Eelgrass Meadows in the California Channel Islands and Adjacent Coast Reveal a Mosaic of Two Species, Evidence for Introgression and Variable Clonality


Department of Marine Benthic Ecology and Evolution, Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands, University Herbarium, University of California, Berkeley, California, USA, Marine Science Institute, University of Santa Barbara, California, USA and Instituto de Investigaciones Oceanológicas, Universidad Autónoma de Baja California, Ensenada, Baja California, Mexico

Received: 10 June 2007 Returned for revision: 30 August 2007 Accepted: 1 October 2007 Published electronically: 14 November 2007

Background and Aims Seagrasses are important facilitator species in shallow, soft-bottom marine environments worldwide and, in many places, are threatened by coastal development and eutrophication. One narrow-leaved species (Zostera marina) and one wide-leaved species, variously designated as Z. marina, Z. pacifica or Z. asiatica, are found off the California Channel Islands and adjacent California–Mexico coast. The aim of the present study was to confirm species identification genetically and to link patterns of genetic diversity, connectivity and hybridization among and within the populations with historical sea levels (Ice Age) or the contemporary environment.

Methods Samples (n = 11–100) were collected from 28 sites off five California Channel Islands and six sites off the adjacent coast of southern California and Baja California, Mexico. DNA polymorphisms of the rDNA-ITS (internal transcribed spacer) citron (nuclear), the nuK intron (chloroplast) and nine microsatellite loci (nuclear) were examined in a population genetic and phylogeographic context.

Key Results All wide-leaved individuals were Z. pacifica, whereas narrow-leaved forms were Z. marina. Microsatellite genotypes were consistent with hybridization between the two species in three populations. The present distribution of Z. pacifica follows a glacial age land mass rather than present oceanographic regimes, but no link was observed between the present distribution of Z. marina and past or present environments. Island populations of Z. marina often were clonal and characterized by low genotypic diversity compared with populations along the Baja California coast. The high level of clonal connectivity around Santa Catalina Island indicated the importance of dispersal and subsequent re-establishment of vegetative fragments.

Conclusions The pristine environmental conditions of offshore islands do not guarantee maximum genetic diversity. Future restoration and transplantation efforts of seagrasses must recognize cryptic species and consider the degree of both genetic and genotypic variation in candidate donor populations.

Key words: Clonality, eelgrass, genetic structure, introgression, Last Glacial Maximum, seagrass, Zostera asiatica, Z. marina, Z. pacifica.

INTRODUCTION

The structure and connectivity of seagrass populations rarely have been examined among groups of islands in which the degree of insularity is influenced by geological processes, Ice Age sea-level changes, contemporary oceanographic processes and different levels of anthropogenic disturbance (but see Alberto et al., 2006 for the Canary Islands). Like other seagrasses, Zostera marina (eelgrass) is an aquatic angiosperm that reproduces sexually through seeds and asexually through horizontal rhizomes and leaf shoots. Although dispersal of pollen and individual seeds is limited in eelgrass, genetic studies have shown that the seed-bearing spathes can be transported from one area to another via rafting of detached plants or inflorescences (Reusch, 2002). For example, microsatellite-based studies of Z. marina along the contiguous Northern European coast revealed high levels of gene flow among populations at distances of 34–50 km (Reusch, 2002). Similarly, significant gene flow was documented at distances <100–150 km (Olsen et al., 2004) and also along the western Mexican coast (Baja California, Gulf of California) among populations separated by <220–250 km (Muñiz-Salazar et al., 2005). On the other hand, genetic structuring was evident within 5 km in the edge populations off Portugal (Billingham et al., 2003) and 2–10 km among populations in a restricted water flow area of Baja California (Muñiz-Salazar et al., 2006). Consequently, eelgrass is an excellent species in which to test hypotheses regarding connectivity of populations within an island group.

Along the west coast of North America, eelgrass is found from southeastern Alaska to southern Baja California, Mexico, typically in protected bays and estuaries from the low intertidal to a depth of approx. 20 m (Green and Short, 2003; J. M. Engle and K. A. Miller, unpubl. res). The eight California Channel Islands (3–250 km²) form a mixed continental and oceanic island system off the coast of southern California, separated from one another by 8–80 km and from the mainland by 20–100 km (Fig. 1). Tectonic and faulting activities over the past 40 million years have created a complex bathymetry of deep
submarine valleys, submarine mountains and 14 basins with depths >250 m (Browne, 1994). Superimposed on this area are the recent changes in sea level stemming from the Last Glacial Maximum (LGM) approx. 18 500 years BP, with concomitant changes in the size and position of the islands relative to each other and the mainland (Vedder and Howell, 1980; Graham et al., 2003). During periods of lower sea level in the LGM, some islands coalesced and were nearly connected to the mainland, whereas others remained essentially unchanged in their degree of insularity (Fig. 1). The present bathymetry of the Southern California Bight has fostered a complicated intermixing of oceanic water masses and currents, which broadly determines distributions of nearshore marine species (Browne, 1994; Engle, 1994).

Eelgrass has been recorded at 37 sites on six California Channel Islands over the past 25 years, during which natural extinction (seven sites) and colonization (two sites) have been documented (Engle and Miller, 2003). Areal cover ranged from <0.01 to >10 ha and was remarkably constant for most meadows. The island populations of eelgrass are especially important because coastal development has severely depleted mainland populations within the Southern California Bight and elsewhere (Short and Wyllie-Echeverria, 1996; Green and Short, 2003).

Distinct differences in eelgrass leaf morphology have been observed among the islands. A wide-leaved form was reported from Santa Cruz, Santa Rosa and San Nicolas, and a narrow-leaved form from San Clemente and Santa Catalina. At one site on Santa Catalina, populations of wide- and narrow-leaved forms were adjacent to one another (Engle and Miller, 2003). At all locations, the differences in leaf width were temporally and spatially stable, not correlated with depth and generally graded from wide to narrow in a northwest (colder temperatures) to southeast (warmer temperatures) direction across the Southern California Bight (Engle and Miller, 2003).

The narrow-leaved form observed on the islands and the mainland has been identified as *Z. marina*, whereas the wide-leaved form has been regarded as *Z. marina ‘latifolia’* (Setchell, 1927; Armstrong and Thorne, 1989), *Z. pacifica* (Hickman, 1993; Junak *et al.*, 1995) or *Z. asiatica*.

---

**Fig. 1.** The Southern California Bight. Circles indicate populations sampled: filled, narrow-leaved *Zostera*; open, wide-leaved *Zostera*. The solid black arrow depicts surface flow of the sub-arctic California Current flowing from the north; the solid grey arrow shows the warm, saline central north Pacific water mass flowing from the west; and the broken arrow shows the warm, highly saline Equatorial Pacific water mass flowing in from the south, which becomes warmer in the summer and autumn (Browne, 1994). Approximate LGM boundaries of the 10 m depth contour around Santarosae and San Nicolas are indicated (but not for other areas) (see text and Graham *et al.*, 2003 for details). The insert map shows the location of the Channel Islands and the populations collected from Baja California, Mexico; PB, Estero Punta Banda; SQ, San Quintin Bay.

In this study, populations of Zostera among the California Channel Islands and adjacent areas along the mainland coast were examined and the following questions were asked: (a) Is leaf width variable within a single species or are other species present? (b) Is hybridization present among any of the populations? (c) How do genetic diversity, structure and connectivity of the island populations link with historical sea levels (Ice Age), contemporary sea-water temperature/current patterns, deep-water isolation or anthropogenic disturbances? DNA polymorphisms of nuclear [rDNA-internal transcribed spacer (ITS) cistron] and chloroplast (matK intron) regions, as well as nine nuclear microsatellites were analysed.

**MATERIALS AND METHODS**

**Sample collection**

Samples (n = 11–100) of Zostera were collected from 28 sites (meadows) spread over five California Channel Islands and six sites off the adjacent mainland (Fig. 1, Table 1). Wide-leaf and narrow-leaf populations were found adjacent to one another at San Clemente (Purse Seine Rock) and Santa Catalina (White Cove) Islands, and each was designated as a separate population from the same site. Eelgrass has not been reported from San Miguel and Santa Barbara Islands. Because eelgrass was transplanted to Anacapa Island from Santa Cruz Island in 2002–2003 to replace a population lost due to sea urchin herbivory in the 1980s and 1990s (Alstatt, 2003; Engle and Miller, 2003), Anacapa Island was not included in the analysis. Samples were also collected from sites at Estero Punta Banda and San Quintin Bay, Mexico. Two leaf morphotypes in San Quintin Bay were considered as separate populations; one at the Bay’s mouth consisted of individuals with uniquely curved (expanded ‘W’-shaped leaves) whereas leaves of individuals in a population at the head of the Bay were flat with no midrib (SQmd); whereas leaves of individuals in a population at the head of the Bay were flat with no midrib (= SQfl) and typical of other populations in the Bay (A. Cabello-Pasini, unpubl. res). Samples (n = 50) of Zostera originally identified as Z. asiatica (Phillips and Wyllie-Echeverria, 1990) were collected from populations at Isla Vista and Goleta Bay, both on the mainland coast near Santa Barbara, California. Single individuals from the above sites, as well as single individuals of Z. marina from Popova Island (Northern Sea of Japan), Russia, and various locations in Japan [Mukaihima, Akaguri, Misaki and Asamashi (Honshu); Akkeshi Lake and Notoro Bay (Hokkaido)], were used in the phylogenetic analyses. The phylogenetic analyses also included single individuals of: Z. japonica from Akaguri (Honshu) and Notoro Bay (Hokkaido), Japan; Dadaepo (Goejedo), Korea; Z. asiatica from Akkeshi (Hokkaido), Japan; and Z. noltii from Sylt, Germany.

Mean depths for the inshore and offshore margins of the eelgrass meadows on the California Channel Islands were, respectively, 5 and 11 m for sheltered sites and 12 and 17 m for more exposed sites (Engle and Miller, 2003). For population analysis, divers using scuba collected shoots separated by 1–2 m along linear transects (ranging from 50 to 100 m); each sample was isolated in a separate bag. Samples collected specifically for phylogenetic analysis (Russia, Japan and Germany) were collected by snorkeling or at low tide. After collection, 2–3 leaves from each shoot were blotted dry and cut into 5–10 mm lengths before placement into 1.7 mL plastic tubes filled with silica gel crystals for rapid dehydration and subsequent storage. At least ten individuals were collected from each site for leaf width measurements and preservation as dried voucher specimens that are deposited in the Herbarium, University of California at Berkeley (Engle and Miller, 2003).

**DNA extraction and microsatellite amplification**

Template DNA for polymerase chain reactions (PCRs) was obtained from two to three, 5–10 mm pieces of silica-dried leaves. Microsatellite primers used were Zosmar CT3, CT12, CT17H, CT19, CT20, CT35, GA2, GA3 and GA6 (Reusch, 1999; Reusch, 2000; Olsen et al., 2004). Details of DNA extraction, PCR amplification and genotyping are described elsewhere (Coyer et al., 2004; Olsen et al., 2004).

**Sequencing**

Sequences of rDNA-ITS and matK were obtained from individuals of Z. marina, Z. asiatica, Z. japonica, Z. noltii and Phyllospadix torreyi (GenBank accession numbers provided on Figs 2 and 3). Two individuals of Z. asiatica and P. torreyi were sequenced for each region, and the matK sequences for Z. asiatica were compared with the published sequence (AB0996161).

The 214 bp rDNA-ITS1 region was amplified with primers ITS3 (forward) and ITS2 (reverse) (White et al., 1990); primers for the 263 bp rDNA-ITS2 region were P5 (forward) and G4 (reverse) (Saunders and Druehl, 1993). The 718 bp matK chloroplast intron was amplified with forward primer matK-F (5’ACATTTCCCTTTTGG AGGA) and reverse primer matK-R (5’CAGAAATCGATAATCAGTCCA) (S. Ferber, unpubl. res.). PCRs (50 µL total volume) for the ITS regions contained 5 µL of the Sephaglas resuspension (Coyer et al., 2004) as a DNA template, 1× Taq polymerase buffer (Promega), 2 mM MgCl2, 0.2 mM of each dNTP, 1 µM of each primer, 0.01 % bovine serum albumin (BSA) and 0.025 U of Taq polymerase (Promega). PCR conditions for matK were the same as for ITS except that 1.6 µL of each primer was used. PCR was performed with a PTC-100 (MJ Research) or Mastercycler Gradient (Eppendorf) thermocycler for the ITS regions (94 °C for 2 min; followed by 30 cycles of 94 °C for 1 min, 37 °C for 2 min and 72 °C for 2 min; and a final extension at 72 °C for 10 min) and the matK intron (94 °C for 3 min; followed by 40 cycles of 94 °C
Genetic diversity and clonality in *Z. marina* (bold) and *Z. pacifica*

<table>
<thead>
<tr>
<th>Code</th>
<th>n</th>
<th>G</th>
<th>$\hat{A}$</th>
<th>R</th>
<th>$G &gt; 1$</th>
<th>nR</th>
<th>$H_{exp}$</th>
<th>$F_{IS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goleta Bay</td>
<td>GB</td>
<td>50</td>
<td>1.639 ± 0.002</td>
<td>0.689 ± 0.003</td>
<td>8</td>
<td>4.9 (3–8)</td>
<td>0.236 ± 0.259</td>
<td>0.091</td>
</tr>
<tr>
<td>Isla Vista</td>
<td>IV</td>
<td>50</td>
<td>–</td>
<td>0.261 ± 0.003</td>
<td>4</td>
<td>11.5 (2–33)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Santa Cruz Island</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prisoner’s Harbor</td>
<td>PR</td>
<td>11</td>
<td>1.693 ± 0.001</td>
<td>0.90</td>
<td>1</td>
<td>– (2)</td>
<td>0.247 ± 0.265</td>
<td>0.148</td>
</tr>
<tr>
<td>Scorpion Anchorage</td>
<td>SC</td>
<td>77</td>
<td>0.379 ± 0.003</td>
<td>7</td>
<td>10.7 (3–29)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Smuggler’s Cove</td>
<td>SM</td>
<td>77/55</td>
<td>2.111 ± 0.005</td>
<td>0.942 ± 0.002</td>
<td>15</td>
<td>2.4 (2–5)</td>
<td>0.304 ± 0.303</td>
<td>–</td>
</tr>
<tr>
<td>Forney’s Cove</td>
<td>FC</td>
<td>50/1</td>
<td>0.00</td>
<td>1</td>
<td>– (50)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cañada del Agua</td>
<td>CA</td>
<td>48/22</td>
<td>1.844 ± 0.003</td>
<td>0.751 ± 0.003</td>
<td>10</td>
<td>3.5 (2–7)</td>
<td>0.255 ± 0.307</td>
<td>–0.185</td>
</tr>
<tr>
<td>Agua de Escondido</td>
<td>AE</td>
<td>11/4</td>
<td>0.30</td>
<td>2</td>
<td>4.5 (3, 6)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Santa Rosa Island</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Johnson’s Lee</td>
<td>JL</td>
<td>42/6</td>
<td>–</td>
<td>0.390 ± 0.002</td>
<td>6</td>
<td>6.8 (2–14)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Old Ranch Canyon</td>
<td>OR</td>
<td>48/18</td>
<td>1.509 ± 0.003</td>
<td>0.649 ± 0.004</td>
<td>7</td>
<td>5.1 (2–10)</td>
<td>0.183 ± 0.204</td>
<td>0.117</td>
</tr>
<tr>
<td>Belcher’s Bay</td>
<td>BB</td>
<td>100/45</td>
<td>2.026 ± 0.006</td>
<td>0.877 ± 0.003</td>
<td>25</td>
<td>3.2 (2–9)</td>
<td>0.280 ± 0.272</td>
<td>0.060</td>
</tr>
<tr>
<td>San Nicolas Island</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coast Guard Beach</td>
<td>SN</td>
<td>19/5</td>
<td>–</td>
<td>0.307 ± 0.002</td>
<td>3</td>
<td>5.7 (3, 7, 7)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>San Clemente Island</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Rock</td>
<td>WR</td>
<td>25/2</td>
<td>–</td>
<td>0.00</td>
<td>2</td>
<td>12.5 (10, 15)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Purse Seine Rock (narrow)</td>
<td>PN</td>
<td>54/1</td>
<td>–</td>
<td>0.00</td>
<td>1</td>
<td>– (54)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Purse Seine Rock (narrow)</td>
<td>PS</td>
<td>54/1</td>
<td>–</td>
<td>0.00</td>
<td>1</td>
<td>– (54)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Northeast Airstrip</td>
<td>NA</td>
<td>22/3</td>
<td>–</td>
<td>0.00</td>
<td>3</td>
<td>7.3 (2, 18)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fish Hook Cove</td>
<td>FH</td>
<td>50/2</td>
<td>–</td>
<td>0.00</td>
<td>2</td>
<td>25.0 (7, 43)</td>
<td>0.312 ± 0.338</td>
<td>–</td>
</tr>
<tr>
<td>San Catalina Island</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>East End</td>
<td>EE</td>
<td>50/21</td>
<td>1.646 ± 0.005</td>
<td>0.592 ± 0.004</td>
<td>6</td>
<td>20.8 (2–16)</td>
<td>0.176 ± 0.280</td>
<td>0.262*</td>
</tr>
<tr>
<td>White Cove (wide)</td>
<td>WCw</td>
<td>20/5</td>
<td>–</td>
<td>0.335 ± 0.002</td>
<td>4</td>
<td>4.8 (2–7)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Palisades</td>
<td>PA</td>
<td>61/21</td>
<td>1.642 ± 0.004</td>
<td>0.635 ± 0.004</td>
<td>11</td>
<td>4.6 (2–19)</td>
<td>0.131 ± 0.244</td>
<td>–0.042</td>
</tr>
<tr>
<td>Emerald Bay</td>
<td>EB</td>
<td>19/8</td>
<td>–</td>
<td>0.562 ± 0.002</td>
<td>6</td>
<td>2.8 (2–5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>White Cove (narrow)</td>
<td>WCN</td>
<td>44/1</td>
<td>–</td>
<td>0.00</td>
<td>1</td>
<td>– (44)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Button Shell Beach</td>
<td>BS</td>
<td>50/2</td>
<td>–</td>
<td>0.039 ± 0.001</td>
<td>2</td>
<td>25.0 (2, 48)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rippers Cove</td>
<td>RC</td>
<td>51/6</td>
<td>–</td>
<td>0.120 ± 0.003</td>
<td>2</td>
<td>23.5 (2, 45)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Empire Landing</td>
<td>EL</td>
<td>48/3</td>
<td>–</td>
<td>0.062 ± 0.002</td>
<td>2</td>
<td>23.5 (2, 45)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Big Geiger Cove</td>
<td>BG</td>
<td>47/2</td>
<td>–</td>
<td>0.077 ± 0.001</td>
<td>2</td>
<td>23.5 (5, 42)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Big Fisherman Cove</td>
<td>BF</td>
<td>50/6</td>
<td>–</td>
<td>0.337 ± 0.003</td>
<td>5</td>
<td>9.6 (2–19)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Catalina Harbor</td>
<td>CH</td>
<td>50/10</td>
<td>2.256 ± 0.003</td>
<td>0.363 ± 0.003</td>
<td>5</td>
<td>9.2 (3–27)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>San Diego (Zunaga Jetty)</td>
<td>SD</td>
<td>50/35</td>
<td>2.034 ± 0.004</td>
<td>0.910 ± 0.002</td>
<td>9</td>
<td>2.6 (2–5)</td>
<td>0.337 ± 0.296</td>
<td>0.357*</td>
</tr>
<tr>
<td>Baja California</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estero Punta Banda</td>
<td>PB</td>
<td>44/22</td>
<td>3.318 ± 0.009</td>
<td>0.810 ± 0.003</td>
<td>7</td>
<td>3.4 (2–7)</td>
<td>0.539 ± 0.250</td>
<td>0.181*</td>
</tr>
<tr>
<td>San Quintin (midrib)</td>
<td>SQmd</td>
<td>38/4</td>
<td>–</td>
<td>0.204 ± 0.002</td>
<td>3</td>
<td>12.3 (5, 7, 25)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>San Quintin (flat)</td>
<td>SQfl</td>
<td>49/35</td>
<td>3.963 ± 0.012</td>
<td>0.893 ± 0.003</td>
<td>9</td>
<td>2.8 (2–5)</td>
<td>0.541 ± 0.232</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Eight loci amplified for *Z. pacifica* and nine (+CT20) for *Z. marina*, but only eight loci were used for all samples to normalize comparisons.

- n, number of ramets; G, number of genotypes; $\hat{A}$, allelic richness standardized to $n = 10$ (s.e.); R, genotypic richness standardized to minimum $n = 11$ (s.e.); $G > 1$, number of genotypes with more than one ramet; nR, mean number of ramets per genotype (range): $H_{exp}$, Nei’s gene diversity; $F_{IS}$, inbreeding coefficient.

* Indicates a significant $F_{IS}$ value (e.g. departure from Hardy–Weinberg equilibrium, $P \leq 0.05$) with 1000 permutations. Only genotypes ($n \geq 10$) were considered for estimates of $H_{exp}$, $F_{IS}$ and $\hat{A}$. 

Downloaded from https://academic.oup.com/aob/article-abstract/101/1/73/93025 by guest on 31 December 2017
for 1 min, 57 °C for 2 min and 72 °C for 1 min; and a final extension at 72 °C for 10 min).

Amplification products were purified either by filtration using the GenElute PCR Clean-Up Kit (Sigma) or through Sephadex G-50 (Amersham). Both forward and reverse strands were sequenced directly using the dGTP BigDye Terminator Kit and visualized on the ABI 377 autosequencer (Applied Biosystems). Forward and reverse sequences were aligned and edited with SequenceNavigator software (Applied Biosystems) and by eye.

**Genets and ramets**

In seagrasses, a genetic individual (the genet) consists of several shoots (ramets) (Harper, 1977). During the life history of a genetic individual, ramets can become separated by breaks in the rhizome and grow independently. As genets and ramets cannot be distinguished in the field, genotyping is necessary for identification. Inclusion of multiple ramets into population analyses is equivalent to resampling genotypes (genets), leading to spurious positive deviations from Hardy–Weinberg equilibrium (HWE) and downwardly biased estimates of genetic diversity (Reusch et al., 1999; Reusch, 2001). Nevertheless, it is also possible that individuals with the same genotype could be identical simply by chance. Accordingly, the program GENCLONE 1.0 (Arnaud-Haond and Belkhir, 2007) was used to estimate the probability that ramets with identical genotypes were identical (clones) or different, both within and among populations, based on the eight microsatellite

---

**Fig. 2.** Phylogenetic tree based on 477 bp concatenated nrDNA ITS1 and ITS2 sequences. Numbers above and below the line are Bayesian posterior probability values and MP bootstrap values (1000 replications), respectively. Optimal model of evolution = HKY, burn-in = 9000, generations = 2 000 000 (see text). As each member of a multiple member clade has identical sequences, a GenBank accession number (EF prefix) is provided for only one member; sequences of other species (AY or AB prefix) were obtained from GenBank.
loci that amplified in all Channel Islands, southern California mainland and Mexican populations (locus CT20 was excluded because it did not amplify in all populations). All ramets reported as identical, therefore, were identical due to clonality, not chance (P < 0.05), and finding identical genotypes across considerable geographic distances implies dispersal.

Data analysis

Descriptive statistics were based on eight microsatellite loci (excluding locus CT20). Allelic richness (A) and genotypic richness (R) were estimated using GENCLONE 1.0 (Arnaud-Haond and Belkhir, 2007). For both estimates, sample sizes were standardized to allow comparison across populations with different sample sizes: allelic richness to n = 10 (equivalent to the smallest number of genotypes at Prisoner’s Harbor on Santa Cruz and Catalina Harbor on Santa Catalina), and genotypic richness to n = 11 (equivalent to the smallest data sets from Prisoner’s Harbor and Aguaje Escondido on Santa Cruz). For both estimates, sample units were randomly resampled 1000 times in order to obtain standard errors. Within- and among-locality genetic diversity (number of alleles; times in order to obtain standard errors. Within- and among-locality genetic diversity (number of alleles; observed heterozygosity, Ho; Nei’s gene diversity, Heq) (Nei, 1978), estimator of FIS (Wright, 1969) and f (Weir and Cockerham, 1984) were estimated using GENETIX 4.02 (Belkhir et al., 2001). The significance of FIS estimates was tested using permutations (n = 2000) and sequential Bonferroni corrections (Rice, 1989).

Pairwise genetic distances among all population pairs among the Channel Islands, southern California mainland and Mexico were calculated from allele frequency data (eight loci) using the Cavalli-Sforza and Edwards’ chord distance (Cavalli-Sforza and Edwards, 1967), because this measure has been shown to generate higher probabilities of obtaining the correct tree topology (Takezaki and Nei, 1996). Neighbor-joining was used to construct the tree in NEIGHBOR, and bootstrap resampling (1000 replications) was performed using SEQBOOT and CONSENS. All programs are a part of the software package PHYLIP 3.5 (Felsenstein, 1994).

The degree of admixture among populations was determined through the analysis of eight microsatellite loci and STRUCTURE (Pritchard et al., 2000). All analyses were replicated ten times to ensure proper convergence of the MCMC with the parameters: ancestry model = admixture (to account for recent divergence and shared ancestral polymorphisms); frequency model = independent; burn-in = 1 000 000; MCMC length = 2 000 000 after burn-in; and K = 2.

Sequence analysis

Aligned sequences (indels excluded) were analysed with Bayesian maximum likelihood using MrModeltest 2.1 (Nylander, 2004). As the optimal model was HKY for both ITS regions, the two sequences were concatenated. The optimal model for matK was GTR. Two independent MCMC searches were run for each data set using different random starting points (number of generations = 2 000 000). Convergence was examined visually by plotting likelihood vs. generation for the two runs. Based on this analysis, the burn-in was set to 9000. The ITS tree was rooted with the sister species P. torreyi based on previous ITS analysis (Les et al., 2002); the matK tree was rooted with P. torreyi (Kato et al., 2003) and Elodea nuttallii.

RESULTS

Species identity and introgression

Bayesian maximum likelihood analysis of ITS (nuclear) and matK (chloroplast) sequences revealed that the wide-leaved (mean ± s.d.: 12.4 ± 1.5 mm) individuals collected from Isla Vista and Goleta Bay, California, which were formerly identified as Z. asiatica (Phillips and Wyllie-Echeverria, 1990), did not cluster with Z. asiatica collected from Japan (Figs 2 and 3). Furthermore, all eight microsatellite loci developed for Z. marina amplified in the putative Z. asiatica populations from California, but only four loci (CT12, CT19, GA2 and GA6) amplified in ten individuals of Z. asiatica collected from Hokkaido, Japan (mean leaf width ± s.d.: 12.8 ± 1.4 mm; data not shown). Thus, individuals identified as Z. asiatica by Phillips and Wyllie-Echeverria (1990) are not Z. asiatica.

Wide- and narrow-leaved Zostera could not be distinguished with ITS or matK sequences (Figs 2 and 3), but were distinguished with microsatellite loci. For nearly all populations of Zostera, the CT20 locus was correlated with leaf morphology: the locus did not amplify in wide-leaved individuals (range = 8.3–15.4 mm), but did amplify in narrow-leaved individuals (range = 1.8–5.9 mm; Fig. 4). The only exception was the narrow-leaved (3-30 mm) CT20-negative Emerald Bay population from Santa Catalina Island.

The use of eight microsatellite loci (excluding locus CT20) strongly resolved (bootstrap = 93%) the wide-leaved, CT20-negative populations (including the populations from Isla Vista and Goleta Bay, California) from the narrow-leaved, CT20-positive populations at the Channel Islands, mainland southern California and Baja California, Mexico (Fig. 5). Consequently, the wide-leaved eelgrass was identified as Z. pacifica and the narrow-leaved eelgrass as Z. marina (see Discussion). Leaf widths of Z. marina were more variable. For example, populations on Santa Catalina and San Clemente collectively were narrower [mean (s.d.) = 3.0 (1.1) mm] relative to eight sites (n = 10 each) in Japan [5.4 (1.5) mm]. Mean width of the Estero Punta Banda population [3.6 (0.3) mm] was slightly wider than the those of the islands, but narrower than the SQmd [5.4 (0.3) mm] and SQf [5.9 (0.9) mm] morphotypes from San Quintin Bay (approx. 150 km south of Estero Punta Banda).
Based on blade widths, the two species of eelgrass co-occurred only at White Cove on Santa Catalina Island (Figs 4 and 6), where both species were present in adjacent meadows with no intermixing. Genetic analysis, however, revealed introgressed or hybridized individuals at Big Fisherman Cove on Santa Catalina Island, Purse Seine Rock on San Clemente Island, and San Diego (Fig. 7).

**Richness and clonality**

In general, genetic diversity of _Z. pacifica_ was greater than that of _Z. marina_. Allelic richness (≥10 genotypes) of _Z. pacifica_ populations ranged from 1.5 (Santa Rosa, Old Ranch Canyon) to 2.1 (Santa Cruz, Smuggler’s Cove; Table 1). Because of the much higher degree of clonality in _Z. marina_, allelic richness could be calculated for only one island (Santa Catalina: 2.3) and two mainland populations (Mexico: 3.3 and 4.0). Genotypic richness of nearly all populations of _Z. pacifica_ was higher than that of island populations of _Z. marina_ (Table 1). Mainland populations of _Z. marina_ (San Diego, Mexico) displayed much higher richness values than island populations (Santa Catalina, San Clemente) and were comparable with, or higher than, those of several populations of _Z. pacifica_.

Clones were present at every site, but the largest were confined to three islands (Table 1). Four populations were composed entirely of a single genotype: _Z. marina_ at White Cove, Santa Catalina (44 ramets), Purse Seine Rock, San Clemente (two narrow-width populations, each with 54
**FIG. 4.** Leaf widths of *Z. marina* and *Z. pacifica*. Measurements are from ten individuals at each site (with 95% CI indicated); abbreviations are listed in Table 1.

**FIG. 5.** Neighbor-joining tree illustrating the relationships among California Channel Islands and Baja California populations of *Z. marina* and *Z. pacifica*. The tree was based on pairwise Cavalli-Sforza and Edwards’ chord distance (Cavalli-Sforza and Edwards, 1967) between genotypes only, using eight microsatellite loci (locus CT20 excluded). Bootstrap values were derived from 1000 resamplings. Abbreviations are listed in Table 1.
ramets) and *Z. pacifica* at Forney’s Cove, Santa Cruz (50 ramets). Six other populations of *Z. marina* consisted of two or more genotypes, with one being dominant (>43 ramets): one on San Clemente and five on Santa Catalina. The largest mainland clones were found at Isla Vista (*Z. pacifica*, 33 ramets) and the mouth of San Quintin Bay (SQmd), Baja California (*Z. marina*, 25 ramets). According to the method of sampling used, minimal linear dimensions of the largest clones are 50–100 m.

**Genotype sharing**

Only three genotypes (1.7% of 177 genotypes present) were shared among the 12 island populations of *Z. pacifica*: between widely separated Prisoner’s Cove (Santa Cruz) and Coast Guard Beach (San Nicolas) (approx. 95 km), Cañada del Agua (Santa Cruz) and Old Ranch Canyon (Santa Rosa) (approx. 32 km), and Forney’s Cove (Santa Cruz) and Old Ranch Canyon.
(approx. 5 km; Fig. 6). No genotypes were shared between island populations and the mainland populations at Goleta Bay, Isla Vista and San Diego (data not shown). Among the four populations of *Z. pacifica* on Santa Catalina, however, 11 genotypes (31% of 32 genotypes present) were shared among Palisades and East End (separated by 10 km) and White Cove (separated from East End by approx. 10 km), with two genotypes found in all three locations (Fig. 6). None of these genotypes was shared with the eight genotypes from Emerald Bay (narrow-leaf, CT20-positive) 20 km to the north (Fig. 6), and no *Z. pacifica* genotypes on Santa Catalina were shared with any island or mainland populations of *Z. pacifica* (data not shown).

No genotypes were shared among *Z. marina* populations off Santa Catalina, San Clemente or Baja California (data not shown). One genotype was shared (25% of four genotypes present) among the three southern populations of *Z. marina* on San Clemente (separated by 5–10 km) (Fig. 8). Two genotypes were shared (using nine microsatellite loci; 6.7% of 30 genotypes present) among all six of the *Z. marina* populations (separated by 2–20 km) on the leeward coast of Santa Catalina, but none was shared with the single population on the windward coast (Fig. 6). No genotypes were shared among the Baja California populations separated by 8–150 km (data not shown).

A strongly resolved (bootstrap = 83%) cluster on Santa Catalina consisted of three contiguous populations (Rippers Cove, Button Shell Beach and White Cove; spanning 8 km) and one disjunct population (Big Geiger; 9 km from Rippers Cove) (Figs 5 and 6). One Santa Catalina population (Empire Landing) aligned with a strongly resolved (bootstrap = 90%) San Clemente group, approx. 63 km distant. Two Catalina populations (Big Fisherman Cove and Catalina Harbor) were distinct from the others on San Clemente and Santa Catalina; both islands were moderately resolved (bootstrap = 73%) from the three Baja California populations. Among the Baja California populations, SQmd and SQfl were strongly resolved (bootstrap = 97%) from Estero de Punta Banda, but not from each other.

**DISCUSSION**

**Species identity**

Early floristic treatments identified the wide-leaved *Zostera* as *Z. marina ‘latifolia’* (Morong, 1886) and later as *Z. latifolia* (Morong, 1893). The wide-leaved form was also described as *Z. pacifica* (Watson, 1891), which has nomenclatural priority over *Z. latifolia*. More recently, wide-leaved *Zostera* populations occurring along the central to southern California coastline (including the present Isla Vista site) have been identified as *Z. asiatica* based on leaf and seed morphology, depth distribution and phenology (Phillips and Wyllie-Echeverria, 1990). These specimens were morphologically similar to *Z. asiatica* as originally described from Hokkaido, Japan by Miki (1932), but substantially

![Fig. 7. Microsatellite detection of admixture (introgression) using the program STRUCTURE (Pritchard et al., 2000). Each individual is represented in the figure by a vertical bar partitioned into dark or light grey segments. The length of each segment is proportional to the individual’s membership in each of two clusters (K) representing the parental species, thereby providing a quantitative illustration of introgression. Dark grey represents *Z. pacifica*, light grey *Z. marina*. Populations are separated by the vertical black lines; abbreviations are given in Table 1.](https://academic.oup.com/aob/article-abstract/101/1/73/93025)

![Fig. 8. Genotype sharing among populations of *Z. marina* on San Clemente Island. See the legend for Fig. 6.](https://academic.oup.com/aob/article-abstract/101/1/73/93025)
different from *Z. marina*. Ecological and morphological characters of *Z. asiatica* are similar to those of *Z. pacifica*, however, and some authors have considered the two species to be identical, with the name *Z. pacifica* having priority (Hickman, 1993; Junak et al., 1995).

Comparative sequence analysis of *Z. marina* and *Z. asiatica* from sites in Japan, Alaska, Oregon and California, using the 5.8S rDNA-ITS cistron (approx. 520 bp) and a portion of the *matK* intron (348 bp), revealed that the wide-leaved ‘*Z. asiatica*’ collected from Isla Vista (identical to the present site) differed from herbarium specimens of *Z. asiatica* from the type locality in Japan, but were indistinguishable from wide-leaved *Z. marina* collected from Monterey Bay in central California (Talbot et al., 2006). The present sequence data, using concatenated ITS1 and ITS2 regions (474 bp) plus a larger region of the *matK* intron (718 bp), support the finding of Talbot et al. (2006) that the California ‘*Z. asiatica*’ is not *Z. asiatica*. Inclusion of microsatellite data provided additional species resolution, in terms of both differences in allele frequencies and the presence of a locus dropout (a locus that does not amplify in some populations). In the present case, one locus (CT20) did not amplify in the wide-leaved populations, but did amplify in the narrow-leaved *Z. marina*. Analysis with eight additional microsatellite loci (excluding locus CT20) also strongly resolved the wide-leaved from narrow-leaved populations. Because the wide-leaved form is not *Z. asiatica* (nuclear and chloroplast data) and differs from the narrow-leaved *Z. marina* (microsatellites), the wide-leaved form is considered to be *Z. pacifica*.

The inability of nuclear and chloroplast markers to resolve the wide-leaved ‘*Z. asiatica*’ from Isla Vista and wide-leaved *Z. marina* from Monterey Bay (Talbot et al., 2006) is because *Z. pacifica* has historically been misidentified as *Z. marina* in Monterey Bay. Indeed, microsatellite analysis of 38 wide-leaved *Zostera* individuals collected from Del Monte Beach in Monterey Bay [mean (s.d.): 11.7 (2.9 mm)] revealed a dropout of locus CT20 (J. A. Coyer, unpubl. res.). Patterns of locus dropout for microsatellites (e.g. CT20 +/−) have been shown to convey a similar or greater phylogenetic signal than scored alleles (Amos, 2006). Microsatellites, therefore, can distinguish sympatric species of *Zostera* as they have for species among the intertidal seaweed genus *Fucus* (Coyer et al., 2002; Bergström et al., 2005; Billard et al., 2005; Engel et al., 2005).

Chloroplast sequence data (*matK*) suggest that *Zostera* diverged 3–6 Mya, with *Z. marina* and *Z. asiatica* confined to the northern hemisphere (Kato et al., 2003). It is likely, therefore, that the much closer affinity of *Z. pacifica* for *marina* (rather than *Z. asiatica*), as revealed by nuclear and chloroplast sequences, coupled with evidence of hybridization between the two species (see below), reflects a relatively recent divergence of *Z. pacifica* from *Z. marina* along the California coast and/or the northern Channel Islands.

**Hybridization and introgression**

Hybridization and introgression were apparent among some populations of *Zostera*. For example, two of the 26 populations could not be reliably assigned to either *Z. marina* or *Z. pacifica*. The Emerald Bay (Santa Catalina) population was characterized by narrow leaves (= *Z. marina*) but, unlike other narrow-leaved populations, was CT20-negative (= *Z. pacifica*). A clonal population (one genet with 54 ramets) at Purse Seine Rock (San Clemente) was narrow-leaved and CT20-positive (= *Z. marina*), but aligned between the well-resolved *Z. pacifica* and *Z. marina* clusters. Although no genetic evidence of introgression was apparent at Emerald Bay, extensive levels of introgression were observed in the San Diego population, and, to a lesser extent, the Big Fisherman Cove population on Santa Catalina. In both areas, introgression may have occurred by anthropogenic mixing of the two species. For example, >20 transplantation efforts were conducted in the San Diego area from 1976 to 1997 (Anonymous, 1997), any of which could have mixed the two species and promoted hybridization. At Big Fisherman Cove (a popular boat mooring site), the combination of the meadow’s young age (10 years; Engle and Miller, 2003) and the highest genotypic diversity for any population on the Channel Islands suggests recent hybridization of the two species. At the same time, however, co-occurrence of genetically distinct (using microsatellites) *Z. marina* and *Z. pacifica* at White Cove implies that some barriers to hybridization must exist.

Hybridization among seagrass species has not been discussed in recent reviews (Hemminga and Duarte, 2000; Larkum et al., 2006; Procaccini et al. 2007) and, to our knowledge, has not been examined for any seagrass species. Nevertheless, the possibility of hybridization has been suggested between *Z. asiatica* and *Z. marina* in California (Phillips and Wyllie-Echeverria, 1990) and in areas where *Z. capricorni*, *Z. mucronata* and *Z. muelleri* overlap in Australia (Den Hartog and Kuo, 2006). The present data indicate that hybridization in seagrasses, especially among sympatric *Zostera* spp. in Japan, Australia and California, should be examined more closely.

**Paleoclimatology, oceanography or humans?**

*Zostera pacifica*. Contemporary sea surface temperature and surface current regimes have not influenced the connectivity of *Z. pacifica* populations. If contemporary regimes were important, one would predict the cold-water populations on San Nicolas and the northern shores of Santa Rosa and Santa Cruz (all influenced by the cold California Current) to form a cluster differing from that formed by the warm-water populations on the southern shores of Santa Rosa and Santa Cruz (influenced by the warmer California Counter Current, especially in summer/autumn). No differentiation among these populations was apparent using eight neutral microsatellite loci, even though the species composition of sub-tidal communities differs substantially between the cold and warm water areas (J. Engle and K. A. Miller, unpubl. res.). Similarly, if the degree of spatial isolation was important, the San Nicolas population should be distinct, yet it was indistinguishable from the Santa Cruz and Santa Rosa populations 95 km distant. The strong differentiation of the Isla Vista
and Goleta Bay populations, as distinct from the San Nicolas/Santa Cruz/Santa Rosa cluster, illustrates that a distance of only 20–30 km results in effective isolation.

Likely determinants of present levels of connectivity among *Z. pacifica* populations are the sea level and oceanic currents associated with the LGM, when the four northern Channel Islands were consolidated into one large island, Santarosae (Orr, 1968), that was only 6–8 km offshore (Junger and Johnson, 1980; Vedder and Howell, 1980; Graham et al., 2003). To the south, San Nicolas was much larger during the LGM, with an extensive and shallow shelf extending northward, nearly connecting with a similar shelf extending southward from Santarosae (Fig. 1 in Graham et al., 2003). In contrast, the sizes of Santa Catalina (where *Z. pacifica* occurs in only a few areas, see below) and San Clemente during the LGM were essentially unchanged from the present because both are volcanic in origin (middle Miocene) and rise steeply from the ocean floor (Vedder and Howell, 1980). Thus, the contemporary distribution of *Z. pacifica* closely corresponds with a land mass that existed during the LGM. Although the south-flowing California Current may have been weaker before the LGM (Herbert et al., 2001), the weakened flow still would have oceanographically ‘connected’ the extended shelves of Santarosae and San Clemente.

Populations of *Z. pacifica* on Santa Catalina are highly differentiated as revealed by the 100 % resolution of the three eastern-most populations (Palisades, East End and White Cove) and the long branch length between this cluster and other *Z. pacifica* populations. The long branch length of the eastern populations is consistent with a degree of geographical separation (100–150 km) where isolation by distance becomes apparent in *Z. marina* (Olsen et al., 2004). Furthermore, the high degree of relatedness among the eastern Santa Catalina populations, with six genotypes shared between Palisades and East End (separated by 10 km) and two genotypes found in populations spanning nearly 20 km (Palisades and White Cove), was much greater than among three similarly separated populations on Santa Cruz (Prisoner’s Harbor, Aguaje Escondido and Cañada del Agua) and undoubtedly was due to long-term connectivity. Genotype sharing cannot arise by dispersal of seeds, but instead must result from either fragmentation of an earlier and much larger meadow spanning all three areas or dispersal and reattachment of ramets (rhizomes with meristems and leaf shoots). The fragmentation hypothesis on Santa Catalina would require the ‘founder’ meadow to be at least 20 km in length and >35 000 years old, assuming the East End population as the centre of growth and mean rhizome elongation rates of 26 cm year−1 for *Z. marina* (Marbà and Duarte, 1998). Because the hypothesized age pre-dates the LGM and is far older than the 1000-year-old *Z. marina* clone in the Baltic (Reusch et al., 1999a), it is more plausible that the high degree of genotype sharing between the three eastern populations is due to dislodged and subsequently reattached ramets. An earlier experimental study conducted in San Diego (Mission Bay) concluded that natural re-establishment of *Z. marina* vegetative fragments was very rare and not likely to contribute substantially to eelgrass dispersal (Ewanchuk and Williams, 1996). The study also acknowledged, however, that patterns of fragmentation and re-establishment might be different in higher energy environments.

Although dispersal of seeds would not result in the sharing of genotypes, it would increase relatedness among populations via gene flow. Gene flow by rafting of seed-bearing shoots of *Z. marina* is likely, as wrack-bearing seeds have been found up to 34 km from the nearest source (Harwell and Orth, 2002) and assignment tests using microsatellites have demonstrated that novel genotypes arrived from non-local populations at the 30–54 km scale (Reusch, 2002). Natural means of dislodgement and dispersal among the Channel Islands include storms and foraging bat rays (*Myliobatis californica*). Anthropogenic mechanisms for disruption and dispersal (via seeds and vegetative fragments) also are likely, especially as the leeward or northern coast of Santa Catalina Island has been a popular recreational fishing area since the 1800s. Eelgrass meadows are preferred anchoring areas, and dislodging/entangling by anchors can lead to dispersal. Anthropogenic introductions also may be important over a time frame of centuries; *Zostera* was used for food or ceremony by coastal cultures in the Northwest Pacific and Gulf of California well before European contact (Wyllie-Echeverria and Ackerman, 2003). Coastal dispersal of both seeds and vegetative fragments undoubtedly contributes to the high levels of gene flow observed among Santa Catalina Island populations of *Z. pacifica*.

*Zostera marina.* It is difficult to link the present distribution of *Z. marina* with either changing sea levels or sea surface temperatures. Because *Z. marina* is present well north (cold-water North Pacific) and south (warm-water of southern Channel Islands, Baja California Peninsula and Gulf of California) of *Z. pacifica*’s range (Green and Short, 2003; Muñiz-Salazar et al., 2005), temperature, degree of isolation and past sea levels do not correlate with its present distribution.

Complex patterns of gene flow are evident among populations of *Z. marina* on both Santa Catalina and San Clemente. On Santa Catalina, two genotypes formed a substantial portion of each of the six leeward populations. While genotype sharing among three adjacent populations spanning 8 km (Rippers Cove, Button Shell Beach and White Cove) may represent fracturing of a former larger meadow, it is again difficult to explain genotype sharing among all six populations collectively spanning 20 km of shoreline. The sharing of genotypes between populations from Empire Landing and Rippers Cove (separated by 1–2 km) is probably due to meadow fracturing, but the significant inclusion of Empire Landing in a highly resolved cluster of *Z. marina* populations from San Clemente approx. 63 km distant can arise only by transfer of seeds, as no genotypes were shared between Santa Catalina and San Clemente. Transfer of eelgrass seeds over 63 km of open sea via favourable currents is within the realm of possibility (Harwell and Orth, 2002). Furthermore, the Big Fisherman Cove population displayed the highest
genotypic richness of the 11 Z. marina populations on both Santa Catalina and San Clemente, and was genetically distant from other Z. marina populations on both islands, despite being only 10 years old (Engle and Miller, 2003). Multiple introductions from multiple sources have undoubtedly occurred at Big Fisherman Cove during the last decade, involving both seeds and re-establishment of vegetative fragments. Thus, the patterns of connectivity exhibited among Z. marina populations on Santa Catalina and San Clemente Islands can best be understood by assuming that natural and anthropomorphic mechanisms frequently introduce seeds and vegetative fragments to new areas (Reusch, 2002; Rhode and Duffy, 2004) and that reattachment of vegetative fragments is more important than previously realized.

Conservation and mitigation

Recent experiments with Z. marina show that genotypic diversity enhances ecosystem productivity and recovery from disturbance (Reusch and Hughes, 2006). Consequently, the genetic diversity of donor populations is an essential consideration for successful transplant programmes (Anonymous, 1995; Williams and Davis, 1996). Eelgrass has been recognized as a significant habitat in California by the National Marine Fisheries Service, the US Fish and Wildlife Service and the California Department of Fish and Game, which collectively have formulated a comprehensive mitigation policy for eelgrass transplantation (Anonymous, 1991). One requirement for transplant mitigation is that donor plants should be collected from the area of direct impact (whenever possible), as well as from ‘...two additional distinct sites to better ensure genetic diversity of the donor plants’. (Anonymous, 1991).

Future transplant programmes of eelgrass in southern California need to consider three additional concerns: (a) the presence of two species; (b) the possibility of hybridization between donor and native species; and (c) the degree of clonality in donor populations. Zostera spp., as for most seagrass species, have a two-tiered level of genetic variation: genetic/allelic and genotypic/clone (reviewed in Procaccini et al., 2007). Thus, a donor population may be genetically diverse (as measured by the classic diversity measures of $H_{\text{exp}}$ and number of alleles per locus), but genotypically depauperate if all individuals in a donor population are members of one genet or clone.

Due to their distance from urban development, it is commonly assumed the Channel Islands Zostera meadows are the most ‘natural’ in the Southern California Bight and, therefore, the most genetically diverse. In fact, however, many of the populations of Z. marina on Santa Catalina (an easily accessible source of donor plants for transplant programmes in southern California) are composed of one or two clones and are not appropriate candidates for donor populations. While the most diverse populations of Z. pacifica are found on Santa Cruz and Santa Rosa, the most diverse populations of Z. marina are off Baja California Mexico. Clearly, an added requirement for major mitigation projects involving the transplantation of any seagrass species should be a thorough genetic analysis of potential donor populations to insure that their genetic structure (e.g. high genotype richness/diversity, low clonality, levels of heterozygosity or $H_{\text{exp}}$ close to HWE, high number of alleles per locus) will maximize success of the restoration effort.

ACKNOWLEDGEMENTS

We thank volunteers of the Channel Islands Research Program for assistance in collecting, particularly S. Adams, J. Alstatt, H. Chomeau, J. Klaib, S. Lee and J. Wibble, as well as the crew of the R/V Cormorant, J. Chomeau, H. Chomeau, C. Bungener and C. Gotschalk. We also acknowledge the cooperation of the Channel Islands National Park, especially G. Davis and D. Richards. For additional collections, we thank S. Anderson (Goleta Bay/Isla Vista); B. Reed (San Diego); H. Kawai (Popova Island, Russia); and S. M. Boo (Korea). J.A.C. is grateful to H. Kawai and his laboratory at Kobe University, especially T. Hanyuda and S. Uwai, for innumerable courtesies. Thanks also to M. Kamiya, M. Aoki, S. Nishiuichi, T. Masahide, H. Mukai and M. Nakaoka for logistical support in Japan; T. B. H. Reusch for manuscript comments; M. Chevolot for discussions; and S. Ferber for the matK primer sequences and assistance with data analysis. Supported by a research grant from the University of Groningen Breedingstrategie (J.A.C.), a Netherlands Organization for the Advancement of Research (NWO) travel grant to Japan (J.A.C.) and the Tatman Foundation (K.A.M. and J.M.E.).

LITERATURE CITED


Nylander JAA. 2004. MrModeltest 21.1. johan.nylander@ebc.uu.se, Uppsala.


Rhode JM, Duffy JE. 2004. Relationships between bed age, bed size, and genetic structure in Chesapeake Bay (Virginia, USA) eelgrass Zostera marina L. Conservation Genetics 5: 661–671.


