GENETICS OF REMONTANCY IN OCTOPLOID STRAWBERRY (*Fragaria* × *ananassa*)

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ABSTRACT

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Flower initiation in strawberry genotypes is primarily determined by two environmental factors: photoperiod and temperature. Commercially grown strawberries are generally classified as remontant (repeat flowering) or short day types based on their photoperiod requirement for flower initiation. However, both types will flower in any photoperiod when temperatures are sufficiently cool and flower initiation is inhibited beyond a temperature threshold. The currently available remontant genotypes do not perform well in the extreme heat of midwestern summers. Therefore it is necessary to develop remontant cultivars tailored to the midwestern environmental growing conditions by incorporating heat tolerance and/or other sources of remontancy.

This research was designed to identify the regions of the genome that regulate heat tolerance and remontancy in a population developed from 'Honeoye' × 'Tribute', where 'Tribute' is a remontant parent and as a result the progeny segregated for remontancy. A SSR-based linkage map was generated and the QTL associated with remontancy and duration of flowering were identified using phenotypic data collected in multiple environmental conditions (MI, MN, MD, OR, CA) and multiple years (2005, 2006, 2011). In addition, the same population was grown under different temperatures in the greenhouse to observe segregation of heat tolerance in the progeny. Flowering phenotypic data collected from the different temperature environments were used to identify QTL associated with heat tolerance.

The ‘Honeoye’ × ‘Tribute’ linkage map consisted of 34 linkage groups (LG) and heat tolerance QTL were identified on 8 linkage groups. Five of the heat tolerance QTL co-located with remontancy QTL indicating that the commonly observed photoperiodic response in the field may actually be due to differences in heat tolerance. Remontancy QTL from all 5 field locations overlapped at 8 chromosomal locations. QTL associated with remontancy in the cooler western states (CA and OR) co-located in three LG regions and QTL for remontancy in the warmer states (MI, MN, MD) co-located in two LG regions. Duration of flowering QTL co-located with several remontancy QTL indicating that our way of phenotypic categorization of remontant vs non-remontant trait was able to identify regions of the genome that determine extended flowering season. Duration of flowering QTL co-located with heat tolerance QTL suggesting that the ability of a plant to have an extended flowering season is dependent on its ability to tolerate extreme summer temperatures. Five markers associated with the heat tolerance trait were identified and several progeny that were both heat tolerant and remontant were identified. These markers associated with heat tolerance should be validated on a larger panel before their use in marker-assisted breeding. However, the most heat tolerant, remontant progeny may be used in further crosses to develop cultivars better suited to the hot, midwestern climate.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction and objectives

Strawberries are among the most commercially important small fruit crops in the world. Most of the commercial production is in the Northern Hemisphere, although the environmental conditions in the southern hemisphere are also suitable for growing strawberries (Hummer and Hancock, 2009). United States is the leading producer of strawberries, producing 1,270,690 metric Tons in 2009 (http://faostat.fao.org). The total value of fresh and processed strawberries produced in US in 2009 was $158,665,000 (USDA Economics, Statistics, and Market Information System). California is the largest producer of strawberries and produced 2,485.6 million pounds of strawberries in 2009 (USDA Economics, Statistics, and Market Information System). Michigan produced 4.6 million pounds of strawberries the same year. The commercial strawberry, *Fragaria* × *ananassa*, is an octoploid derived from hybridization of *F. virginiana* and *F. chiloensis*.

Flower initiation in strawberries is affected by photoperiod and temperature. A wide range of cultivars have been developed that have been categorized from short-day to remontant based on their photoperiodic requirement for flowering (Durner *et al.*, 1984; Hancock, 1999). Over 60% of the commercially grown cultivars in California are remontant, while most grown in the eastern US are short-day (Hancock, 1999). Floral initiation in remontant cultivars is not affected by photoperiod. They produce crops about 60 days after planting, regardless of season, and they can have several crops during the growing season. On the other hand, short day cultivars initiate flowers in the shorter days of winter and as a result they bear fruits only at the beginning of spring. In the Californian growing regions, where there is an extended growing season, short day cultivars are grown from Jan-Apr and remontant types are grown from Apr-Oct (Hancock,

1999). Many growers in the midwestern and eastern US would prefer remontant cultivars because they produce multiple harvests. However, flower initiation in the currently available remontant cultivars is generally inhibited by the extremely hot temperatures during summer in the midwestern and eastern US. Therefore, the available remontant cultivars do not perform well in these conditions and it will be necessary to incorporate new sources of remontancy or develop heat tolerant remontant cultivars that are better suited to the extreme temperatures.

Genetic control of remontancy has been debated and several hypotheses have been proposed that range from single to multiple gene control. Weebadde *et al.* (2008) identified several QTL determining remontancy in *F.* × *ananassa* ('Honeoye' x 'Tribute') in a multi-location study and proposed that heat tolerance QTL (Quantitative Trait Loci) may be acting along with photoperiod perception QTL in determining flower initiation. Bradford *et al.* (2010) determined that temperature plays a crucial role in determining whether the plant initiates flowers under short or long days. Both these studies concluded that in order to have a better understanding of the regulation of remontancy, it is important to identify the loci regulating temperature tolerance/sensitivity in the genome. In addition, since there are several sources of remontancy that are available for breeding, it is important to determine whether these sources share the same QTL or whether additional remontancy loci may be available that can be pooled together to develop new heat tolerant remontant cultivars for midwestern and eastern US climates.

The specific objectives of this project were to:

Quantify the effect of temperature on flower and runner production in a population segregating for remontancy ('Honeoye' x 'Tribute').

Create a genetic linkage map of octoploid strawberry *Fragaria* x *ananassa* using SSR markers.

Identify QTL linked to heat tolerance, remontancy, and duration of flowering in the 'Honeoye' x 'Tribute' population.

1.2 Ploidy in the genus *Fragaria*

Commercial strawberry belongs to the genus *Fragaria* in the family Rosaceae and sub-family Potentilloideae (Hummer and Hancock, 2009). The genus includes 24 species that range in ploidy from diploid to decaploid (Staudt 1989, 2009; Hummer *et al.*, 2009) (Table 1.1). All of the species, except *F. chiloensis* are native to the northern hemisphere (Hancock *et al.*, 1991; Hancock, 1999; Potter *et al.*, 2000). *Fragaria ves*ca is the most common diploid species and is the most widely distributed in the world (Staudt, 1989; Hancock, 1999). *F. vesca* is native to regions in Europe, Asia, and North and South America. Other diploid species include *F. viridis* Duch. (native to Europe and Western Asia), *F. daltonica* (Sikkim, Himalayas), *F. nilgerrensis* Schlecht (south Asia, Sikkim, China), *F. nubicola* Lindl. ex Lacaita (Central Asia, Himalayas), *F. gracilisa* Lozinsk (North China), *F. pentaphylla* Lozinsk (North China), *F. mandshurica* Staudt (Siberia, Mongolia, Manchuria, Korea), *F. innumae* Makino (central and northern Japan), *F. yezoensis* Hara. (North Japan), and *F. nipponica* Lindl. (Japan) (Staudt, 1989; Hancock *et al.*, 1991; Hancock, 1999). All known tetraploids are native to regions in Eurasia: *F. orientalis* Lozinsk (Siberia, Mongolia, Manchuria), *F. corymbosa* (North China), and *F. moupinensis* (French.) Card. (China) (Staudt, 1989; Hancock *et al.*, 1991; Hancock, 1999). Only one hexaploid species has been described, *F. moshchata* Duch., and it is native to north and central Europe (Staudt, 1989; Hancock *et al.*, 1991; Hancock, 1999). Octoploid species include *Fragaria chiloensis* (L.) Duch. (native to North and South America), and *F. virginiana* Duch.

(native to Central and North America) (Staudt, 1989; Hancock, 1999). *F. iturupensis* (native to Iturup Island, Japan) was initially classified as an octoploid (Staudt, 1989; Hancock, 1999, Staudt 2009), but subsequent flow cytometry analysis revealed that it is a decaploid (Hummer *et al.*, 2009). *Fragaria* × *ananassa* is the cultivated strawberry and is grown in many regions of the world. It was formed by hybridization between Chilean *F. chiloensis* and North American *F. virginiana* that were growing in proximity in Europe (Hancock, 1999). Natural hybrids of *F. chiloensis* and *F. virginiana* have also been found in the western parts of North America by Nuttal and described as *F.* × *ananassa* nm *cuneifolia* (Staudt, 1989; Hancock 1991).

Table 1.1 Ploidy levels in *Fragaria* species along with their genomic models as proposed by Rousseau-Gueutin *et al.* (2009) and their geographical origin.

Species	Ploidy (2n=)	Genome model (Rousseau-Gueutin *et al.*, 2009)	Location
F. vesca	2x	Y1	Europe, Asia, North and South America
F. viridis Duch.	2x	Y2	Europe and Western Asia
F. daltonica	2x	X1	Sikkim, Himalayas
F. nilgerrensis Schlecht	2x	X2	South Asia, Sikkim, China
F. nubicola Lindl. ex Lacaita	2x	X1	Central Asia, Himalayas
F. gracilisa Lozinsk	2x		North China
F. pentaphylla Lozinsk	2x	X1	North China
F. mandshurica Staudt	2x	Y1	Siberia, Mongolia, Manchuria, Korea
F. innumae Makino	2x	Z	North and Central Japan
F. yezoensis Hara.	2x	X1	North Japan
F. nipponica Lindl.	2x	X1	Japan
F. orientalis Lozinsk	4x	Y1Y1Y1Y1	Siberia, Mongolia, Manchuria
F. corymbosa	4x		North China
F. moupinensis(French.) Card.	4x		China
F. moshchata Duch.,	6x	Y1Y1Y2Y2Y2Y2 or Y1Y1Y1Y1Y2Y2	North and central Europe
Fragaria chiloensis (L.) Duch.	8x	Y1Y1Y1Y1ZZZZ	North and South America
F. virginiana Duch.	8x	Y1Y1Y1Y1ZZZZ	Central and North America
F. iturupensis	10x		Iturup Island (Japan)

1.3 Genomic model of *F.* × *ananassa*

Cultivated strawberry, *Fragaria* × *ananassa*, is an allo-octoploid with chromosome number 2n=8x=56. Cytogenetic studies have proposed three genomic models for the cultivated strawberry: AABBBBCC (Federova, 1946 in Hancock, 1999), AAA'A'BBBB (Senanayake and Bringhurst, 1967), and AAA'A'BBB'B' (Bringhurst 1990). In the original model, the A genome was thought to be contributed by *F. orientalis*, B genome by *F. nipponica*, and C genome by *F. vesca*. However, later models concluded that the A genome came from *F. vesca* or *F. viridis*, while the origin of the B genome was unknown. Hancock (1999) suggested that *F. vesca, F. viridis*, or *F. nubicola* may have contributed the A and A' genome. He also suggested that the B genome may have originated from *F. innumae* because it has glaucous leaves that is seen in some octoploids and can be crossed with *F.* × *ananassa*. Several other studies have also concluded that *F. vesca* is one of the diploid ancestors of *F.* × *ananassa*. Bringhurst and Khan (1963) identified naturally occurring hybrids of *F. chiloensis* and *F. vesca* from the coastal regions of California. Staudt (2009) discussed the origin of octoploid *F. virginiana* and *F. chiloensis* based on his studies of ploidy levels, stolon branching, pollen morphology, and sex expression in 24 species of *Fragaria*. He proposed that *F. daltonica* may be an ancestor of *F. chiloensis* based on similarities in the leaf morphology and fruit color of the two species. In addition, Staudt (2009) proposed *F. innumae* and *F. chinensis* may be progenitors of *F. virginiana* from eastern and western N. America.

In recent years, molecular phylogenetic approaches have been used to determine the inter-relationship among the various species of *Fragaria*. Harrison *et al.* (1993) studied the relationship between 30 *Fragaria* accessions from 9 species using RFLP (Restriction Fragment

Length Polymorphism) markers on the chloroplast genome. Their study concluded that *F. innumae* was ancestral to the other species because it had one mutation that was common with *Potentilla fruticosa* which was the outgroup in this analysis. Potter *et al.* (2000) studied variations in the non-coding regions of chloroplast and nuclear DNA in 43 *Fragaria* accessions from 14 species that included representatives of naturally occurring ploidy levels. They concluded that *F. vesca* and *F. nubicola* were the most closely related to the octoploids *F. virginiana* and *F. chiloensis*. They also concluded that *F. virginiana* and *F. chiloensis* originated from a common octoploid ancestor. Some accessions of *F. virginiana* (*F. virginiana* subsp. *platypetala*) were more closely related to accessions of *F. chiloensis* than to *F. virginiana*. Similar observations were made in an earlier study by Harrison *et al.* (1997) when they used RAPD (Random Amplified Polymorphic DNA) markers to identify variation between octoploid *F. virginiana* and *F. chiloensis* and observed that *F. virginiana* ssp. *platypetala* had closer similarity to *F. chiloensis* than with other subspecies of *F. virginiana*.

In the most recent study (Rousseau-Gueutin *et al.*, 2009), the sequences of two nuclear genes (*GBSSI-2* and *DHAR*) were compared from diploid, tetraploid, hexaploid, and octoploid *Fragaria* and it was concluded that the diploid *Fragaria* species can be divided into three main clades: X, Y, and Z (Table 1.1). The tetraploids originated from diploids of clades X and Y. The three octoploid species were shown to have allopolyploid constitution and originated from the Y and Z lineages. Their study demonstrated that the commercial octoploid strawberry that originated from *F. virginiana* and *F. chiloensis* consists of the Y and Z genomes. The Y genome represents *F. vesca* and the Z genome was contributed by *F. innumae*, making it the other likely diploid progenitor of *F.* x *ananassa*. Although strawberry is an octoploid, at least two studies (Lerceteau-Kohler *et al.*, 2003 and Rousseau-Gueutin *et al.*, 2008) concluded that the genome is

largely diploidized based on segregation patterns of AFLP (Amplified Fragment Length Polymorphism) and SSR (Simple Sequence Repeat) markers in a mapping population.

1.4 Flowering types in strawberry

Flower initiation in strawberries occurs in response to environmental conditions and strawberry genotypes have been typically classified based on their flowering response to photoperiod as short day, long day, day-neutral and everbearing types (Figure 1.1). Among these, what has been called short day genotypes is the most clearly defined group. They initiate flowers in the late summer through winter and then flower only once in early spring (Hancock *et al.*, 1991; Taylor, 2002; Stewart and Folta, 2010). The time of flowering in short day types varies with their specific chilling requirements (Hancock *et al.*, 1991). The other flowering types are not so clearly defined (Taylor 2002). The strawberry genotypes that are thought to have no specific photoperiod requirement have been referred to as day-neutrals. The day-neutrals have several cycles of flowers from early spring through late summer until the plant goes dormant. These genotypes are perhaps more appropriately described as remontant because of their repeated cycles of flowering (Bradford *et al.*, 2010). The day-neutral or remontant types are often confused with what have been called everbearing and long day types (Bringhurst *et al.*, 1989; Taylor, 2002; Hancock *et al.*, 1991). The term everbearing has been given to those genotypes that have an extended flowering season. These genotypes are variously described as being photoperiod sensitive or insensitive. The long day types are thought to flower in response to the long (>14 hr) photoperiods in summer. These genotypes may also have an extended flowering season from mid to late summer and are sometimes also categorized as everbearing (Hancock *et al.*, 1991; Stewart and Folta, 2010). In this dissertation, the term remontant will be used to refer

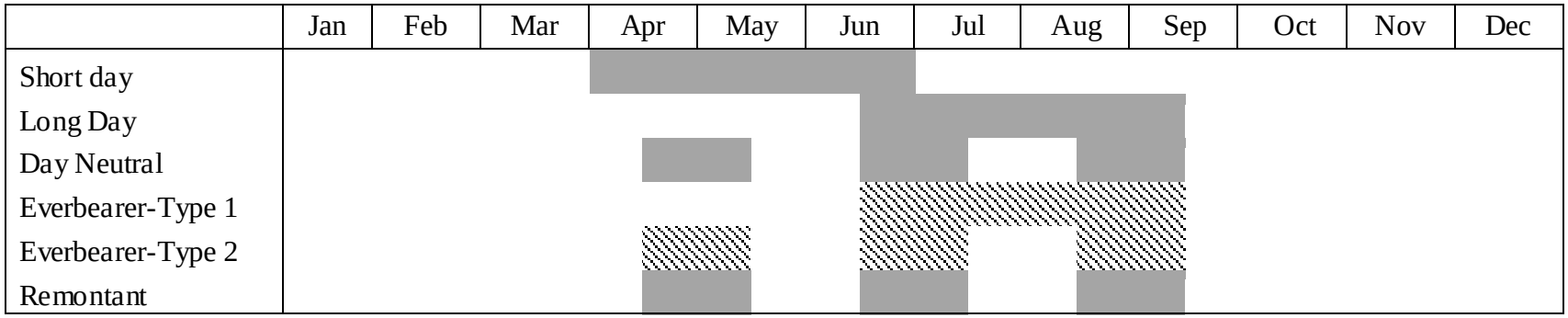
to any octoploid genotype that has extended or multiple cropping seasons and flowers under both long and short days. Although there are multiple flowering responses in the octoploid strawberries, the diploid strawberry has clearly defined flowering types: short day type *F. vesca vesca* and repeat flowering type *F. vesca semperflorens* (Taylor 2002).

Taylor (2002) summarized the processes involved in flower formation in strawberry in three stages: induction, when environmental and growth conditions create a stimulus to flower, and the plant transitions to the reproductive stage; initiation, when the floral primordia are differentiated following physiological and morphological changes caused by the stimulus; and differentiation, when the floral primordia develop into flowers. Photoperiodic response in strawberry depends on three factors, temperature, genotype, and chilling (the minimum duration of cold temperature required for a plant to break dormancy) (Stewart and Folta, 2010).

Based on their flowering habits, growers in midwestern and north-eastern US would prefer remontant genotypes to extend the fruiting season and get maximum yield in the short growing season. However, as Dale *et al.* (2002) highlighted, several factors impede breeding for remontant cultivars adapted to the northern regions of North America. Among them are variations in segregation ratios when remontant and non-remontant genotypes are crossed. This is a clear reference to the fact that the remontant trait is a multi gene trait. Dale *et al.* (2002) also pointed out that the extreme high temperatures during summer and the short growing season affects expression of the trait and as a result the phenotypic ratios observed may not be accurate. In addition, fruit quality in the remontant genotypes is affected by the environment and this makes it necessary to make selections in the environment where the genotypes will be grown. They also pointed out that those cultivars that have been bred in other environments often yield

soft, small, dark red fruits when grown in the northern regions. Another major difficulty in breeding for a remontant cultivar is that remontant genotypes have few or no runners. Since strawberry cultivars are propagated clonally, it becomes necessary to use micropropagation or crown separation, both of which are labor-intensive and expensive.

Figure 1.1 Bloom patterns in Short day, Day neutral, Long Day, Everbearing, and Remontant strawberry. Short day genotypes initiate flowers in the short days of winter and flower during early spring/summer. Long day genotypes initiate flowers in the long days of summer and flower from mid to late summer. Day neutral types are photoperiod insensitive and flower in repeated cycles from early to late summer until the plant goes dormant. Everbearing is a term given to genotypes that have an extended flowering season and has been applied to both long day and day neutral types**.** In this research, the term 'remontant' is used for any genotype that has multiple flowering cycles, because temperature plays an important role in regulating flowering, not just photoperiod.



1.5 Photoperiod and temperature control of remontancy

While most octoploid strawberry cultivars have been traditionally classified as short-day and day-neutral, several studies have demonstrated that temperature plays a critical role in the photoperiodic control of flowering in strawberry (Darrow, 1936; Serçe and Hancock, 2005a; Bradford *et al.* 2010). Darrow (1936) compared floral responses of strawberry cultivars growing in different photoperiods (13.5-16 hr) and different temperatures (~13°C to 21°C) and concluded that short days promote flowering and inhibit runner formation. Long days have the opposite effect on flower and runner formation. Variations in temperature affected flower and runner formation. Runner formation was favored by higher temperatures. Flower formation was optimal at 21°C, and at the lowest temperature (13.8°C) flower formation was inhibited.

Durner *et al.* (1984) compared the effect of photoperiod and diurnal variations in temperatures. They studied what they classified as short day, day-neutral, and everbearing genotypes under three light treatments: 9 hr, 16 hr, and 9 hr with night interruption, and four temperature treatments: day/night temperatures: 18/14°C, 22/18°C, 26/22°C, and 30/26°C. When grown under long days with night interruption, short day plants did not form any runners. The everbearers were unaffected by the night interval, but flowered under long photoperiods. The day-neutrals flowered in all photoperiods. Short day plants had the most flowers at low temperatures 18/14°C and at 22/18°C with night interruption. Day-neutrals flowered in all photoperiods when temperatures were 18/14°C. Higher temperatures, 26/22°C and 30/26°C inhibited flowering in day-neutrals. Short day plants produced runners in all temperature conditions, but there were more runners at higher temperatures. In everbearers, 22/18°C was the most favorable for runner formation. Day-neutrals produced the most runners at 26/22°C.

Bradford *et al.* (2010) did a similar study where they grew what they classified as remontant 'Tribute', short day 'Honeoye', and remontant *F. virginiana* RH30 under short and long photoperiods and different temperatures from 14-29°C. 'Tribute' and RH30 had previously been classified as day-neutral. They were able to identify specific 'permissive' temperature (14-17°C) at which the genotypes flowered at similar rates under both short and long photoperiods. The genotypes had a temperature threshold beyond which flowering was photoperiod dependent; and flowering was inhibited above 23-26°C. Very few runners were formed under short days. Under long days there were increasing numbers of runners with increasing temperatures.

All the above studies clearly showed that temperature and photoperiod interact to determine the flowering and vegetative response of genotypes. At lower temperatures, flowers are initiated in all photoperiods, regardless of whether a plant is categorized as photoperiod sensitive. Photoperiod-mediated flowering response is only observed above a particular threshold temperature. Both Durner *et al.* (1984) and Bradford *et al.* (2010) observed that flowers are inhibited above extremely high temperatures, while runner formation is favored at higher temperatures and under long photoperiod. Because temperature has such a strong influence on flowering and runnering in the octoploid strawberry, the term remontant is much more appropriate than day-neutral to describe genotypes that have extended or multiple cropping seasons.

1.6 Genetic control of flower remontancy (often described as day-neutral or everbearing)

Three major sources of remontancy are known among strawberry cultivars: 1: Seedling of 'Gloede' which is the source of remontancy in European cultivars; 2. Clonal mutation in 'Bismarck'; 3: *F. virginiana glauca* from Utah which is the source of remontancy in Californian

cultivars (Bringhurst *et al.*, 1989; Ahmadi *et al.*, 1990; Sakin *et al.*, 1997). Hancock (1999) reported that the first remontant cultivar in Europe was 'Climax', but it did not perform as an everbearer in warm climates. In addition, he reported that European remontant types may have derived this trait from *F. vesca*. Sakin *et al.* (1997) also identified several sources of remontancy among *F. virginiana* accessions collected from the Rocky Mountains, although these sources have not yet been incorporated into commercial remontant cultivars. Inheritance of remontancy is a complicated debate with studies reporting remontancy as a single gene, two genes, or multiple gene trait (Table 1.2). The differences in opinion possibly arise because of variations in classifying remontant/short day types (Bringhurst *et al.*, 1989; Serçe and Hancock, 2003), and variations in test environments that are caused by ambient temperature, chilling requirement, inconsistent cultural systems, and earliness of fruiting (Bringhurst *et al.*, 1989). Serçe and Hancock (2003) compared four methods of evaluating day-neutrality. They categorized genotypes as day-neutral based on whether they flowered within 100 days of germination in the greenhouse, whether two year old seedlings growing in the greenhouse and in the field flowered under short and long photoperiods, and whether the seedlings flowered in the summer that they were planted in the field. They concluded that when the seedlings were observed in the greenhouse for the second year, their assessments on day-neutrality were highly correlated with field observations. They also concluded that scoring day-neutrals based on whether they flower within 100 days of germination is the least reliable method. Another potential source of conflicting results in inheritance studies occurs when progeny from multiple crosses are pooled together to determine segregation ratios, instead of analyzing each cross separately (Powers *et al.*, 1954; Bringhurst *et al.*, 1989).

Single gene trait: Bringhurst *et al.* (1989) proposed that day-neutrality is a dominant trait controlled by a single gene. They crossed the heterozygous day-neutral cultivar 'Selva' with four short day genotypes: 'Chandler', 'Douglas', 83.91-3, and 83.91-31. They also made a set of day-neutral × day-neutral crosses by selfing 'Selva' and crossing with other day-neutrals CN-25, 83.91-27, and 83.94-9. They germinated the seeds in July, planted them in Sept, and recorded the yield at intervals of 6 weeks from Apr to Jul in the following year. They were able to identify day-neutral and short day progeny based on the fruit yield during early and late summer. In the day-neutral × day-neutral crosses (two heterozygous parents), they observed 3:1 segregation between day-neutral and short day types. In the day-neutral × short day progeny, they observed a 1:1 segregation as would be expected from a test cross. Thus they concluded that the trait is determined by a single major dominant gene.

Ahmadi *et al.* (1990) also proposed that day-neutrality is controlled by one major dominant gene based on their segregation ratios from a diallel cross made with four short day and four day-neutral cultivars, and with interspecific crosses between *F.* × *ananassa, F. vesca, F. viridis, F. virginiana,* and *F. chiloensis.* They categorized their plants as day-neutral based on 4 selection criteria: flowering in short and long photoperiods, flower initiation in seedlings 3-5 months after germination, repeated flowering cycles in 2 year old plants, and segregation pattern of progeny derived by crossing with a short day parent. However, they used different methods to categorize progeny from different crosses making it impossible to compare the efficiency of each of the methods, and making it difficult to compare segregation patterns of progeny derived from different crosses. They generated short day × day-neutral populations of *F.* × *ananassa* over 4 years (total of 28,000 progeny) and evaluated the progeny based on whether or not they flowered in late summer in the second year. Almost half of the progeny derived from heterozygous day-

neutral x short day were day-neutrals (1:1 segregation) and 75% of the progeny from day-neutral x day-neutral crosses were day-neutral (3:1 segregation) indicating that the trait is controlled by a single major gene. Homozygous day-neutral genotypes derived by selfing 'Fern' and 'Mrak' crossed with short day octoploid genotypes resulted in progeny that were all day-neutral, again confirming that this trait is controlled by a single dominant gene. Selfed day-neutral octoploids crossed with diploid short day genotypes of *F. vesca* and *F. viridis* resulted in 50% day-neutral progeny, further confirming the single dominant gene hypothesis.

Multi-gene trait: Darrow (1937) reported that in crosses between everbearing × everbearing cultivars in Canada, the progeny segregated into 88 everbearing and 66 June-bearing which fits the 9:7 ratio of two dominant complementary genes. Everbearing × short day crosses resulted in 257 everbearing and 788 June bearing in a 1:3 ratio, again confirming that the trait is controlled by two dominant genes. Unfortunately, he did not provide details on the criteria he used to categorize the progeny as everbearing.

In an extensive study in New Jersey (Clark, 1937), 4000 progeny from 61 crosses were evaluated based on whether or not they had an extended bloom (everbearers) or whether they flowered only in early summer (short day). He observed that while most of the crosses indicated that the everbearing trait was inherited as a dominant single gene trait, there were three parents in which the trait was regulated differently. The genotype 'New Jersey 1' did not produce any everbearing progeny when it was selfed or crossed with another parent. 'New Jersey 8' also resulted in a very low percentage of everbearer progeny: 11% when selfed and ~8% when crossed with other parents. Another genotype 'New Jersey 220' was not an everbearer but produced 11.8% everbearing progeny when crossed with a non-everbearer ('Dorsett'). They

speculated that either the everbearing trait in 'New Jersey 220' is controlled by a recessive trait, or both the parents have one copy of a complementary gene. The progeny from all the remaining everbearer x non-everbearer crosses were pooled together and consisted of 1104 everbearers and 705 non-everbearers, similar to a 9:7 ratio of two complementary genes. He concluded that the trait is controlled by dominant genes which interact. This study demonstrated that the everbearing trait was being differentially regulated in different parents. Although there was evidence of two complementary genes controlling the trait in most of the crosses, and possible recessive gene control in 'New Jersey 220', the authors did not make any conclusions about the genetic makeup of 'New Jersey 1' and 'New Jersey 8'.

Powers *et al.* (1954) proposed that the everbearing trait is controlled by at least three dominant and recessive genes. They crossed 10 genotypes (3 everbearers and 7 non-everbearers) in 45 combinations and observed segregation of the trait in the progeny. They categorized the progeny that flowered only in May and Jun as non-everbearers and those that flowered in Jul, Aug, and Sept as everbearers. It is however unclear whether the everbearers were also in bloom in early summer (May and Jun) which would make them truly photoinsensitive, or whether they were actually long day plants flowering only in late summer. Only one out of the three everbearers they tested resulted in segregation ratios that would fit single dominant gene model for everbearing trait. Selection '473' (everbearer) crossed with non-everbearing selections '471', '472', 474', '477', '478', and '4710' resulted in 1:1 segregation in the progeny. Segregation ratios for the other two everbearers (Selections '475' and 476') when selfed or crossed with each other did not fit the 3:1 ratio that would be expected for single dominant gene control. Instead, 33.3%, 42.3%, and 35.6% non-everbearering progeny were obtained by selfing '475', selfing 476', and crossing 475 and 476. Powers *et al.* (1954) suggested that the everbearing trait may be

controlled by two dominant genes. Progeny ratios from crossing the everbearers with non bearers supported this observation because they mostly fit the 9:7 ratio indicating that the everbearing trait is regulated by two dominant genes. However they did observe deviations from the expected 9:7 ratio in three crosses with '475' as a parent, and two crosses with '476' as a parent, and concluded that this was a result of presence of modifying genes in the progeny. They proposed that the everbearing trait is determined by at least 6 pairs of genes (dominant and recessive) with cumulative effect.

A study by Ourecky and Slate (1967) reported complementary gene action controlling the everbearing trait. In this study 25 combinations derived by crossing 9 non-everbearing and 4 everbearing genotypes were evaluated for the everbearing trait based on whether they flowered in late summer (Sept and Oct). They compared the segregation ratios of the progeny with octoploid segregation ratios for single dominant gene and multiple gene control. The expected ratio for single dominant gene control aaaaaaaA (everbearer) × aaaaaaaaa (noneverbearer) is 1:1. If there are two dominant genes controlling the trait, the ratio of progeny from aaaaaaAA × aaaaaaaa would be 11:3. The same ratios for everbearing × everbearing would be 3:1 for single dominant gene control: aaaaaaaA × aaaaaaaA, and 25:3 for two dominant genes control: aaaaaaAA × aaaaaaAA. The segregation ratios from their crosses mostly fit the two dominant genes model. However, they also observed that the progeny from some crosses had a higher percentage of everbearers and proposed that there may be additional loci determining everbearing trait.

Barritt *et al.* (1982) evaluated 3944 progeny from 54 crosses (day-neutral × day-neutral and day-neutral × short day) by recording presence of flowers from mid-June to mid-Sept in two and

three year old progeny . They observed that the percentage of day-neutral progeny in day-neutral × day-neutral crosses ranged from 70-100%. The percentage day-neutral progeny in day-neutral × short day crosses ranged from 43-100%. This indicated that day-neutrality was not controlled by a single major gene. They also pointed out that the percentage day-neutral progeny depends on the length of the growing season and the percent progeny categorized as day-neutral depends to a large extent on whether they were early flowering. Progeny from one or both day-neutral parents had earlier bloom dates than progeny from one or both short day parents. They acknowledged that some late flowering day-neutral seedlings may have been misclassified if they flowered after their 1 Sept cut-off date

Shaw (2003) selfed 10 day-neutral genotypes that were selections in the University of California breeding program, and that were expected to be heterozygous for the trait because they were produced by crossing day-neutral and short day genotypes. They selfed each day-neutral genotype and scored the progeny as day-neutral based on whether or not they flowered in May and Aug and found that the percent day-neutral progeny ranged from 41.8% to 84.8%, which is a significant deviation from the expected 75% if there was one major gene determining the remontancy trait. In addition, the pooled segregation ratio for all the crosses resulted in 70.9% day-neutrals and this was also a significant deviation from the expected 75%.

Similarly, Serçe and Hancock (2005b) crossed several day-neutral and short day genotypes belonging to both *F. x ananassa* and *F. virginiana* and categorized a progeny as day-neutral if it flowered in early and late summer. When they combined the progeny from different crosses, they observed that 71% of progeny were day-neutral in the day-neutral x day-neutral crosses and this was a significant deviation from the expected 3:1 ratio. Most crosses involving only *F.* ×

ananassa parents fit into the single gene model. However crosses involving *F.* × *ananassa* × *F. virginiana* parents had 88% day-neutral progeny. The *F. virginiana* × *F. virginiana* progeny pooled together had 48% day-neutrals. Overall, 30-87% of the progeny from day-neutral × short day cross, and 22-93% of progeny from day-neutral × day-neutral cross were day-neutral. Such segregation patterns led them to conclude that the day-neutral phenotype is under the control of multiple genes. They also obtained different proportions of day-neutral progeny when they crossed different day-neutral parents to the same short day parent. For example, 'Tribute' (day-neutral) × 'Chandler' (short day) resulted in 74% day-neutrals, and 'Aromas' (day-neutral) × Chandler resulted in 55% day-neutrals. Their results lead to the conclusion that the day-neutral trait is most likely controlled by multiple loci, and the proportion of day-neutral progeny from a cross depends on the dosage of day-neutrality alleles in the parents.

In another extensive experiment involving 30 crosses generated from 45 parents, Shaw and Famula (2005) compared the day-neutral vs short day ratio after combining the progeny from all the crosses. They identified day-neutrals based on whether they were flowering in Aug and Sept. They compared the segregation ratios to fit into three genetic models: multi gene inheritance along with environmental effect, dominant major gene with other additive genes and environmental effects, and single major gene with partial dominance along with additive genes and environmental effects, and concluded that the third model most accurately represents the genetic control of the trait

Weebadde *et al.* (2008) developed one of the first octoploid linkage maps using progeny from the cross between 'Honeoye' (short day) and 'Tribute' (remontant). This study was an important step towards identifying regions of the genome that control the day-neutral trait. Their study was

unique in that replicate populations from the cross were grown in 5 states (MI, OR, CA, MD, and MN) and hence the interaction with the environment could also be detected. In the eastern/mid-western states, almost 50% of the progeny were day-neutral. However, in the western states (CA and OR), 80% and 87% of the population were day-neutral. Clearly there was a strong interaction with the environment and based on previous studies on interaction of photoperiod and temperature, the authors explained that this was probably due to presence of a heat sensitivity locus in the genome that complicated flowering response. The eastern states had warmer summer temperatures in comparison to the western states. This environmental effect was also reflected in the fact that different QTL were identified using phenotypic data from different locations. There was one QTL on linkage group 28 that was common to all the eastern states (MI, MN, MD). MI had another QTL on the same linkage group. Only one QTL was identified for CA and three additional QTL were detected for MN. Identification of multiple genomic regions determining day-neutrality was a clear indication that day-neutrality in this population was a multigenic trait. The QTL identified in this study explained ~11-36% of the phenotypic variation.

Table 1.2 Summary of reports on inheritance of remontancy in published literature.

Genetics	Reference
1 gene- dominant	Bringhurst *et al.*, 1989; Ahmadi *et al.*, 1990
2 dominant complimentary genes	Darrow, 1937
Multiple genes-dominant and recessive	Clark, 1937
Multiple genes with cumulative effect-dominant and recessive	Powers *et al.*, 1954
2 complementary genes + modifier genes	Ourecky and Slate, 1967
1 major gene (partial dominance) + additive genes + environmental effects	Shaw and Famula (2005)
Multi gene	Barritt *et al.*,1982; Shaw, 2003; Serçe and Hancock, 2005
Multi gene: photoperiod loci + possible heat tolerance loci	Weebadde *et al.*, 2008

1.7 Genetics of remontancy in diploid *Fragaria*

Diploid *F. vesca* genotypes are typically short day, although some everbearing ('Alpine') types exist (Brown and Wareling, 1965; Hancock 1999). Genetic control of the everbearing trait was studied in a short day wild *F. vesca* accession, and in two everbearing *F. vesca* cultivars 'Baron Solemacher' and 'Bush White', by observing segregation of the trait in F2 and backcross populations (Brown and Wareling, 1965). It was concluded that in the diploid strawberry, the everbearing trait is controlled by a single recessive gene. In addition, runner formation is controlled by a dominant gene present in the seasonal flowering types, while the everbearing types have the recessive form of the gene. They suggested that additional additive or minor genes affect runner formation in the absence of the major runnering gene.

In another study, Ahmadi *et al.* (1990) proposed that day-neutrality or photoinsensitivity in diploid Californian *F. vesca* is controlled by three major genes in which day-neutral is recessive to short day. They crossed day-neutral Alpine *F. vesca* from Europe with short day *F. vesca* from California. Seedlings were categorized as day-neutral based on whether or not they flowered 3-5 months after germination. All the F_1 seedlings were day-neutral. The F_2 populations segregated as 1:63, and the BC_1 population segregated 1:7 for day neutrality. This led them to the conclusion that the North American *F. vesca* have diverged significantly from the European *F. vesca* in the genetic control of photoperiod sensitivity. In addition, day-neutral Alpine *F. vesca* crossed with short day *F. chiloensis* resulted in all short day progeny, further confirming that the trait is under a recessive gene control in the diploid.

1.8 Marker-assisted breeding for remontancy

Kaczmarska and Hortynski (2002) performed a preliminary bulk segregant analysis to identify the association between RAPD markers (Williams *et al.*, 1990) and 'photoinsensitive' (day-neutral/remontant) trait in *F.* × *ananassa*. Although they identified one RAPD marker that they described as 'likely' associated with the trait, there were no follow-up reports confirming the marker-trait association. In an attempt to develop markers associated with flowering in diploid *Fragaria*, Cekic *et al.* (2001) identified an ISSR (Inter Simple Sequence Repeat) marker (Zietkiewicz *et al.*, 1994) located at 2.2 cM from the *SEASONAL FLOWERING LOCUS* using a bulk segregant analysis on a backcross population of 168 plants developed from *F. vesca* (short day) x *F. vesca* (remontant). As discussed above, the genetics of remontancy in diploid strawberry is significantly different from that of octoploid strawberry because the trait is controlled by a single gene in the diploid, whereas it is a multi-gene trait in the octoploid. Therefore, markers developed for the single major diploid gene are not likely to be applicable in breeding for remontancy in commercial octoploid strawberry where the trait is under a more complex genetic control. Weebadde *et al.* (2008) identified QTL associated with remontancy in *F.* × *ananassa* using AFLPs, but the markers associated with the QTL were not validated and converted to transferable markers for wider application in breeding. Sugimoto *et al.* (2005) developed a population from octoploid 'Ever Berry' (everbearing) and 'Toyonoka' (short day) and used bulk segregant approach on 191 F_1 seedlings that segregated 1:1 for the everbearing trait. They identified two RAPD markers associated with the everbearing trait at 11.8 and 15.8 cM from the locus. However, they did not use the markers in marker-assisted breeding. There are reports that the private company, Driscoll Associates, has used markers in remontancy breeding; however, few details have been provided (Chen Niu, personal communication).

1.9 Genetics of flowering in *Arabidopsis* and how it relates to strawberry

Much of the research on the genetic regulation of flowering has focused on the diploid model plant *Arabidopsis*. In this section the major events in the process of flower initiation in *Arabidopsis* are summarized based on several extensive reviews (Aukerman and Amasino, 1996; Putterill *et al.*, 2004; Boss *et al.*, 2004; Kobayashi and Weigel, 2004; Samach and Wigge, 2005; Wilkie *et al*., 2008; Albani and Coupland, 2010; Srikanth and Schmid, 2011). In *Arabidopsis*, flower formation is regulated by environmental (photoperiod, vernalization, ambient temperature) and endogenous (autonomous, hormonal, and developmental) signals. Figure 1.2 is reproduced from Albani and Coupland (2010) and presents the complex interaction among the major flowering pathways in the floral meristem. All the pathways are integrated at the gene *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS*) through *FT* (*FLOWERING LOCUS T*) and *FD* (*FLOWERING LOCUS D*) (photoperiodic pathway), *FLC* (*FLOWERING LOCUS C*) and *SVP* (*SHORT VEGETATIVE PHASE*) (vernalization and autonomous pathway), miR156, *SPL3* (*SQUAMOSA PROMOTER BINDING PROTEIN LIKE 3*), and *SPL9* (developmental pathway), and gibberellin-mediated activation (hormonal pathway). *SOC1* acts upstream of floral meristem identity genes *LFY* (*LEAFY*) and *AP1* (*APETALA1*).

Photoperiodic flower induction in *Arabidopsis* occurs in response to long photoperiods that initiates expression of *FT* in the leaves and upregulation of *FD* in the meristem, which interact to activate *SOC1*. *FT* is activated by a zinc finger protein called *CO* (*CONSTANS*). *CO* is regulated by the circadian clock and the level of expression of *CO* oscillates in a 24 hr cycle. The oscillation in expression of *CO* is affected by other circadian pathway genes, *GI* (*GIGANTEA*), *FKF1* (*FLAVIN-BINDING, KELCH REPEAT, F-BOX 1*), and *CDF1* (*CYCLING*

DOF FACTOR1). Three photoreceptor genes, *CRY1* (*CRYPTOCHROME 1*), *CRY2*, and *PHYB* (*PHYTOCHROME B*) also maintain the expression of *CO*. *FT* is the floral signal that moves from the leaves, where the photoperiodic signal is received, to the shoot apical meristem, where floral differentiation occurs. *FT* interacts with *FD* and upregulates the floral meristem identity gene *AP1*.

Although the mechanism by which plants measure and perceive ambient temperature is not understood, studies have measured the effects of ambient temperature on plants (Samach and Wigge, 2005). In *Arabidopsis*, lower temperatures delay flowering and the genes *FVE* and *FCA* have been identified as candidate genes that prevent flower initiation under low temperatures. *FVE* maintains *FLC* expression through a histone modifying complex. Certain photoreceptor genes also require specific temperatures to function. *PHYB* inhibits flowering under high temperatures (23°C). In addition, *Arabidopsis* genotypes that contain the *FRI* (*FRIGIDA*) gene require prolonged treatment in cold temperatures (vernalization) to flower. Presence of the *FRI* gene increases expression of the flowering inhibitor *FLC*. *FLC* is a MADS box protein that acts as a transcriptional regulator and inhibits expression of the *SOC1* gene.

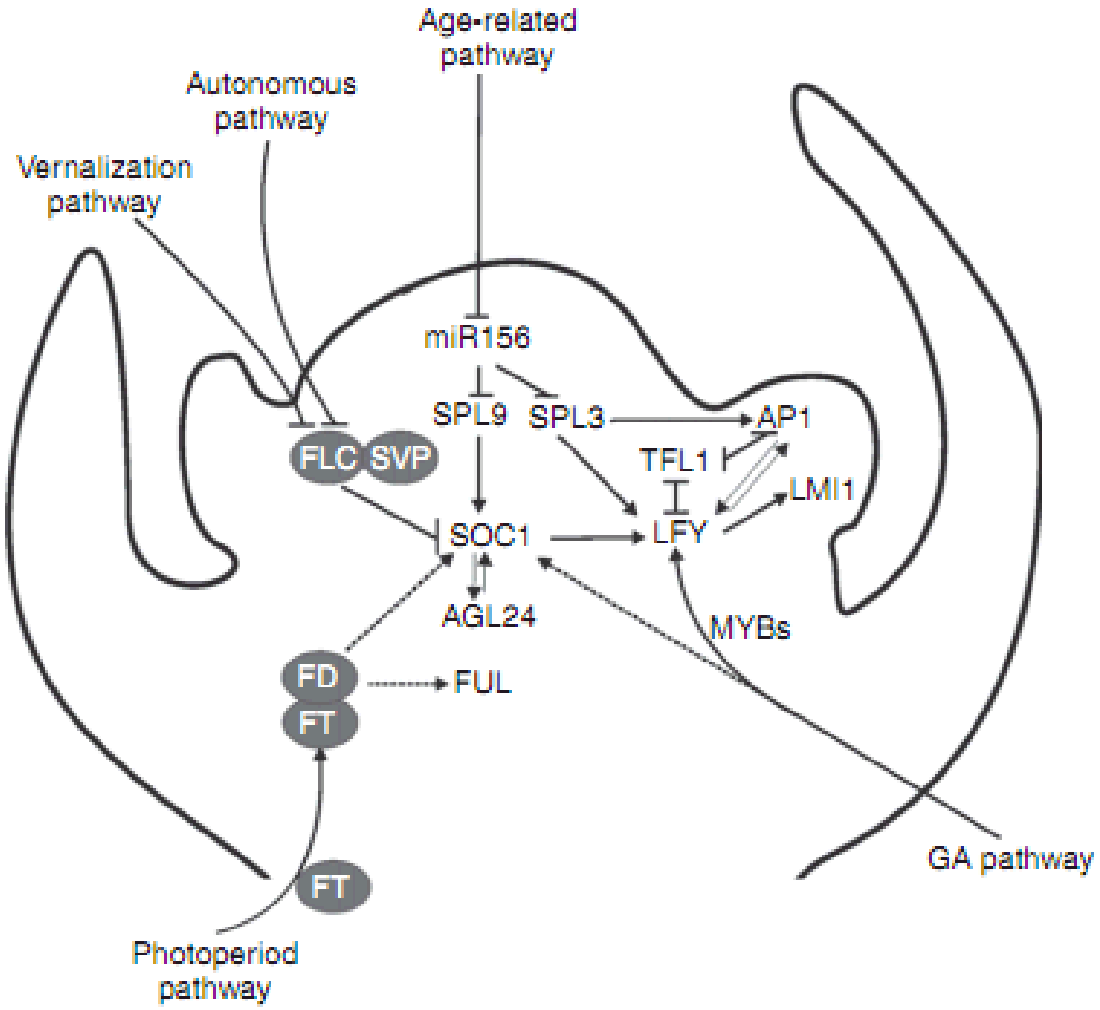
The key difference between annual plants like *Arabidopsis* and perennial plants such as strawberry is that annuals undergo floral transition only once in their life cycle, while perennials go through repeated vegetative and reproductive phases over the years (Wilkie *et al.*, 2008; Albani and Coupland, 2010). However, flower initiation in perennials may involve some of the same pathways that stimulate reproductive growth in annuals. There is evidence of photoperiodic and temperature regulation of flower initiation in strawberry (Section 1.5 above). Most strawberry genotypes have a chilling requirement before the dormant flower buds can

resume differentiation (Hancock *et al*., 1991), and this may be comparable to the vernalization pathway in annual plants like *Arabidopsis*.

There has been little investigation on how the regulation of flowering in strawberry relates to that of *Arabidopsis*. Mouhu *et al.* (2009) identified homologs of 66 *Arabidopsis* flowering time genes from *Fragaria vesc*a EST sequences. These homologs represent genes from all the major flowering time pathways suggesting that the same major flowering pathways may be present in diploid strawberry. However, when they compared expression patterns of some of these genes in everbearing short day *F. vesca*, they were unable to detect any differences, suggesting that they probably did not identify the homolog of the *SEASONAL FLOWERING LOCUS* (Cekic *et al.*, 2001), the major gene responsible for the everbearing trait in diploid strawberry. Thus, little is known about the regulation of flowering in the diploid strawberry and there are no published studies on the octoploid to date. Previous studies have demonstrated that remontancy in diploid strawberry is a qualitative trait under the control of a major gene, whereas in octoploids there are multiple loci determining the remontant phenotype. In order to develop an understanding for genetic control of flower initiation in commercial *F.* × *ananassa*, studies will have to be done using the octoploid species.

Figure 1.2 Diagrammatic representation of regulation of flowering time in *Arabidopsis*.

(Reprinted from Albani and Coupland (2010) Current topics in Developmental Biology 91: 323-348 with permission from Elsevier).



1.10 Conclusions and thesis introduction

Developing heat tolerant remontant cultivars is critical for the midwestern market to extend the harvest season. However, we have little information on the regulation of flowering in strawberry and few molecular markers have been developed for marker-assisted breeding for remontancy in strawberry. We can perform marker-assisted breeding for remontancy without specific knowledge of the genes regulating the characteristic, but we will need tightly associated markers to use this strategy. To have maximum utility, it is important for the marker associated with the trait to be transferable across populations.

This study was designed to identify regions of the octoploid strawberry genome that are associated with remontancy and heat tolerance, and identify marker-trait association that would help develop marker-assisted breeding in strawberry. SSRs were used as an efficient low cost transferable marker. The population was selected so that phenotypic data from multiple locations and years can be used to validate the QTL. In addition, the study was also designed to observe the effect of temperature in a population segregating for remontancy and identify genotypes that are heat tolerant by growing the replicates of the same population under different temperature conditions.

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CHAPTER 2

EFFECT OF TEMPERATURE ON FLOWER AND RUNNER NUMBER IN A STRAWBERRY POPULATION SEGREGATING FOR REMONTANCY

Abstract

Flower initiation in strawberry has been typically classified by photoperiod sensitivity; however, several recent studies indicate that temperature also plays a major role in determining flower initiation. In this study, a population developed from the non-remontant (short day) 'Honeoye' and remontant 'Tribute' were grown in three temperature conditions (17, 20, and 23°C) under a long photoperiod in the greenhouse and the differences in flower and runner formation among the progeny was compared. In addition, the same population was grown under field conditions in Michigan and Oregon and remontant vs non-remontant phenotype in the progeny was compared with the extent of heat tolerance (numbers of flowers at 17 vs 23°C) observed in the greenhouse. Level of heat tolerance among the progeny was a continuous distribution. All the progeny that produced more flowers at 23°C than at 17°C in the greenhouse were remontant in MI and most were also remontant in OR. Flower initiation in both the parents was reduced at 23°C, but 'Tribute' performed better than 'Honeoye' at the higher temperature. Most remontant progeny had few runners, even at higher temperatures, although one remontant progeny had a high level of heat tolerance, and produced >20 runners. The results indicate that temperature tolerance plays a critical role in flower initiation. The most heat tolerant genotypes identified in this study will be useful in breeding new cultivars better adapted to hot mid-western climates.

2.1 Introduction

Strawberry genotypes have been traditionally classified according to their flowering response to photoperiod as short day (June-bearers), day-neutral, long-day, and everbearing. Everbearing genotypes are actually long day because they initiate flowers under long photoperiods (Durner *et al.*, 1984), although day-neutrals are also sometimes referred to as everbearers because of their

repeated cycles of flowering. Runner production is reported to be photoperiod-dependent, and is favored by long day photoperiods (Darrow, 1936; Bradford *et al.*, 2010).

The classification of strawberry genotypes based on their photoperiodic response is complicated by the effect of temperature (Darrow, 1936; Durner *et al.*, 1984; Serçe and Hancock, 2005; Weebadde *et al.*, 2008; Sonsteby and Heide, 2008; Bradford *et al.*, 2010). What have been classified as short-day genotypes will flower under long days when temperatures are cool, and genotypes that have been called day-neutral will not flower under hot conditions. For this reason, day-neutrals are more accurately described as remontant and short-day types as non-remontant.

Darrow (1936) studied the effect of photoperiod (13.5 hr, 14hr, 16 hr), along with different temperatures, 70°F (~21°C), 60°F (~15.5°C), and 55°F (~13.8°C) on flower and runner formation in several strawberry cultivars. His observations showed that while flowering is a photoperiodic response, differences in temperatures affected the number of flowers/runners that were produced.

In another extensive study, Durner *et al.* (1984), studied the flowering response of what they classified as short day, day-neutral , and everbearing genotypes grown under different photoperiod (9 hr, 16 hr, and 9 hr with night interruption-NI), and different temperatures (day/night temperatures: 18/14°C, 22/18°C, 26/22°C, and 30/26°C). Their short day types produced more flowers at 18/14°C and night interruption was more effective in producing flowers at 22/18°C. In their day-neutrals, there was no effect of photoperiod at 18/14°C. However, at 22/18°C and 26/22°C, there were more flowers under night interruption. Higher temperatures 26/22°C and 30/26°C inhibited flowering in all photoperiods. On the other hand,

runner production was favored by warmer temperature and long days or NI. Their short day plants produced more runners at all temperatures under NI and SD, although maximum runner production was at 26/22°C with night interruption and SD with 30/26°C. Their day-neutrals produced the most runners at 26/22°C with NI.

Serçe and Hancock (2005) studied what they classified as day-neutral genotypes of *F.* × *ananassa* ('Aromas', 'Fort Laramie', 'Ogallala', 'Tribute', 'Quinalt') and *F. virginiana* (Brighton-3, LH30-4, LH39-15, LH40-4, LH50-4, RH23, RH30, RH43, RH45, Frederick-9) under different temperatures regimes (18°C, 22°C, 26°C, and 30°C) in growth chambers with 12 hr photoperiod and identified 'Fort Laramie' as the most heat tolerant, day-neutral genotype.

Weebadde *et al.* (2008) identified QTL associated with what they classified as day-neutrals by evaluating the progeny from a 'Honeoye' × 'Tribute' cross at 5 field locations across North America (MI, OR, CA, MN, MD). They found that 80 and 87% of the progeny flowered in both the spring and summer in the cooler western states OR and CA where the average maximum temperatures in mid-late summer were 26°C and 21°C. In the warmer mid-western and eastern states, MI, MN, and MD, average maximum temperatures in mid-late summer were 29°C, 28°C, and 30°C. The same set of progeny growing in these warmer states had 49%, 50%, and 48% repeat flowerers in MI, MN, and MD respectively. The authors speculated that a heat tolerance QTL exists along with photoperiod response QTL and flower initiation depends on both photoperiod sensitivity and heat tolerance.

All of these studies demonstrated that flower initiation is affected by both temperature and photoperiod, and that flower initiation is inhibited at higher temperatures. To further study the interaction between temperature and photoperiod, Bradford *et al.* (2010) observed the effect of 8

hr and 16 hr photoperiod, along with temperatures 14°C, 17°C, 20°C, 23°C, 26°C, and 29°C on 'Honeoye', 'Tribute', and a wild *F. virginiana* selection, RH30. Their study clearly showed that these genotypes were photoperiod sensitive only above a particular threshold temperature. Although 'Honeoye' is classified as a short day genotype, it had more flowers under long days when temperatures were 14°C and 17°C. There were fewer flowers at 20°C and 23°C, and flowering was inhibited at 26°C. 'Honeoye' had more flowers under short days when temperature was 20-26°C, although flowering was inhibited above 23°C. This indicates that 'Honeoye' performed as a short day plant when temperature was above 20°C. Similarly, RH30 was photosensitive above 23°C. RH30 was also more heat tolerant because it had some flowers at 29°C. 'Tribute', which had previously been classified as day-neutral, had more flowers under long days than under short days. However, flower formation decreased above 26°C.

Runner production in strawberries is also affected by both photoperiod and temperature. In the study of Serçe and Hancock (2005), what they classified as day-neutral genotypes 'Aromas', 'Tribute', Frederick 9, and 'Fort Laramie' did not form any runners, even under long days. Other genotypes such as CFRA0368 and 'Quinalt' formed runners only under short days, while LH50-4 and RH30 formed more runners under long days than under short days. Genotypes also varied in their runner production abilities at different temperatures. While some genotypes (LH 40-4, RH 23, and RH 45) did not form any runners at all, others (LH50-4) formed runners at all temperatures. In the study by Bradford *et al.* (2010), 'Honeoye' produced no runners and 'Tribute' produced only a few runners under short days. However, the number of runners increased with increasing temperatures under long days. 'Honeoye' had the maximum number of runners at 26°C and 'Tribute' had the most runners at 23°C.

While the effect of temperature on flower and runner production has been well studied on individual strawberry genotypes, there has been little work on the genetics of these traits. All the previous work on the environmental regulation of remontancy has been conducted in the field, where both temperature and photoperiod were variable. In this study, the permissive and inhibitive temperatures identified by Bradford *et al.* (2010) were maintained in a greenhouse to determine the heat tolerance of segregating individuals from the same cross used by Weebadde *et al.* (2008), non-remontant 'Honeoye' × remontant 'Tribute'. Runners from these progeny were also grown in the field in Michigan and Oregon to determine how a genotype's heat tolerance was related to field performance.

2.2 Material and Methods

2.2.1 Selection of the segregating population

A segregating population of 54 individuals from a F_1 cross between 'Honeoye' and 'Tribute' was used for the greenhouse study. This population was also planted in the field in MI and OR for phenotypic evaluation of plant, flower, and fruit traits under different environmental conditions. In addition, these genotypes were included in the development of an SSR linkage map of octoploid strawberry that was used to identify QTL associated with remontancy and heat tolerance (Mookerjee *et al.*, in review)**.**

2.2.2 Growth conditions

Seedlings from the 'Honeoye' × 'Tribute' cross were propagated by rooting runners under mist in 10 cm pots containing BACCTO High Porosity Professional Potting Mix (Michigan Peat

Company, Houston, TX). After 4 weeks, when the roots of the runners were firmly established, the runners were disconnected from the parent plant.

Greenhouse: The rooted runners were transferred to 3.8 L pots filled with the same potting mix. The experiment was set up as a single block with two treatments: temperature and genotype. Three replicates of each genotype were grown in temperature controlled greenhouses at 17 °C, 20°C, and 23°C**,** and under 16 hr photoperiod using supplemental lights (400W high-pressure sodium lamps- P.L. Light Systems Inc. Beamsville, ON, Canada). Average Daily Light Integrals in the greenhouses are listed in Table 2.1. The plants were watered using distilled water injected with 125 ppm constant feed (14.7% N, 3.2% P_2O_5, 14.1% K_2O, 7.6% Ca, 1.4% Mg, 0.117% Fe, 0.059% Mn, 0.059% Zn, 0.117% Cu, 0.035% B, and 0.012% Mo). Predator mites (SPIDEX, Koppert Biological Systems, Howell, MI 48843), Volck (petroleum oil), Sulphur, Terragurard (triflumizole), and Floramite (bifenazate) were used for pest and disease control when necessary.

The plants were grown in greenhouses under long day photoperiods to ensure that the differences in flowering responses were an effect of temperature and not due to inductive photoperiod. The selection of the three temperature levels was based on the study by Bradford *et al.* (2010) where they observed that 17°C was conducive to flower formation under all photoperiods, 20°C was the critical temperature beyond which flowering was photoperiod dependent, and 23°C was the lowest temperature at which flower formation was inhibited.

Field: The same set of plants propagated through runners was planted in the field with two replicates in MI (Southwest Michigan Research and Extension Center) on Aug 12, 2010 and in OR (Corvallis) on Aug 26, 2010. The plants were set at a spacing of 0.9 m by 0.9 m between

plants at both locations. These plants were contained in a larger planting that included 960 genotypes for phenotypic assessment of commercial traits.

Air temperature in MI was warmer than in OR. Table 2.2 lists the number of days in Apr, May, Jun, and Jul 2011 that had temperatures >20°C and >30°C and the mean air temperatures at the two locations.

Table 2.1 Average Daily Light Integral measured as mol $m^{-2}d^{-1}$ in the greenhouses at Michigan State University, East Lansing.

	17°C	**20**°C	**23**°C
Nov 2010	10.66	9.51	13.29
Dec 2010	8.54	7.13	11.22
Jan 2010	9.38	6.05	12.09
Feb 2010	14.63	12.35	14.02
Mar 2010	14.6	16.75	16.59

Table 2.2 Air temperature at MI (Benton Harbor) and OR (Corvallis) field locations

	Number of days >20°C		Number of days >30°C	
	MI	OR	MI	OR
Apr	1	2	0	0
May	12	5	1	0
Jun	26	23	4	0
Jul	29	29	12	5
Avg min temp (Apr-May)	7°C	6°C		
Avg max. temp (Apr-May)	16°C	15°C		
Avg min. temp (Jun-Aug)	17°C	12°C		
Avg max. temp (Jun-Aug)	27°C	23°C		

2.2.3 Phenotypic observations

Greenhouse: The plants were maintained under treatment conditions for 45 days before data were collected. This was to ensure that all phenotypic observations were a result of the treatment conditions, and not an effect of prior growing conditions. The total number of flowers, inflorescences, and runners were counted every week from Dec 1, 2010 to Mar 30, 2011. All open flowers and runners were removed after counting every week. All dead leaves present at the time of data collection were removed from the plant.

Field: Presence/absence of flowers was recorded every week from May 2011 through Aug 15, 2011. Progeny that flowered both in the spring and in the long days of summer after Jul 23 were categorized as remontant. Progeny that only flowered early in the season were categorized as non-remontant.

2.2.4 Data collection and analysis

Greenhouse air temperature was monitored and recorded every 10 s using thermocouples connected to a CR10 data logger (Campbell Scientific, Logan, UT). Graphs were plotted using MSExcel. ANOVA analysis was done with R 2.1.2.2.

2.3 Results and discussion

2.3.1 Flower formation: Segregation for heat tolerance in the greenhouse

There was a significant effect of temperature, genotype, and genotype × temperature on total number of flowers (α=0.01) and on number of inflorescences (α=0.01) (Table 2.3). Figure 2.1a-c shows the distribution of total number of flowers among the 54 progeny at 17°C, 20°C, and 23°C. In general, the distributions were skewed towards individuals with lower flower production. Figure 2.2a-b shows the distribution of number of flowers at 17°C minus the number of flowers at 23°C, as a representation of the extent of heat tolerance among the progeny. The total flowers in all the progeny and the parents under the three treatment conditions are shown in Appendix 2.1. The level of heat tolerance varied continuously among the progeny (Figure 2.2a-b, Appendix 2.1). Some progeny, such as, HT5 and HT18, were highly heat tolerant and performed much better at 23°C than at 17°C. Others such as HT31 and HT37 were weakly heat tolerant and had only a few more flowers at 23°C than at 17°C. Still others such as HT2 and HT42 had flower numbers that were only slightly lower at 23°C than at 17°C, and some such as HT8 and HT25 were greatly affected by the higher temperature. These variations in total flower number were more strongly regulated by differences in inflorescence number than by flowers per inflorescence, as the total number of flowers per inflorescence was relatively constant across treatments. Both 'Honeoye' and 'Tribute' had fewer flowers at 23°C than 17°C and therefore were heat sensitive, although 'Tribute' performed better than 'Honeoye' at 23°C. Bradford *et al* (2010) also found 'Tribute' to be more heat tolerant than 'Honeoye' and RH30 (a wild selection of *F. virginiana*) and that inflorescence numbers per plant varied more widely between treatments than flowers per inflorescence.

Table 2.3 ANOVA analyses showing significant effect of temperature, genotype, and temperature × genotype interaction on the number of flowers, number of inflorescences, and number of runners in 'Honeoye' × 'Tribute' progeny and the parents growing at 17°C, 20°C, and 23°C in a greenhouse in East Lansing, MI .

		Probability		
	df	Total flowers	Total inflorescence	Total runners
Genotype (54 progeny + 2 parents)	55	<0.01	<0.01	<0.001
Temperature	2	<0.01	<0.01	<0.001
Genotype x Temperature	110	<0.01	<0.01	<0.001

Figure 2.1a-c Distribution of progeny with different numbers of flowers in the 'Honeoye' × 'Tribute' population. (a) Distribution of total flowers at 17°C, (b) Distribution of total flowers at 20°C, (c) Distribution of total flowers at 23°C. Number of flowers in the parents are indicated: Honeoye: Shaded arrow, Tribute: Black arrow.

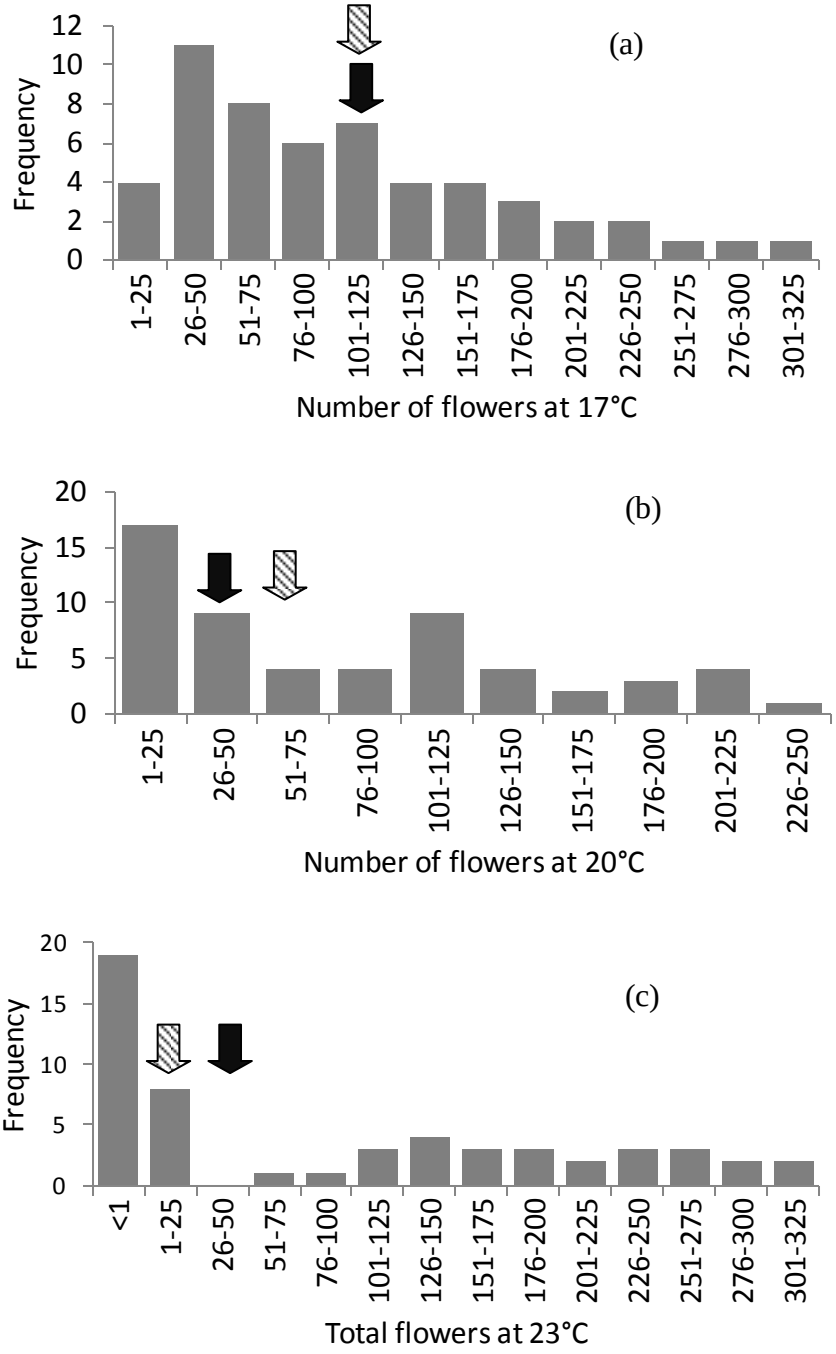


Figure 2.2a-b Distribution of total flower numbers at 17°C minus total flower numbers at 23°C (y-axis) in the 'Honeoye' (Hon) × 'Tribute' (Tri) progeny and the parents. (a) Distribution of total flowers at 17°C minus total flower numbers at 23°C in progeny that had more flowers at 17°C than at 23°C (heat sensitive). The parents are included for reference. (b) Distribution of total flowers at 17°C minus total flower numbers at 23°C in progeny that had fewer flowers at 17°C than at 23°C (heat tolerant). Remontant/Non-remontant phenotypes from the field observations at MI and OR are included with the genotype names on the x-axis. The progeny that were remontant at one location and non-remontant at the other are labeled as RM(m)=Remontant in MI, NR(o)= Non-remontant in OR.

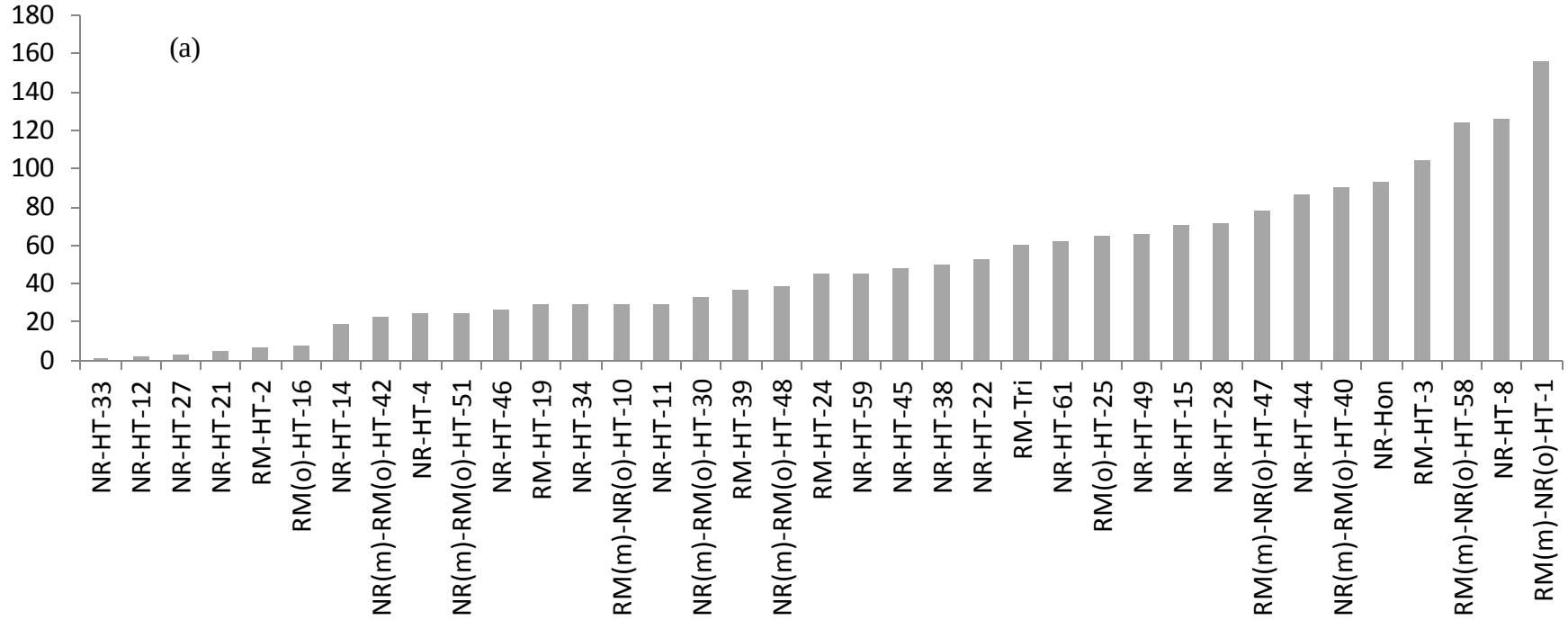
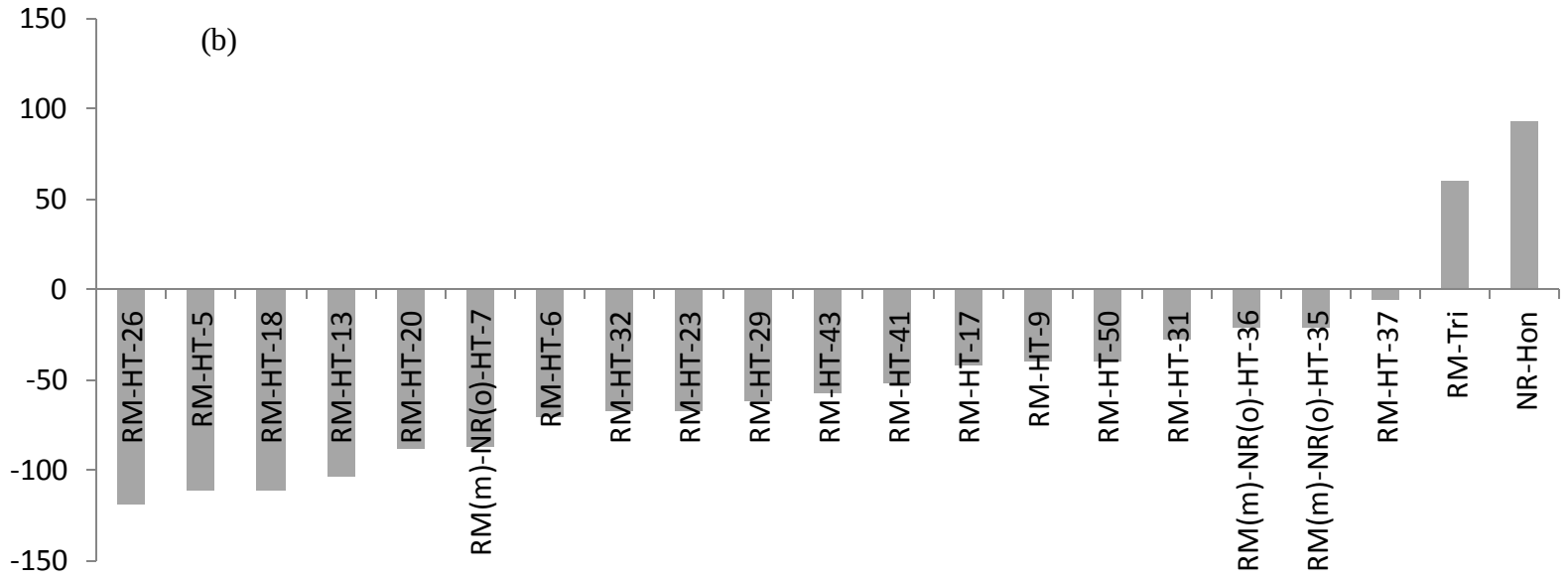


Figure 2.2 (cont'd)

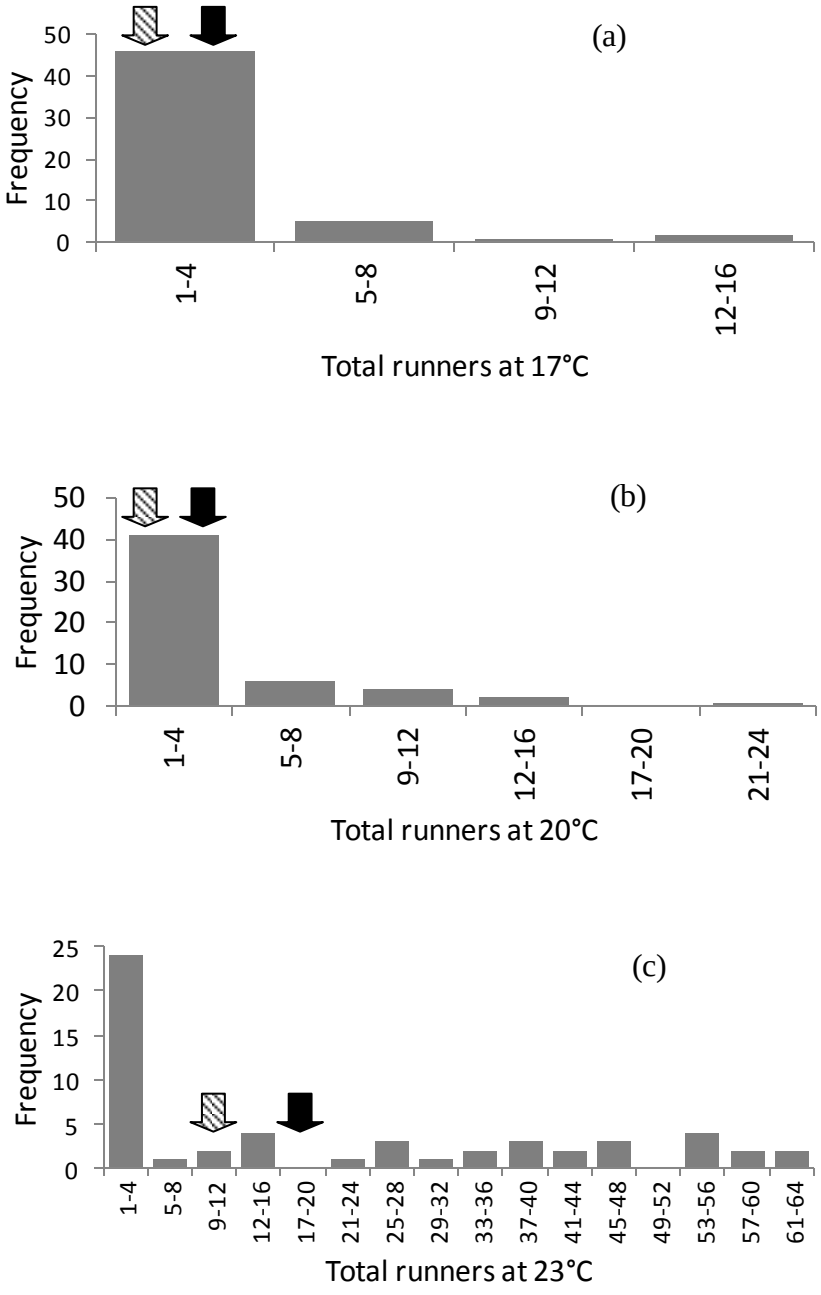


2.3.2 Runner formation in the greenhouse

There was a significant effect of temperature, genotype, and genotype × temperature on number of runners (α=0.001) (Table 2.3). Figure 2.3a-c shows the distribution of progeny and parents with different numbers of runners at 17°C, 20°C, and 23°C. The total number of runners in each progeny and the parents is displayed in Appendix 2.2. In general, there were fewer runners at lower temperatures than at 23°C. Most of the progeny with floral sensitivity to heat had more runners at 23°C than at the lower temperatures, similar to the phenotype of the parents. The progeny with the highest floral heat tolerance generally had very few or no runners at 23°C; however, one progeny (HT43) was heat tolerant and had >20 runners at 23°C. This genotype is of breeding and commercial interest, as it has the potential to produce crops in extreme heat and still produce runners for propagation.

Serçe and Hancock (2005) also reported that the genotypes with the most floral heat tolerance had few runners, if any. Their most floral heat tolerant genotype, 'Fort Laramie', did not form any runners, even under long days. Bradford *et al.* (2010) observed that under long days, 'Honeoye' formed runners above 20°C, but runner formation was inhibited above 26°C. 'Tribute' produced runners under long days above 23°C, although the number of runners was less than 'Honeoye' and RH30. In our experiment only three temperature conditions were tested, but 'Tribute' produced more runners than 'Honeoye' in all treatments. In the Bradford *et al.* (2010) study, although 'Tribute' had more runners than 'Honeoye' at 17°C, 'Honeoye' had more runners than 'Tribute' at 20°C and 23°C, and there was little difference in runner numbers between the two genotypes at 20°C and 23°C.

Figure 2.3a-c Distribution of progeny with different numbers of runners in the 'Honeoye' (H) × 'Tribute' (T) population grown in a greenhouse at 17°C, 20°C, and 23°C. (a) Distribution of total runners at 17°C, (b) Distribution of total runners at 20°C, (c) Distribution of total runners at 23°C. Numbers of runners in the parents are indicated: Honeoye: Shaded arrow, Tribute: Black arrow.



2.3.3 Remontancy in the field

Out of the 54 progeny planted in the field in MI, 52 survived and 28 (54%) were remontant. Among the progeny in OR, all survived and 28 (51%) were remontant. 21 progeny were remontant at both locations, while 5 progeny were remontant in OR and non-remontant in MI, and 7 progeny were remontant in MI and non-remontant in OR.

All the field planted remontants in Michigan were heat tolerant in the greenhouse screen, while 75% of the remontants in Oregon were heat tolerant (Appendix 2.1).

While ~50% of the progeny in MI and OR were remontant in this study, in Weebadde *et al* (2008), 80% of the progeny in OR and 50% in MI were remontant. It is unclear why the proportion of remontants was so much lower in OR in 2005 than 2011. A comparison of average maximum air temperatures in early and late summer in OR in 2005 vs. 2011 shows it was 3°C cooler during the latter study. However, the fruiting season in OR was 3 weeks later this year than in other years (Chad Finn *pers comm*.). Therefore it is possible that some of the remontant types were incorrectly identified as short day because they did not flower by the Aug 15 cutoff date. The population size used in this study was much smaller than that used in the Weebadde *et al.* (2008) study, making it also possible that the differences in the percent remontant progeny were an artifact of small population sizes.

2.4 Overall conclusions

These data clearly demonstrate that the progeny of 'Honeoye' × 'Tribute' segregate for degree of floral heat tolerance, suggesting that it is a heritable trait. The degree of heat tolerance is likely quantitatively controlled as the difference between flowers produced at 23°C vs 17°C varied

continuously among genotypes. While neither of the parents was heat tolerant, a continuum in heat tolerance was observed among the progeny, with 19 of them having more flowers at 23°C than at 17°C.

Overall, our experiment demonstrates that when photoperiodic conditions are non-inductive, flower formation is largely determined by a genotype's level of heat tolerance. Phenotypic evaluations of these progeny under field conditions showed a clear relationship between remontancy and heat tolerance. All the progeny that had high levels of heat tolerance in the greenhouse experiment were remontant under field conditions in MI, and most were in OR. The most heat tolerant progeny will likely be useful in breeding new remontant cultivars for hot mid-western climates. The ability of a remontant genotype to form runners is also an important consideration for a clonally propagated crop. At least one progeny in this experiment had the ability to form flowers under high temperatures and also produce runners.

The results of this experiment show that ambient temperature is an important factor when classifying genotypes based on their photoperiod requirements. The importance of heat tolerance in the expression of remontancy may explain why the remontant cultivars bred for the milder temperatures of CA flower weakly in the more extreme summer temperatures of the Midwest. Our most heat tolerant, remontant genotypes could be used to breed cultivars better adapted to the Midwestern conditions.

APPENDIX

Appendix 2.1

Figure 2.4. Total flowers (y-axis) at 17°C, 20°C, and 23°C in the 'Honeoye' × 'Tribute' progeny (HT1-54) and the parents. Remontant/Non-remontant phenotypes from the field observations at Benton Harbor, MI and Corvallis, OR are included with the genotype names on the x-axis. The progeny that were remontant at one location and non-remontant at other are labeled as RM(m)=Remontant in MI, NR(o)= Non-remontant at OR. (a) Total flowers at 17°C, 20°C, and 23°C in the heat tolerant progeny. (b) Total flowers at 17°C, 20°C, and 23°C in the heat sensitive progeny.

For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

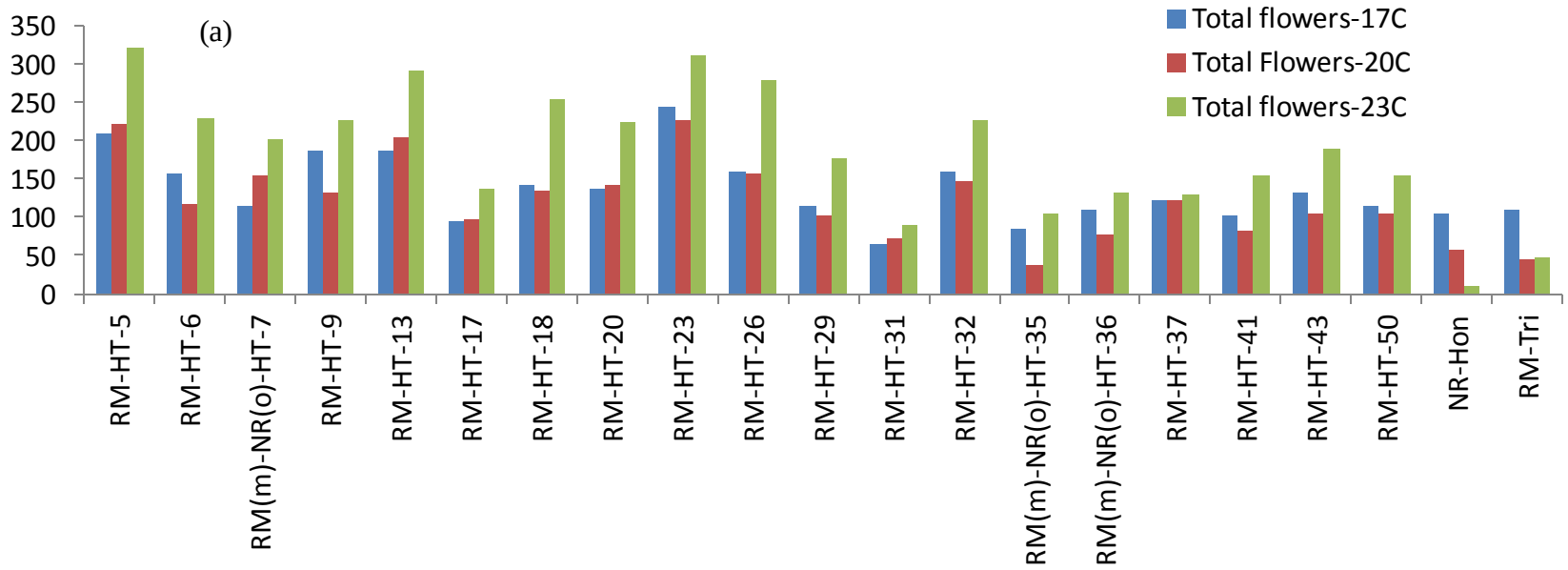
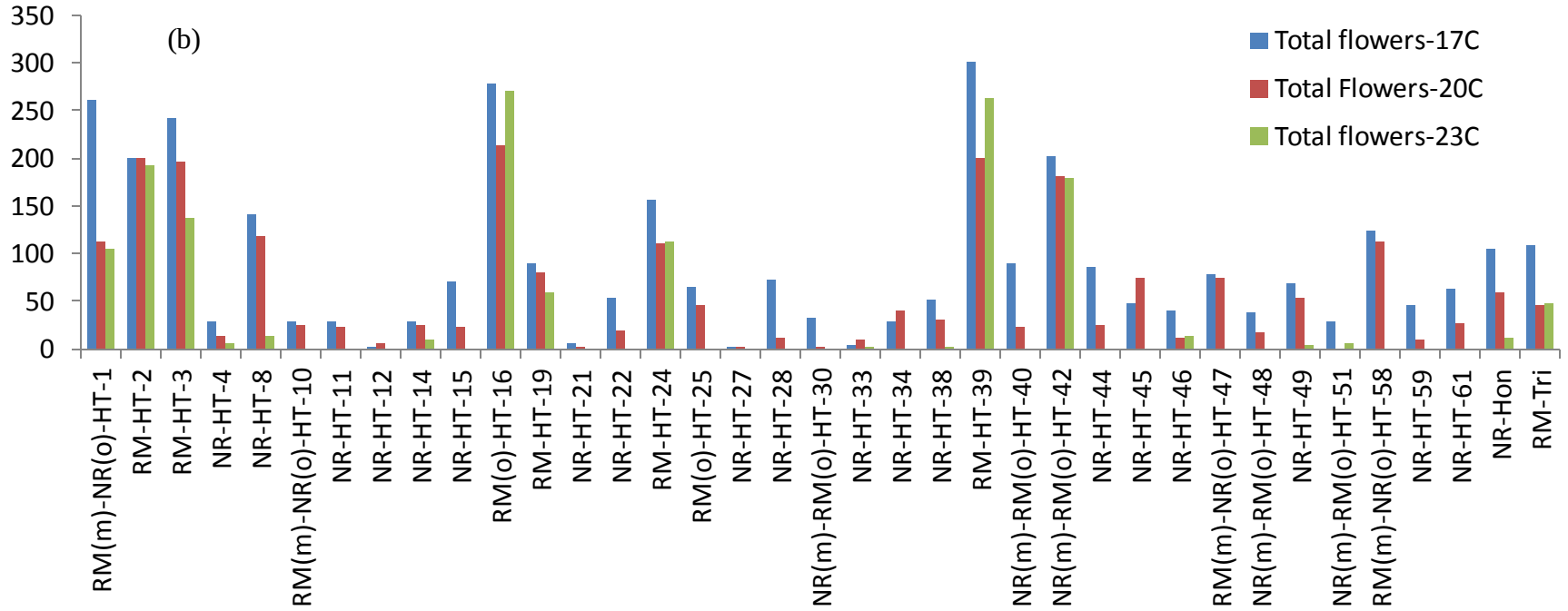
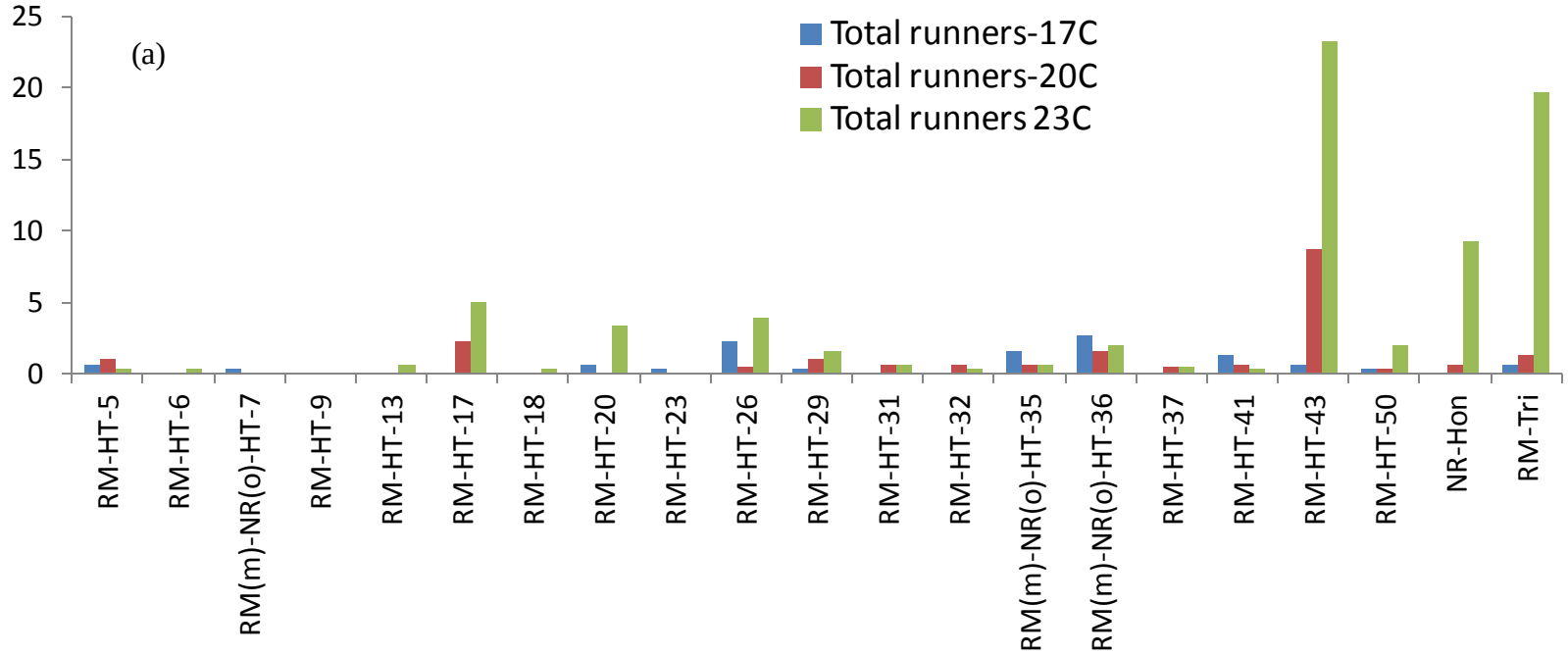


Figure 2.4 (cont'd)



Appendix 2.2

Figure 2.5 Total runners (y-axis) at 17°C, 20°C, and 23°C in the 'Honeoye' × 'Tribute' progeny (HT1-54) and the parents. Remontant/Non-remontant phenotype from the field observations at Benton Harbor,MI and Corvallis, OR are included with the genotype names on the x-axis. The progeny that were remontant at one location and non-remontant at the other are labeled as RM(m)=Remontant in MI, NR(o)= Non-remontant at OR. (a) Total runners at 17°C, 20°C, and 23°C in the heat tolerant progeny. (b) Total runners at 17°C, 20°C, and 23°C in the heat sensitive progeny.



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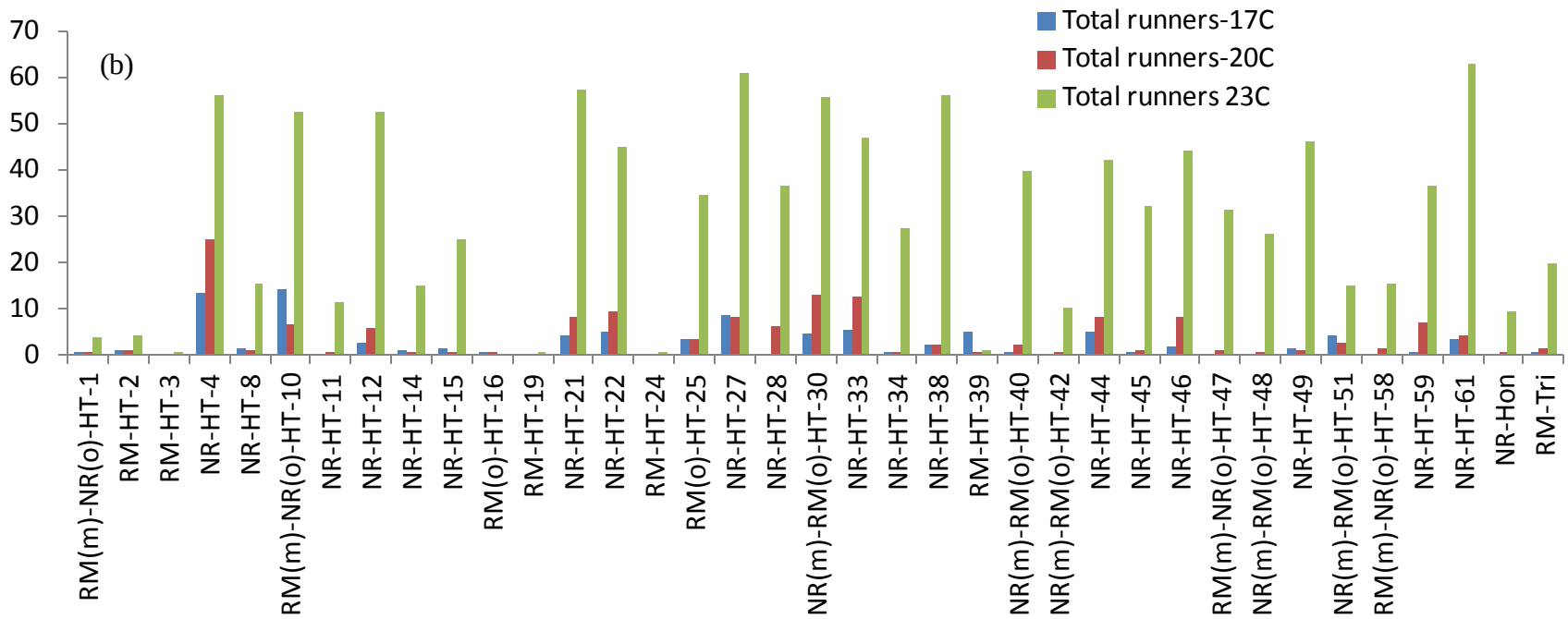

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CHAPTER 3

IDENTIFICATION OF QTL ASSOCIATED WITH HEAT TOLERANCE AND REMONTANCY

Abstract

Flower formation in strawberry is typically considered to be determined by photoperiod, although several studies have demonstrated that temperature plays an important role in the process. Remontancy or repeat flowering is determined by multiple loci and it has been proposed that some of these loci may be determining floral heat tolerance. In this study, a segregating population developed from 'Honeoye' (non-remontant) × 'Tribute' (remontant) was used to develop a linkage map using SSR markers. Phenotypic observations were collected from replicated sets of this population growing at 5 locations (MI, OR, CA, MN, and MD) over two years (2005 and 2006). An additional population developed from the same parents and replicated sets were grown in the greenhouse under three temperature conditions (17, 20, and 23°C) to observe effect of temperature on flowering. This second population was also replicated and grown in the field at MI and OR and phenotypic observations were collected in 2011. Phenotypic data (remontant vs non-remontant, weeks of flowering, and total flowers at 17, 20, and 23°C) was used to identify QTL. Heat tolerance QTL were identified on 8 linkage groups and 5 of these overlapped with remontancy and weeks of flowering QTL. Co-location of heat tolerance QTL with remontancy and weeks of flowering QTL indicated that temperature tolerance is an important aspect of the repeat flowering trait of remontant genotypes. One allele was identified that resulted in significantly higher numbers of flowers at 23°C.

3.1 Introduction

Genetic control of remontancy has been debated and several hypotheses have been proposed including single dominant gene (Bringhurst *et al.*, 1989; Sugimoto *et al.*, 2005), single major gene with modifier genes (Shaw and Famula, 2005), dominant complementary genes (Ourecky

and Slate, 1967), and multiple gene control (Shaw, 2003; Serçe and Hancock, 2005a; Weebade *et al.*, 2008). It has been demonstrated that flower initiation in strawberry is determined by both photoperiod and temperature (Darrow, 1934; Durner *et al.*, 1984; Serçe and Hancock, 2005b; Sonsteby and Heide, 2008; Bradford *et al.*, 2010). However, previous studies exploring inheritance of remontancy have not considered the presence of heat tolerance loci. In the recent study by Weebadde *et al.* (2008), a multi-location (MI, OR, MD, CA, MN) study was conducted to identify QTL (Quantitative Trait Loci) associated with remontancy. Several QTL were identified, some of which were location specific. They proposed that there may be heat tolerance QTL in addition to photoperiod response QTL that determine flowering in strawberry.

Development of heat tolerant remontant genotypes is critical for the midwestern market where the extreme summer temperatures inhibit flower initiation and adversely affect yield. To date there have been no studies to identify chromosomal regions that determine heat tolerant flowering response in cultivated strawberry. Identification of QTL affecting remontancy and heat tolerance will be the first step towards developing markers linked to these traits for marker-assisted breeding.

There have been several studies identifying genes and observing variations in gene expression associated with various developmental processes in strawberry, including flower formation, fruit development, fruit ripening, and disease responses (Aharoni and O'Connell, 2002; Blanco-Portales *et al.*, 2002; Rosin *et al.*, 2003; Mehli *et al.*, 2004; Fuente *et al.*, 2006; Figueroa *et al.*, 2009; Chatterjee *et al.*, 2011), but there has been little effort to identify allelic variants and marker-trait associations. The first report on association between a molecular marker and a phenotypic trait was by Haymes *et al.* (1997) who used a bulk segregant analysis approach to

screen RAPD (Random Amplified Polymorphic DNA) markers for association with resistance to *Phytophthora fragariae* var. *fragariae*, the pathogen causing red stele disease in strawberry. They identified seven RAPD markers linked to red stele resistance in octoploid strawberry, although these markers have not been used for marker-assisted breeding in the public sector.

Lerceteau-Kohler *et al.* (2004) used a population derived from 'Capitola' and 'CF1116' to identify QTL associated with fruit traits. They studied 34 traits and they were able to identify 37 QTL associated with 18 traits related to fruit maturity, color, and firmness, sugar and acid content. In a later report, (Lerceteau-Kohler *et al.*, 2006), they identified 30 QTL associated with sugar and acid content in strawberry fruits. Since of these studies used an AFLP (Amplified Fragment Length Polymorphism) map, the markers would need to be converted to transferable markers to be applicable in breeding programs.

Zorilla-Fonatesi *et al.* (2011) studied a population derived from *F.* × *ananassa* selection lines '232' and '1392' and identified 33 QTL associated with 14 (out of 17 studied) agronomic and fruit quality traits. Their linkage map included several genic SSRs and they were able to identify associations between gene function and QTL locations. For example, ARSFL-099 was derived from the *Fa-Exp-2* gene and was associated with the QTL for fruit firmness. The *Fa-Exp-2* gene is an expansin gene identified in strawberry that is associated with softening of the fruit cell-wall during ripening (Civello *et al.*, 1999). Razavi *et al.* (2011) used an association mapping approach to identify marker trait associations by correlating 24 EST (Expressed Sequence Tag) markers and 369 AFLP marker profiles of 23 *Fragaria* genotypes (20 *Fragaria* × *ananassa*, 2 *F. vesca*, and 1 *F. chiloensis*) with phenotypic observations associated with water deficit. Although they were able to identify several marker-trait associations, they acknowledged that their sample

size was too small to be conclusive and these identified markers would need to be validated in larger sample sets.

There have been few reports on QTL identification and marker-trait associations of flowering traits in strawberry. Three SCAR (Sequence Characterized Amplified Region) markers were developed from ISSRs (Inter Simple Sequence Repeat) associated with the *SEASONAL FLOWERING LOCUS* (*SFL*) in *F. vesca* (Albani *et al.*, 2004). The *SFL* locus is associated with flowering pattern in diploid *F. vesca*. Genotypes that have the dominant allele of this locus are short day plants. Genotypes that have two recessive alleles of this locus are remontant (Brown and Wareing, 1965; Albani *et al.*, 2004). In another study, Sugimoto *et al.* (2005) used a population derived from *F.* × *ananassa* 'Ever berry' and 'Toyonoka' to identify RAPD markers associated with everbearing trait. They identified 5 RAPD markers associated with the everbearing trait: OPE07-1 (11.8 cM) was the closest marker and 88.9% everbearers had this marker. Other RAPD markers associated with the trait were: OPB05-1 (15.8 cM), OPG11-1 (13.9 cM), OPD20-2 (15.1 cM), and OPG09-1 (24.3 cM). However, none of these markers have been exploited in commercial marker-assisted breeding.

Weebadde *et al.* (2008) developed an AFLP linkage map using a population segregating for remontancy and used this map to identify QTL based on phenotypic observations collected in 5 states across US with different environmental conditions. Proportion of remontant progeny in the population varied with the location. While in MI, MN, and MD approximately 50% progeny were remontant, the cooler western states (OR and CA) had >80% remontant progeny. This indicated that there is a strong environmental effect on the remontant trait. They identified 5 QTL using phenotypic observations from the eastern states, and 2 QTL for the western states. In

addition to providing conclusive evidence of remontancy being a multi-genic trait, their study demonstrated that environmental factors determine whether or not the genotype will be remontant.

Construction of a genetic linkage map is the first step towards identification of QTL related to any phenotypic trait. Although mapping efforts with octoploid strawberry have been limited until recently, the diploid *Fragaria* provided the attractive alternative for developing reference maps for studying colinearity within *Fragaria* species (Rousseau-Gueutin *et al.*, 2008; Sargent *et al.*, 2008, 2011). The first linkage map for diploid strawberry was developed by Davis *et al.* (1997) using an F_2 population of 80 individuals from *F. vesca* 'Baron Solemacher' × *F. vesca* 'WC6'. However, the exclusive use of RAPD (Random Amplified Polymorphic DNA) markers in this map limited its transferability and use. The most complete SSR (Simple Sequence Repeat)-based diploid *Fragaria* linkage map was developed by Sargent *et al.* (2004, 2006, 2007, 2008, and 2011). They developed a linkage map using an F_2 population from *F. vesca semperflorens* 815 × *F. bucharica* (previously referred to as *F. nubicola* ((Hook.f.) Lindl. ex Lacaita). Their initial map (Sargent *et al.*, 2004) was developed using a population of 94 seedlings and consisted of 68 SSRs, 1 SCAR (Sequence Characterized Amplified Region), 3 morphological markers, and 6 gene-specific markers, covering 448 cM on 7 linkage groups. This map was later improved by addition of 109 SSRs (Sargent *et al.*, 2006), 29 loci linked to functional genes (Sargent *et al.*, 2007), and an additional 38 new SSRs and 4 markers linked to a fruit ripening gene from *Prunus* (Sargent *et al.*, 2008). The following year, Sargent *et al.* (2009) added additional markers to bring the map coverage up to 568.8 cM. This included 348 published transferable genetic markers, including SSR, and gene-specific markers (173 SSRs, 31 gene-specific and STS markers, 40 RFLPs and 1 SCAR). Ruiz-Rojas *et al.* (2010) used T-DNA

insertional mutagenesis to develop mutants in F1 and then used the T-DNA flanking sequences to map 74 polymorphic loci on the same mapping population. After the availability of the diploid *F. vesca* genome sequence (Shulaev *et al.* 2011), Sargent *et al.* (2011) developed SSR markers for those regions of the genome that did not have adequate coverage in their earlier map. They developed an additional 152 SSR markers and placed these in addition to 42 published SSRs on the map. The *F. vesca* genome sequence was also divided into pseudochromosomes based on the map locations of SSR loci and their corresponding positions on the sequence scaffolds. The latest map has 444 SSR markers covering 442.8 cM.

Until recently, octoploid *Fragaria* linkage maps were mostly developed using non-transferable AFLP (Amplified Fragment Length Polymorphism) markers (Lerceteau-Kohler *et al.* 2003; Weebadde *et al.*, 2008) and as a result have limited use for identifying markers for marker-assisted breeding. The first octoploid strawberry map was developed by Lerceteau-Kohler *et al.* (2003) using AFLP markers on a population of 119 individuals derived from 'Capitola' × 'CF116'. The female map included 235 markers (out of 257) in 43 linkage groups covering 1604cM with marker density of 8.4cM. The male map included 283 markers (out of 293) in 43 linkage covering 1496 cM and an average marker density of 6.3cM. A second AFLP-based map was developed by Weebadde *et al.* (2008) on a population of 127 individuals derived from a F1 cross between 'Honeoye' × 'Tribute'. They mapped 427 SDRF (Single Dose Restriction Fragments) (Wu *et al.*, 1992) on 43 linkage groups as a consensus map. Although the average marker density was 0.3 markers/cM, the map consisted of 42 linkage groups (more than the expected number: 28) indicating that the more markers were needed to link the groups together.

In recent years, at least three SSR-based octoploid linkage maps have been developed. Zorilla-Fonatessi *et al.* (2011) developed a linkage map using a population of 95 F_1 individuals from selection lines '232' and '1392' of *F.* × *ananassa*. This map has 338 markers (from 146 SSR primers) on 37 linkage groups covering 1259.8cM with average marker spacing 4.3 cM. Rousseau-Gueutin *et al.* (2008) added 306 markers from 79 SSR primers to the AFLP map developed by Lerceteau-Kohler *et al.* (2003). The resulting female map covered 2582 cM on 28 linkage groups. The male map covered 2185 cM on 26 linkage groups. As with the diploid, Sargent *et al.* (2009, 2012 submitted) most recently constructed the most comprehensive octoploid linkage map with transferable SSR markers. They used an F_1 population of 188 seedlings developed from 'Redgauntlet' × 'Hapil'. The initial map was developed using 71 SSRs, 5 gene specific primers, 11 RAPDs and 10 AFLP primers combination (Sargent *et al.*, 2009). The female ('Redgauntlet') map consisted of 32 linkage groups with 170 loci covering 1675.3 cM. The male ('Hapil') map consisted of 37 linkage groups with 182 loci covering 1440.7 cM. Sargent *et al* (2012 submitted) added another 331 SSR loci to this map resulting in a map covering 2140.3 cM in 28 linkage groups and with 550 marker loci (491 SSRs from 241 primer pairs). In addition to the cultivated strawberry, linkage map has also been developed for the native octoploid *F. virgininana* (Spigler *et al.*, 2008). The *F. virginiana* map consists of 42 linkage groups with 210 SSR markers (from 100 primers) markers and covers 2373 cM.

The availability of several SSR-based linkage maps makes comparison of QTL across different populations possible. Weebadde *et al.*, (2008) identified 8 QTL linked to remontancy using a 'Honeoye' × 'Tribute' population. However, markers associated with these QTL cannot be compared across maps developed with other parents because the AFLP markers on the map were not developed into SCAR markers. This mapping population is an excellent resource to study

remontancy and other commercially important traits associated with remontant cultivars. In this study, the 'Honeoye' × 'Tribute' population was used to develop a transferable SSR-based linkage map and this map was used to identify QTL associated with heat tolerance and remontancy using phenotypic data collected in the greenhouse (Chapter 2), and in the field (Chapter 2 and Weebadde *et al.*, 2008). These different data sets provided several routes to verify QTL associated with remontancy (Lander and Kruglyak, 1995; Collard *et al.*, 2005, Pelgas *et al.*, 2011). Since remontant genotypes are characterized by flowering in early and late summer, and an extended flowering season, both the traits were used to identify QTL in this study.

3.2 Material and Methods

3.2.1 Mapping population

An F_1 population of 174 progeny of 'Honeoye' × 'Tribute' was used to build the linkage map. 'Tribute' is a remontant cultivar released in 1981 by Draper *et al.* (1981) in Maryland. 'Honeoye' is a short day cultivar released in 1979 in New York (Ourecky, 1979). This population was selected because it segregates for remontancy and had earlier been used by Weebadde *et al.* (2008) to identify QTL determining remontancy. Out of the 174 individuals in our mapping population, 112 had been phenotyped by Weebadde *et al.* (2008) and 62 were used in their AFLP map. The additional 62 progeny were generated from a cross made in 2009.

3.2.2 DNA extraction

Young leaf samples from the parents and progeny were collected from greenhouse grown plants and placed on ice while transporting to the lab. The leaves were stored at 4 °C for a maximum of 2 days until DNA extraction. When DNA was not extracted immediately, the leaves were stored

at -80°C. The leaf samples were ground to a fine powder with liquid nitrogen and DNA was extracted using the DNeasy Plant mini kit (Qiagen) following the manufacturer's protocol. DNA was eluted in a volume of 200 µL. DNA quality was determined by running 4 µL of the eluted DNA on a 0.8% (w/v) agarose (Invitrogen, Carlbad, California 92008) gel containing 0.5 mg/mL ethidium bromide, in 1x TBE buffer (90mM Trizma Base (Sigma-Aldrich Corp, St Louis, MO) + 90mM Boric acid (J.T. Baker, Phillipsburg NJ 08865) + 2mM EDTA (Invitrogen, Carlbad, California 92008), pH 8.0). DNA samples were loaded along with a loading dye (30% glycerol, 0.25% Bromophenol blue). The gels were run at 100V for 20 min and visualized using the ChemiDoc^{TM} XRS+ system (Biorad, Hercules, CA 94547). The DNA was diluted 1:4 with Nuclease free water (Promega Corporation, Madison, WI 53711) for PCR amplification.

3.2.3 Genotyping

Selection of SSRs: SSR loci developed from *F.* × *ananassa*, *F. vesca, F. nubicola*, and *F. viridis*, in previously published literature (Sargent *et al.*, 2003; Cipriani and Testolin, 2004; Lewers *et al.*, 2005; Bassil *et al.*, 2006; Bassil *et al.*, 2006b; Sargent *et al.*, 2006; Gil-Ariza *et al.*, 2006; Njuguna 2010; Zorrilla-Fontanesi *et al.*, 2011) were screened for polymorphism in a subset of 54 progeny and the parents. 157 SSR markers were screened and 99 were selected based on polymorphism and distinct scorable bands on the 6% polyacrylamide gel. These 99 SSR primer sets were used for genotyping the population. These included 70 (out of 115 tested) derived from *F.* × *ananassa*, 20 (out of 26 tested) from *F. nubicola*, 7 (out of 13 tested) from *F. vesca*, and 2 (out of 3 tested) from *F. viridis*. Approximately 60 per cent of the SSR markers mapped were EST derived (Lewers *et al.*, 2005; Gil-Ariza *et al.*, 2006; Bassil *et al.*, 2006; Bassil *et al.*, 2006b; Zorrilla-Fontanesi *et al.*, 2011) and therefore can potentially be associated with a gene function. Appendix 3.1 lists the SSR markers used to genotype the population, along with

their primer sequences and the putative functions associated with the EST-SSRs based on BLAST searches reported in the original literature.

DNA amplification: DNA amplification was performed in 20 μL reactions containing 1 x GoTaq® Green Master Mix (Promega Corporation, Madison, WI 53711), 0.5 mM of forward and reverse primer, and 1 μL of diluted DNA template. Amplifications were done in C1000TM Thermal Cycler (Biorad, Hercules, CA 94547) using the following PCR cycle: Initial denaturation: 95°C for 2 min; 34 cycles of 1 min at 95°C, 60 s at annealing temperature (=T_m + 2°C), 1.5 min at 72°C; and a final extension step of 10 min at 72°C, hold at 15°C.

Polyacryamide Gel Electrophoresis: PCR amplified products were separated using a 6% denaturing polyacrylamide gel (15 mL of 40% Acrylamide/Bis Solution (BioRad, Hercules, CA 94547), 10 mL 10 x TBE buffer, 42 g Ultra Pure Urea (Invitrogen, Carlbad, California 92008), 500 μL 10% APS, 100 mL TEMED (BioRad, Hercules, CA 94547). The PCR amplicons were denatured (95°C for 5 min, hold at 4°C) and loaded on to 38 cm x 50 cm Sequi-Gen GT system (BioRad, Hercules, CA 94547) that was preheated for 20-30 min. The gels were run at 80W for 3.5 hrs. Silver staining (Bassam *et al.*, 1991) was used to visualize the products and the fragment sizes were estimated by comparing with 10 and 50 bp ladders (Invitrogen, Carlbad, California 92008).

3.2.4 Linkage map

The Single Dose Restriction Fragment (SDRF) (Wu *et al.*, 1992) approach was used for scoring the markers. In this approach, each segregating fragment was treated as an individual allele and the genotypes are scored for presence/absence of the allele. Each gel was scored twice to

minimize errors. Markers present in both the parents that segregate in a 3:1 ratio were coded as dominant markers. Markers present in only one parent that segregate in a 1:1 ratio were coded as codominant markers. Both the dominant and codominant markers were used to develop the linkage map using Joinmap 3.0 (Stam 1993). The linkage map was developed using a minimum threshold LOD score value of 3.0, maximum recombination frequency of 0.3, and Kosambi mapping function.

The linkage groups created by Joinmap were visualized using MapChart 2.2 (Voorips, 2002). The SSR markers were color coded to reflect whether they were present in 'Honeoye', 'Tribute', or both (Figure 3.1). Marker names included the name of the SSR locus as in the original publication, followed by the band size in bp. When SSR primers resulted in duplicated bands, the marker names included sizes of all the cosegregating bands. Markers with segregation distortion were indicated with an asterisk (*).

Identification of homeologs: The linkage groups identified in the 'Honeoye' × 'Tribute" population were compared with the diploid (Sargent *et al.*, 2011) and octoploid (Sargent *et al.*, 2012) maps to identify their homeologous groups. In addition, locations of the SSR markers were identified on the *F. vesca* pseudochromosomes (Shulaev *et al*, 2011; Sargent *et al.*, 2011) using the BLASTn function in the PFR strawberry genome server www.strawberrygenome.org. The locations on the diploid pseudochromosomes was considered conclusive only when >20 bp of both the forward and the reverse primer sequence had a complete match with only one pseudochromosome. This criterion was used to ensure BLASTn was not picking up random similarities with the short oligonucleotide sequences. The locations of the markers on the diploid map were indicated on the octoploid linkage groups in parenthesis (Figure 3.1).

3.2.5 Phenotypic evaluation

Greenhouse: 54 progeny were propagated and grown in the greenhouse under three temperature conditions: 17°C, 20°C and 23°C under a 16 hr photoperiod as described in Section 2.2.2 (Chapter 2). Number of flowers for each of the 54 progeny in the greenhouse was counted every week from Dec 2010 to Mar 2011 as described in 2.2.3 (Chapter 2).

Field observations: 62 progeny from 'Honeoye' × 'Tribute' cross made in 2009, including the 54 that were used in the greenhouse study, were planted in the field at MI and OR in Aug 2010 as described in Section 2.2.2 (Chapter 2).

In addition, phenotypic data for a set of 112 progeny that were collected by Weebadde *et al.* (2008) from 5 locations in MI, MN, MD, OR, CA was used for QTL validation. The growth conditions for these plants are described in Weebadde *et al.* (2008).

The average minimum and maximum temperatures in early and late summer at all the field locations and in all the years of study are listed in Table 3.1.

Remontant vs non-remontant phenotype was recorded for each of the 62 progeny planted at MI and OR. Presence/absence of flowers was recorded every week from May, 1 2011 to Aug 15, 2011. Progeny that flowered in the spring and then again after July 22 were categorized as remontant. In these genotypes, flower initiation had occurred both in short days (early summer flowering), and long days (late summer flowering). The genotypes that only flowered in the spring were considered non-remontant.

The phenotypic data collected by Weebadde *et al.* (2008) was also used to identify QTL. Information on whether the genotypes were remontant or non-remontant was available from OR, MN, MD, and CA in 2005, and from MI in 2005 and 2006. Weeks of flowering data was also available in 2005 from May 6-Jul 6 for MI, May 2-Aug 25 for CA, and Mar 10-Aug 25 for OR. In 2006, weeks of flowering data was collected in MI from Apr 27-Aug 2. The differences in data collection dates reflect the differences in growing seasons. Weeks of flowering information for 2005 was not available for MN and MD.

3.2.6 Distribution graphs

Phenotypic distribution histograms were prepared using MSExcel.

3.2.7 QTL identification

MapQTL5 (Van Ooijen, 2004) was used for QTL identification using the MQM or Composite Interval Mapping approach. The population was derived from two heterozygous parents and was coded as CP to include the three types of marker data: 1: codominant markers segregating in 'Honeoye', 2: codominant markers segregating in 'Tribute', 3: Dominant markers present in both parents. Markers identified as significant by the Kruskal-Wallis test were used as cofactors. The significant LOD score at $p \leq 0.05$ was determined from 1000 permutations with the dataset. Significant QTL regions along with the linkage groups were visualized using MapChart 2.2 (Voorips, 2002). Only those regions that had a variance of >10% are reported. The location of the highest peak in the QTL region is reported in the Appendix 3.6, 3.7, and 3.8, and the entire range of QTL region is represented in Figure 3.1 for comparison of all QTL locations. Regions with significant LOD scores that were separated by less than 10 cM were considered to be the same QTL.

Table 3.1 Average minimum and maximum temperatures at the field locations (MI-Benton Harbor, MN-St Paul, MD-Beltsville, OR-Corvallis, CA-Watsonville) in the different years of study (2005, 2006, 2011).

Location	**MI**			**MN**	**MD**	**OR**		**CA**
Year	**2005**	**2006**	**2011**	**2005**	**2005**	**2005**	**2011**	**2005**
Avg min temp (Apr-May)	6	6	7	3	2	6	6	7
Avg max. temp (Apr-May)	18	17	16	17	20	18	15	19
Avg min. temp (June-Aug)	17	15	17	18	14	10	12	11
Avg max. temp (June-Aug)	29	26	27	28	30	26	23	21
Percent remontant plants	49.2	45	50	50	48	80	50	87.3

3.3 Results and discussion

3.3.1 Linkage map

The 99 SSR primer pairs resulted in the amplification of 258 segregating markers and ~556 monomorphic markers. Out of the 258 segregating markers, 77 were present only in 'Tribute', 115 were present only in 'Honeoye', and 66 were present in both parents. The segregation type and Chi square values of the markers are listed in Appendix 3.2.

Initially, markers heterozygous in either of the parents were used to develop separate male and female maps. However, these maps had very limited coverage. The 'Honeoye' map had 103 markers in 23 linkage groups, and the 'Tribute' map had 78 markers in 22 linkage groups (Appendix 3.3). Only 7 male and female groups had common markers. Therefore, a consensus linkage map with all the markers was developed. The consensus map includes 130 (out of 258 markers) in 34 linkage groups and covers 1028 cM with an average marker density of 1 marker per 7.9 cM (Figure 3.1). This map length has slightly less coverage than the *F. x ananassa* SSR map developed by Zorilla-Fonatesi *et al.* (2011): 1259.8 cM on 37 linkage groups. Their map was also denser with an average of 4.3 cM between markers. The map coverage is approximately half of the map developed by Sargent *et al.*(2012 submitted): 2140.3 cM in 28 linkage groups, and the *F. virginiana* SSR map developed by Spigler *et al.*(2008): 2373 cM in 42 linkage groups.

Segregation distortion was observed for 34% of the markers (Appendix 3.4). Out the markers that showed segregation distortion, 40 were in 'Honeoye', 24 were in 'Tribute', and 24 were in both the parents. Segregation distortion has been observed in the diploid and octoploid *Fragaria* maps developed to date. Sargent *et al.* (2004) observed segregation distortion in 54% of the markers they used to assemble the diploid map. They attributed it to the fact that they were

using progeny from an interspecific cross (*F. vesca semperflorens* x *F. bucharica*), and that one of the parents was self incompatible. Nier *et al.* (2006) observed significant segregation distortion on 3 linkage groups while mapping 33 SSRs using a backcross population (*Fragaria vesca* × (*F. vesca* × [*F. vesca* × *F. viridis*]). Ruiz-Roja *et al* (2010) observed segregation distortion in 42% of the markers developed through T-DNA insertional mutagenesis. Twenty eight percent of the markers in the octoploid *F.* x *ananassa* map developed by Sargent *et al.* (2009) had segregation distortion. In the case of the other octoploid species, 30% of the markers in the *F. virginiana* map (Spigler *et al.*, 2008) showed distortion.

Sargent *et al.* (2009) observed that the markers with distorted ratios were evenly distributed across the linkage groups. In the present 'Honeoye' × 'Tribute' map, the distorted markers were distributed in 22 groups. Removal of the markers with distorted segregation did not change the groups formed by the markers. Spigler *et al.* (2008) speculated that the distortions are a result of PCR amplification errors rather than a biological phenomenon. However, Ruiz-Roja *et al* (2010) discussed the possibility of gametophytic selection, non-homologous recombinations, transposons, and the structure of mapping population contributing to the distorted segregation. Similar speculations were made by Zorilla-Fonatessi *et al.* (2011) who explained that segregation distortions may be the result of genes that lead to low survival of some genotypes.

Figure 3.1 Consensus 'Honeoye' × 'Tribute' linkage map and the QTL associated with remontancy (rem), weeks of flowering (wks), and heat-tolerant/sensitive floral responses (FL23C, FL20C, FL17C). Map distances are in cM. Only the groups with significant QTL regions are shown. The significant LOD score at $p \leq 0.05$ was determined from 1000 permutations with the dataset. The markers are color coded to indicate whether they segregate in Honeoye (red), in 'Tribute' (green), or both (blue). Locations of the markers in the diploid map are indicated in parenthesis. Markers with segregation distortion are indicated with asterisk (*).

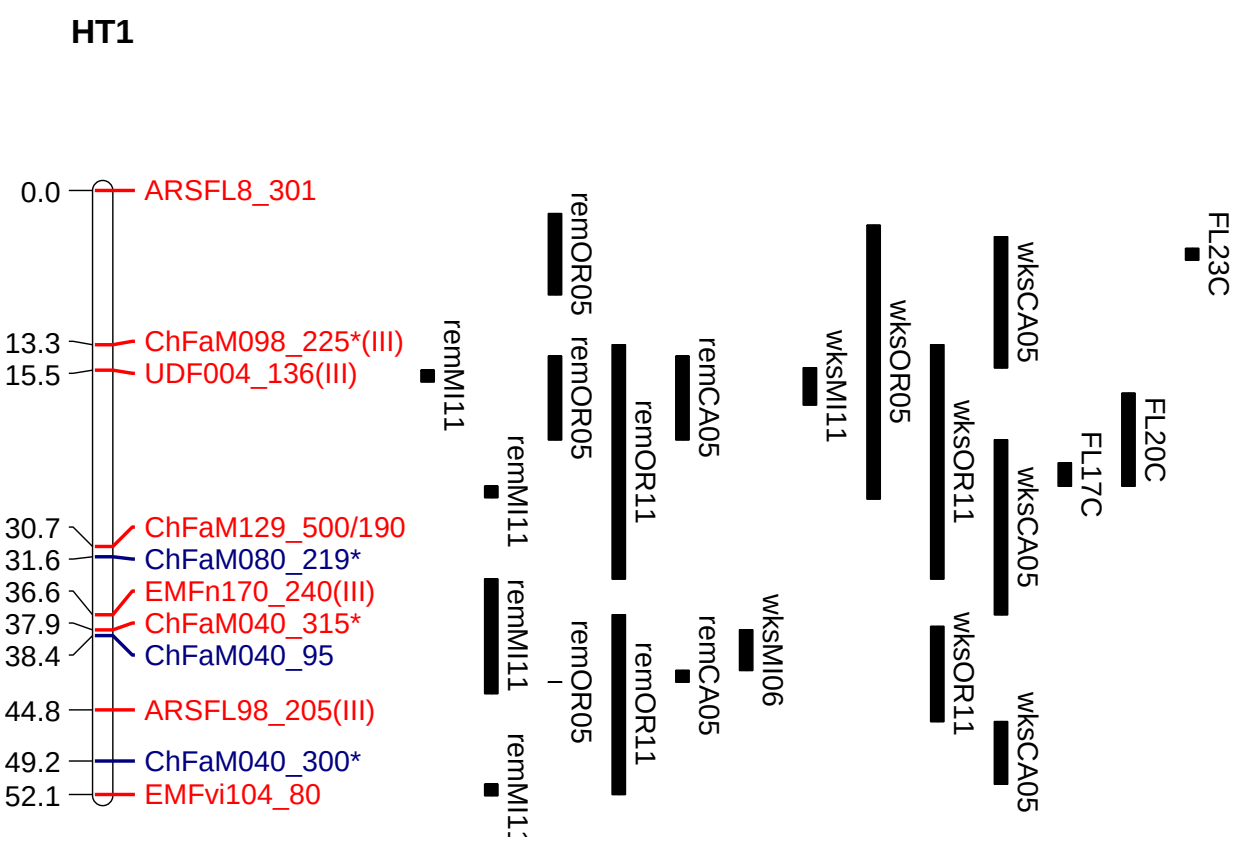


Figure 3.1 (cont'd)

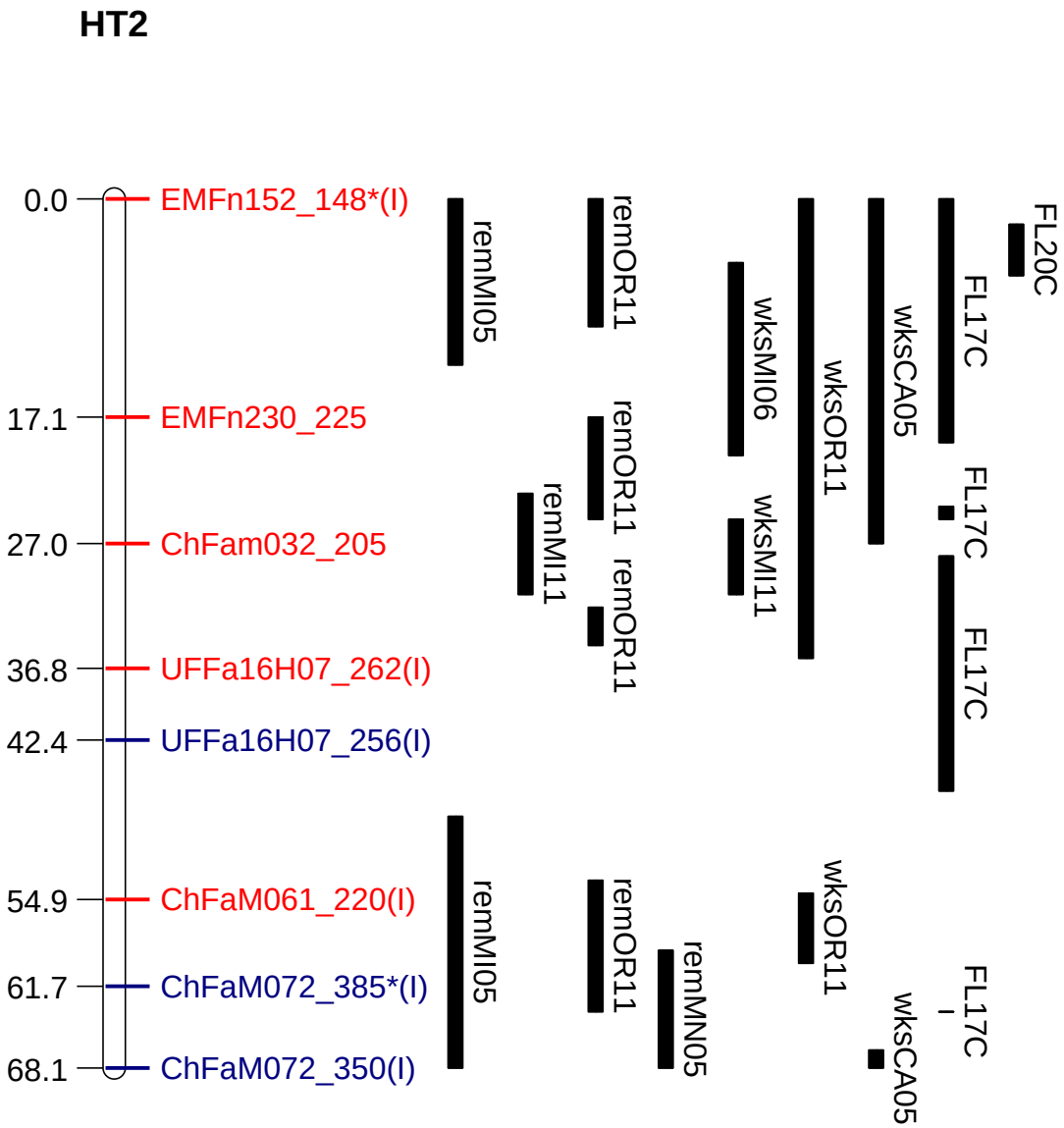
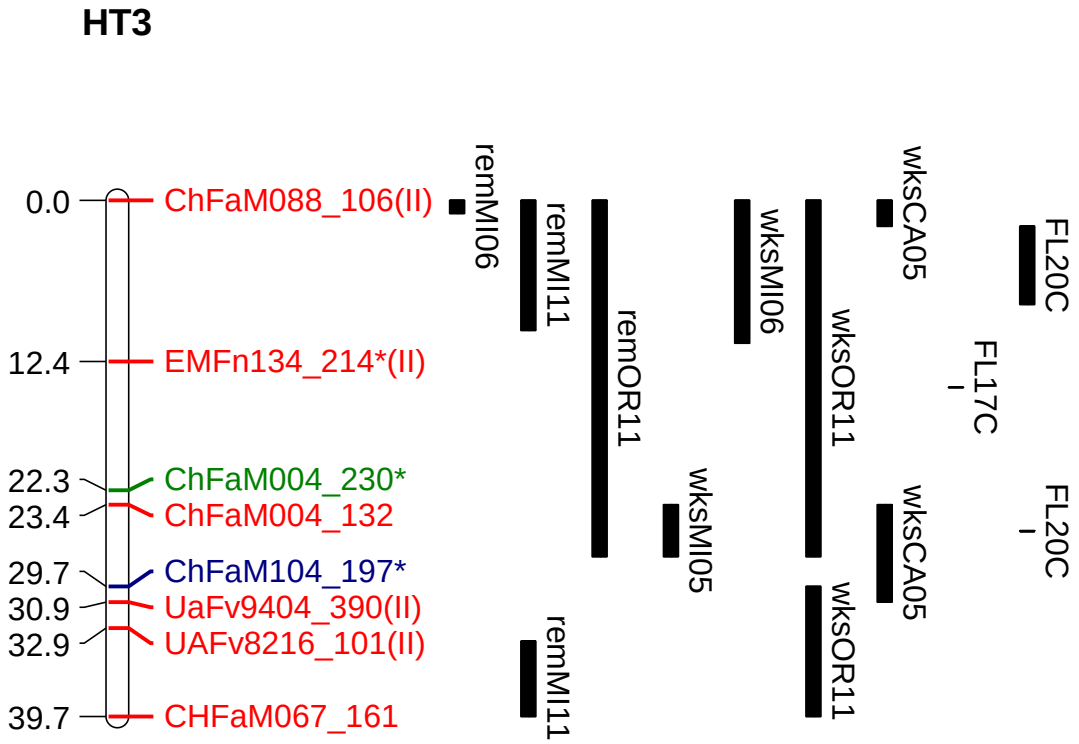


Figure 3.1 (cont'd)



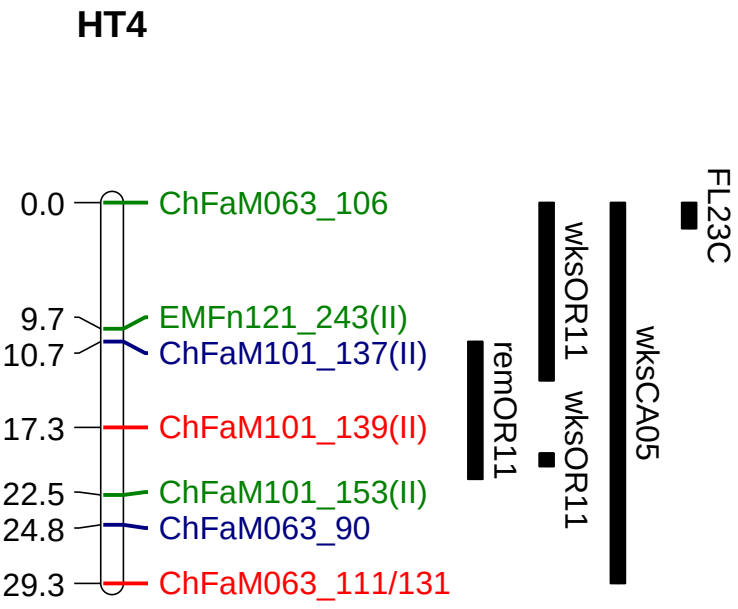


Figure 3.1 (cont'd)

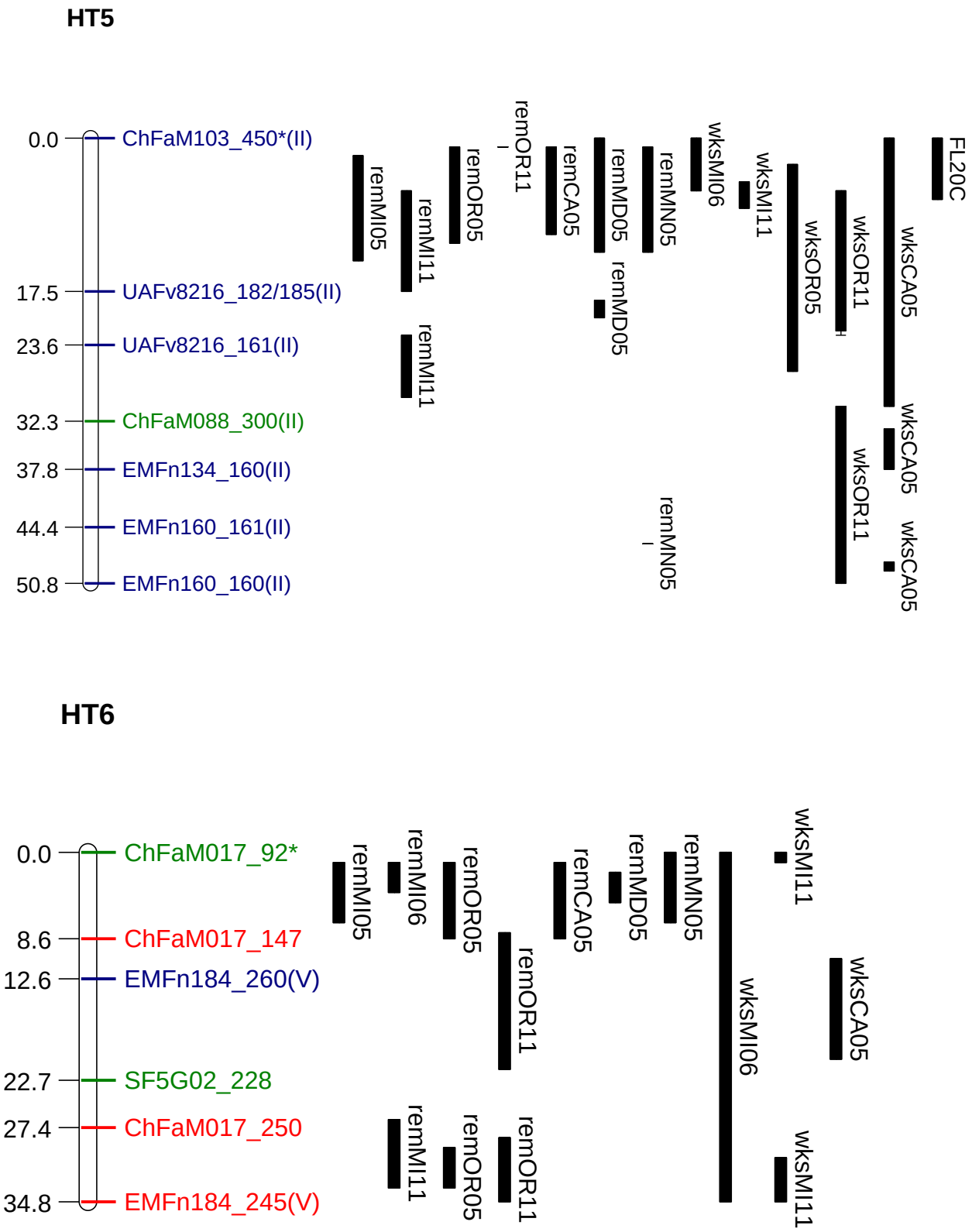


Figure 3.1 (cont'd)

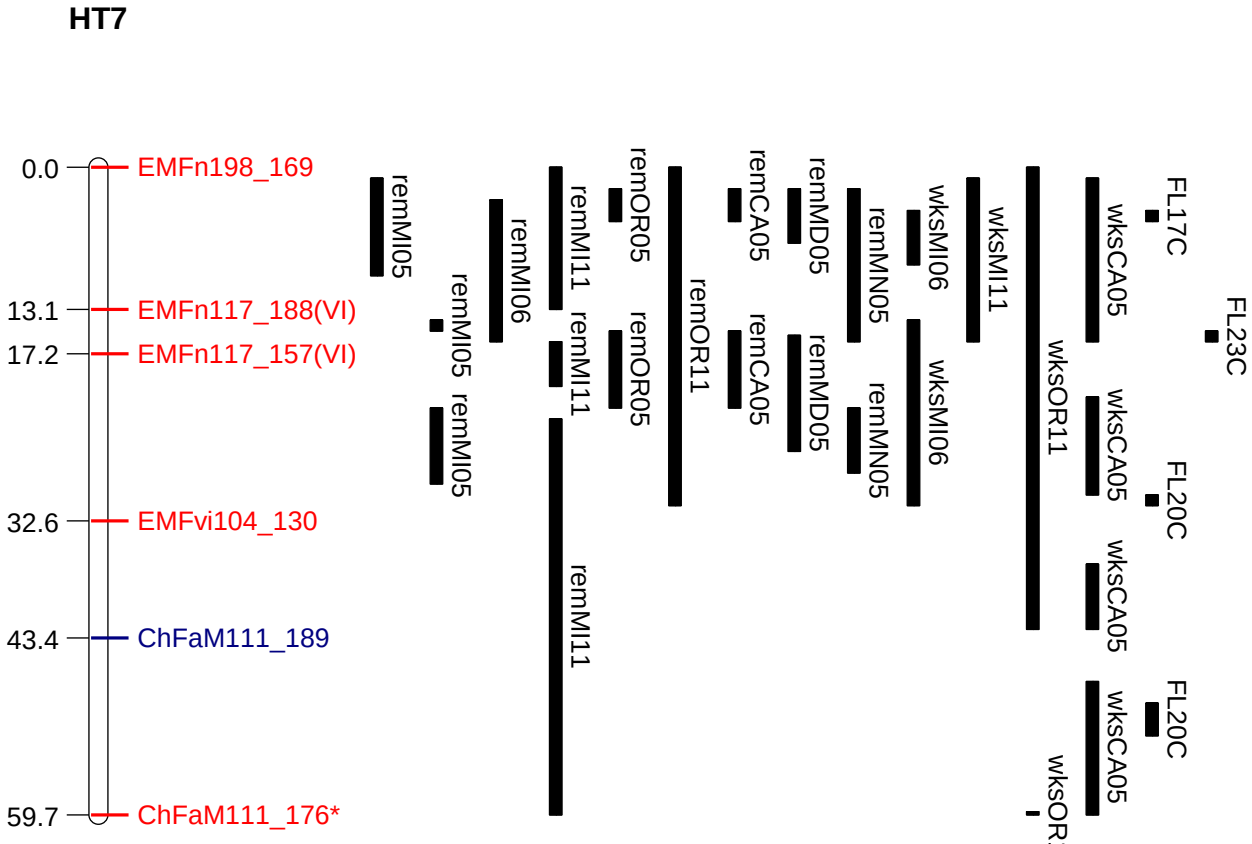


Figure 3.1 (cont'd)

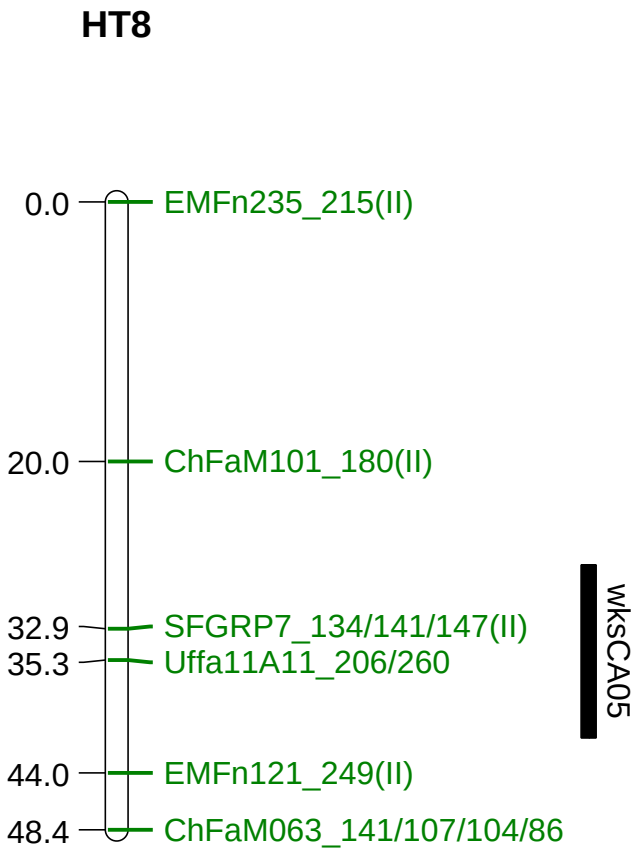


Figure 3.1 (cont'd)

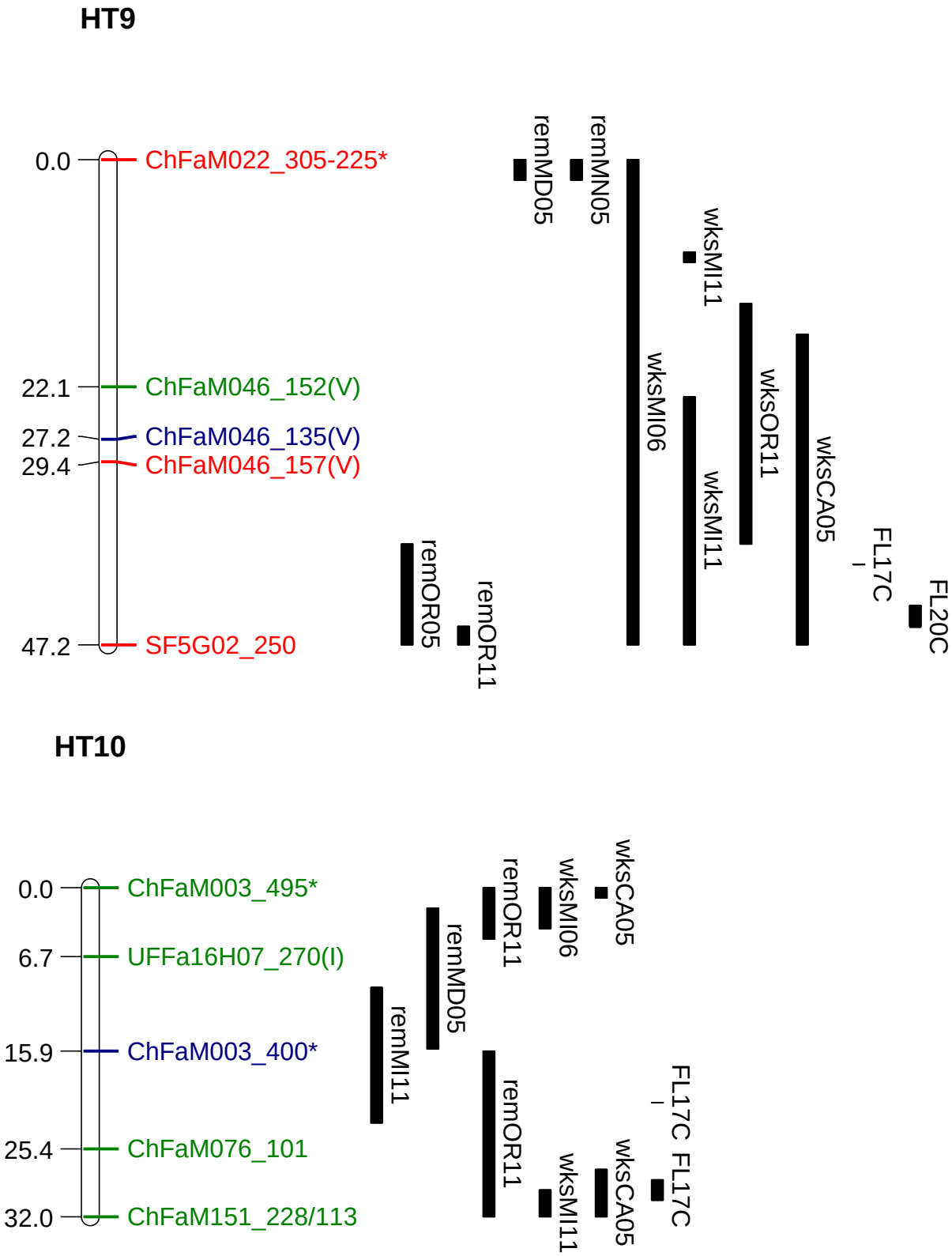


Figure 3.1 (cont'd)

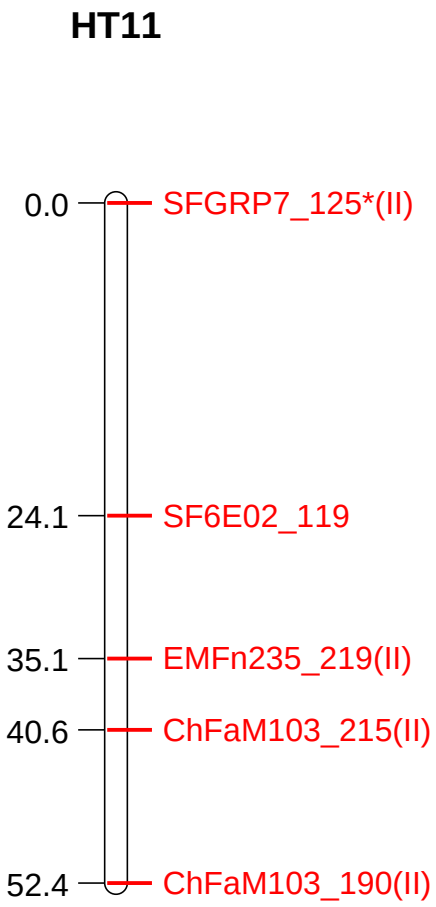
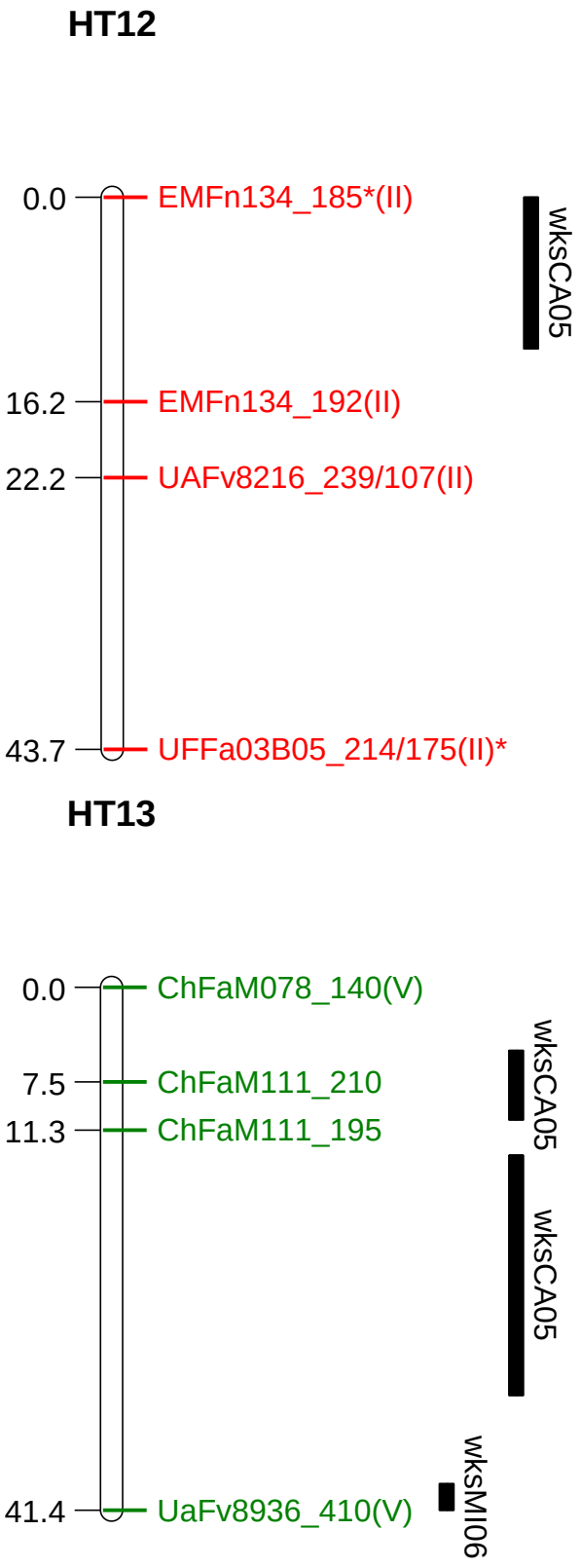


Figure 3.1 (cont'd)



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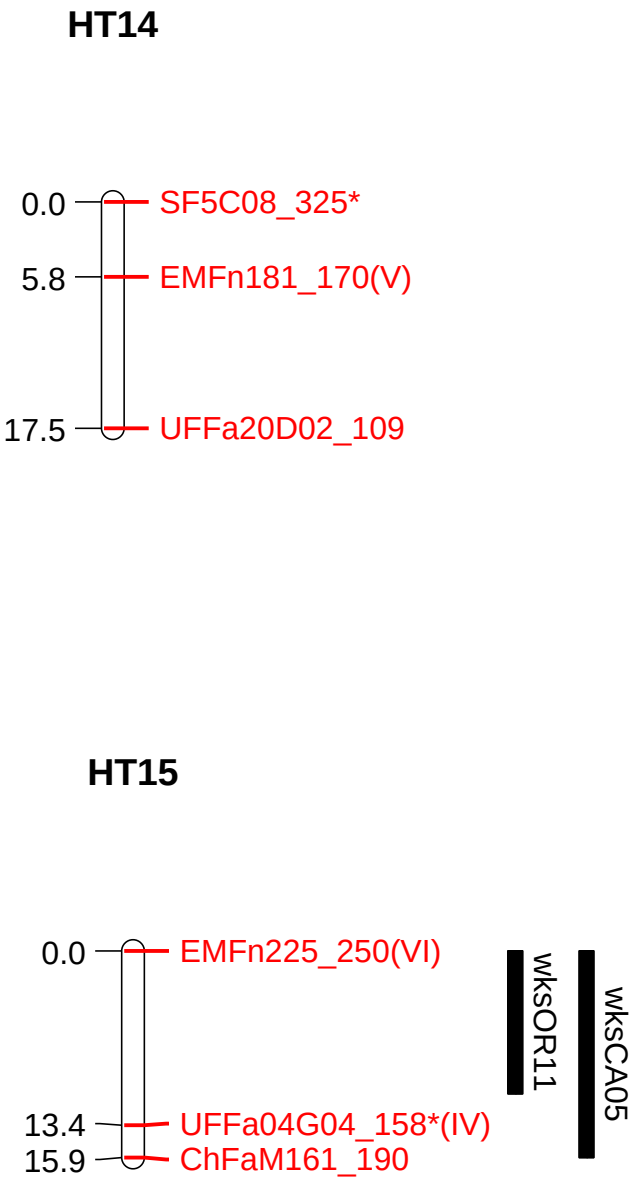

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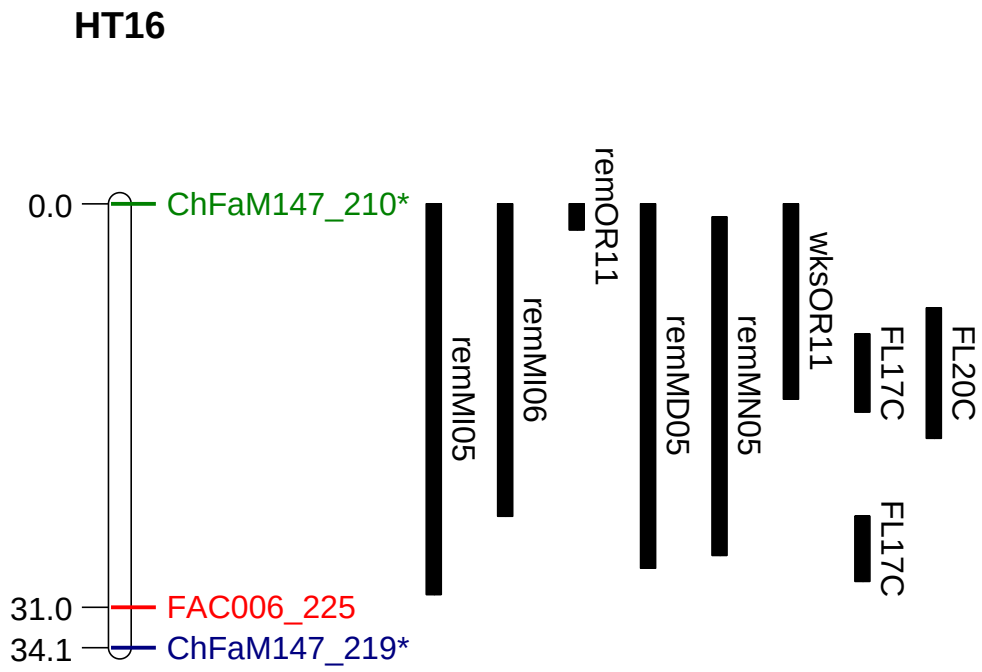

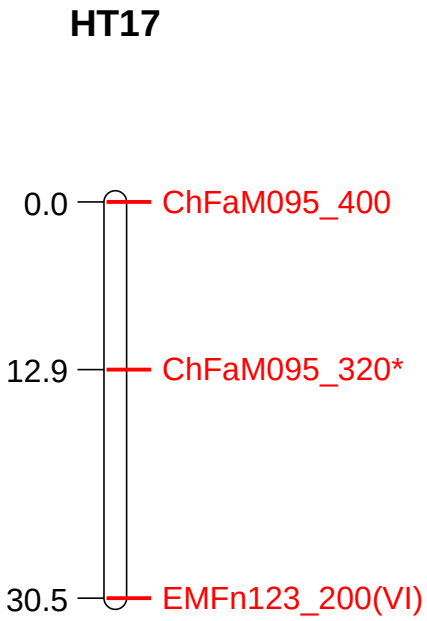

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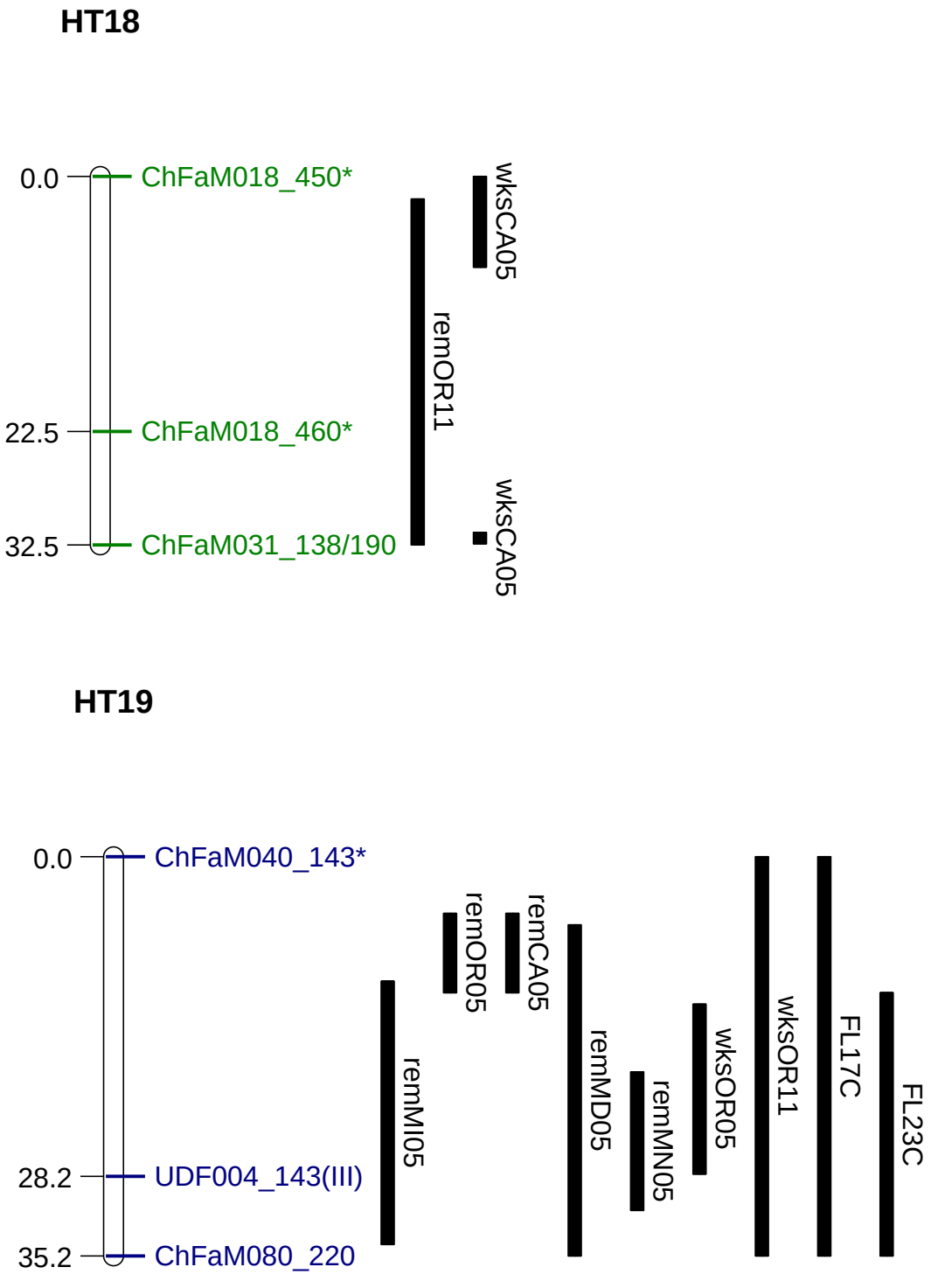

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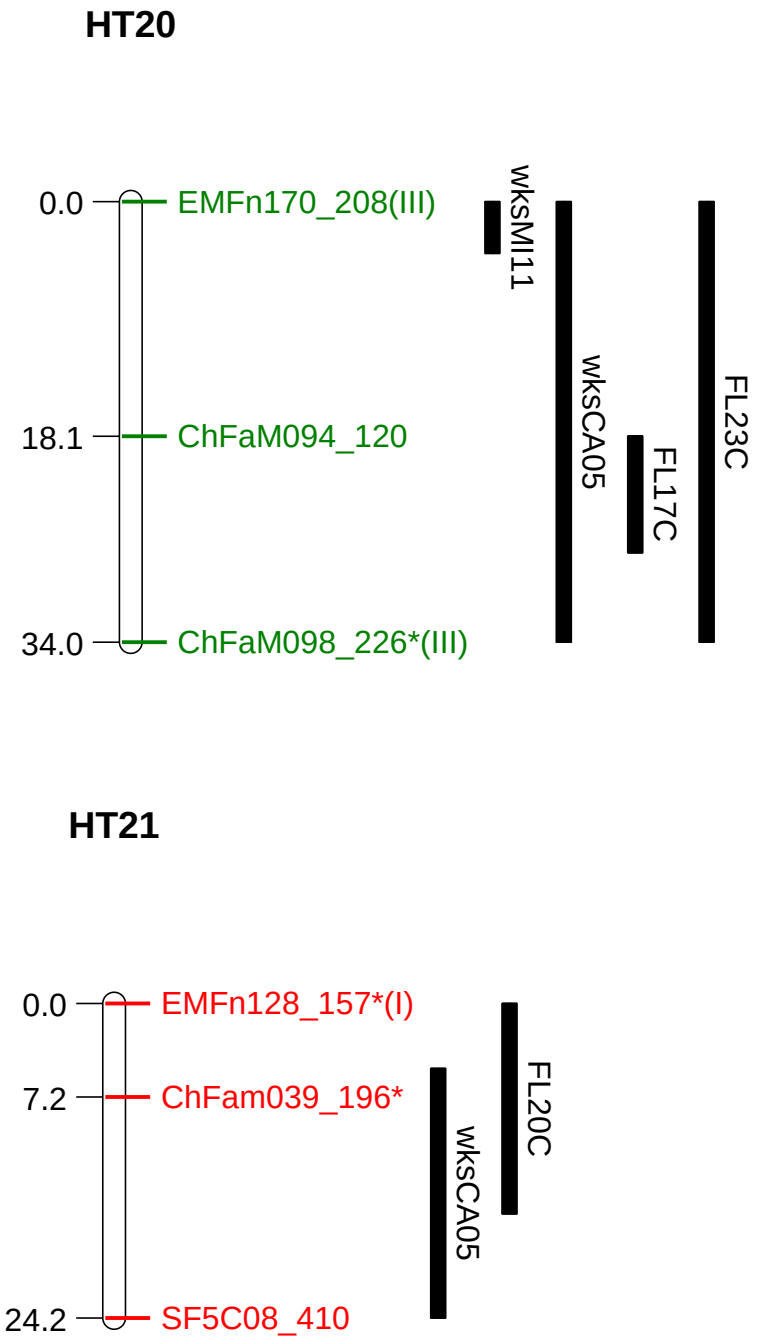

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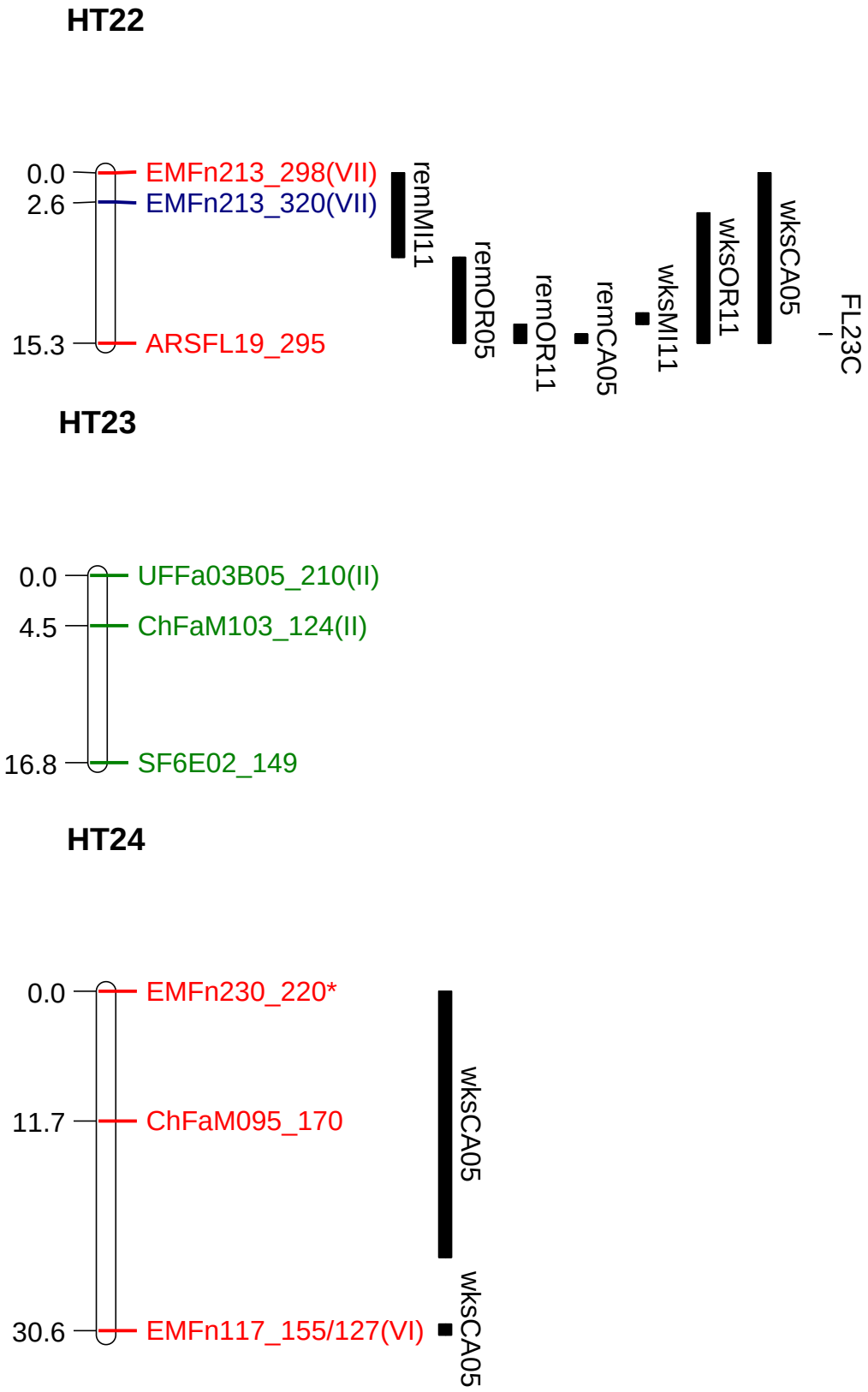

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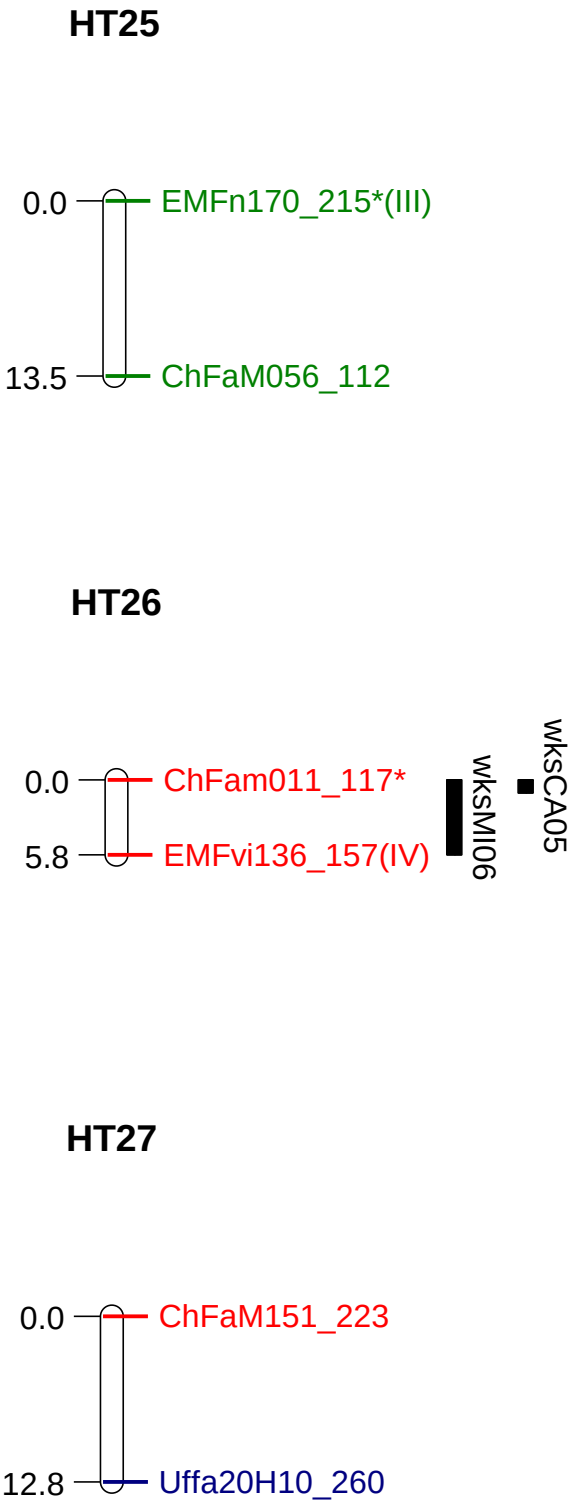

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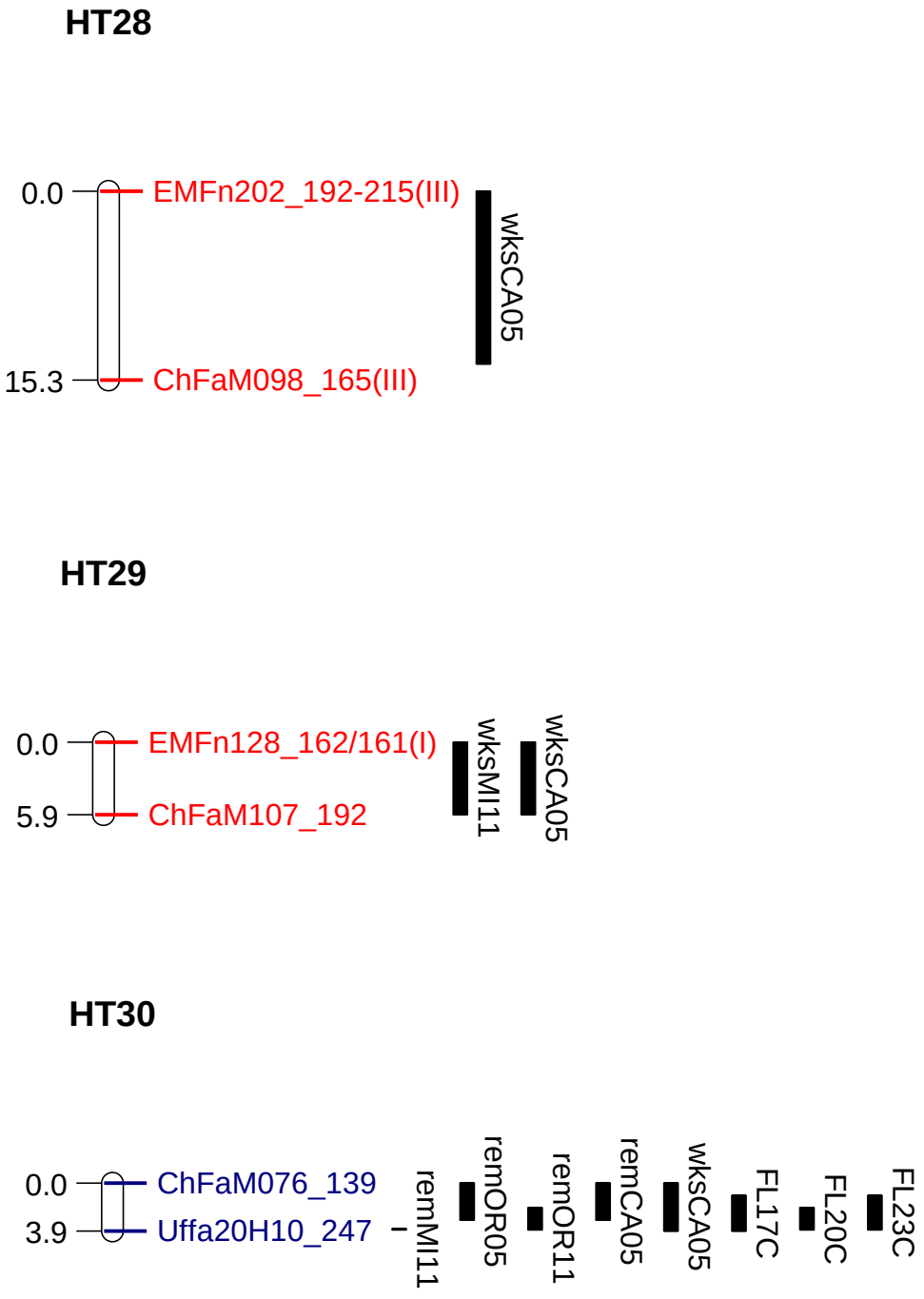

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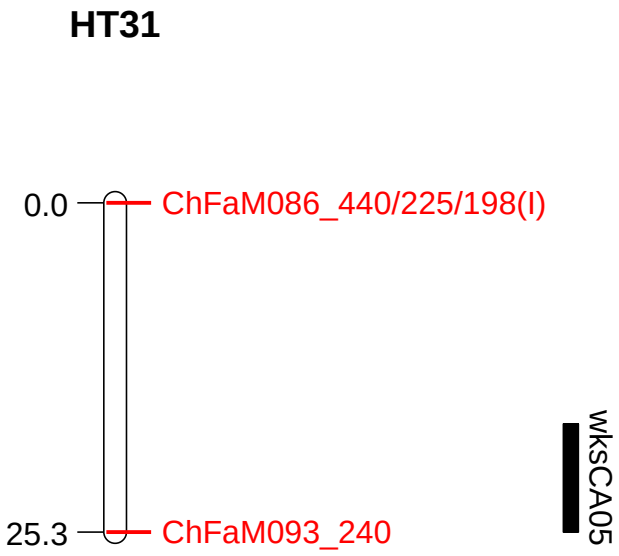

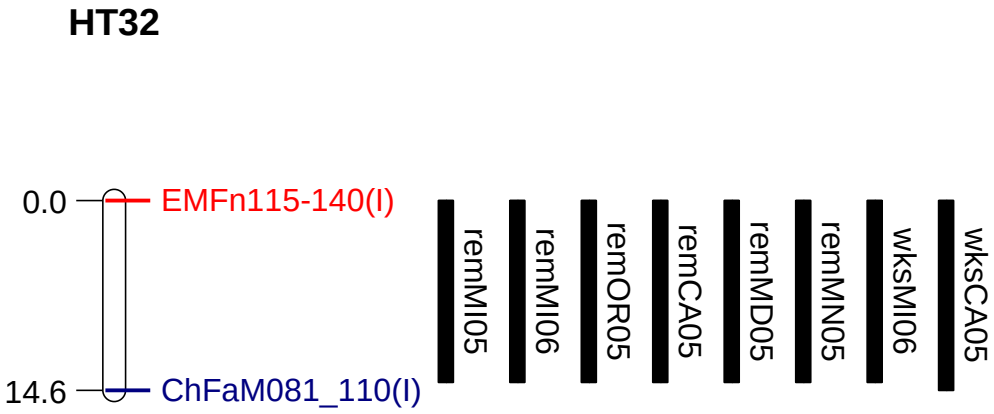

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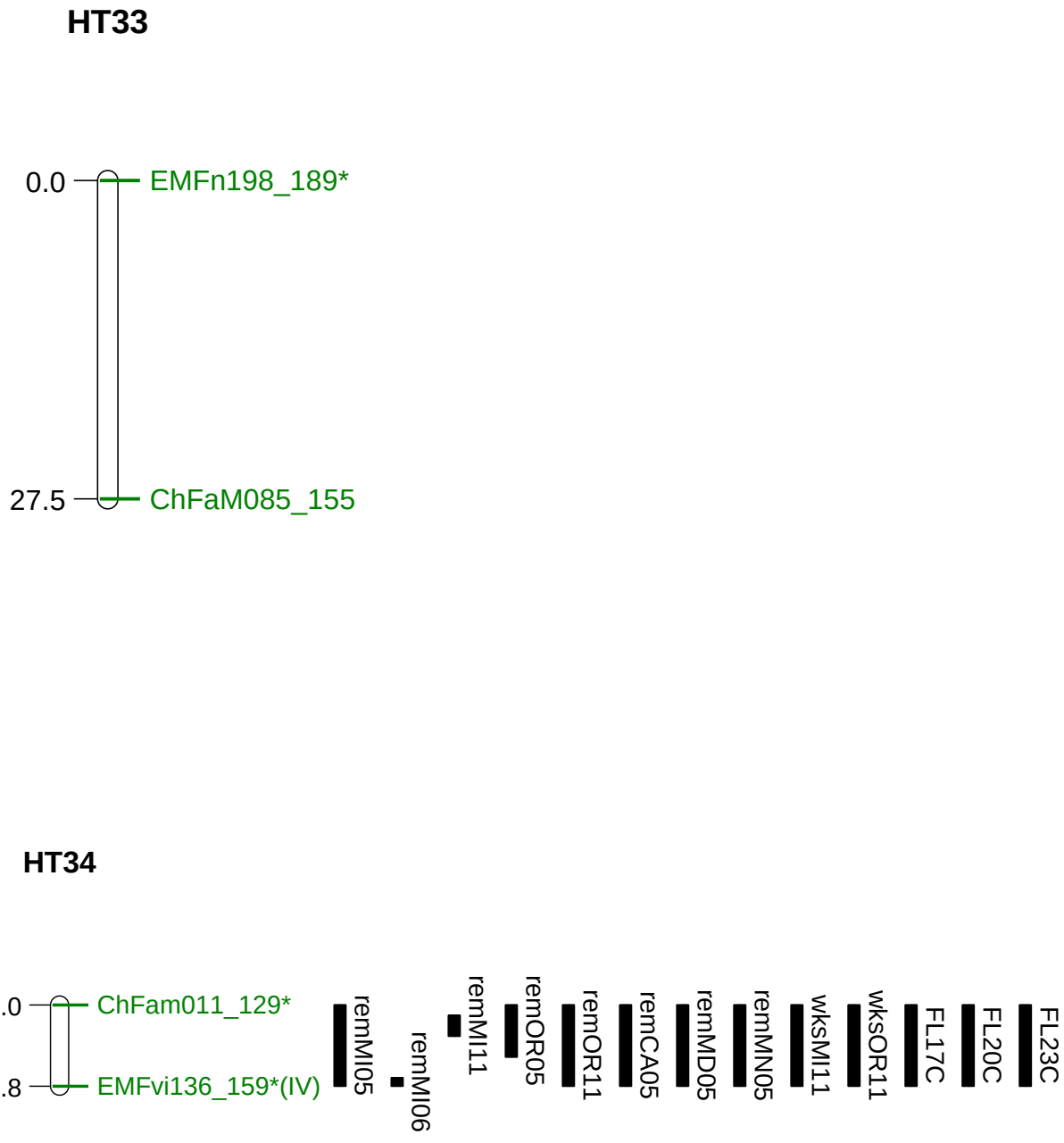


3.3.2 QTL identification

To identify the most robust QTL regulating remontancy, fifteen phenotypic data sets were available that were collected on the same segregating population ('Honeoye' × 'Tribute') at multiple field locations and in different years. Remontancy was evaluated in two ways, as flowering in both spring and summer and by duration of flowering. The co-localization of floral heat tolerance QTL with remontancy QTL was also documented using data from a controlled greenhouse study. Table 3.2 summarizes the QTL identified from all the datasets on the 'Honeoye' x 'Tribute' linkage groups.

QTL identification with 2005-2006 data from CA, MD, MI, MN and OR

Phenotypic data collected from 5 locations in 2005 and 2006 (Weebadde *et al.*, 2008) and the SSR linkage map was used to identify QTL associated with remontancy and weeks of flowering. The remontancy QTL identified along with nearest marker and percent variance explained are listed in Appendix 3.6. Phenotypic distributions of weeks of flowering were continuous (Figure 3.2a-f), although the distribution was skewed to longer durations in CA, indicating a longer fruiting season at this location. The weeks of flowering QTL identified along with the nearest marker and percent variance are listed in Appendix 3.7. The significant QTL regions are also represented on Figure 3.1 along with the linkage groups and all other QTL identified.

Several region-specific remontancy QTL were identified (Figure 3.1; Appendix 3.6). These included two QTL each for MI (HT2, HT3), MD (HT5, HT10), and OR (HT6, HT9), and one for MN (HT5). Three regions specific to the western states (OR and CA) were identified in groups HT1, HT22, and HT30 and two regions associated with the three eastern states (MI, MN, MD) were identified in groups HT16 and HT19. Five regions were identified where QTL for all 5

states collocated: HT5, HT6, HT7, HT32 and HT34. One region was identified with QTL for MN and MD (HT9) and one region for MI and MN (HT2). Variances of the QTL identified using 2005/2006 data ranged from 10.1% to 69%.

These observations were similar to those of Weebadde *et al.* (2008) who also identified region-specific QTL and some QTL common to multiple datasets. They identified one QTL common to the eastern states MN, MI, and MD, in addition to three QTL specific to MN, and one QTL each specific to MI and CA, and none for MD. However, our study found many more QTL using the same phenotypic data. It is possible that we identified more QTL because the population size (112 progeny) used in our study was larger than the population (65 progeny) used by Weebadde *et al.*, (2008) for QTL identification. It is also possible that we observed more QTL because we had fewer markers and as a result our linkage groups were more fragmented. Many of our QTL were placed at the end of linkage groups, which might have been joined with more markers. Although Weebadde *et al.* (2008) did not find any QTL common to all locations, we did identify regions where QTL for remontancy at all 5 locations overlapped, and, there were two regions specific for western states that were not identified in the earlier study. Unfortunately, the earlier map was developed using AFLP markers and it is not possible to directly compare those QTL with the ones identified here.

Most 'weeks of flowering' QTL overlapped with the remontancy QTL (HT1, HT2, HT3, HT5, HT6, HT7, HT9, HT10, HT16, HT19, HT22, HT30, HT32, and HT34) (Figure 3.1; Appendix 3.6 and 3.7). This suggests that our method of categorizing remontant vs non-remontants was able to identify regions of the genome that determine extended flowering season that is typical of remontant genotypes. Several regions were identified specific to weeks of flowering in CA on

groups HT8, HT12, HT13, HT18, HT21, HT24, HT28, HT30, and HT31. Two regions, HT16 and HT19, had regions specific for weeks of flowering in OR. QTL for weeks of flowering in all three states (MI, OR, and CA) collocated on groups HT1, HT2, HT3, HT7, HT9, HT22. This suggests that there is significant environmental effect on determining the duration of flowering in genotypes, but a few QTL are represented across locations. Only one QTL was identified for weeks of flowering at MI in 2005. This is likely because the data was collected only until early July, whereas in the other states the data was collected until Aug. Variances for the QTL identified ranged from 15.6-36.4% for MI 2006, 28.4-61.1% in OR2005, and 23-54.5% in CA2005 (Appendix 3.7).

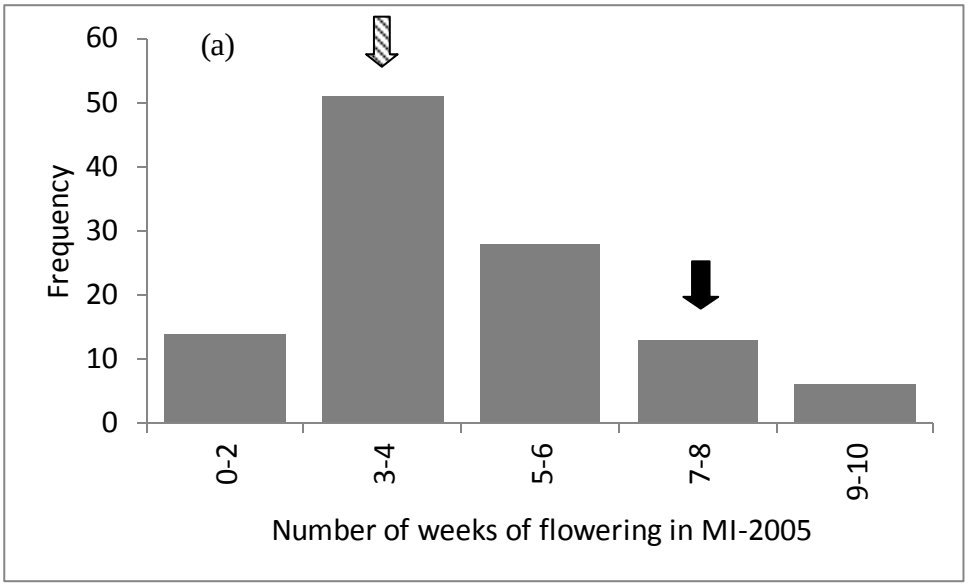
Table 3.2 QTL regions associated with remontancy, weeks of flowering, and flower number at different temperatures (17, 20 and 23 °C) in the 'Honeoye' × 'Tribute' population. . The place and time of the phenotypic observations are listed by the initials of the state and the year of collection. LG = linkage group. 'x' indicates presence of QTL on the linkage group (HT1-HT34).

LG	Remontancy QTL								Weeks of flowering QTL						Temp QTL		
	MI05	MI06	MI11	OR05	OR11	MN05	MD05	CA05	MI05	MI06	MI11	OR05	OR11	CA05	23	20	17
HT1			x	x	x			x		x	x	x	x		x	x	x
HT2	x		x		x	x				x	x		x	x		x	x
HT3		x	x		x				x	x			x	x		x	x
HT4					x								x	x	x		
HT5	x		x	x	x	x	x	x		x	x	x	x	x		x	
HT6	x	x	x	x	x	x	x	x		x	x			x			
HT7	x	x	x	x	x	x	x	x		x	x		x	x	x	x	x
HT8								x									
HT9				x	x	x	x			x	x			x		x	x
HT10			x		x		x			x	x			x			x
HT12														x			
HT13										x				x			
HT15													x	x			
HT16	x	x			x	x	x						x			x	x
HT18					x									x			
HT19	x			x		x	x	x				x	x		x		x
HT20											x			x	x		x
HT21														x		x	
HT22			x	x	x			x			x		x	x	x		
HT24														x			
HT26										x				x			

Table 3.2 (cont'd)

LG	Remontancy QTL								Duration of flowering QTL						Temp QTL		
	MI05	MI06	MI11	OR05	OR11	MN05	MD05	CA05	MI05	MI06	MI11	OR05	OR11	CA05	23	20	17
HT28														x			
HT29											x			x			
HT30			x	x	x			x						x	x	x	x
HT32	x	x		x		x	x	x		x				x			
HT34	x	x	x	x	x	x	x	x			x		x		x	x	x

Figure 3.2a-f Distribution of weeks of flowering in 'Honeoye' x 'Tribute' progeny with different flowering durations. Weeks of flowering in the parents are indicated: 'Honeoye': shaded arrow, 'Tribute': dark arrow. (a) Weeks of flowering in MI-2005, (b) Weeks of flowering in MI-2006, (c) Weeks of flowering in OR-2005, (d) Weeks of flowering in CA-2005, (e) Weeks of flowering in MI-2011, (f) Weeks of flowering in OR-2011



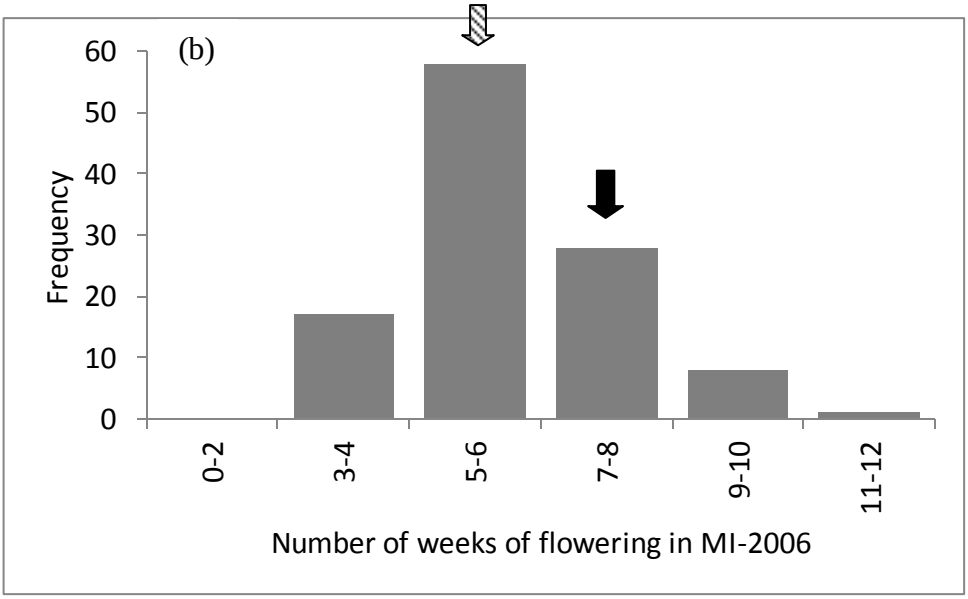
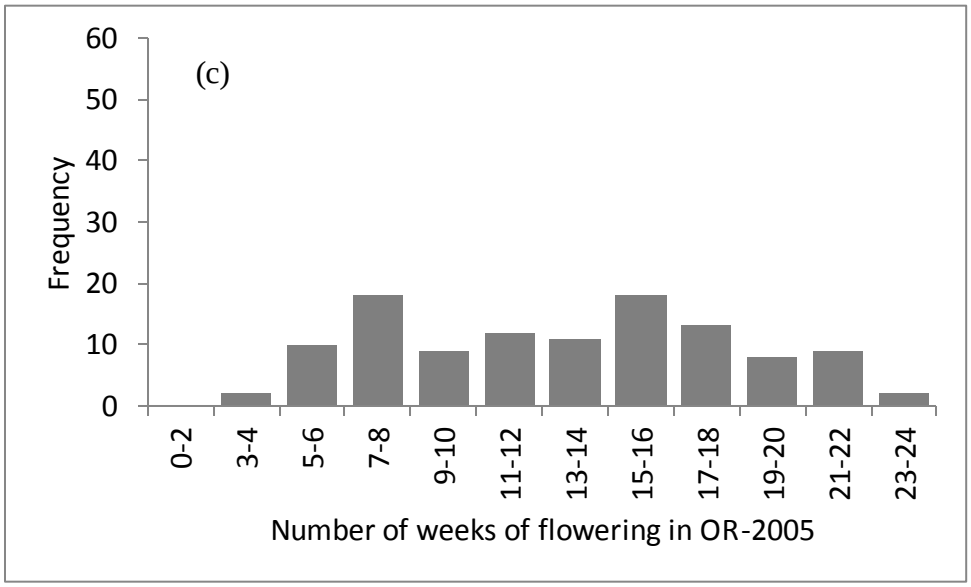
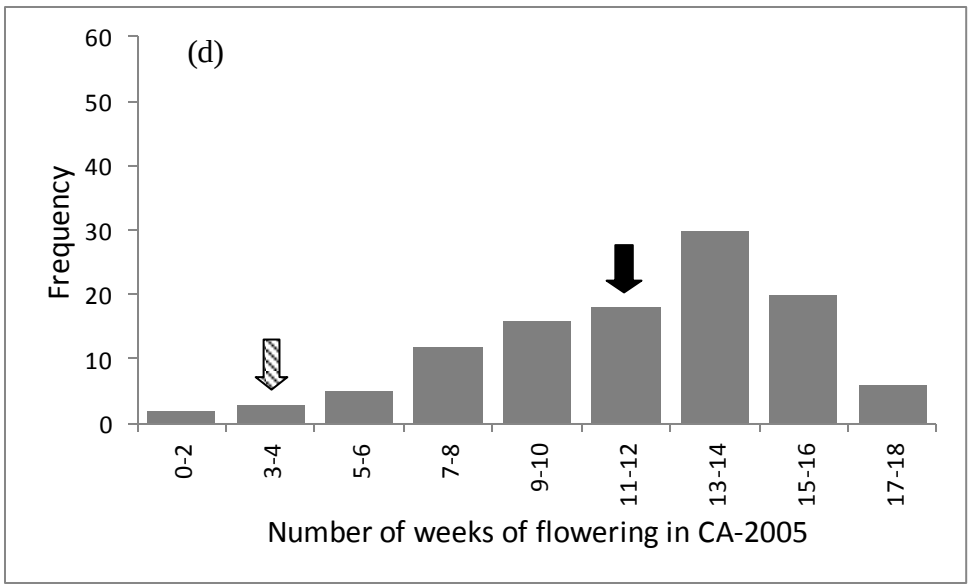


Figure 3.2 (cont'd)





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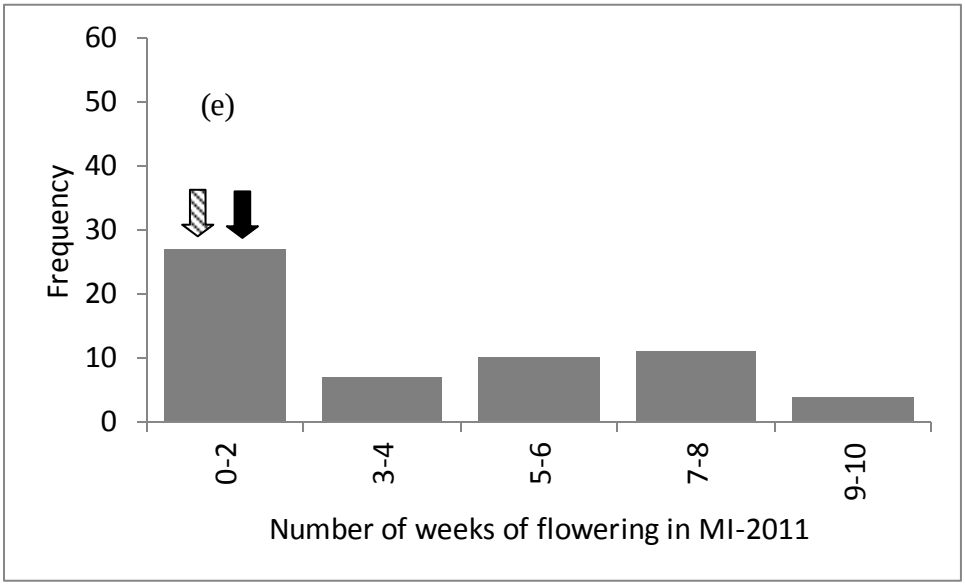
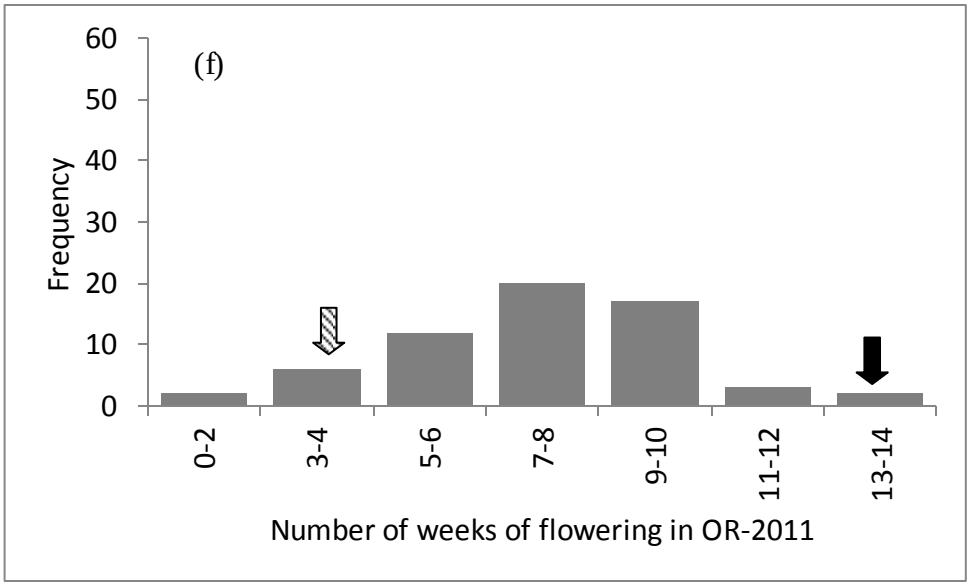




QTL identification with 2011 data from MI and OR

Field observations on the remontant/non-remontant flowering habit of the 'Honeoye' × 'Tribute' population planted in 2010 was used to further validate the QTL from MI and OR. Half of the progeny in both MI and OR in 2011 were remontant. This was unexpected based on observations by Weebadde *et al.* (2008) that 80% of the progeny in OR were remontant in 2005 and the 2011 air temperature in late summer in OR was 3°C cooler than in 2005. However, it was an unusually late growing season in OR in 2011 and it is possible that some very late flowering remontants were missed.

The significant QTL regions are listed in Appendix 3.6, and shown in Figure 3.1 for comparison with other QTL. Significant QTL regions associated with remontancy in MI (2011) were identified on 11 linkage groups (Appendix 3.6). Variances of these regions ranged from 39.7 to 51.3%. Significant QTL regions associated with remontancy in OR (2011) were identified on 13 linkage groups (Appendix 3.6) with variances from 45.4 to 69.7%.

QTL for remontancy in MI in 2011 were identified in groups HT1, HT3, HT5, HT6, HT7, HT10, HT22, HT30, and HT34 (Figure 3.1; Appendix 3.6). Among these, the regions on HT3 overlapped with the QTL for MI 2006, the region on HT5 overlapped with the QTL identified for MI 2005, and the QTL for all three years overlapped on groups HT7 and HT34. QTL for remontancy in OR (2011) were identified in HT1, HT2, HT3, HT4, HT6, HT7, HT9, HT10, HT16, HT18, HT22, HT30, HT34. The regions in groups HT1, HT5, HT6, HT7, HT9, HT22, HT30, and HT34 had QTL for both years of study (2005, 2011) indicating that these QTL are among the most robust. The MI (2011) QTL in group HT1 overlapped with QTL from the western states. Some regions were identified that were specific for a particular location and year

such as MI (2011) on HT5 and OR (2011) on HT18. MI (2011) QTL on group HT5, HT6, HT7, and HT34 overlapped with the QTL identified using 2005 and 2006 data, as well as QTL from all 5 states, suggesting these QTL are also very robust.

Weeks of flowering QTL for MI 2011 were identified on groups HT1, HT2, HT5, HT6, HT7, HT9, HT10, HT20, HT22, HT29, and HT34 with variances ranging from 15.8-59.6% (Figure 3.2, Appendix 3.7). Weeks of flowering QTL for OR 2011 was identified in groups HT1, HT2, HT3, HT4, HT5, HT7, HT9, HT15, HT16, HT19, HT22, and HT34) (Figure 3.1; Appendix 3.7). As with the remontancy QTL, weeks of flowering QTL for OR were identified in many more groups with 2011 data than with 2005 data. Weeks of flowering QTL for OR in 2005 were identified in only 2 groups and both of these overlapped with the 2011 QTL indicating that these are stable regions. Weeks of flowering QTL in MI for 2006 and 2011 collocated on groups HT5, HT6, HT7, and HT9.

QTL associated with heat tolerant floral response in a controlled greenhouse study

Significant QTL regions were associated with flower numbers at 17°C, 20°C, and 23°C (Figure 3.1; Appendix 3.8). Ten linkage groups had QTL associated with flower numbers at 17°C (Figure 3.1, Appendix 3.8). Some of these QTL regions (example on group HT2) were spread out over a wide range (Figure 3.1), likely due to inadequate marker coverage. The variance explained by the QTL ranged from 14.5% to 47.3%, indicating that both major and minor QTL were present. QTL associated with flower number at 20°C were identified in 9 linkage groups and their variance ranged from 32-44% (Appendix 3.8). QTL for flower number at 17°C and 20°C collocated on three linkage groups (HT1, HT2, and HT16). These regions are probably associated with the regulation of flower formation at lower temperatures.

QTL associated with flower number at 23°C were identified on 8 linkage groups with variances from 12.2 to 50.3% (Figure 3.1; Appendix 3.8). QTL for flower number at all three temperatures collocated on one linkage group (HT30 and HT34). These regions are probably associated with the general process of flower formation, instead of ambient temperature perception. QTL for flowering at 17°C and 23°C collocate on two groups (HT19 and HT20).

QTL for flower numbers at 17°C and 20°C (low temperature QTL) collocated with remontancy QTL from MI, OR, and CA in group HT1, with MI, OR, MN in group HT2, with all remontancy QTL in group HT5, HT7, with MI, OR, MD, and MN in group 16. All three temperature QTL overlapped with QTL from MI, OR, and CA in group HT30, and with QTL from all locations in group HT34. Interestingly there were two regions (HT19 and HT20) where QTL for flower numbers at 17°C and 23°C overlapped. In addition, all heat tolerant progeny had higher flower numbers at 17°C. It is possible that QTL in these regions were related to the increased flower numbers in the heat tolerant progeny.

Weeks of flowering QTL for MI, OR, and CA collocated with the heat tolerance QTL on group HT1, HT2, HT4, HT7, HT19, HT20, HT22, HT30, and HT34 (Figure 3.1), suggesting that heat tolerance most likely plays an important role in determining whether a genotype continues flowering over several weeks.

3.3.3 Phenotypic distribution of markers associated with heat tolerance/sensitivity

The phenotypes of the individuals carrying those alleles that were present in the QTL regions associated with flowering at high temperature (23°C) were compared to examine whether these markers can be exploited for marker-assisted selection for temperature tolerant genotypes. Means of total number of flowers at 17°C, 20°C, and 23°C in progeny with and without the allele

were compared (Figure 3.3 and Table 3.3). The marker profiles of the parents along with their phenotypes are listed in Table 3.4. Only those alleles that resulted in higher flower numbers are shown in Table 3.3 and Figure 3.3. Three alleles in group HT1 (ARSFL8_301, ChFaM098_225, ChFaM040_315), and one allele each in group HT7 (EMFn117_157), and in group HT20 (EMFn170_208) were associated with higher flower numbers at 23 °C, although the difference was significant for only one of these, HT20 (EMFn170_208). This allele would be a good candidate for marker assisted breeding. While the effects of the other alleles (Figure 3.3) were non-significant, they remain potential candidates for marker assisted breeding as the overall levels of variance were quite high due to high numbers of non-flowering genotypes. These alleles need to be further validated using a larger panel. The alleles on groups HT1 and HT7 were associated with several of the QTL associated with remontancy and weeks of flowering. HT7 is particularly interesting because the remontancy QTL from all years and 5 states, and the weeks of flowering QTL from 3 states collocate with the heat tolerance QTL on this group. The QTL associated with the allele EMFn170_208 partially overlapped with the total flowers at 17 °C allele, but did not collocate with any of the other remontancy QTL. However, this region is associated with weeks of flowering at MI and CA.

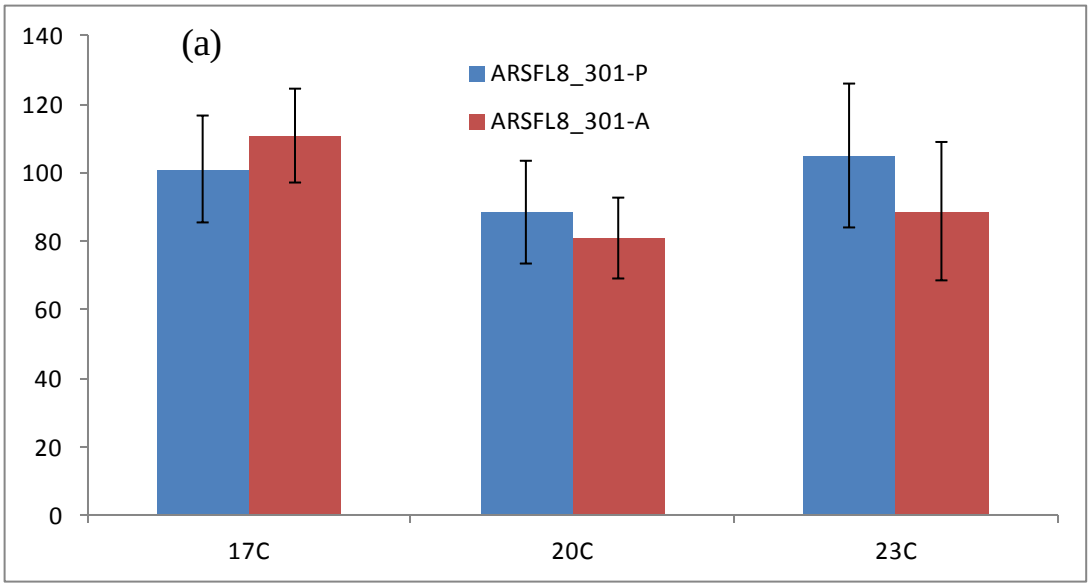
Table 3.3 Alleles associated with 'Total flowers at 23°C' QTL and the phenotypic observations associated with them. Presence and absence of the allele are indicated by 'p' and 'a'. Mean flower numbers at 17°C, 20°C, 23°C in the progeny with and without the allele are listed.

Allele	Group	Temperature		
		17°C	**20°C**	**23°C**
ARSFL8_301-a (28)	HT1	110.7	80.79	88.63
ARSFL8_301-p (26)		100.95	88.35	104.88
ChFaM098_225-a (31)	HT1	109.54	83.48	82.24
ChFaM098_225-p (23)		101.24	85.7	115.62
ChFaM040_315-a (31)	HT1	101.62	76.42	85.65
ChFaM040_315-p (23)		111.91	95.22	111.03
EMFn117_157-a (33)	HT7	97.67	75.41	88.72
EMFn117_157-p (21)		119.1	98.6	108.62
EMFn170_208-p (35)	HT20	120.4	96.3	115.8
EMFn170_208-a (19)		79.6	62.5	60.8

Table 3.4 Genotype of the parents and associated phenotypic observations for the alleles ARSFL8_301, ChFaM098_225, ChFaM040_315, EMFn117_157, and EMFn170_208. Total flower numbers are the mean of three replicates.

Group	Allele	'Honeoye'	'Tribute'
HT1	ARSFL8_301	H	A
HT1	ChFaM098_225	H	A
HT1	ChFaM040_315	H	A
HT7	EMFn117_157	A	H
HT20	EMFn170_208	A	H
Total flowers-17°C		104.67	108.67
Total flowers-20°C		58.33	45.67
Total flowers-23°C		11.33	48.00

Figure 3.3a-e Phenotypic distributions associated with presence of the alleles located in regions with significant QTL for flower formation at 23°C. Blue bar represents presence of the allele; brown bar represents absence of the allele. (a) Phenotype associated with ARSFL8_301, (b) phenotype associated with ChFaM098_225, (c) phenotype associated with ChFaM040_315, (d) phenotype associated with EMFn117_157, and (e) phenotype associated with EMFn170_208



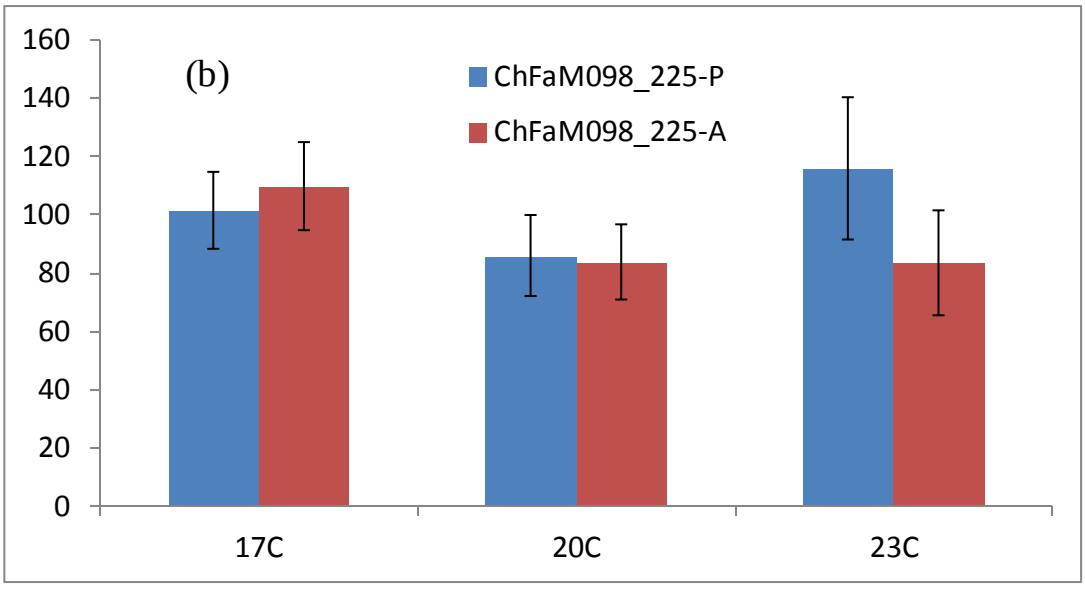
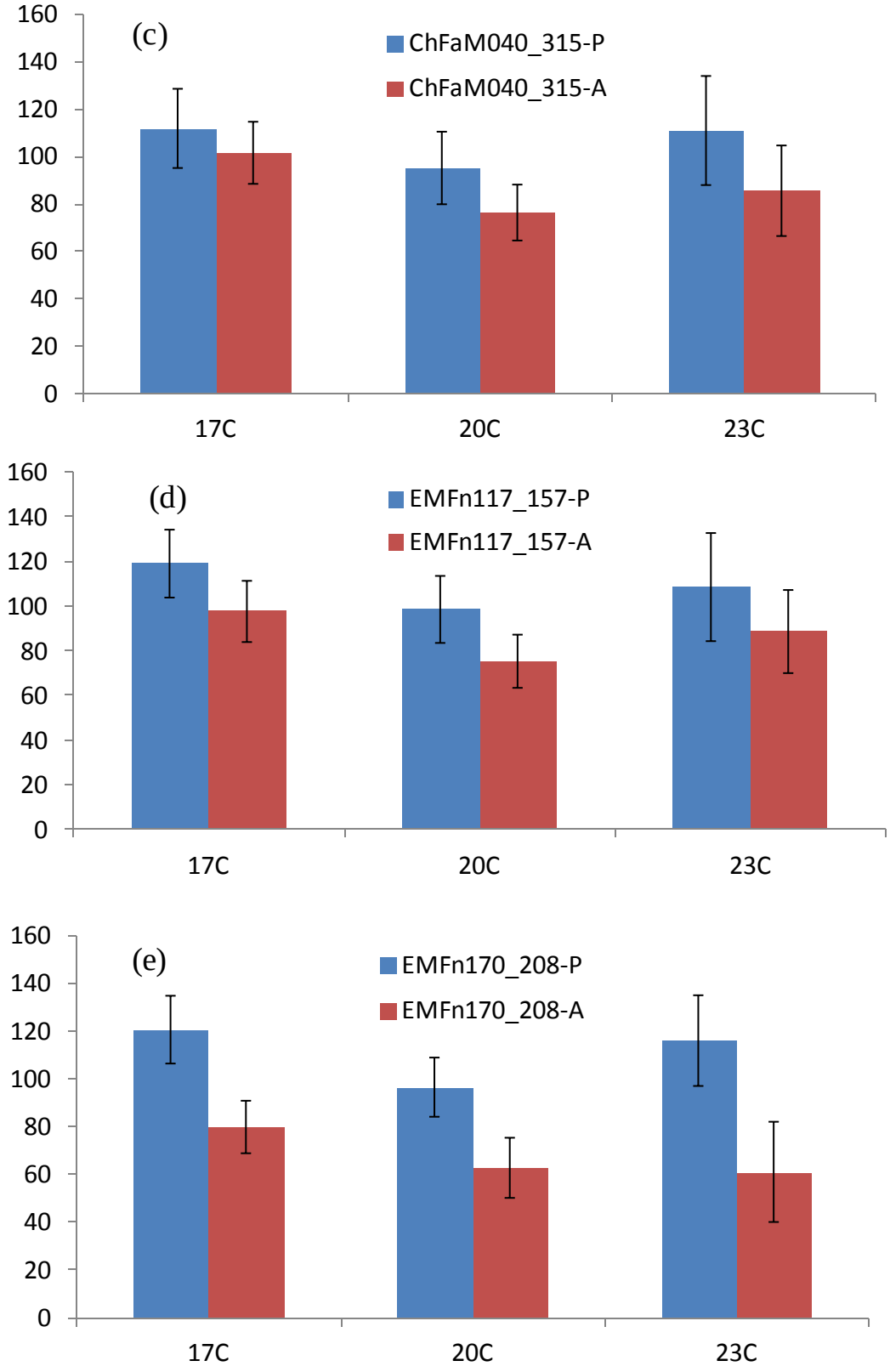


Figure 3.3 (cont'd)



3.3.4 Overall conclusions

In many instances, remontancy QTL from several datasets co-located on the linkage groups, indicating they are quite robust. Many of the remontancy QTL also co-located with heat tolerance QTL. The QTL region on HT7 is perhaps the most interesting region, because remontancy QTL from all 5 states overlapped in this region, as well as the QTL identified using multiple years' data from MI and OR. In addition, QTL for weeks of flowering also co-located on HT7. This region is also associated with heat tolerant floral response (QTL for flowers at 23°C). A comparison of the mean flower numbers at 23°C associated with presence and absence of this allele (EMFn117_157) suggests that this allele contributes to higher flower numbers.

Several remontancy QTL regions from multiple data sets were spread over wide ranges with few markers (Figure 3.1) making it difficult to associate a specific marker to the trait. Adding markers to the regions of interest identified in this study will probably narrow down the QTL regions. The availability of the diploid *F. vesca* genome sequence (Schulaev *et al.*, 2011) provides an excellent resource for identifying and developing markers associated with the regions of interest.

Regardless, this study was able to identify QTL associated with ambient temperature perception, remontancy, and weeks of flowering using data from controlled and natural environment in multiple years and locations. At least one allele (EMFn170_208) was identified whose presence has resulted in significantly higher flower numbers at 23°C compared to 17°C. This QTL was specific to heat tolerant floral response, and also collocated with weeks of flowering QTL. It could prove to be useful in marker assisted breeding of remontant cultivars.

APPENDIX

Appendix 3.1

Table 3.5 SSR loci used for genotyping the mapping population with their source, primer sequences, and putative functions of associated ESTs.

SSR	Developed in	Putative function/BLAST hit (as reported in original publication)	F-primer	R-primer	Reference
ARSFL19	*F.* x *ananassa* 'Earliglow'	N.S. (no significant match)	GCGAAACCGAAGAAGAACAAATGC	GCGGCCCAAACGGACAAGA	Lewers *et al.*, 2005
ARSFL2	*F.* x *ananassa* 'Earliglow'	N.S.	GCGAAGCGAAGCGGTGATG	GCGAACGTCGAGGAGCATTCTCAT	Lewers *et al.*, 2005
ARSFL31	*F.* x *ananassa*	Pectate lyase B	CGACCCAGCGACTACATTG	ACTTTAACCGCCACCAACTG	Lewers *et al.*, 2005
ARSFL8	*F.* x *ananassa* 'Earliglow'	N.S.	GCGGACCCAAGATGACCTCACCC	GCGTTAGCCGAGAATGTTCTACTG	Lewers *et al.*, 2005
ARSFL98	*F.* x *ananassa* 'Elsanta'	Metallothionen-like protein	CCCCTATTCGACAACCAATG	TGGCTACCAAAGAACACGAA	Lewers *et al.*, 2005
ChFam003	*F.* x *ananassa*	Iron(III)-zinc(II) purple acid phosphatase	CCTCCCCAACTGATTCTTCA	TGCCATGGTTGTTCCTTCAC	Zorrilla-Fontanesi *et al.*, 2011
ChFaM004	*F.* x *ananassa*	Anthocyanin regulatory C1 protein	CCCAGCATATACTTTGCCGTA	TCCTTTCTTCATCCCCTCCT	Gil-Ariza *et al.*, 2006
ChFaM010	*F.* x *ananassa*	Unknown protein	TATCGCCTGCAATTCATCTG	GCTGGCTCTGTGGAGTGAGT	Zorrilla-Fontanesi *et al.*, 2011

Table 3.5 (cont'd)

SSR	Developed in	Putative function/BLAST hit (as reported in original publication)	F-primer	R-primer	Reference
ChFam011	*F.* x *ananassa*	N-acetylglucosaminyl transferase	TCCTCTCCTTCTTTCCCTTCA	CGAGATCTCCCGAGACTGAG	Zorrilla-Fontanesi *et al.*, 2011
ChFaM017	*F.* x *ananassa*	N.S.	CTCACTCTCTGCGAACTTGC	CAACTCACCTTGCACCGATT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM018	*F.* x *ananassa*	N.S.	AGCCGCATCCCTCTTTTCTA	CTAGGGATTGAGGACCGACA	Zorrilla-Fontanesi *et al.*, 2011
ChFaM022	*F.* x *ananassa*	L-Asparaginase	GGGCCACTCCTACTTCTTCA	TTGGCCTTGAGAGCTTCGAT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM030	*F.* x *ananassa*	Thioredoxin H	CCATGAAGCAGTGAAGTCCA	AGAAAATCCCGAGAGCCTTT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM031	*F.* x *ananassa*	Avr9/Cf-9 elicited protein 146	GCTAGCAAAGCCCTAAGCAA	ACGGTGGGCACACTTAAAGA	Zorrilla-Fontanesi *et al.*, 2011
ChFaM032	*F.* x *ananassa*	Hydrophobic protein LTI6A	GGTCCCTGCTTCTTCTTCTTT	TTCAGCCCCATTTTCCAGTA	Zorrilla-Fontanesi *et al.*, 2011
ChFaM035	*F.* x *ananassa*	N.S.	AACCCACTTCCACAGGTGAC	AGGAAAAAGAGGGCTTGGAG	Zorrilla-Fontanesi *et al.*, 2011

Table 3.5 (cont'd)

SSR	Developed in	Putative function/BLAST hit (as reported in original publication)	F-primer	R-primer	Reference
ChFaM039	*F.* x *ananassa*	Mitochondrial carrier protein	GTGGTTTTTGTTGGGCAAAG	AACGGCTTATCATCCTGCAT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM040	*F.* x *ananassa*	60S ribosomal protein L18a	AGTGGTCATCAGCACCATCA	TAACCGGGAACGGTACTCTG	Zorrilla-Fontanesi *et al.*, 2011
ChFaM044	*F.* x *ananassa*	Dynamin-like protein	CGCTGAGTCGTTCTAATTTCA	TTTTGTTGACGAGCGAGATG	Zorrilla-Fontanesi *et al.*, 2011
ChFaM046	*F.* x *ananassa*	Lil3 protein	CCATTTCCATGGCCTTGTTT	GGCCTTGTTGGGTCTGAGAG	Zorrilla-Fontanesi *et al.*, 2011
ChFaM056	*F.* x *ananassa*	Nuclear acid binding protein	AAAACGTCGTCGTTCAGGAT	CGTACTGCTGTTGCTGCTGT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM060	*F.* x *ananassa*	N.S.	TGAGCTACCACCAAGAACCC	AATACCCTTGGTACCCCTCG	Zorrilla-Fontanesi *et al.*, 2011
ChFaM061	*F.* x *ananassa*	N.S.	GTGCTCAAGAAACCCTTTCG	GCGCTAGCAACAGTAAGGTG	Zorrilla-Fontanesi *et al.*, 2011
ChFaM063	*F.* x *ananassa*	Cysteine proteinase RD19A	GACGTCTCCGATCCGTTGAT	CTGGCTCGCGTACGACTTTC	Zorrilla-Fontanesi *et al.*, 2011

Table 3.5 (cont'd)

SSR	Developed in	Putative function/BLAST hit (as reported in original publication)	F-primer	R-primer	Reference
ChFaM065	*F.* x *ananassa*	Unknown protein	GACCGGGAGAGATAACAGCA	ATAGAAGCCAATGCGTGATG	Zorrilla-Fontanesi *et al.*, 2011
ChFaM067	*F.* x *ananassa*	N.S.	AGAACCAGCAAGAGCAGCAC	CAGCTCTGTGTATGCCTGGA	Zorrilla-Fontanesi *et al.*, 2011
ChFaM072	*F.* x *ananassa*	N.S.	TGGCAGAAATTTCCAAAAGG	CTCCCCCAGAAGTCCAGATT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM076	*F.* x *ananassa*	TINY transcription factor	GCCTCCATGGAAAACCTAAA	CTCGGCAGCTCCACTATCTC	Zorrilla-Fontanesi *et al.*, 2011
ChFaM077	*F.* x *ananassa*	Plasma Membrane H+ ATPase	GAAAGGGCTGGACATGGATA	ATGTTGTTATTTGGCCTGCT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM078	*F.* x *ananassa*	N.S.	CAGCCTCATTGCAAATCTGA	CTTACCGGTTTCGATGTGGT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM080	*F.* x *ananassa*	Unknown protein	TTCGGTGCCGGTAAAGATAC	AAGTTCCACCACCATGCAAT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM081	*F.* x *ananassa*	Prenylcystein oxidase	AACTGAGCTCTCGGCAAGTC	GAATACTCGCGGAGGAAGTG	Zorrilla-Fontanesi *et al.*, 2011

Table 3.5 (cont'd)

SSR	Developed in	Putative function/BLAST hit (as reported in original publication)	F-primer	R-primer	Reference
ChFaM085	*F.* x *ananassa*	Purple acid phosphatase	AGATGGGTCATTTTCTGACGA	GTAGTGCATGTCCGCCACTT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM086	*F.* x *ananassa*	N.S.	TTTGGAGCTCAATCCCATCTG	ATTTGGCCAGCCTCCGTCT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM088	*F.* x *ananassa*	Unknown protein	GGTGGCAAAACTCATGGAGA	GGGAAGCGAAGTTGAAGAGG	Zorrilla-Fontanesi *et al.*, 2011
ChFaM089	*F.* x *ananassa*	4-coumarate-CoA ligase	GAAGGATGGTTGCACACAGG	GAGAGGTTGGGATGGGAGAT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM092	*F.* x *ananassa*	N.S.	ACCCAAGTTCCCTTCGACTC	ATGCGCTTTGCATAACAGGT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM093	*F.* x *ananassa*	N.S.	CGCCCTCAAATCCCTCTAAC	GAAGTGAGTGTTCCGCTGCT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM094	*F.* x *ananassa*	tRNA isopentenylpyrophosphatase	ATGGAGGGCGCTACTGAAAA	AATGGCGAGCTTGGACTTTC	Zorrilla-Fontanesi *et al.*, 2011
ChFaM095	*F.* x *ananassa*	Signal peptidase protein	GCCAGAAGCAAAAACCAGAA	GGGAAGTTGAAATTGTCGGA	Zorrilla-Fontanesi *et al.*, 2011

Table 3.5 (cont'd)

SSR	Developed in	Putative function/BLAST hit (as reported in original publication)	F-primer	R-primer	Reference
ChFaM098	*F.* x *ananassa*	Unknown protein	GTGAGAGTCAGCCCACCCTA	GCGACGAGGATGAAGAAGAG	Zorrilla-Fontanesi *et al.*, 2011
ChFaM101	*F.* x *ananassa*	3-Methylcrotonyl-CoA Carboxilase	GGAGTAAGCTGATCACTCTGT	ACTCCGAGGCTGTAATCCCT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM103	*F.* x *ananassa*	Delta-7-sterol-C5(6)-desaturase	CATCTCTTCTCCTTTCCGATCT	GAGCACAATGCGGTTGTAGA	Zorrilla-Fontanesi *et al.*, 2011
ChFaM104	*F.* x *ananassa*	N.S.	CAGTCATTTTTGGCTTCACC	TTGGTCTGTTCCTTTCCTTG	Zorrilla-Fontanesi *et al.*, 2011
ChFaM107	*F.* x *ananassa*	N.S.	TGCCAAACAAACAAATGTTGA	CATATCGATGTCCTTCATAGGG	Zorrilla-Fontanesi *et al.*, 2011
ChFam111	*F.* x *ananassa*	N.S.	GCCCAACCGAGTCTCTCTCT	CGGGCTTCAATTTGCTCAAT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM129	*F.* x *ananassa*	N.S.	AGATCAACATCGCCTCCAAC	TGCTCGTTGTCCATAACCTG	Zorrilla-Fontanesi *et al.*, 2011
ChFaM147	*F.* x *ananassa*	NADH dehydrogenase	ACGAGGGTCACCTGAGACTG	CCAGGAGAAGGTACCGAAGG	Zorrilla-Fontanesi *et al.*, 2011

Table 3.5 (cont'd)

SSR	Developed in	Putative function/BLAST hit (as reported in original publication)	F-primer	R-primer	Reference
ChFaM148	*F.* x *ananassa*	GalUR	CCCTCCATCAAAGCCAGTT	CATTAGACCCCGACTTGTCA	Zorrilla-Fontanesi *et al.*, 2011
ChFaM151	*F.* x *ananassa*	Unknown protein	ACCACCACCGTTTTCTCCTC	ACCACCGACTCGTCCTTCTT	Zorrilla-Fontanesi *et al.*, 2011
ChFaM161	*F.* x *ananassa*	NAD-dependent glucuronic acid epimerase	CGAGGCCTTGTCTTCTTTGT	GCGGAGGTAGCTGTTGTAGC	Zorrilla-Fontanesi *et al.*, 2011
EMFn115	*F. nubicola*		TGGAGATGATGGTCAAGACG	GACAAGACCACGAAAACACG	Sargent *et al.*, 2006
EMFn117	*F. nubicola*		ATCGGATCAACAAGCAAAGC	ATGGATGAGGGGAGAAGAGG	Sargent *et al.*, 2006
EMFn121	*F. nubicola*		GGTCCCTAAGTCCATCATGC	GAGTGGATGCAAACATGAGC	Sargent *et al.*, 2006
EMFn128	*F. nubicola*		CATCAACATTCACATGAATTTACC	CGGCGGATCTAGTTTTGAGG	Sargent *et al.*, 2006
EMFn134	*F. nubicola*		TGATTCTTTGAAAGGCTTTGG	AAAACAACCCCCTCTCATCC	Sargent *et al.*, 2006
EMFn148	*F. nubicola*		TTACCTGCACAGAAACAACG	CAACTTCCTCCTCACTCACC	Sargent *et al.*, 2006
EMFn152	*F. nubicola*		GGGCCAAAATGAGTATCTTGC	TTAGAGCGAGGTGGTAATGC	Sargent *et al.*, 2006
EMFn153	*F. nubicola*		CTCGAGCTCCCTTTCTATCG	TGGCCAAATGTTCTCACTAGC	Sargent *et al.*, 2006
EMFn160	*F. nubicola*		GCATCCTTGGGAAATTAATGC	TTGGGAAGGATCATAAAAACC	Sargent *et al.*, 2006

Table 3.5 (cont'd)

SSR	Developed in	Putative function/BLAST hit (as reported in original publication)	F-primer	R-primer	Reference
EMFn170	*F. nubicola*		CAGTTTGCCCAACAACAAGG	TTGATGGCAACAAATCACG	Sargent *et al.*, 2006
EMFn181	*F. nubicola*		CCAAATTCAAATTCCTCTTTCC	GCCGAAAAACTCAAACTACCC	Sargent *et al.*, 2006
EMFn184	*F. nubicola*		GATGAGAATTGTTTGAGTGAAGG	TGACCAGCGGATTCATAAGG	Sargent *et al.*, 2006
EMFn198	*F. nubicola*		CCAAATTGTCCTTGATGTCG	CACCTGCTTCAAAGCAAACC	Sargent *et al.*, 2006
EMFn201	*F. nubicola*		CAGCTCAGAAAAGCTCACAGC	TAGAACGCCAATCACAAACC	Sargent *et al.*, 2006
EMFn202	*F. nubicola*		CTCTCTCCCTCAACCTCTCG	TGGACCAATATCTCCCTTGC	Sargent *et al.*, 2006
EMFn213	*F. nubicola*		AGCGTGATTTTGCCTTTGTT	CACAGTAAAGAACAGGAGGGAGAT	Sargent *et al.*, 2006
EMFn225	*F. nubicola*		AAGGAAAAATGCTCAAATACCC	TACGTGCGACGTTAGAGTCC	Sargent *et al.*, 2006
EMFn230	*F. nubicola*		AATGACTACGACAACGACAGTCT	AGGGAAAATGCCCAAATACC	Sargent *et al.*, 2006
EMFn235	*F. nubicola*		AGGAACAAGAGCTGGCAATG	CTCAAGTATCAGGCCTCCAAG	Sargent *et al.*, 2006
EMFvi104	*F. viridis*		TGGAAACATTCTTACATAGCCAAA	CAGACGAGTCCTTCATGTGC	Govan *et al.* (2008)
EMFvi136	*F. viridis*		GAGCCTGCTACGCTTTTCTATG	CCTCTGATTCGATGATTTGCT	Sargent *et al.*, 2003

Table 3.5 (cont'd)

SSR	Developed in	Putative function/BLAST hit (as reported in original publication)	F-primer	R-primer	Reference
FAC001	*F. ananassa*	Cinnamyl alchohol dehydrogenase	AAATCCTGTTCTTGCCAGTG	TGGTGACGTATTGGGTGATG	Lewers *et al.*, 2005
FAC002	Chandler	FaEG3 gene for end beta 1,4 glucanase	TCATCCTCTTTCACCTCCACTT	TCAAAAGACTTGGAAATGTTGC	Lewers *et al.*, 2005
FAC006	*F. ananassa*	Pectate Lyase	ACTGGTGGAGGAGAGGACTGTA	TGTGGAGCAGAGAGAATTGAAG	Lewers *et al.*, 2005
SF4B12	*F.* x *ananassa* 'Strawberry Festival'		GCAAAGTCGGAGAGAGATAGA	CTGAAGAAGGTGTTGAGGAA	Njuguna 2010
SF5C08	*F.* x *ananassa* 'Strawberry Festival'		TCTCTTTCTCTTCTCTCACTCTC	AAACATTCAACCAAACAAA	Njuguna 2010
SF5G02	*F.* x *ananassa* 'Strawberry Festival'		CTTTTGCTGCTAGCTCTTTGTG	TACGTACTCCACATCCCATTTG	Njuguna 2010
SF6E02	*F.* x *ananassa* 'Strawberry Festival'		GAAGGAGCATAGAGTTGTGGAGA	TGATCTCACTCTCGGTTTCAGA	Njuguna 2010

Table 3.5 (cont'd)

SSR	Developed in	Putative function/BLAST hit (as reported in original publication)	F-primer	R-primer	Reference
SFGRP7	*F.* x *ananassa* 'Strawberry Festival'		ATCTAGACGGCCGTAACATCAC	:CCACTTCCATAGCTACCACCTC	Njuguna 2010
UaFv8150	*F. vesca* 'Yellow Wonder'	*A. thaliana* metallo-beta-lactamase family protein mRNA (gi|42568732)	CCACCTCTCTCTCCATTTCC	AGCGGTGTGAAGACTTGAGG	Bassil *et al.*, 2006b
UaFv8216	*F. vesca* 'Yellow Wonder'	*Nicotiana tabacum* mRNA for nucleic acid binding protein (nbp1 gene) (gi|15594034)	GGTAATGCAGCACCAAATGA	GGAAGCGAAGCAGTTATGGA	Bassil *et al.*, 2006b
UaFv8936	*F. vesca* 'Yellow Wonder'	*A. thaliana* expressed protein (gi:18378999)	GTGACTTTGACGCTGACC	TGAGAGTGGTTCTGTTCCTC	Bassil *et al.*, 2006b
UaFv9092	*F. vesca* 'Yellow Wonder'	*A. thaliana* similar to dihydroflavonol reductase mRNA (gi|14596184)	ACCACAATCCTCCGCCATT	AGTCGTGCTTGATGTTGAG	Bassil *et al.*, 2006b
UaFv9404	*F. vesca* 'Yellow Wonder'	A. thaliana PHD finger protein — like	AGTCGTGCATCATGGATCAG	CATTAGTTGGCCACACACCA	Bassil *et al.*, 2006b
UDF-004	*F. vesca*		GCTTGCATTTCAATAGCTGGA	TTTACTGATGCAGGAGTAGAATGA	Cipriani and Testolin (2004)

Table 3.5 (cont'd)

SSR	Developed in	Putative function/BLAST hit (as reported in original publication)	F-primer	R-primer	Reference
Uffa01E03	*F.* x *ananassa* 'Strawberry Festival'	DegP protease	ACCCCATCTTCTTCAAATCTCA	GACAAGGCCAGAGCTAGAGAAG	Bassil *et al.*, 2006; Sargent *et al.*, 2006
Uffa01H05	*F.* x *ananassa* 'Strawberry Festival'	Similarity to pollen major allergen 2 protein (*Juniperus ashei*)	GGGAGCTTGCTAGCTAGATTTG	AGATCCAAGTGTGGAAGATGCT	Bassil *et al.*, 2006; Sargent *et al.*, 2006
Uffa02F02	*F.* x *ananassa* 'Strawberry Festival'	Similar to histone H1-3,(*Lycopersicon pennellii*)	CTTTGCAGCTGAAGAACTCTGA	CAGCAGCTGCCTTAGTCTTAGT	Bassil *et al.*, 2006; Sargent *et al.*, 2006
Uffa03B05	*F.* x *ananassa* 'Strawberry Festival'	Response regulator 7 (ARR7)	GGAATCCAAGTTACAGGCTTCA	AAGGAGCCTCTCCAATAGCTTC	Bassil *et al.*, 2006; Sargent *et al.*, 2006
Uffa04G04	*F.* x *ananassa* 'Strawberry Festival'	Nucleotide sugar epimerase	ACGAGGCCTTGTCTTCTTTGTA	GCTCCAGCTTTATTGTCTTGCT	Bassil *et al.*, 2006; Sargent *et al.*, 2006
Uffa11A11	*F.* x *ananassa* 'Strawberry Festival'	EST-SSR-function not reported	ACGAGGCTCCAATAGAGTTCTG	CTGAGCAGAAGCCATAGTATCAC	Bassil *et al.*, 2006

Table 3.5 (cont'd)

SSR	Developed in	Putative function/BLAST hit (as reported in original publication)	F-primer	R-primer	Reference
Uffa14A11	*F.* x *ananassa* 'Strawberry Festival'	EST-SSR-function not reported	ATGAAAGAAGTAGC CACTGAGC	TACGAGAGATACTAGG CGTGCTA	Bassil *et al.*, 2006
Uffa16H07	*F.* x *ananassa* 'Strawberry Festival'	EST-SSR-function not reported	CTCTACCACCATTCA AAACCTC	CACTGGAGACATCTAG CTCAAAC	Bassil *et al.*, 2006; Sargent *et al.*, 2006
Uffa18H04	*F.* x *ananassa* 'Strawberry Festival'	EST-SSR-function not reported	CCTTCGTTACTCTAG TAGCTCCA	GTGATGAAGACGATGA TGAGGT	Bassil *et al.*, 2006
Uffa20D02	*F.* x *ananassa* 'Strawberry Festival'	EST-SSR-function not reported	CTCCATCTCCACAAA TCCTCTC	GGCTAGAGTGCATGAG ATGTAGT	Bassil *et al.*, 2006
Uffa20H10	*F.* x *ananassa* 'Strawberry Festival'	EST-SSR-function not reported	GATGTGCTAGGACTC ATACTTGG	TAAAAGACGAGGCCAT CTGA	Bassil *et al.*, 2006

Appendix 3.2

Table 3.6 Segregation type and Chi square (X2) values of the markers in the 'Honeoye' × 'Tribute' SSR map. Segregation type <lm × ll>: codominant marker segregating in 'Honeoye', <nn × np>: codominant marker segregating in 'Tribute', and <hk × hk>: dominant marker present in both parents. Markers with segregation distortion are labeled with *.

Locus	Segregation type	X2	Segregation Distortion
ARSFL19_295	<lm×ll>	2.1	-
ARSFL2_200-248	<lm×ll>	1.7	-
ARSFL2_203	<nn×np>	0.1	-
ARSFL31_178-225	<lm×ll>	0.5	-
ARSFL8_301	<lm×ll>	0.3	-
ARSFL98_205	<lm×ll>	0.1	-
ChaM093_725	<lm×ll>	4.9	*
ChFaM003_400	<hk×hk>	3.8	*
ChFaM003_495	<nn×np>	18.4	*
ChFaM004_132	<lm×ll>	0.3	-
ChFaM004_230	<nn×np>	3.7	*
ChFaM010_290	<lm×ll>	1.3	-
ChFam011_117	<lm×ll>	2.7	*
ChFam011_129	<nn×np>	11.7	*
ChFaM017_147	<lm×ll>	1.7	-
ChFaM017_211	<lm×ll>	2.8	*
ChFaM017_250	<lm×ll>	2.6	-
ChFaM017_525	<lm×ll>	22.4	*
ChFaM017_86	<hk×hk>	8.5	*
ChFaM017_92	<nn×np>	3.1	*
ChFaM018_450	<nn×np>	6	*
ChFaM018_460	<nn×np>	3.1	*
ChFaM022_305-225	<lm×ll>	3.1	*
ChFaM030_205	<lm×ll>	1.8	-
ChFaM030_210	<nn×np>	8	*
ChFaM030_230	<lm×ll>	7.6	*
ChFaM030_500	<nn×np>	2	-
ChFaM031_138-190	<nn×np>	0.1	-
ChFam032_205	<lm×ll>	0	-
ChFam032_210	<hk×hk>	2.8	*

Table 3.6 (cont'd)

Locus	Segregation type	X2	Segregation Distortion
ChFaM035_245	<nn×np>	0	-
ChFam039_196	<lm×ll>	3.3	*
ChFam039_205	<lm×ll>	10.6	*
ChFaM040_100	<hk×hk>	7.9	*
ChFaM040_143	<hk×hk>	13.7	*
ChFaM040_155	<hk×hk>	22.4	*
ChFaM040_300	<hk×hk>	113.8	*
ChFaM040_315	<lm×ll>	12.8	*
ChFaM040_95	<hk×hk>	0.3	-
ChFaM044_129	<lm×ll>	0.1	-
ChFaM044_135	<nn×np>	8.2	*
ChFaM044_240	<hk×hk>	0.7	-
ChFaM046_128	<lm×ll>	13.1	*
ChFaM046_135	<hk×hk>	1.4	-
ChFaM046_152	<nn×np>	0.4	-
ChFaM046_157	<lm×ll>	0	-
ChFaM056_112	<nn×np>	1.3	-
ChFaM056_130-220	<lm×ll>	0.5	-
ChFaM060_110	<hk×hk>	0.1	-
ChFaM060_140	<lm×ll>	12.2	*
ChFaM060_200-230	<lm×ll>	0.6	-
ChFaM061_220	<lm×ll>	0.8	-
ChFaM061_225	<nn×np>	2.6	-
ChFaM063_106	<nn×np>	0	-
ChFaM063_111	<lm×ll>	1.5	-
ChFaM063_131	<lm×ll>	7.9	*
ChFaM063_141-107	<nn×np>	0	-
ChFaM063_90	<hk×hk>	0	-
ChFaM065_160	<lm×ll>	1.1	-
CHFaM067_161	<lm×ll>	0	-
CHFaM067_176	<hk×hk>	0.4	-
ChFaM072_350	<hk×hk>	0.1	-
ChFaM072_385	<hk×hk>	3	*
ChFaM076_101	<nn×np>	2.1	-
ChFaM076_103	<lm×ll>	2.4	-
ChFaM076_139	<hk×hk>	0	-
ChFaM076_140	<hk×hk>	2.6	-

Table 3.6 (cont'd)

Locus	Segregation type	X2	Segregation Distortion
ChFaM077_220	<hk×hk>	12.4	*
ChFaM078_140	<nn×np>	1.1	-
ChFaM080_219	<hk×hk>	3	*
ChFaM080_220	<hk×hk>	0.8	-
ChFaM081_110	<hk×hk>	1.4	-
ChFaM081_152-300	<hk×hk>	5.7	*
ChFaM085_155	<nn×np>	0	-
ChFaM086_230	<lm×ll>	5.4	*
ChFaM086_360	<lm×ll>	8.1	*
ChFaM086_440-225-198	<lm×ll>	0.1	-
ChFaM088_106	<lm×ll>	0.1	-
ChFaM088_300	<nn×np>	0.5	-
ChFaM089_390	<nn×np>	0.1	-
ChFaM092_140	<nn×np>	1.8	-
ChFaM093_200	<hk×hk>	10.8	*
ChFaM093_240	<lm×ll>	0.1	-
ChFaM093_750	<nn×np>	4.3	*
ChFaM094_120	<nn×np>	0	-
ChFaM095_135	<nn×np>	0	-
ChFaM095_150	<hk×hk>	0.1	-
ChFaM095_170	<lm×ll>	0.1	-
ChFaM095_320	<lm×ll>	2.8	*
ChFaM095_400	<lm×ll>	0.6	-
ChFaM098_108	<hk×hk>	0.3	-
ChFaM098_165	<lm×ll>	0	-
ChFaM098_225	<lm×ll>	4	*
ChFaM098_226	<nn×np>	4.3	*
ChFaM098_600	<lm×ll>	0.6	-
ChFaM101_136	<nn×np>	0.9	-
ChFaM101_137	<hk×hk>	2.3	-
ChFaM101_139	<lm×ll>	0	-
ChFaM101_153	<nn×np>	1.5	-
ChFaM101_180	<nn×np>	1.5	-
ChFaM103_124	<nn×np>	0.1	-
ChFaM103_190	<lm×ll>	1.3	-
ChFaM103_215	<lm×ll>	2.3	-
ChFaM103_450	<hk×hk>	31.5	*

Table 3.6 (cont'd)

Locus	Segregation type	X2	Segregation Distortion
ChFaM104_196	<nn×np>	38.3	*
ChFaM104_197	<hk×hk>	5	*
ChFaM104_209	<lm×ll>	1.1	-
ChFaM104_220	<hk×hk>	3.8	*
ChFaM107_192	<lm×ll>	1.3	-
ChFaM111_150	<lm×ll>	2.9	*
ChFaM111_176	<lm×ll>	4.2	*
ChFaM111_189	<hk×hk>	1.1	-
ChFaM111_195	<nn×np>	0.3	-
ChFaM111_210	<nn×np>	0.7	-
ChFaM111_400	<lm×ll>	0.3	-
ChFaM111_650	<hk×hk>	1.1	-
ChFaM111_675	<hk×hk>	1.6	-
ChFaM129_189	<nn×np>	2.4	-
ChFaM129_500-190	<lm×ll>	0.1	-
ChFaM147_210	<nn×np>	14.7	*
ChFaM147_219	<hk×hk>	17.8	*
ChFaM147_275	<lm×ll>	5.2	*
ChFaM148_156	<nn×np>	11.4	*
ChFaM148_161	<lm×ll>	43.5	*
ChFaM148_162	<nn×np>	2.4	-
ChFaM151_210	<hk×hk>	6.3	*
ChFaM151_218	<lm×ll>	6.4	*
ChFaM151_223	<lm×ll>	0	-
ChFaM151_228	<nn×np>	2	-
ChFaM151_325	<lm×ll>	12.3	*
ChFaM151_375	<nn×np>	56.2	*
ChFaM151_475	<lm×ll>	19.9	*
ChFaM151_500	<nn×np>	16	*
ChFaM161_190	<lm×ll>	1.9	-
ChFvM049_153	<nn×np>	9	*
EMFn115-133	<nn×np>	0.1	-
EMFn115-140	<lm×ll>	0	-
EMFn115-163	<nn×np>	0.5	-
EMFn117_129	<nn×np>	1.9	-
EMFn117_155-127	<lm×ll>	1.7	-
EMFn117_157	<nn×np>	0.8	-

Table 3.6 (cont'd)

Locus	Segregation type	X2	Segregation Distortion
EMFn117_188	<nn×np>	0.1	-
EMFn121_243	<nn×np>	0.6	-
EMFn121_249	<nn×np>	0.1	-
EMFn123_154	<nn×np>	19.6	*
EMFn123_163	<hk×hk>	29.1	*
EMFn123_200	<lm×ll>	0.1	-
EMFn128_146	<nn×np>	0.7	-
EMFn128_155	<hk×hk>	8.3	*
EMFn128_157	<lm×ll>	2.8	*
EMFn128_162-161	<lm×ll>	0.1	-
EMFn134_160	<hk×hk>	0	-
EMFn134_185	<lm×ll>	8.8	*
EMFn134_192	<lm×ll>	1.1	-
EMFn134_214	<lm×ll>	7.9	*
EMFn148_189	<lm×ll>	2.4	-
EMFn152_148	<lm×ll>	5.3	*
EMFn160_160	<hk×hk>	0.2	-
EMFn160_161	<hk×hk>	0.1	-
EMFn170_190	<hk×hk>	0.1	-
EMFn170_208	<nn×np>	1.7	-
EMFn170_215	<nn×np>	2.9	*
EMFn170_240	<lm×ll>	0.1	-
EMFn181_155	<hk×hk>	0.2	-
EMFn181_170	<lm×ll>	1.6	-
EMFn181_221	<hk×hk>	5.1	*
EMFn181_240	<nn×np>	0.5	-
EMFn184_239	<hk×hk>	0.4	-
EMFn184_245	<nn×np>	1.3	-
EMFn184_260	<hk×hk>	1.2	-
EMFn198_161	<hk×hk>	0.1	-
EMFn198_169	<nn×np>	0.4	-
EMFn198_189	<nn×np>	4.6	*
EMFn201_201	<hk×hk>	0.4	-
EMFn201_240	<hk×hk>	5.6	*
EMFn202_192-215	<lm×ll>	0.7	-
EMFn202_205	<lm×ll>	11.8	*
EMFn213_298	<lm×ll>	0.1	-

Table 3.6 (cont'd)

Locus	Segregation type	X2	Segregation Distortion
EMFn213_310	<nn×np>	2.4	-
EMFn213_320	<hk×hk>	0.1	-
EMFn225_250	<lm×ll>	0.4	-
EMFn225_259	<lm×ll>	3.5	*
EMFn230_220	<lm×ll>	9	*
EMFn230_225	<lm×ll>	0.4	-
EMFn235_210	<lm×ll>	1.9	-
EMFn235_214	<lm×ll>	0	-
EMFn235_215	<nn×np>	2	-
EMFn235_219	<lm×ll>	0.2	-
EMFvi104_127	<lm×ll>	4.5	*
EMFvi104_130	<nn×np>	0.3	-
EMFvi104_80	<lm×ll>	0	-
EMFvi136_157	<lm×ll>	0.1	-
EMFvi136_159	<nn×np>	11.1	*
FAC001_182	<hk×hk>	0.1	-
FAC001_211	<hk×hk>	0.2	-
FAC001_258	<hk×hk>	0.9	-
FAC002_270	<lm×ll>	1.3	-
FAC006_225	<lm×ll>	0.2	-
SF4B12_600	<lm×ll>	0	-
SF5C08_325	<lm×ll>	8.1	*
SF5C08_410	<lm×ll>	2.4	-
SF5G02_228	<nn×np>	0.2	-
SF5G02_250	<lm×ll>	1.1	-
SF5G02_260	<lm×ll>	1.5	-
SF5G02_273	<lm×ll>	0.2	-
SF5G02_275	<nn×np>	1.3	-
SF6E02_119	<lm×ll>	1.9	-
SF6E02_149	<nn×np>	0	-
SFGRP7_125	<lm×ll>	3.9	*
SFGRP7_134-141-147	<nn×np>	1.1	-
UaFv8150_130	<nn×np>	1.1	-
UAFv8216_101	<lm×ll>	0.1	-
UAFv8216_161	<hk×hk>	0.9	-
UAFv8216_182-185	<hk×hk>	24.3	*
UAFv8216_239-107	<lm×ll>	2.4	-

Table 3.6 (cont'd)

Locus	Segregation type	X2	Segregation Distortion
UaFv8936_320	<lm×ll>	0.3	-
UaFv8936_410	<nn×np>	2.7	-
UaFv9092_122	<lm×ll>	0.7	-
UaFv9094_390	<lm×ll>	2.5	-
UaFv9094_495	<lm×ll>	14.9	*
UaFv9094_700	<hk×hk>	2.2	-
UDF004_130	<hk×hk>	8.3	*
UDF004_136	<lm×ll>	0	-
UDF004_143	<hk×hk>	0.1	-
UDF004_150	<hk×hk>	0.6	-
UFFa01E03_135	<hk×hk>	58.3	*
UFFa01E03_165	<hk×hk>	1.8	-
UFFa01E03_175	<hk×hk>	1.5	-
UFFA01H05_250	<lm×ll>	21.3	*
UFFA01H05_251	<hk×hk>	1.6	-
UFFa02F02_106	<lm×ll>	0.1	-
UFFa02F02_209	<hk×hk>	0.5	-
UFFa02F02_500	<nn×np>	3	*
UFFa02F02_505	<nn×np>	0.9	-
UFFa02F02_510	<nn×np>	1.6	-
UFFa03B05_210	<nn×np>	0.4	-
UFFa03B05_214	<lm×ll>	12.4	*
UFFa04G04_158	<lm×ll>	2.8	*
UFFa04G04_161	<nn×np>	10.4	*
UFFa04G04_162	<lm×ll>	16.5	*
Uffa11A11_206-260	<nn×np>	1.9	-
Uffa11A11_225	<lm×ll>	4.4	*
Uffa14A11_105	<nn×np>	1.2	-
Uffa14A11_114	<lm×ll>	4.9	*
UFFa16H07_250	<nn×np>	1.8	-
UFFa16H07_256	<hk×hk>	0	-
UFFa16H07_262	<lm×ll>	0.2	-
UFFa16H07_270	<nn×np>	1.1	-
UFfa18H04_120	<lm×ll>	1.9	-
UFfa18H04_121	<nn×np>	0.4	-
UFfa18H04_143	<hk×hk>	0.6	-
UFFa20D02_109	<lm×ll>	0.7	-

Table 3.6 (cont'd)

Locus	Segregation type	X2	Segregation Distortion
UFFa20D02_97	<hk×hk>	0.9	-
Uffa20H10_247	<hk×hk>	0.9	-
Uffa20H10_260	<lm×ll>	0.2	-
Uffa20H10_273	<lm×ll>	1.6	-

Appendix 3.3

Figure 3.4 The male and female parent maps. Distances on linakge groups are in cM. The 'Honeoye' map had 103 markers in 23 linkage groups and the 'Tribute' map had 78 markers in 22 linkage groups.

Honeoye Map:

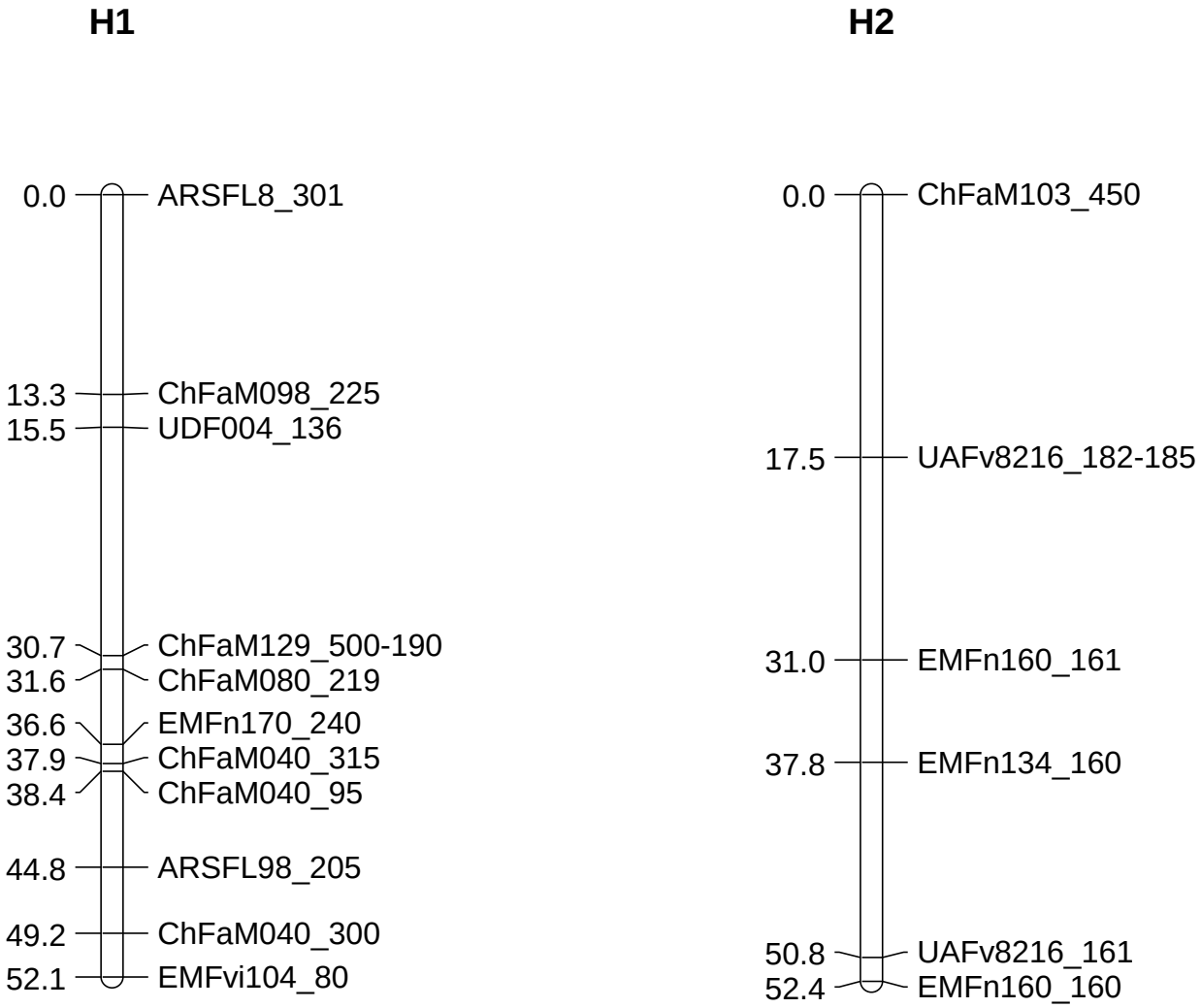


Figure 3.4 (cont'd)

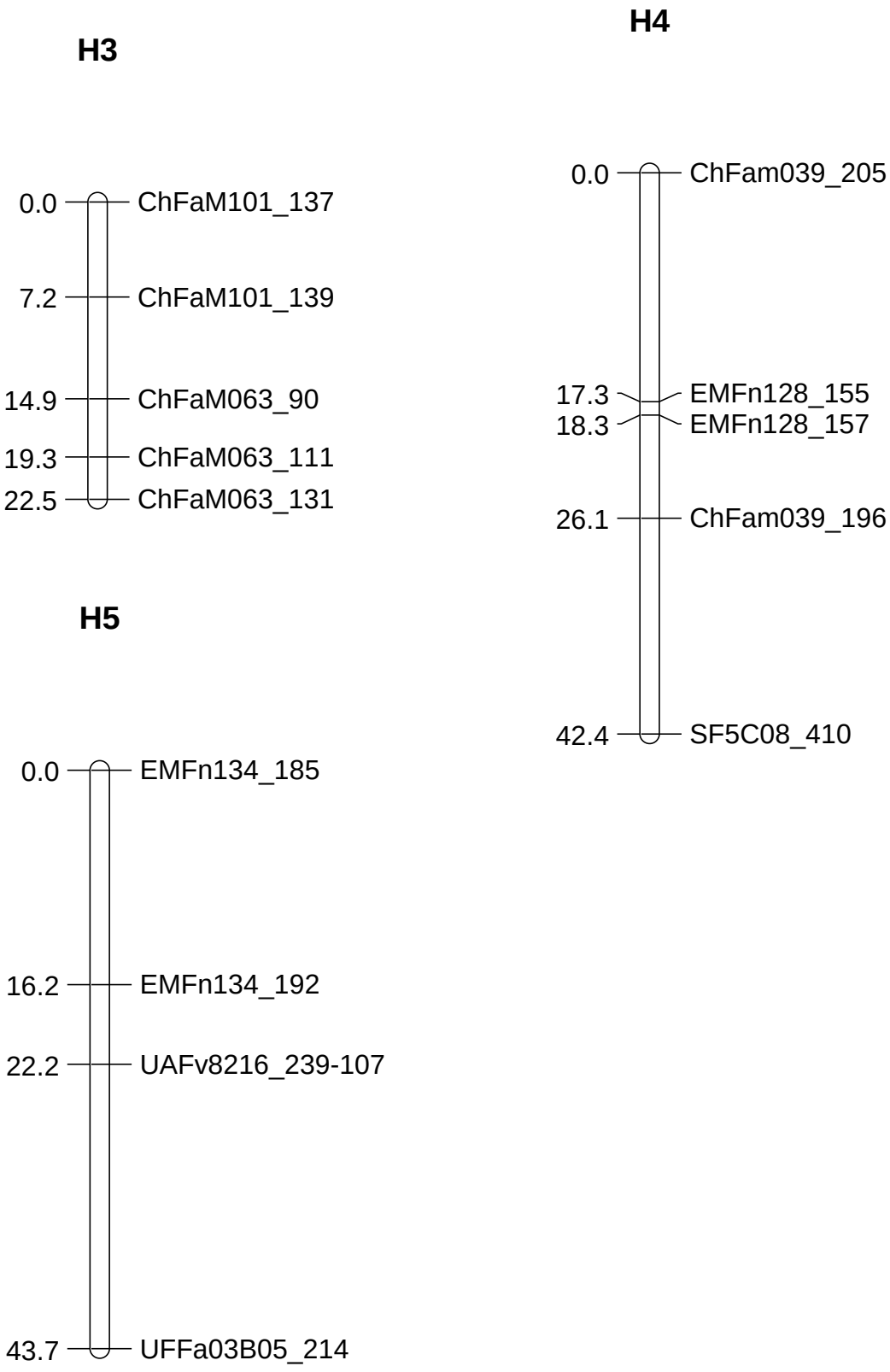


Figure 3.4 (cont'd)

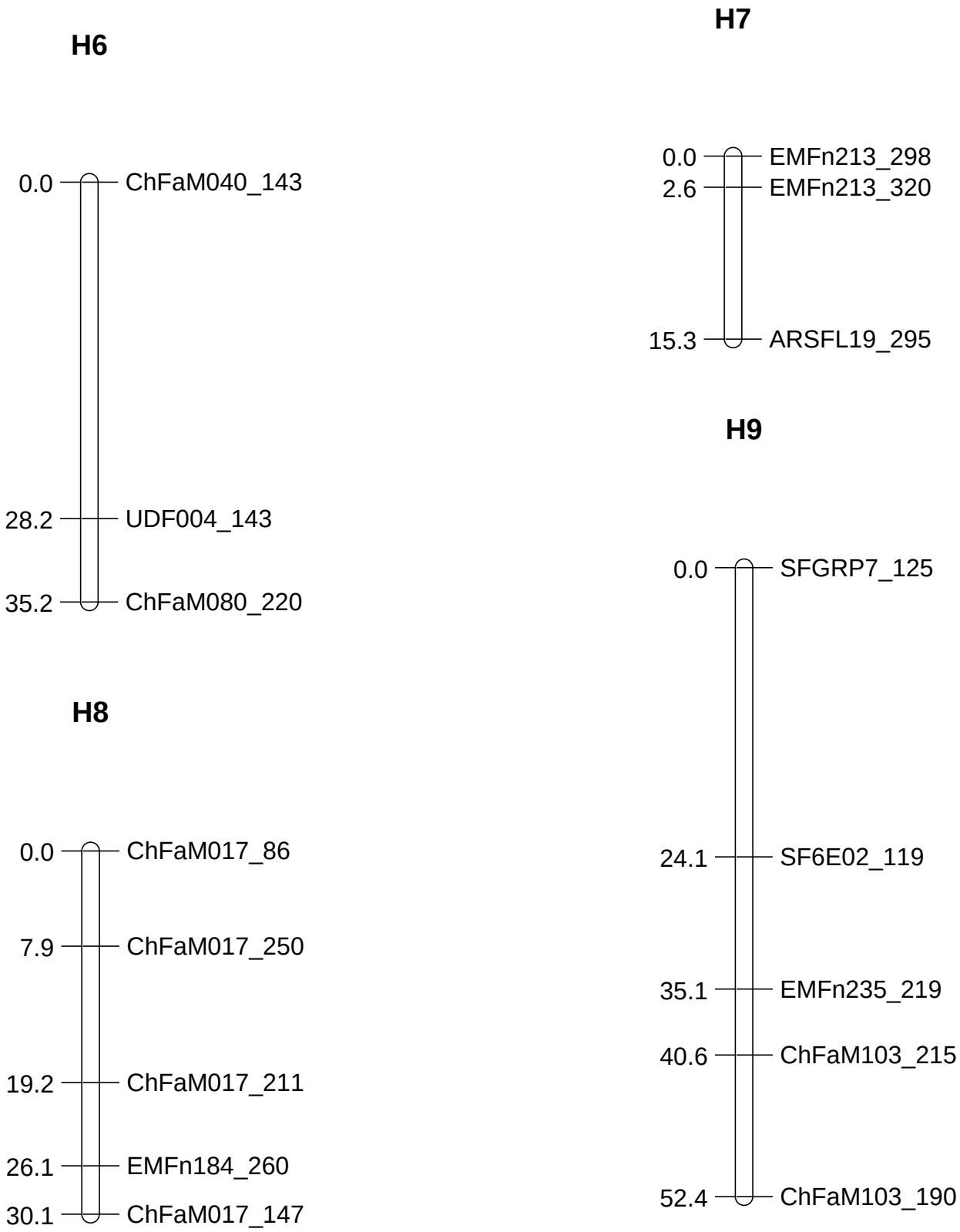


Figure 3.4 (cont'd)

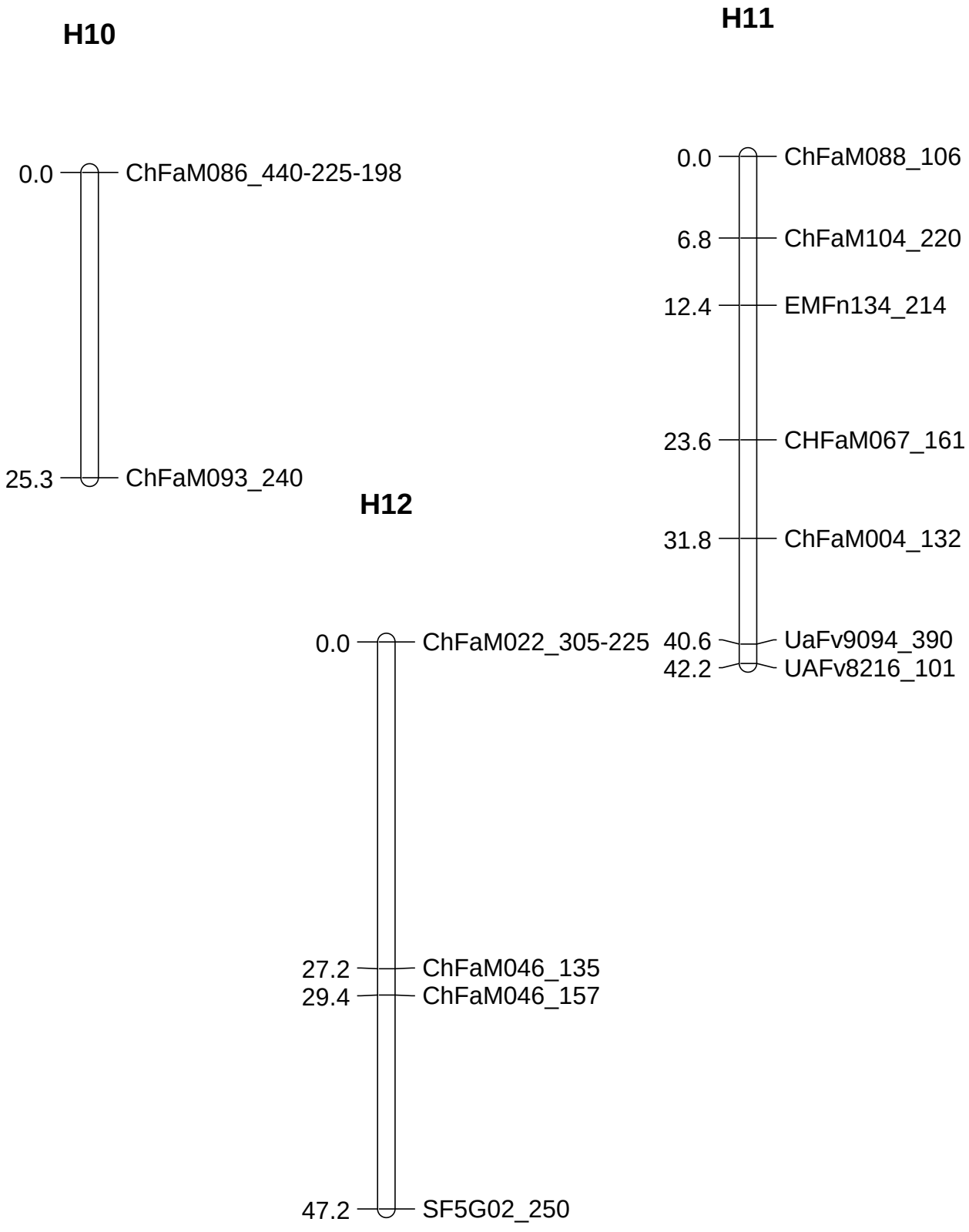


Figure 3.4 (cont'd)

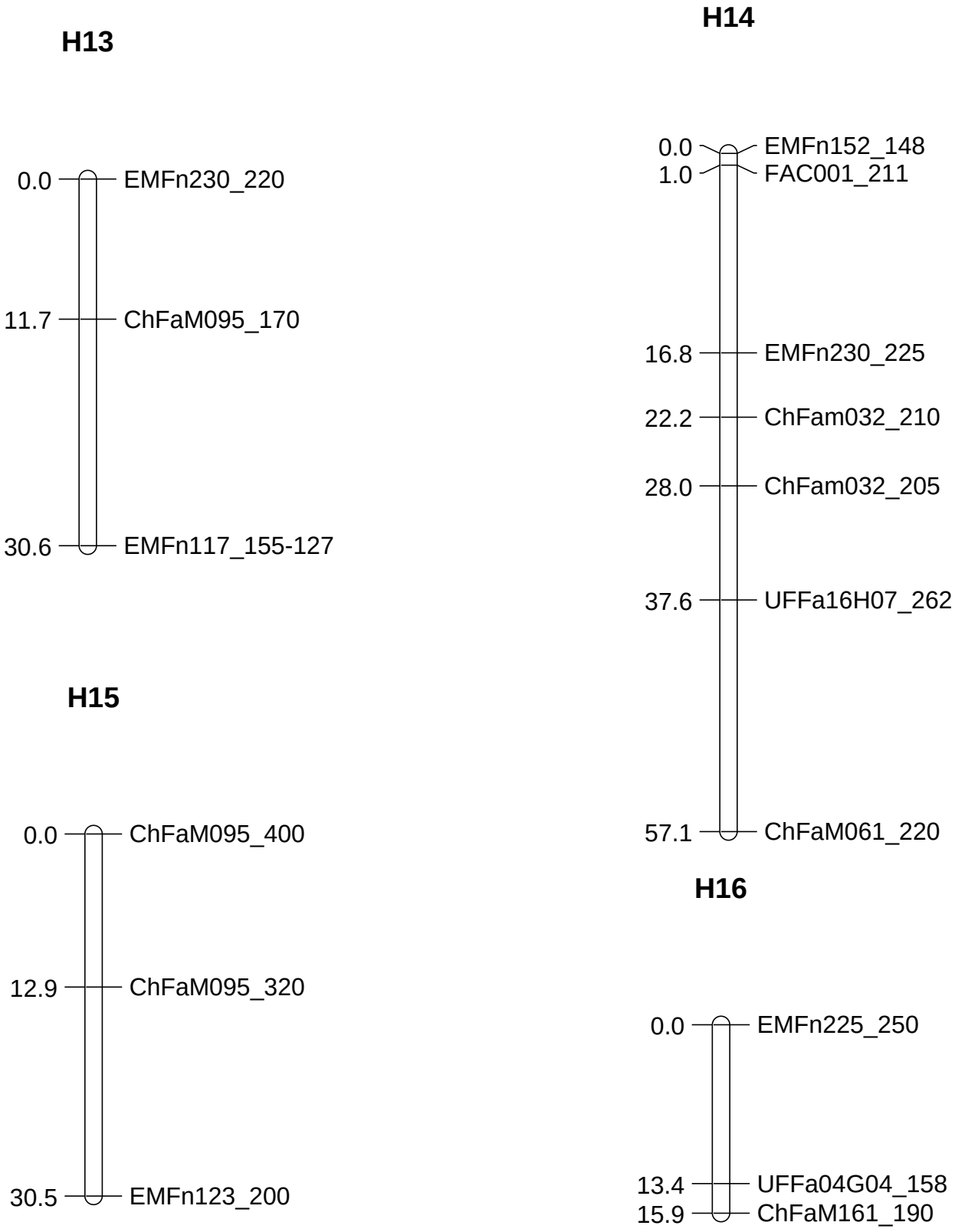


Figure 3.4 (cont'd)

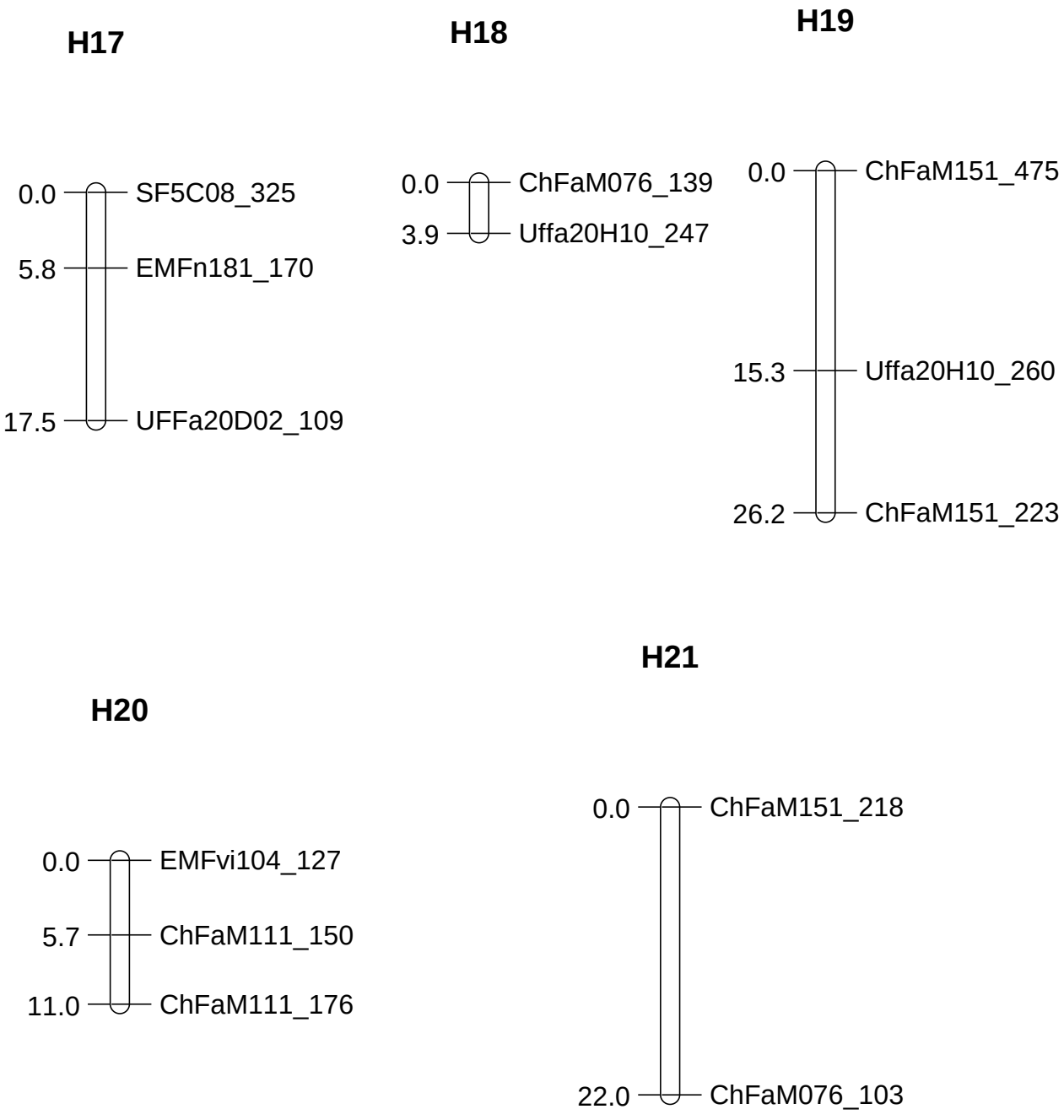


Figure 3.4 (cont'd)

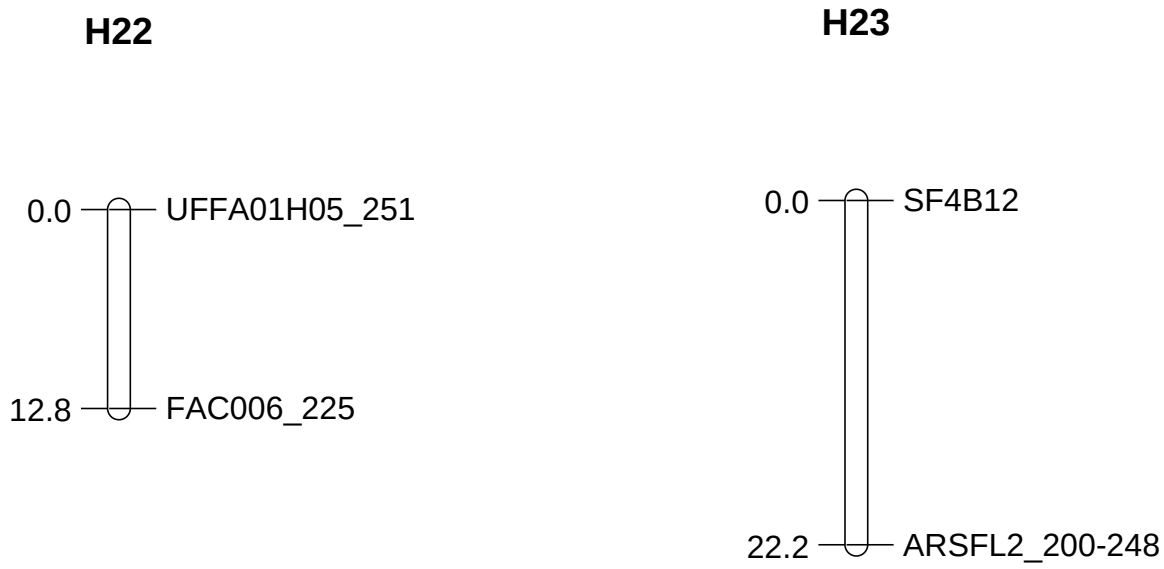


Figure 3.4 (cont'd)

Tribute Map:

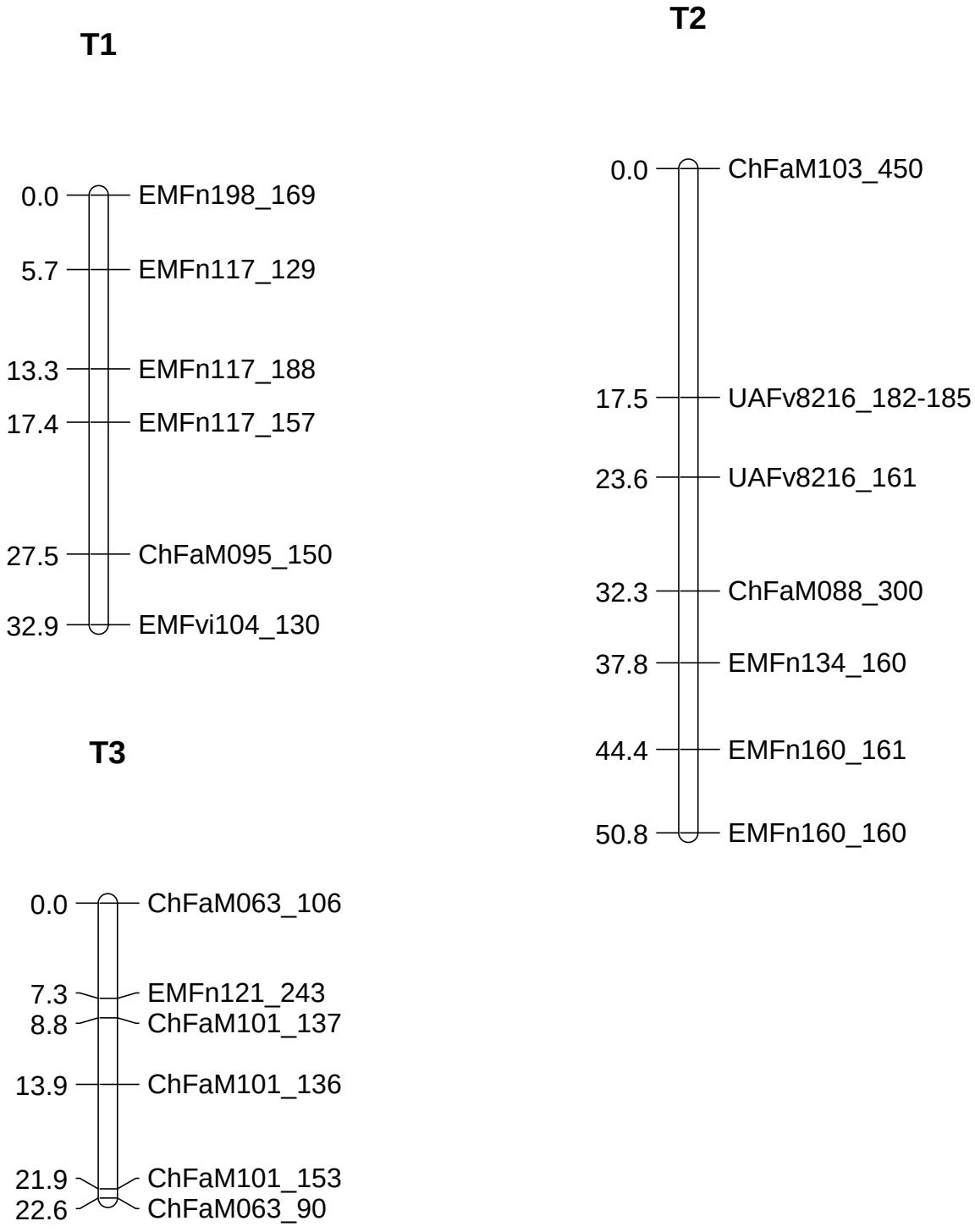


Figure 3.4 (cont'd)

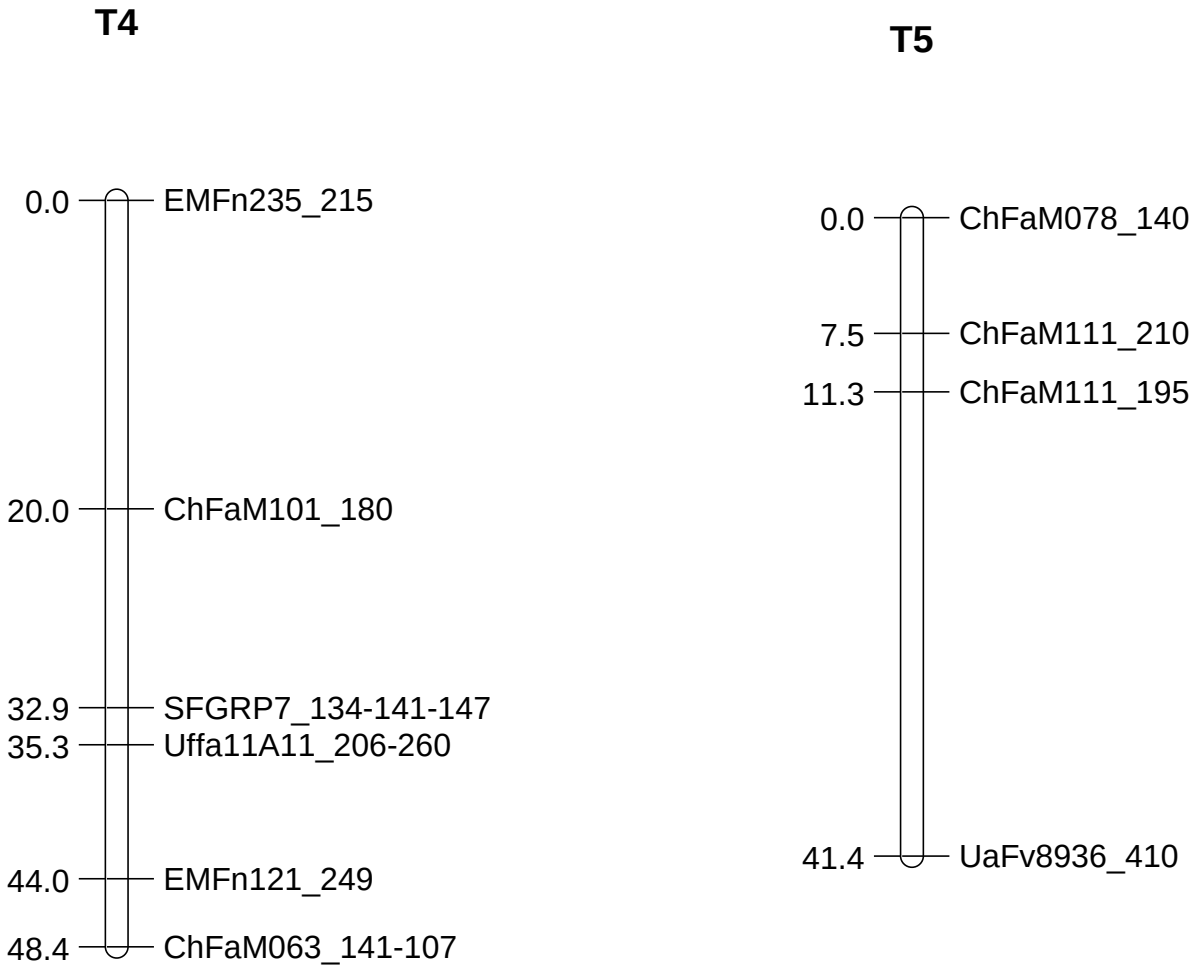


Figure 3.4 (cont'd)

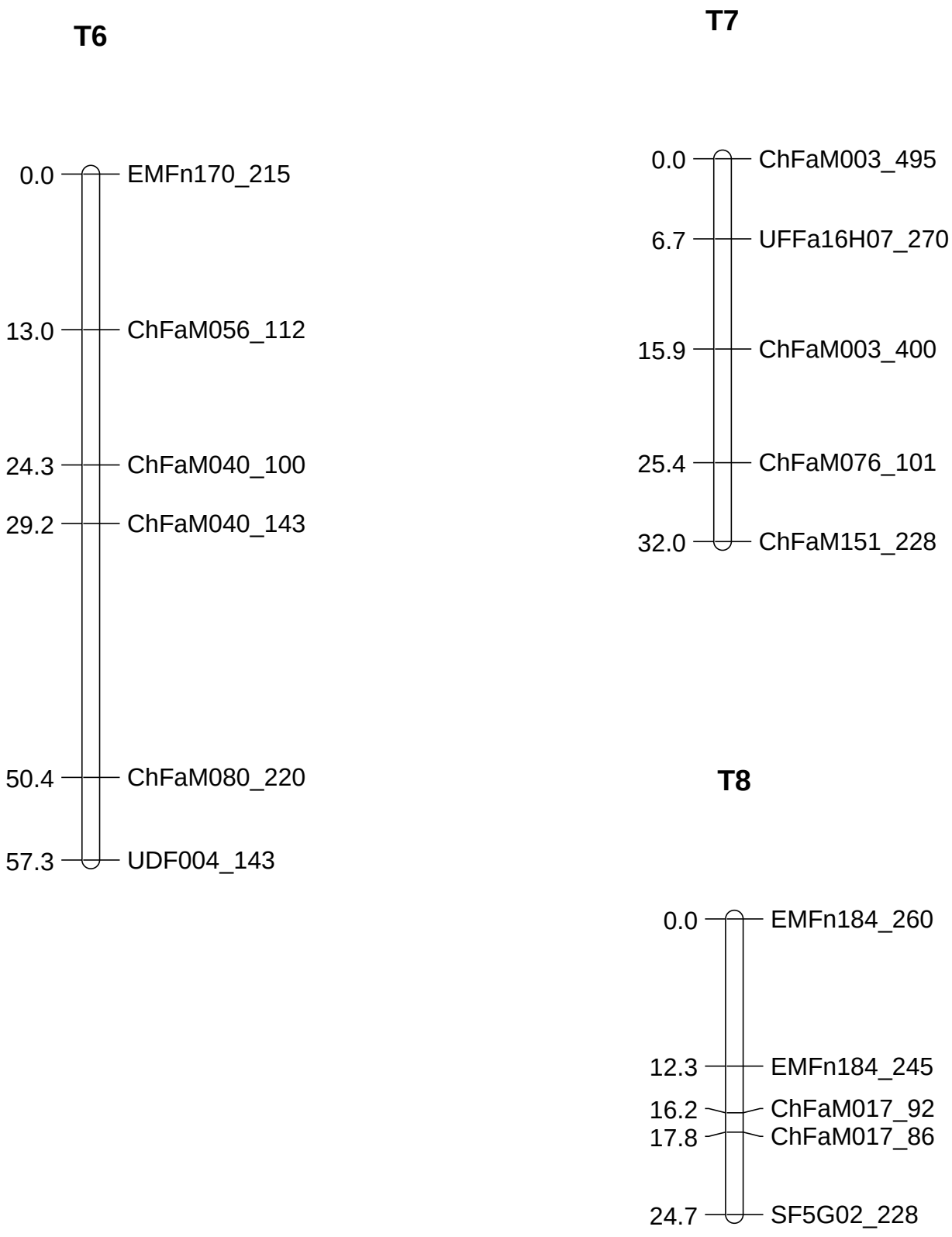


Figure 3.4 (cont'd)

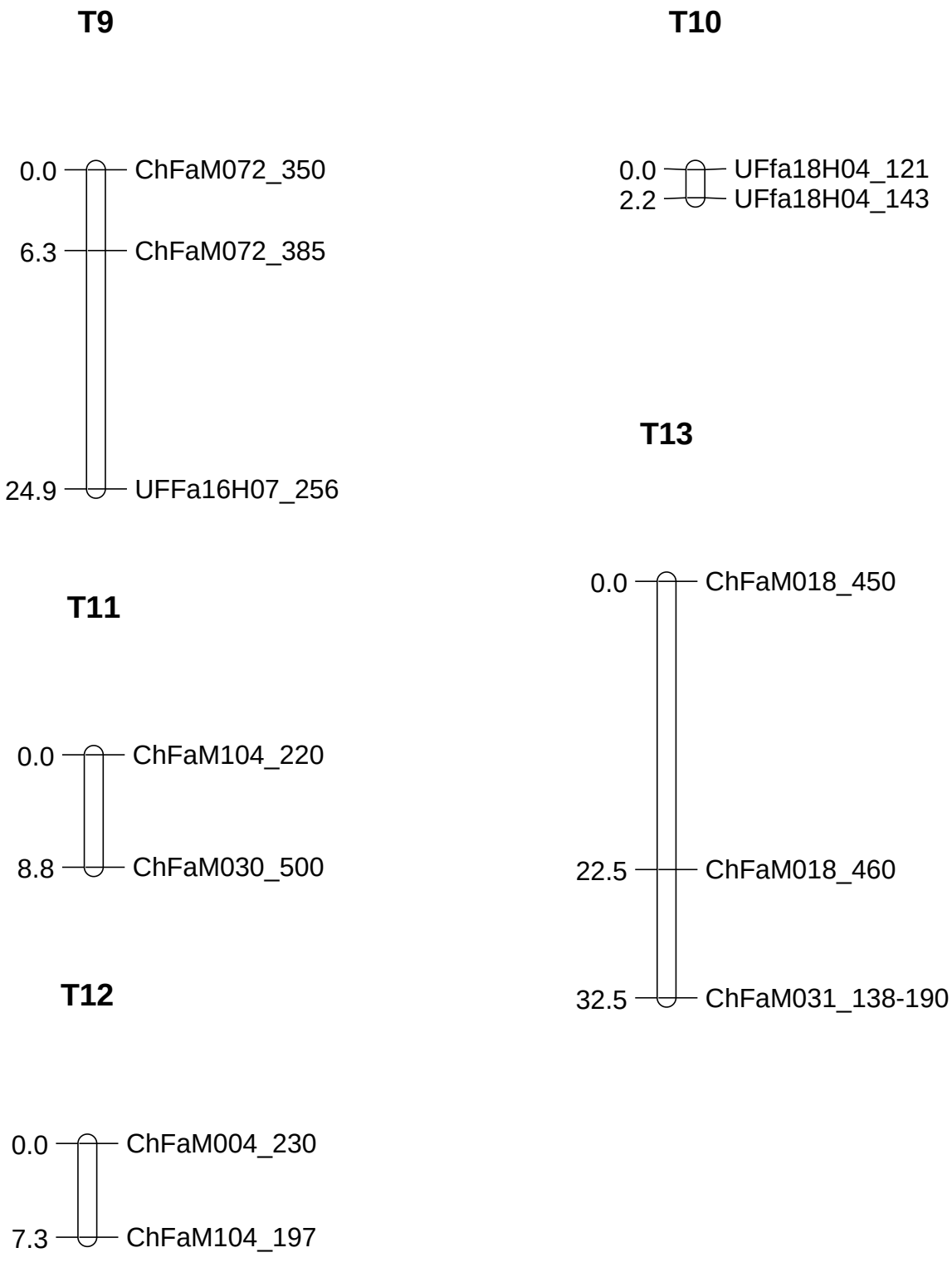


Figure 3.4 (cont'd)

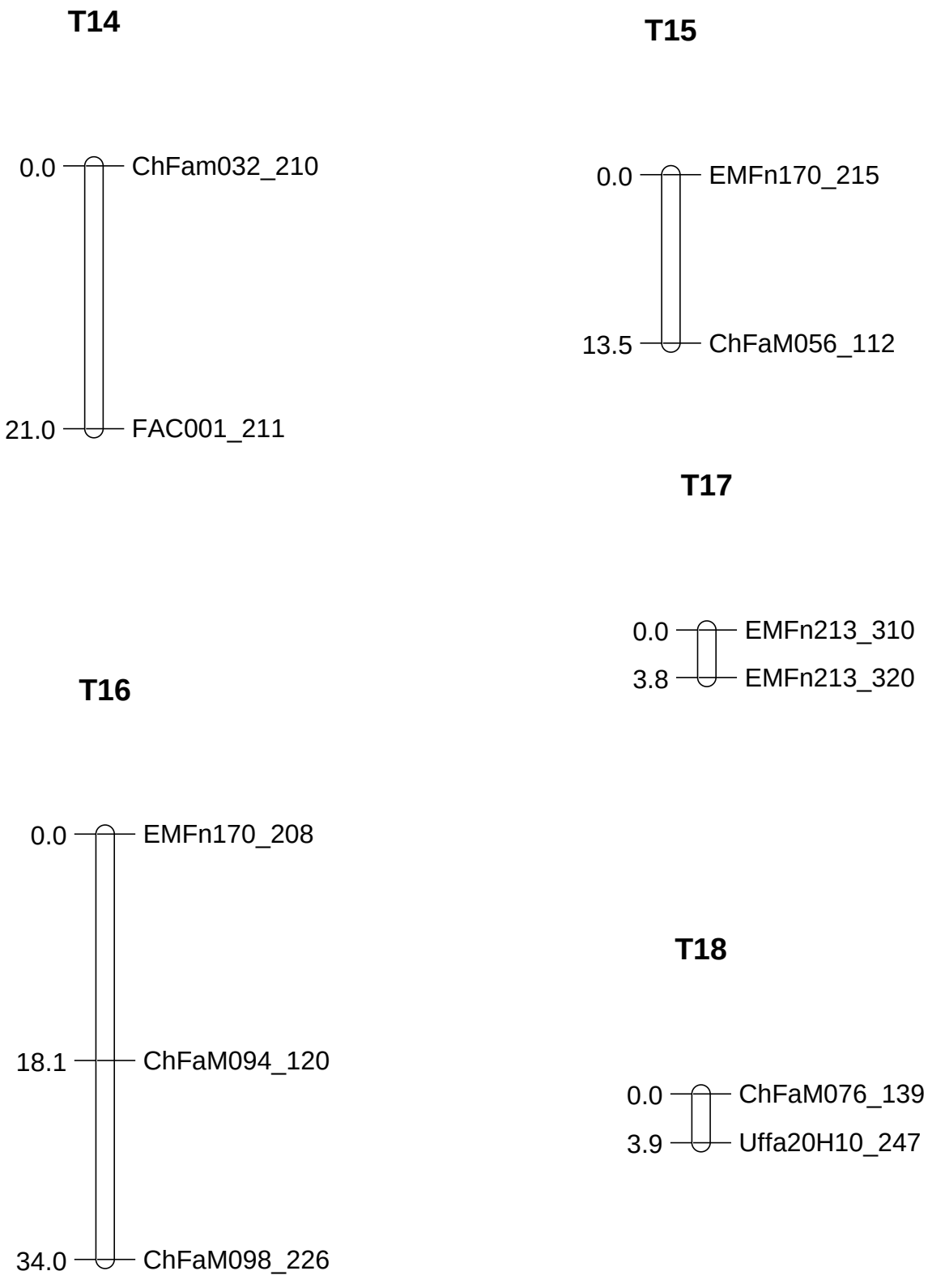
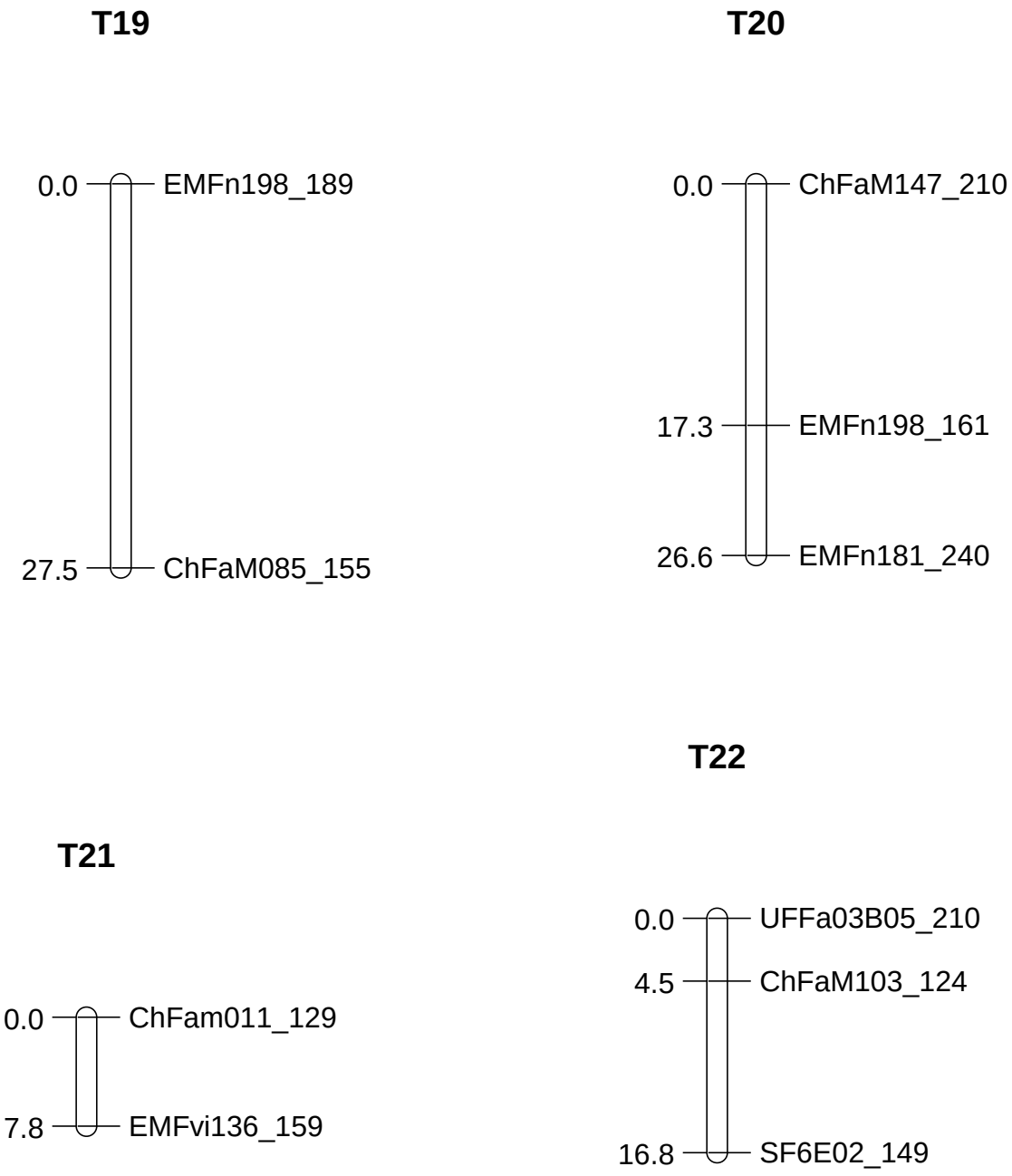


Figure 3.4 (cont'd)



Appendix 3.4

Multiplex segregation ratios of SSR markers

The markers with distorted segregation ratios were tested to see if they fit into any of the multiplex segregation ratios as described by Lerceteau-Kohler *et al.* (2003). Markers segregating in only one parent were tested for multiplex ratios 3:1 (disomic, SD-single dose x ND-null dose), 7:1 (disomic, TD-triple dose x ND), 15:1 (disomic, 4D-four dose x ND), and 11:3 (octosomic DD-double dose x ND). Markers segregating in both parents were tested for multiplex ratios: 7:1 (disomic, DD x SD), 15:1 (disomic TD x SD), and 25:3 (octosomic, DD x SD). Only 15% (13 out of 88 markers with distortion) fit into multiplex segregation ratios at the 0.05 level. Five markers that segregated in both parents fit into the multiplex disomic ratio 7:1. Out of these, two also fit into the ratio 25:3. Six markers segregating in 'Honeoye' fit multiplex segregation ratio 3:1 and three of these also fit into 11:3 ratio. Two markers segregating in 'Tribute' fit the multiplex disomic ratio 3:1. Lerceteau-Kohler *et al.* (2003) could not fit 28 (out of 892) markers into any segregation ratios. Of the remaining, 72 fit into both simplex and multiplex ratios. 8% of the markers they used fit into multiplex ratios, whereas, in this study, 5% of the markers fit multiplex ratios. Overall, 65% of the markers in the 'Honeoye' × 'Tribute' fit simplex segregation ratio indicating that the genome is largely diplodized, as reported by Lerceteau-Kohler *et al.* (2003). Only 33% of the markers displayed multiplex segregation ratios, indicative of polysomic inheritance.

Table 3.7 Multiplex segregation ratios of SSR markers with segregation distortion.

Locus	Seg.type	h-	kk	X2 (3:1)	X2 (7:1)	X2 (25:3)	Df
Present in both parents				simplex (SD × SD)	multiplex disomic (DD × SD)	multiplex octosomic (DD × SD)	
UDF004_130	<hk×hk>	98	15	8.3	0.062	0.774	1
ChFaM040_100	<hk×hk>	145	27	7.9	1.608		1
ChFaM151_210	<hk×hk>	144	29	6.3	2.874		1
EMFn181_221	<hk×hk>	92	17	5.1	0.955	2.716	1
ChFaM104_197	<hk×hk>	126	26	5	2.947		1

Locus	Seg.type	lm	ll	X2 (1:1)	X2 (3:1)	X2 (11:3)	Df
Present in Honeoye				simplex (SD × ND)	multiplex disomic (DD × ND)	multiplex octosomic (DD × ND)	
ChFaM148_161	<lm×ll>	116	35	43.5	0.267	0.275	1
UFFA01H05_250	<lm×ll>	78	30	21.3	0.444	2.586	1
UFFa04G04_162	<lm×ll>	40	11	16.5	0.320	0.001	1
ChaM093_725	<lm×ll>	38	21	4.9	3.531		1
Uffa14A11_114	<lm×ll>	34	18	4.9	2.564		1
Uffa11A11_225	<lm×ll>	33	18	4.4	2.882		1

Locus	Seg.type	np	nn	X2 (1:1)	X2 (3:1)	X2 (11:3)	Df
Present in Tribute				simplex (SD × ND)	multiplex disomic (DD × ND)	multiplex octosomic (DD × ND)	
ChFaM104_196	<nn×np>	111	36	38.3	0.020	0.818	1
ChFaM147_210	<nn×np>	81	39	14.7	3.600		1
							1

Appendix 3.5

Colinearity in Octoploid map

Homeologous groups could be identified for 29 out of 34 groups based on comparisons with the diploid map of Sargent *et al.* (2011), octoploid map of Sargent *et al.* (2012), and the *F. vesca* genome sequence (www.strawberrygenome.org; Shulaev *et al.*, 2011). Only one group (HT15) had markers from more than one diploid group (VI and VI). This group has only 3 markers covering 15.9 cM, but it is possible that it represents a translocation at this location. In his denser map, Sargent *et al.* (2009) observed duplicated loci in homeologous groups I and VI.

Sargent *et al.* (2012) suggested that there was "almost complete colinearity" between diploid and octoploid marker locations, except for two chromosomal inversions on three homeologs (I, III, and IV). In their earlier paper, Sargent *et al.* (2009) also reported that there were some regions on the octoploid map where the marker order was not collinear with the diploid. For example, CVFCT032 and BFAC045 were not collinear with respect to other markers in group RG3-A and BFACT002, and EMFn214 and CFVCT015 were not collinear on group RG2-B. Rousseau-Gueutin *et al.*, (2008) also suggested that overall the marker colinearity is conserved between diploid and octoploid genome. However, they identified two regions (homeolog II and IV) where there were potential inversions. In our 'Honeoye' × 'Tribute' map, linkage group (HT15) consisted of markers from two different homeologs (IV and VI), which could be due to a translocation.

When we compared the physical locations of markers on the pseudochromosomes of the diploid physical map (www.strawberrygenome.org) to the octoploid, we observed much less colinearity than was described by Sargent *et al.* (2009 and 2011). The figure below shows a comparison

between the physical marker locations on the diploid map and the 'Honeoye' × 'Tribute' octoploid groups. Markers from diploid homeolog I were found in 6 octoploid linkage groups, markers from homeolog II were found in 7 groups, markers from homeolog III were found in 5 groups, markers from homeolog IV were found in 4 groups, markers from homeologs V and VI were found in 4 groups each, and markers from homeolog VII were found in one group. BLASTs with the primer sequences placed ChFaM098 in diploid group II, even though it was placed in homeolog III in the octoploid map by Sargent *et al.* (2012), and ChFaM098 cosegregated with EMFN170 and EMFn202, both present in homeolog III. Some of the markers that were located close together on the diploid pseudochromosome (Shulaev *et al.*, 2011) and in the diploid map (Sargent *et al.*, 2004, 2006, 2011) were found in separate groups in the octoploid. For example, diploid group I has EMFn128 and ChFaM081 next to each other separated by 10,00,000 bp. However, in the octoploid map, EMFn128 was located on two linkage groups HT21 and HT29, while ChFaM081 was located in group HT32. In diploid homeolog II, ChFaM103 was separated from UFFa03B05 by 500,000 bp on the diploid physical map; however, these two markers cosegregated in only one octoploid group (HT23), even though ChFaM103 was found on two of other octoploid linkage groups (HT5 and HT11).

Diploid homeologous group II had the highest density of markers in our octoploid map, and therefore was the most useful linkage group to evaluate colinearity. There were several locations on the octoploid map where the marker order was not collinear with the diploid. For example, diploid group II had the marker order SFGRP7, EMFn235, EMFn121, while the homeolog HT8 has the order EMFn235_215, SFGRP7_134/141/147, and EMFn121_249. Similarly, the marker order ChFaM103, UFFaB05, ChFaM088, UaFV8216, and EMFn134 in the diploid group was

not repeated in the octoploid HT5 which had markers in the order: ChFaM103_450, UaFV8216_182/185, UaFv8216_161, ChFaM088_300, and EMFn134_160.

We also found instances where closely linked diploid markers were found on different linkage groups in the octoploid. Based on comparison with Sargent *et al.* (2011) diploid SSR map, UFFa04G04 belongs to homeologous group VI, while EMFn225 belongs to homeologous group V in Sargent *et al.* (2012 submitted) octoploid map. Sargent *et al* (2004) observed that 2 out of the 75 SSRs segregated at two loci in their octoploid map, and Sargent *et al.* (2009, 2011) found several additional SSR markers that mapped to 2 locations. For example, CFVC032 mapped to homeologs IV and VII in the octoploid 'Redgauntlet' map, although it was located in group III in the diploid map. Similarly, EMFn181 markers mapped to homeologs III, IV, V, and VI on the 'Redgauntlet' map, but were located on group V in the diploid map. We also found 4 alleles that amplified from EMFn181; however only one of them assembled into linkage groups, so it is not known whether the other alleles would have mapped to different homeologs.

Only 130 out of the 258 markers used to assemble our map were placed in linkage groups and as a result our octoploid map was much less dense than that of Sargent *et al.* (2006, 2009, 2011). However, the separation of closely linked markers into separate groups and the differences we observed in marker order between the diploid and the octoploid suggests that colinearity between the two ploidy levels in our mapping population is much more limited than that described by Sargent and his group. It is unknown why our data provide such a different picture about colinearity, unless a more dense map of 'Honeoye' × 'Tribute' will combine many of our smaller linkage groups and provide greater evidence of colinearity.

One of the factors limiting the density of our map was that we were unable to accurately evaluate allele dosage (van Djik *et al.* 2010) because polyacrylamide gel electrophoresis was used to visualize PCR amplicons.

Figure 3.5 Comparison of ‘Honeoye’ × ‘Tribute’ linkage groups (HT1-HT34) with diploid linkage groups (I-VII) developed based on physical distances on pseudochromosomes. Diploid map distances are in x 10,00,000 bp. Octoploid distances are in cM. The markers on the octoploid linkage groups are color coded to indicate whether they segregate in ‘Honeoye’ (red), in ‘Tribute’ (green), or both (blue). Marker names are abbreviated to include the first letter of the SSR locus name and the band size. Original marker names are shown in Figure 3.1

Figure 3.5 (cont'd)

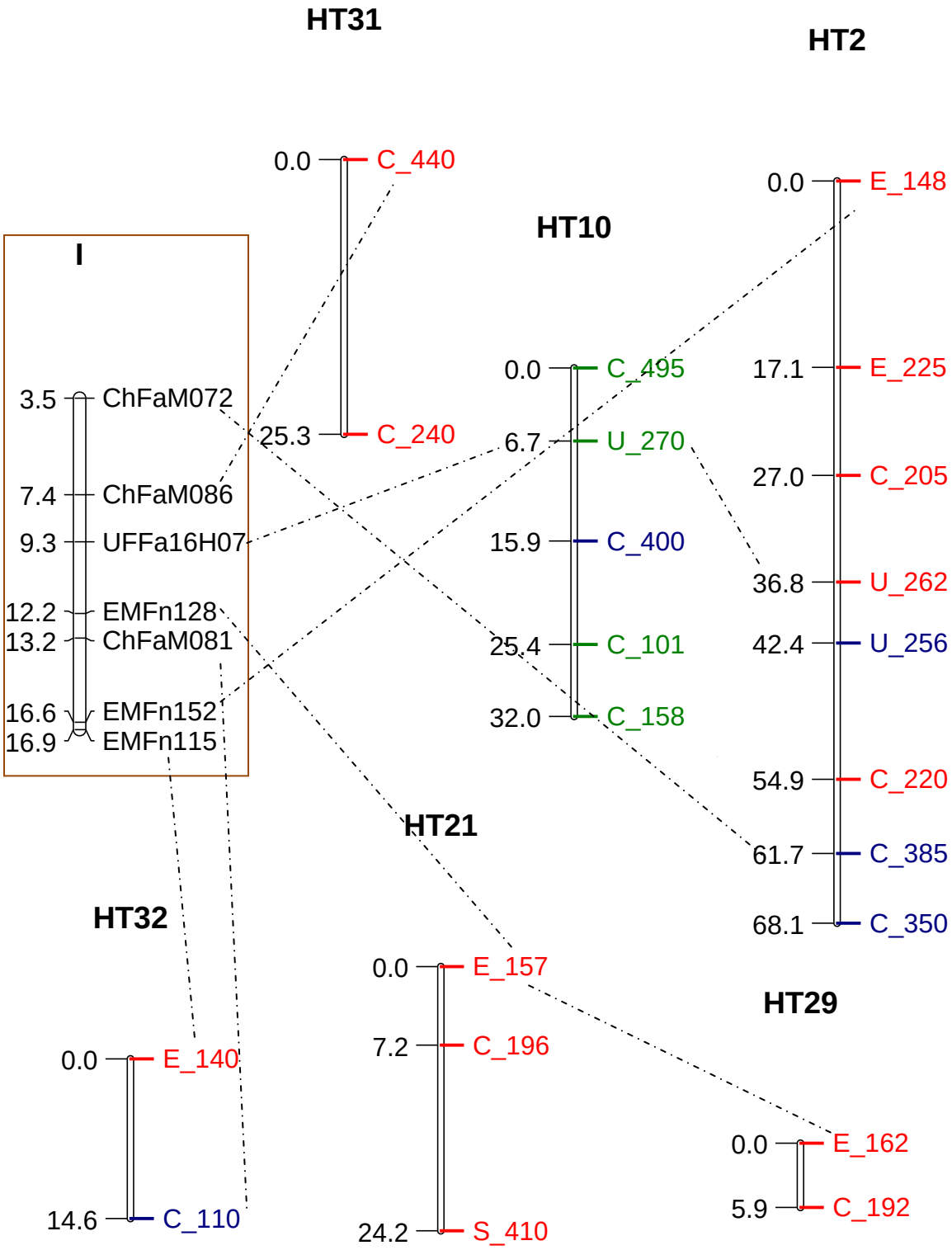


Figure 3.5 (con t'd)

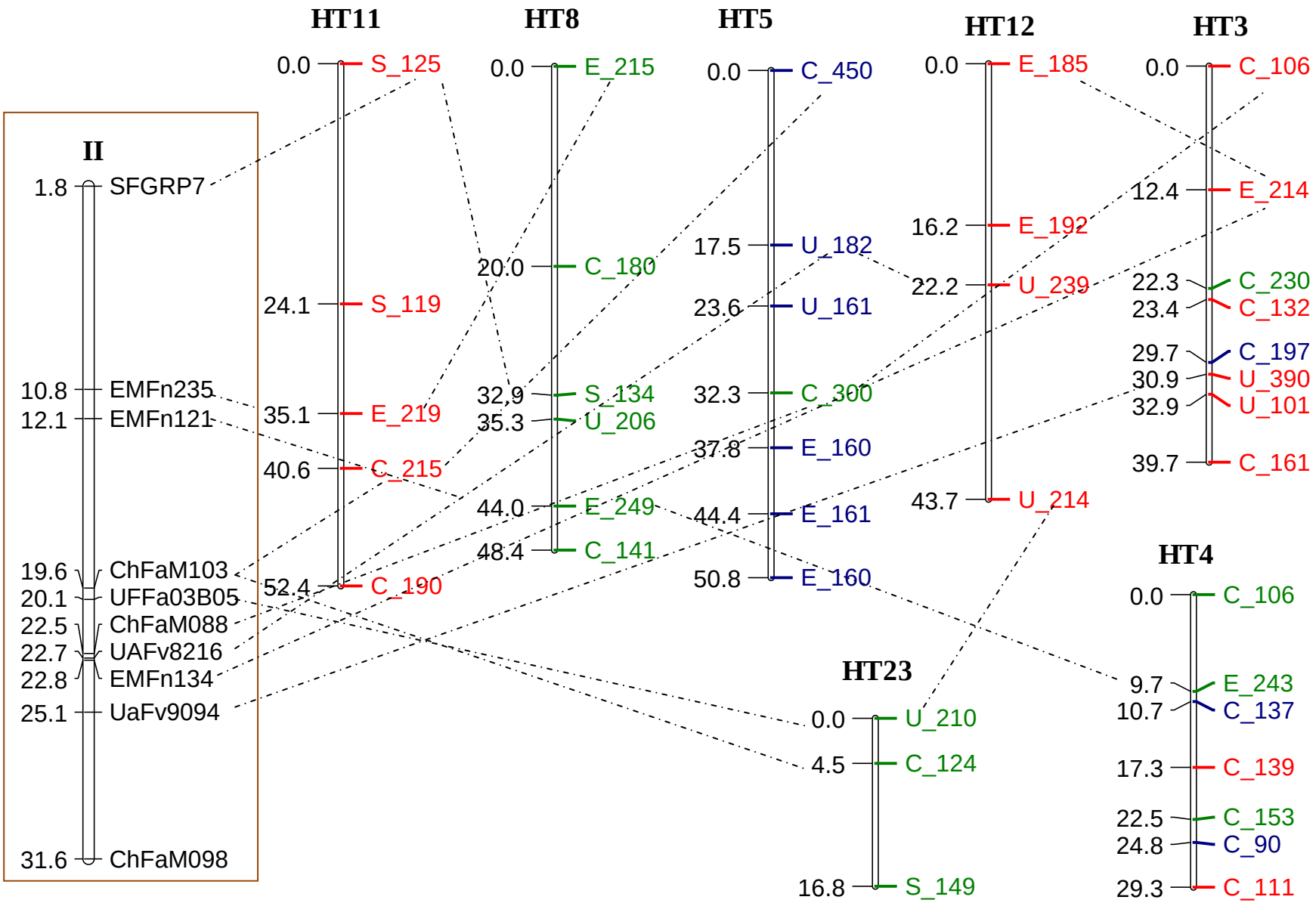


Figure 3.5 (cont'd)

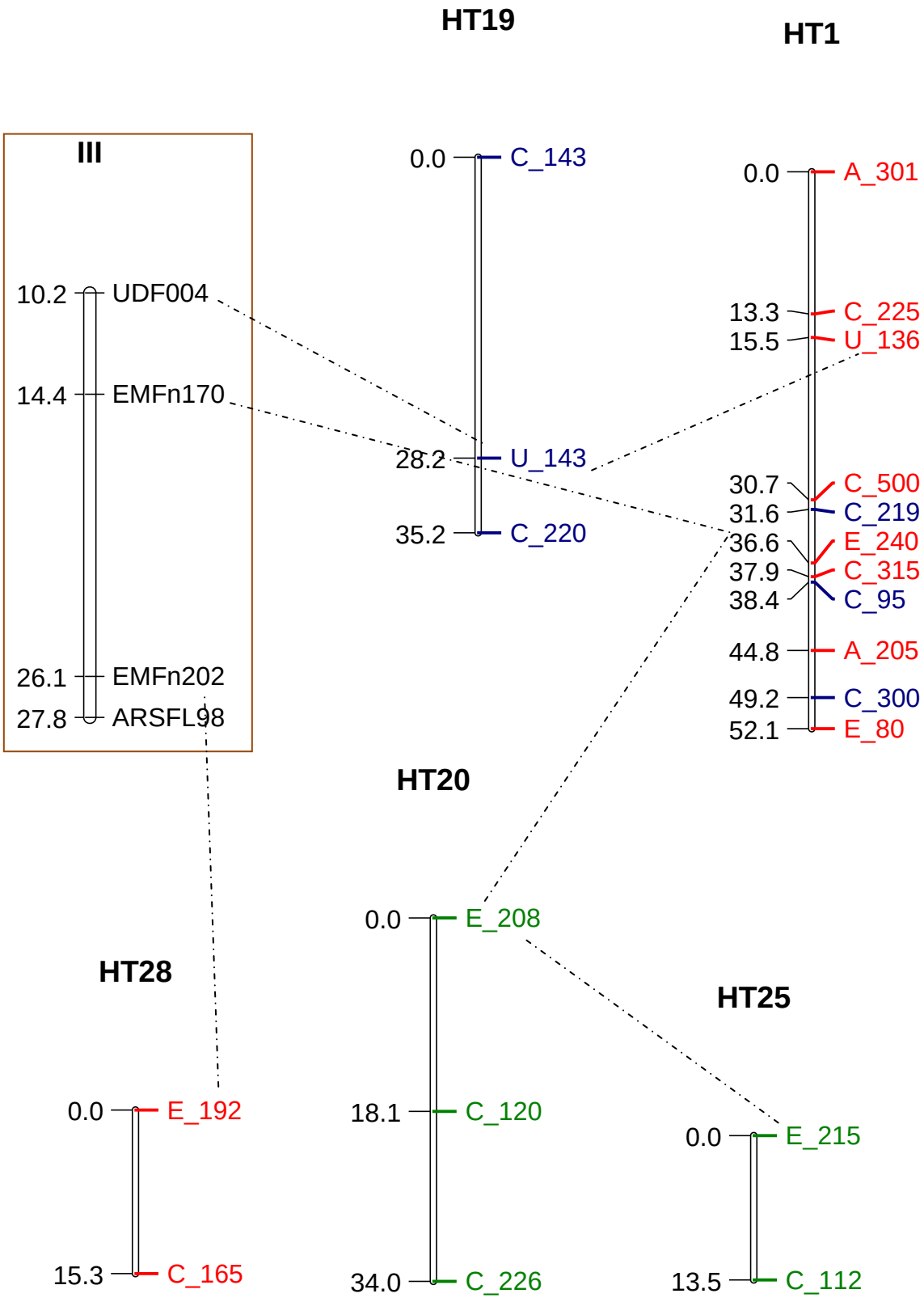


Figure 3.5 (cont'd)

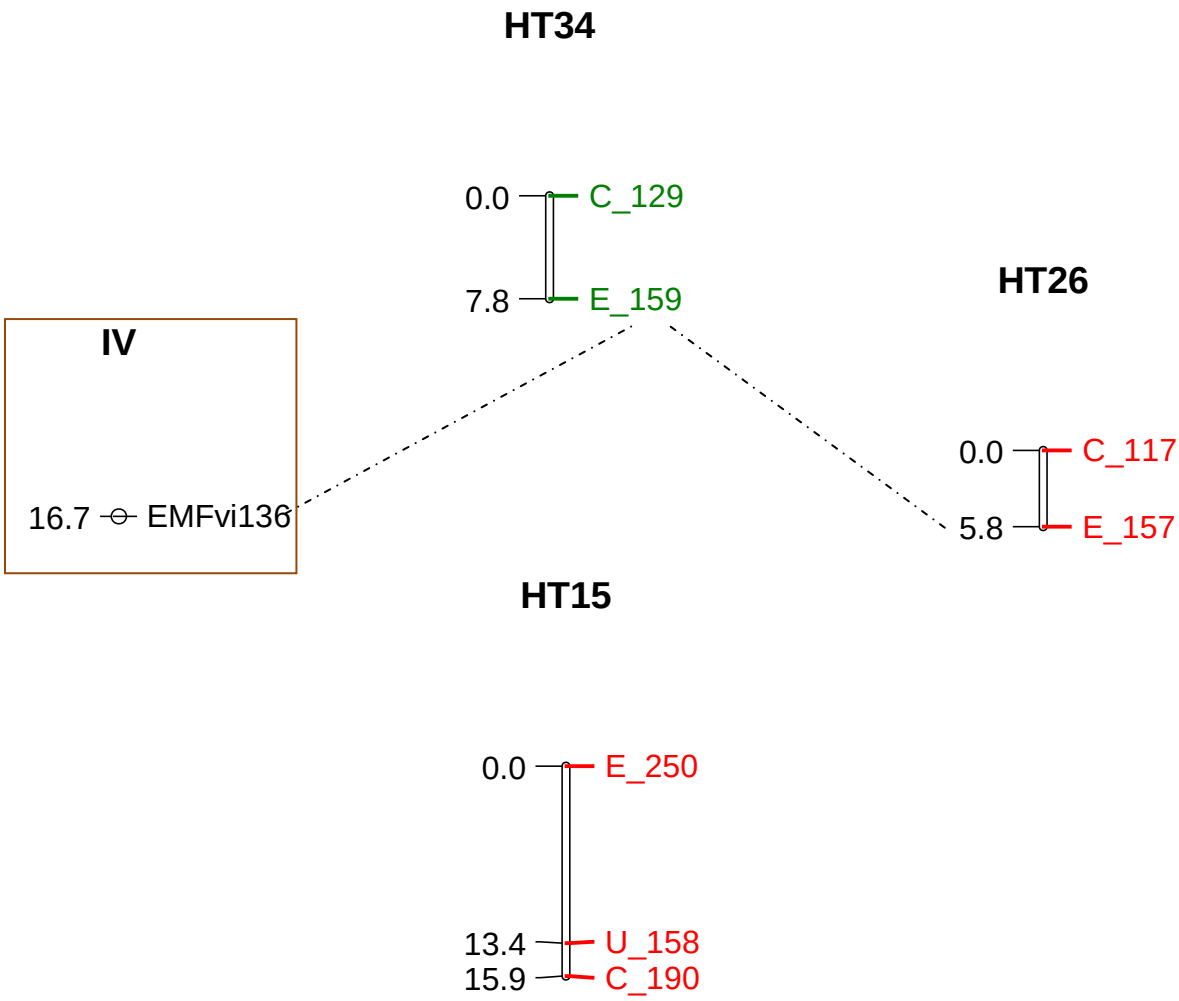


Figure 3.5 (cont'd)

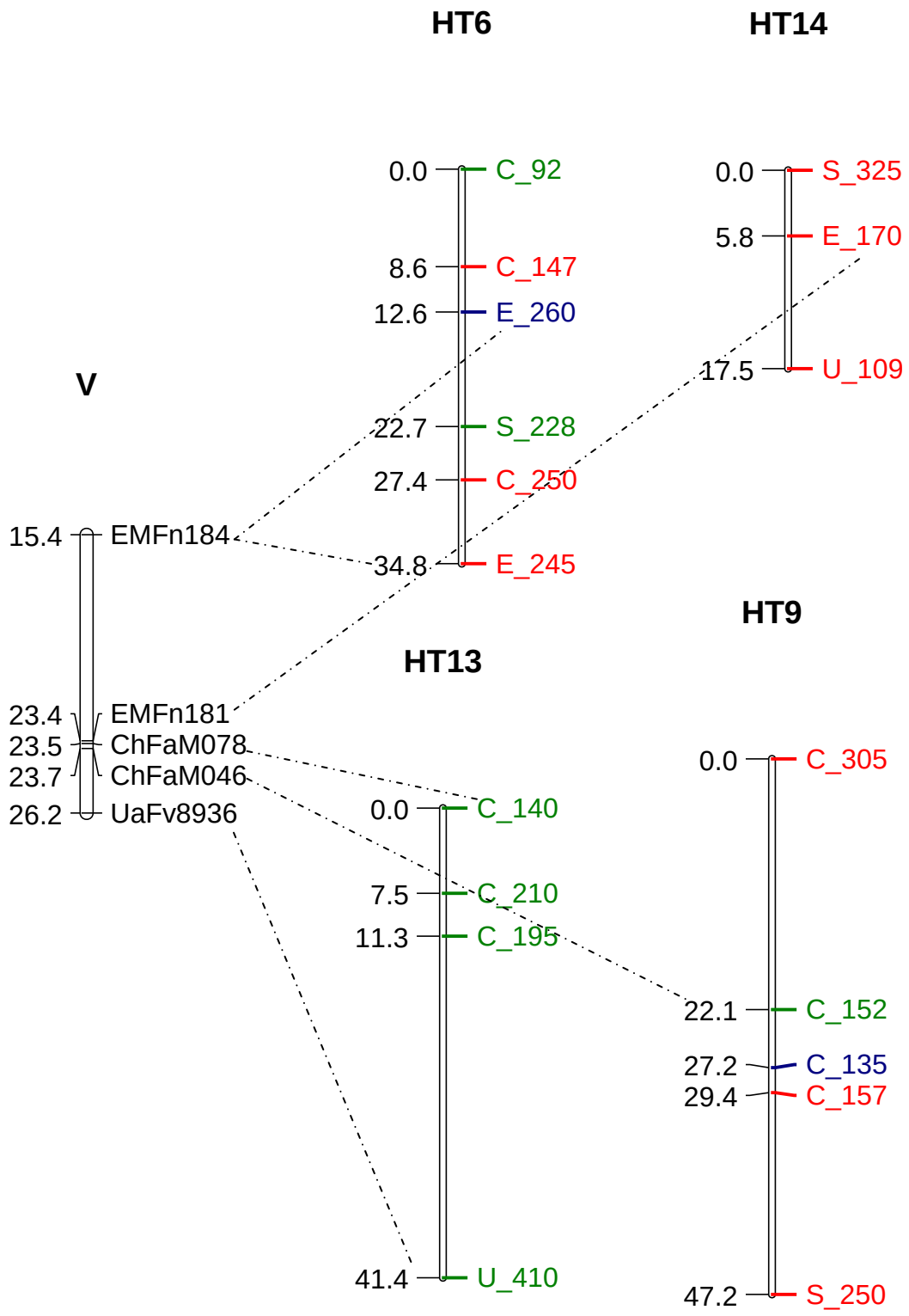


Figure 3.5 (cont' d)

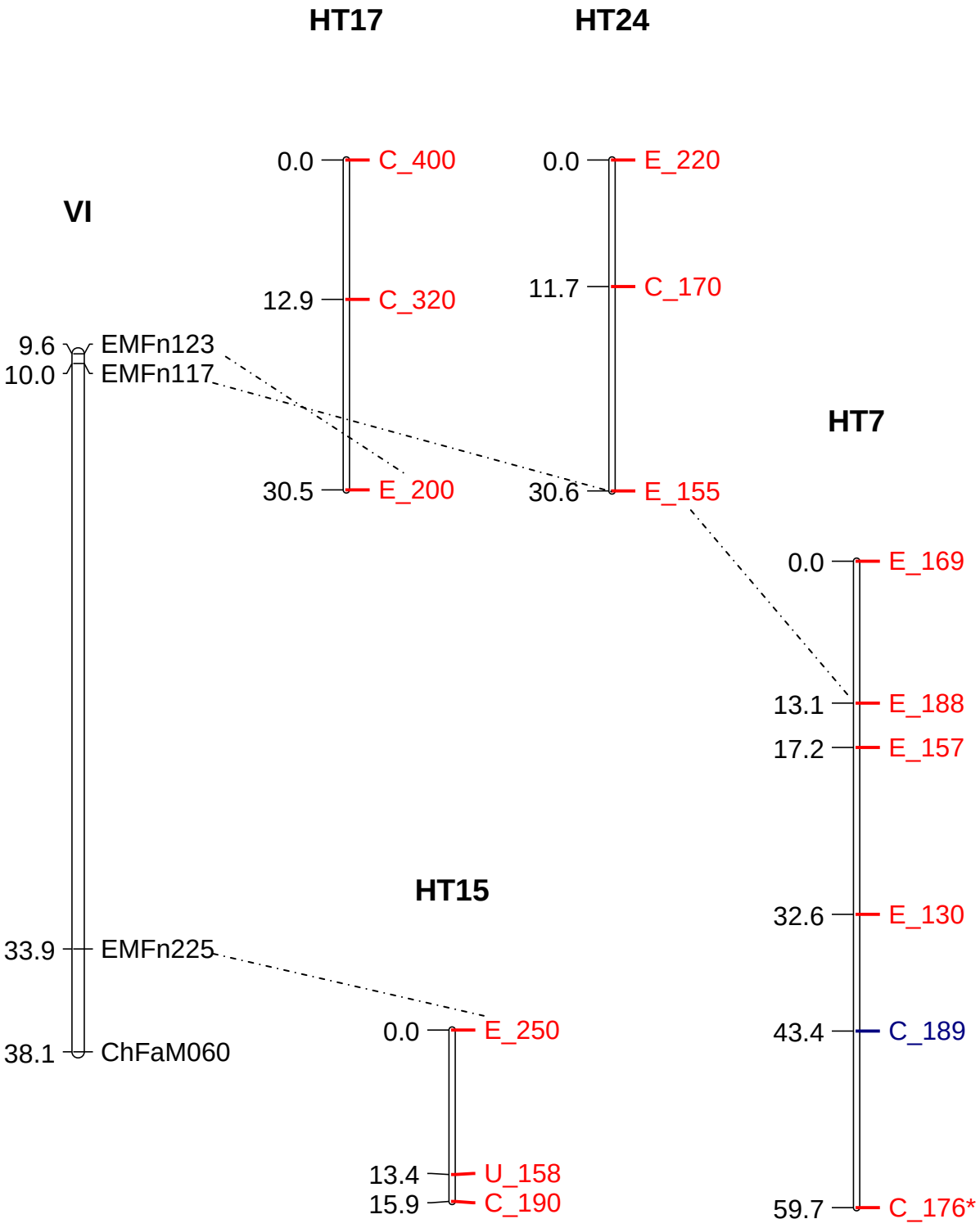
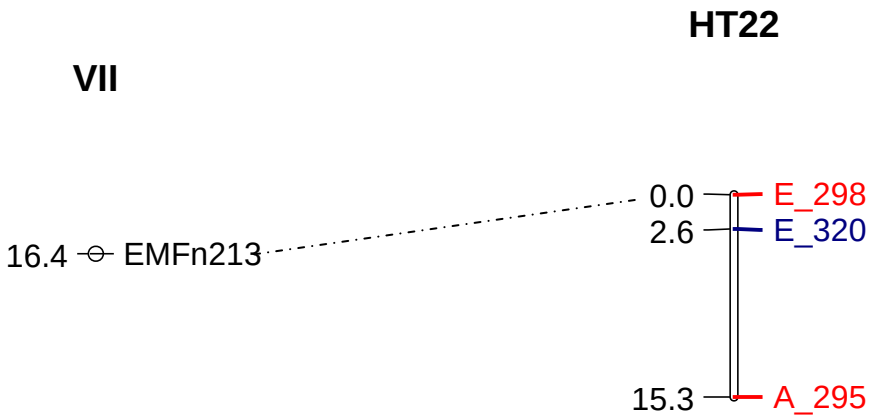


Figure 3.5 (cont'd)



Appendix 3.6

Table 3.8 QTL regions associated with remontancy (rem) in MI, OR, CA, MN, and MD in 2005, 2006, and 2011 in 'Honeoye' × 'Tribute' population. The position of the highest peak is represented where the significant regions were spread over a range. Only regions with significant LOD values are represented. Significant LOD value at $p \leq 0.05$ was determined from 1000 permutations with the dataset.

Trait	Group	Position	Nearest Locus	Locus position	% Expl.
rem MI2005	2	6	EMFn152_148	0	54.4
	2	61.7	ChFaM072_385	61.7	54.4
	5	2	ChFaM103_450	0	54.4
	5	13	UAFv8216_182/185	17.5	54.4
	6	7	ChFaM017_147	8.6	54.4
	7	5	EMFn198_169	0	54.4
	7	15.1	EMFn117_188	13.1	54.4
	7	26.2	EMFvi104_130	32.6	54.4
	16	0	ChFaM147_210	0	54.4
	16	20	FAC006_225	31	54.4
	19	16	UDF004_143	28.2	54.4
	19	32.2	ChFaM080_220	35.2	54.4
	32	5	EMFn115-140	0	63.9
	34	4	ChFam011_129	0	17
rem MI2006	3	0	ChFaM088_106	0	53.6
	6	4	ChFaM017_92	0	53.6
	7	7	EMFn117_188	13.1	53.6
	16	0	ChFaM147_210	0	53.6
	16	15	EMFn117_157	17.2	53.6
	32	0	EMFn115-140	0	63.7
	32	14	ChFaM081_110	14.6	63.7
	34	7	EMFvi136_159	7.8	10.1
rem MI2011	1	16.5	UDF004_136	15.5	51.3
	1	26.5	ChFaM129_500/190	30.7	51.3
	1	37.6	ChFaM040_315	37.9	51.3

Table 3.8 (cont'd)

Trait	Group	Position	Nearest Locus	Locus position	% Expl.
	1	51.2	EMFvi104_80	52.1	51.3
	2	31	ChFam032_205	27	51.3
	3	2	ChFaM088_106	0	51.3
	3	33.9	UAFv8216_101	32.9	51.3
	5	27.6	ChFaM088_300	32.3	51.3
	6	29.4	ChFaM017_250	27.4	48.8
	7	4	EMFn198_169	0	51.3
	7	18.2	EMFn117_157	17.2	51.3
	7	28.2	EMFvi104_130	32.6	51.3
	7	38.6	ChFaM111_189	43.4	51.3
	7	55.4	ChFaM111_176	59.7	51.3
	10	14.7	ChFaM003_400	15.9	51.3
	13	12.3	ChFaM111_195	11.3	51.3
	22	4.6	EMFn213_320	2.6	51.3
	30	3.9	Uffa20H10_247	3.9	51.3
	34	2	ChFam011_129	0	39.7
rem OR2005	1	4	ARSFL8_301	0	54.6
	1	18.5	UDF004_136	15.5	54.6
	1	42.4	ARSFL98_205	44.8	54.6
	5	5	ChFaM103_450	0	54.6
	6	3	ChFaM017_92	0	46.1
	6	31.4	EMFn184_245	34.8	36.4
	7	5	EMFn198_169	0	54.6
	7	15.1	EMFn117_188	13.1	54.6
	9	42.4	SF5G02_250	47.2	30.9
	19	8	ChFaM040_143	0	63
	22	15.3	ARSFL19_295	15.3	54.6
	30	2	ChFaM076_139	0	54.6
	32	5	EMFn115-140	0	54.6
	34	0	ChFam011_129	0	14.1
rem OR2011	1	13.3	ChFaM098_225	13.3	55.2
	1	44.8	ARSFL98_205	44.8	56
	2	23.1	ChFam032_205	27.3	56
	2	54.9	ChFaM061_220	54.9	56
	3	0	ChFaM088_106	0	56

Table 3.8 (cont'd)

Trait	Group	Position	Nearest Locus	Locus position	% Expl.
	3	23.4	ChFaM004_132	23.4	56
	4	16.7	ChFaM101_139	17.3	56
	5	1	ChFaM103_450	0	56
	6	8.6	ChFaM017_147	8.6	56
	6	18.6	SF5G02_228	22.7	47.9
	6	32.4	EMFn184_245	34.8	56
	7	4	EMFn198_169	0	56
	7	19.2	EMFn117_157	17.2	56
	9	47.2	SF5G02_250	47.2	56
	10	2	ChFaM003_495	0	56
	10	24.9	ChFaM076_101	25.4	56
	16	0	ChFaM147_210	0	56
	18	2	ChFaM018_450	0	56
	18	22.5	ChFaM018_460	22.5	56
	30	2	ChFaM076_139	0	45.4
	34	7.8	EMFvi136_159	7.8	69.7
rem CA2005	1	4	ARSFL8_301	0	54.6
	1	14.3	UDF004_136	15.5	54.6
	1	41.4	ChFaM040_95	38.4	54.6
	5	5	ChFaM103_450	0	54.6
	6	3	ChFaM017_92	0	46.1
	7	2	EMFn198_169	0	54.6
	7	15.1	EMFn117_188	13.1	54.6
	19	8	ChFaM040_143	0	63
	23	15.3	ARSFL19_295	15.3	54.6
	30	2	Uffa20H10_247	3.9	54.6
	32	4	EMFn115-140	0	54.6
	34	0	ChFam011_129	0	14.1
rem MN2005	2	62.7	ChFaM072_385	61.7	56.6
	5	46.4	EMFn160_161	44.4	56.6
	6	0	ChFaM017_92	0	56.6
	7	2	EMFn198_169	0	56.6
	7	16.1	EMFn117_157	17.2	56.6
	7	26.2	EMFvi104_130	32.6	56.6
	9	2	ChFaM022_305/225	0	35.9

Table 3.8 (cont'd)

Trait	Group	Position	Nearest Locus	Locus position	% Expl.
	16	1	ChFaM147_210	0	56.6
	16	19	FAC006_225	31	56.6
	19	23	UDF004_143	28.2	56.6
	32	8	ChFaM081_110	14.6	62.3
	34	3	ChFam011_129	0	15.1
rem MD2005	5	5	ChFaM103_450	0	47.7
	5	20.5	UAFv8216_182/185	17.5	47.7
	6	3	ChFaM017_92	0	47.7
	7	3	EMFn198_169	0	47.7
	7	16.1	EMFn117_157	17.2	47.7
	7	26.2	EMFvi104_130	32.6	47.7
	9	0	ChFaM022_305/225	0	29.2
	10	3	ChFaM003_495	0	47.7
	10	15.7	ChFaM003_400	15.9	47.7
	16	5	ChFaM147_210	0	47.7
	16	19	FAC006_225	31	47.7
	19	10	ChFaM040_143	0	47.7
	19	23	UDF004_143	28.2	47.7
	32	5	EMFn115-140	0	57.2
	34	6	EMFvi136_159	7.8	65.5

Appendix 3.7

Table 3.9 QTL regions associated with weeks of flowering in MI, OR, and CA in 2005, 2006, and 2011 in 'Honeoye' × 'Tribute' population. The position of the highest peak is represented where the significant regions were spread over a range. Only regions with significant LOD values are represented. Significant LOD value at $p \leq 0.05$ was determined from 1000 permutations with the dataset.

Trait	Group	Position	Nearest Locus	Locus position	% Expl.
Weeks-MI2005	3	24.4	ChFaM004_132	23.4	20
Weeks-MI2006	1	37.9	ChFaM040_315	37.9	24.4
	2	11	EMFn230_225	17.1	65.6
	3	2	ChFaM088_106	0	24
	3	9	EMFn134_214	12.4	24
	5	3	ChFaM103_450	0	24.3
	6	5	ChFaM017_147	8.6	15.6
	6	13.6	EMFn184_260	12.6	25
	6	28.4	ChFaM017_250	27.4	24.4
	7	5	EMFn198_169	0	25
	7	25.2	EMFn117_157	17.2	25
	9	8	ChFaM022_305/225	0	24.7
	9	22.1	ChFaM046_152	22.1	20.3
	9	42.4	SF5G02_250	47.2	23.9
	10	3	ChFaM003_495	0	25
	13	41.3	UFFa03B05_214/175	43.7	24.7
	26	2	ChFam011_117	0	36.4
	32	0	EMFn115-140	0	24.7
	32	9	ChFaM081_110	14.6	25
Weeks MI2011	1	16.5	UDF004_136	15.5	45.1
	2	28	ChFam032_205	27	45.6
	5	6	ChFaM103_450	0	43.8
	6	0	ChFaM017_92	0	35.9
	6	34.8	EMFn184_245	34.8	15.8
	7	3	EMFn198_169	0	47

Table 3.9 (cont'd)

Trait	Group	Position	Nearest Locus	Locus position	% Expl.
	7	11	EMFn117_188	13.1	46.4
	9	9	ChFaM022_305/225	0	39.9
	9	27.2	ChFaM046_135	27.2	42.2
	9	42.4	SF5G02_250	47.2	43.5
	10	31.4	ChFaM151_228/113	32	45
	20	2	EMFn170_208	0	35.7
	22	13.6	ARSFL19_295	15.3	39.4
	29	3	ChFaM107_192	5.9	37.6
	34	5	EMFvi136_159	7.8	59.6
Weeks OR2005	5	10	UAFv8216_182/185	17.5	28.4
	5	20.5	UAFv8216_161	23.6	28.7
	19	14	ChFaM040_143	0	49.8
	19	27	UDF004_143	28.2	61.1
Weeks CA2005	1	8	ChFaM098_225	13.3	54.5
	1	29.5	ChFaM129_500/190	30.7	29.9
	2	1	EMFn152_148	0	30.8
	2	67.7	ChFaM072_350	68.1	30
	3	2	ChFaM088_106	0	31.1
	3	25.4	ChFaM004_132	23.4	31.8
	4	23.5	ChFaM101_153	22.4	32.6
	5	49.4	EMFn160_160	50.8	32.8
	6	12.6	EMFn184_260	12.6	30.2
	7	5	EMFn198_169	0	44.5
	7	58.4	ChFaM111_176	59.7	31.3
	8	35.3	Uffa11A11_206/260	35.3	30.2
	9	41.4	SF5G02_250	47.2	32.2
	10	30.4	ChFaM151_228/113	32	23.9
	12	2	EMFn134_185	0	29.5
	13	7.5	ChFaM111_210	7.5	28.7
	13	23.3	ChFaM111_195	11.3	31.3
	15	7	UFFa04G04_158	13.4	29.9
	16	30	FAC006_225	31	32.2
	18	1	ChFaM018_450	0	29.4
	20	7	EMFn170_208	0	23
	20	33.1	ChFaM098_226	34	29.1

Table 3.9 (cont'd)

Trait	Group	Position	Nearest Locus	Locus position	% Expl.
	21	24.2	SF5C08_410	24.2	30.7
	22	13.6	ARSFL19_295	15.3	32.6
	26	0	ChFam011_117	0	26.8
	28	8	ChFaM098_165	15.3	27.3
	29	4	ChFaM107_192	5.9	30.4
	32	3	EMFn115-140	0	31.7
Weeks OR2011	1	18.5	UDF004_136	15.5	32.2
	1	31.6	ChFaM080_219	31.6	31.4
	2	1	EMFn152_148	0	32
	2	23.1	ChFam032_205	27	32.2
	2	57.9	ChFaM061_220	54.9	30.6
	3	2	ChFaM088_106	0	32
	3	24.4	ChFaM004_132	23.4	29.1
	4	0	ChFaM063_106	0	31.7
	4	11.7	ChFaM101_137	10.7	31.1
	5	21.5	UAFv8216_161	23.6	31.6
	5	37.3	EMFn134_160	37.8	14.8
	5	50.8	EMFn160_160	50.8	23.1
	7	3	EMFn198_169	0	32.2
	7	30.2	EMFvi104_130	32.1	32.2
	7	59.7	ChFaM111_176	59.7	35.8
	9	17	ChFaM046_152	22.1	30.5
	9	26.1	ChFaM046_135	27.2	30.8
	15	4	EMFn225_250	0	16.1
	16	8	ChFaM147_210	0	32
	19	3	ChFaM040_143	0	30.7
	19	32.2	ChFaM080_220	35.5	29.5
	22	9.6	ARSFL19_295	15.3	30.1
	34	4	EMFvi136_159	7.8	24.3

Appendix 3.8

Table 3.10 QTL regions associated with flowering at 17°C, 20°C, and 23°C in 'Honeoye' × 'Tribute' population. in 'Honeoye' × 'Tribute' population. The position of the highest peak is represented where the significant regions were spread over a range. Only regions with significant LOD values are represented. Significant LOD value at $p \leq 0.05$ was determined from 1000 permutations with the dataset.

Trait	Group	Position	Nearest Locus	Locus position	% Expl.
Total Flowers at 17°C	1	23.5	ChFaM129_500/190	30.7	47.3
	2	4	EMFn152_148	0	45.9
	2	17.1	EMFn230_225	17.1	46.6
	2	24.1	ChFam032_205	27	46.9
	2	33	UFFa16H07_262	36.8	46.6
	2	63.7	ChFaM072_385	61.7	45.2
	3	14.4	EMFn134_214	12.4	44.7
	7	4	EMFn198_169	0	46.1
	9	39.4	SF5G02_250	47.2	41.4
	10	20.9	ChFaM076_101	25.4	44.5
	10	30.4	ChFaM151_228/113	32	44.7
	16	14	ChFaM147_210	0	40.4
	16	24	FAC006_225	31	47.5
	19	18	UDF004_143	28.2	45
	19	35.2	ChFaM080_220	35.2	43.3
	20	18.1	ChFaM094_120	18.1	14.5
	30	2	Uffa20H10_247	3.9	40.2
	34	5	ChFam011_129	0	31.9
Total Flowers at 20°C	1	25.5	ChFaM129_500/190	30.7	43
	2	6	EMFn152_148	0	42.5
	3	5	ChFaM088_106	0	44
	3	25.4	ChFaM004_132	23.4	39.2
	5	0	ChFaM103_450	0	33.4
	7	30.2	EMFvi104_130	32.6	42.3

Table 3.10 (cont'd)

Trait	Group	Position	Nearest Locus	Locus position	% Expl.
	7	50.4	ChFaM111_189	43.4	43.9
	9	45.4	SF5G02_250	47.2	44.1
	16	14	ChFaM147_210	0	45
	30	3.9	Uffa20H10_247	3.9	37.3
	34	4	ChFam011_129	0	32.4
Total Flowers at 23°C	1	5	ARSFL8_301	0	49.1
	4	0	ChFaM063_106	0	46.6
	7	15.1	EMFn117_157	17.2	48.8
	19	12	ChFaM040_143	0	47.7
	19	29.2	UDF004_143	28.2	47.1
	20	6	EMFn170_208	0	12.2
	20	18.1	ChFaM094_120	18.1	19.7
	22	14.6	ARSFL19_295	15.3	47.4
	30	2	Uffa20H10_247	3.9	47.1
	34	6	ChFam011_129	0	50.3

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CHAPTER 5

CONCLUSIONS AND FUTURE RESEARCH

The goal of this research was to measure the interaction between heat tolerance and photoperiod sensitivity in regulating flowering in the octoploid strawberry. Previous research had shown that photoperiodic response of strawberry genotypes is modified by the ambient temperature (Darrow, 1936; Durner *et al*., 1984; Serçe and Hancock, 2005a; Weebadde *et al*., 2008; Sonsteby and Heide, 2008; Bradford *et al*., 2010). This research demonstrated that a population segregating for remontancy also segregated for heat tolerance/sensitivity. Flower initiation in plants growing under unfavorable photoperiod (long days) depended on their relative temperature (17°C, 20°C, 23°C) tolerance. Although both the parents ('Honeoye' and 'Tribute') were heat sensitive and had fewer flowers at 23°C than at 17°C, the progeny included both heat tolerant and sensitive responses. The extent of heat tolerance varied among the progeny. In addition, almost all heat tolerant genotypes had very few runners at high temperatures. Bradford *et al*. (2010) previously concluded that runner formation was favored by high temperatures and long photoperiod; however, they studied three genotypes ('Honeoye', Tribute', and RH30) that had heat sensitive floral responses.

All the heat tolerant progeny had the remontant phenotype when grown under field conditions in MI, and most were remontant in OR. This suggests that heat tolerance is required for repeat flowering in the Midwestern US during the warm summer. This hypothesis is supported by the previous work of Weebadde *et al*. (2008) who observed a higher percentage of remontant progeny in the cooler western states (CA and OR) than in the warmer eastern states (MI, MD, MN). Only one genotype (HT43) had a heat tolerant floral response in the greenhouse, remontant phenotype in the field, and produced a significant number of runners. In a clonally propagated crop like strawberry, runner production is an important trait and remontant genotypes typically do not produce many runners making it necessary to use labor intensive methods of

micropropagation or crown separation. Therefore, the genotype HT43 combines two desirable traits: heat tolerance and ability to propagate vegetatively.

An octoploid linkage map for 'Honeoye' x 'Tribute' was developed using SSR markers. The SSR markers selected were derived from diploid and octoploid *Fragaria* species (*F.* x *ananassa*, *F. vesca*, *F. viridis*, and *F. nubicola*), and a large majority of the mapped SSRs were generated from ESTs. Although the map was not as dense as other recently published SSR maps (Zorilla-Fonatessi *et al.* 2011; Sargent *et al.*, 2011), it was the first one that targeted a population segregating for remontancy, and therefore provided a framework for identifying QTL associated with remontancy and heat tolerance.

Phenotypic observations taken on the population growing in three temperature conditions in a greenhouse were used to identify regions in the genome that are associated with flowering at higher temperatures (23°C). In addition, remontancy vs. non-remontancy was assessed in the same progeny previously grown at 5 locations (MI, MN, MD, OR, CA) in 2005, at MI in 2006, and at MI and OR in 2011 to identify QTL associated with remontancy. Availability of phenotypic observations from multiple environmental conditions and multiple regions ensured that stable loci would be identified. Locations of remontancy QTL were compared with the heat tolerance QTL to identify whether the remontancy phenotype is associated with heat tolerance. QTL for flowering at 23°C (heat tolerance) were identified on 8 linkage groups and out of these, the QTL on groups HT7 and HT19 overlapped with the remontancy QTL for all 5 states. In addition, remontancy QTL from different environmental conditions and years overlapped on several linkage groups, and some QTL specific to eastern and western states were identified. This observation was similar to Weebadde *et al* (2008) who reported that some QTL are specific

to geographical locations and some are common to multiple environments. However, this study identified many more QTL than Weebadde *et al.* (2008) using the same phenotypic data. Numerous other inheritance studies have also suggested that the remontant trait is under complex, multigenic control (Barritt *et al.* 1982; Shaw, 2003; Serçe and Hancock, 2005b; Shaw and Famula, 2005).

The small population sizes used in our study may have caused statistical artifacts that resulted in the identification of spurious QTL in individual experiments (Beavis, 1998; Xu, 2003; Holland, 2007). In addition, quantitative traits are known to be greatly affected by environmental conditions (Patterson *et al.*, 2003; Collard *et al.*, 2005; Kenis *et al.*, 2008). We used fifteen different phenotypic data sets from replicated populations growing under multiple environmental conditions in the field and in the greenhouse to identify the most robust QTL. In addition, we developed an independent population from the same parents used by Weebadde *et al.* (2008) and identified QTL using phenotypic data from both the populations to confirm our data and identify robust chromosomal regions associated with our trait of interest. This is the approach recommended by many authors including Lander and Kruglyak (1995) and Pelgas *et al.* (2011).

The phenotypes associated with the markers flanking the heat tolerant QTL were compared to see if the phenotypic data supported the hypothesis that the presence of these markers resulted in the heat tolerant floral response. Five markers were identified where the presence of the allele produced the heat tolerant floral response; however, flower numbers associated with only one of these (EMFn170_208) was significant. Since there was considerable variance in flower number with many genotypes producing no flowers at all, all 5 of these markers should be tested using larger population sizes to determine how robust they are for marker-assisted selection.

Flower initiation in plants is a complex process that has been shown to involve many induction pathways (photoperiodic, circadian, autonomous, developmental, ambient temperature, vernalization) with multiple genes functional in every pathway in both dicots (*Arabidopsis*) and monocots (rice, wheat, barley) (Boss *et al.*, 2004; Henderson and Dean, 2004; Greenup *et al.*, 2009). Although homologs of several *Arabidopsis* flowering genes have been identified in strawberry (Mouhu *et al.*, 2009), the process of flower induction and differentiation in perennial plants is complicated by extended juvenility, dormancy, and repeated reversion of shoot apical meristem from floral to vegetative states (Albani and Coupland 2010). Homologs of the *Arabidopsis* genes such as *LFY*, *AP1*, *TFL1*, *FT* have been identified in perennial crops like apple (Hattasch *et al.*, 2008), poplar (Igasaki *et al.*, 2008), grape (Carmona *et al.*, 2007), and citrus (Nishikawa *et al.*, 2010). However, in most cases, there are multiple homologs of the *Arabidopsis* gene that have additional divergent functions in flower development (Hattasch *et al.* 2008; Mimida *et al.*, 2009). These findings suggest that there might be multiple homologs of the *Arabidopsis* flowering genes in the octoploid strawberry. The complexity of the process and the numerous loci involved in the process of flower initiation is probably reflected in the fact that QTL associated with remontancy were identified in multiple linkage groups in the 'Honeoye' × 'Tribute' population.

The immediate next step in this project should be validating the markers associated with heat tolerance on a wider panel of heat tolerant/sensitive and remontant/non-remontant genotypes. Once the markers are identified as tightly linked to heat tolerance, they may be developed for use in capillary electrophoresis for high throughput genotyping. In addition, more tightly linked markers should be added to those linkage groups that did not have dense marker coverage but had significant heat tolerance and remontancy QTL. The existing diploid and octoploid SSR

maps provide an excellent resource for selecting markers that are likely to map in the targeted linkage groups. The availability of the *F. vesca* genome sequence (Shulaev *et al.*, 2011) provides an additional resource for designing markers for specific homeologous groups. Mouhu *et al.* (2009) identified several homologs of *Arabidopsis* flowering genes in strawberry. SSR markers derived from these EST sequences can be mapped to the 'Honeoye' × 'Tribute' linkage map to see if they collocate with the remontancy or heat QTL.

The marker-trait associations may also be tested in populations derived from other remontant genotypes to determine whether they share the same QTL, and to identify new QTL of interest that can be pooled together when developing remontant cultivars. Several populations derived from remontant genotypes ('Seascape', RH30, and 'Fort Laramie') are available and have been evaluated for remontant vs non remontant phenotype in MI and OR in 2011. These populations would be the ideal for screening the marker-trait associations. The populations include: 'Earliglow' × 'Seascape', 'Seascape' × 'Honoeye', 'Seascape' × MSU56, MSU49 × 'Seascape', MSU56 × RH30, 'Earliglow' × RH30, MSU49 × RH30, 'Honeoye' × RH30, 'Fort Laramie' × MSU49, 'Fort Laramie' × Earliglow, 'Fort Laramie' × 'Honeoeye' and 'Fort Laramie' × MSU56.

Fruit quality is the most important trait when developing a horticultural fruit crop. The 'Honeoye' × 'Tribute' population has been evaluated for fruit quality traits in 2011 and repeat phenotypic observations will be collected over the next two years. The SSR linkage map developed in this study may be used to identify fruit quality QTL in this population and can be compared with the fruit quality QTL identified in *F.* × *ananassa* selection lines '232' and '1392'

(Zorilla-Fonatesi *et al*. 2011). In addition, the fruit quality assessments will be useful in selecting the heat tolerant, remontant genotypes for cultivar development.

In conclusion, the results provided here demonstrate that seasonal patterns of flowering in strawberry may be more strongly regulated by temperature than photoperiod. In cultivars that have historically been considered short day plants, mid-summer temperatures may actually be more important in regulating mid-summer flowering than photoperiod. In this study, remontant genotypes had varied levels of heat tolerance. However, all the genotypes that were remontant in the warmer midwestern environment were heat tolerant, indicating that heat tolerance is an important attribute for a plant to continue flowering. Five alleles associated with heat tolerance were identified. These alleles are potential candidates for validation on a larger panel and subsequent use in marker-assisted breeding.

Availability of tightly linked markers to important phenotypic traits will allow the strawberry breeders to take advantage of the benefits of marker assisted breeding (Collard *et al.*, 2005). All breeding programs are limited by the amount of land available for phenotypic evaluations of crosses. Since heat tolerance is a primary trait necessary for strawberry cultivars developed for the midwestern market, the ability to use markers to screen seedlings for heat tolerance will make selection much more efficient. Seedlings could be screened for heat tolerance before they are field planted, allowing the breeders to use the available land for phenotypic evaluation of only the heat tolerant progeny. The markers could also be used in parent selection to identify non-remontant genotypes that are carrying some QTL for remontancy but do not flower in mid- to late summer because they are heat sensitive. In addition, availability of markers will allow breeders to reliably select for a trait that is affected by environmental conditions. The heat

tolerant progeny from ‘Honeoye’ × ‘Tribute’ will likely be used to introduce heat tolerance in future crosses, and markers associated for heat tolerance have immediate application in marker-assisted breeding (after validation).

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