20. Strawberry Genomics: Botanical History, Cultivation, Traditional Breeding, and New Technologies

Kim E. Hummer and James Hancock

1 Origin, Speciation, and Evolution

> The Origin of Strawberries – Cherokee Nation
>
> When first man was created, he lived with the mate Creator gave him. When they began to quarrel, first woman left her husband. The man followed, sad and crying, but first woman kept going and never looked behind. Unetlanv, the Creator, took pity on first man and asked him if he was still angry with his wife. He said he wasn't, so Unetlanv asked him if he would like to have her back. He answered, 'yes!'
>
> Unetlanv put a patch of the ripest huckleberries in the path of first woman, but she passed right on by. A little further, he put a big clump of blackberries, but she didn't notice these, either. One by one, Unetlanv put fresh fruits in her path, but these she also refused to see. Suddenly, she saw a patch of large, ripe strawberries in front of her. She had never seen these before. She bent down to gather a few to eat, and as she picked them up, and she thought, 'My husband would love to eat these!' She gathered a bunch of the finest berries and started back along the path to give them to him. He met her with joy, and together they went home.
>
> Today, strawberries are often kept in traditional homes. They remind us not to argue, and are a symbol of good luck.
>
> (Cherokee Nation, 2007).

Strawberries, genus *Fragaria* L., are the most economically important soft fruit worldwide. This genus is a member of the Rosaceae, sub-Family Potentilloideae (formerly classified in Rosideae), has *Duchesnea* and *Potentilla* as close relatives. While Mabberley (2002) proposed reuniting, i.e., submerging, *Fragaria* under *Potentilla*, further research (Eriksson et al., 2003) has suggested that Mabberley was premature in this judgment, and in present majority botanical opinion the genus status has been retained.

The common dessert strawberry, *Fragaria* × *ananassa* Duchesne ex Rozier nothosubsp. *ananassa*, is a regular part of the diet of millions of people and is cultivated in the arable regions of the globe from the arctic to the tropics. More than

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K.E. Hummer (✉)
USDA ARS National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333-2521, USA

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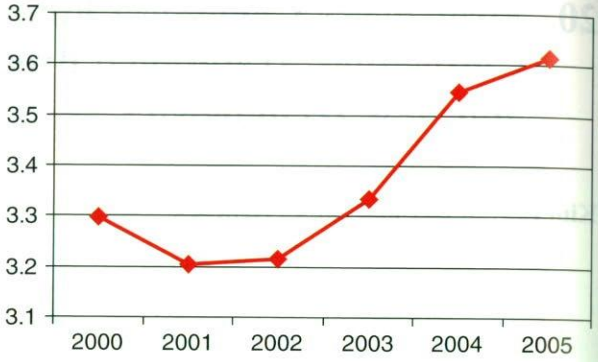

Fig. 1 World strawberry production (FAO, 2007). MT per year

75 countries have significantly reportable amounts of strawberry production (FAO, 2007). Annual world production of strawberries has more than doubled in the last 20 years to over 3.6 million metric tons (Fig. 1). Most of the production occurs in the northern hemisphere (98%), though no genetic or climatic barriers prevent expansion to the south.

Fragaria includes 21 species (Table 1) distributed in the north temperate and holarctic zones (Staudt, 1989, 1999a, b; Rousseau-Gueutin et al., 2008). An accurate synonymy and phylogeny of the strawberry species is emerging. European and American species of *Fragaria* have been rigorously defined by Staudt (1989, 1999a), who, with colleagues, is proceeding to examine Asian species (Staudt 1999b, 2003, 2005; Staudt and Dickoré, 2001). Chinese species are under study (Dai et al., 2007; Lei et al., 2005) but require further collection and examination in light of global taxonomy. The distribution of specific ploidy levels within certain continents reflects the history and evolution of these species (Staudt, 1999a).

2 Diploid Species

Diploid strawberries can be distinguished by their plant habit, foliage, inflorescence structure, flowers and fruit (Table 2). *Fragaria viridis* has one of the smallest genomes of vascular plants (Hodgson, 2007). The *F. vesca* genome is also small. At 164 Mb (Akiyama et al., 2001) it is only slightly larger than that of *Arabidopsis thaliana*.

Although 12 diploid ($2n = 2x = 14$) species are native to Eurasia (Table 1), only *F. vesca* is indigenous in northern Eurasia and North America. It is also the only diploid species of North America. Along with other factors, the broader distribution of *F. vesca* suggests that it originated during the Cretaceous (Staudt, 1989). Its ancestor may be basal for the genus.

Although Darrow (1966) and subsequent authors (Hancock, 1999) describe *F. vesca* as native circumpolar boreal, and their distribution maps show *F. vesca* throughout Northern Europe, Asia and North America, this diploid species is not

Table 1 World strawberry (*Fragaria* L.) species. After Hancock (1999) and Rousseau-Gueutin et al. (2008)

Species	Ploidy	Geographic distribution
F. bucarica	2*x*	Western Himalayas
F. daltoniana J. Gay		Himalayas
F. gracilis A. Los.		North China
F. innumae Makino		Japan
F. mandshurica Staudt		North China
F. nilgerrensis Schlect.		Southeastern Asia
F. nipponica Lindl.		Japan
F. nubicola Lindl.		Himalayas
F. pentaphylla Lozinsk		North China
F. vesca L.		Europe, Asia west of the Urals, North America
F. viridis Duch.		Europe and Asia
F. yezoensis		Japan
F. corymbosa	4*x*	Northern China
F. gracilis		Northwestern China
F. moupinensis (French.) Card		Northern China
F. orientalis Losinsk syn. = *F. corymbosa* Lozinsk		Russian Far East/ China
F. tibetica spec. nov. Staudt		China
F. × *bringhurstii* Staudt	5*x*	California
F. moschata Duch.	6*x*	Euro-Siberia
F. chiloensis (L.) Miller	8*x*	Western N. America, Hawaii and Chile
F. virginiana Miller		North America
F. × *ananassa* Duch. ex Lamarck		Cultivated worldwide
F. iturupensis Staudt	10*x*	Iturup Island, Kurile Islands

native east of the Urals to Kamchatka (Hultén, 1927–1930), Hokkaido, Japan (Makino, 1979), western Alaska (Hultén, 1968) or Hawaii (Degener, 1975). This species has been recently introduced, i.e., since the time of European explorers, into these regions. *F. vesca* most likely initially arrived in North America from the east, i.e., Europe, and dispersed to the west developing into the North American subspecies (*F. v.* subsp. *americana* and *F. v.* subsp. *bracteata*).

While diploid strawberries have some barriers to inter-fertility, they can be crossed, and meiosis is regular even where interspecific hybrids are sterile (Hancock, 1999). At least three overlapping interfertile groups of diploid species have been suggested (Bors and Sullivan, 1998): (1) *F. vesca, F. viridis, F. nubicola* and *F. pentaphylla,* (2) *F. vesca*, *F. nilgerrensis*, *F. daltoniana* and *F. pentaphylla,* (3) *F. pentaphylla, F. gracilis and F. nipponica. Fragaria iinumae* may belong in group 3, as no fertile seeds have been recovered when it was crossed with either *F. vesca*, *F. viridis* or *F. nubicola*, but it has not been sufficiently artificially crossed with other species to accurately classify it. *Fragaria iinumae* does, however, have a glaucous leaf trait that is unique among the diploids, and its chloroplast RFLPs clusters it with *F. nilgerrensis* in a group that is isolated from the rest (Harrison et al., 1997).

Table 2 Characteristics of diploid *Fragaria* species (modified from Sargent et al., 2003 and Folta and Davis, 2006)

Species	Plant and stolon habit	Foliage	Inflorescence structure	Flowers	Fruit
F. daltoniana	Small; low-growing; short petioles; few hairs on all plant parts; deciduous habit Stolons deep red; sympodial	Small waxy leaflets; prominent petiolules	Single flowered; fully erect in flower and fruit; flowers above foliage; peduncles absent	Small; ovate, prominently veined and widely spaced petals; calyx clearly visible; round receptacle; large flat anthers; short stamens	Conic to cylindrical; bright pink skin; purple to black achenes; white flesh; woolly texture; no aroma
F. gracilis	Vigorous	Tri to penniform five foliate leaves with adpressed soft hairs	Self-incompatible,	Lanceolate sepals that reflex upon fruit ripening	Elongated ovoid
F. iinumae	Small; stout plants; thick leathery petioles and stolons; annual habit;leaves go dormant during the winter Stolons pinky-red; thick and leathery; sympodial	Glabrous leaves with large serrations; short petiolules	Few flowers per inflorescence; few inflorescences per plant; short peduncles	Medium-sized; long slender petals, always more than five; small receptacles; large numbers of anthers; short stamens	Conic; bright red skin; soft watery flesh; bright yellow achenes; unpleasant, blackcurrant-like flavor; acidic
F. *mandschurica*	Sympodial with short spreading hairs	Bright green leaves	Flowers bisexual, hermaphroditic, inflorescence mostly surpassing leaves, drooping with fruit	Petals very widely ovate to depressed ovate, almost orbicular, overlapping to not touching	Ovoid to broadly ovoid, sometimes globose. Achenes yellow-green to light brown when mature, in shallow pits or superficial

Table 2 (continued)

Species	Plant and stolon habit	Foliage	Inflorescence structure	Flowers	Fruit
F. nilgerrensis	Robust; vigorous; long, thick leathery petioles; thick pubescence on all plant parts; evergreen habit	Thick, leathery leaves; leaflets almost round; deep, prominent veins; distinct petiolules	Complex inflorescence, supporting many flowers; thick peduncles, erect to semi-erect in fruit	Medium sized; prominent, large round receptacles; narrow petals do not overlap; calyx clearly visible; large flat anthers	Globose-conic fruit; white skin; deeply pitted; firm flesh; small brown achenes; highly aromatic, peach-like aroma; clasping to spreading calyx
	Stolons deep-red; thick and leathery; sparsely produced; sympodial				
F. nipponica	Small; compact deciduous habit. Stolons pinky-red; slender and filiform; support a large number of daughter plants; monopodial	Many leaves that fold upwards with a bluish hue; insignificant petiolules	Simple inflorescence; peduncles droop when in fruit	Medium-sized; very small receptacle; almost circular petals; long prominent outer filaments	Short wedge-shaped berries; pinky-red skin; pleasant aroma like that of cultivated strawberry; long prominent calyx
F. nubicola	Small; not vigorous; few leaves; short petioles; remontant flowering habit; deciduous habit	Light-green leaflets, becoming darker around veins; short, almost absent petiolules	Simple inflorescence; few inflorescences per plant; peduncles clothed in thick pubescence; lie along ground in fruit; remontant flowering habit	Large; prominently veined petals small receptacle	Globose, necked berries; flattened on top; dark wine-red skin; tightly clasping calyx
	Stolons deep red; sympodial				

Table 2 (continued)

Species	Plant and stolon habit	Foliage	Inflorescence structure	Flowers	Fruit
F. pentaphylla	Compact; densely leafed; wavy petiolesStolons deep red; monopodial	Leaflets shiny, almost leathery; petioles support tertiary leaflets	Relatively simple inflorescence; slender peduncles; flowers above foliage	Large; large, contorted, wrinkled petals	Long conic berries, almost rectangular; knobbly appearance; pink to orangy-red skin; sunken achenes.White fruited form has larger, rounder berries
F. vesca	Robust and vigorous; long, gracile petioles; evergreen habit; *americana* subsp. is in general smaller, more gracile and less vigorous	Leaflets large; often curled under at edges	Complex inflorescence; peduncles stand erect in flower; *americana* subsp. has characteristically long pedicels and very short peduncles	Small; round, prominently veined petals; large receptacle; small anthers held on short filaments;Subsp. *americana* has smaller flowers	Long-conic to globose; usually bright red; distinctive, powerful aroma; large achenes raised or in shallow pits; clasping to reflex calyx
	Stolons green to brown; many slender, filiform stolons; sympodial				
F. viridis	Slender and erect; long, slender petioles; deciduous habit	Yellowish leaflets; petiolules almost absent	Complex inflorescence; peduncles above leaves in flower but lie along ground in fruit; remontant flowering habit	Large; medium sized receptacles; large, overlapping petals	Oblate to globose berries; pale green skin with a red blush; firm flesh; acidic apple-like aroma; very large achenes
	Stolons pinky-red; monopodial				

3 Higher Ploidy Species

Polyploidy in *Fragaria* probably arose through unification of 2*n* gametes. Unreduced gametes are relatively common (Hancock, 1999; Hancock et al., 2007). Bringhurst and Senanayake (1966) found frequencies of giant pollen grains (a result of unreduced gametes) to be about 1% of the total. Over 10% of the natural hybrids generated of these two species resulted from unreduced gametes. Staudt (1989) observed restitution in microsporogenesis of a F_1 hybrid of *F. virginiana* × *F. chiloensis*.

From the biogeography of the genus, the pattern of occurrence of the polyploids, and the distribution of specific characteristics, Staudt (1999) speculated on *Fragaria* origin and evolution. He suggested that East Asia is a center of origin for diploid strawberries. The tetraploid species ($2n = 4x = 28$) are also East Asian natives. More recently Rousseau-Gueutin et al. (2008) examined phylogeny of *Fragaria* using *GBSSI-2* and *DHAR* nuclear genes sequences (Fig. 2). They concluded that their results provided ‘evidence of the occurrence of multiple polyploidization events within *Fragaria* and of the allopolyploid origin of the hexaploid and octoploid species.’ However, they could not discriminate between autopolyploid versus allopolyploid origins for the five tetraploid species.

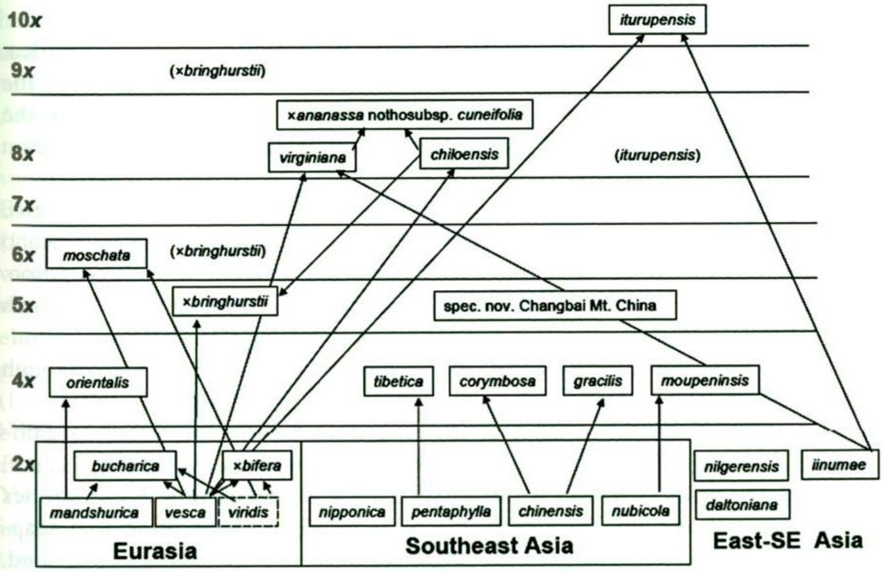

Fig. 2 Phylogenetic hypothesis for intrageneric relationships in *Fragaria* based on *GBSSI-2* and *DHAR* nuclear genes sequences. Each *line* represents species from a specific ploidy level (diploid to octoploid). Bracketed species present congruent data between the two genes. Species in the same bracket belong to the same clade. *Dashed lines* indicate a soft incongruence between the two datasets.

They suggested that ;the analysis of additional variable nuclear genes might provide the needed information to better resolve the relationships between closely related diploid *Fragaria* species, to clarify the position of *F. bucharica* and *F. viridis*, and to better understand the origin of all *Fragaria* polyploid species.'

The tetraploids may have spread to the periphery of diploid *Fragaria's* ranges. *Fragaria orientalis* is probably an autopolyploid of *F. mandschurica* (Staudt, 1959, 2003). The tetraploid *F. corymbosa* Los. has been submerged under *F. orientalis* (Staudt, 2003). *F. nilgerrensis* is probably the diploid progenitor of the tetraploid *F. moupinensis* (Darrow, 1966; Staudt, 1999). *Fragaria tibetica* seems to be a tetraploid descendent of *F. pentaphylla*. Heteroecy occurs in strawberries in association with doubling of the chromosome number. Diploid *Fragaria* species are not dioecious, though tetraploids (Staudt and Dickoré, 2001), and octoploids are. Tetraploid *Fragaria* species are interfertile (Hancock, 1999).

Wild, naturally occurring pentaploid ($2n = 5x = 35$) strawberry species have been observed in California (*F.* × *brinhurstii* Staudt) and Jilin, China (Lei et al., 2005). These strawberries produce no fertile offspring.

The hexaploid ($2n = 6x = 42$), *F. moschata*, is solely European. The musk strawberry, as it is commonly known, is a dioecious, tall vigorous plant that produces few runners. Leaves are large, dark green, rugose, rhombic, prominently veined and pubescent. The flowers are large and the inflorescence is superior to the foliage but droops with ripe berries. The fruit is purplish red, soft, irregularly globose and has a strong flavor. The calyx reflexes. Red and white fruited forms are cultivated (Hancock, 1999).

Native octoploid strawberries are found primarily in North and South America, however, a small distribution of one octoploid species occurs on Iturup, one of the Kurile Islands (Staudt 1989). This pattern of distribution could be explained if the first hypothetical octoploid arose in East Asia and migrated via an Alaskan-Siberian land bridge to North America.

A limited distribution of a few colonies of an Asian decaploid *F. iturupensis*, (Hummer et al., 2009) grows on the rock skree on the eastern flank of Volcano Atsunupuri, on Iturup, in the Kurile Islands, now part of the Russian Federation. This location might have provided a refugia from the most recent glaciation, which is reported to have come only as far south as the northern part of Iturup Island. *F. iturupensis* has fruit similar in shape and flavor components to those of *F. vesca*, though its plant and leaves resemble *F. virginiana* subsp. *glauca* (Staudt, 1989, 2008).

After arriving in Northwestern America, the hypothetical octoploid may have differentiated into two ecologically distinct groups such as are present today (Staudt, 1999). *Fragaria chiloensis* and *F. virginiana* may be extreme forms of one species that separated during the Pleistocene, and subsequently evolved differential adaptations. One group, *F. chiloensis*, became adapted to coastal habitat; the second, *F. virginiana*, to montane continental conditions. While spreading along the coast, *F. chiloensis* developed the typical shiny, coriaceous, glabrous leaves of the species that we know today. The dispersal of *F. chiloensis* to Hawaii and Chile may have occurred via bird migrations from North America (Hancock, 1999). Potter et al. (2000) found that *F. virginiana* and *F. chiloensis* carry similar cpDNA restriction fragment mutations. Nucleotide sequences of the nuclear internal transcribed spacer (ITS) region and two contiguous non-coding regions of cpDNA also support a sister relationship that may indicate a monophyletic origin for these two species. Staudt (1999) proposed that an ancient *F. vesca* and a hypothetical octoploid *Fragaria* ancestor could have been members of the Arcto-Tertiary flora present from Alaska to Greenland and Siberia that occupied temperate upland areas at middle latitudes in North America during the Eocene. Arcto-Tertiary flora invaded lowlands as temperatures decreased in the Miocene. Towards the end of the Miocene, many species moved developed that are closely related to those of the present day (Wolfe, 1969; Ritchie, 1984).

Staudt (1999) postulated that *F. iturupensis* may be a primitive form related to *F. virginiana* subsp. *glauca*. Thus far molecular analyses have concurred (Njuguna et al., 2007). Strawberries are reported on multiple islands surrounding Hokkaido and in the greater and lesser Kuriles. Further exploration and study of strawberries of northern Pacific Islands is needed to determine where other higher ploidy strawberry colonies exist and what their phylogenetic role may have been.

4 Cultivated Octoploid Strawberries

The most economically important strawberry, *F.* ×*ananassa* nothosubsp. *ananassa,* has $2n = 8x = 56$ chromosomes. It is an accidental hybrid of two octoploids and arose in the mid-1700's when plants of *F. chiloensis* imported from Chile were planted in France near *F. virginiana* transplanted from the eastern North America.

Three hypothetical genome formulae have been suggested for the wild and cultivated octoploids: AAAABBCC (Federova, 1946), AAA'A'BBBB (Senanayake and Bringhurst, 1967), and AAA'A'BBB'B' (Bringhurst et al., 1990). Bringhurst et al.'s (1990) suggestion reflects the contention that octoploids are completely diploidized with strict disomic inheritance. *F. vesca* is likely to be the A genome donor; *F. innumae*, the B genome donor (Davis et al., 2006). Hybrids of *F. iinumae* that had been chromosome doubled and *F.* ×*ananassa* were highly fertile. Studies of the AdH gene also confirm that ancestors of *F. iinumae* could be linked to the 'B' genome (Davis et al., 2006). *F. viridis* may also be part of the background of octoploid strawberries. Like *F. vesca*, its chromosomes pair regularly with those of *F. chiloensis*, *F. virginiana* and *F.* ×*ananassa*. *F. viridis* could represent the A' genome. Davis and Yu (1997) demonstrated that *F. nubicola* or *F. pentaphylla* could also be represented in the A' group because each are interfertile with *F. viridis*. The chromosomes of these species share high levels of homology.

Inheritance patterns of the octoploids are in dispute. Lerceteau-Köhler et al. (2003) concluded that *F.* ×*ananassa* has mixed segregation ratios using amplified fragment length polymorphism (AFLP) markers, as they found that the ratio of coupling vs. repulsion markers fell between the fully disomic and polysomic expectations. However, three other studies evaluating isozyme, simple sequence repeat

(SSR) and restriction fragment polymorphism (RFLP) segregation observed predominantly disomic ratios, indicating that the octoploid strawberry is completely diploidized (Arulsekar and Bringhurst, 1981; Ashley et al., 2003).

The incorporation of traits from a number of lower ploid species has been accomplished through pollinations with native unreduced gametes or by artificially doubling chromosome numbers. The utility of this approach has been shown for a wide range of species in *Fragaria* and in the related genus *Potentilla* (Hancock, 1999). Particular success in incorporating lower ploidies into the background of *F.* × *ananassa* has come through combining lower ploidy species and then doubling to the octoploid level (Bors and Sullivan, 1998).

5 Intergeneric Hybrid

Recently *Potentilla palustris* (L.) Scop. was crossed with *Fragaria* ×*ananassa* Duchesne ex Rozier to produce inter-generic hybrid plants that resemble a strawberry but have pink flowers. These plants are everbearing, with strawberry like fruit. Hammer and Pistrick (2003) have proposed the name *F.* ×*rosea* (Mabb.) K. Hammer et Pistrik for this artificial hybrid. Cultivars of these hybrids have been named and patented (Ellis, 1989).

6 History of Cultivation

6.1 Classical

'*Fraga*' is the Latin word for the strawberry. Linnaeus chose this as a derivative for the genus name. Roman poets Virgil and Ovid wrote of '*fraga*' in their poetry. Virgil (70 to 19 BCE), wrote '*humi nascentia fraga*' [child of the earth] in his third *Eclogue*. Virgil confirms that strawberries were not cultivated during his time when he writes only a warning to children picking wild strawberries to beware of serpents lurking in the grass. Ovid wrote of the '*arbuteos fructus mon-tanaque fraga*' [(They gathered) Arbutus berries and mountain strawberries] in his *Metamorphoses*, book I, v. 104, as furnishing a food of the golden age and again in the 13th book, '*mollia fraga*.' Pliny separates the, '*terrestribus fragis*,' [ground strawberry] from the arbutus tree in his lib. xv, c. 28. Cato, a Roman Senator (234-149 BCE) mentioned the medicinal uses of strawberries.

The Greeks, Theophrastus, Hippocrates, Dioscorides and Galen, however, did not mention strawberries; nor did other Latin writers on agriculture, Varro, Columella or Palladius. The strawberry is cited in Apuleius Platonicus for its medicinal value (Hedrick, 1919). Strawberries are not mentioned in the Bible, nor do they appear in any Egyptian or Greek art. This could be because of their northernly distribution of the species.

In the 12th century an abbess named Saint Hildegard von Binger declared strawberries unfit for consumption because they grew along the ground where snakes and toads most likely crawled upon them. Her words had such an effect on the local political figures that they, too, made similar declarations, discouraging the population from eating the berries. Among Europeans, this belief held for several years. In the mid 18th century, Charles Linnaeus, the Swedish botanist, put this superstition to rest by switching to a diet consisting only of strawberries to prove them edible (Darrow, 1966).

6.2 Old World

Fragaria vesca, the alpine strawberry or fraise de bois, was the first strawberry domesticated in the old world. The ancient Romans and Greeks originally cultivated it in gardens, and by the 1300's, this plant was being grown across Europe (Darrow, 1966). *F. vesca* had its widest popularity in the 1500's and 1600's in Europe before the introduction of strawberry species from the New World.

The musk-flavored *F. moschata* (hautbois or hautboy) was also planted in gardens by the late 15th century, along with the green strawberry, *F. viridis*. *F. viridis* was used solely as an ornamental all across Europe, while *F. moschata* was utilized for its fruit by the English, Germans and Russians.

6.3 New World

Fragaria vesca dominated strawberry cultivation in Europe, until *F. virginiana* from eastern Canada and Virginia began to replace it in the 1600's. Jacques Cartier, who discovered the St. Lawrence River in 1523, was most likely the first to bring *F. virginiana* to the Old World. Cartier mentioned strawberries numerous times in his diary (Hancock, 1999; Wilhelm and Sagen, 1974). The clones that arrived in Europe were wild because the aboriginal peoples of North America did not cultivate strawberries.

A Chilean clone of *F. chiloensis* was brought into Europe in the early 1700's by a French spy, Captain Amédée Frézier (Darrow, 1966; Wilhelm and Sagen, 1974). This strawberry had been domesticated in Chile for about 1,000 years by the indigenous Mapuches, and was spread widely by the Spanish during their colonization period (Hancock, 1999).

6.4 New World Species Brought to Europe

Unfortunately, after arriving in Europe, the Chilean strawberry did not bear fruit for several years and early reports on it were negative. The plants were barren because Frézier had brought back pistillate plants and the need for cross pollination was not recognized. The young French Botanist Antoine Nicholas Duschesne discovered that the 'Chili' would produce fruit when pollinized by *F. moschata* or *F. virginiana,* two other higher ploidy plants. The 'Chili' did not cross with the diploid *F. vesca*. The Chilean strawberry reached its highest acclaim in Brittany, and by the mid-1800's, probably more *F. chiloensis* was cultivated in France than in its native country.

Unusual seedlings with unique combinations of fruit and morphological characteristics began to appear in the gardens of Brittany after *F. chiloensis* was brought to France. While the origin of these seedlings was initially mysterious, Duchesne determined in 1766 that they were hybrids of *F. chiloensis* × *F. virginiana* and he named them *Fragaria* × *ananassa* to recognize the perfume of the fruit as smelling like pineapple (*Ananas*). The first hybrids of the 'Pineapple' or 'Pine' strawberry may have been selected early in the commercial fields of Brittany, and in botanical gardens across Europe.

The dessert strawberry, *F.* ×*ananassa*, now dominates strawberry cultivation and is grown in the arable regions of the world. *F. vesca* is generally restricted to home gardens where the small, aromatic fruit are considered a delicacy; most of the cultivars grown are everbearers. *F. chiloensis* is currently grown to a small extent in Chile, but has been largely replaced by *F.* ×*ananassa*. Neither *F. viridis nor F. moschata* is of current commercial importance.

6.5 Economic Importance

The USA is the leading producing nation with approximately 25% of the world's crop, followed by Spain, Japan, Poland, Italy and the Korean Republic. California dominates the strawberry industry in the USA with over 80% of the total production. The industries in Spain, the Korean Republic and the USA have grown steadily over the last two decades, while production in Japan, Italy and Poland have declined in the last decade, after dramatic increases in the 1970's and 1980's.

6.6 Nutritional Components

The strawberry is widely appreciated for its delicate flavor, aroma and nutritional value. Ripe strawberries are composed of approximately 90% water and 10% total soluble solids (Hemphill and Martin, 1992), and contain numerous important dietary components. They are extremely high in vitamin C and a standard serving of strawberries (10 fruit) supplies 95% of the recommended dietary requirements (Maas et al. 1996). The main soluble sugar components in strawberries are glucose and fructose, which are over 80% of the total sugars and 40% of the total dry weight (Wrolstad and Shallenberger, 1981). The primary organic acid is citric acid, which composed 88% of the total acids (Green, 1971). The strawberry also contains significant levels of ellagic acid, which is thought to be an anticarcinogenic (Maas et al., 1991).

Red color develops through the production of anthocyanins, primarily pelargonidin-3-glucosidase (Wrolstad et al., 1970; Kalt et al., 1993), although at least eight pelargonidin- and two cyanidin-based anthocyanins have been detected in strawberry juice (Bakker et al., 1994). Cyanidin 3-glucoside is the second most common anthocyanin. The total concentration of anthocyanins varies 16-fold across cultivars, and there is some variation in anthocyanin composition, although no clear associations between individual anthocyanins and color have been observed (Bakker et al., 1994).

Glucose, fructose and sucrose are the major soluble sugars found in the fruit of strawberries during all stages of ripening. Glucose and fructose are found in almost equal concentrations (Maas et al., 1996), and they rise continuously during fruit development from 5% in small green fruit to 6–9% in red berries (Kader, 1991). Sucrose levels are generally much lower, and show little accumulation until about the middle of fruit development (Forney and Breen, 1985). Invertases probably play an important role in regulating sweetness, by regulating hexose and sucrose levels (Ranwala et al., 1992; Manning, 1998).

Strawberry flavor is a complex combination of sweetness, acidity and aroma. The most intensely flavored fruits generally have high levels of both titratable acidity (TA) and soluble solids, while the blandest fruit are low in both these components (Kader, 1991). The primary components of flavor have not been completely elucidated, but strawberry aroma is thought to originate from a complex mixture of esters, alcohols, aldehydes and sulfur compounds (Dirinck et al., 1981; Pérez et al., 1996). Hundreds of volatile esters have been correlated with strawberry ripening and aroma development, with methyl- and ethyl-esters of butanoic and hexanoic acids being among the most prevalent (Larsen and Poll 1992; Pérez et al.; 1992, 1996). Other components in high concentration are trans-2-hexenyl acetate, trans-2-hexenal, trans-2-hexenol and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol). Concentrations of these volatiles vary widely among cultivars and produce large variations in aroma quality (Hirvi, 1983; Shamaila et al., 1992; Pérez et al., 1996, 1997).

Aroma and fragrance content also varies across species. Several researchers consider 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) and 2,5-dimethyl-4-methoxy-3(2H) furanone (mesifurane) as being particularly important aroma contributors (Pyysalo et al. 1979; Larson and Poll 1992; Sanz et al. 1994; Pérez et al., 1996). The wild species *F. vesca* and *F. virginiana* have much stronger aroma than the cultivated types (Hirvi and Honkanen, 1982). *F. vesca* contains high amounts of ethyl-acetate, but low amounts of methyl-butyrate, ethyl-butylate, and furanone. *Fragaria nilgerrensis* contains high levels of ethyl-acetate and furanone, but low levels of methyl-butyrate and ethyl-butyrate. Hybrids between *F. vesca* and *F. ×ananassa* have intermediate levels of fragrance and aroma, while crosses between *F. nilgerrensis* and *F. ×ananassa* more closely resemble *F. nilgerrensis*.

7 Traditional Breeding

The dessert strawberry is an outcrossing crop that is sensitive to inbreeding (Morrow and Darrow, 1952; Melville et al., 1980). It is asexually propagated by runners, so most breeding programs have been based on pedigree where elite parents are selected for intercrossing each generation. The strawberry germplasm base is relatively narrow (Sjulin and Dale, 1987), but if adequate population sizes are maintained, changes in levels of homozygosity across generations appear to be minimal (Shaw, 1995). Since highly heterozygous genotypes can be propagated as runners, few breeding programs have developed hybrid cultivars using inbred lines, although a few cultivars have been developed in this manner.

Selfing has been used in a number of instances to concentrate genes of interest (Hancock et al., 1996) and backcrossing can incorporate specific traits. Barritt and Shanks (1980) moved resistance to the strawberry aphid from native *F. chiloensis* to *F.* ×*ananassa*. Bringhurst and Voth (1978, 1984) transferred the day neutrality trait from native *F. virginiana* subsp. *glauca* to *F.* ×*ananassa*. About three generations were necessary to restore fruit size and yield to commercial levels.

In 1817, formal strawberry breeding was initiated in England by Thomas A. Knight (Darrow 1966; Wilhelm and Sagen 1974). He was one of the first systematic crop breeders. He used clones of both *F. virginiana* and *F. chiloensis* in his crosses. He produced 'Downton' and 'Elton' cultivars, noted for their large fruit, vigor and hardiness. Michael Keen, a market gardener near London, also became interested in strawberry improvement about this time and developed 'Keen's Imperial' whose offspring, 'Keen's Seedling' is in the background of many modern cultivars. This cultivar dominated strawberry acreage for about 100 years.

Thomas Laxton of England was the most active breeder during the later part of the 18th century. He released 'Noble' and 'Royal Sovereign'. These two cultivars were grown on both sides of the Atlantic, and were popular until the middle of the 20th century. 'Nobel' was known for earliness, cold hardiness and disease resistance. 'Royal Sovereign' was popular because of earliness, productivity, flavor, attractiveness and hardiness.

In 1836, Charles Hovey, of Cambridge, Massachusetts, produced the first important North American strawberry, 'Hovey', by crossing the European pine strawberry, 'Mulberry' with a native clone of *F. virginiana*. This was the first American fruit cultivar produced from an artificial cross. For a while this strawberry was the major pomological product in the country (Hedrick 1925).

Albert Etter of California developed dozens of cultivars around the turn of the century with native *F. chiloensis* clones (Fishman 1987). His most successful cultivar was Ettersburg 80 (1910), which was widely grown in California, Europe, New Zealand and Australia. It was renamed 'Huxley' in England and was popular until 1953. Ettersburg 80 was extremely drought resistant, of high fresh and processing quality, because of the solid bright red color. Other outstanding Etter cultivars were 'Ettersburg 121', 'Fendalcino' and 'Rose Ettersburg'. While his releases were very successful as cultivars, their greatest lasting impact was as breeding parents. Most California cultivars (and many others) have an Ettersburg cultivar in their background (Darrow 1966; Sjulin and Dale 1987).

In the middle of the 20th century, a number of particularly successful breeding programs emerged in Scotland, England, Germany and Holland. In Scotland, Robert Reid developed a series of red stele resistant cultivars utilizing American 'Aberdeen' as a source of resistance. His cultivar 'Auchincruive Climax' dominated acreage in Great Britain and northern Europe until its demise due to June yellows in the mid-1950's. In England, D. Boyle produced a large series of cultivars with the prefix 'Cambridge'. 'Cambridge Favorite' (1953) became the most important of the group and dominated the acreage in Great Britain by the 1960's. In Germany, R. von Sengbusch's produced a 'Senga' series, of which 'Senga Sengana' (1954) became paramount. 'Senga Sengana' was widely planted for its processing quality and is still important in Poland and other eastern European countries. In the Netherlands, H. Kronenberg and L. Wassenaar's released several cultivars, of which 'Gorella' (1960) made the greatest impact. It was noted for its size, bright red glossy skin and red flesh. B. Meelenbroek who followed in this program released 'Elsanta' (1981), considered the ideal fresh market cultivar for its bright color, flavor and regular size.

Many breeding advances in the eastern United States have come from the U. S. Department of Agriculture (Hancock, 1999). George Darrow at Beltsville, Maryland, developed 'Blakemore' which became the major southern US cultivar in the mid-1930's and 'Fairfax' was widely planted in the middle of this century from southern New England to Maryland and westward to Kansas. These two cultivars were used extensively in breeding, finding their way into the ancestry of a diverse array of cultivars grown in all parts of the US. Other important releases from Darrow were 'Pocahontas', 'Albritton', 'Surecrop' and 'Sunrise'. D. H. Scott, A. D. Draper and G. J. Galletta followed Darrow and released 'Redchief' (1968), 'Earliglow' (1975), 'Allstar' (1981), and 'Tribute' and 'Tristar' (1981). All of these cultivars are still grown today. Tribute and Tristar were the first day-neutrals widely grown in the eastern US and remain the leaders today. An active USDA breeding program has also been conducted at Corvallis, Oregon, initially by Darrow, G.F. Waldo and F.J. Lawrence, and now C. Finn. Some of the more important cultivars emerging from this program were 'Siletz' (1955) and 'Hood' (1965). 'Hood' is considered the premier berry for processing.

Several other state and federal supported programs have released important cultivars in the USA and Canada. Some of the most significant ones from the USA were 'Honeoye' and 'Jewel' (New York), 'Raritan' (New Jersey) and 'Sweet Charlie' (Florida). From Nova Scotia came 'Bounty', 'Glooscap' and 'Kent'.

H. Thomas and E. Goldsmith's of the University of California released the important cultivars 'Lassen' and 'Shasta' in 1945. 'Shasta' was widely grown in the central coast of California in the 1950's and 1960's because of its large size, firmness and long season. 'Lassen' was grown extensively in southern California about the same period, prized for its short rest period and high productivity. R. Bringhurst and V. Voth took over the California-Davis program in the 1950's and generated a succession of internationally important, Mediterranean adapted cultivars have including 'Tioga' (1964), 'Tufts' (1972), 'Aiko' (1975), 'Pajaro' (1979), 'Chandler' (1983), 'Selva' (1983), 'Camarosa' (1992) and 'Seascape' (1991). Most recently, Doug Shaw has released 'Diamonte' from this program.

The greatest concentration of breeding activity outside of Europe and the USA has been in Japan. Two very important cultivars were produced there: H. Fukuba's 'Fukuba' (1899), noted for its large size and high flavor (Darrow, 1966), and K. Tamari's 'Kogyoku' (1940), respected for its vigor, earliness and fruit size. 'Fukuba' was the most important cultivar in forcing culture until the early 1970's. 'Kogyoku' was one of the leading field grown cultivars after World War II, until it lost importance to the American import 'Donner' in the 1950's.

8 Structural Genomics

Marker systems have been developed in strawberry for genetic linkage mapping and QTL analysis (Sargent et al., 2004, 2007; Hadonou et al., 2004). These are broadly applicable across strawberry species, although SSRs developed from other Rosaceae species show only limited amplification. Davis and Yu (1997) provided the first diploid map of *F. vesca*, using RAPD markers and isozymes, plus some morphological traits. They crossed *F. vesca* f. *semperflorens* 'Baron Solemacher' and a wild clone of *F. vesca* ssp. *vesca* from New Hampshire, and developed an 80-marker map in the F2 population that represented the seven linkage groups and was 445 cM long. Unusually high levels of segregation distortion were noted (47%) that were skewed toward 'Baron Solemacher'. Davis and Yu speculated that the segregation distortion was caused by the maternal cytoplasm favoring maternal genes. Davis also used a candidate gene approach to determine the molecular basis of the yellow fruit color locus (c) in diploid strawberry. Using PCR they degenerated primer pairs to examine segregation patterns in intron length polymorphisms of genes involved in the anthocyanin biosynthetic pathway. They studied F2 progeny populations of a wild clone of northern California *F. vesca* × *F. vesca* 'Yellow Wonder' and 'Yellow Wonder' × *F. nubicola* from Pakistan, and were able to place five genes into their previously published map. They found F3H, the gene encoding flavanone 3-hydrolase, to be the likely candidate for the yellow fruit color locus.

Most recently, a diploid map of 78 markers was constructed from a hybrid population of *F. vesca* subsp. *vesca* f. *semperflorens* × *F. nubicola* (Sargent et al., 2004). The authors used a combination of SSRs, SCARs, gene specific markers and morphological markers that came from the GenBank data base and other studies. The seven linkage groups were identified in their 448 cM map. Segregation distortions were noted at 54% of the loci that were skewed toward the paternal parent F. nubicola. They speculated that the segregation distortions were due to meiotic irregularities or the self-incompatible nature of *F. nubicola*. Only one octoploid map has been published to date. Lerceteau-Köhler et al. (2003) used 727 AFLP markers and 119 individuals to build both a female and a male map from the cross of 'Capitola' × CF1116 [Pajaro × (Earliglow × Chandler)]. The female map was built with 235 markers and was 1604 cM long, while the male map was 1496 cM long with 280 markers. Only 3.2% of the markers displayed distorted segregation ratios. They detected 30 linkage groups on the female and 28 on the male side, but did not develop a consensus map of the two parents. The female genome was estimated at 2870 cM, while the male was 1861 cM. Two abstracts have described work on octoploid genetic maps, Viruel et al. (2002) and Weebadde et al. (2008). Viruel et al. used 300 SSR and RFLP markers and 86 progeny to build a consensus linkage map with 17 linkage groups and a total distance of 627 cM. 120 markers were unlinked or linked to only one marker, suggesting the need for more markers to build a complete map. Only 10% of the markers showed distorted segregation ratios. Weebadde et al. genotyped fifty-seven individuals of the cross 'Tribute' × 'Honeoye' with AFLP markers. Out of 611 polymorphic bands obtained using 52 primer combinations, 410 single dose fragments (SDRFs) were identified and 23 linkage groups. Most of the markers (255 out of 410) remained unlinked, indicating the need for more markers and larger population sizes to build a map with wide genome coverage.

9 Functional Genomics

Only a few strawberry QTL analyses have been conducted. In Weebadde et al. (2008), two AFLP markers were significantly associated with segregation of the day-neutrality trait at a 0.01% level and five at a 0.1% level. Several of these markers were not linked, indicating that day-neutrality is a quantitative trait in the octoploids. Haymes found AFLP markers linked to three red stele resistance genes (Hokanson and Maas, 2001). Lerceteau-Köhler et al. (2003) found fourteen QTL associated with seven characters (fruit height, ratio fruit height/diameter, fruit color, firmness, malate content, glucose content and ratio fructose to glucose). The percentages of phenotypic variance explained by the QTLs ranged from 12 – 20%.

10 Biotechnological Approaches to Genetic Improvement

Currently, two transgenic herbicide resistant cropping systems are common for soybean, maize, rapeseed, and cotton: RoundupReady® (active agent: glyphosate) and Liberty Link® (active agent: glufosinate). These systems may have application for strawberry. Weed infestation in fields is one of the major problems in all small fruit crops, particularly strawberry. To control weeds in many crops, non-selective and broad-spectrum herbicides, such as glyphosate and phosphinothricin, are used although they can only be applied as a directed spray under the bushes and avoiding any contact with the green tissues. Herbicide-resistant plants can be expected to broaden the application of non-selective herbicides and to provide a simple, inexpensive, potent, and environmentally friendly management for weed control.

With the impeding ban on methyl bromide (MB) fumigation in strawberry production, growers have lost the most effective control for weeds and soil pathogens.

Yields in today's cultivars are reduced by 50% in non-fumigated soil. Limited genetic variability for resistance has been found to the broad range of pathogens controlled by MB fumigation (Particka and Hancock, 2005). Herbicide resistant strawberries with the *CP4.EPSP* synthase gene or *PAT* would allow for the effective control of weeds, and a transgene like *pcht28* that provided resistance to a broad range of fungal pathogens could restore considerable yield potential. To minimize marketers concerns about the acceptance of transgenic fruit, targeted expression of these genes to just vegetative tissue would likely be required. The use of marker-free selection systems might also prove beneficial.

The incorporation of transgenes for fruit rot resistance and increased firmness in strawberries, such as the antisense of genes for pectate lyase and polygalacturonase-inhibiting protein, would be highly beneficial if the public will accept them. Strawberries are highly perishable, and even with controlled atmosphere storage and refrigeration, a high proportion of fruit are lost due to softening and fungal disease. In particular, *Botrytis* and *Phytopthora* are sources of substantial crop losses.

Transgenic breeding could also provide resistance to major virus diseases such as strawberry crinkle and yellows. In addition to expression of coat protein for protection against these diseases, application of RNA interference (RNAi) technology could be exploited to obtain virus-resistant plants (Tenllado et al. 2004; Hoffmann et al. 2006).

The distribution of small fruit cultivation is often restricted by low temperature stress. Frost tolerance during bloom and winter cold hardiness may reduce crops. While some genetic variability for these characteristics exists, very little improvement has been made by plant breeders. The incorporation of genes such as *CBF1* could provide the necessary genetic variability to improve this trait, if phenotypic effects could be limited by targeting expression to floral tissues or cold periods (Kasuga et al. 2004).

Cool temperatures during flowering also limits yields. When temperatures are low during bloom, pollinator activity is greatly reduced leading to poor seed set, reduced fruit size, irregularly shaped fruits and low yield. The incorporation of a gene that induces parthenocarpic fruiting like the *defH9-iaaM* auxin-synthesizing gene would be very beneficial in areas where cool conditions prevail during pollination.

Transgenic approaches might also be employed to modify the secondary metabolism of strawberries to improve their nutritional quality and flavor (Scalzo et al., 2005). Successful modification of the nutritional value of tomatoes through metabolic engineering and transformation has provided a novel example of the use of organ-specific gene silencing to enhance the nutritional value of fruits. Biochemical pathways might be altered through transgenic approaches to enhance anthocyanin production and strengthen aroma. Numerous genes have been identified in strawberry that are associated with aroma (Aharoni et al., 2000, 2004) and flavonoid metabolism (Manning, 1998).

Several obstacles are working against the acceptance of transgenic strawberries. The economic value of these fruit crops is limited compared to many of the agronomic crops and as a result there is only modest private stimulus to develop new biotechnological products. A second issue is that strawberries outcross and have widespread, native relatives in close proximity to cultivated fields. Most transgenic releases to date have been with species that do not have nearby congeners, greatly reducing the risk of the movement of the transgene into wild species populations. The release of transgenic strawberries will require more scrutiny and in-depth ecological studies than have been required of previous releases. A third issue is reluctance of the fruit industry to introduce products with a potential negative backlash from people leery of consuming transgenic crops.

A strong influx of federal and state funds, along with a careful analysis of what people's perceptions are regarding transgenic fruit is needed to stimulate strawberry biotechnology research. Until this happens, transgenic strawberries will remain as a research tool without commercialization. Using marker-free transformation systems and targeted expression of transgenes will minimize public concern, but the fear of the technology must be abated before transgenic strawberries will be commonly accepted.

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