Assessing Differences in Genetic Variation Among Invasive/Non-native and Native Populations of *Andropogon virginicus*

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Abstract

Introduced populations may be less genetically variable than native populations because of the founder effect. Alternatively, if they are invasive and spread widely, they may be more variable, allowing them to exploit a wide range of environments. We examined genetic variation in native and non-native populations of *Andropogon virginicus* (broomsedge), a common grass native to the eastern U.S., which is naturalizing in California's Central Valley and invasive in Hawaii. We extracted DNA from plants grown from field-collected seed, and conducted PCR using ISSR primers on 20 individuals each in 4-8 populations from each region, with a total of 19 polymorphic loci. We calculated the percent polymorphic loci and Nei's gene diversity for each population. Hawaiian populations had somewhat lower percent polymorphism (means: native, 44.9%; California, 49.1%, Hawaii 31.4%), and included the lowest values (21.1% in two of the four populations). However, ANOVA did not detect significant variation among the regions (P=0.18). The gene diversity results also indicated no difference among the three regions (means: native, 0.12; California, 0.14; Hawaii, 0.11). Hawaii is very remote from the native range, and a strong founder effect may be expected. However, broomsedge grows there in variable niches ranging from xeric lava flows to pastures and submontane forests, so a high level of genetic variation may also be expected. In California, broomsedge is found in the seasonally dry Central Valley, where it is restricted to wet soils of riparian zones, seeps, and lakeshores. This narrower niche predicts less genetic variation. More ISSR loci will provide additional data to further test these predictions.

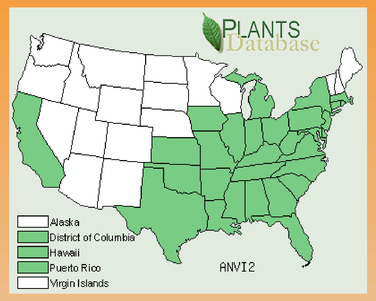
Introduction

Invasion ecology is an important field of biology that benefits from a population genetics approach. In order to better understand the factors that determine plant invasiveness, we are characterizing the fundamental genetic features of native vs. non-native/invasive grass populations. We aim to determine the role of genetic diversity in the invasion processes within natural plant populations.

We are analyzing *Andropogon virginicus*, a C_4 grass that is a widespread, ecologically dominant species in many open field communities in the eastern U.S. It has been introduced to California, where it is naturalizing, and to Hawaii, where it is considered an invasive species that promotes fire in montane woodlands. In both these areas, invasion by this perennial grass decreases usable rangeland, due to the plant's unpalatability. The widespread distribution of *A. virginicus* and its significant influence on community structure make it an ideal model species for a study of invasion ecology. By assessing genetic diversity within non-native populations and comparing it to that of native east coast populations, we will determine whether invasive populations are more or less genetically homogenous than native populations.

We hypothesized two possible situations for population genetic diversity levels. 1) Genetic diversity may be lower in non-native than in native populations due to a founder effect. 2) Alternatively, high genetic diversity levels in non-native populations may help to facilitate invasion, by allowing for a greater ability to exploit a wide range of niches.

We assessed the level of genetic diversity within a population using Inter-Simple-Sequence-Repeats (ISSRs). We grew plants from field-collected seed taken from eight native populations ranging from Pennsylvania to North Carolina, four California populations from the Central Valley, and four Hawaiian populations from the Big Island and Maui. After extracting DNA, we did PCR with two ISSR primers to generate several different fingerprints for each individual. We used these data to determine two measures of population genetic diversity: percent polymorphism and Nei's genetic diversity.



Current range of *A. virginicus*, showing both native and non-native populations.

Native old-field population in Virginia

Non-native population in California, along water course

Non-native population in Hawaii Volcanoes National Park, by steam vents

Methods

Plant collection:

- Systematically collected mature fruiting stalks of *A. virginicus* plants in 2006 from a series of locations throughout the eastern states, California, and Hawaii; removed the seeds and stored in the light at 4^0C until planting in the greenhouse.

Seed germination and plant culture:

- Chose eight populations from eastern states, and four each from California and Hawaii).
- Planted seeds from 36 individual mother plants from each population in flats of pots containing soil planting mix; placed the flats into trays and sub-irrigated for continuous moisture. Plants grew for several months in the greenhouse until adequate foliage was available for DNA extraction.

DNA extraction:

- Extracted genomic DNA from 20 plants per population using the DNeasy extraction kit (Qiagen); stored at -20°C.

ISSR Analysis:

- Conducted PCR (polymerase chain reaction) with two ISSR primers so far, "ISSR2" (5'-gagagagagagagagayc-3') and "ISSR10" (5'-aacaacaacaacaacaacaacaac-3'); used Promega GoTaq Green Master Mix in 25 uL reaction volumes.
- Separated PCR products with agarose gel electrophoresis and imaged the resulting bands under UV light.
- Visually scored band presence/absence, counting as present only those bands that appeared in two replicate PCRs
- Analyzed loci using the program AFLP-SURV 1.0 to determine percent polymorphism and Nei's gene diversity within populations.

Left: *Andropogon virginicus plant.*
Top: planting scheme set up for DNA extraction

Results:

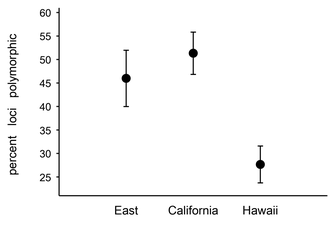


Figure 1. Percent polymorphic loci

Mean ± SE of the populations' values of percent of loci that were polymorphic across 20 individuals (sample sizes: eastern, n = 8 ; California and Hawaii, n = 4). Results were derived from 19 polymorphic loci from 2 ISSR primers. Differences in percent polymorphism were analyzed with ANOVA on arcsine-transformed data.

There was no significant difference among the regions for percent polymorphism ($F_{(2,13)}$ = 4.09, P = 0.04. However, Hawaiian populations had the lowest mean value and included the lowest values measured (21.1% in two of the four Hawaiian populations).

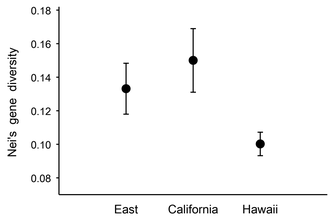


Figure 2. Nei's gene diversity

Mean ± SE of the populations' values of Nei's gene diversity, measured in the same samples as in Figure 1.

These data indicate a marginally significant difference in gene diversity among the regions ($F_{(2,13)}$= 2.19, P = 0.15, log10 transformed).

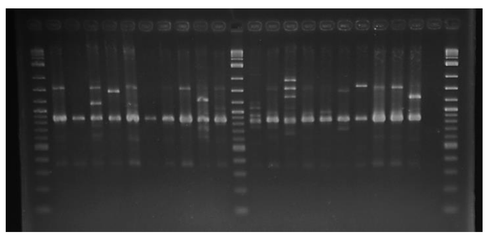


Figure 3. Sample gel

Example of a gel from this study, from Maui (population MA9). Each lane represents an individual plant (with the exception of the three ladders and the negative control in lanes 1, 12, 23, and 24) that has been run in a PCR reaction with the ISSR2 primer. The banding pattern created is the genetic finger print, with each band being a locus.

Discussion

Our results show that non-native, invasive populations of *A. virginicus* in Hawaii have significantly less genetic variation than do the native eastern populations and the naturalizing California populations.

Two possible causes of reduced genetic variation in Hawaii are likely. First, since Hawaii is a remote archipelago there may have been a genetic bottleneck during the initial introduction of this species, in which the few genotypes of founding individuals play a larger role in the overall genetic makeup of the population. Second, the physical niche available to *A. virginicus* in Hawaii is more restricted than on the mainland. In large areas where it is found, the substrate consists of relatively unweathered, xeric lava flows and fire frequency is high. Only genotypes that can withstand these conditions will survive.

Californian *A. virginicus* are found in the seasonally dry Central Valley, where plants are restricted to growing alongside rivers and lakes and other low-lying areas, in order to obtain adequate moisture year-round. This requirement should predict a lower level of gene diversity among Californian populations due to a narrower niche than in the native range. While this may have been the case initially, long periods of time post-introduction and/or multiple introduction events from several areas, may have allowed genetic diversity to increase back to levels similar to those of native populations.

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