

Review Article

The Fungal Holobiont: Immunity, Individuality, and the Evolutionary Foundations of Fungal Innate Immunity

Jalal Soltani[✉], Mahrouyeh Ketabchi^{ID}

Department of Plant Protection, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran

doi 10.22092/mi.2026.373959.1356

ABSTRACT

Fungi are increasingly recognized as hosts of persistent microbial associations, including endohyphal bacteria and mycoviruses, raising important questions about fungal individuality and the extent to which fungal-microbial associations can be considered holobionts, i.e. complex assemblages composed of a host and its associated microbiota. This conceptual shift, rooted in long-standing philosophical debates on biological individuality, challenges traditional organism-centered views and repositions particular fungal-microbial systems as potentially integrated physiological-immunological and ecological units. This analysis synthesizes current knowledge on fungal endosymbionts and examines how these often cryptic partners modulate fungal phenotypes, influencing growth, reproduction, metabolism, stress tolerance and pathogenicity. Drawing on both biological and philosophical perspectives, we critically analyze criteria for fungal individuality, the immunological integration of fungal holobionts, and the extension of physiological individuality across generations through heritable microbial symbionts. We propose that the fungal immune system, particularly NLR-based surveillance and regulated cell death pathways, plays a central role in dynamically defining the boundaries of the fungal self by determining which microbes are tolerated as symbionts and which are rejected as pathogens. Adopting a fungal holobiont perspective provides a framework for understanding fungal biology and offers insights into the evolutionary and ecological significance of persistent fungal-microbe associations.

KEYWORDS

Biological Individuality, Endohyphal Bacteria, Mycoviruses, NLR Proteins, Regulated Cell Death.

INTRODUCTION

The concept of biological individuality has been the subject of sustained philosophical debate since the early nineteenth century, with plants serving as a central test case (Clarke 2012, Baedke 2024). Unlike animals, plants exhibit pronounced modularity, vegetative propagation and genetic mosaicism, challenging traditional criteria used to define biological individuals (Toepfer 2011, Gilbert et al. 2012). These debates have gained renewed relevance with the growing recognition that plants, and indeed all eukaryotic organisms, harbor diverse microbial communities that profoundly influence their physiology, development and fitness (Pradeu 2012, Bordenstein and Theis 2015).

Fungi, which interact closely with plants as pathogens, mutualists and commensals, raise closely related conceptual challenges (Kobayashi and Crouch 2009, Frey-Klett et al. 2011). A fungal mycelium may be extensive, genetically heterogeneous and capable of fusing with genetically distinct individuals, resulting in chimeric or mosaic entities (Rayner 1995, Glass et al. 2004). Vegetative incompatibility between genetically distinct mycelia contributes to the maintenance of mycelial integrity and restricts the spread of deleterious cytoplasmic elements, and may function as a form of prophylactic defense (Debets et al. 1994, Diepeningen et al. 1997). The widespread occurrence of persistent microbial endosymbionts, including endohyphal bacteria (EHB) and mycoviruses, adds an additional layer of complexity and further challenges classical organism-centered notions of individuality (Pakvaz and Soltani 2016, Pawlowska et al. 2018, Partida-Martínez 2024).

Received: 15 Aug. 2026

Revised: 11 Oct. 2026

Accepted: 15 Oct. 2026

Published online: 19 Oct. 2026

✉ Corresponding Author: Jalal Soltani; Email: Soltani@basu.ac.ir

Mycologia Iranica is licensed under a "Creative Commons Attribution 4.0 International (CC-BY 4.0)"



Published by Iranian Mycological Society (IRMS)—<https://mij.areeo.ac.ir>

The term *holobiont* was introduced by Margulis and Fester (1991) to describe symbiotic assemblages composed of a host organism and its associated microorganisms. Originally applied to corals and later extended to plants and animals, the holobiont concept has gained traction as evidence has accumulated for the ubiquity and functional importance of host-associated microbiomes (Vandenkoornhuys et al. 2015, Sánchez-Cañizares et al. 2017). Within this framework, particular fungi can be considered holobionts when persistent microbial partners substantially contribute to their physiology or ecological interactions (Bonfante and Desirò 2017, Deveau et al. 2018). However, the presence, persistence and functional significance of microbial partners may vary among fungal individuals and species. This nested organization, microbiomes within microbiomes, appears to be a general feature of biological systems (Moran et al. 2008, O'Malley 2015).

The study of fungal endosymbionts has a rich history predating the holobiont concept. Bacterial endosymbionts in fungi were first observed via microscopy in 1970, with the description of bacteria-like structures associated with mycorrhizal fungi (Mosse 1970, Bianciotto et al. 1996). Mycoviruses were formally described in 1962, when virus-like particles were observed in cultivated mushrooms suffering from La France disease (Hollings 1962, Ghabrial et al. 2015). However, it is only during the past two decades, with the advent of molecular techniques and high-throughput sequencing, that the true diversity and functional importance of fungal-associated microbes have begun to emerge (Myers et al. 2020, Kelliher et al. 2023).

In this analytic review, we synthesize current knowledge on fungal holobionts, with emphasis on both bacterial and viral endosymbionts and their implications for understanding fungal individuality and immunity. We address the following four key questions: (1) How does the holobiont concept challenge traditional notions of fungal individuality? (2) What are the criteria for recognizing fungal holobionts as biological individuals? (3) How do fungal immune systems shape the boundaries of the fungal self? (4) How do endohyphal bacteria and mycoviruses modulate fungal phenotypes?

CRITERIA FOR FUNGAL HOLOBIONT INDIVIDUALITY

Several criteria have been proposed for recognizing holobionts as biological individuals (Chiu and Eberl 2016, Suárez and Stencel 2020). Applying these criteria to fungal holobionts reveals the complexity of fungal individuality as follows (Table 1).

1. Physiological integration

The host and its symbionts function as a coordinated physiological unit, with metabolic interdependencies and shared biochemical pathways (Stencel and Proszewska 2018, Triviño and Suárez 2020). In fungal holobionts, EHB contribute to nitrogen fixation, vitamin synthesis, and toxin production, while receiving carbon and other nutrients from their hosts (Lackner et al. 2011, Sharmin et al. 2018). Mycoviruses may impose metabolic costs or provide benefits under specific conditions (Espino-Vázquez et al. 2020, Valadez-Cano et al. 2024). Genome-scale metabolic modelling of the *Rhizopus microsporus* holobiont has revealed metabolic integration among the fungus, its bacterial endosymbiont *Mycetohabitans rhizoxinica*, and its viral symbionts (Narnaviruses), predicting metabolic dependencies and resource exchanges that may not be apparent when individual components are studied in isolation (Valadez-Cano et al. 2024).

2. Immunological tolerance

The host immune system can tolerate persistent microbial associations while responding to other interacting organisms, providing one possible framework for considering the boundaries of an immunological individual (Pradeu 2016, 2019). In fungi, NOD-like receptors (NLR)-based surveillance systems have been shown to monitor the integrity of key cellular components and to participate in self/non-self recognition and allorecognition (Heller et al. 2018, Daskalov et al. 2020, Greslehner 2020, Schneider 2021). However, direct evidence that these systems generally distinguish tolerated symbionts from rejected pathogens in fungal holobionts remains limited. The observation that bacteria-free *R. microsporus* can be reinfected with *Mycetohabitans* suggests that the association can be re-established, indicating that host-symbiont boundaries may be dynamic rather than fixed (Partida-Martinez et al. 2007, Mondo et al. 2017). Similarly, mycoviruses that establish persistent infections interact with fungal defense mechanisms (Uehling et al. 2017, Daskalov 2023).

3. Vertical transmission

Symbionts transmitted across host generations contribute to the continuity of the holobiont lineage (Bright and Bulgheresi 2010, Funkhouser and Bordenstein 2013). Many fungal endosymbionts are vertically transmitted through spores, ensuring their persistence across generations (Bianciotto et al. 2004, Pawlowska et al. 2018, Espino-Vázquez et al. 2020). In *R. microsporus*, both *Mycetohabitans* bacteria and Narnaviruses are inherited in sporangiospores and zygospores, maintaining the symbiotic assemblage through fungal reproduction (Partida-Martinez et al. 2007, Espino-Vázquez et al. 2020). Studies on endophytic fungi have also documented the presence of EHB over several rounds of subculturing, suggesting persistent vegetative transmission routes (Pakvaz and Soltani 2016).

4. Co-evolution

Host and symbionts exhibit signatures of co-evolution, including genome reduction in symbionts and the evolution of specialized interaction mechanisms (Naito et al. 2015, Torres-Cortés et al. 2015). The genomes of obligate EHB show extreme reduction (0.7–1.2 Mb) and dependence on host functions (Kuo 2015, Naito et al. 2017). Some endobacteria harbor genes from their fungal hosts, and strikingly, these genes often carry heterokaryon incompatibility (HET) domains, suggesting ancient exchanges between immune and symbiotic systems (Naito et al. 2015, Torres-Cortés et al. 2015). Mycoviruses also show signatures of co-evolution with their fungal hosts, including codon usage adaptation and the evolution of persistent lifestyles (Roossinck 2011, Ghabrial et al. 2015).

Table 1. Criteria for fungal holobiont individuality.

Criterion	Evidence in fungal holobionts	Description	References
Physiological integration	EHB dependence on host; metabolic modeling of <i>R. microsporus</i> holobiont	Metabolic interdependencies between host and symbionts	Lackner et al. (2011), Sharmin et al. (2018), Stencel and Proszewska (2018), Triviño and Suárez (2020), Valadez-Cano et al. (2024)
Immunological tolerance	NLR-based surveillance; re-establishment of symbiosis in cured fungi	Immune system tolerates symbionts while responding to pathogens	Partida-Martinez et al. (2007), Mondo et al. (2017), Pradeu (2016, 2019), Greslehner (2020), Schneider (2021)
Vertical transmission	EHB and Narnaviruses transmitted through spores in <i>R. microsporus</i> ; vertical transmission in AMF	Symbionts transmitted across host generations	Bianciotto et al. (2004), Partida-Martinez et al. (2007), Bright and Bulgheresi (2010), Pawlowska et al. (2018), Espino-Vázquez et al. (2020)
Co-evolution	Genome reduction in obligate EHB; host-derived genes in endobacterial genomes	Signatures of co-evolution between host and symbionts	Kuo (2015), Naito et al. (2015), Torres-Cortés et al. (2015), Naito et al. (2017)

ENDOHYPHAL BACTERIA: KEY PROKARYOTIC SYMBIONTS

Endohyphal bacteria (EHB) are bacteria that live within fungal hyphae, establishing intimate associations with their hosts, ranging from facultative to obligate (Hoffman and Arnold 2010, Pakvaz and Soltani 2016). Based on their phylogenetic affiliations and genomic features, EHB can be classified into three major classes (Desirò et al. 2014, Shaffer et al. 2016).

1. Class 1 EHB: Mollicutes-related endobacteria (MRE)

These obligate endobacteria are observed primarily in arbuscular mycorrhizal fungi (AMF) and other members of *Mucoromycota* (Naumann et al. 2010, Desirò et al. 2014). They have highly reduced genomes (approximately 0.7–1.2 Mb), reflecting metabolic reliance on their fungal host (Kuo 2015, Naito et al. 2015). MRE are globally distributed and vertically transmitted through fungal spores (Desirò et al. 2014).

2. Class 2 EHB: *Burkholderiaceae* and related bacteria

Class 2 EHB include members of the family *Burkholderiaceae*, including the genus *Mycetohabitans*, that associate with fungi from *Glomeromycota*, *Mortierellomycota*, and *Mucoromycota* (Bianciotto et al. 2003, Santos et al. 2018). These bacteria have larger genomes (1.4–3.8 Mb) and can be transmitted both vertically and horizontally (Bianciotto et al. 2004, Arendt et al. 2016).

3. Class 3 EHB: Diverse facultative associates

Class 3 EHB comprise a phylogenetically diverse group of bacteria that associate with hyphae of diverse Dikarya fungi (*Ascomycota* and *Basidiomycota*) (Barbieri et al. 2000, 2002, Bertaux et al. 2005, Sharma et al. 2008). These bacteria have the largest genomes (3.9–8.5 Mb) and show no significant genome reduction, indicating facultative associations with horizontal transmission (Hoffman et al. 2013, Arendt et al. 2016, Boynton et al. 2019). Studies on endophytic fungi from *Cupressaceae* have also revealed diverse EHB with bioactive potential (Pakvaz and Soltani 2016, Soltani 2017).

4. Functional roles

EHB can influence fungal phenotypes, affecting growth, reproduction, metabolism, stress tolerance, and interactions with other organisms (Mondo et al. 2017, Arora and Riyaz-Ul-Hassan 2019). In AMF, the EHB *Candidatus Glomeribacter gigasporarum* enhances fungal ATP synthesis, detoxification of reactive oxygen species, and sporulation (Salvioli et al. 2016). The *Rhizopus-Mycetohabitans* interaction provides a well-characterized example, with bacterial control of toxin production and asexual reproduction (Partida-Martinez and Hertweck 2005, Partida-Martinez et al. 2007). EHB can also modulate fungal stress tolerance (Lupini et al. 2022), alter carbon substrate use (Shaffer et al. 2017), and enhance the production of phytohormones such as indole-3-acetic acid (Hoffman et al. 2013). A summary of well-characterized EHB and their effects on fungal hosts is presented in Table 2.

Table 2. Functional effects of endohyphal bacteria on fungal hosts phenotypes.

Fungal host	EHB partner	Functional effect	References
<i>Rhizopus microsporus</i>	<i>Mycetohabitans rhizoxinica</i>	Toxin production, asexual reproduction, sexual success	Partida-Martinez and Hertweck (2005, 2007), Partida-Martinez et al. (2007), Mondo et al. (2017)
<i>Gigaspora margarita</i> (AMF)	<i>Candidatus Glomeribacter gigasporarum</i>	Enhanced ATP synthesis, ROS detoxification, sporulation	Salvioli et al. (2016), Sharmin et al. (2018)
<i>Mortierella elongata</i>	<i>Mycoavidus cysteinexigens</i>	Metabolic integration (vitamin B12, biotin)	Sharmin et al. (2018)
<i>Fusarium keratoplasticum</i>	<i>Chitinophaga</i> sp.	Altered carbon substrate use	Shaffer et al. (2017)
<i>Pestalotiopsis neglecta</i>	<i>Luteibacter</i> sp.	Enhanced IAA production	Hoffman et al. (2013)
Endophytic fungi from <i>Cupressaceae</i>	Diverse EHB	Antifungal and antibacterial activity	Pakvaz and Soltani (2016)

MYCOVIRUSES: THE VIRAL COMPONENT OF FUNGAL HOLOBIONTS

Mycoviruses are viruses that infect fungi, residing within fungal cells as persistent endosymbionts (Ghabrial and Suzuki 2009, Pearson et al. 2009, Golpayegani et al. 2026). Since their first formal description in 1962 (Hollings 1962), mycoviruses have been found in all major fungal phyla (Ghabrial et al. 2015, Myers et al. 2020, Golpayegani et al. 2026). Most known mycoviruses possess RNA genomes, either double-stranded (dsRNA) or single-stranded (ssRNA), with only a few having single-stranded DNA (ssDNA) genomes (Yu et al. 2010, Kondo et al. 2022).

1. Major groups and transmission

dsRNA mycoviruses belong to several families including *Partitiviridae*, *Totiviridae*, and *Chrysoviridae*, typically forming icosahedral particles (Chiba et al. 2018, Vainio et al. 2018a). (+)ssRNA mycoviruses include *Hypoviridae*, which lack true virions and are enclosed in host-derived lipid vesicles (Hillman and Suzuki 2004, Nuss 2005). (-)ssRNA mycoviruses, though less common, have been increasingly recognized (Kondo et al. 2013, Liu et al. 2014), while ssDNA mycoviruses (*Genomoviridae*) represent a recent discovery with significant biocontrol potential (Yu et al. 2010, Krupovic et al. 2015). Mycoviruses lack extracellular transmission mechanisms and depend on horizontal transmission through hyphal anastomosis or vertical transmission through spores (Pearson et al. 2009, Ghabrial et al. 2015).

2. Functional effects on fungal hosts

Mycoviruses can exert diverse effects on their fungal hosts, ranging from cryptic infections to significant phenotypic alterations. Table 3 provides a comprehensive summary of major mycoviruses and their effects on fungal phenotypes. The most celebrated effect is hypovirulence, i.e. reduced pathogenicity of fungal plant pathogens. *Cryphonectria hypovirus 1* (CHV1) infection of *Cryphonectria parasitica* reduces fungal virulence, allowing infected chestnut trees to recover from blight (Nuss 2005, Bryner et al. 2012, Rigling and Prospero 2018). Similarly, *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1) converts *Sclerotinia sclerotiorum* from a necrotrophic pathogen into a beneficial endophyte (Yu et al. 2010, Zhang et al. 2020).

Some mycoviruses can induce hypervirulence, increasing fungal pathogenicity. In *Magnaporthe oryzae*, *Totivirus* infection enhances production of tenuazonic acid mycotoxin (Ninomiya et al. 2020, 2021). In the entomopathogenic fungus *Beauveria bassiana*, *Polymycovirus* infection increases pathogenicity toward insect hosts (Kotta-Loizou and Coutts 2017, Filippou et al. 2018).

A striking example of mycovirus-mediated stress tolerance comes from the endophytic fungus *Curvularia protuberata*, which colonizes panic grasses in geothermal soils (Márquez et al. 2007). The fungus confers heat tolerance to its host plant only when infected with *Curvularia* thermal tolerance virus (CThTV) (Márquez et al. 2007, Roossinck 2011). This three-way symbiosis shows that mycoviruses can be essential for holobiont function in particular ecological systems.

Mycoviruses can also alter fungal secondary metabolism. In *Saccharomyces cerevisiae*, the L-A virus and its satellite M dsRNA confer the killer phenotype, where infected yeasts produce mycotoxins that kill competing sensitive strains (Schmitt and Breinig 2002, 2006). In *Aspergillus fumigatus*, multiple mycoviruses cause phenotypic alterations through host-specific mRNA silencing (Özkan et al. 2017, Zoll et al. 2018).

Table 3. Functional effects of mycoviruses on fungal phenotypes.

Fungal host	Mycovirus	Family	Phenotypic effect	References
<i>Cryphonectria parasitica</i>	Cryphonectria hypovirus 1 (CHV1)	Hypoviridae	Hypovirulence	Nuss (2005), Rigling and Prospero (2018)
<i>Sclerotinia sclerotiorum</i>	Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1)	Genomoviridae	Hypovirulence; conversion to endophyte	Yu et al. (2010), Zhang et al. (2020)
<i>Rosellinia necatrix</i>	Rosellinia necatrix megabirnavirus 1 (RnMBV1)	Megabirnaviridae	Hypovirulence	Chiba et al. (2009), Kanematsu et al. (2014)
<i>Heterobasidion annosum</i>	Heterobasidion partitivirus (HetPV13-An1)	Partitiviridae	Growth debilitation; reduced virulence	Hyder et al. (2013), Vainio et al. (2018b)
<i>Magnaporthe oryzae</i>	Magnaporthe oryzae virus (MoV)	Totiviridae	Hypervirulence; enhanced mycotoxin production	Ninomiya et al. (2020)
<i>Beauveria bassiana</i>	Beauveria bassiana polymycovirus 1 (BbPmV-1)	Polymycoviridae	Hypervirulence toward insects	Kotta-Loizou and Coutts (2017), Filippou et al. (2018)
<i>Saccharomyces cerevisiae</i>	Saccharomyces cerevisiae virus L-A (ScV-L-A)	Totiviridae	Killer phenotype	Schmitt and Breinig (2002, 2006)
<i>Aspergillus fumigatus</i>	Aspergillus fumigatus mycoviruses	Various	Altered sporulation; reduced pigmentation	Özkan et al. (2017), Zoll et al. (2018)
<i>Curvularia protuberata</i>	Curvularia thermal tolerance virus (CThTV)	Unassigned	Thermal tolerance symbiosis	Márquez et al. (2007), Roossinck (2011)
<i>Rhizopus microsporus</i>	Rhizopus microsporus 20S/23S Narnaviruses	Narnaviridae	Reduced asexual sporulation; influence on sexual reproduction	Espino-Vázquez et al. (2020), Valadez-Cano et al. (2024)

THE *RHIZOPUS MICROSPORUS* HOLOBIONT: A MODEL SYSTEM

Rhizopus microsporus has emerged as a well-studied example of a fungal holobiont, harboring both bacterial and viral endosymbionts that influence its biology (Espino-Vázquez et al. 2020, Partida-Martinez 2024). Research on rhizoxin-producing strains revealed that the biosynthetic genes required for rhizoxin production are not of fungal origin, but belong to the bacterial endosymbiont *Mycetohabitans rhizoxinica* (Partida-Martinez and Hertweck 2005, 2007, Lackner et al. 2009). The fungus provides enzymes for the biosynthesis of the 2,3-oxirane ring of the rhizoxin precursor, indicating close metabolic cooperation (Scherlach et al. 2012).

Strains of *R. microsporus* that lack their bacterial symbionts can no longer reproduce asexually (Partida-Martinez et al. 2007, Mondo et al. 2017). Sexual reproduction is also influenced by the presence of both bacterial and viral symbionts, as zygospore production is compromised if both symbionts are eliminated (Espino-Vázquez et al. 2020, Valadez-Cano et al. 2024). The Narnaviruses associated with *R. microsporus* reduce asexual spore production, imposing a metabolic cost on the fungus while being vertically transmitted alongside the bacterial symbionts (Espino-Vázquez et al. 2020). This system demonstrates the complex interdependencies that can evolve within particular fungal holobionts.

Currently, *R. microsporus*-*Mycetohabitans* should be considered a well-characterized model system rather than a universal representation of fungal-bacterial associations. The occurrence, stability, transmission and functional significance of microbial partners vary among fungal lineages and among host individuals.

FUNGAL INNATE IMMUNITY AND THE DEFINITION OF SELF

Fungal cells possess sophisticated mechanisms for recognizing and responding to non-self elements, maintaining cellular integrity, and coordinating regulated cell death (RCD) (Uehling et al. 2017, Daskalov 2023). This recognition system serves multiple functions, including defense against pathogens and predators, limiting mycoviral transmission, maintaining individual integrity, and potentially regulating symbiotic associations.

1. NOD-like receptors (NLR)-based immunity

Fungal NOD-like receptors (NLRs) are tripartite proteins containing a central nucleotide-binding domain (NBD), an N-terminal effector domain, and a C-terminal repeat domain. Fungal NLRs exhibit remarkable diversity, with at least 12 N-terminal effector domains (Dyrka et al. 2014, Wojciechowski et al. 2022). A subset of functionally validated *het* (heterokaryon incompatibility) genes encodes NLR proteins that control allorecognition. In *Neurospora crassa*, the PLP-1 NLR recognizes the essential SNARE protein SEC-9, and perturbation of SEC-9 by incompatible interactors can trigger RCD (Daskalov 2023, 2026, Daskalov et al. 2017, 2020, Heller et al. 2018).

The “guard model,” originally developed for plant immunity, provides a mechanistic framework for understanding fungal NLR function (Jones and Dangl 2006, Daskalov et al. 2020). According to this model, NLRs “guard” essential host proteins (guardees) against perturbation by pathogens or incompatible interactors. When a guardee is disrupted, the NLR initiates RCD, eliminating compromised cells. Experimental evidence in fungi supports NLR-mediated surveillance of essential cellular components, including SEC-9 surveillance during allorecognition (Heller et al. 2018). However, whether such surveillance generally functions as a guard mechanism for distinguishing tolerated symbionts from pathogens in fungal holobionts remains to be experimentally established.

2. Evolutionary connections

Fungal immune components exhibit striking evolutionary relationships with cell-death pathways in other kingdoms. Fungal gasdermin-like proteins (fGSDMs) controlling allorecognition in *Podospora anserina* and *N. crassa* share deep evolutionary connections with pyroptosis in mammals (Daskalov et al. 2020, Clavé et al. 2022). The HeLo domain, found in fungal cell-death effectors like HET-S, shares structural similarity with the four-helix bundle domain of MLKL, the executioner of necroptosis in mammals (Daskalov et al. 2016, Wojciechowski et al. 2022). The key components of fungal innate immune systems, including NLRs, HET/TIR domains, HeLo domains, and gasdermins, are summarized in Table 4.

3. The iceberg model

Paoletti and Saupe (2009) proposed that allorecognition systems may have evolved from pre-existing gene networks with roles outside allorecognition, likely dedicated to xenorecognition. This hypothesis is represented as a conceptual iceberg, with the visible portion corresponding to characterized allorecognition systems, whereas the submerged portion corresponds to broader immune networks from which these systems emerge (Uehling et al. 2017, Daskalov 2023). This model provides a conceptual framework for considering how fungi may regulate interactions with beneficial and harmful organisms, although its relevance to general symbiont tolerance remains to be established experimentally.

Table 4. Key components of fungal innate immune systems.

Component	Domain architecture	Fungal examples	Homologs in other kingdoms	Function	References
NLRs	N-terminal effector, NB domain, C-terminal repeats	PLP-1 (<i>N. crassa</i>), NWD2 (<i>P. anserina</i>)	Animal NLRs, plant R proteins	Pathogen sensing, allorecognition	Dyrka et al. (2014), Uehling et al. (2017), Heller et al. (2018)
HET/TIR domains	~150–200 amino acid domain	HET domain proteins (<i>N. crassa</i> , <i>P. anserina</i>)	TIR domains (TLRs, SARM1)	NAD ⁺ hydrolase, signalling	Paoletti and Saupe (2009), Daskalov (2023)
HeLo/HELL domains	Four-helix bundle	HET-S (<i>P. anserina</i>)	MLKL 4HB domain (mammals)	Membrane permeabilization	Daskalov et al. (2016), Wojciechowski et al. (2022)
Gasdermins (fGSDMs)	Pore-forming domain + inhibitory domain	HET-Q1 (<i>P. anserina</i>), RCD-1 (<i>N. crassa</i>)	Mammalian gasdermins	Pyroptosis-like cell death	Daskalov et al. (2020), Clavé et al. (2022)

IMMUNOLOGICAL INDIVIDUALITY AND SYMBIONT TOLERANCE

The (dis)continuity theory of immunity, proposed by Pradeu (2012, 2019), offers a framework for understanding how symbionts may become immunologically integrated into holobionts. According to this theory, immune responses are triggered by sudden changes in molecular motifs interacting with immune receptors, rather than by self/nonself discrimination per se. Microbes that do not trigger such changes are tolerated and may become integrated as parts of the physiological individual (Pradeu and Carosella 2006, Pradeu 2010).

Applying this framework to fungi provides a conceptual model for considering how endohyphal bacteria and mycoviruses might become immunologically integrated (Greslehner 2020, Schneider 2021). However, direct empirical evidence linking the (dis)continuity theory specifically to fungal symbiont tolerance remains limited. Their presence does not necessarily elicit strong immune responses because they may maintain continuity of molecular interactions with fungal cells. In contrast, pathogens or incompatible conspecific strains that disrupt this continuity may trigger RCD aimed at restoring cellular integrity. In the *Rhizopus* holobiont, the association with *Mycetohabitans* provides a useful system for examining these questions (Partida-Martinez and Hertweck 2005, Lackner and Hertweck 2011). The observation that bacteria-free fungi can be reinfected with *Mycetohabitans* suggests dynamic rather than fixed immunological boundaries (Partida-Martinez et al. 2007, Mondo et al. 2017).

TRANSGENERATIONAL IMMUNITY AND HOLOBIONT CONTINUITY

Fungi display forms of physiological and defense-related individuality that may extend across generations through non-genetic inheritance mechanisms (Veigl 2022a, b). Growing evidence indicates that epigenetic processes, particularly RNA interference (RNAi) mediated by small interfering RNAs (siRNAs), can generate heritable phenotypic variation in fungi, including defense- and resistance-associated traits, without changes to the underlying DNA sequence (Luna et al. 2012, Yu et al. 2013, Cooper and Ton 2022). Although such processes do not constitute immune memory in the classical sense described for animals, they nevertheless demonstrate that prior environmental or biotic interactions can influence defense-related phenotypes in subsequent generations.

These forms of transgenerational epigenetic inheritance have important implications for the fungal holobiont concept (Veigl 2022a, b). Beyond host-derived epigenetic effects, vertically transmitted endosymbionts may contribute to intergenerational continuity by carrying their own molecular patterns across host generations, thereby shaping host-symbiont interactions over extended evolutionary timescales. Consequently, the fungal defense landscape may

reflect not only the contemporary symbiont community but also persistent associations inherited across generations. In *Rhizopus microsporus*, vertical transmission of both the *Mycetohabitans* bacterial endosymbiont and Narnaviruses has been demonstrated, ensuring continuity of the association across generations (Partida-Martínez et al. 2007, Espino-Vázquez et al. 2020).

Such persistent, multigenerational host-symbiont associations have significant implications for understanding fungal individuality, adaptation and evolution (Booth 2014, Suárez 2020).

PART-DEPENDENT ACCOUNTS OF HOLOBIONT INDIVIDUALITY

Suárez and Stencel (2020) have proposed a part-dependent account of holobiont individuality, recognizing that different components of a holobiont may exhibit different degrees of integration. Applied to fungal holobionts, this perspective suggests that:

From the fungal-host perspective, the holobiont may be seen as an immunological individual, with endosymbionts contributing to host immunity and fitness (Suárez and Stencel 2020). Fungal NLRs and RCD pathways may contribute to the maintenance of cellular integrity and the regulation of interactions with other organisms (Uehling et al. 2017, Daskalov 2023).

From the endosymbiont perspective, the fungal holobiont may appear as a loose ecological community, with bacteria and viruses pursuing their own evolutionary interests while contributing to collective functions (Chiu and Eberl 2016, Skillings 2016). Endosymbiont genomes show signatures of adaptation to the host environment while retaining elements of autonomy (Moran and Sloan 2015, Douglas and Werren 2016). For mycoviruses, this perspective is particularly relevant, as viral replication depends entirely on host cellular machinery while viral genomes evolve independently (Roossinck 2011, Ghabrial et al. 2015).

An alternative interpretation is that physiological integration between a fungal host and its microbial partners does not necessarily imply evolutionary individuality. From the host perspective, persistent symbionts may contribute to an integrated physiological system, whereas from the symbiont perspective, the same association may retain features of an ecological community in which different partners maintain partially independent evolutionary interests (Moran and Sloan 2015, Chiu and Eberl 2016, Douglas and Werren 2016, Skillings 2016). Thus, the fungal holobiont concept can accommodate different degrees of integration without requiring that every host-microbe association constitute a single biological individual.

PHILOSOPHICAL IMPLICATIONS: RETHINKING FUNGAL INDIVIDUALITY

The fungal holobiont concept challenges traditional notions of biological individuality by emphasizing that fungal boundaries may be neither fixed nor purely genetic. Rather, fungi (at least some characterized model fungi) can be understood as integrated physiological and defense-related units whose boundaries may be influenced by immune processes and persistent symbiotic associations. The unit of physiological integration in fungi is therefore not the fungal mycelium alone, but the fungal holobiont comprising the host and its enduring microbial partners. This perspective aligns with historical traditions that emphasized the open organization and aggregative character of plants and, more broadly, of fungi (Plessner 1928, Gilbert and Epel 2015).

Second, immunity may contribute to defining biological boundaries. Fungal immune mechanisms, including NLR-based surveillance and RCD pathways, can contribute to the recognition of genetically distinct cells and the maintenance of cellular integrity, and may therefore influence interactions with microbial associates (Pradeu 2012, Heller et al. 2018, Daskalov 2023). Whether these mechanisms generally determine which microbial associates are tolerated as symbionts versus rejected as pathogens remains an open empirical question.

Third, vertical transmission of symbionts and heritable immune responses may extend fungal physiological individuality across generations. This transgenerational continuity provides an important perspective for understanding host-symbiont associations, although it does not by itself establish that fungal hosts and their symbionts constitute single evolutionary units (Suárez 2020, Veigl 2022a, b).

Fourth, the nested nature of holobionts, with fungi serving as both hosts and symbionts within larger assemblages, suggests that biological individuality may be multi-layered and context-dependent. A fungal host may participate in a fungal-microbial association while simultaneously forming part of larger ecological systems (e.g. plants). Thus, individuality may emerge from specific physiological and ecological relationships rather than being an intrinsic property expressed identically at every biological level.

The holobiont concept also raises questions concerning fungal species concepts. Because microbial associates may vary among individuals within a fungal species, the presence of a particular bacterium or mycovirus cannot necessarily be treated as a defining criterion of fungal species identity. The holobiont perspective may therefore complement rather than replace existing fungal species concepts by drawing attention to biologically important host-microbe associations that occur within species boundaries.

CONCLUSIONS

1. Fungi can form persistent and functionally important associations with microbial partners, including endohyphal bacteria and mycoviruses. These associations provide empirical support for considering fungal-microbial systems as holobionts that has profound implications for understanding fungal biology. However, currently, this do not establish that every fungal individual is necessarily a holobiont or that every holobiont constitutes a single biological individual.
2. Key criteria for fungal holobiont individuality include physiological integration, immunological tolerance, vertical transmission of symbionts, and signatures of co-evolution between host and microbial partners.
3. Both endohyphal bacteria and mycoviruses can modulate fungal phenotypes, affecting growth, reproduction, metabolism, stress tolerance, and pathogenicity. The diversity of reported effects indicates that microbial partners can substantially influence fungal biology in particular systems, although the direction and magnitude of these effects vary among host-microbe associations.
4. The fungal immune system, through NLR-based surveillance of essential cellular components and regulated cell death pathways, contributes to self/non-self recognition and cellular integrity. Whether these mechanisms generally determine which microbes are tolerated as symbionts versus rejected as pathogens remains to be established.
5. Vertical transmission of microbial symbionts and heritable epigenetic immune responses may extend fungal physiological individuality across generations, providing continuity to particular host-symbiont associations.
6. A part-dependent perspective allows the fungal holobiont to be viewed as an integrated physiological-immunological system from the host perspective while retaining features of a multispecies ecological community from the perspective of its microbial partners.
7. The nested architecture of host-microbe associations suggests that biological individuality can be multi-layered and context-dependent, with the degree of integration depending on specific physiological, ecological and evolutionary relationships.

ACKNOWLEDGMENTS

JS work is supported by grants from Bu-Ali Sina University (BASU), Iran. We are grateful to Prof. Dr. Jan Baedke (Department of Philosophy I, Ruhr University Bochum, Germany) for philosophical discussions on biological individuality and the holobiont concept during JS's research visit to his group in 2023, which was co-funded by the Deutsche Forschungsgemeinschaft (DFG), Germany, and BASU, Iran.

DEDICATIONS

JS dedicates this work to the memory of Avicenna (Ibn Sina), a timeless source of inspiration for scientific and philosophical thought.

AUTHOR CONTRIBUTION

JS conceptualized the idea, designed methodology, performed analytical review, and wrote the manuscript. MK contributed to analytical discussions, editing and reviewing the manuscript drafts and revisions. Both authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were generated in this study.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. JS lab is supported and regularly funded by the Bu-Ali Sina University, Hamedan, Iran.

DECLARATION

The authors declare no competing interests.

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals by any of the authors.

REFERENCES

- Arendt KR, Hockett KL, Araldi-Brondolo SJ, Baltrus DA, Arnold AE. 2016. Isolation of endohyphal bacteria from foliar Ascomycota and in vitro establishment of their symbiotic associations. *Applied and Environmental Microbiology*. 82: 2943-2949. <https://doi.org/10.1128/AEM.00452-16>
- Arora P, Riyaz-Ul-Hassan S. 2019. Endohyphal bacteria: the prokaryotic modulators of host fungal biology. *Fungal Biology Reviews*. 33: 72-81. <https://doi.org/10.1016/j.fbr.2018.07.001>
- Baedke J. 2024. The plant individuality problem in historical perspective. *History and Philosophy of the Life Sciences*. 46: 1-18. <https://doi.org/10.1007/s40656-023-00598-0>
- Barbieri E, Potenza L, Rossi I, Sisti D, Giomaro G, ... et al. 2000. Phylogenetic characterization and in situ detection of a Cytophaga–Flexibacter–Bacteroides phylogroup bacterium in *Tuber borchii* Vittad. ectomycorrhizal mycelium. *Applied and Environmental Microbiology*. 66: 5035-5042. <https://doi.org/10.1128/AEM.66.11.5035-5042.2000>
- Barbieri E, Riccioni G, Pisano A, Sisti D, Zeppa S, ... et al. 2002. Competitive PCR for quantitation of a Cytophaga–Flexibacter–Bacteroides phylum bacterium associated with the *Tuber borchii* Vittad. mycelium. *Applied and Environmental Microbiology*. 68: 6421-6424. <https://doi.org/10.1128/AEM.68.12.6421-6424.2002>
- Bertaux J, Schmid M, Hutzler P, Hartmann A, Garbaye J, ... et al. 2005. Occurrence and localization of bacterial microflora in the mycelium of the ectomycorrhizal fungus *Hebeloma cylindrosporum*. *Microbiological Research*. 160: 1-9. <https://doi.org/10.1016/j.micres.2004.06.005>
- Bianciotto V, Schüßler A, Bauer R, Bonfante P. 1996. Presence of an arbuscular mycorrhizal fungus in the cytoplasm of a plant-parasitic fungus. *Nature*. 381: 297-299. <https://doi.org/10.1038/381297a0>
- Bianciotto V, Lumini E, Lanfranco L, Minerdi D, Bonfante P, ... et al. 2003. Detection and identification of bacterial endosymbionts in arbuscular mycorrhizal fungi belonging to the family Gigasporaceae. *Applied and Environmental Microbiology*. 69: 4597-4606. <https://doi.org/10.1128/AEM.69.8.4597-4606.2003>
- Bianciotto V, Giovannetti M, Lumini E, Becard G, Bescher J, et al. 2004. Presymbiotic growth and spore germination of an arbuscular mycorrhizal fungus are promoted by a bacterium isolated from its spores. *Applied and Environmental Microbiology*. 70: 3149-3155. <https://doi.org/10.1128/AEM.70.5.3149-3155.2004>
- Bonfante P, Desirò A. 2017. Who lives in a fungus? The diversity, origins and functions of fungal endobacteria living in Mucoromycota. *The ISME Journal*. 11: 1727-1735. <https://doi.org/10.1038/ismej.2017.21>
- Booth A. 2014. Symbiosis, selection, and individuality. *Biology & Philosophy*. 29: 657-673. <https://doi.org/10.1007/s10539-014-9454-y>
- Bordenstein SR, Theis KR. 2015. Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biology*. 13: e1002226. <https://doi.org/10.1371/journal.pbio.1002226>
- Boynton PJ, Peterson CN, Pringle A. 2019. A novel method for the establishment of stable fungal-bacterial endosymbioses. *mBio*. 10: e02467-19. <https://doi.org/10.1128/mBio.02467-19>
- Bright M, Bulgheresi S. 2010. A complex journey: transmission of microbial symbionts. *Nature Reviews Microbiology*. 8: 218-230. <https://doi.org/10.1038/nrmicro2262>
- Bryner SF, Rigling D, Brunner PC. 2012. Invasion history and demographic pattern of Cryphonectria hypovirus 1 across European populations of the chestnut blight fungus. *Ecology and Evolution*. 2: 3227-3241. <https://doi.org/10.1002/ece3.421>
- Chiba S, Salaipeth L, Lin YH, Sasaki A, Kanematsu S, ... et al. 2009. A novel bipartite dsRNA mycovirus from the white root rot fungus *Rosellinia necatrix*: molecular and biological characterization, taxonomic considerations, and potential for biological control. *Journal of Virology*. 83: 12801-12812. <https://doi.org/10.1128/JVI.01429-09>
- Chiba S, Castón JR, Ghabrial SA, Suzuki N. 2018. ICTV Virus Taxonomy Profile: Quadrviridae. *Journal of General Virology*. 99: 1480-1481. <https://doi.org/10.1099/jgv.0.001139>
- Chiu L, Eberl G. 2016. Microorganisms as scaffolds of biological individuality: an eco-immunity account of the holobiont. *Biology & Philosophy*. 31: 819-837. <https://doi.org/10.1007/s10539-016-9541-3>
- Chiu L, Gilbert SF. 2020. Niche construction and the transition to herbivory: phenotype switching and the organization of new nutritional modes. In: Levine H, Jolly MK, Kulkarni P, Nanjundiah V, editors. *Phenotypic Switching: Implications in Biology and Medicine*. Academic Press, UK, pp. 459-482. <https://doi.org/10.1016/B978-0-12-817996-3.00019-9>
- Clarke E. 2012. Plant individuality: a solution to the demographer's dilemma. *Biology & Philosophy*. 27: 321-361. <https://doi.org/10.1007/s10539-012-9308-4>
- Clavé C, Dyrka W, Turcotte EA, Granger-Farbos A, Ibarzola L, ... et al. 2022. Fungal gasdermin-like proteins are controlled by proteolytic cleavage. *Proceedings of the National Academy of Sciences of the United States of America*. 119: e2109418119. <https://doi.org/10.1073/pnas.2109418119>
- Cooper A, Ton J. 2022. Immune priming in plants: from the onset to transgenerational maintenance. *Essays in Biochemistry*. 66: 635-646. <https://doi.org/10.1042/EBC20210077>
- Daskalov A. 2023. Emergence of the fungal immune system. *iScience*. 26: 106793. <https://doi.org/10.1016/j.isci.2023.106793>

- Daskalov A. 2026. Regulated cell death in fungi from a comparative immunology perspective. *Cell Death & Differentiation*. 33: 244-258. <https://doi.org/10.1038/s41418-025-01570-z>
- Daskalov A, Dyrka W, Saupe SJ. 2020. NLR function in fungi as revealed by the study of self/non-self recognition systems. In: Benz JP, Schipper K, editors. *Genetics and Biotechnology*. Springer, Switzerland, pp. 123-141. https://doi.org/10.1007/978-3-030-49924-2_6
- Daskalov A, Habenstein B, Sabaté R, Berbon M, Martinez D, et al. 2016. Identification of a novel cell death-inducing domain reveals that fungal amyloid-controlled programmed cell death is related to necroptosis. *Proceedings of the National Academy of Sciences of the United States of America*. 113: 2720-2725. <https://doi.org/10.1073/pnas.1522361113>
- Daskalov A, Heller J, Herzog S, Fleißner A, Glass NL. 2017. Molecular mechanisms regulating cell fusion and heterokaryon formation in filamentous fungi. In: Heitman J, Howlett BJ, Crous PW, Stukenbrock EH, James TY, Gow NAR, editors. *The Fungal Kingdom*. ASM Press, pp. 215-229.
- Debets F, Yang X, Griffiths AJ. 1994. Vegetative incompatibility in *Neurospora*: its effect on horizontal transfer of mitochondrial plasmids and senescence in natural populations. *Current Genetics*. 26: 113-119.
- Desirò A, Salvioli A, Ngonkeu EL, ... et al. 2014. Detection of a novel intracellular microbiome hosted in arbuscular mycorrhizal fungi. *The ISME Journal*. 8: 257-270. <https://doi.org/10.1038/ismej.2013.151>
- Deveau A, Bonito G, Uehling J, Paoletti M, Becker M, et al. 2018. Bacterial-fungal interactions: ecology, mechanisms and challenges. *FEMS Microbiology Reviews*. 42: 335-352. <https://doi.org/10.1093/femsre/fuy008>
- Diepeningen AD, Debets AJM, Hoekstra RF. 1997. Heterokaryon incompatibility blocks virus transfer among vegetatively incompatible strains of *Aspergillus niger*. *Mycological Research*. 101: 1451-1455. <https://doi.org/10.1017/S0953756297004305>
- Douglas AE, Werren JH. 2016. Holes in the hologenome: why host-microbe symbioses are not holobionts. *mBio*. 7: e02099-15. <https://doi.org/10.1128/mBio.02099-15>
- Dyrka W, Lamacchia M, Durrans P, Kobe B, Daskalov A, ... et al. 2014. Diversity and variability of NOD-like receptors in fungi. *Genome Biology and Evolution*. 6: 3137-3158. <https://doi.org/10.1093/gbe/evu247>
- Espino-Vázquez AN, Bermúdez-Barrientos JR, Cabrera-Rangel JF, Córdova-López G, Cardoso-Martínez F, ... et al. 2020. Narnaviruses: novel players in fungal-bacterial symbioses. *The ISME Journal*. 14: 1743-1754. <https://doi.org/10.1038/s41396-020-0636-0>
- Filippou C, Garrido-Jurado I, Meyling NV, Quesada-Moraga E, Coutts RHA, ... et al. 2018. Mycoviral population dynamics in Spanish isolates of the entomopathogenic fungus *Beauveria bassiana*. *Viruses*. 10: 665. <https://doi.org/10.3390/v10120665>
- Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, ... et al. 2011. Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. *Microbiology and Molecular Biology Reviews*. 75: 583-609. <https://doi.org/10.1128/MMBR.00020-11>
- Funkhouser LJ, Bordenstein SR. 2013. Mom knows best: the universality of maternal microbial transmission. *PLoS Biology*. 11: e1001631. <https://doi.org/10.1371/journal.pbio.1001631>
- Ghabrial SA, Suzuki N. 2009. Viruses of plant pathogenic fungi. *Annual Review of Phytopathology*. 47: 353-384. <https://doi.org/10.1146/annurev-phyto-080508-081932>
- Ghabrial SA, Castón JR, Jiang D, Nibert ML, Suzuki N. 2015. 50-plus years of fungal viruses. *Virology*. 479-480: 356-368. <https://doi.org/10.1016/j.virol.2015.03.007>
- Gilbert SF, Epel D. 2015. *Ecological Developmental Biology: The Environmental Regulation of Development, Health, and Evolution*. Sinauer Associates, USA.
- Gilbert SF, Sapp J, Tauber AI. 2012. A symbiotic view of life: we have never been individuals. *The Quarterly Review of Biology*. 87: 325-341. <https://doi.org/10.1086/668166>
- Glass NL, Rasmussen C, Roca MG, Read ND. 2004. Hyphal homing, fusion and mycelial interconnectedness. *Trends in Microbiology*. 12: 135-141.
- Golpayegani S, Pakdel A, Soltani J. 2026. Mycovirome: biology, diversity, structures, advantages, and applications in plant protection. *Mycologia Iranica*. 13: 1-23. <https://doi.org/10.22092/mi.2026.371143.1331>
- Greslehner GP. 2020. Not by structures alone: can the immune system recognize microbial functions? *Studies in History and Philosophy of Biological and Biomedical Sciences*. 84: 101336.
- Heller J, Clavé C, Gladioux P, Saupe SJ, Daskalov A. 2018. NLR surveillance of essential SEC-9 SNARE proteins induces programmed cell death upon allorecognition in *Neurospora crassa*. *Proceedings of the National Academy of Sciences of the United States of America*. 115: E2292-E2301. <https://doi.org/10.1073/pnas.1718842115>
- Hillman BI, Suzuki N. 2004. Viruses of the chestnut blight fungus, *Cryphonectria parasitica*. *Advances in Virus Research*. 63: 423-472.
- Hoffman MT, Arnold AE. 2010. Diverse bacteria inhabit living hyphae of phylogenetically diverse fungal endophytes. *Applied and Environmental Microbiology*. 76: 4063-4075. <https://doi.org/10.1128/AEM.02928-09>
- Hoffman MT, Gunatilaka M, Wijeratne EMK, Gunatilaka AAL, Arnold AE. 2013. Endohyphal bacterium enhances production of indole-3-acetic acid by a foliar fungal endophyte. *PLoS ONE*. 8: e73132. <https://doi.org/10.1371/journal.pone.0073132>

- Hollings M. 1962. Viruses associated with a die-back disease of cultivated mushroom. *Nature*. 196: 962-965. <https://doi.org/10.1038/196962a0>
- Hyder R, Pennanen T, Hamberg L, Vainio EJ, Piri T, ... et al. 2013. Two viruses of *Heterobasidion* confer beneficial, cryptic or detrimental effects to their hosts in different situations. *Fungal Ecology*. 6: 387-396. <https://doi.org/10.1016/j.funeco.2013.04.003>
- Jones JD, Dangl JL. 2006. The plant immune system. *Nature*. 444: 323-329. <https://doi.org/10.1038/nature05286>
- Kanematsu S, Shimizu T, Salaipeth L, Yaegashi H, Sasaki A, Ito T, Suzuki N. 2014. Genome rearrangement of a mycovirus Rosellinia necatrix megabirnavirus 1 affecting its ability to attenuate virulence of the host fungus. *Virology*. 450-451: 308-315. <https://doi.org/10.1016/j.virol.2013.12.002>
- Kelliher JM, Robinson AJ, Longley R, Johnson LYD, Hanson BT, ... et al. 2023. The endohyphal microbiome: current progress and challenges for scaling down integrative multi-omic microbiome research. *Microbiome*. 11: 192. <https://doi.org/10.1186/s40168-023-01623-4>
- Kobayashi DY, Crouch JA. 2009. Bacterial/fungal interactions: from pathogens to mutualistic endosymbionts. *Annual Review of Phytopathology*. 47: 63-82. <https://doi.org/10.1146/annurev-phyto-080508-081729>
- Kondo H, Chiba S, Toyoda K, et al. 2013. Evidence for negative-strand RNA virus infection in fungi. *Virology*. 435: 201-209. <https://doi.org/10.1016/j.virol.2012.09.008>
- Kondo H, Botella L, Suzuki N. 2022. Mycovirus diversity and evolution. *Annual Review of Phytopathology*. 60: 307-330. <https://doi.org/10.1146/annurev-phyto-021021-095744>
- Kotta-Loizou I, Coutts RHA. 2017. Studies on the virome of the entomopathogenic fungus *Beauveria bassiana* reveal novel dsRNA elements and mild hypervirulence. *PLoS Pathogens*. 13: e1006183. <https://doi.org/10.1371/journal.ppat.1006183>
- Krupovic M, Dolja VV, Koonin EV. 2015. Plant viruses of the Amalgaviridae family evolved via recombination between viruses with double-stranded and negative-strand RNA genomes. *Biology Direct*. 10: 12. <https://doi.org/10.1186/s13062-015-0050-0>
- Kuo C. 2015. Common trends in two facultative endosymbionts of fungal hosts: genome reduction and horizontal gene transfer. *PeerJ*. 3: e778. <https://doi.org/10.7717/peerj.778>
- Lackner G, Scherlach K, Korfist C, Schmidt T, Hertweck C. 2009. Nonpathogenic bacteria living in a fungal host: genome reduction, host dependence, and horizontal gene transfer. *mBio*. 1: e00005-10. <https://doi.org/10.1128/mBio.00005-10>
- Lackner G, Moebius N, Hertweck C. 2011. Endofungal bacterium controls its host by an Hrp type III secretion system. *The ISME Journal*. 5: 252-261. <https://doi.org/10.1038/ismej.2010.126>
- Lackner G, Hertweck C. 2011. Impact of endofungal bacteria on infection biology, food safety, and drug development. *PLoS Pathogens*. 7: e1002096. <https://doi.org/10.1371/journal.ppat.1002096>
- Liu L, Xie J, Cheng J, Fu Y, Li G, Yi X, Jiang D. 2014. Fungal negative-stranded RNA virus that is related to Bornaviruses and Nyaviruses. *Proceedings of the National Academy of Sciences of the United States of America*. 111: 12205-12210. <https://doi.org/10.1073/pnas.1401786111>
- Luna E, Bruce TJA, Roberts MK, Flors V, Ton J. 2012. Next-generation systemic acquired resistance. *Plant Physiology*. 158: 844-853. <https://doi.org/10.1104/pp.111.187468>
- Lupini S, Peña-Bahamonde J, Bonito G, Rodrigues DF. 2022. Effect of endosymbiotic bacteria on fungal resistance toward heavy metals. *Frontiers in Microbiology*. 13: 822541.
- Margulis L, Fester R, (eds.). 1991. *Symbiosis as a Source of Evolutionary Innovation: Speciation and Morphogenesis*. MIT Press, USA.
- Márquez LM, Redman RS, Rodriguez RJ, Roossinck MJ. 2007. A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. *Science*. 315: 513-515. <https://doi.org/10.1126/science.1136377>
- Mondo SJ, Lastovetsky OA, Gaspar ML, Schwardt NH, Barber CC, Riley R, Sun H, Grigoriev IV, Pawlowska TE. 2017. Bacterial endosymbionts influence host sexuality and reveal reproductive genes of early divergent fungi. *Nature Communications*. 8: 1843. <https://doi.org/10.1038/s41467-017-02052-8>
- Moran NA, McCutcheon JP, Nakabachi A. 2008. Genomics and evolution of heritable bacterial symbionts. *Annual Review of Genetics*. 42: 165-190. <https://doi.org/10.1146/annurev.genet.41.110306.130119>
- Moran NA, Sloan DB. 2015. The hologenome concept: helpful or hollow? *PLoS Biology*. 13: e1002311. <https://doi.org/10.1371/journal.pbio.1002311>
- Mosse B. 1970. Honey-coloured, sessile Endogone spores: II. Changes in fine structure during spore development. *Archiv für Mikrobiologie*. 74: 129-145. <https://doi.org/10.1007/BF00446901>
- Myers JM, Gillespie AF, Kuo HC, Patterson WI, Fountain JC, ... et al. 2020. A global survey of mycoviruses in fungi. *Nature Communications*. 11: 1-11. <https://doi.org/10.1038/s41467-020-16355-w>
- Naito M, Morton JB, Pawlowska TE. 2015. Minimal genomes of obligate fungal endobacteria reveal evolutionary adaptation to an intracellular lifestyle. *Genome Biology and Evolution*. 7: 2403-2417. <https://doi.org/10.1093/gbe/evv151>

- Naito M, Desirò A, González JB, Tao G, Morton JB, Bonfante P, Pawlowska TE. 2017. 'Candidatus *Moenioplasmaglomeromycotorum*', an endobacterium of arbuscular mycorrhizal fungi. *International Journal of Systematic and Evolutionary Microbiology*. 67: 1177-1184. <https://doi.org/10.1099/ijsem.0.001785>
- Naumann M, Schüßler A, Bonfante P. 2010. The obligate endobacteria of arbuscular mycorrhizal fungi are ancient heritable components related to the Mollicutes. *The ISME Journal*. 4: 862-871.
- Ninomiya A, Urayama S, Suo R, ... et al. 2020. Mycovirus-induced tenuazonic acid production in a rice blast fungus *Magnaporthe oryzae*. *Frontiers in Microbiology*. 11: 1641. <https://doi.org/10.3389/fmicb.2020.01641>
- Nuss DL. 2005. Hypovirulence: mycoviruses at the fungal-plant interface. *Nature Reviews Microbiology*. 3: 632-642. <https://doi.org/10.1038/nrmicro1207>
- O'Malley MA. 2015. Endosymbiosis and its implications for evolutionary theory. *Proceedings of the National Academy of Sciences of the United States of America*. 112: 10270-10277. <https://doi.org/10.1073/pnas.1421378112>
- Özkan S, Mohorianu I, Xu P, et al. 2017. Profile and functional analysis of small RNAs derived from *Aspergillus fumigatus* infected with double-stranded RNA mycoviruses. *BMC Genomics*. 18: 416. <https://doi.org/10.1186/s12864-017-3773-6>
- Pakvaz S, Soltani J. 2016. Endohyphal bacteria from fungal endophytes of the Mediterranean cypress (*Cupressus sempervirens*) exhibit in vitro bioactivity. *Forest Pathology*. 46: 569-581. <https://doi.org/10.1111/efp.12274>
- Paoletti M, Saupe SJ. 2009. Fungal incompatibility: evolutionary origin in pathogen defense? *BioEssays*. 31: 1201-1210. <https://doi.org/10.1002/bies.200900073>
- Partida-Martinez LP. 2024. Fungal holobionts as blueprints for synthetic endosymbiotic systems. *PLoS Biology*. 22: e3002587. <https://doi.org/10.1371/journal.pbio.3002587>
- Partida-Martinez LP, Hertweck C. 2005. Pathogenic fungus harbours endosymbiotic bacteria for toxin production. *Nature*. 437: 884-888. <https://doi.org/10.1038/nature03997>
- Partida-Martinez LP, Hertweck C. 2007. A gene cluster encoding rhizoxin biosynthesis in '*Burkholderia rhizoxina*', the bacterial endosymbiont of the fungus *Rhizopus microsporus*. *ChemBioChem*. 8: 41-45. <https://doi.org/10.1002/cbic.200600393>
- Partida-Martinez LP, Monajembashi S, Greulich KO, Hertweck C. 2007. Endosymbiont-dependent host reproduction maintains bacterial-fungal mutualism. *Current Biology*. 17: 773-777. <https://doi.org/10.1016/j.cub.2007.03.051>
- Pawlowska TE, Gaspar ML, Lastovetsky OA, Mondo SJ, Real-Ramirez I, ... et al. 2018. Biology of fungi and their bacterial endosymbionts. *Annual Review of Phytopathology*. 56: 289-309. <https://doi.org/10.1146/annurev-phyto-080417-050030>
- Pearson MN, Beever RE, Boine B, Arthur K. 2009. Mycoviruses of filamentous fungi and their relevance to plant pathology. *Molecular Plant Pathology*. 10: 115-128. <https://doi.org/10.1111/j.1364-3703.2008.00500.x>
- Plessner H. 1928. *Die Stufen des Organischen und der Mensch*. De Gruyter.
- Pradeu T. 2010. What is an organism? An immunological answer. *History and Philosophy of the Life Sciences*. 32: 247-268.
- Pradeu T. 2012. *The Limits of the Self: Immunology and Biological Identity*. Oxford University Press, UK.
- Pradeu T. 2016. The many faces of biological individuality. *Biology & Philosophy*. 31: 761-773.
- Pradeu T. 2019. Philosophy of biology: immunology and individuality. *eLife*. 8: e47384. <https://doi.org/10.7554/eLife.47384>
- Pradeu T, Carosella ED. 2006. The self model and the conception of biological identity in immunology. *Biology & Philosophy*. 21: 235-252. <https://doi.org/10.1007/s10539-005-0436-y>
- Rayner ADM. 1995. Fungal holomorphs and the concept of the individual. *Mycological Research*. 99: 1131-1140. [https://doi.org/10.1016/S0953-7562\(09\)80781-3](https://doi.org/10.1016/S0953-7562(09)80781-3)
- Rigling D, Prospero S. 2018. *Cryphonectria parasitica* and chestnut blight: a global perspective. *Annual Review of Phytopathology*. 56: 451-472. <https://doi.org/10.1146/annurev-phyto-080417-050053>
- Roossinck MJ. 2011. The good viruses: viral mutualistic symbioses. *Nature Reviews Microbiology*. 9: 99-108. <https://doi.org/10.1038/nrmicro2501>
- Salvioli A, Ghignone S, Novero M, Navazio L, Bagnaresi P, et al. 2016. Symbiosis with an endobacterium increases the fitness of a mycorrhizal fungus, raising its bioenergetic potential. *The ISME Journal*. 10: 130-144. <https://doi.org/10.1038/ismej.2015.91>
- Sánchez-Cañizares C, Jorrián B, Poole PS, Tkacs A. 2017. Understanding the holobiont: the interdependence of plants and their microbiome. *Current Opinion in Microbiology*. 38: 188-196. <https://doi.org/10.1016/j.mib.2017.05.004>
- Santos P, Palmer M, Chávez-Ramírez B, Beukes C, Steenkamp ET, et al. 2018. Whole genome analyses suggest that *Burkholderia* sensu lato contains two additional novel genera (*Mycetohabitans* gen. nov. and *Trinickia* gen. nov.): implications for the evolution of diazotrophy and nodulation in the Burkholderiaceae. *Genes*. 9: 389. <https://doi.org/10.3390/genes9080389>
- Scherlach K, Busch B, Lackner G, Paszkowski U, Hertweck C. 2012. Symbiotic cooperation in the biosynthesis of a phytotoxin. *Angewandte Chemie International Edition*. 51: 9615-9618. <https://doi.org/10.1002/anie.201204338>
- Schmitt MJ, Breinig F. 2002. The viral killer system in yeast: from molecular biology to application. *FEMS Microbiology Reviews*. 26: 257-276. <https://doi.org/10.1111/j.1574-6976.2002.tb00614.x>

- Schmitt MJ, Breinig F. 2006. Yeast viral killer toxins: lethality and self-protection. *Nature Reviews Microbiology*. 4: 212-221. <https://doi.org/10.1038/nrmicro1347>
- Schneider T. 2021. The holobiont self: understanding immunity in context. *History and Philosophy of the Life Sciences*. 43: 99. <https://doi.org/10.1007/s40656-021-00451-1>
- Shaffer JP, Sarmiento C, Zalamea P-C, Gallery RE, Davis AS, Baltrus DA, Arnold AE. 2016. Diversity, specificity, and phylogenetic relationships of endohyphal bacteria in fungi that inhabit tropical seeds and leaves. *Frontiers in Ecology and Evolution*. 4: 116. <https://doi.org/10.3389/fevo.2016.00116>
- Shaffer JP, U'Ren JM, Gallery RE, Baltrus DA, Arnold AE. 2017. An endohyphal bacterium (Chitinophaga, Bacteroidetes) alters carbon source use by *Fusarium keratoplasticum* (*F. solani* species complex, Nectriaceae). *Frontiers in Microbiology*. 8: 350. <https://doi.org/10.3389/fmicb.2017.00350>
- Sharmin D, Guo Y, Nishizawa T, Ohshima S, Sato Y, ... et al. 2018. Comparative genomic insights into endofungal lifestyles of two bacterial endosymbionts, *Mycovoidus cysteinexigens* and *Burkholderia rhizoxinica*. *Microbes and Environments*. 33: 66-76. <https://doi.org/10.1264/jsme2.ME17153>
- Sharma M, Schmid M, Rothballer M, Hause G, Zuccaro A, ... et al. 2008. Detection and identification of bacteria intimately associated with fungi of the order Sebaciniales. *Cellular Microbiology*. 10: 2235-2246.
- Skills D. 2016. Holobionts and the ecology of organisms: multi-species communities or integrated individuals? *Biology & Philosophy*. 31: 875-892. <https://doi.org/10.1007/s10539-016-9547-x>
- Soltani J. 2017. Endophytism in Cupressoidae (Coniferae): a model in endophyte biology and biotechnology. In: Maheshwari D, editor. *Endophytes: Biology and Biotechnology. Sustainable Development and Biodiversity*, vol. 15. Springer, Cham, pp. 127-143.
- Stencel A, Proszewska AM. 2018. How research on microbiomes is changing biology: a discussion on the concept of the organism. *Foundations of Science*. 23: 603-620. <https://doi.org/10.1007/s10699-017-9543-x>
- Suárez J. 2020. The stability of traits conception of the hologenome: an evolutionary account of holobiont individuality. *History and Philosophy of the Life Sciences*. 42: 11. <https://doi.org/10.1007/s40656-020-00305-2>
- Suárez J, Stencel A. 2020. A part-dependent account of biological individuality: why holobionts are individuals and ecosystems simultaneously. *Biological Reviews*. 95: 1308-1324. <https://doi.org/10.1111/brv.12612>
- Toepfer G. 2011. Individuum. In: *Historisches Wörterbuch der Biologie*. J.B. Metzler, Germany, pp. 159-180.
- Torres-Cortés G, Ghignone S, Bonfante P, Schübler A. 2015. Mosaic genome of endobacteria in arbuscular mycorrhizal fungi: transkingdom gene transfer in an ancient mycoplasma-fungus association. *Proceedings of the National Academy of Sciences of the United States of America*. 112: 7785-7790. <https://doi.org/10.1073/pnas.1501540112>
- Triviño V, Suárez J. 2020. Holobionts: ecological communities, hybrids, or biological individuals? A metaphysical perspective on multispecies systems. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences*. 84: 101323. <https://doi.org/10.1016/j.shpsc.2020.101323>
- Uehling J, Deveau A, Paoletti M. 2017. Do fungi have an innate immune response? An NLR-based comparison to plant and animal immune systems. *PLoS Pathogens*. 13: e1006578. <https://doi.org/10.1371/journal.ppat.1006578>
- Vainio EJ, Chiba S, Ghabrial SA, et al. 2018a. ICTV Virus Taxonomy Profile: Partitiviridae. *Journal of General Virology*. 99: 17-18. <https://doi.org/10.1099/jgv.0.000985>
- Vainio EJ, Jurvansuu J, Hyder R, Kashif M, Piri T, et al. 2018b. Heterobasidion partitivirus 13 mediates severe growth debilitation and major alterations in the gene expression of a fungal forest pathogen. *Journal of Virology*. 92: e01744-17. <https://doi.org/10.1128/JVI.01744-17>
- Valadez-Cano C, Olivares-Hernández R, Espino-Vázquez AN, Partida-Martínez LP. 2024. Genome-scale model of *Rhizopus microsporus*: metabolic integration of a fungal holobiont with its bacterial and viral endosymbionts. *Environmental Microbiology*. 26: e16551. <https://doi.org/10.1111/1462-2920.16551>
- Vandenkoornhuyse P, Quince C, Young JPW, Poole PS. 2015. The importance of the microbiome of the plant holobiont. *New Phytologist*. 206: 1196-1206. <https://doi.org/10.1111/nph.13312>
- Veigl SJ. 2022a. Adaptive immunity or evolutionary adaptation? Transgenerational immune systems at the crossroads. *Biology & Philosophy*. 37: 41. <https://doi.org/10.1007/s10539-022-09869-8>
- Veigl SJ. 2022b. Do heritable immune responses extend physiological individuality? *History and Philosophy of the Life Sciences*. 44: 67. <https://doi.org/10.1007/s40656-022-00549-0>
- Wojciechowski JW, Tekoglu E, Gasior-Glogowska M, Coustou V, Szulc N, ... et al. 2022. Exploring a diverse world of effector domains and amyloid signaling motifs in fungal NLR proteins. *PLoS Computational Biology*. 18: e1010787. <https://doi.org/10.1371/journal.pcbi.1010787>
- Yu X, Li B, Fu Y, Jiang D, Ghabrial SA, ... et al. 2010. A geminivirus-related DNA mycovirus that confers hypovirulence to a plant pathogenic fungus. *Proceedings of the National Academy of Sciences of the United States of America*. 107: 8387-8392. <https://doi.org/10.1073/pnas.0914503107>
- Yu A, Lepère G, Jay F, Wang J, Bapaume L, ... et al. 2013. Dynamics and biological relevance of DNA demethylation in *Arabidopsis* antibacterial defense. *Proceedings of the National Academy of Sciences of the United States of America*. 110: 2389-2394. <https://doi.org/10.1073/pnas.1215404110>

- Zhang H, Xie J, Fu Y, Cheng J, Qu Z, et al. 2020. A 2-kb mycovirus converts a pathogenic fungus into a beneficial endophyte for *Brassica* protection and yield enhancement. *Molecular Plant*. 13: 1420-1433. <https://doi.org/10.1016/j.molp.2020.07.022>
- Zoll J, Verweij PE, Melchers WJG. 2018. Discovery and characterization of novel *Aspergillus fumigatus* mycoviruses. *PLoS ONE*. 13: e0200511. <https://doi.org/10.1371/journal.pone.0200511>

Accepted manuscript

هولوبیونت قارچی: ایمنی، فردیت و مبانی تکاملی ایمنی ذاتی قارچ‌ها

جلال سلطانی[✉]، مهرویه کتابچی

بخش بیماری‌شناسی گیاهی، گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه بوعلی سینا، همدان، ایران

doi[®] 10.22092/mi.2026.373959.1356

چکیده

قارچ‌ها به‌طور فزاینده‌ای به‌عنوان میزبان مجموعه‌ای از ارتباطات میکروبی پایدار، از جمله باکتری‌های درون‌هیفی و مایکوپلاسما، شناخته می‌شوند. این موضوع پرسش‌های مهمی را درباره فردیت قارچی و نیز این مسئله مطرح می‌کند که تا چه اندازه می‌توان برهم‌کنش‌های قارچ-میکروب را هولوبیونت در نظر گرفت؛ یعنی مجموعه‌ای پیچیده متشکل از یک میزبان و میکروبیوتای همراه آن. این تغییر نگرش مفهومی که ریشه در مباحث فلسفی دیرینه درباره فردیت زیستی دارد، دیدگاه‌های سنتی مبتنی بر محوریت موجود زنده را به چالش می‌کشد و برخی سامانه‌های قارچ-میکروب را به‌عنوان واحدهایی بالقوه یکپارچه از نظر فیزیولوژیک، ایمنی‌شناختی و بوم‌شناختی بازتعریف می‌کند. مقاله حاضر، دانش کنونی درباره همزیست‌های درون‌سلولی قارچ‌ها را جمع‌بندی کرده و به کنکاش و تحلیل این موضوع می‌پردازد که چگونه این شرکای اغلب ناشناخته و پنهان، فنوتیپ‌های قارچی را تعدیل می‌کنند و بر فرایندهایی همچون رشد، تولیدمثل، متابولیسم، تحمل تنش و بیماری‌زایی قارچ میزبان تأثیر می‌گذارند. با بهره‌گیری هم‌زمان از دیدگاه‌های زیست‌شناختی و فلسفی، در این مقاله معیارهای تعریف فردیت قارچی، یکپارچگی ایمنی‌شناختی هولوبیونت‌های قارچی و گسترش فردیت فیزیولوژیک در طول نسل‌ها از طریق همزیست‌های میکروبی وراثت‌پذیر به‌صورت انتقادی بررسی می‌شوند. ما بر این دیدگاه تأکید می‌کنیم که سیستم ایمنی قارچ، به‌ویژه پایش مبتنی بر گیرنده‌های NLR و مسیرهای مرگ سلولی تنظیم‌شده، نقشی محوری در تعیین و تنظیم‌گری پویای مرزهای «خود» قارچی دارد. این سامانه با تعیین اینکه کدام میکروب‌ها به‌عنوان همزیست قارچ تحمل شوند و کدام‌یک به‌عنوان بیمارگر دفع شوند، به‌صورت پویا حدود «خود» قارچی را تعریف می‌کند. اتخاذ دیدگاه هولوبیونت قارچی چارچوبی برای درک بهتر زیست‌شناسی قارچ‌ها فراهم می‌آورد و درک عمیق‌تری از اهمیت تکاملی و بوم‌شناختی ارتباطات پایدار میان قارچ‌ها و میکروارگانیسم‌ها به دست می‌دهد.

واژگان کلیدی: باکتری‌های درون‌هیفی، پروتئین‌های NLR، فردیت زیستی، مایکوپلاسما، مرگ سلولی تنظیم‌شده.