

Introducing DNA-based methods to compare fungal microbiota and concentrations in indoor, outdoor, and personal air

Choa An · Cheolwoon Woo · Naomichi Yamamoto 

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Abstract Inhalation of airborne fungi is known to cause respiratory illnesses such as allergies. However, the association between exposure and health outcomes remains largely unclear, in part due to lack of knowledge about fungal exposure in daily life. This study aims to introduce DNA-based methods such as high-throughput sequencing (HTS) and quantitative polymerase chain reaction (qPCR) to compare fungal microbiota and concentrations in indoor, outdoor, and personal air. Five sets of concurrent indoor, outdoor, and personal air samples were collected, each with duration of 4 days. Sequencing analysis revealed greater species richness in personal than indoor air for four out of the five sets, indicating that people are exposed to outdoor species that are not in indoor air. The personal–indoor (*P/I*) and personal–outdoor (*P/O*) ratios of total fungi were 1.2 and 0.15, respectively, suggesting that personal exposure to total fungi is better represented by indoor than outdoor concentrations. However, the ratios were taxon dependent, highlighting the complexity of generalizing personal exposure to the diverse kingdom Fungi. These results

demonstrate that the HTS/qPCR method is useful for assessing taxon-specific fungal exposure, which might be difficult to achieve effectively using conventional, non-DNA-based techniques.

Keywords Bioaerosols · Mold · Next-generation sequencing · Mycobiota · Aerobiology

1 Introduction

The kingdom Fungi is diverse with an estimated 1.5 million unique species (Hawksworth 2001), being found ubiquitously in both indoor and outdoor environments (Amend et al. 2010; An and Yamamoto 2016; Fröhlich-Nowoisky et al. 2009; Tedersoo et al. 2014). An estimated 28–50 Tg of fungal material is released into the global atmosphere each year (Elbert et al. 2007; Heald and Spracklen 2009). Deposition of allergenic fungal spores in the respiratory system results in immunoglobulin E (IgE)-mediated type I hypersensitivity in sensitized individuals (Denning et al. 2006). To date, around 150 allergenic fungal species have been reported (Simon-Nobbe et al. 2008), and 3–10 % of the global population are thought to be sensitized to them (Horner et al. 1995). However, the association between fungal exposure and health outcomes remains largely unclear (Mendell et al. 2011). To elucidate the elusive mechanisms

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C. An · C. Woo · N. Yamamoto (✉)
Department of Environmental Health Sciences, Graduate
School of Public Health, Seoul National University,
Seoul 08826, South Korea
e-mail: nyamamoto@snu.ac.kr

underlying fungal allergies, it is important to characterize how people are exposed to airborne fungi in daily life.

People are exposed to airborne fungi both indoors and outdoors, since people spend time in both of these environments (Klepeis et al. 2001; Yang et al. 2011). In exposure assessments for airborne contaminants, personal exposure is often assessed based on personal sampling (Shinohara et al. 2004; Yamamoto et al. 2007, 2015b). An advantage of personal sampling is its representativeness of personal exposure, since sampling is conducted continuously in the proximity of a human subject; For instance, Adhikari et al. (2004) used the Button Sampler for personal air sampling followed by microscopic analyses for fungi collected in agricultural confinements. Lee et al. (2006a) used a system consisting of a respirator and sampling probe for personal air sampling followed by microscopic and growth-based analyses for fungi collected in agricultural environments. Lee and Liao (2014) used the same system for personal air sampling in agricultural environments and analyzed the collected samples for (1→3)- β -D-glucan by limulus amoebocyte lysate assay. However, few studies have quantified fungal exposure in daily life (Mitakakis et al. 2000). Instead, studies have quantified exposure in specific occupational settings (Adhikari et al. 2004; Eduard et al. 2001; Lee et al. 2006a; Lee and Liao 2014; Madsen 2006). Thus, more studies are needed to assess exposure to airborne fungi in daily life.

Traditionally, culture-based methods have been used to quantify airborne microorganisms (Andersen 1958; Shelton et al. 2002; Takahashi 1997; Yao and Mainelis 2007). However, such methods have several disadvantages. First, they are biased by selection of culture media (Takahashi 1997) and incapable of detecting fastidious species. Literature reports that only 10–40 % of airborne fungi are culturable (Palmgren et al. 1986; Peccia and Hernandez 2006). Moreover, dead and dormant microorganisms are undetectable even though they may retain biologically active materials such as allergenic proteins or toxic secondary metabolites (Kauffman and van der Heide 2003). Alternatively, chemical or immunological assays can be used to detect specific fungal components. However, these methods suffer from lack of sensitivity and specificity, and are incapable of species identification (Pasanen 2001).

Emerging DNA-based techniques can circumvent such disadvantages; For instance, such methods are capable of detecting fastidious species. Additionally, they can detect dead and dormant microorganisms, as long as DNA is present. Polymerase chain reaction (PCR) is a technique to amplify DNA markers of interest, being highly sensitive down to detection of one fungal spore from environmental samples (Yamamoto et al. 2011). To analyze fungal diversity, PCR-based approaches such as cloning followed by Sanger sequencing and nucleic acid hybridization-based technologies such as DNA microarrays have been used (Mitchell and Zuccaro 2006). In the last decade, high-throughput sequencing (HTS) technologies have been introduced to analyze fungal communities in environments including soil (Tedersoo et al. 2014) and house dust (Amend et al. 2010) and revealed high diversity of fungi in indoor and outdoor air (Adams et al. 2013; Lee et al. 2016; Yamamoto et al. 2012, 2015a). HTS is not quantitative, but instead provides relative fractions of taxa in a population. However, HTS can be combined with quantitative PCR (qPCR) to provide taxon-specific concentrations and aerodynamic diameters of airborne fungi (Dannemiller et al. 2014a; Yamamoto et al. 2014). Alternatively, taxon-specific PCR or qPCR assays are available (Cruz-Perez et al. 2001; Halstensen 2008; Vesper 2011; Wu et al. 2002), but their taxonomic coverage is limited, since each assay requires its own primers and probe(s). We hypothesized that the HTS/qPCR method would be useful to achieve broader coverage of taxon-specific assessment of personal exposure to airborne fungi.

This study aims to introduce the HTS/qPCR method to compare fungal communities and concentrations in indoor, outdoor, and personal air. The HTS/qPCR method is expected to determine representative exposure to fungi in daily life; For instance, traditional impactor sampling followed by culture-based detection suffers from short sampling duration to avoid particle overloading and reduction of microbial viability. Moreover, impactors are not easily portable and are therefore not suitable for personal sampling. Use of air filter sampling followed by DNA-based analyses overcomes such difficulties and provides deep insights into how people are exposed to airborne fungi in daily life.

2 Materials and methods

2.1 Air sampling

Five sets of concurrent indoor, outdoor, and personal air samples were collected from February to October 2015 for a human subject who is a researcher of this study. Meteorological conditions during the sampling periods are summarized in Table S1. Each set of concurrent air samples was collected for duration of 4 days during weekdays. Each air sample was collected on a 25-mm membrane air filter (1.2- μm pore size; Zefon International, Inc., USA) loaded into the inhalable IOM sampler (SKC Inc., PA, USA) (Mark and Vincent 1986) connected to a minipump with air flow rate adjusted at 21 min^{-1} . MP- Σ 300, MP- Σ 300NII, and MP- Σ 150S minipumps (Sibata Scientific Technology, Ltd., Tokyo, Japan) were used for indoor, outdoor, and personal air samplings, respectively. The MP- Σ 150S minipump for personal air sampling has an internal rechargeable battery that can last at least 8 h without power supply from an AC adapter. The MP- Σ 300NII minipump is a successor to the MP- Σ 300 minipump, with similar specifications in terms of air flow rate and requirement for a stationary power source.

Indoor air samplings were conducted at height of 1 m from the floor in the bedroom of the subject's apartment on the third floor of the building. Outdoor air samplings were performed by attaching the IOM sampler to a window fence by extending tubing from the pump placed inside the apartment. Personal air samplings were conducted by bringing the sampler with the subject in a business bag, from which the IOM sampler was extended via tubing (Yamamoto et al. 2007). As far as possible, the sampler was brought to rooms and locations where the subject was present. During sleep, the sampler was placed in the subject's bedroom. The battery was recharged using a QC-10N quick charger (Sibata Scientific Technology) when the subject was in a room where a power source was available. Time activity logs were kept throughout the sampling periods (Fig. S1). On average, 60, 35, and 5 % of the time was spent at home, in other indoor environments such as office, and outdoors, respectively. The filters were brought to the laboratory after air sampling, and stored at $-20\text{ }^{\circ}\text{C}$ until DNA extraction.

This study involved a human subject who is a researcher of this study. The study was not invasive and did not involve biomarker collection. Therefore,

approval by the institutional review board was not required by Seoul National University.

2.2 DNA extraction and sequencing

DNA was extracted according to a protocol reported elsewhere (Hospodsky et al. 2010). Briefly, the PowerMax[®] soil DNA isolation kit (Mobio Laboratory, Carlsbad, CA, USA) was used with some modification. A one-quarter section of each sampled air filter was cut using sterile scissors and enclosed in a 2-ml tube containing the kit's power beads (1.0 g) and extraction solution along with additional 0.1-mm-diameter glass beads (300 mg) and 0.5-mm-diameter glass beads (100 mg). The samples were homogenized for 3 min using a bead beater (BioSpec Products, Bartlesville, OK, USA), from which DNA was subsequently extracted, purified, and eluted into 30 μl TE [10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0] in accordance with the kit protocol.

DNA libraries were prepared according to the method reported by An and Yamamoto (2016). Internal transcribed spacer 1 (ITS1) region was PCR-amplified using universal fungal primers ITS1F and ITS2 (Gardes and Bruns 1993; White et al. 1990) attached with adapter sequences for Illumina MiSeq. Only ITS1 was sequenced in this study because full-length ITS might be too long for $2 \times 300\text{ bp}$ paired-end sequencing by Illumina MiSeq. The amplicons were purified using AMPure XP beads (Beckman Coulter, Inc., Brea, CA, USA). The amplicons were indexed using the Nextera XT Index kit (Illumina, Inc., San Diego, CA, USA), purified using AMPure XP beads, normalized to 4 nM in 10 mM Tris HCl (pH 8.5), and pooled with internal control PhiX (30 %). The pooled amplicons were loaded into a v3 600 cycle-kit reagent cartridge (Illumina), and $2 \times 300\text{ bp}$ paired-end sequencing was carried out by Illumina MiSeq. Raw sequence data were submitted to the Sequence Read Archive of the National Center for Biotechnology Information (NCBI) under project number SRP101537.

2.3 DNA sequence processing and analyses

DNA sequence processing and analyses were done by the method reported by An and Yamamoto (2016). Sequences with read quality score below 20 were removed using MiSeq Reporter v2.5 (Illumina).

Poly(N)-containing sequences due to reverse adaptor sequences or ambiguous base calls were further excluded using Trimmomatic-0.33 (Bolger et al. 2014). The read 1 and 2 sequences were joined with minimum allowed overlap of 10 bp ($-j\ 10$) in QIIME (Caporaso et al. 2010). Sequences shorter than 100 bp were excluded by tool filter sequences as part of Galaxy tool version 16.07 (Blankenberg et al. 2010; Giardine et al. 2005; Goecks et al. 2010). Chimeric sequences were identified using a reference database containing named fungal ITS sequences (Nilsson et al. 2009) and removed using the “chimera.uchime” command (Edgar et al. 2011) in mothur v.1.35.1 (Schloss et al. 2009). A total of 485,582 valid sequences were obtained, with numbers ranging from 10,680 to 75,624 reads per library (Table S2).

The resultant sequences were taxonomically assigned using BLASTN2.2.28+ (Altschul et al. 1990) against the fungalITSdatabaseID.fasta database (Nilsson et al. 2009) and classified by FHiTHINGS (Dannemiller et al. 2014b). For taxonomic assignment, the sequences were not clustered into operational taxonomic units (OTUs), since no consensus threshold is available for fungal ITS (Yamamoto and Bibby 2014). Identification was made only down to genus level because of possible ambiguity of identification down to species level (Lee and Yamamoto 2015). For diversity analyses, the sequences were clustered into OTUs based on 97 % sequence similarity (Buée et al. 2009; O'Brien et al. 2005; Yamamoto et al. 2012). From each library, 10,680 sequences were subsampled, which was the smallest number of sequences per library (Table S2). For α diversity analysis, the observed numbers of OTUs were determined using mothur v.1.35.1. For β diversity analyses, the Jaccard indices (community membership) and Yue and Clayton theta similarity coefficients (community structure) were calculated. Using these distance indices, the parsimony test (P test) was performed using mothur v1.35.1.

2.4 Quantitative PCR

Quantitative PCR (qPCR) was performed to quantify gene copy numbers (GCNs) of fungal ITS (Kumari et al. 2016). The same primers as used for the high-throughput sequencing analyses (i.e., ITS1F and ITS2) were used for qPCR. Each of the 20- μ l PCR mixtures contained 1 $\times$ Fast SYBR Green Master Mix reagent (Clontech Laboratories, Inc., Mountain View, CA,

USA), 10 μ M forward and reverse primers, and 1 μ l template DNA. A 7300 real-time PCR system (Applied Biosystems, Inc., Foster City, CA, USA) was used. Standard curves were generated based on the ITS1 amplicon with known GCN concentration. The standard was prepared by amplifying the ITS1 regions of the DNA extract from *Aspergillus fumigatus* ATCC MYA4609 by using the universal primers ITS1F and ITS2 with conventional PCR. The concentration of the standard was quantitated using the Quant-iT PicoGreen dsDNA reagent kit (Life Technologies, Carlsbad, CA, USA). Tenfold serial dilutions of the standard from 1 to 10^6 GCN μ l $^{-1}$ were used to calibrate qPCR. Each qPCR measurement was triplicated, and PCR inhibition was checked using the method reported by Hospodsky et al. (2010). No inhibition was found. DNA extraction efficiency of 10 % was assumed to calculate GCN per 1 m ^{3} of air (Hospodsky et al. 2010).

3 Results

3.1 Alpha diversity

Species richness was characterized in terms of the numbers of observed OTUs based on the ITS sequences at 97 % similarity (Fig. 1). The numbers ranged from 78 to 138 for indoor air, from 139 to 299 for outdoor air, and from 60 to 196 for personal air. The mean numbers were 192, 130, and 116 for outdoor, personal, and indoor air, respectively. Species richness was greater in outdoor than indoor air for all five sets of samples. Similarly, species richness was greater in personal than indoor air for four out of the five sets of air samples.

3.2 Beta diversity

The similarities of fungal communities across samples are shown in Fig. 2. Fungal community structures as characterized by the Yue and Clayton theta similarity coefficients appeared to pattern by sampling location, whereas fungal community memberships characterized by the Jaccard indices appeared to pattern by sampling date. However, no significance was confirmed for these patterns ($p > 0.05$, P test).

3.3 Taxonomic compositions

In each library, 14–32 % of the sequences were ambiguous at genus rank, which might be in part

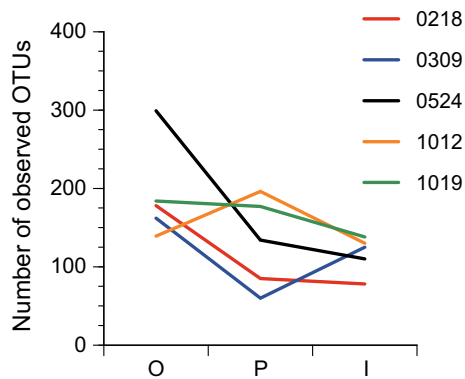


Fig. 1 Numbers of observed OTUs based on ITS sequences at 97 % similarity. Results based on 10,680 sequences subsampled from each library. Legends indicate sampling start dates. O outdoor; I indoor; P personal

because only ITS1 was sequenced and analyzed. Nonetheless, a total of 493 fungal genera were detected. Relative abundances of the 40 most abundant genera are shown in Fig. 3. These 40 genera represent 54 % of all high-quality sequences analyzed. The 20 most abundant genera and their mean relative abundances for all samples were *Pseudocercospora* (7.9 %),

Catenulostroma (4.2 %), *Alternaria* (4.1 %), *Fusarium* (3.6 %), *Cladorrhinum* (2.5 %), *Microcyclospora* (2.5 %), *Piedraia* (2.5 %), *Phoma* (2.3 %), *Cryptococcus* (2.2 %), *Teratosphaeria* (2.1 %), *Monodictys* (1.7 %), *Chaetomium* (1.6 %), *Mortierella* (1.4 %), *Hygrophorus* (1.3 %), *Preussia* (0.97 %), *Bullera* (0.79 %), *Acremonium* (0.77 %), *Aspergillus* (0.75 %), *Pseudallescheria* (0.69 %), and *Eudarlucella* (0.67 %).

3.4 Concentrations

Total fungal concentrations were quantified by universal fungal qPCR (Fig. 4). The geometric mean concentrations of all five sets of measurements were 9.3×10^4 , 1.4×10^4 , and 1.3×10^4 GCN m^{-3} for outdoor, personal, and indoor air, respectively. The outdoor concentrations were notably high in the samples collected from May 24 and October 12, i.e., 2.7×10^6 and 5.4×10^5 GCN m^{-3} , respectively.

3.5 I/O, P/O, and P/I ratios

The indoor–outdoor (I/O), personal–outdoor (P/O), and personal–indoor (P/I) ratios of total fungal concentrations for the five sets of measurements are shown in Fig. 5. The geometric means of the I/O, P/O, and P/I ratios were 0.12, 0.15, and 1.2, respectively. The genus-specific ratios were also determined by using the HTS/qPCR method (Dannemiller et al. 2014a) (Fig. 6). Only 4 out of the 22 selected genera showed P/O ratio in the range of 0.5–2. Meanwhile, 12 out of the 22 genera showed P/I ratio in the range of 0.5–2. However, the remaining 10 genera showed P/I ratio outside the range of 0.5–2.

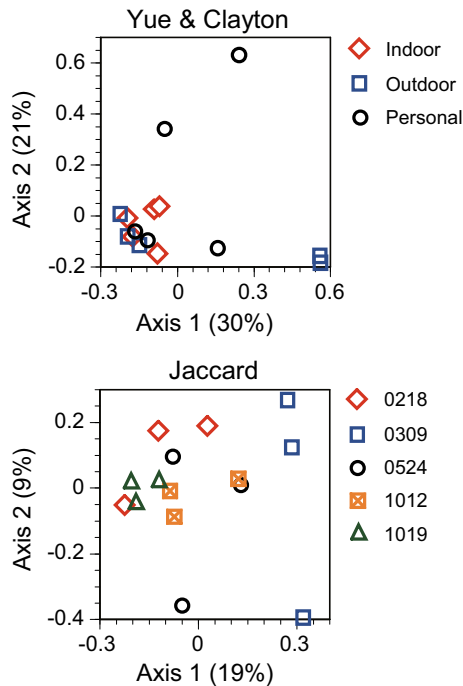


Fig. 2 Principal coordinate analysis plots based on Jaccard indices and Yue and Clayton theta similarity coefficients based on OTUs with ITS sequences at 97 % similarity

4 Discussion

DNA-based methods were introduced to compare fungal biota and concentrations in indoor, outdoor, and personal air. Each sample was collected for duration of 4 days, remarkably longer than in reported fungal exposure studies (Adhikari et al. 2004; Eduard et al. 2001; Lee et al. 2006a; Lee and Liao 2014; Madsen 2006). Since the subject carried the sampler for the entire durations, the results reported herein are likely spatiotemporally representative of his exposure to airborne fungi in daily life.

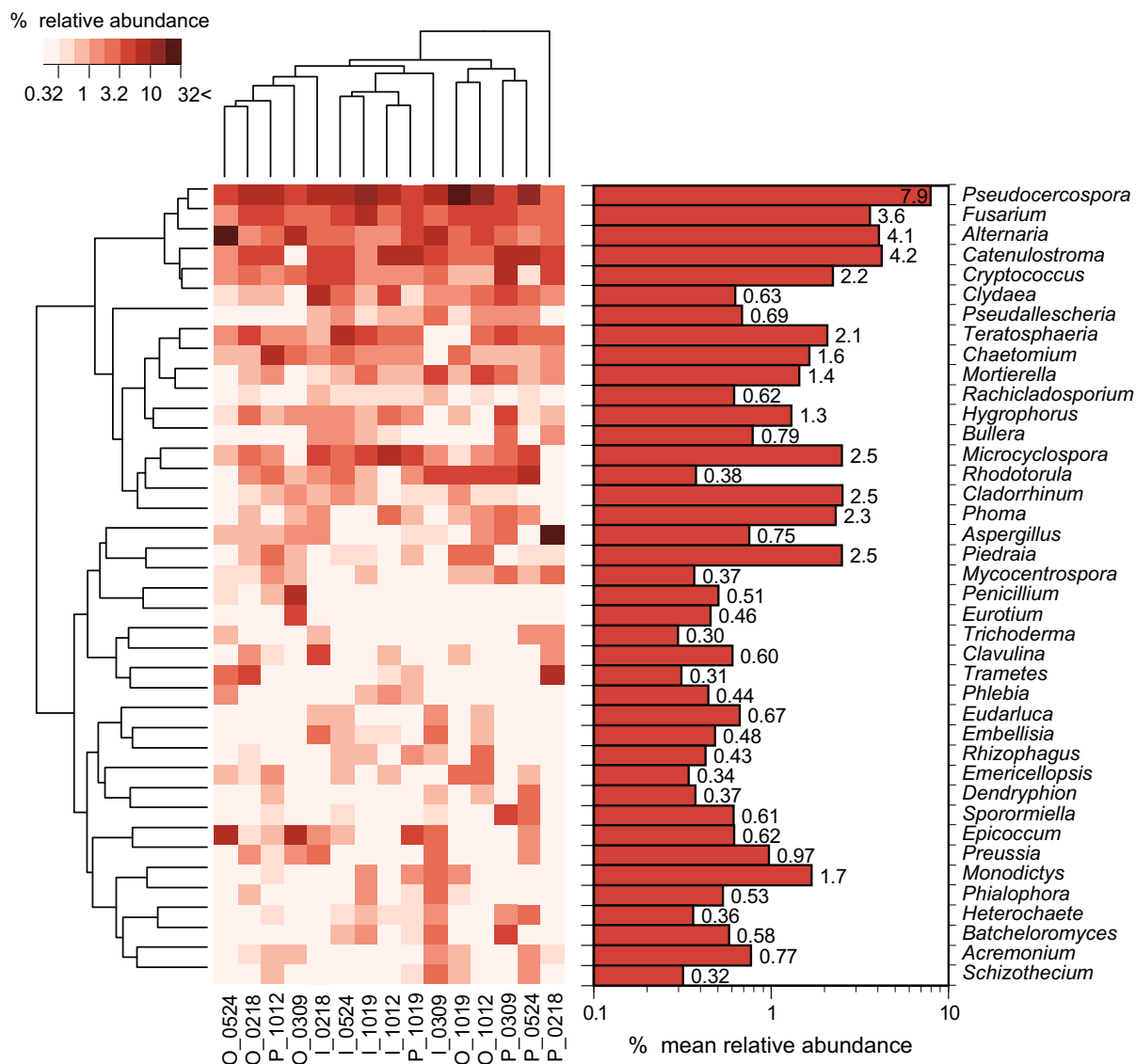


Fig. 3 Fungal compositions and relative abundance of the 40 most abundant fungal genera. Taxa shown represent 54 % of all high-quality sequences analyzed. Mean relative abundances

across all samples are calculated for each genus. Euclidean distances based on log-transformed relative abundance values are shown

We used qPCR to determine fungal concentrations in indoor, outdoor, and personal air. The concentrations were based on GCN of ITS1, from which the number of fungal spores cannot be obtained due to strain-dependent variation in the number of ITS copies per genome (Herrera et al. 2009; O'Donnell and Cigelnik 1997), strain-dependent variation in the number of nuclei per cell (Yuill 1950), and strain-dependent variation in the number of cells per spore (Pringle 2013). Nonetheless, we could determine *I/O*,

P/O, and *P/I* ratios of GCN-based airborne fungal concentrations. The *I/O*, *P/O*, and *P/I* ratios for total fungi were 0.12, 0.15, and 1.2, respectively (Fig. 5). Lee et al. (2006b) reported a geometric mean of *I/O* equal to 0.32 for airborne fungal spores, based on microscopic enumeration, collected from five occupied and one mostly unoccupied home in the Cincinnati metropolitan area in the USA. Haas et al. (2014) reported *I/O* ratios ranging between 0.2 and 2.0 in homes in Austria based on culture techniques. Using

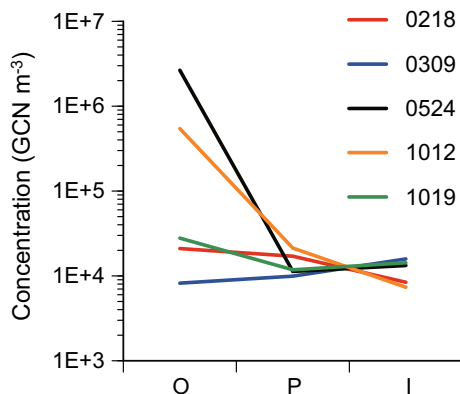


Fig. 4 Indoor, outdoor, and personal concentrations of airborne total fungi measured by universal fungal qPCR. *Legends* indicate sampling start dates. *O* outdoor; *I* indoor; *P* personal

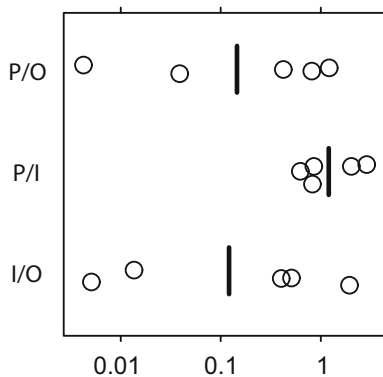


Fig. 5 Distributions of *I/O*, *P/O*, and *P/I* ratios of airborne total fungal concentrations. *Lines* indicate geometric means of the ratios of the five sets of measurements

culture-independent qPCR, Yamamoto et al. (2015a) reported *I/O* ratios of 0.21 and 0.89 under vacant and occupied indoor conditions, respectively. The *I/O* ratio observed in this study is smaller than the ratio measured under vacant conditions by Yamamoto et al. (2015a), though our ratio was based on vacant and occupied conditions combined. This difference might be attributable to the types of building studied. While we measured for a residential building with no mechanical ventilation system, Yamamoto et al. (2015a) measured for school classrooms with mechanical ventilation systems with filtration. We speculate that background concentrations might be high in rooms with mechanical ventilation relative to vacant rooms with stagnant air without disturbance of settled particles caused by mechanical ventilation, since particles may be resuspended from inner surfaces of

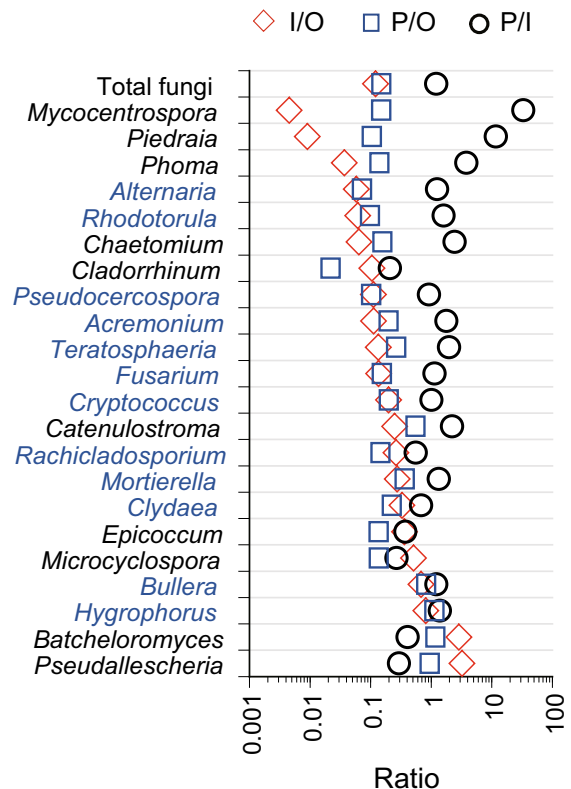


Fig. 6 *I/O*, *P/O*, and *P/I* ratios of airborne fungal concentrations. Geometric means of the ratios of the five sets of measurements are shown. Taxa shown are selected from those shown in Fig. 4. Taxa were excluded if there were data for which the ratios could not be calculated because of 0 GCN m^{-3} for both the antecedent and consequent concentrations. 1 GCN m^{-3} is assumed for 0 GCN m^{-3} if either one of the antecedent or consequent concentration is 0 GCN m^{-3} . Taxa in blue have *P/I* ratio in the range of 0.5–2

ventilation ducts by passing airflow (Zhou et al. 2011) and/or particles in rooms may be resuspended by disturbance caused by air mixed by mechanical ventilation.

Species richness was characterized in terms of the number of observed OTUs (Fig. 1). The greatest richness was observed in the outdoor sample collected from May 24, 2015. The total fungal concentration was also highest in this sample (Fig. 4). The greatest richness and concentration observed in this sample might be seasonally associated (Yamamoto et al. 2012). However, the reasons remain unclear due to the limited number of seasonally collected samples. Meanwhile, species richness was greater in outdoor than indoor air (Fig. 1). This observation is reasonable since indoor fungi are considered to be of outdoor

origin if no fungal infestation is present indoors (Adams et al. 2013; Burge 2002). In the sampled home, moldy spots, which are thought to be minor, were found in kitchen, bathroom, and balconies, but visible sporulation was not confirmed. Meanwhile, species richness was greater in personal than indoor air in four out of the five sets of measurements (Fig. 1), indicating that people are exposed to outdoor species that are not present in indoor air. Additionally, the subject might be exposed to fungi previously deposited onto and released from his clothes (McDonagh and Byrne 2014) and/or fungi desquamated and resuspended from his skin (Milstone 2004).

Overall, species richness was high in the samples collected in this study, i.e., numbers of observed OTUs ranging from 60 to 299 per sample (Fig. 1). The numbers of fungal species were likely larger than the numbers of observed OTUs since sequence clustering reduces taxonomic diversity (Yamamoto and Bibby 2014). A total of 493 genera were detected, some of which are not commonly identified from indoor and outdoor air. Indeed, 29 out of the 40 abundant genera detected in this study (Fig. 3) were not reported by previous culture-based studies (Shelton et al. 2002; Takahashi 1997). Such genera included *Pseudocercospora* and *Catenulostroma*. *Pseudocercospora* is an anamorphic state of the genus *Mycosphaerella*, and contains plant pathogenic species (Taylor et al. 2003). *Catenulostroma* is a genus closely related to *Mycosphaerella*, and contains plant and human pathogenic species (Crous et al. 2007). Morphology-based identification might be difficult for these genera, since sporulation is poorly induced by conventional culture techniques (Takahashi 1997). However, HTS is capable of identifying such species, and therefore useful for assessing their potential health impacts. It is possible that their importance has been neglected because of the difficulty in identification by conventional, culture-based techniques.

Some genera detected in this study are commonly identified from indoor and outdoor air (Shelton et al. 2002). Such genera included *Alternaria*, *Fusarium*, *Rhodotorula*, *Epicoccum*, *Aspergillus*, *Chaetomium*, *Penicillium*, *Acremonium*, *Phialophora*, *Trichoderma*, and *Eurotium* (Fig. 3). The two most abundant genera and their mean relative abundances were *Alternaria* (4.1 %) and *Fusarium* (3.6 %). *Alternaria* includes an allergenic species that is associated with development of asthma in children (Halonen et al. 1997). *Alternaria*

produces large multicellular conidia with reported aerodynamic diameter greater than 10 μm (McCartney et al. 1993; Yamamoto et al. 2014). Particles of this size settle rapidly under gravity. Consequently, *Alternaria* is found in low concentrations under vacant indoor conditions without human disturbance (Yamamoto et al. 2015a). Our study also showed low *I/O* ratio (0.058) for *Alternaria* (Fig. 6). *Fusarium* was another abundant genus, containing human and plant pathogenic species (O'Donnell et al. 2009). *Fusarium* produces micro- and macroconidia with size of $8\text{--}16 \times 2\text{--}4$ and $27\text{--}52 \times 4.4\text{--}6.8$ μm , respectively (Cole and Samson 1984) and mesoconidia with size larger than that of microconidia but smaller than that of macroconidia (Guarro and Gené 1992). It is saprotrophic and found abundantly in soil (Li et al. 2014). *Fusarium* is also found in indoor environments such as kitchens and bathrooms (An and Yamamoto 2016; Hamada and Abe 2009) and in outdoor air (Dannemiller et al. 2014a; Hoseini et al. 2013). The *I/O* ratio of *Fusarium* was 0.14 (Fig. 6), considerably smaller than the ratios found by Yamamoto et al. (2015a), who reported *I/O* ratios of 3.5 and 15 under vacant and occupied conditions, respectively. The reasons are unknown. One possible explanation is that the relative source strengths (indoor versus outdoor) might vary across the two studies' sites, especially for genera such as *Fusarium* that can inhabit and infest both indoor and outdoor environments.

In exposure assessment studies for airborne contaminants, including fungi, stationary indoor or outdoor monitoring has often been used to estimate personal exposure (Adhikari et al. 2004; Delfino et al. 1997; George et al. 2010; Williams et al. 2000). However, limited evidence is available regarding whether stationary monitoring is representative of personal exposure to airborne fungi (Adhikari et al. 2004). We found that only 4 out of the 22 genera showed *P/O* ratio in the range of 0.5–2 (Fig. 6), suggesting that outdoor concentrations are not representative of personal exposure to most airborne fungal genera. Meanwhile, 12 out of the 22 genera showed *P/I* ratio in the range of 0.5–2 (Fig. 6), suggesting that indoor concentrations are representative of personal exposure to the majority of fungal genera. Similar tendencies were observed for total fungi, with *P/O* and *P/I* ratios of 0.15 and 1.2, respectively (Fig. 5). However, several genera showed *P/I* ratio greater than 2 (Fig. 6), indicating that the subject was likely exposed to fungi in nonhome indoor

environments, such as office, or in outdoor environments. Such taxon-specific variabilities in relative source strength (e.g., indoor versus outdoor) add to the complexity of generalizing personal exposure to the diverse kingdom Fungi. Additionally, it should be noted that extra air sampling in office, which was not conducted in this study, might help us dissect the personal exposure in office, where the subject stayed for about 35 % of his time.

Cladosporium is thought to be an abundant genus in indoor and outdoor environments (Amend et al. 2010; An and Yamamoto 2016; Yamamoto et al. 2012), but it was not abundant in this study, with mean relative abundances of 0.02, 0.14, and 0.30 % for indoor, outdoor, and personal air, respectively. Although we do not know the reasons why *Cladosporium* was not abundant, there are two possible explanations: (1) *Cladosporium* was truly not abundant, and/or (2) the ambiguously identified sequences contained sequences of *Cladosporium*. We found that 3.3–16.3 % of total sequences were ambiguously identified at genus level but identified as the sequences of the class Dothideomycetes that contains the genus *Cladosporium*. This indicates that up to 3.3–16.3 % of total sequences might be from *Cladosporium* if all the ambiguous sequences were from *Cladosporium*. If this were the case, reducing the number of ambiguous sequences, for instance, by sequencing full-length ITS, might be needed for more accurate quantification of taxa for which identification tends to be ambiguous.

The goal of this study was to demonstrate an approach using the combined HTS/qPCR method to compare fungal communities and concentrations in indoor, outdoor, and personal air. To this end, we collected air samples longitudinally for the same subject. All five sets of air samples were collected during weekdays with similar time activity pattern (Fig. S1). We chose this sampling strategy to minimize confounders and identify taxonomic patterns across measurement sets. As expected, the concentrations and species richness differed considerably according to the sampling date (Figs. 1, 4). Although this study focused on testing the combined HTS/qPCR method and trying to extract fungal biota patterns for one human subject, future research is warranted to understand between-subject variability, which is thought to be important when applying this method in future exposure assessment studies. In addition, personal cloud effects are important for personal exposure to air pollutants (Allen

et al. 2007; Licina et al. 2017). However, this effect might be neglected in this study, since the subject did not wear the personal sampler directly around his body, but instead carried a bag containing the sampler continuously in his vicinity. In future studies, this effect should be scrutinized in order to characterize personal exposure to airborne fungi in daily life more accurately.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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Supplementary Materials of:

Introducing DNA-based methods to compare fungal microbiota and concentrations in indoor, outdoor, and personal air

Submitted to:

Aerobiologia

Choa An, Cheolwoon Woo, Naomichi Yamamoto^{*}

Department of Environmental Health Sciences, Graduate School of Public Health, Seoul National University, Seoul 08826, South Korea

* Corresponding Author.

Naomichi Yamamoto, Department of Environmental Health Sciences, Graduate School of Public Health, Seoul National University, Seoul 08826, South Korea.

Tel: +82-2-880-2837 Fax: +82-2-762-2888 E-mail: nyamamoto@snu.ac.kr

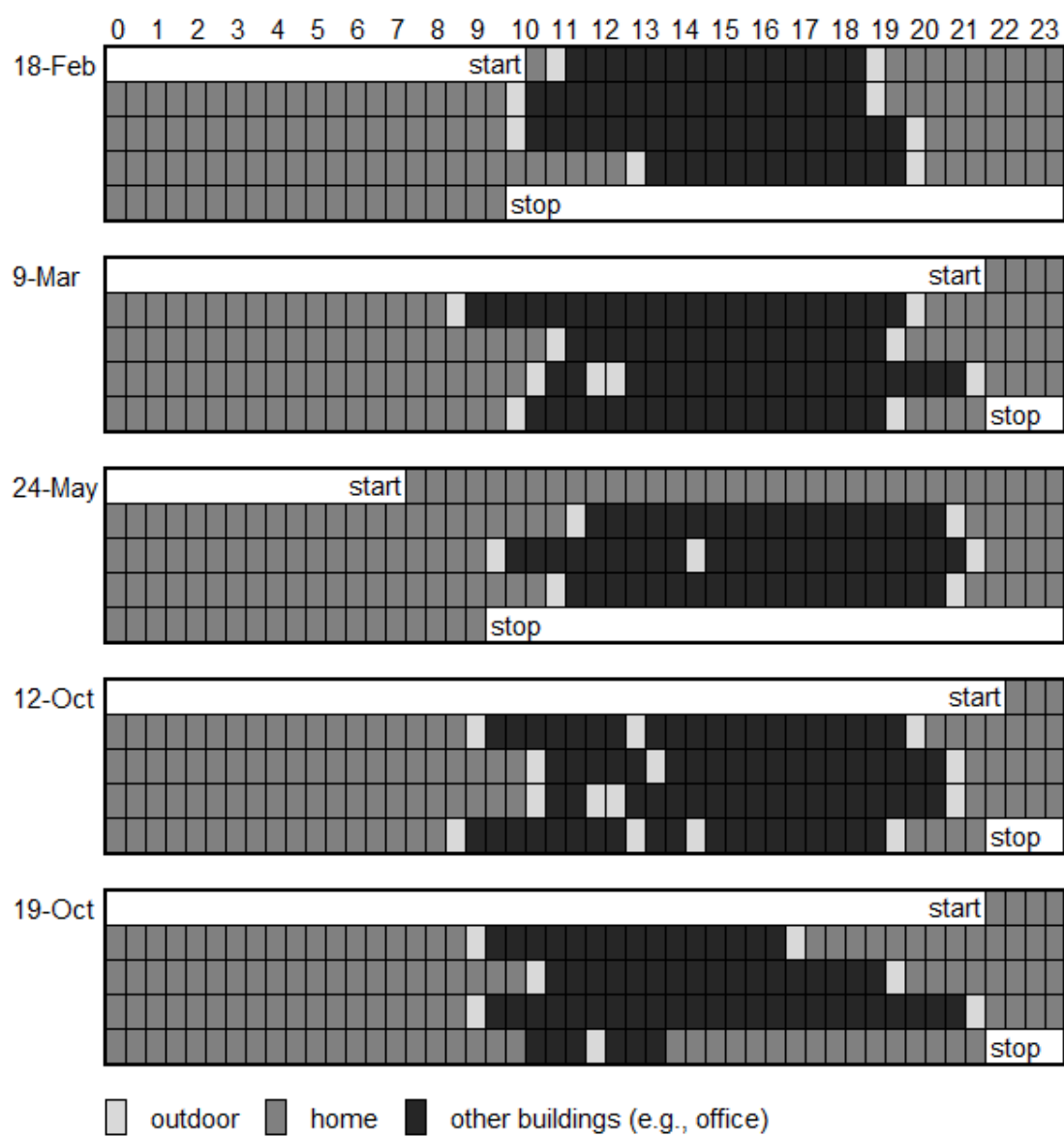


Fig. S1 Time activity patterns.

Table S1 Climatological conditions during the 4-day sampling periods.

Start date (MM/DD/YYYY)	Temperature (°C)			Relative humidity (%)			Wind velocity (m s ⁻¹)		Precipitation (mm) ^b
	Highest ^a	Average	Lowest ^a	Highest ^a	Average	Lowest ^a	Highest ^a	Average	
02/18/2015	11.4	3.5	-2.7	79	70	55	7.3	2.7	10.5
03/09/2015	8.2	1.4	-6.9	45	41	31	9.1	4.6	0.1
05/24/2015	32.2	22.2	14	53	40	27	6.6	2.4	0
10/12/2015	26.1	16	7.8	71	64	57	7.1	2.1	0
10/19/2015	25.1	18	13.6	73	69	65	4.9	2.0	0

^a The values are based on 1-min measurement interval.

^b The values are based on the entire 4-day sampling periods.

Table S2 Numbers and lengths of filtered high-quality sequences produced by 2×300 bp Illumina MiSeq.

Sample	Number of sequences	Mean sequence length (bp)
I_0218	42698	279
I_0309	75624	272
I_0524	27338	275
I_1012	10680	275
I_1019	18930	253
O_0218	12666	265
O_0309	33481	273
O_0524	72885	280
O_1012	38846	273
O_1019	18313	273
P_0218	27625	286
P_0309	25825	248
P_0524	36940	272
P_1012	15598	273
P_1019	28133	260