Morphological Variation in *Medicago polymorpha* Pods

Lauren Otaguro, Wendy Vu, Maren Friesen

Advisor: Sergey Nuzhdin

USC WiSE Undergraduate Research Fellowship

Introduction

Nitrogen is more often than not a limiting factor in plant growth. Consequently, legumes of the genus *Medicago* have developed a way to better assimilate nitrogen; by forming a symbiotic relationship with N-fixing rhizobium bacteria, they are able to grow in soils with harsh conditions, like saline and serpentine soils. *Medicago* could have the ability to remedy nutrient depleted croplands and create more sustainable lands, and is potentially ecologically important.

This project focuses on the species *Medicago polymorpha*, named for the many morphological variations seen in its pods, one of which is a gradient of spine length. Spine length could potentially affect pod distribution by influencing how much a pod sticks to animals, their main means of migration. Pods with long spines are more likely to travel further and grow in a different soil type than its parent plant, whereas smooth pods are more likely to stay in the same environment. Understanding this will help to shed light on the adaptability of *Medicago*, and if spine length is connected to its ability to disperse effectively.

Methods

Studying this trait both ecologically, by setting up transects simulating the natural variation in pod morphology to measure migration rates, and genetically, by performing RAD sequencing, will help to understand pod distribution rates across Catalina Island and what genes are responsible for spine length. Salinity readings were taken to understand the extent of soil heterogeneity in which *Medicago* is distributed.

Transects for pod distribution rates:

Figure 1, right, shows one of the six transects near the Wrigley Institute for Environmental Studies. Each transect holds 100 pods (50 with spines and 50 with spines cut off) in the innermost circle. Pods were painted with UV paint and counted nightly. Figure 2, below, is a diagram of the quadrants in the transect.

Fig 1

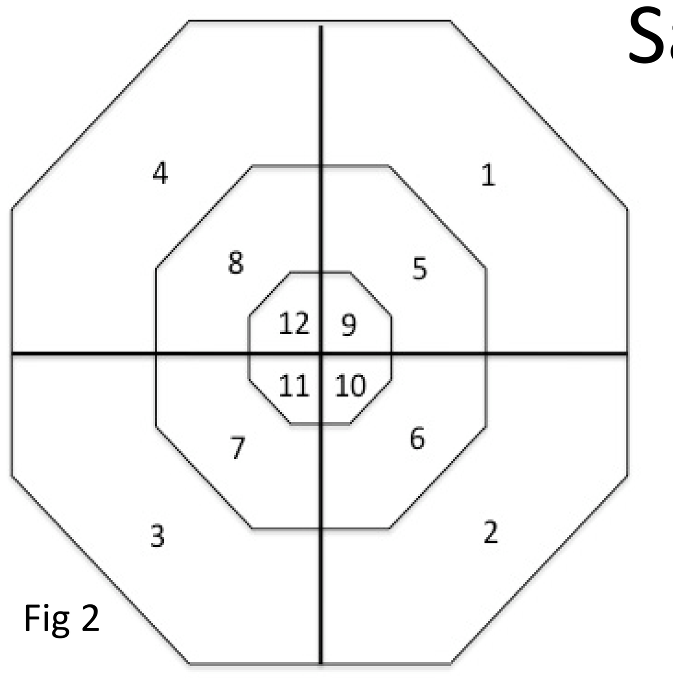

Fig 2

Salinity Readings:

Soil samples were collected from:

- Catalina Cove
- Parson's Landing
- Little Harbor
- Shark Harbor
- Hill above Shark Harbor
- Ben Westin

Salinity levels were measured using an EC meter

RAD Sequencing:

RAD sequencing uses restriction enzymes to simplify complex genomes so that target genes can be effectively sequenced using an Illumina sequencer, and various adapters and barcodes.

Seed Prep: Scarify → Sterilize → Inbibe → Germinate

↓

Plant in a/c formiculite and Cassion Nutrient Solution → Lyse Plant Tissue → Extract & Purify DNA

↓

RAD Sequencing Protocol

1. GBS 96-Plex Protocol
2. Illumina Sequencing

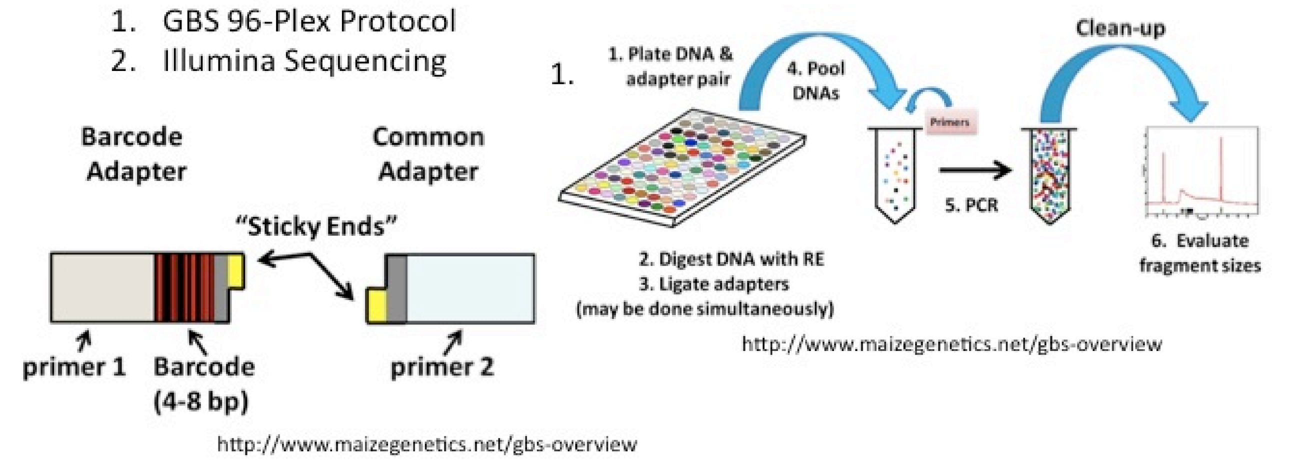

Figure 3, below, shows the pods from Starlight Beach, ranging from spineless on the left to very spiny on the right, whose seeds have been grown and will eventually undergo RAD sequencing. Letters identify the pod and numbers represent the spiny-ness rating from 1 to 10.

Fig 3

Results

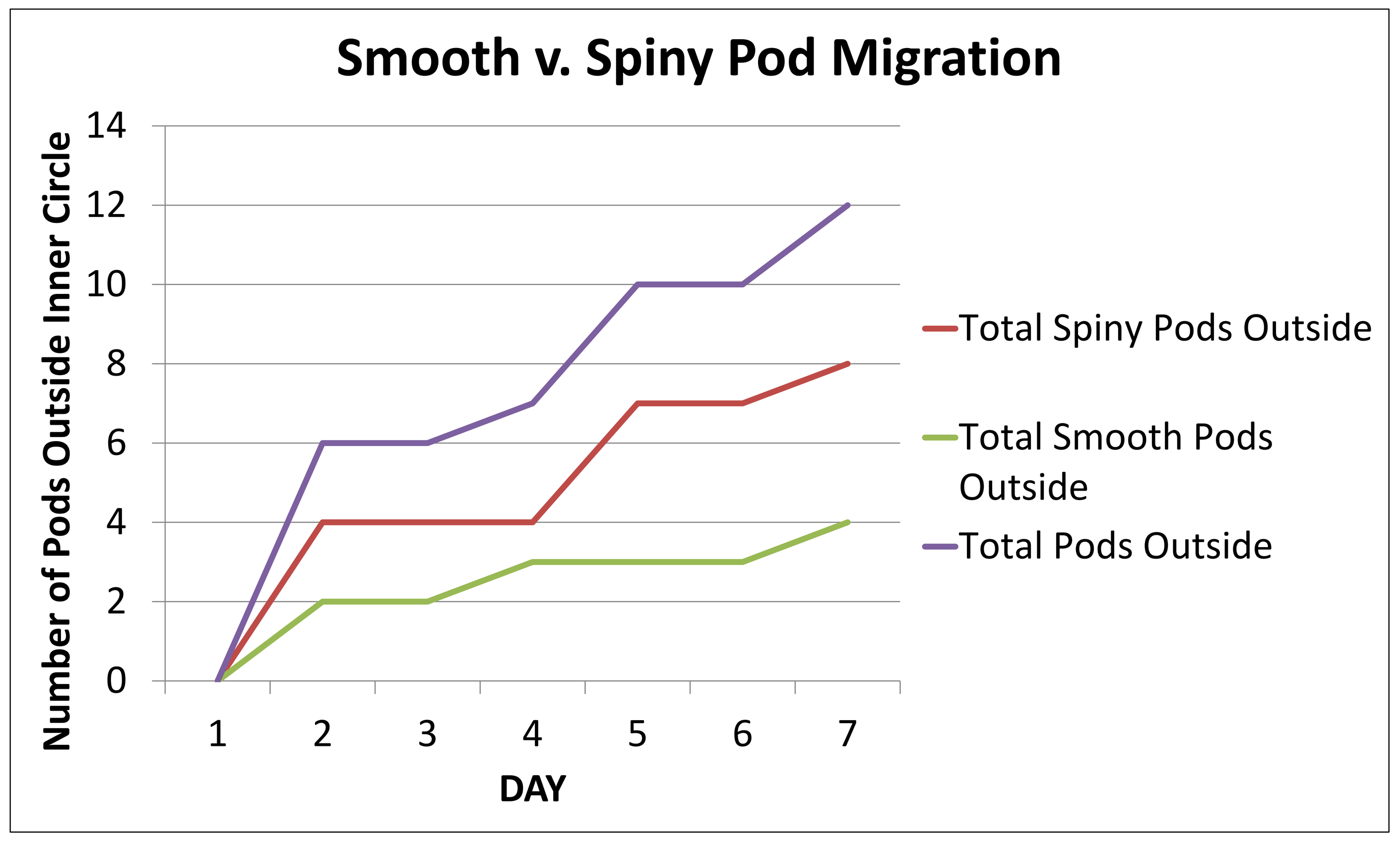

Fig 4

Figure 4 shows transect results after one week. Results are preliminary and show total numbers for all six transects. Twice as many spiny pods as smooth pods migrated outside the initial circle, supporting the hypothesis that spines help pods move farther.

Figure 5, right, is a picture of the pods at night under the UV light.

Fig 5

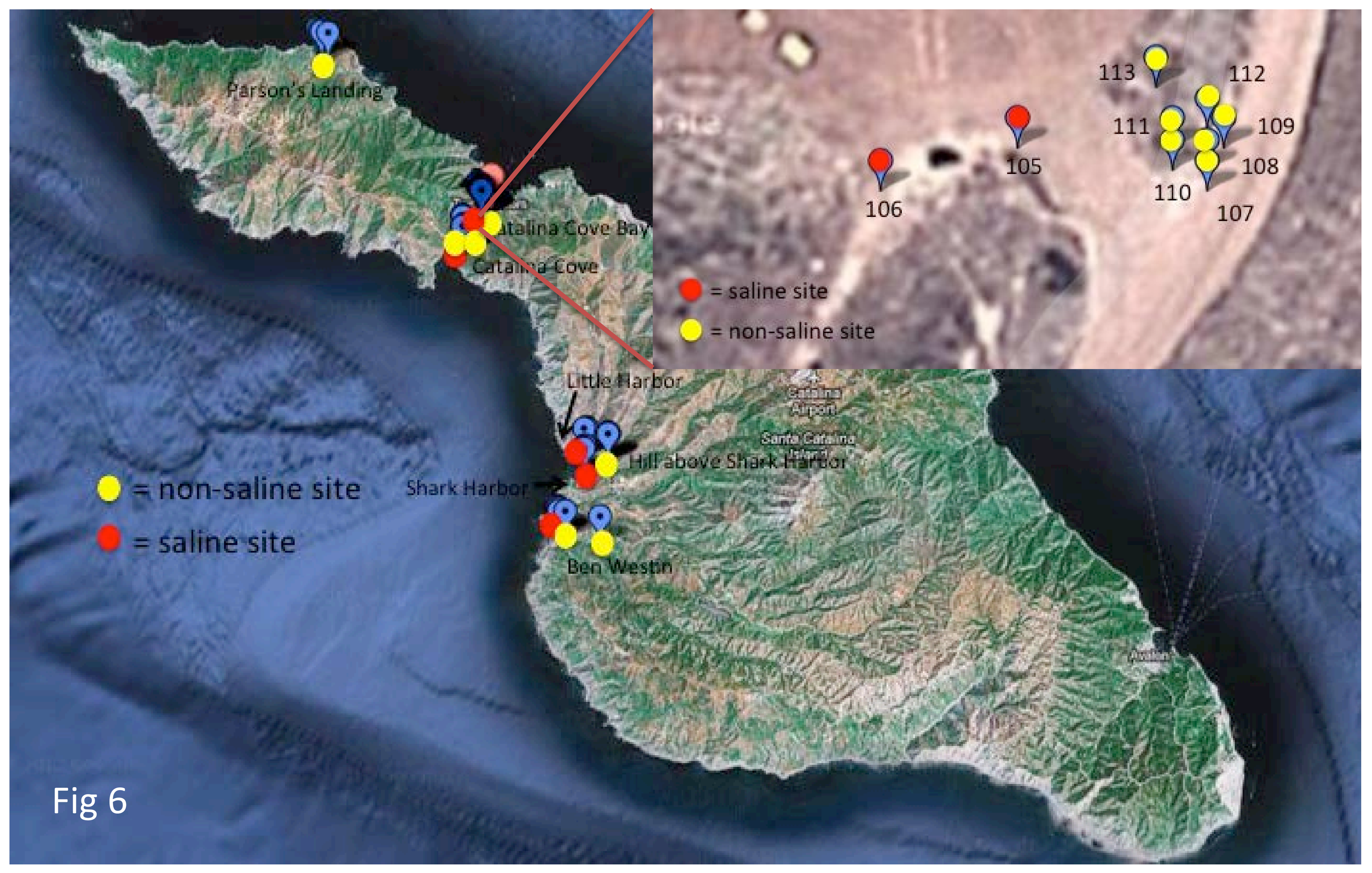

Fig 6

Table 1

Site	GPS #	EC Rdg
Catalina Cove	105	3240 µS
Catalina Cove	106	1991 µS
Catalina Cove	107	280 µS
Catalina Cove	108	175.6 µS
Catalina Cove	109	209 µs
Catalina Cove	110	212 µS
Catalina Cove	111	191 µS
Catalina Cove	112	247 µS
Catalina Cove	113	111.2 µS

Figure 6, above, shows salinity results of soil samples tested from around Catalina. There was much variation in salinity levels even in one site, as shown in the table and the inset map, which is a zoomed in portion of Catalina Cove. GPS points 105 and 106 are lower in the valley, while the others are higher on the hill.

Discussion

Salinity data has shown that *Medicago* grows in both saline and non-saline soils that are sometimes in the same area. This means that rate of pod dispersal is important because *Medicago* must be able to adapt to different environments if carried elsewhere, and that pod spine length could be an important factor affecting this.

Seeds with different morphologies are currently growing, so RAD sequencing is the next step in determining the genes that control spine length. The transect data will continue being collected in order to obtain more conclusive results.