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**AFLP MAPPING AND QTL IDENTIFICATION FOR DAY NEUTRALITY IN THE OCTOPLOID STRAWBERRY (*Fragaria* x *ananassa*)**

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# AFLP MAPPING AND QTL IDENTIFICATION FOR DAY NEUTRALITY IN THE OCTOPLOID STRAWBERRY (*Fragaria* x *ananassa*)

**By**

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## ABSTRACT

AFLP MAPPING AND QTL IDENTIFICATION FOR DAY NEUTRALITY IN THE OCTOPLOID STRAWBERRY (*Fragaria* x *ananassa*)

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Day-neutrality in strawberry is a highly desirable trait to increase productivity. Day neutral (DN) cultivars developed in California have not been successful in continental regions of the US, because they are poorly adapted to high summer temperatures. Studies on the inheritance of this trait are also contradictory and support a number of models from single gene to quantitative inheritance. A linkage mapping approach was used to determine if day neutrality is qualitatively or quantitatively inherited. Amplified Fragment Length Polymorphic (AFLP) markers were used to build a genetic linkage map from a segregating population created from a cross between the DN cultivar, 'Tribute' and the short day (SD) cultivar, 'Honeoye'. One hundred and twenty seven progeny were genotyped and 387 single dose restriction fragments (SDRFs) were mapped to obtain a consensus map of 1310.7 cM with 42 linkage groups and an average marker density of 0.3 markers/cM. Individuals of the mapping population were observed for their flowering habit throughout the growing season in Michigan (MI), Minnesota (MN), Maryland (MD), Oregon (OR) and California (CA), and quantitative trait loci (QTL) for day-neutrality were identified from the phenotypic data collected at each of the locations. A number of QTL were identified which were either shared or were location specific, but none of these QTL explained over 40% of the phenotypic variation, indicating that

inheritance of day-neutrality is not regulated by a single, major gene. Of the QTL identified for the eastern states (MI, MN, MD), one on linkage group 17 was found in all eastern states, suggesting that a gene or genes regulating day neutrality is found in this linkage group. However, only one significant QTL was identified in the two western states (OR and CA) and it was located on another linkage group (LG 7) in California. These results suggest that different genes may be responsible for regulating day-neutrality in eastern and western climates. The western climates of OR and CA, have much milder temperatures than the eastern ones of MI, MN and MD during the production season and generated higher percentages of DN progeny. This could be one reason why DN cultivars developed in California do not perform as well in the eastern climates.

I dedicate this work to three wonderful people in my life,

my parents and my husband,

for their undying support and encouragement towards my success.

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# LITERATURE REVIEW

# Introduction

Most cultivars of the strawberry, *Fragaria* x *ananassa* are classified as one of two types: day neutral (DN) or short day (SD). Wild type SD plants (also known as June-bearers) are facultative and initiate flower buds either under short day conditions (less than 14hrs of day length) or when temperatures drop below $15^0$C (Darrow, 1936). The DN genotypes are photoperiod-insensitive and produce flower buds approximately three months after planting regardless of day length (Hancock, 1999). The DN genotypes also produce floral meristems cyclically throughout the growing season, although the production of floral meristems is inhibited at high temperatures. There are also long day (LD) strawberry cultivars which flower during the middle months of summer, but these are rarely grown commercially and none have been released in the last 50 years.

*Fragaria* x *ananassa* is an autoallopolyploid with a chromosome number of 8x = 2n = 56. It was created by an accidental hybridization in the 1700s between *Fragaria virginiana* and *Fragaria chiloensis* in a French botanical garden. There is little firm evidence available as to how the octoploid species themselves arose, although most investigators believe that the diploid strawberry species *Fragaria vesca* was a progenitor. Photoperiod insensitivity is also observed in *F. vesca*, and is governed by a single recessive gene at the *SEASONAL FLOWERING LOCUS (SFL)* (Brown and Wareing, 1965; Cekic et.al., 2001; Albani et.al., 2004). As will be more fully described later, photoperiod insensitivity in the octoploid appears to be controlled by more than one gene

and is at least partially under dominant control (Clark, 1937; Powers, 1954; Ourecky and Slate, 1967; Ahmadi et.al., 1990, Sugimoto et.al., 2005).

About 25 percent of the world strawberry production is supplied by the United States (US), of which nearly 80 percent comes from California, due in a large part to their higher output per acre and their intense cultural systems (D. Bertelsen, 1995). Cultural practices such as an annual planting system that use soil fumigation to eradicate pests and the use of new and higher yielding DN varieties with an extended harvest season, have contributed immensely toward California's dominance. Over 60 % of the Californian strawberry production areas use DN cultivars (Hancock, 1999). Nursery plants are set out each year in California in a winter (October-November) or summer planting system (late July- September) and are replaced the following year. The plants are grown in clear polythene mulch that warms the soil and stimulates early plant growth, giving California a 12 month growing season, compared to about a 5 month growing season in other strawberry growing states in the US (D. Bertelsen, 1995). In the Northwestern, Midwestern and Eastern States of the US strawberries are grown as a perennial crop with harvesting carried over 4 - 5 years after planting.

Day-neutrality was incorporated into modern commercial strawberry cultivars (*Fragaria* x *ananassa*) by Bringhurst and Voth (1984), using a native genotype of *Fragaria virginiana* ssp. *glauca* from the Wasatch Mountains of Utah. Compared to the traditional short-day (SD) cultivars that flower only once a year in spring, photoperiod-insensitive DN cultivars flower in any season three months after planting and cyclically thereafter

throughout the growing season. When Californian DN genotypes are grown in continental climates of northern America, their cyclic flowering habit is hindered due to summer heat. DN plants either remain vegetative failing to produce floral meristems at 30/26 $^{0}$C day/night temperatures (Galletta et al., 1981; Durner et al., 1984), or if they do flower during the middle months of summer, they have reduced yields and small, soft fruits (Draper et al., 1981) with little economic value. Efforts have not been successful to develop commercially acceptable DN cultivars for continental climates using the Wasatch source of day-neutrality.

The low success rate at breeding for better DN cultivars for continental climatic regions has stimulated increased interest in studying the inheritance of the trait and determining the environmental cues associated with flowering (Serçe and Hancock, 2005a). A search has also been initiated for new and stronger sources of day-neutrality for incorporation into northern cultivars (Hancock et.al., 2001a). The goals of this study were to use a map based approach to determine whether day-neutrality in octoploid strawberries is a quantitative or a qualitative trait and to identify Quantitative Trait Loci (QTL) for day-neutrality that can be used in marker assisted selection.

# Literature Review

This literature review will focus on the various studies done on genetics and physiology of flowering in strawberry and how this information is related to the dissertation research.

## Physiology of flowering in strawberry

The most important factors that determine flowering in the strawberry are temperature and photoperiod. Darrow (1936) was the first to measure the inter-relationship between temperature and photoperiod in the production of runners and flower buds of June bearing strawberries. He discovered that short days stimulate flower bud production at all temperatures, although the most favorable temperatures for flower production varied with day length. He noted that a short day period of at least 14 hrs was necessary for inducing flower buds at 21 $^0$C. He stated that photoperiods of much less than 14 hrs and temperatures lower than 16 $^0$C were most favorable for initiation of flower buds, although under these conditions flower bud initiation proceeded at a slower rate. Darrow also concluded that cultivars adapted to different geographical regions have their own characteristic temperature day-length responses. Hartman (1947a, 1947b) verified Darrow's findings by discovering that flower bud initiation in June bearing strawberries was promoted if temperatures were less than 15 $^0$C, even under long day conditions. When temperatures were at 21 $^0$C, he found that flowers were only formed during short days.

Downs (1955) compared the photoperiodic effect on flowering in June bearing as well as what he called everbearing types. He tested the effect of 11, 13, 15 and 17 hr day lengths on flowering. The June bearing types responded as expected with flowers being promoted under shorter day lengths (11 and 13 hr days) and being inhibited at longer day lengths (15 and 17 hr days). The everbearers were able to produce flower buds under all of the above day lengths, but produced fewer flower buds during short days. He did not consider temperature regulation in his studies, as all his cultivars were subjected to the same fluctuating temperatures in a greenhouse.

Ito and Saito (1962) studied the effects of temperature and photoperiod on flower initiation in a June bearing strawberry under a number of controlled temperature and photoperiod conditions. What was unique about their work was that they provided the temperature and photoperiodic treatments in a phytotron with tight control of both temperature and photoperiod, and after each treatment, transferred the plants to a continuously illuminated greenhouse held at 24 $^{0}$C. This avoided any effects that natural temperature and photoperiod variation might have had on flower formation. They found that when the temperatures were as low as 9 $^{0}$C, flowers were formed regardless of day length, while at temperatures of 17 $^{0}$C or 24 $^{0}$C, flowers were only formed during short days of 8 hrs. When they maintained plants under four different temperatures (9, 17, 24 and 30 $^{0}$C) and seven different photoperiods (0, 4, 8, 12, 16, 20 and 24 hr day lengths), they found that at 9 $^{0}$C, 10 or more cycles of 8 - 24 hr day lengths stimulated flower buds whereas at 17 $^{0}$C, flower buds were only formed after 8 or more cycles of 4 - 12 hr day lengths. Further increases in temperature to 24 $^{0}$C increased the number of cycles of 4 -

12 hr day length necessary for flowering to 10 and at temperatures of 30 $^0$C, flower buds were not formed even with 20 cycles of short day conditions. They also found that providing daily high temperatures of 24 $^0$C for 12 hrs under continuous illumination prevented flower bud formation, even if the rest of the day was held at 9 $^0$C. They concluded that for optimal flower bud formation in the strawberry, temperatures should be below 12 - 14 $^0$C and as the temperatures rise, shorter and shorter day lengths are required to induce flowering.

Durner et.al. (1984) tested temperature and night interruption (NI) effects on flower bud formation in June bearing, DN and everbearing (LD) plants. LDs and NI inhibited flowering in the June bearers at 21 $^0$C, and they concluded that flowering in the June bearers was regulated by photoperiod. On the other hand, flowering in the everbearers was enhanced by LD or NI, but was not completely inhibited by short days. The DN cultivars flowered regardless of photoperiod. It was also noted that the DN plants behaved more like LD plants when the temperatures were at or above 22 $^0$C/18 $^0$C day/night temperatures.

Nicoll and Galletta (1987) classified cultivars and selections by flower response type to see if flowering response patterns had a relationship to morphological variation. They studied plants belonging to five flowering response types, which belonged to a continuum of photoperiod and temperature response types: Strong DN plants (DNs), Weak DN plants (DNw), Older everbearers (OEB), Reverted or very weak DN plants (DNr), and June bearers (JB). As in the Durner (1984) study, they used short day (9 hr) photoperiod

conditions along with a 3 hr NI to separate June bearers and everbearers. In their growth chamber studies, the day/night temperatures were controlled at 22 $^0$C/18 $^0$C, whereas in their greenhouse studies, temperatures were varied from a minimum of 19 $^0$C to a maximum of 35 $^0$C. In both the growth chambers and the greenhouse, all the DNs, DNw and the OEB flowered at least once after NI, whereas flowering in the DNr (only 27 % flowered) and JB genotypes (none flowered) was limited by NI.

Based on their studies, Nicoll and Galletta classified cultivars into four photoperiodic classes and three agricultural classes. The four photoperiodic classes were, strong DN plants (including DNs), intermediate DN plants (including DNw and OEB), weak DN plants (including the DNr) and the JB types. The three agricultural classes were single cropping (including JB and DNr that do not produce any summer crop), intermediate everbearer (including DNw and OEB that produce both summer and fall fruits in small quantities) and strong everbearers (including DNs that produce summer and fall fruits in commercial quantities). They suggested that the terms DN and everbearer can be used interchangeably if plants are classified in this manner.

Nicoll and Galletta also observed that the strength of everbearing was linked to the growth habit of the plant and proposed three models to explain the different photoperiodic types. For strong DN plants, they suggested that there is a high inflorescence: crown ratio with few leaves intercalated between terminal inflorescences. Every new shoot terminates growth by initiation of a flower bud, resulting a smaller meristem pool. In the intermediate DN plants, the branch crowns terminate in an

inflorescence. Therefore, the plant had many crowns and leaves with a 1:1 inflorescence: crown ratio. For DNw, the main axis remains vegetative with axillary buds turning into runners. These may produce a branch crown that rarely ends up in an inflorescence during summer, although under short day conditions, the plants are able to produce inflorescences on the main axis.

Manakasem and Goodwin (2001) also studied the responses of DN and June bearing strawberries to temperature and day length. They used two day-length conditions (9 and 15 hrs) and three day/night temperature conditions (18/13, 21/16, and 30/26 $^0$C) to study the flowering response to temperature and photoperiod, and used 15/10, 18/13, 21/16, 24/19, 27/22 and 30/25 $^0$C day/night temperatures under natural daylight conditions to test the effect of temperature on flower initiation and development. As was observed in the previous studies, a significant cultivar x temperature x photoperiod interaction was observed. They observed consistent flowering in the DN plants over a wider range of temperatures and photoperiods compared to the SD cultivars. As expected, the SD types did not initiate flowers during LDs, but flowers initiated previously during SDs, continued to develop well into the LDs. In the DN plants, flower initiation occurred during both LDs and SDs, resulting in continuous flowering. A day/night temperature of 21/16 $^0$C was found to be optimum for initiation of inflorescences in the SD cultivars, although the greatest floral initiation occurred at 18/13 $^0$C. In DN cultivars, they found that the optimum day/night temperature for floral initiation depended on the cultivar and varied with photoperiod.

Serçe and Hancock (2005a) tested the effect of temperature and photoperiod on flowering and runnering of SD and DN representatives of the cultivated strawberry, *Fragaria* x *ananassa*, and its wild progenitors, the scarlet strawberry, *Fragaria virginiana,* and the beach strawberry, *Fragaria chiloensis*. When they measured the critical day length (CDL) for floral induction of SD genotypes, a quantitative SD model fit the cultivar Honeoye, where the length of the photoperiod was significantly correlated with flower number, but the other SD cultivars and wild genotypes did not show consistent trends. The wild genotype Eagle 14 (*F. virginiana*), that had previously been categorized as a SD type in the field, produced the most flowers during LDs in the growth chamber, suggesting it is actually a LD type. They also found that there was a difference in the number of flowers produced by the different DN types in response to photoperiods of 8 vs.16 hr day lengths. Some DN plants produced the same number of flowers regardless of day length (eg. Frederick 9 of *F. virginiana)*, while other genotypes produced the most flowers under LD conditions (eg. cultivar 'Tribute'), and still others produced more flowers under SD conditions (eg. RH 30, also a scarlet strawberry). This indicates that there is a continuous distribution in response to photoperiod among strawberry genotypes. Serçe and Hancock also tested the effects of temperature on flowering of DN and LD plants, in an attempt to select genotypes that can initiate flowering under high temperatures. All of the genotypes had significantly lower productivity under high (30 $^0$C) than low (18 $^0$C) temperatures, except the old cultivar Fort Laramie.

In conclusion, the studies on the physiology of flowering in the strawberry performed to date, show a clear interaction between cultivar x temperature x photoperiod, and there is a

continuum in photoperiod response from short day to intermediate to fully DN plants. Based on this range of response, it is likely that the photoperiod regulation of flowering is a complex genetic trait.

## Genetics of flowering in strawberry

So far, no clear consensus exists on the inheritance of photoperiod insensitivity in strawberry. Various attempts at studying the inheritance of this trait have resulted in several different models: 1) regulation by a single gene (Ahmadi et. al., 1990 and Sugimoto et.al., 2005); 2) regulation by two complementary genes (Clark, 1937); 3) regulation by two complementary genes with interactions (Ourecky and Slate, 1967), and 4) regulation by at least 6 dominant and recessive genes (Powers, 1954). However, it is difficult to directly compare these studies, as they used different sources of day-neutrality, dissimilar terminology to explain temperature/photoperiod interactions, grew the plants under variant environmental conditions, and had diverse selection criteria for DN plants. The older studies also considered all multiple cropping genotypes to be "everbearing", and they did not distinguish long-day flowering genotypes from DN ones that flowered under both long and short days.

Clark (1937) made the first observations on the genetics of the everbearing trait in a New Jersey breeding population. He observed different degrees of expression of the trait, with some everbearers having continuous blooming patterns, while others produced only a few flowers during summer. He found that two of his parent plants (New Jersey No.1 and

New Jersey No.2) transmitted the trait to different percentages of progeny even when they were crossed with the same individual. The phenotypic data he obtained by combining several crosses was close to a 9:7 ratio, indicating that two complementary genes may govern the everbearing trait. However, he believed that the breeding behavior of the everbearing strawberries was not uniform and interactions with many genetic and environmental factors could influence segregation patterns.

Powers (1954) studied the genetics of everbearingness in populations made by self and cross-pollination of three selected everbearers (473, 475 and 476) and seven June bearers (471, 472, 474, 477, 478, 479 and 4710). Altogether, he examined 10 self-pollinated and 45 cross-pollinated families. In the progeny populations of self pollinated 473, and the cross between 473 x 475, he obtained a 3:1 ratio of everbearers to non-everbearers which he concluded could have arisen if 473 and 475 were heterozygous for a dominant gene conditioning everbearingness. In crosses between 473 and the June bearer types 471, 472, 474, 477, 478 and 4710, he obtained a 1:1 ratio, supporting the hypothesis that 473 was heterozygous for a single dominant gene conferring everbearingness. However, he did not observe the 3:1 ratio expected if the trait was regulated by a single dominant gene in the cross between 473 and 476. When Powers combined all the progeny he obtained by selfing 475 and 476 and by crossing 475 and 476, he observed a 37.5:62.5 ratio of June bearers: everbearers suggesting 475 and 476 were heterozygous for two pairs of dominant genes conditioning the trait rather than just one. Likewise, self pollinated progenies of 475 and 476, and the cross pollinated progeny of 475 and 476 did not produce the expected 25 % non-everbearer types if both these parents were heterozygous for one pair

of genes conditioning the everbearing trait. When he crossed each of 475 and 476 with the seven June bearers, he observed that the total progeny obtained fit a 9:7 ratio of non-everbearers: everbearers. He did not calculate if each of the above populations fit the same 9:7 ratio when taken individually; however, he suspected that in his combined analyses numbers could have been balanced by individual crosses with too many or too less everbearers because none of the crosses involving 475 or 476 were homogenous. He further stated that some of the crosses involving these two parents deviated substantially from simple genetic ratios and the only way the situation could be explained was to assume that some of the non-everbearing types carry modifying genes. He also obtained some everbearers from a number of self and cross-pollinations between June-bearing types, which would not be expected if inheritance was regulated by a dominant gene.

Powers concluded that the flowering genes carried by the June bearers may have varied in their dosage response, depending on the genetic background. He suggested that the genes regulating SD in his June bearers may have been recessive to a dominant gene carried by 473, but they may not have been recessive to those genes carried by 475 and 476. From these observations, he concluded that both dominant and recessive genes conditioned the trait, and that there was an unequal contribution of dominant alleles toward the trait. He further stated that the effects of all the genes are cumulative and that there are at least 6 different pairs of genes with different dosage relationships.

Ourecky and Slate (1967) studied the segregation of the everbearing trait in progeny of nine June bearing and four everbearing genotypes that were crossed in 25 different

combinations. They observed a wide range in percent everbearing progeny produced either when the same everbearing type was crossed with a variety of June bearing types, or when the same June bearer was crossed with different everbearing types. They also found that only a few of the populations gave a 1:1 ratio of everbearing: June bearing progeny, eliminating the possibility that a single gene governs the trait. When they averaged the percent progeny obtained in the crosses involving each everbearing parent, the percentage of everbearing progeny ranged from a 30.8 to 54.5 %. They hypothesized that the trait was controlled by two dominant complementary genes with interactions. They also suggested that there could be other genes for everbearingness not included in their model, because a cross between one of their everbearing parents 'Geneva' and a June bearer produced a higher percentage of everbearers than expected.

Ahmadi et.al. (1990) suggested in a study of a California breeding population that day-neutrality in the octoploid strawberry is regulated by a dominant allele at a single locus. He drew his conclusions from crosses made between DN and SD genotypes, selfs of the hybrids and backcrosses to the short-day parents. He did not display the segregation patterns of individual crosses and his chi square analyses were done on the combination of all the crosses belonging to one type. For example, he tested whether the total of all SD/SD x DN/SD and DN/SD x SD/SD crosses would result in an expected 1:1 ratio of DN: SD progeny, all DN/SD x DN/SD crosses would result in an expected 3:1 ratio of DN: SD progeny, all SD/SD x SD/SD crosses would not result in any DN progeny, and all DN/DN crosses to either SD/SD or DN/SD would result in 100 % DN plants. Although he used a large number of progeny and his ratios support single gene control,

there is a chance that by combining several populations belonging to one type, individual crosses having too many DN plants got balanced by those that had fewer than expected, as was suggested by Powers (1954). In a more recent study done on the California breeding population using selfed progenies of 10 DN parents, Shaw (2003) found three families that did not fit an expected 3:1 ratio; two produced lower numbers than expected and one produced higher number of DN plants than expected. This suggests that day-neutrality is not controlled by a single dominant gene in the California breeding population.

Ahmadi et. al. (1990) defined day-neutrality in a number of different ways, depending on the type of population they were evaluating: 1) plants that had flowers on mothers as well as runners during summer and fall under nursery conditions, 2) plants that flowered three to five months after germination, 3) plants that were continuously flowering after being established in the field the fall of previous year and 4) classifying the parent plants from observing their progeny obtained by hybridizing with the SD *F. chiloensis* and rating the progeny as in # 2.

Single gene control for the everbearingness was recently found by Sugimoto et.al. (2005) in Japan using the DN scoring method of Ahmadi et. al. (1990) and Barrit et. al. (1982) where flowers on mother and the first runner plant were considered DN. When they selfed populations of the everbearing genotype 'Ever Berry', and the June bearer 'Toyonoka,' they found that the progeny segregated in the 3:1 and 0:1 ratios of everbearers: June bearers expected if 'Ever Berry' is heterozygous for the dominant gene

conferring the everbearingness. They also obtained the expected 1:1 ratio for the cross between the 'Ever Berry' and 'Toyonoka' and identified Randomly Amplified Polymorphic DNA (RAPD) markers linked to the everbearing gene.

Polygenic inheritance of day-neutrality was suggested by Hancock et. al. (2001b) to explain the inheritance patterns observed in crosses of SD and DN representatives of *Fragaria virginiana* from the wild with DN and SD *Fragaria* x *ananassa* cultivars. The progeny of the crosses were planted at three different locations; Michigan, Minnesota and Ontario and the plants were classified as DN if they flowered under both SD and LD conditions. A wide range in the percentage of DN progeny was observed among the different SD *F.* x *ananassa* and SD *F. virginiana* families, which is not consistent with control by a single dominant gene, particularly since most ratios deviated from a 1:1 ratio. In addition, they observed a significant difference in the expression of day-neutrality between the locations that indicated an environmental interaction. They found that their segregation ratios fit expected ratios of a single dominant gene control when the DN *F.* x *ananassa* genotypes were crossed with SD genotypes, but when many of the DN *F. virginiana* genotypes were crossed to the SD *F.* x *ananassa* genotypes the progeny populations did not fit monogenic ratios. This indicated that the two species were either under different genetic control for the trait or that the trait is regulated in both species quantitatively but as a threshold trait. To rigorously test whether multiple germplasm sources of day-neutrality exist, Hancock et al. (2001b) suggested testing segregating populations of di-allele crosses among a broad range of DN and SD genotypes with

known responses to temperature and photoperiod. They also suggested the establishment of a uniform method to characterize DN plants.

Serçe and Hancock (2003) compared the 5 different methods commonly used to identify DN plants (Ahmadi et. al., 1990; Barritt et. al., 1982) and found that the highest proportion of DN plants was detected by growing plants in the field under short and long day conditions. In other work by Serçe and Hancock (2005b), partial di-allele crosses were made between 12 selected parental genotypes of the three octoploid strawberry species to obtain 35 families of more than 50 individuals each that segregated for the day-neutrality trait. Analysis of the flowering habit revealed wide ranges in the percentage of DN progeny in various families (30 – 87 % in DN x SD crosses and 22 – 93 % in DN x DN crosses). These results suggested that day-neutrality is a quantitative trait and that there is more than one source of day-neutrality in the native strawberry populations.

In conclusion, the conventional inheritance studies that have been performed to date indicate that there is likely a small number of major genes regulating day-neutrality in strawberries, but there are numerous other genes which have an influence on the trait depending on the source of day-neutrality and the test environment. To clearly elucidate the genetics of day-neutrality in strawberries, it will be necessary to study a diverse array of the same segregating populations in several different environments (locations/year). It would also be helpful to use a map based QTL approach to study the genetics of day-neutrality, to identify how many genes are associated with day-neutrality and to calculate their relative contributions to the phenotypic variability.

The goal of this dissertation was to use such a map-based approach to identify the QTL associated with one segregating population planted in several different environments. A mapping population was selected from the previous Serçe and Hancock (2005b) study to build a genetic linkage map using Amplified Fragment Length Polymorphic (AFLP) markers. This linkage map consisted of 387 markers and 42 linkage groups. The mapping population was observed for flowering during the SDs of spring and LDs of summer to identify DN genotypes from that of SD types in Michigan in the year 2005. The same mapping population was also observed in four other locations; Minnesota, Maryland, Oregon and California, for their flowering phenotype. The phenotypic data was analyzed along with the molecular marker data to map Quantitative Trait Loci (QTL) for day-neutrality.

The results indicated that different genes may be responsible for day-neutrality in the eastern and western states, as a QTL observed in all the three eastern locations Michigan, Minnesota and Maryland, was not seen in Oregon or California. Numerous other site-specific QTL were identified. Since none of the QTL identified in any of the locations explained over 40 % of the phenotypic variation, it appears that day-neutrality is not controlled by a single dominant gene.

## References


Ahmadi H., R.S. Bringhurst and V. Voth. 1990. Modes of inheritance of photoperiodism in *Fragaria*. J. Amer. Soc. Hort. Sci. 115:146-152.

Albani M.C., Battey N.H., Wilkinson M.J. (2004). The development of ISSR-derived SCAR markers around the SEASONAL FLOWERING LOCUS (SFL) in Fragaria vesca. Theor Appl Genet 109:571-579.

Barrit B.H., R.S. Bringherst, and V. Voth 1982. Inheritance of early flowering in relation to breeding day-neutral strawberries. J. Amer. Soc. Hort. Sci. 107:733-736.

Bringherst R.S. and V. Voth 1984. Breeding octoploid strawberries. Ipwa State J. Res. 58:371-381.

Brown T., Wareing P.F. (1965). The genetical control of the everbearing habit and three other characters in varieties of *Fragaria vesca*. Euphytica 14:97-112.

Berstelsen D. 1995. The U.S. Strawberry Industry. Commercial Agriculture Division, Economic Research Service, U.S. Department of Agriculture. Statistical Bulletin No. 914. http://www.nal.usda.gov/pgdic/Strawberry/ers/ers.htm

Cekic C., Battey N.H., Wilkinson M.J. (2001). The potential of ISSR-PCR primer pair combinationsfor genetic linkage analysis using the SEASONAL FLOWERING LOCUS in Fragaria vesca as a model. Theor and Appl Genet 103:540-546.

Clark J.H. 1937. Inheritance of so-called everbearing tendency in the strawberry. Proc. Amer. Soc. Hort. Sci. 35:67-70.

Darrow G.M. 1936. Interaction of temperature and photoperiodism in the production of fruit buds and runners in the strawberries. Proc. Amer. Hort. Sci. 34:360-363.

Downs R.J. and A.A. Piringer, 1955. Differences in photoperiodic responses of evberbearing and june-bearing strawberries. Proc. Amer. Soc. Hort. Sci. 66:234-236.

Draper A.D., G.J. Galletta and H.J. Swartz. 1981. 'Tribute' and 'Tristar' everbearing strawberries. HortScience 16:794-796.

Durner E.F., J.E. Barden, D.G. Himelrick and E.B. Poling. 1984. Photoperiod and temperature effects on flower and runner development in day-neutral, junebearing and everbearing strawberries. J. Amer. Soc. Hort. Sci. 109:396-40.

Galletta G.J., A.D. Draper and H.J. Swartz. 1981. New everbearing strawberries. HortScience 16:726.

Hancock J.F. 1999. Strawberries. CAB International, Wallingford, UK.

Hancock J.F., P.W. Callow, A. Dale, J.J. Luby, C.E. Finn, S.C. Hokanson and K.E. Hummer. 2001a. From the Andes to the Rockies: native strawberry collection and utilization. HortScience 36:221-225.

Hancock J.F., J.J. Luby, A. Dale, P.W. Callow, S. Serçe and A. El-Shiek. 2001b. Utilizing wild *Fragaria virginiana* in strawberry cultivar development: Inheritance of photoperiod sensitivity, fruit size, gender, female fertility and disease resistance. Euphytica 126:177-184.

Hartman H.T., 1947a. Some effects of temperature and photoperiod on flower formation and runner production in the strawberry. Plant Physiol. 22:407-420.

Hartman H.T., 1947b. The influence of temperature on the photoperiodic response of several strawberry varieties grown under controlled environment conditions. J. Amer. Soc. Hort. Sci. 50:243-245.

Ito H. and T. Saito 1962. Studies on the flower formation in strawberry plant. I. Effects of temperature and photoperiod on the flower formation. Tohoku J. Agr. Res. 13:191-203.

Manakasem Y. and P.B. Goodwin 2001. Response of day neutral and junebearing strawberries to temperature and day length. J. Hort. Sci. & Biotech. 76 (5):629-635.

Nicoll M.F. and G.G. Galleta 1987. Variation in growth and flowering habits of june bearing and everbearing strawberries. J. Amer. Soc. Hort. Sci. 112:872-880.

Orecky D.K. and G.L. Slate 1967. Behavior of the everbearing characteristics in strawberries. J. Amer. Soc. Hort. Sci. 91:236-241.

Powers L., 1954. Inheritance of period of blooming in progenies of strawberries. Proc. Amer. Soc. Hort. Sci. 64:293-298.

Shaw D.V., 2003. Heterogeneity of segregation ratios from selfed progenies demonstrate polygenic inheritance for day neutrality in strawberry (*Fragaria ×ananassa* Duch.). J. Amer. Soc. Hort. Sci. 128:504-57.

Serçe S. and J.F. Hancock, 2003. Assessment of day-neutral scoring methods in strawberry families grown in greenhouse and field environments. Turk. J. Agric. For. 27:191-198.

Serçe S. and J.F. Hancock, 2005a. The temperature and photoperiod regulation of flowering in *Fragaria chiloensis*, *F. virginiana*, and *F. ananassa* genotypes. Sci. Hortic. 103:167-177.

Serçe S. and J.F. Hancock, 2005b. Inheritance of day-neutrality in octoploid species of *Fragaria*. J. Amer. Soc. Hort. Sci. 130:580-584.

Sugimoto T., Tamaki, K., Matsumoto, Y., Shiwaku, K., and K. Watanabe, 2005. Detection of RAPD markers linked to the everbearing gene in Japanese cultivated strawberry. Plant Breeding 124:498-501.

# CHAPTER 2

# BUILDING A GENETIC LINKAGE MAP FOR THE OCTOPLOID STRAWBERRY USING AFLP MARKERS

# Introduction

Most traits of agronomic importance are controlled by multiple loci and show a continuous distribution in phenotype. Such traits are called complex or quantitative traits (QTs) and efficient breeding for them requires an understanding of the inheritance of the genes involved in determining the trait. Often, such genes interact among themselves and/or with the environment, which modifies trait expression, complicating inheritance studies. Furthermore, these genes can be scattered across the genome, making their concentration into a single genotype difficult.

Similar to all other breeders, strawberry breeders often struggle to improve QTs. One such commercially important trait in strawberry that has proven recalcitrant is day-neutrality or photoperiod insensitivity. The inheritance studies previously done for day-neutrality have indicated that the trait may be under a wide array of control from single gene to multi-gene (Clark, 1937; Powers, 1954; Ourecky and Slate, 1967; Ahmadi et. al., 1990; Hancock et. al., 2001; Shaw 2003; Serçe and Hancock, 2005 and Sugimoto et.al., 2005).

In order to determine how many genes govern QTs, a linkage mapping approach can be used in combination with phenotypic analyses. In this approach, a genetic linkage map is generated using a mapping population that segregates for the QT of interest. This map is then used to locate quantitative trait loci (QTL) that make a significant contribution to the

expression of a complex trait, by identifying molecular markers on the map that co-segregate with the trait of interest. Once chromosomal regions are identified that contain the genes of interest, further fine mapping can be done to find markers that are closer to the QTL. These can then be used in marker-assisted selection (MAS) in crop breeding efforts.

Linkage mapping and QTL approaches are attractive to study the inheritance of day-neutrality in strawberries for several reasons. In classical genetic studies, progeny segregation ratios are used to determine how many genes govern a trait. Often due to small sample size, numbers that depict contrasting phenotypes can fit several different genetic ratios complicating conclusions. Furthermore, QTs are often controlled not only by a few major genes, but many minor genes that modify the phenotype depending on the environmental conditions. When this happens, the final phenotype that is scored can vary greatly regardless of what the major genes are. In a QTL analysis, an association is sought between the phenotype and the genotype depicted by molecular/DNA markers (Collard et.al., 2005) and as a result, the number of genes determined to be associated with the trait is not based solely on phenotypic ratios. It is even possible to detect genes that have relatively limited affects on the phenotype, if the markers are closely linked to the gene of interest.

The cultivated strawberry (*F.* x *ananassa*), is an octoploid species (2n = 8x = 56) and the species ploidy level complicates linkage map construction, due to the possibility of multisomic relationships and different loci carrying the same allele. However, the

limitations of genetic map construction in polyploids can be overcome by the use of single dose restriction fragments (SDRF) (Wu et. al., 1992). An SDRF is a fragment present in a single dose in a parent, and segregates as a single gene trait in the progeny. In strawberry, when a fragment is present in a parent, it can be one of +-------, ++------, +++-----, ++++----, +++++---, ++++++-- or +++++++- (where + and - signify presence and absence of the marker respectively). Previous mapping experience with strawberry has indicated that it has primarily disomic inheritance and about 120 individuals are adequate to identify SDRFs (Lerceteau-Köhler et. al., 2003).

Complete linkage maps are only just beginning to emerge for *F.* x *ananassa.* Two of the currently published maps were created with SDRFs of Amplified Fragment Length Polymorphic (AFLP) markers and were used by Parisy (2001) and Lerceteau-Köhler et. al. (2003). Parisy (2001) used 518 SDRFs to generate female and male maps containing 44 and 46 linkage groups, respectively. The female map had a length of 1745 cM, whereas the length of the male map was 1876.6 cM. Parents of his mapping populations were not mentioned. The Lerceteau-Köhler group (2003) also developed separate female and male maps of 'Capitola' x clone CF1116 using a total of 727 SDRFs, which contained 43 linkage groups each, with lengths of 1604 cM (with 235 SDRFs) and 1496 cM (with 280 SDRFs), respectively. They were able to identify 30 and 28 linkage groups on the female and male sides, and reported the genome sizes of the female to be 2870 cM and the male, 1861 cM. Viruel et. al. (2002) crossed clones P1 and P5 (cultivar names not mentioned) and used 177 Simple Sequence Repeat (SSR) and 123 Restriction Fragment Length Polymorphic (RFLP) markers to develop a consensus map of 17

linkage groups and a length of 627 cM. He used 65 *Prunus* probes in his RFLP work, but states that the probes generated low levels of polymorphism in strawberry compared to *Prunus*. Additional information on where the probes and SSRs were derived was not published.

If a QTL approach is going to be used to map the genes determining day-neutrality in strawberry, we will need to examine a progeny population segregating for that trait. In the currently published maps, where parents of the mapping population are indicated, there is no mention that the population segregates for day-neutrality. Therefore, we cannot use existing maps to do a QTL analysis of our trait of interest. The major goal of this research was to develop a linkage map of a breeding population of *F.* x *ananassa* that was segregating for day-neutrality.

We choose to build our map using AFLPs, as they are highly polymorphic, relatively inexpensive to develop and only a few RFLP and SSR markers have been used in the octoploid strawberry to date (Ashley et.al., 2003, Folta et.al., 2005). We included the AFLP primer combinations used in the Lerceteau-Köhler et.al. (2003) study to search for common markers. Our goal is to initially use AFLP markers to locate QTL for day-neutrality, and then as more SSRs are developed, add these markers to build a dense map that is also transferable across cultivars. AFLPs have also been used in other Rosaceous crops such as apple to create saturated maps, with the inclusion of SSRs to make the maps transferable among populations and species (Liebhard et. al., 2003). A few SSRs have been reported in the diploid strawberry (Viruel et. al., 2002 and Sargent et.al., 2003,

Hadonou et. al., 2004), but their segregation patterns in the octoploid strawberry have not yet been studied. We are currently collaborating with Dr. Kim Lewers at the fruit Laboratory of the USDA in Beltsville, Maryland to develop strawberry SSRs from published EST sequences. We also hope to determine if *F. vesca* and *Prunus* SSRs segregate as SDRFs in our mapping population, to include them in our map.

## Material and Methods

### Mapping population

Six different populations were evaluated from the factorial experiment of Serçe and Hancock (2005) to identify the best population for mapping through selective genotyping. All six populations were segregating for the day-neutrality trait. The crosses were, 'Tribute' x 'Honeoye', 'Tribute' x 'Allstar', 'Tribute' x Eagle 14 (native genotype of *F. virginiana*), Frederick 9 (native genotype of *F. virginiana*) x 'Honeoye', Frederick 9 x 'Allstar' and Frederick 9 x Eagle 14. Ten individuals, five day neutral (DN) and five short day (SD) types, along with parents of each population were initially genotyped in each population using 15 AFLP primer combinations.

The segregating mapping population that we selected to build the linkage map was 'Tribute' x 'Honeoye'. This population had the highest number of polymorphic fragments per primer combination (an average of 17) and the highest proportion of markers that

segregated in expected SDRF ratios (about 70 %). 'Tribute' is one of the two DN cultivars released in the last 20 years for the eastern USA, while 'Honeoye' is the most popular SD variety grown in the Midwestern and Northeastern USA.

**DNA isolation**

For reproducible AFLP analyses in strawberry, highly purified DNA is required as strawberry DNA is often contaminated with large amounts of polysaccharides that tend to inhibit digestion and amplification reactions in the AFLP technique. Young leaves were obtained from plants maintained in the greenhouse and were placed in coolers containing ice before being transported to the lab. The Haymes (1996) method of DNA extraction was used with the addition of two steps of chloroform / isoamyl alcohol (24:1) extractions instead of one.

Leaf samples were lyophilized for 48 hrs after being frozen overnight at – 80 $^{0}$C. 200 – 400 mg of lyophilized leaf tissue of each genotype was placed in a 2 ml tube with two 4 mm glass beads (Fischer scientific, Pittsburgh, PA) and 5 mg of polyvinylpyrrol-iodine 40 (PVP-40) (Sigma-Aldrich, St. Louis, MO). The samples were ground into a fine powder using the Fast prep FP20 (Savant Instruments Inc., Hallbrook, NewYork). The powder was then transferred into a 15 ml centrifuge tube (Corning Incorporated, Corning, NY) to which 6ml of pre-warmed extraction buffer (to about 65 $^{0}$C) was added. After mixing the contents well, the tubes were incubated in a water bath at 65 $^{0}$C for an hour with further mixing by inversion every 10 minutes. The samples were taken out of the

water bath and allowed to cool to room temperature before adding an equal volume of chloroform / isoamyl alcohol (24:1). Then, the tubes were placed horizontally on a shaker and were mixed for about 30 minutes. The aqueous and the organic layers were separated by centrifuging at 6500 rpm for 20 minutes. The aqueous supernatant was separated into a clean sterile 15 ml centrifuge tube (Corning Incorporated, Corning, NY) and the chloroform/Isoamyl step was repeated. DNA was precipitated from the resulting aqueous supernatant by the addition of an equal volume of ethanol-acetate solution (96 ml of 200 % ethanol with 4 ml 3M sodium acetate solution) and gentle mixing by inversion. The DNA precipitated as a white mass which was hooked using a sterile glass rod, was washed twice with 500 µl of 70 % ethanol before being air-dried overnight.

The DNA pellet was dissolved in 150 µl of double distilled, autoclaved water and kept at 4 $^{0}$C for two weeks before an RNase treatment (Roche- RNase DNase free) with 1 µl of a 10 mg/ml Rnase solution for each sample. DNA was quantified using the Hoefer DyNA Quant 200 Fluorometer (Amersham Pharmacia Biotech) and 100 ng/µl dilutions were made for each sample of DNA. The quality of the DNA was tested by running 5µl of the DNA sample with 10 µl water and 2 µl loading dye in a 1 % agarose gel for an hour under 100 volts.

The Vos et al. (1995) protocol for AFLP analysis was performed on 127 individuals of the segregating population using the modifications of Hazen et.al. (2002), Vallejo and Kolkman (2002). 10 µl of each DNA sample (of 100 ng/µl DNA concentration) was used for each digestion reaction. 72 AFLP primer combinations were evaluated using *EcoR1*

and *Mse1* (Table 2.1). One selective nucleotide from each of the primers was used in the pre amplification step and two additional selective nucleotides were used in the selective amplification step except for the combination, M + CG_ with E + AGT. After the selective amplification, 8 µl of the formamide loading dye (98 % formamide, 10 mM of EDTA, 1 mg/ml xylene cyanol and 1 mg/ml bromophenol blue) was added to each PCR product and all products were denatured for 5 minutes at 98 $^0$C. After the denaturation, the samples were immediately placed on ice to minimize renaturation before gel loading. 4.5 µl of each sample was loaded onto a 6 % denaturing polyacrylamide gel which was pre equilibrated with pre heated 1 x TBE buffer. The gel was allowed to run for about 150 minutes after which the plates were separated and silver-stained. The plates were then dried over night before the polymorphic fragments were scored over a light box. The size of every polymorphic fragment scored was estimated by comparing it with 10 and 50 base pair ladders run on either side of the parents and progeny.

**Map construction and visual presentation**

Strawberry is highly heterozygous and is an outcrossing species and hence, a double pseudo test cross strategy was used for mapping (Hemmat et. al., 1994). A total of 127 individuals of the cross 'Tribute' x 'Honeoye' was utilized in the segregation analyses. The linkage mapping was done with those SDRFs that differed between parents and segregated in a 1:1 (presence: absence) ratio and those that were present in both parents and segregated in a 3:1 ratio. $\chi^2$ analyses were performed to test goodness of fit at 5 % level and only markers that fit were used in linkage analyses. Marker names were

selected to include the AFLP primer combination used, the size of the polymorphic fragment and whether the fragment was present only in 'Tribute' (T), only in 'Honeoye' (H) or in both parents (B).

Joinmap 3.0 (Van Ooijen J.W. & R.E. Voorrips, 2001) was used to perform the linkage analyses with a minimum LOD score of 3.0 and a maximum recombination fraction of 0.3. Map distances were calculated using the Kosambi map function and were expressed in centi-Morgans (cM). Markers were excluded if their segregation pattern conflicted with other markers in the same linkage group. MapChart software (Voopris, 1999-2002) was used to draw the maps for the linkage groups.

## Results and Discussion

Sixty nine AFLP primer combinations were used to genotype the 127 individuals in the mapping population. Thirty-two of these primer combinations were among the 40 combinations used by Lerceteau-Köhler et. al. (2003). Table 2.1 presents a summary of the primer combinations used and the number of polymorphic fragments (#PF) that were generated from each primer combination. Of the 1065 polymorphic fragments scored, 279 markers were excluded from the map because they were resolved in fewer than 100 of the genotypes. Out of the remaining 786 markers, 539 or 69 % significantly fit the 1:1 or 3:1 ratios expected for SDRFs and were used to build the genetic linkage map. Of the

markers not included in the map, 16 % (247) significantly fit multiplex segregation ratios [7:1 (49), 11:3 (12), 13:1 (18), 25:3 (29), 27:1 (1 3), 31:1 (5)], and 15 % did not fit any discrete segregation ratio. Thirty-eight markers significantly fit more than one complex ratio.

Of the 539 SDRF markers, 383 segregated in a 1:1 fashion and 156 segregated in a 3:1 fashion. Three hundred and eighty seven of these markers were placed on the consensus map, which consisted of 42 linkage groups. The rest of the SDRF markers were either not linked to any of the linkage groups recognized or were not included because their segregation pattern conflicted with other markers in the same linkage group at a LOD score of 3.0.

While over 75 % of the AFLP primer combinations used by the Lerceteau-Köhler group (2003) were included in our analysis, we were unable to locate any of their polymorphic fragments in our population. It is possible that our parents did not carry the same polymorphisms, but another reason might have to do with how we modified the Vos et. al. (1995) protocol. At the beginning of this study, several steps were taken to see if the Vos et. al. (1995) protocol modified by Hazen et.al. (2002), Vallejo and Kolkman (2002) would work well for strawberry. In this attempt, several different *Taq* polymerase enzymes were tested (by Promega, Invitrogen etc.) to select the best enzyme for amplification reactions in the strawberry. Surprisingly, the banding patterns generated by the different enzymes were not identical even when the rest of the protocol was

unchanged. Sigma *Taq* DNA polymerase was used by the Lerceteau-Köhler group and we used Promega *Taq* DNA polymerase for our PCR amplifications.

Our map is more dense than the previous ones published for the octoploid strawberry. Average marker density for our consensus linkage map was 0.3 markers/cM and the length of the linkage map was 1310.7 cM. We also had an average of 9.2 markers per linkage group (LG). Parisy (2001) developed female and male maps of 1745 and 1876.6 cM respectively and had one marker at each 7 cM with an average of 5.8 markers per linkage group. Lerceteau-Köhler et.al. (2003) created maps of 1604 cM (female) and 1496 cM (male). They had an average of 5.5 markers per linkage group with an average marker density of 8.4 (±7.9) markers/cM and 6.3 (±7.0) markers/cM for the two maps (Köhler et al, 2003).

The longest linkage group of our map is LG 1, which is 122 cM in length with 31 markers (Figure 2.1 and Table 2.2). This group had a marker density of 0.25 markers/cM and an average distance of 3.9 cM between markers. The shortest linkage group was LG 20, which had only two markers that mapped to the same locus.

The most dense of our linkage groups were LG 21, which had a marker density of 1.05 markers/cM, but only two markers.

Considering there are 28 linkage groups expected for the strawberry genome, our map is far from being complete. It is likely that many of our linkage groups represent fragmented portions of chromosomes and more markers are needed to link some of the groups. We are currently developing SSR markers for this purpose in collaboration with Dr. Kim Lewers in the Fruit Laboratory of the USDA in Beltsville, Maryland. However, the objective of this work was to develop a sufficiently dense linkage map to determine whether day-neutrality is a quantitative or qualitative trait. As will be described in Chapter 3, this map was adequate to identify multiple QTL for day-neutrality in strawberry.

**Table 2.1**: AFLP primer combinations and the number of polymorphic fragments (PF) scored in a progeny population of 'Tribute' x 'Honeoye' segregating for day-neutrality. The primers previously used by Lerceteau-Köhler et. al. (2003) are indicated with an asterisk.

E = EcoR1 primers, M = Mse1 primers

| Primer | E+aa- | #PF | E+at- | #PF | E+ac- | #PF | E+ag- | #PF |
|---|---|---|---|---|---|---|---|---|
| **M+ca-** | aag/cag | 11 | ata/cac | 19 | acc/cag | 13 | agt/caa | 19 |
| | | | atg/cag* | 17 | acc/cac | 11 | agt/cat* | 8 |
| | | | atg/cac* | 15 | acc/cag | 13 | agt/cag* | 14 |
| | | | atg/caa* | 15 | aca/caa | 16 | aga/cat* | 11 |
| | | | | | aca/cag* | 12 | agg/cag | 12 |
| | | | | | acc/caa* | 19 | agc/cag | 14 |
| | | | | | aca/cat* | 8 | agg/cat* | 17 |
| | | | | | act/cag | 14 | aga/cag* | 4 |
| | | | | | | | aga/caa* | 17 |
| | | | | | | | aga/cac* | 17 |
| | | | | | | | agg/caa | 23 |
| **M+ct-** | aag/cta | 16 | atg/ctg* | 18 | acc/cta | 19 | aga/ctc | 16 |
| | aag/ctt | 22 | atg/cta* | 19 | acc/ctg* | 8 | agt/cta* | 25 |
| | aag/ctc | 27 | ata/ctc* | 27 | act/cta | 17 | aga/ctt* | 22 |
| | aac/ctg | 17 | atg/ctt* | 20 | acc/ctt | 14 | agt/ctc* | 8 |
| | aag/ctg | 8 | ata/ctt | 21 | act/ctt | 18 | agg/ctt | 10 |
| | | | atg/ctc* | 17 | act/ctc | 15 | agg/ctg | 15 |
| | | | | | acc/ctc* | 8 | aga/ctg* | 24 |
| | | | | | act/ctg* | 19 | agc/ctt | 11 |
| | | | | | aca/ctc* | 13 | agt/ctg* | 13 |
| | | | | | | | agg/ctc | 11 |
| | | | | | | | agg/cta | 4 |
| | | | | | | | aga/cta* | 18 |
| | | | | | | | agc/ctg* | 8 |
| | | | | | | | agt/ccc | 10 |
| | | | | | | | agc/ctg* | 10 |
| **M+cc-** | | | ata/ccg | 18 | act/ccg | 10 | | |
| | | | ata/cca | 20 | | | | |
| | | | ata/cct | 18 | | | | |
| | | | ata/ccc | 27 | | | | |
| **M+cg-** | | | ata/cgc | 7 | | | agt/cga | 8 |
| | | | atg/cg-* | 19 | | | agg/cga | 14 |
| | | | ata/cgt | 7 | | | | |

**Figure 2.1**: AFLP consensus genetic linkage map for the 'Tribute' x 'Honeoye' mapping population. Markers on the right are identified by the AFLP primer combination, the fragment size and whether the marker was only present in 'Tribute' (T), only present in 'Honeoye' (H) or, if marker was present in both parents (B).

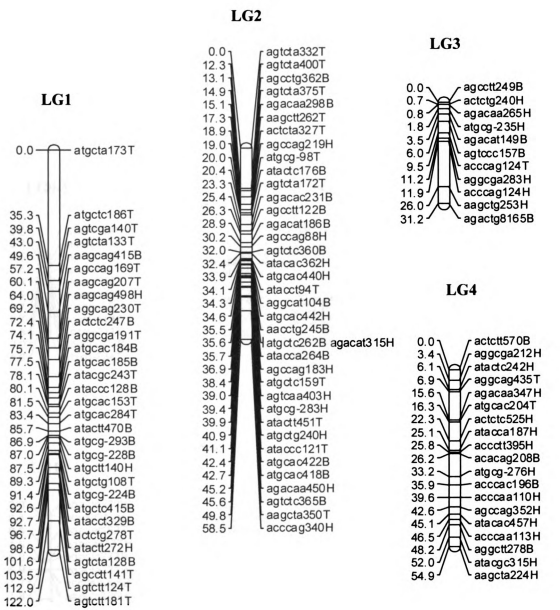


**Figure 2.1** (Cont'd).

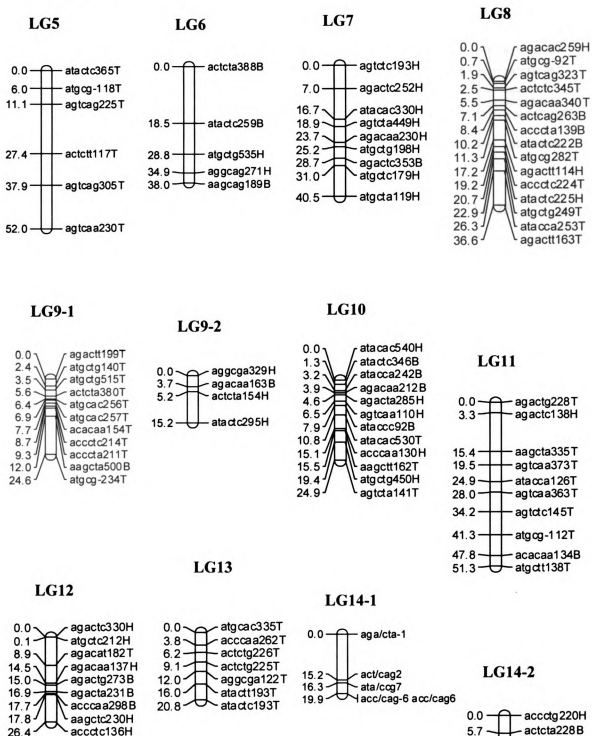


**Figure 2.1** (Cont'd).

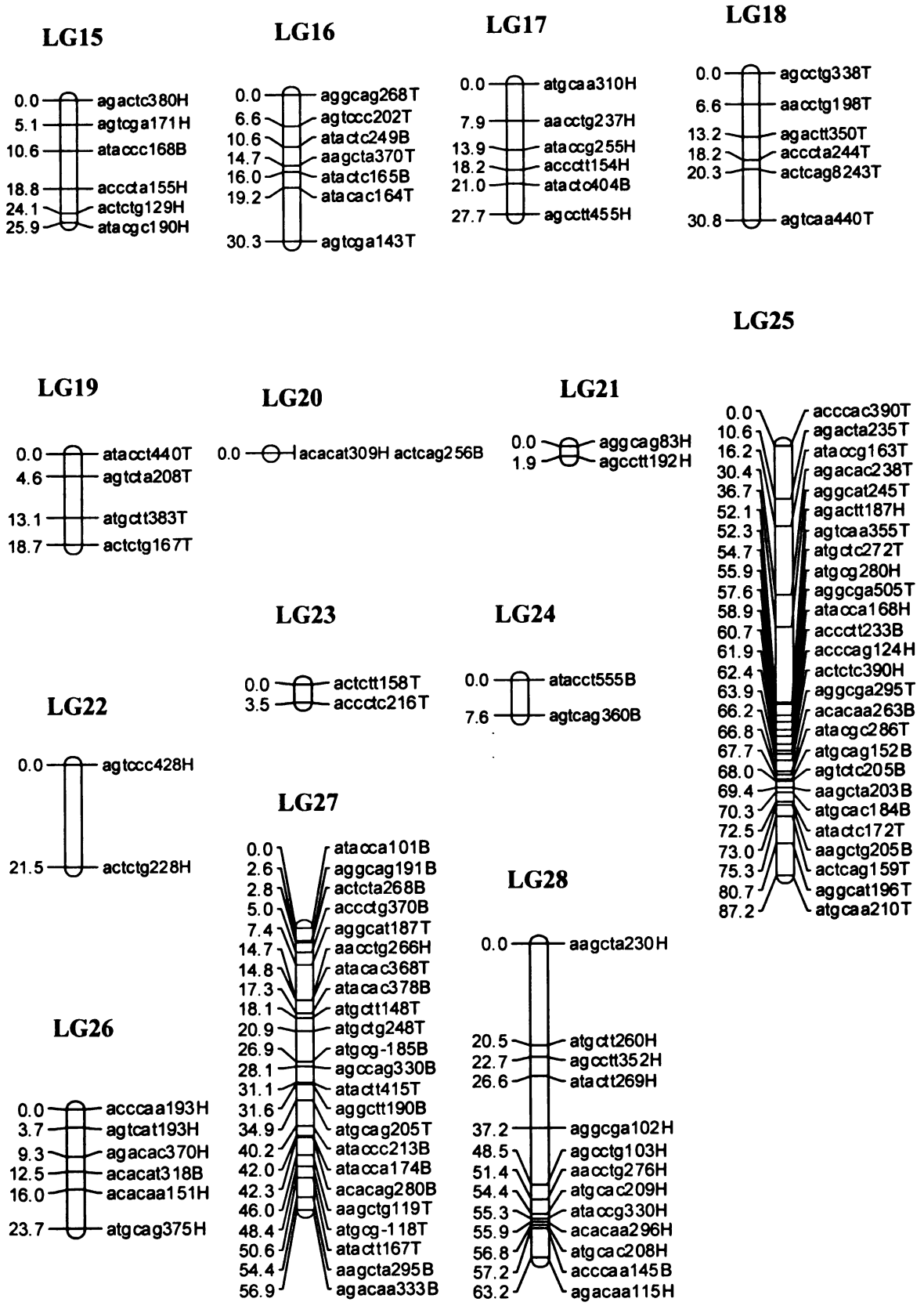


**Figure 2.1** (Cont'd).

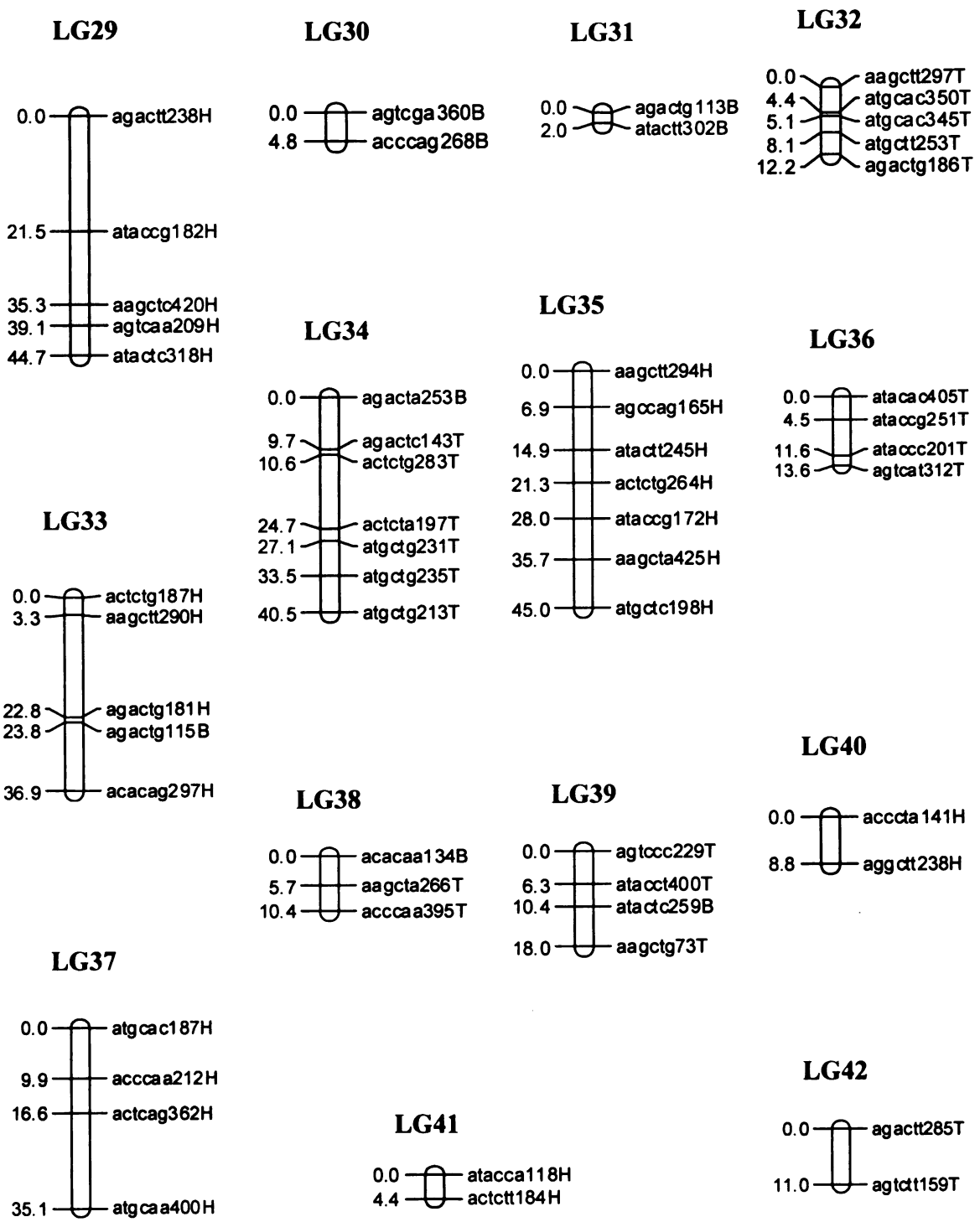


**Table 2.2**: Detailed presentation of the number of markers, length, marker density, and gaps for each linkage group of the consensus genetic linkage map constructed from the progeny population of the cross 'Tribute' x 'Honeoye'.

| Linkage group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9-1 | 9-1 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **AFLP markers** | 31 | 38 | 11 | 19 | 6 | 5 | 9 | 15 | 11 | 4 | 12 |
| **Length in cM** | 122 | 58.5 | 31.2 | 54.9 | 52 | 38 | 40.5 | 36.6 | 24.6 | 15.2 | 24.9 |
| **Marker density Markers/cM** | 0.25 | 0.65 | 0.35 | 0.35 | 0.12 | 0.13 | 0.22 | 0.41 | 0.45 | 0.26 | 0.48 |
| **Average distance between markers** | 3.9 | 1.5 | 2.8 | 2.9 | 8.7 | 7.6 | 4.5 | 2.4 | 2.2 | 3.8 | 2.1 |
| **Largest gap** | 35.3 | 12.3 | 14.1 | 8.7 | 16.3 | 18.5 | 9.7 | 10.3 | 12.6 | 10 | 5.5 |

| Linkage group | 11 | 12 | 13 | 14-1 | 14-2 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **AFLP markers** | 10 | 9 | 7 | 5 | 3 | 6 | 7 | 6 | 6 | 4 | 2 |
| **Length in cM** | 51.3 | 26.4 | 20.8 | 19.9 | 7.8 | 25.9 | 30.3 | 27.7 | 30.8 | 18.7 | 0 |
| **Marker density Markers/cM** | 0.19 | 0.34 | 0.34 | 0.25 | 0.38 | 0.23 | 0.23 | 0.22 | 0.19 | 0.21 | 0 |
| **Average distance between markers** | 5.13 | 2.93 | 2.97 | 3.98 | 2.6 | 4.32 | 4.33 | 4.62 | 5.13 | 4.68 | 0 |
| **Largest gap** | 12.1 | 8.8 | 4.8 | 15.2 | 5.7 | 8.2 | 11.1 | 7.9 | 10.5 | 8.5 | 0 |

**Table 2.2** (Cont'd).

| Linkage group | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **AFLP markers** | 2 | 2 | 2 | 2 | 26 | 6 | 23 | 13 | 5 | 2 | 2 |
| **Length in cM** | 1.9 | 21.5 | 3.5 | 7.6 | 87.2 | 23.7 | 56.9 | 63.2 | 44.7 | 4.8 | 2.0 |
| **Marker density Markers/cM** | 1.05 | 0.09 | 0.57 | 0.26 | 0.3 | 0.25 | 0.4 | 0.21 | 0.11 | 0.42 | 1 |
| **Average distance between markers** | 0.95 | 10.75 | 1.75 | 3.8 | 3.35 | 3.95 | 2.47 | 4.86 | 8.94 | 2.4 | 1 |
| **Largest gap** | 1.9 | 21.5 | 3.5 | 7.6 | 15.4 | 7.7 | 7.3 | 20.5 | 21.5 | 4.8 | 2 |

| Linkage group | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **AFLP markers** | 5 | 5 | 7 | 7 | 4 | 4 | 3 | 4 | 2 | 2 | 2 |
| **Length in cM** | 12.2 | 36.9 | 40.5 | 45 | 13.6 | 35.1 | 10.4 | 18 | 8.8 | 4.4 | 11 |
| **Marker density Markers/cM** | 0.4 | 0.14 | 0.17 | 0.16 | 0.29 | 0.11 | 0.29 | 0.22 | 0.23 | 0.45 | 0.18 |
| **Average distance between markers** | 2.44 | 7.38 | 5.79 | 6.42 | 3.4 | 8.78 | 3.47 | 4.5 | 4.4 | 2.2 | 5.5 |
| **Largest gap** | 4.4 | 19.5 | 14.1 | 9.3 | 7.1 | 18.5 | 5.7 | 7.6 | 8.8 | 4.4 | 11 |

# References


Ahmadi H., R.S. Bringhurst and V. Voth. 1990. Modes of inheritance of photoperiodism in *Fragaria*. J. Amer. Soc. Hort. Sci. 115:146-152.

Ashley M.V., Wilk J.A., Styan S.M.N, Craft K.J., Jones K.L., Feldheim K.A., Lewers K.S., Ashman T.L. 2003. High variability and disomic segregation of microsatellites in the octoploid Fragaria virginiana Mill. (Rosaceae). Theor Appl Genet URL http://www.springerlink.com/app/home/content/asp?wasp=hpb9jdh...

Basten C.J., Weir B.S. and Z.B. Zeng 2002. QTL Cartographer, Version 1.16. Department of Statistics, North Carolina State University, Raleigh, NC.

Clark J.H. 1937. Inheritance of so-called everbearing tendency in the strawberry. Proc. Amer. Soc. Hort. Sci. 35:67-70.

Collard B.C.Y., Jahufer M.Z.Z., Brouwer J.B., and E.C.K. Pang 2005. An introduction to markers, quantitative trait loci (QTL) maooing and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142:169-196.

Folta K.M., Staton M., Stewart P.J., Jung S., Bies D.H., Jesdurai C. and D. Main 2005. Expressed sequence tags (ESTs) and simple sequence repeat (SSR) markers from octoploid strawberry (*Fragaria* x *ananassa*). BMC Plant Biol. 5:12.

Hadonou A.M., Sargent D.J., Wilson F., James C.M., and D.W. Simpson 2004. Development of microsatellite markers in *Fragaria*, their use in genetic diversity analysis, and their potential for genetic linkage mapping. Genome 47:429-438.

Hancock J.F., Luby J.J. , Dale A., Callow P.W. , Serçe S. and A. El-Shiek. 2001. Utilizing wild *Fragaria virginiana* in strawberry cultivar development: Inheritance of photoperiod sensitivity, fruit size, gender, female fertility and disease resistance. Euphytica 126:177-184.

Haymes K.M. 1996 mini-prep method suitable for a plant breeding program. Plant molecular biology reports, 14-280-284.

Hazen S.P., Leroy P. and R. Ward 2002. AFLP in *Triticum aestivum* L.: patterns of genetic diversity and genome distribution. Euphytica 125 (1): 89-102.

Hemmat M., Weeden N.F., Manganaris A.G. and D.M. Lawson, 1994. Molecular markers linkage map for apple. J. Hered. 85:4-11.

Lerceteau-Köhler E., Guérin G., Laigret F. and B. Denoyes-Rothan. 2003. Characterization of mixed disomic and polysomic inheritance in the octoploid

strawberry (*Fragaria ×ananassa*) using AFLP mapping. Theor. Appl. Genet. 17:619-628.

Liebhard R., Koller B., Gianfranceschi L. and C. Gessler 2003. Creating a saturated reference map for the apple (Malus x domestica Borkh.) genome. Theor. Appl. Genet. 106:1497-1508.

Orecky D.K. and G.L. Slate 1967. Behavior of the everbearing characteristics in strawberries. J. Amer. Soc. Hort. Sci. 91:236-241.

Parisy V. 2001. Establishment of the strawberry, *Fragaria* x *ananassa* Duchesne, genetic map; search for molecular markers of agronomic characters. The French Institute for Agranomic Research (INRA), Fruit and wine research station, Bordeaux, France. (Abstract) URL http://genetics.biology.kyushu-u.ac.jp/pgen/cv/vipar/fragaria%20project.html

Powers L., 1954. Inheritance of period of blooming in progenies of strawberries. Proc. Amer. Soc. Hort. Sci. 64:293-298.

Sargent D.J., Davis T.M., Tobutt K.R., Wilkinson M.J., Battey N.H. and D.W. Simpson 2004. A genetic linkage map of microsatellite, gene-specific and morphological markers in diploid *Fragaria*. (Abstract) Theor Appl Genet (in press) URL http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=disply&db=pubmed&uid=15290052&dopt=abstract

Serçe S. and J.F. Hancock, 2005. Inheritance of day-neutrality in octoploid species of *Fragaria*. J. Amer. Soc. Hort. Sci. 130:580-584.

Sugimoto T., Tamaki, K., Matsumoto, Y., Shiwaku, K. and K. Watanabe, 2005. Detection of RAPD markers linked to the everbearing gene in Japanese cultivated strawberry. Plant Breeding 124:498-501.

Vallejo V. and J. Kolkman (2002), http://www.css.msu.edu/bean/PDF/AFLP_protocol.pdf

Van Ooijen J.W. & R.E. Voorrips, 2001. Join map Version 3.0, Soft ware for the calculation of Genetic linkage map. Plant Research International, Wageningen, the Netherlands

Viruel M.A., Sánchez D. and P. Arús 2002. An SSR and RFLP linkage map for the octoploid traswberry (*Fragaria* x *ananassa*). Plant & Animal Genome *X* Conference, San Diego, CA. (Abstract)

Voorrips R. E. 2002: MapChart, Software for the graphical presentation of linkage maps and QTLs. J. Heredity 93, 77—78.

of linkage maps and QTLs. J. Heredity 93, 77—78.

Vos P., Hogers R., Bleeker M., Reijans M., Theo van de Lee, Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. and M. Zabeau, 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acid Research 23:4407-4414.

Wu K.K., Burnquist W., Sorrels M.E., Tew T.L., Moore P.H. and S.D. Tanskly., 1992. The detection and estimation of linkage in polyploids using single dose restriction fragments. Theor Appl Genet 83:294-300.

# CHAPTER 3

# QTL IDENTIFICATION FOR DAY-NEUTRALITY IN THE OCTOPLOID STRAWBERRY (*Fragaria* x *ananassa*)

# Introduction

Day-neutrality was first introduced into to short day (SD) strawberry cultivars in California by Bringhurst and Voth (1984) using a native genotype from the Wasatch Mountains of Utah. Over 60 % of the California production areas are now planted with day neutral (DN) cultivars to extend the production season. Wild type SD plants or June-bearers initiate flower buds either under short day conditions (less than 14 hrs of day length) or at lower temperatures of below 15 $^{0}$C (Darrow, 1936). The DN plants are photoperiod insensitive and flower early (floral meristems produced after a shorter vegetative phase) and cyclically throughout the growing season.

The cyclic flowering habit of DN genotypes would be useful to extend the harvest season in other regions of the US. However, when the DNs developed in California are grown in continental climates of North America, they remain primarily vegetative when day/night temperatures are above 30/26 $^{0}$C and any fruit that are produced during the warmest months of summer are small and soft (Galletta et.al., 1981, Durner et.al., 1984).

Attempts at studying the inheritance of day-neutrality in octoploid strawberries have resulted in several different models being presented including: 1) regulation by a single dominant gene (Bringhurst and Voth, 1978, Ahmadi et. al., 1990, Sugimoto et. al., 2005); 2) regulation by dominant complementary genes (Clark, 1937 and Orecky and Slate, 1967); and 3) quantitative inheritance (Powers, 1954; Hancock, 2001c; Serçe and

Hancock, 2005). However, these studies are difficult to compare as they utilized different sources of day-neutrality, dissimilar terminology to explain the different temperature/photoperiod interactions, a variety of environmental conditions and a wide array of selection criteria for what constituted DN plants.

Studies done by Hancock et. al. (2001) investigated the inheritance of day-neutrality in strawberry by crossing a diverse group of SD and DN representatives of *Fragaria virginiana* from the wild with SD and DN *Fragaria* x *ananassa* cultivars. Cultivar representatives were used from all the US breeding programs and the progeny were evaluated in three different locations; Michigan, Minnesota and Ontario. In their crosses of DN *F. virginiana* x SD *F.* x *ananassa,* most of the progeny ratios deviated significantly from a 1:1 ratio, indicating that photoperiodic insensitivity is not controlled by a single dominant gene in these populations. They detected a wide variation in the percentage of DN plants produced by each DN *F. virginiana* parent and DN progeny were generated from crosses of SD *F. virginiana* and SD *F.* x *ananassa*; all these observations are consistent with polysomic inheritance. A significant difference in the expression of day-neutrality was also observed across locations, indicating a strong environmental component.

In a comparison of the five techniques commonly used to identify DN genotypes, Serçe and Hancock (2003) found that the highest percentage of DN plants was discovered when segregating populations were grown in the field for a full season, and plants were classified as DN, only if they flowered under both short and long day conditions. Serçe

and Hancock (2005) also made partial diallele crosses between 12 selected SD and DN genotypes of California cultivars, Eastern cultivars and a group of wild genotypes that had previously been shown to produce different frequencies of DN progeny. The segregating populations were analyzed for whether they flowered under SDs (<14 hr) in the spring (before 30$^{th}$ of May) and under LDs (>15 hr) in the summer (after 24$^{th}$ July). They found a wide, almost continuous range in the percentage of DN progeny produced across families, from 30 – 87 % in DN x SD crosses and from 22 – 93 % in DN x DN crosses.

Serçe and Hancock (2005) made a number of additional observations that suggested that day-neutrality in octoploid strawberry is inherited as a complex trait. Overall, their DN x DN crosses produced 71 % DN progeny, significantly lower than the expected 75 % if day-neutrality was controlled by a single dominant gene. Their SD x DN crosses produced 58 % DN progeny, which is significantly higher than the 50 % expected. The selfed DN progeny from the strong DN cultivar 'Tribute' produced the highest percentage of DN progeny (88 %) compared to any other DN x DN cross. In addition, different percentages of DN plants were produced when different DN parents were crossed to the same SD genotype, and some of the DN sources appeared to be stronger in their ability to generate DN progeny. They also found that none of the DN x DN crosses produced 100 % DN progeny as would be expected if they were homozygous for a dominant gene, and only half the families fit a segregation ratio of a single dominant gene model.

In another study of the genetics of day-neutrality using California breeding populations, Shaw (2003) selfed populations of 10 DN *F.* x *ananassa* genotypes and observed a continuous range in the percent DN progeny produced by the various selfed DN parents. He also found that 3 of the families did not segregate in the 3:1 ratio expected for heterozygous DN parents carrying a single dominant gene that controls the trait. Out of the three families, two produced lower numbers of DN plants than expected, while the third produced an excess. He concluded that day-neutrality in the California breeding population is not controlled by a single locus.

Based on these most recent field studies of a wide range of germplasm, we hypothesized that day-neutrality is governed by a few major genes (controlling a major component of the phenotype) and many minor genes or modifiers that alter the phenotype depending on the environment, as has been detected for many quantitative traits (Patterson et. al., 1991). To directly test this hypothesis, a linkage mapping approach was used to determine if there are multiple QTL for day-neutrality that map at different locations. A suitable segregating population for day-neutrality for this approach was selected from the previous partial diallele crossing experiment by Serçe and Hancock (2005), and was used to develop a genetic linkage map. Phenotypic data on flowering was obtained on a weekly basis in Michigan during both SD and LD conditions and were analyzed with marker data for detecting QTL for day-neutrality/cyclic flowering trait.

It was realized that the QTL associated with day-neutrality would need to be verified before using them in strawberry breeding efforts. There are several ways to verify QTL:

1) by testing the same mapping population in multiple environments, 2) searching for the same QTL in independent populations, 3) developing near isogenic lines (NILs) that differ only in the QTL region, 4) through high-resolution QTL mapping and 5) by cloning and transforming the putative QTL into varieties lacking them to see if the introduced QTL gives expected expression. Since strawberry is a highly heterozygous, outcrossing species, we are unable to develop near isogenic lines, and our map was not sufficiently dense to perform high-resolution QTL mapping or cloning. Therefore, we verified the QTL using multiple environments and independent populations. In this paper, we report on the identification of QTL for day-neutrality in Michigan, and our efforts to verify the QTL in Minnesota, Maryland, Oregon and California.

## Material and Methods

### Obtaining phenotypic data

The first 62 individuals of the mapping population (initial population or IP) were obtained from a previous study (Serçe and Hancock, 2005). Mother plants were dug from the field in the fall of 2003, potted in 15 cm diameter pots, and maintained in an unheated greenhouse over the winter. During summer of 2004, we expected to collect runner plants from these mother plants to be set in the field in fall of 2004 at Benton Harbor, MI. However, most of the mother plants did not produce any runners, so we propagated the IP using a single crown separated from each of the mother plants. The plants were then

grown in a heated greenhouse over the winter and set in the field at Benton Harbor in spring of 2005.

Because the IP did not have sufficient individuals for map construction (Chapter 2), we re-constructed the mapping population (re-constructed population or RP) using the same parents in spring and summer of 2003. Anthers of fresh flowers of the pollen parent, 'Honeoye' were collected into 2.5 cm diameter petri dishes and were allowed to dehisce overnight at room temperature in the lab. Some of the petri-dishes containing the pollen were sealed with parafilm and stored at –16 $^0$C for future use. In the female plant 'Tribute', mature flower buds were selected for crossing that were about to open and all stamens were removed using sharp tweezers to avoid self pollination. Crosses were made using a camel hairbrush that was used to transfer the pollen on to the pistils of the flower.

Seeds were hand picked from harvested fruits and were placed on trays containing sterile moist soil. The trays were then kept in a growth chamber maintained at 4 $^0$C with continuous fluorescent light for a period of 3 months, by which time the seeds were beginning to germinate. The trays were transferred to a growth room with a temperature of about 18 $^0$C with continuous fluorescent light to enhance germination of seeds. Once the seedlings were at four to six leaf stages, plants were potted in 7.6 x 7.6 cm cell packs and allowed to grow in the growth room until they were acclimated for potting in a green house. A total of 360 plants were recovered from the seeds. The plants were transferred into 14 x 12 x 12 cm pots in April 2004 and were maintained in the greenhouse, where they were allowed to runner.

About 10 runners were collected from each of the mother plants during the first week of July 2004. Runners of each of the genotypes were separated from each other and planted into 2.5 x 2.5cm cell packs filled with a commercial soil mix and were maintained for two weeks in a mist house to induce roots. The rooted runner plants were then transferred to a greenhouse for acclimation and were held for two weeks with watering every day. In the last week of July, each of the runner plants with 2 - 4 leaves, were packed into separate plastic Ziploc bags with a tag identifying the plant number. The plants were held at 12 $^{0}$C over night and were shipped to four different strawberry growing states in the US; Minnesota (St.Paul), Maryland (Beltsville), Oregon (Corvallis) and California (Watsonville), for testing in those sites the next day. The plants were sent to our collaborators of this project; James Luby (R342, Alderman Hall, Department of Horticultural Science, University of Minnesota, 1970, Folwell Ave., St. Paul, MN 55108), Kimberly Lewers (USDA-ARS, Fruit Lab, R 210, 10300, Baltimore Ave., Building 010A BARC- West, Beltsville, MD 20705-2350), Chad Finn (USDA-ARS, Horticultural Crops Research Laboratory, 3420, NW Orchard Ave., Corvallis, OR 97330) and Tom Sjulin and Jill Bushakra (Driscoll Strawberry Associates inc., 404, San Juan Road, Watsonville California 95077).

In Michigan, one set of runner plants were potted in 14 x 12 x 12 cm pots with a commercial soil mix and were held in the greenhouse at East Lansing for three weeks before they were planted in a Completely Randomized Design at the SWMREC Research Farm of Michigan State University at Benton Harbor. They were set at 1.2 m x 1.2 m in

the field and were planted in the fall of 2004. The IP was planted at the same site with the same dimensions in the spring of 2005.

The RP were planted in a similar field design in Maryland and Oregon in the fall of 2004 after being potted and held in a greenhouse for 3 weeks. In Minnesota, plants were also set in the field after being maintained in the greenhouse for 3 weeks, but at 0.45 m x 0.45 m spacing on black plastic. In California, the plants were maintained in a screen house for 4 months before they were set in the field at 0.36 m x 0.36 m in a green semi-opaque plastic mulch.

Flowering response was evaluated by recording the presence of open flowers at weekly intervals at all locations starting the first week of May 2005 and continuing until the end of August 2005. Plants were considered to be DN, if they flowered both under SD and LD conditions in the field as suggested by Serçe and Hancock (2003). Since flowers initiated under SD conditions may still develop well into the LDs (Manakasem and Goodwin, 2001), we considered a plant to be day-neutral only if it flowered after the 15$^{th}$ of June, allowing over a month for any SD initiated flowers to develop. At all locations, days become longer than 14 hrs after the first week of May. From previous studies, it is known that strawberry plants take from 7 - 22 days to initiate flowers depending on the temperature (Hartman, 1947b). The photoperiod requirement of each genotype at each site was rated by assigning either number 1 or 2, depending on whether they flowered only during SDs (1), or flowered under both SDs and LDs (2).

## Genetic linkage map

The genetic linkage map previously generated using the 62 progeny of the IP and 65 of the RP was used to detect QTL (Chapter 2). This map consisted of 387 markers and 42 linkage groups.

## QTL Analysis

The phenotypic data and molecular marker data for each individual of the mapping population was analyzed using the WIN QTL CARTOGRAPHER software (Basten, C.J., B.S. Weir and Z.-B. Zeng, 2002). Since this software is only capable of analyzing one type of maker data at one time, three different maps were constructed, two maps each using 1:1 segregating markers that were present in 'Tribute' and absent in 'Honeoye' and vice versa, as well as a map using 3:1 segregating markers that were present in both the parents. For each of these three maps, QTL mapping was performed as composite interval mapping (CIM) available with the WIN QTL CARTOGRAPHER (Basten, C.J., B.S. Weir and Z.-B. Zeng, 2002) software program. CIM gives better precision for QTL compared to the interval mapping (IM) method since CIM tests for QTL in one interval at a time and so, has the ability to control background effects of other QTL out side of the interval being tested. For all of the analyses, model 6 (standard model) of CIM was used with a window size of 10 cM. The background control marker number was kept to 5 markers, which were detected through a forward and backward stepwise regression. The LOD threshold for detecting QTL for each of the locations was tested by performing

1000 permutations. Two data sets were analyzed in Michigan; (1) phenotypic data on only the 65 progeny of the RP planted in the fall of 2004 and (2) phenotypic data on only the 62 IP planted in the spring of 2005.

The phenotypic variation explained by each QTL was obtained from the output of the analysis from the WIN QTL CARTOGRAPHER software (Basten C.J., B.S. Weir and Z.-B. Zeng, 2002). The estimation for the proportion of the phenotypic variation explained by each QTL was made using the square value of the partial correlation coefficient ($R^2$).

## Results and Discussion

In the previous studies done by Serçe and Hancock (2003 and 2005), genotypes were considered DN if they flowered during the SDs of spring and the LDs of summer from July 24th to August 24th. However, there was a chance that some DN genotypes were misclassified that flowered before this cutoff date. Some DN plants that are more sensitive to higher temperatures may begin flowering for a second time before the end of July when the longer day-lengths begin, but temperatures are not as high as in late July. Therefore, in 2005, we took data on flowering on a weekly basis from the first week of May until the last week of August. While less than 3 individual genotypes were found in

Michigan and Minnesota that flowered before July 24th, this number ranged from a 5 - 15 at the other locations.

Table 3.1 gives a summary of the QTL detected in Michigan, Minnesota, Maryland, Oregon and California. Any QTL that was observed above a LOD score of 2.0 is reported; however, an asterisk is used to denote those QTL that were above the threshold LOD score determined by the permutation test. The LOD thresholds determined at the 1 % significance level for Michigan, Minnesota, Maryland, Oregon and California were 3.2, 2.4, 4.01, 3.8 and 3.3 respectively. All the QTL associated with day-neutrality were derived from the cultivar 'Tribute'.

In the eastern states (Michigan, Minnesota and Maryland), a number of QTL were identified that were associated with day-neutrality, and all of these states shared a QTL on linkage group 17. For RP in Michigan, two QTL were identified (Table 3.1, Figure 3.1), both of which were on linkage group 17 and had $R^2$ values of 26.1 and 21.9 %. These two QTL were closest to markers aggcat187 and atgcag205. One QTL was detected for IP in Michigan over a LOD threshold of 3.2 (Table 3.1, Figure 3.2). This QTL was located on the linkage group 8 closest to marker atgctg249 and was responsible for 25 % of the total phenotypic variation. In Minnesota, 4 QTL were detected above the LOD threshold of 2.4 that were located in the linkage groups 7, 17, 20 and 28 (Table 3.1, Figure 3.3). The QTL on linkage group 7 was closest to marker agtcag305 and had an $R^2$ value of 14.43 %. The QTL detected on linkage group 17 was closest to the aggcat187 marker and was responsible for 20.1 % of the phenotypic variation. $R^2$ values of 11.48

and 13.04 % were obtained for the QTL detected on the linkage groups 20 (closest to marker agacaa174) and 28 (closest to marker agactc179). One QTL was detected in Maryland on linkage group 17, which was closest to marker aggcat187 (Table 3.1, Figure 3.4) and was responsible for 36 % of the phenotypic variation for day-neutrality.

These data suggest that there is likely a gene or genes on linkage group 17 that regulates day-neutrality in eastern US climates. It is unlikely that it is a single major gene, as it was responsible for at most, 36 % of the phenotypic variation. Several other location specific QTL were also identified, indicating the regulation of day-neutrality is multigenic, with a strong environmental interaction. A QTL on linkage group 17 was not identified in the Michigan IP, but it was handled quite differently to RP, being planted in the spring rather than the fall and as crowns instead of runners.

Only one significant QTL was identified in the segregating population evaluated in the western states (Oregon and California) of the US. A highly significant QTL was identified in linkage group 7 in California (Table 3.1, Figure 3.6) that was closest to marker agtcag225 and was responsible for 22 % of the phenotypic variation. A QTL in this linkage group was also identified in Minnesota and Michigan RP. No significant QTL were detected in Oregon at the threshold LOD score of 3.8 (Table 3.1, Figure 3.5), although one on Chromosome 14 (closest to marker aggctg217) was just below the 3.13 LOD threshold cut off point at the 5 % significance with a LOD score of 3.11.

It is not known why fewer QTL were identified in the western than eastern states of USA, but it can be speculated that the different temperature patterns found in these regions may have played a role. From studies done on the physiology of flowering, it is known that there is a strong temperature/photoperiod interaction that determines flowering in the strawberry. When average high temperatures are below 15 $^0$C, all genotypes tend to behave in a photoperiod insensitive manner (Hancock, 1999). Michigan, Minnesota and Maryland had average mid-summer temperatures ranging above this threshold from 16 to 18 $^0$C and they had similar percentages of DN progeny (49.23 %, 50 % and 48 % respectively). Oregon and California had much lower temperatures during the LDs of mid-summer that were below the threshold (11 and 13 $^0$C) and they had a much higher percentage of DN plants (80 and 87 %). Twenty-seven of the genotypes that did not flower under LDs in the eastern states flowered in the west because of this difference in temperature patterns.

This suggests that different genes may be responsible for regulating day-neutrality in the eastern vs. western states of the US. There may be a gene or genes regulating temperature insensitivity that must be present to allow for DN flowering in the warmer eastern climates. The QTL identified on LG 17 may be such a gene. In the cooler western states, this gene may not play a role in the expression of day-neutrality, as temperatures are too mild to need its expression for the trait. This may contribute to the reason why the California DN cultivars have not performed as well in eastern strawberry growing states than western ones. Depending on environmental conditions, different loci in the genome of strawberry may become important for flowering.

In conclusion, day-neutrality does not appear to be under single major gene control, as we did not observe any one QTL that explained more than 40 % of the phenotypic variation at any location. Day-neutrality may indeed be quantitative in nature; as we identified numerous QTL with modest effects ranging from 15 – 40 %. It is possible that a strong, dominant locus regulating day-neutrality does exist, and we didn't uncover a marker tightly linked to it as in Sugimoto et. al. (2005); however, the regulation of day-neutrality still has to be considered under polygenic control, as multiple QTL were detected at the various locations.

Considering there are 28 chromosomes in the strawberry genome and we have identified a higher number of linkage groups (42), many more segregating markers will need to be added to the map to have complete genome coverage. Better map coverage with more AFLP markers and SSR markers, will allow us to more precisely estimate QTL locations and may result in the identification of more genes regulating day-neutrality. An evaluation of our total population of 422 individuals will also lead to better map resolution. We have already started genotyping the remaining of the 422 individuals of the mapping population with AFLPs and the entire RP has been phenotyped in the field. The population will again be observed for their weekly flowering habit next year (2006) in Michigan, allowing us to test the reproducibility of the QTL in multiple years. In addition, work has been initiated by one of our collaborators, Kim Lewers, to genotype the population using SSRs developed from strawberry EST libraries. These data will

allow us to produce a much denser map and transfer our QTL results between different segregating populations.

**Table 3.1**: QTL for day-neutrality that were detected in a segregating population of 'Tribute' x 'Honeoye' grown in five different states. All QTL over a LOD of 2.0 are listed, but an asterisk is used to denote QTL with a LOD value significant at 1 % level. In Michigan, two separately planted populations were evaluated, one that had been studied in previous work (initial population - IP) and one newly reconstructed for the present study (reconstructed population - RP). In Minnesota, Maryland, Oregon and California, only RP was evaluated. The percent phenotypic variation is indicated by the square value of the partial correlation coefficient ($R^2$). QTL are named according to the population, state, year and a number assigned in descending order of the LOD value.

| Environment | QTL | LG | Closest Marker | LOD | R2 |
|---|---|---|---|---|---|
| **Michigan (IP)** | IPMI05-1* | 8 | atgctg249 | 3.3644* | 25% |
| **Michigan (RP)** | RPMI05-1* | 17 | aggcat187 | 6.252* | 26.10% |
| | RPMI05-2* | 17 | atgcag205 | 5.3382* | 21.90% |
| | RPMI05-3 | 17 | atgctg248 | 2.6339 | 12% |
| | RPMI05-4 | 7 | agtcag225 | 2.3618 | 19.70% |
| | RPMI05-5 | 8 | atacca253 | 2.3497 | 11.50% |
| | RPMI05-6 | 2 | agtcta172 | 2.1343 | 7.90% |
| **Minnesota** | RPMN05-1* | 17 | aggcat187 | 4.8158* | 20.10% |
| | RPMN05-2* | 7 | agtcag305 | 2.7796* | 14.43% |
| | RPMN05-3* | 28 | agactc179 | 2.7679* | 13.04% |
| | RPMN05-4* | 20 | agacaa174 | 2.7569* | 11.48% |
| **Maryland** | RPMD05-1* | 17 | aggcat187 | 6.4563* | 36% |
| | RPMD05-2 | 29 | atgcag135 | 2.4505 | 11.70% |
| | RPMD05-3 | 1 | acacag380 | 2.3314 | 13% |
| **Oregon** | RPOR05-1 | 14 | aggctg217 | 3.1127 | 15% |
| | RPOR05-2 | 17 | atgcag205 | 2.8089 | 14.60% |
| **California** | RPCA05-1* | 7 | agtcag225 | 4.6717* | 22% |
| | RPCA05-2 | 10 | agactg228 | 2.6086 | 11% |
| | RPCA05-3 | 5 | atacgc286 | 2.2766 | 8.60% |

**Table 3.2**: Average minimum and maximum temperatures, and percent day-neutral progeny observed at the various study locations used to detect QTL for day-neutrality in a segregating population of 'Tribute' x 'Honeoye'. The various study sites were located at Benton Harbor, Michigan (MI), St. Paul, Minnesota (MN), Beltsville, Maryland (MD), Corvallis, Oregon (OR) and Watsonville, California (CA).

| **Location** | **MI** | **MN** | **MD** | **OR** | **CA** |
|---|---|---|---|---|---|
| **% DN plants** | **49.23%** | **50%** | **48%** | **80%** | **87.30%** |
| **Temp. SDs (April)** | | | | | |
| average min | $5^0$C | $5^0$C | $4^0$C | $4^0$C | $7^0$C |
| average max | $17^0$C | $16^0$C | $17^0$C | $16^0$C | $19^0$C |
| **Temp. LDs (July)** | | | | | |
| average min | $16^0$C | $18^0$C | $17^0$C | $11^0$C | $13^0$C |
| average max | $29^0$C | $29^0$C | $28^0$C | $27^0$C | $22^0$C |

**Figure 3.1**: QTL for day-neutrality detected in the RP progeny of 'Tribute' x 'Honeoye' evaluated in Benton Harbor, Michigan, 2005. This population was newly constructed for the present study (see text for details). Two QTL were detected at the LOD threshold of 3.2 in linkage group 17 closest to markers aggcat187 and atgcag205.

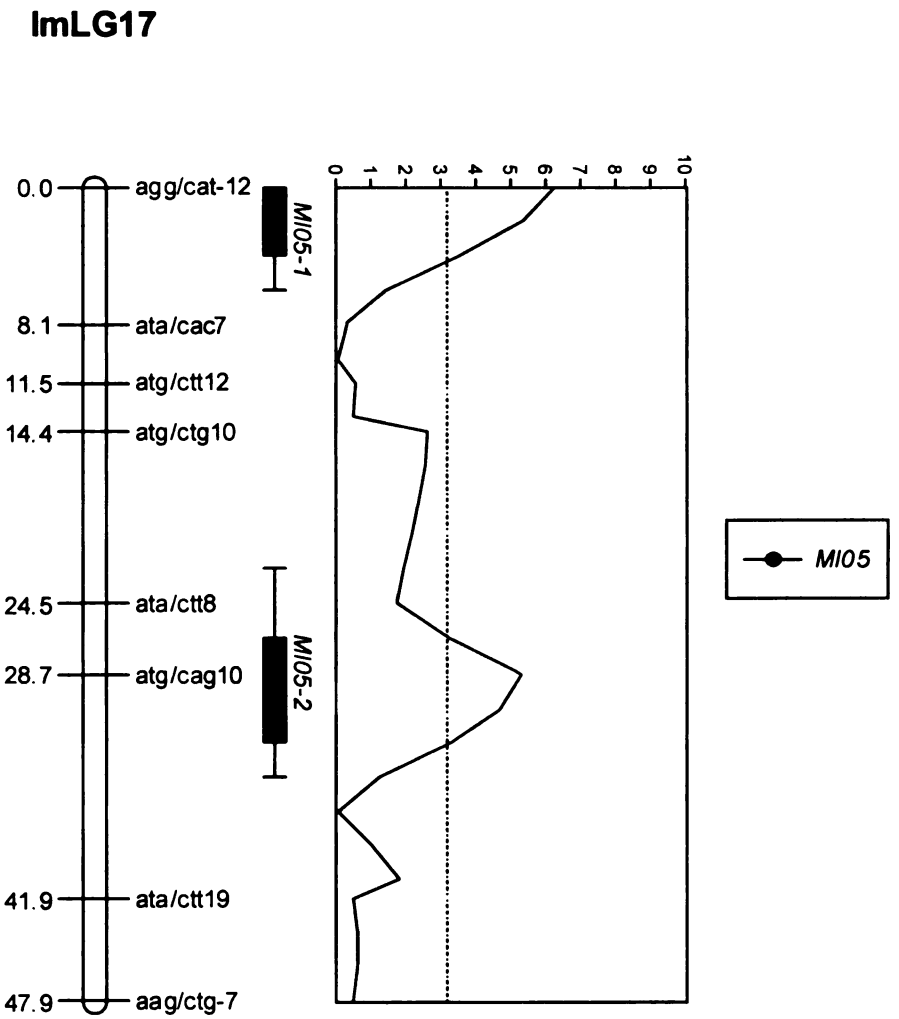


**Figure 3.2**: QTL for day-neutrality detected in the IP progeny of 'Tribute' x 'Honeoye' evaluated in Benton Harbor, Michigan, 2005. This population had been studied in previous work (see text for details). One QTL was detected at the LOD threshold of 3.2 in linkage group 8 closest to marker atgctg249.

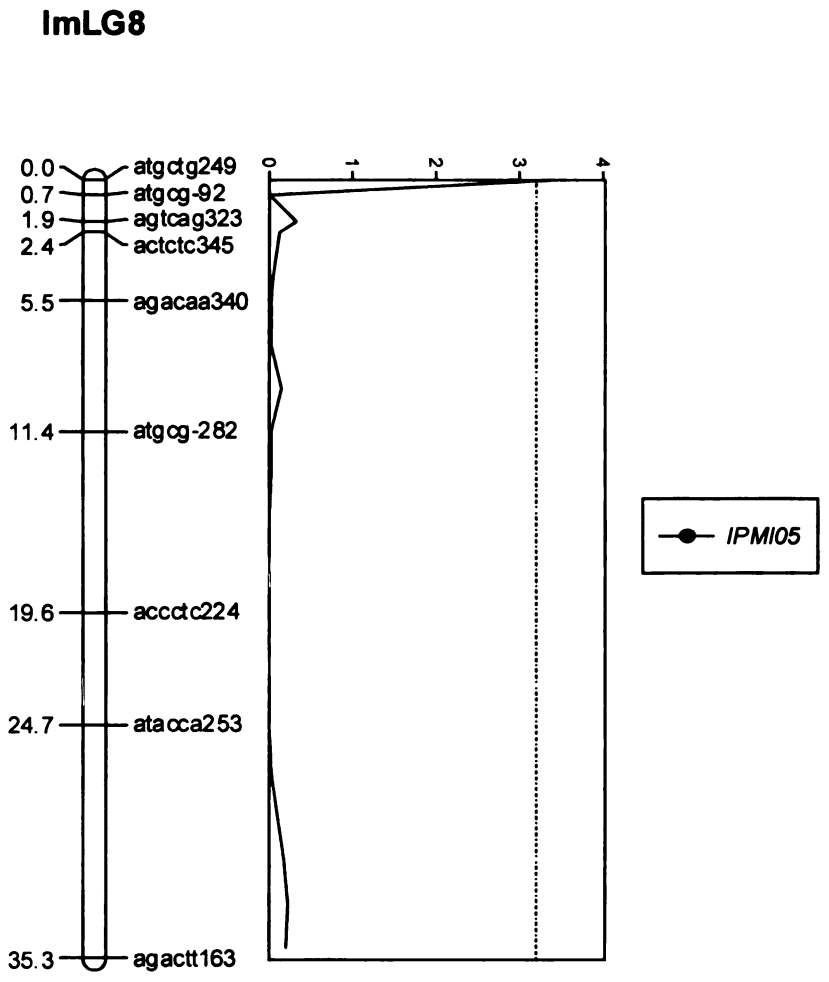


**Figure 3.3**: QTL for day-neutrality detected in the RP progeny of 'Tribute' x 'Honeoye' evaluated in St. Paul, Minnesota, 2005. This population was newly constructed for the present study (see text for details). Four QTL were detected at the LOD threshold of 2.4 in linkage groups 7, 17, 20 and 28 closest to markers agtcag305, aggcat187, agacaa174 and agactc179 respectively.

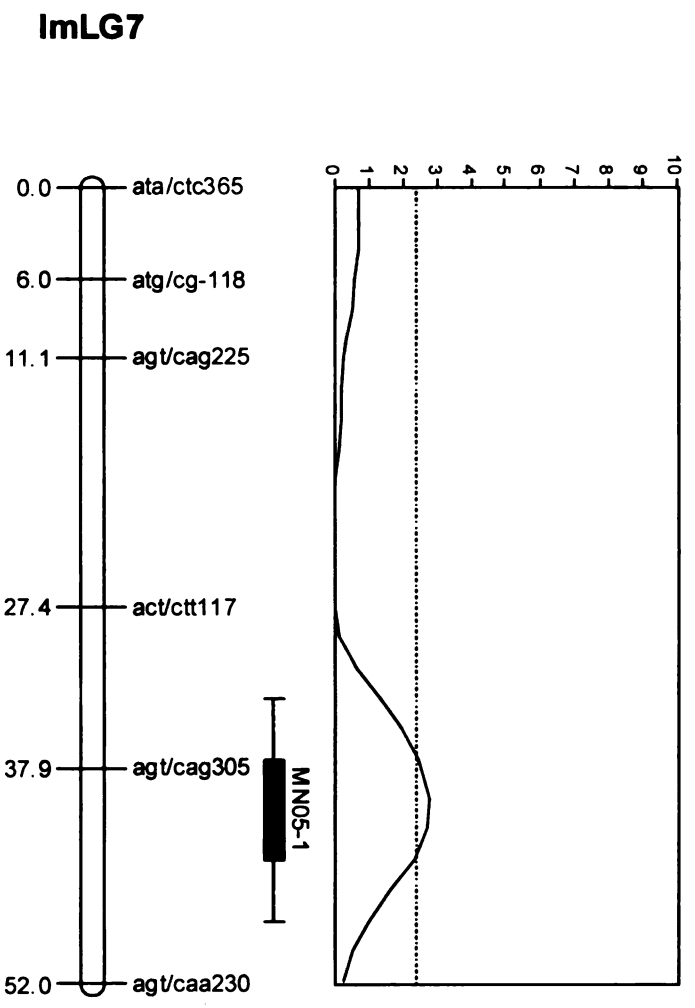


**Figure 3.3** (Cont'd).

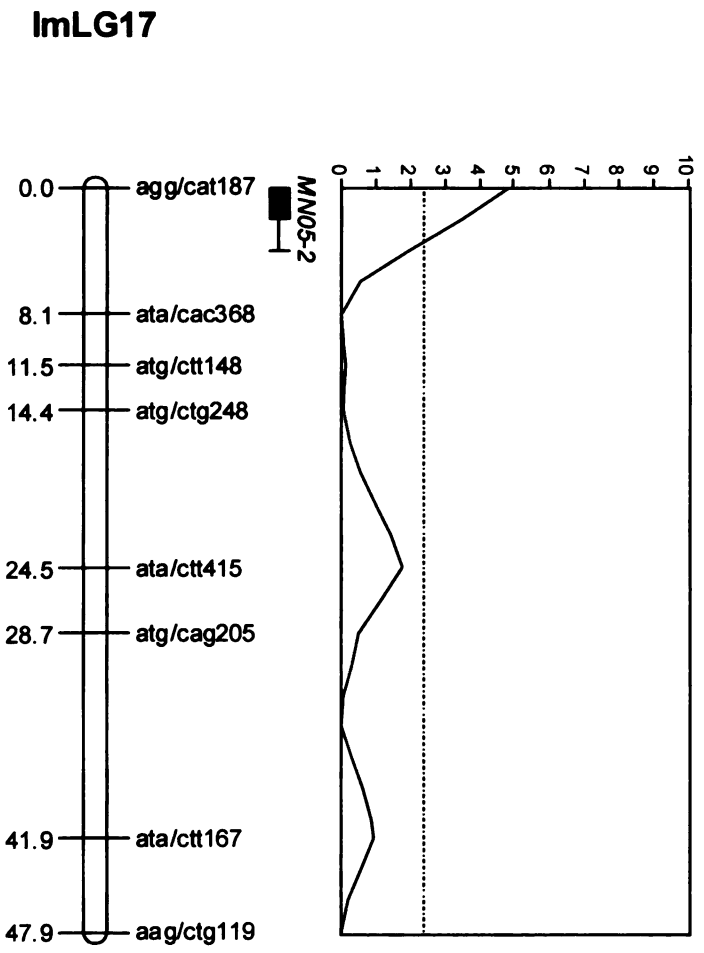


**Figure 3.3** (Cont'd).

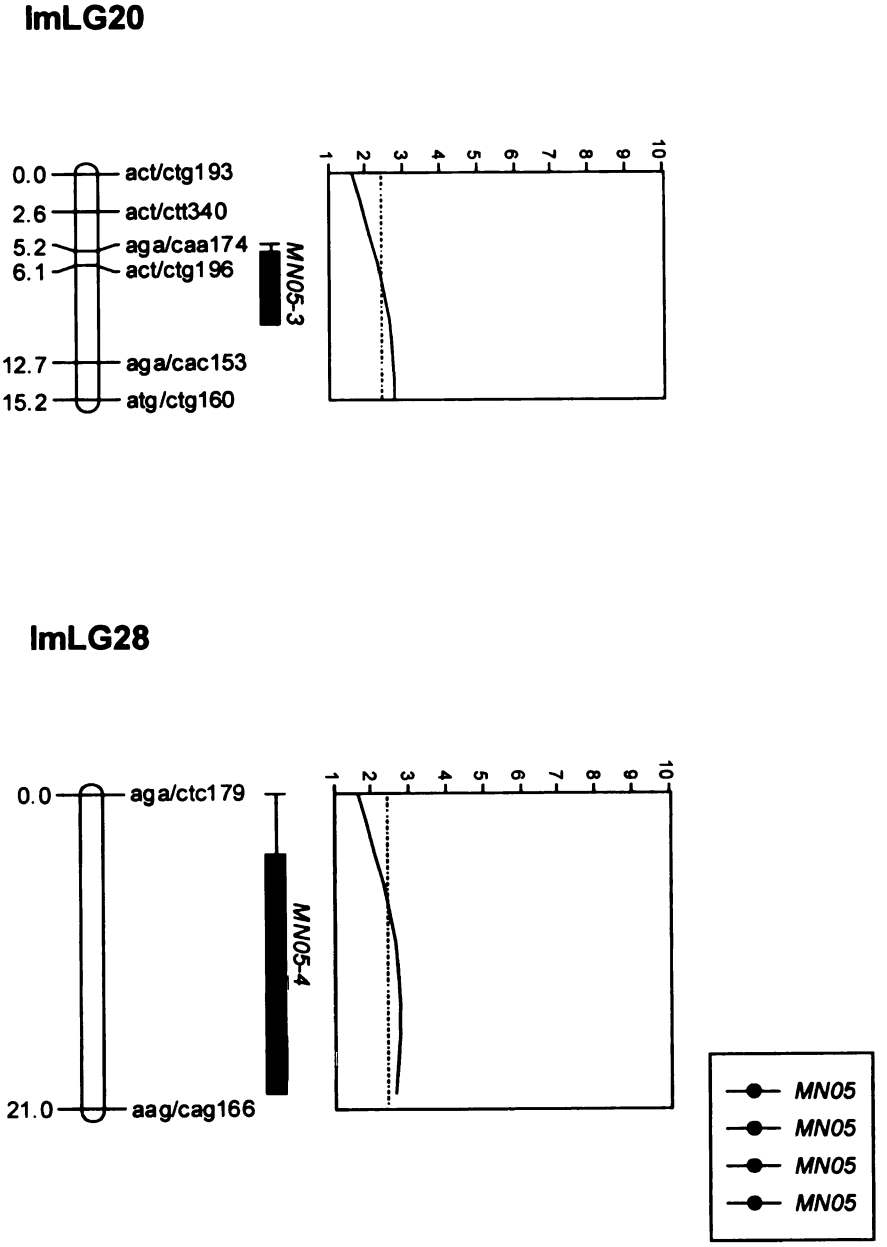


**Figure 3.4**: QTL for day-neutrality detected in the RP progeny of 'Tribute' x 'Honeoye' evaluated in Beltsville, Maryland, 2005. This population was newly constructed for the present study (see text for details). Only one QTL was detected at the LOD threshold of 4.01 in linkage group 17 closest to marker aggcat187.

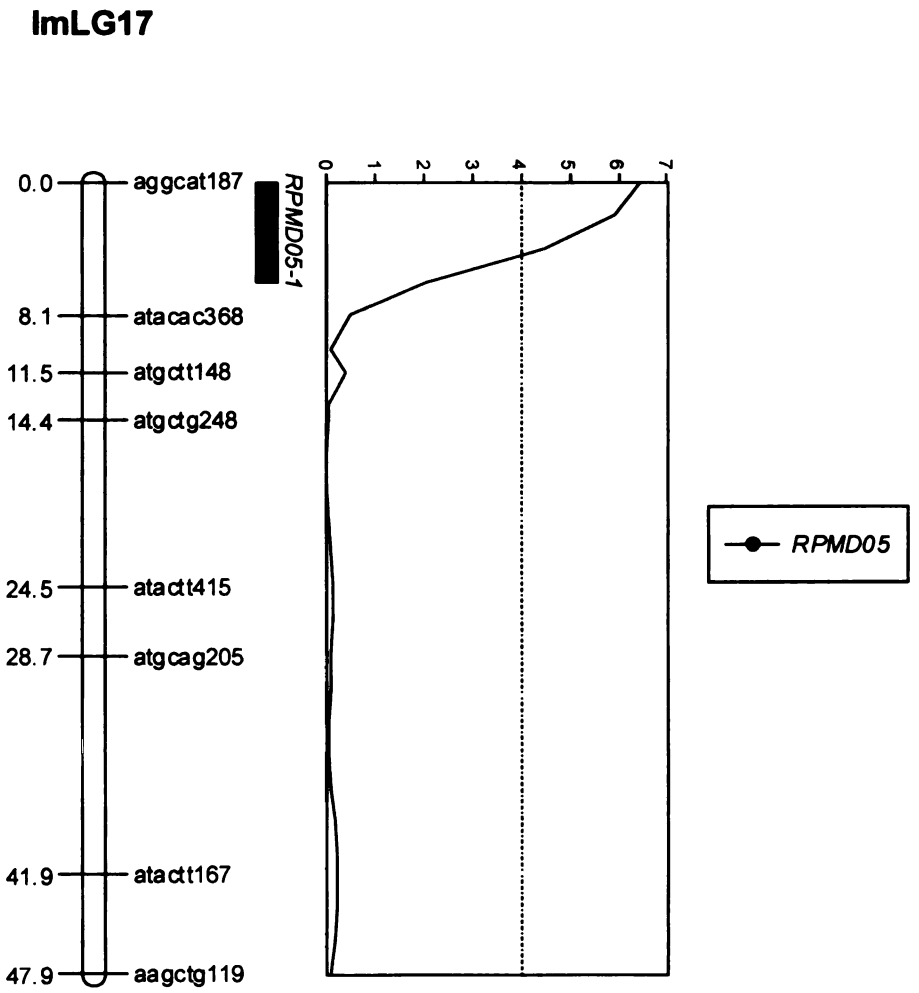


**Figure 3.5**: QTL for day-neutrality detected in the RP progeny of 'Tribute' x 'Honeoye' evaluated in Corvallis, Oregon, 2005. This population was newly constructed for the present study (see text for details). Two QTL were identified with a LOD score above 2.0, but they were below the 5% LOD threshold of 3.13.

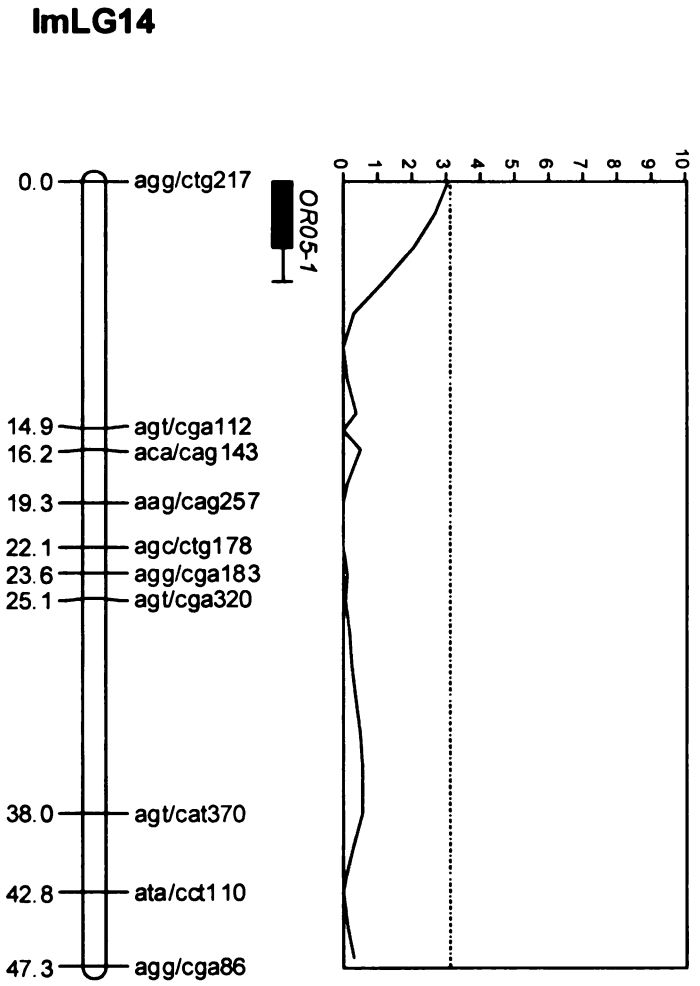


**Figure 3.5** (Cont'd).

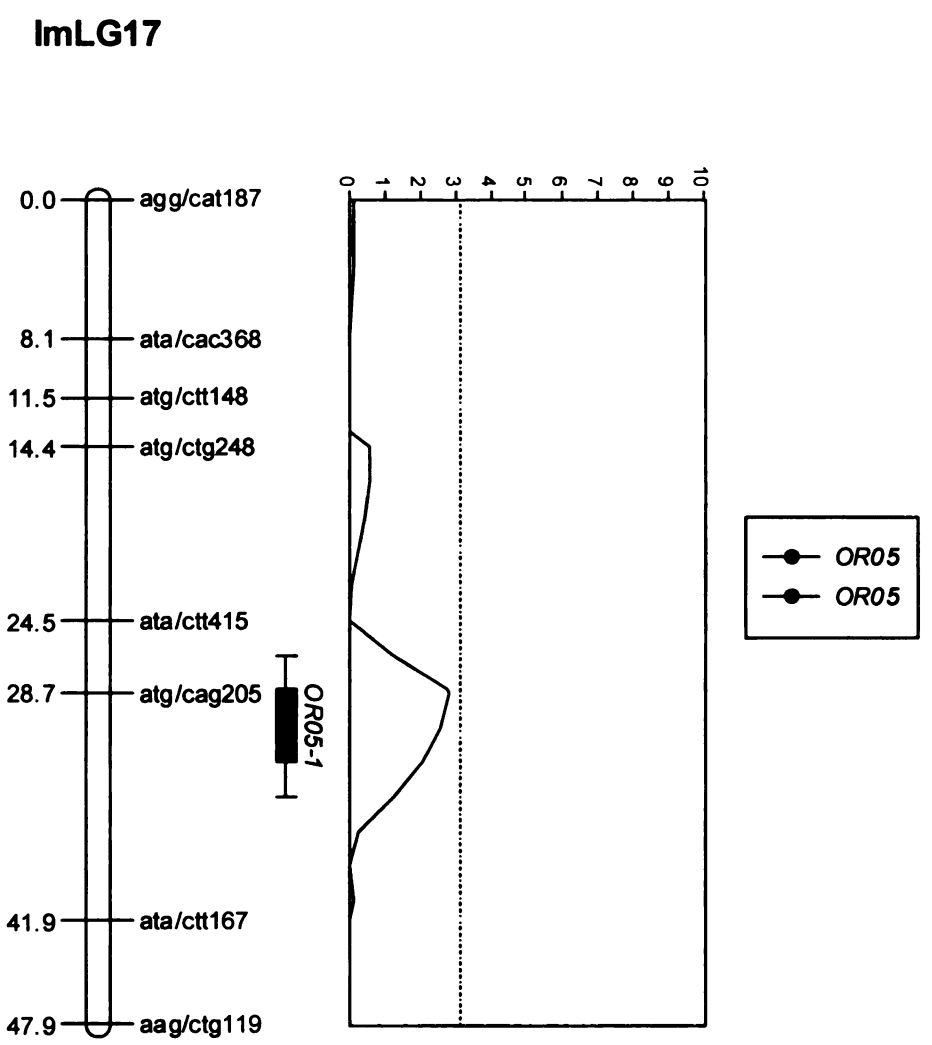
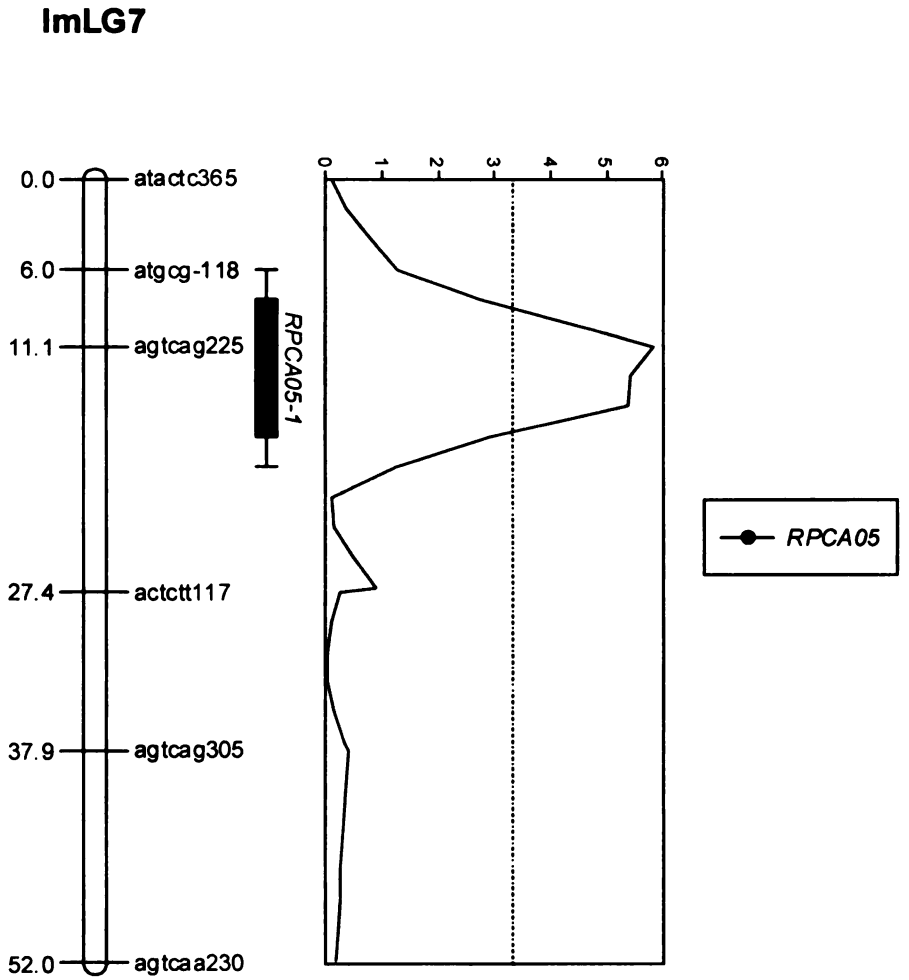


**Figure 3.6**: QTL for day-neutrality detected in the RP progeny of 'Tribute' x 'Honeoye' evaluated in Watsonville, California, 2005. This population was newly constructed for the present study (see text for details). Only one QTL was detected at the LOD threshold of 3.3 in linkage group 7 closest to marker agtcag225.



## References


Ahmadi H., R.S. Bringhurst and V. Voth. 1990. Modes of inheritance of photoperiodism in *Fragaria*. J. Amer. Soc. Hort. Sci. 115:146-152.

Basten C.J., B.S. Weir and Z.-B. Zeng (2002). QTL Cartographer, Version 1.16. Department of Statistics, North Carolina State University, Raleigh, NC.

Bringhurst R.S. and V. Voth. 1978. Origin and evolutionary potentiality of the day-neutral trait in octoploid *Fragaria*. Genetics 90:510 (Abstract).

Bringherst R.S. and V. Voth 1984. Breeding octoploid strawberries. Iowa State J. Res. 58:371-381.

Clark J.H. 1937. Inheritance of so-called everbearing tendency in the strawberry. Proc. Amer. Soc. Hort. Sci. 35:67-70.

Durner E.F., J.E. Barden, D.G. Himelrick and E.B. Poling. 1984. Photoperiod and temperature effects on flower and runner development in day-neutral, junebearing and everbearing strawberries. J. Amer. Soc. Hort. Sci. 109:396-40.

Galletta G.J., A.D. Draper and H.J. Swartz. 1981. New everbearing strawberries. HortScience 16:726.

Hancock J.F. 1999. Strawberries. CAB International, Wallingford, UK.

Hancock J.F., J.J. Luby, A. Dale, P.W. Callow, S. Serçe and A. El-Shiek. 2001. Utilizing wild *Fragaria virginiana* in strawberry cultivar development: Inheritance of photoperiod sensitivity, fruit size, gender, female fertility and disease resistance. Euphytica 126:177-184.

Hartman H.T., 1947. The influence of temperature on the photoperiodic response of several strawberry varieties grown under controlled environment conditions. J. Amer. Soc. Hort. Sci. 50:243-245.

Manakasem Y. and P.B. Goodwin 2001. Response of day neutral and junebearing strawberries to temperature and day length. J. Hort. Sci. & Biotech. 76 (5):629-635.

Orecky D.K. and G.L. Slate 1967. Behavior of the everbearing characteristics in strawberries. J. Amer. Soc. Hort. Sci. 91:236-241.

Patterson A.H., Daman S., Hewitt J.D., Zamir D., Rabinowitch H.D., Lincoln S.E., Lander E.S., and S.D. Tanksley, 1991. Mendelien factors underlying quantitative traits in tomato: comparison across species, generations and environments. Genetics 127: 181-197.

Powers L., 1954. Inheritance of period of blooming in progenies of strawberries. Proc. Amer. Soc. Hort. Sci. 64:293-298.

Shaw D.V., 2003. Heterogeneity of segregation ratios from selfed progenies demonstrate polygenic inheritance for day neutrality in strawberry (*Fragaria ×ananassa* Duch.). J. Amer. Soc. Hort. Sci. 128:504-57.

Serçe S. and J.F. Hancock, 2003. Assessment of day-neutral scoring methods in strawberry families grown in greenhouse and field environments. Turk. J. Agric. For. 27:191-198.

Serçe S. and J.F. Hancock, 2005. Inheritance of day-neutrality in octoploid species of *Fragaria*. J. Amer. Soc. Hort. Sci. 130:580-584.

Sugimoto T., Tamaki, K., Matsumoto, Y., Shiwaku, K., and K. Watanabe, 2005. Detection of RAPD markers linked to the everbearing gene in Japanese cultivated strawberry. Plant Breeding 124:498-501.