

ABSTRACT

SPENCER, JESSICA ADRIENNE. Molecular Marker Analysis of Primocane-Fruiting Traits in Raspberry. (Under the direction of Dr. Gina Fernandez and Dr. Bryon Sosinski).

Raspberry, or *Rubus idaeus*, is a perennial shrub whose roots produce biennial canes. While commercially valuable, characteristic softness of the fruit limits its shelf life while the length of its fruiting season also limits its local fresh market availability. Most cultivars of raspberry are grown in a two year system with vegetative first year canes (primocanes) pruned and trellised after the second year canes (floricanes) have fruited and been removed. However, there are some raspberry cultivars that produce fruit on the primocanes. These primocane-fruited varieties produce fruit later than floricane fruited varieties and have the added advantage that they can be easily mown to the ground each year after harvest. There is great potential in using primocane fruited varieties to extend the raspberry season for local fresh markets. Unfortunately, most primocane fruited cultivars yield less than their biennial counterparts. Efforts in breeding and selection are underway to overcome these problems, but the primocane fruited trait is irregular and not well understood, while the selection process can be slow. This research aims to clarify the genetics behind the primocane fruited trait by developing a genetic linkage map using AFLP's and SSR's to identify associated QTL(s). The mapping population consisted of 130 individuals from a cross between the primocane fruited selection NC 493 and floricane fruited variety 'Chilliwack'. The primocane fruited trait was found in half the population. In addition, there was segregation within the primocane fruited population for lateral branch fruited, tip fruited, and tip and lateral fruited in a 1:2:1 ratio. A total of 208 AFLP and SSR markers were found and a final map of 9 linkage groups was constructed from 116 of these markers. Linkage groups (LG)

of the parents showed significant molecular marker loci for primocane fruiting on linkage group 7 when analyzed with MapQTL 5. When lateral and tip fruiting was analyzed, separate loci appeared on LGs 5 and 7, respectively. This suggests that there are three genes for the primocane fruiting trait; one which controls the primocane fruiting ability and two which control whether fruit appear on the tips and/or lateral branches of the canes. In addition, markers normally found on LG 5 were instead found on LG 1 for NC493, indicating a possible chromosomal rearrangement that could be related to the formation of the primocane fruiting trait. Additional traits of vigor, growth habit, and cane spread were also measured on a subjective 1-9 scale. Significant loci were found on LG's 1 for spread and vigor, and LG 3 for spread and growth habit. These data will help create a solid base for future research to find markers for MAS and may one day aid in finding the specific genes responsible for these traits.

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Molecular Marker Analysis of Primocane-Fruiting Traits in Raspberry

by
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DEDICATION

To Mama and Keifie, of course. With all my love.

BIOGRAPHY

Jessica Adrienne Spencer was born and raised in High Point, North Carolina. Although a self-described fantasy/sci-fi book worm, she was always most curious about the natural sciences, greatly enjoying field trips to learn about local flora and fauna and often reading fiction about survival in the wild. Biology was a favorite subject in high school at Westchester Academy where she was the only one in her school to take the AP Biology exam. In 2000, she chose to attend North Carolina State University over UNC to pursue a degree in Biology with more of a plant/agronomy focus. She graduated in May 2004 with a B.S. in both Biology and Biochemistry and a minor in Biotechnology. Although hoping to obtain a job working with plants, her first job was working at BASF with insects, her second at LabCorp in cytogenetics, and her third was at the UNC Arthritis Research Center working with mice. Still harboring a desire to work with plants, and also a secret desire to put plants on Mars, she applied to the NCSU Plant Breeding program. Through this program she received a Monsanto Fellows scholarship and was accepted to the Horticultural Science graduate program, choosing to work with Gina Fernandez and Bryon Sosinski in raspberry breeding. The Monsanto scholarship provided her with many excellent opportunities to learn more about commercial breeding programs, meet other plant breeding students, and also have an experiential learning internship with Seminis in Davis, California. After obtaining her Master's, she plans to travel to Costa Rica to visit her friend Ramon Molina-Bravo's breeding program at the National University of Costa Rica and brush up on her Spanish before entering the job market.

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CHAPTER I

Literature Review

Introduction

Raspberry (*Rubus idaeus L.*) is one of the world's most important berry crops and global production has steadily increased for the past fifty years (FAOSTAT, 2011). It is a popular fruit due to its flavor, bright color, and its recent positive health associations (Halvorsen et al., 2002; Ross et al., 2007; Scheuller et al., 2006). Approximately 38 raspberry breeding programs in 21 countries have released more than 160 cultivars of raspberry in the past 30 years (Dossett et al., 2009). Breeding programs aim to develop new raspberry cultivars that are high yielding and adapted to more diverse production environments. Most raspberry breeding is done using traditional methods, which can often take 5 to 10 years. Novel breeding methods can decrease the turnaround time in producing new cultivars by using molecular markers (Lande & Thompson, 1990). Although markers have not yet been used comprehensively in raspberry breeding, it is inevitable that they will soon be used to facilitate the more rapid development of raspberry cultivars. Herein the literature to date on raspberry breeding and genetics is reviewed as it pertains to the molecular markers discovered thus far, with particular emphasis placed on primocane fruiting genetics.

A Brief Overview of *Rubus idaeus*

Red raspberry is a popular fruit with ancient origins, first described by Pliny the Elder around 45 A.D. as “ida” fruits gathered near Mount Ida in Turkey, from which originates its classification *Rubus idaeus* (Jennings, 1988). Seeds buried in Roman ruins around Britain may represent the first attempts at cultivation and breeding, although physical documentation of cultivation did not occur until the 16th century. Today’s varieties are mainly European and Asian in origin, but many cultivars also have the North American subspecies *strigosus* in their ancestry. *Rubus idaeus* has a genome of approximately 275Mb comprised of 7 diploid chromosomes ($2n=2x=14$) (Jennings, 1988). This small genome makes raspberry especially suitable for genetic research.

Most commercial varieties of raspberry are deciduous shrubs with perennial roots and crowns that produce biennial canes. In the first year the canes are called primocanes and are vegetative. After overwintering, these canes produce flowers, consequently named floricanes, and die shortly after harvest. A few species will produce fruit on the primocane, mainly on the tips of the canes, and are accordingly called primocane-fruiting. The aggregate fruit is composed of many drupelets around a central core (receptacle) which separates from the fruit when ripe; a unique trait which classifies it into the subgenus *Idaeobatus*. *Rubus idaeus* usually produces bright red fruit, although some cultivars have a range of ripe colors from bright yellow and orange to dark red. The coloration of raspberry fruit is mostly due to concentrated anthocyanins, one of many antioxidants in raspberry fruit. Research shows that berry fruits have levels of antioxidants 4 times greater than non-berry fruits and 10 times

greater than vegetables (Halvorsen et al., 2002). Phenolic compounds found in berries, which also contribute to color, can inhibit cancer cell proliferation in vitro and have anti-inflammatory and antimicrobial effects (Ross et al., 2007; Scheuller et al., 2006).

Traditional Breeding

There are almost 200 wild and cultivated species in the *Idaeobatus* subgenera, of which 40 or more have been incorporated into raspberry breeding (Dossett et al., 2009). The pedigrees of 137 cultivars released since 1961 trace back to 50 original parental clones, and only 15 of these contributed more than 1% of their genetic material (Dale et al., 1993). This shows that the gene pool of raspberries is relatively limited and that the genetic base needs to be broadened if new cultivars are to be developed. Wild raspberry species represent a large source of genetic diversity for developing improved traits and have been utilized in the past to develop new raspberry cultivars. For example, *R. kuntzeanus* and *R. parvifolius* were successfully used to breed for traits such as low chilling requirement and adaptation to warmer temperatures (Ballington, JR, 2008; Jennings, 1988). Another example is the primocane fruiting trait that has been incorporated into *R. idaeus* through several wild species such as *R. illecebrosus*, *R. odoratus*, and *R. arcticus* (Keep, 1988). However, interspecific breeding can still be difficult and time consuming, and bringing in a desirable trait from another species also brings with it many undesirable traits that must then be eliminated over subsequent selection cycles. Also, raspberry cultivars often show inbreeding depression and self-incompatibility, making selfing and backcrossing challenging if not impossible, or force the breeder to use a slower modified backcrossing scheme (Jennings, 1988). These issues are

not only a problem for interspecific breeding, but intraspecific raspberry breeding as well, and each selection cycle for raspberry may take up to 2 or more years while raspberry plants reach maturity. Modern molecular techniques, such as genetic markers, could speed up this process significantly by enabling the breeder to screen for traits while the raspberries are still seedlings.

Molecular Breeding

Molecular markers

Molecular markers are DNA fragments, proteins, or other molecules that can provide a means of identifying the approximate location of genes without genome sequencing, a method which, at present, is costly and time consuming. There are several types of DNA molecular markers and the most common of these include amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), random amplification of polymorphic DNA (RAPD), and single nucleotide polymorphism (SNP) (Pang et al., 2005). Most of these markers are created through variations in the procedure of amplifying DNA of an organism through polymerase chain reaction (PCR) and sorting the products by size, usually by visualizing the markers as bands on a gel.

In order to link traits to specific molecular markers, this process must be repeated among several closely related organisms, most often siblings, to create a genetic profile of each related organism. The resultant bands of the same molecular size are markers, and their

presence or absence between siblings and between other markers forms the basis for genetic mapping. During meiosis, crossover events between homologous chromosome pairs may occur, and the further apart markers are on a chromosome, the higher the chance that this will occur. When several markers appear together across 50% or more of the siblings, these can be inferred to be linked due to a high likelihood of being present on the same chromosome. The lower the occurrence of crossover events, then the closer together these markers lie.

These linked markers are called a linkage group, and in this way a rough genetic map of a chromosome may be formed. Similarly, when numeric, phenotypic data for a trait of interest from the segregating population is included in the analysis, the relationships between traits and markers can be revealed. This marker trait analysis, often called quantitative trait loci (QTL) analysis, does not identify the genes themselves but a general area where related genes may lie. These markers are tested against other individuals in that species and are deemed transferable if they reliably appear in association with the investigated trait. These can then be used in marker assisted breeding (MAB) or selection (MAS). Marker assisted breeding has already proven its utility in several crop breeding programs by allowing selection for traits that may vary with season or environment, and by reducing linkage drag by selecting against undesirable alleles. This time saving method can be especially valuable in perennials where years may pass before a fruit crop is produced (Dirlewanger et al., 2004). In addition, markers will help locate genomic regions where the actual genes may lie once an organism is sequenced (Bernardo, 2008).

Molecular markers have been developed for raspberry and were used to identify the genetic diversity between and within groups of wild and cultivated *Rubus idaeus* genotypes

(Marshall et al., 2001). This research confirmed previous studies on the diversity of cultivated raspberry by showing that wild raspberry is much more genetically diverse than cultivated raspberry with little evidence for gene flow between the two. Molecular markers were also used to construct the first genetic linkage map of red raspberry. Thus far there have been four linkage maps of raspberry produced from four separate research groups (Graham et al., 2009; Molina Bravo, 2009a; Pattison et al., 2007; Sargent et al., 2007). In three of these maps, SSR's were used to provide a consensus between the linkage groups of the separate maps.

The first molecular map of raspberry was produced using 94 progeny from a 'Glen Moy' × 'Latham' (GMxL) cross (Graham et al., 2004). A combination of 432 AFLP, SSR, and EST-SSR primers were used to create a 465.8 cM map with 9 linkage groups, although later maps consolidated these to 7 linkage groups (Woodhead et al., 2008). The first QTL in raspberry were found when spininess, root sucker density and root sucker spread were analyzed and mapped in the same population (Graham et al., 2004). Spininess in raspberry is mainly controlled by dominant gene *s*, with spineless raspberries only occurring when the raspberry is homozygous recessive for gene *s* (Jennings and Brydon, 1990). Other sources of spinelessness, such as that of a spineless sport from the cultivar 'Willamette', were also found but the method of its inheritance was never made clear. For example, crosses made between spine-free 'Willamette' and homozygous *S* created spiny and spineless progeny, although crosses between spine-free 'Willamette' and spiny 'Willamette' resulted in only spiny offspring (Jennings and Brydon, 1990). In the GMxL cross used to form the genetic map, all progeny produced spines even though 'Glen Moy' is spine-free, suggesting

‘Latham’ is homozygous for gene *s*. While this means that the exact location of gene *s* could not be determined, variation in the number of spines among progeny was mapped to two locations near several markers on linkage group (LG) 2 (Graham et al., 2004). Later research in the same population also mapped the spininess trait to LG 2 and also to LG 3 near gene *H*, which controls cane hairiness (Graham et al., 2006). The sucker production gene is simple in comparison, possibly controlled by three genes *sk1*, *sk2*, and *sk3*. It was theorized that suckers are produced when gene *sk1* is recessive or when *sk2* and *sk3* are both present (Knight and Keep, 1960). Graham et al., (2004) mapped root sucker spread to two locations on LG 8, one of which overlapped with sucker density, a pattern that corresponds with the actions of genes *sk1*, *sk2*, and *sk3*.

Using traditional breeding methods, Jennings (1990) explored the genes that control cane hairiness and anthocyanin production in stems and fruits, named *H* and *T* respectively. Both of these are usually present at intermediate frequencies, suggesting a linkage to a recessive lethal trait. Gene *H* is also shown to be associated with small, abundant spines and fungal resistance (Jennings and Brydon, 1990). In molecular marker research, gene *H* was shown to be located on linkage group 2 and confirmed to be associated with resistance to cane botrytis and spur blight (Graham et al., 2006). Other fungal resistances for cane spot and yellow rust studied on the GMxL progeny showed no association with gene *H*, and mapped to LG 2 and 4, and LG 3 and 5, respectively.

A second genetic map was made from a first generation backcross population derived from a resistant (‘Latham’) × susceptible (‘Titan’) cross to find QTL for phytophthora root rot resistance (PRR). Pattison et al. (2007) first used statistical analysis of their population

(generation means) to hypothesize that PRR is controlled by 2 dominant genes. Using AFLP, RAPD and RGAP (Resistant Gene Analog Polymorphism) markers to map the population, Pattison et al. showed that most PRR resistance variance is located on two linkage groups, which corresponds with their previous population analysis. However, Pattison's mapping methods did not use any previously mapped codominant markers so a consensus map between other existing maps is unavailable.

A third genetic map was created from 94 seedlings from a cross between 'Malling Jewel' × 'Malling Orion' (Sargent et al., 2007). This 505 cM genetic map of 7 linkage groups was made using 95 AFLP and 22 SSR markers in order to map the A_1 resistance gene and dw , the dwarf phenotype described as possibly being linked to genes H and T .

Amphorophora idaei, the large raspberry aphid, is a vector for four viruses: Rubus yellow net virus, Raspberry leaf spot virus, Raspberry leaf mottle virus and Black raspberry necrosis virus. There are four described biotypes of this aphid and one resistance gene, A_1 , is widely used although it only imparts resistance to the first biotype, which is now nearly extinct. In response, biotype 2 aphids are more prevalent. By mapping the A_1 gene, alternate sources of resistance can be found. Sargent et al. mapped A_1 to LG 3 in the same area as rust resistance in the Graham et al map. Gene dw mapped to LG 6, contradicting the hypothesis that dw is linked to gene H (Sargent et al., 2007).

The most recent molecular map was produced by Molina-Bravo (2009) utilizing a population from a cross between the selection NC 497 (*R. parvifolius* × 'Tulameen') × 'Qualicum' (NCxQ). AFLP and SSR markers were used to create a map to identify quantitative trait loci associated with chilling requirements and heat tolerance. The map

contained seven linkage groups which were found to be analogous to an existing map produced by Graham (2006) through SSR markers. Using chlorophyll fluorescence to assess heat tolerance, three QTL located on LGs 1, 5 and 7 were found that explained 15.9, 10.4 and 8.8% of the variation within the mapping population. Variability of chilling requirements was explained by up to 69% through several QTL mainly located on LGs 1, 3 and 7.

The NCxQ population also segregated for spiny and completely spineless raspberry (Molina-Bravo, 2009). Gene *s* was mapped to LG 7, and while this does not correspond to Graham's finding of spine density on LGs 2 and 3, it is important to note that the Graham population did not contain any spine-free progeny (Graham et al., 2006). This does, however, confirm the hypothesis that spines are a complexly inherited trait and could use clarification with further research. Molina-Bravo (2009) also located QTL for fruit size and shape on LGs 1, 3, 4 and 5. These data are in accord with findings of fruit quality QTL in the GMxL population which located on LGs 1, 4, and 5 and fruit size on LG 5 (Graham et al., 2008).

Specific Trait of Interest

The work described in this thesis uses molecular markers to discover QTL associated with primocane-fruiting in raspberries. The following section provides an overview of this trait and the genetic and molecular data in literature to date.

Primocane Fruiting

Most raspberries produce fruit from second year canes during the early to mid-summer, limiting the availability of local fresh fruit to one short season in locations where raspberries can be readily grown. During non-peak seasons and in areas unsuited to raspberry production, consumers must rely on raspberries shipped from other regions; long distance transportation of raspberry fruit is problematic, particularly due to the fruit's short shelf life. Ideally raspberries would be grown close to markets and for an extended growing season. Producing raspberries in a greenhouse would solve both these problems but is costly in relation to field production. To grow raspberries close to local markets outside of greenhouses, cultivars need to be bred for those climates. As described earlier, work is being done on traits such as heat tolerance and chill requirements, which will result in raspberry cultivars adapted to grow in warmer climates. Recent popular methods of lengthening the growing season use row covers, which can force earlier ripening, and tipping, which can delay ripening, but these only extend the fruiting season by a few weeks (Pritts, 2008).

A solution to extending the fruiting season is introducing more varieties of primocane-fruiting raspberry, which can also be subjected to row cover and tipping methods. Primocane-fruiting (PF) raspberries produce fruit on first year canes in the late summer through the autumn, usually beginning on the tips of the primocane (Keep, 1961). Primocane fruiting raspberries have the benefit of being less labor intensive as they can be mowed to the ground each year eliminating hand pruning at the end of each season. Alternatively, some PF raspberries are capable of "dual cropping" and can bear two seasons of fruits: one late in the first season from the upper parts of the primocanes, then another crop from the lower half of

the floricanes in the spring after overwintering. Using these cultivars along with other methods could provide fresh fruit to local markets for the majority of the year.

This valuable trait occurs naturally in several wild species, including *R. idaeus*, *R. occidentalis*, and *R. strigosus*, although yield is often very low and limited to the tips of the canes in late fall (Keep, 1988). Despite the low yield, primocane fruiting types have been cultivated in gardens across Europe and America for over 200 years (Hall et al., 2009). This trait had no commercial importance until primocane-fruiting ‘Lloyd George’ (*R. idaeus*) and various *R. strigosus* selections were interbred in 1934 at the New York State Experiment Station, Geneva (Keep, 1988). There are now many popular primocane fruiting varieties, such as ‘Heritage’, ‘Ruby’, ‘Autumn Bliss’, ‘Caroline’ and ‘Chinook’. However, PF cultivars still yield less when compared with floricanes fruiting raspberries and PF often ripen over several weeks. For this reason, the majority of breeding for PF cultivars has been focused on increasing yield.

There is still much debate about the genetics behind the primocane-fruiting trait as it does not reliably occur from year to year in some genotypes and can also partially occur on primocanes of the same plant (Keep, 1988). Crosses and selfs done by Keep (1961) of ‘Lloyd George’ showed the PF trait to be present in the majority of ‘Lloyd George’ progeny, with the amount of PF canes dependent on whether the cross contained any PF traits. Fejer (1977) suggested that PF inheritance is additive and also influenced by the fruiting season of the PF parents. Ourecky (1976) confirmed this additive effect and also showed a correlation between fruit size, branching and ripening; when raspberries bearing larger fruits were selected, there was a reduction in lateral fruiting branches and ripening occurred later in the

season, while genotypes expressing more fruiting laterals produced smaller fruit. Lawrence (1976) also showed a large correlation between extensive branching and early flowering in PF raspberry, which he attributed to two complimentary genes. Further evidence that the expression of the PF trait is linked to plant architecture was documented by Keep (1988), who showed that selecting for early bearing PF raspberries resulted in the selection of shorter or dwarf plants (Keep, 1988).

It appears that primocane-fruiting is a complex trait controlled by both genetic and environmental factors. Traditional breeding is slowly improving primocane-fruiting traits, but this process could be enhanced through earlier screening methods, specifically those using genetic markers (Hall et al., 2009). Further research in genetic markers for the PF trait will not only give insight into the genetics behind it, but could also lead to the development of markers for use in rapid screening methods.

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CHAPTER II:

Molecular Marker Analysis of Primocane-Fruiting Traits in Raspberry

Introduction

Raspberry is an economically important fruit crop due to its flavor, bright color, and beneficial nutritional components. Berry fruits contain on average four times more antioxidants than non-berry fruits and ten times more than vegetables, creating a large consumer demand for berries as concerns over health increase (Halvorsen et al., 2002). New ways of meeting this demand must be found as the monetary and environmental costs of shipping increase. One way that local growers can help meet the demand for raspberries is by using primocane-fruiting plants to extend the harvest season.

Most commercial raspberry cultivars are biennial with berries produced in the mid to late summer on second-year canes (floricanes) after overwintering. Primocane-fruiting (PF) varieties produce fruit in late summer and fall on the first year canes (primocanes) without vernalization. PF cultivars differ from floricane-fruiting (FF) in several aspects. Firstly, this shorter growing cycle means less maintenance for growers as each year the canes can be mown to the ground after harvest instead of hand pruning dead floricanes to allow room for growing primocanes. Additionally, no dormancy period must be overcome by chilling requirements, which makes winter killing of dormant buds or late frosts less of a threat.

Moreover, PF usually occurs from the tip of the most mature canes and moves down the cane until cold temperatures set in, creating a prolonged harvest when compared to floricane fruiting raspberries. Parts of the cane that have not budded will overwinter to produce fruit the next spring. This prolonged harvest may be an asset to hand-picked operations, but is not as desirable for larger operations. The largest reason that PF varieties are not used extensively is the fruit lacks in quality and yield in comparison to FF varieties (Graham and Woodhead, 2009).

While the PF trait is found in several wild species, it has only been bred into commercial varieties within the past century. Attempts have been made to describe the genetics behind the primocane fruiting trait, which was originally believed to be due to a single recessive gene designated *af* for autumn fruiting, but its complexity and the apparent randomness in its expression has led researchers to believe that environment plays a large role (Keep, 1988). The PF character is not complete in all plants, with some individuals often producing fruit on only part of the canes and this count varying from year to year. PF canes are often shorter and more numerous than summer-fruiting types as apical flowering stops the vertical growth of the cane. Elizabeth Keep (1988) theorized that the PF trait is additive and dependent on the earliness, vigor and maturity of the plant and local conditions such as climate, nutrition, hydration, and day length, an observation also made previously by Ourecky (1976) and Fejer (1977).

Although primocane-fruiting raspberries have come a long way in terms of earliness, yield, and quality when compared to their wild relatives, traditional breeding methods could be accelerated with the use of molecular markers for marker assisted selection (MAS). To date, there are several linkage maps of *Rubus idaeus* available, mapping traits such as spininess, color, anthocyanin content, fungal resistance, dwarfing, aphid resistance, heat tolerance, chill requirement, height, fruit set timing, and ripening stages. The goal of this study is to add to this knowledge by analyzing a population segregating for PF traits and locating loci that will give insight into how the PF mechanism functions and help identify useful markers associated with the trait for MAS.

Materials and Methods:

Field Research:

Plant Materials

In 2004, a cross was made between a primocane fruiting selection NC 493 (*R. parvifolius* × ‘Cherokee’) and a floricanne fruiting cultivar ‘Chilliwack.’ The resulting seedlings were planted in 2006 at the Sandhills Research Station, Jackson Springs NC (N. 35° 11.1471 W. 0.079° 40.629 elevation 567’ (173 m)). This population was shown to segregate for primocane fruiting, fruit color, spine density, growth habit, and other horticulturally important traits. In the spring of 2008, young leaf samples from the 131 individuals and NC 493 were collected and frozen at -80°C. ‘Chilliwack’ young leaf samples were received from the USDA National Clonal Germplasm Repository in Corvallis, Oregon.

Primocane Fruiting Evaluation:

In 2008, NC 493 and the 131 progeny of NC 493 × ‘Chilliwack’ were assessed on a biweekly basis from July to September for the presence or absence of primocane fruiting by determining whether flowers or fruit were present on the primocanes. Two individuals died over the winter, and in 2009 the rest of the population and the PF parent NC 493 were again evaluated for primocane fruiting on a weekly to biweekly basis from 1 June to 3 August. Notations were also made as to whether the plants were fruiting on the tip of canes, the lateral branches, or both as a distinct segregation occurred. The relative fruitfulness was also scored as being more or less than 15 nodes that were bearing flowers.

Other Screening:

The architecture and growth of canes were scored as spread, habit, and vigor in August 2009. Each trait was scored on a scale of 1-9, with 1 being least spread/most compact growth of canes, least vigorous growth, and canes that grew low to the ground. A score of 9 indicated a large spread of canes, very vigorous growth, and erect growth of canes.

Linkage map development:*DNA Extraction*

Genomic DNA was extracted from young freeze dried leaves using the 2% CTAB method described by Graham (2003). Approximately 20 mg of ground tissue was added to 1 ml 2x CTAB buffer then heated at 65°C for 30 minutes. To this sample, 800 ul of 24:1 chloroform-isoamyl alcohol was added, the sample was agitated for 5 minutes, then centrifuged at 9000 rpm at room temperature. The supernatant was then transferred to a new

tube with an equal volume of ice-cold propanol. After incubating for 15 minutes, DNA was pelleted by centrifugation at 3500 rpm for 10 minutes. The supernatant was decanted and the pellet allowed to dry before the DNA was suspended in 50 μ l TE buffer and heated to 65°C for 5 minutes. 0.5 μ l RNase A (10 mg/ml) was added and samples were incubated at 37°C for one hour before being stored in the refrigerator or -80°C freezer.

AFLP Analysis:

Amplified fragment length polymorphisms were generated using 250 to 500 ng of DNA from the parents and progeny based on the protocol described by Vos et al. (1995). Modifications of DNA digestion include using New England Biolabs (Ipswich, MA) Buffer 4, 8 U of EcoRI, and 5 μ g of BSA for a total 30 μ l reaction. The ligation step was modified by adding 5 μ l of solution containing 60 U of T4 ligase per reaction and NEB T4 buffer. The rest of the amplification process was performed as described by Vos et al. (1995) with the exception of using IRDye™ 800 (LICOR, Lincoln, Nebraska) infrared dye labeled to the 5' end of the EcoRI selective primer in the second amplification step to visualize the fragments. Selective primer combinations with three base extensions (Table 1) were chosen according to the number of polymorphic markers seen in screening plates and from previous work by Molina-Bravo (2009). AFLP products were denatured and visualized in an 8% acrylamide gel that ran in a 4300 DNA Analysis System (LI-COR, Lincoln, Nebraska) for approximately 3 hours. Markers were scored by hand with the help of Cross Checker (J.B. Buntjer, 1999) and a genotype table was produced using population type CP with parents present. Segregation of markers in the population were tested using χ^2 analysis (P=0.05, df=1,2). Markers were discarded by the Cross Checker program when no significant χ^2 was found,

and in cases where both segregation types possessed significant χ^2 , the highest score was used.

SSR Analysis:

Simple sequence repeat (SSR) markers developed by Graham et al. (2004) were used to anchor linkage groups to maps produced by Graham et al (2006), Sargent et al (2007) and Molina-Bravo (2009). Polymerase chain reactions (PCR) for SSRs were carried out using the three-primer tailing system described by Schuelke (2000), where either 6-FAM, VIC, NED, or PET fluorescent dyes were bound to the 5' end of the M13 universal primer. Each 10 μ l PCR reaction was comprised of 20 to 50 ng of DNA, 0.2 pmol forward primer, 1.0 pmol reverse primer, 1.0 pmol M13-universal fluorescent dye labeled primer, 0.2 mM of dNTPs, 1X standard buffer with MgCl₂, 0.5 U *Taq* DNA polymerase (Roche). Due to unclear PCR results using temperatures suggested in literature, a gradient temperature trial was performed using parental DNA with temperatures ranging from 50°C to 65°C in order to find ideal annealing temperatures. PCR's were then run in a Gene Amp PCR System 9700 thermal cycler (Applied Biosystems) for 15 cycles with the steps as follows: denaturing at 94.0°C for 30 s, annealing at ideal temperature for 30 s, extension at 72.0°C for 1 min. This was followed by 25 cycles of: denaturing at 94.0°C for 30 s, annealing at 50°C for 30 s, an extension at 72.0°C for 1 min, and a final extension at 72°C for 7 min. To visualize DNA fragments, 0.5 μ l of PCR reaction was added to 9.5 μ l of formamide with size standard, denatured, and ran on a 3730 DNA Analyzer (Applied Biosystems, Carlsbad, CA).

Of the 48 SSR primer pairs tested on the parents for polymorphisms, 23 were run on the entire population and parents. Scoring was performed using Genemarker 1.95 software by SoftGenetics LLC (*State College, PA*).

Linkage and QTL Analysis:

Segregation analysis of 208 polymorphic markers on 129 individuals of the population was performed using JoinMap 4 (Van Ooijen, 2006). Settings include population type CP for cross pollinated crops and default calculation option with the exceptions of Kosambi's mapping function instead of Haldane's and use of Maximum Likelihood (ML) mapping instead of Regression Mapping. Linkage groups were formed at a minimum logarithm of odds (LOD) of 5.0. Quantitative trait loci analysis was conducted using MapQTL 5 under default conditions (Van Ooijen, 2004). A Kruskal-Wallis test for single point analysis was used to identify the most likely areas for trait loci and was followed by interval mapping to calculate the explained variation and LOD scores. Permutation testing was performed using 500 permutations to identify significant LOD levels.

Results and Discussion

Linkage Mapping:

A total of 208 markers were found for the population. Seven AFLP primer combinations produced 173 markers. Of the 48 SSR primer pairs tested on the parents for polymorphisms, 23 were run on the entire population plus parents although only 12 were clear enough to be confidently scored across the entire population for use in linkage analysis (Table 2). Most alleles are comparable in size with those found by Graham (2006) in

‘Latham’ and ‘Glen Moy’ (Table 3). Two or more polymorphisms appeared on each SSR primer product and while most could be scored as codominant, errors involving linkage phases were resolved when they were also scored as dominant markers. These SSRs made up the rest of the 208 markers used in analysis and were used to anchor the map to linkage groups previously identified by Graham et al. (2006).

Maximum likelihood (ML) mapping and regression mapping were both performed on the population but only ML maps were used in the final analysis. Regression mapping was used first to create a linkage map of the entire population. This first map did not produce linkage group (LG) 2 and produced two LGs 4 as well as three small unidentified LGs. When parental maps were produced, these linkage groups did not differ significantly in marker placement from the whole population maps except for LG 2, which resolved itself from ‘Chilliwack’. The three unknown linkage groups remained. In addition, when these linkage groups were applied to QTL mapping, results between Kruskal-Wallis (KW) and Interval Mapping (IM) analyses were inconsistent.

While ML mapping is limited to creating maps of the parents, more markers were placed and the three unknown linkage groups were either absorbed by LG 5 or dissolved when ML mapping was performed. Results from QTL analysis using this map explained more variance and the KW and IM analyses were in consensus. This evidence plus previous research suggesting ML is more powerful and precise than regression mapping led to the use of ML linkage maps being used for the final analysis (Kao, 2000). The only disadvantage found was that marker distances were much greater than in regression mapping and did so

did not concur with map distances found by other researchers. A comparison can be found in table 4.

The final map consisted of 9 linkage groups divided between NC 493 and 'Chilliwack'. NC 493 produced linkage groups 1, 3, 4, 6, and 7 and placed 82 markers while 'Chilliwack' produced LGs 2, 4, 5, and 7 and only placed 34 markers. This uneven placement of markers is not unusual as Molina-Bravo (2009) and Graham (2006) also found that marker segregation favored one parent, likely due to one parent being more heterozygous and therefore contributing more polymorphisms to the progeny. The female parent of NC 493 was selected from an open pollinated population of *Rubus parvifolius* (Figure 1). The mapping population is therefore at least 12.5% *R. parvifolius*, and may partially explain the increased heterozygosity of NC 493 observed relative to 'Chilliwack' based on the number of segregating molecular markers.

The majority of SSR markers segregated according to previously found linkage groups with one exception; linkage group 1 from NC 493 contained markers from both LG's 1 (Rub166b and Rub210a) and 5 (Rub35a) from the previous published maps. Grouping at a LOD of 7.0 separated out Rub166b and 7 other markers, but Rub210a and Rub35a stayed firmly linked. The 'Chilliwack' map did not map LG 1 but did map LG 5 with both Rubus35a and Rubus105b contained therein.

Primocane Fruiting:

The population segregated 1:1 for primocane fruiting, suggesting that NC 493 was heterozygous for the PF trait assuming monogenic inheritance. Within primocane fruiting plants, it was also noted that there was segregation between lateral branch fruiting, tip

fruiting, and tip and lateral fruiting plants in a 1:2:1 ratio, respectively (Figure 2). Thus the PF trait seems to be influenced by three genes; one which controls whether PF appears and two others which control whether PF occurs in the tips or laterals. A χ^2 analysis supports this three gene hypothesis (Table 5). Additionally, this three gene effect may explain the additive nature of PF found by prior researchers. Tip fruiting appeared in a 3:1 ratio, suggesting that the trait is heterozygous for both NC 493 and 'Chilliwack', which corresponds to the finding of significant loci on LG 7 of 'Chilliwack' as well as that of NC 493. Lateral fruiting appeared in a 2:1 ratio, suggesting heterozygosity in NC 493 only.

A Kruskal-Wallis analysis revealed several significant markers, but the most significant K*-values for primocane fruiting fell on LG 7 of NC 493. When only lateral fruiting was analyzed, significant loci were found on the 'Chilliwack' LG 5, and when only tip fruiting was analyzed, significant loci were found on the upper half of LG 7 of NC 493. Interval mapping was also performed and showed a QTL on LG 5 and two QTL on LG 7 of NC 493 for the PF trait. A short significant group of markers also appeared on the 'Chilliwack' LG 7 using the interval mapping method. Table 6 displays the mapped traits, significant LOD scores, and the percent variation explained by these QTL.

To date, few traits have been reported to be associated with linkage group 7. Some loci for heat tolerance, chilling requirement, and spine were found by Molina-Bravo (2009). However, several traits have been mapped to LG 5, including flower timing, ripening rates, and plant height from Graham (2009) and heat tolerance, chilling requirement and berry color, shape, and size from Molina-Bravo (2009). Takeda et al.(1993) found that in the PF cultivar 'Heritage', the more chilling hours experienced by the root stock, the fewer days

until flowering and the fewer nodes per cane were produced. This also resulted in shorter plants which concurs with previous research showing that primocane fruiting plants selected for earlier ripening show more lateral branching and are often shorter or dwarfed (Keep, 1988; Ourecky, 1976; Lawrence, 1976). This evidence suggests that height and chilling likely play important roles in the PF mechanism. In addition, loci on LG 1 and 5 have been associated with cane height by Graham (2009) and from the appearance of Rubus35a on LG1 in NC493, there is evidence that a crossover event occurred at some point between LGs 1 and 5 in this population. It is possible that this event caused a deletion of a gene involved in the signaling of flower bud initiation, helping to give rise to primocane-fruiting traits.

Other Traits:

The spread of canes, growth habit, and vigor of growth were also mapped. Kruskal-Wallis analysis showed spread linked to loci on LG 1, although less significant K-values also showed a few loci on LG's 3, 4, and 5. Subsequent interval mapping showed QTL at LGs 1 and 3. Spread also displays a bimodal distribution within the population, possibly suggesting two competing genes for this trait (Figure 3).

Vigor also mapped to LG 1, although since a large spread of canes is considered a sign of vigorous growth, it is no surprise that spread co-localized with vigor on the map. The NC 493 × 'Chilliwack' population was grown in the same location and scored at overlapping times as the population from Molina-Bravo (2009). He found heat tolerance loci on LG 1, so a correlation between the heat tolerance and vigor seems reasonable.

Growth habit also mapped to LG 3, close to the location where spread occurred. Molina-Bravo (2009), mapped the growth habit trait as well, but QTL for that population were found on LG's 2 and 5. While growth habit for other populations has not been examined, QTL for height, which could be related to growth habit, were found on LG 3 by Graham (2009).

Conclusion

The primocane fruiting trait in raspberry is a desirable trait yet was poorly understood until now. While this research only looks at a single population, the segregation within the trait and the identification of the single PF gene and the two modifier genes will clarify further research in primocane fruiting genetics for other populations while confirming these findings. In addition, there is a large link between the chilling of root stock and primocane fruiting that needs to be explored, so more populations in more locations or controlled environments need to be combined with more markers to saturate those regions identified with primocane fruiting. It is inevitable that the *Rubus idaeus* genome will be sequenced, and this data will be able to help identify the specific gene sequences for the primocane fruiting and other growth traits.

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TABLES AND FIGURES

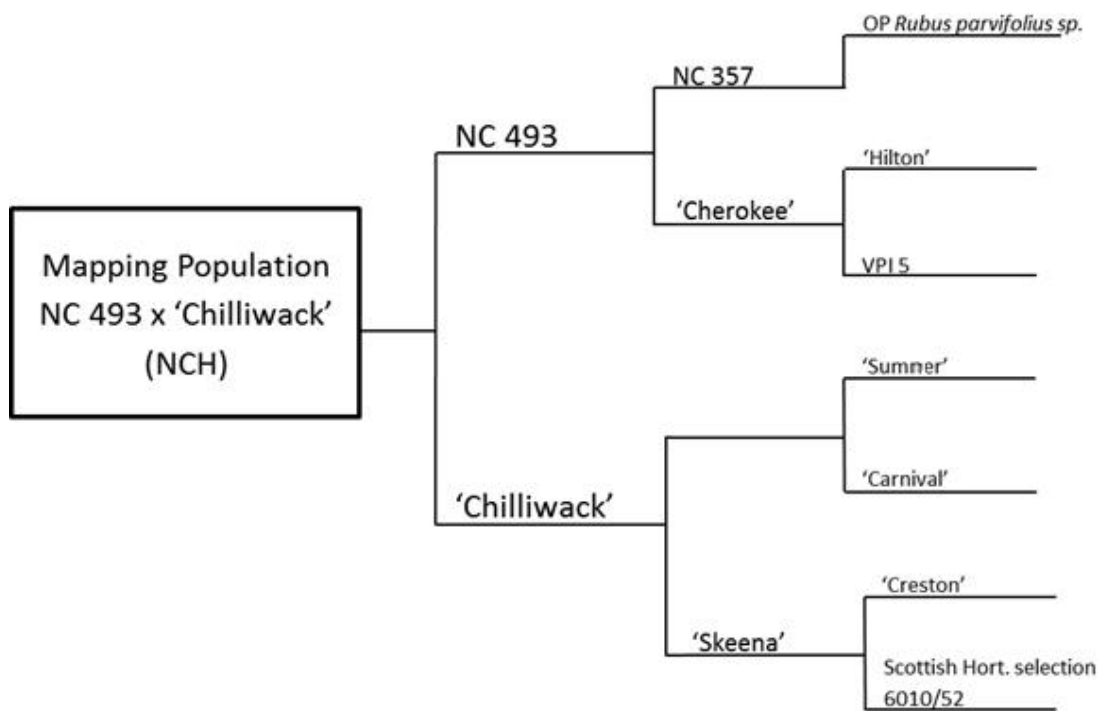


Figure 1. Dendrogram of mapping population ancestry

Table 1: AFLP primer codes and combinations used in NC493 x ‘Chilliwack’ (NCH) in comparison with Molina-Bravo's (2009) NC497 x *Qualicum* population (NQ).

Code	Combination		NCH	NQ
E32M32	EcoRI+AAC	MseI+AAC		x
E32M48	EcoRI+AAC	MseI+CAC	x	
E35M32	EcoRI+ACA	MseI+AAC		x
E36M35	EcoRI+ACC	MseI+ACA		x
E38M36	EcoRI+ACT	MseI+ACC	x	x
E38M40	EcoRI+ACT	MseI+AGC	x	x
E41M31	EcoRI+AGG	MseI+AAA	x	x
E41M50	EcoRI+AGG	MseI+CAT	x	
E42M32	EcoRI+AGT	MseI+AAC		x
E43M32	EcoRI+ATA	MseI+AAC		x
E44M33	EcoRI+ATC	MseI+AAG	x	x
E45M33	EcoRI+ATG	MseI+AAG		x
E45M35	EcoRI+ATG	MseI+ACA		x
E45M37	EcoRI+ATG	MseI+ACG	x	x

Table 2: List of polymorphic SSR markers ran in NCH as compared to Molina-Bravo's (2009) NC497 x Qualicum population (NQ) and the associated linkage groups (LG). "X" indicates mapping data acquired.

Code	NCH	NQ	LG
Rub166b	x	x	1
Rub210a	x		1
Rub107h		x	2
Rub157		x	2
Rub194h	x	x	2
Rub103a		x	3
Rub228a	x	x	3
Rub259f	x	x	3
Rub126b	x		4
Rub236b		x	4
Rub105b	x	x	5
Rub214b		x	5
Rub222e		x	5
Rub25a		x	5
Rubr35a	x	x	5
Rub118b	x		6
Rub123a	x	x	6
RubLeaf102		x	6
RubLeaf97		x	6
Rub116a	x	x	7
Rub263f		x	7
Rub26a	x		7

Table 3: SSR allele size and annealing temperature data from research population (in italics) for comparison with allele sizes from Graham et al (2004).

Code	Repeat motif	Primer sequences	Allele Sizes				Annealing Temp °C
			Latham	Glen Moy	<i>NC493</i>	<i>Chilliwack</i>	
Rubus116a	(ct)12-(t)10	L: ccaacccaaaaacctcaac R: gttgtggcatggcctttat	185, 190	193	<i>201, 298</i>	<i>211, 289</i>	58
Rubus166b	(tc)15	L: ccgcaagggtgtatcctaa R: gcatgagggcgatataaagg	215, 221	219, 224	<i>221, 229</i>	<i>229</i>	56
Rubus123a	(ag)8	L: cagcagctagcattttactgga R: gcactctccaccatttcat	170, null	140, 150	<i>152, null</i>	<i>162</i>	51.1
Rubus228a	(ga)41	L: tggacagctttgtgcagagt R: gcttgctgtatctccattgc	118, 128	150	<i>147, 165</i>	<i>165</i>	60
Rubusr35a	(ct)8	L: ttggaagcaciaaagcgata R: gcgacagccaaaacaaaagt	210, 226	226	<i>238</i>	<i>226, 232</i>	60.2
Rubus126b	(ct)31(ca)22	L: cctgcattttctgtattttgg R: tcagttttctcccaggtta	150, 164	164, 201	<i>187, 203</i>	<i>157, 183</i>	56.2
Rubus259f	(ct)4-(ag)8	L: tggcacaagaagcctgtaac R: tccatatccctcagcattc	244, 252	247	<i>264, 269</i>	<i>264</i>	60.2
Rubus194h	(ga)12	L: tgtgtttgtctctgcaacca R: agcccttactttctctgcaa	100, 111	111, 115	<i>188, null</i>	<i>188, 224</i>	60.2
Rubus118b	(ct)25	L: ccgcaaaacaaaaggtaag R: ggattcttgccaaagtcgaa	104, 112	137	<i>100, 121</i>		58
Rubus105b	(ag)8	L: gaaaatgcaaggcgaattgt R: tccatcacaacaccaccta	158, 190	158, 164	<i>190, 211</i>	<i>182</i>	56
Rubus26a	(ct)11(ca)29	L: aacaccggcttctaaggctc R: gatcctggaaagcgatgaaa	122, 150	150	<i>132, 138</i>	<i>138</i>	60.2
Rubus210a	(ct)25	L: tcctgatggtgtctggttg R: ttcgaggctttcagaaaca	101, 115	101, 115	<i>93, 131</i>	<i>119</i>	52.5

Table 4: Comparison of linkage group sizes from mapping using linear regression analysis (LR) of the population NCH and parents NC 493 and Chilliwack, and maximum likelihood (ML) analysis. Linkage group sizes from Molina-Bravo's 2009 map (NQ) and Graham's 2007 map (LGM) are also included.

LG	LR NCH	LR NC493	LR Chill	ML	NQ	LGM
1	88	88.2	0	383.5	63.7	133.4
2	0	0	87.7	292.4	90.5	118.6
3	94.1	97	0	143.1	101.3	157
4	65.9/86	86	65.9	129.0/463.4	54.7	183.7
5	16.4	0	66	509	90.7	91
6	102.1	102.1	0	273.4	35/44.2/28.7	117.7
7	54.1	90.6	0	198.3/370.2		
Unk	56.2/57.5/35.6	57.7	59.4/74.9		69.3	82.6
Total	653.2	515.6	353.9	2909.291	577.4	884

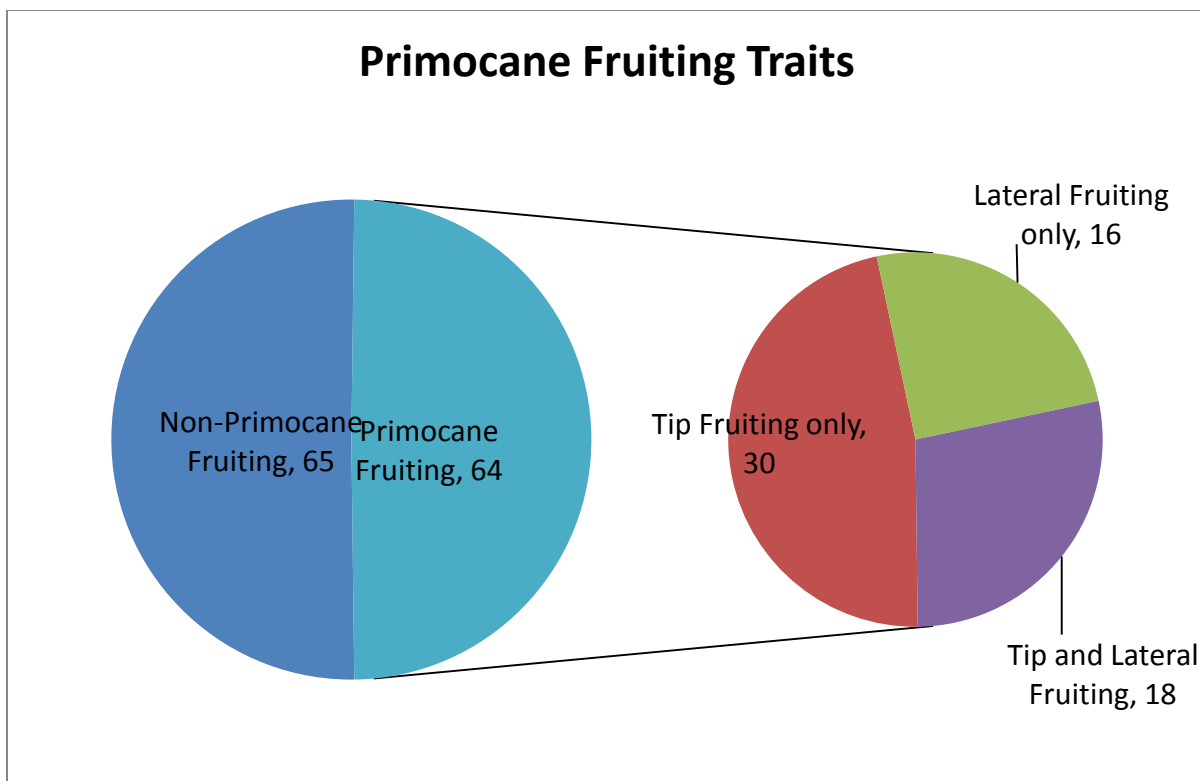


Figure 2. Comparison of non-primocane fruiting and primocane-fruiting divisions within the population NCH.

Table 5: PF numbers and % by type, expected % and Chi-square test for 1:3:1 segregation ratio.

PF type	n	%	Expected %
Total	64	48.85	50
lateral	16	12.21	12.5
tips	30	22.9	25
both	21	16.03	12.5

Chi-Square Test for Specified Proportions	
Chi-Square	1.5802
DF	3
Pr > ChiSq	0.6639

Table 6: Summary of QTL detected for traits primocane fruiting (PF), tip fruiting (Tips), lateral fruiting (Lats), Vigor, spread of canes (Spread) and growth habit (Growth) along with the associated linkage group (LG), significant LOD scores and the percent variation explained by the QTL.

Traits	LG	LOD	%
PF	7-NC493	24.04	94.3
Lats	5-Chilliwack	3.64	31
Tips	7-Chilliwack	4.74	28.7
Tips	7-NC493	13.07	43.7
Spread	1-NC493	2.28	11
Spread	3-NC493	3.69	22.3
Vigor	1-NC493	3.3	13.5
Habit	3-NC493	5.59	22.3

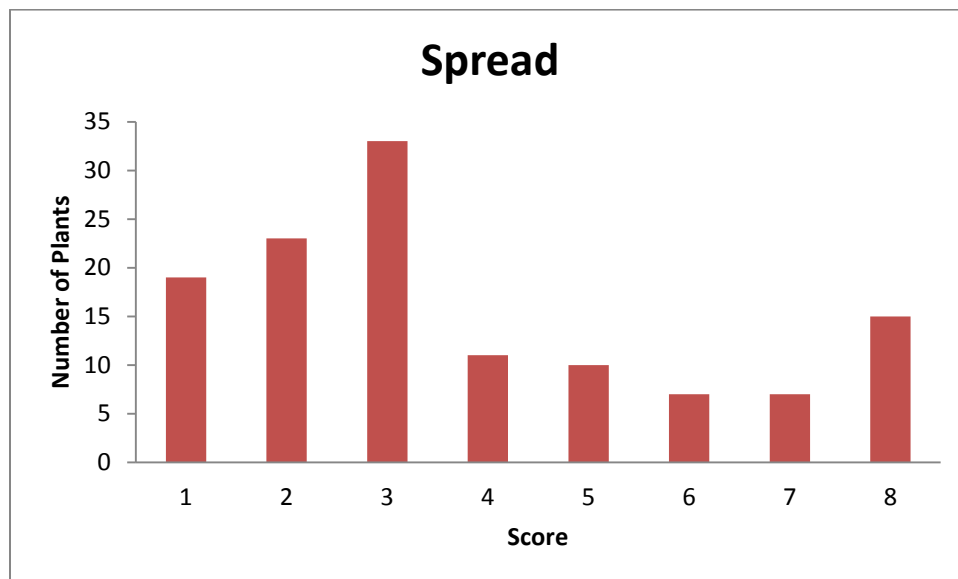


Figure 3. Spread of primocanes outward from center of plant. 1= no spread, 8= very wide spread.

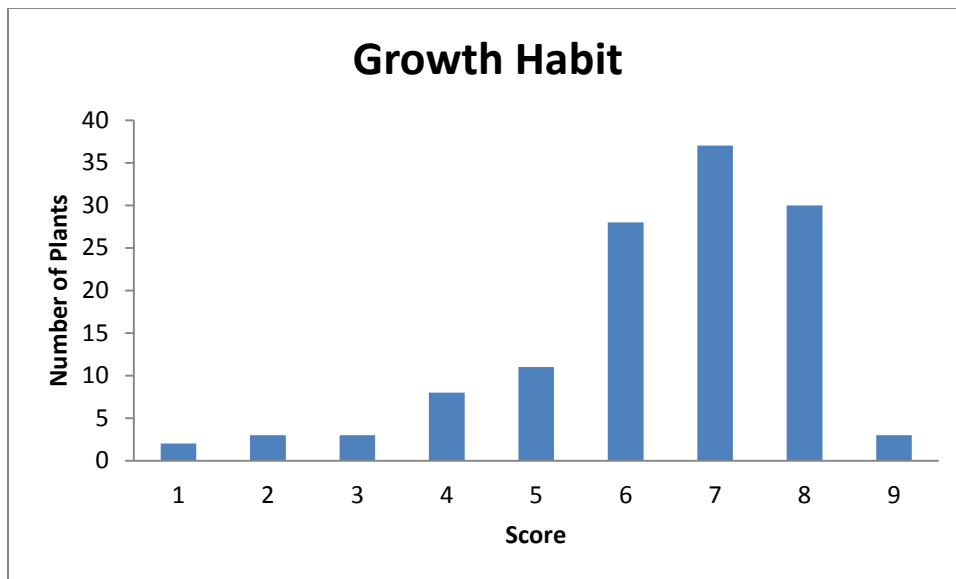


Figure 4. Growth habit, from decumbent to erect. 1= decumbent, 9= erect.

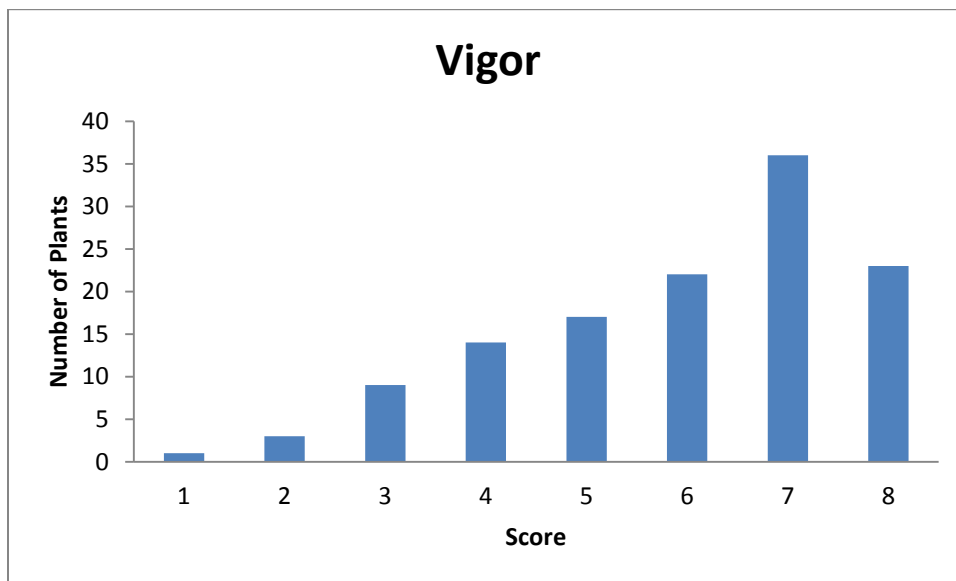
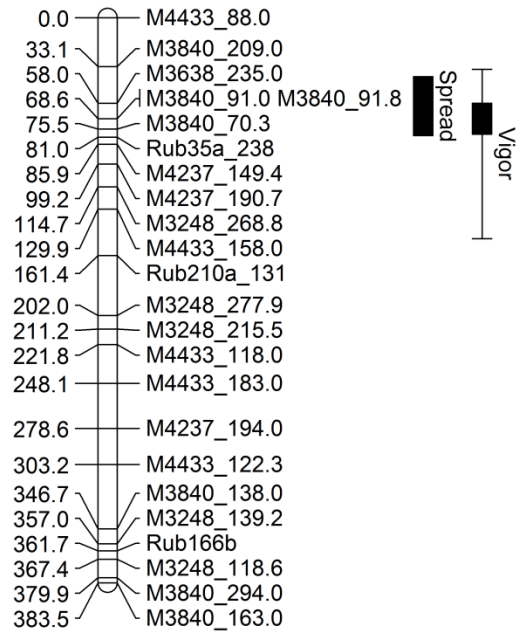


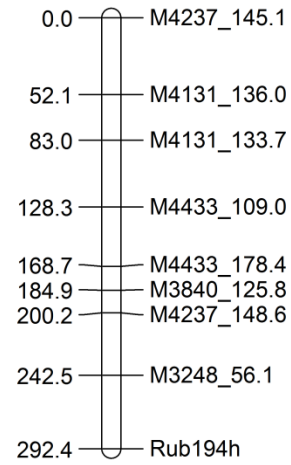
Figure 5. Subjective vigor of plants. 1= weak/struggling, 8= very vigorous growth.

Figure 6. Genetic linkage maps (cM) and relative QTL positions (1 LOD intervals, 2 LOD for error bars) for Vigor, spread of canes (Spread), growth habit (Growth), primocane fruiting (PF), tip fruiting (Tips) and lateral fruiting (Lats).

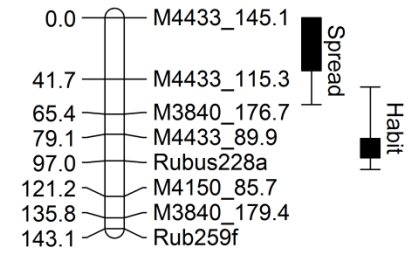
1-NC493

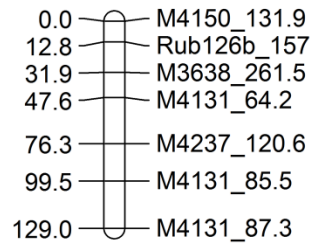
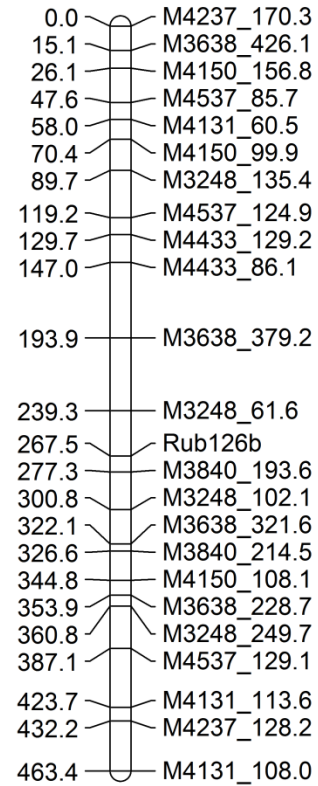
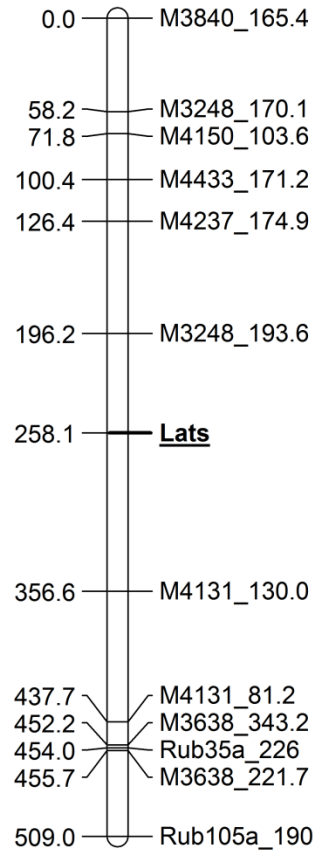
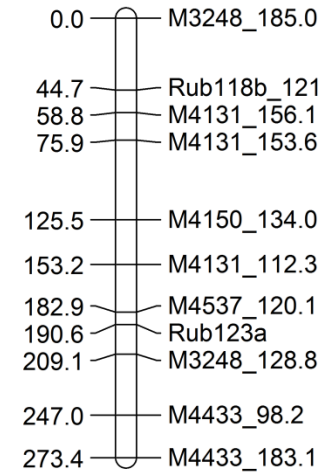


2-Chilliwack

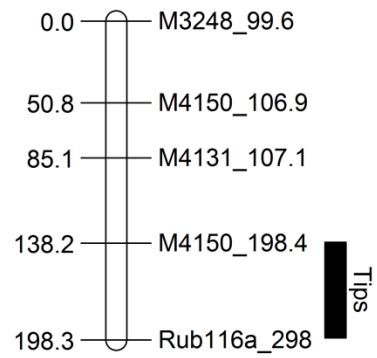


3-NC493

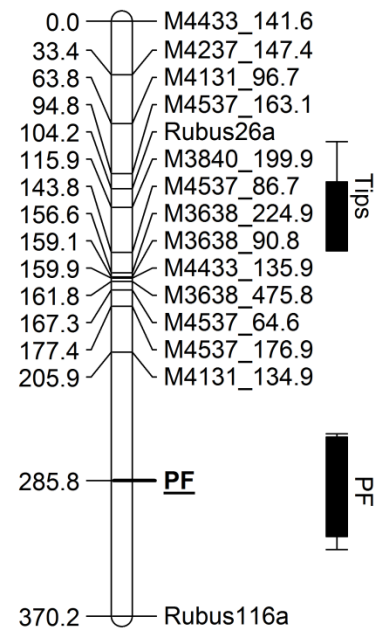


4-Chilliwack**4-NC493****5-Chilliwack****6-NC493**

7-Chilliwack



7-NC493



APPENDIX

QTL Mapping of Hue, Chroma, Anthocyanins and Phenolics

Introduction

Recent research has focused on anthocyanins and other phenolic compounds which not only give fruit their color but also impart many healthful benefits. Anthocyanins and other antioxidant compound in berry extracts have been found to inhibit cancer cell proliferation in vitro and have anti-inflammatory and antimicrobial effects (Ross et al., 2007; Scheuller et al., 2006). Raspberry (*Rubus idaeus*) is a popular fruit known to have significant levels of these beneficial compounds (Moyer et al., 2002), but research into the controlling genetics behind color and antioxidants is incomplete.

Gene *T* is the most significant gene controlling anthocyanin content and therefore color in raspberry, with the recessive homozygous *tt* displaying yellow fruits and stems and the least amount of anthocyanin (Jennings, 1963). Kassim et al. (2009) analyzed 8 anthocyanins from the 'Glen Moy' x 'Latham' (GML) population and mapped them to locations on LG 1 and 4; however, no color analysis was performed and this population produced fruit only in the red color range. Further research with the GMxL population analyzed ripening through color characteristics and hypothesized that results would be similar to those of Kassim et al, but instead markers were identified on LG 5 with significant QTL's also found on LGs 2, 3 and 6 (Graham et al., 2009). Molina-Bravo's (2009) population from the cross (R. parvifolius x 'Tulameen') x 'Qualicum' (NCQ) segregated for a range of fruit colors, from yellow blush to dark red. These colors were measured at different ripening stages, and two significant QTL were found on LG 5 and 6, with several QTL for size and shape also located

on LG 5. These conflicting data indicate complex genetics behind color and anthocyanins, with color traits perhaps more related to ripening than the anthocyanin genes themselves.

Materials and Methods

The population, plant materials collected, DNA extraction, linkage map development, and QTL analysis were all previously described in Chapter II.

Fruit Color:

Fruit samples of approximately 15 berries were collected in the summer of 2009. A total of 82 samples were first evaluated using a Royal Horticultural Society color chart, and then whole berries were analyzed by an Ultrascan Pro by HunterLab which produced CIE LAB scores and wavelength intensity scores. The LAB scores were then converted into hue and chroma for ease of reference. Berries were scored as ripe, under ripe or overripe before being measured.

Total Anthocyanins and Phenolics:

Analysis of anthocyanins and phenolics from 73 freeze dried samples was performed courtesy of Christine Bradish and Dr. Penelope Perkins-Veazie at the NCRC, PHHI, Kannapolis NC.

Results

Color varied widely from a yellow-blush to deep red, but was also dependent upon ripeness. Yellow fruits still retained some yellow when overripe but red fruits ranged from light red when underripe to sometimes purple color when overripe. On average, underripe berries scored higher in both hue and chroma while overripe berries scored lower where hue

describes the relative color and chroma is the lightness of that color. Figure 7 gives a general idea of this concept with regards to the analysis results.

QTL for hue were found on LGs 1, 4, and 6 of NC493, although the most significant scores were on LG 6. Significant LOD scores for chroma were only found on LG 6. There were no appreciable differences in QTL scores or locations when underripe, ripe, and overripe berries were accounted for.

Average phenolics of the raspberry samples were 2078.9 mg/kg FW (fresh weight) and 590.1 mg/kg FW for anthocyanins. Significant QTL were found on LGs 1 and 4 for both components. A summary of QTL and their significant LOD score along with percent variation explained can be viewed in table 6.

Discussion

Anthocyanins make up a portion of phenolic compounds and are also responsible for a large component of fruit color, mainly in shades of red. To this end there should be a relationship between the hue and chroma of fruit, which were also measured at different stages of ripeness, and anthocyanin content. While differences in ripeness could not be seen genetically, the fact that chroma, anthocyanin and phenolic QTL were found on LG's 1 and 4 confirms the relationship between them. These data also coincide with Kassim et al. (2009) who mapped 8 anthocyanins from the 'Glen Moy' x 'Latham' (GML) population to locations on LG 1 and 4 as well, and partially coincide with the fruit color analysis by Molina-Bravo (2009) that found QTL on LGs 5 and 6. Further in depth analysis would be needed to see if individual markers also coincided.

It is also significant to note that the GML population contained no yellow fruit, while the NCH contained some yellow fruit and NCQ contained even more. Anthocyanin is a general name for several pigment components so it is not difficult to imagine that different anthocyanin components could also be located on LG 6. Further research to identify this possibly missing color anthocyanin would be needed to verify this supposition.

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Ross, H.A., G.J. McDougall, and D. Stewart. 2007. Antiproliferative activity is predominantly associated with ellagitannins in raspberry extracts. *Phytochemistry* 68(2):218-228.

Scheuller, H.S., R. Lee, D. Sand, Y. Zhang, D. Heber, L.S. Adams, and N.P. Seeram. 2006. Blackberry, Black Raspberry, Blueberry, Cranberry, Red Raspberry, and Strawberry Extracts Inhibit Growth and Stimulate Apoptosis of Human Cancer Cells In Vitro. *J. Agric. Food Chem.* 54(25):9329-9339.

Tables and Figures

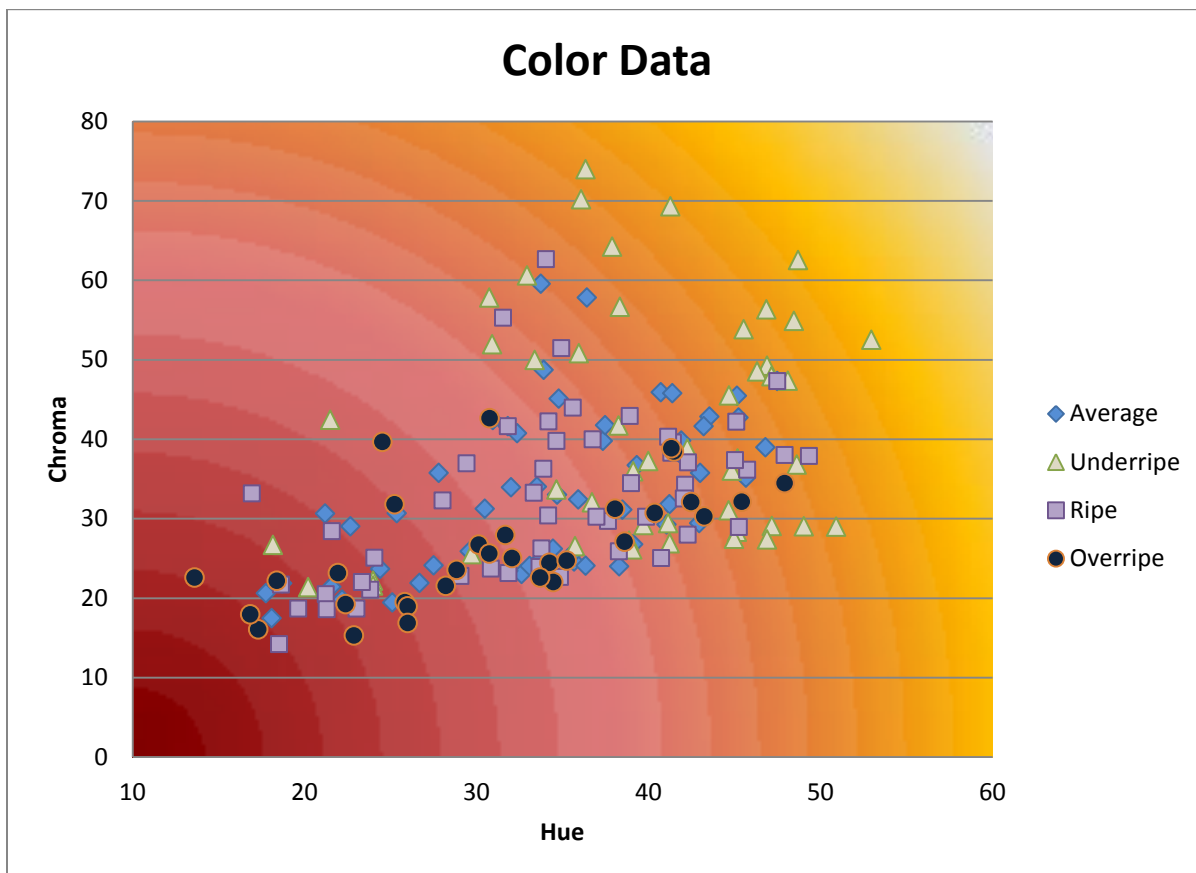


Figure 7. Hue and chroma scatter plot for data taken for underripe, ripe and overripe berries and their average. Background conveys a sense of the general color of individual berries.

Table 7. Summary of QTL detected for Hue, Chroma, Anthocyanins and Phenolics, along with the associated linkage group (LG), significant LOD scores and the percent variation explained by the QTL

Traits	LG	LOD	%
Hue	6-NC493	7.72	38.8
Hue	4-NC493	3.51	19.3
Hue	1-NC493	3.49	20.7
Chroma	6-NC493	3.08	19.4
Anthocyanins	1-NC493	3.26	21.6
Anthocyanins	4-NC493	2.39	16.5
Phenolics	4-NC493	2.13	19.6