


Seagrasses: Biology, Ecology and Conservation

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Anthony W.D. Larkum,
Robert J. Orth
and Carlos M. Duarte



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Seagrass Evolution, Ecology and Conservation: A Genetic Perspective

Michelle Waycott*

School of Tropical Biology, James Cook University, Townsville, Qld 4811, Australia

Gabriele Procaccini

*Stazione Zoologica 'A. Dohrn', Laboratorio di Ecologia del Benthos,
Punta S. Pietro, 80077 Ischia (Napoli), Italy*

Donald H. Les

*Department of Ecology and Evolutionary Biology, The University of Connecticut,
75 North Eagleville Road, U-43, Storrs, CT 06269-3043, USA*

Thorsten B. H. Reusch

Max-Planck-Institut für Limnologie, August-Thienemann-Street 2, 24306 Plön, Germany

I. Introduction

The study and characterization of natural systems involves the evaluation of their diversity and the identification and definition of processes and fluxes operating at different temporal and spatial scales. Ecological studies in general are limited by their ability to infer these different scales of process. The use of genetic analysis to provide insight into scales of process in ecology has increased as the basic tools to undertake such studies have improved and become more widely available. A large number of genetic based approaches are available today but most commonly utilized are DNA markers to assess the relationships among individuals at hierarchical levels ranging from fine scale population processes to the phylogenetic relationships of species, genera, and higher taxa. To date, most studies of seagrass genetic diversity have been aimed toward the comprehension of ecological and evolutionary processes and, as such, this is the main subject of this chapter.

However, we also provide insight into how seagrass genetic diversity can be assessed and utilized for biodiversity conservation, although the use of this approach has been very limited to date (Waycott, 2000a). As genetic diversity data provide insight into such an array of evolutionary and ecological processes it represents valuable information for conservation management strategies (e.g. Ehrlich and Wilson, 1991; Faith, 1994; Moritz and Faith, 1998). The widespread use of genetic data in conservation (e.g. Frankel and Soule, 1981; Frankel et al., 1995; Holsinger et al., 1999; Frankham et al., 2002) has allowed dramatic improvement in our ability to interpret patterns of genetic diversity in this context.

Seagrasses represent difficult ecological subjects in marine ecosystems. The biology of seagrasses is constrained by the environment they inhabit leading to convergence in morphologies. For example the majority of seagrass species possess flexible strap-like leaves (den Hartog, 1970), although they do not represent a single evolutionary lineage (Les et al., 1997). This leaf form allows the plants to thrive in their fluidic, tide- and wave-driven

* Author for correspondence, email: michelle.waycott@jcu.edu.au

environment. The evolutionary trends that have lead to these, and other convergent characteristics, are difficult to interpret without adequate insight into their origins in both an ecological and evolutionary context. Significantly, the suite of seagrass adaptations (see "Foreword") represents dramatic evidence of the adaptive capacity of flowering plants to evolve and survive in extreme environments and as such warrants careful study.

Our understanding of seagrass ecology, physiology and adaptation has been constrained by our ability to develop a clear understanding of many biological features of these plants such as their ability to pollinate in water (Cox, 1993; Ackerman, 1995; Verduin, 1996; Ackerman, Chapter 4). In contrast the details of seagrass photosynthetic mechanisms (as inferred through the application of PAM, e.g. Larkum et al., Chapter 14) during different phases of their daily, weekly or monthly growth are rapidly being unravelled due to the now widespread use of new technologies. However, some of the most basic questions of biology such as evolutionary relatedness, plant (genet) age and growth, selection for adaptive traits, breeding system, and disturbance response strategies, are poorly studied. It is now possible to investigate these questions using a range of readily available DNA based markers (for review see Reusch, 2001b). With these techniques we can now study almost any level in the hierarchy of seagrass relationships and population processes (Les et al., 1997; Waycott, 2000a; Waycott, 2000b; Reusch, 2001b).

The use of genetic markers to study questions of seagrass evolution and ecology began during the 1980–1990s (Les, 1988; McMillan, 1991; Triest, 1991a). These earlier studies largely concluded that seagrasses were remarkable in their genetic uniformity indicating that the application of allozyme data to the study of population processes would not be fruitful. However, later studies (Laushman, 1993; Ruckelshaus, 1995; Waycott, 1995; Williams and Davis, 1996; Waycott et al., 1997), demonstrated that greater sampling efforts could better detect genetic polymorphism thus improving the utility of allozyme markers. The introduction of readily applied DNA markers provided significantly greater sensitivity (Alberte et al., 1994; Procaccini et al., 1996; Reusch et al., 1999c).

In this chapter, we discuss the outcomes of genetic approaches to the study of seagrass biology. Our aim is to provide insight into the outcomes gained from

the use of powerful molecular based methodologies. We do not attempt to explain these methods, as many other reviews are available (e.g. Bachmann, 1994; Jarne and Lagoda, 1996; Mueller and Wolfenbarger, 1999; Ouborg et al., 1999; Bachmann, 2001; Reusch, 2001b; Gibson, 2002; Judd et al., 2002) and we direct the reader to these sources for further details and additional literature. This chapter includes two major sections. The first discusses molecular systematics and insights obtained from its application, as an approach independent of earlier exclusively morphological based methods. This section deals first with the systematic placement of families and insights gained from recent large-scale molecular phylogenetic analyses of the entire monocotyledon subclass (Alismatidae) that contains the seagrasses. We then discuss the current state of molecular based intrageneric studies and the insights they provide into seagrass evolution. The second section is concerned with genetic approaches to the investigation of ecological and population processes including seagrass growth, reproduction and adaptation.

II. The Evolution of Seagrass Diversity

A. Classification and Higher Level Relationships

Early impressions of seagrass relationships reflected superficial morphological comparisons to other plant groups. These crude concepts are illustrated by the 1792 generic name *Phucagrostis* Cavolini (literally 'seaweed grass'), now a synonym of *Cymodocea* K. D. Koenig. Agardh (1821) actually included *Amphibolis antarctica* among the algae (Sauvageau, 1891) and the marine angiosperms remained less studied than most algae throughout the mid 19th Century (Ascherson, 1867). Eventually, their angiospermous affinity was elucidated, but misconceptions regarding their precise relationships persisted. Delpino (1870) regarded *Zostera* as an aquatic aroid (Araceae) and *Posidonia* as an aquatic grass (Poaceae) (Schenck, 1886/2003). However, by the early 20th Century, authors consistently accepted the placement of all seagrasses within the monocotyledonous Helobiae (subclass Alismatidae).

Endlicher (1836–1840) included *Thalassia* among the genera of 'Fluviales' such as *Althenia*, *Cymodocea*, *Halodule*, *Posidonia*, *Ruppia*, and *Zostera*. However, other authors placed *Enhalus*,

Table 1. Comparison of selected marine angiosperm (seagrass) classifications.

Marine genera	Ascherson and Graebner (1907)	Arber (1920)	Sculthorpe (1967)	Den Hartog (1970)	Cronquist (1981)	Tomlinson (1982)	Dahlgren et al. (1985)	Thorne (1992)	Les et al. (1997)
<i>Enhalus</i>	HYD	HYD	HYD	HYD	HYD	HYD	HYD	HYD	HYD
<i>Halophila</i>	HYD	HYD	HYD	HYD	HYD	HYD	HYD	HYD	HYD
<i>Thalassia</i>	HYD	HYD	HYD	HYD	HYD	HYD	HYD	HYD	HYD
<i>Amphibolis</i>	POT	POT	ZAN	POT	CYM	CYM	CYM	CYM	CYM
<i>Cymodocea</i>	POT	POT	ZAN	POT	CYM	CYM	CYM	CYM	CYM
<i>Halodule</i>	POT	POT	ZAN	POT	CYM	CYM	CYM	CYM	CYM
<i>Heterozostera</i>	—	—	ZOS	POT	ZOS	ZOS	ZOS	ZOS	ZOS
<i>Lepilaena</i> ^a	POT	—	ZAN	—	ZAN	ZAN	ZAN	ZAN	ZAN
<i>Phyllospadix</i>	POT	POT	ZOS	POT	ZOS	ZOS	ZOS	ZOS	ZOS
<i>Posidonia</i>	POT	POT	POS	POT	POS	POS	POS	POS	POS
<i>Ruppia</i>	POT	POT	RUP	POT	RUP	POT	POT	POT	RUP
<i>Syringodium</i>	POT	—	ZAN	POT	CYM	CYM	CYM	CYM	CYM
<i>Thalassodendron</i>	POT	—	—	POT	CYM	CYM	CYM	CYM	CYM
<i>Zostera</i>	POT	POT	ZOS	POT	ZOS	ZOS	ZOS	ZOS	ZOS

^aNot identified as marine, but included in Zannichelliaceae by Les et al. (1997).

CYM = Cymodoceaceae; HYD = Hydrocharitaceae; POS = Posidoniaceae; POT = Potamogetonaceae; RUP = Ruppiaceae; ZAN = Zannichelliaceae; ZOS = Zosteraceae. — = not considered by author.

Thalassia and *Halophila* within Hydrocharitaceae (Richard, 1811; Rohrbach, 1871; Balfour, 1879). Bentham and Hooker (1883) and Ascherson and Graebner (1907) maintained the division of seagrass species into separate families (Hydrocharitaceae, Potamogetonaceae). This arrangement was later adopted by Arber (1920), and the distinction of seagrass species in the Hydrocharitaceae has been followed since. Yet, even though seagrasses were divided among separate families, they were believed by some to retain a relatively close relationship. Balfour (1879) considered *Halophila* as the link between the families Potamogetonaceae and Hydrocharitaceae, Arber (1920) and Setchell (1920) regarded these same families as 'nearly related'.

With the exception of den Hartog (1970), who simply adopted Ascherson and Graebner's (1907) two family system, classifications increasingly partitioned seagrass genera among several additional families by the later part of the 20th Century (Table 1). den Hartog (1970) did, however, remark that Ascherson and Graebner's concept of Potamogetonaceae was heterogeneous and needed to be subdivided. Major similarities and differences in the historical family groupings of seagrass genera are shown by the selected classifications in Table 1. It is noteworthy that greater taxonomic consistency underlies these examples, because Ascherson and Graebner's dual family system (followed by Arber and den Hartog) subdivided Potamogetonaceae into tribes corresponding to Cymodoceaceae (Cymodoceaceae), Posidoniaceae (Posidoniaceae), Pota-

mogetonaceae (Potamogetonaceae), Zannichelliaceae (Zannichelliaceae) and Zosteraceae (Zosteraceae), thus yielding the same six taxa recognized by many of the later classifications. These examples illustrate that a relatively stable seagrass classification has existed for more than a century.

However, the nature of scientific evidence in support of the assorted marine angiosperm classifications shown in Table 1 is quite variable. The earliest classifications essentially represented opinions of natural groups based on an author's perception of which taxa were most similar morphologically and anatomically. The incorporation of evolutionary theory in the 20th Century inspired the adoption of classifications that also strived to be meaningful phylogenetically with most modern classifications now being implicitly phylogenetic (Judd et al., 2002).

In early phylogenetically based studies, relationships were ascertained by identifying groups that shared a number of distinctive features, arguably indicating their common ancestry. Bessey (1915) incorporated the concept of character state polarity to help differentiate groups as relatively primitive or advanced. Otherwise, this approach did not differ materially from that used to establish putatively 'natural' classifications other than assuming that similarity resulted from a common evolutionary ancestry. Efforts to better quantify group similarity led to the incorporation of 'phenetic' approaches, which performed cluster analyses of character matrices using computerized algorithms. Such an approach was taken by Clifford (1970) who

demonstrated that Cymodoceaceae, Posidoniaceae, Ruppiaceae, Zannichelliaceae and Zosteraceae were quite similar as a group, but differed considerably from Hydrocharitaceae.

The comprehensive and highly influential work by Cronquist (1981) allegedly was based on an integration of all data available at that time. That work depicted phylogenetic relationships within subclasses and among orders using tree-like diagrams; however, these were not produced using an explicit method. Similar approaches were taken by authors of other synthetic works (e.g. Dahlgren, 1980; Takhtajan, 1980). Comparable methods were used to develop quite detailed classifications of seagrasses (e.g. Tomlinson, 1982).

The incorporation of cladistic methodology led to an important refinement in phylogenetic classification, that is it established an objective methodology for reconstructing phylogenetic hypotheses. Unlike previous approaches, cladistic analyses could provide explicit details regarding the character states that defined particular relationships, which could then be depicted as a hierarchical, tree-like diagram.

The first detailed cladistic analysis of seagrass relationships was conducted by Dahlgren et al. (Dahlgren and Rasmussen, 1983; Dahlgren, 1985) as a part of their study of monocotyledons. Within the subclass Alismatidae (which contains all seagrasses), they compared the distribution of states for 56 morphological characters and provided the first explicit evolutionary framework on which to base a phylogenetic seagrass classification (Fig. 1). Their results depicted seagrasses within three distinct clades: (1) Cymodoceaceae/Zannichelliaceae, (2) Posidoniaceae/Zosteraceae and (3) Hydrocharitaceae (Ruppiaceae were merged with Potamogetonaceae). Within Hydrocharitaceae, *Enhalus* was allied with *Vallisneria* (subfamily Vallisnerioideae), whereas *Halophila* and *Thalassia* were each placed in separate subfamilies (Halophiloidae, Thalassioideae). Dahlgren (1985) also argued against the inclusion of Cymodoceaceae (incl. *Amphibolis*, *Cymodocea*, *Halodule*, *Syringodium*, *Thalassodendron*) within Zannichelliaceae (*Athenia*, *Lepilaena*, *Zannichellia*). Cox and Humphries (1993) conducted a morphological cladistic analysis of some seagrasses, which indicated the common origin of Posidoniaceae, Cymodoceaceae, and Zosteraceae. However, their analysis excluded many non-seagrass families necessary to evaluate this question adequately, thereby yielding unfounded re-

sults (see Les et al., 1997). Les and Haynes (1995) reanalysed the Alismatidae data of Dahlgren (1985) using a computer program capable of recovering additional maximum parsimony reconstructions and also performed new analyses using corrected data. Although the results were similar to Dahlgren's original study, a number of discrepancies indicated that morphological data alone could not be relied on to provide compelling support for relationships in this group.

Incorporation of molecular data in cladistic analyses provided the next major refinement in phylogenetic reconstructions of seagrass relationships. Les et al. (1993) conducted preliminary cladistic analyses of Alismatidae using *rbcL* gene sequence data from 8 families, but included only a few seagrass genera, which did not provide much insight into their relationships. In an *rbcL* analysis expanded to include 25 genera from all 15 alismatid families, Les and Haynes (1995) began to disclose consistent seagrass clades representing (1) Hydrocharitaceae, (2) Posidoniaceae/Ruppiaceae/Cymodoceaceae, (3) Zosteraceae, and (4) Zannichelliaceae (*Lepilaena*). Seagrass relationships were evaluated further in more comprehensive *rbcL* analyses of 55–69 alismatid species (eventually including 23 seagrass species from all 14 marine genera) which specifically addressed the question of the number of seagrass origins (Waycott and Les, 1996; Les et al., 1997) (Fig. 1).

The *rbcL* survey by Les et al. (1997) indicated that marine angiosperms have evolved in at least three separate lineages. Another independent lineage (Zannichelliaceae), which contains *Lepilaena marina*, represents a fourth marine angiosperm origin given that this species (with several others in the genus) is known to occupy marine habitats (Womersley, 1984). To date, the study by Les et al. (1997) remains the most complete molecular phylogenetic analysis of seagrass interrelationships at the genus and family level. Procaccini et al. (1999b) used *trnL* intron sequence data to study relationships among six marine genera in five families and recovered distinct clades containing (1) *Cymodocea*/*Posidonia*/*Ruppia*, (2) *Zostera*/*Phyllospadix* and (3) *Halophila*, thus in agreement with the *rbcL* analysis by Les et al. (1997).

Molecular phylogenetic analyses have begun to clarify some finer details of seagrass relationships. First, the emerging pattern of phylogenetic relationships indicates that seagrasses should be divided

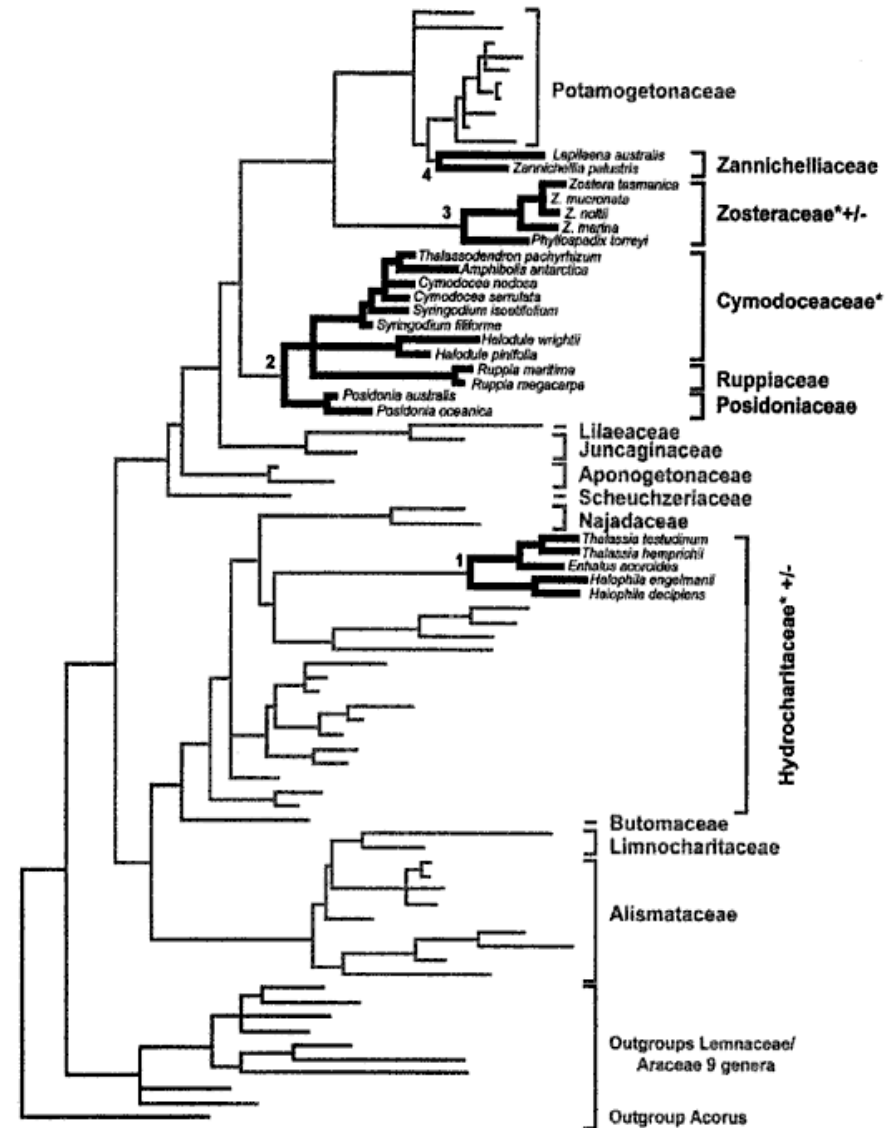


Fig. 1. Alismatid phylogeny based on *rbcL* cpDNA sequence data (adapted from Les et al., 1997). Family designations are shown defined in Les et al. (1997) and seagrass species are listed, for full details see Les et al. (1997). Seagrass taxa exhibiting a dioecious breeding system are indicated (*).

among six separate families (Table 1, last column) in preference over classification systems that recognize fewer families. Moreover, it is now evident that Ruppiales are closely allied with Posidoniaceae and Cymodoceaceae rather than representing a subdivision of the freshwater family Potamogetonaceae. Molecular analyses also confirm the distinctness of the Zosteraceae and remote relationship of marine Hydrocharitaceae to all other seagrasses. Finally, molecular studies have provided compelling evidence that marine Hydrocharitaceae occur within a single, monophyletic clade, thereby invalidating classifications of the family that divide the three marine genera otherwise among the freshwater representatives.

At this time, higher-level seagrass relationships appear to be clarified quite adequately, at least well enough to provide a meaningful, defensible classification. Additional sequence data could corroborate the integrity of seagrass clades further, but would unlikely result in any major alterations of phylogenetic relationships already disclosed at higher levels. Instead, more emphasis should be made to continue refining phylogenetic relationships at lower, i.e. interspecific levels within seagrass genera (see below).

B. Interspecific Relationships

Cladistic analyses of molecular and non-molecular data have been applied to questions of seagrass species relationships, but this approach only has been taken relatively recently and many species remain unstudied. Overall, seagrasses are not diverse with most genera being quite species poor.

Enhalus (Hydrocharitaceae) is regarded as monotypic, comprising the sole species *E. acoroides*. Araño et al. (2003) reported a complete lack of detectable RFLP variation among *E. acoroides* populations from different geographical regions, which would support the existence of a single *Enhalus* species. However, a recent comparison of ITS sequences from Chinese and Australian material of *Enhalus* show that they are quite distinct (Les and Moody, unpublished). Further investigation of *Enhalus* is warranted and may disclose the existence of additional species.

Four marine genera (*Amphibolis*, *Syringodium*, *Thalassia*, *Thalassodendron*) each contain only two species, thus showing unequivocal intrageneric phylogenetic relationships if the genera are truly monophyletic. Les et al. (1997) included both species

of *Syringodium* and *Thalassia*, which confirmed the monophyly of *Thalassia*, but not *Syringodium* (paraphyletic with *Cymodocea*). The monophyly of *Thalassia* and its subdivision into two species (Old and New Worlds) has been demonstrated further by molecular sequence data from nuclear (18S, ITS) and other cpDNA (*trnL*) loci. The monophyly of *Amphibolis* and *Thalassodendron* (sister genera in *rbcl* analyses) could be tested similarly by obtaining *rbcl* sequences for *Amphibolis griffithii* and *Thalassodendron ciliatum* to include within the data in Les et al. (1997). Only two of the four *Cymodocea* species were included in the *rbcl* analysis and it would be desirable to obtain data for the unsampled species (*C. rotundata*, *C. angustata*) as well. Further details of relationships in the *Amphibolis/Cymodocea/Syringodium/Thalassodendron* clade should be investigated by sampling all 10 species using *rbcl* and additional molecular loci as well as incorporating a complete morphological data set. Precise delimitation of generic boundaries in this group of seagrasses remains unsettled.

Halodule (three species, plus) has been well investigated using nuclear (ITS) and cpDNA (*rbcl*, *trnL*) markers (Les et al., 1997; Waycott, unpublished), yet relationships remain complicated. All markers (ITS, *rbcl*, *trnL*) examined thus far show the genus to be monophyletic. Within the genus, nuclear (ITS) sequence data analysis yielded one monophyletic clade comprising the New World *H. wrightii* and another containing the Old World *H. pinifolia* and *H. uninervis* (Fig. 2A). Populations of the latter two species overlap and are not clearly differentiated by ITS data. However, cpDNA (*trnL*) data show considerable overlap among populations of all three species, thus complicating the interpretation of the molecular analyses (Fig. 2B). *Halodule* would benefit from a genus wide intensive study of population level relationships.

Posidonia (five species) has been studied cladistically using morphological data and molecular sequence data from nuclear (ITS) and cpDNA (*rbcl*; *trnL*) loci. Phylogenetic analyses of these different data sets are consistent and indicate a similar overall pattern of relationships in the genus. Combination of these congruent data sets (Waycott and Les, unpublished data) suggests that the Mediterranean *P. oceanica* (the outlier geographically) is basal, with the Australian taxa derived (Fig. 3). *Posidonia sinuosa*, *P. australis* and *P. angustifolia* are relatively

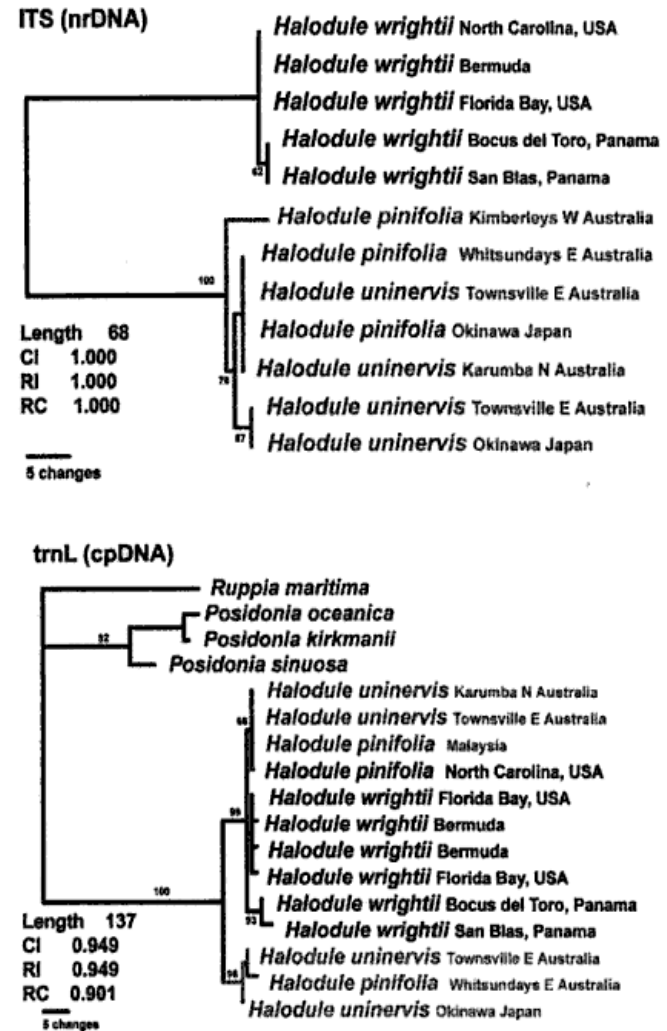


Fig. 2. (a) Above ITS and (b) below *trnL* parsimony phylogram of the genus *Halodule* (bootstrap support shown adjacent to nodes), localities of collections shown next to species name (Waycott and Barnes unpublished).

distinct (the latter two representing closely related sister species); however, the five remaining Australian taxa (*P. coriacea*, *P. ostenfeldii*, *P. robertsonii*, *P. denhartogii*, *P. kirkmanii*) appear to represent only minor variants of a single

species and are indistinct both morphologically and genetically.

A phylogenetic study of *Halophila* has been conducted by Waycott et al. (2002) who analysed ITS data from 36 accessions of 11 recognized species.

ITS (nrDNA) and trnL (cpDNA) combined

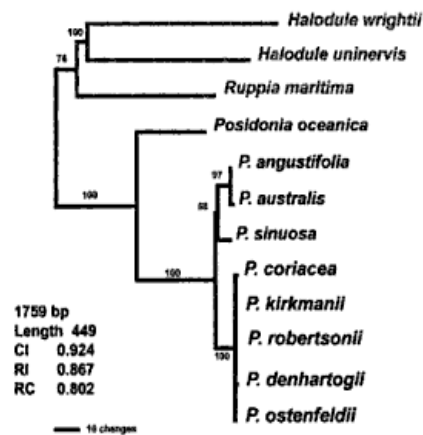


Fig. 3. Combined data of ITS and *trnL* parsimony phylogram of *Posidonia* (bootstrap support shown adjacent to nodes), localities of collections shown next to species name (Waycott and Les unpublished).

Their results dispute the taxonomic limits of *H. ovalis*, wherein some populations are closely related to *H. australis*. The rare *H. johnsonii* and *H. hawaiiiana* were not separable from *H. ovalis* by ITS data and perhaps represent only minor variants of a single species. However, most other species appear to be distinct and are associated with a pattern of vegetative reduction proceeding phylogenetically from complex leaf arrangements to reduced, simplified phyllotaxy (Fig. 4). Two *Halophila* species (*H. capricorni*, *H. baillonis*) remain unsurveyed for ITS. It has also been observed that rDNA can accumulate pseudogenes, making the verification of sequences important before use in analyses of relationships (Ruggiero and Procaccini, 2004). Additional loci (especially cpDNA) should be surveyed to seek further support for relationships disclosed by the initial ITS data analysis.

Systematic relationships within *Phyllospadix* (five species) have not been investigated in any detail. Isozyme patterns have been compared for three species, with higher similarity reported between *P. scouleri* and *P. torreyi* than between either species or *P. serrulatus* (Triest, 1991b). *Phyllospadix* would benefit from a thorough evaluation of taxonomic limits and relationships using both molecular and morphological data sets.

Relationships within *Zostera* (nine species including *Heterozostera*) have now been studied in some detail. Chromosome morphology is distinct between subgenera with those of subgenus *Zostera* smaller than those of subgenus *Zosterella* (Uchiyama, 1996). Similarly, isozymes show different patterns between *Z. marina* (subg. *Zostera*) and species of subgenus *Zosterella*; however, only slight isozymic differences were observed among *Z. capensis*, *Z. capricorni*, *Z. muelleri*, and *Z. novaezelandica* (subgenus *Zosterella*) (Triest, 1991b). Uchiyama (1996) conducted a molecular analysis of three *Zostera* species using 18s rDNA RFLP data. His results also showed differences between species of different subgenera, but sampling was insufficient to address relationships in detail. Les et al. (1997) surveyed three *Zostera* species (representing both subgenera) in their *rbcL* analysis of Alismatidae. Again, species from the different subgenera were considerably divergent. Les et al. (1997) also provided evidence that the taxon formerly recognized as a separate genus (*Heterozostera tasmanica*) falls within the genus *Zostera* phylogenetically and should be included within *Zostera*. (Les et al., 2002) performed a morphological phylogenetic analysis of all *Zostera* species (including *Heterozostera*) and also evaluated systematic relationships among eight taxa using a combined data set consisting of DNA sequences from ITS, *rbcL*, and the *trnK* intron. Results of this study reinforced earlier work that indicated significant morphological and molecular divergence between the two subgenera of *Zostera* (Fig. 5). However, there was no phylogenetically defensible structure to accessions sampled for *Z. capensis*, *Z. capricorni*, *Z. muelleri*, and *Z. novaezelandica*, leading to the recognition of only one variable species (*Z. muelleri*) in that group. This study verified that *Heterozostera* should be merged with *Zostera*. Recently, Les and Moody (unpublished) obtained ITS sequences from an extremely broad-leaved accession of *Zostera* from California (USA), which has been referred to as *Zostera latifolia* by some authors. Setchell (1927) regarded *Z. latifolia* to be an ecological variant of *Z. marina*. Les and Moody detected only a single substitution in the entire ITS region between the broad-leaved form from California and narrow-leaved material of *Zostera marina* from the east coast (Connecticut, USA), evidence that supports Setchell's merger of these taxa, despite conspicuous morphological difference in leaf size. These results are supported by the work of Tanaka

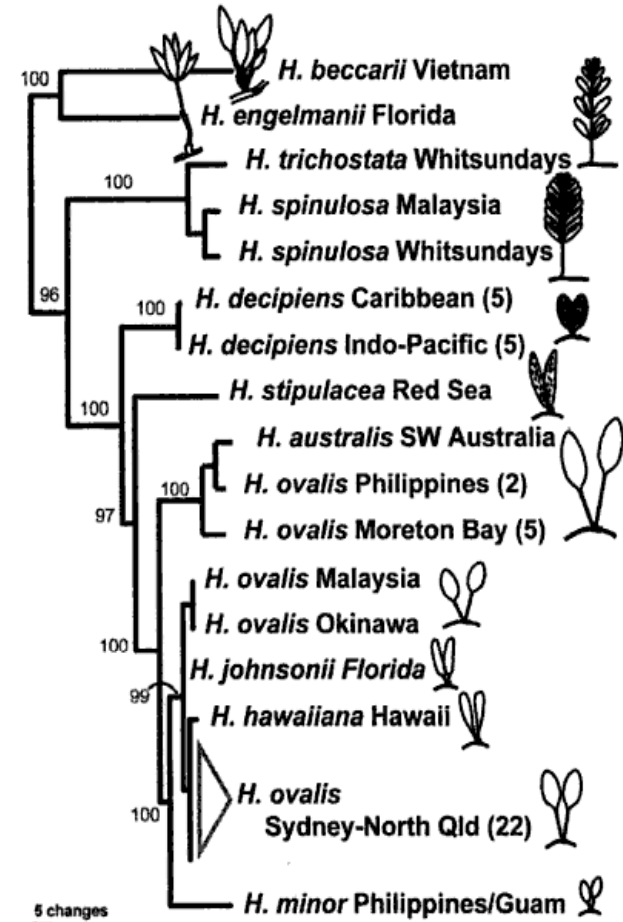


Fig. 4. ITS parsimony phylogram of *Halophila* (bootstrap support shown adjacent to nodes), localities of collections shown next to species name (adapted from Waycott et al., 2003).

et al. (2003) who analysed the chloroplast gene region *matK*. Their results suggest that *Z. noltii* and *Z. japonica* are sister to the combined taxon *Z. muelleri* (= *Z. capricorni* of Les et al., 2002). Further exploration of the intrageneric relationships would be useful to provide insight into suggestions of creating additional genera within the family by Tomlinson and Posluszny (2001).

The taxonomy and systematics of *Ruppia* is in serious need of study. Until recently, *Ruppia* had

been placed within the family Potamogetonaceae, usually as a separate subfamily. Les et al. (1993) showed that *rbcL* data placed *Ruppia* closer to Cymodoceaceae (*Syringodium*). The alliance of Cymodoceaceae, Ruppiaceae, and Posidoniaceae was verified in later, more comprehensive studies (Waycott and Les, 1996; Les et al., 1997). The actual number of *Ruppia* species is not known with any certainty. Cook (1996) reported 2–10 species worldwide. The study by Les et al. (1997) included only

Combined ITS, trnK analysis

CI=0.97

RI=0.97

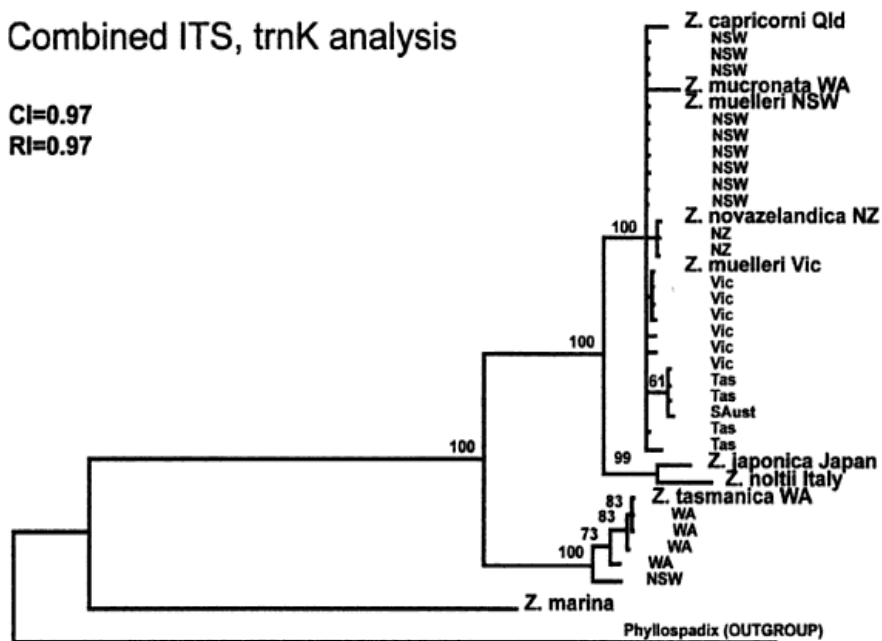


Fig. 5. Combined data of ITS and *trnK* parsimony phylogram of *Zostera* (bootstrap support shown adjacent to nodes), localities of collections shown next to species name (adapted from Les et al., 2002).

two species (*R. maritima* from North America, *R. megacarpa* from Australia) whose *rbcl* sequences were fairly distinct. Guha and Mondal (1999) studied pollen morphology in *Ruppia* and concluded a worldwide revision of *Ruppia* which includes a full appraisal of morphological characters as well as molecular phylogenetic analyses will be necessary before further systematic details such as species boundaries and relationships can be ascertained in this genus.

Lepilaena (five species; Zannichelliaceae) also has not been studied systematically in any great detail. Molecular (*rbcl*) data clearly showed *Lepilaena australis* as related to *Zannichellia*, but other species await study. Two species (*Lepilaena marina*; *L. cylindrocarpa*) are truly marine (Womersley, 1984) and it would be informative to determine whether the marine habit is basal or derived in this genus of otherwise freshwater species. Although quite likely, the monophyly of *Lepilaena* has not yet been verified by phylogenetic analysis.

These studies have all verified the utility of detailed analysis of the intraspecific variation across a wide geographic range for seagrass species using DNA sequence data and careful phylogenetic analysis. Broader scale studies may require considerable effort in obtaining samples from extremes of the range of species to better describe the finer scale evolutionary trends within genera and species. These types of study begin to impinge upon the traditional population genetic approach to understanding relatedness of seagrass populations as will be discussed in the following sections. The intersection of these two fields of study represents the investigation of species phylogeography (e.g. for review see Avise, 2000). This field of research has barely been touched in seagrasses and while at present markers that adequately detect the historical biogeographic processes are unavailable (Schaal et al., 1998), future research will prove invaluable to the study of broad scale evolution of seagrass species. The field of molecular systematics is undergoing continuous and rapid

development and this will provide fertile ground for future research activities.

III. Ecological and Reproductive Processes

A. Species-Wide Population Genetic Studies

1. Introduction

Seagrasses are distributed in coastal areas of all continents and belong to nine species assemblages (floras), six of which are exclusively temperate (Duarte, 2001). Distributional ranges of single species can differ radically; ranging from species whose distribution is limited to a single flora (e.g. *Posidonia oceanica* in the Mediterranean) to widely distributed species such as *Zostera marina*. The present distribution of seagrass species is the result of processes occurring at a number of different temporal scales and may reflect events that occurred a very long time ago (tens of thousands of years or more). However, the actual distribution of populations is also the result of ongoing extant dynamic processes of colonization/extinction. When population distributions are viewed using a genetic perspective, higher genetic differentiation among populations reflects lower genetic exchange (gene flow) and indicates longer-term isolation. The application of molecular markers to study levels of population connectivity potentially can provide significant insights into the factors influencing present-day distribution of seagrass species, particularly when applied across broad spatial scales.

Almost half of the roughly 65 papers published on seagrass population genetic studies attempt to quantify gene flow among distinct populations (Table 2). To date studies addressing the broad scale distribution genetic diversity has only been documented for a few species (Table 2). However, there are many studies in progress on different species with new and potentially more polymorphic molecular markers. When viewed across all the broader scale studies, levels of population connectivity are not always related to differences in dispersal mode or reproductive characteristics among species, and do not appear to be linked to their phylogenetic relatedness.

Only few species have been studied in detail: within the genus *Posidonia*, only the Australian endemic *P. australis* and the Mediterranean endemic

P. oceanica; within the genus *Zostera*, only *Zostera marina* from Europe and North America and in the genus *Thalassia*, only the Caribbean species, *Thalassia testudinum* (these species are the subject of individual chapters in this volume, see Chapters 16, 17 and 18).

2. *Posidonia australis*

A broad scale study of 20 *P. australis* populations has been performed with RAPD and allozyme markers (Waycott et al., 1997; Waycott, 1998). Populations have been sampled along their geographical range, from the north-western populations of Shark Bay, in warm-subtropical waters, to the eastern populations of Lake Macquarie. RAPDs detected higher genotypic variability compared to allozymes, although in a few cases the authors observed the opposite result. In general, populations showed high variability, with average RAPD Dg values of 0.91. Nevertheless, the analyses showed the existence of low population connectivity ($G_{st} = 0.623$) at the scale surveyed and identified regions with significant differences in genetic diversity. Meadows sampled in the south-western region show highest allozyme diversity, whereas lower diversity was recorded at the extremes of the range. Interestingly, regions with higher genetic diversity corresponded to regions of highest species diversity within the genus (Kuo and Cambridge, 1984; Waycott et al., 1997). Explanations for this pattern of species diversity may reflect the selective pressures experienced by the Australian species of *Posidonia*, which reached their current distribution from refugia occupied following Gondwanan break up millions of years ago. Selective pressures may have facilitated speciation and high genetic variability within some of the species. A South-North oriented secondary expansion from few genotypes would explain the low allozyme diversity of the north-western populations. Patterns of phylogeographic relationships show a clear distinction between these localities and the southern and eastern populations. Ongoing local reproduction and recruitment could explain the existence of molecular based genetic diversity in such localities (Waycott et al., 1997; Waycott, 2000a). This study demonstrates the utility of sampling over a very wide range to infer broader processes such as the observation of low diversity in some populations and high diversity in others (Waycott, 1998).

Table 2. Seagrass population genetic studies. The aim of each study has been classified as either: W = within population genetic diversity, B = between population genetic diversity, MS = mating system analysis, T = marker development, R = restoration studies.

Study species	Methodology	Study aim	References
<i>Amphibolis antarctica</i>	Allozymes, DNA fing.	W, B	1
<i>A. griffithii</i>	Allozymes, DNA fing.	W, B	1
<i>Cymodocea nodosa</i>	RAPD, SSR	W, T	2–5
<i>Halodule wrightii</i>	RAPD	W, B	6
<i>Halophila decipiens</i>	RAPD	W, B	7
<i>H. engelmannii</i>	RAPD	W, B	7
<i>H. hawaiiiana</i>	SSR	B	8
<i>H. johnsonii</i>	RAPD	W, B	7,9
<i>H. stipulacea</i>	RAPD, DNA seq.	W, B	10–11
<i>Posidonia australis</i>	Allozymes, RAPD	W, B, MS	12–15
<i>P. coriacea</i>	Allozymes	W	16
<i>P. oceanica</i>	Allozymes, RAPD, SSR, DNA fing.	W, B, T, R, MS	17–33
<i>Thalassia testudinum</i>	RAPD	W, B	34–37
<i>Thalassodendron ciliatum</i>	RAPD	W, B	38
<i>Zostera marina</i>	Allozymes, DNA fing., SSR, DNA seq.	W, B, T, R, MS	39–64
<i>Z. noltii</i>	SSR	W, B, T	65–66

DNA fing. = multilocus DNA fingerprinting; RAPD = Randomly Amplified Polymorphic DNA; SSR = Simple Sequence Repeats (microsatellites); DNA seq. = DNA sequencing.

1. Waycott et al. (1996); 2. Alberto et al. (2001); 3. Alberto et al. (2003b); 4. Procaccini and Mazzella (1996); 5. Ruggiero et al. (in press); 6. Angel (2002); 7. Jewett-Smith et al. (1997); 8. McDermid et al. (2003); 9. Freshwater et al. (2003); 10. Procaccini et al. (1999a); 11. Ruggiero and Procaccini (2004); 12. Waycott (1995); 13. Waycott et al. (1997); 14. Waycott and Sampson (1997); 15. Waycott (1998); 16. Campey et al. (1999); 17. Alberto et al. (2003a); 18. Capiomont et al. (1996); 19. Dalmazio et al. (2002); 20. Franconi and Baraccia (1995); 21. Jover et al. (2003); 22. Orsini et al. (2001); 23. Procaccini et al. (1996); 24. Procaccini and Mazzella (1996); 25. Procaccini and Mazzella (1998); 26. Procaccini and Waycott (1998); 27. Procaccini et al. (2000); 28. Procaccini et al. (2001); 29. Procaccini and Piazzi (2001); 30. Procaccini et al. (2002); 31. Raniello and Procaccini (2002); 32. Ruggiero et al. (2002); 33. Sandmeier et al. (1999); 34. Kirsten et al. (1998); 35. Schlueter and Guttman (1998); 36. Waycott and Barnes (2001); 37. Davis et al. (1999); 38. Bandeira and Nilsson (2001); 39. Alberte et al. (1994); 40. Billingham et al. (2003); 41. de Heij and Nienhuis (1992); 42. Fain et al. (1992); 43. Gagnon et al. (1980); 44. Hämmerli and Reusch (2002); 45. Hämmerli and Reusch (2003a); 46. Hämmerli and Reusch (2003b); 47. Hämmerli and Reusch (2003c); 48. Laushman (1993); 49. Olsen et al. (2004); 50. Reusch et al. (1999a); 51. Reusch et al. (1999b); 52. Reusch et al. (1999c); 53. Reusch et al. (1999d); 54. Reusch (2000); 55. Reusch et al. (2000); 56. Reusch (2001a); 57. Reusch (2002); 58. Reusch (2003); 59. Ruckelshaus (1995); 60. Ruckelshaus (1996); 61. Ruckelshaus (1998); 62. Williams and Davis (1996); 63. Williams and Orth (1998); 64. Williams (2001); 65. Coyer et al. (2004); 66. Coyer et al. (submitted).

For species level allozyme studies see also: McMillan (1981); (McMillan (1982) and McMillan (1991).

3. *Posidonia oceanica*

Contrasting levels of genetic diversity have also been observed within the Mediterranean basin for the congeneric species *P. oceanica*. Studies have been conducted on 33 meadows sampled along the entire distribution range of the species using microsatellite analysis. Overall genetic diversity seems to be low [average $D_g = 0.727$; (Procaccini et al., 2001; Procaccini et al., 2002)], with some identical genotypes present in different populations. Based on the results from published studies, *P. oceanica* appears as a genetically depauperate species as result of evolutionary processes or recent anthropogenic influence.

Populations from the western basin were genetically more polymorphic than those from the east-

ern Mediterranean Sea, where North Adriatic meadows were represented by only one clone (Ruggiero et al., 2002). Meadows are distinct genetically, with overall $\theta = 0.451$ ($N_m \theta = 0.304$). A clear genetic sub-division seems to exist within the basin, with the presence of three main groups representing north-western, south-western, and eastern populations (Fig. 6). The existence of defined population groups may result from processes acting on different time scales. Present surface circulation patterns of surface currents may affect gene flow and/or colonization of different areas and could account for the existence of genetic structure over the Mediterranean Sea as a whole. The genetic structure existing in the Tyrrhenian Sea, with the presence of a clear distinction between northern and southern populations (Ruggiero et al., 2002) (Fig. 6), reflects the



Fig. 6. Distribution of the genetic variability of *Posidonia oceanica* in the Mediterranean basin. Analysis is based on data from six microsatellite loci. Tree is based on $(\delta\mu)^2$ distance values (modified from Procaccini et al., 2002).

presence of seasonal circulation gyres in this area (Astraldi and Gasparini, 1994). The Mediterranean Sea has experienced dramatic changes of environmental parameters related to glaciations and hypersalinity crises (Maldonado, 1985; Thiede, 1978). The evolution of the basin was controlled by the interplay between geological processes and environmental parameters determining species loss and recolonisation in particular areas. Dramatic fluctuations of more than 100 m of sea water level during last glaciation surely have caused the disappearance of *Posidonia* from some areas. Thus, the actual distribution could have originated by recolonisation from populations persisting in relict zones and the low genetic variability detected in some areas could have originated from a founder effect. The latter possibility seems to be the case for the uniclinal patches of the North-Adriatic, where locally adapted genotypes are now present in the area (Ruggiero et al., 2002).

New polymorphic di-nucleotide microsatellite loci recently selected (Alberto et al., 2003a) are showing higher polymorphism levels. Preliminary analysis at basin level is confirming at broad scale the separation between eastern and western Mediterranean populations. Intra-population analyses instead are showing higher heterozygosity and clonal diversity values (Alberto et al., 2003a; Arnaud-Haond personal communication).

4. *Zostera marina*

This species has had the greatest number of publications in the genetic analysis literature for seagrasses (Moore et al., Chapter 16). Nonetheless, only re-

cently a first study has been produced that documents the genetic diversity and genetic structure of the species throughout almost its entire geographical range (Olsen et al., 2004). This shortcoming may be due in part to the particularly widespread distribution of this species which occurs across the majority of available habitats in the northern hemisphere. Studies along the Pacific coasts of North America date back to 1992–1994 and have been carried out using RFLP (Fain et al., 1992), allozymes (Laushman, 1993) and DNA fingerprinting analyses (Alberte et al., 1994). Of these studies, DNA fingerprinting showed higher polymorphism within meadows, as expected for this marker type. More recently, other studies have utilized allozymes to compare distinct *Z. marina* meadows in California and Chesapeake Bay, showing F_{st} values ranging from 0.06 to 0.335, depending on the geographic distance among meadows (Williams and Davis, 1996; Williams and Orth, 1998). In the last few years, genetic diversity in *Z. marina* has been studied extensively along the northern coasts of Europe using polymorphic microsatellite loci (Reusch, 1999c among the others). The use of the same markers shows high allelic richness in the Pacific populations in respect to the Atlantic ones and clear genetic distinction between southern and northern east-Pacific populations (Olsen et al., 2004). Unfortunately, the values of polymorphism observed using microsatellites could not be compared directly with results obtained from markers utilized previously.

The aforementioned study on the distribution of microsatellite genetic diversity along the whole geographic range of the species (Olsen et al., 2004)

provides a much comprehensive, comparable picture of the overall phylogeographic pattern of this species. Among the previous broader scale studies on *Zostera marina* to date, pronounced genetic sub-structuring was observed among eight European populations, with a strong linear relationship of genetic differentiation along geographic distances of 12–4,500 km (Reusch et al., 2000). However, on a larger geographic scale the observed pattern was counterintuitive, with two North American populations clustering with those from the Baltic Sea and North Sea. New data support recent genetic exchange in *Z. marina*, between the east Pacific, west and east Atlantic, suggesting a still-active trans-Arctic connections (Olsen et al., 2004). Instead, a weak correlation of genetic and geographic distance was found in populations sampled in the northern Wadden Sea and south-western Baltic Sea, indicating enhanced metapopulation dynamics in the area (Reusch, 2002). Recolonisation in the two areas may have occurred recently, masking any signal resulting from recent gene flow among populations. In general, populations showed high connectivity ($\theta = 0.018$), despite the fact that populations are annual in the Wadden Sea and perennial in the Baltic. Theoretically annual Wadden Sea populations may show higher connectivity due to greater propagule production and stronger tidal currents. The vectors of such high gene flow are still unclear. Assignment tests conducted on rafting reproductive shoots shows that they can have an important role in dispersing genotypes up to 50 km (Reusch, 2002).

5. *Thalassia testudinum*

This species occurs throughout the Caribbean and Gulf of Mexico (Tussenbroek et al., Chapter 18), where periodic die-off of above ground biomass has been observed (Robblee et al., 1991). Studies on genetic diversity among different localities have been conducted using allozymes (Schlueter and Guttman, 1998), RAPD (Kirsten et al., 1998) and AFLP markers (Waycott and Barnes, 2001); microsatellite markers are currently under development (Waycott, unpublished; Kor-Jent unpublished). As for the other species discussed above, a comparison among the results obtained is difficult to make because of differences in the level of genotypic polymorphism detected by the different markers utilized. Eighteen meadows have been sampled in the lower Florida Keys and allozyme analyses show, as expected, low

genetic variation within and between sites ($G_{st} = 0.050$). Although these low values of genetic variability could be due to low inherent polymorphism of the markers, the general trend indicated seems to be real. RAPD and AFLP markers, in fact, also confirm high population connectivity with higher, but still low, within-site genetic variation. In both RAPD and AFLP analyses the partitioning of genetic diversity was distributed similarly, with a higher percent of variation (81.2–96.6%) within meadows. No AFLP differentiation was found between the two regions analysed (overall mean $F_{st} = 0.03$). In general, *T. testudinum* appears to be a species with weak genetic structure and high homogeneity within its distributional range. The nature of that uniformity is still uncertain, although possibly the result of a recent colonization of the region, following changes in habitat occurring since the Eocene (Waycott and Barnes, unpublished data). Studies on the effects of heavy human impact in the area, the importance of *Thalassia* for the coastal dynamics and more detailed genetic analyses using co-dominant molecular markers (microsatellites) are underway.

6. Other Studies

A modicum of additional information exists on the distribution of genetic diversity in other seagrass species. The introduced species *Halophila stipulacea* has high RAPD diversity in meadows sampled along the Sicilian coasts and high population connectivity between distinct localities (Procaccini et al., 1999a). Absence of clear differentiation in the ITS regions between Mediterranean and Red Sea populations supports the hypothesis of a recent Lessepsian introduction in the Mediterranean (Ruggiero and Procaccini, 2004). The congeneric *H. johnsonii* shows very high clonality and genetic homogeneity in south-eastern Florida, with the same RAPD phenotype present in more than 50% of the samples (Freshwater et al., 2003). In a recent analysis of morphological and genetic variation of *Halophila hawaiiiana* (McDermid et al., 2003), no genetic structure was revealed, although the authors utilized a chloroplast DNA homopolymer that may not yield more than species level variability (Waycott and Freshwater, unpublished). *Cymodocea nodosa* seems to occur only as one or few genotypes in Ria Formosa (southern Portugal) (Alberto et al., 2001); whereas, higher genetic variability was found in meadows in the central/southern

Tyrrhenian Sea (Mediterranean Sea) and in the Cadiz Bay, Spain (Atlantic Sea) by means of microsatellite analyses (Alberto et al., 2003b; Ruggiero et al., in press; Ruggiero and Procaccini, unpublished). *Zostera noltii* seems to possess a moderate degree of variability, but very low population connectivity in populations sampled in central/southern Tyrrhenian Sea (Procaccini and Ruggiero, unpublished). Ongoing studies on the complete distribution range of the species are showing significant clustering among distinct geographical regions and high degree of population differentiation at smaller regional scale (Coyer et al., submitted). More data on both *Cymodocea nodosa* and *Zostera noltii* will soon be available using the same markers from several distinct localities inside and outside Mediterranean (Coyer et al., in press). *Halodule wrightii* appears to be distributed in genetically distinct meadows, with high variability, along Texas and Florida coasts (Angel, 2002). *A. antarctica* seems to be represented by a single clone along the whole southern Australian coast (Waycott et al., 1996).

To date, studies generally confirm that patterns of genetic diversity in seagrass species are determined by a series of interconnected factors that are not always easy to distinguish. There is no readily identifiable trend common among species belonging to the same genus, or those theoretically having similar or identical theoretical dispersal capabilities. To more completely understand factors causing genetic structure over wide geographical ranges requires the knowledge of local environmental factors, current regimes, human impact and historical colonization events of the area. Difficulties in understanding and predicting population structure often arise from an inadequate knowledge of the factors mentioned above.

B. Population Structure and Reproductive Strategies

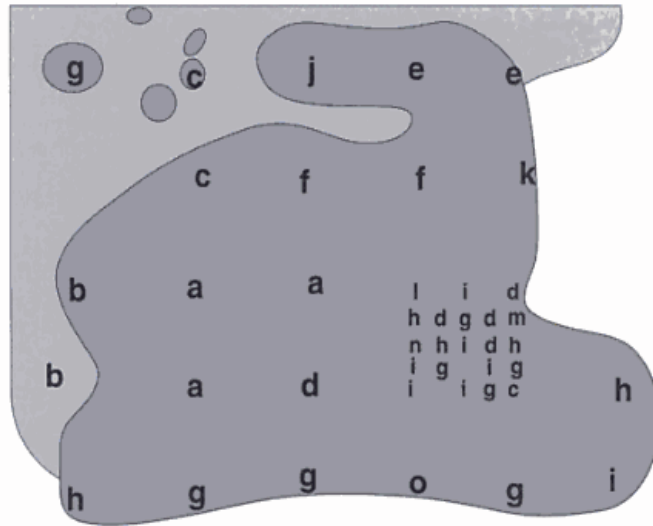
As many of the other chapters in this book will describe, vegetative growth through rhizome extension is a dominant feature of seagrasses. This growth habit allows great flexibility for a single genetic individual to survive in both space and time through clonality resulting from vegetative fragmentation. Most seagrass populations exhibit a mixture of clonal growth along with sexual reproduction and understanding the structure of these populations has

proved challenging using standard ecological approaches. The clonal growth form of many seagrass species results in a hierarchy of different organisational levels. The most elementary level of organisation is the ramet (sensu Harper, 1977), the potentially independent individual. In seagrasses, this individual typically is the leaf bundle, a piece of rhizome, and a root bundle (Tomlinson, 1974). Depending on the longevity of rhizome connections between ramets, several ramets can form physiologically integrated clusters, the second level of organization. The size of these clusters varies greatly between species, and may comprise several hundreds of leaf shoots in the genus *Posidonia*. The sexual individual, or genet, are all ramets or ramet clusters which originated from the same zygote. From an evolutionary point of view, it is only the genet that matters and eventually transmits genes to the next generation. Since genets may be exceedingly large, the determination of genet fitness, reproductive output, and longevity poses formidable practical problems. Most ecological studies have ignored the potential genetic identity of leaf shoots or ramets when selecting experimental plants, or plots. Whether or not this has resulted in biased ecological conclusions remains unclear because quantification of heritable differences in ecological performance of clones is lacking in almost all species (but see Hämmerli and Reusch, 2002).

Since physical connections between ramets persist only for a limited time interval, the identification of the ramet/genet organisation in field populations is possible only using genetic markers. While traditional allozyme markers often provide too little polymorphism, modern DNA based molecular markers such as RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism) or DNA microsatellites allow precise assignment of leaf shoot samples to ramets and genets based on multi-locus genotypes. As an additional advantage, the Mendelian inheritance of DNA microsatellites allows the calculation of error probabilities for an incorrect assignment of ramets to genets (Reusch et al., 1999d). To date, information on the clonal structure of nine seagrass species is available (*Cymodocea nodosa*, *Halophila stipulacea*, *Halophila johnsonii*, *Posidonia oceanica*, *Posidonia australis*, *Thalassia testudinum*, *Thalassodendron ciliatum*, *Zostera marina*). In only a few studies were genotypes mapped in space (cf. Waycott, 1995; Kirsten et al., 1998) (e.g. Fig. 7). This

Allozymes

15 genotypes/43 samples



RAPDs

15 genotypes/23 samples

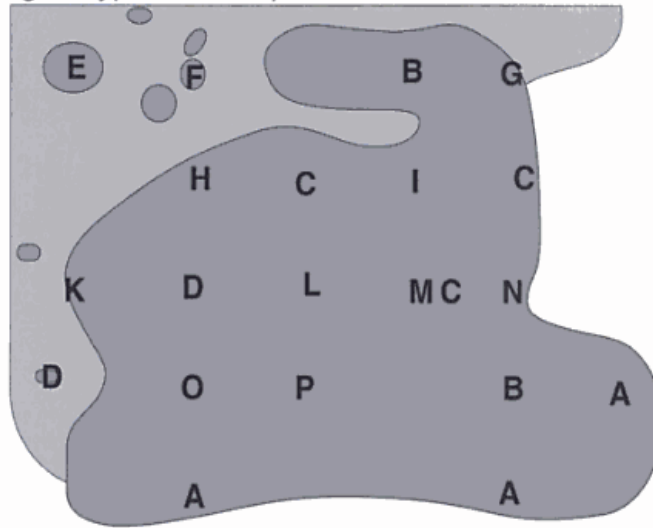


Fig. 7. Clone map of *Posidonia australis* from Western Australia, letters correspond to matching genotypes and are equivalent to ramets of the same clone (from Waycott, 1995).

lack of spatially explicit information is unfortunate as it can be utilized to infer clone sizes age, as well as to characterize the genetic neighborhood of genets within populations (see below).

The large differences in sampling effort, physical distances among samples, and in the resolution of genetic markers employed make comparisons among studies assessing clonal structure difficult. Nevertheless, the use of high-resolution genetic markers has repudiated earlier notions of clonal uniformity in several species, notably *Thalassia testudinum* (Kirsten et al., 1998), *Posidonia australis* (Waycott, 1998) and *Zostera marina* (Reusch et al., 1999d). Previously all three species were thought to comprise only a few clones based on allozyme markers, while DNA based markers revealed several distinct genotypes at the m-scale.

A second major finding was that a widely varying degree of clonal diversity occurs among locations within species. For example, in *Posidonia oceanica*, there are sites in the Adriatic Sea with only a single detectable genotype (Ruggiero et al., 2002); whereas, other populations in more central areas of the Mediterranean are multi-clonal (Procaccini et al., 2001). A similar range in clonal diversity has been observed in the Australian species *Posidonia australis* (Waycott, 1998). The northern-temperate species *Zostera marina* shows an even wider range of clonal diversity across locations, from monoclonal stands to populations where each genotype is unique (Reusch et al., 2000).

The causes of widely varying clonal diversities across locations and species are largely unknown. The proximate reason for the variation observed is probably a shift in the relative success of sexual vs. vegetative reproduction. Although almost nothing is known regarding how variation in reproductive output in seagrass populations translates into rates of successful recruitment, some evidence points to extrinsic forcing favouring sexual recruitment. There is limited evidence from eelgrass (*Zostera marina*) that clonal diversity is higher at sites with greater physical disturbance (Hämmerli and Reusch, 2003b). This is a plausible scenario because the chances of establishment in closed seagrass canopies are low, while gaps may facilitate the emergence of seedlings and hence, new genotypes to the local population (Eriksson and Fröberg, 1996). A recent study by Hämmerli and Reusch (2003b) also found that genotypes with a higher individual heterozygosity, measured at nine polymorphic DNA microsatellite loci,

were larger on average than more inbred clones. Over time, clonal competition seems to favour those genets, which have a higher genetic diversity at the level of individuals. Thus, the local diversity and size distribution of clones also has a deterministic component.

Populations at the distribution limit may also reveal low clonal diversity because sexual reproduction at marginal sites may be reduced or even completely prevented ('geographic parthenogenesis' of Bierzychudek (1985). Such a scenario may apply to seagrasses. For three different seagrass species findings from DNA based markers indicate that populations at the distributional margin may consist of very large clones [*Cymodocea nodosa*: (Alberto et al., 2001); *Posidonia oceanica*: (Ruggiero et al., 2002); *Zostera marina*: (Reusch et al., 1999a); or possess significantly reduced genetic diversity as in *Posidonia australis*: (Waycott et al., 1997)]. As a corollary, successful sexual reproduction at entirely clonal sites may be practically undetectable, although it must have occurred at one point in time if we are not to invoke re-establishing of rafting vegetative fragments as an explanation. Clearly, much more experimental work at the within-population scale, coupled with censuses at the geographic scale are needed to explain extent and variation in clonal diversity among seagrasses, both at the species, and the population level.

The clonal life-history strategy has important ramifications for processes within populations (see Duarte et al., Chapter 11), in particular for the mating system. In the centre of large clones, focal ramets may be surrounded by members of the same clone (Handel, 1985). In monoecious (*Zostera* spp.) and hermaphroditic (*Posidonia* spp.) seagrass genera, the opportunity for outcrossing is thus constrained by the spatial architecture of the genets in a meadow. Not surprisingly, seeds in focal flowering shoots in monoecious *Zostera marina* show selfing rates up to 65% due to between-ramet geitonogamy when growing in a selfing neighborhood (Reusch, 2001a). Selection against selfed progeny effectively restores Hardy-Weinberg-equilibrium in the adult population. Since fitness costs associated with inadvertent between ramet selfing are substantial (Ruckelshaus, 1995; Reusch, 2001a), these findings may provide one possible explanation for the evolution of dioecy in seagrasses. Evidently, within-clone selfing is impossible in dioecious seagrasses, as there are separate male and female plants. However, the high

incidence of dioecy may also reflect the sexual conditions of monocotyledonous plants ancestral to seagrasses (Waycott and Les, 1996; Les et al., 1997).

Long-term demographic censuses are often unavailable for many seagrass populations. Unravelling the spatial pattern of ramets and genets in a seagrass meadow uncovers parts of its local demographic history. Hence, as a potential alternative to census data, important demographic information may be inferred from a genetic analysis of the spatial arrangement of clones. For example, the detection of identical multi-locus genotypes among distant vegetation patches may indicate the fragmentation of previously continuous meadow. Moreover, minimal estimates of meadow age can be inferred from rates of lateral spread of clones in conjunction with the aerial extension of clones (Reusch et al., 1999d). In several northern Baltic Sea populations of *Zostera marina*, vegetation patches are composed of shoots with one identical genotype (TBH Reusch and C. Boström, unpublished data), suggesting that the initiation of patches often starts with a single seedling.

Within-population genetic structure not only comprises the ramet-genet dichotomy but also involves the dispersal capability of pollination and sexual progeny. Given that seeds and pollen are thought to typically travel only a few meters (Ruckelshaus, 1996) (but cf. Harwell and Orth, 2002), germinating seedlings will often be related genetically to nearby plants. Thus, additional population structure can be expected at the scale of meters. Spatial autocorrelation techniques are a powerful tool to detect such structure (Heywood, 1991). In autocorrelation analyses, the genetic similarity among pairs of ramets is analysed as a function of their pairwise distance. If autocorrelation values are positive, genetic relatedness is higher as expected under random mating, and *vice versa*. As clonal plants, seagrasses require special attention because the inclusion of members of the same clone into an autocorrelation analysis inflates small-scale kinship structures (Reusch et al., 1999b). In a recent study using a high-resolution spatial sampling grid (Hämmerli and Reusch, 2003a), a significant positive spatial autocorrelation in kinship coefficients was found at distances between 2 and 5 m even when counting each clone only once. Significant positive spatial autocorrelation was also found between 1 and 11 m in *Cymodocea nodosa* and between 2 and 7 m in *Zostera noltii* (Ruggiero and Procaccini, unpublished). Thus, for any focal shoot,

the landscape of neighbouring plants is complex and consists of several levels of genetic sub-structuring. Clone affiliation, clone spatial arrangement and local kinship patterns interact to influence mating patterns, clonal competition and reproductive output in apparently homogeneous seagrass meadows.

C. Mating Systems

Plant mating systems are one of the most significant factors in the transmission of genetic diversity between generations (Clegg, 1980). The measurement of plant mating systems is complicated since an overwhelming majority of angiosperm species possess both male and female flowers on the same plant (Richards, 1997). These bisexual plants are therefore capable of self-pollination either from the same flower (autogamy) or separate flowers on the same plant (geitonogamy). Most seagrass species are dioecious (individuals of separate sex) (Waycott and Les, 1996; Les et al., 1997) and as such cannot self-pollinate. However, even dioecious plants can be subject to inbreeding through the mating of siblings or parent-offspring. In this context, two components of seagrass mating systems are particularly significant, first the movement of pollen between flowers within a meadow and second the genetic structuring of plants within populations as described in the preceding section.

Sexual reproduction in the marine environment is limited by the ability of pollen to reach stigmas (Pettitt et al., 1981; Cox, 1988; Ackerman, 1995; Verduin, 1996; Reusch, 2003; Ackerman, Chapter 4). Seagrass pollination occurs by hydrophily, that is, water mediated, abiotic pollination (Fægri and van der Pijl, 1979). Our understanding of pollination in seagrasses is limited by the interaction of the timing of pollen release/capture and the hydrodynamic environment in which these events occur. A correlation between hydrodynamic environment and the efficiency of pollen movement between flowers, and thus outcrossing, has been proposed by Waycott and Sampson (1997) for *Posidonia australis*. However, the spatial arrangement of flowers in a seagrass meadow is critical to the outcome of any mating event as the greater the area a flowering genet covers the greater the chance there will be self-pollination as found by Reusch (2001a) where outcrossing rate was positively correlated with clonal diversity (Fig. 8). For example in populations of Northern European *Zostera marina*, pollen limitation has been

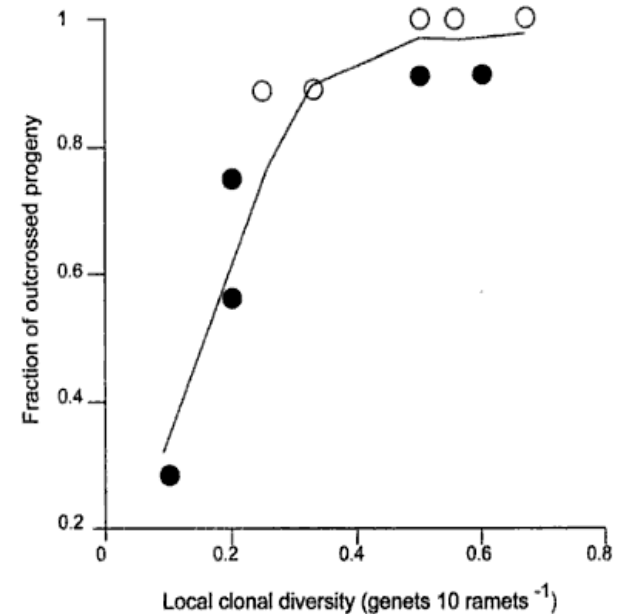


Fig. 8. Relationship between clone number per patch of seagrass and outcrossing rate in *Zostera marina* from two populations in northern Europe (redrawn from Reusch, 2001a).

experimentally demonstrated in a field experiment, where decreased seed set when the density of flowering shoots was below 5 shoots m⁻² (Reusch, 2003). Interestingly, *Zostera marina* adjusts its instantaneous sex ratio (i.e. the ratio of male to female flowering stages) as to maximize the chances to receive outcrossing pollen, as demonstrated in a mesocosm experiment. This way, the negative effects of a monoclinal genetic neighbourhood on the outcrossing rate can be minimized (Hämmerli and Reusch, 2003c).

Assessment of plant mating systems is primarily done in bisexual species as the analyses detect the genotype of the ovule donor (maternal genotype) and document the number of times genetic information must have been derived from a non-maternal source. Detection of inbreeding among related individuals in a dioecious species, although possible, is difficult as the detection of relatedness requires large sample size and fine scale analysis of genetic relatedness. Thus, among the seagrasses we are limited to assessing the mating system (i.e. frequency of selfing) of only three genera: *Posidonia*, *Zostera* and two species of *Halophila*. It is also extremely difficult

to estimate outcrossing rates from non-codominant markers although there have been attempts to do so in other plant species (e.g. Krauss, 1999). Thus, studies are usually limited to those markers that detect codominant genotypes as alleles present in both ovule donor and pollen donor such as allozymes and microsatellites (SSRs).

To date, mating system studies, or those that utilize outcrossing estimates, have been conducted for *Zostera marina* (Ruckelshaus, 1995; Ruckelshaus, 1996; Ruckelshaus, 1998; Reusch, 2000; Reusch, 2001a; Hämmerli and Reusch, 2003b), and *Posidonia australis* (Waycott and Sampson, 1997). In *Zostera marina*, outcrossing rates (*t*) are usually high (*t*; 0.61–0.97); however, estimates using microsatellite markers were higher than allozyme markers as would be expected given the increased sensitivity of the DNA marker system (Reusch, 2001a).

The normally high outcrossing rates in *Zostera marina* are reduced in populations where there are fewer clones per patch (Reusch, 2001a). Conversely, genetic diversity of *Posidonia australis* populations did not have an effect on the outcrossing rate

(Waycott and Sampson, 1997) although the exact structure of the population sampled for mating system analysis was not documented. It should be noted however that a large proportion of seagrass populations studied to date have mixed population structure (see preceding sections this chapter) and in such populations there will be a high probability that seed production will result from outcrossing in these populations. However, it is important to document fine scale population structure alongside the mating system in seagrasses to fully appreciate the implications of any measurement of outcrossing (or by implication inbreeding). In addition, the hydrodynamics of pollen movement needs to be understood better to account for pollen movement and the interaction of the biotic and abiotic factors affecting successful sexual reproduction (Cox et al., 1992; Verduin, 1996; Ackerman, 1997).

Mating system estimates provide a more detailed understanding of population processes such as small scale gene flow, the frequency of inbreeding and estimates of inbreeding depression (e.g. Ruckelshaus, 1995; Hämmerli and Reusch, 2003b). The consequences of inbreeding can significantly impact a species primarily due to reduction in progeny fitness (Charlesworth and Charlesworth, 1987; Ellstrand and Elam, 1993). For this reason alone, estimates of inbreeding are valuable and should be obtained for a greater range of seagrass species.

IV. Concluding Remarks

A. Inferences for Ecology, Conservation and Rehabilitation

Outcomes from population genetic analysis can substantially influence the way we perceive how seagrass ecosystems interact and can identify where important reservoirs of genetic diversity may exist. Different scales of gene flow can be very important for assessing the real significance of geographic isolation and habitat fragmentation in the context of protecting and managing seagrass meadows. Many recent results suggest that seagrasses are capable of at least some long distance dispersal (e.g. Reusch et al., 2000; Waycott and Barnes, 2001; Les et al., 2002; Reusch, 2002; Waycott et al., 2002). These results based on broader scale population genetic and phylogeographic studies imply that the perception of seagrasses as isolated disconnected populations

requires reassessment. Considerable insight will be gained from studies that explore these processes using both an ecological and genetic approach. In addition to the outcomes for an improved understanding of population ecology such studies enhance our ability to reconstruct historical processes that have led to isolation of populations.

Determining population genetic isolation is particularly important for defining correct strategies for collecting transplants to re-establish lost seagrass meadows, a practice now widespread (Fonseca et al., 1998). Broad scale studies identify areas with lower genetic diversity and lower exchange with other localities. The definition of genetic distinction among populations facilitates selection of appropriate donor beds for transplantation programs, according to their relative levels of polymorphism and genetic distinctiveness. In such studies, genetic diversity needs to be carefully defined, as it has two levels in seagrasses, genotypic diversity and genetic diversity. Previous studies were often unable to distinguish between both levels since markers were not polymorphic enough. However, this may be critical when selecting donor beds. For example, a few large heterozygous clones may be ideal starting material for transplantations, yet they could be assessed as genetically uniform when clonal diversity cannot be separated from genetic diversity. A transplant study on *Posidonia oceanica* has suggested, in fact, that transplants coming from genetically polymorphic populations have a greater probability of survival and perform better in common garden experiments (Procaccini and Piazzi, 2001). In addition, understanding the genetic consequences of transplantation may allow us to better infer the ability of those beds to survive in longer time frames (e.g. see Williams, 2001).

B. Interactions of Scale and the Appropriate Use of Molecular Markers

The preceding sections of this chapter present a broad range of scale in genetic analyses of seagrasses from phylogenetic relationships at the sub-class level to centimetre scale measures of gene flow. The interpretation of processes should be made with these differences in scale in mind. For example, it is clear that *Zostera marina* represents a distinct evolutionary lineage to the southern hemisphere *Zostera* species based on molecular phylogenetic results (Les et al., 2002). However, these analyses were done with

relatively few individuals sampled for each species as is appropriate for phylogenetic analysis. In comparison, fine scale estimates of inbreeding and population structure within populations in Europe and the USA have provided insight into the interaction between ecology and evolutionary strategies in this species (Ruckelshaus, 1998; Williams and Orth, 1998; Reusch et al., 1999d; Reusch, 2001a; Olsen et al., 2004). However, the connection between the broadest scale process and the evolution of this species is only now being revealed through studies using high resolution, species-wide population genetic analysis (Olsen et al., 2004).

Thus, a significant issue in the interpretation of genetic data are the level of diversity detected using particular genetic markers. This issue has been exemplified in the early studies that utilized allozymes to detect genetic diversity (e.g. McMillan, 1991) and which revealed very high levels of genetic homogeneity (see Les, 1988 for discussion). Although subsequent studies on the same species using allozymes revealed significantly greater polymorphism than early studies (Ruckelshaus, 1995; Waycott, 1995; Ruckelshaus, 1996; Waycott et al., 1997; Waycott and Sampson, 1997; Ruckelshaus, 1998; Waycott, 1998), DNA based markers revealed even higher levels of variability (Waycott, 1995; Waycott et al., 1996; Waycott, 1998; Reusch et al., 1999d; Reusch et al., 1999c; Reusch et al., 2000).

New approaches are being developed continuously that allow improved interpretations of the different hierarchical processes of interest (e.g. Zhang and Hewitt, 2003). For example, there is now a growing interest in studying adaptive genetic polymorphism at the DNA level. To undertake such studies, however, polymorphism in genes of adaptive significance must be analysed. There are a number of candidate genes to begin these studies, for example genes related to photosynthesis (García-Gil et al., 2003) or to heat stress (Heckathorn et al., 1996), which can be inferred from model plants. Marker loci provide one view on the measurement of genetic diversity; the future of many genetic studies will be to understand the effects, polymorphism and fitness consequences of loci that selection will actually operate upon (see, for example, Mitchell-Olds, 2001 and references therein). It is therefore worthwhile to invest in the most variable and informative marker (currently microsatellite markers for population analyses and DNA sequences of multiple loci for

phylogeny) available within the constraints of time and budget for a project.

To ensure adequate future survival of seagrasses, globally, a greater understanding of the relationships between species, the baseline genetic variability of species, their reproductive biology and unique adaptive traits is needed and such knowledge will become increasingly available, as discussed in this chapter.

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