The Genetic Dissection of Fruit Texture Traits in the Apple Cultivar Honeycrisp

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Dedication

This dissertation is dedicated to the memory of Lara-Karena Bitenieks Kellogg (1968-2007), a continuing source of inspiration and perspective.

Abstract

The commercially successful cultivar Honeycrisp, released by the University of Minnesota in 1991, is known for its high degrees of crispness and juiciness. This cultivar has been incorporated into numerous breeding programs in an effort to duplicate its desirable texture traits in conjunction with such other traits as disease resistance and improved tree vigor. This study characterizes several apple fruit texture traits within a large breeding population over several years, combining the established protocols of incomplete block design, sensory evaluation panels, and best linear unbiased prediction. Five full-sib families, all of which share 'Honeycrisp' as a common parent, were assayed using a variety of molecular markers, and genetic maps were constructed for each of the five families. The five genetic maps were aligned to produce a consensus genetic map for 'Honeycrisp'. Predicted genotype values from each of the five families were coupled with the corresponding molecular data and the genetic maps to identify quantitative trait loci (QTLs) for each family-by-year combination, which were compared relative to the consensus genetic map. Several intervals were identified within the map over which QTLs for multiple families and multiple years were collocated, reflecting consistent and robust QTLs. Results are largely in accordance with previous studies of other apple cultivars with notable exceptions, which are discussed in the context of the recently published apple genome sequence.

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1 Introduction

Domesticated apple (*Malus pumila* Mill. (Rosaceae), synonymous with *Malus* x *domestica* Borkh.; Mabberley *et al.* 2001, Velasco *et al.* 2010) is among the most commercially important temperate tree fruits in the world, with more than 62 million metric tons produced globally in 2005 (FAOSTAT 2005). A large number of globally important apple cultivars (e.g., 'Delicious', 'Golden Delicious', 'Granny Smith') are "found" genotypes or the products of open-pollinated seed, often dating to the 19th century. In contrast, a small number of more recent, successful introductions (e.g., 'Fuji', 'Gala', 'Cripps Pink', 'Honeycrisp') are the products of breeding programs.

Numerous aspects of apple biology, however, hinder the rapid development and adoption of new cultivars. Apple is highly heterozygous, typically self-incompatible, and suffers substantially from inbreeding depression (e.g., Broothaerts *et al.* 2004, Vanwynsberghe *et al.* 2005). Additionally, an apple seedling does not produce fruit for five to ten years in quantities sufficient for preliminary evaluation. The identification of markers corresponding to traits of interest would enable the molecular screening of many genotypes at the small seedling stage, before large amounts of time, land, and money have been allocated to bringing those seedlings to maturity (e.g., Luby and Shaw 2001, Liebhard *et al.* 2003b, Tartarini and Sansavini 2003). Consequently, apple has been identified as a promising candidate for the application of marker-assisted breeding (MAB) and selection (MAS; e.g., Kellerhals *et al.* 2000a,b, Frey *et al.* 2004, Patocchi *et al.* 2009ab).

The development of a successful MAS program entails several components.

First, the phenotypic traits of interest must be well characterized and the heritability of said traits must be estimated in order to assess the feasibility of MAS. Second, populations of interest must be characterized with respect to molecular markers. Third, the relationships between the phenotypic traits and the molecular traits must be assessed, allowing for the identification of molecular markers correlated with the phenotypic traits of interest. Only then can markers correlated with phenotypic traits of interest be identified for screening of future populations (e.g., Dirlewanger *et al.* 2004, Gardiner *et al.* 2007, Patocchi *et al.* 2009ab).

The commercially successful cultivar Honeycrisp, released by the University of Minnesota in 1991, is known for its high degrees of crispness and juiciness, as well as for its long storage capacity (e.g., Janick *et al.* 1996, Allan-Wojtas *et al.* 2003, Mann *et al.* 2005). This cultivar has been reported to command the highest wholesale price of all apple cultivars marketed in the United States (Hanson 2005) and has thus been incorporated into numerous breeding programs in an effort to duplicate its desirable texture traits in conjunction with such other traits as disease resistance and improved tree vigor. Several thousand seedlings have been created at the University of Minnesota by crossing 'Honeycrisp' with numerous other genotypes. These populations provide an opportunity to characterize several full-sib families with 'Honeycrisp' as a common parent and to identify molecular markers from this highly desirable cultivar for inclusion in future marker-assisted selection programs.

2 Literature Review

2.1 Characterization of Apple Fruit Texture Traits

Fruit texture traits, such as crispness, firmness, juiciness, and mealiness, are cited as primary components of consumer choice when purchasing fresh fruit (Péneau *et al.* 2006), so improved texture characteristics are central objectives in apple breeding programs worldwide. Like other quantitative traits, fruit texture traits are assumed to be under multigenic control (e.g., Soglio *et al.* 2009), further impeding rapid cultivar development. MAS provides the opportunity to identify genotypes containing desirable alleles prior to phenotypic expression, allowing for screening and removal of undesirable genotypes at early stages of cultivar development (Knapp 1998). The benefits of MAS are greatest when trait characterization is difficult or expensive or when heritability is low, otherwise phenotypic selection would be adequate (Knapp 1998, Luby and Shaw 2001). Consequently, the precise characterization of the traits of interest and the estimation of the corresponding heritability are necessary before potential markers and their benefits in an MAS program can be assessed.

The complex nature of fruit texture traits and the importance of consumer perception of these traits result in sensory evaluations being utilized in conjunction with instrumental studies (e.g., Mohamed *et al.* 1982, Barreiro *et al.* 1998, Szczesniak and Ilker 1988, Karlsen *et al.* 1999, Allan-Wojtas *et al.* 2003, Liebhard *et al.* 2003a, Mehinagic *et al.* 2004, Oraguzie *et al.* 2009, Chauvin *et al.* 2010). Sensory panels have been employed to characterize the texture traits of various foods (e.g., Vickers and Christensen 1980, Fillion and Kilcast 2002) and to dissect the characteristics of a small

number of apple genotypes, usually named cultivars (e.g., Dever *et al.* 1995, Daillant-Spinnler *et al.* 1996, Karlsen *et al.* 1999, Harker *et al.* 2002, Varela *et al.* 2005). Sensory panels are also incorporated in later stages of fruit breeding programs to estimate such genetic parameters as heritability, combining ability, and variance components (e.g., Oraguzie *et al.* 2001b, Alspach and Oraguzie 2002, Liebhard *et al.* 2003a, Velasco *et al.* 2010) or to assess consumer preferences within advanced selections (Hampson *et al.* 2000). In each of these circumstances, however, all panelists typically evaluate all genotypes, restricting the number of samples that can be evaluated in a single study. Furthermore, this method requires numerous samples for each genotype, a condition that typically cannot be met in the first several years of fruit production, especially in the early stages of a breeding program when genotypes are represented by single trees.

Fruit quality is a complex commercial trait for apple and its close relative pear, consisting of a variety of characteristics such as flavor, aroma, appearance, texture, and shape, each of which is a complex trait in its own right. Texture traits have been classified by Jowitt (1974) as falling into three broad categories relating to "the behaviour of the material under stress or strain, … relating to the structure of the material, … [and] relating to 'mouthfeel' characteristics," a number of which can be experienced when eating apple fruits. Among the first category are such characteristics as firmness, crispness, and chewiness, whereas the second category encompasses such traits as mealiness, grittiness, and coarseness. The third category, which is often not thought of as describing texture, contains such traits as juiciness, greasiness, and mushiness. Despite these many components of texture traits, it is not unusual to encounter publications in

which vague or undefined “texture” is discussed (Bell and Janick 1990, Durel *et al.* 1998, King *et al.* 2000).

2.1.1 Physiology and Genetics of Apple Fruit Texture

Apple fruit texture changes throughout the fruit development and ripening process. Changes during fruit ripening and the immediate pre-ripening stages are of substantial commercial interest, because consumer purchasing decisions are largely dependent upon perceived texture (Péneau *et al.* 2006). Unfortunately for growers, however, the ripening process does not cease upon harvest of apple fruit. Consequently, a detailed understanding of the physiological changes occurring during ripening, and their roles with respect to texture traits, is a valuable tool for determining when to harvest highly perishable fruit like apples.

Harker *et al.* (1997, 2002) and Allan-Wojtas *et al*. (2003) described three forms of tissue failure. The first form is cell fracture in which cells break near the equator, observed in very firm apples and first described for unripe avocado and carrot. The second form is called cell rupture, in which cells fracture in a small region of the cell, as in ripe watermelon and apples of intermediate firmness. The third form is cell-to-cell debonding, in which adjacent cells are pulled apart from each other at the middle lamella without significant cell fracture, as in soft apples, ripe muskmelon and ripe banana.

The softening of the cortex tissues is the principle textural change that occurs during the apple fruit maturation process. This softening is closely associated with increased rates of respiration and ethylene production, a condition termed climacteric

(e.g., Alexander and Grierson 2002). This relationship is exploited by the suppression of ethylene production, and hence the extension of storage life, in controlled atmospheres (e.g., Jobling and McGlasson 1995, Johnston *et al.* 2002b). Some apple cultivars, however, do not soften for extended periods (e.g., Luby and Bedford 1992, Wakasa *et al.* 2006). Further investigations have demonstrated that not all apple cultivars produce elevated levels of ethylene and some cultivars do not respond to elevated levels of ethylene synthesis with the usual rapid softening (e.g., Oraguzie *et al.* 2000, Johnston *et al.* 2002b, Harada *et al.* 2000, Costa *et al.* 2005). This has instigated research into the underlying genetics of variable ethylene production and response.

The biosynthesis of ethylene occurs via a rather short pathway in which the methionine-derived compound S-adenosyl-L-methionine (AdoMet or SAM) is converted to 1-aminocyclopropane-1-carboxylic acid (ACC), which is in turn converted to ethylene (e.g., Harada *et al.* 2000, Giovannoni 2004, Oraguzie *et al.* 2004, Costa *et al.* 2005). These two reactions are controlled by the families of ACC synthase (ACS) enzymes and ACC oxidase (ACO) enzyme, respectively. Additionally, the ACS by-product 5'-methylthioadenosine is salvaged, leading to the resynthesis of methionine, the AdoMet precursor, thus allowing for ethylene biosynthesis to occur again (Alexander and Grierson 2002). Examining polymorphic alleles of the apple ACC synthase gene *Md-ACS1* and the ACC oxidase gene *Md-ACO1*, Harada *et al.* (2000) and Costa *et al.* (2005), respectively, determined that allelic variation of these genes seem to be largely accountable for reduced levels of ethylene production in apple fruit, a conclusion that was supported by post-transcriptional gene silencing (e.g., Dandekar *et al.* 2004). Oraguzie *et*

al. (2004) determined that *Md-ACS1* is inherited in a Mendelian fashion. Multiple ACO and ACS genes have been identified, and numerous cultivars have subsequently been characterized with respect to ACS, ACO, or both (Oraguzie *et al.* 2007, Zhu and Barritt 2008). Additionally, Nybom *et al.* (2008) have reported a substantial shift of allelic ratios for *MD-ACS1* in apple cultivars introduced in the 20th century, relative to older cultivars, presumably due to selection pressure for apple texture traits.

Additional genes are involved in fruit softening; primary among them are expansin genes and polygalacturonase genes associated with the enzymatic disassembly of cell walls and the middle lamella (e.g., Brummel and Harpster 2001, Wakasa *et al.* 2003, 2006, Costa *et al.* 2008, 2010, Mann *et al.* 2008). A variety of genes have also been identified that interact with various aspects of ethylene-regulated fruit softening (Tatsuki *et al.* 2007, Wang *et al.* 2007, Li *et al.* 2010) and cold-regulated fruit softening (Tacken *et al.* 2010). Cevik *et al.* (2009) identified candidate genes from Arabidopsis related to fruit development and found a significant relationship between these genes and apple fruit firmness. Undoubtedly, the recent release of the apple genome sequence (Velasco *et al.* 2010) will only enhance the feasibility of such studies.

Reduced cell turgor can result in perceived softening of apple tissue as cell walls are more likely to tear than to rupture (Johnston *et al.* 2002a, Tong *et al.* 1999, Iwanami *et al.* 2008). The relationship between turgor and perceived juiciness, however, is less clear (e.g., Szczesniak and Ilker 1988). Allan-Wojtas *et al.* (2003) demonstrated that, all other factors being equal, juicy apple "samples were composed of large cells with thin, brittle cell walls." In fact, Mann *et al.* (2005) found cell size to be the only statistically

significant predictor of perceived juiciness. Cell wall strength, however, is likely to affect perceived juiciness; if cell walls are stronger than middle lamellae, tissue failure between cells, rather than cell rupture, will occur and juice will not be released (Boulton *et al.* 1997). Consequently, juiciness is likely to be directly influenced by many of the same physical characteristics that determine crispness and firmness.

2.1.2 Sensory Evaluation of Apple Fruit Texture Traits

The sensory perception of texture is an important commercial trait for apple, yet the complexity of texture makes it a difficult trait to quantify. The characterization of texture is neither simple nor standardized, and, consequently, the genetic characterization of texture traits is not well-established. Many different studies define individual components of texture, such as crispness, firmness, hardness, crunchiness, chewiness, mushiness, crumbliness, and mealiness. In contrast, it is not unusual for studies to simply refer to an undefined, general "texture" (e.g., Bell and Janick 1990, Durel *et al.* 1998).

Focusing on the trait of crispness, numerous different, and even contradictory, definitions are common. Several studies have defined crispness with respect to the rupturing of tissue when bit with the incisors, reserving chewing with the molars for the evaluation of firmness, a generalization of hardness and softness (Jowitt 1974, Barrett *et al.* 1994, Harker *et al.* 2002, Allan-Wojtas *et al.* 2003). Mehinagic *et al.* (2003, 2006) evaluated crunchiness, using the term crispness interchangeably, and chewiness in a corresponding manner. Conversely, Karlsen *et al.* (1999) and King *et al.* (2000, 2001) relate crispness to chewing and hardness to biting. These examples illustrate that the

sensation of crispness is not uniformly accepted.

Different researchers, even when they agree about the sensation, often disagree about the underlying mechanism. Alspach and Oraguzie (2002) and Harker *et al.* (2002) differentiate crispness and firmness as relating to the sound and force, respectively, of biting, whereas Barrett *et al.* (1994) restrict their definition of crispness to force of biting. In contrast, Seymour and Hamman (1988) define crispness with respect to sound of biting, reserving hardness to represent the "force required to bite through [a] sample." Hoehn *et al.* (2003), however, combined firmness and crispness into a single parameter due to their preliminary studies, which indicated that sensory panelists could not differentiate between the two sensations.

Sensory evaluations by trained experts, although often expensive, are fairly standard, but even with trained panelists, terminology must be explicitly defined or there is no guarantee that the same concept is being measured (Roudaut *et al.* 2002). The culture- and language-dependent nature of the terminology only confuses things further (Roudaut *et al.* 2002). For example, "croustillant" is the French word closest to the English word "crisp," yet it is not applied to fruits or vegetables, but, being derived from the same source as "crusty," is applied to cereal products (Dacremont 1995, Roudaut *et al.* 2002, Giboreau *et al.* 2007). In fact, Deletre and Roudot (2003) propose the term "croustillance sèche", loosely translated as "dry crustiness," as the French equivalent for crispness, yet they list lettuce and celery is illustrations of the trait. Edmister and Vickers (1985) divided crisp foods into "wet crisp" and "dry crisp," reflecting the earlier distinction between "crisp" and "brittle" by Jowitt (1974). Nishinari *et al.* (2008)

identified finer distinctions in Japanese terminology specifically associated with apple texture: karikari ("crisp, sound emitted by biting hard foods"), sakusaku ("short, easily broken by biting"), and shakishaki ("sound emitted by biting hard foods"). In contrast, Varela *et al.* (2008) observed that more than 50% of consumers surveyed in Spain did not differentiate between crispy and crunchy. These studies illustrate the necessity of researchers clearly stating the definitions and protocols used in food texture studies (e.g., Giboreau *et al.* 2007).

Some studies have investigated the inheritance patterns of sensory measures of pome fruits (primarily apple and pear, subtribe Pyrinae, tribe Pyreae, synonymous with subfamily Maloideae or Pomoideae; Potter *et al.* 2007) in the absence of physical measures (e.g., Alspach and Oraguzie 2002, White *et al.* 2000b), whereas others have examined the inheritance patterns of physical measures of apple fruit texture in the absence of any sensory evaluation (e.g., Johnston *et al.* 2001b, Liebhard *et al.* 2003a, Oraguzie *et al.* 2001b). The past several years have witnessed a number of studies, however, in which the researchers have investigated the relationships between various physical and sensory values (Karlsen *et al.* 1999, Tong *et al.* 1999, King *et al.* 2000, 2001, Harker *et al.* 1997, 2002, Allan-Wojtas *et al.* 2003, Hoehn *et al.* 2003, Mehinagic *et al.* 2003, 2004, Mann *et al.* 2005).

2.1.3 Instrumental Evaluation of Apple Fruit Texture Traits

As with sensory measures of texture, physical measurements of texture traits are not straightforward. As just described, various authors have described crispness with

respect to sound or force during biting or chewing, once again differentiating between incisors and molars. Consequently, a variety of different mechanical devices have been employed to measure these various traits. Puncture tests have been widely applied to estimate firmness (e.g., Liebhard *et al.* 2003a). Originally designed for analyzing foamed products, penetrometers are typically used for puncture tests, in which a small probe is plunged into a sample at a slow and constant speed, measuring the force per distance required to fracture the tissue (e.g., Roudaut *et al.* 2002). In fact, penetrometer tests are widely used in studies on postharvest softening in apple (Jobling and McGlasson 1995, Johnston *et al.* 2002b, Oraguzie *et al.* 2004, Pre-Aymard *et al.* 2005, Costa *et al.* 2005, Wakasa *et al.* 2006).

Another physical test applied to the study of fruit texture is the triple-beam snapping test, a tensile measurement test (Bourne 1982). In snapping tests, uniform samples of tissue are suspended across two beams and a blunt blade descends at a constant rate. The force required to maintain this constant rate is recorded, capturing the changes in force associated with tissue failure (e.g., Vickers and Christensen 1980, Vincent 1998, Karlsen *et al.* 1999, Tong *et al.* 1999, Alvarez *et al.* 2000, Johnston *et al.* 2001, Vincent *et al.* 2002, Hoehn *et al.* 2003, Mann *et al.* 2005, Harker *et al.* 2006).

Data collected from either penetrometers or snapping tests can be used to generate force-deformation curves that relate the force (typically in Newtons) relative to the displacement (in millimeters). Various characteristics of these curves can be extracted, relating to firmness and fracture behavior (e.g., Vincent 1998, Camps *et al.* 2005, Duizer and Winger 2006). For example, Vincent (1998) defines crispness ranking as "the ratio

between total force drop during fracture and the total length of the fracture path," namely vertical drop versus horizontal displacement in a force curve, whereas firmness is described by the maximum force measured. In contrast, Mann *et al*. (2005) found total work to be the best predictor of sensory crispness.

A number of different physical texture traits have been measured instrumentally and related to several sensory traits (e.g., Vickers and Christensen 1980, King *et al.* 2000, 2001, Harker *et al.* 2002, Hoehn *et al.* 2003, Mehinagic *et al.* 2003, 2004, Mann *et al.* 2005). Tensile and puncture test data modeled sensory firmness and crispness fairly consistently (e.g., Vickers and Christensen 1980, King *et al.* 2000, Harker *et al.* 2002, Mehinagic *et al.* 2003, Mann *et al.* 2005), but to varying degrees between studies. Mann *et al.* (2005) found that cell number and cell size also contributed significantly to sensory crispness models, whereas Vickers and Christensen (1980) found that loudness of biting and chewing contributed to crispness models. Several studies found that force-deformation values, namely gradient of initial force and maximum force, predicted sensory juiciness well (Harker *et al.* 2002, Mehinagic *et al.* 2003, 2004), whereas Mann *et al.* (2005) found no such significant relationship, but rather that cell size was the only significant predictor of sensory juiciness. Interestingly, Harker *et al.* (2002) found that although both puncture and tensile tests yielded significant predictors for both crispness and firmness, they also found that the rankings of the correlations reversed in consecutive years.

2.1.4 Heritability of Apple Fruit Texture Traits

The benefits of MAS are greatest when trait characterization is difficult or expensive, such as in trees with long juvenility periods, or when heritability is low, because phenotypic selection would be adequate otherwise (Knapp 1998, Luby and Shaw 2001). Specifically, since most quantitative traits are oligogenic or polygenic in origin, MAS has the potential of identifying genotypes in which numerous beneficial alleles are present, yet which do not express superior phenotypes, because they are lacking a few members of the optimal genetic suite (Knapp 1998). Consequently, the heritability of the traits of interest must be estimated before the potential benefits of a marker-assisted selection program can be assessed.

Historically, analysis of variance (ANOVA) models have been constructed in order to estimate variance or covariance terms, from which heritability estimates can be calculated (e.g., Falconer and Mackay 1989, Bell and Janick 1990, Abe *et al.* 1995, Tancred *et al.* 1995, Oraguzie *et al.* 2001b, Bernardo 2002, Rumpunen and Kviklys 2003). The incorporation of multiple traits and unbalanced experimental designs, however, limit the usefulness of traditional ANOVA models. Numerous studies have thus adopted restricted maximum likelihood (ReML) (e.g., Durel *et al.* 1998, Currie *et al.* 2000, White *et al.* 2000a,b, Bus *et al.* 2002, Alspach and Oraguzie 2002).

King *et al.* (2000) present narrow-sense heritability values of 0.57 and 0.52 for crispness and hardness [firmness], respectively, but it must be noted that these are both sensory rather than physical, or instrumental, traits. Alspach and Oraguzie (2002) found that heritability of sensory texture traits, although differing between families, was

consistent across years, with firmness ranging from 0.26 to 0.59, and crispness ranging from 0.12 to 0.15. Their families, although including no clonal replicates, did contain mixed half-sib and full-sib families from open-pollination, and families were distributed across three sites. They hypothesized that their relatively low heritabilities were in part due to their lack of control regarding individual fruit ripeness, having only a single harvest, regardless of variability in ripening dates across the trees. Liebhard *et al.* (2003a) also possibly obscured inheritance patterns in texture traits by having only a single harvest date. Durel *et al.* (1998), in contrast, relied primarily on controlled crosses and utilized all known pedigree information to estimate a narrow-sense heritability of 0.33 for undefined texture, which was in turn correlated with both flavor and juiciness. Bell and Janick (1990), working with pear, estimated narrow-sense heritability of 0.30, despite their use of a general "texture" trait; they accomplished this, in part, by having multiple harvest dates within a single season and only using the ripest fruit from each tree. In addition to the necessity of comparing fruit at similar stages of ripeness, uniform storage time prior to evaluation is highly desirable as well, since fruit textural characteristics change as storage time increases (see Tong *et al.* 1999, Johnston *et al.* 2001, 2002b, Mann *et al.* 2005, Watkins *et al.* 2005).

2.2 Molecular Characterization of Apple Populations

2.2.1 Molecular Marker Availability for Apple

The genus *Malus* (apple and crabapple; Rosaceae) has been a focus of molecular studies since at least the mid-1980s, when Weeden and Lamb (1986) used isozymes as a

means of discriminating between different apple cultivars. As new marker types have been developed, apple geneticists have readily adopted them. Early studies implemented anonymous markers (e.g., Goulão *et al.* 2001b, Guilford *et al.* 1997, Hokanson *et al.* 1998, Koller *et al.* 1993, Mulcahy *et al.* 1993), but markers for genes of known or suspected function are increasingly used (e.g., Broothaerts 2003, Calenge *et al.* 2005, Silfverberg-Dilworth *et al.* 2005, 2006, Chagné *et al.* 2007, Igarashi *et al.* 2008, Sargent *et al.* 2009). Whole genome sequencing in apple promises to greatly expand the availability of markers for the genus (e.g., Sosinski *et al.* 2009).

Molecular markers have been widely applied to evolutionary and pedigree studies in apple, applied to wild *Malus* species (Dickson *et al.* 1991, Lamboy *et al.* 1996, Richards *et al.* 2009) as well as to domestic cultivars (e.g., Gardiner *et al.* 1996a, Goulão *et al.* 2001, Hokanson *et al.* 2001, Oraguzie *et al.* 2001, Cabe *et al.* 2005, Evans *et al. In press*). Additionally, markers developed for apple have been fairly widely applied to other pome species, and vice versa, notably pear (*Pyrus* spp.; Yamamoto *et al.* 2001, Hemmat *et al.* 2003), quince (*Cydonia oblonga* Mill.; Yamamoto *et al.* 2004), and mountain-ash (*Sorbus torminalis* (L.) Crantz; Oddou-Muratorio *et al.* 2001). Simultaneously, linkage groups have been constructed for, and aligned across, a number of domestic cultivars (e.g., Hemmat *et al.* 1994, Conner *et al.* 1997, Maliepaard *et al.* 1998, Liebhard *et al.* 2003a, Kenis and Keulemans 2005, N'Diaye *et al.* 2008, Fernández-Fernández *et al.* 2008, Celton *et al.* 2009a, Patocchi *et al.* 2009ab). The successful transferability of molecular markers across species, and even genera, has provided insights into the frequency of chromosomal synteny across species within and

beyond the Rosaceae (e.g., Dirlewanger *et al.* 2004, Sutherland *et al.* 2008, Sargent *et al.* 2009).

The number of published SSR primers developed specifically for apple has grown rapidly in recent years (e.g., Guilford *et al.* 1997, Gianfranceschi *et al.* 1998, Hokanson 1998, Liebhard *et al*. 2002, 2003b). The development of SSRs from expressed sequence tags (ESTs; e.g. Newcomb *et al*. 2006) has increased the pace of new marker development for pome fruits (e.g., Chagné *et al*. 2008, Celton *et al*. 2009, Nishitani *et al*. 2009). Numerous researchers have investigated the transferability of SSR primers developed for different species. SSR primers have been shown to be widely transferable among several genera within the pome fruits, particularly between *Malus* (apple) and *Pyrus* (pear), but also including *Amelanchier*, *Cotoneaster*, *Cydonia*, *Crataegus*, *Mespilus*, and *Sorbus* (e.g., Oddou-Muratorio *et al.* 2001, Yamamoto *et al.* 2001, 2004, Liebhard *et al.* 2002, Hemmat *et al.* 2003, Pierantoni *et al.* 2004). Consequently, SSR primers are also available from a number of species closely related to apple (Oddou-Muratorio *et al.* 2001, Yamamoto *et al.* 2002a,b,c).

The transfer of SSR primers between various pome fruits (subtribe Pyrinae) and the genus *Prunus* (almond, apricot, cherry, peach, and plum; tribe Amygdaleae) has been less successful (e.g., Liebhard *et al.* 2002, Yamamoto *et al.* 2002c). This supports the conclusions of Maliepaard *et al.* (1998) and Evans and Campbell (2002) that the pomes are more likely to be descended from a narrow hybridization event within the tribe Spiraeeae than from a wider spiraeoid-by-amygdaloid hybrid.

In addition to traditional markers such as SSRs and amplified fragment length

polymorphisms (AFLPs), primers for numerous genes of interest have been developed. For example, primers targeting polymorphisms in *Md-ACS1* and *Md-ACO1,* genes responsible for the production of enzymes in the ethylene production sequence, have been developed (Harada *et al.* 2000, Costa *et al.* 2005). *Md-ACS1* maps near the previously identified 'Fiesta' x 'Prima' linkage group 10 QTL for fruit firmness, whereas *Md-ACO1* maps to the same region of the 'Fiesta' x 'Prima' linkage group 15 as the mass spectrometric peak putatively associated with ionized ethylene (King *et al.* 2000, Maliepaard *et al.* 2001, Costa *et al.* 2005, Zini *et al.* 2005). Broothaerts (2003) has published primer sequences for numerous polymorphisms distinguishing apple *S* alleles responsible for gametophytic incompatibility.

In recent years, genomic methods have been embraced by apple researchers (e.g., Velasco *et al.* 2010). The enhanced ability to study gene expression has resulted in new understandings of developmental processes (Ban *et al.* 2007, Espley *et al.* 2007) and plant physiological responses to pathogens (Norelli 2009), as well as the further development of new molecular markers (e.g. Chagné *et al.* 2008, Igarashi *et al.* 2008). The further development of high-throughput genetic technologies and the recent publication of the apple genome sequence (Velasco *et al.* 2010) has resulted in the identification of numerous alleles potentially related to the fruit maturation process, and additional alleles will undoubtedly continue to be identified, enhancing our understanding of genetics within apple and its relatives (e.g., Shulaev *et al.* 2008, Cevik *et al.* (2009, Sosinski *et al.* 2009).

2.2.2 Molecular Marker Selection and Linkage Map Construction

The choice of molecular marker types has historically depended upon technological and financial resources. Dominant marker types, such as AFLPs and random amplified polymorphic DNA (RAPDs) have been frequently used in map construction, as their application does not require prior information regarding marker amplification within the population of interest (Welsh and McClelland 1990, Vos *et al.* 1995). Dominant markers, however, simply express presence/absence of fragments, in contrast to more informative codominant marker types, such as SSRs, that allow for allelic phenotyping (e.g., Gianfranceschi *et al.* 1998).

Codominant marker development generally requires significantly more research effort. For example, SSR marker development might require discovery of hypervariable repeat sites through library screening or analysis of genome or EST sequence, followed by design and optimization of PCR primers (e.g., Guilford *et al.* 1997, Hokanson *et al.* 1998, Gianfranceschi *et al.* 1998). In contrast, dominant markers such as RAPDs or AFLPs require no *a priori* knowledge of genome structure or sequence (e.g., Welsh and McClelland 1990, Vos *et al.* 1995). SSRs yield more reproducible results than either RAPDs or AFLPs (Goulão and Oliveira 2001) and are more easily transferable across populations and species. Consequently, fewer codominant markers are required than dominant markers in order to construct linkage maps. Whereas the earliest published apple and pear linkage maps primarily utilized dominant marker types (Hemmat *et al.* 1994, Maliepaard *et al.* 1998, Iketani *et al.* 2001), the increased availability over time of codominant markers for apple and pear has resulted in their increased use (e.g.,

Gianfranceschi *et al*. 1998, Liebhard *et al*. 2002, Hemmat *et al*. 2003, Pierantoni *et al*. 2004). The increased availability of molecular sequence data in the form of expressed sequence tags (ESTs) have enabled the development of single nucleotide polymorphism (SNP) markers (e.g., Chagné *et al*. 2008). Publication of the apple genome sequence (Velasco *et al*. 2010) will undoubtedly further enable the development of more SNPs and other codominant markers.

Most recently published apple and pear linkage maps have employed a combination of dominant and codominant markers (e.g., Yamamoto *et al*. 2002c, Liebhard *et al*. 2003b, Calenge *et al*. 2004, Dondini *et al*. 2004, James *et al*. 2004, Kenis and Keulemans 2005, Soriano *et al*. 2009,). The joint utilization of both marker categories for mapping studies is likely to increase in the near future with the development of high-throughput marker systems for both marker categories.

In linkage mapping, typically, parental DNA and a subset of offspring DNA are first screened in order to identify segregating markers prior to assaying the complete population (Grattapaglia and Sederoff 1994, Maliepaard *et al.* 1997, Lin *et al.* 2003), as well as providing a means of identifying mistaken parentages (e.g., Cabe *et al.* 2005). Markers previously mapped in other populations are increasingly applied so as to ensure optimal marker coverage for the resulting linkage groups (e.g., Howad *et al*. 2005, Chagné *et al*. 2008, Sargent *et al*. 2009).

A number of linkage maps have been constructed for members of the Rosaceae. Within the pome fruits, linkage maps have been constructed for apple (e.g., Hemmat *et al.* 1994, 2003, Maliepaard *et al.* 1998, Liebhard *et al.* 2003b, Kenis and Keulemans

2005) and for pear (e.g., Yamamoto *et al.* 2002c, Pierantoni *et al.* 2004). Linkage groups have been aligned across a number of domestic cultivars (e.g., Hemmat *et al.* 1994, Conner *et al.* 1997, Maliepaard *et al.* 1998, Liebhard *et al.* 2003a, Kenis and Keulemans 2005, N'Diaye *et al.* 2008, Fernández-Fernández *et al.* 2008, Celton *et al.* 2009a, Patocchi *et al.* 2009ab), and across multiple genera from within the Rosaceae (e.g., Dirlewanger *et al.* 2004, Pierantoni *et al.* 2004, Celton *et al.* 2009ab, Gasic *et al.* 2009, Sargent *et al.* 2009, Mnejja *et al.* 2010).

2.3 Identification of Quantitative Trait Loci in Apple

The pome fruits have been studied extensively with respect to the genetic underpinnings of numerous traits. The identification of molecular markers associated with specific genes are widely sought in commercial crops as essential for the development of marker-assisted selection and breeding programs (Gianfranceschi *et al.* 1996, King *et al.* 1999, Kellerhals *et al.* 2000a,b, Belfanti *et al.* 2004, Frey *et al.* 2004, Gardiner *et al.* 2007, Zhu and Barritt 2008, Patocchi *et al.* 2009ab).

Numerous quantitative trait loci (QTLs) have been identified and mapped in apple and pear, the majority for disease and pest resistance (Koller *et al.* 1994, Gardiner et al 1996b, Iketani *et al.* 2001, Durel *et al.* 2003, Liebhard *et al.* 2003c, Dondini *et al.* 2004, James *et al.* 2004, Calenge *et al.* 2004, 2005, Khan *et al.* 2006, Calenge and Durel 2006, Peil *et al.* 2007, Pierantoni *et al.* 2006, Moriya *et al.* 2010), growth and development characteristics (Conner *et al.* 1998, Segura *et al.* 2007, 2009, Kenis and Keulemans 2007, van Dyk *et al.* 2010), or fruit quality traits (Costa *et al.* 2005, 2008, 2010, Harada *et al.*

2000, Zhu and Barritt 2008, Zini *et al.* 2005, Davey *et al.* 2006). A few studies, however, have investigated QTLs for fruit texture traits (King *et al.* 2000, 2001, Liebhard *et al.* 2003a, Kenis *et al.* 2008).

King *et al.* (2000), working with apple, mapped QTLs for three physical measurements (two penetrometer-derived firmness values and one acoustic resonance-derived stiffness measure), as well as seven sensory measures. Among their results, they identified several putative QTLs for the sensory traits of crispness and hardness (in their study, chewing and biting, respectively). Following their first study, King et al (2001) then mapped QTLs for a larger number of physical parameters derived from tensile strength and compression tests. Upon comparison of the two sets of QTLs, the authors identified several QTLs on linkage group 16 corresponding to several sensory traits, including crispness, hardness, juiciness, and several physical measures, primarily derived from the tensile tests. In both studies, however, they identified significant genotype x environment interaction terms. Liebhard *et al.* (2003a), investigating physical texture traits, found results consistent with those of King *et al.* (2000, 2001).

Repeatability across years and robustness across populations are of primary interest if QTL-associated markers are to be used in MAB or MAS programs. Working with pear, Abe *et al.* (1995) investigated the repeatability of narrow-sense heritability with respect to fruit firmness as measured by a compression test, seeing h^2 values of 0.17, 0.14, and 0.56 in three consecutive years. Foulongne *et al.* (2003), investigating the consistency of QTLs for powdery mildew across generations and environments, identified QTLs in several independent evaluations and compared the results, finding

some that were common to the different populations. Kenis *et al.* (2008) identified QTLs for penetrometer-derived apple fruit firmness that were common across two years of observation. To date, however, theirs is the only published study to examine fruit texture QTL stability in apple.

3 Prediction of Genotypic Values for Apple Fruit Texture

3.1 Introduction

Domesticated apple (*Malus pumila* Mill. (Rosaceae), synonymous with *Malus* x *domestica* Borkh.; Mabberley *et al.* 2001, Velasco *et al.* 2010) is among the most commercially important temperate tree fruits in the world, with more than 62 million metric tons produced globally in 2005 (FAOSTAT 2005). A large number of globally important apple cultivars (e.g., 'Delicious', 'Golden Delicious', 'Granny Smith') are "found" genotypes or the products of open-pollinated seed, often dating to the 19th century. In contrast, a small number of more recent, successful introductions (e.g., 'Fuji', 'Gala', 'Cripps Pink', 'Honeycrisp') are the results of breeding programs. Numerous aspects of apple biology hinder the rapid development and adoption of new cultivars, including self-incompatibility, high heterozygosity, extended juvenility, and long generation times.

Fruit texture traits, such as crispness, firmness, juiciness, and mealiness, are cited as a primary component of consumer choice when purchasing fresh fruit (Péneau *et al.* 2006), so improved texture characteristics are central goals in apple breeding programs world-wide. The complex nature of fruit texture traits and the importance of consumer perception of these traits result in sensory evaluations being utilized in conjunction with instrumental studies (e.g., Mohamed *et al.* 1982, Barreiro *et al.* 1998, Szczesniak and Ilker 1988, Karlsen *et al.* 1999, Allan-Wojtas *et al.* 2003, Liebhard *et al.* 2003a, Mehinagic *et al.* 2004, Chauvin *et al.* 2010). Sensory panels have been used to characterize the texture traits of various foods (e.g., Vickers and Christensen 1980,

Fillion and Kilcast 2002) and for dissecting the characteristics of a small number of apple genotypes, usually named cultivars (e.g., Dever *et al.* 1995, Daillant-Spinnler *et al.* 1996, Karlsen *et al.* 1999, Harker *et al.* 2002, Varela *et al.* 2005). Sensory panels are also incorporated in later stages of fruit breeding programs to estimate such genetic parameters as heritability, combining ability, and variance components (e.g., Oraguzie *et al.* 2001b, Alspach and Oraguzie 2002, Liebhard *et al.* 2003a, Velasco *et al.* 2010) or to assess consumer preferences within advanced selections (Hampson *et al.* 2000). In each of these circumstances, however, all panelists typically evaluate all genotypes, restricting the number of samples that can be evaluated in a single study. Furthermore, this method requires numerous samples for each genotype, a condition that typically cannot be met in the first several years of fruit production, especially in the early stages of a breeding program when genotypes are represented by single trees.

The commercially successful cultivar Honeycrisp, released by the University of Minnesota in 1991, is known for its high degrees of crispness and juiciness, as well as for its long storage capacity (e.g., Janick *et al.* 1996, Allan-Wojtas *et al.* 2003, Mann *et al.* 2005). This cultivar has been incorporated into numerous breeding programs in an effort to duplicate its desirable texture traits in conjunction with such other traits as improved disease resistance and tree vigor. Several thousand seedlings, exhibiting a wide range of texture traits, have been created at the University of Minnesota by crossing ‘Honeycrisp’ with numerous other cultivars and a number of trees have been identified that exhibit the exceptional crispness for which ‘Honeycrisp’ is known. This study characterizes several apple fruit texture traits within this large breeding population using instrumental and

sensory methods and estimates heritability values for each of the traits.

3.2 Materials and Methods

3.2.1 Plant Material

Five full-sib families with ‘Honeycrisp’ as a common parent (Table 3.1) were selected from amongst the breeding population at the University of Minnesota’s Horticultural Research Center in Chanhassen, Minnesota, USA (44º52′ N, 93º36′ W). Criteria for inclusion were number of fruit-bearing genotypes, high levels of variability in texture traits observed in previous years, reciprocal crosses within families, when possible, and no common ancestors other than through the ‘Honeycrisp’ parent.

The five full-sib families comprised more than 600 trees from crosses made between 1992 and 1998. The other parents included two unnamed advanced selections from the University of Minnesota breeding program, MN-1702 and MN-1764, two named North American cultivars, ‘Jonafree’ and ‘Monark’, and ‘Pitmaston Pineapple’ X 692, an accession of an English cultivar (hereafter referred to as ‘Pitmaston’). Reciprocal crosses were available for the first three families. Subsequent molecular data revealed that some of the progeny were inconsistent with their putative parentage. These problematic progeny were removed from the families, but retained in the study, assigned to two groups: an “other family,” reflecting a set of trees consistent with ‘Honeycrisp’ as one parent and a common unknown genotype as the second parent, and non-progeny group otherwise. Additionally, several parental genotypes and purchased cultivars were included, also placed within the non-progeny group. Because fruit from genotypes in the

non-progeny group had been evaluated simultaneously with the rest of the genotypes and were of value with respect to the prediction of panelist effects, those data were retained within the data analyses.

3.2.2 Harvest, Sample Preparation, and Randomization

Apples were harvested weekly, starting in August, in 2005, 2006, and 2007. Prior to harvest, fruit from each tree in the study were examined for maturity using the Blanpied and Silsby (1992) starch-iodine scale. Those trees that consistently scored in the 5 to 7 range on a given day were then designated as mature and a minimum of four fruit per tree were collected. All harvested fruit was stored in labeled paper bags and refrigerated at 4°C as promptly as possible on the day of harvest.

Within one week of harvest, four apples per tree were evaluated by sensory panelists with respect to sensory traits. Quadrants were removed from the same portion of each apple relative to the sun-exposed portion of each fruit. Unpeeled wedges were cut parallel to the core from each fruit and central core tissue was removed. Wedges were halved, producing two 45º wedges per fruit, which were presented to panelists as a single sample.

Fruit samples were randomized according to incomplete randomized block (IRB) designs (e.g., Fu *et al.* 1999), with panelists designated as blocks. In 2005, cyclic designs were constructed each week (John and Williams 1995), whereas in 2006 and 2007, Latinized α-designs were constructed as blocks using CycDesigN 2.1 (John and Williams 1995, Whitaker, Williams and John 2004, Piepho *et al.* 2006a). In this manner, a large

number of trees could be evaluated without requiring that all genotypes be evaluated by all panelists. Frequently, genotypes were replicated within panelists to address consistency of panelists within genotypes (two apples from the same tree) or within apples (four samples from the same apple). Genotype identity was retained for each apple section, but not revealed to panelists. Four fruits per genotype were evaluated by the sensory panel, except as noted below. Samples were presented to panelists in white paper baking cups arrayed in three rows and four columns on white foam supermarket food trays. Apple wedges were presented with peel side down. Because panelist effects were to be directly estimated, control genotypes were not included in the samples.

Panelists were recruited from the students, staff, and affiliates of the University of Minnesota Department of Horticultural Science and the Applied Plant Sciences graduate program. Randomization schemes were constructed on the same days as the panels, allowing for panelists to confirm their availability on each evaluation date.

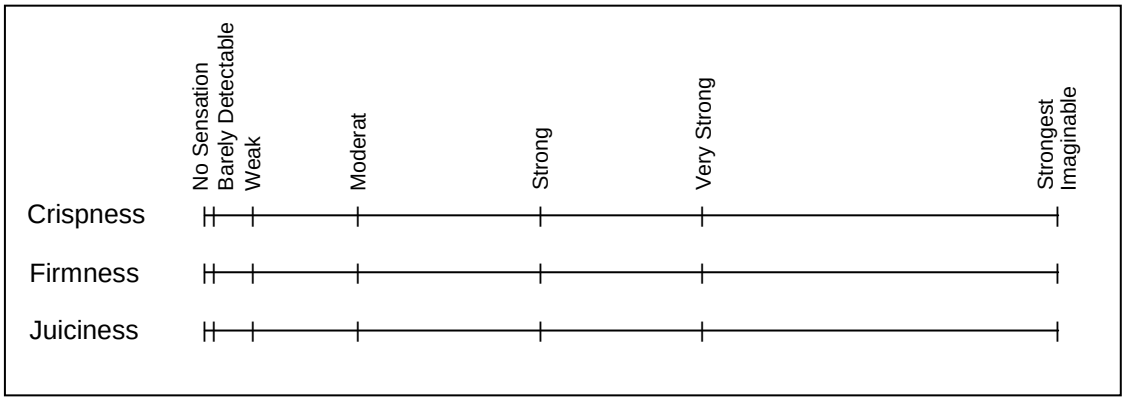


Figure 3.1. Labeled magnitude scale used by sensory evaluation panels.

3.2.3 Sensory Evaluation

Sensory panels assessed three sensory traits for each apple: crispness, firmness, and juiciness. Panelists evaluated crispness during the process of biting each apple wedge once with the incisors, and then they evaluated firmness and juiciness by chewing each section five to ten times with the molars (Allan-Wojtas *et al.* 2003, Harker *et al.* 2003). Panelists were instructed to hold the peel away from their mouths, and to not bite through the peel. Panelists were instructed to assess crispness by biting the samples and then to assess firmness and juiciness by chewing the samples (Vickers and Christensen 1980).

A 16 cm generalized Labeled Magnitude Scale (gLMS) was selected in order to allow for comparisons across panelists of variable sensitivities (Figure 3.1; e.g., Green *et al.* 1993, Bartoshuk *et al.* 2004). Panelists were instructed to make vertical marks along the scale relative to benchmarks. Locations of rating marks were later measured and divided by 16 cm to derive values between 0.00 and 1.00.

3.2.4 Texture Analyzer

Following the sensory panels, the sun-exposed quarters of the same fruits were assayed with a TA.XT2i texture analyzer (Stable Micro Systems LTD, Surrey, UK). Most samples were assayed within one day of the sensory panels. A 6 mm diameter cylindrical plug was removed from the portion of the apple parallel to the central core of the fruit and a triple-beam snapping test with a blunt blade was performed (e.g., Bourne 1982, Tong *et al.* 1999, Mann *et al.* 2005). The blade was applied at constant velocity of

0.8 mm/sec until the apple samples fractured. Force (gf, gram-force), time (sec), and distance (mm) data were recorded and total work was calculated using the formula: 1 Joule = 1 newton x m = 1.01972 x 10^5 gf x mm.

When adequate numbers of fruits were available, additional intact fruits from the same genotypes were included in the texture analyzer samples. These fruits allowed for an investigation into the change in total work of non-intact fruit relative to the intact fruit, in turn clarifying the relationship between the sensory values and the instrumental values.

The texture analyzer recorded the force required to maintain this constant velocity, allowing for the construction of a force-deformation curve and the estimation of a number of different parameters, including total force and total work, as well as smaller fracture events prior to failure of the sample (e.g., Bourne 1982, Tong *et al.* 1999, Mann *et al.* 2005, Vincent 1998, Karlsen *et al.* 1999, Johnston *et al.* 2001, Roudaut *et al.* 2002).

3.2.5 Statistical Analyses

Best linear, unbiased predictor (BLUP) models, also known as mixed-effects models, were constructed for each of the three sensory traits and the total work variable. In this manner, fixed effects, such as progeny and direction of reciprocal cross, were estimated, whereas random effects, such as genotype, year, and panelists, were incorporated solely as additional sources of variability (e.g., Næs and Langsrud 1998, Pinheiro and Bates 2002, Diggle *et al.* 2002, Piepho *et al.* 2003, 2008), as were interaction terms (e.g., Pineau *et al.* 2007). All statistical analyses were performed using R (R Development Core Team 2007).

Prior to analysis, the data were re-parameterized using dummy, or indicator, variables, because of the highly unbalanced nature of the population with respect to the families and the directions of reciprocal crosses. For example, rather than a single variable indicating the five families, five binary variables were constructed for each progeny, indicating presence and absence. Similar indicator variables were constructed for each of the nesting and interaction terms. This re-parameterization prevented estimation of extraneous variables and allowed for highly specific statistical queries (Piepho *et al.* 2006b).

Because the three sensory traits were constrained to values between zero and one, the logit transformation, where logit(y) = ln(y/(1-y)), was applied prior to model fitting. Extreme observed values were adjusted to 0.005 or 0.995, because the logit function is undefined at zero and one. Values of the instrumental work variable were not transformed.

An analysis of variance (ANOVA) model was constructed for each of the three sensory traits in order to assess the consistency of panelists' evaluations of multiple fruit per genotype. Only those data corresponding to multiple fruit per genotypes evaluated by a single panelist were included. The logit-transformed variables were modeled with two nested variables, and a residual term, ε, via the equation $\text{logit}(y) = \eta / \psi + \varepsilon$, where $\eta \sim N(\mu_\eta, \sigma_\eta^2)$, $\psi \sim N(\mu_\psi, \sigma_\psi^2)$, and $\varepsilon \sim N(0, \sigma^2)$. The nested variables, η and ψ, represent panelist and genotype-within-panelist fixed effects. The residual term reflected the variability of multiple fruit from the same tree.

Similarly, an ANOVA model was constructed for each of the three sensory traits to assess the consistency of panelists' evaluation of multiple sections from individual fruits. The models were equivalent to the previous models with the exception that the residual term was reflected the variability of multiple samples from within the same fruit. Model assessment was performed in an analogous manner.

For each trait, a full, or saturated, model was constructed, and a backwards-stepwise regression method was used to sequentially remove statistically nonsignificant terms (Piepho *et al.* 2006b, Pinheiro and Bates 2002). The initial full model for each sensory trait was $\text{logit}(y) = \alpha/\beta/\gamma + \delta + \eta + ((\alpha/\beta/\gamma)\times\delta) + (\eta\times\delta) + \varepsilon$, where $\alpha \sim N(\mu_\alpha, \sigma^2_\alpha)$, $\beta \sim N(\mu_\beta, \sigma^2_\beta)$, $\gamma \sim N(0, \sigma^2_\gamma)$, $\delta \sim N(0, \sigma^2_\delta)$, $\eta \sim N(0, \sigma^2_\eta)$, and $\varepsilon \sim N(0, \sigma^2)$. The first term, $\alpha/\beta/\gamma$, represents family (α), cross nested within family (β), and genotype nested within cross (γ), respectively. The next two terms, δ and η, represent year and panelist effects, respectively, both of which are modeled as random effects. The last three terms, $(\alpha/\beta/\gamma)\times\delta$, $\eta\times\delta$, and ε, model the genetic-by-year interactions, with nesting as before, panelist-by-year interaction, and the residual term. Family and cross were modeled as fixed effects, while all other terms were modeled as random effects. Nesting the various genetic components allows for nonconstant variance across family/cross combinations. The inclusion of panelists as a random term reflects their role as blocking structures. Decisions regarding model selection were based on likelihood ratio tests (constructed via restricted maximum likelihood (ReML)), and the Akaike Information Criterion (AIC); both methods weigh the goodness of fit of the models as well as their complexity (Pinheiro and Bates 2002). Significance levels were

established at $p<0.05$.

The initial model for the total work variable was equivalent with a few modifications. First, the work variable was not transformed with the logit function. Second, all panelist terms were excluded, since these data were not collected via the sensory panels. Third, a new term μ, nested within the interaction term, was added to reflect whether a sample came from an intact fruit or from a fruit that had been sampled for the sensory panels. The resulting model is $y = \alpha/\beta/\gamma + \delta + \left((\alpha/\beta/\gamma)\times\delta\right)/\mu + \varepsilon$, where $\alpha \sim N(\mu_\alpha, \sigma^2_\alpha)$, $\beta \sim N(\mu_\beta, \sigma^2_\beta)$, $\gamma \sim N(0, \sigma^2_\gamma)$, $\delta \sim N(0, \sigma^2_\delta)$, $\mu \sim N(0, \sigma^2_\mu)$, and $\varepsilon \sim N(0, \sigma^2)$.

Heritability terms were estimated using a bootstrap method (Piepho and Möhring 2007) because of the complexity of estimation of variance components for highly unbalanced data sets. First, the final fitted model for each of the traits was used to simulate a data set by means of the **`simulate`** function contained within the lme4 package for R (Bates 2007). These simulated populations incorporated the variance-covariance structure of the fitted linear model and reflects the unbalanced nature of the original data set. The simulated data set was, in turn, fitted according to the same linear model, the fitted values were retained, and the correlation value was calculated for the simulated data set and its corresponding fitted values. This procedure was repeated 1000 times for each trait, yielding 1000 correlation values. The mean of these simulated correlations is asymptotically equivalent to the broad-sense heritability, H^2, and the bootstrap sample provides standard errors for this estimate as well (Piepho and Möhring 2007).

Panelist-adjusted values were calculated by subtracting model-derived predictions of panelist and panelist-by-year terms from the predicted values of each apple. Correlation values were calculated among the observed and panelist-adjusted values for the three sensory traits and observed values for the instrumental trait. Comparisons were made at the level of individual apples as well as at the levels of genotypic means. All sensory trait values were logit-transformed.

3.3 Results

3.3.1 Data Collection

Over three years, a total of 515 trees were sampled from the more than 600 contained within the five families, reflecting the sporadic nature of fruit-bearing in young apple trees (Table 3.1). Weekly sample sizes ranged from 8 to 102 genotypes, with an average of 42.0. A total of 23 panelists participated over the course of the study, 5 for a single year, 9 for two years, and 9 for all three years. Week-to-week variation in panelist availability yielded average annual panel sizes of 13.8, 13.0, and 10.6 for the three years, respectively. Variation in both weekly sample sizes and weekly panel sizes resulted in samples per panelist per week ranging from 2 to 36, with an average of 15.3 fruit per panelist per week.

2.3.2 Prediction of Genotypic Values

Panelists' evaluations of multiple apples from within a single tree were very consistent, relative to evaluations of apples from multiple trees on the same date. For

each of the three sensory traits, the variability across genotypes was significantly greater than the variability within genotypes (nested within panelists; $p<0.001$). Similar results were observed with respect to multiple samples from with the same fruit (nested within panelist; $p<0.001$). In other words, panelists were much more able to differentiate between genotypes than between samples from the same genotype.

Table 3.1. Number of trees within each family-by-cross combination.

Family	'Honeycrisp' as Maternal Parent (No.)	'Honeycrisp' as Paternal Parent (No.)	Total (No.)	Distinguishing trait of non-'Honeycrisp' parent
MN-1702	21	35	56	Firm and long-storing
MN-1764	3	130	133	Crisp texture, softens rapidly in storage
'Jonafree'	13	46	59	Scab-resistant
'Monark'	86	0	86	Fruit matures early
'Pitmaston'	57	0	57	Distinctive flavor and russetting
"Other Family"	NA	NA	32	
Non-Progeny	NA	NA	92	
Total			**515**	

NA: Not applicable, as 'Honeycrisp' was not a parent.

Family effects and cross direction within family were both statistically significant for crispness ($p<0.001$ and $p=0.006$, respectively; Table 3.2), but year did not contribute significantly to the variability ($p=1.000$), nor did the interactions of year with family or cross **(**$p=1.000$ and $p=0.930$, respectively**)**. Interactions of genotype and panelist with

year did both contribute significantly ($p<0.001$ for both) to the variability of the data. The final model for crispness was $\text{logit}(y) = \alpha / \beta / \gamma + \eta + \alpha / \beta / (\gamma \times \delta) + \eta \times \delta + \varepsilon$.

Neither firmness nor juiciness exhibited significant ($p=0.113$ and $p=0.801$, respectively) contributions from the within-family cross terms. Otherwise, the final models for both traits were the same as for crispness:

$\text{logit}(y) = \alpha / \gamma + \eta + \alpha / (\gamma \times \delta) + \eta \times \delta + \varepsilon$.

Family-by-year and tree-by-year interaction terms were statistically significant ($p<0.001$ for both) for the total work, but cross-by-year was not ($p=0.400$). Unlike the three sensory traits, year of harvest contributed significantly ($p<0.001$) to the variability of this instrumental trait. The final model for total work was

$y = \alpha / \gamma + \delta + \alpha / (\gamma \times \delta) / \mu + \varepsilon$.

The large sample sizes allowed for the identification of statistically significant, albeit small, correlations among the raw sensory and instrumental traits at the individual apple level (Table 3.4a). Comparisons at the genotype level yielded larger correlation values (Table 3.4c). Adjusting the sensory trait values for panelist effects further increased the correlation values at both the individual apple level (Table 3.4b) and the genotype level (Table 3.4d).

3.3.3 Heritability Estimation

Despite the identification of numerous sources of variability during the model fitting process, the bootstrap analysis estimate yielded broad-sense heritability terms in

excess of 0.7 for all four traits (Table 3.3). The variability of these estimates was uniformly small, with standard deviations ranging from 0.016 to 0.026.

Table 3.2. Significance levels of terms for each BLUP analysis.

	Crispness	Firmness	Juiciness	Total Work
Family	p<0.001	p=0.002	p<0.001	p<0.001
Cross within Family	p=0.006	p=0.113	p=0.801	p=0.472
Year	p=1.000	p=1.000	p=0.661	p<0.001
Year by Family	p=0.982	p=0.720	p=0.745	p<0.001
Year by (Cross within Family)	p=0.930	p=0.992	p=1.000	p=0.400
Year by (Genotype within Cross within Family)	p<0.001	p<0.001	p<0.001	p<0.001
Year by Panelist	p<0.001	p<0.001	p<0.001	NA
Intact Status	NA	NA	NA	p<0.001

NA: Intact status not relevant for sensory traits; panelists not used for total work.

Table 3.3. Broad-sense heritability estimates, represented as mean bootstrapped correlations, and standard deviations. Crispness, Firmness, and Juiciness terms were calculated using back-transformed data.

	Mean Correlation (Standard Deviation)
Crispness	0.752 (0.016)
Firmness	0.803 (0.017)
Juiciness	0.723 (0.026)
Total Work	0.767 (0.018)

Table 3.4. Correlations between sensory traits (observed and panelist-adjusted) and instrumental trait (intact and non-intact fruit; observed values). a and b) Correlations based upon individual fruit values. c and d) Correlations based upon genotypic mean values. All sensory traits are logit-transformed.

a.	Crispness	Firmness	Juiciness
Firmness	0.730 ***		
Juiciness	0.465 ***	0.398 ***	
Work (Non-Intact)	0.255 ***	0.332 ***	0.043 **

b.	Adjusted Crispness	Adjusted Firmness	Adjusted Juiciness
Adjusted Firmness	0.862 ***		
Adjusted Juiciness	0.640 ***	0.496 ***	
Work (Non-Intact)	0.366 ***	0.432 ***	0.119 ***

c.	Crispness	Firmness	Juiciness
Firmness	0.843 ***		
Juiciness	0.605 ***	0.468 ***	
Work (Non-Intact)	0.403 ***	0.525 ***	0.081 *
Work (Intact)	0.378 ***	0.485 ***	-0.030

d.	Adjusted Crispness	Adjusted Firmness	Adjusted Juiciness	Work (Non-Intact)
Adjusted Firmness	0.859 ***			
Adjusted Juiciness	0.642 ***	0.501 ***		
Work (Non-Intact)	0.445 ***	0.532 ***	0.132 ***	
Work (Intact)	0.420 ***	0.507 ***	0.034	0.740 ***

*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

3.4 Discussion

The inclusion of three of the parental genotypes in this study's population provides a standard by which to assess the performance of the BLUP analysis to predict phenotypic values (Table 3.5). The predicted phenotypic values of three parental cultivars reflect, in part, the reasoning behind their inclusion in the University of Minnesota apple breeding program. 'Honeycrisp' expressed above average crispness and exceptionally high juiciness levels, but low firmness and very low total work to fracture, relative to other genotypes. In contrast, MN-1702 expresses higher levels of crispness and firmness, much higher levels of total work to fracture, and reduced juiciness relative to 'Honeycrisp'. MN-1764, only evaluated in 2005, is only notable for its juiciness, at least when considering this suite of texture traits.

In order to compare fruit at similar stages of maturity, the starch-iodine test (Blanpied and Silsby 1992) was selected as an easily performed and replicable proxy assessment of maturity, rather than as an explicit measure of edibility. 'Honeycrisp' expresses an unusual relationship between starch levels and tissue softening (Tong *et al.*

Table 3.5. Percentiles of panelist-adjusted predicted phenotypic values of parental genotypes, averaged across years.

	'Honeycrisp'	MN-1702	MN-1764*
Crispness	57.5	64.7	14.2
Firmness	33.5	72.9	8.1
Juiciness	93.9	56.5	69.7
Total Work**	18.7	74.7	5.8

* MN-1764 only evaluated in 2005.
** Total work to fracture representing non-intact fruit (see text).

1999), relative to those of other cultivars (e.g., Smith *et al.* 1979, Reid *et al.* 1982, Blanpied and Silsby 1992, Brookfield *et al.* 1997), and a wide range of fruit maturation patterns are likely to exist within these 'Honeycrisp' offspring (e.g., Peirs *et al.* 2002, Wang *et al.* 2009b, Watkins *et al.* 2005). Consequently, variability in the relationship between starch levels and texture are likely to have been an additional source of variation among the individuals in the study.

The employment of multiple sensory panels, reflecting the multiple harvests, reduced variability due to post-harvest physiological effects by allowing for uniform storage time prior to evaluation (e.g., Tong *et al.* 1999, Johnston *et al.* 2001, 2002b, Mann *et al.* 2005, Watkins *et al.* 2005). The employment of panelists throughout the duration of the study eliminated any confounding of panelist effects with week-of-harvest effects.

Apple genotypes with early maturing fruit are well-known to be generally less crisp and less firm than later maturing genotypes (e.g., Bunyard 1929, Juniper and Mabberley 2006) and to soften relatively quickly (Johnston *et al.* 2001, 2002ab). The single harvest per genotype, however, resulted in genotypes being nested within week-of-harvest each year. This study was not designed specifically to investigate early versus late fruit maturation patterns, so week-of-harvest was not explicitly addressed in this analysis because of this confounding.

The inclusion of multiple fruits from the same tree in samples presented to individual panelists revealed that panelists were consistent in their ratings at the daily level. Panelist-by-year interaction terms were significant ($p<0.001$) for all three sensory traits, yet random effects due to year were statistically insignificant ($p>0.65$; Table 3.2).

Further examination of panelist-by-year interaction terms revealed no systematic inter-annual variation common to panelists (data not shown), suggesting that comparisons among panelists were not uniform across years. This variability is not surprising given the absence of standards or controls with an untrained panel, and justifies the removal of panelist-by-year terms as part of the calculation of the panelist-adjusted predicted values.

Khan and Vincent (1993) and Harker *et al.* (2002) have demonstrated that systematic variation in tissue density relates to position with respect to the exposed and shaded portions of the fruit. Thus, data collection was standardized relative to the sun-exposed side of each fruit. The inclusion of multiple sections per fruit and multiple fruit per genotype in panelists' samples, however, introduced insignificant variability relative to samples from multiple genotypes. This result suggests that effective trait characterization may be possible with even fewer fruit, each fruit providing samples for multiple panelists. This conclusion is further supported by Alspach and Oraguzie's (2002) statement that a sample of only two fruits per tree was adequate for their own sensory evaluations. This observation suggests that large-scale population characterizations can occur when trees are young and crops are small.

Collection of the sensory data necessitated the removal of a quarter of each fruit, potentially initiating wound-induced texture changes (Lougheed and Franklin 1974). As a result, same-fruit comparisons of sensory traits with instrumental traits were restricted to non-intact fruit. The time lag between sensory evaluations and the instrumental assay, however, may have provided an opportunity for texture changes to initiate and accumulate. The inclusion of intact fruit from the same genotypes in 2005 and 2006 permitted an assessment of the relationships between the sensory traits and the

instrumental trait, but at the genotype level rather than at the same-fruit level. Despite the high correlation between intact and non-intact values ($r=0.740$, $p<0.001$), all three sensory traits were more highly correlated to the non-intact fruit values than to the corresponding intact fruit values. This suggests that variation due to wound-induced texture changes is less than variation between fruit, even within genotypes, and supports the usefulness of the data collected from non-intact fruit that can be kept in cold storage for at least 24 hours.

Mann *et al.* (2005) reported total work as a statistically significant predictor of sensory crispness ($r^2 = 0.67$), and force at fracture a predictor for firmness ($r^2 = 0.43$). Corresponding values for this study were substantially smaller, the strongest relationship between a sensory value and an instrumental value being that between panelist-adjusted firmness and total work for non-intact fruit ($r^2 = 0.532$ and $r^2 = 0.283$, respectively; Table 2d). This discrepancy may reflect additional variability introduced to this study by the greater number of genotypes assayed (515 versus 10), the greater number of sensory evaluation dates for fresh fruit (22 dates over three years versus a single date), and the greater number of panelists enlisted (23 versus 12). Additionally, the calculations for instrumentally measured work to fracture differ between Mann *et al.* (2005) and the current study; in the prior study force at time of fracture was multiplied by distance traversed, whereas in the current study, work was calculated for each time step, the cumulative sum yielding total work. The latter method is more likely to reflect total energy expended as it does not assume constant force throughout the probe's motion. The current study did not address instrumental force at fracture.

King *et al.* (2000) presented narrow-sense heritability values of h^2=0.57 and h^2=0.52 for sensory crispness and hardness [firmness] traits, respectively, within a full-sib family. Alspach and Oraguzie (2002) reported heritability of sensory firmness ranging from 0.26 to 0.59 and crispness ranging from 0.12 to 0.15, hypothesizing that these relatively low heritability estimates were in part due to their lack of control regarding individual fruit maturity, having only a single harvest, regardless of variability in fruit maturation dates across the trees. It should be noted that their population encompassed much broader genetic material, containing more than100 families of between 9 and 209 individual genotypes. Liebhard *et al.* (2003a) also possibly obscured inheritance patterns in texture traits by having only a single harvest date. Kouassi *et al.* (2009), in contrast, relied primarily on controlled crosses and utilized all known pedigree information to estimate narrow-sense heritabilities for sensory crispness, firmness, and juiciness (h^2=0.26, h^2=0.16, and h^2=0.35, respectively). The estimates of broad-sense heritability, H^2, in the current study are consistently greater than previously published narrow-sense estimates. This is to be expected, since H^2 is greater than or equal to h^2; the latter term only incorporates additive and additive epistatic variances, whereas the former term also incorporates dominance and other epistatic variances. The inclusion of multiple families in the study would be expected to increase genetic variability relative to phenotypic variability, and lack of common ancestry among any of the parental genotypes would only exaggerate this effect. The implementation of multiple harvests would be expected to decrease phenotypic variability, further increasing heritability.

Although estimates of narrow-sense heritability are more informative for estimating the breeding value of an individual, broad-sense heritability is very useful

parameter for clonally propagated crops, individual *per se* performance being the basis of selecting among individuals (e.g., Poehlman and Sleper 1995). Long generation times restrict the number of recombination opportunities, and thus linkages between genes are likely to be retained in a breeding program, relative to annual crops. The asexual propagation of most tree fruit crops further ensures that desirable linkages are retained within desirable phenotypes so long as a substantial fraction of the phenotypic variation is genetically determined. In other words, total genetic variance contributing to high broad-sense heritability, regardless of the relative proportions of its constituent variance components, can be maintained via asexual propagation, resulting in high levels of crop uniformity,

Historically, ANOVA models have been constructed in order to estimate variance or covariance terms, from which heritability estimates can be calculated (e.g., Falconer and Mackay 1989, Bell and Janick 1990, Abe *et al.* 1995, Tancred *et al.* 1995, Oraguzie *et al.* 2001, Bernardo 2002, Rumpunen and Kviklys 2003). The incorporation of complex population structures and unbalanced experimental designs, however, limit the usefulness of traditional ANOVA models. Mixed effects linear models incorporating ReML have been employed to address such analyses in numerous studies (e.g., Durel *et al.* 1998, Currie *et al.* 2000, White *et al.* 2000a,b, Bus *et al.* 2002, Alspach and Oraguzie 2002). The simple structure of the current study's population does not rely on any pedigree assumptions other than the explicit nature of the full-sib families and 'Honeycrisp' as a common parent. The choice of populations with no known common ancestors, coupled with the limited knowledge of pedigree of 'Honeycrisp' (Cabe *et al.* 2005), limits the utility of recent pedigree-based methods (e.g., Bink *et al.* 2008, Kouassi

et al. 2009). The simple nested nature of the population is captured in its entirety by the indicator variables and does not explicitly require the calculation of coefficient of coancestry terms (e.g., Piepho and Möhring 2007) or pre-specified variance components (e.g., Kouassi *et al.* 2009).

3.5 Conclusions

The rapid development of new apple cultivars requires the efficient, early characterization of traits of interest within the breeding population, as well as heritability estimates for the same traits. Heritability estimates for a population can provide guidance in the design of subsequent crosses within a breeding program. The large space and time requirements for breeding tree crops results in many genetic studies being based upon active breeding programs, as opposed to populations especially designed to address genetic questions. 'Honeycrisp' is represented in many different crosses, including several reciprocal crosses. The other parents in these crosses, in contrast, are of lesser interest, primarily with respect to their potential for contribution of particular genetic traits into the 'Honeycrisp' background, so these other parents are rarely crossed with each other. As a result, the existing structure of this breeding program does not contain diallel-type population designs as are frequently developed for genetic studies. Nonetheless, breeding populations can yield valuable information, particularly with respect to phenotypic characterization.

Protocols that aid in the collection of phenotypic data can have substantial financial and labor impacts for a breeding and research program. This study combined the established protocols of incomplete block design, sensory evaluation panels, and

BLUP to characterize several fruit texture traits. Randomized incomplete block design allowed for the evaluation of many genotypes by moderately sized sensory evaluation panels and small quantities of fruit per genotype. The inclusion of replicated genotypes within individual panelists' samples confirmed that between genotype variability far exceeded within genotype variability. The repeated participation of panelists in successive panels permitted the evaluation of panelist effects and subsequent adjustment of phenotypic values. The inclusion of parental genotypes in the analyses provided a basis by which to assess the predicted values. Taken together, these methods have enabled the evaluation a large number of genotypes from an active breeding population to be evaluated at a young age. Coupled with the high estimates of broad-sense heritability identified in this study, the further development of a marker-assisted selection program for texture traits derived from 'Honeycrisp' is clearly warranted.

4 Linkage Map Construction for the Apple Cultivar Honeycrisp

4.1 Introduction

The genus *Malus* (apple and crabapple; Rosaceae) has been a focus of molecular studies since at least the mid-1980s when Weeden and Lamb (1986) used isozymes as a means of discriminating between different apple cultivars. Molecular markers have been widely applied to evolutionary and pedigree studies in apple, both wild *Malus* species (Dickson *et al.* 1991, Lamboy *et al.* 1996, Richards *et al.* 2009) and domestic cultivars (e.g., Gardiner *et al.* 1996a, Goulão *et al.* 2001a, Hokanson *et al.* 1998, 2001, Oraguzie *et al.* 2001, Cabe *et al.* 2005, Evans *et al. In press*). Linkage groups have been aligned across a number of domestic cultivars (e.g., Hemmat *et al.* 1994, Conner *et al.* 1997, Maliepaard *et al.* 1998, Liebhard *et al.* 2003a, Kenis and Keulemans 2005, N'Diaye *et al.* 2008, Fernández-Fernández *et al.* 2008, Celton *et al.* 2009a, Patocchi *et al.* 2009ab), and even across multiple genera from within the Rosaceae (e.g., Dirlewanger *et al.* 2004, Celton *et al.* 2009ab, Gasic *et al.* 2009, Sargent *et al.* 2009, Mnejja *et al.* 2010).

As new marker types have been developed, apple geneticists have readily adopted them. Early studies implemented anonymous markers (e.g., Goulão *et al.* 2001b, Guilford *et al.* 1997, Hokanson *et al.* 1998, Koller *et al.* 1993, Mulcahy *et al.* 1993), but markers for genes of known or suspected function are increasingly used (e.g., Broothaerts 2003, Calenge *et al.* 2005, Silfverberg-Dilworth *et al.* 2005, 2006, Chagné *et al.* 2007, Igarashi *et al.* 2008, Sargent *et al.* 2009). Whole genome sequencing in apple promises to greatly expand the availability of markers for the genus (e.g., Sosinski *et al.* 2009).

The construction of linkage maps has facilitated the identification of molecular

markers associated with numerous phenotypic traits. Among the traits examined to date are resistance to apple scab (caused by the fungus *Venturia inaequalis* Cooke; Koller *et al.* 1994, Gardiner et al 1996b, Liebhard *et al.* 2003b, Calenge *et al.* 2004) and fire blight (caused by the bacterium *Erwinia amylovora* Burrill; Khan *et al.* 2006, Peil *et al.* 2007), columnar growth habit (Conner *et al.* 1998), and several fruit quality traits (e.g., King *et al.* 2000, 2001, Kenis *et al.* 2008). The identification of such molecular markers is widely sought in commercial crops as essential for the development of marker-assisted selection and breeding (MAS and MAB) programs (Gianfranceschi *et al.* 1996, King *et al.* 1999, Kellerhals *et al.* 2000a,b, Belfanti *et al.* 2004, Frey *et al.* 2004, Gardiner *et al.* 2007, Zhu and Barritt 2008, Patocchi *et al.* 2009ab).

Like other quantitative traits, fruit texture traits are assumed to be multigenic (e.g., Soglio *et al.* 2009) in origin, further impeding rapid cultivar development. MAS provides the opportunity to identify genotypes containing desirable alleles prior to phenotypic expression, allowing for screening of undesirable genotypes at early stages of cultivar development and either preferentially retaining desirable genotypes (positive selection) or discarding undesirable genotypes (negative selection) (Knapp 1998). The benefits of MAS are greatest when trait characterization is difficult or expensive, as in the case of extended juvenility and long generation times, or when heritability is low, otherwise phenotypic selection would be adequate (Knapp 1998, Luby and Shaw 2001). Consequently, the precise characterization of the traits of interest and the estimation of the corresponding heritability are necessary before potential markers and their benefits in an MAS program can be assessed.

Typically a single large full-sib family derived from genotypes of interest is evaluated with respect to marker segregation in order to construct linkage maps. In contrast to populations specifically developed for genetic studies, active apple breeding programs often do not have large full-sib families. Instead, numerous, smaller full-sib families are typical of breeding populations, often with full-sib progenies forming half-sib families with each other through a common parent. While small full-sib families may not individually provide adequate information to construct linkage maps, multiple full-sib families with a single common parent can provide adequate information to generate linkage maps for the common parent.

Highly heterozygous and obligate outcrossing species, such as apple, necessitate the use of the pseudo-testcross strategy (Grattapaglia and Sederoff 1994) for linkage group construction. Traditional testcross scenarios implement crossing heterozygous individuals with highly inbred, essentially homozygous individuals; all subsequent segregation can then be attributed to the heterozygous parent. In contrast, the pseudo-testcross scenario implements crossing two highly heterozygous individuals and identifying those markers that are fully informative with respect to the segregation of DNA contributed by the parent of interest. Codominant markers are readily useable in linkage map construction, as long as they are heterozygous for the parent of interest. In highly heterozygous populations, dominant markers, on the other hand, are generally only valuable if they are heterozygous for the parent of interest and homozygous for the second parent. This pair of requirements can substantially restrict the number of useable markers, and thus reduce the ability to successfully construct linkage maps. The inclusion of multiple families in the current study, however, provides an opportunity for

the utilization of doubly-heterozygous markers, those markers heterozygous for both parents of a full-sib family, in the construction of a multiple-family consensus linkage map. In other words, even those markers that are heterozygous for all parents can be added to family-specific linkage maps and assessed with respect to the consistency of their map locations across the families. In contrast, such markers could not be mapped with any confidence had only a single family been incorporated into the study population.

The commercially successful cultivar Honeycrisp, released by the University of Minnesota in 1991, is known for its high degrees of crispness and juiciness, as well as for its long storage capacity (e.g., Janick *et al.* 1996, Allan-Wojtas *et al.* 2003, Mann *et al.* 2005). This cultivar has been incorporated into numerous breeding programs in an effort to duplicate its desirable texture traits in conjunction with such other traits as disease resistance and improved tree vigor. Several thousand seedlings have been created at the University of Minnesota by crossing 'Honeycrisp' with numerous other cultivars. These trees provide an opportunity to generate and align linkage maps for 'Honeycrisp' from several full-sib populations, developing a consensus map for this important cultivar (Schouten *et al. Submitted*).

4.2 Materials and Methods

4.2.1 Plant Material

Five full-sib families with 'Honeycrisp' as a parent, initially comprising more than 600 trees, were selected from within the University of Minnesota's apple breeding population at the University of Minnesota's Horticultural Research Center in Chanhassen, Minnesota, USA (44º52′ N, 93º36′ W; (Table 4.1). Criteria for inclusion

included the number of fruit-bearing genotypes, high levels of variability in texture traits observed in previous years, and no common ancestors other than through the 'Honeycrisp' parent.

The other parents included two unnamed advanced selections from the University of Minnesota breeding program (MN-1702 and MN-1764) two named North American cultivars ('Jonafree' and 'Monark') and 'Pitmaston Pineapple' X 692 (USDA PI 279323, believed to be an accession of an English cultivar), hereafter referred to simply as 'Pitmaston'.

4.2.2 DNA Extraction and Genotyping

An initial DArT array had been constructed for apple by Diversity Arrays Technology staff using germplasm from a historic apple collection in Australia (J. Carling, personal communication). The DArT array was subsequently extended through a collaboration of Diversity Arrays Technology, the University of Minnesota apple breeding program, the Department of Agriculture and Forestry in Western Australia, and the Plant Research Institute at Wageningen University and Research Centre in the Netherlands. Contributed genotypes included 'Honeycrisp', the other five parents in this study, several ancestors, and numerous other heirloom and contemporary apple cultivars, as well as several breeding lines; several of the DNA samples contributed by the University of Minnesota were derived from tissue samples initially obtained from the USDA National Germplasm Repository in Geneva, New York (Schouten *et al. Submitted*).

Leaf tissue was collected during spring flush, placed on ice, and lyophilized. A CTAB-chloroform-isoamyl alcohol protocol, modified from Doyle and Doyle (1987) and Aljanabi *et al.* (1999), was used for genomic DNA extraction (Diversity Arrays Technology (DArT) Pty. Ltd.; http://www.triticarte.com.au/pdf /DArT_DNA_isolation.pdf). DNA samples were converted to DArT targets and hybridized to an apple-derived array by Diversity Arrays Technology (DArT) Pty. Ltd. (Yarralumla, Australia; Jaccoud *et al.* 2001, Wenzl *et al.* 2004, Wittenberg *et al.* 2005, Schouten *et al. Submitted*).

Molecular similarity was estimated for the six parental genotypes based on the DArT marker data. The Canberra dissimilarity metric was implemented to reflect that the absence of a marker from two linkage groups was uninformative, in contrast to the presence of the marker in one or both linkage groups (e.g., Krzanowski 1988). 'Pitmaston' was identified as the parent most dissimilar to 'Honeycrisp', so the 'Pitmaston' family was selected for further examination. DNA samples from the 'Pitmaston' family were assayed with respect to single sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers by Plant and Food Research, Palmerston North Research Center, Palmerston North, New Zealand, using a LightCycler 480 instrument (Roche Diagnostics, Indianapolis, IN, USA) as in Chagné *et al.* (2008).

Additionally, DNA samples from all five families were genotyped with SSRs at the Forest Molecular Genetics Laboratory, Department of Genetics, University of Pretoria, South Africa, using ABI Prism 310 and 3130 (16-capillary array system) Genetics Analyzers (Applied Biosystems, Foster City, CA, USA), as in van Dyk *et al.* (2010).

Molecular phenotypes were examined with respect to all marker data, comparing offspring with their putative parents. Some genotypes were identified that were inconsistent with their putative parentage, probably due to mislabeling of seeds or seedlings, or due to previously undetected open pollination events. All such offspring were excluded from the study prior to linkage map construction, as were many genotypes that did not bear fruit over the course of the study, reducing the number of genotypes to 419.

4.2.3 Linkage Group Construction and Alignment

This study implemented a three stage analysis to construct a consensus map for the apple cultivar Honeycrisp. First, preliminary linkage groups were constructed using no prior information. Second, intermediate linkage groups were constructed based on similarity across families of the preliminary linkage groups with respect to marker composition. Finally, consensus linkage groups were constructed by aligning intermediate linkage groups.

The construction of preliminary linkage groups implemented two separate approaches, utilizing different data sets. The first approach was a pseudo-testcross method, based on a pooled data set from the five families. All markers homozygous for 'Honeycrisp' were excluded. Codominant marker data were coded so as to reflect 'Honeycrisp'-derived segregation information only. Additionally, only those DArT marker data from families in which the non-'Honeycrisp' parent was homozygous were included in this pooled data analysis. For example, if a DArT marker was heterozygous for 'Honeycrisp', homozygous for three non-'Honeycrisp' parents, and heterozygous for

the remaining two non-‘Honeycrisp’ parents, then only those data from the former three families were included in the analysis, and the remaining two families were treated as if they had not been assayed. The resulting data set facilitated the construction of linkage groups that solely reflected the segregation of markers derived from ‘Honeycrisp’.

The second approach was to generate preliminary linkage groups separately for each of the five families, again excluding markers homozygous for ‘Honeycrisp’. A two phase process was implemented for each family. First, only those markers homozygous for the non-‘Honeycrisp’ parent were incorporated into the mapping process according to the pseudo-testcross mapping strategy. Next, the markers heterozygous for both ‘Honeycrisp’ and the second parent were added to the analysis, retaining marker orders from the pseudo-testcross maps. In this manner, doubly-heterozygous markers were included, even they their segregation patterns undoubtedly derived from both parents.

In both approaches, and in all subsequent analyses, JoinMap 4.0 (van Ooijen 2006) was implemented, using the cross-pollinated (CP) population type. Minimum logarithm of the odds (LOD) scores of 3.0 and the Kosambi mapping function were applied for map construction. All linkage group images were generated using Map Chart 2.1 (Voorrips 2002). Family-specific linkage groups of length less than 0.5 cM were retained for alignment as their constituent markers did not always co-segregate equally across families.

A cluster analysis was next performed to determine which preliminary linkage groups corresponded across the five families. An indicator matrix was constructed, comprised of one column per preliminary linkage group, one row per marker, and values of one and zero indicating which markers mapped to each preliminary linkage group. All

five family-specific sets of linkage groups and the pseudo-testcross set of linkage groups were included. Hierarchical, agglomerative cluster analysis was performed on the indicator matrix using the statistical software R 2.6.1 (R Development Core Team 2007), sequentially merging preliminary linkage groups using the group average method. The Canberra metric was implemented to reflect that the absence of a marker from two linkage groups was uninformative, in contrast to the presence of the marker in one or both linkage groups (e.g., Krzanowski 1988). Merging concluded when the number of clusters equaled the number of preliminary pseudo-testcross linkage groups.

The resulting clusters formed the basis for constructing intermediate linkage groups. For each cluster, a new data set was constructed reflecting the subset of markers present in the constituent linkage groups within the cluster. As before, pseudo-testcross linkage groups were constructed for each cluster. Similarly, family-specific linkage groups were constructed for each cluster, using the pseudo-testcross maps as guides with respect to marker order.

Finally, the family-specific linkage groups were integrated to create a consensus map corresponding to each of the original clusters. Again, the marker orders from the pooled data set were used as guides for the alignment. The resulting consensus maps were assessed with respect to codominant markers, as these markers had been previously mapped in other studies. Consequently, some consensus groups were split because of the presence of markers from multiple previously published linkage groups, and other consensus groups were merged based on similar knowledge. In this case, the data sets for the different consensus linkage groups were pooled and linkage group construction and integration was repeated. Finally, individual markers present in multiple consensus

linkage groups were evaluated and removed from linkage groups when the impact was minimal, sometimes being removed from all linkage groups.

4.3 Results

Between 53 and 157 trees were genotyped for each of the five full-sib families, cumulatively representing 419 trees (Table 4.1). A total of 960 segregating DArT markers were identified for the five families. Of these, 322 were homozygous for 'Honeycrisp', and thus not used for linkage map construction. The remaining 638 were heterozygous for 'Honeycrisp', and 478 were homozygous for at least one of the other parents (Table 4.2). A total of 53 SSRs and 29 SNPs were identified as heterozygous with respect to 'Honeycrisp' (Tables 4.2 and 4.3).

The pooled data set of fully informative markers yielded 33 linkage groups, approximately twice the haploid chromosome number for apple (seventeen). Four of the linkage groups were estimated as less than 0.5 cM in length, with an average of 8.8 unique loci and an average length of 48.5 cM (Table 4.4). In contrast, the individual family data sets yielded larger numbers of smaller linkage groups. The five family-specific data sets yielded between 43 and 62 preliminary linkage groups with mean linkage group sizes of 17.4 cM to 33.3 cM (Table 4.4).

The alignment of the family-specific linkage groups to create consensus maps yielded a total of twenty linkage groups of known correspondence to previously published maps (Figure 4.1, Table 4.5). Four fragments tied to linkage group 7 could not be resolved into a single linkage group; all other fragments coalesced into a single consensus map per published linkage group. The prefix "HC" was applied to the linkage

Table 4.1. Number of genotyped trees in each of the five full-sib families included in linkage group construction. Families indicated by non-'Honeycrisp' parent.

Family	Total
MN-1702	53
MN-1764	157
'Jonafree'	60
'Monark'	83
'Pitmaston'	66
All Families	419

group names to indicate "'Honeycrisp' consensus" and numbering was in accordance with Maliepaard *et al*. (1998).

4.4 Discussion

Codominant markers, including SSRs and SNPs, are very informative on a per marker basis as they allow for allelic phenotyping. Numerous publicly available codominant markers have been developed for apple and its relatives, despite the substantial work required for their development (e.g., Guilford *et al.* 1997, Hokanson *et al.* 1998, Gianfranceschi *et al.* 1998, Chagné *et al.* 2008, Han *et al.* 2009, Sargent *et al.* 2009, van Dyk *et al.* 2010). Dominant markers are frequently easier to develop, but only allow for presence/absence data with respect to band lengths (e.g., AFLPs) or DNA:DNA hybridization (e.g., DArT markers; Welsh and McClelland 1990, Vos *et al.* 1995, Jaccoud *et al.* 2001, Wenzl *et al.* 2004). Consequently, more dominant markers are required than codominant markers in order to construct linkage maps, and codominant markers are frequently preferred in map construction for their increased information per marker. The earliest published apple and pear linkage maps primarily utilized dominant marker types (Hemmat *et al.* 1994, Conner *et al.* 1997, Maliepaard *et al.* 1998, Iketani *et*

al. 2001), but the increased availability over time of codominant markers for apple and pear has resulted in their increased use (e.g., Gianfranceschi *et al.* 1998, Liebhard *et al.* 2002, Hemmat *et al.* 2003, Pierantoni *et al.* 2004). Most recent apple and pear linkage maps, however, have employed a combination of dominant and codominant markers (e.g., Yamamoto *et al.* 2002c, Liebhard *et al.* 2003b, Calenge *et al.* 2004, Dondini *et al.*

Table 4.2. Distribution of markers with respect to marker type and level of information across families.

	DArT Markers (No.)	SA SSRs (No.)	NZ SSRs* (No.)	NZ SNPs* (No.)	All Markers (No.)	Mapped Markers (No.)
Fully informative for all five families	13	7	NA	NA	20	11
Fully informative for four families	48	15	NA	NA	63	36
Fully informative for three families	62	8	NA	NA	70	64
Fully informative for two families	149	3	NA	NA	152	123
Fully informative for one family	206	13	10	26	255	174
Fully informative for no families, but partially informative for at least one	160	1	0	3	164	122
Uninformative	322	10	10	13	355	0
Total number of markers	960	57†	20†	42	1079	
Total number of mapped markers	458	43††	5††	24		530

Fully informative refers to markers heterozygous for 'Honeycrisp' and homozygous for the second parent.
* New Zealand markers applied only to 'Pitmaston' family
† Four SSRs assayed by both laboratories
†† Two mapped SSRs assayed by both laboratories

Table 4.3. Distribution of codominant markers segregating with respect to 'Honeycrisp', identified by source

Marker Type	Citation	Segregating Markers (No.)	Mapped Markers (No.)
SSR	Gianfranceschi *et al.*1998	1	0
	Yamamoto *et al.* 2002b	1	1
	Liebhard *et al.* 2002	18	11
	Silfverberg-Dilworth *et al.* 2006	16	7
	Celton *et al.* 2009	3	1
	van Dyk *et al.* 2010	34	26
	Total	73	46
SNP	Chagné *et al.* 2008	13	4
	Chagné, unpublished	7	3
	IASMA, unpublished	22	17
	Total	42	24
	Total	115	70

Table 4.4. Summary statistics of preliminary linkage groups for each data set. Minimum resolution of 1 cM assumed.

Family	Total Number of Preliminary Linkage Groups	Number of Preliminary Linkage Groups >0.5 cM*	Mean linkage group length (cM)*	Average Number of Markers per linkage group	Average Number of Unique Loci per Linkage Group**	Average distance between adjacent unique loci (cM)
Pooled data set	33	29	48.5	16.2	8.8	7.0
MN-1702	62	39	17.4	11.7	5.1	4.6
MN-1764	43	31	35.5	16.5	7.5	7.7
'Jonafree'	52	40	24.2	10.2	5.2	7.6
'Monark'	54	36	22.3	9.7	4.4	6.7
'Pitmaston'	49	32	33.3	14.2	7.0	5.9

*Linkage groups of length 0.0 cM excluded.
**Multiple collocated markers may be represented by a single unique locus.

2004, James *et al.* 2004, Kenis and Keulemans 2005, Kitahara *et al.*2005, Fernández-

Fernández *et al.* 2008, Igarashi *et al.* 2008, N'Diaye *et al.* 2008).

The predominance of the dominant DArT markers in this data set resulted in hundreds of preliminary linkage groups. The application of cluster analysis methods allowed for a data-driven alignment process, while substantially reducing the number of comparisons of linkage groups across families. For example, simply comparing the preliminary pseudo-testcross linkage groups with those of any single family would have required more than a thousand pairwise comparisons. In contrast, the cluster analysis eliminated the comparisons of linkage groups with no common markers and permitted the alignment of multiple linkage groups simultaneously, reducing the total number of alignments to a few dozen.

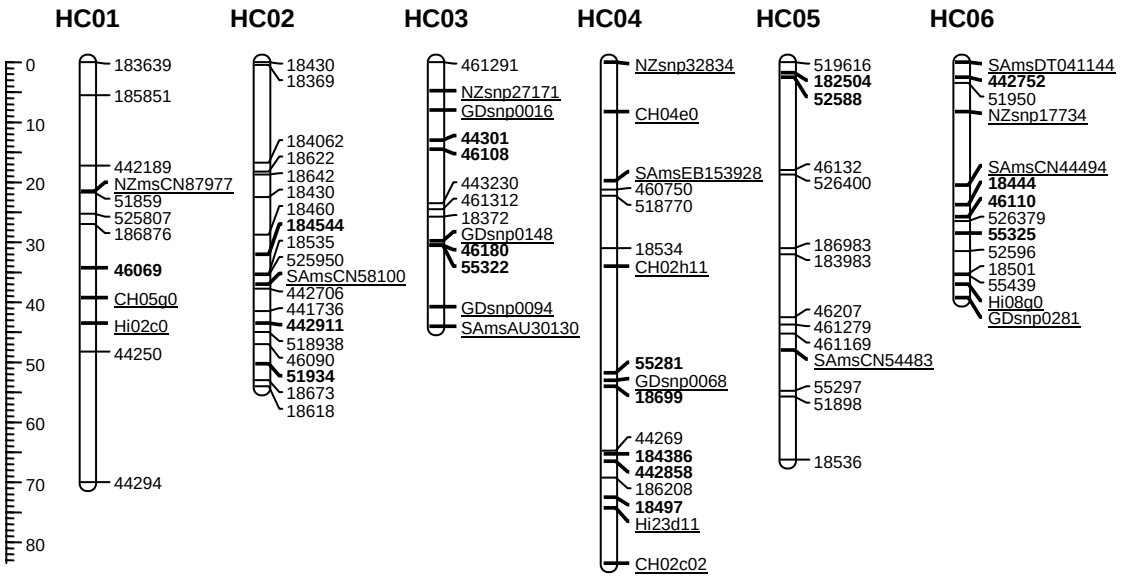


Figure 4.1. Final consensus maps for 'Honeycrisp' (resolution of 1 cM). Bold indicates markers homozygous for no parents; bold and italic indicates marker data available for only one family and heterozygous for both parents. Underlining indicates codominant markers. Single asterisks indicate that multiple markers were collocated. Double asterisks indicate DArT marker 461033, mapped onto two fragments of linkage group 7.

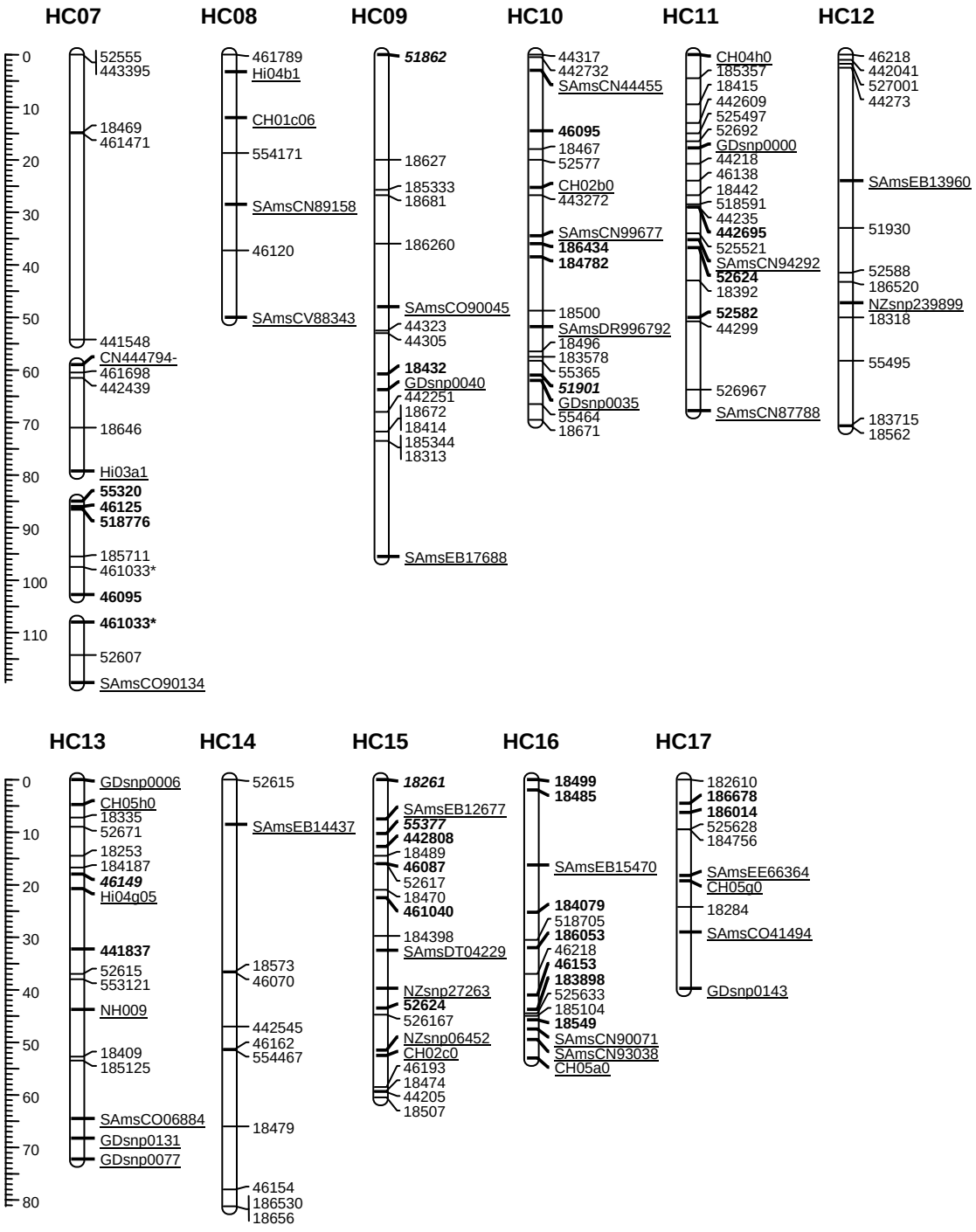


Figure 4.1 (continued).

The construction of the pooled pseudo-testcross data set capitalized on the large cumulative number of 'Honeycrisp'-derived trees in the study to construct robust preliminary and intermediate linkage groups. Marker orders in these linkage groups allowed for the incorporation of additional markers that were heterozygous for both parents. When common patterns were expressed across multiple families, these markers helped facilitate the alignment process (Fig. 4.2).

The length of the 'Honeycrisp' consensus map compares favorably with the integrated genetic map that was a product of the recently published genome sequence (Velasco *et al.* 2010), the 'Honeycrisp' genetic map exhibiting a total length equal to 83% of the integrated genetic map (Table 4.6). 'Honeycrisp' linkage groups correspond to an average length of 81% and a median length of 78% of their analogous integrated map linkage groups. Two 'Honeycrisp' linkage groups exceed 110% of their analogs (HC04 and HC09), and four do not surpass 60% in length of their analogs (HC03, HC06, HC15, and HC17).

The pedigree of 'Honeycrisp' does not contain any known common ancestry with any previously mapped cultivars (Cabe *et al.* 2005). Consequently, markers derived from these cultivars may not segregate in 'Honeycrisp'-derived materials, but are more likely to segregate than would markers derived from other species. The inclusion of DNA from 'Honeycrisp' in the development of the DArT marker array, however, ensured considerable numbers of segregating markers. This is consistent with the same DArT array yielding fewer segregating markers when applied to unrelated material (M.M. van Dyk, personal communication).

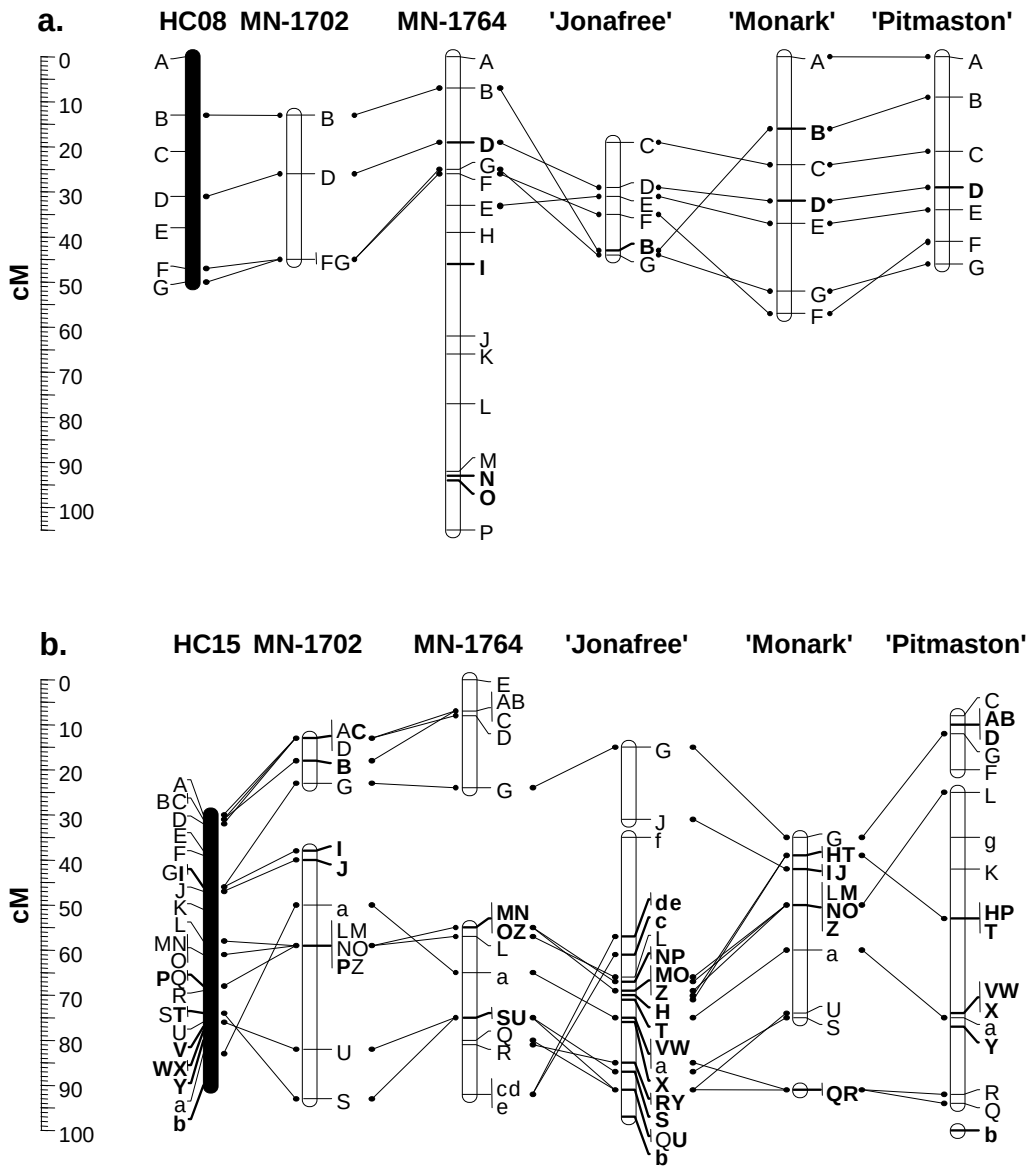


Figure 4.2. Illustration of alignment of preliminary family-specific linkage groups. Linkage groups correspond, from left to right, to the final consensus map, MN-1702 family, MN-1764 family, 'Jonafree' family, 'Monark' family, and 'Pitmaston' family, respectively. Bold font indicates homozygous for non-'Honeycrisp' parent. Homologous loci illustrated only between adjacent maps. a. Linkage group 08 (length of consensus map = 50 cM). Long tail of MN-1764 family linkage group (loci H-P) eventually mapped to another consensus linkage group. b. Linkage group 15 (length of consensus map = 60 cM). Locus labels do not correspond across the two images.

The limited number of previously published markers renders comparison with other genetic maps difficult. Common SSRs permitted the comparison of 'Honeycrisp' consensus maps to their analogs from the cultivar Anna (van Dyk *et al.* 2010). Five pairs of linkage groups had more than two common SSRs (Figure 4.3). Three linkage groups, HC08, HC10, and HC13, demonstrate common marker orders across the two cultivars. The remaining two, HC04 and HC16, exhibit putative inversions. HC16 (Figure 4.2), exhibits a large discrepancy in SSR order when compared to the 'Anna' map, whereas the marker order in HC04 only differs in the order of two terminal markers. The recently published integrated consensus map, produced in conjunction with the apple genome sequence (Velasco *et al*. 2010), also agrees with marker order in the 'Honeycrisp' map. Again, a paucity of common markers limits the comparison; four of the seventeen linkage groups have no common markers between the two maps, and only one of the HC07 fragments has any common markers. Marker order is perfectly conserved, however, in all four linkage groups with three or more markers are common to the two projects. These results suggest that the 'Honeycrisp' consensus maps are largely consistent with other published maps. While additional markers might resolve observed inconsistencies, they might also reinforce the different marker orders, a common characteristic of cross-cultivar linkage map comparisons (e.g., Kenis *et al*. 2008, N'Diaye *et al*. 2008, Van Dyk *et al*. 2010). Additional markers would likely resolve the relationships between the four fragments corresponding to HC07.

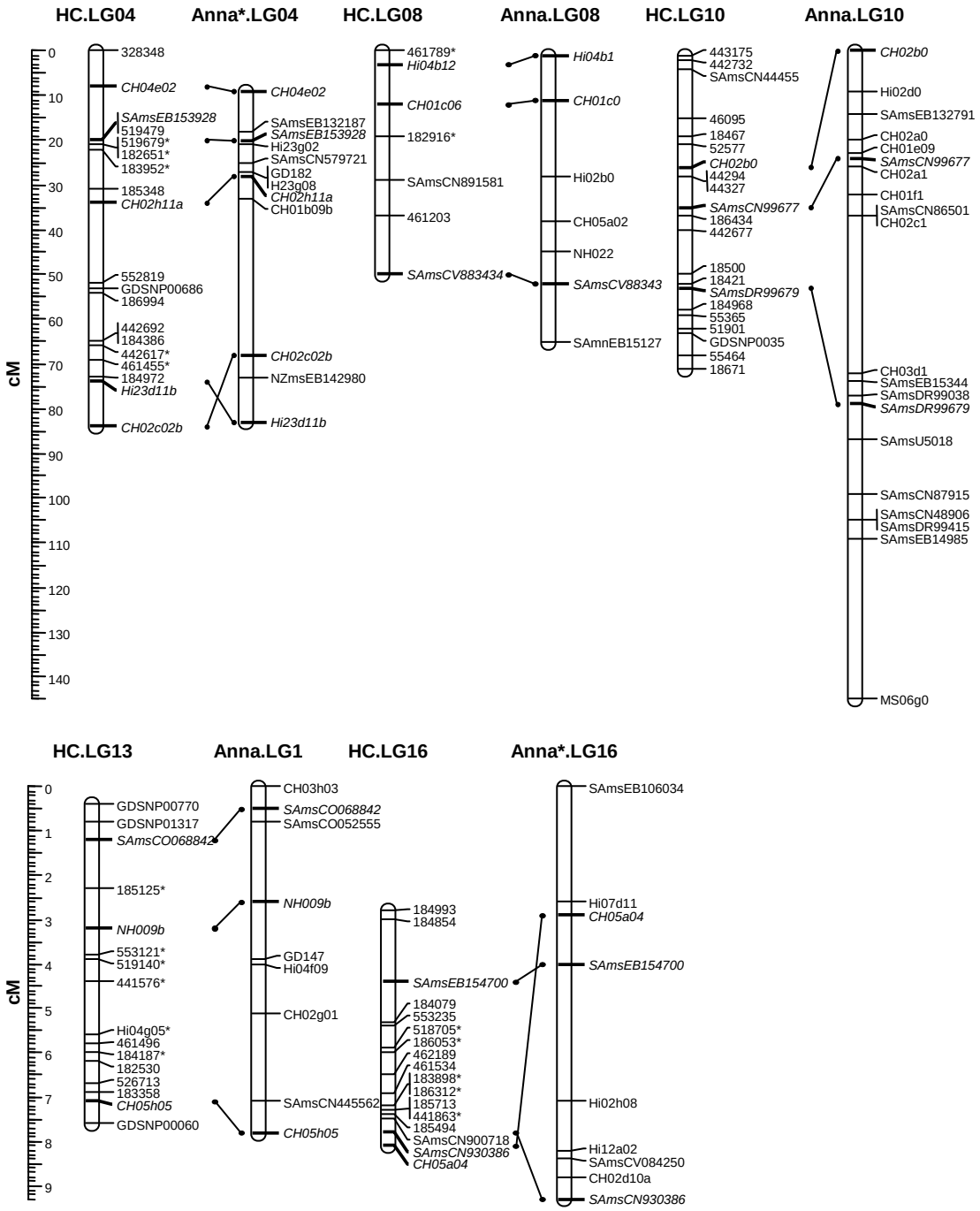


Figure 4.3. Comparison of linkage groups from ‘Honeycrisp’ and ‘Anna’ (van Dyk *et al.* 2010). HC refers to consensus ‘Honeycrisp’ linkage map, whereas Anna and Anna* refer to maps derived from ‘Golden Delicious’ x ‘Anna’ and ‘Sharpe’s Early’ x ‘Anna’ crosses, respectively.

The common ‘Honeycrisp’ parent across the five full-sib families resulted in high levels of contributions of each family to the consensus maps. Ukrainetz *et al.* (2008) noted highly variable family contributions when using multiple full-sib families of Douglas-fir and speculated that either variability in their DNA quality or variable levels of heterozygosity within the families were likely causes. In the present study, the absence of any known relatedness between the parents, coupled with the inclusion of the parental DNA in the development of the DArT array, resulted in comparable levels of heterozygosity across the five families. Additionally, all tissue samples were collected simultaneously from the same orchard, reducing environmentally induced variability in DNA quality across the five families. Finally, the current study implemented stricter criteria for inclusion of markers into linkage groups than did Ukrainetz *et al.* (2008).

4.5 Conclusions

This study demonstrates that existing breeding populations can be utilized for the construction of linkage maps of previously unmapped genotypes, in this case the apple cultivar Honeycrisp. N’Diaye *et al.* (2008) have constructed and aligned family-specific linkage maps from full-sib families with common parents, but the populations in their study had been used previously for map construction. In the present study, a large number of dominant markers of unknown map location were assayed across all five families, and supplemented with a small number of codominant markers selected specifically for their distribution across the linkage groups (Figure 4.1, Table 4.3). Additionally, the populations in the current study were fairly small, averaging 83.8

genotypes per family, compared to an average of 169.0 genotypes per family in N'Diaye *et al.* (2008).

An iterative procedure was performed in which first only fully informative marker data were utilized in linkage map construction, and subsequently partially informative marker data were incorporated. During the first phase, a pooled family data set was constructed and mapped, followed by the construction and mapping of five family-specific data sets. The family-specific maps were largely concordant with the pooled family map, providing support for marker order prior to alignment.

This process allows for the construction of a consensus map from a set of families that are individually inadequate to construct a meaningful map. The inclusion of more than one family increased the number of useful markers, relative to what would be expected with a single family, by increasing the likelihood that a marker would be informative for at least one family. Additionally, the inclusion of more than two families increases the likelihood that a marker is informative for more than one family, facilitating the alignment of the family-specific linkage groups. Together, these facts enabled the mapping of the cultivar Honeycrisp using relatively small, pre-existing mapping populations, rather than a large, specially constructed mapping population.

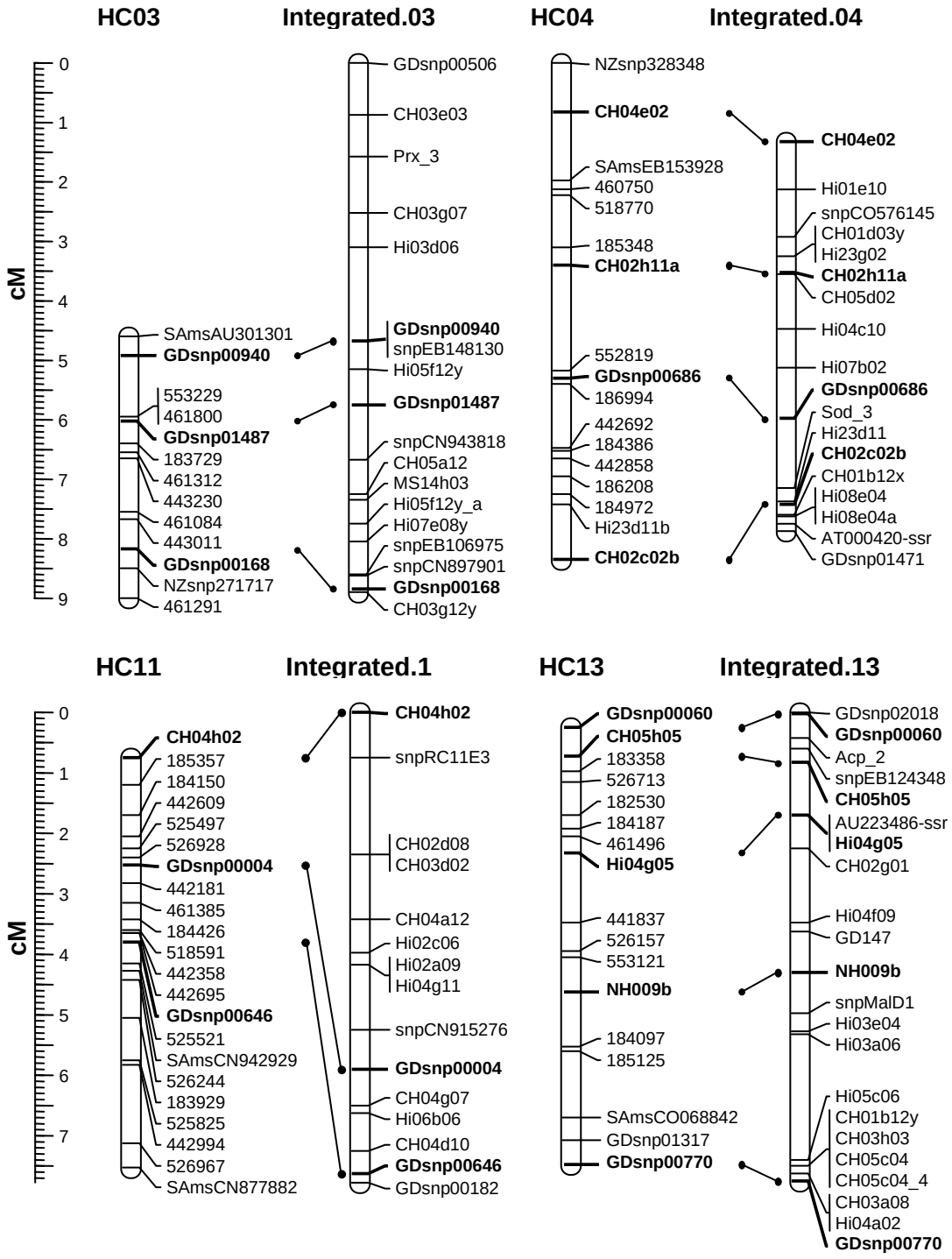


Figure 4.4. Comparison of 'Honeycrisp' consensus map and Integrated Consensus map (Velasco *et al*. 2010). Only those linkage groups with three or more common markers shown. Bold font indicates common markers. Vertical axis in cM.

Table 4.5. Summary statistics for consensus linkage groups.

Final LG	Length (cM)	Number of Markers	Number of Loci*	Density (cM/Locus)
HC01	70	30	14	5.00
HC02	54	37	20	2.70
HC03	44	23	13	3.38
HC04	84	40	16	5.25
HC05	66	41	15	4.40
HC06	39	21	12	3.25
HC07A	54	13	4	13.50
HC07B	20	10	5	4.00
HC07C1	18	15	6	3.00
HC07C2	11	3	3	3.67
HC08	50	11	8	6.25
HC09	95	24	13	7.31
HC10	70	41	20	3.50
HC11	68	44	23	2.96
HC12	71	40	13	5.46
HC13	72	23	16	4.50
HC14	81	22	9	9.00
HC15	60	30	19	3.16
HC16	53	35	15	3.53
HC17	40	27	13	3.08
Total	1120	530	257	4.36

* Locus location resolution of approximately 1 cM.

Table 4.6. Comparison of linkage group lengths between 'Honeycrisp' and integrated map (Velasco *et al*. 2010)

Linkage group	'Honeycrisp' (cM)	Integrated (cM)	Relative length (%)
LG01	70	90	78%
LG02	54	85	64%
LG03	44	89	49%
LG04	84	66	127%
LG05	66	92	72%
LG06	39	76	51%
LG07	103*	59	92%
LG08	50	74	68%
LG09	95	73	130%
LG10	70	95	74%
LG11	68	78	87%
LG12	71	74	96%
LG13	72	78	92%
LG14	81	77	105%
LG15	60	117	51%
LG16	53	58	91%
LG17	40	75	53%
Total	1120	1356	83%

* Sum of four fragment lengths

5 Identification of Quantitative Trait Loci for Apple Fruit Texture Traits

5.1 Introduction

Domesticated apple (*Malus pumila* Mill., synonymous with *Malus* x *domestica* Borkh.; Mabberley *et al.* 2001, Velasco *et al.* 2010) has inspired considerable interest in the application of marker-aided or marker-assisted selection (MAS; e.g., Luby and Shaw 2001, Tartarini and Sansavini 2003, Dirlewanger *et al.* 2004, Gardiner *et al.* 2007, Hancock *et al.* 2008, Bus *et al.* 2009, Moriya *et al.* 2009). MAS has the potential to aid in the identification of genotypes in which numerous desired alleles are present, yet which do not express superior phenotypes due to incomplete representation of an optimal genetic suite (Knapp 1998); whereas such genotypes may not be commercially viable, they can have potential value as parental material within breeding programs.

Members of the genus *Malus* (apple and crabapple; Rosaceae) are highly heterozygous, typically self-incompatible, and suffer substantially from inbreeding depression (e.g., Broothaerts *et al.* 2004, Vanwynsberghe *et al.* 2005). Additionally, apple does not produce fruit when grown from seed for five to ten years in quantities sufficient for preliminary evaluation. The identification of markers corresponding to traits of interest would enable the molecular screening of many genotypes at the small seedling stage, before large amounts of time, land, and money have been allocated to bringing those seedlings to maturity (e.g., Luby and Shaw 2001, Liebhard *et al.* 2003b).

The domesticated apple, other *Malus* species, and several other members of the pome fruits (subtribe Pyrinae, tribe Pyreae, synonymous with subfamily Maloideae or Pomoideae; Potter *et al.* 2007) have been studied extensively with respect to the genetic underpinnings of numerous traits. Numerous quantitative trait loci (QTLs) have been

identified and mapped in apple and pear, the majority for disease and pest resistance (Iketani *et al.* 2001, Durel *et al.* 2003, Dondini *et al.* 2004, James *et al.* 2004, Calenge *et al.* 2004, 2005, Khan *et al.* 2006, Calenge and Durel 2006, Pierantoni *et al.* 2006, Soriano *et al.* 2009, Moriya *et al.* 2010), growth characteristics (Conner *et al.* 1998, Segura *et al.* 2007, 2009, Kenis and Keulemans 2007, van Dyk *et al.* 2010), or fruit quality traits (Costa *et al.* 2005, 2008, 2010, Harada *et al.* 2000, Zhu and Barritt 2008, Zini *et al.* 2005, Davey *et al.* 2006). A few studies, however, have investigated QTLs for fruit texture traits (King *et al.* 2000, 2001, Liebhard *et al.* 2003a, Kenis *et al.* 2008). King *et al.* (2000), working with apple, mapped QTLs for three physical measurements (two penetrometer-derived firmness values and one acoustic resonance-derived stiffness measure), as well as seven sensory measures. Among their results, they identified several putative QTLs for the sensory traits of crispness and hardness (in their study, chewing and biting, respectively). Following their first study, King *et al.* (2001) then mapped QTLs for a larger number of physical parameters derived from tensile strength and compression tests. Upon comparison of the two sets of QTLs, the authors identified several QTLs on linkage group 16 corresponding to several sensory traits, including crispness, hardness, juiciness, and several physical measures, primarily derived from the tensile tests. In both studies, however, they identified significant genotype x environment interaction terms. Liebhard *et al.* (2003a), investigating physical texture traits, found results consistent with those of King *et al.* (2000, 2001).

Fruit texture traits, such as crispness, firmness, juiciness, and mealiness, are cited as primary components of consumer choice when purchasing fresh fruit (Péneau *et al.* 2006), so improved texture characteristics are central goals in apple breeding programs

worldwide. Like other quantitative traits, fruit texture traits are assumed to be under multigenic control (e.g., King *et al*. 2000, 2001, Liebhard *et al*. 2003a, Kenis *et al.* 2008, Soglio *et al.* 2009), impeding rapid cultivar development.

The commercially successful cultivar Honeycrisp, released by the University of Minnesota in 1991, is known for its high degrees of crispness and juiciness, as well as for its long storage capacity (e.g., Mann *et al.* 2005). Several thousand seedlings, exhibiting a wide range of texture traits, have been created at the University of Minnesota by crossing 'Honeycrisp' with numerous other cultivars. This study examines five full-sib families from among these seedlings to identify and map QTLs for the parental 'Honeycrisp'.

5.2 Materials and Methods

5.2.1 Plant Materials

Five full-sib families with 'Honeycrisp' as a common parent were selected from amongst the breeding population at the University of Minnesota's Horticultural Research Center in Chanhassen, Minnesota, USA (44º52′ N, 93º36′ W). Criteria for inclusion were number of fruit-bearing genotypes, high levels of variability in texture traits observed in previous years, reciprocal crosses within families, when possible, and no common ancestors other than through the 'Honeycrisp' parent.

The five full-sib families comprised more than 600 trees from crosses made between 1992 and 1998. The other parents were MN-1702 and MN-1764, 'Jonafree',

Table 5.1. Number of genotypes and molecular markers included in QTL analysis and their distribution across the five full-sib families and across three years, plus discriminating traits behind the inclusion of non-'Honeycrisp' parents into the University of Minnesota apple breeding program.

Family (Non-'Honeycrisp' parent)	Genotypes included in QTL analysis (No.)	Distribution of genotypic representation across years (2005/2006/2007)	Frequency of phenotypic evaluation per genotype (1yr/2yrs/3ys)	Number of markers included in QTL analysis (Dominant /Codominant)
MN-1702	50	33/41/27	15/19/16	114 (108/6)
MN-1764	115	77/76/56	41/54/20	136 (100/36)
'Jonafree'	53	42/22/42	10/33/10	128 (103/25)
'Monark'	73	37/62/24	35/26/12	121 (96/25)
'Pitmaston'	53	38/35/24	17/28/8	151 (102/49)
Cumulative	344	227/236/172	118/160/66	232 (169/63)

'Monark', and 'Pitmaston Pineapple' (hereafter referred to as 'Pitmaston'). Only those progeny confirmed to be consistent with their putative parentage were retained. The five non-'Honeycrisp' parents were initially included in the breeding program for a variety of different phenotypic traits, including, but not limited to, texture (Table 5.1). 'Monark', for instance, was included in the program because it is a cultivar with early fruit maturation, whereas 'Pitmaston Pineapple' X 692 was incorporated for its unusual flavor.

5.2.2 Phenotypic Data

Phenotypic traits assayed for QTLs were three sensory traits (crispness, firmness, and juiciness) and one instrumental trait (total work to fracture). Phenotypic values for each genotype-by-year combination were derived from a best linear, unbiased prediction

(BLUP) analysis (e.g., Bernardo 2002, Piepho *et al*. 2003, 2008), in which pedigree, temporal, and panelist data were incorporated to calculate predicted values for these four traits (e.g., Segura *et al.* 2009). The three sensory traits were adjusted by removing panelist effects. The data underlying the instrumental trait were those derived from the same fruits as had been assayed by the sensory panelists, referred to as non-intact fruit in Chapter 3. All traits were assayed for each of the three years. (See Chapter 3 for more details.)

5.2.3 Molecular Data

Molecular phenotypic data were collected for a combination of DArT markers, SSRs, and SNPs, and were then used develop a consensus map across the five families using JoinMap (Van Ooijen 2006). Marker location data, linkage phase, and classification estimates for each linkage group were extracted for each family from the JoinMap output files; these data reflect the different contributions of each family to the consensus map. Linkage groups were designated as "HC" for "'Honeycrisp' Consensus" and numbered in agreement with those presented by Maliepaard *et al.* (1998). (See Chapter 4 for details.)

5.2.4 QTL Analysis

Data were pooled in two manners for QTL analyses. Multiple-year values were calculated by subtracting the year and year-by-genotype predicted values from the BLUP analysis (e.g., Segura *et al.* 2009) and arithmetically averaging the adjusted values. Additionally, an all-family composite data set was constructed for simultaneous QTL

analysis. For each family, only those marker data for which 'Honeycrisp' was heterozygous and the second parent was homozygous were selected, and the five resulting data sets were pooled. All genotypes were included for which both texture traits and molecular data were characterized.

Prior to QTL analyses, linkage maps were simplified by selecting representative collocated markers, reducing the marker density to no more than 5 cM. Markers were preferentially retained on a family-by-family basis if they were homozygous for the non-'Honeycrisp' parent. Markers common to multiple families were also preferentially retained to allow for later comparison of QTLs across families.

Single-trait QTL analyses were performed for each of the five family-specific data sets as well as for the multiple-family data using MapQTL (Van Ooijen 2004). Interval mapping (IM) was performed, followed by restricted multiple QTL model (rMQM) mapping, with a step size of 1.0 cM. Discrete intervals with maximum LOD scores of 3.0 or greater during the IM stage were deemed putative QTLs, which were then assessed as cofactors for the multiple subsequent iterations of rMQM mapping (e.g., Davey *et al.* 2006, Durel *et al.* 2009, Segura *et al.* 2009, van Dyk *et al.* 2010). Family-specific QTLs were identified when maximum LOD values exceeded the 95% genome-wide (GW) significance level, as identified by the 95^{th} percentile of 1000 permutations (Van Ooijen 2004). Genotypic effects for the 'Honeycrisp'-derived markers were estimated as described in Van Ooijen (2009), allowing for the identification of linkage group intervals over which correlations between markers and phenotypic values were consistently positive or consistently negative across families or years. The results of the

separate family-by-year-by-trait combinations were graphically displayed by box-and-whisker plots (Voorrips 2002).

5.3 Results

Restricted multiple QTL model (rMQM) mapping yielded QTLs distributed across all of the linkage groups except for linkage groups HC08 and HC15 (Figure 5.1, Table 5.2). Additionally, two linkage groups, HC06 and HC13, exhibited QTLs for only one trait within a single family. The remaining thirteen linkage groups exhibited QTLs at for least two traits within at least two families. Furthermore, every linkage group that expressed any QTLs contained at least one with a maximum LOD score in excess of the corresponding 99% genome-wide significance level, as expressed by solid bars (Figure 5.1).

Among these were several sets of putative QTLs corresponding to overlapping linkage group intervals, yet distributed across subsets of the five families, three years, and four traits. Inclusion of linkage phase information complicates the picture but numerous collocated QTLs share common linkage phase status. Among the most striking illustrations are linkage groups HC02, HC11, HC14, and HC16, all of which contain collocated QTLs with common linkage phase, representing at least two years and at least two families (indicated by gray shading in Figure 5.1).

The strict pseudo-testcross strategy applied to the pooled, five family data set yielded only five QTLs, distributed across only three linkage groups (Figure 5.2). In addition to being less informative than the single family analyses, these QTLs are inconsistent with the single family analyses. Most inconsistent are two QTLs associated

with a single, terminal marker on a fragment of HC07 which is otherwise devoid of QTLs, bringing into question the validity of these results.

5.4 Discussion

The stability of QTLs across years, across locations, and across genetic environments is essential if QTLs are to be incorporated into marker-assisted selection programs. Without an understanding of QTL stability, MAS decisions can be based upon spurious observations, result in a non-directed reduction in genetic variability (e.g., Knapp 1998). Repeatability of QTLs across years and locations (physical environments) can yield markers for selection within a family, whereas consistency of QTLs across families (genetic environments) can identify robust markers for broader breeding populations. This study, with multiple families assayed over multiple years within a common set of physical environments, allows for the assessment of the robustness of 'Honeycrisp'-derived QTLs for fruit texture traits.

Multiple-year or multiple-environment assays are well-established components of mapping studies, especially with respect to disease resistance traits (e.g., King *et al.* 1998, Calenge and Durel 2006, Pierantoni *et al.* 2007). King *et al.* (2000) assayed several apple fruit texture traits, using instrumental methods, and identified QTLs common over two years. Similarly, Kenis *et al.* (2008) identified QTLs for instrumentally measured fruit firmness, as well as other fruit quality traits, that were common across two years.

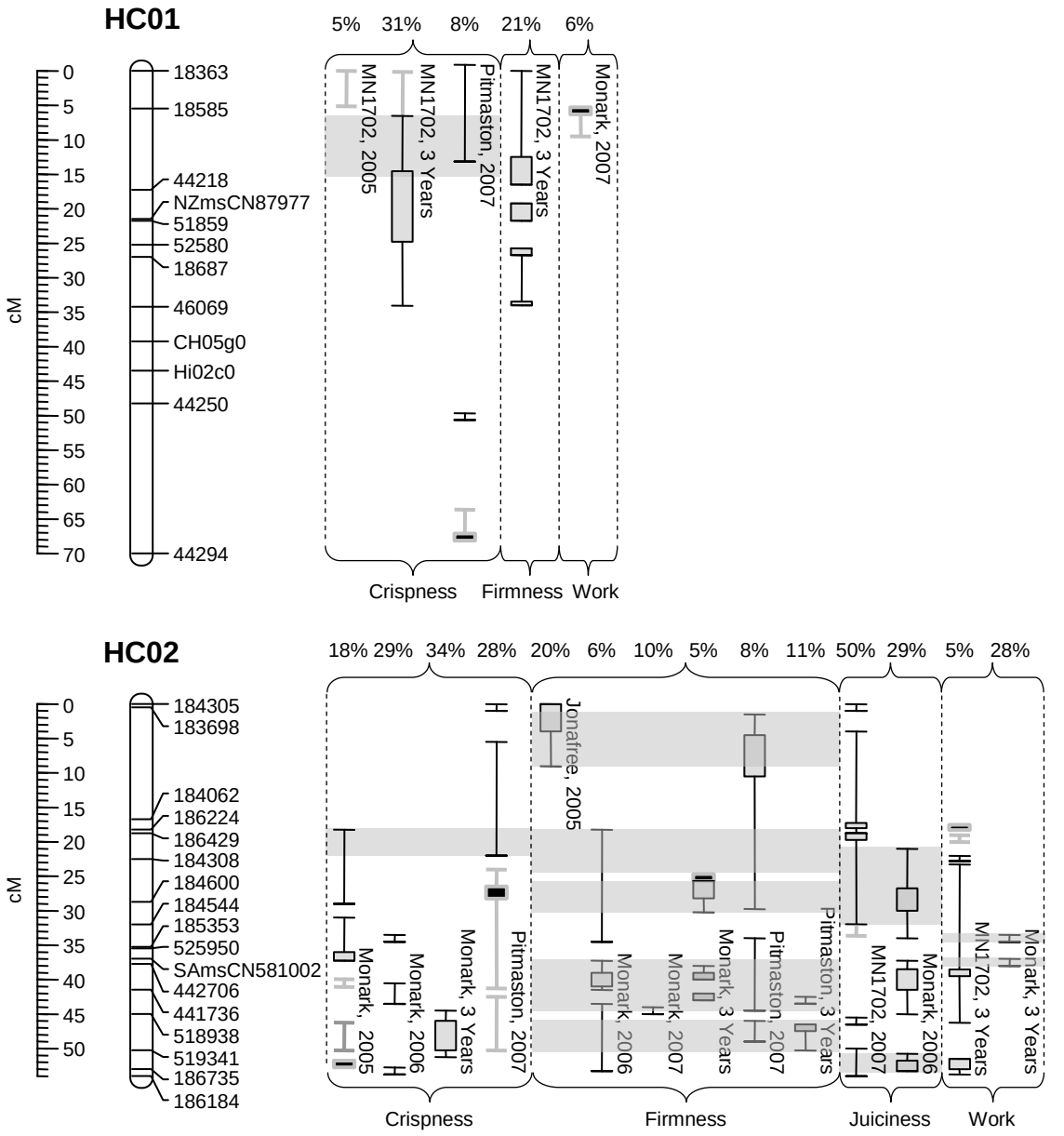


Figure 5.1. QTL distribution across linkage groups. Brackets separate traits. Boxes indicate intervals over which LOD scores exceed genome-wide 99% confidence level and are within one unit of the maximum LOD value for the linkage group. Whiskers represent intervals with LOD scores in excess of the genome-wide 95% confidence level. Thin black boxes and whiskers indicate positive correlation between marker presence and phenotypic trait, whereas thick gray boxes and whiskers indicate negative correlation. Horizontal shading indicates intervals of common linkage phase across at least two families and at least two years. Note that linkage groups HC08 and HC15 exhibited no QTLs and are not presented. Also note that linkage group HC17 is presented with HC09 and HC10. Percentage of variability explained by QTLs expressed across the top of brackets. Vertical scale in cM.

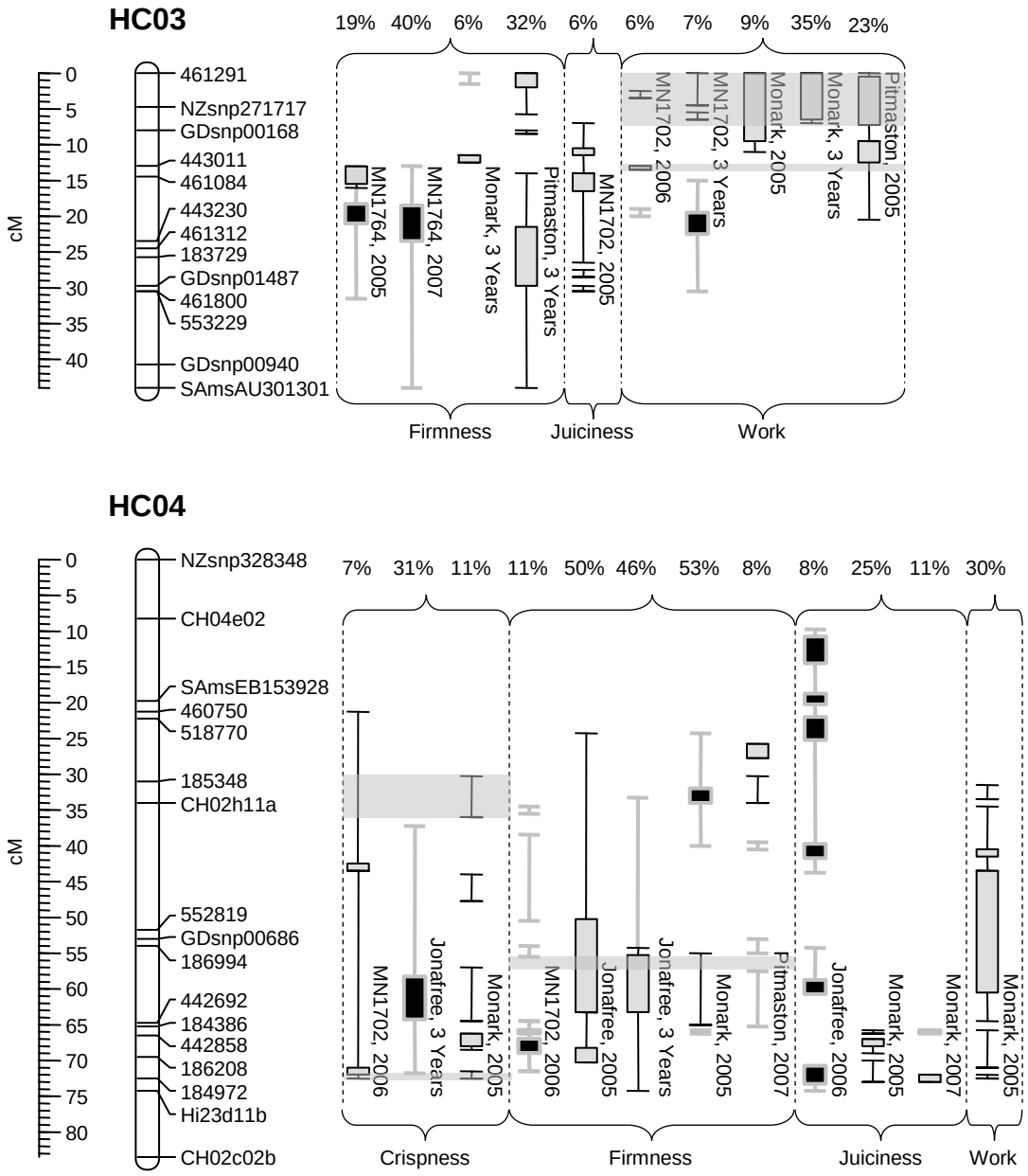
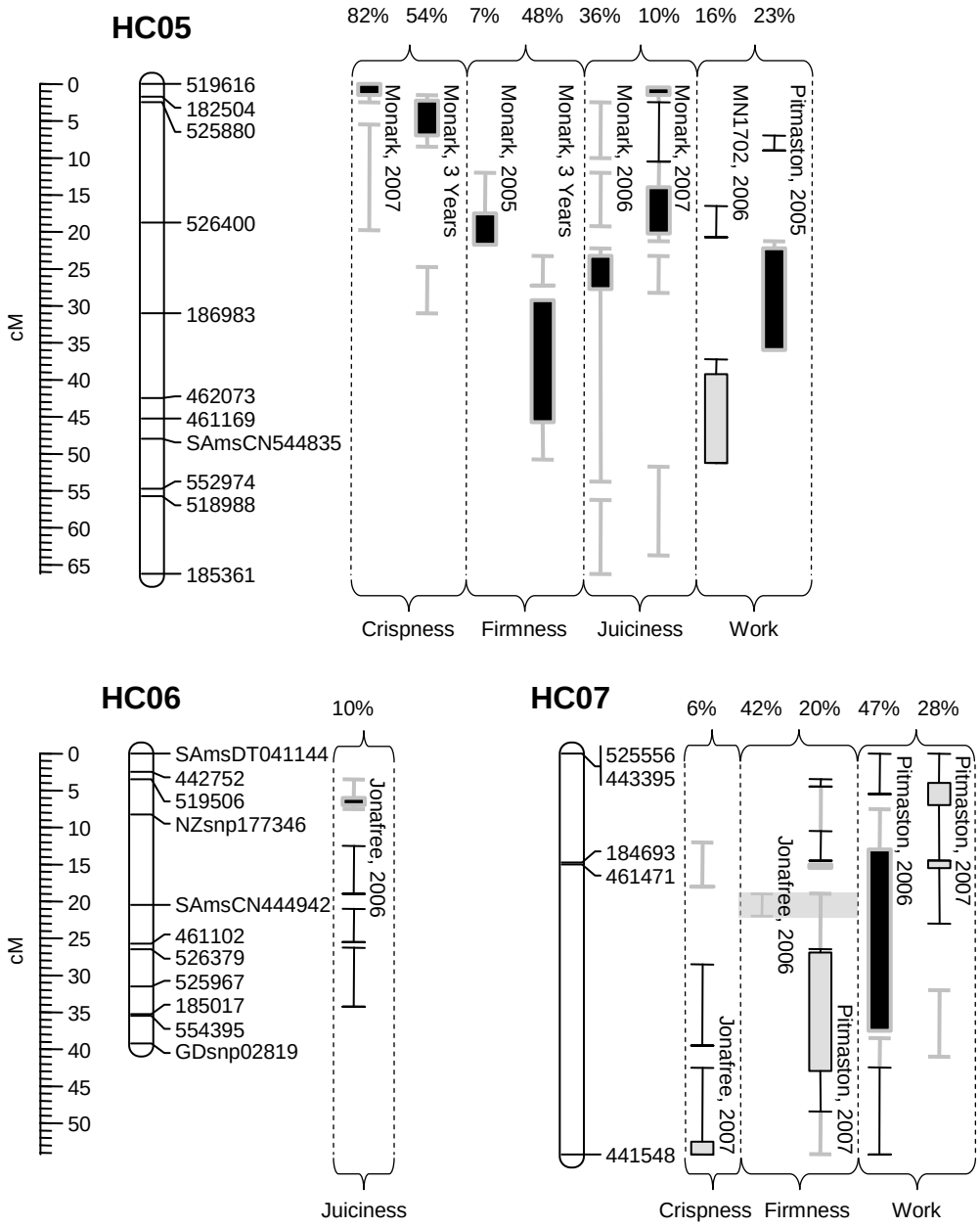


Figure 5.1. (Continued)



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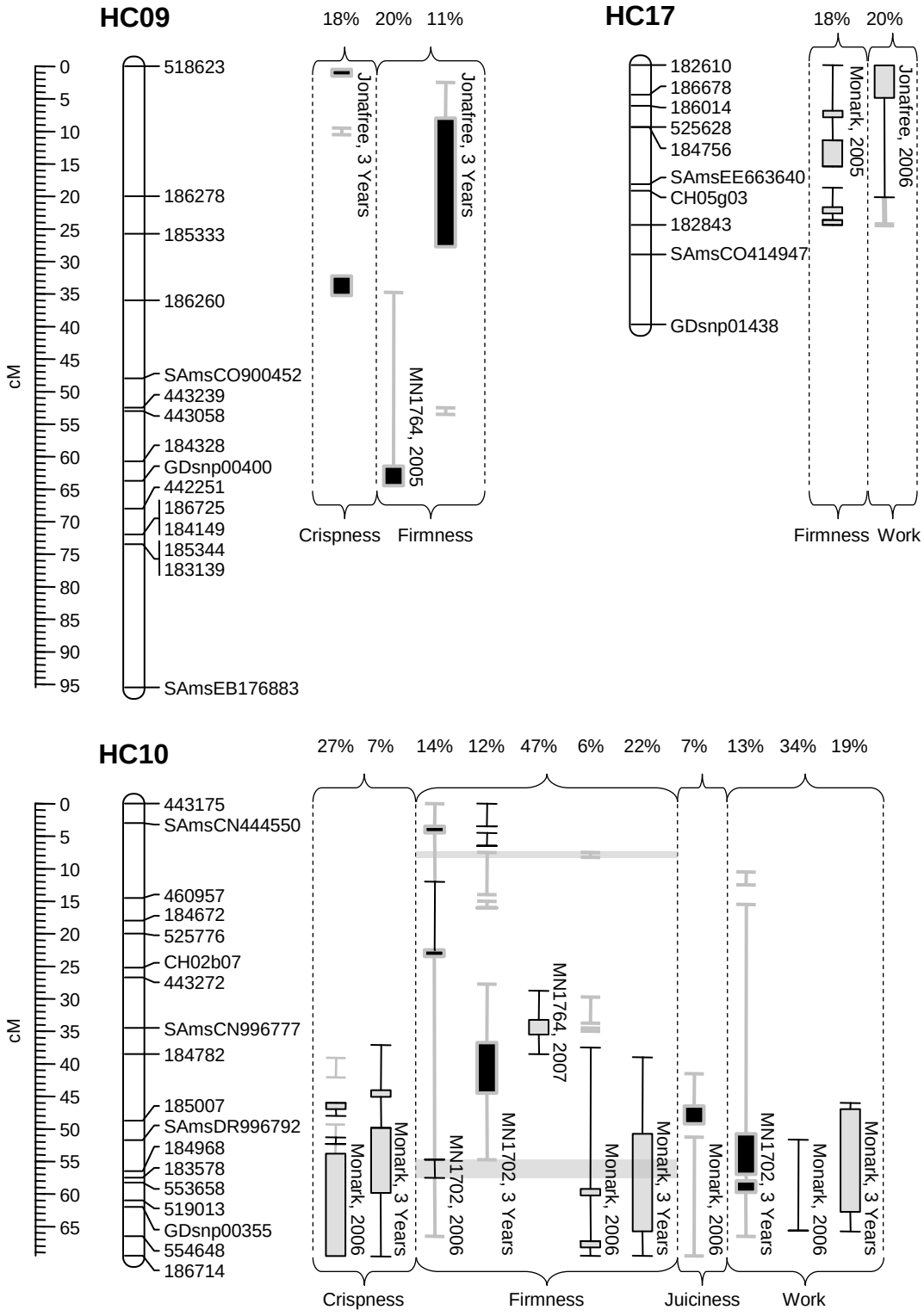

Figure 5.1. (Continued)

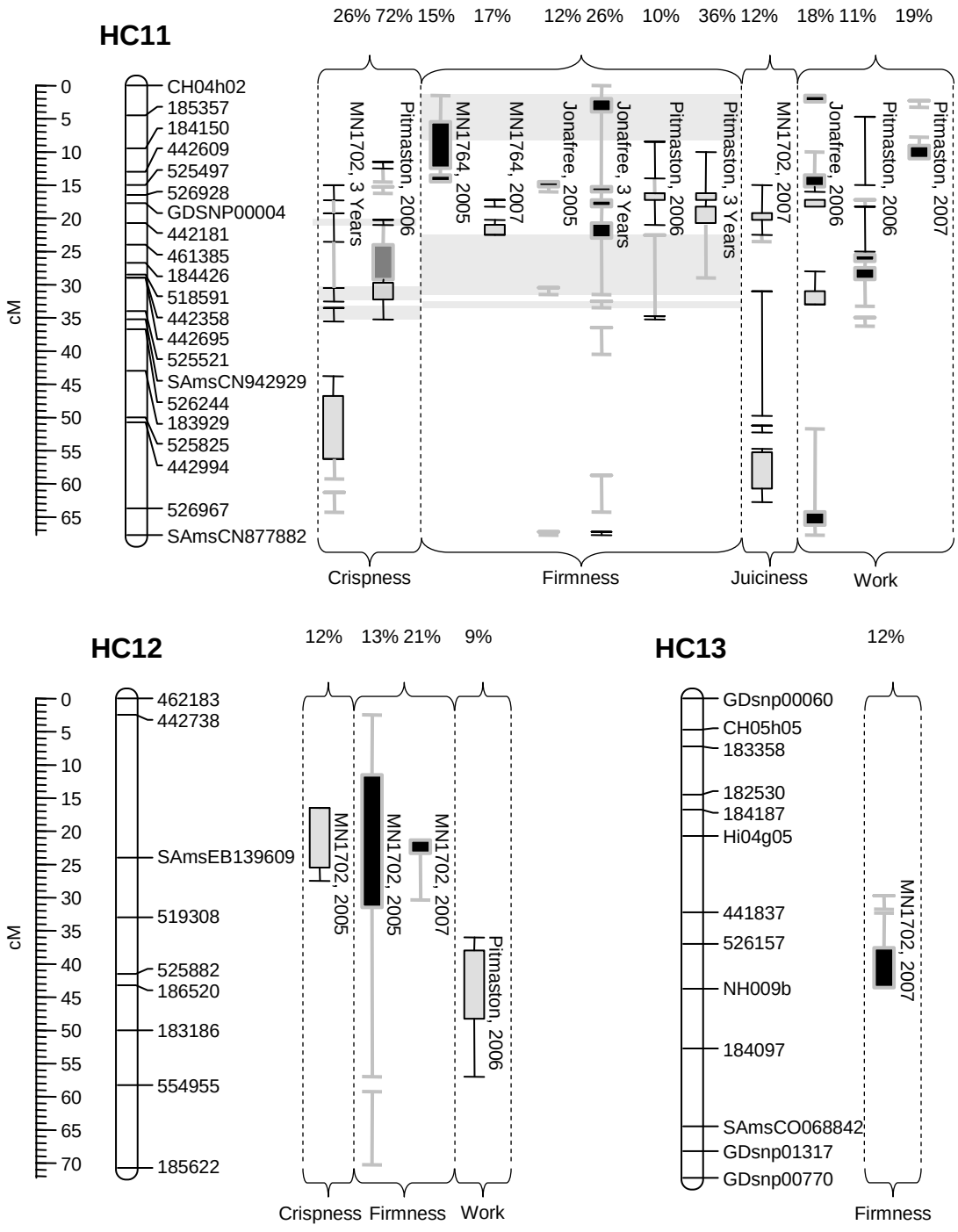

Figure 5.1. (Continued)

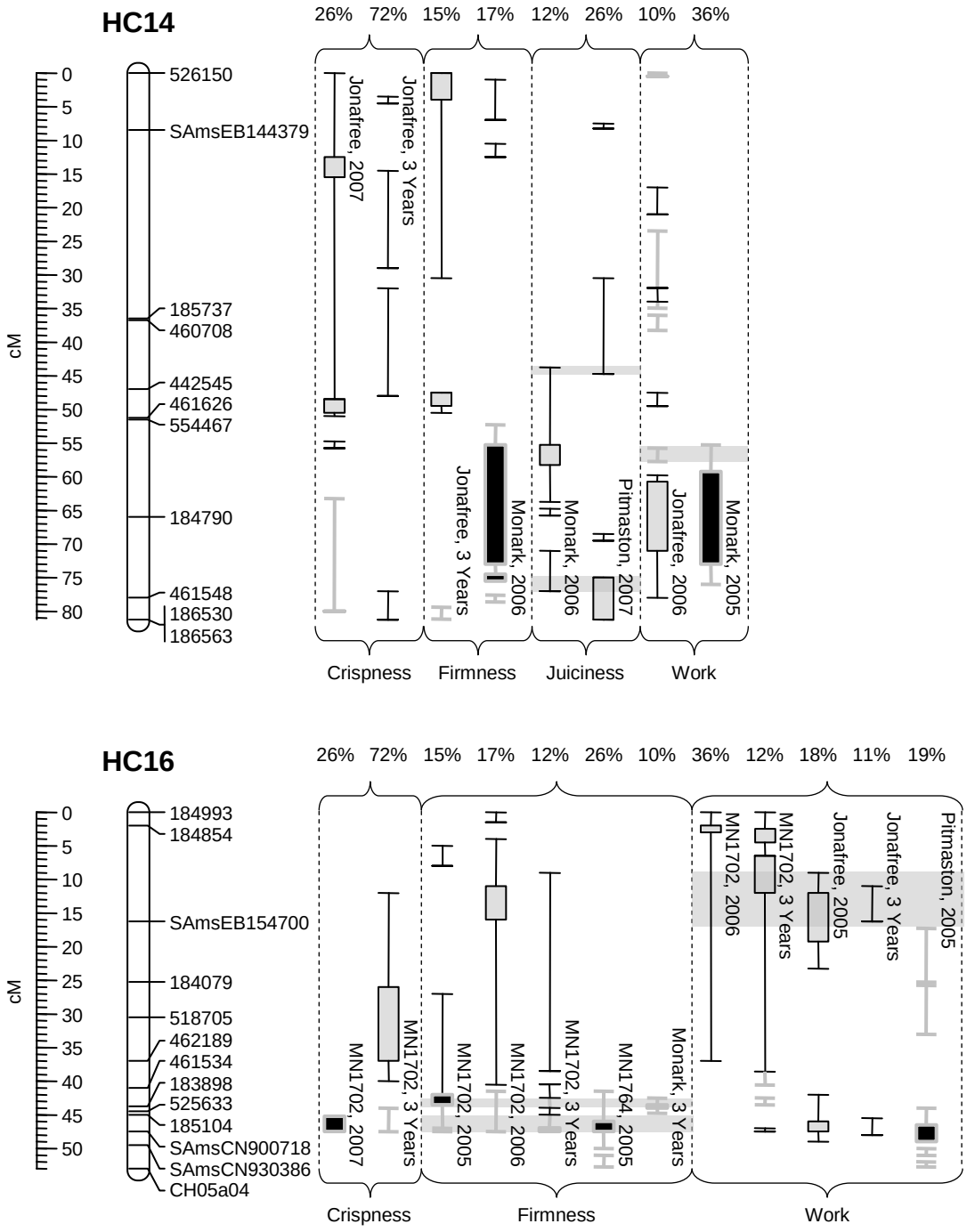

Figure 5.1. (Continued)

Table 5.2. Estimated percentage of variability accounted for by putative QTLs, by trait, linkage group, family, and year. All5 refers to pooled, multiple family QTL analysis. 3Years refers to pooled, multiple year QTL analysis. Linkage groups HC08 and HC15 exhibited no QTLs and are not presented.

	Crispness				Firmness				Juiciness				Work			
HC01	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years
MN1702	5.1%			30.9%				21.2%								
MN1764																
Jon																
Mon											5.7%					
Pit			8.3%													
All5																
HC02	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years
MN1702											50.2%					4.6%
MN1764																
Jon					19.5%											
Mon	17.9%	28.9%		33.7%		6.3%	9.9%	5.2%		29.3%						28.3%
Pit			28.0%				8.3%	11.1%								
All5																5.6%
HC03	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years
MN1702									6.4%					5.8%		7.0%
MN1764					18.5%		39.8%									
Jon																
Mon								5.6%					8.9%			34.9%
Pit								32.2%					23.4%			
All5					21.2%			7.5%								
HC04	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years
MN1702		6.8%				10.9%										
MN1764																
Jon				31.4%	49.6%			45.7%		7.9%						
Mon	11.1%				53.3%				24.7%		11.0%		30.0%			
Pit							7.8%									
All5																

Table 5.2. (Continued)

	Crispness				Firmness				Juiciness				Work			
HC05	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years
MN1702														15.7%		
MN1764																
Jon																
Mon			82.0%	54.8%	7.3%			47.8%		35.7%	9.5%					
Pit													23.3%			
All5																
HC06	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years
MN1702																
MN1764																
Jon										10.1%						
Mon																
Pit																
All5																
HC07	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years
MN1702																
MN1764																
Jon			6%*			42.2%*										
Mon																
Pit							20.2%*							47.4%*	28.2%*	
All5			10%**	6.3%**												
HC09	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years
MN1702																
MN1764					20.1%											
Jon				18.4%				10.5%								
Mon																
Pit																
All5																

Table 5.2. (Continued)

HC10	Crispness				Firmness				Juiciness				Work			
	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years
MN1702						13.6%		12.1%								12.8%
MN1764							47.4%									
Jon										6.7%						
Mon		26.8%		6.5%		6.3%		22.2%						33.6%		19.0%
Pit																
All5																

HC11	Crispness				Firmness				Juiciness				Work			
	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years
MN1702				25.9%							12.3%					
MN1764					14.9%		16.6%									
Jon					11.9%			25.5%						18.3%		
Mon																
Pit		72.4%				10.2%		35.8%						11.0%	18.5%	
All5																

HC12	Crispness				Firmness				Juiciness				Work			
	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years
MN1702	12.1%				13.0%		21.0%									
MN1764																
Jon																
Mon																
Pit														9.0%		
All5																

HC13	Crispness				Firmness				Juiciness				Work			
	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years	2005	2006	2007	3Years
MN1702			12.1%													
MN1764																
Jon																
Mon																
Pit																
All5																

Table 5.2. (Continued)

Crispness

HC14	2005	2006	2007	3Years
MN1702				
MN1764				
Jon			10.0%	23.2%
Mon				
Pit				
All5				

HC16	2005	2006	2007	3Years
MN1702			24.4%	11.4%
MN1764				
Jon				
Mon				
Pit				
All5				

HC17	2005	2006	2007	3Years
MN1702				
MN1764				
Jon				
Mon				
Pit				
All5				

Firmness

	2005	2006	2007	3Years
				35.7%
		10.9%		

	2005	2006	2007	3Years
	5.9%	16.4%		34.2%
	36.5%			
				5.4%

	2005	2006	2007	3Years
	30.9%			

Juiciness

	2005	2006	2007	3Years
		9.7%		
			16.8%	

	2005	2006	2007	3Years

	2005	2006	2007	3Years

Work

	2005	2006	2007	3Years
		11.9%		
	17.4%			

	2005	2006	2007	3Years
		12.4%		12.5%
	49.8%			25.2%
	6.1%			

	2005	2006	2007	3Years
		23.4%		

* Linkage group fragment HC07A.

** Linkage group fragment HC07B. See Chapter 4, Figure 4.1.

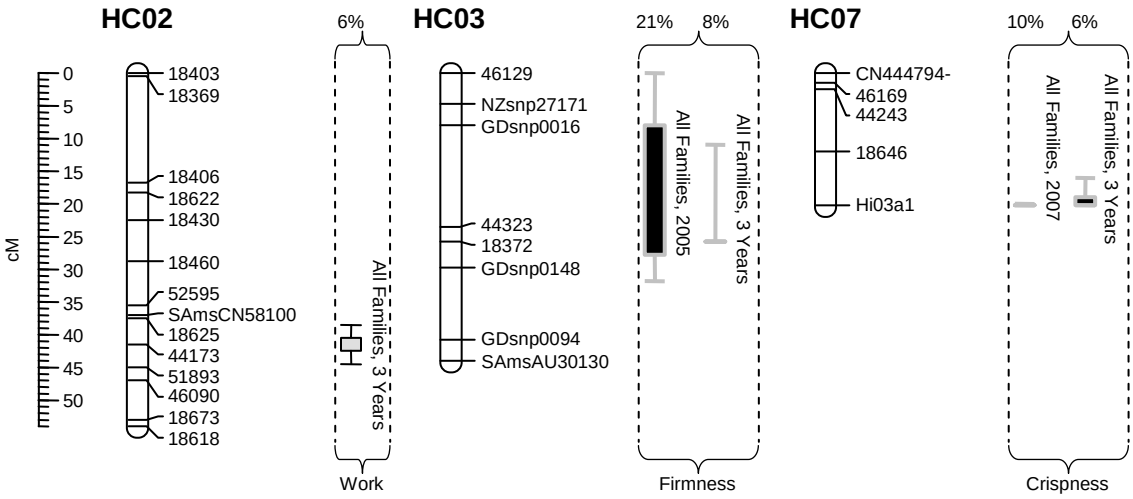

Figure 5.2. QTL distribution across linkage groups for pooled, five family data set. See Figure 5.1 for comments. Note that HC07 is a separate fragment from that illustrated in Figure 5.1.

Approximately 8% of all possible trait-by-family-time period-by-linkage group combinations (100 out of 1360) expressed putative QTLs. Undoubtedly, some of the single-year QTLs are likely statistical artifacts (false positives; e.g., Bernardo 2004), especially when the differing patterns of linkage phase are observed. Some of the single-year QTLs, however, likely reflect inter-annual variability in fruit texture traits, consistent with the statistically significant year-by-family interactions identified in the best linear unbiased prediction (BLUP) analysis (see Chapter 3).

The interpretation of the percentage of variability for which the robust QTLs account is speculative, due to the variability of estimates associated with the single-year (and three year average) QTLs (Table 5.2). For example, the robust crispness QTL on HC01 represents two constituent QTLs estimated to account for 8.3% and 30.9% of total variability. When the three other robust QTLs (on HC02, HC04, and HC11) are included

for consideration, the summed percentages range from 58.9% to well in excess of 100%, an impossible scenario. When one considers the infrequency with which QTLs align across families and time periods, it is probably prudent to err on the side of caution, and assume that the lowest estimates are the most likely.

The QTLs on linkage group HC03 for total work to fracture stand out as an example of collocated single-year and multiple-year QTLs. Particularly noteworthy is that although these QTLs were derived from three families, they all originate from 'Honeycrisp'. Within the interval of overlap, three of the QTLs have LOD scores in excess of their corresponding 99% genome-wide significance levels, a highly unlikely scenario if these QTLs were indeed false positives. In contrast, the solitary QTL on linkage group HC06 for sensory juiciness in the 'Jonafree' family in 2006 stands out as a likely false positive, its LOD scores exceeding the 99% genome-wide significance level over only an interval of only 1 cM in approximate length.

Two thirds of single-year QTLs corresponded with pooled three-year QTLs (such as sensory firmness for the MN-1702 family on linkage group HC16: Figure 5.1). Collocated single-year and multiple-year QTLs typically expressed common linkage phase status, but some notable exceptions exist (such as sensory crispness for the 'Monark' family on linkage group HC02). Similarly, more than one third (37%) of pooled three-year QTLs did not correspond to any single-year QTLs. This scenario suggests that while the single-year and pooled three-year QTLs largely correspond with each other, the pooled three-year data set, with its greater sample size, had greater statistical power (fewer false negatives) to detect QTLs relative to the single-year data sets. Nonetheless, single-year QTLs are valuable as well, since several robust QTLs were

indicated exclusively by single-year QTLs from multiple families. For example, no pooled three-year QTLs were identified for juiciness, yet two robust QTLs were indicated by overlapping single-year QTLs.

As with multiple-year studies, multiple-family studies are well-established features in QTL studies. Working with fruit texture traits in very closely related tomato lines, Lecomte *et al.* (2004) observed epistatic interactions between QTLs and different genetic environments. In a subsequent study, Chaïb *et al.* (2006) confirmed that, despite such interaction, many QTLs were consistent across related genetic backgrounds. Foulongne *et al.* (2003) similarly identified QTLs consistent across three generations for disease resistance in an interspecific *Prunus* cross. In contrast to the tomato and *Prunus* populations, the five 'Honeycrisp'-derived families of this study were not constructed with genetic studies in mind, but, rather, are a subset of a large, ongoing apple breeding program. The lack of known common ancestry among the six parental genotypes provided a more diverse population for identifying consistent QTLs, despite the likely presence of similar epistatic interactions between 'Honeycrisp'-derived QTLs and different genetic environments. Taken as a whole, the five full-sib families in this study allow for the assessment of QTL robustness.

Most QTL studies in apple use a single cross to identify QTLs in the genetic backgrounds of the two parents (e.g., King *et al.* 2000, Calenge and Durel 2006, Kenis *et al.* 2008). Van Dyk *et al.* (2010) included two full-sib families with the cultivar Anna as a common parent, permitting them to investigate consistency of QTLs across the three parental genotypes. Instead, the authors identified QTLs on integrated F_1 maps for each

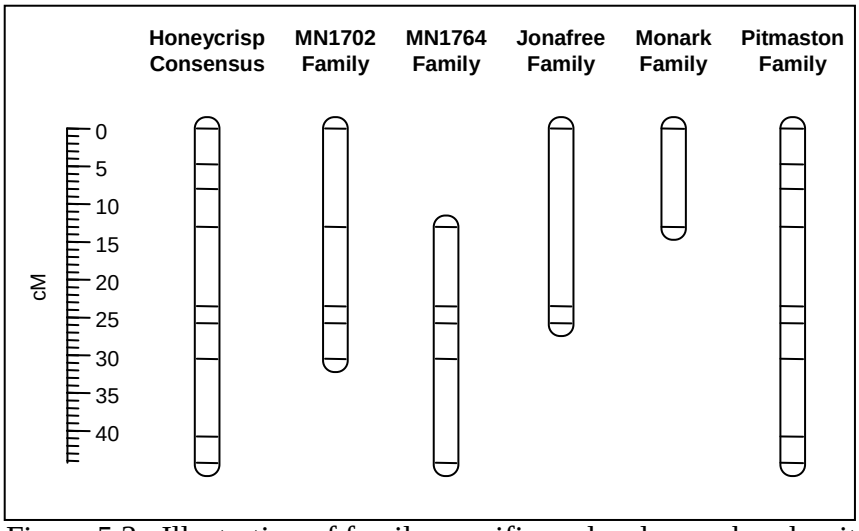

Figure 5.3. Illustration of family-specific molecular marker density and distribution across ‘Honeycrisp’ consensus linkage group 3.

of the two crosses. In contrast, this study used five families to identify QTLs for the same genotype, ‘Honeycrisp’, as expressed across the five genetic environments

Several linkage group intervals were identified with overlapping QTLs from multiple traits, multiple families, and multiple years. For example, HC02 exhibited QTLs for several years for crispness and firmness for the ‘Monark’ and ‘Pitmaston’ families, as well as for juiciness for the MN-1702 and ‘Monark’ families (Figure 5.1); although only approximately 5 cM in length, this interval merits further investigation, as different fruit texture traits are likely affected by common stages of various biosynthetic pathways.

In no instance were collocated QTLs for a single trait identified for all five families. As mentioned above, small family sizes and potential epistatic interactions between QTLs and familial genetic backgrounds could produce this result. Additionally, differences in molecular characterization of the five non-’Honeycrisp’ parents resulted in variable marker coverage across the five families, illustrated for linkage group 3 in Figure 5.3. For example, a marker could be uninformative in one family (e.g., homozygous

Table 5.3. Direction of correlation between markers and texture traits as represented in robust (multiple family, multiple time period) QTLs. Note that HC10 expresses two robust QTLs for firmness, one of each sign.

	Trait			
Linkage Group	Crispness	Firmness	Juiciness	Total Work
HC01	Negative			
HC02	Negative	Negative	Negative	Negative
HC03				Negative
HC04	Negative	Negative		
HC05				
HC06				
HC07		Positive		
HC08				
HC09				
HC10		Both		
HC11	Negative	Positive		
HC12				
HC13				
HC14			Negative	Positive
HC15				
HC16		Positive		Negative
HC17				

dominant for the non-'Honeycrisp' parent) and fully informative in another (e.g., homozygous recessive for another non-'Honeycrisp' parent). The prevalence of dominant DArT markers, as opposed to codominant SSRs and SNPs, in these data resulted in unequal representation of markers across linkage groups in the different families, which in turn yielded linkage group intervals with very low marker density. In the case of terminal intervals, uninformative markers even truncated some linkage groups for some families.

QTL analyses performed on the pooled five-family data sets, however, were of limited value. Only five putative QTLs were identified in this manner (Figure 5.2), and

two of them were not supported by further examination of the detailed family-by-year analyses (HC07; see earlier discussion). This result is undoubtedly due, in large part, to the exclusion of all molecular marker data for which the non-'Honeycrisp' parent was heterozygous, substantially reducing the size of the data set relative to the cumulative sample size of the five separate family data sets.

Markers associated with a majority of the robust QTLs are negatively correlated with the fruit texture traits (Table 5.3). The implications of this pattern is that these markers can be used to screen and cull the large populations of seedlings, discarding many genotypes for which there is little reason to expect desirable texture traits. Such negative selection would permit the generation of larger numbers of seeds, because a smaller percentage of the resulting seedlings would be retained long enough to even be planted out in the orchards.

Several of the linkage groups identified in this study as containing QTLs correspond to previously published reports. Most prominent among these are linkage groups HC02, HC11, HC14 and HC16, all of which displayed multi-trait QTLs in the current study. Linkage group 16, in particular, has been identified as an important location for QTLs associated with sensory crispness, firmness, and juiciness, as well as several instrumental measures of force and work (King *et al.* 2000, 2001

Notably, Kenis *et al*. (2008) identified four linkage groups as containing QTLs associated with instrumental measures of firmness, linkage groups 2, 10, 14, and 16 from a 'Telemon' by 'Braeburn' cross. All four of the corresponding linkage groups in this study have been identified as containing multiple-year, multiple-family QTLs for a variety of texture traits. Other linkage groups for which this study yielded similar results

to prior studies (Liebhard *et al*. 2003a, King *et al*. 2000, 2001) are linkage groups HC01, HC03, HC07, and HC11. The current study has identified a QTL for sensory crispness on HC04, a linkage group for which no texture QTLs have been previously published. In contrast, three linkage groups, HC06, HC12, and HC15, have been reported in prior studies as containing texture QTLs, but none have been identified in the current study (Liebhard *et al*. 2003a, King *et al*. 2000, 2001). Differences in the genetic backgrounds of the various cultivars studied likely explain most of these discrepancies, yet variations in protocols and definitions of fruit texture traits could also play a role.

Sensory juiciness is the trait for which the current study diverges the most from other studies. Only two QTLs have been identified for the trait in 'Honeycrisp', located on linkage groups HC02 and HC14. King *et al*. (2000, 2001), in contrast, identified QTLs for the trait on linkage groups 1 and 16. The current study did not identify any single-year, single-family QTLs for juiciness on these linkage groups, much less any robust QTLs. Allan-Wojtas *et al.* (2003) demonstrated that, all other factors being equal, juicy apple "samples were composed of large cells with thin, brittle cell walls." In fact, Mann *et al.* (2005) found cell size to be the only statistically significant predictor of perceived juiciness. Cell wall strength, however, is likely to affect perceived juiciness; if cell walls are stronger than are middle lamellae, tissue failure between cells, rather than cell rupture, will occur and juice will not be released (Boulton *et al.* 1997). Additionally, sensory juiciness is significantly correlated with sensory crispness and firmness (see Chapter 3 for details). Consequently, juiciness is likely to be directly influenced by many of the same physical characteristics that determine crispness and firmness, justifying further study to determine the mechanisms underlying this highly desirable trait.

Recent years have seen the development of numerous markers for genes involved in the maturation process of fruits, notably with respect to ethylene. The biosynthesis of ethylene occurs via a rather short pathway in which the methionine-derived compound S-adenosyl-L-methionine (AdoMet or SAM) is converted to 1-aminocyclopropane-1-carboxylic acid (ACC), which is in turn converted to ethylene (e.g., Harada *et al.* 2000, Giovannoni 2004, Oraguzie *et al.* 2004, Costa *et al.* 2005, Wang *et al*. 2009a). These two reactions are controlled by the families of ACC synthase (ACS) enzymes and ACC oxidase (ACO) enzyme, respectively. The expansin protein encoded by the gene *MdExp3* is active during the fruit ripening process, whereas several other expansin genes are active during fruit development (Wakasa *et al*. 2003) and postharvest storage (Mann *et al*. 2008). The endopolygalacturonase gene *Md-PG1* (Wakasa *et al*. 2006, Mann *et al*. 2008) and several other genes have also been implicated in changes in apple fruit texture during the fruit maturation process (e.g., Wang *et al*. 2007, 2009b, Cevik *et al*. 2009, Li *et al*. 2010). Markers for several of the genes have been developed (e.g., Harada *et al.* 2000, Costa *et al.* 2005, 2008, 2010, Wakasa *et al.* 2003, 2006, Tatsuki *et al*. 2007, Mann *et al.* 2008, Li *et al*. 2010), but few have been mapped to date.

Significantly, the apple-derived genes *Md-ACO1* and endopolygalacturonase gene *Md-PG1* have been mapped to linkage group 10, the *Md-ACS1* has been mapped to linkage group 16, and the expansin gene *Md-Exp7* has been mapped to linkage group 1 (Costa *et al.* 2005, 2008, 2010). All three of these linkage groups contain multiple-year, multiple-family texture QTLs in the current study. As stated earlier, a paucity of common markers prevents detailed comparison with published linkage maps (e.g., Baldi *et al.* 2004, Calenge *et al.* 2005, Costa *et al.* 2005, Silfverberg-Dilworth *et al.* 2006,

Fernández-Fernández *et al.* 2008). 'Honeycrisp' is heterozygous with respect to both *Md-ACS1* and *Md-ACO1*, but the five non-'Honeycrisp' parents employed in the current study have not been characterized (Zhu and Barritt 2008), but one or both of these genes are likely to be important in the expression of fruit texture traits in 'Honeycrisp' and its progeny.

Only two linkage groups, HC08 and HC15, expressed no QTLs for any traits. It is interesting to note that these two linkage groups have been identified as largely homoeologous (Velasco *et al.* 2010), possibly reflecting the genome duplication event central to the evolution of the pome fruits. At present, no direct assessment of potential homoeology of this study's QTLs is possible due to a lack of sequence data for the DArT markers. Sequencing DArT markers and increasing marker density on targeted 'Honeycrisp' linkage groups would enable finer resolution comparisons of the QTLs identified in this study with those in previously published studies. Additionally, increased sequence information will increase the value of the published apple genome sequence.

Using genes involved in fruit development in *Arabidopsis*, Cevik *et al*. (2009) identified several MADS-box genes associated with fruit texture traits in apple and mapped them to linkage groups 6, 9, and 14. Although only one of these three linkage groups contains a QTL in the current study, the family of MADS-box genes has recently been expanded substantially, providing numerous opportunities for further study (Velasco *et al*. 2010).

5.5 Conclusions

The use of multiple full-sib families with 'Honeycrisp' as a common parent has provided several previously unidentified QTLs for fruit traits, namely for sensory juiciness on linkage groups 2 and 14, a single QTL for all four traits on linkage group 4, and a single QTL for crispness, firmness, and work on linkage group 11. The current study also provides further support for previously reported QTLs, most notably on linkage groups 10 and 16, the first of which likely corresponds to at least one known fruit softening gene, and the second of which has been identified as containing QTLs associated with a variety of fruit texture traits. This study introduces new opportunities for future targeted gene studies, especially in light of the recently announced apple genome sequence (Velasco *et al.* 2010). For example, closely spaced SNPs can be identified with the objective of equalizing the density of informative markers across multiple families and enhancing marker density relative to other marker types.

The current study identified several QTLs that were observed repeatedly across years and consistently across familial genetic backgrounds. Because this study utilized families from an ongoing breeding population, rather than a specifically designed mapping population, this study did not include replicated trees in multiple locations. The multiple years of fruit harvest and evaluation did, however, reflect three rather different weather regimes. Significant interannual variability in fruit bearing among the genotypes and non-uniform molecular characterization across the families resulted in substantially smaller sample sizes than would normally be implemented in a standard QTL analysis. Nonetheless, the multiple families, and multiple years of phenotypic data, served reinforce each other through the identification of robust QTLs.

Because ‘Honeycrisp’ was the subject of this study, the ‘Honeycrisp’ consensus linkage map was utilized, rather than the pre-aligned, family-specific linkage maps. A single, larger population, replicated across sites, would have provided greater statistical power for the identification of QTLs, but existing breeding programs already have large numbers of smaller families. As long as no intentional selection has already occurred within such families, they can provide valuable resources for the identification of markers for MAS. Standard cultural practices, such as pruning and flower and fruit thinning can reduce interannual variability in sample sizes by reducing irregular fruiting. Increasing such practices in an active breeding program would require additional effort and expense, yet the use of pre-existing breeding material benefits from the trees having already passed out of their long juvenility stage.

6 Conclusions

Five full-sib families were employed simultaneously to identify QTLs associated with the common parent, 'Honeycrisp'. The phenotypic evaluation of these five families over three years, coupled with the molecular characterization of the trees, allowed for the identification of molecular markers that were correlated with traits of interest in at least two families and over at least two years. A majority of the markers that were initially identified as potential QTLs in at least one family did not present consistent patterns across multiple families or over multiple years. Spurious markers may explain this inconsistency in part, reflecting the random variability found within highly heterozygous populations. Small sample sizes can also contribute to the identification of spurious QTLs, suggesting that perhaps even more stringent genome-wide significance levels for LOD scores are appropriate (e.g., Bernardo 2004).

Another potential explanation is that different gene interactions are being expressed in the different populations. The five non-'Honeycrisp' parents represent a diverse genetic background, likely generating a wide array of genetic configurations within the progenies. In all likelihood, each of these five parental genotypes contributes unique allelic diversity to this breeding population, a consideration that is somewhat masked by the dominant nature of the DArT markers. Whereas the presence of a common marker between any two parental genotypes indicates a common allele, the absence of a marker in two parental genotypes is uninformative. It is not surprising then that a marker can be positively correlated with a trait of interest in one family and negatively correlated in another, reflecting the potential gene interactions between the amplified markers, derived from 'Honeycrisp', and varying non-amplified corresponding DNA segments from the

other parents.

Finally, marker coverage is not equal across the families, affecting the ability to detect QTLs in all five families. The genetic variation represented within the parental genotypes essentially guarantees the infrequency of markers heterozygous for 'Honeycrisp' and homozygous recessive for the other five parents, a condition required for inclusion in all five family maps. Consequently, the absence of common QTLs across all five families should be expected, and should not be interpreted contradictory to the presence of important QTLs.

Nonetheless, markers have been identified that yield consistent correlative behavior with traits of interest. These markers hold promise for utilization in marker-assisted breeding and selection programs. Most of the markers associated with these robust QTLs are negatively correlated with the texture traits. Consequently, these markers hold promise for their utility in the identification and discard of seedlings with the framework of a marker-assisted selection program. These markers also merit further examination in order to clarify the manner in which their presence is related to the development and maintenance of fruit texture traits. Further characterization of these markers, within 'Honeycrisp' and beyond, coupled with the increasing availability of apple genomic information (e.g., Velasco *et al.* 2010), holds much promise for elucidating the mechanisms underlying important fruit quality traits.

7 Bibliography

Abe, K., Y. Sato, T. Saito, A. Kurihara, and K. Kotobuki. 1995. Narrow-sense heritability of fruit characters in Japanese pear (*Pyrus pyrifolia* Nakai). Ikushu-gaku Zasshi (Japanese Journal of Breeding) 45: 1-5.

Aljanabi, S.M., L. Forget, and A. Dookun. 1999. An improved and rapid protocol for the isolation of polysaccharide- and polyphenol-free sugarcane DNA. Plant Molecular Biology Reporter 17: 1-8.

Allan-Wojtas, P., K.A. Sanford, K.B. McRae, and S. Carbyn. 2003. An integrated microstructural and sensory approach to describe apple texture. Journal of the American Society for Horticultural Science 128: 381-390.

Alspach, P.A., and N.C. Oraguzie. 2002. Estimation of genetic parameters of apple (*Malus domestica*) fruit quality from open-pollinated families. New Zealand Journal of Crop and Horticultural Science 30: 219-228.

Alvarez, M.D., D.E.J. Saunders, and J.F.V. Vincent. 2000. Fracture properties of stored fresh and osmotically manipulated apple tissue. European Food Research and Technology 211: 284-290.

Baldi, P., A. Patocchi, E. Zini, C. Toller, R. Velasco, and M. Komjanc. 2004. Cloning and linkage mapping of resistance gene homologues in apple. Theoretical and Applied Genetics 109: 231-239.

Ban, Y., C. Honda, Y. Hatsuyama, M. Igarashi, H. Bessho, and T. Moriguchi. 2007. Isolation and functional analysis of a MYB transcription factor gene that is a key regulator for the development of red colouration in apple skin. Plant and Cell Physiology 48: 958-970.

Barreiro, P., C. Ortiz, M. Ruiz-Altisent, V. De Smedt, S. Schotte, Z. Andani, I. Wakeling, and P.K. Beyts. 1998. Comparison between sensory and instrumental measurements for mealiness assessment in apples. A collaborative test. Journal of Texture Studies. 29: 509-525.

Barrett, A.H., A.C. Cardello, L.L. Lesher, and I.A. Taub. 1994. Cellularity, mechanical failure, and textural perception of corn meal extrudates. Journal of Texture Studies 25: 77-95.

Bartoshuk, L.M., V.B. Duffy, B.G. Green, H.J. Hoffman, C.-W. Ko, L.A. Lucchina, L.E. Marks, D.J. Snyder. 2004. Valid across-group comparisons with labeled scales: the gLMS versus magnitude matching. Physiology and Behavior 82: 109-114.

Bates, D. 2007**.** lme4: Linear mixed-effects models using S4 classes. R package version 0.99875-9.

Belfanti, E., E. Silfverberg-Dilworth, S. Tartarini, A. Patocchi, M. Barbieri, J. Zhu, B.A. Vinatzer, L. Gianfranceschi, C. Gessler, and S. Sansavini. 2004. The *HcrVf2* gene from a wild apple confers scab resistance to a transgenic cultivated variety. PNAS 101: 886-890.

Bell, R.L., and J. Janick. 1990. Quantitative genetic analysis of fruit quality in pear. Journal of the American Society for Horticultural Science 115: 829-834.

Bernardo, R. 2002. Breeding for quantitative traits in plants. Stemma Press: Woodbury, Minnesota.

Bernardo, R. 2004. What proportion of declared QTL in plants are false? Theoretical

and Applied Genetics 109: 419-424.
Bink, M.C.A.M., M.P. Boer, C.J.F. ter Braak, J. Jansen, R.E. Voorrips, and W.E. van de Weg. 2008. Bayesian analysis of complex traits in pedigreed plant populations. Euphytica 161: 85-96.
Blanpied, G.D., and K.J. Silsby. 1992. Predicting harvest date windows for apples. Cornell Cooperative Extension Information Bulletin 221: 1-12.
Bourne, M.C. 1982. Food texture and viscosity. Academic Press, Inc.: New York.
Brookfield, P., P. Murphy, R. Harker, and E. MacRae. 1997. Starch degradation and starch pattern indices; interpretation and relationship to maturity. Postharvest Biology and Technology 11: 23-30.
Broothaerts, W. 2003. New findings in apple S-genotype analysis resolve previous confusion and request the re-numbering of some S-alleles. Theoretical and Applied Genetics 106: 703-714.
Broothaerts, W., J. Keulemans, and I. Van Nerum. 2004. Self-fertile apple resulting from S-RNase gene silencing. Plant Cell and Reports 22: 497-501.
Brummell, D.A., and M.H. Harpster. 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Molecular Biology 47: 311-340.
Bunyard, E.A. 1929. The anatomy of dessert. Dulau & Company: London.
Bus, V.G.M., P.A. Alspach, M.E. Hofstee, and L.R. Brewer. 2002. Genetic variability and preliminary heritability estimates of resistance to scab (*Venturia inaequalis*) in an apple genetics population. New Zealand Journal of Crop and Horticultural Science 30: 83-92.
Bus, V.G.M., D. Esmenjaud, E. Buck, and F. Laurens. 2009. Application of genetic markers in Rosaceous crops. Pages 563-599 *in* K.M. Folta and S.E. Gardiner (Eds.). Genetics and Genomics of Rosaceae. Springer: New York, NY.
Butcher, P.A., E.R. Williams, D. Whitaker, S. Ling, T.P. Speed, and G.F. Moran. 2002. Improving linkage analysis in outcrossed forest trees – an example from *Acacia mangium*. Theoretical and Applied Genetics 104: 1185-1191.
Cabe, P.R., A. Baumgarten, K. Onan, J.J. Luby, D.S. Bedford. 2005. Using microsatellite analysis to verify breeding records: a study of 'Honeycrisp' and other cold-hardy apple cultivars. HortScience 40: 15-17.
Calenge, F., and C.-E. Durel. 2006. Both stable and unstable QTLs for resistance to powdery mildew are detected in apple after four years of field assessments. Molecular Breeding 17: 329-339.
Calenge, F., A. Faure, M. Goerre, C. Gebhardt, W.E. Van de Weg, L. Parisi, and C.-E. Durel. 2004. Quantitative trait loci (QTL) analysis reveals both broad-spectrum and isolate-specific QTL for scab resistance in an apple progeny challenged with eight isolates of *Venturia inaequalis*. Phytopathology 94: 370-379.
Calenge, F., C.G. Van der Linden, E. Van de Weg, H.J. Schouten, G. Van Arkel, C. Denancé, and C.-E. Durel. 2005. Resistance gene analogues identified through the NBS-profiling method map close to major genes and QTL for disease resistance in apple. Theoretical and Applied Genetics 110: 660-668.

Camps, C., P. Guillermin, J.C. Mauget, and D. Bertrand. 2005. Data analysis of penetrometric force/displacement curves for the characterization of whole apple fruits. Journal of Texture Studies 36: 387-401.

Celton, J.-M., D. Chagné, S.D. Tustin, S. Terakami, C. Nishitani, T. Yamamoto, and S.E. Gardiner. 2009b. Update on comparative genome mapping between *Malus* and *Pyrus*. BMC Research Notes 2: 182.

Celton, J.M., D.S Tustin, D. Chagné, and S.E. Gardiner. 2009a. Construction of a dense genetic linkage map for apple rootstocks developed from *Malus* ESTs and *Pyrus* genomic sequences. Tree Genetics and Genomes 5: 93-107.

Cevik, V., C.D. Ryder, A. Popovich, K. Manning, G.J. King, and G.B. Seymour. 2009. A *FRUITFULL-like* gene is associated with genetic variation for fruit flesh firmness in apple (*Malus domestica* Borkh.). Tree Genetics and Genomes 6: 271-279.

Chagné, D., C.M. Carlisle, C. Blond, R.K. Volz, C.J. Whitworth, N.C. Oraguzie, R.N. Crowhurst, A.C. Allan, R.V. Espley, R.P. Hellens, and S.E. Gardiner. 2007. Mapping a candidate gene (MdMYB10) for red flesh and foliage colour in apple. BMC Genomics 8: 212.

Chagné, D., K. Gasic, R.N. Crowhurst, Y. Han, H.C. Bassett, D.A. Bowatte, T.J. Lawrence, E.H.A. Rikkerink, S.E. Gardiner, and S.S. Korban. 2008. Development of a set of SNP markers present in expressed genes of the apple. Genomics 92: 353-358.

Chaïb, J., L. Lecomte, M. Buret, and M. Causse. 2006. Stability over genetic backgrounds, generations and years of quantitative trait locus (QTLs) for organoleptic quality in tomato. Theoretical and Applied Genetics 112: 9374-944.

Chauvin, M.A., C.F. Ross, M. Pitts, E. Kaupferman, and B. Swanson. 2010. Relationship between instrumental and sensory determination of apple and pear texture. Journal of Food Quality 33: 181-198.

Conner, P.J., S.K. Brown, and N.F. Weeden. 1997. Randomly amplified polymorphic DNA-based genetic linkage maps of three apple cultivars. Journal of the American Society for Horticultural Science 122: 350-359.

Conner, P.J., S.K. Brown, and N.F. Weeden. 1998. Molecular-marker analysis of quantitative traits for growth and development in juvenile apple trees. Theoretical and Applied Genetics 96: 1027-1035.

Costa, F., S. Stella, W.E. Van de Weg, W. Guerra, M. Cecchinel, J. Dallavia, B. Koller, and S. Sansavini. 2005. Role of the genes *Md-ACO1* and *Md-ACS1* in ethylene production and shelf life of apple (*Malus domestica* Borkh.). Euphytica 141: 181-190.

Costa, F., C.P. Peace, S. Stella, S. Serra, S. Musacchi, M. Bazzani, S. Sansavini, and W.E. van de Weg. 2010. QTL dynamics for fruit firmness and softening around an ethylene-dependent polygalacturonase gene in apple (*Malus* x *domestica* Borkh.). Journal of Experimental Botany 61: 3029-3039.

Costa, E., W.E. van de Weg, S. Stella, L. Dondini, D. Pratesi, S. Musacchi, and S. Sansavini. 2008. Map position and functional allelic diversity of *Md-Exp7*, a new putative expansin gene associated with fruit softening in apple (*Malus* x *domestica* Borkh.) and pear (*Pyrus communis*). Tree Genetics and Genomes 4:

575-586.
Currie, A.J., S. Ganeshanandam, D.A. Noiton, D. Garrick, C.J.A. Shelbourne, and N. Oraguzie. 2000. Quantitative evaluation of apple (*Malus* x *domestica* Borkh.) fruit shape by principal component analysis of Fourier descriptors. Euphytica 111: 219-227.
Daillant-Spinnler, B., H.J.H. MacFie, P.K. Beyts, and D. Hedderley. 1996. Relationships between perceived sensory properties and major preference direction of 12 varieties of apples from the southern hemisphere. Food Quality and Preference 7: 113-126.
Dandekar, A.M., G. Teo, B.G. Defilippi, S.L. Uratsu, A.J. Passey, A.A. Kader, J.R. Stow, R.J. Colgan, and D.J. James. 2004. Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. Transgenic Research 13: 373-384.
Davey, M.W., K. Kenis, and J. Keulemans. 2006. Genetic control of fruit vitamin C contents. Plant Physiology 142: 343-351.
Deletre, K. and A.-C. Roudot. 2003. Terminologie comparée français-anglais en analyse de texture. Sciences des Aliments 23: 463-479.
Dever, M.C., M.A. Cliff, and J.W. Hall. 1995. Analysis of variation and multivariate relationships among analytical and sensory characteristics in whole apple evaluation. Journal of the Science of Food and Agriculture 69: 329-338.
Dickson, E.E., S. Kresovich, and N.F. Weeden. 1991. Isozymes in North American *Malus* (Rosaceae) – hybridization and species differentiation. Systematic Botany 16: 363-375.
Diggle, P., P. Heagerty, K.-Y. Liang, and S. Zeger. 2002. Analysis of longitudinal data. Oxford University Press: New York.
Dirlewanger, E., E. Graziano, T. Joobeur, F. Garriga-Calderé, P. Cosson, W. Howad, and P. Arús. 2004. Comparative mapping and marker-assisted selection in Rosaceae fruit crops. Proceedings of the National Academy of Sciences of the United States of America 101: 9891-9896.
Diversity Arrays Technology Pty. Ltd. Plant DNA extraction protocol for DArT. http://www.triticarte.com.au/pdf/DArT_DNA_isolation.pdf. Accessed 24 June 2010.
Dondini, L., L. Pierantoni, F. Gaiotti, R. Chiodini, S. Tartarini, C. Bazzi, and S. Sansavini. 2004. Identifying QTLs for fire-blight resistance via a European pear (*Pyrus communis* L.) genetic linkage map. Molecular Breeding 14: 407-418.
Doyle, J.J., and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11-15.
Duizer, L.M., and R.J. Winger. 2006. Instrumental measures of bite forces associated with crisp products. Journal of Texture Studies 37: 1-15.
Durel, C.-E., C. Denancé, and M.-N. Brisset. 2009. Two distinct major QTL for resistance to fire blight co-localize on linkage group 12 in apple genotypes ‘Evereste’ and *Malus floribunda* clone 821. Genome 52: 139-147.
Durel, C.E., F. Laurens, A. Fouillet, and Y. Lespinasse. 1998. Utilization of pedigree information to estimate genetic parameters from large unbalanced data sets in apple. Theoretical and Applied Genetics 96: 1077-1085.

Durel, C.E., L. Parisi, F. Laurens, W.E. Van de Weg, R. Liebhard, and M.F. Jourjon. 2003. Genetic dissection of partial resistance to race 6 of *Venturia inaequalis* in apple. Genome 46: 224-234.

Edmister, J.A., and Z.M. Vickers. 1985. Instrumental acoustical measures of crispness in foods. Journal of Texture Studies 16: 153-167.

Espley, R.V., R.P. Hellens, J. Putterill, D.E. Stevenson, S. Kutty-Amma, and A.C. Allan. 2007. Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. Plant Journal 49: 414-427.

Evans, R.C., and C.S. Campbell. 2002. The origin of the apple subfamily (Maloideae; Rosaceae) is clarified by DNA sequence data from duplicated GBSSI genes. American Journal of Botany 89: 1478-1484.

Evans, K.M., A. Patocchi, F. Rezzonico, F. Mathis, C.E. Durel, F. Fernández-Fernández, A. Boudichevshaia, F. Dunemann, M. Stankiewicz-Kosyl, L. Gianfranceschi, M. Komjanc, M. Lateur, M. Madduri, Y. Noordijk, and W.E. van de Weg. *In press*. Genotyping of pedigreed apple breeding material with a genome-covering set of SSRs: trueness-to-type of cultivars and their parentages. Molecular Breeding.

Falconer, D.S., and T.F.C. Mackay. 1989. Introduction to quantitative genetics. Prentice-Hall: New York.

FAOSTAT. 2005. Food and Agricultural Organization of the United Nations. http://faostat.fao.org

Fernández-Fernández, F., K.M. Evans, J.B. Clarke, C.L. Govan, C.M. James, S. Marič, and K.R. Tobutt. 2008. Development of an STS map of an interspecific progeny of *Malus*. Tree Genetics and Genomes 4: 469-479.

Fillion, L., and D. Kilcast. 2002. Consumer perception of crispness and crunchiness in fruits and vegetables. Food Quality and Preference 13: 23-29.

Foulongne, M., T. Pascal, F. Pfeiffer, and J. Kervella. 2003. QTLs for powdery mildew resistance in peach x *Prunus davidiana* crosses: consistency across generations and environments. Molecular Breeding 12: 35-50.

Frey, J.E., B. Frey, C. Sauer, and M. Kellerhals. 2004. Efficient low-cost DNA extraction and multiplex fluorescent PCR method for marker-assisted selection in breeding. Plant Breeding 123: 554-557.

Fu, Y.-B., A.D. Yanchuk, and G. Namkoong. 1999. Incomplete block designs for genetic testing: some practical considerations. Canadian Journal of Forest Research 29: 1871-1878.

Gardiner, S.E., H.C.M. Bassett, C. Madie, and D.A.M. Noiton. 1996a. Isozyme, randomly amplified polymorphic DNA (RAPD), and restriction fragment-length polymorphism (RFLP) markers used to deduce a putative parent for the 'Braeburn' apple. Journal of the American Society for Horticultural Science 121: 996-1001.

Gardiner, S.E., H.C.M. Bassett, D.A.M. Noiton, V.G. Bus, M.E. Hofstee, A.G. White, R.D. Ball, R.L.S. Forster, and E.H.A. Rikkerink. 1996b. A detailed linkage map around an apple scab resistance gene demonstrates that two disease resistances classes both carry the V_f gene. Theoretical and Applied Genetics 93: 485-493.

Gardiner, S.E., V.G.M. Bus, R.L. Rusholme, D. Chagné, and E.H.A. Rikkerink. 2007. Apple. In Kole, C. (Ed.). Genome mapping and molecular breeding in plants. Volume 4. Fruits and nuts. Spring-Verlag: Berlin. Pp. 1-62.

Gasic, K., Y. Han, S. Kertbundit, V. Shulaev, A.F. Iezzoni, E.W. Stover, R.L. Bell, M.E. Wisniewski, and S.S. Korban. 2009. Characteristics and transferability of new apple EST-derived SSRs to other Rosaceae species. Molecular Breeding 23: 397-411.

Gianfranceschi, L., B. Koller, N. Seglias, M. Kellerhals, and C. Gessler. 1996. Molecular selection in apple for resistance to scab caused by *Venturia inaequalis*. Theoretical and Applied Genetics 93: 199-204.

Gianfranceschi L., N. Seglias, R. Tarchini, M. Komjanc, and C. Gessler. 1998. Simple sequence repeats for the genetic analysis of apple. Theoretical and Applied Genetics 96: 1069-1076.

Giboreau, A., C. Dacremont, C. Egoroff, S. Guerrand, I. Urdapilleta, D. Candel, and D. Dubois. 2007. Defining sensory descriptors: towards writing guidelines based on terminology. Food Quality and Preference 18: 265-274.

Giovannoni, J.J. 2004. Genetic regulation of fruit development and ripening. The Plant Cell 16: S170-S180.

Goulão, L., L. Cabrita, C.M. Oliveira, and J.M. Leitão. 2001a. Comparing RAPD and AFLP™ analysis in discrimination and estimation of genetic similarities among apple (*Malus domestica* Borkh.) cultivars. Euphytica 199: 259-270.

Goulão, L., and C.M. Oliveira. 2001b. Molecular characterization of cultivars of apple (*Malus* x *domestica* Borkh.) using microsatellite (SSR and ISSR) markers. Euphytica 122: 81-89.

Grattapaglia, D., and R. Sederoff. 1994. Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137: 1121-1137.

Green, B.G., G.S. Shaffer, and M.M. Gilmore. 1993. Derivation and evaluation of a semantic scale of oral sensation magnitude with apparent ratio properties. Chemical Senses 18: 683-702.

Guilford, P., S. Prakash, J.M. Zhu, E. Rikkerink, S. Gardiner, H. Bassett, and R. Forster. 1997. Microsatellite in *Malus* x *domestica* (apple): abundance, polymorphism and cultivar identification. Theoretical and Applied Genetics 94: 249-254.

Hampson, C.R., H.A. Quamme, J.W. Hall, R.A. MacDonald, M.C. King, and M.A. Cliff. 2000. Sensory evaluation as a selection tool in apple breeding. Euphytica 111: 79-90.

Han, Y., D. Chagné, K. Gasic, E.H.A. Rikkerink, J.E. Beever, S.E. Gardiner, and S.S. Korban. 2009. BAC-end sequence-based SNPs and Bin mapping for rapid integration of physical and genetic maps in apple. Genomics 93: 282-288.

Hancock, J.F., J.J. Luby, S.K. Brown, and G.A. Lobos. 2008. Apples. In J.F. Hancock. Temperate Fruit Crop Breeding: Germplasm to Genomics. Pp. 1-38.

Hanson, B. 2005. Best apples to buy and grow. Brooklyn Botanic Garden: Brooklyn, New York.

Harada, T., T. Sunkao, Y. Wakasa, J. Soejima, T. Satoh, and M. Niizeki. 2000. An allele for 1-aminocyclopropane-1-carboxylate synthase gene (*Md-ACS1*) accounts for

the low ethylene production in climacteric fruits of some apple cultivars. Theoretical and Applied Genetics 101: 742-746.
Harker, F.R., K. Lau, and F.A. Gunson. 2003. Juiciness of fresh fruit: a time-intensity study. Postharvest Biology and Technology 29: 55-60.
Harker, F.R., J. Maindonald, S.H. Murray, F.A. Gunson, I.C. Hallett, and S.B. Walker. 2002. Sensory interpretation of instrumental measurements 1: texture of apple fruit. Postharvest Biology and Technology 24: 225-239.
Harker, F.R., M.G.H. Stec, I.C. Hallett, and C.L. Bennett. 1997. Texture of parenchymatous plant tissue: a comparison between tensile and other instrumental and sensory measurements of tissue strength and juiciness. Postharvest Biology and Technology 11: 63-72.
Harker, F.R., A. White, F.A. Gunson, I.C. Hallett, and H.N. de Silva. 2006. Instrumental measurement of apple texture: a comparison of single-edge notched bend test and the penetrometer. Postharvest Biology and Technology 39: 185-192.
Hemmat, M., N.F. Weeden, and S.K. Brown. 2003. Mapping and evaluation of *Malus* x *domestica* microsatellites in apple and pear. Journal of the American Society for Horticultural Science 128: 515-520.
Hemmat, M., N.F. Weeden, A.G. Manganaris, and D.M. Lawson. 1994. Molecular marker linkage map for apple. Journal of Heredity 85: 4-11.
Hoehn, E., F. Gasser, B. Guggenbühl, and U. Künsch. 2003. Efficacy of instrumental measurements for determination of minimum requirements of firmness, soluble solids, and acidity of several apple varieties in comparison to consumer expectations. Postharvest Biology and Technology 27: 27-37.
Hokanson, S.C., W.F. Lamboy, Szewc-McFadden, and J.R. McPherson. 2001. Microsatellite (SSR) variation in a collection of *Malus* (apple) species and hybrids. Euphytica 118: 281-294.
Hokanson, S.C., A.K. Szewc-McFadden, W.F. Lamboy, J.R. McFerson. 1998. Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus* x *domestica* Borkh. core subset collection. Theoretical and Applied Genetics 97: 671-683.
Howad, W., T. Yamamoto, E. Dirlewanger, R. Testolin, P. Cosson, G. Cipriani, A.J. Monforte, L.Georgi, A.G. Abbott, and P. Arús. 2005. Mapping with a few plants: using selective mapping for microsatellite saturation of the *Prunus* reference map. Genetics 171: 1305-1309.
Igarashi, M., Y. Abe, Y. Hatsuyama, T. Ueda, T. Fukasawa-Akada, T. Kon, T. Kudo, T. Sato, and M. Suzuki. 2008. Linkage maps of the apple (*Malus* x *domestica* Borkh.) cvs 'Ralls Janet' and 'Delicious' include newly developed EST markers. Molecular Breeding 22: 95-118.
Iketani, H., K. Abe, T. Yamamoto, K. Kotubuki, Y. Sato, T. Saito, O. Terai, N. Matsuta, and T. Hayashi. 2001. Mapping of disease-related genes in Japanese pear using a molecular linkage map with RAPD markers. Breeding Science 51: 179-184.
Iwanami, H., S. Moriya, N. Kotoda, and K. Abe. 2008. Turgor closely relates to postharvest fruit softening and can be a useful index to select a parent for producing cultivars with good storage potential in apple. HortScience 43: 1377-1381.

Jaccoud, D., K. Peng, D. Feinstein, and A. Kilian. 2001. Diversity Arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Research 29: e25.

James, C.M., J.B. Clarke, and K.M. Evans. 2004. Identification of molecular markers linked to the mildew resistance gene *Pl-d* in apple. Theoretical and Applied Genetics 110: 175-181.

Janick, J., J.N. Cummins, S.K. Brown, and M. Hemmat. 1996. Apples. Pages 1-77. *In* J. Janick and J.N. Moore (Eds.). Fruit breeding, Volume I: Tree and tropical fruits. John Wiley & Sons, Inc.: New York.

John, J.A., and E.R. Williams. 1995. Cyclic and computer generated designs. 2nd Edition. Chapman and Hall: London. 255 pp.

Johnston, J.W., E.W. Hewett, N.H. Banks, F.R. Harker, and M.L.A.T.M. Hertog. 2001. Physical change in apple texture with fruit temperature: effects of cultivar and time in storage. Postharvest Biology and Technology 23: 13-21.

Johnston, J.W., E.W. Hewett, and M.L.A.T.M. Hertog. 2002a. Postharvest softening of apple (*Malus domestica*) fruit: a review. New Zealand Journal of Crop and Horticultural Science 30: 145-160.

Johnston, J.W., E.W. Hewett, M.L.A.T.M. Hertog, and F.R. Harker. 2002b. Temperature and ethylene affect induction of rapid softening in 'Granny Smith' and 'Pacific Rose™' apple cultivars. Postharvest Biology and Technology 25: 257-264.

Jowitt, R. 1974. The terminology of food texture. Journal of Texture Studies 5: 351-358.

Juniper, B.E., and D.J. Mabberley. 2006. The story of the apple. Timber Press: Portland, Oregon.

Karlsen, A.M., K. Aaby, H. Sievertsen, P. Baardseth, and M.R. Ellekjær 1999. Instrumental and sensory analysis of fresh Norwegian and imported apples. Food Quality and Preference 10: 305-314.

Kellerhals, M., E. Dolega, E. Dilworth, B. Koller, and C. Gessler. 2000a. Advances in marker-assisted apple breeding. Acta Horticulturæ 538: 535-540.

Kellerhals, M., L. Gianfranceschi, N. Seglias, and C. Gessler. 2000b. Marker-assisted selection in apple breeding. Acta Horticulturæ 521: 255-265.Kenis, K., and J. Keulemans. 2005. Genetic linkage maps of two apple cultivars (*Malus* x *domestica* Borkh.) based on AFLP and microsatellite markers. Molecular Breeding 15: 205-219.

Kenis, K., and J. Keulemans. 2005. Genetic linkage maps of two apple cultivars (*Malus* x *domestica* Borkh.) based on AFLP and microsatellite markers. Molecular Breeding 15: 205-219.

Kenis, K., and J. Keulemans. 2007. Study of tree architecture of apple (*Malus* x *domestica* Borkh.) by QTL analysis of growth traits. Molecular Breeding 19: 193-208.

Kenis, K., J. Keulemans, and M.W. Davey. 2008. Identification and stability of QTLs for fruit quality traits in apple. Tree Genetics and Genomes 4: 647-661.

Khan, A.A., and J.F.V. Vincent. 1993. Anisotropy in the fracture properties of apple flesh as investigated by crack-opening tests. Journal of Materials Science 28: 45-51.

Khan, M.A., B. Duffy, C. Gessler, and A. Patocchi. 2006. QTL mapping of fire blight resistance in apple. Molecular Breeding 17: 299-306.

King, G.J., F.H. Alston, L.M. Brown, E. Chevreau, K.M. Evans, F. Dunemann, J. Janse, F. Laurens, J.R. Lynn, C. Maliepaard, A.G. Manganaris, P. Roche, H. Schmidt, S. Tartarini, J. Verhaegh, and R. Vrielink. 1998. Multiple field and glasshouse assessments increase the reliability of linkage mapping of the V_f source of scab resistance in apple. Theoretical and Applied Genetics 96: 699-708.

King, G.J., J.R. Lynn, C.J. Dover, K.M. Evans, and G.B. Seymour. 2001. Resolution of quantitative trait loci for mechanical measures accounting for genetic variation in fruit texture of apple (*Malus pumila* Mill.). Theoretical and Applied Genetics 102: 1227-1235.

King, G.J., C. Maliepaard, J.R. Lynn, F.H. Alston, C.E. Durel, K.M. Evans, B. Griffon, F. Laurens, A.G. Manganaris, E. Schrevens, S. Tartarini, and J. Verhaegh. 2000. Quantitative genetic analysis and comparison of physical and sensory descriptors relating to fruit flesh firmness in apple (*Malus pumila* Mill.). Theoretical and Applied Genetics 100: 1074-1084.

King, G.J., S. Tartarini, L. Brown, F. Gennari, and S. Sansavini. 1999. Introgression of the V_f source of scab resistance and distribution of linked marker alleles within the *Malus* gene pool. Theoretical and Applied Genetics 99: 1039-1046.

Kitahara, K., S. Matsumoto, T. Yamamoto, J. Soejima, H. Komatsu, and K. Abe. 2005. Parent identification of eight apple cultivars by S-RNase analysis and simple sequence repeat markers. HortScience 40: 314-317.

Knapp, S.J. 1998. Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. Crop Science 38: 1164-1174.

Koller, B., L. Gianfranceschi, N. Seglias, J. McDermott, and C. Gessler. 1994. DNA markers linked to *Malus floribunda* 821 scab resistance. Molecular Plant Biology 26: 597-602.

Koller, B., A. Lehmann, J.M. McDermott, and C. Gessler. 1993. Identification of apple cultivars using RAPD markers. Theoretical and Applied Genetics 85: 901-904.

Kouassi, A.B., C.-E. Durel, F. Costa, S. Tartarini, E. van de Weg, K. Evans, F. Fernandez-Fernandez, C. Govan, A. Boudichevskaja, F. Dunemann, A. Antofie, M. Lateur, M. Stankiewicz-Kosyl, A. Soska, K. Tomala, M. Lewandowski, K. Rutkovski, E. Zurawicz, W. Guerra, and F. Laurens. 2009. Estimation of genetic parameters and prediction of breeding values for apple fruit-quality traits using pedigreed plant material in Europe. Tree Genetics and Genomes 5: 659-672.

Krzanowski, W.J. 1988. Principles of multivariate analysis. Oxford Science Publications: Oxford.

Lamboy, W.F., J. Yu, P.L. Forsline, and N.F. Weeden. 1996. Partitioning of allozyme diversity in wild populations of *Malus sieversii* L. and implication for germplasm collection. Journal of the American Society for Horticultural Science 121: 982-987.

Lecomte, L., P. Duffé, M. Buret, B. Servin, F. Hospital, and M. Causse. 2004. Marker-

assisted introgression of five QTLs controlling fruit quality traits into three tomato lines revealed interactions between QTLs and genetic backgrounds. Theoretical and Applied Genetics 109: 658-668.

Li, J., H. Zhu, and R. Yuan. 2010. Profiling the expression of genes related to ethylene biosynthesis, ethylene perception, and cell wall degradation during fruit abscission and fruit ripening in apple. Journal of the American Society for Horticultural Science 135: 391-401.

Liebhard, R., L. Gianfranceschi, B. Koller, C.D. Ryder, R. Tarchini, E. Van de Weg, and C. Gessler. 2002. Development and characterization of 140 new microsatellites in apple (*Malus* x *domestica* Borkh.). Molecular Breeding 10: 217-241.

Liebhard, R., M. Kellerhals, W. Pfammatter, M. Jertmini, and C. Gessler. 2003a. Mapping quantitative physiological traits in apple (*Malus* x *domestica* Borkh.). Plant Molecular Biology 52: 511-526.

Liebhard, R., B. Koller, L. Gianfranceschi, and C. Gessler. 2003b. Creating a saturated reference map for the apple (*Malus* x *domestica* Borkh.) genome. Theoretical and Applied Genetics 106: 1497-1508.

Liebhard, R., B. Koller, A. Patocchi, M. Kellerhals, W. Pfammatter, M. Jertmini, and C. Gessler. 2003c. Mapping quantitative field resistance against apple scab in a 'Fiesta' x 'Discovery' progeny. Phytopathology 93: 493-501.

Lin, M., X.-Y. Lou, M. Chou, and R. Wu. 2003. A general statistical framework for mapping quantitative trait loci in nonmodel systems: issues for characterizing linkage phases. Genetics 165: 901-913.

Lougheed, E.C., and E.W. Franklin. 1974. Ethylene production increased by bruising of apples. HortScience 9: 192-193

Luby, J.J., and D.V. Shaw. 2001. Does marker-assisted selection make dollars and sense in a fruit breeding program? HortScience 36: 872-879.

Mabberley, D.J., C.E. Jarvis, and B.E. Juniper. 2001. The name of the apple. Telopea 9: 421-430.

Maliepaard, C., F.H. Alston, C. van Arkel, L.M. Brown, E. Chevreau, F. Dunemann, K.M. Evans, S. Gardiner, P. Guilford, A.W. van Heusden, J. Janse, F. Laurens, J.R. Lynn, A.G. Manganaris, A.P.M. den Nijs, N. Periam, E. Rikkerink, P. Roche, C. Ryder, S. Sansavini, H. Schmidt, S. Tartarini, J.J. Verhaegh, M. Vrielink-van Ginkel, and G.J. King. 1998. Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. Theoretical and Applied Genetics 97: 60-73.

Maliepaard, C., J. Jansen, and J.W. van Ooijen. 1997. Linkage analysis in a full-sib family of an outbreeding plant species: overview and consequences for applications. Genetical Research 70: 237-250.

Mann, H., D. Bedford, J. Luby, Z. Vickers, and C. Tong. 2005. Relationship of instrumental and sensory texture measurements of fresh and stored apples to cell number and size. HortScience 40: 1815-1820.

Mann, H.S., J.J. Alton, S.H. Kim, and C.B.S. Tong. 2008. Differential expression of cell-wall-modifying genes and novel cDNAs in apple fruit during storage. Journal of the American Society for Horticultural Science 133: 152-157.

Mehinagic, E., G. Royer, D. Bertrand, R. Symoneaux, F. Laurens, and F. Jourjon. 2003. Relationship between sensory analysis, penetrometry and visible-NIR spectroscopy of apples belonging top different cultivars. Food Quality and Preference 14: 473-484.

Mehinagic, E., G. Royer, R. Symoneaux, D. Bertrand, and F. Jourjon. 2004. Prediction of the sensory quality of apples by physical measurements. Postharvest Biology and Technology 34: 257-269.

Michelmore, R.W., I. Paran, and R.V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregation populations. Proceedings of the National Academy of Science of the United States of America 88: 9828-9832.

Mnejja, M., J. Garcia-Mas, J.-M. Audergon, and P. Arús. 2010. *Prunus* microsatellite marker transferability across rosaceous crops. Tree Genetics and Genomes 6: 689-700.

Mohamed, A.A.A., R. Jowitt, and J.G. Brennan. 1982. Sensory and instrumental measurement of food crispness: II – In a high moisture food. Journal of Food Engineering 1: 123-147.

Moriya, S., H. Iwanami, N. Kotoda, A. Takahashi, T. Yamamoto, and K. Abe. 2009. Development of a marker-assisted selection system for columnar growth habit in apple breeding. Journal of the Japanese Society for Horticultural Science 78: 279-287.

Moriya, S., H. Iwanami, A. Takahashi, N. Kotoda, K. Suzuki, T. Yamamoto, and K. Abe. 2010. Genetic mapping of the crown gall resistance gene of the wild apple *Malus sieboldii*. Tree Genetics and Genomes **6**:195-203.

Mulcahy, D.L., M. Cresti, S. Sansavini, G.C. Douglas, H.F. Linskens, G.B. Mulcahy, R. Vignani, and M. Pancaldi. 1993. The use of random amplified polymorphic DNAs to fingerprint apple genotypes. Scientia Horticulturae 54: 89-96.

Næs, T., and Ø. Langsrud. 1998. Fixed or random assessors in sensory profiling? Food Quality and Preference 9: 145-152.

N'Diaye, A., W.E. Van de Weg, L.P. Kodde, B. Koller, F. Dunemann, M. Thiermann, S. Tartarini, F. Gennari, and C.E. Durel. 2008. Construction of an integrated consensus map of the apple genome based on four mapping populations. Tree Genetics and Genomes 4: 727-743.

Nelson, M.N., J. Nixon, and D.J. Lydiate. 2005. Genome-wide analysis of the frequency and distribution of crossovers at male and female meiosis in *Sinapis alba* L. (white mustard). Theoretical and Applied Genetics 111: 31-43.

Nishinari, K. F. Hayakawa, C.-F. Xia, L. Huang, J.-F. Meullenet, and J.-M. Sieffermann. 2008. Comparative study of texture terms: English, French, Japanese and Chinese. Journal of Texture Studies 39: 530-568.

Nishitani, C., S. Terakami, Y. Sawamura, N. Takada, and T. Yamamoto. 2009. Development of novel EST-SSR markers derived from Japanese pear (*Pyrus pyrifolia*). Breeding Science 59: 391-400.

Norelli, J.L., R.E. Farrell, Jr., C.L. Bassett, A.M. Baldo, D.A. Lalli, H.S. Aldwinckle, and M.E. Wisniewski. 2009. Rapid transcriptional response of apple to fire blight

disease revealed by cDNA suppression subtractive hybridization analysis. Tree Genetics and Genomes 5: 27-40.

Nybom, H., J. Sehic, and L. Garkava-Gustavsson. 2008. Modern apple breeding is associated with a significant change in the allelic ratio of the ethylene production gene *Md-ACS1*. Journal of Horticultural Science and Biotechnology 83: 673-677.

Oddou-Muratorio, S., C. Aligon, S. Decroocq, C. Plomion, T. Lamant, and B. Mush-Demesure. 2001. Microsatellite primers for *Sorbus torminalis* and related species. Molecular Ecology Notes 1: 297-299.

Oraguzie, N., P. Alspach, R. Volz, C. Whitworth, C. Ranatunga, R. Weskett, and R. Harker. 2009. Postharvest assessment of fruit quality parameters in apple using both instruments and an expert panel. Postharvest Biology and Technology 52: 279-287.

Oraguzie, N.C., S.E. Gardiner, H.C.M. Bassett, M. Stefanati, R.D. Ball, V.G.M. Bus, and A.G. White. 2001a. Genetic diversity and relationships in *Malus* sp. germplasm as determined by randomly amplified polymorphic DNA. Journal of the American Society for Horticultural Science 126: 318-328.

Oraguzie, N.C., M.E. Hofstee, L.R. Brewer, and C. Howard. 2001b. Estimation of genetic parameters in a recurrent selection program in apple. Euphytica 118: 29-37.

Oraguzie, N.C., H. Iwanami, J. Soejima, T. Harada, and A. Hall. 2004. Inheritance of the *Md-ACS1* gene and its relationship to fruit softening in apple (*Malus* x *domestica* Borkh.). Theoretical and Applied Genetics 108: 1526-1533.

Oraguzie, N.C., R.K. Volz, C.J. Whitworth, H.C.M. Bassett, A.J. Hall, and S.E. Gardiner. 2007. Influence of *Md-ACS1* allelotype and harvest season within an apple germplasm collection on fruit softening during cold air storage. Postharvest Biology and Technology 44: 212-219.

Patocchi, A., F. Fernández-Fernández, K. Evans, D. Gobbin, F. Rezzonico, A. Boudichevskaia, F. Dunemann, M. Stankiewicz-Kosyl, F. Mathis-Jeanneteau, C.E. Durel, L. Gianfranceschi, F. Costa, C. Toller, V. Cova, D. Mott, M. Komjanc, E. Barbaro, L. Kodde, E. Rikkerink, C. Gessler, W.E. van de Weg. 2009a. Development and test of 21 multiplex PCRs composed of SSRs spanning most of the apple genome. Tree Genetics and Genomes 5: 211-223.

Patocchi, A., A. Frei, J.E. Frey, and M. Kellerhals. 2009b. Towards improvement of marker assisted selection of apple scab resistant cultivars: *Venturia inaequalis* virulence surveys and standardization of molecular marker alleles associated with resistance genes.

Peil, A., T. Garcia-Libreros, K. Richter, F.C. Trognitz, B. Trognitz, M.-V. Hanke, and H. Flachowsky. 2007. Strong evidence for a fire blight resistance gene of *Malus robusta* located on linkage group 3. Plant Breeding 126: 470-475.

Peirs, A., N. Scheerlinck, A.B. Perez, P. Jancsók, and B.M. Nicolaï. 2002. Uncertainty analysis and modeling of the starch index during apple fruit maturation. Postharvest Biology and Technology 26: 199-207.

Pekkinen, M., S. Varvio, K.K.M. Kulju, H. Kärkkäinen, S. Smolander, A. Viherä-Aarnio, V. Koski, and M.J. Sillanpää. 2005. Linkage map of birch, *Betula pendula* Roth,

based on microsatellites and amplified fragment length polymorphisms. Genome 48: 619-625.
Péneau, S., E. Hoehn, H.-R. Roth, F. Escher, and J. Nuessli. 2006. Importance and consumer perception of freshness of apples. Food Quality and Preference 17: 9-19.
Piepho, H.P., A. Büchse, and K. Emrich. 2003. A hitchhiker's guide to mixed models for randomized experiments. Journal of Agronomy and Crop Science 189: 310-322.
Piepho, H.P., A. Büchse, and B. Truberg. 2006a. On the use of multiple lattice designs and alpha-designs in plant breeding trials. Plant Breeding 125: 523-528.
Piepho, H.P., and J. Möhring. 2007. Computing heritability and selection response from unbalanced plant breeding trials. Genetics 177: 1881-1888.
Piepho, H.P., J. Möhring, A.E. Melchinger, and A. Büchse. 2008. BLUP for phenotypic selection in plant breeding and variety testing. Euphytica 161: 209-228.
Piepho, H.P., E.R. Williams, and M. Fleck. 2006b. A note on the analysis of designed experiments with complex treatment structure. HortScience 41: 446-452.
Pierantoni, L., K.-H. Cho, I.-S. Shin, R. Chiodini, S. Tartarini, L. Dondini, S.-J. Kang, and S. Sansavini. 2004. Characterisation and transferability of apple SSRs to two European pear F_1 populations. Theoretical and Applied Genetics 109: 1519-1524.
Pierantoni, L., L. Dondini, K.-H. Cho, I.-S. Shin, F. Gennari, R. Chiodini, S. Tartarini, S.-J. Kang, and S. Sansavini. 2006. Pear scab resistance QTLs via a European pear (*Pyrus communis*) linkage map. Tree Genetics and Genomes 3: 311-317.
Pineau, N., C. Chabanet, and P. Schlich. 2007. Modeling the evolution of the performance of a sensory panel: a mixed-model and control chart approach. Journal of Sensory Studies 22: 212-241.
Pinheiro, J.C., and D.M. Bates. 2002. Mixed effects models in S and S-Plus. Springer Verlag: New York.
Poehlman, J.M., and D.A. Sleper. 1995. Breeding field crops. Iowa State Press: Ames, Iowa.
Potter, D., T. Eriksson, R.C. Evans, S. Oh, J.E.E. Smedmark, D.R. Morgan, M. Kerr, K.R. Robertson, M. Arsenault, T.A. Dickinson, and C.S. Campbell. 2007. Phylogeny and classification of Rosaceae. Plant Systematics and Evolution 266: 5-43.
Powell, W., M. Morgante, C. Andre, M. Hanfey, J. Vogel, S. Tingey, and A. Rafalski. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular Breeding 2: 225-238.
Pre-Aymard, C., E. Fallik, A. Weksler, and S. Lurie. 2005. Sensory analysis and instrumental measurements of 'Anna' apples treated with 1-methylcyclopropene. Postharvest Biology and Technology 36: 135-142.
R Development Core Team. 2007. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.
Reid, MS, C.A.S. Padfield, C.B. Watkins, and J.E. Harman. 1982. Starch iodine pattern as a maturity index for Granny Smith apples. 1. Comparison with flesh firmness

and soluble solids content. New Zealand Journal of Agricultural Research 25: 229-237.

Richards, C.M., G.M. Volk, A.E. Reilley, A.D. Henk, D.R. Lockwood, P.A. Reeves, and P.L. Forsline. 2009. Genetic diversity and population structure in *Malus sieversii*, a wild progenitor species of domesticated apple. Tree Genetics and Genomes 5: 339-347.

Roudaut, G., C. Dacremont, B. Vallès Pàmies, B. Colas, and M. Le Meste. 2002. Crispness: a critical review on sensory and material science approaches. Trends in Food Science and Technology 13: 217-227.

Rumpunen, K., and D. Kviklys. 2003. Combining ability and patterns of inheritance for plant and fruit traits in Japanese quince (*Chaenomeles japonica*). Euphytica 132: 139-149.

Sargent, D.J., A. Marchese, D.W. Simpson, W. Howad, F. Fernández-Fernández, A. Monfort, P. Arús, K.M. Evans, and K.R. Tobutt. 2009. Development of "universal" gene-specific markers from *Malus* spp. cDNA sequences, their mapping and use in synteny studies within Rosaceae. Tree Genetics and Genomes 5: 133-145.

Schouten, H.J., W.E. van de Weg, J. Carling, S.A. Khan, S.J. McKay, M.P.W. Van Kaauwen, A.H.J. Wittenberg, H.J.J. Koehorst-van Putten, Y. Noordijk, Z. Gao, D. Jaccoud, M. Consodine, and A. Kilian. *Submitted*. Diversity Arrays Technology (DArT) markers in apple for genetic linkage maps and diversity studies. Molecular Breeding

Segura, V., C. Denancé, C.-E. Durel, and E. Costes. 2007. Wide range QTL analysis for complex architectural traits in a 1-year-old apple progeny. Genome 50: 159-171.

Segura, V., C.-E. Durel, and E. Costes. 2009. Dissecting apple tree architecture into genetic, ontogenetic and environmental effects: QTL mapping. Tree Genetics and Genomes **5**:165-179.

Shulaev, V., S.S. Korban, B. Sosinski, A.G. Abbott, H.S. Aldwinkle, K.M. Folta, A. Iezzoni, D. Main, P. Arús, M. Dandekar, K. Lewers, S.K. Brown, T.M. Davis, S.E. Gardiner, D. Potter, and R.E. Veilleux. 2008. Multiple models for Rosaceae genomics. Plant Physiology 147: 985-1003.

Silfverberg-Dilworth, E., S. Besse, R. Paris, E. Belfanti, S. Tartarini, S. Sansavini, A. Patocchi, and C. Gessler. 2005. Identification of functional apple scab resistance gene promoters. Theoretical and Applied Genetics 110: 1119-1126.

Silfverberg-Dilworth, E., C. Matasci, M. Walser, V. Soglio, L. Gianfranceschi, W.E. van de Weg, C.E. Durel, S. Tartarini, M.P.W. Kaauwen, L.P. Kodde, T. Yamamoto, C. Gessler, and A. Patocchi. 2006. Development of a new set of apple (*Malus* x *domestica* Borkh.) microsatellite markers. Tree Genetics & Genomes 2: 202-224.

Smith, R.B., E.C. Lougheed, E.W. Franklin, and I. McMillan. 1979. The starch iodine test for determining stage of maturation in apples. Canadian Journal of Plant Science 59: 725-735.

Soglio, V., F. Costa, J.W. Molthoff, W.M.J. Weeman-Hendriks, H.J. Schouten, and L. Gianfranceschi. 2009. Transcription analysis of apple fruit development using DNA microarrays. Tree Genetics and Genomes 5: 685-698.

Sosinski, F., V. Shulaev, A. Dhingra, A. Kalyanaraman, R. Bumgarner, D. Rokhsar, I. Verde, R. Velasco, and A.G. Abbott. 2009. Rosaceous genome sequencing: perspectives and progress. In Folta, K.M., and S.E. Gardiner (Eds.). Genetics and Genomics of Rosaceae. Plant Genetics and Genomics: Crops and Models, Vol. 6. Pp. 601-615.

Sutherland, B.G., K.R. Tobutt, A. Marchese, G. Paternoster, D.W. Simpson, and D.J. Sargent. 2008. A genetic linkage map of *Physocarpus*, a member of the Spiraeoideae (Rosaceae), based on RAPD, AFLP, RGA, SSR and gene specific markers. Plant Breeding 127: 527-532.

Szczesniak, A.S., and R. Ilker. 1988. The meaning of textural characteristics – Juiciness in plant foodstuffs. Journal of Texture Studies 19: 61-78.

Tacken, E., H. Ireland, K. Gunaseelan, S. Karunairetnam, D. Wang, K. Schultz, J. Bowen, R.K. Atkinson, J.W. Johnston, J. Putterill, R.P. Hellens, and R.J. Schaffer. 2010. The role of ethylene and cold temperature in the regulation of the apple *POLYGALACTURONASE1* gene and fruit softening. Plant Physiology 153: 294-305.

Tancred, S.J., A.G. Zeppa, M. Cooper, and J.K. Stringer. 1995. Heritability and patterns of inheritance of the ripening date of apples. HortScience 30: 325-328.

Tartarini, S., and S. Sansavini. 2003. The use of molecular markers in pome fruit breeding. Acta Horticulturæ 622: 129-140.

Tatsuki, M., A. Endo, and H. Ohkawa. 2007. Influence of time from harvest to 1-MCP treatment on apple fruit quality and expression of genes for ethylene biosynthesis enzymes and ethylene receptors. Postharvest Biology and Technology 43: 28-35.

Tong, C., D. Kruegger, Z. Vickers, D. Bedford, J. Luby, A. El-Shiekh, K. Shackel, and H. Ahmadi. 1999. Comparison of softening-related changes during storage of 'Honeycrisp' apple, its parents, and 'Delicious'. Journal of the American Society for Horticultural Science 124: 407-415.

Ukrainetz, N.K., K. Ritland, and S.D. Mansfield. 2008. An AFLP linkage map for Douglas-fir based upon multiple full-sib families. Tree Genetics & Genomes 4: 181-191.

Van Dyk, M.M., M.K. Soeker, I.W. Labuschagne, and D.J.G. Rees. 2010. Identification of a major QTL for time of initial vegetative budbreak in apple (*Malus* x *domestica* Borkh.). Tree Genetics & Genomes 6: 489-502.

Van Ooijen, J.W. 2004. MapQTL® 5: Software for the calculation of quantitative trait loci in experimental populations. Kyazma B.V.: Waginengen, the Netherlands.

Van Ooijen, J.W. 2006. JoinMap® 4: Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V.: Waginengen, the Netherlands.

Van Ooijen, J.W. 2009. MapQTL® 6: Software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma B.V.: Waginengen, the Netherlands.

Vanwynsberghe, L. K. De Witte, E. Coart, and J. Keulemans. 2005. Limited application of homozygous genotypes in apple breeding. Plant Breeding 124: 399-403.

Varela, P., A. Salvador, and S. Fiszman. 2005. Shelf-life estimation of 'Fuji' apples: sensory characteristics and consumer acceptability. Postharvest Biology and Technology 38: 18-24.

Varela, P, A. Salvador, A. Gámbaro, and S. Fiszman. 2008. Texture concepts for consumers: a better understanding of crispy-crunchy sensory perception. European Food Research and Technology 226: 1081-1090.

Velasco. R., A. Zharkikh, J. Affourtit, A. Dhingra, A. Cestaro, A. Kalyanaraman, P. Fontana, S.K. Bhatnagar, M. Troggio, D. Pruss, S. Salvi, M. Pindo, P. Baldi, S. Castelletti, M. Cavaiuolo, G. Coppola, F. Costa, V. Cova, A. Dal Ri, V. Goremykin, M. Komjanc, S. Longhi, P. Magnago, G. Malacarne, M. Malnoy, D. Micheletti, M. Moretto, M. Perazzolli, A. Si-Ammour, S. Vezzulli, E. Zini, G. Eldredge, L.M. Fitzgerald, N. Gutin, J. Lanchbury, T. Macalma, J.T. Mitchell, J. Reid, B. Wardell, C. Kodira, Z. Chen, B. Desany, F. Niazi, M. Palmer, T. Koepke, D. Jiwan, S. Schaeffer, V. Krishnan, C. Wu, V.T. Chu, S.T. King, J. Vick, Q. Tao, A. Mraz, A. Stormo, K. Stormo, R. Bogden, D. Ederle, A. Stella, A. Vecchietti, M.M. Kater, S. Masiero, P. Lasserre, Y. Lespinasse, A.C. Allan, V. Bus, D. Chagné, R.N. Crowhurst, A.P. Gleave, E. Lavezzo, J.A. Fawcett, S. Proost, P. Rouzé, L. Sterck, S. Toppo, B. Lazzari, R.P. Hellens, C.-E. Durel, A. Gutin, R.E. Bumgarner, S.E. Gardiner, M. Skolnick, M. Egholm, Y. Van de Peer, F. Salamini, and R. Viola. 2010. The genome of the domesticated apple (*Malus* x *domestica* Borkh.). Nature Genetics 42: 833-839.

Vickers, Z.M., and C.M. Christensen. 1980. Relationships between sensory crispness and other sensory and instrumental parameters. Journal of Texture Studies 11: 291-307.

Vincent, J.F.V. 1998. The quantification of crispness. Journal of the Science of Food and Agriculture 78: 162-168.

Vincent, J.F.V., D.E.J. Saunders, and P. Beyts. 2002. The use of critical stress intensity factor to quantify “hardness” and “crunchiness” objectively. Journal of Texture Studies 33: 149-159.

Voorrips, R.E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. The Journal of Heredity 93: 77-78.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414.

Wakasa, Y., Y. Hatsuyama, A. Takahashi, T. Sato, N. Niizeki, and T. Harada. 2003. Divergent expression of six expansin genes during apple fruit ontogeny. European Journal of Horticultural Science 68: 253-259.

Wakasa, Y., H. Kudo, R. Ishikawa, S. Akada, M. Senda, M. Niizeki, and T. Harada. 2006. Low expression of an endopolygalacturonase gene in apple fruit with long-term storage potential. Postharvest Biology and Technology 39: 193-198.

Wang, A., D. Tan, A. Takahashi, T.Z. Li, and T. Harada. 2007. MdERFs, two ethylene-response factors involved in apple fruit ripening. Journal of Experimental Botany 58: 3743-3748.

Wang, A., J. Yamakake, H. Kido, Y. Wakasa, Y. Hatsuyama, M. Igarashi, A. Kasai, T. Li, and T. Harada. 2009a. Null mutation of the MdACS3 gene, coding for a ripening-specific 1-aminocyclopropane-1-carboxylate synthase, leads to long shelf life in apple fruit. Plant Physiology 151: 391-399.

Wang, A., D. Tan, M. Tatsuki, A. Kasai, T. Li, H. Saito, and T. Harada. 2009b. Molecular mechanism of distinct ripening profiles in 'Fuji' apple fruit and its early maturing sports. Postharvest Biology and Technology 52: 38-43.

Watkins, C.B., M. Erkan, J.F. Nock, K.A. Iungerman, R.M. Beaudry, and R.E. Moran. 2005. Harvest date effects on maturity, quality, and storage disorders of 'Honeycrisp' apples. HortScience 40: 164-169.

Weeden, N.F., and R.C. Lamb. 1985. Identification of apple cultivars by isozyme phenotypes. Journal of the American Society for Horticultural Science 110: 509-515.

Welsh, J., and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Research 18: 7213-7218.

Wenzl, P., J. Carling, D. Kudrna, D. Jaccoud, E. Huttner, A. Kleinhofs, and A. Kilian. 2004. Diversity Arrays Technology (DArT) for whole-genome profiling of barley. Proceedings of the National Academy of Science of the United States of America 101: 9915-9920.

Wenzl, P., H. Li, J. Carling, M. Zhou, H. Raman, E. Paul, P. Hearndon, C. Maier, L. Xia, V. Caig, J. Ovesná, M. Cakir, D. Poulsen, J. Wang, R. Raman, K.P. Smith, G.J. Muehlbauer, K.J. Chalmers, A. Kleinhofs, E. Huttner, and A. Kilian. 2006. A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7: 206.

Whitaker, D., E.R. Williams, and J.A. John. 2004, CycDesigN: a package for the computer generation of experimental designs. Version 2.1. CSIRO Forestry and Forest Products: Canberra, Australia.

White, A.G., P.A. Alspach, R.H. Weskett, and L.R. Brewer. 2000a. Heritability of fruit shape in pears. Euphytica 112: 1-7.

White, A.G., L.R. Brewer, and P.A. Alspach. 2000b. Heritability of fruit characteristics in pears. Acta Horticulturæ 538: 331-337.

Yamamoto, T., T. Kimura, Y. Sawamura, K. Kotobuki, Y. Ban, T. Hayashi, and N. Matsuta. 2001. SSRs isolated from apple can identify polymorphism and genetic diversity in pear. Theoretical and Applied Genetics 102: 865-870.

Yamamoto, T., T. Kimura, Y. Sawamura, T. Manabe, K. Kotobuki, T. Hayashi, Y. Ban, and N. Matsuta. 2002a. Simple sequence repeats for genetic analysis in pear. Euphytica 124: 129-137.

Yamamoto, T., T. Kimura, M. Shoda, Y. Ban, T. Hayashi, and N. Matsuta. 2002b. Development of microsatellite markers in Japanese pear (*Pyrus pyrifolia* Nakai). Molecular Ecology Notes 2: 14-16.

Yamamoto, T., T. Kimura, M. Shoda, T. Imai, T. Saito, Y. Sawamura, K. Kotobuki, T. Hayashi, and N. Matsuta. 2002c. Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. Theoretical and Applied Genetics 106: 9-18.

Yamamoto, T., T. Kimura, J. Soejima, T. Sanada, Y. Ban, and T. Hayashi. 2004. Identification of quince varieties using SSR markers developed from pear and apple. Breeding Science 54: 239-244.

Zhu, Y., and B. H. Barritt. 2008. Md-ACS1 and Md-ACO1 genotyping of apple (*Malus* x *domestica* Borkh.) breeding parents and suitability for marker-assisted selection.

Tree Genetics and Genomes 4: 555-562.
Zini, E., F. Biasioli, F. Gasperi, D. Mott, E. Aprea, T.D. Märk, A. Patocchi, C. Gessler, and M. Komjanc. 2005. QTL mapping of volatile compounds in ripe apples detected by proton transfer reaction-mass spectrometry. Euphytica 145: 269-279.

8 Appendix

Table 8.1. Phenotypic sampling frequencies of genotypes by year.

Family	Parental Role of 'Honeycrisp'	2005	2006	2007	Total
MN-1702	Paternal	17	27	12	32
	Maternal	19	19	17	24
	Total	**36**	**46**	**29**	**56**
MN-1764	Paternal	3	1	3	3
	Maternal	82	86	63	130
	Total	**85**	**87**	**66**	**133**
'Jonafree'	Paternal	9	6	12	13
	Maternal	37	18	36	46
	Total	**46**	**24**	**48**	**59**
'Monark'	Paternal	42	74	25	86
	Maternal	0	0	0	0
	Total	**42**	**74**	**25**	**86**
'Pitmaston'	Paternal	39	38	26	57
	Maternal	0	0	0	0
	Total	**39**	**38**	**26**	**57**
"Other Family"		17	23	14	**32**
Non-Progeny		58	51	46	**93**
	Total	**322**	**343**	**254**	**515**

Table 8.2. Phenotypic sampling frequencies of genotypes by number of years.

Family	Parental Role of 'Honeycrisp'	Number of Years Sampled			
		1 Year	2 Years	3 Years	Total
MN-1702	Maternal	4	10	7	21
	Paternal	15	9	11	35
	Total	**19**	**19**	**18**	**56**
MN-1764	Maternal	0	2	1	3
	Paternal	50	59	21	130
	Total	**50**	**61**	**22**	**133**
'Jonafree'	Maternal	3	6	4	13
	Paternal	7	33	6	46
	Total	**10**	**39**	**10**	**59**
'Monark'	Maternal	44	29	13	86
	Paternal	0	0	0	0
	Total	**44**	**29**	**13**	**86**
'Pitmaston'	Maternal	20	28	9	57
	Paternal	0	0	0	0
	Total	**20**	**28**	**9**	**57**
"Other Family"		15	12	5	**32**
Non-Progeny		40	41	11	**92**
	Total	**196**	**229**	**88**	**515**

Table 8.3. Phenotypic sampling frequencies of genotypes across panel dates and panelists.

		Panel Dates													
		2005					2006								
		0830†	0906†	0913†	0920†	0927†	0815†	0822†	0829	0905	0912	0919	0926	1005	1010
Panelists	A	20	22		28		22		12	12	16	16	24	12	8
	B	20				2									
	C	19	20	24	28	3		11	12			16	24	12	8
	D		23	24		2									
	E	20	21							12	16	16	24	12	8
	F			24	27	2		11	12	12		16	24	12	8
	G	19	23	24	28	4	21		12	12	16	16	24	12	8
	H		22	24	28	4		11		12	16	16	24		8
	I	19	23	24	28	3									
	J								12	12	16	16	24	12	8
	K		22	24	27					12	16	16			8
	L	18	23	24	27	2									
	M	18	20	23		3		12	12	12	16	16	24	12	8
	N							11	12		16	16	24	12	8
	O			24	27	3									
	P	19	23	24	28	3	21	10	12	12	16	16	24		8
	Q	19			26			12	12	12	16			12	
	R		22	25	26			12	12	12	16	16	24	12	8
	S	19		24	26	4		12	12	12	16	16	24	12	8
	T	18	22	24	27	4		10	12	12	16	16	24	12	8
	U			24	27	3									
	V						21		12		16	16		12	8
	W							10	12	12	16	16	24		8
# Apples per Panel		228	286	360	408	42	85	122	168	168	240	256	336	156	128
# Panelists per Panel		12	13	15	15	14	4	11	14	14	15	16	14	13	16

† Gaps maintained in sample trays. * Panel on 25 Sept. 2007 assessed apples collected over preceding two weeks.

Table 8.3. (continued)

		Panel Dates								# Apples per Panelist	# Panels per Panelist
		2007									
		0821	0828	0904	0911	0925*	1002	1009	1016		
Panelists	A		8	8	8	36	16	12	8	288	18
	B									22	2
	C	8	8	8	8	36	16	12	8	281	19
	D									49	3
	E									129	8
	F	8				36	16		8	216	14
	G			8	8	36	16	12	8	307	19
	H									165	10
	I		8	8		36		12	8	169	10
	J	8		8	8	36	16	12	8	196	14
	K									125	7
	L									94	5
	M	8	8	8	8	36	16	12	8	280	20
	N			8	8	36	16	12		179	12
	O									54	3
	P									216	13
	Q		8	8			16	12		153	11
	R	8		8	8	36	16	12	8	281	18
	S	8	8	8	8	36	16	12	8	289	20
	T	8			8	36	16	12	8	293	19
	U									54	3
	V	8		8	8				8	117	10
	W			8	8	36	16	12	8	186	13
# Apples per Panel		64	48	96	88	432	192	144	96	4143	
# Panelists per Panel		8	6	12	11	12	12	12	12		271

Table 8.4. Counts of marker segregation types across full-sib families.

		Heterozygous for 'Honeycrisp' Only	Heterozygous for Both Parents
MN-1702 Family	DArT	230	408
	SA SSRs	4	8
MN-1764 Family	DArT	207	432
	SA SSRs	20	23
'Jonafree' Family	DArT	160	478
	SA SSRs	7	25
'Monark' Family	DArT	137	501
	SA SSRs	5	30
'Pitmaston' Family	DArT	213	415
	SA SSRs	7	30
	NZ SSRs	9	2
	NZ SNPs	24	4

*New Zealand SSRs and SNPs assayed for 'Pitmaston' family only.