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Phragmites australis haplotypes present in two populations on San Salvador Island, Bahamas

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HNR 499 – Dr. Timothy Evans

Abstract

Phragmites australis, common reed, is an invasive species present throughout North America. It is also found on San Salvador Island in the Bahamas. The spread of *Phragmites* is a serious issue for the health and biodiversity of wetland habitats throughout the world, but invasive haplotypes are threatening such communities as they choke out other species, including native *Phragmites*. This study sought to discover the *Phragmites australis* haplotypes present on San Salvador Island, and consequently determine the risk of invasion for the island wetlands. Of the 10 samples from two separate sites that were taken, all were the Gulf Coast (I) haplotype, one that is likely native to the region. Therefore it is not likely that the *Phragmites* populations on San Salvador Island are currently under invasion by nonnative haplotypes.

Introduction

Wetlands are one of the most important ecosystems in North America, and exotic invasive species pose the second greatest threat to their species diversity (Keddy 2000). However, defining specific factors that affect invasive and native species distribution is difficult (Zuiderveen, in press). *Phragmites australis*, known as common reed, is a grass with both native and nonnative genetic forms. *P. australis* is one of the most prevalent species in US coastal wetlands, and is considered the most invasive plant species in wetland regions along the East Coast (Rudrappa et al. 2007). This perennial wetland grass can tolerate both freshwater and brackish conditions (Saltonstall 2003c). It is predominantly found along lakes, ponds and rivers and is present in the Bahamas on San Salvador Island. However, no studies have been conducted to identify which haplotypes are present in this area.

The spread of *Phragmites* has been due to both anthropogenic and natural causes, including wrack, marine vegetation that has been cast ashore, and edaphic factors (Minchinton

2002). Due to its harmful spreading, the plant is used as an indicator of wetland disturbance, and has been described by Zuiderveen (in press) as a nuisance species, despite its previously minor presence in marshes. Through its tolerant seeds and rhizomes, *Phragmites* is able to colonize areas quickly, forming dense monocultures (Saltonstall 2003c). The invasive form is detrimental to wetland ecosystems, as it chokes out other species and replaces biodiversity with single species communities (Rudrappa et al. 2007). While control efforts have been made, the nondiscriminatory removal of the species results in the destruction of not only the invasive form but also native haplotypes and other biologically important species (Saltonstall 2003a).

Several different nonnative genetic varieties of *Phragmites* exist, each with a specific set of markers in its chloroplast genome. Although a cryptic invader, meaning that the distinct *Phragmites* haplotypes are difficult to tell apart visually, genotyping is a reliable way to discern among the different forms. Sequencing of two chloroplast DNA (cpDNA) markers allows for the separation among types, as variation has been found in chloroplast DNA at the intraspecific level, and a total of 27 haplotypes have been identified worldwide (Saltonstall 2002). Based on the sequencing of this cpDNA, Saltonstall (2002) concluded that a single, nonnative lineage of *Phragmites* was introduced to North America, likely causing a rampant invasion of native *Phragmites* habitats. Through this, five different cpDNA mutations were found to distinguish between native and invasive haplotypes. The goal of this study is to use the differences in the genome to identify the haplotypes that are present on San Salvador Island, and to determine whether or not the communities on the island are at risk, or currently suffering from, its invasion.

Methods

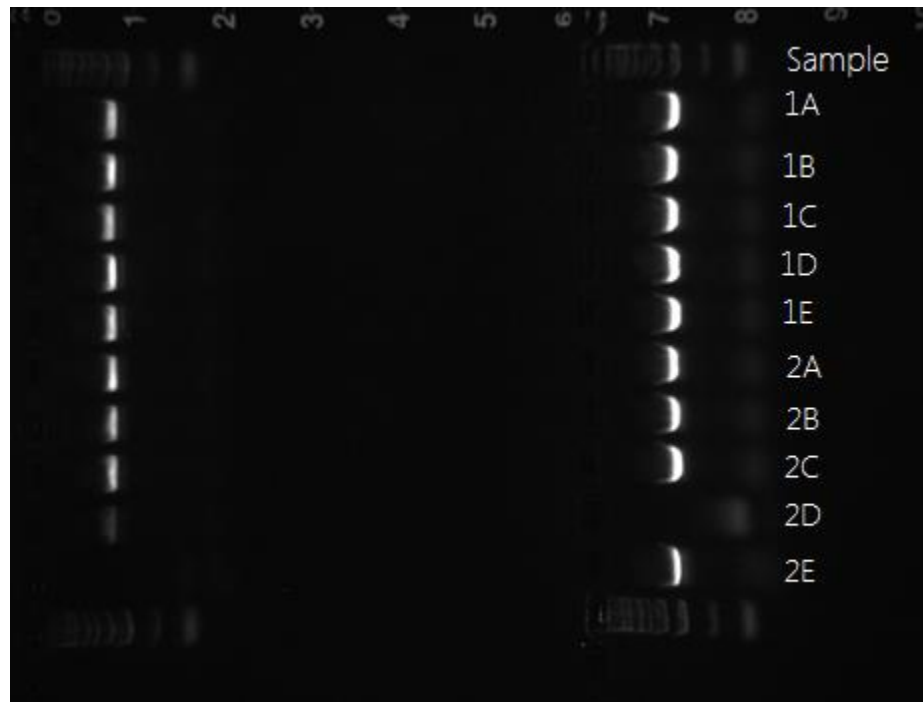
We collected *P. australis* samples from two distinct populations on San Salvador, one at the northern and one at the southern edge of the island. Five leaf samples, each from an individual plant, were taken from each location and immediately desiccated in bags of silica gel. The leaf samples came from flowering specimens with visible reproductive structures. Additionally, a corresponding herbarium sample was cut down, pressed and dried for the Grand Valley State University herbarium. Both sample sites were roadside communities. The northern site was denser, monospecific and adjacent to a small lake. The southern site was less dense, with various plant species interspersed within the *Phragmites* population.

We extracted DNA from each leaf tissue sample using the Qiagen DNeasy Plant Mini Kit. Once extracted, the chloroplast genes *trnL* and *rbcL* were amplified using the primers defined in Saltonstall (2002). Afterward, we digested the *trnL* and *rbcL* fragments with restriction enzymes *RsaI* and *HhaI* respectively, and then distinguished among the possible haplotypes using a 2% agarose gel.

Results:

All ten samples collected represented members of the Gulf Coast Haplotype (I). The fragments for both the *trnL* gene and the *rbcL* gene fall, uncut, at approximately 350 base pairs, the fragment size Saltonstall (2003a) found to be present when these two genes remained uncut by the *HhaI* and *RsaI* enzymes. Of the possible haplotypes, the Gulf Coast (I) is the only one that results in both genes remaining uncut by their respective enzymes. The gene *rbcL* for sample 2D did not amplify, but it is reasonable to assume that this sample would be of the same haplotype as all the other plants from its same population, and that of the population at site 1.

Figure 1: Agarose gel showing the *trnL* (left) and *rbcL* (right) genes of 10 *P. australis* samples from two sites. Numbers represent different populations (1 and 2) and letters represent individuals from these populations.



Discussion:

Both populations of *Phragmites* appeared to be of a single form, the Gulf Coast (I) haplotype, as they were not cut by either restriction enzyme. Not only were the haplotypes the same among all individuals sampled within the populations, but the same haplotype was represented in both populations. This is not to say that this is the only haplotype present on the island of San Salvador, but it certainly is worth noting that, despite the varying conditions and location between the populations, there was no difference in this portion of the genetic makeup.

Although the Gulf Coast haplotype cannot be definitively assigned as native or introduced to North America, as it is also found in South American and Asia, it is possible that it

originated in this region (Saltonstall 2002). Therefore, its presence likely indicates that the wetland habitats of *Phragmites* have not yet been disturbed by invasive forms. Haplotype I is genetically distinct from all North American native forms as well as the introduced haplotype (M) (Saltonstall 2002). This is promising for the ecological health of region, as the invasive haplotypes can cause serious damage to the biodiversity of habitats by choking out the native species (Rudrappa *et al.* 2007). It is not possible to discern whether or not a species will become invasive when it enters a new community, but genetic diversity in a species can play a role in determining possible outcomes. Nonnative genotypes may have unseen, genetic advantages, and thus continued vigilance on the haplotypes of *Phragmites* is important for health among wetland communities (Saltonstall 2002).

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