

GENETIC VARIATION IN *PUERARIA LOBATA* (FABACEAE), AN INTRODUCED, CLONAL, INVASIVE PLANT OF THE SOUTHEASTERN UNITED STATES¹

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Pueraria lobata (kudzu), a clonal, leguminous vine, is invading the southeastern United States at a rate of 50000 ha per year. Genetic variability and clonal diversity were measured in 20 southeastern U.S. populations using 14 allozyme loci. Within its U.S. range, 92.9% of the loci were polymorphic and overall genetic diversity was 0.290. Such high levels of genetic diversity are consistent with its history of multiple introductions over an extended period of time. The average proportions of polymorphic loci and genetic diversity within populations were 55.7% (range = 28.6–85.7%) and 0.213 (range = 0.114–0.317), respectively. The proportion of total genetic diversity found among populations was similar to species with equivalent life history characters ($G_{ST} = 0.199$). No regional patterns of variation were seen. The number of putative genotypes in each population ranged from 2 to 26. Mean genotypic diversity was 0.694, ranging from 0.223 to 0.955. Such high levels of genotypic diversity indicate that local sites are often colonized by several propagules (most likely seeds) and/or that sexual reproduction occurs within populations after establishment. An excess of heterozygosity was observed in populations with few unique genets, implying that selection for highly heterozygous individuals may occur in populations of *P. lobata*.

Key words: allozymes; clonal; Fabaceae; genetic diversity; invasive; kudzu; *Pueraria lobata*; vine.

Blanketed fields of kudzu, *Pueraria lobata* Ohwi, are a familiar sight throughout the southeastern United States. Since its introduction in the 1870s, kudzu has covered an estimated 3 million ha of land and currently spreads at a rate of 50000 ha per year (K. Britton, personal communication). *Pueraria lobata*, a leguminous, twining vine with large trifoliate leaves, dominates by climbing over and shading everything in its path, killing native herbaceous and woody species. It roots easily from nodes and has a large tuberous root system, producing extensive clonal spread. Little biomass is allocated for structural support, allowing *P. lobata* to invest its resources into vine expansion and increased photosynthetic area (Sasek and Strain, 1988). *Pueraria lobata* was repeatedly introduced into the southeastern United States throughout the late 19th century and the first half of the 20th century. Introductions, first as an ornamental and subsequently as fodder and a means of erosion control, were widespread and usually undocumented. Although some research has been conducted on physiological aspects and methods of eradication of *P. lobata* (Wechsler, 1977; Miller, 1985, 1988; Sasek and Strain, 1988; Sharkey and Loreto, 1993), studies of the genetic composition of kudzu populations in the southeastern United States do not exist.

The genetic composition of an introduced, invasive species, such as *P. lobata*, is influenced by its history of introduction as well as its life history characteristics. Naturalized populations of species that have been intentionally introduced multiple times over an extended period should have more genetic diversity than species that were unintentionally introduced once or only a few times; each new introduction should increase the probability of introducing additional genetic vari-

ability. Ornamental species such as *Lathyrus latifolia* (Godt and Hamrick, 1991), *Lonicera japonica* (Schierenbeck, Hamrick, and Mack, 1995), and *Albizia julibrissin* (Hamrick, unpublished data), which were intentionally introduced into the southeastern United States multiple times, generally have high levels of genetic variation in their naturalized range. In contrast, unintentionally introduced species such as *Bromus tectorum* (Novak and Mack, 1993), *Xanthium strumarium* (Moran and Marshall, 1978), and *Abutilon theophrasti* (Warwick and Black, 1986) often have little genetic variation in their naturalized populations. Based on its history of multiple introductions, populations of *P. lobata* in the United States should have relatively high genetic variation.

The intentional introduction of a plant species may also affect regional patterns of genetic variation if genetically differentiated propagules were introduced into different geographic regions. Such introductions could have been due to attempts to match native habitats to recipient sites (i.e., founder selection) or to the haphazard introduction of genetically differentiated propagules into these sites (i.e., founder effects). In either case, such foci of introductions may impact the distribution of genetic variation among present-day populations. Alternatively, with multiple nonselective introductions in each region followed by substantial gene exchange among populations, no such regional patterns should currently exist. Since *P. lobata* was repeatedly introduced throughout the southeastern states over many decades (1870s–1940s), we would not expect there to be genetically identifiable foci of introduction.

The genetic composition (number and frequency of genotypes) of relatively recently established local populations of an invasive, colonizing species such as *P. lobata* is of interest since it may provide insights into the mode of local population establishment. Given the extended time since the last intentional introduction (1940s), and the phenomenal spread of *P. lobata* populations since that time, the vast majority of extant populations are likely naturally established. Current popula-

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tions may generally consist of one or only a few genotypes, with high levels of genetic heterogeneity among populations. This pattern would suggest that new populations are established by a few propagules (vegetative or sexual), which then grow primarily vegetatively. Alternatively, current populations may consist of many genotypes with relatively little heterogeneity among populations. This would suggest that after initial population establishment (by few or many propagules) additional genotypes are derived by sexual reproduction within the population or by secondary long-distance propagule recruitment from other populations. Based on viable seed production by *P. lobata* (Abramovitz, 1983; R. Pappert and J. Hamrick, personal communication), and successful seedling recruitment in a Maryland population (Abramovitz, 1983), we expect populations of *P. lobata* to contain many genotypes with relatively low levels of genetic differentiation among populations.

Genetic analyses of intentionally introduced species provide evidence for the maintenance of many unique genotypes within populations and low among-population heterogeneity. *Lathyrus latifolia* (Godt and Hamrick, 1991) and *Lonicera japonica* (Schierenbeck, Hamrick, and Mack, 1995), both clonally spreading, introduced, invasive species, maintain high levels of genotypic diversity within their populations and relatively low genetic differentiation among populations. Furthermore, reviews of the plant allozyme literature for highly clonal species (Ellstrand and Roose, 1987; Hamrick and Godt, 1989) indicate that such species often maintain significant levels of gene and genotypic variation within their populations.

This paper represents an allozyme analysis of 20 southeastern U.S. populations of *P. lobata*. We address the following questions: (1) Does *P. lobata*, as expected from its history of multiple introductions, maintain high levels of genetic diversity?, (2) Can distinct foci of introduction be identified based on differences in the genetic composition of populations within different geographic regions?, and (3) Do local populations of kudzu consist of one or a few genetically distinct clones or do its populations often consist of many unique genotypes?

MATERIALS AND METHODS

Sample collection and electrophoresis—Samples were collected from twenty populations of *P. lobata* throughout its southeastern U.S. range (Fig. 1). Populations were separated geographically by at least 100 km. Most populations ranged between 1 and 2 ha, although ATH was much larger (~30 ha). At each site, 48 samples of fresh leaf material were collected, and the general location of each sample within the population was noted. To increase the likelihood of picking leaves from different clones, samples were chosen at least 6 m apart. Samples were taken from edges, as well as the interior of populations when accessible. Leaves were kept on ice for a maximum of 48 h until further processing. Leaves were ground to a wet paste with a mortar and pestle using a small amount of sea sand and phosphate extraction buffer (Mitton et al., 1979), which solubilized and stabilized the enzymes. The extract was then absorbed onto Whatman filter paper wicks, placed in microtiter plates, and stored at -70°C until used.

Starch gel electrophoretic techniques were used to assess genetic diversity. Extracted proteins were run on 9% starch gels. We resolved 14 loci, using 11 enzyme systems. The following enzymes were assayed: menadione reductase (MNR, one locus), triose-phosphate isomerase (TPI, three loci), F-1,6-diphosphate (F16, one locus), shikimic dehydrogenase (SKDH, one locus), malic enzyme (ME, one locus), aconitase (ACO, one locus), fluorescent esterase (FE, one locus), diaphorase (DIA, two loci), mannose-6-phosphate (MPI, one locus), peroxidase (PER, one locus), and isocitrate dehydrogenase (IDH, one locus). Buffer systems included Poulik (MNR, TPI, ME, DIA, and PER) from

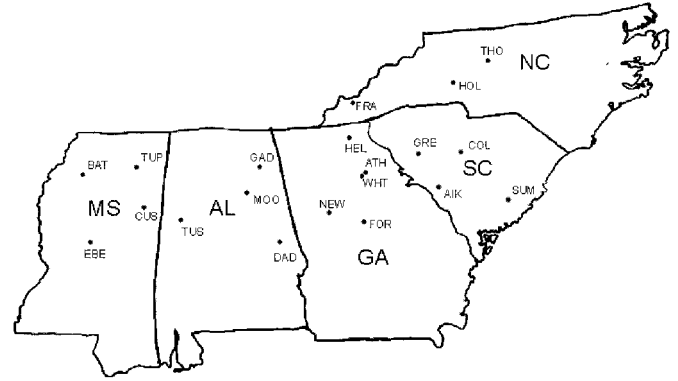


Fig. 1. Locations of *Pueraria lobata* populations. Locations include populations from NC (Franklin: FRA, Mt. Holly: HOL and Thomasville: THO), SC (Aiken: AIK, Columbia: COL, Greenwood: GRE and Summerville: SUM), GA (Athens: ATH, Athens: WHT, Forsythe: FOR, Helen: HEL and Newnan: NEW), AL (Dadeville: DAD, Gadsden: GAD, Moody: MOO and Tuscaloosa: TUS), and MS (Batesville: BAT, Columbus: CUS, Ebenezer: EBE and Tupelo: TUP).

Mitton et al. (1979) and 8 (FE), 4 (IDH, ACO), and 11 (F16, MPI and SKDH) from Soltis et al. (1983). Enzyme staining procedures followed Soltis et al. (1983) and Cheliak and Pitel (1984). The genetic basis of allozyme banding patterns was inferred from electromorph patterns with reference to typical subunit structure (Weeden and Wendel, 1989).

Genetic diversity analyses—Standard parameters of genetic diversity were used to describe genetic diversity at both the species and population levels (Berg and Hamrick, 1997). These parameters included percentage of polymorphic loci (P) and mean number of alleles per locus (A) and per polymorphic locus (AP). Effective number of alleles per locus (A_e) was also calculated using Hedrick's method (1983) for the "ramet" and "genet" data sets described below.

The "ramet" data set included genotypic data from all samples collected ($N = 960$). The "genet" data set gave a minimum estimate of the putative number of clones per population (range = 2–26). Samples with identical multilocus genotypes were considered to be the same clone and were only counted once per population ($N = 202$). Expected heterozygosity (H_e), observed heterozygosity (H_o), Wright's fixation index (F), as well as the chi-square test (Li and Horowitz, 1953) for deviations from Hardy-Weinberg expectations were calculated for the "ramet" and "genet" data sets. Values for H_e were averaged over all loci to obtain a mean value for each population. Each parameter was calculated at the species (subscripted by s) and at the individual population level (subscripted by p). As suggested in McClintock and Waterway (1993), calculations of genetic diversity parameters for the "ramet" and the "genet" data sets indicate how data from clonal organisms affect this standard genetic analysis.

Total genetic diversity can be separated into within- and among-population components. Heterogeneity in allele frequencies among populations was tested with X^2 (Workman and Niswander, 1970). Partitioning genetic diversity within and among populations was determined using Nei's genetic diversity statistics (1973, 1977), with the "genet" data set only. Total genetic diversity, H_T , was partitioned into within-population genetic diversity, H_S , and among population genetic diversity, D_{ST} . The proportion of genetic variation explained by among population variation, G_{ST} , was calculated as: $G_{ST} = D_{ST}/H_T$. Two indirect estimates of gene flow (Nm) were obtained. The first estimates the number of migrants using Wright's (1931) F_{ST} ($= G_{ST}$; Nei, 1977). The second method, the "private" allele method, is based on the mean frequency of alleles found in a single population (Slatkin, 1985; Barton and Slatkin, 1986).

Nei's identity (I_n) and distance values (D_n) were calculated for each locus and population pair and averaged over all loci (Nei, 1972). An unweighted pair-group clustering phenogram (UPGMA) and a nonmetric multidimension-

TABLE 1. Genetic diversity for populations of *Pueraria lobata*. Forty-eight samples were collected in each population.^a

Pop.	<i>P</i>	<i>AP</i>	Ramets			Genets		
			$A_e^{\text{“ramet”}}$	H_o (SD)	H_e (SD)	$A_e^{\text{“genet”}}$	H_o (SD)	H_e (SD)
HEL	71.4	2.20	1.36	0.202 (0.047)	0.193 (0.060)	1.39	0.218 (0.072)	0.215 (0.057)
ATH	78.6	2.27	1.54	0.202 (0.045)	0.277 (0.062)	1.56	0.195 (0.059)	0.284 (0.063)
FOR	57.1	2.13	1.32	0.272 (0.040)	0.172 (0.059)	1.37	0.243 (0.155)	0.218 (0.054)
NEW	50.0	2.00	1.43	0.417 (0.017)	0.222 (0.067)	1.47	0.417 (0.079)	0.236 (0.069)
GRE	71.4	2.60	1.53	0.260 (0.054)	0.280 (0.063)	1.53	0.245 (0.073)	0.277 (0.063)
AIK	50.0	2.00	1.45	0.446 (0.019)	0.230 (0.067)	1.47	0.464 (0.094)	0.241 (0.067)
FRA	28.6	2.25	1.21	0.123 (0.031)	0.108 (0.057)	1.21	0.125 (0.120)	0.114 (0.053)
COL	71.4	2.10	1.44	0.371 (0.055)	0.251 (0.054)	1.42	0.342 (0.168)	0.251 (0.051)
SUM	57.1	2.00	1.35	0.172 (0.043)	0.200 (0.058)	1.37	0.188 (0.107)	0.217 (0.056)
HOL	57.1	2.25	1.51	0.389 (0.039)	0.244 (0.069)	1.54	0.355 (0.137)	0.269 (0.065)
WHT	64.3	2.33	1.32	0.188 (0.040)	0.175 (0.058)	1.37	0.194 (0.083)	0.197 (0.058)
TUP	57.1	2.13	1.39	0.245 (0.044)	0.206 (0.063)	1.38	0.236 (0.113)	0.212 (0.060)
BAT	71.4	2.20	1.59	0.352 (0.054)	0.298 (0.061)	1.62	0.335 (0.092)	0.317 (0.061)
EBE	28.6	2.00	1.26	0.242 (0.025)	0.135 (0.060)	1.20	0.186 (0.096)	0.116 (0.052)
CUS	85.7	2.08	1.43	0.237 (0.060)	0.252 (0.049)	1.37	0.235 (0.112)	0.229 (0.048)
TUS	35.7	2.00	1.32	0.315 (0.018)	0.165 (0.065)	1.33	0.321 (0.094)	0.170 (0.064)
GAD	35.7	2.00	1.26	0.186 (0.020)	0.136 (0.059)	1.26	0.186 (0.076)	0.141 (0.057)
MOO	28.6	2.00	1.29	0.280 (0.013)	0.144 (0.062)	1.26	0.250 (0.094)	0.134 (0.059)
DAD	71.4	2.20	1.39	0.260 (0.055)	0.229 (0.055)	1.46	0.288 (0.123)	0.256 (0.056)
THO	42.9	2.00	1.27	0.177 (0.041)	0.152 (0.057)	1.27	0.180 (0.081)	0.155 (0.054)
Mean	55.7	2.14	1.38	0.267	0.204	1.39	0.260	0.213
SD	2.78	0.16	0.26	0.009	0.014	0.12	0.024	0.013
Species	92.9	2.92	2.79		0.294	1.51		0.290

^a *P* = % polymorphic loci, *AP* = mean number of alleles per polymorphic locus, A_e = effective number of alleles per locus, H_o = observed heterozygosity (± 1 SD), H_e = expected heterozygosity (± 1 SD).

al scaling plot (MDS) were produced using genetic distance values for the “genet” data set using NTSYS-pc to determine unique relationships among populations (Rohlf, 1992).

Clonal diversity analysis—To estimate comparative levels of clonal diversity among populations, two measures of genotypic diversity were calculated (Ellstrand and Roose, 1987). Simpson’s diversity index (*D*) measures the genotypic diversity of clones within a population (Parker, 1979), $D = 1 - \sum [N_j(N_j - 1)/N_r(N_r - 1)]$, where N_j is the number of samples of the *j*th multilocus genotype and N_r is the number of samples collected for that population. Simpson’s *D* varies between 0 in a population composed of a single clone and 1, in which every individual sampled has a unique multilocus genotype.

Fager’s *E* (1972) measures the evenness of genotypic distribution within each population. This value varies from 0, in which all individuals have the same genotype, to 1, in which the population has completely uniform genotype frequencies. Fager’s *E* is calculated as $E = (D - D_{\min})/(D_{\max} - D_{\min})$. The parameters D_{\min} and D_{\max} are given as $D_{\min} = (G - 1)/(2N_r - G)/N_r(N_r - 1)$ and $D_{\max} = N_r(G - 1)/G(N_r - 1)$, where *G* is the number of unique multilocus genotypes in each population and N_r is the total number of samples per population (Eckert and Barrett, 1993). Adjusting for D_{\min} and D_{\max} is necessary since the number of genotypes can be considerably lower than the number of samples per population (Parker, 1979).

RESULTS

Genetic diversity—*Pueraria lobata* maintains high levels of allozyme diversity across the southeastern United States. Thirteen of the 14 loci surveyed (92.9%) were polymorphic in at least one of the 20 populations (Table 1). ME was the only locus monomorphic across all populations. At the species level, there were 2.92 alleles per polymorphic locus (AP_s), the effective number of alleles (A_{es}) per locus was 1.49, and expected genetic diversity (H_{es}), was 0.294.

Within populations, allozyme diversity was considerably lower than at the species level. An average of 55.7% (range = 28.5–85.7%) of the loci surveyed were polymorphic (Table 1). The mean number of alleles per polymorphic locus was

2.14 (range 2.0–2.6), the effective number of alleles was 1.38, and mean expected genetic diversity was 0.204 (range 0.108–0.298) for the ramet data set.

Only 16 of the 202 genotypes occurred in two or more populations. Ten genotypes were in two populations, five were in three populations, and one was in four populations. Although the observed number of multilocus genotypes per population may be somewhat underestimated, we are confident that genotypes were accurately distinguished, as identical genotypes were normally spatially adjacent. Expected heterozygosity values at the species and population levels for the “genet” data set were 0.290 and 0.213, respectively. The effective number of alleles was 1.51 and 1.39 at the species and population levels, respectively.

When using the “ramet” data set, 84 of 280 fixation indices (*F*) were significant ($P < 0.05$). The majority of the significant indices were negative, indicating an excess of heterozygotes. The large number of significant tests using the “ramet” data set is due, in part, to the repetition of genotypes within populations. In contrast, when using the “genet” data set, only 18 of 280 tests were significant. Fourteen of the locus-by-genotype fixation indices were significantly greater than zero, indicating an excess of homozygotes. Four were significantly less than zero, indicating an excess of heterozygotes. Five percent, or 14 of the 280 tests, should deviate from Hardy-Weinberg equilibrium, by chance. No patterns were detected across populations as significance values ranged over populations, as well as loci.

When the populations were arbitrarily divided into those with <10 clones vs. those with ≥ 10 clones, the mean observed heterozygosity of populations with few genotypes was higher (0.288 ± 0.098 ; mean ± 1 SD) than the mean for populations with many clones (0.235 ± 0.053). Perhaps more significantly, differences between mean observed and expected heterozygosity was related to the number of clones observed

TABLE 2. Genetic diversity statistics for 13 polymorphic loci of *Pueraria lobata*, using the “genet” data set.^a

Locus	H_T	H_S	G_{ST}	F_{IS}
DIA-1	0.597	0.463	0.225	-0.074
DIA-2	0.276	0.224	0.188	0.183
MNR-3	0.492	0.421	0.143	-0.246
TPI1-1	0.487	0.404	0.170	-0.089
TPI1-2	0.158	0.132	0.164	0.469
TPI-2	0.218	0.141	0.355	0.150
PER-1	0.393	0.350	0.109	-0.053
FE-1	0.308	0.290	0.060	-0.216
IDH-1	0.282	0.222	0.214	0.015
SKDH-1	0.167	0.127	0.240	-0.410
F16-1	0.117	0.093	0.204	-0.333
MPI-1	0.093	0.072	0.224	-0.356
ACO-1	0.478	0.338	0.293	0.445
Mean	0.313	0.252	0.199	-0.040
SD	0.163	0.134	0.076	0.286

^a H_T = total genetic diversity, H_S = within-population genetic diversity, G_{ST} = among-population variation, F_{IS} = Wright’s fixation index within populations.

in the population (Tables 1 and 3). Populations with few genets have large excesses of H_o while populations with more multilocus genotypes approach Hardy-Weinberg expectations or have a slight deficiency of observed heterozygosity ($r = -0.746$, $N = 20$, $P = 0.0002$, for $H_o - H_e$ vs. G ; Fig. 2).

Chi-square analyses testing allele frequency heterogeneity among populations indicate significant differences ($P < 0.001$) for all 13 polymorphic loci for the “ramet” data set, whereas 12 of the 13 polymorphic loci (all but FE1) were significant (either $P < 0.005$ or $P < 0.001$) when using the “genet” data set. Allele frequencies for each population, at each locus, for both the “ramet” as well as the “genet” data can be obtained from the authors upon request.

Mean genetic diversity at the 13 polymorphic loci was relatively high ($H_T = 0.313$) when calculated with the “genet” data set. Eighty percent of the total diversity occurs within populations ($G_{ST} = 0.199$; Table 2). Average genetic identity between populations was 0.895 (SD = 0.032; Table 3). The highest identity was between MOO and EBE (0.988), and the lowest identity was between GAD and NEW (0.728) (data not shown).

UPGMA clustering, using Nei’s genetic distance values for the “genet” data set, showed no novel relationships among *P. lobata* populations. In many cases, distance values were too low to define specific population clusters at the state or regional level. Similarly, the MDS plot did not show any regional patterning. Thirteen populations were clustered in the center of the two-dimensional MDS plot. Four of the outliers (FOR, HOL, NEW, and AIK) were loosely grouped together due to their low average paired-genetic distances; these four populations were basal on the UPGMA plot as well, due to the low genetic distance averages. BAT, TUS, and GAD were also outliers, however they were scattered at the periphery of the plot, in no distinguishable pattern. BAT, TUS, and GAD remained central in the UPGMA clustering, as their average genetic distances were between that of the 13 populations with low genetic distance averages, and the four populations with higher averages.

Gene flow (Nm) among populations using Wright’s method was estimated to be 1.01. Slatkin’s method, based on two pri-

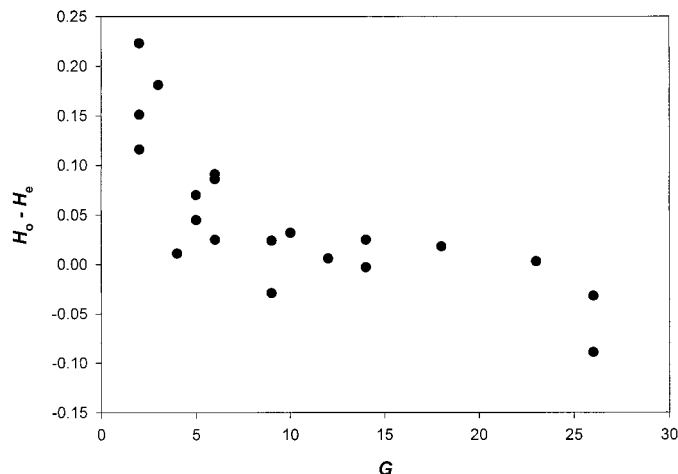


Fig. 2. Relationship between the difference of H_o (observed heterozygosity) and H_e (expected heterozygosity) vs. G (the number of unique genotypes found per population) for 20 populations of *Pueraria lobata*. Data are presented for the “genet” data set only, however the same relationship occurs for the “ramet” data set. See text for explanation.

vate alleles with an average frequency of 0.031, was estimated to be 6.26.

Clonal diversity—Genotypic diversity, or Simpson’s D , averaged 0.694 (SD = 0.226; Table 3). GRE had the highest D value of 0.965, with a total of 26 putative genotypes, whereas MOO (with only two putative genotypes) had the lowest D value of 0.223. Fager’s E averaged 0.753 (SD = 0.188), ranging from 0.263 (FOR) in which the genotypes were unevenly distributed, to 0.932 (GAD), where frequencies of five genotypes were evenly distributed within the population.

TABLE 3. Genotypic diversity parameters and mean genetic identities for populations of *Pueraria lobata*.^a

Pop.	G	Simpson’s D	D_{min}	D_{max}	Fager’s E	I_n
HEL	23	0.954	0.712	0.977	0.913	0.925
ATH	26	0.955	0.776	0.982	0.868	0.911
FOR	6	0.371	0.199	0.851	0.263	0.886
NEW	3	0.407	0.082	0.681	0.542	0.837
GRE	26	0.965	0.776	0.982	0.915	0.915
AIK	2	0.454	0.042	0.511	0.879	0.856
FRA	4	0.637	0.122	0.766	0.799	0.920
COL	6	0.525	0.199	0.851	0.499	0.919
SUM	9	0.783	0.309	0.908	0.791	0.901
HOL	6	0.614	0.199	0.851	0.637	0.849
WHT	14	0.885	0.473	0.948	0.866	0.921
TUP	9	0.795	0.309	0.908	0.812	0.910
BAT	18	0.897	0.588	0.965	0.821	0.856
EBE	5	0.653	0.161	0.817	0.750	0.900
CUS	12	0.874	0.410	0.936	0.882	0.931
TUS	2	0.422	0.042	0.511	0.811	0.839
GAD	5	0.772	0.161	0.817	0.932	0.864
MOO	2	0.223	0.042	0.511	0.388	0.914
DAD	10	0.827	0.343	0.919	0.840	0.917
THO	14	0.876	0.473	0.948	0.848	0.923
Mean		0.694			0.753	0.895
SD		0.226			0.188	0.032

^a G = number of unique multilocus genotypes per population, D = Simpson’s diversity index, Fager’s (1972) E = evenness of genotypic distribution, I_n = mean genetic identity.

DISCUSSION

Consistent with predictions based on its history of multiple introductions, *P. lobata* exhibits a high level of genetic diversity throughout the southeastern United States. Furthermore, the lack of any geographic patterns coupled with relatively little overlap of genotypes between populations is indicative of multiple introductions within local geographic regions followed by subsequent gene exchange and recombination. Viable seeds have been observed in many populations, indicating that gene exchange has occurred among genotypes that may have resulted from separate historical introductions. Such gene exchange could have occurred among adjacent populations with different introduction histories or within populations founded by propagules with varying multilocus genotypes.

Pueraria lobata is more genetically diverse than expected based on the mean values reported by Hamrick and Godt (1989) for species with similar life-history traits. Dicots, long-lived herbaceous perennials, species with regional ranges, and temperate species, on average, have <50% of their loci polymorphic at the species level, while *P. lobata* has 92.9% of its loci polymorphic. Even though levels of polymorphism decrease to 55.7% at the population level, the mean population values of Hamrick and Godt (1989) are again lower for species with this combination of life history characters when compared with *P. lobata*. The same holds for the other genetic diversity parameters.

The proportion of genetic diversity partitioned among *P. lobata* populations ($G_{ST} = 0.199$) is similar to that of outcrossing, animal-dispersed species ($G_{ST} = 0.197$), as well as species with both sexual and asexual reproduction ($G_{ST} = 0.213$; Hamrick and Godt, 1989). Although little is known about the breeding system and seed dispersal mechanisms of *P. lobata*, it reproduces sexually, as well as asexually (Abramovitz, 1983; Forseth and Teramura, 1986; R. Pappert, personal observation). Abramovitz (1983) found extremely low levels of seed set and seed viability, as well as short (≤ 6 m) seed dispersal distances (for *P. lobata* populations located in Maryland). We have observed slightly higher seed germination rates than those reported by Abramovitz, ranging from 10 to 20% per population (Pappert, 1998). Of 245 naturally occurring seedlings observed by Abramovitz (1983) at the periphery of the Maryland populations, only one became established. The Maryland population studied by Abramovitz (1983) is near the edge of the geographic range of *P. lobata*. It is likely that seedling recruitment is higher in more southern populations. Nevertheless, the successful establishment of one or a few seedlings is enough to introduce novel genotypes into a population. Thus, under favorable conditions for seed production and recruitment, sexual reproduction could add significantly to the genotypic diversity of individual populations.

There are no discernible patterns with respect to the levels of genetic diversity, when comparing *P. lobata* with other invasive species with similar introduction histories and life history traits. *Lonicera japonica*, for example, has a significantly lower percentage of polymorphic loci ($P = 75\%$), mean total heterozygosity at polymorphic loci ($H_T = 0.288$), and mean variation within populations ($H_e = 0.258$) than *P. lobata* (Schierenbeck, Hamrick, and Mack, 1995). At the population level, however, *L. japonica* has more polymorphic loci ($P = 53.8\%$), but a lower expected heterozygosity ($H_e = 0.189$). Similarly, *P. lobata* has higher P , H_e , and H_T values than the invasive species *Casuarina cunninghamiana* (Moran, Bell, and

Turnbull, 1989; Moore and Moran, 1989) and *Lathyrus latifolius* (Godt and Hamrick, 1991). However, *Robinia pseudoacacia*, a clonally reproducing, invasive, native tree, has more polymorphic loci, greater total levels of diversity, and higher mean genetic identity among populations than *P. lobata* (Sures, Hamrick, and Bongarten, 1989).

In contrast, *P. lobata* has more genetic diversity than several unintentionally introduced species. As was expected, little genetic variability is found in the naturalized ranges of *Bromus tectorum* ($P = 3.46\%$, $H_e = 0.009$; Novak and Mack, 1993), *Abutilon theophrasti* (fixed banding patterns for most loci; Warwick and Black, 1986) and *Xanthium strumarium* ($P = 15.4\%$; Moran and Marshall, 1978). Additionally, these are primarily selfing species, which further limits the possibility of genetic recombination and subsequent colonization with genetically variable genotypes. Obviously, genetic expectations for species that were intentionally introduced repeatedly as ornamentals or for other specific purposes should be distinguished from those for species that colonize new continents by happenstance. Within this context, interpretation of our results would have been improved if we had sampled from the native range of *P. lobata*. Logistic considerations precluded such collections, however.

Although *P. lobata* spreads vegetatively, very few multilocus genotypes are shared among populations, indicating that populations are not strictly founded vegetatively. Two scenarios could produce such high numbers of genotypes in some populations with so few genotypes in common among populations. First, some populations may have been established by several individuals from diverse sources, starting a population with high levels of genotypic diversity. Secondly, populations may start with relatively few founders, but subsequent pollen flow and seedling establishment introduces additional genotypic diversity into the population. Both scenarios would result in high genotypic diversity within populations. Seed dispersal, although it has not been described at a large geographic scale, is not unlikely. Many populations of *P. lobata* occur on roadsides and railroad embankments, areas where long-distance seed dispersal may be optimized. The high levels of genetic variation observed throughout the southeastern states does not indicate one or even a few points of introduction and subsequent spread from these points. Rather, our data support the conclusion that multiple introductions containing many diverse genotypes occurred in each geographical region.

Populations of *P. lobata* usually consist of more than one genetically distinct individual. Seed set is variable among populations, although many populations flower profusely throughout mid- to late summer. Some populations have copious amounts of seed, whereas others are barren. Of the populations that were revisited after seed set, those with more genotypes had much greater seed set, and subsequent germination (HEL, ATH, GRE), than those with fewer unique multilocus genotypes (MOO, WHT) (R. Pappert, personal observation). This observation suggests that populations with more genotypes are more likely to successfully outcross and set seed. Since environmental factors are also correlated with seed production in *P. lobata* (Abramovitz, 1983), populations growing under favorable conditions may flower more profusely, attract more pollinators, and produce more seed. The reproductive biology of *P. lobata* should be studied more intensively to determine whether widespread patterns of sexual reproduction are evident. Newly established populations also should be identified

to determine the number of genotypes present during and subsequent to the founding event.

An interesting observation from our results is that populations with few genotypes (e.g., TUS, MOO, AIK, and NEW) have higher mean levels of observed heterozygosity and that observed heterozygosities are also higher than Hardy-Weinberg expectations. It is possible that selection has acted in favor of individuals heterozygous at a large proportion of their loci at some or several stages of the life cycle. Although the dominance of few genotypes per population has been suggested based on theoretical studies (Parker, 1979; Ellstrand and Roose, 1987), the mechanism of selection favoring heterozygotes was not proposed. In an experiment implemented subsequent to this study, individuals heterozygous for a high proportion of these allozyme loci accumulated more biomass and leaf area (Pappert, 1998).

In summary, *P. lobata* maintains high levels of genetic diversity throughout the southeastern United States. Factors that influence such high levels of diversity include multiple introductions over approximately five decades, successful sexual reproduction allowing gene flow within and among populations, as well as the founding of populations by more than one genetically distinct individual. *Pueraria lobata* provides a unique opportunity for further research into the actual dynamics and spread of clonal, invasive species. By studying the peripheral spread and mating system of *P. lobata* in greater detail, we could directly determine the general importance of sexual reproduction within populations and whether sexual reproduction is a major component in the spread of *P. lobata* into open habitats.

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