

High levels of gene flow and low population genetic structure related to high dispersal potential of a tropical marine angiosperm

Jent Kornelis van Dijk¹, Brigitta I. van Tussenbroek^{1*}, Karina Jiménez-Durán²,
G. Judith Márquez-Guzmán², Joop Ouborg³

¹Unidad Académica Puerto Morelos, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México (UNAM), Apdo. Postal 1152, Cancún 77500, Quintana Roo, México

²Laboratorio de Desarrollo de Plantas, Facultad de Ciencias, UNAM, Circuito Exterior, Ciudad Universitaria, Del. Coyoacán, México D.F. 04510, México

³Department of Ecology, Radboud University Nijmegen, Tournooiveld 1, 6525 ED Nijmegen, The Netherlands

ABSTRACT: Marine ecosystems are traditionally considered to be highly connected, caused by long-distance movements of propagules in an open fluid medium. But this principle is not universal, and populations of many marine organisms have restricted gene-flow, including ones with drifting propagules. Direct tracking of marine free-floating propagules over large distances is virtually impossible, but long-distance dispersal (LDD) can be deduced from their drifting time and transport rates. Alternatively, LDD can be estimated as genetic exchange with the use of allelic frequency data obtained with genetic markers. The present study compares both approaches of LDD estimation for the tropical seagrass *Thalassia testudinum*. Fruit dispersal potential was measured in Puerto Morelos reef lagoon, Mexico. Between 10 and 15 % of the fruits floated to the surface (floating potential <1 to 10 d), with a derived potential dispersal of <1 to 360 km. The remainder of the fruits dehiscid *in situ* followed by limited seed dispersal (<1 to 10 m). Genetic diversity was characterized using 6 microsatellite loci for 16 populations distributed along a 1350 km long stretch of the Mexican coast, revealing a total of 58 alleles. AMOVA showed that only 9.75 % of the total variation was accounted for by genetic differences between the populations. Isolation by distance was significant, and revealed a panmictic area of 350 km. The results of both approaches of LDD estimation coincided well and the maximum displacement of the fruits of this marine angiosperm exceeded, by 1 to 2 orders of magnitude, the reported seed dispersal by terrestrial seed plants.

KEY WORDS: Connectivity · Genetic diversity · Fruit buoyancy · Hydrochory · Isolation by distance · Microsatellite markers · Long-distance dispersal · Seagrass · *Thalassia testudinum*

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INTRODUCTION

In the marine environment, the life cycle of many sessile or sedentary organisms includes a planktonic or free-floating phase, which allows for transport of progeny outside the donor population (Roughgarden et al. 1988, Siegel et al. 2003). Such free-living propagules of either plants or animals may be larvae, eggs, gametes, spores, rafting vegetative or reproductive plant fragments or seeds (Harwell & Orth 2002, Kinlan & Gaines

2003, Källström et al. 2008). Due to the fluid characteristics of the marine environment, long-distance dispersal (LDD) by oceanic currents is a likely scenario, and in general, the dispersal capacity of the free-living propagules of marine species is thought to be high. Therefore, marine populations, in comparison to terrestrial ones, are hypothesized to be more open with greater dispersal potentials (Carr et al. 2003, Kinlan & Gaines 2003, Kinlan et al. 2005). In specific cases, LDD may be important for the survival, establishment and

*Corresponding author. Email: vantuss@cmarl.unam.mx

local adaptation of spatially distributed populations (Dieckmann et al. 1999, Harwell & Orth 2002, Largier 2003). But in general, LDD is infrequent and stochastic and plays a role in processes with longer evolutionary time scales, such as genetic population connectivity, community responses to climate change, metapopulation dynamics, large-scale disturbance and speciation (Dieckmann et al. 1999, Cain et al. 2000, Kinlan et al. 2005, Procaccini et al. 2007). At present, knowledge of marine connectivity is essential for the design and establishment of marine reserve networks which, in the case of sessile organisms, are connected through transfer of their free-living propagules (Leslie et al. 2003, Palumbi 2003, Shanks et al. 2003, Guichard et al. 2004).

However, dispersal distances in the marine environment vary widely, and may differ by several orders of magnitude between or within taxa (Kinlan & Gaines 2003, Shanks et al. 2003) or species groups occupying a similar ecological niche, such as marine angiosperms or seagrasses. Some seagrasses release their seeds below the sediments, such as *Cymodocea* spp. and *Halodule* spp., but almost 50% of the genera have floating fruits, seeds or inflorescences (Orth et al. 2006). However, the relationship between high or low drifting potential and genetic connectivity between the seagrass populations is not always straightforward (Waycott et al. 2006). For example, *Zostera marina* has been shown to disperse on the order of 100 km by dislodged fruit-bearing rhipidia (Harwell & Orth 2002, Källström et al. 2008), and populations showed high genetic connectivity at an oceanic scale (Reusch 2002, Olsen et al. 2004, Muñoz-Salazar et al. 2005, Ferber et al. 2008), a phenomenon also found for *Zostera noltii* (Coyer et al. 2004). Contrary to this, *Posidonia oceanica* has low genetic migration rates, and a clear E–W genetic division was found in the Mediterranean (Arnaud-Haond et al. 2007). This restricted gene flow is surprising as this species has large hydrochoric fruits (Buia & Mazzella 1991). Inconsistent findings between gene flow and modes of propagule dispersal within this ecological group of marine plants emphasizes the importance of both genetic and more direct measurements of dispersal capacity in understanding the biogeographical patterns and genetic structuring of marine populations.

Long-distance movements of marine organisms are virtually impossible to measure directly (Cain et al. 2000, Cowen et al. 2006). Nevertheless, the dispersal kernel of marine sedentary organisms is related to the residence time of their free-living propagules (Nathan & Muller-Landau 2000, Cowen et al. 2006), and can thus be derived from their drifting time and transport rates. But dispersal will only be successful, and genetically detectible, if the release of a propagule is followed by settlement and establishment, also known as effective

dispersal (Cain et al. 2000). Population genetics studies determine patterns of fixation of imported genes in the populations over many generations, thus indicating effective marine dispersal patterns (Maguire et al. 2000, Núñez-Farfán et al. 2002, Olsen et al. 2004).

The present study aims to compare direct estimates of the dispersal potential of the floating fruits of the dioecious marine angiosperm *Thalassia testudinum* with models of genetic structuring and genetic connectivity. The tropical Atlantic coast of Mexico presents a long stretch of coastline (~1350 km) bordered by almost uninterrupted *T. testudinum* beds. In contrast to islands or isolated populations interrupted by large stretches of unsuitable habitat, the more or less continuous vegetated coastline permits a uniform distribution of suitable sites for seed establishment, and therefore provides an ideal setting for the testing of models of marine dispersal.

MATERIALS AND METHODS

Study region. Study sites were located along the tropical Atlantic coast of Mexico, which borders the Caribbean Sea and the Gulf of Mexico (Fig. 1). The Caribbean coastline is protected by the Mesoamerican Barrier Reef System. The Gulf of Mexico populations were situated at the coastal edge of a shallow, 37 to 220 km broad continental shelf (Hernández-Arana et al. 2003), where lower hydrodynamic regimes allow the development of extensive coastal seagrass beds. Surface rivers are absent in this karstic limestone platform of the Yucatan Peninsula. In the state of Tabasco, high hydrodynamic coastal regimes and the Grijalva watershed inhibit the development of coastal *Thalassia testudinum* beds (Onuf et al. 2003), separating the Veracruz population from the other study sites.

The assessment of the dispersal capacity of *Thalassia testudinum* fruits was assessed in the Puerto Morelos reef lagoon, Mexico (20° 51' N, 86° 55' W), which is bordered on the seaward site by a fringing reef between 500 and 1800 m from the coast. Within this reef lagoon, average currents vary between 10 and 20 cm s⁻¹, their direction generally being from S to N parallel to the coast, although they may invert at times (Coronado et al. 2007). A well-developed seagrass community, dominated by *T. testudinum*, covers the coarse calcareous sands in this reef lagoon (van Tussenbroek 1995, Ruíz-Rentería et al. 1998).

Fruit dispersal. The frequency of fruit detachment was determined during the 2003 fruiting season at the stations Mid-lagoon 1 (20° 52.224' N, 86° 51.472' W), Mid-lagoon 2 (20° 52.248' N, 86° 51.346' W) and Back-reef (20° 51.787' N, 86° 51.503' W), in the Puerto Morelos reef lagoon. At each site, 40 large mature fruits

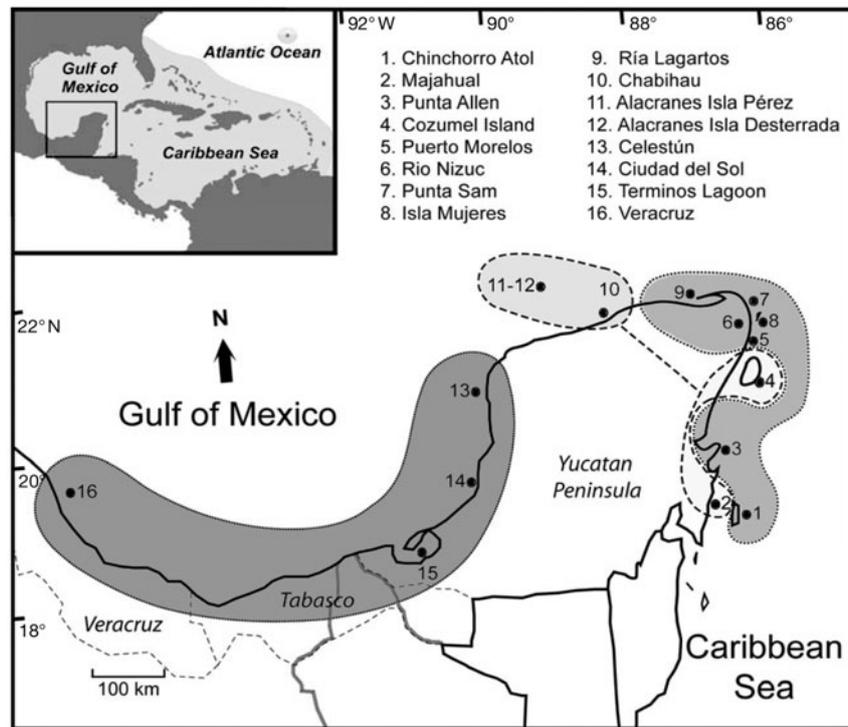


Fig. 1. *Thalassia testudinum*. Sampled populations along the Atlantic coast of Mexico, showing the distribution of the 3 genetic population clusters (indicated by grey shading) determined with STRUCTURE (Pritchard & Wen 2003), and based on the highest membership proportions from Table 4. The light grey area of the inset map indicates the distribution area of *T. testudinum*

(1.4 to 2.6 cm diameter) attached to the mother plant were marked and were inspected every 2 to 4 d to observe whether they had detached or had opened *in situ*, indicated by an open pericarp still attached to the mother shoot. This was repeated on 6 occasions (see Table 1), and the inspections continued until no unopened fruit remained in the area. In 2004, the frequency of fruit detachment was determined, employing a vertical (static) instead of horizontal (dynamic) approach at the back-reef site. On 5 occasions in August and September 2004, between 78 and 84 mature fruits were marked and the number of dehisced or detached fruits was recorded after 6 to 7 d.

To determine the flotation time, collected ripe fruits were placed in sea cages of plastic mesh (0.7 m diameter, 1 m height) or in marine tanks (216×10^3 l) until dehiscence. During September and October, we performed 6 trials in the tanks and 3 in the sea cages, using 20 fruits per trial. A chi-squared (χ^2) test was applied to the pooled data per treatment (sea cage or tank) to verify whether the number of dehisced fruits per day was independent of treatment.

Five field simulations of fruit dispersal were performed in September 2002 and October 2003 (see Table 2). Orange bouncing balls were used as physical models of the fruits, resembling the latter in form, size and density. Density of the models and fruits was veri-

fied from determination of their fresh weight and volume displacement in 20°C distilled water. The field simulation consisted of releasing 50 models at a fixed point and following their trajectory at ~100 m distance by boat during 1.5 to 2 h. At intervals of 20 to 30 min, the 2 extremes of the dispersal plume were marked with a GPS (Magellan 315) by free divers. The mean of these coordinates was considered for the calculation of speed and direction of displacement of the models. Simultaneously, a neutral drifting body consisting of an underwater sail (2×1 m) was released and followed to determine speed and direction of the superficial current. During the first simulation, 50 real fruits were also released to verify whether the models showed similar dispersal patterns to the fruits. Wind velocity and direction during the trials were obtained from an automatic meteorological station (Hydrographic and Meteorological Service, Unidad Académica Puerto Morelos, unpubl. data).

Population genetics. Along the east coast of Mexico, 16 populations were studied; the distance between neighboring populations differed between 7 and 460 km (Fig. 1). The sampling sites consisted of extensive near-coastal *Thalassia testudinum* beds, between 30 and 200 m from the shore, at 0.5 to 1.5 m depth. At each site, a total of 36 shoots were collected at intervals of 1 to 25 m along a ~230 m linear transect, parallel to

the coast. Within 1 h after sampling, a ~2 cm long fragment of the basal sheath tissue of the shoots was preserved in tubes with silica gel. In the present study we refer to a shoot (vertical rhizome with leaves) as a ramet. Ramets that are interconnected by the horizontal rhizome are considered to be a clone, and a genetically unique individual that originated from one zygote is a genet.

In the laboratory, DNA was extracted from rehydrated samples following Doyle & Doyle (1991) with few modifications. The DNA was diluted to a 10 to 20 $\mu\text{g } \mu\text{l}^{-1}$ concentration for amplification with PCR using 6 microsatellite primer pairs (TTMS-GA6, TTMS-GA8, TTMS-TGA39, TTMS-TCT58, TTMS-GGT59, TTMS-GT112) following the conditions described by van Dijk et al. (2007). PCRs in 20 μl reactions were performed on a Flexigene thermocycler (Techne™) using 96 tube plates. On each plate, the last tube contained the original enriched microsatellite vector DNA, which served as a reference for the genotyping. The denatured PCR products were loaded on a 38 \times 50 cm, 4 mm, 6% polyacrylamide gel with 7M Urea - Sequigen GT System (Biorad) for separation. Vector DNA amplicons and a 10 bp DNA ladder (Invitrogen) were used for size determination. All gels were silver stained using the slightly modified protocol of Creste et al. (2001), and bands were sized using a white light trans-illuminator.

Genetically identical ramets were identified with the software program GenClone 2.0 (Arnaud-Haond & Belkhir 2007), as was the total number of unique genets in each population. The probability of clonal identity based on allele frequencies was calculated according to Parks & Werth (1993). Subsequent analyses were only applied to the genets. Calculation of allele frequencies, observed heterozygosity (H_O). Expected heterozygosity or Nei's gene diversity H_E (Nei 1973), average number of alleles (A) and an analysis of molecular variance (AMOVA, 1000 permutations) was performed with ARLEQUIN v.3.1 (Excoffier et al. 2005). The GENEPOP web version (Goudet 1995, 2002, Raymond & Rousset 1995) was used to estimate the inbreeding coefficient (F_{IS}) per population (Weir & Cockerham 1984), applying 2000 permutations. Normal distribution of gene frequencies of each locus was tested with a Shapiro-Wilk test using Analyse-it v.1.73 (www.analyse-it.com). The average allelic richness per locus (R_i) was determined with the use of HP-RARE (Kalinowski 2005) after rarefaction to 15 samples; this because of the unequal number of genets per population due to the high clonality in some populations of this species (see Table 3).

Regional genetic structure and genetic connectivity were determined with 3 analytical procedures. A regression of the pairwise genetic distances between

populations of Rousset (1997) vs. pairwise geographical distances was plotted, and the significance of the relationship was calculated with a Mantel test. The Mantel test was performed with IBD 1.52 (Bohonak 2002) with 10 000 randomizations, using the shortest possible distance over sea between sites. A reduced major axis regression was used to determine slope, intercept and p-value of the IBD graph. A Bayesian assignment procedure (STRUCTURE v.2.1, Pritchard & Wen 2003) was used to determine the optimal number of genetic clusters (K), with K -values initially set from 1 to 16 with an initial burn-in length of 10^5 , followed by a run length of 10^6 assuming admixture and correlated allele frequencies (due to probable high migration rates). The true number of clusters was corroborated employing the ΔK statistic following Evanno et al. (2005) by repeating 20 independent runs. Finally, evidence of recent migration rates between populations was determined with a Bayesian assessment procedure integrated in the software BayesAss v.1.3, using a Markov chain Monte Carlo algorithm (Wilson & Rannala 2003), applying the program default settings.

RESULTS

Fruit dispersal

On average, 10.2 and 15.2% of fruits detached and floated to the surface in 2003 and 2004, respectively (excluding the observations on 22 September 2004, see Table 1). A small percentage was eaten, which was noted by bite marks in the pericarps. No considerable differences in the proportion of detached fruits were detected between sites in 2003 (Table 1). The time the fruits remained intact and afloat was similar for those placed in the sea cages or tanks, the χ^2 of independence being 3.82 (df 9, $0.9 < p < 0.95$, Fig. 2). More than half (52.5%) of the fruits had dehisced and released their seeds within 1 d. After the first day, the rates of fruit dehiscence followed by seed release decreased, and fruits stayed afloat for up to 10 d (Fig. 2).

Displacement of the fruits and models was similar (Table 2), and further observations were realized with models only. Vector conversions of the models and the drifting body displacement allowed derivation of the effect of wind on the displacement of the models (Fig. 3). The effect of wind on fruit displacement was approximately 10% of its velocity, and roughly (although not completely) coincided with its direction (Table 2). In the Puerto Morelos reef lagoon, the mean velocity of the floating fruits from all experiments was 395 m h^{-1} (9.48 km d^{-1}), and the estimated potential transport of fruits, based on this transport rate and floating capacity, is presented in Fig. 4. During the last

Table 1. *Thalassia testudinum*. Fate of marked mature *T. testudinum* fruits in the Puerto Morelos reef lagoon, Mexico. D: date; N: number of fruits; bitten: fruits with bite marks in the pericarp; complete: fruits that remained intact over the observation period in 2004. Fruits opened *in situ* (open) had a disintegrated pericarp, detached fruits had only a pedicel. nr: not relevant; nd: not determined. *: Hurricane Ivan passed with tropical storm strength on 13 September 2004

Site (year)	D _{initial}	D _{final}	Interval (d)	N _{bitten}	N _{complete}	N _{analysed}	% fruits	
							Open	Detached
Mid-lagoon 1 (2003)	18 Aug	08 Sep	2–4	0	nr	40	92.5	7.5
	01 Sep	26 Sep	2–4	0	nr	40	82.5	17.5
	22 Sep	13 Oct	2–4	3	nr	37	89.2	10.8
Mid-lagoon 2 (2003)	22 Sep	14 Oct	2–4	1	nr	39	89.7	10.3
Back-reef (2003)	20 Aug	29 Sep	2–4	0	nr	40	90.0	10.0
	04 Sep	10 Oct	2–4	1	nr	39	92.3	7.7
	19 Sept	13 Oct	2–4	5	nr	35	91.4	8.6
Back-reef (2004)	12 Aug	18 Aug	6	3	59	16	87.5	12.5
	18 Aug	25 Aug	7	4	55	21	85.8	14.2
	25 Aug	02 Sep	6	6	42	35	80.0	20.0
	02 Sep	08 Sep	7	2	40	42	85.8	14.2
	08 Sep*	22 Sep	14	nd	9	74	35.1	64.7

experiment (13 October 2003), the models, released ~50 m from the reef tract, disappeared from the reef lagoon after 30 min and were lost from sight, and were most likely picked up by stronger coastal currents; their estimated displacement is also depicted in Fig. 4.

Population genetics

The basic genetic variables of the studied *Thalassia testudinum* populations are shown in Table 3. None of the populations had unique alleles, but the genets were unique for each population. The majority of the populations did not deviate significantly from HWE, except for Puerto Morelos (heterozygote deficit) and Chabihau (heterozygote excess). All loci in all populations were polymorphic with a total of 58 alleles. The average number of alleles, A , per locus ranged between 3.3 (Terminos Lagoon) to 6.5 (Majahual and Punta Allen; Table 3). Allelic diversity curves indicated that a minimum sample size of approximately 15 genets was required to capture the diversity of the loci,

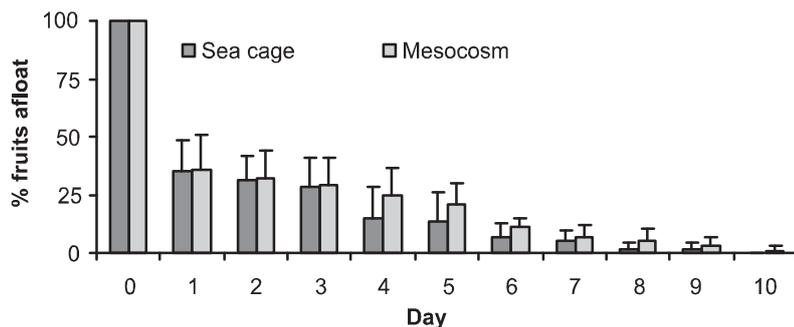


Fig. 2. *Thalassia testudinum*. Mean percentage of fruits afloat (of a total of 20 fruits) in the experimental sea cages (3 trials) and mesocosms (5 trials) during September–October 2003. Error bars are +SD

which was accomplished for most populations, except for the populations at Ría Lagartos (10 genets), Celestún (4 genets) and Terminos Lagoon (10 genets). R_i was similar to A and ranged between 3.7 and 5.8. None of the loci had a normal distribution when tested with the Shapiro-Wilk test ($p < 0.0001$ in all cases), thus the Infinite Allele Model (IAM) for microsatellites could be applied. AMOVA analysis revealed that 9.75% of variance was attributable to genetic variation between the populations and 90.25% was attributable to genetic variation between the samples within the populations. F_{ST} over all populations was 0.098 ($p < 0.000$), indicating a low overall population differentiation.

The STRUCTURE v.2.1 analysis showed only slight clustering with low assignment probabilities, revealing 3 clusters. *A posteriori* probability $P(K/X)$ for these 3 clusters was of 1.00, and the *a posteriori* ΔK statistic of Evanno et al. (2005) also indicated that K was 3. The clusters were not always tightly coupled to geographical areas (Fig. 1, Table 4), and their probability of assignment to any of the 3 clusters was relatively low ($0.363 < p < 0.857$, Table 4). The isolation by distance (IBD) was highly significant (Mantel test, $R^2 = 0.207$, $p < 0.0024$; Fig. 5). Stepwise regression analysis at geographic distance intervals of 50 km revealed significant genetic differentiation at distances >350 km (at $\alpha = 0.05$), which could be considered as the approximate area where gene flow prevented genetic differentiation, or the area of panmixis. The migration values into each population (percentage of direct immigrants and the filial generation [F_1] of immigrants with non-immigrants) estimated by Bayesian based assignment tests of

Table 2. *Thalassia testudinum*. Dispersal of floating fruits and fruit models in Puerto Morelos reef lagoon. Speed and direction of current from drifting body, wind from an automatic meteorological station (Hydrographic and Meteorological Service, Unidad Académica Puerto Morelos). The vector of wind effect was the resultant vector of the vectors of displacement of the models and drifting body (see Fig. 3). Directions were presented indicating destination, not origin. nd: not determined; na: not applicable. *: Models were lost after 30 min observation, and crossed the reef barrier

Date	Module	Module displacement				Current		Wind		Vector wind effect	
		Distance (m)	Time (min)	Speed (m h ⁻¹)	Direction (°)	Speed (m h ⁻¹)	Direction (°)	Speed (m s ⁻¹)	Direction (°)	Speed (m s ⁻¹)	Direction (°)
06 Sep 2002	Fruit	1022	138	444	358	353	358	nd	350	0.026	354
	Model	995	135	443	357						
17 Sep 2002	Model	789	131	360	343	421	10	nd	245	0.053	248
02 Oct 2003	Model	641	91	676	326	331	351	5.83	290	0.051	277
08 Oct 2003	Model	563	106	320	330	284	348	5.23	292	0.048	272
13 Oct 2003	Model	na*	na	na	na	36	147	2.72	293	na	na

origin (BayesAss) of each individual are shown in Table 5. Probabilities of the total migration into each population varied between 1.4% (Alacranes, Isla Pérez) and 31.5% (Punta Sam). A mean of 83% of individuals were assigned to their own population (non-immigrants) (95% CI = 68 to 99%). Chabihau appeared to be the population that contributed most to the immigration into other populations. Neither the Caribbean nor the Gulf of Mexico populations demonstrated a clear direction in gene flow (Table 5).

DISCUSSION

The populations of the marine angiosperm *Thalassia testudinum* distributed along the ~1350 km stretch of Mexican coastline were highly connected. The distribution of genes was homogeneous, and the AMOVA attributed only 9.2% of the total genetic variability to variation between the populations, and the resulting 90.8% to variation of genets within the studied populations. This homogeneous genetic distribution was supported by the low assignment probabilities of the populations into genetic clusters (Table 4), and corresponding poor geographical arrangement of these clusters, with exception of the populations from the southern Gulf of Mexico (Fig. 1). IBD was significant ($p = 0.0024$), but the fit (R^2) was low, and only 21% of the variation could be attributed to the geographical separation. Considering that the distribution of suitable habitat for propagule establishment was almost continuous along the Mexican coastline (with the possible exception of Veracruz, the atolls and Cozumel Island), we expected a more defined pattern of IBD. Instead, some distant populations had low differentiation values (e.g. Punta Allen and Chabihau, $F_{ST} = 0.000$, 94 km), whereas other nearby populations were genetically distinct (e.g. Puerto Morelos and Rio Nizuc, $F_{ST} = 0.127$, 23 km). This suggests that gene flow occurred at different scales, indicating hap-hazard propagule dispersal combined with unequal chances of successful establishment (Ibrahim et al. 1996). Correlations of genetic distance versus geographical distance showed a panmictic unit of 350 km for *T. testudinum* in the sampled area,

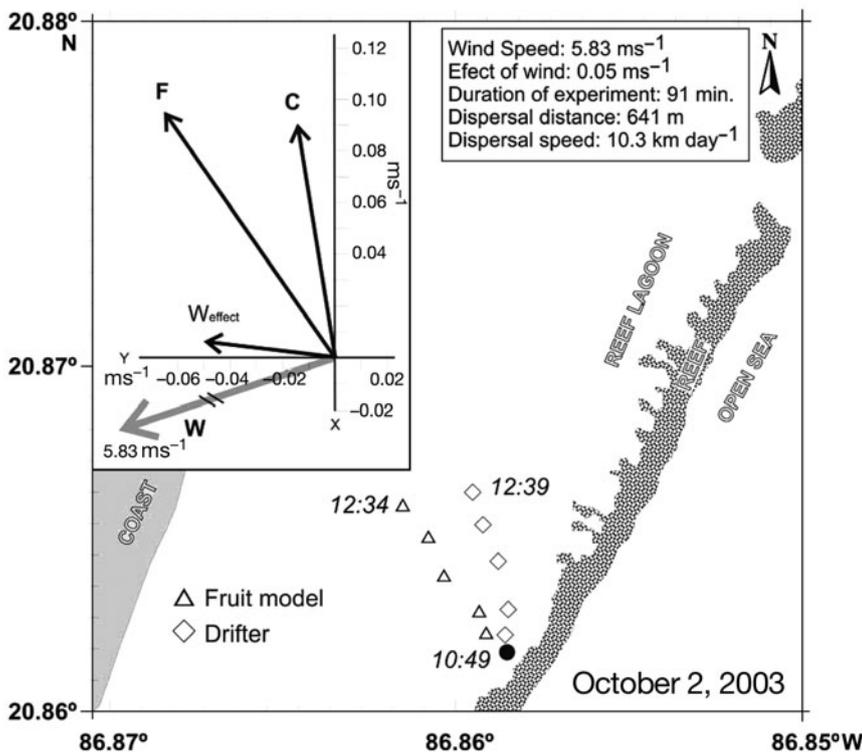


Fig. 3. *Thalassia testudinum*. Displacement tract of 50 physical fruit models and the neutral drifting body in the Puerto Morelos reef lagoon (trial: 2 October 2003, Table 2). The inset indicates the vector map of the models (F), current (C), wind effect (W_{effect}) and wind (W)

supported by the low assignment probabilities of the populations into genetic clusters (Table 4), and corresponding poor geographical arrangement of these clusters, with exception of the populations from the southern Gulf of Mexico (Fig. 1). IBD was significant ($p = 0.0024$), but the fit (R^2) was low, and only 21% of the variation could be attributed to the geographical separation. Considering that the distribution of suitable habitat for propagule establishment was almost continuous along the Mexican coastline (with the possible exception of Veracruz, the atolls and Cozumel Island), we expected a more defined pattern of IBD. Instead, some distant populations had low differentiation values (e.g. Punta Allen and Chabihau, $F_{ST} = 0.000$, 94 km), whereas other nearby populations were genetically distinct (e.g. Puerto Morelos and Rio Nizuc, $F_{ST} = 0.127$, 23 km). This suggests that gene flow occurred at different scales, indicating hap-hazard propagule dispersal combined with unequal chances of successful establishment (Ibrahim et al. 1996). Correlations of genetic distance versus geographical distance showed a panmictic unit of 350 km for *T. testudinum* in the sampled area,

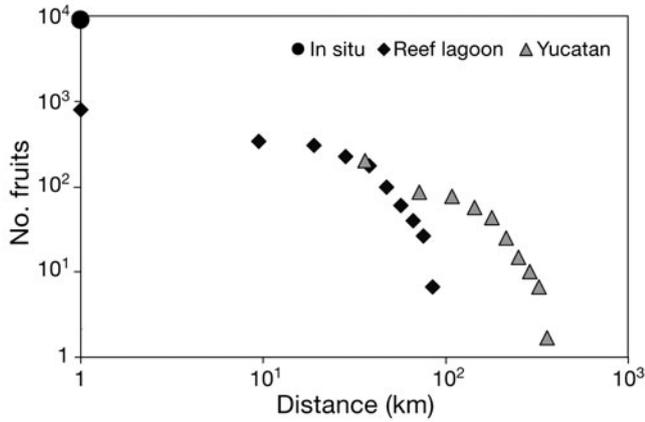


Fig. 4. *Thalassia testudinum*. Potential coastal dispersal of floating fruits, starting with 10 000 fruits (top left; log scale), assuming that 90 % of the fruits dehisced *in situ* and seed dispersal was limited. Dispersal capacity of the 10% of the fruits was deduced from the number of fruits afloat after N days and their estimated displacement. 80% of fruits remained within the reef lagoon, with a mean displacement of 9.5 km d⁻¹ (present study), and 20% moved outside the lagoon, entering the coastal circulation of the Yucatan Peninsula, with a minimum mean displacement of 36 km d⁻¹ (Merino Ibarra 1986)

which was higher than that found for *Zostera marina* (~150 km, Reusch 2002, Olsen et al. 2004) and its congener *Z. noltii* (100 to 150 km, Coyer et al. 2004). The red mangrove *Rhizophora mangle*, with its hydrochoric viviparous floating fruits, has a somewhat similar dis-

persal strategy as *T. testudinum*. However, high values of genetic differentiation ($F_{ST} \sim 0.25$, allozyme-based analysis on 8 populations) have been reported for this species in the same area as the present study (Núñez Farfán et al. 2002). At present, *T. testudinum* is the marine angiosperm with the highest level of genetic connectivity. High genetic homogeneity between populations of this seagrass had already been revealed based on randomly amplified polymorphic DNA analysis by Kirsten et al. (1998) in Florida Bay and Larkin et al. (2006) in Laguna Madre, Texas, and amplified fragment length polymorphism fingerprinting exhibited remarkable resemblance between Panamanian and Bermudan populations (Waycott & Barnes 2001).

The modules of dispersal of *Thalassia testudinum* are the positively buoyant fruits containing seeds. The transport and establishment of vegetative fragments most likely did not occur or was very infrequent, as no repeated genotypes (genets) were encountered at the distinct sites. Other authors (Kaldy & Dunton 1999, Orth et al. 2006) have reported that seeds of *T. testudinum* can float for a limited time (<1 d) when air is trapped under the membrane-like seed coat; however, this rarely occurred in our study area, and was not considered as a dispersal strategy in the present study. Even though the populations of *T. testudinum* along the coasts of Mexico were highly connected, we found that the majority (85 to 90%) of the fruits of *T. testudinum* of the Puerto Morelos reef lagoon dehiscid while attached to the mother plant, liberating the seeds

Table 3. *Thalassia testudinum*. Properties of genetic variability of 16 populations from Mexico. The numbers before the populations correspond with the site numbers from Fig. 1. N: no. ramets; G: no. genets; H_E : expected heterozygosity; H_O : observed heterozygosity; F_{IS} : inbreeding coefficient (*: significant departure from Hardy-Weinberg equilibrium after Bonferroni correction at $p < 0.05$); A: average number of alleles per locus; $R_{t(15)}$: average allelic richness at rarefaction to 15 genets; -: no data ($G < 15$); $p_{gen}(f)_{max}$: maximal probability of clonal identity. Alacranes-IP: Isla Pérez; Alacranes-ID: Isla Desterrada

Population	N	G	H_E (SE)	H_O	F_{IS}	A	$R_{t(15)}$	$p_{gen}(f)_{max}$
Caribbean								
1 Chinchorro	36	26	0.725 (0.140)	0.724	0.001	6.2	5.7	1.40 10 ⁻⁵
2 Majahual	36	26	0.702 (0.174)	0.712	-0.013	6.5	5.8	3.90 10 ⁻⁵
3 Punta Allen	36	29	0.713 (0.108)	0.747	-0.049	6.5	5.6	1.66 10 ⁻⁵
4 Cozumel Island	36	27	0.635 (0.165)	0.599	0.059	5.2	4.7	1.19 10 ⁻⁴
5 Puerto Morelos	35	28	0.549 (0.221)	0.506	0.080*	5.8	5.1	7.80 10 ⁻⁴
6 Río Nizuc	36	15	0.656 (0.143)	0.726	-0.081	5.2	5.2	6.13 10 ⁻⁵
7 Punta Sam	36	23	0.638 (0.188)	0.667	-0.047	6.0	5.6	1.41 10 ⁻⁴
8 Isla Mujeres	35	25	0.684 (0.170)	0.727	-0.064	5.7	5.2	4.53 10 ⁻⁵
Gulf of Mexico								
9 Ría Lagartos	36	10	0.730 (0.146)	0.683	0.067	5.8	-	5.63 10 ⁻⁵
10 Chabihau	36	16	0.697 (0.098)	0.792	-0.141*	5.3	5.3	3.21 10 ⁻⁴
11 Alacranes-IP	36	25	0.582 (0.090)	0.613	-0.055	4.0	3.7	5.55 10 ⁻⁴
12 Alacranes-ID	36	19	0.668 (0.147)	0.614	0.083	5.2	5.0	1.22 10 ⁻⁴
13 Celestún	36	4	0.738 (0.079)	0.833	-0.154	3.8	-	5.24 10 ⁻⁴
14 Ciudad del Sol	35	17	0.638 (0.224)	0.637	0.002	5.2	5.0	5.82 10 ⁻⁵
15 Terminos Lagoon	36	10	0.611 (0.109)	0.650	-0.067	3.3	-	1.86 10 ⁻³
16 Veracruz	36	19	0.602 (0.133)	0.614	-0.021	4.7	4.5	1.13 10 ⁻³

Table 4. *Thalassia testudinum*. Probability of membership of the populations to the 3 clusters ($K = 3$) determined with STRUCTURE v. 2.1 (Pritchard & Wen 2003). Numbers in **bold** indicate highest assignment probability, and are used for the genetic clusters in Fig. 1. Alacranes-IP: Isla Pérez; Alacranes-ID: Isla Desterrada

Population	Inferred clusters		
	1	2	3
1 Chinchorro	0.573	0.230	0.197
2 Majahual	0.298	0.522	0.180
3 Punta Allen	0.420	0.391	0.189
4 Cozumel Island	0.353	0.364	0.283
5 Puerto Morelos	0.643	0.201	0.156
6 Río Nizuc	0.488	0.084	0.428
7 Punta Sam	0.537	0.120	0.344
8 Isla Mujeres	0.448	0.168	0.383
9 Ría Lagartos	0.421	0.283	0.296
10 Chabihau	0.324	0.403	0.274
11 Alacranes IP	0.075	0.827	0.098
12 Alacranes ID	0.162	0.634	0.205
13 Celestún	0.212	0.195	0.593
14 Ciudad del Sol	0.278	0.140	0.582
15 Terminos Lagoon	0.061	0.082	0.857
16 Veracruz	0.088	0.196	0.716

within the bed of origin. Jiménez Durán (2004) reported that seeds liberated within the bed of origin had a very limited dispersal (1 to several meters). The high seed retention of *T. testudinum* in the Mexican populations in the present study is reflected by low values of recent migration between populations. The Bayesian assignment test showed that the proportion of recently established immigrants (direct or F_1 of migrants with non-migrants) was low, and 68 to 99% of the individuals were assigned to their own population (Table 5). Thus, recombination of genotypes within populations

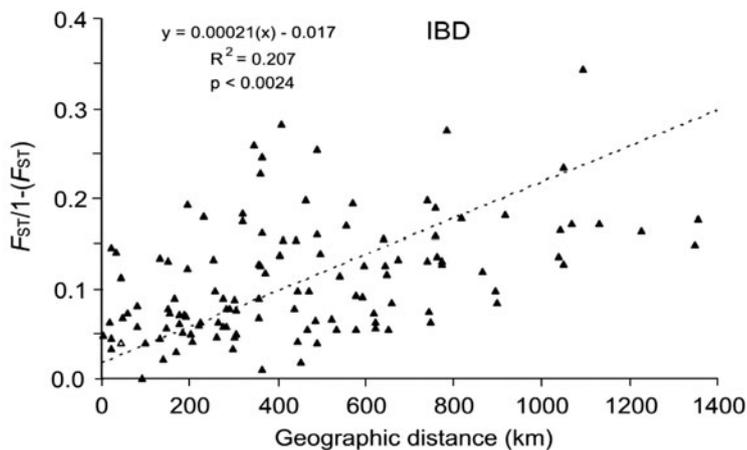


Fig. 5. *Thalassia testudinum*. Isolation by distance (IBD) for 16 Mexican populations along the Atlantic coast, determined with the use of a Mantel test (Bohonak 2002). F_{ST} : degree of genetic differentiation among pairs of populations. The slope, correlation coefficient and p-value were deduced with a reduced major axis regression

is the most likely mechanism for achieving and maintaining local genetic variability. A limited rate of recent migrations does not necessarily imply low genetic connectivity between populations, as low export and import rates of propagules can prevent differentiation between distinct populations (Procaccini et al. 2007).

The transport over larger distances of the fruits which floated to the surface was primarily caused by the direction and speed of surface currents. In the Puerto Morelos reef lagoon, the relationship between current speed and the rate of displacement of the fruit models was almost linear, whereas the wind transferred only ~10% of its speed to these models (Table 2, Fig. 2). The discrepancy in direction between the actual wind and the wind-effect on the displacement of the models (Fig. 3) might be attributed to the effect of waves. On calm days, the floating fruits in the Puerto Morelos reef lagoon traveled at a mean velocity of 395 $m\ h^{-1}$. Considering that 65% of these fruits opened within 1 d, their traveling distance was 9.5 km at most. The maximum recorded flotation time of the fruits was 10 d, and at latter velocity this could result in a potential dispersal of ~95 km within the lagoon system (Fig. 4), but within 1 d, the fruits would most likely either have stranded or have been picked up by a faster coastal current. During one of our fruit dispersal trials (starting point ~100 m from the reef tract), the released fruit models disappeared from the reef lagoon after 30 min, crossing the reef crest into the open sea. This suggests that diffusion into the open sea and incorporation of the fruits into the faster coastal currents is not unlikely, suggesting that even higher dispersal rates should be considered. The direction and velocity of surface coastal transport (seaward side of the coastal lagoon) in this region were determined by

Merino Ibarra (1986) by recapture of beached, neutrally buoyant drifting cards released outside the reef near Puerto Morelos in the month of October, one of the months with major fruiting incidence. Along the Yucatan Peninsula, these drifting cards principally traveled at minimum velocities of 0.2 to 3.0 $km\ h^{-1}$ (mean velocity 1.5 $km\ h^{-1}$) parallel to the coastline (S–N and N–S), with a tendency to drift ashore. Thus bidirectional transport of the floating fruits along the Caribbean coastline of the Yucatan Peninsula is a likely scenario, which is corroborated by the absence of clear directional gene flow in the Bayesian assignment test (Table 5). If we consider bidirectional transport, the resulting maximum dispersal potential of the *Thalassia testudinum* floating fruits is between ~190 and 720 km (corresponding to a drifting period of 10 d within and outside the reef lagoon, respec-

Table 5. Migration values of *Thalassia testudinum* according to the Bayesian assignment test implemented in BayesAss (Wilson & Rannala 2003). The values represent the estimated percentage of individual that migrated into each population from one of the 16 sampled populations along the Mexican Atlantic coast. **Bold** numbers represent the representative migration values (>5%)

	From population															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	96.9	0.3	0.2	0.2	0.3	0.1	0.2	0.2	0.1	0.3	0.4	0.1	0.2	0.2	0.1	0.1
2	0.2	97.8	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.1
3	0.9	2.6	68.0	0.4	1.0	0.3	1.1	0.4	0.4	22.6	0.6	0.4	0.3	0.4	0.3	0.3
4	0.5	0.8	0.4	68.6	1.1	0.4	5.8	0.4	0.4	13.5	6.0	0.4	0.4	0.7	0.4	0.4
5	0.2	0.1	0.1	0.1	98.0	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
6	0.9	0.7	0.7	0.7	4.2	68.6	15.2	0.7	0.7	0.9	0.7	0.7	0.7	3.4	0.6	0.6
7	0.2	0.1	0.1	0.1	0.2	0.1	97.8	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
8	1.1	0.7	0.5	0.5	17.3	0.4	3.8	68.3	0.3	3.2	0.9	0.4	0.5	1.4	0.4	0.5
9	1.8	1.4	1.3	1.0	1.7	0.8	4.1	1.5	70.0	8.8	2.7	0.9	0.9	1.5	0.8	1.0
10	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	97.4	0.2	0.2	0.2	0.1	0.2	0.1
11	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	98.6	0.1	0.1	0.1	0.1	0.1
12	1.1	1.0	0.8	0.7	2.7	0.5	2.2	0.7	0.5	6.0	11.1	69.2	0.6	1.7	0.5	0.5
13	1.7	1.6	1.3	1.4	2.8	1.3	3.0	1.6	1.2	2.5	1.8	1.5	72.5	3.4	1.2	1.4
14	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.1	0.2	0.1	0.2	0.1	97.6	0.1	0.2
15	1.0	1.0	1.0	1.1	1.0	0.9	1.0	1.7	1.1	1.0	1.0	1.0	1.0	15.5	69.7	1.0
16	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	98.3

tively; Fig. 4), which coincides reasonably well with the above-mentioned area of genetic panmixis of 350 km. The maximal coastal dispersal potential of floating fruits of *T. testudinum* along the Mexican coasts far exceeded the estimated dispersal range of the floating fruits and seeds of the same species in Laguna Madre, Texas (<1.5 to 15 km, Kaldy & Dunton 1999), and the recorded displacement of floating fruits of *T. hemprichii* (74 km) and *Enhalus arcoroides* (64 km) in the Philippines (Lacap et al. 2002). Other plant species that share a similar 2-dimensional marine dispersal strategy are mangroves, with estimated dispersal ranges of viviparous propagules of up to 50 km for *Avicennia marina* (Clarke 1993). Kinlan & Gaines (2003) estimated maximum mean dispersal in marine organisms from the slopes of IBD graphs, and if we apply their procedure to the IBD graph of *T. testudinum*, the maximum mean genetic dispersal distance would be 7.7 km, which fits reasonably well with the above-mentioned range of dispersal (maximum 9.5 km) of the majority (65%) of the floating fruits which opened within 1 d.

In conclusion, our results of population genetic structure and connectivity were concordant with the estimates of fruit dispersal, and indicated that the marine angiosperm *Thalassia testudinum* has the potential to disperse seeds over a large range of distances (1 to 10^5 m) along the Mexican tropical Atlantic coast. The dual dispersal strategy (seed release before or after fruit detachment) allowed for high within-population recombination and seed retention combined with the export of genes over large distances, resulting in a highly leptokurtic dispersal curve. Considering its

habitat and the anatomy of the fruits and seeds, this particular seagrass species must be an obligate long-distance disperser. The seeds lack hard seed coats and do not pass through a stage of dormancy, and hence do not form seed banks (Orpurt & Boral 1964, van Tussenbroek et al. 2006). But the populations are subject to elimination by potential major natural perturbations such as hurricanes (Creed et al. 2003), and therefore rely on imported seeds for recolonization. Thus high levels of gene flow were expected and found for *T. testudinum* in the study area. Some other marine angiosperms such as *Zostera* spp. share such high patterns in gene flow, which differ from those found in freshwater plants. Although freshwater angiosperms typically have high dispersal potentials (by waterfowl, Santamaría 2002, Figuerola et al. 2002 or hydrochory, Merritt & Wohl 2002), they tend to be distributed in separated water bodies, and therefore generally show higher levels of genetic structuring between populations (De Meester et al. 2002, Santamaría 2002). Seeds from terrestrial angiosperms dispersed by wind travel on the order of 10 to 10^2 m (Nathan et al. 2002), but mechanistic models estimated that seed uplifting (which occurred in 1 to 5% of the cases) may extend the dispersal distance to 10^3 to 10^4 m in the case of yellow poplar *Liriodendron tulipifera* (Nathan et al. 2002), which is still 1 order of magnitude lower than the maximum dispersal reported for *T. testudinum*. Kinlan & Gaines (2003), in an extensive review contrasting propagule dispersal in marine and terrestrial environments, concluded that the maximum estimates of terrestrial plant dispersal were 1 or 2 orders of magnitude lower than that of marine sedentary species. Our esti-

mates of dispersal of the marine angiosperm *T. testudinum* confirmed this paradigm of openness in the marine environment, emphasizing that marine organisms may exhibit larger dispersal potentials than their terrestrial counterparts.

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