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PATTERNS OF GENETIC VARIATION IN SOUTHERN APPALACHIAN POPULATIONS OF *ATHYRIUM FILIX-FEMINA* VAR. *ASPLENIOIDES* (DRYOPTERIDACEAE)

Kimberly L. Sciarretta,*,† Erin Potter Arbuckle,†,‡ Christopher H. Haufler,[1],§ and Charles R. Werth[2]

*Department of Biology, University of Virginia, Charlottesville, Virginia 22901, U.S.A.; †Mountain Lake Biological Station, University of Virginia, Pembroke, Virginia 24136, U.S.A.; ‡Department of Biology, College of William and Mary, Williamsburg, Virginia 23186, U.S.A.; and §Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, Kansas 66045-2106, U.S.A.

Allozyme variation (17 loci coding 11 enzymes) was investigated in 14 populations of the fern *Athyrium filix-femina* var. *asplenioides* arrayed at differing elevations and latitudes in the southern Appalachians. Allozyme fingerprints showed that *asplenioides* individuals comprise meandering, overlapping clones usually ≤3 m in extent, occasionally forming larger clones of up to 17 m. Levels of genetic variability in populations (means: $A = 2.01$, $P = 32.8$, $H = 0.115$) were near the averages for both ferns and seed plants. General conformance to Hardy-Weinberg expectations indicated a predominantly outcrossing mating system. Hierarchical F statistic analysis and occasional deficits and excesses of heterozygotes indicated population substructure. Similar allele frequencies across all populations resulted in low to moderate F_{ST} values (mean $F_{ST} = 0.069$; range $= 0.013 - 0.112$) and high values of genetic similarity (mean $S = 0.944$; mean $I = 0.992$). Hierarchical analysis indicated that neither regional proximity ($F_{XY} = -0.009$) nor elevation ($F_{XY} = -0.007$) contributed substantially to divergence among populations ($F_{XY} = 0.056$), a result corroborated by UPGMA analysis that clustered together populations from different regions and of different elevational class. Southern Appalachian *asplenioides* differed from more eastern *asplenioides* populations of the piedmont and coastal plain in having higher frequencies of $Pgm\text{-}2^c$ and $Tpi\text{-}2^B$, alleles characteristic of the more northern variety *angustum*. Nonetheless, genetic distinctness of the two varieties was maintained. We hypothesize that higher frequencies of *angustum* alleles in the southern Appalachian *asplenioides* populations are the result of introgression from *angustum* that persisted at high elevations as both taxa migrated northward following the retreat of the Wisconsinan glacier.

Keywords: speciation, ferns, isozymes, introgression, biogeography.

Introduction

The degree to which genetic variation of plant species is structured, i.e., patterned rather than random in space, differs among plants with different life-history attributes, indicating causal links to several factors (Loveless and Hamrick 1984). Among the most influential factors are the dispersibility of propagules that integrate populations genetically and the mode of reproduction that influences the fate of dispersed propagules and the maintenance of genetic variation. Reproduction of many plant species involves a combination of sexual and vegetative reproduction, resulting in challenges to defining and recognizing genetic individuals for population genetic analysis (Cook 1983; Ellstrand and Roose 1987; Wolf et al. 1991; Parks and Werth 1993).

Ferns and other pteridophytes are of special interest because their life-history features may constrain their rates of geographic divergence and speciation. Wind-borne fern spores are capable of dispersing great distances, as evidenced by disjunct occurrences of numerous fern species (Wagner 1972) and multiple long-distance colonizations of some species (Ranker et al. 1994; but also see Vogel et al. 1999). The effectiveness of long-distance dispersal in integrating populations of fern species is suggested by isozyme data sets that show near genetic homogeneity across wide ranges and thus a lack of isolation by distance (Soltis and Soltis 1987; Soltis et al. 1988; Wolf et al. 1991). An exception is the genus *Osmunda*, species of which showed substantial genetic regionalism, perhaps a result of their short-lived green spores (Li and Haufler 1994).

In angiosperms, interspecific isolating mechanisms may evolve rapidly as species-specific interactions between pollinators, stigmatic surfaces, styles, pollen tubes, and flowering times (Arnold 1997), perhaps explaining higher rates of angiosperm diversification associated with biotic pollination modes than with abiotic modes (Ricklefs and Renner 1994; Dodd et al. 1999). In ferns, dispersal of spores is usually abiotic, as are the simple, rather stereotyped sexual apparatus and process, i.e., the simple organs (archegonia and antheridia) borne on gametophytes and water-mediated fertilization by flagellated sperm. These features may combine to constrain the origin of reproductive isolation mechanisms in ferns, as reflected in lower rates of endemism for ferns than for angiosperms. For example, in Hawaii the proportion of

[1] E-mail vulgare@ku.edu.
[2] Deceased.

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Table 1

Locality Information for 14 Investigated Populations of *Athyrium filix-femina* var. *asplenioides*

Region and population	Locality	Elevation (ft)	Collection strategy	Number sampled
Shenandoah area:				
Hawksbill	Virginia, Madison County, Shenandoah National Park along upper Hawksbill trail west of Skyline Drive	3365–4051	Continuous transect along road from summit, arbitrarily divided into three successive subpopulations	67
Big Meadows	Virginia, Madison County, Shenandoah National Park along Big Meadows Nature Trail and Dark Hollow Falls Trail off Skyline Drive	3490	Transects through two naturally separated subpopulations: (1) along Nature Trail, (2) 500 m downslope along Dark Hollow Falls trail	57
Deerfield	Virginia, Bath County, woods and creek side west of junction of routes 629 and 392 near Deerfield	2600	Transect through a small population of ca. 30 m extent; no subpopulations designated	32
Bubbling Spring	Virginia, Bath County, along creek above Bubbling Spring, Forest Road 129 south of junction with route 633, south of Milbourn	1885	Transect through a dense population of ca. 200 m extent; no subpopulations designated	41
Cranberry Mountain area:				
Tea Creek	West Virginia, Pocahontas County, wooded talus and road bank along Willow River Trail at Tea Creek campground	3000	Transect along trail, divided arbitrarily into two contiguous subpopulations	43
Mountain Lake area:				
Wind Rock	Virginia, Giles County, along Appalachian Trail in the vicinity of intersection with route 613	4000	Transects through three contiguous subpopulations separated by road	54
Fern Gully	Virginia, Giles County, along small creek (tributary of Hunters' Branch), southeast side of route 613, 1 mi southwest of Mountain Lake Biological Station	3850	Transect along creek arbitrarily divided into four successive subpopulations	56
Pond Drain	Virginia, Giles County, above Pond Drain and along road to White Pine Lodge northwest of route 613 and Mountain Lake	3700–3850	Transect through two arbitrarily designated subpopulations separated by ca. 100 m	50
Salem	Virginia, Roanoke County, hillside northwest of route 733, north of junction with Wildwood Road north of I-81 and Salem	1625	Transect through small population; no subpopulations designated	25
Southwestern Virginia area:				
White Top Mountain	Virginia, Washington County, woods and roadside at and below summit of White Top Mountain	4800–5200	Transects through three arbitrarily designated subpopulations separated by ca. 1 km	104
Mount Rogers	Virginia, Grayson County, fir forest and blackberry thickets along trail from summit of Mount Rogers to Appalachian Trail shelter on Grayson Highlands side	5200–5730	Continuous transect along trail from summit, arbitrarily divided into four contiguous subpopulations	80
East River Mountain	Virginia, Bland County, along shady stream south-facing slope of East River Mountain, southwest of Bluefield, northeast side of route 662	2300	Continuous transect along stream, arbitrarily divided into two contiguous subpopulations	30
Clintwood	Virginia, Dickenson County, east of state route 637, 1.5 mi north of junction with state route 83, east of Clintwood	1800–2000	Continuous transect, arbitrarily divided into two contiguous subpopulations	41
Chattooga River area:				
Chattooga River	South Carolina, Oconee County, north-facing road bank and ditch on south side of US 76, Sumter National Forest, 0.8 mi east of Chattooga River and Georgia state line	1400	Continuous transect along road bank, arbitrarily divided into two contiguous subpopulations	28

endemics among pteridophytes (69%) is substantially lower than that in angiosperms (89%) (Wagner 1991).

Athyrium filix-femina (L.) Mertens *sensu lato* is a highly variable, globally distributed species complex comprising at least six regional taxa for which taxonomic rank is contentious. The most recent treatment placed four of these taxa at varietal rank within a broadly defined *A. filix-femina* (Kato 1993). Two of these varieties occur in eastern North America, *A. filix-femina* var. *angustum* (Willd.) G. Lawson (northern lady fern) and var. *asplenioides* (Michx.) Farwell (southern lady fern), differentiated by various features including degree of frond tapering, rhizome orientation, and spore morphology and color (Butters 1917; Lellinger 1985; Kato 1993; Kelloff et al. 2002). The present article focuses primarily on *asplenioides* (both taxa will henceforth be referred to by bare epithets for efficiency of wording), which ranges from New York to Florida along the east coast of the United States inland to the eastern portions of Kansas and Texas (Wherry 1961). Its distribution overlaps that of its close relative *angustum* in southern New England westward through southern Pennsylvania to Missouri and southward as outlying occurrences at higher elevations in the Appalachian mountains of Virginia, West Virginia, and Tennessee.

In a recent study comparing *angustum* and *asplenioides*, very strong differences in allele frequencies at four isozyme loci (*Idh-1*, *Pgi-2*, *Pgm-2*, and *Tpi-2*) were observed between these two taxa, including populations near the area of their overlap, in contrast to the substantially less variation among populations within the taxa (Kelloff et al. 2002). Until further sampling is carried out, it remains uncertain whether this pattern reflects differences between distinct species or a steep cline across the transition region between intraspecific taxa. A result indicating that the latter may be the case was the relatively high frequency for characteristically *angustum* alleles $Pgm\text{-}2^B$ and $Tpi\text{-}2^B$ in the only population of *asplenioides* sampled from a high elevation (1300 m), a mountain population from southwestern Virginia. All other *asplenioides* populations sampled were from lower elevations in the southeastern piedmont and coastal plain. As *angustum* has been reported from high elevations as far south as Tennessee (Wofford and Chester 1998), it was hypothesized that the southern Appalachian region could represent a transition zone between *angustum* and *asplenioides* genotypes (Kelloff et al. 2002).

We investigated patterns of allozyme variation within and among southern Appalachian populations of *asplenioides*, both to evaluate this hypothesis of geographic pattern of genetic variation and to explore features of population genetic structure and processes. We sampled from populations arrayed (1) in a latitudinally related hierarchy of geographic scale ranging from adjacent individuals to a maximum separation distance of 594 km and (2) over a range of elevations

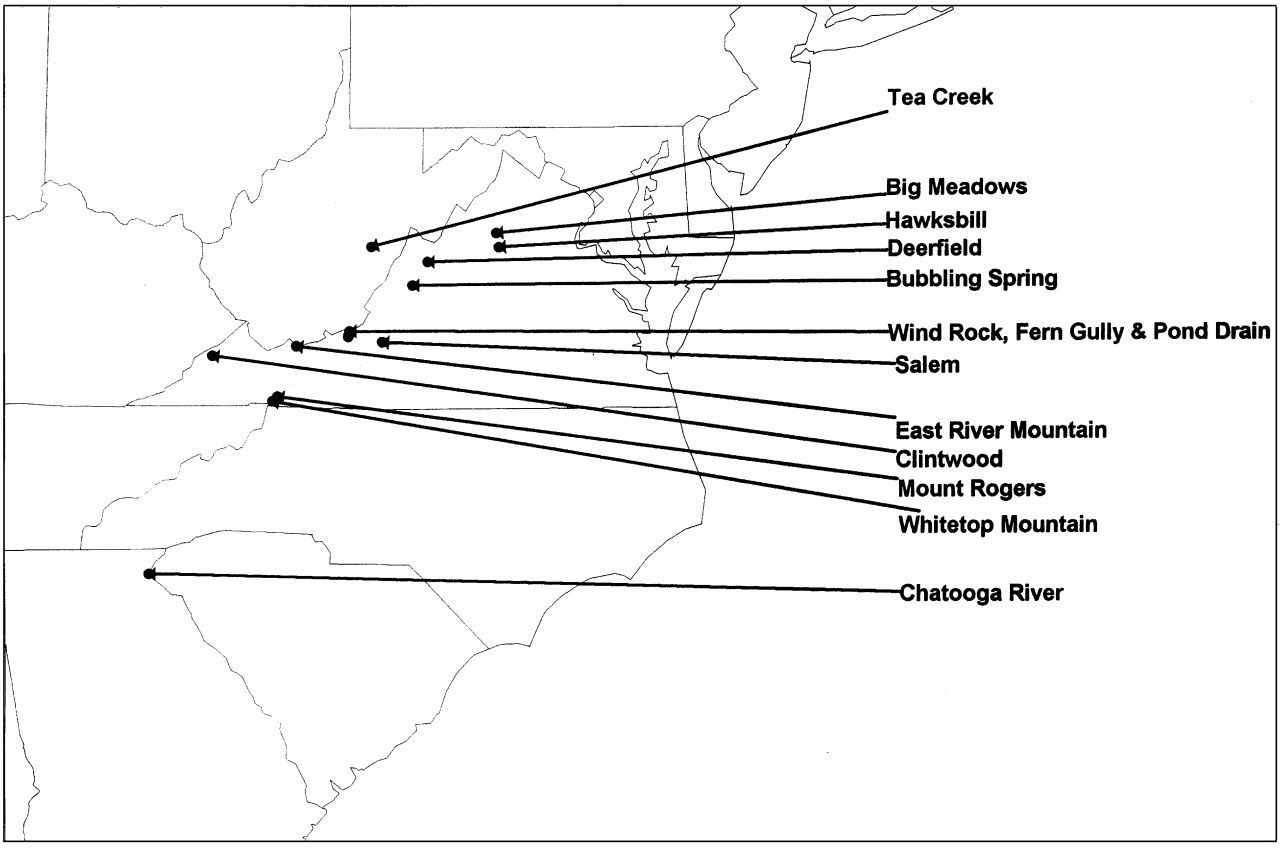

Fig. 1 Map showing distribution of sampling localities for *Athyrium filix-femina* var. *asplenioides* in the southern Appalachians. Most dots represent single localities, the exception being a set of three populations (Wind Rock, Fern Gully, and Pond Drain) occurring on Salt Pond Mountain.

from 430 to 1750 m. Our goal was to determine whether southern Appalachian populations of *asplenioides* are extensively clonal, whether they are predominantly outcrossing or inbreeding, whether they exhibit spatial genetic structure, and whether there are significant differences in the allele frequencies in relationship to latitude and/or altitude, especially as might be influenced by introgression from *angustum*. This article extends and deepens the sampling of *A. filix-femina* populations, and the results reported here complement those of Kelloff et al. (2002). Thus, these represent companion articles that emphasize different facets of the systematics and evolutionary history of this complex taxon.

Material and Methods

Plants of *Athyrium filix-femina* var. *asplenioides* were collected from 14 localities, each designated as a population, occurring along the Appalachian corridor from northern Virginia to South Carolina (table 1; fig. 1). All populations comprised plants exhibiting exclusively *asplenioides* habit and leaf morphology, with the exception of the northernmost population, Hawksbill, Virginia, at which an estimated 5%–10% of the plants exhibited tapering leaf bases suggestive of *angustum*. Two of the *asplenioides* populations, Mount Rogers and White Top, Virginia, included predominantly the broad-leaved form *A. filix-femina* var. *asplenioides* forma *subtripinnatum* Butters characteristic of high elevations in southern Appalachians. Occasional *subtripinnatum* individuals also occurred at the Wind Rock, Virginia, site. Sample sizes ranged from 25 to 104 ramets (a ramet constituted a crown of leaves) per population. At most localities, two to four subpopulations were designated, comprising either natural microgeographic units or arbitrary subdivisions.

Samples consisting of one frond obtained from each ramet were placed in sealable bags and kept refrigerated until isozyme electrophoresis could be performed. The color of the rachis (green vs. red), a single-locus genetic marker encoding dominant (red) versus recessive (green) phenotypes (Andersson-Kottö 1931; Schneller and Schmid 1982), was noted for each sample. Multilocus molecular genotypes were determined for each ramet using isozyme gel electrophoresis on 11%–12%-starch gels (methodology of Werth [1985]) for 11 enzymes as follows: on the lithium hydroxide electrophoretic buffer system (Werth 1985) were assayed hexokinase (HK), leucine aminopeptidase (LAP), and glutamate-oxaloacetate transaminase (GOT); on system 6 of Soltis et al. (1983) were assayed phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and triose phosphate isomerase (TPI); on the morpholine-citrate pH-8.2 system (Werth 1991) were assayed aldolase (ALD), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase

Table 2

Clones Discovered in Southern Appalachian Populations of *Athyrium filix-femina*

Population and ramet numbers	Estimated size	Ramets sampled	Multilocus genotype									Rachis color	P_{gen}
			Hk	*Idh*	*Lap*	*Mdh1*	*Mdh4*	*Pgi-2*	*Pgm2*	*Skdh*	*Tpi-2*		
Hawksbill 5a, 5b	1.5 m		BB	AB	CC	AA	AA	EE	BC	BB	AB	Green	0.0267
Hawksbill 11, 12	3 m		BB		CD	AB	AA	EG	BC	BB	BB	Green	0.000034
Hawksbill 13–17	10 m		BB		BD	AA	AA	EE	BC	BC	AA	Red	0.000005
Hawksbill 18, 19	2 m	2	BB		CC	AA	AA	EE	CC	BB	AB	Red	0.038
Hawksbill 23a, 23b	2 m	2	BB		CC	AA	AA	DE	BC	BB	AA	Red	0.00115
Hawksbill 25a, 25b	2 m		BB	AA	CC	AA	AA	EE	BB	BB	AB	Red	0.025
Bubbling Spring 27a, 27b	2 m		BB	BB	CC	AA	AA	EE	CC	BB	AC	Red	0.00514
Big Meadows 4a–4n	13 m, 25 m^2	11	BB	AA	CC	AA	AA	EF	BB	BB	BC	Red	0.000484
Big Meadows 5b–5l	7 m, 35 m^2	10	BB	AB	DD	AA	AA	EE	BC	BB	AB	Red	0.000251
Big Meadows 6a–6h	3 m, 9 m^2	8	BB	AC	CD	AA	AA	EE	CC	BB	AB	Red	0.000308
Salem 1–3		3	BB	AA	CC	AA	AB	EE	BB	BB	AC	Red	0.00420
Salem 4–5			BB	AA	CC	AA	AB	EE	BB	BB	BC	Red	0.000841
Salem 6–10			BB	AA	CC	AA	AA	EE	BC	BB	AC	Red	0.105
Salem 12–20		8	BB	AA	CC	AA	AA	EE	CC	BC	AC	Red	0.026
Wind Rock Q2 la–1n	17 m, 129 m^2	14	BB	AA	CC	AA	AB	EE	BC	BB	BB	Green	0.000668
Wind Rock Q3 1a–6b[a]	4 m, 16 m^2	12	BBC	AAA		AAA	AAA	EEF	CCC	BBB	ABB	Green	0.000105
Wind Rock Q4 4a, 4b	2 m	2	BB	AA	CC	AA	AA	ED	BB	BB	AC	Green	0.000486
Wind Rock Q4 7a, 7b	2 m	2	BB	AA	CC	AA	AA	EE	BC	BB	AC	Red	0.0213
Fern Gully Q1 1a, 1b	2 m	2	BB	AA		AA	AA	EE	BC	BB	AB	Red	0.12
Fern Gully Q1 4a, 4b	2 m	2		AA		AA	AA	EE	CC	BB	AB	Red	0.029
White Top Q2 7a–7g	3 m, 9 m^2	7	BB	AA	CC	AA	AA	EE	CC	BB	AC	Red	0.018
White Top Q2 10a, 10c, 10d	2 m, 4 m^2		BB	AB	CC	AA	AA	EE	BB	BB	BC	Red	0.000144
White Top Q2 11a, 11b, 11d	2 m, 4 m^2	2	BB	AA	EE	AA	AA	EE	BB	BB	BB	Red	0.000306
White Top Q2 12b, 12c	2 m	2	BB	AA	CC	AA	AA	EE	BC	BB	AC	Green	0.0286

Note. Estimated size, number of ramets sampled, and multilocus allozyme and rachis-color genotype are provided for each clone. Genotypes for loci that vary among clones are provided; all clones were homozygous for the commonest allele at loci not listed.

[a] Wind Rock subpopulation 3 comprised a single clone possessing a triploid genotype, as indicated by asymmetric band intensities at heterozygous loci.

(6PGD), and shikimate dehydrogenase (SKDH). Electrophoretic band patterns, highly comparable to those previously reported in *Athyrium* and related fern genera (cf. Gastony and Darrow 1983; Haufler et al. 1985; Kelloff et al. 2002), were interpreted genetically. Alleles were designated by letters consistent with those previously assigned (Kelloff et al. 2002). To verify allele identities, a standard individual of known genotype was run on each gel.

Data Analysis

Data were analyzed using the BIOSYS computer program (Swofford and Selander 1981) for most analyses and the Matlab program (Mathworks 1997) for the Mantel matrix comparison test. To ensure rational estimation of population genetic parameters, the extent of vegetative reproduction to form clones was evaluated by comparing multilocus genotypes of paired ramet samples, separated by 1–2 m, obtained in many of the populations. In addition, intensive analysis was carried out on several large patches of ramets hypothesized to have derived at least in part from clonal spread. Confidence of clonal identity was evaluated by computing P_{gen}, the probability that two successively sampled ramets with the same genotype could be from different clones, and P_{SE}, the probability of second encounter of a genotype given the sampling effort (Parks and Werth 1993).

Allele frequencies were computed on the basis of the set of genets in each population; i.e., each clone was represented in the data set as one individual, regardless of the number of ramets sampled from that clone. From the allele frequencies, three indices of genetic variability were computed for each population: mean number of alleles per locus (A), percentage of loci polymorphic (P), and mean expected heterozygosity (H). The breeding system of southern Appalachian *asplenioides* was evaluated by testing conformance to Hardy-Weinberg expected genotype proportions using χ^2 analysis (with pooling if more than alleles were present at a given locus) and by computing the fixation index (F) for each polymorphic locus in each population. The χ^2 test was considered valid if at least two of the three genotypic classes were represented by expected values ≥ 5.

The distribution of genetic variation as related to spatial scale and elevation was evaluated using Wright's F statistics (including hierarchical analysis) (Wright 1965, 1978), paired genetic similarity of populations and subpopulations, and evaluation of isolation by distance using matrix comparison. Hierarchical F statistic analysis of subpopulations was used to analyze whether genotypes within populations were distributed randomly in space or were spatially patterned. To evaluate whether genetic similarity of subpopulations is predicted by their geographic proximity, values of Rogers's similarity (S) were computed for each pair of subpopulations, and unweighted pair-group method (UPGMA) clustering was carried out to produce a dendrogram. To evaluate variance of allele frequencies among populations, values of F_{ST} were computed for each locus across the array of 14 populations and were statistically compared to the null hypothesis that $F_{ST} = 0$ using contingency χ^2 analysis. Hierarchical F statistic analysis was carried out to evaluate and compare the relative influence of geographic proximity and elevation on allele frequency divergence among populations. Analysis was carried out separately for populations grouped into their respective regions, as designated in table 1, and into two altitudinal groups, high elevation (>1000 m) and low elevation (≤1000 m). Values for Nei's (1978) genetic identity (I) and Rogers's (1972) genetic similarity (S) were computed for each pair of populations. Populations were clustered using UPGMA on the basis of S values, resulting in a dendrogram displaying associations among the 14 populations. To test a null hypothesis of randomness in relatedness of populations, the relationship between geographic distance of each pair of populations and pairwise values for Rogers's genetic distance ($1 - S$) was evaluated using the Mantel matrix comparison test (Mathworks 1997).

Results

Data were obtained for 17 interpretable loci coding the 11 enzymes assayed. One locus (*Pgi-1*) was monomorphic, while the remaining 16 loci (*Ald*, *Got*, *Hk*, *Idh-1*, *Lap*, *Mdh-1*, *Mdh-2*, *Mdh-3*, *Mdh-4*, *Pgi-2*, *Pgm-2*, *6-Pgd-1*, *6Pgd-2*, *Skdh*, *Tpi-1*, and *Tpi-2*) showed varying degrees of polymorphism. Most individuals possessed conventional one-allele homozygous or two-allele heterozygous phenotypes characteristic of diploid organisms. However, occasional individuals

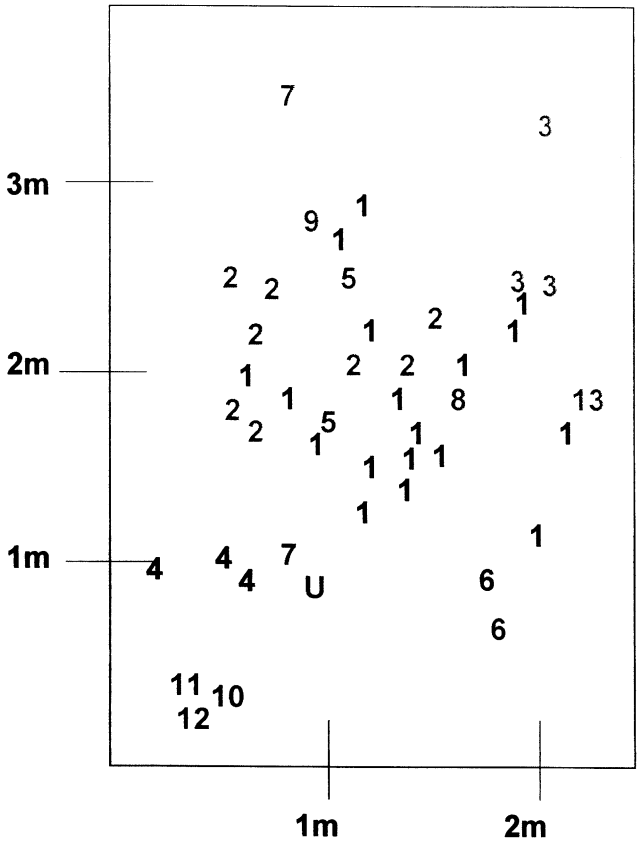

Fig. 2 Map of ramets comprising 13 genets (numbered) in a dense roadside patch of *Athyrium filix-femina* var. *asplenioides* at the Wind Rock locality. The genotype of ramet *U* was not determined.

Table 3

Allele Frequencies in 14 Populations of *Athyrium filix-femina* var. *asplenioides* from the Appalachian Mountains of Virginia, West Virginia, and South Carolina

Locus, allele	Hawksbill, VA	Big Meadows, VA	Deerfield, VA	Bubbling Spring, VA	Tea Creek, WV	Wind Rock, VA	Fern Gully, VA	Pond Drain, VA	Salem, VA	White Top, VA	Mount Rogers, VA	East Rier Mountain, VA	Clintwood, VA	Chattooga River, SC
Ald:														
A	1.000	1.000	0.983	1.000	0.987	1.000	1.000	0.968	1.000	0.987	0.980	1.000	1.000	1.000
B	0	0	0.017	0	0.013	0	0	0.021	0	0.006	0.020	0	0	0
C	0	0	0	0	0	0	0	0.011	0	0.006	0	0	0	0
n	56	28	29	38	38	38	51	47	11	80	75	29	40	28
Got:														
A	0	0.036	0	0	0.012	0	0	0	...	0.005	0.006	0	0	0
B	0.980	0.964	1.000	1.000	0.988	1.000	1.000	0.980	...	0.989	0.975	0.983	0.966	1.000
C	0.020	0	0	0	0	0	0	0.010	...	0	0.019	0.017	0.034	0
D	0	0	0	0	0	0	0	0.010	...	0.005	0	0	0	0
n	25	28	29	16	43	37	53	49	...	92	81	29	29	1
Hk:														
A	0	0	0.024	0	0.035	0	0	0.016	0	0.028	0.065	0	0.025	0
B	1.000	0.964	0.976	0.981	0.942	0.971	0.976	0.953	1.000	0.950	0.812	0.931	0.887	0.909
C	0	0.018	0	0.019	0.023	0.029	0.024	0.031	0	0.022	0.117	0.069	0.050	0.091
D	0	0.018	0	0	0	0	0	0	0	0	0.006	0	0.012	0
E	0	0	0	0	0	0	0	0	0	0	0	0	0.025	0
n	46	28	21	27	43	34	42	32	11	90	77	29	40	22
Idh:														
A	0.667	0.768	0.983	0.724	0.930	0.974	0.877	0.918	1.000	0.993	0.944	0.972	0.938	0.731
B	0.222	0.196	0.017	0.263	0.058	0	0.028	0.061	0	0.007	0.049	0	0.063	0.269
C	0.056	0.036	0	0	0.012	0	0	0.020	0	0	0.006	0.028	0	0
D	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F	0.056	0	0	0.013	0	0.026	0.094	0	0	0	0	0	0	0
n	27	28	29	38	43	38	53	49	11	72	81	18	40	26
Lap:														
A	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	0.018	0.019	0	0.079	0	0	0.014	0.016	0	0.011	0	0	0	0.088
C	0.938	0.759	0.980	0.895	0.869	1.000	0.986	0.935	1.000	0.837	0.870	1.000	0.987	0.912
D	0.018	0.130	0.020	0.026	0.060	0	0	0.032	0	0.033	0.026	0	0.013	0
E	0.027	0.074	0	0	0.071	0	0	0	0	0.114	0.104	0	0	0
F	0	0.019	0	0	0	0	0	0.016	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0	0	0.005	0	0	0	0
n	56	27	25	38	42	22	37	31	11	92	77	29	38	17
Mdh-1:														
A	0.946	0.964	1.000	1.000	1.000	0.934	0.991	0.990	1.000	0.973	0.981	1.000	0.938	0.911
B	0.054	0.036	0	0	0	0.066	0.009	0.010	0	0.022	0.019	0	0.063	0
C	0	0	0	0	0	0	0	0	0	0.005	0	0	0	0.089
n	56	28	13	38	43	38	53	49	11	92	81	29	40	28

Mdh-2:														
A	1.000	0.946	1.000	1.000	1.000	0.974	1.000	1.000	1.000	0.978	1.000	1.000	0.987	1.000
B	0	0.054	0	0	0	0.026	0	0	0	0.022	0	0	0.012	0
n	56	28	13	38	43	38	53	49	11	92	81	29	40	28
Mdh3:														
A	1.000	0.946	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0	0.054	0	0	0	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0	0	0	0	0.012	0
n	56	28	13	38	43	38	53	49	11	92	81	29	40	28
Mdh4:														
A	0.973	1.000	1.000	1.000	1.000	0.987	1.000	1.000	0.909	1.000	1.000	1.00	0.987	0.982
B	0.027	0	0	0	0	0.013	0	0	0.091	0	0	0	0	0.018
C	0	0	0	0	0	0	0	0	0	0	0	0	0.012	0
n	56	28	13	38	43	38	53	49	11	92	81	29	40	28
Pgi-1:														
A	1.00	1.000	1.00	1.000	1.000	1.000	1.00	1.00	1.000	1.000	1.000	1.00	1.00	1.000
n	56		29	38	20	37	53	37		39	62	29	40	28
Pgi-2:														
A	0	0	0	0	0.012	0	0	0	0	0.005	0.012	0	0	0
B	0	0	0	0	0	0.125	0	0	0	0.027	0.012	0	0.012	0
C	0	0	0.017	0	0.012	0	0	0.010	0	0	0	0	0	0
D	0.009	0	0	0	0.023	0.042	0	0.010	0	0	0	0	0	0.071
E	0.857	0.929	0.966	1.000	0.930	0.806	1.000	0.939	1.000	0.951	0.975	1.000	0.938	0.732
F	0.045	0.018	0.017	0	0	0.028	0	0.020	0	0.011	0	0	0.012	0
G	0.089	0.054	0	0	0.023	0	0	0.020	0	0.005	0	0	0.037	0.196
n	56	28	29	38	43	36	53	49	11	92	81	29	40	28
Pgm2:														
A	0	0	0.017	0	0	0.028	0.009	0.020	0	0.016	0	0	0	0
B	0.545	0.464	0.034	0.263	0.256	0.306	0.509	0.388	0.273	0.445	0.438	0.446	0.200	0.393
C	0.455	0.536	0.948	0.737	0.733	0.653	0.481	0.582	0.682	0.533	0.562	0.500	0.800	0.607
D	0	0	0	0	0.012	0.014	0	0	0.045	0.005	0	0.036	0	0
E	0	0	0	0	0	0	0	0.010	0	0	0	0.018	0	0
n	56	28	29	38	43	36	53	49	11	91	81	28	40	28
6Pgd-1:														
A	0	0	0.089	0	0	0.015	0.010	0	0	0	0	0	0	0
B	1.000	1.000	0.911	1.000	1.000	0.985	0.981	1.000	1.000	0.993	0.992	1.000	1.000	1.000
C	0	0	0	0	0	0	0.010	0	0	0.007	0.008	0	0	0
n	41	23	28	27	41	34	52	34	11	67	62	2	40	9
6Pgd-2:														
A	0	0.018	0	0.013	0.058	0.041	0.019	0.021	0	0.054	0.031	0.103	0.037	0
B	1.000	0.964	0.983	0.987	0.942	0.932	0.981	0.947	1.000	0.870	0.951	0.845	0.962	1.000
C	0	0.018	0.017	0	0	0.027	0	0.021	0	0.071	0.019	0.052	0	0
D	0	0	0	0	0	0	0	0.011	0	0.005	0	0	0	0
n	56	28	29	38	43	37	53	47	11	92	81	29	40	19
Skdh:														
A	0	0.036	0	0.013	0.023	0.042	0.028	0	0	0.065	0.007	0.190	0	0
B	0.946	0.911	1.000	0.987	0.942	0.958	0.972	0.990	0.909	0.924	0.986	0.810	1.000	1.000
C	0.054	0.036	0	0	0.35	0	0	0.010	0.091	0.011	0.007	0	0	0
D	0	0.018	0	0	0	0	0	0	0	0	0	0	0	0
n	56	28	29	38	43	36	53	49	11	92	72	29	40	28

Table 3

(*Continued*)

Locus, allele	Hawksbill, VA	Big Meadows, VA	Deerfield, VA	Bubbling Spring, VA	Tea Creek, WV	Wind Rock, VA	Fern Gully, VA	Pond Drain, VA	Salem, VA	White Top, VA	Mount Rogers, VA	East Rier Mountain, VA	Clintwood, VA	Chattooga River, SC
Tpi-1:														
A	0.982	0.893	1.000	0.985	1.000	1.000	1.000	1.000	1.000	0.986	1.000	1.000	0.987	1.000
B	0.009	0.107	0	0.015	0	0	0	0	0	0.014	0	0	0.012	0
C	0.009	0	0	0	0	0	0	0	0	0	0	0	0	0
n	56	28	18	33	39	37	30	49	11	69	20	8	40	23
Tpi-2:														
A	0.500	0.429	0.333	0.539	0.488	0.230	0.483	0.438	0.455	0.407	0.506	0.259	0.462	0.536
B	0.402	0.339	0.074	0.250	0.337	0.419	0.200	0.250	0.091	0.346	0.414	0.155	0.200	0.107
C	0.098	0.232	0.593	0.211	0.163	0.311	0.317	0.313	0.455	0.247	0.074	0.586	0.337	0.357
D	0	0	0	0	0.012	0.041	0	0	0	0	0.006	0	0	0
n	56	28	27	38	43	37	30	48	11	91	81	29	40	28

Note. Sample sizes (*n*) are provided for each locus.

were encountered exhibiting heterozygous phenotypes of three alleles or with asymmetric band intensities. These individuals were inferred to be spontaneous triploids. Sixty-three alleles were observed across all loci, and their frequencies were computed by tallying genotypes of genets, discrimination of which is explained below.

Genetic Variation within Populations

Evaluation of vegetative reproduction. In most cases, paired ramet samples possessed different genotypes, indicating only a limited amount of clonal spread. Among the entire set of ramets sampled were discovered 24 putative clones, i.e., groups of two or more spatially associated ramets with identical multilocus genotypes (table 2). In most cases, probabilities that different clones could independently acquire the same genotype were low ($P_{gen} \ll 0.05$), and in many cases the genotypes of the clones included a rare allele such that P_{gen} was very low ($P_{gen} \ll 0.001$); thus, ramets with the same genotype could be assigned confidently to the same clone.

Clones varied in estimated size, most detected as a pair of ramets $\leq$2 m apart, a few occurring as larger patches of ramets. The largest clone encountered was an oval-shaped patch along the Appalachian Trail at the Wind Rock locality that measured 17 m $\times$ 10.6 m and comprised numerous densely spaced ramets. This patch was sampled along a transect through its longest dimension as well as near its perimeter and was found to comprise only a single genet, marked unequivocally by possession of a rare allele, *Mdh-4*^{B}. A second clonal patch at Wind Rock comprised a set of 12 remotely spaced ramets extending 4 m along talus at the base of the cliff; this clone also was unequivocally marked by possessing a triploid genotype. Additional clonal patches up to 13 m long were discovered at other localities, notably along the nature trail at Big Meadows in the Shenandoah National Park.

To evaluate the nature of interfaces between abutting clones, intensive sampling was used to map genets in a dense patch of ramets occurring on a road bank at the Wind Rock locality. The patch comprised 45 ramets in a space of ca. 3.5 m $\times$ 2.5 m and was believed to include more than one clone, because both red- and green-stiped ramets were observed in the patch. All 45 ramets were scored for stipe color and all but two for allozyme genotype. On this basis, 13 distinct genets were discriminated (fig. 2). The central portion of the patch, where ramet density was greatest, included six genets (1, 2, 3, 5, 8, and 9), of which two comprised only single ramets (genets 8 and 9), while four comprised from two (genet 5) to 16 (genet 1) ramets. External to this dense central portion of the sample area occurred ramets of genets 1 and 3; small distinct genets 4 and 6, comprising three and two ramets, respectively; and genets 10–13, each represented by only a single ramet. In addition, genotype 7 comprised two ramets separated by 2.5 m, one above and one below the dense central subpatch. Even though this genotype included only common alleles, the probability that this genotype would occur spontaneously (P_{gen}) was low (0.016). However, given that this genotype was observed, the chance that it could recur among the sample of 12 additional genets (P_{SE}) was >0.05 (0.176). Thus, it is uncertain whether genotype 7 represents an older clone now fragmented or two different genets independently acquiring the same isozyme genotype.

Allele frequencies and genetic variability in populations. Most loci exhibited similar frequency distributions across all populations, with one very common allele at a frequency of >0.75 and all other alleles at much lower frequencies (table 3). Exceptions were the two most variable loci, *Pgm-2* and *Tpi-2*, for which frequencies were more evenly distributed among two or three alleles and which exhibited a higher

Table 4

Estimates of Genetic Variability at 17 Loci in 14 Southern Appalachian Populations of *Athyrium filix-femina* var. *asplenioides*

Population	Mean sample size per locus	Mean alleles per locus	Polymorphic loci (%)[a]	Mean heterozygosity	
				Observed	Hardy-Weinberg expected[b]
Hawksbill	51.0 (2.5)	2.1 (0.3)	41.2	0.137 (0.051)	0.136 (0.049)
Big Meadows	27.6 (0.3)	2.4 (0.3)	52.9	0.148 (0.041)	0.173 (0.048)
Deerfield	23.7 (1.7)	1.7 (0.2)	17.6	0.075 (0.043)	0.063 (0.032)
Bubbling Spring	35.1 (1.5)	1.6 (0.2)	23.5	0.089 (0.046)	0.102 (0.045)
Tea Creek	40.9 (1.4)	2.2 (0.3)	47.1	0.111 (0.045)	0.113 (0.042)
Wind Rock	35.9 (0.9)	2.1 (0.3)	29.4	0.119 (0.048)	0.121 (0.048)
Fern Gully	48.5 (2.0)	1.8 (0.2)	17.6	0.104 (0.053)	0.094 (0.046)
Pond Drain	45.1 (1.6)	2.5 (0.3)	35.3	0.118 (0.048)	0.112 (0.045)
Salem	10.4 (0.6)	1.4 (0.2)	23.5	0.075 (0.041)	0.084 (0.044)
White Top	83.9 (3.5)	2.8 (0.3)	35.3	0.119 (0.046)	0.132 (0.047)
Mount Rogers	73.8 (3.7)	2.3 (0.3)	29.4	0.115 (0.044)	0.120 (0.044)
East River Mountain	25.5 (2.0)	1.6 (0.2)	29.4	0.110 (0.046)	0.114 (0.048)
Clintwood	39.2 (0.7)	2.1 (0.3)	35.3	0.100 (0.040)	0.104 (0.040)
Chattooga River	23.4 (1.9)	1.6 (0.02)	41.2	0.130 (0.046)	0.143 (0.049)
Mean	40.29	2.01	32.76	0.111	0.115

Note. Values in parentheses are standard errors.

[a] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.

[b] Unbiased estimate (see Nei 1978).

Table 5

Values for the Fixation Index (*F*) for Each Polymorphic Locus in 14 Populations of Southern Appalachian *Athyrium filix-femina* var. *asplenioides*

Population	*Ald*	*Got*	*Hk*	*Idh*	*Lap*	*Mdh-1*	*Mdh-2*	*Mdh-3*	*Mdh-4*	*Pgi-1*	*Pgi-2*	*6Pgd-1*	*6Pgd-2*	*Skdh*	*Tpi-1*	*Tpi-2*
Hawksbill, VA		[−0.020]		0.111	0.105	−0.057			[−0.028]	0.161	−0.332*			−0.057	[−0.014]	0.044
Big Meadows, VA		[−0.037]	[−0.028]	0.325	0.260	[−0.037]	[−0.057]	[−0.057]		[0.469]*	[−0.005]		[−0.028]	−0.065	0.253	0.117*
Deerfield, VA	[−0.018]		[−0.024]	[−0.018]	[−0.020]			[−0.057]		[−0.027]	[−0.042]	[−0.098]	[−0.018]			−0.392**
Bubbling Spring, VA			[−0.019]	0.547**	0.727***						−0.357*		[−0.013]	[−0.013]	[−0.015]	−0.049
Tea Creek, WV	[−0.013]	[−0.012]	−0.046	0.291	0.092					−0.047	0.006		−0.062	0.372*		−0.123
Wind Rock, VA			[−0.030]	[−0.027]		−0.070	[−0.027]		[−0.013]	0.166	0.131	[−0.015]	−0.054	[−0.043]		−0.124
Fern Gully, VA			[−0.024]	−0.027	[−0.014]	[−0.010]					−0.149	[−0.015]	[−0.019]	[−0.029]		−0.171
Pond Drain, VA	[−0.025]	[−0.016]	[−0.038]	−0.071	[−0.046]	[−0.010]				−0.041	[−0.039]		[−0.038]	[−0.010]		−0.092
Salem, VA									[−0.100]		0.405			[−0.100]		[−0.100]
White Top, VA	[0.495]***	[−0.008]	−0.039	[−0.007]	0.467***	−0.023	[−0.022]			0.195	0.002	[−0.008]	0.217*	0.082	[−0.015]	−0.042
Mount Rogers, VA	[−0.020]	[−0.020]	0.156	0.298***	−0.122	[−0.019]				[−0.019]	0.273*	[−0.008]	−0.039	[−0.011]		−0.197*
East River Mountain, VA		[−0.018]	[−0.074]	[−0.029]							−0.041		−0.011	0.215		0.024
Clintwood, VA		[−0.036]	0.280*	−0.067	[−0.013]	−0.067	[−0.013]		[−0.013]	−0.047	0.219		[−0.039]		[−0.013]	−0.068
Chattooga River, SC			[−0.100]	−0.173	[0.634]**	−0.098			[−0.018]	−0.275	0.401*					0.191
Tests showing H-W ratios[a]	0 (4)	0 (8)	3 (8)	7 (4)	4 (4)	5 (4)	0 (4)	0 (2)	0 (5)	7 (2)	7 (3)	0 (5)	4 (6)	4 (6)	1 (3)	10 (1)
Tests showing nonconformance to H-W[a]	0 (1)	0 (0)	1 (0)	2 (0)	2 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	4 (0)	0 (0)	1 (0)	1 (0)	0 (0)	3 (0)

Note. Conformance to Hardy-Weinberg (and therefore statistical difference of F from 0) was evaluated for each case using χ^2 analysis. For cases in which more than two alleles were present, categories were pooled. Tests were considered valid if two of the three genotype classes had expected values >5. Only values marked by asterisks are significant. Results of nonvalid tests (two or more expected classes <5) are in brackets.

[a] Number of nonvalid tests in parentheses.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

degree of variation in frequencies among populations. Certain alleles of very low frequency were found in only one population (private alleles *sensu* Slatkin 1985): Hk^E and $Mdh\text{-}4^C$ in Clintwood, $Mdh\text{-}1^c$ in White Top, $Mdh3^B$ and $Skdh^D$ in Big Meadows, $Pgi\text{-}1^B$ in Wind Rock, and $Tpi\text{-}1^C$ in Hawksbill.

Genetic variability index values for southern Appalachian *asplenioides* (table 4) were of similar magnitude among most populations. Especially high values of H were observed in the two northernmost populations, Hawksbill and Big Meadows, and in the southernmost population, Chattooga River ($H = 0.136$, 0.173, and 0.143, respectively), and especially low values were observed in two small low-elevation populations, Deerfield and Salem ($H = 0.063$ and 0.084, respectively). Mean observed heterozygosity values ($H_{obs} = 0.111$) were similar to the expected heterozygosity values ($H_{exp} = 0.115$) (table 4).

Conformance to Hardy-Weinberg and the breeding system. Of the 139 cases of polymorphic loci across the 14 populations, valid χ^2 analysis of conformance to Hardy-Weinberg expected values could be carried out in 67 cases, while 72 cases could not be validly tested because of skewed allele frequencies (tables 5, 6). Of the valid tests, 53 indicated conformance to Hardy-Weinberg proportions ($P \geq 0.05$), i.e., F not significantly different from 0. Of the 14 cases that deviated significantly from Hardy-Weinberg expectations, 10 had positive F values (*Tpi-2* in Big Meadows, *6Pgd-2* and *Lap* in White Top, *Pgm-2* and *Idh* in Mount Rogers, *Idh* and *Lap* in Bubbling Spring, and *Hk* in Clintwood), indicating deficiencies of heterozygotes and implying some level of inbreeding within the populations. In the other four cases, F values were negative (*Pgm-2* in Hawksbill, *Tpi-2* in Deerfield, *Pgm-2* in Bubbling Spring, and *Tpi-2* in Mount Rogers), showing excess of heterozygotes. Of the 72 invalid tests, 69 indicated conformance to Hardy-Weinberg (the three that did not indicated excess homozygotes), despite the tendency of low expected values to inflate the χ^2 value and falsely indicate significant deviation from expected. Moreover, all populations exhibited a majority of validly tested loci in Hardy-Weinberg.

Table 6

Conformance to Hardy-Weinberg Expected Values

Population	Hardy-Weinberg (H-W) ratios	Heterozygote deficit	Heterozygote excess
Hawksbill, VA	6 (3)	0 (0)	1 (0)
Big Meadows, VA	4 (7)	1 (1)	0 (0)
Deerfield, VA	0 (9)	0 (0)	1 (0)
Bubbling Spring, VA	1 (4)	2 (0)	1 (0)
Tea Creek, WV	7 (2)	1 (0)	0 (0)
Wind Rock, VA	5 (6)	0 (0)	0 (0)
Fern Gully, VA	3 (6)	0 (0)	0 (0)
Pond Drain, VA	3 (8)	0 (0)	0 (0)
Salem, VA	1 (3)	0 (0)	0 (0)
White Top, VA	6 (5)	2 (1)	0 (0)
Mount Rogers, VA	3 (6)	2 (0)	1 (0)
East River Mountain, VA	4 (3)	0 (0)	0 (0)
Clintwood, VA	6 (5)	1 (0)	0 (0)
Chattooga, River, SC	4 (2)	1 (1)	0 (0)
Tests showing H-W ratios	53 (69)	10 (3)	4 (0)
Tests showing nonconformance to H-W	14 (3)		

Note. Conformance to Hardy-Weinberg (and therefore statistical difference of F from 0) was evaluated for each case using χ^2 analysis. Tests were considered valid if two of the three genotype classes had expected values >5. The number of nonvalid tests is in parentheses.

Spatial Distribution of Genetic Variation

Analysis of within-population genetic structure. Of the total variance among all 31 subpopulations ($F_{XY} = 0.068$), roughly half was attributable to variation among subpopulations within their localities ($F_{XY} = 0.034$) and half to variation among localities with respect to the total ($F_{XY} = 0.035$) (table 7). The variance of the subpopulations with respect to the region ($F_{XY} = 0.072$) was similar to that of the subpopulations with respect to total. Thus, the variance among the subpopulations within their populations, while of small magnitude, was as great as the variance among the populations across the total study region.

In the dendrogram resulting from UPGMA clustering based on pairwise values of S, all subpopulations were clustered at $S = 0.92$ or greater (fig. 3). The smallest clusters often consisted of subpopulations from the same or neighboring localities (e.g., Hawksbill I/Hawksbill III/Big Meadows II; Fern Gully II/Fern Gully IV; Mount Rogers III/Mount Rogers IV; Wind Rock I/Wind Rock IV), although there were no cases where all subpopulations from the same locality were placed as nearest relatives, and in many cases subpopulations from distant localities were clustered together. Thus, membership of subpopulations to the same locality was not a strong predictor of genetic relatedness, but relatedness was not random with respect to geographic proximity.

Genetic divergence among populations. Values of F_{ST} ranged from 0.013 to 0.112, with a mean of 0.069 (table 8), magnitudes considered as low to moderate (Hartl 1980), although F_{ST} values were significantly greater than 0 for all loci except *Ald*, *Got*, and *Mdh-1*. The highest values ($F_{ST} > 0.70$) were exhibited by the four most variable loci, *Idh-1*, *Pgi-2*, *Pgm-2*, and *Tpi-2*.

For the hierarchical F statistic analysis of allele frequency divergence among regions (table 9), all of the variation among populations with respect to the total (combined $F_{XY} = 0.056$) was explained by variation among populations within their region ($F_{XY} = 0.064$). The value for region with respect to the total was barely negative ($F_{XY} = -0.009$), interpreted as equivalent to 0, indicating that geographic region explains none of the variance in allele frequency. Similarly, for elevation (table 10), most of the variation among populations with respect to the total ($F_{XY} = 0.056$) was explained by variation among populations within their respective elevational classes ($F_{XY} = 0.049$), with a minuscule value for the variation between the elevational classes ($F_{XY} = 0.007$).

Genetic relatedness of populations was high, with values for S ranging from 0.901 to 0.972, with a mean of 0.944;

Table 7

Hierarchical *F* Statistic Analysis of the Distribution of Genetic Variability among Subpopulations

	Comparison (F_{XY})						
Locus	Subpopulation/ total	Subpopulation/ locality	Locality/ total	Subpopulation/ region	Locality/ region	Region/ total	Total limiting variance
Ald	0.005	0.003	0.002	0.009	0.005	−0.004	0.01356
Got	0.025	0.051	−0.027	0.027	−0.024	−0.002	0.03671
Hk	0.049	0.023	0.026	0.031	0.008	0.019	0.11072
Idh-1	0.125	0.085	0.044	0.075	−0.011	0.054	0.19707
Lap	0.087	0.051	0.037	0.084	0.034	0.003	0.15411
Mdh-1	0.050	0.054	−0.004	0.045	−0.010	0.006	0.05401
Mdh-2	0.038	0.047	−0.010	0.047	0.000	−0.010	0.01489
Mdh-3	0.056	0.047	0.008	0.064	0.018	−0.009	0.00504
Mdh-4	0.027	0.000	0.027	0.040	0.040	−0.013	0.01656
Pgi-2	0.072	0.015	0.058	0.050	0.036	0.023	0.13276
Pgm-2	0.072	0.047	0.026	0.092	0.047	−0.022	0.49314
6Pgd-1	0.019	0.003	0.016	0.031	0.029	−0.013	0.01751
6Pgd-2	0.042	0.037	0.006	0.036	−0.001	0.007	0.09047
Skdh	0.056	0.026	0.031	0.071	0.047	−0.016	0.08304
Tpi-1	0.055	0.000	0.055	0.056	0.056	−0.001	0.02099
Tpi-2	0.058	0.009	0.050	0.078	0.069	−0.021	0.65245
Combined across loci	0.068	0.034	0.035	0.072	0.040	−0.005	

those for I ranged from 0.967 to 1.000, with a mean of 0.992 (table 11). In the dendrogram resulting from UPGMA based on values of S (fig. 4), most clusters did not correspond to groups of populations definable by geographic proximity or elevation, e.g., the association of the southern Virginia, low-elevation Clintwood population with Fern Gully and Pond Drain, two high-elevation populations from Salt Pond Mountain. However, close association of neighboring populations, such as Shenandoah National Park populations Hawksbill and Big Meadows, Mountain Lake populations Fern Gully and Pond Drain, and southwestern Virginia populations White Top and Mount Rogers, as well as the formation of a cluster comprising three low-elevation populations Deerfield, Salem, and East River Mountain, suggest that genetic relatedness of populations is not random. The Mantel test showed a weak positive association ($r^2 = 0.114$) between geographic distance (fig. 2) and genetic distance ($1 - S$; see table 11) that was statistically different from random ($P = 0.030$).

Discussion

The *Athyrium filix-femina* complex includes a number of significantly diverged yet interfertile regional taxa recognized by different authors at the rank of species, subspecies, or variety (reviewed by Kelloff et al. 2002). Thus, this complex seems to represent a lineage "caught in the act" of speciating and providing a useful model for investigating geographic differentiation among populations of ferns. Unlike many angiosperm genera, pteridophyte genera do not tend to form sets of localized, closely related endemic species that have originated within continental subregions such as the southern Appalachians. Exceptions are found primarily among genera with unusual life cycle aspects such as *Botrychium*, which possesses subterranean gametophytes (Wagner and Wagner 1993), and heterosporous *Isoetes* (Brunton and Britton 1999). However, in eastern North America, two genera of ferns with conventional life cycles (i.e., homosoporous and with superficial photosynthetic gametophytes), *Pteridium* and *Athyrium*, show parallel patterns of taxonomic complexity in that each has been divided into northeastern and southeastern taxa that indicate regional geographic divergence. In *Pteridium aquilinum*, a strong north-to-south cline in allele frequencies occurs across the geographic transition zone between the northern variety *latiusculum* and the southern variety *pseudocaudatum*, but the steepest part of this cline occurred within the range of *latiusculum*, indicating a lack of genetic distinction between the two varieties (Speer et al. 1998). In contrast, *A. filix-femina* shows substantial differences in allele frequencies between the two varieties *angustum* and *asplenioides*, whereas populations within the varieties have similar allele frequencies and do not vary with respect to geographic location (Kelloff et al. 2002).

The present study provides a more detailed understanding of the distribution of genetic variation in populations of *asplenioides*, contributing to a broader picture of the geography of genetic divergence in the *A. filix-femina* species complex. The data show that the levels and distribution patterns of genetic variation within and among southern Appalachian populations of *asplenioides* are largely consistent with those observed in other *Athyrium* populations (Schneller and Schmid 1982; Kelloff et al. 2002), as well as in many other widespread diploid fern species. Specifically, (1) sexual reproduction via outcrossing of gametophytes is the predominant reproductive mode in *asplenioides*, giving rise to sporophytes usually capable of limited vegetative expansion, although large clones occasionally form, perhaps through a combination of exceptional genotypes and microhabitat opportunities; (2) the spatial distribution of alleles at most loci appears to be even across the populations, but exceptions indicate that the populations are moderately genetically structured; and (3) within the region studied, *asplenioides* populations show high levels of genetic similarity but are

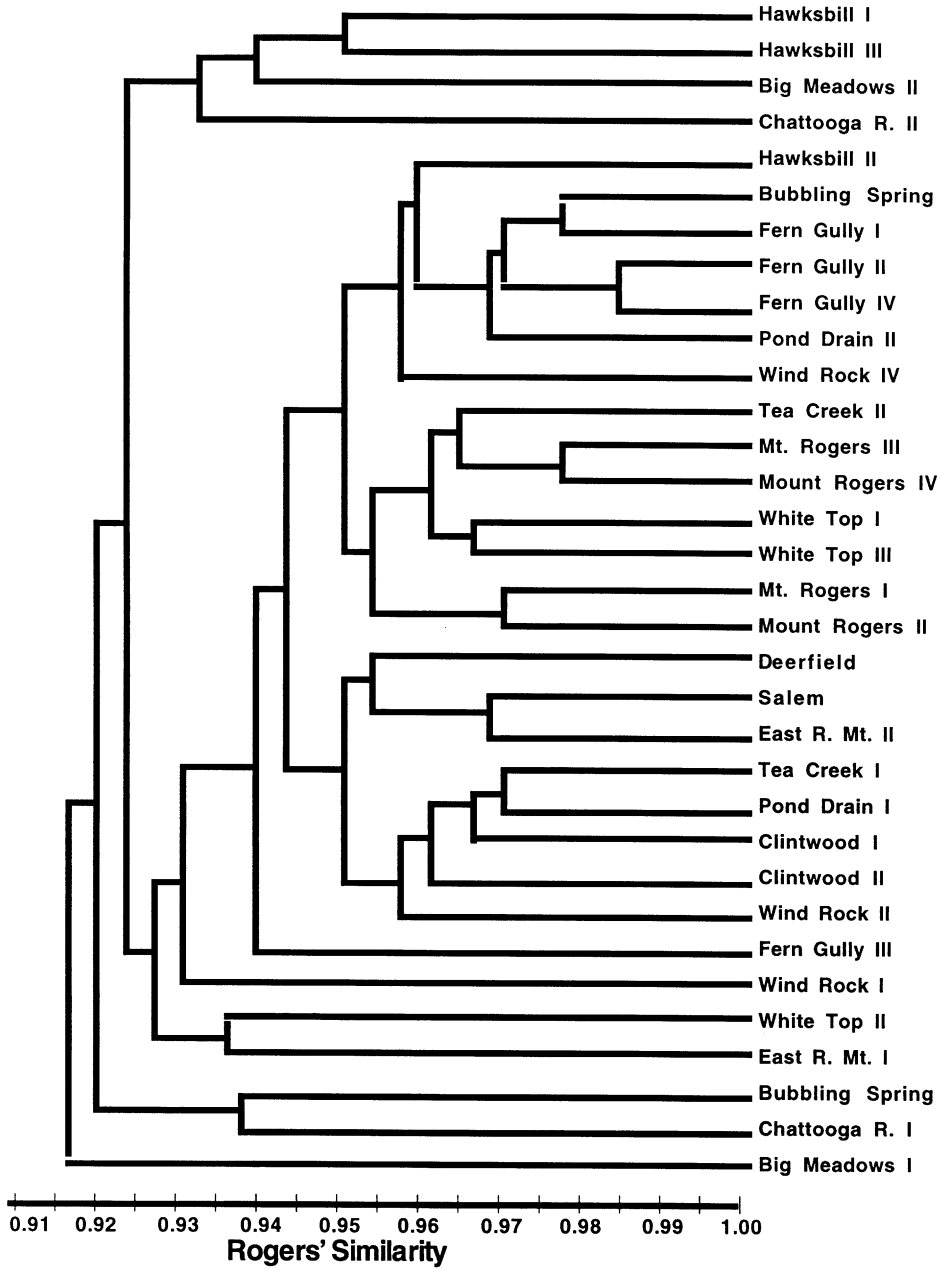

Fig. 3 Cluster analysis of 33 subpopulations using UPGMA method, based on pairwise values of Rogers's (1972) genetic similarity.

differentiated somewhat from populations of piedmont/coastal plain *asplenioides* to the east and more substantially from *angustum* populations to the north.

Levels of Genetic Polymorphism

Athyrium filix-femina sensu lato is well known as one of the most morphologically variable of fern species, as evidenced by the plethora of conspicuous genetically determined sports discovered in nature and propagated in horticulture (Shaver 1954; Kaye 1965; Smith et al. 1980). Natural populations of all subtaxa of *A. filix-femina* possess visible polymorphisms, the best-known example being rachis color, in which dominant and recessive alleles specify red and green phenotypes, respectively (Andersson-Kottö 1931; Schneller 1979; Schneller and Schmid 1982). Variation in features of leaves, such as breadth, degree of tapering, or degree to which pinna tips are acuminate, are readily observed in nature and inferred to have a genetic basis because they exhibit consistency among leaves of single plants yet differences between plants growing in close proximity. Even the distinctive southern Appalachian broad-leaved form *subtripinnatum* apparently represents such a polymorphism rather than a differentiated taxon, because *subtripinnatum* plants were

Table 8

Summary of *F* Statistics at All Loci among 14 Populations of Southern Appalachian *Athyrium*

Locus	F_{IS}	F_{IT}	F_{ST}
Ald	0.048	0.060	0.013ns
Got	−0.025	−0.009	0.015ns
Hk	0.046	0.083	0.038***
Idh-1	0.146	0.241	0.112***
Lap	0.260	0.305	0.062***
Mdh-1	−0.061	−0.022	0.037**
Mdh-2	−0.038	−0.008	0.029**
Mdh-3	−0.057	−0.004	0.050***
Mdh-4	−0.064	−0.011	0.050***
Pgi-2	0.025	0.101	0.078***
Pgm-2	0.036	0.109	0.076**
6Pgd-1	−0.066	−0.009	0.054**
6Pgd-2	0.017	0.054	0.038**
Skdh	0.066	0.124	0.061***
Tpi-1	0.151	0.205	0.063***
Tpi-2	−0.068	0.006	0.070***
Mean	0.024	0.092	0.069***

Note. Statistical departure of F_{ST} from 0 was evaluated using contingency χ^2 analysis.

** $P < 0.01$.

*** $P < 0.001$.

observed growing near plants of more typical morphology at the Wind Rock, Virginia, site, and no association between this form and allozyme genotype was detected. The prevalence of *subtripinnatum* in high-elevation populations such as Mount Rogers and White Top is enigmatic but indicates that it is somehow adaptive for the cooler climatic conditions of these sites.

Despite the impressive amount of morphological variation characteristic of *Athyrium*, levels of allozyme polymorphism were not especially high in southern Appalachian *asplenioides*. Index values (mean $A = 2.01$, range $= 1.4 - 2.8$; mean $P = 32.8$, range $= 17.6 - 52.9$; mean $H = 0.115$, range $= 0.063 - 0.173$) were comparable to the means for seed plants ($A = 1.53$, $P = 34.2$, $H = 0.113$), as compiled by Hamrick and Godt (1989), as well as to those for diploid ferns ($A = 1.65$, $P = 36.0$, $H = 0.109$), as compiled by Li and Haufler (1999). These values are slightly lower than those previously reported for *angustum* and more eastern populations of *asplenioides* (Kelloff et al. 2002). Populations with the highest values of H, i.e., Big Meadows, Hawksbill, and Chattooga River, possessed especially high frequencies of allele $Idh\text{-}1^B$, a characteristically *angustum* allele. The populations with the lowest values of H, Deerfield and Salem, were among the smallest in number of individuals and most isolated. Their low level of genetic variability may reflect founder effect.

Modes of Reproduction

Populations of *asplenioides* are formed through a combination of sexual reproduction via gametophytes, resulting in populations comprising numerous genetically distinct individuals, and vegetative extension of rhizomes, resulting in the formation of clones, a feature shared with many ferns (Sheffield et al. 1989; Parks and Werth 1993). However, most ramets routinely sampled were also distinct genets, indicating that vegetative reproduction has only a minor influence on population structure and that sexual reproduction is the most common process by which sporophyte crowns are produced. While some of the ramet pairs that had identical genotypes could underrepresent the extent of larger clones unknowingly sampled at their margins, the fact that the great majority of paired samples comprised two distinct genotypes indicates that most clonal patches were indeed small. This was verified by the intensive mapping of a multiclonal patch (fig. 2), which revealed the following: (1) clones of limited extent are formed by linear extension and branching of rhizomes in a meandering fashion; (2) growth patterns of clones are essentially independent of one another, as indicated by their substantial overlap and intergrowth; and (3) adjacent ramets are often from different clones. Such clones tend toward "guerrilla" morphology (terminology of Lovett-Doust [1981]) and probably represent the most common expression of vegetative reproduction in *asplenioides*. Dense, uninterrupted patches of single clones up to 17 m in length ("phalanx" morphology; Lovett-Doust 1981) occur, but only occasionally. These observations indicate that clone size and growth form may represent another variable phenotype in *asplenioides* that could have a genetic basis. The degree of clonality, characterized by a spreading horizontal rhizome, may be greater in *asplenioides* than in other geographic taxa of *Athyrium* such as *angustum*, characterized by a shorter rhizome, or the western variety *A. filix-femina* var. *cyclosorum* Ruprecht, which possesses a suberect rhizome.

Despite the potential of homosporous ferns to form hermaphroditic gametophytes, outcrossing is their most common mode of sexual reproduction (Haufler 2002), although a significant minority of ferns is self-fertilizing (Soltis et al.

Table 9

Hierarchical *F* Statistic Analysis of the Relative Importance of Region to the Distribution of Genetic Variability among 14 Populations of Southern Appalachian *Athyrium*

	Comparison (F_{XY})			
Locus	Locality/ total	Locality/ region	Region/ total	Total limiting variance
Ald	0.002	0.008	−0.005	0.01347
Got	0.001	0.002	−0.001	0.02478
Hk	0.026	0.012	0.014	0.10242
Idh-1	0.098	0.054	0.046	0.20507
Lap	0.049	0.051	−0.002	0.14023
Mdh-1	0.027	0.019	0.007	0.05203
Mdh-2	0.015	0.028	−0.013	0.01617
Mdh-3	0.033	0.045	−0.013	0.00762
Mdh-4	0.021	0.033	−0.013	0.02278
Pgi-2	0.066	0.040	0.027	0.13352
Pgm-2	0.062	0.091	−0.032	0.47853
6Pgd-1	0.040	0.056	−0.017	0.01965
6Pgd-2	0.026	0.017	0.010	0.08779
Skdh	0.045	0.061	−0.017	0.09159
Tpi-1	0.049	0.056	−0.008	0.02361
Tpi-2	0.056	0.078	−0.024	0.65277
Combined across loci	0.056	0.064	−0.009	

Table 10

Hierarchical *F* Statistic Analysis of Distribution of Genetic Variability with Respect to Elevation

Locus	Comparison (F_{XY})			Total limiting variance
	Locality/ total	Locality/ elevation	Elevation/ total	
Ald	0.003	0.003	−0.001	0.01347
Got	0.001	0.002	−0.001	0.02478
Hk	0.026	0.031	−0.006	0.10242
Idh-1	0.098	0.113	−0.017	0.20507
Lap	0.049	0.045	0.005	0.14023
Mdh-1	0.025	0.028	−0.004	0.05203
Mdh-2	0.015	0.016	−0.001	0.01617
Mdh-3	0.033	0.038	−0.005	0.00762
Mdh-4	0.021	0.024	−0.003	0.02278
Pgi-2	0.065	0.075	−0.011	0.13283
Pgm-2	0.062	0.049	0.014	0.47864
6Pgd-1	0.040	0.045	−0.006	0.01960
6Pgd-2	0.026	0.031	−0.005	0.08755
Skdh	0.045	0.054	−0.010	0.09144
Tpi-1	0.049	0.055	−0.007	0.02361
Tpi-2	0.056	0.034	0.023	0.65285
Combined across loci	0.056	0.049	0.007	

1988) and the breeding system can vary within species (Schneller et al. 1990). Outcrossing predominates in *A. filix-femina* populations of Europe, which form mainly unisexual gametophytes (Schneller 1979), as well as in *angustum* populations of northeastern North America and in piedmont and coastal plain populations of *asplenioides* in the southeastern United States (Kelloff et al. 2002). The present study shows that outcrossing is characteristic for southern Appalachian *asplenioides* populations as well, with most loci within all populations conforming to Hardy-Weinberg genotype proportions. Nonetheless, the number of valid tests indicating deviations from Hardy-Weinberg expectations (14 out of 67) is too great to attribute to chance alone. In each population for which loci with heterozygote deficits were observed (10 cases), other loci matched Hardy-Weinberg expectations, indicating that the level of inbreeding is not intensive, as expected under self-fertilization, and may be due instead to nonrandom distribution of some alleles within the population (structure) and localized mating (Wahlund effect). The four cases of heterozygote excesses could have resulted from disassortative mating (i.e., mating between individuals with greater-than-random genetic dissimilarity) and/or from greater fitness of heterozygotes. Disassortative mating could result from repeated localized mating between gametophytes arising from massive spore deposits of sporophytes that are homozygous for alternate alleles. The occasional formation of large clones that could saturate an area with the spores of one or a few individuals makes this scenario seem plausible. Higher fitness of heterozygotes is often observed in natural populations (Mitton and Grant 1984) and may be a consequence of genomic heterosis, as has been suggested for *Athyrium* (Schneller and Holderegger 1997) The allozyme genotype itself may not be the proximal cause of higher fitness but rather may mark fitness-conferring genes to which the enzyme locus is tightly linked. Soft selection favoring heterozygotes could be enhanced in gametophytes that form multiple zygotes by differential nurturing of zygotes with higher quality (e.g., more heterozygous) genotypes (Klekowski 1982).

Pattern of Genetic Variation within Populations

The degree of randomness of genotypes within populations is contingent, in part, on the effectiveness of spore dispersal in establishing progeny remote from parental sporophytes. Spore dispersal is known to be capable of effecting long-distance establishment of new populations and, in some cases, of resulting in random allele distributions (Suter et al. 2000). Nonetheless, high degrees of population genetic structure are known to occur in some species of rock-inhabiting ferns (Soltis et al. 1989). A variety of observations indicate that allele frequencies are not randomly distributed within populations of *asplenioides* and that some populations are moderately structured. (1) The occurrence of loci that conform to Hardy-Weinberg expectations along with other loci that show

Table 11

Matrix of Pairwise Genetic Similarity Values among 14 Populations of Southern Appalachian *Athyrium filix-femina* var. *asplenioides*

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Hawksbill		0.949	0.902	0.950	0.943	0.933	0.951	0.947	0.927	0.940	0.947	0.916	0.932	0.943
2. Big Meadows	998		0.901	0.945	0.945	0.929	0.943	0.949	0.919	0.955	0.942	0.919	0.931	0.930
3. Deerfield	967	0.978		0.936	0.940	0.935	0.939	0.946	0.954	0.926	0.922	0.936	0.952	0.915
4. Bubbling Spring	993	0.997	0.986		0.965	0.937	0.959	0.960	0.950	0.942	0.945	0.924	0.955	0.955
5. Tea Creek	991	0.996	0.988	0.997		0.952	0.953	0.970	0.952	0.964	0.966	0.935	0.963	0.933
6. Wind Rock	988	0.992	0.988	0.990	0.995		0.949	0.956	0.948	0.954	0.940	0.940	0.954	0.930
7. Fern Gully	994	0.996	0.982	0.993	0.994	0.993		0.972	0.958	0.958	0.955	0.954	0.956	0.943
8. Pond Drain	994	0.998	0.988	0.996	0.998	0.997	1.000		0.956	0.966	0.966	0.951	0.967	0.949
9. Salem	984	0.991	0.996	0.993	0.996	0.994	0.997	0.999		0.941	0.933	0.950	0.955	0.933
10. White Top	992	0.997	0.981	0.991	0.997	0.996	0.997	0.999	0.994		0.965	0.951	0.946	0.926
11. Mount Rogers	993	0.996	0.975	0.992	0.997	0.992	0.995	0.996	0.989	0.997		0.935	0.949	0.931
12. East River Mountain	980	0.988	0.985	0.983	0.987	0.991	0.994	0.994	0.995	0.993	0.984		0.937	0.917
13. Clintwood	986	0.992	0.995	0.996	0.998	0.995	0.994	0.998	0.999	0.993	0.992	0.989		0.942
14. Chattooga River	993	0.994	0.983	0.995	0.991	0.987	0.993	0.995	0.992	0.988	0.987	0.985	0.993	

Note. Values for Rogers's (1972) genetic similarity *S* are provided above the diagonal and those for Nei's (1978) unbiased genetic identity *I* below the diagonal.

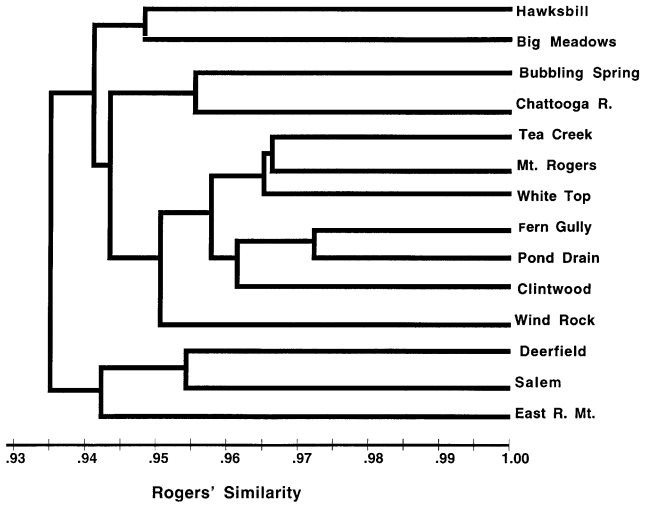

Fig. 4 UPGMA cluster analysis of 14 southern Appalachian populations of *Athyrium filix-femina* var. *asplenioides*, based on pairwise values of Rogers's (1972) genetic similarity.

heterozygote deficits in the same population is best explained by nonrandom distribution of the alleles at these loci. (2) The magnitude of the F_{ST} value was as great among subpopulations within their locality (0.034) as among localities across the entire range of the study (0.035). (3) Clusters resulting from UPGMA never comprised all subpopulations of a single population and often comprised subpopulations from different populations; if there was greater allele frequency homogeneity among the subpopulations of each population, all would form clusters corresponding to localities. (4) Rare alleles were often observed to occur in neighboring individuals rather than randomly dispersed (data not shown). Such structure is most likely the result of leptokurtic dispersal of spore masses from individual plants (Conant 1978; Raynor et al. 1976; Peck et al. 1990), such that neighbor-mating opportunities may be frequent relative to random matings.

Several individuals of *asplenioides* were inferred to be triploid on the basis of their isozyme phenotypes, including a clonal patch at the Wind Rock, Virginia, site. Spontaneous triploids have been observed previously in natural populations of *A. filix-femina* (Wagner and Wagner 1966; Schneller and Rasbach 1984), as well as in *Pteridium aquilinum* (Sheffield et al. 1993) and *Cystopteris protrusa* (Haufler et al. 1985). Such triploids probably arise from spontaneous formation of unreduced gametophytes that mate with normal haploid gametophytes (Schneller and Rasbach 1984; Haufler et al. 1985; Sheffield et al. 1993), although alternative hypotheses have been proposed (Wagner and Wagner 1966). The large size of the Wind Rock triploid clone may have resulted from hybrid vigor due to enhanced heterozygosity, as proposed for a large triploid clone of *P. aquilinum* discovered in England (Sheffield et al. 1993).

Pattern of Genetic Variation among Populations

Populations of *asplenioides* possessed similar allele frequencies across the study region spanning 594 km from northern Virginia to South Carolina, suggesting that these populations are genetically integrated by high rates of gene flow resulting from frequent long-distance dispersal of windborne spores (Soltis and Soltis 1987; Soltis et al. 1988). These similar allele frequencies were reflected in low F_{ST} values, low frequencies for the few private alleles encountered in this study, and high genetic similarities between populations. The high values of S (mean 0.944) and I (mean 0.992) are consistent with those observed for populations within the same species (Gottlieb 1977; Soltis and Soltis 1989). Clustering of taxa based on UPGMA resulted in associations that were not consistently predicted by either geographic proximity or elevation yet did not appear to be random. The departure from random was verified by the Mantel test, which indicated a statistically significant positive association between geographic and genetic distance (isolation by distance), although the effect was weak ($r^2 = 0.114$). Hierarchical F statistic analysis showed that neither regional association ($F_{XY} = -0.011$) nor elevational class ($F_{XY} = 0.009$) explained any part of the differentiation among populations. The mean F_{ST} (0.068) was well below the mean G_{ST} of 0.300 (G_{ST} is roughly equivalent to mean F_{ST}) averaged across 146 species of sexual plants but only slightly lower than the mean G_{ST} of 0.079 for plants with wind-dispersed seeds (Loveless and Hamrick 1984).

Of special interest is the poorly understood role of spore dispersal in contributing to observed patterns of genetic divergence within and among populations of ferns. It is apparent that the effectiveness of spore dispersal in homogenizing allele frequencies among sites, while complete in some species (Wolf et al. 1991; Suter et al. 2000), may be more or less constrained in others (Soltis et al. 1989). Because the sizes of spores and their mode of dispersal are similar among different species of ferns, it becomes necessary to look for other life-history features that can explain these observed differences. Our knowledge of the life history of ferns remains incomplete (Werth and Cousens 1990), even for species as intensively studied as *P. aquilinum*. Research that can directly quantify the dispersal of spores within and among populations, while challenging, would represent a very significant advance in understanding the process of genetic divergence in ferns.

Relationship between asplenioides *and* angustum

The southern Appalachian *asplenioides* populations addressed herein tended to possess higher frequencies for alleles characteristic of *angustum*, especially *Pgm-2*^{B} and *Tpi-2*^{B} and occasionally *Idh-1*^{B}, than did more eastern *asplenioides* populations. To evaluate whether the two taxa remain distinct with the addition of the southern Appalachian populations, pairwise values of S and I were computed for all 23 populations comprising the combined data of the present and the previous study (Kelloff et al. 2002) and were averaged within and between taxa (table 12). Average values for within-*asplenioides* comparisons were slightly lower in the combined data set (mean $S = 0.941$, mean $I = 0.990$) than in the previous averages among eastern populations (mean $S = 0.951$, mean $I = 0.996$), while averages for comparisons between the two taxa were slightly elevated for the combined data sets (mean $S = 0.860$, mean $I = 0.921$) relative to the

Table 12

Means of Pairwise Values for Rogers's Similarity (*S*) and Nei's Genetic Identity (*I*) for Comparisons within and between *Athyrium* Taxa *angustum* and *asplenioides*

Taxon combination	Number of comparisons	*I*		*S*	
		Mean	Range	Mean	Range
angustum/angustum	10	0.996	0.990–1.000	0.948	0.930–0.975
asplenioides/asplenioides:					
Eastern populations[a]	10	0.996	0.990–1.000	0.951	0.932–0.966
Southern Appalachian populations	91	0.992	0.967–1.000	0.944	0.901–0.972
Combined data sets	153	0.990	0.965–1.000	0.941	0.891–0.972
angustum/asplenioides:					
Eastern populations[a]	10	0.911	0.875–0.938	0.848	0.803–0.881
Combined data sets	90	0.921	0.855–0.968	0.860	0.798–0.919

[a] Data of Kelloff et al. (2002). Comparison includes one southern Appalachian population, Pond Drain, Virginia.

previous data set ($S = 0.848$, $I = 0.911$; Kelloff et al. 2002). In addition, UPGMA clustering was carried out based on the matrix of *S* values for the combined data set. In the resulting dendrogram (fig. 5), *asplenioides* and *angustum* formed distinct clusters joined at a slightly higher value of *S* (0.860) than in the previous study (0.848; Kelloff et al. 2002). The value at which all *angustum* populations were joined was unaffected and remained $S = 0.938$, whereas the value at which all *asplenioides* populations were joined was $S = 0.925$, reduced from $S = 0.945$ in the study of Kelloff et al. (2002). Ten of the 14 southern Appalachian *asplenioides* populations were placed in a single cluster that was joined at $S = 0.940$ to a cluster comprising all the eastern populations plus two of the southern Appalachian *asplenioides* populations (Clintwood and Deerfield). The two northernmost southern Appalachian *asplenioides* populations (Hawksbill and Big Meadows) formed a cluster placed outside all other *asplenioides*, to which it was joined at $S = 0.925$. The two populations comprising largely the high-elevation form *subtripinnatum* (Mount Rogers and White Top) were closely associated but did not stand out as a group distinct from other *asplenioides*. There was no evidence of either north-south or altitudinal clines; instead, there was a more haphazard pattern in the variation of frequencies for these alleles. These observations hint that the mountain populations of *asplenioides* may have experienced introgression from *angustum* and currently maintain substantial evidence of *angustum* in the northernmost populations that diminishes but does not vanish southward along the Appalachian corridor.

Nonetheless, the sharp differences in genetic similarity previously seen between *angustum* and *asplenioides* (Kelloff et al. 2002) were only slightly dulled by the addition of the data presented here, with the mean value of *S* between pairs of *asplenioides* populations reduced to 0.941 and the mean *S* value for population pairs of different taxa increased to 0.860. UPGMA analysis of the combined data sets maintained *angustum* and *asplenioides* in separate clusters joined at a similarity ($S = 0.860$) only slightly greater than that between *angustum* and eastern *asplenioides* reported by Kelloff et al. (2002). Within *asplenioides*, most of the southern Appalachian *asplenioides* populations, including two comprising primarily the high-mountain form *subtripinnatum*, were joined to form a single cluster. The two mountain populations that joined the cluster of more eastern populations were Deerfield, Virginia, a small, isolated, and genetically depauperate population, and Clintwood, Virginia, a fairly western population near the Kentucky border, both of which lacked the higher frequencies for some or all of the alleles characteristic of *angustum*. Conversely, the placement of the two northernmost Appalachian *asplenioides* populations sampled, Hawksbill, Virginia, and Big Meadows, Virginia, outside all

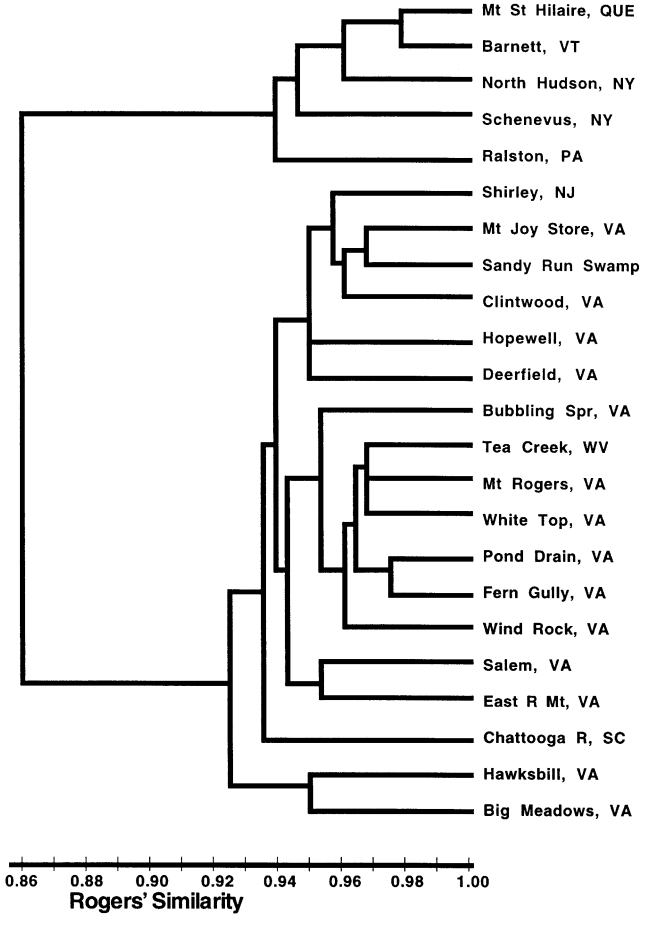

Fig. 5 UPGMA cluster analysis of 23 populations of *Athyrium filix-femina*, representing varieties *angustum* and *asplenioides*, based on pairwise values of Rogers's (1972) genetic similarity.

other *asplenioides* resulted from relatively high frequencies for *angustum*-type alleles, not only $Pgm\text{-}2^B$ and $Tpi\text{-}2^B$ but also Idh^B, Idh^C, and $Pgi\text{-}2^G$. However, only the northernmost Hawksbill population included some individuals resembling *angustum* in morphology.

Most fern species are highly genetically distinct from one another, even if highly similar in morphology, exhibiting low genetic similarities with congeners as compared to angiosperms (Haufler 1987). Almost all interspecific fern hybrids are sterile, producing aborted spores, because of failed meioses characterized by formation of numerous univalents. Whether *asplenioides* and *angustum* ultimately are considered distinct at species or infraspecific rank, they provide a rare opportunity in ferns to study very recently diverged taxa. Although the literature suggests that these two taxa intergrade extensively across their zone of overlap (Benedict 1934; Weatherby 1936; Fernald 1946; Wherry 1961), such statements have not been validated through critical analyses of either morphological or molecular character combinations. Recent evidence from allozymes and spore morphology indicated introgression of *angustum* into a population of *asplenioides* in New Jersey (Kelloff et al. 2002). However, documentation of the existence and nature of a hybrid zone between *angustum* and *asplenioides* will require more detailed investigation combining broader geographic sampling and multicharacter analysis of individual specimens, including morphometrics, spore morphology, and allozyme genotype (Kelloff et al. 2002).

A Biogeographic Hypothesis

The distribution of genetic variation varies in a complex way across the range of *A. filix-femina* in eastern North America. Geographic variation in allele frequencies may have resulted from events of migrational history that override allele frequency homogenization via gene flow. The strongest pattern is, of course, that occurring between *angustum* and *asplenioides*. It is uncertain when the divergence between *angustum* and *asplenioides* initially occurred, but likely it was during one of the glacial maxima. when a number of eastern North American species became geographically subdivided in remote refugia (Parks et al. 1994). Although the pronounced divergence between the two *Athyrium* taxa in numerous morphological features and allozyme frequencies (Butters 1917; Kelloff et al. 2002) indicates separation over an extended time period, the formation of fertile hybrids between *angustum* and *asplenioides* (Schneller 1989; Kelloff et al. 2002) indicates the potential for these diverged taxon gene pools to merge over time.

To account for the increased presence of *angustum* alleles in southern Appalachian *asplenioides* populations relative to *asplenioides* populations farther east, we hypothesize that as *angustum* migrated northward following the retreat of the Wisconsinan glacier, it remained present for an extended period at the higher elevations throughout the Appalachians, as have many woody species (Ware 1999). As *asplenioides* migrated northward into the Appalachian region, its gametophytes frequently may have encountered and mated with *angustum* gametophytes, even at low elevations in valleys where *angustum* spores may have dispersed from the mountaintops. Thus, significant introgression may have taken place between the two taxa, resulting in the lingering elevated frequencies of characteristically *angustum* alleles observed throughout the southern Appalachians. Eastward, on the piedmont and coastal plain, there are no high-elevation refugia for *angustum*; thus, frequencies for the *angustum* alleles are much lower.

Combined with the information contained in Kelloff et al. (2002), data on the patterns of populational variation for *asplenioides* included here provide a sound basis for understanding the current status of the taxonomy and phylogeny of *A. filix-femina* in eastern North America. Genetic and biogeographic data coordinate in building well-supported hypotheses about the origin of diversity and the development of lineages that are distinct in much of their range but whose boundaries are blurred. The relationship between *angusta* and *asplenioides* remains dynamic, and continued recognition of them at varietal or subspecific levels is recommended.

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Literature Cited

Andersson-Kottö I 1931 The genetics of ferns. Bibliogr Genet 8: 269–293.

Arnold ML 1997 Natural hybridization and evolution. Oxford University Press, New York. 215 pp.

Benedict RC 1934 Can anyone readily distinguish the northern and southern lady fern species? Am Fern J 24:117–119.

Brunton DF, DM Britton 1999 Rush quillwort (*Isoetes junciformis*, sp. nov.), a new pteridophyte from southern Georgia. Am Fern J 89: 187–197.

Butters FK 1917 Taxonomic and geographic studies in North American ferns. I. The genus *Athyrium* and the North American ferns allied to *Athyrium filix-femina*. Rhodora 19:169–207.

Conant DS 1978 A radioisotope technique to measure spore dispersal of the tree fern *Cyathea arborea* Sm. Pollen Spores 20:580–593.

Cook R 1983 Clonal plant populations. Am Sci 71:244–253.
Dodd ME, J Silvertown, MW Chase 1999 Phylogenetic analysis of trait evolution and species diversity variation among angiosperm families. Evolution 53:732–744.
Ellstrand NC, ML Roose 1987 Patterns of genotypic diversity in clonal plant species. Am J Bot 74:123–131.
Fernald ML 1946 Some trivial American forms of lady fern. Rhodora 48:389–391.
Gastony GJ, DC Darrow 1983 Chloroplastic and cytosolic isozymes of the homosporous fern *Athyrium filix-femina* L. Am J Bot 70: 1409–1415.
Gottlieb LD 1977 Electrophoretic evidence and plant systematics. Ann Mo Bot Gard 64:161–180.
Hamrick JL, MJW Godt 1989 Allozyme diversity in plant species. Pages 43–63 *in* AHD Brown, MT Clegg, AL Kahler, BS Weir, eds. Plant population genetics, breeding and genetic resources. Sinauer, Sunderland, MA.
Hartl DL 1980 Principles of population genetics. Sinauer, Sunderland, MA.
Haufler CH 1987 Electrophoresis is modifying our concepts of evolution in homosporous pteridophytes. Am J Bot 74:953–966.
——— 2002 Homospory 2002: an odyssey of progress in pteridophyte genetics and evolutionary biology. Bioscience 52: 1081–1093.
Haufler CH, MD Windham, DM Britton, SJ Robinson 1985 Triploidy and its evolutionary significance in *Cystopteris protrusa*. Can J Bot 63:1855–1863.
Kato M 1993 *Athyrium*. Pages 255–258 *in* FNA Editorial Committee, eds. Flora of North America. Vol 2. Pteridophytes and gymnosperms. Oxford University Press, New York.
Kaye R 1965 Variation in *Athyrium* in the British Isles. Br Fern Gaz 9:197–204.
Kelloff C, J Skog, L Adamkewicz, CR Werth 2002 Differentiation of two taxa of eastern North American *Athyrium*: evidence from allozymes and spores. Am Fern J 92:185–213.
Klekowski EJ 1982 Genetic load and soft selection in ferns. Heredity 49:193–199.
Lellinger DB 1985 A field manual of the ferns and fern: allies of the United States and Canada. Smithsonian Institution, Washington, DC.
Li J, CH Haufler 1994 Phylogeny, biogeography, and population biology of *Osmunda* species: insights from isozymes. Am Fern J 84: 105–114.
——— 1999 Genetic variation, breeding systems, and patterns of diversification in Hawaiian *Polypodium* (Polypodiaceae). Syst Bot 24:339–355.
Loveless MD, JL Hamrick 1984 Ecological determinants of genetic structure in plant populations. Annu Rev Ecol Syst 15:65–95.
Lovett-Doust L 1981 Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). I. The dynamics of ramets in contrasting habitats. J Ecol 69:743–755.
Mathworks 1997 Matlab version 5 user's guide. Mathworks, Natick, MA.
Mitton JB, MC Grant 1984 Association among protein heterozygosity, growth rate, and developmental homeostasis. Annu Rev Ecol Syst 15:449–499.
Nei M 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.
Parks CR, JF Wendel, MM Sewell, Y-L Qiu 1994 The significance of allozyme variation and introgression in the *Liriodendron tulipifera* complex (Magnoliaceae). Am J Bot 81:878–889.
Parks JC, CR Werth 1993 A study of spatial features of clones in a population of bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae). Am J Bot 80:537–544.
Peck JH, CJ Peck, DR Farrar 1990 Influences of life history events on formation of local and distant fern populations. Am Fern J 80: 126–142.
Ranker TA, SK Floyd, PG Trapp 1994 Multiple colonizations of *Asplenium adiantum-nigrum* in the Hawaiian archipelago. Evolution 48:1364–1370.
Raynor GS, EC Ogden, JV Hayes 1976 Dispersion of fern spores into and within a forest. Rhodora 78:473–487.
Ricklefs RE, SS Renner 1994 Species richness within families of flowering plants. Evolution 48:1619–1636.
Rogers JS 1972 Measures of genetic similarity and genetic distance. Stud Genet 7 Univ Tex Publ 7213:145–153.
Schneller JJ 1979 Biosystematic investigations on the lady fern (*Athyrium filix-femina*). Plant Syst Evol 132:255–277.
——— 1989 Remarks on hereditary regulation of spore wall pattern in intra- and interspecific crosses of *Athyrium*. Bot J Linn Soc 99: 115–123.
Schneller JJ, CH Haufler, TA Ranker 1990 Antheridiogens and natural gametophyte populations. Am Fern J 80:143–152.
Schneller JJ, R Holderegger 1997 Vigor survival of inbred and outbred progeny of *Athyrium filix-femina*. Int J Plant Sci 158: 79–82.
Schneller JJ, H Rasbach 1984 Hybrids and polyploidy in the genus *Athyrium* (pteridophyta) in Europe. Bot Helv 94:81–99.
Schneller JJ, BW Schmid 1982 Investigations on the intraspecific variability in *Athyrium filix-femina* (L.) Roth. Bull Mus Natl Hist Nat Sect B Adansonia Bot Phytochim, ser 4, 4:215–228.
Shaver JM 1954 Ferns of Tennessee with the fern allies excluded. George Peabody College for Teachers, Nashville.
Sheffield E, PG Wolf, CH Haufler 1989 How big is a bracken plant? Weed Res 29:455–460.
Sheffield E, PG Wolf, FJ Rumsey, DJ Robson, TA Ranker, SM Challinor 1993 Spatial distribution and reproductive behavior of a triploid bracken (*Pteridium aquilinum*) clone in Britain. Ann Bot 72:231–237.
Slatkin M 1985 Rare alleles as indicators of gene flow. Evolution 39: 53–65.
Smith DK, BE Wofford, JL Collins 1980 An atypical *Athyrium* from eastern Tennessee. Am Fern J 70:31–32.
Soltis DE, CH Haufler, DC Darrow, GJ Gastony 1983 Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Am Fern J 73:9–27.
Soltis DE, PS Soltis 1989 Isozymes in plant biology. Vol 4. Dioscorides, Portland, OR.
Soltis PS, DE Soltis 1987 Population structure and estimates of gene flow in the homosporous fern *Polystichum munitum*. Evolution 41: 620–629.
Soltis PS, DE Soltis, KE Holsinger 1988 Estimates of intragametophytic selfing and interpopulational gene flow in homosporous ferns. Am J Bot 75:1765–1770.
Soltis PS, DE Soltis, BD Ness 1989 Population genetic structure in *Cheilanthes gracillima*. Am J Bot 76:1114–1118.
Speer WD, CR Werth, KW Hilu 1998 Relationships between two infraspecific taxa of *Pteridium aquilinum* (Dennstaedtiaceae). II. Isozyme evidence. Syst Bot 23:313–325.
Suter M, JJ Schneller, JC Vogel 2000 Investigations into the genetic variation, population structure, and breeding systems of the fern *Asplenium trichomanes* subsp. *quadrivalens*. Int J Plant Sci 161: 233–244.
Swofford DR, R Selander 1981 BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J Hered 72:281–283.
Vogel JC, JA Barrett, FJ Rumsey, M Gibby 1999 Identifying multiple origins in polyploid homosporous pteridophytes. Pages 101–117 *in*

P Hollingsworth, R Bateman, R Gornall, eds. Advances in plant molecular systematics. Taylor & Francis, London.

Wagner WH Jr 1972 Disjunctions in homosporous vascular plants. Ann Mo Bot Gard 59:203–217.

Wagner WH Jr, FS Wagner 1966 Pteridophytes of the Mountain Lake area, Giles Co., Virginia: biosystematic studies, 1964–65. Castanea 31:121–140.

——— 1993 *Ophioglossaceae*. Pages 85–106 *in* FNA Editorial Committee, eds. Flora of North America. Vol 2. Pteridophytes and gymnosperms. Oxford University Press, New York.

Wagner WL 1991 Evolution of waif floras: a comparison of the Hawaiian and Marquesan archipelagos. Pages 267–284 *in* EC Dudley, ed. The unity of evolutionary biology. Proceedings of the Fourth International Congress of Systematic and Evolutionary Biology. Vol 1. Dioscorides, Portland, OR.

Ware S 1999 Paleoclimatic causes of altitudinal and geographical distribution of high elevation woody species in Virginia. Pages 45–57 *in* RP Eckerlin, ed. Proceedings of the Appalachian biogeography symposium. Virginia Museum of Natural History, Martinsville.

Weatherby CA 1936 A list of varieties and forms of the ferns of eastern North America. Am Fern J 26:130–136.

Werth CR 1985 Implementing an isozyme laboratory at a field station. Va J Sci 36:53–76.

——— 1991 Isozyme studies on the *Dryopteris* "*spinulosa*" complex. I. The origin of the log fern *D. celsa*. Syst Bot 16: 446–461.

Werth CR, MD Cousens 1990 Summary: the contributions of populations studies on ferns. Am Fern J 80:183–190.

Wherry ET 1961 The fern guide: northeastern and midland central states and adjacent Canada. Doubleday, Garden City, NY.

Wofford BE, EW Chester 1998 A comparison and reconciliation of the checklist and atlas of Tennessee vascular plants with published volumes of the Flora of North America. Castanea 63: 466–473.

Wolf P, E Sheffield, C Haufler 1991 Estimates of gene flow, genetic substructure and population heterogeneity in bracken (*Pteridium aquilinum*). Bot J Linn Soc 42:407–423.

Wright S 1965 The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395–420.

——— 1978 Evolution and the genetics of populations. Vol 4. Variability within and among populations. University of Chicago Press, Chicago.