IGERT: Reverse Ecology

Untangling Morphotype and Latitudinal Variation in *Spartina Alterniflora*

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Introduction

Salt marshes fringe the land-water interface across the Atlantic and Gulf coasts of North America, supporting biologically productive, estuarine ecosystems. The perennial cordgrass *Spartina alterniflora* dominates the intertidal of these marshes, creating both habitat and marsh sediment structure. As an ecologically important species, it is imperative that we understand the population structure and climactic adaption present.

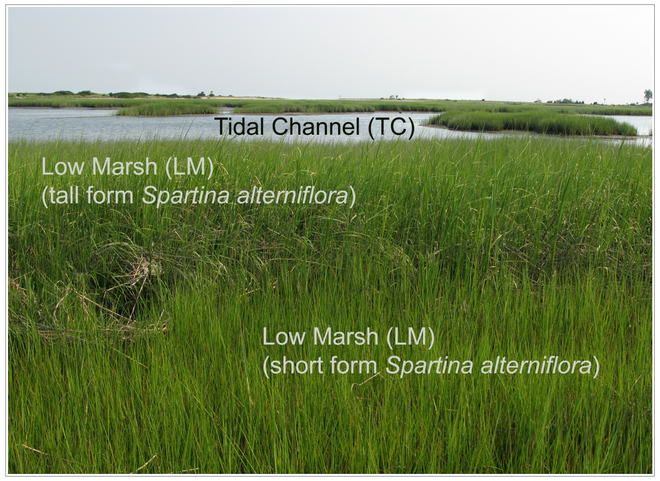

Figure 1: Spartina Alterniflora: Short Form and Tall Form. Photo credit to: `http://bit.ly/11xf5rD`

- Two distinct growth forms across latitudinal range; short form (10-40cm) and tall form (1-3m)
- Tall grows along edge of tidal creeks, short grows further inland
- Much uncertainty surrounding environmental/genetic underpinnings of two observed growth forms Gallagher et al. (1988); Freshwater (1988); Proffitt et al. (2003)
- Genetic variation across latitudinal range suggested by Freshwater (1988); Blum et al. (2007) as well

In this study we aim to resolve the morphotype variation in *S. alterniflora* along its latitudinal cline, from Georgia to Massachusetts using a double digest RAD-tag sequencing approach Peterson et al. (2012).

Materials and Methods

Overview

We collected leaf tissue samples of both tall and short form *S. alterniflora* across a wide latitudinal range (280 total samples) in order to look at differences in SNPs across their genomes.

In general, sequencing and assembling an entire genome is costly in both resources in time. We hence used double digest Restriction Associated DNA sequencing (ddRADseq), a protocol that sequences only regions generated by double digests. This reduces the fraction of each individual genome sequenced, but allows for increases in the number of individuals sampled to make possible comparing differences in SNPs across many individuals.

Field Protocol

Sites	# Short Samples	# Tall Samples
Plum Island (MA)	20	20
Great Sippewissett (MA)	20	20
Shelter Island (NY)	20	20
Tuckerton Peninsula (NJ)	20	20
Oyster Marsh (VA)	20	20
South Carolina	20	20
Georgia	20	20

Table 1: Seven sites along US Eastern Coast

- Collected leaf tissue samples of *S. alterniflora*
- 20 individuals each of tall and short collected at 7 sites along east coast
- Tall criteria: $> 1.00m$ in height
- Short criteria: $< 40cm$ in height
- All samples taken at least $5m$ apart to avoid resampling clones
- At least $20cm$ green leaf tissue collected for each plant
- Samples kept at -80^oC

DNA Extractions

- Approximately 50mg frozen tissue prepared for extraction
- Used Qiagen TissueLyser II to lyse
- Used Qiagen DNeasy Plant Mini Prep kit according to protocol for extraction
- Determined final DNA concentrations with Nanodrop spectrophotometer

Double Digest Restriction Associated DNA (ddRAD) Sequencing

Protocol

- In-depth protocol can be found at `http://www.bit.ly/ddRAD`
- Selected possible enzyme pairs for double digest based on code from `https://github.com/brantp/rtd`
- Desired size range of fragments was 250-350bp, to be selected for using PippinPrep
- Used wheat genome (*Triticum Aestivum*) as reference genome for simulation
- Confirmed **NiaIII-MluCI** as final enzyme pair based on lab work and bioanalyzer results
- Chose 24 barcodes on adapter P1 and 12 indices on adapter P2 to combinatorially barcode each individual sample
- Barcodes will be annealed during ligation before size selection
- Indices will be annealed during PCR

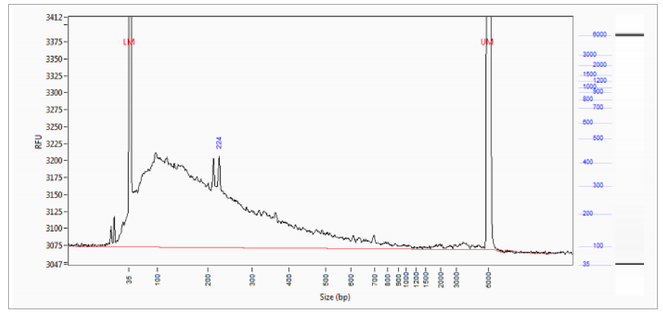

Figure 2: Bioanalyzer results from sample digest

Peak	Size (bp)	Conc. (ng/uL)	Molarity (nmole/L)	Peak Height (RFU)	Corr. Peak Area	Norm. MT (mm:ss)
1	35 (LM)	0.0682	3.184	4499	21.849	20:04
2	224	0.3369	2.473	135	8.997	26:03
3	6000 (UM)	0.0500	0.014	3954	16.022	44:05
	TIC:	0.3369	2.473			
	Total Conc.	1.5519				

Table 2: Bioanalyzer results

Future Work

- Finish preparing samples for sequencing according to ddRADseq protocol
- Send samples in for sequencing
- Perform SNP analysis across regions sequenced

References

Blum, M.J., K.J. Bando, M. Katz, and D.R. Strong. 2007. Geographic structure, genetic diversity and source tracking of *Spartina alterniflora*. *J Biogeogr* 34:2055–2069.

Freshwater, D.W. 1988. Relative genome size differences among populations of *Spartina alterniflora* loisel (poaceae) along east and gulf coasts of u.s.a. *J Exp Mar Biol Ecol* 120:1078–1083.

Gallagher, J.L., G. F. Somers, D.M. Grant, and D.M. Seliskar. 1988. Persistent differences in two forms of *Spartina alterniflora*: A common garden experiment. *Ecology* 69:1005–1008.

Peterson, B.K., J.N. Weber, E.H. Kay, H.S. Fisher, and H.E. Hoekstra. 2012. Double digest radseq: an inexpensive method for *De Novo* snp discovery and genotyping in model and non-model species. *PLoS ONE* 7:1–11.

Proffitt, C.E., S.E. Travis, and K.R. Edwards. 2003. Genotype and elevation influence *Spartinal alterniflora* colonization and growth in a created salt marsh. *Ecol Applications* 13:180–192.

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