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Using a linkage mapping approach to identify QTL for day-neutrality in the octoploid strawberry

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Abstract

A linkage mapping approach was used to identify quantitative trait loci (QTL) associated with day-neutrality in the commercial strawberry, *Fragaria* × *ananassa* (Duch ex Rozier). Amplified Fragment Length Polymorphism (AFLP) markers were used to build a genetic map with a population of 127 lines developed by crossing the day-neutral (DN) 'Tribute' with the short-day (SD) 'Honeoye'. The population was genotyped with AFLP markers and 429 single dose restriction fragments (SDRF) were placed on a consensus map of 1541 cM with 43 linkage groups. Individuals from 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). Eight QTL were found that were either location specific or shared among locations. None of these QTL explained >36% of the phenotypic variation, indicating that the inheritance of day-neutrality is likely a polygenic trait.

Key words: *Fragaria* × *ananassa* — everbearing strawberries — photoperiod insensitivity — AFLPs — QTL mapping

Two primary types of commercial strawberries are grown, short-day (SD) and day-neutral (DN). SD genotypes or June-bearers, initiate flower buds either under SD conditions (< 14 h of day length) or at temperatures below 15°C, while DN genotypes are photoperiod insensitive and will initiate flowers under any photoperiod conditions as long as temperatures are moderate (Darrow 1966, Hancock 1999). Day-neutrality was most recently introduced into modern cultivars by Bringhurst and Voth (1984), using a native genotype of *F. virginiana* (Mill) ssp. *glauca* (S. Watson) Staudt from the Wasatch Mountains of Utah.

To date, the genetics of day-neutrality in strawberries have remained elusive. Several different models have been proposed including: (i) regulation by a single dominant gene (Bringhurst and Voth 1978, Ahmadi et al. 1990); (ii) regulation by dominant complementary genes (Ourecky and Slate 1967); and (iii) quantitative inheritance (Powers 1954, Hancock et al. 2001). The reason why these studies generated different hypotheses may be that they utilized different sets of parents and were conducted in different environments. The study of Ourecky and Slate (1967) was conducted in New York using material that had not recently had any new *F. virginiana* germplasm incorporated. The studies of Powers (1954) and Hancock et al. (2001), were performed in Wyoming and Michigan, respectively, using DN parents that carried genes from *F.* × *ananassa* and wild clones of *F. virginiana* that were different from the Wasatch source. The studies of Bringhurst and Voth (1978) and Ahmadi et al. (1990) were performed in CA using University of California-Davis breeding parents carrying the Wasatch source of day-neutrality. There was one study in CA that suggested day-neutrality may have a quantitative basis (Shaw 2003), but it was later refuted by a more extensive statistical analysis of a greater number of progeny populations (Shaw and Famula 2005). Sugimoto et al. (2005) found a RAPD-marker linked to a dominant gene regulating day-neutrality in a Japanese breeding population carrying the Wasatch source of day-neutrality.

To evaluate the performance of a more diverse set of genotypes in a wider range of environments, Hancock et al. (2001) crossed SD and DN representatives of native *F. virginiana* with SD and DN *F.* × *ananassa* cultivars from several US breeding programs and then evaluated the progeny in Michigan (MI), Minnesota (MN) and Ontario (ON). They detected a wide variation in the percentage of DN plants that were produced by each DN parent, which is consistent with polysomic inheritance. They also observed a significant difference in the expression of day-neutrality across locations, indicating a strong environmental component. The highest proportion of DN progeny was found in ON, which also had the coolest summer temperatures. Serçe and Hancock (2005) then made partial dialled crosses among 12 of the SD and DN genotypes of California cultivars, eastern cultivars and a group of wild genotypes that had been shown in Hancock et al. (2001) to produce different frequencies of DN progeny. Wide ranges in the percent of DN progeny were again observed among the families, suggesting quantitative inheritance. Several two-gene models fit more of the DN : SD ratios in more of the families than the single locus model, but none of these simple models fit the DN segregation ratios at the ends of the distribution range.

Herein, we use linkage mapping and a QTL approach to determine the number of loci regulating day-neutrality in one of the families studied by Serçe and Hancock (2005) that did not deviate significantly from a 1 : 1 progeny ratio of

DN : SD. We first generated a map using amplified fragment length polymorphism (AFLP) markers and then phenotyped replicate plants at five different locations in the USA including CA, MD, MI, MN and OR. Our data support the polygenic inheritance of day-neutrality, as a number of QTL were identified that were either shared or location specific, and none of these QTL explained >36% of the phenotypic variation.

Materials and Methods

Mapping population: The mapping population of strawberry (*Fragaria xananassa* Duch ex Rozier) was 'Tribute' × 'Honeoye'. 'Tribute' is thought to have received its genes for day-neutrality from a CA breeding parent derived from the Wasatch genotype of *F. virginiana* (Galletta et al. 1981), and is one of the two DN cultivars released in the last 30 years for the eastern USA. 'Honeoye' has been one of the most popular SD cultivars grown in the mid-western and north-eastern USA over the last 2 decades.

A total of 127 genotypes were evaluated to build the map. Sixty-two individuals came from the original population of Serçe and Hancock (2005) and another 65 genotypes were from a newly generated population using the same parents. The plants were maintained together in a single greenhouse in East Lansing, MI, USA. DNA was extracted from young, lyophilized leaves according to Haymes (1996), with the addition of an extra chloroform/isoamyl alcohol (24 : 1) extraction.

The Vos et al. (1995) protocol was used for AFLP analysis, with the modifications of Vallejo and Kolkman (2002). Sixty-nine AFLP primer combinations were evaluated using *Eco*R1 and *Mse*1 (Table 1). We also examined 32 of the primer combinations used by Lerceteau-Köhler et al. (2003) to generate their linkage map of strawberry, but none of these primers produced the same segregating markers in our population. 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 + ATG. Each sample was loaded onto a 6% denaturing polyacrylamide gel, which was allowed to run for about 150 min after which the plates were separated and silver-stained. The size of every polymorphic fragment scored was estimated by comparing it with 10 and 50 bp ladders run on either side of the parents and progeny.

Map construction and visual presentation: The linkage mapping was performed with those AFLP markers that segregated as single dose restriction fragments (SDRF). These were the markers 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. A statistical analysis was performed to test goodness of fit at 5% level and only those 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 and 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 (Voorrips 2002) was used to draw the maps of the linkage groups.

Obtaining phenotypic data: Seeds from the newly generated cross of 'Honeoye' × 'Tribute' were germinated in January 2004 and when the resulting seedlings had 4–6 leaves they were transplanted into a commercial soil mix in 14 × 12 × 12 cm pots. The plants were maintained in a greenhouse in East Lansing and allowed to runner. On 10 July, at least five replicate runners from each genotype was collected and moved to a mist house where they were set into

Table 1: AFLP primer combinations and the number of polymorphic fragments (PF) scored in a progeny population of 'Tribute' × 'Honeoye' segregating for day-neutrality

Primer[2]	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*[1]	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
							aga/cac*	4
					act/cag	14	aga/cag*	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				

[1]The primers previously used by Lerceteau-Köhler et al. (2003) are indicated with an asterisk.
[2]E = *Eco*R1 primers, M = *Mse*1 primers.

2.5 × 2.5 cm cell packs filled with a commercial soil mix. The plants were maintained in the mist house for a week until they were rooted, and then transferred to a greenhouse for two more weeks. In the last week of July, each of the runner plants with 2–4 leaves were packed into separate zipper bags and shipped overnight to CA (Watsonville), OR (Corvallis), MN (St Paul) and MD (Beltsville).

The runner plants were held for three weeks in a greenhouse before field planting in MD (the USDA–ARS Beltsville Agricultural Research Center in Beltsville), MI (Southwest Michigan Research and Extension Center, Benton Harbor), MN (University of Minnesota Horticultural Research Center, Victoria) and OR (Oregon State University, Department of Horticulture Vegetable Farm, Corvallis). In CA, the plants were kept for 4 months in a screen house before being field planted at Aromas, The Company Ranch (TCR). Plants were set at 1.2 × 1.2 m spacing in MI, and OR. In MD and MN, plants were set 0.3 × 0.3 m apart and 0.45 × 0.45 m apart, respectively, on black plastic, while in CA, the plants were set at 0.36 m × 0.36 m spacing in a green, semi-opaque plastic mulch. A completely randomized-planting design was used at all locations.

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 LD (Manakasem and Goodwin 2001), we considered a plant to be DN only if it flowered after the 15 June, allowing over a month for any SD initiated flowers to develop. At all locations, days become longer than 14 h after the first week of May. From previous studies, it is known that strawberry plants take from 7 to 22 days to initiate flowers depending on the temperature (Hartman 1947). The photoperiod requirement of each genotype at each site was rated by assigning a number of 1 or 2, depending on whether they flowered only during SD (1), or flowered under both SD and LD (2).

There was a distinct separation between the two groups of DN and SD progeny with respect to whether the plants repeatedly flowered. The DN progeny were not evaluated as strong or weak, or according to the number of weeks they flowered, as performed in the previous studies, because we wanted to identify only those genes associated with photoperiod sensitivity and not those regulating the strength of flowering. The only shared condition in across sites was the day length; hence, the current method of analysis of the two groups using the number of seasons flowered.

QTL analysis: The phenotypic and molecular marker data for each individual of the mapping population was analysed using the WIN QTL CARTOGRAPHER software (Wang et al., 2007). As this software is only capable of analysing one type of marker data at a 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 software program. For each analysis, CIM model 6 (standard model) was used with a window size of 10 cM. The background control marker number was kept to five, which was detected through a forward and backward stepwise regression. The LOD threshold for declaring QTL significant for each of the locations was determined with 1000 permutations (Churchill and Doerge 1994). 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

Genetic linkage map

Sixty-nine AFLP primer combinations were used to genotype the 127 individuals in the mapping population (Table 1). 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 SDRF 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 (4.8%) significantly fit more than one complex ratio.

Of the 539 SDRF markers uncovered, 383 segregated in a 1 : 1 fashion and 156 segregated in a 3 : 1 fashion. Four hundred and twenty-nine of these markers were placed on the consensus map, which consisted of 43 linkage groups (based on the chromosome number 28 linkage groups are expected). The rest of the SDRF markers were either not linked to any of the recognized linkage groups or were not included because their segregation pattern conflicted with other markers in the same linkage group at a LOD score of 3.0.

The longest linkage group (LG) of our map was LG 5, which is 94 cM in length with 19 markers (Fig. 1). This group had a marker density of 0.20 markers/cM and an average distance of 4.9 cM between markers. The shortest linkage group was LG 21, which had only two markers that mapped to the same locus. The densest of our linkage groups were LG 25 and LG 31, which had a marker density of 1.0 markers/cM, but there were only two markers on each of these linkage groups.

Phenotypic and QTL analysis

The proportion of DN progeny varied greatly across locations. In the eastern states, MD, MI and MN, the proportion of DN plants did not vary significantly ($P < 0.05$) from a 1 : 1 ratio, with 48% to 50% of the progeny being DN. In the western states, the ratios were significantly skewed towards DN, with the DN proportions being 80% in OR and 87% in CA.

Figure 2 summarizes the QTL detected in MI, MN, MD, OR and CA. Although three different individual maps had to be constructed to be compatible with the QTL analysis software, markers of these individual maps could be aligned with the consensus map (Fig. 1) using common markers. In all but a few cases, the markers were ordered similarly in the individual and consensus maps. Therefore, the linkage groups of Fig. 2 are labelled with the corresponding group of the consensus map. The LOD thresholds determined at the 1% significance level for MI, MN, MD, OR and CA were 3.2, 2.4, 4.0, 3.8 and 3.3, respectively. All the QTL associated with day-neutrality were derived from 'Tribute'.

Five QTL were identified for day-neutrality in the eastern states (MI, MN and MD). In MI, two QTL were identified, both of which were on LG 28 (Fig. 2) and had R^2 values of 26.1% and 21.9%, respectively. These two QTL were closest to markers aggcat187T and atgcag205T. In MN, four QTL were detected above the LOD threshold of 2.4, which were located on LG 6-2, 28, 1-2 and 3-1 (Fig. 2). The QTL on LG 6-2 was closest to marker agtcag305T and had an R^2 value of 14.4%. The QTL detected on LG 28 was closest to the aggcat187T marker and was responsible for 20.1% of the phenotypic variation. R^2 values of 11.5% and 13.0% were obtained for the QTL detected on LG 1-2 (closest to marker agacaa174T) and 3-1 (closest to marker agactc179T). One QTL was detected in MD on LG 28, which was closest to marker aggcat187T

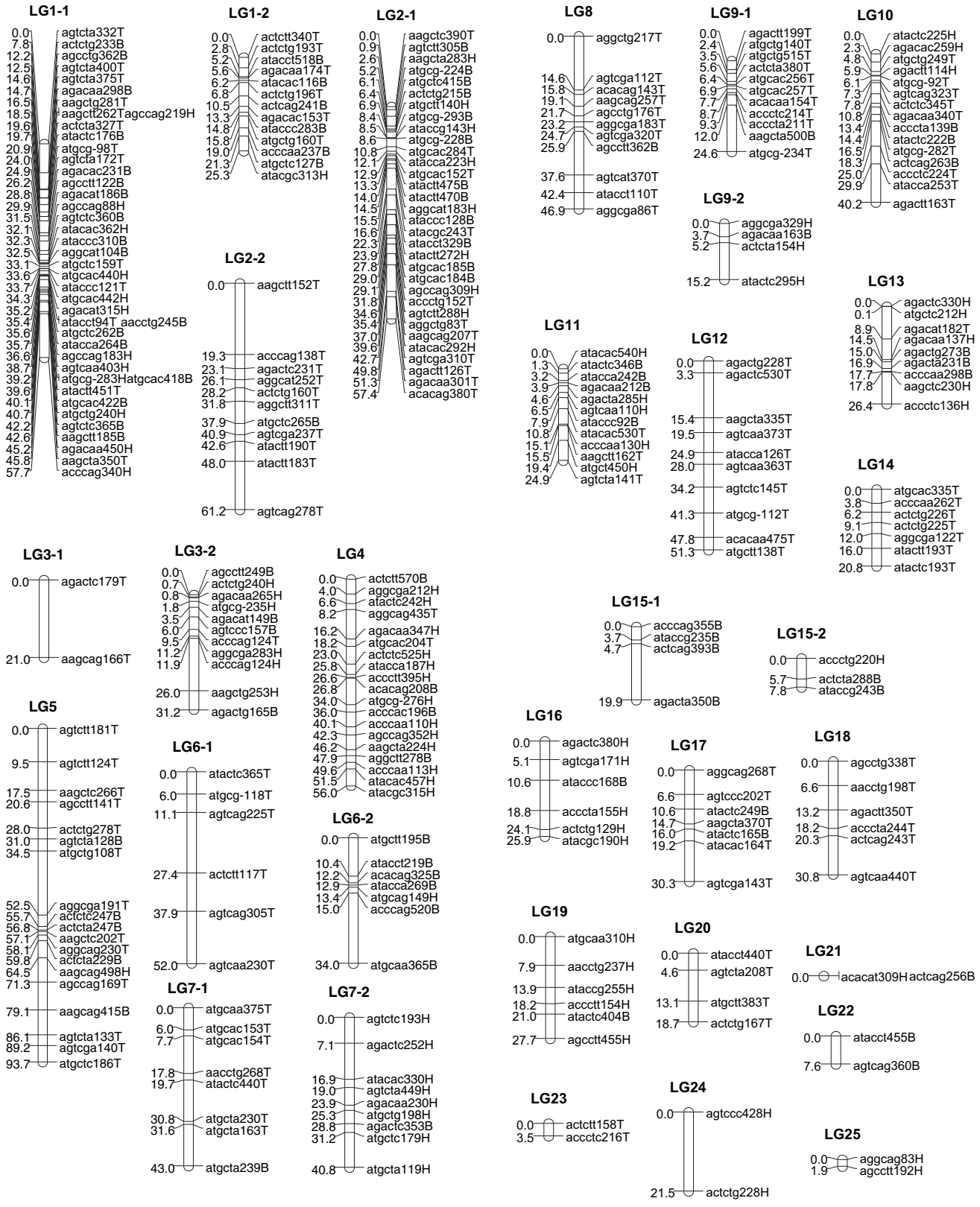

Fig. 1: AFLP consensus genetic linkage map for the 'Tribute' × 'Honeoye' mapping population. Markers on the right are identified by the AFLP primer combination, the fragment size and whether the marker was only present in 'Tribute' (T), only present in 'Honeoye' (H) or, if marker was present in both parents (B)

(Fig. 2) and was responsible for 36.0% of the phenotypic variation for day-neutrality. This QTL was identified in all three eastern states.

Only one significant QTL was identified in the segregating population evaluated in the western states of OR and CA. This QTL was found on LG 6-2 in CA (Fig. 2), close to marker

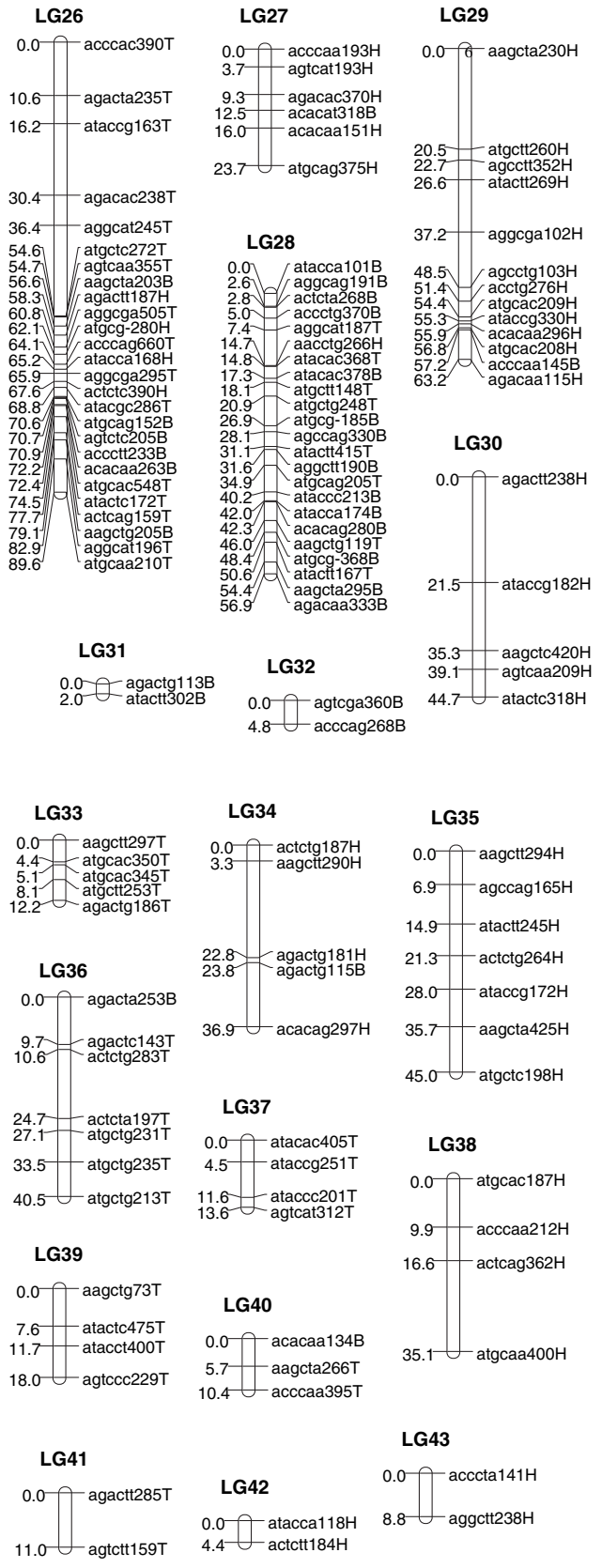

Fig. 1: Continued

agtcag225T and was responsible for 22.0% of the phenotypic variation. A LOD peak of (LOD 2.36 and $P < 0.2$) was identified at this same location in MI with an R^2 value of 19.70% although it was not above the LOD threshold. The QTL located on linkage groups 6–2 for MN was not in the same region as that of CA. No significant QTL were detected in OR at the threshold LOD score of 3.8, although one on LG 8 (closest to marker aggctg217T) was just below the 3.13 LOD threshold cut off point at the 5% significance with a LOD score of 3.11 ($P < 0.063$).

Discussion

Day-neutrality is a polygenic trait in the population we evaluated. A number of QTL were identified that were either shared or were location specific, and none of these QTL explained more than 36% of the phenotypic variation. At all three eastern sites, one QTL was identified on LG 28 that was a strong regulator of day-neutrality; additional QTL were identified in MI on LG 28 and in MN on LGs 6-2, 1-2 and 3-1. In the Western states, only one significant QTL was identified on LG 6-2 which accounted for 22% of the phenotypic variation in CA. This same peak was uncovered with a P-value <0.2 in MI.

These data indicate that regulation of day-neutrality in octoploid *F.* × *ananassa* is likely more complex than in its diploid progenitor, *F. vesca*. The *F. vesca* everbearing cultivars 'Baron Solemacher' and 'Bush White' have been shown to contain a homozygous recessive gene for day-neutrality (Brown and Wareing 1965), and molecular markers have been identified that are closely linked to the seasonal flowering locus in this species (Cekic et al. 2001). In preliminary trials, this marker did not segregate in our *F.* × *ananassa* population.

Several recent studies have focused on developing genetic linkage maps for diploid *Fragaria* species, assuming that the diploid map can be used as a genomic model for predicting behaviour of the octoploid cultivated species (Sargent et al. 2004, 2006). While several octoploid SSR markers have been mapped in the diploid species (Davis et al. 2006), to our knowledge little effort has been made to transfer the diploid SSR markers to the octoploid species. It is our intention to map the diploid *Fragaria* SSR markers into the octoploid genetic linkage map published in this paper, and we intend to determine whether the SCAR markers developed for the seasonal flowering locus in *F. vesca* (Albany et al. 2004) will co-localize with the QTL identified in our segregating octoploid population. This attempt will confirm whether the diploid *Fragaria* species can be used as a model species for the cultivated octoploid strawberry.

The reason why the QTL on LG 28 was so prominent in all three eastern states and absent in CA and OR is not known. However, we speculate that different loci regulate day-neutrality in the various areas due to climatic variation. There is a strong temperature/photoperiod interaction that determines flowering in the strawberry. When temperatures are below 15°C, all genotypes tend to behave in a photoperiod insensitive manner (Darrow 1966, Hancock 1999) and when temperatures are above 26°C, flowering is inhibited regardless of the photoperiod (Durner et al. 1984). In the summer months of 2005, MD, MI and MN had average maximum mid-summer temperatures at 28°C or higher (Table 2), whereas in OR and CA maximum temperatures were at 26°C and 21°C, respectively (Source of climatic data MD: http://cirrus.dnr.state.sc.us/cgi-bin/sercc/cliMONtmxt.pl?md0700, MI: http://www.agweather.geo.msu.edu/mawn/station.asp?id=swm&rt=24, MN http://climate.umn.edu/hidradius/radius.asp OR:

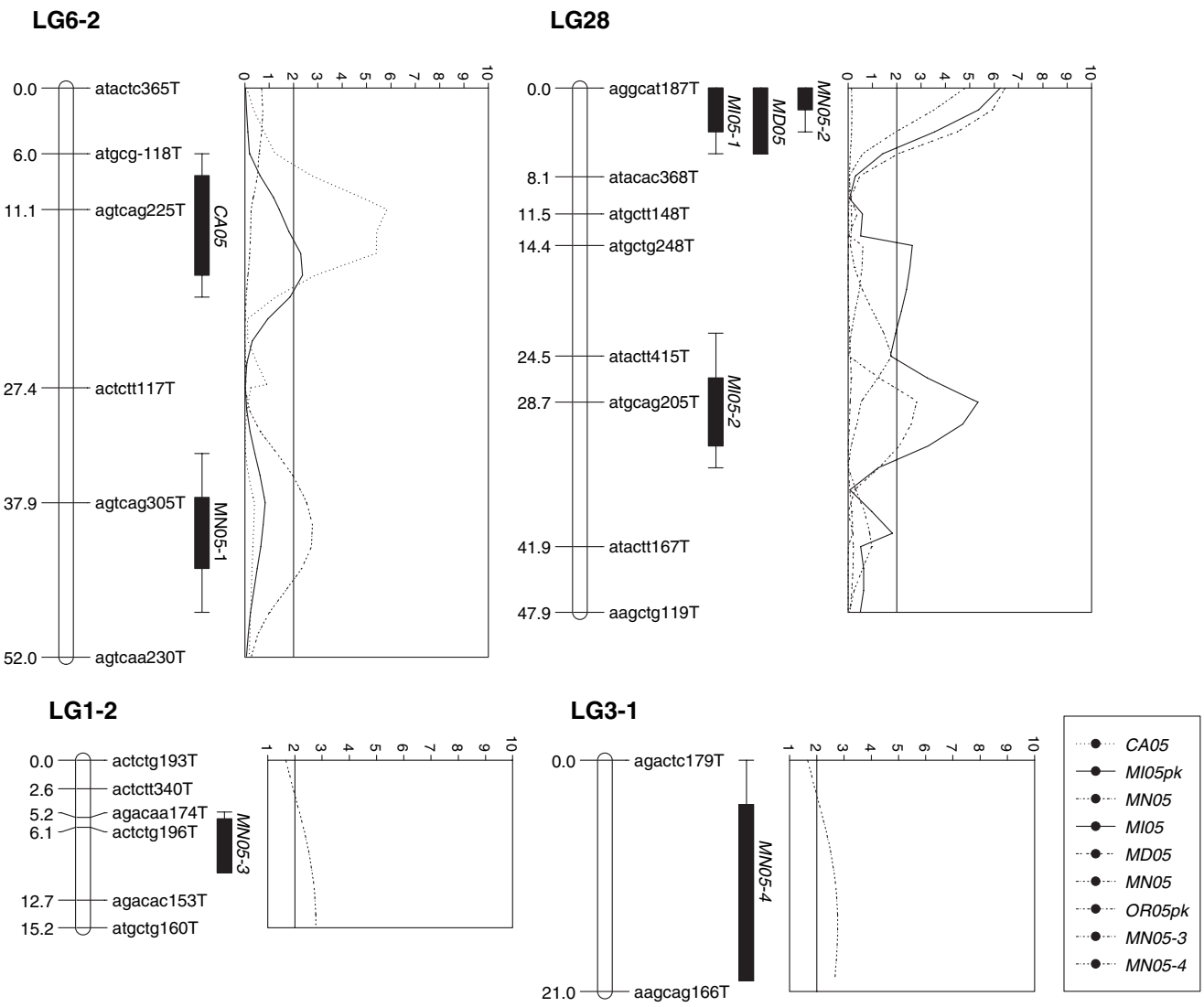

Fig. 2: QTL for day-neutrality detected in the newly generated progeny of 'Tribute' × 'Honeoye' evaluated in MI, MN, MD, CA and OR, 2005. Any peak that was observed above a LOD score of 2.0 is indicated. However, those peaks above the LOD thresholds were considered as QTL (see text for details). All the QTL associated with day-neutrality were derived from the cultivar 'Tribute'

http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?orcorv, CA:TCR Weather Station, Aromas and). The QTL on LG 28 may be required for floral initiation under the hot summer conditions found in eastern continental climates. 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 explain why the percentages of DN progeny were so much higher in the western states (80–89%) than the eastern ones (49–50%).

The dramatic differences in the inheritance patterns of day-neutrality found in previous studies is likely due to variation in the test environments and the specific QTL carried by the parents. The test environment is particularly critical, as very different levels of expression have been observed depending on temperature (Durner et al. 1984, Hancock et al. 2001, Serçe and Hancock 2005).

There may be a wide array of genes determining day-neutrality in strawberry that have differing strength, and it may take a threshold level of these genes to impart day-neutrality. One or more of these genes could be associated with heat tolerance, while others may be associated with variations in rates of floral development, rest period require-

Table 2: Average minimum and maximum temperatures, and percent DN progeny observed at the various study locations used to detect QTL for day-neutrality in a segregating population of 'Tribute' × 'Honeoye'

Character	MI	MN	MD	OR	CA
% DN plants	49.23	50	48	80	87.30
Temp. SD (April and May), °C					
average min	6	3	2	6	7
Average max	18	17	20	18	19
Temp. LD (June, July and August), °C					
Average min	17	18	14	10	11
Average max	29	28	30	26	21

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).

ments and patterns of vegetative growth. A large number of genes have been linked to flowering response in molecular studies of other plant species (Hayama and Coupland 2004, Putterill et al. 2004, Esumi et al. 2005). Perhaps what has been described as DN flowering pattern in strawberries is better defined as remontancy where multiple genes influence repeat flowering in addition to those regulating photoperiod insensitivity.

The genetics of flowering is very complex, as has been shown in *Arabidopsis thaliana*, especially in relation to environmental inputs such temperature or light quality. Photoperiodic flowering in this species appears to incorporate the external coincidence model of Bunning (1936), which suggests that photoperiod responses result from the interaction of an external light signal with an endogenous timekeeping mechanism (i.e. circadian clock). In this model, the circadian clock sets up a light-sensitive phase each day that coincides, depending on the daylength, with either light or darkness. However, it is also clear that, at least in Arabidopsis, photoperiodic flowering involves supplemental mechanisms that refine and reinforce timekeeping (Hayama and Coupland 2004). The mechanisms of photoperiodic flowering in Arabidopsis can be conceptualized as three linked modules. The central core is the endogenous oscillator, which maintains an innate rhythm of approximately 24 h. Upstream, this oscillator is entrained by light and temperature signals. Downstream, the oscillator regulates levels of proteins including CO. High levels of CO are required for transcription of FT, a gene that integrates photoperiod signals with other flowering pathways. To date, nearly twenty genes/proteins have been described that are involved in photoperiodic flowering (Putterill et al. 2004). Disruption of proper expression of any of these can partially or completely eliminate photoperiod flowering and functionally similar genes in other species are thus excellent candidates for QTL affecting photoperiodic flowering.

In addition, characterization of the so-called *heading date* QTL in rice have revealed that at least three of these are homologous to components of the photoperiodic flowering mechanism in Arabidopsis (Hd1/CO, Hd3a/FT, and Hd5/CKII; Yano et al. 2000, Takahashi et al. 2001, Kojima et al. 2002). In addition, the rice se5 mutation, which abrogates production of functional phytochrome, leads to photoperiod-insensitive flowering (Izawa et al. 2000), suggesting that (as in Arabidopsis) phytochromes are essential for measuring day length. Analysis of the nearly sequenced rice genome has also revealed that most of the remaining components of photoperiodic flowering identified in Arabidopsis are also conserved in rice, and reversed-genetic characterization of some of these revealed that they are regulated in a similar manner. For example, proper expression of a rice GI homolog, OsGI, is dependent on functional SE5, and altering OsGI expression in transgenic rice disrupts photoperiodic flowering (Hayama et al. 2002, 2003). The opposite effects of photoperiod on flowering in Arabidopsis and rice appears to be due to divergence in the mechanism of photoperiodic flowering downstream of CO/HD1; Hd1 shows a diurnal pattern of expression similar to that of CO (i.e., accumulating to high levels during the light in LD) yet clearly plays a repressive, rather than activating, role in the downstream expression of Hd3a (Kojima et al. 2002). The molecular biology of photoperiodic flowering has also been addressed in the SD dicot *Pharbitis nil*. Constitutive expression of a CO-like gene from this species in Arabidopsis was sufficient to trigger flowering under non-inductive photoperiods (Liu et al. 2001). The available data from rice and *Pharbitis* strongly suggests that flowering genes and their function are strongly conserved across Angiosperm species. Given the evolutionary distance between Arabidopsis and rice, this degree of homology would represent a minimum expected for a Arabidopsis-strawberry match. Where the strawberry target is present in multiple copies or as a member of a closely related family, sequence polymorphism should allow detection of each copy as a discreetly sized fragment, and this will provide for additional markers useful for linkage mapping.

Our map was relatively diffuse and as such it is quite possible that we missed other QTL that regulate day-neutrality. However, we uncovered a sufficient number of QTL with modest effects to feel confident that day-neutrality is under polygenic control. It is possible that there is a major dominant gene for day-neutrality that we missed, but regardless, it is clear that numerous genes have at least modifying effects. To obtain better genome coverage, we are in the process of identifying many more markers and plan to evaluate their segregation patterns in an expanded population of over 320. Furthermore, with a larger population size and a denser linkage map, more QTL will be uncovered. Our focus will be on using simple sequence repeats (SSR) that are not genome and population specific. We also plan to search for QTL in another segregating population with DN *F. virginiana* as a parent.

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