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Genetic variation detected by use of the M13 "DNA fingerprint" probe in *Malus*, *Prunus*, and *Rubus* (Rosaceae)

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Summary. Recently, "DNA fingerprints" have been reported in a wide array of organisms. We used the M13 repeat probe on several genera and species in the angiosperm family Rosaceae. Four apple cultivars could be differentiated when any one of five restriction enzymes was used to analyze minisatellite DNA. Similarly, four individual trees of *Prunus serotina* (black cherry) exhibited different "fingerprints" with each of four enyzmes. A total of 14 *Rubus* (blackberries and raspberries) plants representing four species were investigated with two enzymes. Extensive inter- and intraspecific variation was found. However, some closely growing plants had identical "fingerprints", probably due to their being derived through vegetative propagation.

Key words: DNA fingerprint – Apple – Cherry – Blackberry – Raspberry

Introduction

The development of suitable genetic markers is essential for many areas of both applied and basic research. A recent major advance has been the discovery of minisatellite DNA sequences that are hypervariable in many species of animals and plants. Such sequences consist of a series of tandem repeats of a core consensus sequence. These sequences are often dispersed throughout the genome and thus represent many loci. At a given locus numerous alleles, differing in the number of core repeats, may occur. The minisatellite sequences thus yield a genetic "fingerprint" when hybridized with a radioactively labeled probe (Jeffreys et al. 1985a, b).

Several genera in the angiosperm family Rosaceae include economically important species. Here we demonstrate that the M13 repeat probe, shown previously to reveal "DNA fingerprints" in some angiosperm species (Rogstad et al. 1988; Ryskov et al. 1988), detects genetic variation in economically important cultivars and naturally occurring species of the family. One genus was examined from each of the three different subfamilies Pomoideae, Prunoideae, and Rosoideae, namely *Malus* (apple), *Prunus* (cherry), and *Rubus* (blackberry and raspberry), respectively.

Materials and methods

Malus

Four commonly grown apple cultivars of *Malus* × *domestica* Borkh. were chosen: Golden Delicious, Jonathan, Red Delicious, and Rome Beauty. Three trees were sampled from each of the cultivars growing in an orchard near Grafton/IL. Since apples grown for fruit production are always vegetatively propagated by budding or grafting, all trees of a cultivar have the same genotype, barring somatic mutations.

Prunus

Four naturally occurring trees of the wild species *Prunus serotina* Ehrh. (black cherry) were sampled in St. Louis County/MO. Two of the trees grew only a few meters apart, whereas the remaining occured several kilometers from one another and from the first ones mentioned.

Rubus

A total of 14 plants were sampled in Missouri, representing four species collected at three localities in south St. Louis County, Hawn State Park, ca. 110 km south of St. Louis, and Shaw Arboretum, ca. 70 km west of St. Louis (Table 1).

The DNA was extracted (Saghai-Maroof et al. 1984) and separate aliquots of 8–10 μg digested with a set of different

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Table 1. *Rubus* collections

Species	St. Louis County	Hawn State Park	Shaw Arboretum
R. allegheniensis Porter Highbush blackberry		2 stands; 2 ramets in each	1 stand; 2 ramets
R. flagellaris Willd. Dewberry	1 stand; 2 ramets		2 stands; 2 and 1 ramets, resp.
R. occidentalis L. Black raspberry		1 stand; 1 ramet	
R. pensilvanicus Poir. Highbush blackberry			1 stand; 2 ramets

Ramets within a stand were 0.5–5 m apart, whereas different stands were >100 m apart

enzymes: DraI, HaeIII, HinfI, RsaI, and TaqI. Digested DNA was electrophoresed in 20-cm long 0.8%–0.9% agarose gels, except in one case when a 23-cm gel was used. The gels were Southern blotted onto Micron Separations hybridization transfer nylon. For all hybridizations, a probe derived from the M13 bacteriophage, hexamer-labeled with ^{32}P-dCTP, was utilized (Rogstad et al. 1988). Filters were hybridized and washed according to Westneat et al. (1988), and autoradiographs were made at −80 °C for 5–10 days with intensifying screens.

In apples, intracultivar variation was studied using all three trees collected from each cultivar. These samples were digested with RsaI. Only one tree per cultivar was used in the other apple investigations. For *Prunus* and *Rubus*, all samples were used in each set of digestions.

Results and discussion

Malus

Intracultivar variation was determined by comparing samples from individual trees of each cultivar. Only Rome Beauty showed intracultivar variation (Fig. 1), with all three trees being dissimilar. Possible explanations for the Rome Beauty variation could be (1) that leaf material was accidentally collected from the root stock instead of from the scion variety (i.e., Rome Beauty), (2) that at least two of the sampled trees were, in fact, not Rome Beauty, or (3) that Rome Beauty has acquired some somatic mutations.

In the analyses of intercultivar variation, only one arbitrarily chosen sample of Rome Beauty was used. Similarly, one sample of each of the other cultivars was used. Comparisons among cultivars were conducted using five different endonucleases. For each enzyme used, a similarity index D was calculated, expressing the probability that a fragment in one cultivar is also found in another (Jeffreys et al. 1985b; Wetton et al. 1987), as well as the probability that two cultivars have indistinguishable fragment profiles (Table 2).

Table 2. Analysis of M13 probe hybridization with endonuclease fragments from Rosaceae taxa

Enzyme	No. of informative fragments	No. of fragments per genotype	Probability that fragment in A is also in B	Probability that A and B share all fragments
Apple cultivars (four genotypes)				
DraI	20	7.75 (0.50)	0.27 (0.25)	4.2×10^{-5}
HaeIII	7	3.50 (0.58)	0.37 (0.26)	0.031
HinfI	12	6.50 (1.29)	0.48 (0.20)	8.0×10^{-3}
RsaI	14	5.75 (1.50)	0.32 (0.35)	1.4×10^{-3}
TaqI	16	6.50 (1.73)	0.29 (0.34)	3.5×10^{-4}
Prunus serotina (four genotypes)				
DraI	17	7.50 (1.00)	0.31 (0.23)	1.6×10^{-4}
HaeIII	8	2.50 (1.29)	0.10 (0.16)	3.4×10^{-3}
HinfI	12	4.50 (1.29)	0.27 (0.20)	2.8×10^{-3}
TaqI	24	9.74 (0.96)	0.34 (0.17)	3.0×10^{-5}
Rubus (eight and seven genotypes, respectively)				
HaeIII	32	8.75 (3.11)	0.25 (0.15)	5.5×10^{-6}
HinfI	50	14.00 (4.24)	0.31 (0.11)	7.4×10^{-8}

No. of informative fragments for the genotypes investigated; average number of informative fragments per genotype; probability that fragment in genotype A is also in genotype B calculated as the average of all pairwise comparisons $D_{AB}=2\times$ no. shared fragments/(no. fragments$_A$ + no. fragments$_B$); and probability that A and B share all fragments (calculated as the average D_{AB} raised to the average number of fragments per genotype); standard deviations within parentheses

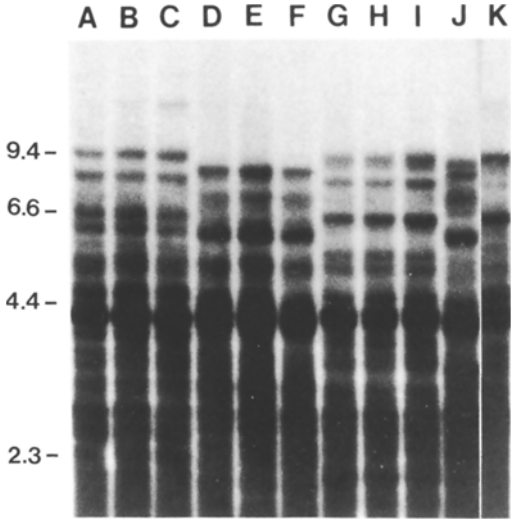

Fig. 1. Samples from two to three trees each of four apple cultivars digested with RsaI; *lanes A–C* – Golden Delicious; *lanes D–F* – Jonathan; *lanes G–I* – Red Delicious; *lanes J, K* – Rome Beauty (a third tree was investigated on another gel and found to be different from the present two). Several autoradiographs of varying intensity were used for evaluation of the fragment patterns. Size markers were derived from λDNA cut with HindIII

The origins of the cultivars investigated are not known and predate modern plant breeding technologies. However, these data suggest that Jonathan is relatively divergent from the other, since the individual similarity index values resulting from comparisons of this cultivar with any of the others are comparatively low (Table 3).

These results clearly show that apple cultivars are highly divergent in their minisatellite profiles. Genetic variation among cultivated apples has been documented also by using protein polymorphisms (Weeden and Lamb 1985; Bournival and Korban 1987). However, usually several enzymes must be examined in order to differentiate between cultivars. With the M13 minisatellite, probe cultivars are resolved with any one of the restriction enzymes used by a single analysis.

Prunus

All four *Prunus* trees could easily be distinguished with any one of the enzymes used (Table 2). The two trees growing close together did not appear to be more similar than those found in any of the other possible comparisons.

Rubus

Separate samples of DNA were digested with HaeIII and HinfI (Fig. 2). In all cases plants sampled within a stand, i.e., at the most a few meters apart, exhibited an identical fragment pattern. Vigorous vegetative propagation in the genus by means of root suckering or tip layering is common (Phelouzat 1982; Nybom 1987). Apomictic seed production (i.e., seeds produced without fertilization) may also be involved in the heptaploid *R. flagellaris* (Einset 1951), whereas *R. allegheniensis* and *R. occidentalis* are usually reported as being diploid and sexual (Longley 1924; Einset 1951). The reproductive mode of *R. pensilvanicus* has not been reported, but its pollen stainability data clearly suggest that it is apomictic (Nybom and Schaal unpublished results).

Comparisons were made only among different genotypes in the subsequent analyses. In one case, a genotype did not show a clear fragment pattern and was not included in the calculations. The endonuclease HinfI seems to reveal the most variation (Table 2). However, this analysis was done on a 23-cm gel, which gives a higher level of resolution.

Since high levels of variation between genotypes were obtained even when comparisons were made within a locality, analysis of minisatellite DNA may prove useful for delimiting clones, as also found in a more extensive *Rubus* investigation (Nybom and Schaal unpublished results).

If extensive detectable minisatellite variation exists among different genotypes in the facultatively apomictic blackberry species, as suggested here for *R. flagellaris*, minisatellite DNA might also be useful for distinguishing sexually derived offspring from apomictically derived offspring. Minisatellite analyses could, thus, be a powerful new tool for studies of the complicated mechanisms involved in pseudogamous, facultative apomixis as found in *Rubus* (Nybom 1988).

Here we have shown that the M13 repeat probe can reveal inter- and intraspecific genetic variation in taxonomically distant Rosaceae taxa and can document cultivar identity. Although the data presented here involve only a few species, the levels of variation detected are among the highest revealed for angiosperms with any known marker. Thus, these results suggest that minisatellite DNA analyses will be useful for genetic investigations

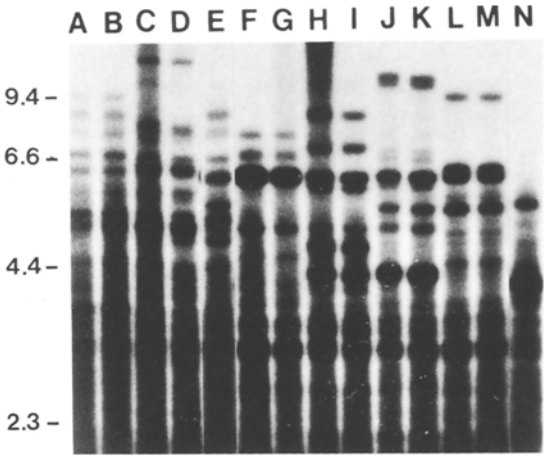

Fig. 2. Samples from 14 *Rubus* plants digested with HinfI; *lanes A, B – R. flagellaris,* Shaw Arboretum stand A; *lanes C, D – R. flagellaris,* St. Louis County; *lane E – R. flagellaris,* Shaw Arboretum stand B; *lanes F, G – R. pensilvanicus,* Shaw Arboretum; *lanes H, I – R. allegheniensis,* Hawn State Park stand A; *lanes J, K – R. allegheniensis,* Hawn State Park stand B; *lanes L, M – R. allegheniensis,* Shaw Arboretum; *lane N – R. occidentalis,* Hawn State Park. Several autoradiographs of varying intensity were used for evaluation of the fragment patterns. Size markers were derived from λDNA cut with HindIII

Table 3. Probability that a fragment in genotype A is also in genotype B calculated for all pairwise comparisons of the apple cultivars

	Golden Delicious	Jonathan	Red Delicious
Jonathan	0.19		
Red Delicious	0.48	0.04	
Rome Beauty	0.50	0.18	0.69

Probabilities given above are an average of the probability values resulting from digestion with DraI, HaeIII, HinfI, RsaI, and TaqI, respectively. For Rome Beauty, tree no. 3 was used in all digests except the one with DraI, where instead tree no. 2 was used

of numerous angiosperms, including cultivar or lineage typing, studies of variation (or lack of) arising from sexual or apomictic (e.g., clonal) means, detection of hybridization and outcrossing, and for providing new genetic markers for linkage analyses.

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