Chloroplast DNA Diversity in Raspberry

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Abstract. The relationships among raspberry (Rubus spp.) clones were investigated using southern hybridization. Total DNA from 22 clones were digested with Bam III and Eco RI and hybridized with two sequences from a Pst I tomato (Lycopersicon esculentum Mill.) chloroplast library. A total of 40 different restriction fragments were distinguished for the four enzyme probe combinations. These fragments distinguished seven groups of clones with members of each group having identical fragment patterns. Clones with R. idaeus L. maternal ancestry were distinct from those with R. occidentalis L. or R. parvifolius L. ancestry. Differences were detected between R. idaeus vulgatus Arrhen. and R. idaeus strigosus Michx.

No commercial cultivars had chloroplast DNA patterns that were the same as an accession of the R. idaeus strigosus subspecies.

Cultivated raspberries are not a single taxonomic entity. Present red raspberry cultivars of major importance are primarily derived from the species Rubus idaeus. This species is divided into two subspecies, R. idaeus vulgatus, European red raspberry, and R. idaeus strigosus North American red raspberry. The two subspecies are completely interfertile, and most cultivars have both subspecies in their lineage. Several cultivars also include the black raspberry (R. occidentalis) ‘Cumberland’ in their ancestry. Additionally, R. arcticus L., R. chamaemororus L., R. cockburnianus Hems., R. kunzeanus Hems., R. odoratus L., R. parvifolius, and R. ursinus Cham. & Schlecht. are in the pedigrees of raspberry cultivars released since 1960 (Dale et al., 1993). However, some listed parents may not actually be in the ancestry, since they are not in the same subgenus as raspberry and have differing ploidy levels.

Although there are pedigree records for modern raspberry cultivars, the actual relationships among clones are not known. According to Jennings’ (1988) history of the evolution of domesticated raspberries, these have been cultivated since at least the fourth century. By the 1920s, many cultivars were being grown. However, most raspberry stocks were mixtures; a single cultivar might be known by several names, or several cultivars could be grown under the same name (Grubb, 1922). Relationships among the ancestors of modern cultivars are not clear, and modern cultivars with pedigree records tracing to different ancestors may be descended from the same cultivar under different names. The true amount of genetic diversity among modern cultivars is not known.

Darrow (1920) attempted to determine the subspecies composition of several raspberry cultivars using morphological characteristics. Ten cultivars were considered to be of subspecies strigosus origin, seven of subspecies strigosus that may have a trace of R. occidentalis, 16 as subspecies vulgatus, and nine as hybrids between the subspecies.

The use of morphological characters can result in unclear results. DNA restriction fragment length polymorphisms (RFLP) can be used to characterize clones with less ambiguity. Waugh et al. (1990) used variation in chloroplast DNA to construct a phylogenetic tree of 20 Rubus genotypes including red raspberry, blackberry, hybrid berries, and wild relatives. No variation was detected among the three raspberry cultivars included.

The objectives of this research were to examine the variability in chloroplast DNA among cultivated raspberries of diverse maternal ancestry and to determine the species or subspecies origin of their chloroplasts.

Material and Methods

Plant material. Twenty-two Rubus clones were used in this study (Table 1). Pedigrees used in a previous study (Dale et al., 1993) were used to identify maternal ancestors. All plant material was from the Washington State Univ. breeding program collection or from the National Clonal Germplasm Repository, Corvallis, Ore. Cultivars with diverse maternal ancestors, according to pedigree records, were selected rather than the most widely grown raspberry cultivars.

DNA isolation. Total DNA was isolated from fresh leaf material by the CTAB procedure of Saghafi-Marooof et al. (1984).

Probe DNA. P-2 and P-4 from the Pst I tomato chloroplast library were used as probes (Phillips, 1985a). P-2 is a 21.9 kilobases (kb) fragment and P-4 is a 19.4 kb fragment that are adjacent to each other (Phillips, 1985a) and the large subunit of RUBP carboxylase (EC 4.1.1.39) maps to the juncture of these two fragments (Phillips, 1985b). These two fragments comprise >25% of the chloroplast genome. Plasmid DNA was isolated by the alkaline/SDS method (Maniatis et al., 1982) and purified by CsCl centrifugation. Inserts were labeled using the oligolabelling method (Feinberg and Vogelstein, 1983).

Gel electrophoresis and southern analysis. 1 to 5 µg of total DNA was digested with Bam HI, Eco RI, Eco RV, Hae III, or Hind III according to manufacturer’s (BRL, Bethesda, Md.) recommendations. Samples were electrophoresed in 1% agarose gels and the DNA transferred to a nylon membrane (GeneScreen Plus, DuPont, Boston) using an alkaline transfer method (Chomczynski and Qasba, 1984). Membranes were hybridized using the protocol recommended by the membrane manufacturer. X-ray film (X-Omat AR film, Kodak, Rochester, N.Y.) was exposed at -70C using intensifying screens.

Data analysis. The presence or absence of each fragment was determined for each Rubus clone for each enzyme-probe combination. The proportion of shared DNA fragments (F) (Nei and Li, 1979) was determined.
1979) was calculated for each pair of Rubus clones. Clones with identical restriction fragment patterns for all enzyme and probe combinations were represented by one clone for statistical purposes.

Results

‘Lloyd George’ was selected as a representative of R. idaeus vulgatus and Dalhousie Lake 4 (Daubeney and Stary, 1982) was selected as a representative of R. idaeus strigosus. Bam HI and Eco RI digested the chloroplast DNA into differing sizes between these raspberry clones with P-2 and P-4 as probes and were used for all 22 Rubus clones.

Probe P-2. Probe P-2 hybridized to DNA fragments in both Bam HI and Eco RI digests that appeared on the autoradiographs to be present at lower concentrations than the other fragments (Figs. 1 and 2). These fragments were consistent, and digestion with lo-fold higher enzyme amounts did not alter their presence. Although these fragments could be used to discriminate among clones, they were not included in the analyses.

The Bam HI digest resulted in five fragments per sample with a total of seven different fragments (Table 1). The bands for ‘Brandywine’, except for 4.8 and 4.4 kb fragments, instead of the 5.0 and 4.5 kb fragments present in ‘Brandywine’.

A total of 40 different restriction fragments were scored for the four enzyme probe combinations. These fragments distinguished seven groups of clones with members of each group having identical fragment patterns (Table 2). This is a limited number of bands and conclusions about the relationships among clones based on this grouping must be regarded as tentative. The proportion of shared fragments (F) between groups does follow the taxonomic groupings. The F values ranged from 0.462 to 0.944 (Table 3). The F values between members of groups 1 to 4 were all 0.800 or higher, indicating high levels of similarity between these groups.

Groups 1 to 4 include all of the cultivars of R. idaeus maternal ancestry. These clones are quite distant from groups derived from the other Rubus species. Groups 1 to 3 had very similar restriction fragment patterns with F values between group members of 0.865 to 0.944. All of the cultivars with R. idaeus vulgatus maternal ancestry are in these groups. Group 4 is Dalhousie Lake 4, a representative of the strigosus subspecies. It is distinct from groups 1 to 3, but closer to those clones than to those derived from other Rubus species in groups 1 to 3 (Table 3). None of the commercial raspberry cultivars included in this study had chloroplast fragments of subspecies strigosus as represented by Dalhousie Lake 4. Although this subspecies has been used in breeding, it has been primarily used as a male, and none of the cultivars in this study are thought to be maternally descended from subspecies strigosus.

| Clone            | Maternal ancestor          | Origin of maternal ancestor  |
|------------------|----------------------------|-----------------------------|
| Avelma           | Unknown                    | Illinois, about 1915        |
| Brandywine       | Logan                      | England, before 1817        |
| Chilcotin        | Hudson River Antwerp       | England, before 1817        |
| Chilwiack        | Hudson River Antwerp       | B.C., Canada about 1950     |
| Comox            | Creston                    | Quebec, Canada 1975         |
| Dalhousie Lake 4 | R. idaeus strigosus        | Scotland, before 1935       |
| Dormanred        | R. parvifolius             | Pennsylvania, before 1896   |
| Glen Moy         | Burnetholm                 | England, before 1869        |
| Glen Prosen      | Cumberland                 | England, 1919               |
| Indian Summer    | English Globe              | England, about 1877         |
| Lloyd George     | Unknown                    |.extensions                |
| Malling Landmark | Superlative                | New York, 1871              |
| Mandarin         | R. parvifolius             | England, about 1877         |
| Munger           | Shaffer                    | New York, before 1896       |
| Norma            | Superlative                | Ohio, 1888                  |
| Southland        | R. parvifolius             | Quebec, Canada 1924         |
| Success          | Morrison                   | Norway, before 1900         |
| Tahoma           | Thompson                   | England, before 1817        |
| Trailblazer      | Newman                     | Quebec, Canada 1924         |
| Veteran          | Asker                      |                            |
| Viking           | Hudson River Antwerp       |                            |
| Willamette       | Newman                     |                            |

Probe P-4. The Bam HI digest resulted in four to six fragments per sample with a total of 11 different fragments (Fig. 3). These fragments distinguished five groups of clones (Table 2).

The Eco RI digest resulted in three to six fragments per sample with a total of 11 different fragments (Fig. 4). These fragments distinguished five groups of clones (Table 2). ‘Munger’ (not shown) had the same fragments as shown for ‘Brandywine’, except for 4.8 and 4.4 kb fragments, instead of the 5.0 and 4.5 kb fragments present in ‘Brandywine’.

A total of 40 different restriction fragments were scored for the four enzyme probe combinations. These fragments distinguished seven groups of clones with members of each group having identical fragment patterns (Table 2). This is a limited number of bands and conclusions about the relationships among clones based on this grouping must be regarded as tentative. The proportion of shared fragments (F) between groups does follow the taxonomic groupings. The F values ranged from 0.462 to 0.944 (Table 3). The F values between members of groups 1 to 4 were all 0.800 or higher, indicating high levels of similarity between these groups.

Groups 1 to 4 include all of the cultivars of R. idaeus maternal ancestry. These clones are quite distant from groups derived from the other Rubus species. Groups 1 to 3 had very similar restriction fragment patterns with F values between group members of 0.865 to 0.944. All of the cultivars with R. idaeus vulgatus maternal ancestry are in these groups. Group 4 is Dalhousie Lake 4, a representative of the strigosus subspecies. It is distinct from groups 1 to 3, but closer to those clones than to those derived from other Rubus species in groups 1 to 3 (Table 3). None of the commercial raspberry cultivars included in this study had chloroplast fragments of subspecies strigosus as represented by Dalhousie Lake 4. Although this subspecies has been used in breeding, it has been primarily used as a male, and none of the cultivars in this study are thought to be maternally descended from subspecies strigosus.
Group 5 consists of ‘Munger’. ‘Munger’ was selected from open pollinated seed of ‘Shaffer’ by Timothy Munger in about 1890 (Hedrick, 1925). ‘Shaffer’ is described as a R. idaeus strigosus x R. occidentalis hybrid selected as a chance seedling (Hedrick, 1925). However, Hedrick (1925) further comments that ‘Munger’ does not show any of the characters of its reputed parent (‘Shaffer’). The chloroplast DNA fragments support the position that ‘Munger’ had a R. occidentalis maternal ancestor rather than a strigosus maternal ancestor. However, its R. occidentalis ancestor is distinct from those in the ancestry of ‘Brandywine’, ‘Success’, and ‘Glen Prosen’.

Group 6 includes ‘Brandywine’, ‘Success’, and ‘Glen Prosen’, all with R. occidentalis maternal ancestors. ‘Brandywine’ and ‘Success’ are considered purple raspberries. ‘Success’ has ‘Morrison’ (R. occidentalis) as a maternal parent and ‘Brandywine’ has another R. occidentalis clone, ‘Logan’ (not ‘Loganberry’), as a maternal ancestor four generations removed. Although ‘Glen Prosen’ has the appearance of a red raspberry, its maternal ancestry includes the R. occidentalis clone, ‘Cumberland’, seven generations removed. These results support at least a predominantly maternal inheritance of chloroplast DNA in raspberry. Group 7 consists of ‘Dormanred’ and ‘Mandarin’, both with R. parvifolius as their maternal ancestors.

Discussion

Waugh et al. (1990) did not detect any differences in chloroplast DNA among R. idaeus clones ‘Creston’, ‘Malling Promise’, and ‘Willamette’. Although all three clones were not used in the present study, ‘Willamette’ and ‘Comox’ (which has ‘Creston’ as a maternal ancestor) were used. In the present study, ‘Willamette’ and ‘Comox’ could be distinguished using different probes and restriction enzymes than used by Waugh et al. (1990). The only differences found here between these two cultivars were by using Bam HI with P-4 as a probe. Waugh et al. (1990) used Eco RI and Eco RV in their analyses. In the present study, ‘Willamette’ and ‘Comox’ were not distinguishable using Eco RI, only with Bam HI. The lack of variability found by Waugh et al. (1990) and the low levels found in this study indicate that R. idaeus vulgatus shows little variability in chloroplast DNA.

There is good agreement between the maternal ancestry based on pedigree information and chloroplast fragment patterns with two exceptions. Possible causes of the differences could be incorrectly identified plant material during propagation, incorrect pedigrees, or occasional paternal inheritance of chloroplast DNA. Although reciprocal crosses should be identical for nuclear genes, they are not identical for cytoplasmic DNA. The parents for a cross may be easily reversed when recorded, resulting in the incorrect maternal ancestor.

According to pedigree records, ‘Newman’ is the maternal ancestor of both ‘Trailblazer’ and ‘Willamette’. With maternal inheritance of chloroplast DNA, ‘Trailblazer’ and ‘Willamette’ should have the same chloroplast restriction fragment patterns. However, using Bam HI with P-4 as a probe, there were differences. There was no evidence to doubt the authenticity of the plant materials before this study.
The maternal ancestor of ‘Southland’ is *R. parvifolius*. ‘Dormanred’ and ‘Mandarin’ are derived from *R. parvifolius* and have distinct chloroplast DNA patterns from *R. idaeus* cultivars (Figs. 14). However, the chloroplast DNA patterns of ‘Southland’ are identical to the three cultivars with ‘Hudson River Antwerp’ as the maternal ancestor (Tables 1 and 2). The plant material representing ‘Southland’ was obtained from breeding plots from the Washington State Univ. breeding program. A second accession representing ‘Southland’ was obtained from a commercial nursery (Ahrens Nursery, Huntingburg, Ind.) with identical results. There was no evidence to doubt the authenticity of this plant material before this study. After this study was completed, Cousineau and Donnelly (1992) reported that, based on isoenzyme analyses, ‘Southland’ from the National Clonal Germplasm Repository in Corvallis was mislabeled. This supports the position that the ‘Southland’ clones used in this study were incorrect.

Chloroplast RFLP analysis can provide evidence to clarify doubtful pedigree records. ‘Veten’ was released as ‘Asker’ × ‘Lloyd George’. However, Oydvin (1969) suggested that ‘Veten’ is a selection of ‘Preussen’ × ‘Lloyd George’. ‘Norna’ is from

| Cultivar                  | P-2       | P-4       | Bam HI | Eco RI | P-2       | P-4       |
|---------------------------|-----------|-----------|--------|--------|-----------|-----------|
| Anelma                    | A         | B         | A      | A      | A         | A         |
| Glen Moy                  | A         | B         | A      | A      | A         | A         |
| Indian Summer             | A         | B         | A      | A      | A         | A         |
| Malling Landmark          | A         | B         | A      | A      | A         | A         |
| Norma                     | A         | B         | A      | A      | A         | A         |
| Willamette                | A         | B         | A      | A      | A         | A         |
| Chilcotin                 |           |           | A      | A      | A         | A         |
| Chilliwack*               |           |           | A      | A      | A         | A         |
| Southland                 |           |           | A      | A      | A         | A         |
| Viking                    |           |           | A      | A      | A         | A         |
| Lloyd George*             | A         | B         | A      | A      | A         | A         |
| Tahoma                    | A         | B         | A      | A      | A         | A         |
| Trailblazer               | A         | B         | A      | A      | A         | A         |
| Comox*                    | A         | B         | A      | A      | A         | A         |
| Dalhousie Lake 4*         | A         | B         | B      | B      |           |           |
| Munger                    | B         | C         | C      | C      |           |           |
| Bandywine*                |           |           | C      | D      | D         | D         |
| Success                   |           |           | C      | D      | D         | D         |
| Glen Prosen               |           |           | C      | D      | D         | D         |
| Dormanred*                |           |           | A      | E      | E         | E         |
| Mandarin                  |           |           | A      | E      | E         | E         |

2 Restriction fragment patterns for cultivars indicated by (*) are shown in Figs. 1, 2, 3, and 4. 3 Cultivars followed by the same letter within columns have identical restriction fragment patterns for a given restriction enzyme/DNA clone combination.

Table 3. Proportion of shared fragments (F) between cluster groups of raspberry cultivars digested with *Bam HI* and *Eco RI* and hybridized with chloroplast probes P-2 and P-4.

| Group number | 2 | 3 | 4 | 5 | 6 | 7 |
|--------------|---|---|---|---|---|---|
| 1            | 0.944 | 0.919 | 0.872 | 0.526 | 0.579 | 0.595 |
| 2            | 0.865 | 0.821 | 0.800 | 0.462 | 0.513 | 0.632 |
| 3            | 0.488 | 0.750 | 0.579 | 0.595 | 0.600 | 0.564 |
| 4            | 0.615 | 0.750 | 0.579 | 0.595 | 0.600 | 0.564 |

4 Cultivar composing groups are listed in Table 2.
Preussen' × 'Lloyd George' and 'Malling Landmark' is from 'Preussen' × 'Baumforth A'. 'Norna' and 'Malling Landmark' have the same restriction patterns as 'Chilliwack' for Bum HI using P-4 as a probe and 'Veten' has a different pattern, which is the same as that of 'Lloyd George' (Fig. 3). The chloroplast restriction pattern supports the original pedigree of 'Veten' as 'Asker' × 'Lloyd George'.

'Newman', the ancestor of many cultivars, was selected from a mixture of open pollinated seed of 'Herbert', 'King', 'Loudon', 'Cuthbert', and 'Eaton' (Hedrick, 1925). Although it is not possible to identify the parent from the sample of cultivars included in this study, it may be possible to determine the parent of 'Newman' by analysis of carefully selected cultivars.

Raspberry chloroplast DNA had low levels of variability in this study. Because of the mode of inheritance of chloroplast DNA, clones that are derived from the same maternal ancestor can not be distinguished. For these reasons, analysis of nuclear DNA can be more sensitive and can be more useful in clone identification (Nybom et al., 1989; Nybom and Schaal, 1990). Variation was detected among cultivars with differing species chloroplasts, between subspecies within the species R. idaeus, and within the subspecies vulgatus. No cultivar had chloroplast DNA patterns that were the same as an accession of the subspecies strigosus. The variation patterns generally supported maternal inheritance of chloroplast DNA, but this assumption needs to be rigorously tested (Smith, 1989).

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