Comparison of genetic variation in healthy and diseased populations of the old-field grass *Andropogon virginicus*

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Abstract

Some natural plant populations may exhibit epidemic levels of disease while other populations of the same host species appear disease-free. Host population genetic structure may contribute to this variation in disease prevalence. Genetically homogeneous populations should be more likely to host disease because a pathogen that successfully infects a population with few, common host genotypes can spread easily and/or can readily evolve virulence on the common genotypes. We studied healthy and infected populations of *Andropogon virginicus* (broomsedge), a common old-field grass in the eastern U.S, where 50% of populations are infected with the smut fungus *Sporisorium ellisii*. This pathogen reduces or eliminates reproduction and increases mortality of *A. virginicus*, which also could result in lower host variation in diseased populations. We extracted DNA from plants grown from field-collected seed, did PCR for ISSR markers on 20 individuals each from four healthy and four infected populations, with 19 polymorphic loci, and calculated percent polymorphic loci and Nei's gene diversity for each population. Percent polymorphism ranged from 26-67% in the healthy populations and 26-63% in the infected populations, with no significant difference (means: healthy = 48%, infected = 42%). Gene diversity ranged from 0.08-0.15 in the healthy populations and 0.09-0.16 in the diseased populations, again with no significant difference (means: healthy = 0.125, diseased = 0.120). Infection of populations by *S. ellisii* appears not to be related to host genetic variation, although planned additional markers and populations will provide more information. Environmental variables may be stronger drivers of disease development in this plant-pathogen system.

Introduction

The study of plant-pathogen interactions in natural communities would benefit from the use of population genetics, yet little work has been done using this approach. In our study, we are characterizing and comparing the fundamental genetic features of populations of the grass *Andropogon virginicus* that in order to better understand the importance of host genetic diversity in its interaction with a smut fungus disease.

Andropogon virginicus is a C_4 grass that is a widespread, ecologically dominant species in many open field communities. In about half of the populations in its native range of the east coast of the United States, *A. virginicus* is infected with the smut fungus *Sporisorium ellisii*. This type of pathogen infects the grass inflorescence and reduces or eliminates seed production, ultimately decreasing reproduction while increasing mortality. We know that artificially homogenous populations, i.e. crop populations, are especially vulnerable to disease epidemics, but less is known about plant disease in the wild. We hypothesize that infected host populations are more genetically homogeneous than those that remain uninfected. Genetically homogeneous populations should be more likely to host disease because a pathogen that successfully infects a population with few, common host genotypes can spread easily and/or can readily evolve virulence on the common genotypes. By assessing the genetic diversity of infected and uninfected populations, we seek to understand the role that genetic diversity plays in plant pathogen interactions in the wild.

We can assess the level of genetic diversity within a population with an Inter-Simple-Sequence-Repeat, or ISSR, analysis. We grew plants from seeds that were collected from populations across the east coast that are both infected with *S. ellisii* and uninfected. We then extracted the DNA from these plants and performed an ISSR analysis using a variety of primers to generate several different fingerprints for each individual. This will allow us to see which populations have high genetic diversity and which have low genetic diversity.

Acknowledgements. We thank the U.S. Dept. of Agriculture, the Garden Club of America, The Mentored Undergraduate Summer Experience of The College of New Jersey, the TCNJ Department of Biology, the TCNJ School of Science, and the National Science Foundation's Undergraduate Research and Mentoring in the Biological Sciences program for funding. Thanks to Scott Morrison Melman for field assistance, and to TCNJ alumnus Lauren Frazee for developing ISSR protocols.

Methods

Plant collection :

• Systematically collected mature seed stalks of *A. virginicus* in 2006, from four infected and four healthy populations in the eastern U.S (PA, NJ, VA, and NC), removed the seeds and stored in the light at 4^{o} C until planting in the greenhouse.

Seed germination and plant culture :

• Planted seeds from 20 individual maternal plants from each population in flats 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 DNA from one offspring from each maternal plant per population using the DNeasy extraction kit (Qiagen); stored DNA at -20°C.

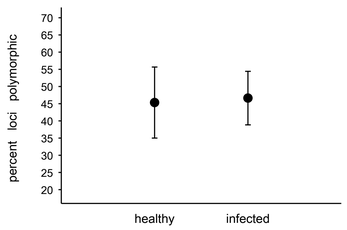
ISSR analysis :

• 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.

Infected

Uninfected

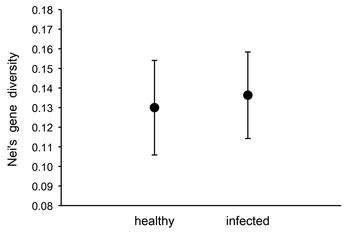
Results



Percent polymorphism.

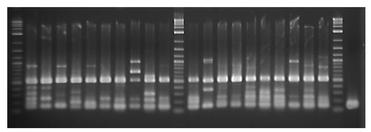
Genetic variation expressed as the percent of loci in a population that were polymorphic was very similar in *A. virginicus* populations that were healthy and those that were infected with the smut fungus (mean ± SE, n = 4).

Results were based on 19 polymorphic loci from 2 ISSR primers. The difference among healthy and infected populations was analyzed with analysis of variance (ANOVA), on arcsin-transformed data: $F_{(1,6)} = 0.01$, P = 0.92.

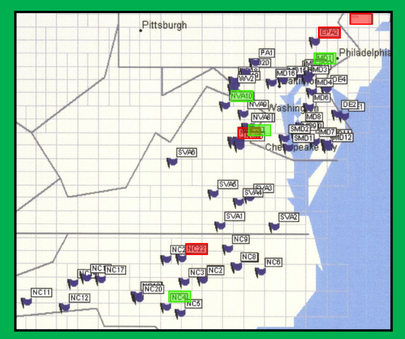


Nei's gene diversity

Average gene diversity per locus also exhibited no significant difference between the healthy and infected populations. ANOVA: ($F_{(1,6)} = 0.04$, P = 0.85



An example of a gel used in the study, from northern Virginia population NVA10. 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.



Sampling locations of *A. virginicus* populations. About 50% were infected. Red labels indicate infected populations used in this study; green labels indicate healthy populations used in this study.

Andropogon virginicus in a hillside pasture in North Carolina.

Discussion

Our results suggests that there is no difference in genetic diversity among *Andropogon virginicus* populations that are infected with *Sporisorium ellisii* and those that are uninfected. We hypothesized that infected populations would exhibit lower genetic diversity if development of the smut fungus disease in the host population depends on host genetic homogeneity, as is seen in agricultural plant populations. Instead, there must be other reasons why the smut fungus disease is only found in about half of all sampled populations. Plant-pathogen interactions in natural communities occur in a much more heterogeneous environment than in agricultural systems, so it is possible that environmental variables are strong drivers of variation in disease development among *A. virginicus* populations.

We also would expect infected populations to be less variable than healthy populations if the disease has selected against susceptible genotypes. The similarity of genetic diversity we observed suggests either that *Sporisorium ellisii* is not a strong selective agent, and/or that variation in the genome-wide ISSR markers does not correspond to possible resistance genes.

These data are based on just 19 loci from 2 ISSR primers, so we will continue to build our data set with additional primers to further examine our findings.