ASSESSMENT OF GENETIC VARIATION IN *PHRAGMITES AUSTRALIS* POPULATIONS ALONG THE HUDSON RIVER USING INTER SIMPLE SEQUENCE REPEAT (ISSR) ANALYSIS

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

Phragmites australis (common reed) has a cosmopolitan distribution and is abundant in marsh lands and along the borders of lakes, ponds, and rivers. It is a perennial grass that reproduces primarily through vegetative growth and sometimes through seed dispersal. Fossil records show that common reed has been present in the United State for 40,000 years. Over the past 150 years its distribution and relative abundance has increased dramatically, particularly along the Atlantic coast. Botanical records suggest that prior to the 1900's, common reed was not common. Today it is distributed all over the mainland U.S. as well as through southern Canada and is considered an indicator of wetland disturbance. It has been suggested that its rapid expansion could be the result of human activities causing habitat disturbances and/or stresses such as pollution, changes in hydrologic regimes, increased soil salinity and the introduction of aggressive genotypes. We hypothesized that natural populations of common reed have high levels of genetic variation that have allowed the species to expand its range into a variety of sediment habitats, including freshwater-to-saline and polluted- to-non-polluted environments. Three Inter Simple Sequence Repeat (ISSR) primers were used to characterize 153 culms from a total of 8 sites along the Hudson River, Rye, Berry's Creek, and Staten Island. ISSR banding patterns indicated that there are high levels of genetic variation within and among populations. None of the collection sites were clonal, suggesting that seed dispersal may be a more important mechanism for dispersal than previously thought. A UPGMA phenogram also suggests that populations from brackish and saline sediments were more closely related to each other than those from freshwater environments. The common reed populations from the more northern collection sites, Tivoli Bays, the Iona Marsh and Constitution Marsh had the most unique ISSR banding patterns.

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INTRODUCTION

Phragmites australis (giant reed or common reed) is a tall perennial grass with a world-wide distribution and is considered one of the most productive species of plants (Hartog 1989). Today it is distributed all over the mainland United States as well as through southern Canada (Wilcox et al. 2003). In many areas it has become the dominant plant of freshwater and brackish marshes at the exclusion of other species such as *Typha spp.* and *Spartina spp.* (Stanne et al. 1996). The common reed frequently grows in a monoculture, thereby reducing the biodiversity of wetland habitats. Even though fossil records show that the plant has been in the U.S. for about 40,000 years, its abundance has increased dramatically since the early 1900's (Saltonstall 2002).

This plant has had great ecological success because it can colonize disturbed soils rapidly and alters the habitat to promote its own growth. It can reproduce through vegetative growth of vertical and horizontal rhizomes and dispersal of seed and rhizome fragments (Wilcox et al. 2003). It has been reported that common reed modifies the sulfide, carbon, oxygen and moisture characteristics of soils as its populations become established (Osgood et al. 2002). Once established, common reed populations can maintain dense stands (200-300 culms/m^2) through the propagation of its rhizomes. Biomass of the rhizomes can exceed the biomass of living plants, resulting in a thick mat that not even new common reed can penetrate (Haslam 1971). Not only does common reed affect the type of plants that grow in its region, it also affects the animal life as well. A study done by Wilcox et al. (2003) suggests that common reed has greatly reduced the number of ducks, waders, gulls, and terns along Lake Erie, Ontario. Many researchers agree that this plant is a nuisance species and reduces the biodiversity of wetlands (Marks et al. 1994, Chambers 1999, Myerson et al.

2000). Many regions around the country have started to implement programs to monitor and reduce the expansion of the common reed because it is perceived to be a threat to important endemic food resources and habitats. However, not all agree that such action is needed, as many species do use common reed as a habitat (Kiviat and MacDonald 2003).

Common reed has at least 42 different phenotypes (Marks et al. 1999) and high levels of genetic variability have been observed among populations (Okoli 1997, Zeidler 1994, Pellegrin and Hauber 1999). Zeidler (1994) observed high levels of among population variation using three Restriction Fragment Length Polymorphism (RFLP) probes and four restriction enzymes. Sixteen populations were studied in a small geographic area along the shoreline of Lake Ammersee, Germany. They observed eight highly differentiated genets (genetically unique individuals within modular growing plants) and the populations were highly structured.

Pellegrin and Hauber (1999) examined 37 common reed populations using allozyme electrophoresis around the U.S. but primarily focused their attention to the populations in the South and Gulf Coast states. Overall, they observed high levels of variability [consistent with those of Zeidler (1994)], identifying 21 multilocus genotypes. Levels of genetic variation were highest along the East Coast and among populations in the Midwest. Zeidler's results suggest that populations of common reed from the east coast are older and that the populations in the South may have a separate origin.

It has been hypothesized that the dramatic increase in common reed has been due to an invasive variety. Recent genetic studies have focused on testing this hypothesis. Research done by Saltonstall (2002) using chloroplast DNA (cpDNA) confirmed that non-native varieties of common reed have spread through the U.S. Saltonstall sequenced two

non-coding regions of the cpDNA from samples collected around the world and from historical herbarium collections. Sequencing results strongly suggested that an aggressive invasive variety (cpDNA haplotype M) from Europe and Asia has dramatically displaced many native U.S. varieties since the 1940s (Saltonstall 2002). The invasive varieties are most prominent in the northeastern parts of the U.S. where they displaced most native genotypes. In most plants, cpDNA is maternally inherited and evolves at a slow rate. Therefore, cpDNA studies may leave an incomplete assessment of the genetic structure of the population and cannot be used to assess if the European/Asian varieties formed hybrids with native plants.

Saltonstall (2003) addressed these problems by examining 10 microsatellite loci. The results of this study were consistent with the cpDNA study in that populations from the different continents were highly structured. The North American populations were similar to European samples. Moreover, there did not appear to be evidence of extensive hybridization between native and non-native genotypes. The highest levels of genetic variation were observed among the Atlantic Coast populations, suggesting that the invasive varieties moved east to west (Saltonstall 2003).

None of these studies examined common reed populations from the Hudson River. The Hudson River estuary provides a variety of habitats, salinities, and soil types across its geographic range. It is also located in the region that should have high levels of genetic variation. There is the potential for common reed genotypes to form structured populations as those observed in the Lake Ammersee study (Zeidler 1994). In theory, genetic variation allows populations to adapt to a variety of local conditions. It is reasonable to assume that common reed samples collected from saltwater, freshwater, polluted, and non-polluted environments will be composed of different genets.

We hypothesized that high levels of genetic variability in common reed populations have allowed them to adapt to a variety of conditions found along the Hudson River. In addition, their life history patterns of reproducing rapidly and by vegetative growth will allow the species to form highly structured populations. Common reed populations were examined using Inter Simple Sequence Repeat Analysis (ISSR). ISSR markers were first published in 1994 (Zietkiewicz et al. 1994, Gupta et al. 1994). ISSR is a PCR based technique that amplifies hypervariable regions from nuclear DNA. ISSR primers are designed to work at higher annealing temperatures than RAPDs, thus avoiding many of the problems associated with RAPDs. ISSR has tremendous potential for studies on natural plant populations because it is inexpensive and easy to do (Wolfe and Liston 1998).

METHODS

Common reed samples were collected from eight sites in and near the Hudson River (Table 1). Levels of genetic variation were examined using Inter Simple Sequence Repeat analysis (ISSR). Twenty 20 to 25 culms were collected per site. Each sampling site was divided into 1 to 4 sub-sites and samples were taken along 10-30 meter transect. Leaves were stored at -70°C until processing.

One-hundred milligrams of plant tissue was ground in liquid nitrogen to fine powder in a mortar. DNA was extracted from ground tissue using plant DNeasy extraction kit according to the manufactures protocol (Qiagen Inc.). An ISSR primer kit was obtained from the University of British Columbia (UBC). Twenty UBC ISSR primers were tested to determine if they would be useful in examining population level differences in common reed. Three primers sets, 811, 851, and 857 were optimized to examine common reed from the

eight sites listed in Table 1. PCR was carried out using approximately 50 ng of DNA, 1μM of primer and dNTPs, 10X buffer and Taq polymerase according to manufacturers' recommendations (Promega Inc.) The PCR cycling conditions were as follows: 95°C for 5 minutes, 35 cycles of 95°C for 1 minute, 50°C for 1 minute and 72°C for 2 minutes, with a final extension of 72 °C for 7 minutes.

ISSR bands were scored on a present or absent basis from photographs of ethidium bromide stained agarose gels. Levels of genetic diversity, exact tests of population differentiation and a UPGMA phenogram using 1000 bootstrap replicates were constructed using the program TFPGA (Miller 1997).

Table 1: Sampling Sites and Number (N) of Culms Sampled per Site

Sites (N)	Habitat
Tivoli Bays (15)	Fresh Water
Constitution Island Marsh (23)	Fresh Water
Iona Island Marsh (20)	Fresh Water
Piermont Marsh (20)	Fresh Water
Berry's Creek (19)	Brackish
Passiac River (19)	Brackish
Rye Marsh (19)	Salt Water
Staten Island (18)	Salt Water

RESULTS

DNA was amplified from a total of 153 culms from the eight sites. Although a minimum of 20 culms were collected, fifteen to twenty-three culms per site yielded high quality DNA needed for the PCR reactions. A total of 27 fragments were observed using the three ISSR primers. Figure 1 shows an example of an ethidium bromide stained agarose gel.

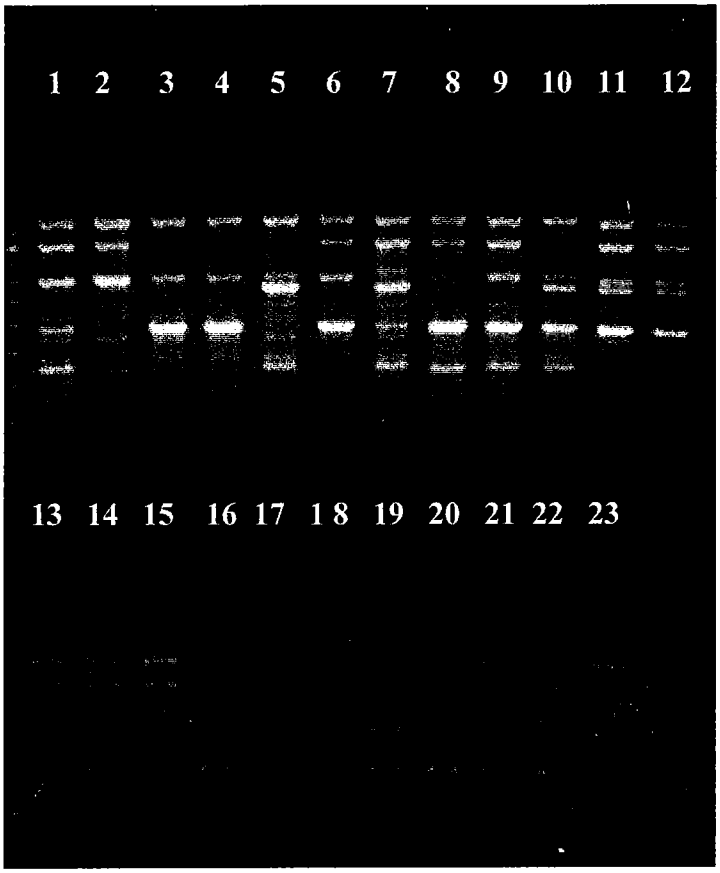

Figure 1. Ethidium bromide stained gel of 23 common reed culms using ISSR primer 857. Culms were isolated from Rye (lanes 1-5), Passaic (lanes 6-12), Berry's Creek (lanes 13-17) and Richmond Creek (lanes 18-23).

The ISSR banding patterns suggest there are high levels of genetic variation within and between sites. No collection was composed of fewer than seven clones. The number of genets per site ranged from 7 to 15. A pair-wise genetic distance matrix was constructed using TFPGA (Miller 1997). The genetic distance values ranged from 0.0483 to 0.3256. Table 2 illustrates the pair-wise genetic distance values were lowest among comparisons from collection sites south of the Tapan Zee Bridge. The Iona and Constitution Marshes had

the highest pair-wise genetic distance values of all sites examined. Exact tests of population differentiation were highly significant among all pair-wise comparisons (Table 2).

Table 2. Pair-wise matrix of Nei's genetic distance and exact test of population differentiation. Genetic distance values are shown above the diagonal and P-values for the exact test are shown below the diagonal.

	Rye	Passaic	Berry's	Tivoli	Richmond	Piermont	Iona	Constitution
Rye	*****	0.0519	0.0483	0.0797	0.0179	0.0804	0.1526	0.1802
Passaic	0.0002	*****	0.0888	0.0816	0.0553	0.1227	0.2299	0.1847
Berry's	0.0000	0.0000	*****	0.1704	0.0627	0.0537	0.1764	0.1892
Tivoli	0.0000	0.0000	0.0000	*****	0.0921	0.2389	0.3585	0.2691
Richmond	0.0151	0.0082	0.0000	0.0000	*****	0.0873	0.1125	0.1474
Piermont	0.0000	0.0000	0.0000	0.0000	0.0000	*****	0.1338	0.1736
Iona	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*****	0.3256
Constitution	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*****

The ISSR banding patterns were used to construct a UPGMA phenogram of common reed populations (Figure 2). The bootstrap values on the UPGMA tree strongly supported (96%) the node connecting the Rye and Richmond populations. There was moderate support (57%) for the Berry's Creek and Piermont node. The remaining bootstrap values were below 50% indicating strong population structuring among the other collection sites.

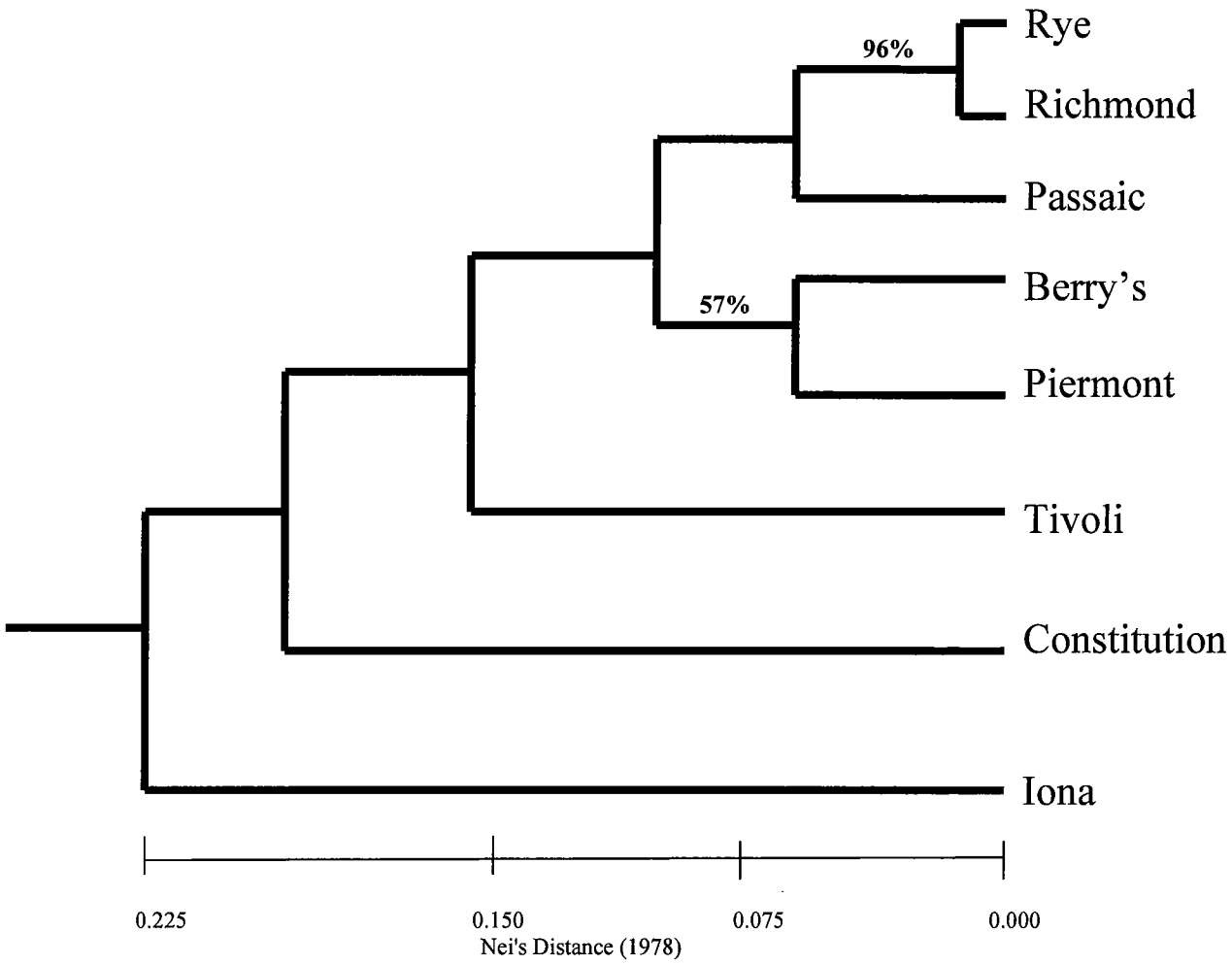

Figure 2: UPGMA Tree of common reed populations based on ISSR Data

DISCUSSION

This study supports the hypothesis that common reed populations have high levels of genetic variation and are highly structured. The sampling strategy employed maximized the likelihood of uncovering genetic variation within each sampling site. Each site was sub-divided based on field observations of differences in terrain and by looking for areas where there was separation among stands of common reed. The high genetic distance values observed in pair-wise comparisons were similar to the values reported by the European studies on common reed (Zeidler 1994, Koppitz 1999). These studies used DNA fingerprinting and RAPDs, two genetic markers that evolve rapidly and should be appropriate in examining population level differences. Exact tests of population differentiation strongly suggest that the eight sampling sites in this study were genetically distinct from one another. It was expected that populations around the Hudson River would be different due to the variety of habitats found along the estuary. Sediments will have a variety of salinities, nutrients and pollutants. Perhaps certain genotypes will grow better under different environmental conditions and the variety of habitats along the Hudson River allows a great number of genotypes to survive. However, the high-degree of structuring was unexpected between sites in close proximity such as Piermont and Iona and Berry's Creek and Passaic.

The UPGMA phenogram suggests there are groupings that could be the focus of future studies. Rye and Richmond were grouped together in 96% of the bootstrap replicates. These two sampling sites are in a salt-water tidal marsh and their grouping suggests that the habitat's salinity plays a role in the selection of certain genotypes. Additionally, the

UPGMA tree grouped the northern populations (Constitution, Tivoli and Iona) from the more southern populations (Rye, Richmond, Passaic, Berry's and Piermont). These habitats south of the Tappan Zee Bridge have higher levels of salinity and different types of pollutants than the more northern sites. However, these relationships should be studied with additional ISSR primers and other genetic markers before any definitive conclusions can be reached.

The most recent genetic studies of common reed in the U.S. have not focused on examining population level differences. These studies examined the question of whether there has been a cryptic invasion in the U.S. by European and Asian varieties (Saltonstall 2002, Saltonstall 2003). Pellegrin and Hauber (1999) used isozyme electrophoresis to examine population-level differences among common reed populations in the southeast and Gulf Coast. Although this study did observe high levels of genetic variation, the ISSR markers used by this study uncovered greater levels of variation, most probably due to the higher mutation rate of the microsatellite regions that ISSR primers amplify.

A surprising outcome of the ISSR data is that common reed populations are not composed of single or even a few genets. Most studies have concluded that common reed reproduces primarily through vegetative growth and thus most collection sites should be composed of one or a few individuals. Our findings suggest that seed dispersal could be a more important route of reproduction than previously thought. Based on our data, it is possible that a disturbed area maybe colonized by a great number of seeds, causing high levels of genetic variation within the site. Over time some genets would be lost but the surviving genets would become intermingled within the site and thus yield high levels of within site variation. This conclusion is consistent with the observations of Koppitz (1999) in that different clones co-exist next to each other. Also, Koppitz found in a more recent

study (2000) that many different clones were observed within newly developing common reed populations. She suggested that over time, sites become dominated by one or a few clones that adapt to the conditions in that habitat. In light of these findings, our study suggests that the high levels of genetic variation observed within the Hudson River populations indicate that they are newly developing populations.

In conclusion, common reed has become the dominant plant in many habitats along the Hudson River, much to the dismay of wildlife enthusiasts because it offers little cover or food for animals. One of the many species it affects is the narrow-leaved cattail, *Typha angustifolia* (Stanne et al. 1996). The narrow- leaved cattail is a dominant plant in the marshes of the Hudson River. Its rhizomes are a primary food for many animals such as muskrats. The population genetic structure of common reed must be clearly understood as part of a monitoring program for this nuisance species. Furthermore, clear understanding of its population genetics may elucidate mechanisms by which it has been able to spread so rapidly. The results of this study suggest that there are high levels of genetic variation within and between the sites. Controlling and monitoring this species could be much harder than expected due to common reed potential to adapt to different environments.

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