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Implications of Phylogenetic Studies for Conservation of Genetic Diversity in Shiitake Mushrooms

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Abstract: *We consider the impact of recent molecular phylogenetic analyses on conservation issues for shiitake mushrooms. Based on mating compatibility, shiitake has been considered a single species, *Lentinula edodes* sensu lato. Phylogenetic analyses of ribosomal RNA internal transcribed spacers (Hibbett et al. 1995), however, suggest that shiitake is composed of four distinct lineages. Thus, under a phylogenetic species concept, four species of shiitake should be recognized. A classification of shiitake based on a phylogenetic species concept would convey information about evolutionary relationships and distribution of genetic variation, whereas a classification based on a biological species concept would lack such information. This is a clear case in which new insights into phylogenetic relationships can be used to target areas for conservation action and formulate international agricultural policies. At the heart of our argument is the belief that the basic units of both taxonomy and conservation should be unique evolutionary lineages of organisms identified through phylogenetic studies.*

Implicaciones de estudios filogenéticos en la conservación de la diversidad genética de hongos shiitake

Resumen: *En este trabajo consideramos el impacto de análisis filogenéticos moleculares recientes en la conservación de hongos shiitake. Con base en la compatibilidad reproductiva el hongo es considerado como una sola especie, *Lentinula edodes* sensu lato. Sin embargo, el análisis de espaciadores transcritos internos del ARN ribosomal sugiere que el shiitake está compuesto por cuatro linajes distintos. Así, de acuerdo con el concepto filogenético de especie, se deben reconocer cuatro especies distintas de shiitake. Una clasificación de shiitake basada en el concepto filogenético de especie proporcionaría información acerca de las relaciones filogenéticas y la distribución de la variación genética, mientras que una clasificación basada en el concepto biológico de especie carecería de tal información. Este es un claro ejemplo de como se pueden utilizar nuevas percepciones de las relaciones filogenéticas para detectar áreas en las cuales desarrollar acciones de conservación, así como formular políticas agrícolas internacionales. En el centro de nuestra argumentación yace la creencia de que las unidades básicas de la taxonomía y de la conservación deben ser los linajes evolutivos identificados por medio de estudios filogenéticos.*

Cultivation and Taxonomy of Shiitake

Shiitake mushrooms are wood-decaying basidiomycetes that are commonly used in northeast Asian cooking. In China a primitive form of shiitake cultivation was in practice perhaps as long as 1000 years ago (Chang &

Miles 1987; Ito 1978). Modern shiitake cultivation has been made possible by advances in the understanding of basidiomycete life cycles and improvements in culturing techniques (Przybylowicz & Donoghue 1988). In contemporary shiitake farming "spawn" is produced by propagating vegetative fungal mycelium on sawdust-based media at centralized facilities that are equipped with large-scale autoclaves, microbiological culturing facilities, and other modern laboratory equipment. The spawn is then used to inoculate specially harvested logs or sawdust-based fruiting media in plastic bags. For mushroom production inoculated logs or bags are typi-

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cally placed in outdoor laying yards, such as in woodlots or bamboo groves (Fig. 1), or under thatch structures. In some cases fruiting takes place inside environmentally controlled mushroom houses.

With the advances in mushroom-growing technology and the increasing sophistication of the international palate, shiitake cultivation has spread far beyond its traditional areas in northeast Asia. Worldwide production of shiitake in 1991 was estimated at 526×10^3 metric tons, making it the third largest mushroom crop, behind the cultivated button mushroom, (*Agaricus bisporus*) (1590×10^3 metric tons) and the oyster mushroom, (*Pleurotus ostreatus sensu lato*) (917×10^3 metric tons) (Chang 1994). The 1991 figure for shiitake represents a 64.4% increase in production since 1986 (Chang 1994). Thus, shiitake farming is a rapidly expanding, increasingly global industry.

The geographic distribution of shiitake in nature extends beyond northeast Asia, but the exact limits are uncertain. The minimum range, based on reliable, well-documented reports, extends from northern Japan as far south and east as Tasmania and New Zealand and as far west as the Himalayan regions of Bhutan, Nepal, and India (Hongo in Kobayasi et al. 1973; Komatsu & Kimura 1968; Mori et al. 1974; Pegler 1983; Shimomura et al. 1992 and references therein). Determining the western limit of the distribution has been particularly problematic. Samgina (1981) reported that shiitake occurs in Kazakhstan, where the mushrooms were said to have been growing on conifer wood. However, shiitake is typically known only to occur on logs of broadleaf trees, especially Fagaceae (Tokimoto & Komatsu 1978; Przybylowicz & Donoghue 1988), which suggests the fungi in Kazakhstan may have been misidentified. The distribution of shiitake in the Pacific islands north and east of Papua New Guinea, such as New Caledonia, is also poorly documented.

Fungal taxonomists have disagreed about the number of species of shiitake that should be recognized across this broad range (reviewed by Hibbett 1992). There are

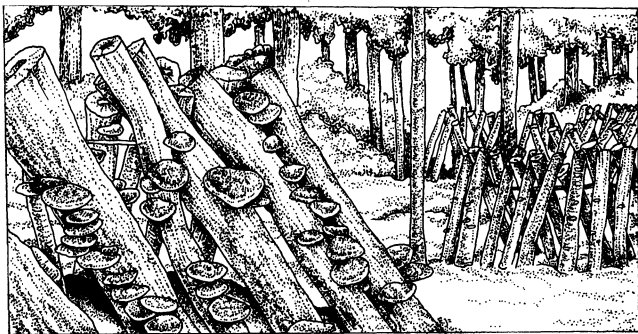


Figure 1. Outdoor log cultivation of shiitake. Drawn after a photograph of a shiitake laying yard in Tottori, Japan.

currently two major competing species-level classifications in shiitake. One is based on a traditional morphological species concept (Pegler 1983), and the other on a form of the biological species concept in which emphasis is placed on demonstration of mating ability in controlled laboratory crosses rather than on evidence of actual gene flow in nature (Shimomura et al. 1992). Morphological differences correlated with geographic distribution led Pegler (1983) to recognize three species of shiitake: *L. edodes* in northeast Asia, *L. lateritia* in southeast Asia and Australasia, and *L. novaezelandiae* in New Zealand. Other mycologists have questioned whether Pegler's species are in fact morphologically distinct (Shimomura et al. 1992; A. Tsuneda, personal communication). Pegler's species concepts have also been challenged on the basis of mating criteria. Isolates of shiitake from Japan, Papua New Guinea, Borneo, Nepal, and New Zealand have been shown to be mating compatible in laboratory crosses (Shimomura et al. 1992), which has led some mycologists to recognize only one species of shiitake. If only one species of shiitake is recognized, the conventions of botanical nomenclature dictate that it be called by the oldest available name, which is *L. edodes*.

We recently completed a phylogenetic study based on ribosomal DNA internal transcribed spacer (ITS) sequences that bears directly on the species controversy in shiitake (Hibbett et al. 1995). Our results suggest there are at least four major lineages of shiitake in Asia-Australasia (Figs. 2 & 3). These groups (lineages) are correlated with the geographic distribution of the isolates that we examined: group 1 occurs in Japan, Borneo, and Thailand; group 2 occurs in Papua New Guinea and Tasmania; group 3 occurs in New Zealand; and group 4 occurs in Papua New Guinea (Figs. 2 & 3). Groups 2 and 4 are sympatric in Papua New Guinea. Our ability to discern four major lineages of shiitake suggests that classifications that lump all of shiitake into a single species lack precision and do not accurately reflect diversity in shiitake. At the same time, the ITS groupings are only partially congruent with the morphology-based, three-species classification of shiitake by Pegler (1983). Our ITS-based phylogenetic trees suggest that *L. edodes* and *L. novaezelandiae sensu* Pegler are monophyletic, but that *L. lateritia* is a paraphyletic assemblage from which multiple lineages of shiitake have been derived (Fig. 2).

There are still certain ambiguities that limit the interpretation of our molecular phylogeny for shiitake (discussed in detail in Hibbett et al. 1995). Briefly, these relate to problems of undersampling of isolates from certain geographic areas and disagreements with some conclusions of a previous phenetic analysis of mitochondrial DNA restriction fragment length polymorphisms (Fukuda et al. 1994). We have therefore not yet proposed formal taxonomic changes for shiitake. Nevertheless, our ITS-based hypothesis is the most rigorous estimate of phylogenetic relationships in shiitake to date,

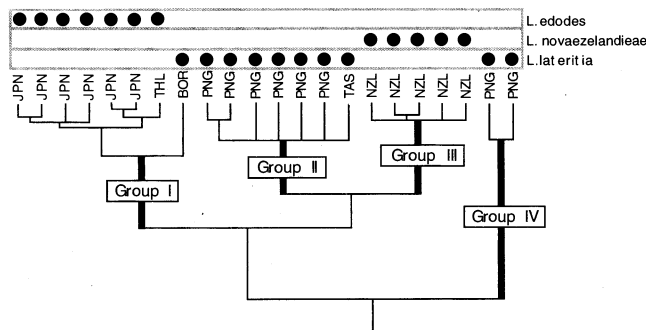


Figure 2. Phylogenetic relationships among shiitake isolates based on analysis of ITS sequences of Hibbett et al. (1995). The lineages designated as groups I–IV, drawn with heavy lines, are strongly supported as monophyletic by bootstrap and decay index measures of robustness. Dots indicate morphological species designation following Pegler (1983). Note that *L. lateritia* is a paraphyletic entity that includes members of groups I, II, and IV. Geographic origin of isolates is indicated by the following codes: JPN = Japan; THL = Thailand; BOR = Borneo; PNG = Papua New Guinea; TAS = Tasmania; NZL = New Zealand.

and we suggest it be used to structure systematic research priorities and guide conservation efforts. Specifically, we should refine our understanding of the various lineages of shiitake, move toward formal recognition of those lineages as species, and take action to protect them in their natural habitats. We anticipate resistance from the shiitake industry to splitting shiitake into multiple species. There is, however, much to be gained for mushroom growers as well as conservationists by recognizing independently evolving groups in shiitake as distinct species. These benefits include (1) a heightened understanding of the distribution of genetic variation in shiitake; (2) a classification system that reflects evolution; and (3) a rational basis for formulating agricultural policies to protect indigenous populations of shiitake.

Our work has a number of practical implications for the shiitake industry. Each of the four lineages of shiitake can be viewed as a potential repository of genes that could be of great value for cultivar development (Chang et al. 1995; Tokimoto & Komatsu 1995). As far as we know, commercial strains of shiitake are derived entirely from northeast Asian stocks, suggesting that breeders have drawn on only a small fraction of the genetic diversity present across the range of shiitake (Royse & May 1987). Happily, because all strains of shiitake tested to date have been intercompatible, the genes resident in the various lineages of shiitake are readily accessible to mushroom breeders through standard crossing methods. The potential value of wild strains for cultivar development has already been demonstrated in a number of plant crops (e.g., Iltis 1988). One example is found in

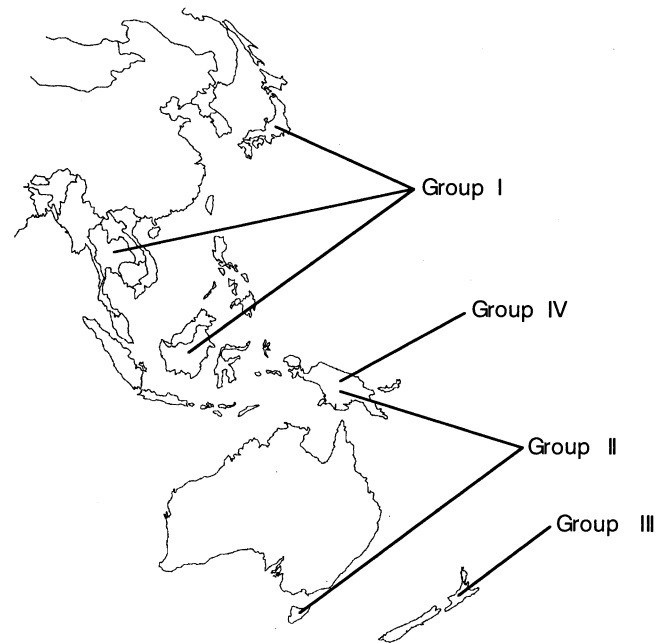


Figure 3. Geographic origins of shiitake groups I–IV identified in Fig. 2.

rice: a wild strain from one region in India provided resistance to the grassy-stunt-virus that at one time threatened the entire African rice crop (Vaughan & Stich 1990). For shiitake, desirable traits that should be sought in wild genomes might include resistance to fungal pathogens, such as *Trichoderma* spp., which cause great losses in shiitake crops (Przybylowicz & Donoghue 1988; Tokimoto & Komatsu 1995).

The shiitake industry should be deeply concerned that many indigenous populations of shiitake, and their virtually untapped genetic resources, are in danger of being destroyed before they can be utilized. The primary threat to wild shiitake populations is habitat loss through deforestation. The burgeoning shiitake industry itself, however, also poses an insidious threat to indigenous shiitake populations. Typical outdoor shiitake farming involves production of unnaturally high densities of spore-producing fruiting bodies (Fig. 1). Under such circumstances it seems likely that cultivars could escape. There is already an extensive literature documenting gene flow between domesticated crop plants and their wild relatives (e.g., Doebly 1990; Ellstrand et al. 1990; Wilson 1990). In fungi there is strong evidence that cultivars of the button mushroom, *Agaricus*, have escaped cultivation and become naturalized to the extent that in some areas the indigenous genotypes are now rare or absent (Kerrigan & Ross 1989; Kerrigan 1995). For these reasons we are concerned that escaped shiitake cultivars could replace or interbreed with indigenous populations, eventually reducing genetic diversity in wild populations. A final hazard of exposing indigenous shiitake

populations to exotic genotypes is the possibility of outbreeding depression, which has been documented in both plant (Waser 1993) and animal systems (Templeton 1994). Although the focus of this discussion is on protection of indigenous populations of shiitake, it should be noted that even in areas without local shiitake populations escaped cultivars of shiitake pose an environmental danger because they could compete with elements of the native mycota. As yet, we are unaware of wild populations of shiitake in North America or Europe, but if shiitake follows the same course as *Agaricus*, it seems probable that they will eventually become established.

Recommendations

This is a critical period for shiitake. The dramatic growth of the shiitake industry, the corresponding expansion of shiitake cultivation into new geographic regions, and the ongoing loss of native shiitake habitats all pose threats to indigenous shiitake populations. This unique combination of factors also endangers the long-term health of the shiitake industry, which ultimately relies on wild genetic diversity for strain development. Given the present situation, we present a number of suggestions for action that can be broken down into two broad, somewhat overlapping categories: basic biological research and agricultural initiatives for biodiversity conservation. These actions should be carried out in combination with efforts to halt deforestation and protect native shiitake habitats.

Basic Biological Research

To understand and protect shiitake genetic resources, it will be critical to assess the existing biodiversity of shiitake, establish culture collections for the preservation of germplasm (but see Hamilton [1994] for a critique of the use of "seed banks" to preserve genetic variation), and continue to identify and delimit the lineages of shiitake. This will require taxonomic research in the field and herbaria, and laboratory-based efforts in molecular systematics. The combination of field and laboratory research we envision would provide data to assess biogeographic and phylogenetic relationships of shiitake and identify particularly critical localities for conservation. Our work has suggested that Papua New Guinea, with its two distinct, co-occurring lineages of shiitake, may harbor an unusually high level of genetic diversity and, therefore, deserves special attention.

In addition to systematic and floristic studies, research within a population biology framework could help assess risk to indigenous populations and formulate intelligent agricultural policies. For example, studies that determine the importance of spore dispersal for establish-

ment and maintenance of populations could help assess the danger of escape by cultivars.

Agricultural Initiatives

Governmental agricultural agencies and the shiitake industry can do much to safeguard indigenous shiitake populations while providing benefits to both the industry and the people who live in areas with indigenous shiitake populations. We encourage shiitake breeders to look to isolates from outside of northeast Asia as sources of useful genes for strain improvement and to compensate local landowners and governments for the isolates or the privilege of seeking them. This would provide new genetic material for strain development and would demonstrate to governments the value of their forests, thereby increasing the likelihood of their preservation.

Environmentally friendly mushroom farming techniques should be developed that would limit the release of cultivar genotypes, especially in areas with indigenous shiitake populations. One possibility would be for mushroom growers in sensitive areas to avoid using imported cultivars, but instead choose local strains whenever possible. There are, however, two problems with this idea. First, it may be prohibitively expensive for small enterprises to develop cultivars that grow well and are appealing to international consumers. Second, cultivation of high concentrations of a single cultivar genotype, even if derived from native strains, could still result in reduction of local genetic diversity through escapes. One promising idea for farming in sensitive areas is to develop sporeless cultivars. A sporeless mutant of shiitake has already been discovered that produces normal fruiting bodies but that has defective spore maturation (Hasebe et al. 1991). Applied molecular genetic studies of basidiosporogenesis might identify target genes which could be knocked out to create other sporeless mutants. Recent developments in protoplast fusion technology, as well as older techniques of di-mon matings, should make it possible to bypass traditional breeding methods and incorporate sporeless mutants into commercial cultivars.

Implementation of these recommendations will require cooperation between governments, academic researchers, and the shiitake industry. Broad-based funding will be needed to undertake the basic research outlined here and to develop and disseminate new agricultural technologies. For example, it is unreasonable to expect fledgling shiitake industries in developing nations to support biotechnology enterprises aimed at producing environmentally friendly cultivars. To accomplish the goals described above, some changes are needed in the standard operating procedures of the shiitake industry. In particular, novel agricultural methods and shiitake cultivars developed through public-private-academic partnerships should be made available to a

broad, international community of users at minimal or no charge. This may be perceived as a radical concept by some members of the shiitake industry, where typically great emphasis has been placed on development and protection of proprietary cultivars (e.g., Royse et al. 1983). Nevertheless, the threat to wild shiitake populations is serious enough to warrant creative solutions and novel alliances. International cooperation will be essential to conduct even the most basic collecting and survey work, requiring permits and agreements on locations of culture collections, deposition of voucher specimens, etc. Indeed, this situation clearly demonstrates how conservation issues transcend political boundaries.

Phylogeny and Conservation

Our work in shiitake provides an example of an application of phylogenetic methods in conservation biology that we think is very promising, namely, the use of phylogenetic trees to identify separately evolving lineages—equivalent to phylogenetic species (Donoghue 1985)—that should be targets of conservation efforts. Other examples of this sort of integration of phylogeny and conservation include the recent studies by Taylor et al. (1994) on wombats, Bowen et al. (1993) on sea turtles, Baker et al. (1993) on humpback whales, Ruvolo et al. (1994) on primates, and Gottelli et al. (1994) on Ethiopian wolves. In each case insights about evolutionary lineages gained through phylogenetic analyses have been used to identify groups that are in need of protection. The uses of phylogenetic inference for conservation purposes in these studies can be contrasted with other, arguably more esoteric applications in which formulas have been devised to generate numerical rankings of terminal taxa, clades, or sets of taxa (e.g., faunal assemblages) to be used in setting conservation priorities. In general such methods assign numerical rankings based on cladogram branching patterns alone or in conjunction with branch length information (May 1990; Vane-Wright et al. 1991; Williams et al. 1991; Faith 1992, 1994; Crozier 1992; Nixon & Wheeler 1992; Stiasny 1992). It is not our purpose here to evaluate these approaches to integration of phylogeny and conservation (Faith 1994; Krajewski 1994; Williams et al. 1993). Nevertheless, we speculate that the simple application of phylogenetic methods to species-level classification may have a more immediate impact on conservation policies than these other methods. This is because the concept of “species” is accessible to legislators and is already emphasized in the language of conservation policy, whereas notions such as “taxonomic dispersion” or “higher taxon richness” will require considerable explanation and justification before they can be brought to bear in policy making.

The shiitake situation also demonstrates how the kind of species concept that is adopted can have a great impact on conservation policy (Woodruff 1989). In the

case of shiitake three kinds of species concepts have been advocated (Pegler 1983; Hibbett 1992; Shimomura et al. 1992). Each divides the group differently and each has different implications for management. Adherence to the biological species concept has been used to lump all of shiitake into a single species (Shimomura et al. 1992). A one-species classification would hinder legislation to protect indigenous populations of shiitake. For example, under the biological species concept, extirpation of shiitake in New Zealand and Papua New Guinea due to habitat loss would most likely be viewed simply as a contraction of the range of a single species. In contrast, under a phylogenetic species concept such events would mean the extinction of two separate species (and range contraction of a third; Figs. 2 & 3). Arguments for habitat preservation will be more compelling if the fate of an entire species is at stake, rather than merely a range contraction of a single widespread species. The division of shiitake into three morphological species, as proposed by Pegler (1983), has greater promise for preserving genetic diversity than does the classification of shiitake as a single biological species. Our results based on ITS data, however, suggest there are four distinct lineages in shiitake, not three, and that one of Pegler’s morphological species, *L. lateritia*, is a paraphyletic entity that includes members of three different lineages, two of which co-occur in Papua New Guinea (Fig. 2).

It should not be construed that we prefer a phylogenetic species concept for shiitake simply because its application would result in the greatest possible number of species of shiitake being recognized. We prefer the phylogenetic species concept because it reflects our understanding of independently evolving lineages, regardless of the retained plesiomorphic ability to interbreed or morphological similarities among lineages. Because independent lineages are expected to contain uniquely derived genes and combinations of genes, conservation efforts based on a phylogenetic taxonomy have the best chance of preserving genetic diversity. Similarly, a taxonomy based on phylogeny would benefit the shiitake industry by virtue of its ability to predict patterns of distribution of genetic variation. In contrast, the biological and morphological species concepts are misleading for predictions of the distribution of genetic diversity in shiitake. Pegler’s (1983) classification for shiitake, based on a morphological species concept, is positively incongruent with the lineages recognized through our phylogenetic analyses (Hibbett et al. 1995). The biological species concept for shiitake, advocated by Shimomura et al. (1992), is also misleading because it lumps shiitake into a single species and thereby conveys a false impression of homogeneity across all populations of shiitake, whereas our phylogenetic studies suggest that genetic diversity in shiitake has a discontinuous distribution that is partitioned according to lineages and correlated with geographic distribution.

Finally, this work demonstrates the impact of molecular genetic techniques on systematics and, potentially, on conservation biology (Templeton 1991). The availability of molecular data, coupled with rigorous analytical methods, has made it possible to resolve lineages of organisms with greater precision than ever before. The gains in systematic power are especially significant for morphologically cryptic groups, such as fungi. Molecular phylogenetics and the corresponding application of phylogeny-based species concepts will be instrumental in bringing taxonomically obscure organisms to prominence in conservation policy.

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