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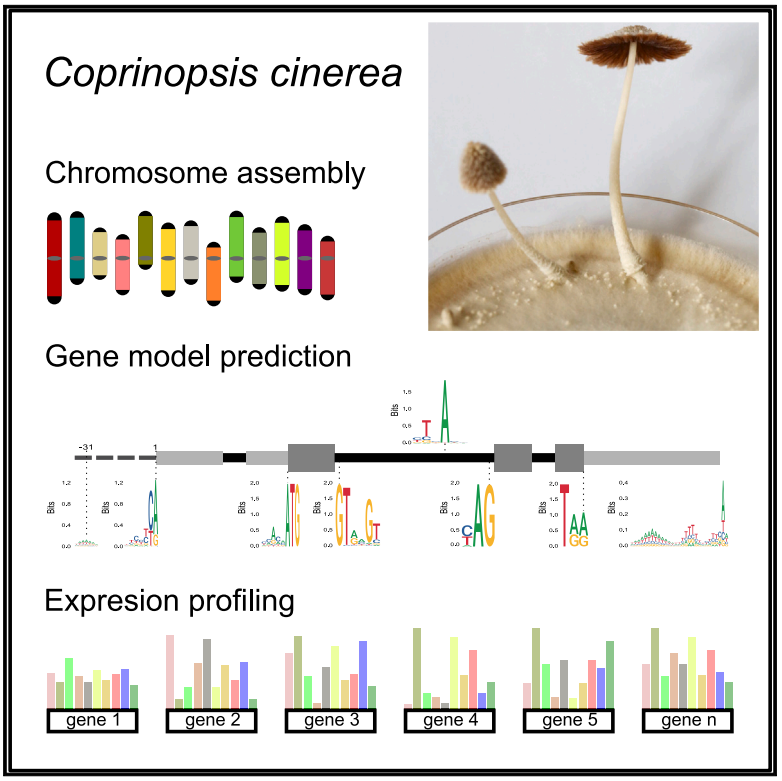
Article

Morphogenesis, starvation, and light responses in a mushroom-forming fungus revealed by long-read sequencing and extensive expression profiling

Graphical abstract



Authors

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In brief

Hegedus et al. produced a high-quality gene annotation for the mushroom-forming fungus *C. cinerea*. A new assembly, long-read cDNA, and RNA-seq data yielded an annotation with key transcriptomic insights into starvation, light induction, and mycelial differentiation. The data are available at https://mushroomdb.brc.hu/, a resource for mushroom biology.

Highlights

- High-quality annotation of *C. cinerea* protein-coding genes
- *C. cinerea* may be the best annotated mushroom-forming fungus
- Alternative polyadenylation site profiling using 3′ mRNA end sequencing approach
- Transcriptomics capture genes for starvation, light, and differentiation

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Morphogenesis, starvation, and light responses in a mushroom-forming fungus revealed by long-read sequencing and extensive expression profiling

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SUMMARY

Mushroom-forming fungi (Agaricomycetes) are emerging as pivotal players in several fields of science and industry. Genomic data for Agaricomycetes are accumulating rapidly; however, this is not paralleled by improvements of gene annotations, which leave gene function notoriously poorly understood. We set out to improve our functional understanding of the model mushroom *Coprinopsis cinerea* by integrating a new, chromosome-level assembly, high-quality gene predictions, and functional information derived from broad gene-expression profiling data. The new annotation includes 5′ and 3′ untranslated regions (UTRs), polyadenylation sites (PASs), upstream open reading frames (uORFs), splicing isoforms, and microexons, as well as core gene sets corresponding to carbon starvation, light response, and hyphal differentiation. As a result, the genome of *C. cinerea* has now become the most comprehensively annotated genome among mushroom-forming fungi, which will contribute to multiple rapidly expanding fields, including research on their life history, light and stress responses, as well as multicellular development.

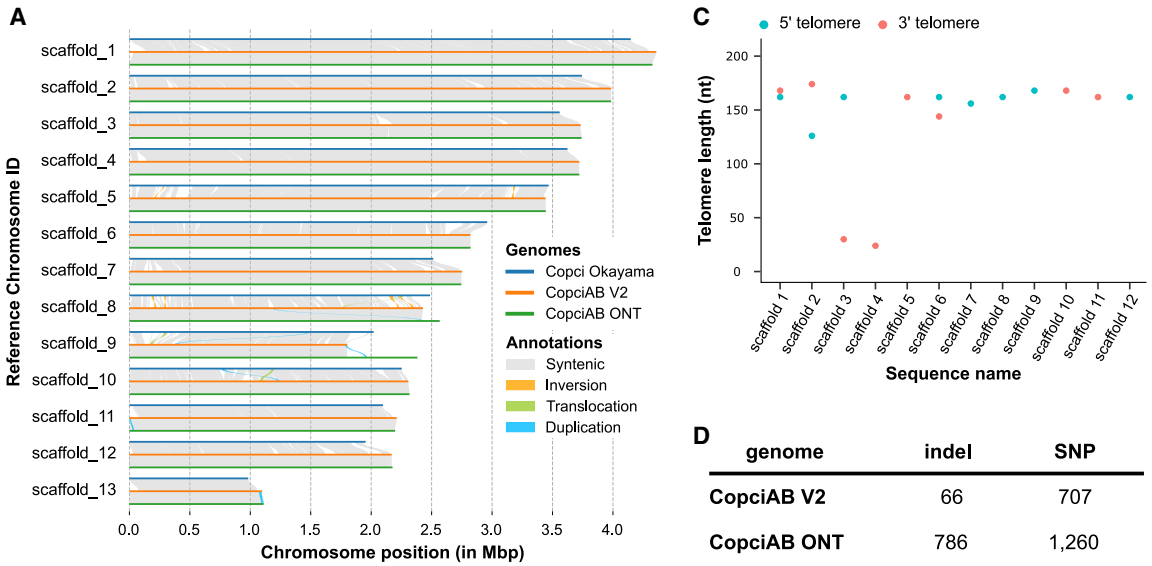
INTRODUCTION

Global concern for sustainability increased interest in environmentally friendly solutions for old industrial problems, for which the kingdom Fungi offers remarkable biological agents. The class Agaricomycetes (mushroom-forming fungi) contains the most powerful degraders of plant lignocellulosic biomass and species that produce the most morphologically complex fruiting bodies, which are harnessed by the rapidly expanding mushroom industry.[1,2]

These two traits, among others, have fueled Agaricomycetes genomics, which led to the availability of >500 draft genomes in various repositories, such as MycoCosm.[3] However, a limitation in Agaricomycetes, as compared to Ascomycota, genomics is the paucity of high-quality annotations and the shortage of knowledge on gene function. This stems from the large diversity of basidiomycete model systems,[4] the lack of systematic gene deletion projects, and the difficulty of homology-based extrapolation of gene function from well-researched organisms, such as *Saccharomyces cerevisiae*. Recently, several chromosome-level assemblies of various Agaricomycetes have been published.[5–11] While these represent a step in understanding the biology of these organisms, improvements of gene annotations did not follow. To the best of our knowledge, there is no Agaricomycetes species in which full-length transcripts, untranslated regions (UTRs), intergenic regions, upstream open reading frames (uORFs), or polyadenylation sites (PASs) have been determined with high precision.

Fungal genomes are relatively compact, usually with short intergenic spaces and few and short introns.[12] Tight gene spacing often causes UTRs of adjacent genes to overlap. Overlaps of 3′ UTRs of adjacent genes are widespread in fungi and can directly or indirectly regulate each other's expression.[13,14] The situation is further complicated by prevalent polycistronic transcription,[15,16] which could be due to transcription through weak termination signals.[15] The identification of the UTRs is important because they can contain (un)structured elements, such as uORFs in the 5′ UTR that may modulate translation initiation and stalling.[17–19] The 3′ UTR may contain elements that regulate mRNA stability, translation, or localization,[19] which is dependent also on alternative polyadenylation (APA).[20–22]

Coprinopsis cinerea is one of the main model mushroom species[23] of principles of mushroom development, meiosis



D

genome	indel	SNP
CopciAB V2	66	707
CopciAB ONT	786	1,260

B

genome	full genome size (bp)	13 chromosome size (bp)	contig/scaffold number
Copci Okayama	36,294,355	35,810,191	94 (13 Chr + 81 U)
CopciAB V2	37,269,365	36,827,329	23 (21 merged: 13 Chr + 8 fragment)
CopciAB V1	34,118,803		944
CopciAB ONT	38,698,142	37,536,632	31 (13 Chr + 18 fragment)

Figure 1. Characterization of the new genome assembly in the light of the previous assemblies

(A) Observed structural variance between the 13 chromosomes of the three *C. cinerea* genomes. The Nucmer function from the MUMmer v.4.0 package was used for the comparisons. SyRI was used to identify genomic rearrangements and local sequence differences. Visualization was performed with plotsr.

(B) Comparison of the genome size and scaffold numbers of the available *C. cinerea* genomes. Copci Okayama stands for the short-read based *C. cinerea* Okayama assembly; CopciAB V1, V2, and ONT for the short-read, PacBio, and ONT-based assembly of the *C. cinerea* Amut1Bmut1 strains, respectively.

(C) Identified telomere repeat sequence length distribution.

(D) The number of indels and SNPs occurring in the two chromosome-level CopciAB genome assemblies. Variants were called in the assemblies based on Illumina genomic read mapping. See also Figure S1.

and mitosis, and photobiology, among others, and is currently one of the main model species of fungal multicellularity[24] and developmental biology.[2,23,25] *C. cinerea* is a litter decomposer (LD); it feeds on non-woody lignocellulosic plant biomass, such as grasses, straw, or manure.[26] Two chromosome levels and a draft assembly for the two most relevant strains (Okayama7 #130 and Amut1Bmut1 #326) have been published.[25,27,28] The widely used Amut1Bmut1 #326 strain is self-compatible due to a mutation in mating type genes and produces dikaryotic cells with two identical nuclei. In response to light and nutritional cues the dikaryon forms sexual fruiting bodies, in which meiosis and spore formation take place.[23] While the importance of light and starvation on fruiting body development are well known,[29] the molecular underpinnings of these are poorly understood.

Here, we assembled a chromosome-level genome for *C. cinerea* Amut1Bmut1 #326, sequenced full-length transcripts using Oxford Nanopore (ONT) and PacBio isoform sequencing (IsoSeq), and profiled its transcriptome across a broad panel of conditions using QuantSeq. Long Nanopore and PacBio reads allowed us to improve gene models, annotate uORFs, intergenic regions, 5′ and 3′ UTRs, and PASs. Long reads also helped to correctly identify microexons,[30] which can have regulatory roles in vertebrates,[31–34] insects,[35,36] and plants[37–39] but are barely studied in fungi. Using these data, we show that events preceding fruiting body development of *C. cinerea* comprise a mixture of starvation and light responses as well as morphogenesis. We provide consensus gene lists underlying starvation, light response, and the differentiation of aerial and submerged mycelium. Finally, we made these resources openly available as a website (https://mushroomdb.brc.hu/) for use by the community.

RESULTS

New assembly for *C. cinerea*

We sequenced and assembled the 13 chromosomes of the *C. cinerea* Amut1Bmut1 #326 strain into 23 scaffolds using PacBio reads, with an estimated 75× coverage (Figure 1A). Based on the scaffold length distribution, a nearly 10-fold decrease is observable between the 15th-longest scaffold (1,097,509 bp) and the following scaffold (162,061 bp). After comparison of the draft genome with the available chromosome-level Okayama

Table 1. BUSCO-based comparison of the three available *C. cinerea* AmutBmut genomes

Description	CopciAB V1	CopciAB V2	CopciAB ONT
Complete BUSCOs (C)	1,723 (97.7%)	1,761 (99.8%)	1,698 (96.3%)
Complete and single-copy BUSCOs (S)	1,715 (97.2%)	1,750 (99.2%)	1,681 (95.3%)
Complete and duplicated BUSCOs (D)	8 (0.5%)	11 (0.6%)	17 (1%)
Fragmented BUSCOs (F)	23 (1.3%)	1 (0.1%)	50 (2.8%)
Missing BUSCOs (M)	18 (1%)	2 (0.1%)	16 (0.9%)

Completeness of the annotation of CopciAB strains was determined using the basidiomycota_db10 database of BUSCO.

7 #130[25] and Amut1Bmut1 #326[28] genome assemblies, we found that the 15 largest scaffolds corresponded to the 13 chromosomes of *C. cinerea*, with two chromosomes each broken into two contigs. These were joined, based on a previous Nanopore-based assembly,[28] using a linker sequence of 1,000 unknown nucleotides (N1000). The new assembly (CopciAB V2) is slightly shorter than the previous Nanopore-based assembly; however, both of them are longer than the Okayama 7 and the Illumina-based Amut1Bmut1 assembly (CopciAB V1) (Figure 1B).

Whole-genome alignments showed a 99.11% and 99.98% average identity between CopciAB V2 and the Okayama and Nanopore-based *C. cinerea* assemblies, respectively. Analyses of structural variations between the three assemblies revealed high synteny (Figure 1A). Mapping Illumina reads from genome resequencing[28] on the Nanopore-based AmutBmut assemblies revealed that our new assembly contains at least 10-fold fewer insertions or deletions (indels) and 2-fold fewer SNPs than the Nanopore-based assembly (Figure 1D).

In 12 of the 13 chromosomes, telomeric repeats on at least one end were identified; four chromosomes have both telomeres (Figure 1C). On average, the telomere sequences are 150 bp long (25 complete consecutive repeats), and unlike the budding and fission yeast, the sequence [5′-$(CCCTAA)_n$/$(TTAGGG)_n$-3′] is identical to repeats from other filamentous fungi[40] and the typical human sequence,[41–43] similar to *Ustilago maydis* (also 150 bp), *Armillaria ostoyae*, *Pleurotus ostreatus*,[44–46] and other fungi.[47,48]

Genome annotation using Nanopore and PacBio cDNA-seq

To create the most complete annotation possible, we used Nanopore and PacBio IsoSeq cDNA sequencing (cDNA-seq) datasets obtained from libraries enriched for full-length transcripts. We also integrated information from the two available annotations of *C. cinerea* Amut1Bmut1 #326 genomes,[27,28] followed by manual checking and correction of misidentified splice sites, transcript flanking regions, and coding DNA sites (CDSs), among others. In this way, we compensated for most of the weaknesses of the two sequencing techniques, such as the lower read output of the PacBio IsoSeq, the lower fraction of longer transcripts in the Nanopore output,[49,50] and the truncation of transcripts with internal poly(A) or poly(T) runs by both techniques.[51,52]

For the ONT dataset, samples were collected from five *C. cinerea* developmental stages, while for IsoSeq, transcript diversity was captured by pooling various tissue types. We improved ONT reads by Illumina reads.[24] As a result, the quality of polished reads approaches that observed for PacBio reads (Figure S1B). After polishing, we obtained approximately 4 million reads per sample—in total ∼20 million reads—using Nanopore. Over 4 million reads were gained from IsoSeq (Figure S1A). The median read length in the ONT dataset is roughly half (943 bp) that of the IsoSeq dataset (1,867 bp), likely because PacBio's IsoSeq protocol is optimized for transcripts centered around 2 kb.

For gene model prediction, we combined numerous sources of information and manual curation steps (see STAR Methods), with ONT and IsoSeq transcripts forming the core of the annotation, supplemented with gene models from previous *C. cinerea* Amut1Bmut1 #326 annotations.[27,28] As a result, 13,617 gene models with 14,750 transcripts were predicted. Of these, 11,583 had support from at least one long-read dataset. The resulting number of genes is slightly lower than those in previous annotations,[27,28] possibly because we excluded genes that had no functional annotations, detectable expression, or homology outside *C. cinerea*. BUSCO indicates that the new annotation is nearly complete, with minor improvements over previous *C. cinerea* genomes (Table 1). The average number and length of CDSs, exons, and introns are higher than those in previous annotations (Table 2). The long-read cDNA data allowed us to annotate UTRs, with 12,011 and 12,130 genes having 5′ and 3′ UTRs, respectively (Table 2).

Microexons are the hallmark of complex gene models

We identified several extremely short, internally located exons, often referred to as microexons.[30] Correct identification of these can be particularly important as their misannotation can break gene models. In the new annotation 1,165 gene models (∼9%) have at least one internal microexon of ≤15 nt; in 835 cases (∼6%), the omission of one of these would create a premature stop codon (Figure S2A). Notably, 89 gene models have at least one exon ≤3 nt (1 nt, 13 exons in 13 genes; 2 nt, 57 exons in 50 genes; 3 nt, 27 exons in 26 genes), and of these, an alternative stop codon is created in 63 gene models when the microexon is omitted. We found that microexons are enriched in certain gene families, for example, in cytochrome P450 encoding genes (Figure S2B), consistent with previous reports from fungi.[53]

Conserved genomic elements in the new *C. cinerea* annotation

We identified conserved genomic elements located around protein-coding genes. First, in ±50 nt flanking the transcription start site (TSS) of long-read-based gene models with 5′ UTR (n = 11,483), we identified patterns consistent with the eukaryotic initiator sequence (Inr) minimal consensus (C/T)(A/G), where A/G is the TSS (Figure 2A), in line with previous results in other organisms.[54–58] Within 50 nt upstream of the TSS, 20% (n = 2,323) of genes have at least one TATA-box-like sequence (TATANN), 1,376 of which had a canonical TATA box (TATATA or TATAAA).

Table 2. Properties of *C. cinerea* protein-coding gene models of CopciAB V2 compared with prior genome annotation versions

Properties	CopciAB V1	CopciAB V2	CopciAB ONT	Okayama
Gene				
Number	14,242	13,617	15,250	13,393
Average length	1,748.24	1,981.68	1,796.85	1,763.01
CDS				
Number	75,485	75,118	80,939	75,320
Average number	5.30	5.52	5.31	5.62
Average length	1,300.33	1,351.49	1,281.34	1,385.96
Exon				
Number	77,000	78,990	83,580	75,796
Average number	5.41	5.80	5.48	5.66
Average cumulative length per gene	1,435.96	1,667.23	1,485.25	1,426.82
Intron				
Number	62,758	65,373	68,510	62,403
Average number	4.41	4.80	4.49	4.66
Average cumulative length per gene	312.28	314.44	311.61	336.19
5′ UTR				
Number of genes with UTR	5,759	12,011	5,560	2,071
Percentage of genes with UTR	40%	88%	36%	15%
Average UTR length of genes with UTR	153.76	147.54	253.60	89.53
3′ UTR				
Number of genes with UTR	5,728	12,130	5,100	2,111
Percentage of genes with UTR	40%	89%	33%	16%
Average UTR length of genes with UTR	182.62	208.36	329.23	171.39

The term "genes" is defined as the complete, unmatured mRNA transcript, which consists of exons and introns. Exons describe the mature part of the mRNA, which may or may not code for a polypeptide. The CDS is the coding part of the exon. The length is given in base pairs (bp).

Of the identified canonical TATA boxes, 83% (n = 1,151), start between −38 and −31 nt (most frequent: −34 nt). Noncanonical TATA boxes were also enriched in these locations. The location of TATA boxes is similar to that observed in *Aspergillus nidulans*[57] and *Schizosaccharomyces pombe*,[58] where TATA boxes are located −45 to −30 nt or −32 to −25 nt upstream of the TSS, respectively.

We also identified signals for the Kozak consensus sequence in the vicinity of the most likely AUG start codon in the top 10% of all transcribed genes based on long-read support (n = 1,096) (Figure 2A), which is similar to that of other fungal species.[54] Regarding splice sites, 99.8% of those found in 10,890 gene models with at least one splice site belonged to the GT-AG, GC-AG, and AT-AC subtypes (Figure 2A), which is consistent with the literature.[59] The translation termination site is represented by one of the three stop codons (UAA, UGA, UAG), and for the long-read-based annotations, these codons were almost evenly used (30.4%, 37.8%, and 31.8%, respectively) (Figure 2A). We detected motifs around the transcription termination site (TTS) (Figure 2A) similar to those observed in *S. pombe*,[60,61] *Magnaporthe oryzae*,[62] *Fusarium graminearum*,[16] and *S. cerevisiae*,[61] as well as in other multicellular organisms.[63]

uORFs

5′ UTR annotations provided an opportunity to investigate the occurrence of uORFs in *C. cinerea*. We considered all alternative ORFs whose start codon is within the 5′ UTR as uORFs as long as they encode at least one amino acid before a stop codon (i.e., at least 9 nt long).[64] We identified a total of 8,704 uORFs, with median predicted peptide length of 19 aa (mean: 29.22 aa), found in 26% (n = 2,982) of the 11,483 predicted genes. The largest proportion (46%) of these genes have a single uORF. The observed proportion of genes possessing uORFs[54,64,65] and the median peptide length of the uORFs are not unusual compared to previously described uORFs.[64] We identified a putative homolog of the arginine attenuator peptide (AAP), first described in the 5′ UTR of the *arg-2* gene of *Neurospora crassa*,[66,67] in the 5′ UTR of the orthologous *C. cinerea* gene (CopciAB_446268) (Figure S3). These data indicate that, similarly to other fungal and eukaryotic genomes, uORFs are widespread in 5′ UTRs of *C. cinerea* genes and that some uORFs are highly conserved across fungi.

UTRs

Across all gene models (n = 13,617), 88% and 89% of the genes have a 5′ UTR and a 3′ UTR annotation, respectively. The median length of the 3′ UTRs is at least twice as long as that of 5′ UTRs (5′ UTR, 68 nt; 3′ UTR, 141 nt) (Figure 2B). We found 1,738 long-read-based adjacent gene pairs with overlapping UTR annotations, with a median overlap of 99 nt. 1,517 of these overlaps were found between convergently oriented gene pairs (+><−), whereas in other orientations, the overlap was considerably less frequent (Figure 2C). Overlapping UTRs in convergently

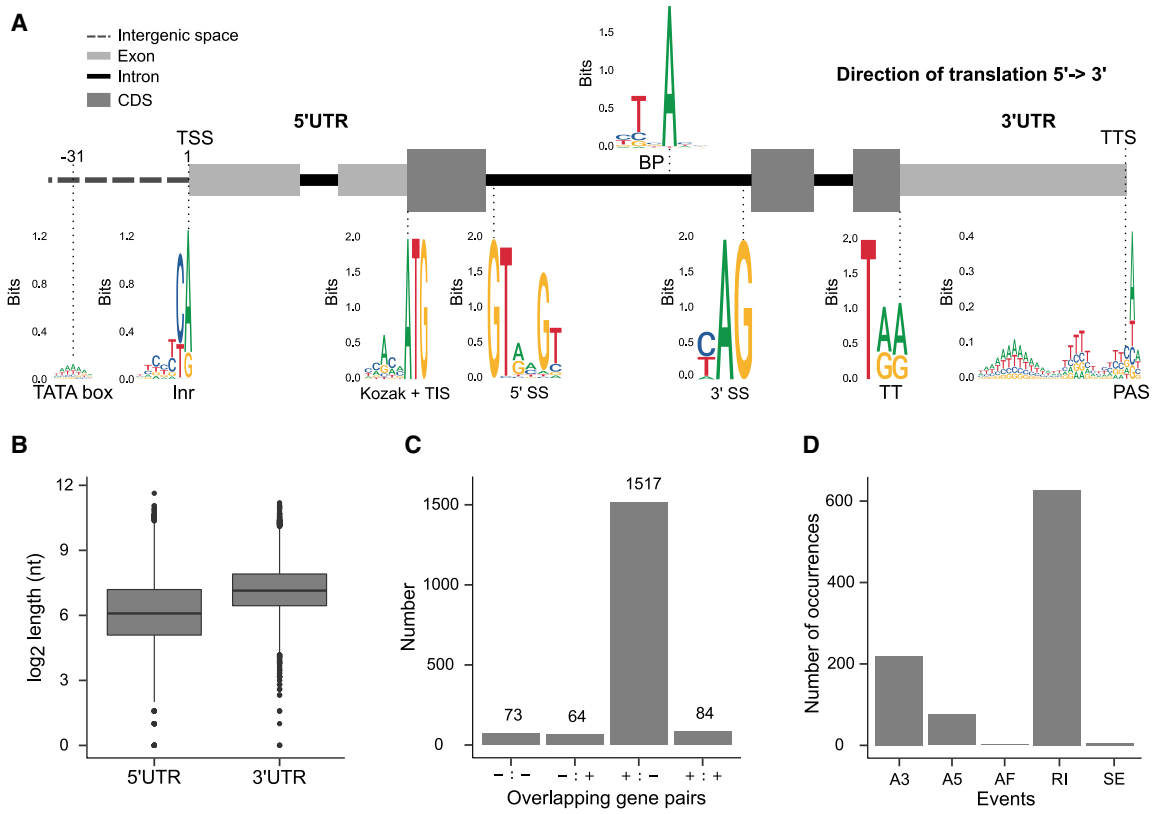


Figure 2. Presentation of the characteristic features of the predicted gene models of *C. cinerea*

(A) Schematic representation of a typical *C. cinerea* protein-coding gene model, highlighting conserved motifs. TATA box and initiator (Inr) consensus motifs typically associated with highly expressed promoters and located in close proximity to transcription start site (TSS). Kozak sequence context localizes adjacent to the translation initiation site (TIS) and regulates its selection. Conserved 5′ splice site (5′ SS), branchpoint (BP), and 3′ splice site (3′ SS) motifs coordinate the spliceosome activity. Translation termination (TT) marks the sequence context defined by the stop codons. The transcription termination site (TTS) marks the 3′ end of the transcript, which is defined by the polyadenylation site (PAS).

(B) UTR length distributions plotted as boxplots. The bottom of the box represents the first quartile of the data, the black line in the middle of the box represents the median of the data, and the top of the box represents the third quartile of the data.

(C) The distribution of the overlapping gene pairs in four possible combinations of gene orientation. The combinations were indicated in the following way: −/− expressed from the antisense strands in the same direction, +/+ expressed from the sense strands in the same direction, −/+ expressed divergently from antisense and sense strands, and +/− expressed convergently from the sense and antisense strands.

(D) Distribution of the alternative splicing events across alternative 3′ SS (A3), alternative 5′ SS (A5), alternative first exon (AF), retained intron (RI), and skipping exon (SE).

oriented gene pairs is common in fungal genomes and can affect transcription and translation.[13,14,68,69]

Transcript isoforms and alternative splicing

As described earlier, an appropriately sensitive method will eventually detect splice variants for every multi-exon gene,[70] not all of which may be biologically relevant. Therefore, only gene isoforms with a read support of at least 10% of total reads for each gene were considered here. Using this conservative approach, 1,053 genes, 7.7% of all genes, were found to have at least one splice isoform, which is consistent with previous studies[71] but considerably lower than Illumina-based figures.[24,72] The relative frequencies of splicing events were consistent with those reported earlier,[16,71,73] with retained introns being most common (67.5%), followed by alternative 3′ splice site (23.5%), alternative 5′ splice site (8.2%), exon skipping (0.5%), and alternative first exon (0.2%) (Figure 2D).

Alternative polyadenylation

To explore the use of PASs in *C. cinerea*, we profiled gene expression across 67 different conditions covering several aspects of the biology of the fungus. We used QuantSeq, a 3′ sequencing approach that yields information on the junction of 3′ UTRs and poly(A) tail[74] and thus can provide PAS information.[54] In the first round, all available QuantSeq reads were used to identify 339,309 PASs. Because we applied stringent filtering criteria (see STAR Methods), we could use only 356 million of the 27 billion QuantSeq reads. Nearby PASs were clustered into PAS clusters (PACs) by allowing a maximum of 10-nt intra-cluster distance, yielding 76,879 PACs. Within a PAC, the PAS with the highest read support serves as the representative PAS for the cluster (Figure 3A), and we used its genomic coordinate for defining a specific PAC.

PACs are enriched in the "sense" orientation in 3′ UTRs (31.28%) and CDSs (25.54%) of the gene models (Figure 3B).

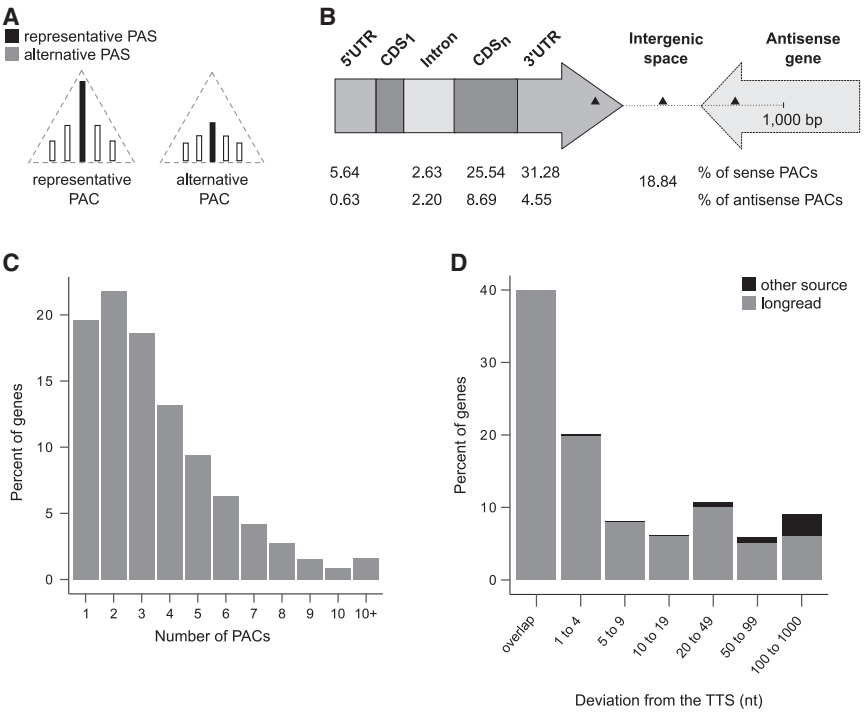


Figure 3. Description of the relationship between the predicted gene models and the PACs

(A) Schematic diagram illustrating the hierarchy of PASs within PACs. The representative PAS with the largest read support is colored black. The best-supported PAC in the case of APA is marked by gray background.

(B) Proportions of 76,879 PACs mapping to parts of a gene model and intergenic region. 40,994 PACs, marked with black triangle, were assigned to the genes when located in the 3′ UTR of the gene, in the intergenic region or in the genic region of downstream antisense genes <1,000 bp from the gene's TTS. Note that if the gene is followed by another in the same orientation, then different rules were used (see STAR Methods).

(C) Proportion of genes with different number of PACs.

(D) Percentage of genes whose TTS is within a certain distance OF the PAC site in genes of different origins in the annotation. See also Table S1.

PACs positioned in non-3′ UTR parts of the gene models may be results of internal priming or template switching at sites with >3–5 adenines,[52] to which the QuantSeq protocol is susceptible. The presence of spurious PACs is suggested by the ~3× higher median expression of the representative PAS of PACs in the 3′ UTRs than that in the other annotation entries (Table S1). Therefore, we further filtered PACs based on localization and orientation, keeping in mind that, due to the frequent overlap of 3′ UTRs between fungal genes, relevant PACs can also be located in neighboring genes. We obtained 40,994 PACs, which were assigned to 11,628 gene models. Over 80% of the genes with an assigned PAC have more than one PAC, and thus, we considered them as genes with putative APA (Figure 3C). For each APA gene, the PAC whose representative PAS had the highest count is hereafter referred to as the best-supported PAC (Figure 3A).

TTS coordinates of genes from the long-read-based annotation perfectly overlap with the coordinates of the best-supported PAC in ~40% of genes and deviates from it by, at most, 4 nt in another 19.75% (Figure 3D). In contrast, the distances of non-long-read-based gene models from their best-supported PAC are considerably larger (Figure 3C), perhaps due to the absence or misannotation of the 3′ UTR.

To understand PAC usage in APA genes across biological conditions, we calculated the evenness in each experiment for each APA gene with an expression ≥10 counts per million (CPM). Figures 4A–4C show that there is a significant negative correlation between evenness and gene expression level; that is, the higher the gene's expression, the less diverse its PAC usage is. This could indicate that, for each gene, a certain PAC is preferred over others, which is especially easy to notice for highly expressed genes. To identify the preferred PAC among the different biological conditions, we ranked PACs based on their relative expression.[75] We found that most genes used the same, most highly expressed (rank 1) PAC under all biological conditions tested (Figure 4C) and that this correlation held strong for highly expressed genes (Figure 4D).

When we checked the mean 3′ UTR length variation in the first four APA ranks with that of non-APA genes (≥10 CPM), we found that the non-APA genes had shorter 3′ UTRs followed by the highest expressed PAC (rank 1) and other PACs in order of decreasing read support (ranks 2–4) (Figure 4E). Thus, there is a clear correlation between PAC use frequency and distance from the CDS. That is, the higher the relative expression of the PAC, the shorter the resulting 3′ UTR. This suggests that *C. cinerea* most often expresses the transcript with the shortest 3′ UTR version. At the same time, we did not find significant variation of PAC usage across biological conditions, which suggests that APA is a prevalent, though probably not influential, phenomenon in *C. cinerea*. This is consistent with APA being "transcriptional noise" and not an actively regulated process.[75]

Collectively, the annotated polyadenylation clusters correspond very closely with the predicted TTS of the gene models derived from long reads, indicating that our long-read-based gene models, including their 3′ UTRs, represent robust annotations. In terms of APA, it appears that most genes, especially highly expressed ones, prefer one PAC and mainly the one that results in the shortest 3′ UTR.

Expression data disentangle light and starvation responses and morphogenesis

In contrast to Ascomycota, where extrapolations from model organisms and a long tradition of both forward and reverse genetics studies yielded functional information for a considerable proportion of genes, Basidiomycota gene function is notoriously

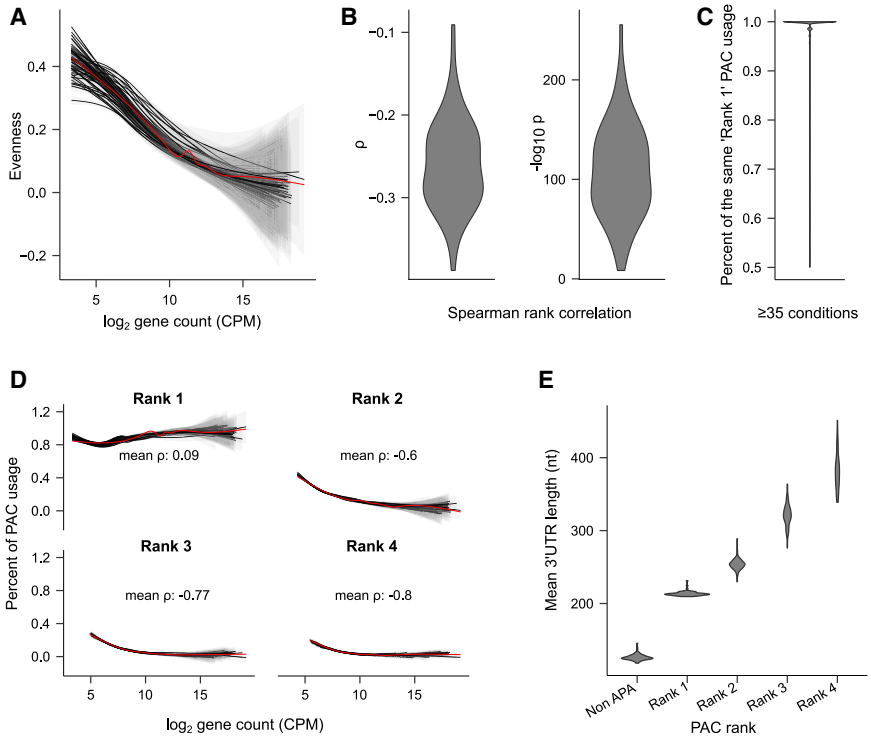


Figure 4. Characterization of PAC use preference in *C. cinerea*

(A) Negative correlation between evenness of PAC site usage and gene expression in APA genes. Note that the higher the expression the less diverse PAC usage is. Lines represent 67 biological conditions analyzed by QuantSeq. The red line represents a mean across all biological conditions.

(B) Violin diagrams of the distribution of the Spearman rank correlation rho (ρ) and the $-\log_{10}$ *p* values of the correlation between evenness and $\log_2$ gene count across the 67 biological conditions.

(C) Violin diagram showing the relative usage of the PAC with highest read support (rank 1) for APA genes expressed in at least 35 biological conditions. Only APA genes with at least one PAC having >10 CPM were considered.

(D) The correlation between the frequency of a given PAC rank and gene expression. The black lines represent 67 biological conditions. The red line represents a mean across all biological conditions.

(E) The association between PAC usage and mean 3′ UTR length across non-APA and APA genes. Note the clear correlation between rank (i.e., decreasing expression) and 3′ UTR length. Violin plots represent 3′ UTR length distributions across biological conditions.

poorly understood. To mitigate this, we profiled gene expression under 67 conditions (201 samples), covering basidiospore and oidium germination, mycelium growth in the dark and after light induction, hyphal knot, sclerotium and fruiting body development, and carbon starvation, as well as 11 stress conditions. Differential gene-expression analyses identified a total of 12,569 differentially expressed genes (DEGs; Benjamini-Hochberg [BH] adjusted $p \leq 0.05$, fold change ≥ 2) (Figure S4), indicating that the 67 conditions induced significantly altered expression of 92.3% of all genes.

Here, we focus more closely on densely sampled time course data for light induction, morphogenesis, and starvation (Figure 5A). Fruiting body development in the basidiomycetes happens in response to changing environmental variables,[29,76] of which the most important are carbon starvation and light.[29] However, under standard fruiting conditions, distinguishing the specific gene-expression responses attributable to starvation and light from those associated with morphogenesis has not been possible. To this end, we obtained consensus gene lists by comparing DEGs in two starvation-inducing experiments: after light induction and two mycelial types (aerial and attached mycelium). Figure 5B shows how comparisons of DEGs from these experiments yielded functional gene groups, which we designate as core starvation response, core light response, and core mycelium differentiation genes.

Carbon starvation

We induced starvation by growing *C. cinerea* on yeast-malt-glucose (YMG) plates with reduced (0.2%) glucose content (called standard experiment)[27] and by transferring 60-h-old dark-grown colonies to agar containing no nutrients (SWAT experiment) (Figure 5A). In both the standard and SWAT experiments, DEG analyses were run with the 60-h dark-mycelium sample as reference. In the standard experiment, nutrients deplete gradually, whereas in the SWAT experiments, nutrient depletion is abrupt, which is expected to induce a stronger starvation response. Accordingly, in SWAT experiments, most gene-expression changes are observable at 8 h, whereas in the standard experiment, upregulation patterns are more diverse, which could stem from a combination of milder starvation, light irradiation, and tissue differentiation. We found 3,242 and 2,348 significantly upregulated genes ($p \leq 0.05$, fold change ≥ 2) in the standard and SWAT experiments, respectively. Of these, 1,759 genes were upregulated in both experiments and are hereafter refer to as core starvation response genes (Table S2).

Gene Ontology (GO) enrichment analyses provided a clear signal for the upregulation of genes encoding plant cell wall-degrading enzymes (PCWDEs) and transporters, among others (Figure S5). Based on these findings and previous considerations of starvation, we prioritized PCWDEs and transporters, as well as autophagy and fungal cell wall-degrading CAZymes (FCWDEs) for deeper scrutiny.

Out of the 213 PCWDEs and 375 transporters of *C. cinerea*, 136 and 134 were upregulated in at least one of the starvation experiments and 96 and 55 (Figure S6) were among the core starvation response genes, respectively. We also compared these gene lists with *C. cinerea* genes upregulated in carbon-catabolite repressor (*cre1*, CopciAB_466792) deletion strains[77] and found that starvation-induced PCWDEs and transporters overlap considerably with those upregulated in the Δ*cre1* strains. Of the 62 PCWDEs and 19 transporters upregulated in Δ*cre1*, 52 (83.9%) and 10 (52.6%), respectively, were also part of the core starvation response.

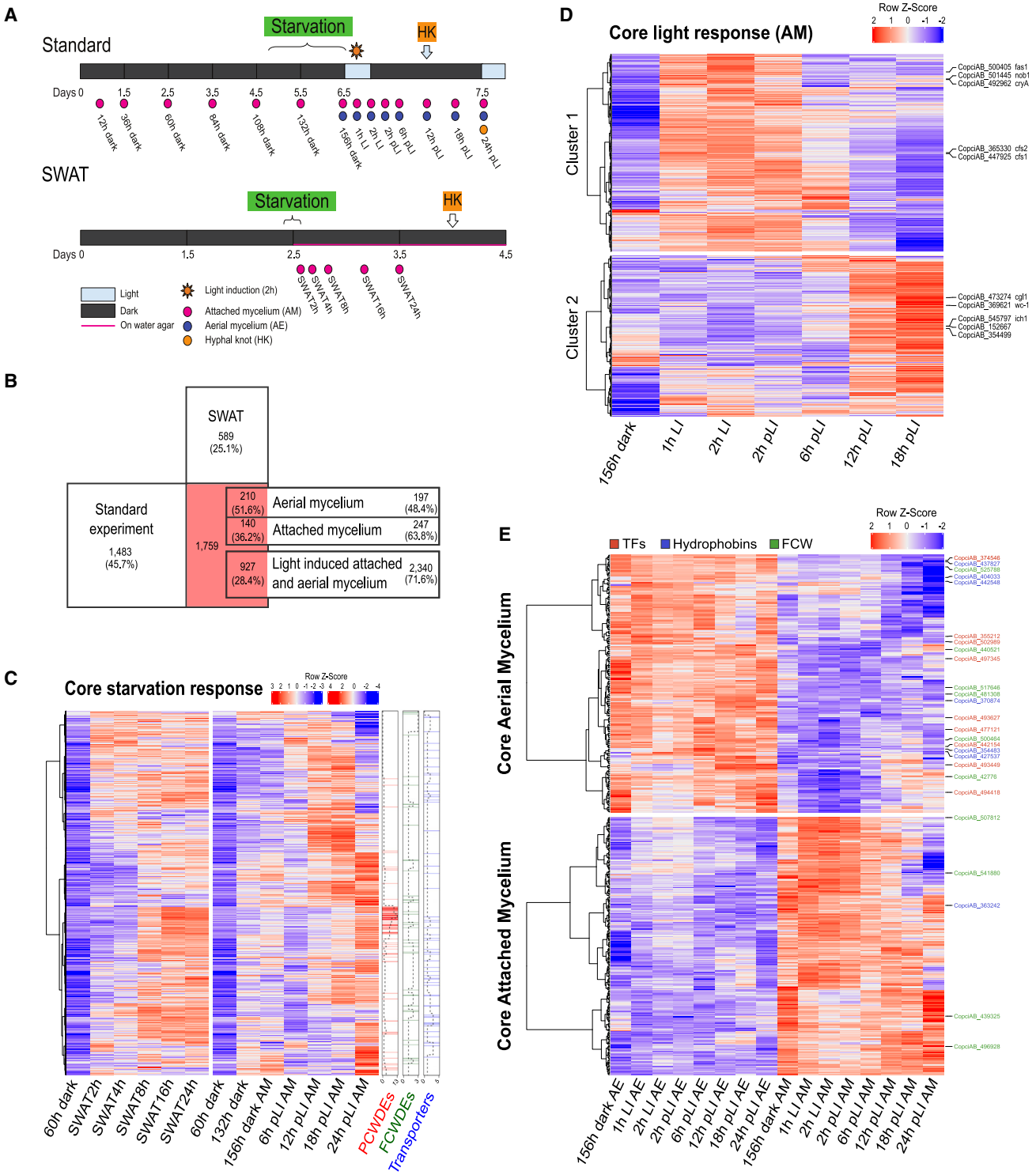


Figure 5. Illustration of the experimental design used to define core gene sets whose expression profiles are shown in the form of a heatmap

(A) Graphical representation of the experimental conditions used for light induction, starvation, and mycelial type. The day-light cycles are marked on the strip with dark-light segments. The reference times are marked below the strip. The collected samples are shown with a colored dot below the time references, where the colors represent the tissue type of the collected sample.

(B) The stylized Venn diagram illustrates the overlaps between the sample sets that were made. The red square marks the core starvation response, which is present to some extent in all sample groups. The size and proportion of the different sections are also indicated.

(legend continued on next page)

Core starvation-responsive PCWDEs included 67 acting on cellulose (e.g., AA9, CBM1, GH3, GH5, GH6, GH7, expansins), 30 hemicellulose (e.g., GH11, GH31, GH93), 16 pectin (GH28, GH88, various PL families), but none of the few lignin-degradation-related genes of *C. cinerea*. Of the 364 transcription factors (TFs) identified in *C. cinerea*, the ortholog (CopciAB_447627) of the cellulase-activating *roc1* TF of *Schizophyllum commune*,[78] together with an additional 39 TFs, was part of the core starvation response. Five of these TFs genes, including *roc1*, were also upregulated in the Δ*cre1* strains.

The core starvation response contained many members of the major facilitator superfamily (MFS), including putative orthologs of experimentally characterized Ascomycota glucose and xylose transporters, including HGT1/HGT-1 of *Candida albicans* and *N. crassa* (CopciAB_501903) or XtrD of *A. nidulans* (CopciAB_358584). MFS transporters in the core starvation response and upregulated in the Δ*cre1* strain included CopciAB_496081 and CopciAB_496145, which encode putative orthologs of the *N. crassa* cellodextrin transporter CLP1 and hexose transporter CDT-2[79,80] (Figure S7).

The core starvation response contains several genes considered essential for fruiting body formation. The *dst-2* gene (CopciAB_361584)[81] is a hypothesized blue-light photoreceptor in *C. cinerea*. Its upregulation in the SWAT experiments is surprising given that these experiments did not receive light. Two fungal galectins, Cgl1 and Cgl2 (CopciAB_473274, CopciAB_488611),[82] which are considered to be involved in hyphal knot formation, were responsive to starvation. This is in line with publications[82,83] showing a link between starvation and fruiting body induction. None of these genes was upregulated in the Δ*cre1* strain.

One of the hallmarks of starvation is autophagy and hyphal autolysis, which may be associated with increased expression of autophagy and fungal cell wall remodeling CAZyme genes,[84–86] respectively. In *C. cinerea*, we found 12 putative orthologs (reciprocal best hits) of 35 autophagy genes of *S. cerevisiae*.[86] Eight of these were upregulated 2 h after transfer to water agar (Figure S8); however, only one of them (CopciAB_375414) was significantly differentially expressed. Of the 138 *C. cinerea* genes encoding CAZymes with an activity on fungal cell wall, 40 were part of the core response (Figure S6). This suggests that there is active remodeling and/or digestion of the fungal cell wall, which could indicate autolytic processes in starving *C. cinerea* cultures.

We defined a core set of starvation-induced genes that can be used to understand how the fungus prepares for fruiting body development, which is induced by nutrient depletion. These indicated that upon starvation, *C. cinerea* was probably released from carbon-catabolite repression, transcribed PCWDE and morphogenetic genes, and showed signs of autophagy or autolysis. These observations are similar to those made in starving Ascomycota cultures[84,86,87] and the basidiomycete *Paxillus involutus*.[85] In the ascomycetes, the carbon starvation response overlaps with the response to lignocellulosic carbon sources in that it comprises the induction of several PCWDEs.[84,88,89] We found similar patterns in *C. cinerea* and hypothesize that this is because, upon glucose depletion, the fungus explores available alternative carbon sources by expressing a broad panel of hydrolytic enzymes. Such scouting enzymes may generate simple sugars that transporters import into the cell, which may trigger positive regulatory feedback loops that strengthen the transcription of genes related to the carbon source found.[87,90]

Light-induced genes

We examined the effects of light induction by using a synchronized growth protocol,[27] with a 2-h light induction followed by 24 h in the dark and then alternating 12-h light/dark cycles. We sampled aerial and attached mycelium separately for eight time points (Figure 5A) and identified light-induced genes by comparing all time points to 6.5-day-old dark-grown aerial or attached mycelium as reference (called 156-h dark; see Figure 5A). We identified 2,453 and 2,065 DEGs in the attached and aerial mycelium samples (Table S3), respectively. Aerial and attached mycelia shared 1,251 DEGs, indicating high similarity in light response irrespective of mycelial type. We used the union of 2,453 and 2,065 genes and removed genes that belong to the core starvation response to identify 2,340 genes specifically induced by light irrespective of mycelial type but not by starvation. We hereafter refer to these as core light-induced genes (Figure 5B; Table S3).

To understand light-induced gene-expression changes, we analyzed the expression of 2,340 core light-induced genes in attached mycelium samples (Figure 5D). We note that aerial mycelia show similar, though noisier, patterns (see Figure S9). An expression heatmap revealed two clear waves of gene upregulation, one evident 1 h after the start of light induction and decreasing expression after 6 h post light induction (pLi) and another wave starting around 12 h pLi (Figure 5D). Hierarchical clustering recovered two clusters corresponding to these two waves. We hereafter refer to cluster 1 (1,291 genes) and cluster 2 (1,049 genes) as early and late genes, respectively, which probably correspond to the two waves of light-responsive gene induction reported earlier.[83] The expression of early genes started to decline relatively quickly, consistent with photoadaptation mechanisms that inhibit white collar complex (WCC) activity. We note that the first hyphal knots emerged at 12 h pLI, so it is

(C) Heatmap representation of the $\log_2$CPM expression values of the core starvation response genes of the water-agar transfer (SWAT, left) and half-glucose YMG medium-induced starvation (standard, right) experiments. The columns on the right mark the position of the plant cell wall-degrading enzyme genes (PCWDEs, red), fungal cell wall-related enzymes (FCWDEs, green) and transporter-encoding genes on the heatmap. To visualize the distribution of the genes, a rolling window with a width of 20 genes and a length of 20 steps was used and is shown with a dashed line.

(D) Heatmap representation of the $\log_2$CPM expression values of the core light response genes in the AM samples. Genes were hierarchically clustered into two groups using the Ward D method based on the Euclidean distance between the *Z*-score-normalized $\log_2$CPM gene-expression values. Genes identified in the literature as early and late light-induced genes are marked on the right.

(E) Heatmap representation of the $\log_2$ CPM expression values of the core aerial and attached mycelium specific genes in the aerial and attached mycelium samples. Putative TF-, hydrophobin-, and FCWDE-encoding genes are marked with red, blue, and green colors on the right. See also Figures S5–S15 and Tables S2–S4.

possible that late genes include ones related to hyphal knot development.

GO analyses revealed a significant effect of early light induction (Figure S10). Of the significant GO terms, chromatin remodeling has been shown to be important for sexual development of *C. cinerea*.[91] Late light-induced genes were enriched for different GO terms, including those related to transcription regulation (GO:0006355), fungal-type cell wall (GO:0009277), DNA packaging (GO:0044815), or G-protein signaling (GO:0007186) (Figure S10), consistent with the appearance of hyphal knots 12 h pLi.

Among the early light-induced genes we found *fas1*, *nod1*, *cfs1*, and *cfs2*, which were reported earlier to respond quickly to blue light.[83,92] The ortholog of the *A. nidulans* blue-light sensor/photolyase *cryA* (CopciAB_492962) belongs to early genes, while the blue-light-sensor gene *dst1* (CopciAB_369621) belongs to late genes. Similarly, *cgl2* (CopciAB_488611), which was proposed to be a marker gene of primary hyphal knots,[23] was among the early light-induced genes, whereas *cgl1* (CopciAB_473274), a proposed marker of secondary hyphal knots, only followed later, 12 h pLi. Wc-2 (CopciAB_8610)[93] was not upregulated in the tested samples.

We also identified diverse genes related to DNA repair (GO:0006281) (Figure S11), possibly for alleviating the effects of DNA damage caused by the ultraviolet light. For example, we detected an ortholog of the *S. pombe* UV-endonuclease *uvdE* (CopciAB_359391) and a *cryA* ortholog photolyase (CopciAB_492962), which is WCC activated in *Ustilago*,[94] *Schizophyllum*,[95] and *Neurospora*[96] and protects from phototoxicity. These observations suggest that these and other early genes might be WCC activated in *C. cinerea* and may form a conserved light-responsive DNA-repair network. We hypothesize that the broad upregulation of DNA-repair genes is related to repairing DNA damage caused by UV or reactive oxygen species (ROS) generated by photons.[97]

Early light induction resulted in the upregulation of diverse lipid metabolism and genes related to glycosylphosphatidylinisotol-anchor synthesis. Of the nine predicted fatty acid desaturases, which include linoleic acid synthases, five can be found among early genes (cluster 1) and only one among late genes (cluster 2). The upregulation of putatively linoleic acid producing fatty acid desaturase genes suggests that membrane fluidity is regulated in response to light, similar to what was found in fruiting bodies.[76]

In summary, our data show that light induces broad transcriptional changes in *C. cinerea*. The observed patterns are similar to those reported in *N. crassa*, where an early wave of light induction is regulated by the WCC. We note that the early wave of gene expression in *C. cinerea* (0–6 h pLi) is considerably slower than that of *N. crassa* (45 min),[98] and more research is needed to clarify whether they represent homologous responses. We hypothesize that *C. cinerea* early genes are also regulated by the WCC, even though motif analyses did not recover a motif similar to that reported in *N. crassa*[99] or *Lentinula edodes*.[100] It is also noteworthy that late genes show up in a consensus catalog of conserved fruiting-related genes,[76] indicating that illumination induces light-responsive genes also during fruiting body development. Finally, it should be noted that here we specifically analyzed upregulated genes, and analyses of light-repressed genes might yield additional insights.

Genes related to mycelial type

Fungal hyphae might be submerged in or attached to the substrate or be erect, forming the aerial mycelium. Fruiting bodies develop from hyphal knots, which in turn emerge by the branching of erect hyphae,[23,101,102] indicating that aerial mycelium may have an important role in fruiting body formation. The transition from attached to aerial mycelium, in contrast to fruiting body development, has scarcely been analyzed.[101–103] Therefore, we analyzed gene expression separately for the aerial vs. attached mycelia at each time point in our light-induction experiment (Figure 5A). Since matched pairs of samples received the same light treatment, we expected DEGs to reflect differences mainly related to tissue type. We identified 501–1,275 and 574–1,135 DEGs upregulated genes in attached and aerial mycelium, respectively, across the time course (Table S4).

GO enrichment analysis revealed that diverse metabolic processes dominated DEGs upregulated in attached mycelia, whereas in aerial mycelium, terms related to transcription regulation (GO:000635), the cell wall (GO:0009277), and transporters (GO:0055085), among others, were consistently enriched (Figures S12 and S13). This suggests that the two mycelial types perform divergent functions. The term fungal-type cell wall (GO:0009277), which is linked, among others, to hydrophobin (IPR001338) upregulation, was enriched in both mycelial types but considerably more strongly in aerial mycelium (Figures S12 and S13). Aerial mycelia further showed GO enrichment signal for lipid (GO:0006629), steroid (GO:0006694), and sphingolipid (GO:0006665) metabolism (Figures S12 and S13). To follow up the GO results, we analyzed the expression of genes encoding hydrophobins, cell wall synthesis and remodeling enzymes, and TFs. Aerial mycelia displayed a considerably stronger hydrophobin gene expression than attached mycelium (Figure S14) in line with previous studies.[101,104,105] Hydrophobin and FCWDE-related CAZyme gene upregulation in the aerial mycelium became stronger as time progressed, possibly indicating increasing specialization at the cell surface. Generally, more TFs were upregulated in the aerial than in the attached mycelium, although the numbers converged as time progressed, and at 24 h pLi, the attached mycelium had more upregulated TFs than the aerial mycelium.

We next attempted to identify a core set of genes that are consistently upregulated in attached or aerial mycelium. This yielded 407 and 387 upregulated in at least five out of eight comparisons in the aerial and attached mycelia, respectively. After removing genes that are also members of the core starvation response, we obtained 197 and 247 genes (Figures 5E and S15; Table S5). Among these, aerial mycelia had nine TFs, six hydrophobins, and six FCW genes upregulated, whereas attached mycelia had no, one, or four upregulated genes in these categories, respectively. The nine TFs consistently upregulated in aerial mycelium suggest there are specific regulatory patterns in this tissue type. One of these is orthologous to *hom1* of *S. commune* (CopciAB_493627), which was reported to regulate vegetative growth and fruiting body development.[106,107]

Website

The *C. cinerea* 2.0 genome has been deposited in MycoCosm[3] and in a new website (https://mushroomdb.brc.hu/). The latter offers the possibility to identify the desired gene based on sequence similarity and keywords from the functional annotation and incorporates a genome viewer and browser (long-read alignments, Illumina, and QuantSeq read coverage), data on gene conservation, orthology in other mushroom-forming fungi, and tools to analyze gene-expression levels in new and published datasets.[24,108–110] The website is centered on *C. cinerea*, as the currently best-known mushroom-forming model species, but includes data from several industrially relevant and commercially produced mushroom-forming species too.

DISCUSSION

In this study, we produced a high-quality annotation of protein-coding genes of *C. cinerea* Amut1Bmut1 #326 by combining a new, chromosome-level assembly, Nanopore and PacBio IsoSeq cDNA-seq data, information from two available annotations, and several rounds of manual correction. To improve functional descriptions of genes, expression profiling was done under dozens of conditions, which we used to glean novel insights into transcriptome states related to starvation, light induction, and mycelial differentiation.

The new *C. cinerea* genome eliminates several problems associated with previous genomes of this species and Agaricomycetes in general. First, most Agaricomycetes genomes are drafts, and only a few are chromosome-level assemblies.[5–11] In addition, gene annotation is usually based on short-read assisted automatic pipelines, which fail to capture key gene features (e.g., APA, uORFs, UTRs) and often yield imprecise gene models.[15,16] It has been shown that such annotation errors can propagate across genomes[111] and can lead to poor predictions of the biological function of genes and encoded proteins.

The new *C. cinerea* genome has better-quality metrics and higher annotation completeness than previous ones. Of the 13,617 gene models, 85% were supported by at least one long read. 5′ and 3′ UTR annotations were obtained for 88% and 89% of the gene models, respectively. Comprehensive UTR annotations enabled the detection of (un)structured elements, such as uORFs in 2,982 genes. By uncovering these genomic features in *C. cinerea*, this study adds the first multicellular Basidiomycota to the considerably denser array of Ascomycota (e.g., *S. cerevisiae*, *N. crassa*, *S. pombe*, *C. albicans*) in which these features have been analyzed. The improved annotation enabled the detection of conserved sequence motifs (e.g., Inr, TATA box) and will help defining *cis*-regulatory elements. We found a large number of microexons that, if overlooked, can lead to premature stop codons. To further validate gene models, we utilized QuantSeq's ability to capture the junction of the 3′ UTRs and the poly(A) tail. This way, we were able to confirm the PAS of the gene models and also obtained information on APA.

Using gene-expression profiling, we enriched the annotation with expression-derived descriptions of gene function. We defined gene sets representing the core response to starvation (1,759 genes), light induction (2,340 genes), and hyphal differentiation (197 and 247 genes in aerial and attached mycelium). These three factors are usually simultaneously present to varying extents in fruiting experiments,[29] and previous studies could not tease them apart from those of fruiting body morphogenesis. We think these gene sets represent a significant improvement over previous RNA sequencing-based treatments of the basidiomycete life cycle, and we anticipate that, beyond starvation, photobiology, and mycelial differentiation, these data will contribute to a better understanding of the fruiting process. For example, by revealing transcriptomic similarities between fruiting bodies and attached or aerial mycelia, it will eventually reveal the developmental origins of fruiting body cells. We also expect the data to provide further insights into the photobiology of basidiomycetes and enable comparative analyses of light responses of basidiomycetes with those of the Ascomycota (e.g., *N. crassa*).[112]

Some limitations resulting from the employed technologies should be mentioned. While we expect the vast majority of genes to be correctly annotated, the annotation of some genes (e.g., lowly expressed long genes) or features may be improved further. Given our stringent filtering criteria to assemble the gene catalog, it is possible that future studies will discover some, albeit likely not many, additional genes in *C. cinerea*. The expression data generated in this study allowed the identification of genes responsive to light, starvation, and tissue type. However, given the diversity of conditions, we envision that these data could be used for addressing more complex questions on gene expression, such as co-expression patterns or global regulatory networks. An exciting next step toward network-based approaches will be exploring *cis*-regulation in *C. cinerea*, by functional assays (e.g., ATAC-seq, DAP-seq, or chromatin immunoprecipitation sequencing). Regulatory interactions could also be interrogated by directed perturbation of the network by reverse genetics, as shown recently by analyses of the *cre1*, *snb1*, *nsdD1* and *nsdD2* genes.[77,92,113] Another promising avenue is extending these analyses to non-model basidiomycetes beyond *C. cinerea* to enable rigorous comparative analyses across species.

This study has improved genomic resources for *C. cinerea*, making it potentially the most comprehensively annotated species among the Agaricomycetes. We anticipate that this work will serve as a foundational resource for further studies, particularly those in which genome quality and accurate gene model prediction are essential, and will facilitate further research on mushroom-forming and lignocellulose-degrading Agaricomycetes as well as functional genomic comparisons across the fungal kingdom.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, László G. Nagy (lnagy@fungenomelab.com).

Materials availability

No new or unique reagents were generated in this study.

Data and code availability

- The raw sequences from the PacBio long-read genome sequencing have been deposited to the NCBI Sequence Read Archive (SRA) under

accession number PRJNA1076273. Long-read transcriptome sequences, including PacBio IsoSeq and Nanopore sequencing, have been submitted to the NCBI SRA under accession numbers PRJNA1076272 and PRJNA1215360, respectively. The Illumina (QuantSeq) gene-expression data associated with this study have been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSE287886.

- The original code generated for data analysis is available on GitHub (https://github.com/HegedusB/CopciGenomeAnnot, https://doi.org/10.5281/zenodo.15019528).
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

B.H. and L.G.N. designed research. B.H., V.B., M.V., R.R., A.L., M.K., E.S., J.G., K.B., V.N., P.U., A.G., M.F., and I.V.G. performed research. B.H. and V.B. curated data. B.H., N.S., B.B., S.H., Z.M., H.W., X.-B.L., P.U., and L.G.N. analyzed data. B.H. and L.G.N. wrote the paper. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
Blood and Cell Culture DNA Maxi Kit	Qiagen	Cat#13362
Quick-RNA Miniprep kit	Zymo Research	Cat# R1054
SMRTbell Express Template Prep Kit 2.0	PacBio	Cat#100-938-900
AMPure PB Beads	PacBio	Cat#100-265-900
Sequel II Binding kit 2.0	PacBio	Cat#101-789-500
NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module kit	NEB	Cat#E6421
NEBNext High-Fidelity 2X PCR Master Mix	NEB	Cat#M0541
ProNex beads	Promega	Cat#NG2001
TeloPrime Full-Length cDNA Amplification Kit V2	Lexogen	Cat#013.24
Oxford Nanopore Native Barcoding Expansion Kit	nanoporetech	Cat#EXP-NBD104
Ligation Sequencing Kit	nanoporetech	Cat#SQK-LSK109
MinION flow cells	nanoporetech	Cat#FLO-MIN106 R9.4.1
QuantSeq 3′mRNA-Seq Library Prep Kit FWD	Lexogen	Cat#113.96
Deposited data		
Raw sequences from the PacBio long read genome sequencing	This study	SRA: PRJNA1076273
Raw PacBio Iso-Seq transcriptome sequences	This study	SRA: PRJNA1076272
Raw Nanopore transcriptome sequences	This study	SRA: PRJNA1215360
Illumina (QuantSeq) gene expression data	This study	GEO: GSE287886
RNA-Seq data of different developmental stages in *Coprinopsis cinerea #326*	Krizsán et al.[24]	GEO: GSE125184
Illumina dataset for genome resequencing of *Coprinopsis cinerea* #326	Xie et al.[28]	SRR10162428
RNA-Seq data of cre mutant Coprinopsis cinerea #326 strains	Pareek et al.[77]	GEO: GSE205749
Script collection used for this work	This study	https://github.com/HegedusB/CopciGenomeAnnot
Experimental models: Organisms/strains		
Coprinopsis cinerea Amut1Bmut1 #326 strain		FGSC#25122
Software and algorithms		
Racon (version: 1.4.13)	Vaser et al.[114]	https://github.com/isovic/racon
Flye (version: 2.8.1-b1676)	Kolmogorov et al.[115]	https://github.com/mikolmogorov/Flye
BBTools (version: 38.79)	Bushnell et al.[116]	https://sourceforge.net/projects/bbmap/
ITSx	Bengtsson-Palme et al.[117]	https://microbiology.se/software/itsx/
lima	PacBio	https://github.com/PacificBiosciences/pbbioconda?tab=readme-ov-file
Guppy (version: 3.2.4)	nanoporetech	https://nanoporetech.com/document/Guppy-protocol
Pychopper (version: 2.3.1)	nanoporetech	https://github.com/nanoporetech/pychopper
LoRDEC (version: 0.9)	Salmela et al.[118]	https://gite.lirmm.fr/lordec/lordec-releases/-/wikis/home
ORNA (version: 0.4)	Durai et al.[119]	https://github.com/SchulzLab/ORNA
pomoxis (version: 0.3.10)	Nanoporetech	https://github.com/nanoporetech/pomoxis
MUMmer (version: 4.0)	Marçais et al.[120]	https://github.com/mummer4/mummer

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
SyRI (version: 1.6.3)	Goel et al.[121]	https://github.com/schneebergerlab/syri
Plotsr (version: 1.1.0)	Goel et al.[122]	https://github.com/schneebergerlab/plotsr
fastp (version: 0.21.0)	Chen et al.[123]	https://github.com/OpenGene/fastp
bwa (version 0.7.17-r1198-dirty)	Li et al.[124]	https://github.com/lh3/bwa
samtools (version 1.13)	Danecek et al.[125]	https://github.com/samtools/samtools
picard (version: 3.0.0)	Broad Institute	https://github.com/broadinstitute/picard
GATK (version: 4.4.0.0)	McKenna et al.[126]	https://github.com/broadinstitute/gatk
DeSALT (version: 1.5.6)	Liu et al.[127]	https://github.com/ydLiu-HIT/deSALT
samclip	Seemann[128]	https://github.com/tseemann/samclip
TAMA	Kuo et al.[129]	https://github.com/GenomeRIK/tama
ORFik (version 1.16.6)	Tjeldnes et al.[130]	https://github.com/Roleren/ORFik
STAR (version: 2.6.1a_08–27)	Dobin et al.[131]	https://github.com/alexdobin/STAR
SQANTI3 (version: 5.2)	Tardaguila et al.[132]	https://github.com/ConesaLab/SQANTI3
minimap2 (version: 2.24-r1122)	Li et al.[133]	https://github.com/lh3/minimap2
mmseqs (version: R15-6f462)	Steinegger and Söding[134]	https://github.com/soedinglab/MMseqs2
BUSCO (version: 3.0.2)	Waterhouse et al.[135]	https://busco.ezlab.org/
SUPPA2 (version: 2.3)	Trincado et al.[136]	https://github.com/comprna/SUPPA
ggseqlogo (version 0.2)	Wagih[137]	https://github.com/omarwagih/ggseqlogo
branchpointer (version: 1.14)	Signal et al.[138]	https://github.com/signalbash/branchpointer
DPAC (version: 1.4)	Routh[139]	https://sourceforge.net/projects/dpac-seq/
vegan (version: 2.6–4)	Oksanen et al.[140]	https://github.com/vegandevs/vegan
ggplot2 (version: 3.4.0)	Wickham et al.[141]	https://github.com/tidyverse/ggplot2
FastQC (version: 0.12.0)	Andrews[142]	https://github.com/s-andrews/FastQC
MultiQC (version: 1.19)	Ewels et al.[143]	https://github.com/MultiQC/MultiQC
Rsubread (version: 2.6.4)	Liao et al.[144]	https://github.com/ShiLab-Bioinformatics/subread
edgeR (version: 3.34.0)	Robinson et al.[145]	https://bioconductor.org/packages/release/bioc/html/edgeR.html
limma (version: 3.48.3)	Ritchie et al.[146]	https://bioconductor.org/packages/release/bioc/html/limma.html
ComplexHeatmap (version: 2.14.0)	Gu[147]	https://github.com/jokergoo/ComplexHeatmap
InterProScan (version: 5.61–93.0)	Jones et al.[148]	https://github.com/ebi-pf-team/interproscan
dbCAN (version: 4.0.0)	Zheng et al.[149]	https://github.com/linnabrown/run_dbcan
topGO (version: 2.40.0)	Adrian et al.[150]	https://bioconductor.org/packages/release/bioc/html/topGO.html
GO.db (version: 3.17.0)	Carlson[151]	https://bioconductor.org/packages/release/data/annotation/html/GO.db.html

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Experimental model

For all experiments we used the *C. cinerea* #326 Amut1Bmut1 PABA-strain (FGSC#25122), which carries mutations in both mating type factors that overcomes the self-incompatibility of monokaryotic strains.[152]

Growth conditions and DNA/RNA extraction

High molecular weight DNA for PacBio sequencing was extracted from mycelium samples grown in liquid YMG (0.4% yeast extract, 1% malt extract, 0.4% glucose) medium at 28°C without shaking using the Blood and Cell Culture DNA Maxi Kit (Qiagen, catalog number: 13362) following the manufacturer's instructions. RNA for Nanopore and IsoSeq was extracted from mycelium and fruiting body tissues grown according to the Krizsán et al.,[24] using Quick-RNA Miniprep kit (Zymo Research, USA, catalog number: R1054), following the manufacturer's instructions. The same kit was used for RNA extraction for gene expression profiling. Table S5 provides detailed growth conditions and protocols for gene expression profiling experiments.

METHOD DETAILS

Genome sequencing and assembly

Approximately 10 μg of genomic DNA was sheared to 30–50 kb using the Megaruptor 3 (Diagenode). The sheared DNA was treated with exonuclease to remove single-stranded ends, DNA damage repair enzyme mix, end-repair A-tailing mix, and ligated with overhang adapters using SMRTbell Express Template Prep Kit 2.0 (PacBio, category number: 100-938-900) and purified with AMPure PB Beads (PacBio, category number: 100-265-900). Individual libraries were size-selected (20 kb) using the 0.75% agarose gel cassettes with Marker S1 and High Pass protocol on the Blue Pippin instrument (Sage Science). PacBio sequencing primer was then annealed to the SMRTbell template library and sequencing polymerase was bound with a Sequel II Binding kit 2.0 (PacBio, category number: 101-789-500). The prepared SMRTbell template libraries were then sequenced on a Pacific Biosystems Sequel II sequencer using tbd-sample dependent sequencing primer, 8M v1 SMRT cells, and Version 2.0 sequencing chemistry with 1x1800 sequencing movie run times.

Filtered subread data was processed with the JGI QC pipeline to remove artifacts.

The mitochondrial genome was assembled separately with the CCS reads using an in-house tool, used to filter the CCS reads, and polished with two rounds of Racon version 1.4.13[114] racon [-u -t 36].[114] The mitochondria-filtered CCS reads were then assembled with Flye version 2.8.1-b1676[115] [-g 40M –asm-coverage 50 -t 32 –pacbio-hifi] to generate an assembly and polished with two rounds of RACON version 1.4.13[114] racon [-u -t 36].

Ribosomal DNA was assembled separately from a subset of CCS reads identified using kmer matching against the UNITE database with BBTools version 38.79[116] bbduk.sh [k=31 mm=f mkf=0.05 ow=true]. Matching reads were subsampled to 600000bp with BBTools[116] reformat.sh [sampleseed=1 samplebasestarget=600000 prioritizelength=t ow=true] and assembled with Flye version 2.8.1 –py37h4bd9754_0[115] [–hifi-error 0.01 –meta –keep-haplotypes –genome-size 12K -t 16 –pacbio-hifi] and polished with two rounds of RACON version 1.4.13[114] racon [-u -t 36].

Eukaryotic internal transcribed spacer (ITS) was identified and extracted from the rDNA assembly using ITSx version quay.iobio-containersitsx1.1b–2[117] [–detailed_results T –anchor 100 –cpu 16 -t F -o ITS]. Results were used to orient and trim the rDNA contig to 100bp SSU - 1Kb LSU.

PacBio Iso-seq library preparation, sequencing and read processing

Full-length cDNA was synthesized using template switching technology with NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module kit. The first-strand cDNA was amplified and multiplexed with NEBNext High-Fidelity 2X PCR Master Mix using Barcoded cDNA PCR primers. The amplified cDNA was purified using 1.3X ProNex beads. Similar sizes were pooled at equimolar ratios in a designated Degree-of-Pool using PacBio Multiplexing Calculator. The pooled samples were end-repaired, A-tailed and ligated with overhang non-barcoded adaptors using SMRTbell Express 2.0 kit. PacBio Sequencing primer was then annealed to the SMRTbell template library and sequencing polymerase was bound to them using Sequel II Binding kit 2.0. The prepared SMRTbell template libraries were then sequenced on a Pacific Biosystems' Sequel II sequencer using sequencing primer, 8M v1 SMRT cells, and Version 2.0 sequencing chemistry with 1x1800 sequencing movie run times.

PacBio subreads were used as an input to generate the polished Circular Consensus Sequence by pbccs (version 4). In pbccs the accuracy rate and the minimum pass number were set to 98% and 3 (ccs –min-passes 3 –min-snr 4 –max-length 21000 –min-length 10 –min-rq 0.98). For the classification of the css reads into full length category according to the presence of the 5′ primer, 3′ primer and poly(A) tail lima was used. For the further classification only, the full-length reads were kept. In the further steps isoseq3 refine function was used to remove poly(A) tails and cluster function to cluster all the full length css reads.

Oxford nanopore cDNA library preparation, sequencing and read processing

From each of the five purified total RNA samples, 2 μg of RNA was used to prepare full-length cDNA libraries using the TeloPrime Full-Length cDNA Amplification Kit V2 (Lexogen, Vienna, category number: 013.24) protocol. Endpoint PCR was performed using the same kit with 12–18 cycles. The concentration and integrity of the samples were determined using a Qubit 2.0 fluorometer with the Qubit (ds)DNA HS Assay Kit (Thermo Fisher Scientific) and the Bioanalyzer 2100 (Agilent Technologies) with the DNA 7500 chip. The amplified cDNA samples were barcoded using the Oxford Nanopore Native Barcoding Expansion Kit (EXP-NBD104). The proportionally mixed barcoded samples were used to prepare libraries with the Ligation Sequencing Kit (SQK-LSK109, Oxford Nanopore Technologies), which were then sequenced on two MinION flow cells (FLO-MIN106 R9.4.1, Oxford Nanopore Technologies). Base calling and demultiplexing were performed using Guppy v3.2.4. Only reads with a Q value $\geq$ 7 were used in further steps. The base called reads were adapter trimmed and oriented using Pychopper v2.3.1.[153] Any reads shorter than 50 bp were excluded. Errors in ONT reads were polished using LoRDEC v0.9.[118] For the polishing process BBTools reformat.sh[116] was used to randomly sample approximately 20M R1 Illumina reads from a previous dataset.[24] To remove redundant or poor-quality reads from the sampled dataset prior to the polishing step, reads were further sampled using ORNA v0.4.[119] To evaluate the indel and mismatch ratios of the long reads assembled with CopciAB V2, the functions stats_from_bam and summary_from_stats from pomoxis v0.3.10[154] were used, which output summary statistics for each read.

QuantSeq library preparation, sequencing

The libraries were generated using the QuantSeq 3′ mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen, Vienna). To begin, 100 ng of total RNA was used as input for first strand cDNA generation with an oligo-dT primer, followed by RNA removal. Next, second strand synthesis was initiated with random priming and the resulting products were purified with magnetic beads. Finally, the libraries were amplified and barcoded using PCR. The Agilent 4200 TapeStation (Agilent) was used to assess all libraries for the formation of adapter dimers during PCR. The QuantSeq libraries were sequenced on the Illumina NextSeq550 platform, producing 75 bp single-end reads.

Genome comparison, structural variation identification

To study the differences in chromosomes (C13) three genomes were compared using the dnadiff function of the MUMmer v4.0 package[120] with CopciAB V2 as a reference. We identified structural variations (SVs) between the genomes using SyRI v1.6.3.[121] For this purpose, chromosome-level alignment was performed between *C. cinerea* Okayama (reference) and CopciAB V2 (query) genomes, as well as between CopciAB V2 (reference) and ONT-based (query) genomes using Nucmer from the MUMmer package. The alignments were used as input for SyRI to identify the genomic rearrangements and local sequence differences. The output of SyRI was plotted using plotsr v1.1.0.[122]

Determination of indel, SNP variation between the CopciAB V2 and Copci ONT genomes

Genomic Illumina reads (SRR10162428)[28] were trimmed with fastp v0.21.0[123] and then mapped to the CopciAB V2 and CopciAB ONT genomes with bwa v0.7.17-r1198-dirty.[124] After sorting and preprocessing the bam alignment files with samtools v1.13[125] and picard package AddOrReplaceReadGroups v2.18.14-SNAPSHOT function,[155] the indels and SNPs were called using GATK.[126]

Long-read-based gene model prediction

During gene model prediction, the main objective was to integrate data from all available genomes and associated annotations into a more complete long-read based annotation. To accomplish this we iteratively utilized the Illumina,[27] ONT[28] and the new PacBio genome. During each iteration, we incorporated manually curated gene models from the reference genome and newly created ONT based gene models into the resulting annotation. In the final polishing step, we integrated the information carried by the PacBio IsoSeq reads into the reference annotation, resulting in the final form.

In detail, we performed the following steps. Firstly, we separately mapped the polished ONT reads from five different samples to the CopciAB V1 genome[27] using DeSALT v.1.5.6.[127] We chose this aligner due to its higher observed precision in *de novo* mapping of transcripts with shorter exons compared to other long read aligners (minimap2 v2.24-r1122,[133] magicblast: v1.5.0,[156] and GraphMap v0.6.3[157]). The aligned reads were processed using samclip[128] to remove long soft clip sequences. The resulting trimmed alignments were then utilized to predict gene models with the TAMA software package.[129] Using TAMA, we initially collapsed mapped reads by samples [tama_collapse.py -s $inputBAMfile -b BAM -f $refgenomePath -p $outTag -x capped -icm ident_map -a 50 -z 50]. Subsequently, we filtered out collapsed reads with low read support (at least 2 reads in at least 1 sample) [tama_remove_single_read_models_levels.py -b $annotationBedFile -r $readSupportFile -l transcript -k remove_multi -o $outputPrefix]. Finally, we merged the collapsed reads from the different samples [tama_merge.py -f $fileList -p $outputPrefix -a 50 -z 50].

It was observed that TAMA often fails to separate overlapping neighboring gene models. To address this issue, we reviewed the length of each TAMA-suggested transcript isoform for each gene model and. To eliminate exceptionally long, possible erroneous isoforms, we filtered out any isoforms with a length twice as larger as the geometric mean of the gene's isoforms. The remaining isoforms were clustered based on their overlap ratio. If the pairwise overlap was less than 50%, the isoforms were reassigned to a separate gene model represented by the isoform with the highest read support. To preserve all the data, the previously filtered out, especially long isoforms were later added back to the gene cluster with the highest read support. In a further step, ORFik[130] was then utilized to predict coding DNA sites (CDSs) with a minimum length of 30 amino acids on the gene/transcript models. Transcript models without CDS predictions were omitted from further analysis.

To identify the primary transcript isoform of the gene for further manual curation, certain filter criteria were required to be met. Firstly, a transcript must be longer than the 80% of the geometric mean of all transcripts in the cluster. Secondly, if a transcript has at least 4 times higher expression than the second highest expressed transcript, it becomes the primary isoform. Otherwise, the longest isoform is selected. The primary isoforms selected were manually checked. To aid in the curation process, Illumina reads[24] were aligned to the reference genome using the STAR v2.6.1a_08-27 aligner.[131] Non-canonical splice sites, splice junctions (SJ) with low Illumina read support ($\leq$5 reads), or alignment errors (indel, mismatch) around the SJ were given special attention and were manually checked and corrected if necessary. At this point, any gene clusters that were removed due to the above 80% filter were restored. Also, partial primary isoforms were corrected to accurately reflect the CDS. Gene models not present in the primary isoforms, but present in the CopciAB V1 annotations were incorporated.

The primary isoforms defined in the CopciAB V1 genome were mapped onto the ONT-based genome[28] using DeSALT. We included gene models that were exclusively present in the ONT-based genome to the primary isoform set. In case when there was a match between the gene models of the ONT-based genome and CopciAB V1 genome, we gave priority to the ones that originated from the ONT-based genome over the CopciAB V1. We utilized SQANTI3[132] to identify overlapping gene models. We then

used DeSALT to map the extended primary isoform set onto the PacBio-based genome. To detect alignment errors for manual curation, we aligned Illumina reads as previously mentioned.

To further improve annotation, we incorporated PacBio IsoSeq reads. We aligned ONT and PacBio reads to the reference genome using minimap2 v2.24-r1122[133] with the assistance of reference annotation. We excluded long reads that had a perfect SJ match with the reference annotation and only considered them as read support for the corresponding gene. The remaining long reads were utilized for an additional round of transcript model prediction with TAMA.

The second round of TAMA transcript/gene models were compared with the corrected extended primary isoform. If the length of the CDS encoded by the new gene/transcript model was longer than the counterpart in the reference genome, then the new gene/transcript model was accepted. If the CDS was identical to a CopciAB V1[27] or the ONT-based[28] gene in the reference genome then, it was also replaced with the new gene model. If it was missing, it was transferred to the corrected extended primary isoform. The gene models that were taken from the new TAMA run underwent manual checking before being accepted. To support the representation of the long gene models with low expression in the long-read based annotation, the long genes with one PacBio IsoSeq read support were also accepted after consideration.

Transcript isoforms were accepted if they had a read support of at least 10 reads and accounted for at least 10% of the gene's total read support, which includes both ONT and PacBio IsoSeq reads.

To identify falsely predicted gene models, we first identified genes with extremely low expression. We collected 165 RNA-seq samples from five studies, each with at least two biological replicates, including two published,[24,110] one published with the current work (QuantSeq data), and two currently unpublished studies (CPM values available on the mushroomdb.brc.hu webpage), For each gene in each sample, we calculated the mean count per million (CPM). Next, we focused on the highest possible mean CPM of the gene reached per study. We marked genes whose maximum expression fell in the lowest 10% of the expression maxima in all five studies as low expressed genes. Low expressed genes without similarity to proteins outside of *C. cinerea* (mmseqs2 R15-6f462,[134] evalue $\leq$ 1e$-$10, qcov $\geq$0.5), lacking Interpro annotation and long read support, were filtered out from the reference annotation. The completeness of the new annotation was assessed using BUSCO v3.0.2 with basidiomycota_db10 database.[135]

Determination of isoforms

SUPPA2[136] was used for splice isoform classification. To improve the detection of intron retention events (RI), the program was executed in two rounds. Initially, the default parameters were utilized, followed by a more permissive second run using the parameters -b V -t 50. The RI events from the first run were supplemented by the RI events from the second run.

Identification of the conserved genomic elements

To identify the conserved genomic elements around transcription start site (TSS), we retrieved sequences of ±50 nt flanking the TSS position of the long-read-based gene models with 5′ UTR region. For the sequence context of the translation initiation site (TIS), we retrieved sequences of ±12 nt flanking the ATG position of the long-read-based gene models with a minimum 12 nt long 5′ UTR region. In the case of the splice site, 6 nt from the 5′ end and 3 nt from the 3′ end of the intron sequences were retrieved and used to identify 5′ss and 3′ss. Finally, in the case of the transcription termination site (TTS), ±50 nt of sequence flanking the TTS were recovered from long-read-based gene models with 3′ UTR region. The sequences obtained were analyzed using ggseqlogo.[137] For the identification of the branchpoint (BP) the branchpointer v1.14 R package was used.[138]

PASs, PACs identification. Relative PAC usage determination

For polyadenylation site (PAS) identification, the DPAC v1.4 tool[139] was used all corresponding QuantSeq reads. Briefly, the QuantSeq reads were adapter and poly(A) trimmed and the length of the poly(A) tail was recorded. Reads with at least 15 A's were mapped to the reference genome. The 3′ end of all reads was used to identify the position of PASs in the reference genome. PASs with at least 20 reads, each with a poly(A) tail of at least 15 A's, were retained. PASs were further filtered for internal priming and template switching by counting the number of A's in the reference genome immediately downstream of the identified PAS. If at least a 4 nt long poly(A) stretch was found immediately downstream of the PAS or, $\geq$70% of the As were in a 10 nt long downstream window, the PAS was filtered out from the further analysis.

The PAS were further clustered into polyadenylation site clusters (PAC) by allowing a maximum intra-cluster distance of 10 nt. Within a PAC, the PAS with the highest read support serves as the representative PAS of the cluster and lends its read support and genomic coordinate to the PAC (Figure 3A). This common coordinate was used to assign the PACs to the different entries of the genome annotation and to identify the associated gene models by assigning them to the TTS of the closest gene. To further reduce the negative effects of internal priming and template switching on PAC identification, we only accepted those PACs which are located in the 3′ UTR of the associated gene and in the downstream intergenic space, or in the genomic region of the antisense gene within 1,000 nt of the TTS of the associated gene (Figure 3B). A distinction was made between PACs based on read support. The PACs of the gene with the highest read support, the highest representative PAS read support, were considered the best supported PAC.

PAC usage across biological conditions was defined by sample-wise counting of mapped 3′ ends of the previously trimmed QuantSeq reads on the defined PACs. PAC read counts were normalized to CPM. The normalized counts were pooled by conditions for increased sensitivity.

To determine the evenness value of APA genes with ≥10 CPM, the vegan R package[140] was used to calculate the Shannon diversity index for the PAC sites and dividing by the logarithmic value of the number of PAC sites. The line graph showing the correlation between the value of the evenness of the APA gene PAC sites and the value of gene expression was generated using ggplot2.[141] The lines were smoothed using a generalized additive model (GAM).[158]

Gene expression analysis

The new *C. cinerea* Amut1Bmut1 #326 genome and gene model prediction were used as a reference for gene expression analysis. The quality of the raw and trimmed QuantSeq reads was assessed using the FastQC[142] and MultiQC[143] tools. The raw reads were trimmed using the BBduk.sh script from BBtools 38.92[116] and then mapped to the reference genome using STAR version 2.6.1a_08-27.[159] The trimming and mapping parameters were set according to the manufacturer's recommendations (https://www.lexogen.com/quantseq-data-analysis/). The number of reads overlapping the transcript annotations was determined using the featureCounts function of the Rsubread package version 2.6.4.[144] For differential gene expression analysis, the edgeR version 3.34.0[145] and limma version 3.48.3[146] packages were used. To minimize the number of misidentified differentially expressed genes (DEGs), only genes whose expression reached at least 1 CPM in at least one experiment were included in the analysis. Genes with a fold change ≥|2| and a Benjamini-Hochberg (BH) adjusted p value ≤ 0.05 were considered as significantly differentially expressed DEGs. To further minimize the number of misidentified DEGs, we only included those DEGs in the further analysis that had an average of at least 2 CPM in at least one of the conditions during comparison. The ComplexHeatmap R library[147] was used to generate expression heatmaps.

Functional annotation and GO enrichment analysis

InterPro (IPR) and GO functional annotations were defined using InterProScan version 5.61-93.[148] Putative CAZymes were identified using dbCAN[149] and further categorized based on their substrate specificity as described in previous publications.[160] Based on the substrate specificity information, the groups of plant cell wall degrading CAZymes (PCWDEs) and fungal cell wall degrading CAZymes (FCWDEs) were defined (available at mushroomdb.brc.hu). The gene sets for transcription factors (TFs) and transporters were defined using InterPro entry information and evidence from previous publications (available at mushroomdb.brc.hu). GO enrichment analysis was performed using topGO version 2.40.0[150] in conjunction with GO.db version 3.17.0.[151] For the analysis, the weight01 algorithm was used with Fisher statistics, and the significance level was set to ≤0.05. The significantly enriched GO terms were plotted with the R package ggplot2.[141]

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis including Fisher exact test and Spearman correlation test, was performed with R (version 4.1).[161] The statistical details including the test method, threshold and packages can be found in Methods Details and main (or supplementary) figure legends.

ADDITIONAL RESOURCES

To facilitate the use of the data presented in this study, a new website has been created that includes, among many other resource, the genome sequence, gene model, functional annotation, and gene expression data: https://mushroomdb.brc.hu/.