Chloroplast DNA Diversity in Raspberry

Patrick P. Moore¹

Washington State University, Puyallup Research and Extension Center, Puyallup, WA 98371-4998

Additional index words. *Rubus idaeus vulgatus*, *Rubus idaeus strigosus*, restriction fragment length polymorphism

Abstract. The relationships among raspberry (*Rubus* spp.) clones were investigated using southern hybridization. Total DNA from 22 clones were digested with *Bam* HI 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. chamaemorus* L., *R. cockburnianus* Hemsl., *R. kunzeanus* Hemsl., *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 Saghai-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 -70°C 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,

Received for publication 30 Mar. 1992. Accepted for publication 24 Oct. 1992. H/ LA Paper no. 91-19. Project no. 0640. Washington State Univ., College of Agriculture and Home Economics, Pullman, WA. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact. ¹Assistant Horticulturist.

Abbreviation: RFLP, restriction fragment length polymorphisms.

Table 1. Raspberry cultivars included in chloroplast DNA analyses, maternal ancestors, and origin of maternal ancestors.

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

²Unknown.

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 10-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 *Bum* HI digest resulted in five fragments per sample with a total of seven different fragments (Fig. 1). These fragments distinguished three groups of clones (Table 2). Most clones had the banding pattern (A) represented by 'Chilliwick'. 'Munger' (not shown in Fig. 1) had an 8.8 kb fragment and lacked the 9.4 kb fragment present in 'Chilliwick'. The third pattern (C) is represented by 'Brandywine'.

The *Eco* RI digest resulted in five fragments per sample with a total of 11 different fragments (Fig. 2). These fragments distinguished five groups of clones (Table 2). The fragments for 'Munger' (not shown) were the same as that shown for 'Brandywine', except for having a fragment of 8.6 kb instead of the 9.8 kb fragment shown for 'Brandywine'.

Probe P-4. The *Bum* 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 chloroplasts 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.

Fig. 1. Autoradiogram of DNA digested with *Bam* HI from 1) 'Chilliwick', 2) 'Comox', 3) 'Lloyd George', 4) Dalhousie Lake 4, 5) 'Brandywine', and 6) 'Dormanred' hybridized with chloroplast DNA probe P-2. Samples 1, 2, 3, 4, and 6 represent pattern A and sample 5 represents pattern C. Numbers to the left indicate molecular weight in kilobases (kb) for fragments of 'Chilliwick'.

Fig. 2. Autoradiogram of DNA digested with *Eco* RI from 1) 'Chilliwick', 2) 'Comox', 3) 'Lloyd George', 4) Dalhousie Lake 4, 5) 'Brandywine', and 6) 'Dormanred' hybridized with chloroplast DNA probe P-2. Sample 1 represents pattern A; 2, 3, and 4 pattern B; 5 pattern D; 6 pattern E. Numbers to the left indicate molecular weight in kilobases (kb) for fragments of 'Chilliwick'.

Table 2. Banding patterns for raspberry cultivars digested with *Bam* HI and *Eco* RI and hybridized with chloroplast clones P-2 and P-4.

Cultivar	Branding patterns			
	P-2		P-4	
	<i>Bam</i> HI	<i>Eco</i> RI	<i>Bam</i> HI	<i>Eco</i> RI
<i>Group 1</i>				
Anelma	A ^y	B	A	A
Glen Moy	A	B	A	A
Indian Summer	A	B	A	A
Malling Landmark	A	B	A	A
Norna	A	B	A	A
Willamette	A	B	A	A
<i>Group 2</i>				
Chilcotin	A	A	A	A
Chilliwack* ^z	A	A	A	A
Southland	A	A	A	A
Viking	A	A	A	A
<i>Group 3</i>				
Lloyd George*	A	B	A	A
Tahoma	A	B	A	A
Trailblazer	A	B	A	A
Comox*	A	B	A	A
<i>Group 4</i>				
Dalhousie Lake 4*	A	B	B	B
<i>Group 5</i>				
Munger	B	C	C	C
<i>Group 6</i>				
Bandywine*	C	D	D	D
Success	C	D	D	D
Glen Prosen	C	D	D	D
<i>Group 7</i>				
Dormanred*	A	E	E	E
Mandarin	A	E	E	E

^yRestriction fragment patterns for cultivars indicated by (*) are shown in Figs. 1, 2, 3, and 4.

^zCultivars 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	Group number ^z					
	2	3	4	5	6	7
1	0.944	0.919	0.872	0.526	0.579	0.595
2		0.865	0.821	0.526	0.579	0.595
3			0.800	0.462	0.513	0.632
4				0.488	0.537	0.600
5					0.750	0.564
6						0.615

^zCultivar composing groups are listed in Table 2.

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

Fig. 3. Autoradiogram of DNA digested with *Bam* HI from 1) 'Chilliwick', 2) 'Comox', 3) 'Lloyd George', 4) Dalhousie Lake 4, 5) 'Brandywine', and 6) 'Dormanred' hybridized with chloroplast DNA probe P-4. Sample 1 represents pattern A; 2 and 3 pattern B; 4 pattern C; 5 pattern D; 6 pattern E. Numbers to the left indicate molecular weight in kilobases (kb) for fragments of 'Chilliwick'.

'Preussen' × 'Lloyd George' and 'Malling Landmark' is from 'Preussen' × 'Baumforth A'. 'Norna' and 'Malling Landmark' have the same restriction patterns as 'Chilliwick' for *Bam* 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

Fig. 4. Autoradiogram of DNA digested with *Eco* RI from 1) 'Chilliwick', 2) 'Comox', 3) 'Lloyd George', 4) Dalhousie Lake 4, 5) 'Brandywine', and 6) 'Dormanred' hybridized with chloroplast DNA probe P-4. Samples 1, 2, 3, represent pattern A; 4 pattern B; 5 pattern D; 6 pattern E. Numbers to the left indicate molecular weight in kilobases (kb) for fragments of 'Chilliwick'.

variation patterns generally supported maternal inheritance of chloroplast DNA, but this assumption needs to be rigorously tested (Smith, 1989).

Literature Cited

- Chomczynski, P. and P.K. Qasaba. 1984. Alkaline transfer of DNA to plastic membrane. *Biochem. Biophys. Res. Commun.* 122:340-344.
- Cousineau, J.C. and D.J. Donnelly. 1992. Use of isoenzyme analysis to characterize raspberry cultivars and detect cultivar mislabeling. *HortScience* 27:1023-1025.
- Dale, A., P.P. Moore, R.J. McNicol, T.M. Sjulín, and L.A. Burmistrov. 1993. Genetic diversity of red raspberry cultivars throughout the world. *J. Amer. Soc. Hort. Sci.* 118: 119-129.
- Darrow, G.M. 1920. Are our raspberries derived from American or European Species? *J. Hered.* 11:179-184.
- Daubeney, H.A. and D. Sary. 1982. Identification of resistance to *Amphorophora agathonica* in the native North American red raspberry. *J. Amer. Soc. Hort. Sci.* 107:593-597.
- Feinberg, A.P. and B. Vogelstein. 1983. A Technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6-13.
- Grubb, N.H. 1922. Commercial raspberries and their classification. *J. Pomol.* 3:11-35.
- Hedrick, U.P. 1925. The small fruits of New York. New York State Agr. Expt. Sta. Rpt. 1925, Pt. II.
- Jennings, D.L. 1988. Raspberries and Blackberries: Their breeding, diseases and growth Academic Press, London.
- Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular cloning: A

- laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.
- Nei, M and W.H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci.* 76:5269-5273.
- Nybom, H. and B.A. Schaal. 1990. DNA "fingerprints" reveal genotypic distributions in natural populations of blackberries and raspberries (*Rubus*, Rosaceae). *Amer. J. Bot.* 77:883-888.
- Nybom, H., B.A. Schaal, and S.H. Rogstad. 1989. DNA "fingerprints" can distinguish cultivars of blackberries and raspberries. *Acta Hort.* 262:305-310.
- Oydvyn, J. 1969. Repeatabilities for measures of cane production and scores of yield characters in the raspberry (*Rubus idaeus* L.). *Hort. Res.* 9:103-111.
- Phillips, A.L. 1985a. Restriction map and clone bank of tomato plastid DNA. *Current Genet.* 10:147-152.
- Phillips, A.L. 1985b. Localization of genes for chloroplast components in tomato plastid DNA. *Current Genet.* 10:153-161.
- Smith, S.E. 1989. Biparental inheritance of organelles and its implications in crop improvement, p. 361-393, In: J. Janick (ed.). *Plant Breeding Rev.* vol. 6, Timber Press, Portland, Ore.
- Waugh, R., W.T.G. van de Ven, M.S. Phillips, and W. Powell. 1990. Chloroplast DNA diversity in the genus *Rubus* (Rosaceae) revealed by southern hybridization. *Plant Systematics Evolution* 172:65-75.