

**GENETIC VARIATION IN WALNUTS (*JUGLANS REGIA* AND  
*J. SIGILLATA*; JUGLANDACEAE): SPECIES DISTINCTIONS,  
HUMAN IMPACTS, AND THE CONSERVATION OF  
AGROBIODIVERSITY IN YUNNAN, CHINA<sup>1</sup>**

BEE F. GUNN<sup>2,8</sup>, MALLIKARJUNA ARADHYA<sup>3</sup>, JAN M. SALICK<sup>4</sup>, ALLISON J. MILLER<sup>5</sup>,  
YANG YONGPING<sup>6</sup>, LIU LIN<sup>7</sup>, AND HAI XIAN<sup>7</sup>

<sup>2</sup>Missouri Botanical Garden, St. Louis, Missouri 63166 USA; and Research School of Biology, Division of Evolution, Ecology and Genetics, The Australian National University, Bldg. 116 Daley Road, Canberra, ACT, 0200, Australia; <sup>3</sup>U. S. Department of Agriculture, National Clonal Germplasm Repository, 1 Shields Avenue, University of California-Davis, Davis, California 95616 USA; <sup>4</sup>William L. Brown Center, Missouri Botanical Garden, P.O. Box 299, St. Louis, Missouri 63166 USA; <sup>5</sup>Department of Biology, Saint Louis University, 3507 Laclede Avenue, St. Louis, Missouri 63101 USA; <sup>6</sup>Kunming Institute of Botany, CAS, 132 Lanhei Road, Heilongtan, Kunming 650204, Yunnan, China; and <sup>7</sup>Shangri-La Alpine Botanical Garden, P.O. Box 118, Xianggelila County, Diqing Prefecture, Yunnan, China

Walnuts are a major crop of many countries and mostly cultivated in large-scale plantations with few cultivars. Landraces provide important genetic reservoirs; thus, understanding factors influencing the geographic distribution of genetic variation in crop resources is a fundamental goal of agrobiodiversity conservation. Here, we investigated the role of human settlements and kinship on genetic variation and population structure of two walnut species: *Juglans regia*, an introduced species widely cultivated for its nuts, and *J. sigillata*, a native species cultivated locally in Yunnan. The objectives of this study were to characterize sympatric populations of *J. regia* and *J. sigillata* using 14 molecular markers and evaluate the role of Tibetan villages and kin groups (related households) on genotypic variation and population structure of *J. regia* and *J. sigillata*. Our results based on 220 walnut trees from six Tibetan villages show that although *J. regia* and *J. sigillata* are morphologically distinct, the two species are indistinguishable based on microsatellite data. Despite the lack of interspecific differences, AMOVAs partitioned among villages (5.41%,  $P = 0.0068$ ) and kin groups within villages (3.34%,  $P = 0.0068$ ) showed significant genetic variation. These findings suggest that village environments and familial relationships are factors contributing to the geographic structure of genetic variation in Tibetan walnuts.

**Key words:** agrobiodiversity; China; Juglandaceae; *Juglans sigillata*; *Juglans regia*; kinship; microsatellites; population genetics; Tibetan; walnuts; Yunnan.

Walnuts (*Juglans* spp., Juglandaceae) are among the most economically important nut trees in the world. The United States produces about 340 000 metric tons of Persian walnuts (*J. regia* L.), and China exports 250 000 metric tons per annum. In addition to large-scale cultivation, *J. regia* is grown locally in the Balkans, Iran, Turkey, Central Asia, Himalayas, and China. In Tibet and southwestern China, *J. regia* and a native thick-shelled walnut *J. sigillata* Dode are cultivated for their fruits, which are eaten and also used in religious and cultural

activities (Rosengarten, 1984; Weckerle et al., 2005). Among the ethnic communities of northwestern Yunnan, China, *Juglans* spp. are prized for their high quality nuts, medicinal properties, and ritual uses, and are passed down through generations as inheritance by individuals or families. Over the past several thousand years, walnuts have been exchanged continually among Tibetans, a practice that persists today. Consequently, Tibetan walnuts present a unique opportunity to examine the role of human-mediated processes (e.g., dispersal, selection) on their genetic structure.

Tree species that grow in close association with humans are subject to unique evolutionary and ecological processes. For example, artificial selection pressures lead to morphological changes in cultivated populations, dispersal by humans expands the natural range of species, and range expansion can lead to sympatry and hybridization with otherwise allopatric congeneric species. Tree species of significant commercial importance are generally grown in large orchards, whilst those of local importance are often maintained in less intense agricultural systems and consist of more diffuse populations with individuals spread over numerous locations. Recent studies have shown that traditionally managed populations may serve as reservoirs for genetic variation within crop species (Miller and Schaal, 2006; Jarvis, et al., 2008); however, impacts of human settlements and familial relationships on population genetic

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<sup>8</sup> Author for correspondence (e-mail: bee.gunn@anu.edu.au)

structure of traditionally managed tree populations are not well understood.

In the Khawa Karpo region of Tibet, *J. regia* (the Persian or English walnut) and *J. sigillata* (the Chinese iron walnut) occur in sympatry. *Juglans regia* and *J. sigillata* are readily distinguished from one another based on leaflet number (9–11 in *J. sigillata*, 5–9 in *J. regia*) and nut morphology (*J. sigillata* has deep pits or seal-like depressions [sigillatae] on the surfaces of the nuts and dark-colored kernels with tough septa, whereas *J. regia* has wrinkled nut surfaces, light-colored kernels and papery septa). The kernels of *J. regia* are macerated and added to yak butter tea or are made into walnut cakes for festive occasions and are traded commercially. Unlike *J. regia*, *J. sigillata* nuts have thick hard shells (thus “iron walnuts” in China); the fruits are crushed whole and cooked for several hours to extract walnut oil.

Although both *J. regia* and *J. sigillata* are cultivated, eaten, and exchanged among families within Tibet, it appears they have different origins within the region. *Juglans sigillata* is one of three *Juglans* species native to Asia (the others are *J. ailantifolia* and *J. mandshurica*), while *J. regia* is native to western Asia Minor and was presumably introduced into Tibet from Tazig (ancient Persia) during Bön religion prior to Buddhism (Danforth and Johnson, 1997). In genus-wide analyses, Dode (1909) and later Manning (1978) grouped *J. regia* and *J. sigillata* together based on morphological similarities. *Juglans regia* and *J. sigillata* were designated as the sole members of section *Juglans* (section *Dioscaryon* Dode); the other Asian *Juglans* species, *J. ailantifolia* and *J. mandshurica*, are classified within section *Cardiocaryon*. Subsequent molecular work based on chloroplast sequence data supported the sister-group relationship of *J. regia* and *J. sigillata* (Aradhya et al., 2006). However, Wang et al. (2008), using different microsatellite markers, were unable to distinguish between the two species in southwestern China, and they concluded that these two should be considered a single species. We accept this possibility and present two additional alternatives: observed patterns in the microsatellite data presented here could indicate (1) incomplete lineage sorting: *J. regia* and *J. sigillata* represent incipient species in which ancestral lineages have not yet sorted along species boundaries, and/or (2) ongoing gene flow: *J. regia* and *J. sigillata* represent distinct species that exchange genes in areas of sympatry.

Despite the long-term economic and cultural significance of walnuts in Tibet, neither the geographic distribution of genetic variation in these taxa nor the role of humans in structuring genetic variation has been studied. Here, we apply 14 microsatellite loci to characterize genetic variation in Tibetan indigenous walnut populations. The objectives of this study were to investigate whether molecular markers distinguish sympatric *Juglans regia* and *J. sigillata* in Tibet and to compare walnut genotypic variation and population genetic structure among Tibetan villages and kin groups.

## MATERIALS AND METHODS

**Sampling procedure**—A total of 220 Tibetan walnut trees (159 *J. regia* and 61 *J. sigillata*) were collected from six villages located between 2000–3000 meters a.s.l. in the foothills of Khawa Karpo, NW Yunnan, China (Fig. 1, Table 1).

Within each village, we chose three related households (three households with residents who were biologically related to the residents of the other two households; each group of three households constitutes a “kin group”) and independently chose another three related households (a second kin group) with no

known family ties to the first kin group, for a total of six households per village. Leaf samples of all walnut trees (*J. regia* and *J. sigillata*) growing next to each household were collected. The locations of individual trees were recorded using a global positioning system (Garmin eTrex, Garmin Ltd., Kansas City, Missouri, USA). Herbarium vouchers for each walnut tree were collected and deposited in Missouri Botanical Garden (MO) (Appendix 1). Leaf material preserved in silica gel was collected from each tree. Species were determined as either *Juglans regia* L. or *J. sigillata* Dode based on a suite of morphological characters including leaflet number, leaflet-tip shape, petiole and petiolule lengths, bark, nut surface patterns, and thickness of nut sutures (Lu et al., 1999; also see introduction).

**DNA isolation and microsatellite amplification**—Genomic DNA was isolated from leaves dried in silica gel using Qiagen Plant Miniprep Kit (Qiagen, Valencia, California, USA). Fourteen microsatellite loci originally developed for the eastern black walnut, *Juglans nigra* (Woeste et al., 2002), were amplified: WGA4, WGA9, WGA89, WGA118, WGA202, WGA210, WGA237, WGA242, WGA276, WGA318, WGA321, WGA332, WGA338, and WGA331. PCR amplifications were performed in a 10  $\mu$ L reaction mixture containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM MgCl<sub>2</sub> (all included in 10  $\mu$ L of 10 $\times$  PCR buffer), 4–5 pM of each fluorescently labeled microsatellite primer, 200  $\mu$ M of each dNTP, 0.5 U of *Taq* polymerase (PE Applied Biosystems, Foster City, California, USA), and 15–20 ng of template DNA. The PCR conditions were: one cycle of 5 min at 94°C; 30 cycles of 30 s at 94°C, 30 s at 55°C, 40 s at 72°C; and one cycle of 7 min at 72°C for all loci.

Amplified DNA fragments were separated using capillary electrophoresis in an ABI Prism 3100 genetic analyzer. Data were collected with the associated data collection software (PE Applied Biosystems). The fragment analysis was performed with GenTyper, version 2.5 and data assembled as multilocus microsatellite genotypes across the 14 loci. Microsatellite data were reformatted from an excel file to an Arlequin (.arp) file using the program CONVERT (Glaubitz, 2004).

**Data analyses—Characterization of microsatellite loci**—Estimates of genetic variation (observed number of alleles per locus, effective number of alleles, Shannon’s information index) as well as heterozygosity statistics (observed and expected homozygosity and heterozygosity, Nei’s (1973) expected heterozygosity) were generated using the program Popgene version 1.32 (Yeh and Boyle, 1997).

**Genetic differentiation of *J. regia* and *J. sigillata***—An analysis of molecular variance (AMOVA) was conducted separately for each species using the program Arlequin version 3.1.1 (Excoffier et al., 2005). The Bayesian model-based clustering algorithm implemented in the program STRUCTURE (Pritchard et al., 2000) was used to determine if individuals from the same species grouped together. The burn-in period and the number of Markov chain Monte Carlo (MCMC) repetitions were set to 50,000; models used for ancestry and allele frequency were: admixture and “allele frequencies correlated”. *K* was set at 1–13, and the highest *K* value was identified as the run with the highest likelihood value. In addition, *K* values were averaged across 10 iterations. We also explored the population structure using the program BAPS 4.14 (Corander and Marttinen, 2006), which determines the number of clusters first and, subsequently, using the output file for analyses of admixtures compared to STRUCTURE’s simultaneous computation. We then used the probabilities of population assignment results to determine the number of hybrid individuals. We considered any individual with  $\leq 70\%$  probability of assignment to a cluster as a possible hybrid.

**Genetic variation and population structure within and among Tibetan villages and kin groups**—We found insignificant genetic variation between the two species (0.7%;  $P = 0.453$ , see Table 4) from the AMOVA analysis above. On the basis of these results, *J. regia* and *J. sigillata* samples were combined for each of the six villages. We investigated the structure of genetic variation within species among villages, within villages among kin groups, and within individuals. Neighbor-joining analysis of relationships based on Nei’s genetic distance among village populations of *J. regia* and *J. sigillata* was performed on the genetic identity matrix using software packages in R (R Development Core Team, 2005) to visualize relationships among species from different villages. The number of migrants between species per generation,  $(1/F_{ST} - 1)/4$  was estimated using the program GENALEX (Peakall and Smouse, 2006).

**Relationship between genetic variation and geography**—To investigate the relationship between geographic distance and genetic distance, we generated pairwise genetic distances and geographical distances among sampled populations

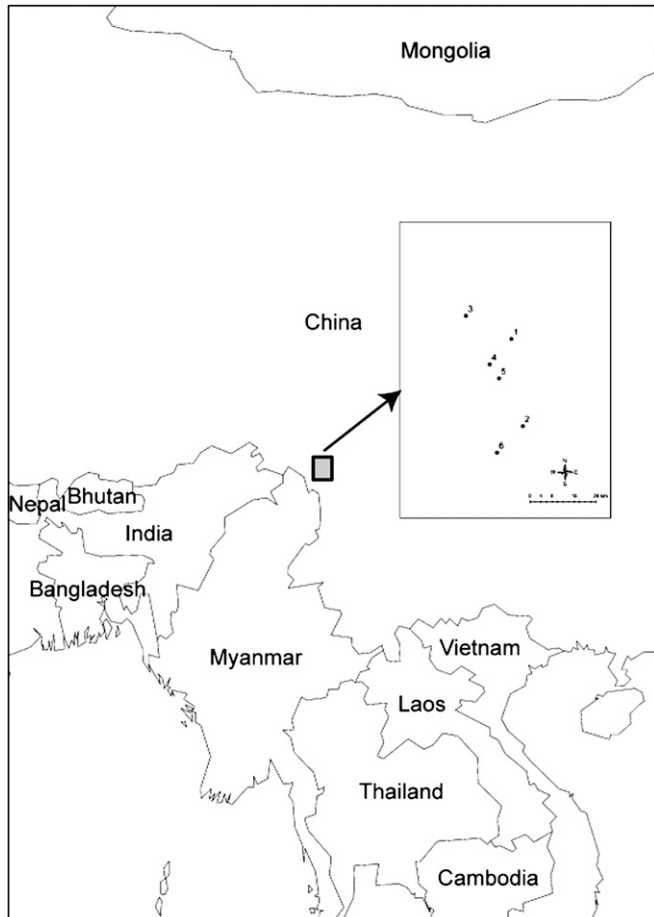


Fig. 1. Locality map of the study region showing village sites in northwestern Yunnan, China. Numbers 1–6 correspond to villages.

in the villages using the ISOLDE program in GENEPOP (Raymond and Rousset, 1995; Rousset, 1997). A Mantel test of the relationship between  $F_{ST}/(1 - F_{ST})$  and the natural logarithm of geographic distance was also performed. The test for isolation by distance was based on 10000 permutations using minimum spatial distance between the sampled populations for each village.

Relationships between estimates of observed heterozygosity and environmental variables were examined to determine if genetic differences among villages were associated with environmental conditions rather than human influences. Measurements of average slope and average aspect were calculated for each village based on field-collected data. In addition, 13 GIS-based environmental variables (30-s resolution) were downloaded from the WorldClim data set (<http://www.worldclim.org>; Hijmans et al., 2005) for each village (Table 1): annual average temperature, temperature seasonality, temperature in the warmest month, temperature in the coldest month, annual temperature range, temperature in the wettest quarter, temperature in the driest quarter, annual precipitation, precipitation in the wettest month, precipitation in the driest month, precipitation seasonality, precipitation in the warmest quarter, and precipitation in the coldest quarter. Temperature data were converted to Kelvin and were  $\log_{10}$ -transformed. Estimates of observed heterozygosity within villages were regressed on each of the 13 climate variables and slope and aspect to assess whether these abiotic measures influenced genetic patterns in the study system. Because multiple tests were performed ( $N = 15$ ), a sequential Bonferroni correction was applied ( $\alpha = 0.05$ ,  $\alpha' = 0.003$ ) (Holm, 1979).

## RESULTS

**Marker summary information**—Microsatellite variation in Tibetan *Juglans* is summarized in Tables 2 and 3. All 14 mic-

TABLE 1. *Juglans regia* and *J. sigillata* individuals collected in each of six villages. The total number of individuals per species is shown with the number of individuals collected per kin group shown in parentheses (kin group 1, kin group 2).

Village	Latitude (N)	Longitude (E)	<i>J. regia</i>	<i>J. sigillata</i>
1	28.56722	98.87083	17 (10,7)	9 (8,1)
2	28.28250	98.90833	55 (26,29)	7 (4,3)
3	28.64296	98.72269	11 (7,4)	1 (1)
4	28.48410	98.80034	37 (19,18)	16 (7,9)
5	28.43855	98.83095	18 (11,7)	14 (8,6)
6	28.19611	98.82416	21 (15,6)	14 (10,4)
Total			159	61

rosatellite loci were polymorphic, and AMOVAs showed that high variation was apportioned within individuals. Genetic variation observed in this data set is consistent with previous studies for *Juglans nigra*, *J. regia*, and *J. sigillata* (Dangl et al., 2005; Victory et al., 2006; Wang et al., 2008).

**Genetic differentiation of *J. regia* and *J. sigillata***— $F_{ST}$  values and AMOVA indicated that only 0.70% of the molecular variance was attributable to differences between *J. regia* and *J. sigillata* ( $P = 0.4537$ ), with 99.30% of variance attributed to within-species variation (Tables 3, 4). A neighbor-joining tree revealed that *J. regia* and *J. sigillata* from the same village were sisters in four cases (villages 1, 2, 5, and 6) (Fig. 2); neither the populations of *J. regia* nor those of *J. sigillata* were monophyletic. The STRUCTURE analysis revealed that  $K = 7$  had the highest likelihood value [ $\text{Ln P(D)} = -6421.52$ ] (data not shown). The BAPS analysis determined seven clusters with likelihood value [ $\text{Ln P(D)} = -6869.3$ ]. The maximum number of populations  $K = 7$  was the same for BAPS and STRUCTURE. We followed the BAPS clustering because it is a more conservative method and also provides the cluster memberships. The number of hybrids estimated overall was 25% (54/220) (Appendix S1, see Supplemental Data with the online version of this article). The number of migrants per generation between *J. regia* and *J. sigillata* was 35.46.

**Genetic variation and population structure within and among Tibetan villages and kin groups**—Levels of genetic variation within villages were slightly elevated for *J. regia* populations relative to *J. sigillata* populations (Table 5). Among village partitioning of variance was significant for both species with AMOVA (*J. regia*: 6.28%,  $P < 0.001$ ; *J. sigillata* 10.00%,  $P < 0.000$ ; Table 6). These data and mean  $F_{ST}$  values (Table 3) revealed that a larger component of the genetic variance was distributed among villages for *J. sigillata* populations ( $F_{ST} = 0.119$ ) compared to *J. regia* populations ( $F_{ST} = 0.088$ ), possibly reflecting human-mediated dispersal of *J. regia*, the more commonly cultivated species (Table 3). Differentiation among kin groups was higher (and nearly significant) for *J. sigillata* (3.58%,  $P = 0.06$ ) than for *J. regia* (0.37%,  $P = 0.386$ ); however, variance due to among-kin group differences was not significant for either species (Table 6).

Given the lack of distinction between microsatellite profiles of *J. regia* and *J. sigillata* individuals (Fig. 2, Tables 3, 4), the two species were grouped to provide a regional assessment of genetic variation and population structure in *Juglans*. Measures of *Juglans* genetic diversity were relatively similar for each of the six surveyed villages; village 2 had the lowest estimates of genetic variation, and village 6 had the highest estimates



of genetic variation (Table 5). Combined Tibetan *Juglans* displayed significant levels of genetic variance partitioned among villages (5.41%,  $P = 0.007$ ), among kin groups within villages (3.34%,  $P = 0.007$ ), and within individuals (88.08%,  $P = 0.003$ ) (Table 6).

**Genetic variation and geography**—The Mantel test failed to reject the null hypothesis of no correlation between the pairwise genetic differentiation [ $F_{ST}$  and  $F_{ST} / (1 - F_{ST})$ ] and geographical proximity among populations at the village level ( $P = 0.092$ ), based on the Spearman rank correlation coefficient statistic. Regression analyses between village estimates of observed heterozygosity and slope, aspect, and 13 environmental variables revealed no significant relationships following sequential Bonferroni correction between genetic variation detected within villages and slope, aspect, or any other environmental variable (Appendix S2, see online Supplemental Data).

DISCUSSION

**Distinguishing sympatric, congeneric taxa using molecular markers**—Data based on 14 microsatellite loci failed to differentiate *J. regia* and *J. sigillata* in the Khawa Karpo region of Tibet. In the field, *J. sigillata* individuals are distinguished from

*J. regia* by their relatively large compound leaves, rugose trunks, thick endocarp, and deeply pitted nut surfaces (Dode, 1909; Stone, 1993). Previous studies based on DNA sequence data from five chloroplast intergenic spacers demonstrated marginal differences between *J. regia* and *J. sigillata* (Aradhya et al., 2006). However, a recently published study of *J. regia* and *J. sigillata* from central and southwestern China (Wang et al., 2008), based on different microsatellite loci than those employed in this study, failed to distinguish *J. regia* from *J. sigillata*, leading Wang et al. to suggest that *J. regia* and *J. sigillata* belong to the same species.

Two independent data sets have failed to differentiate *J. regia* and *J. sigillata* at the molecular level, raising the possibility that *J. sigillata* and *J. regia* should be considered as a single species that includes “*J. sigillata*-type” individuals and “*J. regia*-type” individuals. In maize, morphologically distinct varieties cultivated by different linguistic groups were indistinguishable with allozymes (Perales et al., 2005) and are considered part of the same species. Our study differs from the maize example because it is unclear whether the morphologically distinct *Juglans* types evolved under human influence, in which case, they might be considered cultivars. Although wild (uncultivated) populations have been recorded for both species, those populations were not sampled in this study. Further, it is not known if cultivated *J. regia*-type individuals and *J. sigillata*-type individuals represent distinct cultivated varieties derived from a single domesticated ancestor (as in maize) or

TABLE 2. Summary of genetic variation and heterozygosity statistics for fourteen microsatellite loci amplified from *Juglans regia* and *J. sigillata*.

Taxon	Locus	N	na	ne	I	H <sub>o</sub>	H <sub>e</sub>	Nei	Mean H	
<i>J. regia</i>	A089F	272	10.0000	4.3347	1.6614	0.6765	0.7721	0.7693	0.7171	
	A09F	280	4.0000	1.8843	0.7429	0.4500	0.4710	0.4693	0.4265	
	A237N	254	6.0000	2.1304	0.8527	0.2756	0.5327	0.5306	0.4881	
	A332H	290	5.0000	2.7719	1.1538	0.5448	0.6415	0.6392	0.5689	
	A242N	298	6.0000	3.5889	1.3750	0.6846	0.7238	0.7214	0.6200	
	A276H	308	10.0000	2.4567	1.2899	0.5130	0.5949	0.5930	0.5722	
	A321F	294	9.0000	2.1697	1.2223	0.5102	0.5409	0.5391	0.5173	
	A338N	260	4.0000	1.5179	0.6704	0.3385	0.3425	0.3412	0.3651	
	A004F	244	7.0000	4.7736	1.6545	0.8033	0.7938	0.7905	0.7023	
	A118H	262	7.0000	4.0884	1.5525	0.7481	0.7583	0.7554	0.7050	
	A210N	284	10.0000	3.6172	1.6508	0.8592	0.7261	0.7235	0.7281	
	A202H	260	15.0000	2.6322	1.5957	0.6231	0.6225	0.6201	0.5924	
	A318F	268	8.0000	3.0791	1.4294	0.4776	0.6778	0.6752	0.6284	
	A331N	278	4.0000	2.1609	0.8770	0.5684	0.6241	0.6218	0.5817	
	<b>Mean</b>		<b>275</b>	<b>7.5000</b>	<b>2.9433</b>	<b>1.2663</b>	<b>0.5684</b>	<b>0.6241</b>	<b>0.6218</b>	<b>0.5817</b>
	<b>SD</b>			<b>3.1071</b>	<b>0.9951</b>	<b>0.3563</b>	<b>0.1713</b>	<b>0.1296</b>	<b>0.1291</b>	<b>0.1117</b>
	<i>J. sigillata</i>	A089F	104	9.0000	5.2710	1.8089	0.8269	0.8181	0.8103	0.6102
A09F		110	2.0000	1.9478	0.6797	0.4000	0.4911	0.4866	0.3476	
A237N		106	2.0000	1.9824	0.6887	0.3019	0.5003	0.4956	0.3422	
A332H		116	3.0000	2.6007	1.0204	0.5517	0.6208	0.6155	0.4223	
A242N		114	5.0000	3.1240	1.3182	0.6667	0.6859	0.6799	0.6012	
A276H		122	10.0000	3.3164	1.5553	0.6393	0.7042	0.6985	0.5611	
A321F		112	7.0000	3.0285	1.3998	0.6429	0.6758	0.6698	0.4919	
A338N		106	4.0000	1.5506	0.7174	0.3962	0.3585	0.3551	0.3301	
A004F		110	6.0000	3.8857	1.5045	0.6909	0.7495	0.7426	0.6505	
A118H		106	6.0000	4.0446	1.5375	0.7736	0.7599	0.7528	0.7011	
A210N		112	11.0000	5.0785	1.9218	0.9107	0.8103	0.8031	0.7269	
A202H		112	14.0000	3.5335	1.8039	0.6786	0.7235	0.7170	0.5532	
A318F		106	7.0000	3.6386	1.4722	0.4151	0.7321	0.7252	0.6006	
A331N		116	6.0000	2.2722	0.9816	0.4310	0.5648	0.5599	0.4972	
<b>Mean</b>			<b>111</b>	<b>6.5714</b>	<b>3.2339</b>	<b>1.3150</b>	<b>0.5947</b>	<b>0.6568</b>	<b>0.6508</b>	<b>0.5311</b>
<b>SD</b>				<b>3.4799</b>	<b>1.1207</b>	<b>0.4276</b>	<b>0.1830</b>	<b>0.1339</b>	<b>0.1327</b>	<b>0.1310</b>

Notes: na = observed number of alleles, ne = effective number of alleles (Kimura and Crow, 1964), I = Shannon’s information index (Lewontin, 1972), H<sub>e</sub> = expected heterozygosity were computed using Levene (1949), H<sub>o</sub> = observed heterozygosity, Nei’s (1973) H<sub>e</sub>

TABLE 3. Genetic differentiation between species, and among villages and kin groups within species.

Locus	Between species			Among village						Among kin group					
	$F_{IS}$	$F_{IT}$	$F_{ST}$	<i>Juglans regia</i>			<i>Juglans sigillata</i>			<i>Juglans regia</i>			<i>Juglans sigillata</i>		
				$F_{IS}$	$F_{IT}$	$F_{ST}$	$F_{IS}$	$F_{IT}$	$F_{ST}$	$F_{IS}$	$F_{IT}$	$F_{ST}$	$F_{IS}$	$F_{IT}$	$F_{ST}$
A089F	0.064	0.071	0.007	-0.007	0.098	0.105	-0.085	0.017	0.094	-0.070	0.076	0.136	-0.143	0.02	0.142
A009F	0.132	0.141	0.01	0.007	0.073	0.066	-0.029	0.130	0.154	0.070	0.141	0.076	-0.104	0.028	0.120
A237N	0.432	0.434	0.004	0.322	0.411	0.13	0.146	0.295	0.174	0.358	0.429	0.110	-0.013	0.286	0.295
A332H	0.140	0.145	0.005	0.007	0.152	0.146	0.023	0.183	0.164	-0.041	0.156	0.189	-0.278	0.032	0.242
A242N	0.048	0.052	0.005	-0.029	0.106	0.13	-0.019	0.115	0.131	-0.091	0.061	0.140	-0.097	0.115	0.193
A276H	0.078	0.089	0.012	0.013	0.113	0.102	-0.068	0.079	0.137	-0.038	0.082	0.115	-0.090	0.081	0.157
A321F	0.072	0.082	0.011	0.033	0.106	0.075	-0.032	0.067	0.096	0.007	0.107	0.101	-0.019	0.131	0.147
A338N	0.056	-0.054	0.002	-0.112	-0.075	0.033	-0.188	-0.098	0.076	-0.141	-0.069	0.063	-0.378	-0.136	0.175
A004F	0.022	0.039	0.018	-0.114	0.008	0.109	-0.014	0.161	0.172	-0.177	-0.034	0.121	-0.069	0.235	0.284
A118H	0.013	-0.006	0.006	-0.064	-0.012	0.049	-0.018	0.032	0.049	-0.103	-0.032	0.065	-0.094	0.111	0.187
A210N	0.143	-0.137	0.005	-0.224	-0.162	0.051	-0.107	-0.038	0.063	-0.253	-0.180	0.059	-0.181	-0.003	0.151
A202H	0.034	0.036	0.002	-0.061	0.004	0.062	-0.094	0.037	0.12	-0.112	0.003	0.103	-0.177	0.020	0.167
A318F	0.383	0.390	0.011	0.240	0.303	0.084	0.31	0.401	0.132	0.198	0.286	0.110	0.354	0.473	0.184
A331N	0.221	0.221	0.000	-0.015	0.054	0.069	0.075	0.195	0.129	-0.053	0.041	0.089	0.182	0.325	0.175
Mean	0.095	0.102	0.007	-0.009	0.080	0.088	-0.008	0.112	0.119	-0.043	0.069	0.108	-0.073	0.127	0.186

whether cultivated *J. regia* and cultivated *J. sigillata* populations have separate origins from morphologically distinct native populations. Additional sampling of wild and cultivated populations from a broad geographic range is required to assess the species status of *J. regia* and *J. sigillata*.

A second, possibly related explanation for the lack of molecular differentiation between *J. regia* and *J. sigillata* is the incomplete sorting of ancestral lineages, a phenomenon usually characterizing incipient or recently diverged species. In these cases, the ancestral lineages present in the common ancestor of the diverging taxa have not yet sorted along species boundaries. Identifying instances of incipient speciation is further complicated in lineages where human dispersal, management, and selection play a role. If *J. regia* and *J. sigillata* represent incipient species, it is possible that human actions may influence the speciation process in one of two ways. Human selection for different traits (e.g., taste in *J. regia* and oil content in *J. sigillata*; thin shells and larger kernels in *J. regia*) may promote divergence. Alternatively, similar selection pressures in both species (e.g., selection for fruits that open easily or are early bearing), combined with patterns of dispersal that bring together two formerly allopatric species that exchange genes in sympatry, could blur or reverse the process of divergence. Another consideration is that incomplete lineage sorting may be amplified in tree species. It is well known that trees have slower mutation rates and lower nucleotide substitution and speciation rates than do short-lived herbaceous plants (Smith and Donoghue, 2008). Tree populations are generally heterozygous and have limited differentiation between populations (Petit and Hampe, 2006; Savolainen et al., 2007). Microsatellite markers represent neutral variation that is

subject to evolutionary processes such as drift, but has little (if any) association with adaptive variation (Jump et al., 2006; Mitchell-Olds et al., 2007). Consequently, the morphological variation distinguishing *J. regia* from *J. sigillata* will not be reflected in the microsatellite data if the lineages have not yet sorted. Comparative studies of black walnuts in North America revealed similar results (Victory et al., 2006), where populations in postglacial hydrological regions showed high genetic diversity among individuals but little structure at the population level.

The third possible explanation for the lack of distinction between *J. regia* and *J. sigillata* is that these two taxa may be exchanging genes in northwestern Yunnan. The reported natural species range for *J. regia* is Central Asia, Xinjiang Province (western China), Kazakhstan, Uzbekistan, Kirghizia, Himalayas, Iran, Azerbaijan, Armenia, Georgia, and eastern Turkey; *J. sigillata* is native to Yunnan, Sichuan, southeastern Tibet, Guizhou, Sikkim, and Bhutan. It appears that these two lineages were allopatric until humans introduced the *J. regia* from Xinjiang to the Hengduan Mountains region. In northwestern Yunnan, the high effective number of migrants ( $N_m$ ) between the two species may be a result of human activities. It has been well-documented that the movement of cultivated plants by humans can bring two formerly allopatric species into contact, often leading to serendipitous hybridization as seen in *Leucaena* and *Spondias* (Miller and Schaal, 2005; Hughes et al., 2007). Many tree species are obligate outcrossers that readily hybridize with sympatric congeners when the opportunity arises (Petit and Hampe, 2006). Heterodichogamy or protandrous-protogynous dimorphism is common in Juglandaceae (walnuts, pecans, and hickories), but is a relatively rare syndrome among flowering plants (Renner, 2001). Walnuts are monoecious and heterodichogamous with morphs occurring in a 1:1 ratio; the protandrous individuals shed pollen before their stigma become receptive, whereas protogynous individuals produce female flowers that develop before the male flowers. The temporal separation of male and female function ensures outcrossing in natural populations (Gleeson, 1982; Bai et al., 2006). When members of allopatric species are cultivated together, heterodichogamy may facilitate hybridization in sympatry. This pattern is consistent with the observed lack of differentiation between *J. regia* and *J. sigillata* and the high estimated number of migrants and has been recorded with other walnut hybrids (e.g., *Juglans ×notha*, *J. ×quadrangulata* and *J. ×sinensis*, see Grimshaw, 2003).

TABLE 4. AMOVA for Tibetan *Juglans*.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	P value
Between species	1	0.707	-0.00330	0.70	0.45357
Within species among villages	10	11.980	0.02640	8.75	0.00000
Within villages among individuals	208	60.070	0.01031	3.42	0.13978
Within individuals	220	59.000	0.26818	88.92	0.00391
Total	439	131.757	0.30159		

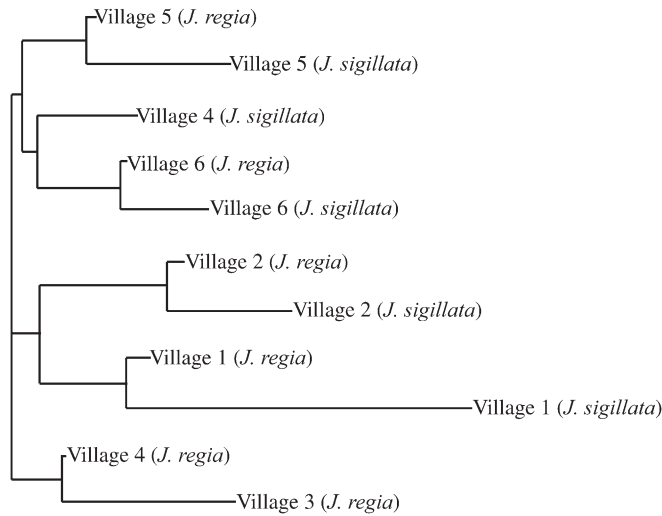


Fig. 2. Neighbor-joining tree of walnut genotypes based on Nei's genetic distance between village populations showing non-monophyly of *Juglans regia* and *J. sigillata* and clustering by villages in northwestern Yunnan, China.

The molecular signature left by ongoing hybridization between morphologically distinct tree taxa (e.g., lack of differentiation between hybridizing species) may persist at neutral loci such as microsatellites because of the slow generation time and/or the rapid decay of linkage disequilibrium (Neale and Savolainen, 2004).

**Human settlements and kin relationships impact population genetic structure**—The role of humans in altering genetic variation within domesticated species has been well documented (Zohary and Hopf, 2000; Breton, et al., 2006; Doebley et al., 2006; Burke et al., 2007); however, the impact of human-mediated evolutionary processes on species grown in traditional agroecosystems is less well known. Previous studies have demonstrated that human management influences forest composition. For example, in southern Mexico, a study examining the

diversity of tree species in 100 rustic coffee plantations showed that human management was one of the factors responsible for the high variation and conservation of wild tree species within the agroforestry system (Bandeira, et al., 2005). Other studies have identified a relationship between ethnolinguistic distinctiveness and morphological diversity. Evidence from maize landraces in upland Chiapas, Mexico suggests that social networks may play an important role in the maintenance of superior morphotypes, through selection for traits well adapted to the environment. Results from reciprocal garden plantings of maize landraces from both Tzeltal and Tzotzil speakers suggested significant differences in yields, phenology, and morphology (Perales et al., 2005).

Data presented here demonstrate that the geographic structure of genetic variation within walnut populations corresponds to human settlements and familial relationships. A significant component of the variance is due to among-village differences, and a small but significant component of variance results from differences among kin groups within villages. In natural populations free from human influence, a pattern of isolation by distance might be expected; however, none was detected for the Tibetan walnuts. Further, no relationship was detected between genetic variation in villages and the environmental variables characterizing those villages, indicating that genetic differences are likely not the result of natural adaptation to local climate. This novel finding demonstrates the impact of human settlements, and specifically of human familial relationships, on the genetic diversity of a tree crop.

Natural dispersal mechanisms for walnuts in the eastern Himalayan foothills are most likely by squirrels and other rodents with scatter-hoarding behavior but to a small extent by water; however, in the Khawa Karpo region of Tibet, most walnut trees are propagated from seeds planted by humans whose ownership of individual trees are known. Grafting of walnut trees is rarely practiced in this region. Walnut landraces are often named after fruit phenotypes (e.g., meyuok da: thin shelled, zhiduok da: thick shelled, or panduok da: hybrid), geographical origin (e.g., Xinjiang) or relatives (e.g., Ajia na da ga: grandmother's walnut). Among the six villages in Kawa Kharpo,

TABLE 5. Diversity statistics by village (mean and standard deviation for 14 loci)

Village	Species (mean sample size)	na	ne	I	H <sub>o</sub>	H <sub>e</sub>	Nei
1	<i>J. regia</i> (28)	4.4286 ± 1.6968	2.7147 ± 0.9250	1.1189 ± 0.3692	0.5936 ± 0.2248	0.6089 ± 0.1602	0.5865 ± 0.1539
	<i>J. sigillata</i> (15)	3.4286 ± 1.2839	2.4924 ± 1.2414	0.9225 ± 0.4617	0.5398 ± 0.2917	0.5406 ± 0.2444	0.5046 ± 0.2287
	All <i>Juglans</i> (43)	4.5714 ± 1.8277	2.7680 ± 1.1109	1.1113 ± 0.4181	0.5772 ± 0.2260	0.5910 ± 0.1868	0.5770 ± 0.1823
2	<i>J. regia</i> (88)	4.6429 ± 1.9848	2.4517 ± 1.1947	0.9784 ± 0.4158	0.5102 ± 0.2398	0.5200 ± 0.1950	0.5140 ± 0.1924
	<i>J. sigillata</i> (12)	3.6429 ± 1.5984	2.6610 ± 1.2494	1.0115 ± 0.4406	0.5582 ± 0.1855	0.6007 ± 0.1967	0.5506 ± 0.1785
	All <i>Juglans</i> (101)	4.7857 ± 2.0821	2.4878 ± 1.1888	0.9987 ± 0.4139	0.5159 ± 0.2265	0.5281 ± 0.1895	0.5228 ± 0.1873
3	<i>J. regia</i> (20)	3.8571 ± 1.6104	2.4169 ± 0.6550	0.9984 ± 0.3071	0.6024 ± 0.2281	0.5896 ± 0.1192	0.5590 ± 0.1118
	<i>J. sigillata</i> (2)	1.7500 ± 0.4523	1.7500 ± 0.4523	0.5199 ± 0.3135	0.7500 ± 0.4523	0.7500 ± 0.4523	0.3750 ± 0.2261
	All <i>Juglans</i> (22)	4.0000 ± 1.6641	2.5032 ± 0.7141	1.0336 ± 0.3315	0.6054 ± 0.2302	0.5983 ± 0.1240	0.5699 ± 0.1186
4	<i>J. regia</i> (67)	5.7143 ± 2.2678	2.9605 ± 0.9369	1.2488 ± 0.3515	0.5712 ± 0.2118	0.6386 ± 0.1196	0.6291 ± 0.1179
	<i>J. sigillata</i> (29)	4.2143 ± 1.9682	2.7027 ± 0.8233	1.0809 ± 0.4322	0.5707 ± 0.3319	0.5977 ± 0.2008	0.5775 ± 0.1943
	All <i>Juglans</i> (97)	5.9286 ± 2.6447	2.9215 ± 0.8824	1.2336 ± 0.3721	0.5709 ± 0.2404	0.6286 ± 0.1348	0.6221 ± 0.1335
5	<i>J. regia</i> (33)	4.5714 ± 1.9890	2.6635 ± 1.1350	1.0788 ± 0.4083	0.5242 ± 0.1746	0.5843 ± 0.1621	0.5654 ± 0.1550
	<i>J. sigillata</i> (25)	3.8571 ± 1.4601	2.6340 ± 1.0047	1.0428 ± 0.3621	0.5507 ± 0.1625	0.5997 ± 0.1404	0.5751 ± 0.1330
	All <i>Juglans</i> (58)	4.9286 ± 2.2348	2.7413 ± 1.1684	1.1091 ± 0.4060	0.5372 ± 0.1519	0.5910 ± 0.1475	0.5803 ± 0.1438
6	<i>J. regia</i> (39)	5.4286 ± 2.3440	2.9664 ± 0.8067	1.2322 ± 0.3563	0.6848 ± 0.1729	0.6530 ± 0.1111	0.6361 ± 0.1081
	<i>J. sigillata</i> (27)	4.9286 ± 1.9793	3.4014 ± 1.4168	1.2843 ± 0.4143	0.6833 ± 0.2137	0.6827 ± 0.1403	0.6577 ± 0.1348
	All <i>Juglans</i> (66)	5.9286 ± 2.3685	3.2221 ± 1.0620	3.2221 ± 1.0620	0.3157 ± 0.1765	0.6655 ± 0.1192	0.6554 ± 0.1172

Notes: na = observed number of alleles, ne = effective number of alleles (Kimura and Crow, 1964), I = Shannon's information index (Lewontin, 1972), H<sub>e</sub> = expected heterozygosity were computed using Levene (1949), H<sub>o</sub> = observed heterozygosity, Nei's (1973) H<sub>e</sub>

TABLE 6. Results of AMOVA partitioning variance within *Juglans regia* and *J. sigillata*, and within all Tibetan *Juglans* combined.

Species	Source of variation	df	Sum of squares	Variance components	Percentage of variation	P value
<i>J. regia</i>	<b>Among villages</b>	<b>5</b>	<b>5.940</b>	<b>0.01777</b>	<b>6.28</b>	<b>0.00098</b>
	Within villages among kin groups	6	1.838	0.00104	0.37	0.38612
	Within kin groups among individuals	147	41.165	0.01580	5.58	0.38612
	<b>Within individuals</b>	<b>159</b>	<b>39.500</b>	<b>0.24843</b>	<b>87.77</b>	<b>0.01466</b>
	Total	317	88.443	0.28304		
<i>J. sigillata</i>	<b>Among villages</b>	<b>5</b>	<b>14.543</b>	<b>0.09099</b>	<b>10.00</b>	<b>0.00000</b>
	Within villages among kin groups	5	5.601	0.03261	3.58	0.05767
	Within kin groups among individuals	50	39.282	-0.00063	-0.07	0.42326
	<b>Within individuals</b>	<b>61</b>	<b>48.000</b>	<b>0.78689</b>	<b>86.48</b>	<b>0.01662</b>
	Total	121	107.426	0.90986		
Tibetan <i>Juglans</i> combined	<b>Among villages</b>	<b>5</b>	<b>9.098</b>	<b>0.1646</b>	<b>5.41</b>	<b>0.00684</b>
	<b>Within villages among kin groups</b>	<b>6</b>	<b>3.865</b>	<b>0.01017</b>	<b>3.34</b>	<b>0.00684</b>
	Within kin groups among individuals	208	59.794	0.00964	3.17	0.20332
	<b>Within individuals</b>	<b>220</b>	<b>59.000</b>	<b>0.26818</b>	<b>88.08</b>	<b>0.00293</b>
	Total	439	131.757	0.30446		

approximately 80 different Tibetan names were recorded. In addition to being cultivated for their fruits, walnut trees are also used to mark boundaries of farm terraces and houses where shade is useful for both humans and animals. Juglone, exuded from the leaves and roots of walnut trees, wards off flies and inhibits growth of weeds.

Walnuts are harvested between July and August, mainly by women and by those who own the trees. Many large, old trees have been inherited through generations, putatively through the female lineage. Walnut oil extraction is an activity shared by women within kin groups. This laborious work of crushing the iron walnut (*J. sigillata*) and cooking in huge woks, stirring for many hours over wood fire is undertaken throughout the harvest season as walnut oil becomes rancid after about 3 months. *Juglans regia* nuts are highly prized and sold for cash to local middlemen who transport walnuts to Sichuan or Dali. Women within families remove husks from the nuts, which are subsequently dried on large woven mats on the flat rooftops of their Tibetan homes. The nuts are graded according to shell thickness and poured into large gunny sacks. Men from the families are responsible for transporting the walnuts on mules or horses, often traveling long distances along mountain trails, to the trading posts situated close to roads. High quality walnuts fetch from 0.5–0.75 USD per 0.5 kg, and families may make approximately 100–150 USD per year from the sale of walnuts.

The Khampa are seminomadic, occupying this rugged mountainous region for millennia, carrying with them walnut germplasm for planting, barter, trade, and religious purposes. The ancient Tea and Horse Caravan trail, from the Tang Dynasty (618–907 AD) followed the deep gorges of the Lan Cang Jiang (Mekong River) in northwestern Yunnan, trading tea from Xishuangbanna, in southern Yunnan and horses from Tibet. Walnut exchanges too may have followed this route, which connected southwestern China to Tibet, Nepal, and India to the west and Sichuan in the east. At present, walnuts are a vital component of the emerging cash economy of northwestern Yunnan.

**Conclusions**—Walnut genotypic diversity in northwestern Yunnan, China is unique, being conserved by Tibetan kinship and culture and may represent an important reservoir of genetic variation for cultivated *J. regia* and *J. sigillata*. Here, Tibetans have shaped agrobiodiversity of walnuts through careful selec-

tion of germplasm for planting and protecting old walnut trees as part of their sacred geography. Results from this study demonstrate the importance of conservation of walnut populations within the villages in the Khawa Karpo region. The future of agrobiodiversity, including walnuts, hinges on the merging of traditional farming knowledge and agricultural crop improvements in participatory breeding collaborations (Gepts, 2006). This study suggests that human village environments and familial relationships play a critical role in the geographic distribution of crop genetic resources and require integration into agricultural programs.

Microsatellite data from this and another independent study (Wang et al., 2008) suggests that *J. regia* and *J. sigillata* are possibly a single species, but a wider geographical survey including wild progenitors of both species is needed to evaluate this possibility.

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APPENDIX 1. Specimen vouchers of *Juglans regia* L. and *J. sigillata* Dode collected and held at the Missouri Botanical Garden herbarium (MO).

Specimen	Accession	Country	Province	Village	Latitude (°N)	Longitude (°E)
<i>Juglans sigillata</i> Dode	BG400	China	Yunnan	4	28.484100	98.800340
<i>Juglans sigillata</i> Dode	BG402	China	Yunnan	4	28.483490	98.802600
<i>Juglans regia</i> L.	BG404	China	Yunnan	4	28.484860	98.800880
<i>Juglans sigillata</i> Dode	BG405	China	Yunnan	4	28.485800	98.803780
<i>Juglans regia</i> L.	BG406	China	Yunnan	4	28.483270	98.803120
<i>Juglans sigillata</i> Dode	BG407	China	Yunnan	4	28.483240	98.802170
<i>Juglans regia</i> L.	BG408	China	Yunnan	4	28.485750	98.804030
<i>Juglans regia</i> L.	BG409	China	Yunnan	4	28.483680	98.802290
<i>Juglans sigillata</i> Dode	BG410	China	Yunnan	4	28.484000	98.802310
<i>Juglans regia</i> L.	BG411	China	Yunnan	4	28.485510	98.804480
<i>Juglans regia</i> L.	BG412	China	Yunnan	4	28.483730	98.800130
<i>Juglans sigillata</i> Dode	BG413	China	Yunnan	4	28.485640	98.798750
<i>Juglans regia</i> L.	BG414	China	Yunnan	4	28.482800	98.801390
<i>Juglans regia</i> L.	BG415	China	Yunnan	4	28.483030	98.801110
<i>Juglans regia</i> L.	BG416	China	Yunnan	4	28.483030	98.801010
<i>Juglans sigillata</i> Dode	BG417	China	Yunnan	4	28.484300	98.799430
<i>Juglans regia</i> L.	BG418	China	Yunnan	4	28.486340	98.798420
<i>Juglans regia</i> L.	BG420	China	Yunnan	4	28.489470	98.805990
<i>Juglans regia</i> L.	BG421	China	Yunnan	4	28.489610	98.806200
<i>Juglans regia</i> L.	BG422	China	Yunnan	4	28.489430	98.806470
<i>Juglans regia</i> L.	BG423	China	Yunnan	4	28.489430	98.806470
<i>Juglans regia</i> L.	BG424	China	Yunnan	4	28.489680	98.806720
<i>Juglans regia</i> L.	BG425	China	Yunnan	4	28.489220	98.806490
<i>Juglans regia</i> L.	BG426	China	Yunnan	4	28.489250	98.806530
<i>Juglans regia</i> L.	BG427	China	Yunnan	4	28.489280	98.806290
<i>Juglans regia</i> L.	BG428	China	Yunnan	4	28.489780	98.806110
<i>Juglans sigillata</i> Dode	BG429	China	Yunnan	4	28.486460	98.800210
<i>Juglans regia</i> L.	BG430	China	Yunnan	4	28.484750	98.802550
<i>Juglans regia</i> L.	BG431	China	Yunnan	4	28.485200	98.800010
<i>Juglans regia</i> L.	BG432	China	Yunnan	4	28.484240	98.801530
<i>Juglans sigillata</i> Dode	BG433	China	Yunnan	4	28.486240	98.798640
<i>Juglans regia</i> L.	BG434	China	Yunnan	4	28.486590	98.798990
<i>Juglans sigillata</i> Dode	BG435	China	Yunnan	4	28.486460	98.798430
<i>Juglans regia</i> L.	BG436	China	Yunnan	4	28.486590	98.798740
<i>Juglans regia</i> L.	BG437	China	Yunnan	4	28.486780	98.798510
<i>Juglans regia</i> L.	BG438	China	Yunnan	4	28.486540	98.798770
<i>Juglans regia</i> L.	BG439	China	Yunnan	4	28.485190	98.804780
<i>Juglans regia</i> L.	BG440	China	Yunnan	4	28.485100	98.804310
<i>Juglans regia</i> L.	BG441	China	Yunnan	4	28.485190	98.804830
<i>Juglans sigillata</i> Dode	BG442	China	Yunnan	4	28.485090	98.804920
<i>Juglans regia</i> L.	BG443	China	Yunnan	4	28.485080	98.804970
<i>Juglans regia</i> L.	BG444	China	Yunnan	4	28.485160	98.804950
<i>Juglans sigillata</i> Dode	BG445	China	Yunnan	4	28.485740	98.804780
<i>Juglans regia</i> L.	BG446	China	Yunnan	4	28.485090	98.804990
<i>Juglans regia</i> L.	BG447	China	Yunnan	4	28.485110	98.805190
<i>Juglans sigillata</i> Dode	BG448	China	Yunnan	4	28.485040	98.804970
<i>Juglans sigillata</i> Dode	BG449	China	Yunnan	4	28.485030	98.804920
<i>Juglans sigillata</i> Dode	BG450	China	Yunnan	4	28.486590	98.798990
<i>Juglans regia</i> L.	BG451	China	Yunnan	4	28.490170	98.799830
<i>Juglans regia</i> L.	BG452	China	Yunnan	4	28.490210	98.799930
<i>Juglans sigillata</i> Dode	BG453	China	Yunnan	4	28.490220	98.799980
<i>Juglans regia</i> L.	BG454	China	Yunnan	4	28.490430	98.800000
<i>Juglans regia</i> L.	BG456	China	Yunnan	4	28.490230	98.800890
<i>Juglans regia</i> L.	BG458	China	Yunnan	4	28.438550	98.830950
<i>Juglans sigillata</i> Dode	BG459	China	Yunnan	4	28.438070	98.832200
<i>Juglans regia</i> L.	BG460	China	Yunnan	5	28.437170	98.830520
<i>Juglans regia</i> L.	BG461	China	Yunnan	5	28.434740	98.836080
<i>Juglans sigillata</i> Dode	BG462	China	Yunnan	5	28.430420	98.825640
<i>Juglans regia</i> L.	BG463	China	Yunnan	5	28.432550	98.836030
<i>Juglans sigillata</i> Dode	BG464	China	Yunnan	5	28.435280	98.834960
<i>Juglans sigillata</i> Dode	BG465	China	Yunnan	5	28.435100	98.835150
<i>Juglans sigillata</i> Dode	BG466	China	Yunnan	5	28.437950	98.832090
<i>Juglans regia</i> L.	BG467	China	Yunnan	5	28.437850	98.832430
<i>Juglans regia</i> L.	BG468	China	Yunnan	5	28.437790	98.832450
<i>Juglans sigillata</i> Dode	BG469	China	Yunnan	5	28.437910	98.832500
<i>Juglans regia</i> L.	BG470	China	Yunnan	5	28.432470	98.836080
<i>Juglans sigillata</i> Dode	BG471	China	Yunnan	5	28.430280	98.825640
<i>Juglans sigillata</i> Dode	BG472	China	Yunnan	5	28.437100	98.832716

## APPENDIX 1. Continued.

Specimen	Accession	Country	Province	Village	Latitude (°N)	Longitude (°E)
<i>Juglans regia</i> L.	BG473	China	Yunnan	5	28.439670	98.828160
<i>Juglans regia</i> L.	BG474	China	Yunnan	5	28.439660	98.830170
<i>Juglans regia</i> L.	BG475	China	Yunnan	5	28.440200	98.831260
<i>Juglans regia</i> L.	BG476	China	Yunnan	5	28.439860	98.831010
<i>Juglans sigillata</i> Dode	BG477	China	Yunnan	5	28.434930	98.834240
<i>Juglans regia</i> L.	BG478	China	Yunnan	5	28.433210	98.836210
<i>Juglans sigillata</i> Dode	BG479	China	Yunnan	5	28.432870	98.837570
<i>Juglans sigillata</i> Dode	BG480	China	Yunnan	5	28.432650	98.836010
<i>Juglans sigillata</i> Dode	BG481	China	Yunnan	5	28.432880	98.837630
<i>Juglans regia</i> L.	BG482	China	Yunnan	5	28.432730	98.837680
<i>Juglans regia</i> L.	BG483	China	Yunnan	5	28.432850	98.837720
<i>Juglans regia</i> L.	BG484	China	Yunnan	5	28.432930	98.837720
<i>Juglans regia</i> L.	BG485	China	Yunnan	5	28.432910	98.837630
<i>Juglans regia</i> L.	BG486	China	Yunnan	5	28.432630	98.837670
<i>Juglans sigillata</i> Dode	BG487	China	Yunnan	5	28.432560	98.837800
<i>Juglans sigillata</i> Dode	BG488	China	Yunnan	5	28.432320	98.837870
<i>Juglans regia</i> L.	BG489	China	Yunnan	5	28.432490	98.838010
<i>Juglans regia</i> L.	BG490	China	Yunnan	3	28.642960	98.722690
<i>Juglans sigillata</i> Dode	BG491	China	Yunnan	3	28.642050	98.724430
<i>Juglans regia</i> L.	BG492	China	Yunnan	3	28.642220	98.724430
<i>Juglans regia</i> L.	BG493	China	Yunnan	3	28.641880	98.724650
<i>Juglans regia</i> L.	BG494	China	Yunnan	3	28.642690	98.721910
<i>Juglans regia</i> L.	BG495	China	Yunnan	3	28.642220	98.724110
<i>Juglans regia</i> L.	BG496	China	Yunnan	3	28.641350	98.723010
<i>Juglans regia</i> L.	BG497	China	Yunnan	3	28.641590	98.722700
<i>Juglans regia</i> L.	BG498	China	Yunnan	3	28.646510	98.721230
<i>Juglans regia</i> L.	BG499	China	Yunnan	3	28.641160	98.723420
<i>Juglans regia</i> L.	BG500	China	Yunnan	3	28.648080	98.720270
<i>Juglans regia</i> L.	BG501	China	Yunnan	3	28.642240	98.724420
<i>Juglans regia</i> L.	LL100	China	Yunnan	6	28.280560	98.925000
<i>Juglans sigillata</i> Dode	LL101	China	Yunnan	6	28.280550	98.925000
<i>Juglans regia</i> L.	LL102	China	Yunnan	2	28.282500	98.908333
<i>Juglans regia</i> L.	LL103	China	Yunnan	2	28.282500	98.908889
<i>Juglans sigillata</i> Dode	LL104	China	Yunnan	2	28.282500	98.908333
<i>Juglans regia</i> L.	LL105	China	Yunnan	2	28.282778	98.908333
<i>Juglans regia</i> L.	LL106	China	Yunnan	2	28.283056	98.909167
<i>Juglans regia</i> L.	LL107	China	Yunnan	2	28.287500	98.907778
<i>Juglans sigillata</i> Dode	LL109	China	Yunnan	2	28.284167	98.907222
<i>Juglans regia</i> L.	LL110	China	Yunnan	2	28.284167	98.908056
<i>Juglans regia</i> L.	LL112	China	Yunnan	2	28.284167	98.908611
<i>Juglans regia</i> L.	LL113	China	Yunnan	2	28.284167	98.908611
<i>Juglans regia</i> L.	LL114	China	Yunnan	2	28.283889	98.908611
<i>Juglans regia</i> L.	LL115	China	Yunnan	2	28.283611	98.908333
<i>Juglans regia</i> L.	LL116	China	Yunnan	2	28.283611	98.908333
<i>Juglans regia</i> L.	LL117	China	Yunnan	2	28.283330	98.907780
<i>Juglans regia</i> L.	LL118	China	Yunnan	2	28.283330	98.907780
<i>Juglans regia</i> L.	LL119	China	Yunnan	2	28.284167	98.908889
<i>Juglans regia</i> L.	LL120	China	Yunnan	2	28.285000	98.907222
<i>Juglans regia</i> L.	LL121	China	Yunnan	2	28.285560	98.907500
<i>Juglans regia</i> L.	LL122	China	Yunnan	2	28.285560	98.907222
<i>Juglans regia</i> L.	LL124	China	Yunnan	2	28.284722	98.909167
<i>Juglans regia</i> L.	LL125	China	Yunnan	2	28.296390	98.909722
<i>Juglans sigillata</i> Dode	LL126	China	Yunnan	2	28.285560	98.906944
<i>Juglans regia</i> L.	LL128	China	Yunnan	2	28.285556	98.906667
<i>Juglans regia</i> L.	LL131	China	Yunnan	2	28.279444	98.912500
<i>Juglans regia</i> L.	LL133	China	Yunnan	2	28.279444	98.912500
<i>Juglans regia</i> L.	LL134	China	Yunnan	2	28.279167	98.912778
<i>Juglans regia</i> L.	LL135	China	Yunnan	2	28.280000	98.907222
<i>Juglans regia</i> L.	LL136	China	Yunnan	2	28.280000	98.913056
<i>Juglans regia</i> L.	LL137	China	Yunnan	2	28.279167	98.913611
<i>Juglans regia</i> L.	LL138	China	Yunnan	2	28.279167	98.913611
<i>Juglans regia</i> L.	LL139	China	Yunnan	2	28.279167	98.914167
<i>Juglans regia</i> L.	LL140	China	Yunnan	2	28.279167	98.914167
<i>Juglans regia</i> L.	LL141	China	Yunnan	2	28.279167	98.914167
<i>Juglans regia</i> L.	LL142	China	Yunnan	2	28.279444	98.914444
<i>Juglans regia</i> L.	LL143	China	Yunnan	2	28.279444	98.914444
<i>Juglans regia</i> L.	LL144	China	Yunnan	2	28.279444	98.914444
<i>Juglans sigillata</i> Dode	LL145	China	Yunnan	2	28.279444	98.914444
<i>Juglans regia</i> L.	LL146	China	Yunnan	2	28.278889	98.913889

## APPENDIX 1. Continued.

Specimen	Accession	Country	Province	Village	Latitude (°N)	Longitude (°E)
<i>Juglans regia</i> L.	LL147	China	Yunnan	2	28.278889	98.913889
<i>Juglans regia</i> L.	LL148	China	Yunnan	2	28.278889	98.913889
<i>Juglans sigillata</i> Dode	LL149	China	Yunnan	2	28.278889	98.913889
<i>Juglans regia</i> L.	LL150	China	Yunnan	2	28.277500	98.915833
<i>Juglans regia</i> L.	LL151	China	Yunnan	2	28.277500	98.915833
<i>Juglans regia</i> L.	LL152	China	Yunnan	2	28.272220	98.921667
<i>Juglans regia</i> L.	LL153	China	Yunnan	2	28.271944	98.921389
<i>Juglans regia</i> L.	LL154	China	Yunnan	2	28.271944	98.921389
<i>Juglans regia</i> L.	LL155	China	Yunnan	2	28.276667	98.915278
<i>Juglans regia</i> L.	LL156	China	Yunnan	2	28.276667	98.914444
<i>Juglans regia</i> L.	LL157	China	Yunnan	2	28.276667	98.914444
<i>Juglans regia</i> L.	LL158	China	Yunnan	2	28.276111	98.920833
<i>Juglans regia</i> L.	LL159	China	Yunnan	2	28.275556	98.921111
<i>Juglans regia</i> L.	LL160	China	Yunnan	2	28.275000	98.921111
<i>Juglans regia</i> L.	LL162	China	Yunnan	2	28.276667	98.916667
<i>Juglans regia</i> L.	LL163	China	Yunnan	2	28.276667	98.916667
<i>Juglans regia</i> L.	LL164	China	Yunnan	2	28.276944	98.916667
<i>Juglans regia</i> L.	LL166	China	Yunnan	2	28.279167	98.916389
<i>Juglans sigillata</i> Dode	LL167	China	Yunnan	2	28.279167	98.916389
<i>Juglans regia</i> L.	LL168	China	Yunnan	2	28.279167	98.916389
<i>Juglans regia</i> L.	LL169	China	Yunnan	2	28.279444	98.915833
<i>Juglans sigillata</i> Dode	LL170	China	Yunnan	2	28.279720	98.916111
<i>Juglans regia</i> L.	LL171	China	Yunnan	2	28.279720	98.916111
<i>Juglans regia</i> L.	LL172	China	Yunnan	2	28.278056	98.913333
<i>Juglans regia</i> L.	LL173	China	Yunnan	1	28.567222	98.870833
<i>Juglans regia</i> L.	LL174	China	Yunnan	1	28.567222	98.870833
<i>Juglans sigillata</i> Dode	LL175	China	Yunnan	1	28.567500	98.871111
<i>Juglans regia</i> L.	LL176	China	Yunnan	1	28.562778	98.869444
<i>Juglans regia</i> L.	LL177	China	Yunnan	1	28.562778	98.869444
<i>Juglans regia</i> L.	LL178	China	Yunnan	1	28.562778	98.869444
<i>Juglans sigillata</i> Dode	LL179	China	Yunnan	1	28.558611	98.873056
<i>Juglans regia</i> L.	LL180	China	Yunnan	1	28.563056	98.867500
<i>Juglans sigillata</i> Dode	LL181	China	Yunnan	1	28.563056	98.867500
<i>Juglans sigillata</i> Dode	LL182	China	Yunnan	1	28.562778	98.867778
<i>Juglans regia</i> L.	LL183	China	Yunnan	1	28.561389	98.868889
<i>Juglans regia</i> L.	LL184	China	Yunnan	1	28.561390	98.868889
<i>Juglans sigillata</i> Dode	LL185	China	Yunnan	1	28.561390	98.868889
<i>Juglans sigillata</i> Dode	LL186	China	Yunnan	1	28.561390	98.868889
<i>Juglans sigillata</i> Dode	LL187	China	Yunnan	1	28.562778	98.866667
<i>Juglans sigillata</i> Dode	LL188	China	Yunnan	1	28.562778	98.866667
<i>Juglans sigillata</i> Dode	LL191	China	Yunnan	1	28.570000	98.820556
<i>Juglans regia</i> L.	LL192	China	Yunnan	1	28.570556	98.851389
<i>Juglans regia</i> L.	LL193	China	Yunnan	1	28.570278	98.819444
<i>Juglans regia</i> L.	LL194	China	Yunnan	1	28.570278	98.819444
<i>Juglans regia</i> L.	LL195	China	Yunnan	1	28.570556	98.822222
<i>Juglans regia</i> L.	LL196	China	Yunnan	1	28.569722	98.821667
<i>Juglans regia</i> L.	LL197	China	Yunnan	1	28.569722	98.821667
<i>Juglans regia</i> L.	LL199	China	Yunnan	1	28.567778	98.821944
<i>Juglans regia</i> L.	LL200	China	Yunnan	1	28.567222	98.822222
<i>Juglans regia</i> L.	LL201	China	Yunnan	1	28.567222	98.821944
<i>Juglans regia</i> L.	LL67	China	Yunnan	6	28.196110	98.824167
<i>Juglans sigillata</i> Dode	LL68	China	Yunnan	6	28.196110	98.757500
<i>Juglans regia</i> L.	LL69	China	Yunnan	6	28.196110	98.823611
<i>Juglans regia</i> L.	LL70	China	Yunnan	6	28.196940	98.825560
<i>Juglans regia</i> L.	LL71	China	Yunnan	6	28.196940	98.825556
<i>Juglans sigillata</i> Dode	LL72	China	Yunnan	6	28.196940	98.825556
<i>Juglans regia</i> L.	LL73	China	Yunnan	6	28.195000	98.821111
<i>Juglans regia</i> L.	LL74	China	Yunnan	6	28.195000	98.821111
<i>Juglans regia</i> L.	LL75	China	Yunnan	6	28.194167	98.821389
<i>Juglans regia</i> L.	LL76	China	Yunnan	6	28.195278	98.820556
<i>Juglans sigillata</i> Dode	LL77	China	Yunnan	6	28.195000	98.820278
<i>Juglans regia</i> L.	LL78	China	Yunnan	6	28.195833	98.823056
<i>Juglans regia</i> L.	LL79	China	Yunnan	6	28.196667	98.823889
<i>Juglans regia</i> L.	LL80	China	Yunnan	6	28.184722	98.816111
<i>Juglans sigillata</i> Dode	LL81	China	Yunnan	6	28.182220	98.814167
<i>Juglans sigillata</i> Dode	LL82	China	Yunnan	6	28.180278	98.812500
<i>Juglans regia</i> L.	LL83	China	Yunnan	6	28.180560	98.811667
<i>Juglans sigillata</i> Dode	LL84	China	Yunnan	6	28.180556	98.811667
<i>Juglans sigillata</i> Dode	LL85	China	Yunnan	6	28.180556	98.811667



## APPENDIX 1. Continued.

Specimen	Accession	Country	Province	Village	Latitude (°N)	Longitude (°E)
<i>Juglans regia</i> L.	LL86	China	Yunnan	6	28.187500	98.811944
<i>Juglans regia</i> L.	LL87	China	Yunnan	6	28.187500	98.811944
<i>Juglans sigillata</i> Dode	LL88	China	Yunnan	6	28.188611	98.813889
<i>Juglans sigillata</i> Dode	LL89	China	Yunnan	6	28.187780	98.811111
<i>Juglans regia</i> L.	LL90	China	Yunnan	6	28.187500	98.810833
<i>Juglans regia</i> L.	LL91	China	Yunnan	6	28.187500	98.810556
<i>Juglans regia</i> L.	LL92	China	Yunnan	6	28.191110	98.809722
<i>Juglans regia</i> L.	LL93	China	Yunnan	6	28.187000	98.810556
<i>Juglans regia</i> L.	LL94	China	Yunnan	6	28.187000	98.810556
<i>Juglans sigillata</i> Dode	LL95	China	Yunnan	6	28.190280	98.811944
<i>Juglans regia</i> L.	LL96	China	Yunnan	6	28.190570	98.813056
<i>Juglans sigillata</i> Dode	LL97	China	Yunnan	6	28.188890	98.811111
<i>Juglans sigillata</i> Dode	LL98	China	Yunnan	6	28.188890	98.812220
<i>Juglans sigillata</i> Dode	LL99	China	Yunnan	6	28.190280	98.813056