5-4-03

Dear Diane,

Thanks again for letting me study Asplenium monanthes at Neversink. I wish my findings were more conclusive to make it more worthwhile, but I still have a lot to figure out about this species. I hope to come back in 2005-2006 to take some more climate readings and count the plants again if I can find someone like Alan who can help me out (I wish I could go down on those ropes myself, but I have no experience...), but I won't ever need to pick any more leaves. I will get in touch with you then to seek permission. I hope you guys can get something useful out of my thesis even though it's pretty inconclusive. Keep up the good work of protecting all these sensitive caves!

Sincerely,
Allison Shaw

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Biogeographic origin, taxonomic status, and conservation biology of *Asplenium monanthes* L. in the southeastern United States

by

Allison Elizabeth Shaw

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Ecology and Evolutionary Biology

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Donald R. Farrar (Major Professor)
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ABSTRACT

Asplenium monanthes L. is an apogamous fern with a wide tropical and subtropical distribution that extends into the temperate zone in the southeastern U.S. These populations are quite distant from the nearest neotropical populations, so the circumstances of their origin (i.e. as pre-Pleistocene relicts or by later long-distance dispersal) were investigated using spore and gametophyte morphology and starch gel electrophoresis. The southeastern U.S. populations of *A. monanthes* can be attributed to multiple long-distance dispersal events during the Pleistocene or Holocene. This recent origin and *A. monanthes*' small population sizes account for southeastern U.S. *A. monanthes*' lack of genetic diversity within and in many cases among populations. The source of the various founding spores cannot be conclusively determined, but evidence favors the Caribbean over Mexico. The southeastern U.S. populations of *Asplenium monanthes* do not warrant distinct taxonomic status. Analysis of genetic structure revealed great diversity in Mexico, so Mexico is suggested as a possible birthplace of allotriploid *A. monanthes*. The rarity and disjunct nature of the southeastern U.S. populations also prompted investigation into their ecology in the temperate zone and their prospects for continued survival. *Asplenium monanthes* survives in the southeastern U.S. by inhabiting shaded gorges, sinkholes, and cave entrances that moderate temperature extremes and maintain reliable moisture levels. The populations are very small, so they are subject to environmental and demographic stochasticity and are fixed genetically for relatively few multilocus genotypes. Several historical populations have disappeared in recent decades and several remaining populations appear to be declining as well. These declines may be a natural part of the species' regional population dynamics or they may be cause for alarm. Regular long-term monitoring is required to determine whether the species is truly at risk of local extinction in the southeastern U.S. and if so, which factors are most responsible for this trend. No management intervention is recommended at this time.

GENERAL INTRODUCTION

Research questions

The fern *Asplenium monanthes* was first described by Linneaus (1767) from the Cape of Good Hope. It was later found to be widespread, covering much of the New World and various oceanic islands as well as much of Africa. In the New World, *A. monanthes*' range is largely continuous, with the exception of its occurrence in the southeastern U.S., separated by approximately 1600 km from the nearest populations in Mexico and the Caribbean. Whereas *A. monanthes*' occurrence on young volcanic islands like Hawaii requires an explanation of relatively recent long-distance dispersal, its occurrence in the southeastern U.S. might be explained either by ancient vicariance, ancient long-distance dispersal, or relatively recent long-distance dispersal. Many other cryptogams[1] share this disjunction between the tropics and the southeastern U.S. The majority are believed to be a result of Pleistocene cooling limiting previously widespread tropical taxa to isolated refugia in the Appalachians (Farrar, 1998). This thesis investigates whether *A. monanthes* shares this ancient origin or is a more recent immigrant to the southeastern U.S.

The southeastern U.S. populations are quite small and few in number, so their conservation is of concern in spite of the species' wide global distribution. The past few decades have witnessed many southeastern U.S. population declines, so this study evaluates *A. monanthes*' prospects for continued survival in the southeastern U.S. The populations' ecology (e.g. associated species, edaphic niche, microclimate, life history, demographics) is described to allow development of appropriate conservation measures to preserve this biogeographical curiosity.

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[1] non-seed plants

Thesis organization

This thesis is divided into two parts. The two parts are preceded by this general introduction that acquaints the reader with the study species and a literature review of topics relevant to both parts of the thesis. Literature reviews of topics specific to a particular part of the thesis (fern biogeography case studies and justification of taxonomic methods in Part I, plant conservation considerations in Part II) are not discussed until that part. Part I investigates southeastern U.S. *Asplenium monanthes'* biogeographic origin and taxonomic status by comparing southeastern U.S. to neotropical populations using morphological and genetic data, and additionally characterizes *A. monanthes'* genetic structure in all regions sampled. Part II of the thesis addresses the southeastern U.S. populations' conservation biology by characterizing their microhabitat and microclimate, life history, demographics, and viability, and provides management recommendations for this locally rare species. Following this is a general conclusion summarizing the main findings of both parts.

Taxonomy of *Asplenium monanthes*

Placement of the Aspleniaceae within leptosporangiate ferns

The Aspleniaceae is a family of ferns with representatives worldwide. In both early and modern phylogenetic schemes proposed for the pteridophytes, the Aspleniaceae has been considered a relatively derived family, possessing the synapormorphies leptosporangia[2], indusia[3], and well-developed perispore[4]. Characters distinguishing the family include linear indusia (with a few exceptions), clathrate[5] (with a few exceptions) rhizome scales, and two back-to-back "C"-shaped vascular bundles in the stipe (petiole) that in smaller species are united to form an "X" shape. According to the review by Smith (1995) of the various morphologically based phylogenetic schemes for the pteridophytes, Wagner, Mickel, and Pichi-Sermolli all placed the Aspleniaceae near the

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[2]Spore-containing structures with walls only one cell thick, developing from a single epidermal cell (as opposed to eusporangia)

[3] Flaps of epidermal tissue covering the sori (groups of developing sporangia)

[4] An additional layer of the spore wall, external to the exospore

terminus of the largest lineage of pteridophytes, allied in various configurations with the Blechnaceae, Davalliaceae, Onocleaceae, and the taxa traditionally clustered into the "Aspidiaceae" (e.g. *Dryopteris, Thelypteris, Polystichum*, etc.), whereas Holttum did not commit to any particular location for the Aspleniaceae in his phylogram.

Evidence from molecular data has not much altered the phylogenetic placement of the Aspleniaceae within the ferns. To date rbcL has been the most popular DNA sequence for elucidating higher-level phylogenetic relationships. Based on rbcL sequences, Hasebe et al. (1995) placed Aspleniaceae sister to a lineage containing the families listed above plus the Polypodiaceae and Grammitidaceae (both formerly considered to be relatively basal lineages).

Placement of Asplenium monanthes within the Aspleniaceae

The Aspleniaceae has at times been segregated into multiple genera, e.g. *Camptosorus, Ceterach, Phyllitis, Pleurosorus, Schaffneria, Holodictyum, Diellia, Diplora, Loxoscaphe, Darea, Boniniella, Sinephropteris, Neottopteris, and Antigramma,* but most pteridologists prefer to recognize only a large (approximately 700 species) and diverse *Asplenium*. The reasons for treating *Asplenium* as a single genus are: (a) the absence of morphological gaps (Wagner et al, 1993), (b) the ability of distantly related species to hybridize (Wagner el al, 1993), and (c) the problem of paraphyly created by recognition of distinct groups as separate genera (Moran, 2001, and Kramer & Viane, 1990). However, an additional segregate genus, *Hymenasplenium*, has recently been readvanced as a valid genus (Murakami, 1995) based upon discovery of anatomical and cytological apomorphies (Murakami & Moran, 1993) sufficient to satisfy concern (a). Concern (c) appears to be satisfied due to the basal location of *Hymenasplenium* within Aspleniaceae (Murakami et al, 1999), leaving the remaining taxa of *Asplenium* a monophyletic group.

In the absence of clear generic limits, *Asplenium* has been subdivided into many informal sections (e.g. no official section name has been designated) for the purpose of grouping similar species, and molecular investigation is underway to determine the

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[5] Cells are transparent except for having dark thick walls, giving the appearance of latticework

sections' monophyly and relationships among one another. Murakami (1999) used rbcL sequence data to elucidate the relationships within *Asplenium*, sampling 21 taxa (3 are different varieties of a single species) of *Asplenium s.s.* plus 5 species of *Hymenasplenium*, but since 26 taxa represent less than 4% of the genus, the results must be considered preliminary. His resulting cladogram shows simple leaves having evolved a minimum of five times within *Asplenium s.l.*, illustrating the caution necessary in using morphological data to formulate phylogenetic relationships in this genus. A much more exhaustive molecular analysis is that of Schneider et al. (in press), who used rbcL and trnL-F spacer sequences to determine the phylogenetic relationships among 71 species of *Asplenium s.l.*

The section containing *Asplenium monanthes* is often called the *Asplenium trichomanes* group. The ferns of the *A. trichomanes* group are terrestrial to epipetric, generally small in stature, with once-pinnate leaves and a shiny castaneous to black rachis. *A. monanthes* (**Fig. 1**) is unique in this group in that most individuals (exceptions are known from Mexico) have sori only on the basiscopic half of each pinna, often only a single sorus there, hence the specific epithet. The *A. trichomanes* group and *A. monanthes* in particular was found to be relatively derived within *Asplenium s.l.* in the Schneider et al. (in press) cladogram; limited sampling and a polytomy prevents detailed assessment of the phylogenetic position of the *A. trichomanes* group in the Murakami et al., 1999a, cladogram.

Cytology of Asplenium monanthes

The base chromosome number for *Asplenium s.s.* is n=36. Chromosome squashes of *Asplenium monanthes* from Madeira and the Azores (islands in the northern Atlantic) (respectively Manton, 1950 and Lovis et al., 1977), Tristan da Cunha (a southern Atlantic island) (Manton & Vida, 1968), and the states of Veracruz (Tryon et al., 1973) and Oaxaca (Smith & Mickel, 1977) in Mexico have all yielded 108 chromosomes in mitosis or 108 pairs in meiosis, indicating triploidy. However Smith & Mickel (1977) found 2n=72II in a sample from Chiapas, Mexico. The rest of *A. monanthes*' extensive range (e.g., the southeastern U.S., the Caribbean, Central America, South America, Hawaii, Africa) has not been cytologically investigated. The sexual tetraploid from Chiapas

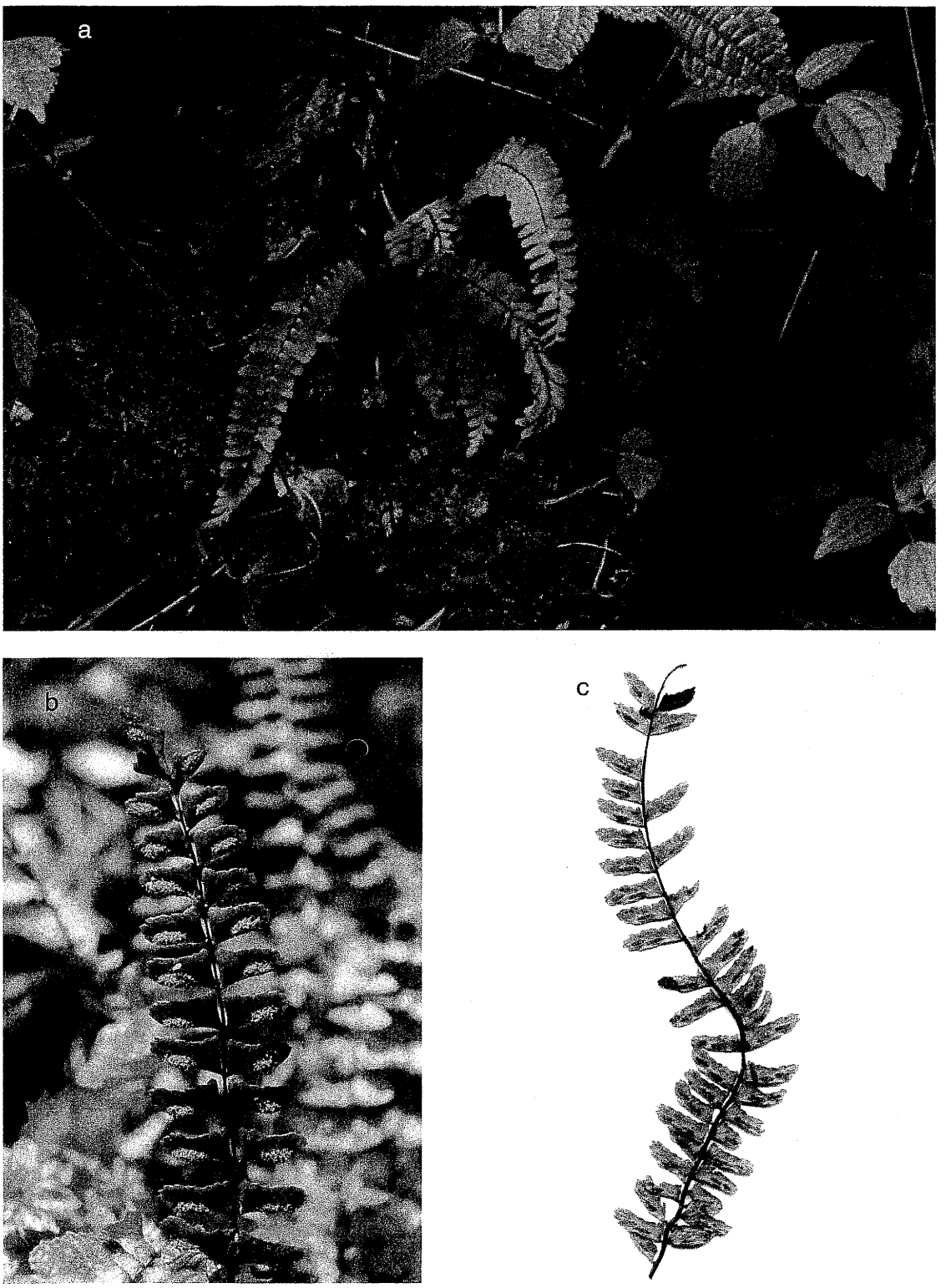

Figure 1: Sporophytes of *A. monanthes*: (a) typical plant, (b) frond with one sorus per pinna (photo by Alan Cressler), (c) frond with multiple sori per pinna

certainly raises the question of whether *A. monanthes* is a monophyletic species, a question already up for debate due to its great morphological variability (Alan Smith, personal communication).

A. monanthes appears to be most frequently triploid, so homologous chromosomes cannot pair normally as bivalents in meiosis. Instead a restitution nucleus is formed when the cells fail to divide, such that each spore (and therefore gametophyte) receives all 108 chromosomes from the parent sporophyte. Gametophytes do not produce sporophytes by successful fusion of gametes as in sexual species but by automatic vegetative sporophyte initiation. The resulting triploid sporophyte matures to produce more triploid spores. This type of asexual lifecycle involving unreduced spores and the absence of fertilization is called apogamy, agamospory, or reproductive apomixis.

Because of the tetraploid record from Chiapas, mitotic chromosome squashes of gametophytes were attempted in this investigation to clarify the ploidy level in the southeastern U.S., the Dominican Republic, and Costa Rica. The mitotic squashes proved unsuccessful, so the plants in these regions will be assumed triploid until meiotic squashes can be performed.

Apogamy

Alternative origins of triploid taxa

Figure 2 shows the four possible origins of triploid species:

(a) From a diploid gamete (i.e. produced by an autotetraploid) mating with a haploid gamete of the progenitor species (e.g. *Cystopteris protrusa* in Haufler et al., 1985; *Polypodium virginianum* in Bryan & Soltis, 1987; *Isoetes echinospora* in Rumsey et al., 1993). The resulting triploid species would contain only alleles found in the single parental species. An individual could potentially have three different alleles at a locus in populations with high genetic diversity, but this is relatively unlikely.

(b) From an autotetraploid crossing with a non-parental diploid (e.g. *Asplenium ruta-muraria* X *A. viride* and *A.* X *germanicum* in Meyer 1960b). Such offspring would have a reasonable probability of three different alleles at a locus.

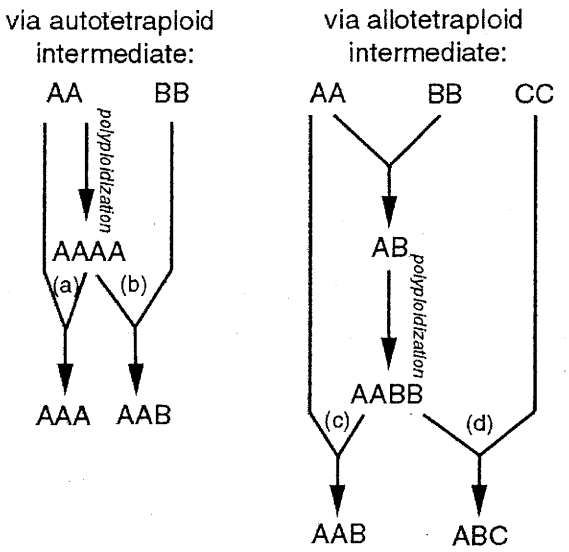

Figure 2: Alternative origins of triploid species: (a) autotetraploid backcrossing with parental diploid, (b) autotetraploid crossing with non-parental diploid, (c) allotetraploid backcrossing with parental diploid, (d) allotetraploid crossing with non-parental diploid.

(c) From an allotetraploid backcrossing with one of the parental diploids (numerous examples). The triploid offspring of this scenario would also have a reasonable probability of having three different alleles at a locus.

(d) From an allotetraploid crossing with a non-parental diploid (several examples in the *Dryopteris marginalis* cluster given in Wagner, 1971, also *Asplenium* X *kentuckiense* in Wagner, 1954). The triploid offspring would be extremely likely to have three different alleles at a locus.

Manton used cytological evidence to assert that *Asplenium monanthes* is an allotriploid (i.e. either origin b, c, or d) like *Dryopteris atrata* which she discusses as follows: "The species here seems to be a triploid hybrid, either formed directly or by descent from some other polyploid in which there is virutally no affinity betweeen the chromosomes of the hybridising parents." (Manton, 1950, p. 183). The electrophoretic phenotypes predicted above for each type of origin rest upon the assumption that gene silencing has not yet occurred. This assumption may not hold for *Asplenium monanthes* because the species' broad geographic distribution implies that it has been in existence

for a long time. Therefore this study is not likely to clarify the mode of origin of *A. monanthes* (inclusion of many candidate parental species and cytological analysis would be necessary for such an investigation), being undertaken instead to determine the biogeographical origin of the SEUS populations.

Mechanisms of apogamy

Any novel triploid organism will remain but a short-lived curiosity in the absence of meiotic compensating mechanisms such as that possessed by *Asplenium monanthes*. There are four proposed compensating mechanisms for the successful production of unreduced spores. These are compared in **Figure 3** along with standard sexual production of reduced spores (**Fig. 3a**). The chromosomes in the 16 spore mother cells cannot be evenly divided by meiosis in triploid species. Within a triploid plant, some sporangia attempt conventional spore production and produce 64 abortive spores, but others utilize one of the compensating mechanisms outlined below (in *A. monanthes*, the majority of spores are viable, so the majority of sporangia utilize a compensating mechanism).

The most common apogamous sporogenesis mechanism is called the Döpp-Manton type (**Fig. 3b**) (Döpp, 1932; Manton, 1950). It differs from regular spore production by bypassing cytokinesis in the forth mitotic division. The replicated chromosomes are thus combined in a "restitution nucleus," producing 8 (instead of the usual 16) spore mother cells with twice the number of chromosomes. The 8 spore mother cells then divide by normal meiosis to produce a total of 32 unreduced spores. This is the mechanism utilized by *A. monanthes* (Manton et al., 1986).

A rarer mechanism (**Fig. 3c**), described by Braithwaite (1964) from *Asplenium aethiopicum*, involves four regular mitotic divisions followed by meiosis of the 16 spore mother cells in which the first meiotic division bypasses cytokinesis. This results in 16 diads of unreduced spores for a total of 32.

Another mechanism (**Fig. 3d**) was proposed by Evans (1964) and is termed ameiotic sporogenesis. As the name implies, it involves simply five successive mitotic divisions, resulting in 16 diads of unreduced spores for a total of 32. Since the end result is the same as that in the Braitwaite mechanism, a fifth cell division must be carefully

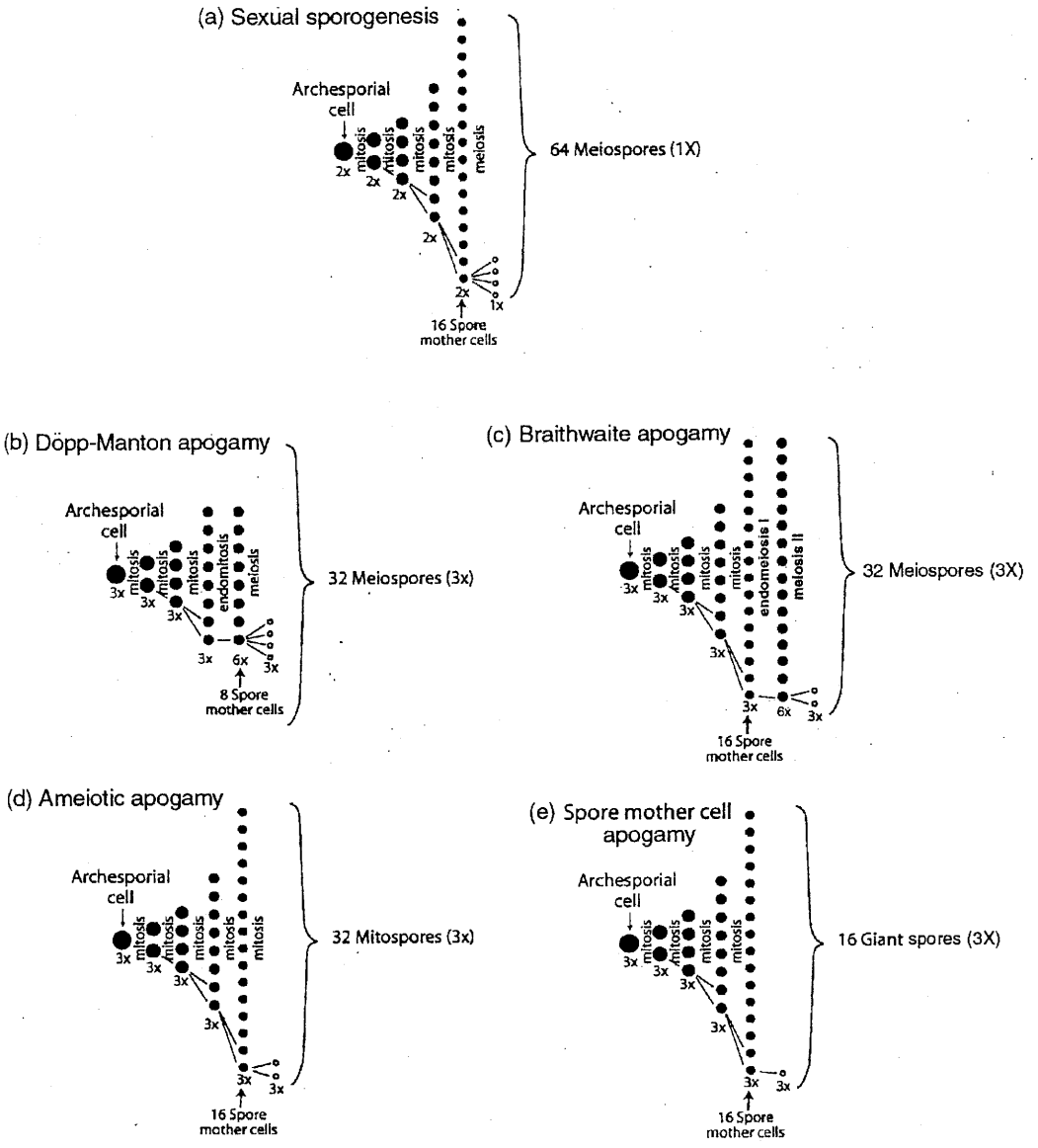

Figure 3: Sporogenesis in (a) sexual ferns and (b-e) various apogamous ferns. *Asplenium monanthes* utilizes mechanism (b). Diagram modified from one provided to the author by Robbin Moran.

documented to distinguish ameiotic from Braithwaite spore production, leaving the existence of this mechanism still in question (Walker, 1966; Lovis, 1977).

A final mechanism (**Fig. 3e**), proposed by Morzenti (1967) and confirmed by Gastony (1986), produces 16 giant unreduced spores that are presumed to be the original 16 spore mother cells.

Consequences of apogamy

Whittier (1970) found that apogamous gametophytes tended to grow faster and also produce sporophytes at a smaller size and earlier date than sexual gametophytes. Apogamous gametophytes were larger than sexual gametophytes by 4 weeks in age in 87% of intrageneric comparisons. Apogamous gametophytes initiated sporophyte development earlier than sexual gametophytes initiated archegonial development (antheridial development was not compared) in 91% of intrageneric comparisons. Despite the faster growth exhibited by apogamous gametophytes, they were still smaller at maturity (sporophyte initiation) than sexual gametophytes were by the time of archegonial development. These relationships, with the exception of increased growth rate in higher ploidy gametophytes (found in 83% of intrageneric comparisons), appear to be unrelated to the polyploidy associated with most apogamous species, as only 31% of intrageneric comparisons of species with increasing ploidy showed earlier maturity and smaller size at maturity in gametophytes with higher ploidy level. Rapid growth and sporophyte initiation in apogamous ferns allows successful completion of the lifecycle in hostile environments with short growing seasons. However this ability is probably unnecessary for *A. monanthes* considering its relatively constant tropical montane climate and moderated microclimate in the southeastern U.S.

Apogamous ferns, unlike sexual ones, do not require water for fertilization and subsequent sporophyte production. This can be a significant advantage in dry environments. Although deserts typically come to mind as the prototypical dry environment, rock substrate in an otherwise moist environment can also become quite dry if it is not permeable to water. *A. monanthes*, in all its occurrences in the southeastern U.S. and in some of its occurrences in the tropics, grows in bryophyte mats on rocks. The bryophytes mats retain some moisture and *A. monanthes* is never found directly on

exposed rock, so presumably the species requires a moderate supply of water for growth if not for reproduction.

The apogamous lifecycle of *A. monanthes* should allow it to form new colonies by long-distance spore dispersal. Colonizing ability in ferns is strongly tied to isolate potential, the ability of an isolated gametophyte to successfully produce a sporophyte in the absence of inter-gametophytic fertilization (Peck et al., 1990). The term "isolate potential" is usually applied to the ability of some sexual gametophytes to become bisexual, self-fertilize, and produce a viable sporophyte (i.e. one without genetic load exposed by inbreeding). Most sexual diploid ferns are outbreeders (Watano & Sahashi, 1992) and have relatively low isolate potential. Sexual species with functionally unisexual gametophytes have zero isolate potential. The concept of isolate potential can also be extended to the ability of apogamous gametophytes to produce viable sporophytes in isolation. *A. monanthes* gametophytes that survive to a certain age are virtually guaranteed to produce sporophytes, so *A. monanthes* should have the ability to colonize all suitable habitats reached by a viable spore. The vast geographical distribution of *A. monanthes* is a logical result of its high isolate potential combined with its ecological flexibility. In contrast, sexual outcrossing species (the majority of ferns) cannot form a distant colony unless a second spore arrives and produces a gametophyte within the lifespan of the original gametophyte.[6]

Gene silencing is a common event in polyploids (which make up the majority of apogamous taxa [Richards, 1997]) with redundant copies of genes that are free to mutate to a non-functional state with minimal fitness consequences. Apogamous polyploids are particularly free to silence genes because genetic recombination is not present to expose non-functional copies. Gene silencing is probably inevitable in an apogamous polyploid given enough time because there should be no selective advantage to maintaining multiple functional copies of a gene. Silencing is presumably random with equal probability of happening to any copy of a gene. Differential silencing causes genetic differentiation among populations as different populations are randomly silenced for

[6] The probability of encountering a second gametophyte can be significantly enhanced by vegetative proliferation of gametophytes (e.g. formation of gemmae) that can significantly lengthen the lifetime of a given gametophyte clone (Dassler & Farrar, 2001).

different copies of each gene (Werth & Windham, 1991). This should compensate somewhat for the absence of genetic recombination in generating genetic variation in apogamous polyploids. Hence appreciable genetic differentiation might be expected among populations and regions of *A. monanthes*. Until silencing occurs, apogamous allopolyploids like *A. monanthes* generally have high fixed heterozygosity so low genetic load.

Some commonly-held assumptions are that asexuality slows the generation of genetic diversity and partitions most genetic diversity among rather than within populations because of the absense of genetic recombination. Various reviews that included facultatively asexual species have for the most part found no such differences between sexual and asexual species. For example Hamrick & Godt (1989) found significantly lower species-level genetic diversity in asexual than sexual plants when measured by some indices but not others, and no significant differences at the among-population and within-population levels. However obligately asexual species have not been as well studied and may actually fit the above assumptions—Ellstrand and Roose (1987) found that species-level genetic diversity decreased as reliance on sexual reproduction decreased, while Pleasants and Wendel (1989) found no such trend. More investigation will be necessary to generalize about the genetic structure of obligately asexual taxa and the forces maintaining their genetic diversity.

Distribution and habitat of *Asplenium monanthes*

Worldwide geographical distribution

Asplenium monanthes' global distribution (taken from Moran & Smith, 2001) ranges in Africa from the Cape of Good Hope along the mountains of southeastern Africa all the way north to Sudan, with a disjunct occurrence in the West African island Bioko. It is found in the Indian Ocean in Madagascar and Réunion (a small island east of Madagascar). It occurs on the South Atlantic islands of Tristan da Cunha and Gough Island and the North Atlantic islands of the Azores and Madeira. *A. monanthes* is known in the Pacific only from Hawaii. *A. monanthes* ranges throughout the mountain ranges of the New World in a basically continuous range from temperate Chile in the South,

through the Andes Mountains to the Cordilleras of Colombia and Venezuela, through the mountains of Central America to the Sierra Madres of Mexico, finally reaching into the southeastern tip of Arizona in the Huachuca Mountains. *A. monanthes* is found in the Caribbean on the island of Hispaniola (in both Haiti and the Dominican Republic) and, at least historically, in Jamaica. The disjunct populations of *A. monanthes* in the southeastern U.S. (in South Carolina, North Carolina, Alabama, and Florida), separated by at least 1600 km from the nearest tropical populations, are the subject of this investigation. **Figure 4** maps *Asplenium monanthes*' range in the northern New World.

Discovery of the SEUS populations

Asplenium monanthes was first discovered in the southeastern U.S. in 1946 by Rufus Morgan in its historically largest Carolina stronghold, the stretch of the Whitewater River below Lower Whitewater Falls (Oconee Co., SC), later submerged by the creation of Lake Jocassee. Botanical exploration continued in the Jocassee Gorges and additional populations have been discovered there almost every decade since the initial discovery. A population was independently discovered in Florida at the San Felasco Hammock (Alachua Co.) by Donald Blake in 1954, the only SEUS population known outside the Carolinas for many decades. The known range of *A. monanthes* in the SEUS was further expanded when Alan Cressler discovered three populations in Jackson Co., Alabama in the late 1980's and a population at Florida Caverns State Park (Jackson Co., Florida) in the early 1990's. **Table 1** lists all known populations and their current status.

Habitat

Asplenium monanthes is found at lower elevations at higher latitudes, ranging from as low as 50-500 m in the southeastern U.S. to as high as 3800 m in the Andes. The majority of *A. monanthes*' range falls within the tropics and subtropics. *A. monanthes* has quite a broad ecological niche there, allowing it to cover an extensive geographic range. While the Costa Rican populations investigated were located in the moist climate regimes of dense cloud forest (**Fig. 5a**) and sub-páramo bamboo thickets (**Fig. 5b**), the Mexican and Dominican populations investigated ranged from dense cloud forest to dry

Table 1: SEUS populations of *A. monanthes*
Top left quarter of table

element occur-rence #*	location	landowner	approx. latitude (N)	approx. longitude (W)	1st historical record
AL 1	Neversink	Southeastern Cave Conservancy	34° 47' 30"	86° 00' 00"	Alan Cressler, late 1980's[1]: ~100 plants
AL 2	Balcony Sink	private	34° 55' 52"	85° 52' 16"	Alan Cressler, late 1980's[1]: "a few immature plants with little regeneration after the really cold winters of the early 1990's"
AL 3	Guess Creek Cave	Three Springs School	34° 47' 30"	86° 13' 00"	Alan Cressler, late 1980's[1]: "large population"
FL 1	San Felasco Hammock	San Felasco Hammock State Preserve	29° 44' 00"	82° 26' 30"	Donald Blake, 1949[2]: 150 plants (30 fertile)
FL 2	Florida Caverns	FL Caverns State Park	30° 48' 54"	85° 14' 09"	Alan Cressler, 1992[1]: 3 fertile plants on W-facing bluff, 4 immature plants in karst feature below Pottery Cave, 3 plants at exit sink, 2 plants at Walt's Misery
NC 1	Maple Springs Branch & Auger Fork Creek	Gorges State Park	35° 05' 28"	82° 53' 49"	Alan Weakley, 1986[3]: Subpop. 1: 5 fertile, 23 immature; Subpop. 2: ~10 immature plants: Subpop. 3: ~20 immature plants; Subpop. 4: 1 immature plant
NC 2	Horsepasture River Gorge	Toxaway gameland (WRC)	35° 03' 31"	82° 56' 30"	L.L. Gaddy & Karin Heiman, 1987[3]: 1-2 immature plants
NC 3	Bearwallow Falls	Gorges State Park	35° 04' 44"	82° 54' 17"	Lewis Anderson, 1957: population size not specified
NC 5	Corbin Creek	Nantahala National Forest	35° 02' 00"	83° 00' 43"	Lewis Anderson, 1949-1951: 6 plants

Table 1 (continued)
Top right quarter of table

element occur-rence #	2nd historical record	3rd historical record	2000 pop. size	2001 pop. size
AL 1			didn't visit	43 clumps with approximately 130 plants
AL 2			didn't visit	2 small clumps of possible tiny plants
AL 3			22 clumps with 29 total plants	14 clumps with 21 total plants
FL 2	E.S. Ford, 1969[2]: 6 plants	Dan Ward & Donald Blake, 1983[2]: 1 young plant	didn't visit	no plants
FL 1			Exit Sink: 8 tiny possible plants (6 later grew enough to be identified as *A. heteroresiliens*); Walt's Misery: 30 tiny possible plants. Possible plants in other locations.	Exit Sink: 18 tiny possible plants and 1 known plant; Walt's Misery: 23 tiny possible plants.
NC 1	Mike Ivey & Dan Pittillo, 1999[3]: Subpop. 1: 3 immature plants; Subpop. 2: none found; Subpop. 3: 6 fertile plants, ~34 immature plants, plus 1 plants a few meters downstream; Subpop. 4: none found; Subpop. 5: 2 fertile plants, 8 immature plants		Didn't visit subpops. 1 & 2. Subpops. 3: >17 plants; Subpop. 4: none found; Subpop. 5: 55 plants	Subpops. 1 & 2 had a few possible A. monanthes juveniles. Subpop. 3: >16 plants; Subpop. 5: 42 plants. Didn't visit subpop 4.
NC 2			no plants	didn't visit
NC 3	Herb Wagner & Steve Leonard, 1972[3]: Curve in creek exposure: over 40 plants; Right side of falls: 2 plants	Steve Leonard & J.H. Moore, 1984[3]: no plants at previous two spots, but 2 plants at left side of falls	no plants	didn't visit
NC 5	Lewis Anderson, early 1960's: 7-8 plants		no plants	didn't visit

Table J (continued)

Lower left quarter of table

element occur-rence #	location	landowner	approx. latitude (N)	approx. longitude (W)	1st historical record
NC Cressler	Upper Whitewater Falls	Nantahala National Forest	35° 02' 08"	83° 01' 02"	Alan Cressler, early 1980's: "very few plants and they maintain a fairly consistent juvenile development"
SC 1, 3, 7	up to a dozen colonies along Whitewater River ~1 mile above and below junction with Thompson River[7]	Duke Power Co. with easement	35° 00' 36"	82° 59' 37"	Morgan & Blomquist, 1946[9]: 2 plants at first colony
SC 2	Thompson River overhang	Duke Power Co. with easement	35° 01' 00"	82° 58' 45"	Donna Ware, 1973[4]: population size not specified
SC 4	lower Coley Creek	Duke Power Co. with easement	35° 01' 09"	82° 58' 28"	Doug Rayner, 1985[4]: Upper subpop.: 2 plants; Lower subpop.: 1 plant
SC 5	mouth of Wright's Creek	Duke Power Co. with easement	35° 00' 23"	82° 58' 38"	Robert Siler & Doug Rayner, 1987[4]: 7 plants
SC 6	main subpop. just south of Table Rock Reservoir, other subpop. at Slicking Falls	Table Rock District of Greenville Watershed: Greenville Water Commission with TNC easement	35° 03' 14"	82° 41' 44"	Steven Hill & Jerry Crisp, 1992[8]: 7 plants at main subpop., 1 plant at Slicking Falls
SC 8	Glade Fern Ravine	Duke Power Co. with easement	35° 02' 23"	82° 56' 37"	L.L. Gaddy, 1987[5]: at least 50 plants
SC 10	upper Coley Creek	Duke Power Co. with easement?	35° 01' 58"	82° 58' 09"	L.L. Gaddy & Robert Siler, 1997[6]: 3 plants
SC 9, 11	Cane Creek	SCDNR	35° 01' 20"	82° 51' 37"	Original subpop.[4]: L.L. Gaddy & Butch Clay, 1998: ~5 plants; New gravel seep subpop.: L.L. Gaddy, Allison Shaw, & Butch Clay, 2000: see column to right
SC Siler	tributary of upper Thompson River	Duke Power Co. with easement?			Robert Siler, 1986[6]: 5-6 plants

**Element occurrence numbers designated by NCNHP for NC records and by SCHTP for SC records. The author assigned numbers arbitrarily to AL and FL records.*

Table J (continued)

Lower right corner of table

element occurrence # NC Cressler	2nd historical record	3rd historical record	2000 pop. size	2001 pop. size
			18 clumps with numerous tiny plants	19 clumps with numerous tiny plants
SC 1, 3, 7	Thomas Darling, 1954[10]: a number of fertile plants	L.L. Gaddy, 1991-2[6]: 1 plant		didn't visit
SC 2	Doug Rayner, 1985[4]: 7 plants	L.L. Gaddy, late 1980's[6]: ~5 plants	10 clumps with 14 plants total	>3 baby plants
SC 4	L.L. Gaddy: late 1980's[6]: same		1 plant in the upper subpop. only	still just 1 plant, in upper subpop.
SC 5			no plants	didn't visit
SC 6			no plants	no plants
SC 8			no plants	>11 possible plants
SC 10			no plants	didn't visit
SC 9, 11			Original subpop.: 25 plants; Gravel seep subpop.: 18 plants	Original subpop.: 24 plants; Gravel seep subpop.: 13 plants
SC Siler			no plants	didn't visit

[1]Alan Cressler, personal communication
[2]Dan Ward and Sam Cole, personal communication
[3]NCNHP, 2000
[4]SCHTP, 2000
[5]Gaddy, 1990
[6]L.L. Gaddy, personal communication
[7]Anderson & Bannister, 1952
[8]Steven Hill, personal communication
[9]Blomquist, 1948
[10]Darling, 1955

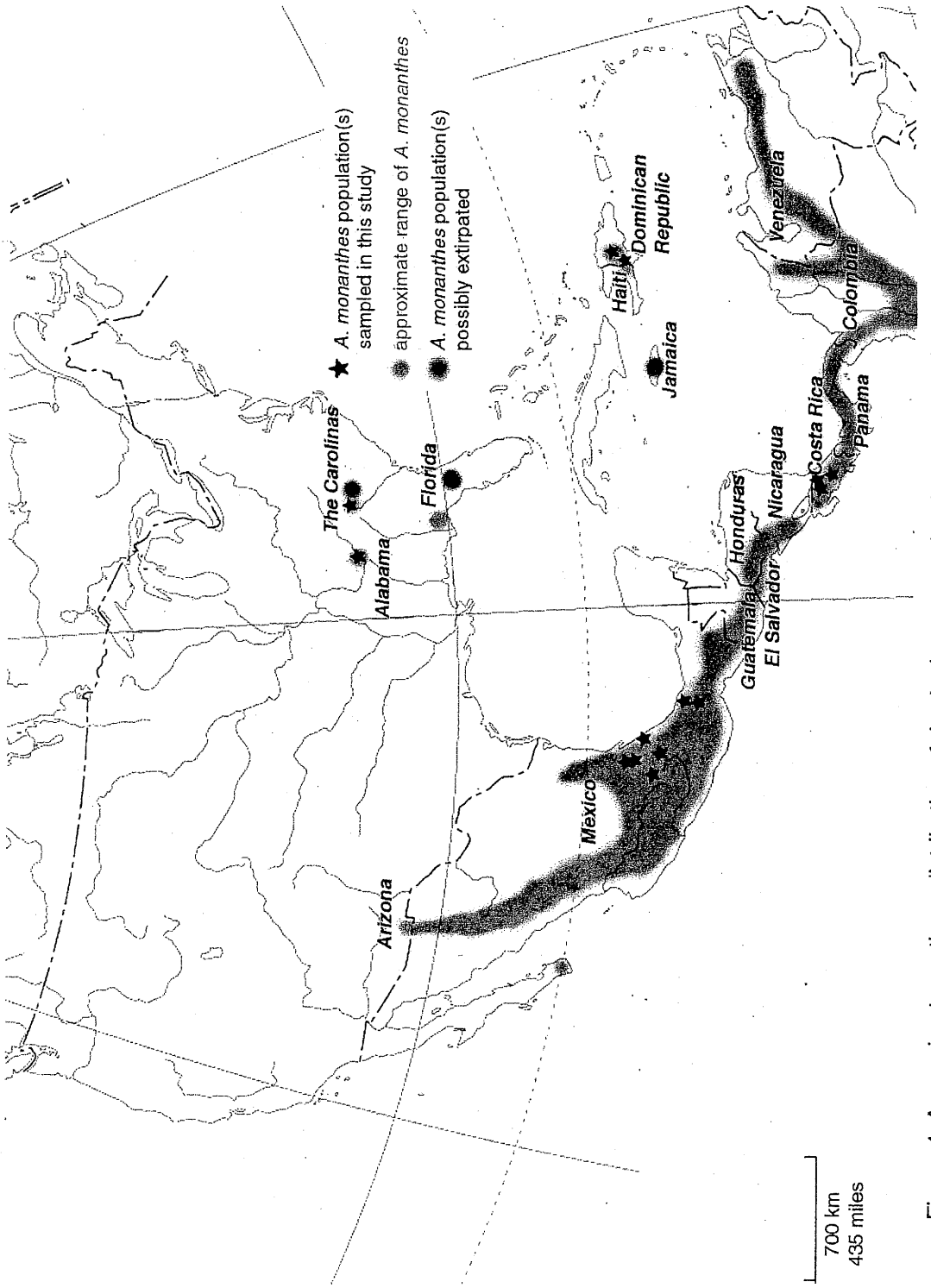

Figure 4: Approximate northern distribution of *Asplenium monanthes* in the New World

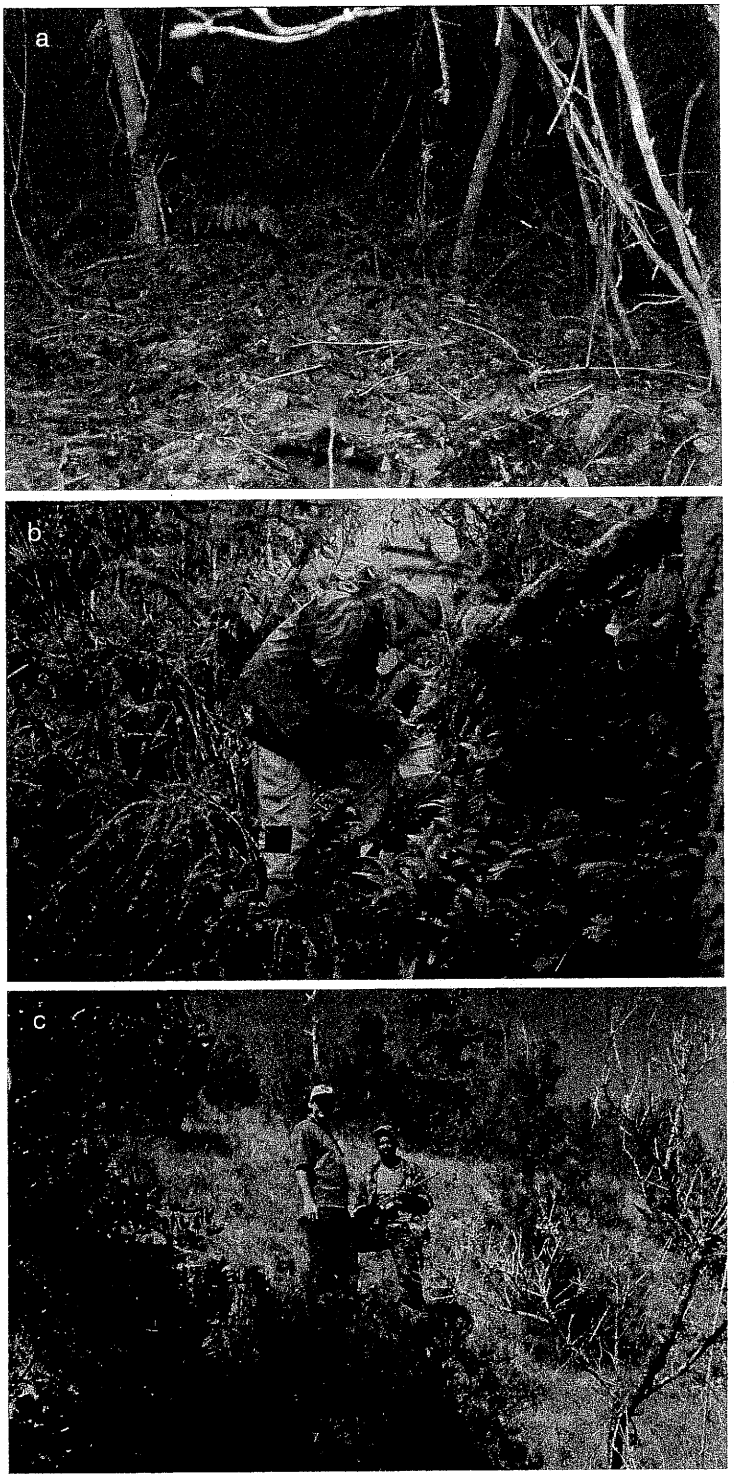

Figure 5: Range of tropical habitats of *A. monanthes*: (a) tropical cloud forest (Sierra de Baoruco, Dominican Republic), (b) sub-paramo bamboo thicket (Cerro de la Muerte, Costa Rica), (c) tropical dry forest (Valle Nuevo, Dominican Republic)

open pine forest (**Fig. 5c**). *A. monanthes* survives in montane dry forest by living in bryophyte mats underneath shrub cover. *A. monanthes* is generally found on steep slopes of hillsides, ravines, or streambanks, and man-made roadcuts if sufficiently undercut and shaded. Although the species is entirely epipetric in the southeastern U.S., it is often terrestrial in the rest of its range.

Asplenium monanthes is found in various climate-moderating habitats in the southeastern U.S. The Jocassee Gorges of the western Carolinas are a series of narrow steep gorges that drain the southern Blue Ridge escarpment. **Figure 6a** shows a typical gorge community. Each gorge is climatically moderated, with a closed canopy on its slopes that is interrupted only narrowly by the creek at the gorge bottom. The canopy is made up of typical cove hardwoods (e.g. *Liriodendron tulipifera, Fagus grandifolia, Betula lenta*) and hemlock (*Tsuga canadensis*). The base of the slope often contains thickets of *Rhodendron maximum, Rhodendron minor*, and *Leucothoe fontesiana*. Rock outcrops are abundant, providing additional shade and protection from the elements for plants living in its crevices, including *A. monanthes*. *A. monanthes* does not seem to require or thrive in the extreme climate moderation of large rockhouses as some tropical disjunct ferns do. Only at one site is it found in the depths of a true rockhouse/grotto, that being Whitewater Falls. At most of the Gorges sites, *A. monanthes* is found in a moist bryophyte mat (**Fig. 7**) on a shaded rock ledge near the creek. One subpopulation at Cane Creek grows several meters away from the creek in a generally dry tributary, where moisture is provided by a gravel seepage instead. Occasionally putative *A. monanthes* plants (differentiation of young plants from related species is difficult) have been observed halfway up the slope from the creek (at Glade Fern Ravine and at Maple Springs subpopulation 1), but this has not yet been confirmed by the presence of mature identifiable plants.

The populations in Alabama and Florida are found in sinkholes or cave entrances, but these features appear to have a stronger microclimatic effect in Alabama than Florida where the macroclimate is already quite mild. Two of the three Alabama populations (Neversink and Balcony Sink) occur on richly vegetated ledges halfway down in giant sinkholes (**Figure 6b**). The Guess Creek population (**Fig. 6c**), located in a richly

Figure 6: Range of habitat of *A. monanthes* in the southeastern U.S.: (a) the steep Jocassee Gorges (Maple Springs Branch, NC; photo by Dan Pittillo), (b) ledge in giant sinkhole (Neversink, AL), (c) boulders outside entrance to Guess Creek Cave (AL; photo by Alan Cressler), (d) entrance to Florida Caverns (FL).

Figure 7: Close-up of typical location hosting *A. monanthes* in the southeastern U.S.: moist bryophyte-covered ledge in vertical rockface (Cane Creek, SC; photo by L.L. Gaddy)

vegetated rocky ravine outside the cave entrance, receives cool moist cave winds throughout the summer. The vegetation association of the Alabama populations is often a mesic island of maple-beech forest associated with the cave or sinkhole within a larger community of oak-hickory-ash forest.

The Florida Caverns cave entrance location (**Fig. 6d**) was only slightly cooler in the summer than the surrounding humid shaded environment, and plants have been observed on bluffs away from any of the park's cave entrances (Alan Cressler, personal communication). Mitchell (1963) describes the community as mixed hardwoods, with "beech, ash, oak, hickory, walnut, elm, linden, Florida maple, magnolia, and an occasional spruce pine" making up the canopy (p. 339). The precise location of the extirpated Florida sinkhole population (San Felasco Hammock) is not known, but no distinct microclimate was detected while exploring the shallow sinkhole that it was known from. The macroclimate of the site is humid and shaded. The mesic hammock community around the ravine/sinkhole in San Felasco Hammock is dominated by

Quercus laurifolia and *Magnolia grandiflora*, while the ravine itself is bottomland forest, where *Quercus michauxii* dominates (Sam Cole, personal communication).

Bioclimatic history of the southeastern U.S.

Tertiary Period

The southeastern U.S. experienced a warmer (by 5°-10° C) more humid climate during the Tertiary than current conditions (Graham, 1993). Graham (1993, p. 60) describes southeastern climates of the Tertiary as having "varied between seasonally dry tropical and humid subtropical." Lower elevations and latitudes were dominated by tropical evergreen broadleaf forest, while several warm temperate trees joined the forest at higher elevations and latitudes (Graham, 1993; Tomlinson, 2001). (*Asplenium* spores are known from lower Eocene palynological samples, so at least one species was present in the Southeast by that point [Berry, 1937].) Global climates gradually started cooling during the Eocene, however, so that forest assemblages were noticeably more temperate in species composition by the middle of the Miocene (Berry, 1937; Graham, 1993). The Pliocene, as the last stage in the transition from the warm, humid climate of the early Tertiary to the cold, dry climate of the Pleistocene, was probably climatically similar to modern times (Berry, 1937).

Quaternary Period

The climate of the southeastern U.S. during full glacial periods of the Pleistocene was slightly cooler (by about 4° C) with seasonal temperature differences marginally larger (also by about 4° C) than those of modern times (Delcourt & Delcourt, 1993). Glacial maxima produced drier conditions than modern conditions (Wright, 1981) with possibly milder tropical storms (Delcourt & Delcourt, 1993). The dominant biomes of the southeastern U.S. during glacial maxima were a narrow belt of mixed conifer/northern hardwoods at approximately 33° N (cf. Macon, Georgia), with boreal forest to the north and a mix of warm temperate deciduous forest and southeastern evergreen forest south to panhandle Florida (Delcourt & Delcourt, 1993; Brown & Lomolino, 1998).

The climate during the Pleistocene's brief interglacial periods was basically that of today, as the Holocene is simply the most recent interglacial period. (However Delcourt and Delcourt (1993) note that the transition from full glacial to interglacial climates created greater seasonality in temperature than either climatic extreme did, so transition periods should not be overlooked as a cause of environmental stress for organisms.) Holocene and Pleistocene interglacial periods have graced the southeastern U.S. with a temperate humid environment with ample precipitation from southerly winds (Billings & Anderson, 1966), elaborated more fully below. The current dominant biomes are temperate deciduous forest across the Cumberland Plateau and Blue Ridge Front and warm temperate southeastern evergreen forest as one descends to the coastal plain (Delcourt & Delcourt, 1993; Brown & Lomolino, 1998). The temperate deciduous forest is further divided by Pittillo et al. (1998) into the following zones, descending from high to low elevations: (a) spruce-fir forest, (b) "northern" hardwood forest, "Grassy Balds," and "Heath Balds" (different topographical and geological conditions dictate different communities within the same elevational range) (c) oak-hickory-chestnut (the latter species recently decimated) forest and pine forest, and finally (d) cove hardwood forest and hemlock forest (both of which are home to *Asplenium monanthes*).

Climate of *A. monanthes*' habitat in the southeastern U.S.

Macroclimate

The general climate of the southeastern U.S. is warm-temperate and humid. The three general areas in which *A. monanthes* is currently found[7], the western Carolinas, northeastern Alabama, and the Florida panhandle, differ in macroclimate as illustrated by data from National Climatic Data Center weather stations. Throughout this paper, Lake Toxaway 2 SW (North Carolina) was the station used for comparison with all Carolina populations, Scottsboro the station for all Alabama populations, and Quincy 3 SSW the station for the Florida Caverns population. These weather stations are respectively located 6-16 km northeast to northwest of the Carolina populations, 15-31 km southeast

[7] The climate of the extirpated population in northern peninsular Florida is not discussed here but is climatically similar to panhandle Florida based on information given in Thomas et al. 1985.

to southwest of the Alabama populations, and 49 km southeast of the Florida Caverns population.

Weather station data from the past 6-11 years' data showed that the Carolinas were the coldest area and Florida the warmest, with Alabama intermediate between the two but closer to the Carolinas. Only the Florida station generally avoids freezing temperatures in winter. The mean growing season for the *A. monanthes* areas in the Carolinas, Alabama, and Florida is 189, 208, and 264 days respectively (from Byrd, 1963; Swenson et al., 1954; Mitchell, 1963 respectively). **Figures 8** and **9** plot average daily maximum and minimum temperatures respectively for each month averaged over the years 1991 (or 1996 in the case of Lake Toxaway) to 2001. The Alabama station showed the greatest seasonality in temperature and Florida the least. Temperatures were within the normal range during the two years (2000-2001) of *A. monanthes* field work based on comparisons with the previous nine years of data, with the exception that winter 2000/2001 was slightly colder than normal.

Figure 10 graphs precipitation over this time period. Precipitation was much higher in the western Carolinas than northeastern Alabama and panhandle Florida, with mean annual values for the period 1991-2001 of 216 cm, 147 cm, and 147 cm at the three respective stations over the 1991-2001 period, as compared to long-term monitoring mean values of 213 cm, 135 cm, and 140 cm, respectively (Byrd, 1958; Swenson et al., 1954; Mitchell, 1963). The high precipitation in the western Carolinas is due to the rapid increase in elevation that cools incoming moist air from the Gulf of Mexico (Billings & Anderson, 1966). No station had a season where precipitation was consistently much higher or lower than the rest of the year except possibly in Florida with somewhat greater precipitation in summer. Only the Florida station had multiple instances where a month passed without precipitation, so at least at the majority of *A. monanthes* populations, moisture should be consistently available. Mean monthly precipitation for 2000-2001 was compared to the long-term mean (**Fig. 11**) and found to be reasonably typical for the Alabama station both years, low in Florida in 2000, and low at the Carolinas station both years, so water relations microclimate data measured in this study may not represent typical values in Florida and the Carolinas.

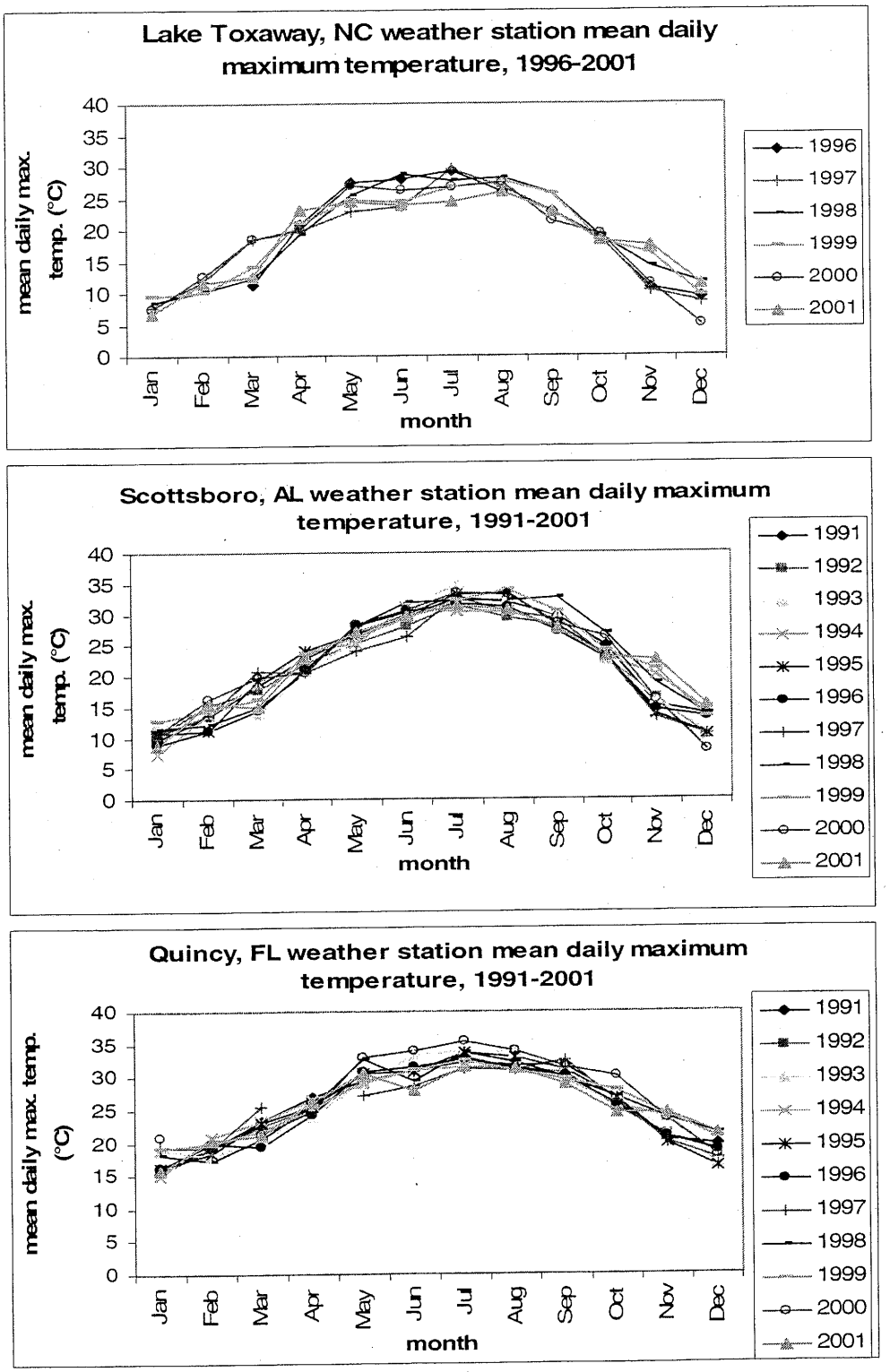

Figure 8: Mean daily maximum temperatures at weather stations in the western Carolinas, northeastern Alabama, and panhandle Florida, respectively

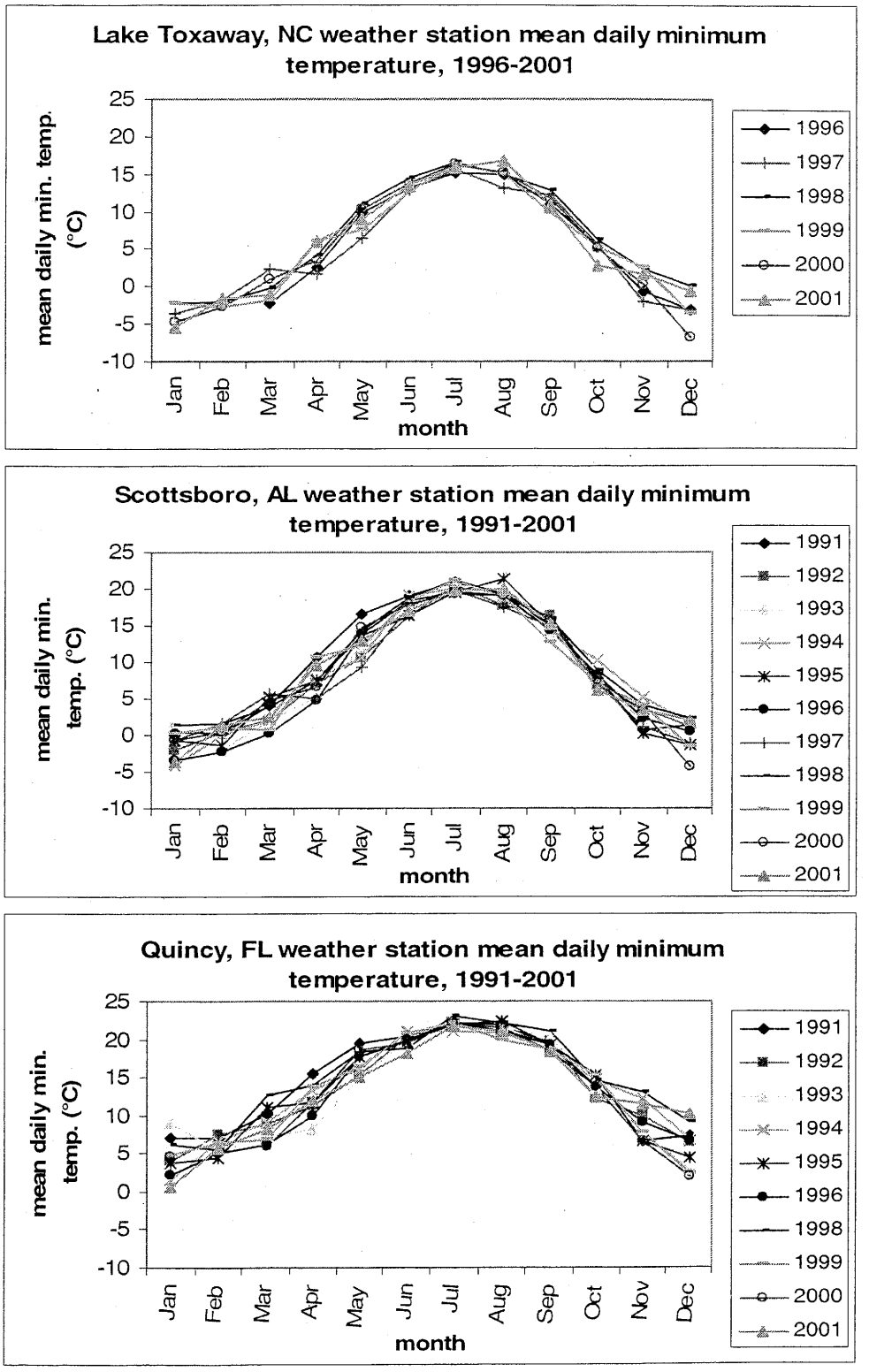

Figure 9: Mean daily minimum temperature at weather stations in the western Carolinas, northeastern Alabama, and panhandle Florida, respectively

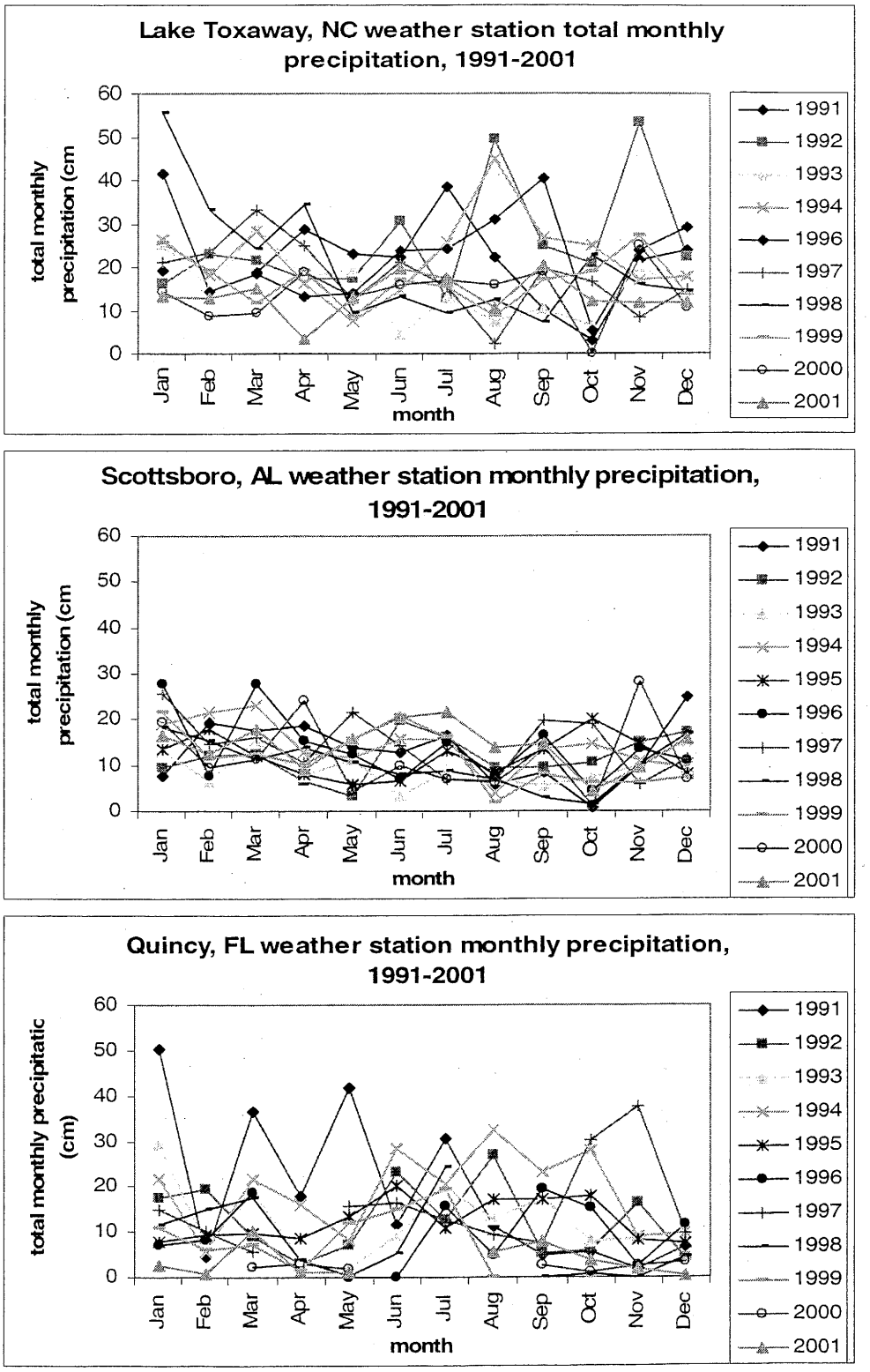

Figure 10: Monthly precipitation at weather stations in the western Carolinas, northeastern Alabama, and panhandle Florida, respectively

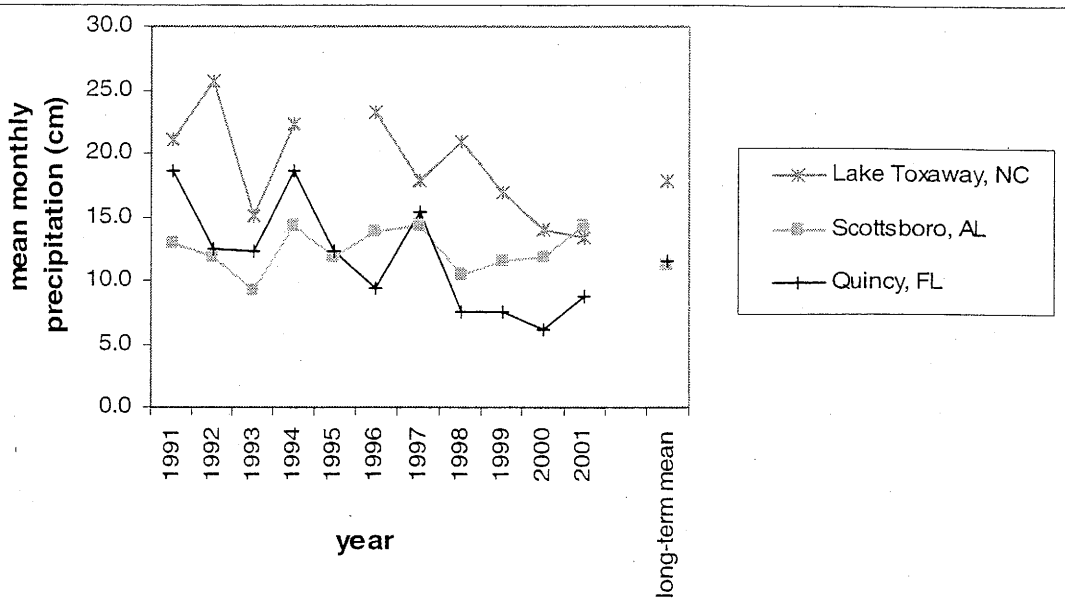

Figure 11: Mean monthly precipitation at weather stations in the western Carolinas, northeastern Alabama, and panhandle Florida for 1991-2001 in comparison with long-term mean

Microclimate and flora of SEUS gorges, sinkholes, and cave entrances

Within the southeastern U.S. there are local features that moderate the environmental conditions experienced by their inhabitants. A narrow gorge or cove can be considered climatically moderated relative to the exposed cliffs or ridges that border it. Several studies have quantified the moderating abilities of the Carolina gorges where *Asplenium monanthes* is found. These gorges often contain smaller topographical features such as rockhouses (sizable recesses in cliffs also known as grottos), but since *A. monanthes* only rarely occurs in these features (e.g. at Upper Whitewater Falls), their microclimate will not be reviewed here[8]. A second topographic feature utilized by *A. monanthes* is the sinkhole. Only one southeastern sinkhole microclimate study was encountered, that of Hemmerly (1967) from central Tennessee. No microclimate studies were encountered of cave entrances, the third type of topographic feature that *A. monanthes* inhabits. The findings of the various microclimate studies are discussed

[8] Walck et al. (1996) and Farrar (1998) reviewed sandstone rockhouse microclimate data and found great temperature moderation and moisture availability but low light availability relative to external conditions.

below with respect to each climatic parameter, but much remains unknown about sinkhole and cave microclimates.

Temperature

Gorges, sinkholes, caves, and other forms of topographic relief minimize both the solar radiation that enters during the day and the heat lost during the night, so temperatures inside remain relatively constant compared to temperatures outside these areas. All microclimate studies reviewed supported this phenomenon.

Gaddy et al. (1984) measured winter temperatures in a cove (cf. gorge) near the Carolinas' Jocassee gorges in which *A. monanthes* is found. The steepest part of the cove had minimum temperatures an average 2.65° C warmer than the flatter mouth of the cove on clear nights. The lowest minimum temperature of the steepest part of the cove was -4.5° C, in contrast to -6.5° C at the mouth. Gaddy et al. found that small herbaceous plants enjoy further temperature moderation from the insulating effect of leaf litter of up to 4° C higher minimum temperatures.

Complementing that winter study, Mowbray & Oosting (1968) measured growing season temperatures in a nearby gorge. Rather than comparing differentially sheltered parts of the gorge, they looked at different points in a single cross-section of a gorge, i.e. the top, middle, and bottom of the North and South-facing slopes. Over the period of March 13-September 25, the average mean daytime temperature at the gorge bottom (where *A. monanthes* is generally found) was 2.6° C cooler than at the tops of the slopes.

Billings & Anderson (1966) compared gorge bottoms to ridges for year-round temperatures. The pairing of Whitewater Gorge and Whitewater Ridge yielded a 4° C mean difference in yearly minimum temperatures (the most extreme temperature of each of three years of data, averaged). The ridge had a mean yearly minimum of -14° C while the gorge bottom had just -10° C (it should be noted that an unpaired gorge bottom, Horsepasture Gorge, had -15° C, so Whitewater Gorge may be exceptionally moderated). The two locations had a difference in mean yearly maximum temperatures of 6° C, with a mean high of 34° C at the ridgetop but only 28° C at the gorge bottom (this same value was observed in Horsepasture Gorge). Billings & Anderson (1966) also measured the temperature in bryophyte mats within the gorges, and found an additional (i.e. beyond the

gorge bottom values) mean winter moderation of 7° C warmer at Whitewater Gorge with a further moderation of 3° C warmer for bryophytes located in rockhouses. Summer readings showed an additional moderation of 4° C cooler in non-rockhouse bryophyte mats plus a further 5° C cooler in rockhouse bryophyte mats at Whitewater Gorge.

Hemmerly (1967) found that a Tennessee sinkhole had a greater temperature-moderating effect in summer than in winter. Winter produced soil temperatures similar in the depths of the sink to the entrance, while winter air temperature was actually colder at greater depths due to cold air drainage. Summer found both soil and air temperatures cooler at greater depths of the sink. The relative depth at which *A. monanthes* would be found (it occurs approximately 30-45% of the way down in its Alabama sinkholes) was only slightly moderated in temperature in this study: soil temperature was comparable to external temperature in winter and notably cooler (by 3.9° C) in summer, while air temperature was just barely moderated (e.g. by approximately 1.1° C) in both winter and summer.

The temperature within caves generally approximates the mean annual temperature of the region and maintains year-round stability. The temperature outside cave entrances involves interactions of this internal air with external air that can vary by season, and no studies were encountered that quantified the resulting temperature fluctuations. Caves with multiple entrances at different heights switch the direction of air flow from winter to summer, so a given cave entrance will only experience significant temperature moderation during one of these two seasons (Geiger, 1965). The *A. monanthes* population at Guess Creek Cave experiences greatest temperature moderation in the summer (Alan Cressler, personal communication). The Florida Caverns Exit Sink subpopulation experiences some temperature moderation year-round but a stronger effect in winter (Mark Ludlow, personal communication).

Water availability

Billings & Anderson (1966) found greater precipitation within various Jocassee gorges than on the ridges abutting them. Whitewater Ridge had mean annual precipitation of approximately 230 cm, already high for the eastern U.S. but surpassed by the mean annual precipitation of approximately 305 cm in Whitewater Gorge. Reflecting

both this precipitation gradient and greater solar radiation at more exposed locations, Mowbray & Oosting reported that gorge bottoms have the least evaporation (a cumulative loss of 187 ml of water from an atmometer over two months), followed by the gorge slopes (423-447 ml over the same period) and finally ridgetops (705 ml over same period).

At a smaller scale, the cool surfaces of rockhouses, sinkholes, and cave entrances and their plant cover often condense moisture from the air during summer (Farrar, 1971). In Hemmerly's sinkhole study (1967), relative humidity increased with greater sinkhole depth in every season except winter, during which there was no consistent trend based on depth. The relatively shallow depth (one-third of the way down) at which *A. monanthes* would be found was somewhat less humid than external conditions in winter but somewhat more humid during the rest of the year. An additional factor in water availability is that both sinkholes and gorges often contain waterfalls that spray mist onto surrounding plants.

Light availability

Light levels were not measured in the studies of Jocassee Gorges microclimates discussed above, but Wolfe et al. (1949) measured light levels within a protected cove in Ohio that is topographically similar to the gorges. They found high winter light levels (49500 lux, 64% of full winter sun) but very low summer light levels (1290 lux, 1.25% of full summer sun) due to a closed canopy. The sinkhole study (Hemmerly, 1967) did not quantify light but noted that the greater depths received only a few hours of sunlight a day. Light intensity in sinkholes and cave entrances remains largely unknown.

Floristic composition

Billings & Anderson (1966) investigated floristic affinities of the mosses in the Jocassee Gorges. Out of 268 species collected, they found 12 (4%) species disjunct from the American tropics, 6 (2%) disjunct from a pan-tropical distribution, and 13 (5%) endemic to the southeastern U.S. but not specified as to higher-level geographic affinity. Zartman & Pittillo (1998) analyzed spray cliff floras (a subset of gorge microhabitat wetter than where *A. monanthes* usually occurs) of the nearby Chattooga Basin's rock

outcrops. They found only 2% of the vascular species but 12% of the non-vascular flora to be tropical disjuncts (no distinction was made between paleotropical and neotropical distributions), with an additional 18% of vascular plants and 12% of the non-vascular plants endemic to the southeastern U.S. (higher-level geographic affiliation not given, but presumably many have close tropical relatives). Several rockhouse ferns of the southeastern U.S. have tropical affinities (Farrar, 1998). The sinkhole microclimate study (Hemmerly, 1967) did not discuss the floristic affinities of the plants of the sinkhole. A Florida site of *Asplenium monanthes*, Florida Caverns State Park, containing multiple caves and more topographic relief than is typical for Florida, was examined floristically (Mitchell, 1963). Eight (9%) of 86 bryophytes present had tropical affinities, while most vascular plants had local or northern affinities with a couple tropical exceptions among the ferns.

Significance of the above site characteristics

To summarize from the studies discussed above, gorges, sinkholes, and cave entrances experience varying but often notable degrees of temperature moderation. This moderation is probably important in allowing tropical plants to persist in the southeastern U.S. The majority of these tropical species are believed to be relicts from the tropical Tertiary flora of the southeastern U.S. rather than recent immigrants (reviewed in Billings & Anderson, 1966 and Farrar, 1998), hence this investigation of *Asplenium monanthes* to determine whether it fits the same pattern.

The SEUS tropical relict plants are generally found only in climatically moderated microhabitats so their ability to withstand temperate climates appears to be limited, but may still surpass that of related species limited to the tropics, via adaptations or preadaptations. Farrar (1971) tested responses to freezing of species of the tropical fern genera *Trichomanes*, including species from Appalachian rockhouses, in a common garden experiment. Sporophytes of tropical species were as tolerant of freezing (i.e. some deaths but generally just setbacks in growth) as Appalachian sporophytes, but tropical gametophytes were not as cold-hardy as Appalachian gametophytes. This suggests that physiological adaptation (or alternatively, pre-adaptation) may have occurred at the gametophyte stage in the Appalachian *Trichomanes*. While Farrar did not

test *Trichomanes*' response to extreme heat, summer temperature moderation could also be important through reduction of evaporative stress.

Water availability is greater in gorges, sinkholes, and cave entrances than in surrounding areas. Most plants of mesic habitat photosynthesize only when evaporative stress is low, so if conditions are too dry, the plants cannot grow and mortality can occur. Farrar (1971) observed the response of Appalachian *Trichomanes* to dessication. Whereas gametophytes of *T. intricatum* (a sporophyteless species) rebounded easily from dessication, sporophytes of *T. boschianum* (gametophytes not tested) generally died. Sporophytes of most tropical disjunct cryptogams are intolerant of desiccation, indicating that the consistant moisture availability in climatically-moderating Appalachian microhabitats is probably important in allowing these tropical taxa to persist.

If tropical plants weather the climate extremes of the southeastern U.S. by living in moderating microhabitats, they must be able to survive at the inherently low light levels there. The tropical habitat of these taxa is generally underneath dense tropical rainforest or cloud forest canopies, so they are pre-adapted to low light levels and therefore make strong competitors in dimly-lit SEUS microhabitats. Light levels under temperate deciduous forest canopies are exponentially higher in winter than in summer due to the open canopy, leading Wolfe et al. (1949) to speculate that understory plants in their Ohio valley study do most of their photosynthesis in late spring and early fall when leaves are not on the trees. Farrar (1971) conducted experiments measuring photosynthesis rates of the rockhouse fern *Trichomanes boschianum* over a range of temperatures and light levels. He concluded that the seasonal trade-off between higher light but lower temperature from late fall to early spring and lower light but higher temperatures during the rest of the year probably results in a relatively constant rate of photosynthesis throughout the year for ferns in these moderated microhabitats.

Therefore climatically-moderated microhabitats, in conjunction with physiological and anatomical adaptations and preadaptations, have allowed a number of tropical plants to survive in the southeastern U.S., in many cases as relicts of tropical Tertiary floras. This phenomenon is largely limited to bryophytes and ferns with clonal gametophytes (Farrar, 1998). Farrar (1998) explains the exclusion of tropical seed plants

by noting the high photosynthetic demands of most species' sporophytes (much energy must be put into the production of photosynthetic sinks like roots, stems, and reproductive structures) relative to gametophytes. Tropical species with the ability to persist as gametophytes (i.e. bryophytes and ferns with clonal gametophytes) in low-light but highly moderated habitats during the cold Pleistocene survived, whereas the sporophyte-dependent lifecycle of tropical seed plants and most tropical ferns prevented their survival. Even if *Asplenium monanthes* is found to be a more recent colonist, the moderated microclimates of gorges, sinkholes, and cave entrances are still responsible for its presence in the southeastern U.S.

PART I.

BIOGEOGRAPHIC ORIGIN AND TAXONOMIC STATUS OF SOUTHEASTERN UNITED STATES *ASPLENIUM MONANTHES* L.

INTRODUCTION

Goals

Asplenium monanthes L. is a fern with a wide geographic distribution that includes many sizable disjunctions. The focus of this investigation is the disjunct populations in the southeastern U.S. (SEUS). These populations are compared to the nearest neotropical populations (the most likely source of the original colonists) for determination of their biogeographical origin, taxonomic status, and genetic structure, utilizing spore and gametophyte morphology and starch gel electrophoresis of enzymes.

Alternative hypotheses for the origin of *A. monanthes* in the southeastern U.S.

The majority of "tropical" plants in the southeastern U.S. appear to be Tertiary relicts (see Billings & Anderson, 1966, Farrar, 1998), so it was of interest to determine whether *A. monanthes* supports this generalization or is instead a more recent colonist. Three questions exist regarding the specific origin of *Asplenium monanthes* in the southeastern U.S. How did it arrive, by long-distance dispersal or by gradual range expansion northward from Mexico? When did it arrive, during the Tertiary, which would have allowed the species to inhabit a continuous range of climatically suitable habitat, or during the Quaternary, during which appropriate target habitat has been rare and isolated? Finally, what was the source of the original colonists? The closest tropical populations, in central Mexico and the Caribbean, are considered the two most probable sources. *A. monanthes* also occurs in western Mexico and Arizona (in Arizona *A. monanthes* is as rare as in the southeastern U.S.), but the somewhat greater geographic distance and the Rocky Mountains as a barrier to wind currents make the western populations an unlikely source for the original SEUS colonists. Therefore western populations of *A. monanthes* were not investigated. The possible combinations of mechanism, timing, and source of colonization of the southeastern U.S. create four colonization scenarios. These possible scenarios and the expected genetic patterns each would produce are outlined below. It is also possible that more than one of these scenarios occurred, e.g. populations in one area of the southeastern U.S. could be pre-Pleistocene relicts of Mexican origin whereas

populations in another area could have been founded by recent colonization from the Caribbean.

Relicts of a large pre-Pleistocene distribution originally founded by Mexican spores (Figure 12a)

Ancient gradual range expansion vs. ancient long-distance dispersal from Mexico cannot be distinguished. *A. monanthes* occurs in only three main areas in the southeastern U.S., so no pattern of progressive isolation by distance from Mexico could be detected even if it had once existed. Therefore the exact mechanism of any ancient colonization of the southeastern U.S. by Mexican spores cannot be determined. It should be noted, though, that this scenario is the only one in which gradual range expansion is a possibility, all other scenarios requiring long-distance dispersal.

If *A. monanthes* once had a continuous range across the southeastern U.S., as would have been possible only during the Tertiary's subtropical climate, the relictual populations should cluster together genetically and show appreciable differentiation from other regions due to a common origin unless multiple tropical colonists from multiple source areas were involved. Genetic drift in subsequent small refugial populations means that widely-separated surviving populations could be fixed for the same historically common genotype or that nearby surviving populations may be fixed for different genotypes, both unlikely if the SEUS populations had a Quaternary origin. A Mexican origin for the original SEUS colonists means that SEUS populations should be more genetically similar to Mexican populations than to any other region and may form a clade within a larger Mexican clade. A Tertiary origin for SEUS populations would have provided sufficient time for genetic differentiation so that no shared multilocus genotypes would be likely between the SEUS and Mexican populations and SEUS populations would probably have evolved some novel alleles and morphological characteristics.

Relicts of a large pre-Pleistocene distribution originally founded by Caribbean spores (Figure 12b)

The island of Hispaniola has been above water for at least 35 million years (Iturralde-Vinent & MacPhee, 1999), so it could easily have had its own populations of

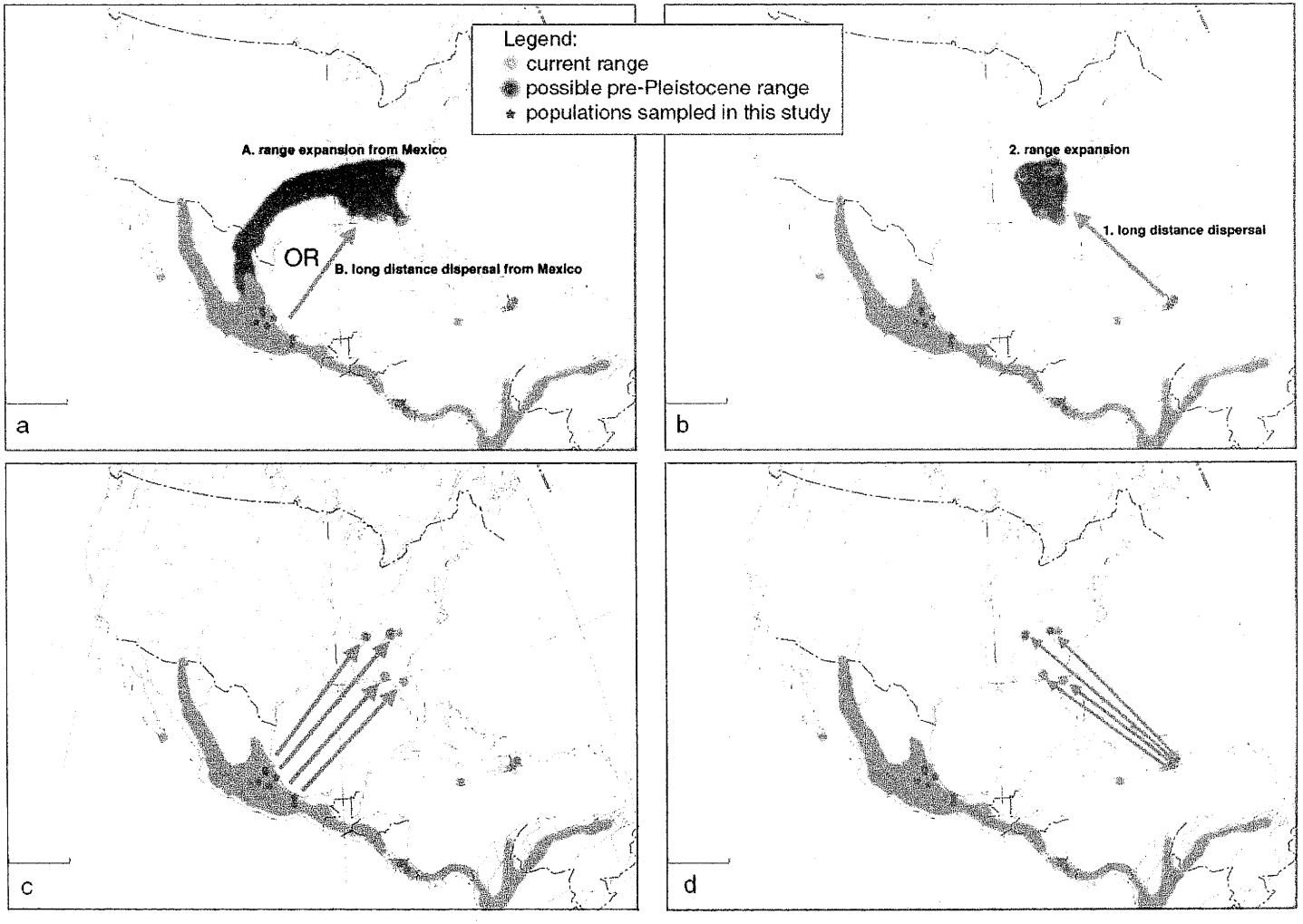

Figure 12: Alternative colonization scenarios to explain *Asplenium monanthes*' current distribution in the southeastern U.S.: (a) pre-Pleistocene colonization from Mexico and later range fragmentation during Pleistocene, (b) pre-Pleistocene colonization from the Caribbean and Pleistocene range fragmentation, (c) multiple colonizations from Mexico during Pleistocene or Holocene, (d) multiple colonizations from the Caribbean during Pleistocene or Holocene

Asplenium monanthes (founded by any of the tropical mainland populations bordering the Caribbean) dispersing spores to North America by the late Tertiary. The subtropical Tertiary climate of the southeastern U.S. would make any *A. monanthes* spores that reached there likely to successfully start a colony. Due to the favorable Tertiary climate, after the initial founding, *A. monanthes* could have quickly spread to form a large continuous range before later restriction to Pleistocene refugia. This scenario would produce the same genetic patterns as expected for the above scenario except that the SEUS populations would be more similar to Caribbean than Mexican populations.

Pleistocene or Holocene long distance colonization from Mexico (Figure 12c)

A gradual range expansion from Mexico to the southeastern U.S. during the Quaternary is unlikely because the moderating topographical features (e.g. steep gorges, sinkholes) that allow *A. monanthes* to survive in the current temperate climate of the U.S. are absent from much of the intervening area. Therefore any Quaternary colonization from Mexico could have occurred only via long-distance dispersal. The interglacial periods of the Pleistocene were approximately similar in climate to our current interglacial period, so appropriate moderated habitat in the southeastern U.S. can be assumed to be present but rare. Therefore an abundance of incoming tropical spores would have been necessary for successful establishment of *A. monanthes* in these small target sites. It would seem unlikely for a given area to host multiple genotypes under this scenario because of the low probability of independent colonization events in the same small area. The distance separating different *A. monanthes* areas (the Carolinas, Alabama, and the two historical Florida areas) in the southeastern U.S., the populations' currently minimal spore output, and their highly sheltered (i.e. minimal wind for spore dispersal) microhabitat suggests that different areas were in fact probably colonized by different tropical colonists as opposed to SEUS spores and should therefore not share genotypes. Therefore this scenario would probably result in SEUS populations being genetically nested among various Mexican populations, showing no regional cohesion. Sufficient genetic sampling might reveal shared or very similar genotypes from Mexico and the southeastern U.S. because of a short time for genetic divergence since

colonization, and the SEUS populations would be unlikely to have generated novel alleles or morphological characteristics.

Pleistocene or Holocene long distance colonization from the Caribbean (Figure 12d)

If Quaternary long-distance dispersal was responsible for the SEUS populations, the Caribbean would be the most probable source for colonists because of regular tropical storms that move across the Antilles towards the southeastern U.S. and probably carry spores. The genetic results of this scenario would be the same as for the previous scenario except that SEUS genotypes would be similar or identical to Caribbean genotypes and would be nested among them in a cladogram.

Causes of biogeographic disjunctions in ferns

The two main phenomena causing biogeographic disjunctions are interruption of a previously continuous range, and colonization of a new far-away site without colonization of the intervening area. These phenomena are respectively called vicariance and long-distance dispersal. Common causes of vicariance include division by a new mountain range or body of water, continental drift, and climate change making the intervening area less hospitable. A pattern of long distance colonization can occur if a species has considerable dispersal capability but the intervening area is inappropriate habitat for the species, or if a species with more modest dispersal capability is assisted by a powerful rare event like a hurricane.

Ferns produce abundant tiny windborne spores, so are potentially capable of much greater dispersal than most seed plants. However, most fern spores fall within a few meters of the source plant (Peck et al., 1990; Penrod & McCormick, 1996), probably because most species live underneath a forest canopy where wind does not frequently penetrate. A further constraint on ferns colonizing new sites is that most species are outbreeding and carry genetic load that prevents self-fertilization (Crist & Farrar, 1983), so only if two spores happen to reach the same site and simultaneously produce gametophytes can a new population be initiated. This consideration does not apply to *A.*

monanthes, being apogamous, but it helps explain why fern species in general show the same distributional patterns as seed plants (Wagner, 1972).

A literature review was performed to see whether vicariance or long-distance dispersal appear to be more common as the cause of geographic disjunctions in ferns. Clearly disjunctions that are occurrences of ferns on remote young volcanic islands (e.g. Hawaii) must be a result of long-distance dispersal, but terrestrial disjunctions could be caused by either phenomenon. The vicariance event that may apply to *A. monanthes* is climatic change that would have limited a possible ancient range in the southeastern U.S. to isolated refugia. Survival in continuously suitable refugia for over a million years and successful dispersal over thousands of miles to a small target area of appropriate habitat are both improbable events, so neither scenario is clearly more likely than the other.

Only eight studies of fern distribution patterns were found which included testable genetic data. The glacial refugia vicariance scenario was the alternative hypothesis to long-distance dispersal for five of the studies (Trewick et al., 2002; Rumsey et al., 1998; Farrar, 1990; Watkins, 1998; Soltis et al., 1997). In the remaining three studies, an exact vicariance event was never postulated (Ranker et al., 1994b), was not suggested because the current range is not disjunct (Schneller et al., 1998), or was irrelevent because long-distance dispersal was the only possible explanation and the question was how many times it had occurred (i.e. an occurrence in Hawaii: Ranker et al., 1994a).

In some cases both phenomena may have occurred. Watkins (1998) inferred ancient long distance dispersal from the Old World followed by range expansion and subsequent restriction to Pleistocene refugia for *Thelypteris pilosa*. Or, following climate change, refugial individuals may colonize newly suitable habitat, at times via long-distance dispersal, as shown for *Asplenium ceterach* by Trewick et al. (2002).

Three case studies (Soltis et al., 1997; Rumsey et al., 1998; Farrar, 1990) found historical limitation to refugia to be the sole cause of disjunction. Genetic results supporting a refugial explanation for the latter two cases were not surprising because both *Trichomanes speciosum* and *Vittaria appalachiana* include populations of gametophytes that have lost the ability to form sporophytes (in the case of *V. appalachiana*, no populations include sporophytes) and must have been reproductively isolated for millenia

for this capability to be silenced. Soltis et al. (1997) invoked multiple refugia for *Polystichum munitum* instead of just one or two refugia with subsequent long-distance dispersal to colonize a third area.

Three case studies exhibited purely long-distance dispersal (Ranker et al., 1994b; Ranker et al., 1994a; Schneller et al., 1998). Western U.S. populations of *Asplenium adiantum-nigrum* were found to be the result of long-distance dispersal rather than vicariance because they were more genetically similar to distant populations (Hawaii) than relatively close populations (Mexico) (Ranker et al., 1994b). Ranker et al. (1994a) also investigated the Hawaiian populations of this species. The great genetic diversity there could be explained only by multiple long distance colonizations of the archepelago, indicating that successful long-distance dispersal is a relatively frequent occurrence for this species. Schneller et al. (1998) investigated the relatively continuous European range of *Dryopteris remota* and found evidence of long-distance dispersal among various distant populations rather than a simple clinal genetic pattern.

Therefore this limited literature review does not show ferns as being either more prone to vicariance (specifically restriction to climatic refugia) or to long-distance dispersal. Both are relatively rare phenomena, and either one could be responsible for the occurrence of *Asplenium monanthes* in the southeastern U.S.

Justification of methods

Sporophyte morphology was not utilized for this investigation. No qualitative morphological differences from tropical plants were mentioned in the literature on SEUS *Asplenium monanthes* and Alan Smith (personal communication), an authority on neotropical *Asplenium*, judged the SEUS collections from this investigation to be well within the range of neotropical *A. monanthes* morphology. Morphometric comparison would be inappropriate in the absense of common garden experiments (impossible because of SEUS plants' protected status) because SEUS sporophytes may be dwarfed for environmental reasons. Instead the generally understudied fern stages, the gametophytes (which were raised under identical lab conditions) and spores, were investigated for possible morphological differences between SEUS and neotropical

plants. Genetic data was obtained from starch gel electrophoresis. These three data sources, although not universally informative, were considered the most promising sources of data for investigation of the SEUS populations' variability, differentiation, and origin.

Spore morphology

Fern spore architecture can be informative in taxonomic investigations. For example, Gastony (1979) observed significant palynological variability within the genus *Trichipteris*, Morbelli & Ponce (1997) found similar variability within Argentinian *Cheilanthes*, and Haufler & Gastony (1978) observed intrageneric variability in *Hemionitis* and *Gymnopteris* although none within *Doryopteris* and *Coniogramme*. Variation in spore architecture even within a putative species (sometimes subsequently used as support for elevating varietal status to specific status) has been documented in *Thelypteris pilosa s.l.* (Watkins, 2000), *T. palustris s.l.* (Tryon, 1971), *Asplenium flaccidum* (Braggins & Large, 1990), *Bommeria hipida* (Ranker, 1989), and *Cystopteris fragilis* (Tryon & Tryon, 1982). Regalado & Sánchez (2002) used spore architecture to clarify species delimitations in three pairs of morphologically (i.e. sporophyte morphology) similar species of *Asplenium* in Cuba. They found minor differences between the spores of *A. cristatum* and *A. myriophyllum*, no differences between *A. erosum* and *A. venustum*, and notable differences between *A. auritum* and *A. monodon*. Sporophyte morphology (and the number of spores per sporangium for *A. auritum* and *A. monodon*) supports the lack of difference between spores of *A. erosum* and *A. venustrum* and the observed difference between spores of *A. auritum* and *A. monodon* (the authors did not compare spore to sporophyte data for *A. cristatum* and *A. myriophyllum*). Therefore spore architecture appears to be largely concordant with more traditional sources of taxonomic data and can strengthen taxonomic decisions.

Gametophyte morphology

Gametophyte morphology has only occasionally been utilized for taxonomic studies of ferns. Gametophytes are difficult to find and identify in natural populations because of their tiny size and the lack of keys for identification. Additionally, many

botanists (e.g. Bower, 1923) believe fern gametophytes to be too developmentally plastic to provide reliable data for taxonomic studies. However recent investigations have uncovered consistant differences between fern taxa, especially at higher taxonomic levels (references in Dassler & Farrar, 1997). At the specific level, differences in gametophyte morphology are less frequent but can occur. Pérez-García et al. (1999) compared the gametophyte morphology of six Mexican *Dryopteris* species and found some differences in growth form and sexuality (i.e. bisexual vs. single-sex vs. apogamous gametophytes), but not enough to be able to distinguish each species. Nester & Schedlbauer (1981) compared *Anemia mexicana* gametophytes to other *Anemia* species and found differences in developmental rate and sexuality but not particularly in gross morphology. Chiou & Farrar (1997) found that *Campyloneurum phlyllitis* shared the developmental path and mature growth forms of *C. angustifolium*, but also included individuals that showed different developmental morphology. Chiou et al. (1998) investigated gametophytes of five species of *Elaphoglossum* and found some differences with respect to rhizoid morphology, general growth form, and sexual systems. Watkins (2000) found differences in developmental rate, antheridiogen response (the induction of male gametangia and dwarfed growth following exposure to female gametophyte secretions), and many morphological characters within *Thelypteris pilosa s.l.* Prada et al. (1995) observed differences between hair production (hair length, density, and developmental timing) in gametophytes of two diploid *Asplenium* species and two varieties of their hybrid derivative. In summary, gametophyte morphology tends to be similar within a genus but sometimes shows species-level differences, so it can be potentially informative but an absence of differences between putative species or varieties does not necessarily mean that taxonomic distinction is unwarranted.

Starch gel electrophoresis

The genetic approach utilized in this investigation was starch gel electrophoresis, also known as protein electrophoresis or isozyme or allozyme analysis. This technique has a long history of utility for a wide range of population genetics questions, so it is reliable and results can be compared to studies of similar organisms. The Mendelian

inheritance and codominant nature of isozyme alleles make them straightforward to interpret. For these reasons recent reviews such as Arnold & Emms, 1998, and Cruzan, 1998, argue that starch gel electrophoresis remains a powerful tool in population genetics despite the increasing popularity of DNA-based molecular markers (e.g. RFLPs, RAPDs, AFLPs, microsatellites, ISSRs).

Preliminary tests revealed sufficient isozyme variability among populations of *Asplenium monanthes*, so starch gel electrophoresis was considered promising for this investigation. Many alternative genetic approaches (DNA sequencing or the above-mentioned molecular markers) could potentially have been carried out in addition to starch gel electrophoresis, but limited resources held us to a single genetic approach and this was chosen as the most reliable one. Many studies (e.g. Fang et al. 1997, Liu & Furnier 1993, Mes et al. 2002, Murakami et al. 1999b, Scribner et al. 1994, Swensen et al. 1995, Van Droogenbroek et al. 2002) have compared isozyme results to those from DNA sequences and various molecular markers for various research questions. These alternative methods gave results usually, although not always, concordant with isozyme results, so little was expected to be gained from trying any additional genetic approaches for this investigation.

MATERIALS & METHODS

Taxa and populations sampled

Table 2 lists the collections used in this investigation, including as outgroups the related species *A. platyneuron, A. polyphyllum, A. resiliens, A. heterochroum,* and the hybrid of the latter two species, *A. heteroresiliens*. This investigation also included samples of *A. hallbergii*, a taxon segregated from *A. monanthes* by Mickel & Beitel (1988). The great morphological similarity between the two taxa led the author to question *A. hallbergii*'s status as a distinct species (a question that will be addressed in a different publication). For the purposes of this investigation, the name "*A. monanthes*"

Table 2: Populations of *Asplenium monanthes* and related species sampled genetically

site	code name	species	voucher(s)	approx. lat. (N)	approx. long. (W)	# plants sampled
United States:						
Florida						
Florida Caverns State Park	heteroresiliens	*A. heteroresiliens*	Shaw 136	30° 48.90'	85° 14.15'	1
Alabama						
Neversink	Neversink	*A. monanthes*	Shaw 146	34° 48.33'	86° 00.28'	7
Guess Creek	Guess	*A. monanthes*	Shaw 1	34° 45.68'	86° 11.33'	6
Guess Creek	resiliens	*A. resiliens*	Shaw 137	34° 47.50'	86° 13.00'	1
Carolinas						
Upper Whitewater Falls	Whitewater	*A. monanthes*	Shaw 7	35° 02.15'	83° 01.08'	1
Thompson River	Thompson	*A. monanthes*	Shaw 8	35° 01.50'	82° 58.97'	1
Coley Creek	Coley	*A. monanthes*	Shaw 9	35° 01.28'	82° 58.45'	1
Cane Creek	Cane	*A. monanthes*	Shaw 10, 123, 18, 120	35° 00.00'	82° 53.00'	9
Maple Springs Branch, Auger Fork Creek	MapleSprings	*A. monanthes*	Shaw 5, 6	35° 05.50'	82° 53.65'	8
Missouri						
Washington Co.	platyneuron	*A. platyneuron*	Farrar 01-04-23	38° 04.66'	90° 41.20'	1
Dominican Republic:						
Cordillera Central						
Valle Nuevo Reserva Cientifica	Valle Nuevo	*A. monanthes*	Shaw 252	18° 47.45'	70° 38.74'	10
Valle Nuevo Reserva Cientifica	heterochroum	*A. heterochroum*	Shaw 303	18° 47.45'	70° 38.74'	1
Sierra de Baoruco						
Palo de Agua	PaloDeAgua	*A. monanthes*	Shaw 157	18° 12.43'	71° 30.72'	11
Caseta Forestal no. 2	Caseta2	*A. monanthes*	Shaw 158	18° 12.37'	71° 33.29'	6
Los Arroyos	LosArroyos	*A. monanthes*	Shaw 159	18° 15.64'	71° 44.14'	7
Mexico:						
Hidalgo						
Zacualtipan	Zacualtipan	*A. monanthes*	Tejero-Diez 4323	20° 39.82'	98° 30.90'	5
Mineral Real del Monte	MineralRealDel Monte	*A. monanthes*	Tejero-Diez 4325	20° 09.75'	98° 41.80'	5

Table 2 (continued)

site	code name	species	voucher(s)	approx. latitude (N)	approx. longitude	# plants sampled
D.F. vicinity						
Magdalena Contreras	RojasMonanthes	*A. monanthes*	Rojas 5505-5509	19° 20.00'	99° 15.00'	5
Magdalena Contreras	RojasHallbergi	*A. hallbergii*	Rojas 5510	19° 20.00'	99° 15.00'	1
Magdalena Contreras	cf.resiliens	*A. hallbergii*	Rojas 5511	19° 20.00'	99° 15.00'	1
Ocuilan	Ocuilan	*A. monanthes*	Tejero-Diez 4326	19° 03.00'	99° 20.22'	4
Ocuilan	polyphyllum	*A. polyphyllum*	Tejero-Diez 4327	19° 03.00'	99° 20.22'	1
Veracruz						
Maltrata	Orizaba-Puebla	*A. monanthes*	Tejero-Diez 4331	18° 52.00'	97° 16.18'	3
Volcan Perote	Jalapa	*A. monanthes*	Farrar 98-10-18	19° 25.66'	97° 06.00'	4
Oaxaca						
Ixtlan	Don'sHallbergii	*A. hallbergi*	Farrar 98-10-12-A	17° 20.00'	96° 30.00'	1
Ixtlan	Ixtlan	*A. monanthes*	Farrar 98-10-12-B	17° 20.00'	96° 30.00'	15
Rancho Tejas	RTmonanthes	*A. monanthes*	no voucher	17° 20.00'	96° 30.00'	3
Rancho Tejas	Rtherringbone	*A. monanthes*	Farrar 98-10-12-D	17° 20.00'	96° 30.00'	13
Llano Verde	LlanoVerde	*A. monanthes*	Farrar 98-10-13	17° 40.00'	96° 20.00'	15
Costa Rica:						
Volcan Barva						
Yurro Seco	Barva	*A. monanthes*	Shaw 106	10° 08.00'	84° 07.50'	11
Volcan Irazu						
steep hillside before Rio Birris	IrazuHill	*A. monanthes*	Shaw 92	9° 58.00'	83° 50.50'	7
Rio Yerbabuena	Yerbabuena	*A. monanthes*	Shaw 101	9° 56.00'	83° 53.00'	2
Cordillera de Talamanca						
km 87, Carretera 2	km87	*A. monanthes*	Shaw 50	9° 35.00'	83° 46.00'	9
torre de TV, Cerro Buenavista	TorreDeTV	*A. monanthes*	Shaw 61	9° 34.00'	83° 45.50'	1
El Tajo, Quebrada Asuncion	ElToja	*A. monanthes*	Shaw 62	9° 34.00'	83° 45.50'	13
Albergue Cuerici (~km 93)	Cuerici	*A. monanthes*	Watkins 24	9° 33.00'	83° 39.00'	1
Total plants						**191**

hereafter refers to *A. monanthes s.l.*, in which *A. hallbergii* is included, unless stated otherwise.

Vouchers for SEUS samples were deposited at Iowa State University (ISC). The historical Jamaican locality for *A. monanthes* was visited but the population was not found, so *A. monanthes* may or may not still exist in Jamaica. Vouchers for Dominican samples were deposited at ISC and at the National Botanical Garden in Santo Domingo (JBSD). Vouchers for Mexican samples collected by Alexander Rojas were deposited at the National Autonomous University of Mexico at Mexico City (MEXU) and ISC. Vouchers for Mexican samples collected by Daniel Tejero Díez were deposited at the National Autonomous University of Mexico at Iztacala (IZTA) and ISC. Vouchers for Mexican specimens collected by Donald Farrar were deposited at ISC. Vouchers for Costa Rican samples collected by the author were deposited at the National University of Costa Rica (USJ) while that collected by James Edward Watkins was deposited at the National Museum of Costa Rica (CR).

Comparative spore morphology

Spore architecture was examined using scanning electron microscopy. The populations used to represent the spores of each region and taxon were as follows:

Southeastern U.S.: Guess, Coley

Dominican Republic: Caseta2

Mexico, *A. monanthes s.s.*, normal form: LlanoVerde, 5506 Rojas

Mexico, *A. monanthes s.s.*, herringbone form: RTherringbone

Mexico, *A. hallbergii*: 5510 Rojas

Costa Rican spores were in poor condition and were not successfully observed.

Spores were kept in a desiccator for several days in preparation for mounting. Double-sided sticky tape was placed on aluminum stubs and spores were sprinkled on. Silver paint was applied around the edges of the field. The stubs were then sputter coated with gold palladium for 120 seconds using a Denton Vacuum LLC Desk II Cold Sputter Unit. The spores were viewed using JEOL 5800LV scanning electron microscope at an accelerating voltage of 10kV. Images were recorded digitally.

SEUS spores were compared with neotropical spores with respect to architecture and size. These spores were also compared to published photomicrographs and/or measurements of *A. monanthes* from Kenya (Viane & Van Cotthem, 1977), Ethiopia (Tryon & Lugardon, 1991), South Africa (Welman, 1970), the Azores (Ormonde, 1987), Tristan da Cunha (Roux, 1992), Argentina (Michelena, 1993), Veracruz, Mexico (Tryon & Lugardon, 1991) and Hawaii (Selling, 1946; Tryon & Lugardon, 1991). *A. monanthes* spore architecture was compared to that documented for *A. castaneum* (Tryon & Tryon, 1982), *A. resiliens* (Michelena, 1993), and *A. trichomanes* subsp. *quadrivalens* (Quieros & Ormonde, 1990) to determine the degree of spore variability within the larger *Asplenium trichomanes* group.

Comparative gametophyte morphology and ontogeny

Petri plate cultures were prepared using 1% agar medium enriched with Bold's macronutrients (Bold, 1957), Nitsch's micronutrients (Nitsch, 1951), and ferric chloride (Farrar, 1974). Spores were sown from all fertile collections listed in **Table 2**. The plates were placed under fluorescent lamps under a constant light regime of approximately 3230 lux at 21°C. The morphology of the resulting gametophytes was documented regularly with drawings (using a drawing tube) and photographs. Gametophytes from SEUS *A. monanthes* spores were compared to those from neotropical spores for morphology and developmental rate.

Starch gel electrophoresis

Grinding protocol

Fresh leaf tissue was kept on ice or in refrigeration for up to three weeks before being ground. The tissue was ground by hand in a phosphate extraction buffer described in Cronn et al., 1997, while all equipment was kept on ice. Sample homogenate was stored in microcentrifuge tubes at a temperature of approximately -80° C for up to three and a half years.

Running protocol

Starch gels were made following the protocol of Murphy et al. (1996) using selected buffer recipes of Soltis et al. (1983). When the gels were ready, the tubes of homogenate were thawed at room temperature, spun in a centrifuge for 5 minutes at 14,000 rpm. Filter paper wicks were saturated with homogenate, then inserted into the gel. Gels were run for 4.5 hours under refrigeration, at 40 amps for buffer systems 7 and 9 and at 55 amps for system 11 (system names from Soltis et al., 1983).

Staining protocol

Each gel was cut horizontally into four slices. Each slice was either immersed in liquid stain or covered with an agarose stain solution. Stain recipes were taken from Soltis et al. (1983). The stains used were as follows: system 7 gels were stained with liquid AAT stain, agarose TPI stain, and liquid PGI stain; system 9 gels were stained with liquid PGM stain, liquid MDH stain, and agarose 6PGD stain; system 11 gels were stained with agarose IDH stain, agarose ACN stain, and agarose SKDH stain. DIA was stained on all three systems but none provided bands sharp enough for scoring, so DIA was not utilized.

Scoring protocol

Banding patterns were assigned an allelic basis whenever possible. The most mobile allele was designated allele 1, the second furthest traveling allele called allele 2, and so on. The enzymes ACN, AAT, and IDH contained multiple overlapping loci with significant genetic variability, so they were not interpretable as alleles and were instead scored as patterns. Pattern assignment was conservative, lumping together variants of a pattern if they could not be consistantly differentiated. Some interpretable enzymes had two scorable loci: TPI, PGI, MDH. The enzymes 6PGD, PGM, and SKDH had one scorable locus each. Thus a total of twelve "loci" were used in this investigation, some actually representing multiple but uninterpretable loci combined as patterns.

Interpretable loci of *Asplenium monanthes* were scored as representing triploid individuals because the majority of chromosome counts recorded for *A. monanthes* show 108 bivalents and because some Mexican and Costa Rican samples showed three

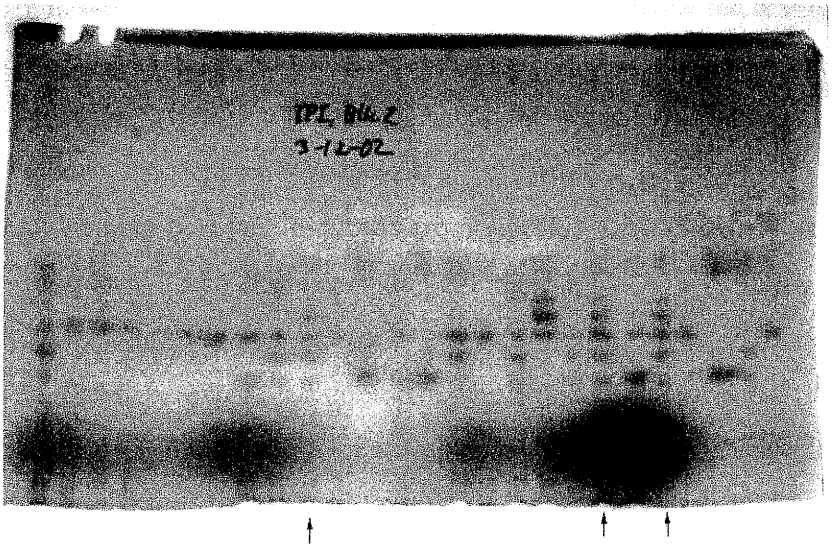

Figure 13: Zymogram showing *Asplenium monanthes* samples with three different alleles at an isozyme locus. Arrows mark samples with a three-allele heterodimer for locus TPI-2.

different alleles at particular loci (**Fig. 13**). *A. hallbergii* has never been investigated cytologically but was scored as triploid based on one sample having three different alleles at a locus (it may actually have a higher ploidy, but there is no evidence to support that). Frequently individuals of both *A. monanthes* and *A. hallbergii* were heterozygous with equal staining intensity for the two alleles present, suggesting that the expected third copy of the gene has been silenced. These samples were not interpreted as diploids or tetraploids because they showed unbalanced heterozygosity at other loci. The diploidized banding pattern was scored as having missing data for the third copy of the gene because it cannot be determined what allele it once expressed.

Homozygous phenotypes presented a greater challenge for scoring; a triploid individual with a homozygous phenotype may have anywhere between one and three copies of the given allele. Apogamy allows silencing of genes with no fitness consequences as long as one functional copy of the gene remains, because meiosis will never occur to produce offspring lacking a functional copy. Because silencing was observed in *A. monanthes* (based on the occurrence of two equal intensity bands in a triploid individual), we cannot assume that a homozygous band represents three copies of the same allele. However to score homozygous individuals as having only one confirmed

copy of an allele would be overly conservative and greatly reduce the resolving power for allele frequency analysis. Therefore all homozygous individuals were scored as having three copies of a given allele, with the caution that the allele frequency analyses are based upon an assumption that has not been tested.

The outgroup taxa ranged in ploidy from diploid (*A. platyneuron*) to triploid (*A. resiliens*) to tetraploid (*A. heterochroum*) to pentaploid (*A. heteroresiliens*) to unknown but greater than triploid (*A. polyphyllum*) (all taken from Lellinger, 1985 except for *A. polyphyllum*, which is based instead upon its complex electrophoretic banding patterns). Because the software (Populations 1.2.26, Langella 2002) used for the allele frequency analysis (see below) did not allow different ploidy levels within an analysis, taxa with more than three genomes were scored as having just three alleles per locus, and relative dosages of alleles were reflected in the scoring when possible. There were a few instances where a sample had more than three different alleles. Such a sample was scored as lacking data for that locus because of the difficulty assigning an allelic basis to such a complicated pattern. The diploid, *A. platyneuron*, was scored as having missing data for the third allele at every locus. Scoring according to an incorrect ploidy may have skewed results for the allele frequency analysis, but fortunately for the allele presence/absence data analysis (see below), ploidy was irrelevent.

Analysis of isozyme data

Geographic distribution of alleles and phenotypic patterns

The geographic range of each allele (in the case of all loci except for AAT, ACN, and IDH) and uninterpretable phenotypic pattern (in the case of the loci AAT, ACN, and IDH) was plotted in a Venn diagram of the four regions sampled. The number of alleles and phenotypic patterns in each region was used to assess the level of genetic diversity in each region. The number of alleles or phenotypic patterns shared with other regions vs. private to a region was used to qualitatively assess the frequency of migration among regions.

Quantitative estimates of genetic diversity and differentiation

Standard measures of genetic diversity and differentiation based on allele frequencies were calculated to allow comparison of *Asplenium monanthes*' genetic structure to species with similar life history or taxonomic affiliation. Genetic diversity was estimated using the species-level percentage of loci diagnostic (loci which are variant from individual to individual, in contrast to "polymorphic loci" which can include invariant fixed heterozygous loci in an asexual species), mean percentage of loci polymorphic at the population and species levels, the mean number of alleles per locus (the three unscorable loci were excluded from this estimate) at the population and species levels, the percentage of populations polymorphic, and the mean number of genotypes per population.

Genetic differentiation was quantified using the percentage of genotypes local (found in only one population) and widespread (found in greater than 75% of populations), Nei's G-statistics, and Nm. The program SPAGeDi 0.1 (Hardy & Vekemans, 2002) was used to estimate Nei's (1973) extension of Wright's F-statistics, Gsr, Grt, and Gst[9]. The three loci unscorable as alleles were excluded from these calculations because they rely upon allele frequencies. The G-statistic values were tested for statistical significance by a permutation test. The effective number of inter-regional migrants per generation, inter-regional Nm, was estimated from the frequency attained by private alleles in each region (Slatkin, 1985) and from the estimate of Grt. Within-region Nm values were estimated from each region's estimate of Gsr. The data was tested for evidence of isolation by distance by regressing pairwise Nei's genetic distance[10] over pairwise geographic distance and over the natural log of geographic distance, both within Mexico (none of the other three regions were sampled sufficiently to test isolation by

[9] These parameters measure reduction in gene diversity from genetic drift due to population subdivision (Nei, 1973). Gsr measures the reduction in gene diversity of a population relative to the regional pool of populations, Grt measures the reduction of a region relative to the pool of regions sampled, and Gst combines these two effects into one parameter.

[10] Alternative genetic parameters for an isolation by distance model involve pairwise Fst and Nm, but these are based on Wright's (1931) island model in which mutation is negligible relative to migration. In a species subject to gene silencing and limited in migration, Nei's genetic distance is a more appropriate measure of pairwise genetic distance.

distance) and for the total study area. A permutation test was used to determine significance of the resulting slope values.

Allele frequency comparison of populations

To compare *A. monanthes* populations to one another, the relative frequencies of alleles in each population were calculated and used for Principle Coordinate Analysis (using the program NTSYS-pc 2.02i by Rohlf, 1998), which represented each population as a point in 3-dimensional space to allow the viewer to group different populations as relatively similar genetically. PCO was first conducted including the five outgroup species to ensure that *Asplenium monanthes* was clustering together and not within the multidimensional genetic space occupied by outgroups. Then it was repeated without the outgroups to maximize the visual spread among *A. monanthes* populations specifically.

In addition to PCO (an ordination method), neighbor joining (a clustering algorithm), was performed on the allele frequency data for *A. monanthes* and outgroups. First Nei's standard genetic distance (1987) was calculated between all pairs of populations based upon differences in allele frequencies. Then the neighbor joining algorithm used these pairwise distances to build a phylogram estimating hierarchical relationships among populations. The neighbor joining analysis was carried out in the program Populations 1.2.26 and was bootstrapped with 1000 replicates.

Presence/absence comparison of genotypes

It can be argued that the functional unit of genetic trends in asexual species is not the population, as for sexual species, but the clone. Sexual populations are coherent units of genetic exchange via sexual reproduction. In contrast, asexual populations are simply aggregates of discrete clones that do not interact except via competition for resources. Comparing allele frequencies of purely asexual populations is probably inappropriate because there is no common gene pool for each population, only changes in relative frequency of various static (in the absence of mutation) clones. In light of this, Gregorius et al. (in review) have formulated the measure Delta which can be used to compare asexual populations. Delta measures differences between populations in both the identity and relative frequencies of clones.

Because each SEUS population sampled was enzymatically monomorphic (presumably representing a single clone) and contained no multilocus genotypes found in the tropical samples, it proved unnecessary to take into account differences in relative clone frequency when comparing populations, only differences in clone identity. Therefore instead of calculating Delta to compare populations, genetic distance was calculated to compare genotypes. This analysis was simpler to perform and interpret yet equally informative for the comparison of SEUS to neotropical *A. monanthes*. Use of genotypes as OTUs created a challenge in comparing results of this analysis to the traditional allele frequency analysis which utilized populations as OTUs because some genotypes were found in multiple populations and some neotropical populations contained multiple genotypes.

To be conservative, individuals were considered to have the same genotype as long as they shared the same set of alleles, even if they appeared to have different relative staining intensity of bands. Therefore scoring for this genotype-based analysis was "present" or "absent" for each possible allele rather than estimating the number of copies of each allele for a given individual. This also avoided the problem discussed above of determining the number of copies of an allele present in homozygous individuals.

A spreadsheet was compiled listing all alleles found in more than 5% of the samples of each taxon and each genotype observed was scored as containing or lacking each allele at a given locus. Thus every allele rather than just every locus is treated as a taxonomic character. This approach requires that one allele per locus be omitted from the data matrix to deal with the problem of non-independence of character states (i.e. if an individual lacks all but one of the possible alleles at a locus, it must be homozygous for the remaining allele, so the different allele states are dependent on one another).

The data matrix was analyzed by Principle Coordinate Analysis using NTSYS-pc. The resulting 3-dimensional plot shows which genotypes appear to be most similar to one another. After confirming that the *A. monanthes* genotypes clustered together away from the outgroup genotypes, PCO was repeated with outgroups omitted to maximize the visual spread of *A. monanthes*.

Neighbor joining was also conducted for the genotype-based presence/absence data set. Dice's similarity/difference coefficient, a distance measure applicable to any type of presence/absence data, was calculated for each pair of genotypes using NTSYS-pc. (Jaccard's difference coefficient, similar to Dice's, was also used but the results will not be reported, being quite similar to those obtained with Dice's coefficient.) The resulting pairwise distance matrix was used to build a neighbor joining tree showing hierarchical relationships between genotypes. The neighbor joining algorithm was carried out by NTSYS-pc and by PHYLIP 3.5c (Felsenstein, 1995) and could not be bootstrapped because of logistical impediments. PHYLIP, while advantageous in showing branch lengths (NTSYS-pc could not), unfortunately could not accomodate all 54 genotypes found within *A. monanthes* and the outgroups. Therefore the five outgroups and three arbitrarily-chosen Mexican genotypes (T, U, NN) were omitted from the PHYLIP tree. The tree made in NTSYS-pc, using all genotypes, was used to determine where to root the *A. monanthes*-only PHYLIP tree. Note that the non-reticulating assumption of dendrogram construction is met when the OTU's are clonal genotypes of apogamous organisms because such genotypes do not intermix with one another as populations do.

RESULTS

Comparative spore morphology

All three regions of *Asplenium monanthes* investigated displayed similar variability in spore architecture (**Figure 14**). As members of the genus *Asplenium*, the spores are monolete, bilaterally symmetric, and perinous. Spores have a smooth exine layer covered by a pillared layer of inner perispore (**Fig. 15**) concealed by the outer perispore. The outer perispore is composed of tall ridges, wide at the base but narrow at the apex, reticulating to form craters between ridges. Samples varied greatly in number of ridges (showing no geographic trends) but all had dentate ridge apices. The surface texture of the craters is foveoreticulate around the margin of the crater, while the center

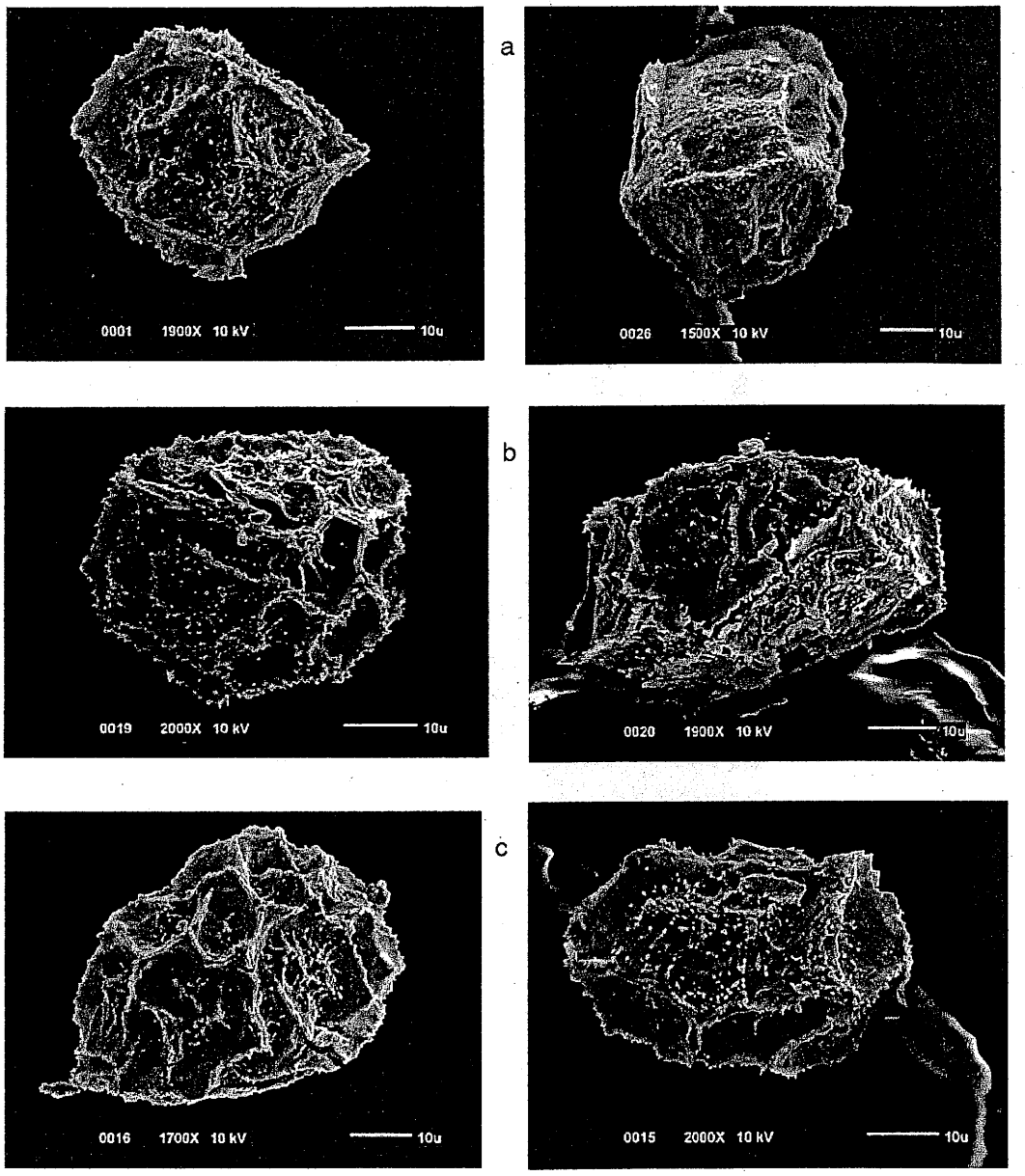

Figure 14: Comparison of *A. monanthes* spores from three of the regions studied: (a) southeastern U.S. (L: Guess, R: Coley), (b) Dominican Republic (Caseta2), (c) Mexico (L: RojasMonanthes, R: LlanoVerde)

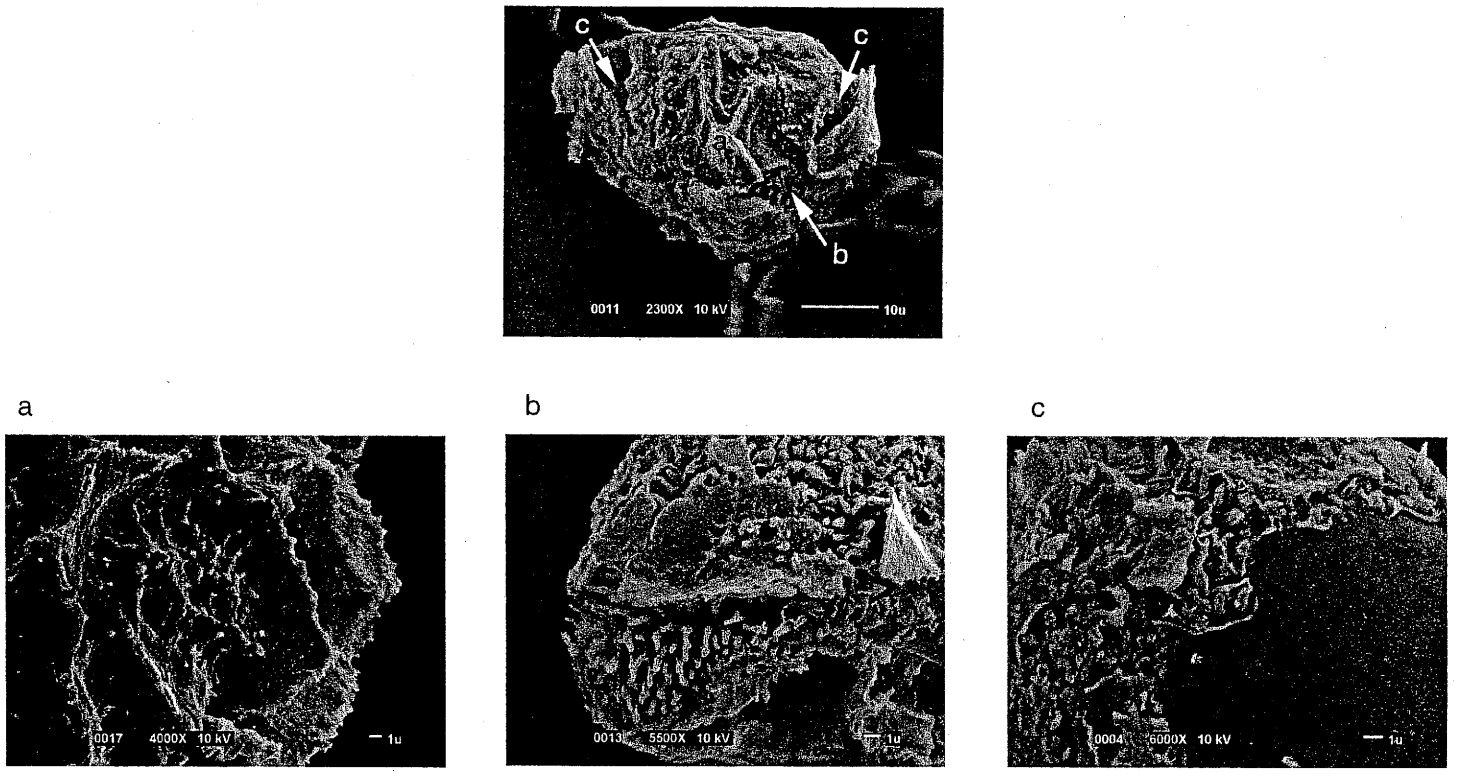

Figure 15: The three spore wall layers of *A. monanthes,* exposed on damaged spore from Llano Verde, Mexico: (a) ridged foveoreticulate outer perispore (close-up: RojasMonanthes, Mexico), (b) exposed pillars of inner perispore (close-up: Llano Verde, Mexico), (c) exposed smooth exospore (close-up: herringbone, Mexico)

of each crater is a convex (usually) area with baculate projections scattered across the foveoreticulate surface.

Spores described in the literature, recorded from Hawaii, South Africa, Tristan da Cunha, and Argentina, in increasing size, ranged in mean size from 26-46.8 micrometers polar diameter and 39-64.5 micrometers equatorial diameter. Not enough spores were sampled in this investigation to reliably estimate spore dimensions, but the few examined were comparable to Hawaii's small spores. (A notable exception was found in one of the collections from Hidalgo, Mexico: plants from Mineral Real del Monte had spores perhaps only half as big as all other collections, thus warranting future cytological examination.)

A. monanthes spore images from the literature (from Mexico, Argentina, Tristan da Cunha, Kenya, Ethiopia, the Azores, and Hawaii) appear qualitatively similar to those examined in this investigation, despite their distant origin, varying mainly in number of ridges (e.g. the Azores spore photo published by Ormonde, 1987, shows an unusual number of closely-spaced ridges).

Published spore images from other species of the *Asplenium trichomanes* group are clearly distinguishable from *A. monanthes* spores. *A. resiliens* spores had an extremely high density of ridges with few holes in the intervening areas. *A. castaneum* spores had a smooth surface texture (lacking holes and projections) with smooth ridge apices. *A. trichomanes* spores have a network of many small ridges in the areas between large ridges.

Comparative gametophyte morphology and ontogeny

Lab-raised *Asplenium monanthes* gametophytes displayed great morphological diversity and several characteristics unusual for *Asplenium*. The majority of *Asplenium* gametophytes have papillate hairs along the margin and follow Nayar & Kaur's (1971) *Aspidium* growth pattern. *Asplenium monanthes,* as a member of the *Asplenium trichomanes* group, is among the hairless *Asplenium* gametophytes and follows Nayar & Kaur's (1971) general *Adiantum* growth pattern with some major modifications: prolonged filamentous growth and multiple thalloid lobes and resulting sporophytes.

Figure 16 shows the two main developmental paths observed, both of which deviate from the standard *Adiantum* pattern.

Growth began with production of a filament of cells. Some gametophytes switched from filamentous to thalloid growth after the first few cell divisions as is typical for all *Asplenium* gametophytes, differentiating ultimately into a heart shape (**Fig. 17a**) with a meristem located in an apical notch between lateral wings. Even these gametophytes differed from traditional *Asplenium* gametophytes by continuing to grow beyond the time of sporophyte formation, as the two wings differentiated to become winged themselves and subsequently produced additional sporophytes from new apical notches. The term "heart-shaped" is used loosely here, as some thalli were quite irregular in form, for example appearing as a circle lacking a notched growth apex, or a reniform to oblong shape (**Figures 17b and 17c,** respectively).

Other *A. monanthes* gametophytes grew as filaments indefinitely, with portions of the filament continuing to divide transversely (**Fig. 18a**). In some cases this intercalary growth switched to 2-dimensional growth to form a thallus (**Fig. 18b**), or the growing tip of the filament finally switched to thallus production (**Fig. 18c**). The resulting thallus developed just as described above from more precocious thalli.

Thus all *A. monanthes* gametophytes appeared to have multiple regions of simultaneous cell division, whether filamentous or thalloid, producing a many-lobed gametophyte with sporophytes developing from each lobe (**Figures 19a and 19b**). Eventually connections between different parts of the gametophyte decayed, separating the various lobes and their sporophytes into independent functional units so that it was hard to determine whether they had ever been linked (**Fig. 19c**). This type of gametophytic growth resulted in multiple sporophytes being produced from a single original spore.

Gametangia were never observed on *A. monanthes* gametophytes during this investigation, although antheridia were reported from earlier trials using the same spores from Oaxaca, Mexico (Erin Heep, personal communication) and are found in many other apogamous fern gametophytes (Sheffield & Bell, 1987). Instead each apogamous thallus produced a 3-dimensional sporophytic proliferation near the apical notch. (Only rarely

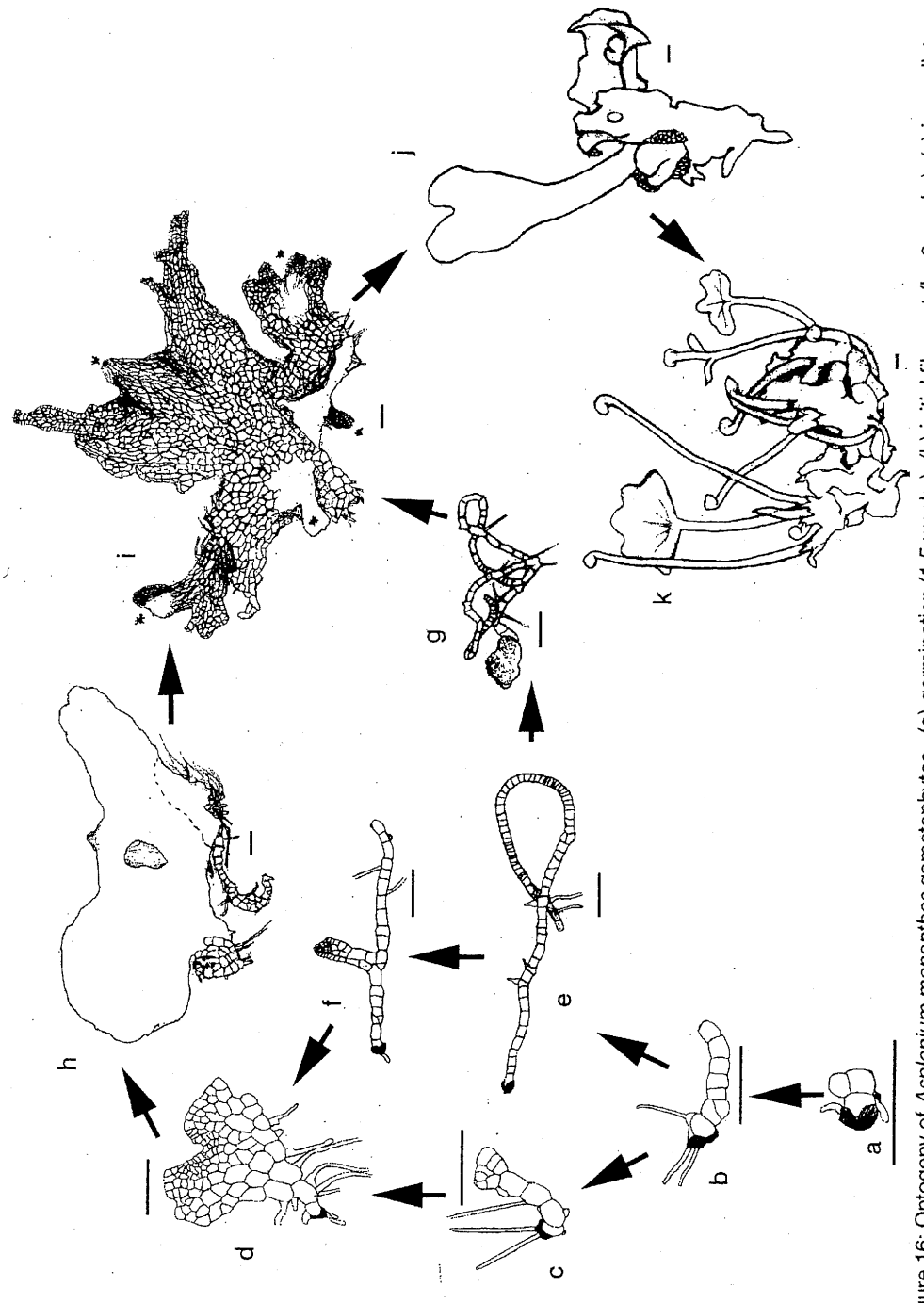

Figure 16: Ontogeny of *Asplenium monanthes* gametophytes. (a) germination (1.5 weeks), (b) initial filament (by 3 weeks), (c) immediate thallus initiation (after 3 weeks), (d) young thallus (6.5 weeks), (e) continued filamentous growth (indefinite), (f) thallus initation from long filament (unpredictable), (g) rare: filament gives rise to bulge directly (unpredictable), (h) winged thallus with bulge (sporophytic growth apex) and, in some cases, "swan neck" extension (10 weeks), (i) wings differentiate to form new zones of sporophyte initiation (asterisks) while original bulge differentiates (by 3 months), (j) bulge covered with clathrate scales, gives rise to first fiddlehead or strap-like extension (3 months), (k) multiple fiddleheads or strap-like extensions, bases of stipes darken, and eventually root develops (3.5 months). Scale bar = 0.1 mm.

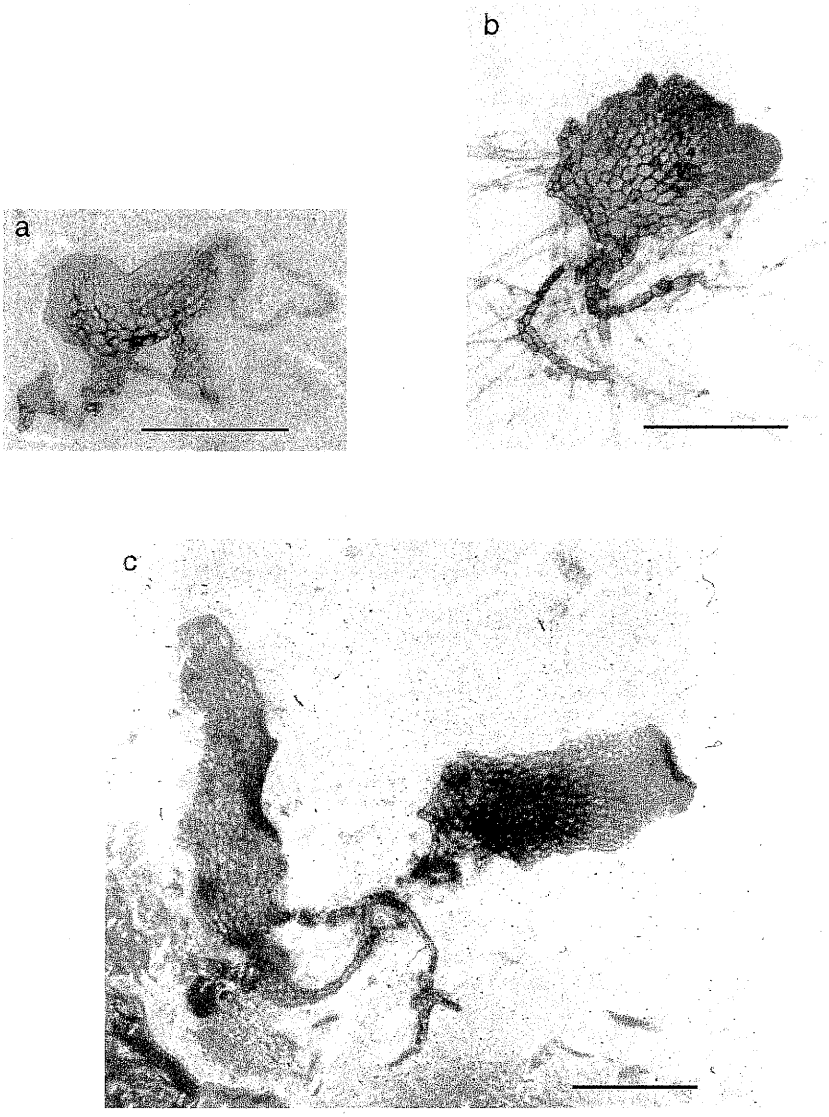

Figure 17: Various thalloid growth forms observed in *A. monanthes* gametophytes: (a) cordate (ValleNuevo, D.R., 9 weeks), (b) rounded, lacking apical notch (Ixtlan, Mex., 7 weeks), (c) oblong or reniform (Coley, SC, 3.5 months). Scale bar = 0.5 mm.

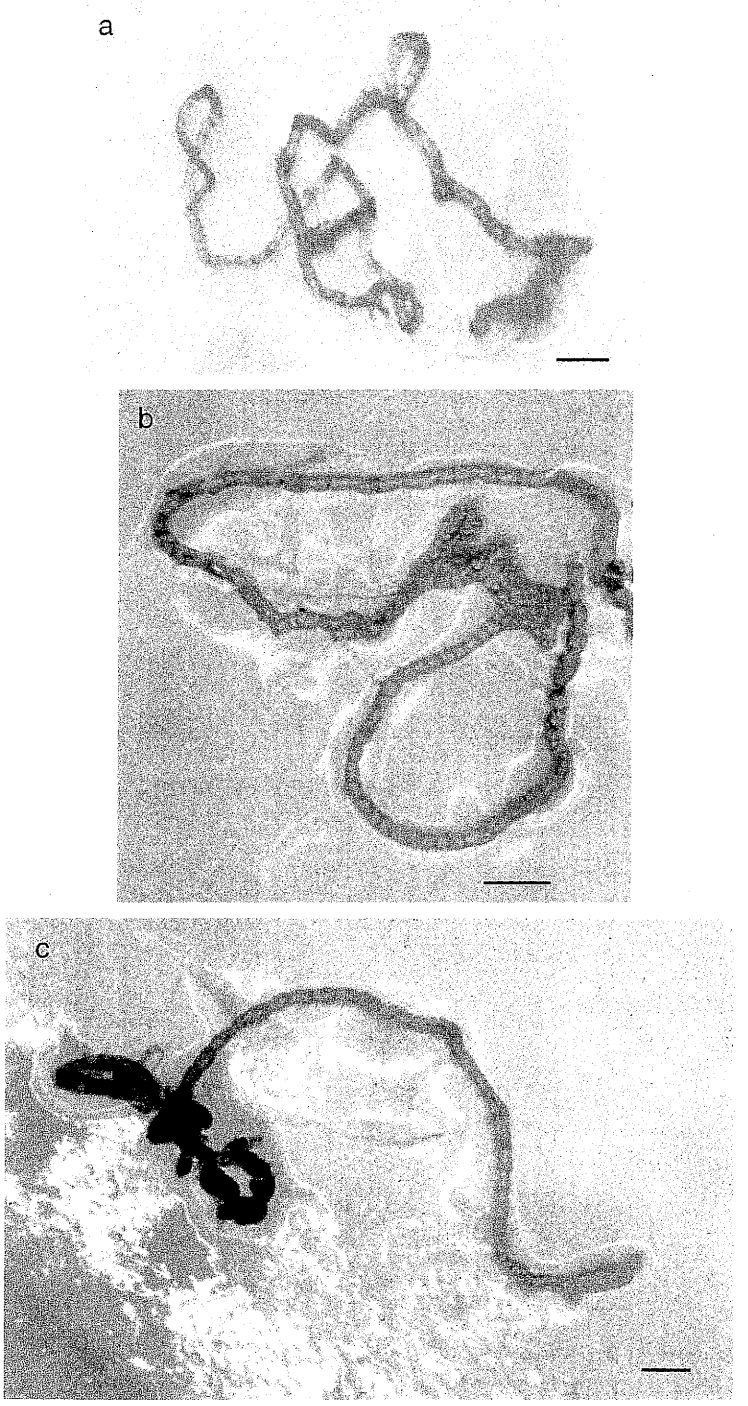

Figure 18: Filamentous gametophytes and eventual thallus initiation: (a) completely filamentous gametophyte (km87, C.R., 6.5 weeks), (b) thallus formed from zone of intercalary growth (Ixtlan, Mex., 6.5 weeks), (c) terminal growth apex widening into a thallus (LlanoVerde, Mex., 11.5 weeks). Scale bar = 0.1 mm.

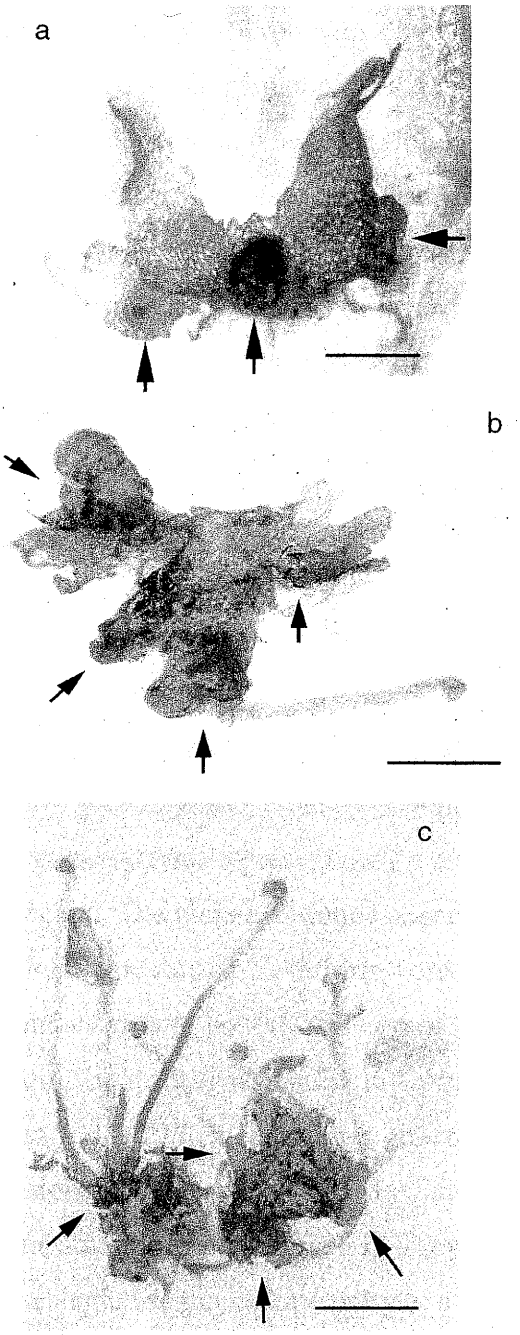

Figure 19: Development of multiple sporophytes from a single gametophyte: (a) gametophyte wings differentiating to form new apical notches and subsequent sporophyte initiation (ElToja, C.R., 3.5 months), (b) subsequent development of sporophytic growth apices (Ixtlan, Mex., 11 months), (c) decay of non-differentiating zones creates multiple tangled but independent sporophytes (Ixtlan, Mex., 11 months). Arrows mark each sporophytic growth apex. Scale bar = 0.5 mm.

were filaments observed to give rise to sporophytes directly, as shown in **Fig. 20a**.) Meanwhile, in some but not all thalloid gametophytes, the growth apex itself also gained 3-dimensional thickness and elongated into an extension reminiscent of a swan neck (**Fig. 20b**) between the two "wings" on either side. The adjacent 3-dimensional proliferation grew and differentiated into a sporophyte shoot and root meristem and produced clathrate scales (a character of *Asplenium* sporophytes) on both surfaces (**Fig. 20c**), fiddleheads from either surface (but more frequently the bottom surface), and roots from the bottom surface. Root initiation occured several weeks after fiddlehead initiation. The first few fiddleheads were completely green but later ones had a light brown base (e.g. **Fig. 21b**). Fiddleheads varied in appearance with no geographical basis to differences. Some were strap-like and differentiated into a broad terminal leaflet (**Fig. 22a**), while others were narrow with a true fiddlehead tip that would unfurl to reveal the first pinnae or terminal leaflet (**Figures 22b and 22c**, respectively).

Spores from each region produced great but similar variability in gametophyte morphology. A sampling of gametophytes from each region are shown for comparison in **Figure 23**, but these few gametophytes represent only a fraction of the diverse growth forms observed in each region. The main difference observed among regions was in the prevalence of the indefinite filamentous growth form versus the immediate thalloid growth form. The filamentous growth pattern was common in Mexican and Costa Rican gametophytes, but was observed only occasionally in Dominican and SEUS gametophytes. Dominican and SEUS gametophytes generally followed traditional thalloid growth patterns, along with about half the Mexican and Costa Rican gametophytes. An unusual structure was observed in a few thalloid Dominican and Costa Rican gametophytes and a single Mexican gametophyte: a toothed raised ridge running along the gametophyte or strap-like fiddlehead (**Fig. 21a and 21b** respectively). The "swan neck" extending from the thallus' growth apex was most common in SEUS gametophytes, but was observed in a few cases in all other regions too. Two SEUS gametophytes, one Mexican gametophyte, and one Dominican gametophyte were observed to have branching fiddlehead-like extensions (**Fig. 24**), but they died before

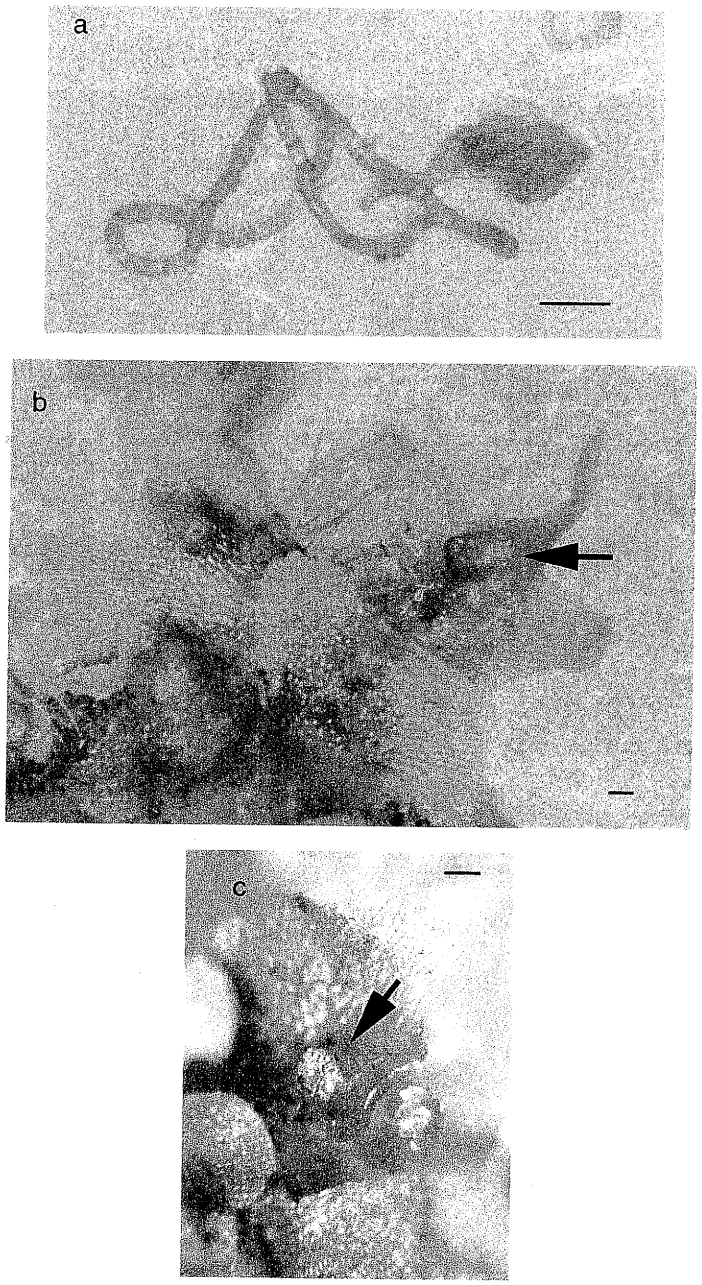

Figure 20: Sporophyte initation: (a) bulbous sporphytic growth apex developing directly on branch of filamentous gametophyte (km87, C.R., 6.5 weeks), (b) cordate gametophyte with "swan neck" extension and adjacent bulge (see arrow) of sporophytic growth apex (Coley, SC, 13.5 weeks), (c) subsequent clathrate scales covering sporophytic growth apex (see arrow) as first fiddlehead emerges from bottom side (at right) (Coley, SC, 13.5 weeks). Scale bar = 0.1 mm.

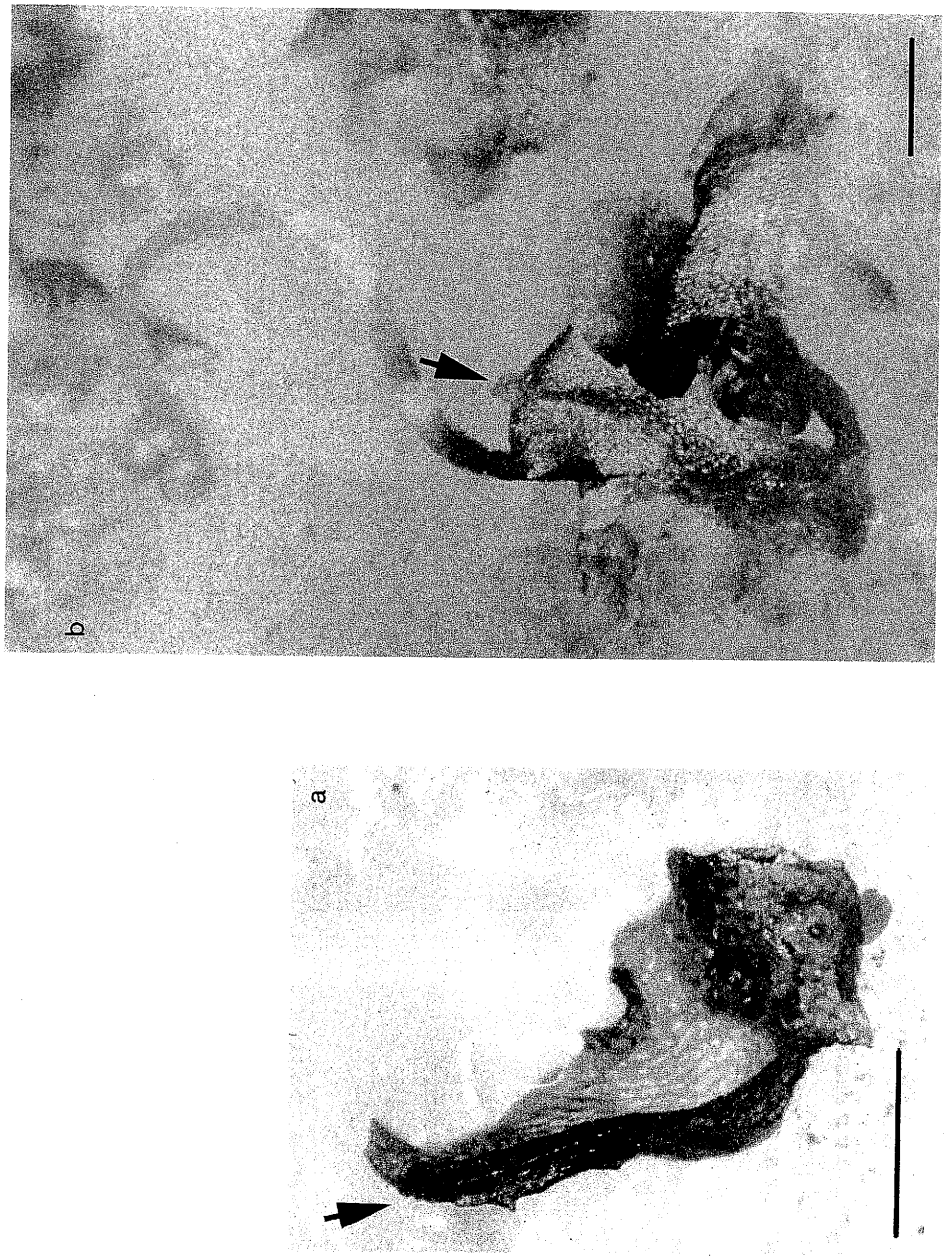

Figure 21: Toothed ridges (see arrows) in: (a) a gametophyte (LosArroyos, D.R., 11.5 weeks), (b) a strap-like sporophytic extension (RojasMonanthes, Mex., 3.5 months). Note also the darkening of the base of the fiddlehead shown in photo (b) to the characteristic brown rachis color of *A. monanthes*. Scale bar = 0.5 mm.

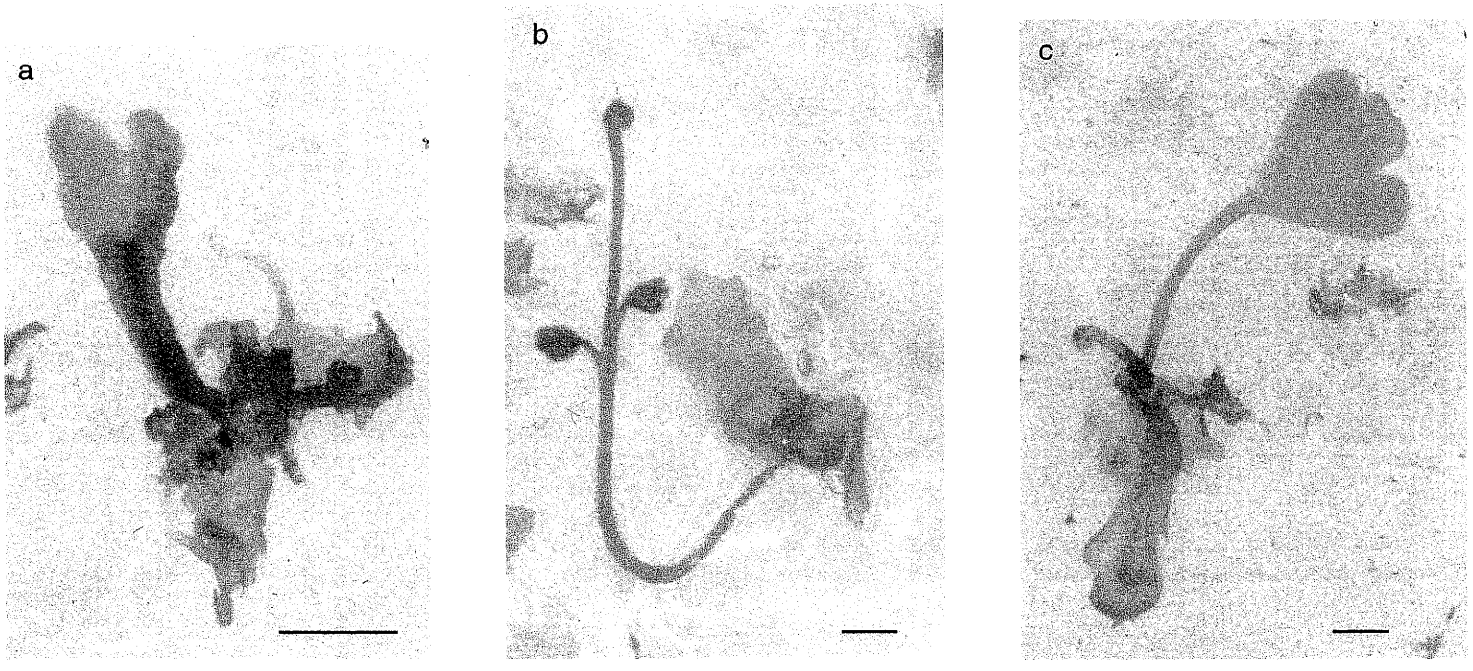

Figure 22: Variations in sporophytes' first fiddleheads: (a) strap-like extension broadening into a leaf (Guess, AL, 5.5 months), (b) fiddlehead with first pinnae (Ixtlan, Mex., 3.5 months), (c) fiddlehead broadening into leaf (LosArroyos, D.R., 3.5 months). Scale bar = 0.5 mm.

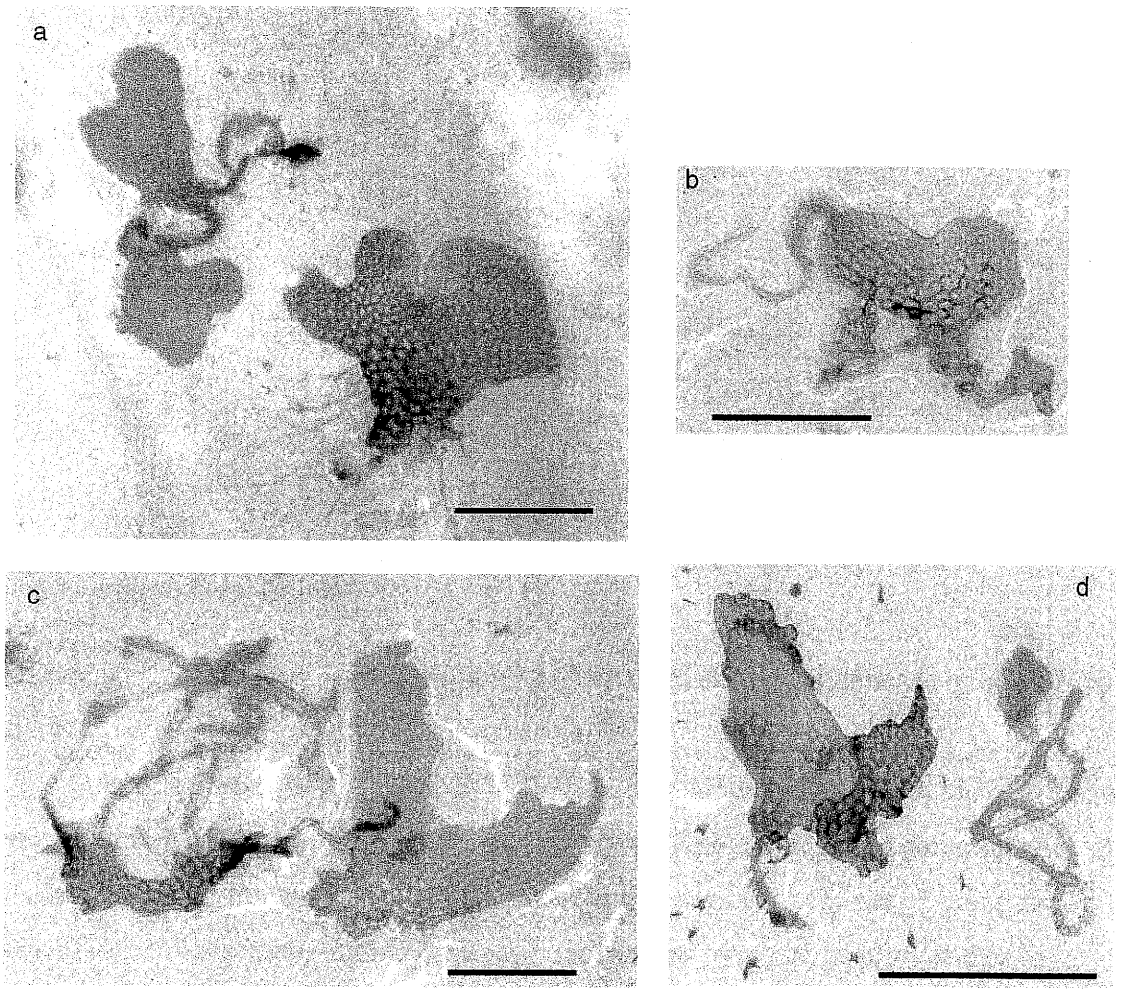

Figure 23: Comparison of select gametophytes from the four regions studied: (a) southeastern U.S. (Cane, SC, 9.5 weeks), (b) Dominican Republic (ValleNuevo, 9 weeks), (c) Mexico (RojasMonanthes, 6.5 weeks), (d) Costa Rica (km87, 6.5 weeks). Scale bar = 0.5 mm.

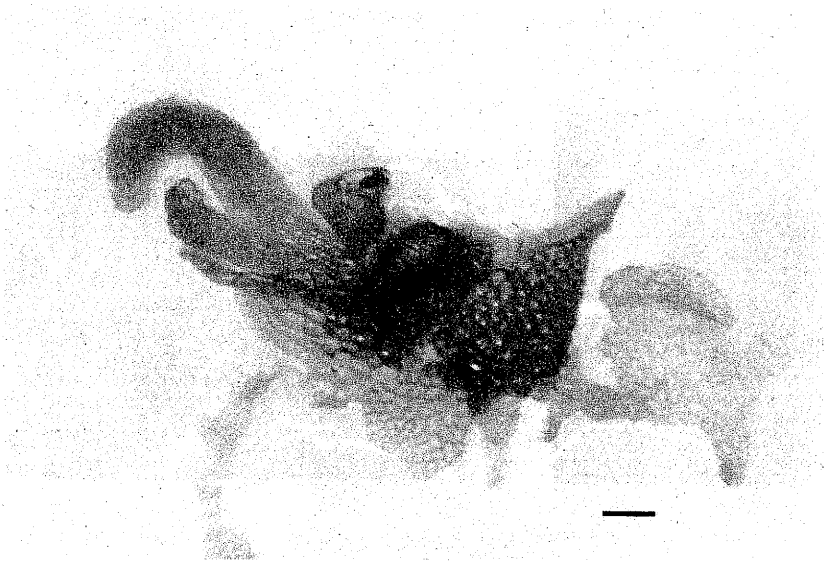

Figure 24: Branching of developing fiddlehead (Guess, AL, 5.5 months). Scale bar = 0.1 mm.

differentiating enough to determine whether they were indeed fiddleheads or something novel.

Gametophytes were extremely variable in developmental rate (but see **Fig. 16** for average timing of various developmental events), with no one region's gametophytes as a whole developing any faster or slower than any other regions'. Gametophytic growth rates of *A. heteroresiliens*, a closely related apogamous fern, and *A. platyneuron*, a somewhat closely related sexual fern, were compared to that of *A. monanthes* and were found to be slightly faster. *A. heteroresiliens* produced its first sporophytic bulge at 9 weeks after sowing and *A. platyneuron* produced its first archegonia at 6.5 weeks, while *A. monanthes* gametophytes ranged from 6.5 to 15 weeks old at first sporophytic bulge production. This shows an exception to Whittier's finding (1970) that asexual fern gametophytes usually reach maturity faster than congeneric sexual gametophytes.

It should be noted that *A. monanthes*' gametophyte developmental pathways, although unusual, are not unique. The closely related apogamous species *A. resiliens* and *A. heteroresiliens* also produced extended filaments (**Fig. 25a and 25b**, respectively) which might lead to mulitple thalli, but more often these two species followed the standard *Adiantum* developmental pathway of a single cordate thallus initiated promptly.

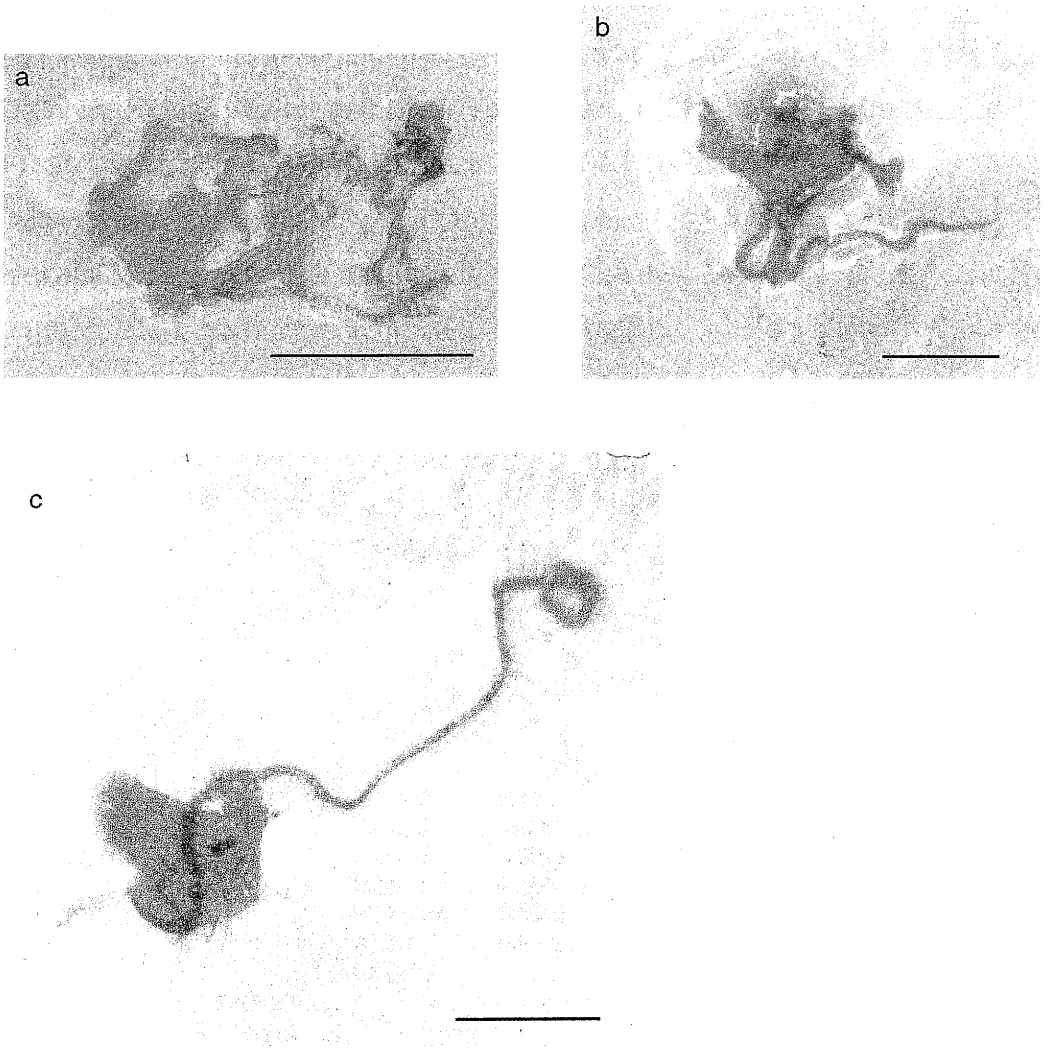

Figure 25: Similar filamentous development in gametophytes of (a) *A. resiliens* (8 weeks), (b) *A. heteroresiliens* (7 weeks), and (c) *A. monanthes* (Cane, SC, 9.5 weeks). Scale bar = 0.5 mm.

Starch gel electrophoresis

Geographic distribution of alleles and phenotypic patterns

To supplement the quantitative analyses discussed below, the allelic composition of the different regions was compared. A Venn diagram **(Fig. 26)** of the four regions was plotted from the ten polymorphic loci (i.e. all but PGI-1 and MDH-2) to show to what extent each region shared alleles or uninterpretable phenotypic patterns with other regions to elucidate current or historical migration patterns. The two regions found to share the most alleles/patterns were Mexico and Costa Rica, with six alleles/patterns

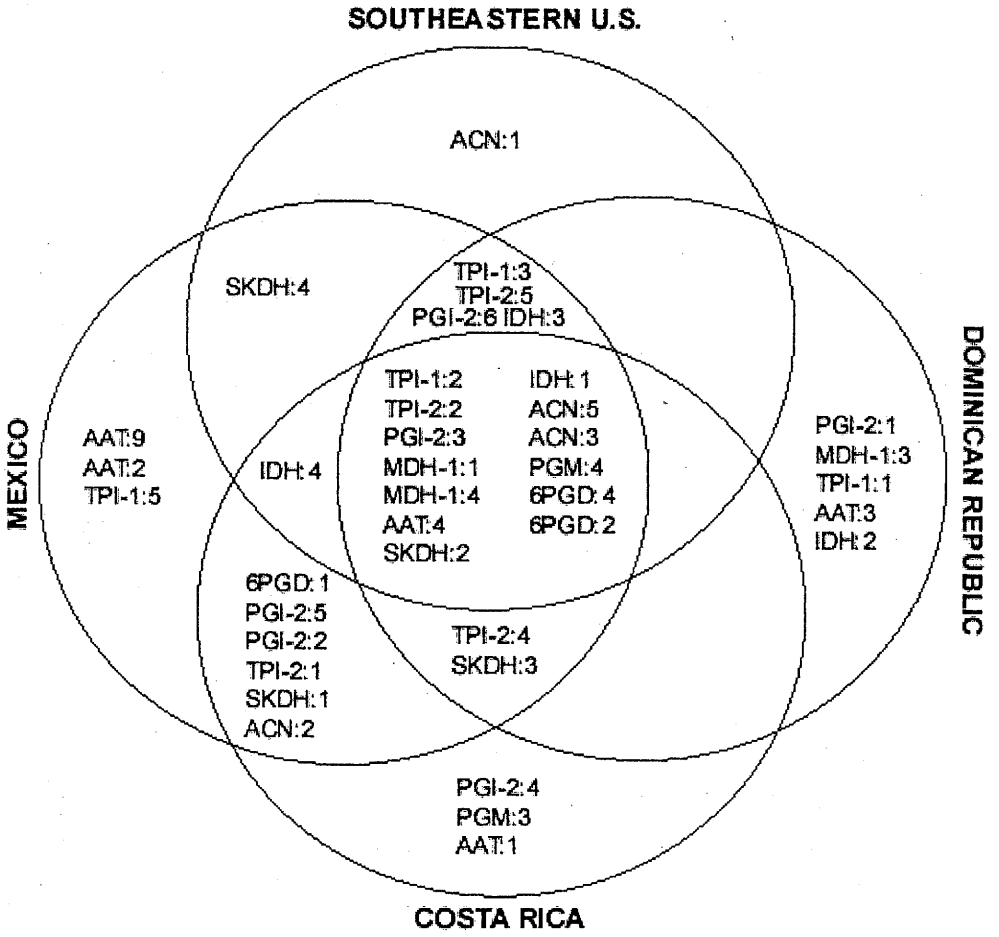

Figure 26: Assignment of each allele or phenotypic pattern of *A. monanthes* to regions to show patterns of similarity among regions. Entries for loci AAT, ACN, and IDH represent uninterpretable phenotypic patterns; all other entries represent alleles. Invariant loci (PGI-1, MDH-2) not pictured.

exclusive to these two regions. Mexico had the greatest diversity of alleles/patterns: 30 out of 39 total (vs. 25 in Costa Rica, 24 in the Dominican Republic, and 20 in the southeastern U.S.) occur in Mexico. The remaining nine alleles/patterns were private to each of the other three regions. Private alleles (at the population or region level) were greatest in the Dominican Republic, with five, and fewest in the southeastern U.S., with only one. The southeastern U.S. had the fewest alleles/patterns in common with Costa Rica and the most with Mexico (two more than with the Dominican Republic).

Quantitative estimates of genetic diversity and differentiation

Table 3 lists various estimates of genetic diversity in different regions of *A. monanthes*' range and pooled for comparisons with various plant groups reviewed in the literature. The data showed notably different values for different regions. Mexico contained sizable genetic diversity within populations as measured by all parameters utilized. Of the remaining three regions, the southeastern U.S. had no within-population genetic diversity (unless fixed heterozygosity is incorporated as in % loci polymorphic and alleles per locus) while the Dominican Republic and Costa Rica had intermediate levels of within-population genetic diversity based on all four parameters.

Likewise, genetic differentiation was estimated using various parameters (**Table 4**), both within each region and overall. The southeastern U.S. displayed the least among-population genetic differentiation for all parameters estimated and Costa Rica the most, while Mexico and the Dominican Republic displayed intermediate values. Among-region genetic differentiation yielded an overall value of Grt = 0.18. Pairwise Grt values were low between the Dominican Republic and Mexico (0.15), the Dominican Republic and the southeastern U.S. (0.18), and Mexico and Costa Rica (0.17) and higher for the remaining comparisons (e.g. 0.23 between Mexico and the southeastern U.S.). Permutation tests showed all G-statistic values to be significantly different from zero, so genetic differentiation has occurred at both the population and regional levels. Slatkin's (1985) private allele method of Nm estimation obtained a value of 0.41 inter-regional migrants per generation, whereas the standard Gst-based derivation of Nm produced a much larger estimate, 1.14 inter-regional migrants per generation. There was a statistically significant logarithmic pattern of isolation by distance in the dataset as a

Table 3: Genetic diversity in *Asplenium monanthes* in comparison with similar plants

		species-level genetic diversity			*within-population genetic diversity*			
reviewed in:	taxa	% loci diagnostic*	% loci polymorphic	mean alleles/ locus	mean % loci polymorphic/ pop.	mean alleles/ locus/ pop.	% pops. polymorphic	mean genotypes/ pop.
	SEUS *A. monanthes*				54	1.12	0	1
	Dominican *A. monanthes*				56	1.54	25	1.5
	Mexican *A. monanthes*				63	1.71	90	4.1
	Costa Rican *A. monanthes*				55	1.62	40	1.8
	overall *A. monanthes*	83	89	3.2	57	1.61	57	2.6
Table 5	apogamous plants	50.3					62.2	6.7
Table 6	all ferns		50	1.9	38	1.5		
Hamrick & Godt, 1989	long-lived herbaceous perennial seed plants		40	1.4	39	1.44		

** % of loci with varying genotypes, in contrast to % loci polymorphic which includes fixed heterozygosity*

Table 4: Genetic differentiation in *A. monanthes* in comparison with similar plants

reviewed in:	taxa	pairwise Grt w/ Dom. Rep.	pairwise Grt w/ Mexico	pairwise Grt w/ Costa Rica	overall Grt[a]	Gsr[a]	Gst[a]	Nm from G-statistics	Slatkin's Nm	% genotypes local[c]	% genotypes widespread[d]	isolation by distance slope[e]	isolation by distance r^{2} [e]
	SEUS *A. monanthes*	0.18	0.23	0.36		0.13		1.62		66	0		
	Dominican *A. monanthes*		0.15	0.20		0.25		0.74		75	0		
	Mexican *A. monanthes*			0.17		0.24		0.42		92	0	0.004	0.019
	Costa Rican *A. monanthes*					0.38		0.81		40	0		
	overall *A. monanthes*				0.18		0.34	1.14[b]	0.41[b]	80	0	0.011	0.037
Table 5	apogamous plants									56.7	26.2		
Table 6	all ferns						0.12						
Hamrick & Godt, 1989	long-lived herbaceous perennial seed plants						0.21						

[a] *All values for A. monanthes highly significant based on 1000 permutations. (Significance not tested for pairwise Grt values.)*
[b] *Estimate of inter-regional migration*
[c] *% of genotypes private to a single population*
[d] *% of genotypes found in over 75% of populations*
[e] *From logarithmic regression of Nei's genetic distance on geographic distance. Slope statistically significant for* A. monanthes *overall but not within Mexico.*

whole but not within Mexico (the other regions were not tested alone). However even the pattern of isolation by distance of the dataset as a whole is probably not biologically significant. The slope was only 0.011 and the r-squared value only 0.037, so it does not appear that A. monanthes has developed a strong pattern of isolation by distance at the scales tested.

A. monanthes values from all regions were pooled and compared to those from other apogamous plants and other ferns, reviewed in **Tables 5 and 6** respectively. In comparison with these two groups and all seed plants, *A. monanthes* exhibited much greater genetic diversity (**Table 3**) than the mean of any group compared at the species level, as measured by species-level % loci diagnostic, % loci polymorphic, and mean alleles per locus. *A. monanthes* had somewhat higher within-population genetic diversity than all ferns and all seed plants. However the parameters used (% loci polymorphic and alleles per locus) incorporate fixed heterozygosity (generally high in an allopolyploid like *A. monanthes*) and do not reveal the fact that *A. monanthes* populations probably contain only a fraction of the number of genotypes found in the predominantly sexual comparison groups. *A. monanthes* can more fairly be compared to other apogamous taxa, and it showed a comparable percentage of populations polymorphic but fewer genotypes per population than other apogamous taxa. *A. monanthes* exhibited greater genetic differentiation (**Table 4**) among populations than the three plant groups compared, as measured by % genotypes local vs. widespread and Gst.

Allele frequency analysis of populations

The allele frequency PCO plot of populations including outgroups (**Fig. 27**) showed the genetic cohesiveness of *Asplenium monanthes s.l.* (i.e. *A. monanthes* and *A. hallbergii*) relative to closely related taxa. Refer to **Table 2** for a key to population code names. When the analysis was repeated with outgroups excluded (**Fig. 28**) for greater resolution of *A. monanthes s.l.,* reasonable clustering of populations by region was found except for significant overlap between the Dominican and SEUS clusters. Notably the Alabama populations, Guess and Neversink, are closer to the Dominican populations Caseta 2 and Los Arroyos and the Mexican population Ixtlan than to the Carolina populations, which are relatively isolated from all other populations.

Table 5: Literature review of genetic diversity and differentiation in apogamous plants

		species-level genetic diversity	*within-population genetic diversity*		*genetic differentiation*		
referenced in:	species	species-level % loci diagnostic*	% populations polymorphic	mean genotypes/ pop.	% genotypes local	% genotypes widespread	mean pops./ genotype
Ellstrand & Roose, 1987; Widen et al., 1994	*Taraxacum obliquum*	0	0	1.0	0	100	2.0
Ellstrand & Roose, 1987, Widen et al., 1994	*Agrostis stolonifera*	10	100	10.2	98	0	1.1
Widen et al., 1994	*Taraxacum hollandicum*	13	0.1	1.4	75	25	2.5
Ellstrand & Roose, 1987; Widen et al., 1994	*Taraxacum officinale*	33	97	9.6	33	39	2.4
Ellstrand & Roose, 1987, Widen et al., 1994	*Pellaea andromedifolia*	63	0	1.0	50	0	1.5
Ellstrand & Roose, 1987; Widen et al., 1994	*Taraxacum tortilobum*	71	90	3.3	53	13	4.3
Ellstrand & Roose, 1987; Widen et al., 1994	*Erigeron annuus*	75	100	14.0	12	59	2.4
Widen et al., 1994	*Taraxacum vindobonense*	88	100	16.0	100	0	1.0
Widen et al., 1994	*Antennaria rosea*	100	73	3.5	89	0	1.1
	mean	**50.3**	**62.2**	**6.7**	**56.7**	**26.2**	**2.0**

**% of loci variable among individuals*

Table 6: Literature review of genetic diversity and differentiation in ferns

		overall genetic diversity		*within-pop. genetic diversity*		*gen. differentiation*	
		species-level % poly-morphic loci	species-level alleles/locus	pop.-level % polymorphic loci	pop.- level alleles/locus	Gst	Nm
Watano & Sahashi, 1992	*Botrychium trianglarifolium*	0	1.0	0			
reviewed in Ranker et al., 2000	*Bommeria ehrenbergiana*	21	1.4				
reviewed in Ranker et al., 2000	*Botrychium virginianum*	22	1.6	15	1.3	0.08	
reviewed in Ranker et al., 2000	*Blechnum spicant*	24	1.4	24	1.4	0.07	
Watano & Sahashi, 1992	*Botrychium nipponicum*	33	1.4				
reviewed in Ranker et al., 2000	*Bommeria elegans*	33	1.4				
reviewed in Ranker et al., 2000	*Bommeria subpaleacea*	39	1.5				
Farrar, 1990	*Vittaria appalachiana*	42		2			
Haufler, 1985	*Bommeria pedata*	46	1.6				
Watano & Sahashi, 1992	*Botrychium multifidum*	56	2.0				
Watano & Sahashi, 1992	*Botrychium ternatum*	56	2.1			0.19	
Chiou et al., 1998	*Elaphoglossum alatum*	56	1.8				
reviewed in Ranker et al., 2000	*Bommeria hispida*	62	2.6				
reviewed in Ranker et al., 2000	*Pteridium aquilinum*	62		35	1.5		
Chiou et al., 1998	*Elaphoglossum crassifolium*	64	1.8				
reviewed in Ranker et al., 2000	*Pellaea andromedifolia*	71	2.1	64			
reviewed in Ranker et al., 2000	*Sadleria pallida*	80	2.8	36	1.6		
reviewed in Ranker et al., 2000	*Sadleria cyatheoides*	86	2.9	43	1.6		
reviewed in Ranker et al., 2000	*Odontosoria chinensis*	94	3.5	27	1.4	0.02	13.1
Suter et al., 2000	*Asplenium trichomanes quadrivalens*			8	1.1		

Table 6 (continued)

		overall genetic diversity		*within-pop. genetic diversity*		*gen. differentiation*	
		species-level % poly-morphic loci	species-level alleles/locus	pop.-level % polymorphic loci	pop.- level alleles/locus	Gst	Nm
reviewed in Ranker et al., 2000	*Dryopteris expansa*			10	1.1	0.21	
reviewed in Ranker et al., 2000	*Asplenium rhizophyllum*			13	1.1		
reviewed in Ranker et al., 2000	*Hemionitis palmata*			19	1.2	0.70	0.1
reviewed in Ranker et al., 2000	*Grammitis hookeri*			26	1.5		
reviewed in Ranker et al., 2000	*Asplenium montanum*			27	1.3		
reviewed in Ranker et al., 2000	*Asplenium platyneuron*			27	1.5		
reviewed in Ranker et al., 2000	*Adenophorus tripinnatifidus*			31	1.4		
reviewed in Ranker et al., 2000	*Grammitis tenella*			39	1.6		
reviewed in Ranker et al., 2000	*Polystichum munitum*			54	2.2	0.05	4.2
Hooper & Haufler, 1997	*Pleopeltis polylepis* var. *erythrolepis*			55	2.0	0.09	2.6
reviewed in Ranker et al., 2000	*Adenophorus tamariscinus*			55	2.2		
reviewed in Ranker et al., 2000	*Polystichum imbricans*			57		0.02	
Hooper & Haufler, 1997	*Pleopeltis wiesbaurii*			58	2.1	0.03	7.6
Hooper & Haufler, 1997	*Pleopeltis complanata*			58	2.3	0.04	6.2
reviewed in Ranker et al., 2000	*Gymnocarpium dryopteris disjunctum*			59	1.8	0.11	
reviewed in Ranker et al., 2000	*Cheilanthes subcordata*			65	1.9		
Hooper & Haufler, 1997	*Pleopeltis polylepis* var. *polylepis*			65	2.4	0.07	3.4
Hooper & Haufler, 1997	*Pleopeltis astrolepis*			68	2.2	0.02	11.6
Hooper & Haufler, 1997	*Pleopeltis crassinervata*			69	2.7	0.04	6.9
	mean	**50**	**1.9**	**38**	**1.5**	**0.12**	**6.2**

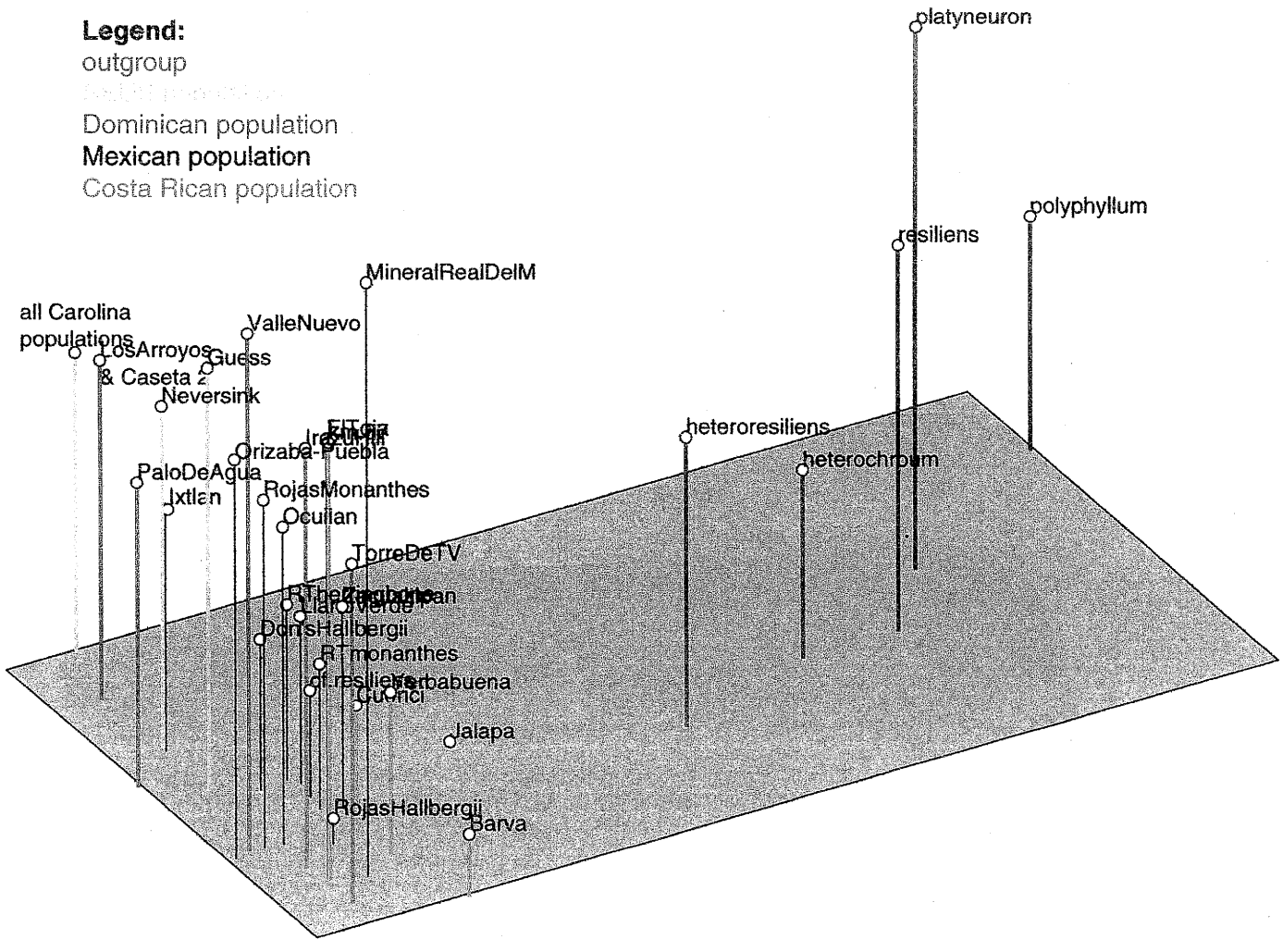

Figure 27: Principle Coordinates Analysis of populations based on allele frequencies. Outgroups included.

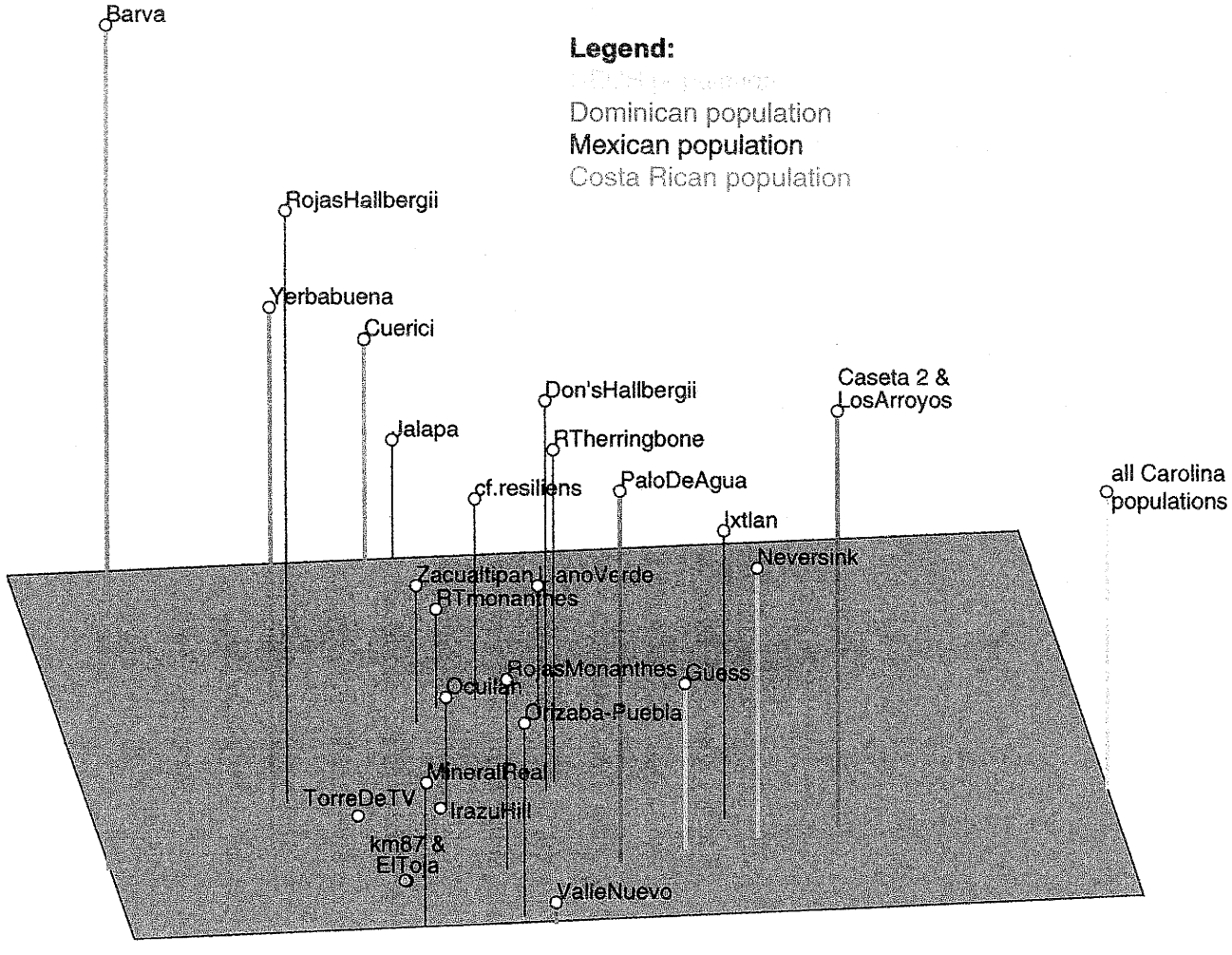

Figure 28: Principle Coordinates Analysis of populations based on allele frequencies. Outgroups excluded.

The neighbor joining allele frequency analysis (**Fig. 29**) showed very little clustering by geographic origin. Costa Rican populations are scattered throughout the tree and Dominican populations are scattered throughout the terminal clusters. As in the PCO analysis, Neversink clusters with Caseta 2, Los Arroyos, and Ixtlan, but this time the Carolinas rather than Guess join this cluster. Guess instead clusters with the Dominican population Valle Nuevo and some other Mexican and Costa Rican populations. Poor bootstrap support for every split in the tree, even those separating outgroup taxa, indicates that this tree is not very robust and its groupings may be uninformative.

Presence/absence comparison of genotypes

The PCO results of the genotype-based allele presence/absence analysis including outgroups (**Fig. 30**) again resulted in *A. monanthes s.l.* samples clustering together separate from the outgroups. **Table 7** lists the population(s) each genotype is found in, allowing attempts at comparison with the results from the population-based allele frequency analyses. (**Table 8** lists the actual allelic content of each genotype). **Figure 31** shows the results of the genotype-based allele presence/absence analysis repeated without outgroups. Regional cohesion was somewhat weaker in this analysis than in the allele frequency analysis discussed above; Costa Rican and Mexican genotypes are scattered thoughout multidimensional genetic space, but the SEUS and Dominican genotypes still form a cluster together. Genotype G (the Carolinas) appears most similar to J (various Dominican populations). Genotype E (Guess) is most similar to various Mexican genotypes and EEE (genotype from misc. Costa Rican populations), and somewhat similar to D (Neversink) and I (Valle Nuevo). Genotype D (Neversink) is most similar to various Mexican genotypes and somewhat similar to E (Guess) and EEE (misc. Costa Rican populations), among others.

The NTSYS-pc-derived neighbor joining presence/absence analysis tree is shown in **Fig. 32**. The PHYLIP-derived tree showing branch lengths, rooted according to the NTSYS-pc tree which included outgroups, is shown in **Fig. 33**. The clusters and their placement are somewhat concordant with the allele frequency population-based tree (**Fig. 29**) discussed above, particularly within the clusters containing SEUS genotypes. The

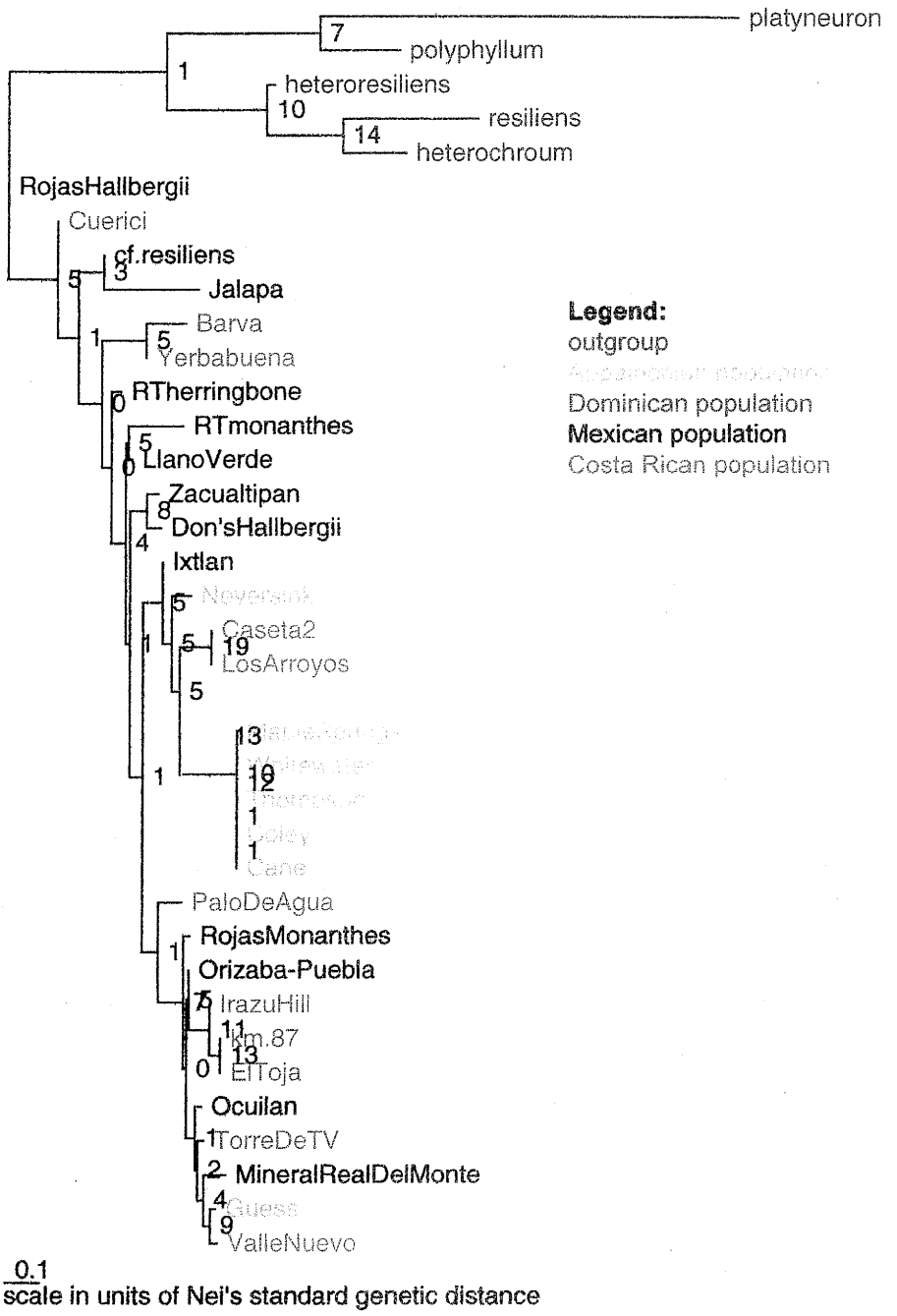

Figure 29: Neighbor Joining tree based on population allele frequencies utilizing Nei's standard genetic distance; Numbers indicate bootstrap support for each node based on 1000 bootstrap replicates.

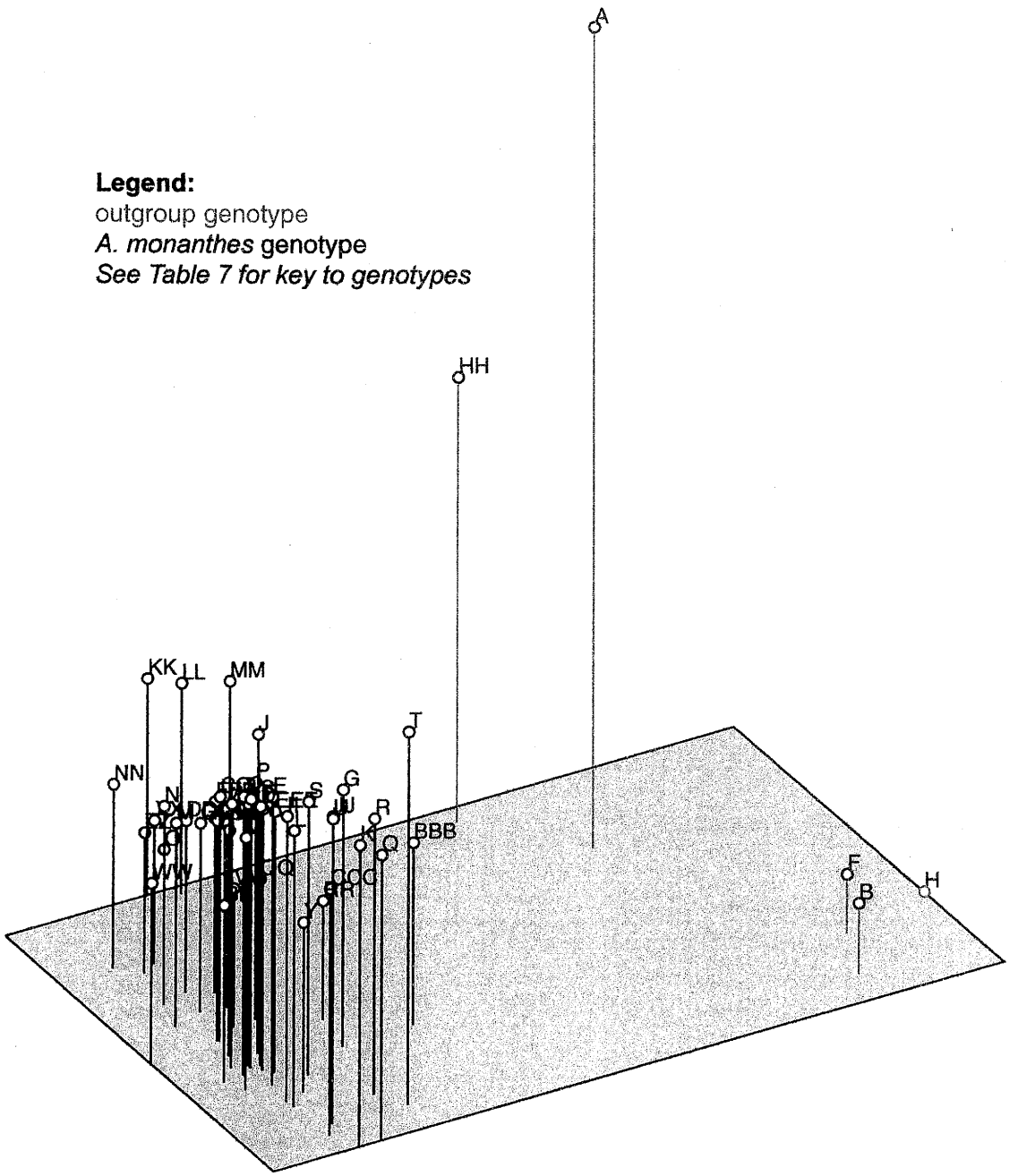

Figure 30: Principle Coordinates Analysis of genotypes based on presense/absence of alleles. Outgroups included.

Table 7: Alphabetical key to genotype codes used in Principle Components Analysis plots and Neighbor Joining trees. Bold type indicates that genotype is from an outgroup.

genotype	population(s) containing genotype	region (species if outgroup)
A	**platyneuron**	***A. platyneuron***
B	**heteroresiliens**	***A. heteroresiliens***
D	Neversink	Appalachians
E	Guess	Appalachians
F	**resiliens**	***A. resiliens***
G	Whitew ater, Thompson, Coley, Cane, MapleSprings	Appalachians
H	**heterochroum**	***A. heterochroum***
I	ValleNuevo	Dominican Republic
J	PaloDeAgua, Caseta2, Los Arroyos	Dominican Republic
K	PaloDeAgua	Dominican Republic
L	PaloDeAgua	Dominican Republic
M	Zacualtipan	Mexico
N	Zacualtipan	Mexico
O	Zacualtipan	Mexico
P	Zacualtipan	Mexico
Q	MineralRealDelMonte	Mexico
R	MineralRealDelMonte	Mexico
S	MineralRealDelMonte	Mexico
T	MineralRealDelMonte	Mexico
U	MineralRealDelMonte	Mexico
V	RojasMonanthes	Mexico
X	RojasMonanthes, Ocuilan	Mexico
Y	RojasMonanthes	Mexico
Z	RojasMonanthes	Mexico
AA	RojasMonanthes	Mexico
BB	RojasMonanthes	Mexico
CC	RojasHallbergii	Mexico
DD	cf.resiliens	Mexico
FF	Ocuilan	Mexico
GG	Ocuilan	Mexico
HH	**polyphyllum**	***A. polyphyllum***
II	Orizaba-Puebla, Ixtlan	Mexico
JJ	Orizaba-Puebla	Mexico
KK	Jalapa	Mexico
LL	Jalapa	Mexico
MM	Jalapa	Mexico
NN	Jalapa	Mexico
OO	Don'sHallbergii	Mexico
PP	Ixtlan, RTherringbone	Mexico
QQ	Ixtlan, RTherringbone	Mexico
RR	RTmonanthes, Llano Verde	Mexico
SS	RTherringbone	Mexico
TT	RTherringbone	Mexico
UU	LlanoVerde	Mexico
VV	LlanoVerde	Mexico
WW	LlanoVerde	Mexico
XX	LlanoVerde	Mexico
ZZ	LlanoVerde	Mexico
AAA	LlanoVerde	Mexico
BBB	Barva, Yerbabuena	Costa Rica
CCC	Barva, IrazuHill, Yerbabuena	Costa Rica
DDD	IrazuHill	Costa Rica
EEE	IrazuHill, km.87, TorreDeTV, ElToja	Costa Rica
FFF	Cuerici	Costa Rica

Table 8: Multilocus isozyme genotypes encountered in each population

population*	geno-type code	N	TPI-2	TPI-1	PGI-2	PGI-1	6PGD	MDH-2	MDH-1	PGM-1	ACN (pseudoalleles)†	AAT (pseudoalleles)†	IDH (pseudoalleles)†	SKDH
outgroups:														
platyneuron	**A**	**1**	**22**	**55**	**77**	**11**	**11**	**12**	**55**	**12**	**444**	**101010**	**55**	**55**
heterochroum**	**H**	**1**	**24**	**234**	**225**	**333**	**311**	**---**	**44**	**555**	**---**	**777**	**999**	**444**
heteroresiliens**	**B**	**1**	**24**	**234**	**73**	**333**	**311**	**---**	**444**	**---**	**555**	**555**	**777**	**---**
resiliens	**F**	**1**	**444**	**34**	**723**	**333**	**111**	**---**	**444**	**111**	**---**	**666**	**888**	**444**
polyphyllum**	**HH**	**1**	**---**	**555**	**222**	**222**	**111**	**25**	**111**	**---**	**222**	**888**	**666**	**444**
SEUS:														
Neversink (AL)	D	7	222	23	336	222	222	334	114	444	555	444	444	222
Guess (AL)	E	6	225	23	336	222	222	334	144	444	333	444	111	222
Whitewater (NC)	G	1	222	23	336	222	422	334	111	444	111	444	333	224
Thompson (SC)	G	1	222	23	336	222	422	334	111	444	111	444	333	224
Coley (SC)	G	1	222	23	336	222	422	334	111	444	111	444	333	224
Cane (SC)	G	9	222	23	336	222	422	334	111	444	111	444	333	224
MapleSprings (NC)	G	8	222	23	336	222	422	334	111	444	111	444	333	224
Dominican Republic:														
ValleNuevo	I	10	25	123	333	222	222	334	444	444	333	444	111	222
PaloDeAgua	J	5	24	23	36	222	222	334	---	444	555	444	333	222
	K	5	24	222	133	222	422	334	444	444	333	333	222	23
	L	1	24	222	133	222	422	334	---	444	555	333	333	23
Caseta2	J	6	24	23	36	222	222	334	13	444	555	444	333	222
LosArroyos	J	7	24	23	36	222	222	334	13	444	555	444	333	222
Mexico:														
Zacualtipan	N	1	112	---	555	222	422	334	144	---	555	444	111	233
	O	1	112	---	235	222	422	334	144	---	555	444	111	233
	M	2	---	222	---	222	422	334	114	---	555	444	111	133
	P	1	224	---	233	222	222	334	111	---	---	444	111	222
MineralReal DelMonte	Q	1	255	23	333	222	222	334	14	---	333	999	---	---
	R	1	444	222	35	222	222	334	444	---	333	999	111	222
	S	1	444	222	333	222	222	334	444	---	333	444	111	222
	T	1	555	222	333	222	222	334	444	---	333	999	111	222
	U	1	255	222	333	222	222	334	444	---	333	444	---	222
RojasMonanthes	V	1	25	222	333	222	---	334	444	---	---	444	444	112
	X	1	222	222	35	222	222	334	444	444	333	444	111	12
	Y	1	244	222	335	222	---	334	114	444	333	---	111	---
	Z	1	12	222	333	222	222	334	14	444	333	444	333	---
	AA	1	12	222	35	222	222	334	14	444	333	444	333	---
	BB	1	224	222	333	222	---	334	144	444	---	444	---	123
RojasHallbergii	CC	1	244	222	---	222	111	334	144	444	555	444	---	13
cf.resiliens	DD	1	222	23	35	222	---	334	144	444	222	444	111	133
Ocuilan	FF	2	122	222	333	222	422	334	444	444	333	444	---	222
	X	1	222	222	355	222	222	334	444	444	333	444	---	12
	GG	1	122	222	223	222	422	334	444	---	555	---	111	---
Orizaba-Puebla	II	2	224	222	333	222	222	334	144	---	333	444	---	122
	JJ	1	255	222	333	222	---	334	444	444	333	444	---	112

Table 8 (continued)

population*	genotype code	N	TPI-2	TPI-1	PGI-2	PGI-1	6PGD	MDH-2	MDH-1	PGM-1	ACON (pseudoalleles)†	AAT (pseudoalleles)†	IDH (pseudoalleles)†	SKDH
Jalapa	KK	1	111	555	25	222	---	334	111	444	222	444	111	333
	LL	1	122	555	555	222	311	334	---	444	222	444	111	333
	MM	1	122	555	---	222	111	334	---	444	222	444	---	333
	NN	1	111	555	---	222	---	334	114	444	222	444	---	333
Don'sHallbergii	OO	1	244	222	356	222	422	334	14	444	555	444	---	13
Ixtlan	PP	12	222	23	333	222	422	334	---	444	---	444	---	23
	II	2	224	222	---	222	222	334	---	444	333	444	---	122
	QQ	1	224	23	233	222	422	334	---	444	555	444	---	133
RTmonanthes	RR	3	122	23	235	222	422	334	444	444	555	222	111	---
RTherringbone	SS	3	222	23	333	222	---	334	144	444	555	444	111	13
	TT	2	124	23	235	222	111	334	144	444	555	444	111	133
	QQ	3	224	23	233	222	---	334	---	444	555	444	---	13
	PP	5	222	23	333	222	422	334	114	444	555	444	444	23
LlanoVerde	UU	7	122	23	333	222	422	334	---	444	222	444	111	234
	VV	1	122	23	235	222	422	334	444	444	555	444	---	333
	WW(a)	1	111	---	355	222	422	334	---	444	---	444	---	233
	XX	2	122	---	225	222	---	334	144	444	555	444	111	233
	WW(b)	1	112	---	355	222	---	334	444	444	555	444	111	233
	RR	2	122	23	235	222	422	334	444	444	555	222	111	333
	ZZ	1	112	---	235	222	---	334	114	444	222	444	111	233
	AAA	1	122	---	235	222	111	334	444	444	555	444	111	133
Costa Rica:														
Barva	BBB(a)	4	444	222	34	222	111	334	444	444	555	111	111	333
	BBB(b)	6	444	222	333	222	111	334	444	444	555	111	111	333
	CCC(a)	1	222	222	333	222	222	334	14	444	333	---	444	12
IrazuHill	DDD	1	244	222	233	222	422	334	244	444	---	444	111	---
	EEE(b)	5	124	222	335	222	222	334	444	344	333	444	444	222
	CCC(a)	1	222	222	333	222	222	334	14	444	333	---	---	12
Yerbabuena	CCC(b)	1	222	222	333	222	222	334	14	444	222	---	---	12
	BBB(b)	1	444	222	333	222	111	334	444	444	555	111	111	333
km.87	EEE(b)	9	124	222	335	222	222	334	444	344	333	444	444	222
TorreDeTV	EEE(a)	1	124	222	335	222	222	334	444	344	333	444	111	---
ElToja	EEE(b)	12	124	222	335	222	222	334	444	344	333	444	444	222
	EEE(c)	1	124	222	333	222	222	334	444	---	333	444	444	---
Cuerici	FFF	1	222	222	23	222	111	334	14	444	---	444	111	133

Note: "---" represents missing data.

† Scoring as "pseudoalleles" means that the allelic basis for this enzyme could not be determined. These enzymes had complex banding patterns involving multiple overlapping loci that could not be differentiated. Therefore each observed banding pattern was given a number which was treated in any genetic analyses as homozygous for a fictional allele of that number.

**see Table A for details of populations' locations*

***These taxa have more than three genomes but were scored as triploids to include as many alleles as possible yet accommodate population genetics software requiring all samples to have the same ploidy.*

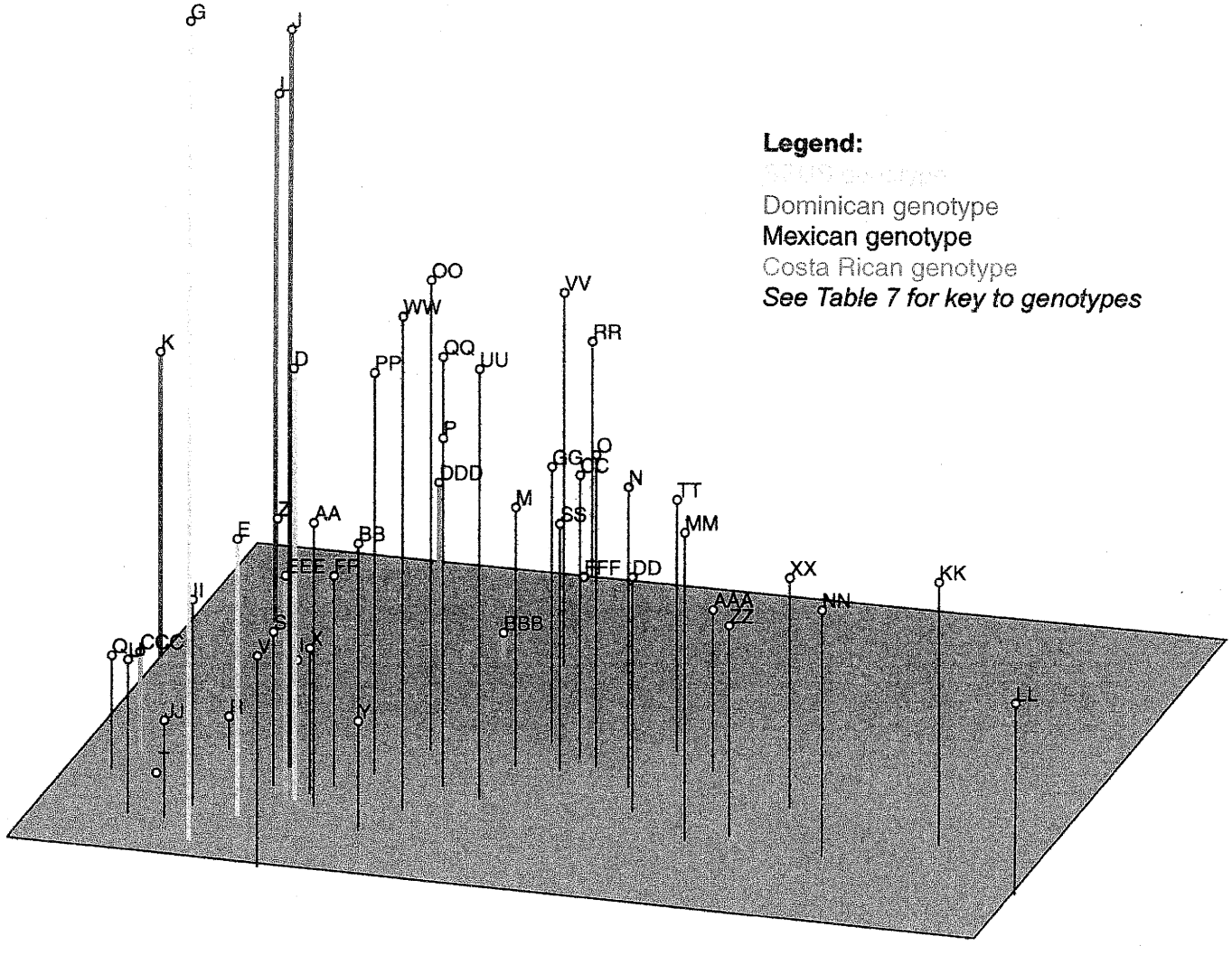

Figure 31: Principle Coordinates Analysis of genotypes based on presense/absense of alleles. Outgroups excluded.

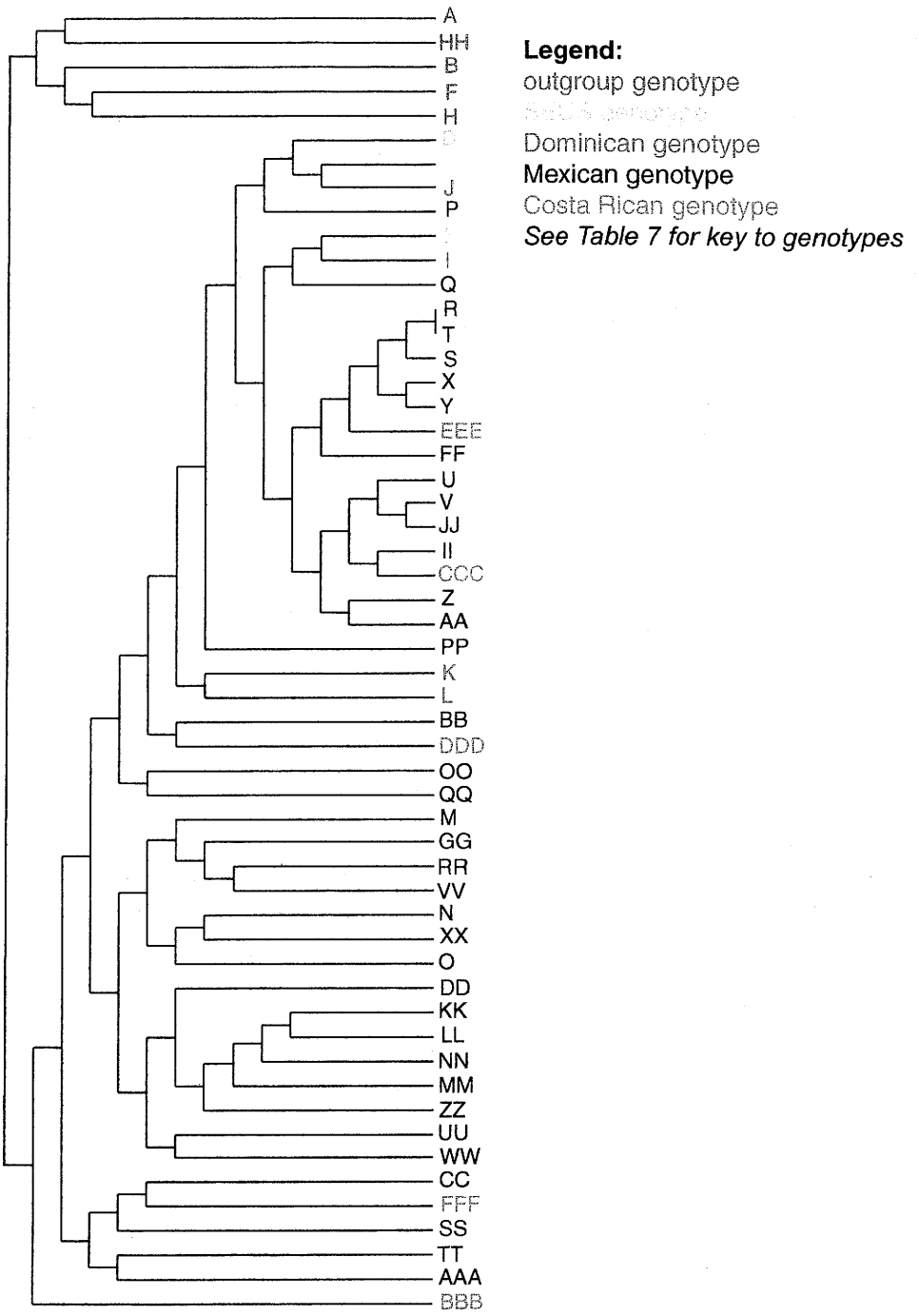

Figure 32: Neighbor Joining tree of genotypes based on presense/absense of alleles, utilizing Dice's dissimilarity coefficient. Outgroups included. Distances not to scale.

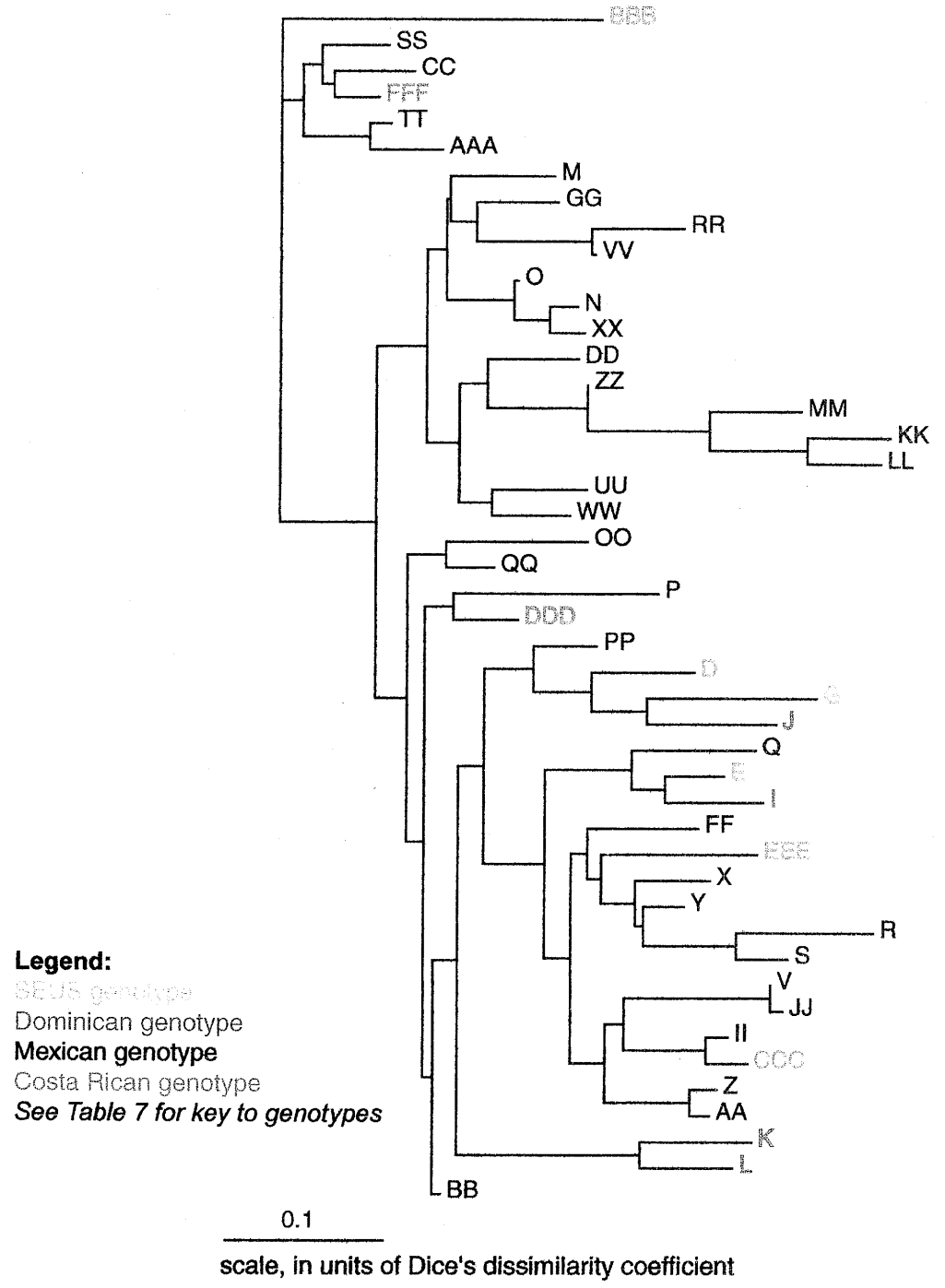

Figure 33: Neighbor Joining tree of genotypes based on presense/absense of alleles, utilizing Dice's dissimilarity coefficient. Outgroups excluded. Distances to scale.

topology of this allele presence/absence tree could not be tested by bootstrapping, but it might well suffer from the same weaknesses as the allele frequency tree.

DISCUSSION

Morphological and genetic diversity and differentiation

Spore morphology

Spores were quite variable in size and ridge density among *A. monanthes* spores sampled in this investigation and from the literature, but that variability did not seem to follow geographic patterns and spores were otherwise similar (e.g. in surface texture and ridge ornamentation). Since even Old World *A. monanthes* spores did not appear notably different from the New World spores sampled, the species appears to be too homogenous in spore architecture for this to be a useful source of taxonomic data for this infraspecific study. However, spores of related species did show marked differences from *A. monanthes* spores, so the general similarity of all *A. monanthes* spores observed argue that *A. monanthes* is a single species despite its great geographic range and variability in sporophytic morphology.

Gametophyte morphology and ontogeny

A. monanthes showed great variability in form and timing of various developmental stages, but most of this variability was found within all regions, showing no geographic trends. However, the literature review of use of gametophyte morphology in fern systematics showed that this does not necessarily preclude taxonomic differentiation.

A. monanthes may be unique among *Asplenium* in having potentially perennial gametophytes, with multiple thalli that can produce sporophytes independently. This was found in gametophytes from all regions. The length of time that a given gametophyte can continue to produce new thalli is not clear because under lab conditions most individuals died from algal or fungal competition, but it is possible that many sporophytes can be derived from a single spore. This capacity for gametophytic clonal reproduction, along with spores' isolate potential discussed earlier, has probably been important for *A.*

monanthes' ability to persist in the southeastern U.S. Several SEUS populations currently lack any mature sporophytes that could produce spores for traditional wind dispersal, and spore dispersal is assumed to be rare in this wind-sheltered habitat, so it may be perennial clonal gametophytes that are maintaining these populations.

A. monanthes gametophytes are also unusual in their ability to grow filamentously for extended periods of time. This trait could allow limited dispersal of gametophyte tissue, similar to the gemmae of other tropical rockhouse ferns, if filamentous segments became tangled around small animals (e.g. insects, salamanders, rodents) for transportation via epizoochory. These animals are unlikely to have large range areas, so such events would probably only spread A. monanthes around the site, but even limited dispersal increases its probability of persistence in limited suitable microhabitat. Strangely the filamentous growth form is much rarer in the southeastern U.S., in which it would seem preadaptive, than in Mexico or Costa Rica, in which the abundance of suitable habitat would make such a mechanism appear unnecessary.

Starch gel electrophoresis

Genetic structure of A. monanthes

More of *A. monanthes*' genetic differentiation appeared to be within regions rather than among them, as the regional Gsr values ranged from 0.13 up to 0.38 versus a value of 0.18 for Grt. The resulting estimates of within-region Nm ranged from 0.42 to 1.62 migrants per generation, lowest in Costa Rica and highest in the southeastern U.S. However the estimation of Nm from G-statistics is inappropriate in many situations due to restrictive model assumptions (Whitlock & McCauley, 1999). The high Nm estimated among SEUS populations does not mean that these populations are currently experiencing migration but reflects a founder effect minimizing genetic differences among populations derived from the same original colonist (i.e. the Carolina genotype). Inter-region Nm was estimated at 1.14 migrants per generation using the same questionable derivation from G-statistic values, but only 0.39 when calculated using Slatkin's (1985) private allele frequency method. There was no strong pattern of isolation by distance overall (although it was statistically significant at this scale) or

within Mexico (other regions were not sampled sufficiently to test within-region isolation by distance). These patterns of less differentiation among than within regions, reasonable genetic differentiation at all scales, and weak isolation by distance suggests that *A. monanthes* is particularly prone to long-distance migration despite migration being somewhat infrequent in general. The lack of geographic pattern in both neighbor-joining trees may partially reflect *A. monanthes*' tendency for long-distance dispersal rather than just failure of these analytical methods to uncover regional patterns, although a literal interpretation of the trees is not recommended (for example, the trees grouped the Carolinas instead of Guess with Neversink even though Neversink shares more alleles with Guess and is geographically adjacent to it)[11]. However both PCO analyses show that regional genetic differentiation has not been obliterated by long-distance dispersal.

A potential biological mechanism for the observed patterns of genetic differentiation would be that spores are only rarely elevated to a strong airstream but once there, they are slow to settle out. Nothing appears particularly unusual about *A. monanthes* spores' size, shape, or architecture that would cause them to stay afloat longer than other fern spores, so *A. monanthes*' apparent propensity for long-distance migration may be due to its ability to found a colony from a single apogamous spore when long-distance dispersal does occur.

Mexico may be responsible for most of the long-distance migrants based on its greater area of *A. monanthes* habitat than any other region sampled. **Figure 26** seems to support this inference, since there were no cases of shared alleles or phenotypic patterns that did not involve Mexico. However if Mexico were the original source of most alleles (see below), it might be difficult to detect subsequent transmission of these alleles from one non-Mexican region to another. Participation in inter-regional migration can also be inferred from the number of private alleles and phenotypic patterns found in each region.

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[11] The greater regional clustering in PCO than neighbor joining analyses may be due to two differences in these analytical methods. Neighbor joining creates a perfectly bifurcating tree from a single ancester, a model that is inappropriate when the OTU's are populations that share migrants (creating reticulation among branches) or when the OTU's are genotypes with multiple hybrid origins. Secondly, neighbor joining loses genetic information by reducing differences at multiple loci to a single measure of genetic distance, whereas PCO utilizes the more nuanced raw data. Either of these limitations might make it difficult for neighbor joining to detect a weak pattern of regional genetic differentiation among *A. monanthes* populations and genotypes.

The Dominican Republic has the most, five, which may mean that it produces few inter-regional migrants. The southeastern U.S. has only one private pattern, but since it is so geographically isolated and has a relatively tiny collective spore output, this probably means that it is too young to have developed unique mutations. Costa Rica and Mexico, with three private alleles or patterns each, appear to be more active in inter-regional migration, especially with each other: they share six alleles/patterns exclusively with each other.

Mexico contained the greatest genetic diversity of any region studied, both measured by allele and phenotypic pattern richness (**Fig. 26**) and genotype richness (**Table 7**). While Mexico's relative allele and pattern richness can probably be explained by greater sampling in Mexico, its relative genotype richness is of a scale that cannot be an artifact of sampling. Sampling of Mexican populations revealed 0.50 genotypes per individuals sampled, whereas the other three regions ranged from 0.09 (southeastern U.S.) to 0.17 (Costa Rica) genotypes detected per sample. This great regional difference in genetic diversity should stimulate investigation of genetic structure in other parts of *A. monanthes*' large geographic range. Mexico is also known for great variability in *A. monanthes*' sporophyte morphology (Alan Smith, personal communication), so it is not surprising that isozyme genotypes reflect the same pattern of greater diversity in Mexico than in other regions sampled.

Moran & Smith (2001) reviewed the pteridophyte taxa found in both the Americas and Africa/Madagascar and speculated that the majority of taxa, including *A. monanthes*, were of New World origin and later colonized Africa/Madagscar via long-distance dispersal, based on greater species richness of each clade in the Americas than in Africa/Madagascar. Considering the great genetic and morphological diversity found there, Mexico may well be the neotropical home of *A. monanthes*. The longer a species' history is in a location, the greater the number of mutations that will have arisen to counteract the initial founder effect. No South American samples were analyzed, so it is possible that even greater genetic diversity occurs there.

Another factor that might explain *A. monanthes*' greater diversity in Mexico would be repeated hybridization between the unknown parental species if Mexico was the

only region sampled in which both parents were present. The tetraploid record from Chiapas and the observation of unusually small spores (possibly representing a diploid) in one of the Hidalgo collections (Mineral Real del Monte) may be evidence of cryptic parental taxa in Mexico. Multiple hybrid origins for a species has been documented in other ferns (e.g. Werth et al., 1985b, Trewick et al., 2002), so this could also have occurred for *A. monanthes* if its parental species have been sympatric for a reasonable length of time. The cohesiveness of *A. monanthes* as a species would not be challenged by multiple hybridizations between the same two parent species assuming that the different individuals involved shared a common pool of alleles. The circumstances of *A. monanthes*' origin have not been investigated, being beyond the scope of this project, so these explanations for regional differences in diversity are purely speculative. An investigation of *Asplenium monanthes*' hybrid origin following the approach of Werth et al. (1985a) would be quite useful for explaining these genetic trends and *A. monanthes*' global geographical distribution.

Comparison with other plants' genetic structure

To place *A. monanthes* in context, a literature survey of genetic structure of apogamous plants, ferns, and long-lived herbaceous perennial (LLHP) seed plants was summarized in **Tables 3 and 4**. Ferns were slightly greater in species-level genetic diversity than LLHP seed plants but similar in population-level genetic diversity. Seed plants as a whole (not listed but available in Hamrick & Godt, 1989) were similar to ferns in genetic diversity at both levels of analysis and the majority of ferns are long-lived herbaceous perennials. Therefore the slight difference observed between ferns and LLHP seed plants in species-level genetic diversity may mean that the four LLHP seed plant species reviewed by Hamrick & Godt (1989) were simply not representative of this type of plant. The general similarity between ferns and LLHP seed plants suggest that the same evolutionary forces govern the generation of genetic diversity at the species level and the maintenance of diversity at the population level in the two groups. Ferns showed greater genetic differentiation among populations than LLHP seed plants, with a mean Fst of 0.21 vs. 0.12. The two groups' difference in genetic differentiation among

populations is probably due to ferns' wind-borne spores versus many LLHP seed plants' less powerful dispersal mechanisms. The difference is not enormous because most ferns, like many seed plants, are limited in forming new populations by low isolate potential.

This literature review does not allow direct comparison of apogamous taxa genetic structure to that of all ferns and LLHP seed plants because of the different parameters generally used to measure asexual species. But it does show that apogamous plants have notable species-level genetic diversity (% diagnostic loci is a more conservative measure of genetic diversity than % polymorphic loci because it excludes fixed heterozygosity) and within-population diversity, and probably intermediate genetic differentiation (in that both widespread and local genotypes were common). The limited nature of this literature review and its heavy bias towards *Taraxacum* invite further investigation of genetic structure in apogamous taxa.

A. monanthes has much greater species-level genetic diversity than the mean for any of the groups compared. This may support the hypothesis given above that *A. monanthes* is the result of multiple hybridization events, each of which could have incorporated different alleles from the parent species' gene pools.

A. monanthes, when averaged over all regions sampled, was slightly higher in within-population genetic diversity than all ferns and LLHP seed plants, although in *A. monanthes* such diversity usually represents fixed heterozygosity within individuals rather than variability among individuals. In comparison with other apogamous plants, *A. monanthes* contained a similar percentage of populations polymorphic but fewer genotypes per population because even polymorphic populations of *A. monanthes* are not extremely polymorphic except in Mexico.

A. monanthes showed high genetic differentiation among populations compared with all three groups, manifested as greater Gst values than all ferns and LLHP seed plants and a higher percentage of local genotypes and the absense of widespread genotypes relative to mean percentages for apogamous plants. These differences may partially reflect the geographical scale that various studies employed; the *A. monanthes* populations sampled covered an area with a diameter of almost 3000 km, whereas the median distance covered in the apogamous plant case studies was between 50 and 500

km, for example. However, the difference may genuinely reflect strong genetic differentiation among *A. monanthes* populations. That 80% of genotypes were private to a single population is independent of the geographic scale of the investigation. It is puzzling why *A. monanthes* would have greater genetic differentiation than most other apogamous plants, the majority of which were also wind-dispersed and all of which have perfect isolate potential and the ability to differentially silence genes. Perhaps this difference is caused by *A. monanthes*' very small populations which are probably quite prone to genetic drift, such that different populations become fixed for different genotypes. The difference between *A. monanthes* and other ferns may also involve genetic drift. In migration-limited asexual taxa, fewer genotypes are expected in each population than in a sexual population of the same size and allelic richness because sexual recombination is not present to reshuffle the allele pool. Thus a given reduction in population size has a greater probability of causing fixation in an asexual population, which again would result in different genotypes becoming fixed in different populations by random chance.

Origin and taxonomic status of the SEUS populations

Timing of A. monanthes' arrival in the southeastern U.S.

Possible evidence for an ancient origin of *A. monanthes* in the southeastern U.S. is the Carolina populations' relative distance from all other populations in the allele frequency PCO ordination. But the Barva (Costa Rica) and Jalapa (Mexico) populations show almost this degree of isolation from other populations in the plot, so genetic isolation doesn't appear to be a particularly unusual phenomenon in *A. monanthes*. The genetic distance from other populations plotted may simply mean that the source of the Carolina colonist was not sampled (perhaps it was from Jamaica, for example), not that the Carolina population has diverged from extended genetic isolation. Furthermore the allele presence/absense genotype-based PCO analysis showed the Carolina genotype (G) only somewhat different from a Dominican genotype (J).

Further evidence that might be invoked for an ancient origin of the SEUS populations is the fact that the SEUS genotypes were not detected among neotropical

samples. This might be taken to mean that SEUS populations have diverged genetically from their neotropical source population(s) due to prolonged isolation. However prolonged isolation would likely have resulted in novel mutations, which were not encountered (with the possible exception of the uninterpretable phenotypic pattern ACN 1). Given the great genetic variability of *A. monanthes*, it is not surprising that the SEUS genotypes were not detected in limited neotropical sampling.

Evidence for a Quaternary origin is that SEUS *A. monanthes* has no unique morphological characteristics. SEUS spores are similar to neotropical spores, although this is not surprising given that more distant disjunct populations (e.g. from Africa or the Atlantic) also show no major differences. SEUS gametophytes differ from Mexican and Costa Rican gametophytes in having a low frequency of filamentous growth forms, but this form is still present. SEUS gametophytes more frequently have a "swan neck" sporophytic extension than other regions' gametophytes, but are not unique in this respect. No SEUS gametophytes were observed to have the jagged sporophytic ridge observed in some neotropical gametophytes, but this was rare there too and may have been overlooked in SEUS gametophytes. The absense of gametophyte differences does not preclude taxonomic differences as concluded from the literature review of gametophytes as taxonomic data sources, but it can add slight support to a decision to keep a taxon intact. SEUS *A. monanthes* populations contain no private alleles and just one private phenotypic pattern (the allelic basis of ACN, AAT, and IDH patterns could not be determined, so a unique pattern may or may not represent a unique allele), whereas all of the other regions have at least three private alleles and/or patterns each.

The lack of novelty in spore architecture, gametophyte morphology, sporophyte morphology (personal communication, Alan Smith), and enzyme alleles of SEUS *A. monanthes* suggest that none of these populations have not been isolated from neotropical ones for very long. In contrast, several other Appalachian "tropical" ferns studied by Farrar (1998) and Watkins (2000) displayed novel isozyme genotypes, spore architecture, leaf shape, and/or the complete loss of the sporophytic phase, indicating a more ancient divergence from tropical relatives. These species' current occurrence and distribution in the Appalachians is explained by a once broad pre-Pleistocene range for each species that

was only preserved in places where suitable refugia existed for them to escape the cooling climate. Evidence is insufficient to support this scenario for SEUS *A. monanthes* and its occurrence is more likely due to Quaternary period long-distance dispersal.

Because different SEUS populations may have had different biogeographic origins, we must examine each area separately to estimate *A. monanthes*' antiquity there, although all are probably from the Pleistocene or Holocene based on the lack of genetic or morphological novelty discussed above. The Carolina genotype has been around long enough to colonize multiple streams in the same gorge system. There are many nearby sites that look like suitable *A. monanthes* habitat yet have not been colonized, so *A. monanthes* habitat might be too sheltered from wind to allow frequent spore dispersal to other gorges. Therefore the original founding of the Carolina genotype probably occurred quite some time ago (probably at least a few hundred years) to allow time for expansion of the initial colony (if the colonist did not arrive until the Holocene) or refugial population (if the arrival was during the Pleistocene) to its current range in the Carolinas.

The two Alabama genotypes differ at only 3 of 12 loci examined and it seems improbable that two rare tropical colonists would become established just 17.5 km apart, so these two genotypes might have a common origin. If so, one could estimate the time expected to produce this degree of genetic divergence between isolated populations. Various models have been proposed for estimating the rate of silencing (e.g. Watterson, 1983 and other models reviewed within, and Nei, 1987) and reciprocal silencing (Werth & Windham, 1991) in polyploids like *Asplenium monanthes*, so these could potentially be used to estimate divergence time between the genotypes if gene silencing was responsible for the differences. But because two (ACN and IDH) of the three loci at which the two Alabama genotypes differ were not scorable as alleles, it is unknown whether silencing can explain the differences and if so how many silencing events occurred, so the associated silencing time cannot be estimated. In the absence of gene silencing information, Nei's genetic distance can be used to roughly estimate divergence time. Nei (1987) provided the formula $t = 5 \times 10^6 D$, where t is the time two populations have been separated and D is Nei's standard genetic distance, given an estimate of 10^{-7} detectible

mutations per locus per year in an average species (also Nei, 1987). This would yield an estimated divergence time of 1.2 million years for the two Alabama genotypes given their genetic distance D=0.238. However Nei warned that this equation is imperfect for electrophoretic data because it ignores an expected increase in back-mutation with time. Furthermore, *A. monanthes'* mutation rate is completely unknown but expected to be higher than average given its duplicated loci. Therefore if the two Alabama genotypes have a common origin, their putative shared ancestor may have been present in Alabama since the early Pleistocene, but this estimate of time could be extremely inaccurate. The current differences between the two genotypes could probably be explained by different paths of gene silencing and/or new mutations and subsequent genetic drift in different Pleistocene refugia.

The two Florida populations are/were on land that was submerged until the Pliocene or even Pleistocene (Randazzo, 1997; Webb, 1990), evidence that they were founded relatively recently. Because the extant Florida population was legally protected from sampling during the period of this investigation, neither population's genetic affinity is known. They could have been colonized either from the tropics or from other SEUS populations. The Florida populations are each approximately three times farther away from the closest neotropical *A. monanthes* populations than from the SEUS populations, but the neotropics have much greater collective spore production and probably greater access to the wind with higher elevations and in some cases more exposed habitat. Further speculation on the origin of the Florida populations is unwarranted until the extant one can be studied genetically.

One might ask how it would have been possible for a tropical fern to have become established in the southeastern U.S. during the Pleistocene, as was suggested for the Alabama populations and possibly others. If *Asplenium monanthes* can maintain itself in the southeastern U.S. during current climatic conditions, it is apparently able to handle reasonably hot summers and cold winters (as moderated by its protective microhabitat) and could have become established during Pleistocene interglacial periods equally well as during the Holocene. If it did arrive during the Pleistocene, however, it would have had to retreat further into rockhouses during full-glacial periods for protection from freezing

temperatures. Light limitation in rockhouses would certainly have slowed sporophyte growth because of all the sporophyte tissue that acts as a photosynthetic sink. Other tropical ferns are believed to have weathered full-glacial periods in Appalachian rockhouses as clonal gametophytes with only occasional sporophyte production (Farrar, 1998), so it is possible that *A. monanthes* utilized the same strategy.

Source of original SEUS colonists

Long-distance colonization of the southeastern U.S. during the Quaternary could have been carried out by either Mexican or Caribbean spores. No morphological traits were found to elucidate the origin of SEUS *A. monanthes*. SEUS gametophyte cultures shared with Dominican cultures a relatively low frequency of filamentous gametophytes, while they lacked a trait, ridged areas, that was most frequent (but still rare) in Dominican and Costa Rican gametophytes.

Shared alleles linked SEUS populations to particular neotropical regions. The Carolinas shared SKDH allele 4 with Mexico alone. Neversink was linked to Mexico and Costa Rica by the presence of IDH pattern 4. No alleles or phenotypic patterns were shared between SEUS and Dominican populations that were not also found in Mexican populations. Therefore analysis of shared alleles and phenotypic patterns supports a Mexican origin for the SEUS populations.

Quantative analysis of the data supported a Caribbean origin instead. Pairwise regional differentiation was calculated and showed greater regional differentiation of the southeastern U.S. from Mexico (Grt=0.23) than from the Dominican Republic (Grt=0.18), although these two values may not be significantly different (the software utilized, SPAGeDi, did not allow such a test). Additionally the two PCO analyses showed SEUS and Dominican clusters overlapping significantly whereas Mexican populations or genotypes were only rarely (e.g. the Ixtlan population) very close to SEUS clusters.

Neighbor joining was performed with the goal of determining specific progenitor-derivative relationships among populations and genotypes, something ordination methods like PCO cannot do. The data gave insufficient resolution for decisive tree-building, based on the universally poor bootstrap values in the population-based allele frequency

neighbor joining tree (the allele presence/absence tree of genotypes could not be bootstrapped, but its similar topology may be similarly statistically non-significant). Therefore the neighbor joining trees are being ignored in the assessment of the biogeographical origin of the SEUS populations.

Reconciliation of the different biogeographical origins suggested by shared alleles and phenotypic patterns and quantitative genetic analysis is difficult. Perhaps the alleles shared by SEUS and Mexican but not Dominican samples exist in Caribbean populations not sampled or now extirpated. If Jamaican populations could have been sampled this study might have been more conclusive, but these historical populations proved elusive during the author's searches and might no longer exist. The PCO results are harder to discount. It is unlikely that chance alone could account for the clustering of SEUS with Dominican populations and genotypes. The only other scenario that could create such a pattern would be spores from the same Mexican populations founding SEUS as Dominican populations. Such a scenario would be highly unlikely given the vast expanse of *A. monanthes* populations across Mexico. Because SEUS populations and genotypes clustered together loosely in PCO analyses, it appears that all were founded by spores from the same geographic region. Therefore after consideration of somewhat equivocal data, the author proposes that *A. monanthes* arrived in the southeastern U.S. via multiple long-distance dispersal events from the Caribbean during the Quaternary period (i.e. **Fig. 12b**). These dispersal events would be notable exceptions to the earlier generalization that Mexico is probably responsible for much more inter-regional migration than the Dominican Republic.

Significance

An important implication of these biogeographical findings is that long-distance dispersal from the tropics to the southeastern U.S. must have occurred successfully two to five times to explain the three genotypes encountered and the two unsampled Florida populations. Long-distance dispersal is generally considered a rare event, but recent studies of other ferns suggest that it can occur multiple times (Schneller et al., 1998; Ranker et al., 1994). Multiple colonizations of the southeastern U.S. by *A. monanthes* in

spite of the scarcity of suitable habitat is remarkable. The fact that multiple spores were able to establish colonies successfully implies that there are more spores of *A. monanthes* (and by extension other neotropical ferns) that reach the southeastern U.S. but do not persist, having landed in inhospitable habitat. This capacity for long-distance dispersal is even more remarkable given *A. monanthes*' somewhat low migration rate within the tropical area studied.

SEUS *A. monanthes* does not warrant any unique taxonomic designation. *A. monanthes* does not appear to have a long history in the southeastern U.S., these populations showing only minimal genetic differentiation from neotropical populations and less morphological and genetic diversity. *A. monanthes* differs from most other Appalachian "tropical" fern species in lacking any novelty with respect to its neotropical relatives and should continue to be considered simply *Asplenium monanthes* L. The many other disjunctions in *A. monanthes*' global range should be investigated genetically and cytologically to confirm that *A. monanthes* is a single allotriploid species and to determine its hybrid and geographical origins, the latter being tentatively proposed as Mexico.

PART II.

CONSERVATION BIOLOGY OF *ASPLENIUM MONANTHES* L. IN THE SOUTHEASTERN UNITED STATES

INTRODUCTION

Goals

Asplenium monanthes L. is a largely tropical fern with small, rare, disjunct populations in the southeastern United States (SEUS) in climatically moderated microhabitats. This paper investigates the SEUS populations' genetic structure, microhabitat and microclimate, historical and current distribution, life history, and demography. These observations and analyses are intended to elucidate SEUS *A. monanthes*' basic biology, strategies for survival in the temperate zone, prognosis for survival, threats to survival, and appropriate conservation measures.

Considerations and strategies for plant conservation

Local rarity of a widespread species

Rabinowitz (1981) created a typology of rarity, distinguishing between species that are limited by geographic range, habitat specificity, and small population size or density. *Asplenium monanthes* as a species cannot be considered rare: tropical *Asplenium monanthes* has an immense geographical range and a wide range of habitat, although generally low abundance where it occurs. *A. monanthes* is certainly rare in the southeastern U.S., living at the edge of its climatic tolerance and therefore severely restricted in acceptable habitat. SEUS *A. monanthes*' rarity would fall into Rabinowitz's category of narrow habitat specificity and small population size but a respectable geographic range spanning much of the southeastern U.S. Rarity is to be expected at the edge of a species' geographical range, so the only thing remarkable about SEUS *A. monanthes* is that it has managed to survive so disjunct from the rest of the species, exposed to a very different climate and limited in migration.

Millar & Libby (1997) suggested that species that are naturally rare may have developed adaptations to rarity that prevent small populations from declining, e.g. great longevity or phenotypic or developmental plasticity. Menges (1997) provides the example that phenotypic plasticity could buffer against environmental plasticity if certain conditions favor growth and others reproduction, such that the plant can turn any

environmental conditions to its advantage. Rabinowitz (1981) found that two rare grasses had greater biomass and yield when grown amongst common grasses than when grown in monoculture, so these species' competitive strategies appear to have adapted to the normal condition of being rare. *A. monanthes* may also have adaptations to rarity because even neotropical populations visited were never particularly large.

It is unknown whether SEUS *A. monanthes* has developed adaptations to the temperate climate, which might warrant conservation attention as a unique ecotype. Millar & Libby (1997) argue that ecotypic diversity of widespread species should be conserved to maintain the evolutionary potential of species that might hypothetically become rare in the event of global climate change, although this is certainly not as pressing as conservation of an already threatened species. *A. monanthes* probably did not arrive in the southeastern U.S. until the Pleistocene or Holocene (based on finding little genetic differentiation between SEUS and neotropical populations), which may not be enough time for selection to act on its physiology particularly if genetic variation was lacking as it is now. No common garden experiments were performed for SEUS and neotropical plants because SEUS plants raised in the lab from spores (*A. monanthes* is too rare in the southeastern U.S. for plants to be taken from natural populations) did not survive to maturity, but this would certainly be interesting to investigate.

Models of regional population dynamics

To conserve rare taxa, it is important to understand which factors most strongly affect population (or metapopulation) persistence and growth. A given taxon can be compared against various idealized models of population dynamics to see which it fits best and conservation measures can be designed accordingly.

The simplest model of population dynamics over time is that of stable equilibrium. This model neglects environmental stochasticity and is inappropriate for populations that have not reached carrying capacity.

Metapopulation theory (Hanski & Gilpin, 1991) was formulated to address taxa in which populations are unstable and of short "lifespan" such that traditional models based on population equilibrium do not apply. Short population lifespan is commonly found in species adapted to frequent disturbance regimes (Eriksson, 1996). Short population

lifespan is also associated with short lifespan of individuals, since populations of long-lived species have overlapping generations which can buffer the effects of temporarily unfavorable environmental conditions (Erikkson, 1996). The metapopulation model also applies to taxa with longer yet finite population lifespan, but such populations fit the simpler stable equilibrium model reasonably well if the scientific monitoring period is dwarfed by the populations' lifespan. Metapopulation theory envisions a patchwork of suitable habitat, and at a given time the species of interest is present at some percentage of suitable sites, and the system as a whole may or may not be in stable equilibrium. As time goes on, populations are lost at some sites and gained at others at a fast enough rate that the functional unit of evolution is the whole metapopulation rather than each individual population. Sufficient dispersal ability and the existence of unoccupied suitable sites are necessary for a species to overcome the frequent local extinctions of the metapopulation model.

Another alternative to the traditional stable population model is that many populations are not at equilibrium with constant recruitment, but instead have extremely sporadic recruitment. This type of population is called a "remnant" population. Its decline is slowed by long plant lifespan, clonal reproduction, and/or a long-lived seed bank that bridge unfavorable periods for recruitment (Eriksson, 1996). The success of this strategy depends on the relative interval between favorable periods—if there is an unusually long unfavorable interval, the population may die out before being "rescued," but it is successful when intervals don't exceed a species' capability for maintaining the status quo. Many plants are known to have somewhat sporadic recruitment (Menges, 2000) and adaptations for population persistance (e.g. long-lived seed banks and clonality) (Eriksson, 1996), so this may be a relatively popular evolutionary strategy.

A fourth model is the source-sink model (Pulliam, 1988), sometimes considered just a variant of the metapopulation model. Instead of the metapopulation and remnant population models, which view all populations in an area as undergoing similar declines or advances, there are two classes of populations. Populations situated on ideal habitat (source populations) regularly produce an excess of individuals, and populations in marginal habitat (sink populations) would decline (due to either reduced survival or

fecundity) in the absence of continued migration from source populations. Habitat-specific birth and death rates may result in different demographic structure and growth rates in the two types of populations. The source-sink model has been more popular with zoologists than botanists, so a scarcity of plant studies prevents any generalizations about the types of plants that tend to fit this model.

Viability analysis

Population viability analysis is an approach applied to rare species to determine whether particular populations are at high risk of extinction. The three main threats to small populations are environmental stochasticity, demographic stochasticity, and genetic stochasticity (Menges, 1997). Environmental stochasticity refers to chance events (e.g. flooding, drought, loss of canopy cover, increases in pathogens) that may eliminate an entire population. Demographic stochasticity refers to the same chance events when they harm only some of the members of a population. In a small population, even a few individuals being lost due to chance events can seriously threaten the population's viability. Genetic stochasticity is another term for genetic drift, that chance differences between individuals in survival and fecundity can have a big impact on the viability of small populations by eliminating genotypes. This can threaten the population if the eliminated genotype was more fit than the remaining one or by causing inbreeding depression in sexual organisms. SEUS *Asplenium monanthes*, as an asexual species with narrow habitat specificity and very small populations, is quite vulnerable to future environmental and demographic stochasticity but not genetic stochasticity (fixation appears to have already occurred in all SEUS populations tested). Furthermore, Hogbin & Peakall (1999) and Lande (1988) suggest that genetic considerations are often redundant to more pressing demographic considerations, even for sexual organisms with larger population sizes. Therefore genetic concerns will not be particularly emphasized in this paper.

Elasticity analysis is a method of determining which life history transitions are the limiting factors for population growth for a given species or specific population. It can be used (Silvertown et al., 1996) to focus conservation efforts on the most effective strategies for increasing population viability for a taxon of concern. Elasticity analysis

first requires the estimation of a stage, size, or age-based transition probability matrix to project future demographic structure. When enough data is available to estimate such a matrix, elasticity analysis can help conservation managers decide whether projects enhancing recruitment, survival, or fecundity are most likely to help the population.

Reintroduction programs for plants

No reintroduction programs for ferns have been documented in recent literature. Transplants of relatively common fern species have resulted in modest success (Larissa Mottl, personal communication). Many reintroduction attempts for rare angiosperm taxa have been documented in the literature. The majority of these efforts resulted in total failure or survival without further recruitment (Morgan 1999, Drayton & Primack 2000, Pavlik & Espeland 1998), while only a few resulted in self-sustaining success (Rich et al., 1999). This suggests that the best-laid plans of botanists can overlook habitat factors or logistical details critical to population persistance, so reintroduction measures are probably worthwhile only when taxa are critically imperiled. Any reintroduction plan must avoid depleting natural populations (Ratcliffe et al., 1993), since natural populations appear to be rare species' best hope for the future.

MATERIALS & METHODS

Substrate and microclimate characterization

Microclimate monitoring locations

At each SEUS site a single representative microhabitat containing *A. monanthes* was measured each year. A second location was a single most exposed nearby location representing the macrohabitat. In the Carolinas this exposed location was a break in the canopy within the gorge, while in Alabama and Florida the location was several meters away from the sinkhole or cave entrance. A few sites were so uniformly climatically moderated that no nearby location could be considered any more exposed than the *A. monanthes* location, so no data was collected for an exposed location there. A third nearby location was also measured each year. In 2000 the third location was one that

looked comparable to the *A. monanthes* location (hereafter denoted as "plausible" locations) but lacked *A. monanthes* plants, in an attempt to evaluate whether *A. monanthes* could potentially expand to occupy more locations at the site. In 2001 the third location was instead the most moderated nearby microhabitat, measured to quantify the range of microclimates available at the site to investigate whether *A. monanthes* sporophytes (gametophytes may exist in these most moderated microclimates but this was not investigated) are limited by light availability. The most moderated location took the form of a dark rock crevice in Carolina sites versus the depths (but only to the depth that bryophytes were still present, indicating incident light) of the sinkhole or cave in Alabama and Florida sites. **Figure 34** illustrates the three locations examined at a prototypical Carolina site. Throughout this paper the following terminology is used: "site" is used synonymously with "population" and refers to the overall locality of a given population, whereas "location" refers to one of three specific microhabitats identified within each SEUS site.

Microclimate protocols

The microclimate of the SEUS *A. monanthes* populations was characterized in detail only in summer due to lack of funds for winter visits or data-loggers. To investigate annual extremes, maximum-minimum thermometers were placed at selected *A. monanthes* sites and read the following summer. One site, Cane Creek, yielded maximum and minimum temperature readings for two years for all three microclimate locations. Two additional sites, Maple Springs Branch subpopulation 3 and Neversink, yielded data only for the specific microclimate of the *A. monanthes* location (measurements were also attempted at other microclimate locations and other sites but thermometers were stolen). The annual maximum and minimum temperatures were compared to those recorded at the NCDC weather stations at Lake Toxaway, North Carolina, for Cane Creek and Maple Springs Branch, and in Scottsboro, Alabama, for Neversink.

In the summers of 2000 and 2001, all SEUS *A. monanthes* populations visited (with the exception of the dwindling Thompson River population in 2001) were measured for multiple microclimate variables. The sites cannot fairly be compared to one another,

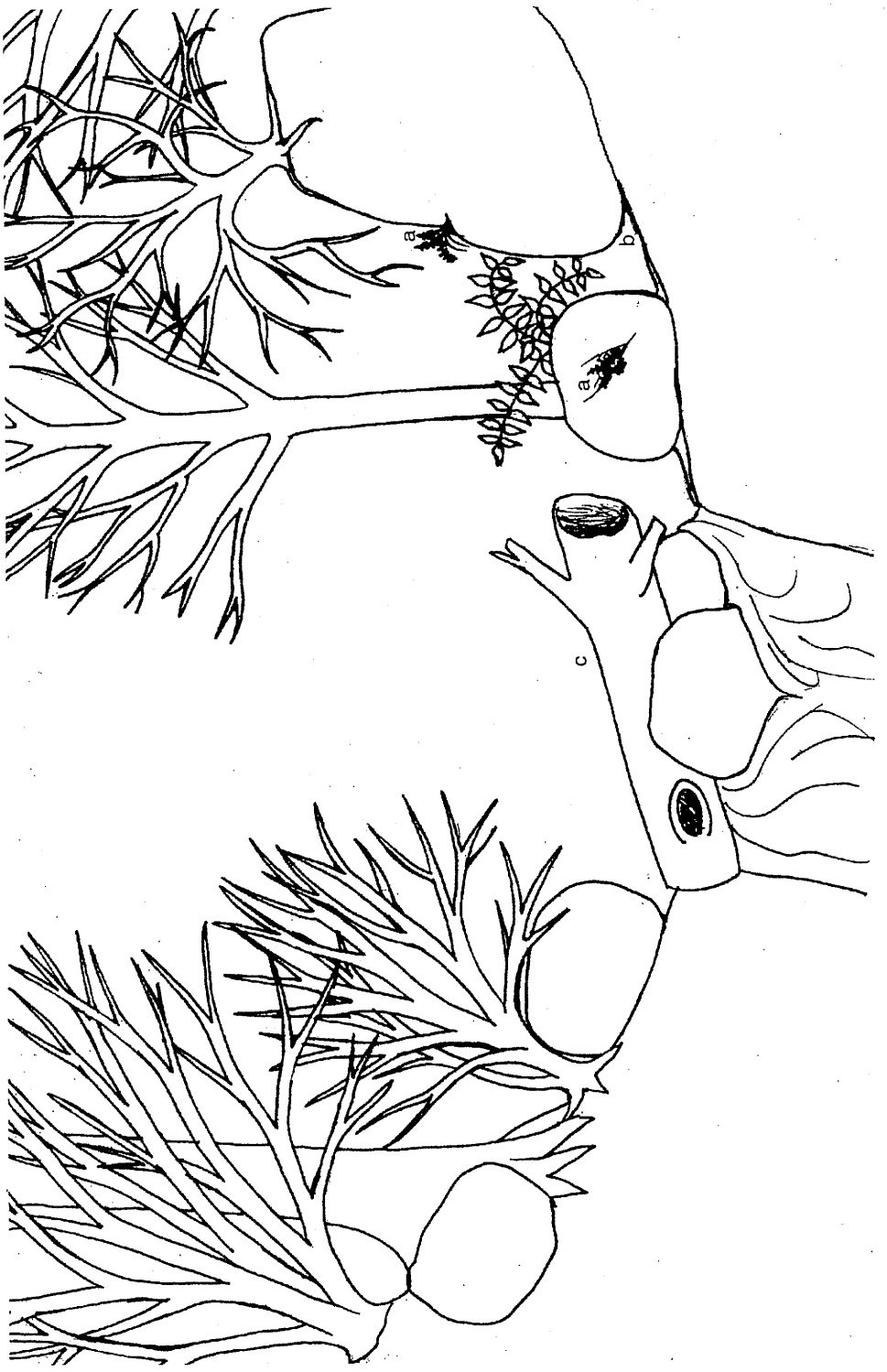

Figure 34: Generalized diagram of an A. monanthes site in the Carolinas showing locations where microclimate data was recorded: (a) *A. monanthes* location, (b) a deep crevice as most moderated location, (c) a break in canopy cover for most exposed location. At Alabama and Florida sites (not pictured because of the diversity of scenarios encountered), the most moderated location was the deepest location inside the cave or sinkhole where bryophytes were still present, while the most exposed location was a spot outside the immediate climatic influence of the sinkhole or cave.

however, because they were measured at different times on different days, so can only be used to show the range of microclimate that *A. monanthes* experiences. However different microclimate locations at the same site can certainly be compared, having been recorded at the same time.

Populations in Costa Rica (June 2001) and the Dominican Republic (January 2002) were measured for the same microclimate variables for comparison with SEUS data, but no more moderated or exposed locations were investigated because the species' neotropical ecology was only indirectly relevant to this study of SEUS *A. monanthes*. No microclimate data was obtained from Mexican populations because the author was not able to visit these sites in person.

The climatological variables recorded at all three locations at each SEUS *A. monanthes* site and at the *A. monanthes* location of each neotropical site were: visible light (lux) and/or photosynthetically active light (quanta), air temperature (°C), substrate (i.e. bryophyte mat) temperature (°C), relative humidity (%), relative substrate moisture (in 2001 only), and rock permeability.

Air temperature was measured with a Taylor mercury maximum-minimum thermometer in 2000 and a digital ExTech hygrothermometer in 2001 (it can fit into smaller crevices) which was also used to measure humidity both years. The SEUS air temperature data was compared to the same day's maximum temperatures recorded at the corresponding NCDC weather station to see if each site as a whole was more climatically moderated than the rest of the general area. Substrate temperature was measured (where a sufficient bryophyte mat existed to warrant measuring) with a Taylor soil thermometer.

Logistical considerations for the light meters limited certain sites to lux readings only (measured with a Sekonic incident light meter) and other sites to quanta only (measured with a Li-Cor light meter), and the two are not interconvertible.
To produce a rough dataset in standardized units to try to compare sites, quanta were converted to a rough estimate of lux by plotting lux against quanta for locations in which both were measured, fitting a regression line, and using the resulting equation to calculate an estimate of lux for remaining quanta-only locations.

Relative substrate moisture was quantified non-destructively for the thin bryophyte mat on each rock by pressing a facial tissue against the substrate for 10 seconds, then sealing it in a fresh ziplock bag. Each tissue was later weighed on a sensitive balance while still moist, then allowed to dry totally and reweighed. The ratio of wet to dry weight was used as the relative moisture parameter.

Rock permeability was another tool used to investigate water relations because humidity and substrate moisture are imperfect tools—humidity is not directly proportional to evaporative pressure (Wolfe et al., 1949) and substrate moisture is subject to time since last precipition. Plants can gain significant water from storage in bedrock pores (Zwieniecki & Newton, 1996), so rock permeability is one factor in water availability that can be successfully measured from a single site visit (as opposed to more comprehensive measures like precipitation, evaporative pressure, or substrate moisture that require multiple site visits for accurate assessment). It is unknown how large a role rock permeability plays for *A. monanthes*' water relations—the bryophyte mat's capacity for water retention may be more significant, but was not investigated, being more complicated to measure. A small representative rock sample was taken from each SEUS and Dominican site (all Costa Rican populations visited were terrestrial rather than epipetric). Each rock's water-holding capacity was measured by immersing it in water for a day, drying off its surface, and weighing it on a sensitive balance, then weighing it again after it had been allowed to dry for a few days. The ratio of wet to dry weight was used as the parameter for permeability.

To determine which of the above microclimate factors affect *A. monanthes*' success at a site, regressions were carried out (using the computer program SAS) between each microclimate variable and the number of sporophytes and fertile sporophytes observed, utilizing data from SEUS sites only and repeated with the inclusion of neotropical sites. The r-squared values were reported and the slope was tested for statistical significance. For these analyses, the SEUS-only dataset and the complete dataset were first tested for a normal distribution, and any variable found to deviate strongly from this distribution was transformed accordingly. A correlation test was also performed for each pair of microclimate variables to determine if any were non-

independent for the combined SEUS and neotropical dataset. If two microclimate variables were correlated with one another, the importance one with the weaker correlation would need to be viewed with suspicion.

Starch gel electrophoresis

Starch gel electrophoresis was used to determine whether SEUS populations were genetically distinct from neotropical populations and to characterize the genetic structure of SEUS populations. Shared genotypes indicate historical or current migration, an important factor in population dynamics. Genetic characterization can be used to prioritize which populations should receive the greatest conservation efforts, being most unique or containing the greatest genetic diversity. Genetic characterization is also crucial for choice of genetic stock for any future restoration programs.

See the preceding chapter for populations sampled and for grinding, running, staining, and scoring protocol. For the purposes of this chapter on conservation biology, the quantitative data analyses (Principle Coordinates Analysis and Neighbor Joining) performed on isozyme genotypes in the preceeding chapter will be largely ignored, being of primarily biogeographical interest.

Gametophyte cultures

Ferns' gametophytic stage is often overlooked in importance because of the difficulties studying it in natural populations. Yet sporophyte production is completely dependent on gametophyte ecology. Because of the difficulties finding microscopic gametophytes inhabiting thick bryophyte mats, natural gametophytes were not studied in this investigation. Instead gametophytes were raised in the lab from spores to characterize SEUS *A. monanthes*' gametophyte morphology and ontogeny. This effort should allow future field identification of *A. monanthes* gametophytes and give a rough idea of gametophyte ontogeny in the wild. An additional purpose for raising gametophytes in the lab was to determine whether sporophytes could be successfully raised for transplantation into declining or extirpated natural populations.

Agar medium developmental studies

Petri plates were prepared using 1% agar medium enriched with Bold's macronutrients (Bold, 1957), Nitsch's micronutrients (Nitsch, 1951), and ferric chloride (Farrar, 1974). Spores were sown from all fertile *A. monanthes* populations visited except Florida Caverns, and from co-occurring *A. platyneuron, A. trichomanes, A. resiliens*, and *A. heteroresiliens* for comparison. The plates were placed under fluorescent lamps under a constant light regime of approximately 3230 lux at 21°C. The morphology and ontogeny of the resulting gametophytes were documented regularly as they grew.

Natural substrate trials

Gametophytes were also grown on soil and rock substrates as a potential way to avoid the observed frequent mortality of agar medium cultures to algal and fungal contamination. The soil, rocks, and spores used for this experiment were from Guess Creek Cave, Alabama. The treatments (four replicates each in most cases) were as follows: (1) spore viability control: agar medium instead of natural substrate, spores sown, (2) autoclave control: substrate autoclaved, no spores sown, (3) spore bank trial: substrate not autoclaved, no spores sown, (4) bryophyte mat trial: substrate included bryophyte mat, not autoclaved, spores sown, (5) *A. monanthes*-only trial: substrate autoclaved, spores sown, and (6) *A. monanthes* supplement trial: substrate not autoclaved, spores sown. The purpose of these trials were respectively to: (1) test that the spores were viable, (2) test the effectiveness of the autoclave treatment, (3) estimate the spore bank in a natural population, (4) see if the live bryophyte mat was critical to gametophyte survival, (5) be sure of the identity of the resulting gametophytes, and (6) see if supplementing the spore bank improved *A. monanthes* recruitment. All plates were kept under constant fluorescent lights and were examined about once a month for *A. monanthes* gametophytes. Unfortunately the plates dried out several times, which may not occur in natural *A. monanthes* habitat.

Population demographics

Census

All historical SEUS populations were visited at least once to search for *A. monanthes* plants. A census was conducted for each extant population. Each plant's number of sterile fronds, number of fertile fronds, and maximum frond length were recorded. In 2001, a few additional parameters were recorded at all populations except Neversink, Maple Springs Branch 3, and Whitewater Falls: the number of new sterile fronds (the current year's fronds can usually be distinguished from old fronds by their light green unweathered pinnae), the number of new pending fertile fronds (sori present), number of dead stipes (actually the stipe plus rachis, which are analogous to petiole plus midrib in angiosperms), and if any dead stipe was longer than the live fronds, it too was measured in length. These additional parameters were measured to get a sense of changes in plants over time. When it was possible to track the same plants from 2000 to 2001 (at Maple Springs Branch 5, both Cane Creek populations, both Florida Caverns populations, and Coley Creek), changes in each plant's size were calculated.

Where many tiny clumped plants made it difficult to keep track of individuals (e.g. Maple Springs Branch 3, Whitewater Falls) for a detailed census, a simpler census was conducted. The number of plants and total fertile fronds in each clump (multiple fertile fronds were likely to be from the same plant, but this was not necessarily so) were counted, and the largest plant in each clump was categorized as small (<6 cm maximum frond length), medium (6-16 cm maximum frond length), or large (>16 cm maximum frond length). In 2001, several parameters were added to this simplified census: for each clump, dead plants were counted, the presence or absence of new growth was recorded, and if a clump contained any dead stipe longer than the longest living frond, the size class (small, medium, or large) of the dead stipe was also recorded.

Demographic analysis

For the SEUS populations studied with the detailed census, each plant was assigned to a developmental stage (unrelated to the size categories used in the simplified census protocol): plants with maximum frond length of 3 cm or less were called

"sporelings" (such plants could turn out to be a different species because many ferns look the same at this stage), plants with a maximum frond length greater than 3 cm were called "juveniles" if they had no fertile fronds or "adults" if they had any fertile fronds. Relative abundance of each stage was calculated for populations containing all three stages (hereafter called "mature" populations) where all plants were likely to be *A. monanthes* (i.e. Maple Springs 5, Guess Creek Cave, both Cane Creek populations, and Neversink; the Florida Caverns populations are mixed with *A. heteroresiliens*, so sporelings cannot be assigned to either species over the other, and Maple Springs 3 was only studied with a size-based census, while the remaining populations did not contain all three stages). Relative abundances (% of total population) of each developmental stage were averaged among mature populations with similar demographic profiles (Guess Creek Cave was excluded because it was unusually skewed towards adults) to create a generalized stage distribution for mature populations. Average mature population size was calculated (excluding Neversink because of its atypically large size, but including Maple Springs 3 because plants were counted even though not assigned to stages), and the average stage distribution was multiplied by this number to derive an average mature population vector. The annual spore production of an average mature population was calculated as shown in **Table 9**. This was added to the average stage distribution as a very rough estimate of the average spore bank. Natural spore dormancy for *A. monanthes* is unknown but probably extends beyond one year because of the protective exospore (see discussion below), however many spores are probably lost immediately to unsuitable habitat. The annual spore production of the population was used as a compromise spore bank estimate.

Segment analysis was then performed on the average mature population vector: survivorship was calculated for each developmental stage, keeping in mind that it takes years for a perennial fern to pass from one stage to the next. Attempts were initially made to estimate the age of plants at the various stages (e.g. using the observed mean change in maximum size from 2000 to 2001 as an estimate of annual length increase, or the observed mean number of new fronds as an estimate of annual frond production) but there was no way to test the validity of these methods for estimating age without long-

term monitoring of marked plants, so these attempts were abandoned. Lab-raised plants could have provided an artificially fast but still useful ontogeny for *A. monanthes* sporophytes, but SEUS plants raised from spores always died upon entering the sporophyte phase.

The three populations (both Cane Creek subpopulations and Maple Springs Branch subpopulation 5) with multiple identifiable plants that were tracked individually from 2000 to 2001 were collectively used to calculate annual transition probabilities based on individual plants' changes (following Lefkovitch, 1965) for a generalized stage-based transition matrix by averaging the transition probabilities observed within each population. If a given plant could not be relocated in 2001, it was assumed to have died. Fecundity was calculated as shown in **Table 9**.

All biologically realistic transition probabilities (i.e. everything except sporeling-to-adult, adult-to-sporeling, sporeling-to-spore, or juvenile-to-spore) were positive with one exception which necessitated an alternative calculation method. The three subpopulations examined showed no cases of advancement from juvenile to adult. This transition must occur occasionally based on the existence of adult plants in these populations, and was observed in a Florida Caverns population plant. This plant was incorporated into the matrix model to make it irreducible (all stages having the possibility of reaching all other stages). No other plants at Florida Caverns could be positively identified, making this transition probability 1.0 with a sample size of one, so averaging

Table 9: Derivation of fecundity estimates

	mean new fertile fronds/ fertile plant/ year		mean pinnae/ fertile frond		mean sori/ pinna of fertile frond		mean sporangia/ sorus		spores/ sporangia			mean fertile plants/ mature pop.
mean spores/ fertile plant/ year =	2.6	x	62	x	1.4	x	80	x	32	=	5.9×10^5	
estimated mean spores/ pop./ year =											5.9×10^5 x 3.3 = 1.9×10^6	

this probability with the zero probabilities from other populations would have skewed the results. Instead the juveniles from all four subpopulations were pooled so that the probability of one out of the 14 total juveniles advancing (=0.07) could be used as this probability.

The only transition probability that could not be estimated was the probability of non-germinated spores remaining viable in the spore bank. The long-term viability of fern spores in nature has rarely been studied; in the one known study, Dyer & Lindsay (1992) found a 40% decrease in viability after two years in a natural temperate spore bank of *Athyrium filix-femina, Blechnum spicant,* and *Dryopteris* spp. However spores kept in various artificial conditions in the lab range widely in their viability period, from several days to several decades (Lloyd & Klekowski, 1970), so species probably also differ in spore lifespan in natural conditions. Because *A. monanthes*' spore lifespan is unknown and there is no consensus on typical values for ferns, a spore-to-spore transition value was chosen to create a stable population size even though there is no evidence that SEUS *A. monanthes* populations are stable in size.

The average population vector was multiplied 100 times by the estimated Lefkovich transition matrix (with various values for the spore-to-spore transition) to determine whether a hypothetical average population was at all close to demographic equilibrium. Because the spore-to-spore transition probability could not be estimated, it is impossible to project future population size, only future demographic ratios.

Elasticity analysis was not performed because the data was assumed not to accurately represent SEUS *A. monanthes* populations because it was based on a single year's transition at only three populations. Instead the model was informally adjusted to determine which transition probabilities would have to change to bring the model into an equilibrium close to the original population vector. These transitions were then considered to be ones limiting population persistence.

Note that these demographic models do not address the gametophyte phase. I am treating this phase as a "black box" connecting the spore stage to the sporeling stage because gametophytes in the populations were not investigated. It was impractical to look for microscopic gametophytes in the thick bryophyte mats in which sporophytes

occur except by taking bryophyte samples back to the lab for examination under a dissecting microscope. This should probably have been attempted but was not. Therefore it remains unknown whether spore germination or sporophyte initiation is the main limiting factor in the complex spore-to-sporeling transition.

To try to determine the long-term stability of populations, changes in population size were investigated by searching for documentation of historical SEUS population sizes over time. Very little information was available, but the findings were examined nonetheless.

For an informal comparison of plant size of SEUS and neotropical plants, fertile plants from all regions were measured for number of sterile fronds, number of fertile fronds, number of dead and broken-off fronds, and maximum frond length. This was performed using live plants in the SEUS but dried specimens for the tropics. The neotropical specimens' original populations were not censused (except for the Dominican population Valle Nuevo) except that population size was estimated, but plants collected were generally representative of the rest of the plants in size.

RESULTS

Substrate and microclimate characterization

Bryophyte associates at SEUS sites

Table 10 lists the bryophytes found at the three locations in each SEUS site sampled. The following bryophytes were found at multiple sites and only in the actual *A. monanthes* location at those sites: *Bryoandersonia illecebra, Ctenidium molluscum/malacodes, Mnium cuspidatum, Myurella sibirica, Plagiochila echinata,* and *Plagiomnium carolinianum. Anomodon attenuatus, Brachythecium oxycladon, Plagiomnium ciliare,* and *Thuidium delicatulum* were found in both actual *A. monanthes* locations and exposed locations. *Bryhnia graminicolor, Cyrtohypnum minutulum, Gymnostomum aeruginosum,* and *Thamnobryum alleghaniense* were found in both actual *A. monanthes* locations and most-moderated locations. Unfortunately the only bryophytes found at more than two *A. monanthes* locations were found at an equal

Table 10: Bryophytes at various locations within SEUS *A. monanthes* populations

Species found in multiple locations are color-coded to facilitate comparison of locations

(sub)population	most moderated location	*A. monanthes* location	exposed location
Florida Caverns Exit Sink subpop. (FL)	*Fissidens taxifolius*, *Eurhynchium pulchellum*, ***Isopterygium tenerum***, *Pallavicinia lyellii*	***Porella pinnata***, *Plagiomnium cuspidatum*, *Brachythecium oxycladon*, *Anomodon rostratus*, *Cyrtohypnum minutulum*	*Anomodon rostratus*, *Eurhynchium pulchellum*
Florida Caverns Walt's Misery subpop. (FL)	*Pallavicinia lyellii*	*Plagiomnium cuspidatum*, ***Haplocladium microphyllum***	
Balcony Sink (AL)	*Bryhnia graminicolor*	*Bryhnia graminicolor*	***Atrichum angustatum***, ***Leucobryum albidum***, *Hypnum curvifolium*, ***Dicranella heteromalla***
Neversink (AL)	***Taxiphyllum deplanatum***	*Plagiomnium carolinianum*, *Gymnostomum aeruginosum*	***Dicranum scoparium***, *Anomodon attenuatus*
Guess Creek Cave (AL)	*Gymnostomum aeruginosum*, ***Mnium stellare***, *Thamnobryum alleghaniense*	***Campylium chrysophyllum***, ***Fissidens dubium***, ***Rhodobryum ontariense***	*Anomodon rostratus*, *Brachythecium oxycladon*, ***Tortella humilis***
Upper Whitewater Falls (NC)	*Thamnobryum alleghaniense*, ***Mnium carolinianum***, ***Fissidens cristatus***, ***Taxiphyllum taxirameum***, *Cyrtohypnum minutulum*, ***Haplohymenium triste***	***Radula sullivantii***, ***Vittaria appalachiana*** (fern gametophyte), *Thamnobryum alleghaniense*, *Myurella sibirica*, ***Mnium hornum***	***Sematophyllum demissum***, ***Dicranum montanum***
Thompson River (SC)		***Dumortiera hirsuta***, *Thamnobryum alleghaniense*	***Climacium americanum***, unidentified lichen
Coley Creek (SC)		***Metzgeria conjugata***, *Ctenidium molluscum/malacodes*, *Cyrtohypnum minutulum*	

Table 10 (continued)

(sub)population	most moderated location	*A. monanthes* location	exposed location
Glade Fern Ravine (SC)		*Thamnobryum alleghaniense*, *Anomodon attenuatus*	
Maple Springs Branch subpop. 1 (NC)		***Plagiothecium cavifolium***	*Thamnobryum alleghaniense*, *Atrichum oerstedianum*
Maple Springs Branch subpop. 3 (NC)	*Thamnobryum alleghaniense*, *Anomodon rostratus*	*Thamnobryum alleghaniense*, *Plagiochila asplenioides*, ***Plagiomnium affine***, *Myurella sibirica*	*Leucodon sciuroides*, ***Hypnum fertile***, *Atrichum oerstedianum*, *Plagiomnium ciliare*
Maple Springs Branch subpop. 5 (NC)	*Anomodon rostratus*, *Thamnobryum alleghaniense*, *Fissidens taxifolius*, *Bryhnia graminicolor*, ***Eurhynchium strigosum***	*Plagiothecium laetum*, ***Mnium marginatum***, *Bryhnia graminicolor*, *Plagiomnium carolinianum*	*Pylaisiadelpha tenuirostris*, ***Platyiomella lescurii***, ***Hygroamblystegium tenax***
original Cane Creek subpop. (SC)	*Thamnobryum alleghaniense*, *Bryhnia graminicolor*	*Bryoandersonia illecebra*, *Plagiochila echinata*	*Hypnum curvifolium*
new Cane gravel seep subpop. (SC)	*Thamnobryum alleghaniense*	*Thamnobryum alleghaniense*, *Brachythecium oxycladon*, *Plagiochila echinata*, *Bryoandersonia illecebra*, *Plagiomnium ciliare*, *Ctenidium malacodes*	

number of exposed or most-moderated locations, so there is no single bryophyte that can be relied upon as a universal indicator of good *A. monanthes* microhabitat. The majority of the bryophytes found in *A. monanthes* locations and almost all species in most-moderated locations are known to be restricted to moist climatically moderated sites, indirect evidence that *A. monanthes*' habitat is climatically moderated year-round.

Rock composition

Table 11 lists the rock types that *A. monanthes* was recorded from and **Table 12** their mineral composition. In Alabama and Florida, *A. monanthes* was always found on limestone, though the particular limestone varied from population to population. In the Carolina gorges in which *A. monanthes* occurs, no limestone is available due to the area's mainly igneous origin followed by metamorphism. The populations here live upon Rosman fault zone breccia, Toxaway gneiss, amphibolite, biotite gneiss, and Brevard schist. Of these rocks, only the first contains calcite (Robert Hatcher, personal communication). Toxaway gneiss and amphibolite contain calcium silicates, but silicates are notoriously difficult to break down, so the calcium remains mostly unavailable to plants. Biotite gneiss and Brevard schist contain no significant sources of calcium but perhaps these sites also contain calcareous rocks that were not sampled, considering that soil samples from the Brevard schist population contained up to 4040 ppm calcium (Gaddy, 1990). The substrates of the Carolina populations suggest that *A. monanthes* has a need for modest amounts of calcium. Rocks from two Dominican populations were also sampled. One was limestone and the other basalt, another slow-weathering calcium silicate rock, so epipetric neotropical populations appear to experience the same range of rock types as SEUS populations.

Soil and pH

Since SEUS *A. monanthes* normally occurs in a bryophyte mat directly upon a rockface, soil characteristics are not directly relevant to its microhabitat. However, soil characteristics are presumed to reflect parent rock characteristics and are often easier to measure, so soil composition and pH were of interest. Soil was sampled from two SEUS (Guess Creek Cave, AL, and Glade Fern Ravine, SC) and two Dominican (Valle Nuevo

Table 11: Identification of rocks hosting *A. monanthes* plants at each population

site	rock identity	source of identification
San Felasco Hammock (extirpated, FL)	Ocala limestone	Sam Cole, pers. comm.
Florida Caverns (FL)	Marianna limestone	Mitchell, 1963
Balcony Sink (AL)	Bangor limestone	Alan Cressler, pers. comm.
Neversink (AL)	Bangor limestone	Alan Cressler, pers. comm.
Guess Creek Cave (AL)	Monteagle limestone	Alan Cressler, pers. comm.
Whitewater Falls (NC)	Toxaway granite gneiss	Robert Hatcher, pers. comm.
Thompson River (SC)	Toxaway granite gneiss	Robert Hatcher, pers. comm.
Coley Creek (SC)	Toxaway granite gneiss	L.L. Gaddy, pers. comm.
Glade Fern Ravine (SC)	Brevard schist	Gaddy, 1990
Maple Springs Branch (NC)	Rosman fault zone breccia	Robert Hatcher, pers. comm.
original Cane Creek subpopulation (SC)	amphibolite	Robert Hatcher, pers. comm.
new Cane gravel seep subpopulation (SC)	biotite gneiss	Robert Hatcher, pers. comm.
Table Rock (extirpated, SC)	biotite gneiss	Garihan & Ranson, 2001
Valle Nuevo (Dom. Rep.)	limestone	Robert Hatcher, pers. comm.
Palo de Agua (Dom. Rep.)	basalt	Robert Hatcher, pers. comm.

and Palo de Agua) populations. **Table 13** lists the samples' levels of phosphorus, potassium, calcium, nitrogen, organic matter, and pH. The Guess Creek Cave and Palo de Agua soils are presumably derived from limestone because limestone was the most common rock at each site. Consequently these two samples show the highest concentration of calcium (5967 and 4264 ppm, respectively) and highest pH (respectively 8.2 and 7.0). Glade Fern Ravine had lower values (3000-4040 ppm calcium and pH 6.5) (Gaddy, 1990) but still elevated in comparison to the standard acidic soil of the Blue Ridge (typical pH values below 5.0) (Pittillo et al., 1998). Valle Nuevo had the lowest concentration of calcium (2519 ppm) and pH (5.9), and may be derived from

Table 12: Mineral composition of the rocks hosting *A. monanthes* listed in Table 11

general type	specific type, if known	mineral composition	calcareous?	reference
limestone	Bangor	bioclastic & oolitic limestone, w/ micrite, shaly argillaceous limestone, calcareous clay shale, earthy dolostone, blocky mudstone	yes	Raymond et al., 1988
limestone	Monteagle	cross-bedded oolitic limestone, w/ micrite, bioclastic limestone, dolostone, dolomitic limestone, argillaceous limestone, clay shale	yes	Raymond et al., 1988
limestone	Marianna	92-95% calcium carbonate	yes	Mitchell, 1963
limestone	Ocala	Upper unit: packstones and grainstones with some wackestones and mudstones	yes	Randazzo, 1997
breccia	Rosman fault zone	quartzite fragments containing tiny white calcite veins	somewhat	Robert Hatcher, personal communication
granite gneiss	Toxaway	plagioclase feldspar, quartz, biotite, w/ a few other things	silicates, so most calcium unavailable	Hatcher & Goldberg, 1991
biotite gneiss		biotite, quartzite, feldspar, sometimes with amphibolite, mica schist, and metagabbro	silicates, so most calcium unavailable	Garihan & Ranson, 2001
amphibolite		hornblende, plagioclase feldspar	silicates, so most calcium unavailable	Robert Hatcher, personal communication
schist	Brevard	"dark or silvery indurated [hardened] mica schist, with interbedded lenses of fine grained augen gneiss, occasional migmatite zones, and minor injected granite"; micaceous quartz-chlorite-muscovite rock containing a small amount of magnesium	not usually	Cazeau, 1967; Robert Hatcher, personal communication
basalt		pyroxene, calcium feldspar, olivine	silicates, so most calcium unavailable	Chernicoff, 1999

Table 13: Soil and bryophyte mat characteristics of select *A. monanthes* sites

site	soil parent material	% organic matter (soil)	% total N (soil)	ppm P (soil)	ppm K (soil)	ppm Ca (soil)	pH (soil)	pH (bryophyte mat)
Glade Fern Ravine* (SC)	Brevard schist					3000-4040	6.5	7.1
Florida Caverns Exit Sink (FL)	limestone							
Guess Creek Cave (AL)	limestone	5.5	0.15	9	181	5967	8.2	7.0
Valle Nuevo (Dom. Rep.)	basalt	23.8	0.78	22	201	2519	5.9	5.5
Palo de Agua (Dom. Rep.)	limestone	18.5	0.75	5	72	4264	7.0	6.5

**information taken from Gaddy, 1990*

conglomerate containing basalt fragments (based on a rock sample identified by Robert Hatcher). This again shows that *A. monanthes* can survive with moderately low calcium availability in certain circumstances.

pH was also measured from the moisture in *A. monanthes'* bryophyte mats (also **Table 13**) of some SEUS and Dominican populations. Values ranged from 5.5 to 7.1 and were somewhat lower than corresponding soil pH values due to the acidity of rainwater. No pH measurements were taken in the Carolinas, unfortunately, but they would likely be similar to Valle Nuevo's 5.5 value, since Valle Nuevo's rock substrate appears to be basalt, yet another calcium silicate.

Year-round microclimate data

Temperature

Figure 35 shows the maximum and minimum annual temperatures recorded at three SEUS sites for the years 2000 and/or 2001. The only site in which thermometers were successfully maintained at a range of microclimates, Cane Creek (SC), showed a notable difference between thermometers for maximum temperatures only between the *A. monanthes* location and the exposed location in 2000-2001. The two locations are quite similar in maximum and minimum temperatures in 2001-2002, both showing much lower maximum temperatures than in the previous year. This is consistant with data from the

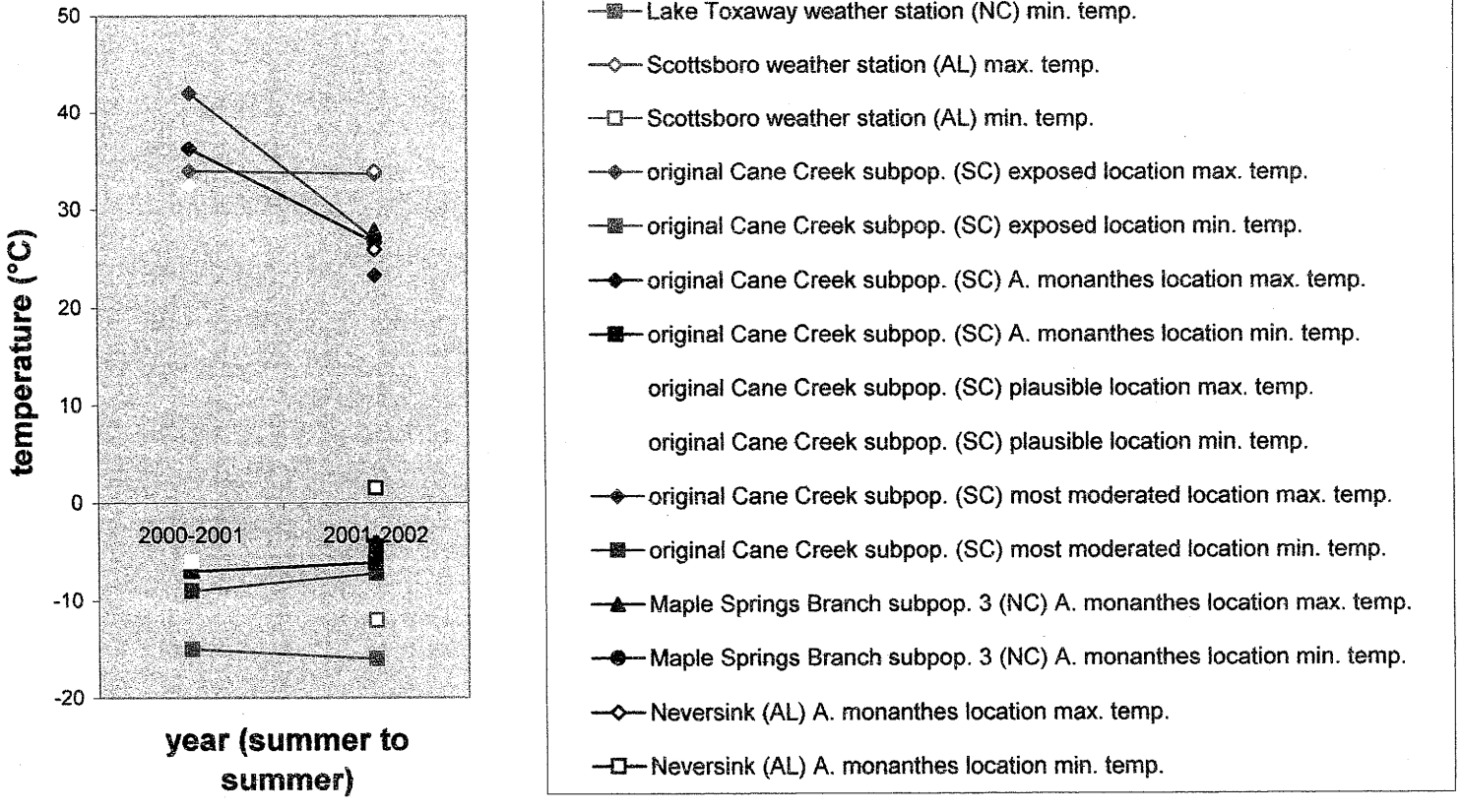

Figure 35: Yearly maximum and minimum temperatures at various locations within *A. monanthes* sites and at corresponding weather stations. Values modified slightly to reveal overlapping points.

NCDC weather station at Lake Toxaway, in the northern part of the Jocassee Gorge area; maximum temperatures there were noticeably lower for 2001 than 2000. A most-moderated location was also monitored starting in 2001, and it showed warmer minimum temperatures and cooler maximum temperatures than the other microclimate locations at that site.

The Cane Creek *A. monanthes* location maximum and minimum temperatures were compared to the other two *A. monanthes* locations monitored. Cane Creek, Maple Springs Branch subpopulation 3 (NC), and Neversink (AL) all showed similar maximum and minimum temperatures with one exception: Neversink had a notably warmer minimum temperature (slightly above rather than notably below freezing) than the two Carolina sites monitored. Because the weather station data shows a similar difference between the Alabama and Carolina minimum temperatures, the difference in microclimate temperatures probably is a simple reflection of macroclimate and sinkholes are not superior to gorges in their ability to moderate extreme temperatures.

The populations' yearly maximum and minimum temperatures were also compared to those of the nearest NCDC weather station (**Fig. 35**). The weather station data should be most comparable to the exposed location data for each population since that location was least sheltered. The only population where the exposed location was successfully monitored was Cane Creek (South Carolina). It showed an inconsistent relationship with the corresponding weather station (Lake Toxaway, North Carolina) as far as maximum temperatures: the exposed Cane Creek location had a much higher maximum temperature than the Lake Toxaway station in 2000 but a much lower one in 2001. However minimum temperatures showed a consistent relationship: both years had warmer minimum temperatures than at Lake Toxaway as expected. The *A. monanthes* locations at all three sites monitored (Cane Creek, Maple Springs Branch 3 and Neversink), were all notably moderated in annual maximum and minimum temperatures relative to their corresponding weather stations (with the same exception of maximum temperature at Cane Creek), as would be expected for *A. monanthes*' protective microhabitat.

Season-specific climatological data

Temperature

All further microclimate data refers to summer conditions in the southeastern U.S. (the hot season) and Costa Rica (the wet season) and winter conditions in the Dominican Republic (the dry season), since no remote measuring devices for these parameters were within the budget of this project. Air and substrate temperatures generally showed the same trends (**Figs. 36** and **37**) between locations at various SEUS sites. Exposed locations usually had the highest temperatures, but occasionally no higher than the other two locations (equal temperatures were more frequent in air temperature than substrate temperature) at each site. Only at Maple Springs subpopulation 3 was the *A. monanthes* location substrate warmer than the exposed location substrate. *A. monanthes* locations and most-moderated locations were generally similar in air temperature and in substrate temperature.

NCDC weather station daily maximum air temperature readings were also compared to the microclimate air temperature of a given day (**Fig. 36**). All Alabama and Florida weather station maximum daily temperatures (from the Scottsboro and Quincy stations, respectively) were much higher than the corresponding population microclimate readings, while Carolina weather station temperatures varied in their relationship to microclimate readings.

Dominican populations had air and substrate temperatures within the low end of SEUS *A. monanthes* locations. Costa Rican populations were much cooler than most SEUS locations in air and substrate temperatures. The inclusion of winter data from the southeastern U.S. would obviously show the opposite relationship.

Water relations

Figure 38 presents relative humidity data for all locations sampled. Relative humidity showed no strong differences among locations at SEUS sites. Surprisingly, exposed locations generally had slightly higher relative humidity than the other two location types. Most-moderated locations were sampled only in 2001, so they have a smaller sample size than exposed or *A. monanthes* locations, but they appeared to be

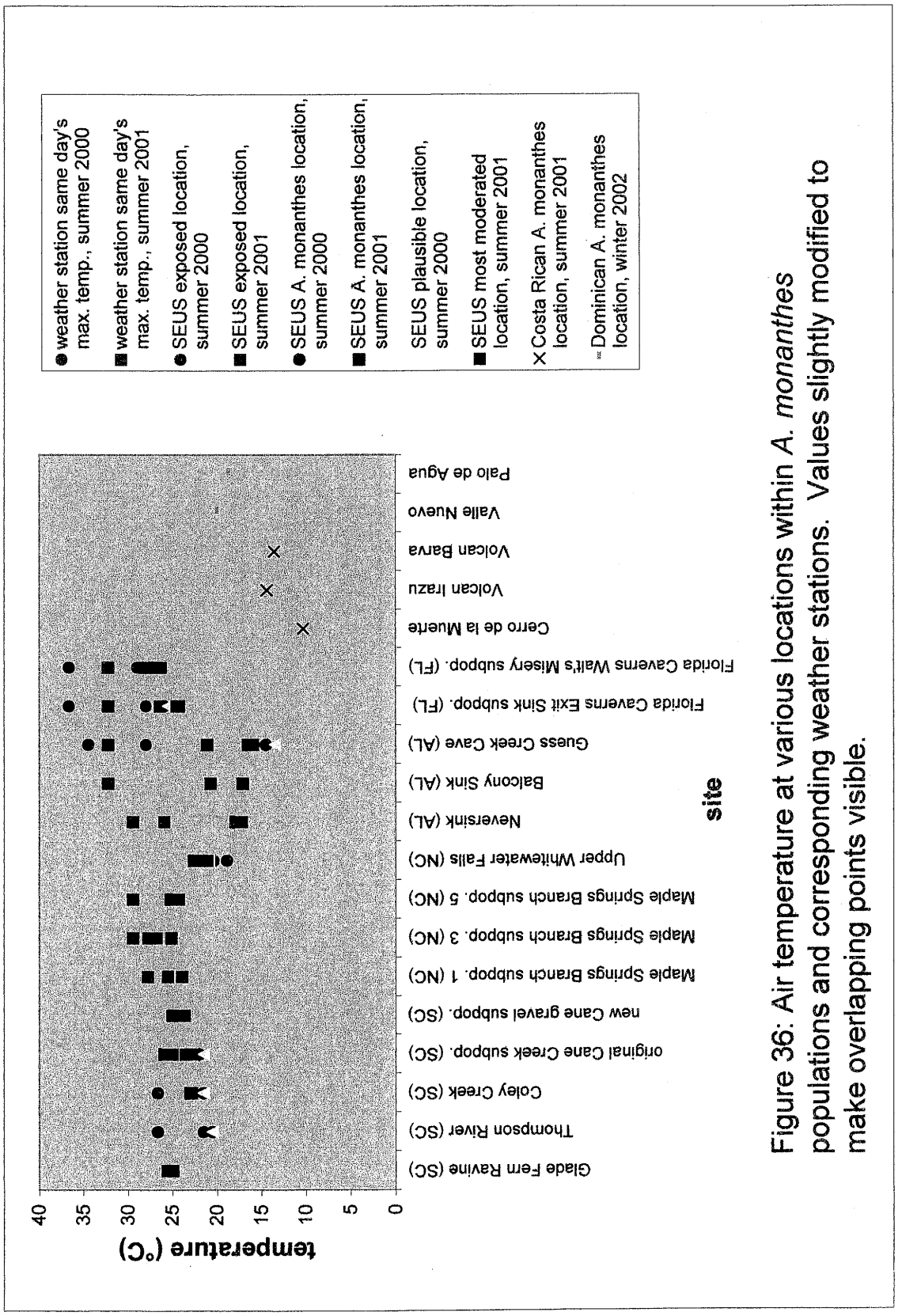

Figure 36: Air temperature at various locations within *A. monanthes* populations and corresponding weather stations. Values slightly modified to make overlapping points visible.

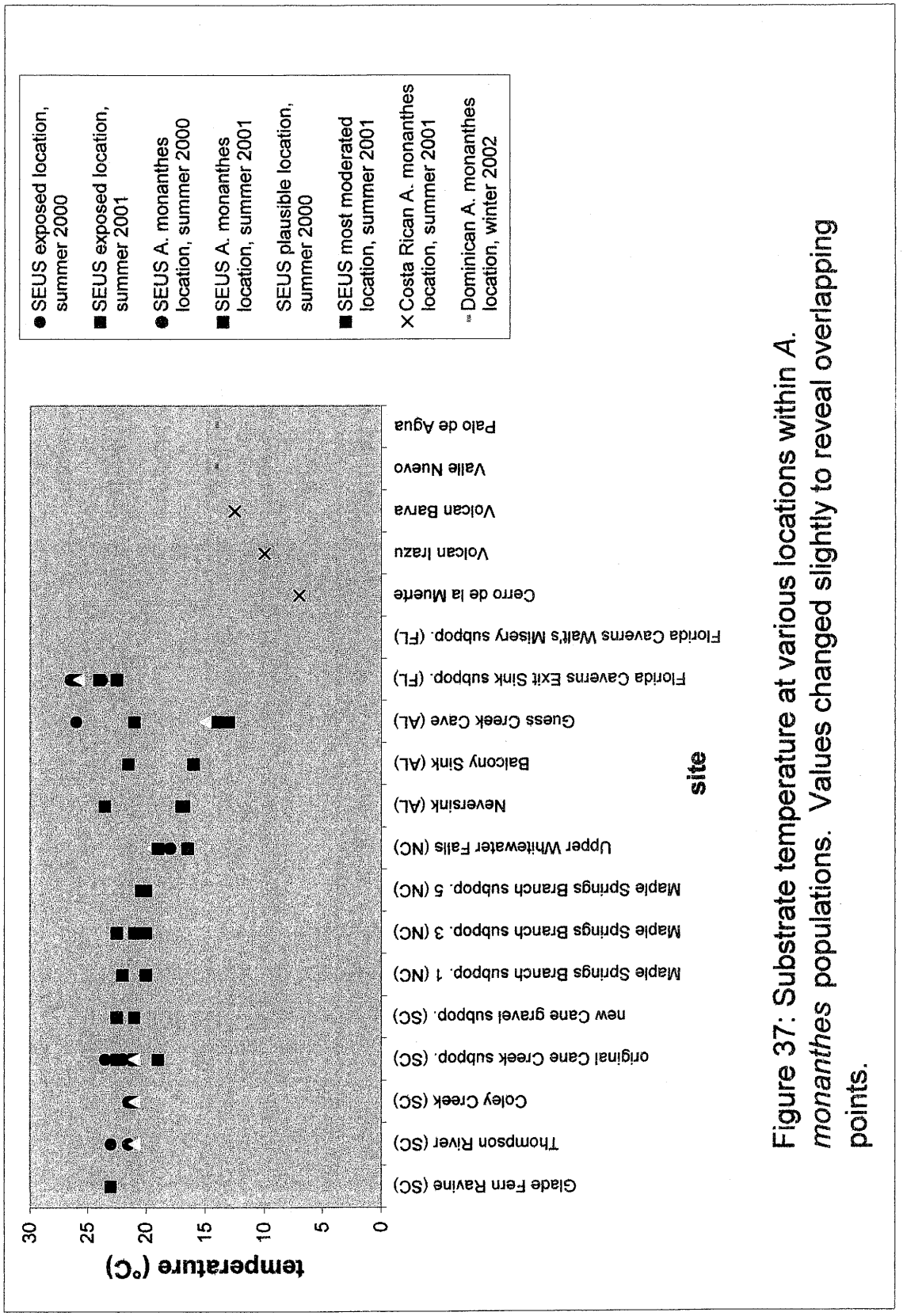

Figure 37: Substrate temperature at various locations within *A. monanthes* populations. Values changed slightly to reveal overlapping points.

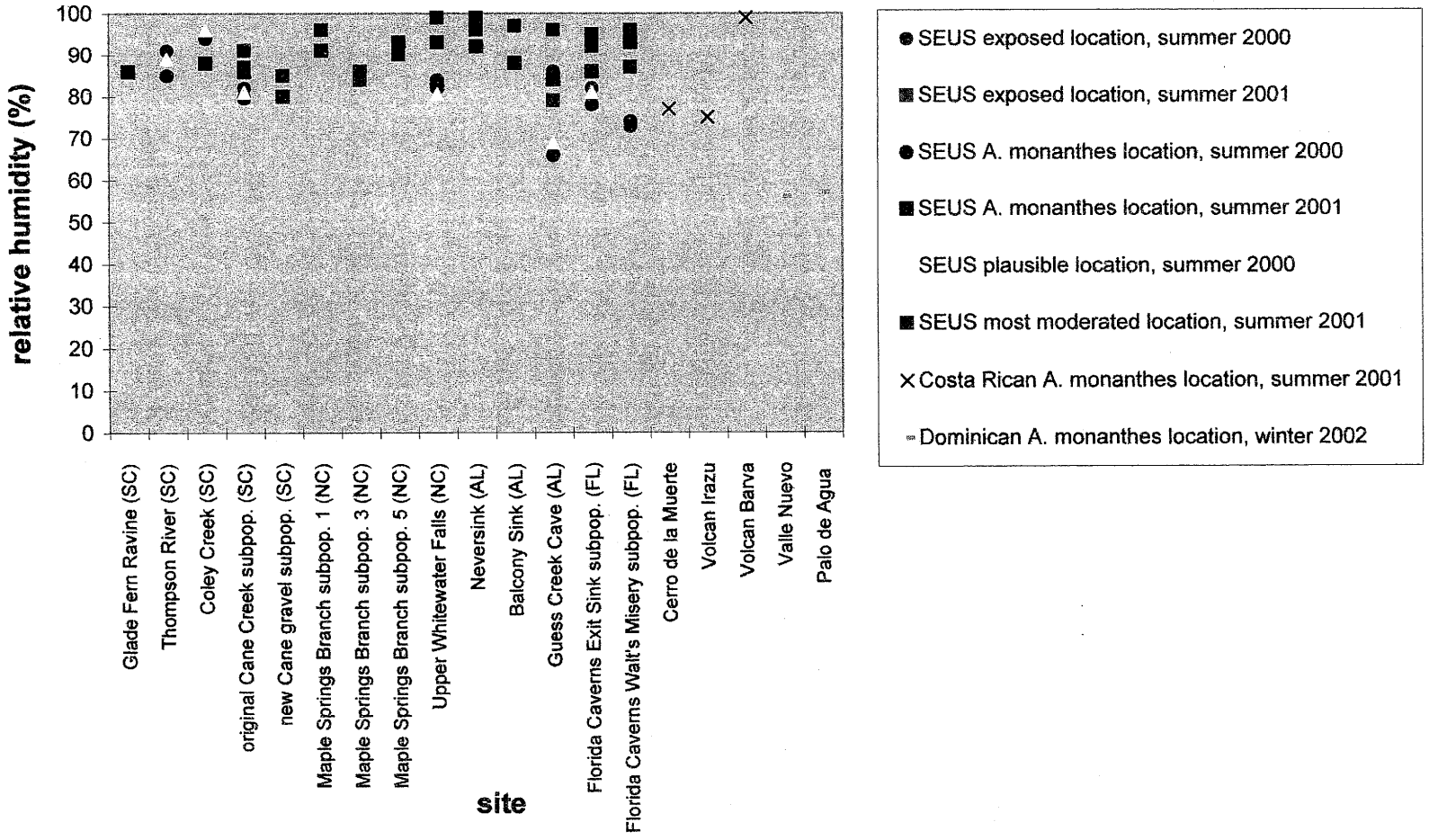

Figure 38: Relative humidity at various locations within *A. monanthes* populations. Values modified slightly to reveal overlapping points.

equal or slightly more humid than *A. monanthes* sites, but not quite as humid as exposed sites. The largest differences between *A. monanthes* and exposed locations were observed in Guess Creek Cave, AL, (large both years) and Upper Whitewater Falls, NC (large only in 2001). Although the *A. monanthes* locations had slightly lower humidity than other locations, they were still reasonably humid: the mean relative humidity for *A. monanthes* locations was 80% in 2000 and 87% in 2001.

The relative humidity of SEUS *A. monanthes* locations was also compared to neotropical *A. monanthes* locations (also **Fig. 38**). SEUS locations were more humid than the pooled neotropical locations. Dominican sites (visited during the dry season) displayed much lower relative humidity, but Costa Rican sites (visited during the wet season) covered the whole range of values observed at various types of SEUS microclimate locations. This limited data suggests that neotropical *A. monanthes* populations may often experience lower relative humidity than SEUS populations, but year-round SEUS data would be necessary to determine whether this is a consistant difference between regions.

Figure 39 plots relative substrate moisture (measured by moisture uptake to a facial tissue as explained above) for the locations sampled. The substrate moisture data comparing microhabitats within sites yielded no conclusive trends largely because of its small sample size and lack of replication. *A. monanthes* locations were no wetter than exposed locations for most sites, but much wetter than either other location at Upper Whitewater Falls (NC) and drier (along with the most moderated location) than the exposed location at the original Cane Creek subpopulation (SC). *A. monanthes* locations were equally moist to slightly drier (or much drier at Maple Springs Branch subpopulation 3 [NC] at which the most moderated location was saturated and therefore much wetter than the other two locations) than most-moderated locations, the exception being the aforementioned Whitewater site at which the *A. monanthes* location substrate was saturated and the other two were not. In general, most locations were far from substrate saturation, as only the *A. monanthes* locations of Coley Creek (SC) and Upper Whitewater Falls (NC) and the most moderated location of Maple Springs Branch subpopulation 3 (NC) were able to quadruple the weight of the absorbing tissue within

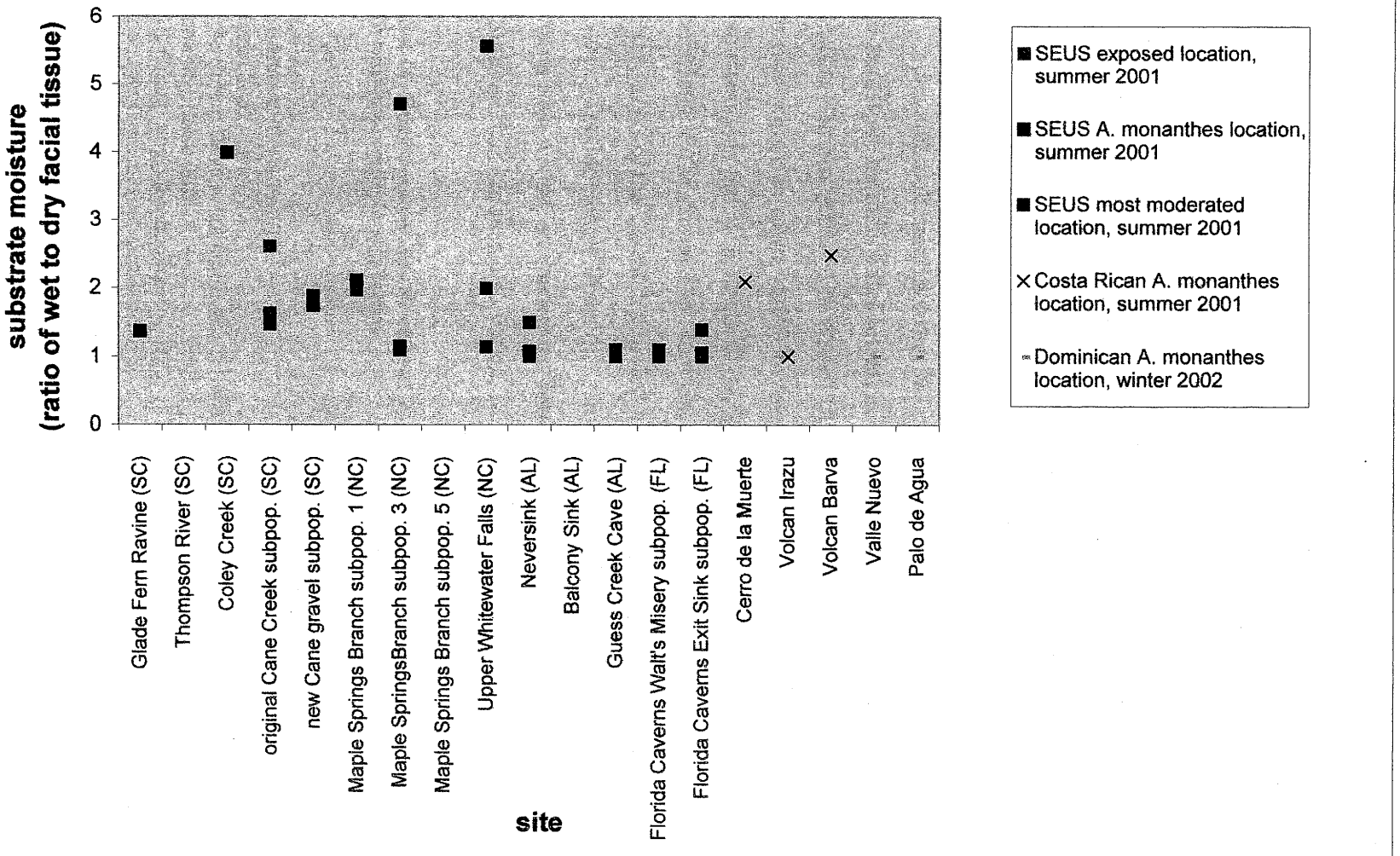

Figure 39: Substrate moisture at various locations within *A. monanthes* populations. Values modified slightly to reveal overlapping points.

the alloted time. Most locations did not manage to double the tissue's weight. With a few exceptions, the whole area at *A. monanthes* sites appears to be roughly equal in moisture availability during the summer, most locations being relatively low in moisture.

SEUS *A. monanthes* locations were also compared to neotropical *A. monanthes* locations for substrate moisture (**Fig. 39**). Dominican locations were at least as dry as SEUS locations, with Costa Rican locations ranging from equally dry to slightly moister. This is not surprising given that the Dominican Republic was sampled during its dry season and Costa Rica during its wet season. Combining these two neotropical countries' observations, it is likely that these populations annually experience the same range of moisture availability as observed in the southeastern U.S.

Several rock samples from SEUS and Dominican populations were compared with sandstone for their water retention capacity (**Table 14**). Florida Caverns' Marianna limestone samples actually surpassed sandstone in water uptake, holding 6.0-9.4% of their own weight in water vs. 2.3% that a sample of sandstone held. This is not surprising given extremely pitted structure of the Marianna limestone samples. All other populations' rocks sampled took up less water than sandstone. The marble, schist, and granite gneiss of the Carolina populations were able to hold 0.8-1.5% of their own weight in water. The Monteagle limestone sampled from Guess Creek Cave, AL, only held 0.5% its own weight, so apparently limestones vary greatly in permeability. The two Dominican rock samples also varied, the limestone holding 1.9% and the basalt only

Table 14: Rock permeability at various *A. monanthes* populations in comparison with highly permeable sandstone

site	rock identification	ratio of wet to dry weight
Valle Nuevo (Dom. Rep.)	basalt	1.002
Guess Creek Cave (AL)	Monteagle limestone	1.005
Maple Spring Branch, subpopulation 5 (NC)	Rosman fault zone breccia	1.007
Thompson River (SC)	Toxaway gneiss	1.008
Cane Creek: original subpopulation (SC)	amphibolite	1.014
Cane Creek: gravel seep population (SC)	biotite gneiss	1.014
Whitewater Falls (NC)	Toxaway gneiss	1.015
Palo de Agua (Dom. Rep.)	limestone	1.019
standard	sandstone	1.023
Florida Caverns Exit Sink subpopulation (FL)	Marianna limestone	1.060
Florida Caverns Walt's Misery subpopulation (FL)	Marianna limestone	1.094

0.2% of their respective weights. No Costa Rican samples were taken, because all populations studied were terrestrial rather than epipetric (whereas only some Dominican populations were terrestrial); rock permeability is therefore not as relevant in the tropics as in the southeastern U.S.

Light

For logistical reasons, some light readings were recorded in lux (for visible light), others in quanta (for photosynthetically active light), and still others in both. This created an unfortunate situation where many populations were recorded in different non-interconvertible measures of light. Therefore interpopulation comparisons can only be made safely where populations were recorded in the same units. **Figure 40** plots all readings in lux, including quanta-only measurements that were roughly converted into lux using a regression line based on locations where both measures were taken (therefore these values should not be taken literally). **Figure 41** plots the original quanta measurements. **Table 15** lists both original lux and quanta values. Within populations, *A. monanthes* locations always received more light (with the exception of Upper Whitewater Falls, NC, where the two locations were approximately the same) than the most moderated locations at the same site. Exposed locations received more light, in many cases much more, than *A. monanthes* locations (with the exception of the quanta scale data only from the Walt's Misery subpopulation at Florida Caverns).

Light was also measured in mid-October for comparison, only at Guess Creek Cave, AL. The light at various *A. monanthes* plants was measured at 1075-3230 lux, about an order of magnitude greater than that recorded during summer visits. The loss of canopy leaves in the fall clearly makes a great difference in light availability at this population and presumably others too.

Light levels at neotropical *A. monanthes* locations spanned the range observed in SEUS *A. monanthes* locations. The Costa Rican population at Cerro de la Muerte greatly exceeded the light levels of SEUS *A. monanthes* populations, being more comparable to SEUS exposed locations. The light intensity at one Dominican population, Valle Nuevo, was slightly higher than at the brightest SEUS *A. monanthes* location. The remaining neotropical populations were near the low end of the range of SEUS *A. monanthes*

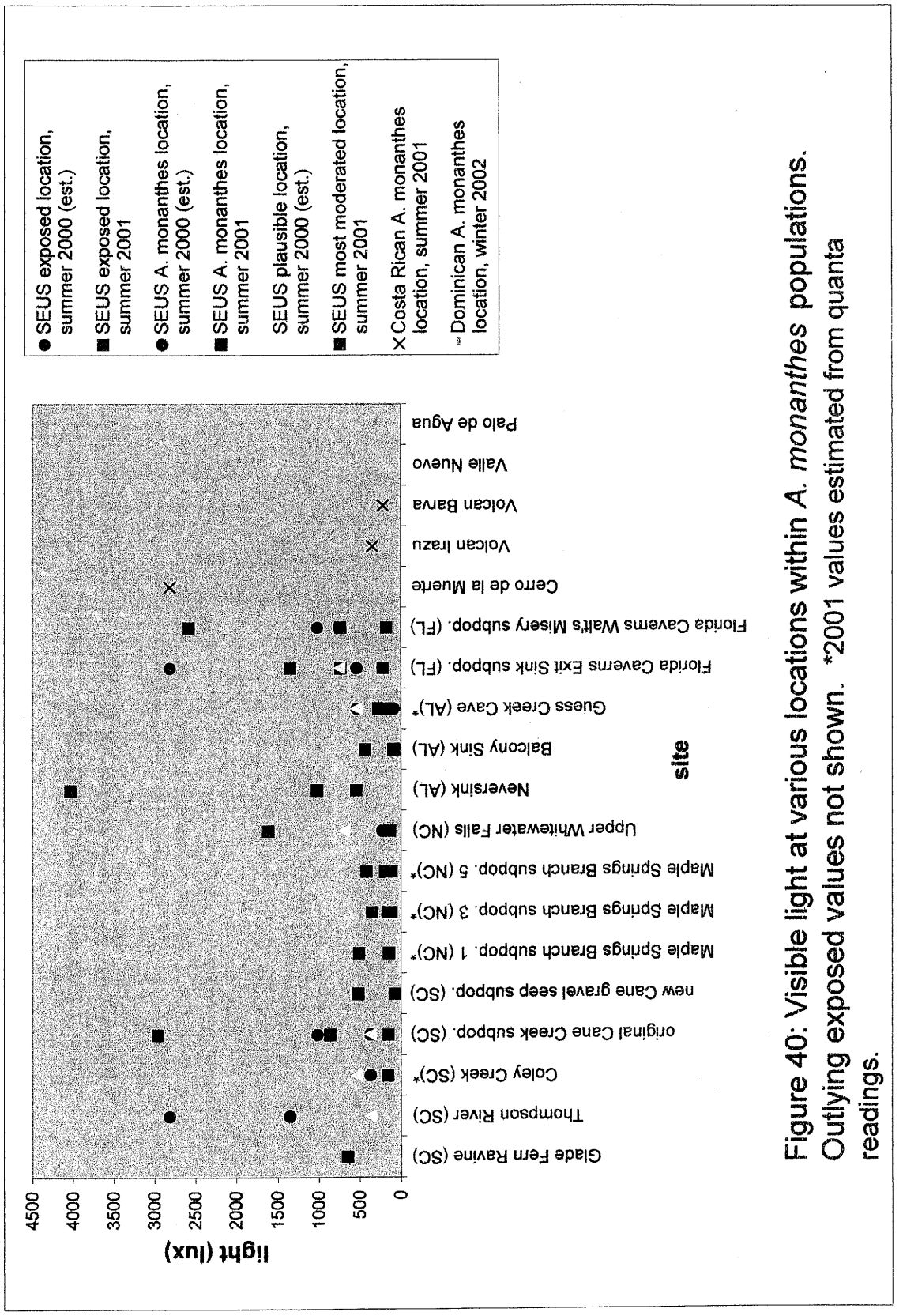

Figure 40: Visible light at various locations within *A. monanthes* populations. Outlying exposed values not shown. *2001 values estimated from quanta readings.

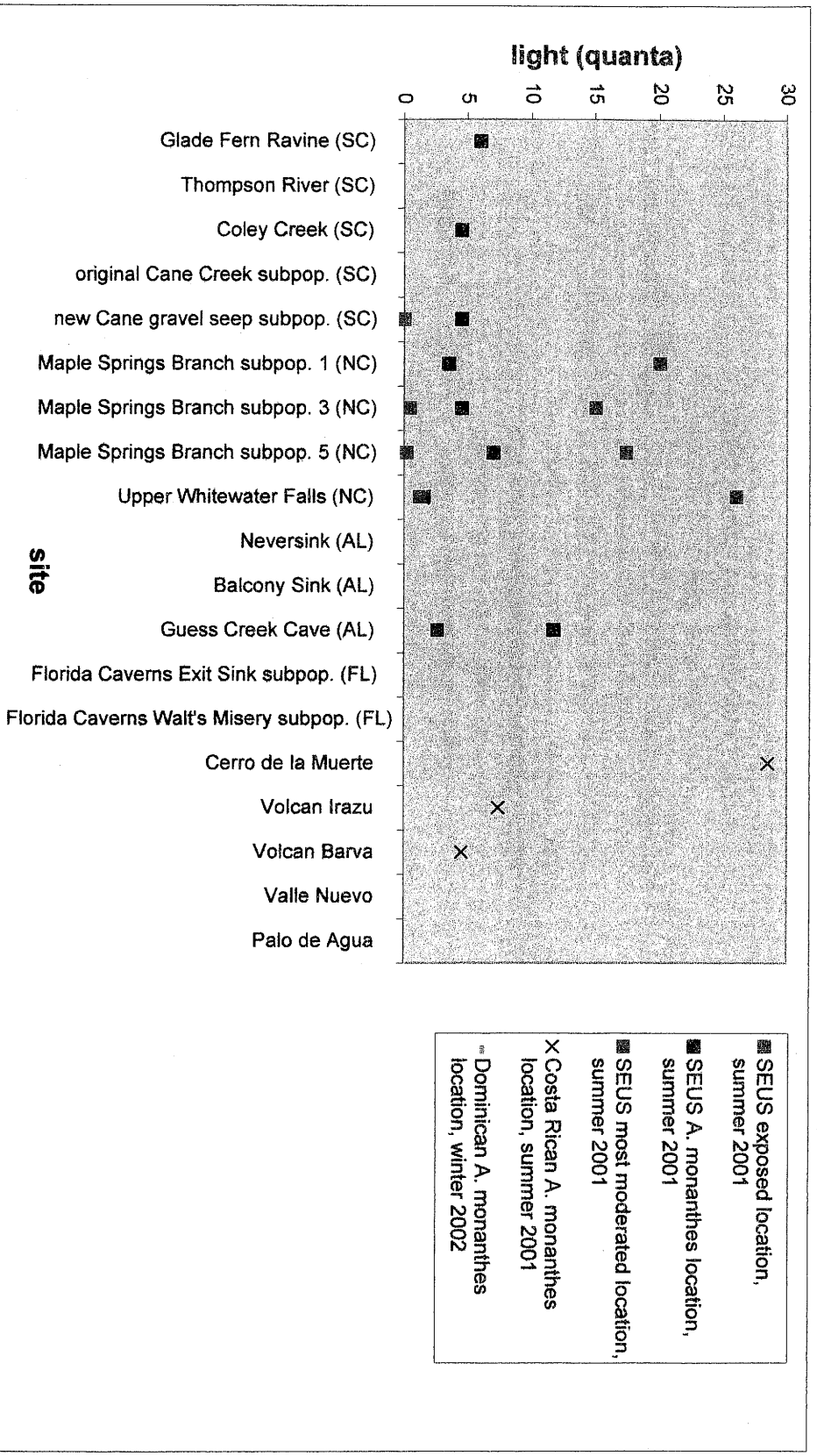

Figure 41: Photosynthetically active light at various locations within *A. monanthes* populations. Values slightly modified to reveal overlapping points. Guess Creek Cave exposed value omitted as outlier.

Table 15: Light intensity at various locations within *A. monanthes* populations

Visible light (lux)							*Photosynthetically active light (quanta)*		
A. monanthes location, summer 2000 (est.)	*A. monanthes* location, summer 2001*	plausible location, summer 2000 (est.)	most moderated location, summer 2001*	exposed location, summer 2000 (est.)	exposed location, summer 2001*	site	*A. monanthes* location, summer 2001	most moderated location, summer 2001	exposed location, summer 2001
	646					Glade Fern Ravine (SC)	6		
1352		368		2816		Thompson River (SC)			
368		535				Coley Creek (SC)	4.5		
368	861	368	151	1019	2959	original Cane Creek subpop. (SC)			
	516		75			new Cane gravel seep subpop. (SC)	4.5	0	
						Maple Springs Branch subpop. 1 (NC)	3.5		20
						Maple Springs Branch subpop. 3 (NC)	4.5	0.45	15
						Maple Springs Branch subpop. 5 (NC)	7	0.18	17.4
226	129	689	151		1614	Upper Whitewater Falls (NC)	1.2	1.2	26
	1022		538		4035	Neversink (AL)			
	86				430	Balcony Sink (AL)			
88		535		535		Guess Creek Cave (AL) (summer)	11.7	2.6	54
	2690		269		3228	Guess Creek Cave (AL) (mid-October)			
535	732	749	215	2816	1345	Florida Caverns Exit Sink subpop. (FL)			
749	732		172	1019	2582	Florida Caverns Walt's Misery subpop. (FL)			
	2816					Cerro de la Muerte (Costa Rica)	28.5		
	344					Volcan Irazu (Costa Rica)	7.4		
	215					Volcan Barva (Costa Rica)	4.5		
	1722					Valle Nuevo (Dom. Rep.) (winter, 2002)			
	301					Palo de Agua (Dom. Rep.) (winter, 2002)			

* *unless timing specified otherwise in site column*

location light levels. The great range in neotropical light levels reflects the fact that *A. monanthes* inhabits a greater range of habitat types in the tropics than in the southeastern U.S. It should be noted that winter light levels in the southeastern U.S. may surpass the high-light neotropical measurements. The only non-summer SEUS reading (at Guess Creek Cave, AL) occurred on October 16, when some canopy foliage was still present, and it was already comparable to the highest light levels observed in the tropics.

Correlation of microclimate variables with population success

Regression analyses were performed between the number of plants, number of fertile plants in a population, and each microclimate variable measured at the *A. monanthes* location, both for SEUS populations only and also with the inclusion of neotropical populations. Such an analysis cannot be used to infer causation of the differences in population size and fertility across populations, but could suggest microclimate correlations with these differences. **Table 16** lists the r-squared values from each test. The greatest r-squared value was found in the analysis using number of fertile plants at all populations (both SEUS and neotropical): substrate temperature appears to be responsible for 46% of the variability observed in the abundance of fertile plants when looking at all populations, with increasing temperature associated significantly with fewer fertile plants. (Air temperature, which was significantly correlated with substrate temperature, also had a statistically significant slope and large r-squared value, as did relative humidity which was not significantly correlated with either temperature measure.) However when this analysis was restricted to SEUS populations, substrate temperature was less powerful than other variables and accounted for only 10% of the variance in fertile plants, perhaps because substrate temperature did not vary as much within the southeastern U.S. This analysis instead had several other variables tied for relatively low r-squared values (none with statistically significant slopes), some showing the opposite relationship from that in the all-population analysis (e.g. increasing air temperature was associated with increasing fertile plants within the southeastern U.S. but decreasing fertile plants in the all-population analysis). The two analyses investigating the number of total plants both revealed substrate moisture to have the strongest effect (a negative effect), though still relatively low at r^2=0.21. Apparently the

Table 16: r^2 values for regressions between population success (measured as number of total plants and number of fertile plants) and various microclimate variables

	all populations included		*SEUS populations only*	
	regression with # of total plants	regression with # of fertile plants	regression with # of total plants	regression with # of fertile plants
visible light (lux)	0.09 (+)	0.02 (+)	0.15 (+)	0.12 (+)
photosynthetically active light (quanta)	0.10 (+)	0.15 (+)	0.05 (+)	0.18 (+)
air temperature	0.01 (-)	0.39* (-)	0.003 (-)	0.16 (+)
substrate temperature	0.01 (-)	0.46* (-)	0.02 (+)	0.10 (-)
relative humidity	0.08 (-)	0.30* (-)	0.05 (-)	0.004 (+)
substrate moisture	0.21 (-)	0.10 (-)	0.21 (-)	0.20 (-)
rock permeability	0.06 (+)	0.01 (-)	0.003 (-)	0.19 (-)

Note: (+) indicates a positive relationship; (-) indicates a negative relationship

**slope for this regression was statistically significant*

microclimate variables observed during my fieldwork do not account for most differences in *A. monanthes*' population success; microclimate at other times of year could be much more relevant.

Because few strong correlations were observed between population success and microclimate variables, correlations among various microclimate variables are not listed, but significant correlations were found between the two light variables, between substrate moisture and both light variables, and between the two temperature variables.

Comparison of actual and plausible locations

Microclimate data was taken in 2000 for locations within each SEUS site that seemed appropriate habitat yet contained no *A. monanthes*, called plausible locations. Spores surely must reach these locations, being within several meters of *A. monanthes* locations, so it was of interest whether these locations have cryptic microclimate differences from the *A. monanthes* locations preventing plant establishment. Of the six populations where this was examined, some plausible locations differed somewhat from the corresponding *A. monanthes* locations for a few of the microclimate variables measured, but rarely by very much (**Figures 35, 36, 37, 38,** and **40**). The only exceptions are that the Thompson River (SC) plausible location was notably darker than the *A.*

monanthes location, the Florida Caverns Exit Sink subpopulation plausible location had a notably higher substrate temperature, and the original Cane Creek (SC) subpopulation plausible location had a notably lower yearly maximum temperature (which would be a benefit if anything to any plants that might grow there). The rarity of these exceptions shows that there is no clear reason for *A. monanthes*' observed limitation to certain moderated locations within a site over others.

Starch gel electrophoresis

Table 8 of the previous chapter lists the multilocus genotypes revealed by starch gel electrophoresis for all populations and regions sampled. Electrophoresis revealed three SEUS genotypes, none of which were observed in neotropical samples. Therefore 2-5 neotropical colonists of unclear origin appear to have independently founded one population in the Carolinas, two in Alabama (which may have diverged genetically from a single original genotype), and possibly two more in Florida (because neither Florida population could be sampled, it could not be determined whether their founders were from the tropics or from other SEUS populations).

While SEUS genotypes differed from neotropical genotypes, they were no more different than one neotropical genotype from another. The SEUS had only one unique phenotypic pattern (ACN pattern 1) whereas each neotropical region had 3 (Mexico, Costa Rica) to 5 (Dominican Republic) unique patterns or alleles. Further genetic and morphological comparisons of SEUS to neotropical *A. monanthes* are presented in the preceding chapter.

At each SEUS population only a single multilocus genotype was observed, so there appears to be no genetic variability within populations. No among-population genetic diversity was found in the Carolinas. This genotype's colonization of several gorges must have occurred during the Holocene such that no subsequent genetic divergence has occurred. The two Alabama populations were each genetically different. The two Alabama genotypes appear slightly more similar to one another than to the Carolina genotype, so they may be descendents of a single ancient colonist of Alabama: differences occurred at three of 12 distinguishable loci analyzed between Neversink and

Guess Creek Cave, five of 12 loci between Neversink and the Carolinas, and six of 12 between Guess Creek Cave and the Carolinas. There was no evidence of migration between Alabama and the Carolinas, not surprisingly for populations separated by long distances of unsuitable habitat. However, no Florida plants were sampled, so evidence of migration within the southeastern U.S. may yet emerge.

For comparison of genetic structure, several Dominican and Costa Rican populations were also genetically monomorphic, but a few populations were polymorphic. Only a few multilocus genotypes were observed in these regions, so interpopulation genetic variability was also somewhat limited. However in Mexico most populations were polymorphic and a very large number of multilocus genotypes (37) were observed. Evidence of local migration was detected in the Dominican Republic (shared genotypes up to 24 km apart) and Costa Rica (up to 35 km apart), and occasionally longer distances (to approx. 190 km apart) in Mexico. This difference in dispersal distance may simply reflect greater probability of encountering shared genotypes by having sampled more populations in Mexico (a total of 12 (sub)populations, vs. only four in the Dominican Republic and seven in Costa Rica), or it may genuinely show fewer barriers to dispersal in Mexico than in the other regions sampled.

Gametophyte cultures

Morphology and ontogeny

A. monanthes gametophytes generally displayed Aspidium-type early development (according to Nayar & Kaur's 1971 classification of fern gametophytes). **Figure 16** in the preceeding chapter illustrates *A. monanthes'* gametophyte ontogeny. Most gametophytes produced first a short filament and then initiated lateral growth from an apical or subapical cell to form a thallus. Alternatively, a few gametophytes maintained filamentous growth indefinitely, or produced a thallus in addition to continuing filamentous growth. Once a thalloid gametophyte reached a certain size, it spontaneously formed a 3-dimensional thickening, generally just below the apical notch of cordate gametophytes. Concurrently many gametophytes began elongation of the

apical notch into a 3-dimensional extension resembling a swan's neck. Meanwhile the thickening bulged out on both sides of the gametophyte and developed clathrate scales typical of a sporophyte's rhizome. The bulge became the shoot and root apex for the developing sporophyte and tiny initial strap-like leaves, fiddleheads, and eventually roots grew from its lower surface and sometimes the upper surface as well. If gametophytes had not succumbed to pathogens by this point, they often continued to produce additional growth apices, for example via development of an apical notch at the tip of each wing or via multiple thalli being produced by filamentous gametophytes.

Morphological comparison with co-occurring gametophytes

Asplenium monanthes gametophytes can be easily distinguished from many co-occurring gametophytes by the absense of hairs. Young *A. monanthes* gametophytes can usually be distinguished from hairless non-*Asplenium* fern gametophytes by various deviations from a perfectly cordate thallus. As shown in **Figures 17** and **18** of the preceding chapter, young *A. monanthes* gametophytes are extremely variable in growth form, only occasionally taking the shape of a perfect heart. Older *A. monanthes* gametophytes can be distinguished by the lack of gametangia and the presence of a bulge showing sporophyte initiation in spite of it.

With respect to co-occurring *Asplenium* gametophytes, *A. monanthes* gametophytes were very similar to *A. resiliens* and *A. heteroresiliens*, somewhat similar to *A. trichomanes*, and quite different from *A. platyneuron* gametophytes. *A. platyneuron*, like most *Asplenium* gametophytes, had noticeable papillae (marginal stubby hairs), while the other four species had no such structures (**Fig. 42**). For approximately the first month and a half of growth it was somewhat difficult to distinguish the different species (**Fig. 43**). *A. monanthes, A. resiliens,* and *A. heteroresiliens* had somewhat irregularly shaped thalli, *A. trichomanes* had perfectly cordate thalli, and *A. platyneuron* gametophytes were composed of two virtually circular broad lobes.

By 8 weeks, *A. platyneuron* and *A. trichomanes* gametophytes had developed numerous archegonia and curled inward to expose a rhizoid-covered underside (**Figures**

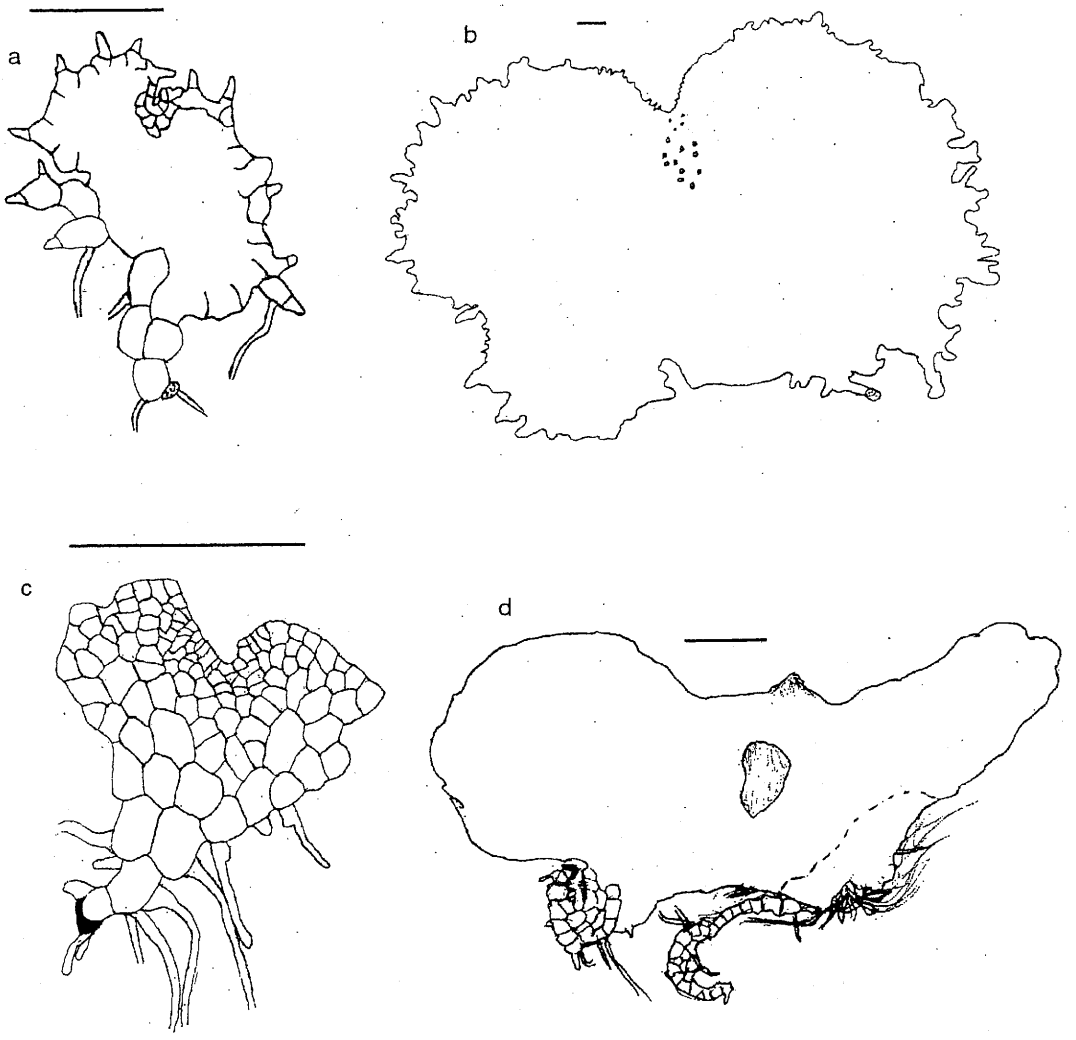

Figure 42: Comparison of papillate to hairless *Asplenium* gametophytes: (a) young *A. platyneuron* gametophyte with papillae drawn in detail, (b) mature *A. platyneuron* gametophyte with frayed-looking margin due to papillae (note also archegonia below apical notch), (c) young *A. monanthes* gametophyte with entire margin, (d) mature *A. monanthes* gametophyte with entire margin (note also sporophytic bulge below apical notch). Drawings of *A. platyneuron* by James Wee. Scale bar = 0.2 mm.

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44a and 44b, respectively) (note that this might not occur on natural substrates), perhaps as a result of a progressively curly margin. *A. resiliens, A. heteroresiliens,* and *A. monanthes* developed no archegonia, growing an apogamous bulge instead, and in some cases also a "swan-neck" extension from the apical notch (**Figures 44c, 44d, and 44e,** respectively). One of the few differences between *A. heteroresiliens* (and possibly also its parental species, *A. resiliens*, but plants died before sporophytes could be observed) and *A. monanthes* is that, at least in agar culture, *A. monanthes* plants did not form a root until well after the first tiny fiddleheads and/or leaves, making rooted plants approximately 5.5 to 7.5 months old. In contrast *A. heteroresiliens* developed roots by approximately 4 months in age. Occasional gametophytes of SEUS *A. monanthes*, *A. resiliens*, and *A. heteroresiliens* remained filamentous (see **Fig. 25** in the previous chapter) rather than following the general cordate path. These three species are very difficult to distinguish as gametophytes.

Natural substrate trials

The only treatment that yielded many identifiable *A. monanthes* gametophytes was the spore viability control (treatment 1) on the agar medium. The autoclave control plate yielded no gametophytes as expected but unfortunately neither did most of plates where spores were sown. Only one of the four autoclaved-substrate-plus-spores plates (treatment 5) yielded any gametophytes, but the filamentous gametophytes found on the single successful culture did manage to produce fiddleheads. The non-autoclaved plates (treatments 4 and 6—treatment 3 already had a rich bryophyte flora) developed a rich carpet of bryophytes, non-*A. monanthes* fern gametophytes, fungal mycelia, and occasionally a few gametophytes that looked like *A. resiliens*. The ones that went on to produce sporophytes were definitely *A. resiliens*, based on the black stipe of initial leaves. Since *A. monanthes* gametophytes are hard to distinguish from *A. resiliens* gametophytes, it is possible that some of the gametophytes that didn't form sporophytes were actually *A. monanthes*, but even if this were the case, they were still rare.

In summary, I found no good way to raise *A. monanthes* sporophytes from spores. Agar cultures usually succumbed to contamination, bare soil or rock was too prone to dessication for many plants to survive, and a live culture of bryophytes and other fern

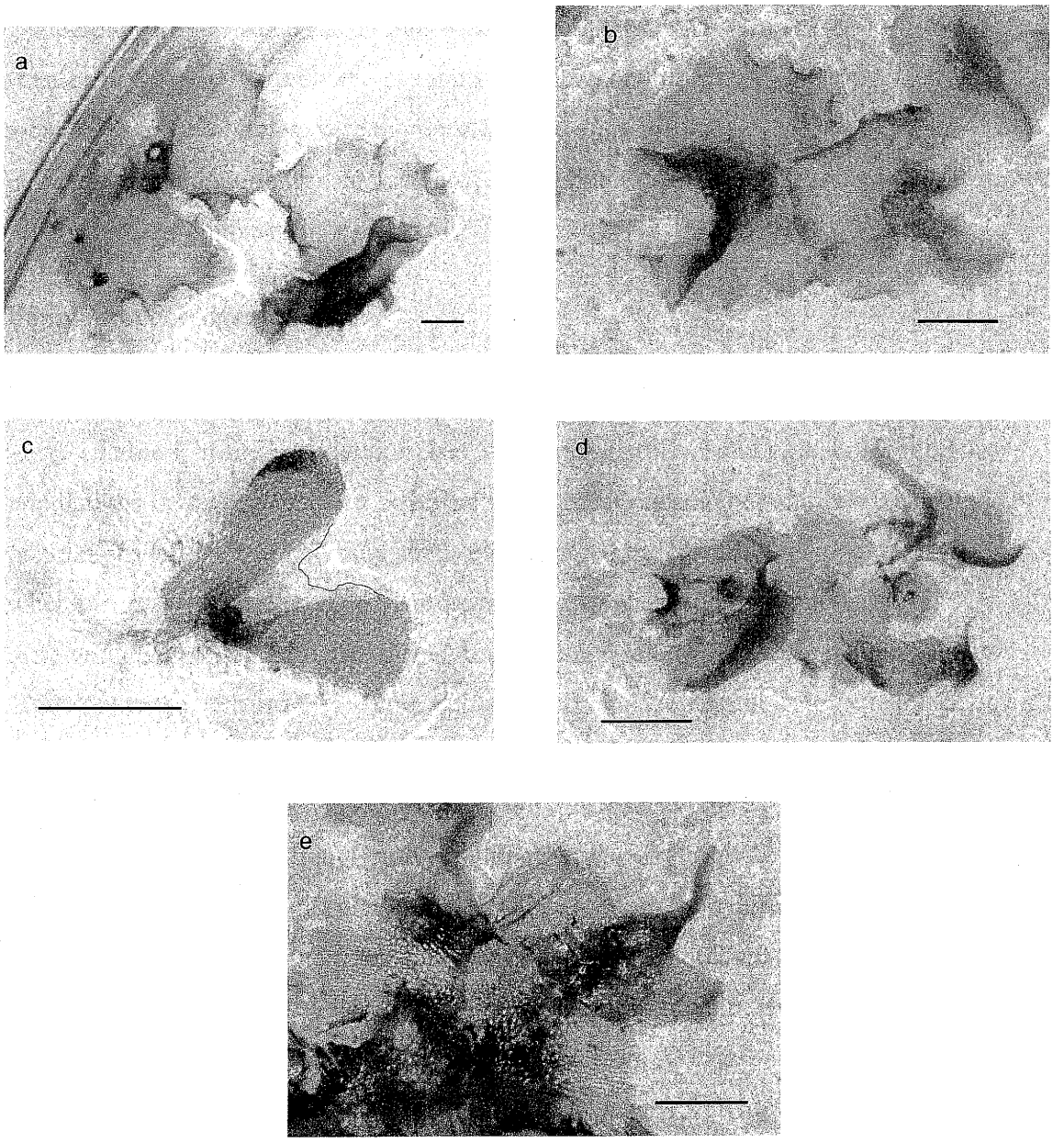


Figure 44: Mature cordate gametophytes of: (a) *Asplenium platyneuron* (8 weeks), (b) *A. trichomanes* (4 months), (c) *A. resiliens* (8 weeks; black outline added to show margin for zone mysteriously lacking pigmentation), (d) *A. heteroresiliens* (8 weeks), and (e) *A. monanthes* (Coley, SC, 13.5 weeks). Note archegonia (dark specks near apical notch) on *A. trichomanes* gametophytes (also present but not visible in *A. platyneuron* photo). Note 3-dimensional sporophytic proliferation (bulge) on *A. resiliens, A. heteroresiliens,* and *A. monanthes* gametophytes. Scale bar = 0.5 mm.

gametophytes maintained moisture better but apparently outcompeted *A. monanthes* gametophytes even when the culture was supplemented with *A. monanthes* spores (treatment 6). Alternatively the problem with the non-autoclaved cultures was that *A. monanthes* gametophytes were more vulnerable than other species to the desiccation all cultures were inadvertently subjected to, and perhaps desiccation never occurs in natural *A. monanthes* populations.

Population demographics

SEUS population demographics

Table 1 of the Introduction lists all known historical and extant *A. monanthes* populations in the southeastern U.S., including locations and population sizes. Only ten populations (14 if differentiating subpopulations) of the historical 17 could be relocated when visited. Of these ten populations, only six (eight if subpopulations are differentiated) contained fertile plants during either year of fieldwork. From 2000 to 2001, Thompson River (SC) decreased drastically in size, Guess Creek Cave (AL), Maple Springs Branch subpopulation 3 (NC), and the new Cane gravel seep subpopulation (SC) decreased noticeably, the Florida Caverns exit sink subpopulation increased noticeably (although many plants may actually be *A. heteroresiliens*), while the remaining populations and subpopulations stayed about the same size.

Table 17 shows the stage and/or size-based demographic profiles of all extant populations (with the exception of the Florida Caverns subpopulations, since young plants there could not be positively distinguished from *A. heteroresiliens*). The (sub)populations at which all three developmental stages were documented (i.e. mature populations: Guess Creek Cave, Neversink [AL], Maple Springs Branch subpopulation 5, and both Cane Creek subpopulations) were compared to one another to see if they shared a general demographic profile. All but Guess Creek Cave had a majority of plants (63-76%) at the sporeling stage, with up to a quarter of plants (12-26%) at the juvenile stage, and an equal or lower proportion of plants (9-18%) at the adult stage. Guess Creek Cave had almost the opposite demographic profile: only 14% of plants were sporelings, 38% juveniles, and 49% adults. Guess Creek Cave appears to represent a different set of

Table 17: Census of SEUS populations

Stage-based census:

(sub)population, year	sporelings	juveniles	adults	total	% sporelings	% juveniles	% adults
Glade Fern Ravine (SC), 2001	5	6	0	11	45	55	0
Thompson River (SC), 2000	5	9	0	14	36	64	0
Thompson River (SC), 2001	2	1	0	3	67	33	0
Coley Creek (SC), 2000 & 2001	0	0	1	1	0	0	100
original Cane Creek subpop. (SC), 2000	14	6	5	25	56	24	20
original Cane Creek subpop. (SC), 2001	17	3	4	24	71	13	17
new Cane gravel seep subpop. (SC), 2000	12	4	2	18	67	22	11
new Cane gravel seep subpop. (SC), 2001	8	4	1	13	62	31	8
Maple Springs Branch subpop. 2 (NC), 2001	6	1	0	7	86	14	0
Maple Springs Branch subpop. 3 (NC), 2000*	lumped with juveniles	48	7	55	--	--	13
Maple Springs Branch subpop. 3 (NC), 2001*	lumped with juveniles	36	6	42	--	--	14
Maple Springs Branch subpop. 5 (NC), 2000	>12	3	2	>17	71	18	12
Maple Springs Branch subpop. 5 (NC), 2001	>13	1	2	>16	81	6	13
Balcony Sink (AL), 2001	1	0	0	1	100	0	0
Neversink (AL), 2001*	~100	18	14	~132	76	14	11
Guess Creek Cave (AL), 2000*	1	11	16	28	4	39	57
Guess Creek Cave (AL), 2001*	7	9	10	26	27	35	38

Size-based census:

(sub)population, year	small†	medium†	large†	total	% small	% medium	% large
Upper Whitewater Falls (NC), 2000	25	0	0	25	100	0	0
Upper Whitewater Falls (NC), 2001	21	0	0	21	100	0	0
Maple Springs Branch subpop. 3 (NC), 2000*	23	16	7	46	50	35	15
Maple Springs Branch subpop. 3 (NC), 2001*	30	9	3	42	71	21	7

**These populations contained many dense clumps of plants in which individual plants could not be differentiated, so the total number of plants at these populations is higher than listed. When a dense clump could not be disentangled to count its plants, the whole clump was categorized as a member of the most advanced stage category found within it. For example, any fertile fronds gave the whole clump a classification as 1 adult plant even if several juvenile plants were also present.*

† Size classes:
small: longest frond less than 6 cm in length
medium: longest frond 6-16 cm
large: longest frond greater than 16 cm

demographic forces, possibly shared by Coley Creek (SC) with its single adult plant, than the other mature populations and will be considered separately.

An average mature population vector was calculated and stage-specific survivorships were calculated from it (**Table 18a**). As might be expected for a plant with a small specialized niche, successful establishment is the major limiting factor. Only a miniscule fraction (2.2×10^{-7}) of the estimated annual spore production advanced to the sporeling stage. Because the intermediate gametophytic stage was treated as a black box, it is unknown whether the main survivorship hurdle is gametophyte establishment, gametophyte survival, or sporophyte initiation. Survivorship to the juvenile stage was 0.25, while survivorship to adulthood was only slightly lower at 0.18. Larger size (i.e. attainment of juvenile status) therefore confers an advantage to *A. monanthes* plants.

A generalized Lefkovitch matrix (**Table 18b**) was calculated and iterated over 100 generations using in the presented example a value of 0.88 for the maintenance of ungerminated spore viability from year to year (**Table 18c**). The resulting model shows sporelings increasing in a sigmoidal curve while juveniles and adults stay approximately the same in number. Smaller spore-to-spore transition values (<0.88) resulted in a peak in sporeling abundance after a few generations and a subsequent decline of all stages (not pictured). Therefore the estimated transition probabilities are inconsistent with stability of the observed average stage distribution: stability was attained only at relative abundances of sporelings significantly higher than ever observed. This suggests (a) that *A. monanthes* populations experience inherent demographic stochasticity and/or (b) that the estimated transition probabilities, based on a single year's transition observations, do not reflect a typical year for *A. monanthes* populations.

The model's transition probabilities were then varied to determine what combination of transition probabilities would result in a stable stage distribution similar to the observed distribution. Multiple combinations exist that create this outcome, but all involve greater advancement of sporelings to juveniles or juveniles to adults and/or greater stasis of juveniles or adults, as opposed to greater fecundity or sporeling recruitment. This tentative observation is supported by Silvertown et al. (1996), who surveyed plants with a range of life history traits and found that iteroparous forest herbs

Table 18: Average SEUS mature population vector, generalized transition matrix, and resulting demographic projection into the future

a. Average mature population vector

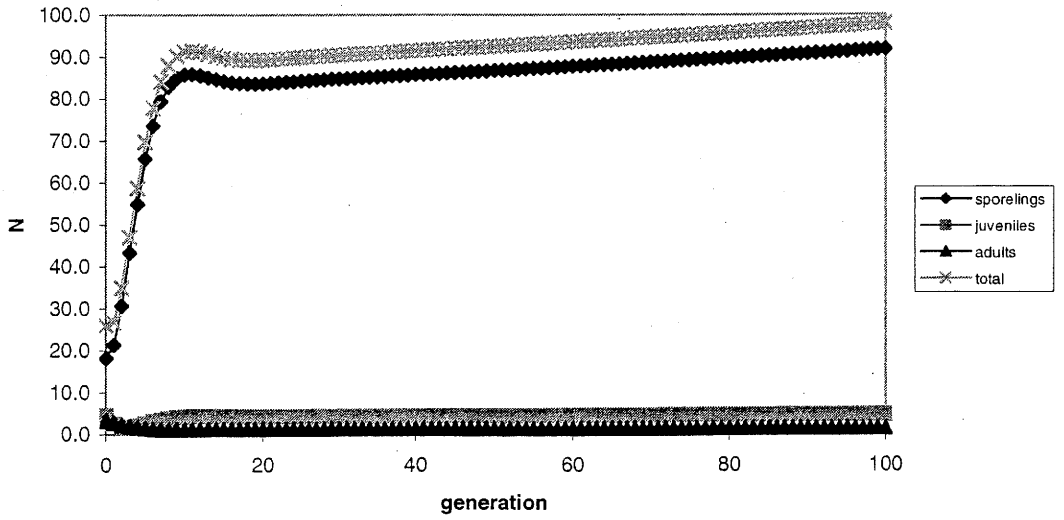
	N	survivorship
spores	1.9×10^6	
sporelings	18.1	
juveniles	4.6	0.25
adults	3.3	0.18

b. Generalized transition matrix based on changes in marked plants from 2000 to 2001

	spores	sporelings	juveniles	adults
spores	0.88*	0	0	5.9×10^5
sporelings	4.5×10^6	0.65	0.19	0
juveniles	0	0.03	0.39	0.07
adults	0	0	0.07	0.77

**this transition probability could not be estimated, so this is an arbitrary value*

c. Demographic profile projected over 100 generations starting with average mature population vector and applying generalized transition matrix



like *A. monanthes* generally had high elasticity values for advancement, a range of values for stasis, and low elasticities for fecundity.

Limited information is available on long-term trends in *A. monanthes* populations. **Figure 45** graphs historical population sizes of six well-documented *A. monanthes* populations. Populations that fell to a small size (e.g. six plants) only rarely increased again, so decline seems to be the most common long-term fate among these populations. Unfortunately the limited sample points were insufficient to reveal whether population trends were constant or stochastic in nature.

Comparison with neotropical population observations

SEUS population size and plant size were also compared to neotropical populations visited and their plants. **Table 19** lists adult plant sizes and mature population sizes for all regions investigated. Neotropical populations had a size range comparable to mature SEUS populations, with 1 to about 50 plants per (sub)population but most populations in the teens. Therefore SEUS *A. monanthes* may have preadaptations to small population size as suggested in the Introduction.

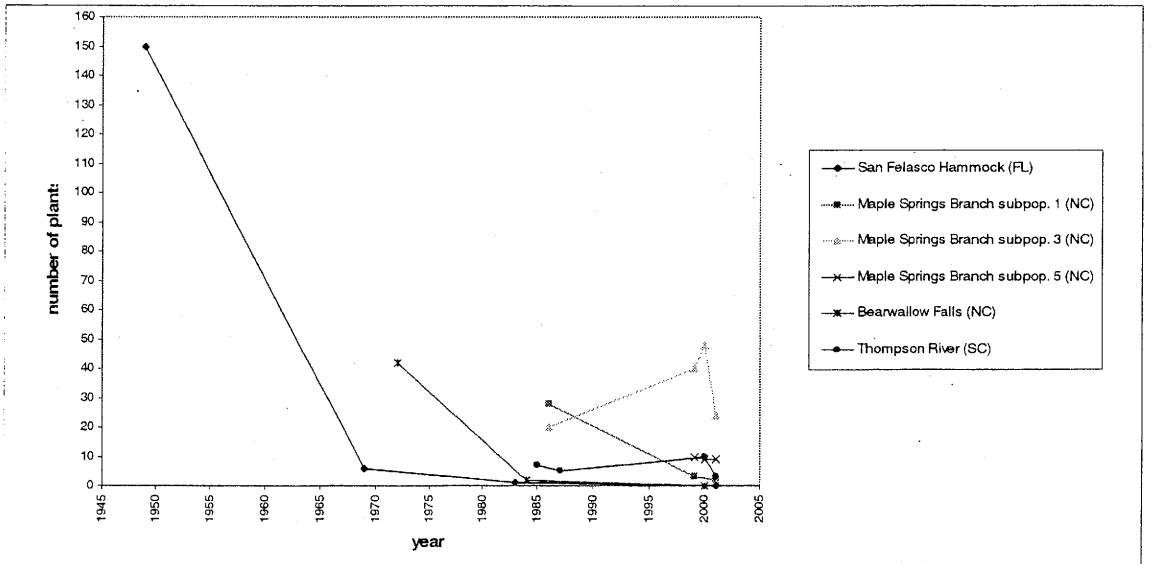


Figure 45: Population size over time at well-documented *A. monanthes* populations

Table 19: Size of fertile plants and their populations

population	max. frond length (cm)	sterile fronds*	fertile fronds*	dead fronds	total live fronds*	total w/ dead fronds	population size
Alabama†:							
Guess Creek Cave	17	5	4	>30	9	39	>21
	12	31	>15	10	46	56	
	17	12	11	10	23	33	
	16	12	7	2	>19	21	
	9	6	1	>3	7	10	
	19	6	9	3	15	18	
	9	3	1	6	4	10	
	12	12	4	2	16	18	
	9	3	7	5	10	15	
	19	16	17	15	33	48	
	9	5	1	15	6	21	
Neversink	22	2	16		18		~132
	23	2	3		5		
	10	8	1		9		
	10	10	2		12		
	15	3	1		4		
	16	3	4		7		
	14	5	5		10		
	17	10	4		14		
	8	4	1		5		
	18	4	1		5		
	18	9	3		12		
	16	5	2		7		
	16	4	1		5		
	20	0	14		14		
Alabama mean	**14.8**	**7.2**	**5.4**	**9.2**	**12.6**	**26.3**	**76.5**
Florida†:							
Florida Caverns Exit Sink subpop.	14	7	3	8	10	18	<20
Carolinas†:							
Coley Creek	27	5	7	9	12	21	1
Maple Springs Branch subpop. 5	20	2	4		6		>15
	18	4	1		5		
new Cane gravel subpop.	25	5	9	9	14	23	23
original Cane Creek subpop.	20	4	2	3	6	9	24
	27	6	3	5	9	14	
	27	11	2	6	13	19	
	18	4	2	2	6	8	
Carolinas mean	**20.5**	**4.6**	**3.5**	**5.7**	**8.1**	**15.7**	**15.8**

Table 19 (continued)

population	max. frond length	sterile fronds*	fertile fronds*	dead fronds	total live fronds*	total w/ dead fronds	population size
Dominican Republic:							
Valle Nuevo	11	1	5	3	6	9	14
	4	1	1	0	2	2	
	4	3	1	0	4	4	
	6	1	2	3	3	6	
	5.5	1	1	0	2	2	
	7	0	3	2	3	5	
	7	1	4	1	5	6	
	5.5	0	4	2	4	6	
	8	0	3	0	3	3	
Palo de Agua	30	0	11	6	11	17	>50
Caseta 2	>31	1	17	6	18	24	10-15
Los Arroyos	16	0	4	3	4	7	~25
D.R. mean	**11.3**	**0.8**	**4.7**	**2.2**	**5.4**	**7.6**	**25.5**
Mexico:							
Mineral Real del Monte	39	0	3	1	3	4	"abundant"
Ocuilan	42	0	4	0	4	4	"somewhat common"
Orizaba-Puebla	14	6	1	0	7	7	~13
Mexico mean	**31.7**	**2.0**	**2.7**	**0.3**	**4.7**	**5.0**	**--**
Costa Rica:							
El Toja	33	0	1	1	1	2	27
km 87	42	0	1	5	1	6	9
	>26	0	1	3	1	4	
	26	0	1	4	1	5	
	31	0	1	7	1	8	
Rio Yerbabuena	30	0	>7	2	7	9	3
Volcan Irazu hillside	29	4	1	3	5	8	11
Volcan Barva	31	0	>5	2	5	7	13
C.R. mean	**31.0**	**0.5**	**2.3**	**3.4**	**2.8**	**6.1**	**12.6**

**Tropical live frond counts are minimums since much breakage occurred while transporting specimens. Alabama frond counts are maximums since many "plants" were actually clumps of indistinguishable multiple plants.*

† SEUS values are from 2001 data

A difference between certain neotropical populations and SEUS populations was apparent in plant density. This was not quantified, but the author noted that SEUS populations are often crowded onto a single rock ledge or boulder because of the inhospitality of the surrounding macroclimate, whereas in neotropical cloud forest, all habitat is equally appropriate for *A. monanthes* so plants may be spread out over a large area. Neotropical dry forest, such as Valle Nuevo in the Dominican Republic, had a clumped distribution similar to that in the southeastern U.S.; plants were clustered together under shrubs where desiccation was less severe. Therefore intraspecific competition is probably greater in SEUS and neotropical dry forest populations than in neotropical cloud forest populations.

Another notable difference between neotropical and SEUS populations was their demographic distribution. SEUS populations were generally dominated by sporelings, with a few juveniles and a few or no adults. In contrast, very few sporelings or juveniles were observed in Costa Rican or Dominican populations. This may be partly because searches were less thorough than in the southeastern U.S. (the plants were generally scattered over a larger area and the populations were not of conservation concern), but one neotropical population (Valle Nuevo, D.R.) was thoroughly censused and still showed a predominance of mature plants. Out of 14 plants found, only two were sporelings and two were juveniles.

Neotropical plants had fewer total fronds, partially due to not counting them until they were dried and transported back to the U.S. and some fronds had broken and were lost. However, adding the number of dead stipes and broken-off stipe bases to compensate for the difference in counting method still resulted in a higher total of fronds for SEUS than neotropical plants, so this appears to be a genuine difference. Additionally, most fronds of neotropical adults were fertile, whereas adult SEUS plants were generally still dominated by sterile fronds (**Table 19**) which they produced even after reaching maturity.

DISCUSSION

SEUS microhabitat and microclimate

Summary of Asplenium monanthes' microhabitat and microclimate

Bryophyte associates

The bryophyte flora at SEUS *A. monanthes* locations includes a mix of grotto specialists and widespread generalists, with more grotto specialists than the exposed locations (Don Farrar, personal communication), suggesting that *A. monanthes*' microhabitat is rarely subject to desiccation or extreme temperatures. The bryophyte flora was extremely variable among locations at a site and from population to population (*T*able 10). There is no bryophyte that serves as a reliable and frequent indicator of good *A. monanthes* microhabitat. *Bryoandersonia illecebra, Ctenidium molluscum/malacodes, Mnium cuspidatum, Myurella sibirica, Plagiochila echinata*, and *Plagiomnium carolinianum* were reliably found only at *A. monanthes* locations but infrequently so (only encountered twice each). Other species were frequent in *A. monanthes* locations but were even more frequent in other microhabitats: *Thamnobryum alleghaniense* was found at four *A. monanthes* locations but also at six most-moderated locations. Therefore botanists searching for *A. monanthes* cannot rely on particular bryophytes as indicators of *A. monanthes* microhabitat and will have to employ a broader microhabitat search image.

pH

A. monanthes can apparently tolerate a range of pH values. In most cases its host rock has some calcium component (Table 12), but in the Carolinas it is bound up in calcium silicates and less available than in the limestone of Alabama and Florida populations. The pH of *A. monanthes* microhabitat, measured from soil or from bryophyte mat moisture, ranged from 5.5 (bryophyte mat in Valle Nuevo, D.R.) to 8.2 (soil from Guess Creek Cave, AL) (Table 13), but further testing is recommended because few sites were examined.

Temperature

Summer temperature moderation was examined on two scales, that of the whole site relative to the external macroclimate and that of differences among locations within a site. The maximum daily air temperature of the Lake Toxaway weather station was in several cases similar to Carolina population temperatures (**Fig. 36**), suggesting that it is just as moderated as *A. monanthes* sites due to its own surrounding topographic relief. This effect is characteristic of the whole Jocassee Gorges area (Billings & Anderson, 1966), so *A. monanthes* could probably grow successfully in many more gorges than it has currently reached. The Alabama and Florida weather stations experience no such topographic relief and accordingly showed a major temperature elevation relative to corresponding *A. monanthes* sites. The Alabama sites minimized summer temperatures especially well so that plants there experience much lower summer temperatures than in any other population. This is due to a summertime reverse chimney effect in the case of Guess Creek Cave (Alan Cressler, personal communication): this cave entrance blows chilled air which enters from an entrance higher up the hillside. In the case of Neversink and Balcony Sink, the deep vertical pits (162 and 136 feet, respectively) collect cool air in the summer. While substrate or soil temperatures were not available for weather stations, substrate temperature generally tracked air temperature at *A. monanthes* populations (compare **Fig. 37** to **Fig. 36**), so the phenomenon of site-wide air temperature moderation is probably shared by substrate temperature as well.

Within a given *A. monanthes* site, the precise *A. monanthes* location was generally slightly cooler or equal to the most exposed location in both air and substrate temperature, but not universally so except at Alabama sites where the difference was quite notable. The *A. monanthes* location was generally slightly warmer or equal to the most moderated location, but again not universally so. Therefore with respect to summer temperatures, it is probably unimportant where *A. monanthes* grows within a site except at Alabama populations where the powerful cave entrance or sinkhole effect is apparently limited to its immediate confines.

Winter temperature, although probably more significant than summer temperature to *A. monanthes*' survival because of potential frost damage, was not investigated in

detail. Instead max-min thermometers were used to measure yearly minimum air temperature in addition to yearly maximum temperature (**Fig. 35**). Relative to corresponding weather stations in the Carolinas and Alabama, *A. monanthes* sites were greatly moderated in yearly minimum and maximum temperature except for the Carolina sites during the summer of 2000 which showed the opposite relationship. Temperature differences within a site were measured at Cane Creek, SC, and found to be consistantly minor for yearly minimum temperatures but quite variable for yearly maximum temperatures. Within-site differences were not measured at Alabama or Florida sites, and may be more significant there. But for Cane Creek and probably other Carolina populations, the exact spot within a gorge does not seem to make a big difference in a plant's exposure to temperature extremes, the whole area being moderated. However the use of simple max-min thermometers did not elucidate the number of times a location dipped below freezing. If locations within a site vary in number and duration of sub-freezing periods, that would make a plant's within-site location quite important.

Water relations

No good method was available for measuring long-term water availability from a single visit. Future studies should utilize multiple site visits and either the substrate moisture protocol used here or a rain gauge. With the three types of measurements that were utilized in this study, comparisons cannot be made to weather stations nor among sites except using rock permeability for the latter type of comparison. Among SEUS rock samples, permeability was greatest for Florida Caverns' Marianna limestone and least for Guess Creek Cave's Monteagle limestone (AL), with the various Carolina silicates intermediate in permeability (**Table 14**). Sandstone, known to be an effective conductor of water (Walck et al., 1996), was somewhat more permeable than the most permeable silicate but only about one-third as permeable than the heavily weathered Marianna limestone. Therefore only the Florida populations are likely to experience significant moisture recharge from their host rocks.

Comparisons among locations within a site can be made from humidity and substrate moisture data. Summertime humidity showed no consistent relationship between locations—at several sites the *A. monanthes* location had the lowest relative

humidity, but at other sites the three locations were virtually equivalent. No explanation is apparent for *A. monanthes* locations having in many cases slightly lower relative humidity than others, but all locations were in the same high range (the mean for *A. monanthes* locations was 85%), so water vapor is abundant throughout *A. monanthes* sites. No patterns were visible from substrate moisture data. Sites that had the greatest moisture at the exposed location had probably had rain recently whereas sites with greatest moisture in moderated locations probably had not received rain recently. Soil moisture ranged from almost none to complete saturation. In summary, *A. monanthes*' water regime is still largely unknown and needs better investigation. But *A. monanthes* populations probably experience generally high moisture availability due to topographic relief at all sites, exposure to moist cave or sinkhole air at Alabama and Florida sites, and water spray at select sites (Thompson River and Whitewater Falls in the Carolinas; Neversink and Balcony Sink in Alabama).

Light

Summer light levels were in some cases somewhat similar for all locations within a site, but in other cases the exposed location had significantly more light than the other two locations. With the exception of these well-illuminated exposed locations, light levels were generally quite low at *A. monanthes* sites, with *A. monanthes* locations receiving an average of 560 lux and 5.4 quanta. Winter light levels are probably about an order of magnitude greater based on autumn readings from Guess Creek Cave, AL, but clearly more cool-season data is needed to elucidate how *A. monanthes* survives on so little light for much of the year.

Which microhabitat/microclimate characteristics are most limiting?

The relative importance of each microclimate variable (there was insufficient data to test substrate variables like pH) to the success of *A. monanthes* populations was investigated by regressing number of total plants and number of fertile plants on each microclimate variable for all populations and for SEUS populations only. These four analyses yielded extremely different results, so apparently a few of the microclimate factors measured play some role but none are uniformly important. For example,

substrate temperature explained 46% of populations' variance (the greatest r-squared value encountered) in number of fertile plants when looking at all populations, but only 2-10% of the variance in the other three analyses. Therefore all microclimate factors tested, at least as measured during the summer in SEUS populations, can probably be considered relatively unimportant in affecting the success of an *A. monanthes* population. Winter microclimate conditions might well prove more important than summer conditions for SEUS *A. monanthes*, so they should be investigated in the future. Further speculation on factors limiting *A. monanthes* populations, including non-climatic factors, can be found later in this section.

Why isn't A. monanthes found at plausible but uninhabited locations?

Comparison of plausible to actual *A. monanthes* locations at each SEUS site yielded only minor differences (with the exception of exposed locations at Alabama sites) for the five parameters measured: yearly temperature extremes, summer air temperature, substrate temperature, relative humidity, and visible light. Therefore these microclimate factors, at least during the time of year they were measured, appear to play no strong role in determining where *A. monanthes* grows within a site. Chance events may instead be largely responsible for *A. monanthes*' distribution within a site. Populations have been known to shift over the years from one part of a site to other locations. At Maple Springs Branch subpopulation 3 (NC), Weakley (1987) documented in 1987 about 20 immature plants on a rockface but did not notice any on a large boulder nearby. My fieldwork in 2000-2001 documented three to seven immature plants on the rockface but over 40 plants, including several fertile ones, on the boulder. Similarly, Bearwallow Falls (NC) lost both plants from the right side of the falls but newly received two plants on the left side from the period 1972-1984 (NCNHP, 2000) before later apparently losing all plants. The Florida Caverns Exit Sink was developed for cave tours in the 1930's, so the particular boulders where *A. monanthes* currently occurs must have been later colonized by spores from nearby plants. Therefore the precise location of *A. monanthes* within a site at a given time appears to be limited by chance as well as specific ecological needs.

Chance events likely also limit the establishment of entirely new populations. Appropriate yet unoccupied habitat well outside current populations may have never

received any colonists or may have also hosted populations at various points in the past thousands of years that have since died out due to chance disturbances (e.g. flooding, drought, historical deforestation).

An implication of this finding is that if reintroduction efforts were to be undertaken for extirpated populations, a botanist familiar with *A. monanthes* microhabitat could make an educated guess about where exactly to reintroduce spores or plants without intensive microclimate characterization. That is not to say that there are no microclimate differences within sites, just that differences are probably not cryptic. The extremes of each site's range of microclimate (i.e. exposed and most moderated monitoring locations) rarely host *A. monanthes* (only at Upper Whitewater Falls is *A. monanthes'* location extremely moderated and some Florida Caverns plants have been recorded in apparently unmoderated locations). It is possible that exposed sites are too subject to desiccation and freezing temperatures for *A. monanthes*, although the limited data from this project do not particularly support this. Most-moderated locations may be too poor in photosynthetically active light to support *A. monanthes* sporophytes.

Comparison of SEUS to neotropical microclimates

SEUS populations appear to experience hotter temperatures (**Figures 36 and 37**) during summer than neotropical populations ever experience. The temperatures experienced are only high enough to increase evaporative stress, and precipitation in the southeastern U.S. probably keeps pace with this evaporation, so these high summer temperatures probably do not pose a major problem for SEUS *A. monanthes*.

Freezing temperatures may present a major challenge to SEUS plants. A weather station near the coldest neotropical population visited, Cerro de la Muerte, Costa Rica, shows temperatures dipping below freezing on just eight occasions during the period March 2000-March 2001 (Valverde, 2001), and only to –2° C on the coldest occasion. SEUS populations' max.-min. thermometers showed yearly minimum temperatures of –5 to –7° C, and it is unknown how many times the temperature dropped below freezing.

Drought stress may not be problem encountered by SEUS *A. monanthes* because plants inhabiting neotropical dry forest during the dry season withstand much drier

conditions than ever occur in the southeastern U.S.[12] For example, the nearest weather station to the dry forest visited in the Dominican Republic, Constanza, has mean monthly precipitation as low as 2.2 cm (during January: Cocco, 2002), whereas the lowest mean monthly precipitation of the three SEUS weather stations was 7.1 cm (**Fig. 10** of Introduction: both at Scottsboro, AL, for August, and in Quincy, FL, for October).

Light levels at SEUS sites appear to be roughly comparable to that at neotropical sites (**Table 15**, **Figures 40** and **41**). Based on my limited sampling, neotropical populations experienced a great range of light depending on the type of habitat: sub-páramo and dry forest populations received much more light, comparable to winter levels in the southeastern U.S., than cloud forest populations, which were similar to summer levels in the southeastern U.S. Therefore *A. monanthes* can probably accommodate both low light intensities in the summer and high intensities in winter in the southeastern U.S.

In summary, the tropical species *Asplenium monanthes* is probably most challenged in the southeastern U.S. by occasional sub-freezing temperatures, but it must have some degree of frost tolerance based on observed yearly minimum temperatures as low as -7° C. Whether this tolerance has recently evolved in the SEUS populations or is shared by all *A. monanthes* can only be resolved by common garden experiments. This would be valuable to determine whether physiological evidence supports genetic and morphological evidence in showing little differentiation of SEUS from neotropical plants. Unfortunately the rarity of SEUS plants precludes such invasive experiments in the absence of successful propagation methods.

Genetic structure in the southeastern U.S.

A lack of genetic and morphological distinction from neotropical populations (see previous chapter for detailed analysis) suggests that *A. monanthes* did not arrive in the southeastern U.S. until the Quaternary. Thus the SEUS populations do not warrant any

[12] It is quite possible that neotropical *A. monanthes* has differentiated genetically into humid versus dry forest ecotypes. If this was the case, the species' habitat in the southeastern U.S. would suggest that it was humid forest-adapted spores that originally colonized the southeastern U.S. and these plants may not be very drought resistant. However, there is no evidence for or against ecotypic differentiation in *A. monanthes*, so the drought-hardiness of SEUS plants would be best determined by direct observation rather than possibly fallacious comparisons with neotropical plants.

taxonomic distinction and the federal Endangered Species Act cannot be used to protect *A. monanthes*.

A. monanthes' apogamy does not preclude genetic variation, based on the impressive genetic diversity observed in Mexico, but this variation may be a result of multiple origins of the allopolyploid *A. monanthes* rather than purely autocthanous generation of genetic variability (see previous chapter for explanation). *A. monanthes*' low genetic diversity in the southeastern U.S., both overall (only three genotypes found) and within populations (no variability observed), may indicate a persistent founder effect from the 2-5 initial colonists and/or genetic bottlenecks from small population sizes and clonal reproduction.

Clear evidence of historical migration within the southeastern U.S. exists only within the Carolinas, where all populations are genetically identical. Florida Caverns and Balcony Sink (AL) should eventually be sampled if population sizes improve to see if they provide any evidence of more powerful migration. Various neotropical genotypes covered a greater geographic range than the Carolina genotype, so neotropical spores appear to experience greater dispersal than SEUS spores. This may be a result of greater neotropical spore production, greater availability of suitable habitat, or simply differences in habitat type (e.g. dry tropical forest is more exposed to wind) or weather patterns.

Plant growth and reproduction in the southeastern U.S.

Significance of apparent clonality

All *A. monanthes* gametophytes, whether filamentous or thalloid, appeared to develop multiple meristematic regions if they did not succumb to pathogens prematurely. This produced a many-lobed gametophyte with sporophytes eventually developing from each lobe. By the time this stage was reached, much of the gametophyte had usually decayed, separating the various lobes and their sporophytes into independent functional units such that it was impossible to determine whether they had ever been linked or not (**Fig. 19** in previous chapter). As a result of this developmental pattern, many sporophytes can potentially develop from a single original spore. The growth of additional lobes was observed by Lindsay & Dyer (1996) in some sexual fern

gametophytes (particularly *Blechnum spicant*) that had lived for quite some time without being fertilized, but polyembryony was not observed when sperm finally reached the gametophyte. Chiou & Farrar (1997) observed both multiple lobes and subsequent multiple sporophytes per gametophyte in various sexual taxa of the Polypodiaceae, and Chiou (1996) observed the same phenomenon in sexual *Elaphoglossum*, so this may somewhat common in epiphytic ferns. But *A. monanthes* is probably unusual among terrestrial and epipetric ferns in this capability.

A. monanthes sporophytes might be able to clone themselves too. Stipe-sprouts were observed on many neotropical plants and a few SEUS plants. A bud develops along the stipe and fronds grow directly from this bud in addition to the fronds growing from the plant's rhizome. In addition to increasing the annual frond production of the plant, this can eventually lead to a second plant when the stipe in question has aged enough to break off and fall to the ground, giving the bud the opportunity to root independently.

Thus *A. monanthes* populations have alternatives to spore production for maintaining themselves. This may explain the persistance of populations with perpetually rare production of fertile plants (e.g. Upper Whitewater Falls and Maple Springs Branch subpopulation 2, North Carolina). Clonal growth may also play a role in sustaining fertile populations, buffering the population against poor years for spore production. Therefore *A. monanthes*' capability for clonal growth may be partially responsible for *A. monanthes*' survival at all SEUS populations, but most significantly at non-fertile populations.

Gametophyte phenology

The phenology of *Asplenium monanthes* gametophytes in natural populations remains unknown. Gametophytes are expected to develop more slowly in the wild in the temperate zone than in culture because of lower light intensity during the summer and lower temperatures and possible snow cover during the winter. Pangua et al. (1994) compared field-grown vs. lab-grown gametophytes (on soil, not agar) of *Asplenium trichomanes*, *A. scolopendrium*, and *A. ruta-muraria* in Scotland. The main difference in developmental rate of their lab and outdoor cultures was a delay in germination in the wild until summer temperatures arrived and outdoor cultures attained the same growth

rate as lab cultures. Cousens (1981) compared the growth rate of natural gametophyte populations to lab cultures of *Blechnum spicant* in the Pacific Northwest and found that lab-raised gametophytes had twice the growth rate of wild gametophytes.

Most phenological studies of natural gametophytes are from cooler climates than the southeastern U.S. In Iowa, spores germinated in late summer to early fall, and gametophytes either produced their first sporophyte leaves by late fall or overwintered as gametophytes and produced sporophytes the following spring (Farrar & Gooch, 1975). In northern Japan, most fern spores germinated in either summer or fall, overwintered as gametophytes, and did not produce their first sporophyte leaves until late the following summer (Sato, 1982). Lindsay & Dyer (1996) observed semi-natural experimental gametophyte populations of four ferns in Scotland, developed from spores sown at various times of year. Germination and early growth progressed faster (1 month vs. 3) for spores sown during summer than other seasons, while sporophyte initiation (fertilization in these sexual species) occurred primarily during the following fall for plants sown in fall or later (sporophyte initiation was not monitored for spores sown in summer). Cousens (1981) found that *Blechnum spicant* in the Pacific Northwest also produced spores in late summer and early fall, quickly germinated, and the majority of archegoniate gametophytes had already produced a sporophyte by January rather than overwintering as gametophytes.

Since all of these studies observed germination during the fall for spores sown in late summer, this is probably a common pattern for cool temperate ferns that may also apply to the warmer temperate populations of *A. monanthes*. Since each study found a different season for sporophyte initiation from spores sown in late summer, no generalizations can be made for the timing of this event. Year-round examination of *A. monanthes*' bryophyte mats for tiny new sporophytes would be required to elucidate the natural timing of sporophyte initiation in SEUS *A. monanthes*. If germination did occur in the fall and growth rate was simply half that observed in lab cultures as in Cousens' study (1981), *A. monanthes* gametophytes would produce sporophytes in late spring, but this is just speculation that requires field investigation.

The difficulty of finding natural *A. monanthes* gametophytes is further complicated by their similarity to *A. resiliens* and *A. heteroresiliens* gametophytes. Studies of natural *A. monanthes* gametophyte populations would therefore be most effective at sites where the other two species do not occur (i.e. any populations except Guess Creek Cave, AL, and Florida Caverns). The similarity of *A. monanthes* to *A. resiliens* and *A. heteroresiliens* gametophytes is probably related to their shared status as apogamous polyploids (Lellinger, 1985, lists *A. resiliens* as triploid and *A. heteroresiliens* as pentaploid) of the *A. trichomanes* group.

Sporophyte phenology

In the southeastern U.S. most new fertile fronds appeared ready to release spores in August, and one plant at Guess Creek Cave, AL, and the single adult plant at Florida Caverns were already doing so by August 16-18 in 2001. Therefore the main spore release (and possibly germination) probably takes place in September. Because fertile fronds remain on the plant for several years and retain many spores (it was these old fronds that were used to harvest spores for sowing in the lab), spores probably continue to be released from open sori throughout the year, as documented by Farrar (1976) for several Iowa ferns. Frond production appears to continue throughout the growing season because new fully-formed fronds as well as fiddleheads were observed when SEUS populations were visited in mid to late summer.

Sporophyte growth

Mean annual frond production was estimated for juvenile and adult plants (sporelings were not measured because they are difficult to track) at all populations thoroughly censused. Growing juvenile plants (only 30% of juveniles grew new leaves) produced an average of 2.5 sterile leaves during the 2001 growing season, whereas growing adults (80% of adults grew) produced an average of 1.5 sterile leaves plus 2.6 fertile leaves. (The remaining juveniles and adults died in some cases, as quantified below, and in other cases just produced no new leaves.) Despite new growth in some plants, new fronds generally did not exceed the length of existing fronds, so 2000-2001 was not a particularly good year for growth.

Adult SEUS plants had more fronds (even when including dead and broken-off fronds) than adult neotropical plants. This may suggest greater lifespan of adult SEUS plants, delayed onset of maturity in SEUS plants, greater annual leaf production (perhaps as a trade-off with proportion of leaves fertile) by SEUS plants, or possibly other explanations. Frond size differed among and within regions. Plants from neotropical cloud forest had larger fronds with more pinnae than SEUS plants. Neotropical dry forest adults (i.e. from Valle Nuevo, Dominican Republic) had smaller fronds than SEUS adults but a similar number of pinnae, which were smaller probably to minimize evaporative loss. Therefore frond size is likely a function of environmental stress. Controlled common garden experiments would be necessary to test these hypotheses and determine whether observed differences were genetic or environmentally-induced, but no experiments were attempted because of the SEUS plants' rarity.

It would be useful to know how many years a sporophyte spends in each stage (sporeling, juvenile, adult) and to be able to estimate the age of a given plant. No general technique has been published for aging ferns. Sharpe (1993), studying the neotropical fern *Danaea wendlandii*, was able to determine plant age by observing frond production in plants over time. Knowing a plant's real age, she was able to test whether various measures of plant size (e.g. number of fronds, maximum frond length) could be used to estimate plant age. This size data is available for all SEUS *A. monanthes* plants visited, so if someone followed the growth of a few plants of various stages for several years, they could estimate age for all plants. However, I followed plants for only one year, so estimation of age from plant size based on my limited observations would be inappropriate at this point.

An alternative approach to aging plants, which also requires following plants for several years, is based on transitions between stages rather than size at a given stage. Cochran & Ellner (1992) estimated mean age of various stages in the plant *Cypripedium acaule* based on a Lefkovitch transition matrix using their model for converting stage-based matrices into age-based matrices. However, the Lefkovitch matrix estimated for *A. monanthes* is based on just a single year (possibly an atypical one) of data, so it would be premature to apply Cochran & Ellner's model to it. For now the mean age of *A.*

monanthes plants at various stages must remain unknown. The onset of fertility appears to take place once the longest frond of a plant is 11-15 cm, but the relationship between frond length and age is unknown.

The annual probability of a sporophyte advancing to the next developmental stage was estimated. Both probabilities, that from sporeling to juvenile and that from juvenile to adult, were quite low, at 0.03 and 0.07 respectively, each representing a single occurrence out of three populations (plus one plant from Florida Caverns) monitored. These low probabilities may mean that it takes decades for a plant to reach maturity, or it may mean that 2000-2001 was simply an unusually bad year for *A. monanthes*.

In contrast to most SEUS populations, neotropical populations were heavily skewed towards adults. It is unknown why neotropical population demographics differ from most SEUS populations. Perhaps disturbance is even more rare in neotropical populations and younger plants rarely find an opening for establishment (as is believed to be the case for the atypical SEUS population Guess Creek Cave) or maturity is reached sooner because conditions are largely conducive to growth year-round.

Fecundity

If adult SEUS plants have more fronds than adult neotropical plants but only a similar number of fertile fronds, this suggests that whereas SEUS plants were observed to produce both fertile and sterile fronds throughout adulthood, neotropical plants probably produce only fertile fronds upon reaching adulthood. The higher fecundity of neotropical populations (more plants reach fertility and neotropical cloud forest plants have many more fertile pinnae per fertile frond) might be due to better growing conditions (i.e. no cold season) or longer photoperiod in the tropics. Greer & McCarthy (2000) studied reproductive allocation in the fern *Polystichum acrostichoides* in Ohio. They concluded that a plant's reproductive strategy was to attempt reproduction only when it could also maintain a certain amount of vegetative growth, to maximize survivorship and therefore long-term fecundity. This strategy might be shared by *A. monanthes*.

Fecundity is probably not a limiting factor for mature SEUS *A. monanthes* populations. The mean annual fecundity of a single adult SEUS plant was 589,000, creating a mean population-level fecundity of 1.9 million spores for fertile populations.

While this sounds like a profuse amount, most fern populations produce several orders of magnitude more (Peck et al., 1990). Many spores may be lost to the population by wind or water transport, but a large number should remain within the population; studies of other ferns show leptokurtic dispersal patterns (Peck et al., 1990; Penrod & McCormick, 1996). Therefore spore abundance is probably not a limiting factor to recruitment in mature *A. monanthes*' populations, but it probably severely limits population growth in populations currently lacking fertile plants, which must rely on clonal gametophytes, spore banks, or occasional spore immigration.

Death

Observed death rates[13] from 2000-2001 were highest for juvenile plants at 42%[14] and lowest for adults at 17%, with sporelings intermediate at 29%. Conversely, the average mature population vector shows a great decrease in number of juveniles relative to sporelings but only a slight decrease in adults relative to juveniles (**Table 18c**). This suggests that juvenile mortality is normally low compared to sporeling mortality, so the stage-specific death rates observed from 2000-2001 were probably atypical. One would furthermore expect mortality rates to decrease with advancing stage because larger plants should have an advantage in competition for light, water, and nutrients.

A. monanthes plants that appear dead may in some cases just be dormant. Of six Costa Rican plants in cultivation that appeared to die of a fungal infection, two later resumed growth. Many SEUS plants were observed to have much longer dead stipes than the existing live fronds, so it is suspected that death of all leaves can be non-fatal and just set the plant back to the previous stage. In light of this observation, two dead SEUS plants were transplanted to the lab, but neither resumed growth. Many setbacks in natural SEUS populations were observed without death of all leaves, as measured in the transition probability matrix. A setback from adult to juvenile stage means that all fertile fronds present the previous year died and were not replaced by new fertile fronds; this occurred in 7% of adults. A setback from juvenile to sporeling status means that the longest leaf of a plant, that had been over 3 cm, was lost and none of the remaining or

[13] = 1 - (setback probability + stasis probability + advancement probability)

replacement leaves were greater than 3 cm long; this occurred in 19% of juveniles. All stage categories are simply theoretical constructs; a setback does not prevent the plant from producing a fertile or long leaf the following year, but it is a way of identifying stress preventing a plant from maintaining its previous vegetative or reproductive output.

SEUS population trends

General decline observed

The transition matrix estimated from 2000-2001 interval data into future generations showed a relative decrease of advanced sporophyte stages with time because advancement (i.e. sporeling-to-juvenile, juvenile-to-adult) transition probabilities were quite low. This shows that 2001 was a bad year for *A. monanthes*, possibly due to slightly colder, drier conditions than usual. Unfortunately the observed transition values may be typical for recent years, since some extant populations (Maple Springs Branch subpop. 1, NC, Glade Fern Ravine, SC) now support many fewer plants than historically and many other populations seem to have disappeared entirely over the past two decades. It is still unclear whether SEUS *A. monanthes* populations (a) are normally stable but currently in a period of sustained decline, (b) normally fluctuate but usually persist it spite of it, or (c) normally fluctuate and die out but a putative metapopulation persists by colonization of new sites. Possibility (b) is unlikely unless seemingly extirpated populations have maintained a spore bank or clonal gametophyte colonies (which continued monitoring would reveal). Possibility (a) would be cause for alarm, while possibility (c) would be the best case scenario given that (b) is unlikely. Clearly more monitoring (additional years and additional populations) will be required to improve the demographic model and resolve this question. A simple Lefkovitch matrix may not be ideal for modelling *A. monanthes*' population dynamics because it does not incorporate environmental stochasticity (e.g. flooding of vulnerable populations), but it should suffice until long-term data on such events can be collected.

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[14] This excludes the Florida Caverns plant added to the model to allow advancement to adulthood.

Causes of decline

Humans have been responsible for the decline of extirpated *A. monanthes* populations in some cases. Many South Carolina subpopulations below Lower Whitewater Falls were permanently flooded by the creation of Lake Jocassee around 1970 and some of the survivors were inadvertently buried during the construction (Bruce & Pittillo, 1974). Lewis Anderson (personal communication) speculates that the Corbin Creek, NC population was poached by unscrupulous fern hobbyists around the same time. Increasing development or logging may cause flash floods or change the water table so that substrate is too wet or too dry (Sam Cole, personal communication regarding San Felasco Hammock population, FL). Flooding may also occur without human involvement. The largest group of plants at Bearwallow Falls (NC) was killed in 1972 by a flood due to their location close to water level (NCNHP, 2000), and it is surprising that the remaining Coley Creek (SC) plant has not suffered the same fate based on its vulnerable location.

Pathogens and parasites may be responsible for limited plant mortality. Misshapen small fronds (some to the extreme of resembling moss or leafy liverworts) were observed occasionally in the Florida Caverns and Maple Springs Branch (NC) populations. One of the two adult plants at new Cane gravel seep subpopulation (SC) died after sawdust-like debris was observed on its fronds, possibly indicating a fungal infection. Algae were observed growing on fronds at Florida Caverns, but no harm appeared to come of this relationship. Aphids were observed on plants at the declining Thompson River, SC population.

Competitive exclusion may occur at some populations. Some plants at Thompson River, the Florida Caverns Walt's Misery subpopulation, and Maple Springs Branch subpopulation 5 were overgrown by Marchantioid liverworts. The liverwort competition was associated at Thompson River with saturation of the substrate, so excessive moisture is probably deleterious just as insufficient moisture may be. Sam Cole (personal communication) speculates that increasing substrate saturation at the now-extirpated San Felasco Hammock population (FL) may have increased competition from the weedy fern *Deparia petersenii* if the *A. monanthes* population was not killed directly by flooding.

Microhabitat instability may threaten populations. At Maple Springs Branch subpopulation 1, NC, Weakley (1987) reported a population of 23 plants, including 5 fertile ones, whereas now only two sporelings (which may not even be *A. monanthes*, since sporelings are impossible to identify) remain. Weakley (personal communication) speculates that this subpopulation may have fallen victim to a poorly attached bryophyte mat sloughing off because this shallow recess was in the direct path of water run-off from the cliff above.

Exposure to extreme cold appears to cause setbacks or death. I visited the new Cane gravel seep subpopulation (SC) in late November and removed leaf litter to census the population. The following summer this population had declined more than the others, possibly due to the absence of leaf litter insulation. Alan Cressler (personal communication) has observed poor regeneration in the Alabama populations after particularly cold winters.

The regional drought that occurred during this project is probably not responsible for the observed decline of SEUS *A. monanthes*. Mean monthly weather station precipitation was examined for each year beginning with 1991 and compared to long-term averages (**Fig. 11** of Introduction). The Scottsboro station (AL) experienced no decrease in precipitation, the Lake Toxaway Station (NC) a 2000-2001 decrease, and the Quincy station (FL) received low precipitation for 1998-2000 or arguably 2001. It is likely that none of the weather stations' values were outside the normal range because the mean annual precipitation over this 11-year period was about equal (Lake Toxaway) to slightly higher (Scottsboro, Quincy) than the long-term annual values obtained from the literature as discussed in this paper's Introduction. So while drought may potentially hurt *A. monanthes* populations (which seems likely based on the poor response to drying out observed in natural substrate gametophyte cultures), the current decline cannot be explained by drought except possibly in Florida, because the recent drought was in general not one of great magnitude.

It is possible that *A. monanthes* may colonize a site in a climatically atypical year and then be unable to withstand the return of more typical conditions in that particular site, e.g. colonizing a dripline during a dry year and dying in later years from

oversaturation. If spores were present at multiple locations within a site (i.e. if the population had once been fertile), loss of plants at any one location would not eliminate the population, but if spores had reached no other suitable habitat at the site (i.e. if the population was newly established), normal climatic fluctuations could threaten the population.

Another possible threat to *A. monanthes* populations is their genetic depauperacy. All SEUS populations sampled appeared to be fixed for a single genotype, but this does not cause genetic load because *A. monanthes* reproduces asexually and maintains a high level of fixed heterozygosity as an allotriploid. The only deleterious consequence of fixation is therefore the loss of evolutionary potential in the absence of genetic variability for selection to act upon. This makes *A. monanthes* less able to adapt to environmental change, a possible threat to *A. monanthes'* long-term survival. Genetic depauperacy seems unlikely to be responsible for *A. monanthes'* current decline because environmental change (e.g. global warming) has been occurring at a rate that even natural selection could probably not keep pace with.

Are current declines permanent or just cyclical?

Reed et al. (2002) recommended waiting until sufficient data is available before trying to assess population viability to avoid inaccurate projections and their possibly detrimental management consequences. However, SEUS *A. monanthes* is unlikely to receive further concerted attention to unsolved questions due to its lack of taxonomic distinction from neotropical *A. monanthes*. Therefore we must make tentative inferences from the existing limited data.

Which model for regional population dynamics (stable equilibrium, remnant, source-sink, metapopulations) does SEUS *Asplenium monanthes* fit? The loss of many historical SEUS populations suggests that many populations do not currently fit the stable equilibrium model. This could still be the populations' historical mode of operation if increased human impact of the past 150 years is responsible for population declines, but it seems doubtful that population sizes were ever large enough to avoid environmental and demographic stochasticity that precludes the attainment of stable equilibrium.

A. monanthes would seem a likely candidate for remnant population dynamics. Its gametophytes have the potential for clonal growth and its sporophytes are long-lived (based on the many fronds of adults combined with slow growth observed), both of which can allow populations to successfully wait out periods unfavorable to recruitment (Eriksson, 1996). Remnant population dynamics can be identified by a demographic profile skewed towards large adult plants at the majority of populations[15], with an absence of recruitment under prevailing conditions (Eriksson, 1996). In contrast to this prediction, sporelings were abundant at most SEUS populations. The main barrier to recruitment seems to occur in transitions from one sporophyte stage to the next (Table 18), but advancement was occasionally observed and the majority of populations contain all sporophyte stages, so recruitment to adulthood does occur slowly. Guess Creek Cave, dominated by large adult plants, is a notable exception, suggesting that mortality is rare and attainment of carrying capacity minimizes opportunities for recruitment. Coley Creek currently hosts just a single adult plant, so recruitment is not occurring there despite the availability of suitable microhabitats. At the remaining populations, there is no evidence of remnant population dynamics because recruitment appears to occur. While this could signify that the SEUS populations are remnant populations currently undergoing a recovery from unfavorable conditions based on observed recruitment, this is unlikely because most populations are in decline relative to historical sizes, some to the point of extirpation. True remnant populations probably also have greater survival probabilities for adults than observed here. While possibly uncharacterically low, the annual stasis transition probability for adults was estimated at 0.77, so with an average of only 2.6 adults per fertile population, all fertile plants would soon be lost in the hypothetical absence of recruitment. Therefore most SEUS populations do not display remnant population dynamics.

The source-sink model would be appropriate if certain populations always remained robust while others remained small and non-fertile based on possible

[15] Populations of species practicing metapopulation dynamics may also become skewed towards adults as the population matures, but many young populations with active recruitment are also present at any given time. In species undergoing remnant population dynamics, most populations lack recruitment most of the time.

differences in site characteristics. This does not appear to be the case. Previously robust populations have declined (Glade Fern Ravine, SC) or disappeared entirely (San Felasco Hammock, FL, Bearwallow Falls and Maple Springs Branch subpopulation 1, NC), while smaller populations have occasionally increased (Maple Springs Branch subpopulation 3). Although many populations have remained small (Coley Creek, SC and many others less severely so) and/or non-fertile (Upper Whitewater Falls, NC), no historically robust fertile populations have remained that way. Additionally one would expect to see a difference in habitat characteristics between small and/or sterile populations and large and/or fertile populations explaining the differences in populations' success. Microclimate variables measured show no consistent correlation with population size or number of fertile plants in a regression analysis, nor do edaphic characteristics examined non-statistically. Populations on limestone in Alabama ranged from the largest and most fertile at Guess Creek Cave and Neversink to small and immature at Balcony Sink, and the original Cane Creek, SC subpopulation is reasonably large despite being on calcium-poor biotite gneiss while other Carolina populations on calcium silicates range in size. Therefore even cryptic abiotic differences among sites cannot explain the differences in population size.

If source-sink dynamics do not apply within the southeastern U.S., one might consider whether they could apply at a much larger scale. Perhaps the southeastern U.S. in general is a sink regularly resupplied by spores from neotropical source populations. While an intriguing idea, this is easily disproven by the paucity of genotypes in the southeastern U.S. If neotropical immigrants were a frequent occurrence, many more genotypes would have been uncovered in the southeastern U.S. than the three known to date.

The metapopulation model would be appropriate if several new populations have become established as others have disappeared. Unfortunately it is impossible to determine whether populations new to botanists are really new or just previously overlooked because of their generally remote locations, small numbers, and similar appearance to other *Asplenium* species. The metapopulation model is most often applied to populations with short "lifespan." *A. monanthes*' main natural disturbances would

probably be floods (not a risk to Alabama populations) and sloughing off of its vertical bryophyte mats, neither of which is believed to occur with great frequency. Therefore *A. monanthes* is not an obvious candidate for the metapopulation model, but may fit it nonetheless. If so, individual populations might occasionally be extinguished by environmental or demographic stochasticity but population extirpation would not be as likely as for the fugitive species that metapopulation theory is most commonly applied to. Metapopulations survive extirpation of individual populations via colonization of new patches. *A. monanthes* has the capability for powerful dispersal of spores but strong winds rarely penetrate its sheltered SEUS microhabitats to carry spores away, so it is not obvious whether the metapopulation model would apply.

Genetic investigation can be used to assess the frequency of migration and determine whether *A. monanthes* is likely to practice metapopulation dynamics. The two Alabama populations sampled appeared monomorphic for different genotypes, so there was no evidence of migration between these two populations (although it is quite possible that my sampling would miss rare migrants since I sampled only 5% of the Neversink population and 21% of the Guess Creek Cave population). Any current migration among Carolina populations cannot be detected with isozymes because all are genetically identical for these markers (they presumably are all the result of dispersal from a single somewhat ancient colonization event from the tropics). Future studies might resolve the migration question by using hypervariable genetic markers to differentiate Carolina populations. For example, Schneller et al. (1998) used RAPDs to reveal genetic variation in the fern *Dryopteris remota* that was not detected by isozymes.

In summary, no single model is a clear fit for SEUS *A. monanthes* given the limited data available. If it is the metapopulation model that is most appropriate, the many observed population extirpations might not be cause for alarm. If it is any other model, there is indeed cause for alarm[16], because population decline would not be balanced by formation of new populations. Therefore continued population monitoring

[16] Although the source-sink model shows no repercussions for loss of sink populations, several of the declining populations were historically too large to be considered expendible sinks.

and further genetic investigation (to test migration) is critical to assessing whether *A. monanthes* is at great risk of regional extinction in the southeastern U.S.

Elucidation of population dynamics will also clarify which conservation strategies are most appropriate. Stable equilibrium and remnant populations are often limited to their existing sites because other sites, though seemingly suitable, are not or are only temporarily so (e.g. are more frequently disturbed than the occupied site) (Parks & Farrar, 1984), so all existing populations should be maintained. Introduction to new sites may also be beneficial if "stable" or remnant populations have limited dispersal, but this is a secondary management priority. Source-sink groups of populations would be best preserved by ensuring the continued viability of source populations rather than trying fruitlessly to bring sink populations up to the size of source populations. Metapopulations can best be preserved by creating new populations (either indirectly by maintaining suitable habitat or directly by sowing or transplanting to uninhabited suitable habitat) rather than placing much effort in preventing the natural loss of existing populations (Menges, 1990).

A. monanthes may not fit any idealized model perfectly, but comparison with idealized models is useful in elucidating which factors (e.g. recruitment, stasis, or immigration) are most responsible for populations' persistence or extirpation so that management decisions can be made accordingly. Until we have a better understanding of *A. monanthes*' population dynamics in the southeastern U.S., the conservative approach would be to protect all occupied sites and a sufficient number of potential sites from human-mediated disturbance.

Conservation recommendations

Monitoring

Simply studying a population can have a deleterious effect on it. There is a possibility that the population declines observed from 2000 to 2001 were a result of this study through handling of fronds and removal of leaf litter during censuses. For this reason, further detailed censuses should be performed only with good reason, for example to create a better transition matrix or to estimate plant age. For general monitoring of

population status, a simple unobtrusive estimate of number of plants at each stage should be sufficient and, if performed regularly, would add much to our understanding of these generally overlooked remote populations.

I would also recommend that apparently extirpated populations be monitored for recurrence of sporophytes. It is possible that *A. monanthes* is still there but lying dormant in the form of a spore bank and/or clonal gametophytes. Some tropical ferns like *Grammitis nimbata* and *Hymenophyllum tayloriae* live in the southeastern U.S. primarily as clonal gametophytes but occasionally produce a sporophyte, so survival as gametophyte colonies is certainly feasible for populations of ferns with clonal gametophytes. A pteridologist familiar with fern gametophytes could visit these extirpated *A. monanthes* populations with the botanists who might remember where exactly sporophytes grew (**Table 1** of the Introduction lists extirpated locations and their respective botanists) to collect miniscule fresh bryophyte samples (e.g. 1 cm^2; there are many rare bryophytes with protected status in *A. monanthes* sites) to search for *A. monanthes* gametophytes with a dissecting microscope.

Management intervention?

The three SEUS genotypes encountered (from Guess Creek Cave, AL, from Neversink, AL, and from the collective Carolinas) were not observed in the tropics. However, because they were qualitatively no more different from neotropical genotypes than one neotropical genotype from another, they do not represent a particularly unique genetic heritage. Therefore although *A. monanthes* should certainly not be ignored by land managers, other rare species can be given a higher priority for scarce conservation resources.

Fortunately none of the three genotypes encountered appear to be in immediate danger of extirpation. The two Alabama populations sampled were reasonably large and robust, and there are enough populations in the Carolinas that that area's genotype is not at risk. If the status of the Alabama populations were to change, however, that would warrant management intervention (e.g. limiting access to the population at Guess Creek Cave or creating additional shade at Neversink to replace a fallen canopy tree), since each Alabama genotype is at the moment known from only one population. The tiny Balcony

Sink population was not sampled, so it could have either Alabama genotype or a novel one. This population's small size relative to Neversink despite similar habitat is puzzling (perhaps different exposures are responsible). No management action can be taken because of its occurrence on private land, but fortunately no anthropogenic threats are present at this site.

The extant Florida population was not sampled genetically due to its small size, but it is probably no more genetically distinct from neotropical *A. monanthes* than the other SEUS populations are. Because of its geographic isolation and small size (an unknown number of sporelings, a few small juveniles, and one small adult), it is the population of greatest conservation concern. No additional conservation measures are feasible for it, unfortunately. One subpopulation is in a location that cannot be exclosed from the public while the other is already off the beaten track. If the Florida Caverns genotype were known and happened to match that of other SEUS populations, it could possibly be supplemented with appropriate spores, but until the population can be examined genetically, it is probably best just left alone. Hopefully several of the unidentifiable sporelings will grow up to be adult *A. monanthes* plants and maintain the population.

If reintroduction methods are ever attempted, what techniques could be used? Transplantation of live plants was the method found to be most successful in angiosperm reintroductions by Drayton & Primack (2000). This was investigated in the lab for mature *A. monanthes* plants harvested from Costa Rican and Mexican populations. Plants survived from a few weeks up to a year before succumbing to fungal pathogens, so this can be considered a small success. No live SEUS plants were harvested for transplantation because of their threatened status, but if it was ever deemed necessary, sporelings and their substrate could potentially be harvested from populations with an excess of sporelings (e.g. Neversink, AL) without causing undue harm. Another option, probably a long shot due to the initial absence of roots, would be to break off the rare frond with a stipe sprout for transplantation. It is unfortunate that this project's attempts at lab propagation from spores proved unsuccessful since this propagation method does not deplete source populations of limited sporophytes and results in a high initial yield

(over 90% of spores germinated on agar medium and many survived to sporophyte initiation). Perhaps further experimentation could yet perfect this approach, e.g. decontamination of spores, use of anti-algal and anti-fungal chemicals in agar cultures or simply more frequent watering of natural substrate cultures. A low-yield but equally population-friendly propagation method would be to simply sow spores directly onto bryophyte mats at reintroduction sites.

In summary, no management intervention is advised at the moment since no successful propagation methods have been developed, most populations already occur in protected and/or remote areas, and the populations are not genetically distinct from neotropical populations. If reintroduction programs are ever attempted (e.g. if *A. monanthes* continued to decline in the southeastern U.S. and land managers become increasingly alarmed), this study's genetic findings will prove useful. Any *ex situ* conservation program would be complete with just a modest amount of spores from Guess Creek Cave, Neversink, any Carolina population, and Florida Caverns (although this population may turn out to be genetically identical to others). Spores from any Carolina plant could safely be used for restoration in the Carolinas (even a site as far away from the others as Table Rock, SC) since all Carolina populations sampled have proved genetically identical. If restoration is attempted for populations in Alabama and Florida that cannot be genetically analyzed (i.e. populations extirpated or nearly extirpated), spores should be taken from the site with the most similar microhabitat (e.g. Balcony Sink would utilize spores from Neversink, San Felasco Hammock would utilize spores from Florida Caverns) to maximize the probability that the imported plants would be well adapted to the host site. For now, land managers should simply monitor the populations and see whether current declines correct themselves or intervention is warranted. While not genetically unique or globally threatened, SEUS *A. monanthes* still deserves some conservation attention as part of the intriguing tropical component of the flora of the southeastern U.S.

GENERAL CONCLUSION

Although the main goal of genetic investigation of *A. monanthes* was to determine the biogeographic origin of the SEUS populations, this investigation also provided information that could be used to determine the species' allopolyploid and geographical origin. There was data suggestive of multiple hybrid origins (a great number of multilocus genotypes) in Mexico but no other region, suggesting that Mexico may be a center of origin of the species. All *A. monanthes* samples clustered together genetically relative to outgroups, suggesting that all hybridizations occurred between the same parental species. This may or may not hold true in other parts of *A. monanthes*' global range. Electrophoretic and cytological studies of *A. monanthes* in distant regions like Hawaii, South America, various Atlantic islands, and Africa would be useful in conjunction with this study to determine *A. monanthes*' allopolyploid origin(s) and global biogeographical history.

Asplenium monanthes appears to have arrived in the southeastern U.S. via multiple long-distance dispersal events, probably from the Caribbean, during the Pleistocene or Holocene. Long-distance colonization may be somewhat frequent in polyploid ferns (demonstrated also in Ranker et al., 1994a and 1994b, and in Schneller et al., 1998), suggesting that successful establishment (i.e. high isolate potential) is probably more limiting than spore dispersal for colonization of distant regions by ferns. This may be partially responsible for the often greater range achieved by polyploid (Vogel et al., 1999) taxa relative to related diploid taxa. Apogamous species would similarly be expected to show a greater geographic range than related sexual species due to high isolate potential, but according to Richards (1997), the evidence for such a trend is less decisive.

A. monanthes' apparent origin from Quaternary Period long-distance dispersal contrasts with the apparent pre-Pleistocene origin of many other tropical plants of the southeastern U.S. There may be other tropical taxa in the southeastern U.S. that likewise did not arrive until the Quaternary; this study shows the importance of investigating each

case rather than assuming that all species with a given biogeographical pattern share the same biogeographical history.

A. monanthes shares with pre-Pleistocene relicts a restriction to climatically moderated microhabitats like gorges, sinkholes, and cave entrances, all of which were characterized climatically in this study. This study also elucidated several other aspects of the SEUS populations' ecology, e.g. bryophyte associates, rock substrates, sporophyte phenology, and typical population demographics. However much remains to be learned of *A. monanthes*' ecology, e.g. winter microclimate, gametophyte phenology, the extent of gametophyte clonality in natural populations, the size of gametophyte populations, plant lifespan, and the relative importance of various environmental factors to *A. monanthes*' success.

Many SEUS populations have declined in recent decades, in many cases for unknown reasons. It is not clear whether the decline of these populations is cause for alarm or just a manifestation of possible metapopulation dynamics. This question can be resolved by continued monitoring of extant and apparently extirpated populations (e.g. including sampling bryophyte mats for gametophytes at extirpated populations) and utilization of hypervariable genetic markers to test for migration among the Carolina populations. For the moment a lack of successful propagation methods precludes any management intervention even if the populations are found to be declining dangerously. Further experimentation with propagation methods might prove fruitful, and this study's characterization of *A. monanthes*' microclimate and substrates would prove useful if reintroduction efforts were ever attempted. The causes of population declines in the southeastern U.S. cannot be remedied because in most cases they remain a mystery. Perhaps long-term monitoring can reveal correlations of declines with climatic conditions, disturbances, or interspecific interactions.

The SEUS populations have not significantly diverged morphologically or genetically from neotropical populations, so they will continue to be recognized taxonomically as *A. monanthes* L. The lack of taxonomic distinction lessens the urgency of conservation of the SEUS *A. monanthes* but does not eliminate it. These populations should be preserved as part of the tropical heritage of the southeastern U.S. It is hoped

that this project catalyzes further study of the SEUS populations that might answer some of the lingering questions about their origin and conservation biology for the benefit of the species' conservation in the southeastern U.S.

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