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Assessment of the DNA Quality in Mushroom Specimens: Effect of Drying Temperature

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Abstract It is generally believed that drying temperature is critical for preserving high quality DNA in biological specimens. Fruit bodies of mushrooms are therefore dried at low temperature, e.g., lower than 60°C. In this study, we assessed the DNA quality in mushroom specimens by using different drying temperatures (35, 52, and 71°C). The quality of DNA was assessed based on gel electrophoresis with whole genomes as well as PCR products from multiple loci. Based on the experiment using a total of seven phylogenetically distantly related species of mushrooms, we conclude that drying temperature does not significantly affect the DNA quality for subsequent studies (PCR, sequencing, etc.). Further studies are necessary to investigate presently unrepresented samples, such as big fleshy mushrooms (e.g., boletes, or Boletales) and gasteromycetes (puffballs, stinkhorns, etc.).

Key words: Agaricomycotina, Basidiomycota, DNA quality, drying temperature, herbarium, PCR, tissue preservation.

Introduction

It is now widely appreciated that herbarium is a great source of genetic materials (Bruns *et al.*, 1990; Taylor and Swann, 1994; Drábková *et al.*, 2002; Erkens *et al.*, 2008; Dentinger *et al.*, 2010). DNA data from the type specimens will be able to solve taxonomical issues in many groups. One particular example is by Nagy *et al.* (2011) who demonstrated that a number of "unidentified" environmental DNA sequences matched with the sequences from the type strains and specimens. Furthermore, herbarium can potentially host a large number of presently unknown or unrepresented lineages. Brock *et al.* (2008) estimated that ca. 70% of the taxonomic diversity in herbarium is not yet represented in the public database, i.e., GenBank.

One of difficulties concerning the DNA from herbarium specimens is that they frequently lack high quality DNA. It is especially true for older, historically important specimens (e.g., types) because DNA in specimens degrades with time (Hummel and Herrmann, 1994; Erkens *et al.*, 2008). The ages of specimens and degrees of degradation in DNA, however, are not strictly correlated (Drábková *et al.*, 2002; Leino *et al.*, 2009). In fact, specimens collected more than 100 years ago often yield good quality DNA whereas much younger specimens (e.g., less than 5 years old) often lack useful quantity and/or quality of DNA (Dentinger *et al.*, 2010). Therefore the quality of DNA depends more on how specimens were collected, prepared, and maintained, than the ages (Bruns *et al.*, 1990; Drábková *et al.*, 2002; Leino *et al.*, 2009).

Because of such difficulties, a variety of DNA extraction methods have been proposed (Bruns *et al.*, 1990; Drábková *et al.*, 2002; Dentinger *et al.*, 2010). However, after DNA highly degrades, there are only limited things we can do unless DNA repair techniques much more efficient than

the ones currently available from the commercial kits (e.g., Sigma, Qiagen, etc.) are invented. Furthermore, many herbarium specimens have been fumigated by a variety of chemicals to prevent insect damage (Whitten *et al.*, 1999; Kigawa *et al.*, 2003). Many widely available fumigants are known to highly efficiently degrade DNA, among which the mixture of methyl bromide and ethylene oxide is demonstrated to be one of the worst in terms of DNA degradation (Kigawa *et al.*, 2003). The authors' herbarium (TNS) has been treated by methyl bromide until relatively recently, and the specimens treated this way (ca. 10 years or older) all have highly degraded DNA. Our initial attempt to amplify shorter fragments (300 bp or shorter) mostly failed (data not shown). For preserving valuable genetic resources, all specimens should be treated by fumigants not affecting DNA, such as sulphuryl fluoride (Whitten *et al.*, 1999) or not fumigated at all.

Most recent attempts therefore focused on preservation of DNA from freshly collected samples, instead of extracting DNA from old materials. A variety of preservation methods are reviewed by Nagy (2010). Among which, cryopreservation in liquid nitrogen or ultra-deep freezer (−80°C) is a single most efficient method for virtually all types of biological materials, but it is not feasible especially during the fieldwork (Seutin *et al.*, 1991; Dawson *et al.*, 1998; Nagy, 2010). Several organic solvents (e.g., ethanol, isopropanol, but especially acetone) efficiently preserve DNA for a long term (Fukatsu, 1999; Nagy, 2010), but air transportation of specimens with such highly flammable substances is not recommended.

Instead, a variety of buffers without organic solvents have been proposed (Nagy, 2010). The efficacy of DNA preservation using different buffers vary among organisms. For example, DMSO-NaCl solution was successfully applied for marine invertebrates and avian samples (Seutin *et al.*, 1991; Dawson *et al.*, 1998), but CTAB-NaCl buffer appears equally efficient for plant tissues (Rogstad, 1992). Laulier *et al.* (1995) reported that guanidium thiocyanate buffer is much more efficient than CTAB-NaCl buffer, and works for a variety of organisms, including virus, fungi, and animals. It is noteworthy that acetone (though flammable) and guanidium thiocyanate buffer can effectively preserve RNA as well as DNA (Laulier *et al.*, 1995; Fukatsu, 1999).

The authors' laboratory routinely uses a modified DMSO-NaCl buffer (Hosaka and Castellano, 2008; Hosaka, 2009) for preserving field-collected mushroom samples with a good success rate. Tissues preserved in this buffer and stored at room temperature for several months usually yield high quality DNA (Hosaka and Castellano, 2008; Hosaka, 2009; Hosaka *et al.*, 2010). However, the process of preparing small tissue fragments in buffer is very tedious during the fieldwork. Since mycologists (and botanists) need to prepare dried specimens regardless of the purpose of studies, it is desirable if we can obtain high quality DNA from dried specimens.

Previous studies all agree that temperature and time for dying specimens are two critical factors for preserving quality DNA (Pyle and Adams, 1989; Bruns *et al.*, 1990; Drábková *et al.*, 2002; Blanco *et al.*, 2006; Erkens *et al.*, 2008). It is generally believed that quickly dried specimens with low heat (ca. 42°C or less) are necessary for subsequent DNA studies (Pyle and Adams, 1989; Bruns *et al.*, 1990; Taylor and Swann, 1994; Johnson *et al.*, 2003; Nagy, 2010). However, some studies reported that drying temperature above 60°C also resulted in good quality DNA (Blanco *et al.*, 2006; Erkens *et al.*, 2008).

By comparing the DNA quality from phylogenetically distantly related mushroom species, which were dried at different temperatures, we here report that drying temperature does not significantly affect the DNA quality for subsequent studies (PCR, sequencing, etc). Although we have tested only seven species of mushrooms, we believe the conclusion is applicable to many other untested species of mushrooms. Field mycologists without storage buffers at hands therefore should dry specimens as quickly as possible (regardless of temperatures) for preserving high quality DNA.

Table 1. Mushroom species investigated in this study

Species	Abbreviation*	Producer
Lentinula edodes	L	Sakuragawa-shi, Ibaraki, Japan
Grifola frondosa	G	HOKUTO Corporation, Nagano, Japan
Flammulina velutipes	F	JA Nakano, Nagano, Japan
Hypsizygus marmoreus	H	HOKUTO Corporation, Nagano, Japan
Pholiota nameko	Ph	JA Tokamachi, Niigata, Japan
Agaricus bisporus	A	JA Ibaraki-Kasumi, Ibaraki, Japan
Pleurotus eryngii	Pl	HOKUTO Corporation, Nagano, Japan

*Abbreviations of species names are used in Figs. 1, 3 and 4.

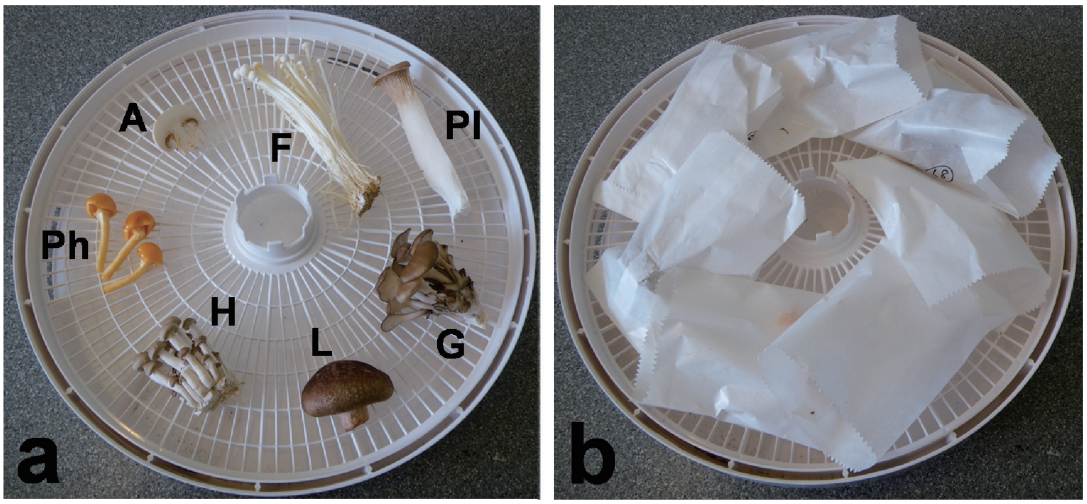

Fig. 1. Drying procedure using a food dehydrator. a. Fresh fruit bodies for drying without paper bags. See Table 1 for abbreviations. B. Fresh fruit bodies for drying in paper bags.

Materials and Methods

Sample Preparation

A total of seven species of edible mushrooms were purchased at the local supermarket on February 15, 2011. All of them were cultivated elsewhere in Japan, and none of the wild mushrooms were investigated in this study. The details of each species are summarized in Table 1.

Fresh fruit bodies of each species were dried using a food dehydrator (Snackmaster Express FD-60, Nesco/American Harvest, WI, USA). The use of food dehydrator is a desirable method for drying mushroom fruit bodies because it circulates warm/hot air for effective drying. The fruit bodies were cut in half (for *Agaricus*, *Lentinula*, and *Pleurotus*; Fig. 1a) prior to drying or dried as a whole (for *Flammulina*, *Grifola*, *Hypsizygus*, and *Pholiota*; Fig. 1a) depending on the size. The drying temperatures were set to 35, 52, and 71°C, respectively (Table 2). For each species at each temperature setting, fruit bodies were dried either with or without paper bag (Figs. 1a, b, Table 2). Drying with enclosed paper bag is a common practice when cross contamination of spores from different specimens is not desirable. This, however, may inhibit effective drying because little air circulation becomes available. The samples were dried for 48 hours. Dried samples were photographed and stored at −20°C until further experiments were conducted.

For each species, two additional treatments

Table 2. Experimental design using different drying temperatures

Experiment No.[1]	Treatment
1	Dried for 48 hours at 71°C without paper bag
2	Dried for 48 hours at 71°C enclosed in paper bag[2]
3	Dried for 48 hours at 52°C without paper bag
4	Dried for 48 hours at 52°C enclosed in paper bag
5	Dried for 48 hours at 35°C without paper bag
6	Dried for 48 hours at 35°C enclosed in paper bag
7	Stored in DMSO buffer[3] at room temperature until DNA extraction
8	Stored at −80°C until DNA extraction

[1] These numbers are shown in Figs. 3 and 4.
[2] See text and Fig. 1b for usage of paper bag.
[3] See Materials and Methods for description of buffer.

were made without drying process (Table 2). One treatment was to store fragments of fruit bodies in DMSO buffer. The composition of buffer was based on Seutin *et al.* (1991) with a minor modification as described by Hosaka and Castellano (2008) and Hosaka (2009). The buffer with sample fragments was stored at room temperature. The other treatment was to freeze fruit bodies at −80°C until further experiments were conducted.

DNA Preparation, PCR and Gel Electrophoresis

DNA extraction was conducted ca. 1 month after the drying procedure. Dried hymenia of ca. 25 mg (dry weight) were soaked in DMSO buffer for 1 hour prior to DNA extraction. DNA from each sample was extracted using a modified CTAB extraction protocol followed by glass milk purification methods as summarized by Hosaka (2009) and Hosaka and Castellano (2008). Briefly, samples were ground in liquid nitrogen using mortar and pestle, incubated in CTAB buffer at 65°C for 1 hour, and proteins were removed using the mixture of chloroform: isoamylalcohol (24 : 1). The materials were further purified using 6 M sodium iodine buffer with glass milk, washed with ethanol/buffer solution, and finally eluted in 100 μl of TE buffer. To assess the DNA quality, 5 μl of genomic DNA was loaded on 1% agarose gels stained with ethidium bromide and visualized under UV light. DNA was initially qualified and quantified using NanoDrop Spectrophotometer (ND-1000, Thermo Scientific, USA), but a small amount of carry-over sodium iodine buffer prevented an accurate measurement (data not shown). The measurement from spectrophotometer was therefore not recorded.

DNA was amplified for ribosomal DNA regions using three different combinations of primers. For amplifying the ITS region (ca. 600 bp), the primer combination of ITS5 and ITS4 (White *et al.,* 1990) was used. For amplifying longer fragments, the combination of ITS5 and LR7 (ca. 1600 bp; Vilgalys and Hester, 1990) or PNS1 (Hibbett, 1996) and LR7 (ca. 3500 bp) was used. In addition, single-copy protein coding genes were amplified for the following loci: RPB1, RPB2, and EF-α. The primer names and sequences were summarized in Table 3.

PCR reactions were carried out using 20 μl reaction volumes each containing: 1 μl genomic DNA, 1 μl dNTP, (4 mM), 1 μl of each primer (8 μM), 0.5 units of Taq polymerase (TaKaRa, Tokyo, Japan), 2 μl $MgCl_2$ (25 mM), 2 μl Bovine Serum Albumin (BSA). PCR products (1 μl) were electrophoresed in 1% agarose gels stained with ethidium bromide and visualized under UV light.

Results

Appearance of Specimens

After 48 hours, all specimens appeared completely dried. There were no signs of incomplete drying. Visual inspections of dried specimens, however, revealed some differences, depending

Table 3. Primers used in this study

	Sequence (5′→3′)	References
[rDNA]		
ITS5	GGAAGTAAAAGTCGTAACAAGG	White *et al.* (1990)
ITS4	TCCTCCGCTTATTGATATGC	White *et al.* (1990)
PNS1	CCAAGCTTGAATTCGTAGTCATATGCTTGTC	Hibbett (1996)
LR7	TACTACCACCAAGATCT	Vilgalys and Hester (1990)
[RPB1]		
gRPB1-Af	GAKTGTCCKGGWCATTTTGG	Matheny *et al.* (2002)
fRPB1-Cr	CNGCDATNTCRTTRTCCATRTA	Matheny *et al.* (2002)
[RPB2]		
fRPB2-5F	GAYGAYMGWGATCAYTTYGG	Liu *et al.* (1999)
bRPB2-7.1R	CCCATRGCYTGYTTMCCCATDGC	Matheny (2005)
[EF-1α]		
EF1-526F	GTCGTYGTYATYGGHCAYGT	Rehner and Buckley (2005)
EF1-2218R	ATGACACCRACRGCRACRGTYTG	Rehner and Buckley (2005)

on the treatment made (Table 2). The most striking difference was discoloration of specimens, especially the cut surface (Fig. 2). Although all specimens with any drying temperatures showed some degrees of discoloration, brownish discoloration was especially severe when specimens were dried at higher temperatures (52 and 71°C (Fig. 2).

Not all species, however, showed apparent discoloration. Dried specimens of *Grifola*, *Pholiota*, and *Pleurotus* did not change their colors regardless of drying temperatures, or only slightly so at the highest drying temperature (71°C) (Figs. 2b, d, e). On the other hand, dried specimens of *Lentinula*, *Flammulina*, *Hypsizygus*, and *Agaricus* showed apparent discoloration (Figs. 2a, c, f, g). The most striking was discoloration observed in *Flammulina*, which became slight pale brownish (at 35°C drying temperature; Fig. 2c) to brownish to reddish brown (at 52 and 71°C drying temperatures; Figs. 2b, c). Comparing with fresh fruit bodies with almost pure white coloration (Fig. 1a), it is apparent that dried specimens totally lost their original color. Although the degrees of discoloration were less severe, *Lentinula* (Fig. 2a), *Hypsizygus* (Fig. 2f), and *Agaricus* (Fig. 2g) all showed apparent discoloration that was otherwise not seen in fresh fruit bodies or dried specimens treated at low (35°C) temperature.

There were generally no clear differences between specimens dried with paper bags and the ones without such bags. However, one species (*Agaricus*) showed apparent discoloration (dark brown) only when specimens were dried in paper bags (Fig. 2h). In addition, discoloration in *Flammulina* seemed to be more severe when dried in paper bags. This difference may be due to slower drying when specimens were enclosed in paper bags. Although specimens were completely dried after 48 hours, we have not checked the status of specimens after shorter period of time (e.g., 6, 12, or 24 hours).

Besides discoloration, differences in shapes and sizes of fruit bodies were observed in some species. Fruit bodies of *Flammulina* have nearly straight stalks when fresh (Fig. 1a) or dried at lower temperatures, but they became curvy or almost curly when dried at higher temperatures (52 or 71°C) (Fig. 2c). At higher drying temperatures, fruit bodies show significant degrees of shrinking (Fig. 2c), that was also observed in *Hypsizygus* (Fig. 2f).

Genomic DNA

After DNA extraction and electrophoresis on agarose gels, all samples produced good quality DNA (Fig. 3). Although there were some differences in brightness of bands, they all showed

Fig. 2. Dried fruit bodies. a–g (from left to right). Fruit bodies dried at 35, 52, and 71°C, respectively. a. *Lentinula edodes*, dried in a paper bag. Note slight discoloration of the cut surface as drying temperature increases. b. *Grifola frondosa* dried without paper bag. c. *Flammulina velutipes* dried in paper bag. Note significant discoloration (brownish) of entire fruit bodies as drying temperature increases. d. *Pleurotus eryngii* dried in paper bag. e. *Pholiota nameko* dried without paper bag. f. *Hypsizygus marmoreus* dried without paper bag. Note slight discoloration and shrinking of fruit bodies as drying temperature increases. g. *Agaricus bisporus* dried with paper bag. Note slight discoloration of the cut surface as drying temperature increases. h. *Agaricus bisporus* dried at 71°C with (right) or without (left) paper bag. The specimen dried in paper bag has a significant discoloration. The same 10 cm scale was placed under fruit bodies in each photo.

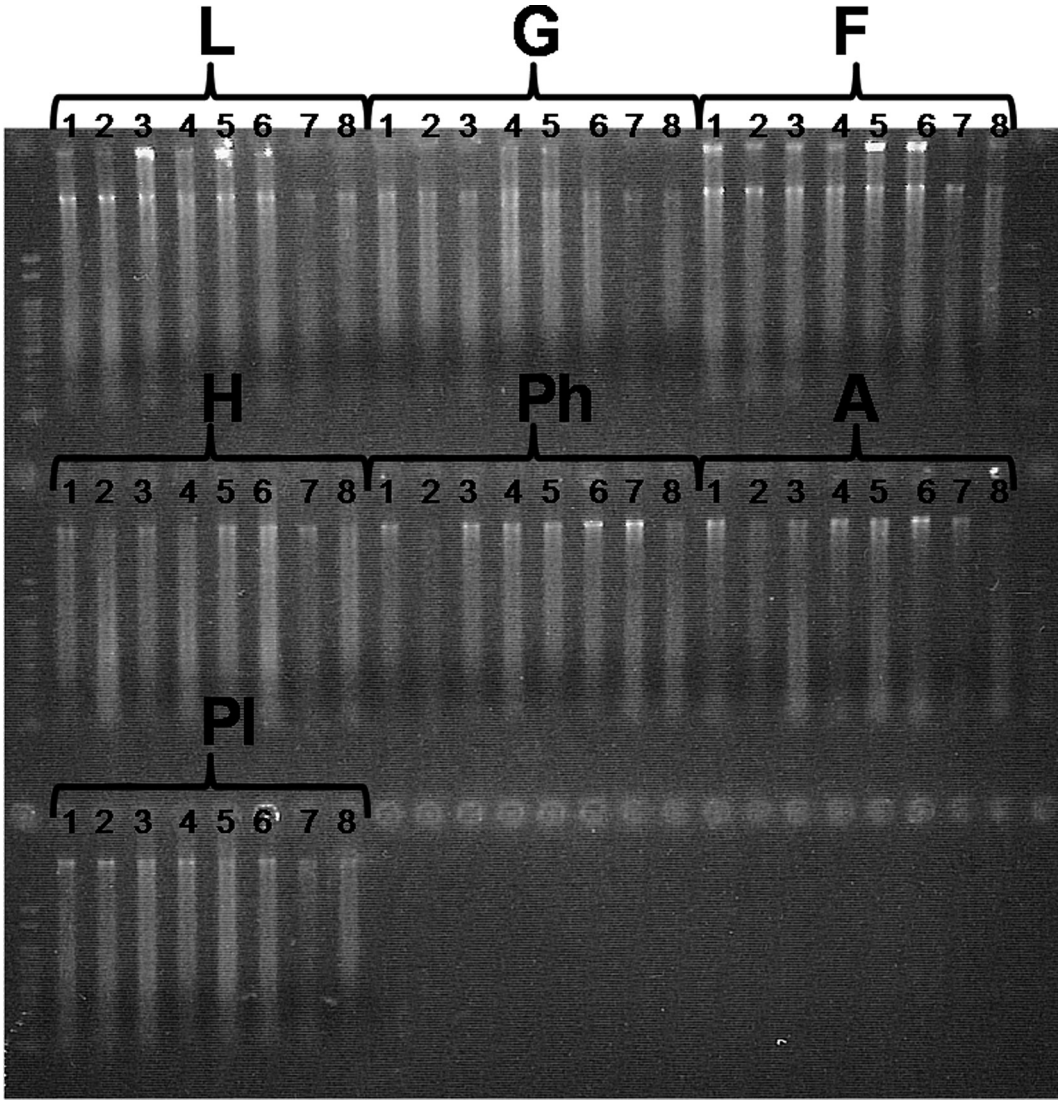

Fig. 3. Comparisons of genomic DNA from mushrooms with various treatments of drying temperatures. For abbreviations (L, G, F, H, Ph, A, and Pl), see Table 1. The numbers represent experimental design summarized in Table 1.

clear bands of large sizes. Presence of smears was apparent for all samples and no obvious differences were seen among different treatments. For most samples, DNA from frozen tissues (at −80°C) showed darker bands than DNA from dried specimens. Differences in brightness of bands appeared negligible among different drying temperatures.

PCR Amplifications

Most samples produced clear bright bands of PCR products with any combinations of primers (Fig. 4). Amplifications of ca. 600 bp (ITS; Fig. 4a) all produced bright single bands and there were absolutely no visible differences in brightness of bands. Amplification of the same ribosomal tandem repeat regions, but with much longer fragments, ca. 1600 bp between ITS5-LR7 (Fig.

4b) and ca. 3500 bp between PNS1-LR7 (Fig. 4c), also showed absolutely no visible differences. The remaining three loci were single-copy protein coding genes (RPB1, Fig. 4d; RPB2, Fig. 4e; EF-α, Fig. 4f). Some weak bands were observed in *Hypsizygus* (Fig. 4d, e; both dried at 71 or 52°C in paper bags) and *Grifola* (Fig. 4e; dried at 52°C without paper bag). Other differences were probably negligible.

Discussion

Despite their importance as genetic resources, herbarium specimens have been prepared and maintained in a variety of ways (Bruns *et al.*, 1990). For example, there is no set standard on how specimens should be dried. The only agreement is that specimens should generally be dried quickly but at lower temperature (Pyle and Adams, 1989; Bruns *et al.*, 1990; Taylor and Swann, 1994; Johnson *et al.*, 2003; Nagy, 2010). It is often claimed that specimens should be dried at 42°C or lower temperature (Taylor and Swann, 1994; Drábková *et al.*, 2002; Johnson *et al.*, 2003) or drying temperature should not exceed 60°C (Nagy, 2010). However, there seems to be no scientific theory behind it because specimens dried at much higher temperature often yield high quality DNA (Blanco *et al.*, 2006; Erkens *et al.*, 2008). The present study also agrees that DNA in specimens dried at high temperature (up to 71°C) was not significantly damaged.

The appearance of specimens varied significantly depending on drying temperatures and presence/absence of paper bags (Fig. 2). This implies that to keep the visual appearance of the specimens, which itself is critical for subsequent taxonomic studies, lower drying temperatures should be used. However, the physical condition of specimens does not necessarily indicate the quality of DNA (Dawson *et al.*, 1998). The same is true for the "greenness" of leaves in plant specimens. Although it is generally assumed that the greenness of leaves depends on how quickly specimens were dried, there was no correlations between the greenness of leaves and the quality of DNA (Erkens *et al.*, 2008). Our study is consistent with their findings that specimens with severe discoloration (Figs. 2c, g, h) yielded equally high quality DNA (Figs. 3, 4).

Biological processes do not stop after specimens are collected. The activity of nucleases is especially problematic because it quickly breaks down DNA molecules to short fragments (Hummel and Herrmann, 1994; Dawson *et al.*, 1998). To stop such enzymatic activities, the elimination of water from specimens should be performed as rapidly as possible (Bruns *et al.*, 1990; Nagy, 2010). In this regard, drying temperature for mushroom specimens should be set high, up to 71°C, to preserve the quality of DNA. We have not tested higher temperatures than 71°C so no conclusions can be drawn on how high drying temperatures can be. It can, however, easily be assumed that very high temperatures (e.g., 100°C or higher) simply burn the samples and probably damage the DNA as well.

Some species of mushrooms (*Grifola* and *Hypsizygus*) showed slightly weaker PCR bands when specimens were dried at 52 or 71°C (Fig. 4). The influence on the presence of enclosing paper bag was not apparent because such a treatment sometimes resulted in weak bands (Fig. 4d) but stronger bands were also observed (Fig. 4e). In this study, we have not prepared replication of each species and we cannot conclude whether the infrequent presence of weak PCR bands is truly due to high drying temperatures. We argue it is probably not the reason because the same species dried at the highest temperature (71°C) in this study showed strong bands (Fig. 4). It is also interesting to note that the frozen samples often yielded less genomic DNA (Fig. 3). We believe it is due to DNA condensation as shown by Johnson *et al.* (2003).

In this study, we have tested the quality of DNA in freshly dried materials. Since DNA in dried specimens degrades with time (Johnson *et al.*, 2003; Erkens *et al.*, 2008), the influence of the storage conditions also needs to be investigated. Specifically, the influence of temperature, humidity, fumigation, and UV light, which are all

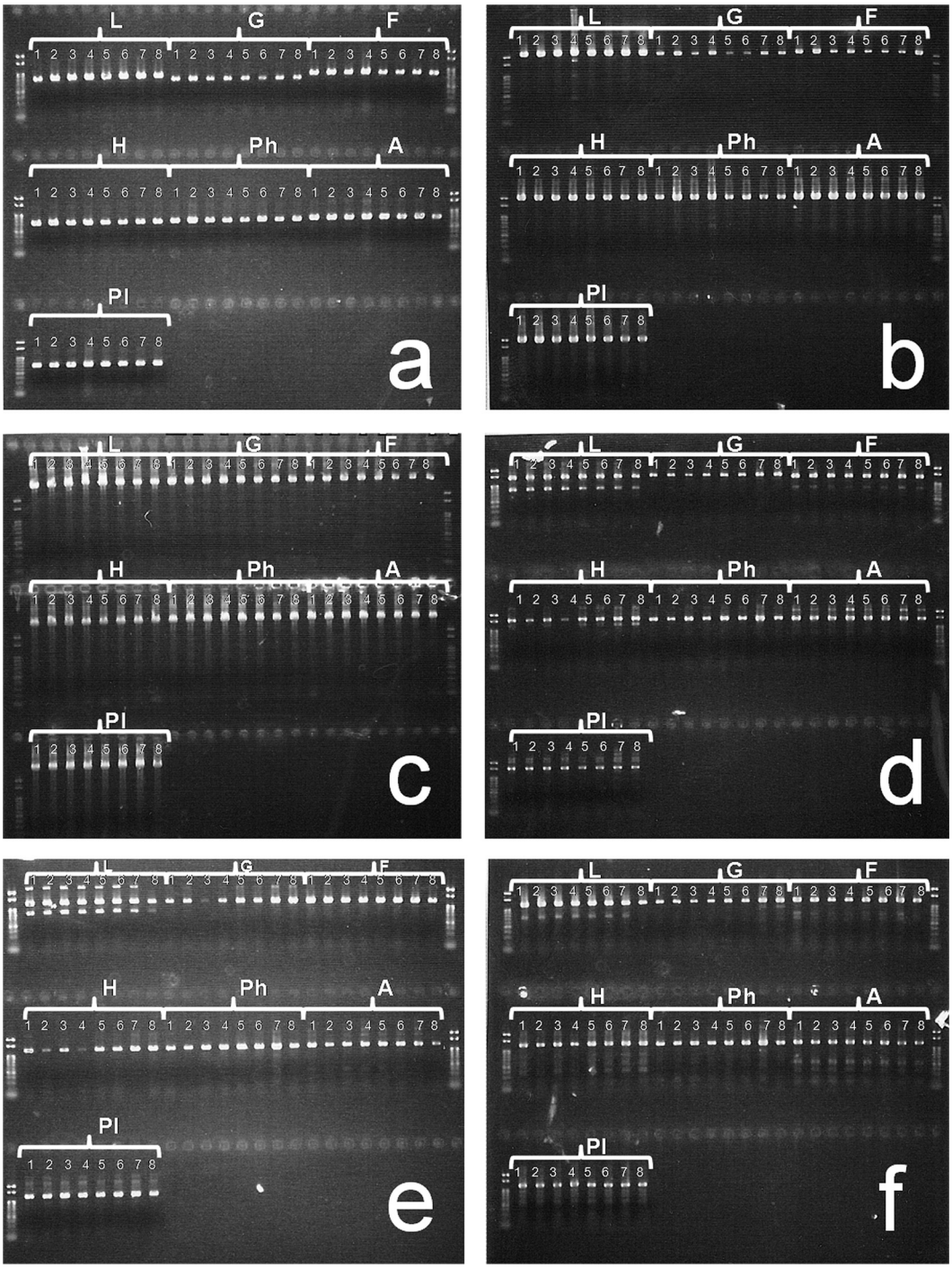

Fig. 4. Amplifications of different loci from mushrooms with various treatments of drying temperatures. For abbreviations (L, G, F, H, Ph, A, and Pl), see Table 1. The numbers represent experimental design summarized in Table 1. a. ITS (ITS5 and ITS4). b. ITS5 and LR7. c. PNS1 and LR7. d. RPB1 (gRPB1-Af and fRPB1-Cr). e. RPB2 (fRPB2-5F and bRPB2-7.1R). f. EF-α (EFa-526F and EF1-2218R). See Table 3 for primer names and sequences.

known to cause DNA degradation, deserves rigorous investigation. In addition, this study has tested only seven species of mushrooms. Some mushrooms of fruit bodies with distinct morphology, such as boletes, gasteromycetes, and conks were not included. However, we believe the conclusion is applicable to many other untested species of mushrooms as long as specimens are dried rapidly. To achieve this, species with big, fleshy fruit bodies need to be cut into thin slices prior to drying.

Our suggestions based on the results of this study are following:

(1) To obtain high quality DNA while maintaining the visual appearance of specimens, drying temperatures should be set low (35°C in this study) as long as specimens can be dried rapidly (within 48 hours).

(2) If drying process takes longer, use higher temperatures (up to 71°C) or cut fruit bodies into thin slices.

(3) Use of paper bags for big, fleshy mushrooms can cause slower drying and therefore should be avoided.

(4) Field mycologists without storage buffers at hands should dry specimens as quickly as possible (regardless of temperatures) for preserving high quality DNA.

(5) DNA from dried specimens should be extracted as soon as possible or pieces of specimens should be stored in freezer until DNA extraction.

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References

Blanco, M. A., Whitten, W. M., Penneys, D. S., Williams, N. H., Neubig, K. M. and Endara, L. 2006. A simple and safe method for rapid drying of plant specimens using forced-air space heaters. Selbyana 27: 83–87.

Brock, P. M., Döring, H. and Bidartondo, M. I. 2008. How to know unknown fungi: the role of a herbarium. New Phytologist 181: 719–724.

Bruns, T. D., Fogel, R. and Taylor, J. W. 1990. Amplification and sequencing of DNA from fungal herbarium specimens. Mycologia 82: 175–184.

Dawson, M. N., Raskoff, K. A. and Jacobs, D. K. 1998. Field preservation of marine invertebrate tissue for DNA analyses. Molecular Marine Biology and Biotechnology 7: 145–152.

Dentinger, B. T. M., Margaritescu, S. and Moncalvo, J.-M. 2010. Rapid and reliable high-throughput methods of DNA extraction for use in barcoding and molecular systematics of mushrooms. Molecular Ecology Resources 10: 628–633.

Drábková, L., Kirschner, J. and Vlček, Č. 2002. Comparison of seven DNA extraction and amplification protocols in historical herbarium specimens of Juncaceae. Plant Molecular Biology Reporter 20: 161–175.

Erkens, R. H. J., Cross, H., Maas, J. W., Hoenselaar, K. and Chatrou, L. W. 2008. Assessment of age and greenness of herbarium specimens as predictors for successful extraction and amplification of DNA. Blumea 53: 407–428.

Fukatsu, T. 1999. Acetone preservation: a practical technique for molecular analysis. Molecular Ecology 8: 1935–1945.

Hibbett, D. S. 1996. Phylogenetic evidence for horizontal transmission of group I introns in the nuclear ribosomal DNA of mushroom-forming fungi. Molecular Biology and Evolution 13: 903–917.

Hosaka, K. 2009. Phylogeography of the genus *Pisolithus* revisited with some additional taxa from New Caledonia and Japan. Bulletin of the National Museum of Nature and Science, Series B 35: 151–167.

Hosaka, K. and Castellano, M. A. 2008. Molecular phylogenetics of Geastrales with special emphasis on the position of *Sclerogaster*. Bulletin of the National Museum of Nature and Science, Series B 34: 161–173.

Hosaka, K., Kasuya, T., Reynolds, H. T. and Sung. G.-H. 2010. A new record of *Elaphomyces guangdongensis* (Elaphomycetaceae, Eurotiales, Fungi) from Taiwan. Bulletin of the National Museum of Nature and Science, Series B 36: 107–115.

Hummel, S. and Herrmann, B. 1994 General aspects of sample preparation. In: Herrmann, B. and Hummel, S. (eds.), Ancient DNA, pp. 59–68, Springer, New York.

Johnson, E. L., Kim, S.-H. and Emche, S. D. 2003. Stor-

age effects on genomic DNA in rolled and mature coca leaves. Biotechniques 35: 310–316.

Kigawa, R., Nochide, H., Kimura, H. and Miura, S. 2003. Effects of various fumigants, thermal methods and carbon dioxide treatment on DNA extraction and amplification: a case study on freeze-dried mushroom and freeze-dried muscle specimens. Collection Forum 18: 74–89.

Laulier, M., Pradier, E., Bigot, Y. and Périquet, G. 1995. An easy method for preserving nucleic acids in field samples for later molecular and genetic studies without refrigerating. Journal of Evolutionary Biology 8: 657–663.

Leino, M. W., Hagenblad, J., Edqvist, J. and Strese, E. K. 2009. DNA preservation and utility of a historic seed collection. Seed Science Research 19: 125–135.

Liu, Y. J., Whelen, S. and Hall, B. D. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16: 1799–1808.

Matheny, P. B. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*; Agaricales). Molecular Phylogenetics and Evolution 35: 1–20.

Matheny, P. B., Liu, Y. J., Ammirati, J. F. and Hall, B. D. 2002. Using RPB1 sequences to improve phylogenetic inferences among mushrooms (*Inocybe*, Agaricales). American Journal of Botany 89: 688–698.

Nagy, Z. T. 2010. A hands-on overview of tissue preservation methods for molecular genetic analyses. Organisms Diversity & Evolution 10: 91–105.

Nagy, L. G., Petkovits, T., Kovács, G. M., Voigt, K., Vágvölgyi, C. and Papp, T. 2011. Where is the unseen fungal diversity hidden? A study of *Mortierella* reveals a large contribution of reference collections to the identification of fungal environmental sequences. New Phytologist DOI: 10.1111/j.1469-8137.2011.03707.x

Pyle, M. M. and Adams, R. P. 1989. *In situ* preservation of DNA in plant specimens. Taxon 38: 576–581.

Rehner, S. A. and Buckley, E. 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97: 84–98.

Rogstad, S. H. 1992. Saturated NaCl-CTAB solution as a means of field preservation of leaves for DNA analyses. Taxon 41: 701–708.

Seutin, G., White, B. N. and Boag, P. T. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69: 82–90.

Taylor, J. W. and Swann, E. C. 1994. 11 DNA from herbarium specimens. In: Herrmann, B. and Hummel, S. (eds.), Ancient DNA, pp. 166–181, Springer, New York.

Vilgalys, R. and Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246.

White, T. J., Bruns, T., Lee, S. and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (eds.), PCR Protocols, pp. 315–322, Academic Press, New York.

Whitten, W. M., Williams, N. H. and Glover, K. V. 1999. Sulphuryl fluoride fumigation: effect on DNA extraction and amplification from herbarium specimens. Taxon 48: 507–510.