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QTL ANALYSIS OF FRUIT COLOR AND ESTIMATION OF GENETIC DIVERSITY USING DNA MARKERS IN SWEET CHERRY (Prunus avium L.)

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By

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ABSTRACT

QTL ANALYSIS OF FRUIT COLOR AND ESTIMATION OF GENETIC DIVERSITY USING DNA MARKERS IN SWEET CHERRY (*Prunus avium* L.)

By

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Fruit color is an important indicator of sweet cherry fruit maturity and distinguishes two major market classes, e.g. yellow skin and fruit with a pink blush on the skin, and dark mahogany colored skin and flesh. Yet, within these extremes, there is a continuum of flesh and skin color types. The genetic control of skin and flesh color in sweet cherry was investigated using a QTL approach with a population derived from a cross between parents representing the two color extremes. Skin and flesh colors were measured from the progeny using a qualitative color card rating in 2006, 2007 and 2008. In 2008, color was also evaluated quantitatively for lightness (L*), redness (a*), and yellowness (b*). The skin and flesh color card ratings for the three years were significantly correlated ($P<0.0001$) and therefore only the 2008 data were used in the genetic analyses. Progeny segregations for the color measurements (card, L*, a*, b*) did not fit normal distributions; instead the distributions were skewed towards the skin color of the dark-skinned parent. A major QTL for skin and flesh color was identified on Linkage Group (LG) 3 and three other QTLs for skin and flesh color were identified on LG5, LG6 and LG8. However, the consistent significance of the QTL identified on LG3 suggests the presence of a major regulatory gene for fruit color development.

The genetic diversity of sweet cherry (*Prunus avium* L.) germplasm historically used in the breeding programs of Pacific North West region in North America was

studied in comparison to a subset of European sweet cherry landraces and a wild cherry (*P. avium*) selection to test the hypothesis of genetic founder effect that occurred when early settlers brought selected subset of sweet cherry germplasm from Europe to the New World. Pacific North West sweet cherry germplasm was defined as a set of 28 landraces, parents and released cultivars. A subset of seven European sweet cherry landraces and a single wild cherry selection were used for the comparison. The genotypic data for all 36 sweet cherry selections were recorded for 77 DNA markers. A total of 300 alleles were detected for 77 markers with an average of four alleles per locus. A total of 52 unique alleles were identified and 40 of them were not present in the Pacific North West sweet cherry germplasm. The 50% of the total alleles detected were rare alleles and 30% of the total rare alleles were not detected in the Pacific North West sweet cherry germplasm. The European landraces were distantly related at 25% of genetic dissimilarity value but Pacific North West sweet cherry parents and cultivars were separated only at 8% of genetic dissimilarity value showing the low level of diversity compared to European sweet cherry landraces and the wild cherry selection. This study shows that Pacific North West sweet cherry germplasm had been subjected to genetic founder effect and implies that the introduction of new germplasm from Europe is necessary to broaden the genetic diversity in the Pacific North West sweet cherry germplasm.

DEDICATION

To my wife Chamila Kumari Pathirana

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LITERATURE REVIEW

GENETICS OF FRUIT SKIN AND FLESH COLOR IN SWEET CHERRY

Importance of fruit color in cherry industry

Fruit color is one of the most important traits in determining consumer demand in sweet cherry (*Prunus avium* L.). Dark mahogany colored sweet cherries are preferred in North America (Turner 2008) and Europe (Wermund and Fearne 2000) and blush colored sweet cherries are preferred in Asia (Miller et al. 1986). The color of fruit skin and flesh is also important to determine the maturity level of fruits (Facteau et al. 1983). Breeding for sweet cherry cultivars with desired fruit colors is challenging, because, the underlying genetics of skin and flesh color traits have not been studied in detail.

Variability of fruit color in sweet cherry

The phenotypic diversity of fruit skin and flesh color of sweet cherry is very high. Fruit skin and flesh colors range from dark mahogany skin and flesh (e.g. cultivar "Bing") and yellow skin and flesh (e.g. cultivar "Gold"). There are blushed fruit cultivars with red/mahogany shades in yellow background and yellow flesh (e.g. cultivar "Rainier"). The classification of sweet cherry skin and flesh into color classes is dependent upon the level of fruit maturity. Dark skinned fruits get darker with time and their flesh follows the same pattern of the color development in skin. In blushed fruits, the red shades get more prominent in the skin and the flesh color remains unchanged with maturity.

Genetics of fruit color in sweet cherry

Classical genetic approaches were used to understand the genetics of fruit color in sweet cherry and postulated that the skin color is controlled by one major factor (*Aa*) and one minor factor (*Bb*) and incomplete dominant epistasis was also suggested for the interaction between *A* and *B*. Factor *A* was also proposed to be responsible for controlling the flesh color (Fogle 1958 and Schmidt 1998). The data from European breeding populations supported this genetic model (Hedtrich 1985; Georgiev 1985; Rodrigues et al. 2008; and Tobutt and Boskovic 1996).

Fruit color pigments in sweet cherry

The color of cherries, either sweet or tart (*P. cerasus* L.) is mainly due to anthocyanins. Red sweet cherry cultivars mainly contain Cyanidin-3-*O*-rutinoside (95% of total anthocyanin) and cyanidin-3-*O*-glucoside. Red sour cherry cultivars such as 'Balaton' and 'Montmorency' have mainly Cyanidin-3-*O*-glucosylrutinoside and cyaniding-3-*O*-rutinoside and minor quantities of cyanidin-3-*O*-glucoside. The blush cultivars have carotenoids such as beta-carotene (Mulabagal et al. 2009).

Factors affecting color development in sweet cherry

Fruit skin and flesh color is affected by environment to a certain degree. The environmental effect on the color development is higher in blush cherries than in dark mahogany colored cherries. Application of gibberellic acid has no significant impact on

the fruit color in cherry (Horvitz 2003). In blush sweet cherries, UV light stimulates the anthocyanin synthesis (Arakawa 1993). This explains the fact that the blush cherries that are located inside the canopy are less colorful than the cherries on the outer canopy, as leaves absorb most of the UV light before reaching the interior canopy.

Variability of fruit color in apple

The fruit color of apple (*Malus* x *domestica*) is well studied, and as apple and cherry belong to the same family, *Rosaceae*, the recent advancements of fruit color genetics in apple are applicable to study the fruit color genetics in sweet cherry. Apple skin color has a wide array of phenotypic diversity ranging from green, yellow and dark purple. The shaded combinations of different colors can also be seen. Lancaster (1992) reported that combinations of carotenoids, chlorophyll and anthocyanins determine the various skin colors in apple.

Genetics of fruit color in apple

The postulated mechanisms for genetics of skin color in apple are not in common agreement. A single dominant gene model was suggested for dark red skin (Brown 1992 and Crane and Lawrence 1933). Klein (1958) found that anthocyanin stripes of apple skin color are controlled by one major gene. White and Lespinasse (1986) suggested two complementary genes, *A* and *B*. Later Lespinasse et al. (1988) proposed a three major

gene model for determination of apple skin color. Schmidt (1988) postulated additional modifying factors.

Biochemistry and molecular genetics of fruit color in apple

The molecular studies on apple color genetics had started in the late 20th century. A RAPD marker was found to be linked to apple skin color (Cheng et al. 1996). Apple has Cynidin 3- *O*-galactoside as the major form of anthocyanin (Lancaster 1992, Tsao et al. 2003). Many genes associated with the anthocyanin biosynthetic pathway have been cloned from apple fruit skin; flavonone 3-hydroxylase (F3H), dihydroflavonol reductase (DFR), anthocyanin synthase (ANS) and UDP-glucose flavonoid 3-Oglucosyltransferase (UFGT) (Honda et al. 2002, Kim et al. 2003). These genes have found to be light induced and highly expressed in red apple skins. Takos et al. (2006), Espley et al. (2007) and Ban et al. (2007) have shown that one or two MYB transcription factors are playing the central role in apple fruit skin color genetics. *MdMYBA*, a cDNA encoding a putative R2R3-MYB protein (Ban et al. 2007), regulated anthocyanin biosynthesis in apple skin, has a huge similarity to MdMYB1 which was independently discovered by Takos et al. (2006). The only marked difference is that these two genes are differentially expressed at young stages of the fruit growth. Another *MYB* gene, *MdMYB10* found by Espley et al. (2007) that has some significant differences in expression relative to *MdMYBA* or *MdMYB1*. Ban et al. (2007) speculated that there would be at least two *MdMYB* loci active in anthocyanin biosynthesis in apple skin. Polymorphism at the *MdMYBa* has been mapped to Linkage Group 9 in apple 'Delicious' (Ban et al. 2007). Chagne et al. (2007)

found that red flesh and foliage color of apple co-segregated. The allele controlling the red color has been named as *Rni,* has been mapped along with *MdMYB10* to a single locus Linkage Group 9 in apple. The expression of these *MYB* genes are UV light defendant and low temperature induced. Espley et al. (2009) showed that a rearrangement in the promoter region of *MdMYB10,* a microsatellite like structure with tandem repeats of 23-bp sequence caused red phenotype in apple flesh and foliage. This motif is a target for the MdMYB10 protein itself and hence provides an autocatalytic regulation. This autocatalytic regulation ensures the accumulation of MdMYB10 protein and accumulation of anthocyain throughout the plant. The MdMYB transcription factor closely interacts with bHLH, another transcription factor that regulates anthocyanin biosynthetic pathway genes. The specific genes targeted by transcription factor complexes in apple have not been found. In Arabidopsis and grapes, such targets have been reported (Borevitz et al. 2000, Tohge et al. 2005, Kobayashi et al. 2002). The molecular genetic information of these studies has an immense importance in studying the fruit color genetics of sweet cherry.

Fruit color studies in other rosaceous crops

Compared to the color work in apple, the skin color of peach and other *rosaceous* fruits has not been studied in detail. Fruit skin and flesh color of peach has very high phenotypic diversity; yellow to red skin and white to red flesh. Connors (1920) described that an allele, *Y,* that controls white flesh is dominant to yellow (*y*) flesh in peach. Beckman et al. (2005) found allele, *h,* (highlighter) suppresses red color. The genetic

correlation between *Y* and *h* is not known. Beckman and Sherman (2003) showed full red phenotype is controlled by *fr*. Dark red flesh is determined by a single gene, *bf* (Werner et al. 1998). Skin color trait has been mapped to linkage group six (Dirlewanger et al. 2004, Yamamoto et al. 2001). Peach color is mainly due to carotenoids and beta carotene is the main form of carotenoid followed by beta-cryptoxanthin (Gil et al. 2002).

The correlation between carotenoid accumulation and the expression of carotenogenic genes in Japanese Apricot (*P. armeniaca* L.) has been established (Kita et al. 2007). Phytoene synthase-1 and lycopene β-cyclase expression is required for carotenoid accumulation. Decrease in lycopene ε-cyclase expression and increase in lycopene β-cyclase causes a metabolic shift from synthesis of β-ε-carotenoid to synthesis of β, β carotenoid with ripening progresses. Ethylene is important for the primary induction of Phytoene synthase-1. Kassim et al. (2009) mapped the polymorphisms of several transcription factors and candidate genes of anthocyanin biosynthetic pathway to QTLs in raspberry, *Rubus idaeus* L., another important *rosaceous* fruit species.

The genetics of fruit color in rosaceous crops is a fast developing area and the advancements in apple, peach, apricot and raspberries could be applied to understand the fruit color genetics of other rosaceous crops such as sweet cherry.

Chapter One: Goal

The aim of the Chapter One was to identify the genomic regions that are associated with fruit skin and flesh color in sweet cherry. This study was the first attempt to utilize the Quantitative Trait Loci (QTL) approach to dissect the genes related to fruit color in cherry. The expected results would enable us to understand the genetic mechanisms of fruit color in cherry and will be useful in marker assisted breeding for sweet cherry varieties with desired fruit colors and to further unravel the molecular genetic basis of fruit color in sweet cherry.

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GENETIC DIVERSITY IN SWEET CHERRY GERMPLASM

Background

The cherry is one of the most important temperate fruit crops in the world. There are two types of cherries. Sweet cherry (*Prunus avium* L.), is eaten fresh and its wild forms (i.e. mazzards) are used as a timber source and sour cherry (*Prunus cerasus* L.) is mainly used in processed food products. 375,000 hectares (Ha) of sweet cherry (with 1,896,000 Metric tons (Mt) of fruit harvest) and 248,000 Ha of tart cherry (with 1,035,000 Mt of fruit harvest) are grown worldwide (FAO 2005). The cost of production for cherry is quite high and various breeding programs around the world are operating to produce improved cultivars (Iezzoni 2008). The breeding for improved cultivars is dependent upon the successful introgression of desired traits from the land races and wild relatives of cherry.

Origin and geographical range

Sweet and sour cherries were originated in Central Asia (Vavilov 1951) and slowly spread to parts of Europe. The natural range of cherries includes temperate regions of Europe and south eastern Russia (Hedrick et al. 1915). Today, sweet cherry is cultivated in more than 40 countries representing temperate to subtropical climates. However, sour cherry is less widely spread compared to sweet cherry, and mainly grown in Europe and U.S.A. (Dirlewanger et al. 2007).

Genetic diversity

The genetic diversity of sweet cherry is represented by wild forest cherries (i.e. mazzards), land races, cultivars, plant materials available from the crosses from the breeding programs, other related species (i.e. sour, ground and duke cherries) and other wild cherry species in family *Rosaceae*. Much of the genetic diversity is available from the wild forms and landraces from the center of origin. The introgression of these exotic germplasm to Pacific North West sweet cherry breeding is important to produce improved cultivars. However, understanding the genetic distance between exotic and Pacific North West sweet cherry germplasms is important for successful introgression.

Sweet cherry is strictly self-incompatible, which promotes 100% out breeding (de Nettancourt 2001), thus, very high genetic heterozygosity is expected within the germplasm. However, vegetative propagation through grafting has fixed heterozygosity within cultivars, limiting the chance events of increasing the diversity in orchards.

The genetic diversity of sweet cherry has been examined for various objectives but none of the studies were aiming to find the genetic distance between Pacific North West and European sweet cherry cultivars (Brettin et al. 2000; Dirlewanger, et al. 2002). The most studied area of the genetic diversity in sweet cherry is the diversity of *S*-alleles. Sonneveld et al. (2003), De Cuyper et al. (2005), Wunch and Hormaza (2004) and Vaughan et al. (2008) reported 31 *S*-alleles (S_1-S_7, S_9-S_{32}) in sweet cherry.

Breeding

Breeding is quite slow compared to other rosaceous fruit crops like apple and peach. The main breeding goals for sweet cherry is large fruit size, high fruit quality, short juvenile phase, self compatibility, rain cracking resistance and pest and disease resistance (Dirlewanger et al. 2007). Even though the classical breeding programs are slow, many cultivars have been made available to the growers and breeders to use them as parent materials. However, these cultivars are selections from the natural populations or just one generation away from the wild progenitors (Iezzoni et al. 1990). The long generation time, self incompatibility and small number of seeds per cross, make cherry breeding a difficult task.

Recently, marker assisted breeding was introduced to address some of the difficulties in breeding but it is still in the developing phase. The most important accomplishment in sweet cherry breeding has been the introduction of self compatibility through mutational breeding (Lewis and Crowe 1954) and the ability to genotype cultivars for *S*-alleles by using DNA fingerprinting to select and grow sufficient number of polleniser-trees in the cherry orchards.

Chapter Two: Goal

The aim of the Chapter Two was to assess the genetic diversity of Pacific North West sweet cherry germplasm in comparison to a set of European sweet cherry land races and a wild cherry selection which have not been introduced to the Pacific North West sweet cherry breeding. This study used allele data from 77 DNA markers, identified unique alleles and constructed graphical genotypes for all the *Prunus avium* selections used. The future marker assisted breeding programs and genetic diversity studies on *Prunus* will be immensely benefited from the findings of this study.

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CHAPTER ONE

QTL ANALYSIS OF FRUIT SKIN AND FLESH COLOR IN SWEET CHERRY

(*Prunus avium* L.)

INTRODUCTION

Sweet cherry exhibits a continuous range of fruit skin and flesh colors from the dark mahogany color skinned and fleshed types, to those that have yellow skin with a red blush and yellow flesh. This variation in sweet cherry fruit skin and flesh color is used to classify different market types and to determine fruit maturity (Facteau et al. 1983). For example, dark mahogany cherries such as 'Bing' are favored in the majority of markets (Miller et al. 1986; Lyngstand and Sekse 1995; Wermund and Fearne 2000; Crisosto et al. 2003, Turner et al. 2007); however, blushed skinned and yellow fleshed sweet cherries such as 'Rainier' are preferred in Asia.

Despite the importance of fruit skin and flesh color in sweet cherry, the genetic control is not well understood. Fogle (1958) and Schmidt (1998) concluded that red skin color is dominant to yellow and proposed the presence of one major (*A/a*) and one minor gene (*B/b*) that exhibit epistasis. *A/a* was also suggested to control flesh color where *A-* and *aa* would confer mahogany and yellow flesh, respectively. The dominance of mahogany over yellow was supported by data from European breeding populations (Hedtrich 1985; Georgiev 1985; Rodrigues et al. 2008; and Tobutt and Boskovic 1996). However, collectively these studies also suggested that the genetic control of cherry skin and flesh color must involve additional minor genes to account for the wide range in color (from light yellow, pinks, reds, to dark mahogany).

To further investigate the genetic control of fruit skin and flesh color in cherry, an existing sweet cherry linkage mapping population that was segregating for these traits, and the available linkage map (Olmstead et al. 2008) were used for QTL analysis. The mapping population was a pseudo testcross between the blush and yellow fleshed Emperor Francis (EF) and dark mahogany skinned and fleshed New York 54 (NY). To facilitate a QTL approach, fruit color was quantified using L*, a* and b* color metrics where L* represents lightness, a* represents red/greenness, and b* represents blue/yellowness. This L*, a* and b* colorimetric system has been used to quantify color pigments in sweet cherries (Crisosto et al. 2003, Clayton and Biasi 2003, Usenik et al. 2005), other *Prunus* species (Gil et al. 2002, Kita et al. 2007) and many other plant samples to include apple (Espley et al. 2007), tomato (Sacks and Francis 2001), and wheat (Zhang et al. 2008).

The objective of this study was to determine the genetic control of fruit skin and flesh color in sweet cherry utilizing a QTL approach.

MATERIALS AND METHODS

Plant material

The QTL analysis was based on a sweet cherry mapping population of 190 pseudo-testcross progeny individuals (~equal numbers from reciprocal crosses) from a cross between a landrace variety 'Emperor Francis' (EF), and a wild 'mazzard' sweet cherry 'New York 54' (NY). A subset of 94 progeny individuals from this population were grafted onto Giesla® 6, a semi-dwarfing precocious rootstock, to provide a clonal replicate. Both the original seedling population and the grafted subset were planted at the Michigan State University Clarksville Horticultural Research Station, Clarksville, Mich., USA. In 2006 and 2007 all the evaluations were from fruits from the original seedlings. However, in 2008 a spring freeze killed the majority of the flowers on trees of the original population, and fruits were only evaluated from a subset of 94 individuals planted in a grafted plot that did not undergo freeze damage. This entire plot of 94 individuals was netted one week prior to fruit harvest to protect the ripening fruit from bird damage and an electric fence was installed around the perimeter of the plot to deter raccoons.

Fruit sampling and evaluation

Five fruits (one to four on the original seedlings if fewer fruit were available) were sampled from the trees. Fruit maturity was judged by observing the luster or dullness of the appearance of cherry fruit skin. However, because of the difficulty in judging maturity, each progeny individual was harvested multiple times, approximately

twice a week for a maximum of four harvest times. The data from the multiple harvests of each tree were compared using ANOVA to identify the maximum color potential to be used in QTL analysis.

In all three years, color card readings were recorded from the darkest location of the fruit skin (skin color 1, SC1), lightest location (skin color 2, SC2) and flesh color (FC). Nine (0-8) and five (1-5) color card categories were used to qualitatively measure skin color and flesh color respectively (Table1.1 and Table 1.2). Color card categories for skin color were defined according to colors previously identified for the Sweet Cherry Maturity Index which was manufactured by Colorcurve Systems, Inc (, East Lansing, Mich.) and color chips from The Flower Council of Holland (FCH), Leiden, The Royal Horticultural Society (RHS), London. Color card categories for flesh color were defined according to Washington State University's Sweet Cherry Flesh Color Index and The FCH Leiden, The RHS, London.

SC1, SC2, and FC were quantitatively evaluated for lightness (L*), redness (a*), and yellowness (b*) using a spectrophotometer (CM-2002, Minolta, Tokyo, Japan). L* measures the range from black (lower values) to white (higher values), a* measures the range from red (higher values) to green (lower values), and b* measures the range from blue (lower values) to yellow (higher values).

Table 1.1: Description of the color card categories for fruit skin color in sweet cherry used for QTL analysis

Color card category[a]	**Color description**	**Correspondent color category in sweet cherry maturity index**[a]	**Color classification in RHS Color Chart**[b]
0	Translucent	-	White – 155 D
1	Pale yellow	-	Yellow – 10 A
2	Orange	-	Grayish orange – 170 D
3	Light red	-	Red – 39 A
4	Red	1	Grayish red – 179 A
5	Dark red	2	Grayish red – 181 A
6	Light mahogany	3	Grayish purple – 183 A
7	Mahogany	4	Grayish purple – 187 B
8	Dark Mahogany	5	Grayish purple– 187 A

[a]Sweet Cherry Maturity Index, Agricultural Engineering Department, Michigan State University, East Lansing, MI 48824. Manufactured by Colorcurve Systems, Inc. Color card categories 1-3 were not included as this Index was developed for dark colored cherries.

[b]The flower council of Holland, Leiden; The Royal Horticultural Society (RHS), London

Table 1.2: Description of the color card ratings for fruit flesh color in sweet cherry used in the QTL analysis

Color card category in the sweet cherry flesh color index[a]	**Color description**	**Color classification in RHS Color Chart**[b]
1	Clear to pale yellow	Yellow – 11 A
2	Pale pink	Red – 37 A
3	Red	Grayish orange – 170 D
4	Mahogany	Grayish red – 182 A
5	Dark mahogany	Grayish purple – 187 A

[a]Washington State University's Sweet Cherry Flesh Color Index

[b]The flower council of Holland, Leiden; The Royal Horticultural Society (RHS), London

Statistical analysis for color measurements

The descriptive statistics of the color data were calculated using the Univariate procedure of SAS version 9.1 (SAS Institute 2006). The differences in color measurements between the two parents were compared using a *t*-test ($P < 0.05$). Pearson correlations for SC1, SC2, and FC using the color card data for 2006, 2007 and 2008 and the L* a* and b* data from 2008 were calculated using the CORR procedure of SAS version 9.1 (SAS Institute 2006). In 2008, estimates of broad-sense heritability were calculated from those seedlings for which measurements were taken from both the original seedling and the grafted replicate using an analysis of variance (ANOVA) Broad-sense heritability was estimated by using variance components with the formula, $H^2=\sigma_g^2 / (\sigma_g^2+\sigma_{g\times r}^2/r)$, where σ_g^2 is the genetic variance of progeny, $\sigma_{g\times r}^2$ is the interaction variance between progeny and plot, and r is the number of plots (i.e. seedling plot and grafted replicate).

QTL analysis

A consensus map of the two individual maps, NY and EF (Olmstead et al. 2008) was used for the QTL analysis. The consensus linkage map has a total of 197 markers, including 102 simple sequence repeat (SSR) markers, 61 amplified fragment length polymorphism (AFLP) markers, 27 gene-derived markers, and 7 sequence related amplified polymorphism (SRAP) markers. QTL analysis was done using MapQTL 5.0 (Van Ooijen 2004). Kruskal Wallis nonparametric test, interval mapping (IM), and multiple QTL mapping (MQM) were performed for each trait. In MQM, the markers closest to the peak of the QTL detected by IM were used as cofactors. The LOD

thresholds were estimated with 1,000 permutation tests for each trait. The QTLs with LOD values higher than the genome wide threshold at $P < 0.05$ were considered most significant, but QTLs with LOD values higher than genome wide threshold at $P < 0.1$ and QTLs with LOD values higher than individual linkage group level at $P < 0.05$ were also reported. QTLs with differing thresholds were reported as the use of phenotypic data that are not normally distributed, can result in unusually high LOD thresholds (Li et al. 2006 and Buil et al. 2005) leading to some real QTLs undetected. The linkage maps and QTL positions were drawn using MapChart (Voorrips 2002).

RESULTS AND DISCUSSION

Color data

Color card readings for SC1, SC2, and FC from the individuals in the linkage mapping population were significantly correlated across all three years ($P < 0.0001$) (Table 1.3) indicating that there was minimal inter-year variation in color. For 2008, the color card, L*, a* and b* values from the 94 seedlings in the clonally replicated mapping population subset were all significantly correlated for both skin (SC1 and SC2) and flesh color (FC) ($P < 0.0001$; Table 1.4). In particular, color card readings for SC1, SC2, and FC exhibited strong significant negative correlations to L* and b*. This reflects increases in darkness (- L*) and increases in blueness (- b*) in the dark mahogany fruit types. The significant correlations across the three fruit measurements suggest that there is a common genetic mechanism controlling skin and flesh color.

For skin color (SC1 and SC2), a* was negatively correlated with the color card data; however, for FC, a* and the card color data were positively correlated. This suggests that a different genetic mechanism may contribute to the variation in a* in the skin versus the flesh.

EF and NY exhibited significantly different color values for all traits except for SC2 a* (Table 1.5). This similarity between the two parents for a* reflects the fact that redness is not so important in lighter side (non blush or yellow) of EF. Collectively these

results indicate that the red – green vector (a*) alone, does not adequately describe the quantitative variation in the cherry fruit and skin color.

Table 1.3: Pearson's correlation coefficients for skin color 1 (SC1), skin color 2 (SC2), and flesh color (FC) card readings from the NY × EF progeny in 2006, 2007 and 2008

Trait	2006 vs. 2007	2006 vs. 2008	2007 vs. 2008
SC1	0.80 [a] (138) [b]	0.78 (85)	0.84 (89)
SC2	0.77 (138)	0.79 (85)	0.85 (89)
FC	0.88 (138)	0.82 (80)	0.91 (85)

[a] all the values are significant at $P < 0.0001$

[b] The number of individuals in each comparison.

Table 1.4: Pearson's correlation coefficients for skin color 1 (SC1), skin color 2 (SC2) and flesh color (FC) card and L*, a*, and b* values for NY × EF progeny evaluated in 2008

	SC1 L*	SC1 a*	SC1 b*	SC2 card	SC2 L*	SC2 a*	SC2 b*	FC card	FC L*	FC a*	FC b*
SC1 card	-0.87[x]	-0.90	-0.90	0.95	-0.84	-0.63	-0.91	0.88	-0.84	0.49	-0.86
SC1 L*		0.90	0.97	-0.84	0.86	0.49	0.88	-0.73	0.72	-0.34	0.80
SC1 a*			0.96	-0.90	0.86	0.70	0.92	-0.86	0.82	-0.36	0.88
SC1 b*				-0.89	0.88	0.56	0.92	-0.79	0.78	-0.37	0.85
SC2 card					-0.88	-0.63	-0.93	0.90	-0.85	0.49	-0.88
SC2 L*						0.44	0.94	-0.76	0.76	-0.38	0.81
SC2 a*							0.61	-0.77	0.68	-0.34	0.70
SC2 b*								-0.86	0.83	-0.43	0.90
FC card									-0.87	0.53	-0.89
FC L*										-0.48	0.89
FC a*											-0.37

[x]All the values are significant at $P < 0.0001$. Each comparison represents 1861 to 1865 individual fruits

Table 1.5: Means and standard deviations for skin color 1 (SC1), skin color 2 (SC2), and flesh color (FC) values for EF and NY in 2008

Tissue	Trait[z]	EF[y]	NY
SC1			8.0 b (0.0)
	Card	3.5 a (0.9)	
	L*	47.4 a (5.1)	27.3 b (0.9)
	a*	35.4 a (2.9)	7.0 b (2.8)
	b*	21.7 a (3.0)	0.6 b (0.6)
SC2	Card	1.3 a (0.5)	7.8 b (0.4)
	L*	69.1 a (6.0)	27.7 b (1.1)
	a*	7.0 a (10.3)	9.0 a (4.0)
	b*	35.3 a (5.7)	1.3 b (1.1)
FC	Card	1.0 a (0.0)	4.8 b (0.4)
	L*	40.8 a (5.5)	20.7 b (2.6)
	a*	3.8 a (1.6)	9.7 b (3.7)
	b*	26.7 a (1.9)	2.6 b (1.6)

[y]Means denoted by same letters in the same row are not significantly different at $P <$ 0.0001.

[z]Units: card (color card categories), L*, a* and b* (colorimeter reading)

Color development

In 2008, the pattern of skin and flesh color development for the parents and progeny were evaluated over four harvest dates to identify the colorimetric values that best represented the maximum color potential of each individual. The skin color metrics for dark mahogany fruits and flesh color metrics of all the fruits exhibited little differences across all four harvest dates (Table 1.6). However, for EF, the majority of the skin color values were significantly different among the four harvest dates. The changes in the EF skin measurements indicated that the fruit skin was becoming less yellow and this change was accompanied by a significant increase in the red blush on the fruit by the last harvest date. However, the flesh color of EF did not exhibit a parallel increase in red pigmentation. Instead, the EF flesh color remained yellow highlighting the importance of carotenoid pigments in the EF blush type cherries compared to the anthocyanin pigments in the red fleshed cherries.

Due to the different final colors between the blush and mahogany cherry types, the progression of color development across harvest date was evaluated using separate groups of seedlings that represented these two color classes. The overall trend was for decreases in L*, a* and b* and increases in card values over time (Table 1.6) that represented a darkening of the fruit skin and flesh. The minimum L*, a* and b* and maximum card values for each seedling were used in the QTL analysis as it represented the maximum color maturity for each seedling.

Table 1.6: The progression of fruit skin and flesh color over harvest data for blush and mahogany classes of NY54 x EF progeny for year 2008

Fruit color class	Location	Color metric[$]	June 20[#]	June 23	June 26	June 30
Blush	SC1	Card	4.7[$] a	5.0 b	5.4 c	5.3 c
		L*	37.3 a	35.1 b	33.8 c	33.5 c
		a*	28.4 a	26.1 b	24.2 c	21.7 d
		b*	13.2 a	10.8 b	9.8 c	8.4 d
Mahogany	SC1	Card	7.8 a	7.9 b	7.9 b	7.9 b
		L*	29.2 a	28.7 b	28.0 c	28.4 c
		a*	12.6 a	9.6 b	7.3 c	5.7 d
		b*	2.0 a	1.3 b	0.9 c	0.6 d
Blush	SC2	Card	3.03 a	3.8 b	3.8 b	4.1 c
		L*	47.5 a	43.6 b	42.9 b	41.2 c
		a*	22.5 a	24.8 b	23.0 c	23.5 c
		b*	20.1 a	18.0 b	16.9 b	15.1 c
Mahogany	SC2	Card	7.6 a	7.8 b	7.7 b	7.8 b
		L*	29.5 a	29.2 a	28.5 b	29.0 b
		a*	14.1 a	11.4 b	9.2 c	8.1 d
		b*	2.9 a	1.8 b	1.8 b	1.5 b
Blush	FC	Card	1.5 a	1.1 b	1.1 b	1.2 b
		L*	33.4 a	35.8 a	35.7 a	35.8 a
		a*	4.3 a	3.4 b	4.4 c	4.4 c
		b*	17.3 a	15.1 b	16.5 c	15.4 d
Mahogany	FC	Card	4.2 a	4.4 b	4.5 b	4.4 b
		L*	21.8 a	21.1 a	21.4 a	21.6 a
		a*	11.7 a	8.0 b	7.8 b	8.6 c
		b*	4.6 a	2.4 b	3.6 c	3.7 c

[$]Means followed by different letters within the same row are significantly different at $P < 0.05$ across the rows. The least square (LS) means are shown here (calculated from General Linear Model Procedure)

[#]Harvest days for 2008 fruiting season, growing degree days calculated from January 1, 2008 with a base temperature of 4.4 C (June 20: 723.2, June 23: 763.8, June 26: 811.3 and June 30: 872.9) and [$]Units: card (color card categories), L*, a* and b*

Data distribution

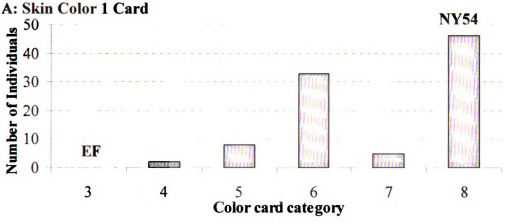
The pattern of the data distribution was examined for the minimum L*, a* and b* and maximum card values of all the individuals for 2008 data. The progeny values for all the color traits were not normally distributed (Figure 1.1) and the Kolmogorov-Smirnov Normality Coefficients (KS) were all significant ($P < 0.01$) (Table 1.7). In addition, the color card and FC L* and b* distributions in particular suggested a 9:7 ratio characteristic of a two locus interaction. These skewed distributions were consistent with the suggestion of Fogle (1958) and Schmidt (1998) that there is at least one major gene controlling the genetic variation in fruit color and possible epistasis. Transgressive segregants were identified for SC2 a* and FC a* whereas for all the other color traits, the progeny had phenotypic values intermediate to the parents. For SC2 a* and FC a* there was an abundance of progeny individuals that had redness values above that of the red fruited NY parent. The transgressive segregants identified for SC2 and FC a* were consistent with the correlation results that suggested a* in the skin and flesh is under different genetic control than the color measured by the color card, L* and b*.

Broad sense heritability estimates (H^2) for all the traits except SC2 a* were higher than 0.80 (Table 1.7). This suggests that the intensity of the red blush on the skin of the light colored cherries may be more sensitive to environmental conditions than the overall skin and flesh color. In particular, the intensity of the red blush is was reduced on fruit from the original seedling block, possibly due to less light interception and slightly immature fruit, compared to the fruit harvested from the clonal orchard where the netting permitted us to harvest fruits at optimum maturity.

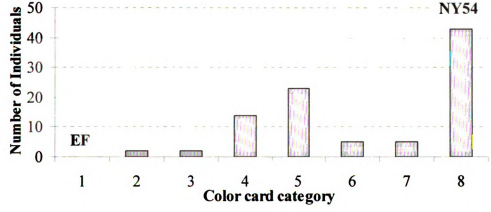
Figure 1.1: A-L Progeny frequency distribution of color traits measured in 2008 (A) SC1 card. (B) SC2 card. (C) FC card. (D) SC1 L*. (E) SC2 L*. (F) FC L*. (G) SC1 a*. (H) SC2 a*. (I) FC a*. (J) SC1 b*. (K) SC2 b*. (L) FC b*. EF and NY parental values are shown.

Fig 1.1 Cont.

A: Skin Color 1 Card



B: Skin Color 2 Card



C: Flesh Color Card

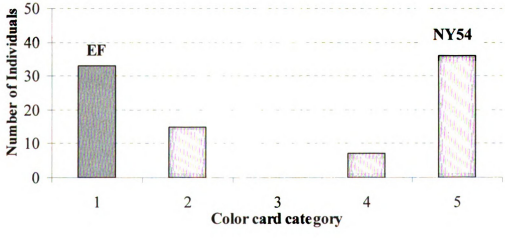
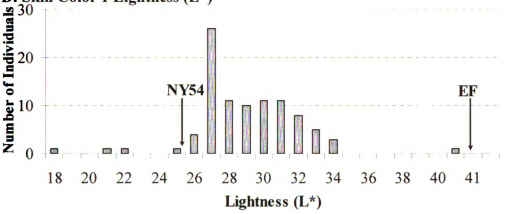
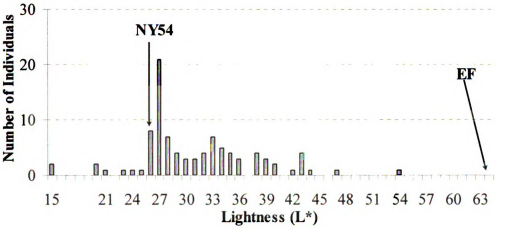


Fig 1.1 Cont.

D: Skin Color 1 Lightness (L*)



E: Skin Color 2 Lightness (L*)



F: Flesh Color Lightness (L*)

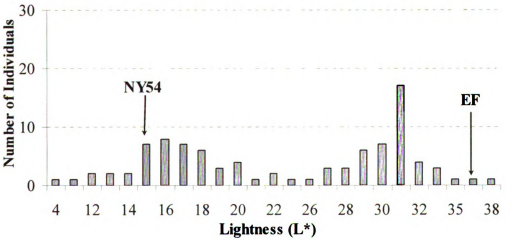
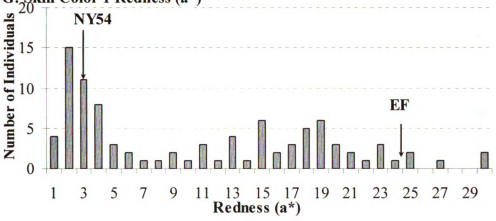
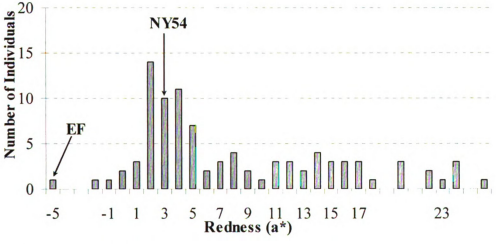


Fig 1.1 Cont.

G: Skin Color 1 Redness (a*)



H: Skin Color 2 Redness (a*)



I: Flesh Color Redness (a*)

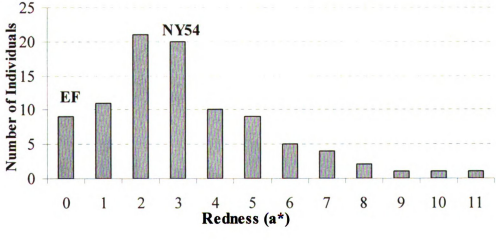
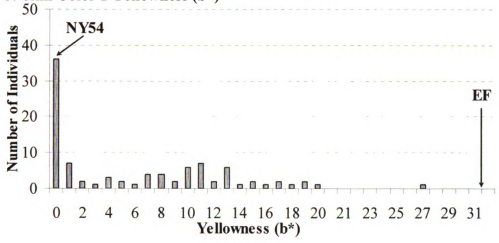
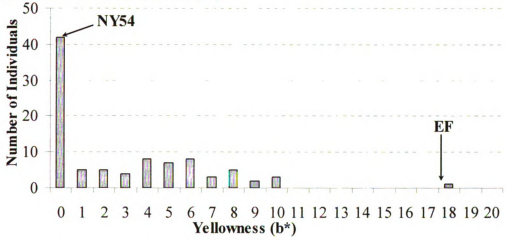


Fig 1.1 Cont.

J: Skin Color 1 Yellowness (b*)



K: Skin Color 2 Yellowness (b*)



L: Flesh Color Yellowness (b*)

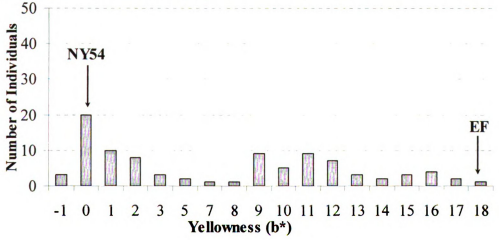


Table 1.7: Summary statistics and heritability of color data for year 2008

Tissue	Color metric	Mean (Standard Deviation)	Broad sense heritability (H^2)	Minimum[$]	Maximum[$]	Kolmogorov-Smirnov Normality Coefficient (KS)***
SC1	Card	6.6 (1.5)	0.96	2.0	8.0	0.1
	L*	31.4 (4.2)	0.82	17.9	54.1	0.2
	a*	15.9 (9.9)	0.95	0.7	42.3	0.1
	b*	5.5 (5.7)	0.90	-0.5	34.3	0.2
SC2	Card	5.8 (2.2)	0.95	1.0	8.0	0.2
	L*	36.0 (9.4)	0.89	15.0	99.2	0.2
	a*	16.4 (9.1)	0.74	-5.1	38.1	0.1
	b*	9.2 (8.9)	0.96	-0.4	45.9	0.2
FC	Card	2.8 (1.8)	0.98	1.0	5.0	0.2
	L*	28.6 (8.3)	0.94	3.6	61.9	0.1
	a*	6.7 (3.9)	0.86	-0.5	22.3	0.1
	b*	9.7 (7.1)	0.96	-0.9	30.9	0.1

[$]Units: card (color card categories), L* (lightness), a* (red/greenness) and b* (yellow/blueness) (colorimeter readings)

*** KS is significant at $P < 0.01$. Significant KS indicates the deviation from normality.

QTL analysis

For 2008 color card and L*, a* and b* data, significant QTLs detected for SC1 card, SC1 a*, SC2 card, SC2 b*, FC card, FC L* and FC b* on linkage group (LG) 3 at 53.7 cM and the average variability (R^2) explained by these QTLs was 87.2% and ranged from 78.4 % for SC2 b* to 94.7% for FC L* and FC b* (Table 1.8, Figure 1.2A) indicating that there is a major QTL on LG3 at 53.7 cM for anthocyanin pigmentation. Significant QTLs for five other color metrics were also located on LG3, however the predicted peak positions ranged from 12.8 cM for FC a* to 40.8 for SC2 a*. The average R^2 for these QTLs was 25.6% and ranged from 13.5% for SC2 L* to 44% for FC a* (Table 1.8, Figure 1.2A). This suggests that there is at least one additional color QTL located on LG3.

To test whether the major QTL on LG3 was significant in 2006 and 2007, QTL analysis was done for these two years using the color card data for SC1, SC2 and FC and (Figure 1.3). Significant QTLs detected for SC1 card, SC2 card and FC card for 2006 and 2007 data on linkage group (LG) 3 at 53.7 cM as in 2008 data. The average R^2 for these color card QTLs were 85.6% and ranged from 73.8% for 2007 SC2 card and 93.8% for 2007 FC card. The identification of a QTL on LG3 at 53.7 cM for two additional years validated our finding that there is a major fruit color QTL on that genomic location.

For the 2008 color card and L*, a* and b* data, three more significant color QTLs were identified, one for SC1 b* and two for FC a*. For SC1 b*, a QTL was detected on

LG6 with an R^2 of 42.8% (Table 1.8, Figure 1.2C). For FC a*, two additional QTLs were detected on LG5 and LG8 (Table 1.8, Figures 1.2 B and D). The QTL on LG5 and LG8 explained 18.7% and 44.0% of the phenotypic variation, respectively. The identification of additional QTLs for FC a* is consistent with phenotypic data that suggested FC a* reflected a different color pattern compared to the other color metrics.

Table 1.8: QTLs for color card values, L*, a* and b* for SC1, SC2 and FC identified in the NY × EF F_1 population in 2008 data

Fruit tissue	Color metric	Linkage group	QTL Peak position (closest marker and T x E bin map position[a])	LOD	R^{2b}
SC1	Card	3	53.7 (PR41, 3:37)	13.3*	87.3
	L*	3	21.0 (UDP97-403, 3:12)	4.8***	21.2
	a*	3	53.7 (PR41, 3:37)	7.9*	80.0
	b*	3	21.0 (UDP97-403 3:12)	6.0***	26.0
	b*	6	15.0 (UDP96-001 ~6:25)	4.1*	42.8
SC2	Card	3	53.7 (PR41, 3:37)	13.5*	87.5
	L*	3	21.0 (UDP97-403 3:12)	3.0*	13.5
	a*	3	40.8 (UDP98-416)	3.9*	23.3
	b*	3	53.7 (PR41, 3:37)	7.7**	78.4
FC	Card	3	53.7 (PR41, 3:37)	28.1*	94.7
	L*	3	53.7 (PR41, 3:37)	11.2*	86.2
	a*	3	12.8 (EAC-MCTA-360)	3.5*	20.8
	a*	5	27.8 (EAT-MCCC-285 ~5:21)	3.2*	18.7
	a*	8	83.3 (PS1H3 – unknown)	4.5**	44.0
	b*	3	53.7 (PR41, 3:37)	13.7*	87.9

[a]QTL peak position is expressed in cM and the closest marker and T × E bin map position is indicated in bracket. QTLs were estimated using multiple QTL mapping (MQM) method of MapQTL 5.0

[b]R^2, percentage of phenotypic variation explained by the QTL

*** The LOD value significant at $P < 0.05$ based on 1000 genome wide permutation tests

** The LOD value significant at $P < 0.1$ based on 1000 genome wide permutation tests

* The LOD value significant at $P < 0.05$ based on 1000 individual linkage group wide permutation tests

Figure 1.2: A-D Locations of QTLs for color card and L*, a* and b* values for SC1 (darkest location of the fruit skin), SC2 (lightest location of the fruit skin) and FC (flesh color) using the multiple QTL mapping method. The variability explained by QTL (R^2%) is shown after the trait name of each QTL. 1-LOD and 2-LOD support intervals of each QTL are marked by thick and thin bars, respectively. Blank bars represent QTLs for color card QTLs. Black bars represent QTLs for L*. Bars filled with one sided hatch lines represent QTLs for a*. Bars filled with two sided hatch lines represent QTLs for b*. Only linkage groups including the QTLs are presented. (A) Linkage group 3, (B) Linkage group 5, (C) Linkage group 6, (D) Linkage group 8. LOD scores and the percentage variability explained by the QTLs (R^2) are presented in the Table 1.8

Fig 1.2 Cont. A

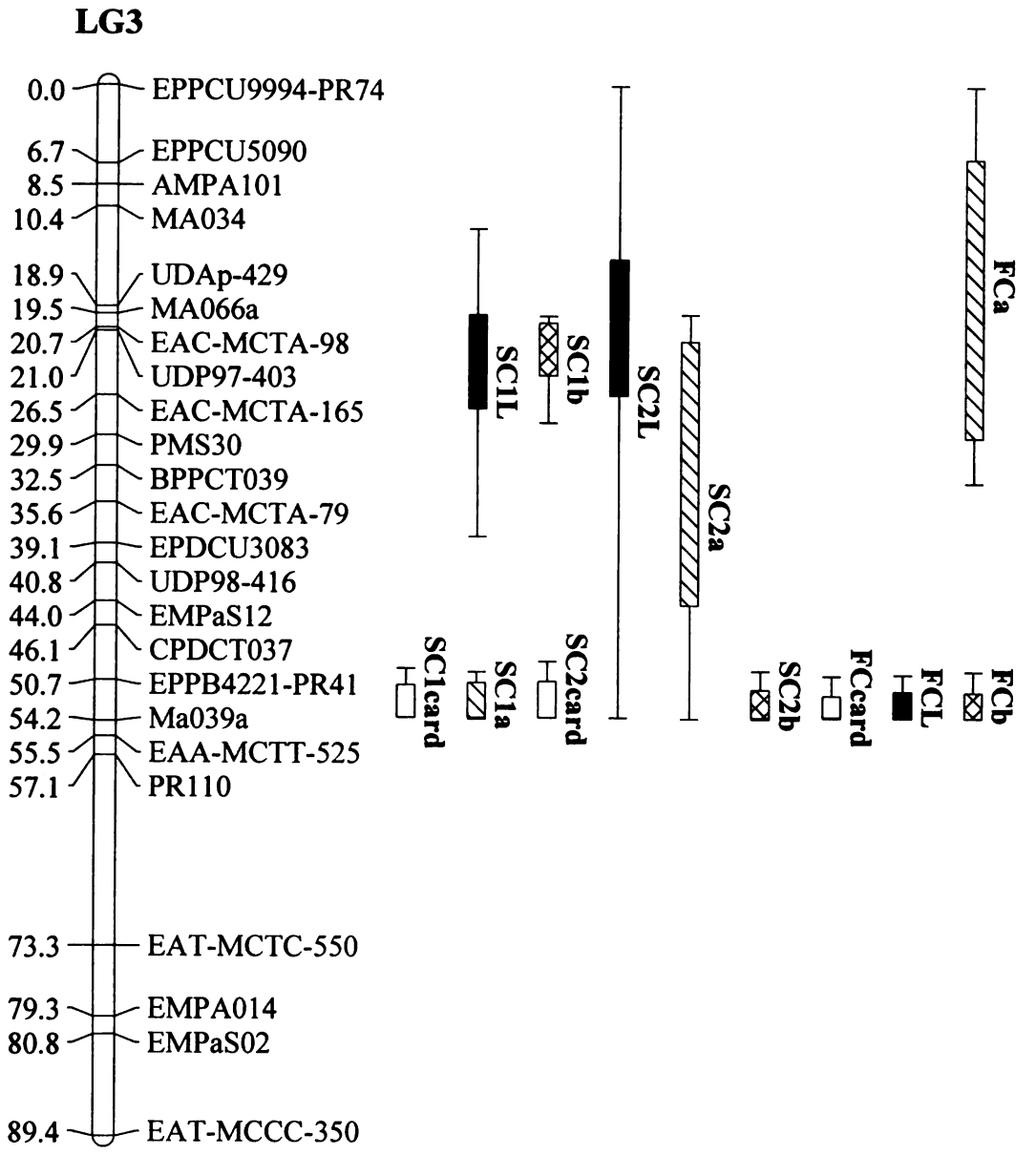

LG3
0.0 EPPCU9994-PR74
6.7 EPPCU5090
8.5 AMPA101
10.4 MA034
18.9 UDAp-429
19.5 MA066a
20.7 EAC-MCTA-98
21.0 UDP97-403
26.5 EAC-MCTA-165
29.9 PMS30
32.5 BPPCT039
35.6 EAC-MCTA-79
39.1 EPDCU3083
40.8 UDP98-416
44.0 EMPaS12
46.1 CPDCT037
50.7 EPPB4221-PR41
54.2 Ma039a
55.5 EAA-MCTT-525
57.1 PR110
73.3 EAT-MCTC-550
79.3 EMPA014
80.8 EMPaS02
89.4 EAT-MCCC-350
SC1card
SC1a
SC2card
SC1L
SC1b
SC2L
SC2a
SC2b
FCard
FCL
FCb
FCa

Fig 1.2 Cont. B

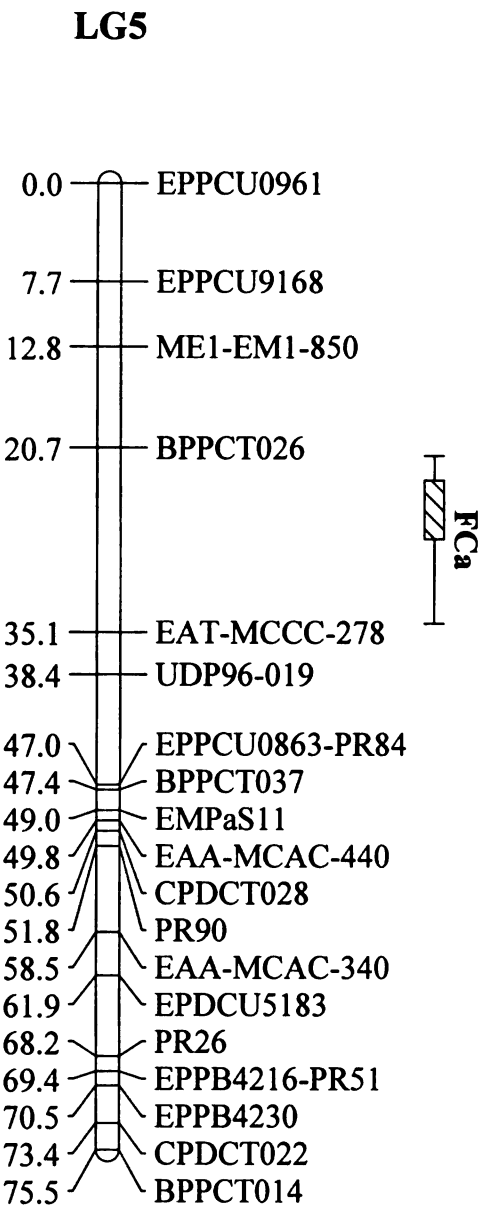

LG5
0.0
EPPCU0961
7.7
EPPCU9168
12.8
ME1-EM1-850
20.7
BPPCT026
FCa
35.1
EAT-MCCC-278
38.4
UDP96-019
47.0
EPPCU0863-PR84
47.4
BPPCT037
49.0
EMPaS11
49.8
EAA-MCAC-440
50.6
CPDCT028
51.8
PR90
58.5
EAA-MCAC-340
61.9
EPDCU5183
68.2
PR26
69.4
EPPB4216-PR51
70.5
EPPB4230
73.4
CPDCT022
75.5
BPPCT014

Fig 1.2 Cont. C **LG6**

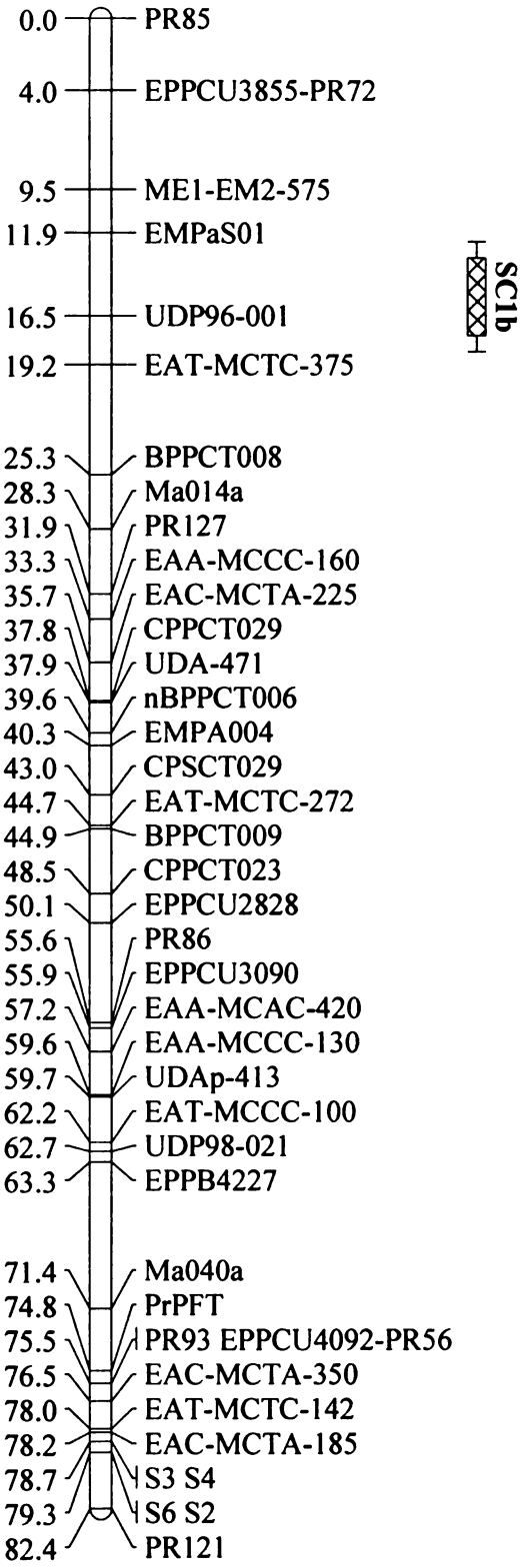


Fig 1.2 Cont. D **LG8**

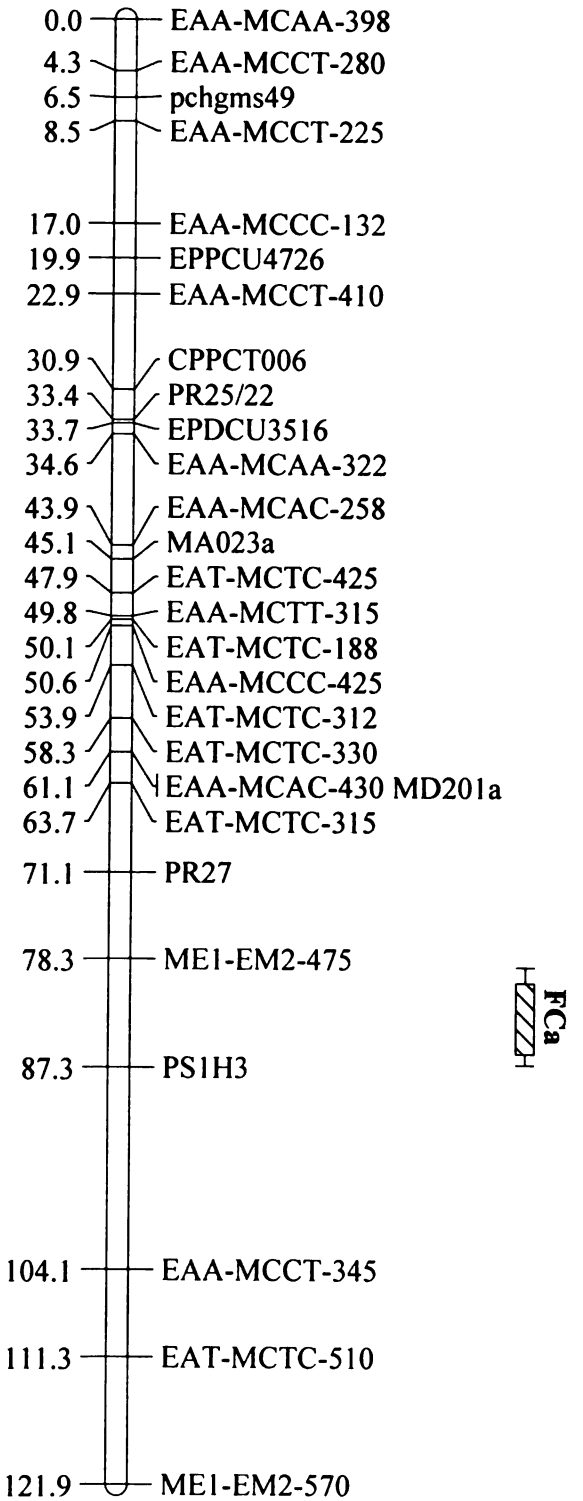
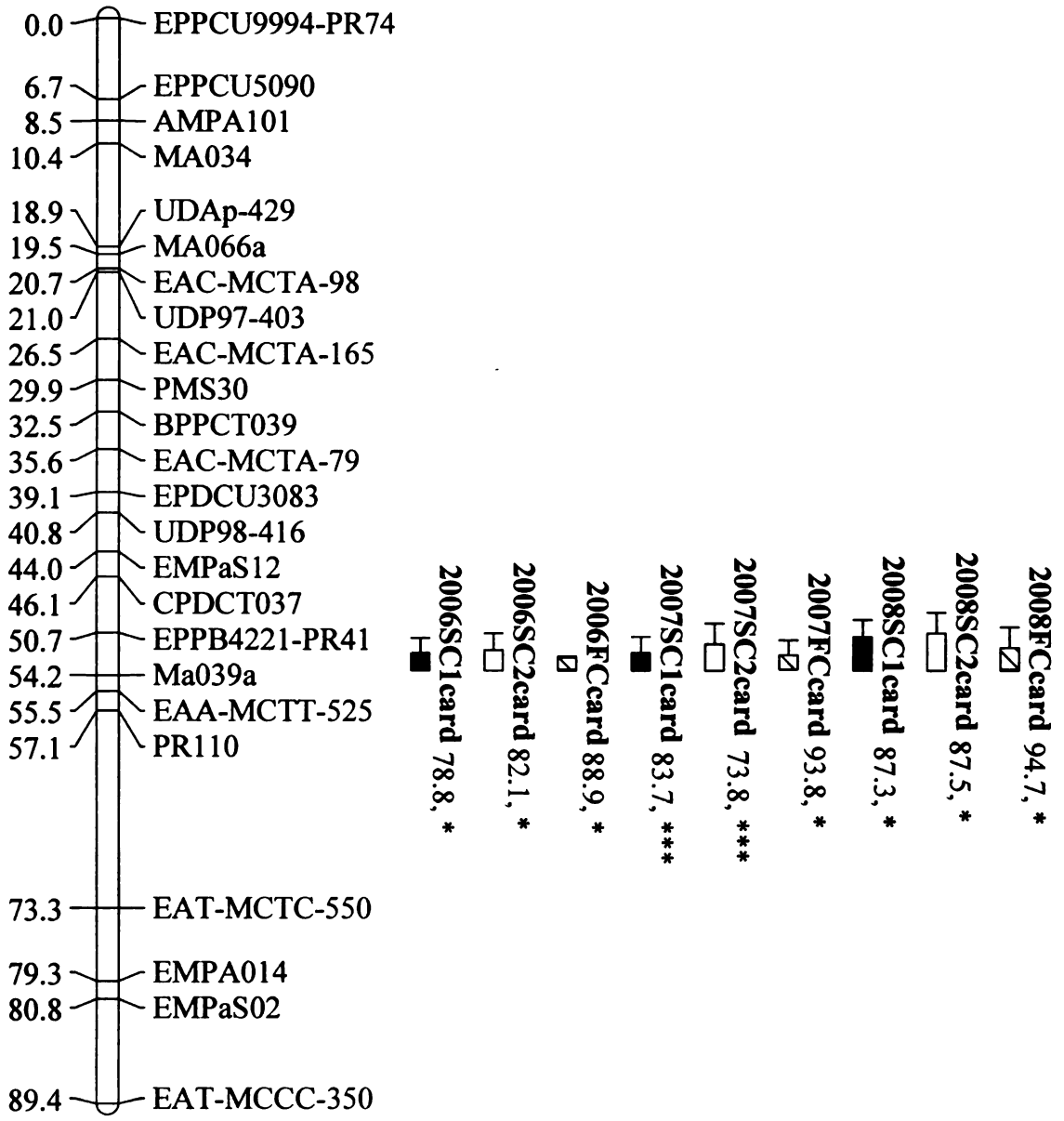


Figure 1.3: Locations of QTLs on LG3 for color card data of 2006, 2007 and 2008 for SC1 (darkest location of the fruit skin), SC2 (lightest location of the fruit skin) and FC (flesh color) using the multiple QTL mapping method. Blank bars represent QTLs. 1-LOD and 2-LOD support intervals of each QTL are marked by thick and thin bars, respectively. The percentage variability explained by the QTL (R^2) and the level of QTL significance in number of stars (***: LOD value significant at $P < 0.05$ genome wide, **: LOD value significant at $P < 0.1$ genome wide, * : The LOD value significant at $P < 0.05$ individual linkage group wide, based on 1000 permutation tests) are shown with the QTLs.


LG3
0.0 EPPCU9994-PR74
6.7 EPPCU5090
8.5 AMPA101
10.4 MA034
18.9 UDAp-429
19.5 MA066a
20.7 EAC-MCTA-98
21.0 UDP97-403
26.5 EAC-MCTA-165
29.9 PMS30
32.5 BPPCT039
35.6 EAC-MCTA-79
39.1 EPDCU3083
40.8 UDP98-416
44.0 EMPaS12
46.1 CPDCT037
50.7 EPPB4221-PR41
54.2 Ma039a
55.5 EAA-MCTT-525
57.1 PR110
73.3 EAT-MCTC-550
79.3 EMPA014
80.8 EMPaS02
89.4 EAT-MCCC-350
2006SC1card 78.8, *
2006SC2card 82.1, *
2006FCcard 88.9, *
2007SC1card 83.7, ***
2007SC2card 73.8, ***
2007FCcard 93.8, *
2008SC1card 87.3, *
2008SC2card 87.5, *
2008FCcard 94.7, *

QTL haplotypes

To further investigate the allele effects of the color QTLs, the progeny individuals were sorted by their parental QTL haplotypes that were defined by the allelic states of at least two linked markers (Table 1.9). The choice of the two markers to represent the QTL haplotype was based on the fact that they should flank the QTL and at least one marker should be heterozygous in one parent. For example, the NY and EF QTL haplotypes for the QTL on LG 3 at 53.7 cM were defined by the alleles for AFLP marker, EAC-MCTA-79 and SSR, Ma039a located at 35.6 cM and 54.2 cM, respectively. EAC-MCTA-79 is heterozygous in NY and homozygous in EF. To facilitate the genetic notation, other allele (i.e. cannot see in the gel and can be considered as a null allele) was designated as '$$' following the format used for FlexQTL (Bink et al. 2008), a software used in pedigree based QTL analysis using multiple populations. Only EF is heterozygous for Ma039a.

For the two QTLs on LG3 and LG6, there were four parental QTL haplotypes (a, b, c and d) as each parent was heterozygous for haplotypes. However, for LG5 and LG8 there were three parental QTL haplotypes as NY was homozygous for the LG5 QTL haplotype and EF was homozygous for the LG8 haplotype.

The progeny color trait means were then calculated for each of the QTL haplotypes. For example, for the QTL on LG3 at 53.7 cM, four progeny classes were defined as "ac", "ad", "bc", and "bd" and the trait means were calculated from 29, 23, 23, and 14 progeny individuals, respectively (Table 1.10). Those progeny individuals that

received the LG3 "a" haplotype from NY had intensified color, most notably it increased darkness (card) and reduced redness (a*) in SC1, increased darkness (card) and increased blueness (b*) in SC2 and increased darkness (card and L*) and increased blueness (b*) in FC as many of the fruits were approaching mahogany color in both skin and flesh. The lighter colored fruit had the LG3 "b" haplotype from NY. As NY has dark mahogany skin and flesh, this finding for the major QTL on LG3, supports the prior observation that mahogany fruit (skin and flesh) is dominant to yellow fruit.

Both EF and NY were heterozygous for the SC1 L*, SC1 b*, SC2 L*, SC2a* and FC a* QTL haplotypes on LG3 at ~21.0 cM (Table 1.11). Those progeny received "a" haplotype from NY had increased darkness (L*) and increased blueness (b*) in SC1 and increased lightness (L*) and reduced redness (a*) in SC2. But the same haplotype "a" increased the redness (a*) in FC suggesting that flesh color is under different genetic control.

Both EF and NY were also heterozygous for the SC1 b* QTL haplotype on LG6 (Table 1.12). Those progeny that received the "d" haplotype from EF had a reduced b* value (increased blueness) compared to those that received the "c" haplotype. However, this effect was only present for those individuals that had the "b" haplotype from NY, not the "a" haplotype. This suggests that the QTL alleles on LG6 may interact.

For the FC a* QTLs on LG5 and LG8 only one of the parents was heterozygous. NY was homozygous for the FC a* QTL haplotype on LG5 (Table 1.13). Therefore the

trait means were calculated from those progeny individuals that received either the "c" or "d" haplotypes from EF. Those progeny individuals that received the "c" haplotype from EF had increased redness compared to those progeny that received the "d" haplotype. EF was homozygous for the FC a* QTL haplotype on LG8 (Table 1.14). Therefore the trait means were calculated from those progeny individuals that received either the "a" or "b" haplotype from NY. The presence of the "a" haplotype as opposed to the "b" haplotype was associated with an increase in red color.

Table 1.9: Definitions of parental haplotypes for five QTL regions on linkage groups 3, 5, 6 and 8

Linkage group	Parent	Haplotype	Molecular marker	
			EAC-MCTA-79	Ma039a
3 at 53.7 cM	NY	a	79[x]	170
		b	$$[y]	170
	EF	c	$$	220
		d	$$	170
			MA066a	BPPCT039a
3 at ~21.0 cM	NY	a	149	138
		b	142	138
	EF	c	142	145
		d	142	138
			BPPCT026	UDP96-019
5	NY	a	164	202
	EF	c	164	205
		d	170	202
			EMPaS01	UDP96-001
6	NY	a	228	129
		b	222	131
	EF	c	228	129
		d	232	115
			MD201a	PS1H3
8	NY	a	250	280
		b	230	270
	EF	c	230	270

[x]Allele fragment size in bp [y]$$: Confirmed null allele for marker, EAC-MCTA-79

Table 1.10: Card and a* of skin color 1 (SC1), Card and b* of skin color 2 (SC2) and Card, L8 and b* of flesh color (FC) of different genotype classes for the major QTL on linkage group 3 at 53.7 cM. Numbers in parenthesis are the number of progeny individuals

Haplotype Combination[a]	Color Metric[b]						
	SC1		SC2		FC		
	Card	a*	Card	b*	Card	L*	b*
ac (29)	7.3 a	8.2 a	6.8 a	4.0 a	3.5 a	21.0 a	4.5 a
ad (23)	7.3 a	7.2 a	6.9 a	3.0 a	3.7 a	21.2 a	3.7 a
bc (23)	6.2 b	15.0 b	5.2 b	9.8 b	2.1 b	27.8 b	9.9 b
bd (14)	6.7 b	13.6 b	6.0 b	6.8 b	2.3 b	25.7 b	8.9 b

Means denoted by the same letters within column are not significantly different at $P < 0.05$ (done using Least Squares Means, General Linear Model SAS 9.1)

[a] a, b, c, and d are the haplotypes as defined in Table 1.9

[b] Units: card (color card categories), L*, a* and b* (colorimeter reading)

Table 1.11: L* and b* of skin color 1 (SC1), L* and a* of skin color 2 (SC2) and a* of flesh color (FC) of different genotype classes for the minor QTL on linkage group 3 at ~21.0 cM. Numbers in parenthesis are the number of progeny individuals

Haplotype Combination[a]	Color Metric[b]				
	SC1		SC2		FC
	L*	b*	L*	a*	a*
ac (14)	27.3 a	0.3 a	26.4 a	4.7 a	4.4 a
ad (32)	28.7 a	2.9 b	31.4 b	7.2 b	3.6 a
bc (13)	30.4 a	4.8 b	32.1 b	9.2 b	2.4 b
bd (09)	29.9 a	3.9 b	32.2 b	14.2 b	1.6 b

Means denoted by the same letters within column are not significantly different at $P <$ 0.05 (done using Least Squares Means, General Linear Model SAS 9.1)

[a]a, b, c, and d are the haplotypes as defined in Table 1.9

[b]Units: card (color card categories), L*, a* and b* (colorimeter reading)

Table 1.12: b* of skin color 1 (SC1) of different genotype classes for the QTL region on linkage group 6. Numbers in brackets are the number of progeny individuals

Haplotype Combination[a]	Color Metric[b] SC1 b*
ac (20)	2.7 a
ad (30)	3.1 a
bc (18)	3.2 a
bd (18)	2.2 a

Means denoted by the same letters within column are not significantly different at $P <$ 0.05 (done using Least Squares Means, General Linear Model SAS 9.1)

[a] a, b, c, and d are the haplotypes as defined in Table 1.7

[b] Units: card (color card categories), L*, a* and b* (colorimeter reading)

Table 1.13: a* of flesh color (FC) of different genotype classes for the QTL region on linkage group 5. Numbers in brackets are the number of progeny individuals

Haplotype Combination[a]	Color Metric[b] FC a*
ac (33)	3.7 a
ad (38)	2.8 a

Means denoted by the same letters within column are not significantly different at $P <$ 0.05 (done using Least Squares Means, General Linear Model SAS 9.1)

[a]a, c, and d are the haplotypes as defined in Table 1.7

[b]Units: card (color card categories), L*, a* and b* (colorimeter reading)

Table 1.14: a* of flesh color (FC) of different genotype classes for the QTL region on linkage group 8. Numbers in brackets are the number of progeny individuals

Haplotype Combination[a]	Color Metric[b] FC a*
ac (31)	3.0 a
bc (33)	3.5 a

Means denoted by the same letters within column are not significantly different at $P <$ 0.05 (done using Least Squares Means, General Linear Model SAS 9.1)

[a]a, b, c, and d are the haplotypes as defined in Table 1.7

[b]Units: card (color card categories), L*, a* and b* (colorimeter reading)

Epistasis

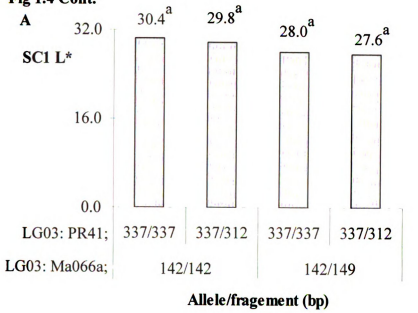
The bimodal pattern of some of the progeny distributions suggested the possibility of epistasis. To investigate this further, the major QTL on LG3 at 53.7 cM was considered as the major factor and the QTLs on LG3 at ~21.0 cM, LGs 5, 6 and 8 were considered separately as the second factor. The trait values for the different allelic states were determined using markers from the QTL peak positions.

The mean SC1 L* and SC2 L* values for genotypic classes defined by PR41 (the marker selected to represent major QTL on LG3) and Ma066a (the marker selected to represent minor QTL on LG3) were not different indicating there is no epistatic interaction for SC1 L* and SC2 L*. The mean SC1 b* values for PR41 genotypes when Ma066a was homozygous were greater than the mean SC1 b* values for PR41 genotypes when Ma066a was heterozygous. This indicates the epistatic interaction for SC1 b* between major and minor QTLs on LG3. The mean SC2 a* was highest for those progeny individuals that were heterozygous for PR41 and Ma066a and lowest for those progeny individuals that werehomozygous for PR 41 and heterozygous for Ma066a. This suggests a possible epistatic interaction for SC2 a* between major and minor QTLs on LG3. The mean FC a* values for PR41 genotypes when Ma066a was heterozygous were greater than the mean FC a* values for PR41 genotypes when Ma066a was homozygous. This indicates the epistatic interaction for SC1 b* between major and minor QTLs on LG3 (Figure 1.4).

The mean FC a* values for those progeny individuals that were heterozygous for PR41 (a marker near the LG3 QTL peak) were similar, irrespectively of differences in the QTL allelic states for LG8 and LG5. However, for those progeny that were homozygous for PR41, the allelic states for PS1H3 (LG8 FC a* QTL) and BPPCT026 (LG5 FC a* QTL) did result in different color outcomes. This suggests that there may be an epistatic interaction between the QTL(s) on LG3 and the QTLs on LG5 and LG8. In contrast, for the LG6 SC1b* QTL, a maximum trait value was obtained for those progeny that had the 131 and 129 bp alleles for UDP96-001, irrespective of the allelic state at the PR41 locus (Figure 1.5).

Figure 1.4: A-E. The two-way inter genomic region interactions between the major QTL and the minor QTL regions on LG3 (A) Inter loci interaction for SC1 L* between PR41 and Ma066a (B) Inter loci interaction for SC1 b* between PR41 and Ma066a (C) Inter loci interaction for SC2 L* between PR41 and Ma066a (D) Inter loci interaction for SC2 a* between PR41 and Ma066a (E) Inter loci interaction for FC a* between PR41 and Ma066a. Means denoted by same letter within each graph are not significantly different at $P < 0.05$ (done using Least Squares Means, General Linear Model SAS 9.1). Units: L*, a* and b* (colorimeter reading)

Fig 1.4 Cont.


A
32.0
16.0
0.0
SC1 L*
30.4a
29.8a
28.0a
27.6a
LG03: PR41;
337/337
337/312
337/337
337/312
LG03: Ma066a;
142/142
142/149
Allele/fragement (bp)

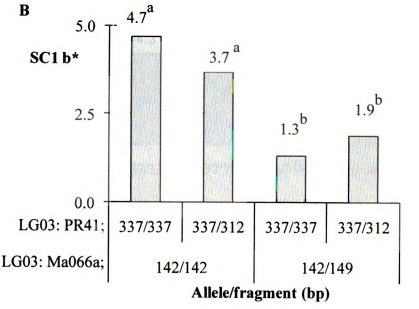
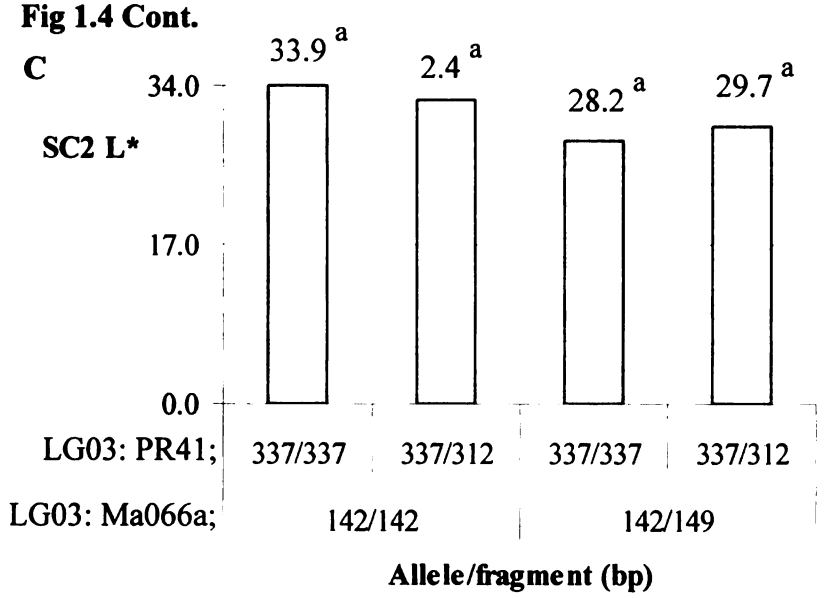

B
5.0
2.5
0.0
SC1 b*
4.7a
3.7a
1.3b
1.9b
LG03: PR41;
337/337
337/312
337/337
337/312
LG03: Ma066a;
142/142
142/149
Allele/fragment (bp)

Fig 1.4 Cont.


C
34.0
SC2 L*
17.0
0.0
33.9 a
2.4 a
28.2 a
29.7 a
LG03: PR41;
337/337
337/312
337/337
337/312
LG03: Ma066a;
142/142
142/149
Allele/fragment (bp)

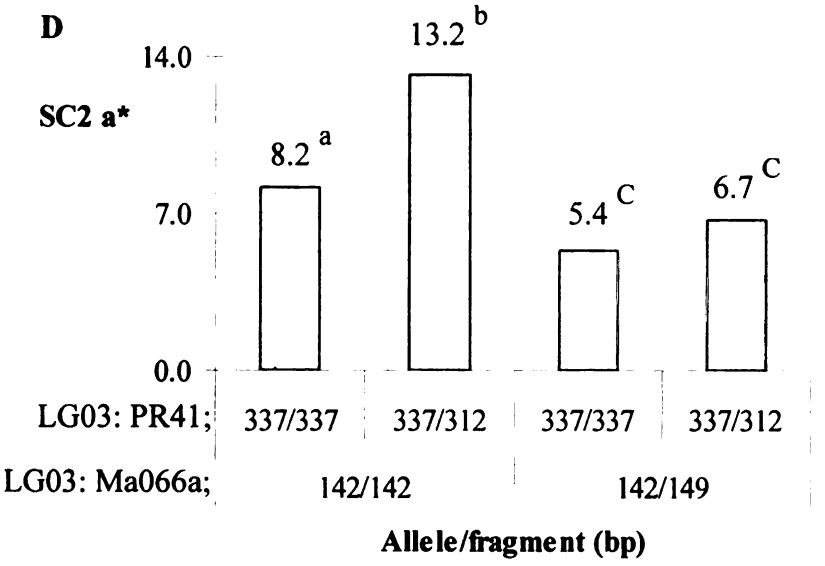

D
14.0
SC2 a*
7.0
0.0
8.2 a
13.2 b
5.4 C
6.7 C
LG03: PR41;
337/337
337/312
337/337
337/312
LG03: Ma066a;
142/142
142/149
Allele/fragment (bp)

Fig 1.4 Cont.

E

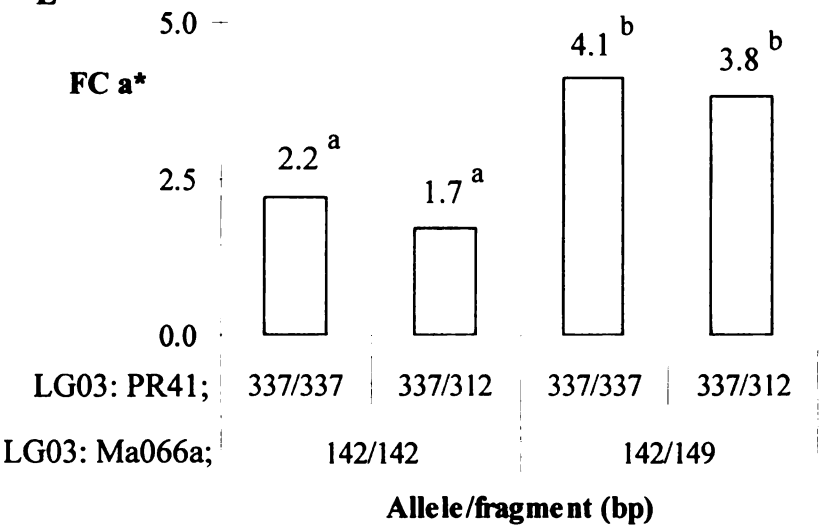
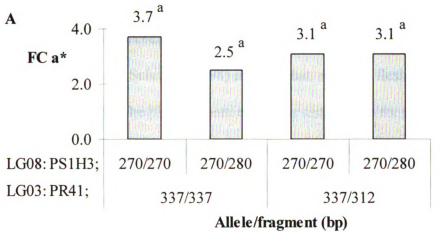
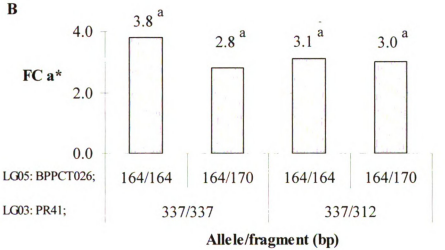
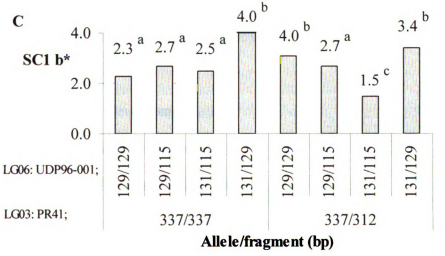

5.0
FC a*
2.5
0.0
2.2 a
1.7 a
4.1 b
3.8 b
LG03: PR41;
337/337
337/312
337/337
337/312
LG03: Ma066a;
142/142
142/149
Allele/fragment (bp)

Figure 1.5: A-C. The two-way inter genomic region interactions between the major QTL region on LG 3 and the other QTLs on LGs 5, 6 and 8. (A) Inter loci interaction for FC a* between PR41 on LG 3 and PS1H3 on LG8. (B) Inter loci interaction for FC a* between PR41 on LG 3 and BPPCT026 on LG5 (C) Inter loci interaction for SC1 b* between PR41 on LG 3 and UDP96-001 on LG6 Means denoted by same letter within each graph are not significantly different at $P < 0.05$ (done using Least Squares Means, General Linear Model SAS 9.1). Units: L*, a* and b* (colorimeter reading)


A
FC a*
4.0
2.0
0.0
3.7 a
2.5 a
3.1 a
3.1 a
LG08: PS1H3;
LG03: PR41;
270/270
270/280
270/270
270/280
337/337
337/312
Allele/fragment (bp)


B
FC a*
4.0
2.0
0.0
3.8 a
2.8 a
3.1 a
3.0 a
LG05: BPPCT026;
LG03: PR41;
164/164
164/170
164/164
164/170
337/337
337/312
Allele/fragment (bp)


C
SC1 b*
4.0
2.0
0.0
2.3 a
2.7 a
2.5 a
4.0 b
4.0 b
2.7 a
1.5 c
3.4 b
LG06: UDP96-001;
LG03: PR41;
129/129
129/115
131/115
131/129
129/129
129/115
131/115
131/129
337/337
337/312
Allele/fragment (bp)

Identification of QTLs for fruit skin and flesh color in sweet cherry is important for marker assisted breeding and to discover the underlying genes. Two previous studies by Fogle (1958) and Schmidt (1998) showed that, skin and flesh color in sweet cherry is a major genic trait. The bimodal pattern of data distribution for fruit color and the higher presence of mahogany skinned and red fleshed individuals in the segregating progenies are in agreement with the these studies. But higher heritability values for all the color metrics except red-green vector of lighter side of the blush cherries (SC2 a*), explain the very high genetic control of the fruit color in sweet cherry. However, this study suggests that minor genes may also control the variation for skin and flesh color in this cross.

The higher correlation between skin and flesh color is in agreement with Fogle (1958) and Schmidt (1998). However, SC2 a* is not correlated to any other color metric. In blush fruits, SC2 a* is not very important as this location is yellow in color. But in mahogany color fruits, a* is important but less important compared to lightness/darkness (L*) and yellowness/blueness (b*) as the visual color is very dark blackish purple and that kind of color is more explained by L* and b*. This implies that SC2 a* is more subjected to environmental effects than any other color vector.

The color development pattern of the skin and flesh with fruit maturity was in agreement with the previous study by Usenik et al. (2005) except in our study we observed that redness, a*, is also decreasing with time. However, Usenik's study didn't include any blush varieties and segregating populations, but the dark varieties he used,

Van, Sunburst and Elisa have different a* development compared to our dark fruited variety, NY54.

The QTL analysis for fruit skin and flesh color in sweet cherry is challenging because the frequency distributions significantly deviate from normality. This has two consequences on the LOD score estimations; an increased Type 1 error rate and the inflated LOD values to levels where they cannot be compared between the different traits (Buil, 2005). The present study encountered these two problems and the MapQTL 5.0 manual (Van Ooijen, 2004) suggested that single marker analysis-Kruskalis Wallis Test could be used to verify the QTLs derived under such conditions. The QTLs presented in this paper were verified with that procedure but care must be taken when interpreting the inflated LOD scores for QTLs.

The detection of a major QTL on LG3 for all the color metrics suggest that, pigmentation is controlled by a major gene on LG3. Four other QTLs on LGs 3, 5, 6 and 8 suggest that genetic control of fruit color is also controlled by other genes with minor effects. However, very high R^2 values for QTLs on LG3 suggest that the QTL on LG3 is the major gene. Previous studies suggested an epistatic interaction between one major and one minor gene. The present study is in agreement with those hypotheses and also suggested that the epistatic interactions would be more complex with different allelic states.

CONCLUSION

This study identified a major QTL for skin and flesh color of sweet cherry on LG 3 and four minor QTLs on LG3, LG5, LG6 and LG8. The genomic regions and parental haplotypes in the QTL regions will serve as a basis for future fine mapping and candidate gene studies designed to determine the genetic change underlying the color QTL. In breeding perspective, this QTL information will expedite the production of sweet cherry varieties with desired skin and flesh colors.

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CHAPTER TWO

GENETIC DIVERSITY ANALYSIS OF SWEET CHERRY (*Prunus avium* L.) CULTIVARS USING DNA MARKERS

INTRODUCTION

Sweet cherry (*Prunus avium* L.), belonging to family *Rosaceae,* is an important temperate fruit crop. *P. avium* originated in central Asia and Europe where wild cherry, also *P. avium,* is an important timber tree. The wild cherries, landraces and improved cultivars represent the genetic diversity of *P. avium* (Iezzoni et al. 2008). Early settlers brought sweet cherry seeds and budwood to the New World from Europe. Most probably they would have brought a small number of selected land races from Europe. Early settlers in the New World selected the best sweet cherry seedlings such as "Bing" and landraces such as "Lambert" from the original material for the large scale planting. The advanced selections and the original material of sweet cherry brought to the New World represent the sweet cherry germplasm in the Pacific North West (PNW) region in North America. The PNW sweet cherry germplasm therefore, may have undergone genetic founder effect when early settlers selected seeds and budwood from the natural habitat to carry with them. Previous studies suggested that the PNW sweet cherry germplasm may have a narrow genetic base. The low genetic polymorphism was reported by Stockinger et al. (1996) and Gerlach and Stosser (1997) with randomly amplified polymorphic DNA (RAPD) markers, and Beaver et al. (1995) and Granger (1993) with isozyme markers. But no studies have been conducted to assess the genetic founder effect with more comprehensive simple sequence repeat (SSR) and gene based markers. If the genetic founder effect could be assessed with DNA markers with reference to the current genomic information of *P. avium*, it would provide a strong platform for germplasm enhancement and crop improvement of sweet cherry and tart cherry (*P. cerasus*), for whom *P. avium* was one of the two parents. We took the advantage of DNA markers and

linkage maps available from various studies for *Prunus avium* and other *Prunus* species (Clarke and Tobutt 2003; Dirlewanger et al. 2002; Joobeur et al. 1998; Dirlewanger et al. 2004; Olmstead et al. 2008) which could be used to estimate and visualize the genetic diversity at genome level.

In the present study, the DNA polymorphisms among PNW sweet cherry germplasm (defined by 28 landraces and cultivars historically used and released in PNW region) (abbreviated as PNW from this point onwards), seven European sweet cherry land races which were not used in PNW sweet cherry breeding (abbreviated as non-PNW from this point onwards) and one wild cherry selection (New York 54) from Germany were compared using DNA markers to test the hypothesis of genetic founder effect in PNW sweet cherry germplasm. The specific objectives of this study were to, 1. Assess the genetic founder effect in PNW sweet cherry germplasm, diversity and genomic relationships among PNW, non-PNW and wild sweet cherry germplasm groups. 2. Examine the level of heterozygosity and allele diversity across the cherry genome. 3. Define a subset (panel of six individuals) of *P. avium* selections for single nucleotide polymorphism (SNP) detection to develop high throughput DNA markers. 4. Recommend a panel of few DNA markers that can effectively be used for in-house *P. avium* DNA fingerprinting purposes without any ambiguity.

MATERIALS AND METHODS

Plant materials

Thirty-six *P. avium* selections were chosen for the study that represented PNW sweet cherry germplasm (defined by 28 landraces and cultivars historically used and released in PNW breeding programs), seven European sweet land races which were not used in PNW sweet cherry breeding and one wild cherry selection (New York 54) (Table 2.1).

Leaves were collected from trees growing at Michigan State University's Clarksville Horticultural Research Station, Clarksville, Michigan and the North West Horticultural Research Station, Traverse City, Michigan or Washington State University's Irrigated Agricultural Research Center, Prosser, Washington. Immature and actively growing leaf samples were collected from all the selections in early spring, placed immediately in dry ice, moved to the laboratory and frozen for 24 hours at 80 ^{0}C. The frozen leaf samples were freeze dried for 48 to 72 hours and stored at -20^{0}C until DNA extraction.

DNA extraction and genotyping

DNA was extracted using the cetyl trimethylammonium method described by Stockinger et al. (1996). PCR conditions were as in Olmstead et al. 2008 except for the EMPA and EMPAS markers, where, touch-down PCR temperature profile was used (Clarke and Tobutt 2003). The *S*-locus was genotyped using the *S*-RNase allele-specific

primers, S_1-S_6 (Sonneveld et al. 2001), S_7-S_9 and S_{12} (Sonneveld et al. 2003). The gene or expression sequence tag (EST) based markers (PR markers) were genotyped according to the method described by Olmstead et al. (2008). SSR markers were size separated in 6% denaturing poly acrylamide gels and visualized using silver stain (Bio-Rad Laboratories, Hercules, CA, USA). The *S*-RNase and PR markers were resolved in 4% agarose gels.

Data analysis

The different alleles for each marker were identified by fragment base pair (bp) size for SSR and PR markers and as nominal data for the *S*-locus. Some of the sweet cherry selections were related, e.g. parents or grandparents, and the marker genotypes for these individuals were checked to verify that these genotypes were consistent with the pedigree relationships (see Table 2.1 for pedigree information). Additionally, three populations were available from the crosses between New York 54 (NY54) and Emperor Francis (EF) (NY x EF: 190 individuals, Olmstead et al. 2008), Powdery Mildew Resistant-1 (PMR1) and Rainier (PMR1 x Rainier: 108 individuals), PMR1 and Bing (PMR1 x Bing: six individuals) and PMR1 and Van (PMR1 x Van: five individuals) (Olmstead 2001). These crosses were used to validate the inheritance pattern of the alleles and to detect the presence of null alleles. The confirmed null alleles were designated as $$ and unconfirmed null alleles which potentially could be homozygous were designated as $ following the format used for FlexQTL® (Bink et al. 2008). The markers, whose putative alleles showed inconsistent segregation patterns, duplicate or multiple loci, or stutter bands/smears were not included in the analysis.

The unique alleles (UA) for each cultivar and for PNW and non-PNW groups were identified. The percentage of heterozygous loci (% H) for each sweet cherry selection was calculated as the proportion of heterozygous loci out of total number of marker loci scored for that cultivar. The frequency of each allele of marker (p_i) was calculated and used to estimate heterozygosity (H) and polymorphic information content (PIC) for the 77 DNA markers. The H and PIC were calculated as explained in Botstein (1980) and Shete et al. (2000) using the following formula: *Pi* is the frequency of i^{th} allele, and *n* is the number of alleles.

$$H = 1 - \sum_{i=1}^{n} p_i^{\,2}$$

$$PIC = 1 - \sum_{i=1}^{n} p_i^{\,2} - \left[\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} p_i^{\,2} p_j^{\,2} \right]$$

The allele frequencies were classified to identify the rare alleles and to see their presence or absence in the PNW sweet cherry germplasm.

Allele data were converted to the binary format (1: presence of the allele and 0: absence of the allele for a given *P. avium* selection) and a dendrogram was constructed using the UPGMA method of McQuitty linkage (McQuitty and Koch 1975) and Absolute Correlation Coefficient Distance (Minitab 15.0) to show the genetic relatedness among the 36 selections.

The distribution of alleles for marker loci along the linkage groups of *P. avium* (Olmstead et al. 2008) was examined to identify the genomic areas where non-PNW and wild type alleles were detected. The %H for all the linkage groups were examined for PNW, non-PNW and wild *P. avium* groups. The graphical genotypes (GGT) for all the linkage groups (LG) of all 36 selections were illustrated to visually represent the alleles of all the loci across the sweet cherry genome.

RESULTS AND DISCUSSION

Unique and rare alleles

A total of 300 alleles were identified for 77 DNA markers from the 36 sweet cherry selections (Table 2.2 and Figure 2.1). 52 unique alleles (UA) (an allele only found in a single sweet cherry selection) out of 300 alleles were identified, 40 of which were not detected in the PNW sweet cherry germplasm. The wild selection, NY54, had the highest number (13) of UA followed by the European landraces, Ambrunus (had nine UA) and Cristobalina (had six UA) (Table 2.1). The higher presence of UA outside the PNW sweet cherry germplasm support our hypothesis that PNW sweet cherry germplasm had been undergone the genetic founder effect when early settlers brought sweet cherry germplasm to the New World.

The frequency for all 300 alleles showed that rare alleles (those alleles with frequency of less than 0.20) were more common (147) and notably 44 out of the 147 alleles (30%) were absent in the PNW sweet cherry germplasm (Table 2.2 and Figures 2.1 and 2.2). The 30% absence of rare alleles also validates the hypothesis of genetic founder effect in PNW sweet cherry germplasm.

Allele diversity and cultivar heterozygosity

A total of 256, 253 and 112 alleles were detected in PNW, non-PNW and wild sweet cherry groups respectively. All three groups shared 31% of total alleles detected) (Figure 2.1). PNW and non-PNW group shared 74% of total alleles showing that they

were evolutionary more related than their individual relationships to wild cherry. The PNW and non-PNW groups shared 32% of total alleles with wild cherry (Figure 2.1).

The percentage of heterozygous loci (%H) was highest in EF. This bias resulted from the initial marker screening to identify allele polymorphism was based on EF and wild cherry, NY54 (Olmstead et al. 2008). However, all the %H values for the other 34 selections and groups were not significantly different (Table 2.1) (statistical analysis is not shown). This is due to the facts that *P. avium* maintain higher level of genetic heterozygosity through the reproductive mechanism of self incompatibility and the heterozygosity for cultivars with no progeny or pedigree data available cannot be exactly determined due to the presence of possible null alleles. Therefore, the cultivar %H is not a good parameter to test the hypothesis of genetic founder effect. The % H for individual linkage groups (LG) of PNW, non-PNW and wild *P. avium* groups were also compared and only LG8 for PNW and non-PNW had lower level of H compared to other LGs and also compared to the LG8 of NY54 (Table 2.3). This could lead to the hypothesis that LG8 may contain many important agronomic traits and therefore, subjected to more intense selection in the processes of domestication and breeding (Table 2.3).

A total of 44 alleles were detected from the eight European landraces and wild cherry which were not present in the PNW sweet cherry germplasm (Tables 2.1, 2.4-2.11 and Figure 2.1). The genomic locations of these 44 alleles were identified and their distribution among the eight *P. avium* linkage groups were graphically displayed along with other alleles (Figure 2.2). A total of 11, 7, 5, 4, 5, 7, 2 and 3 alleles which were

novel to PNW sweet cherry breeding germplasm were detected on LGs 1, 2, 3, 4, 5, 6, 7 and 8 respectively. These allele numbers indicate that LGs 1 to 6 has undergone the genetic founder effect than that of LGs 7 and 8. In figure 2.2 on each LG, the names of European landraces or NY54 which provide the novel alleles are shown. LGs 1, 2 and 6 showed that UA were widely spread along the LGs. Implying the missing DNA diversity as a whole LG8. LGs 7 and 8 were least diverse in terms of UA. Only wild cherry, NY54 and Katalin brought UA to LG7, and NY54, 19-21B and Eugenia brought UA to LG8.

Table 2.1: The *Prunus avium* groups, selections (wild, Non-PNW and PNW), their parents, origins, number of unique alleles (UA) and % of heterozygous loci (H)

Group	Selection	Parent 1	Parent 2	Origin	UA	H	Class H
Wild	NY54	U[a]	U	Germany	13	49.4	49.4
Non-PNW	19-21B	U	U	Ukraine	1	36.9	46.5
	Ambrunes	U	U	Spain	9	50.1	
	Cristobalina	U	U	Spain	6	39.4	
	Eugenia	U	U	N[b]. Europe	4	54.9	
	Katalin	U	U	Hungary	4	43.8	
	Krupnoplodnaya	U	U	Romania	1	50.6	
	Windsor	U	U	N. Europe	2	49.8	
PNW	Emperor Francis	U	U	N. Europe	4	79.8	53.6
	Benton	Stella	Beaulieu	USA	0	57.4	
	Bing	Black-Republican	U	USA	0	63.7	
	Brooks	Rainier	Early-Burlat	USA	0	48.6	
	Chelan	Stella	Beaulieu	USA	0	49.0	
	Chinook	Bing	Gil-Peck	USA	0	52.2	
	Glacier	Stella	Early-Burlat	USA	1	50.8	
	Lambert	U	U	USA	1	45.2	
	Lapins	Van	Stella	USA	0	50.9	
	Napoleon	U	U	Germany	0	59.1	
	Newstar[c]	U	U	Canada	0	49.7	
	PC7147-009	Stella	U	USA	1	49.3	
	PC7903-002	PC7147-4	PC7146-11	USA	0	49.6	
	PC8007-002	Glacier	Cashmere	USA	0	43.6	
	PMR-1	U	U	USA	0	42.7	
	Rainier	Bing	Van	USA	0	58.1	
	Regina	Schneiders	Rube	Germany	4	54.9	
	Sam[c]	V-16U14U	U	Canada	0	51.1	
	Schmidt	U	U	Germany	0	69.3	
	Schneiders	U	U	Germany	0	52.2	
	Selah	P8-79	Stella	USA	0	47.4	
	Stella[c]	Lambert	JI242U	Canada	0	63.3	
	Summit[c]	Van	Sam	Canada	0	39.1	
	Sweetheart[c]	Van	Newstar	Canada	0	51.4	
	Tieton	Stella	Early-Burlat	USA	1	56.9	
	Ulster	Schmidt	Lambert	USA	0	55.3	
	Van[c]	Empress-Eugenie	U	Canada	0	58.1	
	Vic[c]	Bing	Schmidt	Canada	0	52.1	

[a]U: Unknown, [b]N: Northern, [c]Considered as PNW germplasm (closely related to other PNW selections)

Figure 2.1: The number of unique and shared alleles identified in the three groups of sweet cherry used in the study; PNW: 28 cultivars historically used and released in the Pacific North West sweet cherry breeding programs, Non-PNW: seven sweet cherry cultivars from Europe that have not been used in the PNW sweet cherry breeding programs and Wild: one forest (mazzard) cherry (*Prunus avium*) selection (NY54)

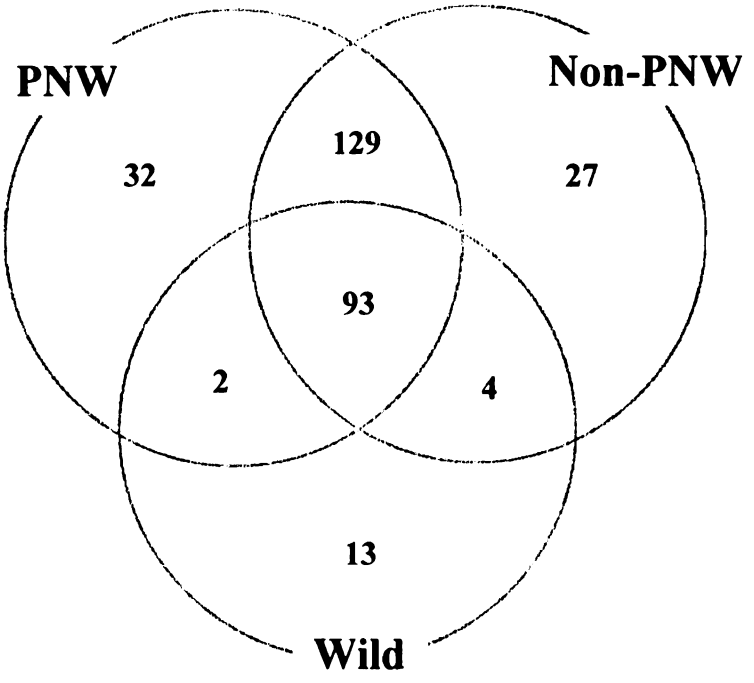


Table 2.2: The relative abundance of alleles with differing frequencies detected for 77 DNA markers

Allele frequency class	Number of alleles within each class	Number of alleles of each class that are not present in PNW
<0.20	147	44
0.20 - <0.40	77	0
0.40 - <0.60	50	0
0.60 - <0.80	22	0
0.80 - <1.00	4	0
Total number of alleles	300	44

Table 2.3: The percentage of heterozygous loci (H) per linkage group

LG	Number of loci per LG	PNW	Non-PNW	Wild (NY54)	Total
1	13	54.8	50.6	30.8	48
2	12	50.5	34.4	58.3	38
3	9	68.9	54.7	22.2	52
4	8	43.4	44.9	37.5	36
5	11	60.6	62.8	50.0	51
6	9	60.2	53.1	66.7	39
7	10	61.3	49.0	70.0	61
8	5	27.4	35.0	60.0	28
Total	77	53.4	48.1	49.4	44

Table 2.4: The possession of marker-alleles by sweet cherry selections - LG1

Map Position (cM)	Marker	Allele (bp)	Number of selections possesing the allele	If Number of selections possesing the allele is less than 3, selection names
0.0	CPPCT016	171	13	
		180	22	
		188	4	
		190	1	Katalin
		198	1	Regina
		200	1	Ambrunus
		204	1	Katalin
		208	16	
1.0	EMPA001	135	1	Cristobalina
		145	1	PC7147-009
		150	5	
		155	11	
		158	1	PMR-1
		160	13	
		165	21	
37.8	EMPA005	225	1	Cristobalina
		240	4	
		245	17	
		255	27	
45.1	EPDCU5100	184	7	
		195	2	EF, Schmidt
		198	7	
		202	1	Cristobalina
47.7	UCD-CH31	145	24	
		150	12	
		155	7	
		165	12	
		170	1	Krupnoplodnaya
50.0	PR33	244	25	
		250	2	Windsor, Sam
		266	24	
63.6	PCeGA59	184	28	
		190	26	
64.9	CPSCT027	204	33	
		216	5	
		218	23	
		220	1	Ambrunus
85.1	PMS67	148	26	
		160	9	
		162	4	
		165	22	
88.1	PR101	146	1	NY54
		152	36	
		158	6	
106.6	CPPCT019	180	31	
		186	8	
		188	1	Ambrunus
		190	1	EF
110.0	EMPA011	240	32	
		245	12	
		250	1	Ambrunus
116.0	EPPB4213	132	1	EF
		140	34	
		146	5	

Table 2.5: The possession of marker-alleles by sweet cherry selections - LG2

Map Position (cM)	Marker	Allele (bp)	Number of selections possesing the allele	If Number of selections possesing the allele is less than 3, selection names
0.0	MA069a	105	1	Wnidsor
		120	36	
		126	14	
		130	1	Lambert
1.5	CPSCT038	190	32	
		192	9	
		204	10	
4.1	UDA-059	134	28	
		138	8	
11	BPPCT034	225	18	
		235	12	
		250	2	Schmidt, Ulster
		255	16	
12.7	MA005c	190	26	
		198	25	
15.8	UDAp-461	159	15	
		178	32	
23.7	BPPCT002	168	1	NY54
		184	36	
		185	9	
		186	14	
32.4	MA007a	93	1	NY54
		104	19	
		110	10	
		116	22	
		126	1	Cristobalina
40.7	UDA-005	218	35	
		222	22	
		224	1	
46.4	UCD-CH12	175	26	
		180	11	
		182	8	
		186	1	19-21B
		190	4	
		192	11	
55.7	PCeGA34	135	12	
		143	9	
		145	1	NY54
		155	1	EF
		165	1	NY54
59.7	CPSCT037	190	7	
		195	13	
		210	32	

Table 2.6: The possession of marker-alleles by sweet cherry selections - LG3

Map Position (cM)	Marker	Allele (bp)	Number of selections possesing the allele	If Number of selections possesing the allele is less than 3, selection names
0.0	EPPCU5990	185	27	
		195	28	
0.1	PaCITA4	140	29	
		143	27	
23.2	PMS30	132	4	
		142	24	
		152	7	
		162	7	
		170	7	
		175	17	
25.8	BPPCT039	128	1	Cristobalina
		134	15	
		138	14	
		140	2	Ambrunus, Regina
		145	23	
32.4	EPDCU3083	145	24	
		153	26	
34.1	UDP98-416	200	18	
		210	33	
39.4	CPDCT037	145	6	
		155	1	Cristobalina
		158	2	Ambrunus, Windsor
		160	10	
		165	8	
		170	25	
47.5	MA039a	170	34	
		216	11	
		220	4	
		222	1	Regina
72.6	EMPA014	220	1	Ambrunus
		225	10	
		230	27	
		232	3	Cristobalina, Napoleon, Lambert
		233	8	
		234	4	
		235	8	

Table 2.7: The possession of marker-alleles by sweet cherry selections - LG4

Map Position (cM)	Marker	Allele (bp)	Number of selections possesing the allele	If Number of selections possesing the allele is less than 3, selection names
0.0	EPPCU3664	115	11	
		122	31	
		125	6	
		130	9	
6.4	EMPA015	220	14	
		222	13	
		225	1	NY54
		240	9	
13.1	AMPA110	135	34	
		138	14	
33.8	BPPCT040	120	11	
		125	12	
		128	1	EF
		130	1	Cristobalina
		135	9	
		145	17	
45.0	UDP97-402	118	1	NY54
		122	1	NY54
		126	3	Cristobalina, EF, Stella
		130	5	
		138	6	
50.0	M12a	180	33	
		185	10	
		190	1	Eugenia
59.5	UDA-037	423	5	
		425	11	
		431	20	
73.7	UDA-027	135	36	
		137	16	

Table 2.8: The possession of marker-alleles by sweet cherry selections - LG5

Map Position (cM)	Marker	Allele (bp)	Number of selections possesing the allele	If Number of selections possesing the allele is less than 3, selection names
0.0	EPPCU0961	146	20	
		148	27	
		150	15	
7.7	EPPCU9168	162	13	
		164	8	
		168	25	
20.7	BPPCT026	164	24	
		170	17	
		178	5	
		186	14	
38.4	UDP96-019	202	22	
		205	22	
47.4	BPPCT037	137	5	
		142	13	
		145	15	
		148	18	
		155	4	
		157	1	Windsor
49.0	EMPaS11	68	16	
		78	22	
		88	3	NY54, 19-21B, Ambrunus
		108	4	
		112	10	
61.9	EPDCU5183	120	20	
		125	2	Cristobalina, Schneiders
		140	4	
		145	1	Krupnoplodnaya
		150	8	
65.0	CPDCT016	150	16	
		160	33	
70.5	EPPB4230	253	9	
		254	7	
		255	1	Ambrunus
		256	24	
		260	7	
73.4	CPDCT022	145	3	NY54, Regina, Tieton
		150	19	
		155	11	
		158	1	Ambrunus
		165	7	
		175	6	
75.5	BPPCT014	190	22	
		192	3	Eugenia, EF, Schmidt
		195	15	

Table 2.9: The possession of marker-alleles by sweet cherry selections - LG6

Map Position (cM)	Marker	Allele (bp)	Number of selections possesing the allele	If Number of selections possesing the allele is less than 3, selection names
0.0	EMPaS01	222	5	
		228	27	
		232	18	
		240	1	Glacier
4.5	UDP96-001	110	1	Ambrunus
		115	7	
		129	28	
		131	15	
13.5	BPPCT008	90	8	
		97	31	
		100	4	
36.6	CPPCT023	170	7	
		171	5	
44	EPPCU3090	172	25	NY54
		180	1	
		185	27	
50.8	UDP98-021	102	27	
		112	24	
		118	1	NY54
51.3	EPPB4227	120	1	Tieton
		125	1	NY54
		130	19	
		135	32	
		145	4	
59.4	MA040a	210	20	
		215	1	Eugenia
		225	12	
		240	6	
66.8	S-Rnase	S-1	9	
		S-2	6	
		S-3	18	
		S-4	21	
		S-5	2	Krupnoplodnaya, PC7903-002
		S-6	3	NY54, Ambrunus, Eugenia
		S-7	1	Eugenia
		S-9	10	
		S-12	2	Katalin, Schneiders

Table 2.10: The possession of marker-alleles by sweet cherry selections - LG7

Map Position (cM)	Marker	Allele (bp)	Number of selections possesing the allele	If Number of selections possesing the allele is less than 3, selection names
0.0	CPPCT022	245	25	
		250	26	
		252	3	NY54, Chinook, Regina
13.1	UDAp-407	205	15	
		215	30	
		217	2	PC8007-002, PMR-1
14.0	CPSCT026	178	17	
		180	5	
14.5	UDAp-401	260	21	
		265	14	
		270	5	
		295	7	
15.5	EPDCU2931	132	15	
		146	3	Napoleon, Windsor, Lambert
		148	5	
		150	1	Katalin
		152	23	
		160	1	NY54
30.3	CPPCT033	145	10	
		148	12	
		149	4	
		150	13	
		152	3	EF, Schmidt, Vic
		158	3	
		164	13	
38.2	PMS2	130	8	
		142	22	
		146	24	
		165	4	
42.4	PS8e08	172	8	
		181	27	
		186	17	
45.0	PCHCMS2	670	18	
		730	28	
49.6	EPDCU3392	110	12	
		115	16	
		123	7	
		129	20	
		135	5	

Table 2.11: The possession of marker-alleles by sweet cherry selections - LG8

Map Position (cM)	Marker	Allele (bp)	Number of selections possesing the allele	If Number of selections possesing the allele is less than 3, selection names
0.0	pchgms49	156	35	
		168	11	
		170	3	Benton, Chelan, Tieton
		173	2	
13.4	EPPCU4726	160	34	
		162	2	
24.4	CPPCT006	188	4	
		190	29	
		204	1	Regina
		206	2	NY54, 19-21B
		208	14	
54.6	MD201a	230	34	
		250	6	
80.8	PS1H3	270	34	
		272	5	
		275	9	
		280	1	NY54
		285	1	Eugenia

Figure 2.2: A-H. The different alleles for the markers and their relative presence in all the linkage groups for 36 sweet cherry selections {wild cherry (gray bar), PNW (white bar) and non-PNW (black bar) groups}. The arrows show the alleles that do not exist in the PNW sweet cherry cultivars and the names of the cultivars are indicated near the arrows. A: linkage group (LG) 1, B: LG2, C: LG3, D: LG4, E: LG5, F: LG6, G: LG7, H: LG8

Fig 2.2 Cont. A: Linkage group 1

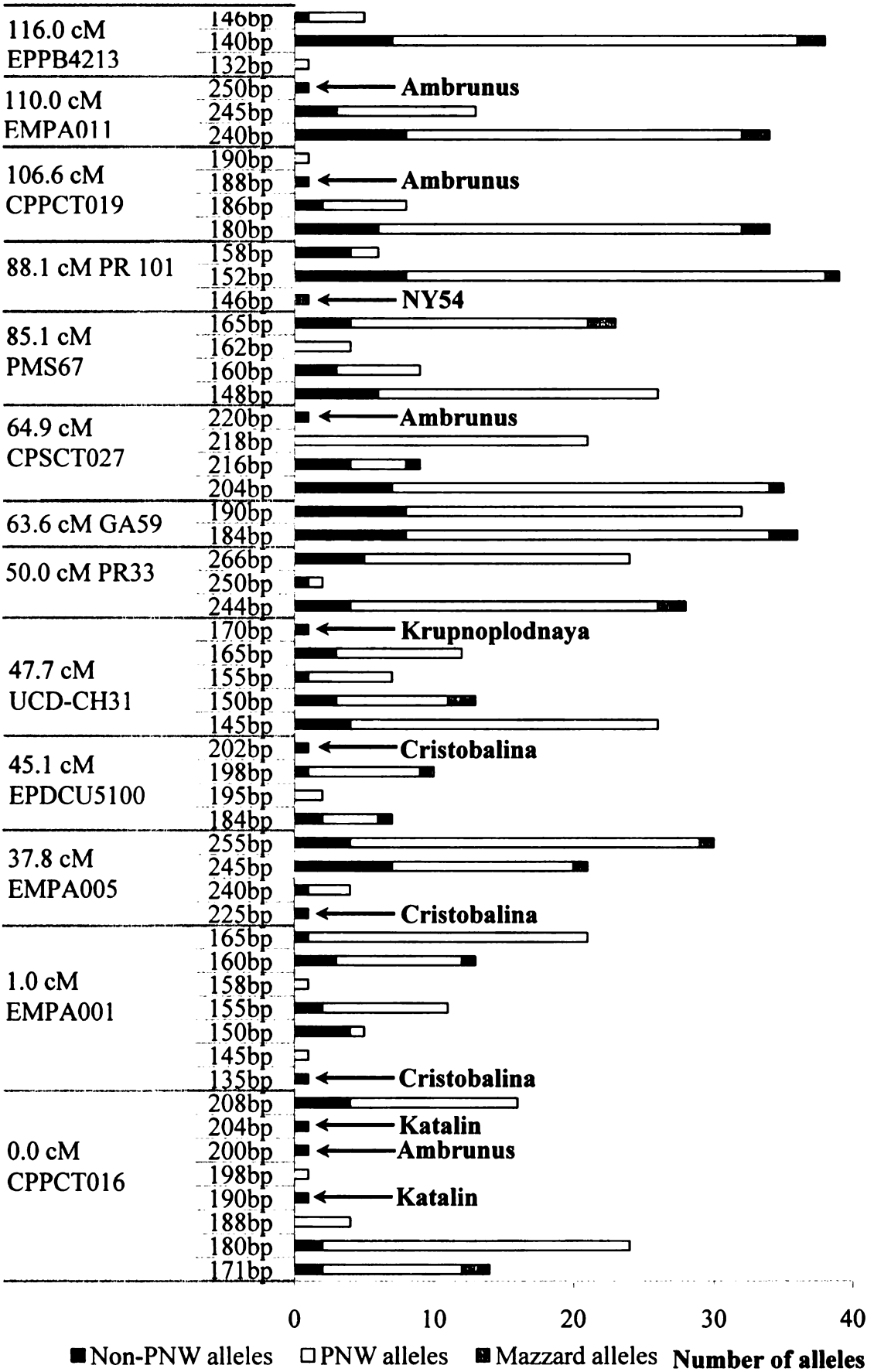


Fig 2.2 Cont. B: Linkage group 2

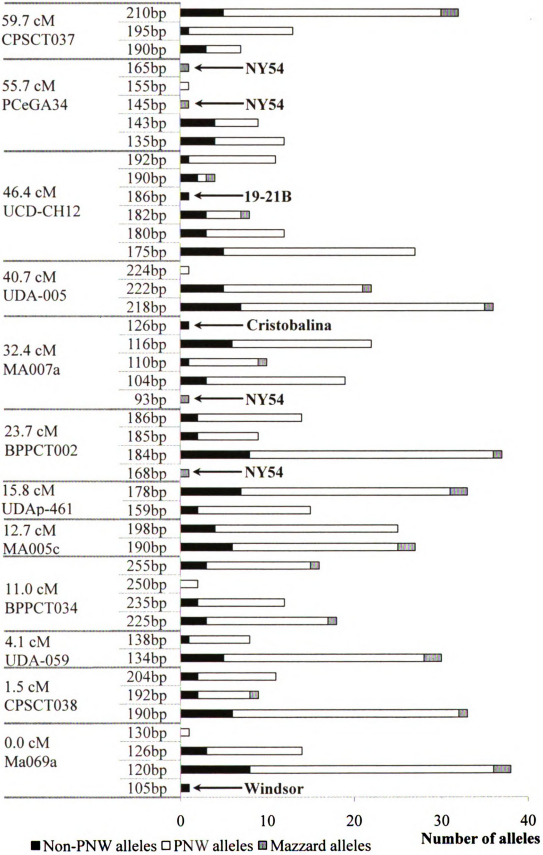


Fig 2.2 Cont. C: Linkage group 3

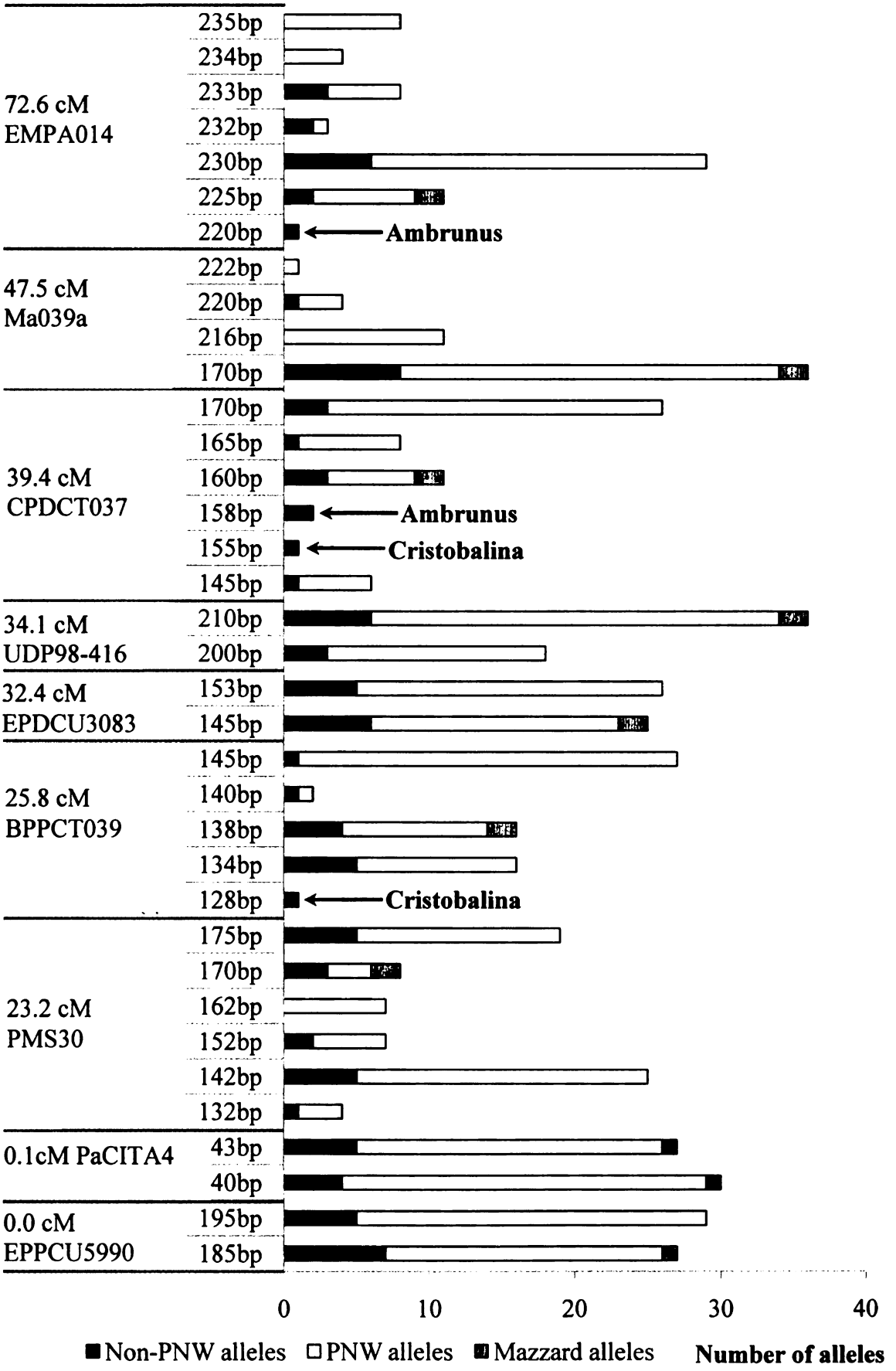


Fig 2.2 Cont. D: Linkage group 4

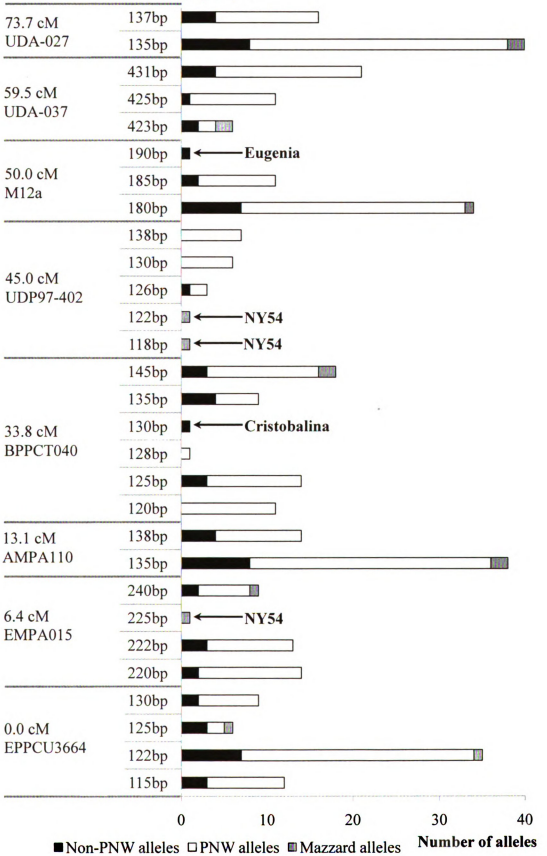


Fig 2.2 Cont. E: Linkage group 5

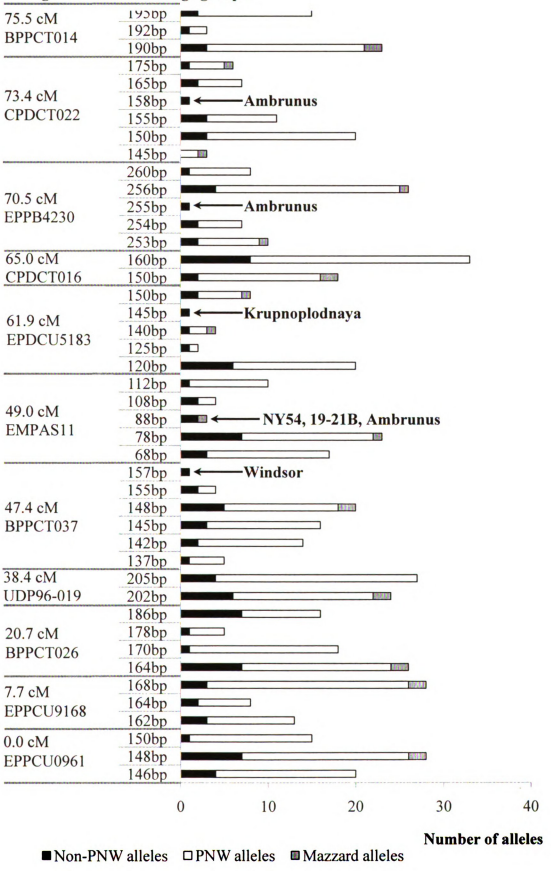


Fig 2.2 Cont. F: Linkage group 6

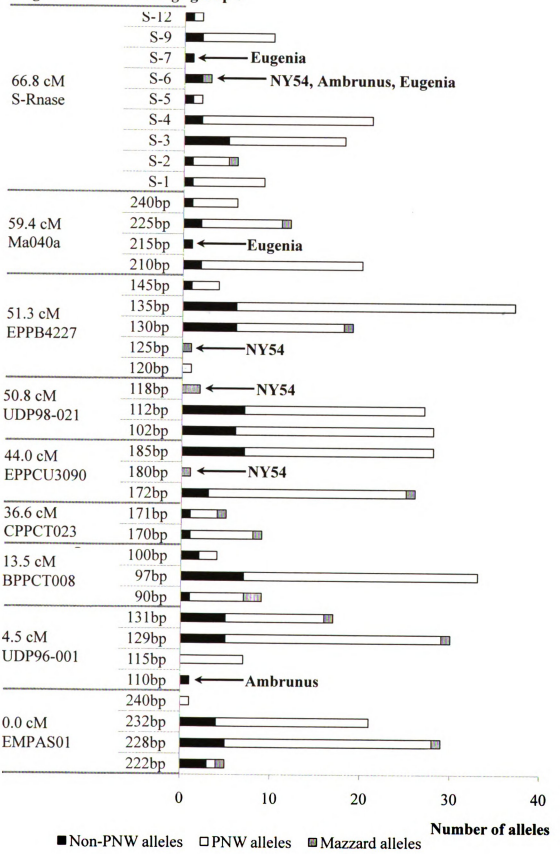


Fig 2.2 Cont. G: Linkage group 7

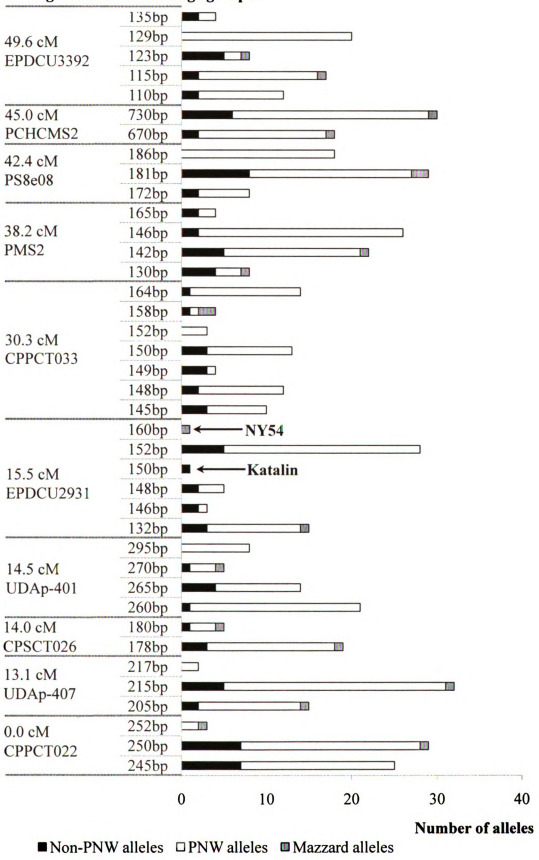
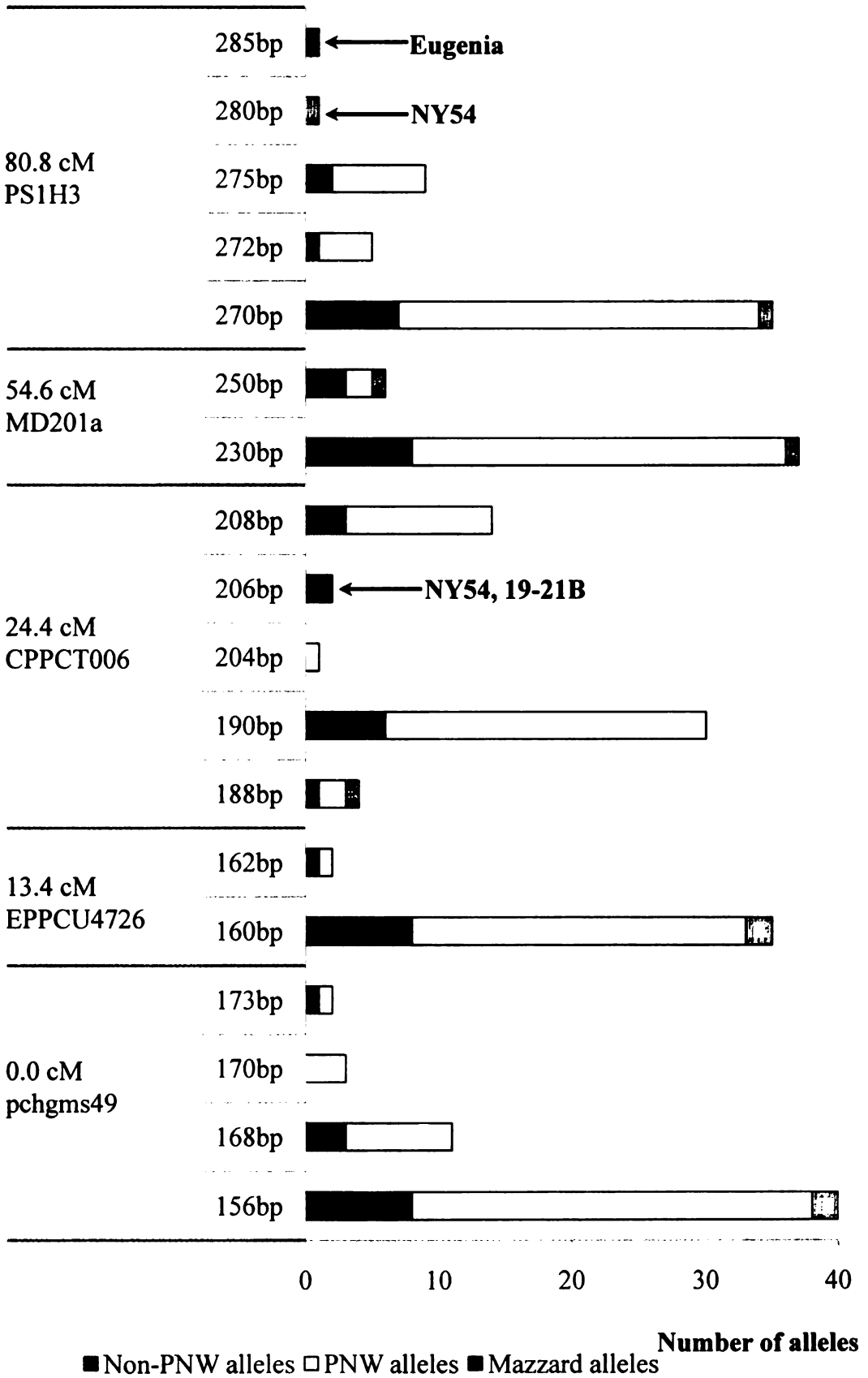


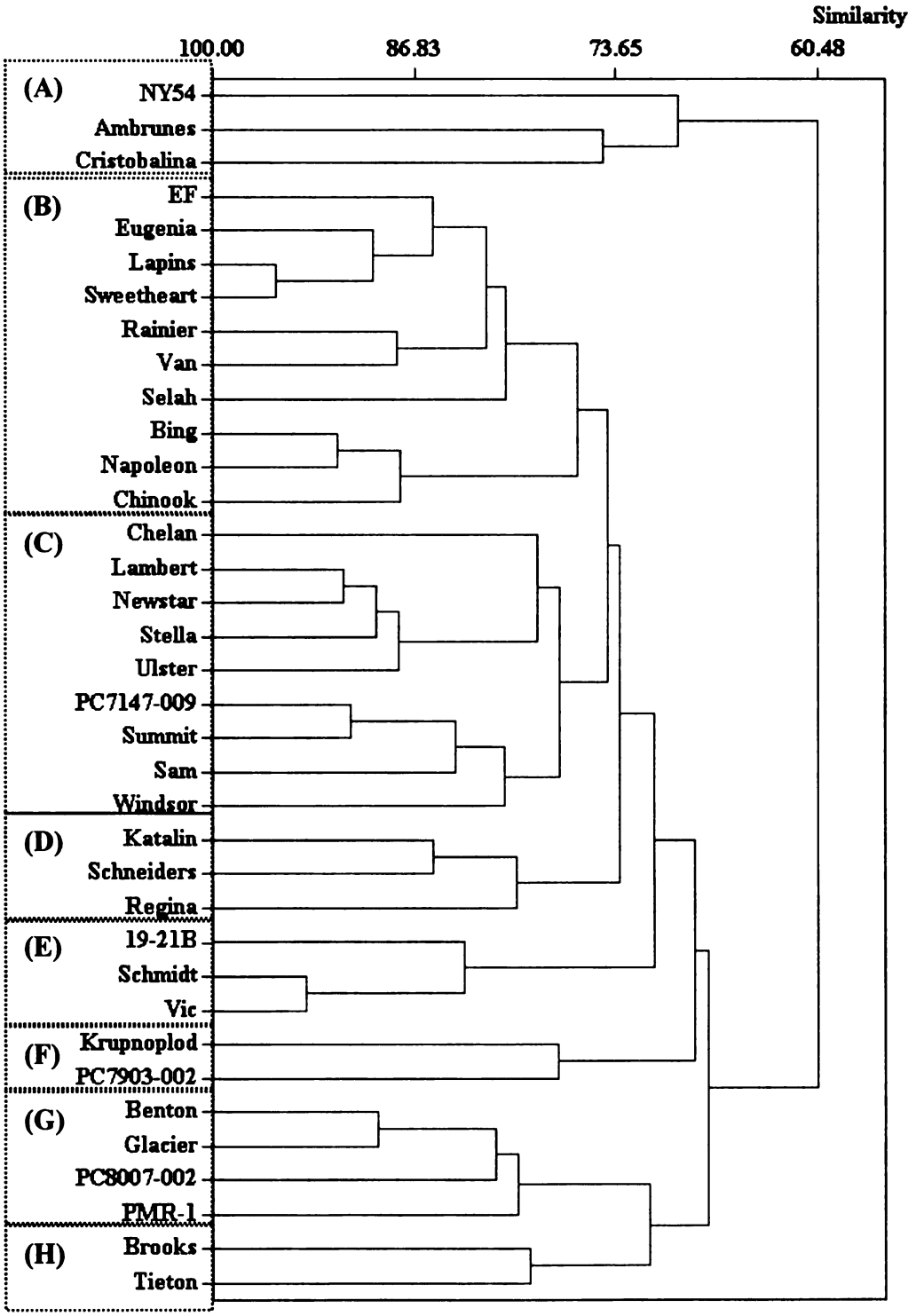
Fig 2.2 Cont. H: Linkage group 8



Genetic diversity structure

The phylogenetic relationships among the 36 cherry selections were determined using the data from the 77 DNA markers (Figure 2.3). At ~75% of genetic similarity value, the selections could be classified into 8 clusters (A-H). The clusters A and D exclusively represent European selections. NY54, a wild cherry is genetically dissimilar from all the other ones studied and has 70% genetic similarity to two Spanish landraces, Cristobalina and Ambrunus. NY54, Cristobalina and Ambrunus as a single cluster (cluster A) were separated from the rest of the cultivars at 40% of genetic dissimilarity value. The cluster E contains Vic, whose parents are Schmidt and Bing. In this study, Vic clustered with Schmidt. The clusters B, C, G and H include PNW sweet cherry breeding germplasm. Grouping of two European selections, Windsor and Eugenia that do not have any known pedigree relationship to the PNW cultivars, with clusters C and B, suggest the close genetic relationship of these selections to the parents that have been used in the PNW sweet cherry breeding.

Figure 2.3: Dendrogram resulting from marker allele based genetic distance analysis of 36 sweet cherry selections. Cluster analysis used McQuitty linkage, Absolute Correlation Coefficient Distance (Minitab 15)


Similarity
100.00
86.83
73.65
60.48
(A)
NY54
Ambrunes
Cristobalina
(B)
EF
Eugenia
Lapins
Sweetheart
Rainier
Van
Selah
Bing
Napoleon
Chinook
(C)
Chelan
Lambert
Newstar
Stella
Ulster
PC7147-009
Summit
Sam
Windsor
(D)
Katalin
Schneiders
Regina
(E)
19-21B
Schmidt
Vic
(F)
Krupnoplod
PC7903-002
(G)
Benton
Glacier
PC8007-002
PMR-1
(H)
Brooks
Tieton

Graphical genotypes for sweet cherry cultivars

The genetic diversity analysis used DNA markers that covered all eight sweet cherry linkage groups. Thus it was possible to visualize the linkage group heterozygosity for the 36 sweet cherry selections and present it as graphical genotypes (GGT) (Figure 2.4). The GGT illustrate the marker alleles for the eight linkage groups using the map positions from the consensus linkage group based on the data of Olmstead et al. (2008). The GGT can be used to search for those selections that have marker alleles that are linked to favorable QTL alleles. For example, Zhang et al. (2009) identified two QTLs for fruit size on LG2 and LG6 segregating in the NY54 x EF mapping population. BPPCT034 was found to be linked to the LG2 fruit size QTL. In our study four alleles were identified for BPPCT034 (PIC of 0.6) indicating that this would be a good marker candidate for fine mapping and validation of the QTL. EPPCU3090 on LG6 was associated with the second QTL identified. In our study three alleles were identified for EPPCU3090 (PIC value of 0.4) therefore, it is also a useful marker to further investigate the LG6 fruit size QTL. Similarly the GGT could be correlated to mapped genes and QTLs to get more insight for the marker haplotype information which will be useful in marker assisted breeding and future functional genomic studies. The only drawback in these GGT is that the exact allele phase (i.e. coupling and repulsion) for markers for some *P. avium* selections cannot be represented as for those selections; marker data for segregating progeny populations are not available.

GGT for sweet cherry cultivars show the allelic states and genomic landscape with respect to studied DNA markers and the consensus linkage map positions. However,

few mapped QTL are available to fully utilize this resource. The extensive phenotyping for important traits in multiple years and locations is necessary to assign breeding values to the linked alleles and marker allele haplotypes. This will allow the GGT to be used in marker assisted breeding and comparative genomics in family *Rosaceae.*

Figure 2.4: A-H Graphical genotypes for 36 sweet cherry cultivars. Eight linkage groups for each cultivar are shown with two homologous chromosomes for each linkage group. The marker positions in centi Morgan (cM) and marker names are shown on the left. In each cell, the allele in base pairs is shown for the SSR and gene based (PR markers and the allele name in number is shown for the *S*-locus. $$ indicates a confirmed null allele and $ indicates an unconfirmed null allele. " -" represents the missing data. The blank cells represent the gaps in the linkage groups. (A) Linkage group 1, (B) Linkage group 2, (C) Linkage group 3, (D) Linkage group 4, (E) Linkage group 05, (F) Linkage group 6, (G) Linkage group 7, (H) Linkage group 8.

Each linkage group has four pages of GGT to represent 36 sweet cherry selections in four separate pages. Page 1 for each linkage group: Wild cherry (NY54) and non-PNW sweet cherry cultivars, Page 2 for each linkage group: second subset of PNW sweet cherry cultivars, Page 3 for each linkage group: third subset of PNW sweet cherry cultivars, Page 4 for each linkage group: fourth subset of PNW sweet cherry cultivars

Fig 2.4 Cont. A: Linkage group 1-page 1

Map Position (cM)	Marker	New York 54		19-21B		Ambrunus		Cristobalina		Eugenia		Katalin		Krupnoplod		Napoleon		Windsor	
0.0	CPPCT016	171	171	208	$	200	$	208	$	180	171	204	190	208	$	180	208	171	$
1.0	EMPA001	160	-	155	150	160	150	150	135	160	-	155	-	-	-	165	160	150	-
37.8	EMPA005	255	245	255	245	255	$	225	-	245	245	255	245	240	245	255	$	245	245
45.1	EPDCU5100	198	184	-	-	-	-	202	184	198	184	-	-	-	-	-	-	-	-
47.7	UCD-CH31	150	150	150	145	155	150	-	-	165	145	165	$	170	145	145	$	165	150
50.0	PR33	244	244	266	244	-	-	266	244	-	-	266	$	266	244	266	$	250	244
63.6	PceGA59	184	184	190	184	190	184	190	184	184	184	190	190	190	190	190	184	184	184
64.9	CPSCT027	216	204	218	204	220	204	218	204	204	$	204	$	-	-	218	204	218	204
85.1	PMS67	165	165	$	148	148	$	160	148	165	160	165	160	148	$	165	148	165	148
88.1	PR101	152	146	152	$	152	158	158	152	152	$	152	158	152	$	152	158	152	$
106.6	CPPCT019	180	180	$	180	188	180	-	-	186	180	180	$	180	$	180	$	186	-
110.0	EMPA011	240	240	240	-	250	240	240	-	245	240	240	-	245	240	240	-	245	240
116.0	EPPB4213	140	140	140	$	140	$	140	$	140	$	146	140	-	-	140	$	140	$

Fig 2.4 Cont. A: Linkage group 1-page 2

Map Position (cM)	Marker	EF		Benton		Bing		Brooks		Chelan		Chinook		Glacier		Lambert		Lapins	
0.0	CPPCT016	208	188	208	180	208	180	180	171	208	180	208	$	180	208	180	180	180	171
1.0	EMPA001	160	-	165	-	165	-	-	-	165	155	165	160	-	-	165	-	165	160
37.8	EMPA005	255	240	255	245	255	255	-	-	255	245	255	$	255	255	255	-	255	245
45.1	EPDCU5100	195	184	-	-	198	198	-	-	-	-	-	-	-	-	-	-	-	-
47.7	UCD-CH31	155	145	-	-	145	145	150	$	-	-	145	$	150	145	145	$	165	145
50.0	PR33	266	244	266	244	266	244	266	244	-	-	266	244	266	244	266	244	-	-
63.6	PceGA59	190	184	190	184	190	184	-	-	190	184	190	184	190	184	190	184	190	184
64.9	CPSCT027	204	204	216	204	218	204	218	204	216	204	218	204	218	204	218	204	218	204
85.1	PMS67	165	148	165	148	160	148	165	148	165	148	148	$	165	148	148	$	165	148
88.1	PR101	152	152	152	$	152	$	152	$	152	$	152	$	152	$	152	$	152	$
106.6	CPPCT019	190	180	180	$	-	-	186	180	180	$	180	$	180	$	180	$	180	$
110.0	EMPA011	240	240	-	-	245	240	-	-	240	-	240	240	240	-	240	-	245	240
116.0	EPPB4213	140	132	146	140	140	140	140	$	146	140	140	$	146	140	146	140	140	$

Fig 2.4 Cont. A: Linkage group 1-page 3

Map Position (cM)	Marker	Newstar		PC7147-009		PC7903-002		PC8007-002		PMR-1		Rainier		Regina		Sam		Schmidt	
0.0	CPPCT016	180	$	180	171	208	171	180	$	180	180	180	171	198	208	180	188	188	208
1.0	EMPA001	165	-	165	145	155	150	165	155	165	158	165	160	160	155	165	155	165	155
37.8	EMPA005	-	-	255	245	245	245	255	$	255	255	255	245	245	245	255	$	255	240
45.1	EPDCU5100	-	-	-	-	-	-	-	-	198	184	198	198	-	-	-	-	195	184
47.7	UCD-CH31	165	145	165	145	155	150	150	145	155	145	150	145	165	155	165	145	155	145
50.0	PR33	-	-	266	$	266	$	266	244	266	244	244	244	266	244	250	244	-	-
63.6	PceGA59	184	184	184	184	190	190	190	184	190	184	184	184	190	190	190	184	190	184
64.9	CPSCT027	218	204	204	204	216	204	218	204	204	218	218	218	216	204	218	204	204	$
85.1	PMS67	148	$	165	$	-	-	148	$	162	148	165	160	165	160	165	148	165	162
88.1	PR101	152	$	152	$	152	$	152	$	152	152	152	152	152	158	152	$	152	$
106.6	CPPCT019	180	$	180	$	180	$	186	$	180	180	180	180	180	$	180	$	186	180
110.0	EMPA011	245	240	240	-	240	-	240	-	240	-	245	245	240	240	-	-	-	-
116.0	EPPB4213	140	$	140	$	140	$	-	-	140	140	140	140	140	$	140	$	140	$

Fig 2.4 Cont. A: Linkage group 1-page 4

Map Position (cM)	Marker	Schneiders		Selah		Stella		Summit		Sweetheart		Tieton		Ulster		Van		Vic	
0.0	CPPCT016	208	$	180	171	180	$	180	171	180	171	180	171	208	180	180	171	188	208
1.0	EMPA001	160	155	160	165	165	-	-	-	165	160	165	155	165	155	165	160	165	-
37.8	EMPA005	255	245	255	245	255	$	255	$	255	245	255	$	-	-	255	245	255	240
45.1	EPDCU5100	-	-	-	-	-	-	-	-	-	-	-	-	198	184	198	198	-	-
47.7	UCD-CH31	165	$	150	145	145	$	165	145	165	145	150	145	155	145	165	150	145	145
50.0	PR33	266	244	244	244	266	244	266	244	266	244	-	-	266	244	266	244	266	244
63.6	PceGA59	190	184	184	184	190	184	190	190	190	184	190	190	-	-	190	184	184	184
64.9	CPSCT027	218	204	218	218	218	204	218	204	218	204	204	$	218	204	218	204	218	204
85.1	PMS67	165	160	160	148	165	148	165	148	165	148	165	148	162	148	165	148	160	162
88.1	PR101	152	158	152	$	152	$	152	$	152	$	152	$	152	$	152	$	152	$
106.6	CPPCT019	180	$	180	$	-	-	180	$	180	$	186	180	186	180	180	$	186	180
110.0	EMPA011	240	-	245	240	240	-	240	-	245	240	240	-	245	240	245	240	245	240
116.0	EPPB4213	140	$	140	$	140	$	140	$	140	$	140	$	140	$	140	$	140	$

Fig 2.4 Cont. B: Linkage group 2-page 1

Map Position (cM)	Marker	New York 54		19-21B		Ambrunus		Cristobalina		Eugenia		Katalin		Krupnoplod		Napoleon		Windsor	
0.0	Ma069a	120	120	120	$	120	$	120	$	126	120	126	120	120	$	126	120	120	105
1.5	CPSCT038	192	190	190	$	190	$	$	192	204	192	190	$	190	$	204	190	190	$
4.1	UDA-059	134	134	134	$	134	$	134	$	138	$	-	-	134	$	-	-	134	$
11.0	BPPCT034	255	225	255	-	225	-	225	$	235	-	255	-	255	-	235	-	225	-
12.7	MA005c	190	190	$	190	190	198	190	198	$	198	$	190	$	190	190	198	-	-
15.8	UDAp-461	178	178	178	-	178	$	178	-	178	159	178	$	178	$	178	159	-	-
23.7	BPPCT002	184	168	$	184	186	184	186	184	185	184	$	184	$	184	185	184	$	184
32.4	MA007a	110	93	116	104	110	$	126	116	104	$	116	104	116	$	116	-	116	$
40.7	UDA-005	222	218	222	218	218	$	-	-	222	218	218	$	222	218	222	218	222	218
46.4	UCD-CH12	190	182	186	175	190	180	190	182	175	$	182	175	180	175	192	180	182	175
55.7	PceGA34	165	145	-	143	143	135	143	-	135	-	-	-	143	-	135	-	135	-
59.7	CPSCT034	210	210	210	-	210	$	210	195	190	$	190	$	190	$	210	$	210	-

Fig 2.4 Cont. B: Linkage group 2-page 2

Map Position (cM)	Marker	EF		Benton		Bing		Brooks		Chelan		Chinook		Glacier		Lambert		Lapins	
0.0	Ma069a	126	120	120	$	126	120	126	120	120	$	126	120	120	$	130	120	126	120
1.5	CPSCT038	204	190	192	190	204	190	190	$	192	190	204	190	190	$	192	190	204	204
4.1	UDA-059	138	134	134	$	138	134	134	$	134	$	138	134	134	$	-	-	-	-
11.0	BPPCT034	255	235	225	-	235	255	255	225	225	-	255	235	235	225	225	-	-	-
12.7	MA005c	190	198	190	198	190	198	-	-	190	198	$	198	$	198	-	-	190	198
15.8	UDAp-461	178	159	178	-	178	159	178	-	178	-	159	$	178	$	178	159	178	159
23.7	BPPCT002	184	185	186	184	185	184	$	184	186	184	185	184	186	184	186	184	185	184
32.4	MA007a	116	104	110	104	116	$$	116	110	110	104	116	$	110	104	104	$	116	$
40.7	UDA-005	222	218	218	$	222	218	222	218	218	$	222	218	218	$	218	$	222	218
46.4	UCD-CH12	180	175	182	175	192	180	182	175	190	175	192	180	180	175	192	175	175	175
55.7	PceGA34	155	143	-	-	-	-	143	-	143	-	-	-	-	-	-	-	135	-
59.7	CPSCT034	210	190	210	-	210	$	210	$	210	$	210	$	210	$	210	195	210	195

Fig 2.4 Cont. B: Linkage group 2-page 3

Map Position (cM)	Marker	Newstar		PC7147-009		PC7903-002		PC8007-002		PMR-1		Rainier		Regina		Sam		Schmidt	
0.0	Ma069a	120	$	120	$	120	$	120	$	120	120	126	120	126	120	120	$	120	$
1.5	CPSCT038	190	$	190	$	190	$	190	$	192	190	204	190	204	190	190	$	192	190
4.1	UDA-059	134	$	134	$	134	$	134	$	134	134	138	134	138	134	134	$	-	-
11.0	BPPCT034	225	-	255	225	225	-	235	225	255	225	255	235	225	-	255	-	250	-
12.7	MA005c	190	198	190	198	190	198	$	198	190	198	190	198	190	198	190	198	$	190
15.8	UDAp-461	178	$	178	$	178	$	-	-	178	159	178	159	178	$	178	159	178	$
23.7	BPPCT002	186	184	186	184	186	184	186	184	184	184	185	184	186	184	184	$	184	$
32.4	MA007a	116	104	116	104	104	$	110	104	110	$$	116	$$	110	104	116	104	116	104
40.7	UDA-005	222	218	222	218	218	$	218	$	218	218	222	218	224	218	222	218	222	218
46.4	UCD-CH12	175	$	175	$	175	$	192	180	192	182	192	175	175	$	175	$	180	175
55.7	PceGA34	135	-	135	-	135	-	-	-	-	-	135	-	-	-	-	-	-	-
59.7	CPSCT034	210	195	210	$	210	195	210	$	195	190	210	210	210	$	195	$	195	190

Fig 2.4 Cont. B: Linkage group 2-page 4

Map Position (cM)	Marker	Schneiders		Selah		Stella		Summit		Sweetheart		Tieton		Ulster		Van		Vic	
0.0	Ma069a	120	$	120	$	126	120	120	$	126	120	126	120	120	$	126	120	120	$
1.5	CPSCT038	190	$	190	$	204	190	190	$	-	-	190	$	192	190	204	190	190	190
4.1	UDA-059	134	$	134	$	138	134	134	$	138	134	134	$	-	-	-	-	134	$
11.0	BPPCT034	255	-	225	-	235	225	255	-	235	-	235	225	250	-	235	255	255	-
12.7	MA005c	$	190	190	198	$	198	190	198	190	198	$	198	-	-	190	198	$	190
15.8	UDAp-461	178	$	-	-	178	159	178	159	178	159	178	$	178	159	178	159	178	$
23.7	BPPCT002	$	184	186	184	186	184	184	$	185	184	186	184	184	$	185	184	184	$
32.4	MA007a	116	104	116	104	104	$	116	$	116	$	110	$	104	$	116	$$	116	$
40.7	UDA-005	222	218	222	218	218	$	222	218	222	218	218	$	218	$	222	218	222	218
46.4	UCD-CH12	175	$	180	175	192	175	175	$	175	$	192	182	192	175	192	175	180	180
55.7	PceGA34	143	-	-	-	135	-	-	-	135	-	143	-	-	-	135	-	-	-
59.7	CPSCT034	210	190	210	195	210	195	210	-	210	195	210	$	210	195	210	$	210	195

Fig 2.4 Cont. C: Linkage group 3-page 1

Map Position (cM)	Marker	New York 54		19-21B		Ambrunus		Cristobalina		Eugenia		Katalin		Krupnoplod		Napoleon		Windsor	
0.0	EPPCU5990	195	185	195	$	185	$	185	$	195	185	195	185	195	185	195	185	185	$
0.1	PaCITA4	140	143	-	-	-	-	143	140	143	140	143	140	143	-	-	-	143	140
23.2	PMS30	170	170	175	175	170	152	142	132	175	142	175	142	170	152	175	142	170	142
25.8	BPPCT039	138	138	138	$	140	134	134	128	138	$	138	134	-	-	138	134	145	134
32.4	EPDCU3083	145	145	145	$	153	-	145	$	145	$	153	145	153	145	153	145	153	$
34.1	UDP98-416	210	210	210	200	210	$	210	200	-	-	-	-	210	$	210	200	210	$
39.4	CPDCT037	160	160	165	160	158	170	$	155	$	160	$	170	$	145	$	160	158	170
47.5	Ma039a	170	170	$	170	$	170	$	170	170	220	$	170	$	170	$	170	$	170
72.6	EMPA014	225	225	230	233	220	225	232	$	230	233	230	$	230	233	230	232	230	225

Fig 2.4 Cont. C: Linkage group 3-page 2

Map Position (cM)	Marker	EF		Benton		Bing		Brooks		Chelan		Chinook		Glacier		Lambert		Lapins	
0.0	EPPCU5990	195	185	195	$	195	185	195	185	195	185	195	185	195	$	195	185	-	-
0.1	PaCITA4	143	140	143	140	143	140	143	140	140	-	143	140	143	140	143	140	143	140
23.2	PMS30	175	142	162	142	175	132	162	132	170	142	175	152	162	142	152	142	175	142
25.8	BPPCT039	145	138	145	134	145	138	145	134	145	$	145	138	145	134	145	134	145	145
32.4	EPDCU3083	153	145	153	$	153	145	153	145	145	$	-	-	153	$	145	-	-	-
34.1	UDP98-416	210	200	210	200	210	200	210	200	210	200	210	200	-	-	210	200	210	$
39.4	CPDCT037	160	170	165	170	160	170	-	-	145	170	160	170	145	170	$	170	$	170
47.5	Ma039a	170	220	$	170	$	170	$	170	170	216	170	216	216	220	170	216	$	170
72.6	EMPA014	230	235	235	225	230	233	230	225	235	225	230	$	230	233	230	232	230	230

Fig 2.4 Cont. C: Linkage group 3-page 3

Map Position (cM)	Marker	Newstar		PC7147-009		PC7903-002		PC8007-002		PMR-1		Rainier		Regina		Sam		Schmidt	
0.0	EPPCU5990	185	$	-	-	195	185	195	185	195	195	195	185	185	$	195	185	195	185
0.1	PaCITA4	143	140	143	140	-	-	-	-	143	140			140	140	140	-	143	140
23.2	PMS30	152	142	142	$	175	162	170	162	162	142	175	132	175	142	170	142	175	142
25.8	BPPCT039	145	$	145	$	145	134	145	134	134	134	145	145	140	138	138	134	145	138
32.4	EPDCU3083	153	145	153	-	153	-	153	145	153	145	153	145	153	$	-	-	153	145
34.1	UDP98-416	210	$	210	$	210	$	210	200	210	210	210	210	210	$	210	200	210	200
39.4	CPDCT037	-	-	$	170	165	170	165	145	145	170	160	170	$	170	165	170	165	170
47.5	Ma039a	170	216	$	170	$	170	170	220	170	216	170	170	170	222	170	216	$	170
72.6	EMPA014	-	-	234	235	230	225	233	225	230	225	230	230	230	233	230	234	234	235

Fig 2.4 Cont. C: Linkage group 3-page 4

Map Position (cM)	Marker	Schneiders		Selah		Stella		Summit		Sweetheart		Tieton		Ulster		Van		Vic	
0.0	EPPCU5990	195	185	195	$	195	185	195	185	195	185	195	185	-	-	195	185	195	195
0.1	PaCITA4	143	140	143	140	143	140	143	140	143	140	143	140	143	140	143	140	143	140
23.2	PMS30	175	142	175	142	152	142	142	$	175	142	162	152	142	142	175	142	175	175
25.8	BPPCT039	145	138	145	138	145	$	145	134	145	145	-	-	145	134	145	145	138	138
32.4	EPDCU3083	153	$	153	145	153	145	153	145	153	$	153	145	153	145	153	145	145	$
34.1	UDP98-416	210	200	210	200	210	$	210	200	210	$	210	$	210	200	210	$	210	200
39.4	CPDCT037	$	170	160	170	$	170	165	170	$	170	145	170	-	-	170	170	160	165
47.5	Ma039a	$	170	170	216	170	216	$	170	-	-	170	216	170	216	$	170	$	170
72.6	EMPA014	230	235	230	$	230	235	230	$	230	$	230	225	235	230	230	233	230	234

Fig 2.4 Cont. D: Linkage group 4-page 1

Map Position (cM)	Marker	New York 54		19-21B		Ambrunus		Cristobalina		Eugenia		Katalin		Krupnoplod		Napoleon		Windsor	
0.0	EPPCU3664	125	122	130	$	125	122	122	115	122	115	125	122	125	122	130	122	122	115
6.4	EMPA015	240	225	-	-	-	-	222	-	-	-	240	-	222	220	240	222	220	-
13.1	AMPA110	135	135	135	$	138	135	138	135	138	135	135	$	135	$	135	$	138	135
33.8	BPPCT040	145	145	145	$	135	125	130	$	135	$	-	-	145	125	135	125	145	135
45.0	UDP97-402	122	118	-	-	-	-	126	-	-	-	-	-	-	-	-	-	-	-
50.0	M12a	180	-	185	-	180	-	180	-	190	180	180	-	185	180	180	-	180	-
59.5	UDA-037	423	423	-	-	423	$	431	423	-	-	431	$	425	$	431	$	431	$
73.7	UDA-027	135	135	135	$	135	$	135	$	137	135	137	135	137	135	135	$	137	135

Fig 2.4 Cont. D: Linkage group 4-page 2

Map Position (cM)	Marker	EF		Benton		Bing		Brooks		Chelan		Chinook		Glacier		Lambert		Lapins	
0.0	EPPCU3664	122	122	122	115	130	122	130	115	122	115	130	122	122	$	122	$	122	122
6.4	EMPA015	222	220	220	-	-	-	240	222	222	-	220	-	240	222	220	-	-	-
13.1	AMPA110	138	135	135	$	135	$	135	$	138	135	135	$	138	135	135	$	138	135
33.8	BPPCT040	128	120	145	120	135	125	-	-	125	120	145	135	145	120	145	125	125	120
45.0	UDP97-402	126	-	-	-	-	-	-	-	-	-	-	-	-	-	138	130	-	-
50.0	M12a	180	-	185	180	180	-	180	-	180	-	185	180	180	-	-	-	180	-
59.5	UDA-037	425	$$	431	423	431	$	425	$	425	423	431	$	-	-	431	$	425	$
73.7	UDA-027	137	135	135	$	135	$	135	$	135	$	135	$	135	$	135	$	137	135

Fig 2.4 Cont. D: Linkage group 4-page 3

Map Position (cM)	Marker	Newstar		PC7147-009		PC7903-002		PC8007-002		PMR-1		Rainier		Regina		Sam		Schmidt	
0.0	EPPCU3664	130	122	122	$	122	115	122	$	115	115	130	-	125	122	122	$	122	115
6.4	EMPA015	222	220	220	-	-	-	240	-	220	-	240	222	240	-	-	-	222	-
13.1	AMPA110	138	135	138	135	135	$	135	$	135	135	135	135	135	135	138	135	138	135
33.8	BPPCT040	-	-	145	120	-	-	145	$	145	120	125	125	145	-	145	125	-	-
45.0	UDP97-402	-	-	138	130	138	130	-	-	138	138	130	130	-	-	138	130	-	-
50.0	M12a	180	-	180	-	185	180	180	-	185	185	180	180	185	180	185	180	180	-
59.5	UDA-037	-	-	431	$	431	$	-	-	431	431	431	425	431	$	431	$	425	$
73.7	UDA-027	137	135	137	135	137	135	135	$	135	135	135	135	137	135	135	$	137	135

Fig 2.4 Cont. D: Linkage group 4-page 4

Map Position (cM)	Marker	Schneiders		Selah		Stella		Summit		Sweetheart		Tieton		Ulster		Van		Vic	
0.0	EPPCU3664	125	122	130	122	122	$	-	-	122	122	122	115	122	115	130	122	122	122
6.4	EMPA015	-	-	220	-	222	220	-	-	220	-	220	-	222	-	240	220	222	-
13.1	AMPA110	135	$	135	$	-	-	135	$	138	135	135	$	135	-	-	-	138	135
33.8	BPPCT040	145	120	145	135	145	120	125	125	125	120	145	135	-	-	125	120	135	$
45.0	UDP97-402	-	-	-	-	138	126	-	-	-	-	-	-	-	-	-	-	-	-
50.0	M12a	180	-	180	-	185	180	180	-	180	-	185	180	180	-	180	-	180	-
59.5	UDA-037	431	$	431	425	431	425	431	$	425	$	431	$	-	-	425	$	431	$
73.7	UDA-027	137	135	135	$	135	135	137	135	137	135	135	$	137	135	137	135	135	$

Fig 2.4 Cont. E: Linkage group 5-page 1

Map Position (cM)	Marker	New York 54		19-21B		Ambrunus		Cristobalina		Eugenia		Katalin		Krupnoplod		Napoleon		Windsor	
0.0	EPPCU0961	148	148	146	$	148	150	146	148	148	$	148	$	148	146	148	$	148	146
7.7	EPPCU9168	168	168	-	-	168	164	164	162	-	-	-	-	-	-	168	162	168	162
20.7	BPPCT026	164	164	186	164	186	164	186	164	178	164	186	164	186	164	170	164	186	186
38.4	UDP96-019	202	202	202	$	205	202	202	$	202	$	202	$	205	$	205	202	205	$
47.4	BPPCT037	148	148	148	155	145	$	148	-	155	145	148	137	148	142	148	142	157	145
49.0	EMPaS11	88	78	88	78	108	88	78	$	108	78	78	68	78	68	78	68	112	78
61.9	EPDCU5183	150	140	140	120	120	$	125	120	120	$	120	$	145	$	150	$	150	120
70.5	EPPB4230	256	253	256	$	255	$	260	254	256	253	-	-	256	254	256	253	-	-
73.4	CPDCT022	175	145	155	150	158	-	155	-	-	-	-	-	165	150	175	150	165	155
75.5	BPPCT014	190	190	-	-	190	-	-	-	192	190	-	-	195	-	195	190	-	-

Fig 2.4 Cont. E: Linkage group 5-page 2

Map Position (cM)	Marker	EF		Benton		Bing		Brooks		Chelan		Chinook		Glacier		Lambert		Lapins	
0.0	EPPCU0961	150	148	150	146	148	146	150	148	148	$	146	148	150	146	148	146	150	148
7.7	EPPCU9168	168	162	-	-	168	162	-	-	-	-	168	162	168	164	168	$	168	$
20.7	BPPCT026	170	164	186	164	178	164	186	170	170	164	178	170	186	164	170	$	170	170
38.4	UDP96-019	205	202	205	$	205	205	-	-	205	202	205	205	205	205	202	$	205	202
47.4	BPPCT037	137	155	142	$	145	142	-	-	142	145	142	145	142	$	148	$	148	145
49.0	EMPaS11	78	68	78	$	112	78	112	78	108	78	112	78	78	$	68	$	112	68
61.9	EPDCU5183	140	120	140	-	120	$	120	$	-	-	150	$	150	$	120	$	120	$
70.5	EPPB4230	256	254	256	253	256	256	-	-	253	253	256	$	-	-	256	$	260	256
73.4	CPDCT022	165	150	-	-	150	$	155	-	175	-	150	$	-	-	150	$	155	150
75.5	BPPCT014	192	190	195	190	-	-	190	-	190	-	195	-	195	190	195	-	195	190

Fig 2.4 Cont. E: Linkage group 5-page 3

Map Position (cM)	Marker	Newstar		PC7147-009		PC7903-002		PC8007-002		PMR-1		Rainier		Regina		Sam		Schmidt	
0.0	EPPCU0961	148	146	148	$	148	$	150	146	148	146	150	146	148	$	148	146	150	146
7.7	EPPCU9168	168	162	168	162	168	162	164	$	168	168	168	162	168	164	168	$	168	164
20.7	BPPCT026	178	164	186	170	-	-	186	186	170	164	170	164	186	164	170	$	170	164
38.4	UDP96-019	-	-	205	202	205	202	205	$	202	202	205	205	202	$	202	$	202	$
47.4	BPPCT037	-	-	148	145	148	145	142	142	148	148	142	145	148	142	148	137	137	155
49.0	EMPaS11	-	-	112	68	112	68	78	$	68	68	112	78	78	78	68	$	108	68
61.9	EPDCU5183	120	-	120	$	-	-	-	-	-	-	120	-	-	-	120	-	120	-
70.5	EPPB4230	260	253	-	-	260	256	-	-	256	256	260	256	256	$	256	254	256	254
73.4	CPDCT022	175	155	165	150	155	150	-	-	150	150	155	150	150	145	165	150	-	-
75.5	BPPCT014	195	190	-	-	190	-	190	-	195	-	195	190	-	-	195	190	192	190

Fig 2.4 Cont. E: Linkage group 5-page 4

Map Position (cM)	Marker	Schneiders		Selah		Stella		Summit		Sweetheart		Tieton		Ulster		Van		Vic	
0.0	EPPCU0961	148	$	150	146	150	148	148	146	150	148	150	148	148	146	150	146	150	146
7.7	EPPCU9168	-	-	168	162	168	$	168	168	-	-	168	162	168	164	168	162	168	164
20.7	BPPCT026	186	164	164	164	170	164	170	$	-	-	186	164	170	164	178	170	170	164
38.4	UDP96-019	-	-	205	202	205	202	205	202	205	202	205	$	-	-	205	205	205	202
47.4	BPPCT037	148	145	148	145	148	142	148	145	148	145	142	$	-	-	145	145	142	137
49.0	EMPaS11	78	$	112	68	78	68	112	68	-	-	78	$	68	$	-	-	78	68
61.9	EPDCU5183	125	-	-	-	150	-	150	120	120	-	150	120	120	-	120	$	-	-
70.5	EPPB4230	256	253	256	$	256	253	260	256	-	-	256	253	256	254	260	260	256	254
73.4	CPDCT022	-	-	150	$	175	150	155	150	155	-	175	145	165	150	155	$	165	150
75.5	BPPCT014	-	-	195	-	195	190	190	-	195	190	190	-	195	190	190	-	-	-

Fig 2.4 Cont. F: Linkage group 6-page 1

Map Position (cM)	Marker	New York 54		19-21B		Ambrunus		Cristobalina		Eugenia		Katalin		Krupnoplod		Napoleon		Windsor	
0.0	EMPaS01	222	228	228	$	222	228	222	228	232	228	228	$	-	-	232	232	232	222
4.5	UDP96-001	129	131	129	$	131	110	129	-	131	131	129	$	129	$	131	129	131	$
13.5	BPPCT008	90	90	97	$	97	$	-	-	97	100	97	$	97	100	97	$	97	90
36.6	CPPCT023	171	170	-	-	-	-	171	170	-	-	-	-	-	-	-	-	-	-
44.0	EPPCU3090	180	172	-	-	185	$	185	$	185	$	185	172	185	172	185	172	185	$
50.8	UDP98-021	118	118	112	102	112	102	-	-	112	102	112	102	102	$	112	102	112	112
51.3	EPPB4227	125	130	130	135	130	145	$	130	$	135	130	135	$	135	130	135	130	135
59.4	Ma040a	225	$$	-	-	-	-	225	$	215	$	210	240	-	-	210	$	225	$
66.8	S-RNase	2	6	2	9	3	6	3	6	3	7	4	12	5	9	3	4	1	3

Fig 2.4 Cont. F: Linkage group 6-page 2

Map Position (cM)	Marker	EF		Benton		Bing		Brooks		Chelan		Chinook		Glacier		Lambert		Lapins	
0.0	EMPaS01	232	228	232	228	232	228	-	-	228	$	232	232	240	228	232	228	228	228
4.5	UDP96-001	115	129	131	129	131	129	-	-	129	$	131	131	129	$	131	129	129	129
13.5	BPPCT008	97	90	97	$	97	$	-	-	-	-	97	$	97	100	97	$	97	$
36.6	CPPCT023	170	170	-	-	171	170	-	-	-	-	-	-	-	-	-	-	-	-
44.0	EPPCU3090	185	172	185	172	185	172	-	-	172	$	185	172	185	172	185	172	185	172
50.8	UDP98-021	112	102	102	$	112	102	-	-	102	$	112	102	102	$	112	102	112	102
51.3	EPPB4227	135	135	130	135	130	135	135	145	$	135	130	135	135	145	130	135	135	135
59.4	Ma040a	210	$$	210	$	210	$$	225	$	-	-	225	210	210	$	210	$	225	210
66.8	S-RNase	3	4	4	9	3	4	1	9	3	9	1	4	4	9	3	4	1	4

Fig 2.4 Cont. F: Linkage group 6-page 3

Map Position (cM)	Marker	Newstar		PC7147-009		PC7903-002		PC8007-002		PMR-1		Rainier		Regina		Sam		Schmidt	
0.0	EMPaS01	-	-	232	228	222	228	232	228	228	228	232	228	232	228	228	$	-	-
4.5	UDP96-001	-	-	115	129	131	129	131	129	129	129	131	129	115	129	129	$	115	129
13.5	BPPCT008	97	$	97	90	97	$	97	100	97	97	97	97	97	$	97	$	97	90
36.6	CPPCT023	-	-	-	-	-	-	-	-	170	170	171	170	-	-	-	-	-	-
44.0	EPPCU3090	185	172	172	$	185	172	185	-	172	172	185	172	185	172	172	$	185	172
50.8	UDP98-021	-	-	112	102	112	102	112	102	102	102	112	102	112	102	102	$	112	102
51.3	EPPB4227	130	135	$	135	$	135	130	145	135	135	135	135	$	135	$	135	130	135
59.4	Ma040a	225	210	210	$	-	-	-	-	210	$$	225	210	225	$	210	240	210	240
66.8	S-RNase	3	4	1	4	5	9	4	9	4	9	1	4	1	3	2	4	2	4

Fig 2.4 Cont. F: Linkage group 6-page 4

Map Position (cM)	Marker	Schneiders		Selah		Stella		Summit		Sweetheart		Tieton		Ulster		Van		Vic	
0.0	EMPaS01	232	228	232	228	232	228	228	$	228	$	232	228	-	-	232	228	232	232
4.5	UDP96-001	115	129	131	129	131	129	129	$	129	$	129	$	-	-	115	129	131	115
13.5	BPPCT008	97	$	97	$	97	$	97	$	97	$	97	90	-	-	97	90	97	90
36.6	CPPCT023	-	-	-	-	-	-	-	-	-	-	-	-	-	-	171	170	-	-
44.0	EPPCU3090	185	-	172	$	185	172	185	172	185	172	185	-	185	-	185	185	172	$
50.8	UDP98-021	112	112	102	$	112	102	112	102	112	102	112	112	-	-	112	-	112	102
51.3	EPPB4227	130	135	$	135	130	135	$	135	135	135	120	135	130	135	130	135	130	135
59.4	Ma040a	240	$	210	$	210	$	225	240	225	210	-	-	210	$	225	$$	210	240
66.8	S-RNase	3	12	3	4	3	4	1	2	3	4	3	9	3	4	1	3	2	4

Fig 2.4 Cont. G: Linkage group 7-page 1

Map Position (cM)	Marker	New York 54		19-21B		Ambrunus		Cristobalina		Eugenia		Katalin		Krupnoplod		Napoleon		Windsor	
0.0	CPPCT022	250	252	250	245	250	245	245	$	250	245	250	245	250	245	250	$	250	245
13.1	UDAp-407	215	205	-	-	-	-	215	$	215	$	215	$	-	-	215	205	215	205
14.0	CPSCT026	180	178	178	-	-	-	180	-	-	-	178	-	-	-	-	-	178	-
14.1	UDAp-401	270	-	265	-	265	-	-	-	270	-	265	-	-	-	265	-	260	-
15.5	EPDCU2931	160	132	152	$	132	148	148	-	152	132	150	$	152	152	146	132	152	146
30.3	CPPCT033	158	158	150	149	150	145	149	$	-	-	145	148	158	149	150	164	145	148
38.2	PMS2	130	142	165	142	146	130	130	$	130	142	142	$	130	165	142	$	146	142
42.4	PS8e08	181	181	172	181	181	$	181	-	181	$	181	$	172	181	181	$	181	$
45.0	PCHCMS2	730	670	730	-	730	-	-	-	-	-	730	670	730	670	730	-	730	-
49.6	EPDCU3392	135	115	-	-	135	123	123	$	123	115	110	$	135	123	123	115	110	$

Fig 2.4 Cont. G: Linkage group 7-page 2

Map Position (cM)	Marker	EF		Benton		Bing		Brooks		Chelan		Chinook		Glacier		Lambert		Lapins	
0.0	CPPCT022	250	245	245	$	250	250	250	245	245	$	252	250	250	245	250	$	250	245
13.1	UDAp-407	215	215	215	205	215	205	215	-	215	-	215	205	205	-	215	205	215	$
14.0	CPSCT026	178	178	-	-	178	-	180	-	-	-	-	-	178	-	180	-	-	-
14.1	UDAp-401	265	260	-	-	265	260	265	260	265	-	265	260	270	-	295	-	260	-
15.5	EPDCU2931	152	148	152	132	152	132	-	-	-	-	152	132	132	$	146	132	152	152
30.3	CPPCT033	152	164	150	164	150	148	-	-	150	145	150	148	150	149	150	$	164	148
38.2	PMS2	146	165	146	142	146	142	-	-	130	142	146	142	146	142	146	142	146	146
42.4	PS8e08	186	172	186	181	181	181	-	-	181	$	181	$	-	-	186	181	186	181
45.0	PCHCMS2	730	670	730	670	730	-	-	-	730	670	730	-	730	670	730	670	730	670
49.6	EPDCU3392	135	129	129	115	115	110	110	$	115	110	-	-	129	115	110	115	129	115

Fig 2.4 Cont. G: Linkage group 7-page 3

Map Position (cM)	Marker	Newstar		PC7147-009		PC7903-002		PC8007-002		PMR-1		Rainier		Regina		Sam		Schmidt	
0.0	CPPCT022	-	-	245	$	245	$	250	$	250	245	250	250	252	245	250	245	250	245
13.1	UDAp-407	215	$	215	$	215	$	217	205	217	205	215	215	215	$	215	205	215	$
14.0	CPSCT026	178	-	-	-	178	-	-	-	178	180	178	178	-	-	178	-	-	-
14.1	UDAp-401	260	-	-	-	265	260	295	-	295	295	295	260	270	26	295	260	265	260
15.5	EPDCU2931	152	$	-	-	152	$	132	148	132	148	152	152	152	$	152	132	152	$
30.3	CPPCT033	-	-	-	-	164	164	150	145	150	164	145	148	158	145	150	164	152	164
38.2	PMS2	-	-	146	165	146	142	146	130	146	142	146	142	146	142	146	142	-	-
42.4	PS8e08	186	$	186	172	186	181	186	181	186	181	186	181	172	181	186	181	186	172
45.0	PCHCMS2	-	-	730	670	670	-	730	-	730	730	730	730	730	670	730	670	730	670
49.6	EPDCU3392	129	115	129	110	129	115	129	110	115	115	129	110	129	123	-	-	129	135

Fig 2.4 G: Linkage group 7-page 4

Map Position (cM)	Marker	Schneiders		Selah		Stella		Summit		Sweetheart		Tieton		Ulster		Van		Vic	
0.0	CPPCT022	245	$	250	245	250	245	250	245	250	245	245	$	-	-	250	250	250	$
13.1	UDAp-407	215	205	215	$	215	205	215	-	215	$	215	205	215	205	215	$	215	$
14.0	CPSCT026	-	-	-	-	178	-	178	-	178	-	-	-	178	-	178	-	-	-
14.1	UDAp-401	265	260	265	260	295	260	260	-	260	-	270	260	260	-	295	260	265	260
15.5	EPDCU2931	152	132	152	$	152	132	152	152	152	$	152	132	-	-	152	152	152	$
30.3	CPPCT033	145	148	164	148	150	164	164	148	164	148	145	164	-	-	145	148	152	148
38.2	PMS2	146	142	146	142	146	142	146	146	146	142	146	130	-	-	146	142	146	$
42.4	PS8e08	172	181	186	186	186	181	-	-	186	181	186	181	-	-	186	181	172	181
45.0	PCHCMS2	730	670	730	670	730	670	730	-	-	-	730	670	-	-	-	-	730	-
49.6	EPDCU3392	129	110	129	$	129	115	129	123	129	115	129	110	129	115	129	115	129	110

Fig 2.4 Cont. H: Linkage group 8-page 1

Map Position (cM)	Marker	New York 54		19-21B		Ambrunus		Cristobalina		Eugenia		Katalin		Krupnoplod		Napoleon		Windsor	
0.0	pchgms49	156	156	156	$	168	156	156	$	173	156	168	156	156	$	168	156	156	$
13.4	EPPCU4726	160	160	160	$	160	$	160	$	160	$	160	$	160	$	160	$	160	162
24.4	CPPCT006	206	188	206	188	190	-	190	-	190	-	208	-	208	190	208	190	190	-
54.6	MD201a	250	230	230	$	230	$	250	230	230	$	250	230	230	$	250	230	230	$
80.8	ps1h3	280	270	270	$	270	$	275	270	285	270	270	$	272	$	270	-	275	270

Fig 2.4 Cont. H: Linkage group 8-page 2

Map Position (cM)	Marker	EF		Benton		Bing		Brooks		Chelan		Chinook		Glacier		Lambert		Lapins	
0.0	pchgms49	173	156	170	156	156	156	156	$	170	156	-	-	156	$	168	156	156	$
13.4	EPPCU4726	162	160	160	$	160	$	160	$	160	$	160	$	160	$	160	$	160	$
24.4	CPPCT006	208	188	190	-	208	190	190	-	208	190	190	-	190	-	190	-	190	190
54.6	MD201a	230	230	230	$	250	230	-	-	230	$	230	$	230	$	230	$	230	$
80.8	ps1h3	270	270	272	$	270	$	275	270	270	$	270	$	272	270	270	$	275	270

Fig 2.4 Cont. H: Linkage group 8-page 3

Map Position (cM)	Marker	Newstar		PC7147-009		PC7903-002		PC8007-002		PMR-1		Rainier		Regina		Sam		Schmidt	
0.0	pchgms49	156	$	168	156	156	$	156	156	156	156	168	156	168	156	156	$	168	156
13.4	EPPCU4726	160	$	160	$	160	$	160	$	160	$	160	$	160	$	-	-	160	$
24.4	CPPCT006	190	$	190	-	190	-	190	-	208	190	208	190	208	204	190	-	208	188
54.6	MD201a	230	$	230	$	230	$	230	$	230	230	230	230	230	$	230	$	230	$
80.8	ps1h3	275	270	275	270	272	270	270	$	272	270	275	270	270	$	270	$	270	$

Fig 2.4 Cont. H: Linkage group 8-page 4

Map Position (cM)	Marker	Schneiders		Selah		Stella		Summit		Sweetheart		Tieton		Ulster		Van		Vic	
0.0	pchgms49	168	156	156	$	156	$	156	$	156	$	170	156	168	156	168	156	156	156
13.4	EPPCU4726	160	$	160	$	160	$	160	$	160	$	160	$	160	$	160	$	-	-
24.4	CPPCT006	208	190	-	-	208	190	190	$	190	-	208	190	190	-	190	-	208	190
54.6	MD201a	230	$	230	$	230	$	230	$	230	$	230	$	-	-	230	$	250	230
80.8	ps1h3	270	$	270	$	270	$	270	$	275	270	270	$	270	$	275	270	270	$

A panel of cultivars for SNP discovery for *P. avium*

The transition from SSR to SNP markers suitable for high throughput genotyping platforms requires the sequencing of a set of selections known to represent the genetic diversity in the germplasm of interest. The results from the SSR diversity study can be used to select such a panel of selections. In our case, goal was to identify six *P. avium* selections as a "SNP detection panel". Such a panel must represent 90% allele diversity, should have wide phenotypic diversity and if possible should have progenies and established linkage maps to further study the genetics. However, still there is a room for polymorphism loss by selecting only six selections but defining a core set is not possible without a comprehensive marker survey and it is not currently feasible to have more than six individuals in the SNP detection panels due to high cost of DNA sequencing.

Table 2.12 lists the six *P. avium* selections for SNP detection panel along with other relevant information. The number of UA is the key criterion which shows the amount of genetic diversity bringing in to the SNP detection panel. The selections that have more than 3 UA were considered for the panel. The *S*-genotype was also considered because it and was the marker locus with highest PIC (Table 2.15). As far as possible many *S*-alleles were recovered in the panel. The cluster positions according to the dendrogram (Figure 2.3) of each selection were considered and as far as possible, most distantly clustered ones were chosen. The individual LG level diversity was also considered and Figure 2.2 and Tables 2.4-2.11 were useful for this purpose as it shows which sweet cherry selections bring UA for each locus and LG. All the eight LGs were represented with UA in selecting the SNP panel. The relevant published information such

as linkage maps and QTLs were considered last to facilitate the quick understanding and application of the new SNP which will be developed based on this panel. Selections EF, NY54, Ambrunus, Cristobalina, Eugenia and Katalin are suggested for a SNP detection panel based on this study. The suggested SNP detection panel brings 40 out of total 52 UA detected in the study and also brings 6 *S*-alleles (S_2, S_3, S_4, S_6, S_7 and S_{12}).

Table 2.12: The panel of selections/individuals for SNP detection in *P. avium*

P. avium selection	Number of UA	*S*-genotype	The LGs where UA located	Published information and remarks
EF	4	S_3S_4	1, 4	Founder parent of many PNW cultivars, LG map available (Olmstead et al. 2008), fruit size (Zhang et al. 2009 in press) and fruit color QTLs reported (this thesis)
NY54	13	S_2S_6	1, 2, 4, 5, 6, 7, 8	Wild cherry, LG map available (Olmstead et al. 2008), fruit size (Zhang et al. 2009 in press) and fruit color QTLs reported (this thesis)
Ambrunus	9	S_3S_6	1, 3, 5, 6	Bring highest number of UA and reported for high fruit quality (Bernalte et al. 1998)
Cristobalina	6	S_3S_6	1, 2, 3, 4	Important to bring DNA diversity for natural self compatibility
Eugenia	4	S_3S_7	4, 6, 8	Important to bring DNA diversity from LG8 (No other sweet cherry selections used in the study does not bring UA to LG8)
Katalin	4	S_4S_{12}	1, 7	Important to bring DNA diversity from LG7 (No other sweet cherry selections used in the study does not bring UA to LG7)

Related studies on genetic diversity in *Prunus*

This study identified 300 alleles for 77 DNA markers for 36 sweet cherry selections with the range of two to nine alleles per locus with an average of five. Struss et al. (2003) identified 48 alleles from 15 SSR markers for 15 sweet cherry cultivars. Boritzki et al. (2000) used ten AFLP markers to characterize 128 sweet cherry accessions but only 128 fragments were found to be polymorphic out of total 712 fragements amplified. This shows the suitability of SSR and gene based markers over AFLP in diversity studies in sweet cherry. Wunsch and Hormaza (2002) used 34 peach SSRs to study 76 sweet cherry cultivars and amplified 24 SSRs but only 14 were polymorphic. Wunch and Hormaza (2004) used 12 SSR markers to fingerprint 28 Spanish sweet cherry genotypes and found 42 informative alleles; which were able to completely classify the genotypes. This clearly shows the present study mined more alleles compared to these previous studies. Ohta et al. (2005) used 85 SSR markers to characterize 144 individuals of flowering cherries (*Prunus* subgenus *Cerasus*), 29 SSR were successfully amplified and they found mean number of alleles per locus of 17.3. Three SSR markers were common to Ohta et al. (2005) and present study (Table 2.13) and for all three SSR, more alleles detected compared to the number of alleles detected in sweet cherry indicating that flowering cherry is more genetically diverse than sweet cherry.

Table 2.13: The comparison of number of alleles per SSR marker and heterozygosity (H) of three SSR between sweet and flowering cherries

SSR Markers	Number of alleles detected in 36 sweet cherry selections	Number of alleles detected in 144 flowering cherry accessions (Ohta et al. 2005)
UDP96-001	4	11
MA007a	6	29
PMS67	4	23

Cantini et al. (2001) identified 107 alleles for 59 accessions of tetraploid tart cherries using 10 SSR markers, the number of alleles on locus ranged from 4 to 16 and 86% of the alleles had less than 0.2 of frequency. The present study found only 55% alleles had a frequency less than 0.2 of frequency. Even though, these parameters are not readily comparable between sweet and tart cherries, there were 6 SSR markers common for study of genetic diversity in tart cherry (Cantini et al. 2001) and the present study (Table 2.14). Except for PS8e8, all the other SSRs exhibit a greater number of alleles in tart cherries compared to sweet cherries.

Table 2.14: The number of alleles detected for sweet and tart cherries for 6 SSR markers

SSR Markers	Number of alleles detected in 36 sweet cherry cultivars	Number of alleles detected in 59 tetraploid tart cherry accessions (Cantini et al. 2001)
PS8e08	4	4
PMS2	5	8
PMS30	7	11
PMS3	7	16
PceGA59	2	10
PMS67	5	13

In current study only nine *S*-alleles were reported, however, to date, 32 *S*-alleles have been reported in sweet cherry [Sonneveld et al. (2003), De Cuyper et al. (2005), Wunsch and Hormaza (2004) and Vaughan et al. (2008)]. De Cuyper et al. (2005) found 17 S-alleles in Belgium wild cherries With the *S*-alleles S_3 (26%), S_1 (16%) and S_2 (13%) the most common and S_1-S_7, S_9, S_{12}-S_{16} also present in that sweet cherry germplasm. S_{10} and S_{17}-S_{22} were unique to wild cherries. In the present study, nine *S* alleles reported (S_1-S_7, S_9 and S_{12}) and S_4 is the most abundant (29%), S_3 (25%), S_9 (14%) and S_1 (13%). S_{13}-S_{16} were not detected in the present study and they occurred in less than 2% in Belgium sweet cherries. Wunsch and Hormaza (2004) reported three new *S*-alleles (S_{23}-S_{25}) and Vaughan et al. (2008) reported six new *S*-alleles in wild cherries (S_{27}-S_{32}) and these alleles were not detected by our study or by De Cuyper et al. (2005). Schuster et al. (2007) studied *S*-allele genotypes of 149 sweet cherry cultivars and clones in Turkey and found 13 different *S*-alleles and 40 genotypes (i.e. *S*-allele combinations). The present study identified nine *S*-alleles and 15 genotypes with S_3S_4 been the highest represented genotype; S_3S_6 is the highest represented genotype. Whereas, S_3S_4 and S_1S_3 are also prominently present in sweet cherry accessions assessed in Turkey (Schuster et al. 2007).

Marker polymorphism

300 alleles were found using 77 DNA markers in 36 *P. avium* selections. The minimum number of alleles for a marker locus was two, the average was four and maximum was nine (for the *S*-locus: S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_9 and S_{12}). The average heterozygosity (H) and the polymorphic information content (PIC) for all the markers

were 0.5 and range was 0.63. The markers which have PIC equal or greater than 0.5 were adequate for linkage mapping, QTL analysis and diversity studies compared to the markers which have PIC less than 0.5. Table 2.15 presents the DNA markers and H and PIC in the studied set of sweet cherry cultivars. This was an important resource to select markers for marker assisted breeding and population genetic studies.

Table 2.15: Heterozygosity (H) and Polymorphic Information Content (PIC) of DNA markers used in the study

Locus	H	PIC
AMPA110	0.33	0.28
BPPCT002	0.50	0.45
BPPCT008	0.34	0.31
BPPCT014	0.55	0.51
BPPCT026	0.70	0.64
BPPCT034	0.68	0.62
BPPCT037	0.75	0.70
BPPCT039	0.67	0.61
BPPCT040	0.76	0.72
CPDCT016	0.46	0.42
CPDCT022	0.73	0.68
CPDCT037	0.67	0.63
CPPCT006	0.57	0.52
CPPCT016	0.73	0.68
CPPCT019	0.29	0.26
CPPCT022	0.54	0.44
CPPCT023	0.46	0.41
CPPCT033	0.82	0.80
CPSCT026	0.33	0.29
CPSCT027	0.55	0.47
CPSCT038	0.47	0.42
EMPA001	0.73	0.68
EMPA005	0.52	0.44
EMPA011	0.42	0.38
EMPA014	0.71	0.68
EMPA015	0.67	0.62
EMPaS01	0.56	0.48
EMPaS11	0.68	0.63
EPDCU2931	0.60	0.55
EPDCU3083	0.50	0.46
EPDCU3392	0.76	0.72
EPDCU5100	0.62	0.58
EPDCU5183	0.60	0.55
EPPB4213	0.16	0.15
EPPB4227	0.51	0.45
EPPB4230	0.65	0.61
EPPCU0961	0.62	0.55
EPPCU3090	0.52	0.40
EPPCU3664	0.61	0.57
EPPCU4726	0.06	0.06
EPPCU5990	0.50	0.45
EPPCU9168	0.56	0.49
M12a	0.40	0.35
MA005c	0.50	0.38
MA007a	0.65	0.58
Ma039a	0.38	0.34
Ma040a	0.60	0.54
Ma069a	0.36	0.31
MD201a	0.16	0.15
PACITA4	0.50	0.46
PCeGA34	0.60	0.55
PceGA59	0.50	0.37
PCHCMS2	0.44	0.34
pchgms49	0.38	0.35
PMS2	0.66	0.59
PMS30	0.76	0.72
PMS67	0.64	0.58
PR101	0.18	0.17
PR33	0.53	0.42
ps1h3	0.42	0.39
PS8e08	0.58	0.51
S-RNase	0.81	0.78
UCD-CH12	0.68	0.65
UCD-CH31	0.70	0.65
UDA-005	0.45	0.36
UDA-027	0.35	0.29
UDA-037	0.56	0.48
UDA-059	0.26	0.23
UDAp-401	0.68	0.64
UDAp-407	0.41	0.35
UDAp-461	0.43	0.38
UDP96-001	0.55	0.48
UDP96-019	0.50	0.38
UDP97-402	0.70	0.65
UDP98-021	0.52	0.42
UDP98-416	0.40	0.32
Mean	0.54	0.48
Standard deviation	0.16	0.16

A panel of DNA markers for *P. avium* DNA fingerprinting

The identification of a minimum number of markers that can differentiate all 36 selections used in this study would be very important for future DNA fingerprinting studies in sweet cherry such as cultivar identification and to solve cultivar mix ups in nurseries and orchards. Based on PIC data (Table 2.15) and the ability to clearly differentiate the fragment size, a subset of five markers (CPPCT016, PMS30, *S*-locus, EPPCU0961 and UCD-CH12) were selected from the 77 markers through several iterations of cluster analysis. The alleles for these five markers together can differentiate all the 36 cultivars in a dendrogram without any ambiguity and overlapping.

Use of DNA markers for diversity studies

The use of DNA markers is currently the most popular approach to study the genetic diversity in living organisms. However, this approach is limited by the availability of a sufficient number of markers that provide genome-wide coverage. In sweet cherry linkage maps are available (Joobeur et al. 1998; Dirlewanger et al. 2004; Olmstead et al. 2008) from which a genome-wide set of DNA markers could be selected. However, sometimes, not all the DNA markers, especially some SSRs, are useful for allele mining in a diverse set of cultivars as SSR alleles can be difficult to resolve due to stutter bands or smears in the gels. The solution is to identify a subset of markers for which alleles can be confidently identified and if possible verified in segregating populations. There are instances, when some markers amplify multiple alleles which cannot be used to mine alleles and the strange alleles which do not agree with Mendelian genetics in pedigree relationships for the studied cultivars. Such markers have to be

discarded from diversity studies. In rare cases, confirmed null alleles (symbol: $$) could be used as normal alleles when confirmatory evidences are available from progeny data.

CONCLUSION

The genetic diversity of 28 PNW, seven non-PNW and one wild sweet cherry groups were analyzed using 77 DNA markers. A total of 300 alleles were identified with an average of four alleles per locus to test the hypothesis of genetic founder effect. A total of 52 unique alleles were identified and 40 of them were not present in the PNW sweet cherry germplasm. A total of 157 rare alleles were identified and 44 of them were absent in PNW sweet cherry germplasm. These results indicate that early settlers brought a limited subset of sweet cherry germplasm to the New World and incorporation of germplasm from the natural habitat would broaden the genetic diversity to provide a better platform for sweet cherry breeding.

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