

# Pollination Research with *Prunus* Species and its Importance to Fruit Growers

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In a recent editorial by Ms. Margaret Roach, she rephrased an old saying, "Curiosity makes the world go round." I believe curiosity is a driving force for humanity and clearly for scientists. As an example the Cassini-Huygens space mission to land on Saturn's moon, Titan, to explore its elements and atmosphere's composition has just sent back enough data from a few minutes of planned sampling by the Huygens rover to keep earth scientists busy for years in determining its relevance to present knowledge. The methane rains on Titan are thought to have provided some of the building blocks for thionolins which are antecedents of amino acids. Physicists are probably more interested in Titan data to elucidate more about the understanding of the solar system, but most geneticists, theologians and even the person on the street are keen to learn what missing links in human's knowledge about biology are filled. Their curiosity is to explore more about Darwinism versus what is now called by many Intelligent Design (ID). Clearly, curiosity is a driving force for humanity.

## Recent Pollination Research and Its Importance To Growers

I will explore here an example from pollination biology research. I think of it as a model for what we should be achieving through our curiosity about fundamental biology questions. Solving some of the 'super questions' can turn our dreams into new cultivars that are truly breakthroughs for human food needs.

Much has been written about pollination. The definitions about this important biological process for all of

plant life are important and straightforward for growers to use in their bag of pomological knowledge. Why bother to increase your knowledge of how these pollination phenomena work? My answer, in a just four words, "curiosity can make money!"

How many individuals in the industry know how many genetically different pollen compatibility groups were thought to exist within sweet cherries in 1990? It was fourteen. Collaborative research since 1990 has proven that the actual number is nearly double that. This is important because there are many more combinations of cultivars that may work effectively in orchard settings to produce regular cropping. Also, in the past decade research has demonstrated how the biochemical signals work that control such incompatibility reactions. Similarly, in 1990, no scientist had yet established how many pollen compatibility groups existed that affected cross-pollination in Japanese plums. A recent Japanese publication reports that there are at least twenty-four separate groups with one of them conferring self-compatibility (Beppu et al. 2003).

Again, this means that growers can use scientific knowledge of which cultivars belong to which groups and which ones will pollinate each other. Tart cherries in production in North America were generally thought by American growers to be self-fertile because Montmorency is self fertile. Dr. Iezzoni's collaborative research with Japanese scientists has demonstrated that different classes of tart-cherry pollination groupings exist (Yamani et al. 2002). This is very important because diversification by growers to plant more nutraceutically beneficial cultivars of tart cherry will require high yields of new cultivars, and

Research over the last 15 years on stone fruit pollination biology has shown that there are many more genetically different pollination compatibility groups than previously thought in both cherry and Japanese plum. This means that there are many more combinations of cultivars that may work effectively in orchard settings to produce regular cropping. Research over the next 15 years may bring answers to other vexing problems with stone fruits.

some of them are known now to be either self-incompatible or only partially self-fertile. Their research demonstrated not only that some tart cherries could be pollinated effectively by some sweet cherries but also demonstrated which sweet cherry cultivars would be effective with which tart cherry cultivars. All of the above sounds to me like precision agriculture instead of hunting in the dark.

I am going to review one of the most recently published pollination research articles. The East Malling research center led the collaborative effort. A graduate student, Dr. Brian Sutherland was the principle. I choose to review this because it presents many important concepts, techniques, definitions; and it's conclusions reach across several *Prunus* crop plants (almonds, apricots, cherries and plums) (Sutherland et al., 2004).

## General Definitions About Pollination Biology

**Pollination** is the movement of pollen among compatible flowering plants (cross pollination) or from anthers to stigmas on the same plant or different plants of the same clone (self-pollination). Note: some plants self-pollinate but set

few fruits; they must be considered as self-pollinated and partially self-compatible; they are not, however, self-fruitful. In regards to temperate-zone tree fruits, self-pollination and achieving some fruit set does not necessarily mean that they are self-fertile to the extent that they would have normal seeds. It is beyond the scope of this article to explore the various reasons for low fertility and poor fruit set. For our purposes it should be recognized that self-pollinated, self-compatible, self-fertile and self-fruitful each have separate definitions and causes.

**Pollinators** - insect vectors that move pollen from stamens to pistil parts of flowers.

**Pollenizers** - male plants that provide pollen that is compatible genetically to female plants and that bloom simultaneously to the female.

**Fertilization** - union of egg cell with gamete from male pollenizer.

**Fruit set** - in most cases, fruits whose seed have been pollinated, fertilized, and matured through the various developmental processes essential to full fruit development.

### Background (taken directly from Sutherland, 2004)

"Self-incompatibility (SI) in flowering plants and cross-incompatibility between cultivars is controlled by the highly polymorphic S-locus. Gametophytic incompatibility occurs when the allele in the pollen matches one of the stylar alleles. *Prunus* S-alleles encode stylar ribonucleases. Most important *Prunus* species are self-incompatible, requiring the planting of suitable pollinator (Note: by our definitions we'd call them pollenizer) cultivars for economic fruit set." (Figure 1).

#### More definitions:

**Allele** - (from Webster) - any of a group of mutational forms of a gene.

**Style & Stylar** - that portion of the flower located between the stigmatic surface and the ovary(ies).

**Ribonuclease** - (from, Albert, et al, 1994) - enzyme that cuts an RNA molecule by hydrolyzing one or more of its phosphodiester bonds.

"Incompatibility genotypes are traditionally identified by controlled pollination, and more recently by stylar ribonuclease analysis (Boskovic and Tobutt 1996, Boskovic et al. 1997, Burgos et al. 1998). Both approaches require plants mature enough to produce blossoms and

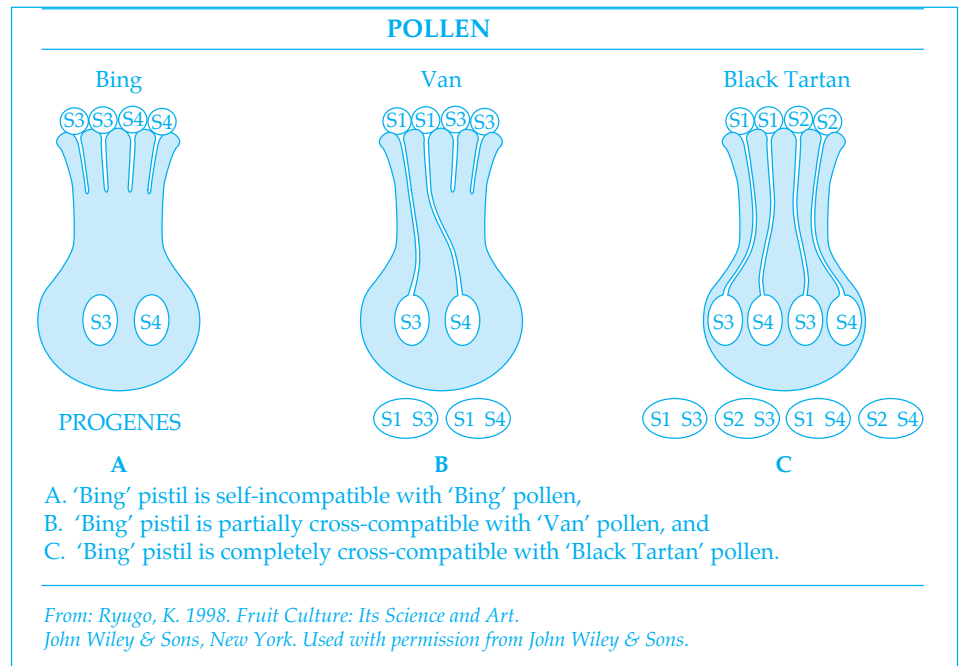


Figure 1. Gametophytic incompatibility in sweet cherry.

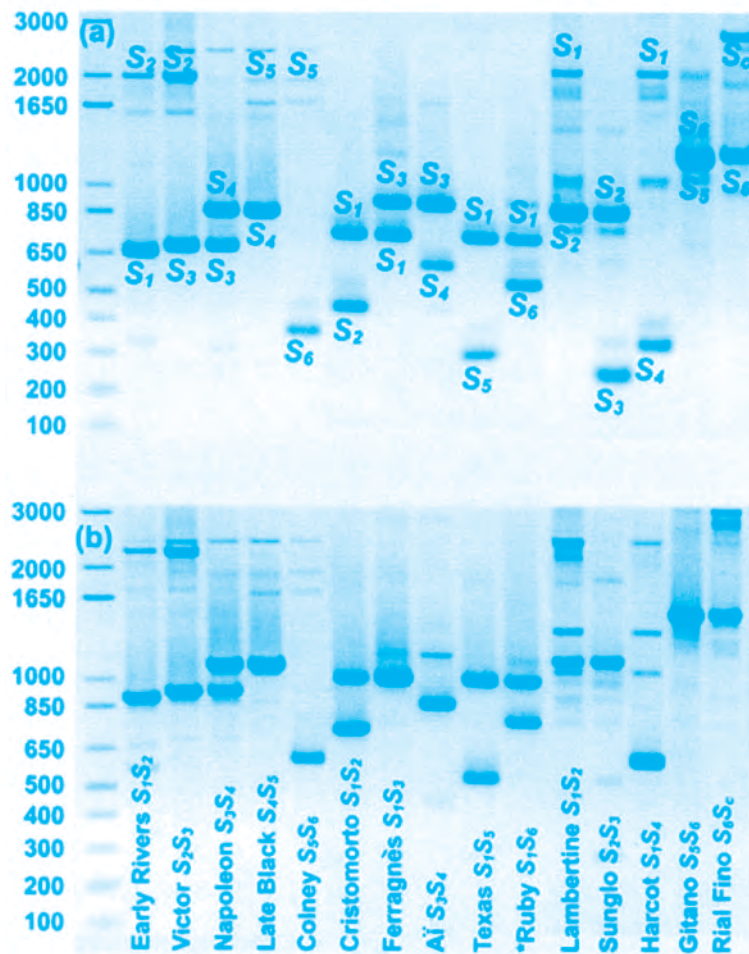


Figure 2 (from Sutherland et al., 2004). S-allele amplification with EM consensus primers in five genotyped cultivars of cherry, almond and apricot (a) amplified with EM-PC2consFD + EM-PC3consRD (b) amplified with EM-PC2consFD + Em-PC5consRD. One kb Plus Ladder (Invitrogen, Carlsbad, California, USA) in lane 1, cherries in lanes 2-6, almonds in lane 7-11, apricots in lane 12-16. \*'Ruby', previously reported as S6S16, subsequently determined as S1S6 (E. Ortega, pers. Comm.). Some alleles, e.g. cherry S5 and Apricot S1 show additional bands.

the latter technique requires a high degree of technical competence. Numerous *Prunus* S-alleles have been at least partially sequenced. They contain two very polymorphic introns."

**More definitions:**

**Intron** - (from Alberts et al, 1994) - noncoding genetic region of a eucaryotic gene that is transcribed into an RNA molecule but is then excised by RNA splicing when mRNA is produced

**Polymorphic** - an adjective describing a gene that has several forms due to past mutational events.

**Sequenced** - a verb describing the technical process of identifying the triplets of nucleotides (codons) that are present in linear order within nucleic acids which determine the specific amino acid to be formed.

"Consensus primers based on conserved regions, especially those flanking the polymorphic second intron, have been developed which distinguish S-alleles on the basis of size of polymerase chain reaction (PCR) product, so that S-genotypes can be deduced from amplification patterns."

**More definitions:**

**Consensus primers** - average or most typical form of a sequence that is produced with minor variations in a group of related DNA, RNA, or protein sequences. The preservation of a consensus implies that the sequence is functionally important.

**Conserved regions** - parts of genetic information that has been proven to exist amongst, and to regulate the same processes in living organisms.

**"PRC"** - polymerase chain reaction - technique for amplifying specific regions of DNA by multiple cycles of DNA polymerization, each followed by a brief heat treatment to separate complementary strands.

Sutherland goes on to state the purpose of his experiments: "Previous work with sweet cherry, almonds and sour cherry developed three consensus primers but these primers were developed from a very small set of allele sequences from just one or two species which could limit their use in detecting S-alleles in a wider range of material." The purpose of Sutherland's work was to produce more versatile consensus primers and he reports having created, ".....three new consensus primers that flank the second intron, designed from alleles from five *Prunus* species and incorporating some degeneracy."

**TABLE 1 (from Sutherland et al., 2004)**

Amplification of S-alleles in sweet cherry, almond and apricot, using consensus primer combinations, two from East Malling and three published previously.					
Trait	EM C2 + C3	EM C2 + C5	Tao C2 + C5 <sup>1</sup>	Tao C2 + C4 <sup>2</sup>	Yamame C2 + C3 <sup>3</sup>
<b>Cherry</b>					
S <sub>1</sub>	+	+	+	+	+
S <sub>2</sub>	+	+	+	-	-
S <sub>3</sub>	+	+	+	+	+
S <sub>4</sub>	+	+	+	+	+
S <sub>5</sub>	+ <sup>4</sup>	+ <sup>4</sup>	-	-	-
S <sub>6</sub>	+	+	+	+	+
<b>Almond</b>					
S <sub>1</sub>	+	+	+	+	+
S <sub>2</sub>	+	+	+	+	+
S <sub>3</sub>	+	+ <sup>4</sup>	+	+	+
S <sub>4</sub>	+	+	+	-	+
S <sub>5</sub>	+	+	-	-	-
S <sub>6</sub>	+	+	+	+	+
<b>Apricot</b>					
S <sub>1</sub>	+	+	+	+	+
S <sub>2</sub>	+	+	-	+	+
S <sub>3</sub>	+	+ <sup>4</sup>	-	-	-
S <sub>4</sub>	+	+	+	+	+
S <sub>5</sub>	+	+	+	+	+
S <sub>6</sub>	+	+	-	+	+
S <sub>c</sub>	+	+	-	-	-

<sup>1</sup>Pru-C2 + Pru-C5 (Tao et al. 1999).

<sup>2</sup>Pru-C2 + Pru-C4R (Tao et al. 1999).

<sup>3</sup>Pru-C2 + PCE-R (Yamane et al. 2001).

<sup>4</sup>Amplification is weak but visible.

**Another definition:**

**Degenerate** -an adjective that describes multiple states that amount to the same thing; different triplate combinations of nucleotide bases (codons) that code for the same amino acid, for example.

Sutherland's results are presented in Table 1 and Figure 2.

He concludes: "The efficacy of the East Malling (EM) primers is attributable to their design from a large set of published sequences drawn from several species and inclusion of degenerate bases where required." He acknowledges that they may need to be redesigned when new alleles are found that can't be picked out by his new EM primer set. He states, "This EM primer set is effective in a range of species, detecting each allele in all cultivars tested. These primers will be suitable for genotyping seedlings in breeding programs, His final statement: "They should also be useful in population genetics and gene-flow studies based on S-alleles."

So, what does the Eastern US stone-fruit growers get out of this (and the collaborative and other preceding experiments about pollen self-incompatibility genetics)? First, they get better and faster knowledge to assist in their planning of new orchard blocks so that they will have

pollination plans that work. Second, they get a scientific approach to phylogenetic sleuthing about the origins of different Rosaceae commercial fruit crops. This may help solve regeneration recalcitrance and brown rot susceptibility of *Prunus*.

**My Dreams**

As I conclude my scientific career I want to ask some questions that have been bugging me and do a little dreaming with the hope that it will inspire future *Prunus* scientists. Dreaming leads to theories which lead to hypothesis which lead to experimentation, and ultimately these dreams produce the basis for business(es) that provide better food and a safer environment.

**1. Why are stone fruits so recalcitrant regarding regeneration of whole plants from masses of cells?** I assert that we have to get serious about the answer to this question if we want to move breeding of *Prunus* to the next level of genetic progress to meet society's food and environmental expectations. Transformation genetics will be kept locked out in the cold until we can clone most *Prunus* plant material at will at the cellular level. I dream of a project that takes the seed physiologists' knowledge of seed dormancy and geneticists' knowledge of

morpho-genetics and then uses this body of knowledge in collaboration with horticultural breeders to solve this dilemma.

**2. Why doesn't brown rot in some form attack apples and pears? Or, conversely, why don't stone fruits get fire blight?** All of the *Prunus*, *Malus* and *Pyrus* are Rosaceae. Where along their evolutionary paths did the various *Prunus* species diverge from *Malus* and *Pyrus* in such a way that the *Prunus* co-evolved in the same eco-systems with brown rot without developing much of a genetic firewall against this terrible fungal disease? Why were apple and pear ancestors spared? Can we assume that botanical geneticists (phylogeneticists) can help us determine which of the living samples of wild accessions of these species are the oldest? Do we have collections in hand that are from geographic sites of intermingled origins of the ancestral homes of apples, pears and stone fruits? If these questions of botanical and pathogen ancestry can't be researched with living accessions, can pollen collections that archeologists use and date for crop plants like maize, wheat and rice also be explored for our most important deciduous tree fruit species? Does the gene-for-gene concept hold potential answers about these questions? I think that we simply have to dream up ways to interest more fundamental sciences to join in exploring our knowledge of food plants and their co-evolution with their pests. I believe that the strong interest in natural foods and their health benefits should be our very best ally in pushing for more science initiatives into the genetics of host-parasite relationships at the molecular level. Who among our scientific community(ies) are working on making these political alliances that will lead to national research initiatives?

**3. Are the metabolic pathway(s) in *Prunus* that important fungicides affect in their control of a disease like brown rot known?** If so, can't we use this knowledge to direct our research? Do other genera within Rosaceae use similar anti-fungal metabolites that occur naturally within their tissues? Do they have conserved genes that guard them from brown rot that *Prunus* does not have? Why? Or, why don't mango fruit get attacked by brown rot? A similar question might be asked for cantaloupe. These two kinds of fruit are emerging as major competitors for the market share that stone fruits want.

They are juicy and full of sugars that would seem to be substrates for brown rot fungi to attack. Do we have any idea what metabolites protect their tissues from brown rot?

**4. Some of my friends from lay backgrounds who love to eat cherries and apricots, and are now seeing 'peento' (flat, doughnut-shaped fruits that are the result of compressed seed shapes in peaches/nectarines). This has made them curious about other seed-related questions. They ask me if we can breed the pit out of stone fruits like they did for seedless grapes?** They hardly think of us doing this for them as being a dream. To them it's a readily achievable goal — because they see that clever grape scientists and watermelon scientists have done so. Well, why not get started on this problem? Seeds aren't necessary to the consumer; they're thought of as something to discard. We know about a few cultivars of plums with edible (soft enough to chew) endocarps, which have sweet seeds (nonpoisonous). We know we can breed edible almond seeds and so-called sweet-kernel apricots. So, we are sure that we could breed seeds that would be healthful, but how about changing the structure of the bony endocarp so it is much more palatable as roughage? How seriously has any stone fruit breeder looked into this question? Would it affect propensity for split pits? Most lay people believe it can be done. Do any of you?

**5. Dr. Dennis Werner at North Carolina State University recently released the first commercial peach cultivar in the USA that did not have 'Chinese Cling' cultivar as its ancestor. How could we have been so narrow in creating the genetic populations from which the USA's peach/nectarine industry is based?** Sweet cherry breeding is still mostly focused on parents that came from Roman Empire sources of old cultivars like Hedelfingen and Napoleon. Dr. Wayne Sherman, Professor Emeritus at University of Florida, dreamed about a low chilling sweet cherry. He used Japanese apricot interspecific hybridization with commercial sweet cherries to get his low chill cherry. These scientists are dreamers and they are my heroes!

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Robert Andersen is a recently retired emeritus professor of Horticulture who specialized in the breeding and culture of stone fruits. He led Cornell's breeding, culture and extension program on stone fruits from 1990 to 2005.