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REVIEW

New markers—old questions: population genetics of seagrasses

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ABSTRACT: Marine angiosperms, or seagrasses, continue to be a major focus of marine biologists because of their important ecological role in many coastal ecosystems. Seagrass population biology could benefit from a population genetic perspective because genetic data enable the extraction of useful demographic information such as isolation and gene flow between demes. Moreover, population genetic processes may contribute to the growing ecological risks of local population extinction. Progress in seagrass genetics is partly driven by novel genetic markers which detect variation at the DNA level and overcome the limited polymorphism of allozymes. Key results of studies in the past decade, mostly using RAPD and microsatellites, were (1) considerable genetic and genotypic (clonal) diversity is present in several species in contrast to earlier notions of low polymorphism detected at allozyme loci, and (2) genetic differentiation among populations seems to be the rule despite earlier reports of genetic uniformity. Pronounced genetic structure was detected between populations of 4 species examined thus far (*Posidonia oceanica*, *P. australis*, *Zostera marina*, *Thalassia testudinum*). The F_{ST} estimates varied widely and ranged from 0.01 to 0.623 across studies and species. Genetic differentiation at a systematic range of scales was only studied in eelgrass *Zostera marina*, where it was positively correlated with geographic distance. The high polymorphism of RAPD or microsatellite markers will allow the augmention of indirect estimates of gene flow by methods detecting individual immigration events through paternity analysis or assignment tests. Important conservation related issues such as the level of inbreeding and the effective population size have also been obtained from genetic marker data, but results are too scarce at the moment to allow generalizations. In *Zostera marina* and *Posidonia australis*, several population genetic attributes such as clonal diversity, mating system and effective population size varied among populations within species, highlighting that there is no 'typical' population. An important gap in our knowledge is whether the effects of natural population fragmentation and patchiness enhance the genetic isolation of populations due to anthropogenic disturbances. It is also unclear whether genetic differentiation displayed at marker loci are correlated with fitness-related plant traits, and whether genetic or genotypic diversity is important for medium- to long-term meadow persistence. An assessment of the genetic and genotypic diversity at marker loci should be combined with experiments on the ecological plasticity and reaction norms of genotypes composing the populations in question. This way, the role of genetic diversity for seagrass population maintenance and growth in the face of changing environmental conditions can be evaluated.

KEY WORDS: Allozyme · Clonal reproduction · Gene flow · Genetic neighborhood · Genetic structure · Plant mating system · Microsatellite · Population genetics · RAPD · Seagrass

INTRODUCTION

Seagrasses, a polyphyletic assemblage of approximately 60 truly marine angiosperms (Les et al. 1997), continue to be a major focus of marine biologists worldwide (Duarte 1999) for good reasons: seagrass meadows provide food and habitat for associated animals in many shallow soft-bottom areas from subartic to tropical regions (den Hartog 1970), they enhance nearshore productivity while buffering waves and cur-

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rents, and many experimentally tractable species interactions have been uncovered in their meadows (overview in Orth 1992). Their direct economic value to humans as a sink for nutrient run-off from land, and as a nursery for commercially important species ranges among the highest of all ecosystems (≥10 000 US$ ha^{-1}, Costanza et al. 1997). Unfortunately, losses of seagrass based coastal ecosystems due to anthropogenic disturbances such as eutrophication, habitat destruction and coastal development have been considerable in past decades (Short & Wyllie-Escheverria 1996).

Population genetic studies have played a relatively minor role in the seagrass research program, but are on the rise with the advent of new technology. Before the early 1990s, seagrass population genetics suffered from methodological shortcomings. Most species revealed disappointingly low levels of electrophoretically separable polymorphism of enzymes (McMillan 1980), the marker of choice in the early days of empirical population genetics.

As a scientific discipline, population genetics lies at the interface of ecology and evolution, quantifying short-term evolutionary changes as a consequence of selection due to ecological factors, and genetic constraints. Since many seagrass beds are monospecific stands, the population biology and genetics of the habitat founding species is inevitably at the core of seagrass research. For a proper management of threatened seagrass populations, we urgently need population genetic information. Is there sufficient genetic variation for seagrass populations to cope with the anticipated changes in the environmental conditions (global change)? Where should donor material come from in the case of deliberate specimen translocation to sites where populations had been destroyed before? In the face of continuing habitat destruction and fragmentation, genetic processes may exacerbate ecological threats, for example through the loss of alleles due to genetic drift or due to the deleterious effects of inbreeding. At present, the necessary data basis for evaluating the role of genetic causes to population extinction is lacking for most seagrass species.

Population genetic information not only is warranted to evaluate and predict the persistence of populations. Another reason for seagrasses as an interesting group of model organisms for plant population geneticists is their diverse reproductive biology. Most species reproduce vegetatively by rhizomes bearing new leaf shoots, and sexually through flowers adapted to true subaqueous pollination. Within sexual reproduction, a multitude of mating system arrangements can be found among the 11 genera which comprise hermaphroditic flowers (genus *Posidonia*), monoecy (e.g. *Zostera*) and dioecy (e.g. *Amphibolis*, *Halodule*, *Thalassia*) (Les 1988). Such a wide array of matings systems calls for a comparative research program of which the first data are now available.

With the advent of high-resolution molecular markers, seagrass population genetics is at a turning point. This review intends to summarize recent progress beginning in the early 1990s with the advent of PCR (polymerase chain reaction)-based technology uncovering the genetic variation directly on the DNA. The review is not meant to be exhaustive, but rather to highlight recent advances and major gaps. Since part of the progress in plant population genetics is method-driven, I begin with briefly reviewing the genetic markers employed.

A BRIEF OVERVIEW OF GENETIC MARKERS EMPLOYED

Genetic markers sensu latu are either gene products, such as the different morphs of enzymes, or polymorphic regions on the DNA itself (Avise 1994). The genetic marker concept implies that they reflect the demography and the diversity of the genome of the population or species in question. The traditional co-dominantly inherited marker in population genetics are allozymes, electrophoretically separable morphs of enzymes which are assumed to be selectively neutral. Because their number and polymorphism is limited, the focus of geneticists is shifting more and more towards characterizing the ample differences on the DNA itself. Generally, DNA based markers are assumed to represent variation at non-coding sites, the so-called 'junk'-DNA. We therefore can reasonably assume that they are selectively neutral.

In single-locus DNA markers such as microsatellites, the stretches on the DNA may be well defined (Jarne & Lagoda 1996). Microsatellite alleles consist of repeats of simple sequence motifs of varying length. When primers upstream and downstream from the microsatellite have been identified, the alleles can be amplified by PCR, separated by gel electrophoresis, and visualized by autoradiography or fluorescence. In contrast, dominant, multi-locus markers, such as random amplified polymorphic DNA (RAPD) (Welsh et al. 1991), are the result of a PCR with unspecific primers, leading to a complex mixture of product fragments from many sites on the target DNA. In all PCR-based methods, very small amounts of plant tissue (≤0.1 mg) are sufficient for a genotypic analysis at several loci due to the exponential increase in copy number during PCR.

A third technique is DNA fingerprinting. Here, the genomic DNA is cut with restriction enzymes. The digested DNA is then blotted onto a membrane and probed with a radioactive oligonucleotide probe which

Table 1. Comparison of within-population (pop.) genetic diversity in seagrasses measured with allozyme polymorphism or DNA-based genetic markers (M-type = genetic marker type, RAPD = random amplified polymorphic DNA, Msat = microsatellites). A = number of alleles per population, H_{obs} = observed heterozygosity, P_D = number of distinguishable genotypes. In the case of several populations arithmetic means are given. na = not applicable, ng = not given

Species	Allozyme polymorphism					DNA-based genetic marker					
	Location (no. of pop.)	A	H_{obs}	P_D	Reference no.	Location (no. of pop.)	M-type	A	H_{obs}	P_D	Reference no.
Zostera marina	Europe (4)	1.1	0.02	ng	(2)	Europe, North America (12)	Msat	4.7	0.48	0.88[a]	(8)
	California (6)	1.3	0.04	0.07	(15)	Europe (3)	Msat	7.5	0.51	1	(7)
	Chesapeake Bay (6)	1.3	0.04	ng	(16)						
	NE Pacific (2)	2.3	0.41	0.38	(9)						
Posidonia oceanica	Mediterranean (3)	1.13	0.065	ng	(1)	Mediterranean (6)	Msat	1.7	0.26	0.33	(5)
						Mediterranean (3)	RAPD	1[b]	na	0.04	(4)
Posidonia australis	Australia (1)	2	0.35	0.35	(11)	Australia (1)	RAPD	25[b]	na	0.73	(11)
	Australia (7)	1.5	0.08	0.57	(13)	Australia (18)	RAPD	28[b]	na	0.91	(14)
	Australia (22)	1.07	0.05	0.43	(12)						
	Australia (18)	ng	ng	0.45	(14)						
Thalassia testudinum	Florida, Jamaica (18)	1.2	0.03	0.15	(10)	Florida, Jamaica (4)	RAPD	29[b]	na	0.79	(3)
Halophila stipulacea	No data					Mediterranean (2)	RAPD	76[b]	na	0.98	(6)

[a]2 monoclonal populations excluded
[b]Number of polymorphic RAPD-bands

Reference: (1) Capiomont et al. (1996); (2) de Heij & Nienhuis (1992); (3) Kirsten et al. (1998); (4) Procaccini et al. (1996); (5) Procaccini & Mazella (1998); (6) Procaccini et al. (1999); (7) Reusch (2000a); (8) Reusch et al. (2000); (9) Ruckelshaus (1998); (10) Schlueter & Guttman (1998); (11) Waycott et al. (1995); (12) Waycott et al. (1997); (13) Waycott & Sampson (1997); (14) Waycott (1998); (15) Williams & Davis (1996); (16) Williams & Orth (1998)

binds to small, unspecific sequence motifs occurring at variable positions on the DNA. In *Zostera marina*, the amount of genetic variation detected in a study by Fain et al. (1992) and Alberte et al. (1994) was relatively small. Large amounts of highly purified DNA are necessary for this method, prohibiting a sample size sufficient for population genetic surveys.

An important advantage of both allozymes and microsatellites is their co-dominant inheritance which allows us to study inbreeding and the mating system. This is not possible by RAPD or DNA fingerprinting because these methods cannot distinguish between homozygous (individuals carry identical alleles at a locus) and heterozygous (individuals carry different alleles) genotypes. When using co-dominantly inherited, single locus markers (microsatellites or allozymes), a rich body of population genetic theory is available to link empirical data to expectations from the neutral model of evolution. Because DNA microsatellites couple co-dominant inheritance with high resolution, they become the marker of choice for many population genetic questions. Currently, microsatellite primers are available for *Posidonia oceanica* (Procaccini & Waycott 1998) and *Zostera marina* (Reusch et al. 1999c, Reusch 2000a). At least in the genus *Zostera*, the identified microsatellite loci are species specific (Reusch 2000a), necessitating relatively labour-intensive development for each target species separately. Because of their advantages compared to other genetic markers, their development is in progress in several other taxa.

DIVERSITY DISPLAYED BY VARIOUS TYPES OF GENETIC MARKERS

Genetic markers are only useful for discriminating genotypes and populations if they display polymorphism within and among populations (Schaal et al. 1991). Very low levels of allozyme polymorphism were found in several seagrass species (Table 1, see also McMillan [1980] and references therein). Based on this evidence, seagrasses were repeatedly described as genetically uniform both within and among populations. To compensate for the limited evolutionary potential, they were thought to be genotypically plastic and have broad reaction norms (Les 1988, Barrett et al. 1993).

The evidence coming from studies involving DNA-based genetic markers, mostly RAPD and

microsatellites, warrants a reconsideration of this view. In Table 1, information on allozyme data and DNA-based marker data are compared. In general, DNA-based markers uncovered a higher genetic and genotypic diversity than allozyme studies in all 4 species where both marker types have been used. For example, in the relatively well-studied eelgrass *Zostera marina*, an average of 1.3 alleles was found in ≤5 polymorphic allozymes (Williams & Davis 1996, Williams & Orth 1998, Ruckelshaus 1998), whereas on average 4.7 to 7.5 alleles per population were discovered in a total of 11 microsatellite loci in 16 populations from North America and Europe (Reusch 2000a, Reusch et al. 2000, and T.B.H.R. unpubl. data). In the Australian seagrass *Posidonia australis* the same individuals were genotyped with allozymes and RAPD, allowing a direct comparison of both types of markers. In a single population of *P. australis*, RAPD-genotyping revealed more polymorphic bands and a higher genotypic diversity (Waycott 1995) than allozymes. This finding was confirmed in a larger survey of 18 populations, albeit with an average sample size of 6.6 ind. per population (Waycott 1998).

In none of the cited allozyme studies could a distinction be made between ramets and genets because the within-population polymorphism displayed by the genetic markers was insufficient. When genets cannot be distinguished from ramets, then both levels of genetic diversity collapse. As a consequence, measures of within-population diversity can be strongly biased downwards if, at a site, genets are large and had been repeatedly sampled (Reusch et al. 1999d). Under these circumstances, estimates of low genetic diversity can be misleading if there is ample variation among genets.

In terrestrial plants, the life-history correlates of genetic structure are well established (Loveless & Hamrick 1984). In contrast, many more data, replicating both populations and species, are necessary for relating the genetic diversity of different seagrass species to their life history, dispersal and breeding system. It is now becoming evident that earlier findings of genetic homogeneity within marine angiosperms in general were methodological shortcomings of the allozyme method, at least for species which now have been studied using DNA-based markers.

IDENTIFYING THE INDIVIDUAL

In clonally and sexually reproducing plants such as seagrasses, the genetic diversity is hierarchically organized. A starting point for population studies is to distinguish morphological individuals, which have arisen from vegetative reproduction (i.e. ramets sensu Harper 1977), from genetic individuals (i.e. genets or clones) which have ultimately stemmed from a zygote. In order to distinguish genets from ramets, genetic markers are needed because morphological connections such as rhizomes disintegrate much earlier than the potential lifetime of genets. Distinguishing genotypes is only possible through DNA-based markers because allozymes have insufficient within-population polymorphism. More distinct genotypes were discovered using DNA-based markers (RAPD) compared to allozyme-based surveys (*Thalassia testudinum*: Kirsten et al. 1998; *Posidonia australis*: Waycott 1998). It is not yet clear whether in these studies a saturation of all distinguishable genotypes was reached, or whether even more distinct genets would have been detectable if a higher polymorphism had been available.

In principle, any genetic marker with sufficient variability (e.g. DNA fingerprinting, RAPD, microsatellites) is useful for excluding individual identity, i.e. for distinguishing different genets. The question becomes more difficult when the aim is to identify the maximal size of clones. In this case, we need to disprove genetic differences between putative clonemates. In allozyme studies seagrass populations were repeatedly found to be genetically homogeneous over large areas (e.g. in *Zostera marina*, de Heij & Nienhuis 1992; in *Amphibolis antarctica*, Waycott et al. 1996). However, this is no convincing evidence for monoclonality because the chosen markers were fixed for certain alleles at all loci over an unknown geographic scale. Only if within-population genetic variation is displayed by the markers at the population level can we safely infer a maximal clone size. Here, DNA microsatellites will undoubtedly play a major role in coming years because they enable the detection of within-population genetic diversity as heterozygosity of individuals. Due to Mendel's fundamental law of genetics, sexual reproduction leads to a segregation of alleles in heterozygous individuals, and hence, to different progeny genotypes. In other words, if the same genotype with an identical heterozygous pattern occurs over and over again in a population, this is strong evidence for membership in an identical genet, in particular if this pattern is found in several loci. The Mendelian inheritance in microsatellites also allows us to give error likelihoods for given assignments of like genotypes to genets. For this purpose, we simply calculate the probabilities of a multi-locus genotype occurring by chance as a consequence of sexual recombination. In eelgrass *Zostera marina* the chances of falsely assigning ramets to the same genet were always <5% across several populations, exemplifying the high resolution obtainable with only 6 microsatellite loci (Reusch et al. 1999a,d). Unfortunately, no expected genotype frequencies can be calculated when using dominant/recessively inherited RAPD.

Recent results from *Posidonia oceanica* (Procaccini & Mazzella 1998), *P. australis* (Waycott 1995), *Thalassia testudinum* (Kirsten et al. 1998), and *Zostera marina* (Reusch et al. 1999a) using either the multi-locus genotypes at microsatellite loci, or RAPD, suggest that genets may stretch over several 10s of meters. At the extreme, entire meadows may be composed of a single large genet (Reusch et al. 1999a), making it questionable to speak of a 'population'. The minimal longevity of genets can be calculated from horizontal rhizome growth rates, assuming a centrifugal growth from a hypothesized genet center. Size and age estimates of genets were found to be heterogeneous within populations, ranging from <5 to 70 yr at a site in the western Baltic Sea (Reusch et al. 1999d). The record is a genet in the central Baltic Sea extending over 160 m which translates into an age estimate of 1000 yr (Reusch et al. 1999a). These findings prompt important questions as to which factors are responsible for the apparent long-term persistence of single genotypes. Such persistence is even more surprising given the stressful and fluctuating environmental conditions in the central Baltic Sea.

Genetic data may also yield surprising results in the opposite direction when uncovering genotypic diversity although sexual reproduction seems very rare. Using RAPD, Procaccini et al. (1999) report considerable genetic diversity in Mediterranean *Halophila stipulacea* populations although female flowers have never been observed at the study sites.

Only a few studies have mapped the location of individuals sampled in a population (Waycott 1995, Williams & Davis 1996, Kirsten, et al. 1998, Reusch et al. 1999d). This is unfortunate because mapped data are prerequisite for assessing the size and spatial arrangement of genets. As soon as more comparative data have been obtained, the size distribution of genets in a population may be utilized for a retrospective demography at sites where no census data are available. For example, relatively large (and possibly old) genets suggest a minimal age of the population equal or greater than the estimated age of the largest genets. Mapping can also provide insight into the autocorrelation of genes and, hence, into the movements of pollen and seeds within the population (Williams & Davis 1996, Ruckelshaus 1998, Reusch et al. 1999b). Spatial autocorrelation techniques are available to utilize such information for detecting the unit of panmixis and the effective population size.

All experimental studies in seagrass ecology (including my own work) have ignored the hierarchical arrangement of seagrasses into ramets and genets. This is not desirable because examples from terrestrial plant species reveal that there are marked differences between clones in almost any trait which can be measured, e.g. flowering time, growth rates, nitrogen demand, and pathogen resistance (e.g. Silander Jr 1985a,b, Schmid 1994). Most likely, many ecological experiments in seagrasses have taken place with only a few genets, or in the extreme case, with only a single genet, with a concomittant loss of generality.

SEXUAL REPRODUCTION AND MATING SYSTEM STUDIES

The mating system of plants describes how male and female gametes unite and transmit hereditary information to the progeny. As such it has a major influence on evolution of populations at all time scales and determines the within- and among-population genetic structure of populations (Loveless & Hamrick 1984). For evaluating genetic processes relevant to population persistence, for example the potential effects of inbreeding, basic information on the breeding system is prerequisite in order to put empirical data into perspective. The available life-history information suggests that most seagrass species are predominantly outcrossing. Among seagrasses we find a much higher proportion of dicliny (separation of male and female sexes as opposed to hermaphroditic flowers) than the average in all flowering plants. An astonishing 94% of all seagrass species are either monoecious or dioecious (den Hartog 1970), compared to a mere 4% in all flowering plants. On theoretical grounds Cox (1983) argued that, as a consequence of subaqueous pollination, aquatic angiosperms are expected to develop diclinous mating systems in order to maximize the outcrossing rate.

For mating system studies, co-dominant markers facilitate the distinction between outcrossed and selfed progeny. The markers of choice for such studies are therefore either allozymes or microsatellites. In seagrasses, mating system information exists for only 2 species, *Zostera marina* and *Posidonia australis*. In monoeceous and protogynous eelgrass (i.e. female flowers ripen after males), the predicted high outcrossing rate was empirically verified in 1 North American (using allozymes, Ruckelshaus 1995) and 4 European populations (using microsatellites, Reusch 2000b, 2001). In the hermaphroditic *P. australis*, Waycott & Sampson (1997) report outcrossing rates ($0 \leq t \leq 1$) between 0.1 and 0.89 in a study using the polymorphism at 7 allozyme loci. On average, the outcrossing rates in *P. australis* are lower than in the diclinous species *Z. marina.* Mean t-values over 6 and 4 populations, respectively, were 0.476 in *P. australis* and 0.862 in *Z. marina*, which is consistent with the expectation that a separation of male and female flowers promotes outcrossing rates. More species and populations within species need to be studied to

identify general mating system patterns related to floral morphology and plant phenology.

Clonal reproduction has previously unappreciated consequences for the mating system of seagrasses. The higher the extent of vegetative (as opposed to sexual) reproduction, and hence, the larger the average size and dominance of particular clones, the fewer the number of distinct genotypes per unit area. Given the physical distance the average pollen grain travels, we would predict a decrease of the outcrossing rate with increasing clone size because more and more pollen is exchanged between ramets belonging to the same genet (Handel 1985). In eelgrass *Zostera marina*, such geitonogamous selfing is indeed positively correlated with increasing clonal reproduction. At 2 sites in the western Baltic Sea, the outcrossing rates dropped sharply below a clonal diversity of 0.5 (i.e. 5 genotypes in 10 sampled ramets per 2 m^2). In addition, there was a tendency on a population scale for clonally less diverse populations to have higher selfing rates among 4 Baltic and North Sea populations (Reusch 2001). Interestingly, the adult populations were very close to Hardy-Weinberg equilibrium (HWE), suggesting very strong selection against inbred genotypes. Based on the approach of Ritland (1990), the relative fitness of selfed progeny in the study populations had to be low (0.32 to 0.56) in order to explain the equilibrium proportion of homozygotes among the adult plants. The consequences of between-ramet selfing for the persistence of entire populations as a function of the clonal diversity have not yet been explored. It is clear, however, that the mating system of seagrasses is affected by their clonal growth patterns in a complex way at several spatial scales.

GENETIC DRIFT AND EFFECTIVE POPULATION SIZE

Seagrass populations become increasingly fragmented and isolated from neighboring meadows (Short & Wyllie-Escheverria 1996). Besides direct ecological threats and environmental stochasticity, small populations face additional genetic extinction risks which we need to evaluate in order to predict the future viability of populations (Hamrick & Godt 1996). Genetic drift is the random loss of alleles through the sub-sampling of the total gene pool in the course of gamete formation and meiosis. Inbreeding, the mating of individuals which are relatives, increases the chances for an individual to carry identical alleles at a locus. The consequence is an excess of homozygous genotypes in the population which often suffer from reduced fitness due to inbreeding depression (Charlesworth & Charlesworth 1987, Frankham 1995).

The effective population size N_e is the number of individuals which represents a conceptual approximation of a real population by a randomly mating population (Wright 1931). Typically N_e is much smaller than the ecological census of a population at a given site. The differences between the ecological census size and N_e stem from restricted gene-flow, fluctuating populations size, unequal sex ratio, and skewed age and size ratios. N_e is a useful parameter because it summarizes the role of genetic drift and the extent of inbreeding in a single number. In each generation, alleles are lost from populations through random genetic drift at a rate proportional to 1/2 N_e. Also, the smaller the N_e, the more matings will occur between close relatives, with a concomittant increase of homozygosity in the population over time, which is commonly quantified using Wright's inbreeding coefficient f.

The only species where information on N_e is available is *Zostera marina* (eelgrass). Here, direct measurements of dispersal distances are augmented by estimates based on genetic data, allowing a cross-validation of both methods. Based on measurements of pollen and seed movement, Ruckelshaus (1996) estimated the neighborhood size of a Pacific eelgrass population at 524 m^2, comprising ≈6200 reproductive leaf shoots (no distinction made between ramets and genets). In the Baltic Sea, an autocorrelation analysis under consideration of genetic similarity due to genetic identity (i.e. clone assignment) revealed no spatial clustering of 6 microsatellite loci representing 37 alleles in an area of 20 × 40 m (= 800 m^2) (Reusch et al. 1999b). Assuming that this is the minimal area of panmixis, this would correspond to 1600 to 3200 reproductive shoots. Taking into account, however, that at this site between 6 and 21 ramets belong to an identical genet, the minimal estimate reduces to an N_e of 76 to 533 genets. This highlights that clonality needs to be taken into account when assessing the effective population size. A recent study based on 8 European eelgrass populations suggests that above calculation based on the autocorrelation of genes within a population is probably too low and should be considered as a minimal estimate. In *Z. marina*, Reusch et al. (2000) also determined the effective population size using the slope of a differentiation-by-distance relationship of several populations sampled at a range of geographic distances (method of Rousset 1997). This method utilizes the fact that the genetic differentiation between subpopulations increases the faster, the smaller the effective population size of a species. The average N_e of 8 European eelgrass populations was between 2440 and 5000 ind., depending on the exact statistic used for quantifying population differentiation. In summary, in multiclonal populations of *Z. marina*, N_e is >500 and

therefore large enough to leave only a minor role for genetic drift (Nunney & Campbell 1993). However, this is likely to be different in eelgrass populations where clones are large and few genotypes participate in the sexually recombining population. N_e is the number of genets, not of the reproductive ramets in the neighbourhood area. Therefore, pronounced clonal reproduction will decrease the effective population size and increase the role of genetic drift. In nearly or completely monoclonal populations the concept of N_e becomes meaningless because the number of distinct genotypes in areas >2000 m^2 was <5 (Reusch et al. 2000).

Since we have no information on N_e in all but 1 seagrass species, we currently do not know the extent of genetic drift and inbreeding in most species under natural conditions. In order to evaluate potential negative effects of genetic erosion in contemporary seagrass populations which are challenged by human activity, we urgently need information on effective population sizes and inbreeding from relatively undisturbed populations as a baseline. In particular, comparative data should be obtained from relatively small, isolated populations versus large, continuous stands.

Besides explicit estimates of the effective population size N_e, some information is available on the closely related issue of the inbreeding coefficient *f*. In fact, an important application of codominantly inherited genetic markers (allozymes, microsatellites) is to compute the inbreeding coefficient of the genotypes in a population and to test statistically whether there is a deviation from the expectations under HWE. A significant positive correlation of alleles within individuals indicates an excess of homozygous individuals, or inbreeding. Population genetic processes leading to more heterozygous individuals than expected are more difficult to explain and may be due to a higher fitness of heterozygous genotypes. Alternatively, they can be a sampling artifact (see below).

Published estimates of deviation from HWE detected at genetic marker loci vary widely among species and populations, and range from an excess of heterozygote individuals (some *Zostera marina* populations, Williams & Davis 1996) to homozygote surplus (*Posidonia oceanica*, Procaccini & Mazzella 1998; *Z. marina*, Ruckelshaus 1998; Williams & Orth 1998). Part of this variation may be due to the failure of previous studies to differentiate between ramets and genets. If, for example, the dominant genet at a site is homozygous at several loci, a spurious positive deviation from HWE will result. Inbreeding will be detected through such sampling artifacts although none occurred. In only 2 studies the duplicate inclusion of ramets of the same genet was avoided prior to the calculation of *f*. In *Z. marina*, deviations from HWE were weak and statistically significant in only 1 of 10 populations (Reusch et al. 2000). In the Mediterranean *P. oceanica*, Procaccini & Mazella (1998) found an excess of homozygote individuals in several populations, indicating pronounced inbreeding.

Another important piece of evidence for an evaluation of genetic erosion would be an assessment of fitness of outcrossed relative to inbred offspring. In most seagrass species we would expect inbreeding to cause strong negative fitness effects given their breeding system adaptations to ensure outcrossing. In obligate outbreeding species, the effects of sudden imposed inbreeding, e.g. through meadow fragmentation or isolation, are strong because deleterious alleles were not purged from the population before (Charlesworth & Charlesworth 1987). Concordant with this prediction, there is evidence for inbreeding depression in predominantly outcrossing populations of eelgrass *Zostera marina* (Ruckelshaus 1995). She found that the abortion rate was higher and offspring were less viable in artificially selfed progeny compared to outcrossed progeny.

SPATIAL VARIATION IN POPULATION GENETIC PROCESSES—THERE IS NO TYPICAL POPULATION

A recent finding is that populations of seagrass species are not uniform but vary in important population parameters such as the relative proportion of clonal versus sexual reproduction, the breeding system, and the effective population size. In the 2 species which have been studied most extensively thus far, *Posidonia australis* and *Zostera marina*, the clonal diversity and the size of genets varied markedly among populations of the same species (Waycott et al. 1997, Waycott 1998, Reusch et al. 2000). In eelgrass, the range of clonal diversity displayed in 12 populations ranged from near mono-clonality to maximal genotypic diversity (i.e. each shoot sampled displayed a unique genotype, Reusch et al. 2000). As a second important population genetic parameter, the mating system also varied across populations in both species which is probably related to water movement and the size of genets. Outcrossing rates ($0 \leq t \leq 1$) in *P. australis* determined with allozyme polymorphism were markedly different among populations and ranged from (t ± SE) 0.1 ± 0.04 to 0.89 ± 0.13 (Waycott & Sampson 1997). It is not clear which factors contribute to such widely varying breeding systems. In *Z. marina*, predominantly sexually reproducing populations from the North Sea displayed an outcrossing rate t close to 1 (i.e. complete outbreeding, Reusch 2000b), whereas populations with a mixture of clonal and sexual reproduction had lower outcrossing rates (t ± SE 0.67 ± 0.03 and 0.85 ± 0.02,

Reusch 2001). Ruckelshaus (1995) found higher outcrossing rates in intertidal as opposed to subtidal *Z. marina* populations at 1 site in the northeastern Pacific.

POPULATION GENETICS OF PATCHY SEAGRASS BEDS

Thus far, the discussion has characterized seagrass beds as continuous areas of vegetation. Very often this is not the case. Instead, many seagrass meadows consist of vegetation patches of varying size and isolation from one another (Bell et al. 1995). Patch sizes need to be precisely defined as they may range in size from a few shoots to 10s of meters in diameter. Patchiness can be caused by several factors. The natural regime of wave- and current-induced disturbances is one, but over the last decades, human disturbances have resulted in additional meadow fragmentation (Short & Wyllie-Escheverria 1996). The principles of landscape ecology which were successfully applied to species interactions within seagrass beds (e.g. Irlandi 1994, 1996, Bell et al. 1995) most likely also apply to population genetic processes within and among patches of seagrass vegetation. Isolated and fragmented seagrass populations may function very differently in terms of their demography and population genetics, as studies in terrestrial plants have revealed (Olesen & Jain 1994). For example, they are more likely to suffer from inbreeding due to the limitation of pollen from unrelated genotypes (e.g. Taylor et al. 1999). Whether and how anthropogenic stresses leading to seagrass bed fragmentation and naturally occurring patchiness act synergistically on population genetic isolation is currently unknown. The interaction of the spatial architecture of seagrass populations, their clonal composition with population genetic processes such as the mating system and genetic drift needs more empirical research. Questions of particular interest are: (1) how many clones are patches typically composed of, (2) what are the distances between vegetation patches that can be travelled by pollen from a neighboring patch, and (3) do plants within patches, as opposed to those in continuous stands, self more due to limited influx of foreign pollen?

DISPERSAL AND ISOLATION IN SEAGRASS POPULATIONS

Gene flow between subpopulations determines the degree of local differentiation and adaptation (Slatkin 1987). Quantifying gene flow between populations as a function of geographic distance is also of immediate ecological relevance for seagrass biologists. The natural rate of recolonization depends on the distances seagrass propagules can travel from vegetated to unvegetated areas. Gene flow estimates are also important to judge the severity of genetic isolation in the face of habitat fragmentation. When selecting donor material for replanting measures, information on natural rates of population exchange are mandatory to avoid the introduction of germplasm which is too foreign to nearby resident genotypes to establish successfully (van Andel 1998).

The population differentiation displayed at neutral marker loci can be utilized to derive an indirect estimate of gene flow. The higher the genetic differentiation between populations, the lower the mean genetic exchange between populations must have been in the previous generations. Population differentiation is commonly estimated as the standardized allelic variance F_{ST} (Wright 1969) which measures the difference in allelic frequencies between populations. For estimating genetic exchange, all authors have used the classical approach of computing the standardized allelic variance $F_{ST} = 1/ (4\ N_e m + 1)$, where $N_e m$ is the product of the fraction of migrants m and the effective populations size N_e. Solving for $N_e m$ yields an indirect estimate of the number of migrants exchanged every generation (Wright 1969) as $N_e m = 1/4\ [1/(F_{ST} - 1)]$. When using microsatellites, an equivalent population differentiation statistic, R_{ST}, has been proposed by Slatkin (1995), which takes account of the stepwise mutation mode of microsatellites. This statistic does compute the mean differences in allelic length between populations and not of frequencies as a genetic distance measure. Which distance measure is better suited for microsatellite data is currently an unresolved issue (e.g. Gaggiotti et al. 1999)

Many studies suffered from such a low level of polymorphism at genetic marker loci that estimates of differentiation and gene flow could not be given. Also, while multi-locus dominant markers such as RAPD and DNA fingerprinting do allow testing for genetic differentiation, a calculation of gene flow is not possible with these markers. This is because there is no theoretical basis to converge genetic distances into gene flow estimates. Table 2 summarizes studies on population differentiation and gene flow using codominantly inherited markers where the genetic variation displayed was sufficient to estimate within- and among population differentiation. Pronounced population subdivision was detected in all species studied, albeit at widely varying spatial scales across studies. The contribution of among population differentiation to the total genetic variance can be as high as 62% in *Posidonia australis* and 53% in *Zostera marina*. These findings are at odds with the widely held belief that

aquatic angiosperms are not genetically differentiated (Les 1988, Barrett et al. 1993).

Pronounced genetic substructuring in plant populations is a common phenomenon (Bradshaw 1984, Linhart & Grant 1996). While the above studies (Table 2) were an important first step towards identifying genetic structure and subdivision in seagrasses, the next step is to determine the scale over which isolation and gene flow operate. This is particularly interesting as many seagrass species are distributed over wide geographical areas. For 2 species, information on population differentiation over a range of geographic distances spanning more than 1 order of magnitude are available (Waycott et al. 1997, Reusch et al. 2000). Unfortunately, in *Posidonia australis* (Waycott et al. 1997) only the overall genetic differentiation was examined. In *Zostera marina*, a strong linear relationship of genetic differentiation with distance was found among 8 European populations 12 to 4500 km distant from one another, supporting a linear stepping-stone model of population subdivision (Reusch et al. 2000). However, on an even larger geographic scale, 2 North American populations were genetically much closer to Baltic and North Sea sites than their distance (>5000 km) would suggest. These findings highlight that the actual patterns of geographic partitioning of genetic variance may be counterintuitive. More studies on scale-dependent subdivision of plant populations are warranted. Ideally, they should employ a nested sampling scheme covering several spatial scales from small (1 to 10 km) to very large (≥10 000 km) geographic distances. Using hierarchical AMOVA (analysis of molecular variance, Weir & Cockerham 1984) the scale(s) at which significant proportions of the genetic variance are distributed can be defined, corresponding to specific levels of genetic isolation and exchange between populations.

In contrast to indirect data derived from genetic markers, direct estimates of seed dispersal are only feasible at very small spatial scales within populations (1 to 10s of meters). In the Chesapeake Bay and the North East Pacific, respectively, Orth et al. (1994) and Ruckelshaus (1996) report seed dispersal distances in *Zostera marina* of only a few meters even in unvegetated areas with high tidal current velocities. These data suggest that natural recolonization rates are slow and historical processes need to be considered to understand seagrass distribution. Within the seagrass meadow, single seeds are probably transported over even shorter distances because the canopy slows down water movement.

In many plant species, the dispersal function of seeds is extremely leptokurtic, i.e. long range dispersal is very rare, but it nonetheless occurs and is extremely important for maintaining genetic exchange between distant populations (Higgins & Richardson 1999). Direct, ecological measurements capture only dispersal events in the relatively frequent range (Loveless & Hamrick 1984). This readily explains why dispersal distances based on ecological measurements often grossly understimate actual re-colonization rates, especially after the last ice age (Slatkin 1987, Higgins & Richardson 1999).

Table 2. Population subdivision and indirect estimates of gene flow in 4 seagrass species, calculated using codominantly inherited genetic markers

Species, location	$N_{populations}$	Genetic marker	Geographic distance (km)	F_{ST}	Gene flow estimate ($N_e m$)	Reference
Zostera marina, California	6[a]	Allozymes	5–55 5–300	0.06 0.14	3.9 1.6	Williams & Davis (1996)
Zostera marina, Chesapeake Bay	8[a]	Allozymes	4–100	0.335	Not given	Williams & Orth (1998)
Zostera marina, NE Pacific	2	Allozymes	14	0.079	2.9	Ruckelshaus (1998)
Zostera marina, Europe	10	Microsatellites	12–4500	Sign. correlation[b] $1/(1-F_{ST}) =$ 0.0002 km + 0.108	Not given	Reusch et al. (2000)
Posidonia oceanica, Mediterranean	6	Microsatellites	40–600	0.14	1.55[c]	Procaccini & Mazella (1998)
Posidonia australis, Australia	22	Allozymes	5–5000	0.623	Not given	Waycott et al. (1997)
Thalassia testudinum, Florida	18	Allozymes	<4 4 4–80	0.01 0.061 0.207	24.7 3.8 0.96	Schlueter & Guttman (1998)

[a]Only non-transplanted populations considered
[b]Linearization of F_{ST} according to Rousset (1997)
[c]Using R_{ST}, the microsatellite equivalent of F_{ST}

Rare dispersal events will influence indirect estimators of genetic exchange, i.e. F_{ST}, because these parameters integrate over many generations. Unfortunatley, F_{ST} based indirect estimates suffer from other important shortcomings. They rely on several assumptions such as drift-mutation equilibrium, the absence of extinction and recolonization, and fixed population sizes. Few (if any) of these are met in natural populations (Bossart & Prowell 1998, Whitlock & McCauley 1999). Therefore, published estimates of gene flow are almost certainly biased. More importantly, they do not reflect contemporary gene flow, the question of interest to the population biologist, but are affected by the past demographic history of the population. Also, the traditional F_{ST} approach cannot distinguish between extinction/recolonization and genetic exchange between stable populations (Wade & McCauley 1988).

An additional problem when using F_{ST} is the need to define a population. The most sensible definition is the area covering 1 panmictic unit (Crawford 1984). Unfortunately, *a priori* information on the genetic neighborhood is lacking for most species. If more than 1 panmictic unit is amalgamated into an arbitrary population, the result may be spurious detection of inbreeding (the 'Wahlund-effect', Crawford 1984). Furthermore, the between-population differentiation is underestimated when the pooled populations in fact represent separate genetic units (for an example see Ruckelshaus 1998). From the findings of widely varying clonal diversity and mating systems among sites discussed above, it follows that the definition of the sampling area is a very difficult practical issue. One way to approach this problem is a small-scale nested sampling scheme in which subareas are successively amalgamated until a significant Wahlund-effect is detectable as an excess of homozygote genotypes (Goudet et al. 1994). Then, the effective populations size is close to the next smallest spatial sampling unit. Another alternative is the application of spatial autocorrelation techniques to identify the area across which genes are not spatially autocorrelated (Reusch et al. 1999b).

The high degree of polymorphism coupled with an almost unlimited number of variable loci in DNA-based genetic markers will result in significant advances of studying gene flow in plant populations. New promising approaches on the small scale are based on paternity analysis. Developing seeds or germinating seedlings in a population can be associated with nearby parent plants based on their multi-locus genotype (Devlin & Ellstrand 1990, Dow & Ashley 1996). These methods have been around for several years, but their application was limited until the advent of high resolution marker loci such as microsatellites. Such approaches are particulary promising in fragmented seagrass populations because here, the number of putative fathers is limited.

An alternative method of studying gene flow with potential application to seagrass population genetics becomes possible with an ever increasing resolution of DNA-based genetic markers. In assignment tests putative immigrant multi-locus genotypes are compared with the genotypic distribution of a hypothetical source population (Waser & Strobeck 1998). Using a maximum-likelihood approach, it is possible to ascribe the individual in question with a certain error likelihood to its most likely source population (Nielsen et al. 1997). An alternative way is to exclude arriving individuals with a certain likelihood from the target population, i.e. to test for misassignment in order to infer recent immigration (Paetkau et al. 1995, Rannala & Mountain 1997). Such direct and individual-based gene flow estimates will provide a major step forward compared to traditional gene flow estimates based on F_{ST} and related statistics (e.g. R_{ST} in the case of microsatellites). Assessing the directionality of gene flow is also possible, a further advantage over the bidirectionality of genetic exchange underlying F_{ST}-related estimates.

In a pilot survey, I compared the genotypic composition of the residential eelgrass *Zostera marina* population with reproductive shoots washed onto the shore at a western Baltic site. The plants were typed for 11 microsatellite loci representing 90 alleles. Assignment tests suggested that the driftline material which carried 2500 seeds m^{-1} shoreline had originated several km from the local population (Reusch 2001). This suggests that rafting seagrass plants carrying ripe seeds can be a major source of medium range dispersal (1 to 5 km), exceeding seed dispersal distances by 2 to 4 orders of magnitude. These data also illustrate the usefulness of PCR-based genetic markers such as microsatellites which allow genotyping of dead driftline material.

WHAT IS THE ECOLOGICAL ROLE OF POPULATION DIFFERENTIATION AND GENETIC DIVERSITY?

It is a common result that seagrasses, just as many other plant populations, are genetically differentiated over a range of spatial scales. What is needed is a proper identification of scales through hierarchical sampling, and, more importantly, the selective correlates for such differentiation (e.g. Podolsky & Holtsford 1995, Lynch et al. 1999). Evidence for genetic differentiation comes either from transplantation experiments (reciprocal or common garden) or from genetic marker data. Despite earlier notions of great phenotypic plasticity and low genetic differentiation in aquatic plants,

there is experimental evidence for local adaptation in seagrasses. In eelgrass *Zostera marina*, salinity and nutrient tolerance (van Katwijk et al. 1999), flowering time and reproductive allocation (van Lent & Verschuure 1995), and plant morphology (Backman 1991) are partly under genetic control. Detecting and quantifying local adaptation is crucial for determining the source populations for planned transplantation measures of seagrasses in order to compensate for losses.

One reason for using genetic markers to study population differentiation is that they allow faster and bigger-scale surveys than time-consuming experiments involving field transplantation or common gardens. The crucial question then becomes what the differentiation at neutral loci tells us about the ecotypic differentiation of populations? Implicitly, the conservation genetic approach assumes that genetic markers reliably reflect variation or uniformity in selectively relevant genes coding for quantitative traits (Milligan et al. 1994). However, based on theoretical arguments, only a weak correlation can be expected between both diversities (Lynch 1996). It cannot be overemphasized that only selectively relevant quantitative traits, which are mostly under the control of several genetic loci, actually matter for adaptive responses and hence, for the persistence of populations. Three scenarios are possible: there is genetic differentiation at marker loci, but no ecotypic differentiation. This is to be expected in similar environments which are geographically widely separated when the markers are truly neutral and not linked to selectively relevant genes. Second, the differentiation at quantitative traits is not paralleled at genetic marker loci. Examples of this are perennial and annual population pairs in the Wadden Sea. Populations in permanently submerged creeks are perennial; those on the tidal flats are entirely annual and reproduce only sexually. Despite such strong differentiation in life-history traits, genetic differentiation at 6 microsatellite loci was small and not larger than the geographic distance would suggest (F_{ST} and R_{ST} < 0.02, Reusch 2001). Finally, the most convenient case the conservation geneticist would be one where differentiation at marker loci reflects the differentiation of heritable morphological, physiological or life-history traits, such that the marker could be used for rapid large-scale screening of genetic attributes of populations (e.g. Podolsky & Holtsford 1995, Lynch et al. 1999). In order to determine whether genetic marker data are useful to infer the differentiation in plant traits, a joint approach is needed, combining experiments in quantitative genetics, i.e. transplantation experiments (Backman 1991), with the genotyping of the experimental populations using high-resolution genetic markers.

A major tenet in conservation biology is that genetically diverse populations will survive better than genetically depauperate ones (Lande 1988). There are 2 diversity levels, genotypic and genetic sensu strictu which need to be carefully distinguished in clonal plants such as seagrasses. Genetically identical ramets belong to a varying number of genetically distinct genets, which in turn comprise the sexually recombining gene pool of the population at a site. One of the most exciting findings in seagrass population genetics is the discovery of very large (>100 m), and possibly very old, genets dominating entire meadows. The consequences of such low genotypic diversity are elusive at the moment. If successful clones are multi-purpose genotypes (Lynch 1984), chances of persistence even in the face of global change could be high. On the other hand, they may represent the competitively superior genotypes which were the fittest under the precise ecological circumstances at the site (frozen-niche hypothesis, Vrijenhoek 1979). In the latter case, such nearly monoclonal meadows are at high risk of extinction if environmental conditions change.

Regarding the second level of genetic diversity, i.e. the genetic diversity distributed among genets, findings in terrestrial plant populations suggest an ecological role for genetic diversity, in particular heterozygosity. Small, fragmented plant populations displayed lower seed set, and greater temporal fluctuations than larger populations which correlated with high inbreeding coefficients and genetic drift (Ouborg et al. 1991, Ouborg & van Treuren 1994). When it comes to field experiments, we know very little about the short-term consequences of genetic diversity (but see Lehberg 1993, Schmid 1994). It is also not clear how a loss of individual fitness affects the population growth rate. Consequently, the ongoing debate on the importance of genetic versus ecological factors affecting population persistence (e.g. Lande 1988, Schemske et al. 1994, Frankham 1995, Montalvo et al. 1997) is based on few empirical data. There is experimental evidence from model organisms that genetically more diverse populations (measured as average heterozygosity and allele number at marker loci) survive longer and have a better adaptive potential. In experimental *Drosophila* species populations, interesting interactions between inbreeding and stress have been identified. When challenged by heat stress, genetically diverse populations experienced a lower risk of extinction than inbred ones (Bijlsma et al. 2000).

Seagrasses are not *Drosophila*, yet the ecological importance of marine angiosperms merits studies on the role of genetic diversity. In addition, seagrass individuals can easily be cloned by vegetative propagation. This way, genetically identical offspring can be produced, which facilitates a separation of environ-

mentally induced and heritable components of plant traits (e.g. Via 1994). Several seagrass species have already been transplanted experimentally, enabling the composition of stands of varying clonal and genetic diversity. There is evidence from *Zostera marina* that populations transplanted for the purpose of habitat restoration are genetically depauperate compared to pristine, unmanipulated stands at sites in southern California (Williams & Davis 1996) but not in the Chesapeake Bay area (Williams & Orth 1998). Unfortunately, the genetic markers used by Williams and co-workers (allozymes) were not polymorphic enough to differentiate between the levels of genotypic and genetic diversity. In a recent study by Procaccini & Piazzi (in press), 3 *Posidonia oceanica* populations were transplanted to a common garden. The population with the highest genotypic diversity performed best in terms of population growth and survival. More such experiments are needed to judge the role of genetic and genotypic diversity in long-term meadow persistence, and to optimize the protocols for transplantation measures.

The fact that many seagrass meadows are monospecific, or are comprised of few different species, inevitably puts the population biology of the habitat-founding species at the center of interest. Seagrass habitats lack the species diversity and redundancy present in terrestrial grasslands or forests (Tilman & Downing 1994, Tilman et al. 1997). It is tempting to postulate that the lack of species diversity results in a strong intraspecific differentiation, leading to pronounced within-population differentiation of genets at a site. In fact, the clones of seagrass populations may occupy only fractions of ecological spaces in a frozen-niche model, just as different grassland species display different ecological amplitudes. Whether seagrass populations can evolutionarily track the predicted environmental changes of, for example, sea surface temperatures depends on the distributions of reaction norms of the genotypes present in the population, as well as on the rate of immigration of new genotypes. Alternatively, the genotypes at a site may be an assemblage of multi-purpose genotypes. In such cases, populations will survive until the genets are beyond the margins of their phenotypic response. Experimental data on this question are warranted if we wish to compose artificial replacement populations in the case of re-transplantation for habitat restoration purposes. Also, data on the distribution reaction norms of genotypes are required as baselines to evaluate the extinction risk of seagrass populations in the face of global change.

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