Raspberries and Blackberries: The Genomics of *Rubus*

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1 Taxonomy

The genus Rubus is comprised of a highly heterozygous series of some 500 species, with ploidy levels ranging from diploid to dodecaploid (Jennings, 1988; Meng and Finn, 2002). Members of the genus can be difficult to classify into distinct species for a number of reasons, including hybridization between species and apomixes (Robertson, 1974; Dickinson et al., 2007; Evans et al., 2007). For a description of the genus and the species contained within see Skirvin et al. (2005). The domesticated subgenera *Idaeobatus* and *Eubatus* contain the raspberries, blackberries, arctic fruits and flowering raspberries, all of which have been utilized in breeding programs. The most economically important *Rubus* species are the raspberries, the European red raspberry, R. *idaeus* L. subsp. *idaeus*, the North American red raspberry *R. idaeus* subsp. *strigosus* Michx and the black raspberry (*R. occidentalis* L.). The red raspberry being diploid has been the focus of most of the developments in molecular genetics.

Rubus subgenus *Idaeobatus* is distributed principally in Asia but also East and South Africa, Europe and North America. In contrast, subgenus *Eubatus* is mainly distributed in South America, Europe and North America (Jennings, 1988). The members of subgenus *Idaeobatus* are distinguished by the ability of their mature fruits to separate from the receptacle.

2 Diversity

Roach (1985) and Jennings (1988) gave accounts of the early domestication of red raspberry (*Rubus idaeus* L). During the 19th Century, the North American red raspberry (*R. idaeus* subsp. strigosus Michx) was introduced into Europe and

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46 subsequently crossed with the European sub-species (R. *idaeus* subsp. vulgatus
47 Arrhen.). Five parent cultivars dominate the ancestry of red raspberry; 'Lloyd
48 George' and 'Pynes Royal' entirely derived from R. *idaeus* var. vulgatus and
49 'Preussen', 'Cuthbert' and 'Newburgh' derived from both sub-species. Controlled
50 crossing began slightly earlier in the US than the UK with the introduction of
51 'Latham' in 1914 (McNicol and Graham, 1992). Domestication has resulted in a
52 reduction of both morphological and genetic diversity in red raspberry (Graham
53 et al., 1996; Haskell, 1960; Jennings, 1988) with modern cultivars being geneti-
54 cally similar (Dale et al. 1993; Graham and McNicol, 1995). Similar work on the
55 genetic relatedness of black raspberries using RAPD markers was carried out and
56 raised similar concerns of a narrow genetic base with the need for more incorpo-
57 ration of more diverse germplasm into black raspberry breeding (Weber, 2003).
58 Relatedness in blackberries has also been examined using pedigree analysis with
59 similar findings recommending the diversification of the gene pool (Stafne and
60 Clark, 2004). This restricted genetic diversity is of serious concern for the future
61 of *Rubus* breeding, especially when seeking durable host resistance to intractable
62 pests and diseases for which the repeated use of pesticides in some regions is inef-
63 fective, unsustainable or unacceptable for certain selected markets, such as 'organic
64 production'. The gene base can and is being increased by the introduction of unse-
65 lected raspberry clones and species material (Knight et al., 1989). However, the time
66 required to produce finished cultivars from this material can be considerable, par-
67 ticularly if several generations of backcrossing are required to remove undesirable
68 traits. Efforts are being made to conserve the biodiversity of berries across Europe
69 (Bartha-Pichler, 2006) and an interest in the conservation of genetic resources
70 has led to studies on wild raspberry populations in various countries. Studies in
71 Scotland have show wild raspberries to be genetically and physiologically differ-
72 entiated from each other and from cultivars (Marshall et al., 2001; Graham et al.,
73 1997, 2003). Similar studies using phenotypic characters have been carried
74 out on twelve wild raspberry populations in Russia (Ryabova, 2007) where wild
75 populations were examined for characteristics which may be useful in cultivated
76 raspberries. Romanian (Rusu et al., 2006a) and Bulgarian red raspberries (Badjakov,
77 personal communication) have been studied to determine their similarity with
78 European and American germplasm using SSR markers. Genetic diversity has been
79 examined in natural populations of black raspberry (*R. coreanus*) in Korea using
80 ISSR markers (Hong et al., 2003) and overall genetic relationships among popula-
81 tions were associated with geographic location. Black raspberry (*R. leucodermis*)
82 populations have also been evaluated for traits of importance for use in red and
83 black raspberry breeding (Finn et al., 2003) With concern over climate change
84 coupled with the desire for limiting 'flown food' to reduce the carbon footprint
85 of agriculture and horticulture, availability of suitable varieties will be crucial for
86 the future success of raspberry commercial cultivation. This conservation of genetic
87 resources may prove to be invaluable in securing germplasm for future breeding
88 program.

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3 Breeding Objectives

Rubus breeding is a long slow process hampered by several genetic problems, which depending on species, include polyploidy, apomixes, pollen incompatability and poor seed germination. As far as we are aware there are 30 *Rubus* breeding programs in 19 countries, almost all of which are in Europe or North America. Breeding programs sponsored by end-users or government aim to develop appropriate germplasm enabling their particular industry to realize its potential and thus goals vary from program to program. As new challenges arise and new production systems are developed, breeding programs are faced with meeting these demands with new cultivars. The core primary objectives in raspberry breeding include: high quality fruit, good yield, shelf life and suitability for shipping, if for the fresh market, suitability for mechanical harvesting for the processing market, adaptation to the local environment and improved pathogen resistance (Graham and Jennings, 2008).

3.1 Fruit Characters

While many characteristics are important in the successful acceptance of new cultivars, fruit quality must be considered the premier factor. Flavor, appearance and shelf life are the main attributes of fresh market quality and are essential for repeat purchase of fruit by consumers. Flavor is a highly subjective trait but can be broken down into multiple descriptors for taste, texture and other sensory characteristics. Good, acceptable flavor in raspberry tends to be fruity, sweet and floral with a desire for some acidity but no bitterness (Harrison et al., 1999). Color, brightness, size and shape contribute to the appearance and success of a variety and are crucial for initial purchase of fruit by consumers. A naturally dark color can be perceived as overripe by fresh market retailers, whereas a darker color is desirable for processing. Large fruit size is an attractive characteristic to both consumers and producers as it is more cost effective to pick.

3.2 Plant Characters

Plant habit is important for plantation management and has a major effect on yield potential. In summer fruiting types, the most important characteristics include the number and height of young canes, consistency of bud break, internode length, lateral length and position of laterals. In primocane fruiting types (where fruit is produced on first year canes) the amount of branching and extent of lateral development on the primocanes are major yield components. In both types erect, spineless canes are desirable (Jennings, 1988).

3.3 Phytochemicals

Raspberries were first used in Europe for medicinal purposes (Jennings, 1988), and once again there is heightened interest focused on these fruit as major sources of antioxidants, such as anthocyanins, catechins, flavonols, flavones and ascorbic acid; compounds that protect against a wide variety of human diseases, particularly cardiovascular disease and epithelial (but not hormone-related) cancers (Deighton et al., 2000; Moyer et al., 2002). There has been a recent explosion of data on berry fruit and their impact on human disease (e.g. Marinova and Ribarova, 2007; Rupasinghe and Clegg, 2007) As a result, the consumption of these berries is expected to increase substantially in the near future as their value in the daily diet is publicized. A concerted effort by the public health authorities in Finland, for example, has promoted the consumption of small berry fruits to their populations (Puska et al., 1990). Recent epidemiological studies have suggested the efficacy of compounds found at high concentrations in berries for the prevention of a number of chronic diseases and studies are now aimed at understanding the mechanisms of action of specific groups of phytochemicals. For a review of the literature on the potential health benefits of berry fruits see Hancock et al. (2007).

3.4 Production

While strawberries remain the best selling soft fruit, other fruits such as raspberry, are gaining popularity because of the increasing all year round availability. Raspberries have always been attractive as fresh dessert fruits or for processing from frozen berries into conserves, purees and juices.

The Scottish bred 'Glen Ample', released in the mid-1990's (www.fruitgate way.co.uk), supercedes older varieties such as 'Malling Jewel', 'Glen Clova', 'Glen Prosen' and 'Glen Moy'. 'Glen Ample', along with 'Tulameen' and recently the new cultivar 'Octavia' dominate the UK market and acreage due to their desirable fresh market characteristics. Serbia is a major world producer and exporter of raspberries, producing one quarter of the world tonnage. 90% of the acreage is dominated by the North American 'Willamette'. Protected cropping and out of season production in European countries is expanding so that in areas of southern Spain nearly 100% of fresh market raspberries are being grown under polytunnels. The early season 'Glen Lyon' has a low chilling requirement which makes it suitable for re-propagation and manipulation of canes and is currently the ideal variety for this production system. These protected cropping systems have been adopted by the UK industry to improve fruit quality and extend the season. Since the majority of fresh market production goes to large supermarket chains, the demand for good fruit quality, flavor and shelf life is high. In other European countries, Pacific Northwest-bred cultivars have led the industry, such as 'Meeker', 'Willamette' and 'Tulameen'. The primocane-fruiting (plants fruit in their first year) 'Heritage' has led the industry in many countries. In Scandinavia, the hardy Norwegian variety

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'Veten' has been the mainstay for many years, now 'Glen Ample' has taken the lead. In the US, 'Meeker' and 'Willamette' developed in the mid-1900s are the primary cultivars although recent publicly developed 'Cowichan' and 'Coho' are being widely planted. Black raspberry (*R. occidentalis* L.) production has traditionally been concentrated almost completely in Oregon with 'Munger' and 'Jewel' being the leading varieties; however, a strong South Korean industry has developed over the past five years. A recent study carried out on the costs associated with blackberries (*Rubus* subgenus *Rubus*) as a crop for the southeastern United States for both the pick your own and wholesale markets (Safley et al., 2006) has shown this could be a profitable venture. Blackberry as a commercial crop is also gaining popularity in Europe, showing one of the biggest rises in cultivation aside from blueberries (Jennings, personal communication.).

Machine harvesting of processing raspberries is the standard practice for most major raspberry production regions around the world and is essential where picking labor is expensive or unavailable. Despite advances in machine technology, it appears that the major improvements in harvesting will come from plant breeding (Cormack, 1989). No single attribute has been found to determine successful machine harvest-ability but a range of interacting traits such as uniform strong vigor and good cane density with an upright habit governs harvest performance. Medium length laterals with good fruit presentation is also desirable. Maturity, physical shape of the berry and receptacle all contribute to ease of pick. This will help ensure that a high percentage of uniform, ripe fruit with acceptable process quality and minimal green fruit are harvested throughout the season (Hall et al., 2002).   AQ1

In the UK and Europe, a move from outside field plantations to protected cropping systems has taken place in an effort to extend the cropping season and to improve fruit quality. Protected cropping and out-of season production in European countries is expanding, so that in areas of southern Spain nearly 100% of fresh market dessert raspberries for early, main and late season are being grown under tunnels (Gillespie et al., 1999) mainly 'Glen Lyon'. Such changes in agronomic   AQ2 practices affect plant growth, seasonality and fruit quality and have implications for a shift in pest and pathogen pressures. Recently, breeding programs in the UK have responded to this change in production by trialing and selecting germplasm under protected cropping systems (FruitGateway.Co.UK).

4 Limitations

Concern over environmental impact and sustainability of agricultural and horticultural practices is leading to a greater emphasis on pest and disease resistance, as well as the ability of plants to withstand local environmental stresses. The changes in environmental, cultural and agronomic practices within the industry will impact strongly on the nature of the germplasm required for the future. Greater conservation of genetic resources and utilization of diverse locally adapted germplasm will be required for future viability of raspberry production.

226   The incorporation of novel resistance/tolerance to pests and diseases is regarded
227   as essential for the development of cultivars suitable for culture under integrated
228   pest management (IPM) systems. Sources of resistance in diverse *Rubus* sp. to many
229   pests and diseases have been identified and exploited in conventional cross-breeding
230   (Keep et al., 1977; Jones et al., 1984; Jennings, 1988; Knight, 1991; Williamson
231   and Jennings, 1992). However, germplasm bearing single resistance genes, when
232   planted over extensive areas, can eventually be overcome by the rapid evolution of
233   new biotypes of pests, so that new types of host resistance are required to sustain
234   plant protection (Birch et al., 2002; Jones et al., 2002). Pest and diseases of raspberry
235   in Europe have been extensively reviewed in Gordon et al., (2006).

5 Structural Genomics

240   Breeding methods used in raspberry have changed very little over the last 40 years
241   or so, and little novel germplasm has made its way into commercial cultivars. How-
242   ever, with the narrowing genetic base coupled with the increasing demands from
243   consumers, new breeding methods are required to meet demands. The speed and
244   precision of breeding can be improved by the deployment of molecular tools for
245   germplasm assessment, management and the development of genetic and physical
246   maps. Red raspberry (*Rubus idaeus*) is a good species for the application of molec-
247   ular and genomics techniques, being diploid ($2n = 2x = 14$) with a very small
248   genome (275 Mbp making it highly amenable to complete physical map construc-
249   tion and map-based gene cloning.

250   The availability of abundant genetic variation in natural and experimental pop-
251   ulations and adaptation to a range of diverse habitats (Keep, 1972; Graham et al.
252   1997, 2003; Marshall et al. 2001; Balciuniene et al., 2005; Ryabova, 2007) offers
253   researchers a rich source of variation in morphology, anatomy, physiology, phenol-
254   ogy and response to a range of biotic and abiotic stressors. The ability to vegetatively
255   propagate individual plants provides opportunities to capture genetic variation over
256   generations and replicate individual genotypes to partition and quantify the environ-
257   mental and genetic components of variation of genetic linkage maps. These are nec-
258   essary to develop diagnostic markers for polygenic traits and, in the future, possibly
259   identify the genes behind the traits. Understanding the genetic control of commer-
260   cially and nutritionally important traits and the linkage of these characteristics to
261   molecular markers on chromosomes is the future of plant breeding. This facilitates
262   the development of diagnostic markers for polygenic traits and the identification of
263   genes controlling complex phenotypes.

AQ3

6 DNA Markers

268   The development and application of molecular markers has been reviewed by
269   Antonius-Klemola (1999), Hokanson (2001) and Skirvin et al. (2005). As well
270   as the deployment of anonymous DNA markers such as RAPDs (Graham et al.,

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271   1994, 1997; Pattison and Weber, 2003) and AFLPs (Graham et al., 2006), SSR and   AQ4
272   EST-SSR markers have been developed (Graham et al., 2002, 2004, 2006; Stafne
273   et al., 2005; Lewers et al., 2005; Lopes et al., 2006; Woodhead et al., 2008) which
274   allow the development of genetic linkage mapping, fingerprinting and assessments
275   of diversity to be undertaken in raspberry. Work is now underway at SCRI to develop
276   single nucleotide polymorphism (SNP) markers for specific genes and transcription
277   factors of interest.

280   # 7 Linkage Mapping

282   To date there are several genetic linkage maps available for raspberry (Pattison and
283   Weber, 2003; Pattison et al., 2007 ; Graham et al., 2004, 2006; Sargent et al., 2007)   AQ5
284   which have largely been constructed in order to identify markers for particular pest
285   and disease resistances, although they can of course be used to identify markers
286   for other traits. Graham et al. (2004) utilised a cross between the phenotypically
287   diverse European red raspberry 'Glen Moy' and the North American 'Latham'.
288   This wide cross segregates for a large number of important traits, thus the same
289   population and this reference map can be used for the major breeding objectives
290   (Table 1). SSR markers from both genomic and cDNA libraries from 'Glen Moy'
291   and 'Autumn Bliss' were used together with AFLP markers to create a linkage map.
292   An enhanced map with further SSR and EST-SSR and gene markers has recently
293   been completed (Graham et al., 2006). This work has highlighted the importance
294   of maps and markers for raspberry breeding with demonstration of a tight associ-
295   ation between Gene H and resistance to two fungal diseases, cane botrytis (*Botry-*

298   **Table 1**   Important plant characters segregating in the 'Glen Moy' and 'Latham' cross utilized for
299   generating a raspberry linkage map (Graham et al., 2004, 2006)

	Glen Moy	Latham
Character		
Canes	Hairy (Hh)	Not hairy (hh)
	Spine free	Spiny
	Green/brown	Brown/purple
Fruit	Large	Small
	Sweet	Sour
	Thimble shape	Round
	Early ripening	Late ripening
	Pale red	Dark red
Pest and Disease		
	Root rot susceptible	Root rot resistant
	Rust Susceptibility	Rust resistance
	Resistance to spur blight	Susceptible to spur blight
	Resistance to cane botrytis	Susceptible to cane botrytis
Other	Not hardy	Hardy

316 *tis cinerea*) and spur blight (*Didymella applanata* (Niessl) Sacc.) on linkage group
317 2 (Graham et al., 2006). This gene is a valuable marker as raspberry breeders in
318 general have limited resources and rarely include a primary screen for fungal dis-
319 eases. It had been reported previously that some disease resistances were associated
320 with distinctive morphological traits, most notably that of cane pubescence (fine
321 hairs) determined by Gene H (genotype HH or Hh), the recessive allele of which
322 gives glabrous canes (genotype hh). Raspberry cultivars and selections with fine
323 hairs (pubescent canes) were reported to be more resistant to cane botrytis (*Botrytis*
324 *cinerea*), cane blight (*Leptosphaeria coniothyrium*) and spur blight than non-hairy
325 ones (Knight and Keep, 1958; Jennings and Brydon, 1989), but more susceptible
326 to cane spot (*Elsinoe veneta*), powdery mildew (*Sphaerotheca macularis*) and yel-
327 low rust (*Phragmidium rubi-idaei*) (Keep, 1968; Anthony et al., 1986; Jennings and
328 McGregor, 1988; Williamson and Jennings, 1992). No association between Gene H
329 and rust or cane spot susceptibility was detected in this recent study. How Gene H
330 contributes to the disease resistance has not been determined, and work to saturate
331 the map region around Gene H is underway using AFLPs, as well as identifying
332 candidate genes in the region (Woodhead, Graham and Smith, personal communi-
333 cation.).

334 Raspberry root rot caused by *Phytophthora fragariae* var. *rubi* is probably the
335 most destructive disease in raspberry plantations (Wilcox et al., 1993; Wilcox and
336 Latorre, 2002) and it has been the focus of several studies (Graham and Smith,
337 2002; Pattison and Weber, 2003; Pattison et al., 2007). Resistance to *Phytophthora*
338 root rot (PRR) is found in cultivars derived from *Rubus idaeus strigosus*, the native
339 North American red raspberry but less so in those derived from *Rubus idaeus vul-*
340 *gatus,* the European red raspberry (Pattison and Weber, 2005). Thus, generating
341 crosses between the two can facilitate the identification of the genes underpinning
342 this resistance. Using a 'Glen Moy' (*R. idaeus vulgatus*) x 'Latham' (*R. idaeus*
343 *strigosus*) mapping population two regions, one on each of two linkage groups have
344 been identified and further research aimed at confirming these in a second popula-
345 tion through glasshouse and field trials has been completed (Graham et al., unpub-
346 lished data). Bac clones have been mapped into the resistance regions and will be
347 tiled across and sequenced to identify any genes in the region (Graham and Smith
348 personal data). Using a RAPD-based linkage map from a cross between 'Latham'
349 and 'Titan', Pattison and Weber (2003) also identified markers clustered on 2 linkage
350 groups which were associated with disease score QTL for PRR using bulk segregant
351 analysis. Recently this work has been extended to include AFLP, RAPD and resis-
352 tance gene analog polymorphism (RGAP) markers in other 'Latham' and 'Titan'
353 populations (Pattison et al., 2007). Considerable progress towards identifying mark-
354 ers and ultimately the gene(s) responsible for resistance to this disease is being made
355 and this can be incorporated into raspberry breeding programs, allowing the rapid
356 identification and selection of durable resistant genotypes.

357 Aphids (particularly *Amphorophora idaei (Borner)* and *Aphis idaei van der*
358 *Goot*) are one of the most damaging arthropod pests in raspberry (Gordon et al.,
359 1997), due to direct feeding damage to susceptible cultivars and because they act
360 as vectors for virus transmission (Gordon et al., 2005). Breeding for host plant

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361 resistance to raspberry aphids, over the past 40 years has reduced the need for
362 pesticides and controlled the spread of aphid borne viruses (Birch et al., 2005). How-
363 ever, over the 10–15 years it takes to produce a new variety by conventional breed-
364 ing methods, insect pests are constantly adapting and overcoming plant resistance
365 genes. Several types of aphid resistance genes, minor/multi-gene and single major
366 genes e.g. A1 and A10 with different mechanisms have been used against *A. idaei* in
367 sequence by raspberry breeders, however in the UK each type of major gene resis-
368 tance has been broken. To date, minor gene-based aphid resistance remains durable
369 in raspberry but it provides only partial resistance (Birch et al., 2005). Efforts to
370 identify new sources of aphid resistance from wild species and other cultivars is
371 underway, as is the development of molecular markers to speed up the selection of
372 promising genotypes (Birch et al., 2005). In addition, efforts to map aphid resis-
373 tance genes by anchoring marker data from appropriate segregating populations to
374 the published raspberry maps are underway (Sargent et al., 2007) with the deter-
375 mination of the linkage group for A1. Determining the map location of a num-
376 ber of aphid resistance genes from various sources will allow the discrimination
377 of different genes and gene pyramiding in new raspberry cultivars. Recent surveys
378 of commercial raspberry crops in the UK have found that many virus diseases are
379 becoming increasingly prevalent (Jones and McGavin, 2004). Another virus Rasp-
380 berry bushy dwarf virus (RBDV) is also causing serious concern. RBDV is a pollen
381 borne virus which has been re-emerging in the past 15 years throughout raspberry
382 growing regions of the world (Martin et al., 2004), mainly because RBDV-immune
383 plants (carrying the Bu gene) are being replaced by RBDV-susceptible plants with
384 superior agronomic traits (Jones et al., 1998; Jones and McGavin, 2004). The dis-
385 ease causes crumbly fruit and yield reduction (Martin et al., 2004), and is there-
386 fore an important target for crop improvement. Several isolates of RBDV have been
387 sequenced (MacLeod et al., 2004) and genetic transformations have been under-
388 taken to produce RBDV resistant plants (MacLeod et al., 2004; Martin et al., 2004).
389 Attempts to develop markers for other viral resistance genes has been carried out
390 for raspberry leaf spot and raspberry vein chlorosis utilizing the 'Glen Moy' x
391 'Latham' cross of Graham et al. (2004). Field screening was carried out to mea-
392 sure symptom production of leaf spot and vein chlorosis in 2 different environments.
393 These traits were analyzed for significant linkages to mapped markers and resistance
394 loci were found on linkage groups 2 and 8 (Rusu et al., 2006b).Work towards the
395 genetic mapping of health-related compounds has been initiated in *Rubus* (Stewart
396 et al., 2007). Berries are extremely high in antioxidants, exhibiting up to 4 times
397 more antioxidant capacity than non-berry fruits, 10 times more than vegetables and
398 40 times more than cereals (Halvorsen et al., 2002). They contain high levels of the |AQ8|
399 antioxidant vitamins A, C and E and very high levels of non-essential but strongly
400 antioxidant phenolic compounds. Phenolics can account for 90% or more of the
401 overall antioxidant capacity found in berry fruit (Deighton et al., 2000), the most
402 readily visible of which are the anthocyanin pigments. These pigments impart the
403 deep, vibrant colors of berries and can be found at concentrations of up to 500 mg
404 100 g FW^{-1}. Berries represent a significant dietary source of anthocyanins, as
405 only 24 out of 100 common foods contain anthocyanins and non-berry anthocyanin

containing foods typically contain less than 100 mg 100 g FW^{-1} (Wu et al., 2006). Progress in mapping anthocyanins has been made by Kassim et al., 2008). Here high performance liquid chromatography (HPLC) was used to quantify eight major anthocyanins cyanidin and pelargonidin glycosides: -3-sophoroside, -3-glucoside, -3-rutinoside and -3-glucosylrutinoside across two seasons and two environments in progeny from a cross between two *Rubus* subspecies, *Rubus idaeus* (cv. Glen Moy) x *Rubus strigosus* (cv. Latham). 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. A chalcone synthase (PKS 1) gene sequence (Zheng et al., 2001; Zheng and Hrazdina, 2008) mapped to LG 7 but did not underlie the anthocyanin QTLs identified. However other candidate genes including bHLH (Espley et al., 2007), NAM/CUC2 (Ooka et al., 2003) like protein and bZIP transcription factor (Holm et al., 2006; Mallappa et al., 2006) underlying the mapped anthocyanins were identified (Kassim et al., 2008). The shift in focus from vitamin C and micronutrients towards the polyphenolics causes something of a challenge for any breeding effort, since the polyphenolics are chemically diverse and the content of individual health-promoting compounds varies in raspberry fruit due to both developmental and genetic factors (Beekwilder et al. 2005). However, 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).

8 Physical Mapping

Large insert genomic libraries (BACS) are both invaluable tools and a source of genomic DNA for physical mapping, positional cloning and as a scaffold for whole genome sequencing. *Rubus idaeus* is an ideal candidate for BAC library construction, since it is diploid ($2n = 2x = 14$) and has a very small genome (275 Mbp). The small genome size of raspberry makes it highly amenable to complete physical map construction, and thereby provides a platform for map-based gene cloning and comparative mapping with other members of the Rosaceae (Dirlewanger et al., 2004).

One of the most challenging steps required for the construction of plant large-insert genomic libraries is the isolation of high molecular weight DNA (HMW-DNA), either in the form of embedded protoplasts or nuclei. Raspberry and other soft-fruit species have, however, proven recalcitrant to standard genomic DNA extractions as they contain very high levels of carbohydrates, particularly polysaccharides, and polyphenolic compounds. They require heavily modified

451 methods for ordinary genomic DNA isolations (Woodhead et al., 1998) and in order
452 to prepare HMW-DNA suitable for the construction of BAC libraries a novel nuclei
453 isolation procedure was developed (Hein et al., 2005). This DNA is of high quality
454 and has been used for the construction of the first publicly available red raspberry
455 BAC library from the European red raspberry, 'Glen Moy'. Currently, the library
456 comprises over 15,000 clones with an average insert size of approximately 130 kb
457 (6–7 genome equivalents). Hybridization screening of the BAC library with chloro-
458 plast (*rbcL*) and mitochondrial (*nad1*) coded genes revealed that contamination of
459 the genomic library with chloroplast and mitochondrial clones was very low (>1%)
460 (Hein et al., 2004).

461 Initial screening of the BAC library employed probes for chalcone synthase,
462 phenylalanine ammonia lyase and a MADS-box gene involved in bud dormancy
463 (Hein and Williamson, personal communication). More recently, the library has
464 been probed with genes involved in epidermal cell fate (Woodhead, Graham and
465 Williamson, personal communication), fruit quality genes (Woodhead and McCal-
466 lum, personal communication) and a peach ever-growing gene (Abbott, personal
467 communication).

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

475 The availability of a detailed genetic linkage map, together with a deep coverage
476 bacterial artificial chromosome library, will be of great value in the identification of
477 the genetic factors that underpin a wide range of commercially important character-
478 istics such as the appearance, texture and the sensory attributes (taste and aroma) of
479 raspberry fruit and the genetic resistance to pests and diseases. The establishment
480 of gene-phenotype relationships will allow gene-based selection in breeding and the
481 functional assignment of genes for commercially important traits.

482
483

484 **9 Functional Genomics**

485

486 Understanding the function of genes and other parts of the genome is known as
487 functional genomics. In raspberry, as with many other species, functional genomics
488 is at an early stage.

489 The advances in genomics technologies have lead to a massive increase in the
490 numbers of DNA sequences held in public databases. However, a search of the
491 NCBI nucleotide database for *Rubus* retrieved only 2239 sequences, a large num-
492 ber of which are actually viral sequences. In comparison, a similar search for the
493 peach yielded 100,021 hits. The number of raspberry sequences is, however, very
494 likely to increase rapidly as efforts are under way to sequence EST libraries gener-
495 ated 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.

Fruit quality characteristics in raspberry are key drivers for breeding programs and a greater understanding of the processes and genes involved in quality will enable more targeted breeding in the future. Genes up-regulated during fruit ripening have been identified using classical plus/minus screening of cDNA libraries (Jones et al., 1999) and RNA fingerprinting techniques (Jones et al., 2000). Amongst the genes identified were cell wall hydrolases involved in fruit softening and ACC oxidase (Jones et al., 2000) involved in the ethylene biosynthetic pathway. Raspberry fruit ripen in response to ethylene (Burdon and Sexton, 1990a, b) but the ripening-related increase in ethylene biosynthesis is not associated with an increase in respiration rate (Jennings, 1988; Perkins-Veazie and Nonnecke, 1992).

The phenylpropanoid pathway is important in raspberry, as end products contribute to the color and aroma of the fruit and are involved in other processes such as lignin production. Aroma and color in raspberry fruit are partly derived from the polyketide derivatives benzalacetone and dihydrochalcone which are formed during fruit ripening as a result of the action of several enzymes, polyketide synthases (PKS), benzalcetone synthase and chalcone synthase (CHS) during fruit development. A number of PKS genes have been characterised from raspberry (Zheng et al., 2001; Kumar and Ellis, 2003). Kumar and Ellis (2003) reported the PKS gene family in *Rubus* consists of at least 11 members and expression analysis of 3 cDNAs showed they exhibited tissue-specific and developmental patterns of expression, with two cDNAs up-regulated during fruit ripening. Work is underway to identify and map these genes in the 'Glen Moy' x 'Latham' mapping population and raspberry BACs containing these PKS genes have been identified and are being characterized (Kassim, Paterson, Graham and Woodhead, personal communication).

Genes encoding 4-coumarate:CoA ligase, an enzyme that activates cinnamic acid and its derivatives to thioesters which then serve as intermediates for the production of phenylpropanoid-derived compounds that influence fruit quality have also been studied. Kumar and Ellis (2003) have characterized the 4-coumarate:CoA ligase (4CL) genes in raspberry found there are three genes which are differentially expressed in various organs and during fruit development and ripening. Based on the expression patterns and substrate utilization profiles of the recombinant proteins, they suggest that 4CL1 is involved in the biosynthesis of phenolics in leaves, 4CL2 in cane lignification and 4CL3 in the flavonoid and/or flavor pathway in fruit. These genes are also being targeted in the *Rubus* mapping programe (Woodhead, Graham and Smith, personal communication).

A further project to characterise bud dormancy phase transition in woody perennial plants at a molecular level generated a total of 5300 ESTs from endodormant (true dormancy) and paradormant (apical dominance) raspberry meristematic bud tissue (Mazzitelli et al., 2007). PCR-products from these cloned cDNA fragments

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have been spotted onto glass slides and have been used in microarray experiments to identify genes that show differential expression. At present, approximately 380 clones exhibit up or down regulation during the endodormancy – paradormancy transition. Some of these ESTs, including one encoding a MADs-box gene, a MYb gene and several containing SSRs have been identified and mapped in the 'Glen Moy' x 'Latham' mapping population and these underlie mapped ripening stage QTL and will form the basis of future studies (Graham personal communication).

10 Future Strategies

Through the creation of genetic and physical map resources in raspberry, there is now the real possibility of linking phenotype with genotype in raspberry. Initial research will focus on mapping key commercial and environmentally important traits. Closer links with other members of Rosaceae community to facilitate sharing of marker data/co-linearity/synteny etc. should allow progress to be made in a timely manner.

Ultimately, the information derived from *Rubus* genomic programs will be adapted and used to drive more efficient, targeted breeding to provide superior cultivars with high fruit quality (flavor, nutrition etc) and durable resistance to pests and diseases as well as a greater understanding of the key processes driving plant and fruit quality characteristics.

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