Environmental Horticulture
1545 Fifield Hall
PO Box 110670
Gainesville, FL 32611-0670
Telephone (352) 392-1831
Fax (352) 392-3870
http://hort.ifas.ufl.edu

30-Nov-09

Lisa Roberts

Executive Director

Florida Wildflower Foundation

Dear Lisa,

The final report for R-003-07 "Establishing provenance, viability-testing standards and enhanced germination for seed production of wiregrass in Florida" is attached. I am pleased to report that a reliable viability testing protocol has been established. Also, the genetic analysis of southeastern wiregrass populations is complete and we are in the process of submitting an article to Conservation Genetics related to wiregrass provenance. A draft copy of this manuscript has been included with this report for your review. I request that the draft manuscript *not* be posted to the FWF website or published in any other context as this constitutes prior publication and voids any publication agreement with peer-reviewed journals. It would also be a great help to postpone distribution of the other data included in this report as they will be used for upcoming manuscripts.

I have included some preliminary germination data from seeds harvested in December 2008. We continue to assemble and analyze the germination data from this harvest. Please note that R-003-07 has been continued as R-003-09 and we will combine germination data from harvests in 2008 and 2009 for final analysis.

Please contact me immediately at 352-392-1831 ext 221 or heperez@ufl.edu if you require further information or would like to discuss any aspect of the report in more detail. We look forward to our continued relationship with the Florida Wildflower Foundation.

Sincerely,

Hector E. Perez, Ph.D.

Assistant Professor

Objective #1 Create a standard viability test of wiregrass – An accurate method to determine viability is an essential prerequisite to germination testing since germination tests need to be corrected for the percentage of viable seeds. In a commercial lab that conducts tetrazolium (TZ) testing of wiregrass, seeds are bisected longitudinally so that the staining pattern can be evaluated. To circumvent this laborious process, which requires a high level of dexterity since the seeds are narrow and the embryos are very small, attempts were made to chemically clear the opaque tissues covering the embryo. However, chemical treatments did not sufficiently clear embryo covering tissues enough to view the embryo.

The challenges of conducting an accurate TZ test led to evaluating a forceps 'press test' for assessing wiregrass seed viability. The press test accurately predicted that a seed was nonviable; however, it did not accurately predict if a seed was viable. In a subsequent work, we modified the forceps 'press test' (pressure applied just below the midpoint of the seed), and used it in combination with a germination test plus post-germination 'press test' to determine the accuracy of this method for evaluating seed viability. Based on the results, the 'press test' in combination with a germination test and post-germination 'press test' accurately predicted the percentage of viable seeds. However, these results, while encouraging, were only preliminary.

The next step was to conduct the press test procedure for seeds of several populations and compare this to germination tests followed by post-germination TZ assay; which is the standard protocol in seed testing labs. The newly developed press test technique was found to be as accurate and more reliable as the standard TZ and germination test (Table 1). Note in Table 1 how the determination of viability is improved by incorporating the press test (compare data in columns under heading Entire Seed Lot to remaining data). More importantly, note how the data in the final column is statistically similar to the previous two columns. This highlights the accuracy and precision of the germination test with the addition of the press test.

Table1. Accuracy and precision of a germination test followed by a postgermination press test to determine viability of firm wiregrass (*Aristida stricta*) seeds.

Location and harvest date	Habitat	Viability of seed sample type[z]: Entire seed lot (%) PreGerm TZ[x]	Entire seed lot (%) Germ + PostGerm TZ[x]	Filled seed only (determined by press test[y]) (%) PreGerm TZ[x]	Filled seed only (determined by press test[y]) (%) Germ + PostGerm TZ[x]	Filled seed only (determined by press test[y]) (%) Germ + PostGerm Press Test[w]
Georgia / Appling County, Moody Forest Natural Area (TNC); 19 November 2008	Moist	43.5 (18.1)	44.0 (9.8)	74.0 (7.0)	66.0 (34.8)	64.0 (3.2)
Seed age at testing[v]		---------- 40 days ----------				30 days
Florida / Liberty County: Apalachicola Bluffs and Ravines Preserve, Unit 1 (TNC); 2 December 2008	Sandhill	28.5 (21.0)	29.0 (33.1)	81.0 (8.4)	71.0 (7.1)	78.0 (8.9)
Seed age at testing		---------- 27 days ----------				17 days
Florida / Charlotte County: Babcock Webb Wildlife Management Area; 25 November 2008	Moist	26.5 (42.0)	27.5 (31.1)	73.0 (5.2)	72.0 (12.0)	73.3 (8.4)
Seed age at testing		---------- 34 days ----------				24 days
Florida / Wakulla County, St. Marks National Wildlife Refuge, Panacea Unit 5; 25 November 2008	Moist	19.4 (37.1)	-----	59.0 (26.8)	58.0 (21.4)	54.5 (3.5)

Seed age at testing		---------- 34 days ----------				24 days
Florida / Osceola County, Disney Wildlife Preserve, Moist Site; 10 December 2008	Moist	36.0 a (24.0)	21.0 b (18.2)	69.0 ab (15.2)	72.0 a (9.1)	53.5 b (15.5)
Seed age at testing		---------- 79 days ----------				84 days
South Carolina / Chester County: Carolina Sandhills National Wildlife Refuge; 20 October 2008	Sandhill	20.0 (18.3)	21.5 (20.6)	77.0 (8.9)	67.0 (14.9)	76.9 (13.5)
Seed age at testing		---------- 11 days ----------				

[z] Except for DWP, within a location and seed sample type, there were no significant differences between or among means as determined by Tukey's studentized range test ($\alpha = 0.05$). Means were arcsine transformed prior to analyses as necessary; nontransformed means are presented, with coefficients of variation in parentheses.

[y] Press test = gentle pressure applied to seed, just below the midpoint, with a pair of fine-tipped forceps under 15-20X magnification. Seeds that did not yield to pressure were deemed firm. Firm seeds were deemed potentially viable prior to pre-germination TZ testing or germination testing. Seeds that remained firm after the germination test (see footnote 'w') were deemed viable.

[x] Testing conducted according to AOSA specifications by Midwest Seed Services, Inc., Brookings, South Dakota. Germination tests were conducted for 14 days in light at 20C. There were four 25 or 50-seed reps for both tests of the entire seed lot, and four 25-seed reps for testing of the firm seeds.

[w] Germination test for 14 days in dark at 15/25C followed by a post-germination press test of seeds for which neither the radicle or cotyledon had emerged. A seed was classified as germinated only if it was deemed to be normal, with normal defined as a seedling with a coleoptile (containing a primary leaf) $\geq$ 5 mm long and a primary root (with root hairs) $\geq$ 3 mm long. A seed in which the primary root and/or coleoptile had emerged and did meet the specifications for a normal seedling was classified as abnormal, and was not counted as germinated. There were four 25-seed reps, except four 50-seed reps for Moody Forest and Babcock Webb.

[v] Upon receipt at the NFREC-Quincy, seeds were stored in the dark at ~30% RH and ~20-25C.

Objective #2 Identify Optimal Regimes to Promote Germination of Wiregrass - Initial viability tests of all seed lots are conducted using the press test techniques generated in Objective 1. Seeds are sown on blotter paper within Petri dishes and incubated at alternating temperature regimes of: 33/24, 29/19, 27/15 and 22/11°C. Alternating temperature regimes simulate environmental conditions throughout Florida during winter, early spring/late fall, early fall/late spring, and summer. Additionally, germination is being investigated at 25°C with the addition of various germination stimulators such as: gibberellic acid, smoke water, and KNO_3. Germination tests run for 28 days. Germination counts are taken every 2 days in order to determine germination rates. Germination is considered as the production of normal seedlings (*e.g.* presence of primary root, hypocotyl, and cotyledon). Total germination percent will be calculated on the proportion of viable seeds at 28 days.

It is interesting to note that seeds from various populations, with the exception of those collected in South Carolina, have a high germination capacity (Tables 2, 3) under simulated Florida seasonal temperatures. Moreover, during the seed cleaning processes it was found that seed fill was low across populations and many seemingly intact seeds succumbed to fungal contamination. Preliminary analysis indicates that this is a seed-borne smut fungus. We are currently trying to identify the fungus to species level and compare this across populations. Gibberellic acid treatments seem to have the most promotive effect on germination independent of the seed source population (Tables 4-6).

Table 2. Germination of *Aristida beyriciana* seeds after 28 days under simulated Florida seasonal temperatures. Seeds were collected throughout the southeastern United States. Seeds received a 12 hour alternating temperature and light regime. Dark incubated seeds were sown in Petri dishes wrapped with two layers of aluminum foil.

	Average germination ± SE %											
	ABRP-1[z]		ABRP-5[y]		St. Mark's[x]		Babcock-Webb[w]		CSNWR[v,u]		Moody[t]	
Temp.	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
33/24°C	95.3 ± 3.4	73.4 ± 2.0	86.5 ± 2.0	88.8 ± 5.0	71.4 ± 14.4	64.1 ± 9.9	97.5 ± 1.5	91.0 ± 2.1	29.4 ± 8.5	4.2 ± 4.2	87.9 ± 4.4	87.3 ± 2.2
29/19°C	89.9 ± 4.0	81.5 ± 4.8	91.7 ± 5.2	80.8 ± 4.3	80.3 ± 4.9	69.4 ± 9.3	97.3 ± 1.6	92.5 ± 0.9	11.1 ± 7.2	68.8 ± 18.8	80.7 ± 4.8	84.1 ± 1.8
27/15°C	83.3 ± 7.3	76.7 ± 7.5	90.1 ± 4.3	76.7 ± 3.7	79.4 ± 10.6	70.3 ± 11.2	93.3 ± 2.6	90.9 ± 2.3	24.2 ± 4.0	5.6 ± 3.3	79.0 ± 6.9	79.5 ± 4.6
22/11°C	56.5 ± 13.5	70.3 ± 6.6	71.7 ± 10.4	67.6 ± 3.2	43.5 ± 6.0	58.3 ± 9.3	91.0 ± 4.2	91.8 ± 1.7	2.1 ± 2.1	3.6 ± 3.6	63.2 ± 4.6	89.6 ± 1.7

[z]Apalachicola Bluffs and Ravines Park Tract 1, Liberty County, FL (panhandle)
[y]Apalachicola Bluffs and Ravines Park Tract 5, Liberty County, FL (panhandle)
[x]St. Mark's National Wildlife Refuge, Wakulla County, FL (panhandle)
[w]Babcock-Webb Wildlife Management Area, Charlotte County, FL (southwest)
[v]Carolina Sandhills National Wildlife Refuge, Chesterfield County, SC
[u]Data based on small sample sizes (e.g. 2-10 seeds per replicate) and should be interpreted cautiously
[t]Moody Forest Natural Area, Appling County, GA

Table 3. Mean time to complete germination for *Aristida beyrichiana* seeds collected throughout the southeastern United States and sown under simulated seasonal temperatures in Florida for 28 days.

	Mean time to complete germination (MTG) ± SE days					
Temp.	ABRP-1	ABRP-5	St. Mark's	Babcock-Webb	CSNWR[z]	Moody
33/24°C	8.3 ± 0.7	9.6 ± 0.7	10.4 ± 1.9	5.3 ± 0.1	2.7 ± 0.5	8.0 ± 0.5
29/19°C	8.8 ± 0.7	12.7 ± 0.5	11.4 ± 0.9	7.3 ± 0.4	3.2 ± 0.1	8.9 ± 1.1
27/15°C	11.0 ± 0.1	12.3 ± 0.9	13.6 ± 1.4	8.5 ± 0.2	3.6 ± 0.3	11.9 ± 0.6
22/11°C	17.2 ± 1.1	17.9 ± 0.5	17.3 ± 1.8	10.5 ± 0.4	4.0 ± 0.0	16.1 ± 0.4

[z]Data based on small sample sizes (e.g. 2-10 seeds per replicate) and should be interpreted cautiously.

Table 4. Effects of increasing concentrations of gibberellic acid (GA_3) on germination percent and rate of Aristida beyrichiana seeds collected throughout the southeastern United States.

GA_3 (ppm)	Average germination ± SE %						Mean time to complete germination (MTG) ± SE days					
	ABRP-1	ABRP-5	St. Mark's	Babcock-Webb	CSNWR[y]	Moody	ABRP-1	ABRP-5	St. Mark's	Babcock-Webb	CSNWR[y]	Moody
0	86.1 ± 8.5	72.1 ± 7.2	--[z]	--	95.0 ± 5.0	89.5 ± 3.7	7.9 ± 0.7	9.4 ± 0.7	--	--	16.9 ± 1.6	7.0 ± 0.5
10	93.9 ± 2.7	83.8 ± 10.1	--	--	100.0 ± 0.0	95.0 ± 3.4	6.2 ± 0.4	9.0 ± 1.1	--	--	11.0 ± 1.0	5.4 ± 0.1
100	95.1 ± 2.9	78.9 ± 8.2	--	--	91.7 ± 8.3	84.6 ± 3.0	5.7 ± 0.2	5.0 ± 0.5	--	--	7.9 ± 0.9	4.3 ± 0.3
250	96.2 ± 2.2	93.8 ± 2.2	--	--	95.0 ± 5.0	95.2 ± 2.1	5.6 ± 0.2	4.8 ± 0.5	--	--	6.4 ± 2.2	3.3 ± 0.3
500	100.0 ± 0.0	40.8 ± 6.6	--	--	81.3 ± 12.0	97.9 ± 2.1	6.3 ± 0.3	5.0 ± 0.2	--	--	9.5 ± 2.5	4.3 ± 0.3
1000	92.0 ± 1.5	42.0 ± 8.1	--	--	100.0 ± 0.0	94.2 ± 3.9	5.1 ± 0.2	4.6 ± 0.6	--	--	13.8 ± 2.3	3.8 ± 0.2

[z]Data being compiled
[y]Data based on small sample sizes (e.g. 3-4 seeds per replicate) and should be interpreted cautiously.

Table 5. Effects of increasing concentrations of potassium nitrate (KNO_3) on germination percent and rate of Aristida beyrichiana seeds collected throughout the southeastern United States.

KNO_3 (%)	Average germination ± SE %						Mean time to complete germination (MTG) ± SE days					
	ABRP-1	ABRP-5	St. Mark's	Babcock-Webb	CSNWR[y]	Moody	ABRP-1	ABRP-5	St. Mark's	Babcock-Webb	CSNWR[y]	Moody
0.0	81.5 ± 9.2	--[z]	--	97.7 ± 2.3	65.8 ± 6.1	69.7 ± 6.3	9.39 ± 0.6	--	--	7.6 ± 0.3	15.4 ± 3.0	7.7 ± 0.7
0.2	89.5 ± 1.6	--	--	95.5 ± 3.0	95.8 ± 4.2	63.8 ± 2.5	7.9 ± 0.3	--	--	5.7 ± 0.2	14.4 ± 1.6	7.5 ± 0.5
0.4	84.2 ± 7.3	--	--	85.6 ± 5.5	85.0 ± 5.0	75.2 ± 3.6	9.0 ± 1.1	--	--	5.9 ± 0.3	16.1 ± 1.4	7.1 ± 0.7
0.8	95.8 ± 4.2	--	--	95.0 ± 5.0	78.3 ± 2.0	78.7 ± 3.6	6.7 ± 0.6	--	--	6.3 ± 0.4	16.3 ± 1.7	7.7 ± 0.4
1.0	93.5 ± 1.5	--	--	90.3 ± 2.1	80.0 ± 8.2	71.7 ± 12.7	8.9 ± 0.8	--	--	6.3 ± 0.3	14.2 ± 1.8	6.6 ± 0.4

[z]Data being compiled
[y]Data based on small sample sizes (e.g. 3-6 seeds per replicate) and should be interpreted cautiously.

Table 6. Germination of *Aristida beyrichiana* seeds exposed to smoked water or untreated water. Seeds were collected in ABRP-1. Germination data for other accessions is being compiled.

	Average germination ± SE %		Mean time to complete germination (MTG) ± SE days	
Temp.	Smoke	Control	Smoke	Control
33/24°C	70.0 ± 4.8	59.0 ± 3.4	7.3 ± 0.7	8.1 ± 0.4
29/19°C	69.0 ± 3.0	64.0 ± 1.6	10.4 ± 0.6	8.1 ± 0.2
27/15°C	73.0 ± 7.7	67.0 ± 3.0	10.6 ± 0.5	10.5 ± 0.6
22/11°C	64.0 ± 6.7	50.7 ± 4.6	13.8 ± 0.8	13.1 ± 0.5

Objective #3 Determine genetic diversity within/among seed sources of wiregrass – see draft manuscript below.

Jyotsna Sharma[1,3]*, Sheeja George[1], Jeff Norcini[1] and Hector Perez[2]

Intra-specific Genetic Diversity of *Aristida stricta* Michx. Native to the Southeastern Coastal Plain in North America

[1] University of Florida NFREC, Quincy, Florida 32351; [2] University of Florida, Gainesville, Florida 32611; [3]Current Address – Department of Plant and Soil Science, Texas Tech University, Lubbock, Texas 79409
*Corresponding Author: jyotsna.sharma@ttu.edu; 806.742.2637 (telephone); 806.742.0775 (facsimile)

Abstract

Aristida stricta Michx. (Poaceae) is a perennial bunchgrass native to the Southeastern Coastal Plain of North America where it can occur as the dominant groundcover in longleaf pine savannas and slash pine flatwoods from southeastern North Carolina to Florida, and westward to the coast of Mississippi. We examined genetic relationships within and among ten populations of *A. stricta* by using eight inter-simple sequence repeat (ISSR) markers to generate band frequency data for 32 individuals from each sampled population. An analysis of molecular variance showed that 38% of the variation resided among populations while 62% was attributable to variation within populations (F_{ST} = 0.377; P = 0.000). Grouping the populations by habitat (F_{CT} = 0.006; P = 0.285) or by geographic location (F_{CT} = 0.007; P = 0.219) did not show significant differentiation between the groups. Overall, pair-wise geographic and genetic distances were not correlated. Data indicate that while individuals within each population are genetically diverse, there seemingly are barriers to gene flow across populations leading to their divergence. Each population contains at least some exclusive loci suggesting that different selection pressures may be resulting in this pattern of localization. Our results, combined with those of the previous studies that presented evidence for local adaptation and phenotypic differences among populations, suggest that there is sufficient variation among populations of this species to warrant: (1) maintenance of the existing genetic diversity at individual sites, and (2) use of local seed and plant sources for conservation projects.

Keywords: inter-simple sequence repeat, long-leaf pine ecosystem, southeastern coastal plain

Introduction

Widely distributed species are generally known to have a diverse genetic base that can help their long-term persistence in a variety of habitats and environmental conditions (Falk and Holsinger 1991). Because of anthropological influences, many species that once had wide distributions have lost their natural habitat, and such fragmentation of populations can lead to local adaptation within populations and/or divergence among the disjunct populations, especially if gene flow is reduced or restricted (Hufford and Mazer 2003). While preservation of the remaining habitats and populations is integral to their survival, restoration of destroyed habitats and augmentation of existing populations are additional strategies for conservation of species in decline (Falk and Holsinger 1991). The latter two strategies require an understanding of the biological consequences of mixing different genotypes, ecotypes, and epitypes of the focal taxon; the resulting mixed populations can either manifest improvement (heterosis) or reduction (outbreeding depression) in fitness in the subsequent generations (Hufford and Mazer 2003). Large-scale conservation and restoration efforts employing translocation of seeds or plants should thus be preceded by genetic and ecological evaluation of the focal species (Hufford and Mazer, 2003). When combined with other biological and ecological attributes of a species, knowledge of the amount and distribution of its genetic diversity can help estimate the evolutionary trajectories of the fragmented populations by providing an insight into the patterns of intra-specific gene flow (Hamrick 1983, Falk and Holsinger 1991, Walters et al. 1994, Godt and Hamrick 1995).

Aristida stricta Michx. (wiregrass; Poaceae) is a perennial bunchgrass native to North America where it can occur as the dominant groundcover across diverse edaphic and climatic conditions in longleaf pine savannas and slash pine flatwoods from southeastern North Carolina to Florida, and westward to the coast of Mississippi (Clewell 1989, Peet 1993). Its native habitat has been greatly reduced due mainly to anthropogenic activities, resulting in fragmented and fewer natural populations than existed before (Clewell 1989, Walters et al. 1994, Earley 2004). Restoration of wiregrass ecosystems has gained importance because of the extensive loss of this plant community across the southeastern United States

(Landers et al. 1995, Gordon and Rice 1998, McCay 2000). Researchers have reported allozyme diversity in a taxonomic context (Walters et al. 1994) and common garden and reciprocal translocation experiments including effects of transplant age and surrounding vegetation (Kindell et al. 1996; Gordon and Rice 1998). The allozyme survey (Walters et al. 1994) showed high within and among population diversity in the species, but the authors recommended a more thorough genetic assessment by sampling a larger number of populations throughout its range. The translocation and common garden studies (Kindell et al. 1996; Gordon and Rice 1998), which showed phenotypic and phenological differences among populations and also surmised that local adaptation was evident at advanced growth stages in *A. stricta*, were restricted to a few populations in Florida leaving a need for range-wide restoration genetics experiments.

Despite the once widespread distribution of this species, several aspects of its biology might explain local differentiation among populations. Both flowering and production of viable seeds are fire dependent, with growing season burns being much more effective than dormant season burns (Parrott 1967, Streng et al. 1993, Outcalt 1994, van Eerden 1997). *Aristida stricta* is also known to reproduce asexually where the main clump expands by producing tillers (Clewell 1989). Tiller internode lengths are short, however, and seed dispersal is within 2 m of the parent plant (Kesler et al. 2003). Although there is no experimental evidence for the reproductive or pollination mechanisms of wiregrass, allozyme studies (Walters et al. 1994) have suggested that it is a wind pollinated, outcrossing species. Since sexual reproduction occurs only after growing season fires, fire frequency has been vastly reduced in recent decades, and the geographic scale of fires has been similarly reduced due to habitat fragmentation and safety issues, gene flow is now limited to relatively local scales (Robbins and Myers 1992). However, we hypothesize that the current genetic structure will still reflect historical gene flow levels given the longevity of individual plants (Clewell 1989).

The taxonomic treatment of *Aristida stricta* has varied over recent decades. Originally referred to as *Aristida beyrichiana* (Trin. & Rupr.) (von Trinius and Ruprecht 1849), it has been a subject of several studies to establish its taxonomic status. Peet (1993) split the original taxon into two based on differences in leaf sheath pubescence among the northern and southern populations. Almost simultaneously Walters et al. (1994) reported high allozyme genetic identity and suggested northern and southern populations to be conspecific. Ward (2001) then followed by conferring a varietal status to the southern populations [*A. stricta* Michx. var. *beyrichiana* (Trin. & Rupr.) D. B. Ward], but the most recent taxonomic treatment (Kesler et al. 2003) based on morphology, anatomy, and common garden studies removed the varietal status of the southern populations leading to the establishment of a single taxon (*A. stricta* Michx.) throughout the range.

Our primary objective for this study was to survey the genetic variation within and among populations of *A. stricta* across its range to develop a component of its conservation and restoration genetics, but we also sought implications of our results for the relationship between the northern and southern populations.

Among the many methods for assessing intra-specific genetic diversity, inter-simple sequence repeat (ISSR) regions of the nuclear DNA, which are hyper-variable dominant molecular markers, are especially useful in detecting diversity in closely related, or even clonal individuals (Zietkiewicz et al. 1994, Esselman et al. 1999, McGlaughlin et al. 2002, Smith and Bateman 2002). We used ISSR polymorphisms to assess the genetic diversity of ten *A. stricta* populations representing its natural range.

Materials and methods

Plant material

Aristida stricta Michx. (Poales; Poaceae) is a native, perennial bunchgrass that grows as the dominant understory species in longleaf pine and slash pine flatwoods (Clewell 1989). It occurs in sandy, infertile soils that are deficient in nitrogen and phosphorus, such as dry sandhills and seasonably wet grass-sedge

flatwoods (Parrott 1967, Clewell 1989). Plants grow as large, dense, tufted clumps with highly involuted leaf blades giving them a wire-like appearance. Maintenance of vertically-held fine leaves, even when senescent, makes this species a critical fuel for carrying fire through these systems (Platt 1988).

Sampling, DNA extraction, and amplification of the ISSRs

Plants from ten naturally occurring populations of *A. stricta* were sampled from one or more xeric sandhill and mesic flatwood sites in North Carolina (NC), South Carolina (SC), Georgia (MFGA, JCGA), Alabama (AL), Mississippi (MS), and Florida (SMFL, DWFL, DDFL, BWFL) from March through September of 2008 (Table 1, Figure 1). Sampling design included collection of tissue across the range by selecting sites toward the distributional extremes of the species; we also included populations which occurred in mesic flatwoods for a comparison with the sandhill sites. Leaf tissues were collected within 10 days of a prescribed burn at each location by gently pulling out approximately 10-12 newly emerging blades from 35 or 36 well-separated plants. Leaf blades from each plant were placed in separate sealable plastic bags that contained moist paper toweling to keep the tissues hydrated during transport; each bag was then placed in a cooler that contained ice packs. Samples were shipped overnight to the laboratory where they were stored at 4°C until the DNA was extracted within 24 h of leaf tissue collection. We used *Poa pratensis* 'Beyond' (Kentucky bluegrass; Poaceae) and *Juncus effusus* L. (soft rush; Juncaceae) as two out-group species. Seeds of *P. pratensis* 'Beyond' were supplied by J. R. Simplot Co. (Jacklin Seeds, Post Falls, ID) and were germinated in a greenhouse. Three weeks after sowing 30 to 40 healthy-looking seedlings were transplanted into individual plastic containers and were grown for one month. Leaf tissues from 15 plants were then collected for DNA extraction. Plants of *J. effusus* were obtained from Superior Trees Inc. (Lee, FL). These were maintained in the same greenhouse as *P. pratensis* 'Beyond' for one week after which we collected leaf tissue from 15 plants for DNA extraction.

Total genomic DNA was extracted from 32 leaf tissue samples from each of the 10 populations of *A. stricta* and 15 samples each from the out-group species *P. pratensis* and *J. effusus* by using the UltraClean

plant DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to manufacturer's instructions. The DNA was stored at –20°C until further use. Primer selection for amplification of the ISSR regions of *A. stricta* was conducted according to Archibald et al. (2006). Of the 21 primers screened, eight primers (Table 2) that produced reproducible and polymorphic bands were selected for the study. Amplification reactions were carried out in an Eppendorf Mastercycler ep. (Eppendorf, Hamburg, Germany) according to the conditions described in Table 2. The PCR products were applied to a 1% agarose gel containing ethidium bromide (2µl/100 ml gel), and were electrophoresed in 1X TBE buffer (pH 8.0) at 85 V for 2 to 2.5 h. Bands were visualized under UV light. Each sample was amplified and electrophoresed at least three times to ensure reproducibility of the banding patterns. Gel images were analyzed by using the GelCompar II software (Applied Maths NV, Keistraat, Belgium). Molecular weights were assigned to each band by using a 1kb ladder. Each ISSR band/ locus was scored as "1" for presence and "0" for absence. Bands that appeared in at least two replicates were considered present. DNA extraction, ISSR amplification, band scoring, and generation of binary (1/0) data were duplicated for individuals sampled from population BWFL to rule out amplification of contaminant DNA, if any.

Data analyses

Intra-population (within population) and inter-population (among population) genetic analyses were conducted independently on two data-sets, each comprising of the binary data from nine populations (NC, SC, MFGA, JCGA, AL, MS, SMFL, DWFL, and DDFL) and either of the binary data sets obtained from BWFL, to test for conformity of results. Because both analyses gave comparable results, we retained only one for presentation and discussion herein.

For the intra-population genetic analyses, the proposal of Lynch and Milligan (1994) for treatment of data from dominant markers was followed for estimation of allele frequencies. Total number of loci, number of loci with frequency ≥ 5%, distribution of loci among populations, number of loci exclusive to a population, and average expected heterozygosity (H_e) were computed by using GenAlEx 6 (Peakall and

Smouse 2006). Number of polymorphic loci was estimated by manually surveying the two-state data and enumerating loci that were present in some individuals while absent in others. We also calculated the Dice similarity coefficient for all pairs of individuals by using the SIMQUAL module in NTSYSpc ver 2.20N (Rohlf, 2005).

Inter-population genetic diversity was assessed by first determining band frequencies for each population by using the FREQ module in NTSYSpc ver 2.20N (Rohlf 2005). Frequency data was bootstrapped by using the SEQBOOT program in PHYLIP (Felsenstein 2004) to generate 1000 replicate data sets. Based on the bootstrapped band frequency data, pair-wise Nei's genetic distances (Nei 1972) between populations were obtained and neighbor-joining trees were generated followed by a majority rule consensus tree (based on pair-wise Nei's genetic distances) by using the GENDIST, NEIGHBOR, and CONSENSE programs in PHYLIP, respectively. An analysis of molecular variance (AMOVA; Excoffier et al. 1992) was performed by using ARLEQUIN ver 3.1 (Excoffier et al. 2006) by grouping the populations in two ways: (1) to examine the effect of habitat, we placed the sandhill populations NC, SC, JCGA, AL, and DDFL in one group and the flatwood populations MFGA, MS, SMFL, DWFL, and BWFL in the second group, and (2) the northern populations NC and SC were placed in one group and southern populations MFGA, JCGA, AL, MS, SMFL, DWFL, DDFL, and BWFL in the second group. A second AMOVA was conducted without grouping the ten populations for comparison with the results obtained after grouping. Significance of the *F* statistics was based on 1000 random permutations. A Mantel test (Mantel 1967) to compare pair-wise geographic distances and pair-wise Nei's genetic distances was conducted by using GenAlEx 6, and its significance was based on 1000 random permutations.

Results

We amplified the ISSR regions with eight primers in 318 individuals from 10 populations of *A. stricta* and 15 individuals each of *P. pratensis* and *J. effusus*. The average number of loci amplified per *A. stricta* population was 102, of which 101 had a frequency of equal to or greater than 5% (Table 3). The percentage of private loci in each population ranged from 7 to 28%. Forty seven percent of the loci with frequency greater than 5% were shared by a given population with one or two other populations, while 80% of loci with frequency greater than 5% were shared by a given population with up to five other populations of *A. stricta*. All loci within a population were polymorphic with respect to band presence or absence. Average expected heterozygosity [Nei's gene diversity (H_e)] in a population was 0.050 with individual values ranging from 0.039 to 0.063; these were statistically different for each population (Table 3).

Based on the AMOVA, 38% of the total variation resides among *A. stricta* populations while 62% is attributable to the differences among individuals within populations. The proportion of total variance residing among populations (F_{ST}) in the absence of any grouping was 0.377 (P = 0.000). When populations were grouped by habitat (xeric sandhill versus mesic flatwood) or by geographic location (northern versus southern populations), the F_{ST} values were 0.377 (P = 0.000) and 0.386 (P = 0.000), while the F_{CT} values were 0.006 (P = 0.285) and 0.026 (P = 0.086), respectively (Table 4).

Pair-wise Nei's genetic distances ranged from 0.014 (between NC and SC, JCGA and AL, and JCGA and SMFL) to 0.028 (between DWFL and DDFL) (Table 5). Geographic distance between the populations (ranging from 1.9 to 1159 km) were not correlated with the corresponding genetic distances (r = 0.17, P = 0.25; Table 5). A consensus neighbor-joining tree based on pair-wise Nei's genetic distances, and rooted by using the out-group species *J. effusus*, showed a cluster formed by NC and SC with bootstrap support of 82%. The branch separating MS from the rest of the populations had a bootstrap value of 60%. All other branches had less than 50% bootstrap support (Figure 2).

Discussion

Genetic structure

Population differentiation and gene flow

Conservation perspectives

We observed significant (38%) inter-population differentiation and a modestly high amount (62%) of intra-population ISSR variation in *Aristida stricta*. Pair-wise similarity coefficients (data not shown) indicated that individuals within a population were more similar to each other than they were to individuals from other populations. This, combined with the large amount of genetic variability within populations and a high percentage of polymorphic loci within and across populations, supports the conclusion of Walters et al. (1994) that *A. stricta* is most likely an outcrossing species. These observations are also in agreement with the significant phenotypic variation observed among plants from the same populations grown in a common garden (Gordon and Rice 1998). Further, when Kalmbacher et al., (2004) tested the establishment of *A. stricta* from seeds by cross-planting between sandhill and flatwood sites within Florida, they observed that while cross-plantings could survive, survival was best when the donor and recipient sites shared similar hydrology. High differentiation (38%) among populations across its range, presence of private loci, and presence of loci shared with only 1 or 2 other populations confirms that local adaptation is likely a range-wide phenomenon in *A. stricta* and phenotypic variation at different life-cycle stages similar to that observed in two Florida populations by Kindell et al. (1996) might also be expected in populations elsewhere in the range.

While local adaptation may be occurring in populations of *A. stricta*, this adaptation appears to be independent of the influence of the hydrology of the macro-sites (i.e., xeric sandhill or mesic flatwood) or the geographic location of the populations. This result is not consistent with the reported phenotypic (Gordon and Rice 1998) or common garden (Kindell et al. 1996, Kalmbacher et al. 2004) results of greater similarity or establishment success of *A. stricta* from sites with similar soils. However, the lack of

dependence on geographic distance was also seen by Gordon and Rice (1996). These apparently conflicting results may potentially be explained by a combination of the historically large-scale fires that would burn through sandhills and flatwoods that would allow periodic gene flow across areas of over millions of acres (Baker 1926, Robbins and Myers 1992) coupled with local adaptation and vegetative growth.

The distribution of the DNA regions amplified in this study was unrelated to the habitat type or geographic location of the populations as shown by the AMOVA, which indicated that grouping populations by habitat or by geographic location did not influence the distribution of genetic variation (Table 4). The pair-wise genetic distances (Table 5) also showed that genetic distances between all pairs of populations were comparable. There was no clear pattern of relatedness when the genetic distances were compared for different habitat types, for example, MS population which is a coastal flatwood site was equally (genetically) distant from any other population regardless of the geographic distance between them or the similarity of habitat (Table 5). Similarly, geographic distances between any two populations did not influence their genetic distance; DDFL and DWFL, two of the geographically closest populations, albeit situated in different habitats, were genetically most distant (Table 5). While the neighbor-joining tree placed NC and SC, the two northern populations, in a cluster separating them from the rest of the populations, neither the branch support for the cluster nor the genetic distance for the pair was sufficient to surmise that these are more similar to each other than several other population pairs, e.g., SMFL and JCGA, or AL and JCGA (Figure 2 and Table 5). As a result, we support the reclassification of all populations of this species as *A. stricta*.

While *A. stricta* is putatively an outcrossing species, intervening ecological features, or urban and/or agricultural practices may be interfering with transport of pollen across populations. Also, the potential phenological differences that can affect gene flow opportunities among populations, for example asynchrony of flowering, could have led to the observed genetic divergence in this species (Walters et al.

1994). Given the lack of correlation between geographic and genetic distances, the low genetic distances for population pairs, and high differentiation, factors other than gene flow could be contributing to the observed genetic differentiation. Species with large geographic ranges (pair-wise geographic distances between sampled populations in this study ranged from 1.9 km to 1159 km) may experience a wider range of climatic and edaphic conditions that could give rise to different selective pressures on populations leading to greater genetic diversity within a species (Godt and Hamrick 1998). Other characteristics of *A. stricta* such as its long-lived individuals (Clewell 1989), low seed viability with rare seedling establishment in the field, short-lived seeds and absence of a persistent seed bank (Parrott 1967, Coffey and Kirkman 2006), fire and season dependent flowering (Parrott 1967), could also be contributing to the observed distribution of variation among the populations.

Our results, combined with those reported by Walters et al. (1994), Kindell et al. (1996), Gordon and Rice (1998), and Kalmbacher et al., (2004), lead us to conclude that both genotypic and phenotypic variability are high in *A. stricta*, and that because of restricted gene flow and selection the distribution of this variation is not uniform. Different selection pressures seem to be operating on populations regardless of their geographic proximity or habitat similarity. Considering these findings, it is recommended that extant sites with *A. stricta* populations be conserved to maintain its overall genetic diversity. Additionally, conservation projects should utilize locally collected seeds representing many different individuals to ensure a wide genetic base for each new or augmented population.

Acknowledgments

This research was supported by grant R-003-07 from the Florida Wildflower Foundation. We are grateful to the following individuals for providing permits, collecting leaf tissue, and/or providing technical or logistical assistance: Susan Cohen (Marine Corps Base Camp LeJeune, NC); Allynne Askins (Carolina

Sandhills National Wildlife Refuge, SC); Kay Kirkman and Melanie Keiser (Joseph W. Jones Ecological Research Center, GA); Rob Sutter and Chuck Martin (The Nature Conservancy, Moody Forest Natural Area, GA); Stephen Lee and Mark Garner (Conecuh National Forest, AL); Durwin Carter (Grand Bay National Wildlife Refuge, MS); Mike Keys (St. Marks Wildlife Refuge, FL); Chris Matson (The Nature Conservancy, Disney Wildlife Preserve, FL); Brian Scofield and Mike Kemmerer (Babcock-Webb Wildlife Management Area, FL); Rebecca Riddle (UF NFREC, Quincy, FL), and Fé Almira (UF, Gainesville, FL). Doria Gordon (The Nature Conservancy, University of Florida) contributed invaluably to the manuscript.

LITERATURE CITED

Archibald, J. K., M. E. Mort, D. J. Crawford, and A. Santos-Guerra. 2006. Evolutionary relationships within recently radiated taxa: comments on methodology and analysis of inter-simple sequence repeat data and other hypervariable, dominant markers. Taxon 55: 747-756.

Baker, H. L. 1926. Forest Fires in Florida. Florida Forestry Association, Jacksonville, Florida.

Clewell, A. F. 1989. Natural history of wiregrass (*Aristida stricta* Michx., Graminae). Natural Areas Journal 9: 223-233.

Coffey, K. L., and L. K. Kirkman. 2006. Seed germination strategies of species with restoration potential in a fire-maintained pine savanna. Natural Areas Journal 26: 289-299.

Earley, L. S. 2004. Looking for longleaf: The fall and rise of an American forest. The University of North Carolina Press, Chapel Hill, NC.

Esselman, E. J., L. Jianqiang, D. J. Crawford, J. L. Winduss, and A. D. Wolfe. 1999. Clonal diversity in the rare *Calamagrostis porterii* ssp. *insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. Molecular Ecology 8: 443-451.

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

Excoffier, L., G. Laval, and S. Schneider. 2006. Arlequin version 3.1: An integrated software package for population genetics data analysis. Computation and molecular population genetics lab, Institute of Zoology, University of Berne, Switzerland. Available for downloading at http://cpmg.unibe.ch/software/arlequin3.

Falk, D. A., and K. E. Holsinger. 1991. Genetics and conservation of rare plants. Oxford University Press, NY.

Felsenstein, J. 2004. PHYLIP (Phylogeny Inference Package), version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, WA.

Futuyma, D. J. 1986. Evolutionary Biology, 2nd ed. Sinauer, Sunderland, MA.

Godt, M. J. W., and J. L. Hamrick. 1995. Allozyme variation in two Great Smoky Mountain endemics: *Cacalia regalia* and *Glyceria nubigena*. Journal of Heredity 86: 194-198.

Godt, M. J. W., and J. L. Hamrick. 1998. Allozyme diversity in the grasses. Pp 11-29, *in* G. P. Cheplick, ed., Population biology of the grasses, Cambridge University Press.

Gordon, D. R., and K. J. Rice. 1998. Patterns of differentiation in wiregrass (*Aristida beyrichiana*): Implications for restoration efforts. Restoration Ecology 6: 166-174.

Hamrick, J. L. 1983. The distribution of genetic variation within and among natural plant populations. Pp 335-348 *in* C. N. Schonewald-Cox and S. M. Chambers, eds., Genetics and wild population management. Addison-Wesley, NY.

Hufford, K. M. and S. J. Mazer. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. TRENDS in Ecology and Evolution 18: 147-155.

Kalmbacher, R., J. Norcini, T. Pittman, S. Pfaff, and F. Martin. 2004. Wiregrass grown from seeds obtained on Florida flatwood and sandhill sites. Native Plants Journal 5: 123-130.

Kesler, T. R., L. C. Anderson, and S. M. Hermann. 2003. A taxonomic reevaluation of *Aristida stricta* (Poaceae) using anatomy and morphology. Southeastern Naturalist 2: 1-10.

Kindell, C. E., A. A Winn, and T. E. Miller. 1996. The effects of surrounding vegetation and transplant age on the detection of local adaptation in the perennial grass *Aristida stricta*. Journal of Ecology 84: 745-754.

Landers, J. L., D. H. van Lear, and W. D. Boyce. 1995. The longleaf pine forests of the southeast: requiem or renaissance. Journal of Forestry 93: 39-44.

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

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27: 209-220.

McCay, D. H. 2000. Effects of chronic human activities on invasion of longleaf pine forests by sand pine. Ecosystems 3: 283-292.

McGlaughlin, M., K. Karoly, and T. Kaye. 2002. Genetic variation and its relationship to population size in reintroduced populations of pink sand verbena, *Abronia umbellata* subsp. *breviflora* (Nyctaginaceae). Conservation Genetics 3: 411-420.

Nei, M. 1972. Genetic distance between populations. American Naturalist 106: 283-292.

Outcalt, K. W. 1994. Seed production of wiregrass in Central Florida following growing season prescribed burns. International Journal of Wildland Fire 41: 123-125.

Outcalt, K. W., M. E. Williams, and O. Onokpise. 1999. Restoring *Aristida stricta* to *Pinus palustris* ecosystems on the Atlantic Coastal Plains, U. S. A. Restoration Ecology 2: 262-270.

Parrott, R. K. 1967. A study of wiregrass (*Aristida stricta* Michx.) with particular reference to fire. MS Thesis, Duke University, Durham, NC. 137 Pp.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288-295. Available for downloading at http://www.anu.edu.au/BoZo/GenAlEx/.

Peet, R. K. 1993. A taxonomic study of *Aristida stricta* and *A. beyrichiana*. Rhodora 95: 25-37.

Platt, W. J. 1988. Fire ecology of the southeastern longleaf pine flatwoods. *Wiregrass Biology and Management: Maintaining Groundcover Integrity in Longleaf*: (eds L.C. Duever and R.F. Noss), pp 5. Proceedings of the Symposium, Valdosta State College, Gainesville, Florida

Robbins, L. E., and R. L. Myers. 1992. Seasonal effects of prescribed burning in Florida: a review. Tall Timbers Research, Inc., Miscellaneous Publication No. 8. Tallahassee, Florida.

Rohlf, F. J. 2005. NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.2. Exeter Software, Setauket, NY.

Smith, J. F., and T. A. Bateman. 2002. Genetic differentiation of rare and common varieties of *Eriogonum shockleyi* (Polygonaceae) in Idaho using ISSR variability. Western North American Naturalist 62: 316-326.

Streng, D. R., J. S. Glitzenstein, and W. J. Platt. 1993. Evaluating effects of season of burning longleaf pine forests: a critical literature review and some results from an ongoing long term study. Pp. 227-263 *in* S. M. Hermann, ed., Proceedings of the Tall Timbers fire ecology conference, No. 18. Tall Timbers Research Station, Tallahassee, FL.

van Eerden, B. P. 1997. Studies on the reproductive biology of wiregrass (*Aristida stricta* Michaux) in the Carolina sandhills. MS Thesis, University of Georgia, Athens, GA. 89 Pp.

von Trinius, C. B., and F. J. Ruprecht. 1849. *Aristida beyrichiana*. Mémoires de l'Académie Impériale des Sciences de St. Pétersbourg, Ser. 6, Sciences et Mathématiques, Second Partie, Sciences Naturelles 7: 104.

Walters, T. W., D. S. Decker-Walters, and D. R. Gordon. 1994. Restoration considerations for wiregrass (*Aristida stricta*): allozymic diversity of populations. Conservation Biology 8: 581-585.

Ward, D. B. 2001. New combinations in the Florida flora. Novon 11: 360-365.

Zietkiewicz, E., A. Rafalski, and D. Labuda. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20: 176-183.

Table 1. Name, location, and habitat type of populations of *Aristida stricta* sampled in 2008 to identify inter-simple sequence repeat (ISSR) polymorphisms.

Population Code	Location	County, State	Habitat
NC	Camp LeJeune	Onslow, NC	xeric sandhill
SC	Carolina Sandhills National Wildlife Refuge	Chester, SC	xeric sandhill
MFGA	Moody Forest Natural Area	Appling, GA	mesic flatwood
JCGA	Joseph W. Jones Ecological Research Center	Baker, GA	xeric sandhill
AL	Conecuh National Forest	Conecuh, AL	xeric sandhill
MS	Grand Bay National Wildlife Refuge	Jackson, MS	mesic flatwood
SMFL	St. Marks National Wildlife Refuge	Wakulla, FL (North)	mesic flatwood
DWFL	Disney Wilderness Preserve	Osceola, FL (Central)	mesic flatwood
DDFL	Disney Wilderness Preserve	Osceola, FL (Central)	xeric sandhill
BWFL	Babcock-Webb Wildlife Management Area	Charlotte, FL (South)	mesic flatwood

Table 2. Primer sequences, components of reaction mixture in a total reaction volume of 12.5 µL, and thermocycler conditions used for PCR-based amplification of the inter-simple sequence repeat (ISSR) regions in *Aristida stricta*.

Primer sequence	**Template DNA (µL)**	**PCR buffer**	**Primer (µM)**	**dNTP (mM)**	**$MgCl_2$ (mM)**	**Taq polymerase (U)**	**Thermocycler conditions**
$(GTG)_3$ GC $(GA)_8$ T $(CA)_8$ G $(GT)_6$ GG	0.5	1X	1.4	200	2	1.0	1 cycle of 94°C for 1.5 min; 34 cycles of 94°C for 40 s, 45°C (annealing temperature) for 45 s, 72°C for 1.5 min; 1 cycle of 94°C for 45 s, 48°C for 45 s, and final extension at 72°C for 5 min
$(TC)_{10}$ A $(AGAC)_4$ GC $(GACA)_4$ GT $(GAG)_4$ GC	1.0	1X	1.4	200	2	1.0	1 cycle of 94°C for 2 min; 35-40 cycles of 94°C for 1.5 min, 50°C (annealing temperature) for 1.5 min, 72°C for 1.5 min; final extension at 72°C for 5 min

Table 3. Number of loci (i.e., bands) amplified and their distribution across the sampled populations of *Aristida stricta* and out-group species *Poa pratensis* (PP) and *Juncus effusus* (JE). Nei's gene diversity (H_e) was calculated based on allele frequencies by using the equation $H_e = 1-\sum p^2$, where p is the frequency of the marker allele [p = 1-q; q = √frequency of null homozygote]. Locus absence indicated a null homozygote, and the frequencies of q and p were calculated by assuming random mating (Lynch and Milligan, 1994). Value of H_e for a population is a mean across all loci and individuals in that population. All computations were conducted by using GenAlEx 6.

Population	Number of individuals sampled	Number of loci	Number of loci with frequency ≥ 5% (%)	Number of private loci (%)	Number of loci shared with ≤ 25% populations (%)	Number of loci shared with ≤ 50% populations (%)	Nei's gene diversity (H_e)	
							Mean	SE
NC	32	105	102 (97%)	13 (12%)	47 (46%)	86 (84%)	0.051	0.005
SC	32	91	91 (100%)	8 (9%)	43 (47%)	76 (84%)	0.049	0.005
MFGA	32	101	100 (99%)	13 (13%)	51 (51%)	82 (82%)	0.050	0.005
JCGA	32	99	99 (100%)	10 (10%)	48 (48%)	84 (84%)	0.048	0.005
AL	32	88	84 (95%)	7 (8%)	42 (50%)	75 (89%)	0.039	0.005
MS	32	100	100 (100%)	21 (21%)	45 (45%)	74 (74%)	0.049	0.005
SMFL	32	99	97 (98%)	11 (11%)	47 (48%)	83 (86%)	0.046	0.005
DWFL	30	103	100 (97%)	22 (21%)	42 (42%)	76 (76%)	0.052	0.005
DDFL	32	109	107 (98%)	18 (17%)	49 (46%)	83 (78%)	0.055	0.005
BWFL	32	125	125 (100%)	28 (22%)	62 (50%)	89 (71%)	0.063	0.006
Mean for A. stricta	*32*	*102*	*101(98%)*	*15(14%)*	*48(47%)*	*81(80%)*	*0.050*	*0.005*
PP	15	91	91 (100%)	18 (20%)	50 (55%)	69 (76%)	0.051	0.006
JE	15	102	102 (100%)	15 (15%)	54 (53%)	83 (81%)	0.047	0.005

Table 4. Analysis of molecular variance (AMOVA) of inter-simple sequence repeat (ISSR) data by using ARLEQUIN ver 3.1 to determine the genetic structure within- and among- populations of *Aristida stricta* sampled in 2008. Populations were grouped by their preferred habitat (xeric sandhill or low mesic flatwood) and region of occurrence (northern versus southern populations). Computations for AMOVA are based on a matrix of pair-wise squared Euclidean distances between individuals. Percentage variation is the distribution of variation at a given level of hierarchy (among groups/among populations within groups/among populations/within populations). The *F* statistic is a measure of genetic structure at different levels of hierarchy with values ranging from 0 to 1. The *P* value is the proportion of 1000 permutations that gave an *F* statistic value ≥ the observed value.

Grouping of populations	**Source of variation**	**Variation (%)**	***F* statistic**	***P***
Without any grouping				
NC, SC, MFGA, JCGA, AL, MS, SMFL, DWFL, DDFL, BWFL	Among populations	37.7	[z] $F_{ST} = 0.377$	0.000
	Within populations	62.3		
Two groups based on habitat type				
Xeric sandhill populations: NC, SC, JCGA, AL, DDFL Mesic flatwood populations: MFGA, MS, SMFL, DWFL, BWFL	Between groups	0.58	[y] $F_{CT} = 0.006$	0.285
	Among populations within groups	37.16	$F_{SC} = 0.373$	0.000
	Among populations	37.74	$F_{ST} = 0.377$	0.000
	Within populations	62.26		
Two groups based on geographic location				
Northern populations: NC, SC Southern populations: MFGA, JCGA, AL, MS, SMFL, DWFL, DDFL, BWFL	Between groups	2.69	$F_{CT} = 0.026$	0.086
	Among populations within groups	35.97	$F_{SC} = 0.369$	0.000
	Among populations	38.66	$F_{ST} = 0.386$	0.000
	Within populations	61.34		

[z] F_{ST} is the proportion of total variance residing among populations
[y] F_{CT} is the proportion of total variance residing in groups
[x] F_{SC} is the proportion of total variance in a group residing among populations in that group

Table 5. Pair-wise geographic distances (km) above diagonal and pair-wise Nei's genetic distance below diagonal for populations of *Aristida stricta* sampled in 2008. Correlation coefficient (r) calculated by using the Mantel test is presented in the last line of each table along with the *P* value.

	NC	**SC**	**MFGA**	**JCGA**	**AL**	**MS**	**SMFL**	**DWFL**	**DDFL**	**BWFL**
NC	-	268.1	578.6	788	966.4	1159	871.3	860.9	862.2	1004
SC	0.014	-	357.1	545.9	710.7	900.5	647.7	730	731.7	875.2
MFGA	0.024	0.021	-	217.8	413.1	605.1	294.2	431.5	433.4	566.6
JCGA	0.020	0.016	0.017	-	199.4	388.5	135.6	456.7	458.6	555.9
AL	0.016	0.015	0.021	0.014	-	193.4	232.3	597.5	599.2	663.8
MS	0.023	0.022	0.021	0.020	0.021	-	382.3	726.4	727.8	761.4
SMFL	0.020	0.018	0.020	0.014	0.017	0.020	-	365.5	367.2	442.1
DWFL	0.026	0.023	0.023	0.018	0.019	0.023	0.021	-	1.9	145.7
DDFL	0.025	0.022	0.025	0.023	0.022	0.026	0.022	0.028	-	144.1
BWFL	0.025	0.019	0.024	0.018	0.018	0.027	0.021	0.024	0.024	-

r = 0.17; *P* = 0.25

Figure 1. Ten populations of *Aristida stricta* across the southeastern US were sampled to assess intra- and inter-population inter-simple sequence repeat (ISSR) polymorphisms. The sampled populations represent the near-boundaries of the species' natural distribution.

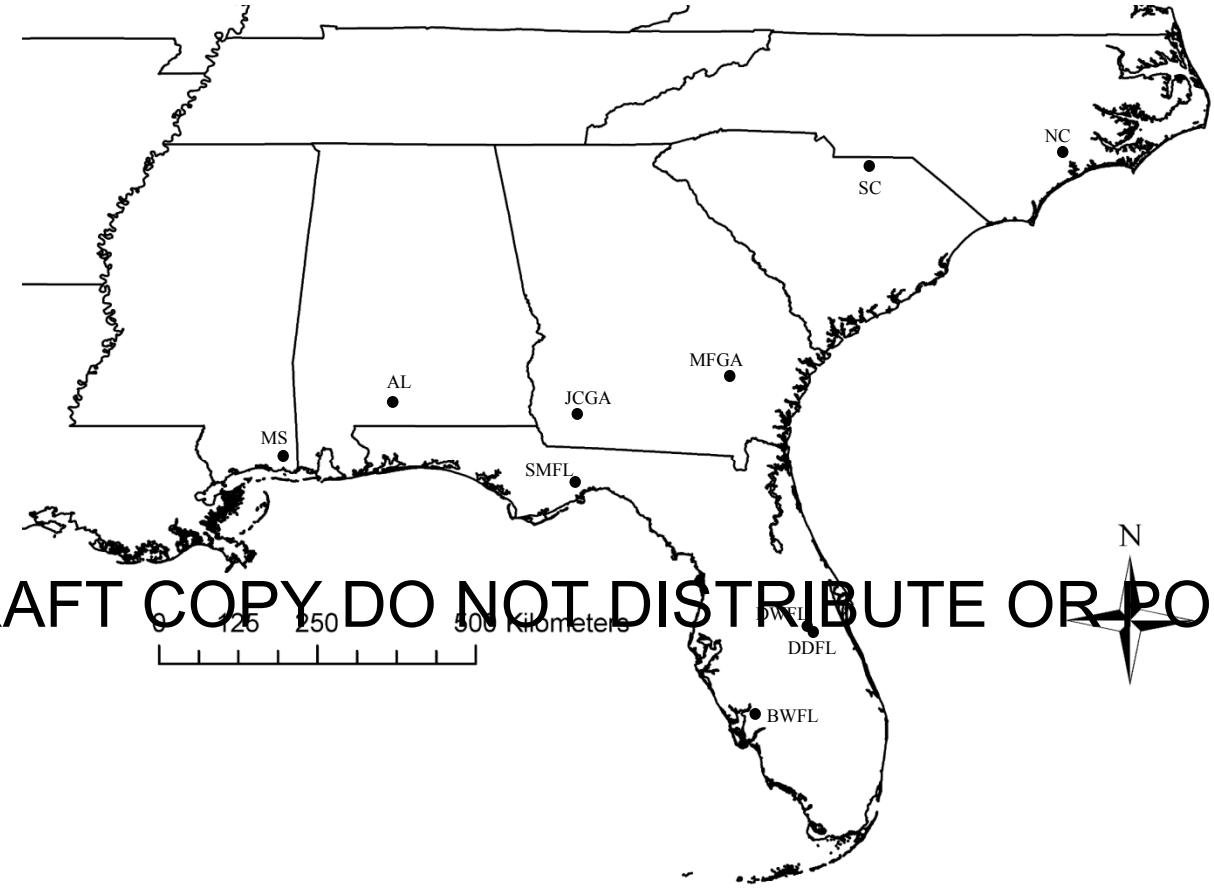

Figure 2. Majority-rule consensus neighbor-joining dendrogram, based on pair-wise Nei's genetic distances, showed phylogenetic relationships among the ten populations of *Aristida stricta* sampled in 2008. The tree is rooted with *Juncus effusus* as the out-group species. Numbers on the branches indicate bootstrap support (%) obtained from analysis of 1000 replicate data sets.