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Zoe E. Gardner
University of Massachusetts, Amherst, herbnerdzoe@gmail.com

Lorna Lueck
University of Massachusetts, Amherst, lolueck@web.de

Erik B. Erhardt
University of New Mexico, erike@stat.unm.edu

Lyle E. Craker
University of Massachusetts, Amherst, craker@umass.edu

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A Morphometric Analysis of *Actaea racemosa* L. (Ranunculaceae)

Z. Gardner[1*], L. Lueck[1], E.B. Erhardt[2], L.E. Craker[1]

[1]Department of Plant, Soil & Insect Sciences, University of Massachusetts, Amherst, MA 01003 U.S.A.
[2]Department of Mathematics and Statistics, MSC01 1115, 1 University of New Mexico, Albuquerque, NM 87131 U.S.A.

*Corresponding author: zoe@psis.umass.edu

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ABSTRACT

Actaea racemosa L. (syn. *Cimicifuga racemosa* [L.] Nutt.), Ranunculaceae, commonly known as black cohosh, is an herbaceous, perennial, medicinal plant native to the deciduous woodlands of eastern North America. Historical texts and current sales data indicate the continued popularity of this plant as an herbal remedy for over 175 years. Much of the present supply of *A. racemosa* is harvested from the wild. Diversity within and between populations of the species has not been well characterized. The purpose of this study was to assess the morphological variation of *A. racemosa* and identify patterns of variation at the population and species levels. A total of 26 populations representative of a significant portion of the natural range of the species were surveyed and plant material was collected for the morphological analysis of 37 leaflet, flower, and whole plant characteristics. In total, 511 leaflet samples and 83 flower samples were examined. Several of the populations surveyed had sets of relatively unique characteristics (large leaflet measurements, tall leaves and flowers, and a large number of stamen), and Tukey-Kramer multiple comparisons revealed significant differences between specific populations for 20 different characteristics. No unique phenotype, however, was found. Considerable morphological plasticity was noted in the apices of the staminodia. Cluster analyses showed that the morphological variation within populations was not smaller than between population and that this variation in not influenced by their geographic distribution.

INTRODUCTION

Actaea racemosa L. (syn. *Cimicifuga racemosa* [L.] Nutt.), Ranunculaceae, commonly known as black cohosh, is an herbaceous perennial medicinal plant native to the deciduous woodlands of eastern North America. The distribution of the plant ranges from Massachusetts to Ontario, Missouri and Georgia (Kartesz, 1999), with the highest density of plants found in the Appalachian Mountains.

Preparations made from *A. racemosa* roots and rhizomes are currently popular medicinal products in the United States and Europe for the relief of menopausal symptoms. In 2005, *A. racemosa* was reported to be the eighth most popular herbal supplement in the U.S. (Blumenthal, 2005). The vast majority, an estimated 96 percent, of the *A. racemosa* sold is collected from the wild (Lyke, 2001). Other slow growing woodland species of North American medicinal plants that have economically valuable roots, such as ginseng (*Panax quinquefolius* L.) and goldenseal (*Hydrastis canadensis* L.), have been harvested to an extent that threatens the species (Robbins, 1999). Since wild populations of the plant are declining and continued dependence on wild sources could easily cause the species to become threatened (Lyke, 2001), efforts are being made to bring *A. racemosa* into commercial cultivation (Popp et al., 2003; Thomas et al., 2001). Baseline data on the naturally occurring morphological variability is one of the prerequisites for establishing defined cultivars that will be needed to produce medicinal products of reproducible and homogenous quality that will be able to compete with the wild crafted material.

A. racemosa has been included in several morphologic studies (Compton, Culham, and Jury, 1998; Compton and Hedderson, 1997; Lee and Park, 1994; Ramsey, 1987). These studies, however, focused on the distinction of *A. racemosa* from related species and did not describe patterns of morphological variation below the species level. The purpose of this study was to assess the morphological variation of *A. racemosa* and identify possible patterns of variation at the population and species levels using morphometric measurements of leaflets, flowers, and habit of plants from geographically distinct populations.

MATERIALS AND METHODS

Plant material. *Actaea racemosa* L. plants from a total of 26 populations in 14 states encompassing a significant portion of the natural range of the plant were identified and sampled during June and July 2002 and July and August 2003 (Table 1, Figure 1). Known populations on public and private land were identified with the assistance of professional contacts. The sampling was randomized in a way that the collector estimated the size of the population and then walked throughout planting, stopping at regular intervals, depending on the spatial size of the population to obtain leaf and flower samples from an individual plant. To minimize the variability of characteristics due to different development stages, flowering plants (early to full anthesis) were sampled.

Only when none or only limited flowering individuals occurred in a population, were plants in earlier (emerging inflorescence) or later development stages (seeds maturing) included. A standardized scale was used to categorize the whole spatial extent for each population due to possible difference in microclimates, especially in large populations. Voucher samples are placed in the University of Massachusetts herbarium.

Table 1. Location and size of sampled populations.

Population[1] (State & Sample)	Sample site[2] (Town or County)	Altitude (m)	Population size	
			(ha)	(plants)
DE-1	Milton	3	0.20	80
DE-2	Smyrna	57	2.02	1500
IN-1	Madison	220	0.40	4000
KY-1	Pulaski Co.	280	2.02	1000
KY-2	Pulaski Co.	332	2.02	800
MA-1	Berkshire Co.	825	2.02	155
MD-1	Grantsville	751	2.02	500
MO-1	Lesterville	265	4.05	20,000
NC-1	Asheville	856	3.24	3000
NC-2	Robbinsville	321	2.83	4000
NC-3	Fontana Village	492	2.02	10,000
NC-5	Morganton	792	8.09	1000
NC-6	Cary	106	2.02	170
NY-1	Katonah	77	4.05	3000
NY-2	South Salem	120	2.02	4000
OH-1	Rutland	204	0.81	200
OH-2	Rutland	147	1.21	400
PA-1	McConnels Mill	312	4.05	500
PA-2	Allensville	220	4.05	15,000
SC-1	Sunset	260	12.14	4000
TN-1	Crandull	1097	4.05	1000
VA-1	Brookneal	137	2.02	550
VA-2	Amherst Co.	762	4.05	15,000
WV-1	Elkview	250	10.12	15,000
WV-2	Chapmanville	171	8.09	550
WV-3	Spencer	213	20.23	50,000

[1]Population codes correspond with those used by Lueck (2003). Morphological data for the population NC4 was not collected.
[2]Due to conservation concerns, only general locations have been used to protect the exact locations of the populations.

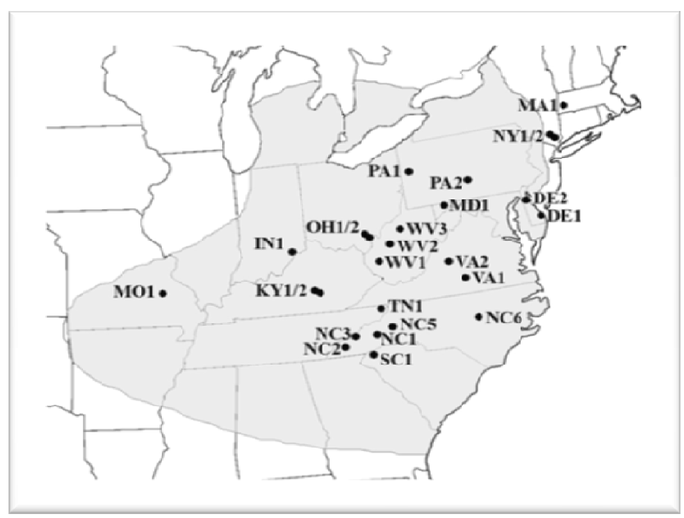

Figure 1. Geographical display of populations.
Grey area indicates approximate range of the species in 1887 (Lloyd and Lloyd , 1887).

Morphological analysis. In each population, 20 plants were examined and morphological data on the height of the mature plant, height of the main compound leaf, number of compound leaves, length of the terminal leaflet, number of inflorescences, height of inflorescences, and the stage of reproduction was recorded at the time of collection (Figure 2). From each plant, the three terminal leaflets of the largest leaf and five flowers were collected. If the desired leaflets were missing or severely deformed, botanically equivalent leaflets, usually from a side branch of the same compound leaf, were collected. Such substitution occurred in approximately 2.5 percent of plants sampled. During collection, the flowers were picked from each flowering plant, immediately pressed flat and allowed to dry in paper envelopes stored in silica gel. Sets of leaflets were picked and kept in a plastic bag until being pressed in newsprint, three to nine hours after collection. The botanical identity of collected plants was verified at the time of collection through the observation of reproductive and vegetative parts and was later confirmed by AFLP fingerprinting (Lueck, 2003).

Based on previous work in this genus (Compton and Hedderson, 1997; Compton, Culham, and Jury, 1998; Lee and Park, 1994; Ramsey, 1987) and initial examination of characteristics that appeared to vary between populations, 13 lengths and 3 angles were measured on each leaflet. Leaflets were measured with the image analysis program Scion Image Beta 4.0.2 (Scion Corp., Frederick, MD). Pressed leaflets were scanned (HP ScanJet 6200) to create digitized images of the leaflets and the digitized images were calibrated using the scanned image of a millimeter grid scanned with each leaflet.

Selected lengths (in cm) and angles (in degrees) in the images were measured using measurement tools in the Scion program. Characteristics of the secondary leaflets were categorized into one of the following five categories: 1) petiolule present, base meeting; 2) petiolule present, base oblique; 3) petiolule absent, base meeting; 4) petiolule absent, base oblique; 5) petiolule absent, base adhering (Figure 3). A leaflet base was considered oblique if the base on one side of the primary vein was more than three millimeters from the base on the opposite side of the primary vein. A leaflet was considered adhering if more than three mm of the base of the leaflet was fused with the petiolule.

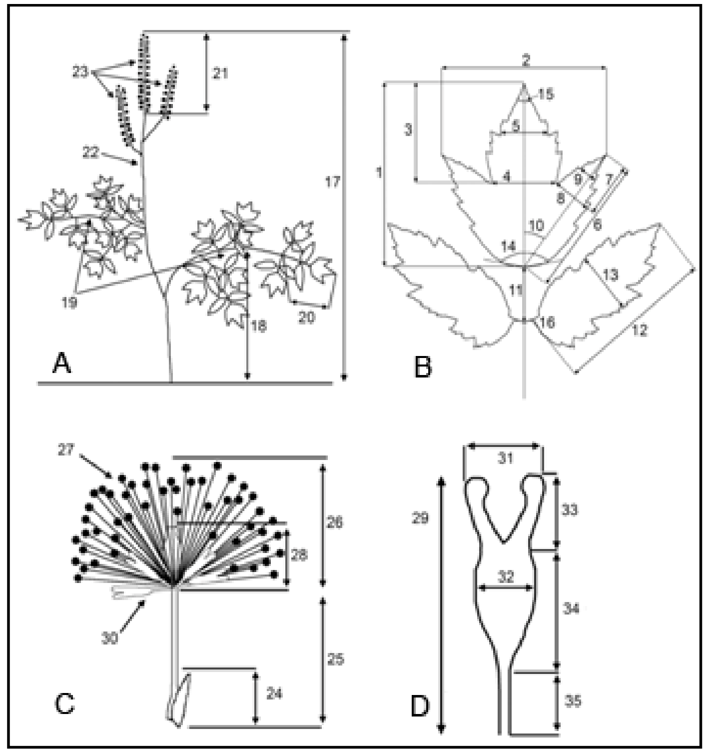

Figure 2. Morphological measurements of plant parts. A=whole plant, B=leaflets, C=flowers, D= staminodia.

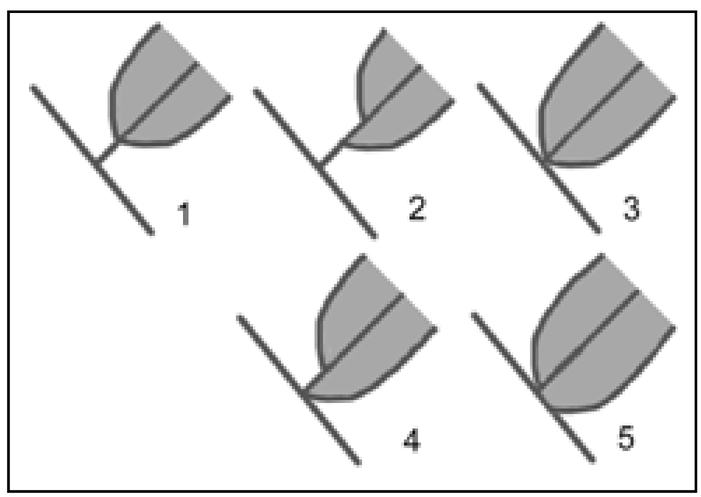

Figure 3. Lateral leaf base categories.

Of the 26 populations sampled, 16 contained flowering plants. Single flowers of five plants per population were examined. Dried flowers were rehydrated for a minimum of 10 min in 70% ethanol. At the time of examination, each flower was placed on a Petri dish and several milliliters of the ethanol solution were added to keep the flower hydrated and easy to manipulate. Under a 10X binocular dissecting scope, the lengths of the flower bract, pedicel, stamens, and pistil were measured using digital calipers (Mitutoyo Plastical digital calipers) and the number of stamens and staminodia were counted. Staminodia were removed with a dissecting needle and stored in 70% ethanol until further examined. Staminodia were placed on a glass slide with several drops of ethanol solution. A graticule in the eyepiece of a 40X binocular microscope was used to measure selected dimensions on each staminodium and the apex and base characteristics of each staminodium were scored (Figure 4).

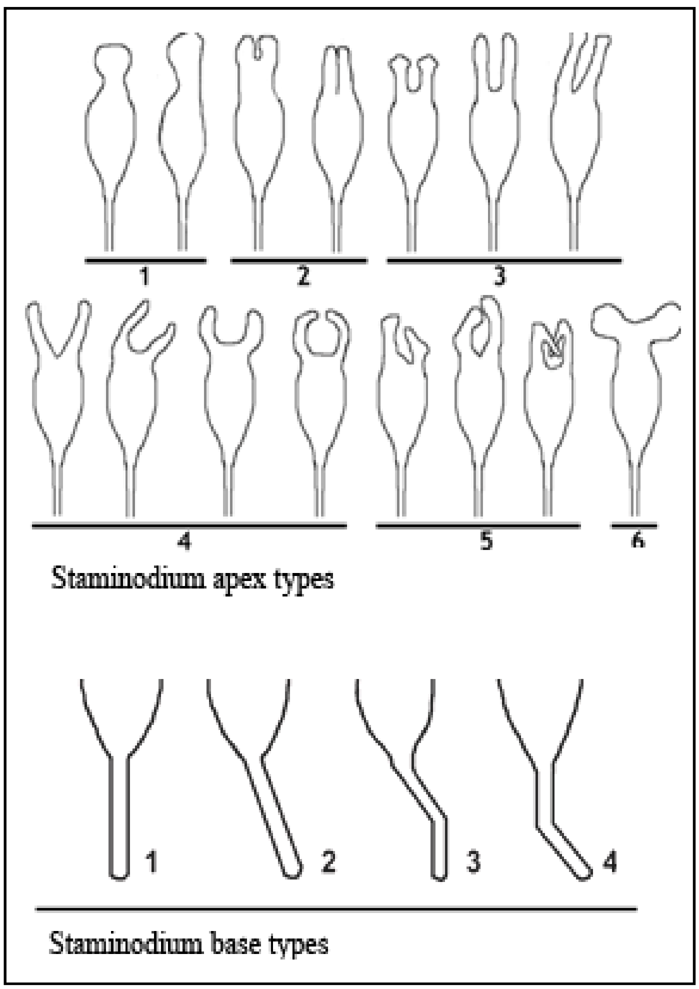

Figure 4. Staminodium apex and base types.

Statistical analysis. Statistical analyses were done using statistical software packages SAS Release 8.00 (SAS Institute Inc., Cary, NC) or Minitab Release 14.20 (Minitab, Inc., State College, PA). Unless otherwise indicated, statistics displayed are for all individuals (rather than population averages of individuals). Descriptive statistics were generated, listing the minimum, maximum, mean, standard deviation, and coefficient of variation for each characteristic. For each quantitative characteristic an analysis of variance (ANOVA) test for equal population means was run, followed with the Tukey-Kramer multiple comparison test (HSD) for unequal sample sizes to indicate pairwise differences of population means using SAS (Kramer, 1956; Tukey, 1953).

Dendrograms were created based on different selections of the available data using the "cluster observations" command in Minitab 14, using the UPGMA algorithm on standardized variables with average linkage and squared Euclidean distances (Lance and Williams, 1967). For consensus, dendrograms were constructed using combinations of linkage, distance, and standardized and unstandardized variables and results were consistent.

In total three datasets were used to create dendrograms: (1) all populations using averages of non-flower characteristics (characteristics labeled 1-23, (2) populations with floral data using all available data (characteristics labeled 1-37) with plant averages of staminodium characteristics (characteristics labeled 31-37) and (3) populations with floral data using floral data only (characteristics 24-37) with plant averages of staminodium characteristics (characteristics 31-37).

RESULTS

An overview of variation for all characteristics measured in the study was established (Table 2) with ANOVA F-statistics indicating the presence of significant differences between at least two populations. For 23 of 37 characteristics, statistical differences between populations were indicated. For the characteristics 7, 9-11, 16, 22, 24, 26, 28-31 and 35-37, no differences between populations were observed. Tukey--Kramer testing of individual characteristics provided groupings that indicate significant pairwise population differences (Table 3).

Table 2. Variability of morphological traits observed in 26 populations.

Morphological trait and measuring units	Number of		Sample measurements			Standard	Coefficient	ANOVA
	Samples	Populations	Minimum	Maximum	Mean	deviation	of variation[1]	F-statistic[2]
Leaflet characteristics							(%)	
1. Terminal leaflet length (cm)	511	20	6.14	16.75	10.42	1.85	17.75	9.75
2. Terminal leaflet width (cm)	511	20	2.86	15.24	7.39	2.19	29.63	5.08
3. Middle terminal lobe length (cm)	511	20	1.93	11.17	5.32	1.42	26.69	4.73
4. Middle lobe width at base (cm)	511	20	0.98	5.98	3.02	0.84	27.81	11.40
5. Middle lobe width at midpoint (cm)	511	20	0.61	5.85	2.31	0.93	40.26	5.79
6. Terminal Leaflet length base to apex (cm)	511	20	4.12	12.69	7.72	1.49	19.30	9.20
7. Lateral lobe length terminal leaflet (cm)	511	20	0.23	3.50	1.05	0.54	51.43	2.63
8. Lateral lobe width at base (cm)	511	20	0.43	3.67	1.79	0.58	32.40	4.68
9. Lateral lobe width at midpoint (cm)	511	20	0.47	7.95	2.63	1.29	49.05	2.18
10. Lateral lobe angle to vertical axis (deg.)	511	20	14.00	52.00	29.00	6.20	21.8	2.48
11. Petiolule length terminal leaflet (cm)	511	20	0.43	5.96	2.72	1.00	36.76	3.72
12. Lateral leaflet length (cm)	511	20	3.50	15.53	9.58	1.71	17.85	9.46
13. Lateral lobe width midpoint (cm)	511	20	1.43	6.55	3.25	0.85	26.15	9.63
14. Lateral lobe angle (deg.)	511	20	37.00	269.0	128.00	44.90	35.10	4.26
15. Terminal leaflet angle (deg.)	511	20	10.00	62.00	26.00	9.30	35.80	10.04
16. Lateral leaflets base characteristics (score)	511	20	N/A	N/A	N/A	N/A	N/A	N/A
Whole plant characteristics[3]								
17. Height (cm)	450	20	45	248	154.07	32.18	20.89	11.39
18. Tallest leaf height (cm)	511	20	27	90	53.60	10.41	19.42	18.76
19. Compound leaves (number)	511	20	1	6	2.21	0.91	41.18	N/A
20. Length three terminal leaflets (cm)	511	20	11	31	19.27	3.48	18.06	8.24
21. Length largest inflorescence (cm)	450	20	17[4]	89	29.67	9.48	31.95	11.65
22. Flower stalks (number)	511	20	0	3	0.38	0.51	134.21	N/A
23. Inflorescences (number)	511	20	0	17	2.70	1.98	73.33	17.31
Flower characteristics[5]								
24. Bract length (mm)	36	15	1.0	5.6	2.66	0.56	21.05	1.31
25. Pedicel (mm)	83	17	2.7	8.2	5.07	1.09	21.50	4.57
26. Stamen length (mm)	83	17	3.6	8.5	6.25	0.99	15.84	2.45
27. Stamens (number)	83	17	43.0	134.0	95.37	15.41	16.16	3.09
28. Pistil length (mm)	83	17	1.7	5.3	3.61	0.49	13.57	2.82
29. Staminodium length (mm)	83	17	2.4	4.7	3.26	0.45	13.80	0.58
30. Staminodia (number)	83	17	8.0	4.4	0	1.73	39.32	1.81
31. Staminodium width top (mm)	350	16	1.0	12.5	6.33	2.07	32.65	3.08
32. Staminodium width midpoint (mm)	350	16	3.0	10.0	5.85	1.15	19.73	11.52
33. Staminodium length top (mm)	350	16	1.0	16.0	5.34	1.80	33.62	7.30
34. Staminodium length midsection (mm)	350	16	5.0	23.5	13.28	2.50	18.83	8.71
35. Staminodium length base (mm)	350	16	5.0	34.0	12.98	3.38	26.02	2.94
36. Apex type (score)	350	16	N/A	N/A	N/A	N/A	N/A	N/A
37. Base type (score)	350	16	N/A	N/A	N/A	N/A	N/A	N/A

[1]Coefficient of variation is a percentage value of the standard deviation divided by the mean.

[2]The between versus within population variation. Values larger than about 3 indicate significant differences between at least two population means.

[3]Whole plant characteristics were recorded for all plants. A total of 61 non-flowering plants were sampled and no total height or inflorescence length was recorded for these plants.

[4]The shortest length in an inflorescence beyond BBCH stage 60 (first flowers open) (Bleiholder et al., 1997).

[5]A total of 83 flowers (collectively having 350 staminodia) were examined. Bracts were separated from many of the dried flowers, but remained attached to 36 flowers (characteristic 24).

N/A = Not applicable.

Whole plant morphology. The largest population mean was roughly twice that of the smallest population mean for all leaflet characters and less than twice for flower characteristics. The tallest individual plants were observed in populations labeled NY-2, IN-1, and WV-3). These populations also had large numbers of inflorescences, the greatest leaf height, more than the average number of leaves, and relatively long inflorescences.

Leaflet morphology. Coefficients of variation for leaflet characteristics ranged from 17.75 to 51.43. The Tukey-Kramer analysis revealed large, overlapping groups of populations with similar ranges. Only populations near the minimum and maximum for certain characteristics were significantly different from each other. For instance, populations MD-1, SC-1, PA-2 and MA-1 had several particularly large leaflet characteristics, while populations NC-6, NC-5, VA-1 and MO-1 were smaller.

Specifically, populations NC-6 and MO-1 had smaller than average leaflet characteristics, including terminal leaflet length, terminal leaflet width, middle lobe length, and middle lobe width at base. Coefficients of variation in these characteristics in these populations were generally smaller than the variation observed in other populations. Population MD-1 had the largest average measurements for many leaflet characteristics, including the terminal leaflet length, terminal leaflet width, middle lobe length of the terminal leaflet, lateral lobe length of the terminal leaflet, lateral lobe angle relative to the vertical axis and lateral leaflet length.

Certain characteristics demonstrated a relatively large amount of variation, as indicated by the Tukey-Kramer analysis for a number of present groupings, including terminal leaflet length, terminal leaflet width, middle lobe width at base, length of terminal leaflet base to lateral lobe apex, lateral leaflet length, lateral leaflet width at midpoint, and angle of terminal leaflet apex. Other characteristics demonstrated a greater amount of uniformity of population means between populations, including length of middle lobe on terminal leaflet, middle lobe width at midpoint, length of lateral lobe on terminal leaflet, lateral lobe angle relative to vertical axis, lateral lobe width at base, lateral lobe width at midpoint, length of petiolule of terminal leaflet and angle of terminal leaflet base. While all terminal leaflets had petiolules, petiolules were present on only 20 percent of lateral leaflets.

Flower morphology. Flower morphology was variable both within and between populations for all characteristics examined. Certain characteristics demonstrated a relatively large amount of variation between population means and statistically significant differences could be observed between populations. These include pedicel length, staminodium midsection width, staminodium tip length, and staminodium midsection length. Other characteristics demonstrated more uniformity among populations and the populations did not differ in flower morphology.

The number of stamens per flower ranged between 43 and 134. The smallest variation in the number of stamens was in population KY-2, with stamen numbers ranging from 71 to 74, population PA-2 had the largest range, 43 to 114, and population SC-1 had the highest mean number of stamens (90 to 134 per flower).

Bracts, present on 40 of the 88 flowers examined, ranged from 1 to 5.6 mm in length. Although all populations were statistically equivalent, population WV-2 had both the longest bract and largest variability. Staminodia demonstrated both within and between population variability, similar to that of other floral traits (Figure 5).

Distinctive populations within traits included population NC-2 with large staminodium width at midpoint, population NC-3 with small and relatively uniform staminodium midsection length, and populations WV-2 and WV-3 with relatively large variation in staminodium base length. The shapes of the staminodium apices and bases demonstrated a surprising amount of plasticity as compared with the summary by Ramsey (1987) that concluded staminadia shapes are stable within the species.

In total, six different types of staminodia apices were recognized. Most populations shared bifid apices that branched into two narrow lobes of similar length, but variable form. In addition, unusual types occurred where the two lobes were nearly or entirely merged, or where the two lobes were enlarged into oval structures. Populations KY-1, MO-1, and WV-2 were most variable in the staminodium apices because all six types were present. The populations NC-2, NY-2, and SC-1 appeared most homogenous in this trait because only three apex types were observed in these populations. Merged lobes were only observed in 9 of the 17 populations, while enlarged lobes were present in even fewer populations (KY-1, MO-1, NY-1 and WV-2).

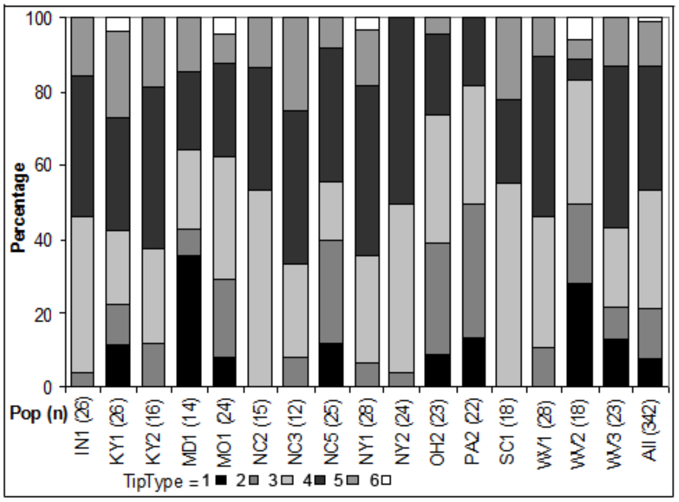

Figure 5.Occurrence of staminodium apex type.

Relationship among populations. Multivariate summaries of population similarity are illustrated by a dendrogram (Figure 6). In the dendrogram, the emerging patterns and groups do not correspond with geographic location or altitude. The groupings depend strongly on the data subsets used and may be the reason dendrograms created on different data sets do not reveal similar patterns. Using vegetative data, populations with a small leaflet width, MO-1, NC-6, and NY-1, form a distinct cluster.

DISCUSSION

Several species of *Actaea* grow in the eastern United States. These species are recognized as being closely related, suggesting a relatively recent evolutionary division (Compton 1982; Ramsey 1986, 1988) and reducing the likelihood of within species differentiation. Even different species are difficult to distinguish when the plants are not in flower, as the leaflet morphology of the different species is very similar (Ramsey 1965). With this level of similarity, elucidating groups with typical morphological traits below the species level can be challenging. Given the wide geographical range of the sampled populations, however, some patterns of morphological variation appear possible.

While the observed variation in this study did not allow delineation of groups based on leaflet morphology, some variation was noted in the different leaflet characteristics, enabling the discernment of certain populations for selected traits. Levels of phenotypic variation detected depend on the characteristics measured and more variation may be expected in leaves than in flowers (Lawrence, 1950; Stace, 1989). In this study, the coefficients of variation for leaflet characteristics observed are generally higher than those observed for flower characteristics.

Means of characteristics in this study are similar to those reported by others examining *A. racemosa*. Ramsey (1987) reported a mean terminal leaflet length of 10.5 cm for *A. racemosa*, and a mean terminal leaflet width of 8.1 cm, as compared to the 10.4 cm terminal leaflet mean length and 7.4 cm mean width in this study. Compton (1982) reports the number of staminodia as 1 to 8, as compared to our 0 to 8, and stamens as 55 to 110 as compared with our 43 to 134.

Commenting on staminodium morphology, Ramsey (1987) noted that staminodia shapes were stable within species. The variation observed in staminodium characteristics in this analysis was much greater than anticipated and greater than reported by Lee and Park (1994) in *A. foetida* and Ramsey (1987) in North American species of *Actaea*. This greater variation is surprising given that morphological diversity appears to be higher in *A. foetida* than *A. racemosa* (Compton and Hedderson 1997).

Ramsey (1965), studying 2000 herbarium specimens of *A. racemosa*, found 16 unique specimens labeled as *dissecta*, a teratological form of the species that has highly dissected leaflets. In sampling a set of populations in this study that cover a significant portion of the geographical range of this species, no such unique individuals or groups that could be classified into forms were observed. This lack of unique individuals is not surprising, as the analysis b Ramsey (1965) included many herbarium specimens that likely represented a significantly higher proportion of the unusual forms than would be found in wild populations.

While *A. racemosa* is typically a plant of deciduous woodlands and most populations observed in this study were growing alongside typical woodland understory plants as *Sanguinaria canadensis* L., *Asarum canadense* L., *Polystichum acrostichoides* (Michx.) Fée., *Adiantum pedatum* L., *Impatiens pallida* Nutt., and *Arisaema triphyllum* (L.) Schott, *A. racemosa* was also observed and collected from atypical sites, such as a hillside clearing with no canopy cover and alongside *Phragmites australis* (Cav.) Trin., *Achillea millefolium* L., and *Verbascum Thapsus* Bertol (population NY2). This observation illustrates the adaptability of the species to different growing conditions and the variability of habitats in which *A. racemosa* grows.

The sampling protocol was designed to exclude variation due to different development stages, while variability due to different microclimates within populations was not excluded. Given the large size of some populations, the protocol procedures enabled insight on the magnitude of variation within populations. While the variation makes differentiation of populations according to morphological traits quite difficult, the naturally occurring variability of the species is reflected. The observed adaptability makes *A. racemosa* more amenable to cultivation than other woodland medicinal plant species, such as *Panax quinquefolius* L. and *Hydrastis canadensis* L.

In addition to ecological variability, the plant breeding system can influence genetic differentiation and cause subsequent morphological differentiation among populations. *A. racemosa* is a slowly reproducing (Baskin and Baskin 1985) and slowly migrating (Matlack, 1994) species, suggesting that differentiation do to distance between populations should be possible. The species, however, is a long-lived perennial, pollinated by insects and by pollen-ovule ratios averaging over 30000:1 (unpublished data), which indicate, according to Cruden (1977), *A. racemosa* is most likely xenogamous. Based on the species longevity, wide distribution, large population sizes, and outcrossing characteristics, gene flow between populations and lower genetic differentiation with subsequently lower morphological differentiation among populations could be expected (Hamrick &Godt 1989).

CONCLUSIONS

This study assessed the morphological variation of *A. racemosa* to identify patterns of variation at the population and species levels. While variation was observed for all characteristics, cluster analyses indicated morphological variation within populations was similar to that between populations and that this variation was not influenced by geographical distribution.

While no unique phenotypes were observed, discernment of some populations based on leaf and flower characteristics was possible, suggesting a starting point the development of possible morphologically defined and homogenous cultivars.

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Table 3.Tukey-Kramer multiple comparisons of morphological characteristics.

1. Terminal leaflet length (cm)

Pop	Grouping	Mean	N
MD1	A	12.16	20
SC1	B A	11.99	20
WV3	B A C	11.97	20
NC1	B A C	11.90	20
PA	BDA C	11.61	20
MA1	EBDA C	11.56	12
KY1	EBDA CF	11.24	20
NC2	EBDA CF	11.10	20
VA2	EBDA CF	11.05	20
TN1	EBDA CF	10.90	20
NY2	EBDAGCF	10.67	20
DE2	EBDAGCF	10.66	20
IN1	EBDAGCF	10.38	20
NC3	EBDHGCF	10.19	20
DE1	E DHGCF	10.13	20
OH1	E DHG F	10.04	20
WV2	E DHG F	10.03	20
WV1	E DHG F	10.00	20
KY2	E DHG F	9.94	20
OH2	E DHG F	9.81	20
NY1	E DHG F	9.80	20
PA1	EI HG F	9.75	20
NC5	I HG F	9.50	17
VA1	I HG	8.96	20
MO1	I H	8.44	20
NC6	I	7.93	20

F Value = 5.08; Pr>F < 0.0001*

2. Terminal leaflet width (cm)

Pop	Grouping	Mean	N
MD1	A	9.60	20
DE2	B A	8.83	20
VA2	B AC	8.49	20
MA1	B AC	8.36	12
PA2	B AC	8.24	20
SC1	B AC	8.13	20
NC2	B AC	7.90	20
WV3	B AC	7.87	20
TN1	B AC	7.86	20
NC3	B AC	7.77	20
NY2	BDAC	7.67	20
KY1	BDAC	7.65	20
OH1	BDAC	7.48	20
NC1	BDAC	7.50	20
VA1	BDAC	7.33	20
IN1	BDAC	7.29	20
DE1	BDAC	7.28	20
KY2	BD C	7.11	20
WV2	BDEC	7.03	20
WV1	BDEC	6.90	20
PA1	BDEC	6.87	20
OH2	BDEC	6.56	20
NC5	BDEC	6.50	17
NY1	DEC	6.40	20
MO1	DE	5.35	20
NC6	E	4.70	20

F Value = 9.75; Pr>F < 0.0001

3. Middle terminal lobe length (cm)

Pop	Grouping	Mean	N
MD1	A	6.57	20
WV3	B A	6.34	20
NC2	B AC	6.09	20
NC1	BDAC	6.06	20
NC3	BDAC	6.05	20
MA1	EBDAC	5.85	12
KY1	EBDAC	5.79	20
SC1	EBDAC	5.76	20
IN1	EBDAC	5.71	20
NY2	EBDAC	5.55	20
TN1	EBDACF	5.49	20
VA2	EBDACF	5.49	20
PA2	EBDACF	5.42	20
DE2	EBDACF	5.38	20
OH1	EBDACF	5.15	20
NY1	EBDACF	5.10	20
KY2	EBDACF	5.04	20
WV1	EBD CF	5.01	20
WV2	EBD CF	4.97	20
DE1	EBD CF	4.89	20
PA1	EBD CF	4.84	20
NC5	E D CF	4.69	17
VA1	E D CF	4.61	20
MO1	E D F	20	
OH2	E F	4.40	20
NC6	F	3.95	20

F Value = 4.73; Pr>F < 0.0001

4. Middle lobe width at base (cm)

Pop	Grouping	Mean	N
PA2	A	3.78	20
MD1	A	3.75	20
VA2	B A	3.62	20
MA1	B A	3.61	12
SC1	B AC	3.56	20
WV3	B AC	3.52	20
NC1	B AC	3.51	20
DE2	B AC	3.48	20
TN1	BDAC	3.34	20
IN1	BDAC	3.34	20
KY2	EBDAC	3.09	20
KY1	EBDAC	3.09	20
NC5	EBDAC	2.98	20
DE1	EBDAC	2.99	20
OH2	EBDAC	2.98	20
OH1	EBD C	2.90	20
NC2	EBD C	2.90	20
WV2	EBD C	2.90	20
PA1	EBD C	2.89	20
NY2	EBD C	2.88	20
WV1	E DFC	2.80	20
VA1	E DF	2.66	20
NY1	EG F	2.33	20
NC3	EG F	2.29	20
MO1	G F	1.99	20

F Value =5.79; Pr>F < 0.0001

5. Middle lobe width at midpoint (cm)

Pop	Grouping	Mean	N
VA2	A	3.24	20
MD1	B A	3.19	20
DE2	B AC	3.13	20
PA2	BDAC	2.80	20
OH2	BDAC	2.62	20
WV3	BDAC	2.61	20
VA1	EBDAC	2.50	20
DE1	EBDAC	2.50	20
MA1	EBDAC	2.47	12
SC1	EBDAC	2.41	20
IN1	EBDACF	2.38	20
NY2	EBDACF	2.38	20
KY2	EBDACF	2.36	20
OH1	EBDACF	2.26	20
WV2	EBDACF	2.26	20
NC1	EBD CF	2.25	20
KY1	E D CF	2.14	20
PA1	E D F	2.10	20
NC5	E D F	2.06	17
MO1	E D F	1.97	20
WV1	E D F	1.94	20
NC3	E D F	1.90	20
NC2	E D F	1.90	20
TN1	E D F	1.88	20
NY1	E F	1.62	20

F Value = 11.40; Pr>F < 0.0001

6. Terminal leaflet length (cm)

Pop	Grouping	Mean	N
SC1	A	9.39	20
MD1	B A	9.02	20
WV3	B A	8.86	20
MA1	B AC	8.78	12
PA2	BDAC	8.56	20
TN1	EBDAC	8.28	20
DE2	EBDACF	8.24	20
VA2	EBDACF	8.18	20
NC1	EBDACF	8.15	20
NC2	EBDACF	8.10	20
KY1	EBDACF	8.07	20
NY2	EBD CF	7.75	20
WV2	EBD CF	7.70	20
WV1	EBD CF	7.66	20
DE2	EBD CF	7.62	20
OH2	EBDGCF	7.58	20
NC3	EBDGCF	7.57	20
KY2	E DGCF	7.36	20
OH1	E DGCF	7.35	20
PA1	E DGCF	7.32	20
NY1	E DG F	7.26	20
IN1	E DG F	7.15	20
VA1	EH G F	6.88	20
NC5	H G F	6.78	17
MO1 6.	H G	20	
NC6	H	5.55	20

F Value = 9.20; Pr>F < 0.0001

12. Lateral leaflet length (cm)

Pop	Grouping	Mean	N
MD1	A	11.13	20
SC1	B A	11.08	20
MA1	B A	11.05	12
WV3	B A C	10.93	20
NC1	BDA C	10.90	20
PA2	EBDA C	10.44	20
KY1	EBDA C	10.38	20
NC2	EBDA C	10.06	20
TN1	EBDA C	9.92	20
DE2	EBDA CF	9.87	20
VA2	EBDA CF	9.80	20
WV2	EBDAGCF	9.60	20
NY2	EBDAGCF	9.59	20
IN1	EBDAGCF	9.52	20
NC3	EBDAGCF	9.44	20
KY2	EBD GCF	9.40	20
OH1	EBD GCF	9.39	20
DE1	E D GCF	9.29	20
OH2	E D G F	9.20	20
WV1	E G F	9.15	20
PA1	E G F	8.97	20
NY1	E G F	8.86	20
NC5	E G F	8.83	17
VA1	HG F	8.19	20
MO1	HG	7.91	20
NC6	H	7.06	20

F Value = 9.46; Pr>F < 0.0001

13. Lateral lobe width at midpoint (cm)

Pop	Grouping	Mean	N
SC1	A	4.28	20
DE2	B A	4.07	20
MD1	B A	4.00	20
MA1	B AC	3.91	12
WV3	BDAC	3.81	20
KY2	EBDAC	3.58	20
NC1	EBDAC	3.48	20
PA2	EBDAC	3.43	20
WV2	EBDFC	3.38	20
VA2	EBDFC	3.38	20
DE1	EBDFC	3.36	20
TN1	EBDFC	3.28	20
VA1	EBDFC	3.28	20
KY1	EBDFCG	3.24	20
OH1	EBDFCG	3.23	20
NY2	E DFCG	3.15	20
OH2	E DFCG	3.14	20
IN1	E DFCG	3.09	20
NC2	E DF G	3.05	20
PA1	E DF G	2.98	20
WV1	E DF G	2.98	20
NC5	E F G	2.95	17
NC3	EH F G	2.83	20
NY1	H F G	2.58	20
MO1	H G	2.40	20
NC6	H	2.06	20

F Value = 9.63; Pr>F < 0.0001

Table 3.Tukey-Kramer multiple comparisons of morphological characteristics (continued).

14. Angle terminal leaflet base (deg)

Pop	Grouping	Mean	N
SC1	A	169	20
WV3	B A	159	20
MD1	B AC	149	20
DE1	B AC	143	20
WV2	B AC	143	20
VA2	B AC	141	20
NC5	B AC	141	17
PA1	B AC	140	20
OH1	BDAC	138	20
DE2	BDAC	136	20
NC3	EBDAC	135	20
VA1	EBDAC	132	20
IN1	EBDAC	131	20
NC2	EBDAC	131	20
TN1	EBDAC	126	20
MA1	EBDAC	126	12
OH2	EBDAC	124	20
WV1	EBDAC	120	20
KY2	EBDAC	119	20
NC1	EBD C	115	20
PA2	EBD C	114	20
KY1	E D C	109	20
MO1	E D C	109	20
NY2	E D C	105	20
NY1	E D	89	20
NC6	E	86	20

F Value = 4.26; Pr> F<0.0001

15. Angle terminal leaflet apex (cm)

Pop	Grouping	Mean	N
DE1	A	38	20
VA1	B A	36	20
MO1	B AC	32	20
DE2	B AC	20	
PA2	BDAC	32	20
IN1	EBDAC	31	20
MD1	EBDACF	29	20
VA2	EBDACF	29	20
NY1	EBD CF	28	20
NY2	EBD CF	28	20
KY2	EBDGCF	27	20
OH2	EBDGCF	27	20
NC5	E DGCF	27	17
MA1	EHDGCF	25	12
KY1	EHDGCF	25	20
WV1	EHDGCF	25	20
WV2	EHDGCF	24	20
NC6	EHDGCF	24	20
TN1	EHDG F	23	20
NC1	EH G F	22	20
SC1	EH G F	22	20
OH1	H G F	21	20
PA1	H G F	20	20
NC2	H G	18	20
WV3	H	17	20
NC3	H	17	20

F Value = 10.04; Pr>F < 0.0001

17. Height of plant (cm)

Pop	Grouping	Mean	N
NY2	A	198.65	20
IN1	B A	179.05	20
WV3	B A	178.35	20
NC1	B AC	174.85	20
SC1	B AC	173.55	20
KY1	BDAC	165.00	20
MO1	BDAC	164.40	20
MD1	BDAC	164.40	20
WV1	BDEC	162.25	20
PA2	BDEC	159.85	20
VA1	BDEC	159.85	20
PA1	BDEC	157.40	20
MA1	FBDEC	151.67	3
OH2	FBDEC	149.65	20
OH1	FBDEC	148.95	20
KY2	FBDEC	148.80	20
DE1	FBDECG	143.38	16
TN1	F DECG	142.05	20
NC6	F DECG	141.95	20
NC3	F DE G	129.45	20
WV2	F DE G	129.06	16
NC2	F DE G	129.00	7
DE2	F E G	127.75	16
NY1	F G	118.00	8
VA2	F G	116.86	7
NC5	G	112.24	17

F Value = 11.29; Pr>F < 0.0001

18. Height of tallest leaf (cm)

Pop	Grouping	Mean	N
NC2	A	71.80	20
NY2	B A	68.90	20
WV3	B C	61.75	20
IN1	B CD	60.90	20
VA1	E CD	59.25	20
PA1	E CD	57.80	20
PA2	E CD	57.60	20
WV1	E CD	57.25	20
SC1	E CD	57.05	20
TN1	EFCD	56.75	20
MA1	GEFCD	55.00	12
MO1	GEFCDH	53.35	20
MD1	GEFCDH	53.35	20
KY1	GEFIDH	52.45	20
KY2	GEFIDH	52.30	20
OH1	GEFIDH	52.00	20
NC1	GEFI H	51.50	20
OH2	GEFI H	51.50	20
DE2	GJFI H	47.95	20
NC6	GJFI H	47.90	20
DE1	GJ I H	46.55	20
NC5	J I H	45.77	17
NC3	J I H	44.60	20
WV2	J I H	44.35	20
NY1	J I	43.70	20
VA2	J	42.35	20

F Value =18.72; Pr>F < 0.0001

19. Number of compound leaves

Pop	Grouping	Mean	N
NY2	A	3.85	20
PA2	B A	3.10	20
SC1	B C	2.70	20
OH1	B CD	2.55	20
WV3	BECD	2.50	20
NC1	FBECD	2.35	20
NC6	FBECD	2.20	20
IN1	FBECD	2.20	20
NC5	FBECD	2.18	17
PA1	FBECD	2.15	20
TN1	FBECD	2.15	20
VA1	FBECD	2.15	20
DE1	F ECD	2.10	20
MD1	F ECD	2.10	20
MO1	F ECD	2.10	20
NC3	F ECD	2.10	20
NY1	F ECD	2.05	20
OH2	F ECD	2.05	20
WV2	F ECD	2.05	20
KY2	F ECD	2.05	20
KY1	F ECD	2.00	20
WV1	F ECD	1.95	20
DE2	F ECD	1.90	20
NC2	F E D	1.70	20
VA2	F E	1.55	20
MA1	F	1.42	12

F Value = 6.51; Pr>F < 0.0001

20. Length three terminal leaflets (cm)

Pop	Grouping	Mean	N
TN1	A	23.40	20
WV3	B A	21.50	20
MA1	B AC	21.25	12
MO1	B AC	21.15	20
MD1	B AC	21.15	20
DE2	BDAC	20.40	20
NC2	BDAC	20.35	20
SC1	BDAC	19.95	20
KY1	BD C	19.60	20
OH1	BD C	19.35	20
OH2	BD C	19.15	20
NC3	BD C	19.00	20
IN1	BDEC	18.95	20
PA2	BDEC	18.80	20
PA1	BDEC	18.80	20
WV2	BDEC	18.45	20
KY2	BDEC	18.45	20
WV1	BDEC	18.45	20
DE1	BDEC	18.35	20
VA1	BDEC	18.05	20
NC5	BDEC	18.00	17
NY2	DEC	17.90	20
VA2	FDE	16.95	20
NC6	FDE	16.90	20
NC1	F E	15.45	20
NY1	F	14.20	20

F Value = 8.24; Pr>F < 0.0001

21. Length largest inflorescence (cm)

Pop	Grouping	Mean	N
WV3	A	38.55	20
NY2	B A	37.60	20
NC3	B A C	36.75	20
NC1	BDA C	34.90	20
SC1	EBDA C	33.95	20
KY1	EBDA C	33.15	20
TN1	EBDA C	32.80	20
PA2	EBDA C	32.50	20
WV1	EBDA CF	31.90	20
KY2	EBDAGCF	30.45	20
IN1	EBDAGCF	29.90	20
NC5	EBDAGCF	29.35	17
VA1	EBDAGCF	29.05	20
MO1	EBDAGCF	28.55	20
MD1	EBDAGCF	28.55	20
NC6	EBDHGCF	27.65	20
OH1	EBDHGCF	27.60	20
OH2	EBDHGCF	27.45	20
MA1	E DHGCF	26.67	3
PA1	E DHGCF	26.35	20
NY1	E DHG F	25.29	7
WV2	E HG F	23.81	16
DE1	HG F	21.13	16
DE2	HG	20.06	16
VA2	H	17.29	7
NC2	I	3.29	7

F Value = 10.39; Pr>F < 0.0001

23. Number of inflorescences

Pop	Grouping	Mean	N
NY2	A	5.35	20
WV3	B A	5.15	20
SC1	B AC	4.50	20
IN1	B AC	4.35	20
PA2	BDAC	4.30	20
WV1	BDEC	3.55	20
PA1	FDEC	3.35	20
VA1	FDEC	3.30	20
NC1	GFDEC	3.25	20
OH2	GFDEC	3.00	20
KY2	GFDECH	2.90	20
OH1	GFDECH	2.80	20
MO1	GFDECH	2.80	20
MD1	GFDECH	2.80	20
KY1	GFDECH	2.75	20
NC3	GFDE H	2.55	20
NC6	GF E H	2.35	20
DE1	GF EIH	1.90	20
NC5	GF IH	1.76	17
TN1	GF IH	1.70	20
WV2	G IH	1.53	19
DE2	IH	1.20	20
NY1	I	0.55	20
MA1	I	0.50	12
VA2	I	0.45	20
NC2	I	0.35	20

F Value = 17.37; Pr>F < 0.0001

Table 3.Tukey-Kramer multiple comparisons of morphological characteristics (continued).

25. Pedicel length (mm)				32. Staminodium width midpoint (mm)				33. Staminodium length top (mm)				34. Staminodium length midsect (mm)			
Pop	Grouping	Mean	N	Pop	Grouping	Mean	N	Pop	Grouping	Mean	N	Pop	Grouping	Mean	N
OH2	A	6.88	5	NC2	A	7.88	16	IN1	A	6.71	26	MD1	A	15.96	14
KY1	B A	6.26	5	SC1	B	6.58	19	WV3	B A	6.57	23	WV2	BA	15.03	18
PA2	B AC	5.80	5	NC3	B	6.46	12	NY1	B AC	6.13	28	KY1	BA	14.94	26
NY1	B AC	5.72	5	IN1	C B	6.35	26	SC1	B AC	6.11	18	NY1	BA	14.89	28
KY2	B AC	5.63	4	PA2	C BD	6.20	23	KY2	BDAC	6.00	16	MO1	BAC	14.31	26
NC5	BDAC	5.48	5	KY1	CEBD	6.09	26	NC2	BDAC	5.73	15	PA2	BAC	14.28	23
IN1	BDAC	5.36	5	NY1	CEBD	6.00	28	NC5	EBDAC	5.66	25	KY2	BDC	13.41	16
TN1	BDAC	5.35	4	KY2	CEBD	5.97	16	WV1	EBDAC	5.57	28	NC2	BDC	12.80	15
VA2	BDAC	5.20	5	OH2	CEBD	5.93	23	NC3	EBDAC	5.46	12	OH2	BDC	12.76	23
MO1	BDAC	4.98	5	WV2	CEBD	5.75	18	MD1	EBDACF	5.04	14	SC1	BDC	12.74	19
WV3	BDAC	4.98	5	NC5	FCEBD	5.68	25	NY2	EBD CF	4.96	24	NC5	DC	12.48	25
MD1	BDAC	4.98	5	WV3	FCE D	5.39	23	KY1	EBD CF	4.87	26	WV3	DC	12.33	23
WV1	BD C	4.74	5	WV1	FCE D	5.33	29	OH2	E D CF	4.70	23	IN1	DC	12.08	26
NY2	BD C	4.36	5	MD1	F E D	5.30	15	MO1	E D F	4.28	25	WV1	D	11.74	29
WV2	D C	4.22	5	MO1	F E	5.09	26	PA2	E F	3.98	22	NY2	D	11.71	24
NC3	D C	4.20	5	NY2	F	4.67	24	WV2	F	3.47	18	NC3	D	11.38	12
SC1	D	3.58	5												
F Value = 4.57; Pr>F < 0.0001				F Value =11.52; Pr>F < 0.0001				F Value = 7.30; Pr>F < 0.0001				F Value =8.71; Pr>F < 0.0001			

[a]For each characteristic, the Tukey-Kramer grouping indicates population means that are not significantly different at a 0.05 family-wise significance level, the sample means in descending order, and the sample size; the ANOVA F-test statistic and p-value for the test that all population means are equal is provided at the bottom of each table. Only characteristics for which the F value was .0001 or lower are shown here. All ANOVA tests were significant at the 0.05 significance level except for the characteristics bract length and staminodium length A complete set for all characteristics is available upon request from the corresponding author.

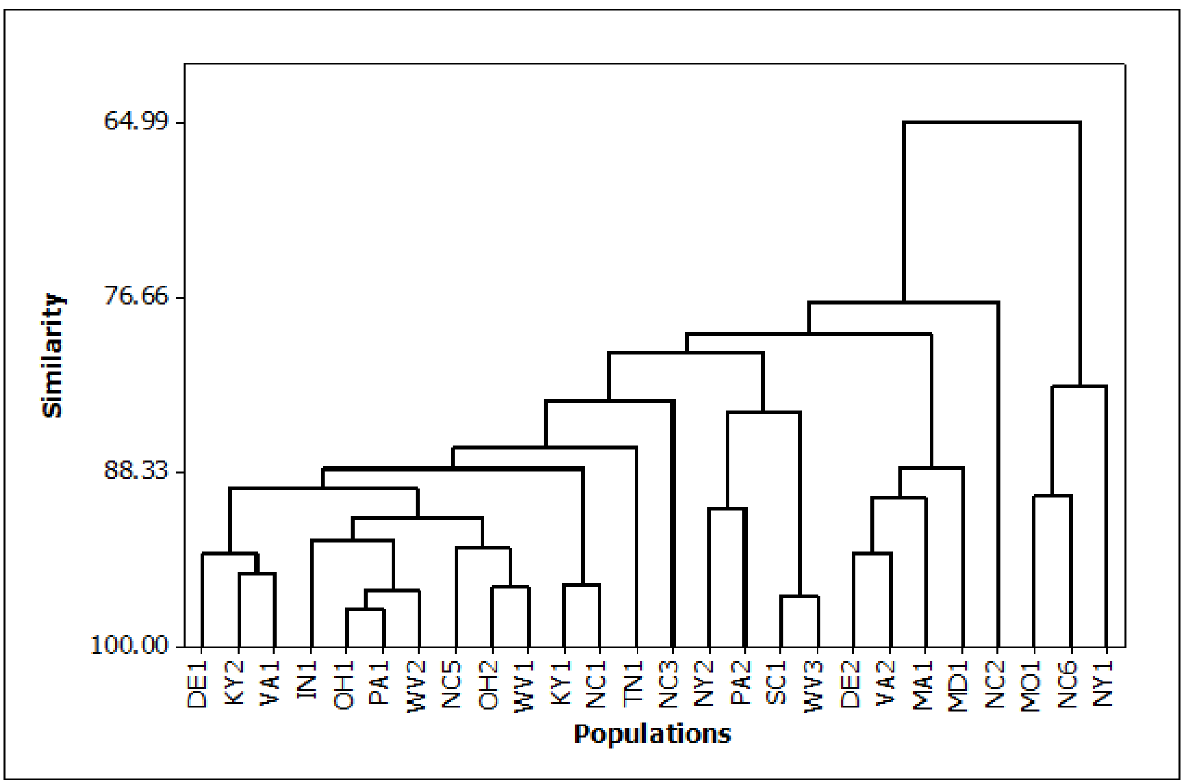

Figure 6. Dendrogram of morphologic relationships of all populations by population.
Developed using averages of non-flower data (characteristics 1-23) and the UPGMA algorithm on standardized variables based on average linkage and squared Euclidean distances.

REFERENCES

Baskin, J.M., and C.C. Baskin. 1985. Epicotyl dormancy in seeds of *Cimicifuga racemosa* and *Hepatica acutiloba*. Bulletin of the Torrey Botanical Club 112(3):253-257.

Bleiholder, H., T. Van den Boom, L. Buhr, C. Feller,H. Hack, M. Hess, R. Klose, P.D. Lancashire, U. Meier, P. Munger, R.Stauss, E. Weber, 1997. *Compendium of Growth Stage Identification Keys for Mono- and Dicotyledonous Plants*. 2nd Ed. Novartis, Basel, Switzerland. 130 p.

Blumenthal, M. 2005. Herb sales down 7.4 percent in mainstream market. HerbalGram. 66:63

Compton, J.A. 1982. *Cimicifuga* L.: Ranunculaceae. The Plantsman 14(2):99-115.

Compton, J.A., T.A.J. Hedderson, 1997. A morphometric analysis of the *Cimicifuga foetida* L. complex (Ranunculaceae). Botanical Journal of the Linnean Society 123:1-23.

Compton, J.A., A. Culham, and S. Jury. 1998. Reclassification of *Actaea* to include *Cimicifuga* and *Souliea* (Ranunculaceae): Phylogeny inferred from morphology, nrDNA ITS and cpDNAtrnL-F sequence variation. Taxon 47(3):593-634.

Cruden, R.W. 1977. Pollen-ovule ratios: A conservative indicator of breeding systems in flowering plants. Evolution 31(1):32-36.

Hamrick, J.L., and M.J. Godt.1989. Allozyme diversity in plant species. *In*A.H.D. Brown,M.T. Clegg, A.L. Kahler, B.S. and Weir, eds. *Plant Population Genetics, Breeding and Germplasm Resources*. Sinauer, Sunderland, MA.pp. 43-63.

Kartesz, J.T. 1999. A synonymized checklist and atlas with biological attributes for the vascular flora of the United States, Canada, and Greenland. *In* J.T. Kartesz and C.A. Meacham, ed. *Synthesis of the North American Flora*. Garden NCB, Chapel Hill, NC.

Kramer, C.Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics 12:307-310.

Lance, G.N, and W.T. Williams. 1967. A general theory of classificatory sorting strategies, I. Hierarchical systems. Computer Journal 9:373-380.

Lee, H. and C. Park. 1994. A systematic study on the *Cimicifuga foetida* L. complex and related species (Ranunculaceae). J. Plant Biol. 37(1):111-124.

Lloyd, J.U., and C.G. Lloyd. 1887. *Cimicifuga racemosa*. *In* Drug plants of North America. Bulletin of the Lloyd Library and Museum of Botany, Pharmacy and MateriaMedica.30 p.

Lueck, L., L.E. Craker, and T. Motley. 2003. Black cohosh - genetic diversity of a medicinal plant at risk. Abstract. HortScience 38(5):863.

Lyke, J. 2001. Conservation status of *Cimicifuga rubifolia*, *C. americana*, and *C. racemosa*. Medicinal Plant Conservation (August issue) pp. 22-24.

Matlack, G.R. 1994. Plant species migration in a mixed-history forest landscape in eastern North America. Ecology 75(5):1491-1502.

Popp, M., R. Schenk, and G. Abel. 2003. Cultivation of *Cimicifuga racemosa* (L.) Nuttal and quality of CR extract BNO 1055. Maturitas 44(Suppl. 1):S1-S7.

Ramsey, G.W. 1965. A biometric study of the genus *Cimicifuga* (Ranunculaceae). Ph.D. dissertation. Univ. of Tennessee, Knoxville, TN.

Ramsey, G.W. 1986. A biometrical analysis of terminal leaflet characteristics of the North American *Cimicifuga* (Ranunculaceae). Virginia Journal of Science 37(1):3-8.

Ramsey, G.W.1987. Morphological Considerations in the North American *Cimicifuga* (Ranunculaceae). Castanea 52(2):129 -141.

Robbins, C.S. 1999. Comparative analysis of management regimes and medicinal plant trade monitoring mechanisms for American ginseng and goldenseal. Conservation Biol. 14(5):1422-1434.

Sneath, P.H.A., and R.R. Sokal.1973. *Numerical Taxonomy*. Freeman, San Francisco.573 p.

Thomas, A.L., D. Lubhan, W. Folk, G. Rottinghaus, J. Miller, S. Woodbury, W. Applequist, L. Havermann, and J. Salick. 2001. Black cohosh cultivation in Missouri, and quantification of its medicinal compounds in response to various cultivation regimens. 2001 Field Day Report.Southwest Center of the Missouri Agricultural Experiment Station. Mt. Vernon, MO. Accessed Dec 12, 2003 at: http://aes.missouri.edu/swcenter/fieldday/page53.stm

Tukey, J.W. 1953. The problem of multiple comparisons.Unpublished manuscript.*The Collected Works of John W. Tukey VIII. Multiple Comparisons: 1948-1983*. Chapman and Hall, NY.