ORIGINAL PAPER

**Hitoshi Murata · Akira Ohta · Akiyoshi Yamada ·
Maki Narimatsu · Norihiro Futamura**

Genetic mosaics in the massive persisting rhizosphere colony "shiro" of the ectomycorrhizal basidiomycete *Tricholoma matsutake*

Abstract The ectomycorrhizal basidiomycete *Tricholoma matsutake* produces commercially valuable fruit bodies "matsutake" on a massive persisting rhizosphere aggregate of mycelia and mycorrhizas called "shiro." Using inter-retrotransposon amplified polymorphism analysis, we attempted to explore the potential diversity within the population of *T. matsutake* isolated from small *Pinus densiflora* woodlands located in various parts of Japan. In general, random phylogenetic relationship was noted among *T. matsutake* tested. The population from each limited sampling area was highly heterogeneous. Even some isolates from fruit bodies produced in the same shiro and those from spores in the same fruit bodies were found to be genetically diverse, indicating the occurrence of genetic mosaics in shiro. In a mosaic shiro, heterologous genets produced their fruit bodies concurrently. Data suggested that the dispersal of spores through sexual reproduction may have been more prevalent than generally accepted in *T. matsutake* to bring mosaicism and coordination of heterologous genets within the shiro. Implementation of management taking such diversity into consideration is urgently needed for the restoration of devastated matsutake fields in Japan. Exploration of individual clones in mosaic fungal resources that promote colonization and fruit body production is necessary for it.

Keywords Basidiomycetes · Ectomycorrhiza · Genetic mosaic · Molecular ecology · Retrotransposons

H. Murata (✉)
Department of Applied Microbiology and Mushroom Science,
Forestry and Forest Products Research Institute,
Matsunosato 1,
Tsukuba 305-8687, Japan
e-mail: murmur@ffpri.affrc.go.jp
Tel.: +81-298-733211
Fax: +81-298-730507

A. Ohta
Shiga Forest Research Center,
978 Kitasakura,
Yasu 520-2321, Japan

A. Yamada
Ibaraki Prefectural Forest Research Center,
Naka,
Ibaraki 311-0122, Japan

M. Narimatsu
Iwate Prefectural Forestry Technology Center,
Oji,
Yahaba 028-3623, Japan

N. Futamura
Department of Molecular Cell Biology,
Forestry and Forest Products Research Institute,
Matsunosato 1,
Tsukuba 305-8687, Japan

Present address:
A. Yamada
Department of Bioscience and Biotechnology,
Shinshu University,
Minami-minowa,
Nagano 339-4598, Japan

Introduction

The ectomycorrhizal basidiomycete *Tricholoma matsutake* produces economically important edible mushrooms "matsutake" in association with *Pinus* sp. plants in the Northern Hemisphere (Hosford et al. 1997; Ogawa 1975). The annual yield of matsutake in Japan has dramatically decreased since the 1940s. The yield of 52 t in the year 2002 was only 0.4% of 12,000 t recorded in 1941 (Ministry of Agriculture, Forestry and Fishery of Japan). The resources of *T. matsutake* are endangered, and scientific countermeasures are urgently needed.

T. matsutake colonizes persistently at the rhizosphere of host trees, and gradually grows on the concentric circle of the host in the form of a unique massive mycorrhizal–mycelial aggregate called "shiro," according to Hamada who monitored shiro for 11 years consecutively (Hamada 1970; Hosford et al. 1997). Unlike rhizosphere colonies of many other ectomycorrhizal basidiomycetes, shiro of *T. matsutake* is such a solid and tight aggregate that the

border between shiro and its surrounding area can be easily recognized. In addition, individual spores of *T. matsutake* hardly germinate in vitro unless they are densely piled around the gill on an agar plate, or treated with (*n-*)butyric acid which does not exist in a major host plant *Pinus densiflora* nor its surrounding soil materials (Ohta 1986; our unpublished record). Thus far, the observation of mycorrhiza synthesis through sexual reproduction in *T. matsutake* has not been scientifically documented. In fact, the importance of sexual reproduction in the life cycle of *T. matsutake* has yet to be clarified. Currently, *T. matsutake* is regarded as a member of fungi that colonize persistently for years to produce a large rhizosphere colony, rather than fungi that colonize shortly and spread through the dispersal of spores (Baar et al. 1994; Gryta et al. 1997; Smith et al. 1992). It is the normal practice of foresters and matsutake hunters to harvest fruit bodies before the dispersal of spores, because such young mushrooms sell at a higher price than the ones that have broken veils. Harvesters relied on shiro for matsutake production, and disregarded the role of spore dispersal from the fruit bodies.

Retrotransposons are retrovirus-like DNA parasites associated with eukaryotic genome (Kumar and Bennetzen 1999). In response to environmental stresses, copies of retrotransposons may be amplified and integrated into other genetic loci of their hosts (Kumar and Bennetzen 1999). This unique replication process often leaves evolutionary footprints in the genomes of eukaryotes (Kumar and Bennetzen 1999; Shimomura et al. 1997). In the rice blast pathogen, *Magnaporthe grisea*, such repetitive elements have been used as genetic markers for clonal analysis on the host plant specificity (Dobinson et al. 1993; Hamer et al. 1989; Levy et al. 1991). Kalendar et al. (1999) demonstrated that inter-retrotransposon-amplified polymorphism (IRAP) can be a powerful molecular tool for the analysis of intraspecific variations, in which polymerase chain reaction (PCR) with outward-facing primers annealing to the long terminal repeat (LTR) of retrotransposons conferred highly polymorphic fingerprints. We reported that IRAP targeting the LTR of the retrotransposon *marY1*, which is abundant in the genome of *T. matsutake*, specified the fungal strains, and demonstrated the phylogenetic relationship among the isolates in a global scale (Murata and Yamada 2000; Murata et al. 2005). In the analysis, *T. matsutake* strains were found to fall into groups reflecting their global origins, such as Asia, Morocco, and Mexico (Murata et al. 2005).

To clarify whether *T. matsutake* colonizes persistently through vegetative growth without the influence of sexual reproduction in the majority of its life cycle, we performed the IRAP analysis on the population of *T. matsutake* from three limited *P. densiflora* woodlands that are distantly located from each other in Japan. The mosaicism found in the populations led us to the conclusion, the view documenting for the first time, that sexual reproduction plays an important role in the life cycle of *T. matsutake*. This information may be useful in managing matsutake-producing forests for the conservation of the fungal resources as well as for the promotion of mushroom production.

Materials and methods

Fungal and DNA samples

T. matsutake strains are listed in Table 1 (see also Figs. 1 and 2 for sampling sites in detail). Strains AT-640, AT-641, and AT-643 were isolated from mycelia germinating from spores. Briefly, after removing the veil from a young fruit body, a piece of mycelia (ca. $5 \times 5 \times 5$ mm^3) containing several gills was cut out axenically, placed on the MNC agar plate, and incubated at 23°C for 7–10days. During incubation, spores reached maturity and were released from basidia, and densely piled around the gills on the agar plate. Mycelia germinated from spores were recovered under a light microscope, and stored as slant cultures. Fungal mycelia were cultured in MMN liquid medium modified by the addition of 1.5% V8 juice, instead of NaCl (Campbell Soup Co., Camden, NJ). Genomic DNA was isolated from frozen mycelia using a lysis buffer containing hexadecyltrimethyl-ammonium bromide and phenol–chloroform (Murata and Yamada 2000).

Polymerase chain reaction

PCR was conducted in 50-µl reaction mixtures containing 250 µM of dNTP, 0.5 µM of the primer, 30 ng of template DNA, 0.5 units of Gene *Taq* NT (Wako Pure Chemicals, Osaka, Japan), and a universal buffer provided by a manufacturer (Murata et al. 2005). The outward facing primer pS1 (5′-GCACCCCCCTAGTCCCCTTACA-3′, T_m=64°C) was designed based on the complimentary strand of the 5′-LTR sequence of the retrotransposon *marY1* (Murata et al. 2005). Cycle reactions were performed as follows; 1×(94°C/2 min), 25×(94°C/30 s, 68°C/30 s, and 72°C/5 min), and 1×72°C/10 min with GeneAmp 9700 (Applied Biosystems, Foster City, USA). PCR products were recognized in TBE-1.8% agarose gel electrophoresis (Nusieve GTG agarose; FMC Bio Products, Rockland, ME).

Phylogenetic analysis

PCR products manifesting reproducible solid bands between 0.15 and 1.5 kbp in the triplicates of the gel electrophoresis were scored via line-by-line comparison according to the theory of "0/1 (= negative/positive)" analysis (Williams et al. 1990). The neighbor-joining (N-J) analysis was conducted with the bootstrap analysis based on 1,000 replications using the CLUSTAL X program (Thompson et al. 1997). Phylogenetic trees were constructed with the Mexican isolate TM-4 as the outgroup control, and visualized using the program TreeView PPC.

Results

PCR generated 75 reliable band positions between 0.15 and 1.50 kbp in the samples tested (Fig. 3). With 75 band lo-

Table 1 *Tricholoma matsutake* strains used in this study

Strains	Sampling site[a]	Sampling date (month/day/year)
Tm024	Shiro # M3, Minakuchi study site, Shiga prefecture, Japan[b] (N34°55′, E136°6′, 320 m)	10/11/1983
Tm027	Shiro # M3, Minakuchi study site	10/14/1983
Tm029	Shiro # S1, Kohnan study site, Shiga prefecture, Japan[c] (N34°55′, E136°7′, 270 m)	10/14/1983
Tm040	Shiro # M7, Minakuchi study site	10/11/1991
Tm043	Shiro # M7, Minakuchi study site	10/14/1991
Tm050	Shiro # S6, Kohnan study site	10/26/1991
Tm068	Shiro # S6, Kohnan study site	10/7/1993
Tm069	Shiro # S6, Kohnan study site	10/2/1994
Tm070	Shiro # S6, Kohnan study site	10/2/1994
Tm071	Shiro # S6, Kohnan study site	10/6/1994
Tm072	Shiro # S6, Kohnan study site	10/6/1994
Tm073	Shiro # S6, Kohnan study site	10/6/1994
Tm074	Shiro # S6, Kohnan study site	10/11/1994
Tm075	Shiro # S6, Kohnan study site	10/11/1994
Tm076	Shiro # S6, Kohnan study site	10/11/1994
Tm077	Shiro # S6, Kohnan study site	10/11/1994
Tm078	Shiro # S6, Kohnan study site	10/11/1994
Tm079	Shiro # S6, Kohnan study site	10/11/1994
Tm080	Shiro # S6, Kohnan study site	10/14/1994
Tm081	Shiro # S6, Kohnan study site	10/14/1994
Tm082	Shiro # S6, Kohnan study site	10/14/1994
Tm083	Shiro # S6, Kohnan study site	10/18/1994
Tm084	Shiro # S6, Kohnan study site	10/21/1994
Tm085	Shiro # S1, Kohnan study site	10/14/1994
Tm086	Shiro # S7, Kohnan study site	10/14/1994
Tm099	Shiro # S9, Kohnan study site	11/4/1998
Tm100	Shiro # S4, Kohnan study site	11/4/1998
Tm114	Shiro # S1, Kohnan study site	10/9/2001
Tm124	Shiro # S10, Kohnan study site	10/14/2003
Tm125	Shiro # S10, Kohnan study site (from pileus)	10/14/2003
Tm126	Shiro # S10, Kohnan study site (from stipe)	10/14/2003
Tm127	Shiro # S5, Kohnan study site	10/21/2003
Tm128	Shiro # S5, Kohnan study site (from pileus)	10/21/2003
Tm129	Shiro # S5, Kohnan study site (from stipe)	10/21/2003
AT-634	The site B1, Morigane study site, Ibaraki prefecture, Japan[d] (N 36° 41′, E 140° 24′, 240 m)	10/6/1997
AT-635	The site B7, Morigane study site	10/8/1997
AT-636	The site B2, Morigane study site	9/30/1997
AT-637	The site B4, Morigane study site	9/30/1997
AT-638	The site B6, Morigane study site	10/8/1997
AT-639	The site B5, Morigane study site	10/6/1997
AT-640	The site B3, Morigane study site	10/6/1997

Table 1 (continued)

Strains	Sampling site[a]	Sampling date (month/day/year)
AT-641	The site B1, Morigane study site (from spore)	10/6/1997
AT-642	The site B5, Morigane study site (from spore)	10/6/1997
AT-643	The site B5, Morigane study site (from spore)	10/6/1997
IW-92604-2	The site W1, Yokkaichi study site, Iwate prefecture, Japan[e] (N39°56′, E141°14′, 370 m from sea level)	9/26/2002
IW-92604-4	The site W1, Yokkaichi study site	9/26/2002
IW-100704-2	The site W1, Yokkaichi study site	10/7/2002
IW-100704-3	The site W1, Yokkaichi study site	10/7/2002
IW-100704-4	The site W1, Yokkaichi study site	10/7/2002
I-84	The site W2, Yokkaichi study site	10/4/2001
I-114	The site W2, Yokkaichi study site	10/4/2001
TM-4	Mexico, obtained from a retailer	10/–/1991

[a] With the exception of TM-4, fruit bodies of all the strains were obtained from *Pinus densiflora* forests. Unless stated otherwise, mycelia were isolated from lamellae
[b] Minakuchi study site stands on the uniform granite field
[c] Kohnan study site stands on the uniform granite field
[d] Morigane study site stands on the uniform granite field
[e] Yokkaichi study site stands on the field composed of peat and andosol

cations, 2^{75} or 9.4×10^{21} types of polymorphisms are theoretically possible based on the "0/1" concept (Williams et al. 1990). Overall phylograms after the N-J analysis of fingerprints exhibited rather random profiles, in which 86% of the branches scored <50% of bootstrap values, not reflecting their local origins (Fig. 4). For example, two genets of "shiro-M7" at the Minakuchi study site, Tm40 and Tm043, did not show any direct phylogenetic relations with each other (Figs. 1, 4). The former strain was related to Tm024 of "shiro-M3" in the same study site, whereas the latter was related to Tm029 of "shiro-S1" at the Kohnan study site in Shiga prefecture (Figs. 1, 4). The population of "shiro-S6" found at the Kohnan study site shared genets with the population of many other shiro in the same area (Figs. 1, 4). It may be noteworthy that fruit bodies of a major genet of shiro-S6 represented by Tm075 occurred rather widely along the periphery of the shiro, while those of a minor genet represented by Tm074 were confined to an edge of the shiro surrounded by the former (Fig. 1). This minor genet of shiro-S6 is closely related with Tm100 of "shiro-S4" and Tm086 of "shiro-S7" with bootstrap values of 30–40%, rather than with the major genet of the apparently same shiro (Figs. 1, 4). Such trends of variations extended further to samples from Iwate prefecture, wherein genets of two small sampling areas that are separated by a valley of ca. 50 m in depth and ca. 200 m in width shared similar genets (Figs. 2, 4). Although some vertically radiating phyloge-

508

Fig. 1 Schematic maps of *T. matsutake* fruit body sampling sites in Shiga prefecture. See Table 1 for sampling dates and field conditions. *Hatched lines* encompass the rhizosphere colony "shiro." The symbol "+" represents the origin of shiro. Samples without information of the specific location of fruit bodies in the shiro are shown in *parentheses*. **A** Location of Minakuchi (N34°55′, E136°6′, 320 m above sea level) and Kohnan (N34°55′, E136°7′, 320 m above sea level) study sites. **B** "Shiro-S6" in 1994. *Solid and open triangles* represent the positions of fruit bodies of two different genets observed in the phylogenetic analysis (see Figs. 3, 4). *Dotted triangles* represent the positions of fruit bodies that are not tested due to unavailability of the cultures. **C** Minakuchi study site. (This study site no longer exists.) The positions of fruit bodies in 1971 only are given. **D** Kohnan study site. *Dotted triangles*, *open circles*, and *solid circles* indicate the positions of fruit bodies observed in 1994, 1996, and 2002, respectively. Strains without information of specific sampling sites within the shiro are given in the *parentheses*
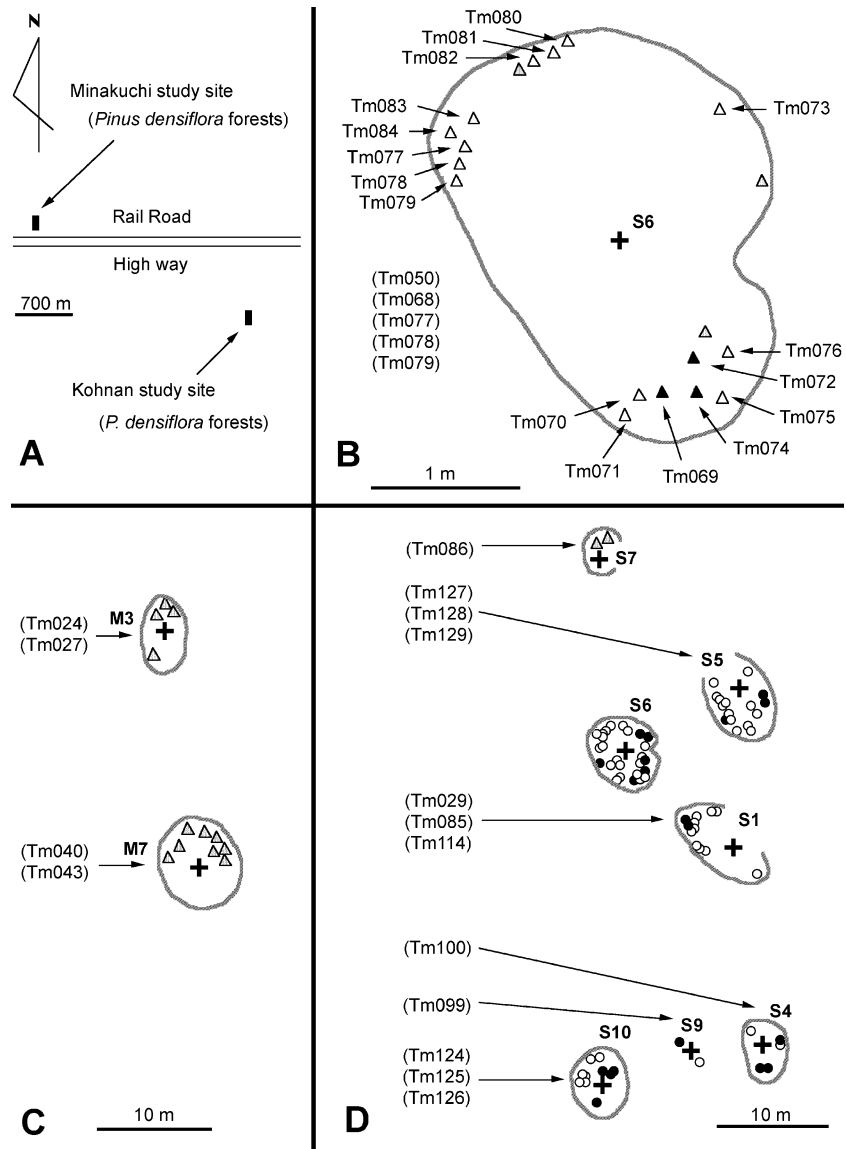
netic relationships were noted in the population of the Morigane study site in Ibaraki prefecture, strains AT-640 and AT-641 showed relatedness with samples from other prefectures (Fig. 4).

Only 14% of phylogenetic branches, all of which were at the terminal ends of the phylogram, exceeded 50% of bootstrap values (Fig. 4). Shiro-S6 contains two different genets represented by Tm074 and Tm075, which formed solid phylogenetic trees of siblings, represented by Tm069 and Tm073, respectively, each scoring nearly 100% bootstrap values within the group (Fig. 4). Similarly, shiro-S1 contains heterologous but closely related genets represented by the isolates Tm085 and Tm114, and shiro-M3 by Tm024 and Tm027 (Fig. 4). Samples from Iwate prefecture also showed that heterologous, but closely related materials coexist, as seen in the cases with IW-100702-2 and IW-100702-4 forming one solid cluster, and IW-92602-2 and IW-92602-4 forming another (Figs. 2, 4). It is interesting to note that AT-634 from the fruit body and its spore isolate AT-641 are genetically heterologous (Fig. 4). Likewise, AT-639 from the fruit body and its spore isolates AT-642 and AT-643 are not identical, although this population exhibits relatively solid phylogenetic relationships as compared with the population of AT-634 and AT-641 (Fig. 4).

Genetically heterologous fruit bodies were produced simultaneously within the same shiro. For example, two heterologous genets of shiro-S6 at Kohnan in Shiga prefecture, one represented by Tm074 and the other by Tm075, produced fruit bodies concurrently, although the former genet was more closely related to Tm100 of shiro-S4 than to the latter (Table 1; Figs. 1, 4). Such apparent coordination among heterologous genets during the fruiting stage was also observed in other shiro, such as M3 and M7 at Minakuchi, as well as limited sampling areas at Morigane and Yokkaichi in other prefectures (Table 1; Figs. 1, 2, 4).
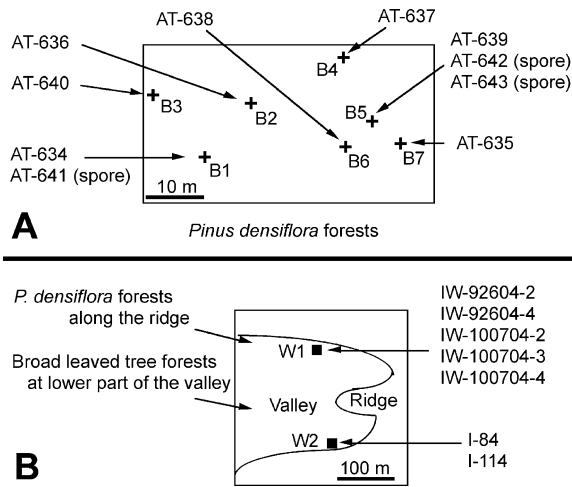
Fig. 2 Schematic maps of the Morigane study site, Ibaraki prefecture (**A**, N36°50′, E140°30′, 350 m above sea level), and the Yokkaichi study site, Iwate prefecture (**B**, N39°56′, E141°14′, 370 m above sea level). See Table 1 for the description of *T. matsutake* strains. The figure is given as in Fig. 1, except for the symbol "+" that represents the positions of fruit bodies of the isolates
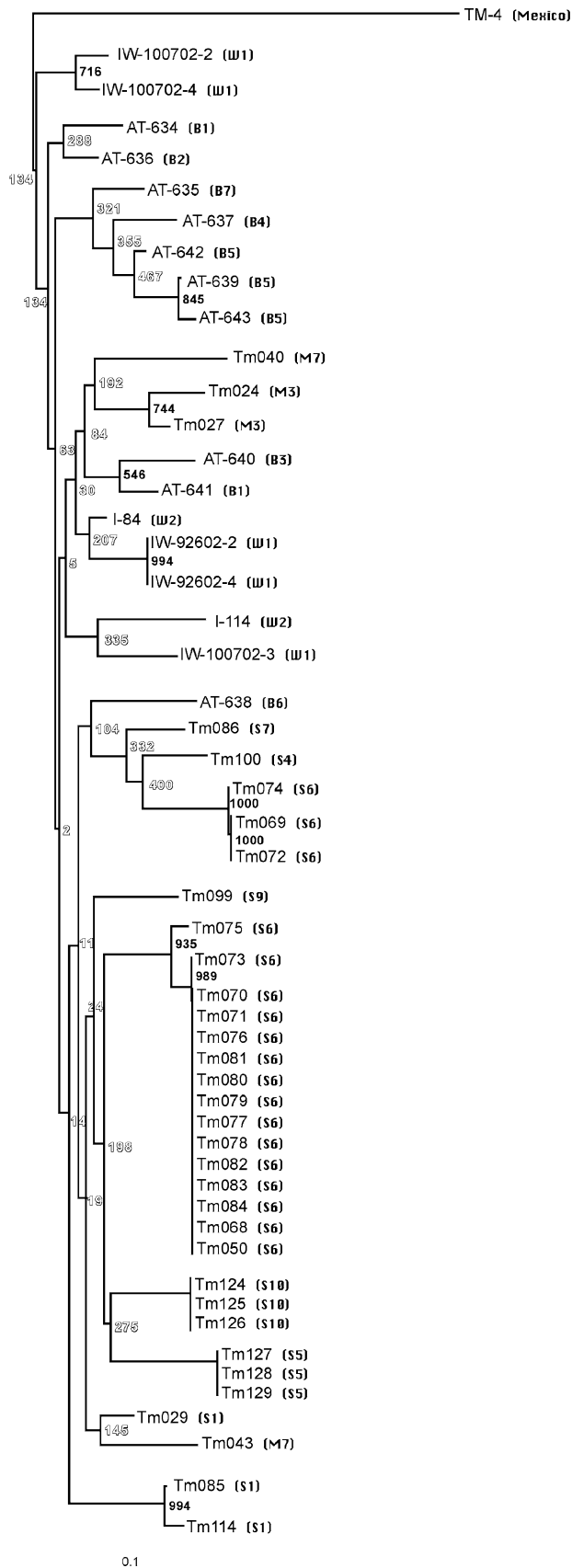
Discussion

Homokaryotic basidiospores are generally produced after meiosis in dikaryotic mycelia that result from mating between two monokaryotic mycelia, or between monokaryotic and dikaryotic mycelia (Babasaki et al. 2003; Buller 1931; Raper 1966). Ectomycorrhizal fungi propagate and expand their colonies through dispersal of such spores produced by sexual reproduction cycle or hyphal spreading through vegetative growth as demonstrated in *Amanita francheti, Cortinarius rotundisporus, Hebeloma cylindrosporum, Laccaria bicolor, Laccaria amethystina, Lactarius xanthogalactus, Russula cremoricolor, Pisolithus tinctorius, Suillus bovinus, S. pungens,* and so on (Anderson et al. 1998; Baar et al. 1994; Bonello et al. 1998; Dahlberg and Stenlid 1990, 1994; Gherbi et al. 1999; Gryta et al. 1997; Redecker et al. 2001; Sawyer et al. 1999). Prior to the experiment, we predicted two optional situations. In one, vertically radiating phylogenetic trees with high bootstrap values could be constructed, showing tight genetic relationships among isolates from shiro in a local area as siblings of a single genet, if vegetative growth has been the major instrument of propagation in *T. matsutake*. On the other hand, if sexual reproduction has been heavily involved,
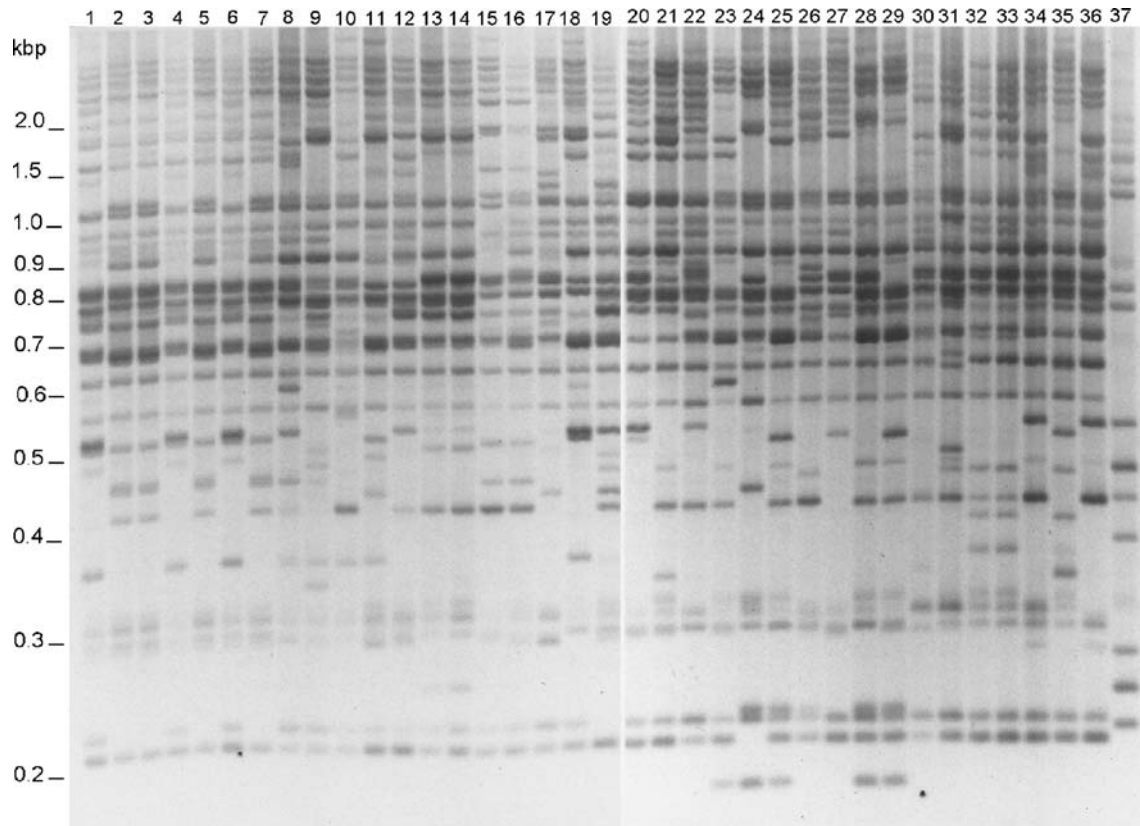
Fig. 3 Inter-retrotransposon-amplified polymorphism of *T. matsutake*. Marker sizes are given on the *left axes. Lane 1* Tm069, *lane 2* Tm070, *lane 3* Tm071, *lane 4* Tm072, *lane 5* Tm073, *lane 6* Tm074, *lane 7* Tm075, *lane 8* Tm124, *lane 9* Tm127, *lane 10* Tm099, *lane 11* Tm100, *lane 12* Tm029, *lane 13* Tm085, *lane 14* Tm114, *lane 15* Tm024, *lane 16* Tm027, *lane 17* Tm040, *lane 18* Tm086, *lane 19* Tm043, *lane 20* AT-634, *lane 21* AT-635, *lane 22* AT-636, *lane 23* AT-637, *lane 24* AT-638, *lane 25* AT-639, *lane 26* AT-640, *lane 27* AT-641, *lane 28* AT-642, *lane 29* AT-643, *lane 30* I-84, *lane 31* I-114, *lane 32* IW-93602-2, *lane 33* IW-93602-3, *lane 34* IW-100702-2, *lane 35* IW-100702-3, *lane 36* IW-100702-4, *lane 37* TM-4. In the separate experiments, we tested Tm050, Tm068, Tm076-084, Tm128-129, and Tm125-126

TM-4 (Mexico)

IW-100702-2 (W1)
716
IW-100702-4 (W1)

AT-634 (B1)
288
AT-636 (B2)
134
AT-635 (B7)
321
AT-637 (B4)
355
AT-642 (B5)
467
AT-639 (B5)
845
AT-643 (B5)
134

Tm040 (M7)
192
Tm024 (M3)
744
Tm027 (M3)
84
63
AT-640 (B3)
546
AT-641 (B1)
30
I-84 (W2)
207
IW-92602-2 (W1)
994
IW-92602-4 (W1)
5
I-114 (W2)
335
IW-100702-3 (W1)

AT-638 (B6)
104
Tm086 (S7)
332
Tm100 (S4)
400
Tm074 (S6)
1000
Tm069 (S6)
1000
Tm072 (S6)
2

Tm099 (S9)
Tm075 (S6)
935
Tm073 (S6)
989
Tm070 (S6)
Tm071 (S6)
Tm076 (S6)
Tm081 (S6)
Tm080 (S6)
Tm079 (S6)
Tm077 (S6)
Tm078 (S6)
Tm082 (S6)
Tm083 (S6)
Tm084 (S6)
Tm068 (S6)
Tm050 (S6)
11
24
Tm124 (S10)
Tm125 (S10)
Tm126 (S10)
275
14
Tm127 (S5)
Tm128 (S5)
Tm129 (S5)
19
Tm029 (S1)
145
Tm043 (M7)

Tm085 (S1)
994
Tm114 (S1)

0.1

Fig. 4 Phylogram of *T. matsutake* based on IRAP. Numbers of bootstrap samplings derived from 1,000 replications that are <50% are *highlighted* by *open bold numerals*, while those >50% are given in *solid bold numerals*. Sampling sites were shown in *parentheses*

horizontally radiating trees could be conferred, exhibiting genetic differentiation proceeding in random and sharing similarities among the isolates from various sampling sites. Retrotransposons can be reliable genetic markers to be used in clonal analysis, because they behave as DNA parasites of eukaryotic genomes and leave evolutionary footprints on the genome through amplification of copies (Dobinson et al. 1993; Hamer et al. 1989; Kumar and Bennetzen 1999; Levy et al. 1991; Shimomura et al. 1997).

Based on our experimental results, we conclude that sexual reproduction plays a much more important role in the propagation and distribution of *T. matsutake* in the main island of Japan, Honshu, giving rise to a significant number of heterologous genets that exhibit rather random phylogenetic profiles. The occurrence of such random phylogenetic profiles may be plausible, for this species has no somatic incompatibility system, unlike *S. bovinus* and *L. bicolor*, which may work as a barrier to mycelial interaction or mating, ensuring the chance of genetic recombination (Baar et al. 1994; Dahlberg and Stenlid 1990, 1994). The fact that a small portion of genets localized in terminal branches exhibited solid phylogenetic relationships implies that vegetative propagation occurs in limited local areas. Our record shows that shiro, which is the solid and tight aggregate of *T. matsutake* mycelia and mycorrhiza in the rhizosphere, grows ca. 15 cm/year in one direction at best in the Kohnan study site, theoretically requiring 10 years to grow into a circle of 3 m in diameter [see, for example, shiro-S6 in 1994 (Fig. 1b) and in 2004 (Fig. 1d)]. However, evolution in the long range must have proceeded heavily involving sexual reproduction.

The observation in both the Kohnan and Minakuchi study sites showed that the characteristics of shiro vary from each other in such traits as number of fruit bodies produced in a specific year and size of shiro grown in a single year (Table 1; Fig. 1). In these study sites, where *P. densiflora* trees dominantly grow on uniform granite soil, no significant environmental difference was noted. Such variations in *T. matsutake* among shiro in the limited area may be attributed to genetic rather than environmental factors. Genetic recombination through the di-mon mating between dikaryotic mycelia from shiro and monokaryotic ones grown from spores, or the ordinary mating between monokaryons from spores in association with shiro, may give rise to new dikaryotic genets on the shiro to form genetic mosaic or mosaicism in the population (Babasaki et al. 2003; Buller 1931; Raper 1966; Fukuda and Fukumasa-Nakai 1996; Peabody et al. 2000). In the mosaicism of wood-decaying homobasidiomycetes, heterologous genets colonized together on the same substrate behave coordinately and exhibit unified characteristics especially during the fruiting stage, rather than phenotypically segregate from each other and express unique individual features (Babasaki et al. 2003; Fukuda and Fukumasa-Nakai 1996; Peabody

et al. 2000). This mycelial coordination in wood-decaying homobasidiomycetes reminds us of the cell density-dependent autoregulation system, or a quorum-sensing system, required for the expression of various genes in prokaryotes and a dimorphic fungus, such as those responsible for pathogenicity, depolymerizing activity, and fruit body formation (Hornby et al. 2001; Miller and Bassler 2001).

While the mosaicism can be a major cause of degeneration of spawns in cultivated mushrooms, it often improves the spawns if superior variants occur, conferring characteristics better than the original with regard to quality as well as quantity of fruit bodies produced (Babasaki et al. 2003). It is noteworthy that fruit bodies of heterologous genets were produced simultaneously in the same *T. matsutake* shiro in Shiga prefecture, as well as at any limited sampling areas in other prefectures, like some wood-decaying homobasidiomycetes (Babasaki et al. 2003; Peabody et al. 2000). Such a mycelial coordination as observed during the fruiting stage may allow the mosaic shiro to grow uniformly on the concentric circle of the host tree on the basis that *T. matsutake* lacks a somatic incompatibility system. This physiology may also be relevant to the spore germination of *T. matsutake*, which occurs in vitro only when spores were densely piled around gills, or treated with the inducing chemical *n*-butyric acid (Ohta 1986; our unpublished record).

Spore germination, and consequent sexual and asexual mycelial interactions in vivo in the symbiotic state must be experimentally proven. However, IRAP-based population analysis provided us with a wider perspective regarding the biology of economically valuable ectomycorrhizal basidiomycete *T. matsutake*, demonstrating that this species substantially relies on dispersal of spores involving sexual reproduction for its persistent colonization and consequent fruit body production. Data that will be available through our further analysis of rhizosphere samples of shiro in the Kohnan study site for 5–10 years to come will provide us with further insights regarding the dynamics of the population structure of *T. matsutake*. Meanwhile, we strongly recommend the execution of management of shiro to promote colonization and fruit body production by keeping a favorable mosaic status. It will be necessary to enforce the control of early harvest to allow spore dispersal. Exploration and evaluation of individual fungal clones may also be required.

References

Anderson IC, Chambers SM, Cairney JWG (1998) Use of molecular methods to estimate the size and distribution of mycelial individuals of the ectomycorrhizal basidiomycete *Pisolithus tinctorius*. Mycol Res 102:295–300

Baar J, Ozinga WA, Kuyper TW (1994) Spatial distribution of *Laccaria bicolor* genets reflected by sporocaps after removal of litter and humus layers in a *Pinus sylvestris* forest. Mycol Res 98:726–728

Babasaki K, Masuno K, Murata H (2003) Interactions of heterologous mycelia colonized in the substrate govern fruit body production in the cultivated homobasidiomycete *Pholiota nameko*. Biosci Biotechnol Biochem 67:100–106

Bonello P, Bruns TD, Gardes M (1998) Genetic structure of a natural population of the ectomycorrhizal fungus *Suillus pungens*. New Phytol 138:533–542

Buller AHR (1931) Research on fungi, IV. Longmans, Green and Co., London, pp. 1–329

Dahlberg A, Stenlid J (1990) Population structure and dynamics in *Suillus bovinus* as indicated by spatial distribution of fungal clones. New Phytol 115:487–493

Dahlberg A, Stenlid J (1994) Size, distribution and biomass of genets in population of *Suillus bovinus* (L.:Fr) Roussel revealed by somatic incompatibility. New Phytol 128:225–234

Dobinson KF, Harris RE, Hamer JE (1993) *Grasshopper*, a long terminal repeat (LTR) retroelement in the phytopathogenic fungus *Magnaporthe grisea*. Mol Plant-Microb Interact 6:114–126

Fukuda M, Fukumasa-Nakai Y (1996) Genetic evidence supporting the multicellular origin of fruiting body formation in the basidiomycete *Lentinula edodes*. Mokuzai Gakkaishi 42:1025–1028

Gherbi H, Delaruelle C, Selosse M-A, Martin F (1999) High genetic diversity in a population of the ectomycorrhizal basidiomycete *Laccaria amethystina* in a 150-year-old beech forest. Mol Ecol 8:2003–2013

Gryta H, Debaud JC, Effosse A, Gay G, Marmeisse R (1997) Fine-scale structure of populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum* in coastal sand dune forest ecosystems. Mol Ecol 6:353–364

Hamada M (1970) Diaries on *Armillaria matsutake* (5). Trans Mycol Soc Jpn 11:81–86

Hamer JE, Farrall L, Orbach MJ, Valent B, Chumley FG (1989) Host species-specific conservation of a family of repeated DNA sequences in the genome of a fungal plant pathogen. Proc Natl Acad Sci U S A 86:9981–9985

Hornby JM, Jensen EC, Lisec AD, Tasto JJ, Jahnke B, Shoemaker R, Dussault P, Nickerson KW (2001) Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. Appl Environ Microbiol 67:2982–2992

Hosford D, Pilz D, Molina R, Amaranthus M (1997) Ecology and management of the commercially harvested American matsutake mushroom. USDA Forest service PNW-GTR412 pp 1–68

Kalendar R, Grob T, Regina M, Suoniemi A, Schulman A (1999) IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. Theor Appl Genet 98:704–711

Kumar A, Bennetzen JL (1999) Plant retrotransposons. Annu Rev Genet 33:479–532

Levy M, Romao J, Marchetti MA, Hamer JE (1991) DNA fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. Plant Cell 3:95–102

Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199

Murata H, Yamada A (2000) *marY1*, a member of the *gypsy* group of long terminal repeat retroelements from the ectomycorrhizal basidiomycete *Tricholoma matsutake*. Appl Environ Microbiol 66:3642–3645

Murata H, Babasaki K, Yamada A (2005) Highly polymorphic DNA markers to specify strains of the ectomycorrhizal basidiomycete *Tricholoma matsutake* based on σ_{marY1}, the long terminal repeat of gypsy-type retroelement *marY1*. Mycorrhiza (in press)

Ogawa M (1975) Microbial ecology of mycorrhizal fungus— *Tricholoma matsutake* (Ito et Imai) Sing. in pine forest. II. Mycorrhiza formed by *T. matsutake*. Bull Gov Forest Exp Station 278:21–80

Ohta A (1986) Basidiospore germination of *Tricholoma matsutake* (I). Effects of organic acids on swelling and germination of the basidiospores. Trans Mycol Soc Jpn 27:167–173

Peabody RB, Peabody DC, Sicard KM (2000) A genetic mosaic in the fruiting stage of *Armillaria gallica*. Fungal Genet Biol 29:72–80

Raper JR (1966) Genetics of sexuality in higher fungi. The Ronald Press Company, New York, pp. 1–283

Redecker D, Szaro TM, Bowman RJ, Bruns T (2001) Small genets of *Lactarius xanthogalactus*, *Russula cremoricolor* and *Amanita francheti* in late-stage ectomycorrhizal successions. Mol Ecol 10:1025–1034

Sawyer NA, Chambers SM, Cairney JWG (1999) Molecular investigation of genet distribution and genetic variation of *Cortinarius rotundisporus* in eastern Australian sclerophyll forests. New Phytol 142:561–568

Shimomura M, Yasue H, Ohshima N (1997) Molecular evidence from retroposons that whales from a clade within even-toed ungulates. Nature 388:666–670

Smith ML, Bruhn JN, Anderson JB (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. Nature 356:428–431

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful genetic markers. Nucleic Acids Res 18:6531–6535