

INVESTIGATION OF THE ORIGIN OF 'CHAMPNEYS' PINK CLUSTER', 'BLUSH NOISETTE' AND 'NAPOLEON' ROSES USING RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS

AMANDA WAGNER, CARMELLA FREDERICK
AND NANCY MORVILLO¹
Florida Southern College
111 Lake Hollingsworth Dr.
Lakeland, FL 33801

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Abstract. Utilizing randomly amplified polymorphic DNA (RAPD) analysis, the parentage of accessions of 'Champneys' Pink Cluster' and 'Blush Noisette' from several nursery sources was investigated. Nearly all polymorphic DNA banding patterns in the profile of the accessions of 'Champneys' Pink Cluster' were accounted for in 'Old Blush' (*Rosa chinensis* Jacquin hybrid) and the musk varieties (*Rosa moschata* Herrmann), and many bands in the 'Blush Noisette' accessions were accounted for in 'Old Blush' and the musks. This confirms the parentage of these accessions. 'Old Blush' was also compared to 'Napoleon' via RAPD analysis to investigate the genetic similarities between them. The profiles showed almost identical banding patterns. This suggests the two species are closely related, consistent with reports that 'Napoleon' is a triploid of 'Old Blush'.

In cases where parentage of horticultural species has come into question, DNA analysis has been useful in conclusively determining the lineage of particular cultivars (Bowers and Meredith, 1997; Bowers et al., 1999; Iwata et al., 2000; Meredith et al., 1999). This technology has significant implications for the horticultural community, especially in the family Rosaceae. The historical record of various accessions of roses is, in some cases, very detailed while in others very sketchy. In the later cases, the only way to conclusively determine parentage is through DNA analysis. Randomly amplified polymorphic DNA (RAPD) analysis is a type of polymerase chain reaction (PCR). The amplification of random sequences of DNA can detect polymorphisms in genomic DNA that can then be used to compare samples. This type of analysis can be extremely beneficial in determining parentage with hybrids (Williams et al., 1990) and with cases of polyploidy.

Utilizing this technology, cultivars can be examined and compared with suspected parents. Since the 'Old Blush' (*Rosa chinensis* Jacquin hybrid) rose is an ancestor of nearly every modern rose (Hash, 2000), DNA analysis can be used to confirm its role in a variety of situations.

One example of where DNA analysis can be used is with the Noisette class of roses. The original 'Champneys' Pink Cluster' was reported to be a cross between the musk rose (*R. moschata*) and 'Old Blush' (Thomas, 1994) and is the first rose hybrid in the Western world to be every-blooming (Hash, 2000). The 'Blush Noisette' roses are said to be accessions of

a hybrid resulting from an open pollinated seeding of 'Champneys' Pink Cluster' (Thomas, 1994). All accessions of 'Champney's Pink Cluster' used in this study are nearly identical morphologically, as are the 'Blush Noisette' accessions, and several (Chamblee Rose, Vintage, Wayside, and ARE, Table 1) are from commercial sources. DNA analysis can confirm or refute the heredity of these hybrids, and help identify whether or not accessions are truly what they are claimed to be when sold by nurseries.

In addition to examining hybrids, RAPD analysis can be used to investigate the possibility that one cultivar is a polyploid form of another. 'Old Blush' is thought to have a triploid form, 'Napoleon,' which has been marketed as 'Old Blush' (M. Manners, Florida Southern College, Lakeland, FL, pers. comm.). 'Napoleon' is similar in many ways to 'Old Blush,' but has larger and thicker petals and leaves, is more vigorous, and produces no fruit. The infertility of 'Napoleon' as well as the other morphological characteristics lends support to the notion that 'Napoleon' is a triploid of 'Old Blush.' 'Old Blush' is known to be a diploid; unfortunately, to the best of our knowledge, a karyotype analysis has not been performed on 'Napoleon.' This would be the most direct way to answer the question of polyploidy.

In this study, RAPD analysis was used to examine the genetic profile of the roses in question and to determine their parentage.

Materials and Methods

Plant Material. Small, unopened leaves were collected from the "Elmwood Single" variety of the musk rose (*R. moschata* Herrmann), 'Napoleon' (*R. chinensis* hybrid), and 'Old Blush' (*Rosa chinensis* Jacquin hybrid), grown on the Florida Southern College Campus in Lakeland, Florida. Samples were used either fresh or stored at -25 °C. Accessions of 'Champney's Pink Cluster' and 'Blush Noisette' (see Table 1)

Table 1. Accessions of 'Champneys' Pink Cluster' and 'Blush Noisette' used in RAPD analysis. HPRG: Hampton Park Rose Garden, Charleston, SC; FSC: Florida Southern College, Lakeland, FL.

Accession	Source
'Champneys' Pink Cluster'	
Antique Rose Emporium	HPRG
Cato's Cluster	FSC
CHP	Bed #4, HPRG
Chamblee Rose	Bed #15, HPRG
Ruth	Bed #5, HPRG
Vintage	HPRG
Wayside	Bed #10, HPRG
'Blush Noisette'	
ARE	Bed #15, HPRG
R	Bed #11, HPRG
Rose Guardian	Bed #15, HPRG
VG	Bed #4, HPRG

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¹Corresponding author.

were graciously supplied by Ruth Knopf and JoAnn Breland (Hampton Park Rose Garden, Charleston, South Carolina) and stored at -25 °C until analyzed.

DNA Isolation. Isolation procedures were as described by Walker and Werner (1997) with modifications. Three leaves were quick frozen in dry ice/ethanol bath and then ground in a chilled mortar and pestle. Ground tissue was mixed with 750 µL of Lysis Buffer (100 mM sodium acetate, 50 mM Na₄EDTA, 500 mM NaCl, 2% PVP40, 1.4% SDS, pH 5.5, with 60 mM β-mercaptoethanol added immediately prior to use). Samples were incubated at 65 °C for 15 min and centrifuged for 10 min at 14,000 g_n. One-third volume of 3M potassium acetate was added to the supernatant and incubated on ice for 15 min. The sample was centrifuged for 10 min at 14,000 g_n. DNA was precipitated by the addition of 0.6 volume cold isopropanol to the supernatant. The sample was incubated at 4 °C for 30 min and then centrifuged for 20 min at 14,000 g_n. The pellet was washed in 70% ethanol and resuspended in 100 µL of TE/RNase buffer (10 mM Tris, 1 mM Na₄EDTA, pH 8.0, and 10 µg·mL⁻¹ RNase). Samples were run on a 0.7% agarose gel and concentrations were estimated by comparison with known DNA standards.

PCR Conditions. RAPD analysis was performed as described by Walker and Werner (1997). PCR reactions contained 1.0× Taq buffer (Roche Molecular Biochemicals, Indianapolis, IN), 2.5 mM MgCl₂, 200 mM each of dATP, dGTP, dCTP, dTTP, 20 µM of primer, 1 unit Taq polymerase (Roche Molecular Biochemicals, Indianapolis, IN), and 0.5 ng of genomic DNA, in a final volume of 50 µL. Primers (OPA-05, OPA-08, OPA-09, OPC-05, OPC-09) were obtained from Operon Technologies, Inc., Alameda, CA. The sample was overlaid with 50 µL of mineral oil. The thermal cycler (model PTC150, MJ Research Inc., Watertown, MA) was programmed as follows: 5 min at 94 °C, then 41 cycles of 1 min at 94 °C, 1 min at 35 °C, and 2 min at 72 °C, held at 4 °C. The PCR results were run on a 1.5% agarose gel, stained with 0.5-µg mL⁻¹ ethidium bromide, and viewed on an ultraviolet transilluminator. Gels were photographed and the resulting banding patterns were compared. Analysis was performed at least twice with each accession and each primer.

Results and Discussion

Hybrid study. Initially, the parentage of several accessions of 'Champneys' Pink Cluster' and 'Blush Noisette' was investigated (Table 1). These varieties are assumed to be hybrids of 'Old Blush' and the musk roses in the former case or, in the later case, 'Old Blush' and another variety. As the musk roses have the same RAPD profile (Frederick et al., 2002), "Elmwood Single" was chosen for analysis.

In determining the parentage of a hybrid, the RAPD profile of the offspring should show half of the DNA pattern inherited from one parent and half inherited from the other. Therefore, every band in the RAPD profile from the 'Champneys' Pink Cluster' accessions should match up with either 'Old Blush' or "Elmwood Single" if they are truly hybrids of these two. Five primers were used in the analysis and each showed similar results (Fig. 1A). Each band in the hybrids could be matched with either 'Old Blush' or "Elmwood Single," leading to the conclusion these are the parents of the accessions tested. In addition, the profiles of all the 'Champneys' Pink Cluster' accessions appear to be either identical or nearly so, indicating no major genomic changes have occurred

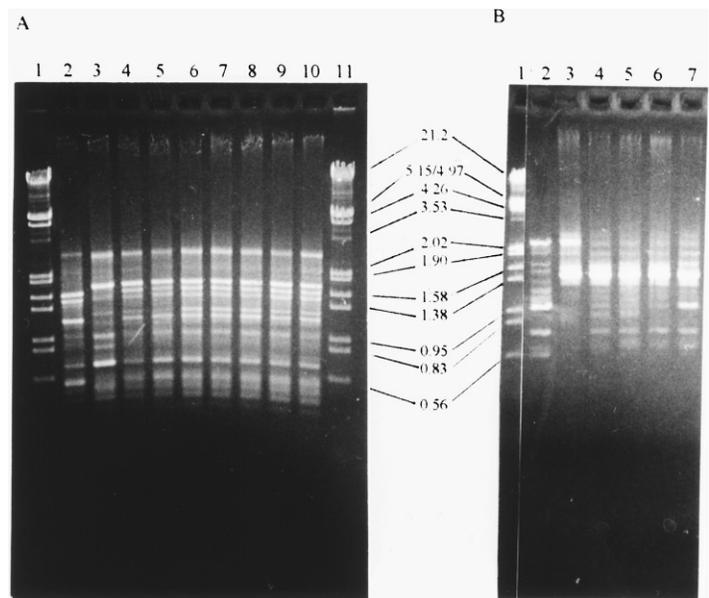


Fig. 1. RAPD analysis of accessions of 'Champneys' Pink Cluster' and 'Blush Noisette'. DNA from young leaves was extracted and subjected to RAPD analysis as described in Materials and Methods. Shown are photographs of the resulting gels. A: Accessions of 'Champneys' Pink Cluster' amplified with primer OPC-05. Lanes 1 and 11: Lambda marker DNA cut with *HindIII* and *EcoRI*; lane 2: "Elmwood Single"; lane 3: 'Old Blush'; lane 4: 'Chamblee Rose'; lane 5: 'Cato's Cluster'; lane 6: 'Wayside'; lane 7: 'Vintage'; lane 8: 'CHP'; lane 9: 'Ruth'; lane 10: 'Antique Rose Emporium'. B: Accessions of 'Blush Noisette' amplified with primer OPA-08. Lane 1: Lambda marker DNA cut with *HindIII* and *EcoRI*; lane 2: "Elmwood Single"; lane 3: 'Old Blush'; lane 4: 'ARE'; lane 5: 'Rose Guardian'; lane 6: 'VG'; lane 7: 'R'. Numbers to the sides of gels indicate size of marker fragments in kilobase (kb) pairs.

among these varieties. The DNA results therefore support the notion from the morphological similarities that all the accessions tested are indeed 'Champneys' Pink Cluster.' One exception was noted with 'Cato's Cluster.' With two of the primers used, a single additional unique band was detected in this accession (data not shown). This slight difference in the profile may explain the slight phenotypic differences seen in 'Cato's Cluster' compared with the other accessions.

In the analysis of the 'Blush Noisette' roses, the RAPD profiles were nearly identical indicating all accessions are truly Blush Noisettes (Fig. 1B). Many bands in the accession profiles matched bands in the 'Old Blush' and musk profiles, indicating the accessions are probably derived from a hybrid of the two (i.e., 'Champneys' Pink Cluster').

Polyploidy study. In cases where one rose variety is a polyploid of another, the RAPD profile of each plant should look identical, as only one genome is present. This is in contrast to a combination of two genomes as in hybridization. Therefore, if 'Napoleon' is truly a polyploid of 'Old Blush,' the two profiles should appear identical in all cases. The RAPD profiles of four primers support this, and the fifth primer (OPA-09) shows extremely similar profiles (Fig. 2). These results support the notion of 'Napoleon' being a triploid of 'Old Blush.' The significance of the variations seen with the OPA-09 primer is not known. These variations may have arisen via mutation after the polyploidy event.

In conclusion, DNA analysis, particularly RAPD analysis, is a powerful tool that can be used to help determine the lineage of particular plants. Work is continuing in our lab to investigate other questions of parentage in the rose family.

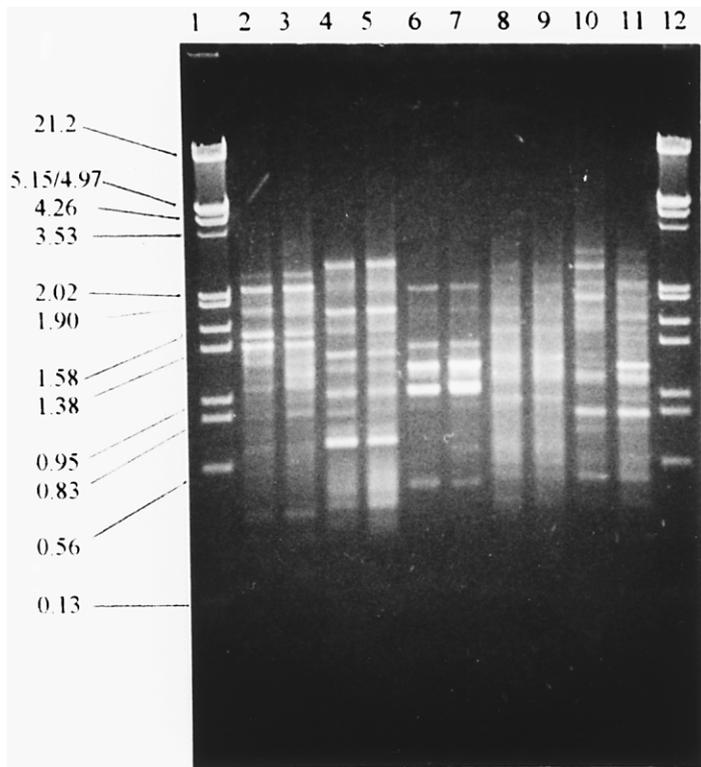


Fig. 2. RAPD profiles of 'Old Blush' and 'Napoleon' using five different primers. Isolation of DNA and amplification via RAPD were as described in Materials and Methods. A photograph of the resulting gel is shown. Lanes 1 and 12: Lambda marker DNA cut with *HindIII* and *EcoRI*; lanes 2, 4, 6, 8, and 10 contain 'Old Blush' DNA whereas lanes 3, 5, 7, 9, and 11 contain 'Napoleon' DNA. Primers were as follows: lanes 2 and 3: OPA-08; lanes 4 and 5: OPC-05; lanes 6 and 7: OPC-09; lanes 8 and 9: OPA-05; lanes 10 and 11: OPA-09. Numbers to the left side of the gel indicate size of marker fragments in kilobase (kb) pairs.

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Literature Cited

- Bowers, J. E. and C. P. Meredith. 1997. The parentage of a classic wine grape, Cabernet Sauvignon. *Nature Gen.* 16:84-87.
- Bowers, J., J.-M. Boursiquot, P. This, K. Chu, H. Johansson, and C. Meredith. 1999. Historical genetics: the parentage of Chardonnay, Gamay and other wine grapes of northeastern France. *Science* 285:1562-1565.
- Frederick, C., A. Wagner, and N. Morvillo. 2002. Randomly amplified polymorphic DNA (RAPD) analysis of the musk roses (*Rosa moschata*). *Proc. Fla. State Hort. Soc.* 115: In press.
- Hash, C. P. 2000. Champneys' triumph: South Carolina's forgotten rose. *Carologue* 16:8-16.
- Iwata, H., T. Kato, and S. Ohno. 2000. Triparental origin of Damask roses. *Gene* 259:53-59.
- Meredith, C. P., J. E. Bowers, S. Riaz, V. Handley, E. B. Bandman, and G. S. Dangl. 1999. The identity and parentage of the variety known in California as Petite Sirah. *Am. J. Enol. Vitic.* 50:236-242.
- Thomas, G. S. 1994. *The Graham Stuart Thomas Rose Book*. Sagapress/Timber Press, Inc., Sagaponack, NY.
- Walker, C. A. and D. J. Werner. 1997. Isozyme and randomly amplified polymorphic DNA (RAPD) analyses of Cherokee Rose and its putative hybrids 'Silver Moon' and 'Anemone.' *J. Amer. Soc. Hort. Sci.* 122:659-664.
- Williams, J. G., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* 18:6531-6535.