

Microsatellites reveal high population connectivity in eelgrass (*Zostera marina*) in two contrasting coastal areas

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Abstract

Populations of marine angiosperms, or seagrasses, are at the basis of productive ecosystems thriving in shallow coastal areas around the world. Genetic markers allow important inferences on the connectivity of seagrass populations. Here, I compare the fine scale geographic structure in eelgrass (*Zostera marina*) at a regional scale (2–60 km) among two adjacent coastal areas, the northern Wadden Sea ($N = 8$), and the southwestern Baltic ($N = 5$). Plants were genotyped for eight highly polymorphic microsatellite loci displaying a total of 114 alleles. Consistent with their clonal mode of reproduction, populations in the Baltic were genetically less diverse compared to those in the Wadden Sea, suggesting an effective population size that is half compared to the Wadden Sea. Although I expected more gene flow among populations in the Wadden Sea where abundant seeds can be transported by strong tidal currents, no quantitative difference in population differentiation was detectable between both coastal areas. To the contrary, in the atidal Baltic, nonsignificant genetic differentiation ($F_{ST} < 0.009$) was present up to waterway distances of 54 km, compared to only 33 km in the Wadden Sea. Correlations between genetic and geographic distances were weak in both areas, and significant only in the Wadden Sea. I examined as one potential mechanism responsible for high population connectivity whether the genotypic composition of rafting reproductive shoots differed from resident Baltic populations ($N = 2$) using assignment tests. At one sampling occasion, at least 130 immigrant seeds m^{-1} shoreline were identified based on their multilocus genotype. The results have implications for coastal management and restoration and suggest that local population history may be less crucial for explaining seagrass distribution than previously suggested.

Despite the notion of the sea being an environment with potentially unlimited dispersal of propagules, there is ample evidence for complex population structure in many marine species (Palumbi 1994; Hilbish 1996). Pronounced population divergence should particularly apply to marine macrophytes, as their dispersal stages (spores or seeds) often travel only in the range of m (Denny and Shibata 1989; Orth et al. 1994). The study of population structure in marine plants, both macroalgae and seagrasses, has undergone a phase of rapid progress, driven by the availability of novel genetic markers, notably RAPD (random amplified polymorphic DNA) and microsatellites. In seagrasses, a polyphyletic group of 60 truly marine angiosperms, such studies have revealed population structures at several scales. Within populations, widely varying degrees of clonal diversity have been reported (Waycott 1998; Reusch et al. 1999). At larger geographic scales, the discovery of marked between-population differentiation (e.g., Procaccini and Mazzella 1998; Reusch et al. 2000; Procaccini et al. in press) has reverted earlier notions of genetic uniformity and a predominance of plasticity versus genetic differentiation in seagrasses (Barrett et al. 1993).

Building on these first pioneer studies, I compared the population structure of eelgrass (*Zostera marina* L.), the ecologically most important seagrass species of the temperate northern hemisphere, among two adjacent coastal areas. In the northern Wadden Sea (Germany) eelgrass is an important habitat forming species in tidal flats, whereas in the southwestern Baltic sea (Germany) meadows are abundant in shallow sublittoral areas. Populations in both areas were chosen to be no more than 60 km apart. Such a regional spatial scale is currently under much scrutiny in metapopulation studies (e.g., Giles and Goudet 1997).

Based on principal differences in the life history and the physical habitat between both coastal areas, I predicted differences in population structure and genetic diversity between the Wadden Sea and the Baltic. In the Wadden Sea, eelgrass populations on the tidal flats display a predominantly annual life cycle, i.e., they only invest into sexual reproduction and overwinter as seeds (den Hartog 1970; Reise et al. 1989). As an exception, in permanently submerged tidal creeks on some Wadden Sea islands (the Halligen) an unknown fraction of plants persists through winter (K. Reise, pers. comm.). In contrast, in the Baltic, populations reproduce both clonally and sexually (Reusch et al. 1999). Clonal reproduction is expected to lower the effective population size (N_e) because fewer genotypes situated in an area of approximate panmixia (Wright's neighborhood size, Wright 1931) contribute to the gene pool. Hence, I examined whether Baltic populations display a lower N_e than Wadden Sea ones.

As a second prediction, genetic differentiation between populations in the Wadden Sea is expected to be lower than in the Baltic at equal geographic distances. Gene flow between populations in the Wadden sea is probably stronger

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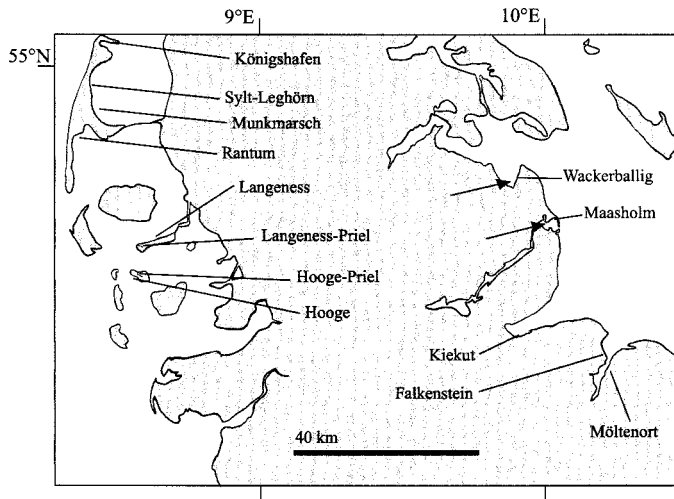


Fig. 1. Map of the sample locations in the northern Wadden Sea and the southwestern Baltic. Arrows indicate the locations where in addition to the population samples, drifting reproductive shoots were sampled.

because more propagules are produced and because strong tidal currents (Backhaus 1993) can disperse propagules more efficiently than in the atidal Baltic Sea.

At medium geographic distances (say, 2–50 km), processes responsible for the transport of propagules are largely unknown in marine plants. Rafting reproductive thallus fragments or shoots have long been suggested as a potential mechanism (den Hartog 1970), yet actual measurements are entirely lacking. Earlier studies have reported substantial gene flow in seagrasses between distant localities using indirect (F_{ST})-based methods (Ruckelshaus 1998), which seem contradictory to direct measurements of seed dispersal in the range of meters (Orth et al. 1994). Therefore, I examined whether drifting reproductive shoots are potential vectors of gene flow using microsatellite genotyping of resident and

(putative) immigrant individuals in conjunction with genetic assignment tests (Paetkau et al. 1995).

Material and methods

Study populations and sampling—*Zostera marina* (L.), or eelgrass, is widespread in shallow water of the entire northern hemisphere (den Hartog 1970). I compared populations situated in two coastal areas in northern Europe: the northern Wadden Sea ($N = 8$) and the southwestern Baltic Sea ($N = 5$) (Fig. 1, Table 1). The northern part of the Wadden Sea is a National Park and of particular importance for two temperate seagrass species, *Zostera marina* (the focal species of this study) and *Zostera noltii*, since 75% of the remaining North Sea populations are found in this area (Reise 2000). In each population, defined as an area of 20×40 m parallel to the shore, tissue (meristem or leaf) of 40 to 50 plants was sampled at distances of ≥ 1 m. In continuous meadows, sampling positions were determined using randomly generated x, y coordinates. Only at Møltenort (Baltic Sea), shoots were sampled haphazardly. In patchy beds (Wackerballig) only one plant was sampled in patches < 1 m², since most patches in this size range are composed of single genotypes (Reusch 2001). The Wadden Sea populations comprise two eelgrass stands with a mixture of annual/perennial plants that are situated in permanently submerged creeks within low-lying islands (800 m and 1,200 m distant from Wadden Sea).

In order to assess the role of drifting reproductive shoots for genetic exchange in eelgrass, I sampled rafting plants at two locations in the Baltic to compare their genotypic composition with the respective resident population. Forty rafting fruiting shoots carrying ripe seeds were sampled close to the shore on 28 June 1999 at Maasholm and on 8 August 2000 at Wackerballig, respectively. At Maasholm, uprooted reproductive shoots had been washed upon the shore, which is situated 150 m from the eelgrass bed. At Wackerballig in 2000, ripe inflorescences were drifting close to the sediment surface directly within the resident meadow.

Table 1. Populations of *Zostera marina* (eelgrass) sampled. The sample size refers to the number of unique genotypes in the sample, i.e., multiple sampled ramets were excluded.

| Region | Population | Geographic position | Sample size | Water depth (m) | Life history |
|------------|------------------|---------------------|-------------|-----------------|------------------|
| Baltic Sea | Falkenstein | 54°24'N 10°12'E | 39 | 2.2–3 | perennial |
| | Maasholm | 54°41'N 10°00'E | 48 | 1–1.6 | perennial |
| | Wackerballig | 54°46'N 9°53'E | 54 | 1–1.4 | perennial |
| | Møltenort | 54°23'N 10°13'E | 32 | 1.8–2.6 | perennial |
| | Kiekut | 54°29'N 9°52'E | 31 | 2.2–2.6 | perennial |
| Wadden Sea | Hooge† | 54°32'N 8°31'E | 47 | intertidal | annual |
| | Hooge-Priel‡ | 54°34'N 8°32'E | 46 | 0.2–0.6 | perennial/annual |
| | Langeness | 54°38'N 8°36'E | 29 | intertidal | annual |
| | Langeness-Priel‡ | 54°39'N 8°32'E | 34 | 0.2–0.6 | perennial/annual |
| | Sylt-Leghörn | 54°58'N 8°22'E | 32 | intertidal | annual |
| | Rantum | 54°51'N 8°19'E | 33 | intertidal | annual |
| | Munkmarsch | 54°54'N 8°22'E | 48 | intertidal | annual |
| | Königshafen | 55°03'N 8°25'E | 25 | 0.4–0.6 | perennial/annual |

* Sylt-Leghörn and Königshafen (Wadden Sea), and Maasholm, Falkenstein (southwestern Baltic) were already analyzed by Reusch et al. (2000) using six microsatellite loci, four of which are among the eight loci used in this study.

† Not identical to population “Hooge” in Reusch et al. (2000), ≈ 2 km distant.

‡ Ramets were randomly sampled along a transect of 50 m length, at ≤ 0.5 m distance.

Genetic analysis—For the genetic analysis I used the polymorphism displayed by microsatellites, codominantly inherited stretches of hypervariable repeat motifs on the DNA. A microsatellite locus corresponds to species-specific sequences flanking the variable repeat (= locus), which can be amplified by PCR, and scored by size (= alleles). A crude extraction of genomic DNA was prepared from 0.01–0.05 g dry tissue mass following the CTAB (cetyltrimethylammonium bromide) protocol. Later (populations Langeness, Wackerballig, Møltenort, Kiekut) I switched to the Qiagen plant DNA extraction kit (Qiagen). The DNA extract was subjected to polymerase chain reactions (PCR), using fluorescently labeled microsatellite primers. I genotyped the plants for eight polymorphic loci following protocols in Reusch et al. (2000) and Reusch (2000) with modifications in primer concentration and primer pooling (*see* Web Appendix 1 at <http://www.aslo.org/lo/toc/vol.47/issue.1/0078a1.pdf>). PCR products were separated electrophoretically on polyacrylamide gels. Scoring of allele sizes was performed on an ABI 377 automated sequencer, using the software GeneScan 3.1 and Genotyper 2.0 (Applied Biosystem).

Data analysis—Prior to the analysis of genetic diversity and genetic structure, all duplicate sampled genotypes (i.e., members of an identical clone) were excluded from the data set. Observed and expected heterozygosities were computed using GENETIX (Belkhir et al. 1998). I tested for linkage disequilibrium among all pairs of loci (a) for each population (b) for all populations within each of the two areas according to the procedure suggested by Black IV and Kraftsur (1985) using GENETIX. Hardy-Weinberg expectations were calculated using the method of Weir and Cockerham (1984), implemented in GENETIX.

Observed heterozygosities were translated into relative differences in the effective population size N_e under the assumption of drift-mutation equilibrium. I used the equilibrium expectations for heterozygosity H_{eq} under a stepwise-mutation model developed by Ohta and Kimura (1973):

$$H_{eq} = 1 - (1 + 8N_e\mu)^{-0.5}$$

where μ is the mutation rate per generation. Since μ is unknown for the specific microsatellites, a comparison of mean heterozygosities between Wadden Sea and Baltic populations only yields the estimated ratio between the average N_e in both seas.

For quantifying genetic distances among populations, I used θ as an estimator for F_{ST} (Weir and Cockerham 1984), assuming that drift and gene flow, but not mutations, were contributing most to genetic differentiation (Estoup et al. 1995). The global estimator of θ was calculated per coastal area using GENETIX and jackknifed over loci to obtain a confidence estimate. Pairwise genetic distances (θ) were tested for significance using 1,000 randomizations (genotypes are permuted between populations) and plotted against pairwise geographic distances. I used geographic distances along the waterways, assuming a stepping-stone model of genetic exchange. In the Wadden Sea, I ignored the dam connecting the mainland with the island Sylt because the genetic/geographic distance matrix lost considerably in determination if considering waterway distances around the northern tip of

Sylt (*see Results*). The genetic-geographic distance matrix was statistically tested for correlation using Mantel's test.

Assignment test—Putative immigrant genotypes sampled in the driftline material were compared to resident genotypes using an assignment test (Paetkau et al. 1995) implemented in the software DOH (Brzustowski 1997–1999, code available at <http://www.biology.ualberta.ca/jbrzusto/Doh.php>). In order to increase the power of the test, at Maasholm, plants were genotyped for additional three microsatellite loci (thus 11 loci in total). The assignment procedure first computes the multilocus likelihood of a given 11-locus genotype to occur in either of the two populations (immigrant or resident), assuming linkage equilibrium. Alleles missing in either population were set at a fixed proportion of 0.01. Individuals are assigned to the population for which they show larger likelihoods. In an initial test, genotypes were permuted 1,000 times between both populations, each time recalculating the assignment. The null-hypothesis that both populations are not differentiated can be rejected when $\geq 95\%$ of the random data sets produce fewer correct assignments than the actual data set. Only if H_0 was rejected, that is both populations were genetically heterogeneous, did I proceed to ask how many of the individuals in the driftline material were immigrants on an individual basis. This was based on the log of odds ratio of both assignment probabilities. A putative immigrant genotype must have a 100 times smaller chance for being assigned to the resident population to count as an individual immigrant. This is a conservative procedure since one may also argue that whenever the global test of population divergence is significant, all immigrant genotypes are foreign to the resident population.

Results

Genetic diversity—Basic data on allele number, heterozygosities, clonal diversity, and Hardy-Weinberg equilibrium are given in Table 2. Baltic populations had a lower heterozygosity, both observed and expected, than Wadden Sea populations (*t*-test, observed heterozygosity $p = 0.0002$, expected heterozygosity $p < 0.0001$). However, the allele number was not significantly different between both areas (*t*-test, $p = 0.18$). Likewise, there was no statistically significant difference in the inbreeding coefficient between both areas (jackknifing over loci, Wadden Sea, $f = 0.064 \pm 0.027$; southwestern Baltic, $f = 0.046 \pm 0.014$). Within the Wadden Sea group, there was no statistical difference in genetic diversity (heterozygosity, allele number) between the two perennial creek populations and the tidal flat meadows (*t*-test, creek vs. flat: observed heterozygosity 0.601 vs. 0.602, $p = 0.9$, allele number 7.2 vs. 8.4, $p = 0.31$).

The difference in heterozygosity between both coastal areas translates to a ratio between average N_e values of Wadden Sea into Baltic populations of 1.73 (based on observed heterozygosity) and 1.85 (expected heterozygosity). Hence, the initial hypothesis of a higher genetic diversity displayed by Wadden Sea populations is confirmed.

I found significant linkage disequilibrium in two Wadden Sea populations, Königshafen and Hooge-Priel (population-wise p both < 0.001). None of the other Wadden Sea pop-

Table 2. Comparison of the clonal and genetic diversity among eelgrass (*Zostera marina*) populations from the northern Wadden Sea ($N = 8$) and southwestern Baltic Sea ($N = 5$). Clonal diversity is given as unique genotypes identified/ramets sampled.

| Region | Population | Clonal diversity | Observed heterozygosity | Expected heterozygosity | Mean number of alleles |
|------------|----------------------|------------------|-------------------------|-------------------------|------------------------|
| Baltic Sea | Möltenort | 0.86 | 0.496 | 0.513 | 6.88 |
| | Falkenstein | 0.77 | 0.503 | 0.525 | 7.00 |
| | Kiekut | 0.94 | 0.522 | 0.518 | 6.97 |
| | Maasholm | 0.33 | 0.422 | 0.475 | 7.13 |
| | Wackerballig | 0.92 | 0.484 | 0.490 | 8.00 |
| | mean Baltic (SE) | 0.764 (0.14) | 0.485 (0.02) | 0.504 (0.009) | 7.19 (0.2) |
| Wadden Sea | Hooge | 1 | 0.621 | 0.634 | 8.62 |
| | Hooge-Priel | 1 | 0.651 | 0.680 | 8.38 |
| | Langeness | 1 | 0.562 | 0.582 | 7.37 |
| | Langeness-Priel | 0.97 | 0.553 | 0.623 | 6.00 |
| | Rantum | 1 | 0.603 | 0.630 | 9.37 |
| | Munkmarsch | 1 | 0.627 | 0.627 | 9.87 |
| | Sylt-Leghörn | 1 | 0.566 | 0.615 | 8.37 |
| | Königshafen | 1 | 0.627 | 0.621 | 6.63 |
| | mean Wadden Sea (SE) | 0.996 (0.005) | 0.601 (0.013) | 0.626 (0.009) | 8.07 (0.5) |

ulations nor any of the Baltic populations revealed linkage disequilibrium. The global test was significant for the Wadden Sea ($p = 0.032$), but clearly not significant in the Baltic ($p = 0.28$).

Population structure—The global θ value was highly significant and virtually identical for both study areas (jackknifing over loci, Wadden Sea, $\theta_{\text{global}} = 0.018 \pm 0.003$; southwestern Baltic, $\theta_{\text{global}} = 0.018 \pm 0.005$; both $p < 0.001$), indicating weak but significant population substructure at a geographic scale of ≤ 60 km. Maximal pairwise genetic distances were approximately similar in both coastal areas ($\theta_{\text{max}} = 0.038$ in Wadden Sea, $\theta_{\text{max}} = 0.045$ in Baltic; Fig. 2A, Table 3). Although the chosen markers offered the statistical power to detect pairwise differentiation as low as $\theta = 0.01$, several population pairs were separated by non-significant genetic distances up to waterway distances of 33 and 54 km in the Wadden Sea and the Baltic, respectively (Table 3). There was also no indication for the Baltic populations revealing a steeper isolation-by-distance graph than the Wadden Sea ones. To the contrary, there was no consistent relationship between genetic and geographic distances in the Baltic at all (Fig. 2A). Thus the initial hypothesis, stating that Wadden Sea populations display a lower average population differentiation, was not supported.

In the Wadden Sea, I a priori restricted the isolation-by-distance graph to tidal flat populations because genetic exchange of the tidal flats with the creeks on Hooge and Langeness is presumably weaker than across intertidal populations (Fig. 2B). A Mantel test on the genetic-geographic distance matrix comprising the remaining six populations was almost significant ($p = 0.07$), and a linear regression equation explained 54% of the variance in genetic divergence.

I further examined all pairwise genetic distances in the Wadden Sea of the two creek populations (Fig. 2C). Qualitatively, there is an indication that genetic distances comprising at least one of the creek populations are higher than those among purely intertidal population at similar geo-

graphic distance. This particularly applies to population pairs comprising Langeness-Priel (cf. Table 3). Nevertheless, a Mantel-test on the full Wadden Sea data set was significant at $p = 0.04$ (equation not shown).

Assignment tests—Figure 3 depicts the assignment likelihoods of individuals to belong to the driftline or resident population at Maasholm (A, 1999), and Wackerballig (B, 2000). Despite a considerable number of cross-assignments between both groups, the driftline population in Maasholm was genetically distinct from the resident population (Fig. 3A). Permutations revealed that most random data sets had a lower proportion of correct assignment than the original data (1,000 randomizations, randomization scheme 2 and 4 in software DOH, $p = 0.006$ and 0.002 , respectively). Based on a log of odds ratio ≥ 100 , there were four out of 39 driftline genotypes that were identified as immigrants on an individual basis. The genotypes of the driftline material were in Hardy-Weinberg equilibrium, suggesting that they originated from a rather localized area. Otherwise, pooling of more than one panmictic unit should have resulted in an excess of homozygotes (Wahlund-effect).

In 2000, at Wackerballig, there was no indication of the drifting reproductive shoots to be genetically distinct from the resident population (Fig. 3B, assignment test: 1,000 permutations, $p = 0.23$).

Discussion

Fast-evolving genetic markers have considerably increased our understanding of genetic patterns resulting from vegetative or generative reproduction at a small scale (dm to 10 m) in macroalgal (Engel et al. 1999) and seagrass species (Waycott 1995, 1998; Reusch et al. 1999). At a larger biogeographical scale, slower evolving markers have yielded insight into possible recolonization routes and biogeographic dispersal patterns (Van Oppen et al. 1994, 1995). The present challenge for understanding marine plant metapopulations are intermediate spatial scales at which several processes,

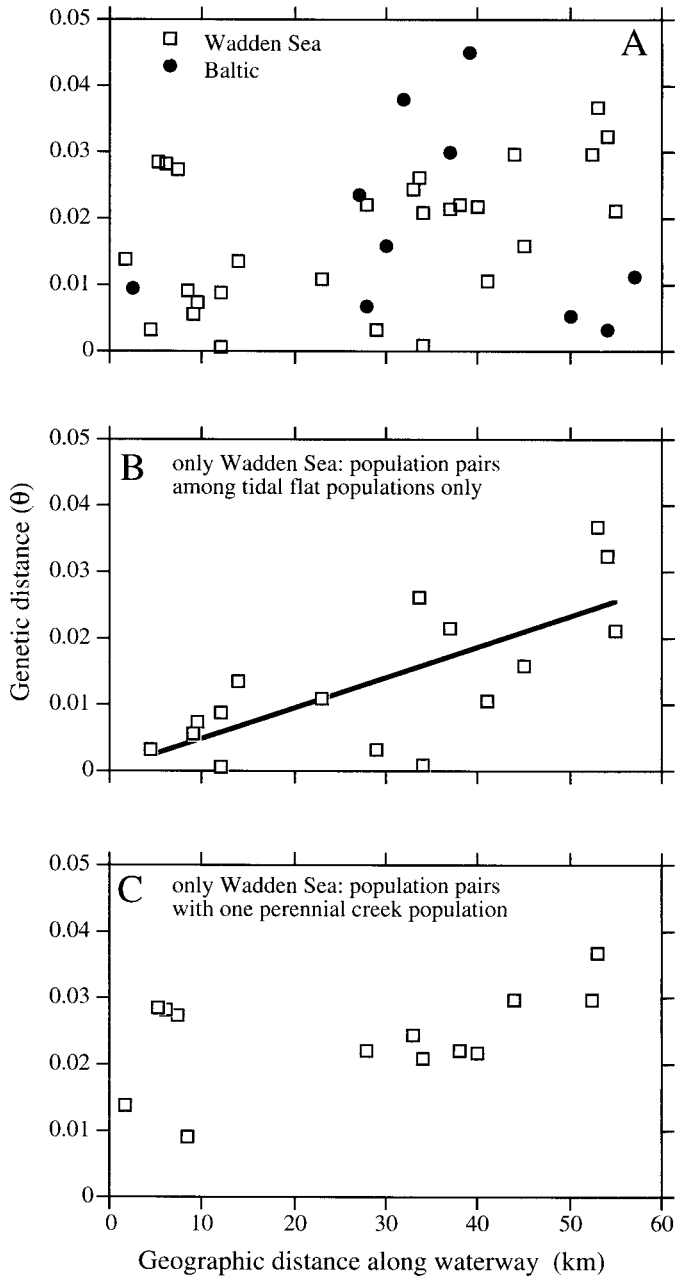


Fig. 2. Correlation between geographic and genetic distance in Baltic and Wadden Sea populations of *Zostera marina* (eelgrass). (A) all populations. (B) Wadden Sea only, populations in permanently submerged tidal creeks on Langeness and Hooge are excluded. The marginally significant correlation (Mantel-test $p = 0.07$) is given according to the equation $\theta = 0.00014 + 0.00047 \times \text{Distance (km)}$, $R^2 = 0.53$. (C) Wadden Sea, only populations pairs including one of the creek populations are depicted.

notably population history such as extinction and colonization, as well as gene flow and genetic drift interact to produce genetic structuring (e.g., Slatkin 1993; Bossart and Prowell 1998; Whitlock and McCauley 1999).

While it is clear that nearly all wild populations studied thus far reveal population substructuring, the examination of differences in population structure between geographical ar-

reas has only recently been undertaken (e.g., Hutchinson and Templeton 1999). Such comparisons may be particularly useful to examine the generality of population structures that have not been replicated in most studies.

In the present study, marked differences between the population genetic structure of two closely adjacent coastal areas could be identified. The hypothesis of Wadden Sea populations displaying a higher N_e than Baltic ones was supported based on observed heterozygosities. This is probably not due to population bottlenecks during recolonization of the Baltic, since differences in allele diversity were not significant between both seas. The observed differences are most consistent with the predominant mode of reproduction in both areas. In the southwestern Baltic, a predominance of clonal spread over sexual reproduction results in clone sizes up to 17 m (Reusch et al. 1999). In contrast, in most Wadden Sea populations, each ramet cluster represented a unique genotype. In meadows with few large clones, as in the Baltic, the number of genotypes per neighborhood area is much lower than in annual populations. Differences in clone size will also lead to a skewed distribution of reproductive output among individuals, again lowering the expected N_e (Loveless and Hamrick 1984). In partly perennial Wadden Sea populations growing in permanently submerged creeks, clones were small (always <1 m) and, consequently, clonal diversity was high. A relatively high genotypic diversity in these populations is the most likely reason that I found no difference in heterozygosity among Wadden Sea creek and tidal flat populations.

At the level of geographic areas, a predominantly sexual or vegetative mode of reproduction was not replicated in this study. Therefore, I cannot exclude that factors specific to the northern Wadden Sea other than the reproductive mode have resulted in higher genetic diversity (heterozygosity). At least for the group of perennial populations, southwestern Baltic populations agree well with perennial meadows outside the Baltic in southeastern Canada and the Mediterranean Sea (mean observed heterozygosity = 0.49, Reusch et al. 2000 and unpubl. data). In summary, there is evidence that the life history of seagrass populations will markedly influence the genetic diversity we may expect at a given site. This needs to be considered when making inferences on the role and possible threats to genetic diversity in eelgrass meadows.

Surprisingly, the connectivity among *Zostera marina* populations was equally good in two rather contrasting coastal areas, the tidal Wadden Sea and the atidal Baltic. Particularly unexpected are findings of statistically nonsignificant differentiation in the Baltic Sea at distances of 54 km waterway. This is remarkable because the detectable magnitude of population differentiation using the set of eight microsatellites is as low as $\theta = 0.01$. Earlier conclusions for the Baltic, based on only one population pair (Maasholm—Falkenstein) using six microsatellite loci, which suggested moderate genetic subdivision at this distance ($\theta = 0.03$, Reusch et al. 2000), were thus not confirmed in the larger data set presented here. Genetic differentiation between populations has to be carefully interpreted since it may be due to other processes than gene flow (Bossart and Prowell 1998; Whitlock and McCauley 1999). Nevertheless, a weak correlation of

Table 3. Pairwise comparison of population differentiation among *Zostera marina* populations using the F_{ST} -estimator θ (above diagonal) in the northern Wadden Sea ($N = 8$), and the southwestern Baltic ($N = 5$). Sampling sites are given from south to north. Statistical significance based on a comparison-wise error rate of $\alpha = 0.05$ (below diagonal). ns = nonsignificant, * $0.05 \geq p > 0.01$, ** $p < 0.01$.

| | Hooge | Hooge-Priel | Langeness | Lange-Priel | Rantum | Munkmarsch | Sylt-Leghörn | Königshafen |
|---------------------|-----------|-------------|-----------|-------------|--------------|------------|--------------|-------------|
| Northern Wadden Sea | | | | | | | | |
| Hooge | — | 0.014 | 0.0073 | 0.027 | 0.001 | 0.010 | 0.016 | 0.021 |
| Hooge-Priel | ** | — | 0.0096 | 0.028 | 0.021 | 0.022 | 0.030 | 0.037 |
| Langeness | ns | * | — | | 0.003 | 0.026 | 0.021 | 0.032 |
| Langeness-Priel | ** | ** | ** | — | 0.022 | 0.024 | 0.022 | 0.030 |
| Rantum | ns | ** | ns | ** | — | 0.006 | <0.001 | 0.011 |
| Munkmarsch | ** | ** | ** | ** | ns | — | 0.0032 | 0.014 |
| Sylt-Leghörn | ** | ** | ** | ** | ns | ns | — | 0.009 |
| Königshafen | ** | ** | ** | ** | * | ** | ns | — |
| | Möltenort | Falkenstein | Kiekut | Maasholm | Wackerballig | | | |
| Baltic Sea | | | | | | | | |
| Möltenort | — | 0.009 | 0.016 | 0.046 | 0.011 | | | |
| Falkenstein | * | — | 0.007 | 0.030 | 0.003 | | | |
| Kiekut | * | ns | — | 0.038 | 0.005 | | | |
| Maasholm | ** | ** | ** | — | 0.024 | | | |
| Wackerballig | * | ns | ns | ** | — | | | |

genetic with geographic distances in addition to nonsignificant differentiation at 30–54 km suggest that gene flow is strong between *Zostera marina* populations in both areas.

The inverse correlation of gene flow and observed population differentiation assumes a constant subpopulation size without extinction/colonization (Slatkin 1993; Whitlock and McCauley 1999), an assumption that is probably not met in the Wadden Sea or the Baltic. Although detailed population censuses are absent, a reasonable assumption is that recent local extinctions have occurred in the past decades given the hydrological changes in the northern Wadden Sea (Reise et al. 1989), as well as the changes in water clarity in the southwestern Baltic (Magaard and Rheinheimer 1973). Hence, observed values of genetic differentiation may also be a result of recolonization rather than gene flow between persisting populations (Wade and McCauley 1988). Terrestrial plant studies explicitly considering a metapopulation dynamic suggest that colonization events enhance rather than decrease genetic differentiation (Giles and Goudet 1997). If this was true in eelgrass, gene flow had rather been underestimated in this study.

A low genetic differentiation identified in this study contrasts with earlier work in eelgrass either using the polymorphism of allozymes (Ruckelshaus 1998; Williams and Orth 1998) or DNA fingerprinting (Alberte et al. 1994). In the northwest Pacific and in the Chesapeake Bay at approximately equal geographic distances, genetic differentiation at allozyme loci was one order of magnitude higher than in the two coastal areas examined here (Ruckelshaus 1998; Williams and Orth 1998). Methodological artifacts associated with the allozyme analysis, for example the possibility of selection at allozyme loci, may partly explain diverging results compared to microsatellites (see examples in Hilbish 1996). Also, allozyme techniques generally do not permit clone assignment due to their low polymorphism, resulting in a confounding of genetic differentiation at the clonal and population level. Both errors may have led to an overesti-

mation of genetic differentiation. We are left with the finding that there is more gene flow than previously appreciated among *Zostera marina* populations in two rather different coastal areas.

A genetic demonstration of high population connectivity is not necessarily at odds with dispersal distances in eelgrass pollen and seed of only a few meters measured by Orth et al. (1994). While such direct measurements cover the left end of a frequency distribution of dispersal distances, they will miss the long tail of the distribution, the medium- to long-range dispersal (Higgins and Richardson 1999; Cain et al. 2000). In this study, I have linked direct observations on potential immigrant genotypes with data from high-resolution genetic markers. In the first application of an assignment test in the marine environment, drifting reproductive shoots were identified as potential medium range (several km) vectors of seeds on one of two occasions. The role of rafting reproductive shoots parallels findings from terrestrial plants where often not the fruit but the entire reproductive organ or plant part is the unit of dispersal (van der Pijl 1982). Wind speeds prior to collection of driftline material at Maasholm were not exceptionally high. They never exceeded 8 m s^{-1} during a 3-d period prior to collecting driftline material (Institute of Marine Sciences Kiel, unpubl. data, available at <ftp://tornado.ifm.uni-kiel.de>). Nevertheless, a considerable proportion of reproductive shoots, carrying 1340 ± 250 seeds per meter shoreline, had been washed upon the shore at Maasholm. Assuming conservatively that only 4/39 driftline genotypes are true immigrants on an individual basis, this translates to approximately 130 seeds per m shoreline that come from a distant location. Exceptional summer storms will have an even stronger impact upon gene flow in *Z. marina* by uprooting and dispersing a large proportion of fruit bearing shoots.

Unfortunately, above quantifications only represent potential gene flow, as no information is available on the fate of the seeds actually ending up in locations favorable for ger-

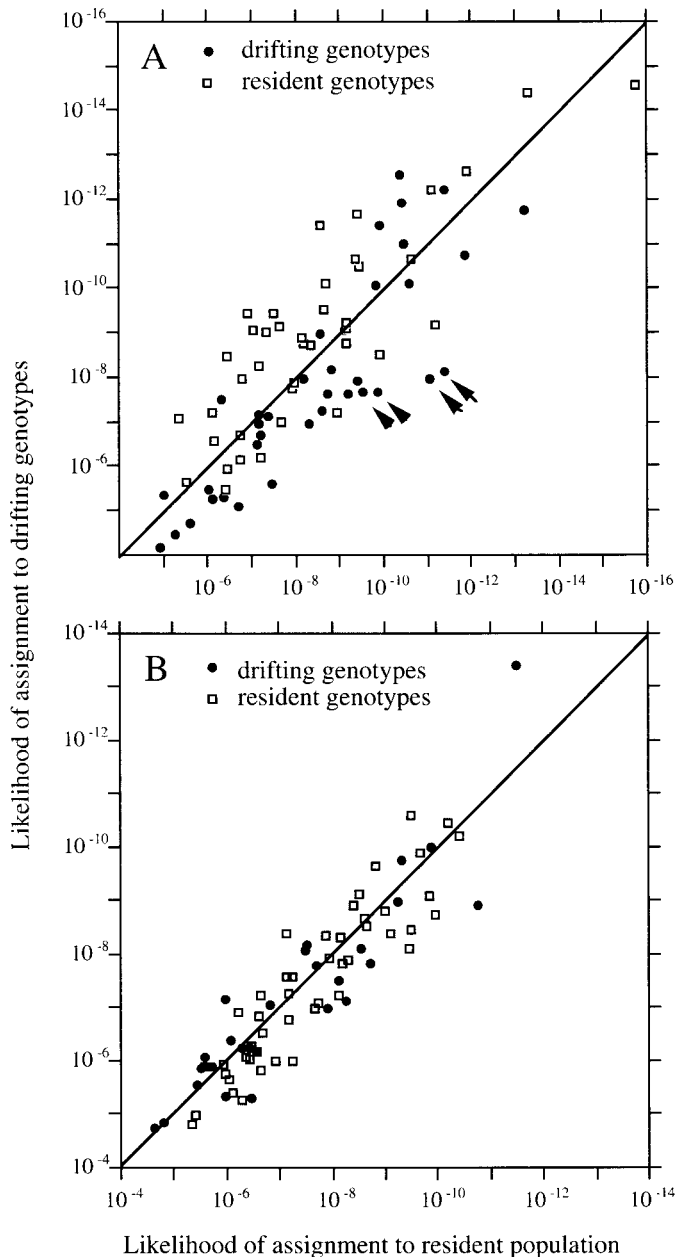


Fig. 3. Multilocus likelihood for *Z. marina* genotypes to belong to the resident population (x-axis) or the driftline population (y-axis) of (A) Maasholm and (B) Wackerballig. Both axes are log-scale and reverse. Individuals above the diagonal are assigned to the drifting population, those below to the resident population. Individuals that are >100 times more likely to be foreign genotypes with respect to the resident plants are assumed to be immigrants on an individual basis (arrows).

mination and survival (Silvertown 1991). Future work will include the genotyping of recently germinated seedlings in order to quantify the realized gene flow. It is also unknown from how far away immigrant genotypes came. Since the relationship between genetic and geographic distances in the southwestern Baltic is not simple (Fig. 2A), many more putative source populations need to be genotyped in order to identify the range of distances traveled by immigrants.

If low population differentiation is mainly the result of high gene flow at scales of ≤ 50 km, this has important implications for conservation. Inferred gene flow was well above one migrant per generation, the threshold beyond which local loss of genetic polymorphism is minimized (Wright 1931). Efficient propagule transport also indicates that the recolonizing ability of eelgrass is higher than previously assumed. This implies that the recent history of local extinctions may not be as important for explaining *Z. marina* distribution than previously thought (Orth et al. 1994). Future work will be directed toward a better understanding of seagrass metapopulations. High-resolution molecular markers will allow a description of the seagrass populations in a coastal area as a spatial network of source and sink populations, connected by episodes of medium- to long-distance gene flow.

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