





Genomic Signatures of Domestication in a Fungus Obligately Farmed by Leafcutter Ants

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Abstract

The naturally selected fungal crop (*Leucoagaricus gongylophorus*) farmed by leafcutter ants shows striking parallels with artificially selected plant crops domesticated by humans (e.g. polyploidy, engorged nutritional rewards, and dependence on cultivation). To date, poorly resolved *L. gongylophorus* genome assemblies based on short-read sequencing have constrained hypotheses about how millions of years under cultivation by ants shaped the fungal crop genome and potentially drove domestication. We use PacBio HiFi sequencing of *L. gongylophorus* from the leafcutter ant *Atta colombica* to identify 18 putatively novel biosynthetic gene clusters that likely cemented life as a cultivar (e.g. plant fragment degradation, ant–farmer communication, and antimicrobial defense). Comparative analyses with cultivated and free-living fungi showed genomic signatures of stepwise domestication transitions: (i) free-living to ant-cultivated: loss of genes conferring stress response and detoxification; (ii) hyphal food to engorged nutritional rewards: expansions of genes governing cellular homeostasis, carbohydrate metabolism, and siderophore biosynthesis; and (iii) detrital provisioning to freshly cut plant fragments: gene expansions promoting cell wall biosynthesis, fatty acid metabolism, and DNA repair. Comparisons across *L. gongylophorus* fungi farmed by 3 leafcutter ant species highlight genomic signatures of exclusively vertical clonal propagation and widespread transposable element activity. These results show how natural selection can shape domesticated cultivar genomes toward long-term ecological resilience of farming systems that have thrived across millennia.

Introduction

Crop domestication involves coevolutionary processes where selectively propagated individuals are actively planted and cultivated in protected growth environments (Purugganan 2022). Across generations, the stringent directional selection imposed by farmers can yield crop adaptations that promote productivity and nutritional optimization while also causing genomic changes that preclude a free-living existence (Araki et al. 2007). Domesticated genomes can thus differ markedly from those of free-living ancestors, with fixation of advantageous alleles (Tenaillon et al. 2023), reduced genetic diversity (Fornasiero et al. 2022), epigenetic changes (Cao et al. 2023), and polyploidy (Wang et al. 2022). While domesticated genome evolution is usually studied in the plant crops that humans artificially selected over the past 10,000 years, there also exist lineages of fungi obligately farmed—and potentially domesticated—by insects over

millions of years (Mueller et al. 2005). We propose that these independent fungicultural origins can provide important insights into whether and how natural selection can drive crop domestication.

Consider the fungus-farming “attine” ants (tribe: Attini) that emerged as the planet’s earliest known farmers over 60 million years ago in the rainforests of South America (Branstetter et al. 2017). In small colonies of perhaps tens to hundreds of workers, the attine ants adopted a lineage of Basidiomycetes fungus that they provisioned with tiny bits of detritus (e.g. insect frass), protected from environmental stress, consumed for food, and vertically transmitted to daughter colonies (Schultz and Brady 2008). These early farming lineages are represented today by the palaeoattine and lower neoattine ants whose fungi generally provide their farmers with undifferentiated hyphae as the food source (Möller 1893; Mueller et al. 2001; Mueller et al. 2005). As the attines spread across

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the Americas, their fungal cultivars acquired domesticated traits that coincided with stepwise transitions toward incrementally greater farm size and complexity in the higher neotattine clade with up to thousands of workers and the crown group leafcutter ants with up to millions of workers (Shik et al. 2014; Branstetter et al. 2017).

Besides a few exceptions, all leafcutter ants are assumed to farm the fungal species *Leucoagaricus gongylophorus*, and we hypothesized that its unique genomic adaptations set the stage for the genus *Atta* to become ecologically important herbivores across diverse ecosystems from southern Argentina to northern Texas (Mueller et al. 2011; Branstetter et al. 2017). These adaptations are expressed most prominently in gongylidia (Aylward et al. 2013; De Fine Licht et al. 2014), the swollen hyphal cells that are otherwise unique in the fungal kingdom and provide both nutritional rewards for ant farmers (Martin et al. 1969; Quinlan and Cherrett 1979; Bolander et al. 2023; Leal-Dutra et al. 2023) and enzymes that ants vector unharmed in fecal droplets to promote degradation and detoxification of provisioned plant material (De Fine Licht et al. 2010; De Fine Licht et al. 2013; Schiøtt and Boomsma 2021). To date, explorations of genomic changes underlying such domesticated traits have been constrained because we have lacked high-resolution genomic resources to delimit syntenic regions and for detecting genetic and structural variations potentially responsible for enabling *L. gongylophorus* to convert plant material that ants cannot eat into a nutritionally optimized and readily digestible diet.

This knowledge gap owes mainly to the genetic complexities of the *L. gongylophorus* because each cell contains up to 17 heterokaryotic nuclei (Kooij et al. 2015; Carlson et al. 2017). This functional polyploidy combined with limited accuracy of existing genomes has precluded studies of changes in genome synteny as well as structural variation within haplotypes. For instance, the short-read *L. gongylophorus* genome of Aylward et al. (2013) isolated from a Panamanian colony of *Atta cephalotes* provides the most current and best cited genome for this fungal species (Floudas et al. 2015; Dentinger et al. 2016; Nygaard et al. 2016; Guo et al. 2023; Martiarena et al. 2023). While the Aylward et al. (2013) genome was the state of the art at the time of its publication, it provides an incomplete and fragmented assembly that cannot delimit key features of genomic change. At an even more basic level, various analytical methods do not even agree on the size of the *L. gongylophorus* genome (Kooij and Pellicer 2020). As a case in point, a second short-read-based assembly of *L. gongylophorus* from a Mexican *Atta mexicana* colony (accession: GCA_022457215.2) was recently deposited on GenBank (Castillo-Alfonso et al. 2024), but it yielded an assembly 1.5 times larger (152 Mb) than the Aylward et al. (2013) assembly (101 Mb).

Moreover, transposable elements (TEs) are thought to promote domestication in human domesticated crops since they can generate useful genomic novelty through altered coding sequences (CDS), chromosome rearrangements, and modified gene regulatory networks (Bourque et al. 2018;

Thieme and Bucher 2018; Muszewska et al. 2019). It was thus intriguing that Clutterbuck (2017) used the Aylward et al. (2013) assembly to suggest that the *L. gongylophorus* genome is also enriched in TEs due to high levels of CpG depletion. Yet, this TE hypothesis remains inconclusive because the sequence data generated by Aylward et al. (2013) do not span complete repeat regions and thus incorrectly resolve the TE landscape by collapsing highly repetitive regions. Current genomic inference thus relies on sequencing data that are too fragmented and incomplete to accurately delimit genomic complexities that have arisen during the domestication process.

In the present study, we aim to resolve the issues of assembly fragmentation and completeness using PacBio HiFi long-read sequencing to produce a high-quality, annotated genome assembly of *L. gongylophorus* from a Panamanian colony of the leafcutter ant, *Atta colombica*. We then harnessed this assembled genome to investigate 4 hypotheses: (i) the genome of *L. gongylophorus* is enriched with TEs; (ii) the ancestral shift toward a mutualistic lifestyle farmed by ant colonies was facilitated by the adaptive evolution or exaptive elaboration of novel biosynthetic gene clusters (BGC); (iii) the major domestication transitions were marked by high levels of gene gain and loss; and (iv) the absence or reduced gene flow and sexual recombination within and across populations of vertically transmitted *L. gongylophorus* clones generates divergent genomic lineages that are highly polymorphic and structurally variable.

Results

High-Quality Genome Assembly

Our long-read genome assembly of *L. gongylophorus* (Ac2012-1) yielded a more contiguous and complete assembly than earlier genomes (Table 1) and enabled a more robust estimate of genome size. From ca. 206,000 HiFi reads (2.47 Gbp) of up to 50 kb length, we generated an initial draft genome of 187.09 Mb with 1,217 contigs (maximum length of 7.91 Mb), 34.51% of GC content and an N50 of 2.18 Mb (supplementary file S1, Supplementary Material online). To reduce the duplication level likely caused by the heterozygosity and polyploidy, we collapsed redundant contigs using Redundans (see Methods) to create a final consensus assembly that we used in the downstream analyses. This final assembly was 74.31 Mb with 39 scaffolds (40 contigs; maximum length of 7.91 Mb), 37.32% of GC content, and a N50 of 6.12 Mb. The genome completeness analysis of the final Ac2012-1 assembly using BUSCO found 89.6% complete Agaricales orthologs, of which 88.1% were single copy and 1.5% were duplicated (Table 1). This assembly exceeded the completeness of the previously assembled cultivars of *A. cephalotes* (73.8% complete Agaricales orthologs) and *A. mexicana* (61.6% complete Agaricales orthologs). Additionally, 4 scaffolds presented telomeric repeats in both ends, while in other 12 scaffolds, these repeats were recovered in only 1 terminus, and no telomeric region was found in the remaining 23 scaffolds.

Table 1 Detailed assembly statistics and BUSCO results for the 3 *L. gongylophorus* assemblies used in this study

	Ac2012-1 v1 ^a	Ac2012-1 final	<i>A. cephalotes</i>	<i>A. mexicana</i>
Assembly statistics				
Largest contig	7,914,281	7,914,281	100,988	109,882
No. of contigs	1217	39	92,785	102,013
Length	187,088,526	74,308,424	101,584,475	152,532,755
No. of contigs ≥ 10 kb	1211	39	346	2222
Length ≥ 10 kb	187,038,405	74,308,424	4,724,447	44,268,675
GC (%)	37.54	37.32	35.03	36.09
N50	2,178,070	6,119,402	2096	5214
L50	22	6	11,283	5662
N90	39,212	2,779,556	699	1136
L90	496	13	43,004	30,597
BUSCO (Agaricales odb: 3,870 orthologs)				
Complete	3,506 (90.6%)	3,467 (89.6%)	2,856 (73.8%)	2,384 (61.6%)
Single copy	99 (2.6%)	3,410 (88.1%)	2,850 (73.6%)	2,334 (60.3%)
Duplicated	3,407 (88.0%)	57 (1.5%)	6 (0.2%)	50 (1.3%)
Fragmented	67 (1.7%)	76 (2.0%)	338 (8.7%)	445 (11.5%)
Missing	297 (7.7%)	327 (8.4%)	676 (17.5%)	1,041 (26.9%)

^aComplete assembly before running through Redundans.

Extensive TE Abundance in the *L. gongylophorus* Genome

Our results support the hypothesis of a high TE content as our long-read genome of *L. gongylophorus* has a total TE content of 67.78% of total genome size (50.36 Mb), with 633 distinct TE classifications. The genome is dominated by retrotransposons, with LTR elements and LINEs comprising 32.3% (24.01 Mb) and 13.1% (9.73 Mb) of total genome size respectively (supplementary fig. S1, table S1, and file S2, Supplementary Material online). Unclassified elements comprise 22.2% of total genome size (16.53 Mb), which can be partially attributed to the general lack of TE surveys in the fungi kingdom. There are few DNA repeats (0.1%), with a complete absence of helitrons (also termed rolling circles) and SINEs, although 1 family of each was found in the pre-Redundans assembly (supplementary fig. S1 and file S2, Supplementary Material online).

Functional Annotation Highlights Novel Metabolic Insights

Analysis with the Funannotate pipeline (Palmer and Stajich 2020) showed that our long-read assembly contained 8,298 gene models, representing 85 tRNA and 8,213 CDS from which 7,511 were assigned with at least 1 functional trait. These gene models provided the basis for further analyses that identified syntenic genes forming BGCs using the fungiSMASH webserver with AntiSMASH v.7.1.0 (Blin et al. 2023).

From this, we detected 20 BGCs, including 2 terpenes with high similarity to (+)- δ -cadinol and 18 that are putatively novel (53 in the pre-Redundans assembly; see supplementary file S3, Supplementary Material online). Within these BGCs, we identified 5 terpenes, 5 NRPS-like, 3 fungal-RiPP-like, 2 indoles, 2 T1PKS, and 1 NI-siderophore. The secondary metabolites produced by these BGCs likely have diverse functions, including communication, antimicrobial activity, and iron uptake (Bingle et al. 1999; Carroll and Moore

2018; K ues et al. 2018). In contrast, the same analysis performed on the 2 short-read *L. gongylophorus* assemblies provided far lower resolution, revealing only 5 gene clusters (*A. cephalotes* isolate) or 1 cluster (*A. mexicana* isolate) (supplementary file S3, Supplementary Material online). Therefore, our assembly improves the resolution of BGC inference and highlights novel molecular mechanisms and evolutionary processes possibly involved in fungal domestication by leafcutter ants.

Linking Domestication Transitions and Genome Evolution

We first reconstructed a consensus phylogenomic tree that resolved relationships between our assembly and those of 7 closely related fungal genomes which were publicly available on NCBI database (Fig. 1; supplementary table S2, Supplementary Material online). We based the tree on the alignment of 527,465 amino acids from 1,136 BUSCO orthologs (of 3,870 total) that were present as complete and single copy in all genomes analyzed. As expected, this analysis recovered the 3 *L. gongylophorus* isolates as a sister clade to *Leucoagaricus* sp. from a colony of the higher neotattine *Trachymyrmex arizonensis* (Fig. 1). The tree also indicates that attine-farmed fungi are polyphyletic, supporting previous phylogenies inferred from only a single protein encoding gene plus 4 ribosomal markers (Mehdiabadi et al. 2012; Mueller et al. 2018). Specifically, a clade with 2 free-living *Leucoagaricus* species (*L. leucothites* and *L. birnbaumii*) separates the fungal mutualist of the lower neotattine *Cyphomyrmex costatus* from the other fungal mutualists (Fig. 1).

We next performed a gene gain and loss analysis to explore genetic modifications during 3 domestication transitions: (i) from free-living to ant-cultivated, (ii) from hyphal food to engorged nutritional rewards in the higher neotattine clade, and (iii) from detrital provisioning to plant fragment provisioning in the leafcutter clade (Fig. 1).

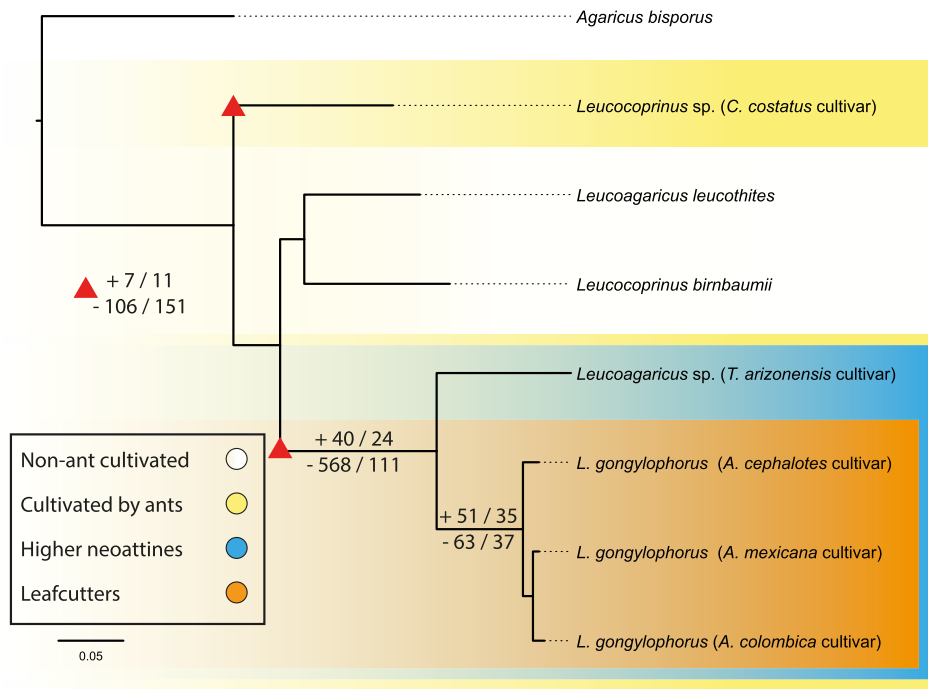


Fig. 1. Maximum-likelihood phylogeny showing domestication transitions in ant-farmed and closely related free-living fungi. The tree was reconstructed from 1,136 amino acid sequences from single-copy orthologs shared by all samples. Domestication transitions indicated by colored boxes: (i) the outermost box (yellow) including all the cultivars: from free-living to ant-cultivated (polyphyletic); (ii) the middle box (blue) including *Leucoagaricus* sp. and *L. gongylophorus*: from hyphal food to engorged nutritional rewards (higher neoattines transition); and (iii) the innermost box (orange) including only *L. gongylophorus*: from detrital provisioning to freshly cut plant fragments (leafcutters transition). Gene gain/expansion (+) and gene loss/contraction (–) are represented for each of these transitions (indicated by the red triangle for the transition to ant-cultivated species). All branches received maximum UFBoot support (100%). Scale bar: amino acid substitutions per site.

The transition to ant cultivation coincided with substantial gene losses relative to non-ant-cultivated fungal clades since 106 orthogroups were lost and 151 gene families were reduced, while only 7 orthogroups were gained and only 11 gene families were expanded (Fig. 1). The domestication transition to specialized nutritional rewards (higher neoattine-leafcutter clade vs. all other clades) coincided with further genetic losses, with 568 orthogroups lost and 111 gene families being reduced and with only 40 orthogroups being gained and 24 gene families expanded. In contrast, the transition to fresh-vegetation provision by leafcutters was associated with less-pronounced genomic changes since similar numbers of orthogroups were lost (63) and gained (51) and similar numbers of gene families were reduced (37) and expanded (35).

We next explored which functions were gained (or expanded) and lost (or contracted) across each domestication transition by performing a functional enrichment analysis within these orthologs (Fig. 2; supplementary file S4, Supplementary Material online). Some genomic trends were consistent across all ant-farmed fungi, including the loss or contraction of genes conferring stress response and detoxification and the gain of genes for macroautophagy and transposition of TEs (Fig. 2a). Subsequent genetic gains or expansion was specific to either the transition to higher neoattines (cellular homeostasis, carbohydrate metabolism, and siderophore biosynthetic pathway; Fig. 2b) and the

transition to leafcutters (cell wall biosynthesis and organization, fatty acid metabolism, and several processes for DNA repair; Fig. 2c). Losses or contraction of gene functions showed similar stepwise changes as the higher neoattine fungal clade lost genes related to carbohydrate-mediated transcription and signaling (Fig. 2b), and the leafcutter fungal clade lost genes related to demethylation, catabolizing certain proteins and macromolecules, and countering osmotic stress (Fig. 2c). These incremental and directional functional gene increases and reductions thus appear to reflect the novel selection pressures encountered across successive domestication transitions.

Genome Evolution under Vertically Transmitted Propagation

While *L. gongylophorus* is considered a single species that is farmed by most species of leafcutter ants, we tested whether this lumping assumption may belie genomic divergence among potentially reproductively isolated strains of *L. gongylophorus* cultivated by different leafcutter species. To quantify pairwise differences between the 3 available *L. gongylophorus* assemblies, we first used a *k*-mer analysis by calculating the Jaccard containment index (JCI) of *k*-mer composition for each assembly. This analysis indicates that our *L. gongylophorus* assembly shares only ca. 35.9% of its *k*-mers with the assembly of the same fungus cultivar species

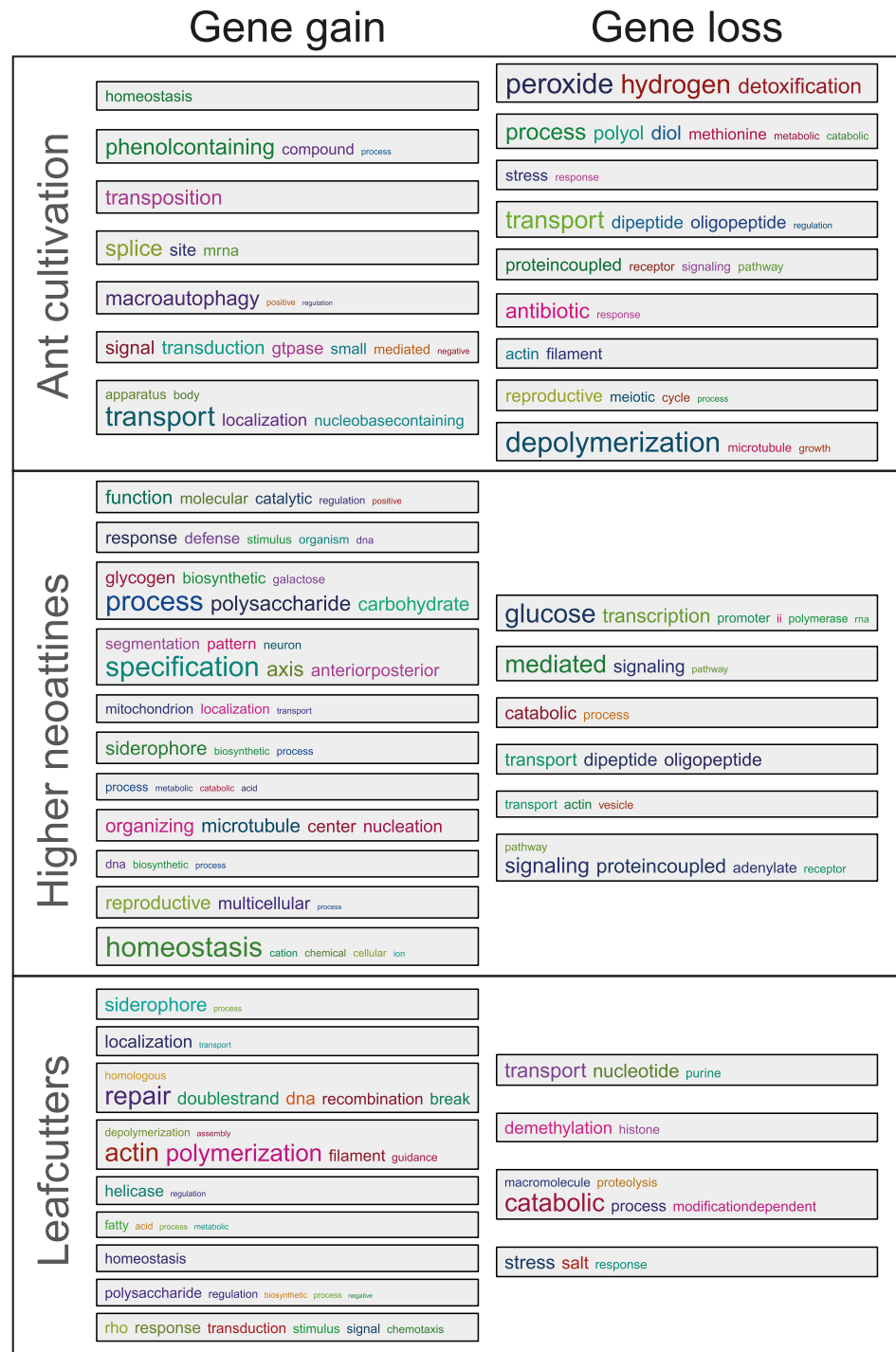


Fig. 2. Word clouds representing the clusters of enriched BP terms from the GO enrichment analysis. Each box clusters significant GO terms from similarity matrices of functional terms corresponding to gene gain (or gene family expansion; left column) and loss (or gene family contraction; right column) through each step of the domestication process noted in Fig. 1.

isolated from of *A. mexicana* and even fewer *k*-mers (ca. 8.4%) with the assembly when isolated from *A. cephalotes* (Fig. 3a). The genomes thus exhibit extensive divergence, reflecting the genetic distance of these vertically transmitted, and thus reproductively isolated, but conspecific fungal lineages.

We note that *k*-mer analyses may overstate levels of evolutionary distance because they conservatively discard all mismatches arising from single or short nucleotide variations such as single nucleotide polymorphisms (SNPs) or insertions or deletions (indels). We thus developed a less conservative “short-read simulation” (SRS) approach that

fragments each assembly to simulate paired-end 150 bp read sequences (PE150) that can then be tested for levels of correspondence when pairwise mapped to each other assembly (see [Methods](#) for more detailed explanation). The SRS test yielded 496,972 fragments from our assembly, 492,852 from the *A. cephalotes* cultivar assembly, and 800,852 from the *A. mexicana* cultivar assembly (Table 2).

We first mapped the fragments of our assembly Ac2012-1 against itself to establish a baseline correspondence level (Fig. 3b). While all the fragments mapped successfully, approximately 32% fragments mapped both “in pairs” and

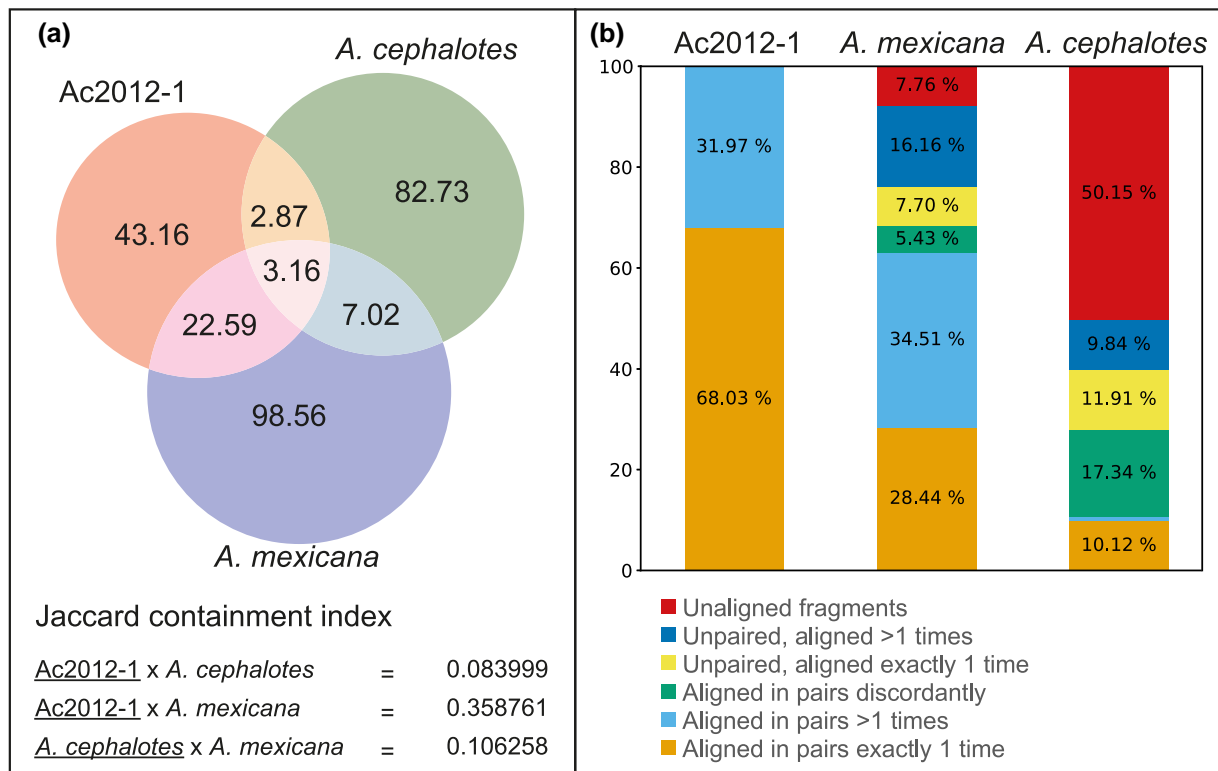


Fig. 3. Genomic similarity comparisons of *L. gongylophorus* assemblies across leafcutter ant species. a) Sourmash k -mer analysis: the Venn diagram displays the number of k -mer ($k = 51$) MinHashes shared between 3 *L. gongylophorus* assemblies, in millions of k -mer MinHashes, alongside their JCI, with the shorter assembly underlined. This analysis indicates that approximately 8.4% and 35.9% of the Ac2012-1 k -mers are found in the genomes of *A. cephalotes* and *A. mexicana*, respectively. The *A. mexicana* cultivar contains approximately 10.6% of k -mers from *A. cephalotes*. b) SRS analysis: the bar chart presents the mapping rate of simulated read fragments from Ac2012-1 aligned to itself and the other reference assemblies. The percentage of fragments aligning to each assembly is color-coded to indicate paired and unpaired alignments, as well as non-alignments. These results suggest large structural variations and a high level of divergence among the assemblies. They also highlight a closer genomic relationship between Ac2012-1 and *A. mexicana* cultivar, reflected by a higher alignment rate, in contrast to the more divergent *A. cephalotes* cultivar.

more than once, possibly reflecting that the genome contains large numbers of TEs and duplicated regions. When we next mapped Ac2012-1 fragments to each other assembly, ca. 7.8% failed to map to the *A. mexicana* assembly and 50.15% failed to map to the *A. cephalotes* assembly. This suggests a high divergence between the assemblies. Additionally, 29.3% of the Ac2012-1 fragment pairs aligned discordantly (i.e. both fragments of the pair aligned but not as a pair) or aligned “unpaired” (i.e. only 1 fragment of the pair) to the *A. mexicana* cultivar, and 39.1% showed the same result when mapped to for the *A. cephalotes* cultivar. This suggests high levels of structural variation (e.g. transposition, duplications, and inversions). Thus, despite high overall levels of genomic divergence, our assembly from *A. colombica* shares more similarities with the geographically distant cultivar of *A. mexicana* than with the cultivar of *A. cephalotes* from the same study locality in Panama.

Discussion

The complex multinucleate genome of *L. gongylophorus* has previously constrained explorations of whether and how continuous directional selection by leafcutter ant farmers has produced a domesticated cultivar. Such inference is of

special interest in this fungus because it exhibits a suite of traits like polyploidy, elaborated nutritional rewards, and obligate dependence on cultivation also seen in plant crops domesticated by humans. In this study, we used PacBio HiFi long-read sequencing to overcome previous analytical issues and produce a highly contiguous megabase-length assembly that opens new perspectives in synteny, TEs, and clusters of coregulated genes and genetic rearrangements across successive domestication transitions. We are further able to confirm the hypothesis that genomic rearrangements such as inversions, duplications, and transpositions set the stage for genetic novelty potentially driving fungal domestication. Moreover, 90% of our consensus assembly is comprised of only 13 scaffolds, and 16 scaffolds present telomeric repeats in at least 1 end, this suggests chromosome-length resolution comparable to the other highest quality available fungal assemblies (e.g. button mushroom *Agaricus bisporus*) (Sonnberg et al. 2016).

TEs Provide Opportunities for Domesticated Trait Evolution

TEs represented around two-thirds of the *L. gongylophorus* genome, which is extremely high for a fungus (Clutterbuck

Table 2 Mapping rate of fragments generated in the SRS analysis

Assemblies	<i>A. colombica</i>	<i>A. cephalotes</i>	<i>A. mexicana</i>
Simulated PE150 fragments from Ac2012-1 from <i>A. colombica</i> (total fragments 496,972)			
Overall fragments alignment rate	100.00%	49.85%	92.24%
Fragments aligned in pairs exactly 1 time	68.03%	10.12%	28.44%
Fragments aligned in pairs >1 times (duplicated)	31.97%	0.68%	34.51%
Fragments aligned in pairs discordantly	0.00%	17.34%	5.43%
Fragments (unpaired) aligned exactly 1 time	0.00%	11.91%	7.70%
Fragments (unpaired) aligned >1 times (duplicated)	0.00%	9.84%	16.16%
Unaligned fragments	0.00%	50.15%	7.76%
Simulated PE150 fragments from <i>A. cephalotes</i> cultivar (total fragments 492,852)			
Overall fragments alignment rate	34.87%	100%	38.61%
Fragments aligned in pairs exactly 1 time	10.45%	84.62%	8.60%
Fragments aligned in pairs >1 times (duplicated)	0.93%	15.38%	5.59%
Fragments aligned in pairs discordantly	15.54%	0.00%	9.20%
Fragments (unpaired) aligned exactly 1 time	4.13%	0.00%	5.83%
Fragments (unpaired) aligned >1 times (duplicated)	3.84%	0.00%	9.39%
Unaligned fragments	64.97%	0.00%	61.39%
Simulated PE150 fragments <i>A. mexicana</i> cultivar (total fragments 800,852)			
Overall fragments alignment rate	76.28%	46.18%	100%
Fragments aligned in pairs exactly 1 time	31.00%	7.91%	27.60%
Fragments aligned in pairs >1 times (duplicated)	9.79%	0.67%	72.40%
Fragments aligned in pairs discordantly	13.58%	12.81%	0.00%
Fragments (unpaired) aligned exactly 1 time	8.35%	13.40%	0.00%
Fragments (unpaired) aligned >1 times (duplicated)	13.56%	11.38%	0.00%
Unaligned fragments	23.72%	53.82%	0.00%

2017; Lebreton et al. 2022). Specifically, 67% of the total TE content was composed of LTRs and LINEs, which have shown high levels of activity throughout the evolutionary history of *L. gongylophorus* but a notable deficiency in recent activity (supplementary fig. S1, Supplementary Material online). This supports an earlier study by Clutterbuck (2017) that indirectly inferred abundant TEs based on CpG depletion levels that are known to silence and immobilize TEs (Gladyshev 2017), which is further supported by the loss of genes related to demethylation that might increase the chances of CpG transitions and fixation of old TEs. This is important because high activity of TEs can set the stage for rapid genome evolution (Raffaele and Kamoun 2012) which has been seen in other domesticated crops of humans (Cai et al. 2022). Abundant TEs are also known in fungal ectomycorrhiza symbioses (Hess et al. 2014; Lebreton et al. 2022).

Given the expansion of genes related to transposition in the first domestication transition (Fig. 2), we further predict that abundant TEs may be a universal feature of all attine-cultivated fungi. Thus, TEs may serve a dual role of promoting genomic variation and driving coevolutionary processes in fungal symbioses. This perspective strengthens the role of TEs in shaping the genomes of symbiotic partners, enhancing the resilience of the symbiosis, and allowing them to thrive in fluctuating environments.

Functional Consequences of Genome Evolution

The annotation made possible by our contiguous assembly not only enhances our understanding of the *L. gongylophorus* genome, but it also enables exploration of potentially novel BGCs and their yet-to-be discovered roles in mutualisms.

Specifically, we identified and annotated 20 BGCs (18 putatively new) with diverse functions. For example, terpenes and indoles are known to mediate fungal communication from the cellular level to interspecific signaling (Kües et al. 2018), potentially enabling the fungus not only to repel fungal competitors or pathogens but also to precisely align its physiological needs with those of the ants. Additionally, some terpenes like (+)- δ -cadinol, and several NRPS, and T1PKS have antifungal and antimicrobial properties (Bingle et al. 1999; Felnagle et al. 2008; Ringel et al. 2022; Vinha et al. 2023). Consequently, these BGCs produce secondary metabolites that could potentially act in colony defense. Furthermore, NI-siderophores may facilitate iron acquisition, essential for Fenton reaction in *L. gongylophorus* fungus gardens known from another leafcutter genus *Acromyrmex* (Schjøtt and Boomsma 2021), allowing the initial breakdown of biomass even when iron is made unavailable by plant-secreted iron chelators (Dellagi et al. 2005; Carroll and Moore 2018). Our high-quality genome assemblies can thus help test hypotheses about evolution of domestication by investigating how metabolic changes potentially favor adaptation and specialization of domesticated symbionts.

Stepwise Genomic Evolution across Domestication Transitions

A specific gene set, shared by all extant cultivars and absent in free-living relatives, was likely a requirement for recruitment for cultivation by ants. In this first step of the domestication process, we highlight the loss of detoxification genes in the cultivars, which could be attributed to the ants' preliminary screening for toxic compounds in the substrate (Herz et al. 2008), which reduces the selective

pressure on the fungi to retain these genes. These results also support the hypothesis that autophagic repurposing of cellular components is important not only for gongylium formation (Leal-Dutra et al. 2023), but likely for other mechanisms in the symbiosis, since autophagy-related genes were gained or expanded across all cultivars.

The evolution of gongylidia as hyphal swellings containing metabolites with multimodal functions was a decisive domestication event in the fungal lineage farmed by higher neotattine and leafcutter ants (Mueller et al. 2001; Schultz and Brady 2008). Gongylidia have clear analogies with enlarged fruits of domesticated plant crops farmed by humans, both for their morphology and nutrient content that would likely be maladaptive in a free-living state and for their polyploid genomic architecture (Alam and Purugganan 2024). Since gongylidia likely ended an avenue for a free-living existence, it is perhaps not surprising that this domestication transition incurred the greatest amount of gene loss (568) we observed. Additionally, among the few gained and expanded gene functions, cellular homeostasis and carbohydrate metabolism might have favored the nutritional stability of gongylidia, while siderophore biosynthesis could support acquisition of iron for Fenton reaction as mentioned before. The loss of genes related to carbohydrate-mediated transcription and signaling may also have facilitated gongylium engorgement since they reduce the cellular response to sugar accumulation (Koch 1996; Jiang et al. 1997) and allow sugar to concentrate without a cellular feedback response.

The *L. gongylophorus* fungus enables the leafcutters to access an herbivorous niche that is otherwise unique among the ants (Hölldobler and Wilson 2011; Shik et al. 2021), and our results help delimit the genomic adaptations that could have enabled this domestication transition. For instance, *L. gongylophorus* is enriched with genes related to cell wall biosynthesis and modification that are upregulated during gongylium formation (De Fine Licht et al. 2014). Fatty acid metabolism genes are also enriched in ways that may produce metabolites that shape ant farming behaviors as exemplified by Khadempour et al. (2021). *Leucoagaricus gongylophorus* is also enriched for DNA repair genes that may be a response to genomic instability caused by the loss of oxidative stress regulatory genes that occurred at the onset of ant cultivation.

Genomic Consequences of Obligate Vertical Crop Transmission

Despite similarities in the assemblies of *L. gongylophorus* samples from 3 different *Atta* colonies observed in the phylogeny using protein encoding genes (Fig. 1), we observed considerable variation at the whole-genome level. This variation likely emanates from the potential reproductive isolation of these fungi since they are propagated vertically as clonal inoculates carried by dispersing foundress queens (Weber 1972; Hölldobler and Wilson 2011; Kooij et al. 2015). This propagation mode has likely promoted founder effects among distinct fungal populations that accumulate

lineage-specific variation (e.g. SNPs, structural variants, and indels). While such variation is expected when comparing allopatric samples from Panama and Mexico, we also observe variation in *L. gongylophorus* farmed sympatrically by 2 different species of *Atta* leafcutter ants. These results strongly support a hypothesis that colonies of different leafcutter ant species are strong barriers to genetic admixture. Further research and the generation of additional high-quality genomes of the attine-cultivated fungi will be crucial to understand the genome evolution patterns across this ant-domesticated fungus.

In conclusion, we use long-read sequencing of the *L. gongylophorus* genome to reveal with novel resolution the intricate genetic changes that accompany each step of fungal domestication, from the initial suitability for ant cultivation to advanced modifications that enabled large-scale leafcutter farming systems. Our findings underscore that fungal genome modifications (e.g. gene gain loss and TE-driven rearrangements) were crucial for expanding the ecological niche and long-term evolutionary stability of leafcutter ants. Moreover, our results provide a robust genomic framework for further research on how domestication can evolve.

Methods

Sample Acquisition

We generated genome sequencing data using *L. gongylophorus* fungus sampled from an *Atta colombica* colony (Ac2012-1) collected from Soberanía National Park, Panama, and maintained at the University of Copenhagen under controlled conditions (25 °C, 70% RH, minimal daylight). We isolated the fungus in axenic cultures in 90 mm diameter Petri dishes with 20 mL potato dextrose agar (PDA) and kept it in the dark at 25 °C. To extract DNA, we optimized fungal biomass production by introducing the PDA-cultivated fungus into 250 mL of potato dextrose broth. This was agitated at 140 rpm in the dark at 25 °C. After 30 d, the mycelia were sieved using a nylon mesh, rinsed with sterile distilled water, and freeze-dried for 48 h.

Available Genomic Data

To evaluate the sequence and structure of our genome assembly, we used 2 publicly available short-read genome assemblies of *L. gongylophorus* from *Atta cephalotes* (Aylward et al. 2013) and *Atta mexicana* [Castillo-Alfonso et al. (2024) for details] colonies for comparisons. We further included 5 other genome assemblies to perform a gene gain and loss analysis across the phylogeny: 1 fungal cultivar from a lower neotattine, *C. costatus* (Nygaard et al. 2016); 1 fungal cultivar from a non-leafcutter higher neotattine, *T. arizonensis* (Beigel et al. 2021); and 3 from non-ant-cultivated fungal species, *A. bisporus* (Morin et al. 2012), *L. birnbaumii* (unpublished, GenBank GCA_027627405), and *L. leucothites* (Floudas et al. 2020). Details about each of these additional genome assemblies are provided in [supplementary table S1, Supplementary Material](#) online.

DNA Extraction and Sequencing

High molecular weight DNA was extracted from fungal tissue using the CTAB-based lysis and precipitation protocol of Jones and Schwessinger (2021). Briefly, about 150 mg of freeze-dried mycelia was ground in liquid nitrogen and re-suspended in 10 mL of lysis buffer (4% CTAB, 1% PVP-40, 100 mM Tris, 20 mM EDTA, 1.2 M NaCl, pH 8) along with RNase A and proteinase K, both at 200 µg per mL. The mixture was placed in a rotating mixer at 10 rpm and incubated at 60 °C for 90 min. These samples were then purified using phenol:chloroform:isoamyl alcohol (25:24:1) followed by an additional purification step with an equal volume of chloroform:isoamyl alcohol (24:1). The DNA was then precipitated by incubating the supernatant with 2 volumes of precipitation buffer (2% CTAB, 100 mM Tris, 20 mM EDTA, pH 8) and rotating at 60 °C for 2.5 h. This mix was centrifuged and the resulting DNA pellet washed with 80% ethanol and centrifuged again. After air-drying, the pellet was resuspended in 150 µL of 10 mM Tris-HCL (pH 8). All centrifugation steps were performed at the speed and temperature guidelines provided by Jones and Schwessinger (2021).

Extracted samples were sent for long-read PacBio HiFi sequencing at the Beijing Genomics Institute (BGI, Hong-Kong, China). A PacBio SMRTbell library was prepared using the SMRTbell Template Prep Kit 2.0 and Sequel II Binding 2.2 (PacBio, CA, USA) and loaded onto the sequencer by diffusion. The sequencing was conducted on a Sequel II platform (PacBio, CA, USA), employing a Sequel II Sequencing Kit 2.0 and a SMRT Cell 8 M Tray, and managed by the instrument control software and the Primary SW (both v.10.1.0.119549). HiFi reads were generated using the *Circular Consensus Sequencing* workflow (CCS) v.6.4.0 (Wenger et al. 2019) with default parameters. Sequencing statistics were performed using Samtools v.1.16 (Danecek et al. 2021).

Genome Assembly

An initial genome assembly was performed using the HiCanu algorithm of Canu software v.2.2 (Koren et al. 2017; Nurk et al. 2020) with default settings and genome size of 40 Mbp, as previously reported by Kooij and Pellicer (2020). Assembly quality and completeness were assessed using QUAST v.5.2.0 (Gurevich et al. 2013) and BUSCO v.5.4.5 (Manni et al. 2021) with the *agaricales_odb10* database, and telomeres were located using *find_telomeres.py* (available at https://github.com/markhilt/genome_analysis_tools).

We next used Redundans v.1.01 (Pryszcz and Gabaldón 2016) to reduce the redundancy of our assembly that generates polymorphic duplicated contigs due to heterozygosity. After testing multiple parameter combinations and assessing the resulting assemblies using BUSCO—while assigning arbitrary weights to single copy versus duplicates, completeness, and assembly size (supplementary table S3, Supplementary Material online)—we set Redundans to collapse shorter contigs with over 82% similarity and a

minimum of 75% overlap with longer contigs. With the final assembly, we identified and removed mitochondrial contigs using MitoFinder v.1.4.1 (Allio et al. 2020).

TE Prediction and Annotation

Next, we curated and annotated TEs in *L. gongylophorus* using the Earl Grey TE annotation pipeline (v.3.0) with default parameters (Baril et al. 2023, 2024). Briefly, de novo consensus sequences were initially identified and subsequently refined in Earl Grey using a “BLAST, Extract, Extend, Trim” process (Platt et al. 2016). Following final annotation, Earl Grey processes TE loci to remove overlapping annotations and to defragment annotations likely originating from the same TE insertion event. The TE consensus library and GFF annotation file are provided in supplementary file S2, Supplementary Material online.

Functional Annotation

The Ac2012-1 genome annotation was performed using the Funannotate pipeline v.1.8.13 aided by the transcriptome and RNAseq reads from the same fungus isolate published in previous study (Leal-Dutra et al. 2023; Leal-Dutra and Shik 2023). This process was done with default Funannotate settings. It incorporated several ab initio gene prediction training tools, including Augustus v3.3.1 (Stanke et al. 2008), CodingQuarry v1.3 (Testa et al. 2015), GeneMark-ES/ET v4.71 (Lomsadze et al. 2014), glimmerHMM v3.0.4 (Majoros et al. 2004), Snap v2006-07-28 (Korf 2004), and tRNAscan-SE v2.0.11 (Chan et al. 2021). These tools utilized splicing information generated by PASA v2.3.3 (Haas et al. 2003). Finally, EvidenceModeler v1.1.1 (Haas et al. 2008) was used to generate consensus gene models. For further functional annotations, Funannotate employed InterProScan v5.55-88.0 (Jones et al. 2014), EggNOG Mapper v2.1.1 (Cantalapiedra et al. 2021), AntiSMASH v7.0.1 (Blin et al. 2023), SignalP v5.0b (Almagro Armenteros et al. 2019), Phobius v1.1 (Käll et al. 2004), and DIAMOND v2.1.6.160 (Buchfink et al. 2021). Functional predictions were assigned by matches to the CAZy (Lombard et al. 2013), InterPro (Paysan-Lafosse et al. 2022), MEROPS (Rawlings et al. 2014), Pfam (Finn et al. 2013), and Swiss-Prot databases (The UniProt Consortium 2022).

The final assembly and annotation of *L. gongylophorus* Ac2012-1 were deposited at NCBI GenBank under the BioProject accession PRJNA879936. Assembly and annotation of the pre-Redundans version of this genome can be found in supplementary file S1, Supplementary Material online.

Phylogeny Reconstruction

We produced a phylogenomic tree to confirm the placement of our Ac2012-1 assembly within the public genomes using an amino acid data set from 1,136 single-copy orthologs shared by the assemblies. We identified these orthologs using BUSCO as described above. To prepare the data set, we first created individual fasta files for each ortholog and aligned the sequences using MUSCLE v.3.8.425 (Edgar 2004) with default parameters. Next, we trimmed each alignment with trimAl

v.1.4.rev15 (Capella-Gutiérrez et al. 2009) using a heuristic selection to decide the optimal method for trimming (setting option -automated1). All the trimmed alignments were concatenated, and a RAxML-style partition file was created. Finally, we used IQ-TREE v.2.1.3 (Minh et al. 2020) to reconstruct a maximum-likelihood phylogenomic tree, first using the built-in ModelFinder (Kalyaanamoorthy et al. 2017) allowing the program to merge partitions to increase model fit followed by tree inference with 1,000 ultrafast bootstraps (UFBoot) (Hoang et al. 2018).

Gene Gain/Loss and Gene Family Expansion/Contraction Analyses

To analyze gene gain or loss and gene family expansion or contraction, we first used the phylogeny reconstructed in this study to delimit the clades into 3 distinct groups, each representing a step in the domestication transition (Fig. 1). We then performed the analyses for each of these groups. First, we looked at the transition from free-living to cultivation by ants, comparing non-ant-cultivated fungi with all cultivars (including the cultivar of the lower neotattine *C. costatus* and the clade of the higher neotattines that includes the cultivar of *T. arizonensis* and the 3 *L. gongylophorus*; yellow box in Fig. 1). Then, we focused on the transition from hyphal food to engorged nutritional rewards, comparing the clade of the higher neotattines cultivars (blue box in Fig. 1) with the free-living and lower attine cultivar. And finally, we tested the transition from detrital provisioning to freshly cut plant fragments, comparing only the *L. gongylophorus* clade (orange box in Fig. 1) with the remaining samples.

It is worth noting that the cultivar of a lower attine, *Mycocepurus smithii*, can produce gongylidia-like structures (Masiulionis et al. 2014). However, it remains unclear whether these gongylidia-like structures are developmentally homologous to the gongylidia produced by the higher neotattine cultivar clades. Due to the lack of a genome sequence for the cultivar of *M. smithii*, we constrain our analyses regarding the appearance of gongylidia to the higher neotattines clade.

We used the protein sequences predicted in all the studied assemblies to assess the number of clade-specific genes across the phylogeny. First, we used Orthofinder v2.5.4 (Emms and Kelly 2019) to infer orthology and set the program to search for sequences using ultra-sensitive DIAMOND, split paralogous clades, and infer maximum-likelihood trees from multiple sequence alignments. The resulting phylogenetic hierarchical orthogroups were further explored to categorize them into single-copy orthogroups (those with 1 copy in at least 75% of the genomes), multi-copy orthogroups (1 or more copies in at least 75% of the genomes, and at least 25% of the genome with 2 or more copies), and taxon-specific orthogroups (present in a single clade or assembly). We also functionally annotated each orthogroup using InterProScan and eggNOG-mapper, setting the filter threshold of bit score to 40 and percentage of identity, query cover, and subject cover to 20%.

We used the inferred orthogroups to retrieve those that are associated with each group of clades, representing different steps in the domestication process. We specifically performed a strict gain/loss analysis to identify group-specific orthogroups that comprise (i) putatively new genes that are only present among all genomes in each group and absent in the other clades and (ii) putatively lost genes that are absent in each group, but present in all the other clades. We also identified gene family expansion and contraction by comparing the number of gene copies present in the species in each group against the remaining clades. We finally performed a gene ontology (GO) enrichment analysis using GO gene sets with biological process (BP) ontology within various orthogroups to highlight the overrepresented functions in genes gained or expanded and lost or reduced across the 3 domestication steps using R package TopGO (Alexa and Rahnenfuhrer 2023). To visualize these enrichments, we used simplifyEnrichment (Gu and Hübschmann 2023) to cluster the significant GO terms with similarity matrices of functional terms and produce a word cloud with the common BP ontology shared by genes within these clusters. It is important to note that although we used a yeast database to assign GO terms to the orthogroups, several terms are derived from animal studies, reflecting the current gaps in our understanding of Agaricales gene functions. Hence, these genes should be further investigated in experimental studies.

Assessing Sequence Similarity across *L. gongylophorus* Assemblies

To evaluate the genomic similarities among the 3 *L. gongylophorus* assemblies, we employed a 2-pronged approach: first, a stringent Jaccard containment-based *k*-mer composition analysis, and second, a more relaxed alignment-based analysis. This dual methodology was chosen to provide a comprehensive and nuanced understanding of the genomic similarities.

The first approach, using the Jaccard containment-based *k*-mer composition analysis, was conducted with Sourmash v.4.8.2 (Brown and Irber 2016). In this method, *k*-mers ($k = 51$) were generated from each genome assembly to create MinHash sketches. These sketches were then utilized to calculate similarity using the JCI. This index is particularly effective for comparing genomes of varying quality and size, as it considers the proportion of shared *k*-mers in relation to the size of the smaller genome set (Pierce et al. 2019). This provides a robust similarity measure, accommodating the differences in the genome assemblies' quality. In this analysis, pairwise containment scores, which ranged from 0 to 1, were compiled for further evaluation. A score of 1 signifies a high degree of similarity, indicating that all 51-mers in the smaller genome were found in the larger genome.

However, considering the unique characteristics of *L. gongylophorus*, which is both polyploid and highly heterozygous, the *k*-mer-based approach has limitations. It restricts similarity searches to exact *k*-mer matches, lacking the capacity to account for nucleotide-level variations

such as base substitutions. To address this, we developed a more lenient method that assesses sequence similarities while ignoring small sequence variations. This involved aligning small fragments from each assembly to the other *L. gongylophorus* assemblies. To circumvent potential misalignments of large contigs that might occur using whole-genome alignment tools, and due to the absence of short reads associated with these assemblies, we evaluated assembly similarities by simulating paired-end 150 bp reads (PE150) derived from each genome. For this, each contig was segmented into 600 bp fragments, further divided into two 450 bp inserts with a 300 bp overlapping region. The initial and final 150 bp of each insert were allocated to the left and right reads files, respectively, with the final 150 bp reverse complemented (supplementary fig S2, Supplementary Material online). We then used Bowtie2 v.2.5.0 (Langmead and Salzberg 2012) to align these short sequences with the assemblies using the default parameters. Since Bowtie2 utilizes an alignment scoring system, which accommodates nucleotide-level variations (e.g. SNPs) and structural variations (e.g. indels, duplications, and inversions), this method provided a more relaxed comparison, capturing lineage-specific mutations.

Therefore, by using both the stringent *k*-mer-based approach and a more relaxed alignment-based analysis, we benefit from their complementary strengths. The *k*-mer-based approach provides a precise and stringent measure of similarity, especially useful when dealing with genomes of different sizes and quality. On the other hand, the alignment-based analysis offers a broader, more inclusive assessment, capturing more general similarities that the stringent method might overlook. Together, these methods enable a more holistic and accurate assessment of the genomic similarities among the *L. gongylophorus* assemblies.

Supplementary Material

Supplementary material is available at *Molecular Biology and Evolution* online and at <https://doi.org/10.17894/ucph.e9980bed-a075-46c8-bc65-5ee3c1a4c2d8>.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

Final assembly and annotation were deposited in NCBI GenBank under the BioProject accession PRJNA879936, which can also be found together with all Supplementary Material at <https://doi.org/10.17894/ucph.e9980bed-a075-46c8-bc65-5ee3c1a4c2d8> (Leal-Dutra et al. 2024).

References

- Alam O, Purugganan MD. Domestication and the evolution of crops: variable syndromes, complex genetic architectures, and ecological entanglements. *Plant Cell*. 2024;**36**(5):1227–1241. <https://doi.org/10.1093/plcell/koae013>.
- Alexa A, Rahnenfuhrer J. topGO: enrichment analysis for gene ontology. R package version 2.54.0. 2023. <https://doi.org/10.18129/B9.bioc.topGO>.
- Allio R, Schomaker-Bastos A, Romiguier J, Prosdociimi F, Nabholz B, Delsuc F. MitoFinder: efficient automated large-scale extraction of mitogenomic data in target enrichment phylogenomics. *Mol Ecol Resour*. 2020;**20**(4):892–905. <https://doi.org/10.1111/1755-0998.13160>.
- Almagro Armenteros JJ, Tsirigos KD, Sønderby CK, Petersen TN, Winther O, Brunak S, von Heijne G, Nielsen H. SignalP 5.0 improves signal peptide predictions using deep neural networks. *Nat Biotechnol*. 2019;**37**(4):420–423. <https://doi.org/10.1038/s41587-019-0036-z>.
- Araki H, Cooper B, Blouin MS. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science*. 2007;**318**(5847):100–103. <https://doi.org/10.1126/science.1145621>.
- Aylward FO, Burnum-Johnson KE, Tringe SG, Teiling C, Tremmel DM, Moeller JA, Scott JJ, Barry KW, Piehowski PD, Nicora CD, et al. *Leucoagaricus gongylophorus* produces diverse enzymes for the degradation of recalcitrant plant polymers in leaf-cutter ant fungus gardens. *Appl Environ Microbiol*. 2013;**79**(12):3770–3778. <https://doi.org/10.1128/AEM.03833-12>.
- Baril T, Galbraith J, Hayward A. Earl Grey. Version 3.0. Zenodo. 2023. <https://doi.org/10.5281/zenodo.5654615>.
- Baril T, Galbraith J, Hayward A. Earl Grey: a fully automated user-friendly transposable element annotation and analysis pipeline. *Mol Biol Evol*. 2024;**41**(4):msae068. <https://doi.org/10.1093/molbev/msae068>.
- Beigel K, Matthews AE, Kellner K, Pawlik CV, Greenwold M, Seal JN. Cophylogenetic analyses of Trachymyrmex ant-fungal specificity: “one to one with some exceptions”. *Mol Ecol*. 2021;**30**(21):5605–5620. <https://doi.org/10.1111/mec.16140>.
- Bingle LEH, Simpson TJ, Lazarus CM. Ketosynthase domain probes identify two subclasses of fungal polyketide synthase genes. *Fungal Genet Biol*. 1999;**26**(3):209–223. <https://doi.org/10.1006/fgbi.1999.1115>.
- Blin K, Shaw S, Augustijn HE, Reitz ZL, Biermann F, Alanjary M, Fetter A, Terlouw BR, Metcalf WW, Helfrich EJM, et al. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. *Nucleic Acids Res*. 2023;**51**(W1):W46–W50. <https://doi.org/10.1093/nar/gkad344>.

- Bolander M, Andersen JE, Conlon BH, Arnan X, Michelsen A, Shik JZ. Reciprocal nutritional provisioning between leafcutter ants and their fungal cultivar mediates performance of symbiotic farming systems. *Funct Ecol*. 2023;**37**(12):3079–3090. <https://doi.org/10.1111/1365-2435.14437>.
- Bourque G, Burns KH, Gehring M, Gorbunova V, Seluanov A, Hammell M, Imbeault M, Izsvák Z, Levin HL, Macfarlan TS, et al. Ten things you should know about transposable elements. *Genome Biol*. 2018;**19**(1):199. <https://doi.org/10.1186/s13059-018-1577-z>.
- Branstetter MG, Ješovnik A, Sosa-Calvo J, Lloyd MW, Faircloth BC, Brady SG, Schultz TR. Dry habitats were crucibles of domestication in the evolution of agriculture in ants. *Proc R Soc Lond B Biol Sci*. 2017;**284**(1852):20170095. <https://doi.org/10.1098/rspb.2017.0095>.
- Brown CT, Irber L. Sourmash: a library for MinHash sketching of DNA. *J Open Source Softw*. 2016;**1**(5):27. <https://doi.org/10.21105/joss.00027>.
- Buchfink B, Reuter K, Drost H-G. Sensitive protein alignments at tree-of-life scale using DIAMOND. *Nat Methods*. 2021;**18**(4):366–368. <https://doi.org/10.1038/s41592-021-01101-x>.
- Cai X, Lin R, Liang J, King GJ, Wu J, Wang X. Transposable element insertion: a hidden major source of domesticated phenotypic variation in *Brassica rapa*. *Plant Biotechnol J*. 2022;**20**(7):1298–1310. <https://doi.org/10.1111/pbi.13807>.
- Cantalapiedra CP, Hernández-Plaza A, Letunic I, Bork P, Huerta-Cepas J. eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. *Mol Biol Evol*. 2021;**38**(12):5825–5829. <https://doi.org/10.1093/molbev/msab293>.
- Cao S, Chen K, Lu K, Chen S, Zhang X, Shen C, Zhu S, Niu Y, Fan L, Chen ZJ, et al. Asymmetric variation in DNA methylation during domestication and de-domestication of rice. *Plant Cell*. 2023;**35**(9):3429–3443. <https://doi.org/10.1093/plcell/koad160>.
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. Trimal: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*. 2009;**25**(15):1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>.
- Carlson AL, Ishak HD, Kurian J, Mikheyev AS, Gifford I, Mueller UG. Nuclear populations of the multinucleate fungus of leafcutter ants can be dekarotized and recombined to manipulate growth of nutritive hyphal nodules harvested by the ants. *Mycologia*. 2017;**109**(5):832–846. <https://doi.org/10.1080/00275514.2017.1400304>.
- Carroll CS, Moore MM. Ironing out siderophore biosynthesis: a review of non-ribosomal peptide synthetase (NRPS)-independent siderophore synthetases. *Crit Rev Biochem Mol Biol*. 2018;**53**(4):356–381. <https://doi.org/10.1080/10409238.2018.1476449>.
- Castillo-Alfonso F, Valadez-Cano C, Cejas-Añón G, Utrilla J, Sigala Alanis J-C, Le Borgne S, Sales-Cruz AM, Viguera-Ramírez G, Olivares-Hernández R. Sequencing, assembly, and genomic annotation of *Leucoagaricus gongylophorus* LEU18496, a dikarya mutualistic species. *Mol Omics*. 2024;**20**:524–531. <https://doi.org/10.1039/D4MO00108G>.
- Chan PP, Lin BY, Mak AJ, Lowe TM. tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. *Nucleic Acids Res*. 2021;**49**(16):9077–9096. <https://doi.org/10.1093/nar/gkab688>.
- Clutterbuck AJ. Genomic CG dinucleotide deficiencies associated with transposable element hypermutation in Basidiomycetes, some lower fungi, a moss and a clubmoss. *Fungal Genet Biol*. 2017;**104**:16–28. <https://doi.org/10.1016/j.fgb.2017.04.005>.
- Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, et al. Twelve years of SAMtools and BCFtools. *GigaScience*. 2021;**10**(2):giab008. <https://doi.org/10.1093/gigascience/giab008>.
- De Fine Licht HH, Boomsma JJ, Tunlid A. Symbiotic adaptations in the fungal cultivar of leaf-cutting ants. *Nat Commun*. 2014;**5**(1):5675. <https://doi.org/10.1038/ncomms6675>.
- De Fine Licht HH, Schiøtt M, Mueller UG, Boomsma JJ. Evolutionary transitions in enzyme activity of ant fungus gardens. *Evolution*. 2010;**64**(7):2055–2069. <https://doi.org/10.1111/j.1558-5646.2010.00948.x>.
- De Fine Licht HH, Schiøtt M, Rogowska-Wrzęsinska A, Nygaard S, Roepstorff P, Boomsma JJ. Laccase detoxification mediates the nutritional alliance between leaf-cutting ants and fungus-garden symbionts. *Proc Natl Acad Sci*. 2013;**110**(2):583–587. <https://doi.org/10.1073/pnas.1212709110>.
- Dellagi A, Rigault M, Segond D, Roux C, Kraepiel Y, Cellier F, Briat J-F, Gaymard F, Expert D. Siderophore-mediated upregulation of Arabidopsis ferritin expression in response to *Erwinia chrysanthemi* infection. *Plant J*. 2005;**43**(2):262–272. <https://doi.org/10.1111/j.1365-3113X.2005.02451.x>.
- Dentinger BTM, Gaya E, O'Brien H, Suz LM, Lachlan R, Díaz-Valderrama JR, Koch RA, Aime MC. Tales from the crypt: genome mining from fungarium specimens improves resolution of the mushroom tree of life. *Biol J Linn Soc Lond*. 2016;**117**(1):11–32. <https://doi.org/10.1111/bij.12553>.
- Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*. 2004;**32**(5):1792–1797. <https://doi.org/10.1093/nar/gkh340>.
- Emms DM, Kelly S. OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biol*. 2019;**20**(1):238. <https://doi.org/10.1186/s13059-019-1832-y>.
- Felnagle EA, Jackson EE, Chan YA, Podelvs AM, Berti AD, McMahon MD, Thomas MG. Nonribosomal peptide synthetases involved in the production of medically relevant natural products. *Mol Pharm*. 2008;**5**(2):191–211. <https://doi.org/10.1021/mp700137g>.
- Finn RD, Bateman A, Clements J, Coggill P, Eberhardt RY, Eddy SR, Heger A, Hetherington K, Holm L, Mistry J, et al. Pfam: the protein families database. *Nucleic Acids Res*. 2013;**42**(D1):D222–D230. <https://doi.org/10.1093/nar/gkt1223>.
- Floudas D, Bentzer J, Ahrén D, Johansson T, Persson P, Tunlid A. Uncovering the hidden diversity of litter-decomposition mechanisms in mushroom-forming fungi. *ISME J*. 2020;**14**(8):2046–2059. <https://doi.org/10.1038/s41396-020-0667-6>.
- Floudas D, Held BW, Riley R, Nagy LG, Koehler G, Ransdell AS, Younus H, Chow J, Chiniquy J, Lipzen A, et al. Evolution of novel wood decay mechanisms in Agaricales revealed by the genome sequences of *Fistulina hepatica* and *Cylindrobasidium torrendii*. *Fungal Genet Biol*. 2015;**76**:78–92. <https://doi.org/10.1016/j.fgb.2015.02.002>.
- Fornasiero A, Wing RA, Ronald P. Rice domestication. *Curr Biol*. 2022;**32**(1):R20–R24. <https://doi.org/10.1016/j.cub.2021.11.025>.
- Gladyshev E. Repeat-Induced point mutation and other genome defense mechanisms in fungi. *Microbiol Spectr*. 2017;**5**(4):10.1128/microbiolspec.funk-0042-2017. <https://doi.org/10.1128/microbiolspec.funk-0042-2017>.
- Gu Z, Hübschmann D. simplifyEnrichment: a bioconductor package for clustering and visualizing functional enrichment results. *Genomics Proteomics Bioinformatics*. 2023;**21**(1):190–202. <https://doi.org/10.1016/j.gpb.2022.04.008>.
- Guo W, Wang W, Tang J, Li T, Li X. Genome analysis and genomic comparison of a fungal cultivar of the nonsocial weevil *Euops chinensis* reveals its plant decomposition and protective roles in fungus-farming mutualism. *Front Microbiol*. 2023;**14**:1048910. <https://doi.org/10.3389/fmicb.2023.1048910>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. *Bioinformatics*. 2013;**29**(8):1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Haas BJ, Delcher AL, Mount SM, Wortman JR, Smith RK, Hannick LI, Maiti R, Ronning CM, Rusch DB, Town CD, et al. Improving the Arabidopsis genome annotation using maximal transcript alignment assemblies. *Nucleic Acids Res*. 2003;**31**(19):5654–5666. <https://doi.org/10.1093/nar/gkg770>.
- Haas BJ, Salzberg SL, Zhu W, Perlea M, Allen JE, Orvis J, White O, Buell CR, Wortman JR. Automated eukaryotic gene structure annotation using EvidenceModeler and the Program to Assemble

- Spliced Alignments. *Genome Biol.* 2008;**9**(1):R7. <https://doi.org/10.1186/gb-2008-9-1-r7>.
- Herz H, Hölldobler B, Roces F. Delayed rejection in a leaf-cutting ant after foraging on plants unsuitable for the symbiotic fungus. *Behav Ecol.* 2008;**19**(3):575–582. <https://doi.org/10.1093/beheco/arn016>.
- Hess J, Skrede I, Wolfe BE, LaButti K, Ohm RA, Grigoriev IV, Pringle A. Transposable element dynamics among asymbiotic and ectomy-corrhizal *Amanita* fungi. *Genome Biol Evol.* 2014;**6**(7):1564–1578. <https://doi.org/10.1093/gbe/evu121>.
- Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS. UFBoot2: improving the ultrafast bootstrap approximation. *Mol Biol Evol.* 2018;**35**(2):518–522. <https://doi.org/10.1093/molbev/msx281>.
- Hölldobler B, Wilson EO. *The leafcutter ants: civilization by instinct.* New York: W. W. Norton & Co; 2011.
- Leal-Dutra CA, Vizueta J, Baril T, Croll D, Shik JZ. Supplementary files for Genomic Signatures of Domestication in a Fungus Obligately Farmed by Leafcutter Ants. University of Copenhagen. 2024. <https://doi.org/10.17894/ucph.e9980bed-a075-46c8-bc65-5ee3c1a4c2d8>.
- Jiang H, Medintz I, Michels CA. Two glucose sensing/signaling pathways stimulate glucose-induced inactivation of maltose permease in *Saccharomyces*. *Mol Biol Cell.* 1997;**8**(7):1293–1304. <https://doi.org/10.1091/mbc.8.7.1293>.
- Jones P, Binns D, Chang H-Y, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, et al. InterProScan 5: genome-scale protein function classification. *Bioinformatics.* 2014;**30**(9):1236–1240. <https://doi.org/10.1093/bioinformatics/btu031>.
- Jones A, Schwessinger B. High-molecular weight DNA extraction from challenging fungi using CTAB for lysis and precipitation V.3. protocols.io. 2021. <https://doi.org/10.17504/protocols.io.bwr8pd9w>.
- Käll L, Krogh A, Sonnhammer ELL. A combined transmembrane topology and signal peptide prediction method. *J Mol Biol.* 2004;**338**(5):1027–1036. <https://doi.org/10.1016/j.jmb.2004.03.016>.
- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermini LS. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods.* 2017;**14**:587. <https://doi.org/10.1038/nmeth.4285>.
- Khadempour L, Kyle JE, Webb-Robertson B-JM, Nicora CD, Smith FB, Smith RD, Lipton MS, Currie CR, Baker ES, Burnum-Johnson KE. From plants to ants: fungal modification of leaf lipids for nutrition and communication in the leaf-cutter ant fungal garden ecosystem. *mSystems.* 2021;**6**(2):e01307–e01320. <https://doi.org/10.1128/mSystems.01307-20>.
- Koch KE. Carbohydrate-modulated gene expression in plants. *Annu Rev Plant Physiol Mol Biol.* 1996;**47**(1):509–540. <https://doi.org/10.1146/annurev.arplant.47.1.509>.
- Kooij PW, Aanen DK, Schiøtt M, Boomsma JJ. Evolutionarily advanced ant farmers rear polyploid fungal crops. *J Evol Biol.* 2015;**28**(11):1911–1924. <https://doi.org/10.1111/jeb.12718>.
- Kooij PW, Pellicer J. Genome size versus genome assemblies: are the genomes truly expanded in polyploid fungal symbionts? *Genome Biol Evol.* 2020;**12**(12):2384–2390. <https://doi.org/10.1093/gbe/evaa217>.
- Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res.* 2017;**27**(5):722–736. <https://doi.org/10.1101/gr.215087.116>.
- Korf I. Gene finding in novel genomes. *BMC Bioinformatics.* 2004;**5**(1):59. <https://doi.org/10.1186/1471-2105-5-59>.
- Kües U, Khonsuntia W, Subba S, Dörnte B. Volatiles in communication of agaricomycetes. In: Anke T, Schöffler A, editors. *Physiology and genetics: selected basic and applied aspects.* Cham: Springer International Publishing; 2018. p. 149–212. https://doi.org/10.1007/978-3-319-71740-1_6.
- Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods.* 2012;**9**(4):357–359. <https://doi.org/10.1038/nmeth.1923>.
- Leal-Dutra CA, Shik JZ. RNA-seq and associated data from *Leucoagaricus gongylophorus* isolated from an *Atta colombica* colony. 2023. <https://doi.org/10.17894/ucph.e6f0438f-e33d-4043-98aa-07de4d9fa9e3>.
- Leal-Dutra CA, Yuen LM, Guedes BAM, Contreras-Serrano M, Marques PE, Shik JZ. Evidence that the domesticated fungus *Leucoagaricus gongylophorus* recycles its cytoplasmic contents as nutritional rewards to feed its leafcutter ant farmers. *IMA Fungus.* 2023;**14**(1):19. <https://doi.org/10.1186/s43008-023-00126-5>.
- Lebreton A, Tang N, Kuo A, LaButti K, Andreopoulos W, Drula E, Miyauchi S, Barry K, Clum A, Lipzen A, et al. Comparative genomics reveals a dynamic genome evolution in the ectomycorrhizal milk-cap (*Lactarius*) mushrooms. *New Phytol.* 2022;**235**(1):306–319. <https://doi.org/10.1111/nph.18143>.
- Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res.* 2013;**42**(D1):D490–D495. <https://doi.org/10.1093/nar/gkt1178>.
- Lomsadze A, Burns PD, Borodovsky M. Integration of mapped RNA-Seq reads into automatic training of eukaryotic gene finding algorithm. *Nucleic Acids Res.* 2014;**42**(15):e119–e119. <https://doi.org/10.1093/nar/gku557>.
- Majoros WH, Pertea M, Salzberg SL. TigrScan and GlimmerHMM: two open source ab initio eukaryotic gene-finders. *Bioinformatics.* 2004;**20**(16):2878–2879. <https://doi.org/10.1093/bioinformatics/bth315>.
- Manni M, Berkeley MR, Seppely M, Zdobnov EM. BUSCO: assessing genomic data quality and beyond. *Curr Protoc.* 2021;**1**(12):e323. <https://doi.org/10.1002/cpz1.323>.
- Martiarena MJS, Deveau A, Montoya QV, Flórez LV, Rodrigues A. The hyphosphere of leaf-cutting ant cultivars is enriched with helper bacteria. *Microb Ecol.* 2023;**86**(3):1773–1788. <https://doi.org/10.1007/s00248-023-02187-w>.
- Martin MM, Carman RM, MacConnell JG. Nutrients derived from the fungus cultured by the fungus-growing ant *Atta colombica* tonsipes1. *Ann Entomol Soc Am.* 1969;**62**(1):11–13. <https://doi.org/10.1093/aesa/62.1.11>.
- Masiulionis VE, Rabeling C, De Fine Licht HH, Schultz T, Bacci M Jr, Bezerra CMS, Pagnocca FC. A Brazilian population of the asexual fungus-growing ant *mycocepurus smithii* (Formicidae, Myrmicinae, Attini) cultivates fungal symbionts with gongyliid-like structures. *PLoS One.* 2014;**9**(8):e103800. <https://doi.org/10.1371/journal.pone.0103800>.
- Mehdiabadi NJ, Mueller UG, Brady SC, Himler AG, Schultz TR. Symbiont fidelity and the origin of species in fungus-growing ants. *Nat Commun.* 2012;**3**:840. <https://doi.org/10.1038/ncomms1844>.
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol.* 2020;**37**(5):1530–1534. <https://doi.org/10.1093/molbev/msaa015>.
- Möller A. *Die pilzgärten einiger südamerikanischer ameisen.* Jena: Gustav Fischer Verlag; 1893.
- Morin E, Kohler A, Baker AR, Foulongne-Oriol M, Lombard V, Nagy LG, Ohm RA, Patyshakuliyeva A, Brun A, Aerts AL, et al. Genome sequence of the button mushroom *Agaricus bisporus* reveals mechanisms governing adaptation to a humic-rich ecological niche. *Proc Natl Acad Sci.* 2012;**109**(43):17501–17506. <https://doi.org/10.1073/pnas.1206847109>.
- Mueller UG, Gerardo NM, Aanen DK, Six DL, Schultz TR. The evolution of agriculture in insects. *Annu Rev Ecol Evol Syst.* 2005;**36**:563–595. <https://doi.org/10.1146/annurev.ecolsys.36.102003.152626>.
- Mueller UG, Kardish MR, Ishak HD, Wright AM, Solomon SE, Bruschi SM, Carlson AL, Bacci M. Phylogenetic patterns of ant-fungus associations indicate that farming strategies, not only a superior fungal cultivar, explain the ecological success of leafcutter ants. *Mol Ecol.* 2018;**27**(10):2414–2434. <https://doi.org/10.1111/mec.14588>.
- Mueller UG, Mikhayev AS, Solomon SE, Cooper M. Frontier mutualism: coevolutionary patterns at the northern range limit of the leaf-cutter

- ant–fungus symbiosis. *Proc R Soc Lond B Biol Sci.* 2011;**278**(1721): 3050–3059. <https://doi.org/10.1098/rspb.2011.0125>.
- Mueller UG, Schultz TR, Currie CR, Adams RM, Malloch D. The origin of the attine ant–fungus mutualism. *Q Rev Biol.* 2001;**76**:169–197. <https://doi.org/10.1086/393867>.
- Muszewska A, Steczkiewicz K, Stepniewska-Dziubinska M, Ginalski K. Transposable elements contribute to fungal genes and impact fungal lifestyle. *Sci Rep.* 2019;**9**(1):4307. <https://doi.org/10.1038/s41598-019-40965-0>.
- Nurk S, Walenz BP, Rhie A, Vollger MR, Logsdon GA, Grothe R, Miga KH, Eichler EE, Phillippy AM, Koren S. Hicaru: accurate assembly of segmental duplications, satellites, and allelic variants from high-fidelity long reads. *Genome Res.* 2020;**30**(9):1291–1305. <https://doi.org/10.1101/gr.263566.120>.
- Nygaard S, Hu H, Li C, Schiøtt M, Chen Z, Yang Z, Xie Q, Ma C, Deng Y, Dikow RB, et al. Reciprocal genomic evolution in the ant–fungus agricultural symbiosis [article]. *Nat Commun.* 2016;**7**:12233. <https://doi.org/10.1038/ncomms12233>.
- Palmer JM, Stajich JE. Funannotate V1.8.1: eukaryotic genome annotation version v1.8.1. 2020. Zenodo. <https://doi.org/10.5281/zenodo.4054262>.
- Paysan-Lafosse T, Blum M, Chuguransky S, Grego T, Pinto BL, Salazar GA, Bileschi ML, Bork P, Bridge A, Colwell L, et al. InterPro in 2022. *Nucleic Acids Res.* 2022;**51**(D1):D418–D427. <https://doi.org/10.1093/nar/gkac993>.
- Pierce N, Irber L, Reiter T, Brooks P, Brown C. Large-scale sequence comparisons with sourmash. *F1000Res.* 2019;**8**:1006. <https://doi.org/10.12688/f1000research.19675.1>.
- Platt RN II, Blanco-Berdugo L, Ray DA. Accurate transposable element annotation is vital when analyzing new genome assemblies. *Genome Biol Evol.* 2016;**8**(2):403–410. <https://doi.org/10.1093/gbe/evw009>.
- Pryszcz LP, Gabaldón T. Redundans: an assembly pipeline for highly heterozygous genomes. *Nucleic Acids Res.* 2016;**44**(12): e113–e113. <https://doi.org/10.1093/nar/gkw294>.
- Purugganan MD. What is domestication? *Trends Ecol Evol.* 2022;**37**(8):663–671. <https://doi.org/10.1016/j.tree.2022.04.006>.
- Quinlan RJ, Cherrett JM. The role of fungus in the diet of the leaf-cutting ant *Atta cephalotes* (L.). *Ecol Entomol.* 1979;**4**(2): 151–160. <https://doi.org/10.1111/j.1365-2311.1979.tb00570.x>.
- Raffaele S, Kamoun S. Genome evolution in filamentous plant pathogens: why bigger can be better. *Nat Rev Microbiol.* 2012;**10**(6): 417–430. <https://doi.org/10.1038/nrmicro2790>.
- Rawlings ND, Barrett AJ, Bateman A. Using the MEROPS database for proteolytic enzymes and their inhibitors and substrates. *Curr Protoc Bioinformatics.* 2014;**48**(1):1.25.21–21.25.33. <https://doi.org/10.1002/0471250953.bi0125s48>.
- Ringel M, Dimos N, Himpich S, Haack M, Huber C, Eisenreich W, Schenk G, Loll B, Brück T. Biotechnological potential and initial characterization of two novel sesquiterpene synthases from Basidiomycota *Coniophora puteana* for heterologous production of δ -cadinol. *Microb Cell Fact.* 2022;**21**(1):64. <https://doi.org/10.1186/s12934-022-01791-8>.
- Schiøtt M, Boomsma JJ. Proteomics reveals synergy between biomass degrading enzymes and inorganic Fenton chemistry in leaf-cutting ant colonies. *eLife.* 2021;**10**:e61816. <https://doi.org/10.7554/eLife.61816>.
- Schultz TR, Brady SG. Major evolutionary transitions in ant agriculture. *Proc Natl Acad Sci U S A.* 2008;**105**(14):5435–5440. <https://doi.org/10.1073/pnas.0711024105>.
- Shik JZ, Kooij PW, Donoso DA, Santos JC, Gomez EB, Franco M, Crumière AJJ, Arnan X, Howe J, Wcislo WT, et al. Nutritional niches reveal fundamental domestication trade-offs in fungus-farming ants. *Nat Ecol Evol.* 2021;**5**(1):122–134. <https://doi.org/10.1038/s41559-020-01314-x>.
- Shik JZ, Santos JC, Seal JN, Kay A, Mueller UG, Kaspari M. Metabolism and the rise of fungus cultivation by ants. *Am Nat.* 2014;**184**(3): 364–373. <https://doi.org/10.1086/677296>.
- Sonnenberg ASM, Gao W, Lavrijssen B, Hendrickx P, Sedaghat-Tellgerd N, Foulongne-Oriol M, Kong W-S, Schijlen EGWM, Baars JJP, Visser RGF. A detailed analysis of the recombination landscape of the button mushroom *Agaricus bisporus* var. *bisporus*. *Fungal Genet Biol.* 2016;**93**:35–45. <https://doi.org/10.1016/j.fgb.2016.06.001>.
- Stanke M, Diekhans M, Baertsch R, Haussler D. Using native and systematically mapped cDNA alignments to improve de novo gene finding. *Bioinformatics.* 2008;**24**(5):637–644. <https://doi.org/10.1093/bioinformatics/btn013>.
- Tenaillon MI, Burbani E, Huynh S, Wojcik A, Thuillet A, Manicacci D, Gérard PR, Alix K, Belcram H, Cornille A, et al. Crop domestication as a step toward reproductive isolation. *Am J Bot.* 2023;**110**(7):e16173. <https://doi.org/10.1002/ajb2.16173>.
- Testa AC, Hane JK, Ellwood SR, Oliver RP. CodingQuarry: highly accurate hidden Markov model gene prediction in fungal genomes using RNA-seq transcripts. *BMC Genomics.* 2015;**16**(1):170. <https://doi.org/10.1186/s12864-015-1344-4>.
- The UniProt Consortium. UniProt: the universal protein knowledgebase in 2023. *Nucleic Acids Res.* 2022;**51**(D1):D523–D531. <https://doi.org/10.1093/nar/gkac1052>.
- Thieme M, Bucher E. Chapter six—transposable elements as tool for crop improvement. In: Mirouze M, Bucher E, Gallusci P, editors. *Advances in botanical research*. London: Academic Press; 2018. p. 165–202. <https://doi.org/10.1016/bs.abr.2018.09.001>.
- Vinha AF, Machado M, Oliveira MBPP. Terpenes from fungi. In: Carochio M, Heleno SA, Barros L, editors. *Natural secondary metabolites: from nature, through science, to industry*. Cham: Springer International Publishing; 2023. p. 497–528. https://doi.org/10.1007/978-3-031-18587-8_15.
- Wang Z, Wang W, Xie X, Wang Y, Yang Z, Peng H, Xin M, Yao Y, Hu Z, Liu J, et al. Dispersed emergence and protracted domestication of polyploid wheat uncovered by mosaic ancestral haploblock inference. *Nat Commun.* 2022;**13**(1):3891. <https://doi.org/10.1038/s41467-022-31581-0>.
- Weber NA. *Gardening ants, the attines*. Philadelphia: American Philosophical Society; 1972.
- Wenger AM, Peluso P, Rowell WJ, Chang P-C, Hall RJ, Concepcion GT, Ebler J, Functammanan A, Kolesnikov A, Olson ND, et al. Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome. *Nat Biotechnol.* 2019;**37**: 1155–1162. <https://doi.org/10.1038/s41587-019-0217-9>.