In Abbott and Shri, (eds.) Molecular Breeding of Fruit Crops. . Springer Science, New York. pg. 1-31.
2. Gradziel. T.M. . 2011. Almond origin and domestication. In J. Janick (ed.) Horticultural Reviews. 38:23-82.
3. Dangl, Gerald S., Judy Yang, Thomas Gradziel, Deborah A. Golino. 2009. A practical method for almond cultivar identification and parental analysis using simple sequence repeat markers. Euphytica 168 (1): 41-48.
4. Arus, Pere, Thomas Gradziel, M. Margarida Oliveira, and Ryutaro Tao. 2009. Genomics of Almond. In K.M. Folta, S.E. Gardiner (eds.), Genetics and Genomics of Rosaceae, Springer Science. NY pp. 187-219.
5. Sorkheh, K., B. Shiran, R. Ahmad, P. Martinez-Gomez, T.M. Gradziel, N.Amirbakhtiar (in press). Analysis of Almond [Prunus dulcis (Miller) D. A. Webb] cultivars and related wild species using SSR and AFLP markers. Journal of Horticultural Biotechnology.
6. Zeinalabedini, M. Khayam-Nekoui, M. Grigorian, V. Gradziel, T. M. Martinez-Gomez, P. 2010. The origin and dissemination of the cultivated almond as determined by nuclear and chloroplast SSR marker analysis. Scientia Horticulturae 125: 593-601.
7. Socias i Company R., O. Kodad, and J.M. Alonso and J.T.M. Gradziel. 2008. Almond Quality: A Breeding Perspective. In J. Janick (ed.) Horticultural Reviews. 34:197-238.
8. Sorkheh, K., B. Shiran, V. Rouhi, E. Asadi, H. Jahanbazi, H. Moradi, F. T.M. Gradziel, Martínez-Gómez (in press). Phenotypic diversity within native Iranian almond species and their breeding potential. Journal of Genetics Resources and Crop Evolution.
9. Sorkheh K, Shiran B, Kiani S, Amirbakhtiar N, Mousavi S, Rouhi V, Mohammady S, Gradziel TM, Malysheva-Otto LV, Martínez- Gómez P (2009) Discriminating ability of molecular markers and morphological characterization in the establishment of genetic relationships in cultivated genotypes of almond and related wild species. Journal of Forestry Research 20 (3):183-194.
10. Sorkheh K., B. Shiran, M. Khodambashi, H. Moradi, T.M. Gradziel, P. Martinez-Gomez. 2010. Correlations between quantitative tree and fruit almond traits and their implications for breeding, Scientia Horticulturae, Scientia Horticulturae 125 (2010) 323–331.
11. Gradziel, T.M. and J.P. McCaa. 2008. Processing Peach Cultivar Development In: D.R. Layne and D. Bassi (eds.). The Peach: Botany, Production and Uses. CAB. N.Y. pg. 175-192.
12. Gradziel, T.M. (in press) Traditional genetics and breeding. In Shri, (ed.) Molecular Breeding of Fruit Crops.
13. Connell, J. H. Gradziel, T. M. Lampinen, B. D.; Micke, W. C.; Floyd, J. 2010. Harvest maturity of almond cultivars in California's Sacramento Valley. Options Mediterraneennes. Serie A, Seminaires Mediterraneens. 94, 19-23.
14. Gradziel, T.M. and B. Lampinen. (in press). Constraints to almond productivity. Proceedings of the International Congress on Almond and Pistaschio. San Liurfa, turkey, 2010.
15. Gradziel, T.M. and P. Martinez-Gomez. (in-press). Almond Breeding. In J. Janick (ed.). Plant Breeding Reviews.