# THE POPULATION BIOLOGY OF *PELLAEA WRIGHTIANA* HOOKER, A FERN DISJUNCT IN NORTH CAROLINA

A Thesis

by

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THE POPULATION BIOLOGY OF
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**ABSTRACT**

**THE POPULATION BIOLOGY OF *PELLAEA WRIGHTIANA* HOOKER,**

**A FERN DISJUNCT IN NORTH CAROLINA. (August 1996)**

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*Pellaea wrightiana* Hooker is a homosporous fern with a main range throughout most of the southwestern United States. In 1956, a small population of *Pellaea wrightiana* was discovered in Alexander County, North Carolina. In 1974, a second population was discovered in Stanly County. Both populations occur on south-facing rock outcrops with similar native plant species. *Pellaea wrightiana* has been confirmed to be a fertile, tetraploid hybrid between two diploid southwestern species, *Pellaea truncata* and *Pellaea ternifolia*. These two North Carolina populations represent the only populations east of the main range, thus genetic analyses of the two populations were conducted to test the following hypotheses: there was only one introduction into the state and one population gave rise to the other, or, there were two separate introductions. Approximately 45 individuals from Alexander County and 75 individuals from Stanly County were examined via

starch gel electrophoresis of enzymatic proteins. Seven enzyme systems were analyzed with a total of eleven loci. While allele additivity from each diploid parental species was detected, no genetic variation was observed either within or between North Carolina populations of *Pellaea wrightiana*. This lack of genetic variation may be attributed to ecological factors and various aspects of fern reproduction, particularly intragametophytic selfing. Because of the complete lack of genetic variation within and between the two populations, evidence supports the hypothesis of a single introduction into the state, possibly by a single spore.

## ACKNOWLEDGEMENTS

There is a song with the lyric, "Nothing can survive in a vacuum; no one can exist all alone." Nowhere is this more true than in academia. First and foremost, I thank Dr. Gary Walker. Gary has done a great deal not only for me, but for all graduate students in our department. His enthusiasm in our goals and willingness to "go to bat" for us often goes unthanked. Gary's time, efforts, lab space, and refusal to give up on me are immeasurably appreciated. I would like to especially thank Dr. John Bond for serving on my committee, turning me on to mushrooms, and for being a role-model teacher. Dr. Bond's zeal and toothy smile will be sorely missed. Dr. Kelly Steele, another committee member, helped with "eyeballing" gels and provided helpful suggestions and encouragement on just about all aspects of this project. I also thank my fourth committee member, Dr. Ken Shull. Dr. Shull, like Gary, refused to give up on me and salvaged what little self confidence I had left. Because of his advice and encouragement, I was pointed in the direction I always wanted to go. Dr. Shull's input to my graduate career will never be forgotten.

I also thank those who worked with me either in the field, lab, or both. My colleague, officemate, and friend, Ed Lickey, had a hand in just about all

aspects of this project. Ed's help and friendship are greatly appreciated. Lonnie Shull risked life and limb by repelling Morgan's Bluff to collect *Pellaea*, and Dana Tamashiro helped with advice on lab techniques and gel scoring. Dr. Jim Matthews at UNC-Charlotte graciously gave me "dibs" on *Pellaea* and served on my committee for a short while. Sam Pearsall, Margit Bucher and all at The North Carolina Nature Conservancy allowed me to repeatedly visit Little Joe Mountain. Mr. and Mrs. Larry Howell of Stanfield allowed me onto their property to access Morgan's Bluff, and Natalie Coffer, Robert Efird, Don Heafner, and Donna Winfree accompanied me on fruitful collecting trips.

Drs. Richard Henson, Wayne Van Devender, Tom Van Devender, Michael Ross, and Gerald Gastony provided fresh material of either *Pellaea wrightiana*, *Pellaea truncata*, or *Pellaea ternifolia*. Their help is much appreciated. Dr. Mike Windham provided advice on how to interpret gels and gave insight into *Pellaea wrightiana* in the southwest. His help was most valuable.

I thank my friends at Appalachian State University: Bennie, Brian and Jen, Tim-O, Carol, Dana, Lonnie, Ed and A., Natalie, Ben, Welch, Wood, Betsy Cobb, Betsy Harris, Judy Williamsen, Kerry Knauf, and all the parents who came along as chaperons! To my brother, I extend my thanks for a place to stay, and my apologies for invading your life.

## DEDICATION

This work is dedicated to my family, my friends, and to the faculty of the Department of Biology at Mars Hill College. Teachers, scientists, friends, and true bacchanalians: Drs. Sam Boggess, JoAnn Croom, Bill Hutt, Scott Pearson, Frank Quick, and particularly Donald E. McLeod, to whom I owe my career.

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# INTRODUCTION

# AND

# REVIEW OF THE LITERATURE

Population disjunctions occur when populations are separated from their main ranges and become established in distant habitats. This can result when geographic events break up large populations into smaller populations, or by long distance dispersal of reproductive propagules. Homosporous pteridophytes may give rise to disjunct populations via long distance dispersal of spores that may be carried extensive distances by winds. If the spores land in suitable habitats, they germinate with each spore giving rise to a free-living gametophyte that may be bisexual. Egg and sperm cells are produced by mitosis and, if fertilization takes place, a new sporophyte is formed.

Wagner (1972) described disjunction patterns of homosporous pteridophytes and concluded their distributions were similar to those of angiosperms and gymnosperms; endemism and long distance dispersal occur in all three groups. Many disjunct populations of pteridophytes in North America may be the result of long distance dispersal of spores rather than historical geographic events (Wagner 1972).

There are several interesting examples of pteridophyte disjunctions in the southeastern United States. Of particular note in the southeastern fern flora is the occurrence of a filmy fern, *Hymenophyllum tunbrigense* (L.) J.E. Smith, in Pickens County, South Carolina. With a main range throughout most of Europe and the West Indies (Wagner 1965; Mickel 1979), South Carolina is its only known North American location (Wagner *et al.* 1970). The most recent record of a pteridophyte disjunction in North Carolina is that of whisk fern, *Psilotum nudum* (L.) Beauvois. Perry and Musselman (1994) discovered a small population growing terrestrially in Chowan County in the Great Dismal Swamp. Long distance dispersal of spores from its subtropical or tropical main range most likely accounts for its occurrence at such a northern latitude.

A west to east disjunct trend is often observed in disjunct pteridophytes of the Carolinas and southeastern United States (Wagner 1972). An example of interest is forked spleenwort, *Asplenium septentrionale* (L.) Hoffmann. With its main range in the Sierra and Rocky Mountain states (Mickel 1979), this species has been observed on several rock outcrops in West Virginia (Lellinger 1985; Bush 1986). In Georgia, wavy cloak fern, *Astrolepis sinuata* (Lagasca ex Swartz) Benham & Windham ssp. *sinuata*, occurs in Meriwether County shaded by some rocks on a roadside (Snyder and Bruce 1986; T.S. Patrick personal communication 1994). Its main range extends from western Oklahoma southward into Arizona and Mexico (Mickel 1979; Lellinger 1985).

In North Carolina, more locally, Tennessee bulblet fern, *Cystopteris tennesseensis* Shaver, occurs on marl outcrops in Craven, Jones, Graham, and Onslow Counties (Wagner 1972; Amoroso and Weakley 1995). With a main range on the Cumberland Plateau of Tennessee, the Ozark Mountains, and Kansas (Wagner 1972; Mickel 1979), individuals of this species represent less widely distributed disjunctions than some western ferns.

The focus of this study is the west to east disjunction of Wright's cliff brake, *Pellaea wrightiana* Hooker, a homosporous, xerophytic fern native to the southwestern United States. Alexander and Stanly Counties, North Carolina (Figure 2) have the only two populations east of the main range (Figure 1), which includes northern Mexico, Arizona, New Mexico, most of Texas, and southern Oklahoma (Tryon 1957; Mickel 1979; Windham 1988). *Pellaea wrightiana* was first discovered in North Carolina in Alexander County by Dr. Albert E. Radford in 1956 (Wagner 1965). The second population was discovered in Stanly County in 1974 (Hood 1978). In North Carolina, *Pellaea wrightiana* is listed as endangered and of special concern by the North Carolina Natural Heritage Program (Amoroso and Weakley 1995).

## SYSTEMATIC BACKGROUND OF *PELLAEA WRIGHTIANA*

Wright's cliff brake occupies sites ranging from 900 to 9000 ft. in elevation throughout its southwestern range. These sites are most commonly dominated by pinyon pine (*Pinus edulis* Engelmann) and juniper (*Juniperus*

**Figure 1.** Distribution of *Pellaea wrightiana* in the United States.

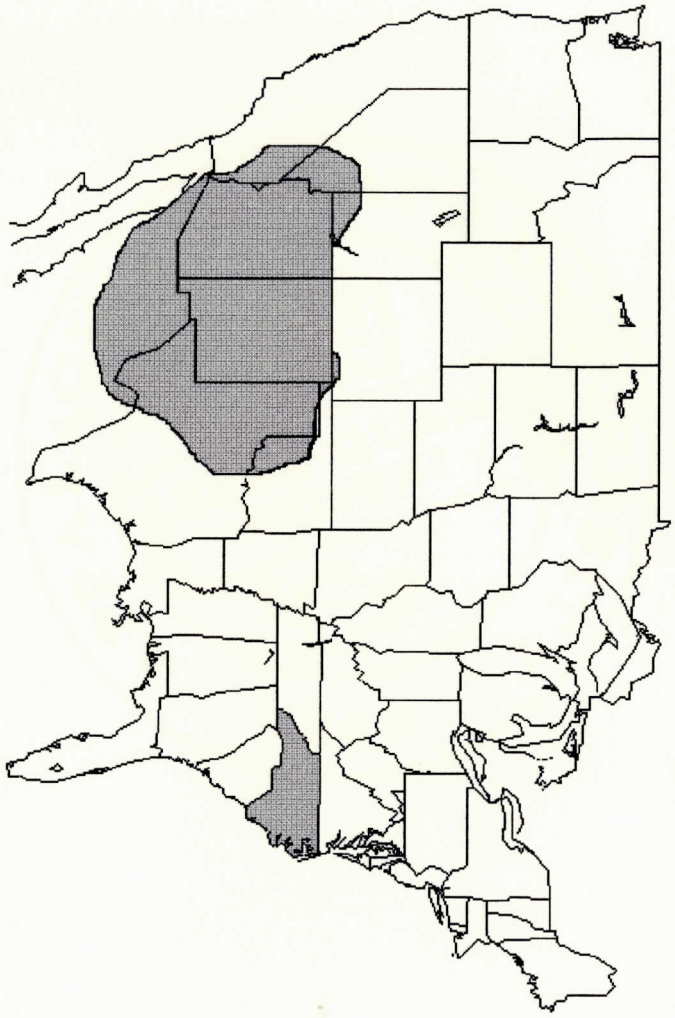
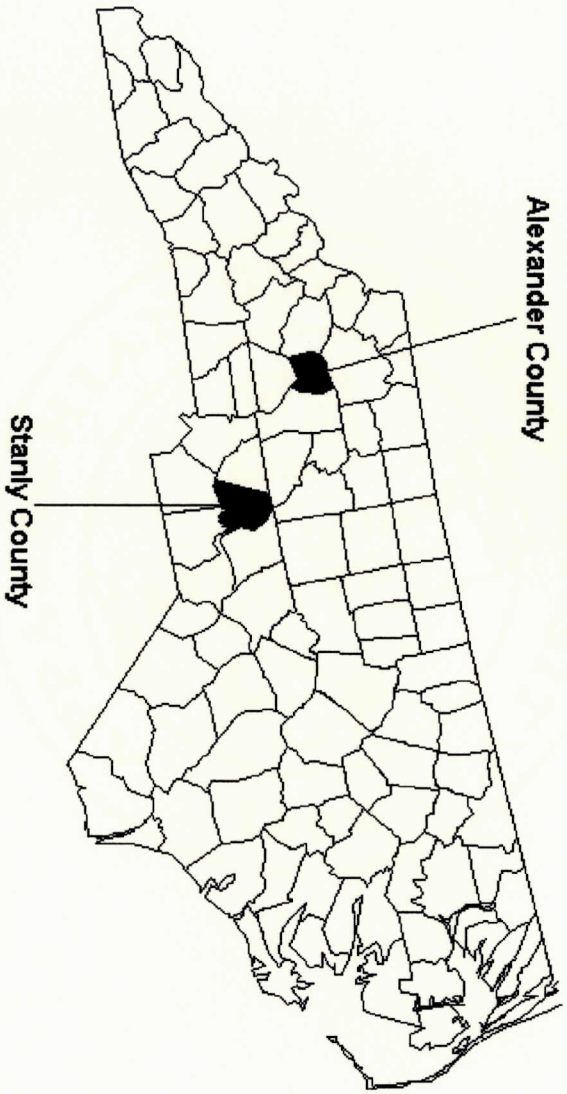


**Figure 2.** Distribution of *Pellaea wrightiana* in North Carolina.

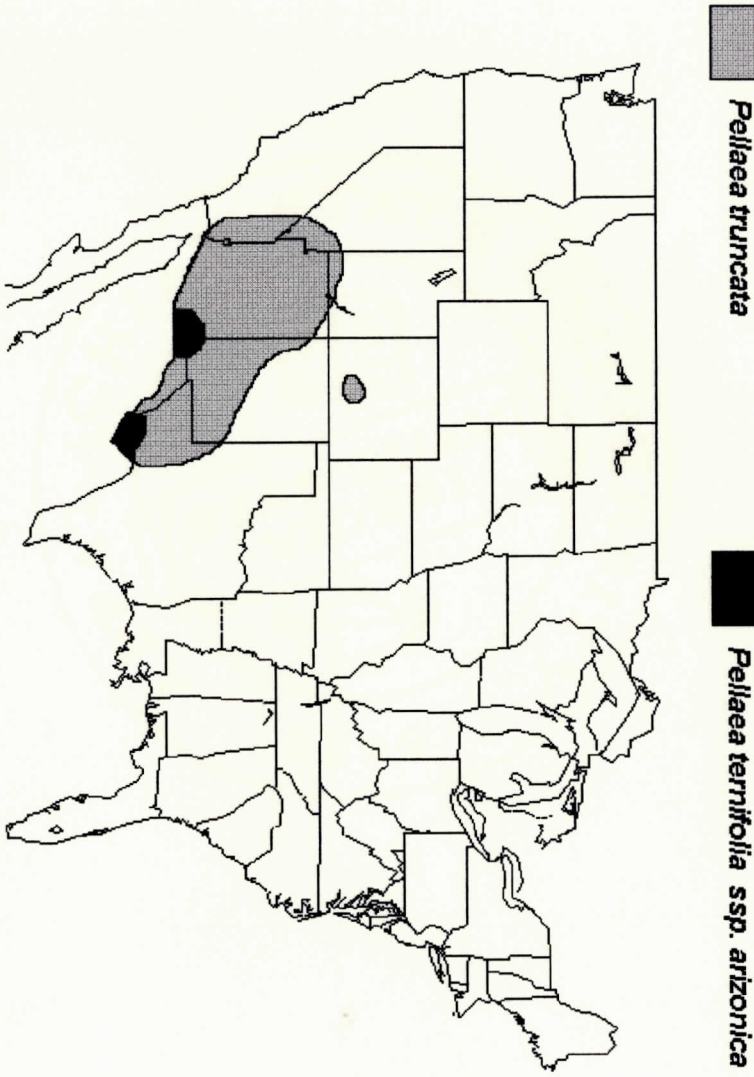

Alexander County
Stanly County

*osteosperma* (Torrey) Little). It is also found in other communities such as desert grassland and chaparral (M.D. Windham personal communication 1994).

*Pellaea wrightiana* has presented taxonomic problems because of its hybrid nature. It was first described by W. J. Hooker in 1858 as he studied the New Mexico fern collections of Charles Wright (Knobloch and Britton 1963; Wagner 1965). Tryon (1957) described *Pellaea wrightiana* as a variety of *Pellaea ternifolia* (Cav.) Link, which has several subspecies ranging from the southwestern United States to Argentina. Knobloch and Britton (1963) presented cytological evidence that *Pellaea wrightiana* contained 29 bivalents, 29 univalents, and 64 variable-sized spores per sporangium, thus supporting that it is a sterile triploid. It was hypothesized to be a hybrid between a diploid cytotype of *Pellaea ternifolia* and diploid *Pellaea longimucronata* Hooker (now called *Pellaea truncata* Goodding (Mickel 1979)).

Following a more detailed investigation into the origin of *Pellaea wrightiana* using both spore morphological and cytological data from Alexander County plants, Wagner (1965) proposed that typical *Pellaea wrightiana* has 58 pairs at diakinesis and develops 64 uniform-sized spores per sporangium, thus indicating that it is a fertile tetraploid. He also concluded that Knobloch and Britton's specimen was a backcross between fertile, tetraploid *Pellaea wrightiana* and one of its parents, *Pellaea truncata*. Further comparisons of leaf cell and frond morphologies made by Wagner (1965) showed that North Carolina *Pellaea wrightiana* was indeed of intermediate form between *Pellaea*

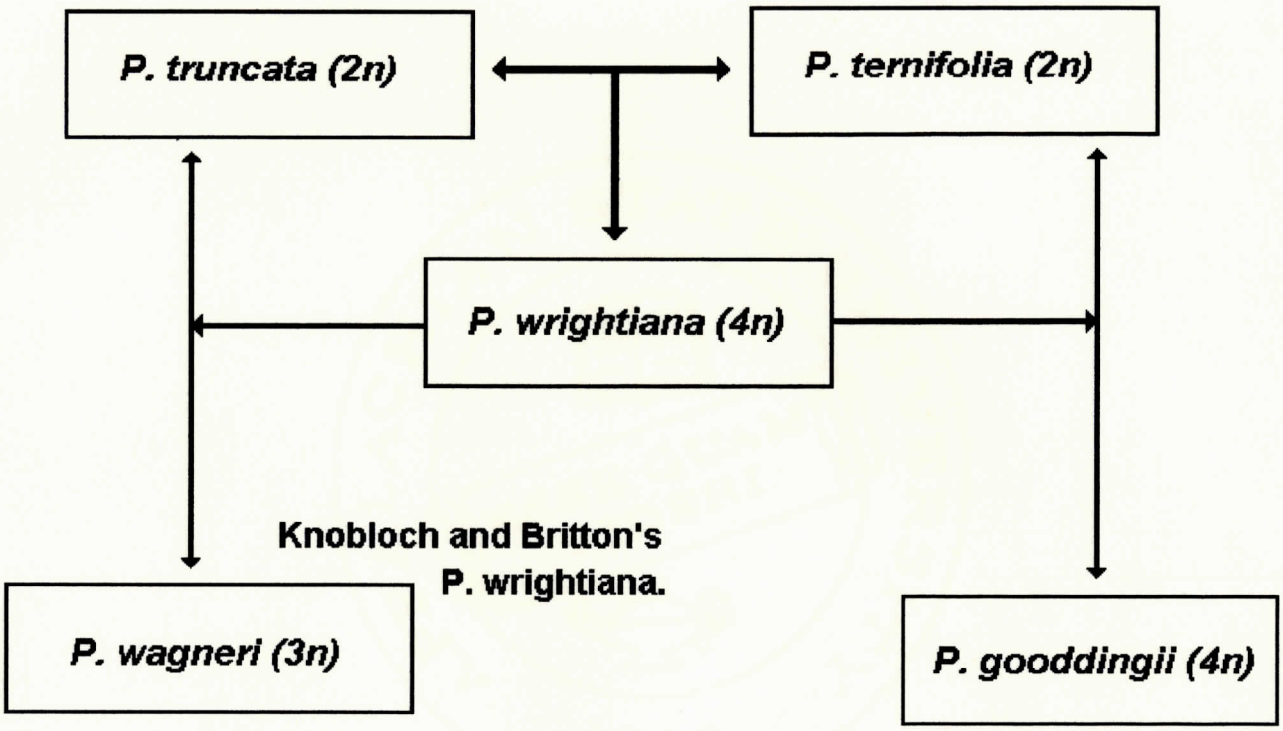
**Figure 3.** Distributions of *Pellaea truncata* and *Pellaea ternifolia* ssp. *arizonica* in the United States.


Pellaea truncata
Pellaea ternifolia ssp. arizonica

*truncata* and *Pellaea ternifolia*. One subspecies of *Pellaea ternifolia*, ssp. *arizonica* Windham, is believed to be the other parental species with *Pellaea truncata* (T.R. Van Devender and M.D. Windham personal communication 1994). Figure 3 represents the distributions of both *Pellaea truncata* and *Pellaea ternifolia* ssp. *arizonica* in the United States. The apparent sexual nature of North Carolina *Pellaea wrightiana* and the geographic overlap of *Pellaea wrightiana* in the southwest with *Pellaea truncata* provided evidence that *Pellaea wrightiana* was a sexually reproducing species, and is part of a problematic species complex.

More extensive cytological and morphological data coupled with electrophoretic data (Windham 1988) provided conclusive evidence that Wagner's (1965) hypothesis of a polyploid complex was accurate and that Knobloch and Britton's (1963) *Pellaea wrightiana* was a sterile backcross. Electrophoretic data further indicated that fertile, tetraploid *Pellaea wrightiana* could backcross with both of its parental species to produce sterile hybrids. *Pellaea* X *wagneri* Windham hybr. nov. was shown to be a sterile triploid hybrid between fertile, tetraploid *Pellaea wrightiana* and *Pellaea truncata* while *Pellaea* X *gooddingii* Windham hybr. nov. was found to be a sterile tetraploid between fertile, tetraploid *Pellaea wrightiana* and diploid *Pellaea ternifolia* (Windham 1988). Neither of these hybrid names have been formally published (M.D. Windham personal communication 1994). A diagram of this complex is shown in Figure 4. Electrophoretic analyses further showed each taxon to be

**Figure 4.** The *Pellaea wrightiana* complex as described by Windham (1988).


P. truncata (2n)
P. ternifolia (2n)
P. wrightiana (4n)
Knobloch and Britton's
P. wrightiana.
P. wagneri (3n)
P. gooddingii (4n)

genetically variable throughout their ranges in the southwestern United States (Windham 1988).

## PREVIOUS FERN GENETIC STUDIES

An ever-increasing body of information exists regarding fern genetics. Many of these studies focus on the cytogenetics of fern species (as in Walker 1979 and citations within) and provide evidence for hybrid complexes, as in the Appalachian *Asplenium* complex (Wagner 1953 and 1954). There also exists a rapidly increasing body of knowledge regarding the population genetics of ferns and fern allies, especially with the utilization of electrophoretic techniques. Electrophoretic data are used in both systematic and population studies of ferns (e.g. Werth *et al.* 1985; Haufler 1987; Windham 1988; Werth 1989). The present study represents the first electrophoretic investigation of both North Carolina populations of *Pellaea wrightiana*.

## PURPOSE OF THIS STUDY

The discovery of *Pellaea wrightiana* over 1000 miles from its main range prompted questions about the origin and genetic relatedness of the two North Carolina populations. This study attempts to answer the following questions.

1. Was there only one introduction of *Pellaea wrightiana* into North Carolina, where one population gave rise to the other?
2. Were there two separate introductions into the state, with neither population giving rise to the other?
3. Are North Carolina populations of *Pellaea wrightiana* sexually reproducing?

These questions can best be answered by examining the genetic structure of these populations via allozyme analyses. If little or no genetic variation is found between the two populations, then the hypothesis of a single introduction is supported. If there is significant variation between the two populations, then the hypothesis of independent introductions is supported. Finding significant amounts of variation will also be an indication of sexual reproduction. In the case of homosporous ferns, however, sexual reproduction may be occurring even if variation is minimal. Evidence that North Carolina populations of *Pellaea wrightiana* are sexually reproducing is indirect. Experiments where two different gametophytes were crossed have produced minimal results (J.F. Matthews personal communication 1993).

## MATERIALS AND METHODS

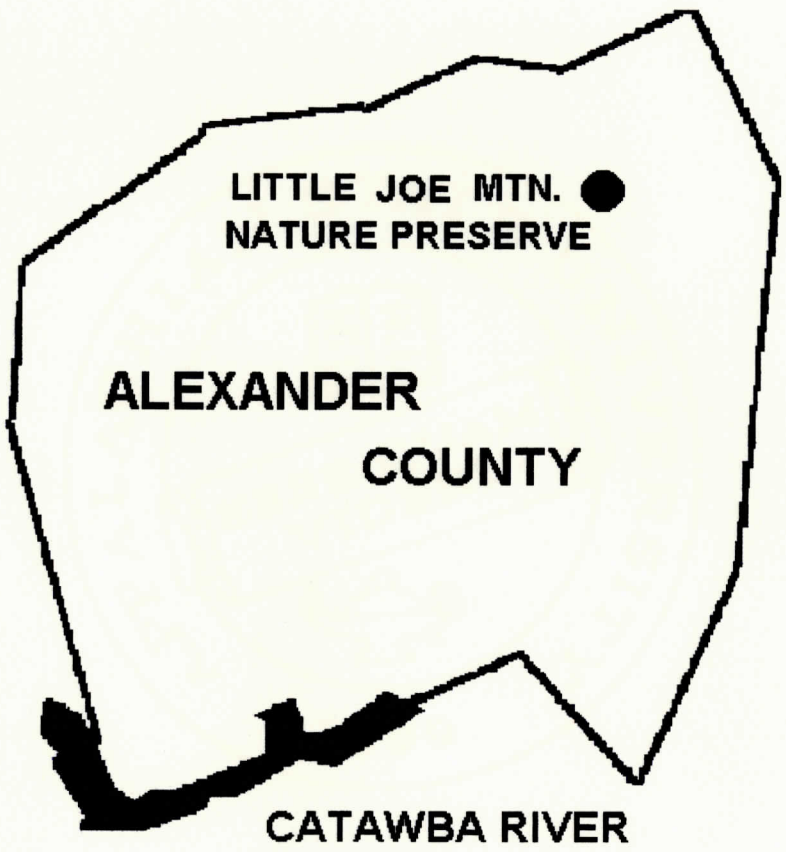
### Description of Collection Sites

#### Alexander County

*Pellaea wrightiana* occurs in Alexander County, North Carolina at Little Joe Mountain Nature Preserve. The approximate location of this site is 36° 01' 00" north latitude and 81° 09' 03" west longitude and is shown in Figure 5. This property is owned by the North Carolina Nature Conservancy. The site on Little Joe Mountain is a 180° south-facing, xeric rock outcrop composed mainly of granitic rock formations and is at an elevation of approximately 457.2 meters (≈1500 ft.). Schafale and Weakley (1990) classified this area as a low elevation granitic dome.

This outcrop supports a diverse assemblage of plant species. The main canopy species on the outcrop itself are eastern red cedar, *Juniperus virginiana* L., and scrub pine, *Pinus virginiana* Miller. Fringe tree, *Chionanthus virginicus* L., is also found scattered across the site. The top of the outcrop grades into mixed hardwood forest consisting of chestnut oak, *Quercus montana* Willdenow, pignut hickory, *Carya glabra* (Miller) Sweet, and other hardwood species. The herbaceous species diversity is especially remarkable for such a xeric habitat. Mats of moss and organic debris

**Figure 5.** Approximate location of Little Joe Mountain Nature Preserve in Alexander County, North Carolina.


LITTLE JOE MTN.
NATURE PRESERVE
ALEXANDER
COUNTY
CATAWBA RIVER

support species such as pineweed, *Hypericum gentianoides* (L.) BSP., fameflower, *Talinum teretifolium* Pursh, dayflower, *Commelina erecta* L., crotonopsis, *Crotonopsis elliptica* Willdenow, and numerous individuals of prickly pear cactus, *Opuntia compressa* (Salisbury) MacBride. Pteridophytes other than *Pellaea wrightiana* include ebony spleenwort, *Asplenium platyneuron* (L.) Oakes, spikemoss, *Selaginella rupestris* (L.) Spring, forming organic mats with mosses, and two species of lip fern: hairy lip fern, *Cheilanthes lanosa* (Michaux) Eaton, and wooly lip fern, *Cheilanthes tomentosa* Link. A few individuals of resurrection fern, *Polypodium polypodioides* (L.) Watt, are evident on branches of *Juniperus*, but occur very sporadically.

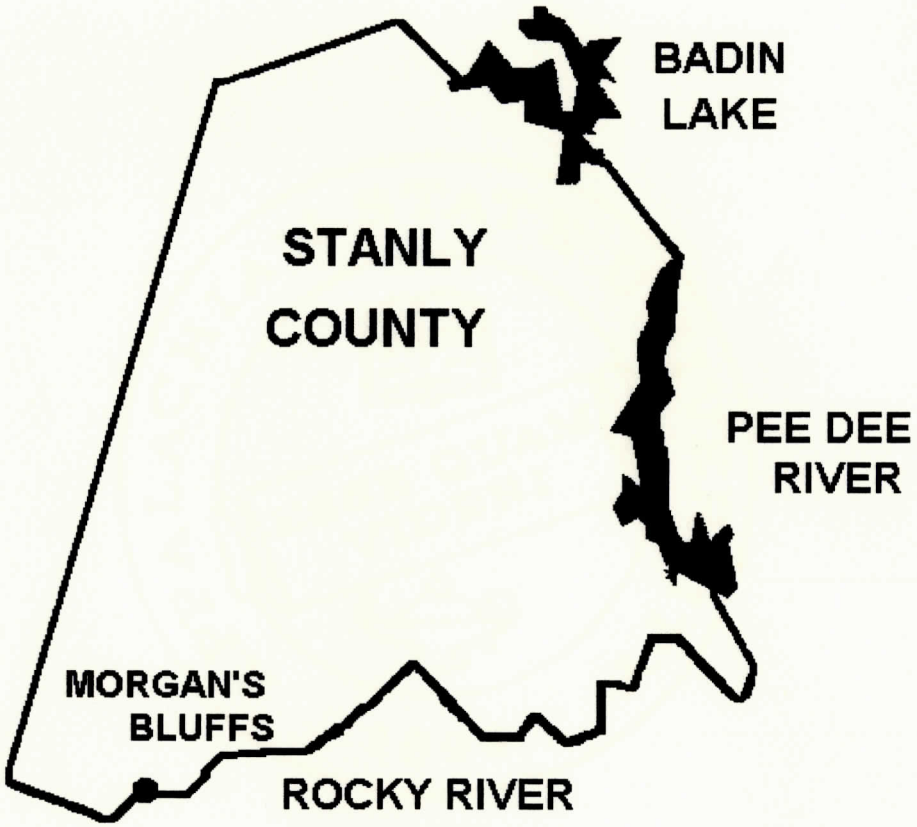
Radford and Wagner estimated the population of *Pellaea wrightiana* to be approximately 100 individual plants in 1964 (Wagner 1965). Since its discovery, the population has decreased to only about 50 individuals (Heafner unpublished data). *Pellaea* is most commonly found in open sun on organic mats in close association with other herbs and may be subjected to intense competition by *Cheilanthes* and various grasses (e.g. *Andropogon sp.*). *Pellaea* can also be found growing out of small cracks on the bare rock, but these plants are usually small and rarely survive the intense summer heat. Robust, heavily sporulating cliff brakes can also be found in microhabitats that are partially shaded by pine or juniper branches, or even rocks.

**Stanly County**

*Pellaea wrightiana* occurs in Stanly County, North Carolina at Rocky River Morgan's Bluff Natural Area. The approximate location is 80° 25' 00" west longitude and 35° 10' 48" north latitude and is shown in Figure 6. This site is currently co-owned by UNC-Charlotte and Davidson College. The Rocky River site is at an elevation of approximately 121.9 meters (≈400 ft,) and is a south-southwest-facing slate escarpment that has been classified by Schafale and Weakley (1990) as a piedmont mafic cliff. Hood (1978) provided a vegetation description of this site and classified it according to Radford's (1977 cited in Hood 1978) Natural Area and Diversity Classification System.

The Stanly County site lies within an area known as the Carolina Slate Belt. The Slate Belt spans almost all the piedmont region of North Carolina and extends out onto the extreme western coastal plain. The Slate Belt also extends northward into Virginia and southward into South Carolina and Georgia (Hood 1978). Hood (1978) described the Carolina Slate Belt based on geologic data of Fullager and Butler (1976 cited in Hood 1978) as being composed of late Precambrian and early Paleozoic metavolcanic and metasedimentary rock. The formations of Morgan's Bluff are mainly of two rock types: mudstone and laminated argillite (Allen 1977 cited in Hood 1978). At its highest point, the escarpment is approximately 17 meters high (Hood

**Figure 6.** Approximate location of Rocky River Morgan's Bluff Natural Area in Stanly County, North Carolina.


BADIN
LAKE
STANLY
COUNTY
PEE DEE
RIVER
MORGAN'S
BLUFFS
ROCKY RIVER

1978) and approximately 300 meters long, running east to west. This approximation takes into account a property boundary on the west end of the bluff.

Morgan's Bluff supports a flora similar to Little Joe Mountain. On the outcrops, the main canopy species are *Juniperus virginiana* and *Pinus virginiana.* There are smaller, scattered hardwoods represented by *Quercus montana*, *Quercus alba* L., and *Carya glabra*. A prevalent shrub species is sparkleberry, *Vaccinium arboreum* Marshall. American holly, *Ilex opaca* Aiton, and flowering dogwood, *Cornus florida* L, are scattered in the subcanopy. The top of the bluff grades into pine-oak-hickory forest.

On the east side, the cliffs gradually lower until they meet the well-developed flood plain, which spans the length of the bluffs. Here, stands of muscle wood, *Carpinus caroliniana* Walter, and chalk maple, *Acer leucoderme* (Small) Desmarais occur. Towards the west end of the flood plain, the dominant canopy species is winged elm, *Ulmus alata* Michaux. Poison ivy, *Toxicodendron radicans* (L.) Kuntze, and trumpet vine, *Campsis radicans* (L.) Seemann, are rooted in the soil on the flood plain and spread up the vertical rock face, sometimes forming dense coverings. The flood plain also supports dense stands of river oats, *Uniola latifolia* Michaux, and various other grasses. Of particular concern is the rapid spread of privet hedge, *Ligustrum sinense* Loureio, an introduced ornamental, along the flood plain. At the height of the growing season, privet and winged elm almost

entirely shade the rock face, thus creating potentially detrimental conditions for *Pellaea*, which is adapted to partial and full sun with a soil substrate.

Herb assemblages are similar to those of Little Joe Mountain in that *Opuntia compressa*, *Cheilanthes tomentosa*, and *Asplenium platyneuron* are present. There are, however, more occurrences of *Polypodium polypodioides*. Dayflower, *Commelina communis* L., and whorled-leaf tick seed, *Coreopsis verticillata* L., are also scattered throughout the area. A rare herb, Missouri rock cress (*Arabis missouriensis* Greene) colonizes areas on both the outcrops and in the more mesic forest.

*Pellaea* occurs most frequently on the vertical rock face, but some individuals occur on the uppermost rocks several meters away from the edge of the cliffs. As in Alexander County, there seems to be intense competition from species such as *Cheilanthes*, various grasses, and especially *Toxicodendron* and *Campsis*. The most significant threat to *Pellaea* at this site, however, is the shading of the rock face by privet and elm. When this site was first described, Hood (1978) estimated the population of *Pellaea* to be 500 individual plants. This population has declined to approximately 150 individuals. The Stanly County plants are usually smaller than the Alexander County plants, and most have ternately compound pinnae the length of the rachis. Fewer sporulating fronds have been observed compared to the Alexander County plants. This is probably due, in part, to the shaded nature of the site.

**Sample Collection**

For electrophoretic analyses, two to three healthy fronds per individual were collected from robust plants in Alexander County. Often, less material was taken in Stanly County, due to the more depauperate nature of plants in that population. Extreme care was taken not to take whole plants or pull plants loose from their substrates. Leaf material was stored in resealable plastic bags containing a moist paper towel for transport to the laboratory. The Alexander County population collection was essentially a census of 45 of the approximately 50 remaining plants while 75 samples were taken from a population of about 150 plants in Stanly County. The total sample size for both populations was approximately 120 individuals. Voucher leaf specimens were deposited in the herbarium at Appalachian State University (BOON).

For spore germination experiments, spores were collected from the Alexander County population by placing sporulating fronds between two sheets of typing paper in a plant press. Fronds were allowed to dry for two weeks in the press, after which, spores were collected in cryogenic storage vials, and stored in a refrigerator until needed. The remaining fronds were deposited in the herbarium as voucher specimens.

**Starch Gel Electrophoresis**

Starch gel electrophoresis of enzymatic proteins was used to estimate genetic variation in North Carolina populations of *Pellaea wrightiana*. For preliminary data runs, leaf tissue was ground using a chilled mortar and pestle with liquid nitrogen. After grinding, an allozyme extraction buffer from Mitton *et al.* (1979) (Appendix A) was added. Once enzyme systems were preliminarily resolved, leaf tissue was ground as above and the phosphate - PVP grinding buffer of Soltis *et al.* (1983) as modified by Windham (1988) (Appendix B) was used. The phosphate grinding buffer has been documented in other electrophoretic fern studies as obtaining maximum resolution of allozymes. Ground leaf/protein slurry was stored in cryogenic storage vials at -80° C until used in electrophoretic analyses.

Twelve percent Sigma starch gels were prepared approximately 24 hours prior to data runs. Four hundred milliliters of appropriate gel buffer (Soltis *et al.* 1983; Haufler 1985; or Werth 1985) (Appendices C and D, respectively) were combined with 48 g of Sigma starch in a 1,000 ml side-arm flask. The gel mixture was heated over an open Bunsen burner flame and swirled constantly until polymerization began. Polymerization was indicated by the sudden silencing and thickening of the gel suspension. The gel mixture was then heated for an additional three minutes (until boiling), removed from the flame, and immediately degassed using side-arm suction for 50 seconds or until all small air bubbles were removed. The hot gel

suspension was then poured into a 400 ml gel mold where any particulate matter or small air bubbles were removed with a glass Pasteur pipette. The gel was allowed to cool and further polymerize for approximately one hour, after which it was wrapped in clear plastic wrap and left on a counter top over night.

To prevent protein denaturation during electrophoresis, gels were sufficiently cooled in a refrigerator approximately one hour prior to electrophoresis. While gels were cooling, protein slurries from each individual were thawed and absorbed through Mira cloth onto three by nine millimeter wicks made of Whatman 3M chromatography paper. The wicks were inserted into an origin consisting of a slit made five centimeters from the bottom edge of the gel. Once wicks were inserted into the gel origin, electrophoresis was carried out using appropriate electrode buffers and electric currents (Haufler 1985; Werth 1985; M.D. Windham personal communication 1994) (Appendices C and D, respectively). Gels run with a marker dye (0.1% bromphenol blue) were not run for a specific length of time, but only long enough to allow the marker dye to migrate a sufficient anodal distance. This, in turn, ensured sufficient migration of proteins. Gels were kept as close to 4° C as possible by placing an aluminum pan filled with ice on top of a glass plate that was, in turn, placed on top of each electrophoretic apparatus. Ice was drained at 20 minute intervals. Frozen

ice packs were placed under the gel between the electrode buffer trays to keep the underside of the gel cold.

After electrophoresis, gels were sliced horizontally to obtain replicate one millimeter slices for a population so several enzyme systems could be assayed in a single data run. Top slices were discarded as resolution was poorest at this level of the gel. Gels were stained by placing a single gel slice in a 15 cm x 20 cm plastic container with appropriate staining solution as prescribed by Soltis *et al.* (1983) or Werth (1985) (Appendices E and F, respectively). Gels were incubated at 37° C while bands developed. After bands had attained maximum resolution, the gel was rinsed with distilled $H_2O$ and placed in a fixative solution made of five parts methanol, five parts distilled $H_2O$, and one part glacial acetic acid. Gels were photographed using Kodak Ektachrome 400 speed slide film on a fluorescent light box.

**Data Analysis**

Banding patterns were compared to electrophoretic data previously collected for *Pellaea wrightiana* (Windham 1988) to detect possible allele differences. Samples of *Pellaea truncata* were run beside *Pellaea wrightiana* to detect isozyme additivity. Consistently resolvable enzyme systems were glutamate oxaloacetate transaminase (GOT, also known as aspartate aminotransferase, AAT), isocitrate dehydrogenase (IDH), malate dehyrdogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGDH),

phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKDH). Enzyme systems and gel and electrode buffer systems are summarized in Table 1.

**Spore Germination**

Spores were sown on agar plates with sterile cotton swabs. The agar was a sterile medium provided by Carolina Biological Supply Company, Burlington, North Carolina. Plates were put into resealable plastic bags to prevent water loss and placed under ordinary fluorescent lights with constant illumination. Gametophytes were observed at different time intervals for formation of gametangia. This would give some indication as to the sexual nature of *Pellaea wrightiana* in North Carolina.

**Table 1. Summary of enzyme systems, gel and electrode buffer systems used for North Carolina populations of *Pellaea wrightiana*.**

| Enzyme Abbreviation | E.C. Number[1] | Gel and Electrode Buffer Systems | Reference |
|---|---|---|---|
| GOT | 2.6.1.1. | System 6 | Soltis *et al.* 1983 |
| IDH | 1.1.1.42. | Tris/Borate pH 8.0 | Werth 1985 |
| MDH | 1.1.1.37. | Tris/Citrate pH 6.3/6.7 | Werth 1985 |
| 6PGDH | 1.1.1.44. | System 11 | Haufler 1985 |
| PGI | 5.3.1.9. | Tris/Borate pH 8.0 | Werth 1985 |
| | | System 11 | Haufler 1985 |
| PGM | 2.7.5.1. | Tris/Borate pH 8.0 | Werth 1985 |
| | | System 11 | Haufler 1985 |
| SKDH | 1.1.1.25. | Tris/Borate pH 8.0 | Werth 1985 |
| | | System 11 | Haufler 1985 |

**1 = Enzyme Commission Number**

# RESULTS

## Electrophoresis

All seven enzyme systems yielded at least one resolvable locus as summarized in Table 2. No genetic variation was found within or between North Carolina populations of *Pellaea wrightiana*. Identical banding patterns were observed for each specific enzyme in both populations.

For IDH, a dimeric enzyme, both Alexander and Stanly County plants exhibited a single banded electromorph (Figure 7). Thus, all individuals in both populations appear homozygous for this allele. GOT, a dimeric enzyme, yielded a three banded electromorph that was exhibited by all individuals in both populations. This pattern consists of a very intense band in the middle, and fainter bands above and below (Figure 8). SKDH, a monomeric enzyme, and 6PGDH, a dimeric enzyme, both exhibited a two banded electromorph. This is shown in Figures 9 and 10, respectively.

Two loci were observed for the monomeric enzyme PGM. PGM-1, the faster locus, exhibited a balanced, two banded electromorph. PGM-2 exhibited the same pattern (Figure 11). These patterns were exhibited by all individuals in both populations. Two loci were observed for PGI, a dimeric

enzyme. PGI-1, the faster locus, exhibited a two banded electromorph in all individuals from both populations. PGI-2 exhibited a single banded electromorph (Figure 12). *Pellaea truncata* also exhibited two loci with a single, fast band showing for PGI-1 and a single band for PGI-2. For MDH, a dimeric enzyme, three putative loci were observed (Figure 13). Initially, MDH-1 was expressed as a single, fast allele. MDH-2 exhibited a three banded electromorph with an intense heterodimer in the middle and two fainter homodimers above and below. MDH-3 exhibited a single faint allele closer to the origin of the gel. As this enzyme was examined further (i.e. longer run times, different concentrations of substrate, etc.), multiple bands appeared in addition to the three previously mentioned loci. These other bands are believed to be heterodimers formed between the three loci. These putative heterodimers are diagramed in Figure 14. In any case, all individuals in both populations exhibited the same pattern.

**Spore Germination**

One hundred percent spore germination was obtained using spores from the Alexander County population. Rhizoids were visible three days after sowing, and full cordate prothalli were visible in six weeks. Gametangia were observed eight weeks after germination. No sporophytes were observed in these cultures.

**Figures 7-9.** Zymograms for IDH, GOT(AAT), and SKDH, respectively. In Figures 7 and 9, all lanes represent *Pellaea wrightiana* from Stanly County. The same patterns were observed in Alexander County plants. In Figure 8, PETR represents *Pellaea truncata* from Pima County, Arizona. "A" and "S" represent Alexander and Stanly County plants, respectively. STAN represents a standard sample from Stanly County. Locus and allele designations hypothesized by Windham (1988) are given on the side.

Figure 7

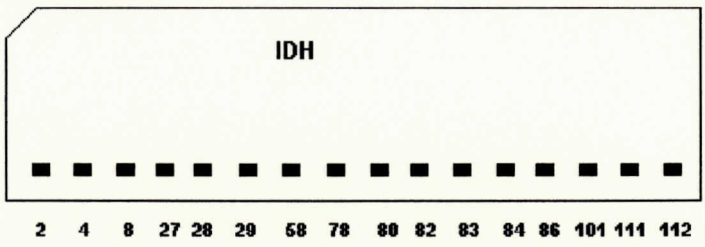



Figure 8

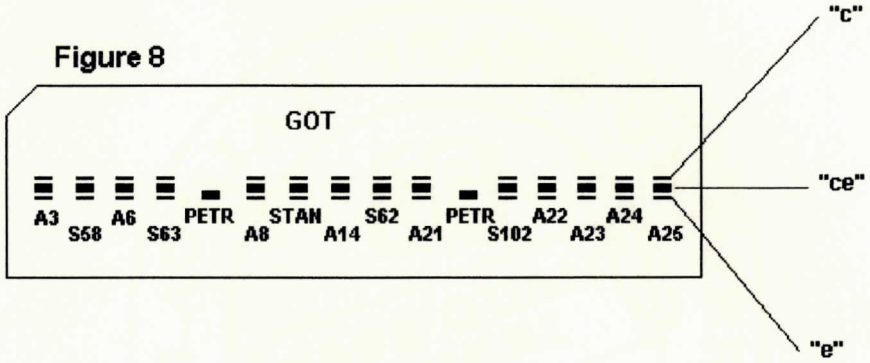
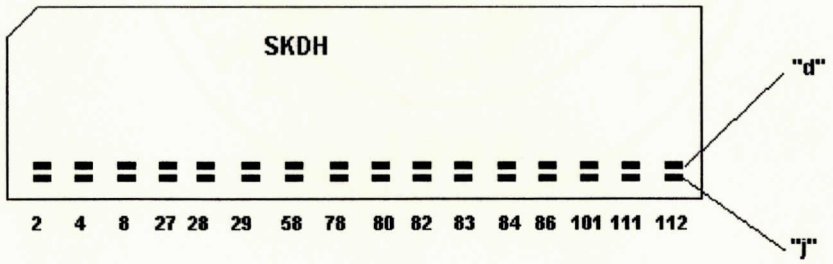



Figure 9



**Figures 10-12.** Zymograms for 6PGDH, PGM, and PGI, respectively. In all lanes, PETR represents *Pellaea truncata* from Pima County, Arizona. STAN represents a standard sample from Stanly County. All lanes in Figures 11 and 12 represent *Pellaea wrightiana* from Alexander County. Stanly County plants exhibited the same patterns. In Figure 10, "A" and "S" represent Alexander and Stanly County plants, respectively. Locus and allele designations hypothesized by Windham (1988) are shown on the side.

Figure 10

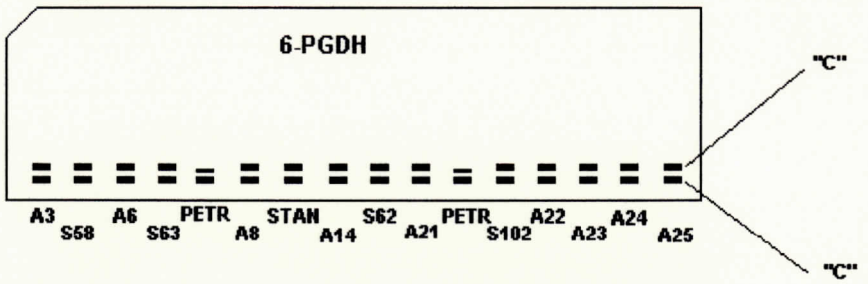



Figure 11

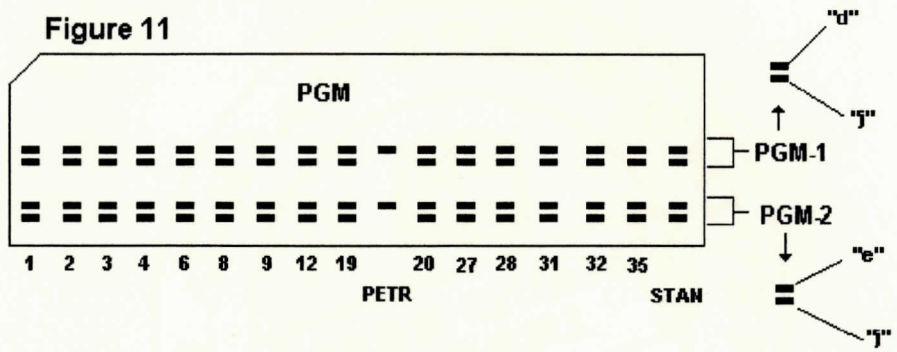
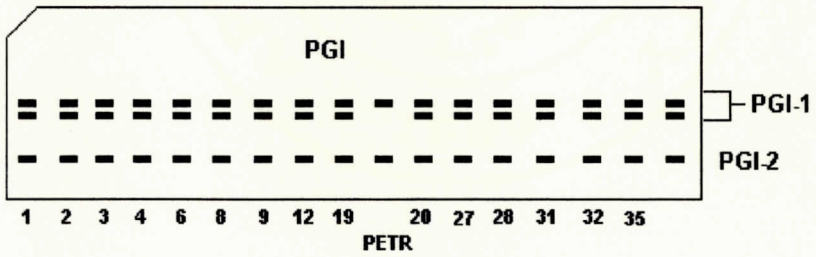



Figure 12



**Figures 13 and 14.** Zymograms for MDH. All lanes in Figure 13 represent *Pellaea wrightiana* from Stanly County. All lanes in Figure 14 represent Alexander County plants. Figure 14 includes a diagram of putative loci observed on some MDH gels. PETR represents alleles possibly donated by *Pellaea truncata*.

Figure 13.

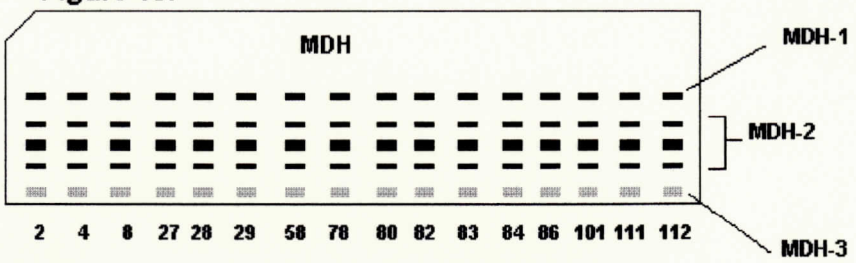
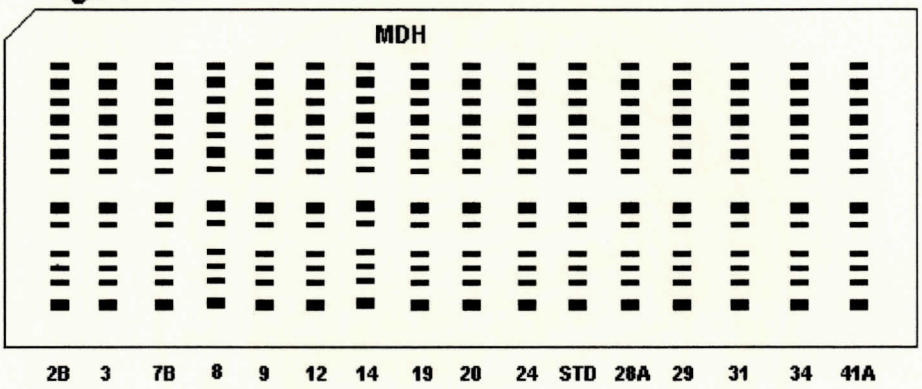



Figure 14.



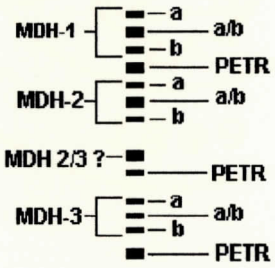



**Table 2. Summary of enzyme systems and number of loci observed in North Carolina populations of Wright's cliff brake.**

| Enzyme | Number of Loci |
|---|---|
| GOT | 1 |
| IDH | 1 |
| MDH | 3 |
| 6PGDH | 1 |
| PGI | 2 |
| PGM | 2 |
| SKDH | 1 |

# DISCUSSION

## ELECTROPHORETIC DATA

Windham (1988) observed 68 different genotypic combinations across 11 loci throughout the southwestern U.S. main range of *Pellaea wrightiana*. In that study, genotype number 67 is based on data collected from only two Alexander County, North Carolina plants. What follows is a discussion of the electrophoretic results of this study in comparison with Windham's observations.

Banding patterns for SKDH, GOT, and 6PGDH were identical to Windham's (1988) observations. He too observed a two banded pattern for SKDH. In the case of allopolyploids, each of the two loci are being donated by different parental species. Windham (1988) found 4 different allele combinations for SKDH in *Pellaea wrightiana*. These were "d*", "bj", "bdjj", and "dj", with the "b" and "d" alleles donated by *Pellaea ternifolia* and the "j" allele donated by *Pellaea truncata*. The asterisk represents a null allele (Windham 1988). Null alleles are genes that no longer code for functional enzymes (Weeden and Wendel 1989). Plants of both North Carolina populations are hypothesized to exhibit the "dj" allele combination.

The three banded pattern observed for GOT also results from additivity of parental alleles. When this occurs, progeny resemble fixed heterozygotes (Werth 1989). Windham (1988) observed three different allele combinations for GOT. These were the "ce", "cc", and "ccce" combinations, where four letters represent segregation in gametophytes. The "c" allele is donated by *Pellaea ternifolia* and the "e" allele is donated by *Pellaea truncata*. Plants of the Alexander County population exhibit the "ce" combination (Windham 1988). Because no variation was observed between populations, Stanly County plants are hypothesized to exhibit the same combination.

Since 6PGDH is a dimeric enzyme, a heterozygous individual would be expected to exhibit a three banded pattern, as in GOT (Kephart 1990). However, the parental plants that formed North Carolina *Pellaea wrightiana* were both evidently homozygous for this locus, each donating a single allele. Windham (1988) observed only two different allele combinations for 6PGDH. These were "cc" and "ac" with the "a" allele being of unknown origin and the "cc" combination occurring most often. Presumably, in this case, both *Pellaea truncata* and *Pellaea ternifolia* donate "c" alleles (Windham 1988). Plants from both North Carolina populations are hypothesized to exhibit the "cc" combination.

Windham (1988) observed a three banded pattern and two very common genotypes for IDH. These were "dh" and "ad", where the "d" allele

is donated by *Pellaea ternifolia* and the "a" and "h" alleles are donated by *Pellaea truncata*. Throughout the entire U.S. range of *Pellaea wrightiana*, nine different allele combinations for IDH were observed (Windham 1988). These were "hi", "ad", "dh", "di", "ai", "d*", and "h*", where the asterisks for "d" and "h" denote null alleles, and "ddhi" and "dhii", which represent segregation in gametophytes (Windham 1988). In addition to the "d" allele, *Pellaea ternifolia* also donates the "i" allele. *Pellaea truncata* also donates an "i" allele. While Windham (1988) reports a three banded pattern for IDH, he shows that some individuals exhibited a single banded electromorph. Only this single banded pattern was observed in plants examined from Alexander and Stanly Counties. Differences in banding patterns of this study compared to Windham's genotype 67 may be attributed to differences in gel and electrode buffer systems used to assay IDH. Windham (1988) observed the "hi" combination in plants from Alexander County. I hypothesize this to be the genotype for Stanly County plants as well.

Banding patterns observed for the monomeric enzyme PGM seem to be consistent with Windham's (1988) observations. He found six different allele combinations for PGM-1. These were "dj", "bj", "gj", "jj", "bgjj", and "dgjj", where the "dj" combination was observed in Alexander County plants. Only two allele combinations were observed for PGM-2. These were "ej" and "e*". According to Windham (1988), Alexander County plants exhibit the "ej" combination. This is hypothesized to be the case in Stanly County

plants as well, since they seem to be genetically identical. *Pellaea truncata* also exhibited two loci for PGM, and at each locus, it appeared to donate the faster allele. This may contradict Windham's generalization of faster alleles being donated by *Pellaea ternifolia* and slower alleles being donated by *Pellaea truncata*.

Windham (1988) scored allelic combinations for only PGI-2 in southwestern U.S. and Alexander County plants. He observed six different allele combinations. These were "ci", "ij", "ii", "I*", "iiil", and "ciii". Alexander County plants exhibited the "ii" combination for PGI-2 (Windham 1988). This is hypothesized to be the case for Stanly County plants as well.

Windham (1988) observed a three banded electromorph and reported allele combinations for MDH-3 only, as this was the more variable MDH locus in southwestern plants. Three different allele combinations were observed. These were "ch", "dh", and "fh", with the "d" allele being of unknown origin (Windham 1988). The "h" allele is donated by *Pellaea ternifolia* and the "c" and "f" alleles are donated by *Pellaea truncata*. Plants from Alexander County exhibited the "ch" combination for MDH-3 (Windham 1988), presumably, so do the Stanly County plants but further study of this enzyme system may be necessary to obtain clearer, more consistent resolution for North Carolina plants.

## GENETIC VARIATION IN FERN POPULATIONS

One aspect of this study was to attempt to determine if there was one introduction of *Pellaea wrightiana* into North Carolina, where one population gave rise to the other, or, if there were two independent introductions into the state. This question was addressed by comparing the genetic variability of the Alexander and Stanly County populations via allozyme analyses. If the two populations are genetically similar, or even identical, evidence may support the hypothesis of a single introduction. If the two populations are genetically dissimilar, evidence may support the hypothesis of two separate introductions. Data of this study provides evidence that supports a single introduction.

Allozyme analyses may also elucidate the mating strategies being employed by these two populations. Mating strategies may explain the genetic composition of fern populations (Klekowski 1972; Soltis and Soltis 1989). Information regarding mating strategies is also of interest since there has been some question as to whether North Carolina populations of *Pellaea wrightiana* are sexually reproducing (as implied in Weakley *et al.* in prep.).

No polymorphisms were detected at any loci for any enzyme system assayed, statistical analyses, such as Wright's F-statistic (Wright 1965), are not possible. What follows is a discussion of factors that may account for the lack of genetic variability in homosporous fern populations. These examples will be used to make inferences about *Pellaea wrightiana* in North Carolina.

The apparent lack of genetic variation within and between North Carolina populations of *Pellaea wrightiana* raises the question: "What accounts for this lack of variation?" Several factors discussed herein may provide explanations.

## MATING STRATEGIES

### SEXUAL REPRODUCTION

Plant population ecologists agree that mating strategies significantly affect the genetic composition of populations (Loveless and Hamrick 1984 and citations within). However, Klekowski (1972) points out some basic differences between homosporous plants and seed plants that affect genetic variation in populations. He notes that seeds are produced and dispersed after fertilization has taken place. When the seed arrives in suitable habitat, by whatever mechanism of dispersal, it will germinate and instantly give rise to a plant (population) that is a genetic sample of the parental sporophyte population. This new plant may contain variation as heterozygous loci. On the other hand, spores are formed and dispersed before gametogenesis and fertilization. When a fern spore lands in suitable habitat, it germinates and gives rise to a free-living, haploid gametophyte. Sperm and egg cells arise mitotically and are therefore genetically identical to the gametophyte (Klekowski 1979). Fertilization takes place away from the parental

sporophyte population, potentially giving rise to a genetically distinct, new population.

Because of the potentially bisexual nature of a single fern gametophyte, one of three scenarios described by Klekowski (1979) regarding sexual reproduction can take place. One possibility is intragametophytic selfing, which occurs when a sperm and egg cell on the same gametophyte unite resulting in a sporophyte that is homozygous at all loci (Klekowski 1972). The new sporophyte could then potentially give rise to a population consisting of genetically identical individuals.

Several studies have been conducted to assess the rates of intragametophytic selfing in populations of homosporous ferns. McCauley *et al.* (1985) showed high rates of intragametophytic selfing in *Botrychium dissectum* forma *obliquum* (Muhlenberg) Fernald, while Soltis and Soltis (1986) obtained similar results for *B. virginianum* L. Their hypotheses were based on the notion that intergametophytic crossing would be extremely difficult in species that have subterranean gametophytes (St. John 1949 cited in McCauley *et al.* 1985). These plants would therefore rely heavily on intragametophytic selfing as a mating strategy. In a study of disjunct populations of ebony spleenwort, *Asplenium platyneuron* (L.) Oakes, Crist and Farrar (1983) provide evidence that intragametophytic selfing is the primary mode of establishment of these plants on abandoned coal spoils in southcentral Iowa. Based on their data gathered by culturing paired and

single gametophytes, *A. platyneuron* appears to be a species that is relatively free of genetic load, the number of lethal recessive alleles that are usually stored in the heterozygous condition and expressed upon intragametophytic selfing. Because of this, ebony spleenwort is capable of long distance dispersal and establishing colonies consisting of only a single plant (Wagner 1972; Crist and Farrar 1983). *Pellaea wrightiana* may also be a species with low genetic load. This could be inferred upon speculation that the North Carolina populations are the result of founder effect, where a new population arises from a single or small number of individuals.

Evidence contradicting these studies has been presented by Soltis and Soltis (1988) using three species of *Lycopodium*, and by Soltis *et al.* (1988) using statistical analyses by Hedrick (1987) to examine populations of *Equisetum arvense* L. Korpelainen and Kolkkala (1996) obtained similar results using fixation indices for populations of *E. arvense* and *E. hymale* L. in Finland.

A second possibility regarding sexual reproduction in ferns is termed intergametophytic selfing, when gametes from two gametophytes formed from the same parental sporophyte unite (Klekowski 1979). This is functionally similar to selfing in seed plants and produces effects similar to intragametophytic selfing, provided there is no mutation and the parental sporophyte has no heterozygous loci (Klekowski 1979). Pteridophyte populations that have been established by plants that go through

intragametophytic or intergametophytic selfing are expected to be genetically lacking (Soltis and Soltis 1990).

The mating strategy that would best promote genetic variation in fern populations is termed intergametophytic crossing and occurs when gametes from two gametophytes of different parental sporophytes unite (Klekowski 1979). Studies have indicated this mating strategy is most commonly exploited by homosporous fern populations. One example, previously mentioned, is that of *Equisetum*. In a study of *E. arvense* intragametophytic selfing was absent (Soltis *et al.* 1988). Estimates of intragametophytic selfing rates based on statistical analyses of Hedrick (1987) were 0.0 for 12 out of 17 populations while the mean number of polymorphic loci for their sample populations was 0.191. Korpelainen and Kolkkala (1996) observed average heterozygosities of *E. arvense* and *E. hymale* to be 0.092 and 0.134, respectively. The often dioecious nature of *Equisetum* gametophyte populations (citations in Soltis *et al.* 1988) very likely facilitates an outcrossing mating strategy.

In an electrophoretic study of *Gymnocarpium dryopteris* (L.) Newman ssp. *disjunctum* (Rupr.) Sarvela, Kirkpatrick *et al.* (1990) found that populations utilized an outcrossing mating strategy. Their mean values for number of polymorphic loci across 15 populations was 0.586. Their mean number of alleles per locus was 1.79, and observed heterozygosity across 15 populations was 0.194. Kirkpatrick *et al.* (1990) also estimated the

intragametophytic selfing rate using statistical analyses of Holsinger (1987) to be 0.0, suggesting complete outcrossing. This highly outcrossing mating strategy has been attributed to several factors including response to antheridiogen, unisexual gametophytes, and inbreeding depression (Kirkpatrick *et al.* 1990).

In a broader review of pteridophyte population genetics, Soltis and Soltis (1989 and citations within) present data from a broad spectrum of continental U.S. species that indicate most homosporous pteridophyte populations exhibit a predominantly outcrossing mating strategy. This has been reported even for species that are highly geographically restricted. In a study of *Adenophorus periens* Bishop, a rare, epiphytic fern endemic to the Hawaiian Islands, Ranker (1994) observed higher levels of genetic variability than levels previously observed in other geographically restricted species. Of 15 loci examined, 12 were polymorphic and observed heterozygosities ranged from 0.80 to 0.464. Based on these data, Ranker (1994) concluded that this single large population is highly outcrossing and was probably established by more than one individual which increases the chance of genetic variability. High levels of genetic variability in this single population may also be attributed to individuals experiencing somatic mutations in the apical meristem cells, perennial life cycles delaying loss of genetic variation, and smaller, outlying populations of *A. periens* not contributing to the genetic variability of this larger population (Ranker 1994). *Adenophorus periens* may

be an example of a species that experiences repeated founder effects and has evolved an outcrossing mating strategy which prevents loss of genetic variability (Carson 1989 cited in Ranker 1994).

If one considers the sexual mating strategies of homosporous ferns, there are several possible scenarios which explain the lack of genetic variation in North Carolina populations of *Pellaea wrightiana*. First, *Pellaea wrightiana* could have arisen in North Carolina via a single spore landing in either the Alexander or Stanly County site. This spore may have arrived from the southwestern United States by prevailing westerly winds. Landing in suitable habitat, this spore germinated when conditions were optimal, and produced a bisexual gametophyte. If intragametophytic selfing occurred, the resulting sporophyte could have produced genetically identical offspring. Spores from this first population may have been subsequently dispersed by wind to the second site, thus establishing the second North Carolina population. Electrophoretic data of the present study showed only one allele combination per locus, which, according to Klekowski (1972), provides compelling evidence for establishment by a single propagule.

A second possibility is that more than one spore from a single sporophyte somewhere in the southwestern United States landed in either Alexander or Stanly County, germinated, and gave rise to a population of either bisexual or unisexual gametophytes. If the gametophytes were bisexual, either intragametophytic or intergametophytic (or both) selfing could

have occurred, thus giving rise to genetically identical sporophytes. If the gametophytes were largely unisexual and intergametophytic selfing occurred, genetically identical sporophytes could have been produced. If spores were then dispersed from this population to the second location, populations of genetically identical plants could be established. This second possibility would be plausible provided there was no variation in this single, parental sporophyte.

Alternative possibilities regarding the alternation of sporophytic and gametophytic generations will be addressed under the heading of ameiotic alternation of generations.

**ASEXUAL REPRODUCTION**

Pteridophytes can reproduce asexually via a variety of mechanisms. These asexual reproductive strategies result in genetically identical plants which could, in turn, give rise to genetically identical populations. One mechanism is by vegetative reproduction from rhizomes. Nearly all pteridophytes are capable of forming colonies through the spreading of underground rhizomes (Raven *et al.* 1992). The rhizomes of *Pellaea* are classified as short and sometimes erect (Radford *et al.* 1968). Due to the rocky substrate that these two populations occupy, vegetative spreading of rhizomes is likely greatly restricted. This provides some evidence that *Pellaea wrightiana* in North Carolina is sexually reproducing, as extensive

colony formation via rhizomatous growth is not possible, even in the shallow soil pockets. Some individuals have been observed to form clumps of two to four plants (Heafner unpublished data). These were assumed to be ramets, or vegetative clones, because of the compact nature of the clumps.

A number of genetic studies on clonal plant species have been conducted and some are reviewed by Ellstrand and Roose (1987). In a study of two temperate, woodland fern species, Hamilton (1992) provides electrophoretic evidence that coincides with the assumption of Hamrick and Godt (1990 cited in Hamilton 1992), that successional status, among other factors, has an effect on genetic diversity in plant populations. Hamilton (1992) found that genetic diversity was higher (16 loci; 7 polymorphic) among ramets of *Deparia acrostichoides* (Swartz) Kato (formerly *Athyrium thelypterioides* (Michaux) Desvaux), a species with a frond emergence that occurs in an early spring flush (Hamilton 1990 cited in Hamilton 1992), than that of ramets of *Diplazium pycnocarpon* (Sprengel) Broun (formerly *Athyrium pycnocarpon* (Sprengel) Tidestrom) (13 loci; 2 polymorphic), which is a species with successive frond emergence throughout the growing season. Hamilton (1992) concluded that species with a single, early spring leaf emergence are late successional species, and will have higher levels of genetic variation than will species that have a continuous emergence pattern, as early successional species. *Pellaea wrightiana* appears to have a successional frond emergence pattern throughout the growing season

(Heafner unpublished data) and would therefore be expected to have low levels of genetic variation.

Vegetative reproduction in ferns is also possible by production of sporophytic gemmae. This has been documented in species of *Botrychium* (Farrar and Johnson-Groh 1990). Gemmae are vegetative propagules produced mitotically off a parent plant. This is well documented in non-vascular plants (Raven *et al.* 1992). Gemmae production has also been well documented in gametophytes of fern species that occur in the southern Appalachians some distance away from their tropical and sub-tropical main ranges (Farrar 1967). This complex, known collectively as "Appalachian gametophyte", is comprised of species of *Vittaria*, *Grammitis*, and *Hymenophyllum*. These tropical ferns have never been observed to form sporophytes in the Appalachians, but reproduce via vegetative gemmae. Farrar (1990) found gametophyte populations of *Vittaria* to be genetically distinct from the nearest sporophyte sources and speculated that evolution may be occurring in these asexual plants. Nevertheless, while extensive morphological analyses of *Pellaea sp.* gametophytes have been done (Pray 1968; Whittier 1968), gemmae production has never been observed for any member of the genus.

## AMEIOTIC ALTERNATION OF GENERATIONS

Pteridophytes are peculiar in that they can exhibit characteristics that depart from the normal alternation of generations life cycles found in land plants (Kingdom Plantae). These processes, termed apospory, apogamy, and agamospory, are discussed below. An explanation of how these processes may or may not relate to *Pellaea wrightiana* in North Carolina is included.

Apospory is defined by Walker (1979) as the production of a gametophyte directly from sporophyte tissue without the production of spores. Walker (1979) further explains that these gametophytes may produce gametangia and fertilization may subsequently occur. However, the chromosome number of this gametophyte is equal to that of the sporophyte. If fertilization does occur, an autopolyploid results. *Pellaea wrightiana* has been shown to have N=58 pairs at meiosis (Wagner 1965; Windham 1988). This has been documented for Alexander County plants (Wagner 1965) as well as for Stanly County plants (J.F. Matthews personal communication 1993). The somatic chromosome number of *Pellaea wrightiana* is 2N=116. If this process were operating in either of the North Carolina populations, some of the plants would be expected to exhibit somatic chromosome numbers of 2N=232, while at meiosis, N would be 116. To date, the phenomenon of autoploidy has never been documented in either North Carolina or southwestern populations of *Pellaea wrightiana*. Furthermore,

there is no evidence to date that shows *Pellaea wrightiana* sporophytic tissue may spontaneously form a prothallus. Walker (1979) points out that generally, apospory is fairly uncommon in nature. This provides some evidence that North Carolina populations of *Pellaea wrightiana* are likely sexually reproducing.

Apogamy is another process by which ferns can produce genetically identical offspring. Apogamy occurs when a sporophyte develops vegetatively from a gametophyte (Walker 1979). The formation of a restitution nucleus prevents haploid spores from being formed (Gastony and Windham 1989). Because of this, the chromosome number of the gametophyte is equal to the sporophyte. The production of unreduced spores, accompanied by a vegetatively-formed sporophyte from an unreduced gametophyte, is termed agamospory (Walker 1979; Gastony and Windham 1989). Several authors have proposed various mechanisms for apogamy/agamospory in different fern species. A summary of only the most common mechanisms is presented here.

In studying the apogamous life cycle of *Polypodium dispersum* Evans, Evans (1964) observed that apogamy can be either meiotic or ameiotic. In ameiotic apogamy, a diploid precursor cell undergoes a series of four mitotic divisions resulting in the formation of 16 diploid spore mother cells. Each of these spore mother cells then undergoes mitosis rather than meiosis. This results in 32 diploid spores that will give rise to genetically identical diploid

gametophytes (Evans 1964). The sporophytes form vegetatively from the gametophyte, thus generating genetically identical progeny. In meiotic apogamy, a process called syndiploidy occurs in which eight temporarily tetraploid (4N) spore mother cells are formed. Meiosis then occurs, resulting in 32 diploid spores. These spores germinate and give rise to diploid gametophytes. Just as in ameiotic apogamy, a new sporophyte will grow vegetatively from the gametophyte (Evans 1964). The new sporophyte will be genetically identical to the parental sporophyte, barring mutation.

Walker (1979) reviews other mechanisms such as the Döpp-Manton scheme, first worked out in *Dryopteris remota* (A. Braun) Druce (Walker 1979). In this sporogenic mechanism, one of two scenarios is possible. Sixty-four haploid, abortive spores can arise in a sporangium or 32 viable spores can arise in a sporangium, each with the same chromosome number as the parent sporophyte (Gastony and Windham 1989). An electrophoretic survey of *D. remota* by Schneller and Holderegger (1994) provides compelling evidence that apogamously reproducing species are essentially clones. They found absolutely no genetic variation in European populations of this species. Genetic variation has been documented in some agamosporous taxa, however. Suzuki and Iwatsuki (1990) were able to distinguish clones of *Pteris cretica* L. in Japan based on two alleles they designated as $Hk^d$ and $PGI\text{-}2^a$. They attributed this variation to recurrent

hybridization between diploid *P. cretica* clones and another sexual taxon, *P. kidoi* Kurata (Suzuki and Iwatsuki 1990).

Although apogamy has been described in other cheilanthoid ferns like *Cheilanthes* (Whittier 1968) and other species of *Pellaea* (Gastony and Gottleib 1985; Gastony 1988), this process has never been observed in *Pellaea wrightiana*. Apogamous sporophytes were not observed in gametophyte cultures of the present study, either. Windham (1993) notes, however, that *Pellaea wrightiana* can hybridize with *Pellaea atropurpurea*, an apogamous triploid (Lellinger 1985), and form an apogamous pentaploid that occurs only in Oklahoma.

**EVIDENCE OF SEXUAL REPRODUCTION**

Despite the fact that no genetic variation was detected either within or between North Carolina populations of *Pellaea wrightiana*, sexual reproduction is apparently occurring. In observing Alexander County plants, Wagner (1965) found meiotic chromosome counts to be N=58 pairs, while mitotic chromosome counts were 2N=116. Windham (1988) observed these same numbers for southwestern plants. Wagner (1965) also observed 64 uniform-sized spores per sporangium, which is indicative of a sexual taxon. These same characteristics were observed in Stanly County plants (J.F. Matthews personal communication 1993).

One hundred percent germination was observed when spores from Alexander County plants were sown. While gametangia did develop, no sporophytes were observed in these cultures. Apparently, sporophyte formation in vitro is relatively unsuccessful. Sporelings have been observed in the Stanly County population. These did not appear to be apogamously-formed sporophytes based on observations made with a 10X hand lens. While apogamous growth does occur in some species of *Pellaea*, it has never been observed in *wrightiana*. Apospory is an uncommon phenomenon, even in nature, and has not been observed in any species of *Pellaea*. In addition, vegetative growth does not appear to significantly contribute to the number of individuals in either population. It is highly unlikely that rhizomatous growth gives rise to large number of individuals due to the rocky substrates and the diminuative, often erect nature of the rhizomes.

## ECOLOGICAL FACTORS

### GENE FLOW

Slatkin (1985) defines gene flow as "mechanisms resulting in the movement of genes from one population to another." For ferns, this is best accomplished by spore dispersal between populations. Soltis and Soltis (1990) discuss the effects of interpopulational and intrapopulational gene flow in fern populations, pointing out that intrapopulational gene flow affects

genetic variation within populations, while interpopulational gene flow affects variation between populations. If gene flow were a significant factor in North Carolina populations of *Pellaea wrightiana*, alleles other than those detected would be expected to be present and detected by electrophoresis despite the fact these eastern populations share common alleles with some western populations. Since both North Carolina populations consist of apparently genetically identical individuals, intrapopulational gene flow would not result in genetic variation within either population. Interpopulational gene flow would be the more efficient mechanism in establishing genetic variation in North Carolina populations. Spores from the southwestern U.S. main range would move alleles from those populations to the North Carolina populations, or, alleles could be exchanged between the Alexander and Stanly County populations. To provide further evidence that gene flow might be occurring in these eastern populations, more than two populations should be analyzed electrophoretically. Unfortunately, this is not possible as no other populations of *Pellaea wrightiana* east of Oklahoma are presently known. Electrophoretic data of this study cannot provide evidence for gene flow between the Alexander and Stanly County populations because it is undetectable as both populations are monogenic. It is, however, more likely that spores could be exchanged over this short distance rather than spores continually being blown from the southwest.

**GAMETOPHYTE SAFE SITES**

Silvertown (1987) explains that seed germination is highly dependent on very specific, soil-level, environmental characteristics. When conditions are conducive to seed germination, these are termed "safe sites". The concept of a safe site can also be applied to ferns. Although many temperate fern species are capable of heavy spore yields each growing season, the species or population will not be perpetuated unless a certain percentage of these spores land in suitable habitat, germinate, and give rise to viable gametophytes. The success of the gametophyte in its habitat is critical to the success of the resulting sporophyte. The physical characteristics of the site a fern population occupies may not only determine the success of spores and gametophytes, but may also determine the genetic structure of the population (Soltis and Soltis 1990). Cousens *et al.* (1988) observed that decaying stumps and logs provided safe sites for the germination of spores and establishment of gametophytes of netted chain fern (*Lorinseria aereolata* (L.) Presl = *Woodwardia aereolata* (L.) Moore), a typically coastal species, while terrestrial sites such as hummocks provided safe sites for sporophyte colonization.

In a study focusing more specifically with xeric-adapted fern species, Pickett (1931) points out that the gametophyte may be more sensitive to the

physical environment of a habitat than the spore. After exposing gametophytes of *Pellaea glabella* and *Pellaea atropurpurea* to five years of desiccation, Pickett (1931) found that up to five percent of both species survived after rewatering. Similar results were also obtained for gametophytes of *Cheilanthes gracillima* Eaton (Pickett 1931).

The availability of safe sites at either Little Joe Mountain or Morgan's Bluff may be a significant factor in the genetic structure of *Pellaea wrightiana* in North Carolina. Safe sites for gametophytes and sporophytes of *Pellaea wrightiana* in North Carolina appear to be soil pockets in partial or full sun, shaded rock crevices, or areas that are semi-shaded by pine and juniper branches. Gametophytes with young sporophytes have been observed at the Stanly County site in small, shaded rock crevices (Heafner unpublished data). These small crevices appear to be safe sites for the gametophytes which, in turn, will produce a new sporophyte. These crevices are, however, scattered throughout each site, and all may not contain gametophytes. This would greatly limit the spread of gametophytes, hence sporophytes, within each population, between the two populations, and limit spore immigration from southwestern populations.

The randomness of these safe sites across each population may also facilitate selection for the best fit genotypes in each population. Some individuals have been observed growing on exposed rock where there is no organic matter. While the crevices in this exposed rock may have been safe

sites for spores and gametophytes, they were not for sporophytes, as they rarely survived the growing season due to the intense heat. Therefore, if there has been any genetic variation within either population, it may have been in individuals that have perished.

## SUMMARY AND SUGGESTIONS FOR FURTHER STUDY

Electrophoretic data indicate no genetic variation either within or between North Carolina populations of *Pellaea wrightiana*. This suggests that there has been only one introduction into North Carolina. The lack of genetic variation in these eastern populations may be attributed to establishment by either a single or small number of individuals with subsequent inbreeding. If a single spore established these populations, then intragametophytic selfing gave rise to a completely homozygous sporophyte, which gave rise to genetically identical offspring. If a small number of spores established these populations, then intergametophytic mating occurred between genetically identical gametophytes; these would give rise to genetically identical sporophytes. As there is evidence, albeit indirect, that North Carolina *Pellaea wrightiana* is sexually reproducing, asexual mechanisms can not likely be factors in the genetic homogeneity of these populations. North Carolina populations of *Pellaea wrightiana* do represent a genetic sample of the species from throughout the southwestern main range. Windham (1988) found 68 different allele combinations across 11 loci for the

species. Genotype 67 represents Alexander County plants. I hypothesize this genotype to also represent Stanly County plants.

The occurrence of *Pellaea wrightiana* in North Carolina provides excellent opportunities for future studies. The entire Carolina Slate Belt should be explored for other *Pellaea wrightiana* populations. Allozyme analyses of additional eastern populations would be of interest in determining the natural history of eastern populations. The extent and mechanisms of spore dispersal in this species would also be of interest. Since *Pellaea wrightiana* belongs to a family characterized by a false indusium (Adiantaceae), the mechanism of spore dispersal is different than most other leptosporangiate ferns. Examining the spore dispersal of this species would provide better insight into how spores might be transported to distant habitats and aid in the understanding of the ecological requirements of the spore during dispersal, or after landing in a new habitat. Gametophyte experiments similar to those of Crist and Farrar (1983) would be useful on evaluating genetic load in *Pellaea wrightiana*, as well as determining whether antheridiogen plays a role in the sexual nature of these populations.

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## APPENDIX A

ALLOZYME EXTRACTION BUFFER

FROM MITTON *ET AL.* 1979.

## APPENDIX A

## ALLOZYME EXTRACTION BUFFER

## FROM MITTON *ET AL.* 1979.

0.093 g germanium dioxide in 65 ml distilled $H_2O$, boil until dissolved.

Cool to room temperature, then add:

0.3 g diethyldithiocarbamic acid, Na salt

4.4 g PVP, avg. molecular weight, 40,000

4.4 g sodium ascorbate

0.33 g sodium metabisulfite

1.21 g sodium borate

8.8 ml 0.16 M phosphate buffer (potassium phosphate monobase), pH 7.0.

Just prior to use, add:

8.8 ml dimethyl sulfoxide (DMSO)

0.8 ml 2-phenoxyethanol

0.18 ml 2-mercaptoethanol

Bring volume to 100 ml with distilled $H_2O$ and place in ice bath.

## APPENDIX B

FERN ALLOZYME EXTRACTION BUFFER

FROM SOLTIS *ET AL.* 1983 AS MODIFIED BY

WINDHAM 1988.

# APPENDIX B

## FERN ALLOZYME EXTRACTION BUFFER

## FROM SOLTIS *ET AL.* 1983

## AS MODIFIED BY WINDHAM 1988.

Phosphate Grinding Buffer - PVP Solution

To prepare 100 ml of phosphate buffer:

Dissolve 1.36 g $KH_2PO_4$ in distilled $H_2O$.

Add 9.0 ml of 1 M NaOH.

Bring volume to 100 ml with distilled $H_2O$, pH 7.5.

To prepare 25 ml of extraction buffer:

0.28 g sodium tetraborate

0.08 g sodium metabisulfite

1.00 g L-ascorbic acid, Na salt

0.07 g diethyldithiocarbamic acid, Na salt

1.00 g PVP, avg. molecular weight, 40,000.

Dissolve gram amounts is 25 ml of phosphate buffer, pH 7.5.

Then add 0.25 ml 2-mercaptoethanol and DMSO to a final concentration of 10% (v/v).

## APPENDIX C

GEL AND ELECTRODE BUFFERS

FROM SOLTIS *ET AL.* 1983 AND HAUFLER 1985

# APPENDIX C

## GEL AND ELECTRODE BUFFERS

## FROM AND SOLTIS *ET AL.* 1983 AND HAUFLER 1985.

### SYSTEM 6

Electrode: 4.0 g NaOH

18.55 g boric acid

Dissolve in 1000 ml distilled $H_2O$ and adjust pH to 8.6.

Gel: 0.92 g Tris

0.4202 g citric acid monohydrate

Dissolve in 500 ml distilled $H_2O$ and adjust pH to 7.8.

Gel ran with marker dye at 250 volts.

### SYSTEM 8

Electrode: 1.64 g LiOH monohydrate

16.23 g boric acid

Dissolve in 500 ml distilled $H_2O$ and adjust pH to 8.0.

Gel: 1.98 g Tris

0.53 g citric acid, monohydrate

0.16 g LiOH monohydrate

0.936 g boric acid

Dissolve in 500 ml distilled $H_2O$ and adjust pH to 7.6.

Gel ran with marker dye at 35 milliamps.

**SYSTEM 11**

Electrode: 117.6 g citric acid, $Na_3$ salt

Dissolve in 1000 ml distilled $H_2O$ and adjust pH to 7.0.

Gel: 0.945 g L-histidine-HCl

Dissolve in 1000 ml distilled $H_2O$ and adjust pH to 7.0.

Gel ran with marker dye at 40 milliamps.

## APPENDIX D

GEL AND ELECTRODE BUFFERS

FROM WERTH 1985.

# APPENDIX D

## GEL AND ELECTRODE BUFFERS

## FROM WERTH 1985.

### Tris-borate, pH 8.0

Electrode: 60.6 g Tris
40.0 g boric acid
6.0 g EDTA disodium salt

Dissolve in 1000 ml distilled $H_20$, final pH 8.0.

Gel: 40 ml electrode buffer in 360 ml distilled $H_20$.

Gel ran at 50 milliamps for 6 hours.

### Tris-citrate, pH 6.3/6.7

Electrode: 27.0 g Tris
18.07 g citric acid monohydrate

Dissolve in 1000 ml distilled $H_20$, final pH 6.3.

Gel: 0.97 g Tris
0.63 g citric acid monohydrate

Dissolve in 1000 ml distilled $H_20$, final pH 6.7.

Gel ran at 100 milliamps for 6 hours or 75 milliamps for 7 hours.

## APPENDIX E

GEL STAINING PROTOCOLS

FROM SOLTIS *ET AL.* 1983.

# APPENDIX E

## GEL STAINING PROTOCOLS

## FROM SOLTIS *ET AL.* 1983.

**GOT (glutamate oxaloacetate transaminase)**
**AAT (amino aspartate transaminase)**

10 ml 1 M Tris-HCl, pH 8.0

90 ml distilled $H_2O$

0.1 g L-aspartic acid

0.1 g $\alpha$-ketoglutaric acid

Adjust pH to 8.0 with 1 M NaOH, then add:

5.0 mg pyridoxal-5'-phosphate

0.1 g fast blue BB salt

**IDH (isocitrate dehydrogenase)**

10 ml 1 M Tris-HCl, pH 8.0

90 ml distilled $H_2O$

0.1 g istocitric acid, $Na_3$ salt

5.0 ml 1 M $MgCl_2$

0.1 g NADP
0.015 g MTT
0.002 g PMS

**MDH (malate dehydrogenase)**

10 ml 1 M Tris-HCl, pH 8.0

10 ml 2 M DL-malic acid, pH 8.0

80 ml distilled $H_2O$

0.01 g NAD
0.01 g MTT
0.002 g PMS

**PGI (phosphoglucose isomerase)**

10 ml 1 M Tris-HCl, pH 8.0

90 ml distilled $H_2O$

1.0 ml 1 M $MgCl_2$

0.03 g fructose-6-phosphate, $Na_2$ salt

40 units glucose-6-phosphate dehyrdrogenase

0.01 g NADP
0.02 g MTT
0.002 g PMS

**PGM (phosphoglucomutase)**

10 ml 1 M Tris-HCl, pH 8.0

90 ml distilled $H_2O$

2.0 ml 1 M $MgCl_2$

0.05 g glucose-1-phosphate, $Na_2$ salt

40 units glucose-6-phosphate dehyrdrogenase

0.01 g NAD
0.01 g MTT
0.002 g PMS

**6PGDH (6-phosphogluconate dehydrogenase)**

10 ml 1 M Tris-HCl, pH 8.0

90 ml distilled $H_2O$

0.04 g 6-phophogluconate, Ba salt

2.0 ml 1 M $MgCl_2$

0.01 g NADP
0.01 g MTT
0.002 g PMS

**SKDH (shikimate dehydrogenase)**

10 ml 1 M Tris-HCl, pH 8.5

90 ml distilled $H_2O$

0.1 g shikimic acid

0.01 g NADP
0.02 g MTT
0.002 g PMS

**APPENDIX F**

GEL STAINING PROTOCOLS FROM

WERTH 1985.

# APPENDIX F

## GEL STAINING PROTOCOLS

## FROM WERTH 1985.

**GOT (glutamate oxaloacetate transaminase)**
**AAT (amino aspartate transaminase)**

25 ml 0.2 M Tris-HCl, pH 7.0

2.5 ml GOT substrate (4% L-aspartate/2% α-ketoglutarate)

1.0 mg pyridoxal-5'-phosphate

75.0 mg fast blue BB salt

**IDH (isocitrate dehydrogenase)**

25 ml 0.2 M Tris-HCl, pH 8.0

2.0 ml 3% isocitrate

1.0 ml 1 M $MgCl_2$

1.0 ml 1% NADP
0.5 ml 1% MTT
0.2 ml 1% PMS

**MDH (malate dehydrogenase)**

25 ml 0.2 M Tris-HCl, pH 8.0

5 ml 2 M DL-malate, pH 7.0

1.0 ml 1% NAD
0.5 ml 1% MTT
0.2 ml 1% PMS

**PGI (phosphoglucose isomerase)**

25 ml 0.2 M Tris-HCl, pH 8.0

1.0 ml 1M $MgCl_2$

1.0 ml 2.5% fructose-6-phosphate

1.0 ml $G_6PDH$ (10 units/ml)
1.0 ml 1% NAD
0.5 ml 1% MTT
0.2 ml 1% PMS

**PGM (phosphoglucomutase)**

25 ml 0.2 M Tris-HCl, pH 8.0

1.0 ml 5% $\alpha$-D glucose-1-phosphate (Sigma G1259)

0.5 ml 1 M $MgCl_2$

1.0 ml $G_6PDH$ (10 units/ml)
1.0 ml 1% NAD
0.5 ml 1% MTT
0.2 ml 1% PMS

**6PGDH (6-phosphogluconate dehydrogenase)**

25 ml 0.2 M Tris-HCl, pH 8.0

1.0 ml 1 M $MgCl_2$

20 mg 6-phosphogluconate, Ba salt

0.1 ml 1% NADP
0.5 ml 1% MTT
0.1 ml 1% PMS

**SKDH (shikimate dehyrdrogenase)**

25 ml 0.2 M Tris-HCl, pH 8.0

10 mg shikimic acid

0.5 ml 1% NADP
0.5 ml 1% MTT
0.2 ml 1% PMS

## VITA

Kerry Donald Heafner was born in Albemarle, North Carolina on 9 March 1970. He is the youngest son of Paul Donald and Gaylene Clark Heafner. He attended primary and junior high school in the Albemarle City School System and graduated from Albemarle Senior High School in June, 1988. The following fall, he entered Mars Hill College and graduated with a B.S. degree in botany in May, 1992. In August, 1992, he entered Appalachian State University to begin work toward the M.S. degree in biology. That degree was awarded in August, 1996. While at Appalachian, Kerry was awarded teaching assistantships and grant money totaling $630.00 for his research. In August, 1996, Kerry will enter Old Dominion University to begin work toward the Doctor of Philosophy degree in ecological sciences under the direction of Dr. Lytton J. Musselman.

During Summer of 1992, Kerry worked as botanical intern for the Nature Conservancy of Georgia at the Altamaha River Bioreserve in Darien. During the 1993 field season, he worked for Dr. Gary Walker assessing gypsy moth habitat through floristic surveys along the Blue Ridge Parkway. Most recently, he has worked for Blue Ridge Electric Membership Corporation and Catawba Valley Community College in Hickory, North

Carolina. Kerry's research interests include taxonomy, ecology, and distribution of vascular plants, particularly pteridophytes. He is a member of the Association of Southeastern Biologists, The Southern Appalachian Botanical Society, The American Fern Society, and The Botanical Society of America. Kerry's home address is 347 Park Road, Albemarle, North Carolina, 28001.