

DIVERSITY AND EVOLUTION OF THE FUNGICULTURE IN FUNGUS-GROWING ANTS (FORMICIDAE: MYRMICINAE: ATTINI)

JORGE VÍCTOR MAURICE-LIRA, JESÚS ROMERO-NÁPOLES, JESÚS PÉREZ-MORENO, SIMÓN MORALES-RODRÍGUEZ, KARLA YOLANDA FLORES-MALDONADO, ARIEL WILBERT FRANCO-GUZMÁN AND HÉCTOR GONZÁLEZ-HERNÁNDEZ

SUMMARY

The fungiculture of ants belonging to the Attini tribe (Formicidae: Myrmicinae) is categorized into five systems with distinctive characteristics. The most advanced system is that of "leaf-cutting higher Attini," followed by "non-leaf-cutting higher Attini." Both cultivate clades of the species *Leucoagaricus gongylophorus* (Möller) (Basidiomycota: Agaricales: Agaricaceae). Subsequent systems, known as "minor fungiculture," "yeast fungiculture," and "coral-type fungus fungiculture," are less advanced. In all cases, except for coral fungi, leucocoprinaceous fungi closely related to *L. gongylophorus* and free-living fungi are cultivated. However, these systems vary in the level of specialization between fungi and their symbiotic ants. Cultivars of higher Attini are obligate symbionts, meaning the cultivated fungus will scarcely survive outside of mutualism,

while fungi cultivated in lower fungicultures are facultative symbionts. Cultivars in the coral-type fungus fungiculture system belong to three species of the genus *Myrmecopterula* (Agaricales: Pterulaceae), which are entirely different from leucocoprinaceous fungi. The characteristics of each fungiculture system vary based on the phylogeny of ants and their cultivars. Understanding the diversity of fungi cultivated by Attini ants and the complexity of their fungiculture systems will elucidate one of the most successful coevolutionary processes between ants and fungi in evolutionary history. This review was aimed to discuss the current knowledge on the fungiculture developed by Attini ants, as well as the level of specialization between cultivated fungi and their relationship with the chronological coevolution of Attini.

Introduction



While humans discovered they could cultivate their own food approximately ten thousand years ago, ants belonging to the

Attini tribe were already engaged in a complex and systematic fungiculture. It is estimated that ant fungiculture first emerged in the southern Neotropics around 50 million years ago (mya), coinciding with the favorable climatic changes of the early Eocene period (Schultz and Brady,

2008). The Attini tribe constitutes a monophyletic and endemic group in America, comprising approximately 245 described species across 20 genera within the Attini tribe (Hymenoptera: Formicidae: Myrmicinae: Attini) (Cardenas *et al.*, 2020; Schultz, 2021).

KEYWORDS / Fungus-Growing Ants / Insect Fungiculture / *Leucoagaricus gongylophoru* / *Myrmecopterula* spp. / Pterulaceae /

Received: 11/12/2023. Modified: 01/03/2024. Accepted: 01/06/2024.

Jorge Víctor Maurice-Lira (Corresponding author). Doctor in Phytosanitary Sciences – Entomology and Acarology, Colegio de Postgraduados (COLPOS), Mexico. Postdoctoral Researcher in Sustainable Forest Management Research Institute, Universidad de Valladolid, Spain. Address: Av. Madrid 44, 34071 Palencia, Spain. e-mail: jvml333@gmail.com; jvmaurice@uva.es.

Jesús Romero-Nápoles. PhD, Northern Arizona University, EE.UU. Full Research Professor, COLPOS, México. e-mail: jnapoles@colpos.mx.

Jesús Pérez-Moreno. PhD, The University of Sheffield, RU. Full Research Professor, COLPOS, México. e-mail: jperez@colpos.mx.

Simón Morales-Rodríguez. Biologist, Benemérita Universidad Autónoma de Puebla, Mexico. Auxiliary Researcher, COLPOS, Mexico. Specialist-in-Charge, Electron Microscopy Unit and Plant Anatomy and Histochemistry Laboratory, COLPOS, Mexico. e-mail: simon.morales@colpos.mx.

Karla Yolanda Flores-Maldonado. Doctor in Phytosanitary Sciences-Entomology and Acarology, COLPOS, México. Senior Researcher, School of Engineering and Sciences, Universidad Autónoma de Tamaulipas, Mexico. e-mail: yflores@docentes.uat.edu.mx.

Ariel Wilbert Franco-Guzmán. PhD, The University of Nottingham, RU. Full Research Professor, COLPOS, Mexico. e-mail: gariel@colpos.mx.

Héctor González-Hernández. PhD, University of Hawaii, Honolulu, EE.UU. Full Research Professor, COLPOS, Mexico. e-mail: hgzzhdz@colpos.mx.

Ants engaged in fungus cultivation can be grouped based on their appearance in evolutionary history. The first ants involved in this behavior emerged approximately 50 to 30 mya and are referred to as "Paleoattinis." Subsequently, around 45 to 30 mya, the "Neoattinis" diverged (Nygaard *et al.*, 2016; Schultz, 2021). Another grouping criterion is based on the fungi they cultivate as symbionts. The majority cultivate leucocoprinaceous fungi (Agaricales: Agaricaceae), with only one genus cultivating coral fungi (Agaricales: Pterulaceae). However, even among ants cultivating leucocoprinaceous fungi, there exists substantial genetic diversity and significant variations within their fungiculture systems (Chapela *et al.*, 1994; Solomon *et al.*, 2011). The third way to categorize fungus-farming ants is based on their fungus cultivation systems, which are closely linked to their coevolutionary processes. Therefore, understanding the evolution of ants and their fungi requires a holistic (ecophysiological) approach that considers the intricate coevolutionary processes of ants and fungi as integral components of more complex systems (Schultz and Brady, 2008; Sosa-Calvo *et al.*, 2018; Schultz, 2021).

In general, cultivated fungi are classified into fungal groups G1, G2, and G3 (Figure 1) (Chapela *et al.*, 1994; Solomon *et al.*, 2011; Nygaard *et al.*, 2016). According to the work of Chapela *et al.* (1994), G1 comprises exclusively cultivars of the species *Leucocoprinus gongylophorus* (Basidiomycota: Agaricales: Agaricaceae), cultivated in the "higher fungiculture" detailed in section 1.2; G2 includes a monophyletic group of "coral fungi" belonging to the family Pterulaceae, described in section 1.4; G3 corresponds to the fungal group closely related to G1, but it exclusively contains cultivars from the most primitive attines, along with free-living fungi and yeast-like Agaricaceae (sections 1.1 and 1.3) (Solomon *et al.*, 2011; Nygaard *et al.*, 2016).

This work presents a detailed exploration of the diversity and complexity of fungus-farming systems employed by fungus-growing ants belonging to the Attini tribe. The objective is to delineate a chronological history of the coevolutionary process that began with an incipient fungus-farming and evolved into an advanced, highly specialized cultivation system. To gain a deeper understanding of the coevolutionary process in the mutualism between fungus-growing ants (Attini

tribe) and their cultivated fungi, this work is divided into two sections. The first section provides a detailed and chronological description of the evolution of fungus-farming developed by ants. The second part focuses on analyzing the primary metabolic capabilities of cultivated fungi that led to the specialization in a specific single crop by a widely distributed group of ants in the American continent. This analysis is grounded in the premise that "mutualistic microorganisms act as a metabolic extension of fungus-growing ants" (Abril, 2011; Khadempour *et al.*, 2020).

Methodology

The first section consisted of a descriptive (non-systematic) review, utilizing the Scopus, Pubmed, and Web of Science databases to gather information. For this part, the sole selection criterion we deemed important was to use primary and secondary sources that had undergone peer review for publication (original articles, reviews, or book chapters). However, for the second part, we conducted a systematic search using the following keywords: "((Myrmecopterula) OR (Pterulaceae)) OR ((*Leucocoprinus gongylophorus*) OR (*Leucoagaricus*

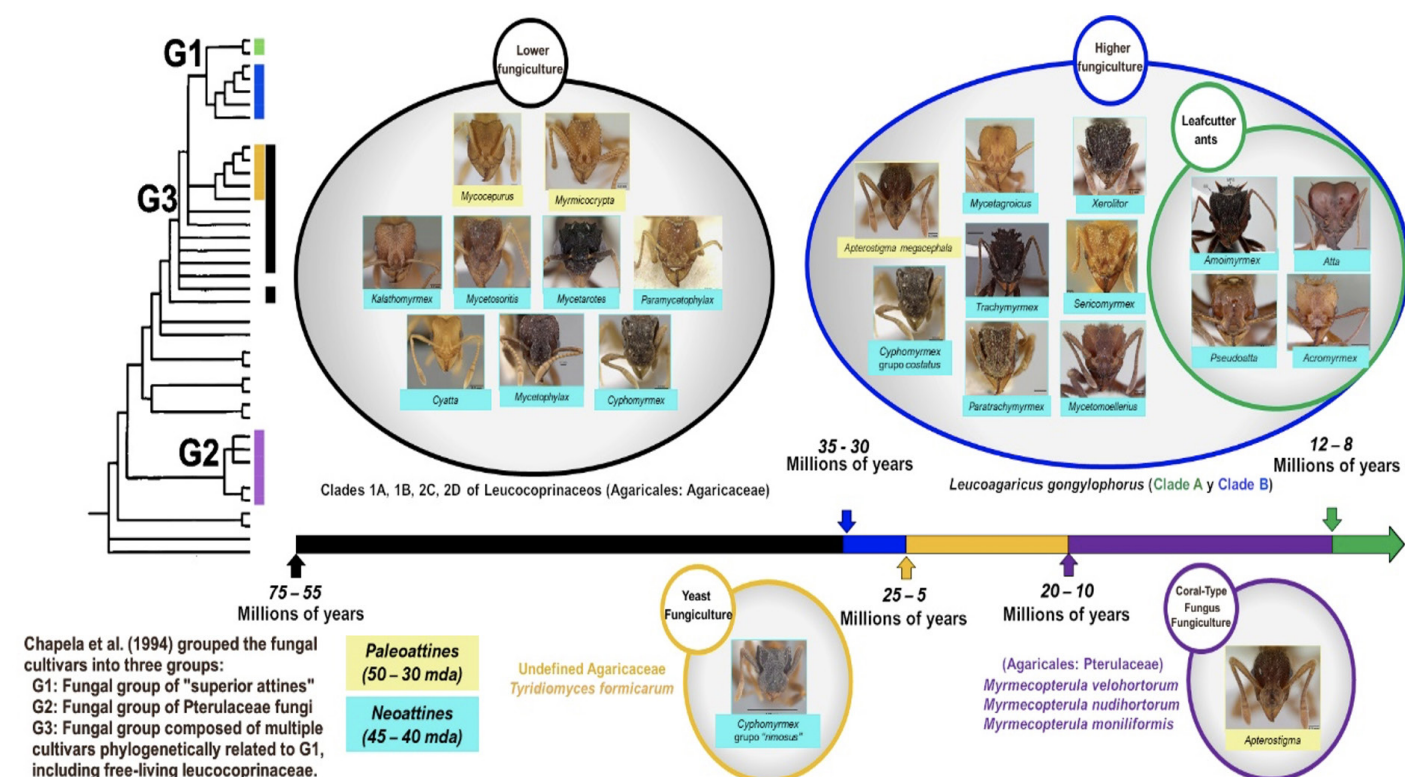


Figure 1. Phylogenetic relationship schematic of fungal cultivars in attines and all ant-developed fungiculture systems (circles). Ant genera enclosed in yellow boxes represent paleoattinis, while genera enclosed in blue boxes represent neoattinis. The approximate time of appearance of different fungiculture systems in history is indicated in the center. Fungal groups (G1, G2, and G3) are presented according to Chapela *et al.*'s (1994) classification to outline their relationship with fungiculture systems. Source: elaborated by the authors.

gongylophorus)) AND (Enzymatic)" in the Web of Science, Pubmed, and Google Scholar databases. The selection of studies for the systematic review in this section of the work was conducted using the PRISMA method (O'Dea *et al.*, 2021). From this process, we identified 58 documents, of which 18 duplicates were removed. Seven were excluded after reviewing the title, and eight were discarded after reading the abstract. Of the remaining 33, we selected nine after a thorough reading of the complete document.

Coevolutionary History

Lower fungiculture (an incipient fungiculture)

It was the first fungiculture, developed by 76 species belonging to nine genera of attines; it appeared approximately 75-55 mya, although recent studies narrow this interval to 60-55 mya (Nygaard *et al.*, 2016). It is termed "lower" because it maintains the most primitive characteristics among all types of fungus production systems within the Agaricaceae family (formerly Lepiotaceae) (Basidiomycota: Agaricales). However, they are considered facultative symbionts as they have been found outside of mutualistic symbiosis (Mueller *et al.*, 1996). This trait indicates micosymbionts that have not reached an advanced level of "domestication" similar to other fungiculture systems, whose mutualistic fungi are obligate symbionts (Mueller *et al.*, 2001; Schultz and Brady, 2008; Nygaard *et al.*, 2016; Kellner *et al.*, 2018; Mueller *et al.*, 2018; Schultz, 2021; Dejean *et al.*, 2023).

In 1998, Mueller *et al.* compared 57 cultivars from lower fungiculture systems with 36 free-living agaricae, demonstrating that cultivars from this system belong to two polyphyletic clades (Clade 1 and Clade 2) within the Agaricaceae family. These clades, in turn, are divided into subclades; within Clade 1, there are two paraphyletic subclades (1A and 1B), while Clade 2 consists of two other paraphyletic subclades (2C and 2D). The phylogenetically closest free-living fungi to these clades are *Lepiota flammeotincta* (Agaricales: Agaricaceae), closely related to Clade 1, and *Leucocoprinus cf. zamurensis* (Agaricales: Agaricaceae), closely related to Clade 2 (Kellner *et al.*, 2013; 2018).

Higher Attini fungiculture (highly specialized advanced fungiculture)

Around 35-30 mya, fungus-farming ants successfully domesticated their cultivar by developing an

advanced fungiculture. This cultivation was so sophisticated that their mutualistic fungi could no longer survive outside of symbiosis, thereby becoming obligate symbionts. This system is termed "higher fungiculture" and is employed by approximately 110 species from the genera *Mycetogroicus*, *Mycetomoellerius*, *Paratrachymyrmex*, *Sericomyrmex*, *Trachymyrmex*, and *Xerolitor* (Solomon *et al.*, 2011; 2019; Jiménez-Gómez *et al.*, 2021; Schultz, 2021).

The exclusive species cultivated in this system is *Leucoagaricus gongylophorus* (Möller) (Basidiomycota: Agaricales: Agaricaceae). However, multiple analyses of genetic diversity have revealed two clades (Clade A and Clade B). Among these, non-leaf-cutting superior attines cultivate Clade B, while leaf-cutting attines (described later) exclusively cultivate Clade A, with some exceptions such as *Atta laevigata* and *A. vollenweideri*, which also cultivate Clade B of *L. gongylophorus* (Ješovnik *et al.*, 2017; Mueller *et al.*, 2018; Solomon *et al.*, 2019; Schultz, 2021; Dejean *et al.*, 2023).

These ants maintain a partially advanced fungiculture system, utilizing plant material found in the organic layer covering the soil, which may already be in various states of decomposition. Additionally, they could use insect and other arthropod excrement to construct the substrate for their fungal gardens, where *L. gongylophorus* is inoculated from an asexual propagule (mycelium). This mycelium is transmitted from parent colonies to their descendants through asexual propagules that have been occurring for millions of years. Thus, it represents a natural cloning process that has persisted to the present day (Mueller *et al.*, 2001; Bizarria *et al.*, 2021).

Within the ants that develop superior fungiculture, there is a small subgroup of four genera of "leaf-cutting ants" consisting of approximately 40 species. This is the latest system to emerge, estimated to have appeared around 12-8 million years ago. They are distinct from other superior attines because their fungiculture system has reached the maximum level of complexity and systematization, comparable to agriculture recently developed by humans (just 10,000 years ago) (Schultz, 2021; Solomon *et al.*, 2019; Bizarria *et al.*, 2021).

Leaf-cutting ants have garnered significant scientific interest due to their specialization in cultivating *L. gongylophorus*. This cultivation process commences with a meticulous selection of fresh plant material, primarily young grass or tree leaves, flowers, and seeds, hence

their designation as leaf-cutting ants. Subsequently, they engage in the preparation of the substrate forming the fungal garden. During this process, the ants triturate and moisten the plant material using a mixture of saliva, salivary enzymes (mainly cellulases and ligninases), and organic acids from their metapleural gland (Abril, 2011; Fernández, 2015; Khadempour *et al.*, 2021; Conlon *et al.*, 2022).

Once the fungal garden substrate is prepared, the ants inoculate the asexual propagule and safeguard it throughout the colony's lifespan, approximately 15 years. This cultivar is considered an obligate symbiont because it does not develop sexual structures. The ants harvest nutrient-rich primordial structures called "gonglydia", preventing the formation of reproductive structures. However, if left unharvested, gonglydia can indeed give rise to a fruiting body. Though few reports exist, these cultivars can develop sporomes (Figure 2) (Möller, 1893; Muchovej *et al.*, 1991; Fisher *et al.*, 1994; Mehdiabadi and Schultz, 2009; Abril, 2011; Fernández *et al.*, 2015; Espinoza *et al.*, 2017; Cardenas *et al.*, 2020; Cristiano *et al.*, 2020; Bizarria *et al.*, 2021; Dejean *et al.*, 2023).

Yeast Fungiculture

Appearing approximately 25 to 5 million years ago, this system is developed by 18 species from the Rimosus group within the genus *Cyphomyrmex*. The cultivars are leucocoprinaceous, closely related to *L. gongylophorus* cultivated by the higher attinis. However, they belong to a different fungal group, designated G3, where cultivars are more closely associated with free-living fungi (Figure 1) (Chapela *et al.*, 1994; Vo *et al.*, 2009; Schultz, 2021).

In contrast to all other fungiculture systems, this system cultivates yeast, not filamentous fungi. For this reason, it was believed to be the first fungiculture system to appear in the evolutionary history of attines (Muller *et al.*, 2001; Mehdiabadi and Schultz, 2009; Vo *et al.*, 2009; Mehdiabadi *et al.*, 2012; Schultz, 2021).

However, due to its relationship with free-living counterparts, the hypothesis is suggested that its cultivars were facultative pleomorphic symbionts, meaning they have the ability to develop filamentously but can switch to yeast-like forms when conditions are adverse (Vo *et al.*, 2009; Schultz, 2021).

Weber (1972) successfully reverted the yeast-like development of the *Cyphomyrmex* cultivar to filamentous

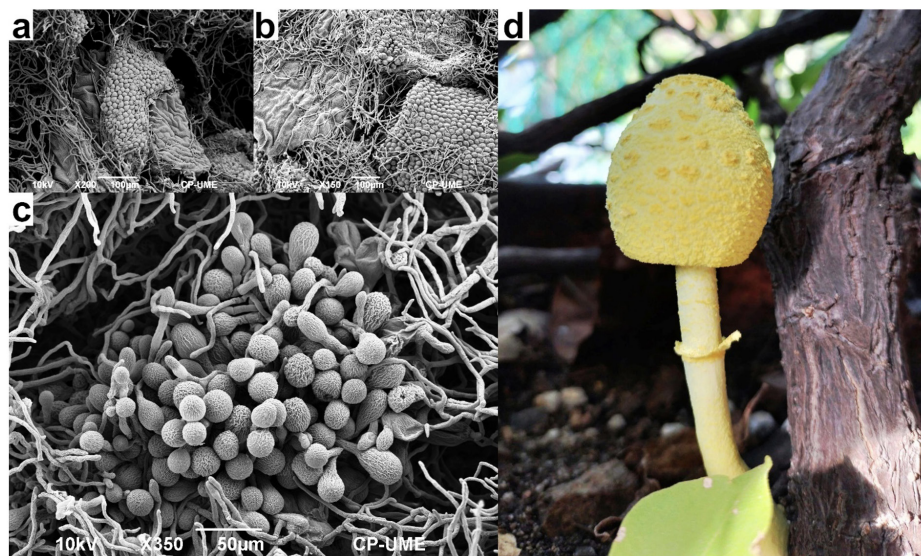


Figure 2. Scanning Electron Microscopy of a fungal garden from *Atta mexicana* Smith, illustrating vegetative material surrounded by *Leucoagaricus gongylophorus* mycelium (a and b), and bunch of developed gonglydia (c). Fruiting body of *Leucocoprinus birnbaumii*, a closely related wild species to *L. gongylophorus* (d). Photo Credit: Teresa N. Maldonado. Source: elaborated by the authors.

growth when provided with optimal laboratory conditions. This could provide insights into how the cultivation by attines transitioned from a free-living saprophyte, capable of surviving in conditions similar to those in primitive fungiculture systems, to becoming an obligate symbiont requiring controlled conditions, as seen in leaf-cutting ant systems (Schultz, 2021). Further in-depth studies are needed to explore the symbiotic fungus's capacity to enter the yeast-like phase as a mechanism of resistance under suboptimal development conditions.

Results from Mueller *et al.* (1996) could support the facultative pleomorphism hypothesis. Through Amplified Fragment Length Polymorphism (AFLP) analysis, they discovered that the diversity of leucocoprinaceous fungi obtained from 13 nests of *Cyphomyrmex minutus* represented various clones dispersed by the ants themselves through vegetative propagules (asexual). Additionally, they observed compatibility with free-living fungi, supporting the idea that leucocoprinaceae from G3 could have been free-living fungi adopted by these attines (Chapela *et al.*, 1994; Schultz, 2021).

Recently, Hanisch *et al.* (2022) reported that *Paramyrmecophylax* maintains the phylogenetically closest position to *Cyphomyrmex* within the rimosus group. This genus *Paramyrmecophylax* does not cultivate yeast-like leucocoprinaceae but rather the obligate symbiont of Clade A (G1) (Mehdiabadi *et al.*, 2012). These

data will enable future research to gain a better understanding of the transition of attines to yeast cultivation.

On the other hand, distinct yeasts from Clade G3 have also been reported (Figure 1). Nair and Hervey (1979) reported a yeast from the genus *Lepiota* sp. cultivated in the fungal garden of *Cyphomyrmex costatus*. Later, Wang *et al.* (1999) identified the yeast *Tyridiomyces formicarum* as the cultivated symbiont by *Cyphomyrmex minutus*. In both cases, the yeasts served additional functions beyond being food; they also synthesized secondary metabolites with antimicrobial potential. For instance, *Lepiota* sp. produced lepioclorin, an antibacterial compound inhibiting *Staphylococcus aureus*, while *T. formicarum* synthesized the antifungal dicetopiperazine, which inhibited the development of yeasts *Saccharomyces cerevisiae* and *C. albicans*. In both cases, it is likely that the cultivars suppressed the development of microorganisms representing competition for nutrients or potentially pathogenic to the fungal garden (Bizarria *et al.*, 2022).

Coral-Type Fungus Fungiculture

A monophyletic group of 34 species from the genus *Apterostigma* within the "pilosum" group engages in "Coral-Type Fungus Fungiculture" cultivating the genus currently known as *Myrmecopterula* (formerly *Pterula*) (Agaricales: Pterulaceae) (Schultz, 2007;

Leal-Dutra *et al.*, 2020; Schultz, 2021; Hanisch, 2022).

The three species within the genus *Myrmecopterula* correspond to two paraphyletic groups within the Pterulaceae family (Dentinger *et al.*, 2009; Leal-Dutra *et al.*, 2020). These clades are referred to as G2 and G4, following the classification proposed by Schultz (2007). Clade G2 corresponds to the cultivar that develops gardens covered by a fungal veil (*M. velohortorum*), while Clade G4 does not produce a fungal veil, resulting in naked gardens (*M. nudihortorum*) (Dentinger *et al.*, 2009). The third species, *Myrmecopterula moniliformis*, was considered the closest free-living coral fungus to Clade G4, as it had not been reported as an attine cultivar. However, Leal-Dutra *et al.* (2020) discovered *Myrmecopterula moniliformis* in nests of *Apterostigma* sp. This species is a facultative symbiont that can produce free-living fruiting bodies without the need for a fungal garden, as the conditions required for its survival are not as specific as in superior fungiculture (Leal-Dutra *et al.*, 2020).

The three species can be distinguished based on the morphology of their sporocarps: *M. moniliformis* exhibits structures of sterile basidiomes resembling strings of beads attached to fertile branching coraloid structures (Dentinger *et al.*, 2009). However, for a more precise identification, it should be noted that fungi of the genus *Myrmecopterula* have a coraloid or filiform (thread-like) fruiting body (basidiome), which appears in a single color or, in some cases, only the stipe is light brown; the fruiting body is densely branched, always with a non-geotropic development. Additionally, it presents a cottony subiculum associated with attine ants (Leal-Dutra *et al.*, 2020).

On the other hand, within the genus *Apterostigma*, there is an exception: the species *A. megacephala* is the only one in this genus that does not cultivate coral fungi but rather *Leucoagaricus gongylophorus*, the same fungus as in superior fungiculture (Schultz *et al.*, 2015; Solomon *et al.*, 2019).

Physiological Capacity of the Cultivated Mutualistic Fungus

To better understand the characteristics that favored ants in selecting a specific species for more advanced fungiculture systems, we must comprehend the metabolic functions developed by the cultivated fungi. Studies focused on other insect-driven fungiculture systems agree that cultivated fungi perform metabolic functions that insects cannot execute on their own. These functions

involve the processing or assimilation of nutrient sources inaccessible to insects, or the biosynthesis of amino acids or microbial metabolites that enhance nutrition or the insect's response to infections by opportunistic microorganisms (Paludo *et al.*, 2018; Cheng *et al.*, 2019).

For example, in some species of stingless bees, fungi such as *Monascus* spp. and *Zygosacharomyces* spp. are cultivated. These species play the primary role of providing food for the larvae, but it has also been confirmed that they produce key metabolites for metamorphosis and the survival of the emerging adults (Menezes *et al.*, 2015; Paludo *et al.*, 2018). In another fungiculture system, certain arboreal ants cultivate fungi of the order Chaetothyriales, serving two known functions to date: a) they serve as the main raw material for the construction of galleries along the stems of myrmecophytic plants (plants hosting ants); in this case, the fungus exhibits high resistance to mechanical and environmental damage, and its ability to rapidly produce biomass makes it a valuable material for colony establishment; b) Chaetothyriales also represent a food source for the larvae of their mutualistic ants. Several studies reveal that, within the colonies, ants cultivate fungal patches where they place the larvae to feed on the young mycelium. It is noteworthy that, in this case, the fungi degrade waste material from the colonies, such as corpses of other insects and excrement, resembling a form of "fertilization" that promotes the development of the fungal patch for consumption by the larvae (Nepel *et al.*, 2014; Leroy *et al.*, 2017; Ruiz-González *et al.*, 2019).

However, the two fungiculture systems described above are not as specialized or systematized as the fungiculture of Attini ants. Additionally, stingless bees and arboreal ants are not obligate mycophages, and their diet is more diverse than the exclusive mycophagy developed by the Attini.

Based on interactions between microorganisms and other insects, two crucial physiological aspects can be suggested that led ants to the selection and specialization of their mutualistic fungi: a) the ability to metabolize organic compounds that ants could not assimilate on their own, and b) a high capacity for fungal biomass production to feed the ants.

In the Attini-cultivated fungus system, the ant performs a pre-treatment of vegetative material using fungal-derived enzymes (mainly proteases, pectinases, and ligninases), which pass through its digestive system without modification (Martin and Martin, 1970; Li *et*

al., 2021). Additionally, ants also produce their own ligninolytic enzymes, but they do so during the mastication of raw material for substrate construction.

In more specialized fungiculture, the fungus *L. gongylophorus* stands out for its extensive capacity to produce enzymes that hydrolyze multiple sources of energy (organic carbon sources) found in the structure of a wide variety of plant tissues from different species foraged by the ants.

Multiple studies have investigated the enzymatic capacity of various strains of *L. gongylophorus*, employing in vitro assays under laboratory conditions or utilizing methodologies associated with omics sciences, primarily proteomics, metaproteomics, and metagenomics (Aylward *et al.*, 2012; Khadempour *et al.*, 2016; 2021). Based on the studies analyzed for the systematic review in this section, was identified that the enzymes most frequently reported are pectinases (15.9%), followed by glucosidases (10.23%), cellulases (7.95%), laccases (7.95%), proteases (6.82%), xylanases (5.68%), and amylases (5.68%) (Figure 3); collectively, these seven enzymes constitute over 60% of the reported enzymes, indicating their potential significance in the metabolic functions of *L. gongylophorus* strains cultivated in the most advanced fungiculture.

Given that the raw material for fungal gardens originates from a wide diversity of plant species, and considering that plant biomass comprises various tissues, the cultivated fungus must be

capable of hydrolyzing the majority of organic compounds in its substrate. Therefore, its broad enzymatic range serves as a decisive factor, acting as a metabolic extension for ants nutrition (Abril, 2011).

However, in addition to the ability to hydrolyze different organic compounds, the cultivated fungus must also efficiently assimilate the hydrolyzed compounds to develop biomass in large quantities, sufficient to feed the colony (Gomes de Siqueira *et al.*, 1998). For example, Gomes de Siqueira *et al.* (1998) and Rønheide *et al.* (2004) agree that pectins are compounds requiring higher enzymatic activity to be hydrolyzed by the symbiotic fungus, but their assimilation rate is very low. Consequently, their degradation involves higher energy expenditure and does not favor the accelerated increase in biomass. This aligns with our results, as pectin was the most reported enzyme in the analyzed studies, suggesting that it is indeed highly produced by the fungus. However, its high production does not guarantee greater assimilation by the fungus. Similar considerations apply to cellulose, which, despite being the most abundant carbon source in plant biomass, has a complex structure and represents a challenging material to hydrolyze. Its utilization rate is very low, making it one of the less important carbon sources for the cultivated fungus (Gomes de Siqueira *et al.*, 1998).

The evidence suggests that starch and xylan are the most important compounds in the nutrition of the

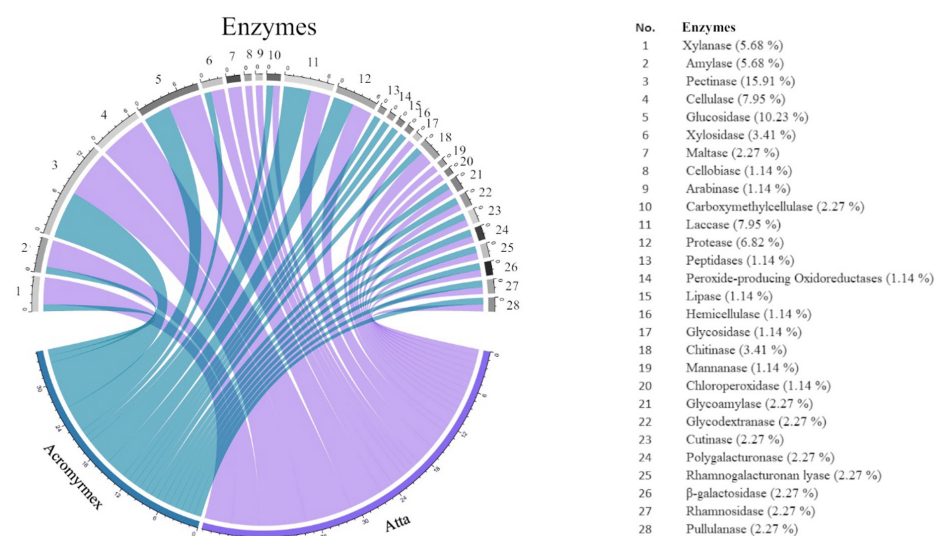


Figure 3. Key enzymes involved in the biodegradation of organic matter in the fungal garden by symbiotic fungus *Leucoagaricus gongylophorus* cultivated by *Atta* spp. and *Acromyrmex* spp. Source: elaborated by the authors.

cultivated fungus. Starch constitutes the main energy reserve compound used by plants, while xylan is a structural polysaccharide that provides rigidity to the cell wall of plant cells and is part of the hemicelluloses. Both compounds are abundant in all plant tissues and are easily hydrolyzed by the symbiotic fungus. Their ability to assimilate them is rapid, making them labile sources of carbon. This results in readily available energy for the fungus to produce biomass more rapidly (Gomes de Siqueira *et al.*, 1998; Abril, 2011).

Gomes de Siqueira *et al.* (1998) and Silva *et al.* (2006) observed that *L. gongylophorus* invests less energy in enzymatic activity and achieves higher biomass production when its carbon sources come from monosaccharides glucose and xylose, compared to polysaccharides such as starch and xylan. This makes sense because monosaccharides are much simpler molecules than the structural polysaccharides found in plant biomass.

The above suggests that the fungus can assimilate simple glucose molecules; however, its ability to degrade complex glucose polymers will depend on the nature of each compound. For example, complex molecules like starch and amylopectins are primarily composed of glucose and are relatively easily assimilated by the fungus. This is not the case with cellulose, despite being essentially composed only of glucose polymers. This is because the glucose polymers in starch and amylopectins are based on α (1 $\rightarrow$ 4) glucosidic bonds, while cellulose has β (1 $\rightarrow$ 4) bonds. Silva *et al.* (2006) confirmed that one of the main enzymes used by ants to convert starch into simple glucose molecules is α -amylase. Therefore, it is likely that much of the enzymatic capacity of *L. gongylophorus* is based on the hydrolysis of α bonds, but not as much on β bonds. Future work should delve further into the hydrolysis capacity of complex compounds based on the types of glucosidic bonds, as this would explain why *L. gongylophorus* is inefficient in utilizing cellulose, despite it being the most abundant source in plant tissues, even when cellulases represent the third most abundant group of enzymes based on our results.

It is likely that fungi cultivated on fresh organic matter (in more advanced fungiculture) require greater enzymatic capacity because the fungus needs to obtain nutrients from a wider variety of plant sources, compared to fungi cultivated on substrates that are already at different stages of decomposition (less specialized fungiculture), in which the main processes of organic matter

biodegradation are carried out by saprophytic bacteria and fungi, not by the cultivated fungus (Moreira-Soto *et al.*, 2017; Khadempour *et al.*, 2020). Recently, studies using metagenomic and proteomic approaches have revealed the presence of hundreds of microbial genes responsible for degrading a wide variety of biomolecules that the symbiotic fungus cannot metabolize. Therefore, it is likely that the answer lies in the fungal garden microbiome and not solely in the cultivated fungus (Khadempour *et al.*, 2021; Schjøtt and Boomsma, 2021).

Our study was limited to discussing the metabolic capacities of *L. gongylophorus* cultivated in the most advanced fungiculture (by leaf-cutting ants); however, future work should include the enzymatic capacity of fungi cultivated in lower-tier systems. Currently, most information has been focused on fungi cultivated by various species of *Atta* and *Acromyrmex*, as they are the species with the widest distribution, and the size of their colonies is larger compared to the rest of the ants in the Attini tribe. While the physiology and metabolic capacity of the cultivation may be crucial factors in the selection of the cultivated fungus, metabolic capacity does not appear to be a limiting factor in such a diverse micro-ecosystem as the fungal garden (Moreira-Soto *et al.*, 2017).

Recently, it has been discovered that the lipid composition varies across the fungal garden, and, more surprisingly, the lipid profiles found in the cultivated fungus gongylidia differ from the lipid profiles of the surrounding substrate. This raises new questions about how the structural fatty acids of plant material are modified so that the fungus can assimilate and translocate them to its storage structures (gongylidia) (Khadempour *et al.*, 2021).

Evidence suggests that the fungus cultivated in higher fungiculture has enhanced its performance in response to the wide variety of plant substrates used by ants, producing a broad range of enzymes in response to the diversity of foraged plants (Khadempour *et al.*, 2016; Shik *et al.*, 2020). This could be explained as follows: in the more specialized fungiculture developed by leafcutter ants, the specialized fungus *L. gongylophorus* has become an obligate mutualist. It has been selected through a coevolutionary process based on the enzymatic and metabolic capacity of the cultivation, capable of obtaining nutrients from different plant tissues from a wide variety of foraged plants (Lange and Grell, 2014; Shik *et al.*, 2020). On the other hand, cultivars from less specialized fungicultures

have less varied substrates (such as leaf litter or waste and vertebrate corpses and exoskeletons). These substrates, being in different stages of decomposition, already have a higher concentration of molecules previously hydrolyzed by the saprophytic microbial communities in the soil (not necessarily from a pre-constructed fungal garden) (Moreira-Soto *et al.*, 2017).

The Metabolic Capacity and Taxonomy of Fungi May Depend on the Cultivation Conditions

The study of the relationship between enzymatic activity and the raw material available for nest construction can also be crucial in the selection of the fungal species. For example, yeasts are fungi that have been proven to produce secondary metabolites with antifungal capacity. Therefore, the cultivation of yeast-like clades, in simple substrates susceptible to infestation by opportunistic saprophytic microorganisms, may provide a greater advantage if yeasts synthesize antimicrobial secondary metabolites, as is the case with other symbiotic yeasts of insects (Smedsgaard and Nielsen, 2005; Aguilar-Colorado and Rivera-Chávez *et al.* 2023).

Moreover, the vast diversity of non-obligate mutualistic clades of leucocoprinaceous fungi or coral-type fungi may depend on the same cultivation system. In a less specific system, their characteristics may not reach the same level of specialization as in the leafcutter ant fungiculture. Therefore, the process of selecting fungi could be part of a virtuous circle, in which the more advanced system requires more specialized fungi (*L. gongylophorus*), and in turn, the more specialized fungi have the ability to degrade a greater variety of organic compounds compared to less specialized fungi. The latter utilize simpler molecules, previously biodegraded by other saprophytic microorganisms in less complex and more susceptible cultivation conditions influenced by the soil microbiome (Abril, 2011; Aguilar-Colorado and Rivera-Chávez *et al.*, 2023; Costa-Santos *et al.*, 2023).

Conclusions and Future Perspectives of Study

Lower attine species employing less developed fungiculture, such as *Cyphomyrmex* or *Apterostigma*, establish their fungal gardens on substrates composed of decomposing plant remains, wood fragments, and arthropod excrement, suggesting that their cultivars are facultative symbionts related to free-living

saprophytic fungi. However, it appears likely that as the fungiculture system becomes more specialized, the cultivar becomes more dependent, as observed in leaf-cutting ants.

Future studies should explore the epigenetics and transcriptome of yeast-like cultivars to understand the molecular processes involved in their yeast-like and filamentous development. This investigation could provide a better understanding of the "domestication" processes of this fungus during its coevolution with attines. Additionally, analyzing the genetic diversity among different attine cultivars with distinct fungiculture systems would be essential.

On the other hand, to gain a deeper understanding of the coevolutionary process in the mutualism of fungus-growing ants (tribe Attini) and their cultivated fungi, was identified that the metabolic capacity of the fungus is crucial. This is because its ability to hydrolyze complex carbon compounds and assimilate them to produce fungal biomass are factors that may have promoted specialization among the more advanced ants and the exclusive fungus. Evidence suggests that in a fungiculture system where ants use more diverse sources of plant material, the symbiotic fungus must have a wide range of metabolic capabilities, including seven key enzymes: pectinases, glucosidases, cellulases, laccases, proteases, xylanases, and amylases. However, the ability of fungus to assimilate hydrolyzed products and its efficiency in biomass production are also important. Our work was limited to analyzing the metabolic characteristics of the cultivated fungus in the most advanced fungiculture system. Still, it is recommended that future studies include the metabolic (enzymatic) characteristics of fungi cultivated in more incipient and less specialized fungiculture systems to gain a deeper understanding of the similarities and differences in enzymatic activities between each cultivation.

REFERENCES

- Abril AB (2011) The Leaf-cutting Ant-plant Interaction from a Microbial Ecology Perspective. En: J. Seckbach y Z. Dubinsky (Eds.), *All Flesh is Grass Plant-Animal Interrelationships* (1st ed.). Springer Dordrecht Heidelberg. London. pp. 39–63. https://doi.org/10.1007/978-90-481-9316-5_2
- Aguilar-Colorado A, Rivera-Chávez J (2023) Ants/Nest-Associated Fungi and Their Specialized Metabolites: Taxonomy, Chemistry, and Bioactivity. *Revista Brasileira de Farmacognosia* 3: 901–923. <https://doi.org/10.1007/s43450-023-00417-3>
- Aylward FO, Burnum-Johnson KE, Tringe SG, Teiling C, Tremmel DM, Moeller JA, Scott JJ, Barry KW, Piehowski PD, Nicora CD, Malfatti SA, Monroe ME, Purvine SO, Goodwin LA, Smith RD, Weinstock GM, Gerardo NM, Suen G, Lipton MS, Currie CR (2012) *Leucoagaricus gongylophorus* Produces Diverse Enzymes for the Degradation of Recalcitrant Plant Polymers in Leaf-Cutter Ant Fungus Gardens. *Applied and Environmental Microbiology* 79: 3770–3778. <https://doi.org/10.1128/AEM.03833-12>
- Bizarria JR, Kooji PW, Rodrigues A (2021) Climate change influences basidiome emergence of leaf-cutting ant cultivar. *Journal of Fungi* 7: 912. <https://doi.org/10.3390/jof7110912>
- Bizarria JR, Pagnocca FC, Rodrigues A (2022) Yeasts in the attine ant–fungus mutualism: Diversity, functional roles, and putative biotechnological applications. *Yeast* 39: 25–39. <https://doi.org/10.1002/yea.3667>
- Cardenas CR, Luo AR, Jones TH, Schultz TR, Adams RMM (2020) Using an integrative taxonomic approach to delimit a sibling species, *Mycetomoellerius mikromelanos* sp. nov. (Formicidae: Attini: Attina). *PeerJ* 9: e11622. <http://doi.org/10.7717/peerj.11622>
- Chapela IH, Rehner SA, Schultz TR, Mueller UG (1994) Evolutionary History of the Symbiosis Between Fungus-Growing Ants and Their Fungi. *Science*. 266: 1691–1694. <http://doi.org/10.1126/science.266.5191.1691>
- Cheng D, Chen S, Huang Y, Pierce NE, Riegler M, Yang F, Zeng L, Lu Y, Liang G, Xu Y (2019) Symbiotic microbiota may reflect host adaptation by resident to invasive ant species. *PLOS Pathogens* 15: e1007942. <https://doi.org/10.1371/journal.ppat.1007942>
- Conlon BH, O'Tuama D, Michelsen A, Crumière AJJ, Shik JZ (2022) A fungal symbiont converts provisioned cellulose into edible yield for its leaf cutter ant farmers. *Biology Letters* 18: 20220022. <https://doi.org/10.1098/rsbl.2022.0022>
- Costa-Santos AC, Ferreira-Borges LD, Coelho Rocha ND, de Carvalho-Azevedo VA, Bonetti AM, Rodrigues dos Santos A, da Rocha-Fernandes A, Cavalcanti-Dantas RC, Ueira-Vieira C (2023) Bacteria, yeasts, and fungi associated with larval food of Brazilian native stingless bees. *Scientific reports* 13: 5147. <https://doi.org/10.1038/s41598-023-32298-w>
- Cristiano MP, Cardoso DC, Sandoval-Gómez VE, Simões-Gomes FC (2020) *Amoimyrmex* Cristiano, Cardoso & Sandoval, gen. nov. (Hymenoptera: Formicidae): a new genus of leaf-cutting ants revealed by multilocus molecular phylogenetic and morphological analyses. *Austral Entomology* 59: 643–676. <https://doi.org/10.1111/aen.12493>
- Dejean A, Azémar F, Naskrecki P, Tindo M, Rossi V, Faucher C, Gryta H (2023) Mutualistic interactions between ants and fungi: A review. *Ecology and Evolution* 13: e10386. <https://doi.org/10.1002/ece3.10386>
- Dentinger BTM, Lodgem DJ, Munkacs AB, Desjardin DE, McLaughlin DJ (2009) Phylogenetic placement of an unusual coral mushroom challenges the classic hypothesis of strict coevolution in the *Apterostigma pilosum* group ant-fungus mutualism. *Evolution* 62: 2172–2178. <https://doi.org/10.1111/j.1558-5646.2009.00697.x>
- Espinoza C, Zavala II, Couttolenc A, Landa-Cadena G, Valenzuela J, Trigos A (2017) In vitro isolation and identification of *Leucoagaricus gongylophorus* from *Atta mexicana* (Hymenoptera: Formicidae) fungal garden. *Scientia Fungorum* 46: 3–8. <https://doi.org/10.33885/sf.2017.46.1170>
- Fernández F, Castro-Huertas V, Serna F (2015) *Hormigas cortadoras de hojas de Colombia: Accromyrmex & Atta* (Hymenoptera: Formicidae). Fauna de Colombia, Monografía No. 5, Instituto de Ciencias Naturales. Universidad Nacional de Colombia. Colombia. 350 pp.
- Fisher PJ, Stradling DJ, Pegler DN (1994) *Leucoagaricus basidiomata* from a live nest of the leaf-cutting ant *Atta cephalotes*. *Mycological Research* 98: 884–888. [https://doi.org/10.1016/S0953-7562\(09\)80259-1](https://doi.org/10.1016/S0953-7562(09)80259-1)
- Gomes De Siquiera C, Bacci, Jr. M, Pagnocca FC, Correa Bueno O, Aparecida Hebling MJ (1998) Metabolism of Plant Polysaccharides by *Leucoagaricus gongylophorus*, the Symbiotic Fungus of the Leaf-Cutting Ant *Atta sexdens* L. *Applied and Environmental Microbiology*. 64: 4820–4822. <https://doi.org/10.1128/aem.64.12.4820-4822.1998>
- Hanisch PE, Sosa-Calvo J, Schultz TR (2022) The last piece of the puzzle? Phylogenetic position and natural history of the monotypic fungus-farming ant genus *Paramycetophylax* (Formicidae: Attini). *Insect Systemics and Diversity* 6: 1–17. <https://doi.org/10.1093/isd/ixab029>
- Ješovnik A, Sosa-Calvo J, Lloyd MW, Branstetter MG, Fernández F, Schultz TR (2017) Phylogenomic species delimitation and host-symbiont coevolution in the fungus-farming ant genus *Sericomyrmex* Mayr (Hymenoptera: Formicidae): ultraconserved elements (UCEs) resolve a recent radiation. *Systematic Entomology* 42: 523–542. <http://doi.org/10.1111/syen.12228>
- Jiménez-Gómez I, Barcoto MO, Montoya QV, Goes AC, Monteiro LSVE, Bueno OC, Rodrigues A (2021) Host Susceptibility Modulates *Escovopsis* Pathogenic Potential In The Fungiculture Of Higher Attine Ants. *Frontiers in Microbiology*. <https://doi.org/10.3389/fmicb.2021.673444>
- Kellner K, Fernández-Marín H, Ishrak HD, Sen R, Linksvayern TA (2013) Co-evolutionary patterns and diversification of ant–fungus associations in the asexual fungus-farming ant *Mycocrepus smithii* in Panama. *Journal of Evolutionary Biology* 26: 1353–1362. <https://doi.org/10.1111/jeb.12140>
- Kellner K, Kardish MR, Seal JN, Linksvayer TA, Mueller UG (2018) Symbiont-Mediated Host-Parasite Dynamics in a Fungus-Gardening Ant. *Microbial Ecology* 76: 530–543. <https://doi.org/10.1007/s00248-017-1124-6>
- Khadempour L, Fan H, Keefover-Ring K, Carlos-Shanley C, Nagamoto NS, Dam MA, Pupo MT, Currie CR (2020) Metagenomics Reveals Diet-Specific Specialization of Bacterial Communities in Fungus Gardens of Grass- and Dicot-Cutter Ants. *Frontiers in Microbiology* 11: 570770. <https://doi.org/10.3389/fmicb.2020.570770>
- Khadempour L, Burnum-Johnson KE, Baker ES, Nicora CD, Webb-Robertson B-JM, White III Ra, Monroe ME, Huang EL, Smith RD, Currie CR (2016) The fungal cultivar of leaf-cutter ants produces specific enzymes in response to different plant substrates. *Molecular Ecology* 25: 5795–5805. <https://doi.org/10.1111/mec.13872>
- Khadempour L, Kyle JE, Webb-Robertson BJM, Nicora CD, Smith FB, Smith RD, Lipton MS, Currie CR, Baker ES, Burnum-Johnson KE (2021) From Plants to Ants: Fungal Modification of Leaf Lipids for Nutrition and Communication in the

- Leaf-Cutter Ant Fungal Garden Ecosystem. *mSystems* 6: e01307-20. <https://doi.org/10.1128/mSystems.01307-20>
- Lange L, Grell MN (2014) The prominent role of fungi and fungal enzymes in the ant–fungus biomass conversion symbiosis. *Applied Microbiology and Biotechnology* 98: 4839–4851 <https://doi.org/10.1007/s00253-014-5708-5>
- Leal-Dutra CA, Griffith GW, Neves MA, McLaughlin DJ, McLaughlin EG, Clasen LA, Dentinger TM (2020) Reclassification of *Pterulaceae* Corner (Basidiomycota: Agaricales) introducing the and-associated genus *Myrmecopterula* gen. nov., *Phaeopterula* Henn. and the corticoid *Radulomycetaceae* fam. nov. *IMA Fungus* 11: 2. <https://doi.org/10.1186/s43008-019-0022-6>
- Leroy C, Jauneau A, Martinez Y, Cabin-Flaman A, Gibouin D, Orivel J, Séjalon-Delmas N (2017) Exploring fungus–plant N transfer in a tripartite ant–plant–fungus mutualism. *Annals of Botany* 120: 417–426. <https://doi.org/10.1093/aob/mcx064>
- Li H, Young SE, Poulsen M, Currie CR (2021) Symbiont-Mediated Digestion of Plant Biomass in Fungus-Farming Insects. *Annual Review of Entomology* 66: 297–316. <https://doi.org/10.1146/annurev-ento-040920-061140>
- Martin JS, Marin MM (1970) The Presence of Protease Activity in The Rectal Fluid of Attine Ants. *Journal of Insect Physiology* 16: 227–232. [https://doi.org/10.1016/0022-1910\(70\)90164-2](https://doi.org/10.1016/0022-1910(70)90164-2)
- Mehdiabadi NJ, Mueller UG, Brady SG, Himler AG, and Schultz TR (2012) Symbiont fidelity and the origin of species in fungus-growing ants. *Nature communications* 3: 840. <https://doi.org/10.1038/ncomms1844>
- Mehdiabadi NJ, Schultz TR (2009) Natural history and phylogeny of the fungus-farming ants (Hymenoptera: Formicidae: Myrmicinae: Attini). *Myrmecological News* 13: 37–55.
- Menezes C, Vollet-Neto A, Marsaioli AJ, Zampieri D, Fontoura IC, Luchessi AD, Imperatriz-Fonseca VL (2015) A Brazilian social bee must cultivate fungus to survive. *Current Biology* 25: 2851–2855. <https://doi.org/10.1016/j.cub.2015.09.028>
- Möller A (1893) *Die Pilzgärten einiger südamerikanischer Ameisen*. Gustav Fischer. Jena, Germany. 127 pp.
- Moreira-Soto RD, Sanchez E, Currie CR, Pinto-Tomas AA (2017) Ultrastructural and microbial analyses of cellulose degradation in leaf-cutter ant colonies. *Microbiology* 163: 1578–1589. <https://doi.org/10.1099/mic.0.000546>
- Muchovej JJ, Della Lucia TM, Muchovej RMC (1991) *Leucoagaricus weberi* sp. nov. from a live nest of leaf-cutting ants. *Mycological Research* 95: 1308–1311. [https://doi.org/10.1016/S0953-7562\(09\)80581-9](https://doi.org/10.1016/S0953-7562(09)80581-9)
- Mueller UG, Kardish MR, Ishak HD, Wright AM, Solomon SE, Brushi SM, Carlson AL, Bacci Jr M (2018) Phylogenetic patterns of ant–fungus associations indicate that farming strategies, not only a superior fungal cultivar, explain the ecological success of leafcutter ants. *Molecular Ecology* 27: 2414–2434. <https://doi.org/10.1111/mec.14588>
- Mueller UG, Lipari SE, Milgroom MG (1996) Amplified fragment length polymorphism (AFLP) fingerprinting of symbiotic fungi cultured by the fungus-growing ant *Cyphomyrmex minutus*. *Molecular Ecology* 5: 119–122. <https://doi.org/10.1111/j.1365-294X.1996.tb00297.x>
- Mueller UG, Rehner SA, Schultz TR (1998) The evolution of agriculture in ants. *Science* 281: 2034–2038. <https://doi.org/10.1126/science.281.5385.2034>
- Mueller UG, Schultz TR, Currie CR, Adams RMM, Malloch D (2001) The origin of the attine ant–fungus mutualism. *The Quarterly Review of Biology* 76: 169–197.
- Nair MSR, Hervey A (1979) Structure of Lepiochlorin, an antibiotic metabolite of a fungus cultivated by ants. *Phytochemistry* 18: 326–327. [https://doi.org/10.1016/0031-9422\(79\)80085-0](https://doi.org/10.1016/0031-9422(79)80085-0)
- Nepel M, Voglmayr H, Schonenberger J, Mayer VE (2014) High Diversity and Low Specificity of Chaetothyrialean Fungi in Carton Galleries in a Neotropical Ant–Plant Association. *PLOS ONE* 9: e112756. <https://doi.org/10.1371/journal.pone.0112756>
- Nygaard S, Hu H, Li C, Schiøtt M, Chen Z, Yang Z, Xie Q, Ma C, Deng Y, Dikow RB, Rabeling C, Nash DR, Weislo WT, Brady SG, Schultz TR, Zhang G, Boomsma JJ (2016) Reciprocal genomic evolution in the ant–fungus agricultural symbiosis. *Nature Communications* 7: 12233. <https://doi.org/10.1038/ncomms12233>
- O’Dea RE, Lagisz M, Jennions MD, Koricheva J, Noble DWA, Parker TH, Gurevitch J, Page MJ, Stewart G, Moher D, Nakagawa S (2021) Preferred reporting items for systematic reviews and metaanalyses in ecology and evolutionary biology: a PRISMA extension. *Biological Reviews* 96: 1695–1722. <https://doi.org/10.1111/brv.12721>
- Paludo CR, Menezes C, Silva-Junior EA, Vollet-Neto A, Andrade-Dominguez A, Pishchany G, Khadempour L, Nascimento FSD, Currie CR, Kolter R, Clardy J, Pupo MT (2018) Stingless bee larvae Require Fungal steroids to pupate. *Scientific Reports* 8: 1122. <https://doi.org/10.1038/s41598-018-19583-9>
- Ronhede S, Boomsma JJ, Rosendahl S (2004) Fungal enzymes transferred by leaf-cutting ants in their fungus gardens. *Mycological Research* 108: 101–106. <https://doi.org/10.1017/S0953756203008931>
- Ruiz-González MX, Leroy C, Dejean A, Gryta H, Jargeat P, Armijos Carrión AD, Orivel J (2019) Do Host Plant and Associated Ant Species Affect Microbial Communities in Myrmecophytes? *Insects* 10: 391. <https://doi.org/10.3390/insects10110391>
- Schiøtt M, Boomsma JJ (2021) Proteomics reveals synergy between biomass degrading enzymes and inorganic Fenton chemistry in leaf-cutting ant colonies. *Ecology Evolutionary Biology* 10: e61816. <https://doi.org/10.7554/eLife.61816>
- Schultz TR (2007) The fungus-growing ant genus *Apterostigma* in Dominican amber. In Snelling RR, Fisher BL, Ward PS (Eds.), *Advances in ant systematics (Hymenoptera: Formicidae): homage to E. O. Wilson – 50 years of contributions*. Memoirs of the American Entomological Institute, 80. pp. 425–436.
- Schultz TR (2021) Fungus-Farming Ants (Attini in Part). In Starr CK (Eds.), *Encyclopedia of Social Insects*. Springer, Cham, Switzerland. pp. 404–411. https://doi.org/10.1007/978-3-030-28102-1_46
- Schultz TR, Brady SG (2008) Major evolutionary transitions in ant agriculture. *PNAS* 105: 5435–5440. <https://doi.org/10.1073/pnas.0711024105>
- Schultz TR, Sosa-Calvo J, Brady SG, Lopes CT, Mueller UG, Bacci Jr M, Vasconcelos HL (2015) The Most Relictual Fungus-Farming Ant Species Cultivates the Most Recently Evolved and Highly Domesticated Fungal Symbiont Species. *The American Naturalist* 185: 693–703. <https://doi.org/10.1086/680501>
- Shik JZ, Kooij PW, Donoso DA, Santos JC, Gomez EB, Franco M, Crumière AJJ, Arnan X, Howe J, Weislo WT, Boomsma JJ (2020) Nutritional niches reveal fundamental domestication trade-offs in fungus-farming ants. *Nature Ecology & Evolution* 5: 122–134. <https://doi.org/10.1038/s41559-020-01314-x>
- Silva A, Bacci Jr M, Pagnocca FC, Bueno OC, Hebling MJA (2006) Starch metabolism in *Leucoagaricus gongylophorus*, the symbiotic fungus of leaf-cutting ants. *Microbiological Research* 161: 299–303. <https://doi.org/10.1016/j.micres.2005.11.001>
- Smedsgaard J, Nielsen J (2005) Metabolite profiling of fungi and yeast: from phenotype to metabolome by MS and informatics. *Journal of Experimental Botany* 56: 273–286. <https://doi.org/10.1093/jxb/eri068>
- Solomon SE, Lopes CT, Mueller UG, Rodrigues A, Sosa-Calvo J, Schultz TR, Vasconcelos HL (2011) Nesting biology and fungiculture of the fungus-growing ant, *Mycetoglyphus cerasidensis*: New light on the origin of higherattine agriculture. *Journal of Insect Science* 11: 12. <https://doi.org/10.1673/031.011.0112>
- Solomon SE, Rabeling C, Sosa-Calvo J, Lopes CT, Rodrigues A, Vasconcelos HL, Bacci Jr M, Mueller UG, Schultz TR (2019) The molecular phylogenetics of Trachymyrmex Forel ants and their fungal cultivars provide insights into the origin and coevolutionary history of ‘higher-attine’ ant agriculture. *Systematic Entomology* 44: 939–956. <https://doi.org/10.1111/syen.12370>
- Sosa-Calvo J, Schultz TR, Ješovnik A, Dahan RA, Rabeling C (2018) Evolution, systematics, and natural history of a new genus of cryptobiotic fungus-growing ants. *Systematic Entomology* 43: 549–567. <https://doi.org/10.1111/syen.12289>
- Vo TL, Mueller UG, Mikhrev AS (2009) Free-living fungal symbionts (Lepiotaceae) of fungus-growing ants (Attini: Formicidae). *Mycologia* 101: 206–210. <https://doi.org/10.3852/07-055>
- Wang Y, Mueller UG, Clardy J (1999) Antifungal Diketopiperazines from Symbiotic Fungus of Fungus-Growing Ant *Cyphomyrmex minutus*. *Journal of Chemical Ecology* 25: 935–941. <https://doi.org/10.1023/A:1020861221126>
- Weber NA (1972) *Gardening ants, the attines*. Memoirs of the American Philosophical Society. Philadelphia, Pennsylvania, USA. 146 pp.

DIVERSIDAD Y EVOLUCIÓN DE LA FUNGICULTURA DE LAS HORMIGAS CULTIVADORAS DE HONGOS (FORMICIDAE: MYRMICINAE: ATTINI)

Jorge Víctor Maurice-Lira, Jesús Romero-Nápoles, Jesús Pérez-Moreno, Simón Morales-Rodríguez, Karla Yolanda Flores-Maldonado, Ariel Wilbert Franco-Guzmán y Héctor González-Hernández

RESUMEN

La fungicultura de las hormigas pertenecientes a la tribu Attini (Formicidae: Myrmicinae) se clasifica en cinco sistemas con características distintivas. El sistema más avanzado es el de las "Attini superiores cortadoras de hojas", seguido de las "Attini superiores no cortadoras de hojas". Ambos cultivan clados de la especie *Leucoagaricus gongylophorus* (Möller) (Basidiomycota: Agaricales: Agaricaceae). Los sistemas posteriores, conocidos como "fungicultura menor", "fungicultura de levaduras" y "fungicultura de hongos tipo coral", están menos avanzados. En todos los casos, salvo en el de los hongos coralinos, se cultivan hongos leucocoprináceos estrechamente relacionados con *L. gongylophorus* y hongos de vida libre. Sin embargo, estos sistemas varían en el nivel de especialización entre los hongos y sus hormigas simbióticas. Los cultivares de Attini superiores son simbiontes obligados, lo que significa que el hongo cultivado

apenas sobrevivirá fuera del mutualismo, mientras que los hongos cultivados en fungicultivos inferiores son simbiontes facultativos. Los hongos cultivados en el sistema de fungicultura de tipo coral pertenecen a tres especies del género *Myrmecopterula* (Agaricales: Pterulaceae), que son totalmente diferentes de los hongos leucocoprináceos. Las características de cada sistema de fungicultura varían en función de la filogenia de las hormigas y sus cultivares. Entender la diversidad de hongos cultivados por las hormigas Attini y la complejidad de sus sistemas fungiculturales permitirá dilucidar uno de los procesos coevolutivos más exitosos entre hormigas y hongos en la historia evolutiva. El objetivo de esta revisión fue discutir el conocimiento actual sobre la fungicultura desarrollada por las hormigas Attini, así como el nivel de especialización entre los hongos cultivados y su relación con la coevolución cronológica de Attini.

DIVERSIDADE E EVOLUÇÃO DA FUNGICULTURA EM FORMIGAS CULTIVADORAS DE FUNGOS (FORMICIDAE: MYRMICINAE: ATTINI)

Jorge Víctor Maurice-Lira, Jesús Romero-Nápoles, Jesús Pérez-Moreno, Simón Morales-Rodríguez, Karla Yolanda Flores-Maldonado, Ariel Wilbert Franco-Guzmán e Héctor González-Hernández

RESUMO

A fungicultura das formigas pertencentes à tribo Attini (Formicidae: Myrmicinae) é categorizada em cinco sistemas com características distintas. O sistema mais avançado é o da "Attini superior cortadora de folhas", seguido pela "Attini superior não cortadora de folhas". Ambos cultivam clados da espécie *Leucoagaricus gongylophorus* (Möller) (Basidiomycota: Agaricales: Agaricaceae). Os sistemas subsequentes, conhecidos como "fungicultura menor", "fungicultura de levedura" e "fungicultura de fungos do tipo coral", são menos avançados. Em todos os casos, com exceção dos fungos de coral, são cultivados fungos leucocoprináceos intimamente relacionados ao *L. gongylophorus* e fungos de vida livre. Entretanto, esses sistemas variam no nível de especialização entre os fungos e suas formigas simbióticas. Os cultivares de Attini superiores são simbiontes obrigatórios, o que significa que o fungo cultivado di-

ficilmente sobreviverá fora do mutualismo, enquanto os fungos cultivados em fungiculturas inferiores são simbiontes facultativos. Os cultivares no sistema de fungicultura de fungos do tipo coral pertencem a três espécies do gênero *Myrmecopterula* (Agaricales: Pterulaceae), que são totalmente diferentes dos fungos leucocoprináceos. As características de cada sistema de fungicultura variam de acordo com a filogenia das formigas e seus cultivares. A compreensão da diversidade de fungos cultivados pelas formigas Attini e a complexidade de seus sistemas de fungicultura elucidarão um dos processos coevolutivos mais bem-sucedidos entre formigas e fungos na história evolutiva. O objetivo desta revisão foi discutir o conhecimento atual sobre a fungicultura desenvolvida pelas formigas Attini, bem como o nível de especialização entre os fungos cultivados e sua relação com a coevolução cronológica das Attini.