Genetics, Genomics and Breeding in Fruit and Vegetable Crops- Berries
Ed. K.Folta

Chapter X: *Rubus*

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Part 1: Rubus: a preamble

1.1 Economic importance, production areas Nutritional composition

Blackberries (*Rubus* sp. L.), red raspberries (*R. idaeus*) and black raspberries (*R. occidentalis* L.) are grown in many areas of the world, but they are most productive in regions

with mild winters and moderate summers. While generally referred to as “brambles” in the eastern U.S., they are generally referred to as “caneberries” in the western U.S.

Blackberries and red raspberries are sold fresh, primarily in clam shell packages, and as processed products, whereas the bulk of the black raspberry crop is processed. The primary processed caneberry products include fruit that is individually quick frozen (IQF), bulk frozen as whole fruit, pureed, juiced, canned, or dried. From these fundamental wholesale products a host of products are made for the retail market and for institutional food service products.

Blackberry production is rapidly increasing (Clark 2005; Clark et al. 2007; Strik 1992; Strik et al. 2007) and there were an estimated 140,292 MT commercially harvested from 20,035 ha in 2005. Europe leads the world in acreage (7,692 ha), while North America has the greatest production (59,123 MT). Serbia (69%) dominates European production however, a number of countries have significant production. In North America, the U.S., particularly Oregon, is the major producer. However, Mexican production has been rapidly increasing, particularly in Michoacan and Jalisco in the past five years. California and Arkansas are the only other states in the US with over 1,000 MT in production. Central American production (1620 ha) is predominantly from Costa Rica and Guatemala where in addition to harvest from managed stands, a great deal is harvested from feral stands. South American production (1597 ha) is predominantly from Ecuador and Chile. Asian production has been rapidly increasing with over 1550 ha of new plantings predominantly in China. Jiangsu is the most productive province, although Liaoning, Shandong and Hubei are increasing their production. The production in Oceania is mainly in New Zealand although the area planted is small with only about 259 ha. African production is only reported in South Africa but has been initiated in Morocco, Algeria and Kenya and possibly others. The bulk of the fruit is grown for processing applications in the

Pacific Northwest U.S., Serbia, and China whereas fresh market sales are the focus of the industry elsewhere.

The major production areas of red raspberries in North America are the Pacific Northwest (Oregon, Washington and British Columbia), California, the eastern US (New York, Michigan, Pennsylvania and Ohio) and a rapidly expanding industry in Mexico. In Europe, red raspberries are grown to the largest extent in Serbia, Russia and Poland, with commercial production scattered all across the European Union. Fresh market production is expanding rapidly in Spain, Portugal and other regions with a climate suitable to mimic the California production system. Average from world production from 2005-2007 was estimated at 602,500 MT from an area of 71,250 ha (wild plants were not included except from the Russian Federation) (Hall et al. in prep).

While adapted to many of the same areas as the other cultivated *Rubus*, Black raspberry (*R. occidentalis*) cultivation is concentrated in Oregon in the western U.S., in Ohio, Pennsylvania and New York in the eastern U.S. and in Korea. Oregon has traditionally been the leading producer with about 600 ha producing ~5,200 MT almost all for processing. The eastern US production is estimated to be around 100 ha and the fruit is grown for fresh and processing markets. While black raspberry production in Korea is estimated to be around 1,332 ha and 5,500 MT (Song J. Yun; personal comm.), this figure may include other *Rubus* sp. and reflects a three-fold increase in acreage since 2003; most of the Korean production is used to make a liqueur called Bokbunja.

In addition to being rich in the traditionally evaluated nutrients (Finn 2008b; Moore et al. 2008), the caneberries have among the highest levels antioxidants/phytonutrients of any fruit crop due primarily to their intense concentration of anthocyanins and phenolic compounds (Moyer et al. 2002). A considerable amount of new research has been performed on variation patterns in the antioxidant capacity of *Rubus* species and crosses. The fact that anthocyanins and polyphenolics are powerful antioxidants has led to a number of investigations that have looked at the nutraceutical/antioxidant levels of raspberries and will be discussed in detail later (Anttonem and Karjalainen 2005; Beekwilder et al. 2005; Dossett et al. 2008; Moore et al. 2008; Moyer et al. 2002; Perkins-Veazie and Kalt 2002; Siriwoharn et al. 2004; Wada and Ou 2002; Weber et al. 2008).

1.2. Academic importance: use as a model plant in genetics, cytogenetics, breeding and genomics works

Biotechnology has resulted in a fundamental shift in detecting and monitoring genetic variation in plant breeding and genetic studies A variety of molecular marker techniques including isozymes, random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP) and others, have been employed in genetic studies of raspberry and blackberry. *Rubus* species have been utilized for the construction of genetic linkage maps and the study of specific mechanisms of pest resistance. Wild Rubus populations have been investigated to elucidate diversity and population dynamics (Graham and

McNicol 1995; Patamsytė et al. 2004) as well as gene flow in wild populations as a model for ecological effects of habitat fragmentation (Graham et al. 2003; Graham et al. 1997) as well as gene flow between wild populations and cultivated varieties. The diversity of the weedy raspberry species *R. alceifolius* Poir. in its native range and in areas where it has invaded as a noxious weed has also been investigated (Amsellem et al. 2001a).

A hydroponic system was developed to directly observe the progression of disease to elucidate root rot in perennial species. Generational means analysis was then combined with molecular markers and QTL analysis to map resistance to *Phytophthora* root rot in a red raspberry population (Pattison et al. 2007). Attempts to develop markers for other viral resistance genes has been carried out for raspberry leaf spot and raspberry vein chlorosis utilizing the 'Glen Moy' x 'Latham' cross of Graham et al. (2004) (Rusu et al. 2006).

Additionally, many genomics tools are becoming available (discussed in Ch. 8). These include the construction of the first publicly available red raspberry BAC library from the European red raspberry, cultivar 'Glen Moy'. Currently, the library comprises over 15,000 clones with an average insert size of approximately 130 kb (6-7 genome equivalents). Additionally there has been several gene expression libraries created from various organs of the plant. Together, these libraries are allowing researchers to identify genes and elucidate their function at a much increased rate compared to the past.

1.3 Brief history of the crop: center of origin, botanical origin and evolution, domestication, dissemination

The center of diversity is considered to be in China where there are 250-700 species of *Rubus* depending on the taxonomist (Thompson 1997). While species are found on all continents except Antarctica, the greatest number of species is found in Eurasia and North America.

Most *Rubus* species were likely food sources wherever humans found them. *Rubus* has been traced back through ancient and historical times through artwork or illustrations of the time (Hummer and Janick 2007). European blackberry and red raspberry plants were mentioned by Ancient Greeks and Romans. At Newberry Crater near Bend, Oregon (USA), artifacts of food remnants containing *Rubus* date to 8,000 BCE. The writings of Aeschylus and Hippocrates from 500-400 BCE include caneberries. By 370 BCE, ancient Greeks were harvesting raspberries. Pompey brought raspberries from southeast of Troy, in current day Turkey, to the Romans around 65 BCE. The Hebrew Bible contained many references to thorny plants native to the Holy Land that some have attributed to *Rubus sanctus* Schreb. or *R. ulmifolius* Schott (Hummer and Janick 2007). The term *sĕneh* used to describe these species is also the term used in Exodus 3:1-5 to describe God's appearance to Moses "in the flame of fire in the bush" (Hummer and Janick 2007). Numerous herbals, particularly Dioscorides' *De Materia Medica* written about 65 CE included the curative properties of blackberry or raspberry (Hummer and Janick 2007). The *Juliana Anicia Codex* from around 512 CE has the first image of *Rubus* that survived antiquity. During the Renaissance *Rubus* was well represented and Hummer and Janick (2007) cite two

paintings by Jan Bourdichon illustrating *Horae ad isum Romanum* and a prayer book of Anne of Bretagne (1503-1508), a drawing of Leonardo da Vinci (1510-1512) and a woodcut from *De Historia Stirpium* (1544), an herbal by Leonhart Fuchs, as particularly good examples.

Raspberries gradually grew in popularity over the centuries and by the 1500s, *R. idaeus* was cultivated all over Europe. In 1829, 23 cultivars were listed in the "History of English Gardening" (Finn and Hancock 2008). North American *R. strigosus* was introduced into Europe in the early 19th century and natural hybrids with *R. idaeus,* resulted in many improvements. Most cultivars dating from this period are hybrids of these two species (Dale et al. 1989; Dale et al. 1993 ; Daubeny 1983).

By the 1600s, blackberries were being mentioned in gardening books (Jennings 1988). Since blackberries were so common around civilization there seemed to be little interest in domestication and identification of superior genotypes, let alone breeding, until the 1800s. Not surprisingly some of the first recorded selections from the wild were oddities such as albino or pink selections (Hedrick 1925).

1.4 Brief history of breeding

Several excellent reviews of blackberry and raspberry breeding have been written in the past couple of years including Clark et al. (2007), Finn (2008), Finn and Hancock (2008) and Finn and Clark (in prep), Kempler (in prep).

1.4.1 Blackberry

Judge James H. Logan of Santa Cruz, Cal. (USA) is usually credited with having the first blackberry breeding effort. ‘Loganberry’, released in 1890, and ‘Black Logan’ were the most successful from his program although ‘Loganberry’, which still is grown commercially more than a century after its release, was selected from open pollinated fruit of the pistillate ‘Aughinbaugh’ presumably crossed with ‘Red Antwerp’ red raspberry (Logan 1955). Luther Burbank who, along with Thomas Edison and Henry Ford, played such a powerful role in the public’s imagination was active in blackberry breeding as well and developed/found “Phenomenal’/’Burbank’s Logan’ that was nearly indistinguishable from ‘Loganberry’ (Clark et al. 2007; Darrow 1925). Byrnes M. Young, an amateur horticulturist in Morgan City, La. could not grow ‘Loganberry’ or ‘Phenomenal’ but who, during correspondences with Burbank, decided to make crosses in 1905 between ‘Phenomenal’ and the locally adapted ‘Austin Mayes’ to produce ‘Youngberry’, which was released in the 1920s, is still commercially grown today, and is a grandparent of the widely grown ‘Marion’ (Clark et al. 2007). The origin of the trailing blackberry ‘Boysenberry’ from a similar era is unknown but Wood et al. (1999) presented a thorough examination of its history. ‘Boysenberry’ was discovered by Rudolph Boysen on the Lubben farm in Napa County in northern California (USA). Later when he moved to southern California, the plants caught the attention George Darrow a plant breeder from the USDA-ARS (Beltsville, MD) who convinced a local grower and nurseryman, Walter Knott, to trial this selection. Knott and Darrow released it as a cultivar bearing the discoverer’s name. Knott went on to develop a thriving business that started as a farm stand serving ‘Boysenberry’ pie and has

now become the Knott's Berry Farm entertainment and food products empire (Wood et al. 1999). While Wood et al.'s (1999) explanation of the historical origins of 'Boysenberry' is well researched there is still no certainty on the genetic origins of 'Boysenberry'. Similar hybrid berries between raspberry and blackberry such as 'Laxtonberry', 'Veitchberry', 'Mahdi', and 'Kings Acre' were developed in Europe (Darrow 1937).

'Thornless Evergreen', a selection of *R. laciniatus*, is the final cultivar that was selected from the wild and is still commercially viable (Waldo 1977). While the original cultivar 'Evergreen' can be traced to the 1800s in Europe, the thornless sport later named 'Thornless Evergreen' was found in Stayton Oregon, in 1926 and quickly became the industry standard. The thornless chimeral form is unstable and commonly reverts to thorny canes with environmental or mechanical injury. 'Everthornless', which is genetically thornless, was developed from somaclonal plants developed from the L_1 layer of 'Thornless Evergreen' (McPheeters and Skirvin 1983; McPheeters and Skirvin 1989, 2000).

Beginning in the early 1900s, publicly funding breeding efforts supported by the land grant university systems or the USDA-ARS began. The Texas Agricultural Experiment Station breeding program was the first blackberry program (Darrow 1937). The primary goals of this program were to develop "hybrid berries" that were adapted to hot climates with low chilling. 'Nessberry', developed there using *R. trivialis* germplasm, had some popularity, but it was even more valuable as a parent of 'Brazos', which until recently was the most popular Mexican cultivar.

The John Innes Horticultural Institute in England and the New York State Agricultural Experiment Station followed by the USDA-ARS in Georgia were the next to develop programs. The John Innes program developed the critically important 'Merton Thornless', which is the primary source of thornless in all tetraploid cultivars. The New York program developed several erect cultivars in the 1950s including 'Bailey', 'Hedrick', and 'Darrow'.

The major concerted breeding efforts worldwide that are still active are the University of Arkansas, the USDA-ARS in Oregon and the private program run by Driscoll's Strawberry Associates (Watsonville, Cal.) (Finn and Knight 2002). Two significant programs that have been discontinued or lost significant funding recently are the USDA-ARS program in Beltsville, MD and the New Zealand Hort Research Inc. program (Motueka).

The USDA-ARS Beltsville program incorporated thornlessness from 'Merton Thornless' into the first outstanding thornless cultivars released in the late 1960s and early 1970s ('Black Satin', 'Smoothstem', 'Thornfree', and 'Dirksen Thornless') (Moore 1997; Scott et al. 1957). The USDA-ARS had a significant effort at their station in Carbondale, Ill in the 1960s until it was closed in the early 1970s and 'Hull Thornless' and 'Chester Thornless' came out of this effort. The last release from these eastern USDA-ARS programs was 'Triple Crown' in the 1990s (Galletta et al. 1998). This group of breeding material and cultivars is called "semi-erect" and the plants are characterized as being thornless, with very vigorous, large erect canes that

grow 4-6 m long from a crown and arch to the ground. Their fruit is similar in quality to the erect blackberries and they are often incredibly productive.

The New Zealand HortResearch Inc. program was one of the most valuable and aggressive programs in the 1980s and 1990s as it developed "Boysenberry-like" cultivars and developed the 'Lincoln Logan' source of spinelessness (S_{fL}) (Hall et al. 1986a; Hall et al. 1986b; Hall et al. 1986c; Hall et al. 2002). 'Ranui', 'Waimate', 'Karaka Black', and 'Marahau' have been the notable recent releases from this program (Clark and Finn 2002; Hall et al. 2003; Hall and Stephens 1999).

Started in 1928, the USDA-ARS program in Oregon is the oldest continuously active program. The efforts there combined wild selections (e.g. 'Zielinski') of the Pacific Coast native, trailing, dioecious *R. ursinus* Cham et. Schlt. with a perfect flowered gene pool including 'Loganberry', 'Youngberry', 'Himalaya', 'Santiam'/''Ideal', and 'Mammoth', and cultivars from elsewhere, to develop cultivars for a whole new industry based on trailing blackberries. These crown-forming types that trail on the ground until lifted onto a trellis have excellent fruit quality but often have poorer winter hardiness than the other types. 'Pacific', 'Cascade', 'Chehalem', and 'Olallie' released from 1942-1950 were instrumental in the industry's establishment (Waldo 1948; Waldo 1950; Waldo and Wiegand 1942). 'Marion' released in 1956 later became the industry standard in the Pacific Northwest (Waldo 1957). During the 1970s and 1980s this program worked to improve the fruit quality in the thornless germplasm pool that had been derived with the 'Austin Thornless' source of thornlessness. The first trailing thornless cultivar

to be released and commercially grown was ‘Waldo’ (Lawrence 1989). Recent releases from this program are being widely planted in the Pacific Northwest, coastal California and other mild climate areas in Europe and South America and these include the thornless ‘Black Diamond’, ‘Black Pearl’, and ‘Nightfall’ as well as the very early ripening ‘Obsidian’ (Finn et al. 2005a; Finn et al. 2005b; Finn et al. 2005c, d).

In addition to the trailing and semi-erect blackberries, the third type of blackberry is the erect blackberry which was largely developed by the University of Arkansas from eastern North American blackberry species. These types produce 1-4 m tall stiff upright canes and the plants sucker to produce a hedgerow. While there are cultivars, such as ‘Eldorado’, that can be traced back to the 1800’s, these really were developed as a viable commercial crop by the University of Arkansas beginning in 1964. The erect blackberry germplasm pool is similar to that of the semi-erects as they are all tetraploid and have comparable fruit quality characteristics. As with the semi-erects, thornlessness from ‘Merton Thornless’ was incorporated into this gene pool. In 1989, ‘Navaho’ was the first thornless erect cultivar to be released. Other cultivars released from this program include ‘Cheyenne’ and ‘Cherokee’ released in the 1970’s, ‘Shawnee’ in the 1980’s, ‘Kiowa’, ‘Apache’ and ‘Chickasaw’ in the 1990’s, and ‘Ouachita’ and ‘Natchez’ in the 2000’s. The Arkansas program, with some input from North Carolina State University, developed primocane fruiting blackberries. The plants are cut to the ground each year and then flower and fruit late in the season on new growth. ‘Prime-Jan’ and ‘Prime-Jim’ are the first of this type. Primocane fruiting was critical to the worldwide expansion of the red raspberry industry and it is hoped that it will have a similar impact on blackberry.

Smaller sized and very productive programs are active elsewhere as demonstrated by the recent development of ‘Tupy’ from Brazil (Clark and Finn 2002), ‘Loch Maree’, ‘Loch Ness’, and ‘Loch Tay’ from the Scottish Crop Research Institute (Clark and Finn 2008; Finn and Clark in prep; Jennings and Brydon 1989), ‘Chesapeake’ from the University of Maryland (Clark and Finn 2002), and 'Cacanska Bestrna' ('Cacak Thornless') from Serbia (Clark and Finn 1999; Stanisavljevic 1999).

1.4.2 Raspberry

Finn and Hancock (2008) along with Jennings (1988) give good overviews of red raspberry breeding and much of the discussion below is a synopsis of their overviews. The first formal breeding work on raspberries was begun in North America. Dr. Brinkle of Philadelphia, Pennsylvania was cited by Darrow (1937) as the “first successful raspberry breeder of this country” (Darrow 1937). ‘Latham’ has been the most enduring cultivar from this early breeding period, introduced by the Minnesota Breeding Farm in 1914, it is still grown. ‘Pruessen’, ‘Cuthbert’ and ‘Newburgh’ were developed in Europe and are hybrids between the North American and European species, along with ‘Lloyd George’ and ‘Pyne’s Royal’, which are pure *R. idaeus,* were the cultivars that played an integral role in early red raspberry breeding.

‘Lloyd George’ was in the direct ancestry of 32 % of the North American and European cultivars in 1970 (Oydvin 1970). This cultivar contributed several important traits including primocane fruiting, large fruit size and resistance to the American aphid. Jennings (1988) speculates that the success of ‘Lloyd George’ hybrids “was possibly achieved because they combined the long-conical shape of ‘Lloyd George’ receptacle with the more rounded shape of the American raspberries”. ‘Willamette’ from a cross of ‘Newburgh’ x ‘Lloyd George’ is an example of a “’Lloyd George’ hybrid” that dominated the industry in western North America for over a half century.

While many programs have released very successful cultivars in the 20th and early 21st Centuries a few programs have been particularly important. The East Malling program in the United Kingdom was responsible for the “Malling series”. A number of selections were made prior to World War II and released in the 1950s, ‘Malling Promise’, ‘Malling Exploit’ and the most successful, ‘Malling Jewel’ (Jennings 1988). This program continues to have an impact with recent release ‘Octavia’ (Finn et al. 2007). In addition to these floricane cultivars, the program has developed a number of very important primocane fruiting cultivars in the “Autumn series”.

The very important “Glen series” was developed at the Scottish Crop Research Institute (Invergowrie, UK). Their first release was ‘Glen Clova’ in 1969 but the release of ‘Glen Moy’ and ‘Glen Prosen’ in 1981 offered great improvements in fruit size and flavor along with

spinelessness. ‘Glen Ample’, released in 1994, is one of the standards in the European wholesale raspberry market.

The breeding programs in the Pacific Northwest of North America at Washington State University (WSU; Puyallup, Wash.), Agriculture and Agri-Foods Canada (AAFC; Agassiz, BC) and the U.S. Dept. of Agriculture-Agricultural Research Service in Oregon (USDA-ARS; Corvallis) have worked closely for decades and with programs in the UK to make fantastic gains. The USDA-ARS’s releases from the mid 1900s, ‘Willamette’ and ‘Canby’, are still commercially important floricane cultivars and their recent release ‘Coho’ has been widely planted for its high yields of IQF fruit (Finn et al. 2001). ‘Summit’ and ‘Amity’, primocane fruiting types from this program have been very important since their release. ‘Summit’ has seen a fantastic resurgence in interest due to its adaptation to Mexican production systems. ‘Meeker’, developed by WSU and released in the 1960s, is still the processing industry standard (Finn 2006). This program also continues to be active and the newest releases ‘Cascade Delight’ and ‘Cascade Bounty’, which are root rot tolerant, and ’Cascade Dawn’ are being widely planted (Moore 2004, 2006; Moore and Finn 2007).

The AAFC program has played a primary role in developing extremely high quality fresh market cultivars over the past few decades. They took full advantage of germplasm exchanges with the U.K. and were very successful at identifying outstanding selections out of crosses between British Columbia selections and some of the “Glen series” particularly ‘Glen Prosen’ (Finn 2006). Their early releases such as ‘Chilcotin’, ‘Skeena’ and ‘Nootka’ had excellent fruit

quality and high yields for a fresh market berry. The program followed these releases with ‘Chilliwack’ in the mid 1980s and ‘Tulameen’ in 1989, which has become the world standard for fresh market fruit flavor and quality. This program remains active and the recent releases ‘Esquimalt’, ‘Chemainus’, ‘Cowichan’, ‘Saanich’ and ‘Nanoose’ are being widely planted (Kempler et al. 2006; Kempler et al. 2007; Kempler et al. 2005a; Kempler et al. 2005b).

In the eastern U.S., Cornell University’s New York State Agricultural Experiment Station (Geneva) has the oldest continuous raspberry-breeding program in North America, dating to the early late 1800’s. Floricane varieties such as ‘Taylor’ and ‘Hilton’ were early staples of the eastern industry. These have been replace in recent decades with varieties such as the large fruited ‘Titan’, released in 1985, early season ‘Prelude’ and very late season ‘Encore’, both released in 1998. ‘Titan’ has proven to be an excellent parent, producing large fruited offspring but is susceptible to Phytophthora root rot.

In the 1950’s primocane fruiting germplasm within the program in combination with material such as ‘Durham’, developed in New Hampshire, was used to produce an excellent primocane fruiting germplasm pool that culminated with the release of ‘Heritage’ in 1969 and ‘Ruby’ (‘Watson’) in 1988 (Daubeny 1997). First viewed as a novelty, the primocane fruiting types have revolutionized raspberry production. They have become the standard in regions where cold winter temperatures caused considerable winter damage to canes of floricane fruiting raspberries, as well as in low chill areas where floricane varieties do not receive adequate chilling to be productive. Private companies in California, such as Driscoll’s Strawberry

Associates (Watsonville, Cal.) have developed cultivars and whole new production systems based around these primocane fruiting types where the plants were only in the ground 18 months (Finn and Knight 2002). These cultivars and management systems have led to the rapid expansion of the California raspberry industry as well as industries in southern Europe, northern Agrica, Australia and South Africa.

While the University of Minnesota program has been recently discontinued, their releases 'Latham' and 'Chief' were valuable commercial floricane cultivars and very valuable parents in further breeding. The University of Maryland has coordinated a breeding program with Virginia Tech University, Rutgers University, and the University of Wisconsin – River Falls, and the primocane fruiting 'Caroline', 'Anne', and 'Josephine' developed in this program have become standards throughout much of North America.

The eastern North America black raspberry (*R. occidentalis*) was not cultivated until the 19th century, probably because of its abundance in the wild and the public's preference for red raspberry (Jennings 1988). Other than at Cornell's New York State Agricultural Experiment Station, there has been no consistent long term breeding effort in black raspberry. The station initiated its efforts in the late 1800s and was the primary center of research for much of the 20th Century (Ourecky 1975; Ourecky and Slate 1966; Slate 1934; Slate and Klein 1952). However by the late 20th Century, there was little breeding effort at Geneva nor or anywhere else; only three cultivars were released in the last half of that century. In the early 21st Century, black raspberry breeding efforts were renewed at the New York State Agriculture Experiment Station,

the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) in Corvallis, Oregon and in Beltsville, Maryland, and with New Zealand HortResearch Inc. In Corvallis, Dossett et al. (2008) evaluated black raspberry families from sibling families from crosses among cultivars and a North Carolina selection to assess variation and inheritance of vegetative, reproductive and fruit chemistry traits in black raspberry. In New Zealand, 'Ebony', the first spineless black raspberry cultivar was released (H.K. Hall, pers. comm.), while in Maryland, an EST library was being fine tuned for use in black raspberry (K. Lewers, pers. comm.), and finally, in New York, a large scale breeding effort was initiated.

Driscoll Strawberry Associates, Inc. (Watsonville, Cal.) has been breeding red raspberries in some manner since the 1930s, but the program was reinitiated in a structured way in the early 1980s and their blackberry program started in 1991. While it may be irrelevant to others what Driscoll's does as the cultivars they develop are kept within the company, since they have played such an important roll in the expansion of the fresh raspberry and blackberry industry and since the acreage devoted to their cultivars in North America and increasingly elsewhere is so large, it is important to recognize their successful efforts.

1.5 Botanical descriptions: taxonomic position, habit, habitat, brief morphology, karyotype, ploidy levels, genome size

The *Rubus* is a diverse group of hundreds of species divided botanically among 15

subgenera, many of which have been used in breeding (Finn 2001; Finn et al. 2002a; Finn et al. 2002b; Jennings et al. 1991; Knight 1993). The subgenus *Rubus* is divided into 12 sections with most of the cultivated blackberries being derived from the *Allehgeniensis*, *Arguti*, *Flagellares, Rubus*, *Ursini*, and/or *Verotrivialis* (Finn 2008a). Red and black raspberries along with many of the wild harvested species from around the world are in the *Idaeobatus* subgenus. The raspberries and blackberries are botanically separated by whether the torus (receptacle) remains in the fruit when picked in which case it is considered a blackberry, or remains on the plant leaving a hollow center to the fruit in which case it is considered a raspberry. The separation leads to some incongruities when something like a 'Loganberry', which has the shape and color of a red raspberry, is by definition a blackberry because the torus picks with the fruit.

The caneberries are typically found as early colonizers of disturbed sites and along forest edges. They are attractive to frugivores who move them quickly into open areas. As a group they are generally found in habitats with decent moisture levels. While they can be found in desert environments, they are always found in close proximity to springs, seeps or streams. Blackberries are typically much more tolerant of drought, flooding and high temperatures while red raspberries are more tolerant of cold winters.

1.5.1 General blackberry and raspberry habit

Plants produce biennial canes from a perennial crown/root system with vegetative canes,

called primocanes growing for the first year. After a dormant period they flower and are called floricanes which produce fruit before dying. Simultaneous with the floricanes fruiting, the plants are producing primocanes for the following year's crop. In any given year, half the canes on a plant are vegetative primocanes and the other half fruiting floricanes. While the plants are perennial, the canes are typically biennial and the floricanes die after fruiting. Primocane-fruiting red raspberry, black raspberry, and blackberry cultivars have been developed that flower and fruit on current season's growth. All cultivated red raspberries and some blackberries produce root initials on spreading roots to form new canes so the plants can spread quite some distance vegetative. All black raspberries and many cultivated blackberries are crown forming and do not spread underground, however, they both can spread vegetatively by tip layering of primocanes. Blackberries are generally larger and more vigorous than raspberries and the cultivated types have prostrate (trailing) to very upright (erect) growth habits with canes up to 5 m tall (Clark et al. 2007). The primary cultivated red raspberry species is very erect while the commercial blackberries have a more varied growth habit.

1.5.2 Blackberry growth habit

While blackberry species range from completely procumbent to very upright, commercial blackberries are classified into three categories based on cane type: trailing, semi-erect, and erect (Strik 1992). Trailing types are crown-forming and the primocanes trail on the ground surface until they are bundled, lifted and tied to a trellis. 'Marion', 'Thornless Evergreen', and 'Black Diamond' are examples of this type of plant. Those with a semi-erect habit are also crown-

forming and require a trellis, with the mature canes growing erect for about 1 m before arching over. Important semi-erect cultivars are ‘Chester Thornless’, ‘Loch Ness’, and ‘Triple Crown’. The erect blackberries grow upright, but less vigorously than the semi-erect types, and instead of being crown forming, they sucker beneath the soil line and will form a continuous row of canes in a managed field. Erect cultivars include ‘Navaho’, ‘Arapaho’, ‘Ouachita’, and ‘Natchez’. Primocanes of erect and semi-erect blackberry cultivars and black raspberry cultivars are tipped in the summer to encourage branching and to increase production, whereas trailing cultivars and red raspberries are usually not tipped.

1.5.3 Raspberry- red, black, and purple growth habit

Red raspberries sucker vegetatively beneath the soil producing new canes from root initials. The primocanes of many raspberry cultivars remain vegetative in the first year and are only harvested in the second year as floricanes. Some of the more popular cultivars of this type are ‘Tulameen’, ‘Glen Ample’, ‘Meeker’ and ‘Willamette’. There are also primocane fruiting red raspberry cultivars that produce fruit in the fall at the top of the current season’s canes and then again in the second year at the base of the cane. Some of the more popular cultivars of this type include ‘Heritage’, ‘Caroline’, ‘Josephine’, ‘Amity’, and ‘Autumn Bliss’. While it is easiest to cut the canes of these cultivars off at ground level each winter after picking the late-summer primocane crop, the canes are sometimes left to over-winter and produce a very early spring crop. Because these primocane fruiting types can be double cropped, they are sometimes called “everbearing raspberries”.

With the exception of ‘Explorer’, all black raspberry cultivars are floricane fruiting. The primocanes that emerge from the crown are tipped in commercial plantings to about 1 m tall to encourage branching. The following year these canes become floricanes and produce the crop. A few of the more widely planted cultivars of black raspberry are ‘Munger’, ‘Jewel’, and ‘Blackhawk’. Purple raspberries, a cross between red and black raspberries, tend to have a great deal of “hybrid vigor” and are also crown forming plants with large, soft fruit. They are generally considered to have only fair quality fresh but truly shine when they are processed. ‘Brandywine’ and ‘Royalty’ are the two most commonly sold cultivars.

Part 2: Classical genetics and traditional breeding

2.1 Classical mapping efforts

Early work on linkage analysis of morphological traits by Crane and Lawrence (1931) and Lewis (1939) documented aberrant segregation ratios among populations segregating for fruit color (T) and pale green leaves (g or ch_1) in red raspberry (Crane and Lawrence 1931; Lewis 1939). Further showed genetic linkage among 5 genes (waxy bloom *b*, apricot or yellow fruit *t*, pale green leaf *g*, red hypocotyl *x* and pollen tube inhibitor *w*), producing the first genetic linkage group for *Rubus* (Lewis 1939, 1940). Sepaloid sx_3 was later added to the linkage group between *b* and *t* (Keep 1964). Crane and Lawrence (1931) and Lewis (1939,1940) also postulated on a linkage between a semi-lethal allele with the unlinked *h* gene. Jennings (1967) added further evidence to this linkage, proposing the symbols *wt* for the locus linked to the fruit color *t* locus and *wh* linked to the hairy locus (*h*) in place of *w* that Lewis (1939) used (Jennings 1967).

Subsequent work in red raspberry has further elucidated the inheritance of hairiness and fruit color as well as numerous other traits. Associations between the *H* allele for cane hairiness and resistance to spur blight, cane *Botrytis* and cane blight have been recognized (Jennings 1988; Keep 1989). This same gene also has been associated with susceptibility to cane spot, powdery mildew and western yellow rust (Jennings and Brydon 1989; Jennings and McGregor 1988). These associations are likely pleiotropic effects rather than genetic linkages Similarly, the

recessive gene *s* for spine-free canes and the dominant *B* for waxy bloom on canes can reduce spur blight incidence (Jennings 1982b, 1988). Again, it is unlikely these genes are genetically linked but rather compliment each other physiologically to reduce disease incidence. However, no other linkage groups of based solely on morphological traits have been proposed. Daubeny (1996) lists 72 individual loci or alleles that have been identified, many of which are part of an allelic series for aphid resistance (Daubeny 1996). Corresponding work in blackberry and other Rubus species has been largely absent, probably due to the complex genetics of blackberry and the relatively unimportant economic impact of other species. Further work on developing linkage maps in will depend on molecular marker to *Rubus*provide whole genome coverage. *Rubus*

2.2 Limitations of classical endeavors and utility of molecular mapping

Prior to the advent of molecular markers, inheritance and genetic mapping studies were limited to simple morphological traits (Jennings 1988; Ourecky 1975). These studies generally utilized phenotypes that are deleterious in the recessive form so that they are undesirable to maintain in breeding programs. The advent of biotechnology has resulted in a fundamental shift in the development of genetic linkage maps and their use in variety development. Classical breeding, which selects parents and their desirable offspring based on an observable phenotype, is being integrated with techniques that can identify and manage genetic variability at the molecular level (protein or DNA). The ability to detect genome wide variability has led to the characterization of genetic variation within, not only coding regions (i.e. genes and their

morphological manifestations), but also in non-coding regions as well, which make up large portions of plant genomes. These developments have enabled the construction of genetic linkage maps of red raspberry containing numerous genetic markers that are phenotypically neutral, which have been used to identify genomic regions associated with phenotype. Corresponding mapping in blackberry and other *Rubus* species has lagged due to their complex genetic make up and/or low economic importance.

A variety of molecular marker techniques including random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP) and others, have been employed in genetic mapping of raspberry.

The first genetic map of raspberry was developed by Graham et al. (2004) utilizing SSR and AFLP markers for a population of 'Glen Moy' x 'Latham'. SSR markers were developed from both genomic and cDNA libraries from the cultivar 'Glen Moy', and AFLP markers further saturated the map for quantitative trait loci (QTL) analysis for complex morphological traits. A genetic linkage map was produced consisting of nine linkage groups with 273 markers covering 789 cM of map distance. QTL analysis for variability in spine density identified two associated regions on linkage group 2. Analysis of the production and spread of root suckers identified two regions on linkage group 8 associated with the spread of root suckers from the mother plant and one similar region associated with sucker production. Graham et al. (2006) later added 20 SSR markers to the 'Glen Moy' x 'Latham' map along with analyzing data on the *H* gene for cane hairiness and resistance to multiple fungal pathogens. The *H* gene was mapped to linkage group

2 and associated closely with resistance to cane *Botrytis* and spur blight. Resistance to cane spot was mapped to a different region of linkage group 2 and linkage group 4, and yellow rust resistance mapped to linkage group 5.

Pattison et al. (2007) combined generational means analysis with molecular markers and QTL analysis to map resistance to *Phytophthora* root rot in a BC_1 population of NY00-34 ('Titan' x 'Latham') x 'Titan'. Separate genetic linkage maps of NY00-34 and 'Titan' were developed using RAPD, AFLP and resistance gene analog polymorphisms (RGAP) and analyzed for QTL associated with various parameters of root rot resistance assayed in a hydroponic system (Pattison et al. 2004). Regions on linkage groups 1, 5 and 7 were associated in multiple parameters of the resistance response. Bulked segregant analysis (BSA) corroborated this conclusion by identifying markers from these regions associated with bulked samples of resistant and susceptible genotypes. Generational means analysis suggests two major genes controlling resistance, possibly corresponding to the two regions on each parental linkage map associated with resistance.

Attempts to develop markers for other viral resistance genes has been carried out for raspberry leaf spot and raspberry vein chlorosis utilizing the 'Glen Moy' x 'Latham' cross of Graham et al. (2004). Field screening was carried out to measure symptom production of leaf spot and vein chlorosis in 2 different environments. These traits were analysed for significant linkages to mapped markers and resistance loci were found on linkage groups 2 and 8 (Rusu et al. 2006).

Recently, Sargent et al. (2007) mapped the *A_1* locus conferring aphid resistance and the *dw* locus conferring dwarfing habit in a population of 'Malling Jewel' x 'Malling Orion' with AFLP and SSR markers. A map of 505 cM with seven linkage groups was produced with *A_1* and *dw* mapping to linkage groups 3 and 6, respectively. Genetic linkage maps have the potential for use in marker assisted selection (MAS) to increase the efficiency of breeding new cultivars and for integrating new traits from related species. Work towards the genetic mapping of health-related compounds has been initiated in *Rubus* (Stewart et al. 2007).

2.3 Breeding objectives: positive (e.g. yield) and negative (e.g. anti-nutritional) factors

While all breeding programs share some common goals they also have specific objectives depending on the type of raspberry or blackberry grown in their region, the use and market for the fruit, and genetic variability available (Clark and Finn 2006). In general terms, yield, fruit quality, abiotic and biotic stress tolerance, and harvest ease are all important traits that have been well addressed by breeders.

Finn and Clark (in prep) identified fruit quality as a primary focus of all programs as it is the main area that can increase blackberry and raspberry consumption (Finn and Clark in prep). Advances in fruit quality from the early wild selections and first improved cultivars have been substantial. In blackberry, this progress has moved it from being viewed as a fruit harvested from

the wild that was often not found on grocers shelves to one that is now found on shelves year round and from all parts of the world. In the fresh market, cultivars with improved flavor and sweetness are foremost in need of enhancement. Progress in this area can be made, and along with manipulation of flavor components, acidity, astringency, and postharvest handling. There is more than adequate genetic variation available to advance blackberries substantially further in quality attributes and displace current cultivars that might deter repeat sales by consumers. Similarly with raspberries, the first goal in breeding for fruit quality of fresh products was to develop genotypes with much firmer fruit that would allow the delicate raspberry to be harvested and shipped around the world. Unfortunately, this has led to the development of very firm berries that are picked immature and do not taste good. More recently, flavor has been reincorporated into commercial red raspberries without a big reduction in fruit firmness and shipability. Cultivars for processing generally have intense flavor and high soluble solid levels. The challenge for breeders is to maintain or improve these characters while thornlessness, machine harvestability, increased yield, and increased fruit firmness are selected for in breeding populations.

Breeding for broader adaptability or for types that will do well in new regions has greatly expanded the industry. In raspberry, California has become the predominant producer due to the development of superior primocane fruiting types that work in an 18 month production cycle. Blackberry production has dramatically expanded in areas with very low chilling, particularly Mexico. While production techniques including defoliation and the use of growth regulators has been a major part of this development in Central, México, the use of the Brazilian cultivar Tupy, has been equally important. Further, the recent introduction of primocane fruiting in blackberry

offers a method to eliminate chilling concerns completely, since these canes do not go through a dormant period prior to initiating flowers.

Adaptation to heat has been a major problem for raspberries and blackberries. While blackberries are generally better adapted to heat than raspberries, high temperatures during fruit ripening can be equally devastating to each crop. While there is genetic variability for this trait, it has not been well characterized.

Overall, variability for most traits such as thornlessness, architecture, disease and insect resistance, adaptation, productivity, fruit quality and size, and other traits is sufficient such that progress can be made if a breeding program focuses on that trait and can assemble the appropriate germplasm. The exception to this is in black raspberry where there is very little variability (Dossett et al. 2008; Weber et al. 2008).

2.4 Classical breeding achievements (yield, quality, and stress resistance)

Blackberries and raspberries have a relatively short history as cultivated crops that have been enhanced through plant breeding. Further, an intensive focus on plant improvement has taken place for less than a century and they are very few generations removed from their wild progenitor species. The improvements that have allowed these plants to be commercial cultivated

crops are well documented: including increased yield, improved harvest efficiency, abiotic and biotic stress tolerance, increased fruit quality for fresh and processed markets, altered plant architecture etc. There are excellent reviews on blackberry and raspberry breeding, genetics and germplasm (Clark and Finn 2008; Darrow 1937; Daubeny 1996; Finn and Clark in prep; Hall 1990; Jennings 1988; Jennings et al. 1991; Kempler in prep; Moore 1984; Oydvin 1970; Sherman and Sharpe 1971; Waldo 1950, 1968).

Part 3: Diversity analysis

3.1 Phenotype-based diversity analysis - Lawrence Alice to write this part

3.2 Genotype-based diversity analysis, molecular markers applied

Many studies have utilized molecular markers to quantify genetic diversity in wild raspberry populations as well as among breeding programs and cultivars. Further use has been made in classifying that diversity and elucidating the relationships among the many hundreds of *Rubus* species (Weber 2003).

Various DNA fingerprinting techniques have been used to investigate diversity and population dynamics in raspberry. Minisatellite -based fingerprints were developed in black raspberry that identified 15 different genotypes from a sample of 20 individuals (Nybom et al. 1990). restricted DNA was probed from 22 red and purple raspberry genotypes with chloroplast DNA probes and determined that maternal ancestry in *R. occidentalis*, *R. parvifolius* and *R.*

idaeus strigosus (Michx.) could be differentiated from *R. idaeus vulgatus* Arrhen., from which arose all of the commercial red raspberry cultivars that they studied (Moore 1993).

Random amplified polymorphic markers (RAPDs) have been used widely in taxonomic studies (Graham and McNicol 1995; Pamfil et al. 2000; Pamfil et al. 1997; Trople and Moore 1999) and germplasm diversity assessments (Badjakov et al. 2006; Graham et al. 2003; Graham et al. 1994; Graham et al. 1997; Patamsytė et al. 2004; Weber 2003) in raspberry species. For example, Graham et al. (1994) used the distribution of markers from nine RAPD primers to produce a similarity index and a hierarchical tree for ten red raspberry cultivars and Graham and McNicol (1995) elucidated relationships among 13 *Rubus* species using RAPDs.

In another use RAPD markers, Graham et al. (1997) examined relationships and spatial diversity among wild populations of *R. idaeus* in Scotland at four sites in comparison to the commercial cultivar 'Glen Clova'. None of the wild populations were closely related to the commercial cultivar. Later, Graham et al. (2003) assayed a wider range of wild *R. idaeus* from 12 sites across a greater area of the United Kingdom and compared the accessions to the cultivar 'Glen Moy'. Again, little gene flow was observed between wild populations and commercial cultivars.

Amplified fragment length polymorphisms (AFLP) have been used to investigate diversity in multiple *Rubus* species. Amsellem et al. (2000c) investigated the weedy raspberry

species *R. alceifolius* Poir. in its native range and in areas were it has invaded as a noxious weed (Amsellem et al. 2001c). Considerably more genetic diversity was detected in its native range with diversity in non-native ranges dependent on distance from the origin. Multiple introduction sites could be identified in some cases. Amsellem et al. (2001b) also used AFLPs to show that reproduction within the native range of *R. alceifolius* is through sexual means while apomixis was common only in non-native locations (Amsellem et al. 2001b). Markling (2006) also found little difference in genetic diversity between natural populations and cultivated populations using chloroplast sequence and SSRs (Marking 2006).

Lindqvist-Kreuze et al. (2003) also used AFLP markers to characterize diversity in six populations of wild arctic raspberry (*R. arcticus* L.) and ten cultivars in Finland. AFLPs were highly effective in distinguishing 78 genotypes from 122 samples. Genetic variation was found to be high within populations indicating a high degree of sexual reproduction but interpopulation gene flow was low as measured by overall diversity among locations. Diversity within the cultivars was high enough that subspecies could be differentiated (Lindqvist-Kreuze et al. 2003).

3.3 Relationship with other cultivated species and wild relatives

Trople and Moore (1999) calculated genetic similarities among 43 *Rubus* species and raspberry genotypes based on marker profiles from six RAPD primers. The similarity indices were relatively low between the species (0.15 to 0.52) with much higher indices for multiple accessions within species (0.62 to 0.82) (Trople and Moore 1999). In another study, 40 species

of *Rubus* were analyzed, including many raspberry types, using RAPD markers and found that molecular classification of species agreed with the traditional classification of *Rubus* in most cases, except for three species in the subgenus Malachobatus that clustered with the raspberry types in subgenus Idaeobatus (Pamfil et al. 2000). However, their RAPD based taxonomy could not explain differential success of interspecific hybridization within each subgenus.

Simple sequence repeats (SSR) were used to compare diversity in the weedy species *R. alceifolius* within native and invasive ranges, and as well could track interspecific hybridization among overlapping *Rubus* species (Amsellem et al. 2001b).

Badjakov et al. (2006) analyzed 28 raspberry genotypes from the Bulgarian germplasm collection including 18 Bulgarian cultivars and breeding lines, eight accessions from outside Bulgaria and two wild species accessions, *R. occidentalis* and *R. adiene* using RAPD markers. They created a genetic similarity tree with two clusters which corresponded to two pedigree groups among the Bulgarian genotypes. They also analyzed the 28 accessions with four SSR loci, demonstrating high levels of diversity within the collection (Badjakov et al. 2006).

3.4 Extent of genetic diversity and relationship with geographical distribution

Research with natural populations has shown genetic diversity in raspberry at levels near 50% for both among and within population estimates (e.g. Lindqvist-Kreuze, et al. 2003 for arctic raspberry). With RAPD markers, Graham et al. (1997) examined spatial diversity in wild accessions of *R. idaeus* from four sites in Scotland. Most of the variability in markers was observed between the collection sites. Within sites, increasing diversity coincided with greater spatial separation. None of the wild populations were closely related to the commercial cultivar. Later, Graham et al. (2003) assayed a wider range of wild *R. idaeus* from 12 sites across a greater area of the United Kingdom and compared the accessions to the cultivar 'Glen Moy'. Again, greater genetic similarity was found within each population collected, which indicates a hindrance of gene movement across geographic locations.

At the species level, Alice and Campbell (1999) found that nuclear sequences were generally consistent with biogeography and ploidy levels and less so with morphological traits. They produced a *Rubus* phylogeny of 57 species including multiple raspberry species based on ribosomal internal transcribed spacer region (ITSR) sequence variation. The *Rubus* subgenus Idaeobatus of the Pacific region was studied in comparison with species from other subgenera to evaluate biogeographic and phylogenetic affinities of *R. macraei*, using chromosome analysis and chloroplast gene *ndhF* sequence (Morden et al. 2003). Their results showed that *R. macraei* is most similar to blackberry species of the subgenus *Rubus*. Moreover they discovered that *R. macraei* and *R. hawaiensis* are derived from separate colonization's from North America and that similarities between them are due to convergent evolution in the Hawaiian environment.

Twenty wild *R. idaeus* accessions from a Lithuanian germplasm collection were examined for genetic diversity using 285 RAPD loci produced from 36 primers (Patamsytė et al. 2004). Genetic distances among the genotypes did not correlate to geographic distances between collection sites; however soil acidity was significantly correlated to observed polymorphisms in the RAPD markers, indicating an environmental effect on diversity within populations.

A study on 63 populations natural populations of *Rubus strigosus* across North America (Marking 2006) using chloroplast sequence and interlocus-simple sequence repeats (I-SSR) found the majority of the variation within populations (79.5%). The total genetic diversity was found to be $\pi = 0.009$ for the chloroplast sequence data, with the genetic variation among populations at $\pi = 0.001$. Expected heterozygosity levels from the nuclear I-SSR marker data ranged from 0.84-1.00. Population specific Fst values ranged from 0.183-0.271, with an average Fst value of 0.208 among all populations. Marking found no evidence of correlations among geographic location and the level of genetic diversity, nor any evidence of increased diversity in naturally existing populations compared to the cultivated populations of North American red raspberry. Weber (2003) analyzed genetic diversity in cultivars of black raspberry (*R. occidentalis*), red raspberry using RAPD markers and found that black raspberry genotypes showed on average 81% genetic similarity. This compared to 70% similarity measured among red raspberry cultivars in Europe (Graham et al., 1994). Of the 16 genotypes investigated, five cultivars accounted for 58% of the observed variability in black raspberry, and none of the black raspberry cultivars were more than 2 generation from at least one wild ancestor.

DNA probes from two variable number tandem repeat (VNTR) loci were utilized to examine diversity in wild populations of *R. moluccanus* L. in the Philippines (Busemeyer et al. 1997). The results were similar to that of Graham et al. (1997, 2003) in *R. idaeus*, finding more similarity was present within populations at each location than between locations. Additionally, apomictic reproduction was ruled out in these populations because no identical VNTR patterns were identified.

Part 4: Linkage Mapping and Molecular Breeding

4.1 A brief history of marker development

The first linkage map for *Rubus* was presented by Lewis in 1939(a,b). He postulated linkage between the genes controlling five traits, namely B (bloomed new stems), T (presence of red pigments in fruit and spines), G (normal vs. pale green leaf), X (green vs. red hypocotyls) and W (responsible for selective fertilization) based on aberrant ratios in a series of more than 60 different progenies of red raspberry using his own data and that of Crane and Lawrence (1931) (Crane and Lawrence 1931; Lewis 1939, 1940). Subsequently, linkage was reported between gene H, controlling cane pubescence, and resistance to cane botrytis (*Botrytis cinerea*) and spur blight (*Didymella applanata*) as well as increased susceptibility to yellow rust (*Phagmidium rubi-idaei*), cane spot (*Elsinoe veneta*) and powdery mildew (*Sphaerotheca macularis*) in red raspberry (Jennings 1982a, b; Keep and Knight 1968; Knight and Keep 1958). Similarly Jennings (1967) and Keep (1968) reported linkages between dwarf habit phenotype (*dw*) and genes H and T, both first described by Crane and Lawrence in 1931 (Crane and Lawrence 1931; Jennings 1967; Keep 1968). We would have to wait until the advent of molecular markers before linkage maps spanning the genome could be developed and some of these hypotheses could be proven or refuted.

4.2 Molecular marker evolution and development in *Rubus*

A number of molecular markers have been employed in the genetic analysis of raspberry, including RAPDs, AFLPs and microsatellites (SSRs). RAPDs have been used for linkage map construction (Pattison et al. 2007), to determine the relationships within *Rubus* species (Graham and McNicol 1995; Stafne et al. 2005), to assess the genetic diversity of *Rubus* species such as black raspberry (Weber 2003), where a lack of genetic variation is a limiting factor in the production of new commercial cultivars and wild *R. idaeus* (Graham et al. 1997), and to identify raspberry cultivars (Parent and Page 1992). Likewise, AFLPs have been employed for linkage map construction (Graham et al. 2004; Graham et al. 2006; Pattison et al. 2007; Sargent et al. 2007; Woodhead et al. 2008), as well as to assess the genetic diversity of both wild and cultivated *Rubus* species (Amsellem et al. 2000; Lindqvist-Kreuze et al. 2003; Marulanda et al. 2007).

However, in recent years, due to their well documented advantages for diversity studies and linkage map construction, SSRs have become the marker of choice for genetic analysis in many crop species, including raspberry. Microsatellites have been developed from a number of different raspberry species, including *R. alceifolius* (Amsellem et al. 2001b), as well as from *R. idaeus* (Graham et al. 2004; Graham et al. 2006; Graham et al. 2002; Lopes et al. 2006), and recently EST-SSRs have been developed in both raspberry and blackberry which have the

advantage of providing functional information at that locus (Lewers et al. 2008; Woodhead et al. 2008).

In addition, SSR markers derived from genera closely related to *Rubus*, such as *Fragaria* have been applied to *Rubus* and have been shown to be transferable and polymorphic, and thus suitable for genetic analysis (Lewers and Hokanson 2005; Stafne et al. 2005).

4.3 Mapping populations used

The most saturated *Rubus* map currently available was constructed from a population derived from a cross between two subspecies of red raspberry: the cultivars *Rubus idaeus* subsp. *idaeus* cv. 'Glen Moy' and *Rubus idaeus* subsp. *strigosus* cv. 'Latham' (L×GM) by Graham et al (2004). The population segregates for a number of phenotypic characters including root-rot resistance. Three maps so far have been published on this progeny. Graham et al (2004) employed SSR and AFLP markers to define a linkage map comprising 273 markers covering 789 cM over nine linkage groups and QTLs were identified for spine density and two measures of plant vigour: the density and spread of root suckers. Subsequently, a further map of the L×GM population was reported (Graham et al. 2006), which contained 349 markers across a total length of 669 cM and eight linkage groups. This second map also contains a map position for gene H in linkage group 2, a gene for cane pubescence that was shown to be associated with resistance to the fungal diseases cane botrytis (*Botrytis cinerea*) and spur blight (*Didymella applanata*

(Niessl), but not to rust (*Phagmidium rubi-idaei*) or cane spot (*Elsinoe veneta*) as previously reported. The latest L x GM map (Woodhead et al. 2008) introduces a set of 25 SSRs derived from EST sequences from raspberry rot and bud tissues. This map covers a total of 884 cM in seven linkage groups and highlights QTL position for fruit and quality attributes.

A number of other linkage maps have been reported for *Rubus* and contain map positions for resistance to pests and diseases of key economic importance in *Rubus*. Pattison et al (2007) raised F1, F2, B1, B2 and S1 populations from a cross between the cultivars Latham and Titan and used these progenies to study the inheritance of resistance to *Phytophthora* root rot (PRR) in *R. idaeus*, caused by the fungus *Phytophthora fragariae* var. *rubi*. A dominant two gene model was shown to be the best fit for the segregation for resistance observed, and a molecular linkage map was constructed of both parents from their B2 population, incorporating138 AFLP 68 RAPD, and 20 resistance gene analogue markers, and spanned seven linkage groups covering 440 cM in Latham and 370 cM in Titan. QTL analysis of the B2 population highlighted two genomic regions on each map that were associated with PRR resistance. Unfortunately this map was not aligned to the LxGM reference map.

Sargent et al. (2007) constructed a linkage map of the progeny 'Malling Jewel' × 'Malling Orion' (MJ×MO) which segregated for the gene A_1 which confers resistance to the aphid *Amphorophora idaei* and for *dw,* a gene for dwarfing habit in *R. idaeus*. The map was produced from and F1 progeny of the cross MJ×MO and contained 95 AFLP and 22 SSR markers in seven linkage groups and covered a total of 505 cM. The two phenotypic traits

mapped to linkage groups 3 and 6 (Graham et al. 2006)and were closely flanked by SSR and RAPD markers.

Part 5: Simply inherited traits

5.1 Aphid resistance

The large raspberry aphids, *Amphorophora idaei in* Europe and *A. agathonica* in North-America, transmit several viruses including Raspberry leaf mottle virus (RLMV), Raspberry leaf spot virus (RLSV), Black raspberry necrosis virus (BRNV) and *Rubus* yellow net virus (RYNV), and thus, breeding for vector resistance is a key goal of many breeding programs. The genetic control of *A. idaei* resistance derived from different sources was elucidated, attributing it to series of single dominant genes (A_1- A_{10}, A_{L518}, A_{K4a}) (Keep and Knight 1967; Keep et al. 1970). However, resistance is strongly dependent upon the *A. idaei* biotype with some genes providing resistance to certain biotypes but not others (Sargent et al. 2007). The widespread growing of cultivars carrying A_1 resistance imposed a strong selection pressure on aphid populations causing biotypes that could overcome this resistance to become predominant in the UK during the 1980s and 1990s (Birch et al. 1997). Resistance to the most common biotypes can be conferred by one of three genes: A_{10} from *R. occidentalis*, A_{L518} from *R. idaeus* subsp. *strigosus* or (A_{K4a}) from a German clone of *R. idaeus* subsp. *vulgatus*. However, an A_{10} resistance-breaking biotype has been reported (Birch et al. 1997) making it ever more important to pyramid of several aphid resistance genes in breeding lines to provide a robust and durable vector resistance. Suitable

molecular markers are essential to determine how many resistance genes are carried by resistant selections as the equivalent effects of many of the reported resistance genes make them phenotypically indistinguishable and indeed possibly synonymous. Thus the development of maps of progenies carrying resistance genes and the identification of molecular markers linked to these genes will provide a key tool in differentiating reported genes, identifying their presence in modern hybrid material and in managing strategies for pyramiding. A_1 has been mapped to LG3 (Sargent et al. 2007) and linked markers to the other genes are needed to enable efficient gene pyramiding.

5.2 Dwarf-habit and pubescent canes

Jennings (1967) reported and depicted a dwarf phenotype which he attributed to the recessive gene *dw* and speculated that *dw* was linked with *H* and *T*, the genes for pubescent stems and for presence or absence of anthocyanin. Keep (1968) attributed this phenotype to the effect of two genes: *d1* and *d2* and accepted Jennings's hypothesis of linkage between this character and the *H* and *T* genes.

Keep (1968) proposed that some dwarf phenotypes could have some interest for self-supporting breeding lines; however, because of other agronomic disadvantages such as reduced fertility and longevity this has not been pursued. However, this recessive gene in still present in modern breeding lines and it becomes apparent in breeding progenies every so often. Therefore it

could be useful to include a linked marker to parental screenings for marker assisted breeding (MAB) thus avoiding unfavourable crosses. The importance of gene *H* (cane pubescence) stems from its linkage with cane disease resistance traits. Mapping (Graham et al. 2006) has confirmed its linkage to QTLs for resistance to two important cane diseases namely spur blight and cane botrytis as had been previously reported (Jennings 1982a, b; Keep 1968 ; Knight and Keep 1958). However, despite breeders observations in breeding material suggesting otherwise, hairy canes did not show association with susceptibility to cane spot or yellow rust in the LxGM progeny. The hypothesis of linkage between *H* and *dw* have also been revised as *H* maps to LG2 (Graham et al. 2006) where as *dw* was mapped to LG6 (Sargent et al. 2007).

Part 6: Molecular mapping of complex traits

6.1 Root-rot resistance

Raspberry root rot, caused by *Phytophthora* species, is a devastating soil-borne disease that has caused serious problems in Europe, N. America and Australia for many years and screening for resistance has been ongoing from the late 1970s (Barritt et al. 1979; Bristow et al. 1988; Duncan and Kennedy 1992; Knight and Fernández-Fernández 2008). Several breeding programmes have resistance to root rot as a major breeding target thus robust and transferable molecular markers to allow reliable identification of genotypes with high levels of resistance to this disease would be very beneficial to breeders. Efforts on both sides of the Atlantic are being devoted to this aim. Pattison et al. (2007) presented an elegant two gene hypothesis for the genetic control of the trait that were mapped to two different linkage groups and ongoing work by Graham (pers. comm.) identified SSR markets linked the QTLs for resistance identified in the LxGM population as well as assessing their transferability to other genetic backgrounds. In view of these parallel efforts it would be beneficial to align both maps.

6.2 Resistance to cane diseases

Cane botrytis (*Botrytis cinerea*) and spur blight (*Didymella applanata*) infect mature or senescent leaves on raspberry primocanes. The infection spreads to the buds where the disease lesions develop. The following spring, buds from infected nodes will break later or not at all constituting a major cause for yield loss, particularly in the case of cane botrytis. A common resistance mechanism to both diseases was proposed (Williamson and Jennings 1986) and Graham et al (2006) identified two major QTL associated with both resistances in LG2 and LG3 of the LxGM progeny suggesting multiple gene control of the trait. Interestingly, the QTL in LG2 collocated with gene *H* previously discussed.

Cane spot or anthracnose (*Elsinoe veneta*) can develop in most raspberry tissues but it is most recognisable in the second year canes where it produces deep lesions that can lead to vascular damage, and therefore reduce yields. The resistance to this pathogen has been associated to the presence of hairy cane (*H*) in European red raspberry but not so in North-American cultivars (Graham et al 2006). Genetic control of the trait has not yet been firmly established although Graham et al (2006) identified two QTL in LG2 and LG4 of the LxGM progeny associated with response to the disease.

Yellow rust (*Phagmidium rubi-idaei*) has increased its prevalence in recent years when the cultivation under tunnels of widespread susceptible cultivars e.g. ‘Glen Ample’ and ‘Tulameen’ has become common. A major resistance gene (*Yr*) from ‘Latham’ was identified by Anthony et al (1986) and Graham et al (2006) postulated this gene to be located in LG3 of the LxGM progeny. The inheritance of complete and incomplete resistance to rust in a half diallel

cross including Boyne was studied, which derives complete resistance from Latham (Anthony et al. 1986). They found that crosses of Boyne to susceptible varieties all segregated for complete resistance and proposed that Boyne was heterozygous for a single resistance gene, designated *Yr*, which was derived from Latham. As the Latham x Glen Moy population also segregates for resistance to rust, Graham et al. (2006) proposed that Latham is also heterozygous for *Yr*, and that this lies on linkage group 3 close to E41M31-147. Anthony et al. (1986) also found variation in the degree of susceptibility among offspring of Boyne without complete resistance, and concluded Boyne to also be a source of incomplete resistance.

In the Latham x Glen Moy cross, there is some evidence, although not highly significant, for a gene on linkage group 5, also from Latham, affecting the susceptibility of the offspring that do not carry the 'resistant' allele on linkage group 3. This area on LG5 is also implicated in spur blight/botrytis resistance. There was no evidence, however, of gene *H* being related to incomplete resistance in this cross. None of the offspring in this cross were as susceptible to rust as the Glen Moy parent. One explanation of this is that there is another resistance gene, for which Latham is homozygous RR and Moy is rr. In this case all offspring would be Rr and so more resistant than Moy.

6.3 Viral Resistance

Attempts to develop markers for other viral resistance genes have been carried out for raspberry leaf spot and raspberry vein chlorosis. Field screening was carried out to measure symptom production of leaf spot and vein chlorosis in two different environments. These traits were analyzed for significant linkages to mapped markers and resistance loci were found on linkage groups 2 and 7 (Rusu et al. 2006).

6.4 Fruit Quality Traits

Progress towards mapping fruit quality traits in terms of both eating quality and their health benefits is underway. Data has been collected on anthocyanins content across seasons and at different environments (Kassim et al. (in press)). Here high performance liquid chromatography (HPLC) was used to quantify eight major anthocyanins, cyanidins, and pelargonidin glycosides: -3-sophoroside, -3-glucoside, -3-rutinoside and -3-glucosylrutinoside in progeny from the Latham x Glen Moy cross. The eight antioxidants mapped to the same chromosome region on linkage group (LG) 1 of the map of Graham et al., (2006), across both years and from fruits grown in the field and under protected cultivation. Seven antioxidants also mapped to a region on LG 4 across years and for both field and protected sites. Candidate genes including bHLH (Espley et al. 2007), NAM/CUC2 (Ooka et al. 2003) like protein and bZIP transcription factor (Holm et al. 2002; Mallappa et al. 2006) underlying the mapped anthocyanins were identified. Volatiles have also been quantified across sites and seasons and mapping is underway here (Kassim pers comm.). Color has been measured both visually and instrumentally and mapped to three linkage

groups (McCallum pers. comm.). Sugars and acids as well as sensory data has been collected from the Latham x Glen Moy population and mapping is underway here (Zait pers. comm.).

Work towards the genetic mapping of health-related compounds has been initiated in *Rubus*. With the emergence of metabolomics the simultaneous analysis of multiple metabolites at specific time points is now feasible. In *Rubus* a metabolomic approach has been used to identify bioactive compounds in a segregating mapping population planted under two different environments (Stewart et al. 2007). As a greater understanding of the relative importance and bioavailability of the different antioxidant compounds is achieved, it may become possible develop and identify those raspberry genotypes with enhanced health-promoting properties from breeding programs (Beekwilder et al. 2005).

6.5 Future prospects and work in progress

Ongoing work at the Scottish Crop Research Institute (UK) aims to further saturate their very well characterized, replicated progeny (LxGM) to map fruit quality characters attributes. A candidate gene approach (Woodhead et al. 2008) is been combined with extensive phenotypic measurements, including metabolomic profiling (Stewart et al. 2007), to identify quality traits and anthocyanin transcription factors (Kassim et al. (in press)) Work is also focusing on identification of gene H through BAC library screening (Woodhead pers. comm.) and

chromosome walking and this sequence characterization is also being applied to the root rot resistance QTL (Graham unreported data).

At East Malling Research (UK), progress is been made to map further aphid resistance genes. The 'Autumn Bliss' x 'M. Jewel' population (ABxMJ), currently being tested with SSRs and RAPDs, segregates for the A_{10} gene of resistance to the large raspberry aphid *A. idaei* (Fernández-Fernández et al unpublished data). This progeny also segregates for primocane fruiting; a trait of great economical importance normally attributed to a combination of major and minor genes. Further progenies are being developed to map A_{L518}, and A_{K4a} (Fernández-Fernández et al unpublished).

Work carried out at North Carolina State University (US) is using the F1 cross (*R. parvifolius* x 'Tulameen') x 'Qualicum' to elucidate the genetics of *Rubus* response to environmental conditions. *R. parvifolius* is of interest to breeders in a time of climate change as a donor of high chilling requirements (allowing germplasm to withstand fluctuations in winter temperature) and, even more interestingly, as a donor of heat tolerance. Both traits and several others of horticultural interest segregate in the (*R. parvifolius* x 'Tulameen') x 'Qualicum' progeny that is currently being analyzed with AFLP and SSR markers (Molina-Bravo unpublished data).

All efforts to date have concentrated in the most economically important of the *Rubus* crops, red raspberry. However, work is being carried out at Cornell University on a black raspberry mapping progeny (Weber unpublished data). We can also expect blackberry, at least in its tetraploid form, to become subject of mapping work in the near future as more resources become available for these species, such as the recent EST library for SSR development published by Lewers et al (2008).

Part 7: Molecular breeding

7.1 Germplasm characterization

The first attempts to 'fingerprint' *Rubus* germplasm using paper chromatography (Haskell and Garrie 1966) were followed by isoenzyme analyses (Cousineau and Donnelly 1989) and more recently by DNA based markers such as minisatellite DNA (Nybom et al. 1990) and RAPDs (Parent and Page 1992; Weber 2003). With their increasing availability for the *Rubus* genus, SSRs are becoming the most commonly used fingerprinting tool and standardised sets of SSRs are needed for use in each crop. A set of *Rubus* SSRs has been identified for use in an EU project Genberry (Denoyes-Rothan et al., 2008) Similar sets have been proposed for other rosaceous crops (Govan et al in press) and when combined with a set of control cultivars, like in the case of Pyrus, (Evans et al in press) allow researchers to readily compare data. The use of markers in breeding to screen germplasm thereby increasing diversity is important in raspberry as five parent cultivars dominate their ancestry; 'Lloyd George' and 'Pynes Royal' entirely derived from the European sub-species and 'Preussen', 'Cuthbert' and 'Newburgh' derived from both European and North American subspecies. Domestication has resulted in a reduction of both morphological and genetic diversity in red raspberry (Haskell and Garrie 1966; Jennings 1988)with modern cultivars being genetically similar (Dale et al. 1993 ; Graham and McNicol 1995). Extensive genetic diversity has been found in wild raspberry germplasm offering scope for expanding the genetic base of cultivated raspberries (Graham et al. 1997; Marshall et al.

2001) and sourcing locally adapted material may become increasingly important in the light of climate change.

7.2 Marker assisted gene introgression and Gene pyramiding:

Gene pyramiding has been successfully achieved for multiple resistance to *A. idaei*. Segregation data for over 500 breeding progenies over the last 25 years indicates the presence of at least 3 independent major genes in the East Malling Research breeding material (Fernández-Fernández unpublished data). This has been achieved with as much thanks to chance as to a rigorous selection for resistant germplasm. Tightly linked markers are needed to improve efficiency and ascertain nature of the resistance in breeding lines. This characterisation will allow researchers to evaluate the response of resistance breaking aphid biotypes to germplasm carrying a combination of resistance genes and therefore plan future breeding strategies. Similarly, the identification of markers linked to root-rot resistance from sources other than 'Latham' would enable a gene pyramiding strategy for this disease.

7.3 Limitations and prospects for Marker Assisted Breeding (MAB) in *Rubus*

At present, there are only a limited number of molecular markers available for *Rubus*, and only a small proportion of those have been shown to be linked to traits of economic importance.

Thus, currently, molecular tools for practical plant breeding and marker assisted selection in *Rubus* are limited.

However, as more and more data are generated from EST libraries for *Rubus* and with the genome sequencing efforts currently underway for *Prunus*, *Malus* and *Fragaria*, there promises to be a wealth of data available for candidate genes that can be used to develop gene-specific markers for *Rubus*. The number of raspberry sequences is increasing rapidly as efforts are under way to sequence EST libraries generated from different tissues and developmental stages. At the Scottish Crop Research Institute, cDNA libraries have been generated from leaves (approximately 6500 clones), canes (approximately 8000 clones) and roots (approximately 7300 clones) and further libraries are being constructed from fruit and shoots (Graham, Smith, Woodhead and McCallum unpublished data). As well as providing sequence information on genes expressed in these tissues, these resources are being used to identify DNA markers (EST-SSRs and SNPs) for use in the genetic mapping programs.

As many traits in *Rubus* have been shown to be under the control of single, or a small number of genes, there is the real possibility of using these gene sequence data to generate markers for traits such as pest and disease resistance, and thus permit the tracking and pyramiding of large numbers of useful genes into single breeding lines or cultivars.

7.4 Transgenic breeding

Genetic transformation is an important component of functional gene analysis studies and holds tremendous potential for crop improvement. The first successful transformation of red raspberry expressing the β-glucuronidase (GUS) marker gene was produced from leaf disks and internodal segments using *Agrobacterium tumefaciens* (Graham et al. 1990). One of the major hurdles to successful transformation was the discovery that kanamycin inhibited organogenesis in raspberry; therefore, the selectable marker gene *nptII* was a poor marker (Graham et al. 1990; Hassan et al. 1993). Later, *A. tumefaciens* transformations of the raspberry cultivars Meeker', 'Chilliwack', and 'Canby' were completed with the gene S-adenosylmethionine hydrolase (SAMase). SAMase lowers ethylene production and hence could potentially increase shelf life of fruit (Mathews et al. 1995). These transformations used hygromycin phosphotransferase (*hpt*), which conferred resistance to hygromycin and lead to a reported 49% transformation efficiency in 'meeker'.

In an attempt to increase yields, the *defH9-iaaM* auxin-synthesizing or parthenocarpic gene using *nptII* as a selectable marker was inserted into 'Ruby' using *A. tumefaciens* with the goal of improving the productivity of raspberry (Mezzetti et al. 2002). Greenhouse trials showed a significant increase in fruit size and yield over two harvest seasons and subsequent field testing showed increases in flowers per plant and per inflorescence translating to a 100% increase in overall yeild (Mezzetti et al. 2004).

To improve virus resistance in raspberry, the 'Meeker' cultivar was transformed using *A. tumefaciens* with six constructs based on the coat protein and movement protein genes of the raspberry bushy dwarf virus (RBDV), the causal agent of crumbly fruit in raspberry (Martin and Mathews 2001). Grafting tests with infected material were performed to test for virus resistance and resulted in 53 of 141 transgenic lines remaining virus free for two rounds of grafting. Additional lines were eventually developed using these genes as well as nontranslatable RNA of RBDV (Martin et al. 2004) resulting in 5 lines of 197 remaining RBDV free for five years in field testing with heavy disease pressure.

While most transformations reported in Rubus have made use of the binary vector *A. tumefaciens* system, alternative methods have been attempted. The cultivar 'Stolicznaya' was transformed by directly introducing a donor plasmid into cells of a callus cell suspension with a 20% glycerol concentration (Friedrich and Váchová 1999). The plasmid carried the isopentenyltransferase gene, that increases cytokinin production, and subsequent testing of callus cells on media containing no cytokinins indicated transgenic lines. Unfortunately, no plants were developed from the lines for further testing.

To date, most successful transformations have taken place in red raspberry, however the arctic raspberry, *R. arcticus*, was transformed with the *gus-int* gene using *A. tumefaciens* (Kokko and Kärenlampi 1998). This was the first and only reported example of successful transformation of any other *Rubus* species other than red raspberry.

As these studies have shown, there is clear potential for improving raspberry cultivars using genetic modification techniques especially for disease resistance and yield improvements. However, by public opposition, especially in Europe, the use of GMO in the human food industry is limited thus preventing the realization of this technology to its full potential. For the time being, future use of these techniques will be limited to the identification and isolation of useful genes to aid breeding/improvement purposes.

Part 8: Genomic Resources

Initial work to identify genes responsible for important economic traits has come from a number of different avenues. As we have indicated previously, there has been some success identifying genes in terms of a breeding sense, however combining traits still remains difficult to achieve especially in high-ploidy level varieties (Clark et al. 2007). Additionally, there has been some success in molecular mapping studies to identify Quantitative Trait Loci (QTL) and marker-trait associations which have been discussed previously. Briefly, those that have been published include QTL for spines, root sucker spread and root sucker density, gene H (associated with both cane pubescence, and resistance to cane botrytis, spur blight, rust, and cane spot), *Phytophthora* root rot resistance, dwarfing, and aphid resistance(Graham et al. 2004; Graham et al. 2006; Pattison et al. 2007; Sargent et al. 2007). In order to identify the specific genes responsible for these traits, identification though map based cloning or identification through expressed sequence tag (EST) libraries will be required. Once identified, genes will need to be analyzed using correlational, mutational and complementation studies in order to elucidate specific gene functions. Additionally, these specific genes may contain Single nucleotide polymorphisms (SNPs) or other marker types that may be superior to conventional markers as

they lie directly on the gene. Additionally, gaining an understanding of the molecular mechanisims responsible for these traits will enable more targeted breeding in the future.

The number of *Rubus* sequences present on the NCBI database (http://www.ncbi.nlm.nih.gov/) has increased rapidly reciently with the number of sequences for *Rubus* spp. increasing from 822 DNA sequences (Jan, 2008) to 3,580 (Aug 2008). Of the 822 sequences identified in Jan 2008, 414 were from *Rubus idaeus*, most of which contained simple sequence repeat (SSR) sequences. Of the remaining species, most sequences were evenly spread among them and were genes that were used traditionally for taxonomy and included *ndhf*, tRNA-Leu (trnL), and ITS 5.8S ribosomal RNA (Alice and Campbell 1999). When this search is compared to the Aug 2008 search, of the 3,580 sequences, 494 are from *R. idaeus*, and 2,678 are ESTs from *Rubus ulmifolius* var. inermis x *Rubus thyrsiger* with the remainder being evenly spread among the other *Rubus* spp. (See Table 1). This large increase of 2,678 sequences from *Rubus ulmifolius* var. inermis x *Rubus thyrsiger* is due to the creation and extensive sequencing of an EST library at the USDA aimed toward SSR marker development (Lewers et al. 2008).

8.1 Genomic Libraries

Genomic Libraries allow the entire genome to be broken down into large chunks (150kb to 70kb) and arrayed out. Genomic libraries can act as a bridge to allow physical mapping, positional cloning of individual genes, and a scaffold for whole genome sequencing. *Rubus idaeus* especially is an ideal candidate for genomic library construction since it is diploid (2n = 2x = 14) and hs a small genome (275 Mbp) (Graham et al. 2004).

Genomic libraries are stored in vectors that are able to hold large DNA fragments and are employed for this purpose. Normally, yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), and genomic libraries require high molecular weight DNA with individual fragments having to be of greater size than 100kb; therefore, specialized DNA extraction protocols were developed for this purpose (Hein et al. 2005). The first publicly available *Rubus* BAC library was created from the European red raspberry, 'Glen Moy'. The library contained over 15,000 clones with an average insert size of 130 kb (almost 8x coverage). Hybridization screening of the BAC library with chloroplast (*rbcL*) and mitochondrial (*nad1*) coded genes revealed that contamination of the genomic library with chloroplast and mitochondrial clones was very low (>1%) (Hein et al. 2004; Hein et al. 2005).

Initial screening of this BAC library employed probes to chalcone synthase, phenylalanine ammonia lyase and a MADS-box gene involved in bud dormancy (I. Hein, pers. comm.; B. Williamson, pers. comm.). More recently, the library has been probed with genes involved in epidermal cell fate (J. Graham, pers. comm.; B. Williamson, pers. comm.; C.E. Woodhead, pers. comm.) and a peach ever-growing gene (A. Abbott, pers. comm.).

A second library has recently been created from the *Rubus idaeus* variety 'Heritage'. Unlike the 'Glen Moy' library, the inserts are smaller at an average of 70kb and have been inserted into Fosmid vectors and will be arrayed out in 24,000 clones (almost 7x coverage)

(Swanson, unpublished data). This variety was chosen to complement several EST libraries that have been created at the United States Department of Agriculture and at the University of Central Arkansas (Lewers, pers. comm., and Swanson, unpublished data). Fosmids were chosen as they allowed some flexibility in DNA extraction, however more clones need to be arrayed compared to a BAC library to represent adequate coverage of the genome.

8.2 Expression Libraries and Gene Identification

Expression libraries provide a tool to allow the identification of genes that are expressed in a certain tissue at a particular time. Quite often clones from these libraries are only partially sequenced to reveal ESTs that are then used in mapping studies or putative gene function is assigned and tested through further experimentation. To date, several expression libraries have been made, most of which are made from Raspberry (Graham, Smith, Woodhead and McCallum unpublished data, Lewers, pers. comm., and (Mazzitelli et al. 2007). However, some libraries are now being created from Blackberry (Jones and Swanson 2008; Lewers et al. 2008).

Initially expression libraries were used for SSR marker creation (Graham et al. 2004; Lewers et al. 2008), but they have recently begun to show promise in functional biology studies (see below). Additionally, subtractive cDNA libraries have recently been employed to aid in the identification of genes that may be involved in prickle development (Jones and Swanson 2008). Subtractive libraries enrich for transcripts that are different between two expression libraries.

Three subtractive libraries have been created and include a 'Heritage' (prickled variety) minus 'Canby' (almost thornless variety) of Raspberry. Siblings showing extreme thorniness minus completely thornlessness of a 'Prime Jim' (thorned) x 'Arapaho' (thornless) cross of blackberry, and an epidermal peel of the raspberry variety 'Heritage' minus the cortical tissue of the same individual. To date, over 400 clones have been sequenced and putative function is currently being assigned.

An additional method for the identification of genes in *Rubus* is to identify genes whose sequence has already been identified in other species. This already available sequence information is used to create degenerate primers to be able to amplify the analogous sequence from *Rubus*. These PCR products are often subsequently screened against a cDNA library to obtain the full length gene sequence. This method was used extensively in phylogenetic studies and the first functional studies of *Rubus* genes (Alice and Campbell 1999; Borejsza-Wysocki and Hrazdina 1996; Kumar and Ellis 2001). The success of this approach has been in some debate with between 65% (raspberry to raspberry) to 30% (raspberry to blackberry) specific SSR markers being transferable among *Rubus* species and much less between *Rubus* and *Fragaria* (26%-31% in Blackberry to Strawberry, and 17%-21% in Raspberry to Strawberry) (Lewers and Hokanson 2005; Stafne et al. 2005). However, in single gene studies, and providing careful attention has been paid in degenerate primer creation, it is possible to alter PCR conditions so that amplification is generally successful (Swanson pers. obs.). Although successful, it must be pointed out that when using this method it is only possible to study genes that have been identified in other species. Therefore, it is very likely that unidentified genes that are specific to

Rubus will be missed unless more random approaches, such as those used in expression libraries, are used.

8.3 Functional Studies

Functional genomics is termed as the study of how genes function. It can be completed at several levels including correlational studies at the transcript or biochemical level, mutational studies where genes or gene products are deleted and the phenotype is observed, and complementational studies where the gene of interest is transformed into a naturally occurring mutant that is deficient in the gene of interest in the hope to recover normal function. The latter two approaches, while being very difficult experimentally, are superior as they show a definite link between the gene and phenotype. The primary studies that have been completed have been involved with fruit ripening, fruit aroma and flavor, disease resistance, and bud dormancy. Functional genomics is, however, still in its infancy in *Rubus*.

8.3.1 Fruit Ripening

The identification of genes involved in fruit ripening has been primarily done at the Scottish Crop Research Institute (SCRI) and has involved both cDNA screening approaches as well as RNA fingerprinting techniques (Iannetta et al. 2000; Jones et al. 1998). These approaches have implicated genes such as pectinmethyl esterase hydrolases (PME), that are

involved in cell wall loosening and thus fruit softening, as well as ACC oxidase, involved in the ethylene biosynthetic pathway (Iannetta et al. 2000). It has long been known that the plant hormone ethylene is involved in fruit ripening and the apparent up-regulation of ACC oxidase seems logical. It was also observed that may of the genes that were up-regulated in the duplets were also up-regulated in the receptacle indicating that both tissues may be responsive to the same environmental cues suggesting that Ethylene is a major factor (Iannetta et al. 2000).

8.3.2 Phenylpropanoid Pathway and Aroma/Color properties of fruit

The end products of the phenylpropanoid pathway contribute significantly to lignin production as well as the color and aroma of *Rubus* fruit (Kumar and Ellis 2001). Members of the Phenylaline Ammonia-Lyase (PAL) gene family were among the first to be studied. Of the two gene family members of PAL identified, one was found to be differentially expressed (3-10 fold) among various tissues. One gene was associated with early fruit ripening events, while the other was associated with later stages of fruit and flower development indicating differing regulatory mechanisms between the two gene family members (Kumar and Ellis 2001).

The enzymes polyketide synthases (PKS), benzalcetone synthase, chalcone synthase (CHS), are expressed during fruit development and are partially responsible for the production of the polyketide derivatives benzalacetone, naringenin chalcone and dihydrochalcone that produce some of the color, sweetness, and aroma in raspberry fruit (Kumar and Ellis 2003b; Zheng and

Hrazdina 2005, 2008; Zheng et al. 2001). A number of PKS genes have been characterized from raspberry (Kumar and Ellis 2003b; Zheng et al. 2001). It is suspected that the PKS gene family in *Rubus* consists of at least 11 members and expression analysis showed two of three cDNA studied were up-regulated during fruit ripening. Additionally, the three studied cDNAs had developmental specific expression patterns, suggesting that a duplication event in *Rubus* gave rise to the independent evolution of regulation and function of these genes (Kumar and Ellis 2003b).

The enzyme 4-coumarate:CoA ligase activates cinnamic acid and its derivatives to thioesters that then serve as substrates for the production of phenylpropanoid-derived compounds that influence fruit quality. Three 4-coumarate:CoA ligase (4CL) genes in raspberry have been identified and are differentially expressed in various organs and during fruit development and ripening (Kumar and Ellis 2003a). Based on the expression patterns and substrate utilization profiles of the recombinant proteins, they found that each of the three genes were expressed in different tissues at different times indicating that the regulation elements may have evolved independently. Furthermore, they suggest that the first gene (4CL1) is involved in the phenolic biosynthesis in leaves, the second gene (4CL2) is involved in cane lignification, and the third gene (4CL3) is involved in the flavonoid and/or flavor pathway in fruit.

8.3.3 Phenylpropanoid Pathway and Disease Resistance

The enzyme 4-coumarate:CoA has also been implicated in fungi resistance as it, in conjunction with malonyl-coenzyme A, forms p-hydroxybenzalacetone (Borejsza-Wysocki and Hrazdina 1996). In this case, benzalacetone synthase was shown to carry out part of this reaction: the product of which, p-hydroxybenzalacetone, was shown to inhibit the mycelia growth of the *Rubus* pathogen *Phytophthora fragariae* var rubi.

Two plant polygalacturonase-inhibiting protein (PGIPs) have also been cloned from raspberry (Ramanathan et al. 1997). PGIPs inhibit endo-polygalacturonases (endo-PGs) that are released by fungi to help degrade the plant cell wall (Johnston et al. 1993). Of the two genes characterized, one was shown to be nonfunctional due to a frame-shift mutation resulting in truncation of the protein. The second gene was shown to be expressed throughout flower and fruit development (Ramanathan et al. 1997).

8.3.4 Bud dormancy release

The first microarray experiment in *Rubus* was conducted to investigate bud dormancy phase transition in woody perennial plants at a molecular level (Mazzitelli et al. 2007). Slides were created using a total of 5300 PCR amplified ESTs from endodormant (true dormancy) and paradormant (apical dominance) raspberry meristematic bud tissue. Over 220 clones were identified that exhibited up or down-regulation during the endodormancy – paradormancy transition. Interestingly, there were a high percentage of genes related to stress tolerance that

were identified. It was attributed that these genes were involved with the activation of multiple mechanisms to allow the bud to survive low temperatures. Additionally, Aquaporins were found to be down-regulated, while cell wall reorganization genes were found to be differentially regulated throughout the time course, and sugar metabolism gene levels also increased. Together, these indicated that water and cell wall reorganization and sugar metabolism were key components of bud dormancy release. Transcription factors, including a SVP-type MADS box transcription factor, and hormone-induced genes were also identified, potentially indicating signaling molecules that may be required to release these buds from dormancy.

8.4 Bioinformatic Resources

As we have already eluded, the advances in genomics technologies have lead to a massive increase in the numbers of DNA sequences held in public databases. However, *Rubus* still trails other Rosaceae with *Rubus* (3580 nucleotides, 127 proteins) trailing Peach (71,730 nucleotides, 304 proteins). However, the number of avalible *Rubus* sequences is increasing very quickly with over a four-fold increase over the past eight months. In order to deal with the increase in genomic and molecular marker data the Genome Database for Rosaceae was developed (Jung et al. 2008). This invaluable tool provides a central bioinformatics resource for not only researchers of *Rubus*, but all Rosaceae species.

8.5 Genomic Resources, the future

Future work will focus on anchoring the physical map to the genetic map, which will enable alignment of the maps and the identification of genomic regions harbouring genes controlling important phenotypes. Some progress has been made here specifically for linkage groups 2, 3 and 6 of the map of Graham et al., (2006) (Graham et al unreported data). An integrated physical/genetic map will also allow the extent of synteny or colinearity of the *Rubus* genome with other members of the Rosaceae to be determined.

With the availability of a detailed genetic linkage map, deep coverage genomic libraries, and several EST libraries we will now be able to identify genetic factors that underpin a wide range of commercially important characteristics. However, the establishment of gene-phenotype relationships will be required. This will require the use of a wide variety of molecular tools including RT-PCR, in situ hybridization, and in vitro analysis. These tools unfortunately will only provide a correlation of gene to trait and it will only be through the implementation of mutational (e.g. RNAi), and complementation studies using transgenic *Rubus* that we can provide solid evidence of gene function *in planta*.

Once these complex pathways of gene function and their respective regulation are elucidated, we will be able to better use gene-based selection in breeding and the functional assignment of genes for commercially important traits. This in-term will aid breeders allowing

for genotype to environment selection so that they may better direct their efforts to create varieties that are able to maximize yield and fruit quality in a wide variety of specific environments.

Part 9: Rubus and Human Health (Joe Scheerens)

9 Heath-promoting Properties of *Rubus* Phytonutrients - Introduction

9.1 Historical and Ethnobotanical Use of *Rubus* spp. as Medicinal Plants (8 papers)
9.2 Phytonutrient Diversity among *Rubus* Species (@20 papers, 1 Table)
9.3 Bioavailability of *Rubus* phytonutrients (6 papers)
9.4 A Decade of Intensive Research Linking *Rubus* Phytonutrients and Human Health – Effects include:
9.4.1 Reduced Oxidative Stress (9 papers)
9.4.2 Anti-inflammatory Activity (11 papers)
9.4.3 Cancer Chemoprevention (33 papers)
9.4.4 Improved Cardiovascular Function (6 papers)
9.4.5 Reduced Risks for Obesity, Metabolic Syndrome and Diabetes (9 papers)
9.4.6 Antinociceptive Activity (pain reduction, 5 papers)
9.4.7 Reduced Edema (1 paper)
9.4.8 Diminished Anaphylaxis (2 papers)
9.4.9 Anti-spasmodic Activity (3 papers)
9.4.10 Anxiolytic Activity (reduced anxiety, 2 papers)
9.4.11 Reduced Risks of Neurodegeneration and Parkinson's Disease (2 papers)
9.4.12 Reduced Bone Loss (1 paper)
9.4.13 Reproductive Health Consequences (3 papers)
9.4.14 Improved Dermatological Conditions (2 papers)
9.4.15 Reduced Levels of Fatigue (1 paper)
9.4.16 Antimicrobial Activity and Protection from Intestinal Pathogens (12 papers)
9.4.17 Antiviral Activity (2 papers)
9.4.18 Improved MRI Imaging (1 paper)
9.5 Factors that Affect Phytonutrient Levels (about 15-20 papers in this section)
9.5.1 Genetic Factors (these may be fully explained in earlier sections)
9.5.2 Abiotic Stress (Light, Heat)
9.5.3 Biotic Stress
9.6 Potential for and Impediments to Improving Phytonutrient Levels
9.6.1 Abundant Genetic Diversity
9.6.2 Apparent Heritability

9.6.3 Lack of Information Necessary to Develop Breeding Objectives – Bioactivity Complex and Synergistic Behavior Among Compounds Unknown
9.6.4 Lack of Information Necessary to Make Horticultural Recommendations to the Producers

Part 10: Future Prospects

As we have shown, *Rubus* has been the focus of many breeding projects contributing to significant advances in the hardiness of the plant (including pathogen resistance and environmental tolerance), fruit quality, fruiting time (primocane flowering), thornlessness, and other growth traits (reviewed by (Clark et al. 2007)). However, combining traits together through traditional breeding approaches still remains a difficult task (Clark et al. 2007). Moreover, application of modern molecular approaches in *Rubus* has been hindered by the lack of knowledge regarding the genes and molecular pathways that specify the preferred traits.

As molecular information is accumulated, it will be imperative to have good communication between and among the breeders, the molecular researchers, the industry and consumers. This will help best direct the future allocation of resources and prevent unnecessary efforts that may not be in the best interest for the crop in general. In particular, molecular markers can provide the ability to greatly aid breeders by allowing early selection of traits of interest. Traditionally many plants are grown in field sights and plants exhibiting those traits are selected. However, with the aid of molecular markers, traits of interest can be selected initially, and the same number of plants can be field tested that already contain the trait of interest therefore the possibility of identifying new, or other rare desired traits can be increased

considerably. This is one example indicating how the community can work together in a synergistic fashion.

Blackberry and red raspberry production has expanded rapidly over the past two decades and, especially with the general interest in berries and specific interest in foods high in antioxidant levels, this expansion will continue for the foreseeable future. There is a strong push to develop raspberries and blackberries that are better adapted to low chilling and/or high temperature regions.

As we have seen, blackberries and raspberries have received a great deal of attention for their nutraceutical value. A large number of publications have now definitively established that the dietary intake of berry fruits has a positive and pronounced impact on human health, performance and disease (Seeram 2008a; Seeram 2008b). The International Berry Health Benefits Symposia held in alternate years beginning in 2005 has provided a forum for some of these discussions (Seeram, 2008a).

While the interest in nutraceutical value catches a great deal of attention, it cannot be forgotten that the caneberries are delightful to eat and good for you. One of the most valuable roles we can play as a *Rubus* community is to develop cultivars that taste better, so that they are more desirable to eat, and that can be grown economically by growers so that, in turn, the crop is available at an affordable price for the consuming public.

Tables

Table 1: Top 20 *Rubus* species with the largest number of nucleotide sequences in GenBank Aug 2008. (www.ncbi.nlm.nih.gov)

Species	Nucleotide	EST	Total Sequences
Rubus alceifolius	8	0	8
Rubus arcticus	12	0	12
Rubus assamensis	8	0	8
Rubus australis	6	0	6
Rubus caesius x Rubus idaeus	12	0	12
Rubus chamaemorus	5	0	5
Rubus corchorifolius	7	0	7
Rubus coreanus	6	0	6
Rubus crataegifolius	9	0	9
Rubus geoides	6	0	6
Rubus hochstetterorum	30	0	30
Rubus idaeus	155	348	503
Rubus longisepalus	6	0	6
Rubus parvifolius	16	0	16
Rubus phoenicolasius	6	0	6
Rubus pungens	6	0	6
Rubus rosifolius	6	0	6
Rubus saxatilis	6	0	6
Rubus trifidus	8	0	8
Rubus ulmifolius var. inermis x *Rubus thyrsiger*	0	2678	2678
All other taxa	236	0	236
	554	3026	3580

References

Alice LA, Campbell CS (1999) Phylogeny of *Rubus* (Rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. American Journal of Botany 86:81-97

Amsellem L, Chevallier MH, Hossaert-McKey M (2001a) Ploidy level of the invasive weed *Rubus alceifolius* Poir (Rosaceae), in its native range and in areas of introduction Plant Syst Evol 228:171-179

Amsellem L, Dutech C, Billotte N (2001b) Isolation and characterization of polymorphic microsatellite loci in *Rubus alceifolius* Poir. (Rosaceae), an invasive weed in La Reunion island. Molecular Ecology Notes 1:33-35

Amsellem L, Noyer JL, Hossaert-McKey M (2001c) Evidence for a switch in the reproductive biology of *Rubus alceifolius* (Rosaceae) towards apomixis, between its native range and its area of introduction. Am J Bot 88:2243-2251

Amsellem L, Noyer JL, Le Bourgeois T, Hossaert-McKey M (2000) Comparison of genetic diversity of the invasive weed *Rubus alceifolius* Poir. (Rosaceae) in its native range and in areas of introduction, using amplified fragment length polymorphism. . Plant Syst Evol 228:171-179

Anthony VM, Williamson B, Jennings DL, Shattock RC (1986) Inheritance of Resistance to Yellow Rust (Phragmidium-Rubi-Idaei) in Red Raspberry. Ann Appl biol 109:365-374

Anttonem MJ, Karjalainen RO (2005) Environmental and genetic variation of phenolic compounds in red raspberry. J Food Comp Anal 18:759-769

Badjakov I, Todorovska E, Kondakova V, Boicheva R, Atanassov A (2006) Assessment the genetic diversity of Bulgarian raspberry germplasm collected by microsatellite and RAPD markers. J Fruit and Ornamental Plant Res 14:61-76

Barritt BH, Crandall PC, Bristow PR (1979) Breeding for root rot resistance in red raspberry. J Amer Soc Hort Sci 104:92-94

Beekwilder J, Hall R, de Vos CHR (2005) Identification and dietary relevance of antioxidants from raspberry. Biofactors 23:197-205

Birch ANE, Georghegan IE, Majerus MEN, Hackett C, Allen JM (1997) Interactions between plant resistance genes, pest aphid populations and beneficial aphid predators. . SCRI Annual Report for 1996 pp 68-72

Borejsza-Wysocki W, Hrazdina C (1996) Aromatic Polyketide Synthases: purification, characterization, and antibody development to benzalacetone synthase from Raspberry fruits. Plant Physiology 110:791-799

Bristow PR, Daubeny HA, Sjulin TM, Pepin HS, Nestby R, Windom GE (1988) Evaluation of Rubus Germplasm for Reaction to Root Rot Caused by Phytophthora-Erythroseptica. Journal of the American Society for Horticultural Science 113:588-591

Busemeyer DT, Pelikan S, Kennedy RS, Rogstad SH (1997) Genetic diversity of Philippine *Rubus moluccanus* L. (Rosaceae) populations examined with VNTR DNA probes. J Trop Ecol 13:867-884

Clark JR (2005) Changing times for eastern United States blackberries. HortTechnology 15:491-494

Clark JR, Finn C (2006) Blackberry and hybrid berry. Register of new fruit and nut cultivars, list 43. . Hortscience 41:1104-1106

Clark JR, Finn C (2008) Trends in blackberry breeding. . Acta Hort 777:41-48

Clark JR, Finn CE (1999) In: WR Okie (ed.). Register of new fruit and nut varieties Brooks and Olmo list no. 39: Blackberries and hybrid berries. . HortScience 34:183-184

Clark JR, Finn CE (2002) Blackberry. In WR Okie (ed). Register of new fruit and nut varieties, list 41. . HortScience 37:251

Clark JR, Stafne ET, Hall HK, Finn CE (2007) Blackberry Breeding and Genetics. Plant Breeding Reviews 29:19-144

Cousineau JC, Donnelly DJ (1989) Identification of Raspberry Cultivars in-Vivo and in-Vitro Using Isoenzyme Analysis. Hort Science 24:490-492

Crane MB, Lawrence WJC (1931) Inheritance of sex, colour and hairiness in the raspberry, *Rubus idaeus* L. Jour Genetics 24:243-255

Dale A, McNicol RJ, Moore PP, Sjulin TM (1989) Pedigree analysis of red raspberry. Acta Hort 262:35-39

Dale A, Moore PP, McNicol RJ, Sjulin TM, Burmistrov LA (1993) Genetic diversity of red raspberry varieties throughout the world. J Amer Soc Hort Sci 118:119-129

Darrow GM (1925) The Young dewberry, a new hybrid variety American Fruit Grower 45:33

Darrow GM (1937) Blackberry and raspberry improvement. . U.S. Dept. of Agr. Yearbook of Agriculture.

Daubeny HA (1983) Expansion of genetic resources available to red raspberry breeding programs. . Proc 21st Int Hort Cong 1:150-155

Daubeny HA (1996) Fruit Breeding. John Wiley & Sons, Inc.,

Daubeny HA (1997) Raspberry, Third edn. ASHS Press, Alexandria, VA

Dossett M, Lee J, Finn CE (2008) Inheritance of phenological vegitative and fruit chemistry traits in black raspberry. J Amer Soc Hort Sci 133:408-417

Duncan JM, Kennedy DM (1992) Raspberry root rot: A summary of recent progress. . SCRI Ann Rep for 1991 pp 89-92

Espley RV, Hellens RP, Putterill J, Stevenson DE, Kutty-Amma S, Allan AC (2007) Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. Plant Journal 49:414-427

Finn CE (2001) Trailing blackberries: From clear-cuts to your table. Hortscience 36:236-238

Finn CE (2006) Caneberry breeders in North America. HortScience 41:22-24

Finn CE (2008a) *Rubus* spp., blackberry. Rubus spp. red and black raspberry., Cambridge, MA

Finn CE (2008b) Temperate fruit crop breeding: Germplasm to genomics.

Finn CE, Clark JR (in prep) Blackberry

Finn CE, Hancock JF (2008) Raspberry

Finn CE, Knight VH (2002) What's going on in the world of *Rubus* breeding? . Acta Hort 585:31-38

Finn CE, Lawrence FJ, Yorgey B, Strik BC (2001) 'Coho' red raspberry. . HortScience 36:1159-

Finn CE, Moore PP, Kempler C (2007) Raspberry cultivars: What's new? What's succeeding?

Finn CE, Swartz HJ, Moore PP, Ballington JR, Kempler C (2002a) Breeders experience with *Rubus* species. .

Finn CE, Swartz HJ, Moore PP, Ballington JR, Kempler C (2002b) Use of 58 *Rubus* species in five North American breeding programs-breeders notes. . Acta Hort 585:113-119

Finn CE, Yorgey B, Strik BC, Martin RR (2005a) 'Metolius' trailing blackberry. . HortScience 40:2189-2191

Finn CE, Yorgey B, Strik BC, Martin RR, Kempler C (2005b) 'Obsidian' trailing blackberry. HortScience 40 2185-2188

Finn CE, Yorgey B, Strik BC, Martin RR, Qian MC (2005c) 'Black Pearl' trailing thornless blackberry. HortScience
40:2179-2181

Finn CE, Yorgey B, Strik BC, Martin RR, Qian MC (2005d) 'Nightfall' trailing thornless blackberry. . HortScience 40 2182-2184

Friedrich A, Váchová J (1999) Transformation of raspberry callus by direct incorporation of plasmid pCB1346 and transfer of this plasmid into apple calluses by cocultivation of these calluses. Acta Hort 484:587-589

Galletta GJ, Maas JL, Clark JR, Finn CE (1998) 'Triple Crown' thornless blackberry. . Fruit Var J 52:124-127

Graham J, Marshall B, Squire GR (2003) Genetic differentiation over a spatial environmental gradient in wild *Rubus idaeus* populations. New Phytol 157:667-675

Graham J, McNichols R, Greig K, Van de Ven WTG (1994) Identification of red raspberry cultivars and an assessment of their relatedness using fingerprints produced by random primers. . J Hort Sci 69:123-130

Graham J, McNicol RJ (1995) An examination of the ability of RAPD markers to determine the relationships within and between *Rubus* species. Theoretical and Applied Genetics 90:1128-1132

Graham J, McNicol RJ, Kumar A (1990) Use of the GUS gene as a selectable marker for Agrobacterium-mediated transformation of Rubus. Theor Appl Genet 90:1128-1132

Graham J, Smith K, MacKenzie K, Jorgenson C, Hackett C, Powell W (2004) The construction of a genetic linkage map of red raspberry (*Rubus idaeus* subsp. *idaeus*) based on AFLPs, genomic-SSR and EST-SSR markers. Theoretical and Applied Genetics 109:740-749

Graham J, Smith K, Tierney I, MacKenzie K, Hackett C (2006) Mapping gene H controlling cane pubescence in raspberry and its association with resistance to cane botrytis and spur blight, rust and cane spot. . Theoretical and Applied Genetics 112:818-831

Graham J, Smith K, Woodhead M, Russell J (2002) Development and use of simple sequence repeat SSR markers in Rubus species. Molecular Ecology Notes 2:250-252

Graham J, Squire GR, Marshall B, Harrison RE (1997) Spatially dependent genetic diversity within and between colonies of wild raspberry *Rubus idaeus* detected using RAPD markers. Mol Ecol 6:1001-1008

Hall HK (1990) Blackberry breeding. Plant Breeding Reviews 8:249-312

Hall HK, Brewer LR, Langford G, Stanley CJ, Stephens MJ (2003) 'Karaka Black': "Another 'Mammoth" blackberry from crossing eastern and western USA blackberries Acta Hort 626:105-110

Hall HK, Cohen D, Skirvin RM (1986a) The inheritance of thornless from tissue culture-derived Thornless Evergreen blackberry. . Euphytica 35 891-898

Hall HK, Hummer K, Jamieson AR, Jennings SN, Weber CA (in prep) Raspberry breeding and genetics. Plant Breeding Reviews

Hall HK, Quazi MH, Skirvin RM (1986b) Germplasm release of 'Lincoln Logan', a tissue culture-derived genetic thornless 'Loganberry'. . Fruit Var J 40:134-113

Hall HK, Quazi MH, Skirvin RM (1986c) Isolation of a pure thornless Loganberry by meristem tip culture. Euphytica 35:1039-1044

Hall HK, Stephens J (1999) Hybridberries and blackberries in New Zealand - breeding for spinelessness. . Acta Hort 505:65-71

Hall HK, Stephens MJ, Stanley CJ, Finn CE, Yorgey B (2002) Breeding new 'Boysen' and 'Marion' cultivars. . Acta Hort 585:91-95

Haskell G, Garrie JB (1966) Fingerprinting raspberry cultivars by empirical paper chromatography. J Sci Food Agr 17:189-192

Hassan MA, Swartz HJ, Inamine G, Mullineaux P (1993) *Agrobacterium tumefaciens*-mediated transformation of several *Rubus* genotypes and recovery of transformed plants. Plant Cell, Tissue and Organ Culture 33:9-17

Hedrick UP (1925) The small fruits of New York. . J.B. Lyon Albany, N.Y.

Hein I, Williamson S, Russell J, Graham J, Brennan R, Powell W (2004) Development of genomic resources for red raspberry: BAC library construction, analysis and screening. Proc Plant and Animal Genome XII Conf, San Diego, CA, USA, p 148

Hein I, Williamson S, Russell J, Powell W (2005) Isolation of high molecular weight DNA suitable for BAC library construction from woody perennial soft-fruit species. BioTechniques 38

Holm M, Ma LG, Qu LJ, Deng XW (2002) Two interacting bZIP proteins are direct targets of COP1-mediated control of light-dependent gene expression in *Arabidopsis*. Genes and Development 16:1247-1259

Hummer KE, Janick J (2007) *Rubus* iconography: Antiquity to the Renaissance. . Acta Hort 759:89-106

Iannetta PPM, Wyman M, Neelam A, Jones C, Taylor MA, Davies HV, Sexton R (2000) A causal role for ethylene and endo-beta-1,4-glucanase in the abscission of red-raspberry (Rubus idaeus) drupelets. Physiologia Plantarum 110:535-543

Jennings DL (1967) Balanced lethals and polymorphism in *Rubus idaeus*. Heredity 22:465-479

Jennings DL (1982a) Further Evidence on the Effects of Gene H Which Confers Cane Hairiness on Resistance to Raspberry Diseases. Euphytica 31:953-956

Jennings DL (1982b) Resistance to Didymella-Applanata in Red Raspberry and Some Related Species. Annals of Applied Biology 101:331-338

Jennings DL (1988) Raspberries and blackberries: Their breeding, diseases and growth Academic Press, London

Jennings DL, Brydon E (1989) Further studies on breeding for resistance to *Botrytis cinerea* in red raspberry canes. Ann Appl biol 115:507-513

Jennings DL, Daubeny HA, Moore JM (1991) Blackberries and raspberries (*Rubus)*. Wageningen

Jennings DL, McGregor GR (1988) Resistance to cane spot (*Elsinoe veneta)* in the red raspberry and its relationship to yellow rust (*Phragmidium rubi-idaei)* Euphytica 37:173-180

Johnston DJ, Ramanathan V, Williamson B (1993) A Protein from Immature Raspberry Fruits which Inhibits Endopolygalacturonases from *Botrytis cinerea* and other Micro-organisms J Exp Bot 44:971-976

Jones CS, Davies HV, McNicol RJ, Taylor MA (1998) Cloning of three genes up-regulated in ripening raspberry fruit (Rubus idaeus cv. Glen clova). Journal of Plant Physiology 153:643-648

Jones N, Swanson J-D (2008) Identification of prickle development genes in Rubus using a subtractive cDNA library. Arkansas Academy of Sciences, Henderson University, AR, USA

Jung S, Staton M, Lee T, Blenda A, Svancara R, Abbott A, Main D (2008) GDR (Genome Database for Rosaceae): integrated web-database for Rosaceae genomics and genetics data. Nucleic Acids Research 36:D1034–D1040

Kassim A, Poette J, Paterson A, McCallum S, Woodhead M, Smith K, Hackett C, Graham J ((in press)) Environmental and seasonal influences on red raspberry anthocyanin and antioxidant contents and identification of QTL. . Mol Nutri and Food Res

Keep E (1964) Sepaloidy in the red raspberry, *Rubus idaeus* L. Can J Genetic Cytol 6:52-60

Keep E (1968) Inheritance of resistance to powdery mildew *Sphaerotheca macularis (*Fr.) Jaczewski in the red raspberry. . Euphytica 17:417-438

Keep E (1989) Breeding red raspberry for resistance to diseases and pests Plant Breeding Reviews 6:245-321

Keep E, Knight RL (1967) A new gene from *Rubus occidentalis* L. for resistance to strains 1,2, and 3 of the *Rubus* aphid, *Amphorophora rubi* Kalt. Euphytica 16:209-214

Keep E, Knight RL (1968) Use of the black raspberry (*Rubus occidentalis L.)* and other *Rubus* species in breeding red raspberries Report of East Malling Research Station for 1967, pp 105-107

Keep E, Knight RL, Parker JH (1970) Further data on resistance to the *Rubus* aphid *Amphorophora rubi* (Kltb.) Report of East Malling Research Station for 1969, pp 129-131

Kempler C (in prep) Raspberry

Kempler C, Daubeny HA, Frey L, Walters T (2006) 'Chemainus' red raspberry. . HortScience 41:1364-1366

Kempler C, Daubeny HA, Harding B, Baumann CE, Finn CE, Moore PP, Sweeney M, Walters T (2007) 'Saanich' red raspberry. . HortScience 42:176-178

Kempler C, Daubeny HA, Harding B, Finn CE (2005a) 'Esquimalt' red raspberry. . HortScience 40:2192-2194

Kempler C, Daubeny HA, Harding B, Kowalenko CG (2005b) 'Cowichan' red raspberry. HortScience 40:1916-1918

Knight RL, Fernández-Fernández F (2008) Screening for resistance to *Phytophthora fragariae* var. *rubi* in *Rubus* germplasm at East Malling Acta Hort 777:353-360

Knight RL, Keep E (1958) Developments in soft fruit breeding at East Malling. . Report of East Malling Research Station for 1957 pp 62-67

Knight VH (1993) Review of *Rubus* species used in raspberry breeding at East Malling. Acta Hort 352:363-371

Kokko HI, Kärenlampi SO (1998) Transformation of arctic bramble (*Rubus arcticus* L.) by *Agrobacterium tumefaciens*. Plant Cell Rep 17:822-826

Kumar A, Ellis BE (2001) The phenylalanine ammonia-lyase gene family in raspberry. Structure, expression, and evolution. Plant Physiology 127:230-239

Kumar A, Ellis BE (2003a) 4-Coumarate:CoA ligase gene family in *Rubus idaeus*: cDNA structures, evolution, and expression. Plant Mol Biol 31:327-340

Kumar A, Ellis BE (2003b) A family of polyketide synthase genes expressed in ripening *Rubus* fruits. Phytochemistry 62:513-526

Lawrence FJ (1989) Naming and release of blackberry cultivar 'Waldo'. In: Agr. USDoAO (ed)

Lewers K, Saski CA, Cuthbertson BJ, Henry DC, Staton ME, Main DE, Dhanaraj AL, Rowland LJ, Tomkins JP (2008) A blackberry (*Rubus* L.) expressed sequence tag library for the development of simple sequence repeat markers. BMC Plant Biology 8

Lewers KS, Hokanson SC (2005) Strawberry GenBank-derrived and genomic simple sequence repeat (SSR) markers and their utility with Strawberry, Blackberry, and Red and Black Raspberry. J Amer Soc Hort Sci 130:102-115

Lewis D (1939) Genetical studies in cultivated raspberries. I. Inheritance and linkage. Jour Genetics 38:367-379

Lewis D (1940) Genetical studies in cultivated raspberries. II. Selective fertilization. Genetics 25:278-286

Lindqvist-Kreuze H, Koponen H, Valkonen JPT (2003) Genetic diversity of arctic bramble (*Rubus arcticus* L. ssp. *arcticus*) as measured by amplified fragment length polymorphism. Can J Bot 81:805-813

Logan ME (1955) The Loganberry. . Mary E. Logan Oakland, Calif.

Lopes MS, Maciel B, Mendonca D, Gil FS, Machado AD (2006) Isolation and characterization of simple sequence repeat loci in *Rubus* hochstetterorum and their use in other species from the Rosaceae family. Mol Ecol Notes 6:750-752

Mallappa C, Yadav V, Negi P, Chattopadhyay S (2006) A basic leucine zipper transcription factor, G-box-binding factor 1, regulates blue light-mediated photomorphogenic growth in *Arabidopsis*. J Biol Chem 281:22190-22199

Marking HJ (2006) "DNA Marker Analysis of Genetic Diversity in Natural and Cultivated Populations of *Rubus strigosus*, American Red Raspberry" Master of Science The Pennsylvania State University

Marshall B, Harrison RE, Graham J, McNicol JW, Wright G, Squire GR (2001) Spatial trends of phenotypic diversity between colonies of wild raspberry *Rubus idaeus*. New Phytol 151:671-682

Martin RR, Keller KE, Mathews H (2004) Development of resistance to raspberry bushy dwarf virus in 'Meeker' red raspberry. Acta Hort 656:165-169

Martin RR, Mathews H (2001) Engineering resistance to raspberry bushy dwarf virus. Acta Hort 551:33-37

Marulanda ML, Lopez AM, Aguilar SB (2007) Genetic diversity of wild and cultivated Rubus species in Colombia using AFLP and SSR markers. . Crop Breeding and Applied Biotechnology 7:242-252

Mathews H, Wagoner W, Cohen C, Kellogg J, Bestwick RK (1995) Efficient genetic transformation of red raspberry, *Rubus idaeus* L. Plant Cell Rep 14:471-476

Mazzitelli L, Hancock RD, Haupt S, Walker PG, Pont SDA, McNicol J, Cardle L, Morris J, Viola R, Brennan R, Headley PE, Taylor MA (2007) Co-ordinated gene expression during phases of dormancy release in raspberry (*Rubus idaeus* L.) buds. Journal of Experimental Botany 58:1035-1045

McPheeters KD, Skirvin RM (1983) Histogenic layer manipulation in chimeral 'Thornless Evergreen' trailing blackberry. . Euphytica 32:351-360

McPheeters KD, Skirvin RM (1989) Somaclonal variation among ex vitro 'Thornless Evergreen' trailing blackberries. . Euphytica 42:155-162

McPheeters KD, Skirvin RM (2000) 'Everthornless' blackberry. HortScience 35:778

Mezzetti B, Costantini F, Chionchetti L, Landi L, Pandolfini T, Spena A (2004) Genetic transformation in strawberry and raspberry for improving plant productivity and fruit quality. Acta Hort

Mezzetti B, Landi L, Spena A (2002) Biotechnology for improving Rubus production and quality. Acta Hort 585:73-78

Moore JN (1984) Blackberry Breeding. Hortscience 19:183-185

Moore JN (1997) Blackberries 3rd edn. ASHS Press, Alexandria, VA

Moore PP (1993) Chloroplast DNA diversity in raspberry. HortScience 118:371-376

Moore PP (2004) "Cascade Delight' red raspberry. Hortscience 39:185-187

Moore PP (2006) 'Cascade Dawn' red raspberry. Hortscience 41:857-859

Moore PP, Finn CE (2007) 'Cascade Bounty' red raspberry. Hortscience 42:393-396

Moore PP, Perkins-Veazie P, Weber CA, Howard L (2008) Environmental effect on antioxidant content on ten raspberry cultivars. . Acta Hort 777:499-504

Morden CW, Weniger DA, Gardner DE (2003) Phylogeny and biogeography of Pacific *Rubus* subgenus Idaeobatus (Rosaceae) species: Investigating the origin of the endemic Hawaiian raspberry R. Macraei. Pacific Sci 57:181-197

Moyer R, Hummer K, Finn C, Frei B, Wrolstad RE (2002) Anthocyanins, phenolics and antioxidant capacity in diverse small fruits: *Vaccinium*, *Rubus* and *Ribes*. . J Agric Food Chem 50:519-525

Nybom H, Rogstad SH, Schaal BA (1990) Genetic variation detected by use of the M13 DNA fingerprint in *Malus*, *Prunus* and *Rubus*. Theoretical and Applied Genetics 79:153-153

Ooka H, Satoh K, Doi K, Nagata T, Otomo Y, Murakami K, Matsubara K, Osato N, Kawai J, Carninci P, Hayashizaki Y, Suzuki K, Kojima K, Takahara Y, Yamamoto K, Kikuchi S (2003) Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*. DNA Res 10:239-247

Ourecky DK (1975) Advances in Fruit Breeding Purdue Univ. Press., West Lafayette, Indiana

Ourecky DK, Slate GL (1966) Hybrid vigor in *Rubus occidentalis*-R. *leucodermis* seedlings. . In: Cong PtIH (ed), p 277

Oydvin J (1970) Important breeding lines and cultivars in raspberry breeding. . St Forsokag Njos 1970:1-42

Pamfil D, Zimmerman RH, Naess K, Swartz HJ (2000) Investigation of *Rubus* breeding anomalies and taxonomy using RAPD analysis. Small Fruits Rev 1:43-56

Pamfil D, Zimmerman RH, Naess SK, Swartz HJ (1997) Taxonomic relationships in Rubus based on RAPD and hybridization analysis (Abst.). . HortScience 31:620

Parent PG, Page D (1992) Identification of raspberry cultivars by non-radioactive DNA fingerprinting. . Hortscience 27:1108-1110

Patamsytė J, Žvingila D, Labokas J, Baliuckas V, Kleizaitė V, Balčiunienė L, Rančelis V (2004) Assessment of diversity of wild raspberries (*Rubus idaeus* L.) in Lithuania. J Fruit and Ornamental Plant Res 12:195-206

Pattison JA, Samuelian SK, Weber CA (2007) Inheritance of Phytophthora root rot resistance in red raspberry determined by generation means and molecular linkage analysis. Theoretical And Applied Genetics 115:225-236

Perkins-Veazie P, Kalt W (2002) Postharvest storage of blackberry fruits does not increase antioxidant levels. . Acta Hort 585:521-524

Ramanathan V, Simpson CG, Thow G, Lannetta PPM, McNicol RJ, Williamson B (1997) cDNA cloning and expression of polygalacturonase inhibiting proteins (PGIPs) from red raspberry (*Rubus idaeus*). Journal of Experimental Botany 48:1185-1193

Rusu AR, Pamfil D, Graham J (2006) Mapping resistance of red raspberry (*Rubus idaeus* subsp. *idaeus*) to viral diseases - leaf spot (RLSV) and vein chlorosis (RVCV) on the genetic linkage map. Buletinul Universitatii de Stiinte Agricole si Medicina Veterinara Cluj-Napoca

Sargent DJ, Fernandez-Fernandez F, Rys A, Knight VH, Simpson DW, Tobutt KR (2007) Mapping of A(1) conferring resistance to the aphid Amphorophora idaei and dw (dwarfing habit) in red raspberry (*Rubus idaeus* L.) using AFLP and microsatellite markers. BMC Plant Biology 7

Scott DH, Darrow GM, Ink DP (1957) 'Merton Thornless' as a parent in breeding thornless blackberries. Proc Am Soc Hort Sci 69:268-277

Seeram NP (2008a) Berry fruits for cancer prevention: current status and future prospects. J Agric Food Chem 56:630-635

Seeram NP (2008b) Berry fruits: Compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. . J Agric Food Chem 56:627-629

Sherman WB, Sharpe RH (1971) Breeding *Rubus* for warm climates. Hortscience 6:147-149

Siriwoharn T, Wrolstad RE, Finn CE, Pereira CB (2004) Influence of cultivar, maturity and sampling on blackberry (Rubus L. hybrids) anthocyanins, polyphenolics, and antioxidant properties. . J Agric Food Chem 52:8021-8030

Slate GL (1934) The best parents in purple raspberry breeding. Proc Am Soc Hortic Sci 30:108-112

Slate GL, Klein LG (1952) Black raspberry breeding. Proc Am Soc Hortic Sci 59:266-268

Stafne ET, Clark JR, Weber CA, Graham J, Lewers KS (2005) Simple sequence repeat (SSR) markers for genetic mapping of Raspberry and Blackberry. J Amer Soc Hort Sci 130:722-728

Stanisavljevic M (1999) New small fruit cultivars from Cacak:1. The new blackberry *(Rubus* sp.) cultivar 'Cacanska Bestrna'. Acta Hort 505:291-295

Stewart D, McDougall GJ, Sungurtas J, Verrall S, Graham J, Martinussen I (2007) Metabolomic approach to identifying bioactive compounds in berries: Advances toward fruit nutritional enhancement. Molecular Nutrition & Food Research 51:645-651

Strik BC (1992) Blackberry cultivars and production trends in the Pacific Northwest. Fruit Var J 46:207-212

Strik BC, Clark JR, Finn CE, Banados P (2007) Worldwide blackberry production. . HortTechnology 17:205-213

Thompson MM (1997) Survey of chromosome numbers in *Rubus* Rosaceae: Rosoideae

Trople DD, Moore PP (1999) Taxonomic relationships in *Rubus* based on RAPD analysis. Acta Hort 505:373-378

Wada L, Ou B (2002) Antioxidant activity and phenolic content of Oregon caneberries. . J Agric Food Chem 50:3495-3500

Waldo GF (1948) The Chehalem blackberry. Oregon Agr Expt Sta Circ 421

Waldo GF (1950) Notice of naming and release of a new blackberry adapted to the Pacific Coast region. In: Notice USDAR (ed)

Waldo GF (1957) The Marion blackberry. Oregon Agr Expt Sta Circ 571

Waldo GF (1968) Blackberry breeding involving native Pacific Coast parentage. Fruit Var J 22:3-7

Waldo GF (1977) Thornless Evergreen-Oregon's leading blackberry. Fruit Var J 31:26-30

Waldo GF, Wiegand EH (1942) Two new varieties of blackberry the Pacific and the Cascade. . Oregon Agr Expt Sta Circ 269

Weber CA (2003) Genetic diversity in Black Raspberry detected by RAPD markers. HortScience 38:269-272

Weber CA, Perkins-Veazie P, Moore P, Howard L (2008) Variability of antioxidant content in raspberry germplasm. Acta Hort 777:493-498

Williamson B, Jennings DL (1986) Common Resistance in Red Raspberry to Botrytis-Cinerea and Didymella-Applanata, 2 Pathogens Occupying the Same Ecological Niche. Annals of Applied Biology 109:581-593

Wood GA, Andersen MT, Forster RLS, Braithwaite M, Hall HK (1999) History of Boysenberry and Youngberry in New Zealand in relation to their problems with Boysenberry decline, the association of a fungal pathogen, and possibly a phytoplasma, with this disease. NZJ Crop Hort Sci 27:281-295

Woodhead M, McCallum S, Smith K, Cardle S, Mazzitelli L, Graham J (2008) Identification, characterisation and mapping of single sequence repeat (SSR) markers from raspberry root rot and bud ESTs. Mol Breeding DOI 10.1007/s11032-008-9198-y

Zheng D, Hrazdina G (2005) Expression and function of aromatic polyketide synthases in Rubus. Abstracts of Papers of the American Chemical Society 229:U34-U35

Zheng D, Hrazdina G (2008) Molecular and biochemical characterization of benzalacetone synthase and chalcone synthase genes and their proteins from raspberry (*Rubus idaeus* L.). Archives of Biochemistry and Biophysics 470:139-145

Zheng D, Schroder G, Schroder J, Hrazdina G (2001) Molecular and biochemical characterization of three aromatic polyketide synthase genes from *Rubus idaeus*. Plant Mol Biol 46:1-15