

## Population genetics of eelgrass (*Zostera marina*) from the Jordan River

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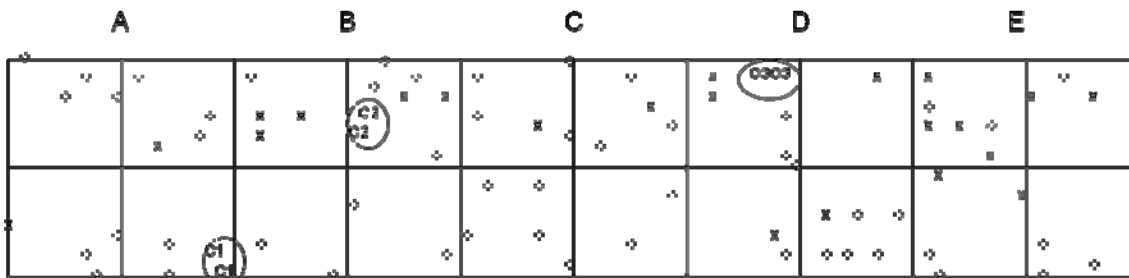
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Populations with high levels of genetic diversity are better able to adapt to change in their environment. Eelgrass from the Jordan River is being used to re-establish eelgrass beds at Hadley Point. Individual eelgrass plants from the Jordan River donor site were genetically characterized. This preliminary investigation found high levels of genetic diversity within the Jordan River.

*Zostera marina* collected from the Jordan River, Hancock County, Maine is being used to restore eelgrass beds at Hadley Point, Bar Harbor Maine. In 2007, 2008, and 2009 eelgrass was collected from the Jordan River and transplanted to protected areas to re-establish eelgrass habitat. Eelgrass communities across all regions of the world are being lost at a rapid rate<sup>8</sup>. This preliminary study investigated the population genetics of the Jordan River eelgrass donor site. Manipulative field experiments<sup>1,4</sup> demonstrate that the greater the genetic diversity in a population of *Z. marina*, the more resistant beds are to changes and disturbances. The success of MDIBL transplant efforts is therefore dependent on the genetic diversity of the Jordan River population. The purpose of this study was to evaluate genetic diversity at the donor site.

In June 2009, 80 samples of *Z. marina* were collected from a sampling grid (grid and sampling method published previously<sup>7</sup>) set up parallel to the shore of the Jordan River. The grid consisted of 5 quadrants, each with an area of 10m<sup>2</sup>. Sixteen samples were taken from each quadrant, with a minimum of 1m between samples to reduce the likelihood of collecting more than one sample from a single ramet<sup>7</sup>. The northeast corner (bottom right corner in figure 1) of the grid was located at 44°28'18.12 N, 68°21'20.20 W.

Following standard CTAB DNA extraction, six polymorphic microsatellite loci<sup>6</sup>, that exhibit high levels of polymorphism between genetic individuals, were individually PCR amplified using fluorescently labeled forward primers and unlabeled reverse primers. Amplified samples were multiplexed on the ABI 3130XL sequencers to determine size in base pairs. All 6 loci were polymorphic, and the proportion of distinct genotypes (non-clones) from the completely genotyped samples is  $G = 0.951$  (Fig. 1). The *Z. marina* population in the Jordan River has relatively high levels of genetic diversity as compared to published data from North American sites, and the Mediterranean and Baltic Seas<sup>2,3,5,9</sup>. The value of  $H_o$  (observed heterozygosity) for the Jordan River is the 21st highest out of 83 sites, and 0.77 standard deviations above the mean (0.4199). The Jordan River donor site also exhibits a high level of intra-site genetic diversity. Most samples (58 of 61 completely genotyped) had unique genotypes. The sampling method was modeled from Reusch<sup>7</sup> who found 12 shared clonal genotypes within 80 samples collected in a similar fashion. For the specific grid site used in this study, the Jordan River appears to have relatively low levels of clonal propagation. The Jordan River displays a low level of asexual reproduction, a high level of genetic diversity relative to other sites, and a higher  $H_o$  than  $H_c$ ; this initial analysis suggests that the Jordan River is an appropriate donor site for transplants.



**Figure 1.** Locations of genotypes within the sampling grid. An open circle represents a genotype that occurs only once. A x represents a sample for which all six microsatellite loci have not been scored and could therefore be either a unique genotype or a clone of another sample. C1, C2 and C3 represent identical genotypes at all 6 loci; these are likely examples of clonal individuals.

This work was supported by a 2009 NSF REU (0453391) award to E. Correa.

1. **Hughes, AR and Stachowicz, JJ.** Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *PNAS*, June 15, 101, 8998-9002, 2004.
2. **Olsen, JL, Stam, WT, Coyer, JA, Reusch, TBH, Billingham, M, Boström, C, Calvert, E, Christie, H, Granger, S, La Lumière, R, Milchakova, N, Oudot-le Secq, M, Procaccina, G, Sanjabi, B, Serrão, E, Veldsink, J, Widdicombe, S, and Wyllie-Echeverria, S.** North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L., *Molecular Ecology*, 13, 1923-1941, 2004.
3. **Reusch, TBH.** Microsatellites reveal high population connectivity in eelgrass (*Zostera marina*) in two contrasting coastal areas. *Limnol. Oceanogr*, 47, 78-85, 2002.
4. **Reusch, TBH, Ehlers, A, Hämmerli, A, and Worm, B,** Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *PNAS*, **102**, 2826-2831. 2005.
5. **Reusch, TBH, Stam, WT, and Olsen, JL.** A microsatellite-based estimation of clonal diversity and population subdivision in *Zostera marina*, a marine flowering plant. *Molecular Ecology*, 9, 127-140, 2000.
6. **Reusch, TBH, Stam, WT, and Olsen, JL.** Microsatellite loci in eelgrass *Zostera marina* reveal marked polymorphism within and among populations. *Molecular Ecology* 8, 317-321, 1999.
7. **Reusch, TBH, Stam, WT, and Olsen, JL.** Size and estimated age of genets in eelgrass, *Zostera marina*, assessed with microsatellite markers. *Marine Biology*, 133, 519-525, 1998.
8. **Waycott, M, Duarte, CM, Carruthers, TJB, Orth, RJ, Dennison, WC, Olyarnik, S, Calladine, A, Fourqurean, JW, Heck Jr. KL, Hughes, AR, Kendrick, GA, Kenworthy, WJ, Short, FT and Williams, SL.** Accelerating loss of seagrass across the globe threatens coastal ecosystems. *PNAS ePub early edition*, July 19, 2009.
9. **Williams, SL. and Davis, CA.** Population Genetic Analyses of Transplanted Eelgrass (*Zostera marina*) Beds Reveal Reduced Genetic Diversity in Southern California. *Restoration Ecology*, 4. 180-193, 1996.