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CHARACTERIZING SPECIMENS OF KUDZU AND RELATED TAXA WITH RAPD's—Kudzu [*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen and Almeida] is a perennial, semi-woody, climbing legume in the tribe Phaseoleae Benth., subtribe Glycininae Benth. (Maesen 1985, Maesen and Almeida 1988, Ward 1998). It is native to China, where an abundance of natural enemies (Pemberton 1988) and its cultivation prevent kudzu from becoming either an important economic or environmental liability. Kudzu was introduced to the United States as an ornamental during middle of the 19th century. During first half of the 20th century, approximately 134,760 ha were planted throughout the southeastern United States to feed livestock and for erosion control (Wheeler 1950). During 1998, kudzu was included by legislators in the United States Congress on a growing list of invasive, exotic plants recognized under the Federal Noxious Weed Law. Presently, it costs commercial forests approximately \$119/ha annually (Beckwith and Dangerfield 1996), it compromises the integrity of valuable natural resources, and dense infestations have interfered with exercises on military bases in North Carolina, South Carolina, and Virginia (Al Cofrancesco, United States Army Corps of Engineers, Vicksburg, Mississippi, pers. comm.).

*Pueraria montana* var. *chinensis* (Ohwi) Maesen and Almeida, and *P. montana* var. *montana* (Lour.) Maesen, are two other varieties of kudzu also distributed throughout southeast Asia. *P. montana* var. *chinensis* has been collected recently in Hawaii (Staples et al. 2002). There is no record of *P. montana* var. *montana* in the United States, but in Asia it has been treated either as a separate species or it has been ignored (Ward 1998). Distinction among the three varieties has relied upon morphological characteristics, including lobed leaflets, wing and keel petals, length of vexil, length of lateral calyx, length of stamen, and margin of leaflet (Chen et al. 1995, Wu et al. 1994). These characteristics demonstrate considerable variation, and distinction among the three varieties in the field is difficult.

Furthermore, kudzu may hybridize with related taxa. Some specimens in his revision confused Maesen (1985), and he treated them as "Material of Uncertain Disposition." Among these specimens are *Henry 13626* and *10931* that he refers to as "near *Pueraria lobata* and *P. edulis*," and Maesen (1985) suggests that, "Perhaps these specimens are simply a form of *P. lobata* var. *lobata*, or, indeed, a hybrid of *P. edulis* or *P. lobata* var. *montana* ..."

Inability to distinguish among the three varieties and their possible hybrids is an obstacle to developing an integrated management program for kudzu and its related taxa. Of particular concern is selecting potential biological control agents because insects and pathogens cannot be reconciled with identity of the plants from which they were collected. Incomplete systematic resolution has been an obstacle to developing integrated management programs for other invasive, exotic plants, including *Cardaria* spp. (Brassicaceae) (Mulligan and Findlay 1974, Bellue 1933), *Vincetoxicum* spp. (Asclepiadaceae) (Sheeley and Raynal 1996), and *Euphorbia* spp. (Euphorbiaceae) (Galitz 1980, Harvey et al. 1988, Crompton et al. 1990).

Using genetic markers for more convenient identification of specimens may be possible. Randomly amplified polymorphic DNA's (RAPD's) have been used successfully to characterize genetic composition and reveal variation among genomic DNA of many important cultivated plants, including wheat (He 1992), soybean (Hui et al. 1996), and tea (Chen and Yang 1998). The objective of this study is distinguishing between kudzu and its related taxa using RAPD's.

Approximately 50 kudzu leaves were collected from plants at 13 sites in the United States and China (Figure 1, Table 1). Their identity has been verified morphologically and specimens have been vouchered by Dr. Teling Wu (South China Institute of Botany, Guangzhou). Extraction of DNA was modified from methods described by Li and Zhou (1999). Fresh leaves contain polyphenol, which can bond with DNA covalently. To prevent oxidation of polyphenol and its interference with activity of Taq polymerase; 2-mercaptoethanol was added to an extraction buffer and insoluble polyvinylpyrrolidone was added to leaves when they were ground. Before raw DNA was used as a template for amplification, it was separated on a 0.7%

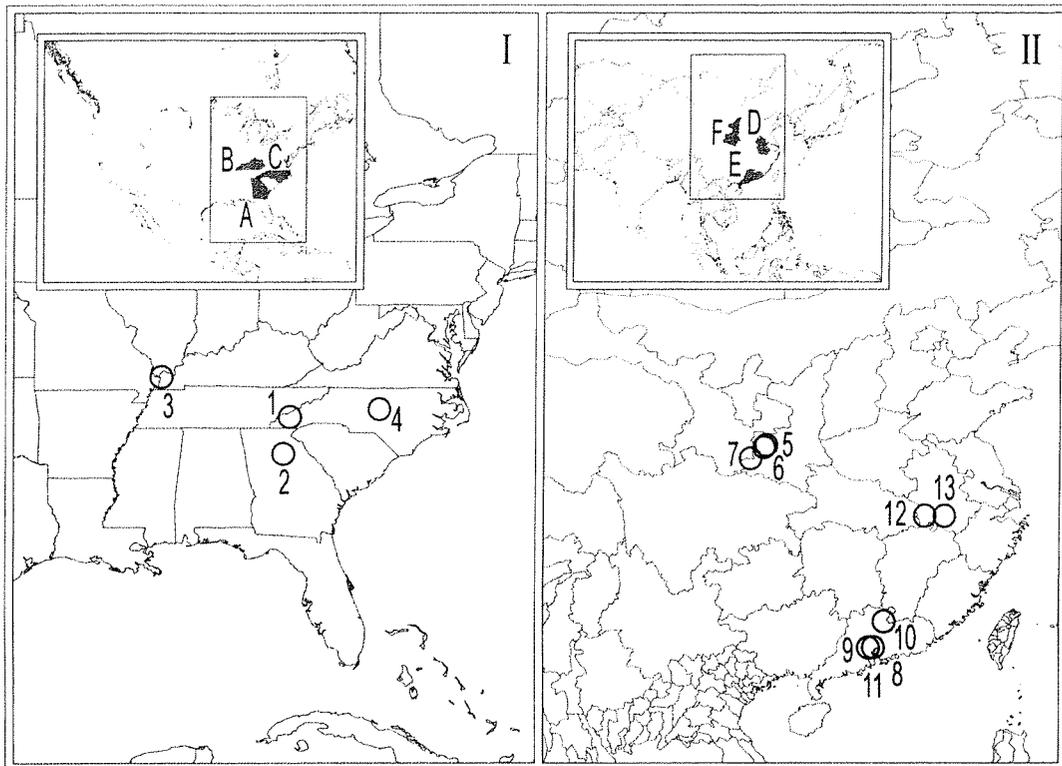


Figure 1. Sites from which specimens were collected. In the United States (I), 4 specimens were collected from 4 sites in 3 states: (A) Georgia, (B) Kentucky, and (C) North Carolina. In China (II), 9 specimens were collected from 8 sites in 3 provinces: (D) Anhui, (E) Guangzhou, and (F) Shaanxi. (Two specimens were collected from site 11.)

agarose gel, visible bands were excised under UV light, and purified with a DNA extraction kit (Sangon Ltd., Ontario, Canada).

PCR amplification was completed in an Express Thermal Cycler (Hybaid Limited, United Kingdom). Each 50  $\mu$ l reaction mixture was comprised of 20 ng DNA template, 0.2  $\mu$ M random primers (Sangon Ltd., Ontario, Canada), reaction buffer (Sangon), 2.5 units Taq polymerase (Sangon, Canada), 2 mM  $MgCl_2$ , 200  $\mu$ M each of dATP, dCTP, dGTP, and dTTP (Sangon, Ltd., Ontario, Canada). Parameters for PCR were: 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 38°C for 1.5 min, and extension at 72°C for 2 min. A final extension at 72°C for 10 min. completed the PCR schedule. Products were separated on 1.7% agarose gel with a 100 bp ladder (MBI). Completed gels were photographed and analyzed by GIS system (Tanon Limited, Shanghai).

Polymorphic bands were identified either as present (1) or absent (0). Relationships were identified with cluster analysis (SAS Institute 1989). A dendrogram (Figure 2) illustrating genetic distance was generated using an unweighted pair group method of arithmetic means (UPGMA), and similarity coefficients between specimens were calculated.

Of 72 random primers, 18 yielded consistent bands between 300 bp and 3 kbp (Table 2, Figure 3). Between 1 and 16 bands were amplified with each primer. Of 171 bands, 151 were polymorphic and 20 were shared among specimens.

Our data support the conclusions of Pappert et al. (2000) that populations of kudzu in the United States demonstrate considerable genetic variation. This conclusion also is supported by the continual and deliberate introduction of kudzu over several decades. Concerning other invasive, exotic plants, a direct relationship between variation and history of introduction has

Table 1. Sites at which specimens were collected

Specimen <sup>1</sup>	Identity <sup>2</sup>	Country	State or Province	Locality	Date <sup>3</sup>	Collector	Latitude (dd)	Longitude (dd)
1	<i>Pueraria lobata</i>	US	North Carolina	Bryson City, Swain Co.	15 Sept. 2000	Kerry Britton	83.45	-35.43
2	<i>Pueraria lobata</i>	US	Georgia	Fort Yargo St. Pk., Barrow Co.	21 Aug. 2000	Kerry Britton	83.72	-33.97
3	<i>Pueraria lobata</i>	US	Kentucky	Wickcliffe, Ballard Co.	14 Sept. 2000	Kerry Britton	89.09	-36.98
4 <sup>4</sup>	<i>Pueraria lobata</i>	US	North Carolina	West of Silver City, Chatham Co.	20 Aug. 1999	K. Kidd/D.B. Orr	79.48	-35.73
5	<i>Pueraria lobata</i>	CN	Shanxxi	Baoji City	07 June 2001	Jianghua Sun	34.30	107.25
6	<i>Pueraria lobata</i>	CN	Shanxxi	Baoji City	07 June 2001	Jianghua Sun	34.26	107.15
7	<i>Pueraria lobata</i>	CN	Shanxxi	Feng County	08 June 2001	Jianghua Sun	33.58	106.30
8	<i>Pueraria thomsonii</i>	CN	Guangdong	Zengcheng, Guangzhou City	23 June 2001	Jianghua Sun	23.16	113.46
9	<i>Pueraria thomsonii</i>	CN	Guangdong	Longdong, Guangzhou City	23 June 2001	Jianghua Sun	23.14	113.15
10	<i>Pueraria lobata</i>	CN	Guangdong	Shixing County	26 May 2001	Jianghua Sun	24.58	114.05
11	<i>Pueraria montana</i>	CN	Guangdong	Longdong, Guangzhou City	23 June 2001	Jianghua Sun	23.14	113.15
12	<i>Pueraria lobata</i>	CN	Anhui	Qiangshan	20 May 2001	Jianghua Sun	30.39	116.35
13	<i>Pueraria lobata</i>	CN	Anhui	Qiangshan	21 May 2001	Jianghua Sun	30.40	117.52

<sup>1</sup> Identity of specimens verified morphometrically by Dr. Teling Wu (South China Institute of Botany, Guangzhou, Guangdong Province, China). In this table, specimens identified as *P. lobata* are recognized in the United States as *P. montana* var. *lobata* (Maesen 1985, Ward 1998). In Asia, *P. montana* is treated as a distinct species or it has been ignored (Ward 1998), but systematists in the United States recognize it as *P. montana* var. *montana* (Maesen 1985). In Asia, systematists treat *P. thomsonii* generally as a distinct species, but Maesen (1985) treated it as *P. montana* var. *chinensis*.

<sup>2</sup> Numbers are same as those in tables 1 and 3, and in figures 1, 2 and 3.

<sup>3</sup> Refers to harvest of leaves or collection of seeds.

<sup>4</sup> Specimens were collected as seed in Chandler Co., North Carolina and were grown in Anhui Province, China.

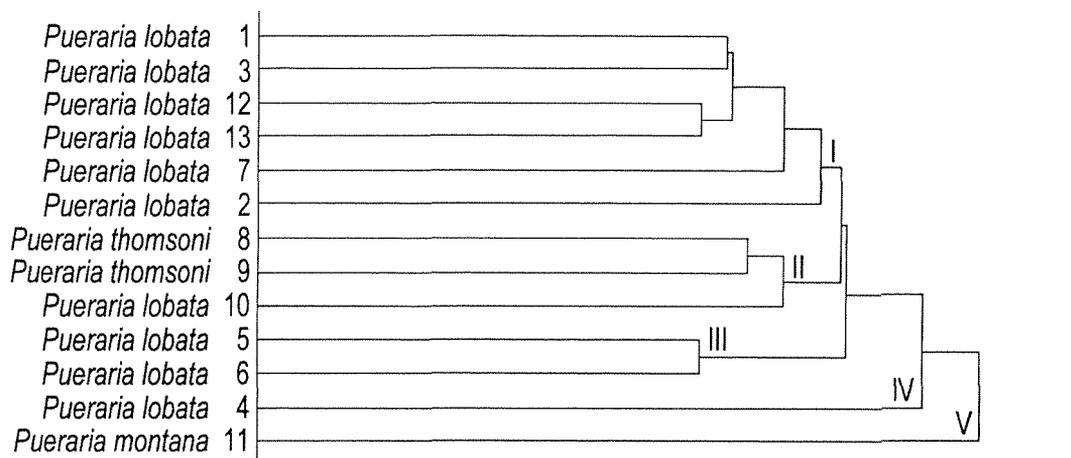


Figure 2. Molecular relationship among specimens.

been reported (Schierenbeck et al. 1995, Novak and Mack 1993, Godt and Hamrick 1991, Moran and Marshall 1978).

Genetic distance among specimens of kudzu is illustrated in Table 3 and in the dendrogram (Figure 2); they resolve into 5 groups. Group I is most inclusive, comprised of specimens 1, 2, 3, 7, 12, and 13. Group II comprised 3 specimens from the same province in China (8, 9, and 10). Group III comprised specimens 5 and 6. Group IV comprised a single specimen—4. Finally, Group V also comprised a single specimen—11 — from the same province as specimens 8, 9, and 10.

Identity of specimens 1, 2, 3, 12, and 13 was verified morphologically as *P. montana* var. *lobata* from different sites in the United States and districts of Anhui Province (Figure 1, Table 1), and molecular profiles reflect their similarity. Like specimens 5 and 6, specimen 7 was collected in Shaanxi Province; but its molecular profile resembles specimens collected in Anhui

Table 2. Molecular profiles expressed as sequence and polymorphisms

Primer	Sequence from 5' to 3'	GC (%)	Number of Total Amplified Bands	Number of Polymorphic Bands	Percent Polymorphic Bands (%)
S10	CTGCTGGGAC	70	11	11	100
S201	GGGCCACTCA	70	4	4	100
S239	GGGTGTGCAG	70	7	7	100
S255	ACGGGCCAGT	70	10	9	90
S266	AGGCCCGATG	70	1	1	100
S510	CCATTCCTCCA	60	2	2	100
S1052	CAGTTCCTCGT	60	8	8	100
S1053	CAGCCGTTC	70	11	10	91
S1056	TCTGGACCGA	60	13	12	92
S1058	GGCTAGGTGG	70	15	13	80
S1060	ACACGTGGTC	60	15	13	86
S1210	TGGGGCTGTC	70	16	16	100
S1302	TCGCAGGTTC	60	11	9	82
S1304	AGGAGCGACA	60	13	9	69
S1306	TTTGGGCCCC	70	3	3	100
S1307	AGCCCCAAG	70	11	9	82
S1308	CTGTCTGTGG	60	11	9	82
S1310	GGTGTTCG	60	9	7	78

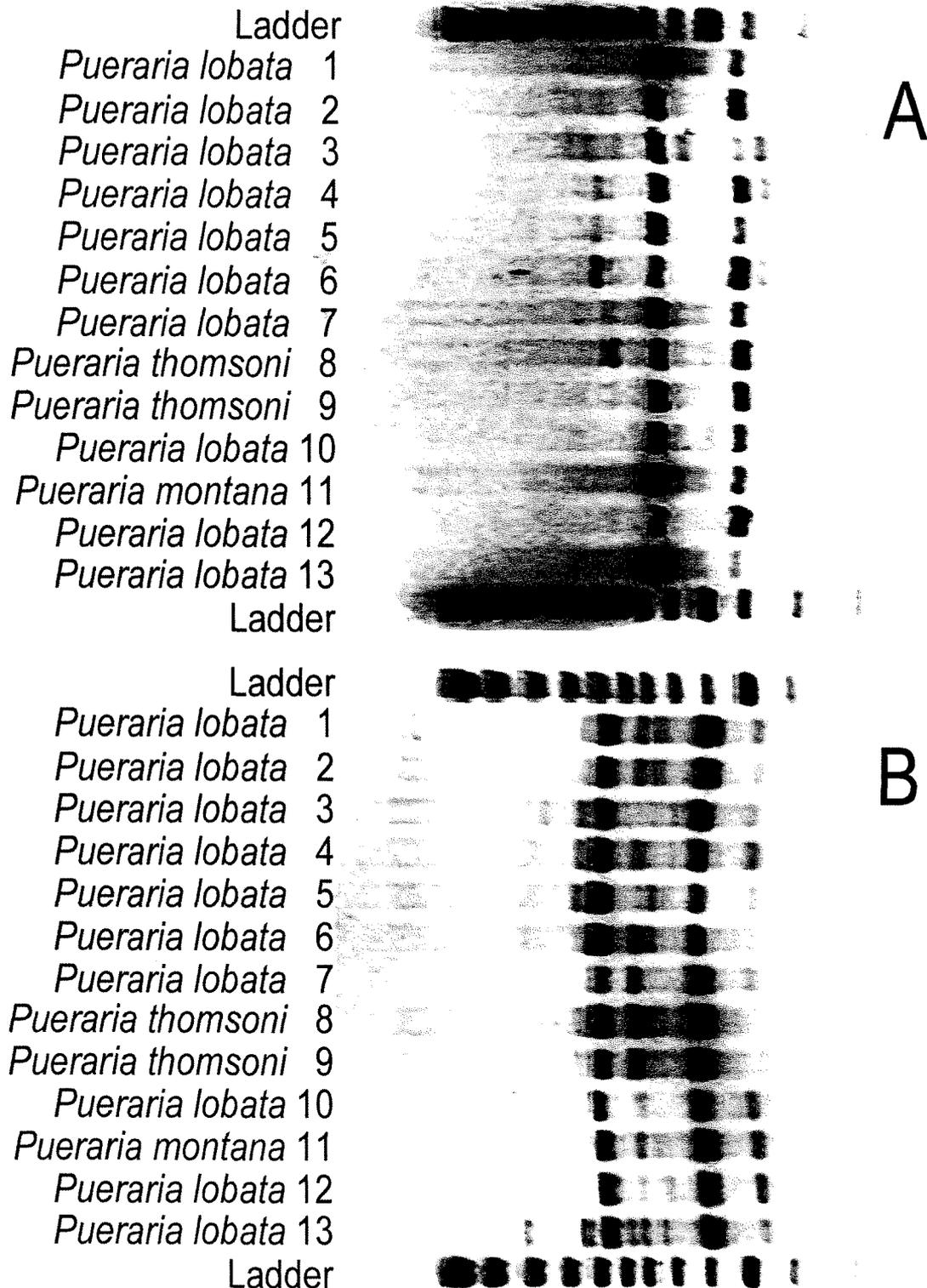


Figure 3. Molecular profile for 13 accessions of *Pueraria* spp. generated with primers S1308 (A) and S1304 (B). "Ladder" is a control comprised of discrete fragments between 100 and 1,000 base pairs at an interval of 100 base pairs. Identification of specimens has been verified by Dr. Teling Wu (South China Institute of Botany, Guangzhou).

**Table 3. Genetic distance among specimens**

1	0.00												
2	23.68	0.00											
3	17.65	23.94	0.00										
4	44.00	34.43	37.14	0.00									
5	28.57	31.43	24.05	30.43	0.00								
6	33.33	31.43	24.05	30.43	15.38	0.00							
7	24.14	28.77	21.95	27.78	23.46	23.46	0.00						
8	27.47	29.87	30.23	47.37	27.06	27.06	29.55	0.00					
9	28.74	34.25	31.71	41.67	30.86	30.86	30.95	20.45	0.00				
10	26.88	36.71	29.55	41.03	31.03	33.33	24.44	21.28	24.44	0.00			
11	46.07	52.00	52.38	48.65	42.17	49.40	41.86	35.56	44.19	36.96	0.00		
12	18.28	29.11	20.45	38.46	31.03	33.33	24.44	27.66	24.44	22.92	50.00	0.00	
13	17.02	27.50	16.85	41.77	29.55	31.82	20.88	24.21	27.47	27.84	46.24	15.46	0.00
specimen	1	2	3	4	5	6	7	8	9	10	11	12	13

Province and in the United States. Specimens 5 and 6 were collected in Shaanxi Province and their identity also was verified as *P. montana* var. *lobata*, but these specimens are genetically distinct from those in group I of the dendrogram (Figure 2). Specimens 8, 9, and 10 were collected in Guangdong Province (Figure 1). Morphologically, identity of two specimens (8 and 9) from this area was verified as *P. montana* var. *chinensis*, but another (10) was verified as *P. montana* var. *lobata*. Molecular profiles, however, fail to distinguish the three specimens (8, 9, and 10) from others of *P. montana* var. *lobata*.

One specimen of *P. montana* var. *lobata* from Guangdong was grouped with two other specimens from the same area identified morphologically as *P. montana* var. *chinensis*. Such genetic similarity may explain the difficulty in separating these two taxa morphologically, and supports Maesen's treatment of them varietally. Considering their close geographic origins, there is the possibility of hybridization in southern China, the only region of sympatry from which specimens were collected. A more comprehensive study is required to test this hypothesis.

Although verified morphologically as *P. montana* var. *lobata*, its molecular profile suggests that specimen 4 is distinct from other specimens in this study. Finally, the identity of specimen 11 was verified morphologically as the only example of *P. montana* var. *montana*; molecular characterization with RAPD's supports its distinction from the other specimens.

This brief study represents continuing development of an integrated management program for Kudzu, and it has been supported by the United States Department of Agriculture, Forest Service International Programs (Washington, D.C.), Forest Health Enterprise Team (Morgantown, West Virginia), and the Southern Research Station (Athens, Georgia). As its development proceeds, more reliable ways of distinguishing kudzu from related taxa and hybrids will be necessary. Additional studies comprised of greater sample size and more out-groups will identify the value of RAPD's to distinguish kudzu from related taxa and hybrids, and to develop an integrated management program.

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