Symbiotic status alters fungal eco-evolutionary offspring trajectories

Authors: Carlos A Aguilar-Trigueros[1,2,3,4]*, Franz-Sebastian Krah[5], William K. Cornwell[6], Amy E Zanne[7,] Nerea Abrego[3,8], Ian C Anderson[4], Carrie J Andrew[9], Petr Baldrian[10], Claus Bässler[5], Andrew Bissett[11], V Bala Chaudhary[12], Baodong Chen[13,14], Yongliang Chen[15], Manuel Delgado-Baquerizo[16,17], Coline Deveautour[18,19], Eleonora Egidi[4], Habacuc Flores-Moreno[7], Jacob Golan[20], Jacob Heilmann-Clausen[21], Stefan Hempel[1], Yajun Hu[22], Håvard Kauserud[23], Stephanie N Kivlin[24], Petr Kohout[9], Daniel R Lammel[1], Fernando T. Maestre[25,26], Anne Pringle[20], Jenna Purhonen[3,27,28], Brajesh K Singh[7,29], Stavros D. Veresoglou[1], Tomáš Větrovský[9], Haiyang Zhang[7], Matthias C. Rillig[1,2+], Jeff R. Powell[4+].

Affiliations:

[1]Freie Universität Berlin, Institute of Biology, 14195 Berlin, Germany.

[2]Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), 14195 Berlin, Germany.

[3]Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35, FI-40014, Finland.

[4]Hawkesbury Institute for the Environment, Western Sydney University, Penrith NSW 2751, Australia.

[5]Department of Conservation Biology, Faculty of Biological Sciences, Institute for Ecology, Evolution and Diversity, Goethe University Frankfurt, Frankfurt am Main D- 60438, Germany

[6]Evolution & Ecology Research Center, School of Biological, Earth, and Environmental Sciences, University of New South Wales, Sydney, Australia.

[7]Department of Biology, University of Miami, 204A, Cox Science Center, 1301 Memorial Dr.

Coral Gables, FL 33146, USA.

[8]Department of Agricultural Sciences, PO Box 27, (Latokartanonkaari 5), FI-00014 University of Helsinki, Finland.

[4]Hawkesbury Institute for the Environment, Western Sydney University, Penrith NSW 2751, Australia.

[9]SLU Swedish Species Information Centre (SLU Artdatabanken), Swedish University of Agricultural Sciences (SLU), Almas Allé 8 E, 756 51 Uppsala, Sweden.

[10]Laboratory of Environmental Microbiology, Institute of Microbiology of the Czech Academy of Sciences, Videnska 1083, 14220 Praha 4, Czech Republic.

[11]Oceans and Atmosphere, CSIRO, Hobart TAS 7000, Australia.

[12]Department of Environmental Studies, Dartmouth College, Hanover, NH, USA.

[13]State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, People's Republic of China.

[14]University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China.

[15]College of Resources and Environmental Sciences, China Agricultural University, Beijing, 100193, People's Republic of China.

[16]Laboratorio de Biodiversidad y Funcionamiento Ecosistémico. Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Av. Reina Mercedes 10, E-41012, Sevilla, Spain.

[17]Unidad Asociada CSIC-UPO (BioFun). Universidad Pablo de Olavide, 41013 Sevilla, Spain.

[18]National University of Ireland, University Road, Galway, H91 TK33, Ireland.

[19]Environment, Soils and Land-Use Department, Teagasc, Johnstown Castle, Y35 Y521, Co. Wexford, Ireland.

[20]Departments of Botany and Bacteriology, University of Wisconsin–Madison, Madison, WI 35706.

[21]Center for Macroecology, Evolution and Climate, GLOBE Institute, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark.

[22]Key Laboratory of Agro-ecological Processes in Subtropical Region & Changsha Research Station for Agricultural and Environmental Monitoring, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan, 410125, China.

[23]Evogene, Department of Biosciences, University of Oslo, P.O. Box 1066, Blindern, N-0316, Oslo, Norway.

[24]Department of Ecology and Evolution, University of Tennessee, Knoxville. Knoxville, TN 37966, USA.

[25]Instituto Multidisciplinar para el Estudio del Medio “Ramon Margalef”, Universidad de Alicante, Carretera de San Vicente del Raspeig s/n, 03690 San Vicente del Raspeig, Alicante, Spain.

[26]Departamento de Ecología, Universidad de Alicante, Carretera de San Vicente del Raspeig s/n, 03690 San Vicente del Raspeig, Alicante, Spain.

[27]Department of Music, Art and Culture Studies, University of Jyväskylä, P.O. Box 35, FI-40014, Finland.

[28]School of Resource Wisdom, University of Jyväskylä, P.O. Box 35, FI-40014, University of Jyväskylä, Finland.

[29]Global Centre for Land Based Innovation, Western Sydney University, Penrith NSW 2751, Australia.

*Correspondence to: calgit@gmail.com.

+joint senior authorship

Author Contributions: CAAT conceived the study together with FSK, WKC, JRP and MCR. WKC downloaded data from Mycobank. FSK developed the text mining algorithm. CAAT, JRP, CD and HZ mined the text data and cleaned spore data entries. CAAT digitized manually the spore size data not present in Mycobank, managed the spore database and assembled the fungal functional database. JRP managed and assembled climatic and geographic data. CAAT and JRP performed statistical analysis with input from WKC and FSK. CAAT wrote the first draft, and all authors contributed to the writing of the paper.

Competing Interest Statement: The authors confirm that there are no competing interests.

Classification: Biological Sciences, Ecology .

Keywords: Offspring size, life-history, symbiosis, fungi, functional ecology

This PDF file includes:

Main Text
Figures 1 to 3
Tables 1

Abstract

Across free-living organisms, the ecology and evolution of offspring morphology is shaped by interactions with biotic and abiotic environments during dispersal and early establishment in new habitats. However, the ecology and evolution of offspring morphology for symbiotic species has been largely ignored despite host-symbiont interactions being ubiquitous in all ecosystems and across all branches of the tree of life. The kingdom Fungi provides an excellent opportunity to address this fundamental knowledge gap since symbiosis has been a major driver in trait evolution of this group. We assembled a database of fungal offspring morphology covering over 26,000 species of free-living to symbiotic fungi, including symbiotic relationships with plants, insects and humans and found more than eight orders of variation in offspring size. Evolutionary shifts in symbiotic status correlated with shifts in spore size, but the strength of this effect varied widely among phyla. Among plant associated fungi, symbiotic status explained more variation than environmental gradients in the current distribution of offspring sizes at a global scale; while being plant-associated limited the dispersal potential of fungal spores: in free-living saprotrophic fungi shifts to smaller spore size correlated with larger species' extent of occurrence while in plant associated fungi this relationship does not hold. Our work advances life-history theory by highlighting how the interaction between symbiosis and offspring morphology shapes the reproductive and dispersal strategies among living forms.

Significance Statement

In life-history theory, offspring size is a trait underpins species' interactions during dispersal, and early colonization. While size has been a predictive trait used in plants and animal research, it has not been tested in fungi, one of the largest eukaryotic kingdom in of the tree of life. Using spore volume as fungal offspring size, our study finds that the ecology and evolution offspring size is linked to symbiotic interactions in this group. Our findings show that the comparative ecology of offspring size can be used to understand distinct selective pressures that microbes face as they transition between asymbiotic to symbiotic life-styles with plant and animal hosts, and add an important driver to life-history theory.

Main text

Introduction

In life-history theory, the ecology and evolution of offspring size is linked to environmental factors that species encounter during reproduction, dispersal, and early-colonization, as well as physiological constraints during their development(1). By providing a common framework where disparate offspring structures such as plant seeds(2), animal ovules(3), avian eggs(4), and mammal size at weaning(5) can be compared, life-history theory aims at discovering general principles behind the drivers shaping the ecology and evolution of species at earlier stages of their life cycle. However, most life forms that have been used to develop this knowledge are free-living macro-organisms, ignoring the large diversity of microbial forms that engage in symbiosis. Conspicuously absent is the Kingdom Fungi, which, with 136,000 described species and an estimated diversity of 3 to 10 million species(6), is a large portion of the tree of life. Furthermore, symbiosis has been a major driver in trait evolution in the fungal kingdom(7, 8) raising the question of how transitions in symbiotic status influenced fungal offspring morphology and function. This dearth represents a fundamental knowledge gap because, as we report here (Fig. 1), variation in fungal offspring size (up to eight orders of magnitude) is as high or higher than that of free-living organism whose comparative offspring studies (e.g., plant seeds and avian eggs) dominate the life-history theory (Fig 1).

Most extant fungi can be placed along a symbiotic spectrum spanning from asymbiotic species, which dominate the decomposition of organic matter in ecosystems, to a variety of symbiotic interactions with hosts in almost all major domains of life(7, 8) (Fig. 2, S3-4). Here, we use a definition of symbiosis that is common in evolutionary biology: the intimate physical living together of distinct species (usually distantly related), whether mutualistic, parasitic, or commensal, including macrobe-microbe interactions, where the

former is considered the “host” and the latter the “symbiont” (9). In addition, we propose the spores of fungi along this symbiotic spectrum as their offspring structures (Fig, 1b) because they represent reproductive output units produced by a mature mycelium (the “parent” fungus) that function as dispersal propagules to colonize novel habitats that are usually distantly located from the parental fungus. Each spore has the potential to develop into a new mycelium which is independent from the parental one in the new habitat. Spore traits, such as total size, are hypothesized to determine the likelihood of colonization based on spore interactions with their environment during their release, movement, attachment/landing, dormancy, and germination. (10-13). Thus, spores are functionally analogous to dispersal offspring propagules of other sessile modular organisms like plant seeds (2) or marine invertebrate eggs (3).

Specifically, to understand whether transitions in symbiotic status explain changes in the size and function of spores across the fungal kingdom, we asked three questions. First, are transitions in symbiotic status correlated with shifts in spore size? To answer this question, we used linear phylogenetic regression to test whether the spore size of symbiotic groups (e.g., insect pathogens, plant pathogens, ectomycorrhizal) shift in size (i.e., increase or decrease) compared to asymbiotic fungi across all major fungal groups. We then focused on plant-associated fungi in the Dikarya to test whether symbiotic groups of obligate lifestyles have larger offspring than symbiotic groups with facultative lifestyles. We focus on fungi associated to plants in this clade because plants are by far the host type with the largest diversification of symbiotic lifestyles in this clade (7). In addition, this hypothesis has been repeatedly used to explain why the spores of some obligate plant pathogenic and mutualistic fungi are so large. This hypothesis posits that obligate symbionts may benefit from the greater resources present in large spores, since these resources represent the only means of surviving during dispersal and initial colonization (i.e., infection) of new hosts until resources can be exchanged with the host plant (14, 15). Second, across global climatic regimes, what is the relative importance of species’ symbiotic status in explaining offspring size variation? We hypothesized that changes in climate variables may be more important than symbiotic status in explaining spore size distribution across communities because fungi have a worldwide distribution (ranging across contrasting climate zones) and their spores, both of asymbiotic and plant associated species, are released and dispersed into the abiotic environment (16). In addition, based on predictions from life-history theory, we expect larger offspring sizes to be associated with higher rates of early survival under limiting environmental regimes(17). Third, does the predicted negative relationship between offspring size and geographic distribution (i.e., extent of occurrence)(18) depend on symbiotic status? One of the main ecological functions of offspring is dispersal and, for several fungal groups, it has been proposed that small offspring should travel farther than large offspring, increasing the dispersal potential of species. If plant associated fungi require large spore sizes, they may have more-limited distributions than asymbiotic fungi.

Unlike macro-organisms such as plants and animals, no databases of offspring morphology for fungi exist. Therefore, to answer our questions, we created and populated a new database by text-mining nearly 100,000 taxonomic descriptions deposited in Mycobank(19) (http://www.mycobank.org/; see Material and Methods for further details). In total, we collected information on spore width and length dimensions for >26,000 accepted species (based on taxonomy from the Catalogue of Life; https://www.catalogueoflife.org/), representing 20% of all described fungal species (Fig. S1). This database includes spore-dimension data from both sexually and asexually produced spores across major fungal lineages at different stages of fungal life cycles (see Material and Methods for details on the spores included in the database). However, we restricted the analysis described below to sexual spore types described as “ascospores” and “basidiospores” (henceforth referred to as “sexual spores”) and asexual spore types described as “conidia” and “sporangiospores” (henceforth referred to as “asexual spores”) because they represent the most frequently occurring types of spores in our dataset and thus can be compared across several fungal lineages and symbiotic groups (Fig. 1c, Fig, S2). We also excluded spores of glomeromycete fungi for our main analyses because their extreme large size may bias the results (see Fig.S2 and Material and Methods for specific spore definitions and nomenclature used in the analysis) (16). We then calculated spore volume using width and length as a proxy for spore size (see Materials and Methods for the specific formula we used). We used volume as a proxy for size because it captures the 3D structure of fungal spores and, based on allometric theory, volume scales with other measurements of size, such as weight. Indeed, volume has been used in life-history research as a proxy for offspring size across several large clades (1). Using this approach, we found that spore size across

species varied by more than eight orders of magnitude (Fig. 1b). We also assembled a symbiotic status database (by mining and crosschecking different functional databases) based on the ecological guilds where fungal species have been reported ranging from asymbiotic guilds (i.e. saprotrophic species that have only been reported as free-living during their whole life-cycle) to different symbiotic guilds (e.g. insect pathogens, mycorrhizal guilds, necrotrophic plant pathogens). For symbiotic species of plant associated fungi, we further classify the level of specialization as either facultative symbiosis (species that are reported to alternate between a free-living and symbiotic phase) or obligate symbiosis (species that have been exclusively reported as symbiotic to complete their life-cycle) based on the biology of their respective build (Fig. 1c, see supplementary material for data sources and details on the criteria used).

Because evolutionary history shapes how and where species are today, the role of this history can be examined by seeing how traits shift across the tree of life. For fungal spores, recent reviews and anecdotal evidence suggest that spore size is expected to differ more widely in some fungal clades than others(20, 21). Thus, we used two phylogenies to test whether transitions in symbiotic status correlate with shifts in fungal offspring size,. The first phylogeny consists of 1644 fungal species whose genome has been fully sequenced as recently published in (22). Focusing our analysis on these species allowed us to incorporate the most robust, species-level phylogenetic tree available to date for fungi (as this tree is based on whole genome data) that captures the entire kingdom (i.e. it is not specific to only a subset of fungal clades). However, because this tree only includes a limited number of species, we also used a taxonomy based phylogeny consisting of 23,000 species from which we obtained taxonomic data from phylum-to-species level (see Materials and Methods).

Results and Discussion

Variation in spore size across different spore types

Spore size variation among sexual and asexual spores was strongly structured by species' evolutionary history (Fig. 1c, Tables S1-S2). For instance, the asexual spores from the glomeromycetes are the largest in the kingdom, from 1.5-to-4 orders of magnitude larger compared to other spores (either sexual or asexual) from other groups and this difference shows strong phylogenetic structure (Pagel´s lambda ~ 0.7 depending on the comparison, see Table S1). These spores of glomeromycetous fungi, however, are unique among other fungi because they contain hundreds of nuclei (an unparalleled feature in the kingdom(23)) which might partly explain their extremely large size(21). Further, we found that sexual spores of basidiomycetes are on average 6 μm^3 smaller than ascomycetes across the tree (Pagel´s lambda = 0.8, see Table S2). While this pattern alone cannot determine the mechanisms behind this size difference, it is consistent with the hypothesis that sexual spores of basidiomycetes are smaller than those of the ascomycetes because the Basidiomycota, as a whole, evolved a spore launching mechanism ("the surface tension catapult") that depends on spore size. In contrast, the launching mechanism of ascomycetes does not(20, 24). This potential mechanism suggests that the size of the spore is dependent on the anatomy and morphology of the reproductive structure of the parental fungus. Such parent-to-offspring regulation has also been observed in other taxa, such as placental mammals, for whom size at birth depends on the anatomical constraints of the reproductive structure where the offspring develops (1).

Transitions in symbiotic status and offspring size

We found support for our hypothesis that transitions in symbiotic status correlate with shifts in the size of both sexual and asexual spores (Table S3-4, Fig. 2). However, the direction and strength of this correlation highly depended on the symbiotic group, spore type and phylum considered. We found that shifts in sexual spore size during transitions from saprotrophic to symbiotic groups were stronger in the Ascomycota compared to the Basidiomycota–specifically, we found shifts to larger spore sizes among insect pathogens, ectomycorrhizal, lichen, and mildew fungi (although statistical support for the last two groups was found on only one phylogenetic regression; see Table S3). For asexual spores, we also observed stronger shifts of size and symbiotic status among groups in the Ascomycota compared to the Basidiomyota, although shifts in asexual spore sizes were more heterogeneous: shifts to larger asexual spores were associated with mildew fungi and necrotrophic plant pathogens, while shifts to smaller

asexual spore sizes were associated with lichen and insect pathogenic fungi (Table S4). Finally, we also detected shifts towards larger asexual spore sizes among insect and necrotrophic pathogens of zygomycetous fungi (Table S4) and for insect pathogens in the Microsporidia. Among plant-associated fungi, we found a global trend towards increased sexual spore size in fungi with more specialized symbioses. The main driver of this trend, though, were mildew and ectomycorrhizal fungi in the Ascomycota (i.e., we found no statistical support for this hypothesis with plant-associated groups in the Basidiomycota). For sexual spores, plant obligate symbionts in the Ascomycota were about 29 µm3 larger than spores of facultative symbionts counterparts, while for asexual spores, obligate symbionts were up to 59 µm3 larger than spores of facultative symbionts (all p-values < 0.001; Fig.3, Table S5). A possible mechanism behind large spores being associated with these groups is that spore reserves or thickening of spore cell walls increase chances of survival when dispersing to a host, overwintering, and/or overcoming initial host resistance (e.g., penetration of the hard cuticle or the epidermal tissue) (25, 26).

Our results are congruent with previous research reporting small differences in spore size across functional groups in Basidiomycota fungi, particularly when comparing the sexual spores of ectomycorrhizal and saprotrophic fungi suggesting than other reproductive traits, such as sporocarp size and shape, might be more functional (27-30). As we show here, this small difference might be due to the already small size of this type of sexual spores of basidiomycete fungi relative to ascomycete fungi, which prompts the hypothesis that for the Basidiomycota the demand for small spores for the launching platform leaves little room for differentiation during evolution of the symbiotic lifestyle. In the case of necrotrophic pathogens or plantendophytes, the overlap in spore sizes with asymbiotic fungi and their relative large variation in sizes (Fig. 2, Fig. S3) may reflect differences in the level of symbiotic specialization (31) that is not captured with the current classification. Plant pathologists have long speculated that larger spores may provide the necessary resources for highly host-specialized necrotrophs to overcome host defenses and infect healthy host tissue, while such resources may be less important among less specialized necrotrophic pathogens that can only infect weakened plants (14). We also found large variation in spore size across asymbiotic saprotrophic fungi (for any group or spore type; Fig. 2, Fig. S3). This variation suggest the existence ofdifferent niches filled by saprotrophic species, such as during decay of different substrates or in different successional stages(32). Finally, we also included in a separate analysis the peculiar case of fungi that cause disease in humans due to their importance. Most of these fungi are described as opportunistic (i.e., causing disease in immuno-compromised individuals(16)) and are commonly found growing as free-living in nature; these fungi are, thus, generally considered asymbiotic rather than symbiotic in the mycological literature(33). Our results, however, show that such fungi, despite their expected asymbiotic nature, have on average smaller sizes than other asymbiotic fungi (a pattern that holds across the phylogeny in some of our models, Table S3-4, Fig. 2). While it is not possible to pinpoint mechanisms, we hypothesize that smaller spores for these fungi may enhance the likelihood to be passively inhaled or ingested (33).

Relative importance of species' symbiotic status in explaining offspring size variation

Symbiotic status was also more important for explaining interspecific variation in spore size than climate variables associated with the distributions of fungal species (Table 1). To obtain climatic information, we first mapped the geographic distributions of fungal species observed in several large-scale, high-throughput DNA-sequencing studies of fungal communities from soil and plant samples covering an extensive breadth of biomes and occurring on all seven continents (Fig. S4; see Material and Methods section for details on how species annotations were performed). Then, we collected climatic data associated with the locations where those species were found, estimated mean values for each species, and compared the ability of those climate variables and each species' symbiotic status to explain variation in spore size. However, mean annual variation was the second most important variable explaining spore size variation across communities. This is congruent with previous research highlighting that in some species of mushroom-forming fungi, thicker spore walls have higher resistance to UV light exposure and freezing temperatures than species with smaller and lighter spores (34). Possibly, for symbiotic fungi, environmental microclimate plays a minor role as the host will buffer these variables (e.g., fungal symbionts of warm-blooded fungal symbionts will be buffered against changes in environmental temperature).

Relationship between offspring size and species' geographic distributions depends on symbiotic status

Finally, we assessed relationships between offspring size and geographic distributions for asymbiotic and plant associated fungal species, which we expect to be negative if smaller offspring size facilitates spread of propagules. To do this, we estimated species' ranges from their mapped distributions in environmental-DNA-sequencing studies by calculating maximum geographical distance among samples in which that species occurred using the ellipsoid method (35) and the estimated area of its range using alpha-hull-derived polygons (36). Spore size was negatively correlated with the geographic range of free-living fungal species (95% credible interval for slope of maximum geographic distance: -0.71 to -0.11; 95% credible interval for slope of range area: -1.01 to -0.14; Fig. 3) but not for symbiotic groups (95% credible intervals for slope of maximum distance: -0.23 to 0.01; 95% credible intervals for slope of range area: -0.25 to 0.09; Fig. 3). In asymbiotic fungi, species with larger spores had a more-limited geographic range compared to species with smaller spores, which may move more easily to new environments. Conversely, geographic range was unrelated to spore size for symbiotic species, for which host-related factors (including the geographic spread of the host itself) may offset any difference in dispersal due to spore size. For example, smaller spore sizes might actually reduce the chances of "landing" on a suitable host because smaller spores remain more easily aloft (18). The role of other spore traits (such as appendage morphology or spore wall ornamentation) must be assessed to fully understand the dispersal of symbiotic fungi (37).

In this study, we uncover massive variation in offspring size in the fungal kingdom whose ecology and evolution is partly explained by transitions in symbiotic status of the species. We also found that for plant associated fungi, changes in spore size impact differently their function during dispersal and early establishment compared to free-living fungi. These result highlights that symbiotic relationships are important drivers in life-history trait evolution particularly in the Fungi. However, our results also show that the direction of this effect (i.e. shifts to smaller or larger spore sizes in symbiotic fungi) and its importance varies widely among symbiotic groups and phyla. Moving forward, two directions of research are clear: first, determining the mechanisms behind correlations between shits in spore size along transition to symbiosis; and second, determining why in some symbiotic groups and clades, spore size does not change along symbiotic gradients. For plant associated fungi, our results provide support to the hypothesis that larger reserves or thicker walls may increase the chances of survival during dispersal to a new plant host and assist early stages of colonization of some groups. However, shifts to smaller spore sizes in other symbiotic groups suggest other mechanisms may be at play. In other host associated taxa, such as parasitic animals, it was proposed that host demographics play an important role in explain offspring size. For example, small-sized offspring structures produced by some parasitic copepods and mollusks increase the chances of transmission when hosts are hard to locate (38), while large offspring produced by tapeworm species assist development on hosts that provide challenging initial growing conditions (38). By testing the extent to which host transmission dynamics impact the reproductive ecology of fungi, we may uncover complex life-history strategies, since reproduction is not limited to the spore structures. For example, among highly host-specialized symbionts, direct host-to-host transmission may lead to less dependence on spore dispersal and instead favor hyphal extension (which can lead to colonization to new hosts, as in the arbuscular mycorrhizal symbiosis(39)) or yeast phenotypes that can be transmitted directly from one host to another (as commonly seen among most insect gut endosymbiont (40)). As host-symbiont specialization is a long-term evolutionary process(9), the age of symbiosis might be a predictor of reproductive trait changes (for both host and symbionts). Thus, the reliance of fungi in other ways of transmission other than spores might explain weaker correlation and symbiotic state we found in some clades. In those cases, variation in spore size might be driven by neutral processes such as drift (which we did not test). Addressing these questions highlights the importance of including more species across different symbiotic lifestyles in phylogenetic studies(41) and the need to populate databases with fungal reproductive traits. Such data would allow tests of even the most fundamental tenets in life-history for the fungal branch of the tree of life, such as the existence of trade-offs in offspring output-offspring size or allometric scaling relationships between parent size and offspring size.

Finally, information on the diversity of dispersal and colonization strategies among asymbiotic and symbiotic fungi will be useful to forecast the impact of global change on ecosystem functions provided by fungi. For example, disease risk caused by fungal plant pathogens is forecasted to change with

increasing global temperature (42). Such changes are likely due to direct effects on survival of spores during dispersal, and indirect effects of changing habitat quality (e.g., host susceptibility). Information on fungal dispersal strategies for symbiotic groups will refine forecasts of pathogen expansions and likelihood of pathogen spillover from natural ecosystems to croplands. Considering that fungi represent the main cause of crop yield losses and are a main threat to animal health (43), such refinements in forecasting are particularly relevant to maintain food security and ecosystem health.

In summary, expanding the realm of life-history analysis beyond plants and animals to other diverse and important clades such as fungi highlights symbiosis as a key biotic driver influencing the ecology and evolution of offspring-size variation. Life-history frameworks are biased toward free-living organisms (1) with relatively limited inclusion of parasitic animals (38). Yet, symbiosis is pervasive through the entire tree of life (including animals and plants) and, as we show here, is a major driver of offspring variation for an entire kingdom. Including symbiosis as a life-history parameter creates the need for new theoretical frameworks to determine, for instance, how much the host controls the offspring traits of the symbionts (as in fungi, and possibly bacteria and protists) and how much the symbionts control the offspring traits of their macro-organism hosts.

Materials and Methods

Assembly of spore trait database

Spore traits provide an opportunity to compare the reproductive ecology of fungi at the broadest diversity level possible to date. This is because spore traits are one of the few types of traits that have been recorded for a wide diversity of fungal species due to their historic taxonomic value. That is, spore morphology has been critical in fungal taxonomy and therefore spore traits such as size are usually reported in fungal species descriptions. In contrast, other morphological traits (hyphal dimensions) or physiological ones (growth rates or enzymatic machinery) only are available for a greatly reduced number of species, usually model fungi or fungi of economic interest.

Given the potential of spore traits, we focused on taxonomic descriptions as our main source of spore-trait information. We first downloaded close to 100,000 species records from Mycobank (accessed in December 2018). Out of the downloaded records we selected the ones containing spore descriptions, resulting in 36,315 unique species-level spore descriptions. To extract information on spore dimensions, we developed a text-mining algorithm in R using the “stringr” package (44). The basic search pattern of the algorithm roughly follows the format “… *Spore ... dimension 1 x dimension 2 µm.“. ". Each of these dimensions consisted of a range from minimum to maximum values. For completeness and accuracy, we reported all values in our database but used only one value per dimension in our analysis as explained below. We used this algorithm to search for spore dimensions across the wide variety of terms that have been used to refer to spores throughout the history of mycology – in particular, the following terms: “ascospores”, “basidiospores”, “zygospores”, “conidia”, “chlamydospores”, “azygospores”, “teliospores”, and “sporangiospores”. Modifications and implementation of the text-mining algorithm optimized for each spore term can be found at https://github.com/aguilart/SporeSizeFungalKingdom).
The variety of spore terms found in the descriptions reflects differences due to morphogenesis (e.g., “condia” vs “sporangiospores”), sexual stage (“conidia” vs “ascospores”), stage of meiotic cycle when they are produced (zygospores vs ascospores or basidiospores), or just taxonomic affiliation (“ascospores” vs “basidiospores”), while other terms are more subjective (e.g., “chlamydospores”) or have been historically used for some groups (“azygospores” for glomeromycetous fungi). Thus, we further standardized all spore types included in our database under a common nomenclature based on reference (16) and expert knowledge as follows: (1) meiospores: walled cells with one to few nuclei that are the immediate products of meiosis (including basidiospores and ascospores); (2) mitospores: walled cells with one to few nuclei that are the immediate products of mitosis (including conidia, sporangiospores, and chlamydospores); (3) multinucleate sexual spores: walled multinucleate cells that precede meiosis (including multinucleated zygospores and binucleated teliospores); and (4) multinucleate asexual spores: walled multinucleate cells that are not involved in a meiotic cycle (including “azygospores” of zygomycetous fungi, and those of arbuscular mycorrhizal fungi). However, we restricted the analysis described in the main text to meiospores (i.e., ascospore and basidiospores) and mitospores (specifically, those described as conidia and sporangiospores) because they represent the largest proportion of spore types collected and can

thus be used for comparative analysis across several fungal lineages and symbiotic groups. For simplicity, in the main text we referred to these meiospores as “sexual spores” and theses mitospores as “asexual spores” (See Figure S2 for representation of different spore types in the dataset based on nomenclature used). For quality control, we manually inspected the data extracted with the algorithm. We supplemented the database with further spore dimensions for 1,345 unique species names provided by other co-authors who are experts in particular groups of fungi and have published their data in previous studies (30, 32)[,(21, 45)], resulting in a total of spore dimension data for 37,660 unique species names. To further check the quality of the data, we correlated spore volume values obtained through the algorithm with values provided by experts and found a strong correlation between the two methods (Fig. S5). Next, we calculated single values for each dimension for each description per name. This calculation consisted of finding the median value for each dimension. Of these resulting values, the larger was considered the spore length and the smaller was considered spore width.

In December 2019 we submitted the 37,660 unique species names in the database to the Catalogue of Life (https://www.catalogueoflife.org/), as it is used as a taxonomic reference database to determine the official number of described fungal species (6), using the R package “taxize” (46). In this database we were able to assign the most recently accepted taxonomical names to our data and to determine which names were synonyms. Based on the updated taxonomy, we established that our spore database represented 25,795 accepted species names. For cases in which a given species was reported with multiple descriptions and/or different synonyms, we calculated average spore dimensions to obtain single values per spore type per species. Finally, we manually digitize spore dimension data for 339 species reported in the phylogenetic tree of reference (22) which were not present in our original dataset (such additions were necessary as we rely on this tree for our comparative analyses), making a total of 26, 134 species. These data have been deposited in the Fun[Fun] database(47).

We focused our analysis described in the next sections to sexual and asexual uninucleate spores because they are the most common type of spores observed across most fungal species and differences is size are not confounded by nuclei count. In addition, only for glomeromycete fungi are multinucleated asexual spores consistently reported for described species and their role as a propagule dispersal structure is well established (48). Finally, multinucleated spores (sexual or asexual) for zygomycetous fungi are rare in our dataset (n=135 species) which is consistent with current knowledge reporting that these spores are rarely observed, and thus are rarely included in taxonomic descriptions that instead rely on asexual uninucleated spores (49).

Calculation of spore volume and aspect ratio

We considered spores to be either perfect spheres or prolate spheroids as reported in references (21, 27). Thus, when there were two different dimensions for a given spore (i.e., length and width), we considered the longest diameter as the polar axis (i.e., spore length) and the shortest diameter as the equatorial axis (i.e., spore width). We then used the formula for the volume of a prolate spheroid as: $(\text{equatorial axis})^2 * \text{polar axis} * (\pi/6)$. Aspect ratio was calculated as the ratio of the polar axis to the equatorial axis.

Assembly of symbiotic status database

We obtained information on symbiotic status by assembling a new dataset from different sources. In this dataset rows indicate a species name and columns indicate symbiotic status. The sources from which the dataset was assembled were species levels entries found in the FunGuild database (50); the USDA host-fungus database (https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm), for which we use the r package *rusda (51)* to select fungal species with an associated plant disease (we specifically searched for the following diseases: “canker”, “spot”, “scorch”, “anthracnose”, “blotch”, “blight”, “damping-off”, “rots” “undefined necrotrophy”, “black mildew”, “mildew”, “rust” and “smut”); the LIAS database (http://liaslight.lias.net/) for lichen and lichenocolus fungi; the DEEMY database (http://www.deemy.de/) for ectomycorrhizal fungi; reference (21) for arbuscular mycorrhizal fungi; reference (52) for entomopathogenic fungi; and reference (53) for human pathogens. In total we obtained information for 7392 accepted species names included in our spore database (representing close to 29% of the spore

database). Further, we manually digitized symbiotic status data for over 300 species present in the phylogenetic tree of reference [22] that were not present in our original dataset. Similar to the spore database, once all species were present in a single matrix, we standardized to a common taxonomy following the Catalogue of Life in order to solve synonymy issues.

We classified symbiotic status, first, as ecological guilds that reflect the biology of a host-symbiont interactions. Twelve guilds were recorded: asymbiotic saprotrophs (for fungal species that have only been reported as free-living saprotrophs, that is, no record of a host was found in any of the database checked), insect pathogens, lichens, plant endophytes, four plant pathogenic guilds (plant necrotrophs, rust fungi, mildew fungi, smut fungi), two mycorrhizal guilds (ectomycorrhizal and arbuscular mycorrhizal), fungal parasites and human pathogens.

Second, we categorized the guilds above based by their level of symbiotic specialization (i.e. the level of dependence of a fungus to establish symbiosis to complete their life cycle) after reviewing the literature on the biology of these guilds. Three levels were used: *1) Asymbiotic fungi* where we included the asymbiotic saprotroph guild mentioned above as well as human pathogens because most of these pathogens have a biology similar to that of saprotrophic fungi and only cause disease under extraordinary circumstances (such as when encountering weak host immune systems) and thus, are not considered symbiotic in many sources (16, 33) (however, due to their importance, we also tested for their differences in spore size with respect to other asymbiotic fungi). 2) *Facultative symbionts* for plant pathogens that cause death of plant tissue (the necrotrophic pathogenic guild) that includes diseases named as “canker”, “spot”, “scorch”, “anthracnose”, “blotch”, “blight”, “damping-off”, “rots” and “undefined necrotrophy” . We consider this group of fungi as facultative because the fungi causing these diseases have been shown to also have a free-living phase where they perform saprotrophic functions (54). *3)*, all mycorrhizal guilds (see reference (55)) and plant pathogens that do not cause tissue death as part of their infection (known as biotrophic pathogens) causing diseases reported as “rusts”, “smuts”, “mildew”, and “black mildew” (see reference (25)).

Assembly of climate and biogeography data

We mapped the geographic distributions of the fungal species for which we assembled trait data by using the data from the GlobalFungi database(56, 57). We also included six additional large-scale, high-throughput DNA sequencing studies, some of which were global in their extent(58), while others were focused on specific regions (e.g. Australia, Brazil, Europe and China: references (59, 60)]). All studies (Fig. S4) targeted the fungal Internal Transcribed Spacer region (ITS) to generate sequence reads. Specific details for how samples were collected and processed and for how DNA sequences were generated and processed can be found in the published studies; for unpublished ones, details are given below. Fungal species-level annotations for each operational taxonomic unit (OTU) were based on the top BLAST result when compared to the UNITE database version 8.2(61); at least a 98.5% match (minimum 90% coverage) was required to accept the species-level annotation as a species hypothesis (SH) for the OTU, and all other OTUs were considered unassigned. We used the sample-OTU tables and geographic origins of samples (decimal degrees, latitude, and longitude) as provided. We calculated the number of sequence reads associated with each species in each sample based on the sum of reads in all SHs assigned to that species.
Details for regional studies are as follows: Australia: sample collection and processing were described as in(62); raw sequencing data can be found under Bioproject ID PRJNA317932, DOI 10.4227/71/561c9bc670099. Brazil: the sampling sites, soil collection, and DNA extraction were described as in(63). PCR amplification was carried out with the primer pair fITS7/ITS4 and sequenced with Illumina MiSeq v3 technology. The sequences were processed within the QIIME2 pipeline (default settings), by using the dada2 processing step. Europe: Soil samples were taken with 2-cm diameter, 10-cm depth soil corers and were stored on dry ice before being transferred to the lab. DNA was extracted with Mo-Bio Powersoil DNA extraction kit and PCR amplification was carried out with the primer pair fITS7/ITS4. The dada2 pipeline was used to process the sequences with default settings.
When matching the species in our spore trait database to those species observed in the environmental DNA sequencing studies, coverage (expressed as a percentage of SHs or reads in a sample for which a trait database match could be found) varied depending on the primer pair used during fungal community

sequencing (Fig. S6). For subsequent analyses, we included samples sequenced using four primer pairs (fITS7/ITS4, ITS1F/ITS2, ITS1F/ITS4, ITS3/ITS4), since they provided coverage of all phyla and spore types (Fig. S7).
Historical bioclimatic variables (related to temperature and precipitation), solar radiation, wind speed, and water-vapor pressure data were obtained from the WorldClim database version 2.0 covering the years 1970 to 2000 (64). We used data collected at 30 seconds (~1 km²) resolution. For each of the sample locations in this study, we extracted and calculated means for the values associated with distances up to 1 km of those geographic coordinates from their respective rasters using functions in the 'raster'(65) and 'rgdal'(66) packages. For solar radiation, wind speed and water-vapor pressure, the rasters contained averages for each month so we calculated minimum, maximum, and coefficients of variation across all months for each variable. For each species, we calculated the mean value associated with each climate variable across all samples in which that species was found.

Analysis of the shifts between symbiotic status and spore size across the phylogeny

Currently there is no species-level fungal phylogenetic time tree that accounts for more than a small minority of the species in the spore dataset. Thus, to visualize general clusters of spore traits against phylogenetic groups of fungi, we collapsed to order-level the recently published phylogenetic tree of (22) (as shown in Fig. 1c). There is no consensus of the higher taxonomic ranking of fungi other than Asocmycota and Basidiomycota (e.g. both taxonomic databases Index Fungorum and Catalogue of Life show different taxonomy for those groups) and new phyla are still proposed (67). Thus, for simplicity, we used rankings and groupings reported in the last kingdom-wise synthesis of fungal biology and taxonomy(15) throughout the text and figures. These terms are as follows: *zygomycetous fungi* for fungi in the proposed phyla Basidiobolomycota, Calcarisporiellomycota, Entomophthoromycota, Kickxellomycota, Mortierellomycota, Mucoromycota and Zoopagomycota; *glomeromycetous fungi* for fungi in the phylum Glomeromycota; *zoosporic fungi* for fungi in the phyla Blastocladiomycota, Neocallimastigomycota and Chytridiomycota; and *microsporidian fungi* for fungi in the phylum Rozellomycota.

For phylogenetic comparative analyses (see below), we used two phylogenetic trees that represent two subsets of species from our database. The first subset is made up of 1346 fungal species that are also present phylogenetic tree of (22). By focusing on these species, we used the most robust species level phylogenetic tree for fungi currently available; this tree is built from whole-genome data and it covers the entire kingdom including most families of fungi (unlike most trees available that are specific to some taxa). The second subset includes 23, 334 species for which we obtained a fully resolved taxonomy from phylum to species level taxonomy (i.e. we removed species without clear assignment to higher level taxonomy or *Incerteae sedis* status). By using these species, we created a cladogram (using the as.phylo function from the ape package in R) where all branches have equal lengths. Although there are methods to add species to a backbone tree to build a single "mega tree" (e.g.https://birdtree.org/), we do not do that because fungal taxonomic resolution below the order level is still largely unknown; any such tree would resolve a relatively low number of genera below the order level, which does not provide much additional information in a phylogenetic analysis.

Independently for each phylum or fungal group (i.e. Ascomycota, Basidiomycota, zygomycetous fungi, zoosporic fungi and microsporidan fungi), we conducted phylogenetic linear regression models where the logarithm of spore volume was the response variable, symbiotic status (based on the 12 symbiotic guilds classified here) was the explanatory variable and either the genome-based phylogenetic tree from (22) or the taxonomy derived cladogram was used to account for phylogenetic relatedness. These phylogenetic regressions were conducted on sexual spores of the Ascomycota and Basidiomycota (i.e. ascospores and basidiospores) and asexual spores of all phyla and fungal groups (as defined above, we only include asexual spores referred as "conidia" or "sporangiospores") separately as they represent two separate traits under different selection. These phylogenetic linear regression models were conducted using the function phylolm from the phylolm package in R (68).

We conducted additional phylogenetic regression models testing whether spore size is bigger for obligate symbionts compared to facultative symbionts for sexual spores of plant associated fungi in the Ascomycota and Basidiomycota and asexual spores of the Ascomycota. As above, this phylogenetic regressions were performed the genome based phylogenetic tree (22) or the taxonomy based cladogram.

Relative importance of symbiotic status against climate variables in explaining spore size variation across communities

For this analysis, we focused on species that in our database are reported to produce only one spore type because it is not possible to determine the spore type associated with environmental DNA sequences.

We assessed the importance of fungal symbiotic status (i.e. whether fungi are free living saprotroph or plant associated) in explaining interspecific variation in spore size relative to other drivers, including spore type (i.e. sexual and asexual) and climate across communities worldwide. Phylogenetic linear regression models were fit using the following predictors: spore type (categorical variable), climate (averages of mean annual temperature and precipitation, temperature and precipitation seasonality, maximum solar radiation and minimum water-vapor pressure calculated across locations in which each species was detected; as continuous variables) and symbiotic status (as a categorical variable —free-living or plant associated). As before, we conducted this analysis using two phylonenetic regression (one using the genome based phylogenetic tree from (22) and the other one using the taxonomy-based tree.

Differences between saprotrophic and plant associated fungi in the relationship between spore size and geographic spread

We assessed the role that fungal lifestyle plays in determining the relationship between geographic range and spore size. As with the previous analysis, we focused on species that in our database are reported to produce only one spore type because it is not possible to determine the spore type associated with environmental DNA sequences.

Geographic range for each species was estimated in two ways: 1) as the maximum distance in meters between samples, in which the species was detected using the ellipsoid method calculated with the distVincentyEllipsoid function from the 'geosphere' package in R(69); and 2) as the range area in square meters using alpha-hull-derived measures incorporating all samples in which the species was detected calculated using the getDynamicAlphaHull function from the 'rangeBuilder' package in R(70). Each estimate of range size was then used as a response variable in linear models to estimate slopes representing the strength of the relationship between geographic range and spore volume for fungi with saprotrophic lifestyles (free-living) and those from plant-associated lifestyles (symbiotic). These models included random intercepts representing the taxonomic order (to account for non-independence among fungal species) and the primer set used to amplify fungal DNA (to account for biases among primer sets in their ability to detect fungal species). Because point-estimates can be sensitive to unbalanced sampling designs and, therefore, are unreliable, we used functions in the 'lme4' (71) and 'brms' (72) packages in R to fit Bayesian models and estimate posterior distributions of the slope parameters and calculated 95%-credible intervals from four MCMC chains (each 2000 iterations with a 1000-iteration burn-in) to assess differences among fungal lifestyles. To assess relationships within individual orders, separate linear mixed-effects models were also fitted for each combination of taxonomic order and fungal lifestyle for which a minimum of five species with spore volume and geographic extent were available.

All statistical analyses were performed using R version 4.0.1(68). Spore volume, projected spore area, and Q-ratio were log10-transformed prior to statistical analyses.

Data availability

Spore data for this paper have been deposited in Fun^{Fun}: https://github.com/traitecoevo/fungaltrait.

Code availability

R code and additional data for text mining (e.g. alogorithms used), assembly, analysis, figures and tables included in this manuscript can be found in https://github.com/aguilart/SporeSizeFungalKingdom

Acknowledgments

We thank J. Antonovics and Tessa Camenzind for helpful comments. Noa Terracina helped sort out plant disease information. We thank Louis Weiss for providing information on the natural history of microsporidian fungi. We also thank anonymous reviewers for helpful comments to this manuscript.

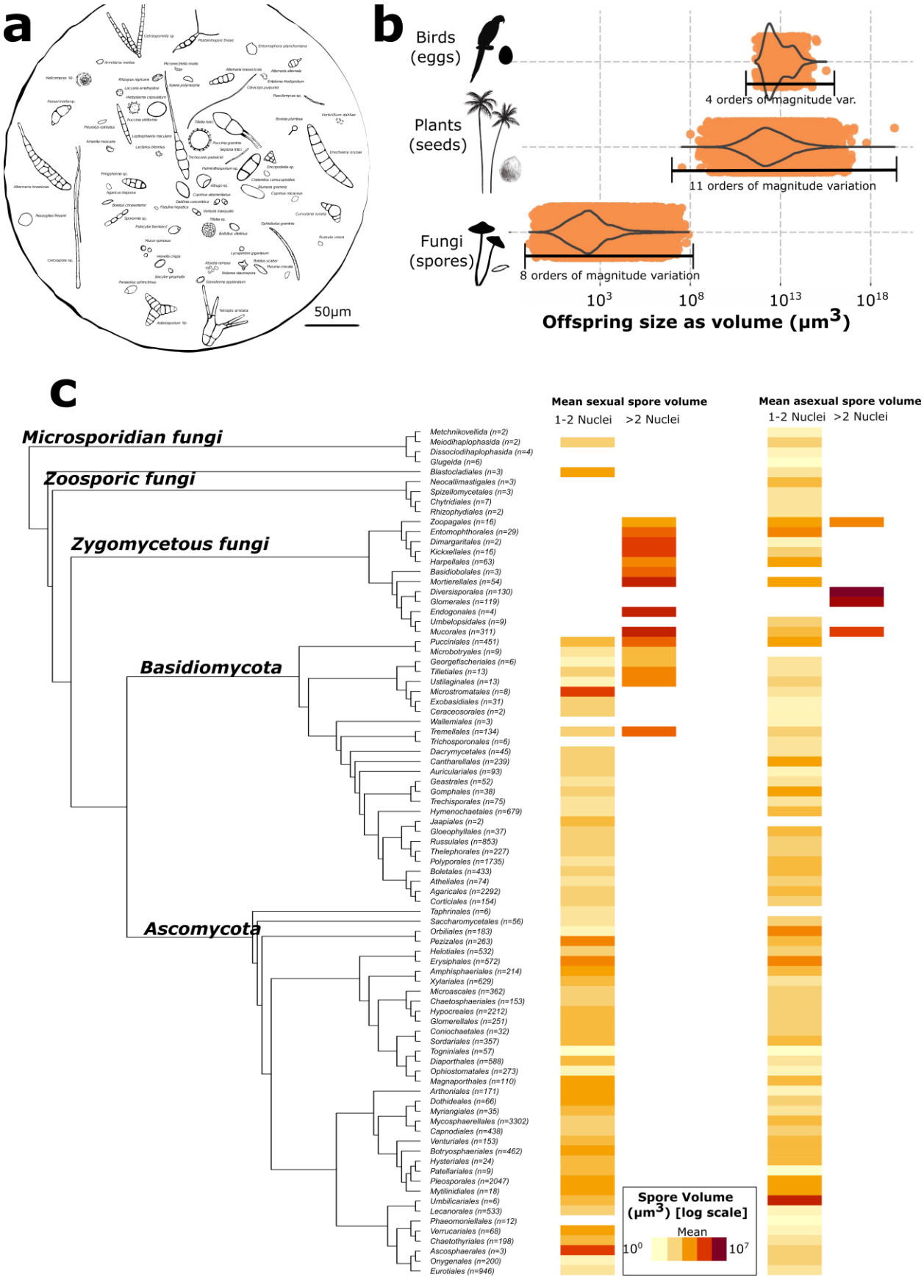
References

1. Stearns S (1992) The evolution of life histories, Oxford Univ. Press. (Oxford. SteamsThe evolution of life histories1992).
2. Moles AT*, et al.* (2005) A Brief History of Seed Size. *Science* 307(5709):576-580.
3. Neuheimer AB*, et al.* (2015) Adult and offspring size in the ocean over 17 orders of magnitude follows two life history strategies. *Ecology* 96(12):3303-3311.
4. Stoddard MC*, et al.* (2017) Avian egg shape: Form, function, and evolution. *Science* 356(6344):1249-1254.
5. Falster DS, Moles AT, & Westoby M (2008) A general model for the scaling of offspring size and adult size. *Am. Nat.* 172(3):299-317.
6. Hawksworth DL & Lucking R (2017) Fungal Diversity Revisited: 2.2 to 3.8 Million Species. *Microbiol Spectr* 5(4).
7. Lutzoni F*, et al.* (2018) Contemporaneous radiations of fungi and plants linked to symbiosis. *Nature communications* 9(1):5451.
8. Naranjo-Ortiz MA & Gabaldon T (2019) Fungal evolution: major ecological adaptations and evolutionary transitions. *Biol. Rev. Camb. Philos. Soc.* 94(4):1443-1476.
9. Chomicki G, Kiers ET, & Renner SS (2020) The Evolution of Mutualistic Dependence. *Annual Review of Ecology, Evolution, and Systematics* 51(1):409-432.
10. Golan J & Pringle A (2017) Long-Distance Dispersal of Fungi. *The Fungal Kingdom*), pp 309-333.
11. Kivlin SN (2020) Global mycorrhizal fungal range sizes vary within and among mycorrhizal guilds but are not correlated with dispersal traits. *J. Biogeogr.* 47(9):1994-2001.
12. Rockett TR & Kramer CL (1974) Periodicity and Total Spore Production by Lignicolous Basidiomycetes. *Mycologia* 66(5):817-829.
13. Halbwachs H (2015) Gone with the wind – a review on basidiospores of lamellate agarics. *Mycosphere* 6(1):78-112.
14. Garrett SD (1970) *Pathogenic root-infecting fungi*.
15. McLaughlin DJ & Spatafora JW (2014) *Systematics and Evolution: Part A* (Springer).
16. Kendrick B (2017) *The fifth kingdom* (Hackett Publishing).
17. Moles AT & Westoby M (2004) Seedling survival and seed size: a synthesis of the literature. *J. Ecol.* 92(3):372-383.
18. Norros V*, et al.* (2014) Do small spores disperse further than large spores? *Ecology* 95(6):1612-1621.
19. Robert V*, et al.* (2013) MycoBank gearing up for new horizons. *IMA Fungus* 4(2):371-379.
20. Ingold CT (2001) Range in size and form of basidiospores and ascospores. *Mycologist* 4(15):165-166.
21. Aguilar-Trigueros CA, Hempel S, Powell JR, Cornwell WK, & Rillig MC (2019) Bridging reproductive and microbial ecology: a case study in arbuscular mycorrhizal fungi. *The ISME journal* 13(4):873-884.
22. Li Y*, et al.* (2021) A genome-scale phylogeny of the kingdom Fungi. *Curr. Biol.*
23. Kokkoris V, Stefani F, Dalpe Y, Dettman J, & Corradi N (2020) Nuclear Dynamics in the Arbuscular Mycorrhizal Fungi. *Trends Plant Sci.*
24. Roper M & Seminara A (2019) Mycofluidics: The Fluid Mechanics of Fungal Adaptation. *Annual Review of Fluid Mechanics* 51(1):511-538.
25. Kemen E & Jones JD (2012) Obligate biotroph parasitism: can we link genomes to lifestyles? *Trends Plant Sci.* 17(8):448-457.
26. Wang C & Wang S (2017) Insect Pathogenic Fungi: Genomics, Molecular Interactions, and Genetic Improvements. *Annu. Rev. Entomol.* 62:73-90.
27. Kauserud H, Colman JE, & Ryvarden L (2008) Relationship between basidiospore size, shape and life history characteristics: a comparison of polypores. *Fungal Ecology* 1(1):19-23.
28. Halbwachs H, Heilmann-Clausen J, & Bässler C (2017) Mean spore size and shape in ectomycorrhizal and saprotrophic assemblages show strong responses under resource constraints. *Fungal Ecology* 26:59-64.
29. Calhim S*, et al.* (2018) Fungal spore diversity reflects substrate-specific deposition challenges. *Sci Rep* 8(1):5356.

30. Bässler C, Heilmann-Clausen J, Karasch P, Brandl R, & Halbwachs H (2014) Ectomycorrhizal fungi have larger fruit bodies than saprotrophic fungi. *Fungal Ecology*.
31. Mengiste T (2012) Plant immunity to necrotrophs. *Annu. Rev. Phytopathol.* 50:267-294.
32. Purhonen J*, et al.* (2020) Morphological traits predict host-tree specialization in wood-inhabiting fungal communities. *Fungal Ecology* 46:100863.
33. Moore D, Robson GD, & Trinci AP (2011) *21st century guidebook to fungi* (Cambridge University Press).
34. Norros V, Karhu E, Norden J, Vahatalo AV, & Ovaskainen O (2015) Spore sensitivity to sunlight and freezing can restrict dispersal in wood-decay fungi. *Ecol Evol* 5(16):3312-3326.
35. Vincenty T (1975) Direct and inverse solutions of geodesics on the ellipsoid with application of nested equations. *Survey review* 23(176):88-93.
36. Edelsbrunner H, Kirkpatrick D, & Seidel R (1983) On the shape of a set of points in the plane. *IEEE Transactions on information theory* 29(4):551-559.
37. Gareth Jones EB (2006) Form and function of fungal spore appendages. *Mycoscience* 47(4):167-183.
38. Poulin R (2011) *Evolutionary ecology of parasites* (Princeton university press).
39. Bielčik M, Aguilar-Trigueros CA, Lakovic M, Jeltsch F, & Rillig MC (2019) The role of active movement in fungal ecology and community assembly. *Movement Ecology* 7(1):36.
40. Gibson CM & Hunter MS (2010) Extraordinarily widespread and fantastically complex: comparative biology of endosymbiotic bacterial and fungal mutualists of insects. *Ecol. Lett.* 13(2):223-234.
41. James TY, Stajich JE, Hittinger CT, & Rokas A (2020) Toward a Fully Resolved Fungal Tree of Life. *Annu. Rev. Microbiol.* 74(1):291-313.
42. Chaloner TM, Gurr SJ, & Bebber DP (2020) Geometry and evolution of the ecological niche in plant-associated microbes. *Nature communications* 11(1):2955.
43. Fones HN, Fisher MC, & Gurr SJ (2017) Emerging Fungal Threats to Plants and Animals Challenge Agriculture and Ecosystem Resilience. *The Fungal Kingdom*), pp 787-809.
44. van der Loo M (2014) The stringdist package for approximate string matching. *The R Journal* 6(1):11-112.
45. Domsch K, Gams W, & Anderson T-H (2007) *Compendium of soil fungi* (IHW-Verlag Eching) Second edition Ed p 672.
46. Chamberlain S & Szöcs E (2013) taxize: taxonomic search and retrieval in R [version 2; peer review: 3 approved]. *F1000Research* 2(191).
47. Zanne AE*, et al.* (2020) Fungal functional ecology: bringing a trait-based approach to plant-associated fungi. *Biological Reviews* 95(2):409-433.
48. Giovannetti M (2000) Spore Germination and Pre-Symbiotic Mycelial Growth. *Arbuscular Mycorrhizas: Physiology and Function*, eds Kapulnik Y & Douds DD (Springer Netherlands, Dordrecht), pp 47-68.
49. Mueller GM (2011) *Biodiversity of fungi: inventory and monitoring methods* (Elsevier).
50. Nguyen NH*, et al.* (2015) FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*.
51. Krah FS*, et al.* (2018) Evolutionary dynamics of host specialization in wood-decay fungi. *BMC Evol. Biol.* 18(1):119.
52. Araujo JP & Hughes DP (2016) Diversity of Entomopathogenic Fungi: Which Groups Conquered the Insect Body? *Adv. Genet.* 94:1-39.
53. Irinyi L*, et al.* (2015) International Society of Human and Animal Mycology (ISHAM)-ITS reference DNA barcoding database--the quality controlled standard tool for routine identification of human and animal pathogenic fungi. *Med. Mycol.* 53(4):313-337.
54. Agrios GN (1997) *Plant Pathology Department of Plant Pathology, University of Florida* (Academic Press).
55. Smith SE & Read DJ (2010) *Mycorrhizal Symbiosis* (Elsevier Science).
56. Větrovský T*, et al.* (2019) A meta-analysis of global fungal distribution reveals climate-driven patterns. *Nature communications* 10(1):5142.
57. Větrovský T*, et al.* (2020) GlobalFungi, a global database of fungal occurrences from high-throughput-sequencing metabarcoding studies. *Scientific Data* 7(1):228.

58. Egidi E*, et al.* (2019) A few Ascomycota taxa dominate soil fungal communities worldwide. *Nature communications* 10(1):2369.
59. Chen Y-L*, et al.* (2017) Plant diversity represents the prevalent determinant of soil fungal community structure across temperate grasslands in northern China. *Soil Biol. Biochem.* 110:12-21.
60. Hu Y*, et al.* (2019) Contrasting latitudinal diversity and co-occurrence patterns of soil fungi and plants in forest ecosystems. *Soil Biol. Biochem.* 131:100-110.
61. Abarenkov K*, et al.* (2020) UNITE general FASTA release for Fungi 2. UNITE Community.
62. Bissett A*, et al.* (2016) Introducing BASE: the Biomes of Australian Soil Environments soil microbial diversity database. *GigaScience* 5(1).
63. Lammel DR, Nüsslein K, Tsai SM, & Cerri CC (2015) Land use, soil and litter chemistry drive bacterial community structures in samples of the rainforest and Cerrado (Brazilian Savannah) biomes in Southern Amazonia. *European Journal of Soil Biology* 66:32-39.
64. Fick SE & Hijmans RJ (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* 37(12):4302-4315.
65. Hijmans RJ & van Etten J (2012) raster: Geographic analysis and modeling with raster data.
66. Bivand R, Keitt T, & Rowlingson B (2020) rgdal: Bindings for the 'Geospatial' Data Abstraction Library.
67. Wijayawardene NN*, et al.* (2018) Notes for genera: basal clades of Fungi (including Aphelidiomycota, Basidiobolomycota, Blastocladiomycota, Calcarisporiellomycota, Caulochytriomycota, Chytridiomycota, Entomophthoromycota, Glomeromycota, Kickxellomycota, Monoblepharomycota, Mortierellomycota, Mucoromycota, Neocallimastigomycota, Olpidiomycota, Rozellomycota and Zoopagomycota). *Fungal Diversity* 92(1):43-129.
68. Team RC (2020) R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna).
69. Hijmans RJ, Williams E, Vennes C, & Hijmans MRJ (2019) Package 'geosphere'. *Spherical trigonometry* 1:7.
70. Davis Rabosky AR*, et al.* (2016) Coral snakes predict the evolution of mimicry across New World snakes. *Nature communications* 7(1):11484.
71. Bates D, Mächler M, Bolker B, & Walker S (2015) Fitting Linear Mixed-Effects Models Using {lme4}. *Journal of Statistical Software* 67:1-48.
72. Bürkner P-C (2017) brms: An R Package for Bayesian Multilevel Models Using Stan. *2017* 80(1):28.

Figures and Tables


a
50µm
b
Birds (eggs)
Plants (seeds)
Fungi (spores)
4 orders of magnitude var.
11 orders of magnitude variation
8 orders of magnitude variation
10^3
10^8
10^{13}
10^{18}
Offspring size as volume (µm^3)
c
Microsporidian fungi
Zoosporic fungi
Zygomycetous fungi
Basidiomycota
Ascomycota
Metchnikovellida (n=2)
Meiodihaplophasida (n=2)
Dissociodihaplophasida (n=4)
Glugeida (n=6)
Blastocladiales (n=3)
Neocallimastigales (n=3)
Spizellomycetales (n=3)
Chytridiales (n=7)
Rhizophydiales (n=2)
Zoopagales (n=16)
Entomophthorales (n=29)
Dimargaritales (n=2)
Kickxellales (n=16)
Harpellales (n=63)
Basidiobolales (n=3)
Mortierellales (n=54)
Diversisporales (n=130)
Glomerales (n=119)
Endogonales (n=4)
Umbelopsidales (n=9)
Mucorales (n=311)
Pucciniales (n=451)
Microbotryales (n=9)
Georgefischeriales (n=6)
Tilletiales (n=13)
Ustilaginales (n=13)
Microstromatales (n=8)
Exobasidiales (n=31)
Ceraceosorales (n=2)
Wallemiales (n=3)
Tremellales (n=134)
Trichosporonales (n=6)
Dacrymycetales (n=45)
Cantharellales (n=239)
Auriculariales (n=93)
Geastrales (n=52)
Gomphales (n=38)
Trechisporales (n=75)
Hymenochaetales (n=679)
Jaapiales (n=2)
Gloeophyllales (n=37)
Russulales (n=853)
Thelephorales (n=227)
Polyporales (n=1735)
Boletales (n=433)
Atheliales (n=74)
Agaricales (n=2292)
Corticiales (n=154)
Taphrinales (n=6)
Saccharomycetales (n=56)
Orbiliales (n=183)
Pezizales (n=263)
Helotiales (n=532)
Erysiphales (n=572)
Amphisphaeriales (n=214)
Xylariales (n=629)
Microascales (n=362)
Chaetosphaeriales (n=153)
Hypocreales (n=2212)
Glomerellales (n=251)
Coniochaetales (n=32)
Sordariales (n=357)
Togniniales (n=57)
Diaporthales (n=588)
Ophiostomatales (n=273)
Magnaporthales (n=110)
Arthoniales (n=171)
Dothideales (n=66)
Myriangiales (n=35)
Mycosphaerellales (n=3302)
Capnodiales (n=438)
Venturiales (n=153)
Botryosphaeriales (n=462)
Hysteriales (n=24)
Patellariales (n=9)
Pleosporales (n=2047)
Mytilinidiales (n=18)
Umbilicariales (n=6)
Lecanorales (n=533)
Phaeomoniellales (n=12)
Verrucariales (n=68)
Chaetothyriales (n=198)
Ascosphaerales (n=3)
Onygenales (n=200)
Eurotiales (n=946)
Mean sexual spore volume
1-2 Nuclei
>2 Nuclei
Mean asexual spore volume
1-2 Nuclei
>2 Nuclei
Spore Volume (µm^3) [log scale]
Mean
10^0
10^7

792

Fig. 1. Interspecific variation in spore size and symbiotic lifestyles across the fungal kingdom: a) Illustration of the diversity of shapes and sizes among all fungal spore types. b) Interspecific spore-size variation is more than eight orders of magnitude across the kingdom, ranging from the mitospores of *Phoma muscivora* of 9.0 $x10^{-2}$ μm^3 to multinucleate spores of the mycorrhizal fungus *Scutellospora scutata* of 7.8 x 10^7 μm^3. This variation is comparable to that of other offspring structures such as angiosperm seeds and bird eggs (to aid comparison, all offspring structures are presented on the same scale [μm^3]). c) Phylogenetic tree with terminal branches representing orders (the number of species per order for which we collected spore data is given in parenthesis). The corresponding heatmap displays order averages (in logarithmic scale) of spore size as volume in yellow-to-red color scale for sexual and asexual spores separating spores types based on the number of nuclei which is a major distinction in spore types for fungi (see main text and Materials and Method for a detailed explanation on descriptions of the biology of these distinct spores). Fungal spores (n= 26, 134 species), avian egg data (n = 1,395 species) were obtained from [7], while seed data (n = 34,390 species) were obtained from the seed database of Kew Botanical Garden (http://data.kew.org/sid/?_ga=2.73581714.1287366807.1501084977-1309187973.1501084964).

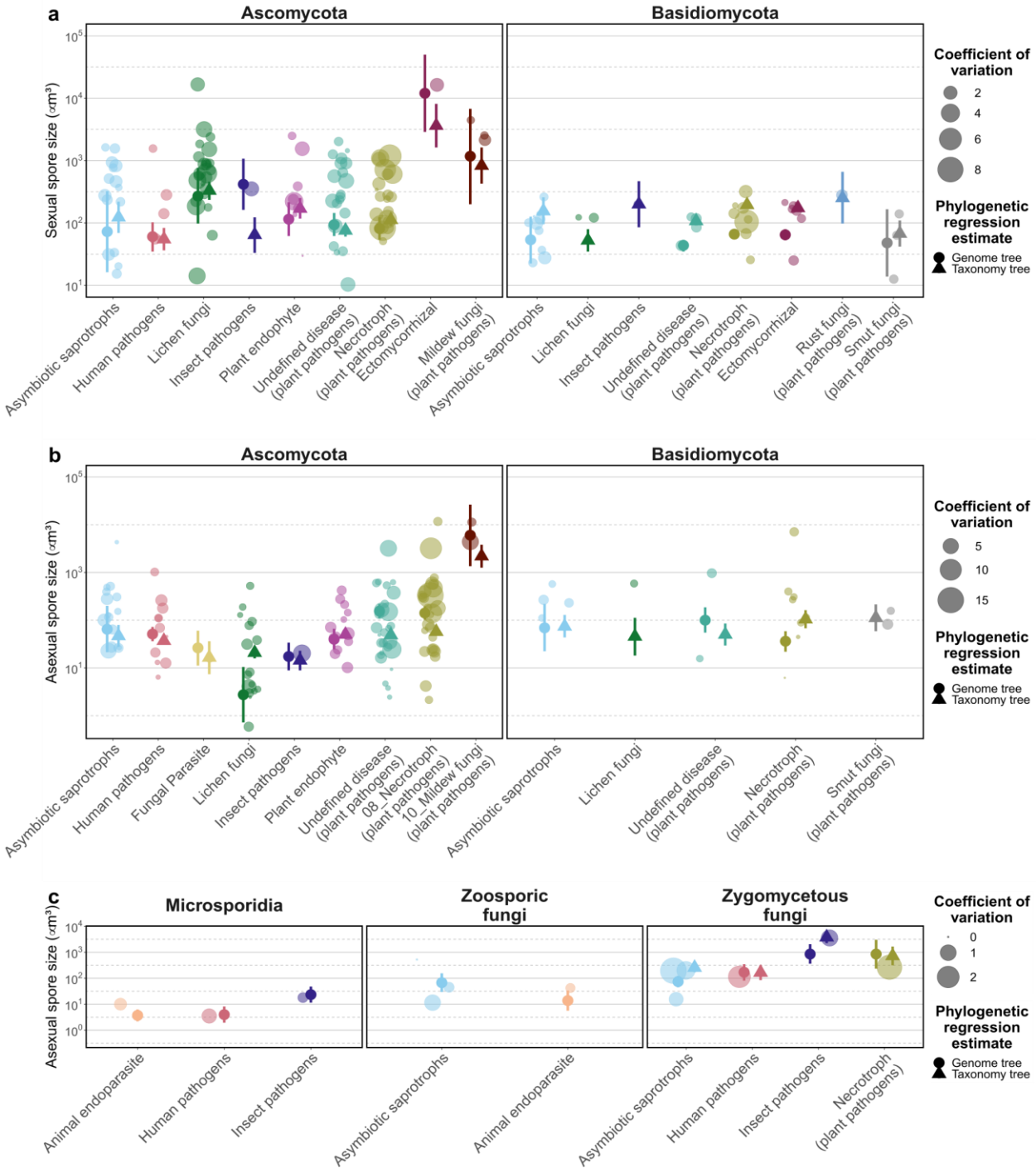


Fig. 2. Bubble plots depicting size shifts of sexual and asexual spores among symbiotic groups across Dikarya and non-Dikarya fungi. Each bubble represents the mean spore value of a taxonomic order and its size represents its coefficient of variation (based on all species within that order). Point ranges show the predicted mean values (points) and associated standard errors (ranges) for each symbiotic group: circle points depict the predictions from phylogenetic linear regression using the genome-based phylogeny, triangular point ranges depict the predictions from phylogenetic linear regression using the taxonomy-based phylogeny.

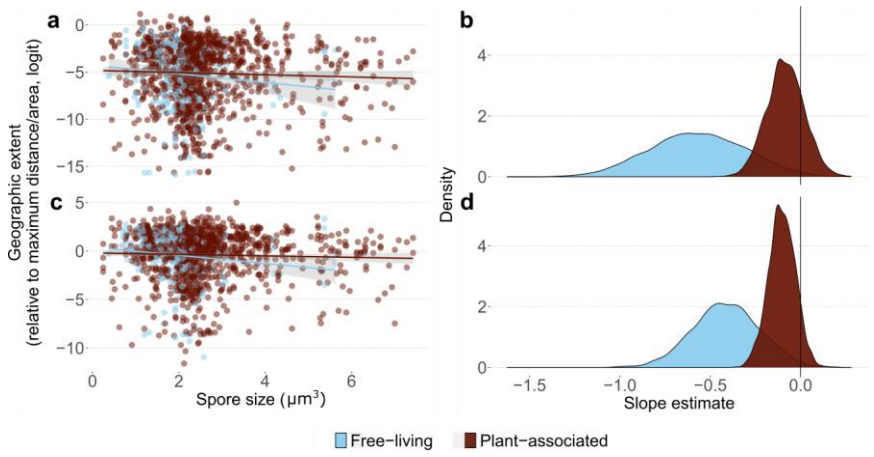


Fig. 3. Asymbiotic fungal species exhibit a negative relationship between spore size and geographic distributions, while species plant associated fungi do not. (a,c). Relationship between spore size and geographic distribution (based on polygon area [a] and the maximum distance between samples in which species were detected [c]) for asymbiotic fungal species and fungal species exhibiting varying degrees of host association. Fungal species were detected in global surveys of environmental DNA from soil and plant material. (b,d) Bayesian models were fitted to estimate posterior distributions of the slope parameters representing the strength of the relationship between geographic extent and spore volume. The density represents the likelihood that a value associated with the slope estimate was present in the posterior distribution. These models included random intercepts representing the taxonomic order and spore type, as well as the primer set used to amplify fungal DNA (Table S6-S8). Only species producing a single spore type were used in this analysis.

Table 1. Relative importance of symbiotic lifestyle versus climatic variables in explaining interspecific spore size variation. The fit of two phylogenetic linear regression models with lifestyle and six climatic variables as explanatory factors is compared to the fit of models in which one of these predictors was removed (indicated in the respective row). The first model uses the phylogenetic tree based on whole genome sequences as provided in (22) which includes 281 species from which we collected climatic data (referred to as the "genome tree model"). . The second model uses a taxonomy-based cladogram for species based on their taxonomy from kingdom to species level (referred to as the "taxonomy tree model") which includes 1137 species from which we collected climatic data. AIC = Akaike's Information Criterion, *d*AIC= delta AIC (difference between the AIC of each model and the one containing all terms). A large delta (e.g., *d*AIC > 10) AIC indicates that dropping that term from the model results in a large decline in model fit. A small (< 2) or negative delta AIC indicates that dropping that term from the model improves model fit.

Phylogenetic regression model	**Adjusted r^2**	**Loglik**	**AIC**	**dAIC**	**Phylogeny used**
All variables	0.21	-336.72	699.43		Genome tree
	0.09	-1328.2	2684.39		Taxonomy tree
(-) Symbiotic lifestyle	**0.18**	**-341.41**	**706.82**	**7.39**	**Genome tree**
	0.07	**-1333.42**	**2690.85**	**6.45**	**Taxonomy tree**
(-) mean annual temperature	0.21	-337.97	699.94	0.51	Genome tree
	0.09	-1328.23	2682.45	-1.94	Taxonomy tree
(-) mean annual precipitation	0.21	-337.25	698.49	-0.94	Genome tree
	0.09	-1328.34	2682.68	-1.71	Taxonomy tree
(-) Temperature seasonality	0.22	-337.02	698.04	-1.39	Genome tree
	0.09	-1328.23	2682.47	-1.93	Taxonomy tree
(-) Precipitation seasonality	0.21	-337	698	-1.43	Genome tree
	0.09	-1329.66	2685.32	0.93	Taxonomy tree
(-) Maximum solar radiation	0.18	-341.29	706.59	7.15	Genome tree
	0.08	-1330.52	2687.05	2.66	Taxonomy tree
(-) Minimum vapor pressure	0.22	-336.72	697.43	-2	Genome tree
	0.09	-1328.25	2682.49	-1.9	Taxonomy tree