DISCOVERY OF A QTL FOR CHERRY LEAF SPOT RESISTANCE AND VALIDATION IN TETRAPLOID SOUR CHERRY OF QTLS FOR BLOOM TIME AND FRUIT QUALITY TRAITS FROM DIPLOID *Prunus* SPECIES

By

Travis Stegmeir

A DISSERTATION

Submitted to
Michigan State University
In partial fulfillment of the requirements
For the degree of

Plant Breeding, Genetics, and Biotechnology – Doctor of Philosophy

2013

ABSTRACT

DISCOVERY OF A QTL FOR CHERRY LEAF SPOT RESISTANCE AND VALIDATION IN TETRAPLOID SOUR CHERRY OF QTLS FOR BLOOM TIME AND FRUIT QUALITY TRAITS FROM DIPLOID *Prunus* SPECIES

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With heterozygous polyploid species, detecting quantitative trait loci (QTL) can be an arduous process, especially in segmental allopolyploids like sour cherry (2n=4x=32) where non-homologous pairing is common. In our sour cherry breeding and genetics program at Michigan State University, we have taken a QTL validation approach for identifying relevant QTLs, whereby QTLs more easily discovered in related diploid species are tested for their association in sour cherry. SNP markers on the Illumina 6K Infinium II array were used for genotyping sour cherry plant materials included in the USDA-SCRI funded RosBREED project (www.rosbreed.org). GenomeStudio polyploidy functionalities were used to score SNP genotypes, including dosage. Previously identified QTLs/candidate genes for several horticulturally important traits (fruit size, fruit flesh color, fruit acidity, fruit firmness and bloom time) were identified from the peach (*P. persica),* almond *(P. dulcis)* and sweet cherry (*P. avium*) literature. SNP markers spanning the target QTL intervals were identified based on synteny with the peach genome sequence, and marker linkage phase was determined based on sour cherry progeny segregation. The different haplotypes identified for these targeted regions were then tested for haplotype trait association. Haplotypes with significant effect on phenotype were identified for marker-assisted breeding. In certain cases, the SNP haplotype was 'converted' to an SSR marker to facilitate future genotyping. Not all regions found to be significant in diploid relatives were significant in sour cherry, indicating either they are absent,

fixed or cannot be detected due to complexity of dosage and more allelic variants compared to diploid species. This approach has been successful for QTLs with fairly large effects, which are good targets for marker-assisted breeding. Since no QTL studies have been done previously with cherry leaf spot (CLS) resistance, we utilized the Bayesian approach, implemented in FlexQTL^{TM} software which allowed us to follow important genotypic regions from multiple populations through generations by including pedigreed parents and grandparents in the analysis. By studying two populations, one with *P. canescens* derived CLS resistance, and one without, we were able to locate a QTL on the top of linkage group (G)4 between SNP markers ss490552303 and ss490552492 (between ~2.9-13.4 cM). When individuals with and without the *P. canescens* haplotype at this region were compared, it was found the *P. canescens* haplotype was significantly associated with disease resistance. The same was found in sour cherry, where all individuals that were resistant to CLS had *P. canescens* haplotypes at this region. In both sweet and sour cherry, however, individuals with the *P. canescens* segment were found that were also susceptible, indicating that this is not the only region important for conferring CLS disease resistance.

ACKNOWLEDEMENTS

I would like to thank my major professor, Dr. Amy Iezzoni, for her guidance, support and editorial assistance, without which, I would not be where I am today. I especially appreciate her willingness to let me partake in several aspects of her breeding program, where I got to use the knowledge I gained throughout my PhD journey to design and implement crosses in the field.

I also appreciated the assistance of my other committee members, Dr. Jim Hancock, Dr. Dechun Wang, and Dr. George Sundin for their valuable suggestions and helpful advice.

Special thanks are also given to past and present members of the Iezzoni lab, especially Audrey Sebolt who was always available to help, and whose knowledge of the lab and procedures was imperative to my success.

Thanks are given also to Mirko Schuster of the Julius Kühn-Institut, Dresden, Germany, for his phenotyping of his sweet cherry populations for Cherry Leaf Spot resistance, and his allowing us to collaborate with him in this project.

The NIFA-funded RosBREED project (www.rosbreed.org) was also pivotal in my research for providing the funding and resources needed to carry out my research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

DISCOVERY OF A QTL FOR CHERRY LEAF SPOT RESISTANCE

Introduction

Cherry leaf spot (CLS), caused by the fungal pathogen *Blumeriella jaapii* (Rehm) Arx (anamorph *Phloeosporella padi* [Lib.] Arx), is a major disease in all humid cherry growing regions worldwide. While sour cherries (*Prunus cerasus*) are generally more prone to infection and the resulting leaf yellowing and defoliation, sweet cherries (*P. avium*) are also affected. When not controlled, CLS can cause early leaf defoliation, which can result in fruit that are poorly colored, soft, and low in soluble solids (Keitt et al., 1937). Premature defoliation can also weaken the tree and reduce winter hardiness which can lead to flower bud freeze damage and even tree death (Howell and Stackhouse, 1973). Studies have shown that fruit have priority over other sinks in *Prunus* (Richards, 1986), so fewer leaves would produce fewer storage carbohydrates for the following year's growth.

As many as seven fungicide applications can be needed each growing season on sour cherry to manage CLS, resulting in substantial cost to growers and a significant amount of pesticides released into the environment. There is also the threat that the pesticides currently being used to control CLS may be removed from the market, jeopardizing the sustainability of the industry. *B. jaapii* has also been found to develop resistance to site-specific sterol demethylation inhibitor fungicides (DMIs) which have been used extensively to control CLS on cherry (Proffer et al., 2006). This developed resistance to a major class of CLS-controlling fungicide increases the need for new varieties that have genetic resistance to CLS. Many studies have shown there are no sour cherry cultivars that have complete resistance to CLS (Sjulin et al., 1989; Schuster and

Tobutt, 2004; Budan et al., 2005); however, in all of these studies, there were some individuals that displayed moderate resistance, indicating some polygenic resistance.

The susceptibility of all current cultivars in *P. avium* and *P. cerasus* germplasm to CLS warranted the need to examine wild cherry species in an attempt to find resistance. One promising candidate to introgress disease resistance was shown to be the wild diploid species *P. canescens* (Wharton et al., 2003; Wharton and Iezzoni, 2005; Schuster and Tobutt, 2004). As a result, *P. canescens* has been used in breeding for CLS resistance in both sweet and sour cherry with diploid and tetraploid populations, respectively, segregating for disease resistance. With the development of an Illumina Infinium® cherry SNP array (Peace et al., 2012), it was possible to locate introgressed chromosome regions from *P. canescens* due to the high marker coverage across all 8 linkage groups. The objective of this study was to determine the inheritance of *P. canescens*-derived resistance to sweet cherry and identify the locations of gene(s) controlling this resistance. Because of the simpler genetics of diploid cherry, the initial investigation was done with *P. canescens*-derived materials from crosses with sweet cherry, followed by validation using *P. canescens*-derived plant materials from sour cherry. In this study, we used the Bayesian approach, implemented in FlexQTL^{TM} software (Bink et al. 2002, Rosyara et al. 2013). This allowed us to follow important genotypic regions from multiple populations through generations by including pedigreed parents and grandparents in the analysis.

Materials and Methods

Plant materials

The sweet cherry plant materials for this study were developed and maintained at the Julius Kühn-Institut (JKI) in Dresden, Germany and included 74 BC_1 progeny individuals (Table 1.1). *P. canescens* was used as a pollen parent and crossed with the sweet cherry selection *(P. avium* M30). From this F_1 population, the CLS-resistant individual F5-18-167 was selected due to its disease resistance to CLS, and was used as a parent to transfer this resistance into one of the populations in this study (Table 1.1).

In sour cherry, *P. canescens* was first incorporated from the triploid grandparent 148-1 (Schmidt and Gruppe, 1988). Seed from this triploid were planted, and from those, a CLS resistant parent '23-23-13' was shown to have sufficient fertility to be used in crosses with sour cherry parents 'Montmorency' and 'Újfehértói Fürtös' ('UF') (Figure 1.1). The resulting 15 sour cherry selections were used in this study and were maintained at Michigan State University's Clarksville Horticultural Experimental Station of Michigan State University in Clarksville, MI, USA (Table 1.1).

Disease rating

The sweet cherry individuals were not sprayed with fungicides for the control of CLS in the years 2008-2011. Selections were scored using the following rating scale modified from Wharton and Iezzoni (2005), where a score of 2 or less was considered resistant:

1 – No chlorotic symptoms, small hypersensitive response at point of infection (Fig. 1.2a)

2 – Scattered pigmented lesions, chlorotic or necrotic points, no visible sporulation (Fig. 1.2b)

3 – Larger lesions, partly with aerial mycelium and stunted sporulating acervuli (Fig. 1.2c)

4 – Sporulating acervuli with chlorotic and necrotic lesions (Fig. 1.2d)

5 – Heavily sporulating acervuli (Fig. 1.2e)

For the sour cherry individuals, CLS was also not controlled in the orchard containing the plant material used for this analysis, resulting in intense disease pressure. In 2010, CLS severity was recorded using the same scale as with sweet cherry. In 2011, the trees were also left untreated for CLS, however individuals were rated only as resistant or susceptible, with those considered resistant if infection did not result in conidia formation and trees did not defoliate (disease score of 2 or less). All individuals on which we observed conidia formation on leaves, or that exhibited defoliation, or both were considered susceptible.

Pedigree confirmation in sweet cherry

P. canescens pedigree confirmation in sweet cherry was done using the SNP data described below and the program KINGROUP (Konovalov et. al., 2004) with R-script. Individuals were considered to have *P. canescens* in their background if the *P*-value comparing marker scores to the parent F5-18-167, which contains *P. canescens*, was equal to or less than 0.05.

SNP data and sweet cherry map construction

For sweet cherry, two sources of *P. canescens*, (one from Michigan State, and one from Germany), 'Namati', F5-18-167, and 72 individuals were genotyped using the RosBREED Illumina Infinium® cherry SNP array of 5,696 SNP markers (Peace et. al., 2012). SNP genotypes were determined using the Genotyping Module of GenomeStudio Data Analysis software v2010.3 (Illumina Inc. 2010). A total 2,949 polymorphic SNPs were found, from which 548 SNPs were selected manually to cover the 8 *Prunus* linkage groups (Figure 1.3). Markers were selected to be spread across each chromosome as equally as possible based on physical map distances previously determined in peach. As the number of true offspring was too small for linkage map construction, the map used for QTL analysis was based on the peach physical map positions (Verde et al. 2013) where the physical map was scaled to a genetic map by conversion factor of 1 Mb = 4 cM.

For sour cherry, a separate GenomeStudio project was done where 'UF', 'Montmorency', '23-23-13', and 15 seedlings and other sour cherry founders and populations (384 individuals) were genotyped the same as above; however, available SNP data from a diverse array of sweet cherry selections and seedlings (105 individuals) were included to aid in the determination of dosage by showing the two homozygous (AAAA and BBBB for sour cherry corresponding to AA and BB in sweet cherry) and balanced heterozygous (AABB for sour cherry corresponding to AB in sweet cherry) classes (Figure 1.4).

QTL analysis and QTL allele identification in sweet cherry

QTL mapping was done initially using the genome-wide set of 548 SNPs selected from those markers found to be polymorphic to equally cover the 8 *Prunus* linkage groups, followed by an

analysis of a single chromosome with a dense map - once a QTL was located on G4, all available polymorphic SNPs found for that linkage group, i.e. 241 markers, were run. The consistency in marker order and distance was verified by comparing expected and observed double cross-over frequency for both maps.

QTL mapping was done with mean disease score for 2008-2011. The parents, grandparents and progenies were included in the pedigree for analysis. QTL analysis was done using a Markov chain Monte Carlo (MCMC) based Bayesian analysis method (Bink et al. 2002, 2008) implemented in the FlexQTL^{TM} software as was done in Rosyara et al. (2013), but with a simulation length of 100,000 iterations with a thinning value of 10, and a simulation length of 200,000 and a thinning value of 20 for all 8 chromosomes, and the dense G4 map respectively.

The haplotypes for the QTL identified were manually constructed for both sweet and sour cherry using the SNP data where linkage phase of the markers could be determined. To determine the effect of the QTL alleles identified, Student's t-tests were performed with all sweet cherry individuals comparing the presence/absence of the *P. canescens* haplotype, and then just within the family with confirmed *P. canescens* pedigree. SSR markers were also used in sour cherry to follow the *P. canescens* chromosome segment when haplotypes were unable to be constructed based on ambiguous SNP calls (Table 1.2).

Results

***P. canescens*-derived cherry leaf spot resistance in sweet cherry**

Thirty four of the 74 progeny individuals were found to have F5-18-167 as a likely parent as the SNPs indicated a high likelihood of relatedness ($P < 0.05$, Table 1.3) confirming that these individuals were derived from this *P. canescens*-containing parent. The other 38 individuals were found to be completely unrelated to *P. canescens*, as marker data showed a low likelihood of relatedness ($P > 0.05$, Table 1.4). These results indicated two distinct populations, one with *P. canescens* ancestry, and one without.

In sweet cherry, the frequency distribution of mean disease scores with all progeny individuals showed a continuous distribution (Figure 1.5). In the population of 34 individuals with confirmed *P. canescens* in their background, and in the populations of 38 individuals without, a bi-modal, and a continuous distribution were observed, respectively (Figure 1.5). The continuous distribution indicated that there may be several genes influencing disease resistance. However, in the population derived from *P. canescens*, the bi-modal distribution suggested one major gene influencing disease score (Figure 1.5). No individuals had a disease score of 1 (hypersensitive response, green leaf) for all of the 4 years of field susceptibility analysis to CLS (Table 1.1). In some instances the fungus was able to infect and produce conidia for secondary infection, resulting in scores of 3 or higher which was considered susceptible. Individuals with disease scores of less than 2 were considered to be resistant.

The Genome-wide QTL analysis showed positive evidence for one QTL on G4 (Table 1.5). Since the Bayes factors for the number and location of the QTL was consistent in the five replications, only that of the first is presented. Once a QTL was located on G4, all 241 polymorphic SNPs found for this linkage group were used in the FlexQTL^{TM} analysis (Figure

1.6). When the QTL analysis was run using all of the polymorphic G4 markers, there was decisive evidence that one QTL was located on the top of G4 between SNP markers ss490552323 and ss490552500 (between 4.0-13.8 cM) (Table 1.6, Figure 1.7a). At over 0.15 at its highest peak, the intensity of this QTL is over the 0.10 threshold, indicating this is a significant QTL. The traceplot for this QTL is also a good indicator that this as a valid QTL (Figure 1.7b). Two new seeds were also used to confirm the first FlexQTL^{TM} run with the same results (data not shown). This QTL was named *CLSR_G4* for CLS resistance found on G4 (Table 1.7).

Haplotype construction and QTL allele identification

To validate this major QTL in sweet cherry, haplotypes were constructed for the QTL region. As *P. canescens* was homozygous at this region and had several unique SNPs, this haplotype was easy to construct as linkage phase between the SNPs was known (Figure 1.8). When comparing mean disease scores for all sweet cherry individuals containing the *P. canescens* haplotype at the G4 QTL region with those that did not, a significantly lower ($P = 0.004$) mean disease score was found for those with this *P. canescens*-derived haplotype than those individuals without the *P. canescens* haplotype at G4, with mean disease scores of 2.3 and 3.2 for individuals with the *P. canescens* haplotype, and those without it, respectively (Table 1.8).

When considering only those 34 individuals with confirmed *P. canescens* lineage (See Table 1.3), those with, and without the haplotype for this region had an even larger difference, with a

mean disease score of 2.3 and 4.1 for those with the *P. canescens* haplotype, and those without it, respectively (Table 1.9).

Not all individuals with this haplotype from *P. canescens* were rated as resistant to CLS (Disease score less than 2), as five of the 15 individuals with this haplotype were susceptible (Table 1.9). No individuals in this family without the *P. canescens* allele at this region were found to be resistant however, indicating that while this region is necessary for CLS resistance, there may be other genes involved.

***P. canescens*-derived cherry leaf spot resistance in sour cherry**

For sour cherry, of the 15 '23-23-13'-derived seedlings screened, 6 had susceptible ratings for CLS, while the other 9 had resistant ratings (Table 1.1). Both 'UF' and 'Montmorency' were susceptible, while the *P. canescens*-containing parent '23-23-13' was resistant (Table 1.1).

Of the 18 sour cherry individuals genotyped, haplotypes for the G4 QTL region could be determined for all parents ('Montmorency,' 'UF' and the *P. canescens*-derived '23-23-13') and nine of the 15 progeny (Figure 1.9 a and b). All of the discerned haplotypes from the progeny of the cross between the resistant parent '23-23-13' and either susceptible parent 'Montmorency' or 'UF' contained the *P. canescens* haplotype R that is associated with disease resistance in sweet cherry. The six individuals for which haplotypes could not be reliably determined were due to either undetermined or ambiguous SNP calls for this region.

To verify *P. canescens* haplotypes, and determine if *P. canescens* is present at the G4 QTL region in those individuals where haplotypes were unable to be constructed, four SSR markers situated within the haplotype and spanning the QTL region between SNP markers ss490552323 (4.0 cM, 1.0 Mb) and ss490552500 (13.8 cM, 3.46 Mb) were designed and run to confirm the presence or absence of the *P. canescens* chromosome (Table 1.2, Figure 1.10). All markers had a unique band representing the *P. canescens* chromosome, which was present in the resistant parent '23-23-13', and in 12 of the 15 seedlings (Figure 1.11 a-d). This allowed us to essentially "tag" the *P. canescens* chromosome at this region, even when haplotypes were unable to be constructed. Due to the unique bands from *P. canescens*, we were confident that no crossovers took place within the QTL region.

The three seedlings which did not contain the *P. canescens* alleles were all susceptible to CLS. There were, however, three individuals which had the *P. canescens* G4 allele, but were also susceptible to CLS (Figure 1.11 a-d). This, as with the case of sweet cherry, shows that the mere presence of this region does not guarantee that the tree will be resistant, but without it, trees are likely to be susceptible.

Discussion

One major QTL controlling CLS resistance, named *CLSR_G4*, was identified on G4. This is in agreement with the phenotypic data in sweet cherry which suggested a major gene effect from *P. canescens* in the population verified to have *P. canescens* in its background. The G4 region has been shown to be a major contributing factor to CLS disease resistance. It appears, however,

that a two gene model where both parents are heterozygous for the second gene may be a better fit (Figures 1.12 and 1.13), as five of the 15 sweet cherry individuals with the *P. canescens* haplotype for the QTL region were susceptible to CLS, and three of the 12 sour cherry seedlings with this G4 *P. canescens* region were found to still be susceptible (Tables 1.10 and 1.11). If only one gene were involved, all 15 sweet cherry, and all 12 sour cherry individuals with this G4 genomic region would be resistant. In sweet cherry, one-third of the individuals with this G4 haplotype from *P. canescens* were still susceptible. While this is close to the one-fourth predicted by the model, since the other parent is unknown, it is possible that some of the seedlings do not share this same parent, perhaps a parent without this second important gene. This would slightly skew the expected ratio. In sour cherry, two-sevenths of the progeny from the cross 'Montmorency' × '23-23-13' with the G4 haplotype from *P. canescens* are susceptible, and one-fifth of the progeny from the cross 'UF' × '23-23-13' with this haplotype are susceptible. Both of these numbers are close to the expected one-fourth of those carrying the resistance haplotype from *P. canescens* exhibiting susceptibility that would be predicted by the suggested model.

Since we had the marker data spanning all 8 linkage groups, a bulked segregant analysis was done in sweet cherry comparing individuals that had the G4 *P. canescens* region and were either resistant or susceptible. No discernible region was determined to be absent only in those individuals that were susceptible, while present in the resistant individuals (data not shown).

Finding individuals within the sweet cherry background without *P. canescens* but still resistant is not surprising as a study on partial resistance to CLS indicated that there were gradients of

infection and subsequent defoliation which was not always associated with high infection rates (Sjulin et. al., 1989). This variation is likely caused by polygenic genes for horizontal resistance present in cherry.

The ambiguity of SNP calls for certain regions in sour cherry is likely due to the segmental allo-polyploid nature of this species (Beaver and Iezzoni, 1993; Beaver et. al., 1995). Cytological studies on sour cherry have revealed that multivalent and univalent formations at meiosis are not uncommon (Schuster, 2000; Schuster and Wolfram, 2005). Any surviving seedling that resulted from an abnormal number of any chromosome would therefore give results that would be ambiguous from the expected results of 4 copies for that region. Due to the SNP dosage ambiguity, the use of the SSR markers aided greatly in this study to allow us to follow the *P. canescens* chromosome for this region. These markers can also be used in subsequent generations in marker assisted breeding (MAB) which would allow the breeder to discard more seedlings at an earlier stage in development to reduce field maintenance costs, and allow for the planting of more superior seedlings for improved chances of CLS-resistant cultivar breeding success. Since sour cherry is more susceptible to CLS than sweet cherry, it is likely that sour cherry has less horizontal resistance either due to lack of the genes, or due to the polyploidy nature of sour cherry, so it is imperative that this G4 region is present if resistance is desired.

Future work in CLS resistance would be important in a larger population to allow for the location of other QTL that contribute to disease resistance. Additional QTL may also be identified for horizontal resistance which would help maintain the integrity of the resistance. This would be

especially beneficial in sour cherry, which is generally more susceptible to CLS, and therefore is likely to carry fewer horizontal resistance genes.

Table 1.1: Plant materials used in this study and cherry leaf spot disease evaluation scores for 2008 to 2011.

				Disease Score				
Plants	Parent 1	Parent 2	Species	2008	2009	2010	2011	Mean
704010-003	F5-18-167	op	Sweet	5	5	4	4	4.5
704010-004	F5-18-167	op	Sweet	2	1	2	2	1.8
704010-008	F5-18-167	op	Sweet	4	2	4	4	3.5
704010-009	F5-18-167	op	Sweet	3	5	4	4	4.0
704010-010	F5-18-167	op	Sweet	4	5	4	4	4.3
704010-015	F5-18-167	op	Sweet	2	2	2	2	2.0
704010-019	F5-18-167	op	Sweet	4	4	4	4	4.0
704010-022	F5-18-167	op	Sweet	3	4	4	4	3.8
704010-025	F5-18-167	op	Sweet	2	1	1	1	1.3
704010-028	F5-18-167	op	Sweet	4	5	5	5	4.8
704010-029	F5-18-167	op	Sweet	4	4	2	4	3.5
704010-034	F5-18-167	op	Sweet	4	5	5	4	4.5
704010-037	F5-18-167	op	Sweet	4	5	5	5	4.8
704010-050	F5-18-167	op	Sweet	-	5	5	4	4.7
704010-057	F5-18-167	op	Sweet	5	5	5	4	4.8
704010-061	F5-18-167	op	Sweet	2	2	2	2	2.0
704010-062	F5-18-167	op	Sweet	2	2	2	2	2.0
704010-066	F5-18-167	op	Sweet	2	1	2	2	1.8
704010-072	F5-18-167	op	Sweet	2	2	2	2	2.0
704010-074	F5-18-167	op	Sweet	4	3	-	-	3.5
704010-078	F5-18-167	op	Sweet	4	5	5	5	4.8
704010-079	F5-18-167	op	Sweet	4	3	2	4	3.3
704010-083	F5-18-167	op	Sweet	2	3	5	-	3.3
704010-084	F5-18-167	op	Sweet	4	5	2	4	3.8
704010-085	F5-18-167	op	Sweet	2	1	1	2	1.5
704010-086	F5-18-167	op	Sweet	3	1	2	4	2.5
704010-087	F5-18-167	op	Sweet	2	2	2	2	2.0
704010-093	F5-18-167	op	Sweet	2	1	2	2	1.8
704010-099	F5-18-167	op	Sweet	-	4	5	-	4.5
704010-125	F5-18-167	op	Sweet	5	5	5	4	4.8
705012-005	F5-18-167	op	Sweet	5	5	5	4	4.8
705012-020	F5-18-167	op	Sweet	4	4	2	4	3.5
705012-025	F5-18-167	op	Sweet	5	5	5	5	5.0
704010-005	Namati	op	Sweet	3	2	2	3	2.5
704010-007	unknown	-	Sweet	4	3	4	4	3.8
704010-012	Namati	op	Sweet	4	2	2	2	2.5

Table 1.1 (cont'd)

Plants	Parent 1	Parent 2	Species	Disease Score				
				2008	2009	2010	2011	Mean
704010-013	Namati	op	Sweet	4	4	4	4	4.0
704010-014	Namati	op	Sweet	2	1	2	2	1.8
704010-016	unknown	-	Sweet	4	5	4	4	4.3
704010-017	Namati	op	Sweet	4	3	2	3	3.0
704010-020	unknown	-	Sweet	2	1	2	3	2.0
704010-024	Namati	op	Sweet	3	1	2	2	2.0
704010-026	unknown	-	Sweet	3	2	2	4	2.8
704010-030	unknown	-	Sweet	3	2	1	2	2.0
704010-032	unknown	-	Sweet	3	1	3	2	2.3
704010-033	Namati	op	Sweet	3	2	2	3	2.5
704010-035	unknown	-	Sweet	3	2	2	4	2.8
704010-036	unknown	-	Sweet	4	2	3	4	3.3
704010-039	Namati	op	Sweet	5	5	5	4	4.8
704010-040	Namati	op	Sweet	4	2	2	4	3.0
704010-043	unknown	-	Sweet	2	2	2	2	2.0
704010-044	unknown	-	Sweet	2	2	2	3	2.3
704010-045	Namati	op	Sweet	2	1	2	2	1.8
704010-047	unknown	-	Sweet	4	1	3	4	3.0
704010-051	Namati	op	Sweet	4	4	4	4	4.0
704010-053	Namati	op	Sweet	2	2	2	2	2.0
704010-054	unknown	-	Sweet	3	2	2	3	2.5
704010-060	Namati	op	Sweet	3	2	2	2	2.3
704010-063	Namati	op	Sweet	4	2	2	3	2.8
704010-064	Namati	op	Sweet	4	4	2	3	3.3
704010-068	Namati	op	Sweet	2	2	2	3	2.3
704010-069	unknown	-	Sweet	3	4	4	4	3.8
704010-071	unknown	-	Sweet	2	2	2	4	2.5
704010-077	Namati	op	Sweet	2	1	2	2	1.8
704010-080	unknown	-	Sweet	4	3	5	4	4.0
704010-081	Namati	op	Sweet	3	1	2	3	2.3
704010-082	unknown	-	Sweet	4	3	3	4	3.5
704010-091	Namati	op	Sweet	3	2	2	2	2.3
704010-092	unknown	-	Sweet	2	2	2	3	2.3
704010-097	unknown	-	Sweet	3	2	2	3	2.5
704010-110	Namati	op	Sweet	5	4	5	4	4.5
705012-002	Namati	op	Sweet	4	2	2	2	2.5

Table 1.1 (cont'd)

Plants	Parent 1	Parent 2	Species	Disease Score				
				2008	2009	2010	2011	Mean
F5-18-167	M30	GerP-can[a]	Sweet	1	1	2	2	1.5
Namati	-	-	Sweet	2	2	3	2	2.3
GerP-can	-	-	P.canescens	-	-	-	-	-
P.canescens	-	-	P.canescens	-	-	-	-	-
148-1	P.canescens	RS	Sour	-	-	2	R[b]	R
23-23-13	148-1	op	Sour	-	-	2	R	R
24-32-17	Montmorency	23-23-13	Sour	-	-	2	R	R
24-32-18	Montmorency	23-23-13	Sour	-	-	2	R	R
24-32-20	Montmorency	23-23-13	Sour	-	-	2	R	R
24-32-21	Montmorency	23-23-13	Sour	-	-	2	R	R
24-32-23	Montmorency	23-23-13	Sour	-	-	3	S[c]	S
24-32-24	Montmorency	23-23-13	Sour	-	-	5	S	S
24-32-25	Montmorency	23-23-13	Sour	-	-	3	S	S
24-32-26	Montmorency	23-23-13	Sour	-	-	5	S	S
24-32-27	Montmorency	23-23-13	Sour	-	-	2	R	R
24-32-37	Balaton	23-23-13	Sour	-	-	2	R	R
24-32-39	Balaton	23-23-13	Sour	-	-	3	S	S
24-32-40	Balaton	23-23-13	Sour	-	-	5	S	S
24-32-41	Balaton	23-23-13	Sour	-	-	2	R	R
24-32-43	Balaton	23-23-13	Sour	-	-	2	R	R
24-32-44	Balaton	23-23-13	Sour	-	-	4	S	S
Balaton	-	-	Sour	-	-	-	S	S
Montmorency	-	-	Sour	-	-	-	S	S

[a] *Prunus canescens* from Germany

[b] Resistant

[c] Susceptible

Table 1.2: SSR markers designed and used to validate the presence of *P. canescens* in the G4 disease resistant region in sour cherry.

Marker Name	Sequence (5' to 3')	Ta	*P. canescens* fragment size (bp)	Scaffold 4 location (bp)[a]
CLS004-F	TGGGCCAGTATTTTACAGGAG	54	230	1414429-1414449
CLS004-R	TTGGCTGGTCTCTCACAAAA			1414644-1414663
CLS005-F	AATTGTGCGGGAGCTACAAG	54	232	1359841-1359860
CLS005-R	GCCATCATCAGGTAGCAATG			1359616-1359635
CLS026-F	AGCCCAACGTCTCATTCACC	Touchdown[b]	180	3456175-3456194
CLS026-R	GGAGATGAAGCAAAAGAGATGC			3456412-3456391
CLS028-F	GAATGCAGTTGGGGAGTTACC	Touchdown	168	3334891-3334911
CLS028-R	CTTCTTGCACCAAAAACAACC			3335078-3335058

[a] Distances according to the Peach v1.0 'dhLovell' genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome (Verde et al. 2013)

[b] Ta of 60°C for 45 seconds, with an extension at 72°C for 60 seconds. For the next 9 cycles, the Ta drops 1°C per cycle, then for the last 24 cycles, Ta remains at 55°C for 45 seconds with the same extension time.

Table 1.3: Pedigree confirmation of 34 individuals found to have *P. canescens* in their pedigree via parent F5-18-167. *P*-values are calculated using the program KINGROUP (Konovalov et. al., 2004) testing a null hypothesis that no parent offspring relation between listed pair in rows vs columns.

	F5-18-167	*P.canescens*
704010-003	0.00	0.18
704010-004	0.00	0.00
704010-008	0.00	0.05
704010-009	0.00	0.02
704010-010	0.00	0.06
704010-015	0.00	0.17
704010-019	0.05	0.28
704010-022	0.00	0.00
704010-025	0.00	0.07
704010-028	0.00	0.05
704010-029	0.00	0.00
704010-034	0.05	0.16
704010-037	0.05	0.09
704010-050	0.00	0.00
704010-061	0.00	0.04
704010-062	0.00	0.01
704010-066	0.00	0.00
704010-069	0.00	0.01
704010-071	0.00	0.02
704010-072	0.00	0.02
704010-074	0.00	0.05
704010-078	0.05	0.17
704010-079	0.00	0.02
704010-083	0.00	0.09
704010-084	0.00	0.07
704010-085	0.00	0.02
704010-086	0.00	0.17
704010-087	0.00	0.03
704010-093	0.00	0.17
704010-099	0.00	0.08
704010-125	0.00	0.13
705012-005	0.00	0.00
705012-020	0.00	0.02
705012-025	0.03	0.09
Namati	1.00	1.00
GerP-can	0.00	0.00

Table 1.4: Pedigree confirmation of 38 progeny individuals found to not have *P. canescens* in their pedigree. *P*-values are calculated using the program KINGROUP (Konovalov et. al., 2004) testing a null hypothesis that no parent offspring relation between listed pair in rows vs. columns.

	P. canescens
704010-005	1.00
704010-007	1.00
704010-012	1.00
704010-013	0.99
704010-014	0.99
704010-016	1.00
704010-017	0.99
704010-020	1.00
704010-024	1.00
704010-026	0.84
704010-030	0.99
704010-032	0.96
704010-033	1.00
704010-035	1.00
704010-036	1.00
704010-039	1.00
704010-040	0.99
704010-043	0.93
704010-044	0.99
704010-045	1.00
704010-047	0.98
704010-051	0.99
704010-053	1.00
704010-054	1.00
704010-057	0.99
704010-060	0.99
704010-063	1.00
704010-064	1.00
704010-068	1.00
704010-077	1.00
704010-080	1.00
704010-081	0.99
704010-082	1.00
704010-091	1.00
704010-092	1.00

Table 1.4 (cont'd)

	P. canescens
704010-097	1.00
704010-110	1.00
705012-002	0.90
F5-18-167	0.00
Namati	1.00
GerP-can	0.00

Table 1.5: Estimates of 2ln Bayes factors[a] for the first replicate of the genome-wide analysis for mean disease resistance identified using the sweet cherry plant material listed in Table 1.1. The number of QTLs being compared in the models are separated by a back slash ("/").

Group	1/0	2/1	3/2	4/3	5/4
1	0.2	0.0	-0.0	NA	NA
2	0.0	0.0	NA	NA	NA
3	-0.1	-0.0	NA	NA	NA
4	4.5	0.2	0.0	NA	NA
5	-0.0	-0.0	NA	NA	NA
6	-0.1	-0.0	NA	NA	NA
7	0.5	0.0	0.0	NA	NA
8	-0.1	-0.0	NA	NA	NA

[a] 2ln Bayes factors for the comparison of two models. Interpretation of the pairwise model comparison 2lnBF range and evidence: 0-2 hardly any, 2-5 positive, 5-10 strong, > 10 decisive, NA not available due to insufficient MCMC draws from one of the two models.

Table 1.6: Estimates 2ln Bayes factors[a] for the first replicate of the G4 analysis for mean disease resistance identified using the sweet cherry plant material listed in Table 1.1. The number of QTLs being compared in the models are separated by a back slash ("/").

Group	1/0	2/1	3/2	4/3	5/4
4	10.9	0.7	0.2	NA	NA

[a] 2ln Bayes factors for the comparison of two models. Interpretation of the pairwise model comparison 2lnBF range and evidence: 0-2 hardly any, 2-5 positive, 5-10 strong, > 10 decisive, NA not available due to insufficient MCMC draws from one of the two models.

Table 1.7: Marker interval and name for the CLS resistance QTL found on G4.

Group	QTL name	Interval (cM)	Peak position (cM)	Marker interval	Physical map interval[a] (Mb)
4	*CLSR_G4*	4.0-13.8	7.0	ss490552323-ss490552500	1.00-3.46

[a] Mb distances according to the Peach v1.0 'dhLovell' genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome) Verde et al. 2013

Table 1.8: Mean cherry leaf spot scores for the presence, or absence of the G4 *P. canescens* haplotype. All sweet cherry progeny individuals are included, those with, and those without *P. canescens* in their background (See Table 1.1). The means are significantly different (P = 0.004) as denoted by different letters in the disease score mean column.

G4 *P. canescens* Haplotype	Number of individuals	Disease Score Range	Disease Score Mean
Yes	16	1.3-4.5	2.3 A[a]
No	58	1.6-5.0	3.2 B

[a] The means are significantly different ($P = 0.004$) as denoted by different letters in the disease score mean column.

Table 1.9: Mean cherry leaf spot score for the presence or absence of the G4 *P. canescens* haplotype. Only individuals confirmed to have *P. canescens* in their background (See Table 1.3) are included. The means are significantly different ($P < 0.0001$) as denoted by different letters in the disease score mean column.

G4 *P. canescens* Haplotype	Number of individuals	Disease Score Range	Disease Score Mean
Yes	16	1.3-4.5	2.3 A[a]
No	18	2.5-5.0	4.1 B

[a] The means are significantly different ($P < 0.0001$) as denoted by different letters in the disease score mean column.

Table 1.10: Disease scores and the presence/absence of the G4 region from *P. canescens* in sweet cherry progeny with confirmed *P. canescens* background (See Table 1.3). Individuals in bold and underlined contain the *P. canescens* haplotype, but are susceptible.

Genotype	Mean disease score	G4 *P. canescens* haplotype
704010-003	4.5	No
704010-004	1.8	Yes
704010-008	3.5	Yes
704010-009	4.0	Yes
704010-010	4.3	No
704010-015	2.0	Yes
704010-019	4.0	No
704010-022	3.8	No
704010-025	1.3	Yes
704010-028	4.8	No
704010-029	3.5	No
704010-034	4.5	Yes
704010-037	4.8	No
704010-050	4.7	No
704010-061	2.0	Yes
704010-062	2.0	Yes
704010-066	1.8	Yes
704010-069	3.8	No
704010-071	2.5	Yes
704010-072	2.0	Yes
704010-074	3.5	No
704010-078	4.8	No
704010-079	3.3	No
704010-083	3.3	Yes
704010-084	3.8	No
704010-085	1.5	Yes
704010-086	2.5	No
704010-087	2.0	Yes
704010-093	1.8	Yes
704010-099	4.5	No
704010-125	4.8	No
705012-005	4.8	No
705012-020	3.5	No
705012-025	5.0	No

Table 1.11: Cherry leaf spot disease scores and the presence/absence of the G4 region from *P. canescens* of sour cherry progeny (See Table 1.1). Individuals in bold and underlined contain the *P. canescens* haplotype, but are disease susceptible.

Genotype	Disease rating	G4 *P. canescens* region
24-32-17	Resistant	Yes
24-32-18	Resistant	Yes
24-32-20	Resistant	Yes
24-32-21	Resistant	Yes
34-32-23	Susceptible	No
24-32-24	Susceptible	Yes
24-32-25	Susceptible	No
24-32-26	Susceptible	Yes
24-32-27	Resistant	Yes
24-32-37	Resistant	Yes
24-32-39	Susceptible	Yes
24-32-40	Susceptible	No
24-32-41	Resistant	Yes
24-32-43	Resistant	Yes
24-32-44	Resistant	Yes
148-1	Resistant	Yes
23-23-13	Resistant	Yes

Figure 1.1: Pedigree of the incorporation of CLS resistant *P. canescens* into the sour cherry background. Individual which are resistant are colored black, susceptible individuals are colored white, and families which are segregating for resistance are grey.

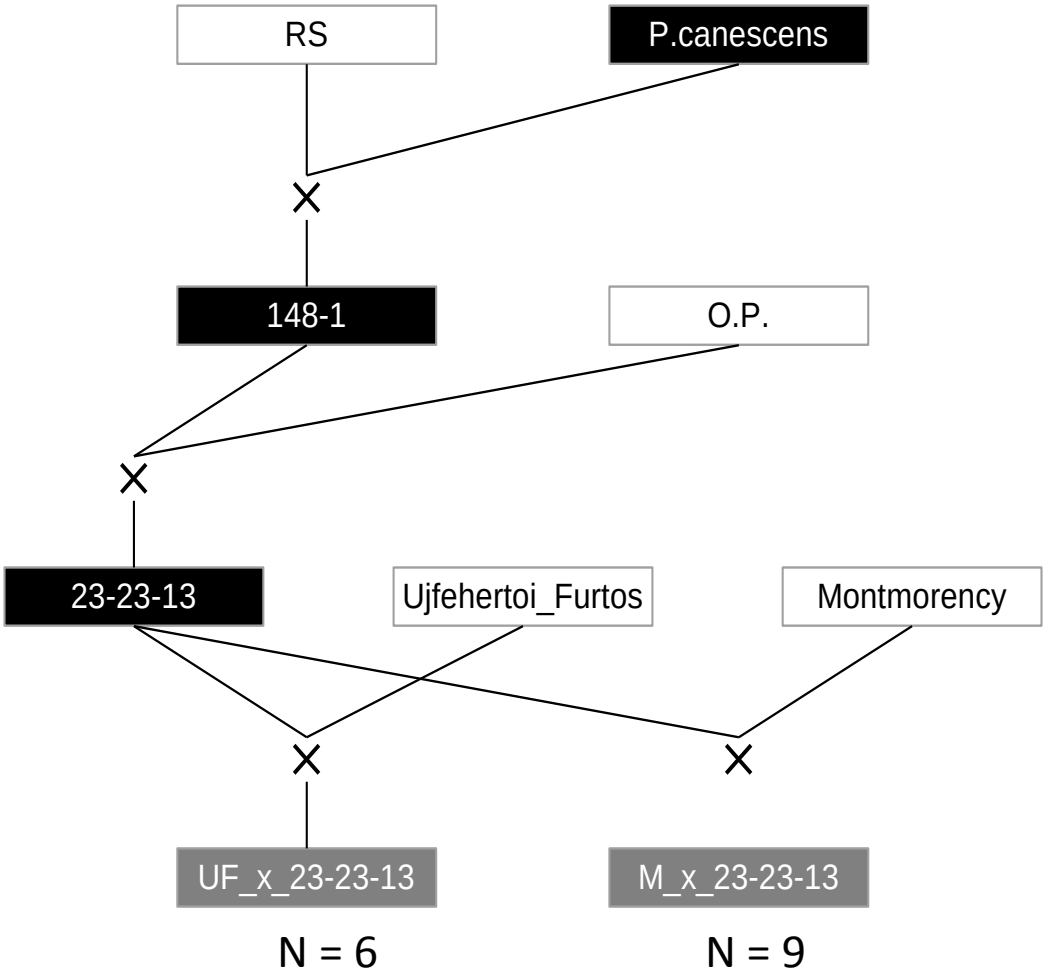


Figure 1.2: Images of cherry leaf spot disease ratings of 1 (a), 2 (b), 3 (c), 4 (d) and 5 (e) on the disease scale used for sweet cherry and *P. canescens* derived diploid individuals. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

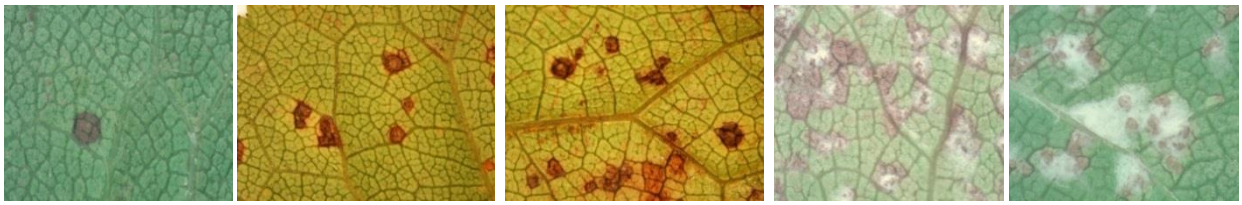


Figure 1.3: Linkage map used for QTL analysis. Large linkage groups were divided into multiple sections (denoted by [ ]) to fit on the page. Marker cM distances were approximated by multiplying marker peach physical map location in Mb by four. A total of 548 markers spanning the 8 linkage groups were used. Markers are a part of the NCBI's dbSNP repository (Sherry et al. 2001).

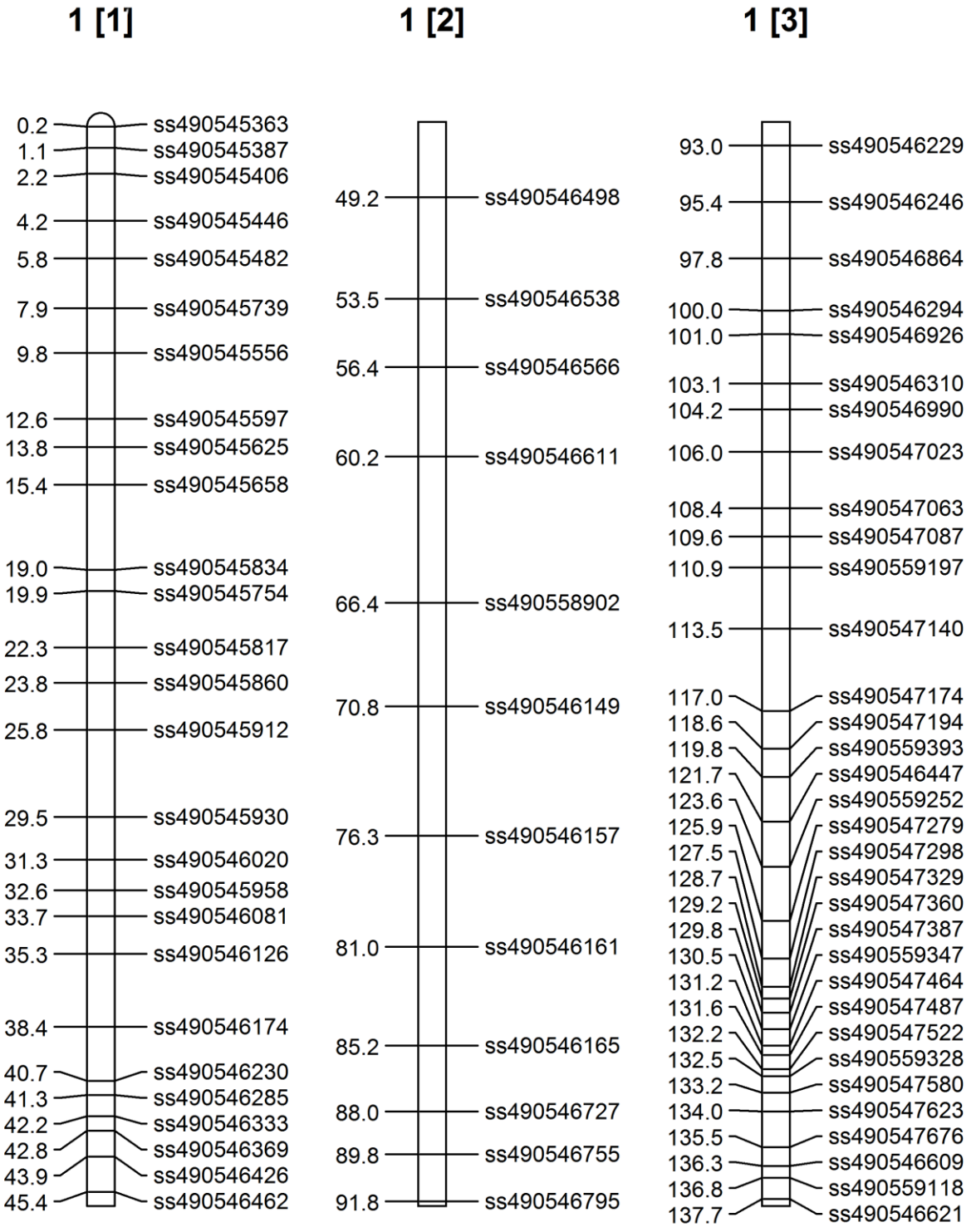


Figure 1.3 (cont'd)

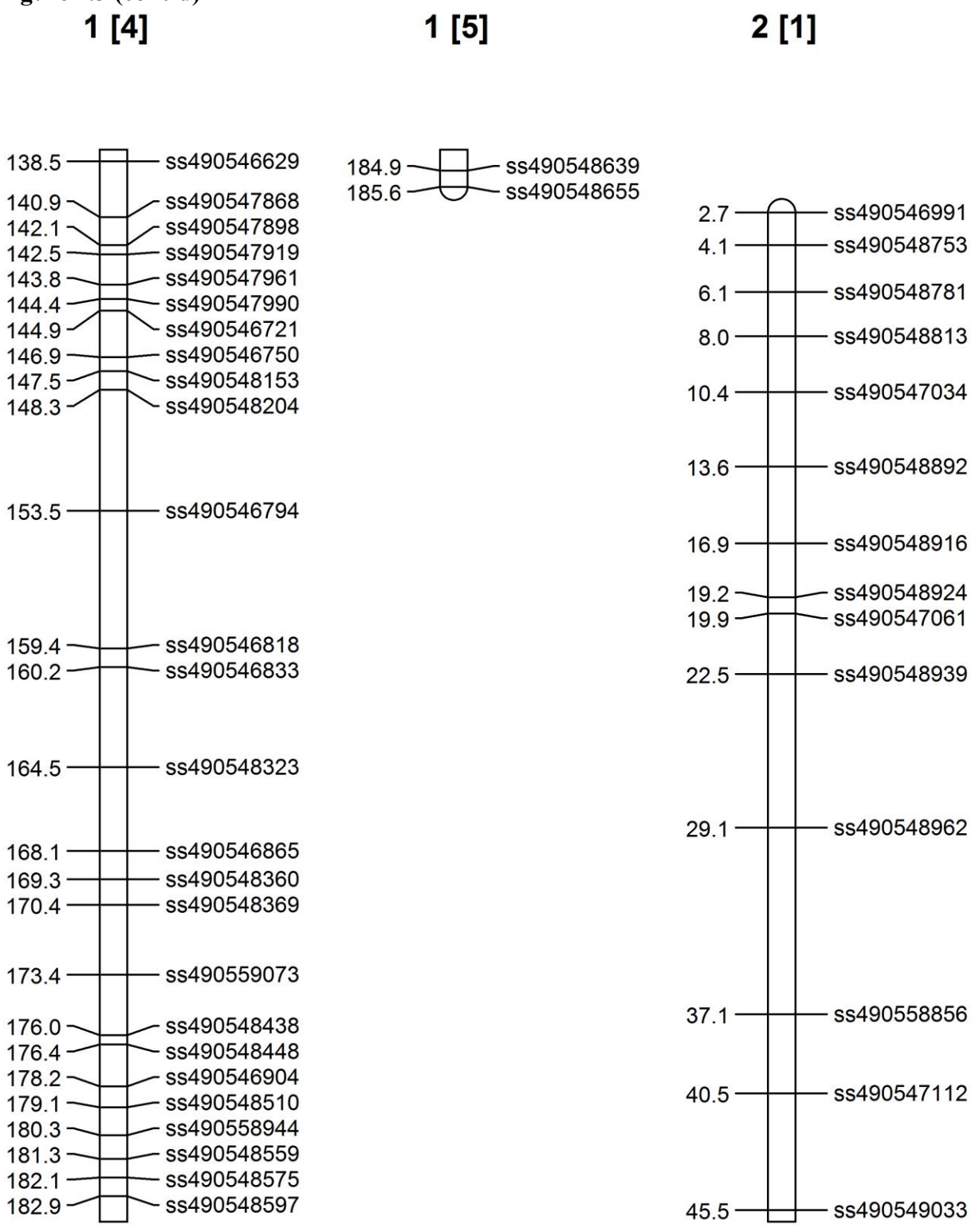


Figure 1.3 (cont'd)

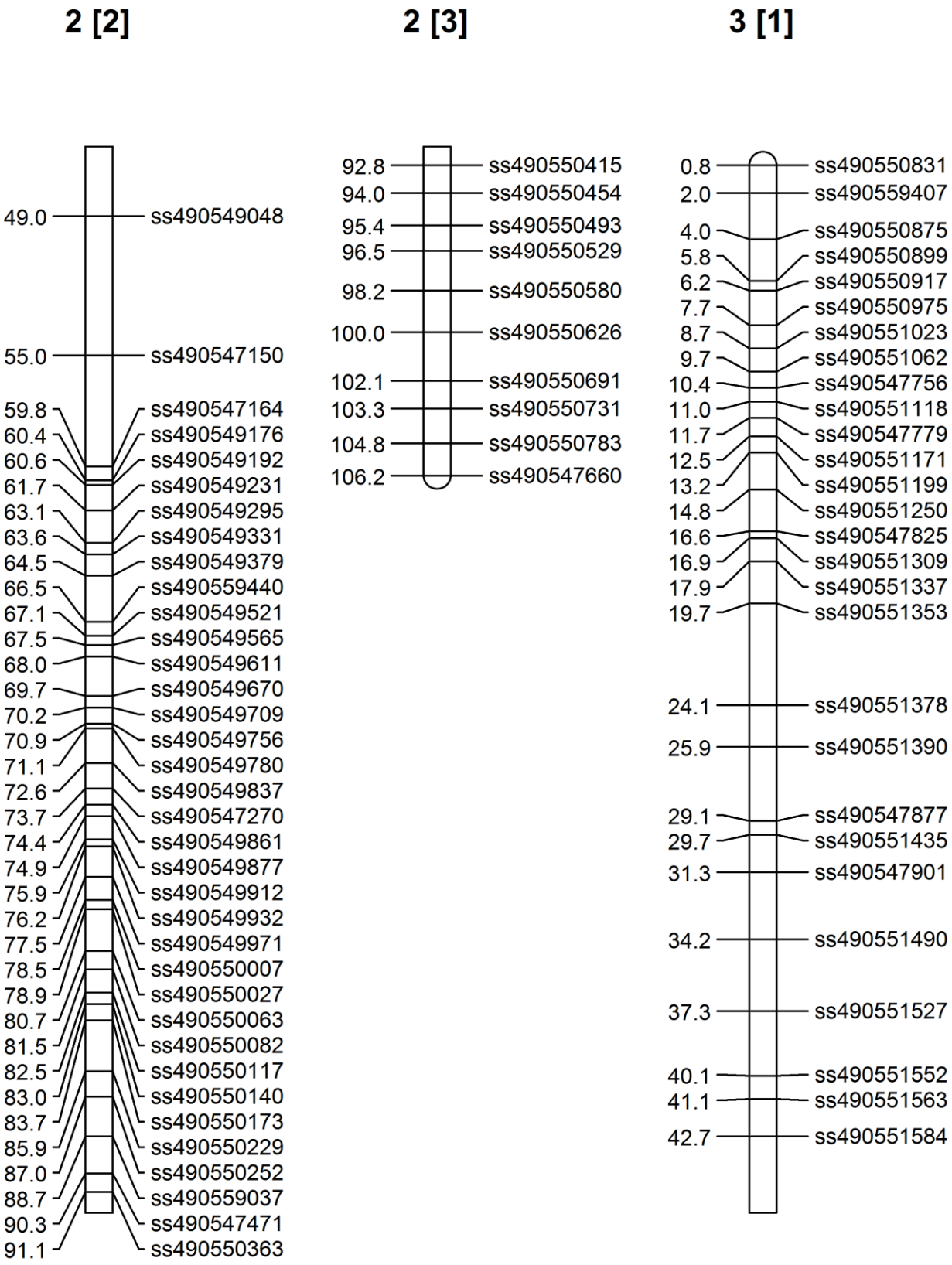


Figure 1.3 (cont'd)

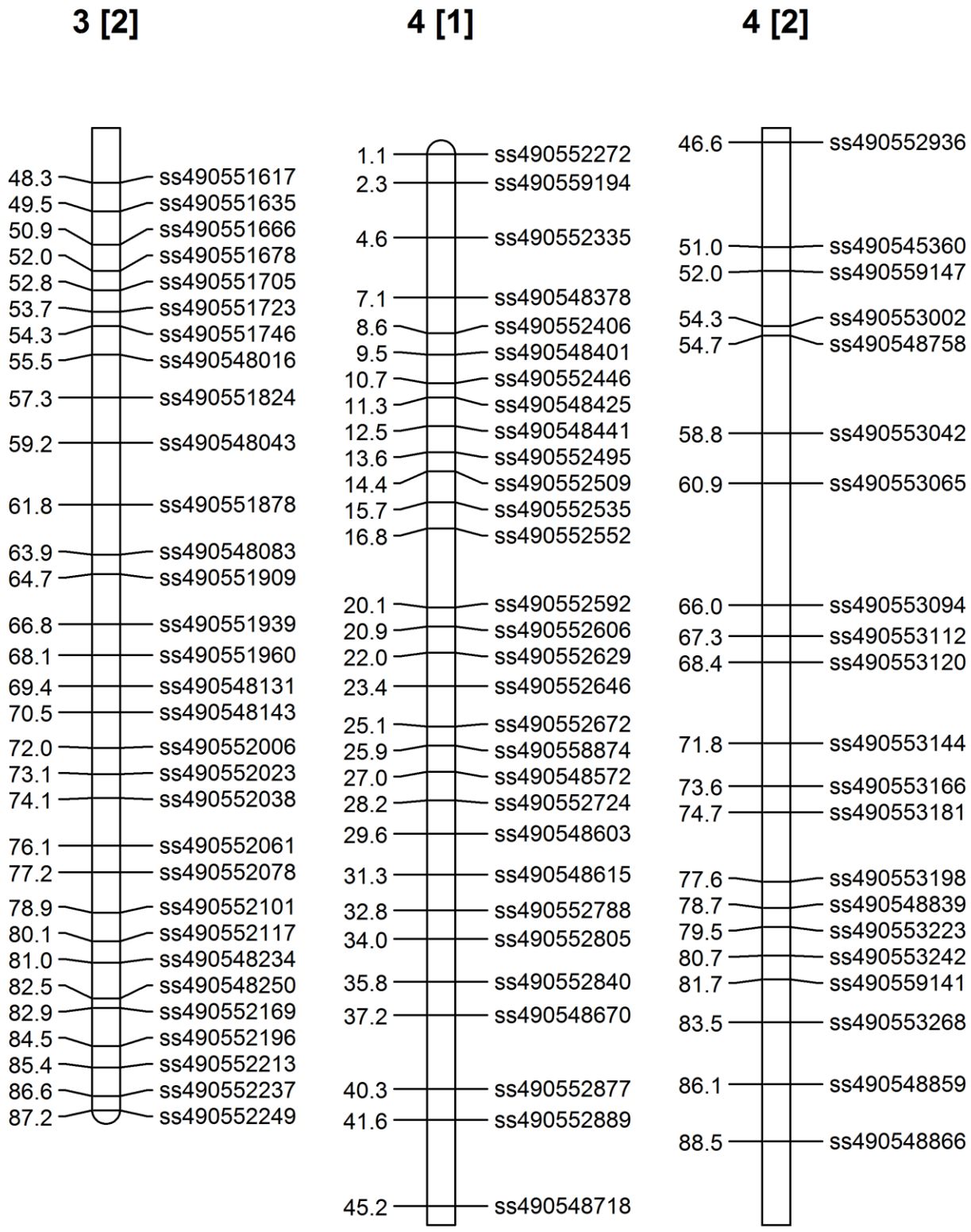


Figure 1.3 (cont'd)

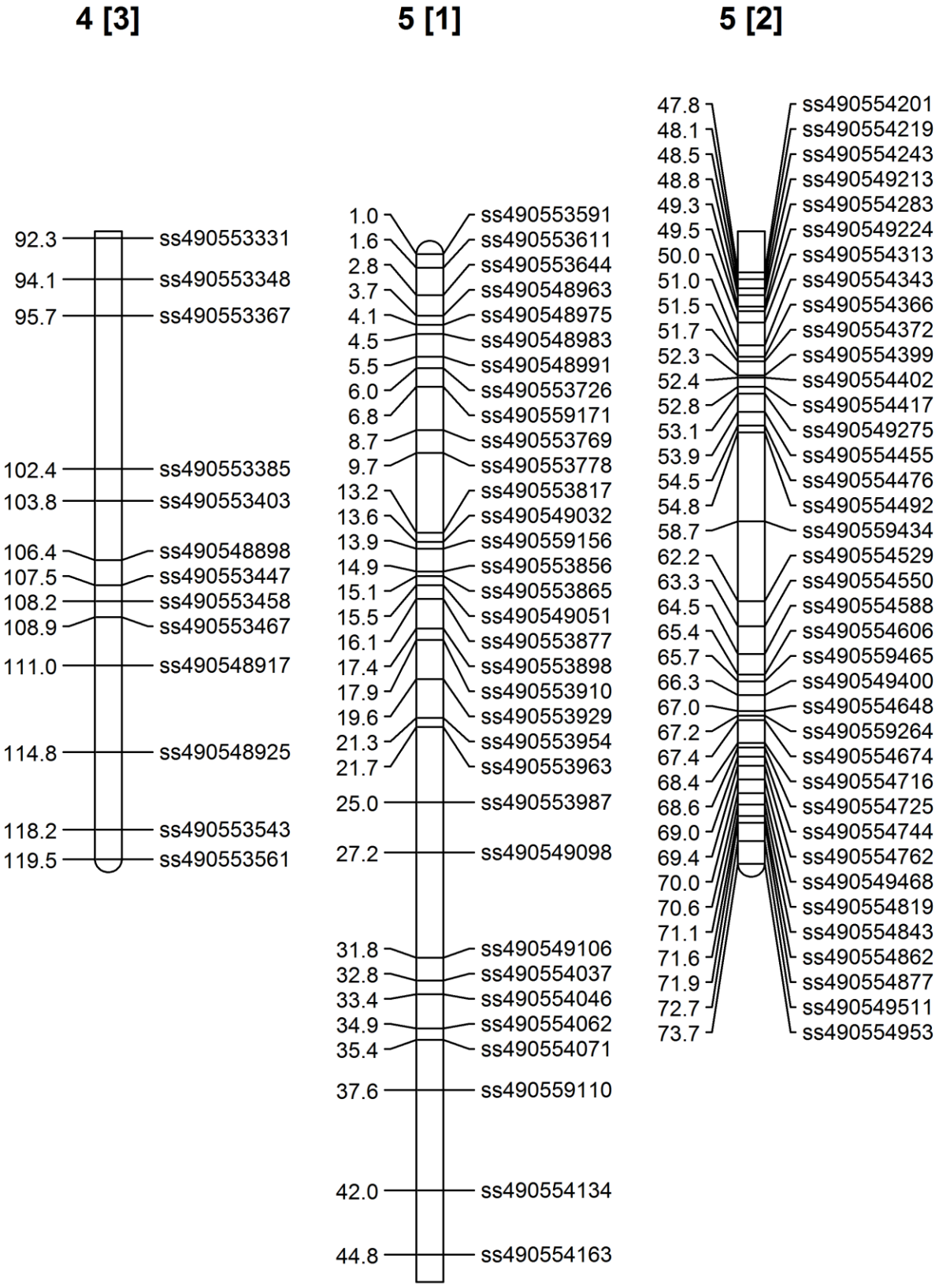


Figure 1.3 (cont'd)

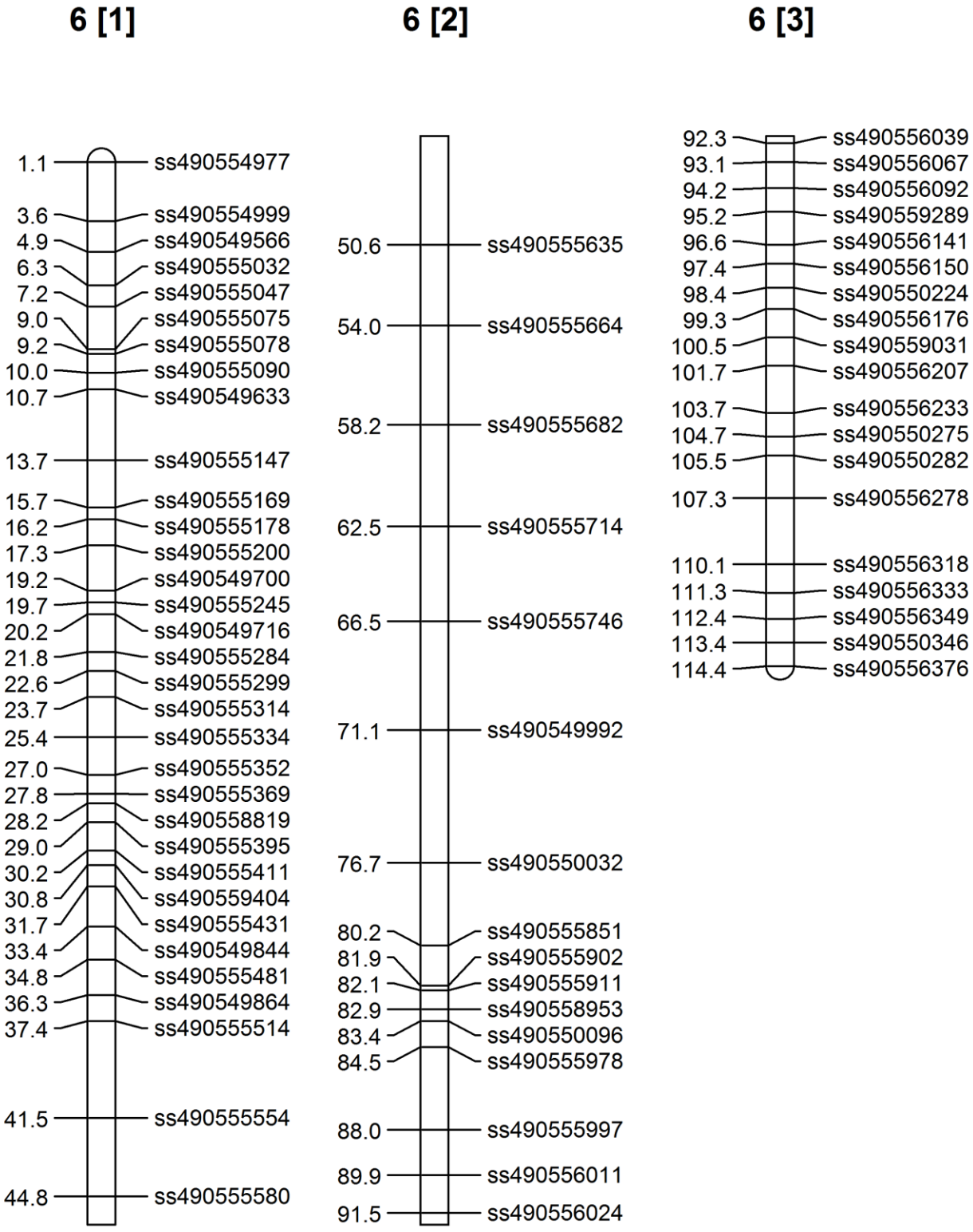


Figure 1.3 (cont'd)

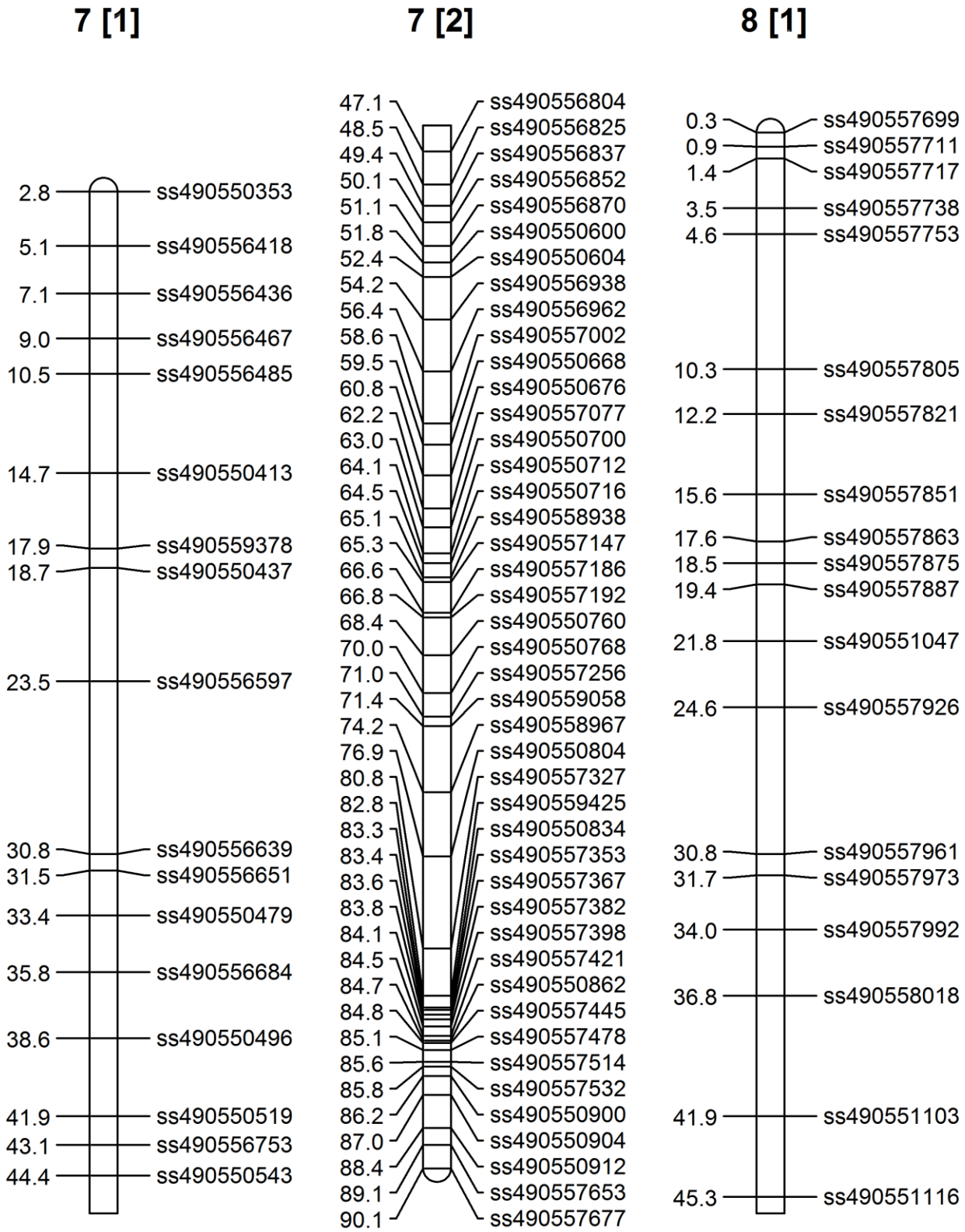


Figure 1.3 (cont'd)

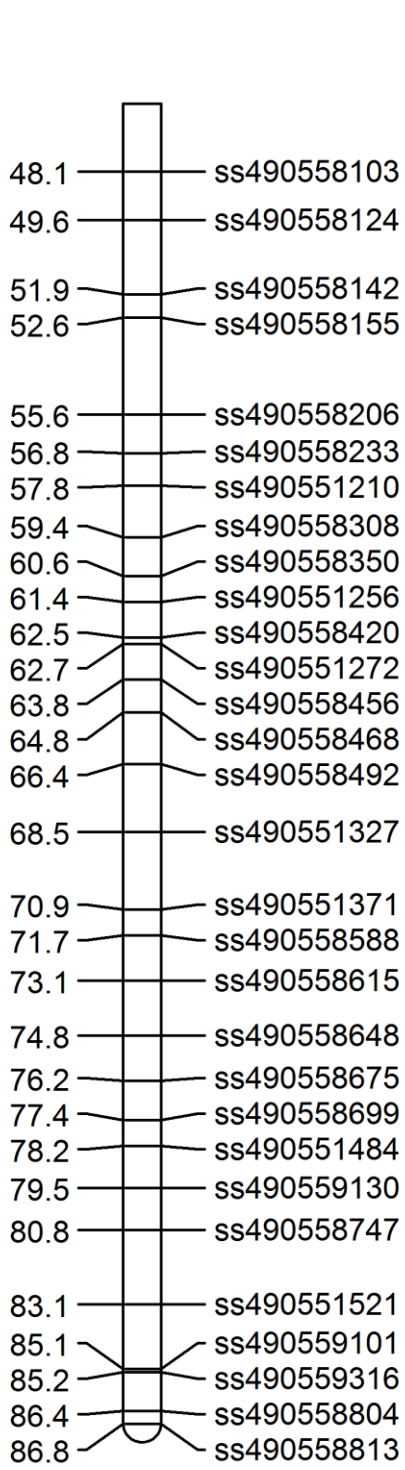


Figure 1.4: Genome Studio (Illumina Inc. 2011) SNP dosage calls were done for each marker. Sweet cherry (yellow) individuals were included to help define the two homozygous (AAAA and BBBB) classes and the balanced heterozygous class (AABB). Determining dosage was necessary to build haplotypes.

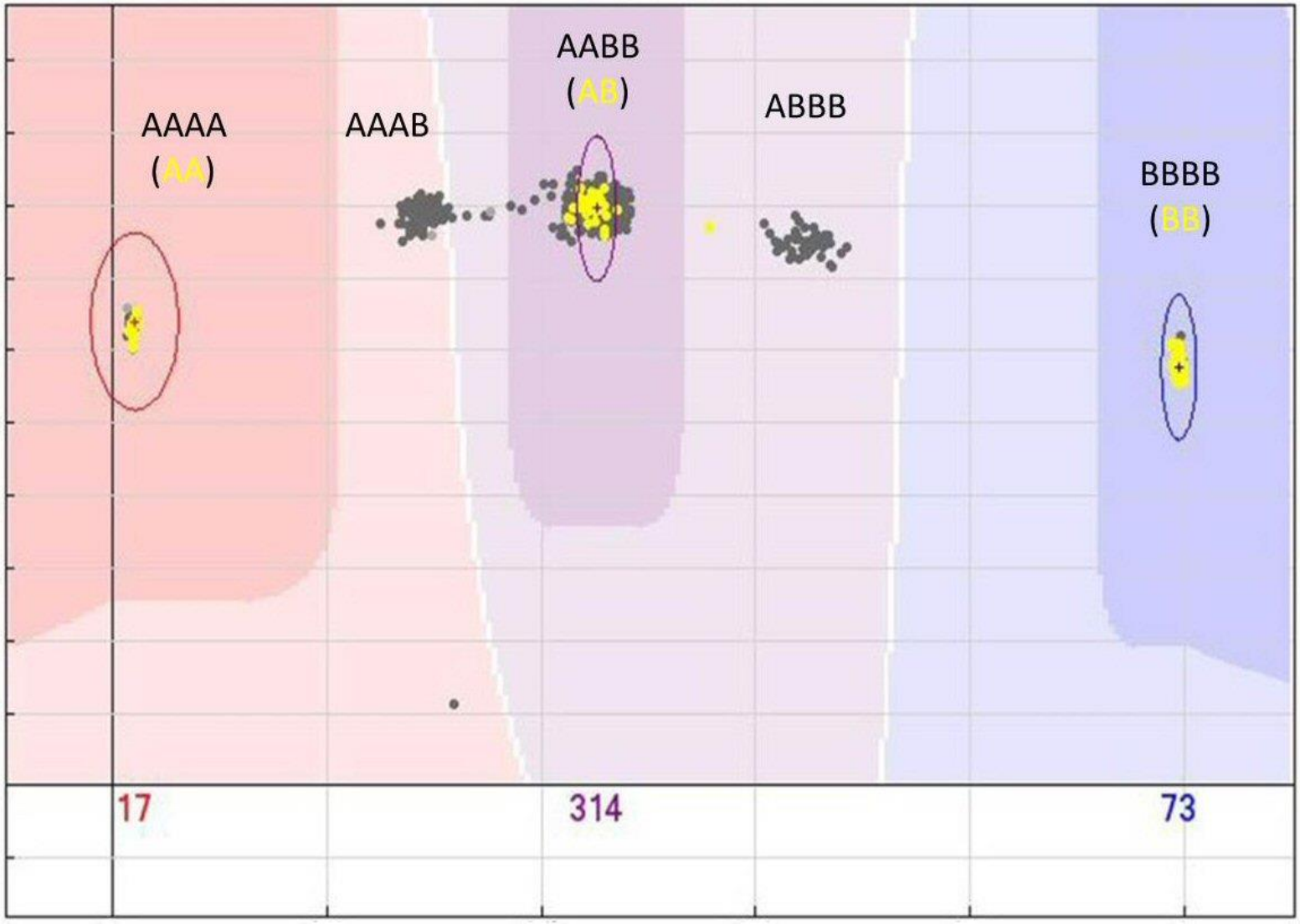


Figure 1.5: Frequency distribution of mean cherry leaf spot disease scores for all sweet cherry individuals (See Table 1.1). The mean disease score for the *P. canescens*-containing parent 'F5-18-167' and 'Namati' were 1.3 and 2.0, respectively.

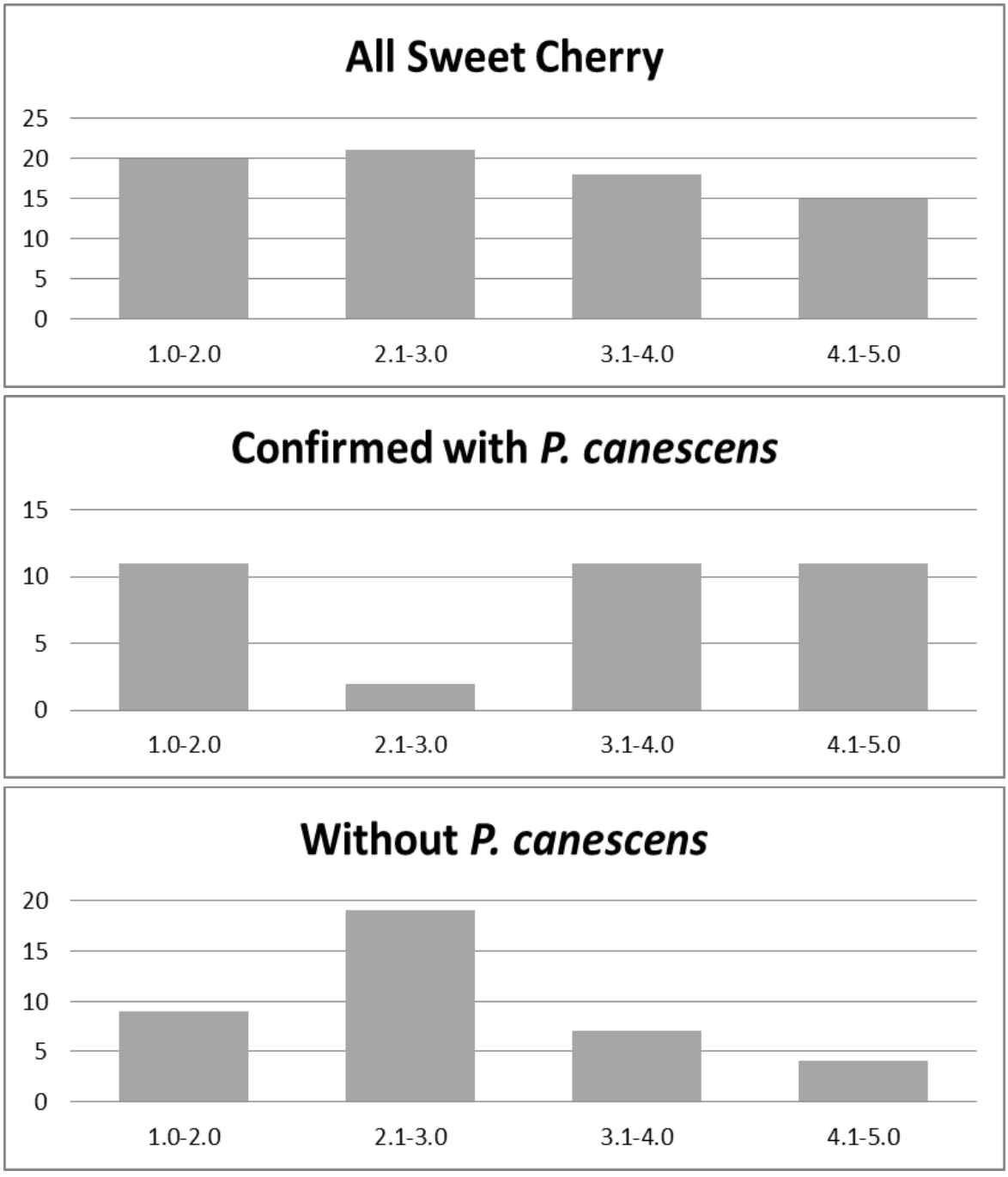


Figure 1.6: Expanded G4 linkage map used for QTL analysis. The G4 linkage group is broken up into multiple sections (denoted by [ ]) to fit on the page. Marker cM distances were approximated by multiplying marker peach physical map location in Mb by four. A total of 241 markers spanning G4 were used. Markers are a part of the NCBI's dbSNP repository (Sherry et al. 2001).

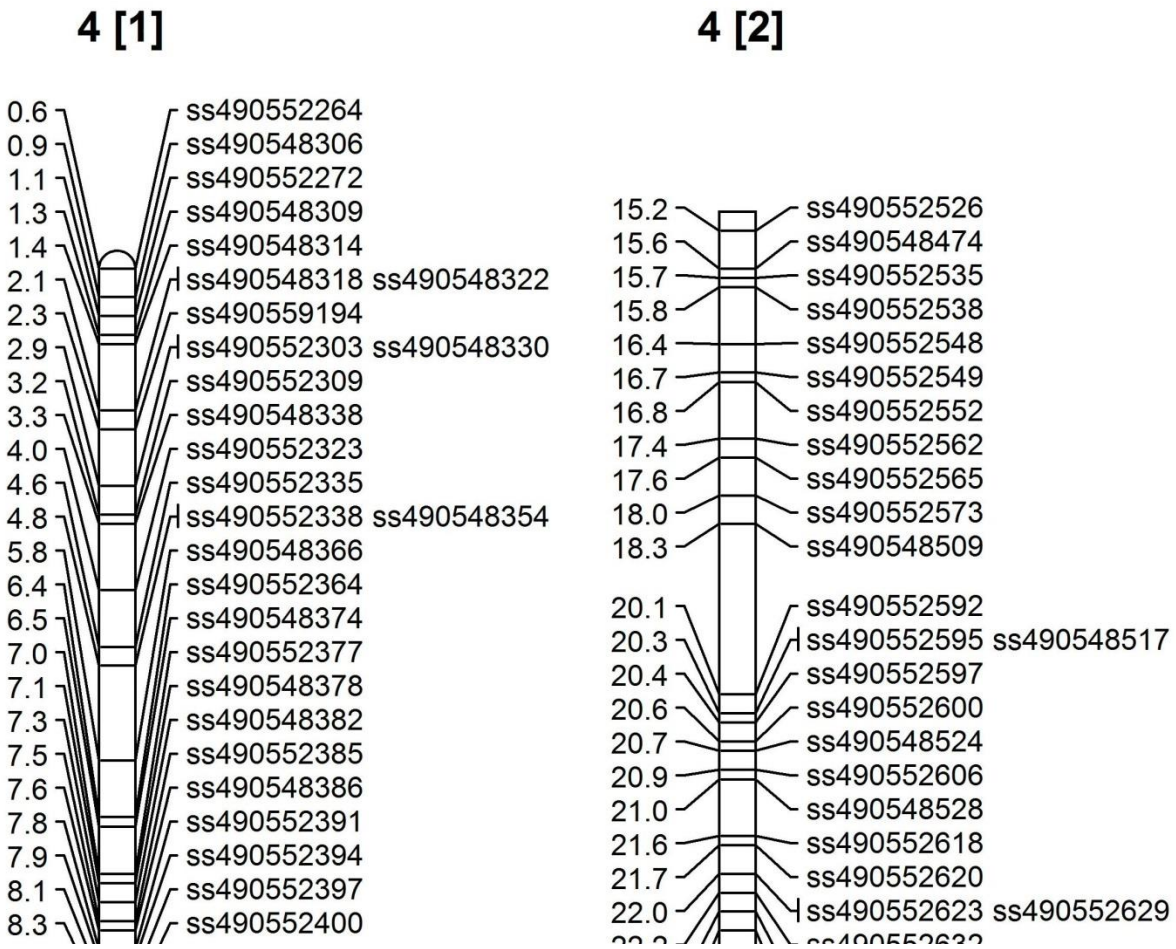


Figure 1.6 (cont'd)

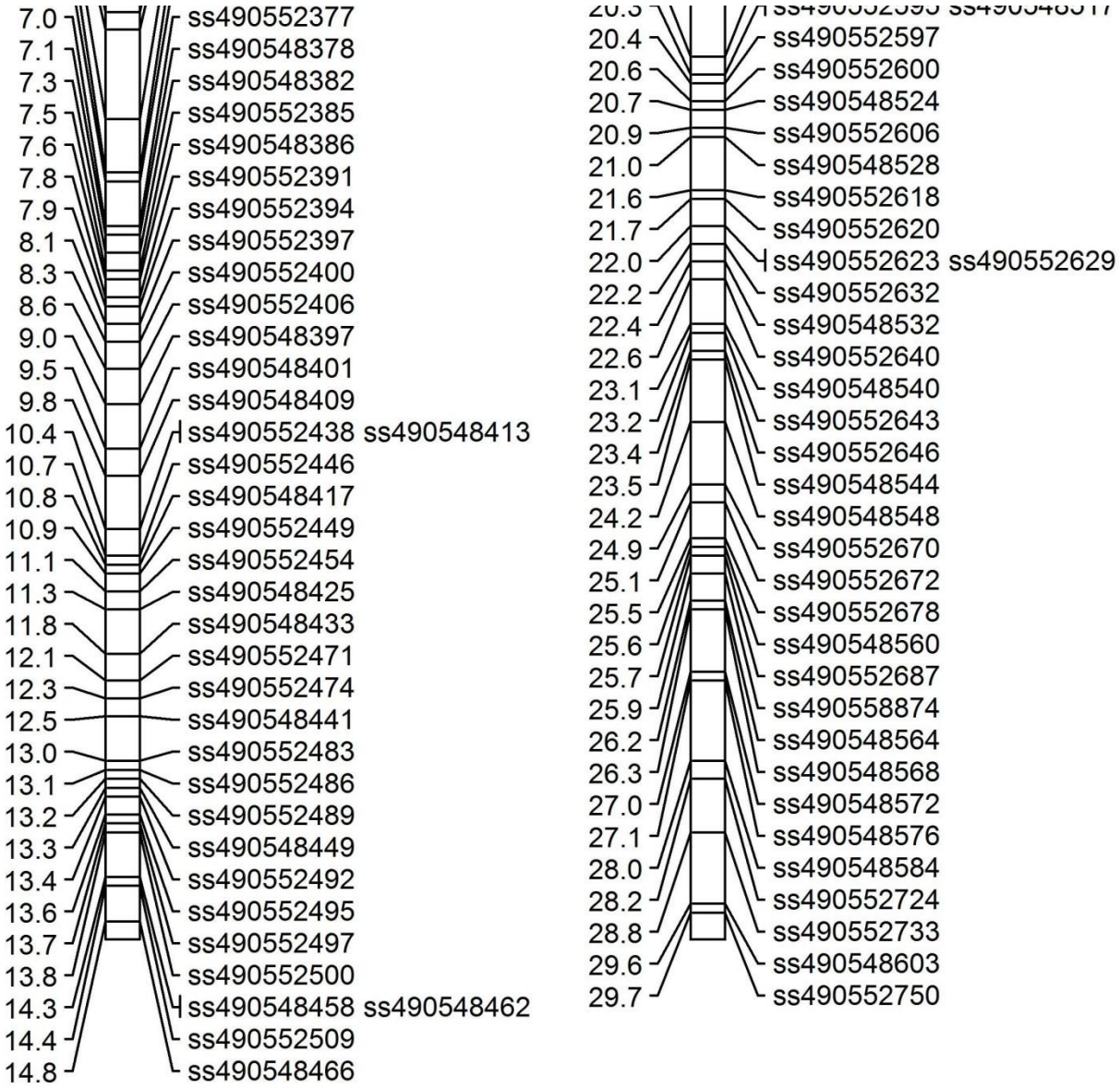


Figure 1.6 (cont'd)

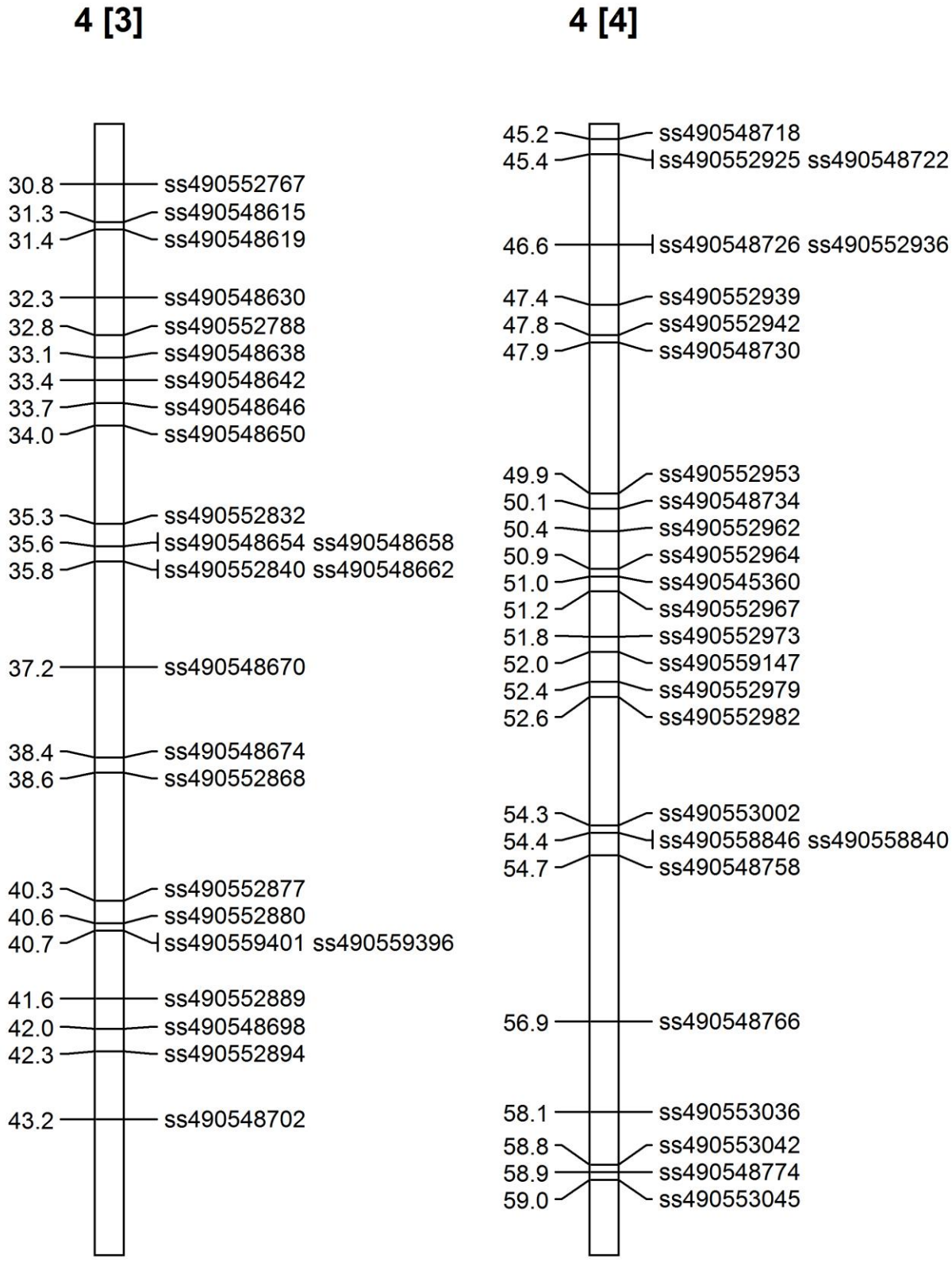


Figure 1.6 (cont'd)

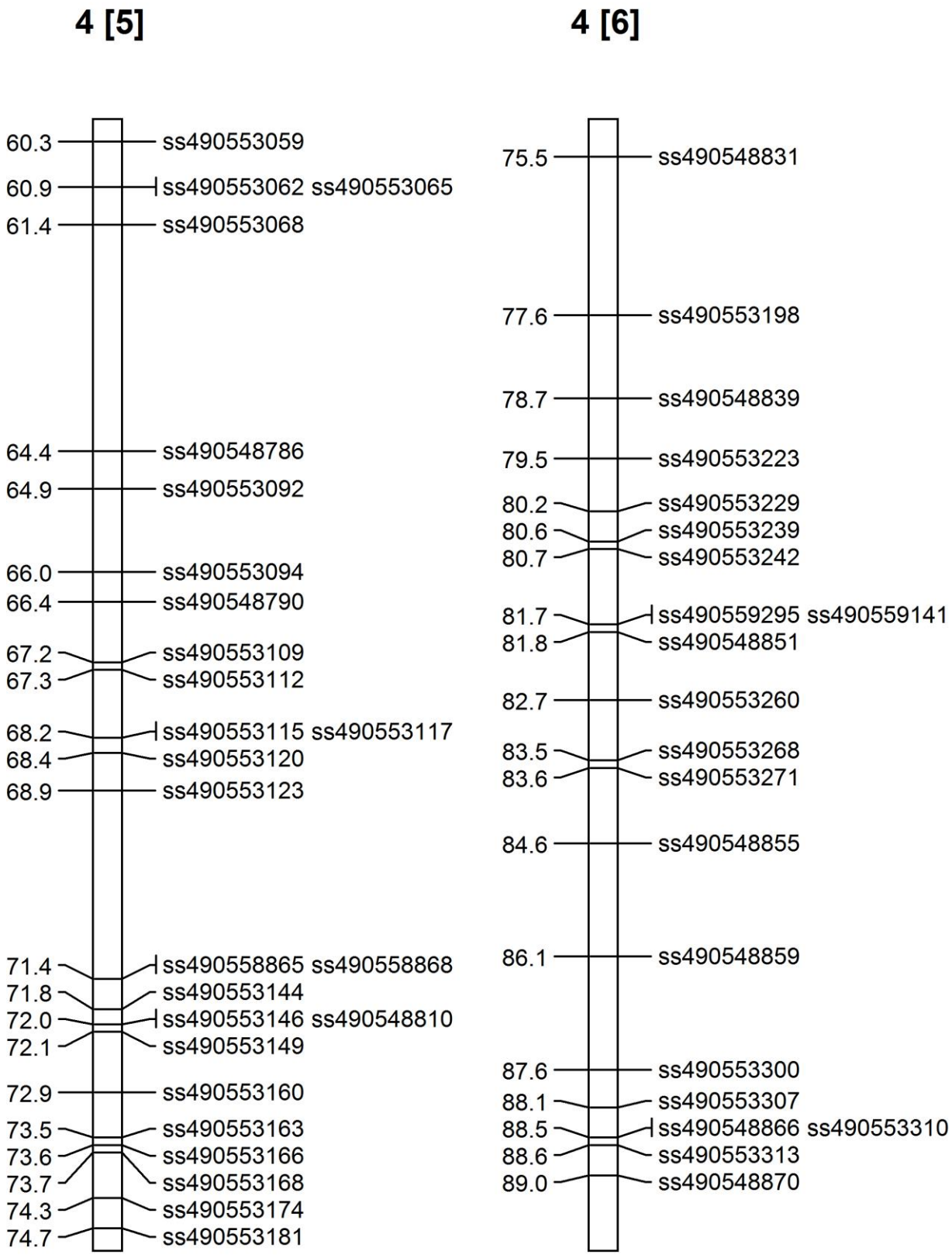


Figure 1.6 (cont'd)

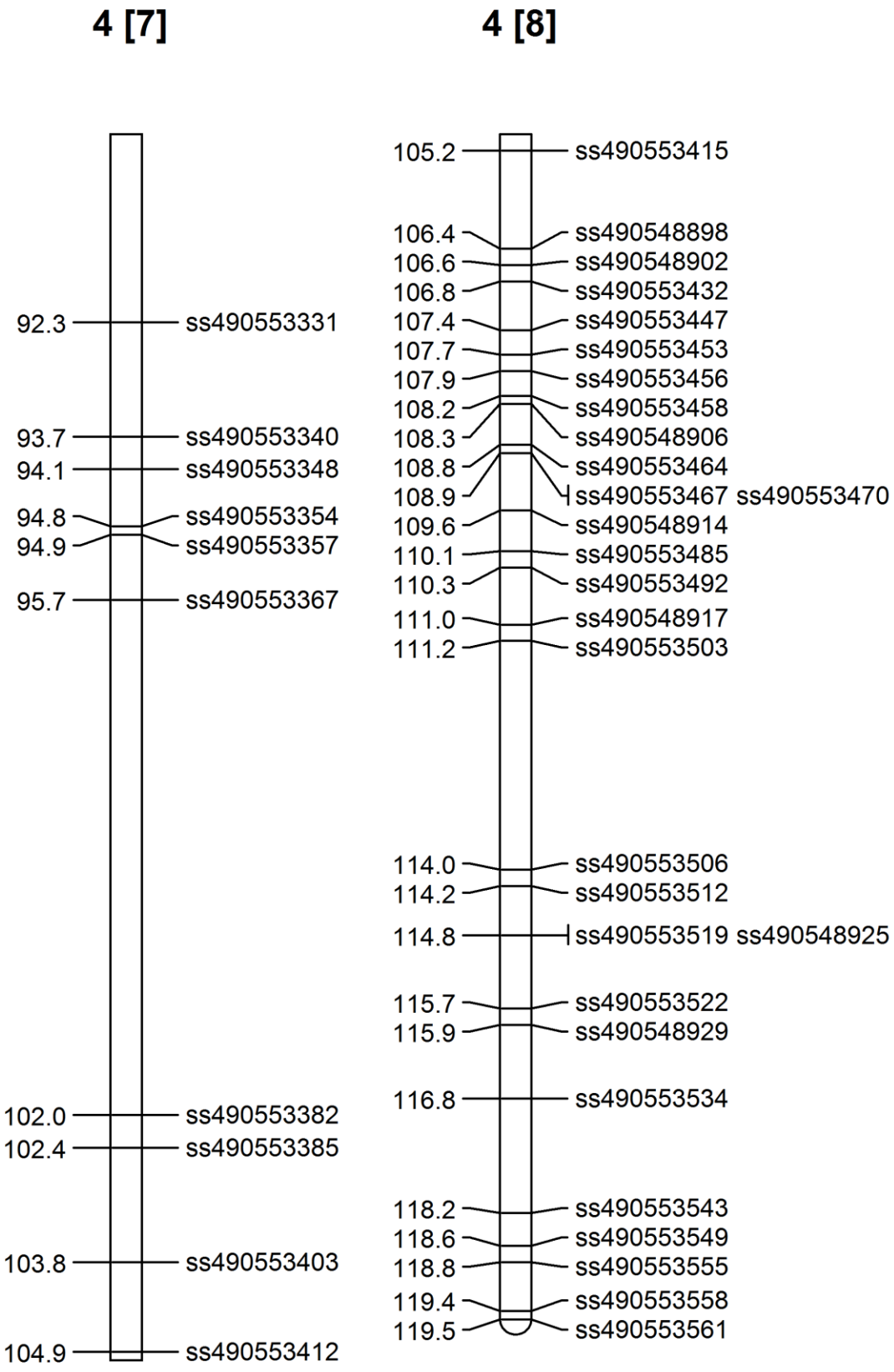
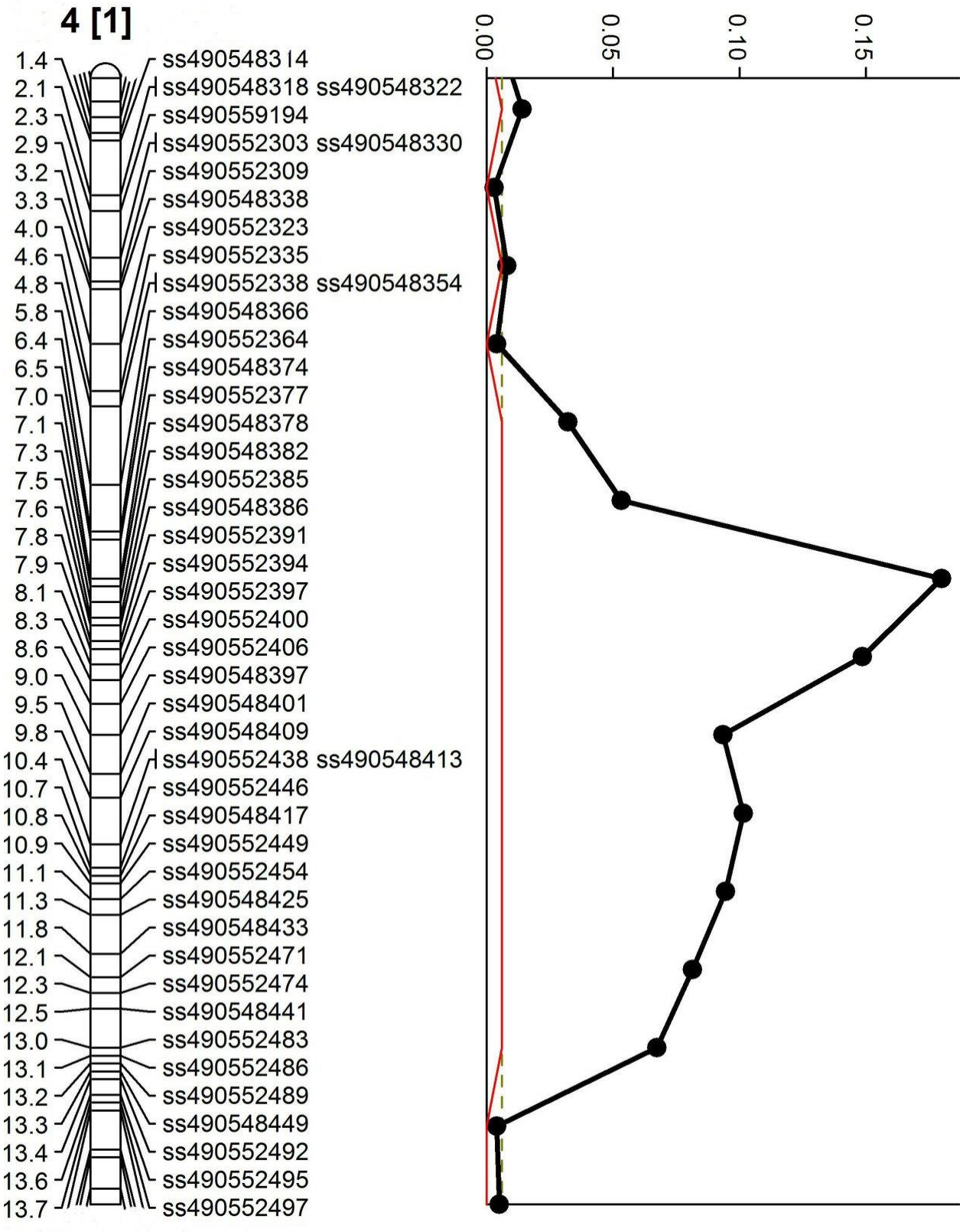
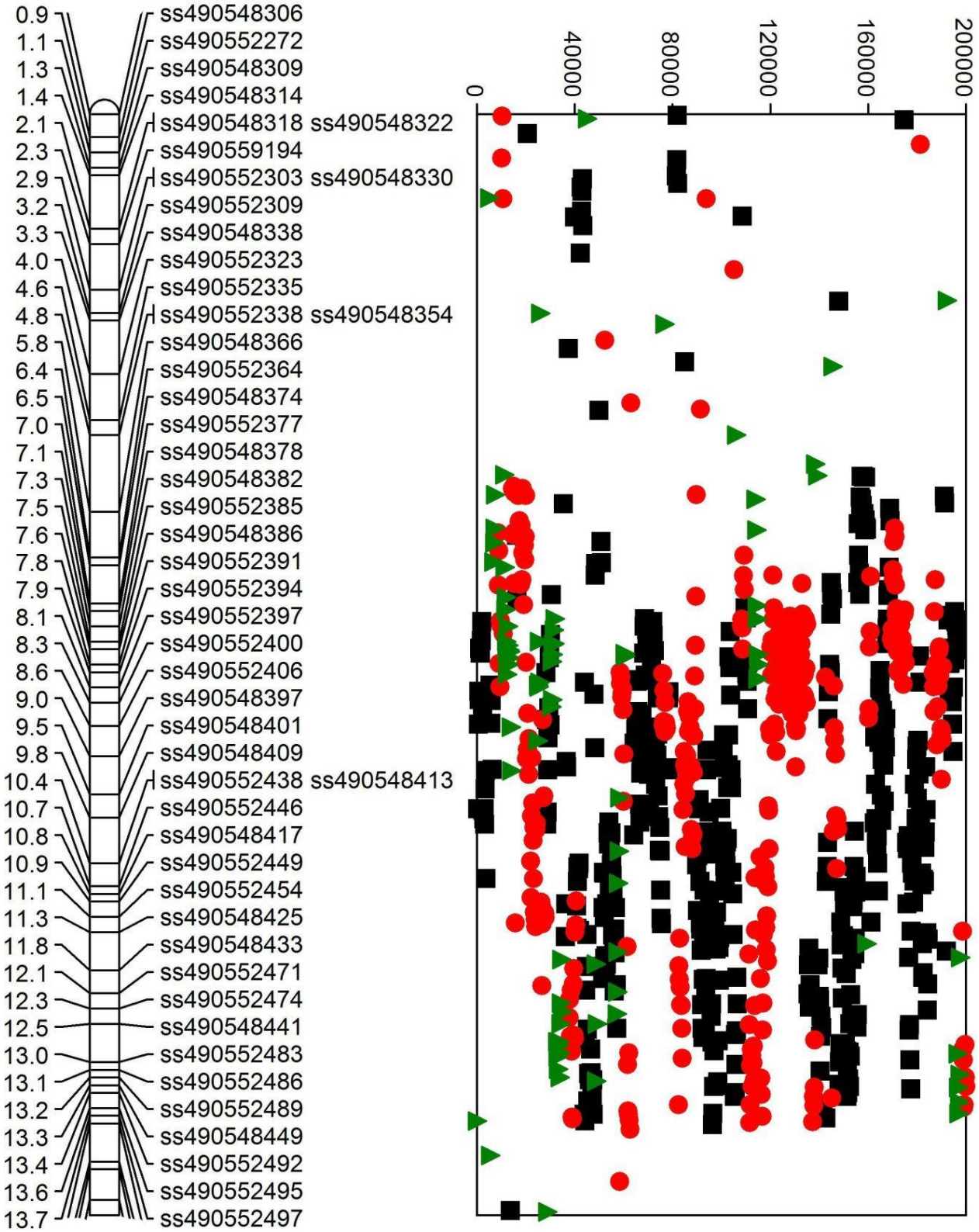


Figure 1.7: a) Disease resistance QTL location on G4 between SNP markers ss490552323 and ss490552500 (between 4.0-13.8 cM). b). Trace plot for the QTL region.



a.

Figure 1.7 (cont'd)



b.

Figure 1.8: Haplotype R is associated with *P. canescens* across the QTL region.

		P. canescens	
NCBI SS#		R	R
ss490552380	RB_S_4_01793880	A	A
ss490552383	RB_S_4_01831038	A	A
ss490552385	RB_S_4_01871597	B	B
ss490552388	RB_S_4_01910142	B	B
ss490548390	RB_T_4_02018251	A	A
ss490552400	RB_S_4_02071162	A	A
ss490552403	RB_S_4_02108244	B	B
ss490552406	RB_S_4_02151854	A	A
ss490548393	RB_T_4_02170096	B	B
ss490548397	RB_T_4_02258698	A	A
ss490552415	RB_S_4_02273965	A	A
ss490552418	RB_S_4_02314853	B	B
ss490552423	RB_S_4_02394394	A	A
ss490552426	RB_S_4_02427939	A	A
ss490548409	RB_T_4_02451708	A	A
ss490552429	RB_S_4_02472320	A	A
ss490548413	RB_T_4_02604396	B	B
ss490552440	RB_S_4_02633721	A	A
ss490552443	RB_S_4_02656936	B	B
ss490552446	RB_S_4_02682092	B	B
ss490548417	RB_T_4_02689311	A	A
ss490552457	RB_S_4_02821081	A	A
ss490559087	RC1422-162_4_02841085	A	A
ss490552460	RB_S_4_02872061	A	A
ss490552463	RB_S_4_02901893	B	B
ss490548433	RB_T_4_02960827	A	A
ss490552466	RB_S_4_02961208	B	B
ss490552469	RB_S_4_02991481	B	B
ss490548437	RB_T_4_03068652	B	B
ss490552474	RB_S_4_03072387	B	B
ss490552477	RB_S_4_03146679	B	B
ss490548445	RB_T_4_03188480	B	B

Figure 1.9a: Sour cherry haplotypes for the G4 QTL region. Parents '23-23-13' and 'Ujfehertoi Furtos' with 5 progeny. Haplotype R is from *P. canescens*.

	23-23-13				UF				24-32-37				24-32-39				24-32-41				24-32-43				24-32-44			
NCBI SS#	w	x	R	z	a	b	c	d	b	c	x	R	b	d	x	R	a	d	w	R	a	d	w	R	b	c	w	R
ss490552385	A	A	B	B	A	A	B	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
ss490548390	A	A	A	B	A	A	A	B	A	A	A	A	A	B	A	A	A	B	A	A	A	B	A	A	A	A	A	A
ss490552400	B	B	A	A	B	B	A	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
ss490552406	A	B	A	B	B	B	B	A	B	B	B	A	B	A	B	A	B	A	A	A	B	A	A	A	B	B	A	A
ss490548393	A	B	B	A	A	B	B	B	B	B	B	B	B	B	B	B	A	B	A	B	A	B	A	B	B	B	A	B
ss490548397	B	B	A	B	B	B	B	A	B	B	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	B	B	A
ss490552426	A	A	A	B	A	A	A	B	A	A	A	A	A	B	A	A	A	B	A	A	A	B	A	A	A	A	A	A
ss490548409	B	B	A	A	B	B	A	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
ss490548413	A	A	B	B	A	A	B	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
ss490552446	B	A	B	B	A	A	B	B	A	B	A	B	A	B	A	B	A	B	B	B	A	B	B	B	A	B	B	B
ss490548417	A	B	A	A	B	B	A	A	B	A	B	A	B	A	B	A	B	A	A	A	B	A	A	A	B	A	A	A
ss490559087	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	B	A	A	A	B	A
ss490548433	B	B	A	A	B	B	A	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
ss490552474	A	A	B	B	A	A	B	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
ss490548445	B	B	B	A	B	B	A	A	B	A	B	B	B	A	B	B	B	A	B	B	B	A	B	B	B	A	B	B

Figure 1.9b: Parents ‘Montmorency’ and ‘23-23-13’ with 4 progeny individuals that were haplotyped. Black regions indicate where a crossover took place, but due to identical SNP markers in that area, the exact location of the crossover is unknown. Haplotype R is from *P. canescens*.

	23-23-13				Montmorency				24-32-17				24-32-20				24-32-24				24-32-27			
NCBI SS#	w	x	R	z	e	f	c	d	f	c	x	R	c	d	x	R	e	c	x	R	f	d	w/x	R
ss490552385	A	A	B	B	A	A	B	B	A	B	A	B	B	B	A	B	A	B	A	B	A	B	A	B
ss490548390	A	A	A	B	A	A	A	B	A	A	A	A	A	B	A	A	A	A	A	A	A	B	A	A
ss490552400	B	B	A	A	B	B	A	A	B	A	B	A	A	A	B	A	B	A	B	A	B	A	B	A
ss490552406	A	B	A	B	A	B	B	A	B	B	B	A	B	A	B	A	A	B	B	A	B	A	A	A
ss490548393	A	B	B	A	A	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	A	B
ss490548397	B	B	A	B	B	B	B	A	B	B	B	A	B	A	B	A	B	B	B	A	B	A	B	A
ss490552426	A	A	A	B	A	A	A	B	A	A	A	A	A	B	A	A	A	A	A	A	A	B	A	A
ss490548409	B	B	A	A	B	B	A	A	B	A	B	A	A	A	B	A	B	A	B	A	B	A	B	A
ss490548413	A	A	B	B	A	A	B	B	A	B	A	B	B	B	A	B	A	B	A	B	A	B	A	B
ss490552446	B	A	B	B	B	A	B	B	A	B	A	B	B	B	A	B	B	B	A	B	A	B	A	B
ss490548417	A	B	A	A	A	B	A	A	B	A	B	A	A	A	B	A	A	A	B	A	B	A	B	A
ss490559087	B	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A
ss490548433	B	B	A	A	B	B	A	A	B	A	B	A	A	A	B	A	B	A	B	A	B	A	B	A
ss490552474	A	A	B	B	A	A	B	B	A	B	A	B	B	B	A	B	A	B	A	B	A	B	A	B
ss490548445	B	B	B	A	B	B	A	A	B	A	B	B	A	A	B	B	B	A	B	B	B	A	B	B

Figure 1.10: Location of SSR markers used to tag the *P. canescens* haplotype in relation to the *CLSR_G4* QTL region of interest between SNP markers ss490552323 (4.0 cM, 1.0 Mb) and ss490552500 (13.8 cM, 3.46 Mb).

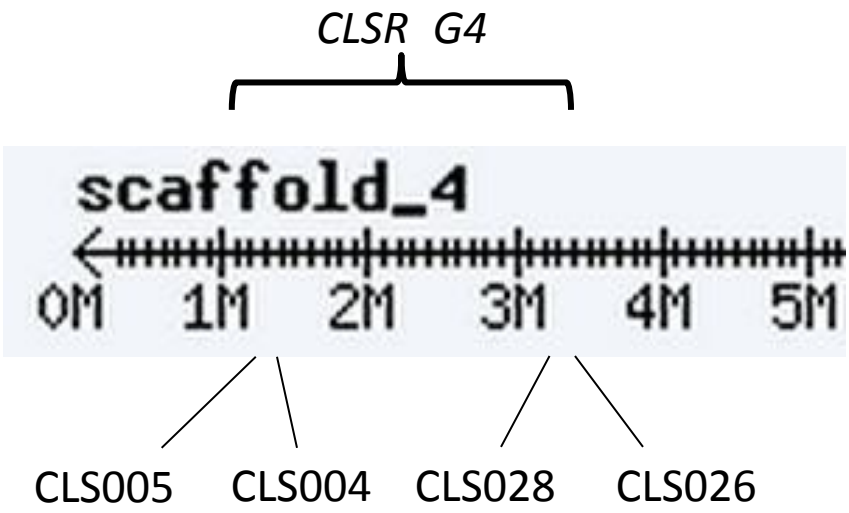
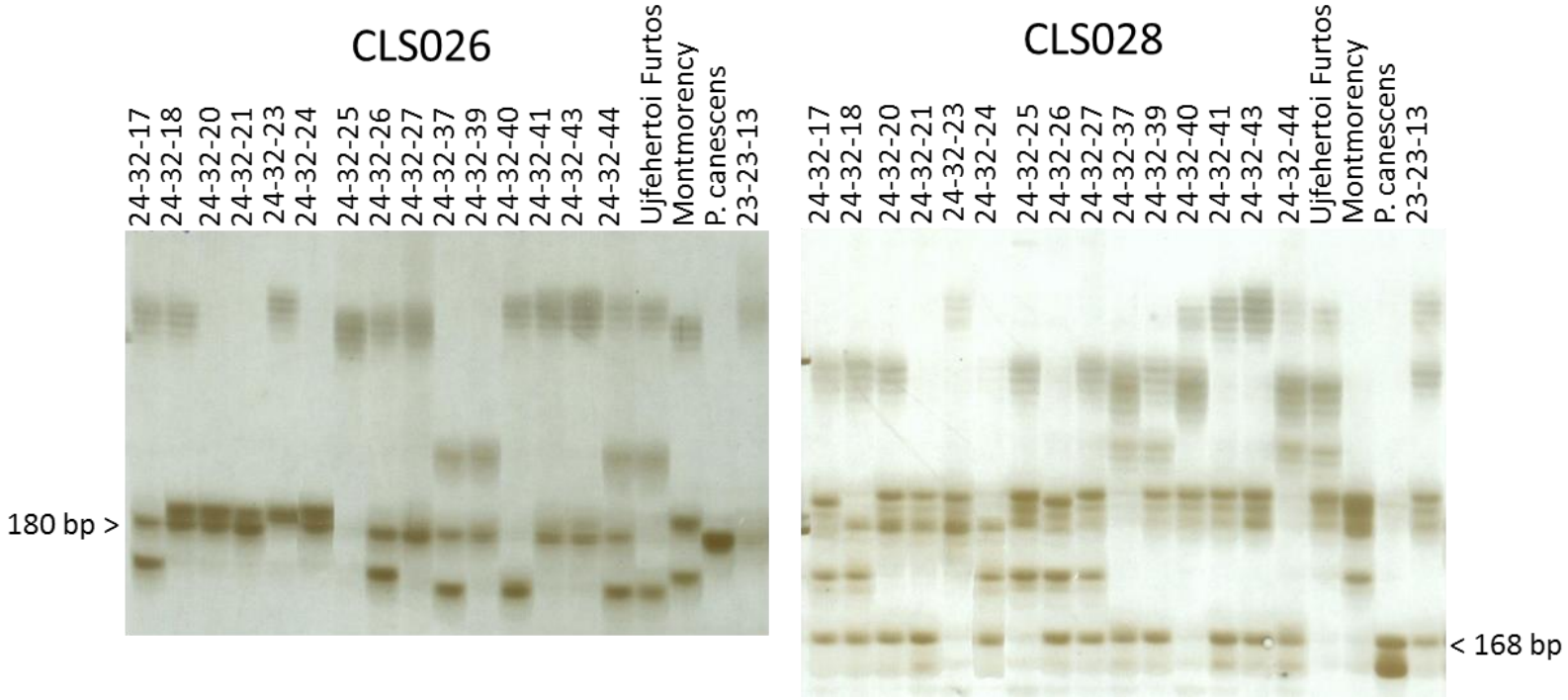


Figure 1.11: SSR fragments for sour cherry individuals (See Table 1.1) for four primer pairs in the G4 cherry leaf spot QTL region: **a)** CLS004 and **b)** CLS005 **c)** CLS026 **d)** CLS028 (See Table 1.2) run on sour cherry individuals for the G4 QTL region. Arrows denote the location and fragment size of the *P. canescens* allele.



a. **b**.

Figure 1.11 (cont'd)

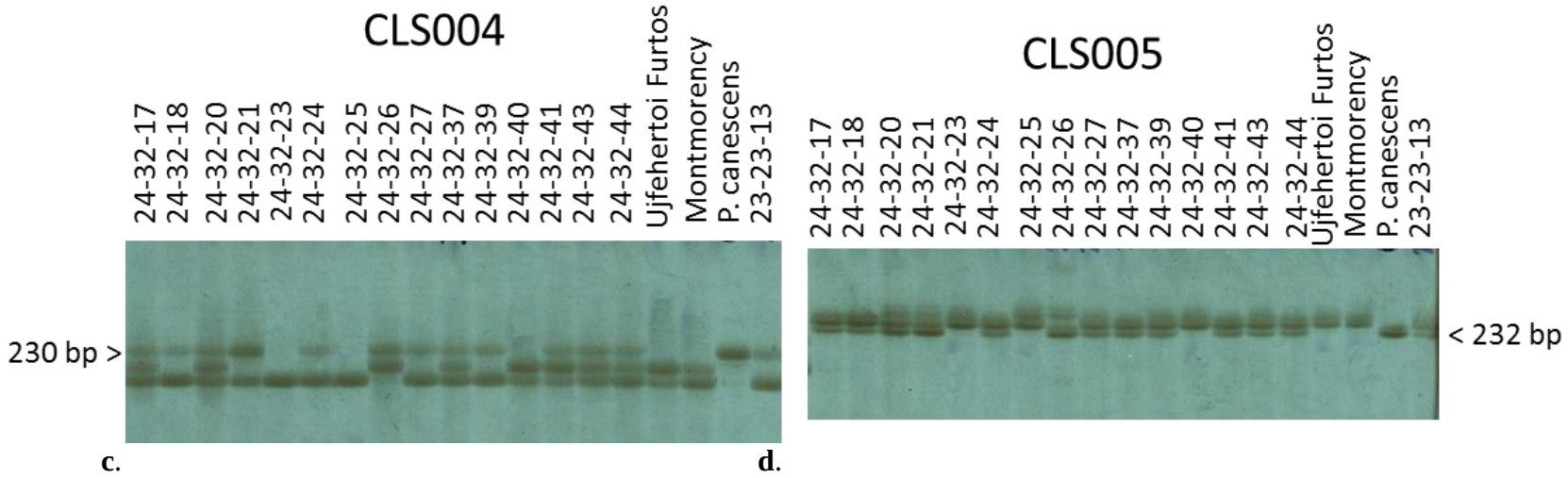


Figure 1.12: Proposed two gene model for disease resistance in sweet cherry, where the individuals are only resistant with dominant genes at both the *P. canescens*-associated G4 "A", and the unknown "B" second loci. Disease resistant parent 'F5-18-167' is shown to be heterozygous at both loci (AaBb) where parent 2 is shown to be homozygous recessive for the G4 "A" locus, and heterozygous for the proposed second locus needed to warrant resistance. Squares highlighted in grey are those that would be resistant.

		F5-18-167 (AaBb)			
		AB	Ab	aB	ab
Parent 2 (aaBb)	aB	AaBB	AaBb	aaBB	aaBb
	ab	AaBb	Aabb	aaBb	aabb

Figure 1.13: Proposed two gene model for disease resistance in sour cherry, where the individuals are only resistant with dominant genes at both the G4 "A", and the unknown "B" second loci. This model assumes preferential pairing within sub-genomes, where sum-genomes are denoted by subscript numbers. Disease resistant parent '23-23-13' is shown to be heterozygous at both loci in only one sub-genome ($A_1a_1a_2a_2B_1b_1b_2b_2$) where both 'UF' and 'Montmorency" are shown to be homozygous recessive for the G4 "A" locus, and heterozygous in one sub-genome for the proposed second locus needed to warrant resistance ($a_1a_1a_2a_2B_1b_1b_2b_2$). Squares highlighted in grey are those that would be resistant.

		23-23-13 ($A_1a_1a_2a_2B_1b_1b_2b_2$)			
		$A_1a_2B_1b_2$	$A_1a_2b_1b_2$	$a_1a_2B_1b_2$	$a_1a_2b_1b_2$
UF & Montmorency	$a_1a_2B_1b_2$	$A_1a_1a_2a_2B_1B_1b_2b_2$	$A_1a_1a_2a_2B_1b_1b_2b_2$	$a_1a_1a_2a_2B_1B_1b_2b_2$	$a_1a_1a_2a_2B_1b_1b_2b_2$
($a_1a_1a_2a_2B_1b_1b_2b_2$)	$a_1a_2b_1b_2$	$A_1a_1a_2a_2B_1b_1b_2b_2$	$A_1a_1a_2a_2b_1b_1b_2b_2$	$a_1a_1a_2a_2B_1b_1b_2b_2$	$a_1a_1a_2a_2b_1b_1b_2b_2$

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CHAPTER 2

VALIDATION IN TETRAPLOID SOUR CHERRY OF QTLS FOR BLOOM TIME AND FRUIT QUALITY TRAITS FROM DIPLOID *Prunus* SPECIES

Introduction

Sour cherry (*Prunus cerasus* L.) is an allotetraploid (2n=4x=32) derived from the hybridization of diploid (2n=2x=16) sweet cherry (*P. avium* L.) and tetraploid (2n=4x=32) ground cherry (*P. fruticosa* Pall.) (Olden and Nybom, 1968; Hancock and Iezzoni, 1988). A high degree of synteny between the two sub-genomes is expected, as *Prunus* species have been found to be highly syntenic (Dirlewanger et al. 2004; Lambert et al. 2004; Arús et al. 2006; Dondini et al. 2007; Olmstead et al. 2008). This indeed, has proven to be the case, as inheritance of isozymes in various crosses of sour cherry also indicated that sour cherry behaves as a segmental allopolyploid (Beaver and Iezzoni, 1993; Beaver et al. 1995) meaning that while there seems to be a preferential pairing of chromosomes within sub-genomes of sour cherry, crossovers, and non-bivalent pairing between sub-genomes is not uncommon (Schuster and Wolfram, 2005). A genetic example of unbalanced chromosomes can be seen when looking at the self-incompatability locus (*S*-locus) on G6 where sour cherry individuals such as 'Rheinische Schattenmorelle' (RS) has three sweet cherry *S*-alleles, and one *P. fruticosa* allele (Hauck et al. 2006). The difficulty in constructing genetic linkage maps of segmental polyploids occurs because marker segregation ratios need to be single-dose restriction fragments (SDRF) or double-dose restriction fragments (DDRF) in order to map (Wu et al. 2002). Linkage mapping in sour cherry showed several markers segregating in ratios other than SDRF or DDRF, which could not be mapped (Wang et al. 1998). This makes genetic studies of sour cherry difficult as alleles are frequently not sub-genome specific and the sub-genomes can be unbalanced.

Due to the mixed mode of inheritance hindering linkage map construction, the understanding of the genetic control of trait variation in sour cherry has lagged behind that of other *Prunus*

species, in particular peach and sweet cherry. Key progress in diploid *Prunus* species has been made in recent years.

Delayed bloom time is a key goal of sour cherry breeding, as the development of new cultivars with later bloom would reduce the chance of pistil damage caused by spring freeze events (Iezzoni 1996). The diversity of bloom times in the sour cherry background is significant (Iezzoni and Mulinix, 1992), probably owing to the *P. fruticosa* background. Within *Prunus*, a number of QTLs have been identified in several different species including almond (Ballester et al. 2001; Silva et al. 2005), peach (Dirlewanger et al. 2012), apricot (Ruiz et al. 2010; Dirlewanger et al. 2012) and sweet cherry (Dirlewanger et al. 2012) making bloom date QTLs a perfect candidate to search for within the sour cherry background.

Knowledge of the genetic control of fruit size is also a major goal in cherry breeding. This, fortunately, has seen some progress in the last year with the discovery that previously found fruit and pit size QTL in sweet cherry (Zhang et al. 2010) are likely due to cell number regulator (CNR) genes which have been found by dissecting the peach genome (De Franceschi et al. 2013). There are, however, several other CNR genes which have yet to be explored as to their possible role in contributing to fruit size. Due to the 3-5 year juvenility period of cherry, seedlings could be screened before planting to determine if they are likely to have adequate fruit size if this important trait was even better understood. It would also allow for the more efficient incorporation of small-fruited wild germplasm for traits such as disease resistance, and allow for the selection for large fruit alleles to minimize the number of generations needed to recover commercial sized fruit.

Fruit flesh color is mainly an industry driven trait for sour cherries in the US, where much of their production is geared toward the industry standard amorello (clear-fleshed) variety 'Montmorency.' This is another area that is not well understood genetically in sour cherries, but work in apple has found MYB10 as a causal gene for red apple color (Espley et al. 2007) and sweet cherry has given us a clear place to start looking through work on QTL mapping of skin and flesh color (Sooriyapathirana et al. 2010). If the industry keeps pushing for amorello varieties, then a better understanding of fruit color will be needed to aid in selection for these types. This is especially important, as colorless flesh and skin color are recessive in sweet cherry (Fogle 1958; Hedtrich 1985; Schmidt 1998), indicating that it will likely be recessive in sour cherry. Much of the sour cherry germplasm is morello (colored flesh), making the understanding of this trait especially important as individuals with multiple dark flesh alleles would be unlikely to give rise to the desired lighter fleshed progeny.

Like flesh color, fruit firmness is also an important trait for the industry. Blemishes from harvest can cause juice loss, and therefore a reduction in harvest weight reducing the profit growers can achieve. Softer, juicier fruit are also preferred by birds, causing an increase in predation to softer cultivars. Utilization of wild germplasm for traits such as disease resistance are also likely to bring with it the softer fruit trait, which warrants an understanding of fruit firmness to allow breeders to bring fruit back to commercial standards. Preliminary data in sweet cherry has indicated some co-localization of fruit weight and fruit firmness QTL, and a negative correlation between these two traits (Quero-García et al. 2010).

The *D*-locus on the top of G5 in peach has been found to be associated with a variation of 83% for malic acid content (Etienne et al. 2002; Boudehri et al. 2009). While the amount of malic acid content is not a huge concern in breeding programs, if the large effect of the *D*-locus in peach were the same in sour cherry, it would be a trait which could be easily controlled if it was warranted in the future.

Because of the complexity of the chromosome pairing, and due to the high degree of synteny in *Prunus*, our strategy to advance our understanding of sour cherry genetics is to test whether QTLs for bloom and fruit quality traits previously identified in sweet cherry or other *Prunus* species control trait variation in sour cherry and to determine if functional allelic variants which exist in sour cherry could then be used in marker-assisted breeding (MAB). With the availability of the cherry 6K Infinium® II SNP array as part of the RosBREED project (www.rosbreed.org) (Peace et al. 2012), many markers can now be screened and dosage can be determined which then allows us to determine the phase of closely linked markers and to follow each of the four individual chromosome segments through inheritance data. This must be done since a comprehensive linkage map is not available in sour cherry. We are also relying on using multiple populations that represent multiple founders and a wide range of the diversity present within breeding populations instead of focusing our attention on just one F1 population for validation.

Materials and Methods

Plant material and phenotyping

A total of 338 cultivars and seedlings from 5 bi-parental populations including parents were used in this study (Figure 2.1). Populations were as follows: 'Újfehértói Fürtös' ('UF') ×'Surefire' (n=76), 'M172' × '25-02-29' (n=111), '25-14-20' × '25-02-29' (n=67), 'Montmorency'× '25-02-29' (53) and 'Rheinische Schattenmorelle' ('RS') × 'Englaise Timpurii' ('ET') (n=23). These individuals are planted at the Michigan State University Clarksville Research Station, Clarksville, Michigan.

Bloom time was taken in 2011 and 2012 and determined when 50% of the flowers were opened on a tree. The day of blooming was converted to Growing Degree Days (GDD) with a base of 4.4 C, as done in Wang et al. (2000), with temperature data collected from Michigan State University's Weather Station "Enviro-weather" resource (www.agweather.geo.msu.edu).

Harvest of fruit was done twice on each tree to better determine maturity. Each harvest consisted of the collection of 30 fruit (of which the best 25 went on to be evaluated), with an additional collection of around 20 fruit on the second harvest to be frozen and later processed for malic acid content.

Fruit firmness (g/mm^2) was measured in 2011 as an average of 25 fruit per harvest using the compression test of BioWorks' FirmTech 2 (Wamego, KS). Compression was done from cheek to cheek (perpendicular to the suture) when fruit were at room temperature.

Color rating for each individual was taken in 2011 and was defined according to the Sweet Cherry Flesh Color Index from Washington State University (WSU) and The Flower Council of Holland, Leiden, The Royal Horticultural Society, London. A visual rating was given from 1-5 representing clear to deeply pigmented color respectively (Figure 2.2).

Fruit size was measured as fruit weight, pit weight and mesocarp weight (mean fruit weight – mean pit weight) in 2011. Fruit and pit weights were taken as the average of the 5 largest fruit during the harvest to capture the maximum genetic potential.

Malic acid content (mg/ml) was evaluated in 2011 and was calculated using an automatic titrator, coupled to an auto-sampler and control unit (Titroline 96, Schott, Germany). Fruit was collected during the second harvest and frozen, then thawed before being strained through a Kim wipe. Juice (10ml) was then placed in 100 ml of water and titrated to a pH of 8.2 using 0.1 N NaOH.

Genotying and Haplotype construction

Four hundred and two sour cherry individuals, including founders, seedlings, and all 338 individuals in the five bi-parental populations were genotyped using the 6K Infinium® II SNP array as part of the RosBREED project (www.rosbreed.org) (Peace et al. 2012). The Illumina® Genome Studio software was used to determine the SNP genotype. Available SNP data from a diverse array of sweet cherry selections and seedlings (105 individuals) were included to aid in the determination of dosage by showing the two homozygous (AAAA and BBBB for sour cherry

corresponding to AA and BB in sweet cherry) and balanced heterozygous (AABB for sour cherry corresponding to AB in sweet cherry) classes (Figure 2.3).

SNP markers which were polymorphic but un-resolved were not used. For each parent and progeny individual, four haplotypes (to represent the four chromosomes in a tetraploid) were built for the target regions of the genome in an Excel spreadsheet by hand based on progeny inheritance and segregation in each of the bi-parental populations. This is in contrast to just two haplotypes which are built for each parent in a diploid species, which can be seen when comparing the S-locus region between sweet cherry parents 'NY54' and 'Emperor Francis' and sour cherry parents 'Újfehértói Fürtös' and 'Surefire' (Figure 2.4 a and b). When more than three ambiguous SNP dosage calls were made in a region for an individual, haplotypes were not built for that individual at that region. Multiple ambiguous SNP dosage calls for a region are likely due to too few or too many chromosomes at that region, or poor quality DNA.

When a region of interest exhibited a large number of haplotypes, haplotypes were condensed either through comparison of SNP calls in a smaller region surrounding the region of interest, or through the use of SSR markers in close proximity with candidate genes/QTL. SSR markers with "null" alleles, or alleles that were not represented with a band, were not analyzed.

Statistical analysis

ANOVA calculations to determine if the target genomic regions were significantly associated with traits were done using a linear additive model test with a user defined design matrix to

consider each haplotype and scores for the presence or absence of those haplotypes as well as the number of times each haplotype is present (to account for dosage), using a modified R-script (version 2.15.1). When a crossover took place within the region of interest, the haplotype was represented as missing data since it was unclear which haplotype would be contributing to the trait.

To confirm the ANOVA calculations, and determine if allele haplotype had a positive, or negative effect on the trait, Student's t-tests were performed comparing groups with, and those without each of the haplotypes that were significant in the linear additive model. Individuals with crossovers between two haplotypes were left out of t-tests comparing the presence or absence of haplotypes only if the crossover took place with a haplotype that was being analyzed. The first t-test determined if haplotypes were significant across all five populations, and an additional t-test was done to determine if haplotypes were also significant within individual families.

For flesh color, the Proc Mixed least squares means statement in SAS (SAS Institute version 9.2) was used to determine if the means of the different haplotypes were significantly different.

Results

Phenotypic variation

Bloom time in 2011 for individuals of all five populations ranged from 256 to 440 GDD (Figure 2.5). All populations exhibited a normal distribution for bloom time except for 'M172' × '25-02-29' which had a narrow bloom time with the bulk of individuals blooming between 256 and 348 growing degree days. Bloom time in 2012 for all populations ranged from 275 to 488 GDD (Figure 2.6). In 2012 there was less of a spread in bloom time GDD. In both years, the populations 'UF' × 'Surefire', 'Montmorency' × '25-02-29', and 'RS' × 'ET' tended to be more late blooming than the populations 'M172' × '25-02-29' and '25-14-20' × '25-02-29'. The correlation between these two years was quite high, with $R^2 = 0.81$.

Fruit weights ranged from 1.6 to 13.0 grams when all populations were considered together (Figures 2.7). The phenotypic distributions for fruit weight in all populations and within individual populations were normally distributed. With the exception of 'M172' × '25-02-29', transgressive segregation with individuals having larger fruit than either parent were found, most notably in the 'RS' × 'ET' population, with one individual far larger than either parent. Pit weight, like bulk weight, was also normally distributed with evidence of transgressive segregation within all families for larger pits, with the exception of the lack of small pit weights among progeny of 'RS' × 'ET' (Figure 2.8). The correlation between pit weight and fruit weight was moderate, with $R^2 = 0.56$. Mesocarp fruit weight showed phenotypic distributions similar to fruit weight (Figure 2.9) which is to be expected as the correlation between these was very high ($R^2 = 0.99$). All populations and the combination of all five populations were normally distributed with all showing some individuals with larger mesocarp size than either parent, with the exception of 'M172' × '25-02-29'. The correlation between fruit weight and mesocarp

weight was very high with $R^2 = 0.998$ and the correlation between mesocarp weight and pit weight was similar to that of pit weight and fruit weight ($R^2 = 0.52$).

Fruit firmness ranged from 103 (soft) to 234 (firm) g/mm^2 and was normally distributed in all individual populations, but when all populations were combined there were higher numbers on the softer end of the scale (e.g. smaller values) (Figure 2.10). The populations with the most firm fruited progeny individuals were populations 'M172' × '25-02-29' and 'RS' × 'ET', with all populations having individuals that were firmer, and softer than the measured parents. The correlation between fruit size and fruit firmness was not significant ($R^2 = 0.01$).

The phenotypic distributions for flesh color ranged from 1 (no red color in the flesh) to 5 (very dark red-purple flesh) but did not show a normal distribution in any of the populations, or when considering all populations together (Figure 2.11). When all populations were considered together, there were fewer individuals in the middle rankings (2, 3 and 4) with higher numbers on both ends (1 and 5). Within individual populations, 'UF' × 'Surefire', '25-14-20' × '25-02-29', and 'RS' × 'ET' tended to skew toward the darker flesh haplotypes, while 'Montmorency' × '25-02-29' had a slight skew to lighter flesh. 'M172' × '25-02-29' had a distribution similar to the combination of all populations, with higher distributions on the light, and dark ends of the scale.

Malic acid content ranged from 0.40 to 2.80 mg/ml when the progeny individuals from all populations were considered together. The phenotypic distributions of malic acid content also

showed a normal distribution (Figure 2.12). Each population had individuals with mean values in excess of either parent. The populations 'UF' × 'Surefire' and 'RS' × 'ET' tended to have more individuals with the highest concentration of malic acid, while progeny of 'Montmorency' × '25-02-29' and 'M172' × '25-02-29' tended to have lower concentrations of malic acid.

Haplotyping to identify different alleles for the target regions

A total of 2058 of the SNP markers evaluated were of sufficient quality for genotyping and polymorphic in the sour cherry materials (Table 2.1). These SNP markers provided the set from which SNPs were chosen to build haplotypes for the regions of interest. Haplotypes were built for all parents and progeny of the 5 bi-parental populations when ambiguous genotypic scores were not encountered. Eight different regions of various sizes were looked at on 6 different chromosomes based on where QTL had been previously found for the traits studied (Table 2.2). Four different SSR markers were used for the construction of haplotypes in on G2 and G6 or as proxy haplotypes to condense haplotypes (Table 2.3). With the exception of few regions in the 'RS' × 'ET' population, all of the populations at all of the regions haplotyped had a small number of individuals which were unable to be haplotyped (Table 2.4). These haplotypes covered eight separate regions on six different chromosomes targeting the QTL regions previously found in *Prunus* studies for the traits of interest (Figure 2.13).

Three different regions were targeted for bloom on G1, G2 and G4. On G1, twelve unique haplotypes were found, and were designated as haplotype 'a' to 'l'. (Figure 2.14). On G2, 21 unique haplotypes were found (Figure 2.15) facilitating the need for an SSR marker

(G2SSR1566) in the region to be used as a proxy haplotype to narrow down the number of haplotypes to just seven haplotypes (2, and 4-9, where haplotypes 1 and 3 were skipped as they were previously classified in sweet cherry in De Franceschi et al. (2013). Dosage for these haplotypes was inferred based on the original haplotypes since dosage could not be determined based solely on SSR banding on the polyacrylamide gel. Seventeen haplotypes were found for the G4 bloom haplotype designated as 'a' to 'p' and 's', where 'q' and 'r' were found to be equivalent to other haplotypes already designated (Figure 2.16).

Haplotypes targeting fruit size and firmness were built for four different regions on G2, G3, G5, and G6. The G2 region was the same as the G2 bloom region, where 21 haplotypes were found, but narrowed down to seven based on a proxy SSR marker, where seven unique fragment sized were found, enabling us to essentially break down the number of proxy haplotypes to seven (Figure 2.15). Sixteen haplotypes (designated 'a' to 'p') were found for the G3 fruit size/firmness region, but this number was reduced to eleven when haplotypes were compared in a narrower region centered around the CNR16 region (Figure 2.17). In condensing the haplotypes, a=i, c=o, d=f, g=m, and k=p. On G5, centered around CNR18 and CNR19, 12 haplotypes were found and designated as 'a' to 'f', and 'h' to 'l' and 'n' with 'g' and 'm' being skipped as they were found to be equivalent to previously designated haplotypes (Figure 2.18). The G6 fruit size/firmness region had 13 unique haplotypes designated as 'a' to 'j' with 'd', 'e', and 'e2', but an SSR marker (G6SSR2206) close to the CNR20 gene was used to condense the number of haplotypes down to just five (1-5) with two null alleles found, which were not analyzed (Figure 2.19).

For flesh color and malic acid content, only one region was targeted for each trait on G3 and G5, respectively. Thirteen unique haplotypes were found centered around three *MYB*10 homologs on the G3 flesh color region with haplotypes designated as 'b' to 'u' skipping 'i', 'j', 'm', 'q', 'r', 's', and 't' due to combining of similar haplotypes (Figure 2.20). On the top of G5 the location of the D-locus in peach, 17 haplotypes were found, designated as 'b' to 'y' (with 'c', 'd', and 't' to 'x' being skipped due to combining of like haplotypes) but that number was reduced to just six (1-6) when just the top of the chromosome that spanned the likely location of the D-locus was considered (Figure 2.21).

Haplotype analysis

Bloom

On G1, the presence or absence of seven of the twelve haplotypes were associated with significant differences in bloom when looked at for all five populations (Table 2.5). The presence of haplotypes c, d, and k lead to earlier bloom, while the presence of f, g, h, and l had mean bloom times which were later than individuals without those haplotypes. All of these haplotypes were significant in both 2011 and 2012 with the exception of haplotype g which was only significant in 2012. None of these haplotypes were significant when looked at within individual families. 'M172' and '25-02-29' each have two G1 haplotypes significantly associated with early bloom ['M172' = ij<u>kk</u>; '25-02-29' = ab<u>cd</u>] and no haplotypes significantly associated with late bloom. This finding is consistent with the 'M172' × '25-02-29' progeny exhibiting the earliest bloom time and absence of late blooming individuals.

Due to the high number of haplotypes for the G2 region for bloom, the SSR marker G2SSR1566 was used to define proxy haplotypes. The presence vs. the absence of all seven haplotypes on G2 was associated with significant differences in bloom over all five populations for both 2011 and 2012 (Table 2.6). Earlier bloom was associated with haplotype 2, 6 and 7, while later bloom was associated with haplotype 4, 5, 8 and 9. When the presence vs. the absence of the haplotypes was compared within individual families, none were significant. As with G1, 'M172' and '25-02-29' each have two haplotypes significantly associated with early bloom ['M172' = 2447; '25-02-29' = 2467] which is consistent with the earlier blooming associated with this family. Haplotype 2 being an early blooming haplotype in sour cherry makes sense as this haplotype is also found in sweet cherry (De Franceschi et al. 2013). Sweet cherry tends to bloom earlier than sour cherry, so it would be expected that a sweet cherry haplotype would be associated with earlier blooming.

Eleven of the 15 haplotypes for G4 were found to be associated with significant differences in bloom time when evaluated across all five populations (Table 2.7). All but one, haplotype h, produced significant differences in both 2011 and 2012, while h was just significant in 2011. Haplotype c, d, e, f, h, and j lead to earlier bloom, while haplotype g, i, k, n, and s lead to later bloom. The largest difference was seen with haplotype k, where the mean of individuals with k was 36 and 35 growing degree days later blooming than those without k in 2011 and 2012 respectively. Both 'UF' and 'Surefire' have this very late k haplotype ['UF' = ahkn; 'Surefire' = giks] which is consistent with this population having a high proportion of later blooming individuals.

When haplotypes were compared within individual families for G4, seven haplotypes were found to significantly affect bloom time (Table 2.8). In the 'UF' × 'Surefire' population, two haplotypes were found to be significant. Haplotype k, when tested over all five populations, led to later bloom in both 2011 and 2012. This haplotype is likely from the founder 'Pandy 38', as 'UF' arose from a mutation from 'Pandy 38' (Figure 1.1). The haplotype is also in Surefire, indicating that 'Pandy 38' may also be in its background. Haplotype a, on the other hand, was found to lead to earlier bloom in just 2012. This haplotype was not found to be significant when tested across all five populations. Two haplotypes were also found to be significant in the population '25-14-20' × '25-02-29'. Haplotype b was found to lead to later bloom within this family only in 2011, while haplotype c was found to lead to earlier bloom only in 2012. Only one haplotype, haplotype e, in the population 'M172' × '25-02-29' was found to significantly affect bloom time. In both 2011 and 2012, the presence of haplotype e led to later bloom. This is the opposite effect that haplotype e had when compared over all five populations. For the populations of 'Montmorency' × '25-02-29' and 'RS' x 'ET', only one haplotype in each population was found to be significant, and only in 2012. In 'Montmorency' × '25-02-29', haplotype i was associated with later bloom, while in 'RS' × 'ET', haplotype m was found to be associated with earlier bloom.

Fruit/pit size

For the fruit size region on G2, two haplotypes, 6 and 8, were found to be significantly associated with fruit and mesocarp weight, while only haplotype 8, was significantly associated

with pit weight (Table 2.9). Haplotype 6 was found to be significantly associated with lower fruit and mesocarp weight, but not pit weight. Only haplotype 8 was significantly associated with fruit, pit and mesocarp weight, where it was associated with lower weights when present. None of the haplotypes produced significant variation within individual families.

On G3, seven of the eleven haplotypes were significantly associated with fruit size and six were associated with pit size (Table 2.10). Haplotypes a, b, c, and n were significantly associated with smaller fruit, while haplotypes e, g, and h were significantly associated with larger fruit. All of these haplotypes were significant for both mesocarp weight and fruit weight. Pit weight was not significantly associated with haplotypes a and e as were fruit and mesocarp weight, but had a significant association with haplotype k, with an average pit weight less when k was present. Haplotypes b, c, n, g, and h had the same associations with pit weight as with fruit and mesocarp weight. No haplotypes were significant within individual families for fruit, mesocarp, or pit weights. 'Montmorency' and '25-02-29' each have three small-fruit haplotypes, and no large fruit haplotypes ['Montmorency' = acjn; '25-02-29' = abcd]. This is consistent with this population being mostly small-fruited individuals.

Only three of the 13 haplotypes built for the G5 fruit/pit size region were significantly associated with fruit size, and two shared significant haplotypes and one unique one being significantly associated with pit size (Table 2.11). Haplotypes f and j were significantly associated with larger fruit, and haplotype a was significantly associated with smaller fruit. Haplotypes a and j were also significantly associated with pit weight in the same direction they were with fruit and mesocarp weight. Haplotype n was also significantly associated with larger pit weight. None of

the haplotypes were significantly associated with fruit, mesocarp or pit weights within individual populations. The three populations that have the largest individuals in them ('UF' × 'Surefire', 'M172' × '25-02-29' and 'RS' × 'ET'), each have one parent that has two large fruit haplotypes: 'UF' = ddfn; 'M172' = dfhj; and 'ET' = djkn. The populations with smaller fruit size ('Montmorency' × '25-02-29' and '25-14-20' × '25-02-29') have zero and one parent with large haplotypes, which is consistent with the phenotypes observed in these families (Figure 2.7).

For the G6 region, only two haplotypes were found to be significantly associated with fruit/mesocarp and pit size, with an additional third for pit size (Table 2.12). Haplotypes 3 and 4 were significantly associated with larger and smaller fruit, mesocarp and pit weights respectively. Haplotype 1 was also associated with smaller pits, but not significantly for fruit and mesocarp weight. None of these haplotypes were significant within individual families.

Fruit firmness

Since fruit firmness was found to be correlated with fruit size in sweet cherry (Quero-García et al. 2010), the haplotypes built for the fruit size regions were also tested for association with fruit firmness. For the G2 region, three haplotypes were associated with fruit firmness (Table 2.13). The three haplotypes significantly associated with firmness were different haplotypes than those associated with fruit size. Haplotypes 5 and 9 were associated with fruit that were less firm than those without the haplotypes, while haplotype 7 was associated with firmer fruit. No haplotypes were significant within individual families. The two populations with the firmest individuals were the two populations where both parents have haplotype 7: 'M172' × '25-02-29' ['M172' =

2447; ‘25-02-29’ = 2467] and ‘RS’ × ‘ET’ [‘RS’ = 4478; ‘ET’ = 4678]. Neither of these families have the haplotypes 5 or 9 which are associated with softer fruit.

The G3 region had three haplotypes which were significantly associated with fruit firmness (Table 2.14). The, haplotypes j and k, were associated with softer fruit while haplotype d was associated with firmer fruit. Haplotypes j and d were not significantly associated with fruit or pit weights, however k was associated with smaller pits as well. No haplotypes were significant within individual families.

For the fruit size CNR region on G5, haplotypes b and j were significantly associated with firmer fruit while haplotypes e, i, and l were significantly associated with softer fruit (Table 2.15). Of those five haplotypes that were significantly associated with fruit firmness, only one of them was also associated with fruit or pit size. Haplotype j was significantly associated with larger fruit and pit weights in addition to being associated with firmer fruit. No haplotypes produced significant variability within individual families. ‘M172’ × ‘25-02-29’ again, like for G2, have only firm haplotypes that were significantly associated with firmness, b in ‘25-02-29’ and j in ‘M172’: [‘M172’ = dfhj; ‘25-02-29’ = abcd] which is consistent with having progeny with firmer fruit. Surefire, on the other hand, has three soft-associated haplotypes at this region [‘Surefire’ = ceil] which is consistent with the ‘UF’ × ‘Surefire’ population have a large number of individuals which are soft fruited, as ‘UF’ also has no haplotypes that are associated with either firm, or soft fruit [‘UF’ = ddfn].

Only one haplotype in the G6 region was found to be significantly associated with fruit firmness (Table 2.16). Haplotype 1 was associated with softer fruit when compared to individuals without this haplotype. This haplotype was not significantly associated with fruit or pit weights. No haplotype were significant within individual families.

Flesh color

When evaluated across all five bi-parental populations, ten of the thirteen G3 flesh color haplotypes were found to be associated with flesh color (Table 2.17). Haplotypes b, d, e, k, l, and p were significantly associated with darker flesh, while haplotypes c, f, n, and o were associated with lighter flesh.

Within individual families, six haplotypes were found to be significant (Table 2.18). In the 'UF' × 'Surefire' population, three haplotypes were significantly associated with flesh color. Haplotype d and c from 'UF' were associated with darker and lighter flesh respectively. From 'Surefire', haplotype e was associated with dark flesh. In the '25-14-20' × '25-02-29' population, there were also three haplotypes which were significantly associated with flesh color. One dark flesh haplotype was from each parent, haplotype d from '25-14-20', and haplotype p from '25-02-29'. The light flesh haplotype o from '25-02-29' was also significant in this population. In 'M172' × '25-02-29', the dark flesh haplotype p, and the light flesh haplotype o from '25-02-29' are significant again, as well as the dark flesh haplotype l from the 'M172' parent. Haplotype p and o from '25-02-29' are once again significant in the 'Montmorency' × '25-02-29' population, with haplotype p being a darker flesh haplotype, and haplotype o a lighter

flesh one. Haplotype o is also present in 'Montmorency'. Only dark flesh haplotypes were found to be significant in the 'RS' × 'ET' population. Haplotype p is from the parent 'RS', while haplotype l comes from 'ET'.

Since each parent carried only one dark flesh haplotype (with the exception of 'ET' which had two) we wanted to investigate if dosage was important in this region. To test this, means were compared within individual families (Figure 2.22). For 'UF' × 'Surefire', individuals with both the d haplotype and the e haplotype have a darker mean flesh score (4.8) than those with only haplotype e (3.0) or with no dark flesh haplotypes (1.9). The mean score of those with both d and e, and those with only d are not significantly different. In the 'M172' × '25-02-29' population, the dark flesh haplotype l came from 'M172' and the dark flesh haplotype p came from '25-02-29'. In each comparison in this family, the mean scores were significantly different from each other. Those with both dark flesh haplotypes had the darkest mean flesh score (4.2), followed by those with only the p haplotype (3.6), those with only the l haplotype (3.2) and lastly those with no dark flesh haplotypes (1.3). In the population '25-14-20' × '25-02-29', dark flesh haplotype d from '25-14-20' was compared with the dark flesh haplotype p in '25-02-29'. Individuals in this population with both d and p had the darkest mean flesh score (4.9) which was significantly different from individuals with just the p haplotype (4.2), and those with no dark flesh haplotypes (1.6). Individuals with just the d haplotype (mean score of 4.4) were not significantly different from those with both p and d, or individuals with only p (4.9 and 4.2 respectively). In the 'Montmorency' × '25-02-29' population, only one dark flesh haplotype was present. The presence of the p haplotype from '25-02-29' in individuals (mean score of 3.9) was significantly

different from individuals with no dark flesh haplotypes (1.5). The family 'RS' × 'ET' was too small to do a comparison, as there were too few individuals in all of the classes to compare.

Malic acid

On G5, three haplotypes were found to be associated with significant differences in the amount of malic acid (Table 2.19). Haplotype 1 was significantly associated with a higher amount of malic acid. When individuals with two copies of haplotype 1 were compared to those with only one, there was also a significant difference, where multiple copies of haplotype 1 had an even higher concentration of malic acid than those with only one copy. Haplotype 4 and haplotype 5 also had significant differences between those with, and those without the haplotype, with those with 4 and 5 having a lower concentration of malic acid. None of these haplotypes were significant within individual families. The families 'UF' × 'Surefire' and 'RS' × 'ET' have the most individuals with higher malic acid. Each of these families has one parent with two high malic acid haplotypes, and the other parent has one: ['UF' = 1236; 'Surefire' = 1123; 'RS' = 1234; 'ET' = 1146].

Discussion

In this study, we used multiple sour cherry populations to validate QTL that have been found in other *Prunus* species. Due to the relatively few sour cherry founders, these populations share many common ancestors, and therefore are inter-related (Figure 2.1). This made comparisons

between the families easier due to the reduced number of unique haplotypes present in this tetraploid species.

In some regions, however, there were still a large number of haplotypes found, such as the G2 region for bloom, fruit size and firmness (Figure 2.15). It was considered that the SNP diversity may over represent the number of functional haplotypes. Therefore the use of the polymorphic SSR marker in this region was important in order to condense the haplotypes to what might better approximate the number of functional haplotypes. Due to the number of important traits in this one narrow region, it is expected that there were so many haplotypes found, with selections taking place in this region, it is likely that unique crossovers in this region to get desired combinations of these traits produced the variety of haplotypes found in this region. SSR markers in all regions could be used in the future to condense all of the regions evaluated.

Bloom

All three of the regions studied were validated by locating haplotypes that were associated with bloom time. The G4 region found in almond (Silva et al. 2005), sweet cherry and peach (Dirlewanger et al. 2012), was expected to have a bloom time QTL in sour cherry. The G4 bloom time QTL was found to explain from 24 to 47 percent of the variance in the sweet cherry population studied (Dirlewanger et al. 2012). It is likely due to the high impact of this region on the bloom phenotype that not only was this region significant when looked at across all populations, but even within individual populations. One haplotype, haplotype e, found only in the early blooming parent ‘M172’, was significantly associated with early blooming when

looked at across all populations, but within ‘M172’ × ‘25-02-29’ it was found to be associated with later bloom (Tables 2.7 and 2.8). A likely explanation of this finding is that while overall haplotype e is an earlier blooming haplotype, within an early blooming family like ‘M172’ × ‘25-02-29’ it is relatively late blooming compared to the other haplotypes present in that family. Of all the significant haplotypes found for the G4 region, haplotype k from ‘UF’ and ‘Surefire’ had the highest impact on bloom time within individual populations. This haplotype was associated with a mean delay in bloom of 39 and 33 GDD in 2011 and 2012, respectively. With such a large effect on bloom time for one haplotype, it is likely that later bloom is dominant to early bloom. Sweet cherry tends to bloom earlier than sour cherry (Iezzoni et al. 1990), which would indicate that sweet cherry-derived haplotypes would also be associated with earlier bloom. This is what was found on G2, where the haplotype 2 and the corresponding haplotype is associated with earlier bloom, and is the only haplotype that is known to be equivalent in sweet cherry (De Franceschi et al. 2013).

For breeding purposes, where later bloom would help individuals avoid freeze damage in the spring, it would be beneficial to focus mostly on the G4 bloom region, as this region has the most impact, keeping in mind that other regions do play a role in contributing to bloom time. For example, on G1 in populations like ‘M172’ × ‘25-02-29’, each parent has two early bloom haplotypes, and no haplotypes that are significantly associated with later bloom. It is unlikely, therefore, for this cross to produce individuals with late bloom.

Fruit/pit size

All four regions that targeted previously identified QTLs for fruit/pit size were validated in sour cherry. Fruit weight and mesocarp weight were always significantly associated with the same haplotypes in all regions studied; however, pit weight was not always found to have the same significance, indicating that not all haplotypes effect fruit weight and pit weights similarly.

On G2, both haplotypes 6 and 8 were found to be negatively associated with fruit size. The two largest fruited parents, 'UF' and 'M172', are the only two parents that do not have either of these negatively associated haplotypes. This could be a contributing factor in that the populations 'UF' × 'Surefire' and 'M172' × '25-02-29' are the two populations with larger-fruited individuals. The haplotype 2 for this region in sour cherry is equivalent to the *PavCNR12* genotype 2 in sweet cherry, where it was found to be associated with smaller fruit in domesticated sweet cherry (De Franceschi et al. 2013). In sour cherry, which are generally smaller than sweet cherry, there was a tendency toward larger fruit (mean fruit weight values of 5.64g vs. 5.30g with and without haplotype 2 respectively) when haplotype 2 was present, however this only significant at the $P = 0.07$ level. While this is opposite to what is found in sweet cherry, the size difference between sweet and sour cherries could account for a negatively associated haplotype in sweet cherry still contributing to larger fruit in sour cherry. This same comparison of sweet cherry haplotypes contributing to larger fruit in sour cherry can also be seen on G6, where the haplotype 3 which is significantly associated with larger fruit (Table 2.12) has also been found to be equivalent to the haplotypes found in sweet cherry (De Franceschi et al. 2013).

The almost total lack of transgressive segregation for fruit size in ‘M172’ × ‘25-02-29’ may partially be explained by the G3 haplotypes, where ‘M172’ has three large-fruit associated haplotypes (e, g and h), and ‘25-02-29’ has three small-fruit associated haplotypes (a, b, and c). No combinations of haplotypes would allow for individuals in this population to have equivalent or greater numbers of either small-, or large-associated haplotypes in this cross, which would not allow for progeny to exceed the parental means in this region.

This study is the first to report on the association between haplotypes across the CNR18 and CNR19 region on G5 and fruit weight. Three of the 4 haplotypes that were significantly associated with fruit size for this region were associated with larger fruit, indicating that this could be a good region to start accumulating positive haplotypes for fruit size. Considering that even the small-fruited parent ‘ET’ (4.11g) has two of the large-associated haplotypes (the same number of large-associated haplotypes as the large-fruited parents ‘M172’ and ‘UF’ with 8.27g and 7.75g fruit weight respectively), it shows that while this may not be a major QTL, progeny ‘27-12-12’ (12.96g) and ‘27-12-13’ (8.09g), the two largest progeny in the ‘RS’ × ‘ET’ population, each have no negative-associated haplotypes for the G5 region, and one and two of the positive associated haplotypes respectively for this region.

Haplotype 6 on G2, haplotype e on G3 and haplotype f on G5 are all significantly associated with fruit size, but not pit size. Conversely, haplotype k on G3, haplotype n on G5 and haplotype 1 on G6 are all significantly associated with pit size but not fruit weight. This indicates that not all haplotypes contribute to both pit and fruit weight in the same manner. When associations between both fruit weight and pit weight do occur from the same haplotype, they are always in

the same direction, where a haplotype negatively associated with fruit weight is also negatively associated with pit weight.

For breeding purposes, large fruit with small pits are desired. To achieve this goal, the accumulation of the larger fruited haplotypes in all the regions validated here, or the exclusion of the smaller fruited ones, would be a good strategy. This, however, may also lead to larger pit sizes. By also focusing haplotypes that only effect pit size, such as small pit-associated haplotypes like k from G3, or haplotype 1 from G6 may help reduced the pit size without adversely affecting fruit size.

Fruit firmness

Fruit firmness was validated for the G2 region, and was discovered to be associated with the other three regions also examined for fruit/pit size on G3, G5, and G6. Of the 14 different haplotypes found to be associated with fruit size for this region, only haplotype k from G3 and haplotype j from G5 were also associated with fruit firmness. In the case of haplotype k from G3, it was linked to softer, smaller fruit, while the opposite effect was found for haplotype j from G5. All other haplotypes significantly associated with fruit firmness were haplotypes that were not significantly associated with fruit size, indicating that these two traits are not affected by the same genetic components. No other regions tested besides these four presented, were important for determining fruit firmness, indicating that there may be other regions of greater importance for this trait, or that many regions affect fruit firmness, so it may be difficult to pin down.

Flesh color

Four haplotypes in total were found to be associated with dark flesh color in sour cherry, validating the QTL in sweet cherry (Sooriyapathirana et al. 2010). Perhaps most surprising is how few dark-flesh haplotypes are present in these 5 populations that cause a range in color from light red to dark mahogany. Haplotype d found in 'UF', and '25-14-20' was found to be associated with the darkest coloration in the two populations it was present in. In both populations, with just this dark flesh haplotype and none of the others, an average color score of 4.4-4.5 was found to be similar to individuals with both d and e in the 'UF' × 'Surefire' population, or with both d and p in the '25-14-20' × '25-02-29' population. This indicates that with just this one haplotype, individuals will be mostly dark fleshed. All of the other dark flesh haplotypes tend to be more moderate in coloration, from a mean color score of 3 for individuals with just haplotype e, to a mean color score of 3.6-4.2 for individuals with one copy of haplotype p. It is unknown how multiple copies of the same haplotype would influence flesh color, as no populations studied had more than one copy of any dark flesh haplotype. Due to the fact that for the moderate flesh color haplotypes e, l and p tend to have an additive affect when found together in the same genotype, it could be inferred that two copies of the same dark flesh haplotype would have a similar result.

Breeding decisions could easily be made to select against dark flesh haplotype d, and select for single copies of the more moderate haplotype l and e if flesh color scores of around 3 were desired. If light-fleshed, industry-standard 'Montmorency'-type flesh color were desired, then selecting against these four dark flesh haplotypes would be the breeding recommendation, as

individuals without any of these four dark flesh haplotypes ranged from 1.3 in the ‘M172’ × ‘25-02-29’ population to 1.9 in the ‘UF’ × ‘Surefire’ population (Figure 2.22).

Malic acid

The D-locus region responsible for variation of malic acid content in peach (Bouderhi et al. 2009) has been validated to also be associated with malic acid content in sour cherry. The families ‘UF’ × ‘Surefire’ and ‘RS’ × ‘ET’ have the most individuals with high malic acid. These also are the families that have the most haplotypes classed as 1 in this study (Figure 2.21). It is likely that since this class of haplotypes had the most individuals (6 of the 17 haplotypes found) that these are haplotypes that belong to the *P. fruticosa* subgenome of sour cherry, rather than the *P. avium* subgenome. Sweet cherry is not known for their high acidity, but *P. fruticosa* is known to be acidic (Iezzoni et al. 1990). Future studies comparing haplotypes found in sweet cherry and haplotypes found in sour cherry for this region could confirm this.

Dosage

While more targeted crosses would need to be undertaken to study the full effects of dosage for all of the traits validated in this study, there were a few notable results that confirmed that dosage can play a role in trait variation in tetraploid sour cherry. Flesh color in the family that had the more moderate flesh colors (‘M172’ × ‘25-02-29’) had increased flesh color when both were present in the same family as opposed to each of haplotype p or haplotype l separately. In this case, flesh color appears to be additive when moderate flesh colors are present. Dosage with

haplotypes with very dark haplotype d makes little difference, as just one copy of d results in very dark fleshed fruit. The effects of dosage can also be seen with malic acid, where multiple copies of haplotype class 1 give rise to an average malic acid content greater than if just one copy of this haplotype were present. In peach where the *D* locus controls fruit acidity, it was found that low acidity is partially dominant (Boudehri et al. 2009). In sour cherry, this would mean that an accumulation of more high-acid haplotypes would help offset low acid ones.

Linkage and breeding implications

G2 was found to be associated with bloom, fruit size, and fruit firmness. Given that the desired cultivar would be a late blooming tree with firm and large fruit, careful consideration is needed when selecting for or against certain haplotypes for this region. For example, haplotype 8 is associated with later bloom, but small fruit. Two of the other haplotypes associated with late bloom, 5 and 9, are also associated with soft fruit. The only haplotype in this region that is associated with later bloom, but has no negative associating with fruit size or fruit firmness is haplotype 4. Haplotype 6 could be selected against, as it is associated with both early bloom and small fruit. As more regions are found to be associated with multiple traits, these kinds of considerations will need to take place in order to avoid negative linkage drag.

Conclusions

Several QTL found in diploid *Prunus* species have been validated in the background of tetraploid sour cherry. While the methods used here of comparing the presence or absence of

haplotypes has proven to be useful as a means of simplifying the genetics of this species, best results seem to be found when looking at regions with a high contribution to phenotypic variation such as flesh color on G3, and bloom time on G4. In using multiple populations with inter-connected pedigrees which still represent the diverse germplasm of sour cherry, we have been able to determine how individual haplotypes perform in different backgrounds. Future studies with targeted crosses to see how dosage plays a role in some of these populations could provide better understanding to these traits in the future.

Table 2.1: SNP informativeness in sour cherry for the eight sets of chromosomes based on whether the SNP was derived from polymorphism in sweet cherry or in one of the two sour cherry subgenomes (i.e., *avium* or *fruticosa*).

Chromosome	SNP source[a]	SNPs chosen[b]	Failed	Monomorphic	Unresolved Polymorphic	Polymorphic
1	Sweet	902 (50)	8 (0)	364 (11)	211 (8)	319 (31)
	Sour	164/161	1/2	14/18	68/62	81/79
2	Sweet	557 (21)	10 (0)	199 (5)	166 (5)	182 (11)
	Sour	92/83	2/0	12/20	40/26	38/37
3	Sweet	434 (18)	4 (0)	165 (4)	107 (6)	158 (8)
	Sour	87/74	1/2	13/12	33/35	40/25
4	Sweet	479 (26)	9 (0)	176 (0)	141 (9)	153 (17)
	Sour	89/73	1/0	8/15	45/28	35/30
5	Sweet	489 (33)	4 (0)	208 (8)	128 (7)	149 (18)
	Sour	66/84	0/0	4/16	30/36	32/32
6	Sweet	508 (32)	13 (0)	188 (3)	145 (11)	162 (18)
	Sour	108/100	0/0	8/14	43/31	57/55
7	Sweet	453 (22)	6 (0)	184 (5)	113 (3)	150 (14)
	Sour	71/75	2/0	2/16	22/29	45/30
8	Sweet	392 (19)	14 (0)	130 (4)	140 (6)	108 (9)
	Sour	75/80	3/1	9/17	28/36	35/26
Total Sweet		4214 (221)	68 (0)	1614 (40)	1151 (55)	1381 (126)
Total Sour		752/730	10/5	70/128	309/283	363/314
Grand Total		5696	83	1812	1743	2058

[a]Numbers of SNPs for the subgenomes of sour cherry are split (/) between *avium* and *fruticosa.*

[b]Numbers in parentheses are totals for RosCOS SNPs derived from sweet cherry and are included in the first number

Table 2.2: Summary of all traits, QTLs and their locations that were validated in this study. The species source and original QTL reference(s) are included.

Linkage Group	Haplotype region built (Mb)[a]	Markers used	Trait	Validation region/marker	No. of significant alleles/No. of alleles	QTL Source/reference
1	45.02-46.75	28 SNPs	Bloom (GDD)	45.02-46.75 Mb	7[b]/12	*P. avium*/Dirlewanger et al. 2012
2	14.93-22.08	122 SNPs, 3 SSRs	Fruit Firmness	SSR marker (G2SSR1566)	3[b]/7	*P. avium*/Quero-García et al. 2010
	14.93-22.08	122 SNPs, 3 SSRs	Fruit size	SSR marker (G2SSR1566)	2[b]/7	*P. avium*/Zhang et al. 2010
	14.93-22.08	122 SNPs, 3 SSRs	Bloom (GDD)	SSR marker (G2SSR1566)	7[b]/7	*P. avium*/Dirlewanger et al. 2012
3	1.14-7.57	75 SNPs	Fruit size	2.74-4.76 Mb	8[b]/11	*P. avium*/Quero-García et al. 2010; Rosyara et al. (in review)
	1.14-7.57	75 SNPs	Fruit Firmness	2.74-4.76 Mb	3[b]/11	*P. avium*/Quero-García et al. 2010
	9.73-15.46	47 SNPs	Flesh Color	10.68-13.41 Mb	4[c]/13	*P. avium*/ Sooriyapathirana et al. 2010
4	7.01-10.83	44 SNPs	Bloom (GDD)	7.31-9.15 Mb	6[c]/15	*P. dulcis*/Silva et al. 2005 Sanches-Perez et al. 2007; *P. avium* /Dirlewanger et al. 2012

Table 2.2 (cont'd)

Linkage Group	Haplotype region built (Mb)[a]	Markers used	Trait	Validation region/marker	No. of significant alleles/No. of alleles	QTL Source/reference
5	0.69-5.43	54 SNPs	Acidity (Malic Acid content)	0.69-1.46 Mb	3[b]/6	P. persica/Boudehri et al. 2009
	16.13-18.10	42 SNPs	Fruit size	16.72-17.76 Mb	4[b]/13	P. persica/De Franceschi et al. 2013[d]
	16.13-18.10	42 SNPs	Fruit Firmness	16.72-17.76 Mb	5[b]/13	-[e]
6	22.12-27.52	69 SNPs, 2 SSRs	Fruit Firmness	SSR marker (G6SSR2208)	1[b]/5	-[e]
	22.12-27.52	69 SNPs, 2 SSRs	Fruit size	SSR marker (G6SSR2208)	3[b]/5	P. avium/Zhang et al. 2010

[a] Mb distances according to the Peach v1.0 'dhLovell' genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome) Verde et al. 2013

[b] Trait values for these alleles were significantly different when all five families were considered together

[c] Trait values for these alleles were significantly different within families

[d] Candidate gene was used instead of a QTL region

[e] QTLs for firmness had not previously been reported but were tested in this study

Table 2.3: SSR markers used in this study and the original SSR reference.

SSR	Species Origin	Peach physical map location (Mb)[a]	Reference
G2SSR1566	*P. persica*	Scaffold 2 (15.66)	De Franceschi et al. 2013
G6SSR2208	*P. persica*	Scaffold 6 (22.08)	De Franceschi et al. 2013
CPSCT038	*P. salicina*	Scaffold 2 (15.05)	Mnejja et al. 2004
BPPCT034	*P. persica*	Scaffold 2 (16.49)	Dirlewanger et al. 2002

[a]Mb distances according to the Peach v1.0 'dhLovell' genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome) (Verde et al. 2013)

Table 2.4: Number of progeny individuals from each bi-parental family for which the four chromosome segments for the target QTL regions in each progeny individual could be identified as haplotypes inherited from its parents. Individuals were not haplotyped when SNPs were ambiguous for dosage, or if individual haplotypes could not be determined.

Linkage group	Region (Mb)[a]	UF[b] x Surefire (n=76)	RS[c] x ET[d] (n=23)	M172 x 25-02-29 (n=111)	25-14-20 x 25-02-29 (n=67)	Montmorency x 25-02-29 (n=53)
1	45.02-46.75	74	23	110	66	48
2	14.93-22.08	72	22	100	62	45
3	1.14-7.57	73	23	100	56	46
3	9.73-15.46	70	23	101	61	46
4	7.01-10.83	74	22	108	58	48
5	0.69-5.43	66	22	105	63	49
5	16.13-18.10	70	23	100	64	51
6	22.12-27.52	71	21	106	60	49

[a]Mb distances according to the Peach v1.0 ‘dhLovell’ genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome) (Verde et al. 2013)
[b]Újfehértói Fürtös, [c]Rheinische Schattenmorelle, [d]Englaise Timpurii

Table 2.5: Phenotypic means for bloom time in 2011 and 2012 for the presence or absence of the G1 haplotypes[a] in sour cherry progeny individuals from the five bi-parental families. Parental genotypes for the G1 haplotypes are: 'M172' (ijkk), 25-02-29 (abcd), 'Montmorency' (aceh), '25-14-20' (adej), 'UF' (bfij), 'Surefire' (ahjj), 'RS' (acdi) and 'ET' (egkl).

	Bloom 2011 (GDD)[b]			Bloom 2012 (GDD)		
	N[c]	Means[d]	*P* value	N	Means	*P* value
G1 haplotype[e]						
a/no a	182/122	$318A^{A}/315^{A}$	0.491	187/127	$368^{A}/365^{A}$	0.406
b/no b	133/170	$317^{A}/317^{A}$	0.956	137/175	$366^{A}/368^{A}$	0.557
c/**no c**	124/173	$309^{A}/322^{B}$	0.0006	131/176	$357^{A}/373^{B}$	<0.0001
d/**no d**	138/164	$311^{A}/321^{B}$	0.007	146/166	$361^{A}/372^{B}$	0.0009
e/no e	60/249	$316^{A}/317^{A}$	0.891	63/256	$362^{A}/368^{A}$	0.14
f/no f	42/269	$339^{A}/313^{B}$	<0.0001	42/279	$390^{A}/363^{B}$	<0.0001
g/no g	12/309	$336^{A}/316^{A}$	0.06	12/321	$384^{A}/366^{B}$	0.046
h/no h	51/252	$333^{A}/312^{B}$	0.0003	50/265	$382^{A}/362^{B}$	0.0006
i/no i	79/235	$317^{A}/317^{A}$	0.953	81/245	$372^{A}/365^{A}$	0.105
j/no j	153/160	$317^{A}/316^{A}$	0.735	158/165	$368^{A}/366^{A}$	0.579
k/**no k**	100/210	$300^{A}/324^{B}$	<0.0001	103/217	$356^{A}/371^{B}$	<0.0001
l/no l	10/310	$339^{A}/316^{B}$	0.025	10/322	$385^{A}/366^{B}$	0.024

[a] Peach physical map distance 45,021,181-46,751,928 bp (see Figure 2.14 for descriptions of the haplotypes).

[b] Growing Degree Days (GDD) with a base of 4.4 °C

[c] Number of individuals

[d] Means with the same letter within a row are not significantly different ($P>0.05$)

[e] The allelic state significantly associated with the increased trait value is identified in bold

Table 2.6: Phenotypic means for bloom time in 2011 and 2012 for the presence or absence of the G2 G2SSR1566 haplotypes[a] in all sour cherry individuals from the five bi-parental families. Parental genotypes for the G2 haplotypes are: 'M172' (2447), '25-02-29' (2467), 'Montmorency' (4488), '25-14-20' (4489), 'UF' (2449), 'Surefire' (4458), 'RS' (4478) and 'ET' (4678).

	Bloom 2011 (GDD)[b]			Bloom 2012 (GDD)		
	N[c]	Means[d]	*P* value	N	Means	*P* value
G2 haplotypes[e]						
2/**no 2**	142/151	$308^{A}/324^{B}$	<0.0001	150/154	$359^{A}/372^{B}$	<0.0001
4/no 4	273/19	$317^{A}/297^{B}$	0.0009	284/19	$367^{A}/354^{B}$	0.0005
5/no 5	32/261	$340^{A}/313^{B}$	0.0002	32/272	$390^{A}/363^{B}$	<0.0001
6/**no 6**	127/166	$309^{A}/322^{B}$	0.0008	134/166	$360^{A}/371^{B}$	0.001
7/**no 7**	137/156	$307^{A}/324^{B}$	<0.0001	144/160	$359^{A}/372^{B}$	<0.0001
8/no 8	142/151	$328^{A}/305^{B}$	<0.0001	145/159	$375^{A}/358^{B}$	<0.0001
9/no 9	70/223	$325^{A}/313^{B}$	0.014	75/229	$373^{A}/363^{B}$	0.02

[a] Peach physical map location 15,666894-15,667,139 bp (See Figure 2.15 and Table 2.3 for haplotype region and SSR marker information)

[b] Growing Degree Days (GDD) with a base of 4.4 °C

[c] Number of individuals

[d] Means with the same letter within a row are not significantly different ($P>0.05$)

[e] The allelic state significantly associated with the increased trait value is identified in bold

Table 2.7: Phenotypic means for bloom time in 2011 and 2012 for the presence or absence of the G4 haplotypes[a] for all sour cherry individuals from the five bi-parental families. Parental genotypes for the G1 haplotypes are: 'M172' (defh), '25-02-29' (abcd), 'Montmorency' (dilo), '25-14-20' (aghj), 'UF' (ahkn), 'Surefire' (giks), 'RS' (bdgi), and 'ET' (amnp).

	Bloom 2011 (GDD)[b]			Bloom 2012 (GDD)		
	N[c]	Means[d]	*P* value	N	Means	*P* value
G4 haplotypes[e]						
a/no a	150/141	$316^{A}/316^{A}$	0.99	153/145	$366^{A}/367^{A}$	0.62
b/no b	121/169	$313^{A}/319^{A}$	0.13	123/175	$363^{A}/370^{A}$	0.07
c/**no c**	108/186	$306^{A}/324^{B}$	<0.0001	112/189	$355^{A}/375^{B}$	<0.0001
d/**no d**	144/147	$308^{A}/325^{B}$	<0.0001	149/150	$358^{A}/375^{B}$	<0.0001
e/**no e**	56/250	$303^{A}/319^{B}$	<0.0001	57/257	$361^{A}/368^{B}$	0.03
f/**no f**	50/248	$294^{A}/321^{B}$	<0.0001	52/254	$349^{A}/370^{B}$	<0.0001
g/no g	58/235	$329^{A}/313^{B}$	0.006	59/240	$376^{A}/365^{B}$	0.01
h/**no h**	112/182	$312^{A}/320^{B}$	0.04	119/183	$364^{A}/368^{A}$	0.28
i/no i	79/226	$332^{A}/310^{B}$	<0.0001	78/235	$382^{A}/361^{B}$	<0.0001
j/**no j**	30/273	$308^{A}/317^{B}$	0.04	33/278	$356^{A}/368^{B}$	0.0001
k/no k	55/253	$346^{A}/310^{B}$	<0.0001	55/261	$395^{A}/360^{B}$	<0.0001
l/no l	18/296	$311^{A}/316^{A}$	0.43	17/309	$359^{A}/367^{A}$	0.25
m/no m	8/313	$319^{A}/317^{A}$	0.84	8/325	$373^{A}/367^{A}$	0.42
n/no n	47/269	$341^{A}/312^{B}$	<0.0001	47/281	$390^{A}/362^{B}$	<0.0001
s/no s	41/275	$334^{A}/314^{B}$	0.002	41/287	$384^{A}/364^{B}$	0.0008

[a] Region analyzed defined by peach physical map location 7,309,282-9,148,953 bp (see Figure 2.16 for descriptions of the haplotypes)

[b] Growing Degree Days (GDD) with base of 4.4 °C

[c] Number of individuals

[d] Means with the same letter within a row are not significantly different ($P>0.05$)

[e] The allelic state significantly associated with the increased trait value is identified in bold

Table 2.8: Phenotypic means for bloom time in 2011 and 2012 for the presence or absence of the G4 haplotypes[a] within individual bi-parental families. Only those populations and haplotypes that were significant in one or both years are presented.

UF x Surefire	Bloom 2011 (GDD)[b]			Bloom 2012 (GDD)		
(ahkn x giks)	N[c]	Means[d]	*P* value	N	Means	*P* value
LG4 haplotypes[e]						
k/no k	55/18	$347^{A}/308^{B}$	0.0002	55/18	$396^{A}/363^{B}$	0.0001
a/**no a**	35/37	$328^{A}/344^{A}$	0.07	35/37	$379^{A}/394^{B}$	0.05

25-14-20 x 25-02-29	Bloom 2011 (GDD)			Bloom 2012 (GDD)		
(aghj x abcd)	N	Means	*P* value	N	Means	*P* value
G4 Haplotypes						
b/no b	32/21	$317^{A}/302^{B}$	0.03	34/24	$361^{A}/355^{A}$	0.23
c/**no c**	29/21	$305^{A}/317^{A}$	0.14	31/24	$351^{A}/365^{B}$	0.02

M172 x 25-02-29	Bloom 2011 (GDD)			Bloom 2012 (GDD)		
(defh x abcd)	N	Means	*P* value	N	Means	*P* value
G4 Haplotypes						
e/no e	56/48	$303^{A}/289^{B}$	0.0007	57/51	$361^{A}/344^{B}$	<0.0001

Montmorency x 25-02-29	Bloom 2011 (GDD)			Bloom 2012 (GDD)		
(dilo x abcd)	N	Means	*P* value	N	Means	*P* value
G4 Haplotypes						
i/no i	28/20	$329^{A}/314^{A}$	0.13	28/20	$374^{A}/354^{B}$	0.04

RS x ET	Bloom 2011 (GDD)			Bloom 2012 (GDD)		
(bdgi x amnp)	N	Means	*P* value	N	Means	*P* value
G4 Haplotypes						
m/**no m**	8/14	$319^{A}/350^{A}$	0.06	8/14	$373^{A}/397^{B}$	0.05

[a] Region analyzed defined by peach physical map location 7,309,282-9,148,953 bp (See Figure 2.16 for a description of the G4 haplotypes.)

[b] Growing Degree Days (GDD) with a base of 4.4 degrees C

[c] Number of individuals

[d] Means with the same letter within a row are not significantly different (*P*>0.05)

[e] The allelic state significantly associated with the increased trait value is identified in bold

Table 2.9: Phenotypic means for fruit, pit and mesocarp weights (g) in 2011 for the presence or absence of the G2 G2SSR1566 haplotypes[a] for all sour cherry individuals from all five bi-parental families. Parental genotypes for the G2 region are: 'M172' (2447), '25-02-29' (2467), 'Montmorency' (4488), '25-14-20' (4489), 'UF' (2449), 'Surefire' (4458), 'RS' (4478) and 'ET' (4678).

	Fruit Weight 2011			Pit Weight 2011			Mesocarp Weight 2011		
	N[b]	Means[c]	*P* value	N	Means	*P* value	N	Means	*P* value
G2 haplotypes[d]									
2/no 2	128/146	$5.64^{A}/5.30^{A}$	0.07	126/145	$0.34^{A}/0.34^{A}$	0.62	126/145	$5.32^{A}/4.98^{A}$	0.06
4/no 4	249/17	$5.41^{A}/5.52^{A}$	0.74	245/17	$0.34^{A}/0.34^{A}$	0.85	245/17	$5.14^{A}/5.18^{A}$	0.89
5/no 5	30/236	$5.55^{A}/5.55^{A}$	0.89	32/239	$0.32^{A}/0.34^{A}$	0.19	32/239	$5.32^{A}/5.11^{A}$	0.52
6/**no 6**	116/158	$5.24^{A}/5.62^{B}$	0.05	116/155	$0.34^{A}/0.34^{A}$	0.51	116/155	$4.91^{A}/5.31^{B}$	0.03
7/no 7	122/152	$5.45^{A}/5.47^{A}$	0.94	122/149	$0.35^{A}/0.33^{A}$	0.11	122/149	$5.10^{A}/5.17^{A}$	0.73
8/**no 8**	139/135	$5.11^{A}/5.38^{B}$	0.0002	137/134	$0.33^{A}/0.35^{B}$	0.007	137/134	$4.81^{A}/5.47^{B}$	0.0003
9/no 9	70/196	$5.21^{A}/5.50^{A}$	0.20	71/200	$0.32^{A}/0.35^{A}$	0.07	71/200	$4.95^{A}/5.21^{A}$	0.24

[a] Region analyzed defined by peach physical map location 15,666894-15,667,139 bp (See Figure 2.13 and Table 2.3 for haplotype region and SSR marker information) Number of individuals

[b] Number of individuals

[c] Means with the same letter within a row are not significantly different (P>0.05)

[d] The allelic states significantly associated with the increased trait value for at least one trait are identified in bold

Table 2.10: Phenotypic means for fruit, pit and mesocarp weights (g) in 2011 for the presence or absence of the G3 haplotypes[a] for all sour cherry individuals for all five bi-parental families. Parental genotypes for the G3 haplotypes are: 'M172' (degh), '25-02-29' (abcd), 'Montmorency' (acjn), '25-14-20' (adjk), 'UF' (ehjk), 'Surefire' (cjjk), 'RS' (acdj), and 'ET' (dgjl).

	Fruit Weight 2011			Pit Weight 2011			Mesocarp Weight 2011		
	N[b]	Means[c]	*P* value	N	Means	*P* value	N	Means	*P* value
G3 haplotypes[d]									
a/**no a**	116/146	$5.04^{A}/5.72^{B}$	0.0003	115/147	$0.33^{A}/0.34^{A}$	0.27	115/145	$4.72^{A}/5.39^{B}$	0.0002
b/**no b**	84/176	$5.18^{A}/5.59^{B}$	0.04	85/175	$0.32^{A}/0.35^{B}$	0.02	84/174	$4.86^{A}/5.27^{B}$	0.03
c/**no c**	25/258	$4.31^{A}/5.53^{B}$	<0.0001	25/258	$0.29^{A}/0.34^{B}$	0.0004	25/255	$4.03^{A}/5.21^{B}$	<0.0001
d/no d	140/146	$5.43^{A}/5.45^{A}$	0.92	139/126	$0.35^{A}/0.33^{A}$	0.23	139/125	$5.10^{A}/5.14^{A}$	0.84
e/no e	91/175	$6.07^{A}/5.09^{B}$	<0.0001	94/172	$0.35^{A}/0.33^{A}$	0.23	91/173	$5.72^{A}/4.78^{B}$	<0.0001
g/no g	39/240	$5.85^{A}/5.29^{B}$	0.009	39/239	$0.39^{A}/0.33^{B}$	<0.0001	39/238	$5.46^{A}/4.98^{B}$	0.02
h/no h	79/197	$6.12^{A}/5.04^{B}$	<0.0001	79/196	$0.37^{A}/0.32^{B}$	0.0001	79/195	$5.76^{A}/4.74^{B}$	<0.0001
j/no j	135/128	$5.37^{A}/5.49^{A}$	0.54	134/129	$0.33^{A}/0.34^{A}$	0.39	133/128	$5.08^{A}/5.15^{A}$	0.69
k/**no k**	73/190	$5.12^{A}/5.53^{A}$	0.07	72/191	$0.31^{A}/0.35^{B}$	0.001	71/190	$4.87^{A}/5.18^{A}$	0.15
l/no l	9/280	$6.42^{A}/5.36^{A}$	0.28	9/280	$0.38^{A}/0.34^{A}$	0.11	9/277	$6.04^{A}/5.05^{A}$	0.29
n/**no n**	25/258	$4.31^{A}/5.53^{B}$	<0.0001	25/258	$0.29^{A}/0.34^{B}$	0.0004	25/255	$4.03^{A}/5.21^{B}$	<0.0001

[a] Region analyzed defined by peach physical map location 2,738,097-4,755,490 bp (See Figure 2.15 for descriptions of the haplotypes)

[b] Number of individuals

[c] Means with the same letter within a row are not significantly different (P>0.05)

[d] The allelic states significantly associated with the increased trait value for at least one trait are identified in bold

Table 2.11: Phenotypic means for fruit, pit and mesocarp weights (g) in 2011 for the presence or absence of the G5 haplotypes[a] for all sour cherry individuals for all five bi-parental families. Parental genotypes for the G5 haplotypes are: 'M172' (dfhj), '25-02-29' (abcd), 'Montmorency' (aceh), '25-14-20' (bdfh), 'UF' (ddfn), 'Surefire' (ceil), 'RS' (bceh), and 'ET' (djkn).

	Fruit Weight 2011			Pit Weight 2011			Mesocarp Firmness 2011		
	N[b]	Means[c]	*P* value	N	Means	*P* value	N	Means	*P* value
G5 haplotypes[d]									
a/**no a**	80/195	$4.91^{A}/5.60^{B}$	0.0002	80/194	$0.31^{A}/0.35^{B}$	0.0009	80/192	$4.60^{A}/5.28^{B}$	0.0002
b/no b	132/137	$5.37^{A}/5.46^{A}$	0.67	132/137	$0.34^{A}/0.34^{A}$	0.53	131/136	$5.04^{A}/5.13^{A}$	0.61
c/no c	161/114	$5.24^{A}/5.62^{A}$	0.06	159/115	$0.34^{A}/0.34^{A}$	0.59	158/114	$4.93^{A}/5.29^{A}$	0.07
d/no d	193/84	$5.44^{A}/5.30^{A}$	0.47	193/83	$0.34^{A}/0.33^{A}$	0.46	192/82	$5.12^{A}/4.99^{A}$	0.52
e/no e	69/206	$5.37^{A}/5.41^{A}$	0.87	70/204	$0.34^{A}/0.34^{A}$	0.91	68/203	$5.03^{A}/5.10^{A}$	0.79
f/no f	115/160	$5.65^{A}/5.21^{B}$	0.03	113/161	$0.34^{A}/0.34^{A}$	0.85	112/160	$5.36^{A}/4.88^{B}$	0.01
h/no h	103/172	$5.18^{A}/5.53^{A}$	0.07	103/171	$0.34^{A}/0.34^{A}$	0.67	102/170	$4.86^{A}/5.21^{A}$	0.06
i/no i	29/246	$5.79^{A}/5.35^{A}$	0.28	29/245	$0.34^{A}/0.34^{A}$	0.78	28/244	$5.55^{A}/5.02^{A}$	0.18
j/no j	58/217	$5.85^{A}/5.28^{B}$	0.003	58/216	$0.37^{A}/0.33^{B}$	0.0001	57/215	$5.48^{A}/4.97^{B}$	0.006
k/no k	8/267	$6.57^{A}/5.37^{A}$	0.28	8/266	$0.39^{A}/0.34^{A}$	0.12	8/264	$6.17^{A}/5.05^{A}$	0.29
l/no l	25/250	$5.84^{A}/5.36^{A}$	0.18	26/249	$0.33^{A}/0.34^{A}$	0.55	25/247	$5.51^{A}/5.04^{A}$	0.17
n/no n	43/232	$5.87^{A}/5.31^{A}$	0.08	43/232	$0.37^{A}/0.33^{B}$	0.02	43/229	$5.50^{A}/5.00^{A}$	0.10

[a] Region analyzed defined by peach physical map location 16,125,708-18,100,331 bp (See Figure 2.16 for descriptions of the haplotypes)

[b] Number of individuals

[c] Means with the same letter within a row are not significantly different (P>0.05)

[d] The allelic states significantly associated with increased trait values for at least one trait are identified in bold

Table 2.12: Phenotypic means for fruit, pit and mesocarp weight (g) in 2011 for the presence or absence of the G6 G6SSR2208 haplotypes[a] for all sour cherry individuals for all five bi-parental families. Parental genotypes for the G6 haplotypes are: 'M172' (3 5 null null), '25-02-29' (1 3 5 null), 'Montmorency' (1 4 5 null), '25-14-20' (2 3 null null), 'UF' (1 3 3 null), 'Surefire' (1 3 5 null), 'RS' (1 2 5 null), and 'ET' (3 5 null null).

	Fruit Weight 2011			Pit Weight 2011			Mesocarp Weight 2011		
	N[b]	Means[c]	*P* value	N	Means	*P* value	N	Means	*P* value
G6 haplotypes[d]									
1/no 1	82/168	5.21^{A}/5.63^{A}	0.06	81/168	0.33^{A}/0.35^{B}	0.03	81/166	4.91^{A}/5.30^{A}	0.07
2/no 2	40/215	5.09^{A}/5.51^{A}	0.15	39/215	0.33^{A}/0.34^{A}	0.33	39/213	4.81^{A}/5.19^{A}	0.19
3/no 3	188/60	5.66^{A}/4.98^{B}	0.0008	188/59	0.35^{A}/0.31^{B}	0.0004	186/59	5.34^{A}/4.67^{B}	0.0006
4/**no 4**	20/245	4.19^{A}/5.53^{B}	<0.0001	20/244	0.29^{A}/0.34^{B}	0.0001	20/242	3.90^{A}/5.21^{B}	<0.0001
5/no 5	174/75	5.47^{A}/5.55^{A}	0.71	174/74	0.34^{A}/0.35^{A}	0.11	172/74	5.15^{A}/5.23^{A}	0.72

[a] Region analyzed defined by peach physical map location 15,666894-15,667,139 bp (See Figure 2.17 and Table 2.3 for descriptions of the haplotypes and SSR marker information)

[b] Number of individuals

[c] Means with the same letter within a row are not significantly different (P>0.05)

[d] The allelic state significantly associated with the increased trait value is identified in bold

Table 2.13: Phenotypic means for fruit firmness (g/mm^2) in 2011 for the presence or absence of the G2 G2SSR1566 haplotypes[a] for all sour cherry individuals from all five bi-parental families. Parental genotypes for the G2 haplotypes are: 'M172' (2447), '25-02-29' (2467), 'Montmorency' (4488), '25-14-20' (4489), 'UF' (2449), 'Surefire' (4458), 'RS' (4478) and 'ET' (4678).

	Fruit Firmness 2011		
	N[b]	Means[c]	*P* value
G2 haplotypes[d]			
2/no 2	125/143	$141^A/142^A$	0.87
4/no 4	251/17	$141^A/145^A$	0.47
5/**no 5**	31/237	$135^A/142^B$	0.02
6/no 6	114/154	$142^A/141^A$	0.65
7/no 7	120/148	$145^A/139^B$	0.02
8/no 8	134/134	$139^A/144^A$	0.10
9/**no 9**	71/197	$137^A/143^B$	0.02

[a] Region analyzed defined by peach physical map location 15,666894-15,667,139 bp (See Figure 2.13 and Table 2.3 for haplotype region and SSR marker information)

[b] Number of individuals

[c] Means with the same letter within a row are not significantly different ($P>0.05$)

[d] The allelic states significantly associated with the increased trait value are identified in bold

Table 2.14: Phenotypic means for fruit firmness (g/mm^2) in 2011 for the presence or absence of the G3 haplotypes[a] for all sour cherry individuals for all five bi-parental families. Parental genotypes for the G3 haplotypes are: 'M172' (efgh), '25-02-29' (abcd), 'Montmorency' (ijno), '25-14-20' (adjk), 'UF' (ehjk), 'Surefire' (jjop), 'RS' (adjo), and 'ET' (fjlm).

	Fruit Firmness 2011		
	N[b]	Means[c]	*P* value
G3 haplotypes[d]			
a/no a	115/148	$144^A/139^A$	0.08
b/no b	85/176	$144^A/140^A$	0.14
c/no c	150/115	$142^A/142^A$	0.94
d/no d	138/128	$144^A/139^B$	0.03
e/no e	93/174	$141^A/142^A$	0.75
g/no g	38/241	$144^A/141^A$	0.34
h/no h	79/197	$142^A/141^A$	0.83
j/**no j**	136/128	$137^A/146^B$	0.001
k/**no k**	75/189	$137^A/144^B$	0.004
l/no l	8/284	$146^A/141^A$	0.70
n/no n	25/261	$142^A/142^A$	0.97

[a] Region analyzed defined by peach physical map location 2,738,097-4,755,490 bp (See Figure 2.15 for descriptions of the haplotypes)

[b] Number of individuals

[c] Means with the same letter within a row are not significantly different (P>0.05)

[d] The allelic states significantly associated with increased trait values are identified in bold

Table 2.15: Phenotypic means for fruit firmness (g/mm^2) in 2011 for the presence or absence of the G5 haplotypes[a] for all sour cherry individuals for all five bi-parental families. Parental genotypes for the G5 haplotypes are: 'M172' (dfhj), '25-02-29' (abcd), 'Montmorency' (aceh), '25-14-20' (bdfh), 'UF' (ddfn), 'Surefire' (ceil), 'RS' (bceh), and 'ET' (djkn).

	Fruit Firmness 2011		
	N[b]	Means[c]	*P* value
G5 haplotypes[d]			
a/no a	80/197	$142^A/142^A$	0.97
b/no b	132/139	$146^A/139^B$	0.01
c/no c	160/117	$143^A/142^A$	0.80
d/no d	195/84	$141^A/144^A$	0.43
e/**no e**	72/205	$137^A/144^B$	0.01
f/no f	114/163	$141^A/143^A$	0.35
h/no h	103/174	$144^A/141^A$	0.33
i/**no i**	32/245	$134^A/143^B$	0.02
j/no j	58/219	$150^A/140^B$	0.008
k/no k	7/270	$164^A/142^A$	0.16
l/**no l**	26/251	$134^A/143^B$	0.003
n/no n	44/233	$138^A/143^A$	0.16

[a] Region analyzed defined by peach physical map location 16,125,708-18,100,331 bp (See Figure 2.16 for descriptions of the haplotypes)

[b] Number of individuals

[c] Means with the same letter within a row are not significantly different (P>0.05)

[d] The allelic states significantly associated with increased trait values are identified in bold

Table 2.16: Phenotypic means for fruit firmness (g/mm^2) in 2011 for the presence or absence of the G6 G6SSR2208 haplotypes[a] for all sour cherry individuals for all five bi-parental families. Parental genotypes for the G6 haplotypes are: 'M172' (3 5 null null), '25-02-29' (1 3 5 null), 'Montmorency' (1 4 5 null), '25-14-20' (2 3 null null), 'UF' (1 3 3 null), 'Surefire' (1 3 5 null), 'RS' (1 2 5 null), and 'ET' (3 5 null null).

	Fruit Firmness 2011		
	N[b]	Mean[c]	*P* value
G6 haplotypes[d]			
1/**no 1**	82/169	$136^A/143^B$	0.006
2/no 2	39/218	$141^A/141^A$	0.99
3/no 3	189/60	$141^A/138^A$	0.32
4/no 4	20/247	$138^A/142^A$	0.35
5/no 5	176/74	$140^A/142^A$	0.59

[a] Region analyzed defined by peach physical map location 15,666894-15,667,139 bp (See Figure 2.17 and Table 2.3 for descriptions of the haplotypes and SSR marker information)

[b] Number of individuals

[c] Means with the same letter within a row are not significantly different (P>0.05)

[d] The allelic state significantly associated with the increased trait value is identified in bold

Table 2.17: Phenotypic means for flesh color[a] in 2011 for the presence or absence of the G3 haplotypes[b] for all sour cherry individuals for all five bi-parental families. Parental genotypes for the G3 haplotypes are: 'M172' (clnu), '25-02-29' (nnop), 'Montmorency' (fhno), '25-14-20' (bdhn), 'UF' (bcdn), 'Surefire' (efgh), 'RS' (khnp), and 'ET' (eglu).

	Flesh color 2011		
	N[c]	Mean[d]	*P* value
G3 Flesh color haplotypes[e]			
b/no b	49/236	$3.63^{A}/3.13^{B}$	0.04
c/**no c**	84/206	$2.71^{A}/3.43^{B}$	0.0002
d/no d	53/217	$4.65^{A}/2.94^{B}$	<0.0001
e/no e	36/233	$4.06^{A}/3.15^{B}$	<0.0001
f/**no f**	65/219	$2.87^{A}/3.36^{B}$	0.02
g/no g	40/250	$3.13^{A}/3.24^{A}$	0.66
h/no h	84/197	$3.06^{A}/3.32^{A}$	0.17
k/no k	14/272	$4.14^{A}/3.20^{B}$	0.01
l/no l	48/220	$3.96^{A}/3.13^{B}$	<0.0001
n/**no n**	235/34	$3.20^{A}/3.79^{B}$	0.03
o/**no o**	98/177	$2.41^{A}/3.75^{B}$	<0.0001
p/no p	97/172	$4.19^{A}/2.76^{B}$	<0.0001
u/no u	107/256	$3.21^{A}/3.30^{A}$	0.59

[a] Washington State University color card rating (See Figure 2.2)

[b] Region analyzed defined by peach physical map location 10,675,150-13,406,263 bp (See Figure 2.20 for the descriptions of the haplotypes)

[c] Number of individuals

[d] Means with the same letter within a row are not significantly different ($P>0.05$)

[e] The allelic states significantly associated with increased trait values are identified in bold

Table 2.18: Phenotypic means for flesh color[a] in 2011 for the presence or absence of the G3 haplotypes[b] for all sour cherry individuals within individual families. Only haplotypes with significant differences are presented.

	Flesh color 2011		
G3 haplotypes[c]	N[d]	Means[e]	*P* value
UF x Surefire (bcdn x efgh)			
d/no d	27/42	$4.63^{A}/2.43^{B}$	<0.0001
e/no e	26/43	$3.92^{A}/2.91^{B}$	0.002
c/**no c**	37/32	$2.62^{A}/4.06^{B}$	<0.0001
25-14-20 x 25-02-29 (bdhn x nnop)			
d/no d	25/33	$4.68^{A}/2.97^{B}$	<0.0001
p/no p	29/29	$4.52^{A}/2.90^{B}$	<0.0001
o/**no o**	27/31	$2.96^{A}/4.35^{B}$	0.0003
M172 x 25-02-29 (clnu x nnop)			
p/no p	37/41	$3.92^{A}/2.20^{B}$	<0.0001
l/no l	35/42	$3.71^{A}/2.48^{B}$	<0.0001
o/**no o**	39/39	$2.13^{A}/3.90^{B}$	<0.0001
Montmorency x 25-02-29 (fhno x nnop)			
p/ no p	20/22	$3.90^{A}/1.63^{B}$	<0.0001
o/**no o**	33/8	$2.33^{A}/4.13^{B}$	0.002
RS x ET (hknp x eglu)			
p/ no p	13/11	$4.62^{A}/3.36^{B}$	0.03
l/no l	13/11	$4.62^{A}/3.36^{B}$	0.02

[a] Washington State University color card rating (See Figure 2.2)

[b] Region analyzed defined by peach physical map location 10,675,150-13,406,263 bp (See Figure 2.20 for the descriptions of the haplotypes)

[c] The allelic states significantly associated with increased trait values are identified in bold

[d] Number of individuals

[e] Means with the same letter within a row are not significantly different (P>0.05)

Table 2.19: Phenotypic means for malic acid (mg/ml) in 2011 for the presence or absence of the condensed G5 haplotypes[a] for all sour cherry individuals for all five bi-parental families. Parental genotypes for the G5 haplotypes are: 'M172' (1236), '25-02-29' (2346), 'Montmorency' (2345), '25-14-20' (1236), 'UF' (1236), 'Surefire' (1123), 'RS' (1234) and 'ET' (1146).

	Malic Acid (mg/ml) 2011		
	N[b]	Means[c]	*P* value
G5 haplotypes[d]			
1/no 1	99/68	$1.55^{A}/1.29^{B}$	0.001
Two 1's/one 1	23/76	$1.84^{A}/1.46^{B}$	0.0004
2/no 2	125/42	$1.48^{A}/1.34^{A}$	0.13
Two 2's/one 2	34/91	$1.41^{A}/1.50^{A}$	0.36
3/no 3	126/41	$1.43^{A}/1.49^{A}$	0.54
Two 3's/one 3	28/98	$1.30^{A}/1.47^{A}$	0.16
4/**no 4**	78/89	$1.29^{A}/1.58^{B}$	0.0003
Two 4's/one 4	10/68	$1.31^{A}/1.29^{A}$	0.88
5/**no 5**	13/154	$1.06^{A}/1.48^{B}$	0.0003
6/no 6	110/57	$1.47^{A}/1.39^{A}$	0.34
Two 6's/one 6	17/93	$1.37^{A}/1.49^{A}$	0.31

[a] Region analyzed defined by peach physical map location 689,941-1,463,960 bp (See Figure 2.21 for descriptions of the haplotypes)

[b] Number of individuals

[c] Means with the same letter within a row are not significantly different ($P>0.05$)

[d] The allelic states significantly associated with increased trait values are identified in bold

Figure 2.1: Pedigrees of the five bi-parental families used in this study. Populations are colored white with the progeny number below. Grandparents are colored grey, while parents are colored black. If individuals are both parents and grandparents, they are colored black.

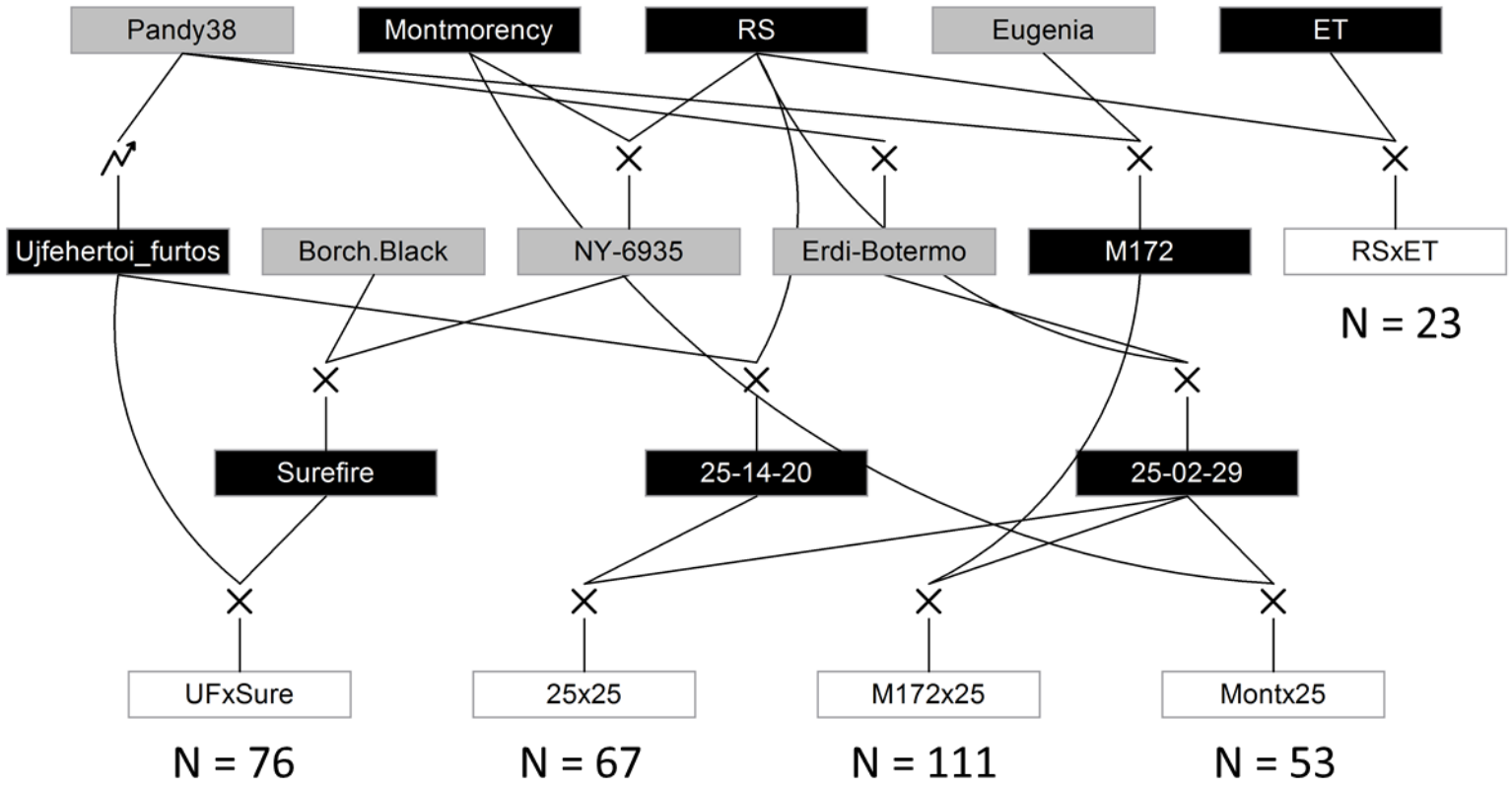


Figure 2.2: Washington State University flesh color card rating scale used to determine flesh color rating for sour cherry individuals.

Figure 2.3: Genome Studio (Illumina Inc. 2011) SNP dosage calls were done for each marker. Sweet cherry (yellow) individuals were included to help define the two homozygous (AAAA and BBBB) classes and the balanced heterozygous class (AABB). Determining dosage was necessary to build haplotypes.

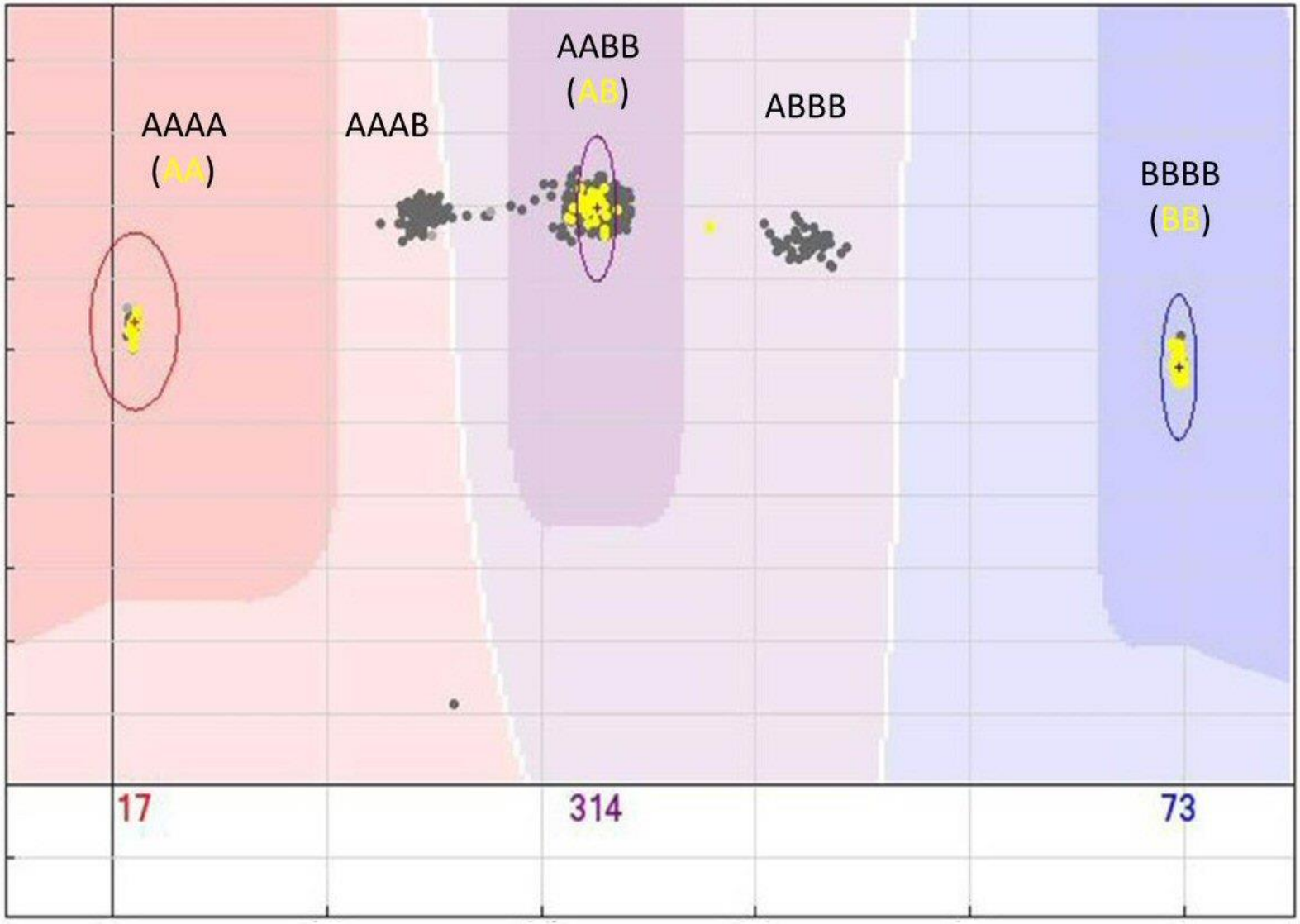


Figure 2.4: Reconstruction of a ~1.2 Mb region spanning the self-incompatibility ***S*-**locus and its inheritance in (a) Sweet cherry, with four parental haplotypes (1–4) and (b) Sour cherry, with eight parental haplotypes (1–8). Identical haplotypes have the same background colors. Haplotypes are shown for five sweet cherry and two sour cherry seedlings. Monomorphic SNPs within cross-over regions are highlighted in grey. Genotypes indicated as "u" are for an unresolved polymorphic SNP in sour cherry (Peace et al. 2013, Figure 4).

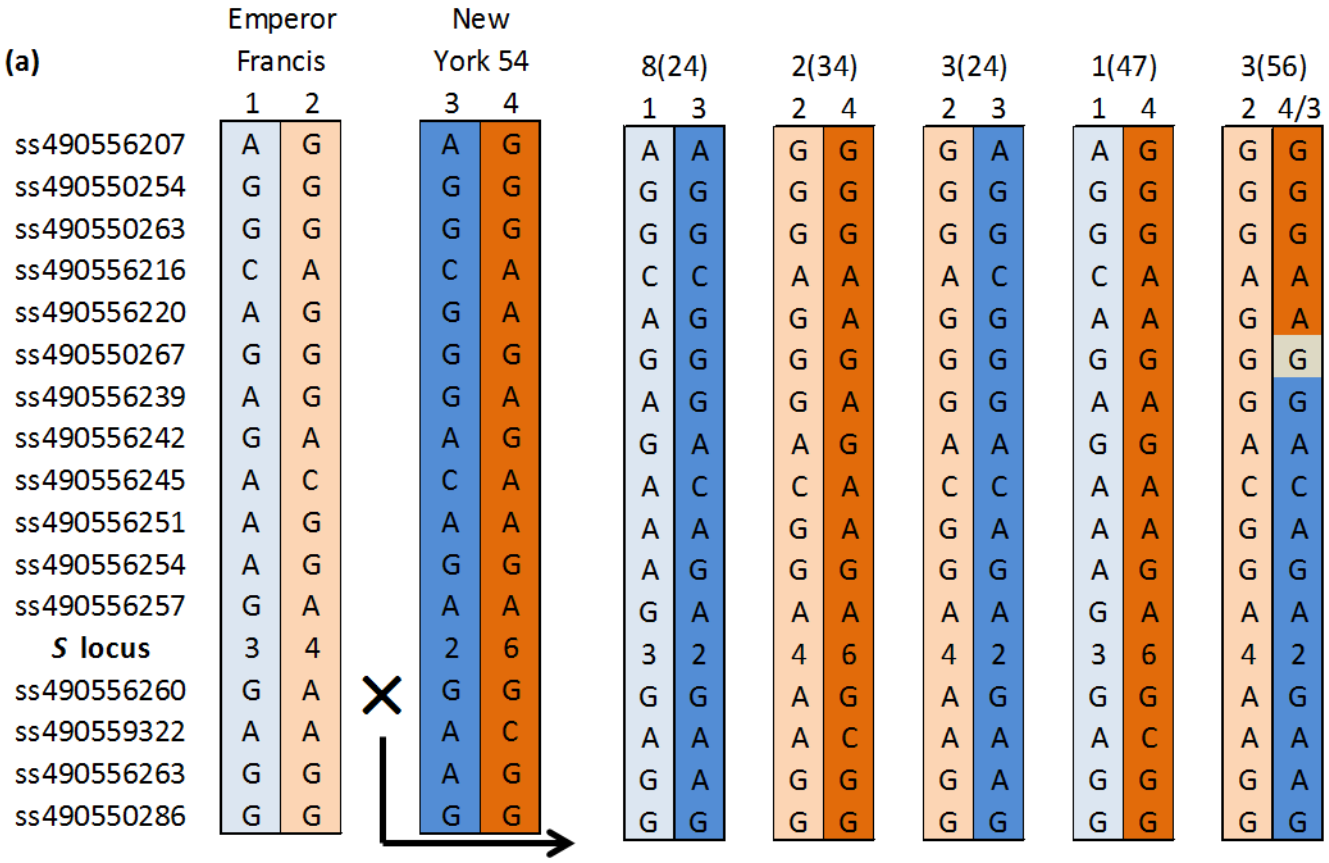


Figure 2.4 (cont'd)

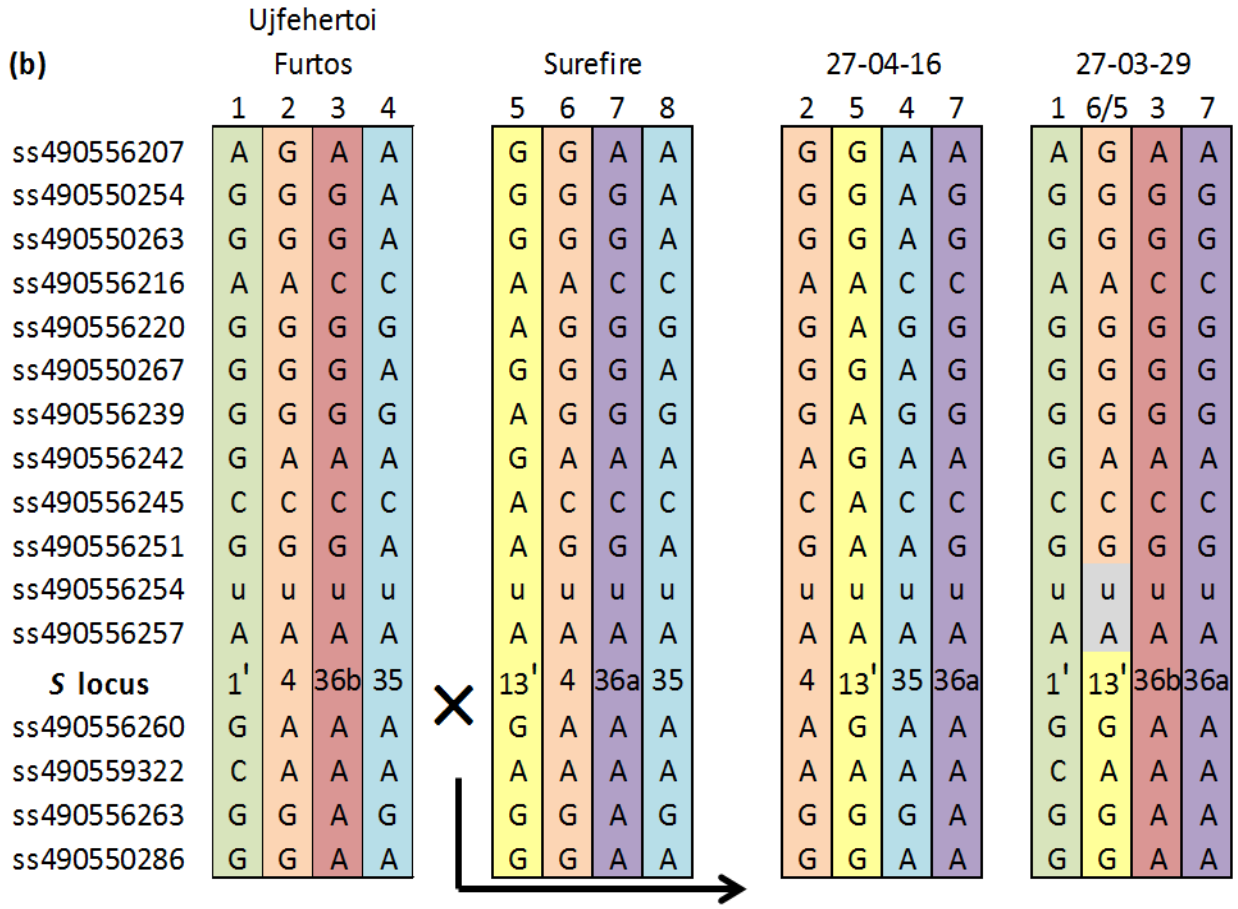


Figure 2.5: Phenotypic distributions for bloom growing degree days (GDD) in 2011 for all populations and each of the five bi-parental populations. Parental values are shown in individual populations when data is available.

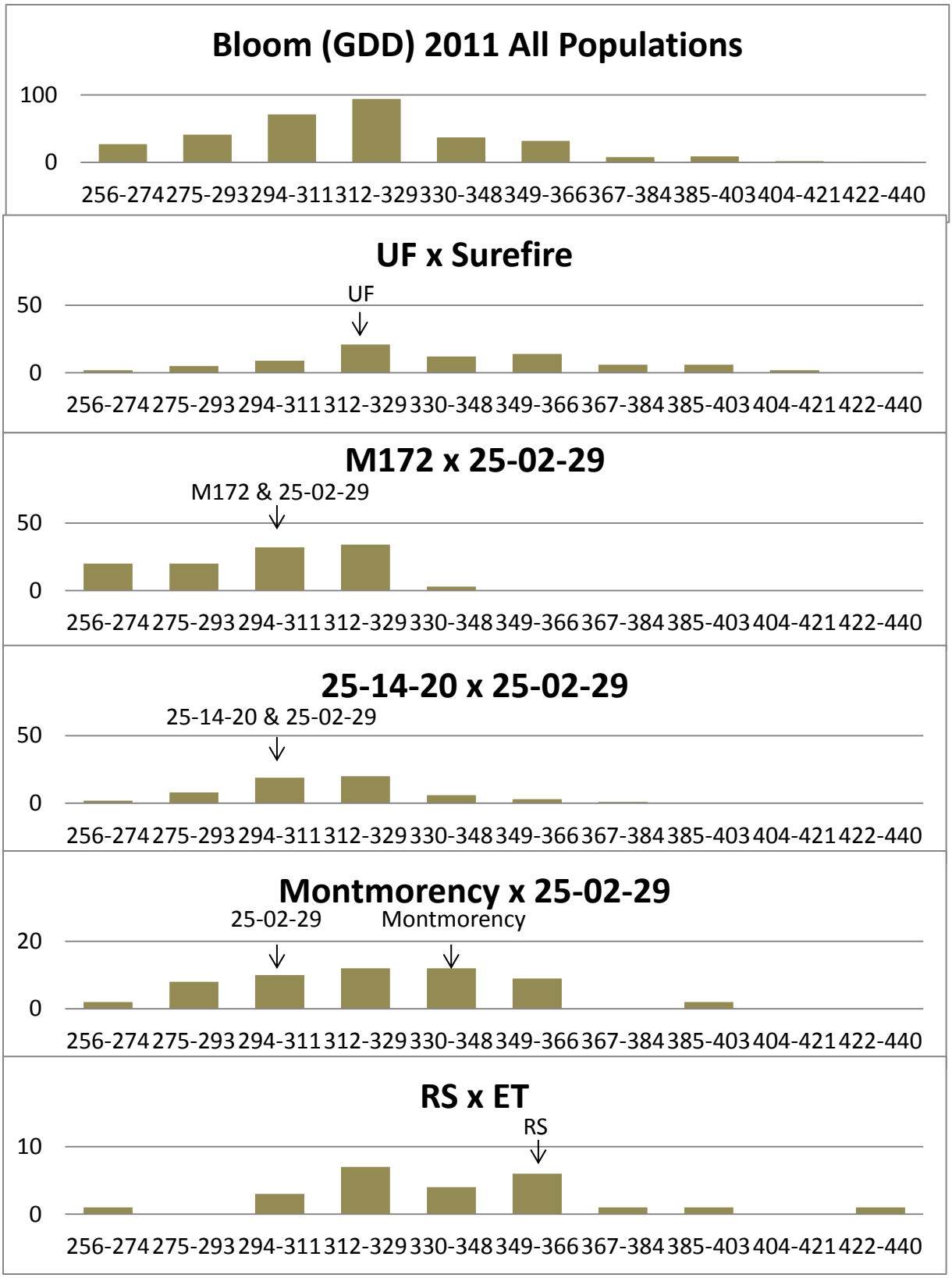


Figure 2.6: Phenotypic distributions for bloom growing degree days (GDD) in 2012 for all populations and each of the five bi-parental populations. Parental values are shown in individual populations when data is available.

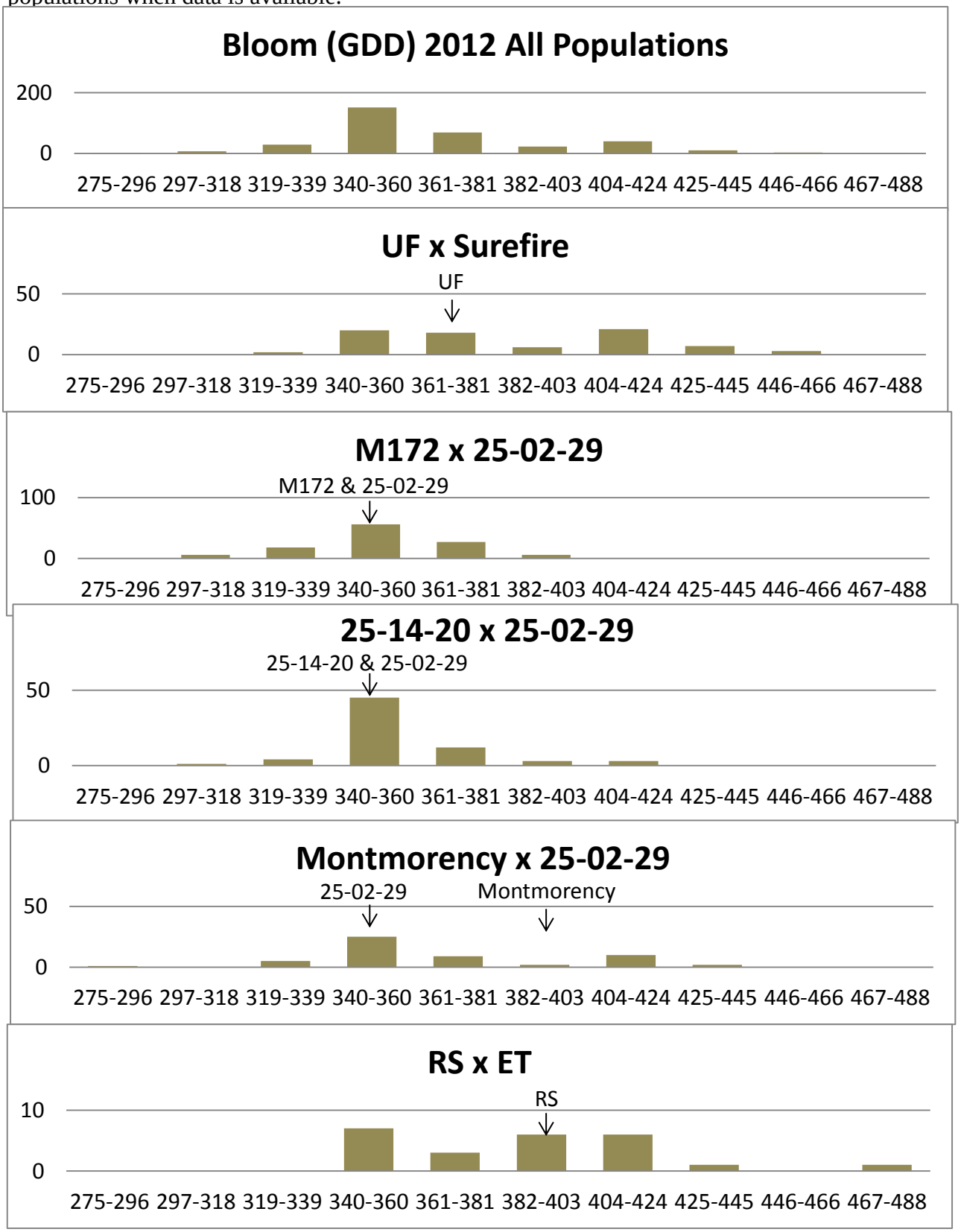


Figure 2.7: Phenotypic distributions for fruit weight (g) in 2011 for all populations and each of the five bi-parental populations. Parental values are shown in individual populations.

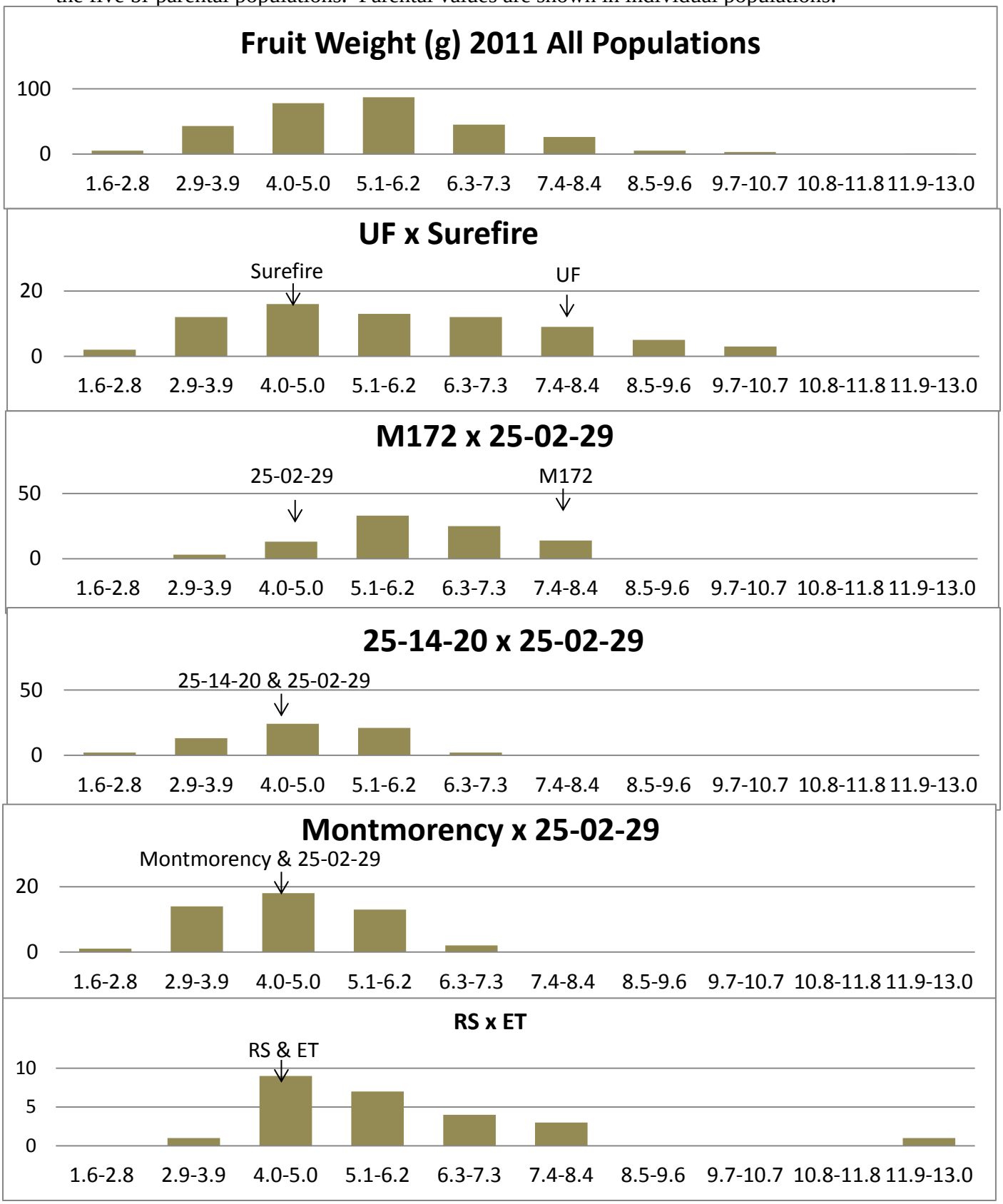


Figure 2.8: Phenotypic distributions for pit weight (g) in 2011 for all populations and each of the five bi-parental populations. Parental values are shown in individual populations.

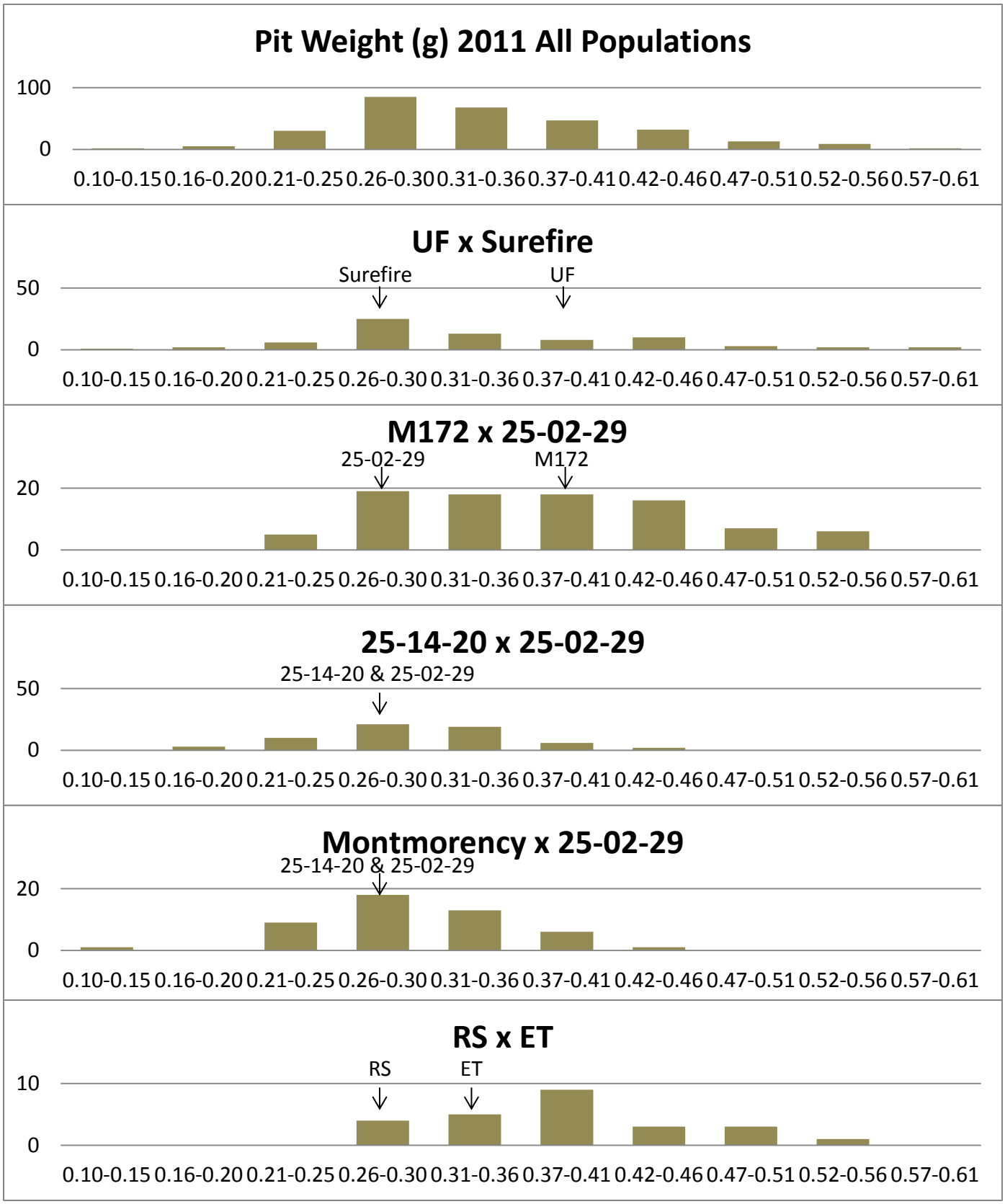


Figure 2.9: Phenotypic distributions for mesocarp weight (g) in 2011 for all populations and each of the five bi-parental populations. Parental values are shown in individual populations.

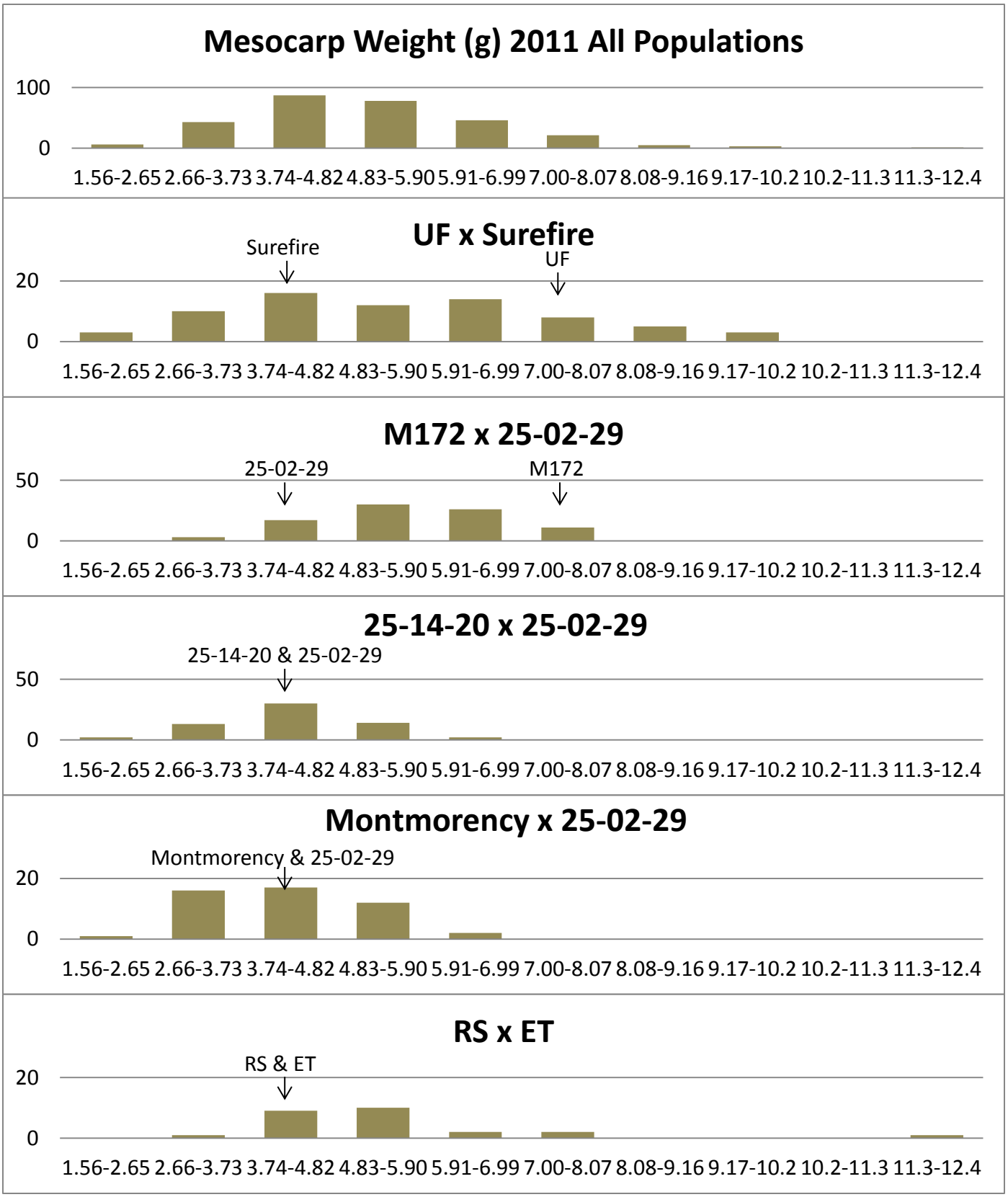


Figure 2.10: Phenotypic distributions for fruit firmness (g/mm^2) in 2011 for all populations and each of the five bi-parental populations. Parental values are shown in individual populations when data is available.

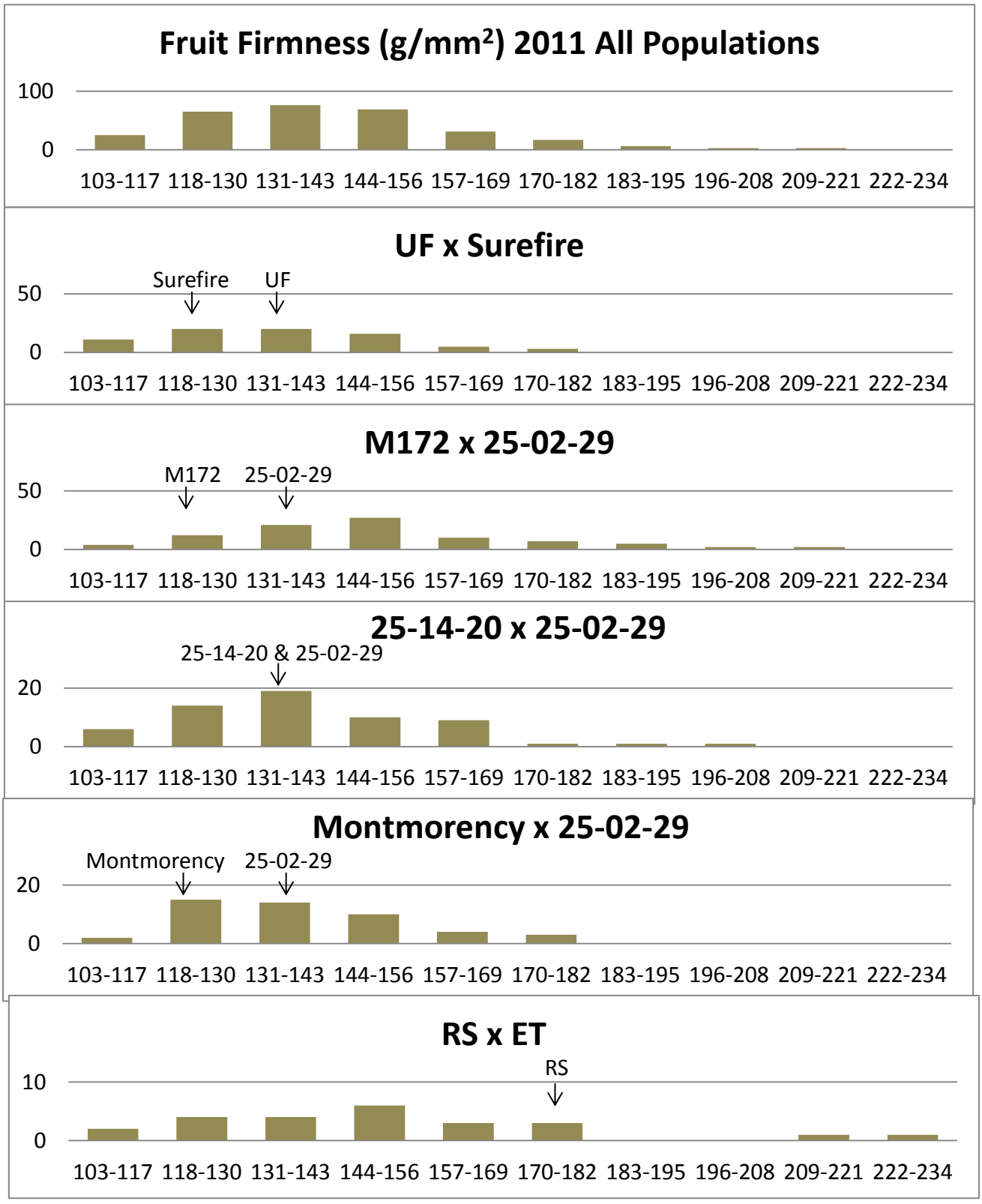


Figure 2.11: Phenotypic distributions for flesh color based on Washington State University's flesh color rating in 2011 for all populations and each of the five bi-parental populations. Parental values are shown in individual populations when data is available.

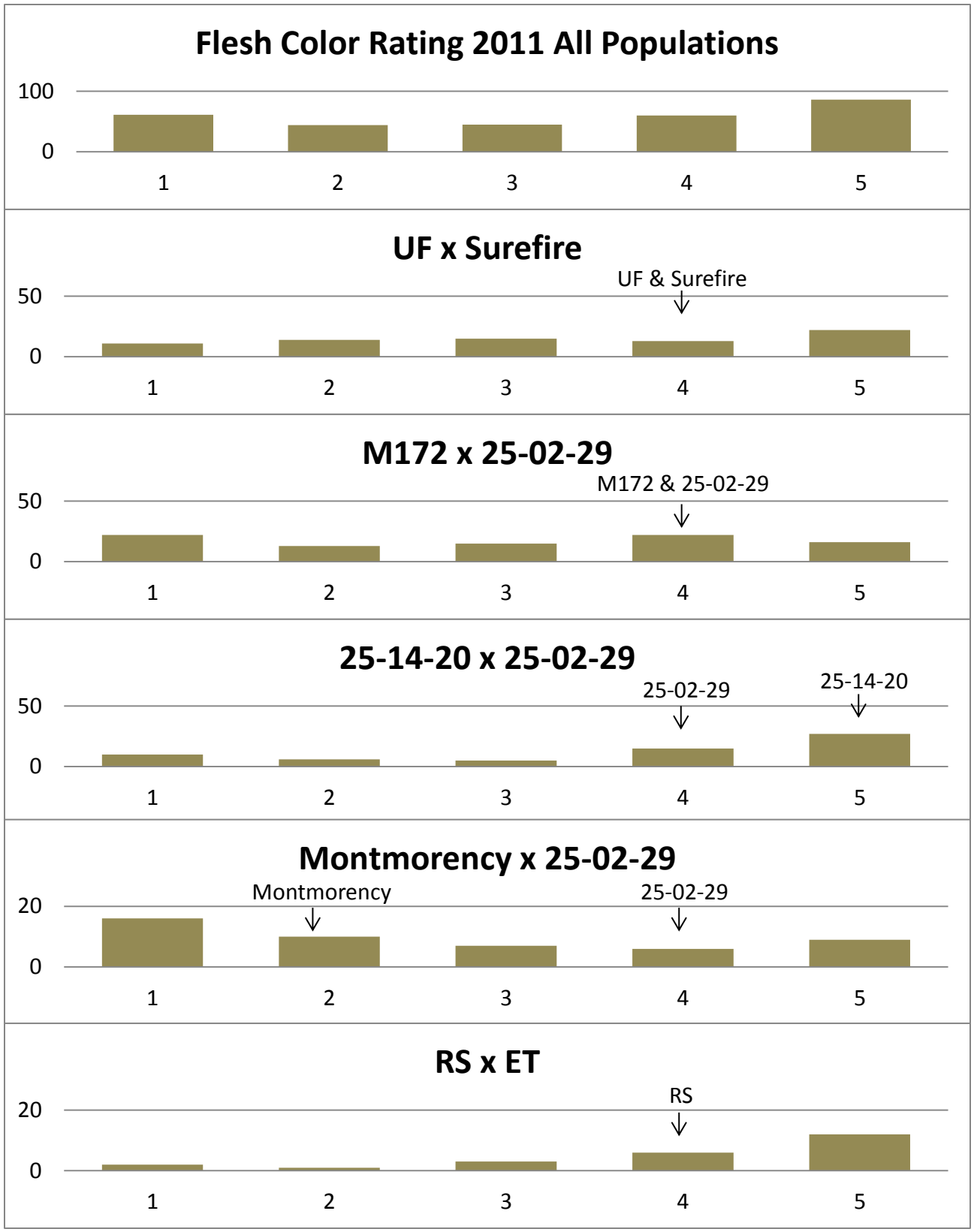


Figure 2.12: Phenotypic distributions for malic acid content (mg/ml) in 2011 for all populations and each of the five bi-parental populations. Parental values are shown in individual populations when data is available.

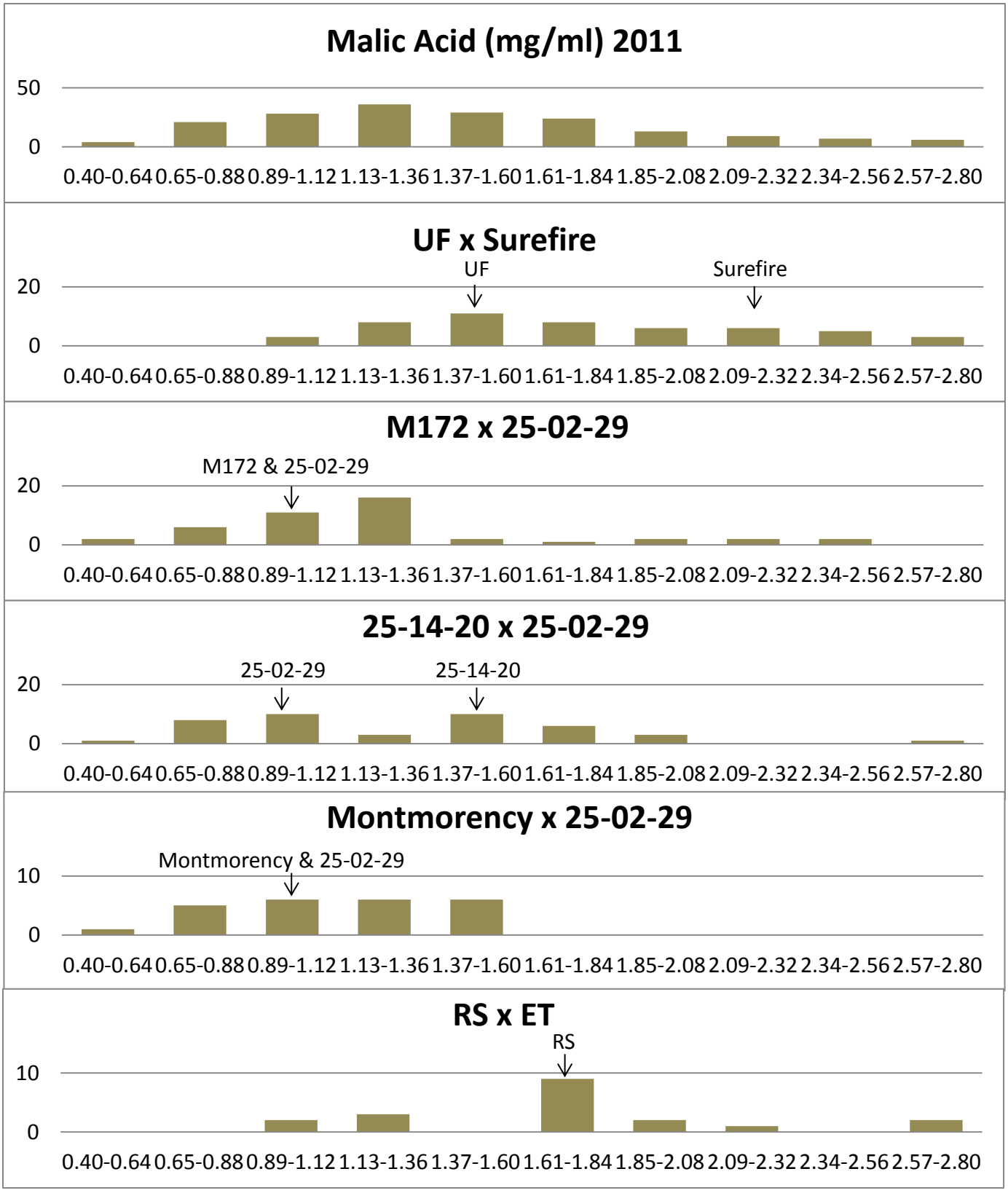


Figure 2.13: Bloom and fruit trait QTLs from diploid *Prunus* (peach and sweet cherry) that were targets of validation in tetraploid sour cherry.

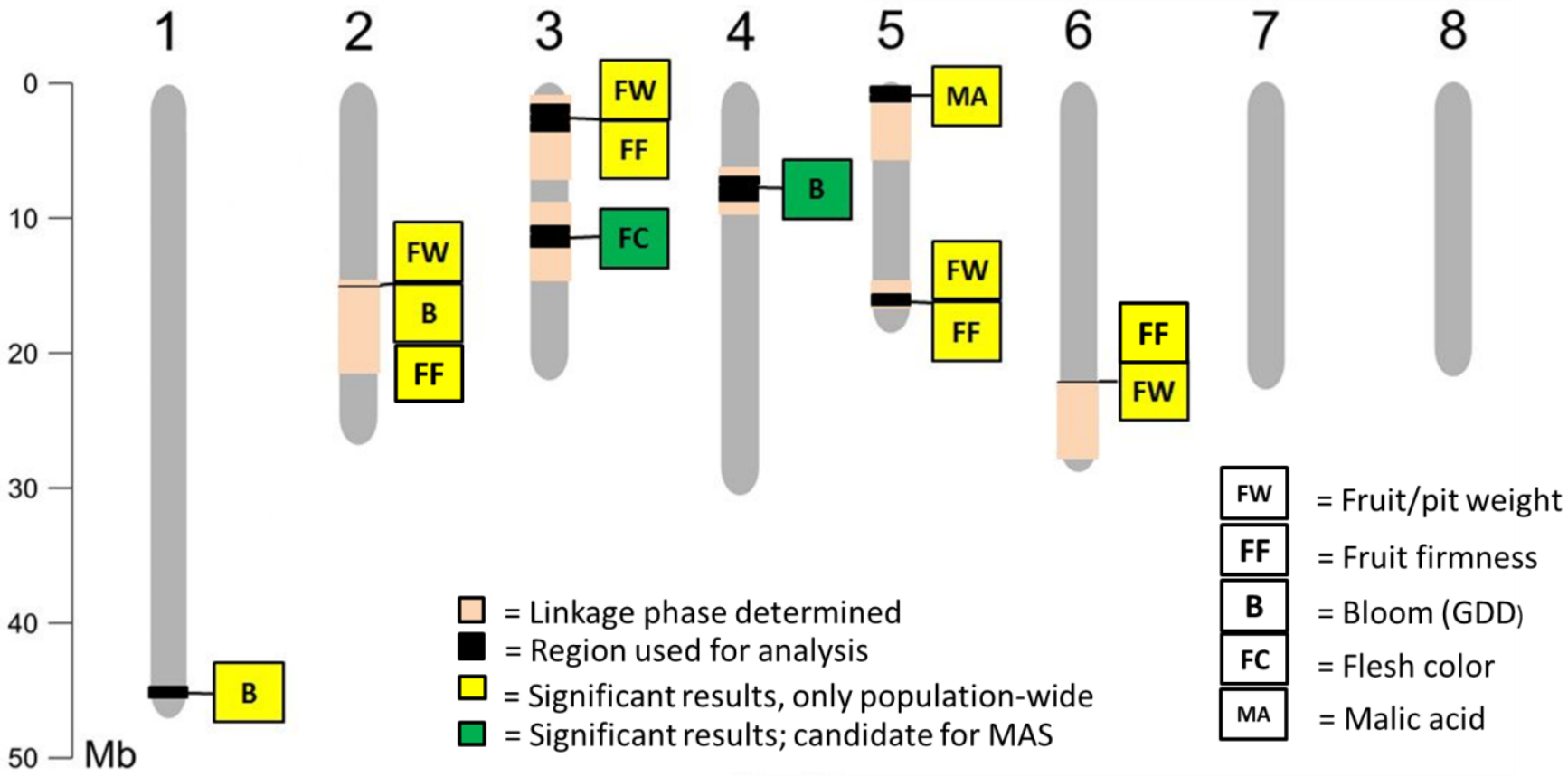


Figure 2.14: The twelve haplotypes identified in sour cherry for the G1 region used to test for the bloom time QTL.

LG1 NCBI SS#	Peach physical map position[a]	a	b	c	d	e	f	g	h	i	j	k	l
ss490548534	45021181	A	B	B	B	A	A	B	B	A	B	B	B
ss490548538	45028492	A	B	B	B	A	A	A	B	A	B	B	B
ss490558944	45077993	A	A	A	B	A	A	B	A	A	B	A	A
ss490548551	45163766	B	B	A	A	B	B	A	A	B	B	A	B
ss490546931	45169388	B	B	B	B	A	A	B	B	B	B	B	B
ss490548555	45210413	B	B	B	B	B	B	B	B	B	A	B	B
ss490559090	45226245	A	A	A	A	A	A	A	B	A	A	B	A
ss490546935	45299782	B	B	B	B	A	A	B	B	B	B	A	B
ss490548559	45322930	A	B	A	A	A	A	B	B	A	B	A	B
ss490548567	45402154	B	B	B	B	B	B	B	A	B	B	B	B
ss490546939	45418879	B	A	A	A	B	B	A	A	B	A	A	A
ss490559081	45469715	A	B	A	A	A	A	A	B	A	A	A	A
ss490548571	45473214	B	B	A	A	A	A	A	B	B	A	A	A
ss490548575	45535084	B	A	B	B	B	A	A	A	B	A	A	A
ss490548589	45633267	B	B	B	A	B	B	A	A	B	A	B	A
ss490548593	45680542	B	A	A	B	B	B	A	A	B	A	A	A
ss490559189	45682217	A	B	B	A	A	A	B	B	A	B	B	B
ss490546951	45748141	B	A	A	A	B	B	A	A	B	A	A	A
ss490548610	45823056	B	B	A	A	B	B	A	A	B	A	A	A
ss490548614	45924398	A	A	B	B	A	A	A	B	A	A	A	A
ss490546967	46207321	B	B	B	B	A	A	B	B	B	B	B	B
ss490548639	46237075	B	A	A	A	B	B	A	A	B	A	A	A
ss490548643	46277304	A	B	B	B	A	A	B	B	A	B	B	B
ss490548655	46402818	B	A	B	B	B	B	B	A	B	A	A	B
ss490546979	46512070	B	B	B	B	A	A	B	B	A	B	B	B
ss490548667	46530908	B	A	B	A	B	B	A	B	B	A	B	A
ss490548680	46635504	B	A	A	A	B	B	A	A	B	A	A	A
ss490548692	46751928	B	A	A	A	B	B	A	A	B	A	A	A

[a] distances according to the Peach v1.0 'dhLovell' genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome) (Verde et al. 2013)

Figure 2.15: The 21 haplotypes identified in sour cherry for the G2 region used to test for bloom time and fruit size/fruit firmness QTLs. Twenty-one unique haplotypes were found, so SSR marker G2SSR1566 was used to "condense" haplotypes based on marker score to 7 haplotypes.

LG2 Marker/ NCBI SS#	Peach physical map distance (bp)[a]	d	b	l	e	h	o	k	r	s	m	a	q	c	g	p	j	n	t	u	f	i
ss490549138	14926622	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A
CPSCT038	15057199	204	185	185	190	190	190	192	-	-	null	185	192	192	192	190	192	192	-	-	190	null
ss490549172	15084429	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	A
ss490549184	15127760	A	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A
ss490549187	15129278	B	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B
ss490549192	15162260	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
ss490549196	15172649	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
ss490549216	15337787	B	A	A	A	A	B	B	A	B	B	A	A	A	A	B	B	B	B	B	B	B
ss490549227	15372418	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B
ss490549238	15492297	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	B	B
ss490549254	15598480	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
(CNR12)[b]	15647989																					
ss490549270	15658996	B	A	A	B	B	B	B	A	B	B	A	A	B	B	B	B	B	B	B	B	B
G2SSR1566	15667139	2	4	4	4	4	4	4	4	4	5	6	7	7	7	8	8	8	8	8	9	9
ss490549287	15747822	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	A	B	B	B	A
ss490549295	15778222	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A
ss490549311	15846482	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
ss490547191	15863936	B	A	A	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
ss490549319	15873315	B	A	A	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
ss490549323	15873418	B	A	A	B	A	A	A	A	A	A	A	A	B	B	B	A	B	A	A	B	B
ss490549331	15894385	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	A	B	A	A	B	A
ss490549335	15894441	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A
ss490547200	16005866	A	B	B	A	A	A	A	B	A	A	B	A	A	A	A	A	A	A	A	A	A
ss490547204	16111179	B	A	A	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B

Figure 2.15 (cont'd)

		d	b	l	e	h	o	k	r	s	m	a	q	c	g	p	j	n	t	u	f	i
ss490549379	16118423	B	A	A	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	A	B
ss490549383	16142700	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A
ss490549411	16229065	B	A	A	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
BPPCT034	16491740	235	218	241	228	206	206	206	-	-	206	210	235	237	237	225	237	237	-	-	255	228
ss490547212	16519837	A	A	A	A	B	B	B	A	B	B	A	A	A	A	A	A	A	A	A	A	A
ss490549435	16530061	B	B	A	B	B	B	B	A	B	B	A	A	B	B	B	B	B	B	B	B	B
ss490549443	16550340	B	B	B	B	B	B	B	B	B	B	B	B	A	A	B	A	A	A	A	A	B
ss490549447	16581454	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A
ss490549451	16583654	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A
ss490549455	16585549	A	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	B
ss490549474	16644104	A	A	A	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
ss490549482	16657611	A	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	B
ss490549494	16671049	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A
ss490549498	16689292	B	B	A	B	B	B	B	A	B	B	A	A	B	B	B	B	B	B	B	B	B
ss490549506	16732373	A	A	B	A	A	A	A	B	A	A	B	B	A	A	A	A	A	A	A	A	A
ss490549514	16738223	B	B	B	B	A	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B
ss490549525	16779535	B	B	A	B	B	B	B	A	B	B	A	A	B	B	B	B	B	B	B	B	B
ss490549529	16801115	B	A	A	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
ss490549537	16827404	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A
ss490547231	16840994	B	B	A	B	B	B	B	A	B	B	A	A	B	B	B	B	B	B	B	B	B
ss490549549	16842231	B	A	A	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
ss490549561	16874016	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A
ss490549565	16875339	A	B	B	A	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	A	A
ss490549569	16879478	B	A	A	B	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	B	B
ss490549573	16885395	B	A	A	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
ss490549590	16918304	B	A	A	B	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
ss490547235	16926980	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A
ss490549615	17026524	B	B	A	B	B	B	B	A	B	B	A	A	B	B	B	B	B	B	B	B	B

Figure 2.15 (cont'd)

		d	b	l	e	h	o	k	r	s	m	a	q	c	g	p	j	n	t	u	f	i
ss490549619	17039549	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A
ss490549623	17047675	A	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	B
ss490547239	17207142	A	A	B	A	A	A	A	B	A	A	B	B	A	A	A	A	A	A	A	A	A
ss490549642	17207160	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A
ss490549646	17244146	A	B	B	A	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	A
ss490547242	17257558	A	B	A	A	B	B	B	A	B	B	A	A	A	A	A	A	A	A	A	A	A
ss490549670	17425495	A	A	B	A	B	A	A	B	A	B	B	B	B	B	A	A	B	A	A	A	A
ss490549674	17426363	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A
ss490549677	17469838	A	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	B	A	A	A	B
ss490549681	17470794	B	B	A	B	A	B	B	A	B	A	A	A	B	B	B	B	B	B	B	B	B
ss490547246	17473563	A	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	A	A	B
ss490549685	17476462	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A
ss490549709	17560969	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B	A	B	A	A	A	A
ss490549720	17571610	A	B	A	A	A	B	B	A	B	B	A	A	A	A	A	A	A	A	A	A	A
ss490549724	17574355	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A
ss490549756	17731307	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B
ss490549760	17736916	B	A	A	B	A	A	A	A	A	A	A	A	B	B	A	A	B	A	A	B	B
ss490549809	17931590	B	B	A	B	B	B	B	A	B	B	A	A	B	B	B	B	B	B	B	B	B
ss490549837	18149310	B	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B
ss490549849	18371629	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A
ss490547270	18433815	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	B	A	B	B	A	A
ss490549857	18522250	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B
ss490549861	18588411	B	A	A	B	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	B	B
ss490558996	18681412	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	B	A
ss490558999	18681519	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A
ss490549869	18683588	A	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	B	A	A	A	B
ss490549873	18702609	A	B	A	B	B	B	B	A	B	B	A	A	A	B	A	B	A	B	B	A	B
ss490547281	18708318	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B

Figure 2.15 (cont'd)

		d	b	l	e	h	o	k	r	s	m	a	q	c	g	p	j	n	t	u	f	i
ss490547285	18753870	A	A	B	A	A	A	A	B	A	A	B	B	A	A	A	A	A	A	A	A	A
ss490549881	18755669	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A
ss490549889	18871385	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B
ss490549897	18897454	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B
ss490547297	18963376	B	A	B	B	A	A	A	B	A	A	B	B	B	A	B	B	B	B	B	B	B
ss490549916	18988718	B	A	A	B	A	A	A	A	A	A	A	A	A	A	B	B	A	A	B	A	B
ss490549920	18997401	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A
ss490549924	19027747	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A
ss490549932	19061737	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	B	A	A	A
ss490547304	19138888	A	A	B	A	A	A	A	B	A	A	B	B	A	A	A	A	A	A	A	A	A
ss490549959	19308510	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A
ss490549971	19364459	A	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	A	B
ss490549975	19384216	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	B	A	B	A
ss490549979	19422560	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A
ss490549987	19477390	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	A	A
ss490547316	19597895	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B
ss490547320	19629831	B	A	A	B	A	A	A	A	A	A	A	A	A	A	B	A	A	A	A	B	B
ss490550011	19643557	A	B	B	B	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	B
ss490550015	19664779	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A
ss490550019	19684679	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	B	A	A	A
ss490550027	19724366	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	B	A
ss490550051	19869360	A	B	A	A	B	B	B	A	B	B	A	A	A	B	A	A	A	A	A	A	A
ss490547331	20041486	A	A	B	A	A	A	A	B	A	A	B	B	A	A	A	A	A	A	A	A	A
ss490550063	20170303	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	A	A
ss490550074	20340464	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A
ss490550082	20371321	B	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	B	B
ss490550097	20458193	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B
ss490547351	20463267	A	A	B	A	A	A	A	B	A	A	B	B	A	A	A	A	A	A	A	A	A

Figure 2.15 (cont'd)

		d	b	l	e	h	o	k	r	s	m	a	q	c	g	p	j	n	t	u	f	i
ss490547354	20470247	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	A
ss490559384	20616108	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B
ss490550125	20694575	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A
ss490550133	20724836	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B
ss490550140	20758268	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	A	A	A	B
ss490550148	20799111	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A
ss490547382	20808955	B	A	B	B	A	A	A	B	A	A	B	B	B	A	B	B	B	B	B	B	B
ss490550156	20834183	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A
ss490550173	20925443	B	B	B	B	B	B	B	B	B	B	B	B	A	B	A	B	A	B	B	B	B
ss490547401	21131678	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B
ss490550213	21264736	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A
ss490547405	21290638	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B
ss490547413	21399347	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B
ss490547416	21611064	A	B	B	A	B	B	B	B	B	B	B	B	A	B	A	A	A	A	A	A	A
ss490547420	21616635	A	A	B	A	A	A	A	B	A	A	B	B	A	A	A	A	A	A	A	A	A
ss490550244	21670225	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	A	B	B	A	A	B
ss490547428	21905412	B	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B
ss490547432	21952248	A	A	B	A	A	A	A	B	A	A	B	B	A	A	A	A	A	A	A	A	A
ss490550273	21965305	B	B	B	B	B	B	B	B	B	B	B	B	A	B	A	B	A	A	B	B	B
ss490547439	22081401	B	B	A	B	B	B	B	A	B	B	A	A	B	B	B	B	B	B	B	B	B

[a] distances according to the Peach v1.0 'dhLovell' genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome)

[b] CNR16 location reported in De Franceschi et al. 2013 (Verde et al. 2013)

Figure 2.16: The 17 haplotypes identified in sour cherry for the G4 region used to test for the bloom time QTL. Haplotype designations q-r were not used.

LG4 NCBI SS#	Peach physical map distance (bp)[a]	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	s
ss490548584	7010787	A	B	B	B	A	B	A	B	A	B	A	B	A	B	A	B	B
ss490552724	7040511	B	B	B	B	B	B	A	B	A	B	A	B	A	B	B	B	B
ss490552727	7065857	B	A	B	B	B	A	B	B	B	B	B	B	B	B	B	A	A
ss490548587	7070007	A	B	A	A	A	B	A	A	A	A	A	A	A	A	A	B	B
ss490548591	7147868	B	B	B	B	B	B	A	B	B	B	B	B	A	B	B	B	B
ss490552741	7309282	A	B	A	B	A	B	A	B	A	B	A	B	A	A	A	B	B
ss490548603	7398453	B	A	A	B	B	A	B	B	B	B	B	A	B	A	B	B	A
ss490552750	7429956	A	A	B	B	A	B	A	B	A	B	A	A	A	B	A	B	A
ss490548607	7434938	A	B	B	B	A	B	A	B	B	B	B	B	A	B	A	B	B
ss490552764	7666480	A	B	B	B	B	B	A	B	B	B	B	B	A	B	A	B	B
ss490552767	7706352	B	A	A	A	B	B	B	B	B	A	B	A	B	B	B	B	A
ss490552770	7731253	A	B	B	B	A	B	A	B	A	B	A	B	A	A	A	B	B
ss490548615	7813828	A	B	B	B	A	B	A	B	A	B	A	B	A	A	A	B	B
ss490552776	7835311	A	B	B	B	A	B	A	B	A	B	A	B	A	A	A	B	B
ss490548619	7861540	B	A	A	A	B	A	B	A	B	A	B	A	B	A	B	A	A
ss490548623	7862326	A	B	B	B	B	B	A	B	B	B	B	B	A	B	A	B	B
ss490548646	8425845	A	A	A	A	B	A	A	A	B	A	B	A	A	A	A	A	A
ss490548650	8495239	A	A	B	B	A	B	A	B	A	A	A	B	A	B	A	B	A
ss490552805	8500917	A	B	B	B	B	B	A	B	B	B	B	B	A	B	A	A	B
ss490552808	8538987	A	B	B	B	A	B	A	B	A	B	A	B	A	A	A	B	B
ss490548658	8893154	B	B	B	B	A	B	B	B	A	B	A	B	B	B	B	B	B
ss490552840	8950735	B	A	A	A	B	A	B	A	B	A	B	A	B	B	B	A	A
ss490548662	8955576	A	A	B	B	A	B	A	A	A	A	A	B	A	B	A	B	A
ss490545355	9145953	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B
ss490552856	9148953	A	B	A	A	A	A	A	B	A	B	A	A	A	B	A	A	A

Figure 2.16 (cont'd)

		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	s
ss490552868	9660471	A	B	B	B	A	B	A	B	A	B	A	B	A	B	A	B	B
ss490548682	9732651	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B
ss490552880	10145480	A	B	A	A	B	B	B	B	B	B	B	A	A	A	A	A	A
ss490559401	10183945	A	B	B	B	A	B	A	B	A	B	A	B	A	B	A	B	B
ss490559398	10184012	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B
ss490552883	10230259	B	B	A	A	B	B	B	B	B	B	B	A	B	A	B	A	A
ss490552889	10402945	B	A	A	A	B	A	B	A	B	A	B	A	B	A	B	A	A
ss490559054	10403896	B	A	A	B	B	B	B	A	B	A	B	A	B	A	B	B	A
ss490548706	10832168	A	B	B	B	B	B	B	B	B	B	B	B	A	B	A	B	B
ss490552912	11034104	A	B	B	B	A	B	A	B	A	B	A	B	A	A	A	B	B
ss490548714	11135825	A	B	B	B	B	B	B	B	B	B	B	B	A	B	A	B	B
ss490552931	11510521	B	A	A	A	B	A	B	B	B	A	B	A	B	A	B	A	A
ss490552933	11588410	B	B	B	B	B	B	B	B	B	B	B	B	A	B	A	B	B
ss490548726	11651018	B	B	B	A	B	A	B	A	B	B	B	B	B	A	B	A	A
ss490552936	11651543	B	B	B	A	B	A	B	A	B	B	B	B	B	A	B	A	A
ss490548730	11979679	A	B	B	B	A	B	A	B	A	B	A	B	A	B	A	B	B
ss490548734	12520610	B	A	A	A	B	A	B	A	B	A	B	A	B	A	B	A	A
ss490548738	12532690	B	A	A	A	B	A	B	A	B	A	B	A	B	A	B	A	A
ss490552959	12567325	A	B	B	B	A	B	A	B	A	B	A	B	A	A	A	B	B

[a] distances according to the Peach v1.0 'dhLovell' genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome) (Verde et al. 2013)

Figure 2.17: The 16 haplotypes identified in sour cherry for the G3 region used to test for the fruit size and firmness QTL. The number of haplotypes was "condensed" to eleven based on the SNP calls for the region in **bold** and underlined. For the analysis, haplotype i = a, o = c, f = d, m = g and p = k.

LG3 NCBI SS#	Peach physical map location (bp)[a]	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p
ss490547685	1137810	A	B	B	B	A	A	B	B	A	A	B	B	B	B	B	B
ss490547689	1182045	A	B	B	B	A	B	B	B	A	B	B	B	B	B	B	B
ss490550887	1257143	B	B	B	B	B	B	A	B	B	B	B	B	B	A	B	B
ss490547697	1409656	A	A	B	B	A	A	B	A	A	A	A	B	B	B	B	A
ss490550895	1414476	B	A	A	A	B	A	A	A	B	A	A	A	A	A	A	A
ss490550899	1439124	A	B	B	B	A	A	B	B	A	A	B	B	B	B	B	B
ss490550906	1476283	A	B	B	B	A	A	B	B	A	A	B	B	B	B	B	B
ss490547699	1487618	B	A	A	A	B	B	A	A	B	B	A	A	A	A	A	A
ss490550910	1494588	A	B	B	B	A	A	B	B	A	A	B	B	B	B	B	B
ss490547703	1496302	B	A	A	A	B	A	A	A	B	A	A	A	A	A	A	A
ss490550917	1544427	A	B	B	B	A	A	B	B	A	A	B	B	B	B	B	B
ss490547720	1841799	A	B	B	B	A	A	B	B	A	A	B	B	B	B	B	B
ss490550975	1933543	B	A	A	A	B	B	A	A	B	B	A	A	A	A	A	A
ss490547728	1953269	A	A	A	A	A	B	A	A	A	B	A	A	A	A	A	A
ss490547732	2129632	A	B	B	B	A	B	B	B	A	A	B	B	B	B	B	B
ss490551023	2181990	B	A	A	A	B	A	A	A	B	B	A	B	A	B	A	A
ss490551026	2200082	B	A	A	A	B	A	A	A	B	B	A	A	A	A	A	A
ss490547736	2203771	A	B	B	B	A	B	B	B	A	B	B	B	B	B	B	B
ss490551030	2262982	B	A	B	B	B	B	A	A	B	A	A	A	A	A	B	A
ss490547744	2265477	A	B	B	B	A	B	B	B	A	A	B	B	B	B	B	B
ss490551038	2307153	A	B	B	B	A	B	B	B	A	B	B	B	B	B	B	B
ss490551054	2387092	B	A	A	A	B	A	A	A	B	B	A	A	A	A	A	A
ss490551062	2431726	A	B	B	B	A	B	B	B	A	A	B	A	B	A	B	B
ss490551066	2449907	A	B	A	A	A	A	B	B	A	A	B	B	B	B	A	B
ss490551074	2512839	B	A	A	A	B	A	B	A	B	B	A	B	B	B	A	A
ss490551078	2532841	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
ss490547752	2598588	A	B	B	B	A	B	B	B	A	A	B	B	B	B	B	B
ss490551094	2615230	B	A	A	A	B	A	A	A	B	A	A	A	A	A	A	A
ss490551106	2679794	B	A	A	A	B	A	A	A	B	B	A	A	A	A	A	A
ss490547760	2698079	A	B	B	B	A	B	B	B	A	A	B	B	B	B	B	B
ss490551110	2699030	B	A	A	A	B	A	B	B	B	B	A	A	B	A	A	A
ss490547764	2735639	A	B	B	B	A	B	B	B	A	A	B	B	B	B	B	B

Figure 2.17 (cont'd)

		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p
ss490547768	**2738097**	A	B	B	B	A	B	B	B	A	B	B	B	B	B	B	B
ss490547771	**2804457**	B	B	A	B	B	B	B	A	B	B	B	A	B	B	A	B
ss490547775	**2820043**	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A
ss490551130	**2845006**	B	B	A	A	B	A	A	A	B	B	B	A	A	B	A	B
ss490551138	**2887683**	B	A	A	A	B	A	A	A	B	A	A	A	A	A	A	A
ss490551142	**2910993**	A	B	B	B	A	B	B	B	A	B	B	B	B	B	B	B
ss490547779	**2912959**	A	B	B	B	A	B	B	B	A	A	B	B	B	B	B	B
ss490551171	**3136656**	B	A	B	B	B	B	B	A	B	B	A	A	B	A	B	A
ss490551175	**3161286**	A	A	B	A	A	A	B	B	A	A	A	B	B	A	B	A
ss490551183	**3196604**	A	B	B	B	A	B	B	B	A	B	B	B	B	B	B	B
ss490547791	**3241000**	A	B	B	B	A	B	B	B	A	A	B	B	B	B	B	B
ss490551199	**3300749**	A	A	B	B	A	B	B	B	A	A	B	A	B	B	B	B
ss490547799	**3318355**	A	A	B	B	A	B	B	B	A	A	B	B	B	B	B	B
ss490551218	**3466624**	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A
ss490547803	**3501764**	B	B	A	A	B	A	A	A	B	B	A	A	A	A	A	A
ss490551226	**3530651**	B	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B
ss490551234	**3593732**	B	B	A	A	B	A	A	B	B	B	B	B	A	B	A	B
ss490547811	**3644850**	B	B	A	A	B	A	A	A	B	B	A	A	A	A	A	A
(CNR16)[b]	**3774129**																
ss490559277	**3792552**	A	A	A	A	A	A	B	A	A	A	A	B	B	A	A	A
ss490547822	**3988721**	A	A	B	B	A	B	B	B	A	A	B	B	B	B	B	B
ss490551275	**4016810**	B	B	B	B	B	B	B	B	B	B	A	A	B	A	B	A
ss490551292	**4123843**	B	B	A	A	B	A	A	A	B	B	A	A	A	A	A	A
ss490551296	**4145092**	B	B	A	A	B	A	A	A	B	B	A	A	A	A	A	A
ss490551305	**4197714**	A	A	B	B	A	B	B	B	A	A	A	B	B	B	B	A
ss490547829	**4224596**	A	A	B	B	A	B	B	B	A	A	B	B	B	B	B	B
ss490547833	**4298083**	A	A	B	B	A	B	B	B	A	A	B	B	B	B	B	B
ss490551321	**4301861**	B	B	B	B	B	B	A	A	B	B	A	B	A	A	B	A
ss490551325	**4334085**	A	A	B	B	A	B	A	A	A	A	A	A	A	A	B	A
ss490551337	**4468725**	A	A	A	A	A	A	B	B	A	A	A	A	B	A	A	A
ss490559471	**4571814**	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
ss490547837	**4633547**	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B
ss490551349	**4755490**	A	A	B	B	A	B	B	B	A	B	B	B	B	B	B	B

Figure 2.17 (cont'd)

		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p
ss490559004	5359437	A	A	A	A	A	A	B	A	A	A	A	B	B	B	A	A
ss490551365	5440880	B	B	B	A	B	A	A	B	B	B	A	A	A	A	B	B
ss490551378	6031878	A	A	B	B	A	B	B	B	A	A	B	A	B	B	B	A
ss490547854	6273459	B	B	A	A	B	A	A	A	B	B	A	A	A	A	A	A
ss490551390	6477319	B	B	A	B	B	B	A	A	B	B	A	A	A	A	A	A
ss490547862	6515224	B	B	A	A	B	A	A	A	B	B	A	A	A	A	A	A
ss490551403	6738267	B	B	A	A	B	A	A	A	B	B	A	B	A	A	A	A
ss490547873	6783562	B	B	B	B	B	B	B	B	B	B	A	B	B	B	A	B
ss490551411	6851920	A	A	B	B	A	B	B	B	A	A	B	B	B	B	B	B
ss490558935	7295951	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B
ss490551446	7572277	B	B	A	A	B	A	A	A	B	A	A	A	A	A	B	A

[a] distances according to the Peach v1.0 'dhLovell' genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome) (Verde et al. 2013)

[b] CNR16 location reported in De Franceschi et al. 2013

Figure 2.18: The twelve haplotypes identified in sour cherry for the G5 region used to test for fruit size and firmness QTL. Haplotype designations g and m were not used.

LG5	Peach physical map											
NCBI SS#	distance (bp)[a]	a	b	c	d	e	f	h	i	j	k	l	n
ss490554588	16125708	A	A	B	B	A	A	B	B	B	A	A	A
ss490549377	16216710	A	A	B	B	A	A	B	B	B	A	A	A
ss490554594	16228253	B	B	B	B	B	B	B	A	B	B	B	B
ss490554600	16320573	A	A	B	B	A	A	B	B	B	A	A	A
ss490549381	16330689	B	B	A	A	B	B	A	A	A	B	B	B
ss490554609	16375840	B	B	B	B	A	B	B	B	B	B	A	A
ss490559465	16429681	A	A	A	A	A	A	B	A	B	A	A	A
ss490549388	16439498	B	B	A	A	A	B	A	A	A	B	A	A
ss490549396	16580379	A	A	B	B	B	A	B	B	B	A	B	B
ss490549400	16585374	A	A	B	B	A	A	B	B	B	A	A	A
ss490549408	16712208	B	B	B	B	B	B	B	B	B	B	B	A
ss490549412	16720675	B	B	B	B	A	B	B	B	B	B	A	A
ss490554652	16768627	B	B	A	A	B	B	A	A	A	B	B	B
ss490559264	16803495	A	A	A	B	A	A	A	A	B	A	A	A
ss490559261	16803595	B	B	A	B	B	B	A	A	B	B	B	B
ss490554664	16812132	B	B	B	B	A	B	B	B	B	B	A	A
ss490554677	16882667	A	A	B	A	A	A	B	B	B	A	A	A
ss490554680	16907514	B	B	A	A	B	B	A	A	A	B	B	B
ss490549417	16911194	A	B	A	A	A	B	A	A	A	B	A	A
ss490554713	17082034	A	A	A	A	A	A	A	A	A	A	A	A
(CNR18)[b]	17128673												
(CNR19)[b]	17130224												
ss490549429	17134242	A	A	B	B	A	A	B	B	B	A	A	A
ss490549433	17175943	B	B	A	A	B	B	A	A	A	B	B	B
ss490549437	17224386	A	A	B	B	A	A	B	B	B	A	A	A
ss490554738	17229379	A	A	B	B	A	A	B	B	B	A	A	A
ss490554744	17255447	B	B	B	A	B	A	B	B	B	B	B	B
ss490549445	17279418	A	A	B	B	A	A	B	B	B	A	A	A
ss490554756	17328363	B	B	A	A	B	B	A	A	A	B	B	B

Figure 2.18 (cont'd)

		a	b	c	d	e	f	h	i	j	k	l	n
ss490554768	17389247	B	A	A	A	A	B	A	A	A	B	A	A
ss490549456	17389482	A	A	B	B	A	A	A	A	A	A	A	A
ss490549460	17404543	B	A	A	A	A	B	A	A	A	B	A	A
ss490554798	17518546	A	B	B	B	B	A	B	B	B	A	B	B
ss490549472	17523276	A	B	B	B	B	A	B	B	B	A	B	B
ss490554819	17647878	B	B	A	A	B	B	A	A	A	B	B	B
ss490554837	17757729	A	A	B	B	A	A	B	B	B	A	A	A
ss490559381	17759765	B	B	B	B	B	B	B	B	B	B	B	B
ss490559292	17779312	B	B	A	B	B	B	A	B	A	B	B	B
ss490549480	17805694	B	B	A	A	B	B	A	A	A	B	B	B
ss490554871	17956179	B	B	B	B	B	B	B	B	B	B	B	B
ss490554877	17977057	A	A	B	B	A	A	B	B	B	A	A	A
ss490549496	17997178	A	A	B	B	A	A	B	B	B	A	A	A
ss490549500	18035286	A	B	B	B	B	A	B	B	B	A	A	B
ss490554898	18100331	A	A	A	B	A	A	B	A	A	A	A	A

[a] distances according to the Peach v1.0 'dhLovell' genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome) (Verde et al. 2013)

[b] CNR18 and CNR19 locations reported in De Franceschi et al. 2013

Figure 2.19: The thirteen haplotypes identified in sour cherry for the G6 region between CNR20 and the S-locus. These haplotypes were "condensed" to five haplotypes based on results of the CNR – linked SSR marker G6SSR2008. This region was used to test for QTLs for fruit size and firmness.

LG6	Peach physical map												
NCBI SS#	distance (bp)[a]	a	b	c	d	d'	e	e'	e2	f	g	h	i	j
(CNR20)[b]	22070836													
G6SSR2208	22083802	3	3	null	1	3	5	5	5	4	null	2	3	1
ss490550132	22122175	A	A	B	B	A	A	A	A	A	B	A	A	B
ss490556003	22194565	B	B	A	A	B	B	B	B	B	A	B	B	A
ss490550135	22481221	B	B	A	A	B	B	B	B	B	A	B	B	A
ss490556011	22483330	B	B	A	A	B	B	B	B	B	A	B	A	A
ss490556014	22578566	B	B	B	B	B	A	A	A	A	B	A	A	B
ss490556018	22682473	A	A	B	B	A	A	A	A	A	B	A	A	B
ss490550143	22731299	B	B	B	A	B	B	B	B	B	B	B	B	A
ss490558923	22953307	A	A	A	A	A	B	B	B	B	A	B	A	A
ss490556027	22969579	A	A	B	B	A	A	A	A	A	B	A	A	B
ss490556030	23001313	B	B	A	A	B	B	B	B	B	A	B	B	A
ss490550163	23103755	B	B	A	B	B	B	B	B	B	A	B	B	B
ss490550167	23117929	A	A	B	B	A	A	A	A	A	B	A	A	B
ss490556045	23121232	B	B	A	B	B	B	B	B	B	A	B	B	B
ss490550171	23125937	B	B	A	A	B	B	B	B	B	A	B	B	A
ss490556048	23138881	B	B	B	B	B	A	A	A	A	B	A	A	B
ss490550179	23368978	A	A	B	B	A	A	A	A	A	B	A	A	B
ss490556080	23460054	B	B	A	A	B	B	B	B	B	A	B	A	A
ss490556083	23466387	B	B	A	A	B	B	B	B	B	A	B	B	A
ss490550183	23491156	A	A	B	B	A	A	A	A	A	B	A	A	B
ss490550187	23515096	B	B	B	A	B	B	B	B	B	B	B	B	A
ss490556092	23550234	A	A	B	B	A	A	A	A	A	B	A	A	B
ss490550192	23617261	B	B	A	A	B	B	B	B	B	A	B	B	A
ss490550196	23656729	B	B	A	A	B	B	B	B	B	A	B	B	A
ss490550200	23717104	B	B	A	B	B	B	B	B	B	A	B	B	B
ss490559356	23740186	A	B	A	A	B	B	B	B	B	A	B	A	A
ss490556117	23776068	B	B	B	A	B	B	B	B	B	B	B	B	A
ss490550208	23799947	A	A	A	B	A	A	A	A	A	A	A	A	B
ss490559289	23810925	B	A	B	B	A	B	B	B	B	B	B	B	B
ss490556126	23851662	A	A	B	B	A	A	A	A	A	B	A	B	B
ss490556129	23925194	A	A	A	A	A	A	A	A	A	A	A	B	A
ss490550216	24251557	B	B	A	A	A	B	B	B	B	A	B	B	A
ss490556147	24311905	B	B	A	A	A	B	B	B	B	A	B	B	A

Figure 2.19 (cont'd)

		a	b	c	d	d'	e	e'	e2	f	g	h	i	j
ss490559115	24324785	B	A	A	A	A	B	B	B	B	A	B	B	A
ss490550220	24433974	B	A	A	A	A	B	B	B	B	A	B	B	A
ss490556163	24593485	A	A	B	B	B	A	A	A	A	B	A	A	B
ss490556173	24757894	A	A	B	B	B	A	A	A	A	B	A	A	B
ss490550235	24770664	A	A	A	B	B	A	A	A	A	A	A	A	B
ss490556176	24822456	B	B	A	A	A	B	B	B	B	A	B	B	A
ss490556182	24914294	B	B	B	B	B	A	A	A	A	B	A	A	B
ss490550239	25019161	B	B	A	A	A	B	B	B	B	A	B	B	A
ss490550243	25034869	A	A	A	B	B	A	A	A	A	A	A	A	B
ss490556190	25113415	A	A	A	A	A	B	B	B	B	A	B	B	A
ss490556194	25146440	B	B	B	B	B	A	A	A	A	B	A	A	B
ss490558886	25325556	B	B	B	B	B	B	B	B	B	B	B	B	B
ss490558890	25325607	B	B	A	A	A	B	B	B	B	A	B	B	A
ss490550250	25413648	B	B	A	A	A	B	B	B	B	A	B	B	A
ss490556207	25429330	A	B	A	A	A	B	B	B	B	A	B	A	A
ss490550254	25441080	B	B	B	A	A	B	B	B	B	B	B	B	A
ss490556210	25528480	A	A	A	A	A	A	A	A	A	A	A	A	A
ss490550263	25606798	B	B	B	A	A	B	B	B	B	B	B	B	A
ss490556216	25631852	A	A	B	B	B	A	A	A	A	B	A	B	B
ss490556220	25713330	B	B	B	B	B	A	A	A	A	B	A	B	B
ss490550267	25761628	B	B	B	A	A	B	B	B	B	B	B	B	A
ss490556239	26089443	B	B	B	B	B	A	A	A	A	B	A	B	B
ss490556242	26149926	B	A	A	A	A	B	B	B	B	A	B	A	A
ss490556245	26206403	B	B	B	B	B	A	A	A	A	B	A	A	B
ss490556251	26322018	B	B	B	A	A	A	A	A	A	B	A	B	A
S-locus	~26447808	1'	4	36b	35	35	13'	13'	13m	6	36a	6	14	26
ss490556260	26484157	B	A	A	A	A	B	B	B	B	A	B	A	A
ss490559322	26505790	B	A	A	A	A	A	A	A	B	A	B	A	A
ss490556263	26537935	B	B	A	B	B	B	B	B	B	A	B	B	B
ss490550286	26634643	B	B	A	A	A	B	B	B	B	A	B	B	A
ss490550290	26800908	A	A	A	B	B	A	B	A	A	A	A	A	B
ss490550294	26801537	A	A	B	B	B	A	B	A	A	B	A	A	B
ss490556278	26816058	A	A	B	B	B	A	B	A	A	B	A	A	B

Figure 2.19 (cont'd)

		a	b	c	d	d'	e	e'	e2	f	g	h	i	j
ss490556284	26929208	A	A	B	B	B	A	B	A	A	B	A	A	B
ss490550302	27072416	A	A	B	B	B	A	B	A	A	B	A	A	B
ss490550306	27138059	B	B	B	A	A	B	A	B	B	B	B	B	A
ss490550310	27359089	B	B	B	A	A	B	A	B	B	B	B	B	A
ss490556318	27518176	B	B	A	A	A	B	A	B	B	A	B	B	A

[a] distances according to the Peach v1.0 'dhLovell' genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome) (Verde et al. 2013)

[b] CNR20 location reported in De Franceschi et al. 2013

Figure 2.20: The thirteen haplotypes identified in sour cherry for the G3 region containing *MYB10*. This region was used to test for the flesh color QTL. Haplotype designations a, i, j, m, q, r, s and t were not used.

LG3 NCBI SS#	Peach physical map distance (bp)[a]	b	c	d	e	f	g	h	k	l	n	o	p	u
ss490551540	9729116	B	B	B	B	B	B	B	B	B	A	B	B	B
ss490547928	9782875	A	A	B	A	A	B	B	A	B	A	B	A	A
ss490551552	10022424	B	B	B	B	B	A	A	B	B	B	B	A	B
ss490551556	10105783	A	B	B	A	A	B	B	A	B	A	B	B	B
ss490551560	10162979	B	A	A	A	A	A	A	A	A	A	A	A	A
ss490551563	10264563	A	A	B	A	A	B	B	A	B	A	B	B	A
ss490551577	10573974	A	B	B	A	A	A	B	A	B	A	B	B	B
ss490547944	10590166	A	B	B	A	A	B	B	A	B	A	B	B	B
ss490551581	10626205	A	B	B	A	A	B	B	A	B	B	B	B	B
ss490551584	10675150	A	B	B	A	A	B	B	A	B	A	B	A	B
ss490551593	10822211	A	B	B	A	A	B	B	A	B	A	B	A	B
ss490547952	10908880	A	B	B	A	A	B	B	A	B	A	B	B	B
ss490547960	12115409	A	B	B	A	A	B	B	A	B	A	A	B	B
ss490551635	12383977	B	A	A	B	B	A	A	B	A	B	A	A	B
ss490551642	12474678	B	B	B	B	B	B	B	B	B	A	B	B	B
ss490551648	12500413	A	B	B	A	A	B	B	A	B	B	B	B	B
ss490547972	12503462	A	B	B	A	A	B	B	A	B	A	B	B	B
ss490547976	12539794	B	B	B	A	A	B	B	A	B	B	B	B	B
3 MYB10 homologs (12.84-12.91 Mb)														
ss490551672	12944437	A	B	B	A	A	B	B	A	B	A	B	B	B
ss490551678	12987920	B	A	B	B	B	A	A	B	B	B	A	A	A
ss490551684	13025963	A	A	A	A	A	B	B	A	B	A	B	A	B
ss490547992	13063792	A	B	B	A	A	B	B	A	B	A	B	B	B

Figure 2.20 (cont'd)

		b	c	d	e	f	g	h	k	l	n	o	p	u
ss490551699	13144730	B	B	B	B	B	A	A	B	B	B	A	B	A
ss490551705	13208005	B	B	A	B	B	A	A	B	A	B	A	B	A
ss490547996	13369328	A	A	A	A	A	A	A	A	A	B	A	A	A
ss490551720	13406263	A	B	B	A	B	A	A	B	A	B	A	B	A
ss490551723	13433848	B	A	B	B	B	A	A	B	A	B	A	B	A
ss490551730	13466702	B	B	B	B	B	A	A	B	A	B	A	A	A
ss490551739	13520194	B	A	B	B	B	A	A	B	A	B	B	B	A
ss490551746	13563908	A	B	B	A	A	B	B	A	B	A	B	B	B
ss490551749	13567593	A	B	B	A	A	B	B	A	B	A	A	B	B
ss490551771	13724726	A	B	B	A	A	B	B	A	B	A	B	B	B
ss490551778	13754793	B	A	A	B	B	A	A	B	A	B	A	B	A
ss490551784	13795019	B	A	A	B	B	A	A	B	A	B	A	A	A
ss490559450	13878008	A	A	B	A	A	A	B	A	A	A	A	A	B
ss490548016	13881088	A	A	B	A	A	A	B	A	A	A	A	A	B
ss490551803	14024780	A	B	A	A	A	A	A	B	B	B	A	A	A
ss490551812	14146853	B	B	B	B	B	B	B	A	B	A	B	B	B
ss490551824	14316165	B	A	A	B	B	A	A	B	A	B	B	A	A
ss490548032	14442011	B	A	B	B	B	A	A	B	A	B	A	A	A
ss490551830	14521488	B	A	A	B	B	A	A	B	A	B	A	A	A
ss490551837	14599590	B	A	B	B	B	A	A	B	A	B	B	A	A
ss490548055	15171728	A	B	B	A	A	B	B	B	B	B	B	B	B
ss490548059	15305145	B	A	A	B	B	A	A	B	A	B	A	A	A
ss490551869	15309954	A	B	B	A	A	B	A	A	B	A	B	B	A
ss490551872	15357433	B	A	A	B	B	B	A	B	A	B	B	A	A
ss490551878	15455662	A	B	B	A	A	B	A	A	B	A	A	B	A

[a] distances according to the Peach v1.0 'dhLovell' genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome) (Verde et al. 2013)

Figure 2.21: The 17 haplotypes identified in sour cherry for the G5 Malic acid QTL region. The Seventeen haplotypes were condensed to just six using the region in **bold** and underlined. Above the haplotypes are what haplotype group each haplotype belongs to (1-6).

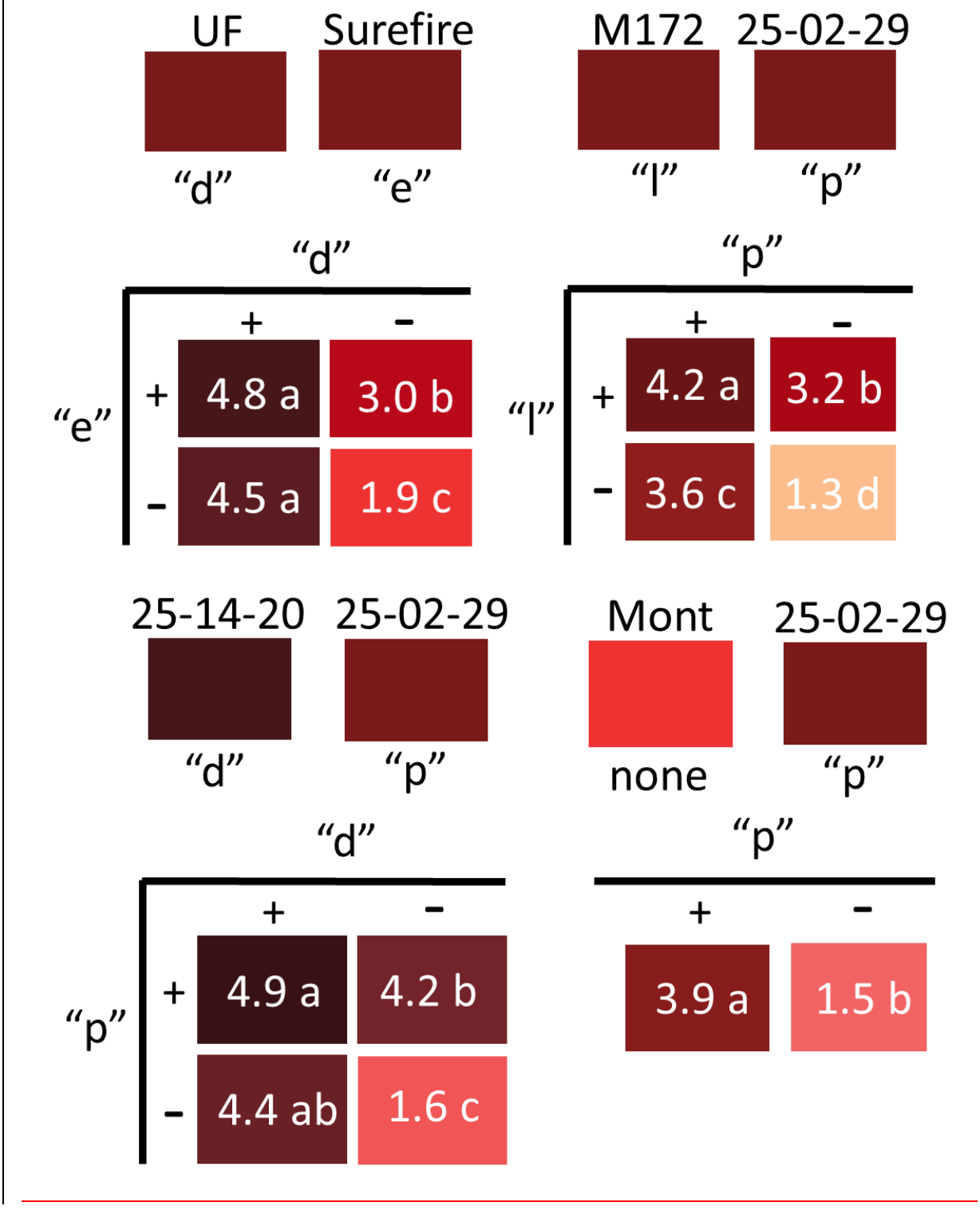
LG5	Peach physical map	1						2		3			4			5	6
NCBI SS#	distance (bp)[a]	b	i	e	f	k	y	s	n	r	g	l	j	p	o	m	h	q
ss490553644	**689941**	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A
ss490553647	**710199**	A	A	A	A	A	A	B	B	A	A	A	A	A	A	A	A	A
ss490553668	**857660**	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A
ss490553674	**934368**	B	B	B	B	B	B	A	A	A	A	A	A	A	A	B	B	B
ss490548963	**935896**	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A
ss490553677	**949123**	A	A	A	A	A	A	B	B	B	B	B	B	B	B	A	A	A
ss490553680	**975724**	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	B
ss490553683	**987941**	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B
ss490548967	**990328**	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A
ss490553686	**1005418**	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B
ss490548971	**1031051**	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A
ss490553696	**1121958**	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B
ss490553708	**1221714**	B	B	B	B	B	B	A	A	A	A	A	B	B	B	A	B	B
ss490553720	**1451354**	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B
ss490548999	**1463960**	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B
ss490553732	1607433	A	A	A	A	A	A	B	B	A	A	A	A	A	A	A	A	A
ss490553738	1645289	A	B	A	A	A	A	A	A	A	A	A	B	B	B	A	B	B
ss490549009	1871057	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A
ss490553772	2236592	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	B	B
ss490549017	2404347	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B
ss490553790	2722408	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
ss490553808	3142214	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A
ss490553814	3248658	A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	A	A
ss490553817	3299986	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	A	A
ss490549028	3394531	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A
ss490553823	3464400	A	A	B	A	B	A	B	B	B	B	B	A	A	A	A	A	A
ss490553829	3492263	B	B	A	B	A	B	A	A	A	A	A	B	B	B	B	B	B
ss490549036	3513593	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	B
ss490553838	3557553	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	B
ss490553844	3631504	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A
ss490553847	3644242	A	A	A	A	A	A	B	B	B	B	B	A	A	A	B	A	A

Figure 2.21 (cont'd)

		b	i	e	f	k	y	s	n	r	g	l	j	p	o	m	h	q
ss490553853	3695755	A	B	B	B	B	A	B	B	B	B	B	A	A	A	B	B	B
ss490553856	3731884	A	A	B	B	B	A	B	B	B	B	B	A	A	A	B	A	A
ss490553865	3767786	A	B	A	A	A	A	A	A	A	A	A	B	B	B	A	A	A
ss490553868	3864042	A	A	A	A	B	A	B	B	B	B	A	B	A	A	A	A	A
ss490549055	3909319	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	B
ss490553871	3917338	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A
ss490553874	4005643	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A
ss490553877	4028824	A	A	B	B	B	A	B	B	B	B	B	A	A	A	A	A	A
ss490549059	4181905	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A
ss490553898	4345439	A	B	A	A	A	A	B	B	B	B	B	A	A	A	A	A	A
ss490553901	4357749	A	A	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B
ss490553907	4413731	A	B	A	B	A	A	A	A	A	A	A	B	B	B	B	B	B
ss490549067	4415391	A	A	A	A	A	A	B	B	A	A	A	A	A	A	A	A	A
ss490553910	4486238	B	B	B	B	B	B	A	A	A	A	A	B	B	B	B	A	A
ss490553922	4755463	A	A	A	A	A	A	B	B	B	B	B	A	A	A	A	B	B
ss490553929	4897952	B	B	A	B	A	A	A	A	A	A	A	B	B	A	B	B	B
ss490553932	4994245	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	A	A
ss490553942	5143453	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B
ss490549078	5238673	A	A	A	A	A	A	A	B	B	B	B	A	A	A	A	A	A
ss490553948	5242696	A	A	A	A	A	A	A	B	B	B	B	A	A	A	A	A	A
ss490549082	5354555	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	B	B
ss490553960	5409519	B	B	A	A	A	A	A	A	A	A	A	B	B	A	B	B	A
ss490553963	5429352	A	A	B	B	B	B	B	B	B	B	B	A	A	B	A	A	B

[a] distances according to the Peach v1.0 'dhLovell' genome assembly (International Peach Genome Initiative; www.rosaceae.org/peach/genome) (Verde et al. 2013)

Figure 2.22: Within population mean comparisons of dark flesh haplotypes d, e, l, and p. Means are significantly different (P< 0.05) if letters after the mean score are different. Colors are representative of the rating.



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