

# Invasion Dynamics and Genotypic Diversity of Cogongrass (*Imperata cylindrica*) at the Point of Introduction in the Southeastern United States

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Nine sites of cogongrass were included in a study of genotypic diversity and spread dynamics at the point of introduction and its adjacent areas in the southern United States. Clones evaluated with two primer pairs yielded a total of 137 amplified fragment length polymorphism (AFLP) loci of which 102 (74.4%) were polymorphic. Genetic diversity was measured as the percentage of polymorphic, Shannon's information index, Nei's gene diversity, and panmictic heterozygosity. Nei's gene diversity ( $H_S$ ) across all nine sites was estimated to be 0.11 and within site gene diversity ranged from 0.06 to 0.16. Bayesian estimate of gene diversity and Shannon's information index were higher (0.17 and 0.17, respectively). The samples from the point of introduction (Pi) had the lowest genetic diversity for all types of estimates. Within site variance accounted for 56% of the total variation and among site variance 44% ( $P < 0.05$ ). Differentiation among sites was assessed using  $F_{ST}$ . The greatest difference was found between the Pi and the others. No relationship was found between genetic and geographic distances. Principal component analysis as well as cluster analysis separated individuals into three main clusters. The Pi formed a separate subcluster. Gene flow ( $Nm$ ), inferred from  $\Phi$ -statistics describing the genetic differentiation between pairs of sites ranged from 0.6 to 5.55. The lack of significant relationship between gene flow and geographic distance as well as genetic and geographic distances suggests that the invasion dynamics of cogongrass into the southern United States is primarily through anthropogenic activities and to the lesser extent through natural forces.

**Nomenclature:** cogongrass, *Imperata cylindrica* (L.) P. Beauv; Brazilian satintail, *Imperata braziliensis* Trin.; satintail, *Imperata brevifolia* Vasey; satsuma orange, *Citrus reticulata* L.; CTAB, cetyltrimethyl ammonium bromide; DNA, deoxyribonucleic acid.

**Key words:** Invasive species, molecular makers, gene diversity, plant introduction.

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The introduction of exotic species is one of the most serious threats to native ecosystems worldwide (Simberloff and Von Holle 1999). Only a fraction of the species either purposefully or accidentally transported from one location to another becomes established and invades the new environment. The magnitude of the problem is such that invasive species threaten most of the species listed in the U.S. Endangered Species Act (D'Antonio et al. 2001). Effort has been spent trying to develop generalizations to determine which species are likely to become successfully invasive (Ellstrand and Schierenbeck 2000). Less frequently, possible genetic correlates have been sought to predict which introduced species might become invasive (Gray 1986). Little attention has been given to understanding the initial spread dynamics of an introduced species.

### Interpretive Summary

Invasive plants are species that, after they have been transported from their native habitat to a new location, have ability to successfully disperse, colonize, spread on their own, and subsequently compete with and disrupt ecosystems. These characteristics rank cogongrass as one of the most noxious weeds in tropical and subtropical regions around the globe. Cogongrass is a serious threat to ecosystems worldwide covering 500 million ha. In southwest Alabama, where an estimated 200,000 ha are dominated by cogongrass, infested areas are increasing rapidly. Controlling this species through herbicides has proven to be expensive (\$400 ha yr) requiring multiple applications per year and several years. While basic ecological and herbicide-based control studies have been conducted, there are still critical information gaps that need to be filled. This study employed amplified fragment length polymorphism (AFLP) analysis as the molecular marker to examine genetic variation among sample sites of cogongrass in the locale of Mobile, AL. The authors demonstrated that the sample site at the point of introduction is not the only source of other infestations in the southern United States. The spread dynamics are greatly influenced by anthropogenic activities and natural factors to a much lesser extent. They have also shown that cogongrass exhibits high genetic diversity in the local environment of its site of introduction. Factors that may contribute such high levels of genetic diversity include cross-compatibility between species allowing genetic exchange within and among sample sites and the founding of the sample site.

The results of this study are essential for the development of appropriate control strategies by government agencies and landowners. The authors are confident that current and future research efforts will help to make the best and cost effective in the eradication program of cogongrass.

Cogongrass [*Imperata cylindrica* (L.) P. Beauv] was accidentally introduced into Alabama near Grand Bay about 1911 as seed in packing materials for Satsuma orange (*Citrus reticulata* L.) rootstock from Japan (Dickens 1974; Tabor 1952). Cogongrass is a rhizomatous, perennial, invasive species that is generally considered a pernicious pest plant because of its ability to successfully disperse and subsequently compete with and displace desirable vegetation and disrupt ecosystems over a wide range of environmental conditions (Dozier et al. 1998; Holm et al. 1977). A few decades after the time of the introduction near Grand Bay, Mobile County, AL., Tabor (1952) estimated that cogongrass could be found on approximately 200 ha (494 ac). Later, estimates increased to nearly 4,000 ha for Mobile County (Dickens 1974). Current estimates exceeded 200,000 ha in southwest Alabama, with northward expansion near the Tennessee border (Faircloth et al. 2003).

The genetic composition of the founding population determines its ability to adapt to the new environment (Tsutsui and Case 2001). However, this constitutes one, but not the only factor that might influence the ability of a species to become invasive. Effective size of an introduction, genetic diversity of the source samples, number of

founding sources, and other characteristics may influence the genetic structure of a successful invasive population. Pappert et al. (2000) reported that populations that have been introduced multiple times over an extended period from different origins should have higher genetic diversity than populations that were introduced once or only a few times. Characterizing the genetics of established sites of the aggressive species cogongrass is of interest because it may provide insights into the spread dynamics and the mode of local population establishment.

In the United States cogongrass has two close relatives: Brazilian satintail (*Imperata braziliensis* Trin.) and satintail (*Imperata brevifolia* Vasey) (Hitchcock 1950). Both Brazilian satintail and satintail are described as native species by Hitchcock (1950) and are distinct in their ranges. Brazilian satintail is native to the pinelands and prairies of the Everglades, southern Florida, and Alabama, while satintail is confined to desert regions of western Texas to southern California. The nonnative cogongrass and the native Brazilian satintail have overlapping ranges, and discriminating these two species can be difficult (Bryson and Carter 1993; Hall 1998). Frequent hybridization between them has been observed (Gabel 1982). Regardless, the introduction of cogongrass has resulted in the release of new genetic material into the U.S. genome (Gabel 1982; Hall 1998). The genetic diversity of cogongrass in the U.S. is unknown (Shilling et al. 1997). Worldwide, Hubbard et al. (1944) describes five varieties of cogongrass, mostly based on geographical distribution, growth habit, and morphological characteristics.

Discrimination of individuals in clonal plants has been one of the methodical problems in plant demography (Ellstrand and Roose 1987) but recent advances in molecular marker techniques have enabled researchers to overcome these limitations (Ellstrand and Roose 1987; Pornon et al. 2000). These markers provide means for assessing genotypic diversity and phylogenetic relationship and have been extensively used to evaluate clonal structure of plants (For example, see Albert et al. 2003; Arens et al. 2005; Douhovnikoff et al. 2004; Eckert et al. 2003; Kreher et al. 2000). The AFLP technique, which is based on selective amplification of restriction fragments from a digest of total genomic DNA, has several advantages over other marker systems currently in use (Vos et al. 1995). It does not require previous knowledge of the species genome, produces a large number of informative polymorphic markers per primer pair, is highly sensitive, and has proven to be robust and reliable (Mueller and Wolfenbarger 1999). Examination of the genotypic diversity of a naturalized species in its new environment provides a first step for understanding the genetic consequences of its introductions and spread (Barrett and Husband 1990). This understanding is strengthened if points of introduction for the species of interest are known and potential

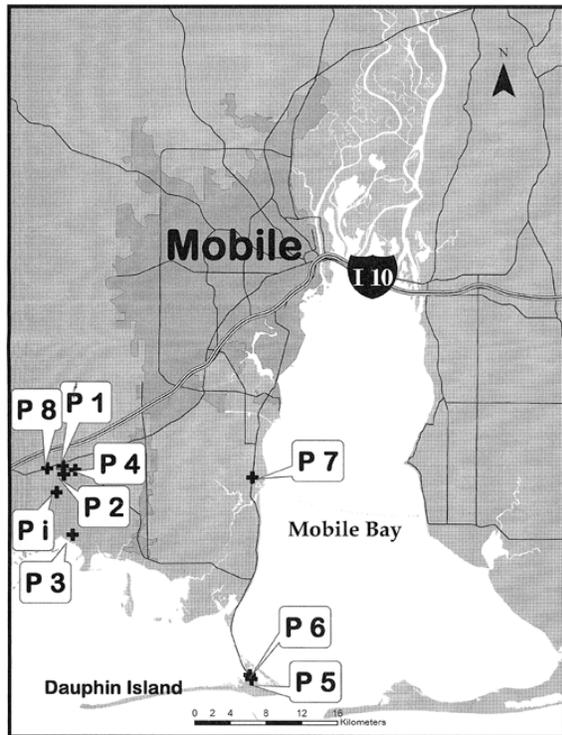


Figure 1. Geographical locations of the nine sample sites of cogongrass studied around Grand Bay, Mobile, Alabama.

source populations are identified (Novak et al. 1993). In this paper we examine the level of genotypic diversity and the invasion dynamics of the introduced cogongrass at the point of introduction and its adjacent areas. We examine the hypothesis that the population at the point of introduction is a source of the other infestations in the southern United States.

### Materials and Methods

**Study Sites and Sampling.** The point of introduction of cogongrass near Grand Bay in Mobile County, AL described by Tabor (1952) was located. We selected eight additional sites in the local Mobile, AL region on the basis of information from records of the U.S. Department of Agriculture Natural Resources Conservation Service (USDA/NRCS) (Figure 1). A site is defined as a single, contiguous population. Distances between collection sites ranged from 1.0 to 60.9 km (0.62 to 37.8 miles). At each site we collected from 10 to 20 individuals along one or two randomly placed transects depending on the size of the site. Samples were collected a minimum of 0.3 and up

to 15 m (50 ft) apart across sites and latitude/longitude was obtained using GPS. A single rhizome was taken from each sampling point, stored in a plastic bag and transported to the greenhouses at the Plant Science Research Center of the Alabama Agricultural Experiment Station (PSRC/AAES) located on the campus of Auburn University. Plants were grown in pots filled with standard growing medium diluted with coarse sand (1:1 v/v).

Genomic DNA was extracted from leaves according to a modified cetyltrimethyl ammonium bromide (CTAB) method of Doyle and Doyle (1990). The present study employed AFLP analysis to examine genetic variation among these nine sites of cogongrass. We performed AFLP analysis according to Vos et al. (1995) with slight modifications with AFLP core reagent and starter primer kits purchased from Life Technology. Each AFLP marker was treated as a unit character and scored as a binary code (1/0). The analysis of molecular variance (AMOVA) was performed as described by Excoffier et al. (1992) using Arlequin 2.000 software (Schneider et al. 2000). AMOVA produces estimates of variance components and fixation index statistics ( $\Phi$ ).  $\Phi$ -statistics are analogous to  $F_{ST}$  and describe genetic differentiation among sample sites. Gene flow ( $Nm$ , number of migrants per generation) among sites was calculated based upon  $\Phi$ -statistics using the method of Wright (1951). An alternative Bayesian approach (Holsinger et al. 2002) was also used to obtain independent estimates of  $F_{ST}$ . The original data matrix was imported into HICKORY, version 1.0 (Holsinger and Lewis 2003) and used for a full model,  $f = 0$  model, Theta-B ( $\theta^B = 0$  model, and  $f$ -free model. We conducted several runs with default sampling parameters (burn-in = 50,000, sample = 250,000, thin = 50) to ensure that the results were consistent. The deviance information criterion (DIC) (Spiegelhalter et al. 2002) was used to estimate the fit between the data and a particular model and to choose among models (Holsinger and Wallace 2004). The  $f$ -free model chooses  $f$  values at random from its distribution while estimating other parameters, resulting in estimates of  $\theta^B$ , which incorporate all of the uncertainty in the prior of  $f$  (Holsinger and Lewis 2003). Recent studies have shown that estimates of  $f$  from dominant markers may be unreliable, particularly in data sets with small sample sizes (Holsinger and Wallace 2004), which is the situation in this study. Thus, the analysis below should be evaluated conservatively and be viewed as a complement to the other analyses.

Within-site genetic diversity was assessed for each site in several ways. We used multiple approaches, as each has a different set of assumptions, strengths, and weaknesses. Where they agree, we can be quite confident in the analysis. The methods are as (1) percentage of polymorphic loci; (2) Nei's (1978) gene diversity ( $H_S$ ), assuming Hardy-Weinberg equilibrium, as implemented in the ARLEQUIN program; (3) Bayesian gene diversity ( $H_B$ ) as implemented in the HICKORY program, which does not assume Hardy-

Table 1. Number of polymorphic fragments and average gene diversity statistics in cogongrass sample sites.

Sample sites	Number of individuals	Polymorphic markers		Average gene diversity		Number of alleles <sup>a</sup>		
		Number	%	Nei's ( $H_S$ )	Bayesian ( $H_B$ )	na	ne	$I$
P1	10	59	43.1	0.12	0.19	1.3	1.2	0.16
P2	10	55	40.1	0.15	0.21	1.4	1.2	0.21
P3	10	68	49.6	0.19	0.25	1.5	1.3	0.25
P4	18	58	42.3	0.12	0.19	1.4	1.2	0.20
P5	17	48	35.0	0.09	0.16	1.3	1.2	0.15
P6	19	59	43.1	0.10	0.17	1.4	1.2	0.16
P7	15	44	32.1	0.12	0.18	1.3	1.2	0.17
P8	17	45	32.8	0.10	0.16	1.3	1.2	0.15
Pi	19	27	19.7	0.06	0.13	1.1	1.1	0.11
Mean				0.11	0.17	1.3	1.2	0.17
Overall	135	102	74.4					

<sup>a</sup>Abbreviations: na, observed number of alleles; ne, effective number of alleles.

Weinberg equilibrium within sites; and (4) Shannon's information index ( $I$ ) implemented in POPGENE (Yeh and Boyle 1997). We also conducted a homogeneity test in POPGENE to evaluate whether allele frequencies were different from each other. Cluster analysis (CLA) was carried out to estimate the relationship based on the banding profile of individuals and assign these individuals to artificial clusters. Genetic similarity coefficients between individuals were calculated according to Nei and Li (1979). Principal component analysis (PCA) was performed to visualize the dispersion of the individuals in relation to the first two principal axes of variation. Computations were done using the procedures in NTSYS-pc version 2.0 (Rohlf 1998). Mantel's tests were calculated with a rejection zone from 100,000 random permutations.

### Results and Discussions

**AFLP and Gene Diversity.** Two AFLP primer pairs identified a total of 137 markers, 102 of which (74%) were polymorphic between two or more ramets (Table 1). The

size of the AFLP fragments, compared to AFLP standard size markers, ranged from 50 to 600 base pairs (bp). Polymorphic fragments were distributed across the entire size range with the major proportion between 75 and 550 bp. Polymorphic markers within sites varied from 27 (19.7%) to 68 (49.6%). Nei's gene diversity ( $H_S$ ) assuming Hardy-Weinberg equilibrium ranged from 0.06 for Pi to 0.19 for P3, with a mean value of 0.11 (Table 1). Estimates of Bayesian gene diversity ( $H_B$ ) using the  $f$ -free model ranged from 0.13 for Pi to 0.25 for P3, averaging 0.17 (Table 1). Shannon's information index ( $I$ ) varied from 0.11 for Pi to 0.25 for P3, with a mean value of 0.17 (Table 1). However, these three estimates of genetic diversity were highly correlated ( $r = 0.99$ ,  $P < 0.001$  for  $H_S$  vs.  $H_B$ ;  $r = 0.96$ ,  $P < 0.001$  for  $H_S$  vs.  $I$ ; and  $r = 0.97$ ,  $P < 0.001$  for  $H_B$  vs.  $I$ ). Homogeneity tests of allele frequencies identified significant ( $P < 0.001$ ) differences across sample sites for a large number of loci (75 out of 137). The genetic distance among sample sites ranged from 0.02 to 0.17 (Table 2).

The lowest value of the genetic diversity in Pi is an indication of low level of heterozygosity among individuals,

Table 2. Sample site pairwise  $F_{ST}$  (above diagonal), genetic distance (below diagonal), and geographic distance (bold; km).

Sample sites	P1	P2	P3	P4	P5	P6	P7	P8	Pi
P1		0.12	0.18	0.44	0.33	0.43	0.43	0.54	0.65
P2	0.04 (1.0)		0.09	0.34	0.27	0.30	0.34	0.49	0.59
P3	0.05 (7.9)	0.02 (6.9)		0.33	0.27	0.27	0.32	0.44	0.57
P4	0.10 (1.5)	0.07 (1.4)	0.08 (6.9)		0.41	0.44	0.35	0.48	0.60
P5	0.07 (32.1)	0.06 (31.3)	0.07 (25.6)	0.08 (60.9)		0.33	0.39	0.52	0.60
P6	0.10 (31.6)	0.07 (30.8)	0.08 (25.4)	0.11 (30.4)	0.06 (0.5)		0.03	0.54	0.62
P7	0.11 (21.3)	0.09 (21.2)	0.11 (21.4)	0.09 (19.9)	0.09 (22.9)	0.07 (22.4)		0.18	0.48
P8	0.16 (1.8)	0.14 (2.0)	0.14 (8.1)	0.12 (3.2)	0.12 (33.1)	0.15 (32.6)	0.03 (23.1)		0.43
Pi	0.17 (3.0)	0.15 (2.2)	0.17 (5.2)	0.14 (3.3)	0.11 (30.4)	0.15 (30.0)	0.08 (22.1)	0.06 (2.9)	

Table 3. Summary of the analysis of molecular variance (AMOVA) within and among sample sites of cogongrass. The significance ( $P$ ) of the  $\Phi_{ST}$  was based on the procedure of 3,000 permutations.<sup>a</sup>

Source of variation	df	Sum of squares	Expected mean squares	Variance		Fixation Index $\Phi_{ST}$	Probability
				Components	Percentage		
Among sample sites	8	753.6	$n \sigma_a^2 + \sigma_b^2$	5.8	43.8	0.44	< 0.05
Within sample sites	126	942.9	$\sigma_a^2$	7.5	56.2	n/a	n/a
Total	134	1,696.5	$\sigma_T^2$	13.3	100		

<sup>a</sup> Abbreviations: n/a, not applicable; df, degrees of freedom.

which suggests high degree of clonality. In contrast, in sites P1 through P8, genetic diversity is high. We suggested four mechanisms that could explain such high genetic diversity. Firstly, multiple different individuals from diverse sources may have established these populations. Secondly, populations may be founded from seedling recruitment. It is likely that long-distance dispersal is driven by seedling recruitment. Many of the infestations of cogongrass occur along rights-of-way bordering highways and railroads and open fields areas where aerodynamic properties of wind dispersed seed from cogongrass may optimize dispersal potential. Thirdly, sites may be founded from the cross between Brazilian satintail and cogongrass as reported by Gabel (1982). It has been shown that genetic diversity at both population and species levels can increase due to natural hybridization (Vilà et al. 2000). We speculate that out-crossing involving the nonnative and native species may have an important impact on the levels of genetic diversity, leading to a number of newly different genotypes.

Fourthly, the variation in gene diversity may also be due to small sample sizes or genotypic dynamics. The number of genets in a clonal population depends on ramet dynamics of existing genets and the recruitment of new genets from seeds (McLellan et al. 1997). If seedling recruitment is infrequent in a clonal population and if increasing ramet density leads to the thinning of genets, genotypic diversity in a population is expected to be greater at the pioneer phase and to progressively diminish with population maturity and closure (McLellan et al. 1997). Population closure through ramets may lead to a dramatic loss of genets and to a selection of highly genetically related genotypes (Pornon et al. 2000). As a result, population closure and maturation could both have reduced genotypic variation more in Pi, the oldest site, than in P1-P8. In general, one could argue that both seedling recruitment and clonal reproduction play an important role in genotypic dynamics in the sites of clonal plants, which may influence the variation and level of genetic diversity. The mechanisms are not mutually exclusive, and all four may contribute to the high genotypic diversity within samples.

**AMOVA and CLA.** The analysis of variance indicated that 56% ( $P < 0.05$ ) of the total genetic variation was found

within sites while the among-site component accounted for 44% ( $P < 0.05$ ; Table 3). CLA (Figure 2) using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Nei and Li's coefficient led to the separation of the individuals into three main clusters. Cluster I grouped sites P1, P2, P6, 9 individuals out of the 10 sampled from P3, and 15 of the 17 from P5. Cluster II grouped 17 of the 18 from P4 and 2 of the 17 from P5. Cluster III captured all individuals of Pi, P8, and 9 of 15 from P7. Within cluster III, Pi formed a separate subcluster. This may indicate that these individuals are highly clonal. As can be seen, an individual of P3 was discernible from the rest as well as one from P4 (Figure 2). Few individuals of P5 and P6 could not be differentiated (Figure 2).

For the nine sites, a Bayesian estimate of  $F_{ST}$  under a random-effects model of sampling ( $\theta^B$ ) based on the  $f$ -free model from the Hickory program (Holsinger and Lewis



Figure 2. Dendrogram generated by UPGMA clustering method based on AFLP markers obtained with two primer pairs.

2003) generated a mean of  $0.39 \pm 0.02$  (95% confidence intervals 0.35 and 0.43) with DIC values of 2,271 and Dbar of 1,839. Results show that the  $f$ -free model yielded estimates of  $F_{ST}$  lower than that of previously reported in the AMOVA. Again, this estimate should be viewed conservatively because of biases associated with too small sample sizes (Holsinger and Lewis 2003).

The value for the within-site variance component was slightly higher. This high within-site estimate may be indicative of out-crossing species. Cogongrass has been described as an obligate allogamous species (Gabel 1982). Viable seed is produced only when cross-pollinated (McDonald et al. 1996). In contrast, in self-pollinated species, one would predict a greater partitioning of diversity among rather than within sites in the absence of human-mediated gene flow among sites. With predictions based on previous evidence of inter- and intraspecific hybridization as a frequent cause (Ellstrand and Schierenbeck 2000), cogongrass exhibits a high level of genetic variation and several genetically differentiated sites around the point of introduction.

In the CLA, the nine sites using UPGMA and Nei and Li's coefficients showed three distinct main clusters. The results indicated that within clusters, there has been relative exchange of migrants among the sample sites at different levels. For instance, in cluster I grouping sites P1, P2, P6, and most of the individuals of P3 and P5, there is no clear subcluster composed of all individuals of a given site as can be seen in cluster III in which all individuals of Pi formed a separate subcluster. The sites P5 and P6 from Dauphin Island are grouped together with most of the sites near the point of introduction, although these sites are farthest away from that point and separated by an environmental barrier. We suggest that anthropogenic activities followed by local migration of cogongrass could be an explanation.

**PCA.** The first two principal components accounted for 70% of the total variation. The scatter-plot representation of the PCA analysis showed a clear-cut separation of the 135 individuals into three main clusters in relation to the first two principal axes of variation (Figure 3). Cluster I comprised all individuals of P1, P2, P3, P6, 5 out of 18 samples from P4, 16 out of 17 from P5, and 4 out of 15 from P7. Cluster II comprised 13 out of 18 from P4, 2 out of 15 from P7, and 1 out of 17 from P5, while cluster III grouped 9 out of 15 from P7 and all individuals of P8 and Pi. Genetic distances among sites ranged from 0.02 to 0.17 with the highest values between Pi and the others. Geographic distances ranged from 1 to 60.9 km (Table 2). As can be seen, most of the individuals are grouped in the same clusters as the three of the previous CLA. We suggest that all of the sampled individuals from P8 and 9 of the individuals from P7 most probably descend from Pi or are closely related.

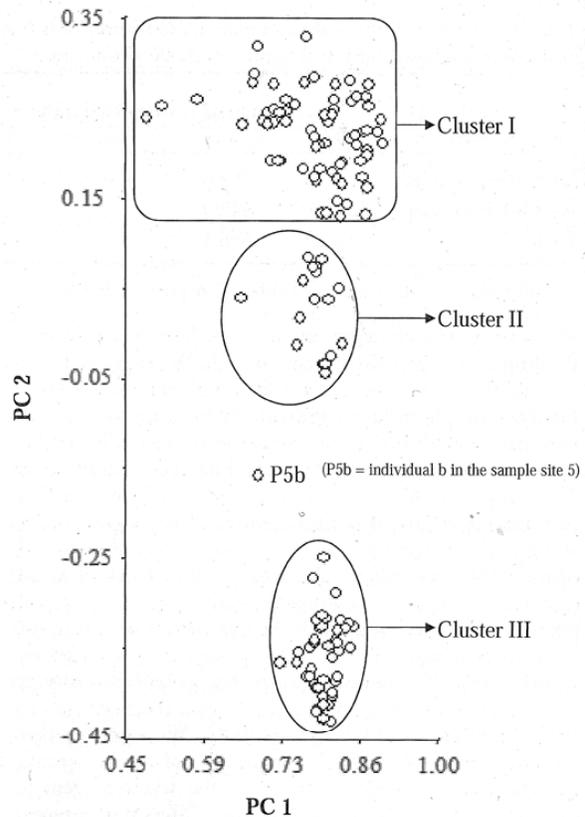


Figure 3. Scatter plot of the first two principal components (PC 1 and PC 2) for 135 cogongrass ramets. Variation explained by the first principal component is 63%; by the second principal component is 7%.

When comparing the dispersion pattern of the individuals within clusters as an indication of individual-level genetic variation, along the first principal axis, individuals of cluster III are less dispersed than those of clusters II and I. The most likely explanation is that clusters with more dispersion pattern may be the result that some sexual reproduction is occurring; thus, indicating the development of new genotypes. Overall, the AFLP approach provided an effective means of examining relationship and genetic similarity within and among sites of cogongrass in the locale of Mobile, AL.

**Site Differentiation.** The results of analysis of  $F$ -statistics as a measure of differentiation among the sites sampled are presented in Table 2. Most pairwise estimates of  $F_{ST}$  (91%) were significant ( $P < 0.05$ ; Table 2), suggesting that each sample site is distinct. The  $F_{ST}$  estimates between Pi and the rest of the sites ranged from 0.43 to 0.65. The data were examined for evidence of the model of isolation-

by-distance (Slatkin 1993). Dispersal is often distance dependant, such that populations near many other populations receive a greater number of migrants, whereas more isolated populations receive fewer (Brown and Kodric-Brown 1977). The geographic distance between pairs of sites ranged from 1 to 60.9 km, averaging 17.0 km. Estimates of gene flow among sites ranged from 0.5 to 5.0, averaging 1.0. Generally, if  $Nm < 1$ , then local differentiation of populations would result, and if  $Nm < 1$ , then there will be little differentiation among populations (McDermott and McDonald 1993). The pattern of gene flow was not related to geographic distance ( $r = 0.09$ ). Lower values ( $Nm < 1$ ) were observed in 27 of 36 pairs of sites (data not shown). The highest value was estimated to be 5.0 between a pair of sample sites separated by 7 km, indicating significant genetic exchange. The lowest gene flow value was estimated to be 0.26 between Pi and P1 distant by 3 km. Accordingly, there was neither significant correlation between the matrices of gene flow and geographic distance (Mantel's test,  $r = -0.13$ ,  $t = -0.70$ ,  $P = 0.24$ ) nor between genetic and geographic distances (Mantel's test,  $r = -0.13$ ,  $t = -0.72$ ,  $P = 0.23$ ). Indeed, the lowest  $F_{ST}$  value observed between sites P4 and P5 may suggest that they have exchanged a greater number of migrants even though they are farther apart. However, these sites may also have received migrants from the same sources. Whitlock and McCauley (1999) reported that a pair of populations that receives migrants from the same source would have a low  $F_{ST}$  even if they exchange no migrants at all.

Model-based tests for site differentiation showed that the cogongrass sample sites are genetically distinct. Both Bayesian and AMOVA estimates of  $F_{ST}$  (0.39 and 0.44, respectively) are high for cogongrass, an out-crossing species. High fixation index was found in the clonal out-crossing species *Pitcairnia geyskesii* L. B. Smith in the family Bromeliaceae (Sarthou et al. 2001). The  $F_{ST}$  estimates for all site pairs showed a high level of genetic differentiation. The subdivision index was lower for the pair P2-P3 ( $F_{ST} = 0.09$ ,  $P < 0.05$ ) and higher for all other pairs with Pi,  $F_{ST}$  ranging from 0.43 to 0.65. This may indicate that Pi has exchanged few migrants with others. The high  $F_{ST}$  values obtained in the analysis may be because of genetic isolation of the sites and the individuals within these sites. This result is surprising, given the geographical proximity of one sample site to another. Such differences among sites suggest that either they have been genetically isolated at some time during their spread or humans have brought together cross-compatible species that had been naturally isolated as acknowledged by Anderson and Stebbing (1954). Thus, the levels of among-site diversity and the difficulty of explaining the observed patterns of site differentiation may be due to varying degrees of interspecific hybridization in some

sample sites and taxonomic misidentification. In future work, it would be important to evaluate the genetic structure of cogongrass along the dispersal routes due to seed being blown by prevailing winds from the coastal to inland areas.

Using molecular markers, our results showed that cogongrass exhibits high levels of genetic variation in the local environment of its site of introduction in the southern United States. That variation showed few patterns geographically, and the site at the point of introduction contained the lowest genetic variation. These patterns lead to the conclusion that our data do not support the hypothesis that the population at the point of introduction is a source of the other infestations in the southern United States. With the exception of P7 and P8, the sites sampled showed little evidence that Pi, the initial point of introduction of the species, played a dominant role in founding the other sites sampled.

The amount of genetic variation within and between sites was quite large given how recently cogongrass was introduced. Factors that may contribute such high genetic diversity include cross-compatibility between species allowing genetic exchange within and among sites and the manner in which the sites were founded. What is clear from this assessment is that seedling recruitment is far more important than has been previously assumed. The spread dynamics of cogongrass is greatly influenced by anthropogenic activities such as soil disturbance and canopy removal, and natural factors influence spread to a much lesser extent. We do not claim that these factors explain the mechanism of invasiveness. They act to accelerate the opportunities for bringing together cross-compatible species that had been previously isolated by natural events such as ecology and geography. This would have influenced the variation and levels of genetic diversity. The results represent an important first step in revealing the mechanisms of invasion of cogongrass and contribute to the growing body of empirical studies of mechanisms and genetic consequences of plant invasions. From a management perspective, the knowledge of genetic diversity and population genetic structure of invasive plants is essential for improving the efficacy of control strategy (Wang et al. 2007). The more alike individuals are, the easier they should be to manage. The genetics should have a larger role in the development of policy to manage and control invasive species through a better understanding of the risk that particular genotypes pose. The concept could also be applied to other invasive clonal species. It would be interesting for future studies to investigate the pattern of distribution of the species along its dispersal route.

#### Sources of Materials

<sup>1</sup> Starter primer kits, Life Technology, Gibco BRL, Gaithersburg, MD, USA.

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## Literature Cited

- Albert, T., O. Raspe, and A. L. Jacquemart. 2003. Clonal structure in *Vaccinium myrtillus* L. revealed by RAPD and AFLP markers. *Int. J. Plant Sci.* 164:649–655.
- Anderson, E. and G. L. Stebbing Jr. 1954. Hybridization as an evolutionary stimulus. *Evolution* 8:378–388.
- Arens, P., C. J. Grashof-Bokdam, T. van der Sluis, and M. J. M. Smulders. 2005. Clonal diversity and genetic differentiation of *Maianthemum bifolium* among forest fragments of different age. *Plant Ecol.* 179:16–180.
- Barrett, S. C. H. and B. C. Husband. 1990. The genetics of plant migration and colonization. Pages 254–277 in A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir, eds. *Plant Population Genetics, Breeding, and Genetic Resources*. Sunderland, MA: Sinauer Associates.
- Brown, J. H. and A. Kodric-Brown. 1977. Turnover rates in insular biogeography: effect of immigration on extinction. *Ecology* 58:445–449.
- Bryson, C. T. and R. Carter. 1993. Cogongrass, *Imperata cylindrica*, in the United States. *Weed Technol.* 7:1005–1009.
- D'Antonio, R., L. Meyerson, and J. Denslow. 2001. Research priorities related to invasive exotic species. Pages 59–60 in M. Soule, G. Orians, and P. Dee Boersma, eds. *Conservation Biology: Research priorities for the coming decade*. Covelo, CA: Island Press.
- Dickens, R. 1974. Cogongrass in Alabama after sixty years. *Weed Sci.* 22:177–179.
- Douhovnikoff, V., A. M. Cheng, and R. Dodd. 2004. Incidence, size and spatial structure of clones in second-growth stands of coast redwood, *Sequoia sempervirens* (Cupressaceae). *Am. J. Bot.* 91:1140–1146.
- Doyle, J. J. and J. L. Doyle. 1990. Isolation of DNA from fresh tissue. *Focus* 12:13–15.
- Dozier, H., J. F. Gaffney, S. K. McDonald, E. R. R. L. Johnson, and D. G. Shilling. 1998. Cogongrass in the United States: history, ecology, impacts, and management. *Weed Technol.* 12:737–743.
- Eckert, C. G., K. Lui, K. Bronson, P. Corradini, and A. Bruneau. 2003. Population genetics consequences of extreme variation in sexual and clonal reproduction in an aquatic plant. *Mol. Ecol.* 12:331–344.
- Ellstrand, N. C. and M. L. Roose. 1987. Patterns of genotypic diversity in clonal plant species. *Am. J. Bot.* 74:123–131.
- Ellstrand, N. C. and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants. Pages 7043–7050 in *Proceedings of the National Academy of Sciences of the United States of America*.
- 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.
- Faircloth, W. H., M. G. Patterson, J. H. Miller, and D. H. Teem. 2003. *Wanted Dead Not Alive: Cogongrass*. Auburn, AL: Alabama Cooperative Extension System Rep. ANR-1241. 4 p.
- Gabel, M. L. 1982. A biosystematic study of the genus *Imperata* (Gramineae: Andropogoneae). Ph.D. dissertation. Ames, IA: Iowa State University. 94 p.
- Gray, A. J. 1986. Do invading species have definable genetic characteristics? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 314:655–674.
- Hall, D. W. 1998. Is Cogon grass really an exotic? *Wildland Weeds* 1:14–15.
- Hitchcock, A. S. 1950. *Manual of the Grasses of the United States*. 2nd ed, revised by Chase A. Washington, DC: U.S. Department of Agriculture. Misc. Pub. No. 200. 1051 p.
- Holm, L. G., P. Donald, J. V. Pancho, and J. P. Herberger, eds. 1977. *The World's Worst Weeds: Distribution and Biology*. Honolulu: The University Press of Hawaii. 609 p.
- Holsinger, K. E. and P. O. Lewis. 2003. HICKORY: a package for analysis of population genetic data V1.0. <http://www.eeb.uconn.edu>.
- Holsinger, K. E., P. O. Lewis, and K. D. Dipak. 2002. A Bayesian approach to inferring population structure from dominant markers. *Mol. Ecol.* 11:1157–1164.
- Holsinger, P. E. and L. A. Wallace. 2004. Bayesian approaches for the analysis of population genetic structure: an example from *Platanthera leucophaea*. *Mol. Ecol.* 13:887–894.
- Hubbard, C. E., R. O. Whyte, D. Brown, and A. P. Gray. 1944. *Imperata cylindrica*: taxonomy, distribution, economic significance and control. Imperial Agricultural Bureaux Joint Publication 7:1–63.
- Kreher, S. A., S. A. Fore, and B. S. Collins. 2000. Genetic variation within and among patches of the clonal species, *Vaccinium stamineum* L. *Mol. Ecol.* 9:1247–1252.
- McDermott, J. M. and B. A. McDonald. 1993. Gene flow in plant pathosystems. *Annu. Rev. Phytopathol.* 31:353–373.
- McDonald, S. K., D. G. Shilling, C. A. N. Okoli, T. A. Bewick, D. Gordon, D. Hall, and R. Smith. 1996. Population dynamics of cogongrass, *Imperata cylindrica*. Page 156 in *Proceedings of the Southern Weed Science Society of America*.
- McLellan, A. J., D. Prati, O. Kaltz, and B. Schmid. 1997. Structure and analysis of phenotypic and genetic variation in clonal plants. Pages 185–210 in H. de Kroon and J. van Groenendaal, eds. *The Ecology and Evolution of Clonal Plants*. Leiden, The Netherlands: Backbuys.
- Mueller, U. G. and L. L. Wolfenbarger. 1999. AFLP genotyping and fingerprinting. *Trends Ecol. Evol.* 14:389–394.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–590.
- Nei, M. and W. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Pages 5269–5273 in *Proceedings of the National Academy of Sciences of the USA*.
- Novack, S. J., R. N. Mack, and P. S. Soltis. 1993. Genetic variation in *Bromus tectorum* (Poaceae): introduction dynamics in North America. *Can. J. Bot.* 71:1441–1448.
- Pappert, R. A., J. L. Hamrick, and L. A. Donovan. 2000. Genetic variation in *Pueraria lobata* (Fabaceae), an introduced, clonal, invasive plant of the southeastern United States. *Am. J. Bot.* 87:1240–1245.
- Pornon, A., N. Escaravage, P. Thomas, and P. Taberlet. 2000. Dynamics of genotypic structure in clonal *Rhododendron ferrugineum* (Ericaceae) populations. *Mol. Ecol.* 9:1099–1111.
- Rolf, F. J. 1998. NTSys-pc, version 2.0: Numerical taxonomy and multivariate analysis system. <http://exetersoftware.com/cat/ntsyspc/ntsyspc>.
- Sarthou, C., S. Samadi, and M. C. Boisselier-Dubayle. 2001. Genetic structure of the saxicole *Pitcairnia geyskesii* (Bromeliaceae) on inselbergs in French Guiana. *Am. J. Bot.* 88:861–868.
- Schneider, S., D. Roessli, and L. Excoffier. 2000. *Arlequin*, Version 2000: Software for Population Genetics Data Analysis. <http://anthropologie.unige.ch/arlequin/>.
- Shilling, D. G., T. A. Beckwick, J. F. Gaffney, S. K. McDonald, C. A. Chase, and E. R. L. Johnson. 1997. Ecology, Physiology, and Management of Cogongrass (*Imperata cylindrica*). Bartow, FL: Florida Institute of Phosphate Research Rep. 03-1070-140. 144 p.
- Simberloff, D. and B. Von Holle. 1999. Positive interactions of nonindigenous species: invasional meltdown? *Biol. Invasions*. 1:21–32.
- Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* 47:264–279.

- Spiegelhalter, D. J., N. G. Best, B. P. Carlin, and A. van der Linde. 2002. Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society, series B* 64:583–639.
- Tabor, P. 1952. Cogongrass in Mobile County, Alabama. *Agron. J.* 44:50.
- Tsutsui, N. D. and T. J. Case. 2001. Population genetics and colony structure of the Argentine ant (*Linepithema humile*) in its native and introduced ranges. *Evolution* 55:976–985.
- Vilà, M., E. Weber, and M. D'Antonio. 2000. Conservation implications of invasion by plant hybridization. *Biol. Invasions* 2:207–217.
- Vos, P., R. Hogers, M. Reijam, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23: 4407–4414.
- Wang, T., Y. Su, and G. Chen. 2007. Population genetic variation and structure of the invasive weed *Mikania micrantha* in Southern China: consequences of rapid range expansion. *Journal of Heredity*. DOI 10.1093/jhered/esm080.
- Whitlock, M. C. and D. E. McCauley. 1999. Indirect measure of gene flow and migration:  $F_{ST} \approx 1/(4Nm + 1)$ . *Heredity* 82:117–125.
- Wright, S. 1951. The genetical structure of populations. *Ann. Eugen.* 15:323–354.
- Yeh, F. C. and T. J. B. Boyle. 1997. Popgene, the user-friendly shareware for population genetic analysis. <http://www.ualberta.ca/~fyeh/index.htm>.

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