

Invasion Success in Cogongrass (*Imperata cylindrica*): A Population Genetic Approach Exploring Genetic Diversity and Historical Introductions

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Propagule pressure significantly contributes to and limits the potential success of a biological invasion, especially during transport, introduction, and establishment. Events such as multiple introductions of foreign parent material and gene flow among them can increase genetic diversity in founding populations, often leading to greater invasion success. We applied the tools and theory of population genetics to better understand the dynamics of successful biological invasion. The focal species, cogongrass, is a perennial invasive grass species significantly affecting the Gulf Coast and southeastern region of the United States. The literature indicates separate, allopatric introductions of material from East Asia (Philippines and Japan) into the U.S. states of Mississippi and Alabama. Molecular analysis of samples from those two states utilized amplified fragment length polymorphism (AFLP) markers on 388 individuals from 21 localities. We hypothesized that previously isolated lineages of cogongrass are present and crossing in the Southeast. We observed genetic variation within localities ($0.013 \leq \text{heterozygosity } (H_e) \leq 0.051$, mean = 0.028 ± 0.001) with significant and substantial population structure ($F_{ST} = 0.534$, $P < 0.001$). Population structure analyses detected two genetically defined and statistically supported populations, which appear to have experienced some admixture. The geographic distribution of those populations was consistent with the two-introduction scenario reported previously. These results are also consistent with contact in the invasive range of previously isolated lineages from the native range.

Nomenclature: *Imperata cylindrica* (L.) Beauv. IMCY.

Key words: AFLP, Alabama, genetic diversity, *Imperata*, Mississippi, Poaceae, population structure.

Propagule pressure is a function of dispersal limitation and is understood as the number and quality of individuals transported and introduced to an area over some focal time period (Eppstein and Molofsky 2007; Lockwood et al. 2005; Lonsdale 1999). Propagule pressure is a significant factor contributing to, or limiting, the successful introduction and establishment of nascent invasive species (Coulatti et al. 2006; Lockwood et al. 2005; Lonsdale 1999). Propagule pressure also is a significant driver throughout all stages of invasion, and is the factor most frequently modified by anthropogenic activities (Catford et al. 2009).

It can be strengthened by greater numbers of individuals per introduction and multiple introductions, both of which increase genetic diversity in a species' introduced range (Catford et al. 2009; Lockwood et al. 2005). Increased propagule pressure has an inherent facilitative effect on the success of biological invasions.

Multiple introductions contribute to propagule pressure by increasing both the total number of individuals introduced *and* the cumulative genetic diversity introduced into a novel range. Propagules can originate from the same parent source or from varied conspecific populations separated by physical distance and/or other barriers to dispersal; source populations need not necessarily coexist in the species' native range. Multiple introductions of exotic propagules may result in gene flow between previously isolated lineages (Lee 2002). Such outcrossing can alter genetic diversity within and among populations, potentially increasing genetic variation to levels greater than parent populations in the native range (Dlugosch and Parker

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Management Implications

Cogongrass is a major invasive weed of forestlands, rights-of-way, agricultural areas, and natural ecosystems of the southeastern United States. The present study investigated patterns of genetic diversity and divergence in cogongrass from areas near the reported sites of this species' initial introduction to the United States. Data from this study provide support for the reports that the species was introduced from two locations in the native range, as we detected two distinct genetic groups: one in central Mississippi and another in southern Mississippi and Alabama. A further insight from this work is the finding that cogongrass appears to rely much more heavily on sexual reproduction than was previously thought. At most of the localized patches examined in this study (19 of 21 localities) each sampled individual had a unique genotype, suggesting that sexual reproduction dominates. Furthermore, population structure suggests a greater reliance on sexual outcrossing throughout the range examined here, than previously believed. Knowledge of cogongrass' genotypic distribution could yield benefits for future management efforts, particularly if management tactics affect the two genetic groups differentially.

2008; Hughes et al. 2008; Lavergne and Molofsky 2007; Lee 2002). In this way, multiple introductions, and gene flow between previously isolated genotypes can impart fitness benefits, such as increased fecundity or adaptive flexibility to biotic and abiotic conditions in the newly encountered environment (Lee 2002).

The structure of genetic variation within and between locations (hereafter population structure) often can reveal admixture that has occurred among previously isolated source populations following secondary contact in an introduced range (e.g., Genton et al. 2005; O'Hanlon et al. 1999; Walker et al. 2003). Population structure can thus be utilized to infer the historical frequency of introduction(s) (Lockwood et al. 2005; Ward et al. 2008). Where multiple introductions remain genetically distinct, the identification of distinct genetic lineages can allow management to target specific source populations during secondary invasion (i.e., during geographic spread of the invader).

Cogongrass [*Imperata cylindrica* (L.) Beauv.] is an invasive, perennial C4 grass with a widespread global distribution, essentially cosmopolitan in the tropical and subtropical regions of the world, and established on every continent with the exception of Antarctica (Bryson and Carter 1993; Hubbard et al. 1944). Documented introductions of cogongrass into the United States point to Asian parent material. In 1912, propagules from Japan were inadvertently introduced into Grand Bay, AL, and in 1921, propagules from the Philippines were purposefully introduced into McNeil, MS for forage (Tabor 1949, 1952). Other introductions of cogongrass might have occurred, but reliable documentation is lacking. Tabor (1949, 1952) did report that cogongrass was anthropogenically and purposefully transported from Mississippi and Alabama into Florida.

Cogongrass is highly variable in its morphology and ecology, known to be phenotypically plastic and morphologically variable among populations, in both the native and introduced ranges (Al-Jaboory and Hassawy 1980; Bryson et al. 2010). Significant genetic diversity, with associated phenotypic and genotypic variation, was found among populations of Taiwanese ecotypes (Cheng and Chou 1997; Chou and Tsai 1999). It is reasonable, therefore, to infer that invasive U.S. cogongrass might also possess substantial genetic variation and population structure, especially considering that phenotypic variation has been observed in Mississippi cogongrass (Bryson et al. 2010).

Most colonizing propagules experience bottlenecks in population size during the introduction process, which generally reduce genetic diversity in the founding population(s) (Dlugosch and Parker 2008; Luikart et al. 1998). Clonal or partially asexual organisms are more able to tolerate losses in genetic diversity than those that solely rely on outcrossing mode of reproduction (Ellstrand and Roose 1987; Pappert et al. 2000), and even in sexual species, bottlenecks might not prevent the spread and success of invasive organisms in the novel environment (Amsellem et al. 2000; Poulin et al. 2005; Salmon et al. 2005; Tsutsui et al. 2000;). Multiple introductions can increase genetic diversity in invasive populations (e.g., Japanese knotweed, Pashley et al. 2007; Walls 2010).

The present research quantified genetic diversity within and among sampled patches of cogongrass from Mississippi and Alabama, with the aim of examining geographic distribution of biological populations, defined here as genetically differentiated clusters of sampled individuals. Analyses utilized an established molecular technique, amplified fragment length polymorphism (AFLP). AFLPs are arbitrarily amplified and a highly reproducible, dominant genetic marker. AFLPs were selected for several reasons: no a priori sequence information was necessary, loci are presumably sampled across the entire genome, and the technique is practical in cost and data generation for studies such as this (Amsellem et al. 2000; Bussell et al. 2005; Campbell et al. 2003; Meudt and Clarke 2007). Additionally, previous AFLP analyses permit comparisons of genetic diversity and structure based on the same marker type. AFLPs are not without their disadvantages relative to other genetic markers, including an inability to directly quantify heterozygosity due to their dominant nature and limitations on the types of population genetic models that can be applied to them (Bonin et al. 2007; Bussell et al. 2005; Meudt and Clarke 2007). Whereas Capo-chichi et al. (2008) conducted a study of cogongrass populations utilizing two AFLP selective primer combinations, this research utilized six selective primer sets in an effort to improve the capture of genetic variation while minimizing the introduction of errors or biases, such as homoplasy.

Homoplasious errors can be avoided by incorporating more AFLP selective primers sets to increase capture of the number of fragments, reducing the likelihood of biases in frequency-based estimation, such as heterozygosity estimates and reducing genetic differentiation (Caballero et al. 2008; Koopman and Gort, 2004; Meudt and Clarke 2007). We discuss below (Materials and Methods) how the resulting data were managed to further avoid errors arising from homoplasy.

We focused this research on Mississippi and Alabama because the literature provides specific details on two introductions of previously isolated parent material into these areas (from Japan and the Philippines, Tabor 1949, 1952). We expected low within-population genetic diversity due to asexual reproductive capacity and reported transport of live rhizome material, assumed to be genetically identical to parent source material, across the region (Holly and Ervin 2006). Capo-chichi et al. (2008) observed most of the genetic variation partitioned within their sample sites near just one point of introduction (Grand Bay, AL). The partitioning of molecular variation detected by Capo-chichi et al. (2008) was unexpected, considering cogongrass' ability to reproduce asexually (see review of expected diversity and life history traits in Nybom 2004), and the authors did not detect an isolation-by-distance (IBD) relationship. They hypothesized that interspecific hybridization with the congeneric *I. brasiliensis* Trin. (Brazilian satintail) confounded expected IBD signals (Capo-chichi et al. 2008). Alternatively, we hypothesized that there were two introductions of distinct genotypes from the native range, and subsequent gene flow between them. A prediction stemming from our hypothesis is that population structure in cogongrass should provide evidence for more than one genetically distinct population (supporting Tabor 1949, 1952) as well as evidence of outcrossing between distinct groups.

Materials and Methods

Sampling. Cogongrass patches occurred in various habitats, including rights-of-way (ROW), forests, and grassy areas. Some location information was provided by cooperators that graciously assisted with tissue location and collection. Sites were identified as contiguous patches of cogongrass, often occurring as circular monocultures in open areas or as long, narrow ones along ROW. The number of genets (genetically identical ramets in the same location) per locality was unknown in the field. Therefore, sampling was conducted in a systematic manner to collect individuals from the edge and the center of patches to ensure a comprehensive snapshot of the genetic diversity of the patch. Not all sites were evenly sampled in terms of the spatial extent, some being over- or undersampled to achieve our aim of 20 sampled "individuals" per location. We

assumed each tiller, or ramet, to be representative of an "individual" (while acknowledging that individual patches can arise from only one to a few colonizing propagules).

Live leaf tissues were collected from cogongrass patches in Mississippi and Alabama during the spring and summer of 2009. We sampled 180 individuals from 11 sites in Mississippi (MS): four from Desoto National Forest (Jones, Greene, and Wayne counties), four from Bienville National Forest (Scott, Jasper, and Smith counties), and three from around the Biloxi metro area of the Mississippi Coast (Harrison County). We sampled 208 individuals from 10 sites in Alabama (AL): six from the vicinity of Mobile Bay (Baldwin, Mobile, and Washington counties), two from the Talladega National Forest (Hale County), one from West Alabama (Sumter County), and one from Auburn (Lee County). Overall, 21 patches were sampled ($N = 388$ individuals, Figure 1). All patches were separated by at least 1 km, with the exception of paired populations AL-2/AL-3 and MS-9/MS-10, which were sampled from large contiguous patches in excess of 4 km². All patches were sampled from the edges and interior, from corner to corner forming an "X", in an effort to capture a representative sampling of genetic diversity. Leaf tissues were stored in individually labeled plastic bags and transported in a cooler with ice or ice substitute for a maximum of 24 h before being placed in a refrigerator or freezer, or dried. Leaf tissues were dried by placing tissues in silica gel with a color indicator. Because cogongrass is a listed Federal Noxious Weed, all sampling was conducted with approval by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Plant Pest Quarantine (Permit #: P526P-12-00211, P526-080721-005).

Tissue Processing and Molecular Analysis. DNA extractions utilized a modified NucPrep[®] Chemistry: Isolation of Genomic DNA from Animal and Plant Tissue protocol (Life Technologies, Carlsbad, CA, USA). Roughly 1 cm² of individual leaf tissue was transferred aseptically into a 2-ml microcentrifuge tube; tissues were then fully disrupted with a Retsch mixer mill. Extracted and purified DNA were transferred into sterile, individually labeled tubes and kept in freezer(s) until analysis (−20 C short-term storage, −80 C long-term storage).

A modified AFLP protocol for capillary electrophoresis was used for molecular analysis, based on technical methodology developed by Vos et al. (1995). Specific reagents are specified in Lucardi (2012). Individually extracted DNA was digested by restriction enzymes, linking primers were ligated, and preselective amplification was carried out by polymerase chain reactions (PCR). Selective amplification generated fragment-based marker sets to allow for detection of polymorphisms. Restriction digest of DNA in 25- μ l reactions were incubated at 37 C for 2 h in a

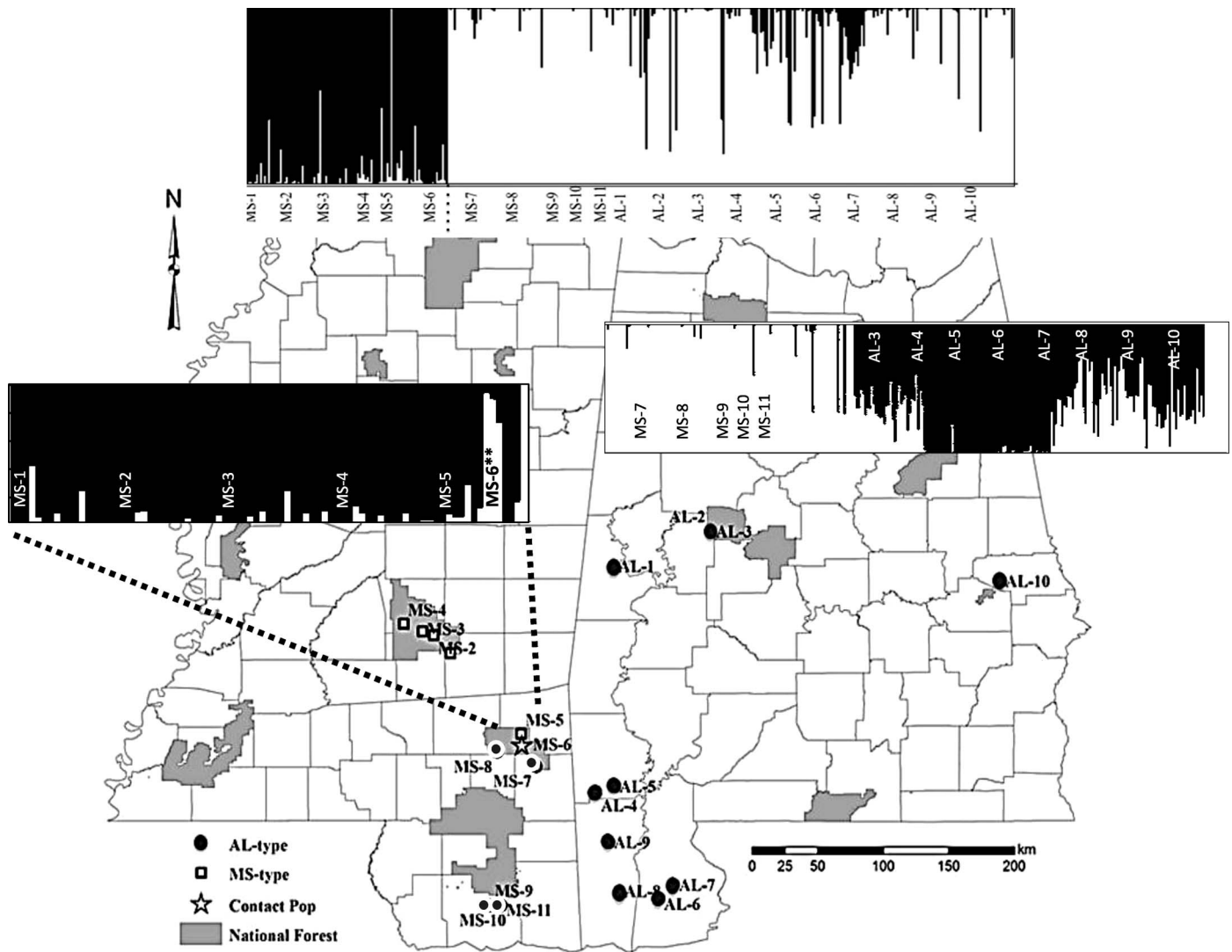


Figure 1. Map of 21 cogongrass sites sampled in Mississippi (MS) and Alabama (AL), USA, with STRUCTURE bar plots ($K = 2$). Top bar plot represents all samples ($N = 388$). MS-type (square symbols) bar plot analyzed localities MS-1 through 5 and 12 individuals from MS-6 ($n = 102$, including samples from Bienville and Desoto national forests). AL-type bar plot (circle symbols) analyzed eight individuals from MS-6, MS-7 through 11, and all Alabama localities. Gray-shaded areas represent National Forest acquisition boundaries. Site MS-6 is represented by a star.

thermal cycler, followed by enzyme denaturation (15 min at 70 C). Eco and Mse linkers were ligated to digested DNA (20- μ l reactions) for 3 h at 37 C or overnight at 16 C. Ligated reactions were stored at -80 C to minimize degradation. Preselective amplifications were conducted in 20- μ l PCR amplification: initial denaturing step 1 min at 94 C, 30 cycles (30 s at 94 C, 1 min at 56 C, and 1 min at 72 C), and final annealing for 2 min at 72 C. Individual pre-selective amplification products were diluted with sterile water (1 : 20). Six selective primer sets were applied to each individual in this study (Table 1). Selective primers sets are fluorescently tagged in different colors, allowing multiple PCR products to be combined and analyzed simultaneously. Each selective primer set consists of a

selective Mse and a fluorescent Eco primer (Table 1). Selective PCR amplifications were conducted in 20- μ l reactions: denaturing step of 2 min at 94 C, 10 cycles (30 s at 94 C, 30 s at 65 C, and 1 min at 72 C, reducing annealing temperature by 1 C/cycle), 30 cycles (30 s at 94 C, 30 s at 56 C, and 1 min at 72 C), and finished with 30 s at 72 C.

Three fluorescently tagged products (1.5 μ l per individual) were combined per well with fluorescent ROX-1000 size standard (0.25 μ l; MapMarker, 50-1000, BioVentures, Inc., Murfreesboro, TN, USA). Individually combined wells were fixed with formamide (10 μ l, Hi-DiTM, Life Technologies, Carlsbad, CA, USA) and pooled fragment products were run on an ABI 3730 capillary sequencer at

Table 1. Primer nucleotide sequences and fluorophore information for modified amplified fragment length polymorphism (AFLP) protocol.

	Sequence(5'–3')	Fluorophore color
Linkers		
<i>EcoR</i> I Linker		
EcoLinker I	CTCGTAGACTGCGTAACC	
EcoLinker II	AATTGGTACGCATCTAC	
<i>Mse</i> I Linker		
MseLinker I	GACGATGAGTCCTGAG	
MseLinker II	TACTCAGGACTCAT	
Pre-Amp		
<i>EcoR</i> I		
Eco+A	GACTGCGTACCAATTC-A	
<i>Mse</i> I		
Mse+C	GATGAGTCCTGAGTAA-C	
Sel-Amp		
<i>EcoR</i> I		
Eco+AXX	^a GACTGCGTACCAATTC- AXX	
<i>Mse</i> I		
Mse+CXX	GATGAGTCCTGAGTAA- CXX	
Selective combinations		
1		
EcoRI-ACT-FAM	MseI-CAT	Blue
2		
EcoRI-AGG-HEX	MseI-CTA	Green
3		
EcoRI-AGC-NED	MseI-CTG	Yellow
4		
EcoRI-ACT-FAM	MseI-CTT	Blue
5		
EcoRI-AGG-HEX	MseI-CTC	Green
6		
EcoRI-AGC-NED	MseI-CAC	Yellow
Size standard		
ROX-MM		Red

^a Fluorophore; **Bold** denotes modifiable codons for selective PCR amplifications.

Arizona State University, DNA Lab, Tempe, AZ. Associated positive and negative control sample replicates accompanied each run. Positive control replicates consisted of a single randomly selected individual following each step of analysis to verify reproducibility. Negative control replicates were absent of genomic DNA to check for potential contamination. The average number of band mismatches or errors were derived from positive control replicates (SE = 0.004; 95% confidence interval of four incorrect bands per individual) which suggested good reproducibility.

Data Management and Analysis. Fragment data were digitally visualized in GeneMarker[®] (SoftGenetics, LLC,

State College, PA, USA); data were exported into general text format for input to Excel 2007 (Microsoft Corporation, Redmond, WA, USA.). Fragments were sorted based on migration size (base pairs) and objectively autoscored utilizing an independently developed procedure (Lucardi 2012; Lucardi and Walker, unpublished methodology) that utilized both Excel 2007 and PASW v.18.0 (SPSS, IBM Corporation, Armonk, NY, USA). Data matrices were created from scored fragment data and autopopulated over several steps in both software programs. We coded “0” for absence and “1” for presence in data matrices. Detected polymorphic loci less than 200 base pairs were removed from statistical analyses to avoid potential effects of

fragment-size homoplasy (the result of comigrating bands during electrophoresis that are not of the same physical locus in the genome), due to the disproportionate number of smaller fragments produced by AFLPs (Bonin et al. 2007; Koopman and Gort 2004).

Data conversions of AFLP data matrices utilized functions within the R-package, AFLPdat source script (Ehrich 2006). Genetic diversity within populations assessed the number of polymorphic and private bands, percentage of polymorphic loci, and expected heterozygosity (biased, H_e and unbiased, UH_e) based on Hardy-Weinberg equilibrium expectations (HWE, Nei 1978). HWE assumptions can reduce accuracy in allele frequency estimations from dominant data, such as AFLPs, but reliable results can be achieved through adequate population sampling and a sufficient number of primer sets to generate a large number of polymorphisms (Bonin et al. 2007; Mariette et al. 2002; Meudt and Clarke 2007). Shannon's Diversity Index (I) was calculated for the 21 sample sites in GenAEx 6.3 (Peakall and Smouse 2006; Sherwin et al. 2006). Cogongrass is able to produce ramets clonally; therefore, we also assessed the number of unique multilocus genotypes present in each patch for a more accurate appraisal of within-patch and overall genetic diversity. We measured the number of unique multilocus genotypes and genotypic diversity with the "Clones" function within AFLPdat. This source package requires an error parameter for the number of band mismatches to account for errors in genotyping and/or scoring (Ehrich 2006). For this purpose and publication, we utilized four mismatches as derived from our positive control replicates. "Clones" function estimates genotype diversity (Nei 1987), effective number of genotypes (Parker 1979), and Nei's gene diversity (1987).

Unbalanced sampling among localities can introduce errors and skew interpretation of frequency-based genetic diversity estimates, especially because fewer individuals would be contributing genetic information. Nonparametric Spearman's correlation values (ρ) were calculated between estimated genetic diversity metrics (H_e , UH_e , I, Nei's gene diversity, and genotype diversity) and locality sample sizes. Significance for Spearman's ρ was set at P value < 0.05, and conducted in R (v.3.0.1; R-project for statistical computing; www.r-project.org) using "cor.test" with Spearman's method.

Genetic distance between all patches with Nei's pairwise unbiased genetic distance (Nei 1972, GenAEx ver. 6.3) and population pairwise F_{ST} (Arlequin ver.3.5; Excoffier and Lischer 2010) were calculated. The relationship between genetic differentiation (pairwise F_{ST}) and spatial distance (km) was determined via linear regression in Excel 2007. Bayesian analysis of population structure with introduction of a priori information was conducted in STRUCTURE (ver.2.3.3, Pritchard et al. 2000). This

method assesses probabilities of the number of clusters (K) that best fits and is most biologically appropriate for the dataset. Therefore, the most appropriate K was initially unknown. We conducted eight simulations per K (1 through 8) to statistically select the most appropriate K using the Evanno et al. (2005) method; other parameters included admixture ancestry model, burn-in of 10,000, and 50,000 MCMC (Markov Chain Monte Carlo) (Pritchard et al. 2000). Posterior probability values from each simulation were used for determination of ΔK (an ad hoc statistic) to infer the most appropriate and "true" K , which is the mode of likelihood distribution acceleration or where ΔK is greatest. One limitation to the Evanno et al. (2005) method, is that the smallest level of structure that can be detected is 2. Equations for calculating ΔK and intermediaries can be found in Evanno et al. (2005). Multiple runs of each K were simulated to allow data convergence and statistical support. Because the Evanno et al. (2005) method finds the highest level of structure in a data set, we conducted additional independent simulations for the two subpopulations that were identified in the global STRUCTURE analysis and evaluated posterior probability values also using Evanno et al. (2005) method to further identify hierarchical population structure. Population structure was further assessed with principal coordinates analysis (PCA, GenAEx ver.6.3) of individual genetic covariance with data standardization ($N = 388$).

Analysis of molecular variance (AMOVA) tests the degree of genetic differentiation based on specific and defined population structure (Excoffier et al. 1992). Two AMOVAs were conducted on the matrix of genetic distances among individuals in Arlequin ver.3.5 (Excoffier and Lischer 2010). We first tested the degree of genetic differentiation between groups based on geographic location (Mississippi or Alabama). We then tested population structure between detectable biological populations as inferred from the above analyses. These were conducted to determine if greater genetic differentiation exists between biologically or geographically designated populations. Stronger population structure between biological populations would suggest population structure is more influenced by genetic mechanisms than by geographic distance.

Results and Discussion

Genetic Diversity. AFLP analysis detected 850 polymorphic loci among all cogongrass individuals sampled from localities in Mississippi and Alabama ($N = 388$). The number of detected polymorphisms exceeds another recommended minimum of "500 AFLP loci" for intraspecific examination of an outcrossing species (Bonin et al. 2007; Mariette et al. 2002; Meudt and Clarke 2007). A larger number of detected AFLP loci achieved through an increase in the number of selective primer pairs improves

accuracy of genetic diversity estimates within and among populations, such that AFLPs are comparable to other molecular markers, such as microsatellites (Mariette et al. 2002; Nybom 2004).

The number of private bands (present in only one locality) observed per site ranged from 0 to 127. The percentage of polymorphic loci detected per location ranged from 4 to 32% with an average value of 14% ($SE \pm 2\%$). Heterozygosity (H_e) was averaged over each patch, resulting in mean values ranging from 0.013 to 0.051 (overall mean $H_e = 0.028$, $SE \pm 0.001$; Table 2). Mean unbiased expected heterozygosity (UH_e) ranged from 0.013 to 0.053, (mean $UH_e = 0.030$, $SE \pm 0.001$). We should note that both heterozygosity values are based on HWE expectations, to which it is unlikely that cogongrass populations conform, especially considering unbalanced sampling among clonal patches. UH_e did not deviate from H_e beyond SE for all sites sampled. Shannon's Information Index (I) ranged from 0.020 to 0.088, with an overall mean of 0.047 ($SE \pm 0.001$; Table 2), resulting in similar relative levels of variation as measures of heterozygosity. Location AL-4 (Washington County, AL), near the Mississippi border (Figure 1), contained the highest genetic diversity among sampled patches ($H_e/UH_e = 0.049/0.051$, $I = 0.088$). Cogongrass persistence and invasion has been attributed to localized spread due to asexual reproduction via resilient, rapidly growing rhizomes (Bryson and Carter 1993; Holly and Ervin 2006). The percentage of detected polymorphisms is consistent with a species that obligately outcrosses, which was somewhat unexpected, given the presumed reliance upon clonal propagation in cogongrass persistence (Gabel 1982; Nybom 2004). However, the mean percentage of polymorphic loci, among all localities, is lower than reported values in other studies also utilizing AFLP analysis in introduced grass species (79%, Båba et al. 2012, *Brachypodium pinnatum* (L.) P. Beauv. BRPI; 90%, Li et al. 2006, *Bromus inermis* Leyss. BRIN2; 28%, Nissar et al. 2010, *Pennisetum ciliare* (L.) Link PEI). Mean heterozygosity was also less than other plant species analyzed with dominant molecular markers, regardless of demographic life history characteristics, including the type of breeding system, breadth of range, dispersal strategy, or successional status (Nybom 2004).

Clonal analysis detected two localities with a genotype diversity less than 0.96, and each sampled ramet represented a unique genotype in half of analyzed patches (Table 3). When genotypic diversity is equal to 1, each individual (ramet) sampled is a unique multilocus genotype (or unique genet); deviations from 1 in this dataset suggest ramets share identical genotypes, indicating some degree of clonality. Overall, 349 genotypes were observed from all 388 individuals analyzed. The effective number of genotypes from this analysis was estimated at 318. The error parameter required for "Clones" was based on the

95% confidence interval (850 ± 4 bands) among positive control replicates.

Two localities seem to have experienced a reduction in genotypic diversity relative to the other sites in this study: MS-8 (genotype diversity = 0.71) and MS-11 (0.87). These sites have likely relied more on clonal propagation than the other locations. The majority of analyzed cogongrass localities consisted of genetically unique individuals possessing high genotypic diversity (genotype diversity > 0.90), and indicative of sexual reproduction and outcrossing (Kreivi et al. 2005). Cogongrass is generally considered an obligate outcrosser (Gabel 1982); however, the rate and frequency of outcrossing is not well-documented, nor understood. Localized cogongrass persistence and expansion has been hypothesized to rely heavily on asexual rhizomatous growth; however, observed levels of genetic and genotypic diversity among samples suggests cogongrass might benefit from reproductive flexibility, with both sexual and clonal propagation being important in the U.S. invasion, as has been shown for other plant invasions (Vellend et al. 2010).

Sample size per locality was disparate, potentially introducing unaccounted variance or disparities in estimating genetic diversity. Therefore, Spearman's ρ correlation tests were conducted to determine if genetic diversity parameters were correlated to sample sizes (n). We found no significant correlations ($P < 0.05$) between genetic diversity estimates (H_e , UH_e , I, Nei's gene diversity, and genotype diversity) and local sample sizes (Table 4); therefore, our estimates and inferences made from genetic and genotypic diversity do not appear to be influenced by incongruent sampling of ramets among localities, and can be considered reliable.

Population Structure. Nei's pairwise unbiased genetic distances between populations (D) ranged from 0.002 to 0.058 (Table 5; Nei 1972). Greatest genetic distance observed was between MS-3 and MS-9 ($D = 0.058$); similar pairwise distances (0.051 to 0.056) were also observed between other localities, indicative of genetic structure among many patches within Mississippi. Larger distances were noted between central Mississippi locations (MS-1 through 6) and Mississippi Gulf Coast sites, near Biloxi (MS-9, 10, 11). This pattern, in concert with genetic diversity estimates, indicates that central Mississippi cogongrass is genetically distant from patches sampled in Alabama and coastal Mississippi.

Pairwise F_{ST} values between sites ranged from 0.022 to 0.816, and all values were statistically significant ($P < 0.05$) (Table 5). The maximum level of genetic dissimilarity between locations was observed between MS-8 and AL-8 ($F_{ST} = 0.816$). Alternatively, the most similar localities were MS-9 and MS-10 ($F_{ST} = 0.022$), AL-4 and AL-5 ($F_{ST} = 0.037$), and AL-8 and AL-9 ($F_{ST} = 0.055$),

Table 2. Genetic diversity indices with location information. Population information, genetic diversity indices (H_e = expected heterozygosity, UH_e = unbiased expected heterozygosity, I = Shannon's information index) for 21 sampled cogongrass populations in Mississippi (MS) and Alabama (AL). Overall totals and averages are in **bold**.^a

Locality	County, state	Other location information	Number of individuals	Number of bands detected	Number of private bands	Polymorphic loci	$H_e \pm SE$	$UH_e \pm SE$	Shannon's information index $\pm SE$
			<i>n</i>			%			I
MS-1	Jasper Co., MS	Bienville NF	20	159	17	17	0.035 \pm 0.003	0.036 \pm 0.004	0.058 \pm 0.005
MS-2	Smith Co., MS	Bienville NF	20	136	10	14	0.035 \pm 0.004	0.037 \pm 0.004	0.056 \pm 0.005
MS-3	Scott Co., MS	Bienville NF	20	150	7	15	0.041 \pm 0.004	0.043 \pm 0.004	0.064 \pm 0.006
MS-4	Scott Co., MS	Bienville NF	10	113	3	11	0.032 \pm 0.003	0.035 \pm 0.004	0.050 \pm 0.005
MS-5	Jones Co., MS	DeSoto NF	20	225	36	26	0.051 \pm 0.004	0.053 \pm 0.004	0.086 \pm 0.006
MS-6	Greene Co., MS	DeSoto NF	20	200	48	23	0.048 \pm 0.004	0.051 \pm 0.004	0.079 \pm 0.006
MS-7	Wayne Co., MS	DeSoto NF	20	88	10	10	0.016 \pm 0.002	0.017 \pm 0.002	0.029 \pm 0.003
MS-8	Wayne Co., MS	DeSoto NF	20	83	6	9	0.013 \pm 0.002	0.013 \pm 0.002	0.024 \pm 0.003
MS-9	Harrison Co., MS	DeSoto NF	10	48	6	5	0.018 \pm 0.003	0.019 \pm 0.003	0.027 \pm 0.004
MS-10	Harrison Co., MS		10	41	2	4	0.013 \pm 0.002	0.014 \pm 0.002	0.020 \pm 0.003
MS-11	Harrison Co., MS		10	64	10	7	0.015 \pm 0.002	0.017 \pm 0.002	0.026 \pm 0.003
AL-1	Sumter Co., AL		20	141	25	16	0.025 \pm 0.003	0.026 \pm 0.003	0.045 \pm 0.004
AL-2	Hale Co., AL	Talladega NF	20	218	34	26	0.043 \pm 0.003	0.045 \pm 0.003	0.077 \pm 0.005
AL-3	Hale Co., AL	Talladega NF	20	99	15	11	0.022 \pm 0.003	0.023 \pm 0.003	0.037 \pm 0.004
AL-4	Washington Co., AL		20	283	127	32	0.049 \pm 0.003	0.051 \pm 0.003	0.088 \pm 0.005
AL-5	Washington Co., AL	Frank Boykin WMA	20	109	0	10	0.025 \pm 0.003	0.026 \pm 0.003	0.040 \pm 0.004
AL-6	Baldwin Co., AL		20	123	4	12	0.031 \pm 0.003	0.032 \pm 0.003	0.050 \pm 0.005
AL-7	Baldwin Co., AL		20	91	0	10	0.022 \pm 0.003	0.023 \pm 0.003	0.036 \pm 0.004
AL-8	Mobile Co., AL		20	85	1	9	0.019 \pm 0.003	0.020 \pm 0.003	0.031 \pm 0.004
AL-9	Mobile Co., AL		20	74	12	8	0.016 \pm 0.002	0.017 \pm 0.002	0.026 \pm 0.004
AL-10	Lee Co., AL	Auburn	28	177	67	20	0.025 \pm 0.002	0.026 \pm 0.003	0.046 \pm 0.004
Overall total or average			388	850		14 \pm 2 SE	0.028 \pm 0.001	0.030 \pm 0.001	0.047 \pm 0.001

^a Abbreviations: SE, standard error; Co., County; NF, National Forest; WMA, Wildlife Management Area.

Table 3. Clonal diversity analysis from AFLP multilocus data. Population identifiers, sample size, and resulting clonal diversity from multilocus amplified fragment length polymorphism (AFLP) data, including number of different genotypes detected, genotypic diversity, effective number of genotypes, and Nei's gene diversity for each of the 21 analyzed locations. Overall totals and averages are in **bold**.^a

Population ^a	Number of individuals	Number of genotypes	Genotype diversity	Effective number of genotypes	Nei's gene diversity
	<i>n</i>				
MS-1	20	20	1	20	0.036
MS-2	20	20	1	20	0.037
MS-3	20	20	1	20	0.043
MS-4	10	10	1	10	0.035
MS-5	20	20	1	20	0.053
MS-6	20	18	0.984	15.385	0.052
MS-7	20	17	0.979	14.286	0.019
MS-8	20	10	0.711	3.077	0.022
MS-9	10	9	0.978	8.333	0.021
MS-10	10	9	0.978	8.333	0.015
MS-11	10	7	0.867	4.545	0.023
AL-1	20	20	1	20	0.026
AL-2	20	18	0.989	16.667	0.049
AL-3	20	20	1	20	0.023
AL-4	20	20	1	20	0.051
AL-5	20	20	1	20	0.026
AL-6	20	20	1	20	0.032
AL-7	20	19	0.995	18.182	0.024
AL-8	20	15	0.963	11.765	0.023
AL-9	20	17	0.979	14.286	0.018
AL-10	28	20	0.963	14	0.032
Overall	388	349		318.858	

^a Abbreviations: MS, Mississippi; AL, Alabama.

MS-3 and MS-4 ($F_{ST} = 0.074$). These four pairs also share county of origin in addition to a high degree of genetic information (see Table 2 and Figure 1). This might suggest a relationship between low genetic differentiation (low F_{ST}) and geographic proximity (i.e., an isolation-by-distance, or IBD, pattern). This pattern of high genetic similarity (low F_{ST}) among paired populations within counties might be due to intracounty maintenance (of ROW, for example) contributing toward within-county spread of propagules.

Low genetic distances among more spatially distant populations might be the result of long-distance gene flow, observable between populations AL-9 (near Mobile Bay) and AL-10 (in Lee County, near Auburn University) ($D = 0.002$), AL-3 and AL-4 ($D = 0.003$), and AL-1 and AL-2 ($D = 0.004$). Pairwise F_{ST} values between locations also indicate genetic similarity among patches sampled from central Mississippi (MS-1 through 6), but genetic differentiation increases between those and all other patches

Table 4. Nonparametric Spearman's correlation values (ρ) between locality sample sizes and genetic diversity metrics. No significant correlations present.

Spearman's correlation coefficient (ρ), P value set at 0.05	Heterozygosity	Unbiased heterozygosity	Shannon's information index	Nei's gene diversity	Genotype diversity
Locality sample size					
<i>n</i>	H^c	UH_c	I		
ρ	0.342	0.316	0.382	0.346	0.150
P value	0.123	0.163	0.087	0.124	0.515

Table 5. Pairwise matrix of Nei's unbiased genetic distances (*D*) (lower left portion of matrix) and pairwise F_{ST} matrix (upper right portion of matrix) among sampled locations.^a

		Pairwise population F_{ST}																					
		MS-1	MS-2	MS-3	MS-4	MS-5	MS-6	MS-7	MS-8	MS-9	MS-10	MS-11	AL-1	AL-2	AL-3	AL-4	AL-5	AL-6	AL-7	AL-8	AL-9	AL-10	
MS-1	*																						
MS-2		0.152																					
MS-3		*	0.139																				
MS-4			*	0.074																			
MS-5				*	0.092																		
MS-6					*	0.163																	
MS-7						*	0.015																
MS-8							*	0.002															
MS-9								*	0.015														
MS-10									*	0.022													
MS-11										*	0.276												
AL-1											*	0.186											
AL-2												*	0.160										
AL-3													*	0.302									
AL-4														*	0.383								
AL-5															*	0.037							
AL-6																*	0.479						
AL-7																	*	0.140					
AL-8																		*	0.357				
AL-9																			*	0.055			
AL-10																				*	0.433		
Nei's Unbiased Genetic Distance																							

^aAll pairwise F_{ST} values are significant ($P < 0.05$). Pairwise values are among 21 locations. Bold values are genetic distances and F_{ST} values between MS-1 through MS-6.

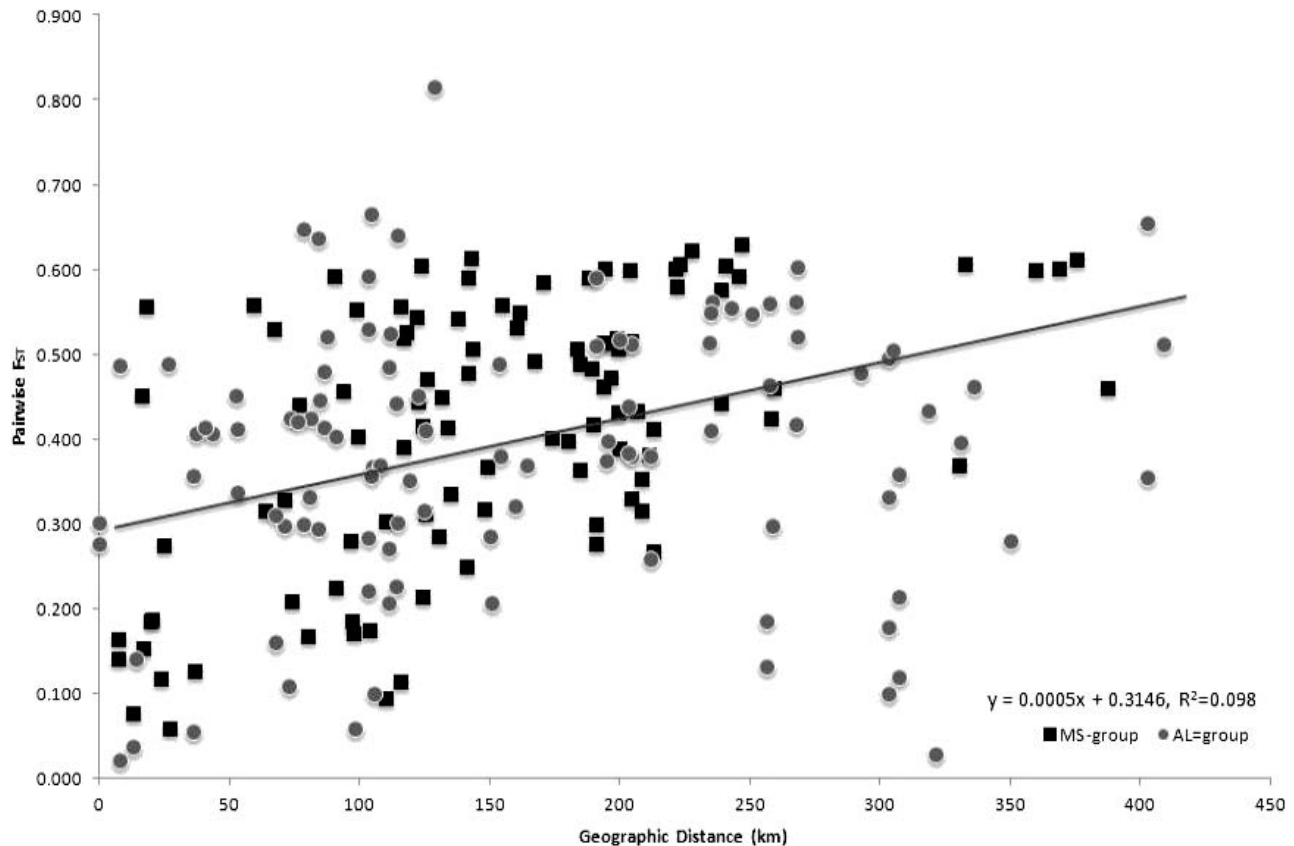


Figure 2. A slight positive but significant relationship between pairwise geographic distances and pairwise F_{ST} values ($R^2 = 0.098$, $P < 0.001$) explaining about 10% of the variation. Geographic distances were straightline and all pairwise F_{ST} values were significant ($P < 0.005$).

(Table 5). The relationship between pairwise genetic differentiation and geographic distance was specifically tested. A statistically significant, but relatively weak positive correlation was found (linear regression; $R^2 = 0.098$, $P < 0.0001$, Figure 2). The low strength of correlation suggested something other than geographic distance explains the majority ($\sim 90\%$) of the variance in F_{ST} among sampled localities.

Relatively high genetic similarity between MS-10 and MS-11 indicates connectedness, which could have been established historically by propagules from the same founding gene pool, as a single or through multiple introductions or via contemporary gene flow between these populations. Furthermore, these populations exhibit connectedness to AL-1 and AL-2, which might indicate origin from the same source propagule pool or contemporary gene flow among these localities, considering their locations and connectivity via highways. Propagule transport among sites probably benefits from human-assisted transport, considering cogongrass' frequency of establishment near roads, reducing the likelihood of detecting an IBD pattern of genetic relatedness (Ervin and Holly 2011).

Two clusters ($K = 2$; estimated Ln likelihood = 51,183.5) were inferred as the uppermost level of structuring present in Mississippi and Alabama, using the Evanno et al. (2005) ad hoc statistic to select K (Figure 3; $N = 388$ from 21 locations). Graphical representation of mean likelihood probability distribution values (i.e., posterior probability or $\text{LnP}[D]$; Pritchard et al. 2000) and ΔK for $K = 1$ through 8 are displayed in Figure 3, with the mode of ΔK denoted by an asterisk. The smallest K that the Evanno et al. (2005) method can infer is two groups, and this is supported in the global analysis by the high probability of assignment of most individuals to one population or the other (Figure 3a). The α -value is generated by each STRUCTURE simulation and is a measure of admixture among analyzed individuals. When α approaches zero, it suggests individuals belong primarily to one of the inferred populations; high α -values (approach or over 1) suggest most individuals being admixed. We observed a mean α of 0.08. All other localities consisted of individuals assigned with a high probability to one cluster or the other. Of 388 total individuals analyzed, 335 were assigned to a single cluster with 90% or greater probability;

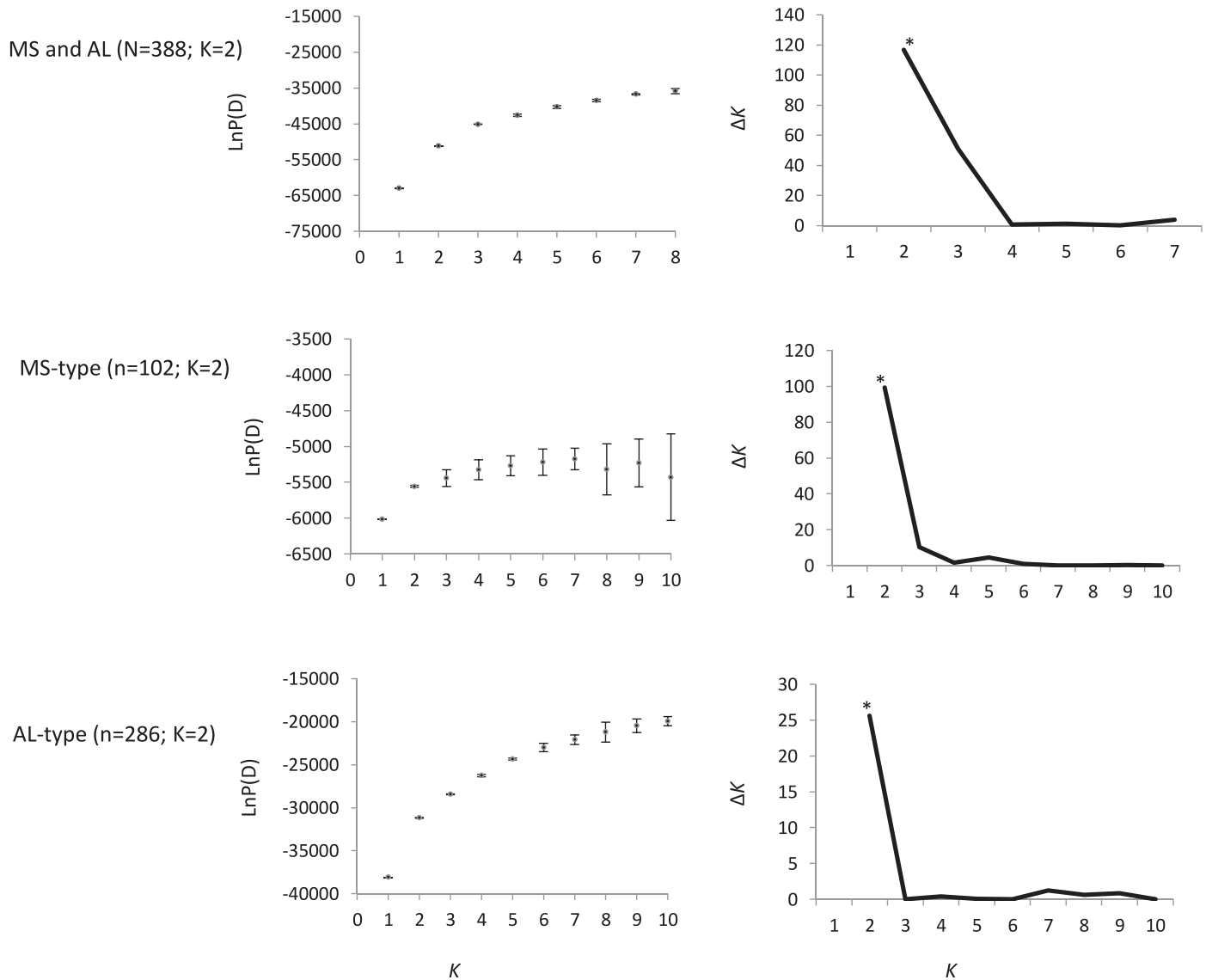


Figure 3. Graphs of mean likelihood probability distribution values (LnP(D)) and second order rate of change in probabilities of the assumed number of clusters (ΔK) from three STRUcTURE analyses. Errors bars represent SD calculated from raw likelihood values per simulation. The top row (a) represents plots from STRUcTURE analysis of all individuals ($N = 388$; K 1 through 8). The second row plots (b) are from STRUcTURE analysis of individuals assigned to MS-type cluster with high probability ($n = 102$; K 1 through 10). Plots in the third row (c) are from AL-type cluster analysis ($n = 286$; K 1 through 10). An asterisk indicates the mode of ΔK . The assumed number of clusters (K) is the independent axis for all graphs. ΔK values are an ad hoc statistic representing the second order rate of change of mean LnP(D) values and incorporate variance from multiple runs at each value of K . Selecting the mode of ΔK statistically supports the most probable and appropriate K for the dataset.

90 to the MS-type and 245 to the AL-type. The remaining 53 individuals exhibited evidence of mixed ancestry, as evidenced by $< 90\%$ probability to one cluster or the other. STRUcTURE analysis resulted in two lineages ($K = 2$; Figures 1 and 3) and around 10 to 20% of individuals suspected of having mixed ancestry.

The selected K of two populations is consistent with documented introduction history and our hypothesized expectations, without introduction of a priori information

as to how genetic information was partitioned among individuals. The first cluster (mostly black; MS-type, $n = 102$) inferred from STRUcTURE includes MS-1, 2, 3, 4, 5, and some individuals from population MS-6. The other cluster (AL-type; mostly white, $n = 286$) includes all individuals from MS-7, 8, 9, 10, 11, all localities sampled from Alabama, and some individuals from MS-6 (Figure 3). Inferred MS- and AL-type clusters (hereafter, “populations”) were further analyzed in separate STRUcTURE



Figure 4. Principle coordinates analysis (PCA) of cogongrass individuals ($N = 388$) sampled from 21 localities in Mississippi and Alabama (with data standardization). The first two axes account for 79% of the variation in this dataset. Individuals from MS-1, 2, 3, 4, and 5, form one cluster in the upper-right quadrant. Individuals from MS-7, 8, 9, 10, and 11, form a tight cluster in the upper-left quadrant and co-occur with individuals from Alabama populations. Individuals from MS-6 are present in both.

runs, again using the ad hoc statistic, ΔK , to select the most appropriate number of clusters. We found $K = 2$ and a similar degree of admixture for both populations (MS-type mean $\text{LnP}[D] = -5556.5$, $\alpha = 0.14$; AL-type mean $\text{LnP}[D] = -31165.5$, $\alpha = 0.14$; Figure 3) as evidenced by mean α values. In the MS-type simulation, 90 of 102 individuals analyzed were assigned with over 90% or greater probability to a single cluster. In the AL-type run, 245 of 286 were assigned with 90% or greater probability to one cluster. Site AL-7, proximal to one of the suspected sites of introduction, contained 7 of 20 individuals with mixed ancestry ($< 75\%$ probability in either cluster).

Principal coordinate analysis (PCA) on this dataset further supports population structure inferred from STRUCTURE analysis. PCA also resulted in two clusters: one well-organized (MS-type) and another less so (AL-type), with some intermediate individuals (Figure 4). The first two

axes explain 79% of individual variation in the data. A distinct break is observed between the two genetically-defined clusters in both the bar plot (Figure 3) and PCA (Figure 4). The larger PCA cluster appears scattered, primarily consisting of individuals from Alabama and the Mississippi Gulf Coast (MS-9, 10, 11), suggesting greater cumulative genetic heterogeneity among these individuals, whereas individuals from the MS-type were more tightly clustered, suggesting comparatively reduced genetic variability (Figure 4). Population structure analyses consistently partitioned all samples into two populations, with one being more diverse and geographically prevalent than the other, suggesting that cogongrass in central Mississippi are genetically divergent from coastal Mississippi and Alabama cogongrass. The central Mississippi population (MS-type) might be geographically constrained to central Mississippi based on our sampling (the Bienville National Forest,

Table 6. Results from two analyses of molecular variation (AMOVA) using F_{ST} : (a) groups defined by location, (b) genetically defined groups (MS- and AL-type populations). Greater F_{ST} observed when tested on biological data (b) than geographic data (a).

Source of variation	df	Sum of squares	Percentage of variation	P value
(a) Groups defined by location ^a				
Among groups	1	622.01	10.37	< 0.001
Among populations within groups	19	2916.34	33.16	< 0.001
Within populations	367	4776.53	54.46	< 0.001
Total	387	8314.87		
(b) Genetically defined groups (MS- and AL-type populations) ^{b,c}				
Among groups	1	1251.15	27.47	< 0.001
Among populations within groups	19	2287.20	22.47	< 0.001
Within populations	367	4776.53	50.06	< 0.001
Total	387	8314.87		

^a F statistics for (a) groups defined by location: $F_{ST} = 0.435$ ($P < 0.001$), $F_{SC} = 0.370$ ($P < 0.001$), $F_{CT} = 0.104$ ($P < 0.001$).

^b F statistics for (b) genetically defined groups: $F_{ST} = 0.499$ ($P < 0.001$), $F_{SC} = 0.310$ ($P < 0.001$), $F_{CT} = 0.275$ ($P < 0.001$).

^c Abbreviations: MS, Mississippi; AL, Alabama.

northwest portion Desoto National Forest; Figures 1, 3, and 4). The second population (AL-type) includes patches sampled from the southern portions of Desoto National Forest, coastal Mississippi, and all of Alabama. Individuals in coastal Mississippi locations, in particular, show very little diversity or mixed ancestry, where all individuals from these patches were assigned to the AL-type population with 99% or better probability.

Two AMOVAs were conducted to test the degree of genetic similarity between: (a) geography, groups based on sample location in Mississippi or Alabama; and (b) genetic information, groups based on statistically supported biological populations (Table 6). The AMOVA grouped by state resulted in significant population structure (Table 6): 54% of genetic variation partitioned within populations, 33% among populations and within groups, and 10% partitioned between groups (defined as MS or AL; $F_{ST} = 0.435$, $P < 0.001$). The second AMOVA, defined by genetic data, resulted in reduced within-populations (50%) and within genetic groups (22%) explaining molecular variation. However, an increase in population structure was observed, where the quantity of molecular variation between the two genetic population was greater than 27% ($F_{ST} = 0.499$, $P < 0.001$; Table 6), better explaining molecular variation. Therefore, population structure was more strongly defined by genetic than by spatial data, indicating that genetic data is more influential than geography in cogongrass.

Partitioning of cogongrass molecular variation (AMOVA) was similar in pattern to Capo-chichi et al. (2008), who also observed the majority of genetic variation within, rather than among, cogongrass populations. However, comparison of F_{ST} and F_{ST} analogs across those same variables (analyzed with dominant markers) resulted

in greater partitioning of molecular variation in our AMOVAs (geographically or genetically defined groups) in this study of cogongrass than with other similar plant species (long-lived perennials, all geographic ranges, mixed breeding system, and wind dispersed; see: Nybom 2004). This pattern, wherein the majority of genetic variation is partitioned within populations, has also been observed in other molecular studies of outcrossing grasses (Huff et al. 1993, *Bouteloua dactyloides* (Nutt.) J. T. Columbus BODA2; Li et al. 2006, *Bromus inermis* Leyss. BRIN2; Mellish et al. 2002, *Agropyron cristatum* (L.) Gaertn. AGCR). Studies in other invasive plant species affecting the United States suggest that multiple introductions can drive high genetic diversity within and among conspecific populations (Baker and Dyer 2011, Mary's-grass *Microstegium vimineum* (Trin.) A. Camus MIVI; Pappert et al. 2000, kudzu, *Pueraria montana* var. *lobata* (Willd.) Maesen & S. M. Almeida PUMOL). In the case of *M. vimineum*, populations in Virginia were found to possess high within-population genetic diversity (AFLP analysis); population structure suggested multiple introductions and secondary contact between differing lineages within the species (Baker and Dyer 2011). Observed within-population variation could be the result of historical and/or extant gene flow among lineages (e.g., among progeny of the original Philippine and Japanese introductions), increasing this species' genetic diversity in the region; however, direct comparison with genetic in the native range is needed (Kolbe et al. 2004; O'Hanlon et al. 1999).

Multiple Introductions and Secondary Contact. Genetic diversity and population structure analysis in this research of invasive cogongrass supported minimally two genetically distinct groups in the Southeast. These two genetically and

statistically supported groups are consistent with our hypotheses and the literature of a two-introduction scenario (Tabor 1949, 1952). We expected further population structure analysis to result in subpopulation structuring, consistent with diverse introduction(s) of propagules. However, results remained consistent, with two lineages and relatively low frequency of admixture among individuals ($\alpha < 0.15$). It should be noted that individuals of mixed ancestry are present in both populations, more so in the AL-type population, suggestive of secondary contact with some admixture (Figure 3 and 4).

The MS-6 locality, located in Greene County, Mississippi, was represented in both populations in the global STRUCTURE analysis but with high probability of assignment of most individuals to just a single population (Figure 3). Individual tissue samples were collected along two sides of a ROW, bordering private land within the Desoto National Forest acquisition boundary. During sampling, 12 individuals were collected first from the north ROW and the remaining eight from the south ROW. Both PCA and STRUCTURE assigned 10 of the 20 individuals (north ROW) within the MS-type population (black), eight (south ROW) within the AL-type population (white), and two of more substantially mixed ancestry (Figure 3 and 4). Of the 20 individuals sampled at site MS-6, 10 were assigned with over 98% probability to the MS-type, eight assigned to the AL-type ($> 99\%$ probability), and two were considered to be of mixed ancestry. One individual at this location was assigned with 75% probability within the MS-type and the other with 88% probability assignment within AL-type. This alignment between genetic information and location suggests that cogongrass at MS-6 is probably one “contact population” of a contact zone, where divergent genetic populations (as determined by this research) co-occur at the same sampling locality. Although other localities possess individuals of mixed ancestry, this site contains both lineages divided by a road and is indicative of physical admixture but not necessarily significant genetic admixture at this time.

Our data are consistent with documented introduction history of cogongrass reported in the literature and are consistent with other recent genetic studies of this species. Vergara et al. (2008) used microsatellites to identify two clades containing cogongrass. Their data placed cogongrass from McNeil, MS (the other suspected, documented site of introduction) in a separate clade from Japanese cogongrass, alleged to have sourced the Alabama introduction (Vergara et al. 2008). Therefore, this research is harmonious with previous research on cogongrass in this region.

Cogongrass invasion in the United States has reached the stages of substantial range expansion, and its perceived negative impact across the invaded range is a threat to native and managed systems (Bryson and Carter 1993; MacDonald 2004). Molecular data from this study support documented introduction history, secondary contact, and

gene flow among genetically differentiated lineages. In addition to an already ruderal and plastic biology, this research suggests rapid expansion and persistence of cogongrass during the last century might have benefitted from a combination of introduced diversity, propagule pressure, secondary contact, and reproductive flexibility in response to varying environmental conditions (Coulatti et al. 2006; Catford et al. 2009).

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