A Comparative Evaluation of the Genetic Structure and Variation within and among three Populations of *Sporisorium ellisii* using ISSR and SCoT Primer Sets.

Lisbeth Disla, Samhita Narendrula, Eva Yarsky, Waez Umer & Navya Yemula

Abstract

Sporisorium ellisii is a systemic, biotrophic parasite of *Andropogon virginicus*. It has not been widely studied as its host plant has no positive nor negative economic value and the fungus is not easily grown. This study investigates genetic differences between different populations of *S. ellisii*. Teliospores were collected from three populations of infested *A. virginicus* from New Jersey, Pennsylvania, and Ohio. PCR analysis was used to study the genetic structure of each population and indirectly asses gene flow among the three populations using ISSR and SCoT primers. AMOVA analysis was run to determine the genetic variance within and among the populations. The study found that there was evidence of genetic variation among the populations, but most variance occurred within populations suggesting little gene flow. Future research aims to investigate genetic recombination within the species using both molecular and microscopic techniques.

Introduction

The biology of putatively asexual *Sporisorium ellisii*, a smut fungus that is a systemic, biotrophic endo-parasite in *Andropogon virginicus* (Broomsedge), has not been examined. The host plant is a very common, early successional bunch grass found mostly in the eastern half of the U.S. This study elucidates the genetic structure of three populations of *S. ellisii* from three states (NJ, PA and OH; see Fig. 1). Microsatellite-based markers provide an informative means to assess the genetic structure of populations of organisms, although sequence targeted methods are increasingly replacing the standard "fingerprinting" techniques (e.g. RAPD, AFLP, ISSR). A study of the genetic structure, distribution of variance and estimation of gene flow was conducted using ISSR and SCoT primers. The latter have rarely, and only recently, been used for fungal population studies (Zhang, *et al*, 2014). The 18-mer primers are designed around the start codon with knowledge of certain, conserved flanking bases up- and downstream from the codon in plants (Bertrand and Mckill, 2009).

Methodology

Spores to DNA

- Collection sites: See Fig. 1
- Infected plant shoots stored at 4°C for ≥ 2 months
- Day 1: Spores were soaked in deionized water
- Day 2 and 3: surface spores were surface-sterilized and spread on PDA + streptomycin plates; the next day, the spore germlings were then plucked and subcultured onto PDA plates.
- Day 10: mycelial plugs were subcultured into PD broth (25°C shaker incubator)
- Day 17-20: DNA phenol extraction
- Day 21: Nanodrop samples and dilute to 12 ng/µl

PCR to band assessment/statistics

- ISSR and SCoT primers run X 24 ng DNA in 15 µl reactions with 0.2 µM primer 2X EmeraldAmp GT pcr master mix (takarabio.com).
- PCR-2 mins. @ 94°C, 30 secs @ 94°C, 45 secs. @ X°C (see Fig. 6), 90 secs. @ 72°C, return to step two 35X, 30 secs @ 94°C, 45 secs @ X°C, 5 mins. @ 72°C, 4°C forever
- 1.5% agarose gel; 80V X 70-80 mins.
- Clear, repeatable bands were identified using GelAnalyzer; no bands over 2000 bp were scored
- Bands were scored as present (1) or absent (0) and this binary data was entered in an excel sheet
- Excel was used to calculate PIC values for each primer
- the GenALEx program was used to run AMOVA

Results

Figure 1. *S. ellisii* collection sites: Monmouth Battlefield State Park in New Jersey (NJ-1 & 2A), Tyler State Park in Pennsylvania (TSP), & Rt. 70 near Zanesville, Ohio (OH)

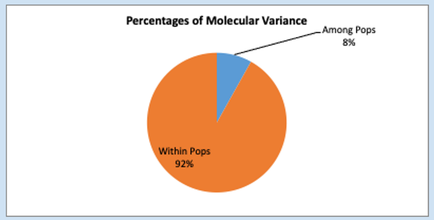

Figure 2. SCoT primer-based Analysis of Molecular Variation (AMOVA) of the genetic variance distributed within and among the Ohio, Tyler State Park, and NJ2A populations of *S. ellisii*.

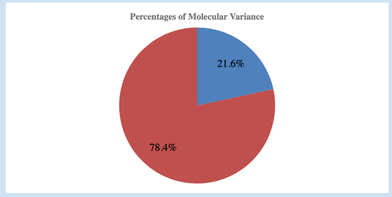

Figure 3. ISSR primer-based Analysis of Molecular Variation (AMOVA) of the genetic variance within and among (blue) the Ohio, Tyler State Park, and NJ2A populations of *S. ellisii*.

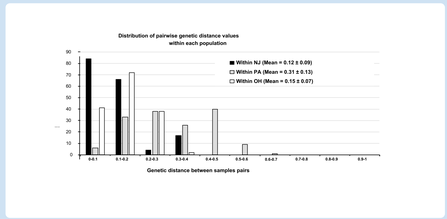

Figure 4. Distribution of pairwise Dice genetic distance values calculated for sample pairs from ISSR data: within NJ populations in Black; within PA populations in grey and within OH populations in white (Hervé Gryta, unpublished, based on data from this study)

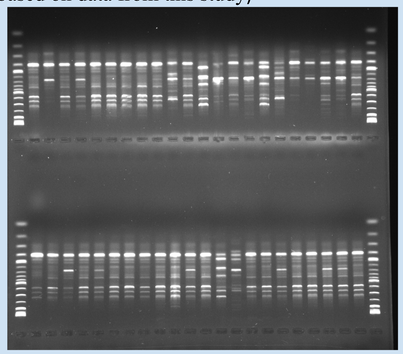

Figure 5. SCoT 14 primer. TSP(aka PA) & NJ 2-log ladder included on each side of gel, in order to determine band-size. Annealing temperatures were previously determined using gradient PCR.

Discussion

- Of the 36 originally published SCoT primers tested 9 were found to yield useful PIC values/polymorphism
- This is only the 3rd and most comprehensive study using SCoT primers to evaluate fungal population biology
- AMOVA analysis showed that the majority of genetic variance was within populations
- Dice pair-wise genetic distance data showed the PA population to be the most genetically diverse
- The initial statistical evidence suggests little gene flow among populations

Future Directions:

- Does *S. ellisii* engage in genetic recombination in spite of the lack of structural and life history evidence for a sexual cycle?

Primer	Sequence	Optimal Annealing Temperature (°C)	Band	(PIC)
F01	ACC ACC ACC ACC ACC ACC CC	53.1	12	0.22
FO2	CCA TGA TGA TGA TGA TGA TG	48.3	7	0.19
FO4	GCA ACA CAC ACA CAC AC	59.1	7	0.15
FO5	CAC ACA CAC ACA CAC AGG	48.3	8	0.34
UBC 807	AGA GAG AGA GAG AGA GT	56.9	17	0.44
UBC 808	AGA GAG AGA GAG AGA GC	50	6	0.22
UBC 809	AGA GAG AGA GAG AGA GG	48.3	7	0.31
UBC 810	GAG AGA GAG AGA GAG AT	48.3	7	0.14
UBC 812	GAG AGA GAG AGA GAG AA	53.1	9	0.38
UBC 820	GTG TGT GTG TGT GTG TC	58.2	9	0.24
UBC 841	GAG AGA GAG AGA GAG ACC	53.1	7	0.29
UBC 842	GAG AGA GAG AGA GAG ACC	53.1	7	0.24
ISSR 7	ATA GCC GCC GCC GCC GCC	58.3	16	0.26
ISSR 29	CAC CCA CCA CCA CCA CCA	53.1	12	0.26
Bis 9	AGA GAG AGA GAG AGA GTC	56.9	13	0.19
Lent 1	GGA GAA GCT CTC TCT CTC T	55.2	5	0.47

Primer	Sequence (5'to 3')	Optimal Annealing Temperature (°C)	Bands	(PIC)
SCoT13	ACG ACA TGG CGA CCA TCG	51.4	14	0.18
SCoT14	ACG ACA TGG CGA CCA CGC	55.2	11	0.24
SCoT15	ACG ACA TGG CGA CCG CGA	51.4	11	0.28
SCoT16	ACC ATG GCT ACC ACC GAC	55.2	7	0.26
SCoT18	ACC ATG GCT ACC ACC GCC	49.1	7	0.33
SCoT22	AAC CAT GGC TAC CAC CAC	51.4	4	0.21
SCoT28	CCA TGG CTA CCA CCG CCA	55.2	5	0.23
SCoT29	CCA TGG CTA CCA CCG GCC	49.1	17	0.28
SCoT34	ACC ATG GCT ACC ACC GCA	49.1	11	0.38

Figure 6. The average Polymorphism Information Content (PIC) based on the polymorphism of DNA fragments amplified by specific SCoT and ISSR primers (range possible 0-0.5)

Literature Cited

Bertrand C. Y. C. & D. J. Mackill. 2009. Start Codon Targeted (ScoT) Polymorphism: A Simple, Novel DNA Marker Technique for Generating Gene-Targeted Markers in Plants. Plant Mol. Biol. Rep. 27:86–93

Zhang, J., B. Zhang, Z. Wu. 2014. Analysis of Genetic Differences among Monokaryon Strains of *Flammulina velutipes* Using SCoT and ISSR Markers. Agricultural Biotechnology 3:46 - 49.

Acknowledgements

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