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Threats to biodiversity caused by traditional mushroom cultivation technology in China

SIU WAI CHIU & DAVID MOORE

Introduction

China is the world's major mushroom producing country. *Agaricus* production is mainly for export but *Lentinula edodes* (shiitake or shiang-gu) is the traditional local product, and now the major crop. *Lentinula edodes* is indigenous to China. It was first cultivated there more than 800 years ago, and today, China accounts for about 70% of world production. In 1997, Chinese production was recorded as 91 500 metric tonnes of the dried crop (drying produces the characteristic taste of the mushroom), ten times that in fresh weight. Shiang-gu (the Chinese name) is presently about the second or third most popular cultivated mushroom in the world, being consumed throughout China, Taiwan, Japan and Korea, and with increasing world-wide popularity. One-third of the Chinese crop is exported. As this amounts to the equivalent of about 300 000 tonnes of fresh mushrooms, the industry is an important earner of foreign exchange as well as making a very significant contribution to the income of peasant-farmers especially of the mountainous regions in China (Chang & Chiu, 1992). In these regions the land is poor in fertility and too distant from reliable transport to make conventional farming of green crops profitable.

Traditional technology

The traditional log-pile cultivation method is still the one that is most frequently used. For this, locally felled logs (oak, chestnut, hornbeam, maple and other trees) over 10 cm diameter (probably about 20 to 30 years old) and 1.5 m to 2 m long are normally cut in spring or autumn of each year. Felling at this time minimises pre-infestation by wild fungi or insects. Holes drilled in the logs (or saw- or axe-cuts) are packed with spawn, and the spawn-filled holes are then sealed with wax or other sealant to protect the spawn from the weather. The logs are stacked in laying yards on the open hillside, or under shelters built of bamboo or other materials, in arrangements which permit good air circulation and easy drainage and temperatures between 24 °C and 28 °C. The logs remain here for five to eight months for the fungus to grow completely through the log before transfer to the raising yard to promote fruit body formation. This is usually done in winter to ensure the lower temperature (12–20 °C) and increased moisture that are required for fruit body initiation. The first crops of mushrooms appear in the first spring after being moved to the raising yard. Each log will produce 0.5 kg to 3 kg of mushrooms, each spring and autumn, for 5 to 7 years. We now have enough information for a 'back of an envelope calculation': if each log produces 5 kg mushrooms per year; total annual production today is probably in excess of one million metric tonnes so we need the equivalent of 200 000 2-m logs. This amounts to between 50 000 and 100 000 trees every year just to maintain current production levels. Traditional usage of natural wood logs has been pursued to the extent that as availability of mature trees has declined attention has turned to younger trees and other tree species. This, combined with other demands for timber and land, has contributed to a loss of 87% of the native forests in China (Anon., 1997). China now faces the problem that the rate of deforestation is much greater than the rate of reforestation in the remaining 13% forest cover (Mackinnon *et al.*, 1996; Loh *et al.*, 1999)! There are regulations about planting and prohibitions on felling young trees, but these are difficult to monitor and 'conservation awareness' is especially low among those poor peasants living in the remote mountainous regions.

Conservation issues

The conservation pressures of *Lentinula* production result from the scale of the industry and from the biological consequences of traditional practices. There has been a minimum four-fold increase in production of *Lentinula* over the past twenty years or so, and every prospect that demand for the crop will continue to increase. The conservation pressure the industry exerts is more likely to increase than to ease. The scale is difficult for Europeans to imagine. The crop is still grown mostly by open outdoor cultivation on a very large number of small farms. One well-informed commentator (Luo, 1998) has claimed that there are *10 million mushroom farmers* in China! These mushroom farms are distributed over the whole of the central highlands in China. This means that the *Lentinula* growing region covers *an area about equal to the entire land area of the European Union*.

The main conservation issues raised by the traditional cultivation practices are:

- the obvious impact on wooded hillsides caused by felling of mature trees (of which only the largest logs are used);
- the fact that outside cultivation is used *and the mushroom crop is harvested after basidiospore release has started* with the consequent (unknown) danger(s) resulting from cross contamination between cultivated and natural populations of *Lentinula edodes*.

Population biology

Over the past several years we have investigated the cultivation physiology of *Lentinula edodes* with a view to providing a good scientific basis for using alternative cultivation substrates (Tan & Moore, 1992, 1995), but we have put greater efforts into studying the population biology of *Lentinula edodes* by examining both the cultivated (Chiu *et al*., 1996) and natural populations (Chiu *et al*., 1998*a*,*b*, 1999*a*,*b*). The research covers a geographical area which is around 1700 km north to south and 700 km west to east (Fig. 9.1), but includes detailed surveys down to individual logs, and deals with phenotypes varying from morphology and palatability to DNA sequences.

Nineteen strains of *Lentinula edodes* which are used for spawn production for farms throughout mainland China were characterised with three arbitrarily-primed polymerase chain reaction (AP-PCR) profiles, seven random amplified polymorphic DNA markers (RAPD) profiles and five restriction patterns (restriction fragment length polymorphism patterns of the PCR-amplified ribosomal DNAs; rDNA-RFLPs). For AP-PCR, 4–14 DNA bands were amplified for a particular strain while 1–9 DNA bands were amplified using RAPD. Among them only three of the strains tested showed different amplification profiles with most of the primers used. The others showed small differences in three or fewer DNA amplification profiles. All strains showed identical rDNA-RFLPs.

This study indicates that cultivated strains of shiang-gu in China are genetically very homogeneous. In part this probably results from the concentrated artificial breeding work that has been done using a limited range of imported Japanese cultivars, originating in a series of breeding experiments with wild isolates collected from China, Taiwan and New Guinea (Mori, Fukai & Zennyoji, 1974). However, genetic homogeneity is also evident in cultivated strains of *Agaricus bisporus* (Loftus, Moore & Elliott, 1988) and *Volvariella volvacea* (Chiu, Chen & Chang, 1995). Loftus *et al.* (1988) could find no RFLP polymorphisms between three commercial cultivars of *Agaricus bisporus.* They noted that the growth conditions and flushing (times of fruiting) patterns for each of the three strains were also very similar, despite the fact that the three strains were marketed by different companies as original and independent products. These authors commented on how remarkable was ' . . . the coincidence of their genetic similarity . . . '. Since the same remarkable genetic similarity between allegedly different commercial cultivars has been encountered in *Volvariella volvacea* (Chiu *et al.*, 1995) and now in *Lentinula edodes*, it is entirely feasible that genetic homogeneity in cultivated mushrooms does not result from any peculiarity of mushroom genetics but most probably from behaviour patterns of mushroom growers around the world.

Although a very narrow gene pool is used in cultivated strains, our survey of diversity of rDNA sequences indicates that China harbours the greatest germplasm resource of the mushroom *Lentinula edodes* (Chiu *et al.*, 2000). The internal transcribed spacer (ITS) regions of rDNA in *Lentinula edodes* are rather conserved and therefore can be used to trace lineage relationships. Hibbett *et al.* (1995) and Hibbett, Hansen & Donoghue (1998) have identified five lineages in Asia–Australasia with two lineages appearing in China. As the sample size of all previous studies is rather small, we carried out a large-scale and more detailed screening in China, focusing attention on three provinces that are not among those in which traditional cultivation is popular (to avoid the danger of contamination with the 'commercial gene pool'). These are central Hubei, north central Shaanxi and the most Southwest Yunnan (Fig. 9.1). Several fruit bodies from single fallen logs and fruit bodies from different logs were collected and used for culture isolation. Biomass grown in pure culture was used for small scale DNA 'mini-preparations' (Chiu *et al.*, 1996), followed

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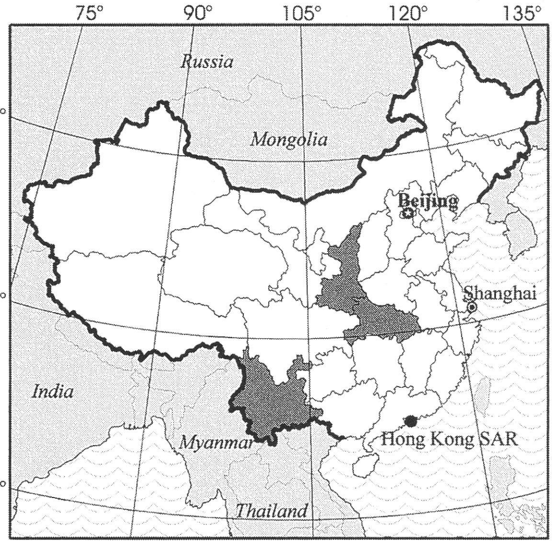
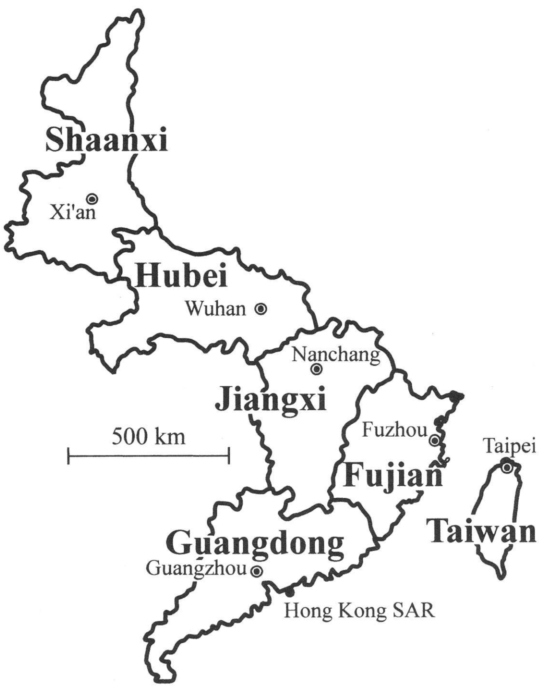
Fig. 9.1. (*opposite*) The experimental area. The three provinces from which field isolates were collected (Shaanxi, Hubei and Yunnan, the latter sharing borders with Myanmar, Laos and Vietnam) are shown shaded in the upper map. In the larger scale map in the lower part of the figure the location of traditional shiang-gu cultivation provinces, Guangdong, Fujian and Jiangxi (and provincial capital cities), is indicated. Commercial cultivars were collected from institutes/universities in Beijing, Hubei, Shanghai, Guangdong, Fujian and Hong Kong.



Modified from Magellan Geographix Map in CorelDRAW 8



by specific polymerase chain reaction using a fungal-specific primer set (ITS4 and ITS5). Direct sequencing was performed with the purified PCR-amplified rDNA fragments.

The results reveal that a dominant group I rDNA lineage appears in these three provinces. Compared with the rest, the Group I lineage has the widest geographical distribution, extending from North Korea and Japan to southern China and Thailand. This could indicate an ancient history for this lineage but as many Japanese and Chinese cultivars belong to the Group I lineage it is more probably the effect of breeding work and subsequent adoption of those cultivars for commercial cultivation in these regions. Similarly, wild isolates found to show group I rDNA sequences that were collected in the traditional shiang-gu growing areas of Fujian and Guangdong provinces may indicate cross contamination of the wild population with cultivar genes or even escape of the cultivar to the wild. The collection made by the authors in remote areas such as reserve areas of national status or mountainous areas far beyond human access could answer this problem. In the three provinces Hubei, Shaanxi and Yunnan, group I rDNA lineage sequences were found in the wild isolates. Thus, group I rDNA lineage occupies the largest territory and China might be the home for this lineage. Isolates of the group IV rDNA lineage previously reported in Nepal and Hubei were found in Shaanxi province instead. However, novel rDNA sequences (not belonging to either of the five lineages previously described) were found in the three provinces. Thus, several additional new rDNA lineages are present in China. Besides, as isolates of different rDNA lineages appeared in one single log, it is evident that genetic recombination is possible among the different lineage populations existing in the remote mountainous areas of China.

To investigate small-scale population structure, a field study was carried out in a remote broad-leaved *Fagus longipetiolata* forest in Shaanxi province. Following spatial mapping, 24 fruit bodies were collected for tissue isolation into axenic culture. Twenty-four 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 that 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. This also applies to *Agaricus* and *Pleurotus* mushrooms which, together with *Lentinula* form the triumvirate of the most popular cultivated species in the world, with China being the main producer and exporter.

Improved technology

The traditional approach to shiang-gu production is demanding and exploitative in its use of both land and trees. For these reasons more industrial approaches are being applied to shiang-gu growing. Hardwood chips, sawdust and other solid agricultural wastes packed into polythene bags as 'artificial logs' provide a highly productive alternative to the traditional technique, and the cultivation can be done in houses (which may only be plastic-covered enclosures) in which climate control allows year-round production. The industry raises other conservation issues that our recent analyses of molecular and conventional genetic markers have addressed. A very limited gene pool is exploited in the cultivated strains in China, yet there is an enormous biodiversity in the species in the wild. Analysis of local populations reveals that *L. edodes* strains show strong somatic incompatibility reactions and individual territories can be small (a few hundred mm). The widespread nature of the species and absence of other means of dispersal indicate that basidiospores are the major, even only, method of natural distribution. As the crop is harvested *after* initiation of basidiospore release and the crop is mostly grown outdoors, cross contamination between wild and cultivated strains is inevitable. This puts the natural gene pool under threat. Protection of the natural environment is still the best strategy for conserving the biodiversity of this important commercial resource. Collection of wild strains for preservation in a culture collection would directly conserve the wild germplasm. Making such a gene bank readily accessible to the public and industry would also generate a commercial resource for exploitation in both cultivation and breeding programmes. It may also reduce the pressure caused by non-professional collection from nature.

Our studies suggest that a move to indoor cultivation, less dependence on multispore spawns and exploitation of a wider range of natural genotypes would better safeguard both cultivated and natural populations of the fungus and avoid denuding hillsides of mature trees. The concept of sustainable management is too novel for the mushroom farmers of a third world country. But villages in China are accustomed to working co-operatively to establish a shared facility, and the advantages of indoor cultivation to the farmer (consistency of yield, much shorter production cycle, use of solid industrial/agricultural wastes in the substrate) can be readily appreciated. An essential step is to educate the public to introduce these ideas. Education is the key.

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