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GENETIC VARIATION IN CHRYSOPSIS FLORIDANA SMALL, THE ENDANGERED FLORIDA GOLDEN ASTER, AS REVEALED BY RANDOM AMPLIFICATION FOR POLYMORPHISM DETECTION (RAPD)

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CERTIFICATE OF APPROVAL

Master's Thesis

This is to certify that the Master's Thesis of

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by

LAURIE LYSLE WALKER MARKHAM

A thesis submitted in partial fulfillment
of the requirements for the degree of
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Co-Major Professor: Bruce J. Cochrane, Ph.D.
Co-Major Professor: Richard P. Wunderlin, Ph.D.

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LIST OF ABBREVIATIONS

A	adenine
AMOVA	analysis of molecular variance
β-ME	beta mercaptoethanol
BBR	Balm-Boyette Road
bp	base pair
BSA	bovine serum albumin
BTG	Bok Tower Gardens
°C	Celsius
C	cytosine
CAS	Casperson Farm
cm	centimeter
CPC	Center for Plant Conservation
CTAB	hexadecyltrimethyl ammonium bromide
DNA	deoxyribonucleic acid
dNTP	deoxynucleic triphosphate
DOT	State of Florida Department of Transportation
EDTA	ethylenediamine tetra acetic acid

ELAPP	Environmental Lands Acquisition and Protection Program
FGA	Florida Goldenaster Scrub
FNAI	Florida Natural Areas Inventory
FWS	US Fish and Wildlife Service
G	guanine
Gst	proportion of genetic variation among populations
hr	hour
Hs	mean genetic diversity within populations
Ht	total genetic diversity averaged over all loci
ITS	internal transcribed spacer
LB	Luria Bertani broth
µ	micron
µg	microgram
µl	microliter
m	milli
mM	milli molar
M	molar
mg	milligram
min	minute
ml	milliliter
mol	mole
n	nano

ng	nanogram
Nm	gene flow
p	pico
PCA	principle component analysis
PCR	polymerase chain reaction
pmol	picomole
PVP	polyvinyl pyrrolidine
RAPD	randomly amplified polymorphism detection
RHO	Rhodine Road
rpm	revolutions per minute
SAF	Saffold Road
sd	standard deviation
sec	second
SHP	Sun City Heritage Park
STR	Sterling Downs
T	thymine
TBE	tris boric acid EDTA
TE	tris EDTA
Tris	(Tris[hydroxymethyl])aminomethane
TSR	template suppression reagent
U	unit
USDA	US Department of Agriculture

USF	University of South Florida
UV	ultraviolet
v/v	volume to volume
w/v	weight to volume

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An Abstract

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December 1998

Co-Major Professor: Bruce J. Cochrane, Ph.D.
Co-Major Professor: Richard P. Wunderlin, Ph.D.

Chrysopsis floridana Small (Asteraceae) is a federally endangered plant endemic to the Tampa Bay area of west central Florida. It is confined to the sand pine scrub community, growing in open, sunny, and sandy areas or in disturbed areas at the edges of scrub. As a means of assessing the genetic variability of this species, eight populations in Hillsborough County were compared with a seed stock collection housed at Bok Tower Gardens in Lake Wales using random amplification for polymorphism detection (RAPD). The objectives of this study were to (1) describe the overall genetic variation among and within populations and subpopulations of *C. floridana*, (2) compare the variation of the seed stock collection to that of the natural populations, and (3) to apply this information to reintroduction programs for this species.

The average genetic diversity was relatively high for an endemic species with geographically isolated populations. The Bok Tower Garden population exhibited a level of variation and polymorphism comparable to those of wild populations. The sampled populations do not appear to be highly structured. The majority of the variation was attributed to within population differences and only 20 percent of the variance could be attributed to genetic variation between the populations. This distribution of variance indicates that this species is rather homogeneous and suggests considerable gene flow occurs between populations.

My results suggest that conservation plans for *C. floridana* should focus on the total number of individuals rather than the number of populations. As there appears to be little genetic differentiation between populations and polymorphism levels are comparable in most populations, seed from different populations could be used in recovery programs

without the fear of loss of genetic information. Preservation and proper management of the natural habitat is also important to insure suitable sites for existing and introduced populations.

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INTRODUCTION

An important part of developing sound conservation programs and appropriate management strategies for sensitive plant taxa is the conservation of genetic diversity. Information about the genetic variation and structure within small populations of rare and endangered plants is instrumental in developing efficient sampling strategies of genetic resources and is crucial to effectively sustain them *in situ* and *ex situ*. This information can be used to select the group of plants that exhibit the maximum genetic variability to be used in recovery programs and to identify the relative importance of evolutionary forces affecting them, such as gene flow, genetic drift and breeding patterns.

Sensitive plant taxa (rare, endangered or threatened) may be described as such in that they often exist in small, geographically restricted populations. The genetic structure within populations may be the result of limited dispersal abilities, local adaptation and the small or isolated neighborhood size. These populations may be small in size as a result of the loss of suitable habitats or fragmentation of those habitats, restricting plants to isolated populations. Dispersal of seed or pollen to more appropriate sites may be difficult or impossible due to habitat fragmentation, and these small populations often experience reduced gene flow between neighboring populations (Ellstrand and Elam, 1993). Life history characteristics may influence the genetic diversity of plant species (Hamrick and Godt, 1990). Geographically restricted or isolated species are expected to have lower

genetic diversity than more widespread species. Endemic dicot species that are animal pollinated and gravity dispersed are thought to be less variable than their counterparts (Hamrick and Godt, 1990). Loss of genetic diversity can threaten the ability of these plant populations to adapt to biotic and abiotic pressures such as changes in environmental conditions or the effects of herbivores and parasites.

Small populations of geographically restricted or endemic taxa, may experience a reduction in genetic diversity due to evolutionary forces such as genetic drift, inbreeding or founder effect. The effect of drift, the random change in allele frequencies due to chance sampling of gametes from generation to generation, is largely dependent on the size of the population. Random genetic drift can cause a reduction in the heterozygosity within populations, as well as an increase in the differentiation between populations. Especially evident in small populations, the decrease in variation will lead to fixation of alleles. And since closely related individuals are more likely to mate in small populations, genetic variation within the populations will tend to decrease. This is also evident in populations that are geographically structured, where pollen and seed dispersal is restricted or limited. Fluctuations in population size, such as bottlenecks or founder effect, may also contribute to the loss of genetic variation. However, gene flow, the movement of genes among populations, may introduce enough new alleles and variation to offset the effects of genetic drift regardless of population size (Hartl and Clark, 1997) and prevent population differentiation.

The objective of this study is to investigate the genetic structure on the molecular level of nine populations of a federally listed endangered plant, the Florida Goldenaster,

Chrysopsis floridana Small (Asteraceae), using Randomly Amplified Polymorphic DNA profiles (Williams, *et al.*, 1990). The questions to be addressed are: Is there significant genetic variation among the populations and subpopulations? Does the variation in the seed stock collection housed at Bok Tower Gardens differ significantly from the remaining wild populations in Hillsborough County? How can this information be used to augment reintroduction programs?

Chrysopsis floridana

Florida is home to 3,834 native or naturalized plant species, of which about 4% are endemic (Wunderlin, 1998). Florida is second only to California in the continental US in the level of endemic species (CPC, 1995). This is due in part to a wide range of habitat types and climates. Florida is both a climatic and geographic bridge between temperate North America and the subtropical Caribbean (Ewel, 1990). One of the most distinctive habitat types is the scrub ecosystem found in the inland and coastal peninsula and in the coastal panhandle on sand ridges and ancient (late Pleistocene) dunes of Florida. These communities are noted for their high rates of endemism (40-60%), with thirteen plant species federally listed as endangered or threatened and 22 listed by the State (Myers, 1990). The Lake Wales Ridge scrub community, in the central peninsula, is home to many rare scrub endemics (Myers, 1990) due to the former island nature of the ridge.

Chrysopsis floridana is a perennial herb endemic to the Tampa Bay area of west central Florida. This plant is unusual in that it occurs west of the endemic-rich Lake Wales Ridge sandhill and sand pine scrub region of inland peninsular Florida. It is confined to the sand pine scrub community, growing in open, sunny, and sandy areas or in

disturbed areas at the edges of scrub. These scrub sites are usually on nutrient poor, very well drained St. Lucie Fine Sand or Lakewood Fine Sand soils and occur on former coastal dune systems formed during the late Miocene through the early Pleistocene (Myers, 1990). These communities are rapidly disappearing due to urban development. The main threat to *C. floridana* appears to be habitat destruction and its inability to disperse into isolated suitable sites.

First described from specimens collected in Manatee County in 1901, *C. floridana* was also collected early this century in Pinellas and Hillsborough counties. Known past occurrences in Manatee (Bradenton Beach) and Pinellas (St. Petersburg Beach) counties are thought to be extirpated due to rapid development. This species now exists in a few populations in Hillsborough County, one population in Manatee County (Lake Manatee State Recreation Area) and four populations in Hardee County. *Chrysopsis floridana* has been introduced into several protected sites in both Hillsborough and Pinellas Counties (Lambert, 1993).

Chrysopsis floridana has a dense, wooly pubescent basal rosette with leaves 4-10 cm long. The stem (0.3-0.7m) is woody toward the base and non-woody above and grows from the basal rosette in late summer. The stem leaves are densely pubescent, slightly clasping and obovate-elliptic and entire. The golden yellow flower heads are grouped into a somewhat flat-topped cluster (~2.5 cm diameter) at the top of the stem. Flowering occurs in November to December. Ray flowers are pistillate and the disk flowers are hermaphroditic and fertile (n=5). The entire genus has an out-crossing breeding system. Reproduction is primarily by seed, but the plant can spread by

vegetative reproduction of the rhizome. Plants are pollinated by insects and seed is thought to be disseminated by gravity or wind.

Chrysopsis floridana was once thought synonymous with the more widespread relative, *C. scabrella*, but has since been recognized as a full species (Semple, 1981, Lakela *et al.*, 1976, and Wunderlin, 1979, 1982, 1998). *Chrysopsis floridana* is distinguished from more common *C. scabrella* by the dense pubescence of the leaves and stems and by smaller clusters of flower heads. *Chrysopsis scabrella* is common on vacant lots, road edges and around buildings in urban areas and appears to be a more effective colonizer. *Chrysopsis floridana* may not be able to disperse across areas of unfavorable habitat, seeming to require relatively open areas for germination and seedling establishment (Lambert and Menges, 1996).

Chrysopsis floridana is presently listed in the Federal National Areas Inventory (FNAI) as critically imperiled globally because of extreme rarity or because of extreme vulnerability to extinction due to some natural or human factor. The US Fish and Wildlife Service (FWS) listed it endangered under provisions of the Endangered Species Act, that is, a species which is in danger of extinction throughout all or a significant portion of its range. The Center for Plant Conservation (CPC) lists this plant as a taxa that could possibly go extinct in the wild within the next ten years without conservation measures. Hillsborough County has purchased sites where *C. floridana* populations are protected under the Environmental Lands Acquisition and Protection Program (ELAPP). However, most of the natural populations are on private property and are subject to disturbance by development, bulldozing, and land clearing (FWS 1988, CPC 1995).

The Center for Plant Conservation (CPC) at the Missouri Botanical Garden in St. Louis, with its participating institutes around the country, has compiled a database of information and conservation strategies for the rare and endangered plant species of the US. The goal of the CPC is to collect and maintain species from the wild in living collections, preserve germplasm and tissue cultures, and to protect the habitats of the wild populations. In Florida, Bok Tower Gardens (BTG) in Lake Wales and Fairchild Tropical Gardens in Coral Gables, are maintaining living collections of 80 of the 179 listed plant species. BTG has 35 of these 80 CPC approved rare taxa in its *ex situ* collection, 34 of which are endemic to Florida. The seed stock collections of *C. floridana* at BTG were made from the Shadow Run residential subdivision, located in southeastern Hillsborough County, prior to its development. Plants propagated from seed and cuttings collected at the Shadow Run subdivision are currently in the living collection at BTG and germplasm is being stored in the USDA seed storage facility in Fort Collins, CO.

Methodology

The purpose of this study was to determine the genetic variation of this species from natural populations in Hillsborough County and compare it to the variation found in the garden population at BTG. I used random amplification for polymorphism detection (RAPD) to assess the genetic variation of *C. floridana*. RAPD is a relatively cost-effective PCR- based method of screening the entire genome without requiring *a priori* knowledge of a species DNA sequences (Williams *et al.* 1990; Huff *et al.* 1993; Hadrys *et al.* 1992). These presumably neutral markers are random with respect to the genome and are useful in describing polymorphisms and genome profiles at the species level.

The RAPD technique requires only small amounts of tissue from which to extract DNA, which is especially important when the species is rare and endangered and material is limited. RAPD uses short random oligonucleotides to amplify polymorphic markers. The bands produced by the random sequence primers are detected in a dominant fashion, as either present or absent. Presence for a band assumes individuals share the same sequence at the priming site, separated by the same number of base pairs. Absence of a band could indicate a change in the priming site or insertions or deletions between the priming sites. Because of the dominant nature of the bands, there is no distinction between homozygotes and heterozygotes for the presence of a band. It is thus not possible to determine allelic information nor Hardy-Weinberg equilibrium. Another assumption is that comigrating fragments are homologous. However, Rieseberg *et al.* (1995) determined that ~13% of the bands are paralogous rather than orthologous. Homologous fragments may be different in size (Rieseberg, 1996) and the number of bands may be overestimated and scored more than once, leading to inaccurate estimates of relationships. Some polymorphic bands may represent better matches to the RAPD primer than others and this competition for primers may result in reduced amplifications of the poorly matched fragments. This could lead to the misinterpretation of homologous bands and the underestimation of relatedness. The problem of reproducibility depends on the primer:template ratio, temperatures, and magnesium concentrations. Therefore, care must be taken to standardize the amplification reactions and the interpretation of the banding patterns produced. Gel resolution is critical for the correct scoring of RAPD fragments (Welsh and McClelland, 1990).

Despite the drawbacks, RAPD has advantages over other techniques (Hadrys *et al.*, 1992). A larger number of polymorphic loci may be detected, randomly sampling the entire genome for presumably neutral markers, relatively quickly and inexpensively, from a small amount of DNA. RAPD techniques have been used in the analysis of: taxonomic relationships (Lifante and Aguinagalde, 1996, Maguire and Sedgley, 1997, Chalmers *et al.*, 1992), mating systems (Fischer and Matthies, 1998, Smith and Pham, 1996, Kirsten *et al.*, 1998), hybrid origins (Rieseberg, 1996, Multani and Lyon, 1995) and population structure (Haig *et al.*, 1996). RAPD has been used to determine the level of genetic variation in sensitive plant taxa (Ayres and Ryan, 1997, Black-Samuelsson *et al.*, 1997, Brunnell and Whitkus, 1997, Fischer and Matthies, 1998, Halward *et al.*, 1997, James and Ashburner, 1997, Maguire and Sedgley, 1997, Martín *et al.*, 1997, Palacios and González-Candelas, 1997, Rossetto *et al.*, 1995, Smith and Pham, 1996, Stewart and Porter, 1995). These RAPD analyses have demonstrated genetic diversity within and among natural plant populations and have been useful in devising conservation strategies for rare, threatened or endangered plant species.

MATERIALS AND METHODS

Population Sampling

Leaf samples of *Chrysopsis floridana* were collected from nine sites (Figure 1 and Table 1): Bok Tower Garden (BTG) in Lake Wales, Sterling Downs (STR), Rhodine Road (RHO), Balm-Boyette Road (BBR), Sun City Heritage Park (SHP), Saffold Road (SAF), State of Florida Department of Transportation Right-of-Way (DOT), Casperson Farm (CAS), and Florida Goldenaster Scrub (FGA) in southeast Hillsborough County. The Bok Tower Garden site consisted of two beds of plants propagated from seed and cuttings, collected in 1986 and 1989, from an area prior to become a housing development, Shadow Run. This development, located in central Hillsborough County, sits between two of the collection study sites, STR (to the north) and RHO (to the south), and a few populations of the plant can be found growing in homeowners' yards near the sites. The STR site consisted of four subpopulations located a few hundred meters from each other. One subpopulation (SDA) abutted a population located on private property in Shadow Run. The RHO population contained seven subpopulations (RRA-G) distributed rather evenly throughout the 600 acres. Located west of Shadow Run, the FGA site consisted of three subpopulations, one located in the open clearing between a borrow pit (BP) and wooded area. The other two subpopulations (OAK and ORC) were located south of BP, near each other, in open sandy areas. The DOT site, just south of FGA,

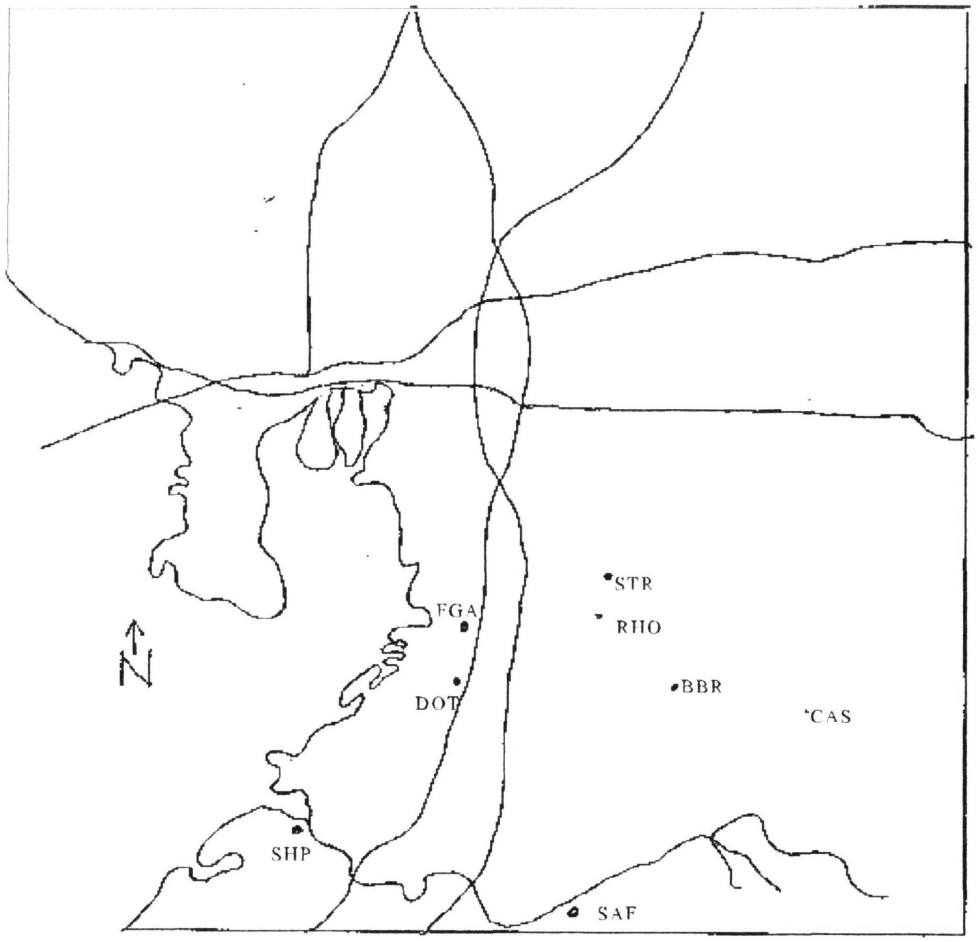

Figure 1. Map of *Chrysopsis floridana* collection sites. This map of Hillsborough County, in west central Florida, shows the location of the eight populations collected for this study.

Table 1. Locations and characteristics of sample collection sites.

Site (Population Designation)	Population/ Subpopulation Abbreviation	Date Collected	Geographic Coordinates	Number of Samples Collected	Estimated Area of Site (m^2)	Estimated Number of Individuals
Bok Tower Garden (BTG)	BTA	Feb-96	27.92 N	27	25	80
	BTB		81.57 W	29	25	80
DOT Right-of-Way (DOT)	DTA	Oct-96	27.79 N	10	75	300
	DTB		82.35 W	10	50	50
Sterling Downs (STR)	SDA	Jul-96	27.83 N	10	7700	100
	SDB		82.29 W	10	3700	100
	SDC			10	7000	100
	SDD			10	19500	100
Rhodine Road (RHO)	RRA	Jul-96	27.83N	10	1000	100
	RRB		82.33W	10		100
	RRC			10		100
	RRD			10		100
	RRE			10		1000
	RRF			10		1000
	RRG			10		1000
Goldenaster Scrub (FGA)	BP	Jun-96	27.80N	15	100	600
	OAK		82.36W	10	50	100
	ORC			10	75	100
Saffold Road	SAF	Jul-96	27.66N 82.30W	10	50	50
Balm-Boyette Road (BBR)	BBE	Jul-96	27.80N	10	50	75
	BBW		82.30W	10	50	50
Casperson Farm	CAS	Jul-96	27.70 N 82.15 W	10	50	50
Sun City Heritage Park	SHP	Jul-96	27.70N 82.46W	10	25	75

consisted of two subpopulations (DTA and DTB). DTA runs along the southbound entrance ramp to I-75 and is located near a large population on private property to the west . DTB is in the middle of the SW quadrant of the interchange. SAF was the southernmost site, located approximately one mile north of the Manatee County line. This site is east of Little Manatee River State Recreation Area, which contains a population not included in this study. SHP, the westernmost site, with one population in the middle of a parking lot of a small county park located on the banks of the Manatee River. A larger population of plants exists near SHP on private property southeast of the park. BBR consisted of two subpopulations, BBE and BBW, located east and west, respectively, of Balm-Boyette Road. This site is southeast of the Shadow Run development in the central county. CAS was the eastern most site. All of the natural populations included in this study, with the exception of DOT and CAS, were located on county property, managed by the Hillsborough County Parks and Recreation Department. No other collections were made on private property. Voucher specimens were deposited in the USF Herbarium.

Approximately 30 samples were collected from each bed from BTG. This sampling allowed for extra material for DNA extraction and amplification tests. Within the sites, STR, RHO, BBR, DOT, CAS, and FGA, ten samples were collected from each subpopulation. Table 1 shows the location and characteristics of the collection sites. In addition, samples from a related, widespread species, *C. scabrella*, were collected from an area between the USF Botanical Garden and the Shriner's Childrens Hospital on the west side of the USF campus. These samples were used to test the initial DNA extraction procedure and subsequent amplification tests.

Two leaves were removed from a mature plant and placed in a sterile bag (Fisher-Brand) with 10 mg of 28-200 mesh grade 12 silica gel desiccant and color indicator 6-16 mesh grade 42 both from Fisher Scientific (Chase and Hills, 1991). The bags were labeled with the three-letter abbreviation indicating the collection site and sample number (e.g. BTA 19). The plants were labeled with the corresponding code with a plastic label, fastened around a lower stem of the plant with a coated wire for later location if necessary. The leaves were removed from the desiccant upon return to the lab, cleaned of debris and hairs by gently scraping with a scalpel blade. Each leaf was coarsely chopped, placed in an appropriately labeled 1.5 µl microcentrifuge tube, and the samples stored at -70 °C until tested.

DNA Extractions

A miniprep DNA extraction procedure was modified from the protocols of Virk *et al.* (1995). One leaf (~25 mg) was ground into a powder in a 1.5 µl microcentrifuge tube using a disposable pellet pestle (Kontes, Owens-Illinois) in a motorized homogenizer (Fisher Scientific, Inc.) with 700 µl of preheated (65 °C) CTAB extraction buffer (4% w/v CTAB (hexadecyltrimethylammonium bromide), 100 mM Tris, 20 mM EDTA, 1.4 M NaCl, and 1% w/v PVP-40, pH 8.0; to each aliquot 2% v/v β-mercaptoethanol and 100 µg/ml Proteinase-K) was added to the ground tissue. (All reagents were of molecular biology grade or better). The tube was vortexed for about 20 sec to ensure thorough mixing and placed in a 65°C water bath. After a 45 min to one hour incubation, one volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added and the tube was gently mixed by inverting for 5 min. The tube was centrifuged at maximum speed for 5

min and 450 µl of the supernatant was transferred to a clean tube. One volume of chloroform:isoamyl alcohol (24:1) was added to the supernatant, the tube was mixed for 5 min and centrifuged for 5 min. The supernatant was recovered to a new tube, to which 1/3 volume of 10 M ammonium acetate and 1/3 volume of ethanol was added to precipitate proteins and polysaccharides. The sample was incubated for 30 min at -20 °C. After the incubation, the tube was centrifuged at maximum speed for 5 min, the supernatant was recovered and 1 ml of ethanol was added to precipitate nucleic acids. The tube was incubated overnight at -20 C and the DNA pelleted by 5 min of centrifugation at maximum speed. The pellet was recovered, washed twice with 70% ethanol, dried in a vacuum and resuspended in 50 µl of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and 0.5 µl of DNase-free RNase A. The extractions were incubated at 37 °C for one hr. Additional purification of the DNA was accomplished by adding 1/3 volume of 10 M ammonium acetate and one volume of isopropanol to the extraction. This was incubated overnight and the pellet recovered and resuspended as before. The DNA was quantified by agarose gel electrophoresis and ethidium bromide staining under UV light. The extracted DNA was stored at 4 °C.

PCR Amplifications

All PCR reactions were performed using a Perkin-Elmer DNA Thermal Cycler 480 and Cycler-Mate heated lid (BioLogic Engineering, Inc.) Initial testing for amplifiable DNA was done with ITS 4 and ITS 5 primers (internal transcribed spacers of 18S ribosome; White *et al.*, 1990) on the local source of *C. scabrella* varying the magnesium concentration from 1.5 to 3.5 mM. A 50 µl volume reaction containing 5 µl of 10X

magnesium-free reaction buffer (50 mM potassium chloride, 10 mM Tris-HCl, 0.1% Triton X-100) supplied by Promega, 5 µg (0.25 µl) bovine serum albumin (BSA), 0.25 mM each dNTP, 50 pmol primer and 10-50 ng template DNA (1 µl), 1U Taq DNA polymerase (Promega), and a gradient of 1.5, 2.0, 2.5, 3.0, 3.5 mM magnesium (3.0 to 7.0 µl). The cycling protocol was 4 min at 93 °C, 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C, followed by 10 min at 72 °C. A blank without DNA template and 4.0 µl (2.0 mM) magnesium was run with the reaction. The amplification products (4 µl) were run on a 1.2 % agarose gel in 1X TBE (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA, pH 8.0) and visualized with ethidium bromide staining.

RAPD primers were tested for amplification optimization using gradients of both magnesium and primer:template ratios with *C. floridana* DNA extractions. PCR reactions were carried out in 25 µl volumes varying the magnesium (1.5, 2.0, or 2.5 mM) and primer (25, 37.5, or 50 pmol) concentrations and 1.25U of Taq DNA polymerase was used in all reactions. A blank without template was run as a control. The cycling protocol was the same as described previously. This experiment was repeated using a 28:1 and a 56:1 molar ratio of TaqStart Antibody (Clontech) to Taq DNA polymerase to reduce background noise. Additional PCR amplifications were performed using 3.75U of Taq with no TaqStart Antibody. All products were run on a 3.0% agarose gel with ethidium bromide staining.

The final PCR reactions were carried out in 50 µl volumes containing 5 µl of 10X magnesium-free buffer, 2.0 mM magnesium, 5 µg BSA, 0.25 mM each dNTP, 3.75 U Taq DNA polymerase, 50 pmol primer and 10-50 ng template DNA (1 µl). A blank without

template was included as a control. The cycling protocol and visualization of the amplification products was conducted as described above. The gels were photographed with Polaroid black and white 667 film for later band scoring.

Scoring and Statistical Analysis

For the RAPDs study, 42 primers from Operon, Inc. and three two-base decamers were screened to determine those primers that produced products. The sizes of the bands were determined using HaeIII-digested ΦX DNA molecular weight marker. Bands were scored as present or absent (1 or 0). Products from prior amplifications were run on gels with later reactions in order to score same-sized bands from amplification to amplification.

A presence/absence table of 31 bands was used to create a distance matrix in the AMOVAPREP software package by Miller (1998). This program was designed to automate the process of preparing dominant marker data input files for use with the WINAMOVA software package by Excoffier *et al.* (1992). The resulting distance (squared Euclidian distance) matrix was analyzed with the WINAMOVA package in order to determine the distribution of molecular variance among groups of populations, within populations and among individuals within populations. This program also computed Bartlett's statistic (heteroscedasticity index) at the population and group levels, as well as population pairwise Φst statistics.

Another software program, POPGENE Ver. 1.21 (Yeh *et al.* 1997) was used for analysis of dominant marker data. This program provided information of genetic distance (Nei's 1972 genetic identity and distance) and Shannon's (1949) information index as a measure of gene diversity. Clustering of the individuals from the subpopulations and

groups of populations was analyzed with principle component analysis (PCA) using SAS software.

Single Fragment Cloning

Single bands produced in two samples (BTA2 and DTA6) during the screening process with primer OPA13 were reamplified for cloning and sequencing. These two bands "picked" by stabbing the gel with a micropipette tip and resuspending the stab in 50 µl of sterile water. These two "new" DNA templates were reamplified with the OPA13 primer using the standard RAPD PCR reaction at 39, 41 and 43 °C for optimization. The final PCR amplification was run at 43 °C.

Restriction enzyme analysis of the PCR products was performed using enzymes that recognize four base pairs restriction sites, CfoI, RsaI, MspI, and HaeIII. A 20 µl digest reaction volume containing 0.25 µl of the enzyme, 2 µl 10X buffer (supplied by Boehringer-Mannheim, Inc.), and 5 µl of the DNA was incubated at 37 °C for two hr. The digest was run on a 3.0 % agarose gel in 1X TBE and visualized with ethidium bromide.

The single band products from both BTG2 and DTA6 were cloned using the TA Cloning™ System (Invitrogen Corp.). A 10 µl volume ligation reaction was performed using 1 µl of 10X ligation buffer, 2 µl pCR®21 vector (25 ng/µl), 1µl (~10 ng) PCR product, and 1 µl (4.0 Weiss units) T4 DNA ligase. All reagents were supplied by the manufacturer. The reactions were incubated overnight at 12 °C.

For each ligation reaction, one tube of One Shot™ Competent Cells (INVαF') was thawed on ice, to which 2 µl (0.5M) β-ME was added. The mixture was gently stirred

and 2 µl of the ligation reaction was added to the tube. After a 30 min incubation on ice, the tubes were heat shocked (42 °C) for 30 sec and placed immediately on ice for 2 min. 250 µl of SOC medium (2% Bacto tryptone, 0.5% Bacto yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose) was then added to the tube and placed in a 37 °C rotary shaking incubator at 225 rpm. After 1 hr, the reaction tube was placed on ice.

From each transformation, 50 µl and 200 µl were spread on two LB medium plates to which 50 ug/ml of kanamycin and 40 µl of X-β-galactose (40 mg/ml) had been added. The plates were incubated for 18 hr at 37 °C and then stored at 4 °C for 2-3 hr for color development. Successfully transformed colonies, those containing the PCR product insert, appeared white while those not transformed were blue. Ten white colonies were picked from each plate (50 and 200 µl) for both PCR products and subcultured.

PCR Cloning Product Sequencing

Successfully transformed bacteria containing the DTA6 and BTG2 OPA13 band inserts were picked for sequencing. Using a sterile toothpick, one colony from each was picked for PCR amplification using the primers, M13 Reverse (from the *lac* promoter) and T7 promoter (from the *lacZα* fragment), that span the inserted region of the vector. A 50 µl volume reaction was set up with 5 µl of 10X buffer, 0.25 mM each of dNTPs, 2.0 mM magnesium, 50 pmol each primers, 0.25 µl BSA, and 1.25 U Taq DNA polymerase. The PCR amplification cycling protocol was 4 min at 94 °C, 30 cycles of 1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C, followed by 7 min at 72 °C. Products were run on a 2% agarose gel with ethidium bromide staining. Inserts were determined to be those products

approximately 1200 base pairs in length (200 bp of vector and 1000 bp of DTA6 OPA13 product) and 1400 bp (200 bp vector and 1200 bp BTG2 OPA13).

Sequencing of the OPA13 inserts was performed on a Perkin Elmer ABI Prism 310 Genetic Analyzer. Two 20 µl volume sequencing reactions were done using 3 µl (30-60 ng) of the PCR product, 8 µl Terminator Ready Reaction Mix (supplied by Perkin Elmer) and 4 µl (0.8 pmol/µl) of either the M13 Reverse or T7 Promoter primer. The cycling protocol was 25 cycles of ramp to 96 °C, hold for 10 sec, ramp to 50 °C, hold for 5 sec, ramp to 60 °C, hold 4 min, ending with a 4 °C soak. The resulting product was purified to remove any unincorporated dye terminator dNTPs. In a 1.5 µl tube, 2 µl 3M sodium acetate, pH6, 50 µl 100% ethanol, and 20 µl of the sequencing reaction were vortexed briefly and incubated on ice for 10 min. The tube was centrifuged at high speed for 15-30 min. The supernatant was aspirated and the resulting pellet was rinsed with 70% ethanol, centrifuged for 5 min, and decanted. The pellet was dried in a speed vacuum and resuspended in 25 µl of Template Suppression Reagent (supplied by Perkin Elmer). The sample was then vortex and heated (95 °C) for 2 min to denature, vortexed again, and the entire volume was transferred to a genetic analyzer 0.5 ml tube for loading into the analyzer for sequencing.

Sequence Analysis and Primer Design

The resulting sequences from the M13 Reverse and T7 Promoter primer products of DTA6 OPA13 insert were entered into the Primer3 Output program (http://www.genome.wi.mit.edu/cgi-b...r/primer3.cgi v 0.2b). The left and right primers within the insert were identified. The primers were 20 bases in length, with a GC

percentage of 50 and 45 for the left and right primer, respectively. A 50 µl volume PCR amplification was set up as described previously using 50 pmol of each primer (named 192 and 193). A cycling reaction of 30 cycles was run with an annealing temperature of 55°C. Products were run on a 2% agarose gel with ethidium bromide staining. In total, six templates were tested for amplification of the band. All primer sequences other than those used for the RAPD experiments are listed in Appendix 2.

RESULTS

RAPD Band Distributions

In total, 45 RAPD primers were tested for amplification, resulting in the detection of 171 bands in 120 *C. floridana* samples. Primers that showed inconsistent banding patterns in independent, replicate amplifications or those whose bands were faint and difficult to score were not used, nor those that did not amplify consistently in all samples. In the final analysis, the three, two-base primers CA919, CT919, and GT923, producing 31 bands in 80 samples, were used (Table 2). A presence/absence (1/0) table (Appendix 1.) was used to create a distance matrix in the AMOVAPREP software program. The resulting matrix was analyzed with the WINAMOVA and POPGENE programs. Samples sizes for the populations used in this study ranged from 15 individuals in BTG to three individuals in CAS (Table 1).

Primer CA919 produced 10 bands ranging in size from 200 to 1400 base pairs (bp). CT919 produced 11 bands (400 to 1350 bp) and GT923 produced 10 bands (325 to 1000 bp). An example of a RAPD primer (CA919) amplification is shown in Figure 2. Two of the 31 bands, CT919-5 and CT919-7, were monomorphic in all 80 samples. CA919-4 band was present in all samples except for one, CAS3. Three bands were present in over 90 percent of the samples. One primer band, CA919-5, was present in only two samples in population STR. No other populations had private alleles (bands

Table 2. Random decamer primers, sequences and number of bands amplified.

Primer	Sequence 5' -> 3'	Total Bands
CA919	CCCACAACCC	11
CT919	CCCTCTTCCC	10
GT923	GGTGGTTGGG	10
OPA11	CAATCGCCGT	1
OPA13	CAGCACCCAC	3
OPA14	TCTGTGCTGG	2
OPA15	TTCCGAACCC	2
OPA16	AGCCAGCGAA	1
OPA17	GACCGCTTGT	1
OPA18	AGGTGACCGT	4
OPA19	CAAACGTCGG	5
OPB1	GTTTCGCTCC	4
OPB3	CATCCCCCTG	2
OPB4	GGACTGGAGT	3
OPB5	TGCGCCCTTC	2
OPB6	TGCTCTGCCC	2
OPB7	GGTGACGCAG	2
OPB8	GTCCACACGG	1
OPB9	TGGGGGACTC	2
OPB10	CTGCTGGGAC	4
OPB11	GTAGACCCGT	3
OPB12	CCTTGACGCA	2
OPB13	TTCCCCCGCT	1
OPB14	TCCGCTCTGG	1
OPB15	GGAGGGTGTT	3
OPB17	AGGGAACGAG	5
OPB18	CCACAGCAGT	1
OPB20	GGACCCTTAC	4
OPF1	ACGGATCCTG	8
OPF2	GAGGATCCCT	4
OPF3	CCTGATCACC	5
OPF4	GGTGATCAGG	7
OPF5	CCGAATTCCC	8
OPF6	GGGAATTCGG	7
OPF7	CCGATATCCC	5
OPF8	GGGATATCGG	1
OPF9	CCAAGCTTCC	2
OPF10	GGAAGCTTGG	7
OPF11	TTGGTACCCC	4
OPF12	ACGGTACCAG	4
OPF13	GGCTGCAGAA	6
OPF14	TGCTGCAGGT	1
OPF15	CCAGTACTCC	2
OPF16	GGAGTACTGG	6
OPF20	GGTCTAGAGG	2
TOTAL 45		171

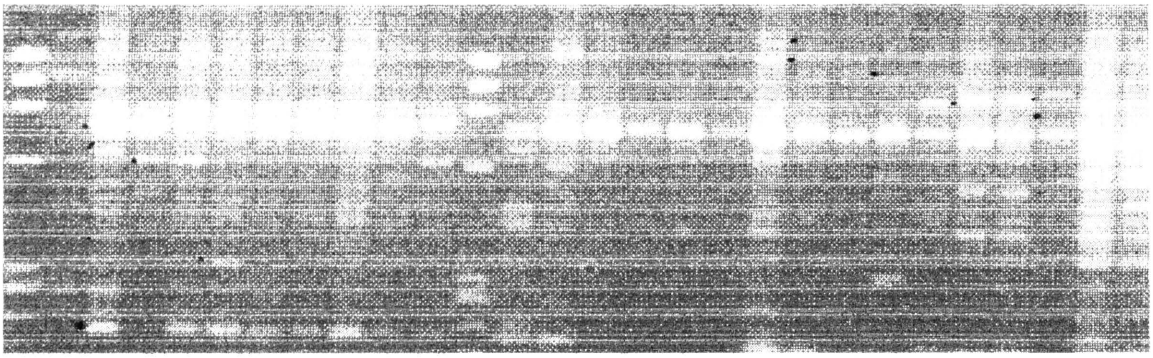

Figure 2. An example of a RAPD band pattern using CA919 primer. Lanes 1 and 12 are the molecular weight marker, ΦX174 DNA cut with HaeIII restriction enzyme. Lanes 3 - 11 and 13 - 19 are BTG samples. DTA samples are in lanes 20 - 23 and DTB samples are in lanes 24 - 28.

exclusive to their populations). In total, there were 79 haplotypes, RAPD phenotypes, with two BTG samples identical in banding patterns. These two individuals, BTB122 and BTB148 were collected from the same bed in the garden and originated from the same seed stock collection.

The largest samples, BTG, STR, and RHO showed the largest number of polymorphic loci (21, 27, and 21, respectively) (Table 3). The percent of polymorphic loci ranged from the highest 87.10 (STR) to lowest 41.94 (SAF). SAF (N=9) had the lowest number polymorphic loci (13 of 31). However, the smallest sample, CAS, (N=3) had 45.16 percent polymorphic loci, the same as a larger sample, DOT (N=8) and larger than SAF. The average band frequency (the number of bands per sample) was 43 percent of the total 31 bands analyzed. Primer CA919 had the highest overall band frequency in

BBR and CAS (0.55) and was lowest in SAF. RHO exhibited the highest overall band frequency for primer CT919 (0.58) and SAF had the lowest frequency (0.27). For the primer GT923, BBR had the highest frequency of bands (0.50) and SAF the lowest (0.34). The garden population of BTG was most similar in both number and percent polymorphic loci to RHO, and both were less polymorphic than the seed stock donor population, STR.

Genetic Diversity

Table 3 also shows the summary of the genetic variation statistics produced by the POPGENE 2.0 program. The mean gene diversity (Nei, 1987) was 0.2419 (sd. 0.1509) for all of the *C. floridana* samples. The mean gene diversity ranged from 0.1246 in SAF to 0.2687 in STR. The smallest sample, CAS, had a mean gene diversity of 0.1779. This was greater than DOT, FGA, SAF, and BBR, all larger sample sizes. BTG showed a gene diversity of 0.1920, lower than both STR and RHO (0.2267). Shannon's Information Index (a measure of gene diversity) ranged from 0.1924 in SAF to 0.4156 in STR, with an average of 0.3850 (sd 0.2021). BTG showed a consistent ranking lower than STR and RHO. The average number of observed alleles was 1.9355, and ranged from 1.4194 in SAF to 1.8710 in STR. The mean effective number of alleles was 1.3739, with a range of 1.2043 (SAF) to 1.4268 (STR). The effective number of alleles is the number of equally frequent alleles that would produce the same homozygosity as that of an actual population (Hartl and Clark, 1997) and can be used to compare the distribution of allele frequencies from different populations.

Gst, the proportion of total variation due to interpopulational differences, was estimated to be 0.2659 (Table 5). The same table shows the analysis of gene diversity in

subdivided populations based on Nei 1987 using the POPGENE program. Total genetic diversity averaged over all loci (Ht) was estimated to be 0.2445. Hs, the mean genetic diversity within populations, was 0.1795. Gene flow (Nm), the number of migrants per generation, was estimated from Gst where Nm =0.5(1-Gst)/Gst. This value was 1.3807, averaged over all loci.

Table 5 gives the pairwise comparison of genetic identity and genetic distance for the nine populations. Gene identity is defined by Nei (1987) as I = 1-h, where h=gene diversity, and is shown above the diagonal. This value is 1 when two populations have identical gene frequencies. Population BTG is most similar in gene frequency to DOT (0.9495) and least similar to CAS (0.8674). The southern most population (SAF) is most similar to STR and least similar to BBR, while BBR was most similar to STR. The westernmost, SHP, was most similar to BTG and least similar to CAS. CAS, the smallest sample size and easternmost population, was least similar to BBR and most similar to RHO.

Analysis of Molecular Variance

The WINAMOVA program was used to construct an analysis of molecular variance (AMOVA) from a pairwise Euclidian squared distance matrix. The AMOVA is a nested analysis in which the variation is partitioned within and between populations and groups of populations (termed subpopulations and populations in this study). Two comparisons were performed: 80 individuals in the nine study populations and 80 individuals in 21 subpopulations of the nine populations. Subpopulation RRE consisted of only one individual and was included with subpopulation RRF for these analyses. This

analysis was performed after completing the band scoring for each primer and again as new samples or primers were added to the data set. The variance partitioning did not change significantly with new data. In the nine population comparison, 80.22 percent of the variation could be contributed to variation within the populations and 19.78 percent to the variation between the nine populations (Table 6). This table also shows the variance partitioning of the 21 subpopulations.

Distance Matrix

Excoffier *et al.* (1992) defined Φ statistics to explain the significance of the variability in their AMOVA program. Φ statistics are similar to Wright's F-statistics and, defined by Excoffier *et al* (1992) to determine the significance of variability in their WINAMOVA program. Φst is the correlation of pairs haplotypes (RAPD phenotypes) drawn from random within populations relative to the species. Φct is the correlation of randomly drawn pairs of haplotypes within a group of populations relative to the species. Φsc is the correlation within populations relative to the species. Φst for the nine populations was 0.198 and for the 21 subpopulations 0.285 (Table 7). Φsc for the 21 subpopulations was 0.181. The Φct statistics for the nine and 21 comparisons were 0.198 and 0.127, respectively.

Distances as measured by Φst for the nine populations ranged from 0.0229 (STR - BBR) to 0.4319 (CAS - SAF) (Table 8). The probabilities of a random distance being greater than the observed distance were significant ($p<0.001$) in all but a few comparisons. Bartlett's test of heteroscedasticity was used to test the null hypothesis of homogeneity of variance (Table 9). All tests were significant at $p<0.001$, except those indicated. The

Table 3. Summary of genic variation statistics for all loci (Nei 1987). Total number of loci = 31. N=sample size. h=Nei's (1973) gene diversity averaged across all loci. I=Shannon's Information Index (Lewontin 1972) averaged across all loci. na=observed number of alleles and ne=effective number of alleles. Standard deviations are shown in parentheses.

Population	N	# Polymorphic loci	% Polymorphic loci	h	I	na	ne
BTG	15	21	67.74	0.1920	0.3014	1.6774	1.3035
				(0.1751)	(0.2519)	(0.4752)	(0.3185)
DOT	8	14	45.16	0.1382	0.2144	1.4516	1.2220
				(0.1763)	(0.2600)	(0.5059)	(0.3172)
STR	13	27	87.10	0.2687	0.4156	1.8710	1.4268
				(0.1554)	(0.2148)	(0.3408)	(0.2945)
RHO	12	21	67.74	0.2267	0.3383	1.6774	1.3973
				(0.2088)	(0.2906)	(0.4752)	(0.4032)
FGA	9	17	54.84	0.1496	0.2393	1.5484	1.2258
				(0.1612)	(0.2424)	(0.5059)	(0.2773)
SAF	9	13	41.94	0.1246	0.1924	1.4194	1.2043
				(0.1777)	(0.2591)	(0.5016)	(0.3187)
BBR	6	15	48.39	0.1538	0.2370	1.4839	1.2462
				(0.1807)	(0.2668)	(0.5080)	(0.3161)
CAS	3	14	45.16	0.1779	0.2614	1.4514	1.3119
				(0.2094)	(0.3011)	(0.5059)	(0.3927)
SHP	5	15	48.39	0.1839	0.2724	1.4839	1.3149
				(0.2048)	(0.2964)	(0.5080)	(0.3734)
TOTAL	80	29	93.55	0.2419	0.3850	1.9355	1.3739
				(0.1509)	(0.2021)	(0.2497)	(0.2890)

Table 4. Analysis of gene diversity in subdivided populations (Nei 1987). Ht=total genetic diversity averaged over all loci. Hs=mean genetic diversity within populations. Gst=the proportion of genetic diversity among populations relative to the total genetic diversity. Standard deviation is shown in parentheses. Nm=gene flow, where Nm=0.5(1-Gst)/Gst.

Ht	Hs	Gst	Nm
0.2445	0.1795	0.2659	1.3807
(0.0223)	(0.0108)		

Table 5. Nei's measures of genetic identity and genetic distance. Above diagonal=Nei's genetic identity. Below diagonal=genetic distance. Nei (1972).

	BTG	DOT	STR	RHO	FGA	SAF	BBR	CAS	SHP
BTG	---	0.9495	0.9436	0.9443	0.9340	0.9349	0.8957	0.8674	0.9328
DOT	0.0518	---	0.9502	0.9420	0.9631	0.9219	0.9101	0.8943	0.9143
STR	0.0581	0.0511	---	0.9491	0.9431	0.9524	0.9344	0.8886	0.9106
RHO	0.0574	0.0597	0.0522	---	0.9170	0.9239	0.8638	0.9135	0.8732
FGA	0.0683	0.0376	0.0586	0.0867	---	0.9272	0.8905	0.9056	0.9202
SAF	0.0673	0.0813	0.0488	0.0792	0.0756	---	0.8864	0.8874	0.8944
BBR	0.1102	0.0942	0.0679	0.1464	0.4460	0.1206	---	0.8265	0.8772
CAS	0.1422	0.1117	0.1181	0.0905	0.0992	0.1194	0.1906	---	0.8426
SHP	0.0696	0.0896	0.0936	0.1356	0.0831	0.1116	0.1310	0.1713	---

Φst distance matrix for the 21 subpopulation comparison is shown in Appendix 3. The distances ranged from 0.0000 to 0.7607. A few of the distances exhibited negative correlations. This indicates that the pair of haplotypes being compared are more related to

each other than those drawn from a group of subpopulations. This could be a function of small sample size (Excoffier *et al.*, 1992).

Table 6. Analysis of molecular variance (AMOVA). Nested analyses comparing the partitioning of variation for 80 individuals within 21 subpopulations in 9 populations, and among and within subpopulations and populations.

Source of Variation	df	SS	MS	Variance	% Total
Among Populations	8	95.7073	11.963	0.600	12.67
Among Subpopulations within Populations	12	70.1773	5.848	0.748	15.81
Within Subpopulations	59	199.916	3.388	3.388	71.52
Among Subpopulations	20	165.885	8.294	1.303	27.79
Within Subpopulations	59	199.916	3.388	3.388	72.21
Among Populations	8	95.7073	11.963	0.938	19.78
Within Populations	71	270.093	3.804	3.804	80.22

Table 7. PHI statistics (Excoffier *et al.* 1992). PHIst=the correlation of random pairs of haplotypes (RAPD phenotypes) within populations relative to the species. PHIct=the correlation within a group of populations relative to the species. PHIsc=the correlation within populations relative tot he group. $P<0.0020$, the probability of a more extreme random value.

Comparison	PHIst	PHIsc	PHIct
9 Populations	0.198	0.000	0.198
21 Subpopulations	0.285	0.181	0.127

Table 8. Distances between pairs of populations in PHIst (below diagonal). Above the diagonal is the probability of a random distance being greater than the observed distance[a].

	BTG	DOT	STR	RHO	FGA	SAF	BBR	CAS	SHP
BTG	---	***	***	***	***	***	***	***	***
DOT	0.1846	---	***	***	***	***	***	***	***
STR	0.1421	0.0918	---	***	***	***	ns	*	***
RHO	0.2049	0.1834	0.1135	---	***	***	***	*	***
FGA	0.1611	0.0860	0.0690	0.2333	---	***	***	*	ns
SAF	0.2978	0.3075	0.1611	0.3466	0.2466	---	***	***	***
BBR	0.2568	0.2852	0.0229	0.3311	0.2175	0.3009	---	ns	***
CAS	0.3012	0.2116	0.1487	0.1611	0.1492	0.4319	0.3604	---	***
SHP	0.1573	0.2356	0.0883	0.2919	0.0946	0.3001	0.1902	0.3122	---

[a]=significance levels - *** p <0.0001, ** p <0.001, * p <0.05, ns=not significant

Table 9. Pairwise Bartlett's statistics (below diagonal) to test the null hypothesis of homogeneity of variance. Above the diagonal is the probability that the observed statistics are not different from random[a]. Bartlett's statistics significance, <0.001996, the probability that random populations and group heteroscedasticity is greater than the observed heteroscedasticity.

	BTG	DOT	STR	RHO	FGA	SAF	BBR	CAS	SHP
BTG	---	***	***	***	*	***	***	***	*
DOT	2.9982	---	***	***	*	***	***	***	***
STR	3.4977	2.3797	---	***	*	***	ns	***	***
RHO	3.9477	2.8531	2.6188	---	***	***	***	*	***
FGA	2.8119	1.6301	1.9672	3.5586	---	***	***	ns	***
SAF	5.1300	3.9355	3.8183	5.6440	3.5299	---	***	***	***
BBR	3.3562	3.0393	1.2733	4.0184	2.4954	3.4875	---	***	***
CAS	2.4657	1.6556	1.6315	1.5487	1.4050	3.1621	2.2616	---	***
SHP	2.0871	2.4118	1.5914	3.1839	1.4394	3.2203	1.8318	1.8859	---

[a]=significance levels - *** p <0.001, ** p <0.01, * p <0.05, ns = not significant

Principal Component Analysis

Principal component analysis (PCA) was performed to determine if there was a natural clustering of the samples similar to that of their grouping by geographic location. This technique identifies a smaller set of variables (eigenvectors) that explain most of the variance among the original set of variables. The first two principal components are then used to plot the location, or pattern, of the samples tested. The first analysis plotted the 80 individuals in this study (data not shown). Three observations were hidden in the plot. Two of the samples in the BTB subpopulation sample, had the same RAPD phenotype and most probably were plotted on the same location. The other hidden observation was from subpopulation SAF. A second analysis was performed, with the nine populations, using the data as an average of band frequencies for all loci. The data were rerun using the input as an average of all individuals in each sample population or subpopulation. The resulting plots, Figs. 3 and 4, show no hidden observations and the values for the first two principal components increased from 24 percent to 48 percent for the nine population summary.

Single Fragment Cloning and Sequencing

One of the primers (OPA13) produced a few, strong bands in two samples (DTA6 and BTG2) during one of the screening runs (Fig. 5). One band (~1000 bp) found in DTA6 was not present in the BTG2 sample. In BTG2, a band (~1200 bp) gave a very strong signal but was very weak in DTA6. Two DNA samples (BTG2 and DTA6) were used with primer OPA13 to test primer:template ratio during initial RAPD amplification optimization. One band, approximately 1200 bp in length, appeared as a strong band in BTG2 and only faintly in DTA6. Another band, 1000 bp, was visible in DTA6, but was

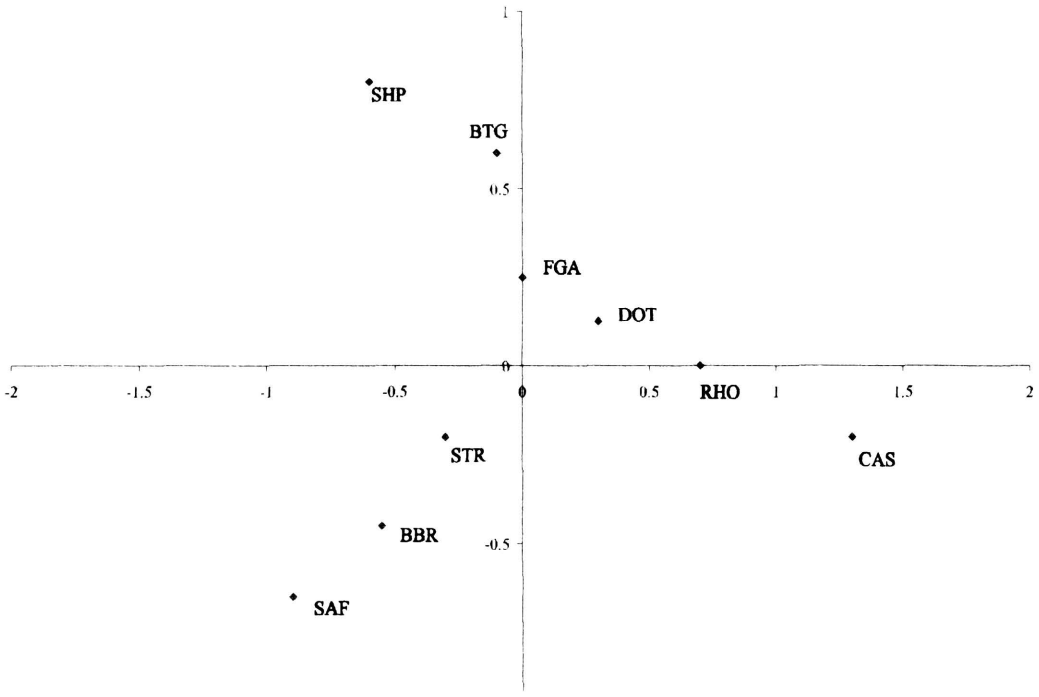

Figure 3. Plot of the first two principal components for nine populations of *Chrysopsis floridana*.

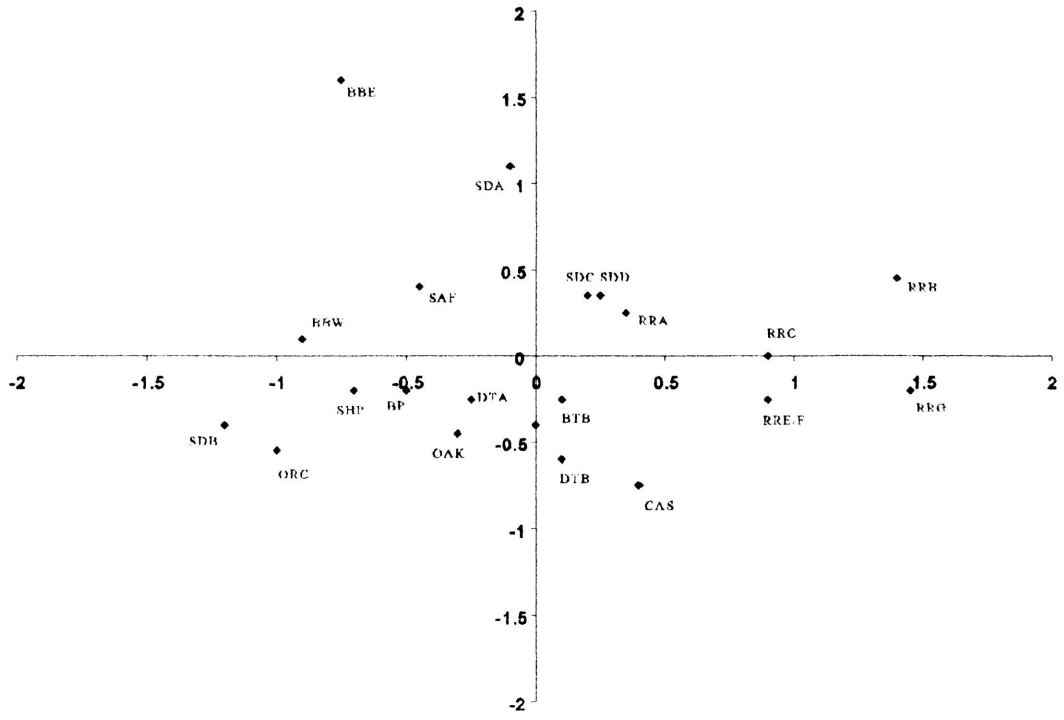

Figure 4. Plot of the first two principal components for the 21 subpopulations of *Chrysopsis floridana*.

absent in BTG2. The single bands produced in a subsequent amplification reaction, were picked from the gel and reamplified. Both templates produced very bright bands of the expected sizes, but also produced additional faint bands of smaller size. The reaction was rerun, with the temperature increased to 41 and 43 °C, to reduce background banding. At 43 °C, there did not appear to be bands other than the two expected (1200 bp in BTG2 and 1000 bp in DTA6) (Fig. 5).

In order to determine whether these bands were from homologous sites in the genome, the products were subjected to restriction analysis using the enzymes CfoI (GCGC), HaeIII (GGCC) and its "-iso" MspI (CCGG), and RsaI (GTAC). In DTA6,

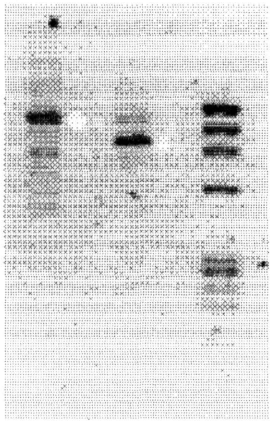

Figure 5. RAPD fragments produced by the OPA13 primer in *Chrysopsis floridana* samples DTA6 and BTA2. The ΦX174 HaeIII molecular weight marker is in lane 3, BTA2 is in lane 2, and DTA6 is in lane 3. The darker bands in lanes 1 and 2 are the RAPD fragments used in the cloning experiment.

CfoI did not cut the PCR product, HaeIII and MspI produced two fragments, and RsaI resulted in three fragments. All bands resulting from the restriction enzyme digests appeared to "add up" in size to the uncut band. In BTG2, only RsaI digested the product, with the bands being equivalent to the uncut product. However, extra faint bands were visible in all four digests, suggesting that additional PCR products may have been present.

The OPA13 RAPD amplifications from BTG2 and DTA6 were cloned using the TA-Cloning Kit and PCR reactions were performed using M13 reverse and T7 promoter primers to amplify the portion of the vector that contained the inserted region. Clones from both BTG2 and DTA6 were identified that contained the inserted RAPD product and sequenced using the ABI Prism 310 sequencer. The RAPD primer sequence (CAGCACCCAC) was located in all four sequencing reactions results (M13R and T7 for primers to amplify the portion of the vector that contained the inserted region. Clones from both BTG2 and DTA6 were identified that contained the inserted RAPD product

and sequenced using the ABI Prism 310 sequencer. The RAPD primer sequence (CAGCACCCAC) was located in all four sequencing reactions results (M13R and T7 for DTA6 and BTG2). Each sequencing reaction generated a sequence of approximately 350 readable bases. The sequences from DTA6 M13R and T7 were used to design a primer located interior to the RAPD priming site of each end of the OPA13 - DTA6 locus (Table 10). These primers, named 192 and 193, were then used in a PCR amplification reaction to amplify the corresponding "OPA13" locus. These primers produced a single product in approximately the expected size, but did not amplify consistently in the samples tested.

Table 10. Sequence data (5' -> 3') of the insert derived from primer OPA13 and template DTA6. Sequences underlined denote the primers designed by Primer3 Output program. Space in center is the unsequenced region of the insert.

Primer 192	5' = CGATGGGACAAAAGAGTCCT
Primer 193	3' = TGAATGCATGGAATGAGAGG

CGCGCAAATTTCTACATTAGCGGAATTTTGATTATTGAATCATCTATGTAAACACGATGGGAC
AAAAGAGTCCTTTTTAGACTTTCCACAGTCAAGCAGATAAATTTTCATTTCAGGAGAATGAAT
TAAAAATGGTTAGGTATATTCAAAACACTTGATTTATTATATATAGGTGCACATGGCCGGATC
GACTTTCTTTGTCTGGTACTCTAGTTATTTAACACAAAACTTATCTCAATCTACATAGGTATTA
TCTTCTTCTAACATAGGACCAA

TTGGGCTTTAAAATAAGTTCCTAATATTTAAATTTGAGATATGGGGTCATTTTAGTTGGAACT
TTAGTAGATAGCGGATATAATACCCTATCTATTTTAATGGGACGTGGCAGGCTGGGGGATTTA
CACCCCATCCATCTGATTGTTGATTCAATATATCATTTTAACTCTATTCTCAAATCCTCTCATT
CCATGCATTCACCCATTCCTTATCCAATTTCAATTCCATTTTCATCTTCAAGTGCTAGTAATCT
AATTTGGAGACATTCAAGTGTGTGTAGTGT

DISCUSSION

The objectives of this study were to (1) describe the overall genetic variation among and within populations and subpopulations of the Florida Goldenaster, *Chrysopsis floridana*, (2) compare the variation of the seed stock collection to that of the natural populations, and (3) to apply this information to reintroduction programs for the endangered species. The average genetic diversity, for the populations sampled of *Chrysopsis floridana*, was relatively high, 0.242. The *Chrysopsis floridana* populations in Hillsborough County seem to exhibit a rather high level of overall genetic diversity for an endemic species with geographically isolated populations. The Bok Tower Garden population is not located near wild populations of *C. floridana*, yet seems to have retained a rather high level of variation and polymorphism. This level is comparable to those seen in remaining wild populations in Hillsborough County. Thus, the initial sampling strategy for the establishment of the garden population at Bok Tower Gardens appears to have captured that of the natural populations.

In comparison, McDonald and Hamrick (1996) found that three Florida scrub species ranged in genetic diversity from 0.104 to 0.219, as measured by allozymes. Rosetto *et al.* (1995) found relatively high variability in the endangered Australian *Grevillea scapigera* (V = 0.32) using RAPD analysis. It is expected, however, that RAPD genetic diversity be greater than the diversity of allozymes due to the higher percentage of

polymorphic loci revealed by RAPD (Brunell and Whitkus, 1997). It is possible that RAPD reveals a larger proportion of polymorphic loci due to the dominant nature of identifying the marker loci (Smith and Pham, 1996). Yet, the loci are detected by sequence differences in priming sites and distances between those priming sites and there may be competition among fragments which may reduce amplifications of the fragments. These factors may contribute to an underestimation of bands and therefore variation among bands.

The percentage of polymorphic loci ranged from 0.419 to 0.871, and was higher than the average expected for an endemic species (Hamrick and Godt, 1990). There did not appear to be a specific pattern in the distribution of polymorphic loci when comparing the populations of *C. floridana* sampled, despite the unequal sample sizes. However, there were some differences that could be seen when comparing samples from centrally located populations and those found further away. The two populations (STR and RHO) nearest the original donor site, Shadow Run subdivision, did appear to exhibit the highest percentage of polymorphic loci. These sites were the largest, in both area and plant numbers, sampled for this study. The smaller or more remote sites tended to have a lower percentage of polymorphic loci. BTG, the garden population, showed a similar frequency to that of the STR and RHO sites. SAF, while not the smallest sample, did exhibit the lowest percentage of polymorphic loci produced by all three primers, whereas CAS, a much smaller sample size, had a larger percentage of polymorphic loci. This population was located nearer to the large STR - RHO complex, and the BBR site, and could receive

a higher input from pollinators visiting those sites. SAF, on the other hand, is more remote and may have experienced limited gene flow into the population.

The range of mean genetic diversity showed similar trends to that of the polymorphic loci data. The STR site showed the highest level of polymorphism. Its location near the large area of the RHO site, and the high percentage of polymorphic loci found in RHO, indicates ample gene flow for maintaining the variation. The more remote site, SAF, had the lowest average diversity. This reinforces the proposed idea of limited or lower gene flow in the SAF site. The intermediate value for polymorphic loci frequency in SHP, could be due to its relative proximity to a population on private land, near the park.

In a review of genetic diversity associated with life history traits, Hamrick *et al.* (1991) found that the average genetic diversity (Ht) among populations was 0.310, where the estimates were based on allozyme data. For endemic species, the average Ht was 0.263. They found that the proportion of diversity attributed to among populations, Gst, was 0.224 for various plant taxa examined and 0.248 for endemic species. The largest portion of variation is expected to be associated with geographic range as endemics are usually restricted in range and may be effected by lower gene flow. This RAPD analysis of the endemic *C. floridana* indicates similar levels of among population genetic variation, 0.269, to those shown in the allozyme review. However, these data are much higher than allozyme data reported for other Florida scrub species (Gst range 0.059-0.137, McDonald and Hamrick, 1996). Godt and Hamrick (1998) found Gst ranges of 0.086 and 0.141 in endangered pitcher plant (*Sarracenia* spp.) species (also allozymes). Other Gst data in the

literature range from 0.008 to 0.372 (Brunell and Whitkus, 1997, Smith and Pham, 1996) based on RAPD analyses of endangered taxa. *Chyrospsis floridana*, therefore, falls well within this range.

While the sampled populations of *C. floridana* exhibited relatively high genetic variation, they were not highly structured. The majority of the variation, from the AMOVA analysis, was attributed to within population differences, 0.8022. This indicates that the 20 percent variation between populations describes a rather homogeneous species, with low genetic differences between populations. Limited gene flow would result in high variation among populations. In comparison, the average for plant taxa based on allozyme data is 0.78 within populations and 0.22 between. The *C. floridana* results are very similar to this value. Other studies of endangered plant species show a wide range of variation distribution. James and Ashburner (1997) found 0.428 among population variation in an Australian species, *Astelia australiana*, while Rossetto et al. (1995) found only 0.13 of the variation attributable to between populations of the endangered *Grevillea scapigera*. While lower genetic diversity is expected in endemic taxa, the proportioning of the variance in endemics does not seem to differ from more widespread species (Hamrick *et al.*, 1990).

There did not appear to be any pattern in the clustering of the samples of populations or subpopulations when the data were analyzed with PCA. The variation between populations (0.20) was low enough to indicate little differentiation as the PCA indicated. I expected that the STR and RHO populations would plot near one another and that of the BTG population, as they are geographically near each other and may have

historically represented a continuous population. The BTG population did not plot near either of these two sites. However, the more outlying samples did show as outliers on the plot (SAF, CAS, and SHP). The DOT and FGA sites are geographically close to one another and also on the plot. In a comparison of the subpopulation samples, only the RHO subpopulation samples clustered near one another. The PCA was created using the first two principal components, presumably those that account for as much of the variation in the data as possible. Yet these eigenvectors only accounted for 48 and 38 percent of the total variation in the nine population and 21 subpopulation analyses, respectively. Therefore, the resulting plotted site relationships may not be as robust as desired for an analysis of the genetic versus geographic relationship of the sampled populations.

The single fragment cloning experiment was designed to produce a locus-specific polymorphic marker. This information would allow for analysis of the level of inbreeding through the identification of heterzygotes, which are not detectable with RAPD analysis. The resulting sequences did not match any sequences in GENBANK. The primers did not consistently amplify the fragments in other *C. floridana* samples, and therefore, the results of this experiment were inconclusive.

CONCLUSIONS

This study shows that the majority of the diversity in the populations of *Chrysopsis floridana* studies was found within the populations and only 20 percent of the variance could be contributed to genetic variation between the populations. This distribution of variance indicates that this species is rather homogeneous and gene flow is likely occurring between the populations. One migrant per generation is sufficient to balance a force, such as drift, that tends to increase divergence (Hartl and Clark, 1997). The introduction of genes via pollination from nearby populations or as a result of the existence of a substantial seed bank has maintained the genetic similarity of the populations. Therefore, conservation plans should focus on the total number of individuals rather than the number of populations. As there appears to be little genetic differentiation between populations, seed from different populations could be used in recovery programs without the fear of loss of genetic information.

Preservation and proper management of the natural habitat of *C. floridana* is also important. The wild populations of *C. floridana* vary in size from <50 to several hundred (Table 1). This species appears to be locally abundant, while quite limited in distribution. Lambert and Menges (1996) found that disturbance of the soil and high light levels are important for germination of *C. floridana* seeds. Presence of litter, which prohibits penetration of the small seeds, shading from nearby or encroaching hardwoods, and

suppression of fire from the fire maintained scrub system, all contribute to reduced germination and suitable habitat for this species. The smaller populations may be a result of the inability of seed to disperse for the reasons described, while the larger populations may exist in more suitable areas. It is possible that there is a considerable seed bank from which recruitment is available in the areas surrounding the existing populations, especially within the larger ones. *Chrysopsis floridana* populations should benefit from periodic disturbances in order to increase dispersal and gene flow within and between the populations.

The Bok Tower Garden collection shows a similar percentage of polymorphic loci and average gene diversity as the sampled sites, Sterling Downs and Rhodine Road. The variation in the Bok Tower Garden population appears to be representative of that of the species as a whole and may not have had enough time to undergo drift. This study shows that there is significant genetic variation within the species as measured by the populations sampled and that the seed stock collection represents this variation. Additional collection of seed from other natural populations may contribute to the capture of rare alleles, but does not appear to be necessary to increase the genetic variability of the seed stock population at present. However, additional seed stock should be collected in order to maintain gene flow in the captive population.

This study could be expanded to address the question of possible hybridization between the closely related *C. floridana* and the wide-spread *C. scabrella* species. There is a question as to whether recent collections in Hardee County are a hybrid between these two since they overlap in distribution (Wunderlin, personal communication). Similarly, a

phylogenetic study of the two restricted species found in the panhandle of Florida could be explored. Resulting information from studies such as these would benefit our understanding of the historical biogeographic and ecological forces that have influenced the present day distribution of members of the genus.

The use of genetics in the conservation of endangered and threatened species focuses on the preservation of genetic variation, or high levels of heterozygosity. Because of the inability of the RAPD technique to detect heterozygosity, it was not possible to determine the extent of Hardy-Weinberg equilibrium nor breeding structure. In an attempt to develop a locus-specific polymorphic marker from which to obtain allelic information, I sequenced two bands produced from a single primer in two samples and designed primers from one of them. The primers did not reliably amplify bands in several attempts with different DNA samples. However, further work with these or similarly developed primers, with microsatellite data, or with restriction fragment length polymorphisms (RFLPs) should be explored to elucidate heterzygote detection in *C. floridana*. Multiple analyses used to detect genome-wide levels of genetic variation may help to detect the factors that influence the species population structure: inbreeding, past population bottlenecks, or past evolutionary history of the populations.

LITERATURE CITED

Arias, D. M. and L. H. Rieseberg. 1995. Genetic relationships among domesticated and wild sunflowers (*Helianthus annuus*, Asteraceae). Econ. Bot. 49(3): 239-248.

Ayres, D. R. and F. J. Ryan. 1997. The clonal and population structure of a rare endemic plant, *Wyethia reticulata* (Asteraceae): allozyme and RAPD analysis. Mol. Ecol. 6: 761-772.

Black-Samuelsson, S., G. Eriksson, L. Gustafsson, and P. Gustafsson. 1997. RAPD and morphological analysis of the rare plant species *Vicia pisiformis* (Fabaceae). Biol. J. of the Linn. Soc. 61: 325-343.

Bonnin, I., T. Huguet, M. Gherardi, J. Prosperi, and I. Olivieri. 1996. High level of polymorphism and spatial structure in a selfing plant species, *Medicago truncatula* (Leguminosae), shown using RAPD markers. Am. J. Bot. 83(7): 843-855.

Brunell, M. S. and R. Whitkus. 1997. RAPD marker variation in *Eriastrum densifolium* (Polemoniaceae): Implications for subspecific delimitation and conservation. Syst. Bot. 22(3): 543-553.

Center for Plant Conservation. 1995. An Action Plan to Conserve the Native Plants of Florida. Southeast Environmental Research Program, Florida International University and Center for Plant Conservation. St. Louis, MO. 50 pp.

Chalmers, K. J., R. Waugh, J. I. Sprent, A. J. Simons, and W. Powell. 1992. Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. Heredity 69: 465-472.

Daniels, R. E., A. F. Raybould, and J. M. Farkas. 1997. Conserving genetic variation in British populations of *Lobelia urens*. Biol. Cons. 79: 15-22.

Ellstrand, N. C. and D. R. Elam. 1993. Population genetic consequences of small population size: Implications for plant conservation. Ann. Rev. Ecol. Syst. 24: 217-242.

Ewel, J. J. 1990. Introduction. In *Ecosystems of Florida*, eds. R. L. Myers and J. J. Ewel. pp. 3-10. University of Central Florida Press, Orlando FL. 765 pp.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Fischer, M. and D. Matthies. 1998. RAPD variation is relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). Am. J. Bot. 85(6): 811-819.

Godt, M.J. W. and J. L. Hamrick. 1998. Allozyme diversity in the endangered pitcher plant *Sarracenia rubra* ssp. *alabamensis* (Sarraceniaceae) and its close relative *S. rubra* ssp. *rubra*. Am. J. Bot. 85(6): 802-810.

Hadrys, H., M. Balick, and B. Schierwater. 1992. Applications of randomly amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol. 1: 55-63.

Haig, S. M., R. Bowman, and T. D. Mullins. 1996. Population structure of red-cockaded woodpeckers in south Florida: RAPDs revisited. Mol. Ecol. 5: 725-734.

Halward, T., A. Hill, and R. Shaw. 1997. RAPD analysis of genetic diversity among and within populations of *Balduina atropurpurea* at Fort Stewart, Georgia. Phytologia 83(1): 22-33.

Hamrick, J. L., Y. B. Linhart, and J. B. Mitton. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Ann. Rev. Ecol. Syst. 10: 173-200.

Hamrick, J. L. 1982. Plant population genetics and evolution. Am. J. Bot. 69(10): 1685-1693.

Hamrick, J. L., and M.J. W. Godt. 1990. Allozyme diversity in plant species. In *Plant Population Genetics, Breeding and Genetic Resources*, ed. A. H. D. Brown, M. T. Clegg, A. L. Kahler, B. S. Weir, pp. 43-63. Sinauer. Sunderland, Mass. 449 pp.

Hamrick, J. L., M.J. W. Godt, D. A. Murawski, and M. D. Loveless. 1991. Correlations between species traits and allozyme diversity: Implications for conservation biology. In *Genetics and Conservation of Rare Plants*, eds. D. A. Falk and K. E. Holsinger. pp. 75-86. New York, Oxford. 283 pp.

Hartl, D. L. and A. G. Clark. 1997. *Principles of Population Genetics*, 3rd. ed. Sinauer Associates, Inc. Sunderland, MA. 542 pp.

Huff, D. R., R. Peakall, and P. E. Smouse. 1993. RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.]. Theor. Appl. Genet. 86:927-934.

James, E. A. and G. R. Ashburner. 1997. Intraspecific variation in *Astelia australiana* (Liliaceae) and implications for the conservation of this Australian species. Biol. Cons. 82: 253-261.

Jewett-Smith, J., C. McMillan, W. J. Kenworthy, and K. Bird. 1997. Flowering and genetic banding patterns of *Halophila johnsonii* and conspecifics. Aq. Bot. 59: 323-331.

Kirsten, J. H., C. J. Dawes, and B. J. Cochrane. 1998. Randomly amplified polymorphism detection (RAPD) reveals high genetic diversity in *Thalassia testudinum* banks ex König (Turtlegrass). Aq. Bot. 61(4): 269-287.

Lakela, O., R. W. Long, G. Fleming, and P. Genelle. 1976. *Plants of the Tampa Bay Area*. Banyan Books, Miami, FL.

Lambert, B. B. and E. S. Menges. 1996. The effects of light, soil disturbance and presence of organic litter on the field germination and survival of the Florida Goldenaster, *Chrysopsis floridana* Small. Florida Scient. 59 (2): 121-137.

Lifante, Z. D., and I. Aguinagalde. 1996. The use of randomly amplified polymorphic DNA (RAPD) markers for the study of taxonomical relationships among species of *Asphodelus* sect. *Verinea* (Asphodelaceae). Am. J. Bot. 83(7): 949-953.

Loveless, M. D. and J. L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. 15: 65-95.

Luque, T., C. Ruiz, J. Avalos, I. L. Calderon, and M. E. Figueroa. 1995. Detection and analysis of genetic variation in Salicornieae (Chenopodiaceae) using random amplified polymorphic DNA (RAPD) markers. Taxon 44:53-63.

Lynch, M. and B. G. Milligan. 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3: 91-99.

Maguire, T. L. and M. Sedgley. 1997. Genetic diversity in *Banksia* and *Dryandra* (Proteaceae) with emphasis on *Banksia cuneata*, a rare and endangered species. Heredity 79: 394-401.

Martín, C., M. E. González-Benito, and J. M. Iriondo. 1997. Genetic diversity within and among populations of a threatened species: *Erodium paularense* Fern. Gonz. & Izco. Mol. Ecol. 6: 813-820.

McDonald, D. B. and J. L. Hamrick. 1996. Genetic variation is some plants of Florida scrub. Am. J. Bot. 83(1): 21-27.

Miller, M. P. 1998. AMOVA-PREP software package. Department of Biological Sciences, Northern Arizona Univeristy. Flagstaff, AZ.

Milligan, B. G. 1992. Plant DNA isolation. In *Molecular Genetic Analysis of Populations A Practical Approach*, ed. A. R. Hoelzel. pp. 59-88. IRL Press at Oxford University Press, Oxford. 315 pp.

Multani, D. S. and B. R. Lyon. 1995. Genetic fingerprinting of Australian cotton cultivars with RAPD markers. Genome 38:1005-1008.

Myers, R. L. 1990. Scrub and high pine. In *Ecosystems of Florida*, eds. R. L. Myers and J. J. Ewel. pp. 150-193. University of Central Florida Press, Orlando, FL. 765 pp.

Nei, M. 1987. Genetic variation within species. In *Molecular Evolutionary Genetics*. pp. 176-207. Columbia University Press, New York. 512 pp.

Palacios, C. and F. González-Candelas. 1997. Lack of genetic variability in the rare and endangered *Limonium cavanillesii* (Plumbaginaceae) using RAPD markers. Mol. Ecol. 6: 671-675.

Palacios, c. and F. González-Candelas. 1997. Analysis of population genetic structure and variability using RAPD markers in the endemic and endangered *Limonium dufourii* (Plumbaginaceae). Mol. Ecol. 6: 1107-1121.

Rieseberg, L. H. 1996. Homology among RAPD fragments in interspecific comparisons. Mol. Ecol. 5:99-105.

Rossetto, M., P. K. Weaver, and K. W. Dixon. 1995. Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera* (Proteaceae). Mol. Ecol. 4: 321-329.

Semple, J. C. 1981. A revision of the goldenaster genus *Chrysopsis* (Nutt.) Ell. nom. cons. (Compositae - Astereae). Rhodora 83: 323-384.

Smith, J. F. and T. V. Pham. 1996. Genetic diversity of the narrow endemic *Allium aaseae* (Alliaceae). Am. J. Bot. 83(6): 717-726.

Stewart, C. N., Jr., and D. M. Porter. 1995. RAPD profiling in biological conservation: An application to estimating clonal variation in rare and endangered *Iliamna* in Virginia. Biol. Cons. 74: 135-142.

Sydes, M. A. and R. Peakall. 1998. Extensive clonality in the endangered shrub *Haloragodendron lucasii* (Haloragaceae) revealed by allozymes and RAPDs. Mol. Ecol. 7: 87-93.

United States Fish and Wildlife Service. 1988. Recovery Plan for Florida Golden Aster. U.S. Fish and Wildlife Service, Atlanta, GA.

Van de Ven, W. T. G. and R. J. McNicol. 1995. The use of RAPD markers for the identification of Sitka spruce (*Picea sitchensis*) clones. Heredity 75:126-132.

Virk, P. S., B. V. Ford-Lloyd, M. T. Jackson and H. J. Newbury. 1995. Use of RAPD for the study of diversity within plant germplasm collections. Heredity 74: 170-179.

Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucl. Acids Res. 18: 7213-7218.

White, T. J., T. Burns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*, eds. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White. pp. 315-322. Academic Press. San Diego, CA. 482 pp.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18(22): 6531-6535.

Wunderlin, R. P. 1979. In *Rare and Endangered Biota of Florida. Vol. 5, Plants.* Ward, D. B. (Ed.). University Presses of Florida, Gainesville, FL.

Wunderlin, R. P., D. R. Richardson, and B. Hansen. 1981. *Chrysopsis floridana.* Status report prepared for U.S. Fish and Wildlife Service. Endangered Species Field Station Files, Jacksonville, FL.

Wunderlin, R. P. 1982. *Guide to The Vascular Plants of Central Florida.* University Presses of Florida. Gainesville, FL. 472 pp.

Wunderlin, R. P., 1998. *Guide To The Vascular Plants of Florida.* University Press of Florida. Gainesville, FL. 806 pp.

Yeh, F. C., R. Yang, and T. Boyle. 1997. POPGENE Version 1.21 Population Genetic Analysis software package. http://www.ualberta.ca/~fyeh.

APPENDICES

Appendix 1. Presence/Absence for 31 RAPD Bands Analyzed

Individual	CT919	CA919	GT923
BTA19	10101011010	1101001101	1101000101
BTA33	10101010010	1101000000	1101001101
BTA40	10101010010	1101010101	0101001101
BTA53	10101010000	0001000000	0111000100
BTA59	11111110011	0101001000	0001000001
BTA65	11111110011	1101001101	0011010101
BTA71	10101010000	1101000111	0011011101
BTB106	10101010000	0101000000	0000000101
BTB110	10101011000	0001000000	0010000101
BTB122	10101011000	1101000101	0101000101
BTB134	10101010000	1101010101	0111010101
BTB136	10101011000	0101000101	0001000101
BTB148	10101011000	1101000101	0101000101
BTB150	10101010000	0101000101	0001000101
BTB166	11101110000	0101001001	0001000101
DTA2	10101010100	0101000001	0101001100
DTA3	11101010110	0001001010	0101001100
DTA8	10101010100	0011000001	0101001100
DTB1	10101010000	0011010101	0101001101
DTB2	11101010000	0011000101	0101001101
DTB3	10101010000	0001000010	0001000100
DTB6	10101010000	0001011101	0001001100
DTB7	10101010000	0101010101	0011000100
SDA6	00001010100	0001011000	0110100000
SDA7	10101010000	1101001000	0110100100
SDA8	11101010101	1011011000	0101001100
SDA10	11001011001	1101000000	1111100110
SDB1	10101110010	1001010000	1111001100
SDB2	11101011000	0001100000	1001011101
SDB3	10101010110	0001100000	1101011101
SDC1	11101010000	0011000000	0110110101
SDC2	10111010110	0101011101	0101010100
SDC3	11101010110	1001010101	0010010101
SDD1	10101010100	0101011101	0101000100
SDD2	11101110000	1011000001	0101000100
SDD3	11101010111	0001001000	0101010100
RRA1	10101010011	1111001000	0101100100
RRA2	11101010000	1101010111	0101000101
RRA3	10101010011	1101011111	0001000100
RRB3	11101010100	1101010011	0101000100

(Continued on next page)

Appendix 1. (Continued)

RRB4	11101010100	1101010011	0110000100
RRC1	10101010010	1101011001	0101000100
RRC2	11101010011	0001011111	0010000110
RRE2	11101010000	0001011101	0110001100
RRF1	11101010000	0001000111	0011000000
RRF2	11101010010	1101010101	0110010110
RRG4	11101010100	0001000111	0111000100
RRG5	11101010100	1101010111	0000000110
BP1	11101010000	0011000000	0111001100
BP2	10101110001	0001000000	0111001100
BP5	10101010010	1101000001	0010001100
ORC1	11101011010	1101010101	0010001101
ORC2	10101010000	0001000000	0111011101
ORC4	11101010000	1001011101	0001001101
OAK1	11101110011	0001000000	0001001100
OAK2	10101010010	0001000000	0001001100
OAK3	10101010010	0001001000	0001011100
SAF1	11101010010	0101000000	0110100100
SAF3	10101010000	0111000000	1111100100
SAF4	10101010000	0001000000	0001000000
SAF5	10101010000	0001000000	1010001100
SAF6	10111010000	0001000000	0010000100
SAF7	10101010000	0111000000	1111000100
SAF8	10111010000	0101000000	0010000110
SAF9	10101010000	0111000000	0111000000
SAF10	10101010000	0111000000	0110100000
BBE1	10111010001	0001001000	0111110100
BBE2	10111010110	1001001000	0111110100
BBE3	10111010000	1011001000	0111110100
BBW1	11101010000	0101001000	0111010101
BBW2	10101011101	0001010000	0101011101
BBW3	10101011100	0101000000	0101011101
CAS1	11101011000	0001001111	0011001100
CAS2	11101010000	0001000001	0010001100
CSP3	11101011010	0010011111	0011010100
SHP2	11101111010	0101000000	0011000101
SHP3	10101011110	0101000000	0111011101
SHP4	10101110110	1111010000	0111001100
SHP5	00101010000	1111011000	0001001101
SHP8	00101010000	0101011000	0011001101

Appendix 2. Primer Sequences used in PCR amplifications.

Primer	Sequence 5' -> 3'
ITS4	TCCTCCGCTTATTGATATGC
ITS5	GGAAGTAAAAGTCGTAACAAGG
192	CGATGGGACAAAAGAGTCCT
193	TGAATGCATGGAATGAGAGG
M13 Reverse	CAGGAAACAGCTATGAC
T7 Promoter	TAATACGACTCACTATAGGG

Appendix 3. Distances between pairs of subpopulations in PHIst. Below diagonal is PHIst distance, above is the probability of a random distance being greater than the observed.

	BTA	BTB	DTA	DTB	SDA	SDB	SDC	SDD	RRA	RRB	RRC	RRE/F	RRG	BP	ORC	OAK	SAF	BBE	BBW	CAS	SHP
BTA	---	0.1038	0.0419	0.0399	0.0000	0.0279	0.0838	0.0818	0.2874	0.1238	0.0878	0.0200	0.1317	0.1297	0.4212	0.0439	0.0000	0.0000	0.0858	0.0419	0.1357
BTB	0.0895	---	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0818	0.0000	0.0000	0.0000	0.0000	0.0399	0.0000
DTA	0.2368	0.4459	---	0.0958	0.0000	0.0000	0.0000	0.3493	0.1058	0.0000	0.0000	0.0000	0.0000	0.1178	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0699
DTB	0.1576	0.1951	0.2143	---	0.0000	0.0479	0.0000	0.1776	0.0459	0.0000	0.0978	0.0120	0.0479	0.1058	0.3473	0.0479	0.0000	0.0000	0.0000	0.0000	0.0000
SDA	0.2187	0.3897	0.2309	0.3128	---	0.0000	0.0499	0.2535	0.0499	0.0000	0.0000	0.0000	0.0000	0.0519	0.0499	0.0000	0.0000	0.0000	0.0519	0.0000	0.0000
SDB	0.1483	0.4246	0.2692	0.3150	0.2575	---	0.0000	0.1058	0.0000	0.0000	0.1497	0.0539	0.0000	0.0140	0.1896	0.0499	0.0000	0.0000	0.0000	0.0000	0.0958
SDC	0.0831	0.2698	0.2500	0.1904	0.1282	0.1765	---	0.4910	0.3054	0.0838	0.5010	0.2894	0.0559	0.1178	0.7385	0.0000	0.0000	0.0000	0.1238	0.3034	0.0479
SDD	0.0851	0.2863	-0.0169	0.1202	0.0425	0.2105	-0.0482	---	0.2715	0.1118	0.7944	0.0539	0.2495	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
RRA	0.0047	0.2847	0.3448	0.2226	0.1435	0.3739	0.1340	0.0132	---	0.1657	0.8882	0.2136	0.1138	0.0000	0.0000	0.0399	0.0000	0.0379	0.0000	0.0000	0.0000
RRB	0.3075	0.5093	0.4950	0.4670	0.2085	0.5263	0.2075	0.1982	0.2500	---	0.0000	0.1397	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.1936	0.0000
RRC	0.0613	0.3732	0.3359	0.1996	0.0809	0.3276	-0.0406	-0.0603	-0.2000	0.2500	---	0.8862	0.0000	0.0000	0.1697	0.0000	0.0000	0.0000	0.0000	0.3633	0.0000
RRE/F	0.1638	0.3468	0.3523	0.1483	0.1812	0.3455	-0.0500	0.0964	0.1910	0.2500	-0.1471	---	0.5010	0.1078	0.4511	0.0000	0.0000	0.0000	0.0000	0.4391	0.0000
RRG	0.2372	0.3890	0.3226	0.2408	0.2053	0.4202	0.0267	0.0560	0.1687	0.0000	0.0000	-0.1129	---	0.0559	0.1158	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
BP	0.1149	0.3601	0.1500	0.1760	0.1016	0.1538	0.1875	0.1266	0.2903	0.4672	0.2075	0.1538	0.3321	---	0.0519	0.2056	0.1118	0.0000	0.0000	0.1277	0.1856
ORC	-0.0312	0.1562	0.2857	0.0456	0.1910	0.1071	-0.0357	0.1429	0.1573	0.3579	0.0502	-0.0135	0.1828	0.0676	---	0.0000	0.0000	0.0000	0.1098	0.4291	0.3713
OAK	0.2109	0.5010	0.3571	0.3291	0.3587	0.2031	0.3438	0.2603	0.4270	0.7607	0.3802	0.4457	0.5967	0.1667	0.2895	---	0.0000	0.0000	0.0000	0.0000	0.0000
SAF	0.3023	0.3718	0.3904	0.3363	0.2157	0.3963	0.3328	0.3294	0.4338	0.5286	0.4198	0.3561	0.4609	0.1151	0.3936	0.4183	---	0.0000	0.0000	0.0000	0.0000
BBE	0.3500	0.6046	0.5955	0.5615	0.1971	0.4950	0.2588	0.3415	0.4796	0.7607	0.4932	0.5143	0.6486	0.4578	0.4953	0.6471	0.4504	---	0.0000	0.0000	0.0000
BBW	0.1620	0.3111	0.2836	0.3370	0.2124	0.1370	0.1176	0.1538	0.3700	0.5435	0.3945	0.3883	0.4466	0.2785	0.1429	0.4400	0.4170	0.5000	---	0.0000	0.0479
CAS	0.2474	0.4128	0.3704	0.1834	0.2944	0.3592	0.1429	0.2159	0.3265	0.4851	0.0830	-0.0476	0.1682	0.2000	0.0548	0.4000	0.4319	0.5673	0.4184	---	0.0000
SHP	0.0749	0.2720	0.2536	0.2565	0.1851	0.1549	0.1690	0.1997	0.2357	0.3885	0.2336	0.3103	0.3720	0.0844	0.0448	0.2577	0.3001	0.4419	0.0959	0.3122	---