USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISMS (AFLP) TO IDENTIFY BLACK COHOSH (*ACTAEA RACEMOSA*)[1]

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Zerega, Nyree J. C., Scott Mori *(New York Botanical Garden, Bronx, NY 10458, USA),* **Charlotte Lindqvist** *(Norwegian Forest Research Institute N-1432 As, Norway),* **Qunyi Zheng** *(Pure World Botanicals, Inc., South Hackensack, NJ, USA), and* **Timothy J. Motley** *(New York Botanical Garden, Bronx, NY 10458, USA).* USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISMS (AFLP) TO IDENTIFY BLACK COHOSH (*ACTAEA RACEMOSA*). *Economic Botany 56(2):154–164, 2002. The rhizome of* Actaea racemosa L., *commonly called black cohosh, is a popular botanical dietary supplement used to treat female health concerns. The rhizomes used in black cohosh products are often collected from the wild. To ensure quality control, it is imperative that plants be correctly identified. This paper examines the use of the DNA fingerprinting technique, AFLP, as an analytical means of identifying* A. racemosa *from three other closely related sympatric species. To this end, 262 AFLP markers were generated, and one unique fingerprint was identified for* A. racemosa, *whereas two, six, and eight unique fingerprints were identified for the closely related species* A. pachypoda, A. cordifolia, *and* A. podocarpa, *respectively. Two commercial black cohosh products were also subjected to AFLP analysis and shown to contain only* A. racemosa. *The results of this study suggest that AFLP analysis may offer a useful method for quality control in the botanical dietary supplements industry.*

DIE VERWENDUNG VON AFLP-MUSTERN ZUR IDENTIFIKATION VON BLACK COHOSH (*ACTAEA RACEMOSA*). *Das Rhizom von* Actaea racemosa L., *allgemein als 'black cohosh' bezeichnet, ist eine beliebte pflanzliche Diätsergänzung, die für weibliche Gesundheitsprobleme benützt wird. Oft sind die in 'black cohosh'-Produkten verwendeten Rhizome in freier Natur gesammelt. Um Qualitätskontrolle zu sichern, ist es zwingend, die Pflanzen richtig zu identifizieren. Diese Studie überprüft den Gebrauch der DNA-Fingerabdrucktechnik, AFLP, als analytisches Mittel der Identifizierung, um* A. racemosa *von drei anderen in ihrer Nähe beheimateten und nah verwandten Spezies zuunterscheiden. Zu diesem Zweck wurden 262 AFLP-Fingerabdrücke erzeugt. Für* A. racemosa *wurde ein einzigartiger Fingerabdruck identifiziert, während für die nah verwandten Spezies* A. pachypoda *zwei,* A. cordifolia *sechs, und* A. podocarpa *acht einzigartige Fingerabdrücke gefunden wurden. Zwei kommerzielle 'black cohosh'-Produkte wurden ebenfalls der AFLP-Analyse unterzogen, wobei nur A. racemosa nachgewiesen werden konnte. Die Resultate dieser Studie zeigen, daß die AFLP-Technik eine nützliche Methode für die Qualitätskontrolle in der pflanzlichen Diätsergänzungsindustrie bieten kann.*

Key Words: AFLP, black cohosh, *Actaea racemosa*; DNA fingerprinting; botanical dietary supplements; Ranunculaceae; *Cimicifuga racemosa.*

Black cohosh, *Actaea racemosa* L. (= *Cimicifuga racemosa* L. (Nutt.); Ranunculaceae) is a North American herb that also has been known by several other common names including black snakeroot, squaw root, bugbane, and rattleroot (Foster 1999). Linnaeus typified black cohosh as *Actaea racemosa* in *Species Plantarum* (Linnaeus 1753). Later, this species was transferred to *Cimicifuga* (Nuttall 1818). Until recently, the genera *Actaea* and *Cimicifuga* were separated based on fruit type (follicles in *Cimicifuga* and berries in *Actaea*). However, Compton, Culham, and Jury (1998) subsumed *Cimicifuga* within *Actaea* based on several lines of morphological and molecular evidence. Their classification is adopted in this paper.

For centuries, people have been using the rhizome of *Actaea racemosa* to treat a broad range of ailments. For example, among the Iroquois, Cherokee, and Algonquians, black cohosh was used to treat rheumatism, colds, consumption,

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constipation, fatigue, hives, backache, and kidney trouble (Foster 1999; Hamel and Chiltoskey 1975; Mooney and Olbrechts 1932; Speck 1917). Black cohosh continued to be an important herbal remedy in North America throughout the 1800s when its medicinal use was expanded. It was reported in 1836 that decoctions of the rhizome were used to treat small pox (Howard 1836), and in the mid-1800s medical writings began to discuss the use of black cohosh for female health problems (Porcher 1849). However, by the end of the 19th century the majority of U.S. physicians showed little interest in this plant as a medicinal. At this time, black cohosh was introduced into Germany and by the 1930s became an accepted curative agent, the efficacy of which was supported by pharmacological and clinical research (Foster 1999). Renewed interest in black cohosh in the United States as a therapeutic preparation has been stimulated due to its success in Germany and because of a general resurgence in the use of dietary supplements in the USA.

Today black cohosh is still touted as a treatment for a wide range of health concerns, but its primary use concentrates on the treatment of menopausal and menstrual problems, and it is used as an alternative to hormone replacement therapies (Foster 1999). Under U.S. law black cohosh is considered a dietary supplement. The laws regulating dietary supplements are less stringent than those regulating drugs.

Several companies manufacture black cohosh supplements in the form of teas, capsules, extracts, and pills. These companies often purchase the rhizomes for their products from collectors. If the plant source is misidentified, it will be difficult if not impossible to remedy this mistake by visually examining the rhizomes. Therefore, additional measures are necessary to assure correct identification. These could include voucher specimens as well as analytical tests.

There currently is an analytical method for identifying black cohosh rhizomes. He et al. (2000) used reverse-phase liquid chromatography with positive atmospheric pressure chemical ionization mass spectrometry to detect different triterpene glycosides produced by *Actaea racemosa* and *A. cimicifuga* L. (= *Cimicifuga foetida* L.), a related Asian species that is sometimes found in black cohosh products on the market. The compound cimicifugoside M is produced only by *A. racemosa,* whereas cimicifugin is only found in *A. cimicifuga.* This is an important technique that has applications in the quality control of commercial black cohosh products. However, *A. racemosa* is usually collected from wild plants in eastern North America where its range overlaps with the closely related *Actaea cordifolia* DC. (= *Cimicifuga rubifolia* Kearney), *A. podocarpa* DC. (= *Cimicifuga americana* Michx.), *A. pachypoda* Elliott, and *A. rubra* (Aiton) Willd. *Actaea pachypoda* and *A. rubra* are genetically and morphologically similar differing only in their pedicel thickness after anthesis, and sometimes in the color of their berries (Compton, Culham, and Jury 1998). No analytical method has been developed for identifying *Actaea racemosa* from these closely related species, which mistakenly could be included in or substituted for black cohosh products on the market.

DNA fingerprinting techniques often are used to detect genetic differences among closely related plant species or among different populations or varieties of a species (Cervera et al. 1998; Hill et al. 1996; Loh et al. 1999; Milbourne et al. 1997; Paran, Aftergoot, and Shifriss, 1998; Yamamoto et al. 1998). Once a DNA fingerprinting profile for a given species, population, or variety has been established it can then be applied to help identify unknown samples. Using a highly reproducible (Jones et al. 1997) DNA fingerprinting technique called Amplified Fragment Length Polymorphism (AFLP) (Vos et al. 1995), this study analyzed black cohosh and the sympatric eastern North American *Actaea cordifolia, A. pachypoda,* and *A. podocarpa.* The purpose of the study was to (1) create AFLP fingerprinting profiles that can be used to identify the four sympatric species of *Actaea,* (2) apply these fingerprinting profiles to test commercial black cohosh products for the presence of *Actaea racemosa* and the absence of *A. pachypoda, A. cordifolia,* and *A. podocarpa,* and (3) determine if different populations of *Actaea racemosa* can be uniquely identified using AFLP analysis to monitor the populations from which black cohosh is being harvested and to help control overcollecting and collecting from protected sites.

Materials and Methods

Plant Material

DNA was extracted from leaves of 37 *Actaea racemosa* individuals representing 11 populations (Fig. 1), from eight *A. cordifolia,* 10 *A. podocarpa,* and five *A. pachypoda* individuals,

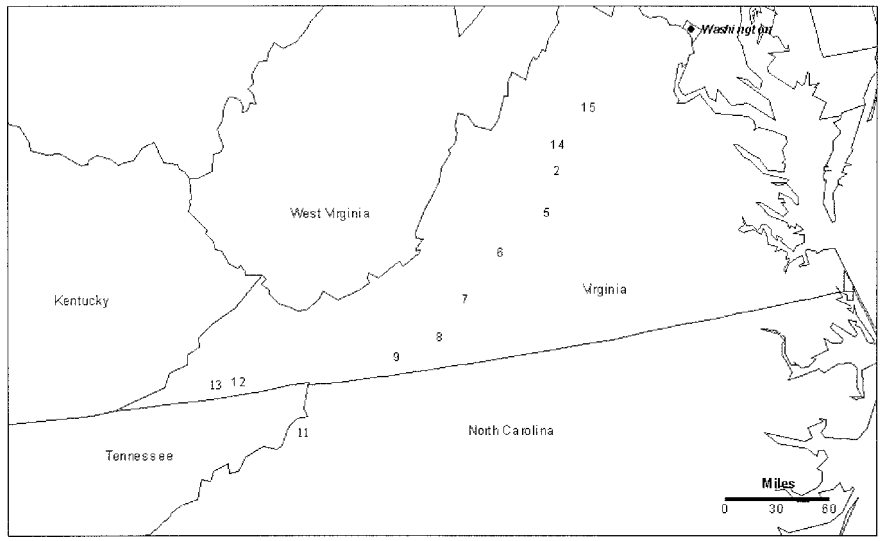

Fig. 1. Locations of the various *Actaea racemosa* populations used in this study are indicated.

and from two outgroup taxa in the Ranunculaceae family, *Aconitum napellus* L. and *Pulsatilla vulgaris* Mill. DNA was also extracted from one *A. racemosa* rhizome collected in New York. The rhizome was air dried and the leaves were dried in silica gel before storing at −80°C. Sample number per *A. racemosa* population varied from two to 10. Vouchers were made for each population. These along with DNA and silica gel-dried leaves of all samples are deposited at the New York Botanical Garden (Table 1). In addition, commercially sold black cohosh dried rhizome pieces, and dietary supplements (Seelect® tea bags, GNC® capsules, and Nature's Way® tablets) were purchased for analysis.

DNA Isolation

DNA was isolated from ca. 1.0 cm^2 of tissue from fresh leaves dried in silica gel and from ca. 40 mg of material from the rhizomes and dietary supplements. DNA extraction was performed as described by Struwe et al. (1998) with minor modifications. Briefly, plant material was ground in Bio 101 Lysing Matrix tubes using a Bio 101 Fastprep machine. Then 500 µl lysis buffer (2 g CTAB; 8.18 g NaCL; 0.745 g EDTA; 10 ml 1 M Tris/HCL, pH 9.5; 1 g PEG 4000, water to 100 ml) and 75 µl β-mercaptoethanol was added to each tube. Samples were incubated at 74°C for 90–120 min. Then 575 µl of chloroform/isoamyl alcohol (24:1) was added to each sample and tubes were mixed for 30–60 min. Cell debris was spun down and 350 µl of supernatant was transferred to tubes containing 20 µl glassmilk (Bio 101) and 1050 µl NaI solution (Bio 101). Tubes were shaken for 60 min and spun down. Supernatant was discarded and the glassmilk pellet was washed three times with 800 µl and once with 150 µl of New Wash solution (Bio 101). The pellets were then resuspended in 20 µl of 10 mM Tris, incubated for 10 min at 45–55°C, centrifuged for 60 sec and the supernatants (DNA containing solution) were transferred to new tubes for storage at −20°C.

AFLP Analysis

AFLP analysis was conducted according to the Applied Biosystems, Inc. plant mapping protocol with some minor modifications. Genomic DNA (0.3 µg) was digested by two restriction enzymes (*Eco*RI/*Mse*I) and simultaneously ligated with *Eco*RI and *Mse*I adapter sequences in a 6.6 µl reaction volume at 37°C for 2 h. PCR reactions were performed after diluting the ligated DNA 17-fold with TE buffer (20 mM Tris-HCL, 0.1 mM EDTA, pH 8.0). Fragments were pre-amplified by 20 PCR cycles (94°C for 1 sec, 56°C or 30 sec, 72°C for 2 min) using *Eco*RI and *Mse*I primers with one selective nucleotide. PCR products were diluted 19-fold and used as templates for the selective amplification using two primers, *Mse*I + three selective nucleotides and fluorescently-labelled *Eco*RI + three selective nucleotides. Selective amplification AFLP

reactions were performed using 94°C for 2 min in the first cycle and for 1 sec in subsequent cycles, 65°C for 30 sec, and 72°C for 2 min, followed by reduction of the annealing temperature at each cycle by 1°C for 9 cycles. The annealing temperature was then maintained at 56°C for the remaining 23 cycles. Selective amplification reactions for 20 *A. racemosa,* and for all *A. cordifolia, A. pachypoda, A. podocarpa,* and outgroup taxa were performed with four primer combinations. Twenty additional samples of *A. racemosa* were selectively amplified with one primer combination (Table 1). The AFLP fragments were separated and visualized using a 5% Long Ranger (FMC Bioproducts) gel on an ABI 377 sequencer. Gel analysis was carried out with Genescan 3.1 and Genotyper 2.1 software packages (Applied Biosystems, Inc.).

Data Analysis

AFLP products were scored as the presence (1) or absence (0) of bands and a binary matrix was constructed. Only AFLP fragments that could be scored unambiguously were included in the analysis. All characters were coded as unordered with equal weight and were analyzed using maximum parsimony criterion (Fitch 1971) with PAUP 4.0 software (Swofford 1998). Heuristic searches were performed using the following settings: sequence addition, simple; starting trees for branch swapping computed by stepwise addition; and swapping algorithm, tree bisection reconnection (TBR). When multiple parsimonious trees were found, a strict consensus tree was computed. Internal branch support was examined by bootstrapping (1000 replicates, full heuristic search, retaining groups with frequency greater than 50%) (Felsenstein 1985).

Results

Four AFLP primer combinations produce a total of 262 unambiguous DNA fragments for the species *Actaea cordifolia, A. pachypoda, A. podocarpa,* and *A. racemosa.* The fragments range in size from 30 to 500 base pairs long. Of these, 258 are polymorphic and only four are monomorphic. No single primer combination produces unique DNA fingerprints that can be used to identify each of the four *Actaea* species. However, combining the results of just two primer combinations is sufficient to uniquely identify each species (Table 2). Primer combination *Eco*RI + ACA/*Mse*I + CTT amplifies five unique fragments for *A. cordifolia,* three for *A. podocarpa,* one for *A. racemosa,* and one shared by *A. cordifolia, A. racemosa,* and *A. pachypoda* (a sample electropherogram indicating some of these unique markers is shown in Fig. 2). Primers *Eco*RI + ACA/*Mse*I + CTG amplify three unique fragments for *A. podocarpa* and one for *A. pachypoda.* Primers *Eco*RI + AAG/*Mse*I + CTC amplify one unique fragment for *A. pachypoda.* Finally, primers *Eco*RI + ACA/*Mse*I + CAT amplify two unique fragments for *A. podocarpa,* two for *A. cordifolia,* and one fragment shared by *A. pachypoda* and *A. racemosa.* Each of the four *Actaea* species can be uniquely identified by combining the fingerprinting results from primer combinations *Eco*RI + ACA/*Mse*I + CTT and either *Eco*RI + AAG/*Mse*I + CTC, or *Eco*RI + ACA/*Mse*I + CTG.

DNA was successfully extracted from commercial black cohosh tea bags, capsules, and dried rhizome pieces, however, extraction was ineffective from black cohosh coated tablets. DNA from the tea bag was too degraded for successful AFLP analysis. However, AFLP analysis was performed successfully on DNA from the black cohosh capsules and commercially sold rhizome pieces. Importantly, the unique marker for *A. racemosa* produced by the primer combination *Eco*RI + ACA/*Mse*I + CTT is present in these commercial products, and markers for the other three species are absent.

Parsimony analysis conducted on four *Actaea* species and two outgroup taxa using data from four AFLP primer combinations found 420 most parsimonious trees with 929 steps, CI (consistency index) = 0.27, and RI (retention index) = 0.70. In a strict consensus of all most parsimonious trees each species forms a monophyletic clade, with *A. racemosa* most closely related to *A. pachypoda* (Fig. 3). These results correspond with previous findings (Compton, Culham, and Jury 1998) and demonstrate that AFLP analysis can be used to resolve the systematic relationships among these taxa.

Parsimony analysis of two populations of *A. racemosa* using AFLP data from four primer combinations found three most parsimonious trees with 270 steps, CI = 0.47, RI = 0.49, and a strict consensus tree was computed (Fig. 4). An additional parsimony analysis of 11 *A. racemosa* populations using data from one AFLP primer found 27 most parsimonious trees with 365 steps, CI = 0.22, and RI = 0.50, and a strict

TABLE 1. PLANT SPECIES, PROVENANCE, AND AFLP PRIMER COMBINATIONS USED IN THIS STUDY. N = NUMBER OF SAMPLES. L&A = LINDQVIST AND ALBERT. ALL VOUCHERS AND DNA ACCESSIONS ARE DEPOSITED AT THE NEW YORK BOTANICAL GARDEN.

Taxon	N	Provenance	Collector and vouchers (DNA accessions used in study)	*Eco*RI primer	*Mse*I primer
Aconitum napellus	1	NY Botanical Garden	Accession 94/2000	ACA	CAT
				ACA	CTT
				ACA	CTG
				AAG	CTC
Actaea cordifolia	8	Virginia	L&A population vouchers 489, 490 (489–493, 496–498)	ACA	CAT
				ACA	CTT
				ACA	CTG
				AAG	CTC
A. pachypoda	2	Virginia	L&A 394 (390, 394)	ACA	CAT
				ACA	CTT
				ACA	CTG
				AAG	CTC
A. pachypoda	3	New York	Mori 24875A, 24875B, 24875C	ACA	CAT
				ACA	CTT
				ACA	CTG
				AAG	CTC
A. podocarpa	10	Virginia	L&A population vouchers 395, 397 (395, 397, 399–406)	ACA	CAT
				ACA	CTT
				ACA	CTG
				AAG	CTC
A. racemosa	10	Virginia, Population 2	L&A population vouchers 366, 367 (358–367)	ACA	CAT
				ACA	CTT
				ACA	CTG
				AAG	CTC
A. racemosa	9	North Carolina, Population 11	L&A population voucher 457 (457–461, 463–466)	ACA	CAT
				ACA	CTT
				ACA	CTG
				AAG	CTC
A. racemosa (rhizome)	1	New York	Mori 25014	ACA	CAT
				ACA	CTT
				ACA	CTG
				AAG	CTC
A. racemosa (rhizome pieces)	1	From Aphrodisia, New York City		ACA	CAT
				ACA	CTT
				ACA	CTG
				AAG	CTC
A. racemosa (capsules)	1	GNC® brand black cohosh capsules		ACA	CAT
				ACA	CTT
				ACA	CTG
				AAG	CTC

TABLE 1. CONTINUED.

Taxon	N	Provenance	Collector and vouchers (DNA accessions used in study)	*Eco*RI primer	*Mse*I primer
Pulsatilla vulgaris	1	NY Botanical Garden	Zerega 204	ACA	CAT
				ACA	CTT
				ACA	CTG
				AAG	CTC
A. racemosa	2	Virginia, Population 5	L&A population vouchers 391 (392–393)	ACA	CTG
A. racemosa	2	Virginia, Population 6	L&A population vouchers 407, 408 (408–409)	ACA	CTG
A. racemosa	2	Virginia, Population 7	L&A population voucher 417 (418–419)	ACA	CTG
A. racemosa	2	Virginia, Population 8	L&A population voucher 427 (428–429)	ACA	CTG
A. racemosa	2	Virginia, Population 9	L&A population voucher 437 (438–439)	ACA	CTG
A. racemosa	2	Virginia, Population 12	L&A population vouchers 467, 468 (468–469)	ACA	CTG
A. racemosa	2	Virginia, Population 13	L&A population vouchers 477, 478, 488 (478–479)	ACA	CTG
A. racemosa	2	Virginia, Population 14	L&A population voucher 501 (502–503)	ACA	CTG
A. racemosa	2	Virginia, Population 15	L&A population voucher 504 (505–506)	ACA	CTG

consensus tree was computed (Fig. 5). Both *A. racemosa* analyses suggest that the separate populations may not be genetically isolated, as members of one population are not necessarily most closely related to other members of the same population.

DISCUSSION

A major goal of this study was to determine if the AFLP fingerprinting technique could be used to identify *A. racemosa* from three other closely related species found in the same geographic range. This proved to be possible and species-specific AFLP profiles were identified (Table 2). These profiles were then successfully applied to verify the presence of *A. racemosa* and the absence of *A. pachypoda*, *A. cordifolia*, and *A. podocarpa* in two commercial black cohosh dietary supplements. DNA from commercial black cohosh rhizome pieces and capsules contain the unique marker for *A. racemosa* and lack all of the species-specific markers for the three other sympatric *Actaea* species. However, we were unable to use the AFLP profiles for verification of the presence or absence of *Actaea* species in black cohosh tea bags and coated tablets. This is likely due to a combination of factors including rhizome drying and storage conditions, processing techniques, and length and quality of storage of the final commercial product. All of these factors could contribute to the degradation of DNA. The success of the AFLP analysis for two dietary supplements (capsules and dried rhizome pieces) suggests that this technique, in combination with other methods, could potentially be used in the quality control of black cohosh products. For this to be most successful, it would be important that rhizomes be dried quickly, stored at a cool temperature, and tested as soon as possible after collection, preferably before being processed into a commercial product. The more deviation from these conditions, the greater the chance that the DNA will become degraded and AFLP markers lost, making the technique less reliable.

In addition to the application of DNA fingerprinting for the identification of species, it has also been employed to study genetic differences among populations of the same species (Cardoso et al. 2000; Escaravage et al. 1998; Krauss 1999). If members of one *A. racemosa* population could be uniquely identified from another, this would have important applications in the

TABLE 2. AFLP FRAGMENTS INFORMATIVE FOR SPECIES DIAGNOSIS. THE NUMBERS REPRESENT BASE PAIR LENGTHS OF THE DIAGNOSTIC DNA FRAGMENTS. NUMBERS IN BOLD ARE UNIQUE TO ONE SPECIES.

Species	AFLP Primer pair combinations			
	E+ACA/M+CAT	E+ACA/M+CTT	E+ACA/M+CTG	E+AAG/M+CTC
A. cordifolia	**63, 144**	51, 58, **88, 94, 154, 223, 364**		
A. pachypoda	177	51, 58	**270**	**254**
A. podocarpa	**176, 320**	**303, 305, 336**	**78, 163, 253**	
A. racemosa	177	51, **52**, 58		

protection of populations from overcollecting and in helping to prevent collecting altogether from protected sites such as national parks. At the level of our sampling, 11 different populations of *A. racemosa* from North Carolina and Virginia do not appear to be genetically distinct as members of different populations, but rather they appear to represent a large interbreeding population (Fig. 4, 5). That is, individuals are not necessarily most closely related to other individuals of their same geographic population. Although members of the same population tend to group with one another in the parsimony analyses, this is not always the case. For example, there is 94% bootstrap support for the close relationship between an *A. racemosa* individual from Virginia (population two) and one from North Carolina (population 11) (Fig. 4). Also, the lack of resolution between populations (Fig. 5) suggests that there are no significant genetic differences between the individuals of the different populations. Additionally, no population

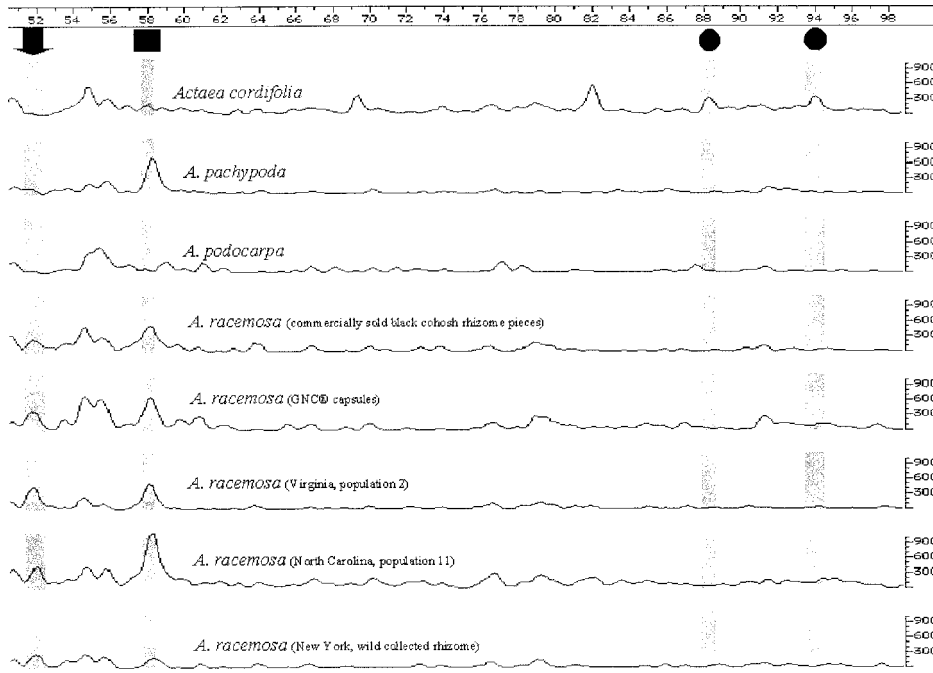

Fig. 2. A portion of the AFLP fingerprinting profiles derived from primer combination E-ACA/M-CTT for *Actaea racemosa, A. cordifolia, A. pachypoda,* and *A. podocarpa.* The numbers across the top indicate the size of the fragment in base pairs. The number scale on the side indicates the relative intensity of the fragment expressed in fluorescent units. Shaded bars indicate fragments of interest. The 52-base-pair fragment is unique to *A. racemosa* and is found in commercial black cohosh products as well as plants from three different populations (indicated by the arrow). The 58-base-pair fragment is shared by all species except *A. podocarpa* (indicated by the square), and the 88 and 94 base pair fragments identify *A. cordifolia* (indicated by the circles).

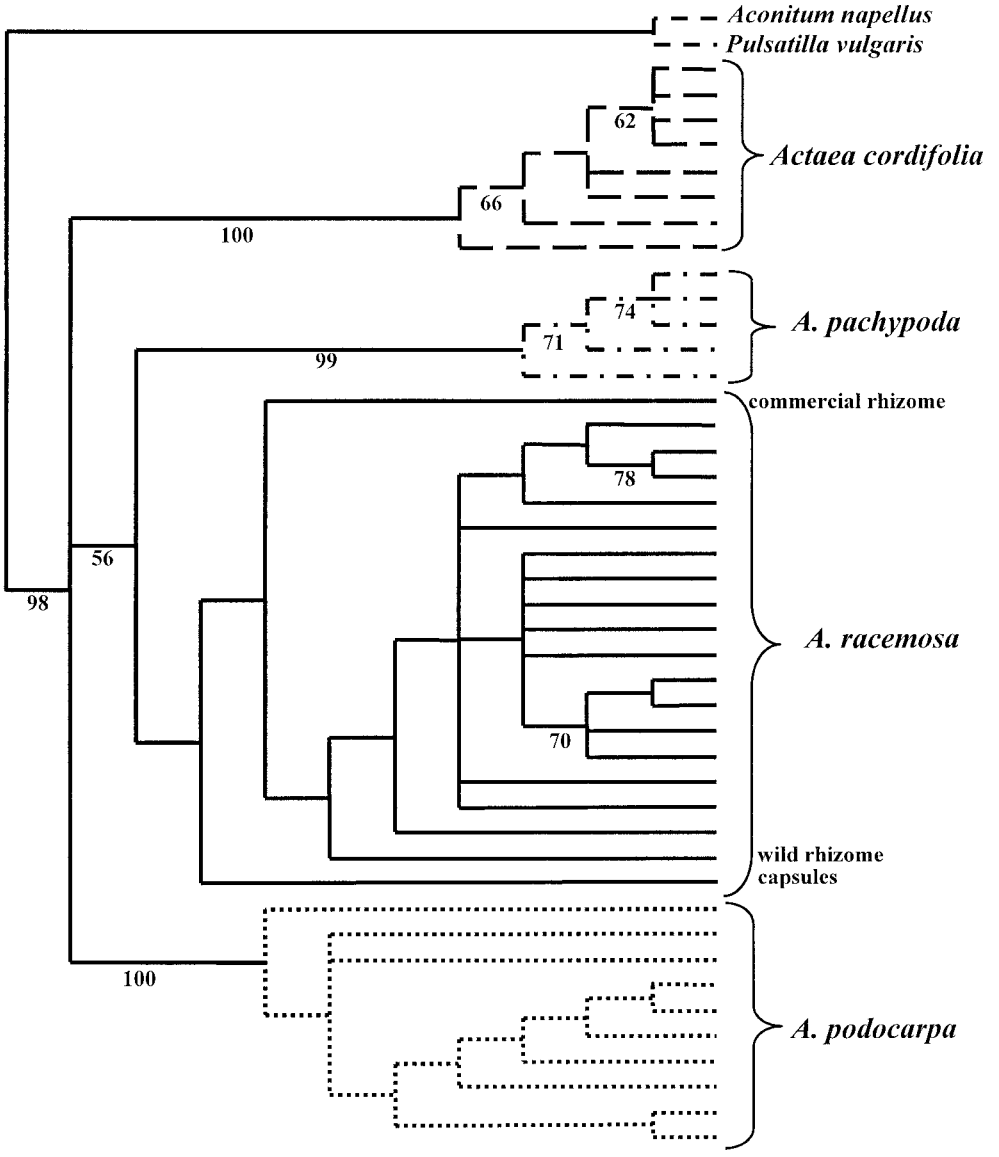

Fig. 3. Strict consensus tree derived from analysis of four AFLP primer combinations. The analysis resulted in 420 most parsimonious trees (929 steps, CI = 0.27, RI = 0.70). Bootstrap values greater than or equal to 50 are indicated below branches.

had any unique AFLP markers defining it. Principle components analysis (PCA) was also employed on the population level data (results not shown), but also lacked any resolution (SAS Institute 2000). This suggests that gene flow is occurring between these populations or that the markers studied have not stabilized enough in populations to allow for detection of differences among populations. Although AFLP analysis is capable of identifying *A. racemosa* from other closely related species, it is not possible, at the level examined here, to differentiate between populations of *A. racemosa.* However, increased sampling within populations and/or testing additional AFLP primer combinations may prove useful in addressing population-level issues.

The fingerprinting data is not only useful for the practical purpose of *Actaea* species identifi-

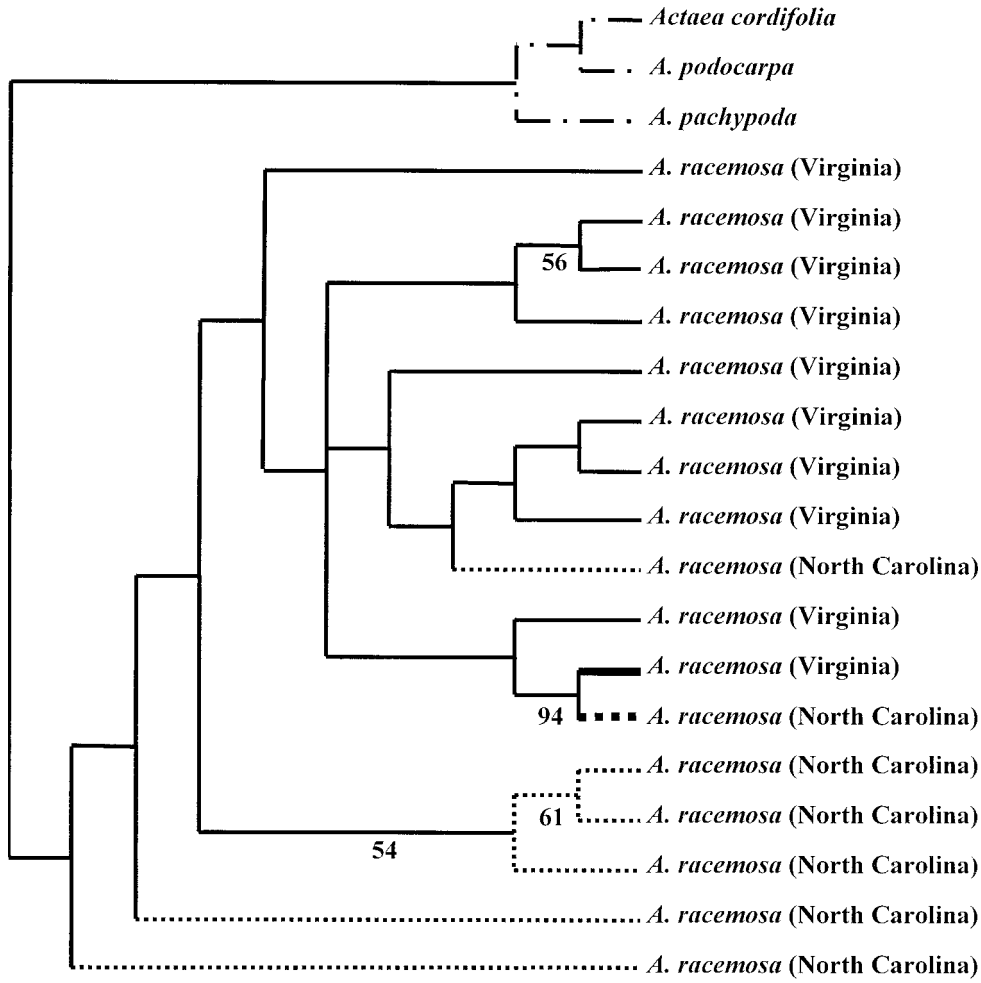

Fig. 4. The strict consensus of three most parsimonious trees generated from an AFLP data matrix of two *A. racemosa* populations (population 2—Virginia, population 11—North Carolina) using four primer combinations (270 steps, CI = 0.47, RI = 0.49). Bootstrap values greater than or equal to 50 are indicated below branches. The clade in bold indicates strong support for the close relationship between *A. racemosa* individuals from two different populations.

cation, but it also provides support for the use of the AFLP technique in phylogenetic analyses among closely related congeneric species. First, each species forms a monophyletic lineage supporting their traditional species delineations (Fig. 3). Second, until recently, *A. racemosa, A. cordifolia,* and *A. podocarpa* were all placed in the genus *Cimicifuga,* but the AFLP data suggest that *A. pachypoda* and *A. racemosa* are the most closely related among these four species (Fig. 5). These results support uniting *Cimicifuga* with *Actaea* as Compton, Culham, and Jury (1998) did based on their morphological and DNA sequence data from 30 taxa. Although the use of DNA fragment data in parsimony analyses has been criticized (Backeljau et al. 1995), the data presented here corroborates other studies demonstrating the utility of AFLP data in creating phylogenetic hypotheses among closely related species by means of parsimony analysis (Kardolus, van Eck, and van den Berg 1998; Qamaruz-Zaman et al. 1998).

The quality control of botanical dietary supplements on the market today can be difficult to regulate. In the case of some commercial botanical supplements, chemical analyses exist to aid

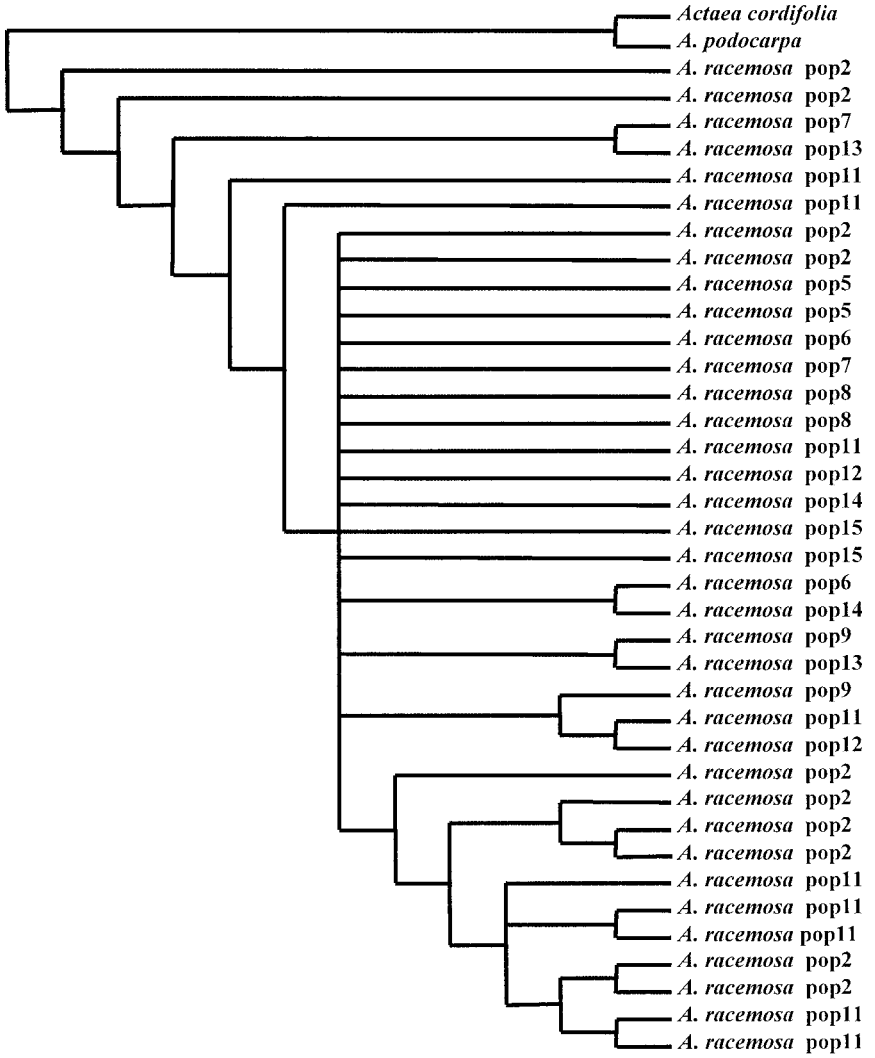

Fig. 5. Strict consensus tree derived from analysis of one AFLP primer combination for 11 populations of *Actaea racemosa.* The analysis resulted in 27 most parsimonious trees (365 steps, CI = 0.22, RI = 0.50).

in the proper identification of species. Although such analyses are important, other species identification methods would be useful. This is particularly true in situations where chemical analyses do not exist or are limited. In the case of black cohosh, an existing analytical test checks for the presence or absence of both *A. racemosa* and *A. cimicifuga* (He et al. 2000). The data presented here provides a test that can be used to distinguish *A. racemosa* from three sympatric species (*A. cordifolia, A. pachypoda,* and *A. podocarpa*). This technique is not limited by chemical production in varying plant parts or by quantitative differences due to ecological components or seasonality of collection. DNA fingerprinting techniques such as AFLP can offer an additional and stable means of verifying the presence of the desired species and the absence of others in commercial black cohosh products, and it may prove to be beneficial for other botanical dietary supplements as well.

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