An integrated study of individualism in *Lentinula edodes* in nature and its implication for cultivation strategy

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A field study was carried out in a remote broadleaved *Fagus longipetiolata* forest in Shaanxi province, China to study the natural local distribution of *Lentinula edodes*. Following spatial mapping, 24 fruit bodies were collected for tissue isolation into axenic culture. 24 genets distributed on fallen tree trunks within a distance of 120 m were identified and clustered into 7 groups using the unweighted pair-group method algorithm using data based on colony morphologies, abilities to degrade aromatic poly-R478 dye, somatic incompatibility reaction patterns and DNA fingerprints. Among the parameters used, the somatic incompatibility reaction, a polygenic phenotype, was the most differentiating, identifying 22 incompatible classes. Two sets of fruit bodies of different genets were so close together that they would otherwise have been described as aggregate fruits of presumed identical origin. Eighteen genets found on the same 5.6 m long tree trunk divided roughly into two clusters, matching their spatial distribution, and a nearby branch bore another distinct cluster. More heterogeneity was encountered between isolates the greater the distance separating them on the original site. Genets on the same tree trunk showed more compatible somatic reactions among themselves, and their DNA fingerprints showed higher similarity. Nevertheless, considering the totality of phenotypic characters, each fruit body is a genet in *L. edodes*. Such features are concluded to result from a reproductive strategy which depends on basidiospore dispersal. Within each cluster of isolates from the collection site genets seemed to have arisen from multiple sib-mating events. Thus, a cluster may represent a lineage of *L. edodes*. Individualism in *L. edodes* is based on a strong somatic incompatibility system. Strong competition from contaminating individuals arriving as air-borne basidiospores could explain decreased and fluctuating crop yields which are now frequently observed in later flushes from the outdoor wood log cultivation system. Further, it would also explain why multispore spawn is not favoured in artificial cultivation of this economically important edible mushroom.

Lentinula edodes (Berk.) Pegler (syn. *Lentinus edodes* (Berk.) Sing.), commonly known variously as the black oak mushroom, shiitake or shiang-gu, is a gilled mushroom with polyporaceous affinities (Pegler, 1983). It was first cultivated in China more than 800 years ago (Chang & Miles, 1987; Zhang & Lai, 1993) and today China accounts for about 70% of world production. This amounted to 91,500 metric tonnes of the dried crop, ten times that in fresh weight, in 1997 (van Nieuwenhuijzen, 1998; Wang, 1998). One-third of the Chinese production is exported, and the earnings make a significant contribution to the income of peasants in China (Yang, 1988; Chang & Chiu, 1992; Zhang & Lai, 1993). Shiang-gu is widely consumed in China and the neighbouring regions including Taiwan, Hong Kong, Japan and Korea with increasing worldwide popularity, being the third or fourth most popularly cultivated one in the world list (Chang, 1993; Royse, 1995). *L. edodes* is valued for its unique flavour, which derives mainly from its content of the modified amino acid lenthionine and the nucleotide guanine-5-monophosphate (Mizuno, 1995; Yang *et al*., 1998). The fruit bodies are rich in minerals, vitamins, essential amino acids (especially lysine and leucine), are high in fibre content but contain less than 10% crude fat (Ho, Hun & Yei, 1994; Mizuno, 1995). Besides this nutritionally valuable mushroom crop, a protein-bound polysaccharide (lentinan) has been extracted from *L. edodes* fruit bodies and found to have clinically-useful immunomodulatory, anti-cancer and anti-viral effects (Chihara, 1993; Mizuno, Sakai & Chihara, 1995; Anon., 1998).

Given the commercial importance of *L. edodes*, it is not surprising that tremendous efforts have been devoted to research on various aspects of its biology, especially cultivation, fruiting and breeding (e.g. Nakai, 1986; Tokimoto & Komatsu, 1995; Ishibashi, Yamazaki & Shishido, 1996; Ikegaya, 1997). In China, cultivation of *L. edodes* has spread from the coastline to central and from the south to the north, there now being over ten million part-time and full-time mushroom farmers (Zhang & Lai, 1993; Luo, 1998; Wang, 1998). Outdoor wood log cultivation was initiated in 1939 and is still the dominant method in small family farms scattered in country and mountainous areas. Decrease in crop yield for the later flushes are frequently encountered and usually ascribed to 'strain degeneration'. However, our results described here suggest that competition from contamination of cultivation sites by airborne spores may contribute to crop

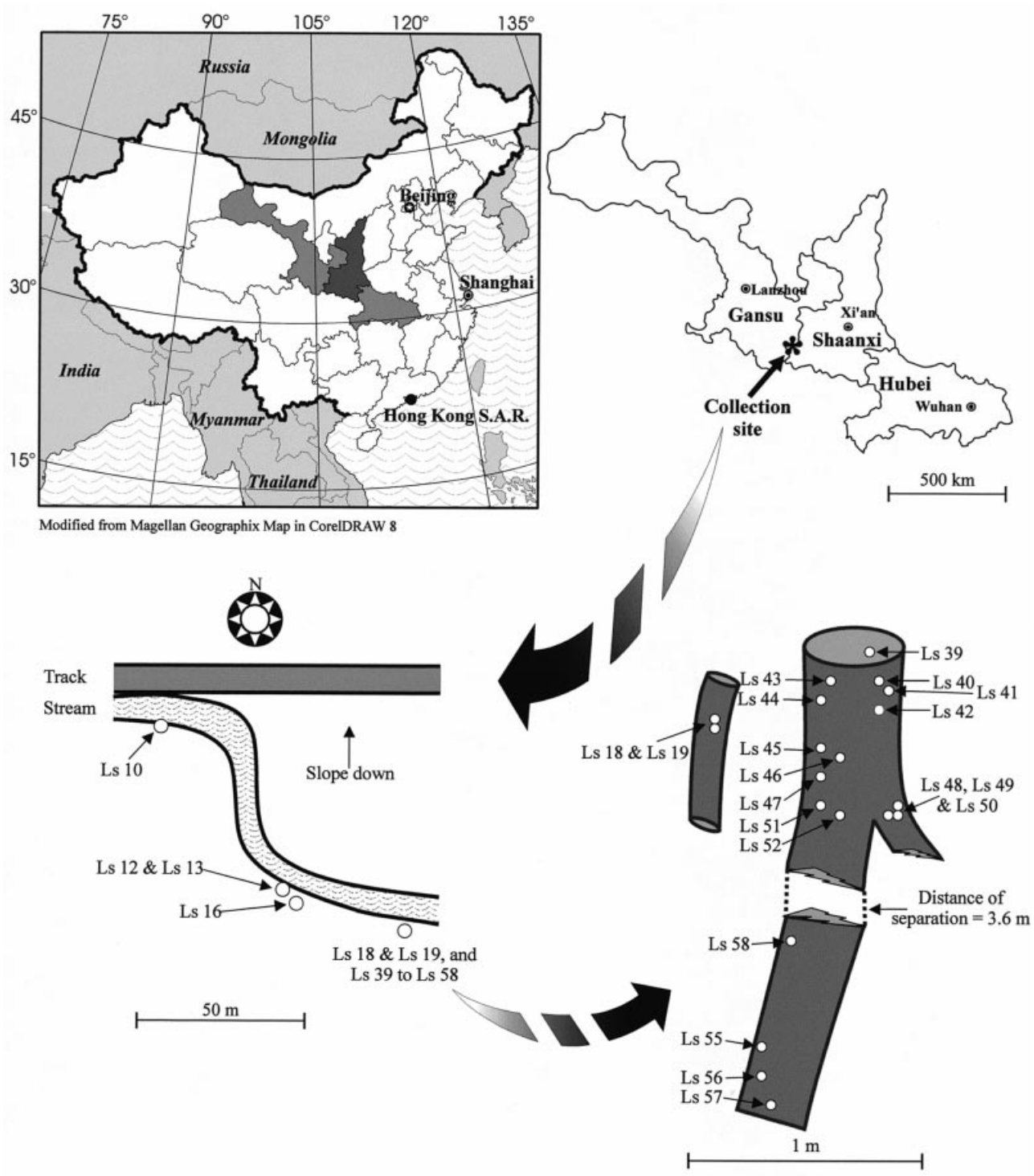

Fig. 1. Location of the wild *Lentinula edodes* strains collected in Shaanxi Province, China.

losses. Studies have been made of the distribution of *Lentinula* on a continental scale (Hibbett, Hansen & Donoghue, 1998), but we are ignorant of the spatial organization of *L. edodes* in the wild on a scale relevant to farming practice.

Strain improvement is a continuous exercise in mushroom farming to respond to consumer demand, expand the market, adapt to changing cultivation technology and counteract strain degeneration. Biodiversity in nature is a key element of the genetic resource for breeding programmes and, consequently, hunting for wild *L. edodes* has been practised for a long time (Mori, Fukai & Zennyoji, 1974; Ellor, 1992; Zhang & Lai, 1993; Pan *et al.*, 1998). Cultivated strains of *L. edodes* collected worldwide reveal genetic heterogeneity (Tokimoto, Komatsu & Takemaru, 1973; Mori *et al.*, 1974; Kwan *et al.*, 1992; Shimomura *et al.*, 1992; Chiu, Kwan & Cheng, 1993; Fox *et al.*, 1994). In contrast to the homogeneity observed in cultivated strains (Chiu *et al.*, 1996), heterogeneity has also been observed in collections of wild isolates made in China (Chiu *et al.*, 1996, 1998*a*, *b*; Hibbett *et al.*, 1998). Biodiversity has been assessed using a variety of genetic information,

usually studied separately. Only an integrated study can give a view of native population structure which is sufficient to direct the sampling strategy needed to collect future breeding strains. In this paper we report the first study of the spatial organization of *Lentinula edodes* in the wild using an approach which integrates studies of classical and molecular markers.

MATERIALS AND METHODS

Isolation of specimens

Fruit bodies of *Lentinula edodes* (Berk.) Pegler were surveyed and collected in a remote mountainous region at the border between Shaanxi and Gansu provinces in central China. The site was approx. 300 km WSW of Xi'an the capital city of Shaanxi Province (Fig. 1). The nearest local cultivation using the outdoor wood log system (with *L. edodes* strain HL7925, DNA fingerprints of which were reported in Chiu *et al*., 1996) was over 50 km away. The site was a broadleaved monotree forest of *Fagus longipetiolata* Seem. Fruit bodies were collected from fallen trunks along a stream at an altitude of 1450 m. The ambient temperature was 16–18 °C with 70 to 80% relative humidity. When a fruit body of *Lentinula edodes* was found, the fruit body size and morphology were measured and recorded. Then it was picked up, put in a paper bag and transported to a laboratory within seven days. The stipe or pileus was cut with a sterilized knife and split open by hand to expose the inner tissues. A segment of the inner tissue was picked up with a sterilized knife or needle, and inoculated onto a PDA medium. The culture was kept at 25 ° for 1 week in darkness. Newly-grown hyphae were transferred to fresh (PDA) medium as a pure culture. Fig. 1 shows the distribution of the isolates. Most of the isolates examined here were collected from a fallen tree trunk. The bark layer of this was intact which, together with the degree of decay of the fallen trunk, suggested that it had been *in situ* for about one to two years. Although fruit aggregates were encountered (Fig. 2), they were treated as putatively different individuals and assigned different strain numbers (i.e. strains Ls 18 and Ls 19, and Ls 48, Ls 49 and Ls 50 shown in Fig. 2).

Di-mon mating to confirm identity. If the identity of a culture was in question, a di-mon mating test was carried out using haploid testers recovered from a commercially cultivated *L. edodes* strain. Two inocula of the culture concerned and the tester were placed 3 mm apart on PDA. The plate was incubated at 25 °. After two to three weeks, sectors of new dikaryons were detected. Dikaryosis was confirmed by microscopic search for the presence of clamp connections.

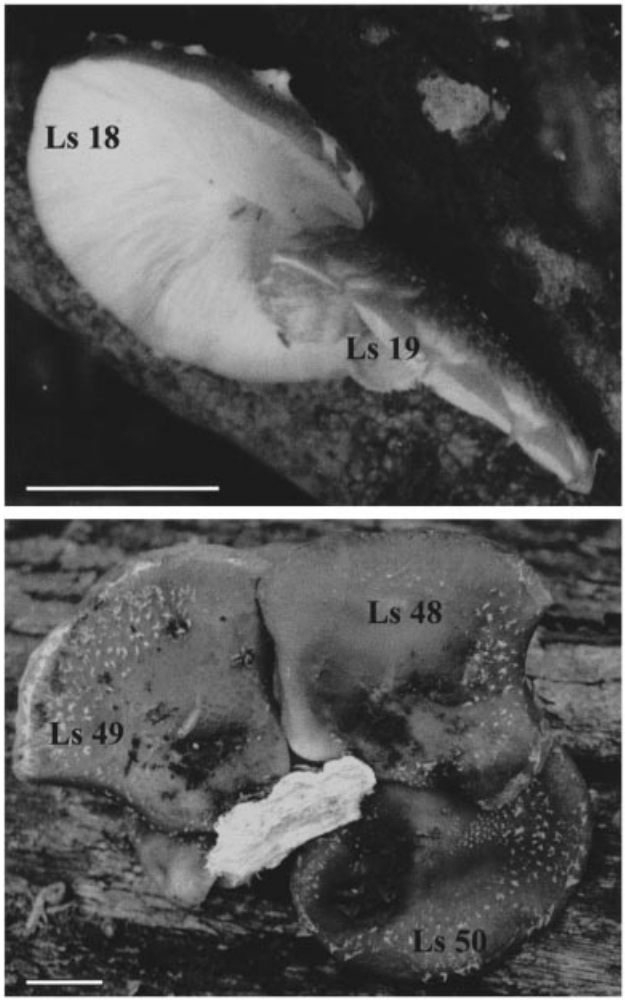

Fig. 2. Aggregates of fruit bodies of wild strains seen on fallen trunks of *Fagus longipetiolata* in Shaanxi Province, China. Scale bars = 2 cm.

DNA extraction

Mycelia were obtained from liquid PD broth cultures under shaking at 100 rpm at 25 ° for 3–6 weeks, depending upon the growth rate of the culture. Genomic DNA was extracted from mycelia frozen in liquid nitrogen, and was purified following a protocol of White *et al*. (1990) with the addition of RNAse treatment and caesium chloride further to purify the DNA (Yoon, Glawe & Shaw, 1991; Yoon & Glawe, 1993). The concentration and purity of a DNA sample were measured by the spectrophotometric absorbance and the ratio of $OD_{260}:OD_{280}$. A sample having a ratio greater than 1.8 was considered suitable for use, and its concentration and purity were further checked by agarose gel electrophoresis using ethidium bromide staining.

AP-PCR

Four arbitrary primers (M13FS, M13RS, *Eco* RI ext and GalK-54) were used (Welsh & McClelland, 1990; Williams *et al*., 1990; Chiu *et al*., 1993, 1998*b*). These sequences are as follows (5′ → 3′): primer *Eco*RI ext, TAGGCGTATCACGAGGCCCT; primer GalK-54, TACGGTCCGGGAGCGCAGCA; primer M13RS, AGCGGATAACAATTTCACACAGGA; and primer M13FS, CGCCAGGGTTTTCCCAGTCACGAC. The reaction mixture contained: 1 × PCR buffer II (Perkin Elmer Cetus), 3.5 mM $MgCl_2$, 200 M each of dNTPs (Perkin Elmer Cetus), 1 M of the primer and 2.5 U *Taq* DNA polymerase (Boehringer Mannheim) and 25 ng of genomic DNA. The thermal program was: 2 low stringency cycles of 94 ° for 2 min, 35 ° for 1 min and 72 ° for 2 min. This was followed by 39 cycles of high stringency: 94 ° for 1 min, 55 °

for 1 min and 72 ° for 2 min and with the last extension time lengthened to 10 min.

Experiments were repeated to verify consistency, and controls without DNA were amplified in parallel to check for contamination or carry-over. DNA fingerprints were resolved on NuSieve gel (FMC) together with 100 bp ladders as molecular size marker (Pharmacia or Bio-Rad; the Pharmacia product has a bright band of double intensity at 800 bp).

Somatic compatibility

Somatic compatibility refers to the ability of vegetative hyphae of the two isolates to intermix freely like hyphae from two inocula from the same colony. Tests were carried out by excising two inocula from the same isolate and inoculating them at the margin and centre of a PDA plate. Another isolate was inoculated similarly, with the central pair of inocula from the two cultures facing each other at a distance of 5 mm. Plates were scored after 3 to 6 weeks incubation at 25 °.

Colony morphology

An agar block of an isolate (5 mm diam.) was cut and transferred to a PDA plate and incubated at 25 ° for 1 month. Colony morphology and any browning of the culture were noted for each isolate.

Dye decolourisation test

Poly-R478 (Sigma P-1900), a lignin model compound, was added at 0.02% (w/w) to a basal medium (BM) which comprised (g l^{-1}): glucose (20); magnesium sulphate (0.5); potassium dihydrogen orthophosphate (0.46); dipotassium hydrogen orthophosphate (1); and agar (15)(Chiu *et al.*, 1998*b*). Ammonium sulphate was added to BM at 26 mM and 2.6 mM to create ammonium-rich and ammonium-deficient media respectively. BM without dye but containing 26 mM ammonium sulphate was used as a control. Inoculation and incubation followed the procedure described above. A decolorized zone appeared when the fungus degraded the dye. Observations were made over 48 days incubation. Three replicates were used for each strain.

Cluster analysis

The data obtained by the above tests (attributes including: colony morphology, DNA fingerprints, somatic incompatibility and dye decolourisation ability) were analysed by algorithms using the software package SPSS v. 8.0 (SPSS, 1998). The UPGMA (unweighted pair-group method algorithm) was used for cluster analysis on the calculated square-Euclidean distances and to construct a dendrogram. The square-Euclidean distance between any two fungi, d_{ij}, was computed using the formula:

$$d_{ij} = \sum_{k=1}^{p} (x_{ik} - x_{jk})^2$$

where i and j represent the ith and jth fungi under comparison, p represents the number of variables observed in all the attributes examined (in this data set, there were 52 variables), and k specifies a particular variable. The original data scale had a range from 0 to 9.545 and was rescaled to the arbitrary range 0 to 25 to draw the dendrogram shown in Fig. 7.

RESULTS

Table 1 summarizes the data obtained for each isolate examined.

Colony morphologies. The 24 strains were grouped according to their production of brown pigmentation during vegetative growth (category 1 shown in Table 1 refers to observation of browning, category 2 refers to white mycelium throughout the observation period), presence of aerial mycelium ('fluffy'), and spreading or aggregating colony morphologies (Table 1). Contrasting colony morphologies were observed with isolates Ls 44 and Ls 45 as well as Ls 55 and Ls 56 (Fig. 3).

DNA fingerprint groups. For the arbitrary primer *Eco*RI ext (Fig. 4), 6 patterns were observed (Table 1). For M13RS (Fig. 5, upper panel), 6 different patterns were observed (Table 1). Similarly, there were 4 patterns for primer GalK54 (Fig 5, lower panel) and 9 patterns for primer M13FS (Table 1). Strains Ls 18, Ls 39 to Ls 51 had essentially identical DNA fingerprints (Figs 4, 5 and Table 1) and DNA fingerprints of Ls 41, Ls 43, Ls 47, Ls 48, Ls 49 and Ls 51 were also identical. Finally, strains Ls 42, Ls 44, Ls 45, Ls 46 and Ls 50 formed a group with identical amplification profiles, and isolates Ls 57 and Ls 58 formed another group.

Somatic incompatibility groups. Somatic incompatibility was reflected as barrage formation, lysis of a confronting colony, formation of brown zone lines and abundance of brownish yellow exudate in the mycelia. Fig. 6 shows the results; there were 22 somatic incompatibility classes (Table 1). Strains Ls 44 and Ls 45 formed one pair of identical incompatibility pattern while strains Ls 55 and Ls 56 were another pair which reacted identically to all isolates. The rest of the isolates were distinguishable on the basis of their patterns of compatibility reactions (Fig 6).

The poly-R478 dye decolourisation abilities. In 48 days incubation, only strain Ls 48 failed to decolourise the dye at both ammonium levels (Table 1).

Assessment of genetic relatedness by sum of characters and cluster analysis. Table 2 shows the calculated squared Euclidean distance among the isolates, and Fig. 7 shows the dendrogram based on UPGMA average distance. The isolates separated into 7 clusters (membership shown in Table 1). Within the same tree trunk, strains Ls 39 to Ls 51 clustered together and so did strains Ls 55 to Ls 58 except strain Ls 52 (Table 1). Interestingly, isolate Ls 13 showed greater similarity to isolate Ls 16, although there was 10 m distance between the two tree trunks from which they were isolated, than to Ls

Table 1. Summary of variables observed in the wild strains of *Lentinula edodes* collected in Shaanxi Province, China

isolate	PCR fingerprint pattern*				Colony pigmentation	Colony morphology	Somatic compatibility group	Dye decolourisation	UPGMA Cluster Group
	M13FS	M13RS	GalK54	*Eco*RI					
Ls10	1	1	1	1	1	1	1	1	1
Ls12	2	2	2	2	1	2	2	1	2
Ls13	3	3	1	3	2	1	3	1	3
Ls16	4	3	1	3	2	3	4	1	3
Ls18	5	2	1	4	1	4	5	1	5
Ls19	6	2	1	5	1	2	6	1	4
Ls39	7	2	1	4	1	1	7	1	4
Ls40	8	4	1	4	2	1	8	1	4
Ls41	4	2	1	4	2	1	9	1	4
Ls42	9	2	1	4	1	1	10	1	4
Ls43	4	2	1	4	1	5	11	1	4
Ls44	9	2	1	4	1	1	12	1	4
Ls45	9	2	1	4	2	6	12	1	4
Ls46	9	2	1	4	1	7	13	1	4
Ls47	4	2	1	4	1	1	14	1	4
Ls48	4	2	1	4	1	4	15	2	4
Ls49	4	2	1	4	1	1	16	1	4
Ls50	9	2	1	4	1	1	17	1	4
Ls51	4	2	1	4	1	1	18	1	4
Ls52	9	3	3	4	1	8	19	1	6
Ls55	4	5	4	6	2	9	20	1	7
Ls56	4	6	4	6	1	2	20	1	7
Ls57	4	6	3	6	1	3	21	1	7
Ls58	4	6	3	6	2	1	22	1	7

*DNA fingerprint patterns as revealed by arbitrarily-primed polymerase chain reaction using four primers, *Eco*RI ext, GalK54, M13FS and M13RS. The cluster group dendrogram is shown as Fig. 7.

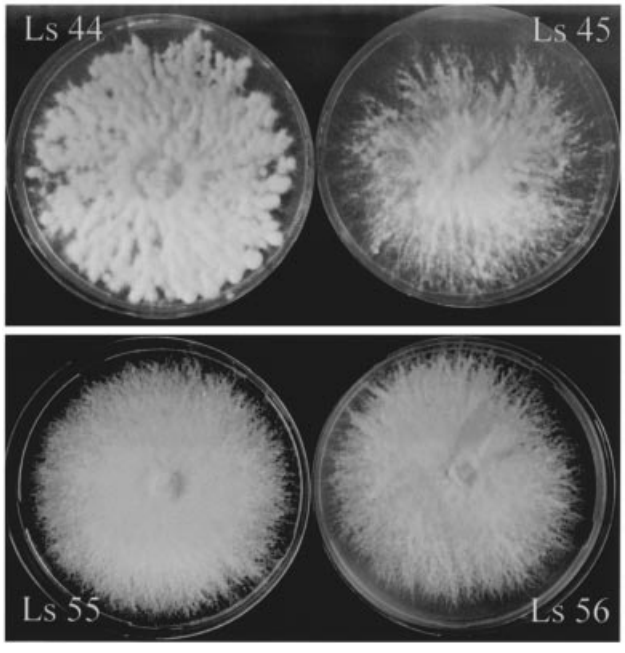

Fig. 3. Different colony morphologies of four of the isolates when grown on 9 cm Petri dishes.

12 which was isolated from the same tree trunk at a distance of 1 m. Also, isolate Ls 18 showed greater similarity to isolates from a tree trunk 40 cm away than to its close neighbour Ls 19 (Figs 1, 2 & 7). Fig. 7 shows that strains Ls 12 and Ls 13 were the most distinct. The two clusters which were most closely related comprised strains Ls 47, Ls 49 and Ls 51 on the one hand and Ls 42, Ls 44 and Ls 50 on the other (Table 1; Fig. 7).

DISCUSSION

From Table 1, 22 somatic incompatibility groups were observed among 24 isolates. Variation in culture behaviour in terms of colony morphology, growth rate, pigmentation and mycelial reactions, and/or DNA fingerprints was common among members of a group (Table 1). Thus, a somatic compatibility group is not equivalent to an individual (Guillaumin *et al*., 1996; Marçais, Martin & Delatour, 1998; Vasiliauskas & Stenlid, 1998). In this case, therefore, all 24 isolates studied represent different genets (= genetic individuals as understood by Anderson & Kohn, 1995; Ramsdale & Rayner, 1997) (Table 1). On this basis, a fruit body is a genet in *Lentinula edodes*. Among all the phenotypes assessed, somatic incompatibility pattern was the most discriminating (Table 1). Unfortunately, the genetic control of this reaction is least well understood in basidiomycetes (Ramsdale & Rayner, 1997; Worrall, 1997). The frequency of somatic incompatibility among full sib-synthesized heterokaryons ranged from 2 to 100% in *Pisolithus*, *Pleurotus* and *Heterobasidion* (Kope, 1992; Kay & Vilgalys, 1992; Hansen, Stenlid & Johansson, 1993; Worrall, 1997) and several multiallelic loci are involved in *Heterobasidion annosum* (Hansen *et al*., 1993). The mitochondrial genome appears to be involved in *Coprinus*

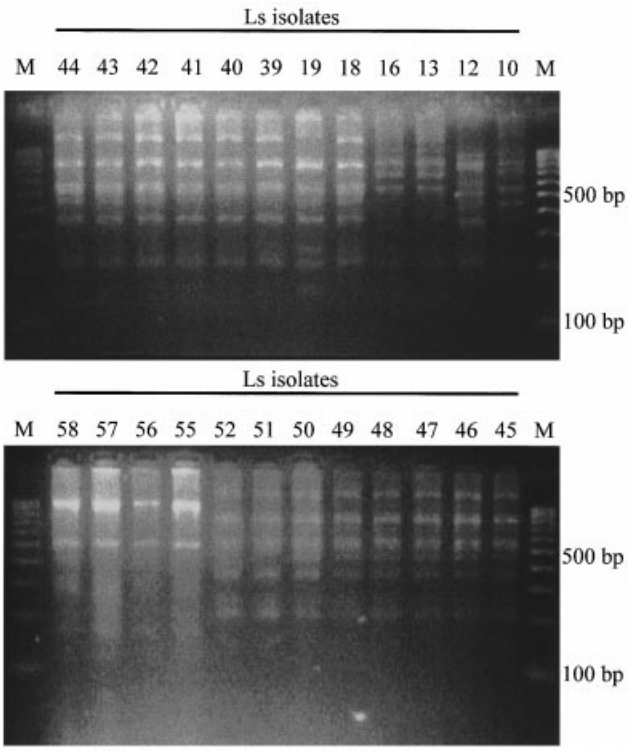

Fig. 4. Amplification profiles of the wild strains of *Lentinula edodes* collected in Shaanxi Province, China by arbitrarily-primed polymerase chain reaction using primer *Eco*RI ext. Lanes marked M were loaded with a 100 bp ladder.

cinereus (May, 1988). Apart from pointing out that the high frequency of somatic incompatibility amongst the isolates tested here indicates a polygenic control for this phenomenon in *L. edodes*, further comment must await detailed segregation analysis.

Neighbouring isolates mostly belonged to different somatic compatibility groups (Figs 1, 6) implying that the territory of *Lentinula edodes* individuals in the wild is very small. In the area studied here, the smallest fruiting territory of *L. edodes* genets proved to be less than 20 cm in diam., this being the maximum distance between adjacent isolates in the log which was sampled (Fig. 1 and Table 1). An important point is the physical scale involved here. The *maximum* distance across the entire sampling site was about 120 m and most of the isolates were recovered from a single log 5.6 m long. Two individuals of *Pleurotus ostreatus* were 1 m apart on the same log in the study of Kay & Vilgalys (1992) and territories of 1 to 200 m^2 have been demonstrated for root pathogenic or mycorrhizal fungi (Baar, Ozinga & Kuyper, 1994; Dahlberg & Stenlid, 1994; Anderson, Chambers & Cairney, 1998; Marçais *et al.*, 1998). For comparison, the largest territory known to be occupied by a single fungal clone is the 150,000 m^2 of the root-rotting honey fungus *Armillaria gallica* (= *bulbosa*)(Smith, Bruhn & Anderson, 1992). The evident small size of the *L. edodes* territory has some important consequences for the mushroom industry as it is currently practised in China.

First, conventional sampling methods used to collect material from the wild grossly underestimate the natural

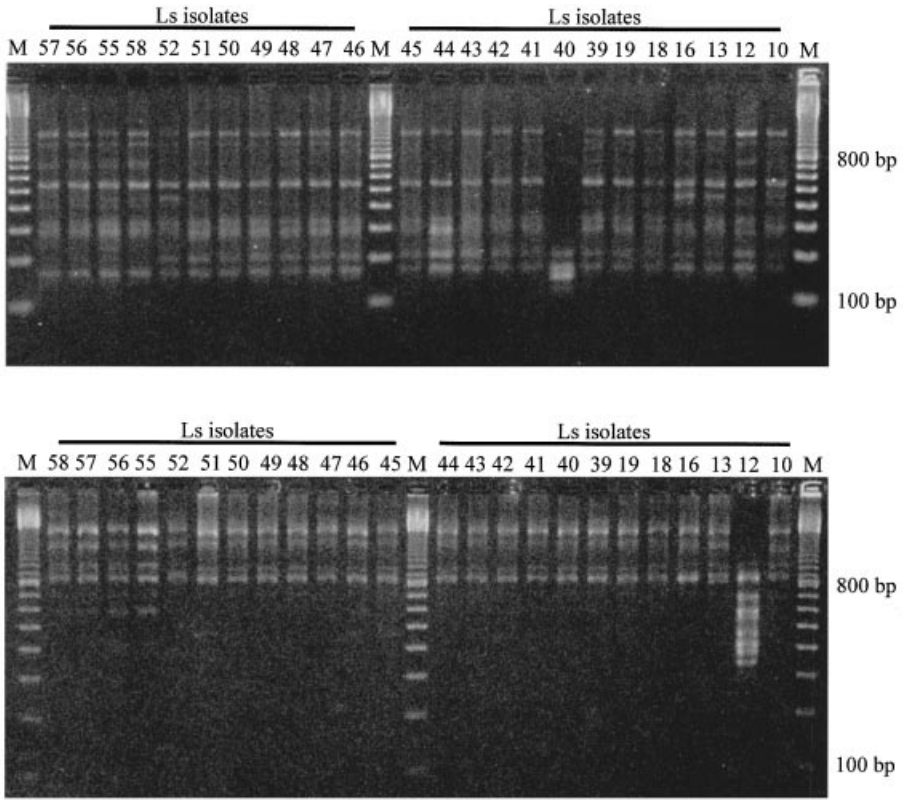

Fig. 5. Amplification profiles of the wild strains of *Lentinula edodes* collected in Shaanxi Province, China by arbitrarily-primed polymerase chain reaction. Upper panel: using primer M13RS; lower panel using primer GalK54. Lanes marked M were loaded with a 100 bp ladder.

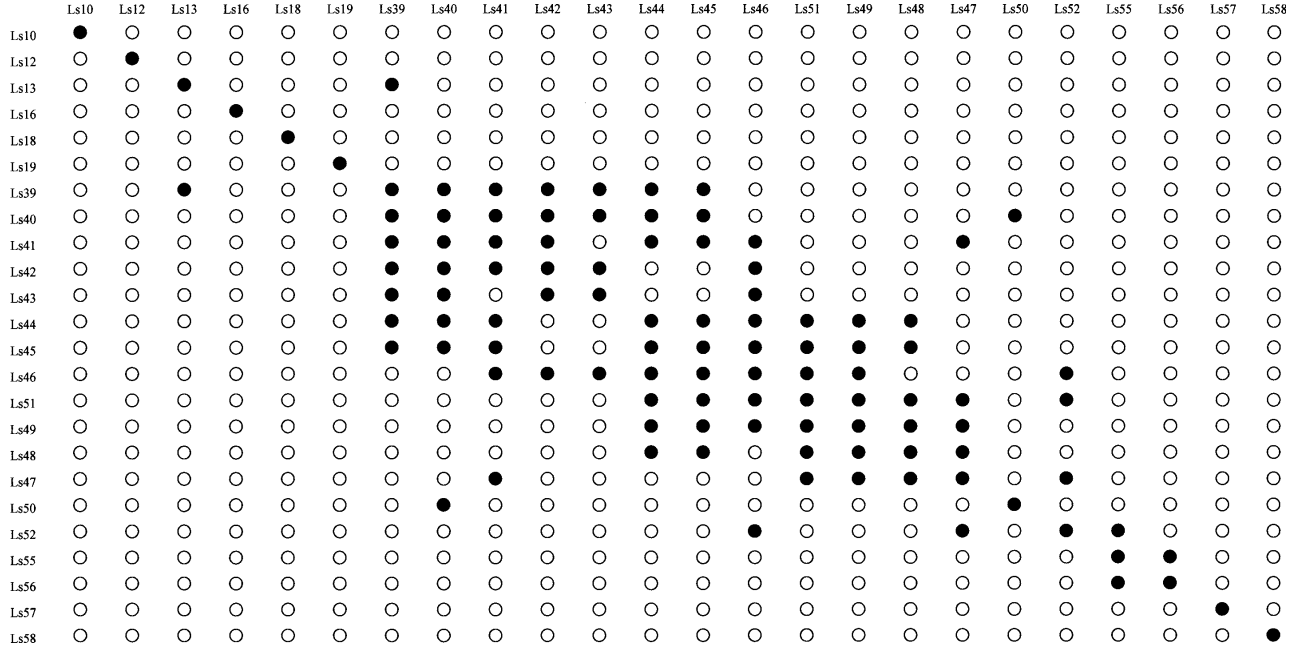

Fig. 6. Somatic compatibility matrix for the wild strains of *Lentinula edodes* collected in Shaanxi Province, China. ● = somatically compatible; ○ = somatically incompatible.

Table 2. Dissimilarity matrix calculated for the wild strains of *Lentinula edodes* collected in Shaanxi Province, China

Case	Ls19	Ls12	Ls16	Ls13	Ls10	Ls52	Ls58	Ls55	Ls57	Ls56	Ls40	Ls45	Ls18	Ls39	Ls43	Ls48	Ls46	Ls42	Ls50	Ls44	Ls41	Ls47	Ls49	Ls51
Ls19		8	11	11	10	11	10	10	10	7	11	8	8	8	8	9	7	7	7	7	9	8	8	
Ls12	8		13	13	12	11	10	10	10	7	13	10	10	10	10	11	9	9	9	9	11	10	10	10
Ls16	11	13		6	11	10	7	7	7	8	10	9	11	11	9	10	10	10	10	10	8	9	9	9
Ls13	11	13	6		9	10	7	9	11	10	8	9	11	9	11	12	10	8	8	8	8	9	9	9
Ls10	10	12	11	9		11	8	10	10	9	9	10	10	8	10	11	9	7	7	7	9	8	8	8
Ls52	11	11	10	10	11		7	9	7	8	10	9	9	9	9	10	8	8	8	8	10	9	9	9
Ls58	10	10	7	7	8	7		4	4	5	7	8	10	8	8	9	9	7	7	7	5	6	6	6
Ls55	10	10	7	9	10	9	4		6	3	9	8	10	10	8	9	9	9	9	9	7	8	8	8
Ls57	10	10	7	11	10	7	4	6		5	11	10	10	10	8	9	9	9	9	9	9	8	8	8
Ls56	7	7	8	10	9	8	5	3	5		10	9	9	9	7	8	8	8	8	8	8	7	7	7
Ls40	11	13	10	8	9	10	7	9	11	10		7	9	7	9	10	8	6	6	6	6	7	7	7
Ls45	8	10	9	9	10	9	8	8	10	9	7		6	6	6	7	5	5	5	3	5	6	6	6
Ls18	8	10	11	11	10	9	10	10	10	9	9	6		6	6	5	5	5	5	5	7	6	6	6
Ls39	8	10	11	9	8	9	8	10	10	9	7	6	6		6	7	5	3	3	3	5	4	4	4
Ls43	8	10	9	11	10	9	8	8	8	7	9	6	6	6		5	5	5	5	5	5	4	4	4
Ls48	9	11	10	12	11	10	9	9	9	8	10	7	5	7	5		6	6	6	6	6	5	5	5
Ls46	7	9	10	10	9	8	9	9	9	8	8	5	5	5	5	6		4	4	4	6	5	5	5
Ls42	7	9	10	8	7	8	7	9	9	8	6	5	5	3	5	6	4		2	2	4	3	3	3
Ls50	7	9	10	8	7	8	7	9	9	8	6	5	5	3	5	6	4	2		2	4	3	3	3
Ls44	7	9	10	8	7	8	7	9	9	8	6	3	5	3	5	6	4	2	2		4	3	3	3
Ls41	9	11	8	8	9	10	5	7	9	8	6	5	7	5	5	6	6	4	4	4		3	3	3
Ls47	8	10	9	9	8	9	6	8	8	7	7	6	6	4	4	5	5	3	3	3	3		2	2
Ls49	8	10	9	9	8	9	6	8	8	7	7	6	6	4	4	5	5	3	3	3	3	2		2
Ls51	8	10	9	9	8	9	6	8	8	7	7	6	6	4	4	5	5	3	3	3	3	2	2	

Dissimilarity is measured using squared Euclidean distance.

genetic diversity. The reason is that conventional wisdom envisages the territory as perhaps encompassing whole logs. The strategy most often used, therefore, is to make isolations and/or collect fruit bodies from different stumps or fallen logs to avoid harvesting closely-related (or identical) individuals. There may be some danger of this, but the more significant result of the observations we report here is that very different individuals can be borne on the same log (e.g. strains Ls 12 and Ls 13) (Figs 1, 7). Therefore, by deliberately avoiding close fruit bodies, the conventional sampling strategy underestimates the extent of genetic diversity harboured in nature.

Second, being distributed over a large number of small territories implies dependence on dispersal by small propagules in *Lentinula edodes*. No asexual spore stage is known, nor are there any reports of soil-colonizing vegetative structures

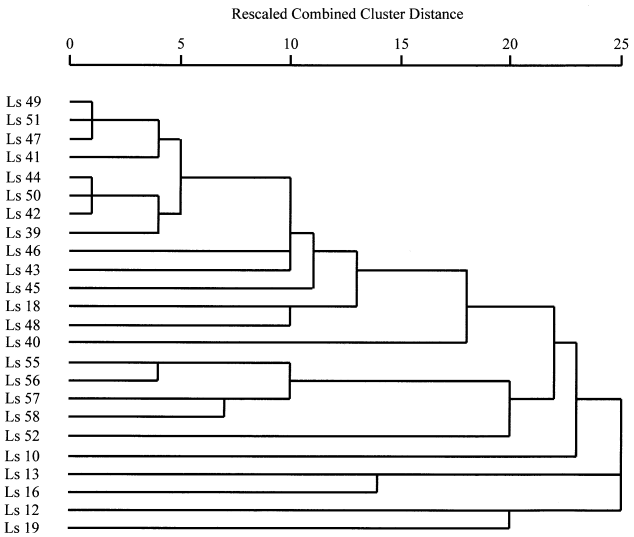

Fig. 7. Estimates of genetic distances between wild strains of *Lentinula edodes* collected in Shaanxi Province, China. Shown as a dendrogram constructed using UPGMA analysis of squared Euclidean distances. The original data scale had a range from 0 to 9.545 and was rescaled to the arbitrary range 0 to 25 for convenience in drawing this dendrogram.

(mycelial cords, rhizomorphs, etc.) (Nakai, 1986; Chiu, unpublished observation). Thus, basidiospore dispersal is the most likely primary mechanism for dispersal (and subsequent dikaryon establishment) in *L. edodes*, as is the case with *P. ostreatus* (Kay & Vilgalys, 1992). Lu (1997) reported that *L. edodes* fruits ranging in size from 11 to 13 cm diam. on a wood log released spores for 61 days at a daily temperature of 3 to 5 ° and 74 to 78% relative humidity. Although one report claims low (0.07 to 10%) spore germination frequency (Fox *et al.*, 1994), in our experience spores of *L. edodes* germinated readily; usually within 24 h from fresh spore prints, and germination frequencies could reach 80 to 100% at 25 ° (Nakai, 1986; Chiu, unpublished observation). Since *L. edodes* has multiallelic A and B mating type factors (Tokimoto *et al.*, 1973; Fox *et al.*, 1994; Chiu *et al.*, 1998*a*) outbreeding is favoured. The distribution and small size of *L. edodes* genets on the fallen trunk studied here suggests that many individuals were formed by sexually compatible mating events among basidiospore germlings which recently colonized the log (Dahlberg & Stenlid, 1990; Ramsdale & Rayner, 1997). If this happens in nature, it will also happen in mushroom farms where basidiomes start spore dispersal before being harvested.

The multispore spawn system is used widely for *Lentinula* (and, for that matter, for *Agaricus*) cultivation. We doubt its value. The approach aims to select for the strongest and fittest clone to propagate in a suitable substrate for mass production of fruit bodies based on the expectation of competition in mating, hybrid vigour and consequent selective advantage. It may be hoped that the multispore spawn system selects for the strongest and fittest clone, but small territories and many genets in nature seems to imply very equal competition between the genets with no single genotype having enough advantage over the others to become dominant. The key to this is SOMATIC incompatibility. As more and more spores alight on the substratum the territories may get smaller and smaller and may decline to the point where each has too little substratum to support fruiting. Death of incompatible dikaryons and antagonism as reflected by brown zone lines and pseudosclerotial plates on artificially inoculated wood logs using mixed strains have been reported (Yang, 1988). In synthetic media, frequent somatic incompatibility and strong antagonistic reactions have been encountered among sib-sib dikaryons. Such colonies remained unfused, lysed or persisted with rich secretion of brown exudates (S. W. Chiu, unpublished observations). When somatic incompatibility tests were carried out *in vitro* with a pair of isolates showing compatible or weakly antagonistic reactions, confronted cultures produced fruits more readily, perhaps as a result of the more readily available nutrients from co-operative digestion of substrates or induction by the stress on the fungus to initiate the multicellular differentiation pathway (Moore, 1998*a*, *b*). Co-operation of different genets to form a single fruit body is only possible if they belong to the same somatic compatibility group (so that their hyphae can intermingle) which our analysis shows to be unlikely. There must be a limit to any such enhancement of fruiting, however, and we consider that adverse competition by immigrant spores may explain the decreased crop yield in later flushes in mushroom farms rather than strain degeneration in the strict sense; i.e. crop degeneration is not equivalent to strain degeneration.

In China, most mushroom farmers are in mountainous regions and use outdoor cultivation. As stated earlier, strain degeneration expressed as decreased crop yield in later flushes is now frequently reported (Professor Y. J. Pan, Director of the Shanghai Institute of Edible Fungi, personal communication). Recent isolations from different 3 year old inoculated wood logs from a mushroom farm in Hubei Province recovered different genets instead of the single inoculated strain which was expected (S. W. Chiu & M. L. Yip, unpublished results). Such a situation must result from contaminating spores from the open air falling onto the cultivation logs and then successfully competing with the established spawn. Importantly, crop quality and quantity cannot be guaranteed in such a situation. Contamination of the cultivated strain must be prevented. Indeed, it is also wise to prevent the unwanted reverse flow of genetic information through escape of the cultivar to contaminate and consequently endanger the germ-plasm resource in the wild. It is essential that more emphasis and encouragement be placed on indoor cultivation and development of breeding strains which can fruit within cultivation containers. A further advantage is that the indoor plastic bag cultivation method can use industrial and agricultural waste and thereby help relieve the serious problem of deforestation in China. For all these reasons action should be prompt.

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