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Genetic Diversity and Structure of *Cordyceps sinensis* Populations from Extensive Geographical Regions in China as Revealed by Inter-Simple Sequence Repeat Markers

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Cordyceps sinensis **is one of the most valuable medicinal caterpillar fungi native to China. However, its productivity is extremely limited and the species is becoming endangered. The genetic diversity of eighteen *C. sinensis* populations across its major distributing regions in China was evaluated by inter-simple sequence repeat (ISSR) markers. A total of 141 markers were produced in 180 individuals from the 18 populations, of which 99.3% were polymorphic. The low average of Shannon (0.104) and Nei index (0.07) of the 18 populations indicates that there are little genetic variations within populations. For all 18 populations, estimates of total gene diversity (H_T), gene diversity within populations (H_S), coefficient of genetic differentiation (G_{ST}), and gene flow (Nm) were 0.170, 0.071, 0.583, and 0.357, respectively. This pattern suggests that the genetic diversity of *C. sinensis* is low and most of the ISSR variations are found among populations with little gene exchange. The 18 populations are divided into five groups based on the genetic distance and the grouping pattern matches with the geographic distribution along the latitudinal gradient. The five groups show obvious difference in the G_{ST} and Nm values. Therefore, the genetic diversification of *C. sinensis* populations may be determined by geographic isolation and the combined effects of life history characters and the interaction with host insect species. The information illustrated by this study is useful for selecting *in situ* conservation sites of *C. sinensis*.**

Keywords: *Cordyceps sinensis*, genetic diversity, genetic structure, ISSR, geographical distribution

Cordyceps sinensis (Berk.) Sacc., a caterpillar fungus of the genus *Cordyceps*, has been used in China as a food and herbal medicine for centuries (The State Pharmacopoeia Commission of P. R. China, 2005) to treat asthma, bronchial and lung inflammation, and kidney disease (Shimitsu, 1978). The fungus parasites on the larvae of Hepialidae and generally exists in two generations (one is asexual stage of mitosporic fungi, and the other is sexual stage of a compound of stroma and dead larvae) (Jiang and Yao, 2003). According to the Regulations of the People's Republic of China on Wild Plants Protection, *C. sinensis* has been ranked as Chinese second class key protected wild plant. In China, *C. sinensis* only distributes in Qinghai-Tibet plateau of western China with 3,000~5,100 meter altitude, mainly in Qinghai, Tibet, Yunnan, and Sichuan provinces. The number of *C. sinensis* natural populations is highly limited, and the hosts, which are specific for the propagation of *C. sinensis*, are also potentially threatened (Zhu and Mou, 2006). In recent years, due to the increasing demand and commercial value, the overexploitation of *C. sinensis* and habitat degradation made the species endangered. Therefore, enacting proper strategy and policy for *C. sinensis* conservation is in urgent demand.

Understanding the genetic diversity and structure of *C. sinensis* populations will provide important information for the conservation policy, e.g. central areas and priority populations to conserve. To date, genetic diversity of *C. sinensis* is only studied on local level and the geographic coverage is very limited. Chen *et al*. (1999) investigated the genetic variation and relationships of 29 individuals (sexual stage of *C. sinensis*) from 10 geographical populations by Random Amplified Polymorphic DNA (RAPD) markers, and high genetic diversity was found among different populations. Zhang *et al*. (1999) estimated the high genetic divergence in *C. sinensis* (8 individuals) and *C. crassispora* (6 individuals) from northwest Yunnan province by using RAPD markers. Kinjo and Zang (2001) analyzed the phylogenetic relationships among the *C. sinensis* materials from 11 localities of southwestern China based on the sequences of ITS1, 2, and 5.8S rDNA regions, and related sequences obtained from GenBank. However, ITS sequences within *C. sinensis* are homologous and display highly conservative evolutionary characters among geographically isolated populations (Chen *et al*., 2004), so the sequences of ITS1, 2, and 5.8S rDNA regions are unsuitable for revealing the genetic variations among *C. sinensis* populations. In summary,

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Table 1. Eighteen *Cordyceps sinensis* populations used in this study

Population and code	Collection site	Altitude (m)	Longitude	Latitude
Maqin (MQ)	Maqin county, Qinghai	4,200	100°16' E	34°29' N
Yushu (YS)	Yushu county, Qinghai	4,500	96°56' E	33°02' N
Zaduo (ZD)	Zaduo county, Qinghai	4,300	95°02' E	32°55' N
Qilian (QL)	Qilian county, Qinghai	2,700	100°13' E	38°01' N
Huangzhong (HZ)	Huangzhong county, Qinghai	2,260	101°34' E	36°29' N
Gangcha (GC)	Gangcha county, Qinghai	3,200	100°10' E	37°19' N
Tianjun (TJ)	Tianjun county, Qinghai	3,200	99°02' E	37°17' N
Gonghe (GH)	Gonghe county, Qinghai	3,200	100°37' E	36°16' N
Xinghai (XH)	Xinghai county, Qinghai	4,300	99°59' E	35°04' N
Guinan (GN)	Guinan county, Qinghai	3,100	100°45' E	35°34' N
Henan (HN)	Henan county, Qinghai	3,600	101°37' E	34°45' N
Milin (ML)	Milin county, Tibet	3,700	94°08' E	29°11' N
Linzhi (LZ)	Linzhi county, Tibet	3,000	94°15' E	29°35' N
Dingqing (DQ)	Dingqing county, Tibet	4,300	95°38' E	31°25' N
Shiqu (SQ)	Shiqu county, Sichuan	4,200	98°04' E	33°01' N
Kangding (KD)	Kangding county, Sichuan	4,200	101°57' E	30°02' N
Shangrila (SG)	Shangrila county, Yunnan	4,500	98°43' E	27°47' N
Deqin (TQ)	Deqin county, Yunnan	3,559	98°56' E	28°29' N

these results are based on a few individuals within populations of *C. sinensis* and the studied areas are limited. Thus, our understanding on the genetic diversity and genetic structure of *C. sinensis* populations on the regional scale across the Qinghai-Tibet Plateau remain insufficient, which significantly hindered the development of a national level conservation policy.

Traditional experiences in medicine proved that the quality, curative effect and the price of *C. sinensis* are strongly associated with the producing area. Previous studies also found that the differences of morphological characters, mannitol, and adenosine contents among *C. sinensis* populations in Tibet, Sichuan, and Qinghai province are related to their geographical distribution (Cai *et al*., 2001, 2003; Liang *et al*., 2005). Therefore, information about genetic relationships among different populations is critical for the pharmaco-

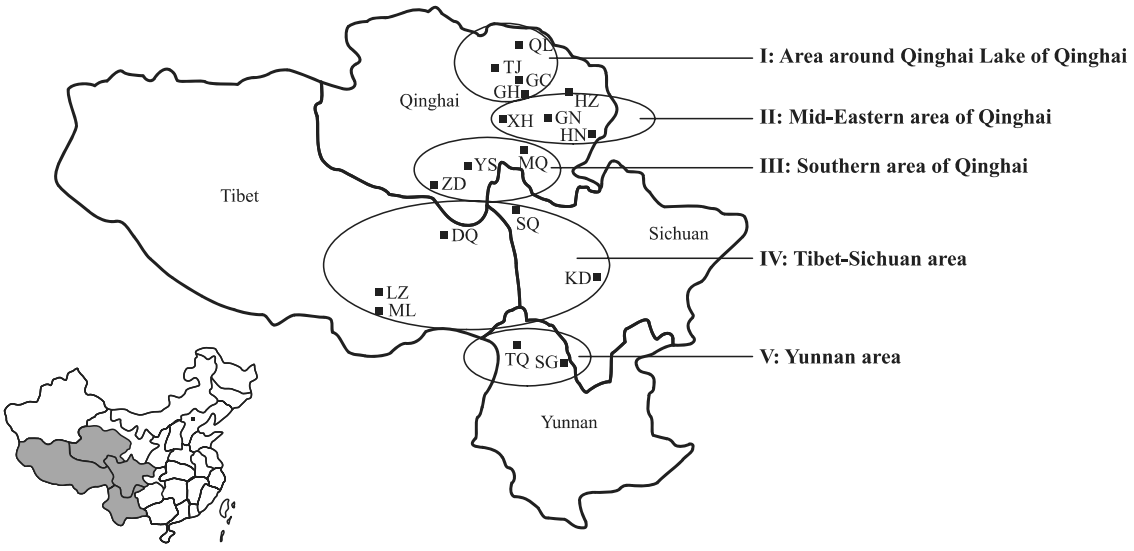

Fig. 1. Sketch maps of 18 *C. sinensis* populations from 4 provinces of Qinghai, Tibet, Sichuan, and Yunnan, China. Population codes are referred in Table 1.

Table 2. ISSR analyses of *Cordyceps sinensis* populations

Primer code	Sequences from 5' to 3'	Annealing temp (°C)	Reproducible bands	Polymorphic bands	Percents of polymorphic bands
UBC807	$(AG)_8T$	52	14	13	93.9
UBC809	$(AG)_8G$	49	20	20	100.0
UBC826	$(AC)_8C$	49	14	14	100.0
UBC835	$(AG)_8Y^aC$	54	12	12	100.0
UBC836	$(AG)_8YA$	51	23	23	100.0
UBC840	$(GA)_8YT$	48	23	23	100.0
UBC841	$(GA)_8YC$	51	27	27	100.0
UBC888	$B^bD^cB\ (CA)_7$	52	10	10	100.0
UBC889	$DBD(AC)_7$	52	8	8	100.0
			Total 141	Total 140	Average 99.3

[a] Y = C, T
[b] B = non-A
[c] D = non-C

logical research, market evaluation, and conservation area instauration. The objective of this study was to investigate the inter- and intra-population genetic diversity among 18 populations of *C. sinensis* across the whole distribution area in China. The results will be helpful to suggest a strategy to conserve and utilize *C. sinensis*. Inter-simple sequence repeat (ISSR) markers (Zietkiewicz *et al*., 1994) were used to reveal the genetic relationships of 18 populations of *C. sinensis* with extensive geographical distribution across Qinghai-Tibet plateau. We selected ISSR markers because for this method, no prior DNA sequence information is needed; development costs are low; and the low reproducibility of RAPD markers can be overcome. As a potential molecular marker system, its uses in the analysis of phylogeny and in population genetics have been documented in various organisms (Belard *et al*., 2005; Kar *et al*., 2005; Casu *et al*., 2006; Spagnuolo *et al*., 2007).

Materials and Methods

Sampling of *Cordyceps siensis*

One hundred eighty samples representing eighteen populations (ten samples for each population) of *C. sinensis* were collected from eighteen different sites across the major distribution area of China (Qinghai, Tibet, Yunnan, and Sichuan province). The sampling sites extensively distributed in the range of N27°47'-38°02' and E94°08'-101°57', and the altitudes of these populations varied from 2,260 to 4,500 m (Table 1). The detailed locations of the samples are presented in Fig. 1. Specimens were preserved in the Herbarium of the Institute of Bioresource and Applied Technology of Tongji University, China.

Genomic DNA extraction and electrophoresis

DNA was extracted from 0.02 g fruit body of *C. sinensis* using the modified method of CTAB protocol (Liang *et al*., 2005). The extracted DNA was separated by electrophoresis in a 0.7% agarose gel in 1× TAE buffer, and stained with ethidium bromide. The quality of the extracted DNA was examined under the UV light. DNA concentration was determined by comparing the fluorescence under the UV light with λ-DNA (*Hin*dIII Digest) of known concentration.

ISSR primer screening and PCR amplification

The UBC SSR Primer Synthesis Project Oligonucletide Set 100/9 (The University of British Columbia) was used in this study. The reaction mixture (20 μl) for PCR consisted of 10 mmol/L Tris-HCl (pH 8.0), 50 mmol/L KCl, 2 mmol/L

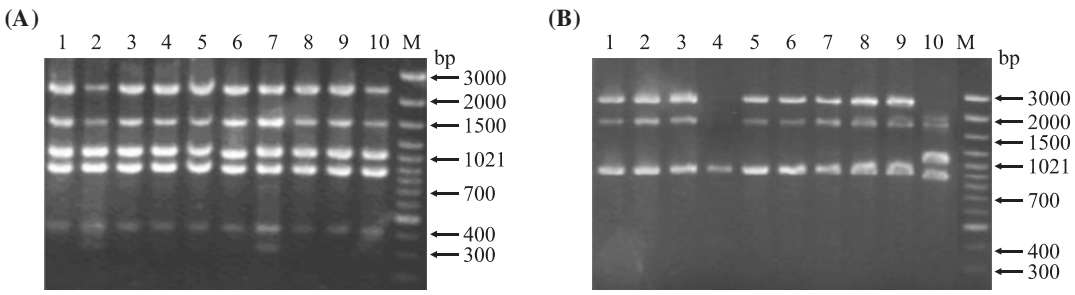

Fig. 2. ISSR band and patterns of *Cordyceps sinensis* from Qilian (A) and Milin (B) counties generated by the primer UBC888. Marker, 100 bp DNA Ladder Plus.

Table 3. Estimates of genetic diversity in each population among *C. sinensis* populations, assuming Hardy-Weinberg disequilibrium

Population[a]	Shannon index I	Nei index H	Number of observed allelesna	Number of effective allelesne	Number of polymorphicloci	Percentage of polymorphic loci (%)
MQ	0.090	0.062	1.159	1.111	37	15.8
YS	0.106	0.072	1.192	1.131	45	19.2
ZD	0.108	0.072	1.218	1.123	51	21.8
QL	0.088	0.058	1.180	1.098	42	17.9
HZ	0.102	0.068	1.209	1.118	49	20.9
GC	0.097	0.065	1.184	1.115	43	18.4
TJ	0.122	0.082	1.226	1.145	53	22.7
GH	0.111	0.078	1.197	1.135	46	19.7
XH	0.109	0.074	1.197	1.131	46	19.7
GN	0.096	0.064	1.192	1.108	45	19.2
HN	0.106	0.070	1.209	1.119	49	20.9
LZ	0.132	0.089	1.239	1.156	56	23.9
ML	0.113	0.075	1.239	1.129	56	23.9
DQ	0.078	0.049	1.184	1.082	43	18.4
SQ	0.101	0.069	1.184	1.122	43	18.4
KD	0.127	0.085	1.231	1.147	54	23.1
SG	0.092	0.062	1.171	1.107	40	17.1
TQ	0.092	0.062	1.171	1.107	40	17.1
Overall	0.270	0.170	1.675	1.263	158	67.5
Ave.	0.104	0.070	1.199	1.121	46.6	19.9

[a] Population codes refer in Table 1

$MgCl_2$, 0.25 mmol/L dNTPs, 0.2 μmol/L primer, 0.75 U Ex *Taq* DNA polymerase (TaKaRa), and 10 ng template DNA. After preheating for 5 min at 94°C, PCR was run for 40 cycles each consisting of a 94°C denaturation for 45 sec, an annealing for 45 sec at 48°C~55°C (annealing temperature following different primer shown in Table 2), and a 72°C extension of 45 sec in a Mastercycler Gradient PCR (Eppendorf, Germany). At the end of the run, a final extension period was appended for 5 min at 72°C, then soaked at 10°C. Amplified products were separated on 1.5% agarose gels in 1× TAE buffer (100 V for 35 min), stained with ethidium bromide and photographed under UV light using UVP-GDS8000 imaging system.

One hundred ISSR primers were screened with four DNA samples randomly selected from all *C. sinensis* individuals. Nine ISSR primers that produced distinctive and reprodu-

Table 4. Nei's genetic diversity parameters (1973) of the all 18 *C. sinensis* populations and subdivided populations of 5 groups

Genetic parameters	All populations	Group I[a]	Group II	Group III	Group IV	Group V
Total gene diversity: H_T	0.170	0.121	0.110	0.101	0.119	0.126
Gene diversity within populations: H_S	0.071	0.070	0.069	0.069	0.072	0.074
Gene diversity among populations: D_{ST}	0.099	0.051	0.042	0.032	0.047	0.052
Ratio of gene diversity within population: H_S/H_T	0.417	0.579	0.624	0.679	0.604	0.587
Coefficient of gene differentiation: G_{ST}	0.583	0.421	0.376	0.321	0.396	0.415
Gene flow: Nm	0.357	0.688	0.830	1.056	0.761	0.704
Number of observed alleles: na	1.675	1.423	1.389	1.342	1.389	1.501
Number of effective alleles: ne	1.262	1.198	1.181	1.169	1.199	1.200
Number of polymorphic loci	158	99	91	80	91	10
Percentage of polymorphic loci: %	67.5	42.3	38.9	34.2	38.9	50.9

[a] Groups divided by the genetic distance and refer to Fig. 3

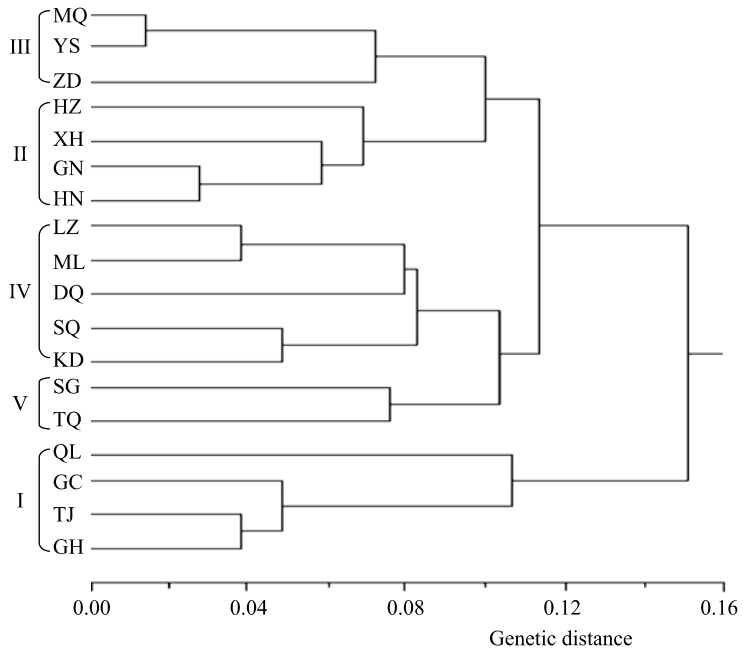

Fig. 3. A dendrogram showing the genetic relationships among 18 populations of *C. sinensis* obtained by the UPGMA clustering method based on the genetic distances. Population codes refer to Table 1. Group of I to V refer to Fig. 1.

cible fragments were chosen to investigate the genetic diversity within and between *C. sinensis* populations in the north-eastern Qinghai-Tibet Plateau. The amplification results were summarized in Table 2.

Data analysis

ISSR amplified bands were scored as 1 (present) or 0 (absent) and stored as a matrix representing the ISSR profile of each sample. Only those amplified fragments that were clear and reproducible were included in the analysis. The 180 individual samples from different locations were clustered firstly based on the Jaccard similarity coefficient. The genetic relationships were very close among individuals collected from same location, and individuals sampled from different locations were well separated from each other (data not shown). This pattern suggests that geographic isolation among the sampled locations is obvious, so it is reasonable to treat samples from each location as from one population. Thus, marker patterns of all individuals from the same location were bulked to study the genetic diversity and structure on population level. Genetic distances among populations were estimated by calculating Nei's unbiased genetic similarity for all pairs of populations (1978). Nei's (1978) genetic distance was used to perform cluster analysis by UPGMA using the Numerical Taxonomy System analytical software NTSYS-PC version 2.0 (Rohlf, 2000) and the dendrogram was generated. POPGENE software 1.31 (Yeh *et al*., 1999) was used to generate the single population and multi-populations descriptive statistics data respectively. In single population descriptive statistics, Nei's (1973) estimates of gene diversity (H) and Shannon index (I) were computed assuming Hardy-Weinberg disequilibrium. Observed number of alleles (na), effective number of alleles (ne) and polymorphic loci (percentage of all loci that were polymorphic regardless of allele frequencies) were calculated. Multi-populations descriptive statistics estimates among- and within-population interlocus correlations for multiple populations. The total genetic diversity (H_T), the within population genetic diversity (H_S), the among population genetic diversity (D_{ST}) and the coefficient of genetic differentiation (G_{ST}) were related by the expression $H_T=H_S+D_{ST}$, and $G_{ST}=D_{ST}/H_T$. The estimate of gene flow from G_{ST} was calculated as $Nm=0.5(1-G_{ST})/G_{ST}$. The genetic diversity parameters based on Shannon index were also calculated.

Results

ISSR profiles

The nine selected ISSR primers produced 141 countable bands, of which 140 bands (99.3%) were polymorphic across the 180 *C. sinensis* samples from 18 populations (Table 2). The amplified DNA bands were in the range from 300 bp to 1,000 bp. 8 (UBC889) to 27 (UBC841) bands were obtained from each primer with a mean of 15.6 bands/ primer. The ISSR bands showed considerable polymorphism among populations from different geographical distribution. Example of ISSR banding pattern of population ZD and QL generated by the UBC888 primer was shown in Fig. 2.

Genetic diversity within and among populations

The genetic diversity-related parameters for each population are summarized in Table 3. The genetic diversity level was different among populations. For example, the percentage of polymorphic loci within populations varied from 15.8% for MQ to 23.9% for ML and LZ populations with a mean of 19.9%. The least genetically diverse population was MQ

with 37 polymorphic loci and 0.062 Nei gene diversity index and the most diverse populations were ML and LZ with 56 polymorphic loci and 0.089 Nei index. The averages of all populations were 46.6 polymorphic loci and 0.07 Nei index (Table 3). The observed number of alleles ranged from 1.158 in MQ to 1.239 in ML and LZ while effective number of alleles ranged from 1.082 in DQ to 1.156 in LZ. The pattern of Shannon index similar to the Nei index. The low Shannon index with a mean of 0.104 and Nei index of 0.07 indicate that there was little within-population genetic differentiation.

The genetic diversity of the all 18 populations is presented in Table 4. The total percentage of polymorphic loci of 18 populations was 67.5%. For the entire 18 populations of *C. sinensis*, the Nei's total genetic diversity (H_T), the within population genetic diversity (H_S), among populations genetic diversity (D_{ST}), the coefficient of genetic differentiation (G_{ST}) and gene flow (Nm) were 0.170, 0.071, 0.099, 0.583, and 0.357, respectively (Table 4). The low H_S (0.071) and the relatively high G_{ST} value (0.583) showed that most of genetic diversity of the total 18 *C. sinensis* populations was found between populations. The partitioning of within-population (H_{pop}=0.104) and among-populations (H_{sp}-H_{pop}=0.166) genetic diversity based on Shannon index also revealed more variation between populations (0.615) than within-population variations (0.385).

Genetic relationship and geographical grouping

A dendrogram was constructed based on the ISSR band variation in the 18 populations of *C. sinensis* (Fig. 3). In the dendrogram, *C. sinensis* populations were divided into five groups. Populations of QL, GC, TJ, and GH from the areas around the Qinghai Lake formed an independent group that was obviously separated from the other groups at genetic distance of 0.16. In this group, the north QL population far from the Qinghai Lake showed distant relationship with the other three populations which were closer to the Lake. Populations of HZ, XH, GN, and HN from mid-eastern Qinghai province and population of MQ, YS, and ZD from southern Qinghai province formed two groups (II and III) at genetic distance of 0.10. All populations from Tibet, Sichuan, and Yunnan provinces were clustered together, but the Yunnan populations (group V) were separated from the populations of the other two provinces (group IV) at genetic distance of 0.10. The five groups distributed along a decreasing latitude with the average of 37°13' N, 35°28' N, 33°29' N, 30°39' N, and 28°08' N, respectively. The grouping pattern seems to match with the geographic distribution along latitudinal gradient.

Genetic diversity in the partitioned groups

The genetic diversity in the five groups partitioned by the genetic distance in the dendrogram (Fig. 3) is summarized in Table 4. Their number of observed alleles, effective alleles, polymorphic loci, and percentage of polymorphic loci were obviously less those of the overall populations. Relatively low G_{ST} values (0.321~0.421) were detected in partitioned groups, indicating that the genetic diversity among populations is less than that within populations within these groups. In each partitioned group, most genetic diversity is detected within populations (Table 4). In spite of the differentiations in the G_{ST} value of all populations and different groups, the within population genetic diversity (H_S) values were almost same (around 0.07). Compared with the low gene flow Nm of 0.357 for all 18 populations, the within group gene flows were relatively higher, and the values were obviously different among groups (0.688~1.056, Table 4).

Discussion

In our study, the genetic diversity of 180 individuals representing 18 populations of *C. sinensis* across the major distributing regions in China was analyzed by ISSR marker. ISSR analysis was proved to be an informative and reproducible approach that is appropriate to trace the genetic diversity and structure for *C. sinensis* populations. In previous researches, the sequences of ITS1, 2, and 5.8S rDNA regions were also used for analyzing the phylogenetic relationships and genetic variations of *C. sinensis* materials (Kinjo and Zang, 2001; Chen *et al*., 2004). However, they are unsuitable for revealing the genetic variations among *C. sinensis* populations, because the ITS-rDNA sequences are usually species specific and highly homologous within *C. sinensis*.

The high polymorphism (99.3%) observed among *C. sinensis* populations in this study can be attributed to the geographically extensive sampling and high sensitivity of ISSR marker. About 73% and 84.7% polymorphism were detected in a few individuals of *C. sinensis* from limited areas using RAPD marker (Chen *et al*., 1997, 1999). In our study, ISSR markers detected genetic variations within and among populations from wide distribution range. The low average Shannon index (0.104) of each population, low gene diversity within populations (H_S=0.071 or H_{pop}=0.104) and among populations (D_{ST}=0.099 or H_{sp}=0.270) reflected low genetic diversity of *C. sinensis*. Even though the detected genetic diversity within or among *C. sinensis* populations was still very low. It is very different from the results of Chen *et al*. (1999), which demonstrated extensive genetic diversity among different geographical populations based on the RAPD markers, although only 29 individuals of *C. sinensis* from 10 sites of 3 provinces were analyzed in that research. The population partition, extracted from whole cordyceps (may include some host DNA) should be considered as the reasons why the high Shannon index (0.4478~1.8435), genetic diversity within populations (H_{pop}=0.7397) and among populations (H_{sp}=1.4547) were obtained by RAPD analysis (Chen *et al*., 1999). An individual cordyceps consists of a stalked fungus fruiting body and the dead larva body of host insect. The mixture of *C. sinensis* and host insect genome DNA can be extracted with CTAB method from the dead larva body in an individual cordyceps (Cheng *et al*., 2007). Usually, *C. sinensis* is considered as one species while about 68 species of host insects from 4 genera had been reported (Yang *et al*., 1996; Li *et al*., 2000; Chen *et al*., 2001, 2004; Jiang and Yao, 2003; Liu *et al*., 2005). Therefore, interfusing host insect DNA would strongly undermine the reliability of genetic analysis results.

Previous studies reported latitudinal genetic differentiation of *C. sinensis* from south to north (Chen *et al*., 1997; Liang *et al*., 2005). Chen *et al*. (1999) divided 29 samples into three

geographical populations, i.e., the northern population, middle population, and southern population. Our results support their conclusion and further clustered the *C. sinensis* populations into five groups along a latitudinal gradient. The populations from Qinghai province were separated into three groups, which respectively distribute in the mid-eastern Qinghai, southern Qinghai, and Qinghai Lake vicinity. Meanwhile, the populations from other three provinces were clustered together as an independent "Tibet-Yunnan-Sichuan" cluster with two separated groups. The grouping patterns match well with the latitudinal distribution of *C. sinensis* across Qinghai-Tibet Plateau. In China, *C. sinensis* only distributes in the altitude of 3,000~5,100 m in Qinghai-Tibet Plateau. The altitude of this region is descending from west to east and most mountains are mainly west-to-east extended. Thus, the geographic isolation can be the major reason to cause the latitudinal grouping pattern. One exception was in Qinghai Lake vicinity, although the population GH and HZ locate at almost same latitude about 36°20' N and not very far from each other (110 km), they are separated in two groups at distance of 0.10, which may mainly caused by the geographic isolation due to Riyue Mountain (3,520 m) between these two groups. In summary, mountain isolation might cause the genetic differentiation of *C. sinensis* and affected the genetic relationship between *C. sinensis* populations.

Gene flow affects genetic diversity level within and among organism populations (Slatkin, 1987). The moderately high coefficient of gene differentiation (G_{ST}=0.583) observed among the overall populations indicated that most of the ISSR variation is found among the populations. However, all 5 groups revealed that their genetic diversity mainly exist within populations. The geographic isolations lead to limited gene exchange among groups of *C. sinensis*. The inter-population gene exchange of *C. sinensis* is mainly mediated by the dispersion of ascospores, which has fairly limited mobility. The spread of *C. sinensis* depends on the scattering of ascospores from perithecium located stroma by wind, which has short spreading distance. Mountains, rivers, and valleys all limit the movement, dispersal, and genetic communication of *C. sinensis* populations. The isolation by mountains or waterbodies makes the gene flow especially difficult among several populations. For example, the least gene exchange exists among the populations from the areas around the Qinghai Lake, probably due to the isolation effect of the salt lake. The gene flow among populations within each group shows different Nm values, but that among all 18 populations were very low with an Nm of 0.357. Therefore, the limited gene exchange among *C. sinensis* populations caused by the geographic isolation led to a high genetic differentiation among populations on regional scale, although the among-populations and within-population genetic diversity level remain low in each group. This observation matches with the theory that spatiotemporal variations of diversifying selection can maintain genetic polymorphism (Hedrick *et al*., 1976; Nevo *et al*., 1988).

C. sinensis is a caterpillar fungus parasitizing on the larvae of the *Hepialus* spp. to finish its life history. Both *C. sinensis* and *Hepialus* spp. are endemic species with specific distributing range in Qinghai-Tibet Plateau, so the relationships and co-evolution with local host species also can affect the gene exchange of *C. sinensis* on regional scale. The host insect is indispensable for the three-stage life history (infection, parasitism, and saprophyte) of *C. sinensis* fungus (Li and Tsim, 2002). Host insects of *C. sinensis* (genus *Hepialus*) are highly diverse, with various morphologic characters and species specific geographical distribution. The active interspecific divergence is attributed to the complex spatial pattern of geographical and environmental factors in the Qinghai-Tibet Plateau (Cheng *et al*., 2007). Yang *et al*. (1996) reported that each species of *Hepialus* spp. distributes in a narrow geographical range, and different species usually distribute in different mountain ranges, and even in different sides or altitudes of the same mountain. The mitochondrial *Cytb* sequences of the host insect of all 18 *C. sinensis* populations in our study indicated that their phylogenetic relationships and distributing pattern were similar to that of *C. sinensis* populations revealed in this study (Cheng *et al*., 2007). This similarity implies that the host insects may play an important role in the genetic differentiation and evolution of *C. sinensis*. In southern Qinghai province, where the mountains are flatter and the host insects are considered as only one species (*Hepialus yushuensis*, Cheng *et al*., 2007), the populations show frequent gene exchange with a relative high gene flow (Nm=1.056). The same host insect of *Hepialus yushuensis* might make the gene exchange easier among these *C. sinensis* populations. Therefore, the adaptation of host species to the habitat environment, and the co-evolution of *C. sinensis* should also be considered as factors influencing gene flow and gene differentiation.

A primary objective of conservation genetics is to estimate the level and distribution of genetic variation in endangered species (Fritsch and Rieseberg, 1996). The low total genetic diversity, genetic diversity within populations, and the low gene flow among the *C. sinensis* populations should be considered as one of the reasons why the species becomes endangered. Reduced gene flow and genetic diversity found in *C. sinensis* populations reflects the cutoff of gene exchange caused by geographic isolation, limited dispersal ability (such as low mobility to migrate), population bottleneck related to host adaptation, and the long term selection pressure in tough, high-altitude environment. The current genetic differentiation and low genetic diversity among populations may result from combined effects of these factors. At present, the productivity of *C. sinensis* is extremely limited and the species is becoming endangered because of the fragility of its preferred habitat and excessive exploitation. The information illustrated by this study implies that the *C. sinensis* resources from Qinghai province, which have high genetic diversity and distribute widely across latitude range (38°01' N to 32°55' N), can be regarded as a conservation center to enforce full scale *in situ* conservation. It is useful for making the strategic plan for effective conservation and sustainable exploitation of this precious traditional medicine resource.

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