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INVESTIGATING THE IDENTITY OF ROSE VARIETIES UTILIZING RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS

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Abstract. **RAPD-PCR analysis was used to answer questions regarding the identity of numerous varieties of roses. It was previously reported that the DNA profile of "Bremo Double Musk" did not match any of the other musk (*Rosa moschata* Herrmann) varieties; however, upon further analysis, it was determined that "Bremo" is indeed a true musk. A parentage analysis of 'Xanadu', a recently registered modern rose, indicates that it probably resulted from a self-pollination of 'Carefree Beauty'. Numerous samples of "Found Noisettes" were analyzed, showing multiple genetic differences among the varieties, but similarities to their assumed ancestors, 'Blush Noisette' and 'Champneys' Pink Cluster'. Utilizing 'Katie Bell's Devonianthus', it was determined that roses grown today as "Tradd Street Yellow" and 'Devoniensis' are very likely the real, original, 'Devoniensis'. Finally, the question of the identity of 'Spray Cécile Brunner'/'Bloomfield Abundance' was investigated, indicating that the plant currently grown under both names is truly a sport of 'Cécile Brunner', and should be classified as 'Spray Cécile Brunner'. As shown here, RAPD-PCR can be a useful tool in determining the heritage of historic and modern roses.**

Anecdotal and historical accounts have led often to inconsistent conclusions with regard to the relatedness of various rose cultivars. Determining aspects such as ancestry in these situations has often proven difficult, if not impossible. Therefore, it is desirable to have a scientific process to examine biological features of these roses. Genetic analysis reduces ambiguities that can arise when examining morphological properties. Although many techniques exist to study DNA, the procedure known as RAPD-PCR allows for examination of whole genomes for comparison purposes. This procedure involves copying many different, and often polymorphic, regions of the DNA using single, short, nonspecific primers (Williams et al., 1990). If results from different varieties are identical or very similar, then these varieties must be genetically identical or closely related. Major differences in the DNA indicate varieties are not closely related. RAPD-PCR is extremely well suited to help determine direct inheritance patterns and has been useful in examining the genetic relatedness among specimens in several plant species, including roses (Frederick et al., 2002; Martin et al., 2001; Wagner et al., 2002; Walker and Werner, 1997), medicinal plants (Fu et al., 2003), grape rootstock cuttings (Wolf et al., 1998), rice (Raghunathachari et al., 2000), and classic wine grapes (Ye et al., 1998). This paper reports the investigation of five questions regarding the identity and lineage of several rose varieties through the use of RAPD-PCR. The findings of these investigations illustrate how useful this type of analysis can be in identifying related members of ornamental varieties, and how well it can be used to confirm or refute historical accounts.

Materials and Methods

Plant Material. Small, unopened leaves from most varieties of roses were obtained from plants grown on the campus of Florida Southern College (FSC) just prior to DNA isolation. The "Found Noisette" varieties were obtained from the Hampton Park Noisette Study Garden in Charleston, S.C. (generous gifts from R. Knopf and J. Breland; see Table 1) and cultivated on the campus of FSC. In instances where specific plants were not grown on the campus, samples were sent via overnight delivery without any special preparation and frozen at -25 °C upon receipt.

DNA Isolation. DNA was isolated from leaves as previously described (Frederick et al., 2002; Wagner et al., 2002). After

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Table 1. Groupings of the "Found Noisettes."

Group 1	Group 2	Group 3
"Mrs. Woods Lavender Pink"	"Narrow Water"	"Fellenberg"
"Chester Cemetery"	"Princess de Nassau"	"Placerville"
"Pleasant Hill Cemetery"	"Nastarana"	"Setzer"
"Wavy Leaf"	"Belle Vichysoise"	"Camelia"
"Manchester Guardian Angel"	"Mt. Vernon"	"Lingo Musk"
"Hickory Hill"	"Mary Washington"	"Alister Stella Gray"
"Natchitoches Noisette"	"Tutta's Pink"	"Jim's Fence Corner"
"Bougainville"		
"Fewell's"		
"Jeanne d'Arc"		

DNA was isolated, two phenol extractions (1:1 v/v) and one chloroform extraction (1:1 v/v) were done prior to the final resuspension in TE/RNase buffer.

PCR Conditions. RAPD-PCR was done as described (Frederick et al., 2002; Wagner et al., 2002) or with the aid of two commercially available kits (PCR Core System II from Promega Corporation, Madison, Wis., and PCR Master Kit from Roche Molecular Biochemicals, Indianapolis, Ind.) as per the manufacturers' suggestions. Five primers were used, corresponding to the primers previously available from Operon Technologies, Inc. (Frederick et al., 2002; Wagner et al., 2002). Sequences of the primers were as follows: OPA-05: 5' AGGGGTCTTG 3'; OPA-08: 5' GTGACGTAGG 3'; OPA-09: 5' GGGTAACGCC 3'; OPC-05: 5' GATGACCGCC 3'; and OPC-09: 5' CTCACCGTCC 3' (Qiagen, Inc., Valencia, Calif.). All cultivars were amplified with all of the above primers in separate reactions. Results were run on 1.5% agarose gels containing ethidium bromide, photographed and analyzed. Only representative samples, and not all results with all primers, are shown in this report. Analyses included a control sample of an unrelated species for comparison purposes (see figure legends).

Results and Discussion

"Bremo Double Musk." A previous study had shown that nine different accessions of the musk rose (*Rosa moschata*) tested via RAPD-PCR analysis showed similar, if not identical, DNA patterns, with the exception of "Bremo Double Musk" (Frederick et al., 2002; see also Fig. 1A). There were questions, however, as to the actual identity of this cultivar, as it did not have the same appearance or characteristics of the other musk roses. The donor suspected that a sample of the wrong plant had been sent. A true, verified sample of "Bremo Double Musk" was obtained from The Center for Historic Plants at Thomas Jefferson's Monticello estate and compared with other known musk varieties (Fig. 1A). The banding pat-

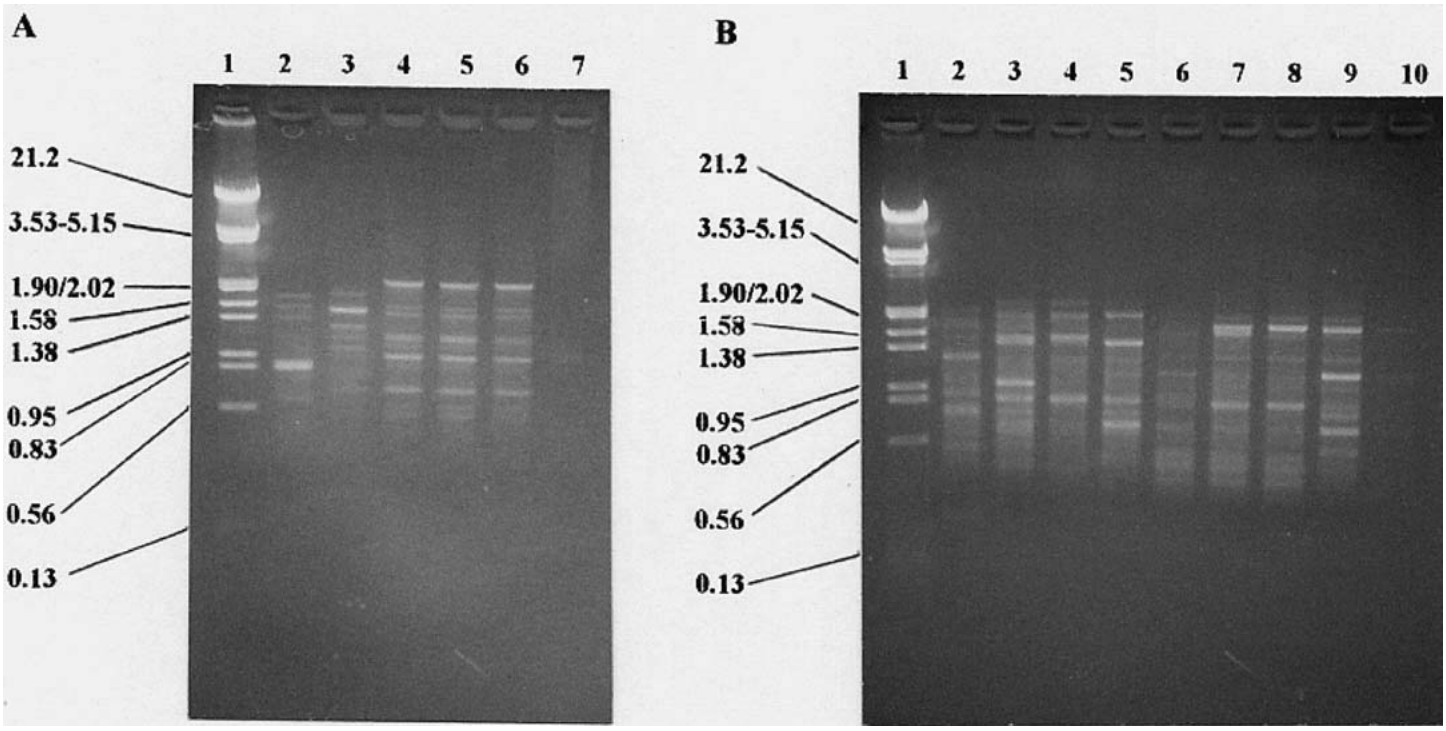

Fig. 1. RAPD analysis of "Bremo double musk" and 'Xanadu' roses. The DNA from all varieties was isolated and subjected to RAPD analysis as described in Materials and Methods. Photographs of the resulting gels are shown. Numbers to the sides of gels indicate size of lambda marker fragments in kilobase (kb) pairs. A: Analysis of *R. moschata* varieties using primer OPA-08. Lane 1: Lambda marker DNA cut with *HindIII* and *EcoRI*; lane 2: *Rosa laevigata* control; lane 3: suspect sample of "Bremo double musk" used in a previous study (Frederick et al., 2001); lane 4: verified sample of "Bremo;" lane 5: "Graham Thomas musk;" lane 6: "Temple musk." B: Parentage analysis of 'Xanadu' using primers OPA-05 (lanes 2-5) and OPC-05 (lanes 6-9). Lane 1: Lambda marker DNA cut with *HindIII* and *EcoRI*; lanes 2 and 6: "Mary Washington" control; lanes 3 and 7: 'Carefree Beauty'; lanes 4 and 8: 'Xanadu'; lanes 5 and 9: 'Fragrant Cloud'.

terns seen in the new sample were identical to other known musk varieties, indicating that "Bremo Double Musk," is, in fact, a true musk rose.

Parentage of a modern rose. RAPD-PCR analysis was also used to answer two questions concerning the identities of open-pollinated seedlings. The first of these questions centered on 'Xanadu', a newly registered seedling of 'Carefree Beauty' grown on the campus of FSC (M. Manners, personal communication). The suspected pollen parent is 'Fragrant Cloud', a rose grown in close proximity to 'Carefree Beauty'. DNA analysis of all three varieties was used to investigate parentage. If 'Xanadu' resulted from a cross-pollination of 'Carefree Beauty' with 'Fragrant Cloud', then half of the bands resulting from the 'Xanadu' RAPD-PCR analysis would match with 'Carefree Beauty', and the other half would match with 'Fragrant Cloud'. As seen in Fig. 1B, every band in 'Xanadu' was found in 'Carefree Beauty', and, while some bands did match up between 'Xanadu' and 'Fragrant Cloud', there were no bands that were unique to these two varieties and not to 'Carefree Beauty'. Therefore, it appears that 'Xanadu' is not a hybrid of 'Fragrant Cloud', but a result of a self-pollination of 'Carefree Beauty'.

Parentage of historic roses. The other investigation into open-pollinated seedlings centered on the varieties known as the "Found Noisettes." These are presumed to be descendents of 'Blush Noisette', itself a descendent of 'Champneys' Pink Cluster' (Hash, 2000). Most direct descendents of 'Blush Noisette' are known as the "Old Noisettes." Many roses that appear to be "Noisettes" have been found growing in back yards and old cemetery plots. Apparently they are descendents of the "Old Noisettes" that have been breeding in the wild for many generations. The Hampton Park Noisette Study Garden in Charleston, S.C., has an extensive collection of "Found Noisettes" (Knopf, 2002). Twenty-four of these varieties were analyzed via RAPD-PCR analysis. These roses have been divided into three main groupings (Table 1) based on their appearances and growth characteristics (R. Knopf, personal communication). As these roses have been growing wild for so many years and are presumably the result of open-pollinated seedlings, it was unlikely that RAPD-PCR would be able to determine exact inheritance patterns in these extended lineages. However, it was still of interest to see how closely related these varieties are. In all comparisons, the PCR analysis showed major differences among varieties (Fig. 2 and data not shown). However, similarities did exist among the varieties and with 'Blush Noisette' and 'Champneys' Pink Cluster'. The number of bands in common among varieties within the three groups was up to 77% and, when compared with 'Blush Noisette' and 'Champneys' Pink Cluster', the percentage of similar bands ranged between 30-62% (Table 3 and data not shown). These results confirm the notion that these varieties are related but only distantly so. Notably, some bands are present in the "Found Noisettes" but not in 'Blush Noisette' and 'Champneys' Pink Cluster' (for example the 0.5 and 0.9 kb polymorphic bands listed in Table 3), providing further evidence that the "Found Noisettes" have other rose genetics in their background and are not simply self pollinated descendants. Further refinement of relationships among the "Found Noisettes" will require more extensive analysis with genetic comparisons of suspected parents.

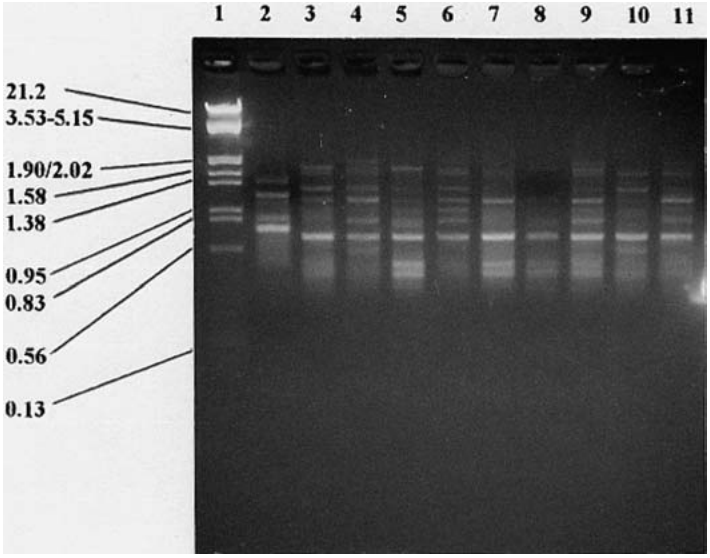

Fig. 2. RAPD-PCR analysis of the Group 3 "Found Noisettes." The DNA from all varieties was isolated and subjected to RAPD analysis using primer OPC-05 as described in Materials and Methods. A photograph of the resulting gel is shown. Lane 1: Lambda marker DNA cut with *HindIII* and *EcoRI*; lane 2: *Rosa laevigata* control; lane 3: 'Champneys' Pink Cluster'; lane 4: 'Blush Noisette'; lane 5: "Fellenberg;" lane 6: "Placerville;" lane 7: "Setzer;" lane 8: "Camelia;" lane 9: "Lingo Musk;" lane 10: "Alister Stella Gray;" lane 11: "Jim's Fence Corner." Numbers to the side of gel indicates size of lambda marker fragments in kilobase (kb) pairs.

'Devoniensis' candidates. 'Devoniensis' is a historic Tea rose variety introduced in its shrub form in 1838 by Foster and the nursery Prince & Company, and in the climbing form in 1858 by Pavitt and Curtis (Cairnes, 1993). Growers in England and California have what they believe to be the original 'Devoniensis', and sell it as such. The rose currently grown appears to

Table 2. Analysis of the Group 3 "Found Noisettes." The varieties tested were as follows: 1: "Fellenberg;" 2: "Placerville;" 3: "Setzer;" 4: "Camelia;" 5: "Lingo Musk;" 6: "Alister Stella Gray;" 7: "Jim's Fence Corner." Data shown are for results with the OPC-05 primer.

		Polymorphism present (+) or absent (-) in	
Polymorphism size (in kb)	Varieties showing the polymorphism	'Champneys' Pink Cluster'	'Blush Noisette'
0.3	1, 3, 4, 5, 6	+	-
0.4	1, 3, 4, 5	+	-
0.5	6	-	-
0.6	all	+	+
0.8	2, 5, 7	-	+
0.9	2	-	-
1.1	2, 3, 5, 7	+	+
1.3	2, 6	+	+
1.9	1, 2, 3, 5, 6, 7	+	+
2.0	5, 7	-	+

Table 3. Comparison of selected Group 3 roses. Rose varieties are as listed in Table 2. Data are shown for the results with the OPC-05 primer.

Rose varieties compared	Total number of bands in both varieties	Number of bands in common	Percentage in common
2, 6	8	3	37.5
5, 7	7	4	57.1
'Champneys' Pink Cluster' with all varieties	10	6	60.0
'Blush Noisette' with all varieties	10	6	60.0

match old paintings and descriptions, but there has been no solid historic link to prove it to be the "real" thing. Charles Walker (North Carolina State University, Raleigh) grew a rose that Katie Bell had gotten from her mother, who had always called it "Devonianthus," undoubtedly a mispronunciation of 'Devoniensis'. Since the original rose was introduced in 1838, the further back in time a person can be found growing the rose under its name, the stronger the evidence becomes to believe that the rose is correctly named. Katie Bell's family had been growing that rose prior to 1884 (C. Walker, personal communication). Another rose found in Charleston, SC, by Ruth Knopf, called "Tradd Street Yellow," appears to be identical to the California form of 'Devoniensis' obtained from Vintage Rose Gardens, Sebastopol, Calif. All three varieties were analyzed via RAPD-PCR. As shown in Fig. 3A, each band in "Katie Bell's Devonianthus" was found in both "Tradd Street Yellow" and 'Devoniensis'. This leads to the conclusion that all three are the same rose and supports the idea that the rose grown commercially is the real 'Devoniensis'.

'Spray Cécile Brunner'/'Bloomfield Abundance'. There has been a long-running debate between rose enthusiasts of Great Britain and of the United States as to the identity of 'Spray Cécile Brunner'. The predominantly American view has been that there are three forms of 'Cécile Brunner': 'Cécile Brunner', a small growing shrub in the Polyantha class cultivated since 1881 (Cairns, 1993); 'Spray Cécile Brunner', a much larger growing rose with huge sprays of flowers that repeats well; and 'Climbing Cécile Brunner', an even larger rose that usually is once-flowering in the spring. The British consider the 'Spray' form actually to be 'Bloomfield Abundance', a hybrid of 'Sylvia' and 'Dorothy Page-Roberts' made by Thomas in 1920 (Cairns, 1993) and classified as a Floribunda. If this view is correct then the DNA profiles of 'Bloomfield Abundance' should be dramatically different from the 'Cécile Brunner' varieties, as they would be totally different classes of roses (Floribunda versus Polyantha). The RAPD profiles of these varieties show tremendous similarities between the shrub and the climbing form (Fig. 3B). Examination of the profile of 'Spray Cécile Brunner'/'Bloomfield Abundance' shows an almost identical profile to the shrub variety (Fig. 3B). Therefore 'Spray Cécile Brunner' is most likely a sport of 'Cécile Brunner' and is probably unrelated to 'Sylvia' and 'Dorothy Page-Roberts'.

RAPD-PCR is a powerful technique that can help to deduce the genetic relatedness of many rose cultivars. In this study, the technique was successful in determining the parentage of 'Xanadu' and the identity of several rose cultivars, namely "Bremo Double Musk," "Katie Bell's Devonianthus," "Tradd Street Yellow," and 'Spray Cécile Brunner'. However, RAPD-PCR is not always useful in identifying the genetic differences among sports, particularly those that may be due to small mutations within the DNA. The employment of this

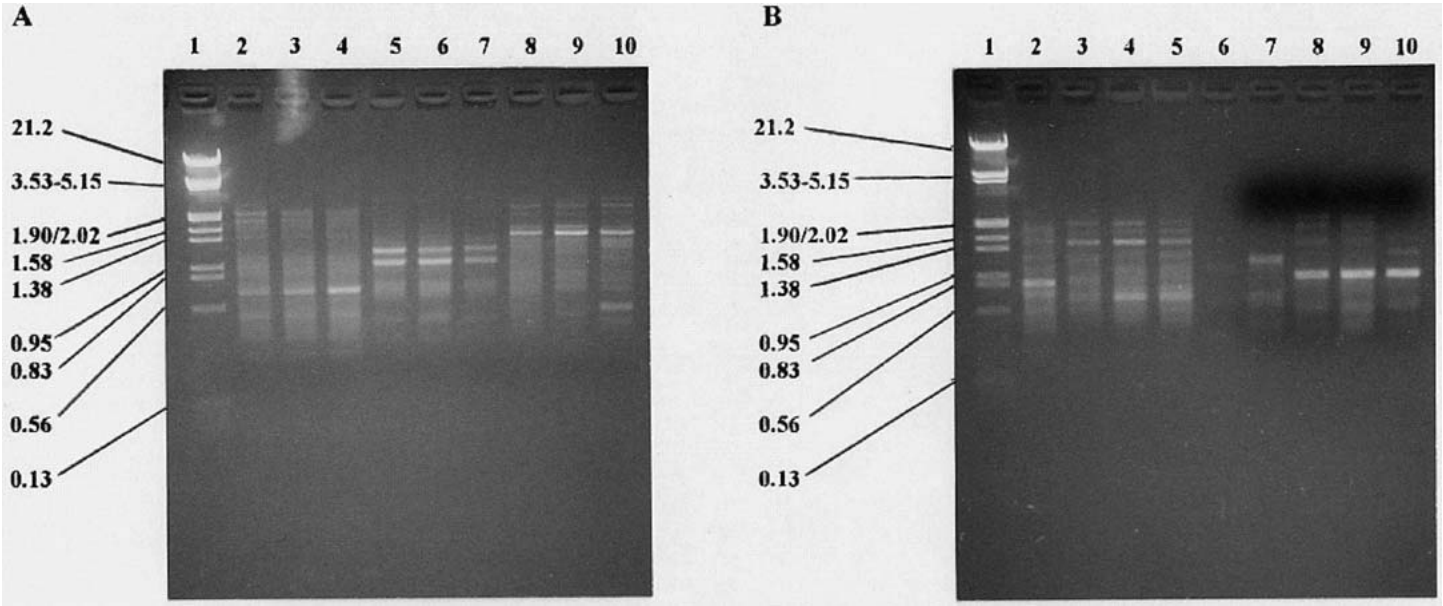

Fig. 3. Comparisons among 'Devoniensis' and 'Cécile Brunner' candidates. The DNA from all varieties was isolated and subjected to RAPD analysis as described in Materials and Methods. Photographs of the resulting gels are shown. Numbers to the sides of gels indicate size of lambda marker fragments in kilobase (kb) pairs. A. Analysis of 'Devoniensis' candidates using primers OPA-09 (lanes 2-4), OPC-09 (lanes 5-7) and OPA-08 (lanes 8-10). Lane 1: Lambda marker DNA cut with *HindIII* and *EcoRI*; lanes 2, 5 and 8: 'Devoniensis'; lanes 3, 6 and 9: "Tradd Street Yellow;" lanes 4, 7 and 10: "Katie Bell's Devonianthus." B. Analysis of 'Cécile Brunner' using primers OPA-05 (lanes 2-5) and OPA-09 (lanes 7-10). Lane 1: Lambda marker DNA cut with *HindIII* and *EcoRI*; lanes 2 and 7: *Rosa leavigata* control; lanes 3 and 8: 'Cécile Brunner'; lanes 4 and 9: 'Climbing Cécile Brunner'; lanes 5 and 10: 'Spray Cécile Brunner'/'Bloomfield Abundance'. Lane 6: no sample loaded.

technique can also allow for comparisons among varieties with a suspected common ancestor, such as the "Found Noisettes," and may aid rosarians in identification and classification. However, due to their long history of breeding in the wild, exact relationships require further genetic analysis to provide a more complete picture of the ancestries of these roses.

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