8 Strawberry

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8.1 Introduction

The genome composition of the octoploid ($2n = 8x = 56$), cultivated strawberry, *Fragaria* ×*ananassa*, is among the most complex of any crop species. The most recently proposed genome composition model for the octoploid *Fragaria* species, AAA′A′BBB′B′ (Bringhurst 1990), implies the presence of up to four distinct subgenomes (A, A′, B, and B′). The genomic complexity of the octoploid species has prompted attention to diploid relatives, such as *Fragaria vesca* ($2n = 2x = 14$), as model systems for strawberry genetics and genomics (Davis and Yu 1997; Sargent et al. 2004). Previous reviews have summarized the history of strawberry breeding and genetics (Darrow 1966; Galletta and Maas 1990; Hancock 1999) and initial developments in strawberry biotechnology (Hokanson and Maas 2001). This review updates the state of progress in the genetic and genomic characterization and manipulation of the cultivated strawberry and its respective diploid model system.

8.1.1 Origin of the Cultivated Strawberry

The genus *Fragaria* is comprised of 23 species variously distributed throughout the northern hemisphere and also extending southward along the western coast of South America and to Hawaii. Historically, several *Fragaria* species and novel hybrids have been brought into cultivation in various parts of the world, including *F. chiloensis* in South America, and *F. moschata* and *F. vesca* in Europe (Darrow 1966; Hancock 1999). However, the current economic significance of all other *Fragaria* species combined is insignificant compared to that of *F.* ×*ananassa*. Therefore, use of the term "cultivated strawberry" in this review will refer specifically to *F.* ×*ananassa* unless otherwise qualified.

The origin of the cultivated strawberry traces to the 1700s, when representatives of the octoploids *F. chiloensis* and *F. virginiana* – previously brought to Europe from South and North America, respectively – were grown in proximity in European horticultural gardens. Cross-pollination produced hybrids that were quickly recognized for their unique and desirable combinations of morphological and fruit characteristics, and were brought into cultivation and breeding (Hancock 1999). This recent origin makes *F.* ×*ananassa* one of the youngest of contemporary crop species. The cultivated species' immediate octoploid progenitors, *F. chiloensis* and *F. virginiana*, may have arisen from a common octoploid ancestor (Potter et al. 2000); however, no lineage has been established connecting the octoploids to any lower ploidy level. Several diploid species have been suggested as possible ancestors of the octoploid species, including *F. vesca*, *F. iinumae*, *F. daltoniana*, and others (Hancock 1999). The need for a comprehensive phylogenetic treatment of *Fragaria* that identifies the diploid ancestors of the cultivated species is becoming increasingly acute, as researchers begin to develop *F. vesca* and perhaps other diploid species as model systems for strawberry genetics and genomics.

8.1.2 Systematics and Phylogenetics

The strawberry genus, *Fragaria,* belongs to the family Rosaceae, subfamily Rosoideae. *Fragaria* has been represented in two molecular phylogenetic studies of the Rosaceae family (Morgan et al. 1994; Eriksson et al. 1998), but these broad studies included only one or two *Fragaria* species, respectively, and provided no insight into species relationships within *Fra-*

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garia. The monophyly of *Fragaria* is considered to be well-supported (Potter et al. 2000), but species relationships within *Fragaria* have not been adequately delineated. Molecular phylogenetic resolution within *Fragaria* has been limited, in part, by the low levels of variability detected in the nuclear ITS (internal transcribed spacer of rDNA) and chloroplast DNA (cpDNA) sequences that have been used for phylogenetic analysis (Harrison et al. 1997; Potter et al. 2000).

The Harrison and Potter studies provide an informative foundation for further, more detailed investigations of *Fragaria* phylogenetics. Significantly, the Potter study draws attention to *F. vesca*, *F. nubicola*, and *F. orientalis*, as possible progenitors to the octoploids. However, neither study discerned the reticulate phylogenetic history expected for the octoploids, or for hexaploid *F. moschata*. Of course, uniparentally inherited cpDNA sequence alone cannot provide evidence of phylogenetic reticulation. However, study of the biparentally inherited, nuclear ITS sequence has also failed to provide evidence of reticulate evolution in the *Fragaria* octoploids. Although ITS has been widely used for phylogenetic analysis at the specific and generic level in angiosperms, concerted evolution can homogenize ITS regions in allopolyploids, potentially erasing the contribution(s) of all but one diploid progenitor (Wendel et al. 1995). Perhaps this has been the case for the ITS region in *Fragaria* octoploids.

8.1.3 Karyotype

The basic chromosome number in *Fragaria* is $x = 7$ (Ichijima 1926). The recognized *Fragaria* species comprise a polyploid series, including twelve diploid ($2n = 2x = 14$) species, four tetraploids ($2n = 4x = 28$), one hexaploid ($2n = 6x = 42$: *F. moschata*), and four octoploids ($2n = 8x = 56$). Synthetic octoploids have been constructed via controlled, interspecific hybridizations accompanied by chromosome doubling, in an effort to broaden the octoploid gene pool available to strawberry breeders (Evans 1977; Bors 2000). The decaploid level has been obtained in controlled crosses accompanied by chromosome number manipulation (Scott 1951; Ahmadi and Bringhurst 1992). Decaploids referred to as *Fragaria* ×*vescana* were derived from crossing *F.* ×*ananassa* ($2n = 56$) with tetraploid forms of *F. vesca* var. *semperflorens* ($2n = 28$) followed by backcrossing to *F.* ×*ananassa* (Bauer 1993). Other decaploids have been derived from crosses involving octoploid cultivars and diploids *F. vesca* or *F. nilgerrensis* (Mochizuki et al. 2002). Many other natural or synthetic hybrids of various even and odd ploidy levels have been described (Darrow 1966; Bringhurst and Gill 1970; Staudt 1999; Staudt et al. 2003), reflecting the broad potential for interspecific hybridization both within and between ploidy levels in *Fragaria*.

Chromosomes are quite small in all *Fragaria* species, and only minor variation in chromosome morphology has been described (Ichijima 1926; Senanayake and Bringhurst 1967; Iwatsubo and Naruhashi 1989, 1991). Satellites have been observed on one chromosome pair in five diploid species: *F. vesca*, *F. iinumae*, *F. nipponica*, *F. nubicola*, and *F. daltoniana* (Iwatsubo and Naruhashi 1989, 1991), and chromosome morphology, *per se*, has provided no basis for differentiating the subgenomes in the octoploids.

8.1.4 The Strawberry Plant

A detailed description of strawberry morphology and physiology is provided in Darrow (1966 – Chap. 18). Strawberries are perennial, herbaceous, low-growing plants. Strawberries are capable of vegetative propagation via the production of runners (stolons), which are trailing, above-ground stems that can take root at their nodes to establish new, clonal daughter plants. A runnerless mutant form is known in *F. vesca*. Strawberry leaves are generally trifoliate; however, pentafoliate leaves occur in the diploid species *F. pentaphylla*, and are sometimes seen in other species as well.

The fleshy red strawberry "fruit" is actually the expanded receptacle of the strawberry flower. The true fruit of strawberry are the seed-like achenes borne on the surface of the receptacle. Each achene is derived from an individual, monocarpelate pistil, and if successfully fertilized contains a single seed. Strawberry flowers typically have five white petals, exceptions including higher petal number in some Asian species (Staudt 1989, 2003, 2005) and pink flowers in certain novelty varieties (e.g., 'Pink Panda'). Typically, strawberry is a short day plant, flowering in response to short day lengths and low temperature (Battey et al. 1998). However, day neutral forms have been identified in octoploid *F. virginiana* and diploid *F. vesca* (Ahmadi et al. 1990; Brown and Wareing 1965; Sakin et al. 1997). The day neutral (everbearing), or "*Sem-*

perflorens" form of *Fragaria vesca* ssp. *vesca* is of European origin, and is often termed the 'Alpine' form.

Sex determination in strawberry varies among species (Hancock 1999). Contemporary cultivars of *F.* ×*ananassa* are hermaphroditic; however, gynodioecy and/or trioecy have been reported in the octoploid species *F. chiloensis* and *F. virginiana* (Ahmadi and Bringhurst 1991; Staudt 1989; Ashman 2003), and in tetraploid *F. orientalis* and hexaploid *F. moschata* (Staudt 1989). A genetic model was proposed for trioecy in the octoploids involving a sex determination locus with three alleles (F, H, and M) (Ahmadi and Bringhurst 1991). According to this model, females are heterogametic (F/H or F/M), hermaphrodites may be heterogametic (H/M) or homogametic (H/H), and males are homogametic (M/M). The diploid species are reportedly all hermaphroditic, except for *F. vesca* ssp. *bracteata*, in which gynodioecy also occurs (Ahmadi and Bringhurst 1991). Gametophytic self-incompatibility occurs in the diploid species *F. viridis*, *F. nubicola*, *F. mandshurica*, *F. nipponica*, *F. yezoensis*, *F. gracilis* and *F. pentaphylla*, while diploids *F. vesca*, *F. iinumae*, *F. nilgerrensis*, and *F. daltoniana* are self-compatible (Staudt 1989; Hancock 1999). Self-incompatibility is not known to occur in the octoploid species.

8.1.5 Breeding

Detailed accounts of the history of strawberry domestication and breeding are to be found in Darrow (1966) and Hancock (1999). Before obtaining the first genotypes of *F.* ×*ananassa* in the 1760s, *F. vesca* and *F. moschata* (Hautbois) were cultivated in Europe. *F. viridis* was also cultivated but was less important than the former two species. In 1764, Duchesne identified clearly the parentage of the modern, cultivated strawberry. This strawberry appeared as a vigorous, perfect hermaphrodite displaying fruit a little smaller than the Chilean and with pineapple aroma. Duchene suspected a cross between the Scarlet strawberry (*F. virginiana*) as pollen source, and the Frutillar (*F. chiloensis*) (Darrow 1966). At the same period, this new species was also reported in England and in Holland.

The first breeding work on modern strawberries was conducted in the middle of the 1800s, mainly in England and in America. In the 1900s and particularly after World War II, breeding programs appeared in public institutions. In 1961, the protection of new plant varieties by an intellectual property right (International Convention for the Protection of New Varieties of Plants in Paris, http://www.upov.int/index.html), allowed private companies to develop their own breeding programs. During the twentieth century, the efficiency of strawberry production and the fruit quality were drastically improved with the development of superior production environments and with the breeding of cultivars specifically adapted to these superior environments. In this context, the breeders have to integrate the research in production physiology and cultural practices in order to optimize their selection strategies.

The methodology of strawberry breeding mainly involves pedigree selection, since strawberry is highly heterozygous as observed in other polyploid species. Large genetic variability among strawberry progenies is the major factor for the selection of desirable characters. Traditionally, the best cultivars are crossed and from their progenies, the best genotypes are selected. The succession of crosses between the best genotypes and the selection in the progenies constitute recurrent breeding associated to pedigree selection in which the choice of the parents and the choice of the best combination are critical.

Selection for a new cultivar starts with the cross, and ends with the release of the new cultivar, which takes about 8–10 years. Usually, the process of the breeding program is as follows. The first cycle is obtained with controlled crosses among selected parents chosen for their phenotype values in the considered location. Since main characters of interest are quantitative ones, the genetic gain is achieved with the choice of the best genotypes phenotypically selected for their desirable traits, then making numerous crosses to promote the best combination of alleles. Selfing to fix character is rarely used since inbreeding depression is observed. However, selfing can reveal genetic potential of some genotypes to be used further as parents. The first year of evaluation is performed on the basis of seedling performance. Approximately, 1–3% of the genotypes are kept and further evaluations are performed on plots of runner plants. After a few years of screening on the desirable characters, the selected genotypes are evaluated in multi-location trials under commercial conditions. Controlled tests are required to analyse some characters in the breeding program, i.e. disease resistance tests or simulation of fruit conservation test.

Although some breeding objectives vary according to the area of cultivation, traditional main breeding objectives are the following (Rosati 1993): a production of relative large berry size in order to limit the cost of harvest, a firmer fruit with regular shape and long shelf life, which is easy to harvest, an increase in the total yield, an improvement in fruit appearance (color, shape, brightness), and disease resistances. When cultivars have to be adapted to specific regions or to specific markets, specific objectives are included in the breeding programs such as developing production for processing, time of ripening (very early or very late ripening). Breeding for good taste and flavor is also an important objective to fit the quality market needs. The evolution of cultural practices leads also to new objectives such as resistance to powdery mildew which is more important in greenhouse production.

8.1.6 Nutritional Composition

Strawberries present many specific nutritional characteristics known to have health benefits. They are particularly rich in vitamin C (60 mg per 100 g fresh fruit corresponding to 75% of the daily need), richer than oranges, and contain a high amount of potassium (180 mg per 100 g of fresh fruit). Besides these essential nutrients, strawberries contain a high content of ellagic acid more commonly found in the form of water-soluble ellagitannins. This phenolic compound is known as a naturally occurring dietary antimutagen and anticarcinogen (Maas and Galetta 1991; Clifford and Scalbert 2000). In vitro, strawberry appears to exert a weaker antioxidant activity as compared to other berries. They are rich in pelargonidin-3-glucoside, the major strawberry anthocyanin, and ascorbic acid, both of which are weak antioxidants (Törrönen and Määttä 2002). However interesting results have been reported on beneficial effects of strawberries in experimental animals (e.g., Joseph et al. 1999).

8.1.7 Economic Importance

Globally, 214,118 Ha of strawberries were cultivated in 2004, representing a worldwide production of 3,113,840 Mt (FAOSTAT data 2004). A large part of the cultivated area is located in Europe (63.3% of the total area), followed by Asia (14.8%) and North and Central America (13.8%). However, Europe and North and Central America have a comparable production level with 1,164,650 Mt and 1,022,521 Mt. The USA is the world's leading strawberry producer with 840,000 Mt. Spain ranks second (285,600 Mt) followed by the Republic of Korea, Japan, Mexico, Italy, the Russian Federation, Turkey, Poland and Germany. During the last ten years, some countries like Turkey, Morocco and Egypt have strongly increased their production. Germany (109,824 Mt) and France (93,591 Mt) are importing a large quantity of strawberries whereas Spain and the USA are exporting a large part of their production (212,327 and 94,666 Mt respectively).

8.2 Genetic Characterization

8.2.1 Genome Composition

The first model of octoploid *Fragaria* genome composition – AABBBBCC – was proposed by Federova (1946). Cytological (meiotic pairing) evidence also provided the basis for Bringhurst's initial, partially differentiated AAA′A′BBBB genome composition model for the octoploid strawberry species (Senanayake and Bringhurst 1967). Subsequent genetic evidence, notably the absence of any indication of polysomic inheritance patterns, prompted the proposal of the prevailing, fully differentiated AAA′A′BBB′B′ model (Bringhurst 1990). This last cytological formula implies that the genome of *F.* ×*ananassa* is highly diploidized. A recent study using CAPS (cleaved amplified polymorphic sequence) markers detected only disomic inheritance in *F.* ×*ananassa* (Kunihisa et al. 2005). The diploidization of the wild octoploid strawberry *F. virginiana*, one of the parents of the cultivated species, was also suggested by studying SSR (single sequence repeat) markers (Ashley et al. 2003). However, a final conclusion regarding the diploidized status of the octoploïd genome requires the analyses of markers spread over the whole genome, as will be further detailed in the linkage mapping section.

8.2.2 Genome Size

C-value determinations based on flow-cytometric measurement were reported for one representative of *F. vesca* (1 C = 164 Mb), and for two *F.* ×*ananassa* cultivars (1 C = 562 Mb and 1 C = 571 Mb) (Akiyama et al. 2001), where 1 C is the DNA content of an unreplicated haploid nucleus. In this study, *Arabidopsis thaliana* was used as a standard, and it was assigned a C-value of 125 Mb based on the length of the *Arabidopsis* genome sequence (Arabidopsis Genome Initiative 2000). However, when Bennett et al. (2003) measured the *A. thaliana* C value via flow cytometry (in comparison to a *Caenorhabditis elegans* standard), they obtained a value of 157 Mb, which is about 25% larger than the 125 Mb value determined on the basis of genome sequencing. Using the *A. thaliana* flow-cytometric C-value of 157 Mb as an appropriate standard for flow-cytometric analysis, the 164 Mb C-value reported for *F. vesca* (Akiyama et al. 2001) should be proportionately corrected upward by 25% to 206 Mb, and the *F.* ×*ananassa* values of 562 Mb and 571 Mb should be corrected to 708 Mb and 720 Mb, respectively. Notably, if the two corrected octoploid C values are divided by four to obtain an average C-value for the "basic" (x = 7) subgenome size in *F.* ×*ananassa*, the resulting values of 177 Mb and 180 Mb, respectively, are less than the corrected 206 Mb size of the *F. vesca* genome.

In a similar study using *A. thaliana* as reference (1C = 157 Mb), the diploid (*F. vesca*), hexaploid (*F. moschata*) and octoploid (*F.* ×*ananassa*) genotypes displayed genome sizes of 264 Mb, 731 Mb and 884 Mb, respectively (Denoyes-Rothan, unpublished results). These values, which are corrected in accord with the reference of Bennett et al. (2003), are slightly higher than the corrected values of Akiyama et al. (2001). In both the Akiyama and Denoyes-Rothan studies, the evident diminution of the size of the octoploid genome relative to the diploid one is similar (12% and 16% less than the size expected if the octoploid genome was four times the size of the diploid one). This discrepancy prompts speculation that the genomes originally contributed to the octoploid species by their diploid ancestors were not of uniform size – some being smaller than the 206 Mb size of *F. vesca*. Alternately, the octoploid subgenomes may have undergone some size reduction since the origin of the octoploid species. This smaller size could be due to events which followed the origination of a polyploid such as loss of DNA segments (reviewed in Osborn et al. 2003). It is evident that a comprehensive survey of C-values in the diploid and polyploid species is needed to provide a basis for future investigations of genome evolution in *Fragaria*.

8.2.3 Gene Nomenclature

This review provides a useful opportunity to consider the status of gene nomenclature in strawberry. No uniform nomenclatural guidelines have been established for strawberry, and very few gene names have been assigned. Nevertheless, some conflicts and inconsistencies have occurred, drawing attention to the need for development of a uniform gene nomenclature system for strawberry.

The first use of gene names in strawberry was the assignment of the symbols *s*, *c*, and *r* to the monogenic recessive traits, respectively, of perpetual flowering, yellow/white fruit color, and non-runnering, in *F. vesca* (Brown and Wareing 1965). In this instance, the single-letter gene symbols correspond to the dominant, wild type forms of the respective traits: seasonal flowering (*S*), colored fruit (*C*), and runnering (*R*). Subsequently, Guttridge (1973) employed the gene symbol *j* with reference to the perpetual flowering trait in *F. vesca* f. *semperflorens* cv 'Baron Solemacher', one of the two everbearing varieties previously studied by Brown and Wareing (1965). Although not explicitly stated, the symbol *j* evidently referenced the dominant, wild type "June bearing" (*J*) or seasonal bearing form. More recently, Albani et al. (2004) introduced the symbol *SFL* (*SEASONAL FLOWERING LOCUS*) in relation to the locus governing seasonal (*SFL*) versus perpetual (*sfl*) flowering in *F. vesca* f. *semperflorens*. Again, although not explicitly stated by the authors (Albani et al. 2004), the chosen gene symbol referenced the dominant, wild type form of the trait (seasonal flowering). Thus, three different gene symbols, *s*, *j*, and *sfl*, have already been introduced for what is probably a single locus conferring the mutant form, perpetual flowering. This example emphasizes the need for the establishment of a genetic nomenclature committee for strawberry at the earliest available opportunity. This committee should be charged with establishing guidelines for gene nomenclature in strawberry, and for resolving existing nomenclatural conflicts. Of even greater benefit would be the adop-

tion of a common nomenclatural system for all of the species within the Rosaceae family.

8.2.4 Morphological Markers

Few monogenic morphological markers have been identified in strawberry, in large part because of the genetic and genomic complexity of the octoploid cultivated species. At the diploid level, a few simply inherited traits have been described in the classical literature (reviewed by Brown and Wareing 1965), but few genes have been named. Other than the *c*, *s*, and *r* loci described in the previous paragraph, the only other named morphological marker at the diploid level is the *arb (arborea)* locus conferring a long stemmed phenotype (Guttridge 1973). At the octoploid level, very few simply inherited traits have been described (Scott and Lawrence 1975; Galetta and Maas 1990) or gene symbols assigned. A series of monogenic, dominant determinants (*Rpf* genes) of resistance to red stele disease (causal organism *Phytophthora fragariae* var. *fragariae*) have been described by Van de Weg (1997), and linkages to molecular markers have been identified for three of these (Haymes et al. 1997; Hokanson and Maas 2001). Another dominant gene, *Rca*2, conferring resistance to *Colletotrichum acutatum* (Denoyes-Rothan et al. 2005) has been described. In contrast, quantitative variation has been assessed in a plethora of traits and studies in *F.* ×*ananassa* (reviewed in Galletta and Maas 1990). Despite their major significance to strawberry breeding, quantitative genetic studies that do not include a molecular or mapping component fall outside the scope of this review.

8.2.5 Isozymes and Molecular Markers

Hokanson and Maas (2001) carefully summarized applications of isozymes and the initial phase of molecular marker (RAPD, RFLP, AFLP and first SSRs) development in *Fragaria*. The investigations of PGI (phosphoglucoisomerase) isozymes by Bringhurst and coworkers provided the first evidence that a single gene could be represented by four distinct loci, all of which could be expressed, in the octoploid strawberry (Arulsekar et al. 1981). RFLP (restriction fragment length polymorphism) markers using probes developed from *Prunus* were used for mapping in the octoploid strawberry (Viruel et al. 2002). Among the 123 probes tested, 27 (22%) revealed polymorphism whereas 60–75% revealed polymorphism in *Prunus*, indicating a low overall level of variability in strawberry in the conditions of the study. RFLP markers, which potentially reveal homologous loci, could be very useful in the study of synteny between species of the Rosaceae family.

AFLP (amplified fragment length polymorphism) markers were utilized exclusively in construction of the first published octoploid map (Lerceteau-Köhler et al. 2003). RAPD (randomly amplified polymorphic DNA) markers were employed in construction of the first *Fragaria* linkage map, in *F. vesca* (Davis and Yu 1997). A remarkable aspect of this mapping study was the development of a method of identifying codominant RAPD markers by heteroduplex analysis (Davis et al. 1995) that resulted in placement of 11 codominant RAPD markers on the map. Nevertheless, concern about the comparatively poor transferability of RAPD markers between mapping populations (Sargent et al. 2004), a concern that also applies to AFLP and other anonymous marker types, has prompted intensive attention to the development of sequence-specific, PCR-based markers such as SSR markers for strawberry.

The high cost of SSR marker development via genomic library screening – the source of the initial wave of *Fragaria* SSRs (Nourse et al. 2002; Ashley et al. 2003; James et al. 2003; Sargent et al. 2003; Cipriani and Testolin 2004; Hadonou et al. 2004; Lewers et al. 2005; Monfort et al. 2006) – has been drastically reduced by the advent of EST (expressed sequence tag) database mining as a means of discovering SSR loci within the rapidly growing body of publicly available cDNA and genomic sequences for strawberry (Lewers et al. 2005; Sargent et al. 2006). Lewers et al. (2005) showed that SSRs developed from the genomic library were only slightly superior to GenBank-derived SSRs in their ability to detect polymorphisms. An anticipated advantage of SSRs was their portability at the intra or inter-generic levels. In Lewers et al. (2005), most of SSRs from various species of *Fragaria* amplified within the genus *Fragaria*. Differential patterns of SRR marker transferability from octoploid to various diploid *Fragaria* species are reported in Davis et al. (2006), who also review the general issues relevant to SSR marker transferability within *Fragaria*. Preliminary studies on transferability between genera of the same Rosoideae tribe such as

Rosa showed that about 30–50% of the microsatellites amplified. However, this transference was very low between genera from different tribes such as between *Fragaria* and *Prunus* (Denoyes-Rothan, unpublished results).

The CAPS technique (Konieczny and Ausubel 1993) has also been explored as a gene-based marker technology for strawberry (Kunihisa et al. 2003, 2005). In this method, PCR primers located in exon sequences flanking one or more introns are used to amplify intron-containing sequences, and the amplification products are subjected to restriction digestion with the goal of detecting sequence polymorphisms that are not readily detectable as intron length polymorphisms. This promising method offers the opportunity to conveniently map genes that do not contain SSRs.

8.3 Linkage Mapping

The first instances of genetic linkage to be described in *Fragaria* each involve an isozyme marker and a single gene morphological trait in *F. vesca*. In a cross between Alpine *F. vesca* cultivars 'Yellow Wonder' and 'Baron Solemacher', the yellow fruit color trait (*c* locus) was linked to the SDH (shikimate dehydrogenase) isozyme locus, with a recombination frequency of 1.1% (Williamson et al. 1995). Analysis of several segregating populations derived from crosses between non-runnering, 'Alpine' cultivars and wild type runnering plants detected a linkage (~18% recombination frequency) between the non-runnering (*r*) locus and the PGI-2 (phosphoglucoseisomerase 2) isozyme locus (Yu and Davis 1995).

The first *Fragaria* linkage map was also constructed in *F. vesca* (Davis and Yu 1997). This map defined the expected number of seven linkage groups, covered a total map length of 445 cM, and consisted of 80 markers of which 75 were RAPD markers. The remaining five markers were the SDH and PGI-2 isozyme markers, which anchored linkage groups I and II, respectively, the *r* locus, the *Adh* (alcohol dehydrogenase) gene detected molecularly as an intron length polymorphism, and the *c* locus, which was not segregating in the cross but was added to the map based upon its known close linkage to the SDH isozyme locus (Williamson et al. 1995).

A subsequent diploid linkage map (Sargent et al. 2004), based on the interspecific cross *F. vesca* × *F. nubicola* (Fv × Fn), consisted of 78 markers and spanned a map distance of 448 cM, nearly identical to the 445 cM length of the initial *F. vesca* map (Davis and Yu 1997). The Fv × Fn map marked the beginning of a shift toward use of SSR markers for mapping in strawberry, and contained 68 SSR markers out of a total of 78 markers mapped. The development of this map is ongoing, and has been expanded to a 182 marker version by the addition of new microsatellite loci (Sargent et al. 2006).

The first reported instance of linkage in the octoploid strawberry was that of Haymes et al. (1997), who used bulked segregant analysis (Michelmore et al. 1991) to identify seven RAPD markers linked to the R*pf1* gene for resistance to *Phytophthora fragariae* (red stele) resistance in *F.* ×*ananassa*. Two SCAR (sequence characterized amplified region) markers closely linked in coupling phase to the R*pf1* gene were subsequently developed and found to be widely associated with resistance in a survey of 133 European and North American cultivars and breeding selections (Haymes et al. 2000).

The first published octoploid map was that of Lerceteau-Köhler et al. (2003) for *F.* ×*ananassa*. This mapping study employed a two-way pseudo-testcross strategy, combined with a single dose restriction fragment (SDFR) analysis applied to 789 AFLP markers. Due to the difficulty of accurately detecting repulsion phase linkage in cases of polyploids with polysomic inheritance, as described in Wu et al. (1992) and detailed recently in Qu and Hancock (2001), a two-step mapping procedure was applied (Grivet et al. 1996; Fregene et al. 1997). In a first step, markers linked in coupling phase were mapped into cosegregation groups and in a second step the data matrix was inverted to test the repulsion phase between markers allowing the definition of linkage groups. Separate female and male maps were constructed, and had total map lengths of 1,604 cM and 1,496 cM, respectively. The female map consisted of 235 markers assigned to 30 linkage groups, or an average of 7.8 markers per group (Fig. 1). The male map consisted of 280 markers assigned to 28 linkage groups, or 10 markers per group. Dividing the total map lengths by the number of linkage groups yields average linkage group lengths of 53.5 cM and 53.4 cM for the female and male maps, respectively, which is slightly less than the average linkage group lengths of 63.6 cM

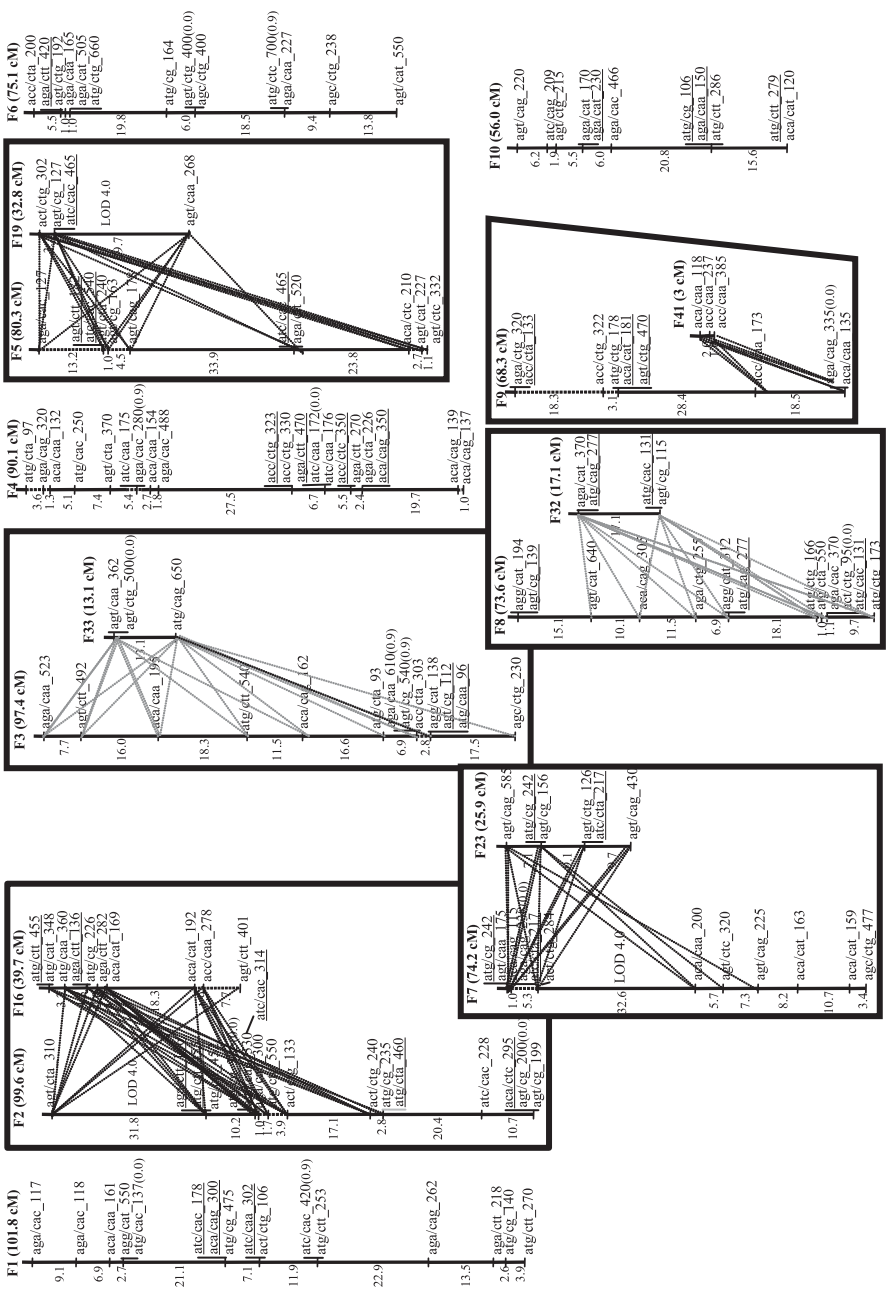

Fig. 1. Linkage map of the female parent (Capitola) (Lerceteau-Köhler et al. 2003). Linkage groups were numbered in order of descending size. Uncertain marker orders in the co-segregation groups (alternate orders not ruled out data LOD = 1) are represented by a *dotted line*. The 3:1 segregating markers are *underlined* and not included in the count of groups with only two markers. Linkage in the repulsion phase between two markers is represented by a *dashed line* (LOD = 3.0). *Boxes* represent linkage groups in the repulsion phase. The genetic distance, in centimorgans, of each marker to its closest neighbouring marker is given in *parentheses*

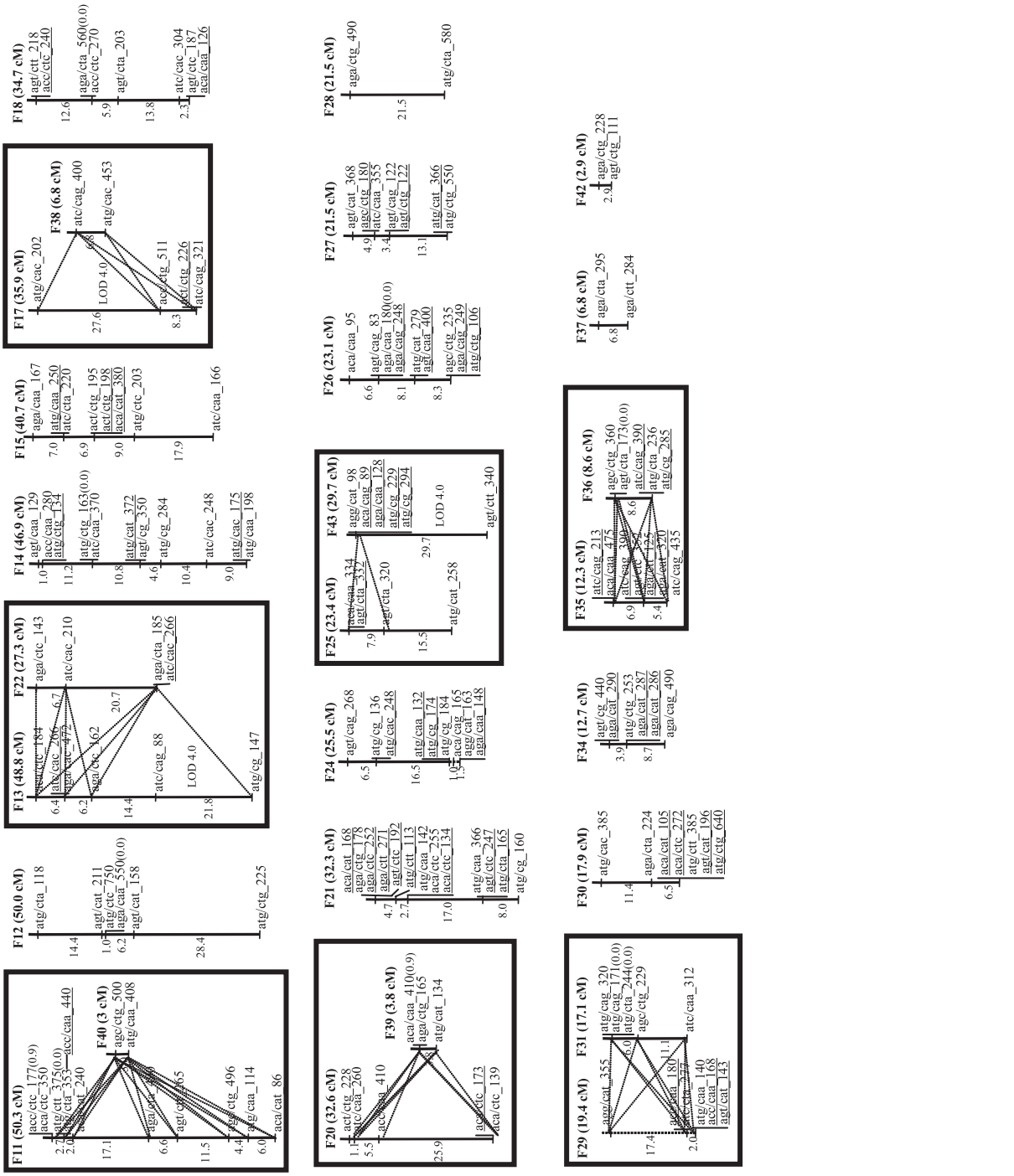

Fig. 1. (continued)

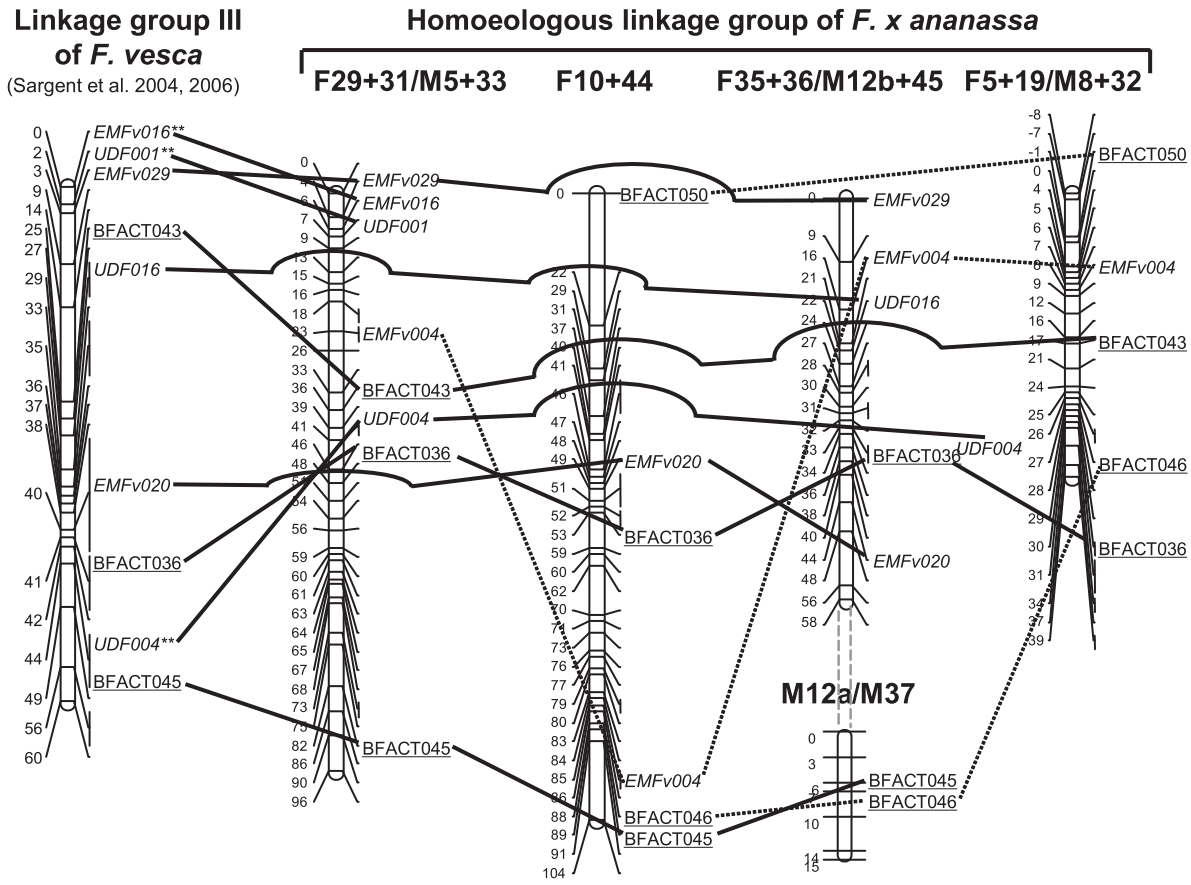

Fig. 2. Comparison of Fragaria vesca and *F.* ×*ananassa* linkage groups (LG). The genetic distances are expressed in map distances (cM) according to Kosambi. Only microsatellites (SSRs) involved in comparisons of linkage groups are indicated (in *underlined*, SSR from *F. x ananassa* and in *italic* SSR from F. vesca). The other markers (SSRs or AFLP) are indicated by *dashes*. Connections between microsatellites located on the four homoeologues LG of *F.* ×*ananassa* and on their homologue LG in *F. vesca* are indicated by *continuous lines*. Connections between microsatellites located exclusively on the four homoeologues LG of *F.* ×*ananassa* are indicated by *dotted lines*

and 64.0 cM calculated, respectively, for the *F. vesca* (Davis and Yu 1997) and *F. vesca* × *F. nubicola* (Sargent et al. 2004) diploid maps described above, both of which had about 11 markers per linkage group. However, since the octoploid map is not fully saturated, the previous figures might be biased and a direct comparison between the diploid and the octoploid maps might be problematic. The analysis of repulsion phase showed that most of the groups were in coupling/repulsion phase reflecting a disomic behavior. However, the presence of some large groups displaying only single dose markers in coupling phase suggested that the entire genome might not be completely disomic.

Recently, a comparison between the octoploid (Lerceteau-Köhler et al. 2003) and the diploid (Sargent et al. 2004, 2006) maps was initiated using microsatellites (Denoyes-Rothan, unpublished results). The first step was the construction of an integrated map in *F.* ×*ananassa*. The pattern of conserved linkages between *F.* ×*ananassa* and *F. vesca* allows the assignment of linkage groups of *F.* ×*ananassa* as potentially homoeologous and homologous to one linkage group of *F. vesca* (Fig. 2).

8.4 Gene Mapping

In the diploid species, *F. vesca*, gene mapping has initially focused on fruit and flowering aspects. A candidate gene mapping approach undertaken by Deng and Davis (2001) discovered an association between the *c* (fruit color) locus and the flavanone 3-hydroxylase (*F3H*) gene in *F. vesca*. Molecular markers linked to the *F. vesca* seasonal flowering locus were identified by Albani et al. (2004). Initially, three ISSR (inter simple sequence repeat) markers (Cekic et al. (2001) linked to the seasonal flowering locus were identified, and were then converted to sequence-specific SCAR markers (Albani et al. 2004). SCAR2 cosegregated with the seasonal flowering locus, which was in turn flanked by SCAR1 (3.0 cM distance) and SCAR3 (1.7 cM distance). The identification of these and other markers linked to the flowering locus provides a starting point for positional cloning of this important locus.

In *F.* ×*ananassa*, gene tagging has so far focused on disease resistances. As previously mentioned, RAPD markers and their derived SCAR markers linked to the R*pf1* gene for resistance to *Phytophthora fragariae* (red stele) resistance have been identified in *F.* ×*ananassa* (Haymes et al. 1997, 2000). The development by Haymes of RAPD markers closely linked to the R*pf3* and R*pf3* red stele resistance genes has also been reported (Hokanson and Maas 2001). A bulked segregant analysis (BSA) was recently used to identify molecular markers linked to the *Rca2* gene conferring resistance to *Colletotrichum acutatum* pathogenicity group 2, which causes anthracnose in the octoploid strawberry *F.* ×*ananassa* (Lerceteau-Köhler et al. 2005). Among the four AFLP markers linked to the resistance gene, two were converted into SCAR markers (STS-Rca2_417 and STS-Rca2_240) and were located at 0.6 cM and 2.8 cM from the resistance gene respectively. Studying the presence of the STS-Rca2_417 marker in 43 cultivars of *F.* ×*ananassa* showed that 81.4% of the resistant/susceptible genotypes were correctly predicted. All these developed SCARs constitute new tools for indirect selection criteria of disease resistance genotypes in strawberry breeding programs (Fig. 3).

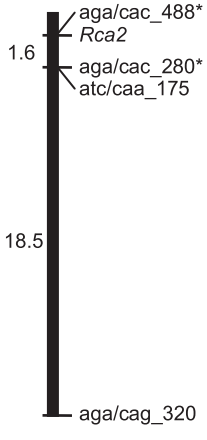

Fig. 3. A genetic map of the chromosome region containing the *Rca2*, *Colletotrichum acutatum*, pathogenicity group 2, resistance gene (Lerceteau-Köhler et al. 2005). The map is based on 62 F_1-individuals from the 'Capitola' × 'Pajaro' cross. AFLP markers labeled with an *asterisk* (∗) were successfully converted into SCAR markers

A complementary approach to the identification and mapping of resistance genes is the use of degenerate PCR primers targeted to conserved sites in the NBS (nucleotide binding site) domain of many plant resistance genes to isolate resistance gene analogs (RGAs) (Leister et al. 1996; Kanazin et al. 1996; Yu et al. 1996). Martinez Zamora et al. (2004) have reported the isolation of RGAs from cultivated and wild strawberries. Seven distinct families of RGAs were described.

8.5 QTL Detection

Only a few QTL (quantitative trait locus) studies have been published to date in strawberry. New approaches such as genetic association have been initiated for studying the relationship between the underlying genotype and the observed phenotype. These studies have their extension in the pedigree approach (Van de Weg et al. 2005). The first QTLs published on strawberry concerned fruit quality (Lerceteau-Köhler et al. 2004) and were detected using a segregating population of 213 individuals of a cross between Capitola and CF1116, two genotypes with many contrasting fruit quality traits. A total of 34 traits involved in fruit quality were evaluated, including developmental and fruit aspect related traits, texture related traits, fruit acidity, sugar and ascorbate concentrations. Amino acid concentrations were quantified using one-dimensional proton NMR spectroscopy. Most of the traits except the amino acid concentrations

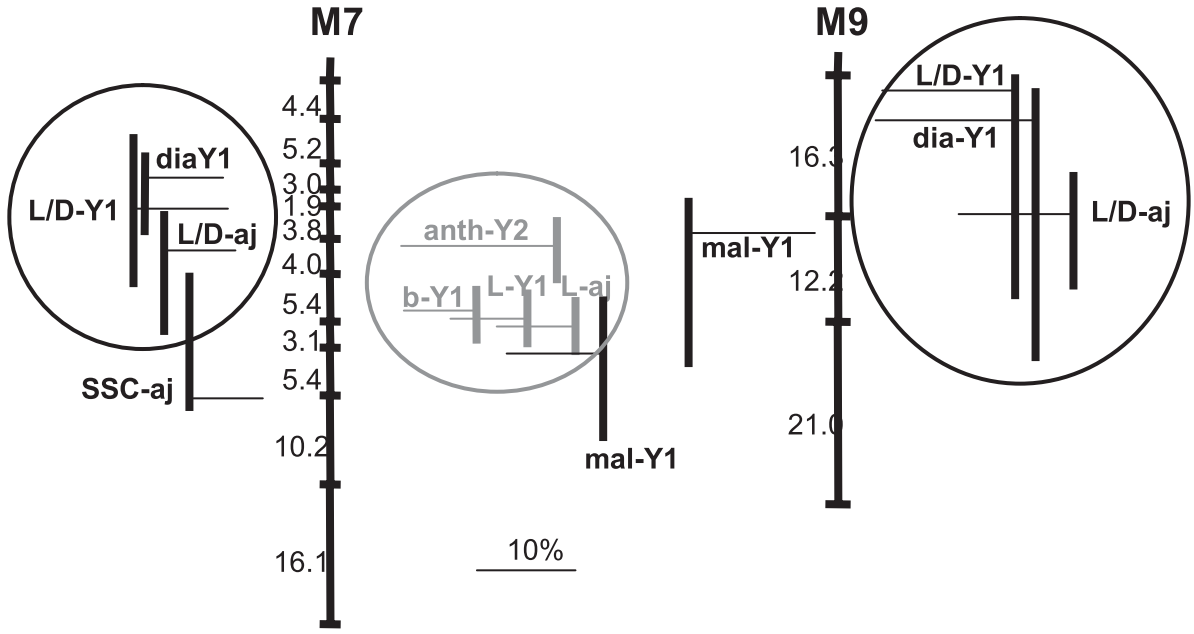

Fig. 4. QTL clusters for fruit quality traits (*mal*, malate; *anth*, anthocyanin; *L/D*, length/diameter ratio; *dia*, diameter; *L* and *a*, external skin color parameters; *SSC*, soluble-solids content) detected on the CF1116 linkage map (Lerceteau-Köhler et al. 2004). Analyses were conducted on two years data (*1* and *2*), and on year-adjusted data. The linkage groups M7 and M9 are in the coupling/repulsion phase. *Horizontal bar* represents the percentage of phenotypic variation explained by a QTL. *Vertical bar* represents the one-LOD support confidence interval

were evaluated during two successive years. A total of 22 significant QTLs were detected by simple interval mapping (LOD > 3.0) in year 1, four on the female map and 18 on the male map, whereas 17 were detected in year 2, ten on the female map and seven on the male map. Only two QTLs could be detected both years. When removing the year effect, 22 QTLs were observed, eight on the female and 14 on the male map. The percentages of phenotypic variance explained by each QTL ranged from 6.5% to 16.0%. An example of QTL cluster of fruit quality-related traits is given in Fig. 4.

QTLs associated with *C. acutatum* and *P. cactorum* resistances were also detected in the same population (Denoyes-Rothan et al. 2004). One hundred eighty five progeny were inoculated with *C. acutatum* by dipping cold stored plants obtained from vegetative multiplication in a conidial suspension adjusted to 2.10^6 conidia per ml. Cold stored plants of the all progeny were also inoculated separately by *P. cactorum* by inserting an agar disk containing mycelium into the crown. Each inoculation was conducted twice, named experiments 1 and 2. For resistance to *C. acutatum*-pathogenicity group 1, five QTLs with LOD scores ranging from 2.0 to 2.8 and spread over three female and two male groups were mapped. The individual QTL effects (R^2) ranged from 5.8 to 12.2%. No QTLs common to experiments 1 and 2 were detected. For resistance to *P. cactorum*, five QTLs with LOD scores ranging from 2.0 to 2.6 and spread over two female and three male groups were mapped. The individual QTL effects (R^2) ranged from 6.5 to 10.2%. Two QTLs, one on female group (F19) and one on male group (M2a), were detected in both experiments. No QTL for *P. cactorum* resistance overlapped the QTLs for *C. acutatum* resistance.

In both fruit quality and disease resistance QTL studies, different putative QTLs were found depending on the technique, the year or the experiment. These differences could be attributed to an environment variation or to a lack of accuracy in the notation. Therefore, before using QTLs in breeding programs, it is necessary to know in which conditions the QTL is expressed. Since there was no QTL for the different studied resistances (*C. acutatum*-pathogenicity groups 1 and 2, and *P. cactorum* resistances), a pyramidal strategy as suggested by Hospital and Charcosset (1997) should be considered for constructing a durable resistance to both pathogens in a breeding scheme.

8.6 Marker-Assisted Selection

The identification of molecular markers linked to QTLs, as well as to qualitative trait loci, enhances the opportunity for use of marker-assisted selection (MAS) in strawberry. Luby and Shaw (2001) have specified criteria that can be used to assess whether MAS will make economic sense in fruit breeding programs. Among these are the requirements for inexpensive marker technologies and for markers that have highly robust marker-locus association. The ultimate test will be whether MAS can provide an economical and substantially improved probability of selecting superior individuals as compared with the best conventional breeding and evaluation practices (Luby and Shaw 2001). Already at least one commercial strawberry breeder is utilizing molecular markers for MAS. RAPD-derived SCAR markers are being used at Driscoll Strawberry Associates in California to screen 1,500 – 30,000 seedlings per year for markers associated with day-neutrality and resistance to *Colletotrichum acutatum* (T. Sjulin, personal communication).

8.7 Development of Genomics Resources

As of July 2005, approximately 7,000 strawberry genomic and cDNA sequence entries were listed in GenBank. By April 12, 2006, this number exceeded 20,000. Although these numbers are small in comparison to the GenBank entry lists for many crop species, they represent a dramatic uptrend over the prior 2 – 3 year period, before which the number of GenBank entries was well under 1,000. Many thousands of additional strawberry EST sequences are in the bioinformatics pipeline. DNA microarray technology has been employed by only one laboratory, resulting in the identification of genes involved in fruit ripening and flavor, including genes *SAAT* (strawberry alcohol acetyltransferase) (Aharoni et al. 2000), and *FaNES1* (*F.* ×*ananassa* Nerolidol Synthase1) and related genes (Aharoni et al. 2004).

Initiation of positional cloning efforts has been hampered by the general unavailability of high molecular weight genomic libraries for strawberry. BAC (bacterial artificial chromosome) and fosmid libraries have been constructed from *F. vesca* genomic DNA at the University of Reading and the University of New Hampshire, respectively, have yet to be described in peer reviewed publication. However, initial sequencing of genomic fosmid clones from *F. vesca* (Davis, unpublished results) suggests that gene density in *F. vesca* is about 1 gene per 6 kb.

8.8 Conclusion and Future Prospects

Despite its genomic complexity, the small size of the basic *Fragaria* genome makes the strawberry a favorable subject for genomics resource development. The next few years should bring rapid progress in strawberry genomics in several areas. These areas include linkage mapping, positional cloning, functional genomics, and possibly the complete sequencing of a basic strawberry genome. Much of the genomics and mapping data being generated for strawberry and other rosaceous crops is being coordinately assembled and disseminated through the Genome Database for Rosaceae (GDR). Details are available at the site http://www.mainlab.clemson.edu/gdr/.

Second generation linkage maps at the diploid and octoploid levels will be constructed using sequence-specified, transferable markers such as gene-based SSRs, CAPS, and other PCR-based marker technologies. Establishment of robust associations, and ideally identities, between gene-based markers or candidate genes and quantitative or qualitative trait loci will promote the wider evaluation, and potentially adoption, of MAS methods by strawberry breeders. The enhanced transferability of gene-based markers, as compared with the anonymous RAPD and AFLP markers used for construction of the first generation diploid and octoploid maps, respectively, will greatly facilitate map comparison between *Fragaria* species within and between ploidy levels. Mapping with gene-based, codominant markers in the octoploid will enhance the opportunity to detect polysomic inheritance, if it exists at all, in the octoploids.

The high gene density evident from preliminary genomic sequence samples in strawberry favors efforts to positionally clone genes known only by phenotype, particularly in the diploid model system. However, for such efforts to move forward, routine methods for constructing BAC libraries with inserts in the ~150 kb range are needed. The primary obstacle here is the isolation in large quantities of high qual-

ity, high molecular weight DNA, which has proven to be a particular problem in strawberry. Efforts to overcome this obstacle are in progress.

EST resource development for strawberry still lags far behind that of many other fruit crops, including other rosaceous species such as apple and peach. The advent of new technologies for economical, high-throughput, short-read sequencing of individual cDNA molecules in the absence of cloning, such as that recently introduced by the private company 454 Life Sciences, promises to open up an enormous opportunity to expand the strawberry EST database, and to extend the opportunity for strawberry microarray analysis beyond the narrow private sector in which it currently resides.

Finally, the prospect of obtaining the complete sequence of a basic strawberry genome looms inevitably in the minds of strawberry genomicists. The less-than 200 Mb size of the basic ($x = 7$) strawberry genome makes it by far the smallest genome of any rosaceous crop species. An inbred line of the diploid model species, *Fragaria vesca*, almost certainly a genome contributor to the octoploids, would be an excellent subject for complete genome sequencing.

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