

Combined analysis of population genetic structures in a living-fossil grass *Brachyelytrum erectum*, and its endophyte *Epichloë brachyelytri*

Christopher L. SCHARDL^{1*}, Padmaja NAGABHYRU¹, Andrew C. TAPIA², Patrick J. CALIE³, Simona FLOREA¹, Neil MOORE², Jerzy W. JAROMCZYK² and Carolyn A. YOUNG⁴

¹Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546, USA

²Department of Computer Science, University of Kentucky, Lexington, KY 40508, USA

³Department of Biological Sciences, Eastern Kentucky University, Richmond, Kentucky 40475, USA

⁴Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, North Carolina 27695, USA

*Corresponding author: chris.schardl@uky.edu

Brachyelytrum is the earliest diverging genus known relative to other genera of grass subfamily Poöideae. *Brachyelytrum erectum* inhabits forest understorey in temperate eastern North America and possesses the endophyte *Epichloë brachyelytri*. A genome sequence of *E. brachyelytri* included genes for biosynthesis of peramine, the loline alkaloid precursor *exo*-1-acetamidopyrrolizidine (AcAP), and the ergot alkaloid precursor chanoclavine. In a survey of 24 *B. erectum* populations in Kentucky and southern Indiana, endophytes were genotyped by PCR for alkaloid genes and mating types. All had *ppzA1* for peramine whereas genes for AcAP and chanoclavine exhibited presence/absence polymorphism. Approximate alkaloid levels in leaves were, per g dw, 4–15 µg peramine, 2000–5000 µg AcAP and 175–300 µg chanoclavine when the corresponding gene clusters were present. The sexual state of *E. brachyelytri* was observed on few plants in only two populations, and the overall ratio of mating types *A* and *B* was 1.0:4.3, indicating very limited

sexual reproduction for this endophyte. We obtained RNA-seq data from 4–12 symbiotic (E+) plants and 2–7 asymbiotic (E–) plants each in seven populations separated by distances of 10–321 km (average = 171 km), then applied our recently described rna-clique method to infer pairwise genetic distance matrices comparing plants in different populations, plants with different endophyte genotypes, and endophytes with different genotypes. Results indicated geographical structure of *B. erectum* that distinguished even the closest populations. Within populations we observed partial structure of plant subpopulations based on symbiotic status (E+ vs. E–) or endophyte genotype. The relationship of endophyte genetic structure to endophyte genotypes was consistent with infrequent sexual recombination. Overall, the results indicate local adaptation of *B. erectum* populations for which its functionally and genetically diverse endophyte species plays a role.