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ORIGINAL ARTICLE

Genetic diversity and population structure of Chinese *Lentinula edodes* revealed by InDel and SSR markers

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Abstract Genetic diversity and population structure of 88 Chinese *Lentinula edodes* strains belonging to four geographic populations were inferred from 68 Insertion-Deletion (InDel) and two simple sequence repeat (SSR) markers. The overall values of Shannon's information index and gene diversity were 0.836 and 0.435, respectively, demonstrating a high genetic diversity in Chinese *L. edodes* strains. Among the four geographic populations, the Central China population displayed a lower genetic diversity. Multiple analyses resolved two unambiguous genetic groups that corresponded to two regions from which the samples were collected—one was a high-altitude region (region 1) and the other was a low-altitude region (region 2). Results from analysis of molecular variance suggested that the majority of genetic variation was contained within populations (74.8 %). Although there was a strong genetic differentiation between populations (F_{ST}=0.252), the variability of ITS sequences from representative strains of the two regions (<3 %) could not support the existence of cryptic species. Pairwise F_{ST} values and Nei's genetic distances showed that there were relatively lower genetic differentiations and genetic distances between populations from the same region. Geographic distribution could play a vital role in the formation of the observed population structure. Mycelium growth rate and precocity of *L. edodes* strains displayed significant differences between the two regions. Strains from region 2 grew faster and fructified earlier, which could be a result of adaptation to local environmental factors. To the best of our knowledge, this was the first study on the genetic structure and differentiation between populations, as well as the relationship between genetic structure and phenotypic traits in *L. edodes*.

Keywords Shiitake mushroom · Population genetics · InDel markers · Mycelium growth rate · Precocity

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Introduction

The basidiomycete *Lentinula edodes* (also called Xianggu or shiitake) is one of the most important and popular edible mushrooms in the world, especially in East Asia. *L. edodes* follows a typical sexual life history, with meiotic basidiospores that develop into monokaryotic mycelium. As a tetrapolar heterothallic basidiomycete, *L. edodes* generates a dikaryon that is able to develop into a fruiting body by the fusion of two monokaryons with different mating alleles at the *A* and *B* mating type loci (Li et al. 2007).

China is the largest producer of *L. edodes*—it produced over 2 million tons of *L. edodes* in 2003, accounting for two-thirds of the total world production (Chang 2005). *L. edodes* is well known not only for its high nutritious values but also for its pharmacological effects. This mushroom presents medicinal constituents including polysaccharides, terpenoids, sterols, and lipids, which participate actively in

immunomodulation as well as anti-tumor, antioxidant, and antiviral activities (Finimundy et al. 2014). Moreover, *L. edodes* is invaluable for effective bioconversion of locally available agricultural wastes (Philippoussis et al. 2007) because it produces hydrolytic and oxidative enzymes to degrade organic substrates (Gaitán-Hernández et al. 2006).

Recently, random amplified polymorphic DNA (RAPD) markers (Sun and Lin 2003), simple sequence repeat (SSR) markers (Xiao et al. 2010a), target region amplification polymorphism (TRAP) markers (Xiao et al. 2010b), and internal transcribed spacer (ITS) sequences (Xu et al. 2005), were used to analyze the genetic diversity of wild *L. edodes* strains in China. Results revealed a high genetic diversity in the natural germplasm of Chinese *L. edodes*, and strains from Yunnan Plateau, Hengduanshan Mountains, Taiwan, South China, and Northeast China showed greater genetic diversity. For Chinese shiitake cultivars, a low level of genetic diversity was uncovered by RAPD, inter-simple sequence repeat (ISSR) and sequence-related amplified polymorphism (SRAP) markers (Fu et al. 2010; Liu et al. 2012b). However, these reports just focused on the construction of cluster dendrograms and estimated genetic parameters within populations. The genetic structure and differentiation between populations are still uncovered in this species. Furthermore, the relationship between genetic structure and important phenotypic traits has not yet been investigated. As a result, the reference basis for further utilization of the germplasm resources is not yet available.

Insertion-Deletion (InDel) markers arise from the insertion of transposable elements, slippage in simple sequence replication, or unequal crossover events (Britten et al. 2003). By simple experimental procedures used in SSR markers, InDel polymorphisms can be easily detected based on polymerase chain reaction (PCR) fragment length polymorphisms (Bhattramakki et al. 2002). InDels are widely spread across the genome and their genomic density are far higher than those of SSRs (Liu et al. 2013; Väli et al. 2008). InDels are also deemed as a major source of gene defects and a significant source of evolutionary change (Britten et al. 2003). Because of their high abundance, reproducibility, co-dominant inheritance, and extensive genomic coverage, InDels have been applied successfully for the analysis of genetic diversity and population structure in plants (García-Lor et al. 2012; Ollitrault et al. 2012; Wu et al. 2014). However, this robust marker system is seldom used in mushroom species. Previously, 15 InDel markers were employed to construct a shiitake genetic linkage map (Gong et al. 2014a). InDels within the ribosomal DNA and β-tubulin sequences were utilized in phylogenetic analysis of *Rhizoctonia solani* (Gonzalez et al. 2006). With the rapid development of mushroom genome sequencing, it is now possible to detect genome-wide InDel polymorphisms among different strains using whole-genome re-sequencing.

Screening and breeding of superior strains are crucial for sustainable production and marketing of *L. edodes*. Although more than 100 commercial *L. edodes* cultivars have been developed in China, their narrow derivation from a limited number of elite strains has caused an increasingly narrow genetic base (Chiu et al. 1996). Therefore, new genetic resources, particularly wild strains, should be introduced into breeding programs to increase the genomic variability. As for mushroom species, population genetic inference can provide a prerequisite for the efficient use of genetic resources in breeding programs (Urbanelli et al. 2003). Therefore, to effectively utilize the wild genetic resources of *L. edodes*, it is necessary to comprehensively explore its population structure and population genetic differentiation.

Fungal mycelium growth rate (MGR) is a widely studied quantitative trait since it is related to yield, inhibitory sexual recognition, and ability against competitors (Salmones et al. 1997; Larraya et al. 2002; Sivolapova et al. 2012; van der Nest et al. 2009). In the breeding scheme of *L. edodes*, strains with a low MGR were usually excluded for further selection. The whole harvest season of *L. edodes* lasts a long time (Royse and Sanchez-Vazquez 2001). Precocity (early formation of primordium and fruiting body) is a crucial target in the genetic improvement of *L. edodes*. Early-fruiting strains could greatly reduce the costs in commercial cultivation. In addition, precocity was reported to be negatively correlated with yield in *Agricus bisporus*, *Pleurotus ostreatus*, and *L. edodes* (Foulongne-Oriol 2012; Larraya et al. 2003; Gong et al. 2014b). Early-fruiting mushroom strains usually have a higher yield.

Here, we employed 68 InDel and two SSR markers to survey the genetic variation of four *L. edodes* populations from two Chinese regions. This study aimed to investigate the genetic diversity and the genetic structure of the Chinese *L. edodes* population, and to infer factors affecting the observed population genetic structure and differentiation. We also determined the MGR and precocity of *L. edodes* strains for breeding purposes and discussed the differences of these traits among individual populations. This study could provide necessary information for efficient conservation and management, as well as subsequent selection and breeding programs of *L. edodes*.

Materials and methods

Lentinula edodes strains

Eighty-eight wild strains of *L. edodes* from different areas in China were used in this study (Supplemental Table S1, Fig. 1). These strains were collected either from natural

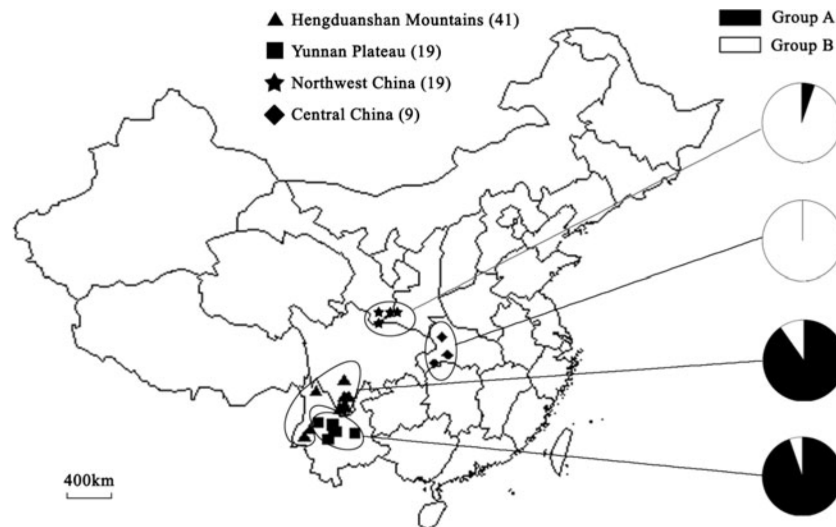

Fig. 1 Geographical distributions of *Lentinula edodes* strains from China. Strains collected from the four populations are marked in different symbols: ▲, localities in Hengduanshan Mountains; ■ in Yunnan Plateau; ★, in Northwest China; and ◆, in Central China. Numbers in brackets indicate the number of strains in the four populations. Pie charts show the relative frequency of strains allocated to each group according to the UPGMA dendrogram of the tested strains (in black, strains assigned to Group A; in white, strains assigned to Group B). Geographical distribution of the 88 *L. edodes* strains are as follows (numbers in bracket indicated the number of strains in each county or city): 1) Hengduanshan Mountains. Mianning (24); Dechang (3); Miyi (5); Huili (2); Yanbian (1); Puge (1); Panzhihua (1); Lijiang (1); Xianggelila (1); Longling (2). 2) Yunnan Plateau. Jingdong (1); Chuxiong (1); Nanhua (4); Yaoan (1); Yangbi (8); Yiliang (3). 3) Northwest China. Liuba (5); Lveyang (1); Kang County (5); Qingchuan (8). 4) Central China. Shennongjia (7); Hefeng(1); Changyang (1)

reserves and remote mountain areas far from cultivation sites or provided by professional research institutes. All the strains were cultivated in experimental mushroom farms at Huazhong Agricultural University. Morphological characteristics of the fruiting bodies indicated that all the tested strains belonged to *L. edodes*. The 88 strains were divided into four geographic populations according to their origins (Supplemental Table S1, Fig. 1) (Sun and Lin 2003), namely the Hengduanshan Mountains (HM), Yunnan Plateau (YP), Northwest China (NC), and Central China (CC). The four geographic populations were then grouped into two regions. HM and YP were defined as region 1 since both are located in high-altitude regions in Southwest China, whereas NC and CC were defined as region 2 since both are located in relatively low-altitude regions.

DNA extraction

The mycelia of each strain were cultured in liquid MYG (malt extract, yeast extract and glucose) broth (2 % malt extract, 2 % glucose, 0.1 % peptone and 0.1 % yeast extract) in the dark at 25 °C for 2 weeks and collected by filtering. Genomic DNA was extracted from approximately 100 mg fresh mycelia via the cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980). The concentration and purity of the DNA samples were determined with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and adjusted to 50 ng/μl.

ITS sequencing

We retrieved the ITS sequences of eight strains (EFISAAS0350, EFISAAS0351, HUB021, HUB028, HUB037, SHX002, GAN057, and GAN059) previously deposited in GenBank (Xu et al. 2006). We then randomly selected 17 representative strains from the two regions and sequenced their ITS sequences. The GenBank accession numbers of the 25 ITS sequences were listed in Supplemental Table S1. PCR amplification of ITS sequence was conducted with primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990). The PCR reactions and amplification program followed the description by Sadfi-Zouaoui et al (2009). The PCR products were purified using beads and were directly sequenced by the Quintarabio Company (Wuhan, China).

Molecular marker analysis

Seven strains (00168, GAN060, HUB039, SHX006, LMLH36, YAASM292, and YAASM1553) from the four populations were randomly selected to screen for 132 InDel loci and 11 SSR loci. A total of 70 polymorphic markers, including 68 InDels and two SSRs, were then selected

(Supplemental Table S2). The 70 molecular markers were designed from our unpublished *L. edodes* genome sequence (Kwan et al. 2012). Those markers were distributed in 63 different scaffolds of the *L. edodes* draft genome.

Both InDel and SSR PCR amplifications were carried out in 20-μl reactions containing 50 ng template DNA, 2 μl 10 × buffer, 1U *Taq* DNA polymerase (Biocolors, Shanghai, China), 0.15 mM dNTP mixture, and 0.4 μL each of 10 μM forward and reverse primers. PCR reaction was programmed as follows: 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C for 1 min, and a final step at 72 °C for 10 min. PCR products were detected by electrophoresis on 6 % denaturing polyacrylamide gel (PAGE), and then stained with silver nitrate solution.

Data analysis

Analysis of ITS sequences

ITS sequences were submitted to GenBank for species identification using BLASTN. ITS sequences of eight *L. edodes* strains, *L. raphanica* (GenBank accession No. AY256687) and *L. aciculospora* (GenBank accession No. JQ247977) retrieved from GenBank, as well as our 17 sequences, were used for phylogenetic analysis. All the ITS sequences were aligned using ClustalW in MEGA 5.05 (Tamura et al. 2011). The aligned sequences were checked by eye and edited. A phylogenetic tree was then constructed in MEGA using the neighbor-joining (NJ) method based on 1,000 bootstrap pseudoreplicates and the Kimura-2-parameter model (Kimura 1980). Among the 25 ITS sequences, the percentage of sequence identity was analyzed using DNAMAN version 8 (Lynnon Biosoft Corp.). The objective of ITS sequence analysis is to detect any cryptic species that might be present in the Chinese *L. edodes* population.

Analysis of genetic diversity

Clear InDel and SSR bands were scored with present (1) or absent (0), and the matrix of marker data was assembled. Alleles of each InDel and SSR locus were numerically coded (e.g., 1, 2, and 3 for each band) in decreasing size order. We analyzed some parameters indicating genetic diversity of the tested strains. For each population, the observed number of alleles (*Na*), effective number of alleles (*Ne*), Shannon's information index (*I*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and percentage of polymorphic loci (PPL) were calculated by POPGENE 1.32 (Yeh 1997). Gene diversity (*H*) and polymorphism information content (PIC) were determined by PowerMarker V3.25 (Liu and Muse 2005). The index of inbreeding (F_{IS}), defined as $F_{IS} = 1-(Ho / He)$, was measured for each population (Urbanelli et al. 2003). Genotype frequencies were tested for departures from the Hardy-Weinberg Equilibrium (HWE) with chi-squared tests implemented in GenAlEx version 6.501. For each locus, *H* and PIC were calculated by the PowerMarker V3.25, whereas *Ho* and *He* were calculated by POPGENE 1.32.

Genetic structure and differentiation

The extent of genetic differentiation was evaluated using Wright's *F* statistics. A global F_{ST} value across all populations and pairwise F_{ST} values between pairs of populations were estimated using GenAlEx version 6.501. Pairwise Nei's genetic distances between populations were calculated by POPGENE 1.32 (Yeh 1997). To compare the genetic variations within and among different populations, the data were also subjected to phi-statistics through the hierarchical analysis of molecular variance (AMOVA). This analysis was implemented in GenAlEx version 6.501. The total genetic variation was partitioned at three levels: within populations (PhiPT), among populations within regions (PhiPR), and among regions (PhiRT) (Xu et al. 2008). The gene flow among populations, or the number of effective migrants, was defined as $Nm = [\ (1/F_{ST})-1\ ]/4$ (Frankham et al. 2002), where F_{ST} represents the degree of population genetic differentiation. F_{ST} values were interpreted as follows: values that ranged from 0 to 0.05 indicated little genetic differentiation, values from 0.05 to 0.15 showed moderate genetic differentiation, values from 0.15 to 0.25 displayed great genetic differentiation, and values above 0.25 showed very great genetic differentiation (Hartl and Clark 1997).

To indicate the genetic relationship among the tested strains, pairwise Dice similarity coefficients between strains were calculated by NTSYS-pc version 2.10e (Rohlf 2000), from which a UPGMA (unweighted pair group method with arithmetic mean) dendrogram was inferred. We also constructed a UPGMA dendrogram based on pairwise Nei's genetic distances between populations to depict the genetic relationship of the four populations.

To further assess the population structure, a Bayesian clustering analysis was performed using STRUCTURE version 2.3 (Pritchard et al. 2009). In order to identify the number of subgroups (*K*), we set *K* from 1 to 10 using admixture model assumptions with correlated alleles. For each *K*, seven independent runs were performed separately, with a burn-in period of 10,000 Markov Chain Monte Carlo (MCMC) iterations and a run length of 100,000. In order to detect the most probable value of *K*, the ΔK method was used as previously described (Evanno et al. 2005). Strains assigned to corresponding genetic groups were graphically displayed using the DISTRUCT software package (Rosenberg

2004). Strains with membership probabilities ≥ 0.55 were assigned to corresponding groups, while those < 0.55 were assigned to a mixed group.

Assays for mycelium growth rate and precocity

The MGR of all 88 strains in the mixed sawdust (SD) medium were determined using methods described previously (Gong et al. 2014a). An inoculum of each strain was placed at the top of each different SD test tube, and the distance colonized by the mycelium (in millimeters) was measured 20 days after inoculation. The MGR was determined as the ratio of the colonized distance to the growth time (20 d). Three repetitions were performed for all the aforementioned samples.

An experiment for precocity was carried out according to the methods described by Gong et al (2014b). *L. edodes* strains were grown in plastic bags including 1 kg (wet wt.) sterilized mixed sawdust medium. After inoculations using corresponding spawn, culture bags were incubated at 24 °C in the dark until the completion of spawn running and formation of brown film. The bags were then transferred to a mushroom house where environmental conditions changed along with the changing natural conditions. The tested strains were allocated according to a randomized-block design with two blocks, and six bags of each strain were contained in each replication. Precocity was indicated by two aspects: (1) the time interval (in days) from incubation to formation of the first primordium (FP); and (2) the time interval (in days) from incubation to harvest of the first fruiting body (FB) (Gong et al. 2014b).

Correlation analysis between MGR and precocity was performed via the Pearson procedure (Larraya et al. 2002). The effects of geographic distribution on MGR and precocity were determined by one-way analysis of variance (ANOVA) among the four geographic populations. Duncan's multiple range tests were further performed to analyze the difference of MGR and precocity among the four populations (Duncan 1975). A *t*-test was carried out to detect the differences of MGR and precocity between two regional populations. All the phenotypic data was analyzed by SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

Analysis of ITS sequences

The 25 ITS sequences examined in this study showed a ≥99 % sequence identity to ITS sequences of *L. edodes* in GenBank by BLASTN analysis (data not shown), confirming their identity as *L. edodes*. In the NJ tree, 19 strains (eight from region 1 and 11 from region 2) formed a cluster whereas six others (three from region 1 and three from region 2) formed another cluster (Fig. 2) supported by a bootstrap value of 100 %. The percentage of sequence identity among the 25 ITS sequences was 97.27 %, while those among strains from region 1 and region 2 were 97.08 % and 97.41 %, respectively.

Genetic diversity of *L. edodes* populations in China

Based on the results of multilocus module in GenAlEx version 6.501 (Peakall and Smouse 2012), no repeated genotypes in our Chinese *L. edodes* population was detected. From all the 88 strains, values of *Ne* (effective number of alleles), *He* (expected heterozygosity), *I* (Shannon's information index), PPL (percentage of polymorphic loci), *H* (gene diversity), and PIC (polymorphism information content) were 2.288, 0.438, 0.836, 100 %, 0.435, and 0.395, respectively, indicating high genetic variations among Chinese *L. edodes* populations. As for the four populations, the *H* index varied from 0.289 (CC) to 0.435 (NC) with a mean of 0.355. The PIC value ranged from 0.255 (CC) to 0.373 (NC) with an average of 0.314. The *I* value varied from 0.510 (CC) to 0. 734 (NC) with an average of 0.641. The HM population had the highest PPL value (100 %), while that in CC was the lowest (82.86 %). Combining all these genetic parameters, the *L. edodes* strains from CC possessed a lower genetic diversity whereas those from NC, HM, and YP had a higher genetic variation. The F_{IS} values varied from 0.007 in NC to 0.291 in YP, with a mean of 0.193 (Table 1).

In the four populations, the numbers of loci that significantly deviated from the HWE ($p < 0.05$) ranged from 24 to 38, with an average of 31 (Table 1). There were 24 and 28 such loci in the NC and CC populations, respectively, fewer than those in the HM (34) and YN (38) populations, pointing to an overall lower level of nonrandom association among alleles in the strains from region 2 compared to those from region 1.

Population genetic structure and differentiation

The overall F_{ST} value across the 88 strains was 0.252, representing a very great differentiation among *L. edodes* populations in China. F_{ST} values between pairs of populations ranged from 0.024 to 0.283 (Table 2). There was little genetic differentiation between the YP and HM populations. By contrast, there were great differentiations between the CC and NC populations, the NC and HM populations, as well as the NC and YP populations. However, very great differentiations existed between the CC and HM populations, as well as the CC and YP populations. The genetic differentiation was positively correlated with the geographical distributions between populations, as the pairwise F_{ST} values between populations within regions were far lower than those between regions.

The pairwise Nei's genetic distances between populations varied from 0.021 to 0.291 (Table 2). Generally speaking, the relationships of genetic distances between populations showed

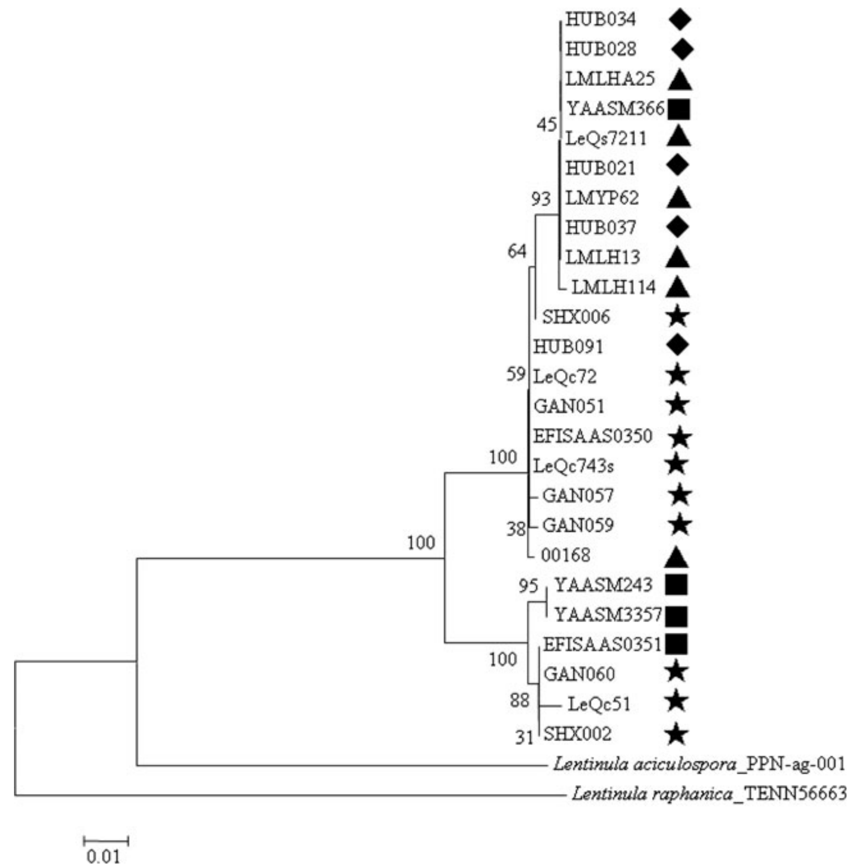

Fig. 2 A neighbor-joining tree of the ITS sequences from 25 strains of *Lentinula edodes*. *Lentinula raphanica* strain TENN56663 (GenBank accession No. AY256687) and *Lentinula aciculospora* strain PPN-ag-001 (GenBank accession No. JQ247977) were used as outgroups. The bootstrap values were calculated from 1,000 pseudo-replications. Strains collected from the four populations are marked in different symbols: ▲, localities in Hengduanshan Mountains; ■ in Yunnan Plateau; ★, in Northwest China; ◆, in Central China

a similar situation with those of the pairwise F_{ST} values. There were relatively smaller genetic distances between populations from the same region, whereas those between populations from different regions were relatively larger.

The analysis of molecular variance (AMOVA) results suggested that all three levels contributed significantly to the overall genetic variation (Table 3). Specifically, the total genetic diversity in *L. edodes* was ascribed to individual variations within populations (74.8 %), to variations among populations within regions (5.7 %), and to variations between populations from different regions (19.5 %).

In the UPGMA dendrogram of *L. edodes*, the 88 strains could be classified into two distinct groups consistent with the two regions, suggesting that the genetic diversity was closely related to the geographical distribution (Fig. 3). Group A, further divided into two subgroups at the similarity level of 0.66, included 55 strains from region 1 and one strain from region 2 (Fig. 3, Supplemental Table S1). Subgroup A_1 consisted of six strains from the YP population and two strains from the HM population. Subgroup A_2 contained 35 strains from the HP population, 12 strains from the YP population, and one strain

Table 1 Estimation of genetic variability in *Lentinula edodes* populations of China

Populations	Regions	*N*	*Na*	*Ne*	*I*	*Ho*	*He*	F_{IS}	*H*	PIC	PPL	No. Of loci out of HWE (out of 70)
HM	Region 1	41	3.771	1.927	0.676	0.260	0.346	0.249	0.346	0.315	100 %	34
YP	Region 1	19	3.157	1.898	0.646	0.249	0.351	0.291	0.351	0.312	91.43 %	38
NC	Region 2	19	2.914	2.023	0.734	0.432	0.435	0.007	0.435	0.373	97.14 %	24
CC	Region 2	9	2.443	1.578	0.510	0.224	0.289	0.225	0.289	0.255	82.86 %	28
Means	/	22	3.070	1.857	0.641	0.291	0.441	0.193	0.355	0.314	92.86 %	31

HM, Hengduanshan Mountains; YP, Yunnan Plateau; NC, Northwest China; CC, Central China

N, Number of strains per population; *Na*, observed number of alleles; *Ne*, effective number of alleles; *I*, Shannon's information index; *Ho*, observed heterozygosity; *He*, expected heterozygosity; PPL, percentage of polymorphic loci; *H*, gene diversity; PIC, polymorphism information content; F_{IS}, the index of inbreeding

Table 2 F_{ST} values and Nei 's genetic distances between pairwise populations

	HM	YP	NC	CC
HM	/	0.021	0.272	0.275
YP	0.024	/	0.291	0.290
NC	0.249	0.242	/	0.147
CC	0.283	0.283	0.151	/

F_{ST} values are shown below, diagonally. Nei 's genetic distances are shown above, diagonally

HM, Hengduanshan Mountains; YP, Yunnan Plateau; NC, Northwest China; CC, Central China

from the NC population. Group B, also classified into two subgroups at the similarity cut-off of 0.53, contained nine strains from the CC population, 18 strains from the NC population, four strains from the HM population, and one strain from the YP population. Subgroup B_1 consisted of 16 strains from the NC population, four strains from the HM population, one strain from the YP population, and one strain from the CC population. Two strains from the NC population and eight strains from the CC population were included in Subgroup B_2. Principal coordinate analysis (PCoA) was also undertaken to depict the genetic relationships among the 88 strains, and the results also identified two groups consistent with that of UPGMA clustering (data not shown).

In the UPGMA dendrogram of the four populations, two distinct groups consistent with the two regions were observed, suggesting that the genetic diversity was closely related to the geographical distribution (Fig. 4). The HM and YP populations were clustered together in the first group, while the NC and CC populations were assigned to the second group.

Analysis of the rate of change, ΔK, over the range of K values showed a clear maximum for $K=2$ ($\Delta K=386.57$) (Fig. 5a), and higher K values did not show secondary peaks. The classification robustly suggested the presence of two genetic groups among the 88 strains (Fig. 5b), in concordance with the results of UPGMA clustering.

Assays for mycelium growth rate and precocity

For the four geographic populations of *L. edodes*, there was no significant difference in MGR between the NC and CC populations. It was also the same case among the NC, HM, and YP populations (Fig. 6a). For the two regions, the MGR of the strains from region 2 was significantly higher than that from region 1 ($p<0.01$) (Fig. 6a).

For the four geographic populations, there was no significant difference between FP and FB of strains from the HM and YP populations. It was also the case between the NC and CC populations. However, FP and FB of the strains from the HM and YP populations were significantly higher than those from the NC and CC populations. When combining all 88 strains in a single population, the FP and FB of the strains from region 1 were significantly higher than that from region 2 ($p<0.01$) (Fig. 6b and c).

Taking the 88 *L. edodes* strains as a whole, the MGR showed a significant negative correlation with FP ($r^2=-0.305$, $p<0.01$) and FB ($r^2=-0.298$, $p<0.01$). Thus, the faster the mycelium of a strain grew in the sawdust, the faster it formed a primordium and fruiting body. FP also showed a significant negative correlation with FB ($r^2=-0.997$, $p<0.01$), indicating that strains forming the primordium earlier frequently harvested a fruiting body earlier.

Discussion

Genetic diversity indicates the total genetic characteristics in the genetic makeup of a species and plays a vital part in the species' survival and adaptability (Given 1994). Analyses of genetic diversity and population structure provide important information for surveying the origin and evolution of a species, and for identifying potential genetic resources for further utilization. In this study, we revealed the genetic diversity and population structure of Chinese *L. edodes* using InDel and SSR markers, which would provide comprehensive insights into further managing and using those genetic resources.

Table 3 Analysis of molecular variance (AMOVA) among populations and within populations of *Lentinula edodes* in China

Source of variation	d.f.	SS	MS	Est. var.	% var.	Phi Statistic	Value	*P*
Among regions	1	339.674	399.674	3.659	19.5 %	PhiRT	0.195	0.001
Among populations within regions	2	109.782	54.891	1.070	5.7 %	PhiPR	0.071	0.001
Within populations	172	2411.804	14.022	14.022	74.8 %	PhiPT	0.252	0.001
Total	175	2861.261		18.752	100 %			

d.f., Degrees of freedom; SS, sum of squared observations; MS, mean of squared observations; Est. var., estimated variance; % Var., percentage of total variance; PhiRT, proportion of the total genetic variance between regions; PhiPR, proportion of the total genetic variance among populations within a region; PhiPT, proportion of the total genetic variance among individuals within populations

Fig. 3 UPGMA dendrogram of 88 *Lentinula edodes* strains constructed using the genetic similarity coefficient based on InDel and SSR data. Strains collected from the four populations are marked in different symbols: ▲, localities in Hengduanshan Mountains; ■ in Yunnan Plateau; ★, in Northwest China; and ◆, in Central China

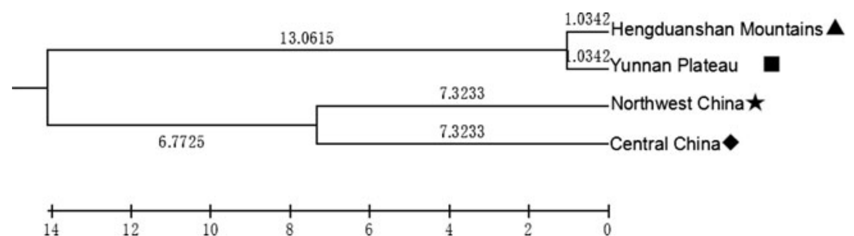

Fig. 4 UPGMA dendrogram based on Nei's genetic distances among four *L. edodes* populations

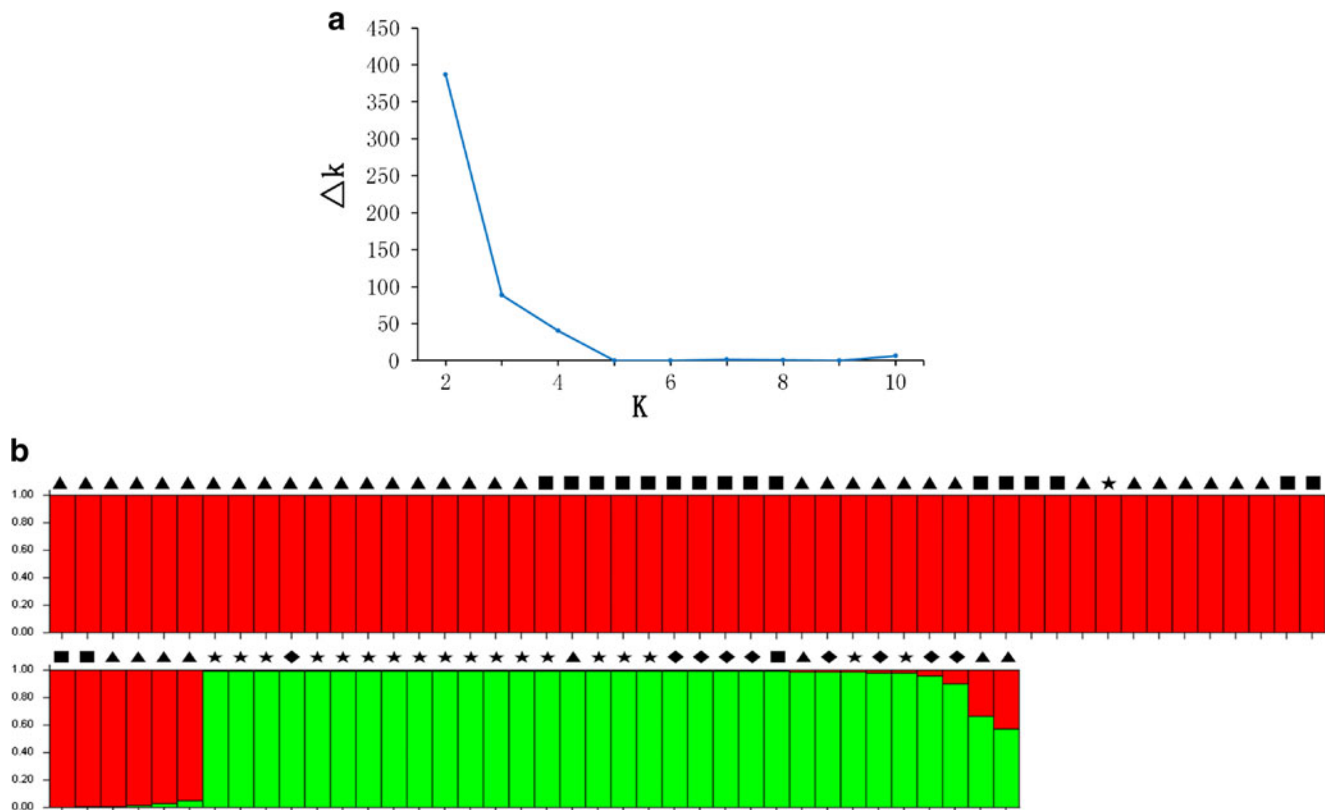

Fig. 5 Results of STRUCTURE analysis. **a** Estimation of the number of populations for K ranging from 1 to 10 by ΔK values. **b** Classification of 88 *Lentinula edodes* strains into two genetic groups. The distribution of the strains assigned to different groups is indicated by the color code (Group A: red, Group B: green). The y-axis quantifies cluster membership, and the x-axis lists the different strains. Strains collected from the four populations are marked in different symbols: ▲, localities in Hengduanshan Mountains; ■ in Yunnan Plateau; ★, in Northwest China; ◆, in Central China

Genetic diversity of *L. edodes* in China

To the best of our knowledge, this study is the first to employ InDel markers to disclose the genetic diversity and population structure of mushroom species. In previous studies, Chinese *L. edodes* was proven to have a high genetic diversity using RAPD, TRAP, and SSR markers as well as ITS sequences, especially for strains from Southwest China, including the Yunnan Plateau and Hengduanshan Mountains, and in Northwest China (Sun and Lin 2003; Xiao et al. 2010a, b; Xu et al. 2005). Results obtained here were congruent with those obtained previously. This, together with the fact that most of the *L. edodes* strains analyzed here were different than those of the previous studies, confirmed that Southwest and Northwest China harbor a high genetic diversity of *L. edodes*.

High levels of genetic diversity are generally expected for organisms that reproduce sexually, have broad ecological niches and a wide geographical distribution (Hamrick and Godt 1990; James et al. 1999). *L. edodes* obviously accords with these characteristics: (1) it possesses a typical sexual life history; (2) it grows on the fallen wood of several broadleaf species belonging to Fagaceae, Betulaceae, and Hamamelidaceae (Sun and Lin 2003); and (3) it is distributed in continental and northeast Asia (Hibbett et al. 1998).

Fourty-eight out of 70 loci (68.57 %) were significantly deviated from HWE, due to obvious heterozygote deficiency. This agrees with previous reports that some loci significantly deviated from HWE at the population level. Kim et al. (2009) used five SSR markers to assess the genetic diversity of 89 *L. edodes* strains from East Asia and reported that genotypes at four loci differed significantly from HWE. The genetic structures of nine populations were analyzed using four SSR loci in *Suillus luteus*, and those markers showed a significant departure from HWE in six populations (Muller et al. 2007). Significant deviation from HWE is always associated with heterozygote deficiencies in *P. eryngii* (F_{IS}=0.235) and *P. ferulae* (F_{IS}=0.262) (Urbanelli et al. 2003). The total F_{IS} value reported here was 0.331, higher than those in *P. eryngii* and *P. ferulae*. However, the inbreeding levels observed here were not high—inbreeding species usually have inbreeding coefficients ranging between 0.80 and 1.00 (Urbanelli et al. 2003). The relatively low F_{IS} values of the total 88 strains reflected a low autogamy rate of the Chinese *L. edodes* population. *L. edodes* possesses a tetrapolar incompatibility

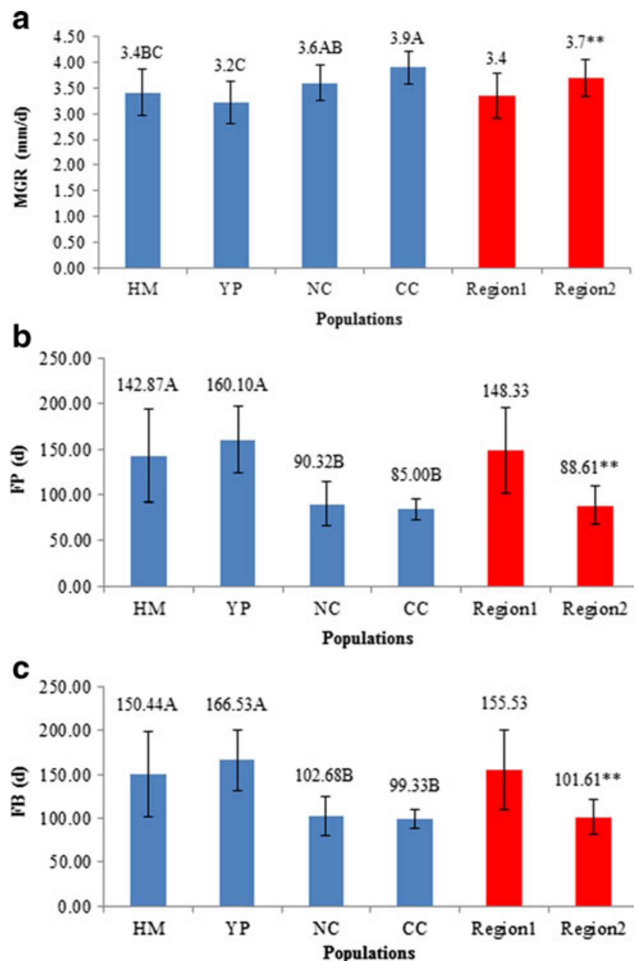

Fig. 6 (**a**) mycelium growth rate (MGR), (**b**) time interval from incubation to formation of the first primordium (FP), and (**c**) time interval from incubation to harvest of the first fruiting body (FB) of four geographic populations and two regional populations. Numbers above the column chart indicate means of (a) MGR, (b) FP, and (c) FB in different populations. Geographic populations are indicated in blue columns and regional populations are indicated in red columns. For the four geographic populations, significant differences at the 0.01 level (uppercase letters) were indicated by different letters using Duncan's multiple comparison. For the two regional populations, ** indicates significant difference at the 0.01 level. Error bars indicate standard deviations

system, which allows mating between basidiospores from the same parent, at a rate of 25 % (Li et al. 2007). As described in some other basidiomycetes, inbreeding could produce local heterozygote deficiencies (Douhan et al. 2011; Vincenot et al. 2012), thus causing the deviation from HWE of some loci.

Cryptic species are common in fungi (Carriconde et al. 2008; Cruse et al. 2002; Kauserud et al. 2006). Generally speaking, fungal intraspecific variability of ITS sequence is no more than 3 % (Nilsson et al. 2008). The variability of ITS sequences from representative strains of the two regions were less than 3 %, thus verifying that the tested strains belong to a single species rather than two cryptic species.

Population genetic structure and differentiation

The entire Chinese *L. edodes* population displayed a high genetic differentiation (F_{ST}=0.252), which is similar to those reported in several other fungal species. For the intercontinental populations of the model basidiomycete *Schizophyllum commune*, the global G_{ST} value was 0.214, indicating a significant differentiation (James et al. 1999). The Chinese *P. eryngii* var. *tuoliensis* population showed a G_{ST} value of 0.218 (Zhao et al. 2013). F_{ST} value in *Tuber melanosporum* strains from Europe was 0.714, suggesting strong genetic differentiation among populations (Sica et al. 2007). A *Russula brevipes* population from the USA also showed strong genetic differentiation, with a F_{ST} value of 0.434 (Bergemann and Miller 2002). The F_{ST} value here was far lower than those from reports that supported existence of cryptic species in other fungi, such as *Tricholoma scalpturatum* (Φ_{ST}=0.44) (Carriconde et al. 2008) and *Blastomyces dermatitidis* (F_{ST}=0.772) (Brown et al. 2013). The evidence of ITS sequence variability also confirmed that there were no cryptic species among the Chinese shiitake population. Despite abundant genetic differentiation between the *L. edodes* populations, most genetic variation was within populations. This is also the case in outcrossing and widespread species, whose genetic diversity mainly exists within populations (Hamrick et al. 1992). The relatively high genetic variation detected within populations is probably due to an efficient gene flow among inter-compatible strains within each population (Ravash et al. 2010). As there are significant genetic differentiations between geographical populations of Chinese *L. edodes*, each population needs to be managed and conserved separately.

We firstly inferred the population structure of *L. edodes* strains in China using the model-based method in the STRUCTURE software. The population structure revealed by STRUCTURE was highly consistent with that of UPGMA and PCoA analyses (Data not shown), strongly supporting that the Chinese *L. edodes* population has two well-differentiated genetic groups separated according to geographical regions. Such a geographically based pattern of genetic structure could result from the restricted dispersal among populations ascribed to geographic factors, which could allow time for genetic differentiations caused by mutation, drift, and selection. Sun and Lin (2003) also deemed that the disparity of natural geography and ecological environment between different regions is one of the factors impacting on the genetic divergence among *L. edodes* strains. The high genetic diversity of *L. edodes* might be caused by the long-term interaction between the varied ecological environments and the genetic systems of *L. edodes*. It is therefore inevitable for genetic variation to be closely correlated to the geographical distribution, and for strains from the close populations to develop less genetic differentiation.

Gene flow counteracts genetic differentiation between populations, and results in a homogeneous population structure. Higher levels of gene flow observed in Italian *P. eryngii* ($Nm = 3.50$) and *P. ferulae* ($Nm = 5.43$) populations caused a lower level of genetic diversity and a more homogeneous population (Urbanelli et al. 2003). A Swiss *Armillaria cepistipes* population, showing little but significant genetic differentiation suggested strong gene flow due to long-distance dispersal of airborne basidiospores. Gene flow seemed to be only partially restricted by the high mountain ranges of the Alps (Heinzelmann et al. 2012). The *Nm* values were grouped into high (>1.0), intermediate (0.250-0.99), and low (0.0-0.249) categories (Govindaraju 1988). The overall *Nm* value in the Chinese *L. edodes* population was 0.742, representing a moderate gene flow among populations. Aerial basidiospores are important in the dispersal of basidiomycetes, and their dispersal is a major factor in shaping the population genetic structure (James et al. 1999; James and Vilgalys 2001). Thus, the natural gene flow of *L. edodes* could be primarily mediated by the dispersal of airborne basidiospores. Gene flow mediated by basidiospore dispersal is sensitive to geographic distance, and increasing geographical distances further restricted the gene flow between populations (Liu et al. 2012a). Therefore, the gene flow between populations from geographically distant populations was lower than those between adjacent populations. Consistently, the genetic differentiations and genetic distances between populations were positively correlated with geographic distribution. In addition, gene flow was also restricted by geographical barriers, such as high mountains (Heinzelmann et al. 2012). Region 1 is located in a high-altitude area, and high mountains might act as a natural barrier to confine basidiospore dispersal, thus confining the gene flow between regions 1 and 2. But the restricted gene flow could be inadequate to offset the genetic differentiations among regions, and Chinese *L. edodes* population consequently formed two well-differentiated genetic groups in consistence with two geographic regions. Therefore, geographic distribution is a key factor in shaping the current genetic structure of *L. edodes* via the influence on the gene flow mediated by basidiospore dispersal.

MGR and precocity of *L. edodes* population in China

Genetic structure analysis uncovered two genetic groups in the Chinese *L. edodes* population that were consistent with the two geographic regions. Strains from the two regions had different phenotypic traits of MGR and precocity. Strains from region 2 grew faster and developed fruiting bodies earlier than those from region 1. Although the results were strictly derived from the laboratory condition, the phenotype is an expression of genotype. The phenotypic traits of the tested strains here, to some extent, could tell part of the story under natural condition. Environmental conditions play a crucial role in the fruiting body formation of basidiomycetes and the different phenotypic traits could result from adaptations to the local environmental conditions. Under the influence of natural selection, strains from region 2 evolved to grow faster and fructify earlier. *L. edodes* strains with their mycelia growing more rapidly could uptake enough nutrients to meet the demand of fruiting body formation. This could interpret the negative correlation between precocity and MGR in *L. edodes*.

As one of the most important edible mushrooms in the world, *L. edodes* plays a vital role in international mushroom markets. It is necessary to develop new shiitake varieties with good quality and high-yield to meet the multiple market demands. This study reported the relationship between genetic structure and the phenotypic traits in *L. edodes* for the first time, thus establishing the foundation for further utilization of strains from different regions. In one natural population and two testcross populations in *L. edodes*, precocity was proven to be significantly negatively correlated with yield (Gong et al. 2014b). Therefore, precocity could be used as an index for breeding improved strains with high yields. To breed faster-growing and earlier-fruiting varieties of *L. edodes*, strains from region 2 should be taken into preferential consideration.

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