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Allozyrne Variation Within and Between Populations of Ruth's Golden Aster, *Pityopsis ruthii* (Small) Small

Steven A. Sloan
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Steven A. Sloan entitled "Allozyrne Variation Within and Between Populations of Ruth's Golden Aster, *Pityopsis ruthii* (Small) Small." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Botany.

E. E. C. Clebsch, Major Professor

We have read this thesis and recommend its acceptance:

Frank McCormick, Gary L. Walker, Gary F. McCracken

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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ALLOZYME VARIATION WITHIN AND BETWEEN POPULATIONS OF RUTH'S GOLDEN ASTER, *Pityopsis ruthii* (Small) Small

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Steven A. Sloan

August 1994

Dedication

This thesis is dedicated to two men; both have greatly influenced my life. First, to my chemical engineer grandfather, Steve Puschaver, a first generation Hungarian-American who sought an education because he did not want to die in the coal mines like his father. His belief in education and his personal success in life continue to provide the medium from which my enthusiasm feeds. Secondly, to Rev. Tom Lee Stewart, a first-generation "free" man. His will to live, hard work, and simple lifestyle provide a high standard by which I judge my own.

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Abstract

Genetic structure of the two known populations of Ruth's golden aster, *Pityopsis ruthii* (Small) Small, was determined. *Pityopsis ruthii* is a rare, endemic plant species restricted to exposed phyllite and graywacke rock outcrops in the flooding zones of the Hiwassee and Ocoee River drainages in Polk County, Tennessee. There are an estimated 10,000 to 15,000 *P. ruthii* plants on the Hiwassee and ≈600 on the Ocoee River. Patterns of variation at three enzyme loci were examined. Two loci, *Pgm-2* and *Prx-1*, were polymorphic in both river populations. Despite limited habitat and subsequent restricted ranges (≈ 5 km along each river), populations of this obligately outcrossed species were moderately substructured (mean F_{ST} = 0.163). Although river populations were separated by ≈ 20 km of mountainous terrain, only 1.05% of the gene diversity was attributable to differences between river populations, while 15.16% was attributable to differences among subpopulations within respective river gorges, and 83.79% was attributable to within subpopulation variation. The low mean F_{IS} value for *P. ruthii* (0.062) may indicate that this species is avoiding inbreeding by predominantly reproducing vegetatively.

What has been traditionally considered two separate, breeding river populations of *P. ruthii* was demonstrated, on the basis of two polymorphic loci, to be many separate "rock populations" within each river population. To determine how many separate breeding groups exist, further allozyme analyses of more rock populations and subsampling within rock populations needs to be conducted. Based on these data each rock outcrop should, unless demonstrated to be the contrary, be considered a "breeding population."

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CHAPTER I

INTRODUCTION

Pityopsis ruthii (Small) Small (Ruth's golden aster) is a federally endangered plant species endemic to two river gorges in Polk County, Tennessee. The *P. ruthii* plants in each river gorge (Hiwassee and Ocoee) have been regarded as separate breeding populations (U.S. Fish and Wildlife Service, 1990). Recovery efforts for Ruth's golden aster include the planned establishment and maintenance of two *ex situ* populations, representing the genetic composition of each river population. Efforts also include the establishment of *P. ruthii* populations on presently unoccupied suitable habitat within the range of the species and the collection of seed for long-term storage (U.S. Fish and Wildlife Service, 1990). Falk (1990) notes that it is important to know the genetic structure of rare plant populations if conservation efforts are to include the collection of seed for the establishment of cultivated populations.

In order to effectively estimate the extent of genetic variation within a population it is necessary to determine at which level of population structure the most variation is maintained. If there were no genetic differentiation between river populations of *P. ruthii* then it would only be necessary to establish and maintain a single population in cultivation. According to Brown (1978), measures of the distribution of genetic variation within a species collectively defines the population genetic structure of the species. These measures include the average genetic diversity of a population, the range in diversity levels among populations, and the extent and variety of genetic differentiation between populations. The purpose of this study is

to determine the genetic structure of *P. ruthii* river populations, based on data obtained from allozyme analyses. The term population used throughout this document refers to all *Pityopsis ruthii* plants occurring within a river gorge (Hiwassee or Ocoee), unless otherwise noted. Subpopulations were defined as plants occurring on single or multiple, adjacent rock outcrops, unless otherwise noted. Allozyme variation was observed in preliminary gel analyses of field collected plants.

Objectives

There were three objectives of this study:

I. To determine the degree of genetic differentiation between river populations of *Pityopsis ruthii*.

II. To determine the degree of genetic differentiation among subpopulations within their respective river populations.

III. To determine the amount of genetic variation contained within a given subpopulation on both the Hiwassee and Ocoee Rivers.

Allozymes and their use in determining the genetic structure of natural populations

The significance of the size of populations relative to genetics, mating structure, and evolutionary dynamics is a present concern of conservation biology, and was first recognized by Wright in 1931 (Barrett and Kohn, 1991). A goal in the management of many threatened and endangered species is the preservation of habitat and the maintenance of existing levels of genetic variation contained within the species (Barrett and Kohn, 1991). Genetic variation within a taxon provides a means by which potential evolutionary change can occur (Huenneke, 1991). Often, methods of measuring genetic variation in a taxon are time consuming (morphometric

and physiological analyses) or expensive (DNA sequencing and polyacrylamide analysis). Starch gel electrophoresis offers several advantages to other methods for determining the genetic variation within and between taxa. It is a relatively fast, inexpensive, and often non-destructive method by which genotype and allele frequencies can be directly estimated (Hamrick *et al.*, 1991). Allozymes are stained enzyme products that vary in electrophoretic mobility as a result of allelic differences at a single locus (Hartl, 1988). They separate on the basis of molecular size, configuration, and charge when migrating through a buffered matrix of hydrolyzed potato starch or other gel medium. Allozymes can be used to describe genetic variation within and between populations and to monitor the loss of genetic variation in populations over time (Hamrick *et al.*, 1991). In rare taxa, there are several factors that may result in changes in genetic variation. Genetic drift (i.e., founder effect, inbreeding followed by a loss of rare alleles, and other stochastic events that change allele frequencies) and intense natural selection toward genetic uniformity in a few restricted environments are the most important (Karron, 1991).

Description of the species *Pityopsis ruthii*

Ruth's golden aster is one of the grass-leaved golden asters in the *Asteraceae*, genus *Pityopsis* (Semple and Bowers, 1985). According to Semple and Bowers (1985), *Pityopsis ruthii* is the most narrowly distributed species of *Pityopsis*. They have considered it to be the most primitive species in the genus because it has features common to both sections, *P.* sect. *Pityopsis* and *P.* sect. *Graminifoliae*. *Pityopsis ruthii* has a chromosome number of n=9. A taxonomic description of the species is

provided by the U. S. Fish and Wildlife Service (1990):

> *Pityopsis ruthii* is an herbaceous, tufted perennial with slender stoloniferous rhizomes. The few to several stems are one to three decimeters (dm) tall, erect to ascending or decumbent, stiffish, and terete. They are silvery-sericious (or partly glabrate) throughout, except for the stipitate glandular peduncles and involucres and the bases of the stems, which retain the brownish, scaly, old leaf bases. The silvery-white appearance is produced by numerous long, appressed hairs that are admixed above on the stem with short, spreading, peg-like glandular hairs. Branches are few to several, upwardly arching, and originating from the mid-stem upward. Leaves are numerous, chiefly cauline, overlapping in tight spirals, ascending or erect, linear, lance-linear or gladiate, mostly 2 to 6 centimeters (cm) long by 2 to 4 millimeters (mm) wide. They are narrowly acute or acuminate and entire; the bases are attenuate and clasping, with surfaces silvered with long, appressed hairs. The lower cauline leaves generally are soon deciduous. Basal leaves, when present, often are tufted but not enlarged. The inflorescence comprises one to several heads in a cyme. The peduncles are usually longer than the heads, upwardly arching, and copiously spreading-glandular-hairy. The heads are broadly campanulate, about 1 cm high (from the base to tip of disc), and 1 cm broad across the top to the involucre. The involucre is 6-10 mm high. The involucral bracts are lance-linear; attenuate-tipped; loosely overlapping in several series; all green with broad, pale, ciliate margins; and the backs are sessile-glandular. Innermost bracts are 7-8 mm long, while the outermost are shorter. There are 8-15 ray florets with a pappus of numerous capillary, dull-white bristles about 4-5 mm long. Corollas are yellow with flattish spreading claws about 3 mm long. Blades are linear-elliptic or oblanceolate, 6-10 mm long. Disc florets are numerous, with pappus similar to that of the rays. Their corollas are yellow, tubular, and about 5 mm long, with a slightly expanded throat. The five corolla lobes are triangular and erect or slightly spreading. Flowering occurs from July to frost, peaking in September. The pale brown achenes are lance-fusiform or linear-fusiform, 3.5 to 4.0 mm long, slightly ribbed, slightly compressed, silvery-pubescent proximally and smooth distally, being narrow at the apex.

Life history and reproduction

Pityopsis ruthii is an obligately outcrossing species (Bowers, 1972). During flowering (1992 and 1993) the predominant insect visitors observed on Ruth's golden

aster were halictid bees; however, it is not known if they are pollinators of *P. ruthii.*

Three likely means of achene dispersal for *P. ruthii* are wind (Gunn, 1990), water (Wofford and Smith, 1980), or gravity (Clebsch, pers. comm., 1992). Seedling establishment may be a rare occurrence in the field. Seven of 71 seedlings (9.86%), naturally dispersed on a rock outcrop in the Hiwassee River basin, survived during 15 months of field observation (unpublished, 1993). Wofford and Smith (1980) have stated that reproduction in natural populations is predominantly vegetative. I have been successful at propagating *P. ruthii* from hormone treated cuttings of aerial stems.

The movement of pollen and seeds among subpopulations and between populations greatly influences the organization of genetic variation contained therein (Hamrick, 1989). For instance, plants with long distance gene dispersal mechanisms are expected to exhibit less population and subpopulation genetic differentiation than plant species with more restricted pollen and seed movement. Obligately outcrossed species generally have evolved or coevolved mechanisms by which gene flow is facilitated. It has been demonstrated, using computer simulations of Wright's isolation by distance models, that in outcrossed plant species near-neighbor mating increases as population size decreases (Turner *et al.*, 1981). Turner *et al.* (1981) suggest that the occurrence of nearest-neighbor pollination alone is enough to explain substantial population differentiation without the inclusion of genetic drift and founder effects. If seedling establishment is rare and reproduction in *P. ruthii* is predominantly via vegetative means, random events may be predominantly

responsible for genetic differentiation observed among subpopulations and between river populations. If sexual reproduction were resulting in the establishment of *P. ruthii* seedlings in these relatively isolated subpopulations then substructuring due to consanguineous mating may be expected.

Habitat

Pityopsis ruthii appears to be restricted to soil-filled crevices of exposed phyllite and graywacke boulder outcrops. This habitat is rare and discontinuous within the species' range. Collins and Gunn (1986) noted that subpopulations of *P. ruthii* are easily distinguished because of several factors: boulders are limited in size, there is a frequent distance of several hundred meters between boulders occupied by *P. ruthii*, and the habitat is sharply defined by the river's edge on one side paralleled by the encroaching forest on the other.

Baskin and Baskin (1988) report that rock outcrop endemics are rare, typically because the habitat which provides their long-term physiological requirements is rare. Also, high intensity sunlight is a requirement common to most outcrop endemics. Supporting this, White (1977) observed that *P. ruthii* does not occur where the light intensity is less than 50% full sunlight.

Population size and extent

Population size of *P. ruthii* on the Hiwassee River is difficult to determine given the relief of the terrain. There have been many attempts, each successively more detailed than the last (Bowers, 1972; White, 1978; Wofford and Smith, 1980; and Haggard and Halback, 1985), to quantify the range and number of *P. ruthii*

plants on the Hiwassee and Ocoee Rivers. On the Ocoee River there is less *P. ruthii* habitat, a greater distance between suitable rock outcrops, and easier access to the population. There are fewer plants in this drainage; therefore, population estimates likely represent an actual census. Bowers (1972) was the first to describe the range of the species. He estimated that the Hiwassee River population (then the only known population of *P. ruthii*) inhabited a stretch of the river 1.6 to 2.4 km in length centered around McFarland, Tennessee. White (1978) published a range extension for the species, and he observed *P. ruthii* along a 4.8 km segment of the Hiwassee River and a 1.6 km section of the Ocoee River. Wofford and Smith (1980) confirmed the 4.8 km range extension for the Hiwassee River population (concurrent with White, 1978). Wofford and Smith (1980) also extended the known Ocoee River population range an additional 3.2 km upon locating *P. ruthii* on several large graywacke boulders at river mile 23. Haggard and Halback (1985) extended the range for the Hiwassee River population 0.8 km farther downstream from previous range estimates upon finding *P. ruthii* plants distributed on boulders at the southeast end of Big Rock Island. I observed no additional *P. ruthii* habitat beyond the present range estimates: 5.5 km on the Hiwassee River, beginning 1.7 km upstream from the Apalachia Powerhouse to a distance 0.6 km upstream from the Wolf Creek inlet (river mile 58), and 4.8 km on the Ocoee River, measuring from 0.4 km upstream from the Ocoee Power Plant No. 2 (river mile 20) to a point 0.2 km downstream from the Short Creek inlet (river mile 23). The Hiwassee River is estimated to support between 10,000 and 15,000 *P. ruthii* plants. The number of *P. ruthii* plants

comprising the Ocoee River population was reported as 631 in a 1987 census by Collins and Gunn (U. S. Fish and Wildlife Service, 1990).

It is not known what is considered an individual in these estimates, because the plants grow as clusters of aerial stems and can reproduce by "stem regeneration or tillering of the subaerial rhizome crown" (Wofford and Smith, 1980). Therefore, in population estimates of *P. ruthii*, previous researchers have assumed that plants within ≈15.24 cm of one another, which share a crack in a rock, are a genet. Preliminary investigations indicated that clusters of aerial stems sharing a soil-filled crevice in a boulder may not represent a single plant.

The Hiwassee and Ocoee Rivers have common and local features that may affect the way in which the genetic variation is apportioned in *P. ruthii*. The natural flooding regimes have been disrupted with the impoundment of the Ocoee in 1939 and the Hiwassee in 1940 (White, 1977; Gunn, 1990; U.S. Fish and Wildlife Service, 1990). The rivers probably have similar climatic conditions; they drain in an east to west direction into a common watershed, and they are in close physical distance to one another. Anthropogenic and stochastic environmental effects may influence these rivers similarly.

Historically, the Ocoee River may have supported more and larger subpopulations than are currently present. The building of a wagon road in the 1850's and ultimately the construction of U.S. Route 64 along the north bank, along with poor water quality from mining activities in the Copper Basin, and recreational activities (kayaking, canoeing, and rafting) may have reduced the frequency and size

of subpopulations on this river (U. S. Fish and Wildlife Service, 1990 and White, 1977). The Hiwassee River has had different factors which may have adversely affected the size of its subpopulations. Gunn (1990), U.S. Fish and Wildlife Service (1990), White (1977), and Bowers (1972) contend that competition with woody and herbaceous plants may be the single most important factor causing the decline of *P. ruthii* on the Hiwassee River. The construction of a railroad along the south bank and the subsequent chemical spills from train derailment into the river gorge may have had adverse effects on the size of the subpopulations on this river (White, 1977 and U.S. Fish and Wildlife Service, 1990).

CHAPTER II

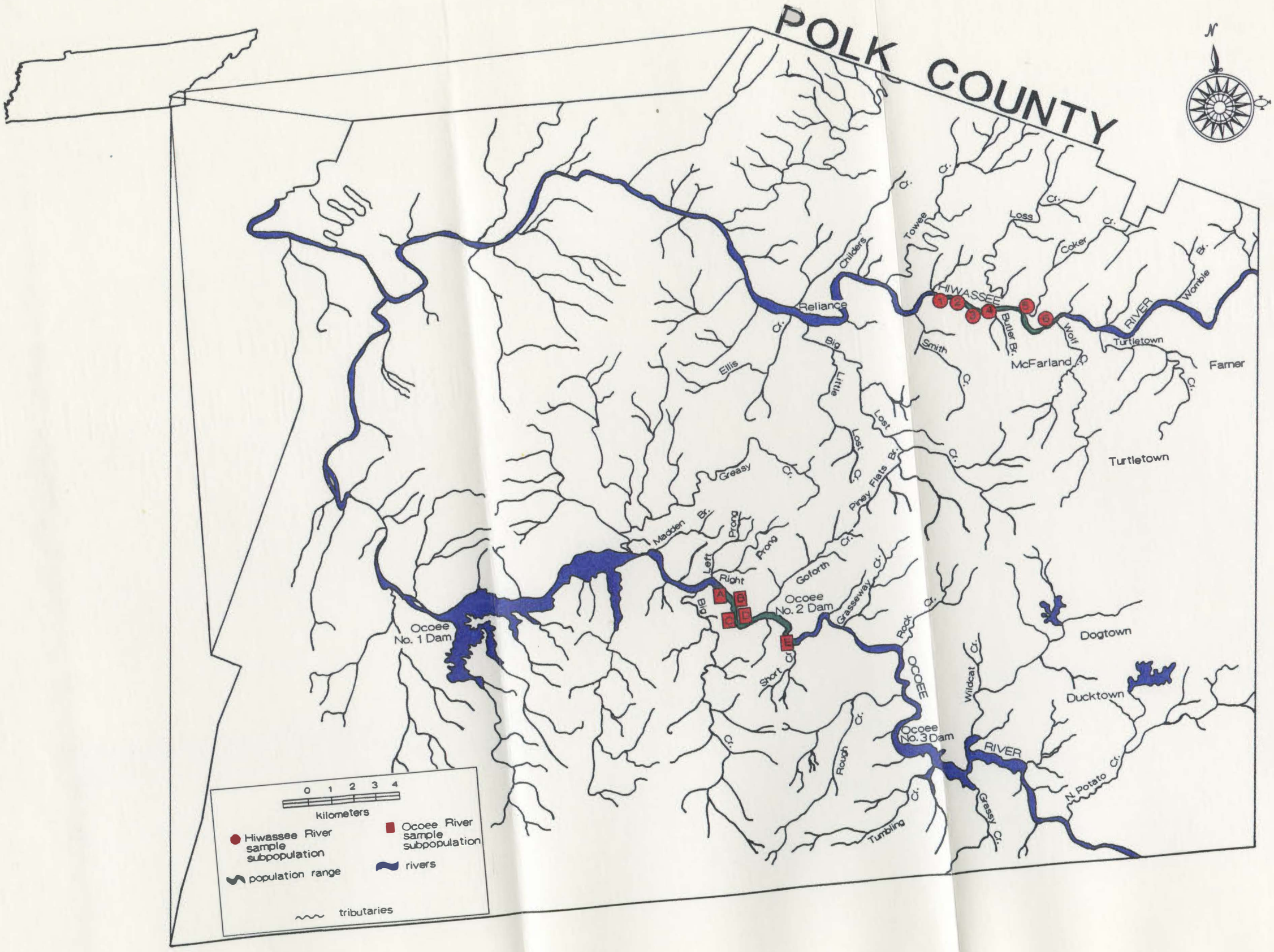
MATERIALS AND METHODS

Field methods

Six subpopulations along the Hiwassee River and five subpopulations along the Ocoee River were sampled for allozyme analyses using starch-gel electrophoresis (Figure 1). Samples ranging from 18 to 31 individuals, occurring on a single or multiple, closely adjacent rock outcrops were obtained from each subpopulation. A sample consisted of five to eight of the most recently formed leaves cut near the stem of a tagged plant. Leaves were put in labeled glass vials, which were subsequently placed on ice in cardboard boxes for transport to the laboratory. The location of each sampled subpopulation is presented in Appendix A.

Sampled subpopulations on the Hiwassee River (**HR**) were coded $\mathbf{HR_1}$ through $\mathbf{HR_6}$ and on the Ocoee River (**OR**) $\mathbf{OR_A}$ through $\mathbf{OR_E}$. Subpopulations were labeled numerically (Hiwassee River subpopulations) and alphabetically (Ocoee River subpopulations) in ascending order from the subpopulation farthest downstream to the subpopulation farthest upstream. All samples except those from subpopulation $\mathbf{OR_B}$ were collected within a three week period, 8-26 May 1993. Subpopulation $\mathbf{OR_B}$ was sampled in August 1993 to increase the number of samples from the Ocoee River population. Two hundred and seventy total samples were collected for allozyme analyses: 145 samples from the Hiwassee River population and 125 samples from the Ocoee.

Figure 1. Map of Polk County, Tennessee with sampling locations of *Pityopsis ruthii* noted on the Hiwassee and Ocoee Rivers. Sampled subpopulations are labeled 1 - 6 on the Hiwassee and A - E on the Ocoee River. Both are labeled consecutively from the subpopulation farthest downstream to the subpopulation farthest upstream.


POLK COUNTY
N
Towee Cr.
Loss Cr.
Coker Cr.
Wombie Br.
HIWASSEE
RIVER
Reliance
Childers Cr.
Ellis Cr.
Big Little Lost Cr.
Lost Br. Cr.
Smith Cr.
Butler Br.
McFarland Cr.
Wolf Cr.
Turtletown
Farner Cr.
Piney Flats Br.
Greasy Cr.
Madden Br.
Left Prong
Right Prong
Goforth Cr.
Grassey Cr.
Big Cr.
Ocoee No. 1 Dam
Ocoee No. 2 Dam
Short Cr.
Rock Cr.
OCOEE
Wildcat Cr.
Dogtown
Ducktown
Ocoee No. 3 Dam
RIVER
Rough Cr.
Tumbling Cr.
Grassy Cr.
N. Potato Cr.
A
B
C
D
E
1
2
3
4
5
6
0 1 2 3 4
Kilometers
Hiwassee River sample subpopulation
Ocoee River sample subpopulation
population range
rivers
tributaries

Laboratory methods

Samples were homogenized and frozen within 36 hours of the time they were collected. Approximately 0.2 g of leaves were cut into strips, placed in chilled spot-plates with glass grinding beads with ≈ 0.20 to 0.35 mls. of crushing buffer (recipe presented in Appendix B), and homogenized with a pestle. The homogenates were put into labeled vials, immersed in liquid nitrogen and stored in the -80°C freezer until analyzed.

Pityopsis ruthii plants grown from seed were maintained at The University of Tennessee to obtain fresh sample material for starch gel electrophoresis. Samples from these plants were used in preliminary electrophoresis surveys to determine which gel/ electrode buffer system combinations would yield resolvable allozymes. The crushing buffer used in preliminary allozyme electrophoresis was selected because it provided an abundance of enzyme activity for eight enzymatic stains with three gel/ electrode buffer systems by Wang (1991): tris-citrate/ NaOH-boric acid (#6), tris-citrate/ lithium hydroxide-boric acid (#8), and a morphaline-citrate buffer system (#M). Electrophoresis techniques and buffer/stain recipes followed Acquaah, 1992; Selander *et al.*, 1971; Soltis *et al.*, 1983; Soltis and Soltis, 1989; Wang, 1991; and Werth, 1985. Twenty-two enzymes were assayed, with as many as 15 buffer systems (see Appendix B for the recipes of scored enzymes).

Data analyses

All computations of allele frequencies, genetic distances, fixation indices, *F*-statistics, and *G*-tests were performed using the microcomputer program "Genes in Populations" by B. May and C.C. Krueger and written in C by W. Eng, Cornell University. This program was also used to produce dendrograms by subjecting Nei's genetic distance to unweighted pair group method cluster analysis (Sneath and Sokal, 1973). Calculations of genotypes were performed in "Word Perfect 5.0" by Word Perfect Corporation. Gene diversity was calculated following the procedures described by Perkins *et al.* (1993). The statistics used to delineate population structure are defined below.

Allele frequency is the proportion of an allele type at a locus. Allele frequency, a quantitative measure of genetic diversity, measures the evenness of allelic distributions within a population.

The fixation index (F_{IS}) is a measure of the deviation in the observed frequency of heterozygotes from those expected given Hardy-Weinberg equilibrium or random mating within populations. Values range from -1, indicating extreme outcrossing, to +1, indicating extreme inbreeding (Wright, 1965).

F-statistic coefficients (Wright, 1965) calculated from *P. ruthii* allozyme data are described as follows: F_{IT} measures the level of heterozygosity in the individual relative to the total population, taking into account the effects of non-random mating within subpopulations and population subdivision. F_{IT} is positive if there is systematic subdivision of the genetic variation (consanguineous mating) and negative if there is

no subdivision of genetic variation (random mating). F_{IS} measures the reduction in heterozygosity due to inbreeding within populations. Values of F_{IS} are generally positive because subdivisions of a population are typically small relative to the total size of the population, and heterozygosity is expected to decrease due to an increase in consanguineous mating. F_{IS} values are negative if there is systematic avoidance of consanguineous mating. F_{ST} measures the amount of genetic variation in the whole population that is attributable to genetic differentiation among subpopulations. For example, $F_{ST} = 0$ when there is no genetic differentiation among subpopulations. In other words, the population is a single, large panmictic breeding population when $F_{ST} = 0$. When $F_{ST} = 1$ there is fixation for different alleles among subpopulations. The relationship among the three *F*-statistic coefficients is presented in the following formula: $1 - F_{IT} = (1 - F_{IS})(1 - F_{ST})$.

The *G*-test is a widely used general statistic, a goodness of fit test, that fits the data to a random mating model. $G = 0$ if the data conform perfectly to the random mating model. In other words, the observed heterozygosity exactly matches that expected given Hardy-Weinberg equilibrium. For comparison, the greater the value of *G*, the closer F_{ST} is to 1. Nei's genetic distances were subjected to unweighted pair group method cluster analysis and dendrograms were constructed. The physical distance was plotted against the genetic distance in a correlation analysis.

CHAPTER III

RESULTS

Enzyme activity

Of the twenty-two enzymes assayed, allozyme banding patterns were scorable for phosphoglucomutase (PGM) and peroxidase (PRX) loci. Other enzymes expressed activity but were not scored because they could not be consistently resolved or effectively separated. Three of the banding patterns for both polymorphic systems were interpreted as monomeric enzyme loci. Two loci were scored for PGM, *Pgm-1* (monomorphic) and *Pgm-2* (polymorphic). Both migrated in an anodal direction from their point of origin. Four alleles were discernable for the *Pgm-2* locus. Enzyme loci and allozymes were numbered beginning with the locus/ allele farthest from the origin.

Although there were many patterns of enzymatic activity for PRX, only one could be interpreted and scored. This zone of activity migrated cathodally from the origin and was labeled *Prx-1*. Two alleles were inferred at this locus. Allozymes of this enzyme were scored on the basis of staining intensity. For instance, if two bands of enzyme activity were present with equal intensity then the allozyme was scored as a heterozygote. If two bands of activity were present but stained unequally, the darker of the two was scored as a homozygote. If only one band was predominant in staining intensity then it too was scored as a homozygote.

Allele frequencies for the two polymorphic loci, *Pgm-2* and *Prx-1*, are presented in Table 1. Enzyme names, abbreviations, and Enzyme Commission numbers are listed in Table 2.

Table 1. Allele frequencies at two polymorphic loci, *Pgm-2* and *Prx-1*. Beneath each dotted line are the sample sizes (N) for the subpopulations. The subscript $_{POP}$ refers to the pooled allele frequencies of all sampled subpopulations within a river drainage.

Hiwassee River

Locus		HR_1	HR_2	HR_3	HR_4	HR_5	HR_6	$HR\text{-}_{POP}$
Pgm-2	**A**	0.540	0.080	-----	0.640	0.286	-----	0.257
	B	0.040	0.080	0.173	0.100	0.071	-----	0.079
	C	0.420	0.840	0.827	0.260	0.643	0.917	0.651
	D	-----	-----	-----	-----	-----	0.083	0.014
	N	25	25	26	25	21	24	146
Prx-1	**A**	0.500	0.500	0.635	0.260	0.750	0.750	0.561
	B	0.500	0.500	0.365	0.740	0.250	0.250	0.439
	N	25	25	26	25	22	24	147

Ocoee River

Locus		OR_A	OR_B	OR_C	OR_D	OR_E	$OR\text{-}_{POP}$
Pgm-2	**A**	0.043	0.231	0.386	0.118	0.450	0.263
	B	-----	-----	-----	-----	0.050	0.013
	C	0.957	0.769	0.614	0.882	0.500	0.725
	D	-----	-----	-----	-----	-----	-----
	N	23	26	22	17	30	118
Prx-1	**A**	0.750	0.231	0.560	0.472	0.538	0.508
	B	0.250	0.769	0.440	0.528	0.462	0.492
	N	24	26	25	18	26	119

Table 2. Enzymes assayed, abbreviations, and Enzyme Commission numbers

Enzyme Assayed	Abbreviation	Enzyme Commission Number
Aconitase	ACO	4.2.1.3
Alcohol dehydrogenase	ADH	1.1.1.1
Aspartate transaminase	AAT	2.6.1.1
Catalase	CAT	1.11.1.6
Fluorescent Esterase	fl-EST	3.1.1.-
Formate dehydrogenase	FDH	1.2.1.2
Fructose-bisphosphate aldolase	FBA	4.1.2.13
Glucose 6 phosphate dehydrogenase	G6PDH	1.1.1.49
Glucose-6-phosphate isomerase	GPI	5.3.1.9
Glutamate dehydrogenase	GDH	1.4.1.2
Hexokinase	HEX	2.7.1.1
Isocitrate dehydrogenase	IDH	1.1.1.42
Leucine aminopeptidase	LAP	3.4.11.1
Malate dehydrogenase	MDH	1.1.1.37
Maleic enzyme	ME	1.1.1.40
Mannose-6-phosphate isomerase	MPI	5.3.1.8
Peroxidase	PRX	1.11.1.7
Phosphoglucomutase	PGM	5.4.2.2
Phosphogluconate dehydrogenase	PGD	1.1.1.44
Shikimate dehydrogenase	SKD	1.1.1.25
Superoxide dismutase	SOD	1.15.1.1
Triose-phosphate isomerase	TPI	5.3.1.1

Preliminary electrophoretic data indicated variation within one cluster of aerial stems. Thus, the assumption that clusters of aerial stems in close physical distance sharing a common crevice represent a genet may not be valid. Also, no differences in electrophoretic mobility were detected that were attributable to age of the ramet sampled or time of year the sample was collected.

Allele frequencies

A total of four alleles was detected at the *Pgm-2* locus across all subpopulations sampled. Two or three alleles were observed in any given subpopulation. Allele **D** of the *Pgm-2* locus was only observed in subpopulation $\mathbf{HR_6}$, the sampling site farthest upstream on the Hiwassee River (Figure 1 and Table 1). Allele **B** of the *Pgm-2* locus was present in all subpopulations on the Hiwassee River except subpopulation $\mathbf{HR_6}$ and was absent from all subpopulations on the Ocoee River except $\mathbf{OR_E}$, the farthest upstream. Hiwassee River subpopulations differed in their most common alleles at both loci. Ocoee River subpopulations differed in their most common allele at the *Prx-1* locus, but the **C** allele of the *Pgm-2* locus was consistency the most frequent allele observed in subpopulations of the Ocoee River.

Genotypes

The genotypes observed in individuals for both loci, the distribution of genotypes among subpopulations, and the cumulative percentage each genotype is reflected in the species is presented in Table 3. Three genotypes represent ≈53% of the individuals sampled. In all three genotypes the homozygote 33 at the *pgm-2* locus was consistently the most frequent genotype. The Chi square value calculated from comparing the observed genotypes to those genotypes expected was not significant, indicating that the genotypes observed conform to those expected in a random mating model (Table 4).

Table 3. Distribution of observed genotypes among subpopulations

Genotypes *pgm-2-prx-1*	HR_1	HR_2	HR_3	HR_4	HR_5	HR_6	OR_A	OR_B	OR_C	OR_D	OR_E	Total observed individuals	Cumulative total (Percent)
33-11	1	4	8		6	13	11	1	4	2	3	53	20.95
33-12	2	8	8	1	3	5	9	3	4	6	3	52	41.50
33-22	2	5		2		2	1	10	1	5	2	30	53.36
13-11	3	2		1	3		2	1	4	1	6	23	62.45
13-22	4			4				5	3		2	18	69.56
13-12	4	1		2	4			3	1	1	1	17	76.28
11-12	5			6	1				1		1	14	81.81
11-22				4				1	2		3	10	85.76
23-12		4	4		1						1	10	89.71
11-11	2				1				1	1	2	07	92.48
12-12	2			2	1							05	94.46
23-22			3								1	04	96.04
23-11			2		1							03	97.23
12-22				3								03	98.42
34-11						2						02	99.21
34-22						1						01	99.61
34-12						1						01	100.00
Total	25	24	25	25	21	24	23	24	21	16	25	253	

Table 4. Chi square value calculated from observed genotypes.

Genotypes	Expected genotypes	Observed genotypes	Chi square
33-11	47.01	53	0.76
33-12	52.83	52	0.01
33-22	35.27	30	0.79
13-11	20.16	23	0.40
13-22	15.13	18	0.54
13-12	22.64	17	1.41
11-12	12.17	14	0.28
11-22	8.12	10	0.44
23-12	6.63	10	1.71
11-11	10.83	7	1.35
23-11	5.89	3	1.42
Other genotypes pooled	16.43	16	0.00
Total	253.11	253.00	X^2 = 9.11 n.s. df(11)

Genetic structure of populations

The extent of inbreeding within subpopulations was estimated using Wright's (1965) fixation index (F_{IS}). Values of F_{IS} are presented for all subpopulations in Table 5. Two subpopulations, $\mathbf{OR_C}$ and $\mathbf{OR_E}$, deviated from Hardy-Weinberg expectations at one locus due to an excess of homozygotes. Mean F_{IS} values ranged from -0.144 to +0.121 in the Hiwassee River subpopulations and from -0.078 to +0.340 in the Ocoee River subpopulations.

The organization and apportionment of genetic variation within and between *P. ruthii* populations was analyzed using *F*-statistics described by Wright (1965). F_{IT}, F_{IS}, F_{ST}, and *G*-test values as well as the total number of alleles per locus are presented in Table 6. Wright (1978) and Hartl (1988) provide a standard by which F_{ST} values can be compared. According to this standard, F_{ST} values ranging from 0 to 0.05 indicate *little* genetic differentiation; 0.05 to 0.15 indicate *moderate* differentiation; 0.15 to 0.25 indicate *great* differentiation; and values greater than 0.25 indicate *very great* genetic differentiation. Single locus F_{ST} values ranged from a maximum of 0.251 for *Pgm-2* in the Hiwassee River population to a minimum of 0.112 for *Prx-1* in the Ocoee River population. All *G*-test values were significant at the 0.001 level, indicating a non-random distribution of alleles into subpopulations ($F_{ST} \neq 0$). Samples from river drainages did not cluster together. Cluster analysis indicates a pattern of less genetic distance among geographically closer samples in the Hiwassee population than in the Ocoee population (Figures 2, 3, and 4). No significant correlation was found between Nei's genetic distance and the physical distance between subpopulations (Figure 5).

Table 5. Fixation indices (F_{IS}) within Hiwassee (**HR**) and Ocoee (**OR**) River subpopulations of *Pityopsis ruthii*.

Locus	HR_1	HR_2	HR_3	HR_4	HR_5	HR_6	OR_A	OR_B	OR_C	OR_D	OR_E
Pgm-2	+0.020	-0.136	-0.209	+0.064	+0.048	-0.091	-0.045	-0.083	+0.137	+0.433	+0.144
Prx-1	-0.040	-0.040	-0.078	-0.143	-0.333	+0.333	-0.111	+0.133	+0.513*	-0.003	+0.536**
Mean	-0.010	-0.088	-0.144	-0.040	-0.143	+0.121	-0.078	+0.025	+0.325	+0.215	+0.340

*=P<0.05 **=P<0.01

Table 6. Organization of genetic variation within and between populations as measured by Wright's (1965) F-statistics. G-tests of the null hypothesis that $F_{ST} = 0$ and the degrees of freedom (df) are presented (Sokal and Rohlf 1981).

	Locus	Number of Alleles and totals	F_{IT}	F_{IS}	F_{ST}	G	df
Hiwassee River Population	*Pgm-2*	4	0.236	-0.020	0.251	148.943***	15
	Prx-1	2	0.074	-0.051	0.119	35.475***	5
	Means	6	0.156	-0.036	0.185	184.418***	20
Ocoee River Population	*Pgm-2*	3	0.240	0.120	0.136	43.471***	8
	Prx-1	2	0.322	0.236	0.112	29.538***	4
	Means	5	0.286	0.186	0.123	73.009***	12
Pityopsis ruthii Species	*Pgm-2*	4	0.243	0.039	0.212	211.691***	30
	Prx-1	2	0.190	0.081	0.118	66.489***	10
	Means	6	0.215	0.062	0.163	278.181***	40

***P<0.001

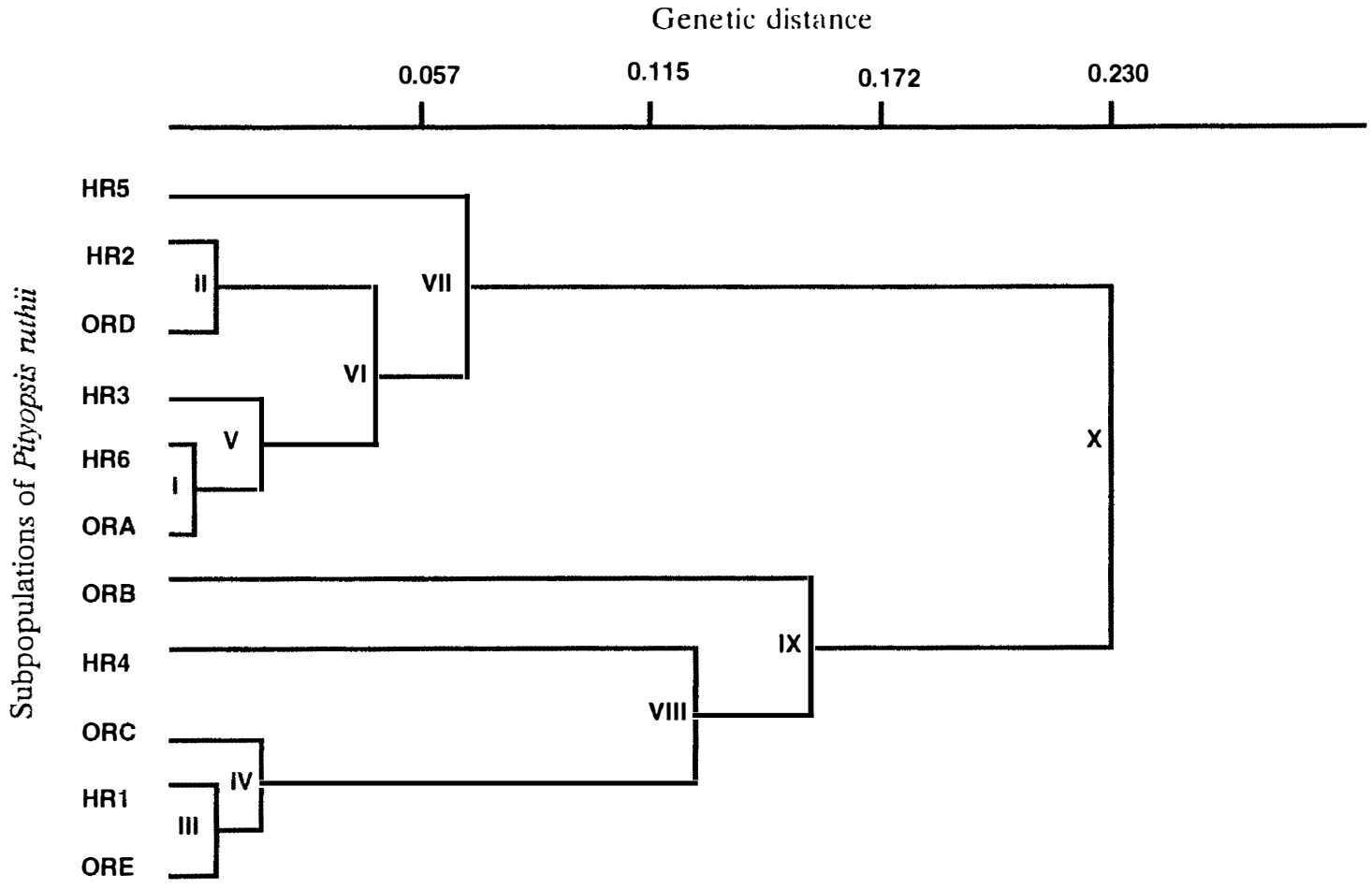


Figure 2 Cluster analysis of both the Hiwassee and Ocoee River subpopulations of *Pityopsis ruthii* using unweighted pair group method. Distance coefficient used is Nei's (1978) genetic distance.

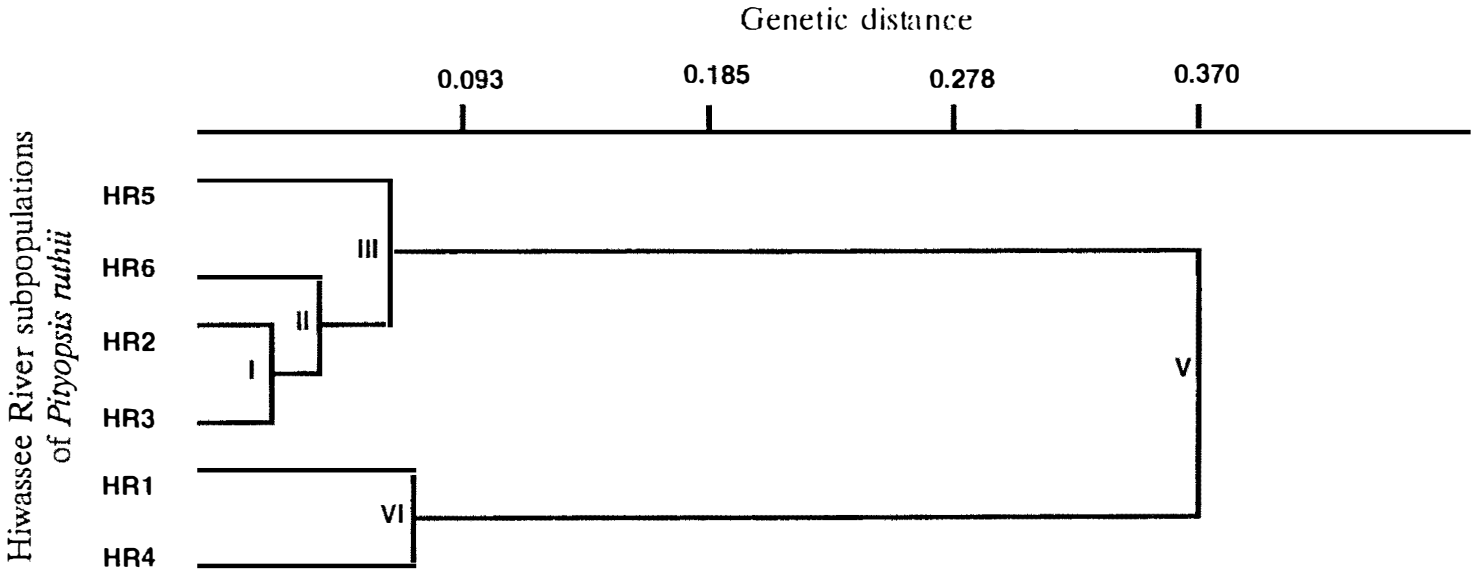


Figure 3 Cluster analysis of Hiwassee River subpopulations of *Pityopsis ruthii* using unweighted pair group method with arithmetic averaging based on Nei's (1978) genetic distance.

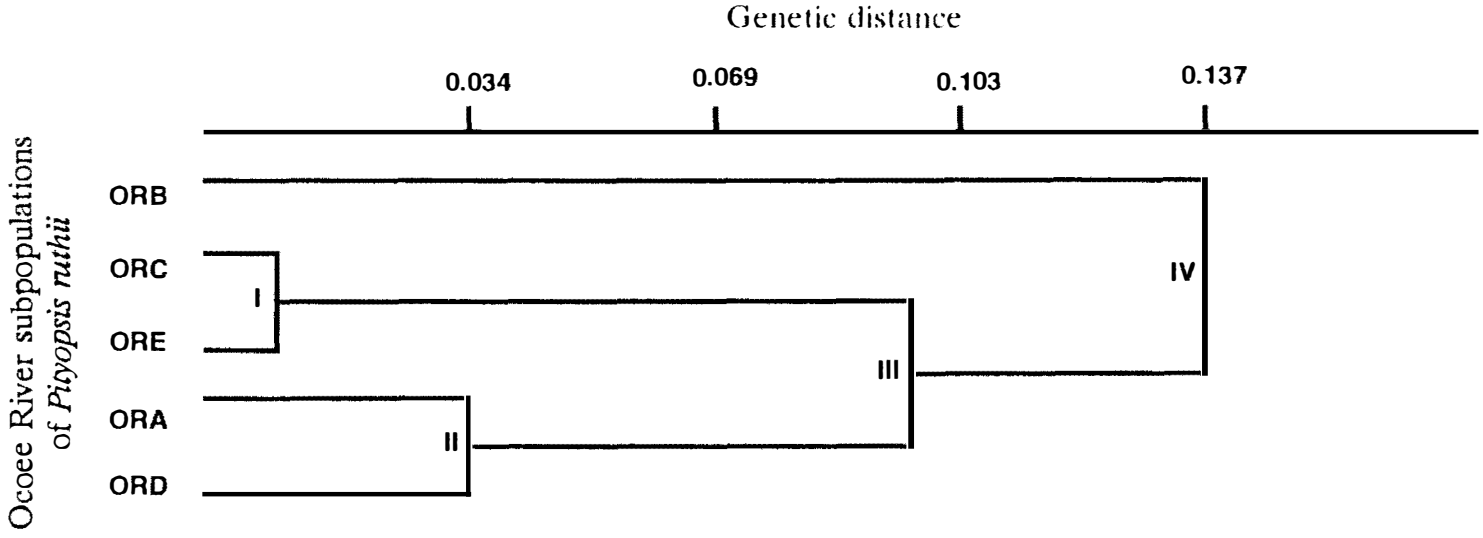


Figure 4 Cluster analysis of Ocoee River subpopulations of *Pityopsis ruthii* using unweighted pair group method with arithmetic averaging based on Nei's (1978) genetic distance.

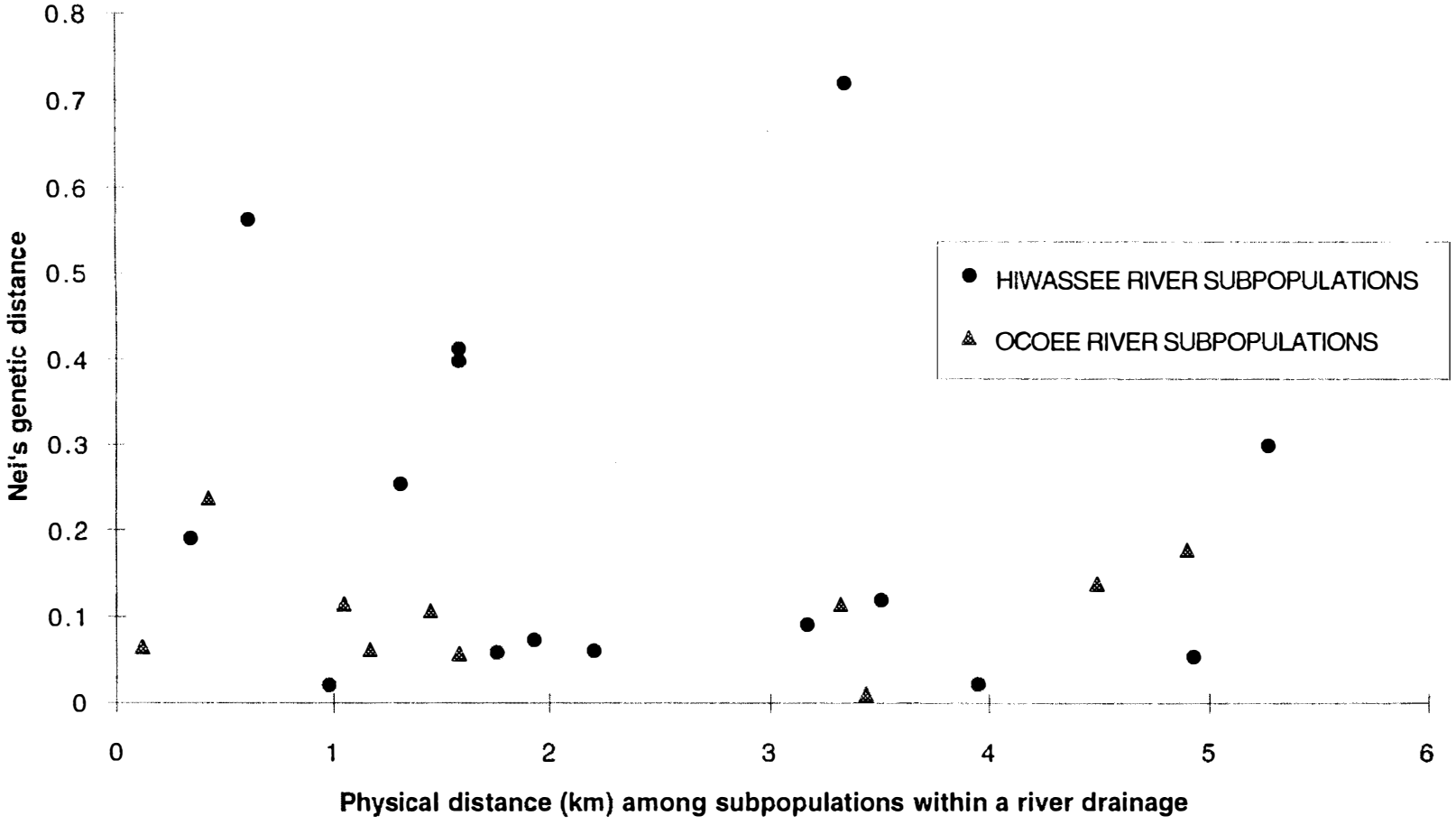


Figure 5. Correlation between Nei's (1978) genetic distance and the physical distance measured among subpopulations of *Pityopsis ruthii*. Hiwassee River subpopulations r = -0.14. n.s.; Ocoee River subpopulations r = 0.08. n.s.

Gene diversity was calculated for *Pityopsis ruthii* populations following a model provided by Perkins *et al.* (1993). 83.79% of the total gene diversity for *P. ruthii* was attributable to variation within subpopulations, 15.16% of the total variation was distributed among subpopulations within a river gorge, and 1.05% of the total heterogeneity of the species was attributable to differences between river populations.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Enzyme activity

This work provides the first study of the population genetic structure of any member of the genus *Pityopsis*, using allozyme analysis. With only two polymorphic loci, important information regarding the population structure of *P. ruthii* was obtained.

Allele frequency

Because there is no historical information on the establishment of these subpopulations, it is impossible to assess precise factors that may have influenced the genetic structure of these populations. However, in isolated subpopulations differentiation among populations and subpopulations is expected to increase with decreasing population size due to genetic drift (Ellstrand and Elam, 1993). Based on allele frequencies, genetic differences exist between river populations of *P. ruthii* and among subpopulations within river gorges. This difference may result from random changes in allele frequencies over time. Relative frequencies of alleles vary greatly among subpopulations on the Hiwassee River for both polymorphic loci and at the *Prx-1* locus among subpopulations on the Ocoee River.

Turner *et al.*, (1981) suggest that nearest-neighbor pollination may explain population differentiation without the effects of genetic drift and founder events. Because there has not been an adequate pollination study for *P. ruthii*, near neighbor pollination cannot be ruled out as an explanation for the subpopulation

differentiation observed in this species. Close agreement with Hardy-Weinberg in small isolated subpopulations of *P. ruthii* may suggest that in the history of these subpopulations sexual reproduction has resulted in, at least, the establishment of the plants sampled.

Genotypes

A likely explanation for the distribution of genotypes observed on the Hiwassee and Ocoee Rivers is that sexual reproduction resulting in the establishment of a new genotype (genet) occurs infrequently due to limited habitat. Given the perennial habit of this species and its mode of vegetative reproduction, a genet may occupy a crevice for many years potentially preventing or limiting the success of seedling establishment. This may slow the effects of inbreeding. The chi square value calculated in Table 4 indicates that the genotypes observed do not vary significantly from what would be expected if the number of genotypes were distributed randomly. A significant chi square value would be expected if vegetative reproduction resulted in the predominance of only a few successful genotypes.

Genetic structure of populations

There are several likely explanations for why subpopulations $\mathbf{OR_C}$ and $\mathbf{OR_E}$ were not in agreement with Hardy-Weinberg expectations. At the 0.05 significance level, one subpopulation in twenty would be expected to deviate by chance alone. Expressed deviation from Hardy-Weinberg may be the result of having sampled from large subpopulations composed of genetically differentiated subgroups, and resulting Walhund effect (Karron *et al.*, 1988). $\mathbf{OR_C}$ and $\mathbf{OR_E}$ were composed of plants from

several closely adjacent rock outcrops; all other sampled subpopulations were from single, isolated rock outcrops. Because it has been suggested that *P. ruthii* can reproduce vegetatively from the root crown rhizome, care was taken to avoid sampling plants sharing a soil filled crevice positioned within ≈15 cm of one another. Also, I have not observed *P. ruthii* roots bridging crevices across exposed boulder surfaces. Based on the care taken when collecting samples, field observations of the growth habit of *P. ruthii*, and the genotype data presented in Tables 3 and 4, I do not believe that ramets of the same genet were sampled.

There were differences observed between river populations of *P. ruthii* as interpreted through *F*-statistics. Single locus F_{IT} values were positive for both Hiwassee and Ocoee River populations of *P. ruthii*, indicating there is substructuring within these river populations. On the Hiwassee River, differentiation among subpopulations (F_{ST}) contributed more to the total inbreeding of the population (F_{IT}) than inbreeding within subpopulations (F_{IS}). On the Ocoee River, the large contribution of F_{IS} to F_{IT} may reflect the sampling of two large subdivided subpopulations, $\mathbf{OR_C}$ and $\mathbf{OR_E}$, or the observed shorter physical distance between many of the subpopulations. F_{ST} values for both loci and for each river population displayed moderate to great genetic differentiation among subpopulations. Great genetic variation (F_{ST} = 0.185) was observed among subpopulations on the Hiwassee River.

The negative mean F_{IS} value calculated from the two polymorphic loci over the six Hiwassee River subpopulations (F_{IS} = -0.036) indicates a slight excess of

observed heterozygotes. The positive mean F_{IS} value calculated from the two polymorphic loci in the five Ocoee River subpopulations (F_{IS} = 0.186) indicates an overall heterozygote deficiency in these samples. In theory, obligately outcrossed species occurring in small isolated rock outcrop populations would undergo inbreeding; but, F_{IS} values of the Hiwassee River subpopulations perhaps reflect the avoidance of consanguineous mating, the predominance of vegetative reproduction, or no reproduction. Random events (i.e., genetic drift, extreme flooding, drought, boulder movement, etc.) may have resulted in a greater differentiation among subpopulations in this drainage than has isolation followed by subsequent inbreeding within subgroups. Only in the much smaller Ocoee River population were lower levels of heterozygosity detected. The F_{IS} values observed in this population may indicate near neighbor mating. All of the Ocoee River subpopulations may have been similarly affected by past events. Fewer low frequency alleles observed on the Ocoee River (Table 1) may have resulted from a combination of many factors: the disruption of natural flooding regimes, road construction, siltation and water contamination from mining activities, and to a lesser extent recreational activities. The genetic structure observed in the Ocoee River population may have resulted from frequent, severe decreases in the size of subpopulations followed by an increase in homozygosity and the loss of rare alleles, genetic bottlenecks, or the chance distribution of alleles in the colonizing population, founder effect.

It is unlikely that close clustering of subpopulations on dendrograms in different river populations indicates gene flow between river gorges. On the contrary, the pairing of subpopulations between river populations is more likely the result of having insufficient polymorphic loci to adequately characterize the two river populations. These dendrograms support the *F*-statistics' results, suggesting the occurrence of little gene flow among subpopulations. Subpopulations within close physical distance were in some cases more genetically distant from one another than subpopulations with greater physical distance between them. Gene flow among subpopulations appears to have been infrequent, as evidenced by the lack of pattern in the clustering of subpopulations regardless of the physical distance between them (Figures 2, 3, and 4).

Even though *P. ruthii* is an obligately outcrossing species there is an expected loss of genetic variation due to genetic drift and near neighbor mating in the Hiwassee River subpopulations. If these subpopulations were sexually reproducing and were isolated from one another, there should have been greater genetic differentiation, due to genetic drift, expected among subpopulations than was observed. Most of the genetic variation in *P. ruthii* is maintained within subpopulations. Because seedling establishment in the field appears to be rare (Gunn, 1990; Wofford and Smith, 1980; U.S. Fish and Wildlife Service, 1990; pers. obs., 1993), the variation observed within these subpopulations may be maintained vegetatively. If *P. ruthii* plants were predominantly reproducing vegetatively, levels of genetic variation could have been maintained in subpopulations. This would imply

that at some time in the past all of the subpopulations on a river were connected as a single panmictic population. A reduction in the natural fluctuation of water on these rivers may have allowed other species less tolerant of natural flooding cycles to become established. Uneven encroachment of vegetation around boulder outcrops toward the river may have effectively isolated these rock outcrops from one another. However, there needs to be further investigation of gene dispersal and more enzyme loci resolved for *P. ruthii*, to substantiate what has been presented here.

Because only two polymorphic loci were resolved in this investigation, broad based comparisons of these data to other studies are inappropriate. Most broad based literature reviews commonly cited for comparisons among species include measures of genetic diversity (% polymorphic loci, mean number of alleles per locus, and the effective number of alleles per locus) based on more than two polymorphic loci (Hamrick *et al.*, 1991; Hamrick and Godt, 1990; Hamrick, 1989; Loveless and Hamrick, 1984). Further allozyme analyses are required in order to determine if *P. ruthii* is comparatively genetically depauprate or rich.

Summary and Conclusions

Based on the allozyme analyses of this study, the following observations can be made. River populations of *P. ruthii* apportion the genetic variation contained within them differently, with greater genetic differences observed among subpopulations on the Hiwassee River and a lesser degree of subpopulation differentiation observed on the Ocoee. Consequently, most of the genetic diversity in *P. ruthii* on the Hiwassee River is maintained among subpopulations. While there

was less genetic differentiation among subpopulations on the Ocoee River, most of the genetic variation was observed within subpopulations. What has been traditionally referred to as a single, breeding river population of *P. ruthii* was shown to be many, somewhat isolated, rock outcrop populations.

The apportionment of genetic variation observed through allozyme analyses may be explained as resulting from a combination of stochastic and anthropogenic effects. The encroachment of vegetation on and around boulder habitat within the Hiwassee drainage may pose a threat in terms of increased competition and serve as a barrier to gene flow between subpopulations. If subpopulations of *P. ruthii* on the Hiwassee River predominantly reproduce vegetatively, substantial levels of genetic variation may have persisted and be capable of continued persistence through time.

Future electrophoretic analyses of *P. ruthii* should emphasize the empirical determination of a crushing buffer that provides scorable enzyme products and not simply enzyme activity. In this study, the latter was assumed and this may be the reason more loci were not resolved. Being careful that all sampled plants root, cuttings from field collected plants should be propagated and maintained as a living sample set. Fresh cultivated material showed more enzymatic activity on gels than frozen, field-collected plant material. Resampling field collected plants was hampered by unpredictable flooding and the difficulty in negotiating the mountainous terrain. Many tagged plants appeared to die as a result of extreme weather conditions, further complicating resampling.

Because it is unlikely that gene flow occurs between river populations, there may have been greater differences detected between river populations if more allozyme loci were resolved. Consequently, management considerations based on these data should be kept in perspective of the number of loci resolved and scored in this study.

The loss of any one rock outcrop population on the Hiwassee River may result in a greater loss of genetic diversity for the species than a loss of any single subpopulation on the Ocoee. Reexamining subpopulation locations mapped by White (1977) revealed the disappearance of several large subpopulations on the Hiwassee River, which may indicate the loss of genetic diversity.

To fulfill sections 6.5 and 7.6 of the *Ruth's Golden Aster Recovery Plan* (U.S. Fish and Wildlife Service, 1990), it may be necessary to obtain seeds from multiple rock outcrops on both of these rivers and maintain each as separate gene pools. Due to the higher degree of genetic differentiation among subpopulations on the Hiwassee River, subpopulations from this river should be given greater consideration and more subpopulations should be represented in cultivation.

Unless future research demonstrates hybrid vigor from the genetic mixing of these subpopulations, their integrity should be maintained. Dinerstein and McCracken (1990) report that the duration of the bottleneck in relation to the species generation time determines how fast erosion of the genetic variation occurs. If subpopulations on the Ocoee River have undergone recent bottlenecks, maintaining large populations in cultivation could prevent the further loss of genetic variation from stochastic events.

What has been traditionally been considered as two separate, breeding river populations of *P. ruthii* was demonstrated, on the basis of two polymorphic loci, to be many separate "rock populations" within each river population. To determine how many separate groups exist, further allozyme analyses of more rock populations and subsampling within rock populations needs to be conducted. Based on these data each rock outcrop should, unless demonstrated to be the contrary, be considered a "breeding population."

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Appendices

Appendix A

Sampled subpopulation locations, number of samples collected and the number of plants tagged in each subpopulation. Longitude and latitude were measured from 7.5 minute US TVA topographic maps, McFarland (Hiwassee) and Caney Creek (Ocoee). Subpopulations are labeled consecutively from downstream to upstream, numerically on the Hiwassee (**HR**) and alphabetically on the Ocoee (**OR**).

Name of subpopulation	Number of samples obtained	Number of plants tagged	Location of subpopulation	
			Longitude	Latitude
HR_1	25	25	84°25'40"	35°11'11"
HR_2	26	27	84°25'24"	35°11'10"
HR_3	27	27	84°24'29"	35°10'47"
HR_4	25	50	84°24'14"	35°10'46"
HR_5	23	52	84°23'34"	35°10'44"
HR_6	22	53	84°23'46"	35°10'07"
OR_A	24	51	84°32'02"	35°05'31"
OR_B	28	28	84°31'57"	35°05'24"
OR_C	26	27	84°31'54"	35°04'56"
OR_D	19	25	84°31'52"	35°04'55"
OR_E	31	31	84°30'51"	35°04'26"

Appendix B

Allozyme stain and buffer recipes adopted from Zhongren Wang Lab manual The University of Tennessee:

Soltis, D., C. Haufler, D. Darrow, and G. Gastony. 1983. Starch-gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Amer. Fern J.* 73:9-27.

Werth, C. 1985. Implementing an isozyme laboratory at a field station. *Virginia J. Sci.* 36:53-76.

Grinding Buffer

The following recipe is good for 8 gels of ground plant material assuming 30 samples per gel.

0.28 g Sodium Tetraborate
0.075 g Sodium Meta-Bisulfite
1.0 g Polyvinylpyrrolidone
1.0 g L-Ascorbic Acid
0.069 g Diethyldithiocarbamic Acid
25 ml *Phosphate crushing buffer
0.25 ml 2-Mercaptoethanol
2.5 ml Dimethyl Sulfoxide **(only used if freezing)
* Phosphate crushing buffer pH 7.5, 1.361 g KH_2PO_4, 90 ml dd H_2O, and bring to pH 7.5 with approx. 9 ml 1M NaOH
bring to 100 ml dd H_2O
**After freezing, add one drop of freshly made crushing buffer to each wick saturated with homogenate, blot and load onto gel.

Electrode and Gel Buffer Recipes

#6 Buffer system

Electrode buffer:
4.0 g NaOH
18.55 g Boric acid
bring to 1000 ml dd H_20 Assay pH 8.6

Gel buffer:

10x concentrate

18.4 g Tris

6.9 g Citric acid

bring to 1000 ml dd H_2O Assay pH 7.8

Staining Buffers

PGM (Phosphoglucomutase)

3.0 ml 0.1M Tris-HCl, pH 8.0

1.0 ml 10% $MgCl_2$

0.25 ml Glucose-6-PO4-dehydrogenase (20 units/ml)

0.4 ml 5% Glucose-1-PO_4-Na_2

0.25 ml 0.5% NADP

0.25 ml 0.5% MTT

0.1 ml 1.0% PMS

6.0 ml 1.0% Agarose

Procedures: Mix all ingredients, add to heated agarose, and pour evenly over gel.

ALLOZYME STAINS ADOPTED FROM WENDEL & WEEDEN IN SOLTIS & SOLTIS (1989) *ISOZYMES IN PLANT BIOLOGY*.

PRX (Peroxidase)

50 ml 50 mM Na-acetate buffer, pH 5.0

0.05 g $CaCl_2$

0.25 ml 3% H_2O_2,

0.025 g 3-Amino-9-ethylcarbazole

2 ml N,N-Dimethylformamide

Procedures: Dissolve 3-Amino-9-ethylcarbazol in the N,N-Dimethylformamide. Add to other ingredients and pour over gel.

VITA

Steven Alexander Sloan was born in Memphis, Tennessee on November 13, 1964. He attended primary and secondary school in the Shelby County public school district (Collierville) and graduated from Collierville High School in May 1983. The following August he entered Memphis State University and in July, 1987 received the degree of Bachelor of Science in Biology with a concentration in Invertebrate Zoology. In 1990, following extensive travel through Europe and Africa, he taught Advanced Level Biology at Founder's High School in Bulawayo, Zimbabwe, to afford an airline ticket back to the U.S.A. via South America. In August, 1991 he entered The University of Tennessee, Knoxville and while there, he was awarded a Graduate Assistantship, a Graduate Teaching Assistantship, a Research Assistantship and the 1993 H. R. DeSelm Fund Award for research.