Measuring Clonal Diversity in Stands of Bamboo at Shelby Farms for Memphis Zoo Pandas

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Abstract

Bamboo serves as an important food source for pandas and has many medicinal and commercial applications. The Memphis Zoo grows a large bamboo farm located in Shelby Farms to feed their two pandas and the pandas in Toronto Zoo, Canada. We studied the genetic variation present in one of the bamboo species, *Phyllostachys bissetii*, from Shelby Farms using Simple Sequence Repeat genetic markers. The study will provide valuable information for the Memphis Zoo and establish the methodology for identifying clonal diversity and genetic variation within other species of bamboo in natural stands located in China.

Introduction

Worldwide, there are over 1,400 species of bamboo. This member of the grass family, Poaceae, is found on every continent except Antarctica and Europe (Yeasmin et al. 2014). Bamboo can be classified into two categories of the subfamily Bambusoideae. These two categories are woody bamboo or herbaceous bamboo. Woody bamboo is the most prevalent of the two and is a staple food source for pandas.

Bamboo reproduces both sexually by flowering and asexually through clonal growth via rhizomes. Most species flower every 60 to 130 years, and it is unknown when or why flowering occurs (Bamboo Botanicals 2014). For most species of bamboo, the entire stand dies after it flowers. There have been recordings of this phenomena occurring in Sichuan, China where 80-90% of its natural stand of *Sinarundinaria fangiana* flowered, leading to a loss of food and habitat for many animals (Taylor 1998). Understanding the genetic variation of a species is necessary to determine the fitness of individuals within a population and the likelihood of extinction (Mandel 2010). It is our goal to develop a method to understand the clonal variation within one species of bamboo and to apply it to other species in the future.

Methods

Bamboo leaf samples, from Shelby Farms, were systematically obtained from each row within a stand of bamboo. A leaf was collected from one stalk every eleven feet on both sides of a row. Each leaf was labeled with a number and letter corresponding to the stalk location (see figure 1). The culm from which the sample was collected was marked with field tape. The plant tissue was placed in a 2.0 ml tube containing three metal pellets and was ground in a SPEX Geno grinder 2000. An OMEGA bio-tek E.N.Z.A. SQ Plant DNA kit and specified protocol was used to extract the DNA of the leaf. One of ten different micro-satellites from Eurofins Genetics were added to the DNA master mix along with one of three simple sequence repeat (SSR) primers, NED, FAM, or VIC. A polymerase chain reaction (PCR) was performed, and the DNA fragments were visualized using gel electrophoresis. The amplified DNA products were sent to the University of Tennessee's Molecular research center for fragment analysis. The fragments were analyzed and scored using the 2.6.3 version of Gene Marker computer software.

Results

Based on fragment analysis, it was determined that all three individuals are clones. At each locus, individuals 1, 2, and 3 either exhibited the same heterozygous or homozygous genotype with the exception of a few DNA failures which are indicated by the white box. Individuals with the same color box contain have the same genotype for that locus. Figure 1 illustrates the bamboo stand displaying each individual's location and color-coded genotype. Below each picture is the name of the genetic marker used to identify the genotype. At least six out of ten markers have been successful.

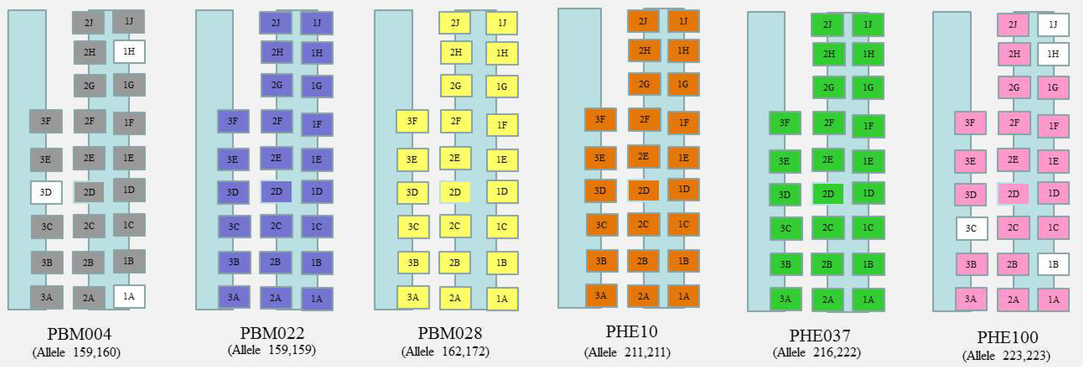

Fig. 1 *P. bissetii* Bamboo Rows 1 and 2

P. bissetii Bamboo

Discussion

One main objective of this experiment was to determine a method that successfully extracts and amplifies DNA from bamboo tissue. Based on PCR and fragment analysis results, our protocol was effective. Establishing a methodology to understand the genetic makeup of a population is important for future research of natural stands of bamboo all over the world. If we can measure the genetic variation of individuals within a small population, we could apply this same methodology toward large natural stands in the wild. This protocol could be applied further to understand flowering patterns. We could determine which individuals would most likely flower at the same time based on genetic similarities and environmental factors. Conservation efforts could then be taken in a situation where mass flowering of one species might occur. It is our assumption based on fragment analysis that all individuals within one stand are clones. However, we can not conclude this until the entire stand is analyzed in the same manner.

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