Systematics of Eastern North American Bracken Fern

William D. Speer

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APPROVED
Khidir W. Hilu, Chair
Duncan M. Porter
Bruce J. Turner

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(ABSTRACT)

The cosmopolitan Pteridium aquilinum (L.) Kuhn is widespread throughout eastern North American, where it is represented primarily by Tryon's (1941) var. latiusculum (Desv.) Underw. and var. pseudocaudatum (Clute) Heller. The taxonomy of Pteridium is controversial. Fourteen isozyme loci and 12 morphological characters were used to assess the taxonomic relationship of these two varieties. Isozyme data indicated a high mean genetic identity (I = 0.976) between eleven bracken populations. Strong patterns of geographic variation for isozyme allele frequencies were also observed. The isozyme results did not separate the two taxa. Numerical analysis of the morphology distinguished the two taxa when the qualitative characters were used alone or in conjunction with some of the quantitative traits. All qualitative characters differed significantly between the two taxa. No perceptible geographic pattern of variation was observed. Morphological distinctiveness was maintained even in those localities where both taxa were present, with few or no intermediates being found. Isozyme evidence suggestive of gene flow between the two varieties was found at Greensboro, NC, where the two morphotypes were easily recognizable. The isozyme evidence strongly indicates conspecificity, while the morphological evidence supports their status at the varietal level.

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Chapter 1

INTRODUCTION

PTERIDOPHYTES

The ferns and the so-called "fern-allies" are collectively referred to as pteridophytes. "Pteridophyte" is a general term used to describe the various seedless vascular plant groups. These plants are free-sporing and have free-living gametophytes. The pteridophytes include plants that are either homosporous (producing a single spore type) or heterosporous (producing microspores and megaspores). They are distinct from bryophytes (mosses, liverworts, hornworts), which are homosporous only and lack vascular tissues, and from spermatophytes, which are all heterosporous, have a pollination biology, have parasitic gametophytes, and produce seeds.

In many early taxonomic systems, pteridophytes presented some serious problems, probably because of their lack of flowers or seeds. Stace (1989) points out that until A. P. de Candolle placed all pteridophytes into a separate plant group in 1813, Lycopodium was considered a moss, Equisetum a conifer, and the aquatic fern Salvinia a liverwort. It is now generally recognized that the pteridophytes do not form a monophyletic group, but rather, comprise four separate evolutionary lineages, which are often recognized at the division level as the Psilophyta (whisk ferns), Lycopodiophyta (lycopods), Equisetophyta (horsetails, also called Sphenophyta), and the Polypodiophyta (true ferns)(Cronquist et al 1966).

Pteridophytes are often described as being either eusporangiate or leptosporangiate. In eusporangiate pteridophytes, each sporangium develops from several initials and produces several hundred to thousands of spores (Foster and Gifford 1974; Jones 1987). This distinguishes them from leptosporangiate plants, in which each sporangium develops from a single initial cell, and usually produces no more than 128 spores (Foster and Gifford 1974; Jones and Luchsinger 1979; Jones 1987). The eusporangiate group includes all non-fern groups and the more primitive fern taxa, while the leptosporangiate group is comprised of ferns only (Sporne 1966; Lellinger 1985).

FERNS

The ferns comprise the largest and most diverse group of living pteridophytes, with around 12,000 species (Jones and Luchsinger 1979). They are usually distinguished from the other pteridophytes by having leaves with branching venation, or megaphylls (Sporne 1966). In contrast, the extant non-fern groups either have microphylls, or leaves with a single vein (Lycopodiophyta); reduced megaphylls (Equisetophyta); or lack true leaves altogether (Psilophyta) (Sporne 1966; Foster and Gifford 1974; Jones 1987).

The largest group of extant ferns is the leptosporangiate ferns, which contain approximately 90 percent of all living fern species (Smith 1972). The leptosporangiate taxa are regarded as being more advanced than the eusporangiate (Sporne 1966; Lellinger 1985).

BRACKEN FERN -THE RESEARCH ORGANISM.

Bracken fern is considered to be the most widely distributed pteridophyte and one of the most widely distributed vascular plants (Page 1976). Bracken is often considered a harmful weed (Tryon 1941; Page 1976; Fletcher and Kirkwood 1979), having a negative impact on the agriculture of many countries (Nelson 1946; Salisbury 1961; Gliessman 1978). This makes bracken not only one of the few fern taxa that is a nuisance to humankind, but also one of the few

weeds that has a natural world range (Page 1976). Its weedy character (i.e. tendency to invade disturbed habitats), however, may be a function of taxonomic biotype and geographic location, as eastern North American bracken rarely presents an agricultural problem (Tryon 1941).

Bracken's economic importance goes beyond being just a weedy nuisance. It contains a number of chemical substances harmful to both humans and livestock (Fletcher and Kirkwood 1979; Hannam 1986; Camus et al. 1991). It has a thiaminase which destroys thiamine (vitamin B_1), thereby causing an avitaminosis B_1 induced neurotoxic syndrome (Evans 1986). It is also known to contain various carcinogenic substances (Evans 1976; Galpin and Smith 1986). One such carcinogen that has been the subject of much research is ptaquiloside, a norsesquiterpene glycoside believed to be responsible for the high incidence of human esophageal cancer in Japan (Kushida et al. 1994; Oelrichs et al. 1995).

Bracken has not always been viewed as a weed or as a harmful plant; it was once considered to be a very useful plant with a rich ethnobotany (reviewed by Rymer 1976). It was an significant source of potash, an important ingredient for the production of soap, glass, and various dyes. In many areas it was used as a fuel to provide warmth, as thatching for houses, and as bedding for livestock. So important was this plant, that laws once existed in Britain that regulated when and where bracken might be cut and harvested.

Sexual Reproduction in Bracken. Bracken is a homosporous leptosporangiate fern. Meiosis in plants results in the production of spores, which develop into haploid gametophytes (Raven et al. 1981). The freeliving gametophytes of homosporous pteridophytes are potentially bisexual, that is, capable of having both male (antheridia) and female (archegonia) gametangia (Raven et al. 1981). The presence of bisexual gametophytes formerly led to the suggestion that sexual reproduction in most ferns was due to intragametophytic matings, i.e., self-fertilization of a single gametophyte (Klekowski and Baker 1966). Subsequent studies have revealed that ferns with hypogeal (below ground) gametophytes do indeed reproduce primarily in this fashion (McCauley et al. 1985; Soltis and Soltis 1986). However, ferns with epigeal (above ground) gametophytes, such as bracken, exhibited a variety of mating systems (Soltis et al. 1988). Studies of the mating system of bracken have reported that sexual reproduction occurs primarily by intergametophytic matings, i.e. outcrossing (Wolf et al. 1988; Korpelainen 1995). With this type of mating system it can be seen that colonization by sexual means, although often infrequent (Conway 1957; Page 1976; Korpelainen 1995), requires the arrival and germination of at least two spores and the subsequent development of gametophytes for the production of a sporophyte.

Several factors are known to increase the likelihood of outcrossing in bracken. One such factor is genetic load, i.e. the accumulation of recessive lethal alleles in the sporophyte (Klekowski 1972). Gametophytic self-fertilization results in the production of sporophytes that are homozygous at all loci. As a result of self-fertilization, a gametophyte having deleterious alleles will generate a sporophyte that is homozygous for these alleles. If these alleles are lethal, the sporophyte will reach reproductive maturity. Outcrossing reduces the possibility that a sporophyte will be homozygous for lethal recessives. Another process contributing to outcrossing in bracken is the production of the hormone antheridiogen (Näf 1979). Antheridiogen is secreted by large, functionally female gametophytes and promotes antheridial development in nearby smaller gametophytes, rendering them functionally male (Näf 1979).

Bracken has a diploid genetic structure expressing disomic inheritance patterns (Wolf et al. 1987). It possesses a considerable level of polymorphism at isozyme loci (Wolf et al. 1991), with most plants being heterozygous for at least one locus (Sheffield et al. 1989).

Variability in bracken is also noticeable at the morphological level. The substantial degree of variation in frond morphology has usually been explained as a result of phenotypic plasticity (Page 1976) or rhizome age (Watt 1976). However, there is a consistent degree of variation between most bracken taxa that has allowed taxonomic discrimination (Tryon) 1941; Page 1976).

This suggests that morphological variation may have a genetic component.

Asexual reproduction in bracken. An important aspect of bracken biology contributing to its spread and success is the propensity of its rhizome for extensive growth (Conway 1957; Oinonen 1967; Gliessman 1978; Parks and Werth 1993). The mean growth rate of a bracken rhizome is approximately 1 m per growing season, but it can be as much as 2.1 m (Fletcher and Kirkwood 1979). Such a rate of growth facilitates the vegetative spread of bracken into suitable areas. The rhizome network of bracken has been variously described. Watt (1976) recognized two types of shoots in bracken: 1) short shoots, which grow upwards and are frond bearing, and 2) long shoots, which can grow in any direction and tend to lack fronds. A three-fold system is illustrated by Conway (1959), consisting of 1) thick shoots, which penetrate deeply into the soil and bear few fronds; 2) thinner shoots, which are close to the soil surface and are the main frond bearing stems; and 3) intermediary, which link the two and can develop into either type. Bracken rhizomes can go as deep as 34 cm into the soil (Watt 1976), making them difficult to eradicate once established. Its rhizomes have vessel elements in their xylary tissues (Mehra and Soni 1971), a trait usually associated with angiosperms (Mauseth 1988). This feature plus its complex polystelic vascular arrangement (Sporne 1966) may explain why bracken is often found in dry, sunny locations, areas that are not preferred by most ferns. In short, its complex rhizome system appears to have made bracken a very successful competitor with flowering plants (Sporne 1966).

Taxonomy of bracken. As pointed out by Page (1976), bracken has always been taxonomically difficult. Linnaeus (1753) placed bracken in the genus Pteris, recognizing two species: P. aquilina and P. caudata. Subsequent taxonomic treatments of bracken have differed from the original Linnaean treatment in two ways. First, while a number of treatments continued to recognize bracken's placement in the genus Pteris (Agardh 1839; Hooker 1858), other treatments separated it from Pteris (Scopoli 1760; Gleditsch 1764; Smith 1875). It was not until Kuhn (1879) defined the genus Pteridium Gled. ex Scop. that bracken was widely recognized as a separate genus (Tryon 1941). Second, the number of recognized bracken species has ranged from one (Diels 1899; Christensen 1938) to as many as five or six (Willdenow 1810; Copeland 1947).

The most widely recognized taxonomic treatment of bracken is that of Tryon (1941), who placed all bracken into a single species, Pteridium aquilinum (L.) Kuhn. Within P. aquilinum he recognized two subspecies and twelve varieties. P. aquilinum subsp. aquilinum, comprising eight varieties, is mainly north temperate in distribution. This subspecies occurs throughout North and Central America, Africa, Eurasia, and the Hawaiian islands. Tryon described it as having adnate ultimate segments which are not farinaceous on the lower surface and being either glabrous or lanuginose on the abaxial surface of the lamina. On the other hand, P. aquilinum subsp. caudatum (L.) Bonap., is comprised of four varieties and is distributed throughout the Southern Hemisphere. It is found throughout Central and South America, southern Asia, Australia, and throughout the South Pacific. Tryon described it as having strong decurrent ultimate segments with a distinctly farinaceous lower surface. The abaxial surface of the lamina tends to have straight, stiff hairs.

There has been some noticeable disagreement with Tryon's monotypic treatment of bracken. On the basis of morphological variation, Lellinger (1985) has recognized the occurrence of two bracken species in North America: Pteridium aquilinum and P. caudatum (L.) Maxon. A more recent treatment is that of Page and Mill (1995a, b). This treatment is interesting because Tryon's var. latiusculum is dealt with as two species, P. latiusculum (Desv.) Hieron and P. pinetorum Page & Mill, which together constitute the P. latiusculum complex.

While the debate continues as to whether or not Pteridium is monotypic, recognition of at least one proposed bracken species has been found to be unwarranted. All bracken taxa have a chromosome number of n = 52 (Manton 1950), although a triploid (3x = 156) clone in Britain has been reported by Sheffield et al. (1993) and a tetraploid (4x = 208) was found by Jarrett et al. (1968) on the Galapagos Islands. However, Löve and Kjellqvist (1972) reported finding bracken

in southern Spain that had a chromosome number of n = 26, which they treated as Pteridium herediae (Clemente ex Colmeiro) Löve and Kjellqvist. Although this new species was widely cited (e.g. Page 1976; Fletcher and Kirkwood 1979), Sheffield et al. (1989) demonstrated that there were no grounds for accepting this new bracken species on the basis of biochemistry, morphology, habitat, or chromosome number.

Bracken is widely distributed throughout much of North America. Systematic studies are needed to evaluate the taxonomy of the North American varieties (Jacobs and Peck 1993). Questions have been raised regarding the taxonomic delimitation of these taxa due to morphological variation, overlap in geographic distribution, and uncertainties regarding the interfertility (e.g. Montgomery and Fairbrothers 1992) of eastern North American taxa.

OBJECTIVES OF THIS STUDY

This study attempts to evaluate the taxonomic status of two North American varieties, var. latiusculum and var. pseudocaudatum, using two approaches. First, a numerical study utilizing both multivariate and univariate approaches was conducted to analyze the morphological variation occurring in these two taxa and to identify the important discriminating characters. Second, an isozyme study aimed at estimating the amount and pattern of genetic variation existing between these two bracken groups was done. Combining these two approaches, this investigation expects to provide additional insight into the taxonomy of bracken.

Chapter 2

MORPHOLOGICAL STUDY

INTRODUCTION

Bracken fern is most widely treated as a single species, Pteridium aquilinum (L.) Kuhn, of cosmopolitan distribution. This taxon has long been recognized as a complex group, comprising a diversity of elements that are morphologically and geographically distinct to varying degrees. However, it is contentious as to the extent to which some of these elements are distinct and the taxonomic rank (species, subspecies, variety) at which they should be recognized.

In the most comprehensive treatment of the genus, Tryon (1941) recognized in his monograph a single, morphologically variable species, P. aquilinum. He subdivided the species into two subspecies [subsp. aquilinum and subsp. caudatum (L.) Bonap.], that were further subdivided into eight and four varieties, respectively. Tryon (1941) reported that the characters used to distinguish varieties tended to be inconsistent and highly variable within and overlapping between varieties. However, substantial disagreement exists with the monotypic treatment of Pteridium (reviewed by Page 1976). A number of authors have indicated that Tryon's (1941) subspecies should be elevated to species rank (e.g. Lellinger 1985). Others have suggested that one or more of the current varieties should be raised to either specific (e.g. Copeland 1947) or subspecific rank (e.g. Page 1989). These viewpoints may gain support from suspected reproductive isolation between co-occurring bracken varieties (Mickel and Beitel 1988).

It has been recognized that detailed systematic studies are needed to assess the status and rank of North American bracken (Jacobs and Peck 1993). The present study addresses the relationship between two bracken varieties of subsp. aquilinum occurring in eastern North America and exhibiting extensive range of geographical overlap: 1) P. aquilinum var. latiusculum (Desv.) Underwood, a nearly circumboreal taxon common through much of eastern North America and occurring as disjunct populations in western North America; 2) P. aquilinum var. pseudocaudatum (Clute) Heller, occurring in southeastern United States and extending to Massachusetts along the Atlantic coast. The distributions of var. latiusculum and var. pseudocaudatum overlap in the mid-Atlantic, east central, and southeastern United States. Variety latiusculum is more common in the northern part of its range and in more mountainous areas, becoming less common in the southern part of its range. Variety pseudocaudatum, on the other hand, is more common in the eastern coastal plain and in the southern part of its range. A third taxon present in eastern North America, P. aquilinum var. caudatum (L.) Sadebeck, whose North American distribution is limited to southern Florida, was not included in this study because of its considerable morphological distinctiveness.

Varieties latiusculum and pseudocaudatum are generally separated on the basis of frond morphology. Tryon (1941) delineated var. latiusculum based on lamina that is usually 3-pinnate or 3-pinnate-pinnatifid and pubescent along the margins and abaxial surface of the axes, and by the apical segments that are 3-7 (usually 4) times longer than wide. On the other hand, he described var. pseudocaudatum as being 2-pinnate-pinnatifid or 3-pinnate (rarely 3-pinnate-pinnatifid), usually glabrous along the margins and abaxial surface of the axes, and with apical segments 6-15 times longer than wide. Tryon (1941) stated that there is a high degree of overlap between most of the characters used to separate the two varieties. He reported extensive morphological intergradation between the two taxa and pointed to the presence of intermediates in areas where both varieties coexist.

Although intermediates did not form a numerically large segment of such bracken populations, he nonetheless considered them as a very significant portion. Tryon concluded that these two varieties are closely related.

Subsequent treatments of these bracken varieties have not always relied completely on the characters used by Tryon (1941). For example, Lellinger (1985) re-defined the states of some of the characters used by Tryon (1941), such as the apical segments of var. latiusculum as 2-4 times longer than wide and the lamina of var. pseudocaudatum as 2-3-pinnate-pinnatifid. He also included other discriminating traits such as the shapes of the small and large pinnae and the ratio of the ultimate segment width to the distance between individual segments. Such incongruencies in defining important morphological characters can present conflicting views on defining the boundaries between these taxa and contribute to the disagreement on their taxonomic status.

The objectives of the present study were to 1) quantitatively examine the patterns of morphological variation in var. latiusculum and var. pseudocaudatum, 2) determine the important morphological characters that contribute to the discrimination between the varieties, and 3) evaluate the taxonomic status of the two taxa.

MATERIALS AND METHODS

Material and Data Collection. The study was based on 262 specimens covering the range of distribution of the two taxa in eastern North America from Quebec to Florida. These specimens comprised both plants collected in the field (vouchers deposited at VPI), as well as specimens from the Massey herbarium at VPI. Using Lellinger's (1985) description, specimens were identified as belonging to either var. latiusculum (126 specimens) or var. pseudocaudatum (136 specimens). No specimen was a priori identified as intermediate. Each specimen was examined for twelve frond characters (Table 1), selected from the treatments of Tryon (1941) and Lellinger (1985). Three of these characters were scored as quantitative (continuous) and nine as qualitative characters (six bi-state and three multi-state).

The vast majority of the specimens used were complete, allowing the scoring of all twelve characters. Missing data made up only 0.8% of the total data matrix. There were 23 individuals which could not be scored for one (22 specimens) or two characters. The stipe length was the predominantly unscorable character in most of the 23 specimens. The impact of this character on this numerical study is minimal (discussed below).

Although the character choice in this study relied on the treatments of Tryon (1941) and Lellinger (1985), the actual character-state definitions deviated in most cases from those used by the above authors. The twelve characters used, character scoring and character states are listed in Table 1. The character-state definitions for the terminal segment took into consideration both treatments and, thus, the ratio of 4 was used as the discriminating value. The definition of character-states for the leaf cut (frond architecture) was determined primarily on the basis of a preliminary examination of bracken morphology in this study. The type and presence of hairs on the abaxial midrib and the lamina margin were treated as separate, bi-state characters since they do not appear to be linked. Character-state definitions for the ultimate segment, large pinnae, and small pinnae closely followed Lellinger (1985).

Table 1. Frond characters measured for numerical analysis of eastern North American Pteridium aquilinum.

Character	Type	Scoring
1. Lamina Width (LWid)	quantitative	Continuous
2. Lamina Height (LHgt)	quantitative	Continuous
3. Stipe Length (Stipe)	quantitative	Continuous
4. Terminal Segment (Term)	qualitative	L/W ratio $\leq$ 4 (0), > 4 (1)
5. Ultimate Segments (Ultim)	qualitative	L/D ratio < 1 (0), $\geq$ 1 (1)
6. Large Pinnae Shape (Lpin)	qualitative	Broadly triangular (0), triangular (1), ovate-lanceolate (2)
7. Small Pinnae Shape (Spin)	qualitative	Narrow triangular (0), oblong (1), oblong-accuminate (2)
8. Leaf Cut (Cut)	qualitative	3-pinnate (0), 3-pinnate-pinnatifid (1), variably 2-3-pinnate-pinnatifid (2)
9. Margin - Villous Hair (MVH)	qualitative	absent (0), present (1)
10. Margin - Pilose Hair (MPH)	qualitative	absent (0), present (1)
11. Abaxial Midrib - Villous Hair (AVH)	qualitative	absent (0), present (1)
12. Abaxial midrib - Pilose Hair (APH)	qualitative	absent (0), present (1)

Numerical Analysis. Both univariate and multivariate statistical approaches were utilized in analyzing the data. Univariate analysis was performed on each character to determine the range of variation between and within taxa. Basic statistics for mean, standard deviation, and analysis of variance, were calculated for each variable using JMP, version 3 (SAS 1994). Analysis of variance was carried out by a t-test of the differences between two means. Multivariate methods were performed to examine the pattern of grouping of the 262 specimens. Cluster analysis, principal components analysis (PCA) and minimum spanning tree (MST) were computed with NTSYS-pc, version 1.80 (Rohlf 1993).

To determine the contributions of quantitative and qualitative characters to the separation of the two taxa, seven different combinations of characters were analyzed in the cluster analysis: 1) all twelve characters (CC1), 2) quantitative characters alone (CC2), 3) qualitative characters alone (CC3), 4) qualitative characters plus stipe length (CC4), 5) qualitative characters plus lamina height (CC5), 6) qualitative characters plus lamina width (CC6), and 7) qualitative characters plus both lamina height and lamina width (CC7). Character combinations 4 through 7 were determined as a result of the statistical data obtained from the univariate analysis.

For each cluster analysis, except for CC3, a similarity matrix was generated using average taxonomic distance. CC3 was composed entirely of qualitative data and, thus, the simple matching coefficient was used. The sequential, agglomerative, hierarchal, and nested (SAHN) clustering (Sneath and Sokal 1973) was performed using the unweighted pair-group method with arithmetic averages (UPGMA). Because clustering methods yield clusters regardless of the data structure (Sneath and Sokal 1973), cophenetic correlation coefficients were used to measure the distortion between the similarity matrix and the resulting phenogram utilizing the matrix comparison application.

PCA was computed for CC3, CC5, CC6, and CC7. Because of the outcome of the cluster analyses, PCA was not computed for CC1, CC2, and CC4. To evaluate proportionality and independence between characters (Sneath and Sokal 1973), the product-moment correlation coefficient option was used to generate a correlation matrix. The first three PCA axes were then extracted from the correlation matrix. OTUs were projected onto the first three PCA axes. To detect local distortion in the PCA, minimum spanning trees (MST) were computed from the similarity matrices of CC3, CC5, CC6, and CC7 and were imposed on the respective PCA plots.

RESULTS

Cluster Analysis. Cluster analysis based on all 12 qualitative and quantitative characters did not result in recognizable separation of OTUs on either a taxonomic, population, or geographic basis. Similar results were obtained when the quantitative characters were used. However, when the qualitative characters alone were used, two very distinct clusters (0.24 similarity level) corresponding to the OTUs identified as var. latiusculum and var. pseudocaudatum were obtained (Fig. 1). In the latter analysis, four OTUs identified as var. latiusculum were placed in the var. pseudocaudatum cluster. This marked difference in clustering with different sets of characters prompted us to use univariate analysis to examine the pattern of variation of both qualitative and quantitative characters in these two groups of OTUs. The results of the univariate analysis, including information on the mean, range and standard deviation of the characters, are summarized in Table 2. There were very significant differences between the two clusters for all qualitative characters ($P < 0.0001$ in each case). This was not the case for the three quantitative characters. There was no significant difference between the two groups for the stipe length ($P = 0.6446$). The

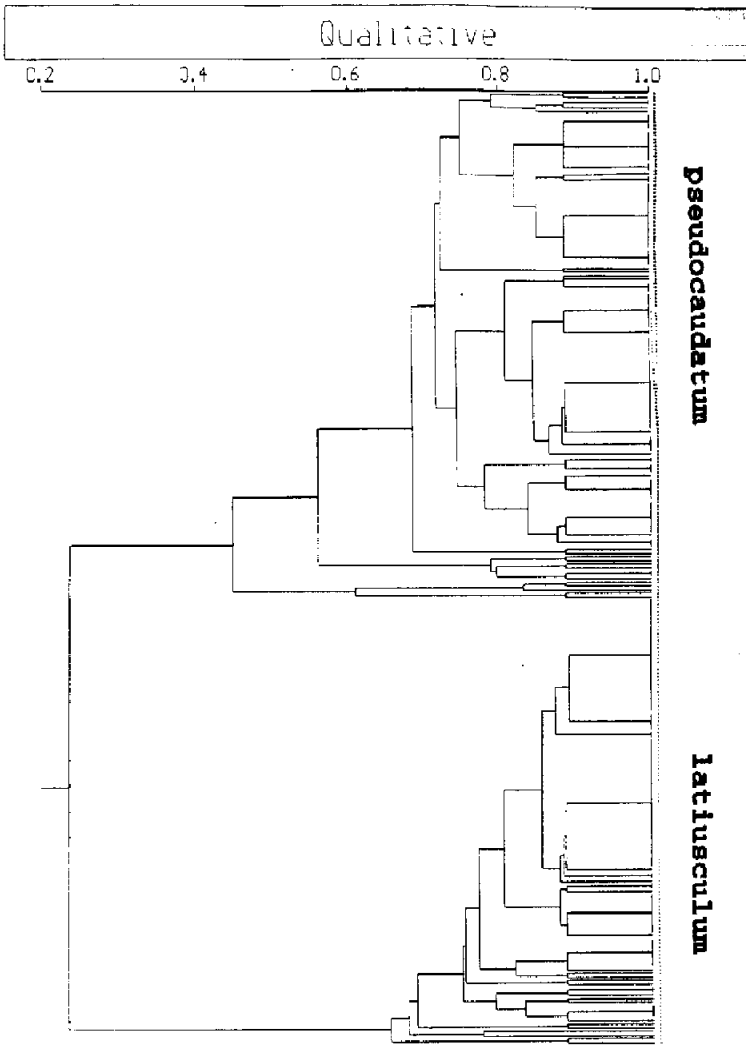

Figure 1. Cluster diagram produced using only qualitative characters. OTUs were grouped into two clusters corresponding to var. pseudocaudatum (top cluster) and var. latiusculum (bottom cluster).

lamina height (P = 0.0516) and lamina width (P = 0.0691), however, were borderline in terms of statistical significance.

Given these statistical outcomes, it was decided to further examine the possible contributions of individual quantitative characters to the systematics of bracken when used in combination with the nine qualitative characters. Using the qualitative characters plus the stipe length (CC4), the phenogram did not segregate the OTUs into recognizable taxonomic or geographic entities. However, when each or both of the lamina height and lamina width were used along with the qualitative characters (CC5, CC6, and CC7), two clusters representing var.

Table 2 . Results of univariate analysis of the twelve morphological characters used in the numerical study. Characters marked with an asterix (*) differed significantly (in all cases, P < 0.0001) between the two taxa using a t-test of two sample means. Qualitative characters (Lwid, Lhgt, and Stipe) were measured in cm. See Table 1 for character abbreviations.

	All OTUs			var. latiusculum					var. pseudocaudatum				
	n	mean	sd	n	mean	sd	min	max	n	mean	sd	min	max
LWid	261	38.82	11.46	126	37.48	11.33	15.00	70.70	135	40. 06	11.49	12.70	63.10
LHgt	260	38.20	10.00	126	36.95	9.89	17.20	66.60	134	39.37	10.00	16.50	75.60
Stipe	243	34.94	13.16	115	34.53	13.32	8.60	69.20	128	35.31	13.07	7.50	78.90
Term*	260	0.62	0.49	124	0.23	0.43	0.00	1.00	136	0.98	0.15	0.00	1.00
Ultim*	262	0.53	0.50	126	0.06	0.23	0.00	1.00	136	0.98	0.15	0.00	1.00
Lpin*	262	1.00	0.92	126	0.35	0.69	0.00	2.00	136	1.56	0.67	0.00	2.00
Spin*	262	1.44	0.54	126	0.96	0.23	0.00	2.00	136	1.88	0.33	1.00	2.00
Cut*	262	1.15	0.81	126	0.50	0.55	0.00	2.00	136	1.78	0.48	0.00	2.00
MVH*	262	0.49	0.50	126	0.96	0.20	0.00	1.00	136	0.06	0.24	0.00	1.00
MPH*	262	0.19	0.40	126	0.03	0.18	0.00	1.00	136	0.35	0.48	0.00	1.00
AVH*	262	0.47	0.50	126	0.94	0.23	0.00	1.00	136	0.04	0.19	0.00	1.00
APH*	262	0.24	0.43	126	0.02	0.15	0.00	1.00	136	0.45	0.50	0.00	1.00

latiusculum and var. pseudocaudatum were obtained. The four OTUs of var. latiusculum that grouped with var. pseudocaudatum in CC3 exhibited similar grouping in CC5, CC6, and CC7.

Principal Components. PCA was carried out for those character combinations that resulted in the segregation of the OTUs into clusters representing the two varieties (CC3, CC5, CC6, and CC7). The two groups of OTUs depicted in the UPGMA analysis segregated well on the first three PCA coordinates. In the PCA plot that was based on the qualitative characters (CC3), separation of the two taxa occurred along the first principal component (Fig. 2a). However, when intra-group variation is considered, the first principal component accounted for most of the variation within var. latiusculum while the second component described most of the variation within var. pseudocaudatum. The first two principal components accounted for 64% and 12% of the variance, respectively. The first principal component had very high loading scores for the two villous hair characters and the ultimate segment character, while the two pilose hair characters had the lowest scores (Table 3). In contrast, the second principal component showed the two pilose hair characters with the highest loading scores, while the two villous hair characters had the lowest scores (Table 3). The two quantitative characters variably used in CC5, CC6, and CC7 displayed the lowest scores on the first principal component and the highest on the second component.

The MST showed primarily within-group nearest-neighbor connections in CC3, CC5, and CC6 (Fig. 2). This pattern was quite evident when the qualitative characters alone (CC3) were used (Fig. 2a). The four OTUs identified as var. latiusculum that appeared in the var. pseudocaudatum cluster were connected to the OTUs of the latter variety by the MST, further substantiating the UPGMA results. In contrast, when the MST matrix was based on CC7, numerous inter-group connections were evident (Fig. 2d). The latter character combination differed from CC3, CC5, and CC6 by including both lamina height and width characters.

DISCUSSION

Using qualitative characters alone or in combination with the lamina height and/or lamina width, both cluster analysis and PCA resulted in two distinct groups. The grouping was strongly supported by the low 0.24 similarity value in the UPGMA, the lack of overlap in PCA, and the extensive intragroup MST linkages. Assignment of plants to each cluster agreed very closely with the prior identification of specimens as either var. latiusculum or var. pseudocaudatum. The results of the multivariate analyses are consistent with the recognition of the two taxa as separate taxonomic entities.

The lack of geographically-based structuring within the clusters could be the consequence of greater variation within populations and regions than between them in the morphological characters used in this study. Bracken frond morphology is not only variable at the population level, but also within a single genet. Oinonen's (1967) attempts to discern individual genets on the basis of frond morphology met with limited success in Finnish bracken. Field observations of frond morphology of both var. latiusculum and var. pseudocaudatum also revealed some variation among fronds of the same rhizome (personal observation). It has been suggested that such variation in frond morphology may be due to rhizome age (Watt 1940; 1947). Watt (1976) observed that the fronds from younger, deeper shoots differed in appearance from those produced by older, shallower shoots.

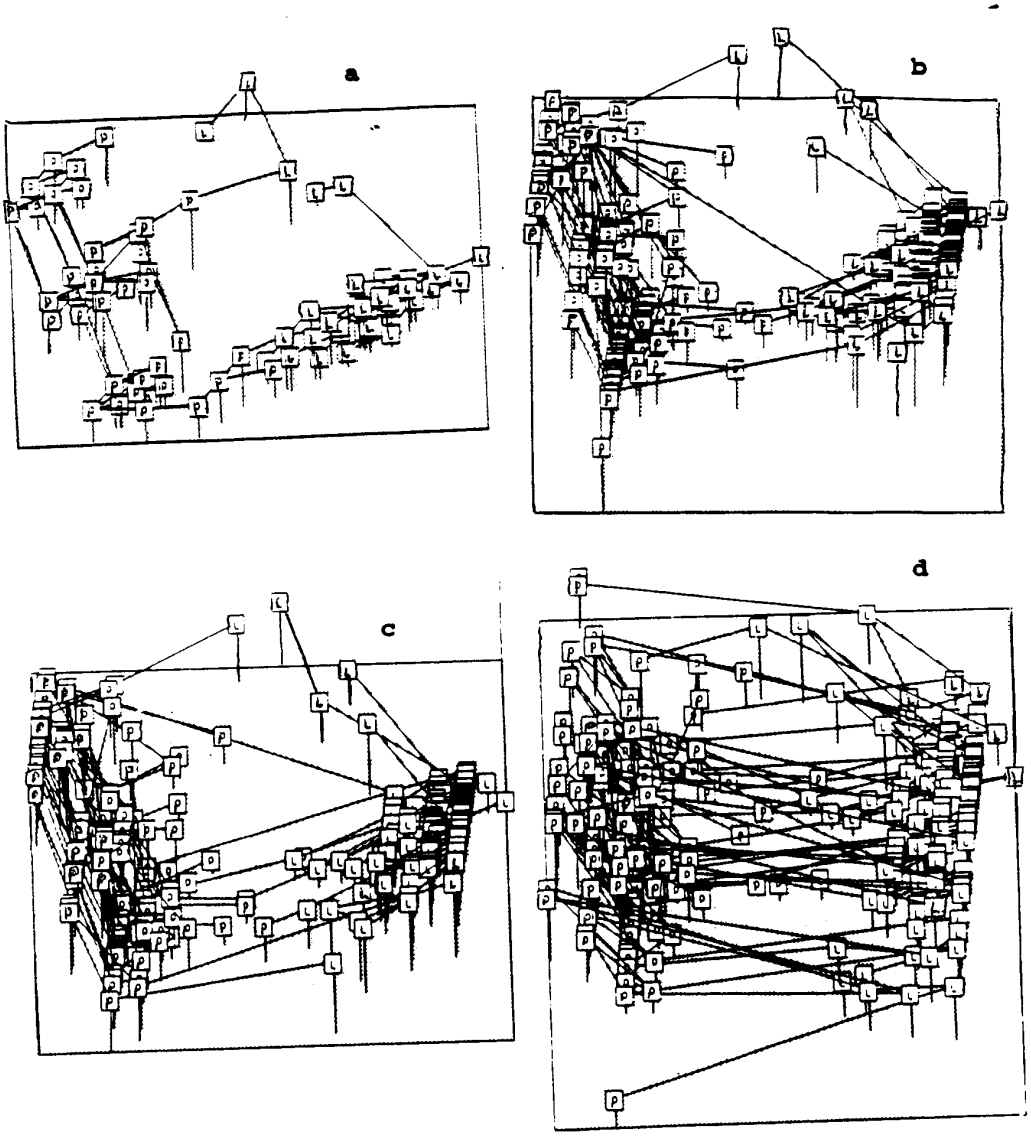

Figure 2 a-d. PCA with MST of different character combinations. 2a. CC3 (qualitative only). 2b. CC5 (qualitative with lamina height). 2c. CC6 (qualitative with lamina width). 2d. CC7 (qualitative with both lamina height and lamina width).

Table 3. Eigenvector matrix for character combinations 3 and 7. PCA axes 1, 2, and 3 represent character combination 3 (qualitative only) and PCA axes 1', 2', and 3' represent character combination 7 (qualitative plus lamina height and lamina width).

Character/PCA axis	1	1'	2	2'	3	3'
LWid	----	0.18	----	0.87	----	0.22
LHgt	----	0.19	----	0.88	----	0.18
Term	0.80	0.80	0.21	0.12	0.06	-0.17
Ultim	0.91	0.91	0.18	0.01	0.00	-0.18
Lpin	0.75	0.75	0.20	0.07	-0.42	-0.17
Spin	0.86	0.86	0.15	0.02	0.02	-0.14
Cut	0.80	0.80	0.15	-0.09	0.26	-0.20
MVH	-0.93	-0.93	0.03	0.08	0.03	-0.01
MPH	0.52	0.51	-0.71	-0.27	-0.38	0.67
AVH	-0.94	-0.93	-0.02	0.06	-0.03	0.04
APH	0.60	0.59	-0.61	-0.27	0.40	0.55

The four OTUs originally identified as var. latiusculum that grouped with the var. pseudocaudatum cluster and appeared in an intermediate area in the PCA tended to follow Tryon's (1941) description of morphological intermediates. Tryon described intermediates as having the leaf architecture or "cut" of one variety but the margin and abaxial midrib hairs of the other. This suggests the possible presence of two types of intermediates: one with a var. latiusculum type of frond, but being either glabrous or pilose on the margins and abaxial surface of the midrib, and the other with a var. pseudocaudatum type of frond and villous hairs along the margin and on the midrib. Both types were represented in this group of specimens. The small number of intermediates found in this study agrees with the point made by Tryon (1941) that intermediates, although significant taxonomically, are not common.

The results of the MST varied according to the character combination used. In CC3 (qualitative characters alone), little or no distortion was detected using the MST. Almost all connections were within group and only two were between group (Fig. 2a). In each case,

the between-group connections involved specimens that were morphologically intermediate and arbitrarily identified as var. latiusculum. Increase in the number of inter-group connections was evident with the inclusion of the two lamina characters. When either lamina height or lamina width was used, the distortion in the PCA was low, as the two groups showed considerably higher number of within-group linkages (Fig. 2b, c). However, that was not the case when both of these characters were used together with the qualitative characters (Fig. 2d). The distortion may be explained by the additive effect of less discriminatory traits, as is evident from the univariate statistical information (Table 2). The P values obtained from the t-test for lamina height and lamina width were only 0.051 and 0.069, respectively. The mean and standard deviation of the lamina height was 36.95 ± 9.89 cm for OTUs identified as var. latiusculum and 39.37 ± 10.00 cm for OTUs identified as var. pseudocaudatum. For lamina width, these values were 37.48 ± 11.33 cm for OTUs identified as var. latiusculum and 40.06 ± 12.70 cm for OTUs identified as var. pseudocaudatum. Therefore, it is not surprising to see the lack of clear cut segregation when these two quantitative characters were used.

The numerical analyses strongly indicate the presence of two taxa. The consistent pattern of variation observed does not support the notion of a single polymorphic taxon as proposed by Willdenow (1810). On the other hand, while there were statistically significant differences between the two morphotypes for all qualitative characters, some overlap was observed. Furthermore, the distinction between the two groups was blurred or disappeared when all the quantitative characters were included in the analysis. The lack of clear distinction does not support recognition of two species. Based on the level of variation, the two recognizable clusters obtained in this study probably represent two infraspecific taxa in bracken, corresponding to Tryon's (1941) var. latiusculum and var. pseudocaudatum. An infraspecific treatment gains support from the isozyme study (see Chapter 3), which found strong genetic continuity between the two taxa in the form of high mean genetic identity (I = 0.976) and evidence of apparent gene flow between the two taxa (see Chapter 3).

Chapter 3

ISOZYME STUDY

INTRODUCTION

This study addresses the genetic relationships of two closely related varieties of bracken (Pteridium Gleditsch ex Scopoli) that occur in eastern North America (United States and Canada): the predominantly northeastern Pteridium aquilinum (L.) Kuhn var. latiusculum (Desv.) Underw., and the predominantly southeastern P. aquilinum var. pseudocaudatum (Clute) Heller. The distributions of the two varieties overlap extensively in the central and southern states of the eastern United States. The taxonomic status of these two taxa has been disputed. (Schkuhr 1809; Willdenow 1810; Hooker 1858; Anonymous 1901; Tryon 1941). Although the two varieties are separated on the basis of frond morphology (Tryon 1941; Lellinger 1985), Tryon (1941) stated that there is a high degree of intergradation between most of the characters used to separate them.

Questions have been raised regarding the interfertility of these two eastern North American taxa. In many areas, such as New Jersey, only sterile (i.e. lacking sporangia) plants are found, making it very difficult to determine whether hybrids occur or if they are fertile (Montgomery and Fairbrothers 1992).

An extensive morphological study was conducted which examined 262 collections of the two varieties for ten morphological characters (see Chapter 2). It was observed that the two varieties were distinct entities when qualitative characters were used, while they were indistinguishable on the basis of the quantitative traits. To further evaluate relationships between these varieties, an isozyme study was undertaken. Isozymes have been used extensively to study the systematics and genetic variability of pteridophytes in general (e.g. Soltis and Soltis 1987; Ranker et al. 1994; Korpelainen and Kolkkala 1996) and Pteridium in particular (e.g. Sheffield et al. 1989; Rumsey et al. 1991; Wolf et al. 1991). The objectives of this study were to 1) estimate levels and patterns of genetic variation as well as degree of divergence among bracken populations of these two varieties, and 2) use this information to assess their taxonomy.

MATERIALS AND METHODS

Collecting. Pteridium aquilinum was sampled from eleven populations distributed along the eastern seaboard from Massachusetts south to Florida (Table 4). To effectively evaluate genetic relations in the context of overlapping distributions of var. latiusculum and var. pseudocaudatum, most collection localities were chosen along two parallel NW to SE transects in Virginia, North Carolina, and South Carolina (Fig. 3). Each transect spanned from the mountains where var. latiusculum predominates, across the piedmont where the two varieties come into contact, through the coastal plain where var. pseudocaudatum predominates (Fig. 3). To establish whether differences that might be discovered between the varieties within this limited area are representative of the taxa as a whole, one outlying population of each taxon was also included, i.e. a var. latiusculum population from Petersham, Massachusetts and a var. pseudocaudatum population from Nassau County, Florida.

Pteridium aquilinum spreads through rhizome to form clones that may extend for several hundred meters (Sheffield et al. 1989; Parks and Werth 1993). To avoid replicating genets, samples were collected at large intervals of at least 400 m and more often at

Table 4. Localities sampled in isozyme study. L = var. latiusculum, P = var. pseudocaudatum

Locality	Taxon present	Geographic description
Petersham, MA (PETE)	L	Harvard Forest, Prospect Hill Tract to Petersham on Hwy. 2.
Mountain Lake, VA (MLBS)	L	Mountain Lake Biological Station, near Pembroke, VA
Martinsville, VA (MART)	L	Henry County area with some specimens from adjacent Patrick and Franklin counties.
Greensboro, NC (GREN)	L, P	Guilford County, with a few specimens from Forsyth and Rockingham counties.
Aberdeen, NC (ABER)	P	Aberdeen, Pinebluff, and the Sandhills Game Mgt. Area.
Southport, NC (SOUT)	P	Bladen, Columbus, and Brunswick counties.
Asheville, NC (ASHE)	L	Buncombe Co. with a few specimens from Avery, McDowell, and Mitchell counties.
Columbia, SC (COLU)	P	Lexington and Calhoun counties. One specimen from Newport county.
Orangeburg, SC (ORAN)	P	Orangeburg and Berkeley counties.
Nassau Co., FL (NASS)	P	Nassau County, FL, west of I-95. A few specimens from adjoining Camden Co., GA.

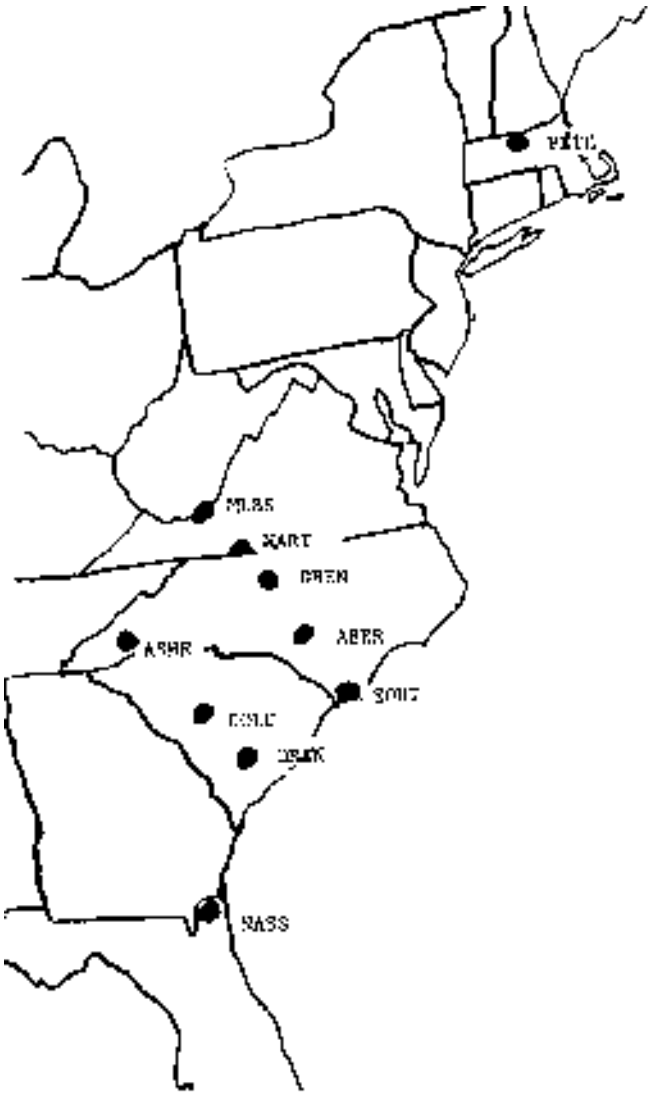

Figure 3. Locations of populations sampled for isozyme study.

intervals of approximately 800 m, resulting in most cases in the collection of genetically different individuals

from adjacent samples. In most cases samples were taken, one per patch, from discrete patches collected along roadsides, although bracken growth was nearly continuous in certain portions of the coastal plain. Previous studies on bracken indicate that randomly mating neighborhoods of this outcrossing organism occur over huge expanses (Wolf et al. 1988, 1991), and the results of Hardy-Weinberg comparisons from the present study (see isozyme results below) validate the appropriateness of treating these extensive collection localities as single populations.

Collections were assigned to one of the two varieties being compared (latiusculum or pseudocaudatum) on the basis of morphological features of the leaf (Tryon 1941, Jacobs and Peck 1993). As the varieties exhibit strong geographic association, only a single variety was encountered at most localities. However, both varieties occurred together at the

Greensboro locality, which was purposefully chosen for this reason; because the goal of this study was to evaluate whether the varieties are distinct, each was treated as a separate population designated Greensboro-L and Greensboro-P for latiusculum and pseudocaudatum respectively.

Samples consisted of entire leaves, which were placed in ziplock bags and kept refrigerated at 4°C until they were used to prepare homogenates for electrophoresis within 3-4 days. Subsequent to homogenate preparation, leaves were pressed and dried for use in the morphological study and deposited as vouchers at VPI.

Genotype detection. Isozyme genotypes were determined through starch-gel electrophoresis of ten enzymes. Leaf tissue homogenates were prepared using "microbuffer" with 5% polyinylpyrrolidone (MW 40,000) and 0.1% 2-mercaptoethanol following Werth (1985). These were electrophoresed in 12% starch gels using three buffer systems and stained followed Soltis et al. (1983), using the "zymecicle" method of Werth (1990). Isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH), and 6-phosphogluconate dehydrogenase (6-PGD) were resolved on the morpholine-citrate system described in Werth (1991). PGM was resolved also on buffer system #6 of Soltis et al. (1983), as were glutamate oxaloacetate transaminase (GOT), phosphoglucose isomerase (PGI), and triosephosphate isomerase (TPI). A lithium hydroxide buffer was also used for GOT, as well as for hexokinase (HK), and leucine aminopeptidase (LAP). Alleles at each locus were designated by numbers with the lowest number representing the most anodal migration. Allele identity was controlled by running as a standard an individual (designated 95ML-12) with known genotypes at each gel. Data were analyzed using BIOSYS-1 (Swofford and Selander 1981) to evaluate patterns of genetic variation within and among populations and between the two varieties.

RESULTS

Fourteen loci coding eight enzymes (GOT, IDH, MDH, PGI, PGM, 6-PGD, SKDH, and TPI) were consistently scored for all populations. Bands observed for two enzymes (HK, LAP) could not be reliably scored and therefore were not included. Twelve of the scorable loci were polymorphic in at least one population (a locus was considered polymorphic when the frequency of the most common allele was not greater than 0.95) with the other two loci (Pgi1 and 6-Pgd1) being monomorphic in all populations.

Levels of genetic variation were evaluated by computing three indices: the mean number of alleles per locus (A), the proportion of polymorphic loci (P), and the expected mean heterozygosity (H_{exp}) (Table 5). The values of these indices differed substantially among populations, ranging from A = 1.5 to 2.4, P = 0.214 to 0.571, and H_{exp} = 0.042 to 0.241. The distribution of values followed a strong geographic trend. Populations of the outer piedmont and coastal plain, predominately var. pseudocaudatum, tended to exhibit substantially less polymorphism than those of the mountains and inner piedmont, these being exclusively var. latiusculum. The highest level of polymorphism was possessed by the northernmost population sampled, i.e. var. latiusculum from Petersham, MA. Within pseudocaudatum, the highest level of polymorphism was possessed by the southernmost population sampled, i.e. that from Nassau Co., FL.

Table 5. Levels of genetic variation using three indices: Alleles/locus (A), Proportion of polymorphic loci (P), HW expected mean heterozygosity (H_{exp}). Standard errors are in parentheses. Taxon present at each population is noted in parenthesis. L is for var. latiusculum and P is for var. pseudocaudatum.

Population	Mean sample size per Locus	A	P	Mean Heterozygosity H_{exp}	H_{obs}
Petersham, MA (L)	23.6 (0.3)	2.4 (0.3)	0.571	0.241 (0.063)	0.207 (0.063)
Mountain Lake, VA (L)	16.6 (1.0)	2.1 (0.3)	0.429	0.210 (0.070)	0.177 (0.065)
Asheville, NC (L)	19.0 (0.0)	1.8 (0.2)	0.571	0.157 (0.050)	0.184 (0.069)
Martinsville, VA (L)	63.4 (0.3)	2.4 (0.3)	0.429	0.164 (0.053)	0.173 (0.056)
Greensboro, NC (L)	20.6 (0.4)	1.6 (0.2)	0.286	0.109 (0.044)	0.102 (0.041)
Greensboro, NC (P)	23.9 (1.1)	1.6 (0.2)	0.357	0.091 (0.034)	0.103 (0.041)
Aberdeen, NC (P)	36.0 (0.0)	1.9 (0.3)	0.357	0.089 (0.032)	0.099 (0.037)
Southport, NC (P)	39.0 (0.0)	1.7 (0.3)	0.357	0.073 (0.030)	0.073 (0.032)
Columbia, SC (P)	21.0 (0.0)	1.5 (0.2)	0.214	0.042 (0.017)	0.041 (0.018)
Orangeburg, SC (P)	22.0 (0.0)	1.6 (0.2)	0.357	0.084 (0.030)	0.052 (0.023)
Nassau Co., FL (P)	20.0 (0.0)	1.9 (0.2)	0.571	0.128 (0.042)	0.143 (0.070)

Allele frequencies. Allele frequencies are shown in Table 6. A number of the polymorphic loci (Got, Idh, Mdh2, Mdh3, Pgi2, Skdh, Tpi2) were weakly so, a single allele predominating in all populations; these loci showed no strong geographic or taxonomic trends. In contrast, those loci with higher levels of polymorphism (Mdh1, Pgm1, Pgm2, 6-Pgd2, and Tpi1) showed striking geographic variation with similar trends (Figs. 4-5): allele frequencies were very similar in the two northernmost populations (Petersham, MA and Mountain Lake, VA), but showed substantial shifts southward and eastward across the transects sampled.

These shifts were especially dramatic for Pgm1, Pgm2, and 6-Pgd2; for each of these loci one of the alleles whose frequency in Petersham and Mt. Lake was less than 0.5 ($Pgm1^2$, $Pgm2^2$, $6Pgd2^1$) became greater than 0.6 in the more southern populations, increasing clinally to values greater than or near 0.9 in the outer coastal plain populations (Table 6, Fig. 3). At Tpi1, allele $Tpi1^2$, while the most frequent allele in the northern and mountain populations of latiusculum, became even more frequent, approaching or exceeding 0.9, in the more piedmont and coastal plain populations of latiusculum and pseudocaudatum.

The Mdh1 locus exhibited a pattern distinct from the other loci. $Mdh1^2$ was the most common allele in all populations, becoming especially frequent in the more southern populations. Allele $Mdh1^3$ was found at a substantial frequency in the northern and mountain populations but declined southward, disappearing in the easternmost populations of the two Carolinas. However, it reappeared in the southernmost population in Nassau County, FL at a 0.500 frequency, the highest for any population.

The strongest and most consistent shift in allele frequencies occurred at the mountain/piedmont boundary between the Mountain Lake and Martinsville populations, allele frequency changes occurring more gradually southeastward.

Genotype proportions within populations. The genotype proportion in each population was evaluated for conformance to Hardy-Weinberg expectations (Table 7). Deviations were quantified as the inbreeding coefficient F (Wright, 1965). Of the thirty-two cases that could be validly tested with chi-square, only six were significantly different from F = 0. The Tpi1 locus at the Petersham population, Pgm1 at Mountain Lake, Pgm2 at Greensboro (P), Idh at Orangeburg, and Tpi1 at Nassau possessed heterozygote deficiencies, while 6-Pgd2 at Asheville had an excess of heterozygotes.

Genetic Identity and Clustering**.** Values for Nei's (1978) unbiased genetic identity (I) were computed for all pairwise combinations of the eleven populations (Table 8). These values were consistently very high, all exceeding 0.9 and averaging 0.976. The genetically depauperate populations of the outer piedmont and coastal plain, because they shared very high frequencies for the same alleles, tended to possess the highest I values when compared, these all exceeding 0.98 and ranging up to 1.000 for the Aberdeen - Southport populations. Other notably high values were I = 1.000 between the Martinsville population of the Virginia piedmont and Asheville populations of the North Carolina Blue Ridge and I = 0.998 between the Petersham, MA and Mountain Lake, VA populations. The lowest value encountered was I = 0.916 between the Petersham, MA and Orangeburg, SC populations. The mean genetic identity among the five populations of var. latiusculum was 0.980 and among the six populations of var. pseudocaudatum was 0.991.

Table 6. Allele frequencies for each locus surveyed in eleven bracken populations. In those cases where a locus has an allele "0", it should be noted that this allele is more anodal than allele "1" and was discovered after allele "1" had been designated.

	Population								
	PETE	MLBS	MART	GREL	GREP	ABER	SOUT	ASHE	COLU
Got									
(N)	24	18	63	21	25	36	39	19	21
1	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000
2	1.000	0.972	0.992	1.000	1.000	0.944	0.987	0.947	1.000
3	0.000	0.028	0.008	0.000	0.000	0.042	0.013	0.053	0.000
Idh									
(N)	24	18	64	21	25	36	39	19	21
1	0.042	0.139	0.031	0.000	0.000	0.028	0.000	0.000	0.000
2	0.958	0.833	0.961	1.000	1.000	0.972	1.000	1.000	1.000
3	0.000	0.028	0.008	0.000	0.000	0.000	0.000	0.000	0.000
Mdh1									
(N)	24	18	64	21	25	36	39	19	21
1	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.000	0.000
2	0.750	0.861	0.836	0.976	0.960	1.000	1.000	0.921	1.000
3	0.250	0.139	0.148	0.024	0.040	0.000	0.000	0.079	0.000

Mdh2

(N)	24	18	64	21	25	36	39	19	21
0	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	0.938	1.000	0.992	1.000	1.000	0.972	1.000	1.000	1.000
2	0.021	0.000	0.008	0.000	0.000	0.028	0.000	0.000	0.000

Mdh3

(N)	24	18	64	21	25	36	39	19	21
0	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	0.979	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Pgi1

(N)	21	18	64	21	25	36	39	19	21
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Pgi2

(N)	21	18	60	21	25	36	39	19	21
1	0.071	0.028	0.000	0.000	0.020	0.000	0.000	0.000	0.000
2	0.857	0.972	1.000	1.000	0.980	1.000	1.000	0.974	1.000
3	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.000

Pgm1

(N)	24	18	64	21	25	36	39	19	21
0	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	0.500	0.389	0.164	0.214	0.080	0.042	0.051	0.105	0.024
2	0.375	0.306	0.781	0.786	0.920	0.958	0.949	0.895	0.976
3	0.104	0.167	0.039	0.000	0.000	0.000	0.000	0.000	0.000
4	0.000	0.139	0.016	0.000	0.000	0.000	0.000	0.000	0.000

Pgm2

(N)	24	18	64	21	25	36	39	19	21
0	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1	0.021	0.000	0.102	0.048	0.080	0.153	0.103	0.079	0.024
2	0.417	0.472	0.617	0.762	0.620	0.764	0.782	0.684	0.952
3	0.000	0.056	0.023	0.000	0.000	0.000	0.000	0.000	0.024
4	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5	0.500	0.417	0.258	0.190	0.300	0.083	0.115	0.237	0.000

6-Pgd1

(N)	24	18	64	21	25	36	39	19	21
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

6-Pgd2

(N)	24	18	64	21	25	36	39	19	21
1	0.271	0.278	0.602	0.762	0.820	0.889	0.897	0.605	0.905
2	0.083	0.083	0.008	0.000	0.000	0.000	0.000	0.000	0.000
3	0.646	0.639	0.391	0.238	0.180	0.111	0.103	0.395	0.095

Skdh

(N)	24	18	64	21	25	36	39	19	21
1	0.000	0.000	0.008	0.024	0.000	0.042	0.013	0.000	0.024
2	0.042	0.000	0.078	0.000	0.060	0.069	0.077	0.053	0.048
3	0.958	1.000	0.914	0.952	0.940	0.861	0.885	0.947	0.929
4	0.000	0.000	0.000	0.024	0.000	0.028	0.026	0.000	0.000

Tpi1

(N)	24	9	62	16	10	36	39	19	21
1	0.250	0.056	0.016	0.031	0.000	0.000	0.026	0.053	0.000
2	0.625	0.778	0.806	0.906	0.900	0.944	0.936	0.684	0.929
3	0.125	0.167	0.177	0.063	0.100	0.056	0.038	0.263	0.071

Tpi2

(N)	24	9	62	21	25	36	39	19	21
1	0.917	1.000	0.984	1.000	0.980	1.000	1.000	0.921	1.000
2	0.083	0.000	0.016	0.000	0.020	0.000	0.000	0.079	0.000

	Population	
	ORAN	NASS
Got		
(N)	22	20
1	0.091	0.025
2	0.909	0.875
3	0.000	0.100
Idh		
(N)	22	20
1	0.205	0.000
2	0.795	1.000
3	0.000	0.000
Mdh1		
(N)	22	20
1	0.000	0.000
2	1.000	0.500
3	0.000	0.500
Mdh2		
(N)	22	20
0	0.000	0.025
1	1.000	0.950
2	0.000	0.025

Mdh3

(N)	22	20
0	0.000	0.000
1	1.000	1.000

Pgi1

(N)	22	20
1	1.000	1.000

Pgi2

(N)	22	20
1	0.000	0.000
2	1.000	1.000
3	0.000	0.000

Pgm1

(N)	22	20
0	0.000	0.000
1	0.000	0.050
2	1.000	0.950
3	0.000	0.000
4	0.000	0.000

Pgm2

(N)	22	20
0	0.000	0.000
1	0.045	0.050
2	0.909	0.900
3	0.000	0.000
4	0.000	0.000
5	0.045	0.050

6-Pgd1

(N)	22	20
1	1.000	1.000

6-Pgd2

(N)	22	20
1	0.886	0.850
2	0.000	0.000
3	0.114	0.150

Skdh

(N)	22	20
1	0.000	0.000
2	0.023	0.050
3	0.955	0.950
4	0.023	0.000

Tpi1

(N)	22	20
1	0.000	0.100
2	0.886	0.825
3	0.114	0.075

Tpi2

(N)	22	20
1	1.000	1.000
2	0.000	0.000

Table 7. Fixation index (inbreeding coefficient) values at each locus for all populations. These were tested for statistical difference from F = 0 using chi-square analysis, with pooling where appropriate. Levene (1949) correction for small population size was employed in the chi-square analysis. The chi-square test was not valid (nv) in some instances due to small expected class values. Dashes (-) indicate monomorphic loci.

	Population										
Locus	PETE	MLBS	MART	GREL	GREP	ABER	SOUT	ASHE	COLU	ORAN	NASS
Got	-	nv	nv	-	-	nv	nv	nv	-	nv	nv
Idh	nv	-0.168	nv	-	-	nv	-	-	-	0.860***	-
Mdh1	0.111	nv	-0.176	nv	nv	-	-	nv	-	-	nv
Mdh2	nv	-	nv	-	-	nv	-	-	-	-	nv
Mdh3	nv	-	-	-	-	-	-	-	-	-	-
Pgi1	-	-	-	-	-	-	-	-	-	-	-
Pgi2	nv	nv	-	-	nv	-	-	nv	-	-	nv
Pgm1	0.373	0.686*	-0.039	-0.273	nv	nv	nv	nv	nv	-	nv
Pgm2	-0.238	nv	-0.038	0.000	0.307*	-0.206	-0.055	-0.233	nv	nv	nv
6-Pgd1	-	-	-	-	-	-	-	-	-	-	-
6-Pgd2	-0.078	0.125	0.002	-0.050	-0.220	-0.125	-0.114	-0.652**	nv	nv	0.216
Skdh	-0.043	-	-0.086	nv	nv	-0.106	-0.095	nv	nv	nv	nv
Tpi1	0.529**	nv	-0.218	nv	nv	nv	nv	0.114	nv	nv	-0.519*
Tpi2	nv	-	-0.016	-	nv	-	nv	nv	-	-	-

* P < 0.05; ** P < 0.01; *** P < 0.001.

Table 8. Matrix of pairwise values of Nei's (1978) unbiased genetic identity between populations of Pteridium aquilinum.

Population	1	2	3	4	5	6	7	8	9	10	11
1 Petersham	-----										
2 Mountain Lake	0.998	-----									
3 Martinsville	0.972	0.979	-----								
4 Greensboro (L)	0.957	0.967	0.997	-----							
5 Greensboro (P)	0.949	0.957	0.996	0.999	-----						
6 Aberdeen	0.926	0.938	0.988	0.997	0.998	-----					
7 Southport	0.929	0.940	0.988	0.998	0.998	1.000	-----				
8 Asheville	0.966	0.971	1.000	0.996	0.996	0.989	0.990	-----			
9 Columbia	0.919	0.932	0.983	0.995	0.993	0.999	0.999	0.987	-----		
10 Orangeburg	0.916	0.934	0.982	0.992	0.991	0.997	0.996	0.986	0.998	-----	
11 Nassau	0.927	0.928	0.979	0.979	0.981	0.978	0.980	0.978	0.981	0.978	----

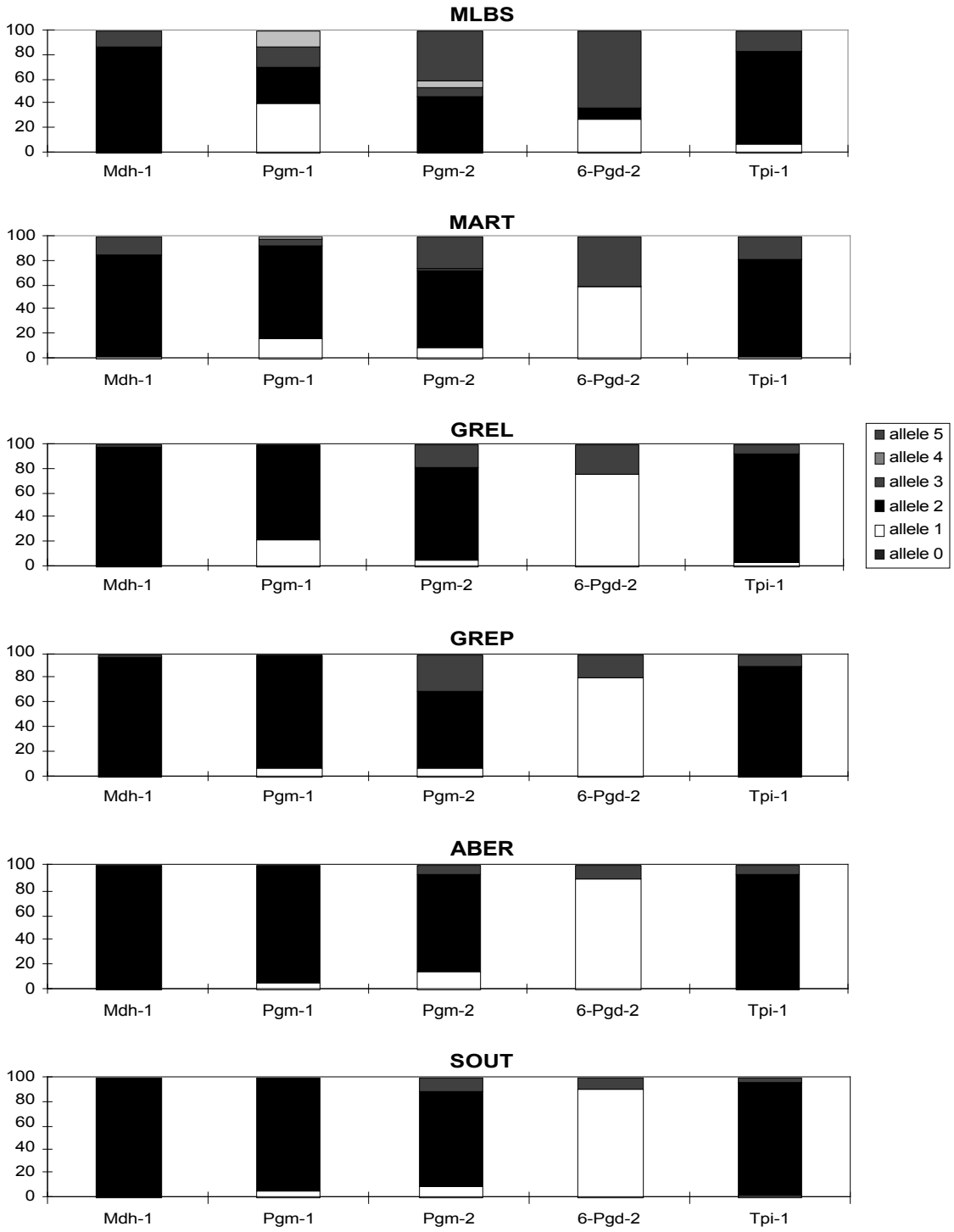

Figure 4. Transect 1; allele frequency changes for Mdh1, Pgm1, Pgm2, 6-Pgd2, and Tpi1

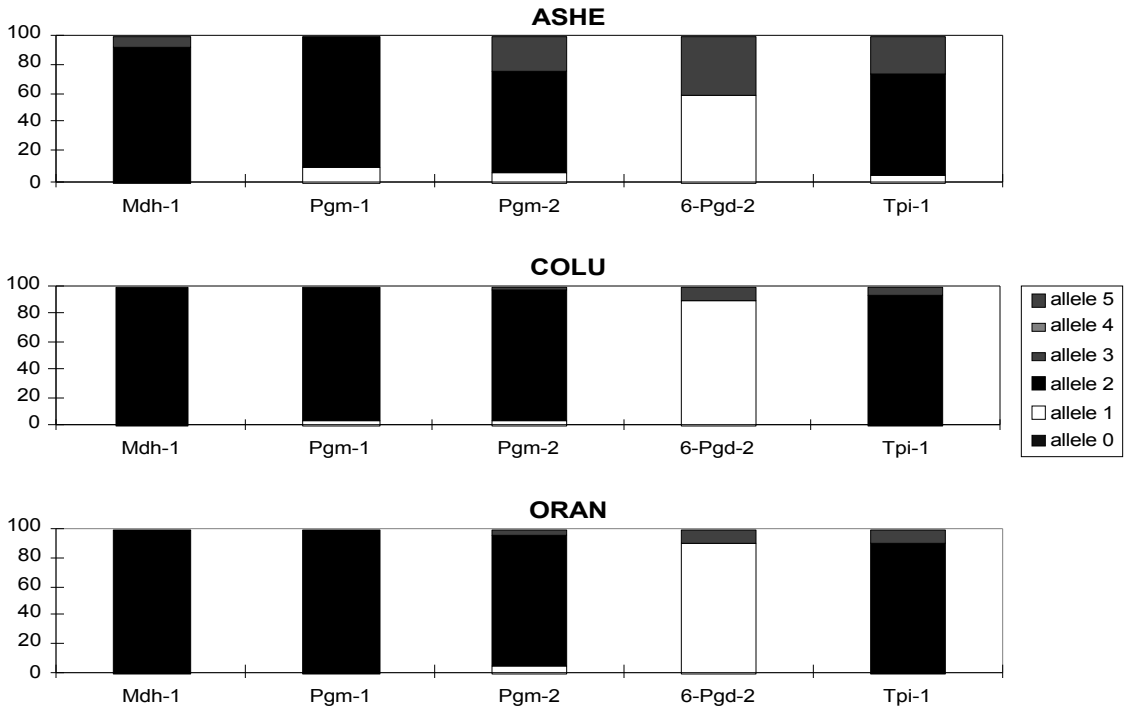

Figure 5. Transect 2; allele frequency changes for Mdh1, Pgm1, Pgm2, 6-Pgd2, and Tpi1.

Clustering was carried out using the Unweighted Pair-Group Method using Arithmetic Averages (UPGMA) algorithm, resulting in a dendrogram with two principle clusters, one comprising the Petersham, MA and Mountain Lake, VA populations, both var. latiusculum, the second cluster comprising all the other more southerly populations with the two varieties intermingled in a complex way (Fig. 6). Within the larger cluster, the Nassau County, FL population of var. pseudocaudatum was placed outside the other populations. Although the remaining pseudocaudatum populations of North and South Carolina coastal plain were placed in a single cluster, pseudocaudatum from Greensboro, NC was clustered as most similar to the sympatrically occurring latiusculum population from this same locality, reflecting the strong genetic affinities indicated by the isozyme data for the two morphologically defined entities co-occurring in this region. Thus, UPGMA failed in any way to separate the two varieties into distinct clusters based on isozymes.

Population differentiation**.** Population differentiation for allele frequencies was quantified by computing F_{ST} (Wright 1969; 1978). Values were computed for the set of all populations, separately for each variety, and for the set of two populations, separated according to variety, co-occurring in the Greensboro, NC area (Table 9). For the comparison of all populations, values of F_{ST} were substantial, at most loci exceeding 0.05, and significantly greater than 0 ($p < 0.05$). Using the criteria of Hartl (1981), most loci exhibited moderate differentiation ($0.05 < F_{ST} < 0.15$). The exceptions were Mdh2, Mdh3, and Skdh for which the frequency of the most common allele

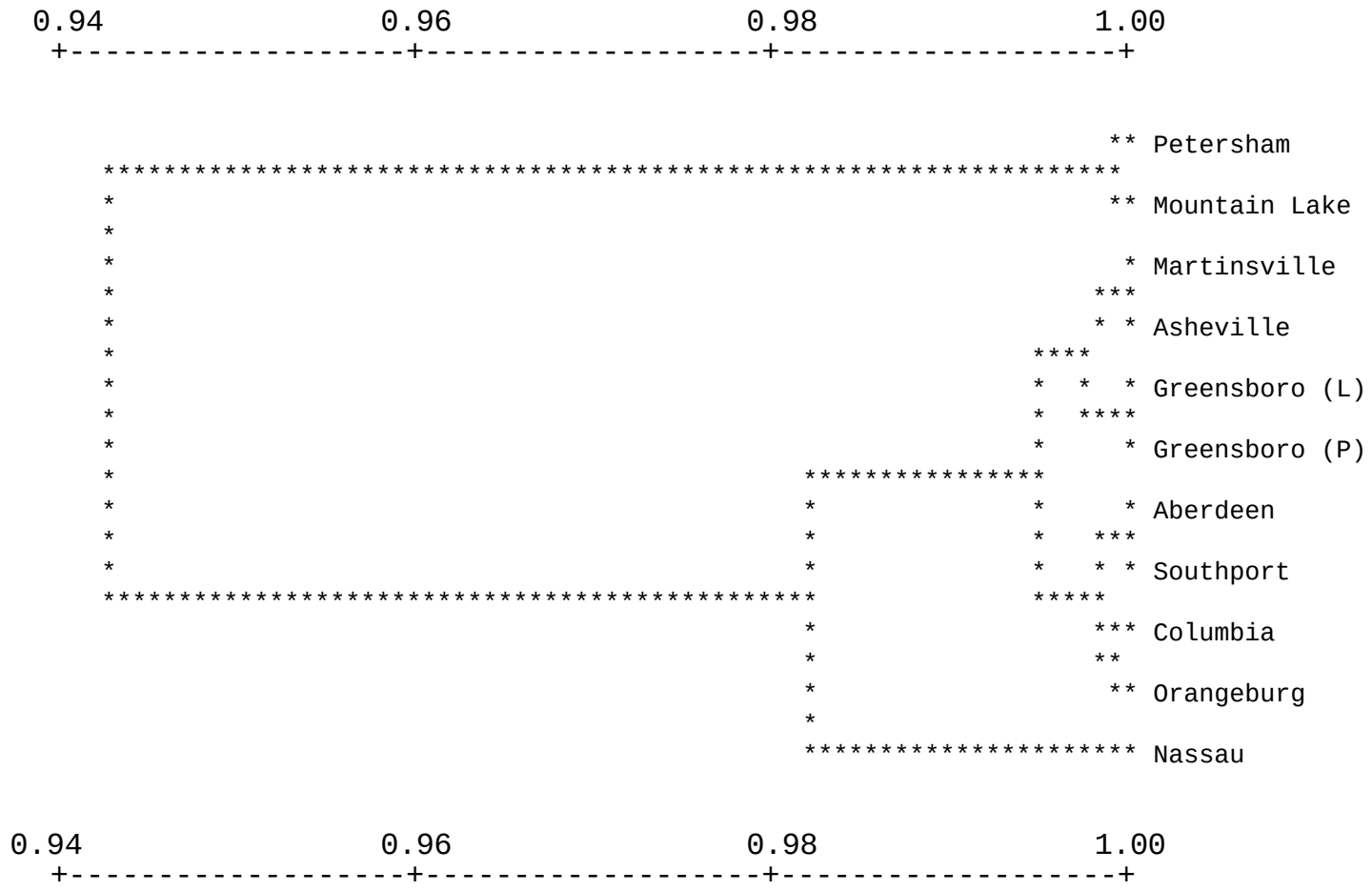

Figure 6. Cluster diagram based on Nei's (1978) unbiased genetic identity values using UPGMA; cophenetic correlation coefficient = 0.888.

Table 9. Values for F_{ST} at all polymorphic loci. Dashes (-) indicate where monomorphic.

Locus	All Populations	latiusculum	pseudocaudatum	Greensboro
Got	0.049***	0.024	0.051***	-
Idh	0.109***	0.068*	0.151***	-
Mdh1	0.222***	0.051	0.413***	0.002
Mdh2	0.029	0.034	0.024	-
Mdh3	0.019	0.017	-	-
Pgi2	0.065***	0.057***	0.017	0.010
Pgm1	0.253***	0.165***	0.016	0.036
Pgm2	0.122***	0.061***	0.077***	0.018
6-Pgd2	0.219***	0.122***	0.008	0.005
Skdh	0.019	0.021	0.013	0.012
Tpi1	0.069***	0.054***	0.020***	0.003
Tpi2	0.053*	0.041	0.017	0.010
Mean	0.152***	0.090***	0.090***	0.015

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

was very high in all populations. The highest F_{ST} values were observed at those loci exhibiting the strongest geographic trends (Mdh1, Pgm1, Pgm2, and 6-Pgd2). The mean value of 0.152 across all loci indicates a considerable amount of genetic differentiation among these populations.

Levels of genetic differentiation among populations of either variety were lower than among all populations, with most values below 0.1, fewer showing statistical difference from 0, and a mean of 0.90 for both varieties. However, values of F_{ST} across the two varieties within the Greensboro locality were very small, ranging from 0.002 for Mdh1 to 0.036 for Pgm1, and none were significantly different from 0 (Table 9).

To compare the separate contributions of geographic differentiation and taxonomic status to genetic variance among populations, two hierarchal analyses were carried out using BIOSYS-1. In the first, populations were grouped into their respective varieties, while in the other, populations were grouped by regions (Table 10). Variance components for variety and locality and for region and locality, respectively, were then computed (Table 11). In both analyses, most of the heterogeneity was attributable to differences among localities while a much smaller portion of the variance was due to either taxonomic or regional differences. Region (F_{XY} = 0.062) explained slightly more of the variance than did variety (F_{XY} = 0.053).

Table 10. Hierarchal arrangement of populations for hierarchal F-statistic analysis.

HIERARCHY 1 (by Taxon)	HIERARCHY 2 (by Region)
Latiusculum	Mountains
Petersham	Petersham
Mountain Lake	Mountain Lake
Martinsville	Asheville
Greensboro-L	
Asheville	Piedmont
	Martinsville
Pseudocaudatum	Greensboro-L
Greensboro-L	Greensboro-P
Aberdeen	Aberdeen
Southport	Columbia
Columbia	
Orangeburg	Coastal Plain
Nassau	Southport
	Orangeburg
	Nassau

Table 11. Variance components and F-statistics combined across all loci for hierarchal analyses.

Comparison			Variance component	F_{XY}
X		Y		
Locality	-	Taxon	0.16745	0.087
Locality	-	Total	0.27441	0.135
Variety	-	Total	0.10695	0.053
Locality	-	Region	0.14964	0.079
Locality	-	Total	0.27489	0.136
Region	-	Total	0.12524	0.062

DISCUSSION

Based on the fourteen isozyme loci examined, genetic relatedness among all populations was very high regardless of taxonomic affinity. The mean Nei's (1978) identity value for the eleven populations, I = 0.976., is well above the mean identity of I = 0.33 reported for congeneric fern species (Soltis and Soltis 1989) but is comparable to the high values observed for conspecific populations of angiosperms ($I \geq 0.95$, Gottlieb 1977; 1981; Crawford 1983) and ferns (Soltis and Soltis 1989). It is substantially greater than the mean genetic identity of I = 0.681, using 21 loci, between European var. aquilinum and North American var. latiusculum (Wolf et al. 1991), this lower value undoubtedly owing to the broad geographic scope among populations included in the average.

Identity values, while useful for indicating amounts of genetic relatedness, should not be used alone to assign rank to putative taxa. For example, interspecific identity values comparable to those of conspecific angiosperm populations were observed between species of Antennaria Gaertner (Bayer 1991) and Menziesia Smith (Wells and Bohm 1994). On the other hand, conspecific Liriodendron tulipifera L. populations exhibited identity values as low as I = 0.62 (Parks et al. 1994), comparable to the mean of I = 0.67 for congeneric angiosperm species (Gottlieb 1981; Crawford 1983). In the present study, a transect-based sampling strategy was designed to detect possible geographic variation that might exist for allozyme frequencies and to evaluate the correlation of such variation with the occurrence of the two putative varieties distinguished on the basis of morphological features. Genetic identities, F-statistics, and indices of genetic polymorphism (A, P, and H_{exp}) were considered collectively to evaluate the taxonomy of the putative bracken varieties.

A strong pattern of among-population variation was observed that reflected geographic distribution rather than taxonomic affinity, and failed to separate the eleven populations into their respective taxonomic groups (Fig. 6). The two northernmost populations from Petersham, MA and Mountain Lake, VA were highly similar despite being geographically distant, while in the southern portion of the range similar north-to-south clines in allele frequencies occurred at five loci (Mdh-1, Pgm1, Pgm2, 6-Pgd2, and Tpi1). These clines were accompanied by reduced genetic variability in the more southern populations that reached an extreme of near monomorphism in the outer coastal plain. The most abrupt shift in allele frequencies seemed to occur within var. latiusculum between the Mountain Lake population and the Martinsville and Asheville populations to its south. Thus, the strong geographic pattern of allozyme exhibited independence of the varietal status of populations. This pattern was reflected in UPGMA clustering that grouped Petersham and Mountain Lake populations, both variety latiusculum, in one cluster and all the other more southerly populations in a second cluster that included both var. latiusculum and var. pseudocaudatum populations intermingled.

Of the localities studied, Greensboro, NC is of special interest because of the sympatry of the two varieties in that locality. There the two populations had nearly identical allele frequencies at all loci except Pgm1 and Pgm2, resulting in a genetic identity of I = 0.999 (Table 8), a complete lack of genetic differentiation as shown by F_{ST} values at all loci statistically equivalent to 0, and concordance to Hardy-Weinberg expectations at all loci of genotype proportions after combining the putative taxa into a single genotype pool (Table 9). Thus, the Greensboro locality exhibits attributes of a single panmictic population, and its tentative separation, for purposes of the present study, into two populations representing the two putative varieties appears to have been artificial.

The cumulative weight of the evidence supplied by both the morphological and isozyme data sets strongly supported an infraspecific treatment of these two taxa. A consistent pattern of variation suggestive of two taxa was observed in the morphological study. The high mean genetic identity for the populations studied was comparable to the mean reported for conspecific angiosperm populations. The information from the Greensboro locality indicates that gene flow occurs between the two taxa and that they are not reproductively isolated. The findings of this study are viewed as being supportive of a varietal treatment for these two bracken taxa.

Chapter 4

SUMMARY AND GENERAL CONCLUSION

This investigation used both morphological characters and isozyme variation to investigate the systematics of bracken taxa treated by Tryon (1941) as var. latiusculum and var. pseudocaudatum. As a result, valuable information required to evaluate the taxonomy of these two eastern North America taxa was obtained. Moreover, it is hoped that this investigation has contributed to the level of knowledge necessary for a more encompassing systematic evaluation of the genus Pteridium.

As with the majority of the world's flora, delineation of pteridophyte species has been largely been based on morphological species concepts, whereby species are distinguished by the consistent morphological differences perceived on specimens. Assignment of taxonomic rank is based the level or amount of variation. A subspecies would be expected to a have a lower level of variation than that of a species. Varieties, on the other hand, would show less variation than a subspecies. In this investigation both allozymes and morphology genetic species were used.

The allozyme data indicate that Pteridium of the southeastern United States (exclusive of var. caudatum of south Florida) comprises a single uninterrupted gene pool. Geographic variation occurs within this gene pool, but does not correspond to varietal status. In contrast, detailed morphological studies showed that the two putative varieties are quite distinct for all qualitative diagnostic features. An understanding of the genetic complexity and evolutionary history of southeastern U. S. bracken requires a single explanation that can accommodate both of these contrasting observations.

Part of the discordance could be explained if the morphological differences between latiusculum and pseudocaudatum constitute a polymorphism having a simple genetic basis. Consider, for example, a single gene exhibiting pleiotropy and dominance so that the presence of a single dominant allele in the homozygous or heterozygous state would result in the expression of the phenotypes that collectively characterize one of the two varieties. If the pseudocaudatum allele was recessive, then the proportion of pseudocaudatum individuals in a population would be the square of the allele frequency, and thus would be rare or absent where the frequency is low (e.g. < 0.1). Geographic variation for allele frequencies only slightly more contrasting than those of allele 6Pgd-2^1 (Table 6) could result in the observed geographic distribution of morphological phenotypes.

Such contrasts could arise also if the phenotypes were subjected to natural selection imposed by habitat differences. This seems quite feasible. The more skeletonized leaf of pseudocaudatum suggests adaptation for reduced evapo-transpiration. This is a useful feature in the southeastern coastal plain where conditions are warmer, sunnier, and drier than those experienced by latiusculum in the cooler, more mesic northeastern and mountain forest region. Selection could cause exaggeration of allele frequency differences with primarily historical causes, as is likely to be the case for the presumably neutral (unless proven otherwise) alleles comprising allozyme polymorphisms. The isozyme evidence indicated that the northern populations were genetically more variable than the southern populations. If a corresponding relationship exists for loci governing morphotype, it is possible that dominant latiusculum alleles would be at very high frequency in the north, with the recessive pseudocaudatum alleles at high frequency, or even fixed,

in the south. This would account for what was seen at Greensboro, in the North Carolina piedmont, where the two varieties appeared in approximately equal frequencies. If a recessive allele was present at a moderately high frequency (ca. 0.7), the dominant and recessive phenotypes would appear in approximately equal proportions.

In this study, the area of geographic overlap between the two morphotypes (i.e. morphologically defined taxa) was predominantly in the piedmont, where bracken tended to be somewhat less abundant than in either the mountains (var. latiusculum) or the southeastern coastal plain (var. pseudocaudatum). The piedmont populations tended to be intermediate between the mountain populations and the coastal plain populations in terms of genetic variability and allele frequencies. It is quite possible that what is seen in the piedmont populations reflects the combined genetic influence of both the mountain and the coastal plain bracken.

The findings of this study contrast somewhat other that compared bracken populations in Britain (Wolf et al. 1991) and in Finland (Korpelainen 1995) in that neither of these two studies reported clinal variation. The North American populations had a slightly lower mean genetic identity ($\underline{I}$ = 0.976) than either the British ($\underline{I}$ = 0.995) or Finnish ($\underline{I}$ = 0.981) bracken populations. The level of genetic differentiation ($\underline{F}_{ST}$ = 0.152) found among eastern North American populations was greater than that reported for British bracken ($\underline{F}_{ST}$ = 0.110), but lower than that reported for the Finnish populations ($\underline{F}_{ST}$ = 0.307). In this investigation it was suggested that the situation in the North American populations was probably the result of secondary contact between two formerly isolated population systems. Wolf et al. (1991) concluded that extremely high gene flow is evident among the British populations, causing them to behave as a single panmictic population. Korpelainen (1995), however, found low levels of gene flow between the Finnish populations, suggesting that genetic drift played more of a role in determining allele frequencies. Unfortunately, the British and Finnish investigations did not examine morphological variation and, therefore, no comparisons can be made along this line. These contrasting findings indicate that very different historical processes account for these three population sets.

Rumsey et al. (1991) were able to identify hybrids between var. aquilinum and var. latiusculum on the basis of fixed differences in alleles between var. aquilinum and var. latiusculum, probably a result of a long period of isolation between the two taxa. No comparable fixed differences were found between var. latiusculum and var. pseudocaudatum. which may suggesting that they have been in contact more recently.

The isozyme evidence strongly suggests that eastern United States bracken (exclusive of var. caudatum of peninsular Florida) comprises a single gene pool exhibiting significant geographic variation. Allele frequencies at several independently coded loci show dramatic parallel north-south clines, and levels of genetic polymorphism decline dramatically toward the southeastern extreme of this cline. The geographic patterning of the two dissimilar morphotypes classified as var. latiusculum and var. pseudocaudatum may be interpreted as resulting from a similar cline at loci governing leaf characters. The biogeographic and evolutionary scenario that accounted for the development of this gene pool may have been complex, and some details are likely lost in antiquity. Nonetheless, it is possible to consider as plausible two contrasting hypotheses that account for the observed clinal patterns in allele frequency, distribution of the morphotypes, and genetic depauperation of the coastal plain populations. These hypotheses are designated the sympatric hypothesis and the allopatric hypothesis. Both hypotheses assume that the present distribution of eastern North American P. aquilinum and the geographic pattern of genetic variation were arrived at relatively recently. The distribution and geographic pattern having been shaped by northward migrations following the Wisconsin glacial maximum, approximately

18,000 years before present (ybp)(Dawson 1992), and the climatic change of the Hypsithermal period of 10,000 to 6,000 ybp (Davis 1983).

The allopatric hypothesis proposes that at some point in time, bracken became geographically subdivided into two allopatric gene pools, one northern and the other southern. These two populations underwent divergence but have since reunited through secondary contact. This subdivision may have occurred during the Wisconsin glacial maximum, an event that is hypothesized to have caused geographic subdivision of numerous species (Pielou 1991), or possibly during an earlier glacial advance. The northern population system, the source of the present-day latiusculum morphotype, would likely have occurred in a broad zone of the mainly boreal forest that occurred south of, but nearly up to, the glacial margin (Delcourt 1979). The southern population system, source of the present day pseudocaudatum morphotype, may have occurred in a refugium near the Gulf of Mexico, as has been proposed for several temperate forest taxa (Sauer 1988). Isolation may have resulted in divergences in allele frequencies at both morphological and allozyme loci. If the southern population system was small, it may well have acquired through genetic drift (and/or natural selection - see below) features that presently characterize the coastal plain populations of bracken, i.e. low levels of genetic variation accompanied by near fixation in a number of loci of one of the alleles comprising a minority or at most plurality in the northern populations. Alternatively, these features may have resulted from bottlenecks accompanying successive northward migrational events following retreat of the ice sheet. In either case, post-glacial migration would have resulted in secondary contact between these formerly isolated population systems, as represented by the present overlap in range between the pseudocaudatum and latiusculum population systems. The observed clinal variation could then have become established via introgression between the northern and southern gene pools.

The sympatric hypothesis proposes that geographic differentiation arose within a single gene pool, i.e. a system of more-or-less contiguous populations among which gene flow was at least moderately frequent. According to this hypothesis, the distribution of eastern United States bracken has continuously been undivided but has encompassed sufficient physical distance and environmental variability as to become genetically subdivided. Divergence among populations could have resulted from a combination of genetic drift and diversifying natural selection imposed by differences in environmental conditions. Selection may also have played a role under the allopatric hypothesis, but it is more critical for explaining divergence of populations experiencing gene flow. Divergence may have been intensified during the Wisconsin glaciation, when distances among populations may have been greater and consequently rates of gene flow smaller. Selection could easily be envisioned as influencing the frequency of both the pseudocaudatum and latiusculum morphotypes, and might also act directly on allele frequencies at enzyme loci. Such a situation has been reported for mannose-6-phosphate isomerase in amphipods (McDonald 1991) and for arginine kinase in marine snails (Tatarenkov and Johannesson 1994). Natural selection can also influence frequencies of selectively neutral allozymes by acting on other loci that are tightly linked to the enzyme loci, as has been reported for the fruit fly Rhagoletis pomonella (Berlocher and McPheron 1996). The sympatric hypothesis does not provide a robust explanation for the genetic depauperation of the coastal plain populations. Possibly these populations became bottlenecked during the Hypsithermal period to a greater degree than the more northern populations, which may have been able to maintain large populations in the higher elevations and more rugged topography of the mountains.

Although neither of the above hypotheses can be considered unequivocally supported over the other, the allopatric hypothesis is perceived in this study as being more likely. The substantial differentiation between the northern and southernmost populations, and their collective maintenance of genetic diversity, seem most consistent with survival by bracken in two or more

refugia during glaciation, as has been proposed for Liriodendron tulipifera L. (Parks et al. 1994). Survival at a single very restricted center would more likely result in global depletion of genetic diversity, as has been suggested for northern populations of both Thuja plicata Donn ex Don. and Pinus monticola Dougl. (Steinhoff et al. 1983). Moreover, the allopatric hypothesis seems somewhat more parsimonious, requiring only an episode of allopatry that might inevitably lead to some divergence, while divergence under the sympatric hypothesis requires reduced gene flow in sympatry combined with diversifying selection. Neither of these requirements of the sympatric hypothesis is well supported by current knowledge of the biology of P. aquilinum. Allozyme data suggest that extensive gene flow, presumably resulting from long-distance dispersal of spores, is capable of integrating bracken plants distributed over many kilometers into essentially a single panmictic unit (Wolf et al. 1991; Jump 1994). Furthermore, plants from both northern and southeastern population systems appear in a wide range of plant community types, as do most elements in the genus (Page 1976, Fletcher and Kirkwood 1979), and exhibit considerable overlap in the soil, light, temperature, and moisture conditions in which they are found (Tryon 1941; Page 1976). The lack of strong ecological differentiation is emphasized in the region of their sympatry, where both latiusculum and pseudocaudatum morphotypes may be found growing in close proximity.

Additional information is needed to further evaluate the systematics of eastern North American bracken. Genetic and morphological data from bracken west of the Appalachians would certainly furnish important information regarding the historical processes accountable for the current level and distribution of genetic variation in bracken. A common garden-plot study should be conducted to determine if the two morphotypes retain their distinctiveness when grown under identical conditions. Studies using nucleic acids (e.g. nDNA, mtDNA) would be another possible source of useful systematic information. RAPDs and RFLPs have been very useful at the population level and for DNA fingerprinting at the species level. Mitochondrial DNA may also be very useful at the population level. Finally, systematic studies of var. caudatum and var. pubescens need to be conducted for a more comprehensive treatment of North American bracken.

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Appendix A

NTSYS-PC MORPHOLOGY DATA MATRIX

Explanation. OTUs are listed by rows and characters are by columns. There are 262 OTUs and 12 characters. The numeral 9 is used to denote missing data. Specimens beginning with the letter “H” are specimens already deposited at the Massey Herbarium and were not collected by this researcher. The first OTU for which data is entered is H46641, the second H46616, the third H46618, and so on.

"BRACKEN Data"
1 262L 12L 1 9
H46641 H46616 H46618 H46626 H46622 H46621 H46624 H46623 H12560 H12564 H12563
H46625 H12555 H12553 H12556 H46620 H46632 H9617 H8766 H2935 H26878 H46617
H46636 H46649 H70587 H67984 H46626 H85407 H11831 H12562 H46645 H2920 H2113
H12554 H9619 H4452 H1205 H5756 H5715 H5143 H5780 H46646 H74900 H83258 H3044
H43992 H46628 H46648 H46640 H46639 H46638 H46637 H46635 H12572 H12557 H42472
H12565 H12558 H46619 H78979 H4519 H46627 H5142 H6077 H4615 H46633 H12566
H12570 H12571 H12561 H12569 GP2E GP2W GP3E GP3W GP4E GP5E GP5W GP6E GP6O GP6W
GP7E FB2E FB2W FB3E FB3W FB4W FB4E FB5N FB5S SS2W SS2E SS3S SS3N SS1S SS4W SS5
SS6E SR1 SR2 SR3W SR3E SR4W SR4E SR5W SR6 CK1 LF1S LF1N PS1 EN1 RW1 RW2 RW4
VG1 FD1 BM1 BM2 DS1 PH1W PH2 PH3 PH4 PC1 PC2 HE1E FS3 PH5 PH6E PL4 SD2 GP1W
GP4W GP7W FB1 SS1N SS4E SS6W DR1S VG2 RW3 GUL2L RAN6P? RAN8L VNL1L SV1SL SV2L
GUL3L GUL4P GUL5L CAS2P ASH3A ASH3B ASH5 AVE1 BUN1 BUN2 MCD1 MCD2 MIT1 MIT2
MIT4 PIS1 SF5 WM1S WM1N WM2N AB1NA AB1NB AB1NC PN1N PN1S PN2N PN2S SH1N SH1S
SH2E SH2W SH3N SH4N SH4S SH5N SH6N SH6S SH7N SH7S SH8S SH8N SH9N SH9S SH10N
SH10S SH11S SH12N SH13N SH13S SH14W SH14E BLA2 BLA7 BLA8 BLA10 BLA11 BLA12
BRU22A BRU22B BRU23 BRU24 BRU25 BRU26 BRU27 BRU28 BRU29 BRU31 BRU1 BRU13 BRU11
NEW2 LEX1 LEX4 CAL3 CAL6 CAL8 CAL10 ORA1 ORA3 ORA4A ORA4B ORA5 ORA6 ORA7A
ORA7B ORA10 BER1 BER2 BER4 BER5 BER6 BER7 BER8GA1 GA2 GA3 GA4 GA5A GA5B GA6
FL1 FL2 FL3 FL4 FL5 FL6A FL6B FL7A FL7B FL8 FL9 FL10 FLA11 FL13 FL14

LWid LHgt Stipe Term Ultim LPin SPin Cut MVH MPH AVH APH
29.9 39.6 39.9 1 1 1 1 2 0 1 0 1
43.8 34.7 9 1 1 2 1 2 0 0 0 1
31.1 31.6 17.3 1 1 1 1 2 0 0 0 0
27.4 34.1 22.9 1 1 0 1 2 0 0 0 0
23.5 19.7 21.4 1 1 0 1 2 0 0 0 1
38.8 38.6 9 1 1 1 2 2 0 0 0 1
54.2 43.5 41.4 1 0 0 2 2 0 1 0 1
22.6 25.6 24.6 1 1 1 2 2 0 0 0 1
42.6 30.7 9 1 1 2 1 2 0 1 0 1
43.0 34.9 23.1 1 1 1 2 2 0 0 0 1
29.2 27.8 9 1 1 2 2 2 0 1 0 1
30.9 36.0 19.8 1 1 1 2 2 0 0 0 0
44.6 36.2 25.7 1 1 1 2 2 0 0 0 0
33.8 29.3 24.6 1 1 1 2 2 0 0 0 0
53.8 34.9 9 1 1 1 2 2 0 0 0 0

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38.6 9    42.2 1 1 0 2 2 0 1 0 1
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28.3 27.6 9    0 0 0 1 1 1 0 1 0
25.4 32.7 43.4 0 0 0 1 0 0 0 0 1
26.9 33.8 9    1 1 2 1 2 1 0 0 1
26.9 33.9 30.0 1 1 0 2 2 0 1 0 0
47.2 39.7 24.7 1 1 0 1 2 1 0 1 0
36.6 31.7 35.6 1 1 2 2 2 1 0 1 0
38.6 28.7 30.7 9 1 0 1 1 1 0 1 0
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26.7 19.6 19.8 9 0 0 1 1 1 0 1 0
45.4 22.7 31.1 0 0 0 1 1 1 0 1 0
25.9 39.6 9    0 0 0 1 0 1 0 1 0
29.0 36.8 39.4 0 0 0 1 1 1 0 1 0
39.7 46.6 40.0 0 0 0 1 1 1 0 1 0
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29.4 33.5 9    0 0 0 1 0 1 0 1 0
45.4 31.3 35.1 0 0 0 1 1 1 0 1 0
19.7 32.6 08.6 0 0 2 1 1 1 0 1 0
38.8 39.9 37.7 0 0 2 1 1 1 0 1 0
35.2 44.2 9    0 0 0 1 0 1 0 1 0
31.8 26.7 25.5 0 0 0 1 1 1 0 1 0
23.3 29.6 25.9 0 0 0 1 1 1 0 1 0
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30.5 26.8 30.0 0 0 0 1 0 1 0 1 0
29.7 40.3 9    0 0 0 1 0 1 0 1 0
50.8 33.9 9    0 0 2 1 1 1 0 1 0
32.0 32.7 9    0 0 2 1 1 1 0 1 0
26.7 26.3 24.1 0 0 0 1 1 1 0 1 0
70.7 44.4 55.5 1 0 2 1 1 1 0 1 0
34.0 29.1 27.7 0 0 0 1 1 1 0 1 0
34.4 32.7 36.2 0 0 0 1 0 1 0 1 0
44.0 27.4 40.2 0 0 0 1 0 0 1 1 0
23.4 17.2 22.3 0 0 0 1 1 1 0 1 0
22.9 27.6 9    0 0 0 1 0 1 0 1 0
52.6 65.3 50.3 1 1 2 2 2 0 1 0 1
22.3 25.1 17.1 1 1 1 2 2 0 1 0 1
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9    9    23.1 1 1 0 1 2 0 1 0 0
21.6 30.9 26.4 1 0 0 2 2 0 0 0 1
53.8 40.5 33.4 0 0 0 1 1 1 0 1 0
26.6 49.9 39.7 0 0 2 1 1 1 0 1 0
26.6 40.5 9    0 0 2 1 1 1 0 1 0
54.8 26.6 28.1 0 0 0 1 1 1 0 1 0
35.6 22.1 25.7 0 0 2 1 0 1 0 1 0
39.4 28.9 20.5 0 0 0 1 0 1 0 1 0
27.8 34.9 22.3 1 0 2 1 1 1 0 0 0
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31.2 18.4 21.6 1 1 1 2 1 0 0 0 0
27.9 26.7 26.5 1 1 2 2 2 0 0 0 0
42.6 36.1 32.4 1 1 0 2 2 0 0 0 1
29.9 27.6 19.2 1 1 2 2 2 0 1 0 1
```

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15.3 16.5 09.7 1 1 2 2 2 0 1 0 1
12.7 16.7 07.5 1 1 2 2 2 0 1 0 1
35.2 38.7 36.6 1 1 2 1 1 0 1 0 1
45.0 40.2 34.2 1 1 2 1 2 0 0 0 0
48.1 41.0 32.6 1 1 2 2 2 0 0 0 1
39.6 41.1 37.2 1 1 2 2 1 0 0 0 0
42.3 42.4 22.9 1 1 2 2 1 0 0 0 0
47.9 45.7 27.4 1 1 2 1 2 0 0 0 1
49.3 44.9 24.0 1 1 2 2 1 0 0 0 1
37.4 51.9 38.1 1 1 1 2 2 0 0 0 1
65.3 43.8 48.1 1 1 2 2 2 0 0 0 1
27.4 31.9 28.4 1 1 1 2 2 0 0 0 0
65.1 42.2 53.4 1 1 2 2 1 0 0 0 0
40.3 37.6 28.1 1 1 2 2 0 0 0 0 0
31.9 25.0 28.5 1 1 2 2 2 0 0 0 1
21.3 18.4 23.3 1 1 2 1 2 0 0 0 0
56.1 40.0 41.7 1 1 2 2 1 0 0 0 1
33.7 35.2 23.3 1 1 2 2 2 0 0 0 0
33.9 36.0 36.8 1 1 2 2 2 0 1 0 1
44.1 48.3 34.4 1 1 2 2 2 0 0 0 0
47.8 42.8 13.1 1 1 2 1 1 0 0 0 0
61.4 52.7 35.1 1 1 2 2 1 0 0 0 0
34.5 40.9 30.3 1 1 2 2 1 0 0 0 0
47.1 36.5 48.4 1 1 2 2 2 0 0 0 0
49.4 44.9 29.7 1 1 2 2 2 0 1 0 0
48.7 51.4 32.2 1 1 0 2 2 0 1 0 1
33.3 36.3 30.3 1 1 2 2 2 0 1 0 1
47.1 36.1 23.9 1 1 2 2 1 0 1 0 1
55.8 58.6 37.9 1 1 2 2 2 0 0 0 0
63.1 42.6 34.7 1 1 2 2 2 0 0 0 1
41.8 53.9 31.7 1 1 2 2 2 0 1 0 1
56.7 45.4 43.7 1 1 2 2 1 0 1 0 0
54.1 43.6 35.1 1 1 2 2 1 0 0 0 0
33.7 22.3 19.8 1 1 1 2 1 0 0 0 0
24.6 39.3 45.1 1 1 2 2 2 0 1 0 0
45.3 39.3 32.2 1 1 2 2 1 0 1 0 0
58.4 44.8 33.5 1 1 2 2 2 0 1 0 0
41.3 30.7 30.3 1 1 2 2 1 0 1 0 1
40.3 49.9 46.5 1 1 1 2 2 0 1 0 1
36.0 36.6 37.1 1 1 0 2 2 0 0 0 1
48.0 49.7 44.6 1 1 2 2 2 0 1 0 1
42.4 34.1 46.2 1 1 2 2 1 0 1 0 1
61.9 52.5 78.9 1 1 2 2 2 0 0 0 1
51.9 59.3 36.6 1 1 2 2 2 0 0 0 1
44.6 44.3 45.6 1 1 2 2 2 0 0 0 0
38.7 40.4 32.9 1 1 2 2 1 0 0 0 0
32.5 44.7 45.5 1 1 2 2 1 0 0 0 0
46.8 56.7 49.1 1 1 2 2 2 0 0 0 0
48.1 40.7 45.8 1 1 2 2 2 0 0 0 0
54.9 48.8 33.5 0 1 2 2 2 0 0 0 0
45.7 40.7 45.3 1 1 2 2 2 0 0 0 0
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35.7 45.7 23.1 1 1 2 2 2 0 0 0 0
60.9 55.8 9    1 1 2 2 2 0 1 0 1
48.3 47.7 32.2 1 1 2 2 1 0 1 0 0
34.5 51.8 32.6 1 1 2 1 2 0 0 0 0
51.5 48.7 53.0 1 1 1 2 2 0 1 0 1
43.8 49.9 35.0 1 1 1 2 2 0 0 0 0
27.8 25.4 36.7 1 1 2 2 1 0 0 0 0
33.2 40.1 28.7 1 1 2 2 2 0 1 0 1
32.8 44.7 43.6 1 1 2 2 1 0 1 0 0
49.1 39.1 62.7 1 1 2 2 2 0 1 0 1
40.1 53.9 45.7 1 1 2 2 2 0 0 0 0
33.3 36.1 43.6 1 1 2 2 2 0 0 0 1
35.8 40.0 57.0 1 1 2 2 2 0 0 0 0
66.8 75.6 70.8 1 1 2 2 2 0 0 0 0
45.7 47.3 43.6 1 1 2 2 2 0 1 0 1
48.9 49.3 42.4 1 1 2 2 2 0 0 0 0
26.1 25.9 34.3 1 1 2 2 2 0 0 0 0
22.3 27.4 37.9 1 1 2 2 2 0 1 0 1
46.7 43.9 61.7 1 1 2 2 2 0 0 0 0
41.2 45.7 20.1 1 1 2 2 2 0 1 0 1
45.7 51.6 77.3 1 1 2 1 0 0 0 0 1
22.4 32.6 38.1 1 1 1 2 2 0 0 0 0
46.4 38.1 32.0 1 1 2 2 1 0 1 0 0
24.9 25.7 23.3 1 1 1 2 2 0 1 0 1
46.6 35.3 43.2 1 1 2 2 2 0 0 0 0
54.2 43.6 36.7 1 1 2 2 2 0 1 0 1
44.7 40.8 29.9 1 1 2 2 2 0 0 0 1
45.9 39.8 55.1 1 1 2 2 2 0 0 0 0
43.2 36.6 72.3 1 1 1 2 2 0 0 0 0
45.7 56.4 46.9 1 1 2 2 2 0 0 0 0
18.4 28.2 23.1 1 1 1 2 2 0 0 0 0
44.4 38.9 22.4 1 1 2 2 2 0 1 0 1
27.7 32.2 27.5 1 1 2 2 2 0 0 0 0
36.5 48.7 44.6 1 1 2 2 1 0 0 0 1
36.1 40.7 35.7 1 1 2 2 2 0 0 0 0
36.6 39.4 28.1 1 1 2 2 2 0 0 0 0
26.8 39.4 43.2 1 1 2 2 2 1 0 0 1
37.4 33.1 26.4 1 1 2 2 2 0 1 0 0
42.1 36.4 29.3 1 1 1 2 2 0 1 0 1
35.1 34.0 29.6 1 1 2 2 2 0 0 0 1
30.3 37.8 34.1 1 1 2 2 1 0 1 0 0
40.7 41.3 26.2 1 1 2 2 2 0 1 0 0
48.1 53.4 33.5 1 1 2 2 2 0 0 0 0
```

Appendix B

BIOSYS-1 ISOZYME DATA MATRIX

Explanation. The 11 OTUs are the 11 populations described in Chapter 3. Specimen number is listed in the first column. The second column is an arbitrarily assigned population code. The remaining 14 columns, beginning with GOT, list the genotype at each locus.

```
BRACKEN INDIVIDUAL GENOTYPE INPUT
NOTU=11,NLOC=14,NALL=6,CRT;
(14(A3,1X))
GOT IDH MD1 MD2 MD3 PI1 PI2 PM1 PM2 6P1 6P2 SKD TP1 TP2
STEP DATA:
DATYP=1,NUMER;
(A4,7X,14(1X,I1,I1))
PETE  Petersham
B001  BO1   22 22 22 11 11 11 22 22 25 11 33 33 23 11
B002  BO1   22 22 22 11 11 11 22 23 22 11 13 33 11 11
B003  BO1   22 22 22 11 11 11 22 11 55 11 33 33 11 11
B004  BO1   22 22 22 11 11 11 22 11 25 11 33 23 11 12
B005  BO1   22 22 22 11 11 11 23 11 25 11 33 33 22 11
B006  BO1   22 22 23 11 11 11 22 12 55 11 33 33 22 11
B007  BO1   22 22 23 11 11 11 22 22 55 11 13 33 22 11
B008  BO1   22 12 22 11 11 11 22 33 25 11 13 33 22 11
B009  BO1   22 12 22 11 11 11 12 11 22 11 33 33 22 11
B010  BO1   22 22 23 11 11 11 12 33 25 11 13 33 22 11
B011  BO1   22 22 22 11 11 11 22 12 25 11 13 33 11 11
B012  BO1   22 22 22 26 16 11 22 12 25 11 13 33 22 11
B013  BO1   22 22 22 11 11 11 13 11 25 11 13 33 13 11
B014  BO1   22 22 22 11 11 11 22 12 15 11 33 33 23 11
B015  BO1   22 22 22 16 11 11 22 22 26 11 12 33 22 12
B016  BO1   22 22 22 11 11 11 22 11 55 11 33 33 13 12
B017  BO1   22 22 22 11 11 11 22 11 26 11 33 33 23 11
B018  BO1   22 22 33 11 11 11 23 12 25 11 12 23 11 11
B019  BO1   22 22 23 11 11 11 22 11 26 11 33 33 22 11
B020  BO1   22 22 23 11 11 11 22 22 55 11 12 33 22 11
B021  BO1   22 22 33 11 11 11 22 16 25 11 12 33 23 11
MA01  BO1   22 22 23 11 11       22 25 11 33 33 22 11
MA02  BO1   22 22 23 11 11       12 25 11 13 33 22 12
MA03  BO1   22 22 23 11 11       12 25 11 13 33 22 11
NEXT
MLBS  Mountain Lake
BC01  MLB   22 22 22 11 11 11 12 11 25 11 33 33 12 11
BC02  MLB   22 12 22 11 11 11 22 11 25 11 13 33 22 11
BC03  MLB   22 23 23 11 11 11 22 11 23 11 13 33 33 11
CC01  MLB   22 12 22 11 11 11 22 22 25 11 13 33 22 11
ML07  MLB   22 22 22 11 11 11 22 11 25 11 12 33
ML08  MLB   22 12 22 11 11 11 22 14 25 11 33 33
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ML09  MLB   22 12 22 11 11 11 22 44 25 11 33 33
ML10  MLB   22 22 22 11 11 11 22 33 55 11 33 33
ML11  MLB   23 22 23 11 11 11 22 22 25 11 13 33
ML12  MLB   22 22 22 11 11 11 22 12 25 11 13 33 22 11
ML13  MLB   22 22 22 11 11 11 22 33 22 11 12 33
ML14  MLB   22 12 22 11 11 11 22 44 25 11 33 33
ML15  MLB   22 22 22 11 11 11 22 33 22 11 12 33
ML16  MLB   22 22 22 11 11 11 22 11 25 11 33 33
MSR1  MLB   22 22 23 11 11 11 22 22 23 11 11 33 22 11
MSR2  MLB   22 22 23 11 11 11 22 12 45 11 33 33 22 11
MSR3  MLB   22 22 23 11 11 11 22 12 45 11 33 33 22 11
MSR4  MLB   22 22 22 11 11 11 22 22 25 11 33 33 23 11
NEXT
MART  Martinsville
BM01  VSP   22 22 22 11 11 11 22 22 25 11 13 33 22 12
BM02  VSP   22 22 22 11 11 11 22 22 12 11 11 33 22 11
CK01  VSP   22 22 22 11 11 11 22 22 25 11 33 33 22 11
DR1S  VSP   22 22 23 11 11 11 22 12 25 11 11 13 23 11
DS01  VSP   22 22 22 11 11 11 22 12 25 11 11 33 22 11
EN01  VSP   22 22 22 11 11 11 22 22 11 11 13 33 23 11
FB01  VSP   22 22 23 11 11 11 22 12 25 11 11 33 22 11
FB2E  VSP   22 22 22 11 11 11 22 22 55 11 13 33 22 11
FB3E  VSP   22 22 22 11 11 11 22 22 22 11 11 33 22 11
FB4E  VSP   22 12 23 11 11 11 22 22 25 11 33 33 22 11
FB5N  VSP   22 22 22 11 11 11 22 22 25 11 11 33 22 11
FD01  VSP   22 22 22 11 11 11 22 12 25 11 33 33 23 11
FM1E  VSP   22 22 22 11 11 11 22 22 12 11 13 33 23 11
FS01  VSP   22 22 22 11 11 11 22 22 22 11 13 33 22 11
FS02  VSP   22 22 22 11 11 11 22 22 22 11 13 33 22 11
FS03  VSP   22 22 22 11 11 11 22 22 22 11 11 23 22 11
FS04  VSP   22 22 22 11 11 11 22 22 22 11 13 33 22 11
GP1W  VSP   22 23 22 11 11 11 22 12 25 11 13 33
GP2E  VSP   22 22 22 11 11 11 22 12 25 11 13 33 22 11
GP3E  VSP   22 22 22 11 11 11 22 12 25 11 11 33 23 11
GP4E  VSP   22 22 23 11 11 11 22 22 25 11 33 33 23 11
GP5E  VSP   22 23 11 11 11       12 12 11 11 33 23 11
GP6E  VSP   22 22 23 11 11 11    22 22 11 33 23 23 11
GP6W  VSP   22 22 22 11 11 11    12 22 11 13 33 22 11
GP7W  VSP   22 22 23 11 11 11 22 12 22 11 13 33
HE1E  VSP   23 22 23 11 11 11 22 22 11 11 11 33 22 11
HE1W  VSP   22 22 22 11 11 11 22 12 22 11 11 33 12 11
LF1S  VSP   22 22 22 11 11 11 22 23 25 11 13 33 23 11
PC01  VSP   22 22 22 11 11 11 22 22 25 11 11 33 22 12
PH1W  VSP   22 22 22 11 11 11 22 12 25 11 13 23 22 11
PH02  VSP   22 22 23 11 11 11 22 12 25 11 13 33 23 11
PH03  VSP   22 22 22 11 11 11 22 11 25 11 11 33 22 11
PH04  VSP   22 11 22 11 11 11 22 22 25 11 33 33 22 11
PH05  VSP   22 22 12 11 11 11 22 22 22 11 11 33 23 11
PH6E  VSP   22 22 22 11 11 11 22 22 22 11 13 33 22 11
PH07  VSP   22 22 22 11 11 11 22 22 25 11 13 33 23 11
PL1R  VSP   22 22 23 11 11 11 22 22 22 11 13 33 22 11
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PL1L  VSP   22 22 23 11 11 11 22 22 22 11 13 33 22 11
PL03  VSP   22 22 22 11 11 11 22 22 22 11 13 33 22 11
PL04  VSP   22 22 22 12 11 11 22 22 25 11 33 33 23 11
PS01  VSP   22 22 22 11 11 11 22 12 22 11 11 33 12 11
RW0   VSP   22 22 22 11 11 11 22 22 33 11 11 33 23 11
RW02  VSP   22 22 23 11 11 11 22 23 25 11 13 33 22 11
RW03  VSP   22 22 23 11 11 11 22 24 25 11 33 23 22 11
RW04  VSP   22 22 23 11 11 11 22 23 12 11 11 33 22 11
SD01  VSP   22 22 22 11 11 11 22 22 22 11 11 33 23 11
SD02  VSP   22 22 22 11 11 11 22 22 22 11 11 33 22 11
SD03  VSP   22 22 12 11 11 11 22 22 22 11 11 33 22 11
SR01  VSP   22 22 23 11 11 11 22 33 22 11 13 33 22 11
SR02  VSP   22 22 22 11 11 11 22 22 11 11 11 33 22 11
SR4E  VSP   22 22 23 11 11 11 22 12 25 11 13 23 22 11
SR5E  VSP   22 22 23 11 11 11 22 12 25 11 13 23 23 11
SR06  VSP   22 22 23 11 11 11 22 22 15 11 13 33 22 11
SS1N  VSP   22 22 22 11 11 11 22 22 25 11 13 33 22 11
SS2E  VSP   22 22 22 11 11 11 22 22 22 11 12 23 22 11
SS2W  VSP   22 22 22 11 11 11 22 22 22 11 13 23 23 11
SS03  VSP   22 12 22 11 11 11 22 22 25 11 33 33 23 11
SS4W  VSP   22 22 22 11 11 11    22 25 11 11 33 23 11
SS05  VSP   22 22 22 11 11 11 22 24 15 11 13 33 23 11
SS6E  VSP   22 22 22 11 11 11 22 22 35 11 33 33 23 11
SS6W  VSP   22 22 22 11 11 11 22 12 22 11 13 33 23 11
SS07  VSP   22 22 22 11 11 11 22 22 12 11 13 23 22 11
VG01  VSP   22 22 23 11 11 11 22 12 25 11 13 33 22 11
VG02  VSP   22 22 22 11 11 11 22 12 22 11 11 23 22 11
NEXT
GREL  Greensboro (L)
DH01  NCL   22 22 22 11 11 11 22 22 22 11 11 13 22 11
GR1N  NCL   22 22 22 11 11 11 22 12 22 11 11 33 22 11
GUL1  NCL   22 22 22 11 11 11 22 12 22 11 11 33 22 11
GUL2  NCL   22 22 22 11 11 11 22 22 25 11 11 33 22 11
GUL3  NCL   22 22 22 11 11 11 22 22 22 11 13 33 22 11
GUL4  NCL   22 22 23 11 11 11 22 22 22 11 13 33 22 11
GUL5  NCL   22 22 22 11 11 11 22 22 22 11 13 33 22 11
RAN1  NCL   22 22 22 11 11 11 22 12 25 11 11 33 23 11
RAN2  NCL   22 22 22 11 11 11 22 12 22 11 11 33 22 11
RAN7  NCL   22 22 22 11 11 11 22 22 12 11 11 33 22 11
RAN8  NCL   22 22 22 11 11 11 22 12 22 11 11 33 22 11
RAN9  NCL   22 22 22 11 11 11 22 12 25 11 11 33 22 11
SV1S  NCL   22 22 22 11 11 11 22 22 22 11 13 33 23 11
SV02  NCL   22 22 22 11 11 11 22 22 55 11 13 34 22 11
SV03  NCL   22 22 22 11 11 11 22 12 25 11 13 33 22 11
VNL1  NCL   22 22 22 11 11 11 22 12 22 11 11 33 12 11
9601  NCL   22 22 22 11 11 11 22 22 12 11 13 33    11
9602  NCL   22 22 22 11 11 11 22 22 22 11 33 33    11
9608  NCL   22 22 22 11 11 11 22 12 22 11 11 33    11
9610  NCL   22 22 22 11 11 11 22 22 25 11 11 33    11
9611  NCL   22 22 22 11 11 11 22 22 25 11 13 33    11
NEXT
```

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GREP  Greensboro (P)
ALA1  NCP   22 22 22 11 11 11 22 22 22 11 11 33 22 11
ALA2  NCP   22 22 22 11 11 11 22 22 25 11 13 33 23 11
CAS1  NCP   22 22 22 11 11 11 22 22 22 11 11 33 22 11
CAS2  NCP   22 22 22 11 11 11 22 22 25 11 11 33 22 11
RAN3  NCP   22 22 22 11 11 11 22 22 22 11 13 33 22 11
RAN4  NCP   22 22 22 11 11 11 22 22 22 11 11 23 22 11
RAN5  NCP   22 22 22 11 11 11 22 22 22 11 13 33 23 12
RAN6  NCP   22 22 22 11 11 11 22 22 25 11 11 33 22 11
RCK1  NCP   22 22 23 11 11 11 22 22 55 11 11 33 22 11
RCK2  NCP   22 22 22 11 11 11 22 22 22 11 13 33 22 11
9603  NCL   22 22 22 11 11 11 22 22 15 11 11 33    11
9604  NCP   22 22 22 11 11 11 22 22 12 11 11 33    11
9605  NCL   22 22 22 11 11 11 12 11 25 11 13 33    11
9606  NCP   22 22 23 11 11 11 22 12 55 11 11 33    11
9607  NCP   22 22 22 11 11 11 22 12 22 11 11 33    11
9609  NCP   22 22 22 11 11 11 22 22 22 11 11 33    11
9612  NCL   22 22 22 11 11 11 22 22 25 11 13 33    11
9613  NCP   22 22 22 11 11 11 22 22 55 11 13 33    11
9614  NCP   22 22 22 11 11 11 22 22 22 11 11 33    11
9615  NCP   22 22 22 11 11 11 22 22 22 11 11 23    11
9616  NCP   22 22 22 11 11 11 22 22 55 11 11 33    11
9617  NCP   22 22 22 11 11 11 22 22 22 11 11 33    11
9618  NCP   22 22 22 11 11 11 22 22 22 11 13 33    11
9619  NCL   22 22 22 11 11 11 22 22 15 11 13 33    11
9620  NCP   22 22 22 11 11 11 22 22 12 11 11 23    11
NEXT
ABER  Aberdeen
AB1N  NCS   22 22 22 11 11 11 22 22 25 11 11 33 22 11
CT01  NCS   22 22 22 11 11 11 22 22 12 11 11 33 23 11
CT02  NCS   22 22 22 11 11 11 22 22 22 11 11 33 22 11
CT03  NCS   22 22 22 12 11 11 22 22 25 11 13 33 22 11
PN1N  NCS   22 22 22 11 11 11 22 22 12 11 11 33 22 11
PN2N  NCS   22 22 22 11 11 11 22 22 12 11 11 23 23 11
SE01  NCS   22 22 22 11 11 11 22 22 22 11 11 13 22 11
SE02  NCS   22 22 22 11 11 11 22 22 22 11 11 23 22 11
SE03  NCS   22 22 22 11 11 11 22 22 25 11 11 33 23 11
SE04  NCS   22 22 22 11 11 11 22 22 25 11 13 33 22 11
SE06  NCS   22 22 22 11 11 11 22 22 12 11 11 33 22 11
SF1N  NCS   22 22 22 12 11 11 22 22 22 11 13 33 22 11
SF1S  NCS   22 22 22 11 11 11 22 12 22 11 11 33 22 11
SF2N  NCS   22 22 22 11 11 11 22 22 12 11 11 33 22 11
SF3N  NCS   22 22 22 11 11 11 22 22 22 11 11 33 22 11
SF3S  NCS   22 22 22 11 11 11 22 22 22 11 11 23 22 11
SF4N  NCS   22 22 22 11 11 11 22 22 22 11 13 33 22 11
SF5N  NCS   23 22 22 11 11 11 22 22 22 11 13 33 22 11
SF6N  NCS   22 22 22 11 11 11 22 22 22 11 11 33 22 11
SH1N  NCS   22 22 22 11 11 11 22 22 12 11 11 23 22 11
SH2E  NCS   22 22 22 11 11 11 22 22 12 11 11 34 23 11
SH2W  NCS   22 22 22 11 11 11 22 22 12 11 11 34 22 11
SH3N  NCS   22 12 22 11 11 11 22 22 12 11 11 13 22 11
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SH3S  NCS   22 12 22 11 11 11 22 22 12 11 13 13 22 11
SH4N  NCS   23 22 22 11 11 11 22 22 25 11 13 33 22 11
SH5N  NCS   23 22 22 11 11 11 22 22 25 11 13 23 22 11
SH5S  NCS   22 22 22 11 11 11 22 22 12 11 11 33 22 11
SH6N  NCS   12 22 22 11 11 11 22 22 22 11 11 33 22 11
SH7N  NCS   22 22 22 11 11 11 22 22 22 11 11 33 22 11
SH9N  NCS   22 22 22 11 11 11 22 22 22 11 11 33 22 11
S10N  NCS   22 22 22 11 11 11 22 12 22 11 11 33 22 11
S10S  NCS   22 22 22 11 11 11 22 12 22 11 11 33 22 11
S11N  NCS   22 22 22 11 11 11 22 22 22 11 11 33 22 11
S13N  NCS   22 22 22 11 11 11 22 22 22 11 11 33 22 11
S14W  NCS   22 22 22 11 11 11 22 22 22 11 11 33 22 11
SO01  NCS   22 22 22 11 11 11 22 22 22 11 11 33 22 11
NEXT
SOUT  Southport
BLA1  NCC   22 22 22 11 11 11 22 22 25 11 11 33 22 11
BLA2  NCC   22 22 22 11 11 11 22 22 22 11 11 33 22 11
BLA3  NCC   22 22 22 11 11 11 22 12 22 11 11 33 22 11
BLA4  NCC   22 22 22 11 11 11 22 22 22 11 13 33 22 11
BLA5  NCC   22 22 22 11 11 11 22 22 25 11 13 33 22 11
BLA6  NCC   22 22 22 11 11 11 22 22 15 11 11 33 22 11
BLA7  NCC   22 22 22 11 11 11 22 22 25 11 11 33 22 11
BLA8  NCC   22 22 22 11 11 11 22 12 25 11 11 33 22 11
BLA9  NCC   22 22 22 11 11 11 22 22 25 11 11 33 22 11
BL11  NCC   22 22 22 11 11 11 22 22 22 11 13 23 22 11
BL12  NCC   22 22 22 11 11 11 22 22 25 11 11 33 22 11
BRU1  NCC   22 22 22 11 11 11 22 22 22 11 11 33 22 11
BRU3  NCC   22 22 22 11 11 11 22 12 12 11 11 33 23 11
BRU4  NCC   22 22 22 11 11 11 22 22 22 11 11 33 22 11
BRU5  NCC   22 22 22 11 11 11 22 22 15 11 13 13 22 11
BRU6  NCC   22 22 22 11 11 11 22 22 22 11 11 33 22 11
BRU8  NCC   22 22 22 11 11 11 22 22 12 11 11 33 22 11
BR10  NCC   22 22 22 11 11 11 22 22 22 11 13 33 22 11
BR11  NCC   22 22 22 11 11 11 22 22 22 11 11 23 22 11
BR12  NCC   22 22 22 11 11 11 22 22 12 11 13 23 22 11
BR13  NCC   22 22 22 11 11 11 22 22 12 11 11 33 22 11
BR14  NCC   22 22 22 11 11 11 22 22 22 11 11 33 22 11
BR15  NCC   22 22 22 11 11 11 22 22 12 11 11 33 22 11
BR16  NCC   22 22 22 11 11 11 22 22 22 11 11 33 22 11
BR17  NCC   22 22 22 11 11 11 22 22 25 11 11 33 22 11
BR18  NCC   22 22 22 11 11 11 22 22 22 11 11 34 22 11
BR19  NCC   22 22 22 11 11 11 22 12 22 11 13 33 23 11
BR20  NCC   22 22 22 11 11 11 22 22 22 11 11 33 22 11
BR22  NCC   22 22 22 11 11 11 22 22 22 11 11 23 22 11
BR23  NCC   22 22 22 11 11 11 22 22 22 11 13 33 23 11
BR24  NCC   22 22 22 11 11 11 22 22 22 11 11 34 22 11
BR25  NCC   22 22 22 11 11 11 22 22 22 11 11 33 22 11
BR26  NCC   22 22 22 11 11 11 22 22 22 11 11 23 22 11
BR27  NCC   22 22 22 11 11 11 22 22 12 11 11 33 22 11
BR28  NCC   22 22 22 11 11 11 22 22 22 11 11 33 22 11
BR30  NCC   22 22 22 11 11 11 22 22 22 11 11 23 22 11
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BR31  NCC   22 22 22 11 11 11 22 22 22 11 11 33 22 11
COL1  NCC   23 22 22 11 11 11 22 22 22 11 11 33 11 11
COL2  NCC   22 22 22 11 11 11 22 22 22 11 11 33 22 11
NEXT
ASHE  Asheville
ASH1  AS1   22 22 22 11 11 11 22 22 22 11 11 23 22 11
ASH2  AS1   22 22 22 11 11 11 22 22 25 11 13 33 22 11
ASH3  AS1   22 22 22 11 11 11 22 22 12 11 13 33 23 11
ASH4  AS1   22 22 22 11 11 11 22 22 12 11 13 33 23 11
ASH5  AS1   23 22 22 11 11 11 22 22 22 11 13 33 23 11
ASH6  AS1   22 22 22 11 11 11 22 22 22 11 11 33 11 11
AVE1  AS1   22 22 22 11 11 11 22 22 25 11 13 33 22 11
BUN1  AS1   22 22 22 11 11 11 23 12 25 11 13 23 23 11
BUN2  AS1   22 22 23 11 11 11 22 12 22 11 13 33 23 11
MAD1  AS1   22 22 23 11 11 11 22 22 22 11 13 33 22 11
MAD2  AS1   22 22 22 11 11 11 22 22 25 11 13 33 23 22
MCD1  AS1   22 22 22 11 11 11 22 22 25 11 11 33 22 11
MCD2  AS1   22 22 22 11 11 11 22 22 22 11 13 33 22 11
MIT1  AS1   22 22 23 11 11 11 22 22 25 11 13 33 23 11
MIT2  AS1   22 22 22 11 11 11 22 22 15 11 13 33 23 12
MIT3  AS1   22 22 22 11 11 11 22 12 22 11 13 33 23 11
MIT4  AS1   22 22 22 11 11 11 22 12 25 11 13 33 22 11
PIS1  AS1   22 22 22 11 11 11 22 22 22 11 13 33 22 11
PIS2  AS1   23 22 22 11 11 11 22 22 25 11 11 33 23 11
NEXT
COLU  Columbia
CAL1  SP1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
CAL2  SP1   22 22 22 11 11 11 22 22 22 11 11 33 23 11
CAL3  SP1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
CAL4  SP1   22 22 22 11 11 11 22 22 22 11 11 23 22 11
CAL5  SP1   22 22 22 11 11 11 22 22 22 11 13 33 22 11
CAL6  SP1   22 22 22 11 11 11 22 22 22 11 11 33 23 11
CAL7  SP1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
CAL8  SP1   22 22 22 11 11 11 22 12 22 11 13 33 22 11
CAL9  SP1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
CA10  SP1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
CA11  SP1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
CA12  SP1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
LEX1  SP1   22 22 22 11 11 11 22 22 22 11 13 33 23 11
LEX2  SP1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
LEX3  SP1   22 22 22 11 11 11 22 22 22 11 11 23 22 11
LEX4  SP1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
LEX5  SP1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
LEX6  SP1   22 22 22 11 11 11 22 22 13 11 11 13 22 11
NEW1  SP1   22 22 22 11 11 11 22 22 22 11 13 33 22 11
NEW2  SP1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
ORA1  SP1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
NEXT
ORAN  Orangeburg
BER1  SC1   11 22 22 11 11 11 22 22 22 11 13 33 23 11
BER2  SC1   22 22 22 11 11 11 22 22 22 11 11 23 22 11
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BER3  SC1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
BER4  SC1   11 12 22 11 11 11 22 22 22 11 13 33 23 11
BER5  SC1   22 11 22 11 11 11 22 22 22 11 11 33 22 11
BER6  SC1   22 11 22 11 11 11 22 22 22 11 11 33 23 11
BER7  SC1   22 11 22 11 11 11 22 22 22 11 11 33 23 11
BER8  SC1   22 11 22 11 11 11 22 22 22 11 13 33 22 11
ORA2  SC1   22 22 22 11 11 11 22 22 22 11 11 33 23 11
ORA3  SC1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
ORA4  SC1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
ORA5  SC1   22 22 22 11 11 11 22 22 22 11 13 33 22 11
ORA6  SC1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
ORA7  SC1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
ORA8  SC1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
ORA9  SC1   22 22 22 11 11 11 22 22 12 11 11 33 22 11
OR10  SC1   22 22 22 11 11 11 22 22 15 11 11 34 22 11
OR11  SC1   22 22 22 11 11 11 22 22 25 11 11 33 22 11
OR12  SC1   22 22 22 11 11 11 22 22 22 11 13 33 22 11
OR13  SC1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
OR14  SC1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
OR15  SC1   22 22 22 11 11 11 22 22 22 11 11 33 22 11
NEXT
NASS  Nassau
FLA1  SU1   22 22 23 11 11 11 22 22 22 11 33 33 22 11
FLA2  SU1   22 22 23 11 11 11 22 22 22 11 11 23 22 11
FLA3  SU1   23 22 23 16 11 11 22 22 22 11 11 33 22 11
FLA4  SU1   23 22 23 11 11 11 22 22 22 11 11 33 22 11
FLA5  SU1   22 22 23 11 11 11 22 22 12 11 13 33 22 11
FLA6  SU1   22 22 23 11 11 11 22 22 12 11 11 33 23 11
FLA7  SU1   23 22 23 11 11 11 22 22 22 11 13 33 22 11
FLA8  SU1   23 22 23 11 11 11 22 22 22 11 11 33 22 11
FLA9  SU1   22 22 23 11 11 11 22 22 25 11 11 33 22 11
FL10  SU1   22 22 23 11 11 11 22 22 22 11 11 33 22 11
FL11  SU1   22 22 23 11 11 11 22 22 22 11 13 33 22 11
FL12  SU1   22 22 23 12 11 11 22 22 22 11 11 33 22 11
FL13  SU1   22 22 23 11 11 11 22 22 25 11 11 33 22 11
FL14  SU1   22 22 23 11 11 11 22 22 22 11 11 33 22 11
GA01  SU1   22 22 23 11 11 11 22 22 22 11 11 33 22 11
GA02  SU1   12 22 23 11 11 11 22 22 22 11 11 33 22 11
GA03  SU1   22 22 23 11 11 11 22 22 22 11 13 33 11 11
GA04  SU1   22 22 23 11 11 11 22 22 22 11 11 33 11 11
GA05  SU1   22 22 23 11 11 11 22 11 22 11 11 33 23 11
GA06  SU1   22 22 23 11 11 11 22 22 22 11 11 23 23 11
NEXT
END;
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Appendix C

ENZYME SYSTEMS USED IN STUDY

Enzyme	Enzyme Commission #	Buffer System	Loci Assayed	Alleles
GOT	E.C. 2.6.1.1	#6*	Got	3
IDH	E.C. 1.1.1.42	Morph	Idh	3
MDH	E.C. 1.1.1.37	Morph	Mdh-1	3
			Mdh-2	3
			Mdh-3	2
PGI	E.C. 5.3.1.9	#6	Pgi-1	1
			Pgi-2	3
PGM	E.C. 2.7.5.1	#6/	Pgm-1	5
		Morph **	Pgm-2	6
SKDH	E.C. 1.1.1.25	Morph	Skdh	4
6-PGD	E.C. 1.1.1.44	Morph	6-Pgd-1	1
			6-Pgd-2	3
TPI	E.C. 5.3.1.1	#6	Tpi-1	3
			Tpi-2	2

* GOT was also resolved using the LiOH buffer system, as were hexokinase (HK, E.C. 2.7.1.1) and leucine aminopeptidase (LAP, E.C. 3.4.11.-). Later, after HK and LAP were dropped due to consistently poor resolution, it was resolved using only the #6 buffer system.

** Although both Pgm-1 and Pgm-2 were detected by buffer system #6, the morpholine-citrate buffer gave much better resolution of Pgm-2. It was not possible to score Pgm-1 on morpholine-citrate due to extremely poor resolution.

VITA

William D. Speer

PROFESSIONAL ADDRESS

Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

Office Phone: (703) 231-8932 Home Phone: (703) 638-7453

PERSONAL SUMMARY

Born August 24, 1957 in Conrad, Montana

Married to Patricia Keith

EDUCATION

M. S., Botany, August, 1994 to May, 1997
Virginia Polytechnic Institute and State University (Virginia Tech),Blacksburg, VA
Thesis: Systematics of eastern North American Bracken Fern
Advisor: Khidir W. Hilu

B. S., Biology; Minor: Chemistry, Cum Laude, May, 1994
Virginia Polytechnic Institute and State University, Blacksburg, VA

A. A. S., Science, Summa Cum Laude, May, 1991
Patrick Henry Community College (PHCC), Martinsville, VA

HONORS/AFFILIATIONS

Phi Theta Kappa (PHCC)
Spire (PHCC)
Phi Sigma Society (Virginia Tech)
Golden Key (Virginia Tech)
Gamma Beta Phi Society (Virginia Tech)
The Honor Society of Phi Kappa Phi (Virginia Tech)
Virginia Tech Honors Program

SCIENTIFIC ORGANIZATIONS

Virginia Academy of Science, May 1995.

American Fern Society, April 1996.
Botanical Society of America, June 1996.
American Society of Plant Taxonomists, June 1996.

RESEARCH INTERESTS

Plant systematics and speciation with special interest in *Pteridium aquilinum*.
Pteridophyte evolution.
Application of molecular techniques to fern studies.

TEACHING INTERESTS

Undergraduate biology and plant science courses

RELATED EXPERIENCE

Research

Studied the isozyme technique under Dr. Charles R. Werth (Texas Tech) at Mountain Lake Biological Station, Pembroke, VA, July 1994.

Conducted population studies of *Pteridium aquilinum* using isozymes. Two transects were made: the first began at Mountain Lake Biological Station, near Pembroke, VA, and moved southeasterly to Southport, NC; the second began in the Asheville, NC area and moved southeasterly toward Charleston, SC. Eight population were examined in these two transects, five in the first and three in the second. In addition to these populations, specimens from two populations not in the transect zones were also collected. The first of these populations was in the Petersham, MA area, while the second was collected along the Georgia (Camden County) and Florida (Nassau County) border, just west of I-95. Isozymes were ran, scored, and photographed at Mountain Lake Biological Station. Dr. Charlie Werth of Texas Tech was present as my "isozyme advisor" during this time, June 6 to August 18, 1995.

Collected *Pteridium* specimens as part of project to map bracken distribution by variety in Virginia. This project is funded by Virginia Botanical Associates. Project is over 50% completed. Summer, 1996.

Conducted population studies of *Pteridium aquilinum* var. *latiusculum* and var. *pseudocaudatum* in the Greensboro, NC area. Isozymes were ran at Mountain Lake Biological Station. July, 1996.

Seminars
March 29, 1996 - (VPI & SU Botany Seminar) - "The World's most common Fern: Bracken and Isozymes."

May 23, 1996 - (Virginia Academy of Science) - "Systematics of Bracken Fern in Eastern U. S.: Isozymes and Morphology."

Teaching
Laboratory Instructor (BIOL 1115 - one section), Department of Biology, Virginia Tech, Blacksburg, VA, August, 1994 - December, 1994
- -Taught one two hour freshman biology laboratory

Teaching Assistant (BIOL 2314 - one section), Department of Biology, Virginia Tech, Blacksburg, VA, January, 1995 - May, 1995
- -Assisted in the instruction of undergraduates in various aspects of plant science
- -Graded lab assignments

Teaching Assistant (BIOL 2314 - one section), Department of Biology, Virginia Tech, Blacksburg, VA, August, 1995 - December, 1995
- -Instructed undergraduates in various aspects of plant science.
- -Planned classes and made specific class assignments.
- -Graded lab assignments.

Teaching Assistant (BIOL 2314 - two sections), Department of Biology, Virginia Tech, Blacksburg, VA, January, 1996 - May, 1996
- -Instructed undergraduates in various aspects of plant science.
- -Planned classes and made specific class assignments.
- -Graded lab assignments.

Teaching Assistant (BIOL 2314 - one section), Department of Biology, Virginia Tech, Blacksburg, VA, August, 1996 - December, 1996
- -Instructed undergraduates in various aspects of plant science.
- -Planned classes and made specific class assignments.
- -Graded lab assignments.

Teaching Assistant (BIOL 2314 - one section), Department of Biology, Virginia Tech, Blacksburg, VA, August, 1996 - December, 1996
- -Instructed undergraduates in various aspects of plant science.
- -Planned classes and made specific class assignments.

-Graded lab assignments.

Other

Botany Seminar Selection Committee (August, 1995 - May, 1996)
- I was one of two graduate students assisting the committee chairperson, Dr. B. C. Parker, in the selection of speakers for the Botany Seminar. Duties included selection, contact, and scheduling of speakers.

GRANTS / FINANCIAL AWARDS

Pratt Fellowship - Research award of $500 to conduct isozyme studies of Pteridium aquilinum at Mountain Lake Biological Station for Summer 1995. Awarded Spring, 1995.

Virginia Tech Department of Biology - Research award of $500 to match Pratt Fellowship award for Summer 1995. Awarded Spring, 1995.

Barbara J. Harvill Fund (Virginia Botanical Associates) - $300 award to cover collection expenses in Virginia. Awarded Spring 1995.

Virginia Tech Department of Biology - Research award of $300 to match Harvill award. Awarded Spring 1995.

ABSTRACTS

Speer, W. D., K. W. Hilu, and C. R. Werth. 1996. Systematics of Bracken Fern in eastern U. S.: Isozymes and Morphology. Virginia Academy of Science meeting, Richmond, Virginia. Virginia Journal of Science 47:105.

PAPERS IN PREPARATION

Speer, W. D. and K. W. Hilu. Systematic studies in Pteridium of the southeastern United States, I. Morphology. To be submitted to Systematic Botany.

Speer, W. D., C. R. Werth, and K. W. Hilu. Systematic studies in Pteridium of the southeastern United States, II. Isozyme evidence. To be submitted to Systematic Botany.