Journal of Berry Research 5 (2015) 107–116
DOI:10.3233/JBR-150095
IOS Press

Identification of QTL associated with flower and runner production in octoploid strawberry *(Fragaria × ananassa)*

Suneth S. Sooriyapathirana[a], Sonali Mookerjee[b], Cholani K. Weebadde[c], Chad E. Finn[d], Kim Lewers[e], Jill M. Bushakra[f], James J. Luby[g], Philip Stewart[h], Stuart Neils[b] and James F. Hancock[b,*]

[a]*Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka*
[b]*Department of Horticulture, Michigan State University, East Lansing, MI, USA*
[c]*Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, USA*
[d]*U.S. Department of Agriculture (USDA)-Agricultural Research Service (ARS), Horticultural Crops Research Unit, Corvallis, OR, USA*
[e]*USDA-ARS, Genetic Improvement of Fruits and Vegetables Lab, BARC-West, Beltsville, MD, USA*
[f]*USDA-ARS National Clonal Germplasm Repository, Corvallis, OR, USA*
[g]*Department of Horticultural Science, University of Minnesota, St. Paul, MN, USA*
[h]*Driscoll Strawberry Associates, Inc., Watsonville, CA, USA*

Received 6 February 2015; accepted 27 April 2015

Abstract.
BACKGROUND: Understanding the genetics of flowering in the strawberry *(Fragaria × ananassa)* will aid in the development of breeding strategies.
OBJECTIVE: To search for quantitative trait loci (QTL) associated with remontancy and weeks of flowering in the strawberry.
METHODS: Previously collected phenotypic data from two non-remontant 'Honeoye' × remontant 'Tribute' strawberry populations and simple sequence repeats (SSR) markers were used to search for QTL associated with repeat flowering, weeks of flowering and runner production, as well as the ability to produce flowers and runners at 17, 20 and 23°C.
RESULTS: As was discovered in other studies, we found a major QTL that regulated remontancy and weeks of flowering on homeologous linkage group IV of 'Tribute'. This QTL also had a negative effect on runner production and a positive influence on flower production under high temperatures. A number of additional QTL were discovered that significantly (LOD >3.0) influenced flower and runner production.
CONCLUSIONS: Remontancy/non-remontancy is controlled by a major gene/locus and several minor modifying ones.

Keywords: *Fragaria*, remontancy, QTL, heat tolerance, flowering, linkage map

1. Introduction

The seasonal flowering response of strawberry cultivars (*Fragaria × ananassa* Duchesne ex Rozier), has classically been described as short day (June-bearers), day-neutral and long-day (everbearing) [1, 2]. However, evidence is accumulating that the classification of strawberry genotypes based on their photoperiodic response is complicated by the effect of temperature [3–7]. Genotypes previously classified as short-day, will flower under long days when temperatures are cool, and genotypes that have been called day-neutral do not flower at all under warm temperatures above a threshold [1, 8]. For this reason, day-neutrals are more accurately described as remontant and short-day types as non-remontant.

*Corresponding author: James F. Hancock, Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA. Tel.: +1 517 974 5161; Fax: +1 517 353 0890; E-mail: hancock@msu.edu.

Genetic control of day-neutrality or remontancy in the octoploid strawberry has long been debated and several hypotheses have been proposed including a single dominant gene [9, 10], a single major gene with modifier genes [11], dominant complementary genes [12], and polygenic control [5, 13, 14].

In the last decade, molecular marker and QTL approaches have been used to identify the genes associated with remontancy in *F.* × *ananassa* and a major gene and several minor genes have been identified. Sugimoto et al. [10] studied a population derived from 'Ever berry' (remontant) and 'Toyonoka' (non-remontant) and identified a RAPD (Randomly Amplified Polymorphic DNA) marker associated with remontancy. Weebadde et al. [5] used amplified fragment length polymorphisms (AFLPs) to identify QTL regulating remontancy in a population of non-remontant 'Honeoye' × remontant 'Tribute' (H × T) phenotyped at five locations across the USA. They identified one QTL associated with remontancy that was common to the eastern states Minnesota (MN), Michigan (MI), and Maryland (MD), in addition to three QTL specific to MN, and one QTL specific to MI and California (CA).

Castro et al. [15] used SSR markers to identify QTL for remontancy and runner production using the phenotypic data collected in the 'Honeoye' × 'Tribute' population of Weebadde et al. [5]. They scored day-neutrality qualitatively as repeat flowering after July 17 and quantitatively as number of weeks of flowering. When qualitatively scored, they found a QTL that mapped on LG IV of the 'Tribute' map, regardless of planting location. QTL for weeks of flowering were also found on LG IV in MD and CA, and for stolon production in MN, MI and OR.

In another population segregating for remontancy (non-remontant 'CF1116' × remontant 'Capitola'), Gaston et al. [16] identified a single major QTL on LG IV that controlled the length of flowering and runner production. The QTL had opposite effects on flowering (positive effect) and runnering (negative effect), suggesting that both traits share a common physiological control. Gaston et al. [16] also found several minor QTL regulating what they called "perpetual flowering" on other linkage groups.

All of the above studies were done in ambient field conditions, so they provide little information on the influence of temperature on the expression of remontancy. To directly test the influence of temperature in the regulation of flowering in *F.* × *ananassa*, Mookerjee et al. [17] made the same cross as Weebadde et al. [5] and evaluated the flowering patterns of a different set of progeny under three temperature regimes (17, 20, and 23°C) in a greenhouse under long days. In addition, replicates of the same genotypes were grown under field conditions in MI and OR and were evaluated for their flowering patterns. Their data indicated that high temperature tolerance plays a role in the expression of remontancy, as most of the progeny that produced more flowers at 23°C than at 17 °C in the greenhouse were remontant in the field in MI and OR.

Herein, we describe a QTL analysis for remontancy and runner production on the two H×T populations generated by Weebadde et al. [5] and Mookerjee et al. [17] using many of the same SSR markers used by Lewers et al. [18] and Gaston et al. [16]. The goals of this study were to: 1) further validate the existence of a major QTL for remontancy and weeks of flowering in *F.* × *ananassa* on LG IV, 2) determine if the level of floral heat tolerance in Mookerjee et al.'s [17] H × T population co-segregates with a QTL determining remontancy and weeks of flowering, and 3) determine whether the QTL for remontancy in the H × T population also regulates runner production as in the 'CF1116' × 'Capitola' of Gaston et al. [16].

2. Material and methods

2.1. Mapping population

DNA from a Pseudo testcross population of 174 progeny of 'Honeoye' (non-remontant) × 'Tribute' (remontant) was used to build the linkage map [5]. Out of this mapping population, 112 genotypes had been phenotyped in the field by Weebadde et al. [5] in 2005 and 2006. We also extracted DNA from the 54 progeny of the same cross made in 2009 by Mookerjee et al. [17], which had also been phenotyped in the field and greenhouse in 2011.

2.2. Phenotypic evaluations

The phenotypic data collected previously by Weebadde et al. [5] were used to determine QTL, along with the more recent data collected in the field and greenhouse in 2011 [17]. The presence and absence of flowers was recorded every

Table 1
Traits subjected to quantitative trait loci (QTL) analyses in two 'Honeoye' × 'Tribute' families. Information is included on the number of progeny in each family, where the families were grown (greenhouse or field), state in the United States where they were evaluated (CA – California, MA – Maryland, MI – Michigan, MN – Minnesota and OR – Oregon) and the year the data were collected

Trait	Description	Location	State and year	No. of Individuals
Flowers at 17°C	Number of flowers produced by the progeny at 17°C	Greenhouse	MI, 2011	54
Flowers at 20°C	Number of flowers produced by the progeny at 20°C		MI, 2011	54
Flowers at 23°C	Number of flowers produced by the progeny at 23°C		MI, 2011	54
Flowers at 17/23°C	Ratio of flowers produced at 17°C vs. 23°C		MI, 2011	54
Repeat flowering	Whether progeny flowered in the spring and again after 23 July	Open field	CA, 2005	65
			MD, 2005	65
			MI, 2005	65
			MI, 2006	65
			MI, 2011	54
			MN, 2005	65
			OR, 2005	65
Weeks of flowering	Number of weeks progeny flowered during the growing season	Open field	OR. 2005	65
			OR, 2011	54
			MI, 2005	65
			MI, 2011	54
Total runner number	Number of runners produced by progeny during the growing season	Open field	OR. 2005	65
			OR, 2011	54
			MI, 2005	65
			MI, 2011	54
Runners at 23°C	Number of runners produced by the progeny at 23°C	Greenhouse	MI, 2011	54

week from the beginning of flowering until at least early August. Progeny that flowered in the spring (May-June) and in the long days of summer after 23 July were categorized as remontant. Progeny that flowered only in the spring (May-July 22) before the longest day of the year were categorized as non-remontant (Table 1).

In the greenhouse studies by Mookerjee et al. [17], three replicates of each genotype were grown in a completely randomized design in temperature controlled greenhouses at 17°C, 20 °C, and 23°C, and under 16 hr photoperiod using supplemental lights. The number of flowers and runners were counted every week from Dec 2010 to Mar 2011. All open flowers and runners were removed after counting every week (Table 1).

2.3. Genotyping

2.3.1. Selection of SSRs

SSR loci developed from *F.* × *ananassa, F. vesca* L.*, F. nubicola* (Hook. f.) Lindl. ex Lacaita, and *F. viridis* Weston, [18–26] were screened for production of polymorphic bands in a subset of 54 progeny and the parents. Of the 157 SSR markers that were screened, 118 were selected based on the presence of polymorphism and distinct scorable bands on 6% polyacrylamide gels.

2.3.2. DNA amplification

Young leaf samples from the parents and progeny were collected from greenhouse grown plants. DNA was extracted using the DNeasy Plant mini kit (Qiagen, Valencia, CA) following the manufacturer's protocol. DNA amplification was performed in 20 μL reactions containing 1 × GoTaq® Green Master Mix (Promega Corporation, Madison, WI), 0.5 mM of forward and reverse primer, and 1 μL of 60 ng/μl DNA template. Amplifications were performed in a C1000TM Thermal Cycler (Biorad, Hercules, CA) using the PCR protocol: Initial denaturation: 94°C for 2 min; 34 cycles of 1 min at 94°C, 1 min at annealing temperature (= Tm+2°C), 1.5 min at 72°C; and a final extension step of 10 min at 72 °C, hold at 15°C.

2.3.3. Polyacrylamide gel electrophoresis

PCR amplified products were size separated using a 6% denaturing polyacrylamide gel (15 mL of 40% Acrylamide/Bis Solution; BioRad, Hercules, CA), 10 mL 10 × TBE buffer, 42 g Ultra Pure Urea (Invitrogen, Carlsbad, CA), 500 μL 10% APS, 100 mL TEMED (BioRad, Hercules, CA). The PCR amplicons were denatured (95°C for 5 min, hold at 4°C) and loaded on to 38 cm × 50 cm Sequi-Gen GT system (BioRad, Hercules, CA) that was preheated for 20–30 min. The gels were run at 80 W for 3.5 hrs, and visualized with silver staining [27]. The fragment sizes were estimated by comparing with 10 and 50 bp ladders (Invitrogen, Carlsbad, CA).

2.3.4. Linkage map

The Single Dose Restriction Fragment (SDRF) [28] approach was used for scoring the markers. Each segregating fragment was treated as an individual allele and the genotypes were scored for presence/absence of the allele. Markers present in both the parents that segregated in a 3:1 ratio were coded as dominant markers and those that segregated in a 1:1 ratio were coded as codominant markers. The dominant and codominant markers were used to develop the linkage map using JoinMap v 3.0 [29], with a minimum threshold LOD score value of 2.8, maximum recombination frequency of 0.3, and the Kosambi mapping function. The linkage groups were visualized using MapChart 2.2 [30].

2.4. QTL identification

MapQTL v 5.0 [31] was utilized for QTL identification using the Multiple QTL Mapping (MQM) or Composite Interval Mapping (CIM) approach. The population was derived from two heterozygous parents and was coded as CP to include the three types of marker data: 1: codominant markers segregating in 'Honeoye', 2: codominant markers segregating in 'Tribute', 3: Dominant markers present in both parents. Markers identified as significant by the Kruskal-Wallis test were used as cofactors. The significant LOD score at $p \leq 0.05$ was determined from 1000 permutations with the dataset. Significant QTL regions along with the linkage groups were visualized using MapChart 2.2 [30].

3. Results and discussion

The 118 SSR markers resulted in the amplification of 704 segregating SDRF. Out of the 704 segregating SDRF, 396 were present only in 'Honeoye' and 308 were present only in 'Tribute'. The 396 SDRF in 'Honeoye' cover 962.8 cM in 19 linkage groups with the average distance between makers of 9.9 cM (Table 2). The 308 markers in 'Tribute' cover 922.3 cM in 23 linkage groups with the average distance between markers of 11.8 cM (Table 2). The linkage groups of 'Honeoye' and 'Tribute' were named from I to VII using the markers previously placed on the diploid *Fragaria vesca* map by Sargent et al. [23] (Table 2).

As in the work of Castro et al. [15], we found a major QTL for repeat flowering and weeks of flowering on LG IV in the families of 'Honeoye' × 'Tribute' generated by Weebadde et al. [5] and Mookerjee et al. [17]. Gaston et al. [16] also found a major QTL on LG IV for the number of inflorescences emerging from the end of May to the beginning of August. The marker locus ChFam011_129 on LG IV was associated with remontancy in the open fields of CA-05, MD-05, MN-05, MI-05, MI-06, MI-11, OR-05, and OR-11 (Table 3). The r^2 varied from 12.6 to 34.0, and the effect ranged from 0.3 to 0.6. The same marker locus was also associated with weeks of flowering in the open fields of MI-05 and OR-05, with r^2 varying from 11.1 to 16.7 and effects ranging from 1.4 to 4.4. In addition, ChFam011_129 was associated with the number of flowers produced in the greenhouse trials at all temperatures, with r^2 ranging from 24.1 to 30.1, and effects varying from 74.5 to 113. Castro et al. (2014) also found a QTL on LG VI associated with remontancy and weeks of flowering in 'Tribute' with the marker ChFam011_129.

It appears that the QTL represented by ChFam011_129 or closely associated genes also regulate runner production antagonistically, as the same marker locus was associated negatively with runner production in the open field trials of MN-05 and OR-11, with r^2 varying from 14.5 to 14.9 and effects ranging from –8.3 to –15.4. In the greenhouse temperature trials, ChFam011_129 was also found to be negatively associated with runner production at 23°C ($r^2 = 34.4$ effect = –25.4) (Table 3).

Besides the major QTL regulating remontancy and weeks of flowering, we identified a number of other QTL regulating these traits in both 'Tribute' and 'Honeoye', suggesting that the control of remontancy is by one major

Table 2

Size and position of SSR bands linked in the mapping population of 'Honeoye' × 'Tribute' (*Fragaria* × *anannasa).* The proposed linkage groups (LG) are based on the diploid *Fragaria vesca* linkage map of Sargent et al., [23]. The markers came from previously published papers including Sargent et al., [19, 23], Zorrilla-Fontanesi et al., [26]

Proposed LG in H × T Pop	Marker	LG in *F. vesca*	GenBank ID	cM position in H or T map[c]	Polymorphic bands mapped (bp size)
I-H-1[a]	EMFn152	I	AM051335.1	0.0	148
	ChFaM032	I	GU815794.1	24.5	210, 205
	EMFn230	–[b]	–	27.7	225
	UFFxa16H07	I	NW_004440457.1	47.4	262
	FAC-001	VII	FAC-001	58.9	211
	ChFam061	I	GU815808.1	72.3	220
I-H-2	ChFaM039	–	GU815799.1	0.0	205, 196
	EMFn128	I	AM051330.1	24.6	157, 155
	SF5C08	–	–	52.8	410
	ChFaM151	I	GU815865.1	101.5	475, 223
	UFFax20H10	–	–	97.7	260
I-H-3	ChFaM003	I	GU815784.1	0.0	400
	ChFaM151	I	GU815865.1	52.5	325, 218, 210
	ChFaM076	–	GU815820.1	52.6	103
	ChFaM086	–	–	59.4	230
II-H-1	UAFv8216	–	–	0.0	101
	ChFaM004	II	–	9.5	132
	UaFv9094		–	17.8	390
	EMFn134	II	AM051331.1	24.8	214
	ChFaM088	II	GU815829.1	37.7	106
	ChFaM067		GU815814.1	47.7	161
	ChFaM104	II	GU815841.1	54.9	197
II-H-2	SFGRP7	–	–	0.0	125
	EMFn198	–	–	20.4	161
	SF6E02	–	–	30.2	119
	EMFn235	II	AM051352.1	42.6	219
	ChFaM103	II	GU815840.1	55.3	215, 190
	EMFn148	II	AM051333.1	67.9	189
II-H-3	ChFaM103	II	GU815840.1	0.0	450
	UaFv8216	–	–	21.4	182
	EMFn134	II	AM051331.1	46.5	160
	EMFn160	II	AM051337.1	63.6	160
II-H-4	ChFaM101	II	GU815839.1	0.0	139, 137
	ChFaM063	–	GU815810.1	21.6	131, 111, 90
II-H-5	EMFn134	II	AM051331.1	0.0	192, 185
	UAFv8216	–	–	26.4	239
	UFFax03B05	II	–	54.3	214
III-H-1	ARSFL027	III	–	0.0	200
	ChFaM056	–	GU815805.1	13.3	130
	BFACT036	III	AM889106.1	23.2	160
III- H-2	ChFaM129	III	GU815855.1	0.0	500
	EMFvi104	–	–	5.8	80
	TRF033	–	–	12.7	310

(Continued)

Table 2
(Continued)

Proposed LG in H × T Pop	Marker	LG in *F. vesca*	GenBank ID	cM position in H or T map[c]	Polymorphic bands mapped (bp size)
	EMFn170	III	AM051339.1	15.0	240
	ChFaM080	III	GU815824.1	16.6	219
	BFACT036	III	AM889106.1	18.8	195
	ChFaM040	III	GU815800.1	20.9	95
	ARSFL98	–	–	25.3	205
III-H-3	ChFaM040	III	GU815800.1	0.0	315, 300
	CFVCT035	III	DQ117042.1	29.7	112
	UDF003	V	–	33.7	300
	UDF004	III	–	40.2	136
	ARSFL8	–	–	58.0	301
III-H-4	ChFaM040	III	GU815800.1	0.0	143
	UDF004	III	–	36.6	143
	ChFaM080	III	GU815824.1	44.3	220
IV-H-1	EMFn225	–	AM051349.1	0.0	259
	ChFaM017	IV	GU815788.1	57.7	525, 250, 211, 147, 86
	EMFn184	V	AM051342.1	54.8	260
IV-H-2	EMFvi136	IV	AJ564350.1	0.0	157
	ChFaM111	II	GU815848.1	23.6	400, 189, 176, 150
	EMFvi104	–	–	38.7	127
V-H-1	ChFaM046	–	GU815803.1	0.0	128
	EMFn010	V	AJ639622.1	18.9	270
	TRF017	–	–	35.6	210
	SF5G02	–	–	55.6	273, 260
V-H-2	UFFax20D02	–	–	0.0	109
	EMFn181	V	AM051340.1	10.3	221, 170
	SF5C08	–	–	19.9	325
VI-H-1	ChFaM095	–	GU815835.1	0.0	400, 320
	EMFn123	VI	AM051328.1	38.4	200
–	SF5G02	–	–	0.0	250
	ChFaM046	–	GU815803.1	20.1	157
–	BFACT050	–	AM889117.1	0.0	202, 184
I-T-1	ChFaM151	I	GU815865.1	0.0	375, 228
	UFFxa16H07	I	NW_004440457.1	36.4	270
	ChFaM076	–	GU815820.1	60.5	101
	ChFaM003	I	GU815784.1	81.9	495, 400
I-T-2	UFFa02F02	I	AJ870441.1	0.0	209
	UFFa18H04	–	–	20.9	143, 121
	ChFaM092	–	GU815832.1	57.2	140
I-T-3	EMFn115	I	AM051324.1	0.0	163, 133
II-T-1	ChFaM104	II	GU815841.1	0.0	197, 196
	ChFaM004	II	–	68.9	230
II-T-2	ChFaM103	II	GU815840.1	0.0	450
	UAFv8216	–	–	26.1	182, 161
	ChFaM088	II	GU815829.1	40.9	300
	EMFn134	II	AM051331.1	46.7	160
	EMFn160	–	AM051337.1	58.5	161, 160

(Continued)

Table 2
(Continued)

Proposed LG in H × T Pop	Marker	LG in *F. vesca*	GenBank ID	cM position in H or T map[c]	Polymorphic bands mapped (bp size)
II-T-3	ChFaM063	–	GU815810.1	0.0	106, 90
	EMFn121	II	AM051327.1	17.5	243
	ChFaM101	II	GU815839.1	26.0	153, 137, 136
II-T-4	ChFaM111	II	GU815848.1	0.0	210, 195
	ChFaM078	II	GU815822.1	12.3	140
	UaFv8936	–	–	44.7	410
II-T-5	SF6E02	–	–	0.0	149
	ChFaM103	II	GU815840.1	14.2	124
	UFFax03B05	II	–	18.9	210
III-T-1	ChFaM040	III	GU815800.1	0.0	155, 143, 100
	EMFn170	III	AM051339.1	56.7	215
	BFACT036	III	AM889106.1	65.9	150
	ChFaM056	–	GU815805.1	71.4	112
III-T-2	ChFaM098	III	GU815837.1	0.0	226
	ChFaM094		GU815834.1	20.0	120
	EMFn170	III	AM051339.1	43.3	208
III-T-3	ChFvM049	III	–	0.0	153
	FvNES1	–	–	40.5	105
IV-T-1	ChFaM011	–	GU815787.1	0.0	129
	EMFvi136	IV	AJ564350.1	8.4	159
V-T-1	EMFn184	V	AM051342.1	0.0	260, 245
	SF5G02	–	–	28.6	228
V-T-2	ChFaM031	–	GU815793.1	0.0	190, 138
	ChFaM018	V	GU815789.1	11.2	460
V-T-3	UFFax20H10	V	–	0.0	198
	ChFaM046	–	–	26.9	152, 135
VI-T-1	ChFaM095	–	GU815835.1	0.0	150
	EMFn198	–	–	9.4	169
	EMFn117	VI	AM051325.1	29.7	188, 157, 129
	EMFvi104	–	–	48.8	130
VI-T-2	ChFaM035	–	GU815796.1	0.0	245
	EMFn123	VI	AM051328.1	69.0	154
VII-T-1	UFFxa14A11	–	–	0.0	105
	EMFn213	VII	AM051347.1	25.9	320, 310
VII-T-2	ChFaM085	VII	GU815828.1	0.0	155
	EMFn198	–	–	34.7	189
–	ChFaM032	I	GU815794.1	0.0	210
–	FAC-001	VII	–	25.3	211
–	ChFaM081	–	GU815825.1	0.0	300, 152
–	ChFaM076	–	GU815820.1	0.0	139
–	UFFax20H10	–	–	4.0	247
–	ChFaM147	IV	GU815863.1	0.0	219, 210
–	EMFn181	V	AM051340.1	78.0	240

[a]H = 'Honeoye', T = 'Tribute'. [b]Band not reported in diploid mapping population of Sargent et al., [23]. [c]Position indicates the distance in cM of QTL from the top of the linkage group.

Table 3
Quantitative Trait Loci (QTL) detected in 'Tribute' with significant ($p \leq 0.05$) LOD scores >2.8. Significance was determined from 1000 permutations with the dataset

Location	Trait	State and year	Marker locus_band (bp)	LG[a] in *F. vesca*	LOD[b]	r^{2c}	Effect[d]
Greenhouse	Flowers at 17°C	MI-11	ChFam011_129	IV	3.2	24.1	+74.5
	Flowers at 20°C	MI-11	ChFam011_129	IV	4.2	30.1	+76.0
	Flowers at 23°C	MI-11	ChFam011_129	IV	3.8	27.4	+113.0
	Runners at 23°C	MI-11	ChFam011_129	IV	5.0	34.4	– 25.4
Open field	Repeat flowering	MI-11	ChFam011_129	IV	5.2	34.0	+0.6
		MD-05	ChFam011_129	IV	7.0	30.6	+0.6
		CA-05	ChFam011_129	IV	2.9	12.6	+0.3
		MI-05	ChFam011_129	IV	7.3	28.5	+0.6
		MN-05	ChFam011_129	IV	7.5	30.1	+0.6
		OR-05	SF5G02_228	V	3.1	13.4	+0.4
		MI-06	ChFam011_129	IV	5.2	21.1	+0.3
		OR-11	ChFam011_129	IV	4.6	28.8	+0.6
	Weeks of flowering	MI-11	ChFam011_129	IV	5.3	33.8	+3.5
		OR-05	ChFaM040_155	III	4.0	15.3	+2.9
		MI-05	ChFaM040_100	III	2.8	10.0	+0.6
		MI-05	ChFam011_129	IV	2.8	11.1	+1.4
		OR-05	EMFn170_215	III	3.8	14.7	– 0.2
		OR-05	ChFam011_129	IV	4.5	16.7	+4.4
		OR-05	ChFaM040_100	III	3.5	13.5	+2.9
	Number of runners	CA-05	ChFaM151_375	I	4.3	18.4	+1.1
		MN-05	ChFam011_129	IV	3.4	14.5	– 15.4
		OR-05	ChFaM104_196	II	3.9	16.3	– 3.1
		OR-11	ChFam011_129	IV	3.5	14.9	– 8.3

[a]LG = Linkage group. [b]LOD is the log-likelihood at that position. [c]r^2 is the percentage of phenotypic variation explained by the QTL. [d]Mean effect on a trait mean value of the presence of one allele at a marker by comparison with the presence of the second allele. The + and − indicates the direction of the additive effect.

Table 4
Quantitative Trait Loci (QTL) detected in 'Honeoye' with significant ($p \leq 0.05$) LOD scores > 2.8. Significance was determined from 1000 permutations with the dataset

Location	Trait	State and year	Marker locus_band (bp)	LG[a] in *F. vesc*a	LOD[b]	r^{2c}	Effect[d]
Greenhouse	Flowers at 17/23°C	MI-11	ARSFL19_295	V	6.8	59.0	+1.8
	Runners at 17°C	MI-11	ChFaM088_106	II	4.3	30.5	–1.1
Open field	Weeks of flowering	MI-11	ChFaM151_210	I	3.1	21.7	–3.1

[a]LG = Linkage group. [b]LOD is the log-likelihood at that position. [c]r^2 is the percentage of phenotypic variation explained by the QTL. [d]Mean effect on a trait mean value of the presence of one allele at a marker by comparison with the presence of the second allele. The + and − indicates the direction of the additive effect.

gene and a number of modifiers as proposed by Shaw and Famula, [11]. In OR-05, a QTL was identified on LG V of 'Tribute' with an r^2 of 13.4% and an effect of +0.4. Several QTL for weeks of flowering were identified in MI-05, MI-11 and OR-05 for weeks of flowering on LG III of 'Tribute'. Their r^2 varied from 10.1 to 15%, and their effects from 0.1 to 2.9 weeks of flowering. A negative QTL for weeks of flowering was identified on LG I of 'Honeoye' with an r^2 of 21.7 and effect of –3.1 (Table 4).

Like remontancy, runner production appears to be regulated as a polygenic trait, as several QTL were identified that regulated this trait. QTL for number of runners were identified on LG I and II of 'Tribute' in the open field trials

in CA-05 and OR-05, with r^2 ranging from 16.3 to 18.4 and effects from –3.1 to +1.1. Two QTL were also identified on LG II of 'Honeoye' at 17 °C in the greenhouse trails. The r^2 values ranged from 29.6 to 30.5 % and their effects ranged from –1.0 to –1.1 (Table 4).

It appears that heat tolerance plays a role in the expression of remontancy and duration of flowering. In the greenhouse temperature trials, the presence of the allele represented by ChFam011_129 had a dramatic effect of +113 on total numbers of flowers at 23°C, and a negative effect of –25.4 on runner production. An antagonistic QTL (marker locus band ARSFL19_295) was found in 'Honeoye' that had an effect of +1.8 on the ratio of flowers at 17 °C vs. 23°C, meaning that fewer flowers were produced at 23°C vs 17 °C in the presence of this allele (Table 4).

In summary, as in the work of Castro et al. [15] and Gaston et al. [16], we identified a major QTL regulating remontancy and weeks of flowering. This QTL or genes closely linked to it also regulated runner production in an antagonistic fashion and influenced the ability to produce flowers under high temperatures. There were also several additional QTL identified regulating flower and runner production, suggesting that these traits are controlled polygenically, with a major gene and several minor modifying genes.

Acknowledgments

The authors thank Dechun Wang for help with the linkage map and QTL analysis, and Pete Callow who helped collect the field data in the previous studies of 'Tribute' × 'Honeoye'. "RosBREED: Enabling marker-assisted breeding in Rosaceae" is supported by the USDA-NIFA-Specialty Crop Research Initiative by a combination of federal and matching funds (grant number 2009-51181-05808).

References

[1] Darrow GM. The Strawberry. History, Breeding and Physiology. Holt, Rinehart and Winston, New York; 1966.

[2] Stewart PJ, Folta KM. A Review of Photoperiodic Flowering Research in Strawberry (*Fragaria* spp.). Crit Rev Plant Sci. 2010;29:1-13.

[3] Durner EF, Barden JA, Himelrick DG, Poling EB. Photoperiod and temperature effects on flower and runner development in day-neutral, Junebearing, and everbearing strawberries. J Amer Soc Hort Sci. 1984;109:396-400.

[4] Serçe S, Hancock JF. The temperature and photoperiod regulation of flowering and runnering in the strawberries, *Fragaria chiloensis*, *F. virginiana*, and *F.×ananassa*. Sci Hortic. 2005a;103:167-77.

[5] Weebadde CK, Wang D, Finn CE, Lewers KS, Luby JJ, Bushakra J, Sjulin TM, Hancock JF. Using a linkage mapping approach to identify QTL for day-neutrality in octoploid strawberry. Plant Breeding. 2008;127:94-101.

[6] Sönsteby A, Heide OM. Temperature responses, flowering and fruit yield of the June-bearing strawberry cultivars 'Florence', 'Frida' and 'Korona'. Sci Hortic. 2008;119:49-54.

[7] Bradford E, Hancock JF, Warner RM. Interactions of temperature and photoperiod determine expression of repeat flowering in strawberry. J Am Soc Hortic Sci. 2010;135:1-6.

[8] Hancock JF. Strawberries. CABI Publishing, Wallingford, U.K; 1999.

[9] Bringhurst RS, Ahmadi H, Voth V. Inheritance of the day-neutral trait in strawberries. Acta Hortic. 1989;265:35-41.

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

[11] Shaw DV, Famula TR. Complex segregation analysis of day-neutrality in domestic strawberry (*Fragaria×ananassa* Duch.). Euphytica. 2005;145:331-8.

[12] Ourecky DK, Slate GL. Behavior of the everbearing characteristics in strawberries. Proc Am Soc Hortic Sci. 1967;91:236-48.

[13] Shaw DV. Heterogeneity of segregation ratios from selfed progenies demonstrate polygenic inheritance of day-neutrality in strawberry (*Fragaria×ananassa* Duch.). J Am Soc Hortic Sci. 2003;128:504-7.

[14] Serçe S, Hancock JF. Inheritance of day-neutrality in octoploid species of *Fragaria*. J Am Soc Hortic Sci. 2005b;130:580-4.

[15] Castro P, Bushakra JM, Stewart P, Weebadde CK, Wang D, Hancock JF, Finn CE, Luby JJ, Lewers KS. Genetic mapping of day-neutrality in cultivated strawberry. Mol Breed. 2014; (In press).

[16] Gaston A, Perrotte J, Lerceteau-Köhler E, Rousseau-Gueutin M, Petit A, Hernould M, Rothan C, Denoyes B. *PFRU*, a single dominant locus regulates the balance between sexual and asexual plant reproduction in cultivated strawberry. J Exp Bot. 2014;64:1837-48.

[17] Mookerjee S, Finn CE, Mathey MM, Zhang Z, Hancock JF. Differential heat tolerance determines flowering in octoploid strawberry (*Fragaria×ananassa*). J Berry Res. 2013;3:151-8.

[18] Lewers KS, Styan SMN, Hokanson, SC. Strawberry Gene-bank-derived and genomic Simple Sequence Repeat (SSR) markers and their utility with strawberry, blackberry, and red and black raspberry. J Amer Soc Hort Sci. 2005;130:102-15.

[19] Sargent DJ, Hadanou AM, Simpson DW. Development and characterization of polymorphic microsatellite markers from *Fragaria viridis*. Mol Ecol Notes. 2003;3:550-2.

[20] Cipriani G, Testolin R. Isolation and characterization of microsatellite loci in *Fragaria*. Mol Ecol Notes. 2004;4:366-8.

[21] Bassil NV, Gunn M, Folta K, Lewers K. Microsatellite markers for *Fragaria* from 'Strawberry Festival' expressed sequence tags. Mol Ecol Notes. 2006a;6:473-6.

[22] Bassil NV, Njuguna W, Slovin JP. EST-SSR markers from *Fragaria vesca* L. cv 'Yellow Wonder'. Mol Ecol Notes. 2006b;6:806-9.

[23] Sargent DJ, Clarke J, Simpson DW, Tobutt KR, Arús P, Monfort A, Vilanova S, Denoyes-Rotham B, Rousseau M, Folta KM, Bassil NV, Battey NH. An enhanced microsatellite map of *Fragaria*. Theor Appl Genet. 2006;112:1349-59.

[24] Gil-Ariza DJ, Amaya I, Botella MA, Munoz Blanco J, Caballero JL, Lopez-Aranda JM, Valpuesta V, Sanchez-Sevilla JF. EST-derived polymorphic microsatellites from cultivated strawberry (*Fragaria*×ananassa) are useful for diversity studies and varietal identification among *Fragaria* species. Mol Ecol Notes. 2006;6:1195-7.

[25] Njuguna W. Development and use of molecular tools in *Fragaria*. PhD Thesis. Oregon State University. 2010.

[26] Zorrilla-Fontanesi Y, Cabeza A, Domínguez P, Medina JJ, Valpuesta V, Denoyes-Rothan B, Sanchez-Sevilla JF, Amaya I. Quantitative trait loci and underlying candidate genes controlling agronomical and fruit quality traits in octoploid strawberry (*Fragaria*×*ananassa*). Theor Appl Genet. 2011;123:755-78.

[27] Bassam BJ, Caetano-Anolles G, Gresshoff PM. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem. 1991;196:80-3.

[28] Wu KK, Burnquist W, Sorrells ME, Tew TL, Moore PH, Tanksley SD. The detection and estimation of linkage in polyploids using single dose restriction fragments. Theor Appl Genet. 1992;83:294-300.

[29] Stam P. Construction of integrated genetic linkage maps by means of a new computer package: Join Map Plant J. 1993;3:739-44.

[30] Voorips RE. MapChart: Software for the graphical presentation of linkage maps and QTLs. J Hered. 2002;93:77-8.

[31] Van Ooijen JW. MapQTL®5, Software for the mapping of quantitative trait loci in experimental populations. Kyazma B.V., Wageningen, Netherlands; 2004.

[32] Shulaev V, Sargent DJ, Crowhurst RN, et al. The genome of woodland strawberry (*Fragaria vesca*). Nature Genetics. 2011;43(2):109-16. doi:10.1038/ng.740