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A Study of Genetic Structure of *Stephania yunnanensis* (Menispermaceae) by DALP

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Abstract Genetic diversity and genetic structure within and among ten populations of *Stephania yunnanensis* H. S. Lo and three populations of *S. epigaea* H. S. Lo from Yunnan province were evaluated by direct amplification of length polymorphism (DALP) markers. Five primer groups were screened, and a total of 287 DNA fragments were amplified, among which 266 were polymorphic, averaging 53.2 polymorphic bands per primer group in *S. yunnanensis.* The percentage of polymorphic bands of *S. yunnanensis* was 92.68% at the species level and 61.92% within the ten populations sampled. At the species level, the observed number of alleles (N_a) was 1.9268 and the effective number of alleles (N_e) was 1.5933; Nei's gene diversity (H) was 0.3414; Shannon's information index (I) was 0.5057. At the population level, $N_a = 1.6192$, $N_e = 1.4001$, $H = 0.2298$, and $I = 0.3401$. Total gene diversity of *S. yunnanensis* was 0.3419. Gene diversity within population was 0.2298, coefficient of gene differentiation was 0.3278, and estimated gene flow was 1.0254. The results indicated that the genetic differentiation was relatively higher among populations of *S. yunnanensis*. DALP markers were an informative and useful method for assaying genetic diversity and authenticating species of *Stephania*. These data could provide basic molecular evidence for establishing a reasonable strategy for protecting and exploiting the resource of *S. yunnanensis.*

Keywords *Stephania yunnanensis* · *Stephania epigaea* · DALP · Genetic diversity · Genetic structure

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Introduction

Stephania yunnanensis H. S. Lo is a climbing shrub belonging to the Menispermaceae, locally called mountain turtle. The genus *Stephania* contains about 60 species, of which 39 species and 1 variety are found in China (Luo 1996), widely distributed in well-drained basins in subtropical areas, especially in Yunnan and Guangxi provinces. Some species of this genus have been used as medical herbs for a long time, for their functions of heat-clearing and detoxifying, tendon-relaxing and activation of blood circulation, regulating qi to alleviate pain, apocenosis, and detumescence. The plants of this genus often have a high alkaloid content, with strong activity and high medicinal value (He et al. 1996; Gao and Xiao 1998), which has been extracted from root tubers of some species recently. Some have been exploited for medical uses, such as l-tetrahydropalmatine (acting as an analgesia), cepharanthine (raising leucocyte count), tetrandrine (depressurization, relaxing muscle), l-dicentrine (an antineurasthenic), and sinomenine (antirheumatic). In Yunnan province, a large number of *Stephania* species have been utilized to extract medical compounds, to the extent that the natural resources of *Stephania* there were overexploited, even threatened by extinction in some regions.

Fortunately, some species of *Stephania*, such as *S. yunnanensis* (Ma and Wang 1993), *S. longipes* (Huang et al. 1998), and *S. dentifolia* (Wang et al. 1991), have survived because they lack or contain less of the medical compounds traditionally utilized. These species are distributed in southern and western Yunnan and possess a high crebanine content (reaching 2.41%, Huang et al. 1998), an alkaloid of the aporphine type and an isoquinoline derivative. Isoquinoline alkaloids, such as tetrandrine (Guan et al. 1998; Li et al. 1996a, b) and berbamine (Guo et al. 1993; Yang et al. 1984), have effects of antiarrhythmia, calcium channel blockage, or other physiological effects on the cardiovascular system. According to animal experimental study, the antiarrhythmia effect of crebanine on rats is evident and quick (Yu et al. 1992). It also affects pain relief, mitigates spasmolysis, is an antihyperspasmia (Li et al. 1989), reverses human multidrug-resistant leukemic cell line K 562/HHT (Zhao and Shi 2000), and has spasmolytic effects on isolated gall bladder and Oddi's sphincter in vitro (Li et al. 1996a, b). It is a bioactive plant certainly worth researching for medical aims.

Previous studies of *Stephania* species were focused mostly on chemistry, pharmacology, and toxicology, especially in the 1970s and 1980s in China, and to a much lesser extent on biological research, such as genealogical classification (Luo 1996), characteristics of leaf epidermis (Hong et al. 2001) and leaf venation (Liu and Li 2000), chromosome numbers (Wang et al. 2004, 2005; Goldblatt 1984, 1985), and cultivation and breeding (Zhang and Liu 1991). But little is known about the basic population biology of *Stephania* species, particularly concerning the population genetic background and genetic structure of *S. yunnanensis.*

Direct amplification of length polymorphism (DALP) is a new technique for molecular marking (Desmarais 1998). Compared with restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD), a great number of fragments can be detected simultaneously by DALP. As opposed to amplified fragment length polymorphism (AFLP), DALP does not involve any

restriction or ligation steps, having only a single PCR followed by electrophoresis; the fingerprints obtained from DALP can be compared and polymorphism can be detected. Moreover, to get the fingerprints, AFLP needs several steps, each of which can generate artificial polymorphisms (e.g., an incomplete cleavage of genomic DNA or an imperfect ligation). This necessitates the use of a good quality and quantified preparations of DNA, which are not necessary for DALP (Marie et al. 2000). The DALP method was designed to obtain nucleotide sequence information of DNA fragments, but it also can be applied as a fingerprint technique. This study obtained fingerprints for species of *Stephania* by using DALP. By using two primers with high annealing temperatures to amplify "random" fragments of DNA, this method enabled easy and rapid generation of polymorphic DNA bands for these species, for which sequence information is not available.

DALP analysis of genetic variation was investigated in ten populations of *S. yunnanensis* and three populations of *S. epigaea* in this study. The purpose is to assess the genetic structure of *S. yunnanensis* and to provide genetic background data for the protection and sustainable exploitation of this medicinal plant. The DALP survey would provide insight into the population genetics of this species and help us select genotypes with high adaptability and high content of useful components for genetic breeding, along with good agricultural products and reasonable conservation of the species. This survey was expected to construct the basis for the DNA fingerprint of *S. yunnanensis* as well.

Materials and Methods

In this study, 100 individuals were sampled from ten natural populations of *S. yunnanensis*, and 32 individuals were sampled from three populations of *S. epigaea* (Fig. 1, Table 1). Fresh root tuber samples of the same 132 individuals were prepared for DNA extraction.

DNA Extraction

Total DNA was extracted according to a modified cetyltrimethylammonium bromide (CTAB) procedure (Zou et al. 2001). Fresh tissue weighing 1.0–1.5 g was cut and peeled from each root tuber and ground with 2× buffer primarily to remove polysaccharides. After centrifugation and removal of the supernatant, 2× CTAB [1.4 mol/L NaCl, 100 mmol/L Tris–HCl (pH 8.0), 20 mmol/L EDTA, 2% CTAB, and 1% 2-mercaptoethanol] was added and incubated in 65°C water bath for 1 h, with shaking every 10 min. The mixture was then suspended in 5 mol/L KAc for 1 h. Proteins in supernatant were extracted twice with chloroform–isoamyl alcohol (24:1, V/V), and the DNA was precipitated with isoamyl alcohol. The pellet was washed with 70% and 100% ethanol, dried, and redissolved in 150 μL TE buffer (10 mmol/L Tris–HCl, 1 mmol/L EDTA, pH 8.0). The total DNA quality and approximate concentration were visually inspected by electrophoresis on 1.0% agarose gel buffered with 1× TAE, stained with ethidium bromide, and photographed with a Kodak gel imaging system.

Fig. 1 Geographic location of ten populations of *Stephania yunnanensis* (numbered 01–10) and three populations of *S. epigaea* (numbered 11–13) in the present study

Reaction System Screening

An orthogonal design was applied to screen the reaction system. DALP assays were performed in a final reaction volume of 20 μL containing 2.0 μL template DNA, 2.5 μL 25 mmol/L $MgCl_2$, 2.0 μL 10× buffer, 2.0 μL 2.5 mmol/L dNTP mixture, 5.0 μL 0.5 U/μL *Taq* DNA polymerase, 3.0 μL 5 pmol/L selective primers, 1.0 μL 5 pmol/L reverse primer, 2.5 μL ultrapure water. A negative control was used with the same reaction volume containing all reagents, except that the template DNA was replaced by ultrapure water. Each mixture was overlaid with mineral oil.

Primer Couples Screening

Primer couples were screened, using the reaction system described above, from 16 selective primers and 2 reverse primers that were assorted randomly as 32 couples. Template DNA from two different populations was used for each primer couple. The DALP bands of amplification products were visually inspected by electrophoresis on 2.0% agarose gel buffered with 1× TAE, stained with ethidium bromide, and photographed with a Kodak gel imaging system. Five primer couples with distinct bands of amplification, high polymorphism, and good reproducibility were screened for amplification of all samples: P_1P_{r1}, P_2P_{r1}, P_3P_{r1}, P_4P_{r1}, P_5P_{r1} (Table 2).

DALP Amplifications

Amplifications were carried out in a Perkin-Elmer 9700 thermocycler (PE Corp., USA), as follows: initial denaturing step for 5 min at 95°C; followed by 12 cycles of

Table 1 Populations of *Stephania yunnanensis* and *S. epigaea* sampled for this study

Species	Pop[a]	Sample size	Locality	Altitude (m)	Long. E	Lat. N	Habitat
S. yunnanensis							
	01	9	Xiaojie, Yun Co, Yunnan	1,360–1,931	100°14′	24°22′	Bushwood, forest fringe
	02	11	Mengsha, Gengma Co, Yunnan	670–1,380	99°38′	23°44′	Bushwood, limestone
	03	8	Shigongli, Gengma Co, Yunnan	1,100–1,200	99°28′	23°35′	Bushwood, limestone
	04	10	Dongpo, Gengma Co, Yunnan	2,300–2,800	99°35′	23°29′	Bushwood, forest fringe
	05	7	Luoyang, Gengma Co, Yunnan	2,000–3,100	99°17′	23°33′	Bushwood, limestone
	06	12	Dayakou, Gengma Co, Yunnan	2,100–2,600	99°36′	23°28′	Bushwood, forest fringe
	07	10	Sipai Mt, Gengma Co, Yunnan	2,000–2,500	99°33′	23°26′	Bushwood, forest fringe
	08	11	Yongan, Changyuan Co, Yunnan	1,200–1,800	99°19′	23°23′	Bushwood, limestone
	09	10	Xiaohei River, Gengma Co, Yunnan	960–1,100	99°34′	23°22′	Bushwood, forest fringe
	10	12	Pingshan, Lianghe Co, Yunnan	1,060–2,000	98°28′	24°50′	Bushwood, forest fringe
S. epigaea							
	11	10	Hanzhuang, Baoshan city, Yunnan	2,250–2,600	99°13′	25°05′	Bushwood, forest fringe
	12	10	Dayan, Lijiang Co, Yunnan	2,200–3,100	100°13′	26°51′	Bushwood, limestone
	13	12	Zhuilijie, Wenshan Co, Yunnan	2,150–2,500	104°23′	23°18′	Bushwood, limestone

[a] Population numbers refer to locations in Fig. 1

30 s at 94°C, 30 s at 61°C, and 60 s at 72°C; 18 cycles of 30 s at 94°C, 30 s at 50°C, and 60 s at 72°C; and a final 10 min at 72°C.

Electrophoresis and Silver Staining

The final PCR products (5 μL per reaction) were electrophoresed on 5% nondenaturing polyacrylamide gel (polyacrylamide:methylene bisacrylamide, 29:1) in 1× TBE buffer for about 150 min at 800 V. (A synthetic molecular weight marker with a range of 100–3,000 bp was used in one lane.) To make the gel, 40 mL of mixing solution was degassed with a vacuum pump for 20 min, then 256 μL $(NH_4)_2S_2O_8$ and 17.6 μL tetramethyl ethylene diamine (TEMED) was added, and the solution was infused immediately and gelled for 2 h.

Table 2 Sequences of DALP primer couples used in this study

Primer	No.	Sequence (5′–3′)
Selective primers	DALP245 (P_1)	GTTTTCCCAGTCACGACCCAC
	DALP244 (P_2)	GTTTTCCCAGTCACGACCTGA
	DALP243 (P_3)	GTTTTCCCAGTCACGACTCGA
	DALP235 (P_4)	GTTTTCCCAGTCACGACCAC
	DALP234 (P_5)	GTTTTCCCAGTCACGACCAG
Reverse Primer	DALPR1 (Pr_1)	TTTCACACAGGAAACAGCTATGAC

The electrophoresed gel was detected by silver staining and computer scanning. First, the polyacrylamide gel was soaked in fixation fluid (10% HAc) until the color of bromophenol blue was removed, washed with ultrapure water three times for 2 min each time. Second, it was stained with a solution of 0.1% $AgNO_3$, 0.4% methanol, V/V, for 40 min and washed with ultrapure water for 2 s. It was then developed in 3.0% Na_2CO_3, 0.4% methanol, and 0.02% 10 mg/mL sodium thiosulfate, V/V, until the bands were clear. Finally, the gel was fixed in 10% HAc for 5 min, washed in distilled water, and dried in open air.

Bands of DALP Fragments

Some of the fingerprints produced by polyacrylamide gel electrophoresis are shown in Fig. 2. Characteristic bands that showed differentiation between *S. yunnanensis* and *S. epigaea* are indicated in pairs by arrows near the corresponding marker, that is, *S. yunnanensis* amplified with primer group P_1P_{r1} (DALP 245) at 900 and 600 bp, P_2P_{r1} (DALP 244) at 1,200 and 900 bp, and P_5P_{r1} (DALP 234) between 200 and 100 bp had characteristic bands that were different from *S. epigaea.* Also, *S. epigaea* amplified with P_1P_{r1} (DALP 245) at 1,000 bp, P_2P_{r1} (DALP 244) between 100 and 200 bp, and P_5P_{r1} (DALP 234) between 400 and 300 bp and near 300 bp had characteristic bands.

Data Analysis

DALP amplified fragments, with the same mobility according to the molecular weight, were scored by eye for the presence (1), negative (0), or absence (.) of homologous bands. The resulting data matrices of the DALP phenotypes were analyzed using PopGene version 1.31 (Yeh et al. 1999) to estimate genetic diversity parameters: percentage of polymorphic bands (PPB), Shannon's index of phenotypic diversity (I), mean observed number of alleles (N_a), mean effective number of alleles (N_e), Nei's gene diversity (H), total gene diversity (H_t), gene diversity within population (H_s), coefficient of gene differentiation (G_{st}), and level of gene flow (N_m). To examine genetic relationships at the species and population levels, dendrograms were constructed by an unweighted pair group method of cluster

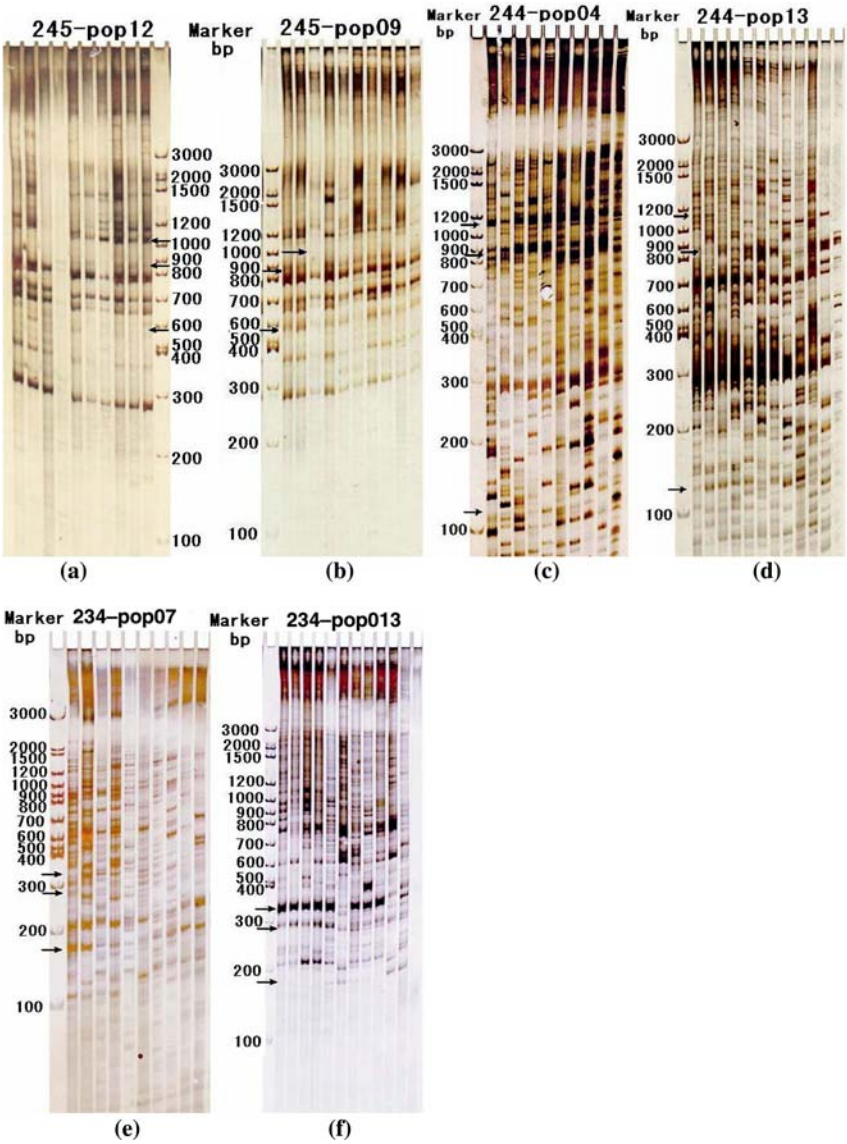

Fig. 2 DNA fingerprints of *Stephania yunnanensis* and *S. epigaea* using five primer groups of DALP. 245-pop12: population 12 of *S. epigaea* amplified by primer group P_1P_{r1} (**a**). 245-pop09: population 09 of *S. yunnanensis* amplified by primer group P_1P_{r1} (**b**). 244-pop04: population 04 of *S. yunnanensis* amplified by primer group P_2P_{r1} (**c**). 244-pop13: population 13 of *S. epigaea* amplified by primer group P_2P_{r1} (**d**). 234-pop07: population 07 of *S. yunnanensis* amplified by primer group P_5P_{r1} (**e**). 234-pop13: population 13 of *S. epigaea* amplified by primer group P_5P_{r1} (**f**)

analysis using arithmetic averages (UPGMA) on the basis of the matrix of Nei's (1978) unbiased genetic distance with PopGene. The tree was subsequently visualized with Treeview (Roderic 2001).

Results

Genetic Diversity

In the ten populations of *S. yunnanensis*, a total of 287 DNA fragments ranging from 100 to 3,000 bp were amplified with five primer groups, averaging 57.4 DNA fragments per primer group. Of those 287 fragments, 266 were polymorphic, with PPB of 92.68% at the species level. The average number of DNA polymorphic bands amplified by each primer group was 53.2 in ten populations, with average PPB of 61.92% at the population level. The ten populations were sorted by PPB, from high to low: pop 07 and pop 04 (68.29%) > pop 02 (65.85%) > pop 10 (64.81%) > pop 01 (64.12%) > pop 06 (60.98%) > pop 08 and pop 09 (57.49%) > pop 05 (56.45%) > pop 03 (55.40%).

At the species level, the diversity measures for *S. yunnanensis* were N_a 1.9268, N_e 1.5933, H 0.3414, and I 0.5057. At the population level, they were N_a 1.6192, N_e 1.4001, H 0.2298, and I 0.3401 (Table 3).

Among the total of 287 DNA fragments surveyed in three populations of *S. epigaea*, 247 were polymorphic, with PPB of 86.06% at the total level, average number of polymorphic bands of 49.4 per primer group, and average PPB of 73.17% within three populations. Total diversity measures for the three populations of *S. epigaea* were N_a 1.8606, N_e 1.5232, H 0.3083, and I 0.4611. Averages of the three populations were N_a 1.7317, N_e 1.4180, H 0.2476, and I 0.3732 (Table 3).

Genetic Differentiation

Genetic differentiation computed for *S. yunnanensis* was H_t 0.3419 and H_s 0.2298 (Table 4). The G_{st} of 0.3278 could be interpreted to mean that 67.22% of the total genetic divergence was within populations and the rest (32.78%) was attributed to among populations. Moreover, these data indicate that genetic divergence was relatively high among populations of *S. yunnanensis*. According to the value of G_{st}, it was estimated that the gene flow of populations (N_m, McDermott and McDonald 1993) was 1.0254 in *S. yunnanensis*. The G_{st} of *S. epigaea* was only 0.1993 (i.e., 19.93% of the total genetic divergence was among the three populations), and many populations must be sampled to satisfy a statistic count for analysis of the genetic differentiation of *S. epigaea.*

Genetic Relationship

Nei's genetic identity and genetic distance were computed for the ten populations of *S. yunnanensis* and the three populations of *S. epigaea* (Table 5). The genetic distance between pop 02 and pop 04 was the lowest (0.0944), and that between pop 05 and pop 08 was the highest (0.2130). The mean genetic distance among the *S. yunnanensis* populations was 0.1598. Among the *S. epigaea* populations the genetic distance varied from 0.1335 to 0.0950, averaging 0.1136. The mean genetic distance between the populations of these two species was 0.2215.

Table 3 Genetic diversity in populations of *Stephania yunnanensis* and *S. epigaea*

Species	Pop[a]	Total bands	Diversity parameter[b]					
			Polymorphic bands	PPB (%)	N_a	N_e	H	I
S. yunnanensis								
	01	287	184	64.11	1.6411	1.4305	0.2448	0.3602
	02	287	189	65.85	1.6585	1.4468	0.2527	0.3883
	03	287	159	55.40	1.5540	1.3378	0.1978	0.3116
	04	287	196	68.29	1.6829	1.4603	0.2632	0.3171
	05	287	162	56.45	1.5645	1.3653	0.2102	0.3130
	06	287	175	60.98	1.6098	1.3634	0.2118	0.3457
	07	287	196	68.29	1.6829	1.4647	0.2645	0.3872
	08	287	165	57.49	1.5749	1.3678	0.2106	0.2956
	09	287	165	57.49	1.5749	1.3674	0.2111	0.3112
	10	287	186	64.81	1.6481	1.3968	0.2316	0.3710
	Mean	/	177.7	61.92	1.6192	1.4001	0.2298	0.3401
	Total	287	266	92.68	1.9268	1.5933	0.3414	0.5057
S. epigaea								
	11	287	209	72.82	1.7282	1.3802	0.2309	0.3533
	12	287	209	72.82	1.7282	1.4443	0.2593	0.3872
	13	287	212	73.87	1.7387	1.4296	0.2525	0.3792
	Mean	/	210	73.17	1.7317	1.4180	0.2476	0.3732
	Total	287	247	86.06	1.8606	1.5232	0.3083	0.4611

[a] Population numbers refer to locations in Fig. 1

[b] *PPB* percentage of polymorphic bands, N_a observed number of alleles, N_e effective number of alleles, H Nei's (1973) gene diversity, I Shannon's information index

Table 4 Nei's analysis of genetic differentiation between populations of *Stephania yunnanensis* and *S. epigaea*

Species	Differentiation parameter[a]			
	H_t	H_s	G_{st}	N_m
S. yunnanensis	0.3419	0.2298	0.3278	1.0254
SD	0.0235	0.0141	\	\
S. epigaea	0.3091	0.2475	0.1993	2.0090
SD	0.0274	0.0201	\	\

[a] H_t total gene diversity, H_s gene diversity within population, G_{st} coefficient of gene differentiation, N_m estimate of gene flow from G_{st} or G_{cs} [e.g., $N_m = 0.5\ (1 - G_{st})/G_{st}$ (McDermott and McDonald 1993)], *SD* Standard deviation

A UPGMA dendrogram describes the relationship of genetic identity among the populations of *S. yunnanensis* and *S. epigaea* (Fig. 3). The populations separated into their respective species groups, indicating that genetic differentiation is obvious

Table 5 Nei's genetic identity and genetic distance among populations of *Stephania yunnanensis* and *S. epigaea*

Pop	01	02	03	04	05	06	07	08	09	10	11	12	13
01	–	0.8370	0.8279	0.8243	0.8445	0.8284	0.8965	0.8171	0.8111	0.8950	0.7766	0.8033	0.8008
02	0.1780	–	0.8864	0.9099	0.8172	0.8713	0.8676	0.8986	0.8691	0.8304	0.7916	0.8285	0.8155
03	0.1888	0.1206	–	0.8886	0.8181	0.8446	0.8638	0.8736	0.8613	0.8162	0.7919	0.8049	0.7977
04	0.1932	0.0944	0.1181	–	0.8296	0.8730	0.8834	0.8935	0.8789	0.8438	0.7944	0.8236	0.8133
05	0.1690	0.2018	0.2008	0.1868	–	0.8241	0.8520	0.8081	0.8332	0.8266	0.7620	0.7808	0.7733
06	0.1883	0.1377	0.1689	0.1358	0.1935	–	0.8603	0.8456	0.8920	0.8262	0.7912	0.8292	0.8024
07	0.1092	0.1420	0.1464	0.1240	0.1602	0.1505	–	0.8803	0.8633	0.8677	0.8160	0.8418	0.8314
08	0.2020	0.1069	0.1351	0.1126	0.2130	0.1678	0.1275	–	0.8535	0.8160	0.7678	0.7972	0.8022
09	0.2094	0.1403	0.1493	0.1291	0.1825	0.1143	0.1470	0.1584	–	0.8264	0.7849	0.8190	0.8156
10	0.1110	0.1859	0.2031	0.1699	0.1904	0.1909	0.1419	0.2034	0.1907	–	0.7828	0.7944	0.8118
11	0.2529	0.2337	0.2333	0.2301	0.2718	0.2342	0.2033	0.2642	0.2423	0.2449	–	0.8937	0.8750
12	0.2191	0.1881	0.2170	0.1941	0.2475	0.1873	0.1722	0.2266	0.1996	0.2302	0.1124	–	0.9094
13	0.2221	0.2039	0.2260	0.2067	0.2571	0.2202	0.1846	0.2204	0.2038	0.2085	0.1335	0.0950	–

Nei's genetic identity values above diagonal; genetic distance values below diagonal. *S. yunnanensis* populations are numbered 01–10. *S. epigaea* populations are numbered 11–13

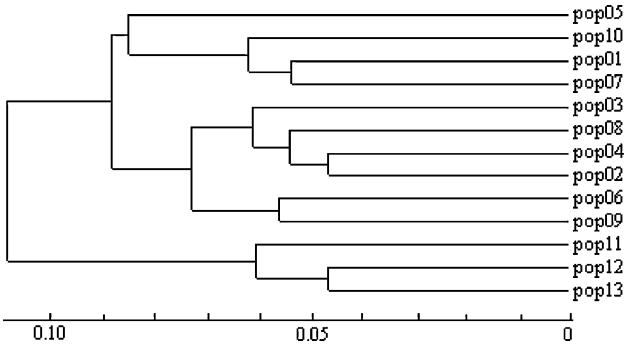

Fig. 3 Dendrogram of ten populations of *Stephania yunnanensis* (numbered 01–10) and three populations of *S. epigaea* (numbered 11–13) by UPGMA

between the two species. The ten populations of *S. yunnanensis* further separated into two major groups, the first including populations 05, 10, 01, and 07, and the second populations 03, 08, 04, 02, 06, and 09. It is suggested that there is no obvious correlation between geographic distance and genetic relationships of the populations in *S. yunnanensis*.

Discussion

The PPB for the ten populations of *S. yunnanensis* ranged from 55.40 to 68.29%, with populations 07 and 04 sharing the highest value (the next highest was pop 02, at 65.85%) and pop 03 having the lowest value. The PPB of the ten populations at the species level was 92.68%, indicating that this species has had abundant genetic diversity and great evolutionary potential, despite its obvious regional characters, mainly in west and southwest Yunnan.

The diversity measures of the ten populations at the species level were N_a 1.9268, H 0.3414, and I 0.5057; whereas these values at the population level were N_a 1.6192, H 0.2298, and I 0.3401. The genetic diversity of *S. yunnanensis* at the population level was much higher than other species with similar life cycle characters (perennial herb, intercross, endemic plants, at the population level, $N_a = 1.8900$, $H = 0.1310$, data based on allozymes by Hamrick and Godt 1989), indicating that *S. yunnanensis* is a polymorphic species. The wide variation among and within populations is probably related to its dioecism, vegetative propagation of root tubers, and geographic distribution.

Stephania yunnanensis has strong vitality. Its root tubers can survive over ten or even scores of years due to their strong adaptability to drought and high temperature tolerance. The tubers, however, grow slowly, so the population can hardly renew once they are overdug. Its gene diversity seems to be correlated positively with species adaptability. If we take measures to protect its habitat and exploit it sustainably, *S. yunnanensis* is expected to be able to recover its population numbers in the near future.

The G_{st} of *S. yunnanensis* is 0.3278, indicating that genetic divergence is relatively evident among populations. According to allozyme and RAPD data, in general G_{st} is about 0.20–0.23 for outbreeding species and 0.50–0.59 for inbreeding species, 0.27 for dicotyledon species, 0.23 for perennial herb species, and 0.23 for amphigenesis species (Hamrick and Godt 1989; Nybom and Banish 2000). For *S. yunnanensis*, a dioecious dicotyledon and perennial vine, G_{st} is obviously higher, exceeding the top limit of outbreeding species (0.23) and indicating a very high level of genetic differentiation among the populations of this species. A high level of population differentiation might be attributed to genetic drift, founder effect, geographic isolation of populations, and selection pressure (Godt and Hamrick 1996). On the other hand, gene flow ($N_m > 1$) can prevent population differentiation resulting from genetic drift; when $N_m < 1$, decreased gene flow is the dominant reason for promoting differentiation among populations (Wright 1931). The N_m value of *S. yunnanensis* was 1.0254, approximating 1, meaning that differentiation among populations has developed to a certain degree. Geographically, *S. yunnanensis* is restricted to the west and southwest of Yunnan province and shows obvious regional differentiation. As we know, *S. yunnanensis* can be propagated from root tubers and is of relatively wide distribution. Moreover, the high medicinal value of the species attracts peasants to collect the plants in fixed sites. Due to its limited distribution and strict demands for habitat, the number of populations is decreasing. In recent decades, owing to the damage to forests and fragmentation of their habitat, gene flow has been hindered among populations, resulting in a sharpening of the differentiation among populations.

Genetic diversity of a species includes not only the level of variation but also the distribution pattern of variation, namely, the genetic structure of the population. Difference of genetic structure of populations is an important appearance of genetic diversity and the evolutionary potential of a species. The adaptability of a species to adverse environments depends on the amount of genetic variance within the species and the genetic structure of its populations as well (Millar and Lobby 1991).

For all pairwise comparisons of the ten populations of *S. yunnanensis*, Nei's (1978) genetic distance ranged from 0.213 to 0.094 (mean 0.160). The cluster phenogram constructed using genetic distance between populations indicates that the genetic divergence sorts into two branches, with populations 05, 10, 01, and 07 in one branch and the others clustered in another branch (Fig. 3). As a result, there is a slight trend that genetic distance increases with geographic separation. There were exceptions, however, such as the genetic distance between pop 05 and pop 08 (0.213), the highest among all the pairwise comparisons although their geographic distance was much closer. Thus it can be seen that the genetic distance is related to a population's habitat, altitude, and other factors, except linear geographic distance.

The active and high content of chemical components (e.g., crebanine, sinoacutine) of *S. yunnanensis* is worth exploitation and utilization, though crebanine, possessing antiarrhythmia and other physiological activity, has not yet been exploited as a new drug. Unfortunately, the natural resources of *S. yunnanensis* have decreased rapidly, because the red juice in its leaves and stem often contains L-dicentrine and has been heavily exploited for medicinal uses. The cross-section of some of the root tubers is also red (often including white, red, or striped with red).

Folk doctors consider them to have great medical value, and thus they have been excessively exploited.

Research data on the genetic background of this important medicinal herb is the basis for induction, acclimatization, cultivation, and sustainable development. The analysis of genetic diversity of *S. yunnanensis* helps us to reveal the genetic cause of variations in the chemical components and provides theoretical evidence for selecting germplasm resources with high content of active components.

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