Genotyping an Invasive Vine: The Hunt for Polymorphic Microsatellite Loci in Kudzu (*Pueraria montana* var. *lobata*)

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Introduction

The climbing vine kudzu, a member of the leguminous pea family (*Fabaceae*), was introduced into the USA from its native Asia in the 1800s. It was initially lauded for efficacy in erosion control along highways and as a high-quality grazing crop for livestock[1]. *P. montana* var. *lobata* has since become a truculent invasive, spreading via vegetative runners and seed dispersal[2]. Seven million acres of the American southeast are now plagued by this vine (Figure 1).

Questions remain: In how many instances was kudzu introduced to the USA? Are there multiple, distinct genetic sources? Elucidating the population genetics of this invader may help us understand its aggressiveness and ultimately inform ecological countermeasures. One way to assess genetic diversity is via microsatellite analysis, and we are undertaking this for kudzu populations from both Asia and the USA.

Objectives

1) Fill holes in the data set for 81 kudzu individuals at microsatellite loci already partially genotyped
2) Augment our suite of available kudzu microsatellite loci & optimize the new primer pairs
3) Genotype the sample individuals at the new loci
4) Use genotype data to investigate genetic variability and population structure of this initial sample

Methods

Previous Efforts:

1) Kudzu sample individuals were collected by A. N. Egan in 2011 - 2014 across native and introduced ranges (Figure 2)
2) DNA & RNA were extracted, and transcriptomes were assessed
3) From transcriptomes, primer pairs were designed by M. S. Hansen with the aim of amplifying 30 specified trinucleotide-repeat microsatellite loci

Current Efforts:

1) Optimize PCR conditions for new primer pairs: gmpc4, PL1 PL11, PP1, PP2 (35 cycles; annealing temperature = 50 – 57° C; template DNA = 1 µL)
2) Perform three-primer PCR amplification of microsatellites with fluorescent-labeled (6FAM, VIC, or PET dye) primers after Culley et al[3]
3) Determine fragment lengths on ABI 3730 DNA Analyzer
4) Genotype alleles using *GeneMapper* to locate peaks in fluorescence along a size spectrum
5) Assign individuals to putative population clusters and visualize population structure using *Structure*[4]
6) Analyze genetic diversity metrics using *Arlequin*[5]

Tables & Figures

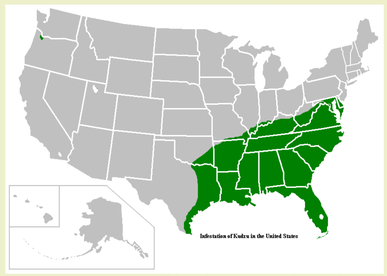
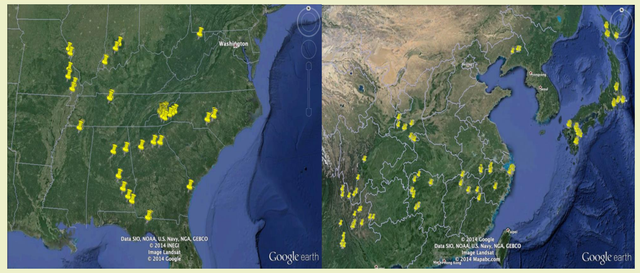


Figure 1: Map of kudzu introduced range in the USA



United States **Asia**

Figure 2: *P. montana* var. *lobata* collection sites in USA, Japan, and China. (Image generated by *GoogleEarth*)

Table 1: Diversity Indices

	USA			China			Japan		
Locus	*H* Exp.	*H* Obs.	# Alleles	*H* Exp.	*H* Obs.	# Alleles	*H* Exp.	*H* Obs.	# Alleles
PL7	0.746	0.560	8	0.775	0.684	8	0.872	0.706	11
PP4	0.561	0.609	4	0.617	0.412	5	0.617	0.444	6
PP10	0.513	0.680	5	0.697	0.647	5	0.446	0.353	5
PP13	0.079	0.080	3	0.435	0.250	7	0.181	0.125	4
PL1	0.241	0.261	4	0.611	0.400	4	0.138	0.143	2
PL11	0.702	0.792	5	0.723	0.737	7	0.506	0.529	3
PP2	0.600	0.667	3	0.851	0.600	8	0.736	0.750	5
Mean	0.492	0.521	4.571	0.672	0.533	6.286	0.499	0.436	5.143
s.d.	0.245	0.255	1.718	0.135	0.180	1.604	0.272	0.248	2.911

H Exp./ *H* Obs. = expected/ observed heterozygosity.

Table 2: AMOVA Results

Source of variation	Sum of squares	Variance components	Percentage variation	
Among Pop'ns	8.221	0.06223	3.11137	F_{ST}: 0.03111
Among Individ'ls	107.555	0.19029	9.51477	F_{IS}: 0.0982
Within Individ'ls	94	1.74747	87.37387	F_{IT}: 0.12626
Total	209.777	2	100	

F statistics are averaged over all loci. F_{ST}: measures genetic drift caused by subdivision of the total population. F_{ST} < 0.05 implies "little" genetic divergence between groups[6]. FIS: the inbreeding coefficient, measures deviation of *H* Obs. from *H* Exp. F_{IT}: measures the loss in heterozygosity for an individual, on average, compared to the total sample.

Table 3: Pairwise F_{ST}

	USA	China	Japan
USA			
China	0.0000 (0.78378)		
Japan	0.00000 (0.99099)	0.03050 (0.00901)	

F_{ST} values < 0.05 imply negligible genetic differentiation. P values are given in parentheses.

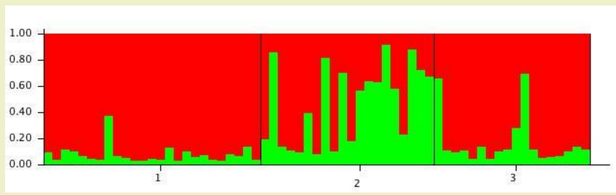


Figure 3: Putative *P. montana* var. *lobata* population structure with *K* = 2, where K is the number of genetic clusters. 1: USA individuals, *n* = 25. 2: China, *n* = 20. 3: Japan, *n* = 18. (Image generated by *Structure*[4])

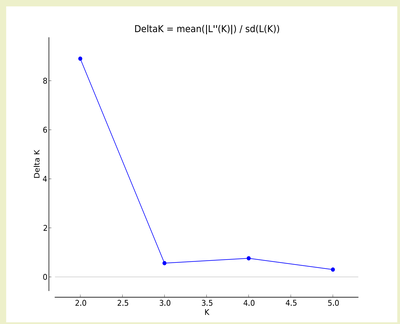


Figure 4: Population probabilities as calculated by Evanno[7] method. (Image generated by *Structure Harvester*[8])

Results & Discussion

In general, China's diversity statistics exceeded those of the other two populations (Table 1), with highest percent of heterozygotes and no. of alleles. China had the most inbreeding (*H* Obs. < *H* Exp.); by contrast, USA exhibited outbreeding. AMOVA compared genetic variation within individuals, within populations, and in the total sample (Table 2). Only 3% of genetic diversity was between populations, implying a small amount of drift between them in the evolutionarily-meager 150 years since isolation. Pairwise F_{ST} (Table 3) is another indication of the genetic distance between subpopulations. Only China and Japan subpopulations showed any differentiation. The USA population may not be old enough to have substantially diverged. *K* = 2 provided the most plausible arrangement of population structure (Figures 3, 4). This implies that USA and Japan individuals fall into the same genetic group.

My main goal was to enlarge the number of known polymorphic microsatellite loci for *P. montana* var. *lobata*. Three new primer pairs & loci were incorporated, and robustness of the data set was improved for numerous individuals through troubleshooting and optimization of primers already in use. Many primers were not included in this analysis for various reasons: lack of polymorphism; PET fluorescent dye indistinguishable from size standard; a haploid cpDNA locus; project timeframe ended before results returned. In sum, 7 microsatellite loci were used to carry out preliminary population genetic analysis of kudzu from the USA and Asia using the 63 individuals with < 50% missing data. The results of this early work cannot be conclusive, but indicate kudzu individuals are genetically diverse and divergence between pop'ns is low. China's pop'n, the oldest and largest, exhibited the greatest diversity. The pop'n structure, with the USA kudzu pop'n most closely related to the Japanese pop'n, supports an hypothesis of a single introduction event, from Japan. On the other hand, the USA pop'n showed an unexpected surfeit of heterozygosity. Could there be ongoing gene flow between the USA pop'n and others overseas? This hypothesis is supported by infinitesimal F_{ST} and pairwise F_{ST}s (Tables 2, 3). An alternative hypothesis, which reconciles USA outbreeding with the pop'n structure results, is that USA kudzu may hybridize with other *P. montana* subspecies.

Future Directions

- The data set can be reanalyzed with the addition of loci that weren't sequenced during project timeframe.
- Primer pairs with currently-incompatible fluorescent label can be developed further.
- Sample size of kudzu individuals can be increased to generate more robust assessments of genetic diversity and population structure.
- Further and more wide-ranging populations can be included; examination of kudzu populations from South America, Australia, and/or Africa could prove interesting.

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