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Genetic Structure of *Halodule wrightii* Populations from the Laguna Madre Region in the Western Gulf of Mexico

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A random amplified polymorphic DNA assay was used to assess genetic variation in populations of the seagrass *Halodule wrightii* (Ascherson) from the western Gulf of Mexico. This region includes one of the world's few hypersaline lagoons (Laguna Madre) and contains the vast majority of seagrasses on the Texas coast. Results indicate a moderate amount of genetic diversity among populations. The highest level ($H_e = 0.33$) was found in a population from Nueces Bay, a disturbed site in the Coastal Bend area, whereas the lowest was found in a Lower Laguna Madre population ($H_e = 0.15$). Genetic differentiation generally followed an isolation-by-distance model. The Nueces Bay population also showed the greatest degree of differentiation, whereas the Redfish Bay and Lower Laguna Madre populations were relatively similar ($\Phi_{ST} = 0.091$). Combined with previous results, we now have a rudimentary picture of genetic variation in this species from the Texas Gulf Coast.

We undertook this study to assess genetic variation in the seagrass *Halodule wrightii* (=*Halodule beaudettei*) from the western Gulf of Mexico. Commonly known as "shoalgrass", *H. wrightii* is a temperate to tropical species whose Atlantic range extends from North Carolina (U.S.A.) through the Gulf of Mexico and Caribbean Sea to the coasts of South America and Africa (den Hartog and Kuo, 2006). *Halodule wrightii* is common throughout the Gulf of Mexico and frequently serves as a pioneer species for the colonization of new or disturbed substrate (Marba and Duarte, 1998; Withers, 2002).

Previous studies have investigated genetic variation in *H. wrightii* using populations from more temperate (northerly) sites on the Texas coast (Angel, 2002; Travis and Sheridan, 2006). However, the majority (approximately 75%) of Texas seagrasses are found in the Laguna Madre of Texas and Tamaulipas, a 460-km-long hypersaline, coastal lagoon that traverses the southernmost third of the state's coast (Tunnell and Judd, 2002; Onuf, 2007). With its warm climate, high salinity, clear, shallow water, and protected shoreline, the Laguna Madre is a very favorable environment for seagrasses. We performed this work as a pilot study to examine genetic variation in *H. wrightii* from the Laguna Madre, and compare the results with those from an area having the second highest concentration of Texas seagrasses, the Coastal Bend. Secondarily, we wished to compare the results with those from a disturbed site where seagrasses have a history of extinction and recolonization. We expected the Laguna Madre population, with its favorable environment and dense concentration of seagrasses, to exhibit the highest degree of genetic diversity. We expected genetic differentiation (Φ_{ST}) among populations to be moderate to high, and follow a model of isolation by distance (Wright, 1943) reflecting the distances involved (20–200 km), putative physical barriers, and presumably low dispersal capacity for *H. wrightii* pollen and seeds (Orth et al., 2006).

MATERIALS AND METHODS

Study Sites.—Three sites were selected for sampling. Redfish Bay (RB: 27°54′18.60″N, 97°07′29.87″W) and Nueces Bay (NB: 27°51′54.64″N, 97°20′45.09″W) are located near Corpus Christi, in an area of the Texas coast known as the Coastal Bend. The Coastal Bend comprises multiple bays and estuaries, which together account for approximately 13% of Texas seagrass beds (Pulich et al., 1997; Pulich, 2007). Bays in this region are relatively shallow (0–2 m), typically receive little freshwater inflow, and experience similar climatic and environmental conditions (mean annual temperature = 22°C, mean annual salinity ~35‰) (Powell et al., 1997; Tunnell and Judd, 2002). NB is adjacent to the nation's sixth busiest port (Corpus Christi) and located at the mouth of the Nueces River. Severe rainfall events periodically inundate the bay with freshwater. Resulting low salinity and turbidity have led to the elimination of seagrasses from its boundaries in the past, though it has subsequently been recolonized from unknown sources (Pulich et al., 1997). Proximity to agricultural and industrial areas has also led to elevated levels of pesticides and metals being found in NB water, sediment, and tissue (oyster) samples (Ward and Armstrong, 1997).

The Lower Laguna Madre site (LLM: 26°15′32.45″N, 97°16′10.72″W) is located in the southern (lower) portion of the Texas Laguna Madre. The LLM is shallow (average depth of ~1 m), has a warmer climate (mean annual temperature = 23°C), and a higher salinity (mean annual salinity ~40‰) compared with the Coastal Bend. Though maintenance dredging and an agricultural drainage ditch contribute to water quality issues, its waters are generally clear and its protective shorelines are relatively undeveloped except for its southern end (Onuf, 1994, 2007; Tunnell and Judd, 2002).

Field and laboratory work.—Sampling occurred at a depth of less than 1 m along a 100-m × 100-m bisect established at each site. All populations were subtidal and substratum consisted either of mud (NB) or muddy sand (RB, LLM). Samples were collected at 5-m intervals along each transect for a total of 40 samples per site. Leaf shoots were placed in tubes with bay water for storage on ice until return to the laboratory. Samples were then frozen in liquid nitrogen and stored at −80°C until use.

DNA was extracted from 100 mg of tissue that had been ground to a fine powder in liquid nitrogen. All extractions used the plant Dneasy™ kit from Qiagen (Valencia, CA) according to the manufacturer's instructions. Samples were quantitated using a PicoGreen™ fluorometric assay (Molecular Probes, Portland, OR), also according to the manufacturer's instructions. A set of 40 random, decamer primers (Operon Technologies RAPD™ 10mer kits L and F) were used to screen for random amplified polymorphic DNA (RAPD) marker production (Williams et al., 1990). Polymerase chain reaction (PCR) DNA amplification reactions (25 µl) consisted of: 2.5 mM MgCl$_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton™ X-100, 1.25 M betaine, 0.2 mM dNTP, 0.2 µM decamer primer, 5–25 ng of DNA, and 2.5 units of Taq DNA polymerase (Promega, Madison, WI). Thermal cycling was performed in a PTC-200 DNA thermocycler (MJ research, Boston, MA). Conditions included an initial denaturation step at 94°C (3 min), followed by 45 cycles of denaturation at 92°C (1 min), annealing at 35°C (1 min), and extension at 72°C (2 min). A final extension step at 72°C (10 min) concluded the program. RAPD amplification products (markers) were separated on 2% agarose gels (1× Tris-acetate–ethylenediaminetetra-acetic acid) containing 0.5 mg ml^{-1} ethidium bromide. Gels were photographed over ultraviolet light using a Kodak EDAS™ 200 documentation system. All samples were analyzed twice with each decamer primer to assess RAPD marker repro-

TABLE 1. Primers used for *H. wrightii* analyses.

Primer	Sequence (5′–3′)	Total no. markers	No. polymorphic markers[a]
F3	CCTGATCACC	2	1
F4	GGTGATCAGG	1	0
F6	GGGAATTCGG	2	2
F7	CCGATATCCC	1	1
F9	CCAAGCTTCC	3	1
F10	GGAAGCTTGG	2	1
F15	CCAGTACTCC	3	2
Total		14	8

[a] Polymorphic markers defined as those with frequency <1 − (3/N), where N = total no. of samples (Lynch and Milligan, 1994).

ducibility. Only markers that appeared in duplicate runs were used for analysis.

Statistical analysis.—The RAPD markers, ranging in size from approximately 200 base pairs to 4 kilobases, were manually scored from digital images as being either present (1) or absent (0) for each sample. RAPD marker scores were used to calculate within-population estimates of genetic diversity typically used for dominant data, including the proportion of polymorphic markers (*P*) and Nei's gene diversity, which was averaged over all loci and reported as mean expected heterozygosity (H_e) (Nei, 1978; Lowe et al., 2004). Genetic differentiation between populations was estimated using the Φ_{ST} statistic (Excoffier et al., 1992), which is analogous to Wright's F_{ST} when binary data are used (Peakall and Smouse, 2005). The distribution of genetic variation, within and among populations, was calculated using analysis of molecular variance (AMOVA) (Excoffier et al., 1992; Lynch and Milligan, 1994). All calculations were performed with the GeneAlEx and ARLEQUIN population genetic analysis software programs (Schneider et al., 2000; Peakall and Smouse, 2005).

RESULTS

RAPD results and genetic diversity.—Although several PCR conditions were manipulated, a number of samples from each population failed to amplify consistently. Fifteen to seventeen samples from each population, however, did consistently amplify and were used for all analyses. Seven primers were found that generated a total of 14 markers across the sample set, eight of which were polymorphic (57%, Table 1). The proportion of markers that were polymorphic (*P*) in each population varied considerably, from a low of 21% in the LLM population to a high of 50% in NB (Table 2).

TABLE 2. Within-population *H. wrightii* genetic diversity estimates.

Population	No. samples	P^a	H_e^b
Redfish Bay	15	0.36	0.27
Nueces Bay	17	0.50	0.33
Laguna Madre	17	0.21	0.15

[a] Proportion of polymorphic loci.
[b] Mean expected heterozygosity.

Genetic diversity for each population was also estimated using mean expected heterozygosity (H_e). These estimates indicated moderate levels of genetic diversity in each population, again with the lowest estimate ($H_e = 0.15$) in the LLM population and the highest ($H_e = 0.33$) in NB (Table 2).

Distribution of genetic variation and genetic differentiation.—An AMOVA was performed to statistically estimate the distribution of genetic variation within and between populations. Our results showed that 73% of the variation was attributed to differences within populations, whereas 27% was attributed to differences among populations (Table 3). These results were supported by our estimates of genetic differentiation (Φ_{ST}) (Table 4). Low differentiation implies some type of active or historical gene flow, typically assumed to involve the movement or exchange of reproductive propagules (pollen, seeds, clones, etc.) (Lowe et al., 2004). Our results showed moderate to high Φ_{ST} values that generally followed a model of isolation by distance. NB consistently showed the highest level of differentiation ($\Phi_{ST} = 0.27$–0.37), whereas it was lowest between the RB and LLM populations ($\Phi_{ST} = 0.09$), even though they are considerably farther apart compared with NB and RB (200 vs 20 km).

DISCUSSION

This study examined genetic variation within and among three populations of *H. wrightii* from the Laguna Madre region in the western Gulf of Mexico. Our results found moderate levels of

TABLE 3. Distribution of genetic variation within and among *H. wrightii* populations.

Source of variation	df	Sum of squares	Est. variance	% of total	P value
Among populations	2	8.4	0.22	27	0.001
Within populations	46	27.0	0.59	73	0.001

TABLE 4. Genetic differentiation estimates between *H. wrightii* populations.

	Redfish Bay	Nueces Bay	Laguna Madre
Redfish Bay	0		
Nueces Bay	0.27^a	0	
Laguna Madre	0.091^b	0.37^a	0

[a] $P < 0.001$.
[b] $P = 0.056$.

genetic diversity in all populations. We found the highest estimates of diversity (P, H_e) in a population from NB, a secondary bay in the Texas Coastal Bend area, followed by a RB population approximately 20 km to the east. The lowest levels of diversity were found in the LLM, where seagrass cover is extensive and many conditions (salinity, water clarity, protected shorelines) are highly favorable for seagrass growth.

Seagrasses are notorious for the variation they exhibit in population genetic diversity, in some instances even within meadows (Waycott et al., 2006; Procaccini et al., 2007). This is typically attributed to differences in the relative amount of sexual, vs vegetative, reproduction. Like most seagrasses, *H. wrightii* is capable of both, and although vegetative reproduction appears to predominate, flowering, fruit, and seed production have been reported throughout its range, including in RB and the LLM (McMillan, 1976, 1981; Johnson and Williams, 1982; Les, 1988; Ferguson et al., 1993; McGovern and Blankenhorn, 2007). If sexual reproduction is higher in the Coastal Bend this could account for the higher heterozygosity (H_e) values. Conversely, genetic drift could account for the lower diversity at the LLM site if it is populated by fewer clones, perhaps as a result of reduced recruitment in its larger, denser beds. In reality multiple factors are likely to be involved. As a recent review has noted, it is becoming evident that seagrass population genetic diversity is a complex result of demographics, mating system, genetic processes (drift, migration, selection, and mutation), and dispersal/recruitment (Procaccini et al., 2007), few data for which are currently available for *H. wrightii*.

We also investigated genetic differentiation among sites. Φ_{ST} estimates generally followed a model of isolation by distance, with the greatest degree of differentiation between the NB and LLM populations. This was generally expected considering the distance involved (ca. 200 km) and low predicted dispersal capacity for *H. wrightii* pollen and seeds, which are poorly

buoyant and released below the sediment surface (McMillan, 1985; Ackerman, 2002; Orth et al., 2006). However, differentiation was also fairly high between NB and the much closer (ca. 20 km) RB population. Physical barriers in NB, including a narrow mouth with an extensive oyster reef, may be one factor (CBBEP, 1998). A history of bed extinction and recolonization in NB could be another. Metapopulation theory, for example, predicts higher differentiation for populations subject to extinction/recolonization dynamics (Slatkin, 1977; Pannell and Charlesworth, 1999). Theory also predicts lower genetic diversity in such populations because of founder effects and drift, but this was not the case found for NB. We cannot, however, rule out gene flow between NB and numerous other populations in the Coastal Bend, or recruitment from a seed bank, both of which could hedge against losses in diversity (Bohrer et al., 2005; Honnay et al., 2008). Honnay et al. (2009), for example, found little evidence of declining genetic diversity over a 3-yr period among a metapopulation of the annual *Erysimum cheiranthoides*, though F_{ST} estimates tripled over this time, a feature they attributed to seed bank activation and high, localized migration rates. Selection may also have a role. NB's unique conditions (periodic low salinity, high turbidity, higher level of metals and pesticide) may exert a selection pressure that contributes to its higher Φ_{ST} estimates.

Genetic differentiation between the RB and LLM populations, in contrast, was relatively low. In fact, it was similar to the value estimated for *Thalassia testudinum* populations from the same general locations (Larkin et al., 2006). This was surprising, given the distance between sites (also ca. 200 km), differences in seed buoyancy, and predicted dispersal capacities for the two species (Kaldy and Dunton, 1999; Orth et al., 2006). However, some factors merit attention. For example, RB and the LLM are connected by a dredged navigation channel, the Gulf Intracoastal Waterway (GIWW). Frequent commercial traffic along the GIWW raises the possibility of reproductive shoot transfer through intermittent attachment to shipping. Second, the Laguna Madre serves as the primary winter habitat for approximately 75% of the world's population of redhead ducks (*Aythya americana*) (Weller, 1964; Woodin and Michot, 2002). The winter forage for these birds consists almost exclusively of *H. wrightii* rhizomes (Cornelius, 1977). Crop surveys have occasionally also shown the presence of *H. wrightii* seeds (M. Woodin, pers. comm.), suggesting at least the potential for redheads to serve as vectors for gene flow. Third, either seed dispersal is much greater than predicted or *H. wrightii* can disperse

by other means, as evidenced by the rapid expansion of *H. wrightii* in the Upper Laguna Madre following completion of the GIWW in 1948 (Quammen and Onuf, 1993; Onuf, 2007). This expansion, approximately 80 km, is unlikely to have occurred via clonal growth alone.

In conclusion, we found that populations from the LLM and Texas Coastal Bend contain moderate levels of genetic variation, similar to those found for other species with comparable life histories and breeding systems (Nybom and Bartish, 2000). Genetic differentiation generally followed a model of isolation by distance, with a population from a disturbed site (NB) showing consistently higher values. Differentiation between two rather widely spaced populations was surprisingly low, contrary to expectations for a species with such a low predicted dispersal capacity. These results complement previous RAPD and amplified fragment length polymorphism studies that examined *H. wrightii* populations from areas north of the Laguna Madre (Angel, 2002; Travis and Sheridan, 2006). The rudimentary picture that emerges is one of low to moderate genetic variation and population differentiation for this species from its range on the Texas Gulf Coast. However, the sampled areas still constitute only a minor fraction of *H. wrightii*'s range and results in this study were obtained with a small number of random, dominant markers (RAPDs). Future studies should seek to verify and extend these results using more robust, codominant markers, work that is currently in progress.

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