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AFLP diversity within and between populations of the Caribbean seagrass *Thalassia testudinum* (Hydrocharitaceae)

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Abstract Genetic variation was assessed in the seagrass *Thalassia testudinum* from three regions of the Caribbean and north Atlantic using allozyme electrophoresis and amplified fragment length polymorphism (AFLP) analysis. Very low allozyme variability was detected among the 196 shoots analyzed from a range of sites in the San Blas region of Panama. AFLP markers detected high similarity (0.87) among the population samples surveyed from Bermuda and Panama across six AFLP primer pairs and over 260 banding positions. High levels of gene flow were detected between all the sites analyzed ($N_m > 1.7$). Significantly complete genotypic similarity was observed between samples from Bermuda and Panama, indicating that long distance vegetative fragment dispersal is highly probable. Very low genetic differentiation between all sites, even Bermuda and Panama, some 2,700 km apart, agrees with other studies and is further evidence of a highly uniform gene pool in *T. testudinum*. High levels of genetic uniformity in *T. testudinum* may be related to long-term environmental change over its geographic range. While AFLP analysis proved useful in determining genetic variation in this seagrass, the application of co-dominant markers such as microsatellites will be more informative in determining the nature of genetic uniformity and its adaptive significance in *T. testudinum*.

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Introduction

Seagrass communities are an essential component of global, nearshore marine habitats (Larkum et al. 1989). As marine angiosperms, seagrasses have adopted both sexual and vegetative methods of recruitment and survival (Inglis 2000). A greater understanding of seagrass population dynamics is essential to understanding long- and short-term factors in how these important communities are established and maintained (Duarte 1999; Waycott 2000a). The vegetative growth habit of seagrasses [via rhizomes in the guerilla habit of Lovett-Doust (1981)] anchors the plants and provides stability and resistance to water currents and wave action (Arber 1920). The fine details of rhizome growth form adopted by various seagrass species are being understood through an integrated approach of modeling and empirical analyses (Marba and Duarte 1998). However, the long-term (i.e. evolutionary) importance of the guerilla vegetative growth strategy in seagrasses remains unexplored, particularly with respect to clonal dispersal and to survival following disturbance (Waycott 2000b).

Early observations among many hydrophilous aquatic plant species of low genetic variability led Les (1988) to suggest that the predominance of vegetative and/or asexual reproduction results from a greater fitness among genetically uniform offspring to preserve adaptive gene complexes. Obviously, testing this concept requires detailed knowledge of not only the genetic diversity of species but also the relationship of genetic diversity to survival and its adaptive significance. Clonal growth might be viewed, therefore, as a significant factor in the slow evolutionary rates among hydrophiles (Les 1988; Waycott 2000b). Genetic diversity studies of seagrass populations, in conjunction with analysis of evolutionary divergence among seagrass lineages, will improve our understanding of the impact of clonality on seagrass evolution.

Genetic variation, and the partitioning of that variation within and between populations, is an informative

measure of population processes in plants (Hamrick and Godt 1989). To date, genetic diversity of seagrass populations has been assessed extensively in only a few species, with studies demonstrating a wide range in genetic variability from zero in *Amphibolis antarctica* (Waycott et al. 1996) to moderate in *Thalassia testudinum* (Kirsten et al. 1998; Davis et al. 1999) and *Posidonia oceanica* (Procaccini and Waycott 1998) to high in *Posidonia australis* (Waycott 1995; Waycott et al. 1997;) and *Zostera marina* (Reusch et al. 1998; Ruckelshaus 1998). Partitioning of variation within and between populations is often high (e.g. *P. australis*) (Waycott et al. 1997), although in many studies to date inadequate population sampling reduces the interpretability of reported results. These observations, at least in part, conflict with earlier studies, thus emphasizing the need for detailed study before we can test models of adaptive gene complexes and the significance of genetic diversity among hydrophiles.

T. testudinum, commonly known as turtle grass, occurs throughout the Caribbean and Gulf of Mexico (den Hartog 1970). Research activities on this seagrass have tended to focus on productivity, community ecology, and ecophysiology with gaps in our understanding of population dynamics (e.g. Zieman et al. 1999). Of particular interest has been the observation of die-off in *T. testudinum* meadows over large areas in Florida Bay, Florida, USA (Robblee et al. 1991). This has led, in part, to the interest in population genetic studies of this species to determine if, perhaps, there is a genetic component to the turtle grass die-off (Kirsten et al. 1998; Davis et al. 1999). Our understanding of population genetic structure, the adaptive significance of observed genetic diversity and the ability of seagrasses to recover from different types of disturbances is only beginning to develop (e.g. Ruckelshaus 1998) and research into this area of seagrass biology is of critical importance.

In this paper, we describe the population genetic diversity of *T. testudinum* from one region in the Caribbean and one region in the north Atlantic using amplified fragment length polymorphism (AFLP) analysis. This relatively new DNA fingerprinting technique (Vos et al. 1995) was, prior to this study, untested in seagrass population genetic analysis. We use these data to describe the extent and distribution of clonal ramets over different spatial scales and to infer population dynamics from the pattern of genetic variation among regions observed.

Materials and methods

Field collections

Shoot samples of *Thalassia testudinum*, sampled quantitatively and considered representative of the population, were obtained from one location within the Caribbean (San Blas Archipelago, Republic of Panama) and one location in the north Atlantic (Bermuda). San Blas collections of *T. testudinum* were made in the northern sector of the San Blas archipelago among coral quays adjacent to Punta San Blas (San Blas Point). Grab samples were collected from an additional site in the Caribbean: Florida Bay, Florida (Fig. 1).

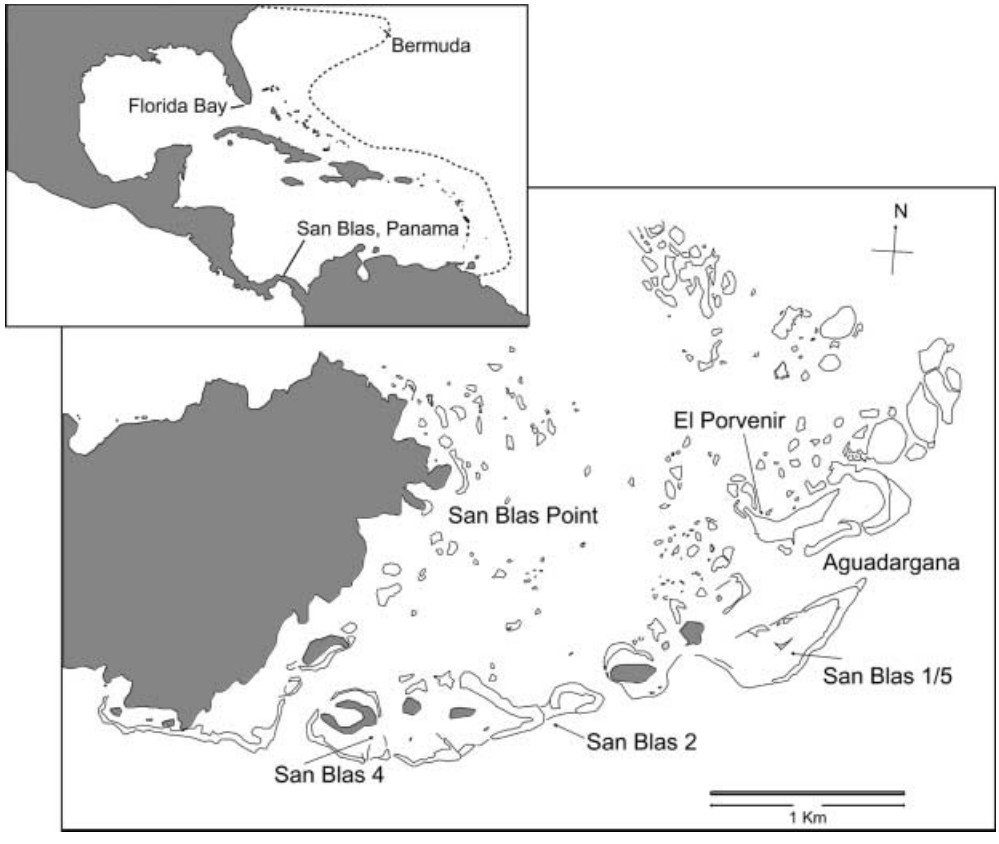

Fig. 1 *Inset* Distribution map of the seagrass *Thalassia testudinum* (*dotted outline*), land is *shaded grey*. The Caribbean and Atlantic collection locations are indicated. *Main map* Collection sites of *T. testudinum* in the San Blas archipelago, Panama, land is *shaded grey*, major coral reefs are *outlined*

Population samples were collected every 2 m within 100 m^2 quadrats, yielding a total of 36 samples per quadrat. This same collection protocol was used at three sites, separated by a maximum of 2 km, in the San Blas Archipelago of Panama (Fig. 1). The entire short shoot was either removed from the rhizome, or collected with a small piece of rhizome attached. Samples were kept cool until return to the field station, where the outer sheath was removed and the shoot was rinsed in clean seawater prior to storage in an airtight plastic bag, with air and excess water removed, in the refrigerator or on ice. All samples were returned, on ice, to the Naos Marine Laboratory (Smithsonian Tropical Research Institute) in Panama City from San Blas and Bermuda. Upon arrival at the Naos laboratory, samples were cleaned and rinsed in distilled water. In preparation for allozyme analyses, the first 1 cm of the shoot meristem was removed, placed in a microcentrifuge tube and stored at –80 °C until required. The remaining shoot tissues were stored in plastic bags at –80 °C until use.

Allozyme analysis

Samples from the three sites at San Blas were ground in a buffer according to Waycott and Sampson (1997); filter-paper wicks were added and kept on ice until loaded into starch gels. Samples were subjected to starch gel electrophoresis following the protocols of Waycott (1995) and Waycott and Sampson (1997). Twenty enzyme systems were screened initially and a total of 14 allozyme loci, from ten enzyme systems, were scored for all 196 samples collected from three sites at San Blas (Table 1). The samples from Bermuda were enzymically degraded when they returned to Panama and were not in condition to be used in allozyme analysis.

DNA extraction

Approximately 1–5 cm of the frozen, basal leaf tissue (previously covered by sheath) was ground in a mortar and pestle with acid-washed sand and SDS grinding buffer (4–5× volumes compared to the weight of tissue, similar to Huff et al. (1993) but with 1% SDS, 1% PVP-40 and 1% PVP-360). The ground tissue in buffer was placed in a 15 ml centrifuge tube and 5 *M* potassium acetate was added to make up 10% of the buffer volume and mixed well. The mixture was heated at 65 °C for 45 min and then placed on ice for 10 min. Cell debris was precipitated by centrifugation (15 min at 5,000 *g*) and the clear, aqueous supernatant was removed to another tube. DNA was precipitated from the supernatant by adding an equal volume of isopropanol and placing at –20 °C for at least 1 h or overnight. The DNA was pelleted by centrifugation (20 min at 5,000 *g*), washed once in 500 µl 70% ethanol (the tube was shaken, the pellet dislodged from the bottom of the tube and then centrifuged to re-pellet) and then alcohol was poured off and excess alcohol removed from the pellet by air-drying. Following resuspension of the DNA in 400 µl of sterile TE (100 m*M* Tris, 5 m*M* Na_2EDTA), the DNA was extracted in isoamylalcohol:chloroform (1:24 v/v). After mixing well and centrifuging at 11,600 *g* for 3 min, the top, aqueous layer was separated into a clean tube and the DNA precipitated in 2 volumes of ice cold ethanol and 0.1 volume 2.5 *M* sodium acetate. If no precipitate formed within 5 min after addition, samples were placed in the freezer for several hours. Samples were microcentrifuged for 1 min at 11,600 *g*, excess alcohol was carefully poured off, followed by a wash in 70% ethanol (as above), and excess alcohol was removed from the pellet by air drying. The final DNA was resupended in 100–200 µl TE; DNA quality and approximate concentration were assessed by agarose gel electrophoresis.

AFLP analysis

The ABI AFLP Plant Mapping Kit with fluorescent tagged primers (Perkin-Elmer product numbers 402004, 402005, 402006) was used at 25% of the recommended volumes; otherwise the recommended protocols were followed. PCRs were conducted in a heated-lid MJ Research PTC-200 thermocycler and the selective amplification products run on a 5% denaturing polyacrylamide gel in an ABI 373 A DNA sequencer. A ROX-100 DNA size standard was run in each lane with the different labeling reactions which had been amplified separately and combined for gel-running purposes. This "multiplexing" did not appear to change the fragment running conditions, although the same primer pairs were always run in the same combinations for each individual. Of the 64 possible primer combinations available in the kit, 24 were screened**;** all 24 showed selective amplification products and of these, 6 were screened across all 92 DNA samples available (Table 2). Replicate samples within, and between, gels were run to check consistency and to allow adjustment for between-run variability. The internal size standard allowed fragment sizes to be calculated and aided between-gel comparisons. A number of DNA samples proved resistant to amplification or contained insufficient DNA (i.e. low yield), despite extra purification by phenol:chloroform extraction; these samples were excluded from further analyses. After analyzing all screening runs using the ABI automatic band size calculations, sufficient variation among banding intensity and run conditions (due to voltage drift during the run) was observed to suggest that the band outputs were unreliable in detecting the presence of a band at a particular position (fragment size). Therefore, scoring was conducted manually by aligning the chromatograms from all samples for a particular primer using an internal ROX-1000 size standard as markers. This method yielded reproducible patterns between the repeat samples, although data from primer pair *Mse*I-CAC and *Eco*RI-ACC were not included as the results were consistently monomorphic and results were often variable between runs of the same sample. A final data set comprising 29 polymorphic bands across 51 samples was used in analysis. The final set of 51 samples included 17 samples from Bermuda, 23 from San Blas site 1, five from San Blas site 2 and six from San Blas site 4.

Table 1 Enzyme systems examined and their electrophoretic conditions. Starch buffer systems (*MC* morpholine citrate (Moran and Hopper, 1983) run at 60 mA for 5 h; *SAC*/*TC* tris citrate modified (Waycott and Sampson 1997) run at 65 mA for 6 h; *LiB* lithium borate (Wendel and Weeden 1990) run at 65 mA for 6 h

Enzyme	Abbreviation	E.C. Code	Buffer system	Number of loci scored
Esterase	EST	3.1.1.–	SAC/TC	1
Glucose phosphate isomerase	GPI	5.3.1.9	LiB	2
Isocitrate dehydrogenase	IDH	1.1.1.42	SAC/TC	1
Malate dehydrogenase	MDH	1.1.1.37	MC	2
Peroxidase	PER	1.11.1.7	MC and LiB	2
Phosphoglucomutase	PGM	5.4.2.2	SAC/TC	2
Phosphogluconate dehydrogenase	PGD	1.1.1.44	MC	1
Shikimic acid dehydrogenase	SDH	1.1.1.25	MC	1
Glucose-6-phosphate dehygrogenase	G6P	1.1.1.49	LiB	1
Diaphorase	DIA	1.6.–.–	SAC/TC	1

Table 2 AFLP primer pairs used in final analysis of all population samples and approximate number of amplification products per primer pair for *Thalassia testudinum* DNA

*Mse*I selective primer extension	*Eco*RI selective primer extension	No. amplification products
–CAA	–AAC	30
–CAA	–ACT	52
–CAC	–ACC	34
–CAC	–AAG	42
–CAC	–ACA	48
–CAG	–ACG	54
Total number of amplification products		260

Data analyses

No analyses were necessary for allozyme electrophoresis data, as virtually no polymorphisms were detected among all samples screened for all 14 loci.

For AFLP analyses, the San Blas sites were treated as populations due to their spatial separation (>1 km). A presence/absence matrix of polymorphic AFLP bands was used as the basis for a dominant marker analysis using POPGENE (Yeh et al. 1997). A similarity matrix, with potential values from 0 to 1 indicating either complete identity (no shared bands) to complete identity (all bands shared), was calculated based on band sharing (Weising et al. 1995). Individuals with similarity of 1 were inferred to have the same genotype (i.e. to be representatives of the same clonal genet). A Similarity Index was calculated for all within, and between, population/site comparisons. In addition, pairwise genetic distances (Euclidian distance of Schneider et al. 1997) were calculated and used in AMOVA (Analysis of MOlecular VAriance, Excoffier et al. 1992) to determine genetic differentiation of the populations and sub-populations surveyed using the program ARLEQUIN (Schneider et al. 1997). The presence/absence data were used also in a non-metric, multidimensional scaling plot of similarity using a Kruskal non-metric analysis (SPSS), and a clustering analysis (Euclidian distance measure) was conducted using PC-ORD (McCune and Mefford 1999) and a dendrogram drawn based on distances between each sample. Neighbor-joining distance analysis, conducted using PAUP4.03b (Swofford 2000), was used to calculate a dendrogram; all samples were treated independently and shared characters were mapped onto the tree.

Results

Allozymes

Allozyme analysis detected only three allozyme variants among the 196 samples screened from the San Blas sites; three single-shoot samples from San Blas site 1 were heterozygous at the PGM-1 locus (data not shown). All other loci were homozygous and monomorphic. The low level of variability meant that allozyme analysis was of little practical use in this study.

Amplified fragment length polymorphism analyses

Twenty-nine polymorphic bands across 51 samples were used in AFLP analyses; the remainder of 226 banding positions were monomorphic. The genotypic similarity between individual samples was high, with a range in Similarity Indices from 0.6 to 1.0. There were three pairs of samples that shared identical genotypes: one pair from Bermuda (BrTh8 and BrTh13; samples 3 m apart), one pair from San Blas (SB4Th6 and SB4Th30; samples 8 m apart) and one pair consisting of an individual sample from each of San Blas and Bermuda (SB1Th19 and BrTh31; samples ~2,700 km apart). Within each of the San Blas and Bermuda populations, it is surprising that more samples did not share a common genotype but, even more surprising, is the sharing of an identical genotype by a sample from San Blas and a sample from Bermuda.

Similarity Indices for within, and between, population comparisons demonstrate a high degree of similarity, with an overall mean of 0.87 (Table 3). It is interesting to note that the Bermuda versus San Blas comparisons generally revealed a higher degree of similarity (mean = 0.865) than the comparisons between sites in San Blas (mean = 0.835). In addition, two of the three Similarity Indices calculated for within sites at San Blas were lower than the overall Similarity Index. Sample size is likely to be the cause of these differences with sites of smaller sample size producing a greater difference in similarity than those of larger sample size. In addition, it should be noted that small sample size in some sites means that conclusions may be general only.

Gene diversity (H) of the entire sample (Nei 1973; calculated using POPGENE) was 0.35 (±0.12). The value of G_{ST} (Nei 1973), again calculated using POPGENE, was 0.19 comparing the four sites independently. Results of AMOVA analysis revealed that no genotypic variation was attributable to differentiation between the two regions, San Blas Panama and Bermuda (Table 4). The majority of variation was within sites (96.6%), with the remainder attributable to between sites within regions (5.4%). Estimations of gene flow between pairs of sites (Table 5) was high ($N_m = 1.7$) to extremely high ($N_m = 31.4$) for a clonal plant species. F_{ST} values indicated low differentiation between most pairs of sites (Table 5).

Table 3 The Similarity Index over all samples and for each pair of sites calculated as the band sharing co-efficient of Weising et al. (1995)

Population group	Average
Overall	0.87
Bermuda	0.90
San Blas 1	0.86
San Blas 2	0.82
San Blas 4	0.92
Bermuda vs San Blas 1	0.87
Bermuda vs San Blas 2	0.84
Bermuda vs San Blas 4	0.91
Bermuda vs San Blas 5	0.84
San Blas 1 vs San Blas 2	0.82
San Blas 1 vs San Blas 4	0.88
San Blas 1 vs San Blas 5	0.82
San Blas 2 vs San Blas 4	0.85
San Blas 2 vs San Blas 5	0.79
San Blas 5 vs San Blas 4	0.85

Table 4 AMOVA (Analysis of Molecular Variance, Excoffier et al. 1992) results, for AFLP variation at four collection sites of *T. testudinum*: three sites in Panama (Caribbean) and one in Bermuda (north Atlantic)

Source of variation	*df*	Percentage of variation	*P*
Regions	1	0 (–2.0)	0
Sites within regions	2	5.4	0.006
Within sites	45	96.6	0.523

Table 5 Population pairwise F_{ST} statistics and gene flow values calculated using AMOVA (Analysis of Molecular Variance, Excoffier et al. 1992) (Schneider et al. 1997)

Population/s	F_{ST}	*P*	N_m
Bermuda vs San Blas 1	0.03	0.00	8.9
Bermuda vs San Blas 2	0.07	0.01	3.2
Bermuda vs San Blas 4	0.01	0.29	31.4
San Blas 1 vs San Blas 2	0.07	0.00	3.3
San Blas 1 vs San Blas 4	0.01	0.26	21.5
San Blas 2 vs San Blas 4	0.13	0.00	1.7
Overall mean	0.03	–	11.5

Population genetic analysis, using a dendrogram based on Euclidian distance clustering analysis of AFLP band presence and absence, reveals that sample genotypes do not group by population (Fig. 2). In fact, while some within-site grouping is in evidence, often the 'sister' sample might be from the farthest site (i.e. Bermuda vs San Blas). Additional evidence that there is little genetic differentiation between sites is seen in the ordination (Kruskal non-metric analysis) (Fig. 3). The scatter of samples is greatest in the site with the greatest number of samples, again indicating that sample size is a significant factor (possibly negatively**:** where the more samples are analyzed the less differentiation is observed) in sample differentiation in this data set. Outlier sample genotypes, such as those from the two San Blas 5 samples and the single Florida Bay sample are from limited collections and may be, by chance, unusual genotypes. Equivalent outlying sample genotypes are present in the samples from San Blas 1, the site with the greatest number of samples analyzed in this data set. We were unable to obtain additional samples from the other sites for this analysis.

Discussion

Less than 2% of the *Thalassia testudinum* San Blas samples had any observable differences in electrophoretic products when assessed using allozyme electrophoresis. This compares well with the study of Schleuter and Guttman (1998) who, studying *T. testudinum* in Florida, observed overall 5% of the population with variation with one homozygous genotype dominating the data set and the remainder being rare genotype combinations. The observed high degree of homozygosity in allozyme genotypes in both Florida (Schleuter and Guttman 1998) and Panama (this study) support the notion that *T. testudinum* is depauperate in genotypic diversity. Other seagrasses and aquatic plants display similar allozymic uniformity (Les 1988; Triest 1991; Waycott et al. 1996), although at this stage there is no observable, unifying trend to indicate why these plants have low variability and others (e.g. Waycott and Les 1996) do not.

The genetic structure of *T. testudinum* populations identified by Schleuter and Guttman (1998) is based on rare allele frequencies and does not take into consideration potential clonally-derived, identical genotypes. Their estimations of genetic diversity and gene flow are based on each collection site being treated as an independent population, however, if ramets of the same genet are present across sites then the concept of what is

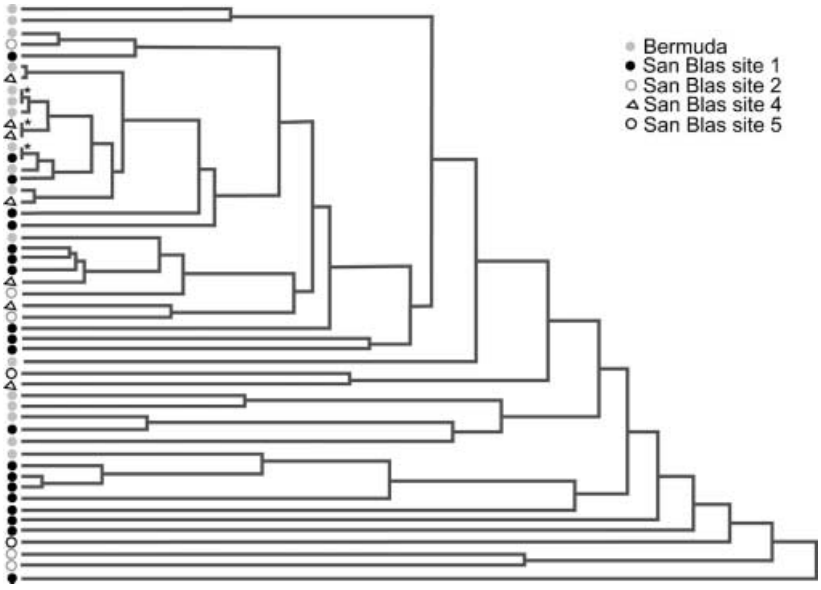

Fig. 2 Dendrogram of the AFLP presence/absence data for all *T. testudinum* samples from three sites in Panama (Caribbean) and one in Bermuda (north Atlantic) based on clustering analysis of a Euclidian distance matrix calculated in the software program PC-ORD (McCune and Mefford 1999). * indicates pairs of samples with identical AFLP genotypes

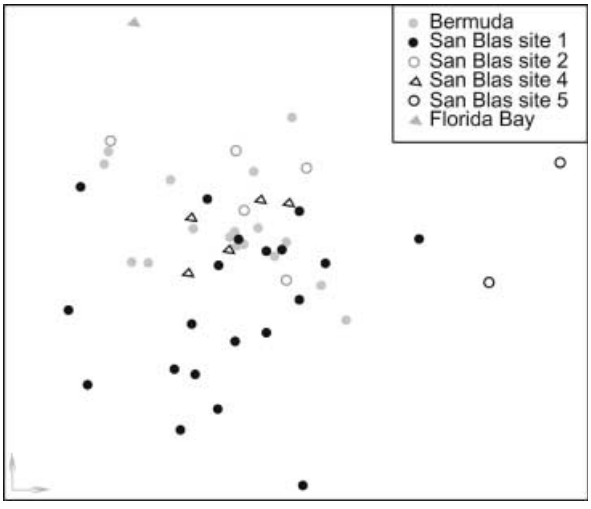

Fig. 3 Non-metric multidimensional scaling two-dimensional plot of the AFLP presence/absence data for all *T. testudinum* samples from three sites in Panama (Caribbean) and one in Bermuda (north Atlantic) based on Euclidian distance matrix calculated in the software program SPSS

to be used as a population in their study should be re-assessed. In addition, the recent study of Davis et al. (1999) demonstrates clonal ramets, detected using a DNA fingerprinting technique (SSR analysis, Davis et al. 1999), within 10 m may be common. The differences in genotypic variability detected using allozymes and DNA fingerprinting techniques support the observation that typically allozyme analysis has limited resolving power to identify clonal siblings in seagrasses (Waycott 1998).

Interestingly, the DNA fingerprinting technique, AFLPs, revealed only 11% of the banding positions as polymorphic (29/260, Table 2). This contrasts with an expected level of variability using AFLPs of 30% in plants (Mackill et al. 1996), although there is considerable variability among studies (Mueller and Wolfenbarger 1999). The polymorphism observed did not partition between populations; most variation was distributed among individuals within populations. However, the total gene diversity (H) (Nei 1973) was 0.35 ($\pm$0.12), a value similar to other studies using a dominant fingerprint markers such as AFLPs (e.g. Palacios et al. 1999).

AFLPs are able to detect a large number of informative markers (Vos et al. 1995) and are therefore ideal for surveying genotypic identity. However, as AFLP markers are assumed to be dominant unless characterized, resolution of relatedness requires a larger number of markers to be utilized than by some other hierarchy techniques (Mueller and Wolfenbarger 1999). By screening six primer pairs in this study, the probability of detecting genotypic differences among samples analyzed is high. This gives us confidence that our finding, of little genetic differentiation between the two widely-separated populations of *T. testudinum*, is a real observation on the level of genotypic variability and shared characters. Indeed, when character states were mapped onto a neighbor-joining distance tree, there were very few characters that were restricted to the major groups evident in the tree (Fig. 2); this is further evidence that there are few population-specific markers.

A study of genotypic variation in *T. testudinum* from southern Florida and Jamaica, using RAPD (random amplified polymorphic DNA) by Kirsten et al. (1998) found the observed partitioning of genetic variation was similarly distributed within populations (~81%) as observed in this study (~96%). These authors suggest that the low differentiation of their sites can be explained by the long-distance movement of vegetative fragments (Kirsten et al. 1998). This would, in fact, be the only probable explanation for the occurrence of the same clone in Bermuda and Panama, as observed in this study. Such fragmentation over large scales would explain the high degree of genotypic homogeneity observed in all studies of *T. testudinum* to date. No empirical evidence has been gathered that supports this notion and it should be considered that different modes of reproduction, such as continued growth, via rhizome extension, into new locations and sexual offspring (seedling) recruitment, may contribute to low differentiation.

On a local scale, the relatively high genetic diversity within populations, observed by Kirsten et al. (1998), may be attributable to rhizome extension, rather than long-distance vegetative propagules. Rhizome extension, which in *T.* is in a guerilla-like fashion (opportunistic spread with long internodes between short shoots), would result in a extremely mixed genotype population structure. These observations imply that the population size of this seagrass may effectively be very high, considerably greater than observed in *Zostera marina* (Ruckelshaus 1996) and more similar to *Posidonia australis* (Waycott et al. 1997).

Davis et al. (1999) demonstrated the presence of mixed clones within 25×25 cm^2 areas, but also that several shoots within the area were probably clonal siblings. In addition, there were clonal siblings found more than 10 m apart. With rhizome extension, young clones will demonstrate a more localized distribution of ramets. When clones have a longer history in a region, there is a greater probability that they will have spread over a larger area and be more distantly spaced from each other. Hence, the results of Davis et al. (1999) suggest both a relatively long history in the region and recent colonization, followed by rhizome extension. This pattern of clone distribution is to be expected, given that the regions studied in Florida Bay have been observed to have frequent die-offs and recolonization events. The results of our study, that over 96% of the genetic variation occurred within *T. testudinum* populations and that little genetic differentiation occurred between the two widely separated populations, agree with those of Kirsten et al. (1998) and Davis et al. (1999). Our results are even more unusual, however, because our comparative populations (Bermuda and Panama) are much more widely separated than those of the previous two

Florida-based studies. The observed genetic similarity may reflect recent colonization of the regions surveyed and possibly the majority of the Caribbean. On an evolutionary time scale, *Thalassia* is thought to have occurred in the Caribbean since the Eocene based on fossil evidence (den Hartog 1970). Since that time, significant marine temperature and habitat changes have occurred (Veron 1995). In addition, rapid colonization, combined with a change in seagrass population dynamics due to the removal of a large number of grazers since human habitation, may explain the high degree of genetic similarity in this species.

An alternative to large-scale disturbance by fragmentation, which must be considered, is that the clonal siblings described in this study are not full clones but are, instead, closely related individuals. Given the low genotypic variability detected in all studies of *T. testudinum*, it is possible (although improbable) that the highly polymorphic fingerprinting methods presented here do not detect complete genetic identity. This scenario then indicates that the sampled range of this seagrass is extremely homogenous and that gene flow occurs at high frequency. Unfortunately, there is extreme difficulty in determining gene flow in long-lived perennial and clonal organisms (Kirsten et al. 1998). As clones are maintained in populations over long periods, and increase their physical size to potentially interact with a greater number of other genets, as described earlier, the concept of migrants per generation becomes difficult to ascertain. Due to the guerilla-like habit of the genets and the difficulty in determining even a working definition of 'population' in these organisms, it makes estimates of gene flow difficult to interpret. For example, in this study gene flow is apparently greatest between the most distant collection sites (Table 5). This counter-intuitive observation may be attributed, in part, to problems of sample size (see earlier discussion). Clearly, it is important to be aware that misleading interpretations of population size and gene flow may result from inadequate sampling or insensitive techniques.

The results of this study, which are in strong agreement with other studies of *T. testudium* population genetic structure, have provided additional evidence of low genetic variability in a homogenous distribution throughout the Caribbean. Given the interest in determining ecological survival and resilience of this seagrass due to its role in nearshore communities along the Gulf of Mexico and elsewhere, it is important to ascertain the nature of this genetic homogeneity and its implications. The use of co-dominant markers with high level of polymorphism (e.g microsatellites) would be particularly useful in unraveling the complex interactions of genetic identity, gene flow and fragmentation and is currently underway in our laboratories.

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