# Characterizing new microsatellite markers for the invasive vine Kudzu, *Pueraria montana*

Smithsonian
*National Museum of Natural History*

Dennis Zhu[12], Matthew Hansen[1], Ashley Egan[1]
1Smithsonian National Museum of Natural History, Department of Botany
2University of Missouri-Columbia, Department of Biochemistry

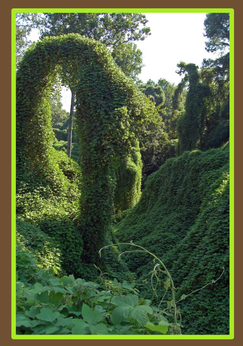
University of Missouri

## Background

Kudzu is the common name for the species *Pueraria montana* var. *lobata*, a vine from the pea family Fabaceae. The genus is native to Asia, but was introduced into the United States a the 1876 (Miller and Edwards 1983). It has since grown to cover over 3 million hectacres of the United States, and in 1997 Congress listed kudzu as a Federal Noxious Weed (Blaustein 2001). The species displays traits that enhance its invasive nature (Forseth and Innis 2004). A previous genetic study of *P. montana* in the U.S. concluded that the populations showed a high level of genetic diversity (Pappert *et al*. 2000).

<u>The objectives of my project are as follows:</u>

- Develop, characterize, and optimize new microsatellite markers for kudzu from amplification to use for fragment analysis
- Demonstrate microsatellite usefulness by performing a preliminary population genetics investigation of a sample of 81 *P. montana* DNA samples



## Methods

1. Collection of *Pueraria montana* samples

United States Asia

Figure: Collection maps of *Pueraria montana* samples in the United States and Asia. Each yellow push-pin is a GPS-recorded collection. Images generated by Google Earth. (Note: Thailand not shown)

2. Sequencing of transcriptome from variety of tissues (leaf and stem)
3. Design of 30 primers for potential microsatellite markers
4. Optimization of PCR methods and testing of newly designed primers
5. Selection of a subsample of 81 *P. montana* individuals across the U.S. and Asia for preliminary screening

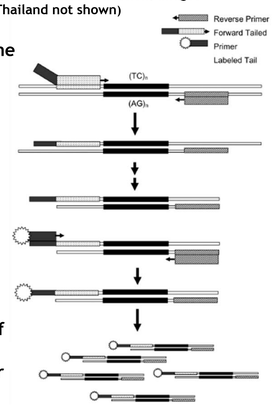
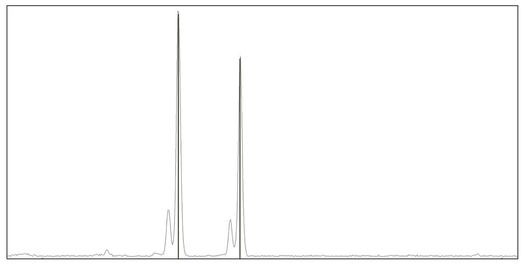



Figure: Concept of the three primer PCR method used for fluorescent labeling . Image from Culley *et al*. 2013

6. Three-primer amplification and fluorescent labeling process
7. Fragment analysis sequencing on an abi3730 sequencer
8. Genotyping microsatellite alleles using GeneMapper
9. Preliminary population genetics analysis
   a. examining population structure using STRUCTURE and Structure Harvester (Pritchard *et al*. 2000; Earl and vonHoldt 2012)
   b. generation of diversity statistics using Arlequin (Excoffier and Lischler 2010)

## Results

| Primer | Tail / Dye | Exp. Size | Obs. Size | Motif |
|---|---|---|---|---|
| PLcpp27_7 | M13/6FAM | ~100 | 100-130 | CTT |
| PL27PP2_4 | M13/6FAM | ~120 | 110-150 | GCT |
| PL27PP2_5 | M13/6FAM | ~120 | 130-150 | CTT |
| PL27PP2_3 | M13A/VIC | ~230 | 170-260 | AAG |
| PL27PP2_10 | M13A/VIC | ~300 | 290-340 | GGT |
| PL27PP2_13 | M13A/VIC | ~270 | 100-300 | GCT |
| PL27PP2_8 | M13A/VIC | ~340 | 350-370 | GGT |

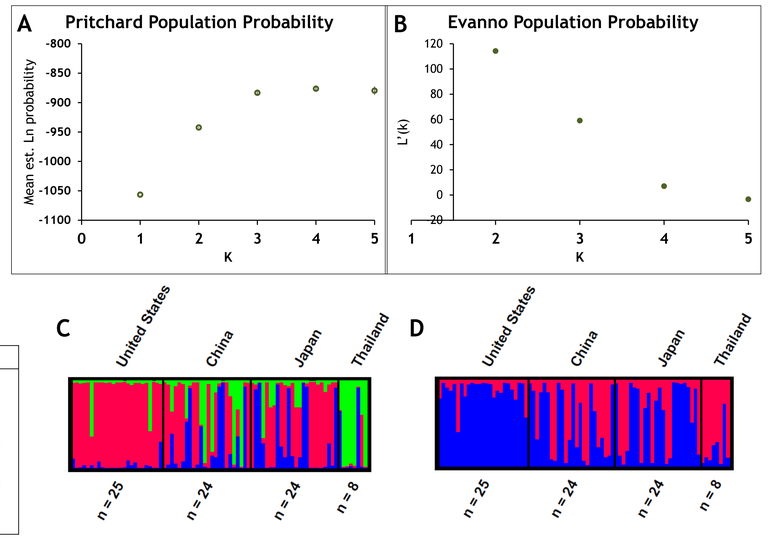



| | United States | | | Asia | | |
|---|---|---|---|---|---|---|
| Locus | AP | Obs. Het. | Exp. Het. | AP | Obs. Het. | Exp. Het. |
| PLcpp27_7 | 6 | 0.522 | 0.744 | 9 | 0.651 | 0.803 |
| PL27PP2_4 | 6 | 0.583 | 0.625 | 8 | 0.408 | 0.723 |
| PL27PP2_5 | 2 | 0.040 | 0.040 | 6 | 0.159 | 0.289 |
| PL27PP2_3 | 3 | 0.913 | 0.530 | 5 | 0.839 | 0.666 |
| PL27PP2_10 | 5 | 0.680 | 0.513 | 8 | 0.410 | 0.750 |
| PL27PP2_13 | 5 | 0.200 | 0.191 | 6 | 0.119 | 0.321 |
| PL27PP2_8 | -* | - | - | 6 | 0.122 | 0.433 |
| Mean (SD) | 4.50 (1.64) | 0.490 (0.320) | 0.441 (0.269) | 6.86 (1.46) | 0.387 (0.279) | 0.569 (0.216) |

**Figures:** (*top-left)* Table of the seven microsatellite loci that were successfully used all the way to the preliminary population genetics test. Sizes list in base pairs. Observed sizes are rounded; (*bottom-left*) AP = number of observed alleles per loci, Obs. Het./Exp. Het. = observed and expected heterozygosity. *PL27PP2_8 loci was completely non-polymorphic for United States samples
(*top*) example of a good signal primer amplification for a polymorphic microsatellite locus as viewed in GeneMapper

**Figures:** (*bottom* ) Pairwise $F_{ST}$ (Fixation Statistic) data between populations in the U.S., China, Japan, and Thailand. Sewall Wright $F_{ST}$ cutoffs are 0-0.05 (little), 0.05-0.15 (moderate), 0.15-0.25 (great), 0.25+ (very great). p-values are listed in parentheses (*left a*) Cluster assignment based on Pritchard 2000, table generated by Structure Harvester; (*left b*) Cluster assignment based on Evanno 2005, table generated by Structure Harvester; (*bottom-left c*) Population structure for *Pueraria montana* with K=3, graphic generated using *distruct* (Rosenberg 2004); (*bottom-left d*) Population structure for *Pueraria montana* with K=2 (Rosenberg 2004)

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| U.S. 1 | - | 0.024 (0.009) | 0.041 (0.000) | 0.212 (0.000) |
| China 2 | | - | -0.002 (0.670) | 0.114 (0.009) |
| Japan 3 | | | - | 0.147 (0.000) |
| Thailand 4 | | | | - |




## Discussion

The primary goal of my project was to test the laboratory effectiveness of primer sequences designed from microsatellites in the transcriptomic library of *Pueraria montana* as genetic markers. Beginning with 30 potential loci, only 7 were successfully used for genotyping and data analysis. Markers were abandoned for various reasons including: amplification of non-target sequences, incapability with our PCR protocol, and lack of polymorphism. Many of these issues can likely be solved via methods troubleshooting, but were not addressed due to the time frame of the project.

A small-scale population genetics study of 81 *Pueraria montana* samples from the U.S. and Asia was used to demonstrate the applications of these new microsatellite markers. Though the sample size and number of loci are too small to draw novel conclusions, the data support previous studies that show U.S. kudzu populations to be genetically diverse. The data were also used to generate a potential population structure for the two different continents.

## Future Work

- Revisiting designed primers that were not successful in this particular project
- Screening the characterized microsatellites across a much larger sample size to increase the power of our population structure results
- Examining the geographical variances within country subpopulations
- Attempting to apply these microsatellites to genetic studies on related species in the genus *Pueraria*

## Acknowledgements

Special thanks to: Virginia Power, Gene Hunt, and Liz Cottrell for directing the NHRE program; to Maggie Halloran for sequencing work; and to Nikki Vollmer for assistance with data analysis

NSF EAR-106292

## References

**Blaustein**, R. (2001). Kudzu's invasion into Southern United States life and culture. *The Great Reshuffling: Human dimensions of invasive species*, 55-62.

**Culley**, T.M., Stamper, T.I., Stokes, R.L., Brzyski, J.R., Hardiman, N.A., Klooster, M.R., and Merritt, B.J. (2013). An Efficient Technique for Primer Development and Application that Integrates Fluorescent Labeling and Multiplex PCR. *Applications in Plant Sciences*, 1.

**Earl**, D.A. and vonHoldt, B.M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 2:359-361.

**Evanno**, G., Regnaut, S., and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology,* 14:2611-2620.

**Excoffier**, L. and Lischler, H.E.L (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564-567.

**Forseth** Jr., I.N. and Innis, A.F. (2004). Kudzu (Pueraria montana): History, Physiology, and Ecology Combine to Make a Major Ecosystem Threat. *Critical reviews in Plant Sciences*, 23:401-413.

**Miller**, J.H., and Edwards, B. (1983). Kudzu: Where did it come from? And how can we stop it?. *Southern Journal of Applied Forestry*, 7:165-169

**Pritchard**, J.K., Stephens, M., and Donnelly, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. *Genetics* 155:945-959.

**Rosenberg**, N.A. (2004). DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, 4:137-138.