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Sara Brauninger

Measuring Clonal Diversity in Stands of Bamboo

Faculty Sponsor
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ABSTRACT

Bamboo is an important food source for wildlife all over the world and has a wide variety of medicinal and economic uses. We studied the genetic variation present in two bamboo species at Shelby Farms, *Phyllostachys bissetii* and *Pseudosasa japonica*, using Simple Sequence Repeat genetic markers. This study provides valuable information for the Memphis Zoo and establishes a methodology for identifying clonal diversity and genetic variation within other species of bamboo. Understanding the genetic variation of a species is essential for conservation, especially if it is susceptible to disease, overuse or, in this case, gregarious flowering. Based on our results, we have concluded that *Pseudosasa japonica* is one genetic individual. *Phyllostachys bissetii* contains more variation within each locus indicating that it is not a clone. Our goal is to study the genetic diversity of all seven species at Shelby Farms.

INTRODUCTION

Over 1400 species of bamboo exist worldwide, and these species are found on every continent with the exception of Europe and Antarctica. Bamboo is a member of the grass family, Poaceae, and is a staple food source for wildlife. China has the largest reserve of bamboo (Tang et al. 2009). In Memphis, TN there is a small field containing seven different species of bamboo, three of which are fed to our own Giant Pandas (*Ailuropoda melanoleuca*) in the Memphis Zoo. One of the seven species is *Phyllostachys bissettii, which* can flower every 60 to 120 years; moreover, often the flowering pattern of this species is gregarious (Tang et al. 2009). Gregarious flowering occurs when an entire stand of bamboo simultaneously flowers and then dies shortly thereafter. Bamboo can reproduce both sexually and asexually. Flowering is a form of sexual reproduction; asexual reproduction occurs through growth from underground rhizomes. This indicates that bamboo is clonal; it can produce genetically identical offspring in the form of stalks.

Gregarious flowering has become a major concern within the past few decades. Very little is known about why bamboo flowers the way it does or what triggers the flowering cycle. There are a few hypotheses that attempt to explain flowering patterns. The first states that gregarious flowering occurs as a predator satiation method (Ananthakrishnan 2012). As a result of mass flowering, large quantities of seeds are dispersed at one time. The wildlife in that area eat their fill of the seeds and then move on to another area. Therefore, producing mass quantities of seeds will ensure the survival of that species. Producing large numbers of seeds requires a lot of energy, however, which may account for why the entire stand dies after flowering.

The second hypothesis is that bamboo gregariously flowers to maintain dominance over other plant species in an ideal area of growth (Ananthakrishnan 2012). If there are a large number of seeds taking over a plot of land, other species will not be able to acquire proper nutrition to survive. A third hypothesis also relates to competition with other plant species but involves fire. The fire cycle hypothesis proposes that environmental disturbances, such as fire or windstorms in areas where bamboo is prevalent promotes its survival and reduces the fitness of other species (Ananthakrishnan 2012). Fire, resulting from a lightning strike for example, would consume the dead stalks, and the seedlings would take over

the available space. There are studies of giant cane in Louisiana that have proven that clonal growth and ramet density increases after a large-scale disturbance such as fire (Gagnon and Platt 2008).

Clonal individuals are more likely to flower at the same time since they are genetically identical. Thus, entire stands composed of the same genetic individual will most likely flower and die at the same time, wiping out that entire bamboo population. Wildlife that rely on that stand for food or shelter will be forced to relocate until new stalks form. Developing a methodology to determine the amount of genetic variation within a species of bamboo could help identify stands that are at risk of gregariously flowering and dying out. Conservation efforts could then be made to plant more stands of bamboo and to monitor flowering cycles.

"Genetic markers provide information on levels of clonal diversity for individuals within a population" (Mandel 2010). Microsatellite markers, or Simple Sequence Repeat markers (SSR), are useful for identifying the genetic diversity of a bamboo species. These markers are short segments of DNA that repeat a variable number of times depending on the individual's genome (Tang et al. 2009). Very few microsatellite markers have been developed for bamboo species; some markers have been created for *Bambusa arundinacea* (Tang et al. 2009). A few studies have shown that there is successful transferability (94.7%) of these markers to *Phyllostachys* species and these primers can be used to detect genetic differences at the same sites in related species (Tang et al. 2009). Ten previously developed primers, that displayed good transferability, were chosen to use in this study.

We hypothesized that the entire bamboo stand for each species, *Phyllostachys bissetii* and *Pseudosasa japonica*, was one genetic individual. Our first goal was to develop a methodology to determine if the ten primers would transfer successfully to our two different species of interest. Our second goal was to determine if there was a significant amount of variation, at ten loci, within a species.

METHODS

Tissue Extraction

Bamboo leaf samples, from Shelby Farms, were systematically obtained from each row within each stand of *Phyllostachys bissetii* and *Pseudosasa japonica* bamboo. For *P. bissetii,* a leaf was collected from one

stalk every 11 feet (3.35 meters) on both sides of a row. A leaf was collected every 13.78 feet (4.2 meters) for *P. japonica*. Each leaf was labeled with a number and letter corresponding to the stalk location (see Figures 1 and 2). The letter "I" was skipped on each row for each species. The culm from which the leaf sample was collected was marked with field tape to keep track of each sample location. The tissue was stored in a refrigerator until the DNA extraction took place. To extract the DNA, the plant tissue was cut into fine strips using a razor blade and the strips were placed in a labeled 2.0 ml tube containing three metal pellets. Ninety percent ethanol was used to clean the blade after cutting each leaf. The tissue was ground in a SPEX Geno grinder 2000 for two minutes at 500 rpm. An OMEGA bio-tek E.N.Z.A. SQ Plant DNA kit and specified protocol was used to extract the DNA from the leaf.

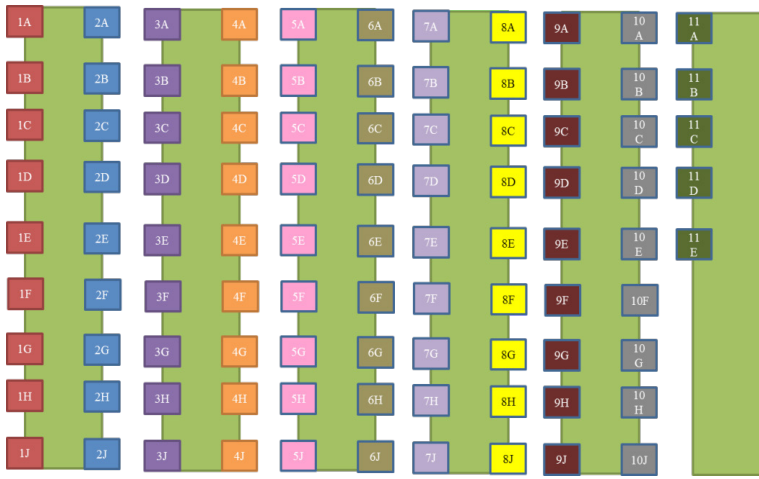

Figure 1: *Phyllostachys bissetii* bamboo stand with sample label

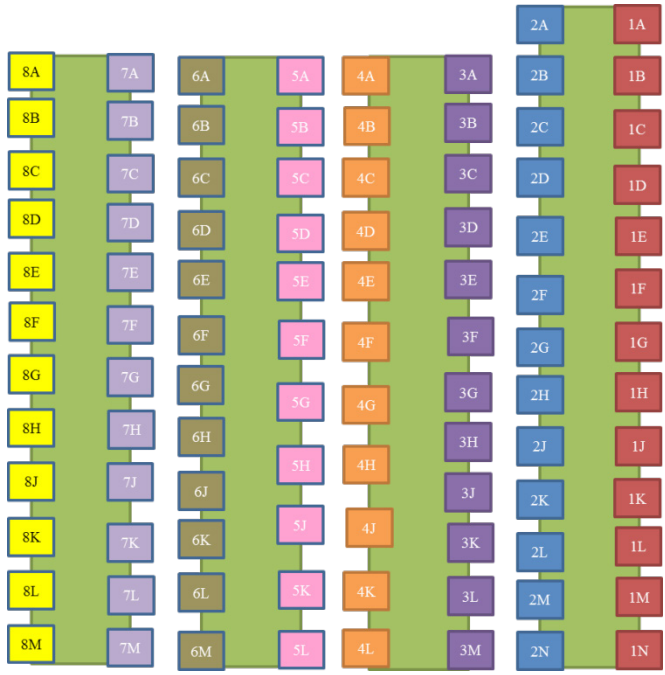

Figure 2: *Pseudosasa japonica* bamboo stand with sample label

DNA Amplification

To amplify the extracted DNA, a master mix comprising of 11.9 µl deionized water, 0.35 µl $MgCl_2$, 1.5 µl buffer, 0.2 µl dNTP, 0.2 µl M13 (VIC, FAM or NED), 0.2 µl forward and reverse primer, and 0.5 µl Taq polymerase was mixed in a micro-centrifuge tube. Each volume was multiplied by 100 to produce enough master mix for 96 individuals. 15µl of the master mix was dispensed into each well of a 96-individual well plate. 1µl of DNA, from only one individual, was added to a well to produce a total of 96 different individuals on each plate. A polymerase chain reaction (PCR) was performed using a thermocycler, program TD55_1min. The TD55_1min PCR program consists of the following temperature cycles:

- 95º C for 3 min
- [94ºC for 30 sec, 65ºC for 30 sec, 72ºC for 1 min] x 9
- [94ºC for 30 sec, 55ºC for 30 sec, 72ºC for 1 min] x 29

- 72ºC for 10 min
- 4ºC for 15 min

The *P. bissetii* PCR products were visualized on 2 % agarose gels using electrophoresis. The gel was run using TAE buffer at 80 volts. The *P. japonica* PCR products were visualized on a 1% agarose gel with Sodium Borate buffer at 200 volts.

Fragment Analysis and Scoring

The PCR products for five of the ten loci, one loci per plate, were diluted with deionized water (15µl per well) and combined in a single plate, depending on the size of the fragment or M13 tag. Tables 1-4 display contents of each dilution plate for both species. The contents of the dilution plates were transferred (1µl from each well) to a run plate containing 12µl of ladder. The ladder contains formamide, which helps keep the DNA denatured. In order to anneal the DNA fragments, the run plates were placed in the thermocycler and the "95 for 5" program was selected. This heats the plates at 95ºC for 5 minutes. The plates were then sent to the University of Tennessee's Molecular Research Center (UTMRC) the same day.

Locus Name	M13 Tag	PCR Product (µl)
PHE 37	FAM	3
PHE 141	FAM	3
PHE 010	VIC	3
PHE 185	NED	3
PBM014	NED	3

Table 1: *P. bissetii* Plate 1 Loci

Locus	M13 Tag	PCR Product (µl)
PBM027	FAM	3
PHE100	VIC	3
PBM028	VIC	3
PBM022	NED	3
PBM 004	NED	3

Table 2: *P. bissetii* Plate 2 Loci

Locus	M13 Tag	PCR Product (µl)
PHE 100	NED	3
PHE185	VIC	3
PHE 37	VIC	3
PHE027	FAM	3
PBM028	FAM	3

Table 3: *P. japonica* Plate 1 Loci

Locus	M13 Tag	PCR Product (µl)
PHE141	FAM	3
PBM022	VIC	3
PBM004	FAM	3
PBM014	NED	3
PHE010 (omitted)	VIC	0

Table 4: *P. japonica* Plate 2 Loci

The amplified DNA products were sent to UTMRC for fragment analysis using an ABI 3130XL Capillary Sequencer. The fragments were scored by eye using the 2.6.3 version of Gene Marker computer software. Once Gene Marker was open and the data from UTMRC was added, the data was run according to the following settings:

Template Selection

- Panel: NONE
- Size Standard: GS500
- Standard Color: Red
- Analysis Type: Fragment (Animal)

Data Process

- Min Intensity: 100
- Max Intensity: 30000

The template selection and data process settings were set to common default settings. The "animal" fragment analysis type was chosen to ensure that the software recognized the samples as diploid. The "min and max intensities" are common settings for peak intensities when scoring data using Gene Marker.

RESULTS

Gel electrophoresis was used to visualize the fragment length at each locus. The first well of each gel image contains a ladder, which acts as a standard to compare fragment lengths. The same individuals were tested for each locus within a species. Different individuals were tested for each species. Gel Images were captured using the MultiDoc-it Digital Imaging System.

P. bissetii individual G12 is a water control for each locus. However, a water control was not used for *P. japonica*. The last individual of each locus contains DNA.

Gel Images: P. bissetii

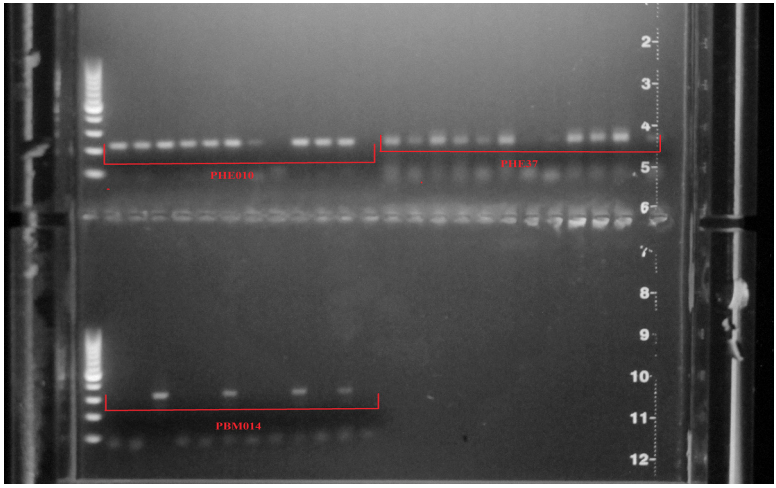

Figure 3: Loci PHE010, PHE037, and PBM014. Individuals G1, F2, E3, D4, C5, B6, A7, J7, H8, G9, F10 and G12 (from wells G1-G12 on a 96 individual well plate). PHE010 fragments were around 220 bp in size, PHE37 fragments were around 230 bp in size, and PBM014 fragments were around 320 bp in size.

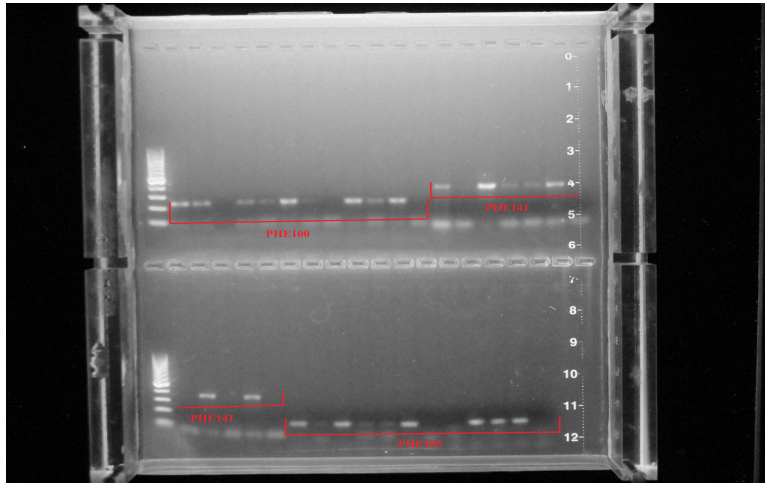

Figure 4: Loci PHE100, PHE141, and PHE185. Individuals G1, F2, E3, D4, C5, B6, A7, J7, H8, G9, F10 and G12 (from wells G1-G12 on a 96 individual well plate). Locus PHE100 was about 220 bp in size, PHE141 was about 330 bp in size, and PHE185 appears to be around 130 bp in size.

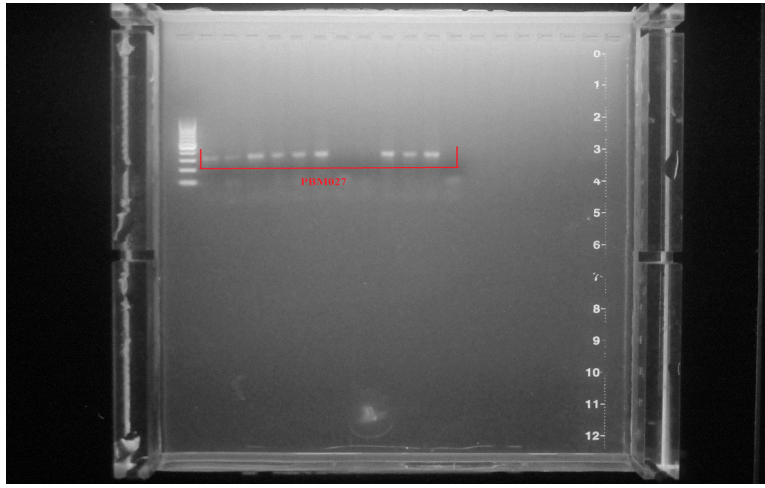

Figure 5: Locus PBM027. Individuals G1, F2, E3, D4, C5, B6, A7, J7, H8, G9, F10 and G12 (from wells G1-G12 on a 96 individual well plate). Fragment length appears to be between 140 and 150 bp in size.

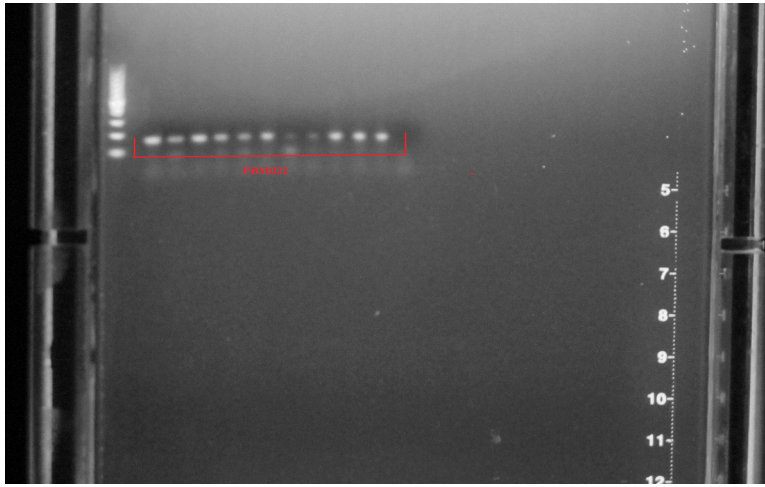

Figure 6: Locus PBM022. Individuals G1, F2, E3, D4, C5, B6, A7, J7, H8, G9, F10 and G12 (from wells G1-G12 on a 96 individual well plate). Fragment length is between 180 and 200 bp in size.

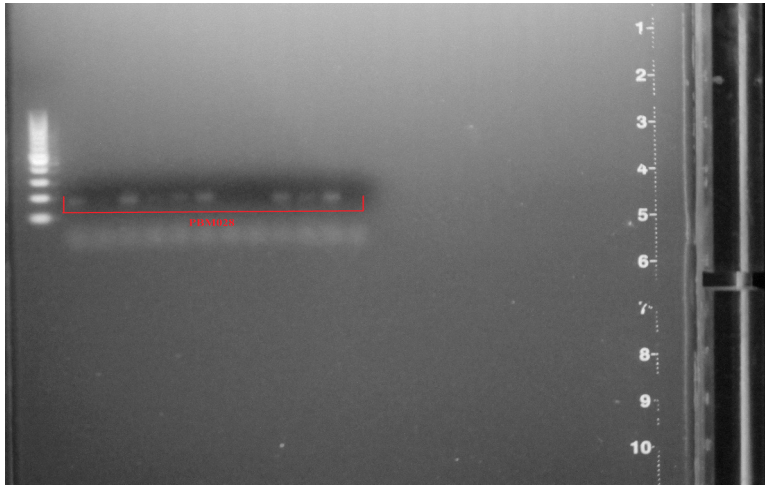

Figure 7: Locus PBM028. Individuals G1, F2, E3, D4, C5, B6, A7, J7, H8, G9, F10 and G12 (from wells G1-G12 on a 96 individual well plate). Bands are faint but are close to 220 bp in size.

Gel Images: P. japonica

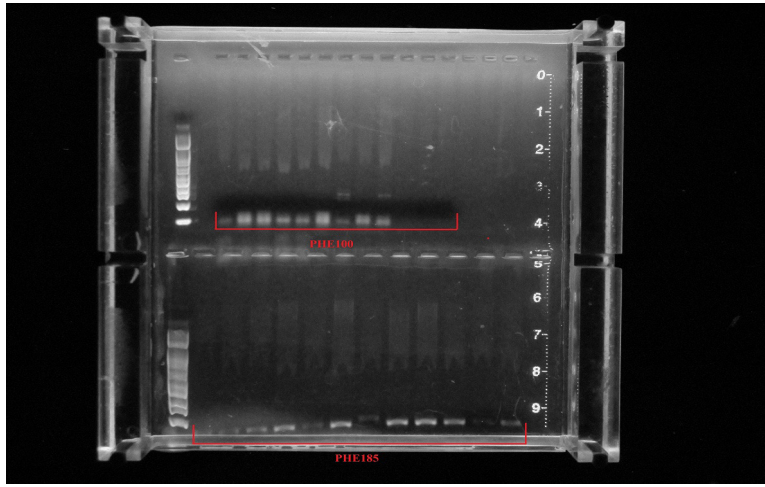

Figure 8: Loci PHE100 and PHE185. Individuals H1, C2, L2, F3, B4, K4, G5, D6, M6, H7, D8, M8 (from wells H1-H12 on plate). PHE 100 fragments seem to be between 400 and 500 bp in size. A few individuals for PHE 185 ran off the gel. However, most seem to be around 120 bp in size.

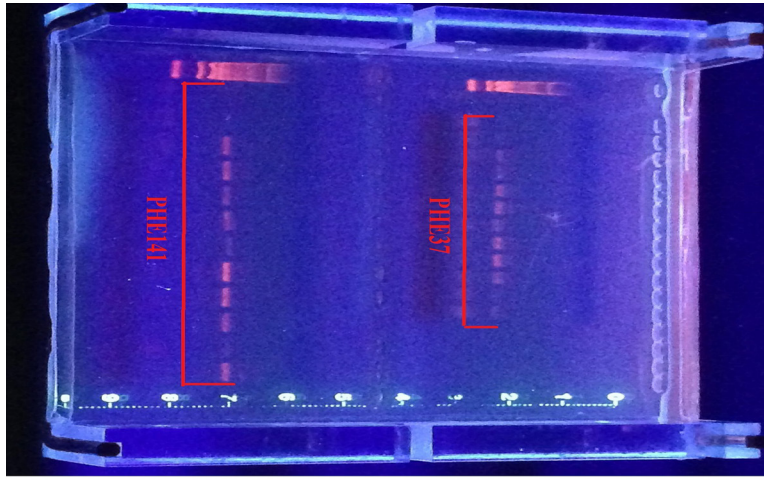

Figure 9: Loci PHE37 and PHE141. Individuals H1, C2, L2, F3, B4, K4, G5, D6, M6, H7, D8, M8 (from wells H1-H12 on plate). PHE37 is between 300 and 400 bp in size. PHE 141 is between 400 and 500 bp in size.

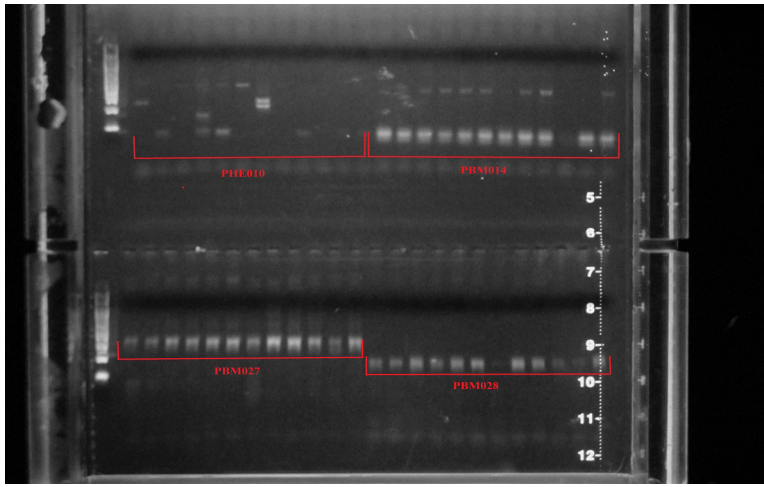

Figure 10: Loci PHE010, PBM014, PBM027, and PBM028. Individuals H1, C2, L2, F3, B4, K4, G5, D6, M6, H7, D8, M8 (from wells H1-H12 on plate). PHE010 was omitted. PBM014 is between 400 and 500 bp in size. PBM027 is around 350 bp in size and PBM028 is about 180 bp in size.

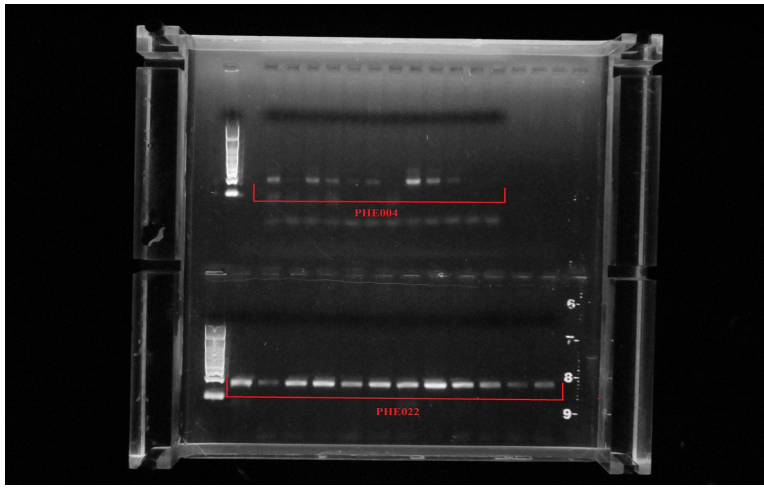

Figure 11: Loci PHE004 and PBM022. Individuals H1, C2, L2, F3, B4, K4, G5, D6, M6, H7, D8, M8 (from wells H1-H12 on plate). PHE004 is about 220 bp in size and PHE022 is around 190 bp in size.

Scored Loci:

The fragments were scored by eye using Gene Marker. Seven loci were successfully visualized for both *P. bissetii* and *P. japonica*. Two loci, PHE100 and PBM014, were large, based off of the gel images, and possibly ran off of the gel when sequencing. PHE010 was omitted from *P. japonica* plates due to its poor quality on the gel (see Figure 10). A "**" symbol on boxes indicates a failure for that individual at that specific locus. Some individuals were not present in the data received from UTMCR; the boxes for those individuals are marked "Missing". Below is an example of the gel image we receive from UTMCR along with the data to be scored.

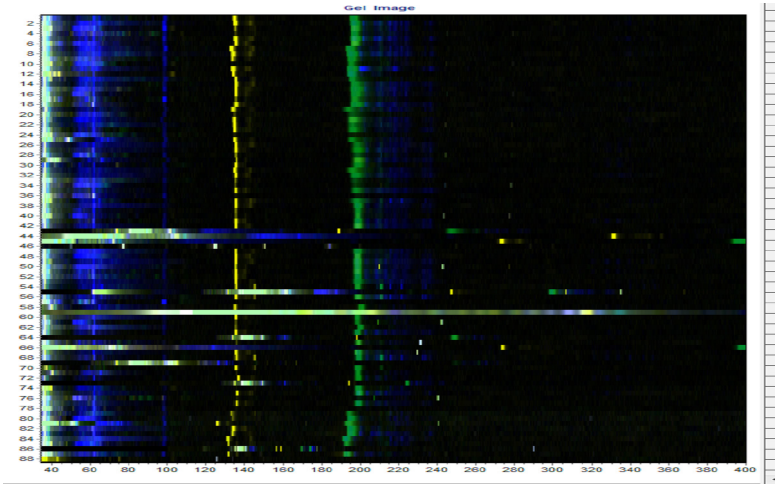

Figure 12: *P. bissetii* Plate 1. Loci PHE010 (yellow) and PHE185 (green) were scored. PHE037 and PHE141 (blue) were too broad to score. PBM014 ran off of the gel (yellow).

Scored Loci: P. bissetii

Individual	Well Position	PBM027	PHE028	PHE100	PBM022	PBM004	PHE010	PHE185
A1	A1	309	**	**	174	**	197	134
B1	B1	**	**	**	174	**	197	135
C1	C1	**	**	**	**	**	197	135
D1	D1	**	191	**	**	**	199	135
E1	E1	**	191	**	**	258	199	135
F1	F1	**	191	**	**	258	200	135
G1	G1	**	**	**	172	**	199	135
H1	H1	300	**	**	**	**	197	**
J1	A2	300	**	**	174	**	197	134
A2	B2	**	OL	**	**	258,260	197	134
B2	C2	**	195	**	**	260	197	135
C2	D2	**	191	**	**	258	**	**
D2	E2	**	**	**	**	**	199	135
E2	F2	309	**	**	**	**	199	135
F2	G2	300	**	**	**	**	199	135
G2	H2	**	**	**	**	258	197	**
H2	A3	**	**	**	**	258	197	134
J2	B3	**	**	**	**	258	197	135
A3	C3	309	**	**	**	**	199	135
B3	D3	**	**	**	**	**	199	135
C3	E3	300	**	**	**	**	199	135
D3	F3	**	**	**	**	**	199	**
E3	G3	**	**	**	**	258	Missing	Missing
F3	H3	**	**	**	**	258,260	Missing	Missing
G3	A4	**	**	**	**	**	197	134
H3	B4	300	**	**	172	**	197	135
J3	C4	300	**	**	172	**	197	135
A4	D4	**	195	226	**	258	197	135
B4	E4	**	191	**	**	258,260	199	135
C4	F4	**	191	**	**	258	199	135
D4	G4	309	**	**	**	**	Missing	Missing
E4	H4	**	**	**	172	**	Missing	Missing
F4	A5	**	**	**	172	**	197	134
G4	B5	**	191	**	**	**	197	135
H4	C5	**	191	**	**	260	199	135
J4	D5	**	191	**	**	258	199	135

A5	E5	**	**	**	**	**	199	135
B5	F5	**	**	**	**	**	199	135
C5	G5	**	**	**	**	**	199	135
D5	H5	**	195	**	**	258,260	197	134
E5	A6	**	191	**	**	258	197	134
F5	B6	**	191	**	**	258	197	134
G5	C6	**	**	**	**	**	197	135
H5	D6	**	**	**	172	**	**	**
J5	E6	**	**	**	**	**	Missing	Missing
A6	F6	**	**	226	**	258	**	**
B6	G6	**	191	**	**	**	199	135
C6	H6	**	191	**	**	**	197	**
D6	A7	309	**	**	174	**	197	134
E6	B7	300	**	**	**	**	197	135
F6	C7	**	**	**	174	**	199	135
G6	D7	**	191	**	**	258	199	135
H6	E7	**	191	**	**	258	199	135
J6	F7	**	191	**	**	258	199	135
A7	G7	**	**	**	**	**	Missing	Missing
B7	H7	300	**	**	174	**	197	**
C7	A8	**	**	**	**	**	197	**
D7	B8	**	**	**	**	**	197	134
E7	C8	**	191	**	**	258	199	135
F7	D8	**	191	**	**	**	**	**
G7	E8	**	**	**	**	**	**	**
H7	F8	**	**	**	174	**	199	135
J7	G8	300	**	**	174	**	**	135
A8	H8	**	191	226	**	258	**	**
B8	A9	**	191	**	**	260	197	134
C8	B9	**	191	**	**	258	197	135
D8	C9	309	**	**	**	**	199	135
E8	D9	300	**	**	172	**	199	135
F8	E9	300	**	**	172	**	199	135
G8	F9	**	195	**	**	**	199	135
H8	G9	**	191	**	**	258	199	137
J8	H9	**	OL	**	**	258	197	134
A9	A10	**	**	**	**	**	197	134
B9	B10	300	**	**	174	**	197	135
C9	C10	**	**	**	174	**	197	135

D9	D10	**	**	226	**	258,260	Missing	Missing
E9	E10	**	191	**	**	258	199	135
F9	F10	**	191	**	**	258	199	135
G9	G10	**	**	**	**	**	199	135
H9	H10	**	**	**	174	**	197	**
J9	A11	300	**	**	**	**	197	134
A10	B11	**	191	226	**	258,260	197	135
B10	C11	**	191	**	**	258,260	199	135
C10	D11	**	191	**	**	258	199	135
D10	E11	309	**	**	172	**	199	135
E10	F11	**	**	**	172	**	199	135
F10	G11	300	**	**	172	**	**	**
G10	H11	**	191	226	**	**	197	134
H10	A12	**	191	**	**	**	197	134
J10	B12	**	191	**	**	258	197	135
A11	C12	**	**	**	**	**	199	135
B11	D12	**	**	**	174	**	Missing	Missing
C11	E12	**	**	**	**	**	**	**
D11	F12	**	**	226	**	258,260	**	**
WATER	G12							

Scored Loci: P. japonica

(PBM014 and PHE100 Ran off gel)

Individual	Well Position	PHE 141	PHE 185	PBM027	PBM028	PBM022	PBM004	PHE037
A1	A1	331	126	290	172	160	346	**
B1	B1	**	126	290	172	160	**	223,234
C1	C1	331	126	290	172	**	346	223,234
D1	D1	**	126	290	172	160	**	223,234
E1	E1	331	126	**	172	160	346	223,234
F1	F1	**	126	290	172	160	**	223,234
G1	G1	331	126	290	172	160	346	223,234
H1	H1	**	126	**	172	160	**	**
J1	A2	331	126	290	172	160	346	223,234
K1	B2	331	126	290	172	160	346	223,234
L1	C2	331	126	290	172	160	346	223,234
M1	D2	331	126	290	172	160	346	223,234
N1	E2	331	126	290	172	160	346	223,234

A2	F2	331	126	290	172	160	346	223,234
B2	G2	331	126	290	172	160	346	223,234
C2	H2	331	126	290	172	**	346	223,234
D2	A3	331	126	290	172	160	346	**
E2	B3	331	126	290	172	160	346	223,234
F2	C3	331	126	290	172	160	346	223,234
G2	D3	331	126	290	172	160	346	223,234
H2	E3	331	126	290	172	160	346	223,234
J2	F3	331	126	290	172	160	346	223,234
K2	G3	331	126	290	172	160	346	223,234
L2	H3	331	126	290	172	160	346	223,234
M2	A4	331	126	290	172	160	346	223,234
N2	B4	331	126	290	172	160	346	223,234
A3	C4	331	126	290	172	160	346	223,234
B3	D4	331	126	290	172	160	346	223,234
C3	E4	331	126	290	172	160	346	223,234
D3	F4	331	126	290	172	160	346	223,234
E3	G4	331	126	290	172	160	346	223,234
F3	H4	331	126	290	172	160	346	223,234
G3	A5	331	126	290	172	160	346	223,234
H3	B5	331	126	290	172	160	346	223,234
J3	C5	331	126	290	172	160	346	223,234
K3	D5	331	126	290	**	160	346	223,234
L3	E5	331	126	290	172	160	346	223,234
M3	F5	331	126	290	172	160	346	223,234
A4	G5	331	126	290	172	160	346	223,234
B4	H5	331	126	290	172	160	346	223,234
C4	A6	331	126	290	172	160	346	223,234
D4	B6	331	126	290	172	160	346	223,234
E4	C6	331	126	290	172	160	346	223,234
F4	D6	331	126	290	**	160	346	223,234
G4	E6	331	126	290	172	160	346	223,234
H4	F6	331	126	290	172	160	346	223,234
J4	G6	331	126	290	172	160	346	223,234
K4	H6	331	126	290	172	160	346	223,234
L4	A7	331	126	290	172	160	346	223,234
A5	B7	331	126	290	172	160	346	223,234
B5	C7	331	126	290	172	160	346	223,234
C5	D7	331	126	290	172	160	346	223,234

D5	E7	331	126	290	172	160	346	223,234
E5	F7	331	126	290	172	160	346	223,234
F5	G7	331	126	290	172	160	346	223,234
G5	H7	331	**	290	**	160	346	223,234
H5	A8	331	126	290	172	160	346	223,234
J5	B8	331	126	290	172	160	346	223,234
K5	C8	331	126	290	172	160	346	223,234
L5	D8	331	126	290	172	160	346	223,234
A6	E8	331	126	290	172	160	346	223,234
B6	F8	331	126	290	172	160	346	223,234
C6	G8	331	126	290	172	160	346	223,234
D6	H8	331	126	290	172	160	346	223,234
E6	A9	331	126	290	172	160	346	223,234
F6	B9	331	126	290	172	160	346	223,234
G6	C9	331	**	290	172	160	346	223,234
H6	D9	331	126	290	172	160	346	223,234
J6	E9	331	126	290	172	160	346	223,234
K6	F9	331	126	290	172	160	346	223,234
L6	G9	331	126	290	172	160	346	223,234
M6	H9	331	126	290	172	160	346	223,234
A7	A10	331	126	290	172	160	346	223,234
B7	B10	331	126	290	172	160	346	223,234
C7	C10	331	126	290	172	160	346	223,234
D7	D10	331	126	290	172	160	346	223,234
E7	E10	331	126	290	172	160	346	223,234
F7	F10	331	126	290	172	160	346	223,234
G7	G10	331	126	290	172	160	346	223,234
H7	H10	331	126	290	172	160	346	223,234
J7	A11	331	126	290	172	160	346	223,234
K7	B11	331	126	290	172	160	346	223,234
L7	C11	331	126	290	172	160	346	223,234
M7	D11	331	126	290	172	160	346	223,234
A8	E11	331	126	290	172	160	346	223,234
B8	F11	331	126	290	172	160	346	223,234
C8	G11	331	126	290	172	160	346	223,234
D8	H11	331	126	**	172	160	346	**
E8	A12	331	126	290	172	160	346	223,234
F8	B12	331	126	290	172	160	346	223,234
G8	C12	331	126	290	172	160	346	223,234

H8	D12	**	126	290	172	160	**	223,234
J8	E12	**	126	290	172	160	**	223,234
K8	F12	331	126	290	172	160	346	223,234
L8	G12	331	126	290	172	160	346	223,234
M8	H12	331	126	290	172	160	346	**

DISCUSSION

Based on our scored data, we can conclude that *P. japonica* is most likely one clone. There was virtually no variation when comparing individuals (see *P. japonica* scores). For example, all individuals at PHE037 were heterozygous 223,234 bp in size while all were homozygous 126 bp at locus PHE185. Loci PBM014 and PHE100 were not scored because they most likely ran off of the gel during sequencing due to their large size. Both loci showed fragment lengths between 400 and 500 bp in size (Figure 10 and 11). On the other hand, *P. bissetii* contained more variation within each successful locus. For example, some individuals at locus PBM004 were heterozygotes with a 258,260 allele. Others were homozygous with either an allele of 258 bp or 260 bp in size for that same locus. PHE010 and PHE185 were the only two loci for *P. bissetii* plate 1 that were successful and contained only a few failures. However, all ten loci for *P. bissetii* had minimal water contamination for plates 1 and 2. There were a few small peaks visible when scoring the data for the water control wells. This indicates that some DNA may have been transferred or leaked into the wells during plate preparation or transport. Even though the contamination was minimal, we would like to re-test *P. bissetii* plates 1 and 2 to ensure that there was no contamination in the remaining wells.

It is our goal to provide the Memphis Zoo with information concerning all seven of their bamboo species. The next step that can be taken with this study is to genotype the remaining five species at Shelby Farms in this same manner. Very little is known about the biology or genetics of bamboo due to its unusual life cycle (Loh et al. 2000). Therefore, any data that is obtained from this experiment or any future studies will provide the Memphis Zoo with valuable information, as well as other organizations interested in the conservation of bamboo. If the remaining stands at Shelby Farms show little variation, like *Pseudosasa japonica*, conservation efforts must be made. Planting more stands would ensure that bamboo would be available should a mass flowering event occur.

This experiment is manageable for small, well-kept stands of bamboo. However, large natural stands, such as those found in China, are much more difficult to test in this manner. The stands are not well kept in rows, like those we tested from, and the terrain might make sampling by hand dangerous and difficult. Remote sensing technologies, such as imaging spectroscopy, may be useful with genotyping large populations. There have been studies performed on trembling aspen in which remote sensing technology is used to distinguish between genotypes through variation in canopy spectral signature (Madritch et al. 2014). This could distinguish different species that may be mixed together over a large area or located in a dense ramet.

Determining the genetic variation of a clonal species is critical not only for the preservation of that species but also for the protection of surrounding wildlife. “Animals, such as the Red Panda (*Ailurus fulgens*) and the Himalayan Black Bear (*Selenarotos thibetanus*), depend on bamboo stands for food and shelter, and 15 Asian bird species exclusively reside in bamboo” (Sertse et al., 2011). A mass flowering event, followed by death of the stand, would threaten the livelihood of those that rely on bamboo for survival. Therefore, it is necessary to study the clonal diversity and genetic variation of bamboo to conserve existing stands as a food source and habitat for wildlife.

References

Ananthakrishnan G. (2012). Gregarious Bamboo Flowering. The Discover Wild Foundation. Retrieved from <http://www.discoverwild.in/publications/articles/13-gregarious-bamboo-flowering.html>.

Gagnon, P., and Platt, W. (2008). Multiple Disturbances Accelerate Clonal Growth in a Potentially Monodominant Bamboo. Ecological Society of America, Vol. 89, pp. 612-618.

Loh, J., Kiew, R., Set, O., Huat, L., Gan, Y. (2000). Study of Genetic Variation and Relationships within the Bamboo Subtribe Bambusinae using Amplied Fragment Length Polymorphism. Annals of Botany, Vol. 85, pp. 607-612.

Madritch, M., Kingdon, C., Singh, A., Mock, K., Lindroth, R., and Townsend, P. (2014). Imaging spectroscopy links aspen genotype with below-ground processes at landscape scales. Philosophical Transactions of the Royal Society B Biological Sciences.

Mandel, J. (2010). Clonal diversity, spatial dynamics, and small genetic population size in the rare sunflower, *Helianthus verticillatus*. Conservation Genetics, Vol.11, issue 5, pp. 2056.

Sertse, D., Disasa, T., Bekele, K., Alebachew, M., Kebede, Y., Eshete, N., Eshetu, S. (2011). Mass flowering and death of bamboo: a potential threat to biodiversity and livelihoods in Ethiopia. Journal of Biodiversity and Environmental Science (JBES), Vol. 1, No. 5, pp. 16-25.

Tang, D., Lu, J., Fang, W., Zhang, S., and Zhou, M. (2009). Development, characterization and utilization of GenBank microsatellite markers in *Phyllostachys pubescens* and related species. Mol Breeding, Vol. 25, pp. 299-311.