14. Sweet and Sour Cherries: Linkage Maps, QTL Detection and Marker Assisted Selection

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1 Introduction

The cherry is one of the most popular temperate fruit crops despite its relatively high price. The fruits are attractive in appearance because of their bright shiny skin color, their subtle flavor and sweetness are appreciated by most consumers. Compared to other temperate fruits, such as apple and peach, breeding improvements for cherries have been slow. The long generation time and the large plant size of cherry trees severely limit classical breeding. Thus, the integration of molecular markers in breeding programs should be a powerful tool to hasten cultivar development. Only a few genetic linkage maps are available for sweet or sour cherry and quantitative trait loci (QTLs) have been reported only for sour cherry. Until now, most of the efforts have concentrated on the use of molecular markers in order to (i) identify the *S*-alleles controlling gametophytic self-incompatibility, (ii) characterize cultivars, and (iii) assess genetic diversity.

1.1 Brief history of the Crop

Prunus avium L. includes sweet cherry trees cultivated for human consumption and wild cherry trees used for their wood, also called mazzards (Webster, 1996). The sweet cherry is indigenous to parts of Asia, especially northern Iran, Ukraine, and countries south of the Caucasus mountains. In Europe, the Romanian and Georgian wild cherry trees appeared to have significantly differentiated from those of central and western Europe (Tavaud, 2002). The Georgian wild cherry trees are the most genetically diverse, suggesting that this area could have been a main glacial refuge. The ancestors of the modern cultivated sweet cherries are believed to have originated around the Caspian and Black Seas, from where they have slowly spread. This

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radiation was driven initially by birds. Sweet cherries are now cultivated commercially in more than 40 countries around the world, in temperate, Mediterranean, and even subtropical regions. Its natural range covers the temperate regions of Europe, from the North part of Spain to the Southeastern part of Russia (Hedrick, 1915). They prefer regions with warm and dry summers, but require adequate rainfall or irrigation during the growing season for production of fruit with appropriate size for marketing. Rainfall at harvest time may reduce the commercial potential of the production by inducing fruit cracking.

Fruit of *Prunus cerasus* L., the sour cherry tree, are mainly used for processed products such as pie filling, jam or liquor. Sour cherry originated from an area very similar to that of sweet cherry, around the Caspian Sea and close to Istanbul. While sour cherry is less widely cultivated than sweet cherry, large quantities of sour cherries are produced in many European countries and in the USA. Most of these are used in processing and processed cherry products are sold worldwide.

Prunus fruticosa Pall., the ground cherry tree, is sometimes used as rootstocks for other *Prunus* species. This species is widespread over the major part of central Europe, Siberia and Northern Asia (Hedrick, 1915).

The duke cherries, which result from crosses between *P. avium* and *P. cerasus*, are cultivated on a much smaller scale. Different names have been given to these interspecific hybrids are such as *Prunus acida* Dum, *Cerasus regalis*, *Prunus avium* ssp *regalis*, but the name used today is *P.* × *gondouinii* Rehd. (Faust and Suranyi, 1997; Saunier and Claverie, 2001). Duke cherry trees are intermediate for their tree and fruit characteristics compared to their progenitors.

1.1.1 Botanical Descriptions

All cherry species belong to the *Cerasus* subgenus of the *Prunus* genus, part of the Rosaceae family. The majority of cultivated cherry trees belong to *Prunus avium* L. and *Prunus cerasus* L. species. Together with *Prunus fruticosa* Pall., these species and their interspecific hybrids constitute the *Eucerasus* section of the *Cerasus* subgenus, based on morphological criteria (Krussmann, 1978; Rehder, 1947). This classification and the monophyletic origin of the *Eucerasus* clade have been confirmed by chloroplast DNA variation analysis (Badenes and Parfitt, 1995).

A large amount of morphological variation is observed among *P. avium*, *P. fruticosa* and *P. cerasus* species. Multivariate analysis on sour cherry revealed continuous variation between the *P. avium* and *P. fruticosa* traits throughout the geographic distribution of the species. In Western Europe, *P. cerasus* trees more closely resemble *P. avium* whereas in Eastern Europe, *P. cerasus* is closer to *P. fruticosa* (Hillig and Iezzoni, 1988; Krahl et al., 1991). This continuum of morphological characteristics makes species assignment difficult when considering only phenotypic traits. The sweet cherry is a deciduous tree of large stature, occasionally reaching almost 20 meters in height, with attractive peeling bark. The sour cherry is a small tree, or more often a deciduous bush, which suckers profusely from the base. It has smaller leaves and flowers than the sweet cherry. Sweet cherries are usually split into three groups on the basis of fruit characters: 1. Mazzards, often wild types with small inferior fruits of various shapes and colors, 2. Guignes, Hearts or Geans, with soft-

fleshed fruit, and 3. the Bigarreaux with hard-fleshed, heart-shaped, light-colored fruit. Sour cherry cultivars are generally classified as Amarelles (or Kentishand) or as Griottes (or Morellos). Amarelles have pale red fruits flattened at the ends and uncolored juice. Griottes have, in contrast, dark spherical fruits and dark-colored juice. A third group of sour cherry cultivars, called Marasca, are characterized by small, very black-red colored and bitter fruit whose juice is of the best quality for making maraschino liquor. Marasca cultivars are sufficiently distinct to have been classified by early botanists as a subspecies of *P. cerasus* (*Prunus cerasus* Marásca (Reichb.) Schneid, Rehder, 1947).

1.2 Genome Content

Prunus avium has a diploid genome (AA, 2n=2x=16) and small haploid genome size (338 Mb) (Arumuganathan and Earle, 1991), bigger than the genome of peach (290 Mb) which is the smallest *Prunus* genome evaluated to date. *Prunus fruticosa*, the ground cherry tree, is a tetraploid wild species (2n=4x=32) believed to be (FFFF). The genome size is still unknown.

Prunus cerasus is an allotetraploid species (AAFF, 2n=4x=32), with a genome size of 599 Mb, allegedly due to natural hybridization between *P. avium* (producing unreduced gametes) and *P. fruticosa* (Fig. 1). This origin was first suggested by Olden and Nybom (1968) who observed that artificial hybrids between tetraploid *P. avium* and *P. fruticosa* were very similar to *P. cerasus*. Isozyme analysis, genomic *in situ* hybridization and karyotype analysis further confirmed the hybrid origin of *P. cerasus* (Hancock and Iezzoni, 1988; Santi and Lemoine, 1990; Schuster and Schreiber, 2000). The patterns of inheritance of 7 isozymes in different crosses of sour cherry indicated that *P. cerasus* may be a segmental allopolyploid (Beaver and Iezzoni, 1993; Beaver et al., 1995). Studies based on cpDNA markers detected two distinct chlorotypes in *P. cerasus* which strongly suggest that crosses between *P. avium* and *P. fruticosa* have occurred at least twice to produce sour cherry (Badenes and Parfitt, 1995; Brettin et al., 2000; Iezzoni and Hancock, 1996). Moreover, these works showed that most of the time, *P. fruticosa* was the female progenitor of *P. cerasus,* but in few cases, *P. avium* was the female parent due to the formation of unreduced ovules. Tavaud et al. (2004) demonstrated that specific alleles in *P. cerasus* were not present in the A genome of *P. avium* and probably came from the F genome of *P. cerasus*. Recent analysis with cpDNA and microsatellite markers show that some *P. cerasus* share the same chloroplastic haplotype as some *P. fructicosa*, and that some microsatellite markers are share by both species (A. Horvath, personal communication). Triploid hybrids through the fusion of normal gametes of *P. avium* and *P. fruticosa* occur naturally but remain sterile. Due to this sterility and many unfavorable *P. fruticosa* traits, these triploids are not clonally propagated by humans (Olden and Nybom, 1968).

P. × *gondouinii* Rehd is an allotetraploid (AAAF, 2n=4x=32) species stemming from the pollinization of sour cherry by unreduced gametes of sweet cherry (Iezzoni et al., 1990). These hybrids are often sterile, due to disturbances during meiosis, but they are clonally propagated by human.

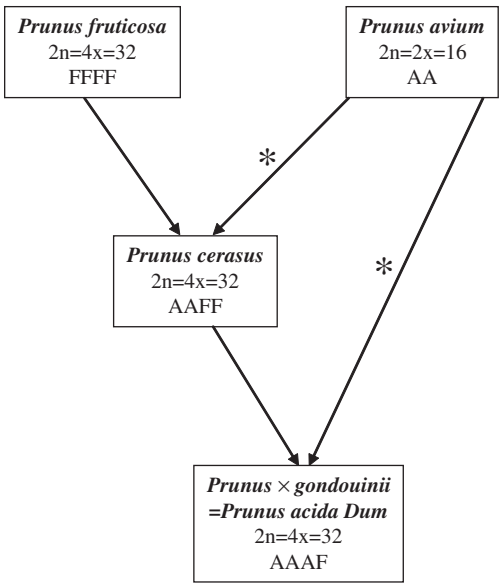


Fig. 1 Relationships and genome constitution among the species of the Eucerasus section

1.3 Economic Importance

Worldwide, 375,000 Ha of sweet cherry and 248,000 Ha of sour cherry are cultivated giving a total production of 1,896,000 Mt and 1,035,000 Mt respectively (FAO, 2005). The main production areas in the world for sweet and sour cherries are located in Europe (953,000 Mt and 711,000 Mt), Asia (653,000 Mt and 208,000 Mt) and North America (228,000 Mt for sweet cherry and 115,000 Mt for sour cherry) (FAO, 2005). However, a huge increase in sweet cherry production occurred 10 years ago in the Southern hemisphere, especially in Chile and Argentina. In Chile, the cultivated area increased by four times in two years and nearly all the production is exported to the USA and Europe. In the Northern hemisphere, sweet cherry production is mainly located in Europe but major shifts are occurring in European production. France was one of the main producers in Europe (100–120,000 Tonnes) but halved its production in 2003 and 2004 (57,000 Tonnes), and at the same time Spain doubled its production, especially with early maturing varieties. In the next following years, Turkey may become the leading world producer of sweet cherries.

1.4 Breeding Objectives

The main breeding objectives for sweet cherry are:

– large, attractive and good-flavored fruits,
– reduced juvenile phase,

- large and constant yields,
- reduced susceptibility to fruit cracking,
- self-compatibility,
- improved resistance or tolerance to diseases, especially bacterial canker induced by *Pseudomonas* mors pv. *prunorum* and *P. syringae*.

Regular yields and superior fruit quality are the two main objectives of sour cherry breeding programs. Breeding for disease resistance in sour cherry is concentrated on resistance to cherry leaf spot caused by *Blumeriella japii.* When not properly controlled, CLS can cause leaf chlorosis and premature defoliation resulting in fruit that is poorly colored, low in soluble solids and softer than fruit on healthy trees (Keitt et al., 1937). Early defoliation can also result in reduced winter hardiness, potentially leading to flower bud loss and tree death (Howell and Stackhouse, 1973).

Yields per hectare vary by the country of production, the commercial use (for fresh market or for industry) and the training system. The average yield ranges from 8 to 10 T/Ha in classical orchards but can reach 30–40 T/ha for an intensive industrial orchard. The highest limitation to the development of cherry culture is the high cost required to manually pick the fruit, as manual picking may account for 70% of the production price. This has led to the selection in some breeding programs of new varieties that can be harvested partially with machines, such as 'Sweetheart' and 'Van' that can be harvested without the stem. At the same time, a better knowledge of the architecture of the tree has led to new approaches to orchard training.

Because of the efforts of classical breeding programs, a large number of cultivars are now available. Within the last 10 years, 20 new varieties have gained wide interest internationally such as 'Earlise' (early season), 'Summit' (middle season) and 'Sweetheart' (late season). Each of these should be widely cultivated in the next 15–20 years.

Classical breeding programs are time consuming because cherry trees take a minimum of 3–5 years of growth before they are capable of flowering and fruit production. Prior knowledge of linkage relationships between marker loci and important flower and fruit characteristics will facilitate and shorten the selection of promising individuals. Consequently, introduction of marker-assisted selection will be especially beneficial for sweet and sour cherry breeding.

2 Construction of Genetic Maps

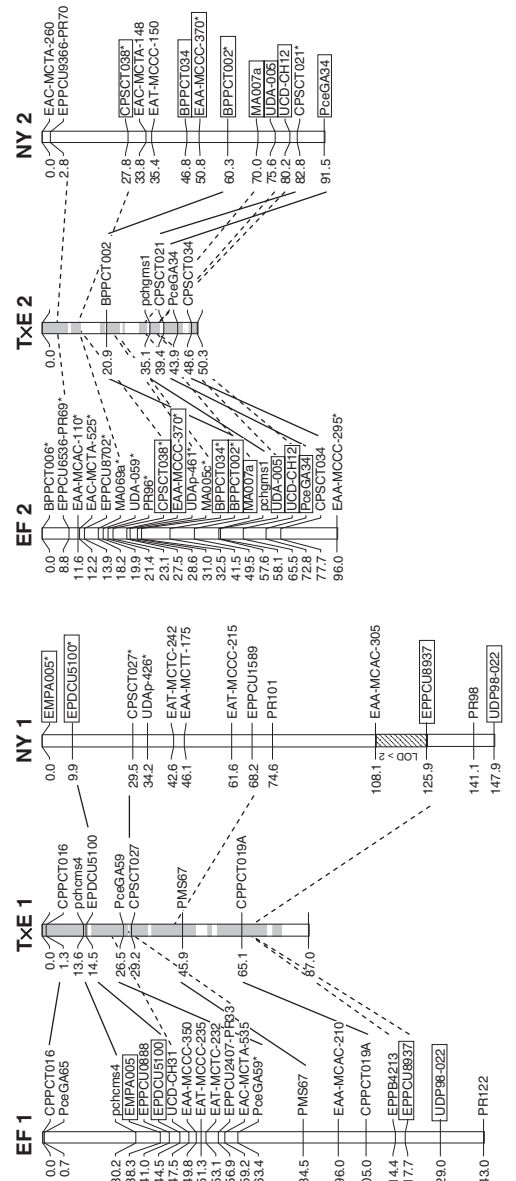
The construction of genetic maps is useful for localisation of important genes controlling both qualitative and quantitative traits in numerous plant species and, then, for improving and shortening breeding selection (Tanksley et al., 1989). In the subgenus *Cerasus*, several maps have been published using five segregating populations. Until recently, only partial maps for sweet or sour cherry were available. The earliest of them was constructed in a sweet cherry using random amplified polymor-

phic DNA (RAPD) and allozyme analysis of 56 microspore-derived callus culture individuals of the cv. 'Emperor Francis' (Stockinger et al., 1996). Two allozymes and 89 RAPD markers were mapped to 10 linkage groups totalling 503 cM. Interestingly, another map integrating isozyme genes exclusively, was obtained using data from two inter-specific F_1 cherry progenies: *P. avium* 'Emperor Francis' × *P. incisa* E621 and *P. avium* 'Emperor Francis' × *P. nipponica* F1292 (Bošković and Tobutt, 1998). This map, one of the most exhaustive ever made with isozyme markers in plants, included a total of 47 segregating isozymes, of which 34 were aligned into seven linkage groups. The East Malling group has continued this research with the construction of an inter-specific cherry map from the cross *P. avium* ' Napoleon' × *P. nipponica* using microsatellite and gene-specific markers (Clark et al., 2009).

Another genetic linkage map is in progress for sweet cherry using an intra-specific F_1 progeny including 133 individuals from a cross between cultivars 'Regina' and 'Lapins' in INRA at Bordeaux (France). These cultivars were chosen as parents for their distinct agronomic characters and especially because they differ for resistance to fruit cracking which is a limiting factor in sweet cherry production ('Regina' is resistant and 'Lapins' is susceptible.) 'Lapins' is a self-compatible cultivar as opposed to 'Regina'. Moreover, they differ for several other characters: blooming and maturity dates, peduncle length, and fruit color, weight, firmness, titratable acidity and refractive index. Preliminary maps of each parent and their comparison with the reference *Prunus* map 'Texas' × 'Earlygold' (T×E) is described in Dirlewanger et al. (2004b). These maps include microsatellite markers, 30 of which are located in the 'Régina' map are anchors marker with T×E map, 28 located in the 'Lapins' map as anchor markers with the T×E map. Only one non-collinear marker was detected, but for all other markers the location was in the homologous linkage group. These results are in agreement with the high level of synteny within the *Prunus* genus (Arús et al. 2006).

An intra-specific sweet cherry genetic linkage map was also constructed at Michigan State University (US) from a F_1 progeny from a cross between a wild forest cherry with small (∼2 g) highly acid dark-red colored fruit (NY54) and a domesticated variety with large (∼6 g), yellow/ pink, sub-acid fruit 'Emperor Francis' (EF) (Olmstead et al., 2007, 2008). The 'EF' and 'NY' maps were 711.1 cM and 565.8 cM, respectively, with the average distance between markers of 4.94 and 6.22 cM (Fig. 2). A total of 82 shared markers between the 'EF' and 'NY' maps and the *Prunus* reference map supported previous findings that the cherry genome is collinear with other *Prunus* genomes. The F_1 population is composed of approximately 600 individuals, including 190 that were used for map construction and initial QTL analysis. The remaining progeny will be used for fine mapping of major QTLs. The objective of the study is to identify QTLs that control the fruit quality traits improved during domestication. In addition, this cross is fully compatible and progeny segregation for the *S*-locus fits the expected 1:1:1:1 ratio (Ikeda et al., 2005).

In sour cherry, linkage maps were constructed at Michigan State University (US) from 86 individuals from the cross of two cultivars, 'Rheinische Schattenmorelle' (RS) and 'Erdi Botermo' (EB). Since sour cherry is a tetraploid, informative restriction fragment length polymorphisms (RFLPs) were scored as single-dose restriction



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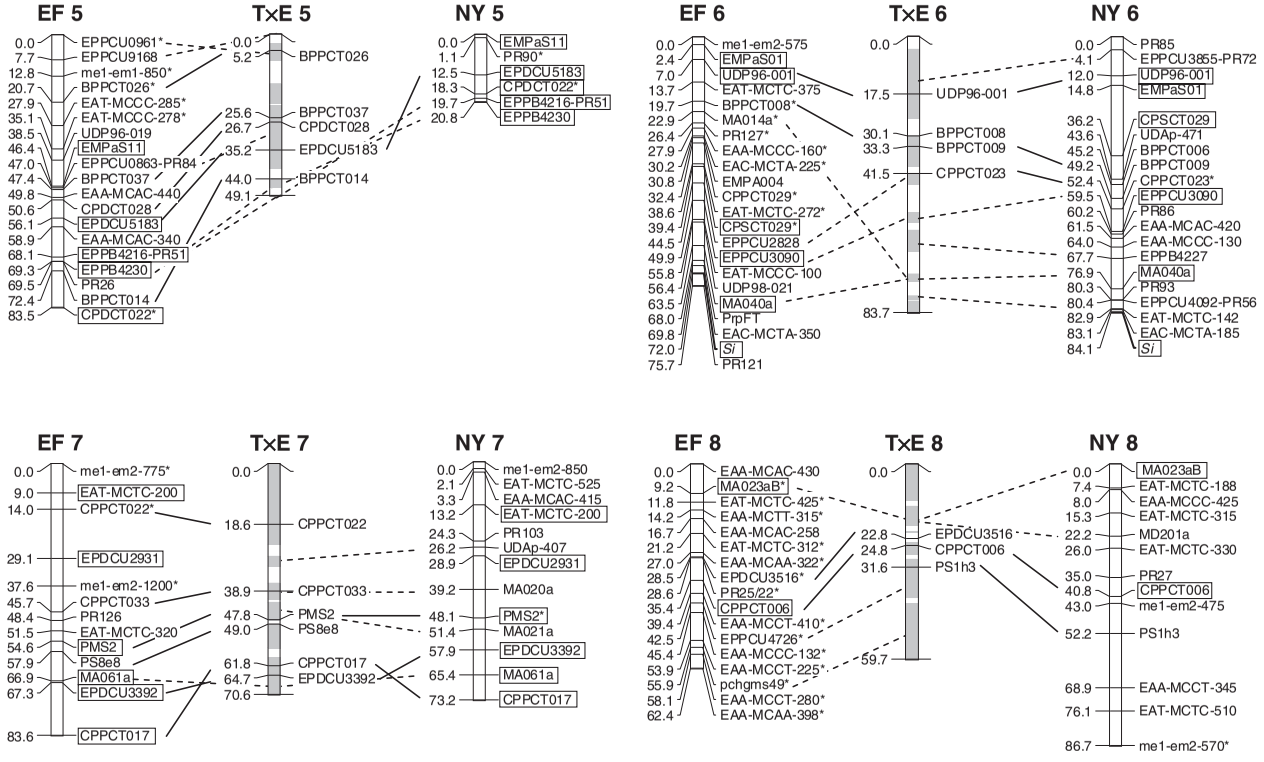


Fig. 2 (continued) Alignment of 'Emperor Francis' (EF) and 'New York 54' (NY) sweet cherry parental maps with the *Prunus* reference and bin map ['Texas' almond × 'Earlygold' peach (T×E)] (Olmstead et al., 2008). Only common markers present on EF and NY maps are presented on the *Prunus* reference map. Shaded areas on the reference map linkage groups indicate bin locations. Solid lines indicate homology between mapped markers; dashed lines indicate markers present in *Prunus* bins. Boxed markers indicate anchor points between the EF and NY parental maps. Markers followed by an asterisk (*) indicate significant deviation ($P<0.05$) from the expected chi-square segregation ratio

fragments (SDRF) according to Wu et al. (1992). A genetic linkage map was constructed for RS that consists of 126 SDRF markers assigned to 19 linkage groups covering 461 cM (Wang et al., 1998). The EB linkage map had 95 SDRF markers assigned to 16 linkage groups covering 279 cM (Wang et al., 1998). Due to the limited number of shared markers between the RS × EB map compared to other *Prunus* maps, putative homologous linkage groups could only be identified in for the *Prunus* LGs 2, 4, 6, and 7. The other linkage groups were arbitrarily numbered from the longest to shortest and therefore the sour cherry linkage groups numbers have not been rigorously aligned with that of the *Prunus* consensus map. The RS × EB population was subsequently screened using 10 *Prunus* microsatellite primer pairs (Canli, 2004a) and a consensus map of 442 cM, less than the previously reported RS map of 461 cM, was constructed. A total of 16 microsatellite markers were added to 10 of the 19 linkage groups; however, the linkage groups were not re-numbered to reflect these markers. In addition, four of the microsatellite primer pairs identified duplicate linked markers. This 'double mapping' of a marker is due to the inclusion of progeny individuals exhibiting tetrasomic inheritance for that linkage group. If this correction had been done by Canli (2004a), it is likely that the number of microsatellite markers added to the map would be reduced to twelve.

The difficulty of identifying SDRFs and eliminating progeny that resulted from non-homologous pairing for the linkage group under study, illustrate the complexity of linkage mapping in a segmental allopolyploid. Hence, future work at Michigan State University will concentrate on linkage map construction in the diploid sweet cherry.

3 Gene Mapping and QTLs Detected

In sour or sweet cherries most of the agronomically important traits have complex inheritance. Only self-incompatibility (SI) in diploid sweet cherry is controlled by a single locus (*S*) with multiple alleles, and fertilization only takes place when the *S* allele in the haploid genome of the pollen is different from the two *S* alleles in the diploid tissue of the style. In contrast, blooming and ripening time, flower bud and pistil death and characters controlling fruit quality are quantitative traits. The self-incompatibility locus is located in the distal part of linkage group 6 in almond (Ballester et al., 1998; Bliss et al., 2002), apricot (Vilanova et al., 2003), and cherry (Olmstead et al., 2008).

Although in peach many major genes (Fig. 3) and QTLs involved in fruit quality (Dirlewanger et al., 1999; Etienne et al., 2002; Quilot et al., 2004) and diseases resistance (Quarta et al., 1998; Viruel et al., 1998; Foulongne et al., 2003) have been reported, the only QTL study published to date in cherry is a QTL analysis of flower and fruit traits using the sour cherry RS × EB linkage mapping population (Wang et al., 1998). Eleven QTLs (LOD>2.4) were identified for six traits (bloom time, ripening time, percent pistil death, percent pollen germination, fruit weight, and soluble solids concentration) (Wang et al., 2000, Fig 4). The percentage of phenotypic

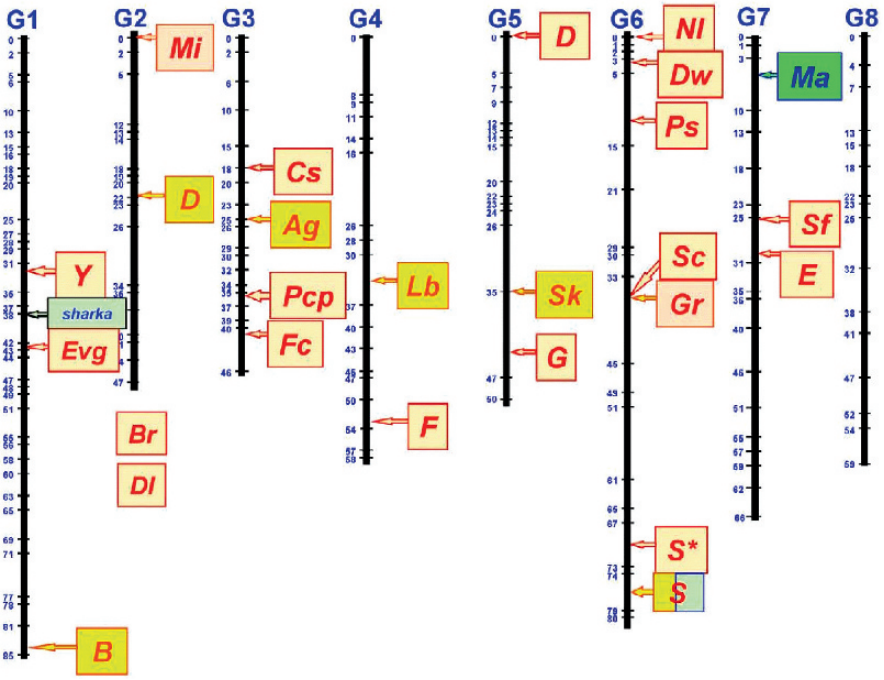
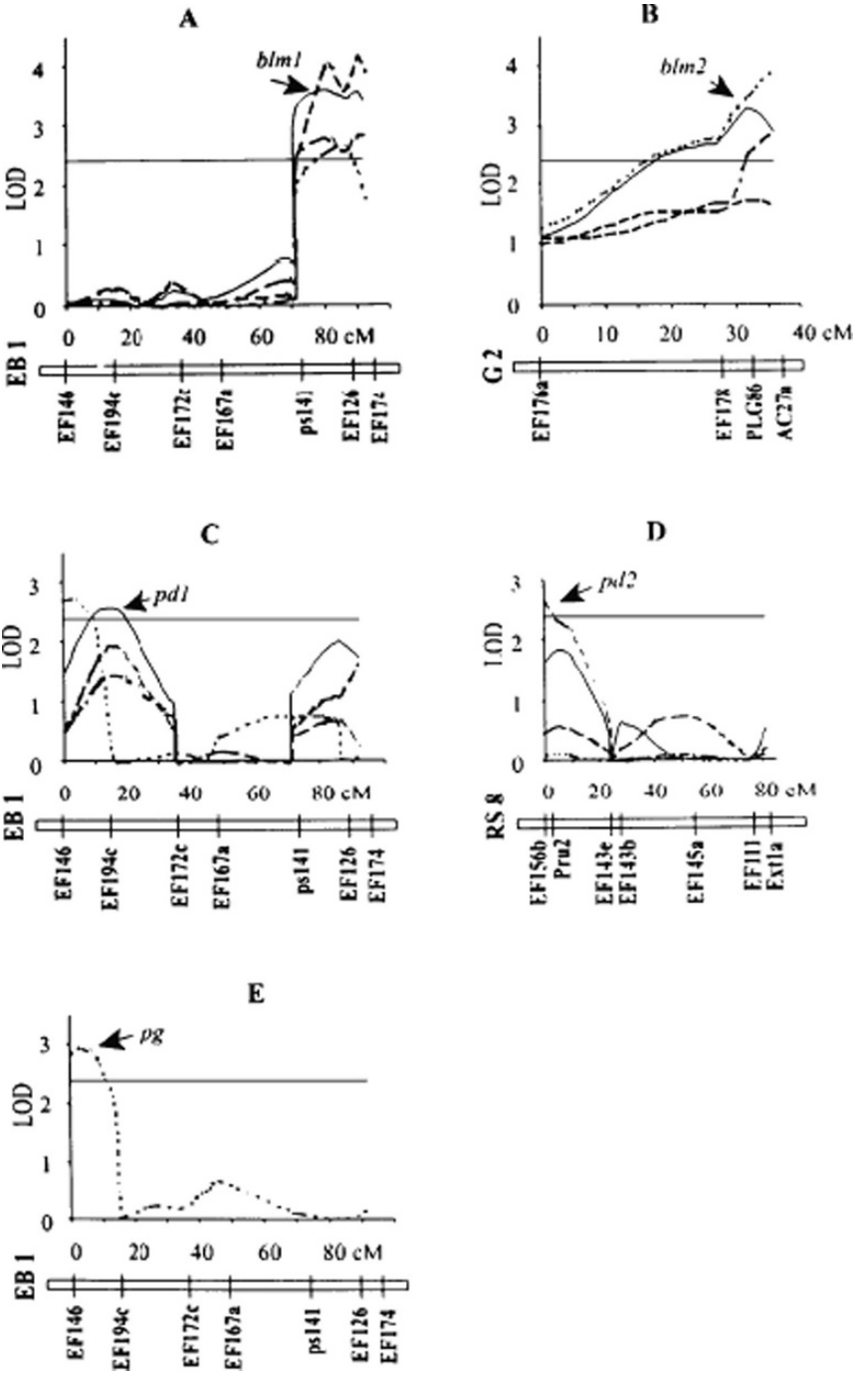


Fig. 3 Approximate position of 28 major genes mapped in different populations of apricot (*blue background*), peach (*orange background*), almond or almond × peach (*yellow background*), and Myrobalan plum (green background) on the framework of the *Prunus* reference map (Dirlewanger et al., 2004b). Gene abbreviations correspond to: *Y*, peach flesh color; *B*, almond/peach petal color; *sharka*, plum pox virus resistance; *B*, flower color in almond × peach; *Mi*, nematode resistance from peach; *D*, almond shell hardness; *Br*, broomy plant habit; *Dl*, double flower; *Cs*, flesh color around the stone; *Ag*, anther color; *Pcp*, polycarpel; *Fc*, flower color; *Lb*, blooming date; *F*, flesh adherence to stone; *D*, non-acid fruit in peach, *Sk*, bitter kernel; *G*, fruit skin pubescence; *Nl*, leaf shape; *Dw*, dwarf plant; *Ps*, male sterility; *Sc*, fruit skin color; *Gr*, leaf color; *S**, fruit shape; *S*, self-incompatibility (almond and apricot); *Ma*, nematode resistance from Myrobalan plum; *E*, leaf gland shape; *Sf*, resistance to powdery mildew. Genes *Dl* and *Br* are located on an unknown position of G2

variation explained by a single QTL ranged from 12.9 to 25.9% (Wang et al., 2000). Subsequently, three microsatellite markers were identified that mapped within the putative location of the previously described QTLs (Wang et al., 2000) for bloom time (*blm2*), pistil death (*pd1*) and fruit weight (*fw2*), respectively (Canli, 2004a). Unfortunately these three microsatellite markers were not used in QTL analyses to determine their location relative to the previously published QTLs.

The identification of bloom time QTL is of particular interest for cherry breeding as the development of new cultivars with late bloom would significantly reduce the probability of spring freeze damage to the pistils (Iezzoni, 1996). Sour cherry exhibits extreme diversity for bloom time with many cultivars blooming exceedingly late in the spring (Iezzoni and Mulinix, 1992). This late bloom character in sour



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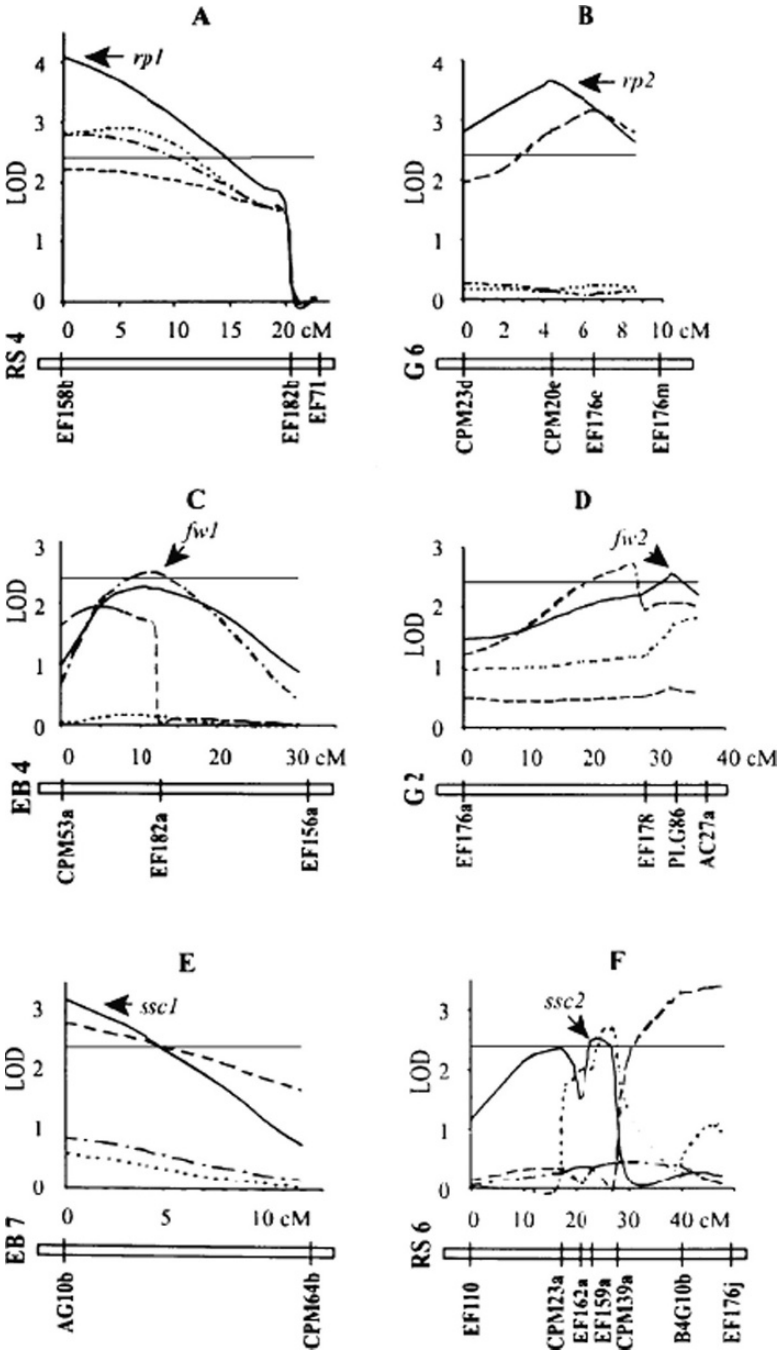

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cherry is likely due to the hybridization and continued introgression with the very late blooming ground cherry, *P. fruticosa*.

Bloom time in cherry is a quantitative trait; however its high broad sense heritability (0.91) led to the identification of two bloom time QTL, *blm*1 and *blm*2, in the 'RS' × 'EB' population (Wang et al., 2000). Unfortunately the genetic effects of these two QTL alleles from 'EB' were to induce early bloom. To identify QTL with alleles conferring late bloom time, a second mapping population between the mid-season blooming 'Ujfehertoi Furtos' and late blooming 'Surefire' has been developed at Michigan State University (US). The population exhibited transgressive segregation for bloom time permitting a bulk segregant approach to identify markers linked to bloom time QTL (Bond, 2004). To date, a third QTL for late bloom, named *blm*3, was identified using AFLP markers that is significantly associated with late bloom using an ANOVA. This QTL allele is present in 'Surefire' and confers late bloom time. Ongoing work attempts to determine the linkage map location of this QTL. Using this same mapping population, two AFLP markers were identified that differed between the early and late bulks (Canli, 2004b). However these markers were never screened over the 'Ujfehertoi Furtos' × 'Surefire' progeny population and the marker results described could not be repeated.

4 Analysis of Self Incompatibility

Sweet cherry, like other Rosaceae species, exhibits a strict self-incompatibility system that has been naturally selected to promote outbreeding (De Nettancourt, 2001). This mechanism disallows the fertilization of flowers of one genotype by its own pollen. As a consequence, commercial fruit set in this species depends upon the presence of other compatible pollinating genotypes or on the utilization of self-compatible cultivars. In sour cherry, self-incompatible as well as self-compatible genotypes have been identified (Lansari and Iezzoni, 1990; Yamane et al., 2001; Hauck et al., 2002). Sour cherry is a tetraploid hybrid of diploid sweet cherry and tetraploid ground cherry and the self-incompatibility mechanism seems to be conserved only in some genotypes.

Fig. 4 (continued) (*Below*) QTLs detected for flower and fruit traits in sour cherry (Wang et al., 2000). LOD scores for bloom date on linkage groups EB 1 (*blm*1) (**A**) and Group 2 (*blm*2) (**B**); pistil death (*pd*) on linkage groups EB 1 (**C**) and RS 8 (**D**); pollen germination percentage (*pg*) on linkage group EB 1 (**E**). Peak LOD scores for each trait are indicated by *arrows*. Linkage groups are shown below the *x-axes*. The *horizontal line* indicates the level of significance at LOD=2.4. *Curves* represent individual years of 1995 (– – –), 1996 (- - - -), 1997 (— - —) and over years (———). LOD scores for ripening date on linkage groups RS 4 (*rp1*) (**A**) and Group 6 (*rp2*) (**B**); fruit weight on linkage groups EB 4 (*fw1*) (**C**) and Group 2 (*fw2*) (**D**); soluble solids concentration on linkage groups EB 7 (*ssc1*) (**E**) and RS 6 (*ssc2*) (**F**)

The type of self-incompatibility operating in the Rosaceae is called gametophytic self-incompatibility (GSI) (De Nettancourt, 2001), and it is shared by other plant families like the Solanaceae and Plantaginaceae. The gametophytic self-incompatibility is controlled by different genes of one polymorphic locus (*S*) that determine the incompatibility response of the pollen and the style (McCubbin and Kao, 2000). In cherries the incompatibility phenotype of the style is determined by a ribonuclease called *S*-RNase (Bošković and Tobutt, 1996; Tao et al., 1999c; Yamane et al., 2001) and the specificity of the pollen is determined by the product of the F-box gene *SFB* (Yamane et al., 2003; Ushijima et al., 2004; Ikeda et al., 2004a). Together the RNAse and SFB protein would interact in an allele specific manner to confer the self-incompatibility reaction. The mechanism of this reaction is such that the growth of the pollen tube is inhibited in the style when the *S*-allele of the pollen factor matches either of the two *S*-alleles of the *S*-RNases expressed in the diploid style tissue. Several models have been proposed to explain how these factors mediate the incompatibility reaction of the *S*-RNase-based self-incompatibility (Luu et al., 2001, Kao and Tsukamoto, 2004; Ushijima et al., 2004; Goldraij et al. 2006; McClure 2006; Hua et al. 2008).

Like sweet cherry, sour cherry exhibits an *S*-RNase based GSI system (Yamane et al., 2001; Hauck et al., 2002; Tobutt et al., 2004; Bošković et al., 2006); however, natural sour cherry selections include both self-incompatible (SI) and self-compatible (SC) types (Redalen, 1984; Lansari and Iezzoni 1990). This genotype-dependent loss of self-incompatibility in sour cherry indicates that genetic changes, not polyploidy per se, cause the breakdown of SI. Instead the genetic control of SI and SC in sour cherry has been shown to be regulated by the accumulation of non-functional *S*-haplotypes according to the 'one-allele-match model' (Hauck et al., 2006b). In this model, the match between a functional pollen-*S* gene produced by the 2*x* pollen and its cognate functional *S*-RNase in the style results in an incompatible reaction. A similar reaction occurs regardless of whether the pollen contained a single functional pollen-*S* gene or two different pollen-*S* genes. The absense of a functional match results in a compatible reaction. Thus for successful fertilization, 2*x* sour cherry pollen must contain two non-functional *S*-haplotypes.

The progress made in the knowledge of the genetic and molecular basis of the self-incompatibility reaction has allowed the application of molecular techniques for two main aspects of sweet cherry breeding, the identification of cross-compatible combinations of different varieties by the identification their S-alleles and the selection of self-compatibility.

4.1 S-Allele Typing

Self-incompatibility in sweet cherry prevents inbreeding but the same mechanism also prevents cross-pollination among varieties with the same *S* alleles. This means that it is necessary to know the *S* haplotypes of each variety to be able to establish which cultivar combinations are compatible and, thus, to select which varieties can

be inter-planted. Varieties that have the same incompatibility alleles and are therefore cross-incompatible, form incompatibility groups. Until the molecular basis of self-incompatibility was characterized, *S* allele typing and incompatibility group assignment was carried out by controlled pollinations followed by recording fruit set (Crane and Brown, 1937; Matthews and Dow, 1969) or by the observance of pollen tube growth in the style by fluorescent microscopy. Since the style *S* factor in GSI was known to be a ribonuclease in Solanaceae (McClure et al., 1989), it was possible to identify *S* alleles in sweet cherry by correlating known *S* alleles with bands obtained from stylar proteins separated by isoelectric focusing and stained for ribonuclease activity (Bošković and Tobutt, 1996). Subsequently this biochemical assay would provide evidence that correlated well with the new incompatibility alleles (Bošković et al., 1997).

The cloning and sequence characterization of the *S-RNases* of sweet cherry (Tao et al., 1999a, b) allowed the development of PCR and RFLP based methods of typing cherry *S*-alleles. Tao et al. (1999c) developed an *S*-allele typing method based in the utilization of two pairs of PCR primers, designed in the conserved regions of the sweet cherry *S-RNase* sequences. These *S-RNase* sequences have two introns varying in length for each different allele and, consequently, PCR amplification with those primers enables differentiation of the different *S*-alleles according to the size of the amplified fragments. Subsequently, other sweet cherry *S*-RNases were cloned and other PCR methods based in conserved sequence primers (Wiersma et al., 2001), allele specific primers (Sonneveld et al., 2001, 2003, 2006) or PCR followed by restriction fragment analysis (Yamane et al., 2000b) have been developed. RFLP profiles have also been used to assign self-incompatibility alleles to different sweet cherry genotypes (Hauck et al., 2001). The identification of the pollen-*S* (SFB) in sweet cherry (Yamane et al., 2003), has also been followed by the cloning and characterization of different cherry *SFB* alleles (Ikeda et al., 2004a; Vaughan et al., 2006; Yamane et al., 2003). The knowledge of the sequence and structure of these alleles has allowed the development of new *S*-allele PCR typing methods based in allele specific primer sets (Ikeda et al., 2005), in sequence conserved primers that distinguish *SFB* alleles by size polymorphisms (Vaughan et al. 2006), and dot-blot analysis using *SFB* sequence polymorphism (Kitashiba et al. 2008). The introduction of molecular methods in sweet cherry *S*-allele typing has allowed a rapid confirmation of the *S*-alleles and incompatibility groups of different cultivars reported previously, the identification of the *S*-genotype of new varieties and the identification of putative new *S* alleles by their correlation with new PCR products (Table 2, Tao et al., 1999c; Yamane et al., 2000a, b; Hauck et al., 2001; Sonneveld et al. 2001; Wiersma et al., 2001; Choi et al., 2002; Zhou et al., 2002; Sonneveld et al., 2003; Wunsch and Hormaza, 2004d; De Cuyper et al., 2005). S-allele typing has also become a useful tool for genetic studies of germplasm collections (Wünsch and Hormaza, 2004c; Marchese et al., 2007a; Schuster et al., 2007; Gisbert et al., 2008) and wild cherry populations (De Cuyper et al., 2005; Schueler et al. 2006).

To date, 31 functional *S*-haplotypes have been characterized in cherry, and due to overlapping studies and the use of different techniques, synonymous alleles have

been subsequently detected and in some cases the number labeling does not follow a chronological order. These *S*-alleles are numbered $S_1 - S_7$, $S_9 - S_{10}$, $S_{12} - S_{14}$, S_{16}, as S_8, S_{11}, and S_{15}, appear to be synonyms of S_3, S_7 and S_5, respectively (Sonneveld et al., 2001, 2003). Three additional alleles, S_{23}, S_{24} and S_{25}, were later characterized from Italian and Spanish cultivars (Wünsch and Hormaza, 2004a). Of these; S_{23} seems to be synonymous to S_{14} (Sonneveld et al. 2003; Vaughan et al., 2008). In wild sweet cherry populations six additional alleles, S_{17} to S_{22} were characterized (De Cuyper et al., 2005), and according to Vaughan et al. (2008) S_{21} seems to be synonymous of S_{25} (Wünsch and Hormaza, 2004a). Allele S_{26} was reported in sour cherry (Hauck et al., 2006b), $S_{27} - S_{32}$ were described in wild sweet cherry (Vaughan et al., 2008), and finally, S_{33} to S_{36} were described in sour cherry (Tsukamoto et al., 2008).

4.2 Breeding for Self-Compatibility

The use of self-compatible varieties in sweet cherry orchards can limit some of the problems incurred from self-incompatibility, such as the cost derived from the need to use pollinator varieties and losses from erratic production (Tehrani and Brown, 1992). As a consequence, obtaining and introducing self-compatible varieties has been one of the main objectives of sweet cherry breeding (Brown et al., 1996). Self-compatibility was induced in sweet cherry by irradiation, giving rise to several self-compatible seedlings (Lewis, 1949). 'Stella' (Lapins, 1970), a descendent of one of these seedlings (JI2420), is self-compatible and has been widely used as a progenitor in self-compatible sweet cherry breeding. Most of the self-compatible varieties currently used derive from 'Stella'. Self-compatibility in these genotypes is caused by a pollen function mutation in the S_4' allele (S_4' standing for mutated S_4 allele), (Bošković et al., 2000). To carry on selection of self-compatible seedlings derived from these genotypes it is necessary to differentiate the genotypes that inherited the S_4' allele. However, since the S_4-RNase in these genotypes is intact, it was not possible to differentiate genotypes with the S_4' mutant allele from genotypes with a functional S_4 allele, using *S*-allele typing methods based on *S*-RNase sequence allele diversity. It was not until the finding of the pollen determinant (SFB) of GSI in *Prunus* (Yamane et al., 2003: Ushijima et al. 2004) that has been possible to establish a method that allows the identification of genotypes carrying the mutated S_4' allele (Ikeda et al., 2004b). This method is based in the identification of a 4 bp deletion in the *SFB* sequence of the S_4' allele when compared with the normal S_4 allele. This deletion has been used to design molecular markers that identify the S_4' allele by PCR followed by polyacrylamide gel electrophoresis or restriction digestion (Ikeda et al. 2004b).

Additional sources of self compatibility, that can broaden the genetic base of cultivated germplasm and that can also be highly useful to understand the mechanism of GSI, are also being studied. Sonneveld et al. (2005) carried out molecular and genetic analysis of the two self-compatible accessions obtained by the radiation of pollen at the John Innes Institute, JI 2420 and JI 2434 (Lewis and Crowe,

1954). As determined by Ushijima et al. (2004), a 4 bp deletion was identified in *S4'-SFB* of JI 2420. On the other side, *S3'-SFB* (*S3'* standing for mutated *S3* haplotype) of accession JI 2434 appeared to be deleted (Sonneveld et al. 2005). Self-compatible progeny derived from JI 2434 can now be selected by detecting this *SFB* deletion through RFLP or PCR analysis of S_3-*SFB* (Sonneveld et al. 2005). Self-compatibility in the Spanish landrace 'Cristobalina' is also being investigated to identify markers that facilitate the introgression of this trait, as analysis in this genotype have shown that self-compatibility is not associated with the *S*-locus (Wünsch and Hormaza, 2004b). On the other hand, self-compatibility in the Sicilian sweet cherry 'Kronio' has been attributed to a pollen part mutation in S_5-*SFB* (thus called $S_{5'}$) caused by a premature stop codon that results in a truncated protein (Marchese et al., 2007b). The presence of a polymorphic microsatellite in the *S-RNase* intron of S_5 and S_5' has allowed developing a marker to identify self-compatible genotypes carrying $S_{5'}$ (Marchese et al., 2007b).

Sour cherry selections that have two non-functional *S*-haplotypes are SC (Hauck et al., 2006b). These non-functional *S*-haplotypes can results from the loss of pollen function (termed pollen-part mutants) or loss of stylar function (termed stylar-part mutants), or both (Tsukamoto et al., 2006). Three of the *S*-haplotypes prevalent in sweet cherry (S_1, S_6 and S_{13}) have been shown to also have non-functional variants in sour cherry that have lost pollen or stylar function (Hauck et al., 2006a: Tsukamoto et al., 2006). Loss of function was due to structural alternations of the *S-RNase*, *SFB* or *S-RNase* upstream sequences.

5 Conclusion and Future Scope of Work

5.1 Genome Mapping and QTL Detection

Genetic mapping and QTL detection will continue, especially in sweet cherry. Since sweet cherry is diploid, it is much easier to develop linkage maps when compared with sour cherry which is tetraploid with an in-complete disomic inheritance, and occasional intergenomic pairing and pre-or post zygotic selection. Because of the high level of synteny demonstrated within *Prunus*, results obtained in sweet cherry will be useful for sour cherry. For the same reason we can expect that cherry will take benefit of knowledge developed in other members of the Rosaceae family. The enormous progress made during the last decade on genetic characterization of the cultivated species of the Rosaceae, and particularly of peach as its more logical model, can be exploited for cherry.

5.2 Self-(in)Compatibility: Molecular Cloning and MAS

Progress in the understanding of the RNase-based self-incompatibility, has allowed the development of molecular methods that accelerate two relevant aspects of cherry breeding: Incompatibility Group assignment through *S*-allele genotyping, and

introgression of self-compatibility through marker assisted selection. At the same time, research in sour and sweet cherry self-incompatibility and self-compatible mutants is greatly contributing to the knowledge of the mechanism operating in the self-incompatibility reaction in the genus *Prunus*. A better understanding of the self-incompatibility reaction from future progress in Rosaceae GSI research, together with the increasing availability of genetic tools in cherry species will provide an appropriate ground for a more efficient cherry improvement.

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