

GENOTYPIC DIVERSITY AND CLONAL STRUCTURE OF EVERGLADES SAWGRASS, *CLADIUM JAMAICENSE* (CYPERACEAE)

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The extent of asexual reproduction and the spatial distribution of clones can influence ecological and evolutionary processes in populations. Sawgrass, *Cladium jamaicense*, which is the dominant ecosystem component in the Florida Everglades, can reproduce sexually and asexually. We examined patterns of genotypic diversity and evaluated the importance of clonal reproduction in Everglades populations of *C. jamaicense*, using allozymes as genetic markers. We sampled plants in a replicated grid along 11-m transects in 18 populations of sawgrass distributed throughout the Everglades. Genotypic diversity was low in Everglades sawgrass populations, compared with other plants (mean [SE] number of multilocus genotypes per population = 4.9 [0.7]), but only one population was monomorphic. Diversity was present even at a small scale; 85 of 108 1-m² quadrats had more than one multilocus genotype. South Florida water management areas did not differ with respect to genotypic diversity, except for Everglades National Park, which had populations with a smaller proportion of polymorphic quadrats. Sawgrass clones were closer together than nonclones on average, although this varied among populations. Despite this observation, we found little evidence for spatial structuring of clones using the method of probability of clonal identity. This result reflected the broad interdigitation of clones along transects. Estimated mean (SE) minimum clone size was 46.2 (5.2) m² and clones may reach over 200 m². Our results indicate that asexual reproduction is common in *C. jamaicense* populations but that genotypic diversity is maintained throughout the ecosystem, even at relatively small scales.

Keywords: allozymes, asexual reproduction, *Cladium jamaicense*, clonal structure, Everglades, genotypic diversity, sawgrass, wetlands.

Asexual reproduction traditionally has been thought to predominate in clonal plants (Janzen 1977). The widespread observation of genetic and genotypic diversity in asexually reproducing plants (Ellstrand and Roose 1987; Hamrick and Godt 1989; Widén et al. 1994), however, indicates that sexual reproduction can also be common in clonal plants. The factors that contribute to the maintenance of dual reproductive modes in clonal plants have provoked considerable interest (Widén et al. 1994; Eriksson 1997).

The extent and pattern of clonal diversity can impact interpretation of a variety of ecological and evolutionary observations (Cook 1983; Handel 1985). Genetic differentiation among populations, for example, can result from asexual propagation of a few genotypes within populations (Hamrick and Godt 1989; Barrett et al. 1993). In some cases, genetic differences among populations are diminished or eliminated when reanalyzed without duplicate genotypes (Ivey and Richards 2001), which underscores the impact of clonal reproduction on genetic structure. In addition, the spatial arrangement of clones can affect mating patterns (Handel 1985). Clusters of genetically identical plants, for example, limit the number of mates available to self-incompatible species with limited pollen dispersal (Eckert and Barrett 1993). To interpret the significance of clonal structure or the mechanisms contributing to

maintenance of diversity, however, extant patterns of diversity must be understood.

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). In the Everglades, which is a vast, freshwater wetland in south Florida, sawgrass is the dominant ecosystem component, reaching 70% cover overall (Loveless 1959). The Everglades ecosystem has been transformed by human activities during the last century, and it is the focus of a 30-yr, \$7.8 billion restoration effort. Restoration may involve replanting some areas of the Everglades with sawgrass (Newman et al. 1996). Thus, knowledge of the population biology of *C. jamaicense* will be fundamental to implementing effective ecosystem restoration plans (Walters and Gunderson 1994) as well as to understanding the ecology of the Everglades.

Sawgrass often occurs in extensive, nearly monospecific stands in the Everglades, which led early workers to assume that asexual reproduction predominated over sexual reproduction in this species (Alexander 1971; Steward and Ornes 1975). Seed-set in *C. jamaicense* is high; populations can produce an estimated 5,000 seeds/m² annually (Alexander 1971). Seedling recruitment, however, is thought to be low (Alexander 1971; Miao et al. 1997). Sawgrass produces copious rhizomes (Alexander 1971) that have been estimated by excavation to generate clonal patches with an average size of 1.5 m² (Brewer 1996). These rhizomes interdigitate (Yates 1974; Brewer

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1996), which indicates that boundaries between sawgrass clones are indistinct. Miao et al. (1998) described a second mode of asexual reproduction in which plantlet propagules are produced within inflorescences. Since the dispersal potential of plantlets is likely to be higher than that of rhizomes, the plantlets seem likely to contribute to patterns of clonal interdigitation as well. Virtually nothing is known, however, about the ecology of these propagules, such as rates of growth, dispersal, recruitment, or the conditions that favor their production.

The genotypic diversity and spatial structure of clones in populations of *C. jamaicense* have not previously been described. The goals of this study were to estimate genotypic diversity and to examine clonal structure in Everglades sawgrass populations. We describe elsewhere (Ivey and Richards 2001) the genetic (allelic) diversity and population genetic structure of sawgrass in south Florida wetlands.

Material and Methods

Plant samples were collected during October 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. Distances between collection sites ranged from 3.3 to 141.2 km.

At each site 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). To estimate ramet density at each site, we counted the number of culms in the third quadrat from each transect. We collected leaf material from the ramet most

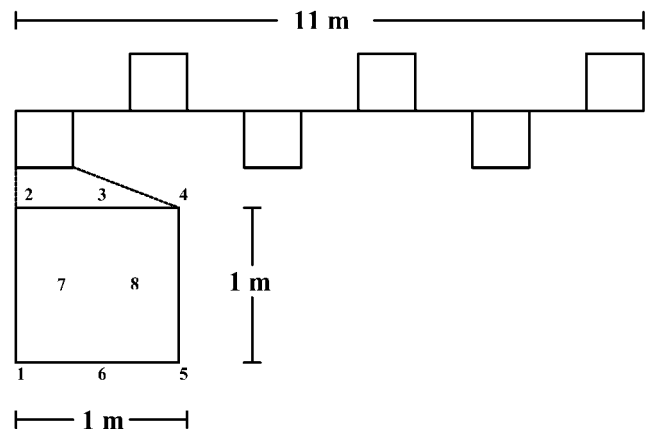


Fig. 2 Design used to sample ramets of *Cladium jamaicense* at 18 sites throughout the Everglades.

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. We used horizontal starch-gel electrophoresis to resolve allozyme genetic markers. Samples with different multilocus allozyme phenotypes were considered to represent different genotypes. Procedures for enzyme extraction and resolution are described by Ivey and Richards (2001).

To characterize genotypic diversity in populations we calculated the number of genotypes per population and the proportion of distinguishable genotypes (G/N , where G = the number of distinct genotypes per population and N = the number of plants sampled; Ellstrand and Roose 1987). We also calculated mean number of genotypes per quadrat in each population and compared populations using a Kruskal-Wallis test. We compared number of genotypes per population among water management areas using a Kruskal-Wallis test. To estimate the evenness of genotypic diversity, we calculated the complement of Simpson's index (D ; Pielou 1969) for each population as $D = 1 - \sum [N_i(N_i - 1)/N(N - 1)]$, where N_i = the number of plants of genotype i and N = the number of plants sampled. We calculated a second estimate of evenness (E ; Fager 1972), which is not influenced by sample size, as $E = (D - D_{\min})/(D_{\max} - D_{\min})$, where $D_{\min} = [(k - 1)(2N - k)]/[N(N - 1)]$, $D_{\max} = [(k - 1)N]/[k(N - 1)]$, and k = the number of multilocus genotypes in the population. To evaluate the power of the data to distinguish clones, we calculated each population's mean probability that ramets with identical multilocus isozyme phenotypes belong to the same genet (Aspinwall and Christian 1992) as

$$\bar{P} = 1 - \left[\frac{\sum_{Q=1}^N \prod_{D=1}^M \left(\frac{X_{DQ}}{P_D} \right)}{N} \right],$$

where Q = an individual from a population, N = the number of individuals in the population, M = the number of polymorphic loci in the population, X_{DQ} = the number of individuals that have the same allozyme pattern at locus D as

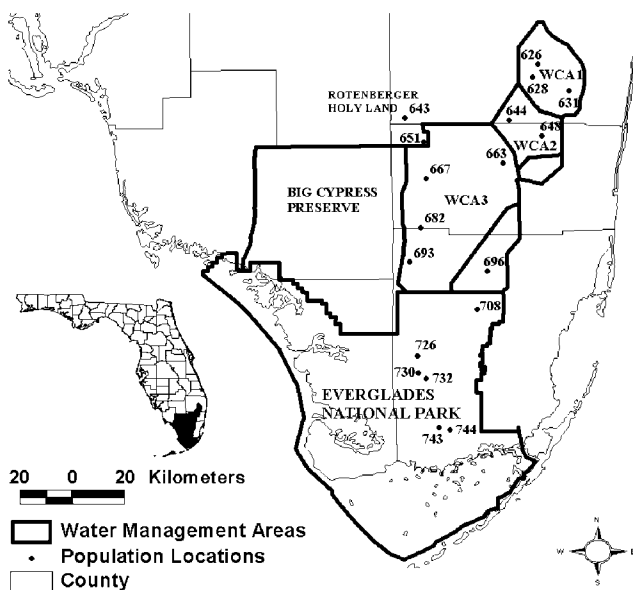


Fig. 1 Locations of *Cladium jamaicense* populations studied in the Everglades.

individual Q , P_D = the total number of individuals analyzed for allozyme locus D . We calculated the maximum number of possible genotypes within each population given the allozyme diversity observed (Parker and Hamrick 1992) as

$$N_g = \frac{\prod_{i=1}^L a_i(a_i + 1)}{2},$$

where a_i = the number of alleles detected at the i th locus and L is the number of polymorphic loci analyzed. For each genotype within each population, we also calculated its expected frequency as

$$p_{\text{gen}} = \left(\prod_{i=1}^L p_i q_i \right) 2^b$$

(modified from Parks and Werth 1993), where p_i and q_i = the population frequency of the two alleles at the i th locus for a given multilocus genotype, L = the number of polymorphic loci in the population, and b = the number of loci that are heterozygous in the given multilocus genotype. We then calculated the probability of observing n individuals of each multilocus genotype by chance in a sample of r ramets, if all individuals were the result of independent recombination (modified from Parks and Werth 1993), as the binomial probability function

$$p_{\text{sex}} = \sum_{x=n}^r \frac{r!}{x!(r-x)!} (p_{\text{gen}})^x (1 - p_{\text{gen}})^{r-x},$$

where r = the number of ramets sampled and n = the number of ramets with a given genotype.

To test for clustering of clones, we calculated the distances among all possible pairs of ramets bearing the same multilocus genotype within each population and compared them to distances among pairs of nonclones. Shorter distances between clones indicate aggregation of ramets of the same genotype. This was tested using a two-way ANOVA, with clonal identity and population as main effects (Burke et al. 2000).

Clonal structuring may change over distance, such that pairs of ramets that are closer together are more likely to be clones than those farther apart. Harada et al. (1997) described a lattice model to examine the probability of clonal identity (F) as a function of distance in populations sampled on a grid, such that

$$F(r) = k \frac{e^{-cr}}{\sqrt{r}},$$

where c represents the slope by which $F(r)$ declines with distance r and k is a scaling constant related to the size of each ramet (Harada and Iwasa 1996). An indirect estimate of the relative success of sexual versus vegetative reproduction can be derived from the slope c of this relationship, as

$$c = \sqrt{\frac{2u}{v\sigma^2}}$$

for which u = the rate of recruitment of sexually produced progeny, v = the rate of asexually produced progeny, and σ^2 = the mean square distance between parent and asexually produced offspring. Thus, c will be large when the success of sexually produced offspring is high relative to clonal reproduction and if vegetative dispersal is small. Furthermore, mean clone size (area) can be estimated by evaluating the integral

$$\int_0^{\infty} 2\pi F(r) r dr = k \left(\frac{\pi}{c} \right)^{1.5}$$

(Harada et al. 1997). This model assumes that populations are in equilibrium with respect to clonal reproduction, that vegetative reproduction can occur in 360°, that populations are spatially homogeneous, that all clones have been detected, and that survivorship is equivalent among clones (Harada et al. 1997). The first three assumptions are likely to hold, since Everglades management has been relatively consistent for >50 yr, rhizome and plantlet dispersal can occur in all directions for *C. jamaicense* (Alexander 1971; Yates 1974), and site heterogeneity over the scale at which we sampled is not likely to be great. Isozyme markers may underestimate true genotypic diversity (see "Discussion"). We have no data on genet survivorship, but this final assumption seems likely to be restrictive.

We used nonlinear least-squares regression to fit the model of Harada et al. (1997) to our data in each population, weighted by the number of observations in each distance class. We used the Gauss-Newton routine in PROC NLIN from SAS (1996) to iteratively estimate parameters until the incremental reduction in the residual sums of squares was minimized. To evaluate data fit and model assumptions, we examined plots of predicted versus residual values for nonrandom patterns in each population and tested the normality of residual distributions using the Shapiro-Wilk test (Shapiro and Wilk 1965). Estimates for slopes were considered to be stable if their 95% confidence intervals did not include zero (Draper 1998).

To estimate the relative success of sexual versus vegetative reproduction (u/v) using slope estimates, an estimate of mean square distance between parent and vegetatively reproduced offspring (σ^2) was required. Brewer (1996) estimated median rhizome length within clones of *C. jamaicense* to be 10.5 cm. This estimate is likely to underestimate σ^2 , since dispersal distances of plantlet propagules are unknown (Miao et al. 1998). Nevertheless, this was the best estimate of σ^2 available to us, so we used it to explore model estimates of the relative importance of clonal reproduction from our data.

Results

Mean sawgrass plant density was 20.9 culms/m² (table 1). At most sites, therefore, our sampling intensity (eight plants/m²) resulted in a subsample of all plants present. After an initial screening of 33 enzymes on seven buffer systems, six polymorphic isozyme loci (G3PDH, IDH, MDH [two loci], PGI, and PGM) were chosen to distinguish plants. These loci, however, were not polymorphic in all populations. Populations had from zero to five polymorphic loci with an average of 2.3

polymorphic loci per population (table 1). The loci were capable of distinguishing an average maximum of 81.2 multilocus genotypes per population (N_g ; table 1). We observed, however, an average of only 4.9 genotypes per population (range = 1–12; table 1), which was an average (SE) of 32.7% (5.6%) of the maximum possible genotypic diversity within populations. The number of genotypes per population did not vary among management areas (table 1; Kruskal-Wallis χ^2 approximation = 3.9, df = 4, $P = 0.1$).

Individual 1-m² quadrats had from one to six genotypes, with a mean (SE) of 2.3 (0.1) genotypes across all populations. This within-quadrat diversity varied among populations (table 1; Kruskal-Wallis χ^2 approximation = 51.0, df = 17, $P = 0.0001$) but not among water management areas (table 1; Kruskal-Wallis χ^2 approximation = 7.8, df = 4, $P = 0.1$). Overall, 85 of the 108 quadrats sampled (79%) had more than one genotype. The proportion of polymorphic quadrats varied among populations from 33% to 100%, with the exception of one population (708). Population 708 had only a single multilocus genotype (table 1); this genotype was heterozygous at two loci. The proportion of quadrats with multiple genotypes varied among management areas (table 1; Fisher's exact test, $P = 0.003$); specifically, populations from Everglades National Park had a lower proportion of polymorphic quadrats.

An average of 11% of the ramets sampled within transects were genetically distinguishable (G/N ; table 1); this indicates that an average of 9.1 ramets were observed for each genotype. Thirty-nine of the 88 genotypes (44%) within populations, however, were represented by only one or two ramets (fig. 3). We observed a total of 36 distinct multilocus genotypes across all populations, the majority of which occurred in only a single population (fig. 4); only one genotype

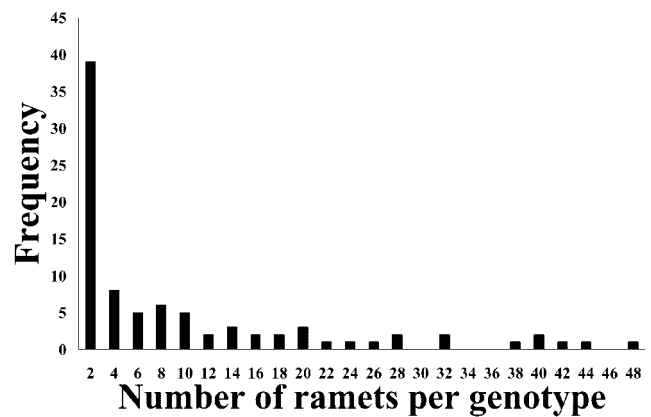


Fig. 3 Histogram of the number of ramets per multilocus isozyme genotype within 18 populations of *Cladium jamaicense* from the Everglades.

was common enough to be observed in >75% of our populations. Genotypes were moderately evenly distributed ($\bar{D} = 0.495$, $\bar{E} = 0.577$; table 1).

Across all populations, mean (SE) probability that ramets with the same genotype belong to the same genet was 0.538 (0.058) (\bar{P} ; table 1). Populations with more polymorphic loci had higher values of \bar{P} ($r_s = 0.70$, $P = 0.001$, $N = 18$). Mean (SE) expected frequency of multilocus genotypes (p_{gen}) throughout all populations was 0.130 (0.013); these values ranged from 0.0002 (663-1; population 663, genotype 1) to 0.563 (726-1 and 628-1; table 2). As expected, genotypes with a higher expected frequency were more commonly observed; we

Table 1

Summary of Genotypic Diversity Estimated by Isozymes in 18 Populations of *Cladium jamaicense* from the Everglades

Site	Mgmt	N	Loci	N_g	G	G/N	\bar{G}/Q (SE)	Prop	D	E	\bar{P}	Density
744	ENP	45	1	3	2	0.044	1.7 (0.2)	0.67	0.236	0.411	0.231	41
743	ENP	45	2	9	2	0.044	1.5 (0.2)	0.50	0.269	0.480	0.394	8
732	ENP	48	4	162	6	0.125	2.2 (0.2)	1.00	0.271	0.110	0.369	39
730	ENP	39	4	162	9	0.231	2.5 (0.6)	0.83	0.682	0.573	0.830	8
726	ENP	47	1	3	2	0.043	1.5 (0.2)	0.50	0.159	0.249	0.278	16
708	ENP	48	0	9	1	0.021	1.0 (0.0)	0.00	0.000	0.000	0.000	23
696	WCA3	48	3	54	7	0.146	2.8 (0.5)	1.00	0.538	0.472	0.562	17
693	WCA3	44	2	9	3	0.068	1.7 (0.4)	0.33	0.212	0.207	0.263	14
682	WCA3	47	2	18	5	0.106	2.8 (0.3)	1.00	0.771	0.928	0.767	8
667	WCA3	42	4	81	6	0.143	2.3 (0.5)	0.67	0.698	0.752	0.774	14
663	WCA3	46	5	972	12	0.261	4.0 (0.6)	1.00	0.814	0.759	0.823	27
651	ROT	44	2	9	5	0.114	3.0 (0.4)	1.00	0.682	0.787	0.664	11
648	WCA2	46	2	18	6	0.130	3.2 (0.2)	1.00	0.673	0.723	0.724	42
644	WCA2	47	1	3	2	0.043	2.0 (0.0)	1.00	0.444	0.857	0.409	21
643	ROT	45	3	27	8	0.178	2.7 (0.4)	0.83	0.605	0.521	0.769	28
631	WCA1	47	2	9	4	0.085	2.7 (0.3)	1.00	0.719	0.926	0.708	11
628	WCA1	46	1	3	2	0.043	2.0 (0.0)	1.00	0.496	0.967	0.473	27
626	WCA1	44	2	18	6	0.136	2.7 (0.4)	0.83	0.635	0.658	0.637	21
Mean		45.4	2.3	81.2	4.9	0.109	2.3	0.79	0.495	0.577	0.538	20.9
SE		0.5	0.3	53.2	0.7	0.016	0.1	0.07	0.058	0.069	0.058	2.6

Note. Mgmt = Water management area (see fig. 1), N = number of sampled ramets, Loci = number of polymorphic loci, N_g = number of multilocus genotypes possible, G = number of multilocus genotypes observed, G/N = proportion of ramets distinguishable, \bar{G}/Q (SE) = mean (SE) number of genotypes per quadrat, Prop = proportion of quadrats with more than one genotype, D = Simpson's diversity index, E = genotypic evenness, \bar{P} = mean probability that ramets with identical multilocus genotypes belong to the same genet, Density = number of culms/m².

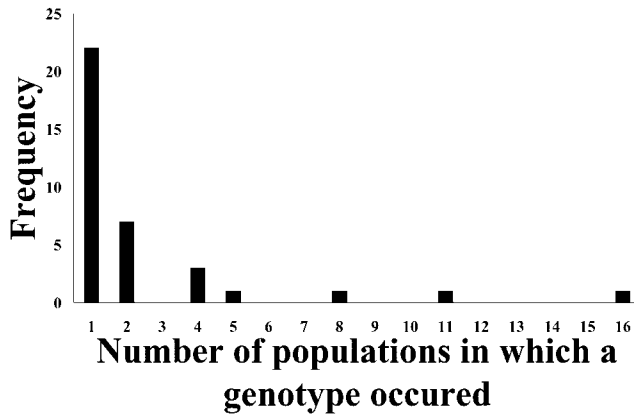


Fig. 4 Histogram of the number of populations in which a multilocus isozyme genotype (of a total of 36) occurred for 18 populations of *Cladium jamaicense* collected from the Everglades.

found a positive relationship between the number of ramets of a genotype and p_{gen} ($r_s = 0.59, P < 0.0001, N = 88$). The overall mean (SE) probability that observed ramet frequency of genotypes could have resulted from sexual reproduction was 0.363 (0.040) (table 2). In general, however, as the number of ramets of a genotype increased, the probability that all ramets resulted from sexual reproduction decreased; we found a negative relationship between p_{sex} and the number of ramets of that genotype ($r_s = -0.60, P < 0.0001, N = 88$). Thus, genotypes with many ramets (e.g., 744-2, 726-2, 651-3, 644-1, 648-3; table 2) probably indicate the occurrence of clonal reproduction, despite a moderately high expected frequency of the genotype, in some cases. In contrast, some genotypes represented by many ramets had a high value of p_{sex} (e.g., 651-2, 628-1, 628-2, 644-2; table 2). The true number of genets within these samples may be higher than what is represented by the number of multilocus isozyme phenotypes that we observed.

Two populations were not included in the analyses of spatial distribution of genotypes, one (708) because it was monomorphic and the second (651) because of a laboratory processing error. Among the remaining 16 populations, plants with the same genotype were closer together on average (mean [SE] distance between clones = 3.92 [0.03] m, nonclones = 4.38 [0.03] m; tables 3, 4). This varied, however, among populations (table 3); distances between clones and nonclones did not differ significantly for 10 of the 16 populations (table 4). In five of the remaining populations, clones were closer together than nonclones (table 4). Nonclones were closer together in one population (726); this population, however, was dominated by a single genotype (table 2), which likely influenced this result. Because we used a replicated sampling design, the significant population term in the ANOVA (table 3) was perhaps unexpected; this term reflects differences among populations in numbers of ramets sampled as well as inequity in genotype evenness.

Nonlinear regression iterations converged on estimates in all populations, and residual errors were small, relative to model mean square values (table 5). We found no patterns in any population upon examination of scatterplots of residuals

versus predicted values (data not shown), and residual values were normally distributed (Wilks-Shapiro test, $W \geq 0.89$) in all populations; thus, model fit appeared to be good and regression assumptions upheld in all populations. Estimates of the scaling constant varied over fourfold and were positive in all populations (mean [SE] $k = 0.617 [0.053]$; table 5). Esti-

Table 2

Number of Ramets (n), Expected Multilocus Genotype Frequency (p_{gen}), and Probability That Observed Number of Ramets Resulted from Independent Recombination (p_{sex}) for 18 Populations of *Cladium jamaicense* Collected from the Everglades

Population/ genotype	n	p_{gen}	p_{sex}	Population/ genotype	n	p_{gen}	p_{sex}
744:				663:			
1	6	0.250	0.955	1	1	0.000	0.000
2	39	0.250	0.000	2	1	0.044	0.609
743:				3	11	0.119	0.007
1	38	0.141	0.000	4	1	0.010	0.078
2	7	0.094	0.056	5	1	0.005	0.022
732:				6	14	0.081	0.000
1	1	0.108	0.971	7	2	0.014	0.027
2	2	0.025	0.119	8	1	0.042	0.581
3	2	0.144	0.977	9	2	0.038	0.257
4	1	0.035	0.497	10	1	0.070	0.840
5	41	0.026	0.000	11	1	0.008	0.056
6	1	0.005	0.020	12	10	0.015	0.000
730:				651:			
1	21	0.067	0.000	1	3	0.102	0.672
2	1	0.022	0.217	2	16	0.307	0.164
3	1	0.068	0.754	3	19	0.230	0.001
4	2	0.058	0.394	4	2	0.154	0.974
5	3	0.052	0.145	5	4	0.115	0.583
6	1	0.003	0.004	648:			
7	7	0.045	0.000	1	2	0.064	0.570
8	2	0.010	0.007	2	4	0.095	0.446
9	1	0.017	0.137	3	24	0.232	0.000
726:				4	10	0.108	0.009
1	4	0.563	1.000	5	5	0.264	0.991
2	43	0.375	0.000	6	1	0.075	0.869
708:				644:			
1	48	0.250	0.000	1	32	0.250	0.000
696:				2	15	0.250	0.106
1	1	0.092	0.943	643:			
2	2	0.136	0.968	1	1	0.054	0.709
3	1	0.044	0.635	2	9	0.138	0.084
4	11	0.136	0.025	3	1	0.154	0.995
5	1	0.044	0.635	4	3	0.088	0.567
6	1	0.044	0.635	5	27	0.098	0.000
7	31	0.044	0.000	6	2	0.026	0.107
693:				7	1	0.065	0.799
1	2	0.475	1.000	8	1	0.046	0.619
2	3	0.194	0.981	631:			
3	39	0.194	0.000	1	13	0.090	0.000
682:				2	19	0.300	0.046
1	18	0.179	0.000	3	10	0.353	0.972
2	8	0.314	0.979	4	5	0.104	0.360
3	9	0.090	0.008	628:			
4	5	0.090	0.242	1	27	0.563	0.317
5	7	0.157	0.461	2	19	0.375	0.245
667:				626:			
1	2	0.026	0.098	1	1	0.039	0.511
2	1	0.151	0.991	2	7	0.216	0.761
3	14	0.227	0.038	3	25	0.162	0.000
4	18	0.101	0.000	4	3	0.054	0.209
5	6	0.151	0.453	5	1	0.161	0.996
6	1	0.026	0.303	6	7	0.121	0.154

Table 3

Results from an ANOVA Testing the Effect of Clonal Identity (Same vs. Different) and Population on Distances between Pairs of Ramets of *Cladium jamaicense* from the Everglades

Source	df	Type III SS	MS	F	P
Clonal identity	1	677.8	677.8	94.8	0.0001
Population	15	254.8	17.0	2.4	0.002
Clonal identity × population	15	1201.0	80.1	11.2	0.0001
Error	16,117	115,210.3	7.1		

mates of the model slope varied among populations from -0.179 to 0.065 , (mean [SE] $c = -0.089$ [0.016]; table 5). The slope estimates for two populations (663 and 682) were not different from zero, and slope estimates were negative for 13 populations (table 5), which indicates that there was little evidence for spatial structuring of clones in these populations. Only one population (730) had a positive estimate of c (table 5); this population also had the largest mean distance separating clones versus nonclones in the ANOVA (table 4). Based on this population's estimate of the decline in clonal identity with distance, the relative success of sexual to asexual reproduction (u/v) in population 730 was 2.33×10^{-5} , implying that an average of 42,937 vegetative shoots were produced for each seed that was successfully recruited. This estimate of u/v , however, is likely to be an underestimate, since dispersal distances of plantlet propagules (Miao et al. 1998) are unknown.

Using the method of Harada et al. (1997), mean clone size in population 730 was 214.7 m^2 . This method produces undefined results for populations with negative slope estimates. We estimated minimum clone size within the other populations as size = $\pi \times (\text{maximum distance between any two sampled ramets of the same genotype within a population}/2)^2$. This assumes that changes in clone size occur radially and at an equal rate in all directions. Using this method, the average (SE) minimum size

of clones across all populations was 46.2 (5.2) m^2 (table 5). Compared to the method of Harada et al. (1997), this method underestimated clone size by over 11 times in population 730 (table 5). Minimum clone size did not vary among management areas (Kruskal-Wallis χ^2 approximation = 3.4 , $df = 4$, $P = 0.5$).

Discussion

Both clonal and sexual reproduction structure Everglades populations of *Cladium jamaicense*. We observed isozyme variation in all but one population across our 22 m^2 sampling transects, and 79% of 1-m^2 quadrats had multiple genotypes. Nearly all populations, however, also had duplicate isozyme genotypes that were unlikely to have been the result of sexual reproduction alone. We found that plants with identical genotypes occurred slightly closer to each other, on average, which likely reflects rhizomatous growth radiating from parental plants. Nonetheless, different genotypes intermingled and boundaries between clones did not appear to be sharp; this observation concurs with previous observations of clonal growth patterns in *C. jamaicense* based on excavation (Yates 1974; Brewer 1996). As a consequence, most populations revealed little evidence for spatial structuring of genotypes over the distance at which we sampled.

At the population and species levels, sawgrass genotypic diversity was low compared to other plants. A review of 47 studies of plant genotypic diversity reported a mean (SE) $G = 10.9$ (1.7), $G/N = 0.27$ (0.04), and $D = 0.75$ (0.04) (Widén et al. 1994), which were 2.2, 2.5, and 1.5 times higher than the values observed for sawgrass, respectively (table 1). Our estimates of *C. jamaicense* genotypic diversity were lower than similar estimates from other sedges (family Cyperaceae); the mean (SE) G , G/N , and D from studies of 10 species of sedges was 13.6 (4.0), 0.43 (0.09), and 0.78 (0.06), respectively (Horak and Holt 1986; Horak et al. 1987; Ford et al. 1991; McClintock and Waterway 1993; von Perger et al. 1994; Jonsson et al. 1996; Steinger et al. 1996). The diversity we observed, however, was greater than the lowest estimates reported, both for all plants (Widén et al. 1994) and for sedges (Horak and Holt 1986; Horak et al. 1987).

Based on Brewer's excavation studies (Brewer 1996), we expected to find an average of over 14 clones of *C. jamaicense* within our 22 m^2 transects. Instead we observed about 33% of that expected diversity (mean = 4.9 per population). Brewer's (1996) estimated mean clone size of 1.5 m^2 was based on observing intact rhizome connections among excavated clones. Our estimates, however, indicate that mean (SE) minimum clone size is 46.2 (5.2) m^2 and may reach over 200 m^2 .

Table 4

Least Square Mean (SE) Distances in Meters between Pairs of Clones and Nonclones for 16 Populations of *Cladium jamaicense* from the Everglades

Population	Clones	Nonclones	P
744	4.14 (0.10)	4.11 (0.17)	0.9
743	4.47 (0.10)	3.87 (0.16)	0.3
732	4.16 (0.09)	4.39 (0.15)	0.9
730	2.97 (0.17)	4.87 (0.12)	0.0001
726	4.39 (0.09)	3.53 (0.20)	0.03
696	3.94 (0.12)	4.46 (0.11)	0.2
693	4.00 (0.10)	5.00 (0.19)	0.0009
682	3.32 (0.17)	4.43 (0.09)	0.0001
667	3.53 (0.17)	4.60 (0.11)	0.0001
663	3.89 (0.19)	4.39 (0.09)	0.8
648	4.24 (0.15)	4.15 (0.10)	0.9
644	4.15 (0.11)	4.34 (0.12)	0.9
643	3.72 (0.14)	4.62 (0.11)	0.0001
631	3.86 (0.15)	4.31 (0.10)	0.8
628	4.09 (0.12)	4.52 (0.12)	0.7
626	3.80 (0.14)	4.47 (0.11)	0.07

Note. P indicates significance of pairwise Tukey's comparisons of distances between clones and nonclones.

Table 5

Summary of Nonlinear Regression to Fit Harada et al.'s (1997) Model Describing the Probability of Clonal Identity as a Function of Distance in 16 Populations of *Cladium jamaicense* from the Everglades

Site	MS-reg _{df}	MS-res _{df}	<i>c</i> (95% CI)	<i>k</i> (95% CI)	Area (SE)
744	27.77 ₂	0.048 ₇	-0.125 (-0.140, -0.110)	0.813 (0.725, 0.900)	76.3 (19.6)
743	28.80 ₂	0.028 ₈	-0.148 (-0.159, -0.138)	0.705 (0.650, 0.761)	57.0 (41.1)
732	22.22 ₂	0.038 ₈	-0.107 (-0.122, -0.091)	0.807 (0.723, 0.891)	36.7 (30.8)
730	3.44 ₂	0.028 ₈	0.065 (0.018, 0.112)	0.639 (0.533, 0.745)	19.1 (12.0)
726	34.43 ₂	0.028 ₈	-0.137 (-0.146, -0.128)	0.830 (0.775, 0.885)	55.4 (42.9)
696	7.84 ₂	0.058 ₈	-0.062 (-0.095, -0.030)	0.608 (0.490, 0.726)	59.8 (28.2)
693	23.39 ₂	0.058 ₇	-0.085 (-0.105, -0.066)	0.939 (0.822, 1.056)	34.1 (30.9)
682	1.40 ₂	0.018 ₈	0.040 (-0.011, 0.092)	0.381 (0.303, 0.458)	38.7 (12.1)
667	3.60 ₂	0.058 ₇	-0.062 (-0.111, -0.013)	0.415 (0.295, 0.534)	40.8 (17.1)
663	1.23 ₂	0.028 ₈	-0.048 (-0.103, 0.008)	0.257 (0.177, 0.338)	39.0 (18.5)
648	5.58 ₂	0.058 ₈	-0.137 (-0.176, -0.098)	0.335 (0.238, 0.432)	45.4 (17.5)
644	13.17 ₂	0.058 ₈	-0.105 (-0.131, -0.080)	0.626 (0.517, 0.736)	92.5 (0.0)
643	5.41 ₂	0.028 ₈	-0.042 (-0.071, -0.014)	0.554 (0.467, 0.641)	44.3 (25.3)
631	4.77 ₂	0.058 ₇	-0.179 (-0.222, -0.136)	0.232 (0.151, 0.313)	48.3 (20.0)
628	9.59 ₂	0.058 ₈	-0.078 (-0.107, -0.049)	0.623 (0.510, 0.735)	89.8 (0.0)
626	4.79 ₂	0.058 ₈	-0.046 (-0.088, -0.04)	0.513 (0.394, 0.633)	41.1 (18.5)

Note. MS-reg_{df} = mean square from regression model and associated degrees of freedom, MS-res_{df} = mean square of residuals and associated degrees of freedom, *c* = regression slope (95% CI), *k* = scaling parameter (95% CI), Area = mean (SE) minimum area (m²) encompassed by clones.

This disparity in size estimates could result from disruption of rhizome connections between sawgrass ramets, and it indicates that Brewer's (1996) assumption that rhizome connections remain intact over time warrants reevaluation.

Although sampling *C. jamaicense* populations over a larger area may unveil greater genotypic diversity at the population level, the increase in diversity with area sampled is likely to be shallow for several reasons. First, we observed a total of only 36 multilocus genotypes throughout a broad study area, despite sampling over 800 plants. In addition, we observed an average (SE) of 60% (5.6%) of the population-level genotypic diversity within 1-m² quadrats (table 1). Thus, a sixfold increase in sampling area increased total genotypic diversity only 1.6 times. Cronberg et al. (1997) also used a hierarchical sampling design to examine genotypic diversity in the moss *Hylacomium splendens*, and they found a similar pattern; the smallest hierarchical scale in their design (10 × 10 cm) contained over 80% of the genotypes that they observed. Thus, the low genotypic diversity that we observed is more likely to be a consequence of low genetic diversity in Everglades sawgrass populations ($H_T = 0.150$ and 0.203 for ramet- and genet-level estimates, respectively; Ivey and Richards 2001), than a reflection of sampling design. This low diversity may be a consequence of the relatively recent origin of these populations (Webb 1990; Ivey and Richards 2001).

One of the difficulties of estimating clonal diversity using molecular markers is that some genetically distinct individuals may possess identical marker phenotypes (discussed in Widén et al. 1994). Thus, molecular markers may underestimate true genotypic diversity. Various statistical approaches, some of which we used, have been developed to evaluate the power of markers to discern genotypes, usually as some function of population allelic diversity. Unfortunately, these approaches are limited as well, since the statistical estimates are based on observed diversity. In other words, if marker allelic diversity is low in a population, low estimates of power to detect ge-

notypes may result, even if all true genets have been distinguished. As marker allelic diversity increases, of course, the power to distinguish individuals increases, so this concern applies only to populations with low genetic diversity. Parks and Werth (1993) have suggested techniques to avoid the inherent circularity of using allele frequencies to estimate statistical power to detect genotypes. The most powerful approach to circumvent this problem, however, is to employ additional, independent genetic markers (Esselman et al. 1999). Because *C. jamaicense* populations possessed low isozyme allelic diversity, we had low statistical power to detect genotypes in some populations. Thus, true genotypic diversity in *C. jamaicense* populations may be higher than our estimates.

Based on our modeling results, we found little evidence for spatial structuring of clones in most *C. jamaicense* populations. Estimates of the decline in the probability of clonal identity with distance (*c*) were negative in all populations but one, which was unexpected. In the model of Harada et al. (1997), a negative slope estimate indicates that the probability of clonal identity declines over short distances and then increases at larger distance classes. Negative slopes may be an artifact of applying this model to populations with extensive clonal interdigitation and weak spatial structure, such as we observed in *C. jamaicense*. Only a few studies have used this model to examine the spatial structure of clonal plants (Harada et al. 1997; Holderegger et al. 1998; Schläpfer and Fischer 1998). One of these (Schläpfer and Fischer 1998) reported negative slopes in 25% of subplots studied but did not further discuss the spatial arrangement of genotypes in these subplots.

Some previous studies of clonal structure in plants have applied spatial autocorrelation analyses to allozyme data to examine patterns of allele frequencies (Chung et al. 1999; Chung and Epperson 2000) or relatedness (Montalvo et al. 1997; Burke et al. 2000) over distance. These analyses, which involve exhaustive sampling of ramets, can provide insight into fine-scale local genetic structure. An advantage of using the Harada

et al. (1997) model to explore clonal structure is that it explicitly examines the structure of multilocus genotypes (clones) over distance, as opposed to the structure of alleles or relatedness. Because it is a lattice model, this information can be gleaned by a more efficient sampling design (i.e., sampling from a grid vs. sampling all ramets). In addition, evidence of structure can be used to infer demographic processes (relative success of sexual vs. asexual reproduction) and average clone size. Thus, the Harada et al. (1997) model is potentially a powerful tool for studies of clonal structure and patterns of reproduction in clonal plants. One constraint of this model is that interpretation of the results is limited to the scale of sampling. Inference beyond the smallest or largest sampling intervals is limited. For *C. jamaicense* populations, therefore, evidence for clonal structure might be found by sampling at a larger spatial scale.

We were unable to use our modeling results to infer the relative importance of sexual to asexual reproduction except in one population. The mean value of G/N (0.109) (table 1), however, is considerably lower than 0.5, which is the value that would indicate equal recruitment rates of sexual and asexual shoots. The estimate of G/N for population 730, furthermore, overestimates model estimates of u/v by four orders of magnitude. These observations indicate that many more plants are produced by asexual reproduction than by sexual reproduction in Everglades *C. jamaicense* populations.

A predominance of asexual reproduction may reflect ecological limits on the success of sexual reproduction. Seedling establishment is thought to be rare in natural populations of *C. jamaicense* (Alexander 1971) because of high seedling mortality. Germination occurs only when soil is exposed during relatively dry periods (Newman et al. 1996; Miao et al. 1997; Lorenzen et al. 2000), which may be rare events. Under favorable field conditions, sawgrass germination rates can reach 250 to 1000/m² (S. L. Miao, South Florida Water Management District, personal communication). Sawgrass seedlings cannot survive complete submersion, however, and periods of dry-down are transient in the Everglades. To our knowledge, recruitment success of *C. jamaicense* seedlings in the Everglades has not been directly estimated. Nonetheless, the patterns of small-scale genotypic diversity reported here indicate that sexual reproduction is common enough to maintain diversity throughout the ecosystem. Although large monomorphic patches can occur (population 708; table 1), these were uncommon in our study.

A lack of demographic data precludes us from inferring what patterns of seed recruitment (*sensu* Eriksson 1997) have led to the establishment of the current patterns of diversity in *C. jamaicense*. Rates of establishment of seeds, ramets, and asexual plantlet propagules are unknown for sawgrass, as are patterns of dispersal, growth, and survival of ramets or genets. At least one other sedge, *Carex bigelowii*, is thought to have

a “repeated window of opportunity” recruitment pattern (Jonsson et al. 1996), in which sexual recruitment is clustered at irregular time intervals (Eriksson and Fröberg 1996). This pattern of recruitment might occur in Everglades sawgrass, if periods of successful sexual recruitment are linked to relatively dry periods during which seedlings remain above water long enough to become established. During intervening periods, asexual reproduction is likely to predominate, since rhizome expansion from parental plants is not hindered by submersion (Yates 1974).

When the current water management system was established in south Florida over 40 years ago, the primary goal was to reduce hydroperiod variation (Light and Dineen 1994). This practice should diminish sexual recruitment of *C. jamaicense* because seedling establishment requires periodic drydown (Newman et al. 1996; Lorenzen et al. 2000). We cannot evaluate this prediction with our data, because there are no estimates of sawgrass genotypic diversity before the establishment of the current water management regime. We found no effect of water management area, however, on genotypic diversity of *C. jamaicense* populations, except when comparing the proportion of polymorphic quadrats among populations. Everglades National Park populations had fewer quadrats with multiple genotypes, on average, than populations in other areas. We also found no evidence for genetic differentiation among water management units (Ivey and Richards 2001).

This study establishes a benchmark for future studies on the effects of restoration efforts on genotypic diversity and clonal structure of Everglades sawgrass (Walters and Gunderson 1994; Newman et al. 1996). Restoration plans should consider the effect that landscape-scale alterations in hydrology could have on the clonal structure and patterns of diversity of this dominant ecosystem species, and replanting efforts need to recognize the local scale of diversity in both source and target populations.

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