Genetic Issues for the Restoration of Seagrass Populations

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Until very recently, seagrasses were viewed as intractable for population genetic study and unlikely to contribute much to the theory and practice of plant population genetics. Seagrass management was focused on getting the vegetation to grow rapidly to support edible critters. Over the past decade, there has been a remarkable transformation in appreciation and knowledge of the importance of fundamental population level processes to seagrass biology and to restoration.

Fundamentally, genetic variation enables evolution, and thus adaptation to changing environmental conditions. Genetic variation arises from mutation, it is enhanced through recombination, and can be neutral, or under selection and thus potentially of adaptive significance. Genetic structure is the nonrandom distribution of genetic variation in space or time. It results from the evolutionary mechanisms of genetic drift and selection and mutation, can be broken down by gene flow, and is influenced by the mating system. The relative influence of each evolutionary mechanism on genetic variation and structure has been the subject of intensive theoretical and empirical research.

There has been considerable debate over the relevancy of population genetics to seagrass restoration that deserves clarification. Transplantations can fail in the short-term due to non-genetic reasons, such as poor site selection, interference from non-native species or stochastic factors like bioturbation or storms. Genetic data add costs to mitigations, and limited research funds require careful prioritization. The argument that genetic issues are not of primary importance in seagrass restoration or application-directed research has been rationalized based on the recognition by conservation biologists that establishing minimum viable population sizes based solely on genetic criteria is probably inadequate. This issue distilled to whether inbreeding effects were more likely to cause extinctions of very small populations than demographic or environmental stochasticity (Lande 1988). Although a minimum population size should be evaluated for seagrass mitigation locally and regionally, the seagrass restoration issue is not extinction, but whether restored populations represent 'natural' ones to the closest degree possible, and whether evolutionary mechanisms proceed 'normally' in restorations. Whether genetic effects play a role in seagrass transplantation failure, along with poor restoration practices and stochastic factors, seems at least worthy of discussion. This is particularly so given recent models demonstrating that inbreeding depression and fixation of new mutations can be as important as demographic and even evironmental stochasticity in increasing extinction risk in small populations (Hart and Haefner 1994, Mills and Smouse, 1994, Frankham 1995, Lande 1994, 1995). The critical issues for seagrass restoration, thus, are the role of genetic effects in successful population establishment and sustainability. Although having little influence on natural selection and mutation, restorationists can strongly influence genetic drift, inbreeding, and possibly gene flow through setting the genetic attributes and likely genetic processes of the transplanted population, e.g., genetic variation through artificial or unconscious selection of stock, population size and siting within a seagrass landscape. Restoration concerns thus go beyond whether a transplantation establishes quickly enough to prevent unacceptable loss of trophic support function. Lack of genetic variation can affect the

long-term potential for seagrass adaptation to a changing environment, e.g., pollution, reduced light, rising sea levels, and thus is related to sustainability.

What is known about the population genetic attributes of diversity and structure for seagrasses? More importantly, what is known about the relationship between seagrass genetic attributes and restoration? There have been 17 published studies since 1990 (Table 1) devoted to the issue but only two of these are devoted specifically to restoration issues (Williams and Davis 1996, Williams and Orth 1998). I will not argue the merits of particular papers or techniques; useful information can be gleaned from each, and careful interpretation is important, as with any body of literature (see "Recent findings..." below). Instead I intend to focus on identification of critical research gaps in seagrass population genetics and the application to management.

Table 1. Summary of Seagrass Population Genetic Studies since 1990. 'Preliminary' refers to studies in the technique development stage or without results to report at present. Contacts for investigators of unpublished data are proviided in a footnote. Citations are provided in the extended abstract "Genetic issues for the restoration of seagrass populations."

Zostera marina (eelgrass)

Study	Locale	# Sites	Technique	Information
1. Backman, 1991	west coast	13	common garden	G x E effects
2. Fain, DeTomaso, Alberte, 1992	CA	4	RFLPs	interpopulation variation
3. Harrison & Durance, 1992	BC	3	allozymes	habitat fragmentation
4. de Heij & Nienhaus, 1992	Europe	2	allozymes	interpopulation variation
5. Laushman, 1993	US	3	allozymes	interpopulation variation
6. Alberte et al., 1994	CA	3	mini-satellites	interpopulation variation
7. Ruckelshaus, 1994	WA	4	allozymes	genetic structure
8. Ruckelshaus, 1995	WA	1	allozymes	outcrossing rates, inbreeding depression
9. Van Lent & Verschurre, 1995	Europe	2	common garden	G x E effects
10. Ruckelshaus, 1996	WA	1	allozymes	genetic neighborhood size
11. Williams & Davis, 1996	so. CA	12	allozymes	transplantation effects
12. Williams & Orth, 1998	Chesapeake	9	allozymes	transplantation effects, interpopulation variation
13. Williams & Hoffman, unpubl.	so. CA	16	allozymes	interpopulation variation
14. Freshwater, unpubl.	US, Japan	11	RAPDs	interpopulation variation
15. Nickl, preliminary	ME		RAPDs	interpopulation variation
16. Rhode, preliminary	Chesapeake		RAPDs	genetic differentiation

Thalassia testudinum (turtlegrass)

Study	Locale	# Sites	Technique	Information
17. Kisten, Dawes & Cochrane, unpubl.	FL, Jamaica	4	RAPDs	interpopulation variation
18. Ruckelshaus, unpubl.	FL	2	allozymes, common garden	interpopulation variation, G x E effects
19. Porter & Hamrick, unpubl.			allozymes	interpopulation variation
20. Cutler & Childers, preliminary	FL		RAMPs	clonal structure

Halophila engelmanii

Study	Locale	# Sites	Technique	Information
21. Jewett-Smith, Garza, Bernal, unpubl.	TX	4	RAPDs	interpopulation variation

Amphibolis antarctica, A. griffithii

Study	Locale	# Sites	Technique	Information
22. Waycott, Walker & James, 1995	w. Australia	3	RFLP, mini-satellites	no intrapopulation variation

Posidonia australia

Study	Locale	# Sites	Technique	Information
23. Waycott, 1995	w. Australia	1	allozymes, mini-sats	intrapopulation variation

Posidonia oceanica

Study	Locale	# Sites	Technique	Information
24. Procaccini & Mazzella, 1996	Italy	1	RAPD	no variation
25. Procaccini, Alberte & Mazzella, 1996	Italy	3	RAPD	little interpopulation variation

Cymodocea nodosa

Study	Locale	# Sites	Technique	Information
26. Procaccini & Mazzella, 1996	Italy	1	RAPD	no variation

Ruppia maritima

Study	Locale	# Sites	Technique	Information
27. Koch & Dawes, 1991	FL, NC	2	common garden	ecotypic differentiation

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Seagrass restorations begin with small population sizes and potentially limited genetic diversity (Williams and Davis 1995, Williams and Orth 1998). Typically, the transplanting units are vegetative material of unknown clonal identity. Successful establishment involves population growth; survival of transplant units, vegetative propagation, and possibly sexual reproduction and seedling recruitment. The hypothetically important genetic effects that could influence the establishment of any plant population reduce to 1) effects in small, possibly isolated, populations, and 2) local adaptation. Genetic drift in small populations and inbreeding can lead to loss of genetic variation due to fixation of alleles, thus reducing the potential for adaptation in the long-term. Inbreeding depression has been documented for eelgrass (Ruckelshaus 1995) but its effects are unknown in seagrass transplantation establishment. Evaluating inbreeding effects on seagrass establishment requires understanding the relative importance of seeds and seedling recruitment to population growth. Demographic population modeling provides a valuable approach (e.g., Horvitz and Schemske 1995), followed by analysis of ecological and environmental factors important in determining survival and reproduction. Elasticity analysis of a demographic model for eelgrass (Ewanchuk 1995) revealed that vegetation reproduction contributed quantitatively more than sexual reproduction to the population growth rate. Seedling recruitment, however, is critical for eelgrass population growth following disturbances (Robertson and Mann 1984, Ewanchuk 1995, Orth pers. com.).

Limited research has been done on genetic effects on seagrass establishment in transplantations. Seeds from an eelgrass population with higher genetic diversity germinated better in the laboratory relative to seeds from a population with lower genetic diversity (Williams unpubl. data). Although more experiments must follow, the result suggests that genetic diversity in seed populations might influence the persistence of transplantations following disturbances, or transplantation establishment if seeds are used or they play a role post-establishment. The genetic diversity of vegetative stock also can influence transplantation establishment. Replicated experimental transplantations of eelgrass with 'high' genetic diversity produced new leaf shoots more rapidly over the first two years relative to the 'low' genetic diversity replicates (Williams unpubl. data).

It seems important to avoid limiting genetic diversity in donor stock for several reasons. Theory predicts transplantations will lose genetic diversity if local selection regimes and genetic drift in small populations override gene flow. The loss will be exacerbated by inbreeding. The argument that natural processes will add to genetic diversity is not well-founded at least for eelgrass. There is no evidence for increasing genetic diversity in older transplantations, and the trend is for loss (Williams and Davis 1995). Genetic diversity was lost during establishment in both high and low diversity transplantations mentioned above. Dispersal via pollen and seed dispersal is limited (Orth et al. 1994, Ruckelshaus 1996) and floating vegetative fragments have little potential to reestablish (Ewanchuk and Williams 1996). Although feasible, later deliberate addition of migrants to established transplantations will not necessarily add genetic variation (Ellstrand and Elam 1993, Mills and Allendorf 1996). The genetic composition of the migrant relative to the population must be known to avoid *reducing* overall genetic variation by adding migrants of identical or common genotypes. Introductions will be effective obviously only if the migrants survive. Application of either theoretical or empirical information is problematic for restoration of seagrass species in which no or virtually no genetic variation has been found even using RAPD and mini-satellite methods to probe DNA structure (Waycott et al. 1996, Procaccini et al. 1996). Further study with larger sampling sizes or more locations might reveal

hidden variation, just as further study did for eelgrass. Until variation is identified, genetic effects on restoration will be difficult to study in such species as *Amphibolis antarctica* and *Posidonia oceanica*. Finally, the data available to date are indicative of discrete genetic variation detectable by markers (e.g., allozymes, RAPDs). Virtually no data are available for quantitative trait variation which is likely to be most important for predicting the likelihood of adaptation.

In the absence of more precise understanding of genetic effects on seagrass transplantation establishment, practical measures can be taken to hypothetically minimize inbreeding depression and loss of genetic diversity from genetic drift. Although some seagrass beds are not single clones and genets can intermingle in areas as small as 1 m² (Lu and Williams, unpublished RAPD data on eelgrass, Alberte et al. 1994, Waycott 1995, Harrison and Durance 1992), rarer genotypes are likely to be excluded just by chance when donor beds are sampled over small spatial scales, i.e., there will be a founder effect in the transplantation (Williams and Davis 1995). If known, genotype frequencies can be used to estimate how many plants should be collected to have a reasonable probability of including rarer genotypes. Knowledge of genetic structure is likewise useful to determining the scales over which rarer genotypes are distributed. For example, we successfully genotyped 1791 eelgrass plants collected over 2500 m². Of these, 1343 were the most common genotype (MDH-GPI loci), 209 were GPI heterozygotes, 191 were MDH heterozygotes, and 48 were rarer genotypes. Transplanted beds and ones resulting from colonization of unvegetated areas (e.g., post-dredging) should be avoided as donor sites because they are likely to have reduced genetic diversity due to founder effects when established (Williams and Davis 1995).

Another critical genetic issue for seagrass restoration is local adaptation leading to 'co-adapted gene complexes' and outbreeding depression. Genetic differentiation over small spatial scales in response to environmental differences is termed ecotypic differentiation. Outbreeding depression in plants is the reduced fitness resulting from pollinations between increasingly distant plants. Ecotypic differentiation is common in plant populations (Bradshaw 1965) and recent research has identified outcrossing depression in wild populations (Waser 1993). The practical issue is whether there is a limit to the distance between donor and transplantation sites beyond which the donor stock will show reduced initial performance or subsequent reproductive success in the transplanted site. How much does the source of the seagrass matter to the successful establishment, growth and persistence of the transplantation? Current ad hoc transplantation protocols to take stock from the closest existing beds implicitly assumes that local adaptation or outbreeding depression occur, i.e., selection is important. Certainlythere are limits to the plasticity of eelgrass morphology (Phillips 1972, Backman 1991), photosynthetic performance (Dennison and Alberte 1986), and possibly flowering (van Lent and Verschuure 1995) that suggest genetic adaptation to local environments is possible. Whether changes in these traits represent adaptation, or instead, developmental canalization or insufficient time for acclimation, cannot be determined until the heritability of the traits is known, e.g., through quantitative genetic experiments employing known matings. Ruckelshaus (1994, 1995) found evidence for outbreeding depression in eelgrass under certain conditions. She also reported that selection influenced the genetic structure of a Puget Sound eelgrass bed more than genetic drift did. Her recent unpublished work on *Thalassia testudinum* transplantations in St. George Sound, FL, and in mesocosms showed significant effects of the source of the turtlegrass in morphology and growth and epiphyte fouling (Ruckelshaus, per. com.). Comparison of survival

and growth rates of *Thalassia* from distant (1 km away) and the transplantation site showed that plants replanted from the vicinity of the transplant site fared best.

The ad hoc policy of selecting nearby donor beds seems prudent in the absence of data on local adaptation and outbreeding depression *unless*, as mentioned above, nearby beds have reduced genetic diversity. Information on genetic structure, or how much of the total genetic variation of a population can be expected in each subpopulation, seems relevant at first glance to guiding donor bed selection. Genetic structure indicates that subpopulations are genetically differentiated from each. Local populations apparently evolved relatively independently of each other but unfortunately the role of selection versus genetic drift in creating genetic structure is not evident without other information (effective neighborhood sizes, gene flow measurements, selection regimes). Local adaptation and outcrossing depression concern selection. The likelihood of inbreeding and outbreeding depression for seagrass restoration rest in part on the genetic relatedness of the donor plants and their mating histories. Another critical consideration is whether the genetic structure measured represents an equilibrium condition (Varvio et al. 1985). In the absence of genetic ata, one strategy would be to 'bet-hedge' by sampling widely within each of several different populations for donor plants.

From this brief discussion it should be clear that accumulating data on population genetic attributes of seagrasses mostly concern eelgrass and virtually none are devoted explicitly to restoration. Restoration genetic research under the current funding arenas will be limited necessarily to population establishment. The long life span of most of the restoration target species prohibits realistic research on sustainability and thus, information on genetic effects on sustainability will be limited to inferences made from older established transplantations. Within these limitations, understanding seagrass population genetics would benefit from research that goes beyond cataloguing genetic diversity and structure to the evolutionary mechanisms that create and maintain genetic diversity and structure. Restorations provide an excellent research setting in which to investigate evolutionary and demographic processes in seagrasses but require understanding and cooperation between practitioners, biologists, and those hlding purse strings. Often small pilot experiments can be planted alongside restorations. Seagrass restoration success has improved with better information on how to select sites and prevent bioturbation, although functional equivalency is still a major issue. It will be unconscionable, however, if restoration practice, no matter how successful in the short-term, leads to reduced evolutionary potential for the future.

Important General Questions:

1. Over what temporal scale does genetic variation matter to the persistence and growth of seagrass populations?

2. Can seagrass restoration be managed without affecting population genetic attributes of seagrasses in ways that would alter their evolutionary potential?

3. What are the effects of seagrass habitat fragmentation on population genetic attributes, population persistence, and evolutionary potential?

4. How much does the source of the donor stock for transplantation matter for its establishment, growth, and persistence? What is the relative effect of genotype versus the environment in determining seagrass 'performance'?

5. How important are seeds for recolonization of transplantations that suffer small-scale disturbances (e.g., bioturbation, burial, poor water quality during critical periods, storm events)? How severe is inbreeding depression in various seagrass species?

6. What are natural levels of gene flow in seagrasses?

7. Does extinction and recolonization by natural events need to be considered in maintaining genetic diversity and structure and population persistence (Ruckelshaus 1994, pers. com.)? How applicable is such information to analogous but human-mediated disturbances and subsequent mitigation?

Recent findings in seagrass population genetics:

1. Populations of some seagrass species comprise several, intermingling clones (Harrison and Durance 1992, Alberte et al. 1994; Williams and Davis 1995; Procaccini and Mazzella 1996; Waycott 1995; *Thalassia*, Dawes pers. com.), although other populations have no detected genetic variation (Procaccini et al. 1996, Waycott et al. 1996).

2. Transplanted eelgrass beds in southern California are reduced in genetic diversity relative to untransplanted beds (Williams and Davis 1995). This pattern was not apparent in transplanted beds in the Cheasapeake Bay (Williams and Orth 1998).

3. Predictions made about genetic diversity and structure from breeding system (sexual vs asexual reproduction, dioecy, life-span) are not always supported by empirical studies (Kisten, Dawes, Cochrane, manuscript on *Thalassia*). For example, the mating system of eelgrass (fast clonal growth, monoecy) is predominantly outcrossed (Ruckelshaus 1995).

4. Inbreeding depression occurs in eelgrass. Sequential temporal development of male and female flowers (protogyny) overlaps sufficiently to allow self-pollination (Ruckelshaus 1995).

5. Dispersal of eelgrass pollen and seeds is more restricted eelgrass than previously thought because pollen and seed dispersal is restricted (Orth et al. 1994, Ruckelshaus 1996) and floating vegetative fragments have a poor probability of re-establishment (Ewanchuk and Williams 1996). These observations imply that transplantations might not be expected to increase naturally in genetic diversity.

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