

GENETIC DIVERSITY OF EVERGLADES SAWGRASS, *CLADIUM JAMAICENSE* (CYPERACEAE)

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Genetic diversity studies of wetland plants are scarce, but estimates of genetic diversity are useful for learning about plant biology or developing appropriate management strategies. We used allozymes to examine patterns of genetic diversity in *Cladium jamaicense*, the dominant plant species of the Florida Everglades. We sampled 18 populations (a total of 818 plants) in a replicated grid pattern. Because *C. jamaicense* can reproduce asexually, we compared estimates of genetic diversity calculated using all sampled ramets to those using only genets within populations. Fewer than half of the 13 loci studied were polymorphic ($P_s = 46.2\%$), with just under two alleles per locus on average ($A_s = 1.9$). Heterozygosity was also low and was lower for ramet-level estimates (ramets: $H_{es} = 0.069$, $H_{ep} = 0.051$; genets: $H_{es} = 0.094$, $H_{ep} = 0.067$). Ramet-level estimates indicated heterozygote excess in eight populations and heterozygote deficiencies in four populations, but genet-level estimates indicated inbreeding in only one population. Ramet-level estimates indicated significant population differentiation ($F_{ST} = 0.268$), but genet-level estimates did not ($F_{ST} = 0.035$). Similarly, mean genetic distance between populations was higher based on ramet-level estimates ($D = 0.024$) than genet-level estimates ($D = 0.016$), and we found a weak correlation between genetic and geographic distance based on ramet-level estimates ($r = 0.15$, $P = 0.02$) but not genet-level estimates ($r = 0.04$, $P = 0.2$). Thus, clonal reproduction resulted in effective genetic differentiation among populations. Observed patterns of population differentiation may reflect high levels of gene flow among populations or patterns established during colonization that persist through long-lived clones. Genetic diversity of Everglades *C. jamaicense* was low compared to other sedges or other species with similar life histories (ramets: $H_T = 0.150$; genets: $H_T = 0.203$). This low genetic diversity may reflect a relatively recent origin of *C. jamaicense* populations in south Florida.

Keywords: allozymes, *Cladium jamaicense*, clonal plant, Cyperaceae, Everglades, Florida, genetic diversity, sawgrass, wetlands.

Introduction

Estimates of genetic diversity offer insight into the biology of a species (Hamrick and Godt 1989; Huenneke 1991) and can provide guidance for management plans. For example, estimates of genetic diversity can guide conservation decisions when choosing among multiple populations for protection or when sampling for *ex situ* collections (Brown and Briggs 1991). Likewise, an understanding of genetic diversity estimates can increase the likelihood of successful restoration plans (Holsinger and Gottlieb 1991). Because wetlands are currently a focal point for conservation and restoration, estimates of genetic diversity in wetland species are of particular interest (Barrett et al. 1993).

Sawgrass (*Cladium jamaicense* Crantz) is a perennial sedge that occurs in wetlands from Virginia to the West Indies and west to Texas (Godfrey and Wooten 1981). It covers 70% of the Everglades (Loveless 1959), a vast, freshwater wetland in southern Florida that has been called “the river of grass” in tribute to sawgrass’s dominance (Douglas 1947). The Everglades ecosystem has been significantly transformed during the

last century, and it is the focus of a 30-yr, \$7.8 billion restoration effort. Knowledge of the reproductive biology of *C. jamaicense* is therefore fundamental to understanding Everglades ecology and to implementing effective ecosystem restoration plans (Walters and Gunderson 1994).

Most previous studies have assumed that *C. jamaicense* predominantly reproduces asexually (Alexander 1971; Steward and Ornes 1975), although seed set is high. Populations of sawgrass can produce an estimated 5000 seeds/m² annually (Alexander 1971). Seedling recruitment, however, is thought to be low (Alexander 1971; Miao et al. 1997). This may be the result of low rates of seed germination, which are <40% under optimal laboratory conditions (Lorenzen et al. 2000); in nature, germination is likely to be considerably lower. Sawgrass produces many spreading rhizomes (Alexander 1971), which have been estimated via excavation to reach an average size of 1.5 m² (Brewer 1996). A second mode of asexual reproduction, in which plantlet propagules are produced within inflorescences, was recently described (Miao et al. 1998). Dispersal distances and recruitment rates of these plantlets are unknown.

Asexual reproduction is common among wetland plants (Sculthorpe 1967). This can complicate interpretations of genetic diversity estimates because multiple plants derived from asexual reproduction have identical genotypes (Barrett et al.

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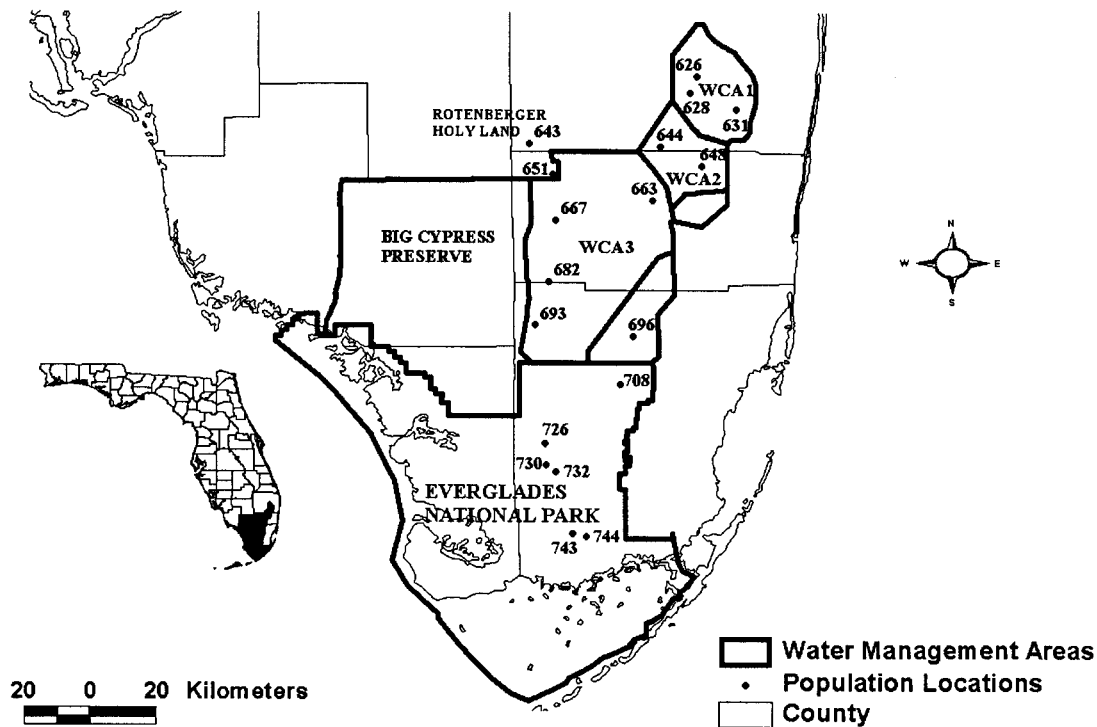


Fig. 1 Collection locations of *Cladium jamaicense* populations

1993). If duplicate multilocus genotypes are removed, rare alleles that distinguish individuals have disproportionately heavy weighting in the data set. Similarly, common alleles may be underrepresented by removing duplicate genotypes, since identical multilocus genotypes composed of common alleles may be expected to occur by chance following recombination. On the other hand, if duplicate multilocus genotypes are not removed, a single genetic individual may be represented several times in the data set, because multiple plants are likely to have arisen from asexual reproduction. An intact data set could be biased because samples are not independent. One strategy to address this problem is to separately calculate genetic diversity estimates with two versions of the data: one in which all sampled ramets are included and a second in which only unique multilocus genotypes are used. These estimates indicate the range of possible genetic diversity values for the species (McClintock and Waterway 1993; Jonsson et al. 1996). Moreover, the differences between the two estimates can indicate how clonal reproduction changes genetic diversity and population structure, particularly with respect to mating opportunities and gene flow (Handel 1985).

The consequences of sexual versus asexual reproduction for genetic diversity and structure of Everglades populations of *C. jamaicense* have not previously been studied. Our objectives for this study were (1) to estimate levels of genetic diversity within and among Everglades populations of *C. jamaicense* and (2) to evaluate the impact of clonal reproduction on genetic diversity estimates. Elsewhere (Ivey and Richards, in press), we describe genotypic diversity and the spatial structure of clonal reproduction in Everglades *C. jamaicense* populations.

Material and Methods

Plant samples were collected between October 1 and October 7, 1999, in conjunction with an Environmental Protection Agency survey of Everglades methyl-mercury contamination and other ecosystem parameters. Survey sites were randomly located throughout the Everglades, and we haphazardly chose a subset of 18 sites from which we collected samples of *Cladium jamaicense* (fig. 1). Sites were distributed among the water management areas included in the survey. Distance between collection sites ranged from 3.3 to 141.2 km.

We collected plants in a replicated grid; eight plant samples were collected in an identical pattern from each of six 1-m² quadrats positioned along alternating sides of an 11-m transect (fig. 2). We collected leaf material from the ramet most closely rooted at each sampling point; if no ramet occurred within a 10-cm radius of the sampling point, we did not collect from that position. Leaf material was labeled with a permanent marker and stored in plastic bags on ice until enzyme extractions could be performed.

Leaves were crushed to a fine powder in mortars using liquid nitrogen and sand. To stabilize the enzymes, a potassium phosphate extraction buffer (Mitton et al. 1979) was mixed with the powder. The extract was filtered through Miracloth (Calbiochem, La Jolla, Calif.) and adsorbed onto 8 × 3-mm wicks cut from Whatman 3M chromatography paper. Wicks were stored at -80°C until electrophoresis was performed.

We used horizontal starch-gel electrophoresis to estimate genetic diversity. After a preliminary screening of 33 enzyme sys-

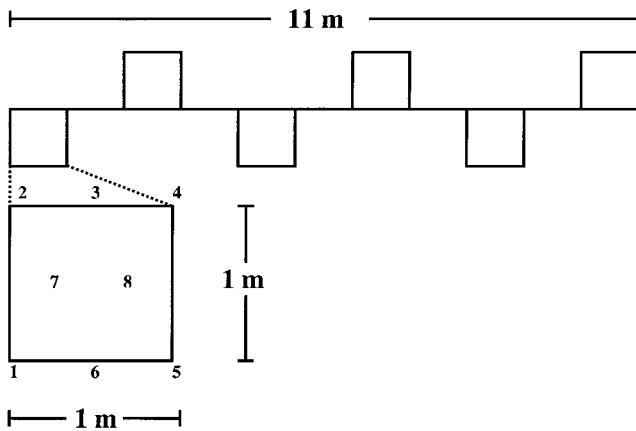


Fig. 2 Design used to sample ramets of *Cladium jamaicense* at 18 sites throughout the Everglades. Eight plants were collected in an identical pattern from each of six 1-m² quadrats along an 11-m transect.

tems on seven buffer systems, we chose 12 markers with consistent banding patterns upon which to focus our analyses. We used a continuous histidine-citrate system (pH 5.7; Wendel and Weeden 1989) to resolve phosphoglucomutase (PGM : EC : 5.4.2.2) and phosphoglucoisomerase (PGI : EC : 5.3.1.9). We used a continuous Tris-citrate buffer (pH 8.0; Wendel and Weeden 1989) to resolve isocitrate dehydrogenase (IDH : EC : 1.1.1.41), menadiene reductase (MNR : EC : 1.6.99.-), and peroxidase (PER : EC : 1.11.1.7). We used a discontinuous histidine-citrate buffer (pH 7.0; Wendel and Weeden 1989) to resolve malate dehydrogenase (MDH : EC : 1.1.1.37), malic enzyme (ME : EC : 1.1.1.40), phosphogluconate dehydrogenase (PGD : EC : 1.1.1.44), and a NAD-specific form of glyceraldehyde-3-phosphate dehydrogenase (G3-PDH : EC : 1.2.1.9). We used a discontinuous system with a lithium-borate electrode buffer and a Tris-citrate gel buffer (pH 8.5; Ivey and Wyatt 1999) to resolve triose-phosphate isomerase (TPI : EC : 5.3.1.1), shikimate dehydrogenase (SHK : EC : 1.1.1.25), and UTP-glucose-1-phosphate uridylyltransferase (UGPP : EC : 2.7.7.9). Stain recipes were modified from Wendel and Weeden (1989) except for UGPP (Manchenko 1994).

All statistics were first calculated using each sampled plant as an individual in the analysis (ramet-level analysis) and then recalculated after removing duplicate multilocus genotypes within each population (genet-level analysis). We estimated the effective number of multilocus isozyme genotypes per population as $N_g = 1/\sum p_i^2$, where p_i is the frequency of the i th genotype in the population. We used a Fortran program written by L. D. Loveless and A. Schnabel to calculate the percentage of polymorphic loci (P), the mean number of alleles per locus (A), the mean number of alleles per polymorphic locus (AP), the effective number of alleles (A_e), observed heterozygosity (H_o), and expected heterozygosity (H_e) within each population. Loci with more than one allele were considered to be polymorphic. We tested for allele frequency heterogeneity among populations within each polymorphic locus using a χ^2 test (Workman and Niswander 1970). We used FSTAT (Goudet 1995) to test for deviation from Hardy-Weinberg equilibrium within populations as well as for deviation from Hardy-Wein-

berg for each polymorphic locus within populations. These tests were based on 23,400 permutations of the data, in which alleles were randomized within populations. Loci were considered to be in Hardy-Weinberg equilibrium if the proportion of randomized data sets resulting in fixation indices (F_{IS}) more extreme than observed exceeded 5%.

We calculated total gene diversity (H_T), within-population gene diversity (H_S), and among-population genetic diversity (G_{ST}) for each polymorphic locus according to Nei (1977). These were averaged arithmetically to estimate species-level parameters (Hamrick and Godt 1989). We used FSTAT to calculate unbiased jackknifed estimates of Wright's (Wright 1965) F statistics for each polymorphic locus and over all loci according to Weir and Cockerham (1984). Positive estimates indicate inbreeding within individuals in populations (F_{IS}) or genetic differentiation among populations (F_{ST}). We tested for deviations from zero within loci using 1300 permutations of the data in which alleles within populations (F_{IS}) or genotypes among populations (F_{ST}) were randomized. Evidence of inbreeding was indicated if fewer than 5% of the randomized data sets resulted in estimates of F_{IS} more extreme than the jackknifed estimate. We used FSTAT to construct 95% bootstrap confidence intervals around the overall estimates to test their significance. Overall estimates were considered to be a significant departure from Hardy-Weinberg expectations if the confidence intervals did not include zero.

To estimate genetic divergence among populations, we calculated Nei's (1972) genetic distance for all possible pairs of populations. We created a phenogram representing Nei's (1972) genetic distance among populations using the unweighted pair

Table 1
Genetic Diversity in Populations of *Cladium jamaicense* Collected in the Everglades

Population	n_{ramet}	n_{genet}	N_g	P	AP	A	$A_{e\text{-ramet}}$	$A_{e\text{-genet}}$
744	45	2	1.30	7.69	2.00	1.08	1.02	1.08
743	45	2	1.36	15.38	2.00	1.15	1.04	1.12
732	48	6	1.37	30.77	2.25	1.38	1.11	1.16
730	39	9	2.94	30.77	2.25	1.38	1.18	1.23
726	47	2	1.19	7.69	2.00	1.08	1.08	1.05
708	48	1	1.00	15.38	2.00	1.15	1.15	...
696	48	7	2.13	23.08	2.33	1.31	1.16	1.19
693	44	3	1.27	15.38	2.00	1.15	1.08	1.06
682	47	5	4.07	15.38	2.50	1.23	1.10	1.10
667	42	6	3.14	30.77	2.00	1.31	1.11	1.12
663	46	12	4.90	38.46	2.40	1.54	1.12	1.21
651	44	5	3.00	15.38	2.00	1.15	1.06	1.11
648	46	6	2.93	15.38	2.50	1.23	1.07	1.12
644	47	2	1.77	7.69	2.00	1.08	1.06	1.08
643	45	8	2.45	23.08	2.00	1.23	1.07	1.17
631	47	4	3.37	15.38	2.00	1.15	1.06	1.09
628	46	2	1.94	7.69	2.00	1.08	1.04	1.05
626	44	6	2.64	15.38	2.50	1.23	1.05	1.13
Mean	45.4	4.9	2.38	18.38	2.15	1.22	1.09	1.12
SD	2.3	2.9	1.09	2.46	0.21	0.13	0.04	0.06
Species	818	36	...	46.15	2.83	1.85	1.11	1.15

Note. N_g = effective number of genotypes per population; P = percent of polymorphic loci; AP = mean number of alleles per polymorphic locus; A = mean number of alleles per locus; A_e = effective number of alleles per locus.

Table 2

Mean (SD) Observed (H_o) and Expected (H_e) Heterozygosity across Loci Within Populations and Within-Population Estimates of Inbreeding (F_{IS}) Based on Analyses of All Sampled Plants (Ramets) and after Duplicate Genotypes Within Populations Had Been Removed (Genets) for *Cladium jamaicense* Collected in the Everglades

Population	Ramets			Genets		
	H_o (SD)	H_e (SD)	F_{IS}	H_o (SD)	H_e (SD)	F_{IS}
744	0.000 (0.000)	0.018 (0.018)	1.000***	0.000 (0.000)	0.038 (0.038)	1.000
743	0.012 (0.004)	0.031 (0.022)	0.624***	0.038 (0.038)	0.067 (0.046)	0.667
732	0.075 (0.011)	0.060 (0.041)	-0.236*	0.077 (0.030)	0.095 (0.048)	0.277
730	0.142 (0.016)	0.102 (0.053)	-0.381***	0.154 (0.033)	0.124 (0.058)	-0.185
726	0.070 (0.010)	0.038 (0.038)	-0.840***	0.038 (0.038)	0.029 (0.029)	0.000
708	0.154 (0.014)	0.077 (0.052)	-1.000***
696	0.131 (0.014)	0.079 (0.052)	-0.648***	0.121 (0.034)	0.088 (0.053)	-0.307
693	0.073 (0.011)	0.043 (0.038)	-0.701***	0.051 (0.035)	0.043 (0.029)	0.000
682	0.072 (0.010)	0.055 (0.040)	-0.293**	0.077 (0.033)	0.060 (0.041)	-0.176
667	0.090 (0.012)	0.066 (0.041)	-0.341**	0.103 (0.034)	0.081 (0.039)	-0.176
663	0.040 (0.008)	0.071 (0.042)	0.446***	0.077 (0.033)	0.106 (0.054)	0.313*
651	0.042 (0.008)	0.040 (0.031)	-0.030	0.062 (0.030)	0.062 (0.043)	0.111
648	0.052 (0.009)	0.046 (0.031)	-0.124	0.066 (0.028)	0.065 (0.045)	0.074
644	0.000 (0.000)	0.033 (0.033)	1.000***	0.000 (0.000)	0.040 (0.000)	1.000
643	0.053 (0.009)	0.053 (0.031)	0.004	0.106 (0.030)	0.094 (0.051)	-0.055
631	0.038 (0.008)	0.044 (0.030)	0.157	0.039 (0.027)	0.054 (0.039)	0.400
628	0.032 (0.007)	0.025 (0.025)	-0.250	0.040 (0.039)	0.030 (0.029)	0.000
626	0.035 (0.008)	0.039 (0.026)	0.113	0.078 (0.031)	0.069 (0.050)	0.016

Note. All P values were obtained in a randomization test of $F_{IS} = 0$ based on 23,400 permutations of the data in which alleles were randomized within populations.

* $P < 0.05$.

** $P < 0.001$.

*** $P < 0.0001$.

group method (UPGMA) of clustering (Rohlf 1994). We tested for genetic isolation by distance among populations by calculating the correlation between pairwise estimates of $F_{ST}/(1-F_{ST})$ and $\log_e(\text{geographic distance})$ (Rousset 1997). This relationship was tested using Mantel's (Mantel 1967) test, based on 1000 permutations of the data, which is a procedure that overcomes problems of nonindependence in such comparisons.

Results

We consistently resolved 13 putative loci from the 12 enzyme systems we analyzed, six of which were polymorphic. We did not confirm the genetic basis of the markers with experimental crosses, but their expression was consistent in subunit structure and genetic interpretation with other plant isozyme studies (Weeden and Wendel 1989). One locus (IDH) was monomorphic and homozygous in all but one individual. Combined, the loci distinguished a mean of 4.9 (SD = 2.9) multilocus genotypes per population. Because genotypes were not evenly distributed within populations, this translated to a mean of 2.38 (SD = 1.09) effective number of genotypes per population (table 1). We found duplicated multilocus genotypes in all populations (table 1). We found, however, only one population (708) in which all sampled ramets expressed the same multilocus genotype. Thus, both sexual and asexual reproduction appeared to be important in Everglades *Cladium jamaicense* populations. Estimates of P , AP , and A were moderate among populations, but A_e estimates calculated from all sampled ramets were relatively low, never reaching above 1.18 in any population (table 1). After duplicate genotypes were

removed from the data, estimates of A_e increased on average (table 1) but were still low. We found significant ($P < 0.001$) differences in allele frequencies across populations for all loci except G3-PDH, IDH (both ramet- and genet-level analyses), and MDH-1 (genet-level analysis; data not shown). When all ramets were analyzed, observed heterozygosity was significantly higher than expected in eight (44.4%) of the 18 populations, whereas for four (22.2%) populations, a significant deficit of heterozygotes was observed (table 2). After duplicate multilocus genotypes had been eliminated within populations, however, these differences were maintained in only one (5.6%)

Table 3

Summary of Genetic Diversity for 18 Populations of *Cladium jamaicense* Collected in the Everglades

Locus	Ramets			Genets		
	H_T	H_S	G_{ST}	H_T	H_S	G_{ST}
G3pdh	0.005	0.005	0.016	0.045	0.042	0.069
Idh	0.001	0.001	0.010	0.011	0.011	0.036
Mdb-1	0.415	0.299	0.280	0.419	0.401	0.043
Mdb-2	0.051	0.041	0.207	0.136	0.089	0.348
Pgi	0.323	0.251	0.224	0.449	0.376	0.163
Pgm	0.106	0.066	0.373	0.160	0.130	0.187
Mean	0.150	0.110	0.185	0.203	0.175	0.141

Note. Estimates are based on analyses of all sampled plants (ramets) and after duplicate genotypes within populations had been removed (genets). H_T = observed total heterozygosity; H_S = within-population heterozygosity; G_{ST} = among-population differentiation; estimates calculated according to Nei (1972, 1973).

Table 4
Estimates of Inbreeding by Locus and Overall for 18 Populations of *Cladium jamaicense*
Collected in the Everglades

Locus	Ramets		Genets	
	F_{IS}	F_{ST}	F_{IS}	F_{ST}
<i>G3pdh</i>	-0.029	0.025*	0.008	-0.023
<i>Idb</i>			0.146	-0.163
<i>Mdb-1</i>	0.051	0.293***	0.280**	-0.092
<i>Mdb-2</i>	0.061	0.219***	0.312	0.291***
<i>Pgi</i>	-0.441	0.232***	-0.082	0.085**
<i>Pgm</i>	-0.839	0.471***	-0.185	0.182**
Overall	-0.161	0.268	0.089	0.035
95% CI	-0.525, 0.0621		-0.112, 0.2691	

Note. Estimates are based on analyses of all sampled plants (ramets) and after duplicate genotypes within populations had been removed (genets). F_{IS} = within-population inbreeding; F_{ST} = among-population differentiation; estimates calculated according to Weir and Cockerham (1984). All P values obtained in a test of $F = 0$ in which alleles within populations (F_{IS}) or genotypes among populations (F_{ST}) were randomized 1300 times. Overall means were based on jackknife estimates; 95% confidence intervals (CI) were constructed by bootstrapping.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

population (663), which had fewer heterozygotes than expected. The deviation from expected heterozygosity was not a function of the number ($r = -0.19$, $P = 0.4$) or the effective number ($r = -0.3$, $P = 0.2$) of multilocus genotypes in the population (*sensu* Pappert et al. 2000). Tests of individual loci within populations for departures from Hardy-Weinberg proportions revealed that, when all ramets were analyzed, 11 out of 43 (25.6%) loci within populations had significantly more heterozygotes than expected, whereas seven (16.3%) populations had a heterozygote deficit. When only genets were analyzed, however, no loci within populations were found to depart significantly from Hardy-Weinberg proportions. Some of these tests, however, should be interpreted cautiously because of the relatively small number of genets within some populations.

Overall genetic diversity (H_T) was low at the ramet level, as was the proportion of genetic diversity residing within populations (H_S ; table 3). At the genet level, these estimates increased slightly but remained low. Estimates of among-population diversity (G_{ST}) were also low; the genet-level estimate was slightly lower than the ramet-level estimate (table 3). Estimates of F statistics for one locus (IDH) were not possible based on the ramet-level analysis, since this locus was homozygous and monomorphic except for a single individual. Individual-locus estimates of inbreeding within populations (F_{IS}) were variable among loci but were not significantly different from zero for any locus at the ramet level; one locus (MDH-1) showed evidence for significantly positive inbreeding within populations at the genet level. Across all loci, no evidence was found for inbreeding within populations, either at the ramet or the genet level (table 4). At the ramet level, significant population differentiation (F_{ST}) was observed at all loci, whereas at the genet level, three loci (MDH-2, PGI, and PGM) contributed to genetic differentiation among populations (table 4). Ramet-level estimates of F_{ST} combining all

loci indicated genetic structuring among populations, whereas genet-level estimates did not (table 4).

Based on the ramet-level analysis, estimates of Nei's (1972) genetic distance among population pairs ranged from 0.0001 to 0.1169 (\bar{D} [SD] = 0.0241 [0.0239]). When only genets were considered, Nei's (1972) genetic distance ranged from 0 to 0.0566 (\bar{D} [SD] = 0.0162 [0.0126]). Water management units did not cluster tightly in UPGMA phenograms based on Nei's (1972) genetic distance at the ramet level (fig. 3A) or the genet level (fig. 3B). Nonetheless, we found a weak but significant correlation between genetic and geographic distance at the ramet level ($r = 0.15$, $P = 0.02$) but not at the genet level ($r = 0.04$, $P = 0.2$).

Discussion

Almost all estimates of genetic diversity for *Cladium jamaicense* were less than the averages reported from other plants with similar life history characteristics at both the species and the population level (table 5; Hamrick and Godt 1989). Only the percentage of loci that were polymorphic (P_s) and the mean number of alleles per locus (A_s) at the species level were above average in *C. jamaicense*. Estimates of total diversity (H_T) and the proportion of total diversity held within populations (H_S) were also considerably lower than the averages reported from species with similar characteristics (table 6). In addition, populations were not as distinct genetically (G_{ST}) as populations among other plants with similar traits, with the possible exception of tropical species (table 6). Genetic diversity in *C. jamaicense* was also less than what has been reported for other sedges (family Cyperaceae), with the exception of *Carex rotundata* ($H_T = 0.148$, $H_S = 0.120$, $G_{ST} = 0.184$; Ford et al. 1991) and *Cyperus esculentus* ($H_S = 0.109$; Horak et al. 1987). Other estimates of genetic diversity in seven



Fig. 3 Phenograms from UPGMA cluster analysis based on Nei's (1972) genetic distance among populations of *Cladium jamaicense*. Analyses are of all sampled plants (A) and after duplicate genotypes within populations had been removed (B). ENP = Everglades National Park; ROT = Rotenberger Holy Land Tract; WCA1–3 = Water Conservation Areas 1–3.

species of *Carex* and *Cyperus* were higher than those observed for sawgrass (Horak et al. 1987; Ford et al. 1991; McClintock and Waterway 1993; Jonsson et al. 1996).

One possible explanation for low genetic diversity in *C. jamaicense* is that a few "general purpose" genotypes have achieved dominance in a wide range of environmental conditions (Baker 1965). We do not know whether this is the case for sawgrass, but to observe low isozyme variation under such a scenario is unlikely since accumulated mutations at presumably neutral isozyme loci would eventually be expected to reveal variation. Alternatively, isozyme patterns may not accurately represent the genetic variation that exists within populations. Studies of other aquatic plants have reported low isozyme variation coupled with high levels of genetically based morphological variation (Marshburn et al. 1978; Sharitz et al. 1980; McMillan 1981). We found wide variation in the morphology of sawgrass plants sampled throughout the Everglades (C. T. Ivey and J. H. Richards, unpublished data). This variation, however, was correlated with environmental variation; larger plants occurred on peat-based soils and in areas where the hydroperiod was longer (C. T. Ivey and J. H. Richards, unpublished data). Thus, the morphological variation we observed seems more likely to be a plastic response to environ-

mental variation than an indication of genetic diversity not revealed in the isozyme patterns.

Plant populations on sites of relatively recent geologic origin can have lower genetic diversity (Lewis and Crawford 1995; Hannan and Orick 2000). Such observations are usually discussed in the context of founder effects during periods of post-glacial recolonization. Genetic bottlenecks have also been used to explain low genetic diversity at the species level (Barrett and Kohn 1991). Although uninfluenced by glaciers, Everglades plant communities were formed ca. 5000 yr B.P. during the middle Holocene (Webb 1990). No communities existed then that were similar to contemporary Everglades communities (Webb 1990). Therefore, low genetic diversity in Everglades sawgrass populations could be a consequence of a relatively recent origin. We do not know the source of colonists for Everglades sawgrass populations, but clonal plants such as sawgrass can establish a new population with one or a few individuals. Comparisons of genetic diversity among populations of *C. jamaicense* occurring outside the Everglades could determine the source population.

We found relatively few multilocus isozyme phenotypes per population (table 1), which raised the concern that isozymes have underestimated true genetic diversity. The use of a second genetic marker sometimes reveals differences among identical isozyme phenotypes (Esselman et al. 1999). This might be possible with *C. jamaicense* since the mean probability of drawing two identical multilocus isozyme genotypes by chance across all populations was 0.29 (SE = 0.06), given observed allelic diversity (Berg and Hamrick 1994). Regardless, isozymes are widely used to estimate genetic diversity, and use of these markers thereby facilitates interpretation of our results in light of other studies (Hamrick and Godt 1989). Sampling more broadly might have uncovered more genotypes, but the increase in diversity is likely to have been slight; we found only 36 different multilocus genotypes in 818 total samples across all populations. Moreover, we observed an average of 60% (SE = 5.6%) of the population-level genotypic diversity within 1-m² quadrats within sampling transects (C. T. Ivey and J. H. Richards, unpublished data). Thus, a sixfold increase in sampling area within transects increased total genotypic diversity within populations by an average of only 1.6 times. This is similar to what was found in the moss *Hylocomium splendens*, in which over 80% of genotypes observed occurred within the smallest hierarchical scale sampled (Cronberg et al. 1997).

The low genetic diversity that we observed was paralleled by estimates of genetic distances among populations, which were somewhat low compared to other plants (Crawford 1983). This was true for estimates of F_{ST} as well, and both results indicate high rates of gene flow among populations. Such observations, however, are not uncommon in wind-pollinated species (Hamrick and Godt 1989) such as *C. jamaicense*. Opportunities for gene flow through seed dispersal, in addition to wind pollination, may be high; sawgrass seeds can disperse by floating (Yates 1974). Vegetative plantlets (Miao et al. 1998) may be dispersed by water, so clonal reproduction may also contribute to gene flow among populations. Although dispersal distances are not known, parts of the Everglades are inundated for most of the year, which would facilitate seed and plantlet dispersal via water. In addition, hurricanes and other heavy rainstorms are likely to play im-

Table 5
Comparison of Genetic Diversity in Everglades *Cladium jamaicense* Populations to Species with Similar Characteristics, Based on a Review of Plant Genetic Diversity Studies

	Species level				Population level			
	P_s	A_s	A_{es}	H_{es}	P_p	A_p	A_{ep}	H_{ep}
Monocots	59.2 (3.4)	2.38 (0.17)	1.27 (0.03)	0.181 (0.015)	40.3 (3.0)	1.66 (0.08)	1.21 (0.03)	0.144 (0.012)
Long-lived, herbaceous perennial	39.6 (16.5)	1.42 (0.13)	1.28 (0.12)	0.205 (0.084)	39.3 (16.2)	1.44 (0.20)	1.14 (0.05)	0.084 (0.028)
Regional geographic range	52.9 (2.1)	1.94 (0.06)	1.20 (0.01)	0.150 (0.008)	36.4 (2.0)	1.55 (0.04)	1.16 (0.02)	0.118 (0.007)
Tropical distribution	49.2 (3.6)	1.81 (0.10)	1.21 (0.03)	0.148 (0.015)	32.7 (3.0)	1.45 (0.05)	1.13 (0.02)	0.109 (0.012)
Sexual-asexual	43.8 (3.7)	1.69 (0.08)	1.20 (0.03)	0.138 (0.016)	29.4 (3.3)	1.47 (0.06)	1.14 (0.02)	0.103 (0.013)
<i>C. jamaicense</i> _{ramet}	46.15	1.85	1.11	0.069	18.38 (2.46)	1.22 (0.13)	1.09 (0.04)	0.051
<i>C. jamaicense</i> _{genet}	46.15	1.85	1.15	0.094	18.38 (2.46)	1.22 (0.13)	1.12 (0.06)	0.067

Source. Hamrick and Godt 1989.

Note. Data presented are mean (SD) of percentage of polymorphic loci (P), mean number of alleles per locus (A), effective number of alleles per locus (A_e), and expected heterozygosity (H_e); subscripts denote estimates for species (s) and means of population estimates (p).

portant roles in long-distance dispersal of seeds and plantlets via flooding and wind. Low estimates of F_{ST} , however, do not necessarily reflect patterns of current gene flow. If individual clones are very long-lived (Steinger et al. 1996), F_{ST} estimates may reflect patterns of differentiation that were established during colonization and that have persisted.

We found a strong effect of clonal growth on estimates of genetic structure. When all ramets were included in the analysis, we found significant differentiation among populations, as well as weak evidence for isolation by distance. When duplicate multilocus genotypes were removed, however, we found no evidence for population differentiation or isolation by distance. Clonal reproduction may have a stronger influence on genetic structure in *C. jamaicense* than on other sedges that have been studied. For example, comparisons in three species of *Carex* found weak effects of clonality on estimates of genetic diversity (McClintock and Waterway 1993; Jonsson et al. 1996). This difference may exist because *C. jamaicense* has

multiple modes of asexual reproduction (Miao et al. 1998). At least three other sedges have more than one mode of clonal reproduction (*Cyperus virens*, Clay 1986; *Eleocharis caespitosissima*, Bruhl 1994; *Eleocharis vivipara*, Severson 1929); there have been no reports, however, on the genetic structure of these species. Clonal propagation may contribute to the success of sawgrass as an ecological dominant in the Everglades, where water levels can vary dramatically among years and can limit opportunities for recruitment of seeds (Lorenzen et al. 2000).

We cannot provide insight into the effects of water management practices and other anthropogenic disturbances, such as nutrient enrichment (Davis 1994), on genetic diversity in *C. jamaicense* populations since genetic diversity estimates prior to the imposition of current management regimes are not available. Reduction of hydroperiod variation, however, was a goal of the water management regime currently used in the Everglades when it was established several decades ago (Light and Dineen 1994). Reduction in hydroperiod variation would affect seasonal drying, which occurred historically and which is known to increase the likelihood of seedling establishment in *C. jamaicense* (Ponzio et al. 1995; Newman et al. 1996; Lorenzen et al. 2000). Nonetheless, we found no evidence for genetic differentiation among water management units (fig. 3). This study establishes a reference point for future studies that seek to evaluate the effects of Everglades restoration efforts (Walters and Gunderson 1994) on genetic diversity in sawgrass. Plans to replant Everglades sites with *C. jamaicense* (Newman et al. 1996) should consider genetic diversity patterns and the importance of sexual and clonal reproduction as well as spatial patterns of clonal growth.

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Table 6

Comparison of Genetic Diversity among Everglades *Cladium jamaicense* Populations to Species with Similar Characteristics, Based on a Review of Plant Genetic Diversity Studies

	H_T	H_s	G_{ST}
Monocots	0.320 (0.019)	0.238 (0.017)	0.231 (0.023)
Long-lived, herba- ceous perennial	0.346 (0.018)	0.282 (0.024)	0.213 (0.144)
Regional geographic range	0.308 (0.010)	0.236 (0.010)	0.216 (0.019)
Tropical distribution	0.278 (0.023)	0.228 (0.017)	0.173 (0.021)
Sexual-asexual	0.305 (0.019)	0.236 (0.018)	0.213 (0.027)
<i>C. jamaicense</i> _{ramet}	0.150	0.110	0.185
<i>C. jamaicense</i> _{genet}	0.203	0.175	0.141

Source. Hamrick and Godt 1989.

Note. H_T = observed total heterozygosity; H_s = within-population heterozygosity; G_{ST} = among-population differentiation; estimates calculated according to Nei (1972, 1973).

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