9 Raspberry

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9.1 Introduction

Rubus is one of the most diverse genera in the plant kingdom, comprising over 400 species (Bailey 1949) subdivided into 12 subgenera (Jennings 1988). Ploidy levels range from diploid to 14-ploid (Nybom 1985). 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). The domesticated subgenera contain the raspberries, blackberries, arctic fruits and flowering raspberries, all of which have been utilised in breeding programs. The most important raspberries are 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.). Rubus subgen. Idaeobatus is distributed principally in Asia but also East and South Africa, Europe and North America. In contrast, subgen. Eubatus is mainly distributed in South America, Europe and North America (Jennings 1988). The members of subgenus Idaeobatus sp. are distinguished by the ability of their mature fruits to separate from the receptacle. The subgenus is particularly well represented in the northern hemisphere.

The place of origin of raspberry has been postulated to be the Ide mountains of Turkey. Jennings (1988) and Roach (1985) have given extensive accounts of early domestication. Records were found in 4th century writings of Palladius, a Roman agriculturist, and seeds have been discovered at Roman forts in Britain; hence, the Romans probably spread cultivation throughout Europe. The British popularized and improved raspberries throughout the middle-ages, and exported the plants to New York by 1771.

Rubus species are prostrate to erect, generally thorny shrubs producing renewal shoots from the ground (called canes). They are perennials only because each bush consists of biennial canes, which overlap in age. Leaves are compound with 3–5 leaflets, the middle one being the largest; margins serrate to irregularly toothed.

Small (0.5–1.5 cm), white to pink flowers are initiated in the second year of planting. The gynoecium consists of 60–80 ovaries, each of which develops into a druplet. There are 60–90 stamens. Raspberries produce copious amount of nectar and attract bees. The flowers of *Rubus* are structurally rather similar to those of strawberries, with five sepals, five petals, a very short hypanthium, many stamens, and an apocarpous gynoecium of many carpels on a cone-like receptacle. Raspberries are an aggregate fruit, composed of individual drupelets, held together by almost invisible hairs. In *Rubus* each carpel will develop into a small drupelet, with the mesocarp becoming fleshy and the endorcarp becoming hard and forming a tiny pit that encloses a single seed. Each drupelet usually has a single seed, though a few have two. Fruiting begins in the second year of planting and can continue for more than 15 years if properly managed. Fruit development occurs rapidly, taking only 30–36 days for most raspberry cultivars.

Canes grow one year and fruit the next, but there are also primocane varieties which fruit in the first year. The biennial growth cycle of raspberry stems begins when a bud from below soil level develops and elongation of the internodes carries the growing point, protected by leaf scales, to the soil surface. At the surface, leaves expand to form a tight rosette around the growing point. Elongation of the shoot starts in spring and continues until autumn, by which time the shoot will have attained a height of 2 to 3 m. In red raspberries (*R. idaeus* L.), shortening days and falling temperatures in late summer cause shoot elongation to cease and dormancy to set in. This is a gradual process extending over several weeks and once a stage of complete dormancy is reached it is not readily reversible. Black raspberries (*R. occidentalis* L.) or purple raspberries (hybrids between red and black raspberries) and most blackberries differ

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from red raspberries both in time when dormancy begins and intensity of dormancy attained. In these fruits, growth continues well into autumn. The initiation of flower buds usually starts at the same time as the canes begin to acquire dormancy. In the spring of the second year, vegetative primocanes become fruiting canes. The fruit is composed of a large number of one-seeded druplets set together on a small conical core (Jennings 1988).

The traditional method of culture harvests fruit annually from each plant, although both non-fruiting vegetative canes (primocanes) and fruiting canes (fructocanes) are present. This main season summer-fruiting crop is usually supported on a post-and-wire system designed to carry the weight of fruits and to protect canes from excessive damage due to wind, harvesting and cultivation. Primocanes are produced in numbers excessive to requirements for cropping in the following season so many must be removed by pruning in winter and early spring to reduce inter-cane competition and create an open crop canopy for efficient light capture. Old dead fruiting canes must also be removed by pruning after harvest. Such pruning operations remove sources of fungal inoculum from the plantation and are important for the long-term health of the crop.

Primocanes and fruiting canes are in close proximity resulting in a complex plant-architecture that provides spatial and temporal continuity for pests and pathogens to colonise a range of habitats (Willmer et al. 1996). The complex nature of the plant architecture also creates a barrier of foliage that impedes spray penetration of plant protection chemicals, thus requiring specialised chemical application equipment (Gordon and Williamson 1988). Healthy plantations are expected to crop productively for more than 10 years, but this is only possible if the planting stocks and soils are free from persistent viral, bacterial and fungal diseases and certain pests, hence the importance of quarantine arrangements and certification schemes to protect the propagation industry and fruit production (Jones 1991; Smith 2003).

In a mature plantation the raspberry roots spread completely across the inter-row space. Young canes ('suckers') developing from root buds (Hudson 1959) in the inter-row space must be removed, to prevent competition of these suckers for light, water and nutrients with the crop. Uncontrolled suckers also represent a reservoir for pests and pathogens. Cultivation of the inter-row space is another alternative way to remove suckers and weeds, but repeated cultivation by machinery leads to loss of soil structure and soil erosion on slopes where raspberries are often grown. Effective weed management by residual herbicides, or cultivation, is essential to remove weeds as alternative hosts for nematodes that are vectors for many viruses (Murant 1981; Harrison and Murant 1996) and to reduce humidity around the base of plants where several pathogens thrive and sporulate at high humidity.

There has been increased interest in the sale of raspberry fruits harvested from 'organic production' – farming based on methods relying entirely on crop rotation and avoidance of pesticide application except certain substances currently permitted by the national regulatory authority for organic farming. However, with woody perennial crops the difficulties of maintaining healthy productive plantations over many years are profound and it is too early to judge the overall success of these ventures in *Rubus* cane fruits.

Increasing popularity of autumn-fruiting raspberries, in which late season fruit is harvested from berries forming on the upper nodes of primocanes, has extended the production season and the period of attack of some foliar and cane pests. Some very early spring fruits with high value can also be obtained from the remaining lower nodes of these over-wintered primocane-fruiting types. Primocane-fruiting raspberries tend to be grown in the warmer areas of Europe where the temperature in autumn is relatively high and there is little risk of early autumn frosts.

Interest has also been shown in extended-season production under glass or under plastic structures in northern European countries, e.g. Belgium (Meesters and Pitsioudis 1993; Verlinden 1995) and the UK (Barry 1995) and now in the Mediterranean fringe, e.g. Spain and Greece, and this trend will affect their pest and disease status. To satisfy these production systems, long primocanes grown in northern regions, such as Scotland, are lifted, chilled and stored for long periods for planting in late spring for late summer harvest under plastic. The concept of extended-season-production would mean that by careful manipulation of plant dormancy cycle and flower initiation it should be possible to produce fresh raspberries in Europe for sale in almost all months.

The genomic number of *Rubus* is seven and species representing all ploidies from diploid to duodecaploid are found in nature. The range in size is from 1 – 4 μm (Jennings 1988). The genome has been estimated to be 275 Mbp.

Self-incompatibility systems occur in some *Rosaceous* species and it is common among many of the diploid *Rubus* species (Keep 1968). In contrast all polyploidy species are self-compatible as are the domesticated forms of the diploid raspberries.

Raspberries are grown in many parts of the world with production estimated at 385,000 Mt (http://faostat.fao.org), Europe is estimated to produce around 316,000 Mt. Cane fruit production, mainly red raspberry (*Rubus idaeus* L.), is an important high-value horticultural industry in many European countries because it provides employment directly in agriculture, and indirectly in food processing and confectionary. Most raspberry production is concentrated in the northern and central European countries, although there is an increasing interest in growing cane fruits in southern Europe e.g. in Greece, Italy, Portugal and Spain. In many production areas, the fruit is grown for the fresh market, but in central Europe e.g. Poland, Hungary and Serbia, a high proportion of the crop is destined for processing. In the UK there has been a major movement away from processing towards fresh fruit production under protected cultivation for the high-value fresh market. Commercial blackberries are also grown, mainly in east Europe, and arctic raspberries (*R. arcticus* L.) are produced commercially on a small-scale in Finland (Koponen et al. 2000).

Fruit has become important in the human diet due to increased consumers awareness of healthy eating practices. In 2003, the global fresh fruit market was valued at £7.6 bn at current prices, having increased by just 3.9% since 1999. The fresh fruit sector accounts for 38.1% of the overall market and is gaining share due to continuing trend towards convenience food. Banana account the largest segment of the fruit sector with 22.5% of the market in 2003. In term of soft fruit, strawberries remain the UK's best selling soft fruit, but other fruit such as raspberry, are gaining popularities because 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. It is interesting to see that raspberries were first used in Europe for medicinal purposes (Jennings 1988), but there is now heightened interest focused on these foods 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; Stewart et al. 2001; Moyer et al. 2002). 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 publicised. 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) and in 2002, a similar initiative was launched in Scotland (Berry Scotland Project www.berryscotland.com) though success here has yet be demonstrated.

Five parent cultivars dominate the ancestry of red raspberry; '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 1960; Jennings 1988) with modern cultivars being genetically similar (Dale et al. 1993; Graham and McNicol 1995). This is of concern as a lack of genetic diversity can lead to inbreeding depression and susceptibility to external stresses. Extensive genetic diversity has been found in wild raspberry germplasm offering scope for expanding the genetic base of cultivated raspberries (Graham et al. 1997b; Marshall et al. 2001; Graham et al. 2003).

The objectives of breeding programs vary from region to region, but certain traits are always considered important. Breeding for high yields of easily harvested, quality fruit remains the priority in any commercial breeding program (www.fruitgateway.co.uk). The incorporation of novel resistance/tolerance to pests and diseases is regarded as essential for the development of cultivars suitable for culture under integrated pest (crop) management (IPM (ICM)) systems (Gordon et al. 2002a, b). The selection of resistant or tolerant cultivars is essential for reduced pest and disease pressure on the raspberry plantation. Careful thought however must be given to the management of reducing chemical applications as these may result in previously well-controlled pests or diseases becoming an unexpected problem in their own right. Additionally specification of cultivars, for example by UK supermarkets where the cultivars Glen Ample and Tulameen have been selected because of their high fruit quality, has lead to increased pesticide use because these cultivars are aphid susceptible (S.C. Gordon personal communication) again challenging the concepts of IPM and highlighting the often conflicting demands breeders face. Most raspberries are produced by small enterprises, frequently lacking the resources to fund adequate support from technical advisory services to manage this complex crop in a low input system. However, a survey in New England, USA in the early 1990s showed that farmers generally knew more about IPM than did consumers, wholesalers and food processors (Hollingsworth et al. 1993). Similarly, when driven by legislation and adequate state support, cane fruit IPM systems can be vibrant and generate considerable local and international interest e.g. Whatcom County (Nootsack) IPM raspberry program in Washington State, USA (MacConnell et al. 2002). Resistance breeding is becoming increasingly urgent due to the withdrawal of and undesirability of remaining chemical control measures. Fruit quality though, determines the ultimate success of a cultivar and these objectives may prove to be conflicting. Initial market acceptance of most fruits is based on color and appearance, as other factors are not evaluated until later when the product is consumed. Usually, the consumer associated eye appeal with quality. For an extensive review of fruit quality parameters in plant breeding see Sistrunk and Moore (1983). There is pressure from the supermarkets and some consumers to develop organic sources of many crops including raspberry. There appears to be no large-scale organic production of cane fruit in Western Europe, except for a few isolated producers. However, several large-scale producers are adopting the 'biodynamic' production system in central Europe. Many growers who have tried organic production in Western Europe in the past have failed due to the lack of control of perennial weeds within the crop and to infestation by raspberry beetle. Although derris (rotenone) sprays applied to the green fruit will give some protection against raspberry beetle, experimental and commercial experience suggests that the level of control is inadequate to satisfy the demands of the consumers. In trials in the early 1970s, comparing the efficacy of different insecticides, derris was considerably less effective than the then standard, malathion (Taylor 1971). Safety concerns of some organically approved products are being raised. Some producers, particularly in Scandinavia are keen to develop organic or very low-input production to exploit the demand. The geographical isolation of plantations coupled with low winter temperatures result in low pest burdens in these areas. Organic production will greatly benefit from cultivars with high levels of resistance or tolerance to the major pests and diseases. The large number of characteristics in any breeding program coupled with long generation times and problems with inbreeding depression have prompted the move towards marker-assisted breeding in red raspberry. For a review of breeding objectives and breeding techniques see Daubeny 1996.

Raspberry breeders have successfully produced cultivars that vary in growth habit, pest and disease resistance, spinelessness, fruit quality and primocane varieties.

9.2 Construction of Genetic Linkage Maps

Breeding methods used in raspberry have changed very little over the last 40 years or so. Little novel germplasm has made its way into commercial cultivars. However, with the narrowing genetic base coupled with the increasing demands from consumers, new breeding methods are required to meet demands. The speed and precision of breeding can be improved by the deployment of molecular tools for germplasm assessment and the development of genetic linkage maps. Such genetic linkage maps can facilitate the development of diagnostic markers for polygenic traits and the identification of genes controlling complex phenotypes. Understanding the genetic control of commercially and nutritionally important traits and the linkage of these characteristics to molecular markers on chromosomes is the future of plant breeding. Red raspberry (*Rubus idaeus*) is a good species the application of such techniques, being diploid ($2n = 2x = 14$) with a very small genome (275 Mbp). Indeed, the haploid genome size of raspberry is only twice the size of *Arabidopsis*, making it highly amenable to complete physical map construction, thereby providing a platform for map-based gene cloning and comparative mapping with other members of the Rosaceae (Dirlewanger et al. 2004). The availability of abundant genetic variation in natural and experimental populations and adaptation to a range of diverse habitats (Graham et al. 1997b; Marshall et al. 2001; Graham et al. 2003) offers researchers a rich source of variation in morphology, anatomy, physiology, phenology and response to a range of biotic and abiotic stress. The ability to vegetatively propagate individual plants provides opportunities to capture genetic variation over generations and replicate individual genotypes to partition and quantify environmental and genetic components of variation of genetic linkage maps. These are necessary to develop diagnostic markers for polygenic traits and, in the future, possibly identify the genes behind the traits. The Rosaceae is an economically important family of perennial fruit bearing crops that includes members of the following genera: *Malus* (apple), *Pyrus* (pear), *Rubus* (raspberry, blackberry), *Fragaria* (strawberry) and *Prunus* (stone fruits). In addition, the family also includes a number of important ornamental plants such as roses, flowering cherry, crab apple and quince. Molecular marker applications have been reviewed in *Rubus* (Antonius-Klemola 1999) and in the small fruits (Hokanson 2001). Linkage maps have been generated in other woody species (Ritter et al. 1990; Grattapaglia and Sederoff 1994; Bradshaw et al. 1994; Bradshaw and Stattler 1995) and in the small (soft) fruit crops a few maps exist. In the diploid strawberry (*Fragaria vesca*) and diploid blueberry (*Vaccinium* spp.) 445 cM and 950 cM or 1,288 cM long linkage maps based on RAPD markers have been constructed (Rowland and Levi 1994; Davis and Yu 1997; Qu and Hancock 1997). Maps of other *Rosaceous* crops include *Prunus* maps (Dirlewanger et al. 1997, 1998; Joobeur et al. 1998, Ballester 2000; Joobeur et al. 2000; Dettori et al. 2001; Aranzana et al. 2003), apple (Hemmat et al. 1994; Maliepaard et al. 1998; Liebhard et al. 2003). Resources are being developed in strawberry to enhance maps based on RAPD markers (Sargent et al. 2003; Graham 2005). The first genetic linkage of raspberry has recently been constructed (Graham et al. 2004b). This 789 cM genetic linkage map was constructed utilising a cross between the phenotypically diverse European red raspberry cultivar Glen Moy and the North American cultivar Latham. SSR markers were developed from both genomic and cDNA libraries from Glen Moy. These SSRs, together with AFLP markers, were utilised to create a linkage map. An enhanced with further SSR and EST-SSR and gene markers has recently been completed (Graham et al. 2006).

9.3 Gene Mapping

Mapping in raspberry is at an early stage. Preliminary work is underway to map genes underlying a number of commercially important traits. Gene *H* in raspberry has recently been mapped to Group 2 of the raspberry map (Graham et al. 2006). Raspberry breeders in general have limited resources and rarely include a primary screen for fungal diseases. It has been reported that some disease resistances are associated with distinctive morphological traits, most notable cane pubescence (fine hairs). Pubescence is determined by gene *H* (genotype *HH* or *Hh*), the recessive allele of which gives glabrous canes (genotype *hh*). Gene *H* is rarely homozygous because it is linked with a lethal recessive gene (Jennings 1988). Raspberry cultivars and selections with fine hairs (pubescent canes) are more resistant to cane botrytis (*Botrytis cinerea*), cane blight (*Leptosphaeria coniothyrium*) and spur blight (*Didymella applanata*) than non-hairy ones (Knight and Keep 1958; Jennings and Brydon 1989) but more susceptible to cane spot (*Elsinoe veneta*), powdery mildew (*Sphaerotheca macularis*) and yellow rust (*Phragmidium rubi-idaei*) (Keep 1968, 1976; Anthony et al 1986; Jennings and McGregor 1988; Williamson and Jennings 1992). How Gene *H* has the large increase or decrease in disease resistance has not been determined. It has been suggested that it is due to linkage with major resistance genes or minor gene complexes that independently contribute to the resistance or susceptibilities of the six diseases affected. An alternative explanation is that the gene itself is responsible through pleiotrophic effects on each of the resistances (Williamson and Jennings 1992). This gene has now been mapped and further mapping of the disease resistance genes is underway (Graham et al. 2006). Other work underway is aimed at identifying the gene(s) responsible for resistance to raspberry root rot (Graham and Smith 2002). Two regions on two linkage groups have been identified and further research aimed at confirming these in a second population through glasshouse and field trials is underway (Graham, Smith and Tierney unpublished data). Efforts to map aphid resistance by anchoring data marker data from appropriate segregating populations to the published raspberry maps are underway (Sargent, Knight Personal Communication).

9.4 Analysis of Quantitative Trait Loci

Preliminary quantitative trait loci (QTL) mapping has been carried out in raspberry using the recently developed genetic linkage map (Graham et al. 2004b, 2006). Morphological data based on the segregation of cane spininess, and root sucker density and diameter were quantified in two different environments. Breeding for spinelessness is a major concern for breeders and there are several major genes that confer this trait (Jennings and Ingram 1983; Jennings 1988). The mapping parents differ for spine morphology with Glen Moy having a spine-free phenotype (being homozygous for gene *s* (Jennings 1988), whereas Latham is a densely spiny cultivar, the genetics of which has not been determined. The progeny generated from the cross were all spiny, though the extent of spines varied continuously from a very sparsely spiny cane to the densely spiny phenotype of the Latham parent. From the phenotypic data it was proposed that two or more genes are involved. This was supported by the mapping data where a number of markers were identified, linked to the spiny phenotypes. These markers mapped onto linkage group 2, and there appeared to be two linked regions within this group accounting for 98% of the variation.

Large differences exist in the extent of root sucker production in cultivated raspberries. Control measures based on the chemical burning of early canes produced from suckers are required in commercial plantations to optimise fruit yield (Jennings personal communication). Roots of red raspberry have adventitious buds, which develop on most roots. The number, density and distance from the mother plant of the root suckers varies between genotypes. Only a proportion of the buds normally develop into suckers. Knight and Keep (1960) have shown that the ability to produce suckers in red raspberry is determined by the recessive gene sk_I or by the complementary genes sk_2 and sk_3. Interestingly, and probably not surprisingly, the measurements of density and spread map to the same linkage group (group 8), with an overlap in the location of the QTLs for the two traits (Graham et al. 2004b).

A number of QTLs for fruit quality parameters have been identified on the raspberry maps and some candidate genes which underlie these traits have been identified. For example a QTL for fruit size has been located (Graham unpublished data) with a vacuolar $^{H+}$-ATPase (Martinoia et al. 2000).

9.5 Marker-Assisted Breeding

A number of DNA-based marker systems have been developed for use in raspberry (Antonius-Klemola 1999; Hokanson 2001; Graham et al. 2002a). Genetic markers have been used to widely to examine genetic variation within and between *Rubus* spp. An M13 bacteriophage probe has been used to examine different *Rubus* spp. and a number of red raspberries (Nybom et al. 1990). A minisatellite probe was used by Kraft et al. (1996) to demonstrate that fingerprints of out-crossing species vary considerably compared to vegetative and apomictic clones. Chloroplast DNA sequence probes were used by Waugh et al. (1990) and Howarth et al. (1997) to examine genotypic and taxonomic relatedness in raspberry. Ribosomal DNA ITS region has been used to construct a phylogenetic tree with representatives from 20 species (Alice and Campbell 1999). RAPD markers have been widely used to examine the relatedness of raspberry cultivars and species (Graham and McNicol 1995; Graham et al.1997b; Coyner 2000).

Marker-assisted selection is developing into a powerful tool for plant breeding, through its ability to select plants with the desired trait(s) accurately and at an early stage of growth. Rather than screening for a particular phenotype (trait), a breeder can screen for a marker tightly linked to the gene of interest that is identified through the construction of a linkage map in a population segregating for that trait. Alternatively, bulked segregant analysis can be used to identify markers linked to a particular trait, the position of which can then be determined on a linkage map (Graham and Smith 2002).

9.6 Map-Based Cloning

Map-based cloning has yet to be carried out in raspberry. Genetic engineering technologies, if they become widely acceptable to customers, could allow high quality cultivars to be transformed with genes conferring resistance to a range of pests and diseases (Watt et al. 1999), thus offering the prospect of reduced pesticide application. Recent research in strawberry has demonstrated that introduction of the Cowpea protease trypsin inhibitor (CpTi) gene resulted in promising levels of control in glasshouse feeding trials and field trials against larvae of *Otiorhynchus sulcatus* (Graham et al. 1997a, 2002b). Use of gene transfer technologies to improve resistance to mites, insects and nematodes would be especially valuable because of the toxicity of acaricides, insecticides and nematicides, many of which are likely to be withdrawn from use in minor crops in the future. Fruit quality and other stress resistance genes would be valuable. However, it is vitally important that these genetically engineered crops are not toxic or pose a serious allergenic risk to humans, do not harm beneficial organisms (e.g., natural enemies of pests, crop pollinators, soil micro-organisms) or affect the wider environment. Large-scale 'risk assessments' of genetically engineered crops such as the Farm-Scale Evaluation of oil seed rape, sugar beet and maize are currently being undertaken in the UK to ensure that, on release, they are environmentally benign.

9.7 Advanced Works

Advanced work for red raspberry is still at an early stage. A search of the NCBI nucleotide database for *Rubus* retrieved only 1,744 sequences a large number of which were actually viral sequences. In comparison, a similar search for the genus *Prunus*, also a member of the Rosaceae family, yielded 325,773 sequences of which 76,619 originated from *Prunus persica* (peach).

The number of raspberry sequences is, however, very likely to increase rapidly as efforts are under way to generate EST libraries from different tissues and developmental stages. At the Scottish Crop Research Institute, cDNA libraries have been generated from leaves (approximately 6,500 clones), canes (approximately 8,000 clones) and roots (approximately 7,300 clones) and further libraries will be constructed from fruit and shoots in the near future (Graham, Smith and Tierney unpublished data). Bacterial colony filters derived from the above libraries have been subjected to hybridization screening to identify simple sequence repeats (SSR) markers and will be partially sequenced.

A further project aims at the characterization of bud dormancy in woody perennial plants on a molecular level and generated in total 5,300 ESTs from endodormant (true dormancy) and paradormant (apical dominance) raspberry meristematic bud tissue (Mazzitelli et al. unpublished data). PCR-products from these cloned cDNA fragments have been spotted onto glass slides and are currently being used in microarray experiments to identify genes that show differential expression. At present, approximately 380 clones exhibit up or down regulation during the endodormancy – paradormany transition.

Large insert genomic libraries (BACS) are invaluble 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). Indeed, the genome size of raspberry is only twice that of the model plant *Arabidopsis*, making it highly amenable to complete physical map construction, and thereby providing a platform for map-based gene cloning and comparative mapping with other members of the Rosaceae.

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 methods for ordinary genomic DNA isolations (Woodhead et al. 1998) and the utilization of activated charcoal in tissue culture to prevent growth inhibition due to excess polyphenolics released into the medium (Millan-Mendoza and Graham 1999). To prepare HMW-DNA suitable for the construction of BAC libraries we have developed a novel nuclei isolation procedure (Hein et al. 2005). The method is based on a modified buffer system including 4% (w/v) PVP-10 described by Peterson et al. (2000) and utilizes a combination of nylon filters and Percoll™ gradients to purify nuclei extracts prior to embedding in agarose plugs. The isolated HMW-DNA is of high quality and has been used for the construction of the first publicly available red raspberry BAC library from the European red raspberry cultivar Glen Moy, which has also been utilised as a parent for the first reported genetic linkage map of *R. idaeus* based on a cross with the phenotypically diverse North American cultivar Latham (Graham et al. 2004b). Currently, the library comprises over 15,000 clones with an average insert size of approximately 130 kb (6–7 genome equivalent). 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. 2004a).

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. An integrated physical/genetic map will also allow the extent of synteny or collinearity of the *Rubus* genome with other members of the Rosaceae to be determined.

The availability of a detailed genetic linkage map, together with a deep coverage bacterial artificial chromosome library, will be of great value in the identification of the genetic factors that underpin a wide range of commercial characteristics such as appearance, genetic resistance, texture and sensory (taste and aroma) attributes of fruit. The establishment of gene-phenotype relationships will allow gene-based selection in breeding and the functional assignment of genes for commercially important traits.

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