Genomewide Selection in Apple: Prediction and Postdiction in the University of Minnesota Apple Breeding Program

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF
THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Dr. Rex Bernardo & Dr. James Luby

October 2019

Acknowledgements

While I have been at the University of Minnesota, it has been my sincere pleasure to be a part of two outstanding labs and departments. My advisors, Dr. Rex Bernardo (Department of Agronomy and Plant Genetics) and Dr. James Luby (Department of Horticultural Sciences), have truly been a privilege to work with. Their guidance, advice, and constant support have been invaluable to me. I would like to thank the members of my committee, Dr. Matthew Clark, Dr. Neil Anderson, and Dr. Aaron Lorenz, for their insights and patience as I moved through the steps of completing my research and dissertation. Additionally, I would like to offer my sincerest gratitude to Dr. Emily Hoover for her mentorship, advice, and constant willingness to lend an ear. I would like to extend a warm thank you to Jack Tillman, whose seemingly bottomless knowledge on apple breeding, fruit research, and lab and field techniques has helped me to grow as a researcher. I would also like to thank all of the members of both of my labs Seth Wannemuehler, Dr. Sofía Brandariz, Emmanuel Adeyemo, Dr. Nick Ames, Dr. Tola Oyetunde, Dr. Sushan Ru, Ashley Powell, Baylee Miller, Laise Moreira, Lu Yin and my APS friends, who are all more like family now. I would finally like the thank my friends and family. My parents and siblings, especially my sister Emily, have helped me through the challenges and obstacles I've faced, and shared in my successes. I cannot put into words how grateful I am for all of the support of everyone mentioned, and many more.

Abstract

Although marker assisted breeding is now considered routine in apple breeding programs, the adoption of genomewide selection is still in its infancy. Genomewide selection offers the potential to be a valuable tool to apple breeders. The first aim of this research was to assess the predictive ability of genomewide selection for fruit traits by testing an additive prediction model, a model fitting heterozygote effects, and a model fitting fixed effects for major QTL. The second aim of this research was to assess the utility of genomewide selection for fruit traits in the University of Minnesota apple breeding program. This comprised two main objectives, a comparison of selections based on genomewide predictions to selections made based on phenotypic selection and an analysis of the impact on predictive ability when full-sibs are included in the training data. This research finds that in general, a simple linear model is the most efficient choice for genomewide selection in apple unless major effect QTL are known, in which case including them as fixed effects may improve predictive abilities. We also confirmed that predictions made based on genomewide selection to be consistent with selections based on traditional phenotypic selection and that including five to 15 full-sibs from the test population in the training population data can improve predictive ability.

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Chapter 1

Literature Review

1.1 Domestication of apple

The main progenitors of the cultivated apple, *Malus domestica Borkh.,* are likely from the species *Malus sieversii* native to Central Asia (Harris et al., 2002; Luby et al., 2003). Following the migration of humans, apples can now be found on each continent except Antarctica. European settlers brought apples to the Americas in the 16th or 17th century (Luby et al., 2003). By the 19th century, apples had begun to be recognized as cultivars that were described by their optimal end use. With the westward movement of settlers in the United States, and the activity of Jonathan Chapman and Peter Gideon, the growth of apples spread to new territories (Nichols, 1975; Luby et al., 2003). The 20th century, with homesteads operating their own orchards and having namesake varieties, held much more diversity for apple in the United States than can be seen in modern cultivation (Luby et al., 2003). Germplasm characterization of the domesticated apple (*Malus domestica Borkh.*) as well as understanding and maintaining diversity in apple is a critical component of continued breeding progress.

1.2 The Rosaceae crop family

The Rosaceae crop family, to which apple belongs, has a domestic value of over $7 billion in the United States and is responsible for vital contributions to human health and rural economies (Iezzoni et al., 2010; Peace et al., 2017).

Apple accounts for approximately half of the Rosaceae crop value with over $3 billion in sales (U.S. Apple Association, 2017; USDA, 2016). An additional $14 billion in downstream economic activity is the foundation of many local economies (U.S. Apple Association, 2017). Despite the importance of the apple industry, breeders face challenges in releasing cultivars to meet the ever-changing demands of the market. The long generation intervals, obligate outcrossing, and extensive amount of space required to reach phenotyping of fruit result in a labor and cost intensive process (Iezzoni et al., 2010; Kumar et al., 2012a).

1.3 Domesticated Apple

Malus domestica Borkh. has an allopolyploid origin and is considered an interspecific hybrid complex (Korban and Skirvin, 1984; Brown, 2012a). Apple belongs to the Maloideae subfamily, within the Rosaceae family (Brown, 2012a; Rohrer et al., 2018). Apple suffers from severe inbreeding depression and employs gametophytic self-incompatibility, all of which necessitates outcrossing and creates a highly heterozygous population (Brown, 2012a). 25-30 species of apple have been reported, with a relatively small but increasing number of species being used in modern breeding (Brown, 2012a). Though, separating and distinguishing species has been problematic and reported numbers vary widely (Luby et al., 2003).

1.4 The Apple Genome

The genome of the first draft of the domestic apple genome, using DNA from ‘Golden Delicious’, was published by Velasco et al. in 2010. In the original draft, the genome was organized into 17 linkage groups (Velasco et al., 2010). In 2017, Daccord et al. published a second draft of the domesticated apple genome representing a chromosome-scale assembly based of DNA from a ‘Golden Delicious’ doubled haploid tree. This assembly is consistent with the expected chromosome number of 17 (Kalkman, 1988). The size of the genome is estimated to be 651 Mb using the ‘Golden Delicious’ doubled haploid and was calculated as 649.7-Mb size in the consensus map created by Daccord et al. (2017) was very close to the 649.7-Mb size in the consensus map. Apple is considered an allopolyploid, but base on behavior is effectively a diploid (Brown, 2012a). As such, apple is frequently described as diploid (Luby et al., 2003; Velasco et al., 2010; Brown, 2012).

1.5 Marker informed breeding in Rosaceae and apple

Developments such as the use of markers in breeding, including arrays of DNA single nucleotide polymorphism (SNP) markers and genomewide selection have the potential to help breeders to overcome these challenges (Iezzoni et al., 2010). The sequencing of the apple reference genome and the development of improved linkage maps are additional advancements that open up new opportunities for apple breeders (Shulaev et al., 2008; Iezzoni et al., 2010;

Velasco et al., 2010; Peace et al., 2014, 2017). With these new opportunities, an effort to bring together breeders and stakeholders led to the RosBREED initiative for improving Rosaceous cultivars (Iezzoni et al., 2010). Utilizing marker assisted breeding has increased the efficiency of apple breeding via its deployment in marker assisted seedling selection, which allows undesirable seedlings to be removed from the program. The Washington State University saved approximately $82,000 and the University of Minnesota saved approximately $40,000 in 2013-2014 alone due to marker-assisted seedling selection in their apple breeding program (Peace et al., 2017). In the second iteration of the RosBREED initiative, advancing genomewide selection was a key research objective (Iezzoni et al., 2014a)

1.6 Genomewide Selection

Genomewide selection (GWS), useful for traits with complex genetic control, is a procedure that makes use of a large, random set of markers in a marker-based selection. In this procedure, there are no tests of significance and the effects of all marker are used in prediction (Meuwissen et al., 2001; Bernardo, 2010). This differs from other types of marker-based selection in that a continuum of effects for all markers can be predicted. Thus GWS predicts which individuals will have the best performance which can be used as the basis for selection (Bernardo, 2010). This makes use of advances such dense marker maps and the increased ease of genotyping large numbers of individuals

(Meuwissen et al., 2001). By accounting for information from all the markers, regardless of size of effect, breeders are able to focus on the improvement of quantitative traits. Such predictive approaches will likely be the future method of choice for breeders (Bernardo, 2008). Despite the promise of GWS, its utilization is still relatively new in apple. However, this could be used in assessing the genetic potential of crosses, enabling more efficient selection, reducing the generations needed for pre-breeding, and reducing the time required in trials of elite selections, in addition to contributing to marker assisted parent or seedling selection (Lorenz et al., 2011; Kumar et al., 2012a; Peace et al., 2017)

1.7 Challenges associated with apple breeding

The research that would enable successful deployment of modern breeding techniques like genomewide selection in apple has been hindered by the complexities of apple breeding. The heterozygosity of apple is maintained by asexual propagation of superior cultivars and a functional gametophytic self incompatibility system (Luby et al., 2003; Brown, 2012b). Large effect quantitative trait loci (QTL) segregate in the breeding germplasm and there are multiple possible alleles at any given locus (Hokanson et al., 2001). However, research in crops that share these characteristics, such as palm-oil (Wong and Bernardo, 2008), eucalyptus (Resende et al., 2012), and grapevine (Fodor et al., 2014), demonstrates the benefit of genomewide selection and genomic tools in breeding programs.

Additionally, apple is self-incompatible and suffers from inbreeding depression (Brown, 2012b). Apple breeding requires large amounts of labor and space, as well as growth in the orchards (Iezzoni et al., 2010; Kumar et al., 2012a). In fact, it can take 10 years or more to begin seeing enough fruit on an apple to tree to begin evaluation (Fischer, 2012; Evans and Peace, 2017).Nybom (1959) cited these hindrances as an obstacle to apple breeding research and they persist to this day.

1.8 Traits

The sensory traits crispness, firmness, and juiciness have been cited as some of the most important traits to consumers who are purchasing apple fruit (Péneau et al., 2006; McKay et al., 2011). Crispness can be described as the rupture or crunching sound that occurs when front teeth bite into fruit and the tissue shatters (Allan-Wojtas et al., 2003). Firmness is similar to crispness, except that it is the rupturing that occurs during chewing rather than biting (Vickers and Christensen, 1980; McKay, 2010) Juiciness is the amount of juice released during chewing of fruit by the molars (Allan-Wojtas et al., 2003; McKay, 2010).

In addition to sensory evaluation, mechanical or instrumental evaluation of fruit is also common and used in conjunction with sensory evaluation (Karlsen et al., 1999; Chauvin et al., 2010). There are many instrumentally measured traits that are considered important to apple breeders and other stakeholders in the

apple industry (Evans et al., 2012; Iezzoni et al., 2014b). Some such traits are described in the following paragraph (s).

Using a homogenized, bulked juice sample from multiple apples from one tree, several important juice traits can be assessed (Evans et al., 2012). Soluble solids content is measured in °Brix and is a proxy for sweetness (Iezzoni et al., 2014a). Additionally, data on acidity can be assessed using pH and titratable acidity (mg/mL malic acid) (CoSeteng et al., 1989; Evans et al., 2012).

Flesh characteristics can also be evaluated instrumentally. Mechanical crispness and firmness were measured using the Mohr Digi-test penetrometer and texture analyzer (MDT analysis, formerly referred to as digi-test analysis). These traits are measured in Mohr Digi-test specific values (Evans et al., 2012) and have been shown to correlate with sensory evaluations of the same traits (Evans et al., 2010). Maturation of fruit is assessed by evaluating fruit using the Cornell starch-iodine scale (Evans et al., 2012). In this procedure, iodine solution is sprayed on fruit flesh, the iodine reacts with starch in the flesh, and the percentage of starchy flesh can be used as an evaluation for maturity (Blanpied and Silsby, 1992).

Metrics assessing the size of fruit are of particular importance to many stake holders in the apple industry. Fruit diameter is measured by either a digital caliper or MDT analysis and is reported in inches or millimeters (Evans et al., 2012). Weight, recorded in pounds (lbs) or grams (g) is also a commonly collected datapoint (Evans et al., 2012).

Fruit appearance is a critical quality which is affected by many traits. An important trait that can negatively affect fruit appearance is russet. Russet occurs when a healthy epidermis is covered by an intact cuticle and appears on fruit skin adjacent to skin areas that are covered by periderm (Bell, 1937; Faust and Shear, 1972; Pratt, 1972; Simons and Chu, 1978; Khanal et al., 2019). Russet coverage, measured as the percentage of skin covered, and russet location, measured as appearance of russet on anatomical locations of the apple, are both traits commonly evaluated as they affect acceptance of apples at market (Evans et al., 2012).

Chapter 2

Fitting heterozygotic and major QTL effects in apple (*Malus domestica Borkh.*)

2.1 Synopsis

New apple (*Malus domestica Borkh.*) cultivars must meet or exceed standards for fruit traits such as flavor, texture, appearance, size and storability. Many of these traits are controlled by many small-effect quantitative trait loci (QTL). However, current genetic tests in apple only allow for the selection of a few traits that are controlled by major QTL. Genomewide selection, though commonly used in some annual crops, is not widely adopted in highly heterozygous, perennial, asexually propagated crops like apple. The objectives were to assess whether accounting for heterozygosity and whether treating major QTL as having fixed effects increases prediction accuracy. Genotyping was conducted using the IRSC apple 8K SNP array v1. One trial used three sensory traits, four half-sib families, 2,507 SNP markers, and approximately 20-90 seedlings per family. The second trial used 1,296 markers and four families consisting of 30-50 full-sib seedlings in each family, selected from the University of Minnesota apple breeding program. This trial analyzed 10 instrumental fruit quality traits relevant to growers and breeders. The models tested were an additive model, a model fitting heterozygote effects, and a model fitting major-QTL as fixed effects. The two test population structures were an untested-family

or an untested-seedling serving as the test population. The results of this research showed no benefit to fitting heterozygote effects but did show that fitting fixed effects for major QTL was beneficial for some traits. In both studies, predictive abilities were higher with the untested-seedling test population analyses, on average. This research shows promise for the use of genomewide selection in apple breeding programs for traits that are critical to cultivar success.

2.2 Introduction

Apple breeders face challenges in releasing cultivars to meet the ever-changing demands of the market. Apple is an obligate outcrossing species due self-incompatibility and inbreeding depression (Brown, 2012b). It requires an extensive amount of space to reach the stage of phenotyping fruit which results in a labor and cost intensive process (Iezzoni et al., 2010; Kumar et al., 2012a). In fact, long generation intervals, space, and resources required to grow and study apples have long been cited as an obstacle to apple breeding research (Nybom, 1959). It can take 10 or more years for an apple tree to begin fruiting (Fischer, 2012; Evans and Peace, 2017). Modern genomic tools, such as genomewide selection, offer a way for breeders to make decisions before fruit and improve the efficiency of their programs.

Genomewide selection uses a large set of genomewide markers in order to make predictions that enable selections on individuals that have not been phenotyped (Meuwissen et al., 2001; Bernardo, 2010). Genomewide selection

was pioneered by the dairy breeding industry as a promising and cost saving technique in the 2000s (Goddard and Hayes, 2007). However, it had already been used in maize (*Zea mays*) without the name genomewide selection as early as 1994 (Bernardo, 1994). Bernardo (1994) used parental RFLP data and yield data to make predictions on the yield of single-cross hybrids. implementation of genomewide selection in dairy cattle has been described as revolutionary as it has doubled the rate of genetic progress in key traits, increased rates of genetic gain, improved selection accuracy, and resulted in substantial cost savings (Garner et al., 2016; Wiggans et al., 2017). Since then, genomewide selection has also been proven to be feasible in wheat (*Triticum aestivum*) (Heffner et al., 2011; Rutkoski et al., 2011, 2012), barley (*Hordeum vulgare*) (Lorenzana and Bernardo, 2009; Zhong et al., 2009), and broiler chicken breeding (*Gallus gallus domesticus*) (González-Recio et al., 2008).

Predictive ability is the correlation between the phenotypic and the marker predicted values (Meuwissen et al., 2001; Bernardo, 2010). Population structure of the training population has been found to impact early success of genomewide selection, however it has little effect on the long-term accuracy and response to genomewide selection (Bastiaansen et al., 2012). Muranty et al. (2015) assessed predictive ability in apple using historical phenotype data looking at traits that were considered selection traits in their program. They found that the predictive ability was highly affected by phenotypic distribution and heritability, but that genomewide selection had the potential to accelerate breeding and decrease

costs (Muranty et al., 2015). Kumar et al. (2012) assessed fruit quality traits in a set of apple seedlings and found that when linkage disequilibrium decay was low, higher predictive abilities were possible and that genomewide selection offers a viable alternative to conventional selection for fruit quality traits. Given that current research supports the implementation of genomewide selection in apple breeding, more research is warranted to investigate potential ways to improve predictive ability and the application of this technology to cultivar development.

In genomewide selection for dairy cattle, some models fit heterozygous SNP effects (Zeng et al., 2013; Sun et al., 2014). Given that a parallel between apple as a species and dairy cattle is the highly heterozygous nature of both genomes, adopting a prediction model in apple that fits heterozygote effects may improve predictive abilities. In some studies, fitting heterozygous effects has been shown to have the potential to increase prediction accuracy in genomewide selection (Sun et al., 2014; Heidaritabar et al., 2016). The first objective in this study was to test the hypothesis that fitting heterozygous effects improves genomewide predictions in apple.

Another possible way to improve predictive abilities is to include QTL that account for a large percentage of trait variation (i.e. major QTL) in the prediction model. When a marker is associated with a major QTL, that marker will still be accounted for in a simple linear prediction model. However, the effect of this marker will tend to be underestimated when shrinkage factors that reduce the error pull the predicted value towards the mean of zero. As such, the major QTL

will have a larger estimated effect when it is included as a fixed effect (Bernardo, 2013a). Studies on *Fusarium* head blight resistance in wheat found some cases where including prior information regarding known QTL improved predictive ability (Rutkoski et al., 2012). Bernardo (2014) confirmed that there are situations in which including known major QTL as fixed effects becomes highly advantageous in genomewide selection. Further, he found that it is rarely disadvantageous to include major QTL as fixed effects. The second objective was to test the hypothesis that including major QTL as fixed effects in the genomewide selection model will improve predictive ability.

2.3 Materials and Methods

2.3.1 Germplasm

Dataset 1 comprised data from McKay et al. (2011) on three sensory traits important to consumer appeal and eating quality of apple: crispness, firmness, and juiciness. The data used represented a cumulative mean data set for data collected over three years of analysis. Dataset 1 included four full-sib families derived from crosses that all included ‘Honeycrisp’ as a common parent and a total of 196 individuals. The other parents were ‘MN1702’, ‘MN1764’, ‘Jonafree’, and ‘AA44’ (Table 2.1). Crosses and the number of seedlings can be found in Table 2.1. Trait data from Dataset 1 were for crispness, firmness, and juiciness as evaluated by a sensory panel. Sensory traits were measured using a 16 cm generalized Labeled Magnitude Scale (gLMS) and converted into units of

millimeters (mm) (McKay et al., 2011). The families hereafter will be indicated by their unique (non-'Honeycrisp') parents. A pedigree map for Dataset 1 is found in the supplemental materials (Appendix 1).

Dataset 2, which was used for most of the analysis, comprised five families derived from crosses that were selected due to their importance to the University of Minnesota breeding program and based on their pedigree. These families were not half-sibs as were the families in Dataset 1. The number of seedlings in each family ranged from 36 to 48 (Table 2.1). Variation in the number of seedlings per cross was due to biennial bearing, fruit dropping before maturity, or too few (fewer than three) representative fruit to warrant harvest and data collection. Trait data for Dataset 2 were collected in autumn 2015, 2016, and 2017. A pedigree map for Dataset 2 is found in the supplemental materials (Appendix 2). Crosses and the number of realized seedlings can be found in Table 2.1.

2.3.2 Trait data and harvest procedure in Dataset 2

All growth and production took place at the University of Minnesota Horticultural Research Center located in Chanhassen, Minnesota. The standard management practices of the research station were used in growing the seedlings. Seedling age varied but was an average of 6-8 years old.

Fruit harvest for Dataset 2 was conducted following weekly checks for the skin ground color change, a trait that is indicative of fruit maturity (Evans et al.,

2012). Harvest of a tree occurred when the ground color began turning from green to yellow and the starch iodine test of the fruit resulted in a rating of 3-5 on the Cornell scale, after an apple sliced in half horizontally was sprayed with iodine to observer the starch pattern (Blanpied and Silsby, 1992; Evans et al., 2012). Harvested fruit were selected to be representative of all other fruit on the tree and were harvested from random and well-exposed areas on the tree avoiding the very top and bottom of the canopy. Ten fruit with no external damage were harvested per tree and the starch iodine rating at the time of harvest was recorded (Evans et al., 2012).

Of the ten harvested fruit for each tree, five fruits were set aside to be phenotyped within 24 hours of harvest. The remaining five apples were put in cold storage at 4°C for 10-weeks, replicating commercial storage. Phenotype data were collected for the following traits: russet (%), weight (g), mechanical crispness (crispness), overall maximum hardness (OMH), overall average hardness (OAH), diameter (mm), starch iodine (score), titratable acidity (mg/mL malic acid), pH, and soluble solids content (% brix).

The percentage of the skin covered in russet was recorded on a 0-10 scale where each one point increase represented 10% of skin covered as determined by visual assessment of two independent raters whose ratings were averaged. Fresh weight was recorded in grams. Diameter, crispness, and firmness were recorded using the Mohr Digi-test penetrometer and texture analyzer (MDT analysis, formerly referred to as digi-test analysis) (Mohr and

Mohr, 2002), hereafter referred to as MDT analysis. The skin on the border of the sun-exposed (blush) and shaded side of the fruit was sliced away prior to MDT analysis to prevent the skin from interfering with the penetrometer probe. The penetrometer mechanically inserted an 11 mm diameter probe to varying depths until the core was reached while constantly recording force necessary to move the probe at a constant speed through the apple flesh (Mohr and Mohr, 2002). The penetrometer probe is inserted in the apple on the perimeter of the shade and blush sides of the apple. Crispness was the energy released during fruit tearing which can be compared to the energy released during a bite (Mohr and Mohr, 2002). OAH was the mean amount of pressure from the entire duration of penetration by the probe. OMH was the maximum pressure recorded during penetration by the probe. When a fruit was too small to undergo MDT analysis, crispness and firmness were not recorded and diameter was recorded manually in millimeters using a digital caliper. The apples were then sliced in half across the horizontal hemisphere. The top half of the apple was sprayed with an iodine solution and compared to the Cornell scale of starch iodine ratings to assess maturity (Blanpied and Silsby, 1992). The bottom half was chopped, bulked with the other apples from that genotype, and juiced. Samples were juiced using a kitchen grade fruit and vegetable juicer into 20 mL vials. Two homogenous juice samples were collected in the 20 mL vials and immediately frozen until titration took place. The same traits were phenotyped after 11 weeks; 10-weeks in cold storage and 1 week at room temperature which replicates the process of being in

commercial cold storage and then being placed on a grocery shelf, to assess the quality as a consumer would experience it. Starch iodine was not evaluated after storage.

To calculate phenotypic values comprised of the data from all three years of data collection in Dataset 2, a combined dataset was created using adjusted arithmetic means from each individual year. For each year, an effect was calculated for each trait-year-dataset combination. This effect was calculated by taking the arithmetic mean of each trait-year-dataset combination and subtracting the grand mean from that trait-year-dataset combination's arithmetic mean. The resulting value was then deducted from the phenotypic observations for the appropriate trait-year-dataset combination. These resulting adjusted phenotypic observations for each year were then combined into one dataset. This new 'all years' dataset was also analyzed using the described methods and is the data that is presented in this chapter.

2.3.3 Marker data and genomewide selection procedures

In both trials, families were genotyped using the Illumina Infinium ® II platform and the IRSC apple 8K SNP array v1 (Chagné et al., 2012). The linkage maps used were Int10 (Howard et al., 2017) for Dataset 1 and Int11, which is a modified version of Int10, for Dataset 2. These maps spanned 1,172 centimorgan across 17 chromosomes (Howard et al., 2017). A subset of 2,507 markers for Dataset 1 and 1,296 markers for Dataset 2 were selected for use in

each respective analysis (Table 2.2). Markers were present on each chromosome with no major chromosomal segments left unrepresented, i.e. the markers were approximately evenly distributed. A separate subset of available markers from the Int10 or Int11 appropriate to the included germplasm was chosen for each trial. Redundant markers were removed by a backwards elimination procedure. Within each chromosome, the correlation between each pair of markers was calculated. When the correlation exceeded 0.80, the marker with the lower minor allele frequency was marked as redundant and removed. This was repeated until all markers on the chromosome had correlations below the threshold and this was repeated for each chromosome.

Genomewide selection models were tested using two test population schemes: 1) using an untested-family as the test population and 2) using a single untested-seedling as a test population. In the untested-family method, genomewide selection was conducted such that one family served as the test population, with the remaining four families serving as the training population. In the untested-seedling method, genomewide selection was conducted such that a single seedling serves as the test population, with the remaining seedlings from all families serving as the training population. For example, if ‘Jonafree’ × ‘Honeycrisp’ was considered as the untested-family, the training population then consisted of the families ‘MN1764’ × ‘Honeycrisp’, ‘MN1702’ × ‘Honeycrisp’, and ‘AA44’ × ‘Honeycrisp’. For an untested-seedling, a single seedling from ‘Jonafree’ × ‘Honeycrisp’ was considered as the test population and the training population

was obtained by pooling the remaining 44 trees in ‘Jonafree’ × ‘Honeycrisp’ and all of the trees in the remaining families. In the untested-seedling procedure, the training population therefore had individuals that were full-sibs of the individual whose performance was being predicted. For both the untested-family and untested-seedling methods, multiple iterations of the analyses were run such that each family or seedling served as the test population in one iteration. Predictive ability was calculated as the correlation between the predicted and observed values for the test population.

For the untested-family and the untested-seedling procedures in each of the two trials, genomewide predictions were obtained via three models. In each trial the additive marker effects were obtained by ridge regression-best linear unbiased prediction (RR-BLUP)(Frank and Friedman, 1993). The three models were an additive model, a model that fit heterozygote effects, and a model that fit fixed effects for major QTL. The additive model was considered the baseline for comparison for the two models with additional parameters. Predictive abilities that were significantly different from the additive model or from zero were noted.

Additive model:

The linear model for the additive model was:

$$y_i = \mu + \sum t_{ij}\beta_j + \varepsilon_i$$

where y_i is the mean phenotypic value corresponding to the *i*th genotype, μ is the overall mean, t_{ij} were marker genotypes coded as 1 or -1 for the two homozygous

genotypes and 0 for the heterozygous genotype, β_j is the effect of the *j*th marker locus, and ε_i is a model residual (Lorenz et al., 2011). The random effects have the following distribution: $t_{ij} \sim N(0, \sigma^2 g)$, and $\varepsilon_i \sim N(0, \sigma^2 e)$ where $\sigma^2 g$ is the variance due to genotype and $\sigma^2 e$ is the error variance. A grid search to select an estimated proportion between 0.01 and 0.99 of the trait variation that is due to genetic effects and cross validation was used to select the shrinkage factor. This shrinkage factor was selected by employing a grid search, where a set of shrinkage factors were tested and the one with lowest error was used in the predictions.

Model fitting heterozygote effects:

The linear model for the modified additive model fitting heterozygote effects was:

$$y_i = \mu + \sum t_{ij}\beta_j + \sum w_{ij}\rho_j + \varepsilon_i$$

where w_{ij} has a value of 1 for a heterozygote and 0 for a homozygote, ρ_j is the effect of the *j*th heterozygous marker locus, and ε_i is a model residual. The random effects have the following distribution: $t_{ij} \sim N(0, \sigma^2 g_{hom})$, $w_{ij} \sim N(0, \sigma^2 g_{het})$, and $\varepsilon_i \sim N(0, \sigma^2 e)$ where $\sigma^2 g_{hom}$ is the variance due to homozygous genotypes, $\sigma^2 g_{het}$ is the variance due to heterozygous genotypes, and $\sigma^2 e$ is the error variance. A grid search to select an estimated proportion between 0.01 and 0.99 of the trait variation that is due to genetic effects and cross validation was used to select the shrinkage factor. This shrinkage factor was selected by employing a

grid search, where a set of shrinkage factors were tested and the one with lowest error was used in the predictions.

Model fitting fixed effects for major QTL:

The marker-trait-association analysis consisted of two steps and was conducted using GMODEL2 (Bernardo, 2019): step one was backwards elimination on a given chromosome to identify significant markers for a trait. The second step was to obtain the final estimates of the marker effects using multiple regression. For one chromosome at a time, after correcting for genomewide marker effects, backwards elimination was used to locate QTL on that given chromosome. To estimate the final marker effects, all markers that were found to have significant effects are analyzed jointly using multiple regression using a significance level of 0.0000001 (Bernardo, 2013b). Marker effects were calculated using the same model as described for RRBLUP.

Criteria were applied to restrict the QTL that would be included in the fixed effect model. QTL effects were considered major if the effect exceeded a (+/-) threshold value based on sizes of effects that would be meaningful in the University of Minnesota apple breeding program, based upon internal standards and personal communication with James Luby (University of Minnesota apple breeder). Thresholds were determined for each trait-evaluation-year combination. A summary of the thresholds used, corresponding QTL effect cutoff values, the

number of QTL detected, and the number that were used as fixed effects in the model can be found in Table 2.3 and Table 2.4.

The linear model for the modified additive model that fit fixed effects for major QTL was:

$$y_i = \mu + \sum t_{if}\beta_f + \sum v_{il}\delta_l + \varepsilon_i$$

where t_{if} are marker genotypes that were coded such that two homozygote class (e.g., AA and BB) were coded as 1 and -1 and the heterozygote class (e.g., AB) and the markers that were treated as fixed effects were coded as 0., β_f was the effect of the *f*th marker locus, v_{il} were marker genotypes corresponding to major effect QTL which were coded as 1 and all other markers were coded as 0, δ_l was the fixed effect of the *l*th QTL, and ε_i was a model residual (Bernardo, 2013a). The random effects were considered to have the following distributions: $t_{if} \sim N(0, \sigma^2 g_{ran})$ and $\varepsilon_i \sim N(0, \sigma^2 e)$ where $\sigma^2 g_{ran}$ was the variance due to random marker effects that were not considered as fixed effects and $\sigma^2 e$ was the error variance. A shrinkage factor was selected in order to regress the estimates back towards zero to control error (Meuwissen et al., 2001; Bernardo, 2010). This shrinkage factor was selected by employing a grid search, where a set of shrinkage factors were tested and the one with lowest error was used in the predictions.

2.4 Results

2.4.1 Model Fitting Additive Effects

In Dataset 1 the results from the standard, additive model resulted in predictive abilities ranging from 0.00 to 0.45 when testing was conducted using the untested-family as a test population. Approximately 50% of these predictive abilities were significantly different from zero (α=0.05) (Table 2.5). Here and in all following cases, a T-test was used, at α=0.05, to test for significant differences. In Dataset 1, when the test population was an untested-seedling, all predictive abilities for the standard model were significantly different from zero. Predictive abilities ranged from 0.23 to 0.38 for the three traits (Table 2.6).

In Dataset 2, the results for the standard, additive model resulted in predictive abilities of -0.24 to 0.53, with seven negative predictive abilities, for the fresh evaluation when testing was conducted using the untested-family as a test population (Table 2.7). It should be noted that negative predictive abilities were not statistically significant than zero and can therefore be considered to 0.00. Approximately 15% of the predictive abilities were significantly different from zero (α=0.05) (Table 2.7). The observed predictive abilities ranged from -0.31 to 0.47, with two negative predictive abilities, for the 10-week storage evaluation. Approximately 16% of the predictive abilities were significantly different from zero (Table 2.7). In Dataset 2, when using the untested-seedling test population, the results from the additive model resulted in predictive abilities ranging from 0.12 to 0.52 for traits in the fresh evaluation (Table 2.8). Approximately 75% of these

predictive abilities were significantly different from zero. The observed predictive abilities ranged from 0.21 to 0.46 for traits in the 10-week storage evaluation. All of these predictive abilities were significantly different from zero (Table 2.8).

2.4.2 Model fitting heterozygote effects

The range of predictive abilities in Dataset 1 values was similar to the additive model when heterozygous effects were fitted in the model, with predictive abilities ranging from 0.03 to 0.45 and approximately 50% being significantly different from zero. Predictive abilities from this model were not significantly different from the predictive abilities from the standard model for any trait (Table 2.5). In Dataset 1 using the untested-seedling as the test population, the predictive abilities were 0.28 for juiciness, 0.27 for crispness, and 0.45 for firmness (Table 2.6). There were no negative predictive abilities and all were statistically significantly different from zero (Table 2.6).

In Dataset 2, using the untested-family as the test population, the results were again similar to those of the additive model (Table 2.7). Predictive abilities in the fresh data ranged from -0.26 to 0.53 with 11 negative values. In the 10-week data, predictive abilities ranged from -0.32 to 0.48 with two negative predictive abilities (Table 2.7). In Dataset 2 using the untested-seedling as the test population, predictive abilities ranged from 0.140 to 0.521 for the fresh data and 0.22 to 0.47 for the 10-week data (Table 2.8). In both the untested-family and the untested-seedling procedures, the models performed similarly to the

additive models. The predictive abilities for untested-seedling procedures were higher than the untested-family procedures.

2.4.3 Model fitting fixed effects for major QTL

In Dataset 1 no QTL were detected for juiciness, two QTL were detected for crispness, and one QTL was detected for firmness (Table 2.4). Detailed information on the QTL included in the model can be found in the supplemental material (Appendices 3-5). Only one crispness QTL met the standard for inclusion, as did the single firmness QTL. The fixed QTL analysis with the untested-family as a test population resulted in predictive abilities ranging from 0.11 to 0.44 with approximately 50% being significantly different from zero (Table 2.5). Predictive abilities from this model were not significantly different from the predictive abilities for the additive model for any traits (Table 2.5). Using the fixed QTL model with an untested-seedling as the test population, in Dataset 1, again all predictive abilities for the additive model were significantly different from zero (Table 2.6). Predictive abilities were 0.27 for crispness and 0.45 for firmness. No predictive abilities for this model were significantly better than those for the additive model (Table 2.6).

In Dataset 2 using the untested-family as the test population, the number of QTL detected ranged from 0 to 36, while the number of QTL included in models ranged from 0 to 9 (Table 2.4). Detailed information on the QTL included in the model can be found in the supplemental material (Appendices 6-8). The

number of QTL included was less than 10 for all traits, ranging from 1 to 9 QTL being included in the fixed QTL model. The fixed QTL analysis resulted in predictive abilities ranging from -0.24 to 0.55 with approximately 38% being significantly different from zero and four being negative for the fresh evaluation. The fixed QTL analysis resulted in predictive abilities ranging from -0.44 to 0.67 with all but one correlation being significantly different from zero and a negative predictive ability for one trait in the 10-week storage evaluation. The correlation of -0.44 was unexpected and deserves deeper investigation. Approximately 9% of the time the fixed QTL model resulted in predictive abilities that were significantly different from those for the additive model for traits in the fresh evaluation, although only one trait (soluble solids) had three of the four families that all had significant improvements. The other significant improvements were isolated families throughout various traits. The traits soluble solids, pH, and titratable acidity (the three traits for which QTL were included) resulted in the fixed QTL model having predictive abilities that were significantly different from those for the standard model for the 10-week storage evaluation (Table 2.7).

In Dataset 2 using the untested-seedling as the test population, the fixed QTL analysis resulted in predictive abilities ranging from 0.098 to 0.531 with all but one of the predictive abilities being significantly different from zero for the fresh evaluation. The fixed QTL analysis resulted in predictive abilities ranging from 0.348 to 0.463 with all predictive abilities being significantly different from

zero for the 10-week storage evaluation. No predictive abilities were significantly different from the additive model for either evaluation (Table 2.8).

2.5 Discussion

There was no trait or training/test population scheme for which fitting heterozygote effects improved predictive ability. In some cases, however, fitting fixed effects for major effect QTL did result in significant increases in the predictive ability. Finally, the inclusion of all additional full-sibs from a given family in the training population when comparing the untested-family and the untested-seedling training/test population schemes also resulted in significant improvements in the predictive ability of the genomewide selection models. The increased relatedness of the training population to the test population when moving from the untested-family to the untested-seedling scheme can be attributed to the benefit of having a training population that is more highly related to the test population. Including even a small number of more related individuals in the training population has been shown to improve predictive abilities in other species (De Roos et al., 2009).

Predictive abilities ranged greatly among traits, both within a given trial and between the two trials, though this range was less pronounced within the untested-seedling results. However, the values and ranges of predictive abilities were consistent with predictive abilities reported for similar fruit quality traits and selection traits (Kumar et al., 2012b; Muranty et al., 2015). The range of

predictive abilities could be due to several factors. Trait heritability and phenotypic distribution of traits could impact the predictive ability of the traits (Muranty et al., 2015). Working with relatively small family sizes could also impact results, as small training sizes have been associated with reduced predictive ability (Habier et al., 2010).

Although estimating non-additive effects is possible and theoretical approaches have been proposed, little practical work has been done to dissect these effects or to apply these approaches (Kumar et al., 2012a). However, including non-additive effects has been found to improve genomewide selection predictive abilities in some species (Su et al., 2012) and Kumar et al. (2012) hypothesized that accounting for dominance effects may be beneficial in apple. Despite this, the results of this study did not find this to be beneficial for the traits evaluated. This result could be due dominance variance being low in some traits or due to sampling error from insufficient data (Sun et al., 2014). It is thus possible that non-additive effects for the set of traits here, or in apple as a species, are minor relative to additive effects. However, more research needs to be conducted on this area to confirm as little is known about the importance of dominance effects in apple. Similar results have also been seen in dairy cattle. One study found that only yield traits benefited from the inclusion of dominance effects in the model, but predictive ability did not increase for non-yield traits with the inclusion of dominance effects (Sun et al., 2014).

Despite the same approach to setting criteria for inclusion in the fixed effect model being used, not all fixed effect models performed better than the additive model. Another study found that major QTL should be fit when only a few major genes are present and each major gene accounts for greater than 10% of the genetic variance (Bernardo, 2013a). Research on the genetic architecture of traits in apple is minimal. For many of the traits studied in this chapter, there is little or no data on heritability or genetic architecture. Kumar et al. (2011) calculated h^2 for firmness, crispness, juiciness, and russet coverage and found moderate heritabilities (ranging from 0.63 at the highest to 0.48 at the lowest). It was also found that genetic architecture was affected by planting on a tree's own roots versus grafting onto rootstock (Kumar et al., 2011). It may be therefore possible that grafting onto different rootstocks, such as is done at the UMN apple breeding program, may also affect genetic architecture and reduce the ability to detect QTL that are effective in the fixed effect model. In many cases, multiple major QTL were detected indicating that there are likely more than one gene that is being captured by these QTL. Kumar et al. (2013) reported major SNPs detected for sensory firmness and titratable acidity, and these two traits also had significant QTL included in the fixed effect model (Kumar et al., 2013). Further, titratable acidity was a trait where predictive ability improved with the fixed effect model in this chapter. It should be noted that we do not have estimates of the genetic variance explained by each QTL so it is likely that although considered major in this study, the QTL may not have an effect large

enough to improve predictive ability for many traits where no improvement was seen.

Although there are other reports of QTL for some of the traits studied here, differences in the markers use, protocols, and evaluation procedures for the traits described can complicate direct comparisons (Kenis et al., 2008). Although Kenis et al. (2008) studied similar traits, they used a different germplasm and phenotyping methods differed. So despite studying similar or the same traits, the QTL detected in this study did not overlap with the QTL detected by Kenis et al. (2008). QTL were detected for weight by King et al. (2001), but none were detected here in the all-years data reported on. Similar findings exited for other traits, as such the decision was made to map QTL specifically for this study to ensure that they were relevant to the germplasm and protocols used in this research. This decision was also applied to traits like titratable acidity, for which a QTL was detected in this research on chromosome 16, which is consisted with reported QTL for this trait in other research (Maliepaard et al., 1998).

2.6 Application

In apple, there is no benefit to fitting heterozygous effects in the genomewide selection models. This finding was consistent for all traits, years, evaluations, and sets of different germplasm. The results are generally consistent with studies in other species, however, this is the first study, to our knowledge, that assesses heterozygous effects in apple. There may be traits for which

including QTL as fixed effects increases the predictive ability of genomewide selection. The importance of fixed effects in the model is likely affected by the size of the effect of the QTL included, the heritability of the trait, and the number of QTL that were included in the model. Predictive abilities varied by trait and relatedness of the test population to the training population, as well as the numbers of individuals in both the training and test population. Including the full-sibs of the test population individual(s), as in with the untested seedling analyses, improved the predictive ability of the models. This research shows promise for the use of genomewide selection in apple breeding programs for traits that are relevant to cultivar success, especially if QTL detection yields markers that can be included as fixed effects and the full-sibs are not included in the training population. This may be particularly useful in assessing seedlings that have not yet fruited for crosses that have siblings that have already fruited or for accumulating data from related families in the program over multiple years to predict related crosses.

Tables

Table 2.1. Parents and number of seedlings (n) within each cross for Dataset 1 and Dataset 2.

Dataset 1		Dataset 2	
Cross	**Seedlings (n)**	**Cross**	**Seedlings (n)**
‘MN1702’ × ‘Honeycrisp’	21	‘Minneiska’ × ‘MN55’	36
‘MN1764’ × ‘Honeycrisp’	91	‘Honeycrisp’ × ‘Minnewashta’	47
‘Jonafree’ × ‘Honeycrisp’	45	‘MN1702’ × ‘Minneiska’	39
‘AA44’ × ‘Honeycrisp’	39	‘Honeycrisp’ × ‘WSU2’	48

Table 2.2. Number of markers (n) used in analysis for Dataset 1 (n=2,507) and Dataset 2 (n=1,296), the total map length (cM), and the number of markers (n) located on each chromosome.

	Dataset 1	**Dataset 2**
Markers (n)	2,507	1,296
Map Length (cM)	1,172	1,172
Chromosome	**Markers/Chromosome (n)**	
1	126	59
2	235	120
3	160	74
4	141	78
5	163	101
6	113	43
7	106	64
8	118	67
9	151	81
10	175	83
11	145	84
12	143	74
13	137	57
14	141	68
15	198	106
16	118	76
17	137	61

Table 2.3. Summary of traits evaluated for Dataset 1 and Dataset 2, respectively. Trait and units, where applicable, are shown on the left. The percentage (%) of one standard deviation and the resulting cut off value for predicted marker effect are in the threshold column and cutoff value column, respectively. The resulting cutoff value is the threshold for inclusion of a QTL as a fixed effect in the model.

Fresh Evaluation						10-Week Evaluation		
Dataset 1			Dataset 2			Dataset 2		
Trait	% of one standard deviation	Cutoff value	Trait	% of one standard deviation	Cutoff value	% of one standard deviation	Cutoff value	
Juiciness	None Detected	---	Soluble Solid Content (°Bx)	0.33	0.49	0.33		0.61
Crispness	0.33	0.028	Crispness (Cn)	0.33	25.87	None Detected	---	
Firmness	0.33	0.026	Diameter (mm)	None Detected	---	None Detected	---	
			Overall Average Hardness (lb.)	0.33	0.64	None Detected	---	
			Overall Maximum Hardness (lb.)	0.33	0.96	None Detected	---	
			pH	0.5	0.1	0.33		0.08
			Russet (%)	0.33	0.31	NA	---	
			Starch Iodine Rating	0.5	0.55	NA	---	
			Titratable Acidity (mg/mL)	0.33	0.82	0.33		0.65
			Weight (g)	None Detected	---	None Detected	---	

Table 2.4. Summary of traits evaluated for Dataset 1 and Dataset 2, respectively. Trait and units, where applicable, are shown on the left. The total number of QTL detected (n) in the marker-trait-association as well as the number of QTL (n) that met criterion for inclusion in the model fitting fixed effects are shown.

Dataset 1			Dataset 2				
				Fresh Evaluation		10-week Evaluation	
Trait	**Total QTL Detected**	**QTL Fixed in Model**	**Trait (unit)**	**Total QTL Detected**	**QTL Fixed in Model**	**Total QTL Detected**	**QTL Fixed in Model**
Juiciness	0	0	Soluble Solid Content (°Bx)	28	7	22	9
Crispness	2	1	Crispness (Cn)	6	3	---	---
Firmness	1	1	Diameter (mm)	---	---	---	---
			Overall Average Hardness (lb.)	1	1	---	---
			Overall Maximum Hardness (lb.)	4	3	---	---
			pH	24	3	21	2
			Russet (%)	28	7	---	---
			Starch Iodine Rating	36	2	---	---
			Titratable Acidity (mg/mL)	31	9	21	5
			Weight (g)	---	---	---	---

Table 2.5. Dataset 1 predictive abilities using an untested-family as the test population. * indicates significantly different from zero at α = 0.05. No predictive abilities between models for a given family were significantly different. Data present represent predictive abilities for the additive model (additive), the model fitting heterozygote effects (Het.), and the model fitting fixed effects for major QTL (Fixed QTL).

Trait	Test Population	Additive Model	Het. Model	Fixed QTL Model
Juiciness	'Jonafree' × 'Honeycrisp'	0.20	0.19	NA
	'AA44' × 'Honeycrisp'	0.30*	0.30*	NA
	'MN1702' × 'Honeycrisp'	0.00	0.03	NA
	'MN1764' × 'Honeycrisp'	0.26*	0.26*	NA
Crispness	'Jonafree' × 'Honeycrisp'	0.36*	0.30*	0.27
	'AA44' × 'Honeycrisp'	0.07	0.07	0.13
	'MN1702' × 'Honeycrisp'	0.14	0.14	0.13
	'MN1764' × 'Honeycrisp'	0.19*	0.19*	0.22*
Firmness	'Jonafree' × 'Honeycrisp'	0.41*	0.39*	0.38*
	'AA44' × 'Honeycrisp'	0.11	0.10	0.11
	'MN1702' × 'Honeycrisp'	0.45*	0.45*	0.44*
	'MN1764' × 'Honeycrisp'	0.43*	0.41*	0.42*

Table 2.6. Dataset 1 predictive abilities calculated using an untested-seedling as the test population. Predictive abilities shown are from the additive model (additive), the model fitting heterozygote effects (Het.), and the model fitting fixed effects for major QTL (Fixed QTL). * indicates significantly different from zero at α = 0.05.

Trait	Additive Model	Het. Model	Fixed QTL Model
Juiciness	0.28*	0.28*	NA
Crispness	0.27*	0.27*	0.27*
Firmness	0.45*	0.45*	0.45*

Table 2.7. Dataset 2 predictive abilities calculated using an untested-family as the test population. Predictive abilities shown are from the additive model (additive), the model fitting heterozygote effects (Het.), and the model fitting fixed effects for major QTL (Fixed QTL). * indicates significantly different from zero at α = 0.05.

		Fresh Evaluation			10-week Storage Evaluation		
Trait (unit)	**Test Population**	**Additive**	**Het.**	**Fixed QTL**	**Additive**	**Het.**	**Fixed QTL**
Soluble Solids Content (°Bx)	'Honeycrisp' x 'WSU2'	0.06	0.07	0.42*	0.16	0.21	0.48*
	'Honeycrisp' x 'Minnewashta'	0.14	0.12	0.32*	0.04	0.04	0.38*
	'MN1702' x 'Minneiska'	0.29	0.28	0.38*	0.08	0.18	-0.44*
	'Minneiska' x ''MN55''	-0.01	0.00	0.29	0.22	0.23	0.53*
Crispness (Cn)	'Honeycrisp' x 'WSU2'	0.05	0.06	-0.24	0.24	0.24	---
	'Honeycrisp' x 'Minnewashta'	0.18	-0.06	0.13	0.29	0.28	---
	'MN1702' x 'Minneiska'	0.03	0.18	0.35*	0.25	0.20	---
	'Minneiska' x ''MN55''	-0.05	0.00	0.21	0.24	0.21	---
Diameter (mm)	'Honeycrisp' x 'WSU2'	0.30*	0.33*	---	0.39*	0.34	---
	'Honeycrisp' x 'Minnewashta'	0.22	0.14	---	0.32	0.31	---
	'MN1702' x 'Minneiska'	0.06	0.14	---	0.36	0.34	---
	'Minneiska' x ''MN55''	-0.03	-0.01	---	0.36	0.24	---
Overall Average Hardness (lb.)	'Honeycrisp' x 'WSU2'	-0.13	-0.1	0.08	-0.01	-0.01	---
	'Honeycrisp' x 'Minnewashta'	0.31*	0.31*	0.40*	0.47*	0.39	---
	'MN1702' x 'Minneiska'	0.10	0.12	0.22	0.16	0.20	---
	'Minneiska' x ''MN55''	-0.08	-0.08	-0.07	0.35	0.36	---
Overall Maximum Hardness (lb.)	'Honeycrisp' x 'WSU2'	-0.02	0.01	0.16	0.12	0.12	---
	'Honeycrisp' x 'Minnewashta'	0.23	0.23	0.23	0.40*	0.41*	---
	'MN1702' x 'Minneiska'	0.11	0.11	0.11	0.47*	0.48*	---
	'Minneiska' x ''MN55''	0.02	-0.01	-0.10	0.46*	0.45*	---
pH	'Honeycrisp' x 'WSU2'	0.38*	0.51*	0.54*	0.17	017	0.48*
	'Honeycrisp' x 'Minnewashta'	0.12	0.19	0.55*	0.27	0.34	0.67*
	'MN1702' x 'Minneiska'	-0.24	-0.05	-0.14	-0.31	-0.32	0.57*
	'Minneiska' x ''MN55''	0.12	0.25	0.40*	0.14	0.17	0.36
Russet (%)	'Honeycrisp' x 'WSU2'	0.21	0.19	0.15	---	---	---
	'Honeycrisp' x 'Minnewashta'	0.53*	0.53*	0.35*	---	---	---
	'MN1702' x 'Minneiska'	0.34*	0.29*	0.21	---	---	---
	'Minneiska' x ''MN55''	0.26	0.14	0.46*	---	---	---
Starch Iodine	'Honeycrisp' x 'WSU2'	-0.13	-0.12	0.03	---	---	---
	'Honeycrisp' x 'Minnewashta'	-0.13	-0.20	0.03	---	---	---
	'MN1702' x 'Minneiska'	0.03	0.03	0.16	---	---	---
	'Minneiska' x ''MN55''	0.08	-0.04	-0.18	---	---	---
Titratable Acidity (mg/mL)	'Honeycrisp' x 'WSU2'	0.05	0.11	0.44*	0.07	0.06	0.61*
	'Honeycrisp' x 'Minnewashta'	0.21	0.28	0.21	0.19	0.17	0.32*
	'MN1702'xMinneiska	-0.18	-0.14	0.37*	0.28	0.18	0.49*
	Minneiskax'MN55'	0.19	0.19	0.29*	0.30	0.17	0.58*
Weight (g)	'Honeycrisp' x 'WSU2'	0.22	0.18	---	0.16	0.16	---
	'Honeycrisp' x 'Minnewashta'	0.47*	0.43*	---	0.09	0.03	---
	'MN1702'xMinneiska	0.04	0.13	---	0.16	0.24	---
	Minneiskax'MN55'	-0.24	-0.26	---	0.09	0.06	---

Table 2.8. Comparison of predictive abilities resulting from the additive model (additive), the model fitting heterozygote effects (Het.), and the model fitting fixed effects for major QTL (Fixed QTL). This data is from Dataset 2 and using an untested-seedling as the test population. * indicates significantly different from zero at α = 0.05. --- Indicates that no data was available to calculate the predictive ability.

	Fresh Evaluation			10-week Storage Evaluation		
	Additive	**Het.**	**Fixed QTL**	**Additive**	**Het.**	**Fixed QTL**
Soluble Solids Content (°Bx)	0.33*	0.33*	0.35*	0.46*	0.47*	0.46*
Crispness (Cn)	0.31*	0.33*	0.34*	0.38*	0.39*	--- ---
Diameter (mm)	0.22*	0.24*	---	0.37*	0.38*	---
Overall Average Hardness (lb)	0.48*	0.49*	0.49*	0.44*	0.44*	---
Overall Maximum Hardness (lb)	0.52*	0.52*	0.53*	0.45*	0.45*	---
pH	0.12	0.17	0.10	0.21*	0.22*	0.35*
Russet (%)	0.20*	0.23*	0.29*	---	---	---
SI	0.16*	0.23*	0.23*	---	---	---
Titratable Acidity (mg/mL)	0.37*	0.38*	0.38*	0.37*	0.37*	0.44*
Weight (g)	0.14	0.14	---	0.40*	0.43*	---

Chapter 3

Potential applications for genomewide selection in the University of Minnesota apple breeding program

3.1 Synopsis

Apple breeders need to develop strategies for routinely implementing genomewide selection in a breeding program. The objectives of this research were to determine (i) if genomewide selection would have successfully identified cultivars and lines from advanced trials that were selected using phenotypic selection, and (ii) if genomewide selection can be used to selected within a cross before all seedlings have fruited and have been evaluated. Thresholds were set for four sensory traits in Dataset 1 and a four mechanically evaluated traits in Dataset 2. The genotype data consisted of 2,507 and 1,296 SNP markers respectively. Five, 10, or 15 full-sibs, which were selected to simulate seedlings that would represented the first year of fruiting for that cross, were randomly selected from the test population and included in the training population. The results of this study indicate that for two sensory traits, crispness and firmness, genomewide predictions did offer the ability to successfully cull non-advanced seedlings while not culling advanced selections. Including as few as five full-sibs in the model improved predictive ability, thus indicating that genomewide selection is useful for circumventing the delay in phenotypic evaluations due to

biennial fruiting and juvenility. Overall, these results support the potential value of routine use of genomewide selection in an apple breeding program.

3.2 Introduction

Apple, *Malus domestica Borkh.*, has a longer generation time than many annual crops, a part of which is the lengthy juvenile phase. During this juvenile phase, the lack of fruit prevents the evaluation that will ultimately determine the potential of the line to become a commercial cultivar. Length of the juvenile phase varies with some genotypes beginning fruiting as soon as three years after the grafting of scion to rootstock in the orchard, but later maturing genotypes may remain in the juvenile phase for up to 10 years (Fischer, 2012; Evans and Peace, 2017). Cultural practices and selection for genetic factors have the potential to shorten the juvenile phase and have been a focus of many apple breeders for years (Visser, 1970; Fischer, 2012). Apple trees from the same cross may exit juvenility several years later than their siblings, due to genetic influence on the trait. A potential tool to overcome the challenges presented by a lengthy juvenility is the adoption of genomewide selection by apple breeding programs. By utilizing genomewide selection in a breeding program, selection decisions can be made before all trees exit juvenility.

Genomewide selection may be a useful tool that enables a breeder to make decisions regarding the fate of seedlings before they have fruited, e.g. selection or culling. Given that relatedness of a training population to a test

population impacts the accuracy of prediction (De Roos et al., 2009; Habier et al., 2010; Kumar et al., 2012a; Lee et al., 2017), including information of fruiting seedlings from a cross in the training data may improve the breeders' ability to make predictions using the non-fruiting siblings of the same cross as the test population. Research shows that adding a relatively low percentage of highly related individuals into the training population can substantially improve prediction accuracies (De Roos et al., 2009). Predicting the value of individuals based on genotype alone (Heffner et al., 2010) allows individuals that have not yet fruited to be evaluated for their potential in the breeding program. The first objective of this study was to determine if increasing the number of fruiting siblings of a cross included in the training data when predicting the performance of non-fruiting seedlings improves predictive ability.

Apple breeding programs routinely utilize marker assisted breeding when determining which parents to cross and ultimately which seedlings will enter field trials, however, the process of releasing a new cultivar still relies heavily on phenotypic selection. As the quality of genomic resources and dense marker maps improves in apple, the possibility of incorporating genomewide selection into apple breeding programs is becoming more and more feasible (Kumar et al., 2012a). In recent years an 8K and, more recently, a 20K SNP chip were made available for apple (Chagné et al., 2012; Bianco et al., 2014). Application of genomewide selection offers breeding programs the chance to improve gains over traditional phenotype based selection as well as to more effectively make

use of more dense marker information (Meuwissen et al., 2001; Bernardo, 2008; Kumar et al., 2012a). Genomewide selection enables selection in a population that has not been phenotyped and has shown promise as a viable tool for selection decisions in apple breeding (Kumar et al., 2012b; a; Muranty et al., 2015). Marker based selection without QTL mapping, such as genomewide selection, has been shown to be a more successful strategy for complex traits (Bernardo, 2008). The use of genomewide selection offers a way to greatly increase the efficiency of cultivar release.

The utility of genomewide selection and its application in apple breeding programs is supported by initial research (Kumar et al., 2012b; Muranty et al., 2015). Yet, it has not been assessed in comparison to traditional phenotypic selection when the goal is identifying superior individuals with commercial potential in a cultivar development effort such as the apple breeding program at the University of Minnesota. Selection traits can be chosen, an idea proposed by Muranty et al. (2015), which are traits that would result in elimination from the University of Minnesota apple breeding program. Advanced selections and successful commercial releases can be used to create test populations in order to test the effectiveness of genomewide selection models using appropriate training data in the University of Minnesota apple breeding program using traditional phenotypic selection as a baseline for comparison. For example, if breeding decisions were made based on genomewide predictions resulting in the culling of a known commercial release, genomewide selection tools may need

more power or refinement. The second objective of this study was to use postdiction (or explanation using hindsight) to determine if genomewide selection in the University of Minnesota apple breeding program would have identified known successes, defined as seedlings selected for advanced testing as well as commercial releases, that were identified via phenotypic selection.

3.3 Materials and Methods

3.3.1 Retrospective analysis

This research was conducted as two sets of analyses. To test this, data from fruit traits important to the advancement of lines through the University of Minnesota apple breeding program were analyzed in two trials, each including a separate set of germplasm. The traits, datasets, and germplasm in this chapter are the same as those described in Chapter 2. Dataset 1 included fruit sensory traits and Dataset 2 included instrumentally measured fruit traits. The data for these two datasets comprised distinct germplasm and SNP markers for each respective dataset. The training populations comprised four families for each trial (Table 3.1). The test populations for each dataset comprised a selection of advanced University of Minnesota apple lines related to the training germplasm and included two commercial releases (‘MN55’ and Minneiska). An additive model was used to make genomic predictions using ridge regression-best linear unbiased prediction (RR-BLUP) for the test population, made up of advanced selections and the two commercial releases, using data from the four related

families to train the models. Based on the results from Chapter 2, the additive model was chosen as it had acceptable predictive abilities and a less computationally intensive process than the model fitting fixed effects.

For this chapter, the commercial releases ‘MN55’, which has fruit sold under the brands First Kiss® and Rave®, and ‘Minneiska’, with fruit sold under the SweeTango® brand, were selected to be included in the test populations. ‘MN55’ was selected because it is derived from one of the crosses in Dataset 1 (‘Honeycrisp’ × ‘AA44’) and shared relatedness with other individuals in both test populations. ‘Minneiska’ was selected because it was derived from one of the crosses in Dataset 2 (‘Honeycrisp’ × ‘Minnewashta’) and also shared relatedness with other individuals in the test populations. Corresponding to Dataset 1, an additional 66 individuals that had been advanced to clonal testing stages in the University of Minnesota breeding program (hereafter referred to as selections) were selected for a total of 68 individuals in the test population. Corresponding to Dataset 2, an additional 96 advanced selections from the University of Minnesota breeding program were included for a total of 98 individuals to be included in the test population. The additional advanced selections were half- or full-sibs to at least one family within the training population of each respective study.

The three sensory traits assessed for Dataset 1 (crispness, firmness, and juiciness) were all considered as selection traits for this trial. For each trait, a threshold or acceptable range was defined to evaluate whether the advanced selections and commercial release would have been culled or kept had selection

been based on genomic predictions alone. Traits and culling thresholds are shown in Table 3.2. The mean of the performance of ‘Honeycrisp’ for each trait was the initial culling threshold. ‘Honeycrisp’ was used as its performance represents the pass or failure standard for fruit quality in the University of Minnesota apple breeding program (James Luby, personal communication). The ‘Honeycrisp’ mean value for each trait was adjusted by the overall standard deviation of all seedlings studied for that trait. The minimum acceptable value for each trait was the ‘Honeycrisp’ mean minus one standard deviation. The standard deviation was the standard deviation of all available seedlings in the study as an approximation of the standard deviation of the population.

For Dataset 2, four traits, titratable acidity (acidity), soluble solids content (soluble solids), and mechanical firmness represented by overall maximum hardness (maximum hardness) and overall mean hardness (mean hardness), were selected to represent traits for Dataset 2 that would be used to cull individuals from the program (Table 3.3). Data on ‘Honeycrisp’ arithmetic mean performance for each trait and the method described for Dataset 1 were used to generate thresholds in Dataset 2 (Table 3.3).

In each dataset, the predicted values of each trait were then compared to the threshold for that trait to determine if the cultivar would have been advanced in the breeding program or culled based on genomewide predicted performance in Datasets 1 and 2 (Table 3.2 and Table 3.3, respectively). By doing this the number of advanced selections that were kept or culled could be determined.

Additionally, whether the known commercial releases had been kept or culled and their relative performance could be assessed.

In addition to assessing the advanced lines' predicted performance by comparing them to a threshold, for each dataset the predicted performance of the seedlings within the training population was also compared to the threshold. The seedlings in the training population comprised seedlings that have not been advanced past the first year of evaluation in the UMN apple breeding program and thus were used as the non-advanced seedlings. Using their genotypic data, a predicted performance was calculated using the additive model. The number of non-advanced seedlings assessed in Dataset 1 was 196 and the number assessed in Dataset 2 was 170. The performance of each seedling in the training population was predicted via the untested-family method as described in Chapter 2 of this thesis. This means that for each family, the performance of each seedling within the family was predicted using the remaining three families as the training population. This was done iteratively such that each family served as the test population once. The predicted values for each trait described above and the training population seedlings, as appropriate for each dataset, were compared to the same thresholds generated for the testing of advanced seedlings to see if they would have been kept or culled. This serves as a test for the stringency of the thresholds and selection protocol.

3.3.2 Comparison of varying levels of relatedness between a training and test population

The second part of this analysis tested the effect of varying the level of relatedness between the training population and test population by adding additional full-sibs to the training data. In each trial, the family from which the commercial release was selected served as the test population, ‘AA44’ × ‘Honeycrisp’ for Dataset 1 and ‘Honeycrisp’ × ‘Minnewashta’ for Dataset 2. These families were selected due to the number of individuals available for each family as well as the importance and success of these crosses in the University of Minnesota breeding program.

Predictive ability is the correlation between the phenotypic and the marker predicted values (Meuwissen et al., 2001; Bernardo, 2010). The predictive abilities of an untested-family and an untested-seedling were determined in the first chapter of this thesis. For this analysis, three treatments consisted of increasing amounts of full-sibs being removed from the test data and added to the training data. To create these new and more related training populations five, 10, and 15 individuals from the test population were selected to be removed from the test population and included in the training population. These individuals were selected at random and 30 iterations at each level of relatedness were conducted. The mean performance of all 30 iterations for each level was calculated. The mean performance was used to compare the performance of genomewide selection between levels of relatedness. The traits in Table 3.2 and

Table 3.3 were used. The minimum, maximum, median, mean, and standard deviation of the predictive ability for all 30 iterations for a given level were compared.

3.4 Results

In Dataset 1, only one advanced line would have been culled based on the predicted performance for crispness, however this line was not a cultivar. Each line in the test population (meaning all advanced selections and both cultivars studied) would have passed the culling test for firmness (Table 3.4). However, none of the lines would have passed the culling test for juiciness (Table 3.4). Accordingly, the 'MN55' and Minneiska cultivars would have passed the culling test in the traits of crispness and firmness, but would have been culled based on juiciness. 'Minneiska', was in the top 25% of seedlings for crispness, the top 35% for firmness, and the top 35% of seedlings for juiciness. 'MN55' was in the bottom 10% of seedlings for crispness, had the lowest predicted performance for firmness, and was in the top 25% of seedlings for juiciness.

In Dataset 2 for soluble solids content and titratable acidity, no selections would have been culled based on the thresholds defined for each trait. For both overall maximum hardness and overall average hardness, the instrumentally measured firmness traits, all seedlings would have been culled (Table 3.4). 'Minneiska' was in the bottom 40% of seedlings for soluble solids, the bottom 5% of seedlings for titratable acidity, the top 30% of seedlings for overall maximum

hardness, and the top 25% of seedlings for overall average hardness. ‘MN55’ was in the top 40% of seedlings for soluble solids, the bottom 30% of seedlings for titratable acidity, the top 55% of seedlings for overall maximum hardness, and the bottom 40% of seedlings for overall average hardness.

Comparing the numbers of advanced selections culled to the number of random/ non-advanced seedlings in Dataset 1, the percentages of seedlings culled increased for crispness (0.02% vs 0.21%), increased for firmness (0.00% vs 0.33%), and decreased for juiciness (100% vs 0.20%). For crispness, there were 41 out of 196 non-advanced seedlings culled compared to only 1 out of 68 advanced selections. For firmness there were 64 out of 196 non-advanced seedlings culled compared to 64 out of 196 advanced selections. However, for juiciness there were only 40 out of 196 seedlings culled compared to 68 out of 68 of the advanced seedling beings culled. In Dataset 2, the percentages of seedlings culled stayed approximately the same. For titratable acidity 0.00% (0 out of 98) of advanced selections were culled, compared to 0.01% (2 out of 170) of non-advanced seedlings. For soluble solids 0.00% (0 out of 98) of advanced selections were culled, compared to 0.00% (0 out of 170) of non-advanced seedlings. For overall maximum hardness and overall average hardness 100% of seedlings were culled, both in the advanced selections and non-advanced seedling comparisons.

When comparing levels of relatedness between the training and test population, for Dataset 1, increasing the number of full-sibs included in the

training population tended to increase the predictive ability of the models (Table 3.5). The exception was in firmness between the 15 and 25 full-sibs included levels, where predictive ability decreased slightly. When the number of added full-sibs increased from five to 15, predictive abilities increased close to the levels of an untested-seedling procedure which corresponded to the maximum level of relatedness we studied (Table 3.5).

When comparing levels of relatedness between the training and test population, for Dataset 2, the difference in predictive abilities between models was minimal. Many of the increases in predictive abilities between models were very small. Adding as few as five full-sibs improves predictive abilities over the untested-family predictive abilities (Table 3.6).

3.5 Discussion

The results from Dataset 1, looking at the comparison of selections made based on genomic predictions with those made using traditional phenotypic selection, have meaningful implications for the future of genomewide selection in the University of Minnesota breeding program. In apple, the taste and mouthfeel, due in part to crispness and firmness, contribute to consumer liking and ultimate commercial success of an apple cultivar (Péneau et al., 2006). Since they were selected based on sensory testing of fruit, the advanced selections and commercial releases represent the goal for performance of genomewide selection, especially for predicting performance of juvenile seedlings. Despite

‘MN55’ and ‘Minneiska’ not being in the top 10% of advanced selections, it should be noted that this is not reflective of their overall rank amongst all seedlings in the population, rather only against other outstanding lines that were also considered for release. Our findings that genomewide selection was consistent with phenotypic selection for advanced selections and commercial releases for certain sensory traits adds to the existing evidence suggesting that genomewide selectin has the potential to assist apple breeders.

The culling thresholds used in this study were based on considered to be stringent based on internal standards for our breeding program. With these standards, ‘MN55’ and ‘Minneiska’ still passed the culling test. However, based on the results with the random/ non-advanced seedlings an increased stringency may be needed for some traits. Within Dataset 1, had the ‘MN55’ commercial release matched the ‘Honeycrisp’ parent standard for crispness, the threshold would have to have gone from 0.35 to 0.41 or greater to cause ‘MN55’ or ‘Minneiska’ to be culled from the program. This means that the culling threshold would have to have been even more strict than the current commercial standard to have culled ‘MN55’ or ‘Minneiska’ based on crispness. The threshold for firmness would have needed to be raised from 0.25 to 0.29, or a change of 0.04 which is almost a 16% increase in stringency before they would have been culled based on firmness.

This was also the case within Dataset 2, both ‘MN55’ and ‘Minneiska’ were several degrees Brix higher than the threshold for soluble solids. The threshold

would have needed to go from 10.67 to over 13.27 for both commercial cultivars to have been culled. This represents an almost 25% increase in the threshold, which also would result in the ‘Honeycrisp’ parent being culled, indicating that such stringency is not realistic. For acidity, the threshold would have needed to change from 1.52 mg/mL malic acid to 2.98 mg/mL malic acid or higher for either cultivar to be culled. This is evidence that the thresholds set were appropriate, and that even at extreme levels of stringency the commercial release and most of the advanced selections would have still been moved forward in the program.

Selections made on the basis of genomewide predictions were consistent with selections made on the basis of traditional phenotypic selection. However, in Dataset 2, the selection procedure based on culling thresholds performed the same in both advanced and non-advanced seedlings. This may indicate that for some traits, this procedure is not discerning enough to be used as the basis for culling in the UMN apple breeding program. However, it is noteworthy that using genomewide predictions as the basis of culling had greater success with sensory traits (Dataset 1) which may be more reflective of traits a field breeder would also base culling decisions on. ‘MN55’ and ‘Minneiska’ performed well and would not have been culled on the basis of genomewide selection for the traits crispness and firmness in Dataset 1 or titratable acidity and soluble solids in Dataset 2. This at least shows that genomewide selection does not appear to cull clear winners within the program. Despite not necessarily performing at the top of the advanced selections, the commercial releases were still moved through the

breeding program based on genomewide predictions. It should be noted that this work examined a small number of traits, and there are many more that may impact success of a line. More traits should be evaluated prior to deployment of using genomewide selection to inform culling. l

All selections would have been culled if juiciness alone had been considered due to predicted values falling below the threshold. This was an indication that, despite its apparent importance to consumer appeal, juiciness is currently not a good candidate for genomewide selection. This may be due to the nature of assessing this trait. Juiciness is the trait which is the most challenging to train panelists in assessing, as such more work may need to be done with this trait before using it in prediction models (McKay, 2010; McKay et al., 2011). Of the traits studied in Dataset 1, juiciness had the lowest heritability (H^2 0.72 compared to point 0.81 for firmness and 0.76 for crispness) that McKay et al. (2011) studied. Juiciness also had the highest standard error (0.026 for juiciness compared to 0.017 for firmness and 0.016 for crispness) (McKay, 2010). Similarly, all advanced seedlings would have been culled based on the test for overall maximum and overall average hardness. These traits are less well researched and may also need more development in terms of procedure and protocol, as well as confirming how well they correlate to sensory firmness, before being utilized in GWS work.

The results from the random/ non-andvanced seedlings were more mixed than with the advanced seedlings. These results indicate that especially based

on the instrumentally measured traits in Dataset 2, culling based on genomewide selection would not have been effective given that either all (for overall maximum and overall average hardness) or effectively no (for soluble solids content and titratable acidity) seedlings were culled. This may indicate that more research is needed before these traits can confidently be used as traits to upon which predictions are used to make culling decisions in the UMN breeding program. For the sensory traits, there were significantly more traits culled in the random/ non-andvanced seedlings than the advanced seedlings. Even though this test did not result in 100% culling, it still resulted in an appreciable number of seedlings being culled and would have represented an increase in efficiency to the breeding program. These traits may therefore be closer to being ready to be used as traits upon which culling can be based. Additionally, these were the sensory traits which may be more reflective of what a field breeder would select or cull in the UMN breeding program which may make them more important to focus on for culling potential selections.

By assessing different levels of relatedness between the training- and test population, we saw that adding in full-sibs helped bring predictive abilities closer towards the maximum predictive ability that we observed which was the untested-seedling predictive ability in general. This is consistent with the prevailing knowledge that relatedness is an important factor in the accuracy of predictions made by genomewide selection (Habier et al., 2010; Kumar et al., 2012a; Lee et al., 2017). It is also consistent with existing research that adding a

low percentage of closely related individuals has strong effects on predictive abilities (De Roos et al., 2009). This indicates that we may be able to confidently predict the performance of selection traits within a cross for unfruiting seedlings after five to 15 of their full-sibs have fruited, been evaluated, and that information added to an appropriate set of training data.

This is consistent with the findings of De Roos et al. (2009) in cattle. It is also supported by other evidence that relatedness is an important factor in the predictive ability of genomewide selection (Habier et al., 2010; Kumar et al., 2012a; Lee et al., 2017). A valuable way that genomewide selection might fit in to the University of Minnesota apple breeding program is to make predictions within crosses using the first lines to begin flowering. These findings show that potentially with even a small number of seedlings from a cross being evaluate at fruiting and added to the training data, the breeding program could begin to make decisions on the remaining selections from the cross (Wannemuehler, 2018). The cost of grafting a seedling is $7 plus $4 to maintain the first year, for a total of $11 dollars the first year in the field. Each additional year costs $4 per year to maintain each selection (Wannemuehler, 2018). This does not assume the costs of then evaluating the fruit, so this is a conservative cost estimate. If we assume five to ten years in the juvenile phase growing in the orchards, $27 to $51 is a reasonable estimate for growing each individual to fruiting. Internal costs of genotyping each individual and processing the resulting information in our program are around $50 per individual using the higher density (8K (Chagné et

al., 2012) to 20K (Bianco et al., 2014) SNP chip) arrays. However, the costs are likely lower when using a mini array or a subset of markers. So genomewide selection is at worst a similar cost to traditional seedling maintenance and evaluation. However, it would allow for more rapid removal of lines without potential in our program meaning we can increase the efficiency by growing only the best seedings. Overall, genomewide selection could be a promising tool in our program with the recommendation, based on this research, that future work focuses on selecting the best traits to use as selection traits for genomewide selection within the program.

Tables

Table 3.1. Number of seedlings (n) and corresponding parents associated with each cross for both Dataset 1 and Dataset 2.

Dataset 1		Dataset 2	
Cross	**Seedlings (n)**	**Cross**	**Seedlings (n)**
‘MN1702’ × ‘Honeycrisp’	21	‘Minneiska’ × ‘MN55’	36
‘MN1764’ × ‘Honeycrisp’	91	‘Honeycrisp’ × ‘Minnewashta’	47
‘Jonafree’ × ‘Honeycrisp’	45	‘MN1702’ × ‘Minneiska’	39
‘AA44’ × ‘Honeycrisp’	39	‘Honeycrisp’ × ‘WSU2’	48

Table 3.2. The ‘Honeycrisp’ arithmetic mean performance for each trait in Dataset 1 and the corresponding value at which a seedling would be culled (culling threshold) for each trait.

	Crispness (mm)	Firmness (mm)	Juiciness (mm)
‘Honeycrisp’ Trait Mean	0.43	0.33	0.43
Culling Threshold	0.35	0.25	0.38

Table 3.3. The ‘Honeycrisp’ arithmetic mean performance for each trait in Dataset 2 and the corresponding value at which a seedling would be culled (culling threshold) for each trait.

	Soluble Solids Content (°Bx)	Acidity (mg/mL)	Overall Maximum Hardness (lb.)	Overall Average Hardness (lb.)
‘Honeycrisp’ Trait Mean	12.17	3.99	95.72	63.12
Culling Threshold	10.67	1.52	92.81	61.17

Table 3.4. Number of advanced selections which served as the test population for Dataset 1 (n= 68) and Dataset 2 (n=98), the traits evaluated in Dataset 1 and Dataset 2 respectively, and the total number of advanced seedlings culled in each dataset for each trait evaluated.

Dataset 1		**Dataset 2**	
Trait	**Culled (n)**	**Trait**	**Culled (n)**
68 Advanced Selections Evaluated		**98 Advanced Selections Evaluated**	
Crispness	1	Acidity (mg/mL)	0
Firmness	0	Soluble Solids Content (°Bx)	0
Juiciness	68	Overall Maximum Hardness (lb.)	98
		Overall Average Hardness (lb.)	98
196 Non-Advanced Seedlings Evaluated		**170[*] or 172 [* *] Non-Advanced Seedlings Evaluated**	
Crispness	41	Acidity (mg/mL) [*]	2
Firmness	**64**	Soluble Solids Content (°Bx) [*]	0
Juiciness	40	Overall Maximum Hardness (lb.) [* *]	172
		Overall Average Hardness (lb.) [* *]	172

Table 3.5. Summary of the mean values for the statistics of minimum, maximum, median, mean, and standard deviation of all 30 iterations of predictive abilities. The levels of relatedness are 5 full-sibs included, 15 full-sibs included, and 25 full-sibs included in the training population. The lower half of the table shows the mean predictive ability for the Dataset 1 traits for the untested-family and untested-seedling training population analyses in Chapter 2. * indicates significantly different from zero at α = 0.05.

	5 Full-sibs Included			**15 Full-sibs Included**			**25 Full-sibs Included**		
	Crispness	**Firmness**	**Juiciness**	**Crispness**	**Firmness**	**Juiciness**	**Crispness**	**Firmness**	**Juiciness**
Minimum	0.02	0.01	-0.18	-0.02	0.12	-0.36	-0.18	-0.08	-0.17
Maximum	0.35	0.40	0.38	0.54	0.50	0.56	0.59	0.58	0.58
Median	0.19	0.22	0.11	0.24	0.28	0.18	0.34	0.30	0.22
Mean	0.18	0.22	0.11	0.23	0.29	0.20	0.29	0.28	0.23
Standard Deviation	0.09	0.09	0.14	0.13	0.10	0.20	0.21	0.21	0.20

	Crispness	**Firmness**	**Juiciness**
Untested-Family	0.19	0.35*	0.19
Untested-Seedling**	0.27*	0.45*	0.28*

**Depending on which family the untested seedling was from, there were 20, 90, 44, or 38 full-sibs included in the training data for said seedling.

Table 3.6. Summary of the mean values for the statistics of minimum, maximum, median, mean, and standard deviation of all 30 iterations of predictive abilities. The levels of relatedness are 5 full-sibs included, 15 full-sibs included, and 25 full-sibs included in the training population. The lower half of the table shows the mean predictive ability for the Dataset 2 traits for the untested-family and untested-seedling training population analyses. * indicates significantly different from zero at α = 0.05.

	5 Full-sibs Included				15 Full-sibs Included				25 Full-sibs Included			
	Acidity (mg/mL malic acid)	**Soluble Solids Content (°Bx)**	**Maximum Hardness**	**Mean Hardness**	**Acidity (mg/mL malic acid)**	**Soluble Solids Content (°Bx)**	**Maximum Hardness**	**Mean Hardness**	**Acidity (mg/mL malic acid)**	**Soluble Solids Content (°Bx))**	**Maximum Hardness**	**Mean Hardness**
Minimum	0.04	0.04	0.19	0.28	-0.08	-0.20	0.08	0.09	-0.20	-0.00	-0.01	0.02
Maximum	0.32	0.28	0.44	0.47	0.53	0.32	0.51	0.59	0.56	0.50	0.63	0.67
Median	0.22	0.12	0.31*	0.41*	0.21	0.17	0.26	0.37*	0.21	0.20	0.39*	0.44*
Mean	0.20	0.14	0.31*	0.40*	0.20	0.14	0.26	0.37*	0.18	0.20	0.35	0.43*
Standard Deviation	0.07	0.06	0.07	0.06	0.14	0.13	0.10	0.11	0.20	0.20	0.15	0.15

	Acidity (mg/mL malic acid)	**Soluble Solids Content (°Bx)**	**Maximum Hardness**	**Mean Hardness**
Untested-Family	0.07	0.12	0.09	0.05
Untested-Seedling**	0.37*	0.33*	0.52*	0.48*

**Depending on which family the untested seedling was from, there were 35, 46, 38, or 47 full-sibs included in the training data for said seedling.

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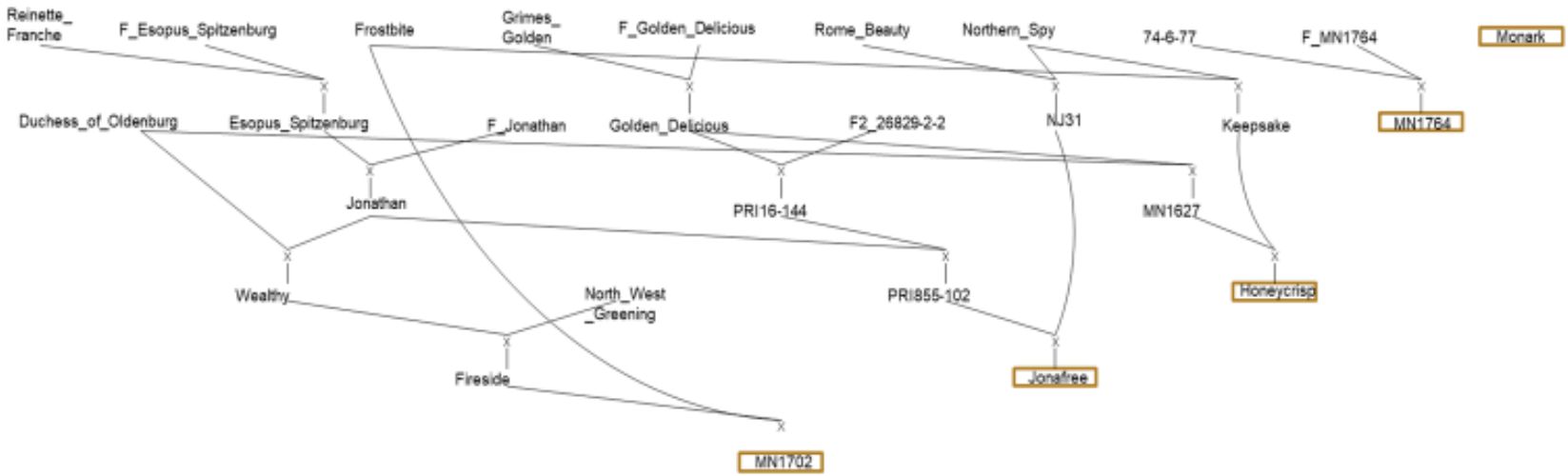
Appendix

Appendix 1. Pedigree map for Dataset 1 which comprised four crosses: Jonafree x Honeycrisp, AA44 x Honeycrisp, MN1702 x Honeycrisp, and MN1764 x Honeycrisp.



Appendix 2. Pedigree map for Dataset 2 which comprised four crosses: MN1914 x MN55, Honeycrisp x Minneiska, MN1702 x MN1914, and Honeycrisp x WSU2.

Appendix 3. Description of the column headers and column content of Appendix 4 and Appendix 5 which describe the QTL included in model fitting fixed effects for Dataset 1.

COLUMN HEADER	COLUMN CONTENT DESCRIPTION
Sort Order ID	This is the identification given to the SNP for a sort order to be used in GWS.
Trait	This is the trait for which the SNP was used as a fixed QTL.
SNP Name	The long name of each SNP marker
SS Number	The SS SNP name used in the Genome Database for Rosaceae (www.rosaceae.org).
8k Marker ID	The marker index numbers from the apple Illumina 8K SNP array.
lg (consensus)	The linkage groups that each SNP belongs to based on consensus groupings between all families used in the map.
cM	Centimorgan position for each SNP.
Position (V1.0 assembly)	This is the original physical position based on V1.0 of the Golden Delicious genome assembly.
Position (V3.0 assembly)	This is the physical position based on the flanking sequence being blasted vs. the V3.0 assembly. NA signifies where a position could not be determined due to multiple conflicting hits, or that there were no hits within the consensus linkage group the SNP was found to belong to in this linkage map.
Flanking Sequence	The flanking DNA sequence for each SNP. These sequences were taken off the the 8K array description from GDR.

Appendix 4. Description of the sort order ID, trait name for which QTL was detected, SNP name, SS number, 8k marker ID, chromosome the QTL is located on (lg), cM, and position within the V1.0 and V3.0 assemblies for the QTL included in model fitting fixed effects for Dataset 1.

Sort Order ID	Trait	SNP Name	SS Number	8k Marker ID	lg (consensus)	cM	Position (V1.0 assembly)	Position (V3.0 assembly)
M227	firm	GDsnp00947	ss475882924	326	9	25.206	11711545	11981411
M2228	crisp	RosBREEDSNP_SNP_CT_ 22205875_Lg4_01026_MAF50_ 1685729_exon4	ss475878069	4375	4	52.683	20040521	29188137
M65	crisp	GDsnp00213	ss475882490	142	14	58.157	27058375	30160527

Appendix 5. Flanking sequences for the QTL included in model fitting fixed effects for Dataset 1.

Sort Order ID	Flanking Sequence
M227	CAACGATCAAGATCTCGATGCATAAAATTAACCGAGTATACCAGAATTTTCGAGCAGGCACATAAATTTGTACAAGC AAATGAGAGATATATAAACATCT[A/G]TACAGCCATGAATTTATTTAATCCGATACAAATTTTGTCGGGTACATAACA AGACCAGCACATATGAACAAGATGATTCTGTTTGACCTCCCTCTATACA
M2228	TTGAGGTTGAAACTGAAAATTGTTTCGTACTTCAGTATACAGTGTTCGATCAGCTAAAACT[T/C]AGACTCCTGAAAA GGAAGAATAAAACGGGCAAGGATTCTTCTCCAGAAGCCCTTTCTGCTT
M65	TGAAAAAAGCAAAAACTTCGGAACTTCATAACCAGCACTCAACAAAATATGCCTAAACAACTCATATAA ACATAATCAACATCAAGGCTAACAAGTTAGT[A/G]CTGTTTGAGACAGCTTCTGGAATGGCCAAAAGTG CTTTAGTGCTTCTTCAACAAGCAAATCCCTGTNCTTTCTTGAAAAGCACTTGAATTTTTAGTAAAG

Appendix 6. Description of the column headers and column content of Appendix 7 and Appendix 8 which describe the QTL included in model fitting fixed effects for Dataset 2.

COLUMN HEADER	COLUMN CONTENT DESCRIPTION
Sort Order ID	This is the identification given to the SNP for a sort order to be used in GWS.
Trait	This is the trait for which the SNP was used as a fixed QTL.
Evaluation	This is the phenotypic evaluation date (fresh harvest or 10-week storage) at which data was collected.
SNP Name	The long name of each SNP marker
SS Number	The SS SNP name used in the Genome Database for Rosaceae (www.rosaceae.org).
8k Marker ID	The marker index numbers from the apple Illumina 8K SNP array.
lg (consensus)	The linkage groups that each SNP belongs to based on consensus groupings between all families used in the map.
lg (apple_assembly_1.0)	This is the original linkage group designation based on V1.0 of the Golden Delicious genome assembly32
cM	Centimorgan position for each SNP.
Position (V1.0 assembly)	This is the original physical position based on V1.0 of the Golden Delicious genome assembly.
Position (V3.0 assembly)	This is the physical position based on the flanking sequence being blasted vs. the V3.0 assembly. NA signifies where a position could not be determined due to multiple conflicting hits, or that there were no hits within the consensus linkage group the SNP was found to belong to in this linkage map.
Flanking Sequence	The flanking DNA sequence for each SNP. These sequences were taken off the the 8K array description from GDR.

Appendix 7. Description of the sort order ID, trait name for which QTL was detected, evaluation, SNP name, SS number, 8k marker ID, chromosome the QTL is located on (lg), cM, and position within the V1.0 and V3.0 assemblies for the QTL included in model fitting fixed effects for Dataset 2.

Sort Order ID	Trait	Evaluation	SNP Name	SS Number	8k Marker ID	lg (consensus)	lg (apple assembly 1.0)	cM	Position (V1.0 assembly)	Position (V3.0 assembly)
604	Soluble Solids Content	Fresh	RosBREEDSNP_SNP_CT_ 34437600_Lg3_02030_MAF20_ MDP0000155220_exon1	ss475877791	4838	3	3	62.536	29244177	34032925
3011	Soluble Solids Content	Fresh	RosBREEDSNP_SNP_AG_1621844 _Lg17_01205_MAF40_967370_exon1	ss475881962	2481	17	17	6.193	1621842	1907980
108	Soluble Solids Content	Fresh	RosBREEDSNP_SNP_AG_27095198_Lg1 _01693_MAF30_332314_exon1	ss475876871	2853	1	1	39.404	21797475	26870224
2698	Soluble Solids Content	Fresh	RosBREEDSNP_SNP_AG_20380867_Lg15 _02080_MAF20_1639356_exon1	ss475881485	2628	15	15	61.177	18398106	21135056
2435	Soluble Solids Content	Fresh	RosBREEDSNP_SNP_AC_12582508_Lg14 _00170_MAF40_248387_exon1	ss475881060	1901	14	14	18.944	10406776	8171471
1554	Soluble Solids Content	Fresh	RosBREEDSNP_SNP_AG_6024891_Lg9 _01603_MAF40_1683715_exon1	ss475879346	3300	9	9	12.412	6027664	6847858
2941	Soluble Solids Content	Fresh	RosBREEDSNP_SNP_TC_14138269_Lg16 _01624_MAF20_MDP0000185660_exon1	ss475881878	7309	16	16	45.547	13010338	14724794
344	Crispness	Fresh	RosBREEDSNP_SNP_GT_25677361_Lg2 _01935_MAF50_1635560_exon1	ss475877316	6938	2	2	45.107	23096320	24763811
2569	Crispness	Fresh	RosBREEDSNP_SNP_GA_1098793_Lg15 _01009_MAF30_84788_exon1	ss475883850	5351	15	15	10.868	1098791	4329961
2229	Crispness	Fresh	GDsnp01855	ss475882417	556	12	12	67.915	31094641	32353530
2858	Overall Average Hardness	Fresh	RosBREEDSNP_SNP_GA_3988516_Lg16 _01179_MAF50_1673516_exon1	ss475881752	6366	16	16	14.73	3988516	6041053
3042	Overall Maximum Hardness	Fresh	RosBREEDSNP_SNP_CT_5689554_Lg17 _00606_MAF40_333837_exon1	ss475882025	5085	17	17	15.698	5489554	5704246
2856	Overall Maximum Hardness	Fresh	RosBREEDSNP_SNP_GA_3981672_Lg16 _01179_MAF50_1629115_exon1	ss475883367	6364	16	16	14.73	3981672	6034237
413	Overall Maximum Hardness	Fresh	RosBREEDSNP_SNP_TC_36462247_Lg2 _00669_MAF50_262678_exon2	ss475877413	8025	2	2	59.201	32994342	34675739
941	pH	Fresh	RosBREEDSNP_SNP_GA_11403959_ Lg5_RosCOS1953_MAF20_MDP0000216403_exon1	ss475878277	5378	5	5	59.012	10008688	37094514

Appendix 7 (con't). Description of the sort order ID, trait name for which QTL was detected, evaluation, SNP name, SS number, 8k marker ID, chromosome the QTL is located on (lg), cM, and position within the V1.0 and V3.0 assemblies for the QTL included in model fitting fixed effects for Dataset 2.

Sort Order ID	Trait	Evaluation	SNP Name	SS Number	8k Marker ID	lg (consensus)	lg (apple assembly 1.0)	cM	Position (V1.0 assembly)	Position (V3.0 assembly)
2821	pH	Fresh	RosBREEDSNP_SNP_TC_1571002 _Lg16_01734_MAF40_216397_exon1	ss475883754	7364	16	16	8.481	1571002	3439690
2640	pH	Fresh	RosBREEDSNP_SNP_GA_8129810_ Lg15_00389_MAF40_1641199_exon1	ss475881365	6634	15	15	34.041	8129810	11267115
2455	Russet	Fresh	RosBREEDSNP_SNP_TG_20660924_ Lg2_RosCOS358_MAF50_1681238_exon2	ss475877280	8503	14	2	37.767	19081898	21025936
81	Russet	Fresh	RosBREEDSNP_SNP_GT_18064090_ Lg1_01783_MAF50_708296_exon1	ss475876793	6838	1	1	23.021	14157757	22285020
1890	Russet	Fresh	RosBREEDSNP_SNP_TC_37072737_ Lg10_00604_MAF10_MDP0000691383_exon1	ss475880013	8043	10	10	77.035	32375828	40965599
484	Russet	Fresh	RosBREEDSNP_SNP_TC_5968340_Lg3 _01263_MAF50_20343_exon1	ss475877555	8201	3	3	7.735	5183030	4548835
298	Russet	Fresh	RosBREEDSNP_SNP_GA_17179364_Lg2 _327287_MAF40_327287_exon1	ss475875821	5630	2	2	32.46	16000338	14850726
3042	Russet	Fresh	RosBREEDSNP_SNP_CT_5689554_Lg17 _00606_MAF40_333837_exon1	ss475882025	5085	17	17	15.698	5489554	5704246
1687	Russet	Fresh	RosBREEDSNP_SNP_TC_867947_Lg10 _00106_MAF40_546402_exon1	ss475879595	8330	10	10	0.276	734700	1189958
1454	Starch Iodine Rating	Fresh	RosBREEDSNP_SNP_CA_32216879_Lg5 _RosCOS2881_MAF20_MDP0000118986_exon2	ss475878440	3789	8	5	43.918	27253603	23884817
58	Starch Iodine Rating	Fresh	GDsnp01500	ss475882271	430	1	1	15.66	11455026	19147458
2640	Titratable Acidity	Fresh	RosBREEDSNP_SNP_GA_8129810_Lg15_ 00389_MAF40_1641199_exon1	ss475881365	6634	15	15	34.041	8129810	11267115
742	Titratable Acidity	Fresh	RosBREEDSNP_SNP_TG_18428562_Lg4_ 00619_MAF20_464560_exon1	ss475877974	8476	4	4	37.316	16263000	25335597
97	Titratable Acidity	Fresh	RosBREEDSNP_SNP_CT_23134240_Lg1 _109586__109586_exon1	ss475875756	4408	1	1	29.279	18236515	24799843
518	Titratable Acidity	Fresh	GDsnp01990	ss475882693	603	3	3	19.302	8983345	8836509

Appendix 7 (con't). Description of the sort order ID, trait name for which QTL was detected, evaluation, SNP name, SS number, 8k marker ID, chromosome the QTL is located on (lg), cM, and position within the V1.0 and V3.0 assemblies for the QTL included in model fitting fixed effects for Dataset 2.

Sort Order ID	Trait	Evaluation	SNP Name	SS Number	8k Marker ID	lg (consensus)	lg (apple assembly 1.0)	cM	Position (V1.0 assembly)	Position (V3.0 assembly)
2271	Titratable Acidity	Fresh	RosBREEDSNP_SNP_TG_3850600 _Lg13_00565_MAF30_1674510_exon2	ss475880718	8653	13	13	10.704	3861812	4317293
2925	Titratable Acidity	Fresh	RosBREEDSNP_SNP_CA_9092272_ Lg16_01730_MAF40_1619732_exon2	ss475881842	3939	16	16	37.565	1.03E+08	12265024
941	Titratable Acidity	Fresh	RosBREEDSNP_SNP_GA_11403959_ Lg5_RosCOS1953_MAF20_MDP0000216403_ exon1	ss475878277	5378	5	5	59.012	10008688	37094514
2512	Titratable Acidity	Fresh	RosBREEDSNP_SNP_CT_29528073_ Lg14_01811_MAF50_159748_exon1	ss475881173	4648	14	14	53.806	25083294	27995212
1300	Titratable Acidity	Fresh	RosBREEDSNP_SNP_GA_25223845_ Lg7_00699_MAF50_1660767_exon1	ss475878923	5927	7	7	43.735	20870240	25715164
2683	Soluble Solids Content	10wk	RosBREEDSNP_SNP_GA_17589314_ Lg15_RosCOS2945_MAF50_1626426_exon1	ss475881460	5642	15	15	52.189	16204568	17541493
2213	Soluble Solids Content	10wk	RosBREEDSNP_SNP_GA_33692967_ Lg12_02008_MAF20_MDP0000291899_exon2	ss475880609	6226	12	12	61.278	29138438	30201756
2812	Soluble Solids Content	10wk	RosBREEDSNP_SNP_CA_1187517_Lg16 _01588_MAF10_545103_exon1	ss475881679	3539	16	16	6.712	1187515	3003499
451	Soluble Solids Content	10wk	RosBREEDSNP_SNP_AC_994373_Lg3_ 00506_MAF40_MDP0000137429_exon1	ss475877474	2269	3	3	0.681	994373	437049
97	Soluble Solids Content	10wk	RosBREEDSNP_SNP_CT_23134240_Lg1 _109586__109586_exon1	ss475875756	4408	1	1	29.279	18236515	24799843
801	Soluble Solids Content	10wk	RosBREEDSNP_SNP_GA_23902093_Lg4_ 00681_MAF40_1663052_exon1	ss475878101	5873	4	4	56.504	21536530	30656131
2017	Soluble Solids Content	10wk	RosBREEDSNP_SNP_TC_26829691_Lg11 _01361_MAF50_1663355_exon1	ss475880228	7738	11	11	48.008	23460198	31708000
1377	Soluble Solids Content	10wk	RosBREEDSNP_SNP_AG_12927423_Lg8_ 01132_MAF50_1636207_exon2	ss475879056	2371	8	8	14.629	11354054	6027440
2685	Soluble Solids Content	10wk	RosBREEDSNP_SNP_GA_17583563_Lg15_ RosCOS2945_MAF40_8165_exon1	ss475881458	5640	15	15	52.189	16198817	17547243
2821	pH	10wk	RosBREEDSNP_SNP_TC_1571002_Lg16_ 01734_MAF40_216397_exon1	ss475883754	7364	16	16	8.481	1571002	3439690

Appendix 7 (con't). Description of the sort order ID, trait name for which QTL was detected, evaluation, SNP name, SS number, 8k marker ID, chromosome the QTL is located on (lg), cM, and position within the V1.0 and V3.0 assemblies for the QTL included in model fitting fixed effects for Dataset 2.

Sort Order ID	Trait	Evaluation	SNP Name	SS Number	8k Marker ID	lg (consensus)	lg (apple assembly 1.0)	cM	Position (V1.0 assembly)	Position (V3.0 assembly)
a2655	pH	10wk	RosBREEDSNP_SNP_CT_12159735_Lg15_00397_MAF30_1653995_exon1	ss475883854	4031	15	15	41.671	11770634	14595292
1488	Titratable Acidity	10wk	RosBREEDSNP_SNP_CA_506758_Lg9_01891_MAF40_MDP0000309381_exon3	ss475879242	3882	9	9	0.188	509533	466032
283	Titratable Acidity	10wk	RosBREEDSNP_SNP_GA_14588942_Lg2_00987_MAF30_1674484_exon1	ss475877223	5546	2	2	26.798	13609915	12868327
2286	Titratable Acidity	10wk	RosBREEDSNP_SNP_AG_5611802_Lg13_RosCOS1918_MAF40_668899_exon1	ss475880740	3280	13	13	18.017	5623016	6124256
2821	Titratable Acidity	10wk	RosBREEDSNP_SNP_TC_1571002_Lg16_01734_MAF40_216397_exon1	ss475883754	7364	16	16	8.481	1571002	3439690
2642	Titratable Acidity	10wk	GDsnp00796	ss475882511	291	15	15	34.304	8352607	NA

Appendix 8. Flanking sequences for the QTL included in model fitting fixed effects for Dataset 2.

Sort Order ID	Flanking Sequence
	CTCATACCATCTCTGCAACTGCAACTGTCAAAGTTTAAACGCAGCGCTGTTGCTCTTTCCT[T/C]
604	CTTTGCTTCCATGGAAAATTGCACAATTACCACATGAATTTTCCACTTTTGTGGTAATTAC
	CTGGTCCAAGAAACTAGAACGTATTCTGTGGATTAACTCTCCTCCGCGGCTCTCGCCTGTA[A/G]
3011	TATGGAGTTTCGTCTATTGTTCTTCTAACGTAATCTATGAACGGCTGAGGTGTTCGAGGAG
	TTCTGTTCTCAGTTAACGAATTGTTCGAAATTGATAGCTATTGTGATGTGCAGCTGAATGC[A/G]
108	GCATACGATGAGCTAACTTCCAAGAGACCTTGCGGGCCAAATAAGAGGGCAATACGCGGAG
	TAGCCTGCCCTGGGTTACAGCGCAAATGAACCTTGGGGCTGTGGAAGTCTTCTACTTTTCC[A/G]
2698	TAGCTTTCGATGTGCCAATTCTTGATTGTTGGGTGGTATTCAGCAACTTCAGAGCAAACAC
	TCGAACACGACCTCCGTCTGGCGCTTGCAGTCGGAGCAATAGGCATCGGACATTTTTAGGT[A/C]
2435	TAAATAATTACACAGTAATCTTATCGGGAAAATGGTGTTTGGGAGGTGAGAAAATTGGAAG
	AAGTTGAGGGTTTGTGCGAGTGCGAAAAATGAGGGTGTTGATGCAGCTGAGGAGGAGAGTA[A/G]
1554	AGGGGAGAGTACTATGCCTGAGAGGTTCAGGTACTTGACCAAGGAAGCTCCTGACCCTCCT
	ATGTCCTTGCTCTTCTTGGAAGGTTCGACAACGCTCGATTTCTTGCGGCGGAGGATGGTGT[T/C]
2941	GCTGGTGGCAACCGCCAACTTTGATTCCCCGCAACCCATTTTTCAATATTTTGTGTGTTGT
	CCCATACATGCCGGAAGCTTAAAGCTCGAGCCGAGCCATGCTGATGATTCCCTCTTCTCTC[T/G]
344	CTTTTAAACCCTGTAATCTCTCTCTCTCTATGTATGTATGTATGTATGTATGTATGTAGAA
	CAAAATTGTAATAAATTAAGAATATTATATTTTTGGAGACTCTCATATGAGCAAGCATCAA[A/G]
2569	CATGGACATCCTGAAATTTTTGAATACAAATAATAAGAATAATTAAGTCTTGAACTCGATG
	AAGAAATGCTATATGATTGCTGCAAGGGCACTTTCGATCGTTTGGGGCGACACTCCTCAATACTGGG
	AATGGATTTCTCTCCCCGACTCGAGGTCTCAAA[T/C]TTCGTTCTTCTTCCTTTCCAAGGAATTGTTATC
2229	TTATTAGCATTCCAAAATAGTCATTTTTTCTACACCCATTCCTCGGAATTGAGGATTGCAGATGATT
	TTATCATCTTTGCCGCTCAGTCGCCTTTTCCTGTTCCTCTTTCTCTCTCTGCTCACTGAGT[A/G]ACCGAG
2858	CAAATGGATAAATTCATCAACCGCGTTGATATTAACCACAATATGTGTGAATTAA
	ACCTTGTGAGTAGTCTGTCTTGAGATGAAGCGGATAGGAGTCCCCTGTTCTCTTCAGCCCT[T/C]CTTA
3042	ACAGGTACGGCAGCACCAACTGTATCGGTCCGAATGGCATGTACTTGCTTACTTGAA
	GGATAGTGGGTAGCATGATCTTCGCCACCGTGGAAGCAGTCGGGTTACAGTAGATGGGCAC[A/G]TC
2856	GTCATCTTCTTCTTCGCCCATGTCAGCGAGGTCGGCAGCAGTGCTCGAAACGACGTCCT
	TATGCTAATTAATATATATTCGTTTAAAAATTTGCTATAAATATTATTGTAAGAGCAAGAG[T/C
413	]ATAAGACACTAGTACGACAGGAGTCAAACAAACCTAGAAATTATCTCCAACAATGGCTACA
	GATTCAGAAGGATTTCACTTTGGCTGCTAGCATTATTCTCTTGCACTTCCACGACTGCGTT[A/G]
941	TGAGAGTAAGCATGCATTCGCGTTAACTTTACTTACGGTTAATAGAACAAAGGCTTAGCAT

Appendix 8 (con't). Flanking sequences for the QTL included in model fitting fixed effects for Dataset 2.

Sort Order ID	Flanking Sequence
	TCGTAGCGACACGGGCTGTGGAGTCGGGTGTGGTTACATGGGTTCGGGTCGGGTGGGGGGA[T/C]AAGCT
2821	TGCAGGCAGAGTCGTAGCAGTGGTAGGGCGAGAATGTAGAGGAGTGGCGGGCGAGG
	TCAATTCTCCTCCTGTGTCCACCGACCACTGCGTTGAAGAAATTTGGTTTGGGACTGTATC[A/G]GTCTTCTTG
2640	ATATGCAAACTGTTCATGGAAGCAGGAGCGGCAGCAGCAACAAGAGGAGCGC
	TCTTTTGGACAATAGCAAAGACAGAGACATCACAGGCCATCTGATGTCAAACCTAATTTAC[T/G]TCTCTAGA
2455	GAAAAAAGCAGGACATCACCCCACCATTTCAAAGCTGGTGCACTTAATGCCCT
	TAACCATTTCAGAAACAATACTATCGTACACACCTCAATATTTGTTGAAACCTTAACGACT[T/G]CATCTGCGCC
81	AAGACTCTTTGCAATCAACAAACGCTCGTCATTCACATCCGCAATGACAAT
	AGAGAAAGCAGCAGAGAAAGAAGAGGAAGATGCGGGACTGTTCCATCAACAACAACACTAG[T/C]TGTTCTT
1890	CGTTAACGAGCCTTCCCACCGAAATTCTTATCGACATCCTCTTTAGGCTACCCG
	TGATACGGATCGTCCATTTGAATGGCTACGTAGAAGAAATCTCACATCCAGTCACTGCTGG[T/C]GAGATCTTG
484	GAAGCAAACCCTAATCATGTTCTCAGCAAACCCAGCTCCCAAGGCGTCGTAC
	TTGAGGGCGGCGACCATGATGCAGTGCTCCTGCTCCTGCTGTATCCGACTCATACGGGAGG[A/G]CATTTTGG
298	TCCAATCAGTCTTCGCTCCAGGGATAGTTTCGGAAAAATACACCGAAAATCGA
	ACCTTGTGAGTAGTCTGTCTTGAGATGAAGCGGATAGGAGTCCCCTGTTCTCTTCAGCCCT[T/C]CTTAACAGG
3042	TACGGCAGCACCAACTGTATCGGTCCGAATGGCATGTACTTGCTTACTTGAA
	CCTGTGGAAACCCAATATCAAAGCGAATCAGAACCGACCGAAATGCCAAGATTTGAGAATT[T/C]GAGGAGAG
1687	AAATTGGGATAATTAGGGGAGTGGGTTTTCCTTGGGGAATTCTCGCTTTTCTT
	CACCGATCAGGATATCCAGAACTACCAATCTATCACTTTCAGCCACCTTCCCATCAACTTC[A/C]TCCACTTGCCA
1454	TGTTTGAGACTAAAAGTCAATTAAAGAGAACACCTGCATTGTGGGGTCCG
	GAAATGTTGTACTTGTCAAAGTCATGAATCATGCCTGAACTGCCCAATTGATAATGGGTTGAAATGGGCTGCACG
	TGCAATGAGATTTGTATCTACATCA[T/C]ATGGGCTAATGGGAGGCTTGAACTGACCATTTGATTCGACTGGTTAA
58	ACTGTTTTTACTCAAGTTTTTCTCTTTATGTTAAAAGAACTTTGTATCTACTT
	TCAATTCTCCTCCTGTGTCCACCGACCACTGCGTTGAAGAAATTTGGTTTGGGACTGTATC[A/G]GTCTTCTTGATA
2640	TGCAAACTGTTCATGGAAGCAGGAGCGGCAGCAGCAACAAGAGGAGCGC
	CCGCTAACAAAATAAGAGTAATAGCTGCAAAAGTGAACTGAAAGTAGACGAGTGATGCCAT[T/G]GGGTAAAAT
742	GGCTCAACCGTTTGAGTCTCAATCGATCCATCGTCACGAAAGTGAGTACTTT
	TCCAAATCCAACAGATAGAATCCAAAAACCCTTATCCCTTACTAGAAAACCCACCTATGCC[T/C]AATCTTCCT
97	TTTTTTACTTGAAAACCCTAAAAGCTAAAAACCCAGAAATATTAGATCCAAC
	CTTCAGCATAAGGTGCCCAGATTATTATGAACTCTTGACTTCCCATACGCAAANNAAATTATCAATTACTATAA
	CAGCATAAAAAATCAGAGTTGTATAC[A/G]TATAAAAACACAGATGGCATTTTCAATGACCGAATCAAGTTCCA
518	TAAATTGAAAAGGTAAACTATACGATATTTTAATGGTGAGCAATCACGAGAAGCAT

Appendix 8 (con't). Flanking sequences for the QTL included in model fitting fixed effects for Dataset 2.

Sort Order ID	Flanking Sequence
	TTTCTTCCAAAAGTGTCATGGTTTTGTACCAGCACCAAGCACCAAAGCCACCTCCATGAAC[T/G]AGGATA
2271	AAATGATTGGTTTCCAGTTCATCAGTCTTCACGTCCTACAAACACAAAAATTGAC
	ACAAGGCGACCAGAGCGATGATTAAATTGCACATAACTATGGTAGTGGTACTGATCCGAGT[A/C]GGCCC
2925	CTTCTTCTTCTTGCTCTGAATTAACAGCGCCTGGGGCTTTATCCGCGCCATGAGCT
	GATTCAGAAGGATTTCACTTTGGCTGCTAGCATTATTCTCTTGCACTTCCACGACTGCGTT[A/G]TGAGAGT
941	AAGCATGCATTCGCGTTAACTTTACTTACGGTTAATAGAACAAAGGCTTAGCAT
	GCAACCGTCACTCACGGCGCACCTTAGATGCCCCCGGCAAACAGTTGCAGCATTGGCTGAG[T/C]CAGTGA
2512	GTGAGCGAGTCAATCGATTAGATGGGTTCGAAAGAGAACTACGGAATCGAGCTTC
	GAAAAAACTAAATACAAATAATTATAAATTAATATTAAAAAATACACAGTAATTTACGACC[A/G]AGAACTC
1300	AGTCCACACGCGGGAAATCGGATAATTCTACACGGGTTTTAGGCGACGGAATAC
	GAGGGAGTCTGAGAGTGAACTCCTCTGAAACTTGAAAGCAGAGATCCCAATTCGCAAATAC[A/G]CAGAAC
2683	CCAAAATCCGTGTCTAAAAATGTCAACAGAGAGAGAAAGGCACGCTTCGCTGCCG
	GCAAGAGTAAGGTCCAAGTCTTCAACGATCCCAGATTCAGCAGCTGATGAATATCCTGTCT[A/G]CAGCAGA
2213	TCATTGTGATTAATGTTTATCAAGATTAAAACCGGAAGATGATTTTTGCACGTC
	TCGCCGTTTTGGTCTCCTTAGCGTCACTCACAACGATCCCGTCAGATCTCTCCGATGGCAT[A/C]TCGGGTAA
2812	TTCGCCGATTTTGTATCTGTTTAGGGTTTCAAATTATGCCATGTTTTAAGTAT
	TCAAGCTTTGGGTAAACGAGGTCGCTGATATCAATCTTGGTCTCGCCGTAGGAGAAGACGA[A/C]AGGGAA
451	CGAGACGCCTCTTTTGGCCTCCATCATCTCTACTATCTCCCGTCTTTGGCGAGAT
	TCCAAATCCAACAGATAGAATCCAAAAACCCTTATCCCTTACTAGAAAACCCACCTATGCC[T/C]AATCTTCCTT
97	TTTTTACTTGAAAACCCTAAAAGCTAAAAACCCAGAAATATTAGATCCAAC
	TGCACAGACTGGAATGGCACGAGTGCACAAGGTAGAGATGAGAATGGAAGACAATGATGGT[A/G]CATTTTC
801	TGACTACATTAACCGCACGAAATTCAAGATCAGTGCTACGACCTCCAATGCTGG
	TAACCAGCTGAATTGAATAACGGTCACCAAGAGTTCCAGTCAGAGAGAGATGTGAGCTTTA[T/C]GAAGACGA
2017	CTCTGCAACTGCAATTGCAAACCCGCAAATGAAACGTTGGGAAATATGCTTTG
	GATCATATCATTCAAACCACAAGCATATCATTTTAGAAAAAGTCGTTATGCTGAAAGCGCT[A/G]TACATGCAT
1377	GTTATGACAATTGAGAAAGATTTTGAAACGCGCGAGTCCAAACATTTGGTTA
	GTAAGAGAAACCCCTATTGTGCAGTAAATCGGCACATTAATTACATCAATTATAATCAATT[A/G]TTATGATCA
2685	GTTACTAATAAACGCGTAGCTAGCTAGATCTACCGCTTTGAAGTTAATATCG
	TCGTAGCGACACGGGCTGTGGAGTCGGGTGTGGTTACATGGGTTCGGGTCGGGTGGGGGGA[T/C]AAGCTTG
2821	CAGGCAGAGTCGTAGCAGTGGTAGGGCGAGAATGTAGAGGAGTGGCGGGCGAGG

Appendix 8 (con't). Flanking sequences for the QTL included in model fitting fixed effects for Dataset 2.

Sort Order ID	Flanking Sequence
	ATAAATAAAATAAAACTGAAATAACGGAATGAAAAATAAAATTCCTCTTATAAAAAGAAAT[T/C]CATAATT
2655	CTTTACAAGGCTAAAAATAAATAAATACAATACTAGCCTCACATCTAATTCGTT
	CCAGTTGGAGACGTGTAAGTAGGACTTAATTCTTTTCGTTTGCTTGCCCTTCGATAACGCA[A/C]CCCTAATTT
1488	TTCCTCGTGATCTCCCTTTGCTCTCTTTTTTCACTAATTCATCATCCTCTGA
	TTTTTACTGTATTCTGAAAATGTCCATGTTATCTGGCACTGCCATGAGTTGCAAACATCGT[A/G]TTCTTCCTGT
283	GCAATGATGAACGTAATAAGAACACAAGAACAAGGGACAAGGTTATAATTC
	GATCGGTGCCTTTCGCGTTTTGAAGCCTGAATTACCACACAGCTAAGCATTAGTATTACAG[A/G]CCGATAAC
2286	AGAAGTTTGTCGATACAAACAGATGAGAAGGGTTGTTCAACTTCTACATACAT
	TCGTAGCGACACGGGCTGTGGAGTCGGGTGTGGTTACATGGGTTCGGGTCGGGTGGGGGGA[T/C]AAGCTT
2821	GCAGGCAGAGTCGTAGCAGTGGTAGGGCGAGAATGTAGAGGAGTGGCGGGCGAGG
	CAATGGGGTGTATGAAATAGAAAAATTACAACCAATCTGGAACTACGCAGAGGCCTTCTGCTGAAGTGTGTAT
	TATTTCGATCGATGTTCAAAATCCCGT[T/C]GGCCAAACCAAGTTCCAACCAGCAGTAGACAAAGCAGGAAGTA
2642	GCTGGTAACTGTCATCAGAAAATTCCTGTTACCGAAAGCTTGAGGAAACAATCGAAA