MOLECULAR FUNCTIONS OF THE EVOLUTIONARILY CONSERVED WETA REGULATOR IN *ASPERGILLUS*.

By

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ABSTRACT

Aspergillus fungi primarily reproduce by forming asexual spores called conidia, whose formation and maturation is governed by the central genetic regulatory circuit BrlA→AbaA→WetA. First, we characterized the WetA function in mycotoxin producer, *A. flavus*. The *Afl*WetA protein is a multi-functional regulator that couples spore differentiation and survival, and governs proper chemical development in *A. flavus*. Loss of function *AflwetA* leads to the formation of conidia with defective cell wall and no intra-cellular trehalose, leading to reduced stress tolerance, a rapid loss of viability, and disintegration of spores. *Afl*WetA is also required for normal vegetative growth, proper hyphal branching, and production of aflatoxins, suggesting that WetA may play a global regulatory role in fungal lifecycle. We further carried out targeted and genome-wide expression analyses in *A. nidulans*, *A. fumigatus*, and *A. flavus*. The comparative RNA-seq studies reveal that WetA exerts feedback control of conidiation and regulates over 40% of each *Aspergillus* genome in conidia. Moreover, over 60% of the WetA-affected genes have orthologs in the three species, indicating the evolutional conservation of the WetA-mediated gene regulatory networks (GRNs). Finally, we identified the WetA response elements (WREs) by investigating the genome-wide WetA-DNA interaction in conidia in *A. nidulans*. The WREs are present in promoter regions of 23% of *A. nidulans* genes, while mRNA levels of only 55% of them were affected in the Δ*AniwetA* conidia, suggesting that the WetA-mediated GRNs may be temporal-/ spatial-specific. Functional category analyses indicate that WetA contributes to stress response, sporulation, cell wall integrity, and carbohydrate metabolic process in conidia. However, even WetA is functionally conserved; the WetA-mediated regulatory effects on the associated GRNs are usually divergent in *Aspergillus*, which suggests the GRNS has rewired during evolution. In summary, WetA is an evolutionarily conserved DNA-binding protein regulating the cellular and chemical development in *Aspergillus*.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SUPPLEMENTARY TABLES

CHAPTER 1

Introduction

1-1 Introduction

Fungi have vital roles in agriculture, medicine, and biotechnology in human lives as well as in the natural ecosystems [1]. Since the 1940s, fungi serve as great genetic model systems in the study of eukaryotic genetics because of the convenience and efficiency of handling and the abundance of available genetic research resources [2]. Among the variety studied filamentous fungi, *Aspergillus* species are popular research targets due to their importance in the areas of human health (*A. fumigatus*), industry (*A. niger* and *A. oryzae*), agriculture (*A. flavus*), and general genetic researches (*A. nidulans*) [3]. Similar to other filamentous fungi, *Aspergilli* produce the asexual spore (conidia) for proliferation, which is the most dominant way to effectively propagate. Asexual development has been studied in *Aspergilli* and other fungi for many years [4–20]. The process of asexual sporulation (conidiation in higher fungi) happens in a timely and complicated manner, which needs to be delicately and precisely controlled. Understanding of the genetic regulatory networks (GRNs) behind this conidiation would provide insights into the developmental and morphological mechanisms on molecular level.

1-2 Asexual development in *Aspergilli*

The general life cycle of *Aspergillus* species initiates from vegetative growth stage. Spores germinate by breaking the dormancy state and transiting to the metabolically active state. The germinated spores then release the germ tubes and extend them in a highly-polarized manner to form the undifferentiated network of the interconnected hyphal mat (mycelium). Under appropriate conditions, the *Aspergilli* shift from the vegetative growth stage to the development stage, the sexual or asexual reproductive process takes place for propagation. All *Aspergilli* are able to reproduce through the asexual sporulation process (conidiation), while only some

Aspergilli can reproduce through sexual development [21]. Conidiation is the most common way for *Aspergilli* and other conidiogenous fungi to rapidly reproduce abundant spores for dispersion to the environment and survival. *Aspergilli* enters into conidiation stage by producing the asexual reproductive structure, conidiophore. Conidiophore development starts from the formation of the thick-cell-wall foot cell, which is differentiated from the vegetative hyphae [22]. An aerial branch (stalk) then get risen from the foot cell, with the apical swelling at its tip (vesicle) [23]. In *A. nidulans*, a layer of uninucleate cells (metulae) buds from the vesicle surface, and another layer of uninucleate sporogenous cells (phialides) formed on the top of metulae [17]. Not all *Aspergilli* contains metulae. The *Aspergilli* species contain both metulae and phialides called biseriate species like *A. nidulans*, while the species contain only contain phialides called uniseriate species like *A. fumigatus*. Some species like *A. flavus* contain both uniseriate and biseriate structures [24]. Chains of conidia are produced by the repetitive mitosis division by the phialides [25] (Fig 1-1).

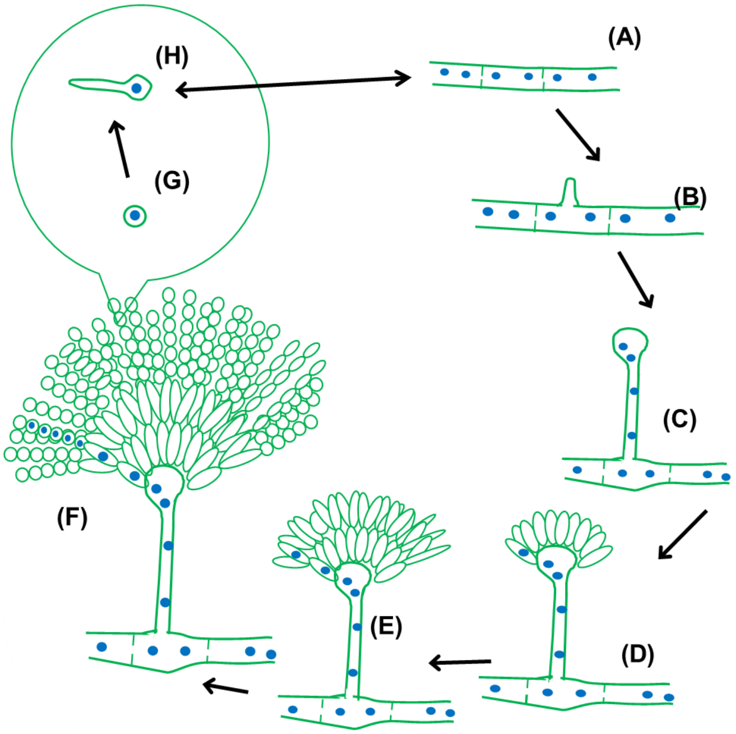

Fig 1-1 Diagram of *Aspergillus* asexual development

In *Aspergilli*, initiate by the differentiation of the foot cell (B) from the vegetative hyphae (A). From the foot cell, a stalk emerges and a vesicle (C) is formed on the top of the stalk. Subsequently, metulae (D), phialides (E), and asexual spores (conidia) (G) bud from the vesicle. The whole structure of (C)-(F) is called conidiophore (G). Conidia then germinate (H) and form vegetative hyphae.
Note: Not all *Aspergilli* contain metulae (D), as mentioned in content.

1-3 Conidia maturation in *Aspergilli*

The life cycle of conidia includes following stages: formation, maturation, dormancy, and germination [26]. Conidia maturation is defined as differentiation occurring after septum formation between phialide and newborn conidium, and the delimitation of this conidium from the phialide. It is a distinct phase of development, and less is known about the details of conidia maturation except for the change of the spore wall structure and the accumulation of certain chemical components during the process. The spore wall differentiation during conidia maturation can be divided into three stages based on the carbohydrate cytochemistry studies [11]. The Stage I conidium is the youngest spore in a chain, which contains two conidial wall layers (outer C1 and inner C2 layers), which is similar to the hyphae cell wall layers (P1 and P2). At Stage II, the conidium has been displaced from the phialide tip by subsequently formed conidia. The C1 and C2 layer are most separated at Stage II. Moreover, C2 layer condenses and the C1 layer becomes crenulated rodlet layer at this stage. At Stage III, the C3 layer is formed between C1 and C2 layers, and then the C4 layer is formed as the innermost layer of the spore wall (See the Fig 1-2). When observing the *A. nidulans* wild type (*Ani*WT) conidia under the transmission electron microscopic (TEM), the C1 layer is electron condense, while C2, C3, and C4 layers are electron light [27]. Previous studies proposed that the four-layer spore wall structure makes the conidia impermeable, which is essential for stress tolerance and maintaining the spore dormancy [28].

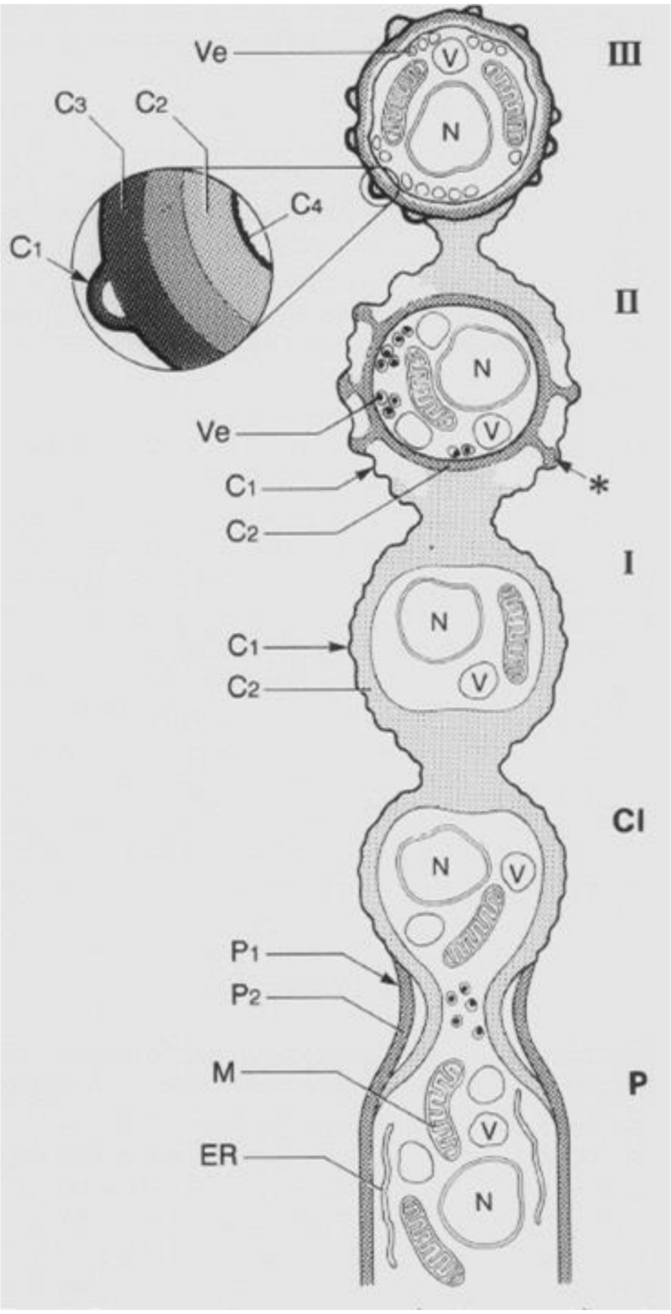

Fig 1-2 Diagrammatic representation of A. nidulans conidium formation and maturation

The stages of conidium maturation (reproduced from Sewall et al., 1990)[11]. Each conidium and phialide (P) contain a nucleus (N), mitochondria (M), endoplasmic reticulum (ER), vacuoles (V), and vesicles (Ve). Three stages (I, II, and III) of conidium maturation are shown after a conidium initial (CI) is delimited from the phialide. Phialide contains two cell wall layers: P1 and P2. The CI and conidium at stage I also contain two cell wall layers: C1 and C2. At stage II, C2 layer condenses to form projections (*) in contact with C1. During stage III, the conidium becomes mature containing four cell layers: C1~C4.

During the maturation, the composition of conidial chemical components also changes. One of the most important conidial content is trehalose. Trehalose is a non-reducing disaccharide which is synthesized in a wide range of organisms, from bacteria to invertebrates. It not only serves as an energy source but also a protectant against the environmental stresses [29]. *Aspergillus* conidia contain a high amount of trehalose (up to 15% of the dry mass), which is quickly degraded during germination, suggests the trehalose catabolism is related to the conidial dormancy [30,31]. Moreover, conidial trehalose functions both as a reserve carbohydrate and a stress metabolite [32,33]. In *Aspergilli*, the conidial trehalose amount is linked with the loss of several genes, such as the trehalose-6-phosphate synthase *tpsA* [30], the *velvet* regulators *vosA* [34] and *velB* [35], and the central regulator *wetA* [5], and all these mutants show impaired conidial viability.

1-4 Regulation of asexual development

Genes involved in the regulation of asexual development are highly conserved in *Aspergilli* [36]. Most of the genetic studies of *Aspergillus* using *A. nidulans* as the research material, thus here we use *A. nidulans* as the model system to illustrate the regulation of conidiation.

The entire process of conidiation is regulated by distinct gene sets, including central regulators, upstream activators, negative regulators, light-dependent regulators, and *velvet* regulators (Fig 1-3) [36,37]. The central regulatory pathway (*brlA* → *abaA* → *wetA*) acts in concert with other conidiation regulators to control conidiation-specific gene expression [3,12]. To kick on the central regulatory pathway, upstream activators (*fluG*, *flbA*-*E*) are activated and the negative regulators (*sfgA*, *vosA*, *nsdD*, *ganB*, *fadA*, *sfaD*, *gpgA*, and *pkaA*) are repressed [37–39]. The velvet regulators (*veA*, *velB*, *velC*, and *vosA*) are able to form diverse complexes with other proteins, which makes their function flexible in coordinating various biological processes [35,40,41]. Moreover, light is a key environmental stimulus of asexual development in *A. nidulans* [42,43]. The light dependent regulators include one red-light receptor FphA and three blue-light receptors LreA, LreB, and CryA [43].

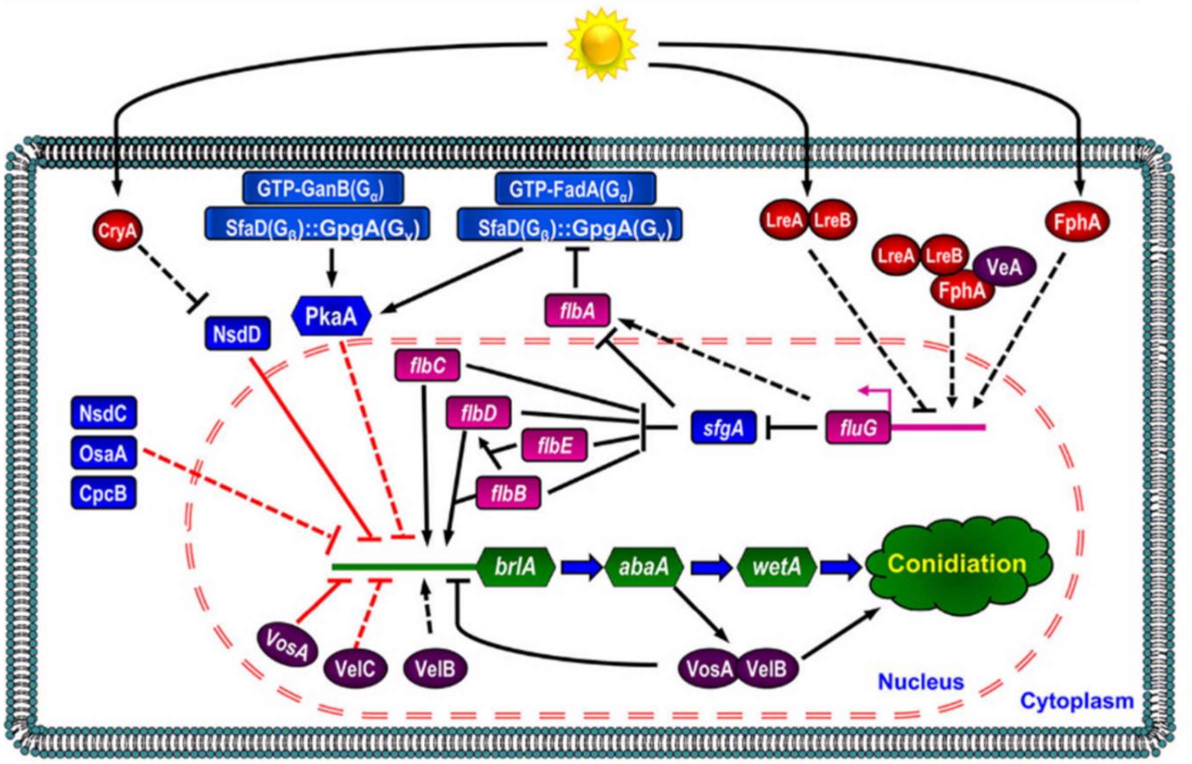

Fig 1-3 Overview of the upstream regulation of conidiation in *A. nidulans*

Regulatory pathway of asexual sporulation in *A. nidulans* (reproduced from de Vries et al., 2017 [36]). Central regulators, upstream activators, negative regulators, *velvet* regulators, and light dependent regulators are illustrated by green, light purple, blue, dark purple, and red icons, respectively.

1-5 Central regulatory pathway

Among the many regulatory pathways, the central regulatory pathway is the key orchestrator of the conidiation event. As summarized in Fig 1-4, the activation of *brlA*, *abaA*, and *wetA* is corresponding to different stages of the conidiation. The *brlA*→*abaA*→*wetA* central regulatory cascade presents in all *Aspergillus* and *Penicillium* genomes, while only one or two central regulators were found in Ascomycotina in general (Fig 1-5) [36]. In most cases, only a single copy of each central regulatory gene presents in the *Aspergillus* genome [36]. Based on the expression levels of the loss of function mutants of *AnibrlA*, *AniabaA*, the conidiation-specific genes can be divided into four classes (Class A-D) (Fig 1-4) [12,16,44]. Class A genes, including *AniyA* and *AnirodA*, are induced by either *AnibrlA* or *AniabaA* or both. Class B genes like *AniwA*, which are also known as conidia-specific genes, are induced by *AniwetA*, but independent with *AnibrlA* or *AniabaA*. Class C and Class D genes require all three central regulators for proper expression, which are also known as phialide-specific genes. The Class C and Class D genes can be further distinguished by their expression patterns during conidiation [12,16,44]. Put together, the central regulators constitute a genetic circuit which governs the conidiation by regulating a large group of conidiation-specific genes.

1-5-1 BrlA

Activation of *brlA* (bristle A) is the key and essential initiation step of conidiation [44,45]. The *AnibrlA* mutants produce the elongated stalk but fail to develop vesicles or any subsequent structures [44,45] (See Fig 1-6, Upper right panel). Overexpression of *AnibrlA* leads to the termination of vegetative growth and causes the formation of conidial spores from hyphae in submerged culture [16,46]. The *AnibrlA* encodes two transcripts, *AnibrlA*α and *AnibrlA*β (Fig 1-7) [47], which provides the translational repression of *AnibrlA* expression and prevents the premature development (Fig 1-7)[48]. As the two transcripts play distinct roles, their mRNA expression levels are differentially regulated at different development stages [48]. While *AnibrlA*α is regulated at the transcription level, *AnibrlA*β is regulated at both the transcriptional and translational levels [48,49]. The activation of *AnibrlA*α requires both *AniabaA* and *AnibrlA*, but overexpression of *AnibrlA*β can induce *AnibrlA*α in the *AniabaA* mutant. The expression of *AnibrlA*β is independent of BrlA but its translation is damped by the expression of *AnibrlA*βμORF, which is a short open reading frame (ORF) located upstream of the *AnibrlA* start codon [48,49]. *AnibrlA* begins to express at 10 h after asexual induction and results in the formation of vesicles (Fig 1-4) [44,50,51]. *Ani*BrlA protein localizes in vesicles, metulae, and phialides, but not in hyphae or mature conidia [44,50,51], suggesting that the main role of *Ani*BrlA is to initiate conidiation.

The *brlA* encodes a C_2H_2 zinc finger transcription factor (TF) [16]. The BrlA response elements (BREs, [C/A][G/A]AGGG[G/A]) has been identified using the yeast system and is widely existed in *A. nidulans* genome [52]. Overexpression of *AnibrlA* leads to the activation of *AniabaA*, *AniwetA*, and other conidiation-specific genes [12,16,44]. Taken together, *AnibrlA* is the key transcription factor, which dominates the initiation of conidiation and termination of vegetative growth.

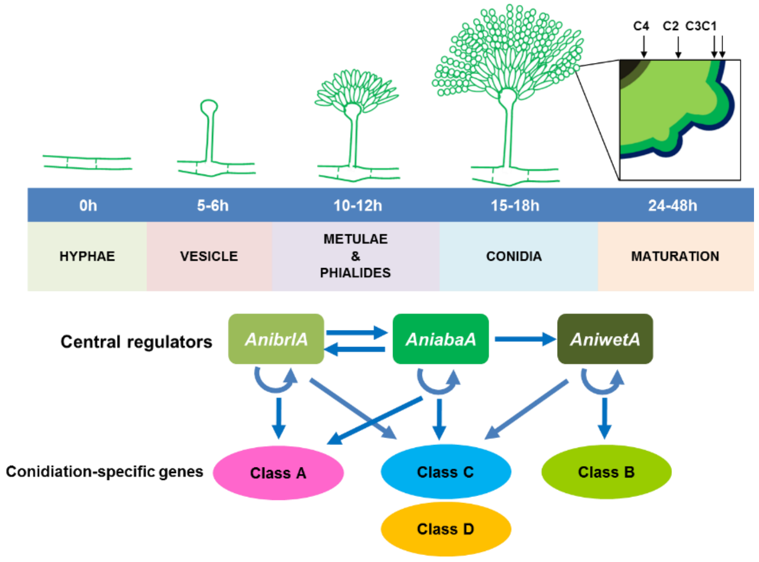

Fig 1-4 Central regulators of *A. nidulans* conidiation

A model for the roles of the central regulators in *A. nidulans* conidiogenesis. The black square illustrates the graphic view of the wall structure of the mature conidium, including the crenulated electron-dense outer layer C1, the carbohydrate-condensed layer C3, the electron-thin layer C2, and the innermost layer C4. The central regulators cooperatively activate other genes (Class A~D) responsible for the morphogenesis of conidiophores.

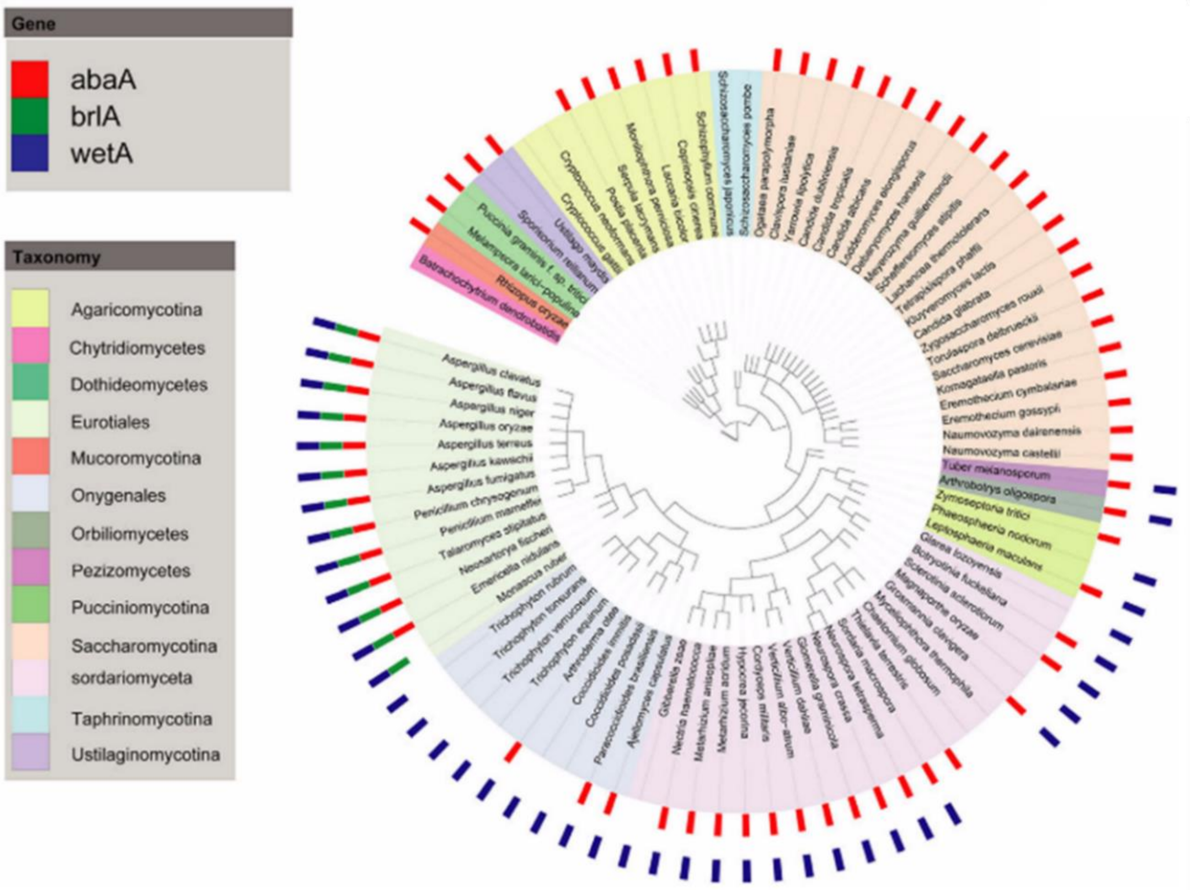

Fig 1-5 The central regulators are highly conserved in *Aspergilli* and *Penicillia*

Distribution of central regulators for asexual sporulation in 85 fungi (reproduced from de Vries et al., 2017 [36]). These fungi are representatives from the phyla Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota. Their genome protein sequences were searched for homologs of AbaA, BrlA, and WetA by BlastP using sequences of A. nidulans AbaA, BrlA, and WetA as queries. Details of these fungi are presented in Central Regulator Strain Information. As shown, BrlA seems to be limited to the Eurotiales group, suggesting a specific role for conidiation in Eurotiales fungi. By contrast, WetA is widely distributed in Pezizomycotina fungi, which suggests a general function for the synthesis of cell wall layers to make conidia mature and impermeable. Surprisingly, AbaA is widespread being found in the phyla Ascomycota, Basidiomycota, and Zygomycota, suggesting that AbaA is involved not only in conidial development but also has other general functions in fungal development

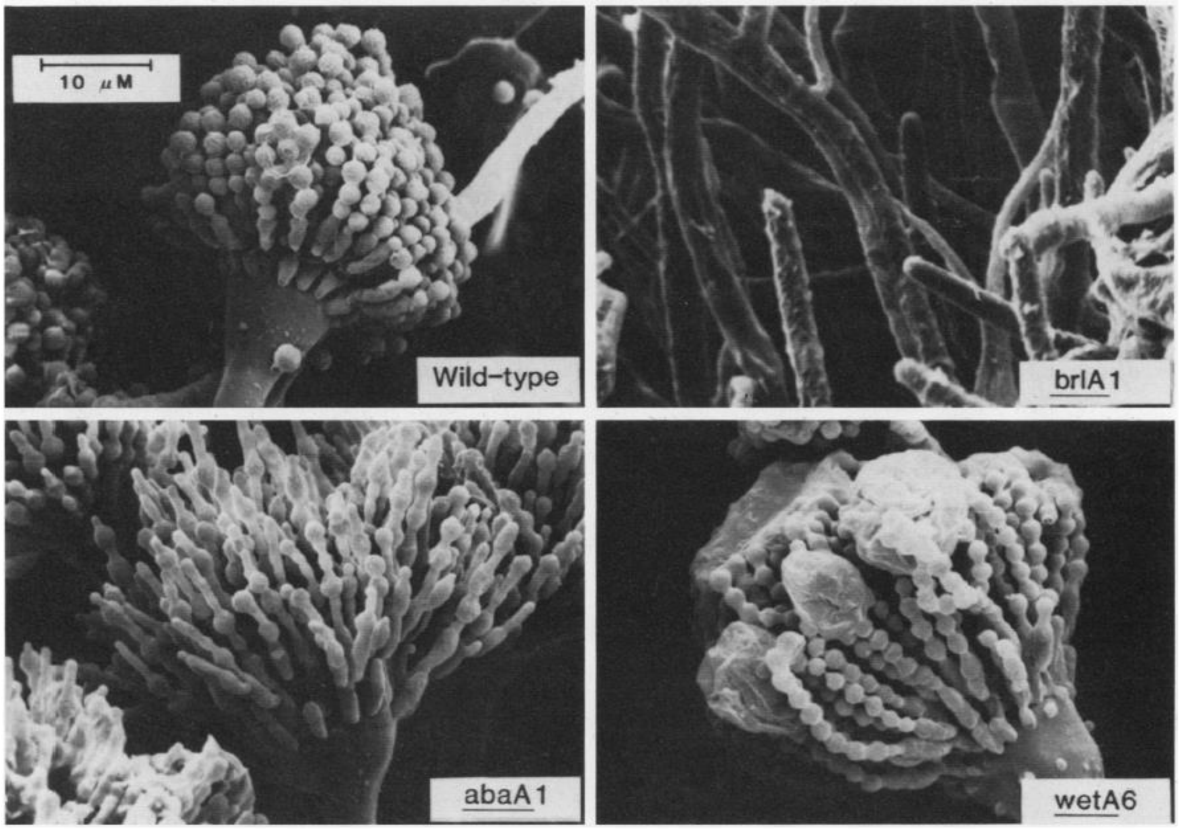

Fig 1-6 Morphology of wild-type and mutant conidiophores in *A. nidulans*

The morphology changes due to the disruption of the central regulator encoded genes in *A. nidulans* (reproduced from Boylan et al., 1987 [44]). Upper left panel: The *Ani*WT conidiophore. Upper right panel: The bristle-like structure in the *AnibrlA*1 mutant. Bottom left panel: The abacus-like structure in the *AniabaA*1 mutant. Bottom right panel: The collapsed conidia chain in the *AniwetA*6 mutant.

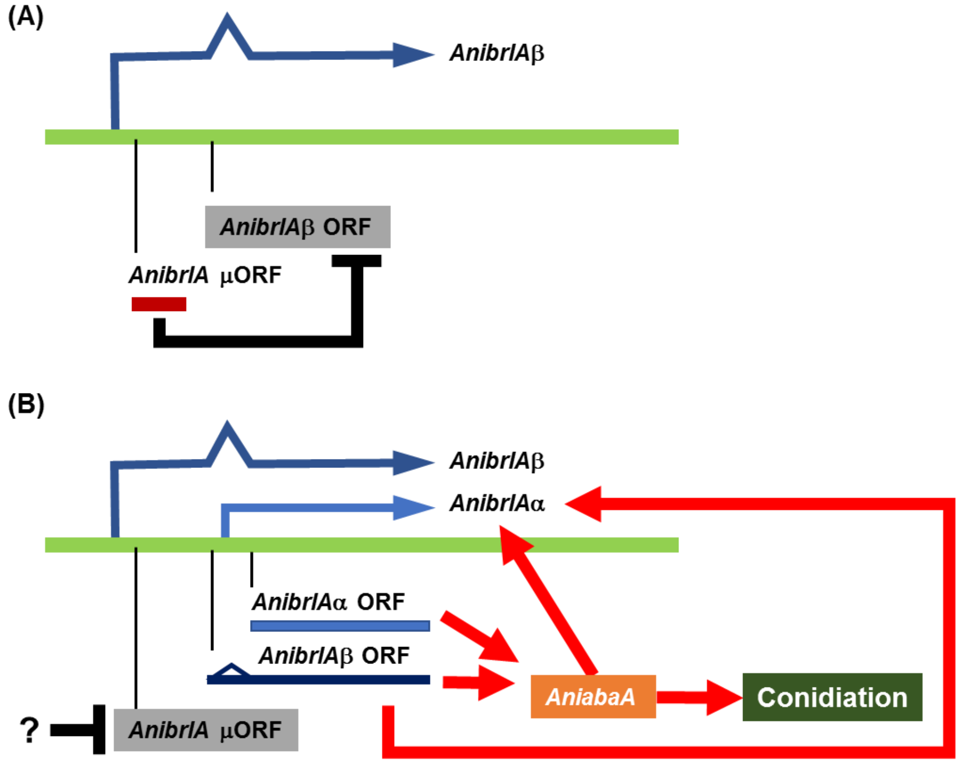

Fig 1-7 Model for differential control of *AnibrlA*α and *AnibrlA*β during conidiation

(A) *AnibrlA*β is constitutively transcribed during *A. nidulans* life cycle. In vegetative growth stage, the translation *Ani*BrlAβ is blocked by the *AnibrlA* μORF. Dark blue arrow: *AnibrlA*β transcript. Brick red box: *AnibrlA* μORF. Grey shaded box: The translation is blocked. (B) Unknown regulators shut down the translation of *AnibrlA* μORF when the conidiation is induced by appropriate environment stimuli signals. *Ani*BrlAα and *Ani*BrlAβ are now able to be properly translated, and then activate the downstream *AniabaA*, which can feedback activate *AnibrlA*α expression. Of note, overexpression of *AnibrlA*β can also activate *AnibrlA*α in Δ*AniabaA* mutant.

1-5-2 AbaA

The *abaA* (abacus A) gene is activated by *brlA* at the middle stage of conidiation. In *A. nidulans*, *AniabaA* begins to express between 10-15 h after conidiation induction, which is after the metulae differentiation (Fig 1-4) [44,53,54]. Loss of *AniabaA* causes repeated sterigmata development and forms abacus-like structure, which is not capable of forming phialides and conidia (Fig 1-6) [3,11]. The abacus-like structure is more metulae-like rather than phialide-like, suggesting that the

AniabaA functions in the differentiation of phialides [55]. Overexpression of *AniabaA* results in the interruption of vegetative growth and the accentuation of cellular vacuolization. However, unlike the overexpression of AnibrlA, overexpression of *AniabaA* does not cause conidiation [50]. Similar to BrlA, AbaA is TF which contains ATTS/TEA DNA binding domain, which recognizes the consensus motif CATTC[C/T] (also known as AbaA response elements, AREs) [54]. The AbaA binding motif is present in the promoter regions of many conidiation regulatory genes, including *AnibrlA, AniwetA, AniyA, AnirodA,* and *AniabaA* itself [3,52]. *AniabaA* induces *AnibrlA* at a certain time during conidiation, although *AnibrlA* has to be expressed first and activates *AniabaA* first. However, the *Ani*AbaA-mediated regulation of *AnibrlA* is more complicated. *AnibrlA* is upregulated in the *AniabaA* null mutant, suggesting that *Ani*AbaA also represses *AnibrlA* during life cycle [50]. Previous study shows the *Ani*AbaA-mediated repression of *AnibrlA* is through the repression of *AnibrlA*β [49]. However, there is no AbaA binding motif present in *AnibrlA*β promoter region [49], indicating that *Ani*AbaA indirectly represses *AnibrlA* through its effect on other genes.

Moreover, the AbaA plays different regulatory roles in conidiation in different *Aspergillus* species. Several *velvet* genes, like *veA*, *vosA*, and *velB*, contain AREs in their promoter regions and are differentially regulated by AbaA in *A. nidulans* and *A. fumigatus* [5,12,19,56]. Loss of *AfuabaA* causes the loss of *AfuvosA* mRNA accumulation and reduced *AfuvelB* mRNA accumulation, while it has no effect on *AfuveA* expression [5]. However, the overexpression of *AniabaA* leads to increased mRNA levels of *AniveA* and *AnivelB*, but not *AnivosA* [12,19]. Taken together, these results suggest that AbaA-mediated GRNs has been genetically rewired in *Aspergilli* during the revolution.

1-5-3 WetA

The *wetA* (wet-white A) gene is activated by AbaA at the late phase of conidiation and is essential for conidial survival [10,11,55]. Interestingly, AbaA is sufficient to activate *wetA* since the overexpression of *AniabaA* can induce *AniwetA* in the *AnibrlA* loss-of-function mutant [12]. Additionally, accumulation of *wetA* mRNA requires *wetA*$^{+}$ activity during conidiation, suggesting that *wetA* is autogenously regulated [10,44]. The transcription of *AniwetA* is initiated at 15 h after conidiation induction, which is corresponding to the timing of conidia formation (Fig 1-4) [12,44]. Moreover, unlike *AnibrlA* and *AniabaA*, *AniwetA* mRNA keeps high levels in conidia [44], which may result from the autogenous regulation of *AniwetA* itself. Taken together, WetA is likely to play more important roles in conidia than just contributes to conidia maturation.

The disruption of *wetA* results in various defects of conidia, including the formation of colorless conidia that undergo autolysis in *A. nidulans* (Fig 1-6) [10–12,15–18,44], *A. fumigatus* [5,20], and *A. oryzae* [8]. The disruption of *AniwetA* causes the loss of the C4 layer of the conidia wall, which makes conidia impermeable and mature [10,11,55]. Moreover, loss of *AniwetA* and *AfuwetA* leads to the failure of the condensation of the C2 layer in Δ*AniwetA* and Δ*AfuwetA* conidia [5,11]. The condensation of the C2 layer along with the formation of the C3 and C4 layers are the final stage of conidial maturation, which contributes to the impermeability of the conidial wall [11]. In addition, overexpression of *AniwetA* in vegetative cells inhibited hyphal growth and resulted in excessive branching and the activation of spore specific genes, however, it does not result in the activation of *AnibrlA* or *AniabaA* activation and never led to premature conidiation [10].

WetA is not only involved in the conidial morphological changes but also associated with the metabolism and expression control of the conidial components. Based on microscopy images, trehalose quantifications, and β-(1,3)-glucan quantifications from our group as well as others, studies proposed that WetA regulates conidial wall integrity and trehalose content [5–7,9,11,19]. The disruption of the metabolism of trehalose and β-(1,3)-glucan usually leads to reduced stress tolerance and viability of conidia, which are both observed in Δ*AniwetA* and Δ*AfuwetA* conidia [5]. WetA is highly and broadly conserved in *Ascomycetes* (Fig 1-5) and has been characterized in several species, including *A. oryzae*, *A. fumigatus*, *A. nidulans*, *Penicillium chrysogenum*, *P. digitatum*, *Beauveria bassiana*, and *Fusarium graminearum* [5–13,15–18,20,44]. The functions of WetA are highly conserved. *P. chrysogenum wetA* can fully complement the *A. nidulans wetA* deletion mutation, suggesting that the WetA-mediated sporulation regulatory mechanisms are conserved in *A. nidulans* and *P. chrysogenum* [13] As the loss of *AniwetA* in *A. nidulans*, the lack of *wetA* also results in abnormal conidia, delayed germination, and reduced stress tolerance in *P. digitatum* and *F. graminearum* [6,7]. Moreover, *F. graminearum* WetA suppresses microcycle conidiation and then further maintains conidial dormancy [6]. In the insect pathogen *B. bassiana*, Δ*BbwetA* mutant produces deficient conidia which are sensitive to environment stresses [9]. Put together, the conserved protein sequence and functions of WetA suggest that WetA plays similar regulatory roles in *Ascomycetes.*

Previous studies report that *Ani*WetA is required to activate the conidia-specific Class B genes and is able to autogenously activate itself [10,12,16,44]. Moreover, all characterized WetA contains a ESC1/WetA-related domain (PTHR22934: SF29) with the putative DNA-binding ability originating near the C-terminus [6,57], which also contains a predicted transcription activation domain (TAD) [58] and a nuclear localization signal (NLS) [59,60], suggesting that WetA is a potential TF, as well as the other two central regulators, BrlA and AbaA. Collectively, we propose that the evolutionarily conserved WetA plays a global regulatory role in bridging spore differentiation and survival in *Ascomycetes* by serving as a transcription factor.

Although WetA is required for trehalose biosynthesis in *A. nidulans*, *A. fumigatus* and *B. bassiana* loss of *wetA* did not alter the trehalose amount in *F. graminearum* and *P. digitatum* [5–7,9]. Moreover, loss of *wetA* in different species results in variable degrees of tolerance to oxidative stress and osmotic stress. The *A. fumigatus* and *F. graminearum wetA* deletion mutants are highly sensitive to H_2O_2 [5,6], while the *B. bassiana wetA* deletion mutants showed WT level tolerance to H_2O_2 [9], and the *P. digitatum wetA* deletion mutants even showed enhanced tolerance to H_2O_2 [7]. Similarly, loss of *wetA* causes reduced osmotic stress tolerance in *A. fumigatus*, *P. digitatum*, and *B. bassiana*, but has not in *F. graminearum* [5–7,9]. Put together, the WetA-mediated GRNs have been rewired during evolution.

1-6 Conclusion and the dissertation overview

In this chapter, we summarized the current understanding of the central regulatory pathway of *Aspergillus* conidiation. As the high degree of cell differentiation and the importance of the fungal reproduction, conidiation in filamentous fungi has long been studied and is still attracting interest from the research society. Even though there many GRNs involved in conidiation have been reported, the systematic transcriptome study of the central regulatory circuit *brlA* → *abaA* → *wetA* is still lack. Although WetA has been reported as a regulator of the completion of conidia maturation, its regulatory mechanism still remains unknown. We hypothesize that WetA is an evolutionally and functionally conserved transcription factor in Ascomycetes, which controls various GRNs involved in biological processes as well as in conidiation.

The ultimate goal of this thesis is to elucidate the roles and regulatory mechanism of WetA in three important *Aspergillus* species, the model organism *A. nidulans*, the human pathogen *A. fumigatus*, and the mycotoxin producer *A. flavus.* While WetA has been characterized in *A. nidulans* and *A. fumigatus*, its functions in *A. flavus* was still unclear. In chapter 2, we characterized the function of *Afl*WetA and carried out transcriptome analysis of the *Afl*WT and Δ*AflwetA* conidia. The result demonstrated that *Afl*WetA bridges cellular and chemical development in *A. flavus*. In chapter 3, we elucidated the conserved and diverged WetA-mediated GRNs in three *Aspergillus* species using comparative transcriptome analysis. Furthermore, we identified the WetA binding motif in *A. nidulans* and predicted potential WetA targeted genes in each species. Chapter 4 provides concluding remarks and ideas for future work on the project. In addition, the reprint of a published book chapter "Epigenetics of fungal secondary metabolism related genes" is included as Appendix 1.

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CHAPTER 2

WetA bridges cellular and chemical development in *Aspergillus flavus*

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2-1 Abstract

Bridging cellular reproduction and survival is essential for all life forms. *Aspergillus* fungi primarily reproduce by forming asexual spores called conidia, whose formation and maturation is governed by the central genetic regulatory circuit BrlA→AbaA→WetA. Here, we report that WetA is a multi-functional regulator that couples spore differentiation and survival, and governs proper chemical development in *Aspergillus flavus*. The deletion of *wetA* results in the formation of conidia with defective cell walls and no intra-cellular trehalose, leading to reduced stress tolerance, a rapid loss of viability, and disintegration of spores. WetA is also required for normal vegetative growth, hyphal branching, and production of aflatoxins. Targeted and genome-wide expression analyses reveal that WetA exerts feedback control of *brlA* and that 5,700 genes show altered mRNA levels in the mutant conidia. Functional category analyses of differentially expressed genes in Δ*wetA* RNA-seq data indicate that WetA contributes to spore integrity and maturity by properly regulating the metabolic pathways of trehalose, chitin, α-(1,3)-glucan, β-(1,3)-glucan, melanin, hydrophobins, and secondary metabolism more generally. Moreover, 160 genes predicted to encode transcription factors are differentially expressed by the absence of *wetA*, suggesting that WetA may play a global regulatory role in conidial development. Collectively, we present a comprehensive model for developmental control that bridges spore differentiation and survival in *A. flavus*.

2-2 Introduction

Coordination of cellular reproduction and survival is fundamental to the existence and propagation of all living organisms. From the simplest single cell organisms to complex multicellular plants and animals, regulatory and signalling systems have evolved to ensure that future viability of the reproductive cells. Fungi primarily reproduce through spore propagation; fungal spores are adapted for dispersal and are resistant to desiccation, heat, oxidative and UV stresses , properties which also render them very capable of establishing infections [1]. Fungal sporulation involves coordinated control of morphological, physiological, and metabolic (chemical) developmental processes.

The genus *Aspergillus* includes several organisms that are commonly found in the human environment. For example, the widely distributed *Aspergillus flavus* is an opportunistic pathogen of plants and humans [2], and can produce the mycotoxin aflatoxin B1 (AFB1), the most potent carcinogen found in nature. The main means of dissemination of this fungus is producing a massive number of asexual spores (conidia), which are dispersed in the soil and air. In agricultural fields, these spores are carried to corn ears by insects or the wind where they grow in maize kernels and produce AFB1 [3]. AFB1 can be present in oil-seed crops, such as corn, cereals, sorghum, and peanuts, and when AFB1 is present in the feed consumed by a cow, it can be metabolised to AFM1 (M for milk), which is also highly toxic and carcinogenic [4]. Consumption of high doses of AFB1 in humans can lead to acute aflatoxicosis, liver necrosis, and even death. Due to their carcinogenicity and toxicity, levels of aflatoxins in foods and feeds are strictly regulated worldwide [5]. As conidiation and AF production are tightly correlated in *A. flavus*, understanding the mechanisms bridging cellular and chemical development may provide novel insights into controlling the dissemination of the fungus and subsequent contamination of crops by AFB1 [6–8].

The asexual reproductive cycle of *Aspergillus* fungi can be divided into two distinct phases: growth and development. The growth phase involves germination of the conidium and formation of an undifferentiated network of hyphal cells that form the mycelium. Once nutritional resources begin to be limiting, some of the hyphal cells stop mycelial growth and begin asexual development (conidiation) by forming complex structures called conidiophores that bear multiple chains of conidia (Fig 2-1A), completing the asexual reproductive cycle [9]. Conidiation in *Aspergillus* involves distinct morphological and chemical processes [9]. For example, a key morphological process is the formation of a large number of conidia with specialized cell walls. Similarly, key primary metabolic processes include the acquisition of pigments and massive biogenesis of trehalose within the spore (up to 15% of dry weight), providing protection and long-term viability [10].

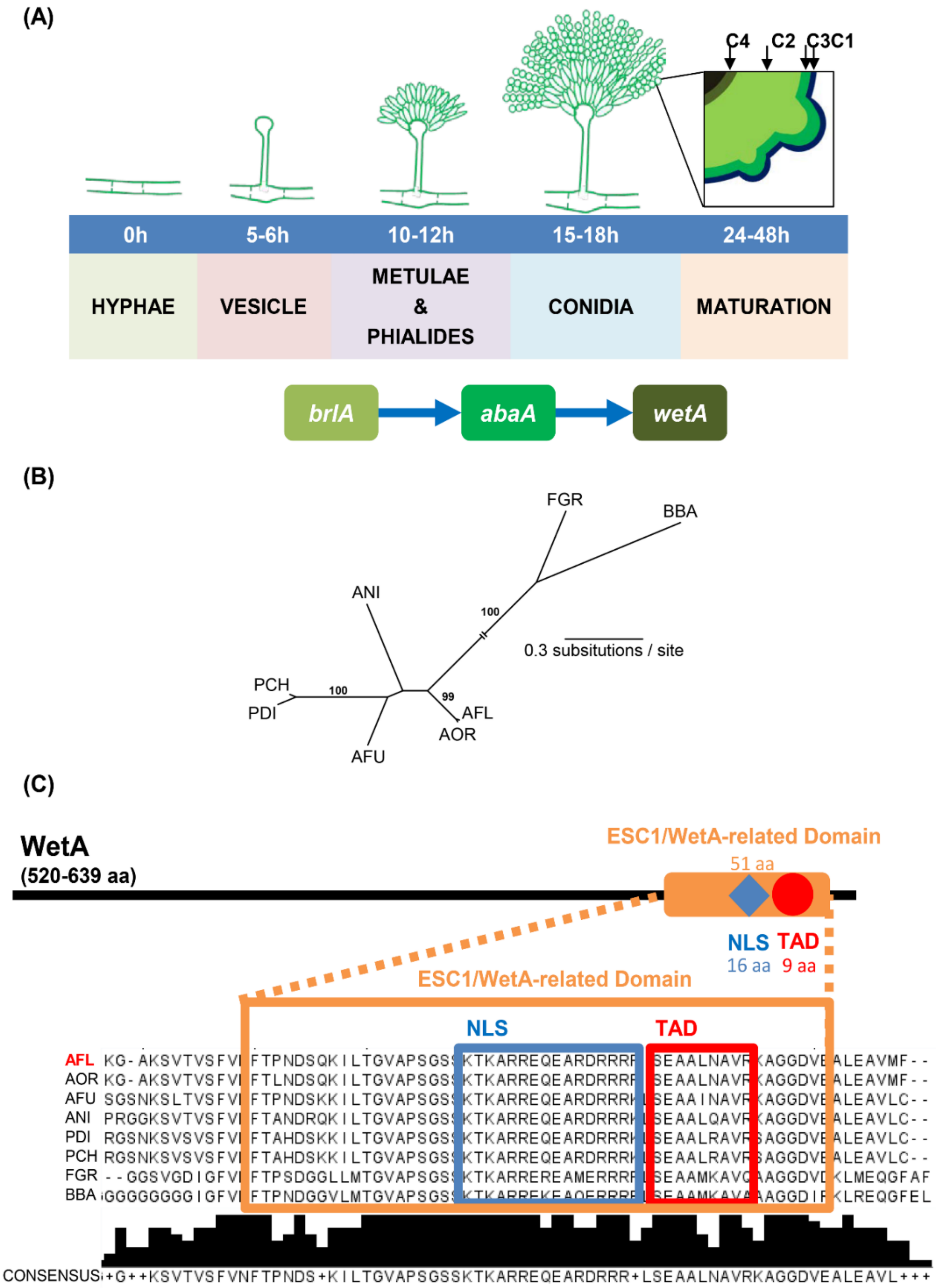


Fig 2-1 WetA is required for proper conidial maturation and contains both a transcription activation domain and a nuclear localization signal in a variety of fungi

(A) A model for the roles of the central regulators in *Aspergillus* conidiogenesis. WetA is activated by AbaA and is responsible for conidia wall maturation. The black square illustrates the graphic view of the wall structure of the mature conidium, including the crenulated electron-dense outer layer C1, the carbohydrate-condensed layer C3, the electron-thin layer C2, and the innermost layer C4. Note: some *Aspergillus* species lack metulae (ex. *A. parasiticus*), and some species can have both metulae-phialides or phialides-only conidiophores (ex. some *A. flavus* variants) [11]. (B) Unrooted phylogeny of WetA amino acid sequences of *A. flavus* NRRL3357 XP_002383329.1 (AFL), *A. fumigatus* Af293 XP_751508.1 (AFU), *A. nidulans* FGSC4 XP_659541.1 (ANI), *A. oryzae* RIP40 XP_001816745.1 (AOR), *Penicillium chrysogenum* Wisconsin 54-1255 XP_002564365.1 (PCH), *P. digitatum* Pd1 XP_014534725.1 (PDI), *Fusarium graminearum* PH-1 I1S0E2.2 (FGR), and *Beauveria bassiana* ARSEF 2860 XP_008599445.1 (BBA) [12–18]. The sequences were aligned using MAFFT, version 7.1.5 [19]. The WetA protein phylogeny was calculated using the maximum likelihood optimality criterion, as implemented in PAUP [20], version 4.0a152; we used the WAG model of amino acid evolution [21], with empirical amino acid frequencies and allowing for rate heterogeneity among sites. Values near internal branches correspond to bootstrap support values (only values above 70% are shown). Branch lengths correspond to the estimated number of amino acid substitutions per site – the internal branch leading to the FGR and BBA sequences has been truncated for optimal visualization. (C) The predicted WetA protein architecture. The red circle and the red box represent the transcription activation domain (TAD) which was predicted by 9aaTAD using the "Less stringent Pattern" setting [22]. The blue diamond and the blue box represent the nuclear localization signal (NLS) predicted by NLStradamus using the 4 state HMM static model [23]. The orange rectangle and the orange box represent the ESC1/WetA-related domain (PTHR22934) predicted by the PANTHER classification system [24]. The consensus sequence and the consensus histogram are shown under the amino acid sequence multiple sequence alignment.

A key and essential step for conidiophore development in *Aspergillus* is activation of *brlA*, which encodes a C_2H_2 zinc finger transcription factor (TF) (Fig 2-1A) [9,25]. Further genetic and biochemical studies identified *abaA* and *wetA* as genes that are also important for conidiation. The *abaA* gene, activated by BrlA during the middle stages of conidiation, has been reported to function in the differentiation and functionality of the cells that produce conidia, which are known as phialides [26,27]. The *wetA* gene is activated by AbaA at the late phase of conidiation and functions in the synthesis of crucial conidial wall components, such as the inner C4 layer, which makes conidia impermeable and mature [28,29]. These three genes define a central regulatory pathway that acts in concert with other genes to control conidiation-specific gene expression and determine the order of gene activation during conidiophore development and spore maturation [30].

In this report, we have characterised the functions of WetA (wet-white A) in an aflatoxigenic A. flavus strain (NRRL3357) employing genetic, analytical, and genomic approaches as a way to better understand the developmental and chemical biology of this important plant pathogen that dramatically impacts human health. Similar to what has been described in *A. nidulans* [29], the deletion (Δ) of *wetA* in *A. flavus* resulted in various defects, including the formation of wet-white conidia that take up water and autolyze rather than undergoing the final stages of maturation. *A. flavus* Δ*wetA* conidia are defective in the formation of a complex cell wall and lack pigments. TEM analysis indicates that many of the Δ*wetA* mutant conidia are misshaped and lack cytoplasm. Moreover, the Δ*wetA* mutant conidia lack trehalose and are highly sensitive to heat and oxidative stress. Importantly, WetA is also necessary for proper vegetative growth and AFB production. RNA-seq analyses of conidia indicate over 5,700 genes are differentially expressed between wild-type (WT) and the mutant conidia including 160 genes predicted to encode (putative) TFs, indicating a global regulatory role of WetA in conidiogenesis. Collectively, we propose that the evolutionarily conserved WetA protein plays a global regulatory role in governing growth, development, and bridging spore differentiation and survival in *A. flavus*.

2-3 Materials and methods

2-3-1 Strains, media, and culture conditions

Aspergillus strains used in this study are listed in Table S2-1. The fungal strains were grown on minimal medium (MM) with appropriate supplements as described previously [31,32] and incubated at 30°C. To determine the number of conidia, WT and mutant strains were point-inoculated and grown on solid MM at 30°C for 2 days. The conidia were collected in ddH$_2$O from the entire colony and counted using a hemocytometer. For liquid submerged cultures, conidia of

WT and mutant strains were inoculated in liquid MM and incubated at 30°C, 220 rpm. Conidiation induction was performed as previously described [33]. *Escherichia coli* strains, DH5α and BL21 (DE3), were grown in Luria-Bertani medium with ampicillin (50 mg/ml) for plasmid amplification.

2-3-2 Generation of *wetA* deletion and complemented strains

The oligonucleotides used in this study are listed in Table S2-1. Double-joint PCR was used to generate the deletion constructs of *wetA* [34]. Briefly, the deletion constructs containing *A. fumigatus pyrG* marker with 5' and 3' flanking regions of *wetA* were introduced into the recipient strain NRRL3357.5 [35]. To generate complemented strains, a WT *wetA* gene region including its upstream 2 kb region and downstream 1 kb region was amplified and introduced into the recipient strain. Multiple *wetA* deletion mutants (Δ*wetA*) in *A. flavus* were generated, which all behaved the same in every assay tested. We also generated three independent complemented strains (C'*wetA*), and they all behaved identically to one another as well. We chose TMY1 (Δ*wetA*) and TMY2 (C'*wetA*) as the testing strains for further experiments.

2-3-3 Nucleic acid manipulation

To isolate genomic DNA, about 10^6 conidia of relevant strains were inoculated in 2 ml liquid MM and stationary cultures at 30°C for 2 days. The mycelial mat was collected, squeeze-dried, and genomic DNA was isolated as described [34]. Total RNA isolation for Northern blot analyses was performed as described [33,34,36]. For RNA-seq, 2-day-old conidia of WT and Δ*wetA* strains were harvested from solid MM. Total RNA was extracted and submitted to ProteinCT Biotechnologies (Madison, WI) and the University of Wisconsin Biotechnology Center (Madison, WI) for library preparation and sequencing.

2-3-4 Conidia viability, autolysis, and stress response test

To check conidial viability, 2-day-old conidia of WT, Δ*wetA*, and C'*wetA* strains were collected and spread onto solid MM and cultured at 30°C. At 2-, 5-, 7-, 14-, and 20-days post-inoculation, conidia were collected from MM plates, and approximately 200 conidia were inoculated onto solid MM and cultured until colonies appeared. Survival rate was calculated as the ratio of the actual colony number to expected colony number. To test for conidial autolysis, approximately 100 conidia of WT, Δ*wetA*, and C'*wetA* strains were inoculated onto solid MM and incubated at 30°C for 4, 7, and 18 days. Conidia from the entire plate were collected and counted. Relative conidial number was compared to the number of conidia derived from a 4-day-old plate of each strain.

Two-day-old conidia of WT, Δ*wetA*, and C'*wetA* strains were collected to examine stress tolerance. For thermal stress tolerance tests, conidia were incubated at 50°C for 0, 10, and 60 minutes, and then spread onto solid MM. For UV stress tolerance tests, conidia were spread on solid MM and exposed to varying UV intensities (0, 100, and 200 J/cm^2). For osmotic stress tolerance tests, conidia were spread onto solid MM with different concentrations (0.0, 0.6, and 2.4 M) of KCl. Finally, for oxidative stress tolerance tests, conidia were spread onto solid MM with different concentrations (0, 2, and 4 mM) of H_2O_2. All plates were incubated at 30°C until colonies appeared. Colony numbers were counted and calculated as a percentage of the untreated control.

2-3-5 Conidia content quantification

Two-day-old conidia of WT, Δ*wetA*, and C'*wetA* strains were collected. Trehalose and β-glucan quantification was performed as previously described [13,37].

2-3-6 Transmission electron microscopy (TEM)

Two-day-old conidia of WT and Δ*wetA* were collected from MM plates. Sample preparation was performed as previous described [31]. TEM analyses were done by the UW Electron Microscope Facility.

2-3-7 Vegetative growth rate and hyphal branching rate tests

Conidia of WT, Δ*wetA*, and C'*wetA* strains were point inoculated onto solid MM and cultured at 30°C. Colony diameter was measured daily until day 7 post inoculation. To measure the hyphal branching rate, conidia of WT and Δ*wetA* strains were inoculated and cultured for 18 hours at 30°C, 220 rpm in liquid MM (V18). The mycelium aggregates were transferred to solid MM and cultured for 8 hours (A8). The average peripheral growth unit (PGU) is defined as the distance between two branching points. At least 15 PGUs were measured for each strain.

2-3-8 AFB1 quantification

Two-day-old conidia of WT, Δ*wetA*, and C'*wetA* strains were inoculated into 100 ml MM (10^3/ml) and cultured for 5 days at 30°C, 220 rpm. Individual liquid cultures were filtered by a single layer of Miracloth. The mycelium aggregates were squeezed with a paper towel to remove as much of the medium as possible. The mycelium mat was placed in a 65°C oven for 2 hours and then its dry weight was quantified. Two milliliters of the culture (with or without filtration to remove the mycelium) were mixed with an equal volume of chloroform, vigorously vortexed, and centrifuged. The chloroform (bottom) layer (750 μl) of each sample was transferred and evaporated in a glass tube overnight. Then each dried sample was dissolved in 500 μl methanol anhydrous and filtrated by a 0.45 μm filter syringe into HPLC vials. Each sample was injected into the HPLC (Agilent 1200 series) at a flow rate of 0.8ml/min with water:acetonitrile anhydrous:methanol anhydrous (20:40:40, v/v), and AFB was detected by a diode array detector at a wavelength of 365 nm [38]. The injected volume was 10 μl and the separation was performed via Agilent HPLC column

Zorbax Eclipse XDB-C18, 5um, 4.6 x 250mm cart.

2-3-9 RNA sequencing

A strand-specific library was prepared from total RNA using the Illumina TruSeq Strand-specific RNA sample preparation system. Briefly, mRNA was extracted from total RNA using poly-A selection, followed by RNA fragmentation. The strand-specific library was constructed by first-strand cDNA synthesis using random primers, sample cleanup, and second-strand synthesis using DNA Polymerase I and RNase H. A single 'A' base was added to the cDNA fragments followed by ligation of the adapters. Final cDNA library was achieved by further purification and enrichment with PCR, then quality checked using a Bioanalyzer 2100. The library was sequenced (SE100bp) using the Illumina HiSeq2500, and over 19 million high-quality reads per sample were achieved. All RNA-seq data files are available from the NCBI Gene Expression Omnibus database (Accession number: GSE95711).

2-3-10 Gene expression analysis

The quality of the raw sequence reads was verified using version 0.11.5 of FastQC [39]. The *A. flavus* genome and gene annotations were downloaded from NCBI (GCF_000006275.2_JCVI-afl1-v2.0_genomic.gff) and used for mapping. Mapping of the raw sequence reads to the genome was carried out with version 2.1.1 of Tophat2 [40], and the default options were used except for the maximum intron length was set to 4,000 bases (--max-intron-length 4000). Most (77-93%) of the reads from each of the samples mapped to the *A. flavus* genome. The alignment BAM files were compared against the gene annotation GFF file, and raw counts for the number of reads mapping to each gene were generated using version 0.6.1 of HTSeq-count [41]. Approximately 70-80% of mappable reads from each of the samples could be assigned to genes. Differential expression analysis of the raw counts was carried out using version 1.14.1 of DESeq2 [42]. Genes were considered differentially expressed between the WT and Δ*wetA* conidia if their adjusted p-value was less than 0.05 and their $\log_2$ fold change was less than -1 or greater than 1.

2-3-11 Functional Enrichment Analysis

GO annotations for *A. flavus* genes were downloaded from the AmiGO 2 website (version 2.4) on February 8, 2017 [43], and terms enriched in either the WetA-activated or -repressed gene lists were detected using version 3.0.3 of the BiNGO application [44] for Cytoscape (version 3.4.0) [45]. Version 1-26-17 of the Gene Ontology (go.obo) [46] was used to establish GO term relationships. GO terms were considered enriched if their p-value, following the Benjamini-Hochberg correction as implemented in BiNGO, was less than 0.05.

2-4 Results

2-4-1 Protein sequence features of WetA in *A. flavus*

The *A. flavus wetA* (XM_002383288.1) ORF comprises 1,692 bp with no introns and is predicted to encode a 563 amino-acid (aa) long protein. BlastP analysis against eight previously characterized WetA amino acid sequences reveals that *A. flavus* WetA has 99%, 61%, 57%, 53%, 53%, 68%, and 35% aa identity with WetA of *A. oryzae*, *A. fumigatus*, *A. nidulans*, *Penicillium chrysogenum*, *P. digitatum*, *Beauveria bassiana*, and *Fusarium graminearum*, respectively. Phylogenetic analysis revealed that *A. flavus* WetA is nested within a clade that also contains the *A. oryzae*, *A. nidulans*, *P. digitatum*, and *P. chrysogenum* WetA sequences, while *B. bassiana* WetA clusters with the *F. graminearum* sequence. Moreover, *A. flavus* WetA is almost identical to *A. oryzae* WetA and is relatively more similar to *A. fumigatus* WetA compared to *A. nidulans* WetA (Fig 2-1B). *A. flavus* WetA, along with all other WetA proteins included in our analyses, has a conserved 51-aa-length ESC1/WetA-related domain (PTHR22934: SF29) with the putative DNA-binding ability originating near the C terminus (Fig 2-1C) [14,47]. This highly conserved domain is further predicted by 9aaTAD and NLStradamus [22,23] to contain a 9-aa-length transcription activation domain (TAD) and a 16-aa-length nuclear localization signal (NLS), suggesting that WetA is a potential TF (Fig 2-1C).

2-4-2 The role of WetA in conidia

To understand the biological functions of WetA, we generated multiple *wetA* deletion mutants and complement strains in *A. flavus*. The *wetA* null mutant forms colourless (white) conidia which start to autolyze (wet) and collapse at 2 to 3 days after conidiation, forming an aggregated sphere structure (Fig 2-2A).

To check the detailed structural defects of the Δ*wetA* conidia, we carried out transmission electron microscopy (TEM) of conidiophores of WT and Δ*wetA* strains. As shown in Fig 2-2B, while WT formed intact conidial chains, the Δ*wetA* mutant showed fewer intact conidia and a high number of lysed conidial remnants. The WT conidial diameter is about 153% longer than that observed with Δ*wetA* (Fig 2-2C). Moreover, the WT conidium shows a crenulated electron-opaque outer layer (C1) and an electron-translucent inner layer (C2), as reported in *A. nidulan*s and *A. fumigatus* [13,29]. Although Δ*wetA* conidium forms the C1 and C2 layers, the C1 outer layer is smooth and the C2 layer is more condensed and thinner than that of WT (Fig 2-2D). Although the TEM results show that the intact Δ*wetA* conidium is as electron-opaque as the WT conidium, the intact Δ*wetA* conidium contains more β-(1,3)-glucan but less trehalose when compared to the WT conidium (Fig 2-2E and 2F).

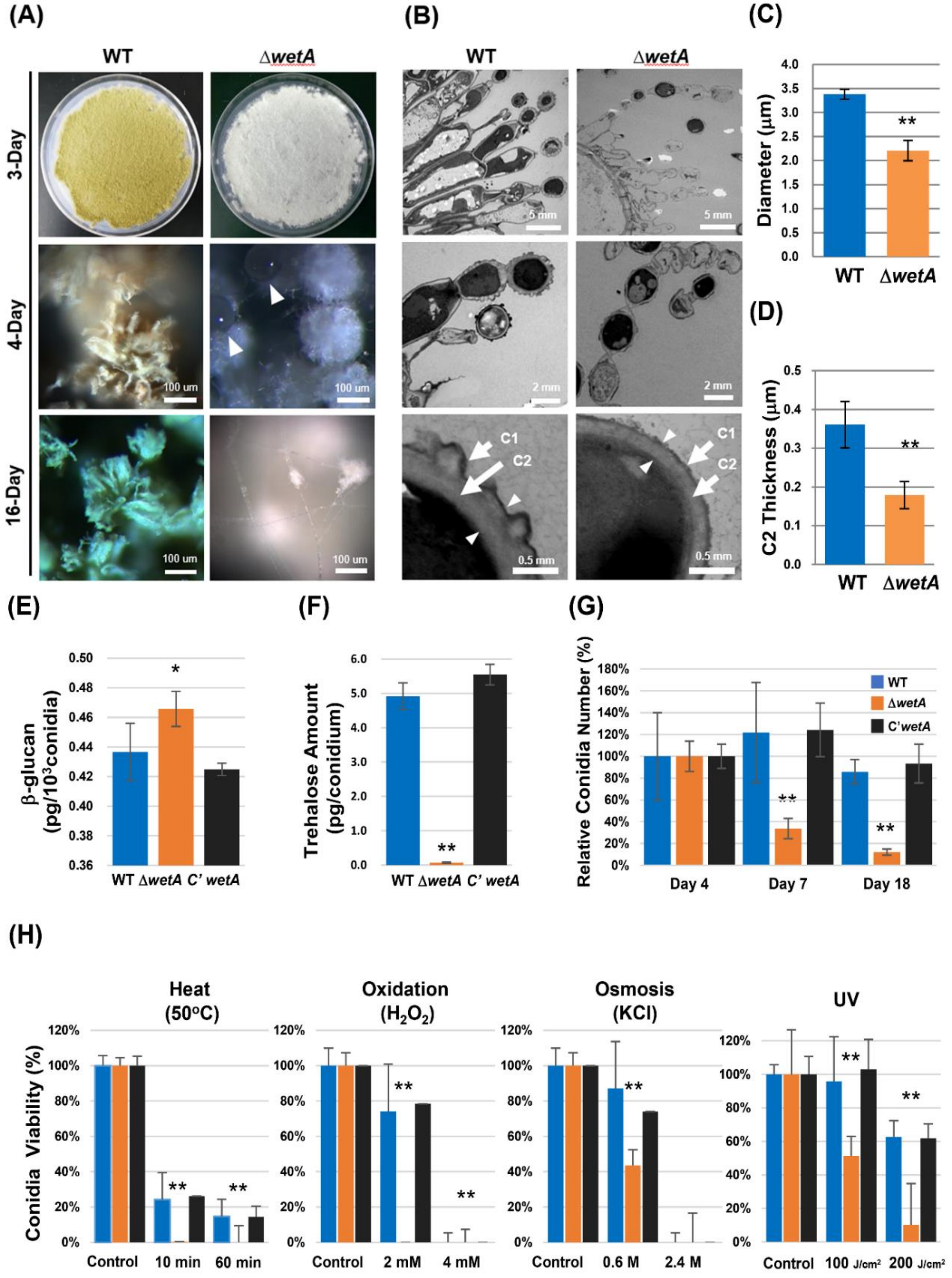


Fig 2-2 WetA is necessary for the proper formation of conidia in *Aspergillus flavus*

(A) Phenotypes of WT (NRRL3357), Δ*wetA*, and C'*wetA* grown on solid MM at 30ºC for 3, 4, 16 days after asexual induction. The white triangles indicate the liquid droplets formed on the autolyzing conidiophores of Δ*wetA* strain. (B) TEM images of 2-day-old conidiophores/conidia of WT and Δ*wetA* strains. Note: the remnant of lysed conidia formed a wet-vesicle-like structure on the top of the conidia chain, and most of the conidiophore/conidia contents were lost in the Δ*wetA* strain. The bottom panels show the conidia wall structures of WT and Δ*wetA* strains. Arrows indicate the locations of the C1 and C2 layers while the arrowheads indicate the C2 layer thickness. (C, D) The average diameter of conidia and thickness of the C2 layer of WT and Δ*wetA* conidia. (E, F) Quantification of conidia content (β-(1,3)-glucan (E) and trehalose (F)) of WT, Δ*wetA*, and C'*wetA* 2-day-old conidia The error bars indicate one standard deviation from the mean and the asterisks the level of significance (*, $p < 0.05$; **, $p < 0.01$). (G) The relative viability of WT, Δ*wetA*, and C'*wetA* conidia grown on solid MM at 30ºC for 4, 7, 18 days after inoculation. The conidial viability at day 4 of each strain was set as 100%. ** ($p < 0.01$). The error bars indicate one standard deviation from the mean viability of triplicates. (H) Tolerance of WT, Δ*wetA*, and C'*wetA* 2-day-old conidia to heat (50ºC), oxidative (H_2O_2), osmotic (KCl), and UV stresses. The control indicates untreated conidia. The viability of the untreated conidia of each strain was set as 100%. ** ($p < 0.01$). The error bars indicate one standard deviation from the mean viability of triplicates.

Somewhat surprisingly, while the loss of *wetA* leads to systemic defects in conidia, about 10% of the total Δ*wetA* conidia appears to be intact (Fig 2-2A) and equally viable as WT (data not shown) even at day 16. However, as the majority of the Δ*wetA* conidia autolyze and disintegrate, the total number of the Δ*wetA* conidia is dramatically decreased at day 7 and 18 post inoculation, whereas no significant changes in the viability of WT and C'*wetA* conidia were observable even at day 18 (Fig 2-2A and 2G). Finally, we examined whether the Δ*wetA* conidia show altered responses to various stresses. As shown in Figure 2H, the *wetA* null conidia are sensitive to osmotic (KCl) and oxidative (H_2O_2) stresses, and highly sensitive to heat (50ºC) and UV stress (Fig 2-2H). Taken together, these results suggest that WetA plays an essential role in the proper maturation and stress tolerance of conidia in *A. flavus*.

2-4-3 The roles of WetA in growth, hyphal branching, AFB production, and developmental control

We further tested the roles of WetA in governing other biological processes. We found that, in addition to conidiation, WetA is associated with proper hyphal growth. The WT and C'*wetA* strains showed higher colony growth rate than the Δ*wetA* mutant on solid minimal medium (MM), regardless of the presence or absence of the light (Fig 2-3A and Fig 2-3C). However, WetA appears to affect conidiation and hyphal development in response to light. Under dark condition, the Δ*wetA* colony exhibits highly reduced conidiation levels (Fig 2-3A). The WT colony edge can be divided into three regions, the single layer vegetative hyphae region (V_s), the multi-layer vegetative hyphae region (V_m), and the dense aerial hyphae region (A_d). In comparison to WT, the Δ*wetA* colony's edge does not contain the V_s region and instead has a sparse aerial hyphae region (A_s) between the V_m and A_d regions (Fig 2-3B). In addition, the A_s region of the Δ*wetA* colony is expanded when grown in the dark environment (Fig 2-3A and Fig 2-3B). Furthermore, the absence of *wetA* results in about 2.5-fold lower hyphal branching rate in both the submerged culture and the solid culture (Fig 2-3D and Fig 2-3E), which is consistent with our previous observation of the same phenotype in Δ*wetA* in *A. fumigatus* [13]. To further elucidate the role of WetA, we examined AFB production in WT, Δ*wetA*, and C'*wetA* strains shake-cultured for 5 days by HPLC. The results show that the Δ*wetA* strain was unable to produce AFB1 and AFB2 in the submerged culture (Fig 2-3F and Fig 2-3G).

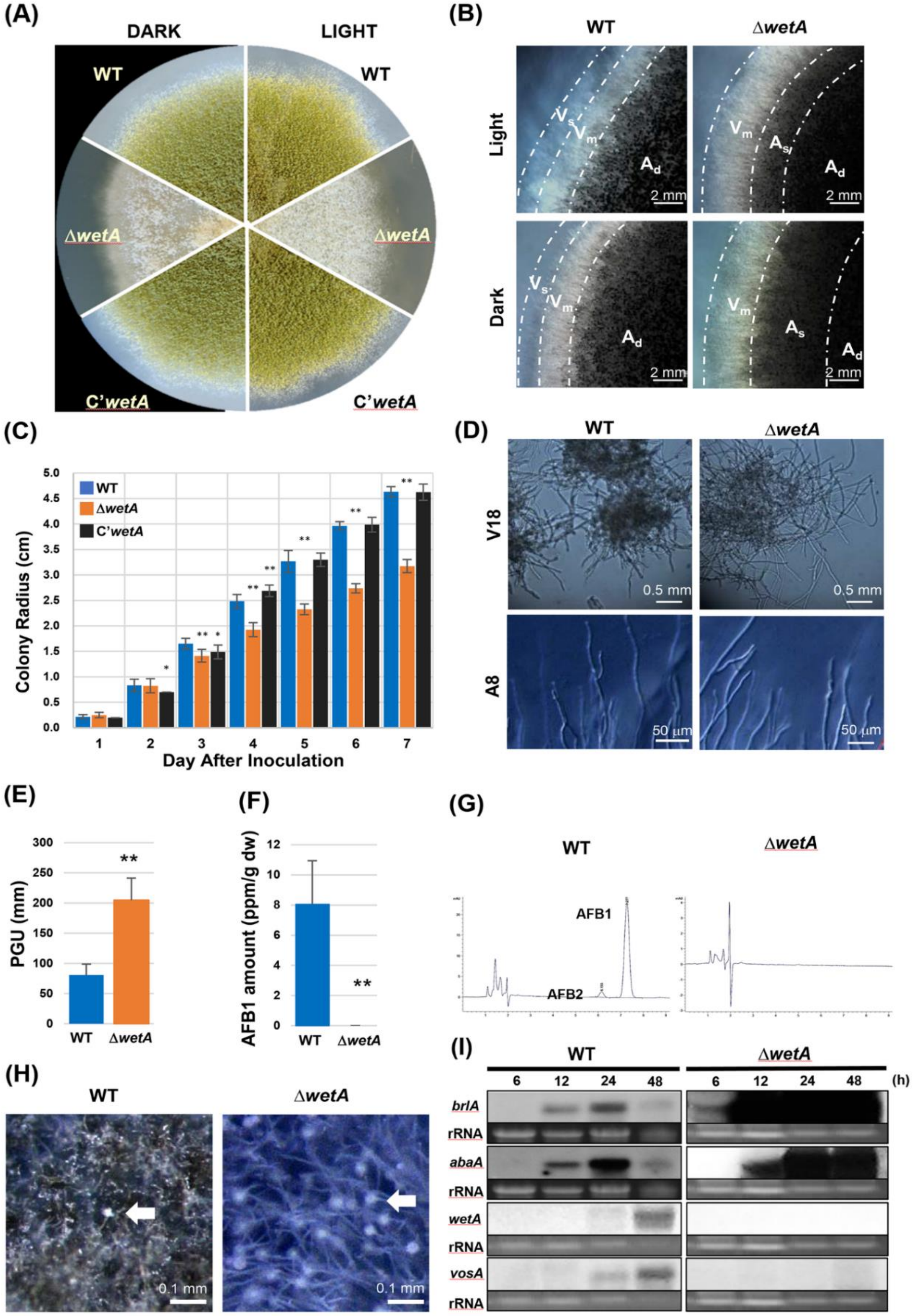


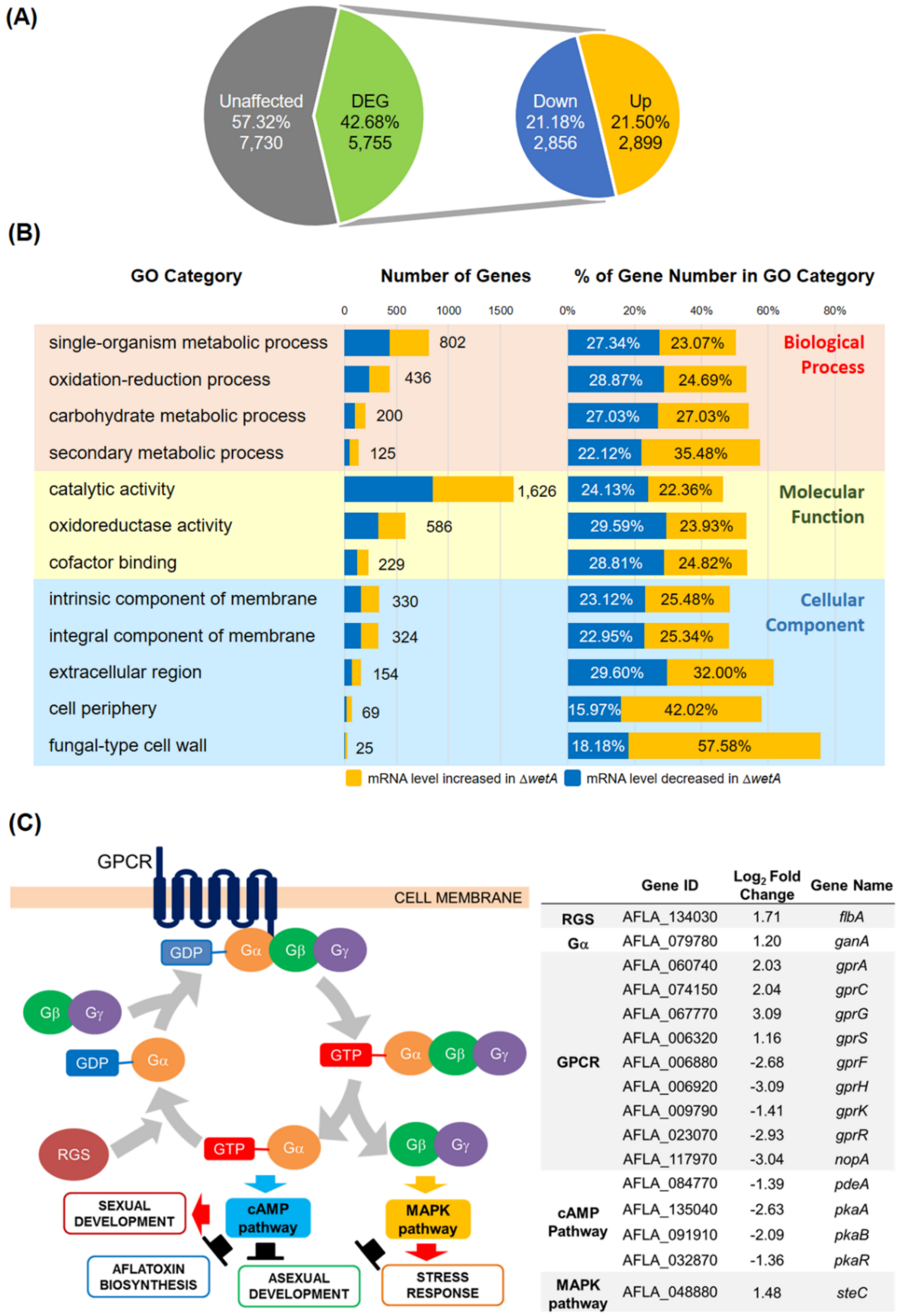
Fig 2-3 Multiple roles of WetA

(A-C) WetA affects vegetative growth. (A) The colony image of WT, Δ*wetA*, and C'*wetA* strains on solid MM at 5 days after point inoculation under light and dark conditions. (B) Colony edge image of WT and Δ*wetA* strains under light and dark conditions. V_s: single-layer vegetative hyphae region. V_m: multi-layer vegetative region. A_s: sparse aerial hyphae region. A_d: dense aerial hyphae region. (C) Colony growth rates of WT, Δ*wetA*, and C'*wetA* strains after point inoculation on solid MM. The error bars indicate one standard deviation. * ($p < 0.05$) and ** ($p < 0.01$). (D, E) Hyphal branching rates of WT and Δ*wetA* strains. (D) Microscopy images show WetA regulates hyphal branching. Loss of *wetA* leads to reduced hyphal branching rate in both solid and submerged cultures. (E) Average PGU values of A8. ** ($p < 0.01$). The error bars indicate one standard deviation. (F, G) Aflatoxin quantification by HPLC of WT and Δ*wetA* submerged culture after 5-days cultivation. (F) AFB1 amount (per g dry weight) in WT and Δ*wetA* vegetative cells. ** ($p < 0.01$). The error bars indicate one standard deviation. (G) The HPLC chromatograms of AFB1 and AFB2 in the culture medium of WT and Δ*wetA* strains. (H) WT and Δ*wetA* strains were induced for asexual development and observed after 8 h incubation at 30°C on solid MM plate. The white arrows indicate conidiophores. Note: the abundant conidiophore formation in Δ*wetA* culture. (I) Northern blot analysis of *brlA*, *abaA*, *wetA*, and *vosA* mRNA levels in WT and Δ*wetA* strains at 6, 12, 24, 48 h after conidiation induction.

We found that the absence of *wetA* resulted in precocious conidiophore development. The Δ*wetA* mutant started to generate abundant conidiophores at 6 h post asexual developmental induction, an hour earlier than WT and complement strains, and by 8 hours the difference in conidiophore production was striking (Fig 2-3H). This observation was corroborated by Northern blot analysis. As shown in Fig 2-3I, loss of *wetA* leads to early accumulation of mRNA from the asexual reproduction-inducing gene *brlA* at 6 h after asexual developmental induction, whereas *brlA* transcript only started to accumulate at 12 h after induction in WT. In WT, mRNA levels of *brlA* and *abaA*, another conidia-inducing regulator, reach their highest at 24 h and drop at 48 h after induction. In contrast, transcript levels of *brlA* and *abaA* dramatically increase at 12 h and stay at high levels at 24 and 48 h after asexual induction in the Δ*wetA* mutant. In WT, the mRNA for the regulator *vosA* started to accumulate at 24 h post-induction, while *vosA* mRNA accumulation was hardly detected in Δ*wetA* strain (Fig 2-3I). These results indicate that in *A. flavus*, WetA is a key feedback negative controller of *brlA* expression and conidiation. Overall, our data suggest that WetA plays multiple roles in cellular and chemical development in *A. flavus*.

2-4-4 Genome-wide expression analyses in conidia

To shed more light on the multiple regulatory roles WetA appeared to play in *A. flavus* biology, we carried out genome-wide expression analyses in WT and mutant conidia using RNA-seq. Poly-A mRNA from three technical replicates of 2-day-old conidia of WT and Δ*wetA* strains were purified and sequenced as described in the methods section; one technical replicate of the Δ*wetA* was discarded following failure in multiple quality control analyses. Examination of global gene expression differences between the WT and mutant *wetA* indicate that WetA plays a broad regulatory role in conidia. Out of the 13,485 mapped *A. flavus* genes, 5,755 (42.68% of the total) showed differential accumulation of mRNAs in the Δ*wetA* conidia in comparison to WT conidia. Among 5,755 differentially expressed genes (DEGs), mRNA levels of 2,856 (21.18%) genes were lower (Down) in the Δ*wetA* conidia compared to WT conidia, and those of 2,899 (21.50%) genes were higher (Up) in the Δ*wetA* conidia compared to WT conidia (Fig 2-4A).



	Gene ID	Log$_2$ Fold Change	Gene Name
RGS	AFLA_134030	1.71	*flbA*
Gα	AFLA_079780	1.20	*ganA*
GPCR	AFLA_060740	2.03	*gprA*
	AFLA_074150	2.04	*gprC*
	AFLA_067770	3.09	*gprG*
	AFLA_006320	1.16	*gprS*
	AFLA_006880	-2.68	*gprF*
	AFLA_006920	-3.09	*gprH*
	AFLA_009790	-1.41	*gprK*
	AFLA_023070	-2.93	*gprR*
	AFLA_117970	-3.04	*nopA*
cAMP Pathway	AFLA_084770	-1.39	*pdeA*
	AFLA_135040	-2.63	*pkaA*
	AFLA_091910	-2.09	*pkaB*
	AFLA_032870	-1.36	*pkaR*
MAPK pathway	AFLA_048880	1.48	*steC*

Fig 2-4 RNA-seq analyses of conidia

(A) The numbers of genes whose mRNA levels were similar (Unaffected, grey), or different between WT and Δ*wetA* conidia (DEG, green), with down (blue) and up (yellow) in the Δ*wetA* conidia compared to WT. A DEG is defined by having a > 2-fold change of mRNA levels between WT and Δ*wetA* conidia and an adjusted *p*-value of less than 0.05. (B) Functional categories of DEGs in conidia. The yellow bars represent genes whose mRNA levels increased in the Δ*wetA* conidia, whereas the blue bars represent those genes whose mRNA levels decreased in the Δ*wetA* conidia. The pink shaded box represents the biological process GO categories; the yellow shaded box represents the molecular function GO categories; the blue shaded box represents the cellular component GO categories. "Number of Genes": the total number of DEGs assigned to the specific GO category. "% of Gene Number in GO": the number of DEGs divided by the total number of genes assigned to the specific GO category in the genome as a whole. (C) The schematic diagram and mRNA expression profile of the G-protein regulatory pathways controlling development, stress response, and aflatoxin biosynthesis.

Functional category analysis was carried out by determining Gene Ontology (GO) terms that were enriched in DEGs. The top enriched biological process GO categories are "single-organism metabolic process", "oxidation-reduction process", "carbohydrate metabolic process", and "secondary metabolic process". The top enriched molecular function GO categories are "catalytic activity", "oxidoreductase activity", and "cofactor binding". The top enriched cellular component GO categories are "intrinsic component of membrane", "integral component of membrane", "extracellular region", "cell periphery", and "fungal-type cell wall" (Fig 2-4B). Of note, over 50% of all genes in the *A. flavus* genome annotated with the GO terms "carbohydrate metabolic process" (54.05%), "secondary metabolic process" (57.60%), and "fungal-type cell wall" (75.76%), were regulated by WetA in our RNA-seq data, consistent with our phenotypic data (Fig 2-4B). The top enriched GO categories for genes whose mRNA levels decreased or increased in the Δ*wetA* conidia are listed in Table S2-2 and Table S2-3, and the top 100 DEGs with decreased/increased mRNA accumulation levels in the Δ*wetA* conidia are listed in Table S2-4 and Table S2-5, respectively.

To explore the molecular roles of WetA in conidiation, we checked mRNA levels of those genes assigned to the GO term "Asexual Development" (GO:0019954) and other known genes related to asexual development [16]. In total, 77 genes related to asexual development are differentially expressed by the absence of WetA: 29 genes (37.66%) and 48 genes (62.34%) showed decreased and increased mRNA levels in the Δ*wetA* conidia, respectively (see Table 2-1 and Table S2-6). These data corroborate our working hypothesis that WetA is an important feedback regulator of conidiation, likely by activating several conidiation repressors and repressing key conidiation activators.

Table 2-1 DEGs of interest

	mRNA level decreased in Δ*wetA*	**mRNA level increased in Δ*wetA***
Asexual Development	*argB, bem1, dewA, fluG, fphA, kex1, nce102, nudG, osaA, pbcR, pkaA, pkaB, pkaR, ppoA, ppoB, rft1, rhbA, ricA, rodB, sfgA, swoM, tcpA, tmpA, tpsA ortholog, tpsC, veA, vosA, wA/pksP, wetA*	*abaA, ams1, atg1, atgH, brlA, cch1, chsA, chsB, chsE, chsF, chsG, crzA, esdC, fbx15, figA, flbA, flbC, ime2, llmB, llmF, medA, midA, mob1, msdS, mtfA, nsdC, nsdD, nudA, odeA, pac2/osaB, pcl1, phnA, ppoC, ppoD, prpA, rgdA, rho1, rodA, sidB, sltA, ssc1, steC, stuA, ugtA, vapA, wsc1, wsc3, zipA*
Transcription Factor*	*aflR, aflYd, amdA, amdR, aro80, atf21, clrA, ctf1B, fcr1, fkh1, galX, metR, nosA, nscR, pbcR, pcaG, prnA, rdr1, regA, scfA, sdrA, sfgA, sfp1, silA*	*abaA, amdX, aoiH, brlA, cnjB, cpcA, crzA, devR/hpa3, egd1, egr2, flbC, glcD, hacA, mtfA, ndtA, nsdC, nsdD, pacC, rap1, rfeB, rfeG, rgdA, rpn4, seb1, sltA, srbA, steA, stuA, zipA*
Aflatoxin Cluster	*aflA, aflR, aflS, aflYd*	*aflB, aflC, aflT, aflU, aflW, alfYa*

*only those genes with annotation are listed

As the GO terms "intrinsic component of membrane" and "integral component of membrane" were enriched in functional analysis, we further examined mRNA levels of the membrane-receptor-encoded-genes like G-protein coupled receptors (GPCRs). A heterotrimeric G-protein pathway can be involved in the activation of cAMP pathways and MAPK pathways, which then lead to the repression of asexual development [48]. The *A. flavus* genome contains 15 GPCRs [49], of which 9 showed altered mRNA levels (4 up, 5 down) in the Δ*wetA* conidia compared to WT (Fig 2-4C). The *ganA* gene predicted to encode a G protein alpha subunit (Gα), and the *flbA* gene predicted to encode a regulator of G protein signaling (RGS) protein showed increased mRNA levels in the Δ*wetA* conidia (Fig. 4C). Genes involved in the cAMP pathway like *pdeA, pkaA, pkaB*, and *pkaR*, showed decreased mRNA levels in the Δ*wetA* conidia (Fig 2-4C). Gene involved in the MAPK pathway, *steC*, show increased mRNA levels in the Δ*wetA* conidia (Fig 2-4C). Our results suggest that the WetA-mediated regulation may be associated with signal transduction pathways.

Next, we checked the expression levels of genes involved in conidia content and conidial wall integrity. As shown in Table 2-2, genes associated with the biosynthesis of trehalose, melanin, and hydrophobins, as well as the degradation of β-glucan, showed decreased mRNA levels in the Δ*wetA* conidia. Conversely, mRNA levels of genes associated with biosynthesis of chitin and β-(1,3)-glucan were increased in the Δ*wetA* conidia. These transcriptomic data and our direct measurement of β-glucan and trehalose indicate that WetA governs the integrity of conidia by coordinating intracellular contents and conidial wall biogenesis (Fig 2-5A, Table 2-2, and Table S2-7).

Table 2-2 DEGs involved in spore maturation

	mRNA level decreased in Δ*wetA*	**mRNA level increased in Δ*wetA***
Trehalose Biosynthesis	*tppB, tppC, rfaB, ccg-9, tpsC,* AFLA_087630	
Trehalose Degradation	*treA*	
Chitin Biosynthesis		*chsA, chsC, chsE, chsF, chsG, chsZ*
Chitin Degradation		*chiA, chiB, cts2, ctcB, nagA,* AFLA_031380, AFLA_107830, AFLA_057680
α-(1,3)-glucan Biosynthesis	*ags1*	*ags2*
α-(1,3)-glucan Degradation	*agnD, agnE*	AFLA_091790
β-(1,3)-glucan Biosynthesis		*fksP, gel1, gel2, gel4, gel5, gel6, gel7*, AFLA_107790, AFLA_064920
β-(1,3)-glucan Degradation	*bgt1, exg1,* AFLA_023650	*engl1, eng3, eng4, eng8, exg0, exg2*
Melanin Biosynthesis	*wA (pksP), ayg1*	
Hydrophobin	*dewA, rodB*, AFLA_063080, AFLA_098980	*rodA*, AFLA_094600

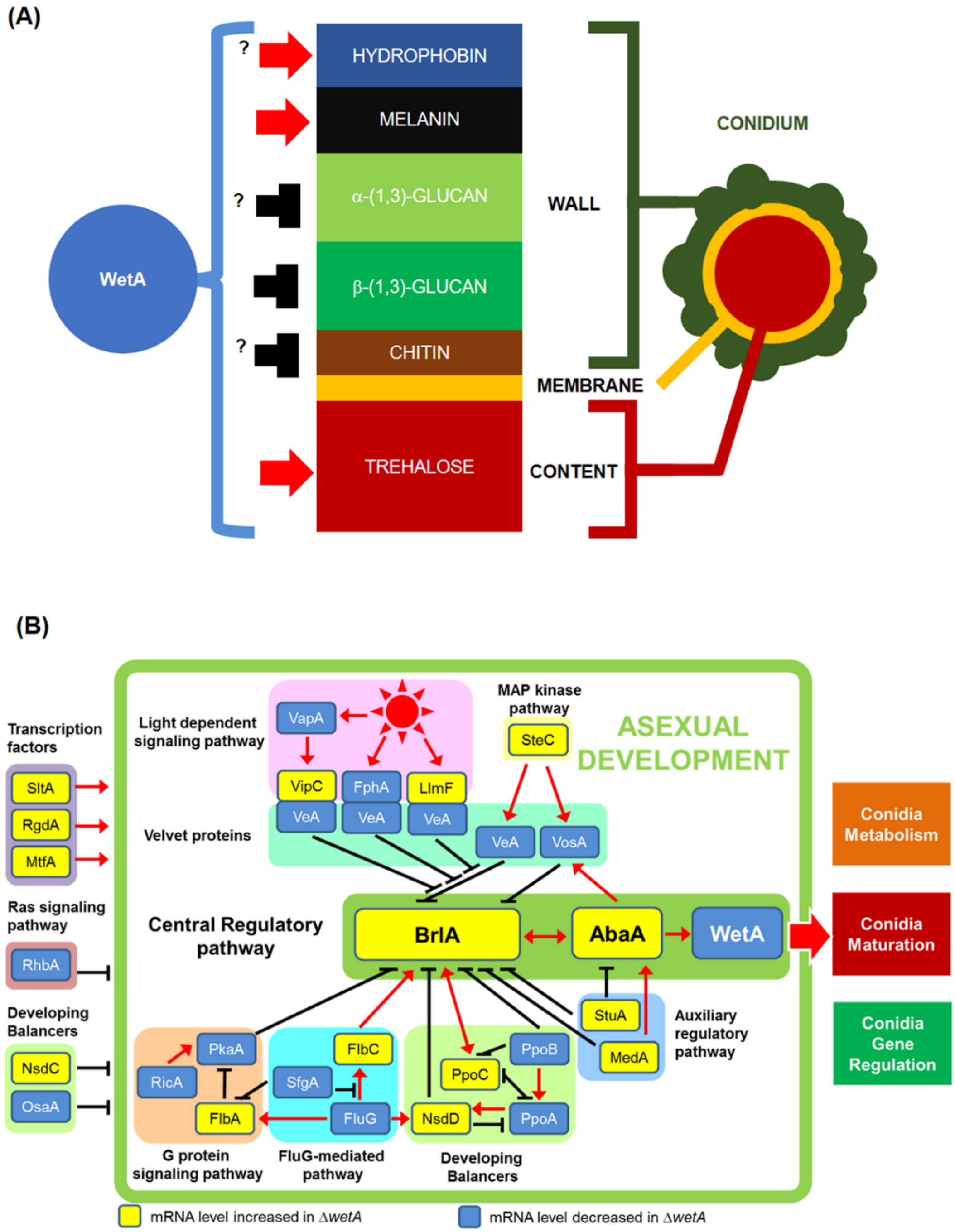


Fig 2-5 Summary of WetA functions and a model for WetA-mediated developmental regulation in *A. flavus*

(A) Schematic diagram of the WetA-mediated regulatory model of conidia architecture. The question mark indicates that the WetA-mediated activation/repression function needs to be verified by further experiments. (B) A comprehensive model for WetA-mediated regulation of asexual development based on transcriptomic, genetic, and biochemical data. In this model, those genes with increased and decreased mRNA levels in the Δ*wetA* conidia are labeled in yellow (WetA-inhibited) and blue (WetA-activated), respectively.

The GO term "secondary metabolic process" is enriched in the WetA-influenced transcriptome. We examined the differentially expressed genes belonging to secondary metabolite gene clusters (SMG clusters). We predicted the *A. flavus* SMG clusters with antiSMASH and used the cluster boundaries identified by Inglis *et al*. for *A. oryzae* clusters if they were conserved in *A. flavus* [50,51]. If the clusters were not identified by Inglis *et al*., we used the default boundaries provided by antiSMASH. There are 660 SMGs distributed in the 74 SMG clusters in *A. flavus* (Table S2-8). We found that 306 genes (46.37%) distributed in 68 SMG clusters (92%) showed altered mRNA levels in the Δ*wetA* conidia (136 down and 170 up, see Table S2-8). All of the genes located in Clusters 23, 35, 41, 46, 48, 52, 54, and 71 showed altered mRNA levels in the Δ*wetA* conidia. Interestingly, all genes in Clusters 23 and 52 showed decreased mRNA levels in the Δ*wetA* conidia, whereas all genes in Cluster 71 showed increased mRNA levels the Δ*wetA* conidia. These data indicate an important role of WetA governing secondary metabolic chemical development in conidia.

Finally, we focused on putative TFs showing altered mRNA levels in the Δ*wetA* conidia as this functional category was enriched among WetA-induced DEGs (Table S2-2. We found that 160 genes predicted to encode TFs exhibited altered mRNA accumulation in the presence and absence of WetA in conidia: 100 (62.50%) showed decreased mRNA levels and 60 (37.50%) showed increased mRNA levels in the Δ*wetA* conidia. Approximately 80% of these putative TFs have a zinc binding domain, including 18 TFs with a C_2H_2 domain and 64 TFs with a $Zn(II)_2Cys_6$ (or C6) domain (Table 2-1 and Table S2-9); important classes for the regulation of fungal development and metabolism. Taken together, WetA governs proper expression of various signaling, regulatory, structural, and metabolic elements that coordinate cellular and chemical development of conidia.

2-5 Discussion

Asexual development has been studied in *Aspergilli* and other fungi for many years [13–18,28–30,52–59]. In addition to *Aspergillus* fungi, the function of WetA is highly conserved in other Ascomycetes. In *P. digitatum*, the lack of *wetA* also results in abnormal conidia, delayed germination, and reduced stress tolerance [15]. Moreover, *P. chrysogenum wetA* can fully complement the *A. nidulans wetA* deletion mutation, suggesting that the WetA-mediated sporulation regulatory mechanisms are conserved in *A. nidulans* and *P. chrysogenum* [18]. In *F. graminearum*, loss of *wetA* causes deficient conidia, reduced oxidative and heat stress tolerance, and reduced chronological spore viability. *F. graminearum* WetA suppresses microcycle conidiation and then further maintains conidial dormancy [14]. In *B. bassiana*, *wetA* null mutants produce deficient conidia which are sensitive to environment stresses [17].

Previous studies suggest that WetA is responsible for activating a set of genes whose products comprise or direct the assembly of the conidial wall layers and ensure proper cytoplasmic status [28]. In *A. nidulans*, WetA together with AbaA activate genes which are expected to encode spore-specific functions (Class B Genes). WetA together with both BrlA and AbaA activate Class C and Class D genes, which are expected to encode phialide-specific functions [28,30,60]. However, WetA alone is sufficient to activate Class B and some Class D genes [28]. At least one gene (*wA*), whose mRNA accumulates in phialide cells instead of in conidia, is activated by WetA, indicating that WetA may regulate genes in these cells as well as in conidia [28]. Additionally, accumulation of *wetA* mRNA requires $wetA^{+}$ activity during conidiation, suggesting that *wetA* is autogenously regulated [12,28].

WetA is functionally conserved and required in *A. flavus* for many aspects of its biology, including spore viability, wall integrity, and stress tolerance. Although the *wetA* null mutant forms the C2 spore wall layer as found in WT, the Δ*wetA* C2 layer is hyper-condensed in *A. flavus* while it fails to condense in *A. nidulans*, *A. fumigatus*, and *P. digitatum* [13,15] (Fig 2-2B and Fig 2-2D). The condensation of the C2 layer along with the formation of the C3 and C4 layers are the final stage of conidial maturation, which contributes to the impermeability of the conidial wall [29]. We observed that the relative conidial viability and tolerance to UV and oxidative stresses are higher in the *A. flavus* Δ*wetA* mutant than the *A. nidulans* Δ*wetA* mutant, which may be due to the hyper-condensed C2 layer of the *A. flavus* Δ*wetA* conidia (Wu and Yu, unpublished data).

WetA is essential for establishing the heat stress tolerance in Ascomycetes [13,15,17]. However, loss of *wetA* in different species results in variable degrees of tolerance to oxidative stress and osmotic stress. The *A. fumigatus* [13], *A. flavus* (Fig 2-2H), *A. nidulans* (Wu and Yu, unpublished data), and *F. graminearum* [14] *wetA* deletion mutants are highly sensitive to H_2O_2. However, the *B. bassiana* [17] *wetA* deletion mutants showed WT level tolerance to H_2O_2 and the *P. digitatum* [15] *wetA* deletion mutants even showed enhanced tolerance to H_2O_2. Loss of *wetA* causes reduced osmotic stress tolerance in *A. nidulans* (Wu and Yu, unpublished data), *A. fumigatus* [13], *P. digitatum* [15], and *B. bassiana* [17], but has not in *F. graminearum* [14]. Somewhat surprisingly, even though the Δ*wetA* conidial structure and stress tolerance were impaired, the small numbers of intact Δ*wetA* conidia that could be isolated showed similar viability as the WT conidia (data not shown). However, only about 30% of the total Δ*wetA* conidia remain intact on day 7 after inoculation, while almost 100% of WT conidia remain intact (Fig 2-2G).

WetA appears to be involved in both the trehalose biosynthetic and degradation pathways in *A. flavus* (Fig 2-2F, Table 2-2, and Table S2-7), both of which are required for conidial stress tolerance and viability. The *velvet* regulator VosA is known as the regulator which couples sporogenesis and trehalose biogenesis, and it activates *wetA* in *A. nidulans* [31]. Our data show that WetA also activates *vosA* in *A. flavus* (Table 2-1 and Table S2-6) and in *A. nidulans* (Wu and Yu, unpublished data), suggesting an inter-dependent activation between the two regulators in conidia. While the loss of *wetA* does not completely block *vosA* expression in conidia (Table S2-6) and vice versa [31], almost no trehalose can be detected in the Δ*wetA* (Fig 2-2F) and Δ*vosA* [31] conidia, suggesting that both WetA and VosA are required for proper trehalose biosynthesis in conidia. Although WetA is required for trehalose biosynthesis in *A. flavus* (Fig 2-2F), *A. nidulans* (Wu and Yu, unpublished data), *A. fumigatus* [13], and *B. bassiana* [17], loss of *wetA* did not alter the trehalose amount in *F. graminearum* [14] and *P. digitatum* [15].

Based on microscopy images, trehalose quantifications, and β-(1,3)-glucan quantifications from our group as well as others, studies proposed that WetA regulates conidial wall integrity and trehalose content [13–15,17,29,58]. Our RNA-seq analyses further expands our understanding of how WetA affects overall conidial wall integrity. More than 75% of the genes assigned to the Cellular Component GO category “fungal-type cell wall” in the *A. flavus* genome were differentially expressed in the Δ*wetA* conidia (Fig. 4B), resulting in this category being statistically enriched amongst WetA-regulated genes. This suggests that WetA plays a global regulatory role in spore wall integrity. The *Aspergillus* conidial wall is composed of chitin, β-(1,3)-glucan, α-(1,3)-glucan, melanin, and hydrophobin [61] (Fig 2-5A). Our transcriptome analyses support the hypothesis that genes involved in the metabolic pathways of the conidial wall components are differentially expressed in the Δ*wetA* conidia (Table 2-2 and Table S2-7). Taken together, we present a summary of the role of WetA in governing conidial maturation by regulating the metabolic pathways of trehalose and conidial wall components in *A. flavus* (Fig 2-5A). However, the WetA-mediated regulatory circuits governing maturation and stress responses of conidia among various fungal species may be genetically re-wired in each fungal species, as is the case for other proteins involved in the regulation of asexual development and secondary metabolism [62]. Our data suggest that WetA is necessary to turn off the conidiation initiation process after the formation of conidiophores. Loss of *wetA* resulted in greatly enhanced levels of *brlA* and *abaA* in *A. flavus* (Fig 2-1D, Table 2-1, and Table S2-6), *A. nidulans* (Wu and Yu, unpublished data), *A. fumigatus* [13], *P. digitatum* [15], and *B. bassiana* [17]. Moreover, our transcriptome analyses indicate that loss of wetA resulted in altered mRNA levels of various regulators of conidiation (Table 2-1 and Table S2-6), implying an upstream regulatory role of WetA in conidia (Fig 2-5B). Consistent with this, we observed earlier conidiation in the Δ*wetA* mutant compared to WT in *A. flavus* (Fig 2-3H). However, the Δ*wetA* mutant showed delayed conidiation in *A. fumigatus* [13], and no differences in *A. nidulans* (Wu and Yu, unpublished data), suggesting that WetA-mediated feedback regulation of conidiation has undergone genetic rewiring in *Aspergilli*.

About 46% of genes positioned in the predicted SMG clusters showed altered mRNA levels in the Δ*wetA* conidia, including 15 genes predicted to encode polyketide synthases (PKSs) and PKS-like proteins, and 19 genes predicted to encode non-ribosomal polyketide synthases (NRPSs) and NRPS-like proteins (Table 2-3 and Table S2-8), suggesting that WetA affects biosynthesis of several secondary metabolites in conidia. The backbone gene, *aflC*, and the transcription factor, *aflR*, of the AF cluster were differentially expressed in the Δ*wetA* conidia (Table 2-1 and Table

S2-8). Although there is no significant difference in AFB amount between WT and Δ*wetA* conidia (data not shown), the loss of *wetA* reduces the amount of AFB1 and AFB2 in submerged culture (Fig 2-3F and Fig 2-3G), suggesting that WetA exerts temporal and spatial regulation of aflatoxin metabolism.

Loss of *wetA* results in reduced radial growth and lowered hyphal branching rates (Fig. 3A, 3C, 3D, 3E). RNA-seq results showed about 50% of the genes in the GO categories, "hyphal tip" and "site of polarised growth", exhibited increased mRNA levels in Δ*wetA* conidia (Table S2-3). Put together, WetA may play a regulatory role in hyphal development. It is possible that WetA regulates both hyphal and conidial cell wall assembly and therefore affects both the radial growth rate and branching rate of *A. flavus*.

Table 2-3 DEGs within the secondary metabolite biosynthesis clusters

BACKBONE GENES				
	mRNA level decreased in Δ*wetA*		**mRNA level increased in Δ*wetA***	
	CLUSTER	GENE ID	CLUSTER	GENE ID
PKS/PKS-LIKE	3	AFLA_127090	6	AFLA_053870
	12	AFLA_079360	10	AFLA_010000
	20	AFLA_116220	24	AFLA_118940
	21	AFLA_116890	24	AFLA_118960
	55	AFLA_006170	50	AFLA_002900
			58	AFLA_137870
			59	AFLA_139410
			71	AFLA_060020
			71	AFLA_060010
			74	AFLA_062820
NRPS/NRPS-LIKE	10	AFLA_010020	11	AFLA_010620
	10	AFLA_010010	26	AFLA_119820
	23	AFLA_118440	27	AFLA_121520
	25	AFLA_119110	41	AFLA_101700
	35	AFLA_038600	44	AFLA_064240
	45	AFLA_064560	46	AFLA_066720
	52	AFLA_004450	48	AFLA_069330
	54	AFLA_005440	61	AFLA_023020
	63	AFLA_028720	69	AFLA_109430
	65	AFLA_105190		
TRANSCRIPTION FACTORS				
	mRNA level decreased in Δ*wetA*		**mRNA level increased in Δ*wetA***	
	CLUSTER	GENE ID	CLUSTER	GENE ID
	18	AFLA_087810	31	AFLA_096370
	31	AFLA_096330	71	AFLA_059960
	40	AFLA_100300		
	59	AFLA_139360		
	63	AFLA_028760		
	66	AFLA_105530		

We observed differential conidial and hyphal development between light and dark conditions in the Δ*wetA* mutant (Fig 2-3A and Fig 2-3B). Transcriptome analyses revealed that several light-sensor-encoding genes were differentially expressed in the Δ*wetA* conidia, including *fphA*, *nopA*, *gprF*, and *gprR*. FphA is a phytochrome that represses sexual development in *A. nidulans* under red-light induction [63,64]. NopA is a fungal opsin type GPCR that represses conidiation in *Neurospora crassa* [65,66], but its function in *Aspergilli* is still vague [49,67]. GprF and GprR repress conidiation under the dark condition [49]. Taken together, WetA may be involved in light-dependent regulatory pathways that affect conidiation and hyphal development.

Our data show that WetA plays multiple roles in governing development (Fig 2-3). Transcriptome analyses showed that WetA affects mRNA levels of 160 genes predicted to encode TFs and 9 genes predicted to encode GPCRs (Fig 2-4C, Table 2-1, and Table S2-9). G-protein signaling governs normal growth, development, and mycotoxin production in filamentous fungi [68]. GPCRs are known to be involved in multiple cell processes, including carbon and nitrogen sensing, aflatoxin repression, germination, quorum sensing, oxylipin sensing, light sensing, and osmotic, acidic pH, ROS, and cell wall stress responses [49]. Taken together, our data suggest that the absence of WetA function results in disturbed expression of TFs and GPCRs leading to downstream pleiotropic effects. As annotation of the *A. flavus* genome improves to the levels of that of *A. nidulans*, *A. oryzae*, *A. fumigatus*, and *A. niger* genomes, we may identify additional putative regulators influenced by WetA in conidia.

In conclusion, we present a genetic model depicting the molecular mechanisms of WetA-mediated regulation in cellular and chemical development in *A. flavus* (Fig 2-5B). WetA affects the pathways of conidial content and conidial wall component metabolism, and further affects conidia viability and stress tolerance. Furthermore, WetA exerts feedback control of conidiation initiation by regulating upstream regulators of asexual development.

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Table S2-1 *Aspergillus* strains and oligonucleotides used in this study

STRAINS		
Name	GENOTYPE	SOURCE
NRRL3357	Wild-type	Fungal Genetic Stock Center
3357.5	*pyrG*$^{-}$	[100]
TMY1	*ΔwetA::AfupyrG*$^{+}$; *pyrG*$^{-}$	This study
TMY2	*ΔwetA::AfupyrG*$^{+}$; *pyrG*$^{-}$; *wetA*	This study

OLIGONUCLEOTIDES		
Name	**SEQUENCE (5’→3’)**	**PURPOSE**
oMY-19	ggagagcagattagcacctg	5’ flanking of *wetA*
oMY-20	ttacctcgacatgccacag	3’ flanking of *wetA*
oMY-21	ccatgcatggttactggagg	5’ nested of *wetA*
oMY-22	atgcgaccacgaatgcatcc	3’ nested of *wetA*
oMY-51	gggtgaagagcattgtttgaggcacgatggtggttccaagctcagctgtc	5' *wetA* with *AfupyrG* tail
oMY-52	tcagtgcctcctctcagacagaatatcggtgttctgatggatccggagtc	3' *wetA* with *AfupyrG* tail
oMY-25	gaccactcgttcaacaacgatg	5’ *wetA*
oMY-26	cgtactgcattaagtgcgg	3’ *wetA*
oJH-84	gctgaagtcatgatacaggccaaa	5’ *AfupyrG* marker
oJH-85	atcgtcgggaggtattgtcgtcac	3’ *AfupyrG* marker

Table S2-2 Top enriched GO categories of decreased mRNA levels in the Δ*wetA* conidia

GO Category	**# of Genes**	**% of Genes in Category**
Biological Process		
metabolic process	846	25.22%
biosynthetic process	298	27.41%
organic substance biosynthetic process	280	27.78%
cellular biosynthetic process	275	28.09%
oxidation-reduction process	235	28.87%
cellular nitrogen compound biosynthetic process	209	33.07%

organonitrogen compound metabolic process	204	30.96%
organic cyclic compound biosynthetic process	161	28.75%
heterocycle biosynthetic process	157	30.13%
organonitrogen compound biosynthetic process	153	31.94%
cellular amide metabolic process	94	33.57%
amide biosynthetic process	86	33.46%
peptide metabolic process	73	33.64%
translation	60	38.22%
organophosphate metabolic process	60	34.29%
cofactor metabolic process	44	35.48%
nucleoside phosphate metabolic process	40	40.00%
coenzyme metabolic process	37	39.36%
pyridine-containing compound metabolic process	19	54.29%
pyridine nucleotide metabolic process	17	54.84%
nicotinamide nucleotide metabolic process	17	54.84%
vitamin metabolic process	16	66.67%
cellular modified amino acid metabolic process	14	51.85%
cellular modified amino acid biosynthetic process	13	59.09%
tetrapyrrole metabolic process	9	69.23%
GTP metabolic process	6	100.00%
molybdopterin cofactor metabolic process	6	85.71%
prosthetic group metabolic process	6	85.71%
Molecular Function		
oxidoreductase activity	1095	29.59%
RNA polymerase II transcription factor activity, sequence-specific DNA binding	167	35.33%
structural molecule activity	115	46.96%
structural constituent of ribosome	97	54.64%
Cellular Component		
extracellular region	74	29.60%
intracellular ribonucleoprotein complex	53	39.85%
ribonucleoprotein complex	53	39.85%
ribosome	53	54.64%
ribosomal subunit	12	63.16%
small ribosomal subunit	8	80.00%

Table S2-3 Top enriched GO categories of genes showing increased mRNA levels in the Δ*wetA* conidia.

GO Category	# of Genes	% of Genes in Category
Biological process		
single-organism process	500	23.89%
transmembrane transport	167	25.97%
response to stimulus	113	29.66%
lipid metabolic process	86	28.67%
secondary metabolic process	77	35.48%
secondary metabolite biosynthetic process	73	35.27%
response to chemical	52	40.00%
cellular response to chemical stimulus	50	40.98%
cell wall organization or biogenesis	39	41.94%
asexual reproduction	38	34.86%
asexual sporulation	37	35.24%
toxin metabolic process	30	38.46%
response to drug	23	41.07%
cellular response to drug	22	41.51%
external encapsulating structure organization	21	48.84%
cell wall organization	21	48.84%
asexual sporulation resulting in formation of a cellular spore	21	42.00%
fungal-type cell wall organization or biogenesis	20	50.00%
chemical homeostasis	18	45.00%
aminoglycan metabolic process	15	57.69%
cation homeostasis	15	57.69%
ion homeostasis	15	51.72%
cell wall polysaccharide metabolic process	15	48.39%
glucosamine-containing compound metabolic process	14	66.67%
amino sugar metabolic process	14	60.87%
metal ion homeostasis	14	58.33%
inorganic ion homeostasis	14	51.85%
chitin metabolic process	11	61.11%
divalent inorganic cation homeostasis	8	66.67%
chitin biosynthetic process	6	85.71%
amino sugar biosynthetic process	6	85.71%
glucosamine-containing compound biosynthetic process	6	85.71%

positive regulation of conidiophore development	5	100.00%
Molecular Function		
catalytic activity	782	22.36%
iron ion binding	60	34.68%
flavin adenine dinucleotide binding	58	30.37%
tetrapyrrole binding	55	35.71%
heme binding	55	35.71%
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	55	32.96%
electron carrier activity	46	32.86%
monooxygenase activity	29	48.33%
acyl-CoA dehydrogenase activity	10	58.82%
chitin synthase activity	6	100.00%
1,3-beta-glucanosyltransferase activity	5	100.00%
Cellular Component		
membrane	277	26.01%
intrinsic component of membrane	173	25.48%
integral component of membrane	170	25.34%
extracellular region	80	32.00%
cell periphery	50	42.02%
plasma membrane	28	44.44%
cell septum	24	57.14%
site of polarized growth	23	50.00%
external encapsulating structure	22	52.38%
hyphal tip	22	50.00%
cell wall	21	53.85%
fungal-type cell wall	19	57.58%
plasma membrane part	10	55.56%
cell surface	9	81.82%

Table S2-4 Top 100 genes showing decreased mRNA levels in the Δ*wetA* conidia

GENE ID	Log$_2$ Fold Change	Description
AFLA_126840	-11.62	conserved hypothetical protein
AFLA_071920	-11.61	class III aminotransferase, putative
AFLA_001980	-11.56	glutamate cysteine ligase, putative
AFLA_007360	-11.52	hypothetical protein
AFLA_116190	-11.46	conserved hypothetical protein
AFLA_102370	-11.30	conserved hypothetical protein
AFLA_124380	-11.18	hypothetical protein
AFLA_075640	-11.09	pigment biosynthesis protein Ayg1
AFLA_116180	-11.04	hypothetical protein
AFLA_120760	-11.01	fatty acid oxygenase, putative
AFLA_124110	-10.99	galactose-proton symport, putative
AFLA_096210	-10.90	catalase, putative
AFLA_077930	-10.90	conserved hypothetical protein
AFLA_126820	-10.90	conserved hypothetical protein
AFLA_004410	-10.89	conserved hypothetical protein
AFLA_066550	-10.87	alcohol dehydrogenase, putative
AFLA_117120	-10.85	hypothetical protein
AFLA_036400	-10.80	conserved hypothetical protein
AFLA_075630	-10.79	conserved hypothetical protein
AFLA_101770	-10.79	conserved hypothetical protein
AFLA_001030	-10.79	lanosterol synthase, putative
AFLA_007350	-10.76	hypothetical protein
AFLA_124890	-10.74	hypothetical protein
AFLA_052030	-10.74	developmental regulatory protein WetA
AFLA_056870	-10.71	conserved hypothetical protein
AFLA_124560	-10.70	conserved hypothetical protein
AFLA_126830	-10.70	conserved hypothetical protein
AFLA_064190	-10.70	hypothetical protein
AFLA_123950	-10.69	ankyrin repeat protein
AFLA_116170	10.67	hypothetical protein
AFLA_009740	-10.66	hypothetical protein
AFLA_023610	-10.65	conserved hypothetical protein

AFLA_126260	-10.59	extracellular 3-ketosteroid 1-dehydrogenase, putative
AFLA_034380	-10.59	catalase, putative
AFLA_007880	-10.59	conserved hypothetical protein
AFLA_085550	-10.55	hypothetical protein
AFLA_117110	-10.50	conserved hypothetical protein
AFLA_073970	-10.48	conserved hypothetical protein
AFLA_104490	-10.46	conserved hypothetical protein
AFLA_126170	-10.41	conserved hypothetical protein
AFLA_004440	-10.38	ABC multidrug transporter, putative
AFLA_101550	-10.38	thioredoxin reductase, putative
AFLA_072030	-10.28	conserved hypothetical protein
AFLA_063400	-10.27	hypothetical protein
AFLA_066540	-10.24	hypothetical protein
AFLA_096220	-10.20	HHE domain protein
AFLA_122950	-10.17	conserved hypothetical protein
AFLA_121110	-10.13	conserved hypothetical protein
AFLA_044800	-10.11	conidiation protein Con-6, putative
AFLA_000870	-10.09	hypothetical protein
AFLA_092300	-10.09	hypothetical protein
AFLA_063080	-10.01	conserved hypothetical protein
AFLA_063110	-9.92	hypothetical protein
AFLA_043550	-9.85	conserved hypothetical protein
AFLA_024660	-9.83	conserved hypothetical protein
AFLA_059700	-9.82	conserved hypothetical protein
AFLA_099050	-9.79	conserved hypothetical protein
AFLA_083110	-9.72	conidiation-specific protein (Con-10), putative
AFLA_034370	-9.67	hypothetical protein
AFLA_014240	-9.66	conserved hypothetical protein
AFLA_002840	-9.66	HHE domain protein
AFLA_117130	-9.61	conserved hypothetical protein
AFLA_003760	-9.59	NACHT domain protein
AFLA_059670	-9.58	conserved hypothetical protein
AFLA_008630	-9.45	hypothetical protein
AFLA_102410	-9.44	conserved hypothetical protein
AFLA_120190	-9.42	67 kDa myosin-cross-reactive antigen family protein
AFLA_077900	-9.41	conserved hypothetical protein

AFLA_057190	-9.38	conserved hypothetical protein
AFLA_033450	-9.36	gamma-glutamylputrescine oxidoreductase, putative
AFLA_010480	-9.33	potassium channel, putative
AFLA_007580	-9.28	MFS sugar transporter, putative
AFLA_102420	-9.20	hypothetical protein
AFLA_123310	-9.17	conserved hypothetical protein
AFLA_048440	-9.13	conserved hypothetical protein
AFLA_071780	-9.06	Dyp-type peroxidase family protein
AFLA_126860	-9.03	cell wall cysteine-rich protein
AFLA_059690	-8.97	conserved hypothetical protein
AFLA_039040	-8.93	conserved hypothetical protein
AFLA_131930	-8.86	conserved hypothetical protein
AFLA_090360	-8.86	conserved hypothetical protein
AFLA_106210	-8.85	efflux pump antibiotic resistance protein, putative
AFLA_010090	-8.85	hypothetical protein
AFLA_096740	-8.85	monoxygenase, putative
AFLA_008410	-8.81	MFS multidrug transporter, putative
AFLA_074740	-8.77	conserved hypothetical protein
AFLA_039050	-8.71	hypothetical protein
AFLA_131600	-8.69	lysine-rich arabinogalactan protein 18 precursor, putative
AFLA_092090	-8.67	oxidoreductase, short-chain dehydrogenase/reductase family
AFLA_016440	-8.67	conserved hypothetical protein
AFLA_124370	-8.64	hypothetical protein
AFLA_126850	-8.63	vacuolar protease A, putative
AFLA_094770	-8.62	conserved hypothetical protein
AFLA_024540	-8.52	sensory transduction histidine kinase, putative
AFLA_015260	-8.50	short chain dehydrogenase/oxidoreductase, putative
AFLA_106220	-8.50	cytochrome P450, putative
AFLA_072380	-8.50	conserved hypothetical protein
AFLA_024610	-8.49	NADP-dependent alcohol dehydrogenase
AFLA_053400	-8.47	hypothetical protein
AFLA_120210	-8.44	conserved hypothetical protein

Table S2-5 Top 100 genes showing increased mRNA levels in the Δ*wetA* conidia.

GENE ID	Log_2 Fold Change	Description
AFLA_121740	10.28	hypothetical protein
AFLA_070470	8.84	conserved hypothetical protein
AFLA_118990	7.89	efflux pump antibiotic resistance protein, putative
AFLA_020010	7.69	hypothetical protein
AFLA_106850	7.56	thioredoxin reductase GliT-like, putative
AFLA_059770	7.54	conserved hypothetical protein
AFLA_064950	7.51	conserved hypothetical protein
AFLA_106040	7.48	conserved hypothetical protein
AFLA_060200	7.45	neutral amino acid permease, putative
AFLA_135310	7.43	hypothetical protein
AFLA_045570	7.40	acetyl xylan esterase, putative
AFLA_134850	7.37	conserved hypothetical protein
AFLA_059950	7.37	oxidoreductase, FAD-binding, putative
AFLA_033410	7.37	conserved hypothetical protein
AFLA_116630	7.35	(S)-2-hydroxy-acid oxidase, putative
AFLA_064840	7.27	conserved hypothetical protein
AFLA_059900	7.21	calcium-binding protein precursor, putative
AFLA_004880	7.10	amine oxidase, flavin-containing superfamily
AFLA_100200	7.01	conserved hypothetical protein
AFLA_001750	6.90	cell surface protein Mas1, putative
AFLA_073230	6.83	conserved hypothetical protein
AFLA_064850	6.83	aminotriazole resistance protein, putative
AFLA_107900	6.82	conserved hypothetical protein
AFLA_104680	6.80	class V chitinase ChiB1
AFLA_137110	6.79	extracellular serine-rich protein, putative
AFLA_043870	6.71	conserved hypothetical protein
AFLA_104880	6.68	glycosyl hydrolase, putative
AFLA_033420	6.61	Mn superoxide dismutase MnSOD
AFLA_060340	6.56	conserved hypothetical protein
AFLA_052490	6.55	C2H2 finger domain protein, putative
AFLA_135300	6.54	hypothetical protein
AFLA_073220	6.51	hypothetical protein

AFLA_008450	6.50	conserved hypothetical protein
AFLA_087030	6.41	conserved hypothetical protein
AFLA_004510	6.39	peroxidase, putative
AFLA_125710	6.37	conserved hypothetical protein
AFLA_065460	6.36	conserved hypothetical protein
AFLA_137940	6.36	conserved hypothetical protein
AFLA_138180	6.34	lysozyme, putative
AFLA_085820	6.32	conserved hypothetical protein
AFLA_129170	6.30	conserved hypothetical protein
AFLA_106070	6.30	conserved hypothetical protein
AFLA_123580	6.28	hypothetical protein
AFLA_023760	6.28	conserved hypothetical protein
AFLA_061710	6.15	calcium/calmodulin-dependent protein kinase type, putative
AFLA_040090	6.14	conserved hypothetical protein
AFLA_053300	6.10	cytochrome P450, putative
AFLA_102200	6.09	conserved hypothetical protein
AFLA_120930	6.03	glycosyl transferase, putative
AFLA_131620	6.00	acyl-CoA desaturase, putative
AFLA_041690	5.98	cysteine synthase B, putative
AFLA_053560	5.90	conserved hypothetical protein
AFLA_060010	5.88	PKS-like enzyme, putative
AFLA_065760	5.87	cytochrome P450, putative
AFLA_001680	5.87	conserved hypothetical protein
AFLA_129490	5.86	hypothetical protein
AFLA_082410	5.84	conserved hypothetical protein
AFLA_122200	5.83	conserved hypothetical protein
AFLA_099940	5.82	conserved hypothetical protein
AFLA_070320	5.80	choline transport protein, putative
AFLA_031380	5.80	class V chitinase, putative
AFLA_039740	5.80	conserved hypothetical protein
AFLA_005370	5.80	conserved hypothetical protein
AFLA_067760	5.78	conserved histidine-rich protein
AFLA_059610	5.68	conserved hypothetical protein
AFLA_100210	5.66	ankyrin repeat-containing protein, putative
AFLA_137860	5.64	conserved hypothetical protein

AFLA_117000	5.64	RNA exonuclease, putative
AFLA_072810	5.62	conserved hypothetical protein
AFLA_110980	5.62	conserved hypothetical protein
AFLA_045580	5.61	hypothetical protein
AFLA_029970	5.60	conserved hypothetical protein
AFLA_116260	5.58	N-hydroxyarylamine O-acetyltransferase, putative
AFLA_034810	5.56	hypothetical protein
AFLA_023250	5.53	conserved hypothetical protein
AFLA_023160	5.53	ankyrin repeat-rich membrane-spanning protein, putative
AFLA_060110	5.52	conserved hypothetical protein
AFLA_001670	5.52	3-demethylubiquinone-9 3-methyltransferase, putative
AFLA_066790	5.51	conserved hypothetical protein
AFLA_137850	5.50	conserved hypothetical protein
AFLA_019000	5.49	conserved hypothetical protein
AFLA_031140	5.49	LipA and NB-ARC domain protein
AFLA_074240	5.48	conserved hypothetical protein
AFLA_075190	5.48	conserved hypothetical protein
AFLA_064810	5.46	extracellular proline-serine rich protein
AFLA_095440	5.45	serine/threonine protein kinase, putative
AFLA_010870	5.43	endo-1,4-beta-xylanase B precursor, putative
AFLA_097190	5.42	conserved hypothetical protein
AFLA_009870	5.41	conserved hypothetical protein
AFLA_134330	5.41	conserved hypothetical protein
AFLA_065050	5.39	Defensin domain protein
AFLA_077720	5.37	conserved hypothetical protein
AFLA_077730	5.36	carnitinyl-CoA dehydratase, putative
AFLA_101360	5.36	cytochrome P450, putative
AFLA_107340	5.35	C4-dicarboxylate transporter/malic acid transport protein, putative
AFLA_078910	5.35	hypothetical protein
AFLA_101190	5.33	conserved hypothetical protein
AFLA_034670	5.31	poly(ADP)-ribose polymerase PARP, putative
AFLA_121660	5.30	conserved hypothetical protein
AFLA_042430	5.28	hypothetical protein

Table S2-6 DEGs related to asexual development

Gene ID	Log2 Fold Change	Common Name
AFLA_034670	5.31	*prpA*
AFLA_033400	4.17	*msdS*
AFLA_021100	3.74	*ppoD*
AFLA_099380	3.60	*wsc1*
AFLA_136030	3.37	*chsE*
AFLA_082850	3.31	*brlA*
AFLA_137320	3.04	*flbC*
AFLA_052510	3.01	*wsc3*
AFLA_029620	2.96	*abaA*
AFLA_008970	2.89	*llmF*
AFLA_031700	2.89	*midA*
AFLA_087350	2.86	*sltA*
AFLA_136410	2.81	*medA*
AFLA_131330	2.72	*nsdC*
AFLA_060590	2.59	*chsG*
AFLA_071090	2.51	*esdC*
AFLA_028750	2.49	*llmB*
AFLA_078290	2.43	*chsF*
AFLA_029150	2.40	*pcl1*
AFLA_086010	2.40	*figA*
AFLA_135550	2.38	*mob1*
AFLA_046990	2.32	*stuA*
AFLA_091490	2.27	*mtfA*
AFLA_102850	2.22	*rgdA*
AFLA_092800	2.17	*nudA*
AFLA_042780	1.95	*chsA*
AFLA_066330	1.85	*odeA*
AFLA_083100	1.81	*zipA*
AFLA_019100	1.74	*fbx15*
AFLA_098380	1.72	*rodA*
AFLA_114760	1.72	*chsB*
AFLA_134030	1.71	*flbA*
AFLA_136540	1.71	*ime2*

AFLA_020210	1.68	*nsdD*
AFLA_093230	1.66	*phnA*
AFLA_037320	1.58	*rho1*
AFLA_127920	1.57	*crzA*
AFLA_068440	1.53	*cch1*
AFLA_132340	1.48	*vapA*
AFLA_048880	1.48	*steC*
AFLA_004760	1.40	*ssc1*
AFLA_091740	1.39	*sidB*
AFLA_110620	1.39	*atg1*
AFLA_020990	1.34	*ugtA*
AFLA_030430	1.23	*ppoC*
AFLA_022400	1.23	*atgH*
AFLA_085200	1.08	*pac2/osaB*
AFLA_086900	1.02	*ams1*
AFLA_028410	-1.02	*pbcR*
AFLA_088670	-1.05	*kex1*
AFLA_110790	-1.07	*ricA*
AFLA_082510	-1.07	*tcpA*
AFLA_065850	-1.09	*fphA*
AFLA_114720	-1.12	*bem1*
AFLA_048000	-1.15	*rhbA*
AFLA_029640	-1.26	*nudG*
AFLA_032870	-1.36	*pkaR*
AFLA_044820	-1.43	*swoM*
AFLA_026790	-1.46	*ppoA*
AFLA_055650	-1.50	*osaA*
AFLA_112660	-1.53	*argB*
AFLA_066460	-1.90	*veA*
AFLA_014260	-2.00	*rodB*
AFLA_091910	-2.09	*pkaB*
AFLA_131370	-2.30	*tpsC*
AFLA_093580	-2.48	*tmpA*
AFLA_039530	-2.59	*fluG*
AFLA_135040	-2.63	*pkaA*
AFLA_087630	-2.65	*tpsA* ortholog

AFLA_005520	-2.82	*sfgA*
AFLA_026900	-3.70	*vosA*
AFLA_060780	-4.32	*dewA*
AFLA_062460	-5.13	*nce102*
AFLA_006170	-5.70	*wA/pksP*
AFLA_074470	-6.25	*rft1*
AFLA_052030	-10.74	*wetA*
AFLA_120760	-11.01	*ppoB*

Table S2-7 DEGs related to conidia maturation

Gene ID	Log_2 Fold Change	Common Name/ Function
AFLA_075640	-11.09	*ayg1*
AFLA_063080	-10.01	putative hydrophobin
AFLA_006170	-5.70	*pksP*
AFLA_060780	-4.32	*dewA*
AFLA_023650	-4.21	endo-1,3-1,4-beta-D-glucanase, putative
AFLA_002830	-3.85	*tpsc*
AFLA_028260	-3.06	*exg1*
AFLA_090490	-2.85	*treA*
AFLA_087630	-2.65	alpha,alpha-trehalose-phosphate synthase subunit, putative
AFLA_068300	-2.36	*bgt1*
AFLA_131370	-2.30	*tppb*
AFLA_098980	-2.28	putative hydrophobin
AFLA_030450	-2.24	*ccg-9*
AFLA_008180	-2.02	*rfab*
AFLA_014260	-2.00	*rodB*
AFLA_077910	-1.86	*agnD*
AFLA_023460	-1.76	*ags1*
AFLA_087640	-1.09	*tppc*
AFLA_004480	1.17	*eng4*
AFLA_129100	1.37	*exg2*
AFLA_091790	1.43	*agnE*
AFLA_091790	1.43	alpha-1,3-glucanase/mutanase, putative
AFLA_098380	1.72	*rodA*
AFLA_108860	1.92	*gel2*
AFLA_052800	1.92	*fksP*
AFLA_042780	1.95	*chsA*
AFLA_006590	2.02	*chiA*
AFLA_107830	2.03	brain chitinase and chia, putative
AFLA_107790	2.09	glucan 1,3-beta-glucosidase precursor, putative
AFLA_095890	2.10	*eng8*

AFLA_033550	2.30	*cts2*
AFLA_078290	2.43	*chesF*
AFLA_121370	2.45	*gel4*
AFLA_060590	2.59	*chsG*
AFLA_058480	2.67	*gel1*
AFLA_052810	2.72	*gel7*
AFLA_041950	2.77	*exg0*
AFLA_064920	2.81	1,3-beta-glucanosyltransferase *gel4* precursor, putative
AFLA_078900	2.97	*nagA*
AFLA_129440	3.02	*gel5*
AFLA_057680	3.18	beta-N-hexosaminidase, putative
AFLA_136030	3.37	*chsE*
AFLA_020630	3.56	*gel6*
AFLA_028950	3.61	*engl1*
AFLA_136040	3.86	*chsZ*
AFLA_013690	3.97	*chsC*
AFLA_101800	4.16	*ctcB*
AFLA_134100	4.21	*ags2*
AFLA_094600	4.57	putative hydrophobin
AFLA_029950	5.25	*eng3*
AFLA_031380	5.80	class V chitinase, putative
AFLA_104680	6.80	*chiB*

Table S2-8 mRNA levels of secondary metabolic clustered genes in *A. flavus*

Cluster	Gene ID	Log_2 Fold Change	Description
1	**AFLA_125780**	-	ATP-binding cassette transporter, putative
1	**AFLA_125770**	-1.76	LysR family regulatory protein, putative
1	**AFLA_125760**	-1.24	squalene-hopene-cyclase, putative
2	**AFLA_126710**	-	polyketide synthase, putative
2	**AFLA_126720**	-	hypothetical protein
2	**AFLA_126730**	-	conserved hypothetical protein
2	**AFLA_126740**	-	lipase precursor, putative
3	**AFLA_126970**	-	arginine permease, putative
3	**AFLA_126980**	-	conserved hypothetical protein
3	**AFLA_126990**	-	conserved hypothetical protein
3	**AFLA_127000**	-	hypothetical protein
3	**AFLA_127010**	-	conserved hypothetical protein
3	**AFLA_127020**	-	monooxygenase, putative
3	**AFLA_127030**	-	conserved hypothetical protein
3	**AFLA_127040**	-	MFS monocarboxylate transporter, putative
3	**AFLA_127050**	-	conserved hypothetical protein
3	**AFLA_127060**	-	conserved hypothetical protein
3	**AFLA_127070**	-	short-chain dehydrogenase, putative
3	**AFLA_127080**	-	conserved hypothetical protein
3	**AFLA_127100**	-	conserved hypothetical protein
3	**AFLA_127110**	-	MFS transporter, putative
3	**AFLA_127120**	-	hypothetical protein
3	**AFLA_127130**	-	conserved hypothetical protein
3	**AFLA_127140**	-	conserved hypothetical protein
3	**AFLA_127150**	-	hypothetical protein
3	**AFLA_127160**	-	NB-ARC and TPR domain protein
3	**AFLA_127170**	-	penicillin-binding protein, putative
3	**AFLA_127090**	-2.42	polyketide synthase, putative
4	**AFLA_128040**	-	efflux pump antibiotic resistance protein, putative
4	**AFLA_128060**	-	polyketide synthase, putative
4	**AFLA_128050**	1.12	conserved hypothetical protein

5	**AFLA_128160**	-	C6 transcription factor, putative
5	**AFLA_128170**	-	NRPS-like enzyme, putative
6	**AFLA_053900**	-	conserved hypothetical protein
6	**AFLA_053910**	-	pantothenate transporter, putative
6	**AFLA_053950**	-	conserved hypothetical protein
6	**AFLA_053880**	-2.39	hypothetical protein
6	**AFLA_053930**	-2.24	multicopper oxidase, putative
6	**AFLA_053940**	-1.99	multicopper oxidase, putative
6	**AFLA_053890**	-1.89	acetylcholinesterase, putative
6	**AFLA_053870**	1.99	polyketide synthase, putative
6	**AFLA_053920**	3.88	hypothetical protein
7	**AFLA_054040**	-	conserved hypothetical protein
7	**AFLA_054070**	-	conserved hypothetical protein
7	**AFLA_054090**	-	polyketide synthase, putative
7	**AFLA_054100**	-	DUF341 family oxidoreductase, putative
7	**AFLA_054080**	-2.74	Na(+)/H(+) antiporter, putative
7	**AFLA_054050**	1.24	ABC multidrug transporter, putative
7	**AFLA_054060**	5.22	ATP/GTP-binding protein, putative
8	**AFLA_054180**	-	conserved hypothetical protein
8	**AFLA_054190**	-	conserved hypothetical protein
8	**AFLA_054200**	-	hypothetical protein
8	**AFLA_054210**	-	ankyrin repeat-containing protein, putative
8	**AFLA_054220**	-	conserved hypothetical protein
8	**AFLA_054230**	-	conserved hypothetical protein
8	**AFLA_054250**	-	conserved hypothetical protein
8	**AFLA_054260**	-	MFS transporter, putative
8	**AFLA_054270**	-	NRPS-like enzyme, putative
8	**AFLA_054280**	-	conserved hypothetical protein
8	**AFLA_054290**	-	aldehyde reductase I (ARI), putative
8	**AFLA_054300**	-	pantothenate transporter, putative
8	**AFLA_054320**	-	NPP1 domain protein, putative
8	**AFLA_054370**	-3.48	short-chain dehydrogenase, putative
8	**AFLA_054310**	-3.24	conserved hypothetical protein
8	**AFLA_054330**	-2.68	conserved hypothetical protein
8	**AFLA_054340**	-1.28	isoflavone reductase family protein
8	**AFLA_054350**	-1.27	actin-binding protein Fragmin, putative

8	**AFLA_054240**	2.53	conserved hypothetical protein
8	**AFLA_054360**	3.36	conserved hypothetical protein
9	**AFLA_008700**	-	monooxygenase, putative
9	**AFLA_008710**	-	monooxygenase, putative
9	**AFLA_008740**	-	oxidoreductase, short chain dehydrogenase/reductase family
9	**AFLA_008760**	-	multidrug resistance protein, putative
9	**AFLA_008770**	-	nonribosomal peptide synthase, putative
9	**AFLA_008780**	-	asparagine synthetase, putative
9	**AFLA_008790**	-	conserved hypothetical protein
9	**AFLA_008730**	-3.17	hypothetical protein
9	**AFLA_008720**	-1.54	conserved hypothetical protein
9	**AFLA_008750**	3.63	hypothetical protein
10	**AFLA_009980**	-	ABC multidrug transporter, putative
10	**AFLA_010030**	-	oxidoreductase, 2OG-Fe(II) oxygenase family, putative
10	**AFLA_010040**	-	conserved hypothetical protein
10	**AFLA_010050**	-3.13	alcohol dehydrogenase, putative
10	**AFLA_010020**	-1.84	nonribosomal peptide synthase, putative
10	**AFLA_010010**	-1.18	nonribosomal peptide synthase, putative
10	**AFLA_010000**	1.98	polyketide synthase, putative
10	**AFLA_009990**	3.05	1-aminocyclopropane-1-carboxylate oxidase, putative
11	**AFLA_010590**	-	siderophore biosynthesis lipase/esterase, putative
11	**AFLA_010600**	-	siderophore biosynthesis acetylase AceI, putative
11	**AFLA_010610**	-	enoyl-CoA hydratase/isomerase family protein
11	**AFLA_010640**	-2.52	MFS siderophore transporter, putative
11	**AFLA_010630**	-2.47	ABC multidrug transporter SidT
11	**AFLA_010620**	1.56	nonribosomal siderophore peptide synthase Sid2
12	**AFLA_079370**	-	hypothetical protein
12	**AFLA_079380**	-	NRPS-like enzyme, putative
12	**AFLA_079390**	-	hypothetical protein
12	**AFLA_079400**	-	NRPS-like enzyme, putative

12	**AFLA_079420**	-	conserved hypothetical protein
12	**AFLA_079440**	-	allantoate permease, putative
12	**AFLA_079460**	-	DNA mismatch repair protein Msh1, putative
12	**AFLA_079360**	-4.14	PKS-like enzyme, putative
12	**AFLA_079430**	-3.82	amidase, putative
12	**AFLA_079450**	-2.39	ureidoglycolate hydrolase, putative
12	**AFLA_079410**	-2.04	MFS transporter, putative
13	**AFLA_080490**	-	polyketide synthase, putative
13	**AFLA_080510**	-	conserved hypothetical protein
13	**AFLA_080550**	-	conserved hypothetical protein
13	**AFLA_080500**	-1.47	cation-transporting ATPase, putative
13	**AFLA_080480**	1.10	HD superfamily hydrolase, putative
13	**AFLA_080520**	1.46	ADP-ribosylglycohydrolase family protein
13	**AFLA_080540**	1.98	DEAD box helicase Mph1, putative
13	**AFLA_080470**	3.03	conserved hypothetical protein
13	**AFLA_080530**	3.07	conserved hypothetical protein
14	**AFLA_082050**	-	epimerase/dehydratase family protein, putative
14	**AFLA_082060**	-	conserved hypothetical protein
14	**AFLA_082070**	-	pantothenate transporter, putative
14	**AFLA_082080**	-	alpha/beta hydrolase, putative
14	**AFLA_082090**	-	conserved hypothetical protein
14	**AFLA_082100**	-	conserved hypothetical protein
15	**AFLA_082150**	-	polyketide synthase, putative
15	**AFLA_082170**	-	efflux pump antibiotic resistance protein, putative
15	**AFLA_082180**	-	hypothetical protein
15	**AFLA_082190**	-	conserved hypothetical protein
15	**AFLA_082210**	1.94	conserved hypothetical protein
15	**AFLA_082230**	2.05	pantothenate transporter, putative
15	**AFLA_082160**	2.47	MFS glucose transporter, putative
15	**AFLA_082220**	2.70	short-chain dehydrogenase, putative
15	**AFLA_082200**	4.14	AAA family ATPase, putative
16	**AFLA_082430**	-	conserved hypothetical protein
16	**AFLA_082440**	-	conserved hypothetical protein
16	**AFLA_082480**	-	NRPS-like enzyme, putative
16	**AFLA_082470**	2.61	AMP dependent CoA ligase, putative

16	**AFLA_082450**	3.29	bicyclomycin resistance protein, putative
16	**AFLA_082460**	4.47	conserved hypothetical protein
17	**AFLA_083210**	-	conserved hypothetical protein
17	**AFLA_083220**	-	ATP dependent RNA helicase (Rok1), putative
17	**AFLA_083230**	-	mitochondrial F1F0-ATP synthase g subunit, putative
17	**AFLA_083260**	-	hypothetical protein
17	**AFLA_083270**	2.12	GABA permease, putative
17	**AFLA_083250**	2.43	dimethylallyl tryptophan synthase, putative
17	**AFLA_083240**	3.57	xylitol dehydrogenase LadA/XdhB
18	**AFLA_087830**	-	DNA excision repair protein Rad2
18	**AFLA_087850**	-	conserved hypothetical protein
18	**AFLA_087820**	-3.25	cell wall integrity signaling protein Lsp1/Pil1, putative
18	**AFLA_087840**	-1.43	WD repeat protein
18	**AFLA_087810**	-1.07	bZIP transcription factor, putative
18	**AFLA_087860**	1.80	cellulose-binding GDSL lipase/acylhydrolase, putative
18	**AFLA_087870**	3.02	endoglucanase, putative
18	**AFLA_087880**	5.24	integral membrane protein
19	**AFLA_114820**	-	polyketide synthase, putative
19	**AFLA_114830**	-	aminopeptidase, putative
19	**AFLA_114840**	-	conserved hypothetical protein
20	**AFLA_116140**	-	putative secreted protein
20	**AFLA_116230**	-	conserved hypothetical protein
20	**AFLA_116300**	-	short-chain dehydrogenase, putative
20	**AFLA_116310**	-	short-chain dehydrogenase, putative
20	**AFLA_116330**	-	O-methyltransferase, putative
20	**AFLA_116190**	-11.46	conserved hypothetical protein
20	**AFLA_116180**	-11.04	hypothetical protein
20	**AFLA_116170**	-10.67	hypothetical protein
20	**AFLA_116200**	-8.39	conserved hypothetical protein
20	**AFLA_116210**	-4.69	O-methyltransferase, putative
20	**AFLA_116290**	-3.47	conserved hypothetical protein
20	**AFLA_116280**	-3.36	dihydroxy-acid dehydratase, putative
20	**AFLA_116270**	-3.06	paraoxonase, putative

20	**AFLA_116220**	-3.01	polyketide synthase, putative
20	**AFLA_116320**	-1.97	hypothetical protein
20	**AFLA_116150**	-1.67	integral membrane protein
20	**AFLA_116160**	1.32	zinc-binding dehydrogenase family oxidoreductase
20	**AFLA_116250**	1.44	conserved hypothetical protein
20	**AFLA_116240**	4.52	hypothetical protein
20	**AFLA_116260**	5.58	N-hydroxyarylamine O-acetyltransferase, putative
21	**AFLA_116840**	-	salicylate 1-monooxygenase SalA
21	**AFLA_116850**	-	Ankyrin domain protein
21	**AFLA_116880**	-	C6 transcription factor, putative
21	**AFLA_116900**	-	conserved hypothetical protein
21	**AFLA_116860**	-5.73	alpha-N-acetylglucosaminidase, putative
21	**AFLA_116870**	-2.05	transferase family protein
21	**AFLA_116830**	-1.28	hypothetical protein
21	**AFLA_116890**	-1.02	polyketide synthase, putative
22	**AFLA_117770**	-	DnaJ domain protein
22	**AFLA_117780**	-	farnesyl-diphosphate farnesyltransferase, putative
22	**AFLA_117760**	2.10	phytase, putative
23	**AFLA_118440**	-4.87	NRPS-like enzyme, putative
23	**AFLA_118430**	-3.82	conserved hypothetical protein
24	**AFLA_118820**	-	short-chain dehydrogenase, putative
24	**AFLA_118830**	-	conserved hypothetical protein
24	**AFLA_118860**	-	hypothetical protein
24	**AFLA_118870**	-	translation initiation inhibitor, putative
24	**AFLA_118880**	-	gibberellin 3-beta hydroxylase, putative
24	**AFLA_118890**	-	eukaryotic translation initiation factor eIF-5A-2
24	**AFLA_118900**	-	conserved hypothetical protein
24	**AFLA_118910**	-	conserved hypothetical protein
24	**AFLA_118920**	-	hypothetical protein
24	**AFLA_118930**	-	hypothetical protein
24	**AFLA_118980**	-	mitochondrial carrier protein Leu5
24	**AFLA_118840**	-4.41	pantothenate transporter, putative

24	**AFLA_118850**	-3.61	2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase, putative
24	**AFLA_118940**	1.18	polyketide synthase, putative
24	**AFLA_118960**	2.48	polyketide synthase, putative
24	**AFLA_118970**	3.45	MAK1-like monooxygenase, putative
24	**AFLA_118950**	4.67	conserved hypothetical protein
24	**AFLA_118990**	7.89	efflux pump antibiotic resistance protein, putative
25	**AFLA_119100**	-	hypothetical protein
25	**AFLA_119090**	-3.44	oxidoreductase, short chain dehydrogenase/reductase family, putative
25	**AFLA_119120**	-3.25	beta-lactamase family protein
25	**AFLA_119110**	-1.86	NRPS-like enzyme, putative
25	**AFLA_119080**	-1.66	hypothetical protein
26	**AFLA_119750**	-	riboflavin aldehyde-forming enzyme
26	**AFLA_119790**	-	beta-xylosidase
26	**AFLA_119810**	-	sensory transduction histidine kinase bacterial, putative
26	**AFLA_119860**	-	pectin lyase, putative
26	**AFLA_119870**	-	iron-sulfur protein subunit of succinate dehydrogenase Sdh2, putative
26	**AFLA_119850**	-2.57	LsdA family protein
26	**AFLA_119770**	-2.49	conserved hypothetical protein
26	**AFLA_119780**	-2.14	neutral protease 2 precursor, putative
26	**AFLA_119880**	-1.51	hypothetical protein
26	**AFLA_119840**	-1.45	hypothetical protein
26	**AFLA_119760**	-1.13	conserved hypothetical protein
26	**AFLA_119800**	1.00	conserved hypothetical protein
26	**AFLA_119820**	1.59	NRPS-like enzyme, putative
26	**AFLA_119830**	2.66	MFS multidrug transporter, putative
27	**AFLA_121400**	-	hypothetical protein
27	**AFLA_121410**	-	hypothetical protein
27	**AFLA_121420**	-	hypothetical protein
27	**AFLA_121430**	-	conserved hypothetical protein
27	**AFLA_121440**	-	fumarylacetoacetate hydrolase, putative
27	**AFLA_121460**	-	hypothetical protein

27	**AFLA_121470**	-	hscarg dehydrogenase, putative
27	**AFLA_121540**	-	MFS multidrug transporter, putative
27	**AFLA_121580**	-	C-4 methyl sterol oxidase, putative
27	**AFLA_121590**	-3.55	zinc-binding alcohol dehydrogenase, putative
27	**AFLA_121450**	-2.35	trihydroxytoluene oxygenase
27	**AFLA_121600**	-1.73	conserved hypothetical protein
27	**AFLA_121550**	1.94	conserved hypothetical protein
27	**AFLA_121560**	1.97	conserved hypothetical protein
27	**AFLA_121570**	2.40	conserved hypothetical protein
27	**AFLA_121520**	2.54	NRPS-like enzyme, putative
27	**AFLA_121500**	2.78	cytochrome P450, putative
27	**AFLA_121490**	2.81	NmrA-like family protein
27	**AFLA_121530**	2.87	NADH-dependent flavin oxidoreductase, putative
27	**AFLA_121480**	3.14	phytanoyl-CoA dioxygenase family protein
27	**AFLA_121510**	3.53	conserved hypothetical protein
28	**AFLA_089620**	-	chalcone synthase, putative
28	**AFLA_089630**	-	choline transport protein, putative
28	**AFLA_089640**	-	conserved hypothetical protein
28	**AFLA_089650**	-	high-affinity iron transporter FtrA
28	**AFLA_089660**	-	ferrooxidoreductase Fet3, putative
28	**AFLA_089670**	-3.14	ferric reductase transmembrane component 4 precursor, putative
29	**AFLA_090190**	-	dimethylallyl tryptophan synthase, putative
29	**AFLA_090200**	-	nonribosomal peptide synthase, putative
29	**AFLA_090180**	3.80	alpha/beta hydrolase, putative
30	**AFLA_090590**	-	alpha-1,2-mannosidase, putative subfamily
30	**AFLA_090600**	-	conserved hypothetical protein
30	**AFLA_090610**	-	hypothetical protein
30	**AFLA_090620**	-	hypothetical protein
30	**AFLA_090630**	-	cytochrome P450, putative
30	**AFLA_090640**	-	geranyl geranyl pyrophosphate synthase, putative
30	**AFLA_090660**	-	conserved hypothetical protein
30	**AFLA_090650**	-1.23	DUF1115 domain protein
30	**AFLA_090670**	1.11	hypothetical protein

30	**AFLA_090680**	1.92	cytochrome P450, putative
30	**AFLA_090690**	2.38	mycelial catalase Cat1
31	**AFLA_096340**	-	hypothetical protein
31	**AFLA_096350**	-	TrkA-N domain dehydrogenase, putative
31	**AFLA_096390**	-	prenyl transferase AtmC
31	**AFLA_096400**	-	FAD-dependent monooxygenase AtmM
31	**AFLA_096430**	-	cytochrome P450, putative
31	**AFLA_096410**	-3.01	PTH11-like integral membrane protein, putative
31	**AFLA_096330**	-2.40	C6 and C2H2 transcription factor
31	**AFLA_096420**	-1.66	hypothetical protein
31	**AFLA_096370**	2.41	Zn2Cys6 transcription factor
31	**AFLA_096360**	3.83	hypothetical protein
31	**AFLA_096380**	4.42	FAD dependent oxidoreductase, putative
32	**AFLA_096700**	-	NRPS-like enzyme, putative
32	**AFLA_096710**	-	NRPS-like enzyme, putative
32	**AFLA_096720**	-	hypothetical protein
32	**AFLA_096730**	-	conserved hypothetical protein
32	**AFLA_096770**	-	polyketide synthase, putative
32	**AFLA_096740**	-8.85	monoxygenase, putative
32	**AFLA_096760**	-7.35	monocarboxylate transporter, putative
32	**AFLA_096750**	-5.37	cytochrome P450, putative
33	**AFLA_017840**	-	NRPS-like enzyme, putative
33	**AFLA_017850**	-4.85	conserved hypothetical protein
33	**AFLA_017860**	-2.98	hypothetical protein
34	**AFLA_018250**	-	conserved hypothetical protein
34	**AFLA_018270**	-	hypothetical protein
34	**AFLA_018280**	-	mitochondrial outer membrane protein (Sam35), putative
34	**AFLA_018290**	-	conserved hypothetical protein
34	**AFLA_018300**	-	cell division control protein 14
34	**AFLA_018310**	-	geranylgeranyl diphosphate synthase
34	**AFLA_018320**	-	Smr domain protein
34	**AFLA_018340**	-	G-protein complex alpha subunit GpaA/FadA
34	**AFLA_018260**	-1.42	DUF543 domain protein
34	**AFLA_018350**	1.72	conserved hypothetical protein

34	**AFLA_018330**	3.00	hypothetical protein
35	**AFLA_038600**	-1.10	nonribosomal peptide synthase, putative
35	**AFLA_038590**	3.93	Ankyrin repeat protein
35	**AFLA_038580**	4.10	RTA1 domain protein
35	**AFLA_038570**	4.44	malonyl CoA-acyl carrier protein transacylase, putative
36	**AFLA_039200**	-	cytochrome P450, putative
36	**AFLA_039210**	-	cytochrome P450, putative
36	**AFLA_039220**	-	cytochrome P450, putative
36	**AFLA_039230**	-	short-chain dehydrogenase, putative
36	**AFLA_039240**	-	aristolochene synthase, putative
36	**AFLA_039270**	-	carboxylesterase, putative
36	**AFLA_039260**	2.02	esterase, putative
36	**AFLA_039250**	2.04	hypothetical protein
37	**AFLA_041590**	-	peroxisomal multifunctional beta-oxidation protein (MFP), putative
37	**AFLA_041600**	-	RING finger protein (Zin), putative
37	**AFLA_041610**	-	NRPS-like enzyme, putative
37	**AFLA_041620**	3.61	aquaporin, putative
38	**AFLA_042330**	-	conserved hypothetical protein
38	**AFLA_042340**	-	conserved hypothetical protein
38	**AFLA_042350**	-	conserved hypothetical protein
38	**AFLA_042360**	-	hypothetical protein
38	**AFLA_042370**	-	conserved hypothetical protein
39	**AFLA_045490**	-	dimethylallyl tryptophan synthase, putative
39	**AFLA_045510**	-	integral membrane protein
39	**AFLA_045500**	4.36	cytochrome P450, putative
40	**AFLA_100320**	-	hypothetical protein
40	**AFLA_100330**	-	FAD dependent oxidoreductase, putative
40	**AFLA_100340**	-	nonribosomal peptide synthase, putative
40	**AFLA_100360**	-	amino acid transporter, putative
40	**AFLA_100370**	-	acetylornithine aminotransferase, putative
40	**AFLA_100380**	-	hypothetical protein
40	**AFLA_100390**	-	conserved hypothetical protein
40	**AFLA_100400**	-	hypothetical protein
40	**AFLA_100410**	-	hypothetical protein

40	**AFLA_100420**	-	amidase, putative
40	**AFLA_100270**	-2.06	aliphatic nitrilase, putative
40	**AFLA_100280**	-1.96	MFS transporter, putative
40	**AFLA_100310**	-1.94	oxidoreductase, putative
40	**AFLA_100300**	-1.44	C6 transcription factor, putative
40	**AFLA_100290**	-1.43	aliphatic nitrilase, putative
40	**AFLA_100350**	3.46	conserved hypothetical protein
41	**AFLA_101740**	-6.59	glycerophosphoinositol/ glycerophosphocholine transporter, putative
41	**AFLA_101750**	-5.66	molybdopterin synthase small subunit CnxG
41	**AFLA_101760**	-4.09	molybdenum cofactor biosynthesis protein Gephyrin, putative
41	**AFLA_101720**	1.27	cytochrome P450, putative
41	**AFLA_101730**	2.08	ferulate-5-hydroxylase, putative
41	**AFLA_101700**	2.24	NRPS-like enzyme, putative
41	**AFLA_101710**	2.37	NmrA-like family protein
41	**AFLA_101690**	3.10	alcohol dehydrogenase, putative
42	**AFLA_102110**	-	conserved hypothetical protein
42	**AFLA_102150**	-	SAM dependent methyltransferase, putative
42	**AFLA_102140**	1.24	conserved hypothetical protein
42	**AFLA_102120**	1.25	hypothetical protein
42	**AFLA_102160**	2.89	lanosterol synthase, putative
42	**AFLA_102130**	3.34	conserved hypothetical protein
43	**AFLA_102480**	-	isoamyl alcohol oxidase
43	**AFLA_102490**	-	conserved hypothetical protein
43	**AFLA_102500**	-	9-cis-epoxycarotenoid dioxygenase, putative
43	**AFLA_102520**	-	phytoene dehydrogenase, putative
43	**AFLA_102530**	-	UMTA methyltransferase family protein
43	**AFLA_102540**	-	hypothetical protein
43	**AFLA_102550**	-	G-patch domain protein, putative
43	**AFLA_102510**	2.71	phytoene synthase, putative
44	**AFLA_064250**	-	phenol 2-monooxygenase, putative
44	**AFLA_064270**	-	gibberellin 2-oxidase, putative
44	**AFLA_064280**	-	N-methyltransferase, putative
44	**AFLA_064290**	-	O-methyltransferase, putative
44	**AFLA_064300**	-	fructosyl amino acid oxidase, putative

44	**AFLA_064330**	-	conserved hypothetical protein
44	**AFLA_064340**	-	conserved hypothetical protein
44	**AFLA_064260**	-1.01	oligopeptide transporter, putative
44	**AFLA_064320**	1.16	oligopeptide transporter, putative
44	**AFLA_064310**	1.21	hypothetical protein
44	**AFLA_064240**	1.54	nonribosomal peptide synthase, putative
45	**AFLA_064510**	-	thioredoxin reductase GliT-like, putative
45	**AFLA_064520**	-	O-methyltransferase GliM-like, putative
45	**AFLA_064610**	-	short-chain oxidoreductase, putative
45	**AFLA_064630**	-	hypothetical protein
45	**AFLA_064560**	-1.39	nonribosomal peptide synthase GliP-like, putative
45	**AFLA_064620**	-1.00	conserved hypothetical protein
45	**AFLA_064500**	1.33	conserved hypothetical protein
45	**AFLA_064580**	2.12	oxidoreductase, putative
45	**AFLA_064570**	2.17	uracil permease, putative
45	**AFLA_064540**	2.31	cytochrome P450 oxidoreductase GliC
45	**AFLA_064600**	2.34	conserved hypothetical protein
45	**AFLA_064550**	2.53	membrane dipeptidase GliJ-like, putative
45	**AFLA_064530**	2.63	glutathione S-transferase GliG-like, putative
45	**AFLA_064460**	2.88	conserved hypothetical protein
45	**AFLA_064440**	3.02	transport protein, putative
45	**AFLA_064590**	3.17	O-methyltransferase, putative
45	**AFLA_064480**	3.28	conserved hypothetical protein
45	**AFLA_064470**	3.31	cytochrome P450, putative
45	**AFLA_064450**	3.74	aminotransferase GliI-like, putative
45	**AFLA_064490**	3.82	conserved hypothetical protein
45	**AFLA_064640**	4.45	oxidoreductase, short-chain dehydrogenase/reductase family
46	**AFLA_066730**	-2.68	alcohol dehydrogenase, putative
46	**AFLA_066710**	1.68	conserved hypothetical protein
46	**AFLA_066700**	2.27	P450 family sporulation-specific N-formyltyrosine oxidase Dit2
46	**AFLA_066720**	2.34	nonribosomal peptide synthase, putative
47	**AFLA_066840**	-	hybrid PKS/NRPS enzyme, putative
47	**AFLA_066850**	-	conserved hypothetical protein

47	**AFLA_066860**	-	conserved hypothetical protein
47	**AFLA_066870**	-	conserved hypothetical protein
47	**AFLA_066900**	-1.60	conserved hypothetical protein
47	**AFLA_066970**	1.28	conserved hypothetical protein
47	**AFLA_066890**	1.36	cytochrome P450, putative
47	**AFLA_066950**	2.04	conserved hypothetical protein
47	**AFLA_066880**	2.08	monocarboxylate transporter, putative
47	**AFLA_066920**	2.36	alcohol dehydrogenase, putative
47	**AFLA_066910**	2.48	conserved hypothetical protein
47	**AFLA_066960**	3.82	hypothetical protein
47	**AFLA_066930**	3.86	cytochrome P450, putative
47	**AFLA_066940**	4.39	O-methyltransferase, putative
48	**AFLA_069340**	-2.33	MSF drug transporter, putative
48	**AFLA_069350**	-1.42	conserved hypothetical protein
48	**AFLA_069330**	1.19	nonribosomal peptide synthase Pes1
49	**AFLA_070860**	-	ACV synthetase PcbAB
49	**AFLA_070900**	-	conserved hypothetical protein
49	**AFLA_070920**	-	NRPS-like enzyme, putative
49	**AFLA_070890**	-3.79	hypothetical protein
49	**AFLA_070870**	1.52	isopenicillin N synthetase PcbC
49	**AFLA_070910**	1.79	aminotransferase, putative
49	**AFLA_070880**	3.90	acyl-coenzyme A:Isopenicillin N acyltransferase PenDE
50	**AFLA_002910**	-	conserved hypothetical protein
50	**AFLA_002930**	-	hypothetical protein
50	**AFLA_002920**	-4.66	flavonoid 3-hydroxylase, putative
50	**AFLA_002890**	-1.06	AMP-binding enzyme family protein
50	**AFLA_002900**	2.44	polyketide synthase, putative
51	**AFLA_004280**	-	C6 transcription factor, putative
51	**AFLA_004290**	-	conserved hypothetical protein
51	**AFLA_004300**	-5.05	prenyltransferase, putative
52	**AFLA_004440**	-10.38	ABC multidrug transporter, putative
52	**AFLA_004430**	-7.35	conserved hypothetical protein
52	**AFLA_004450**	-6.59	nonribosomal peptide synthase, putative
53	**AFLA_005270**	-	conserved hypothetical protein
53	**AFLA_005280**	-	hypothetical protein

53	**AFLA_005300**	-	ankyrin repeat-containing protein, putative
53	**AFLA_005320**	-	polyketide synthase, putative
53	**AFLA_005330**	-	conserved hypothetical protein
53	**AFLA_005340**	-	conserved hypothetical protein
53	**AFLA_005350**	-	hypothetical protein
53	**AFLA_005360**	-	conserved hypothetical protein
53	**AFLA_005380**	-	hypothetical protein
53	**AFLA_005400**	-	ankyrin repeat-containing protein, putative
53	**AFLA_005390**	-3.83	conserved hypothetical protein
53	**AFLA_005290**	-1.14	conserved hypothetical protein
53	**AFLA_005410**	-1.05	5-AMP-activated protein kinase, putative
53	**AFLA_005310**	5.04	vacuolar ATP synthase proteolipid subunit, putative
53	**AFLA_005370**	5.80	conserved hypothetical protein
54	**AFLA_005440**	-1.70	nonribosomal peptide synthase, putative
54	**AFLA_005450**	1.33	conserved hypothetical protein
55	**AFLA_006110**	-	conserved hypothetical protein
55	**AFLA_006130**	-	NADH pyrophosphatase, putative
55	**AFLA_006140**	-	conserved hypothetical protein
55	**AFLA_006150**	-	flavin dependent monooxygenase, putative
55	**AFLA_006160**	-	HDA1 complex subunit, putative
55	**AFLA_006180**	-	conidial pigment biosynthesis oxidase Arb2/brown2
55	**AFLA_006190**	-	multicopper oxidase, putative
55	**AFLA_006240**	-	PHD transcription factor (Rum1), putative
55	**AFLA_006170**	-5.70	polyketide synthetase PksP
55	**AFLA_006100**	-2.45	HypA-like protein, putative
55	**AFLA_006230**	-1.88	1-aminocyclopropane-1-carboxylate deaminase, putative
55	**AFLA_006120**	-1.26	glutamine dependent NAD+ synthetase, putative
55	**AFLA_006220**	-1.22	exopolyphosphatase, putative
55	**AFLA_006210**	1.43	SH3 domain protein (Cyk3), putative
55	**AFLA_006200**	3.15	hypothetical protein
56	**AFLA_006800**	-	DNA damage repair protein (Rad9), putative
56	**AFLA_006810**	-	50S ribosomal protein L27, putative

56	**AFLA_006830**	-	scramblase family protein
56	**AFLA_006860**	-	oxidosqualene:lanosterol cyclase
56	**AFLA_006870**	-	SGT1 and CS domain protein
56	**AFLA_006900**	-	cyclin-dependent protein kinase PhoA
56	**AFLA_006920**	-3.09	cAMP receptor-like protein, putative
56	**AFLA_006880**	-2.68	PQ loop repeat protein
56	**AFLA_006890**	-1.82	SIR2 family histone deacetylase, putative
56	**AFLA_006840**	-1.39	ubiquitin conjugating enzyme (UbcH), putative
56	**AFLA_006850**	1.02	meiosis-specific topoisomerase Spo11, putative
56	**AFLA_006820**	1.64	conserved hypothetical protein
56	**AFLA_006910**	2.74	hypothetical protein
57	**AFLA_135430**	-	cytochrome P450, putative
57	**AFLA_135440**	-	cytochrome P450, putative
57	**AFLA_135450**	-	trichodiene synthase, putative
57	**AFLA_135470**	-	ornithine decarboxylase, putative
57	**AFLA_135480**	-	galactose-proton symport, putative
57	**AFLA_135490**	-	nonribosomal peptide synthase, putative
57	**AFLA_135460**	-1.66	conserved hypothetical protein
58	**AFLA_137790**	-	cell wall protein, putative
58	**AFLA_137800**	-	conserved hypothetical protein
58	**AFLA_137810**	-	pantothenate transporter, putative
58	**AFLA_137890**	-	conserved hypothetical protein
58	**AFLA_137880**	1.22	extracellular triacylglycerol lipase, putative
58	**AFLA_137830**	1.77	hypothetical protein
58	**AFLA_137780**	2.50	ABC transporter, putative
58	**AFLA_137820**	2.92	conserved hypothetical protein
58	**AFLA_137840**	4.15	integral membrane protein
58	**AFLA_137870**	5.11	polyketide synthase, putative
58	**AFLA_137850**	5.50	conserved hypothetical protein
58	**AFLA_137860**	5.64	conserved hypothetical protein
59	**AFLA_139160**	-	aflX/ ordB/ monooxygenase/ oxidase
59	**AFLA_139180**	-	aflV/ cypX/ cytochrome P450 monooxygenase
59	**AFLA_139190**	-	aflK/ vbs/ VERB synthase
59	**AFLA_139200**	-	aflQ/ ordA/ ord-1/ oxidoreductase/ cytochrome P450 monooxigenase
59	**AFLA_139210**	-	aflP/ omtA/ omt-1/ O-methyltransferase A

59	**AFLA_139220**	-	aflO/ omtB/ dmtA/ O-methyltransferase B
59	**AFLA_139230**	-	aflI/ avfA/ cytochrome P450 monooxygenase
59	**AFLA_139250**	-	aflL/ verB/ desaturase/ P450 monooxygenase
59	**AFLA_139260**	-	aflG/ avnA/ ord-1/ cytochrome P450 monooxygenase
59	**AFLA_139280**	-	aflN/ verA/ monooxygenase
59	**AFLA_139300**	-	aflM/ ver-1/ dehydrogenase/ ketoreductase
59	**AFLA_139310**	-	aflE/ norA/ aad/ adh-2/ NOR reductase/ dehydrogenase
59	**AFLA_139320**	-	aflJ/ estA/ esterase
59	**AFLA_139330**	-	aflH/ adhA/ short chain alcohol dehydrogenase
59	**AFLA_139390**	-	aflD / nor-1 / reductase
59	**AFLA_139400**	-	aflCa / hypC / hypothetical protein
59	**AFLA_139440**	-	aflF / norB / dehydrogenase
59	**AFLA_139340**	-3.16	aflS/ pathway regulator
59	**AFLA_139360**	-2.99	aflR / apa-2 / afl-2 / transcription activator
59	**AFLA_139380**	-2.57	aflA / fas-2 / hexA / fatty acid synthase alpha subunit
59	**AFLA_139150**	1.16	aflY/ hypA/ hypP/ hypothetical protein
59	**AFLA_139370**	1.47	aflB / fas-1 / fatty acid synthase beta subunit
59	**AFLA_139410**	2.00	aflC / pksA / pksL1 / polyketide synthase
59	**AFLA_139170**	2.05	aflW/ moxY/ monooxygenase
59	**AFLA_139420**	2.73	aflT / aflT / transmembrane protein
59	**AFLA_139430**	4.24	aflU / cypA / P450 monooxygenase
60	**AFLA_139590**	-	choline dehydrogenase, putative
60	**AFLA_139600**	-	glucose-methanol-choline (gmc) oxidoreductase, putative
60	**AFLA_139620**	-	amino acid permease family protein
60	**AFLA_139630**	-	ABC multidrug transporter, putative
60	**AFLA_139640**	-	hypothetical protein
60	**AFLA_139650**	-	hypothetical protein
60	**AFLA_139660**	-	hypothetical protein
60	**AFLA_139670**	-	nonribosomal peptide synthase, putative
60	**AFLA_139610**	1.75	ceramidase, putative
61	**AFLA_022840**	-	allantoate permease, putative
61	**AFLA_022850**	-	conserved hypothetical protein

61	**AFLA_022870**	-	conserved hypothetical protein
61	**AFLA_023000**	-	Ankyrin domain protein
61	**AFLA_023010**	-	GA4 desaturase family protein
61	**AFLA_023030**	-	cytochrome P450 oxidoreductase GliC-like, putative
61	**AFLA_023040**	-	C6 transcription factor, putative
61	**AFLA_023060**	-	hypothetical protein
61	**AFLA_023080**	-	integral membrane protein TmpA
61	**AFLA_023070**	-2.93	integral membrane protein
61	**AFLA_022990**	-1.83	conserved hypothetical protein
61	**AFLA_022880**	-1.54	oligopeptide transporter, putative
61	**AFLA_022860**	1.73	hypothetical protein
61	**AFLA_023050**	1.99	MFS transporter, putative
61	**AFLA_023020**	4.15	NRPS-like enzyme, putative
62	**AFLA_027210**	-	conserved hypothetical protein
62	**AFLA_027220**	-	hypothetical protein
62	**AFLA_027230**	-	alpha-aminoadipate reductase Lys2, putative
62	**AFLA_027250**	-	aldo-keto reductase, putative
62	**AFLA_027260**	-	conserved hypothetical protein
62	**AFLA_027200**	1.28	xanthine dehydrogenase HxA, putative
62	**AFLA_027240**	1.58	carbonic anhydrase Nce103, putative
63	**AFLA_028730**	-	conserved hypothetical protein
63	**AFLA_028740**	-	coenzyme A transferase, putative
63	**AFLA_028760**	-1.30	C2H2 transcription factor, putative
63	**AFLA_028720**	-1.20	NRPS-like enzyme, putative
63	**AFLA_028710**	2.28	short chain type dehydrogenase, putative
63	**AFLA_028750**	2.49	UMTA methyltransferase family protein
64	**AFLA_105000**	-	DUF636 domain protein
64	**AFLA_105040**	-	conserved hypothetical protein
64	**AFLA_105060**	-	NAD dependent epimerase/dehydratase, putative
64	**AFLA_105080**	-	terpene synthase family protein
64	**AFLA_105030**	-2.57	cytosine deaminase, putative
64	**AFLA_105010**	-2.53	methyltransferase, putative
64	**AFLA_105020**	-1.54	conserved hypothetical protein
64	**AFLA_105050**	-1.45	geranylgeranyl pyrophosphate synthetase

			AtmG, putative
64	**AFLA_105070**	-1.13	cytochrome P450 oxygenase, putative
65	**AFLA_105130**	-	conserved hypothetical protein
65	**AFLA_105140**	-	conserved hypothetical protein
65	**AFLA_105150**	-	extracellular carboxylesterase, putative
65	**AFLA_105160**	-	hypothetical protein
65	**AFLA_105190**	-4.46	NRPS-like enzyme, putative
65	**AFLA_105180**	-2.54	hypothetical protein
65	**AFLA_105170**	-2.39	O-methyltransferase, putative
65	**AFLA_105120**	-1.50	GNAT family acetyltransferase, putative
66	**AFLA_105370**	-	conserved hypothetical protein
66	**AFLA_105380**	-	actin-binding protein, putative
66	**AFLA_105400**	-	kinesin family protein (KlpA), putative
66	**AFLA_105440**	-	cytochrome P450, putative
66	**AFLA_105450**	-	polyketide synthase, putative
66	**AFLA_105460**	-	conserved hypothetical protein
66	**AFLA_105490**	-	phosphoribulokinase/uridine kinase family protein
66	**AFLA_105480**	-5.35	DUF636 domain protein
66	**AFLA_105470**	-3.91	translation initiation factor eIF-2B subunit family protein
66	**AFLA_105540**	-2.34	MFS multidrug transporter, putative
66	**AFLA_105530**	-2.27	C6 transcription factor, putative
66	**AFLA_105390**	-1.20	conserved hypothetical protein
66	**AFLA_105520**	1.07	conserved hypothetical protein
66	**AFLA_105510**	1.33	conserved hypothetical protein
66	**AFLA_105430**	1.37	fatty acid desaturase protein, putative
66	**AFLA_105410**	1.97	thioester reductase family protein, putative
66	**AFLA_105420**	2.16	hypothetical protein
66	**AFLA_105500**	4.17	conserved hypothetical protein
67	**AFLA_107100**	-	conserved hypothetical protein
67	**AFLA_107120**	-	conserved hypothetical protein
67	**AFLA_107130**	-	D-isomer specific 2-hydroxyacid dehydrogenase family protein
67	**AFLA_107140**	-	conserved hypothetical protein
67	**AFLA_107110**	-1.93	conserved hypothetical protein

67	**AFLA_107150**	1.94	conserved hypothetical protein
68	**AFLA_108540**	-	NADH oxidase, putative
68	**AFLA_108550**	-	polyketide synthase, putative
68	**AFLA_108560**	-	O-methyltransferase, putative
68	**AFLA_108570**	-	conserved hypothetical protein
68	**AFLA_108580**	-	cytochrome P450, putative
69	**AFLA_109390**	-	conserved hypothetical protein
69	**AFLA_109400**	-	extracellular protein, putative
69	**AFLA_109410**	-	UV-endonuclease UVE-1
69	**AFLA_109420**	-	actin cytoskeleton organization protein App1, putative
69	**AFLA_109440**	-	long-chain-fatty-acid-CoA ligase, putative
69	**AFLA_109380**	-2.84	alcohol dehydrogenase, putative
69	**AFLA_109430**	4.23	nonribosomal siderophore peptide synthase SidC
70	**AFLA_112780**	-	conserved hypothetical protein
70	**AFLA_112790**	-	acyl-CoA thioester hydrolase, putative
70	**AFLA_112810**	-	trytophan synthase alpha subunit, putative
70	**AFLA_112820**	-	toxin biosynthesis ketoreductase, putative
70	**AFLA_112830**	-	conserved hypothetical protein
70	**AFLA_112840**	-	polyketide synthase, putative
70	**AFLA_112880**	-	2,4-dichlorophenol 6-monooxygenase, putative
70	**AFLA_112900**	-	epoxide hydrolase, putative
70	**AFLA_112910**	-	conserved hypothetical protein
70	**AFLA_112920**	-	mitochondrial cytochrome b2-like, putative
70	**AFLA_112800**	-4.33	L-lactate dehydrogenase
70	**AFLA_112870**	1.11	monooxygenase, putative
70	**AFLA_112890**	1.30	MFS transporter, putative
70	**AFLA_112860**	1.82	conserved hypothetical protein
70	**AFLA_112850**	3.07	O-methyltransferase, putative
71	**AFLA_059980**	3.75	choline dehydrogenase, putative
71	**AFLA_060020**	4.04	PKS-like enzyme, putative
71	**AFLA_059960**	4.33	conserved hypothetical protein
71	**AFLA_059970**	4.65	short-chain dehydrogenase, putative
71	**AFLA_059990**	4.88	O-methyltransferase, putative
71	**AFLA_060000**	5.07	metallo-beta-lactamase domain protein

71	**AFLA_060010**	5.88	PKS-like enzyme, putative
71	**AFLA_059950**	7.37	oxidoreductase, FAD-binding, putative
72	**AFLA_060650**	-	glutaminyl-tRNA synthetase
72	**AFLA_060660**	-	AMP-binding enzyme, putative
72	**AFLA_060670**	-	integral membrane protein
72	**AFLA_060680**	1.60	conserved hypothetical protein
73	**AFLA_062440**	-	conserved hypothetical protein
73	**AFLA_062450**	-	sphingomyelin phosphodiesterase, putative
73	**AFLA_062460**	-	non-classical export protein Nce102, putative
73	**AFLA_062470**	-	pentalenene synthase, putative
73	**AFLA_062480**	-	conserved hypothetical protein
73	**AFLA_062500**	-	mitogen-activated protein kinase MAF1
73	**AFLA_062510**	-	myosin heavy chain, embryonic smooth muscle isoform, putative
73	**AFLA_062520**	-	COPII-coated vesicle protein (Erv41), putative
73	**AFLA_062430**	-5.13	non-classical export protein Nce102, putative
73	**AFLA_062490**	1.25	COPII-coated vesicle protein (Erv41), putative
74	**AFLA_062830**	-	monoxygenase, putative
74	**AFLA_062870**	-	conserved hypothetical protein
74	**AFLA_062880**	-	short chain dehydrogenase/oxidoreductase, putative
74	**AFLA_062890**	-	hypothetical protein
74	**AFLA_062900**	-	glycerol-3-phosphate O-acyltransferase, putative
74	**AFLA_062910**	-	proline-specific permease, putative
74	**AFLA_062920**	-	conserved hypothetical protein
74	**AFLA_062930**	-	glycosyl hydrolase, family 43, putative
74	**AFLA_062840**	-3.46	short chain dehydrogenase/oxidoreductase, putative
74	**AFLA_062860**	-1.15	glycerol-3-phosphate O-acyltransferase, putative
74	**AFLA_062810**	1.15	fatty acid desaturase, putative
74	**AFLA_062850**	3.26	hypothetical protein
74	**AFLA_062820**	3.38	polyketide synthase, putative
74	**AFLA_062800**	3.98	conserved hypothetical protein

Table S2-9 DEGs predicted to encode transcription factors

Gene ID	Log$_2$ Fold Change	Description	Type
AFLA_122410	7.84	Putative TF	bZIP
AFLA_040300	7.03	Putative TF	C_6
AFLA_094010	6.01	*atf21*	bZIP
AFLA_000010	4.85	Putative TF	Zinc-binding
AFLA_049410	4.68	Putative TF	C_6
AFLA_085880	4.19	Putative TF	BTB
AFLA_103640	4.14	*fcr1*	C_6
AFLA_079250	4.13	Putative TF	Zinc-binding
AFLA_120470	4.06	*silA* ortholog	Zinc-binding
AFLA_007370	3.81	Putative TF	Zinc-binding
AFLA_096100	3.73	Putative TF	
AFLA_139560	3.60	Putative TF	C_6
AFLA_001310	3.57	Putative TF	Zinc-binding
AFLA_120480	3.15	*silA*	Zinc-binding
AFLA_120780	3.10	Putative TF	C_6
AFLA_139360	2.99	*aflR*	C_6
AFLA_051880	2.92	*prnA*	C_6
AFLA_097720	2.91	*clrA*	C_6
AFLA_123530	2.86	Putative TF	Zinc-binding
AFLA_096320	2.84	Putative TF	C6 / C2H2
AFLA_005520	2.82	*sfgA*	C_6
AFLA_126910	2.72	Putative TF	C_6
AFLA_059110	2.72	Putative TF	Zinc-binding
AFLA_009580	2.63	Putative TF	C_6
AFLA_065310	2.57	Putative TF	Zinc-binding
AFLA_124010	2.44	Putative TF	C_6
AFLA_064980	2.44	*sdrA*	C_6
AFLA_096330	2.40	Putative TF	C6 / C2H2
AFLA_038210	2.39	Putative TF	C_6
AFLA_122500	2.37	Putative TF	C_6
AFLA_134920	2.36	Putative TF	C_6
AFLA_009690	2.31	Putative TF	Zinc-binding

AFLA_034610	2.28	Putative TF	C_6
AFLA_105530	2.27	Putative TF	C_6
AFLA_033480	2.26	Putative TF	C_6
AFLA_041330	2.22	Putative TF	C_6
AFLA_123540	2.22	Putative TF	Zinc-binding
AFLA_136880	2.19	Putative TF	C_6
AFLA_012100	2.16	*pcaG*	NDT80_PhoG
AFLA_093070	2.15	Putative TF	C_2H_2
AFLA_049640	2.06	Putative TF	Zinc-binding
AFLA_119890	2.01	Putative TF	C_6
AFLA_062330	1.93	Putative TF	C_6
AFLA_076330	1.90	Putative TF	Zinc-binding
AFLA_124630	1.87	Putative TF	Zinc-binding
AFLA_000720	1.85	Putative TF	Zinc-binding
AFLA_123770	1.81	*scfA* ortholog	C_6
AFLA_014270	1.81	Putative TF	C_6
AFLA_080780	1.81	Putative TF	Zinc-binding
AFLA_120290	1.80	Putative TF	C_6
AFLA_000730	1.79	Putative TF	C_6
AFLA_075420	1.78	Putative TF	Homeobox
AFLA_079320	1.77	Putative TF	Zinc-binding
AFLA_138930	1.72	Putative TF	C_6
AFLA_042930	1.71	Putative TF	Zinc-binding
AFLA_025720	1.69	*nosA*	C_6
AFLA_025860	1.65	Putative TF	C_6
AFLA_015850	1.62	Putative TF	C_6
AFLA_125590	1.61	Putative TF	Zinc-binding
AFLA_118080	1.60	*rdr1*	Zinc-binding
AFLA_097920	1.59	*scfA*	C_6
AFLA_053760	1.58	Putative TF	Zinc-binding
AFLA_110020	1.57	Putative TF	C_6
AFLA_097740	1.49	Putative TF	Zinc-binding
AFLA_012010	1.49	*ctf1B*	C_6
AFLA_030600	1.45	*fkh1*	Forkhead
AFLA_073870	1.45	*regA*	C_6

AFLA_100300	1.44	Putative TF	C_6
AFLA_068100	1.43	Putative TF	Zinc-binding
AFLA_049270	1.42	*galX*	C_6
AFLA_036490	1.36	*nscR*	C_6
AFLA_063720	1.33	Putative TF	Zinc-binding
AFLA_028760	1.30	Putative TF	C_2H_2
AFLA_014120	1.30	*scfA* ortholog	C_6
AFLA_048870	1.29	*amdA*	C_2H_2
AFLA_042030	1.29	Putative TF	C_6
AFLA_033160	1.27	*sfp1*	C_2H_2
AFLA_097680	1.26	Putative TF	C_6
AFLA_010880	1.26	Putative TF	Zinc-binding
AFLA_053230	1.26	Putative TF	Zinc-binding
AFLA_015920	1.25	*aro80*	C_6
AFLA_139110	1.24	*aflYd*	Zinc-binding
AFLA_048920	1.24	Putative TF	C_6
AFLA_123500	1.19	Putative TF	C_6
AFLA_024580	1.19	Putative TF	C_6
AFLA_018110	1.18	Putative TF	bZIP
AFLA_076320	1.18	Putative TF	C_6
AFLA_043710	1.17	Putative TF	C_6
AFLA_013890	1.17	Putative TF	C_2H_2
AFLA_083510	1.14	Putative TF	Homeobox/ C_2H_2
AFLA_090160	1.13	Putative TF	Zinc-binding
AFLA_010240	1.13	Putative TF	Zinc-binding
AFLA_121770	1.12	Putative TF	Zinc-binding
AFLA_098130	1.11	Putative TF	Zinc-binding
AFLA_064370	1.08	Putative TF	C_6
AFLA_087810	1.07	*metR*	bZIP
AFLA_028560	1.06	*amdR*	C_6
AFLA_017040	1.03	Putative TF	C_6
AFLA_028410	1.02	*pbcR*	C_6
AFLA_009490	1.01	Putative TF	C_6
AFLA_118300	-1.00	Putative TF	C_6
AFLA_023420	-1.01	Putative TF	Zinc-binding

AFLA_076040	-1.04	Putative TF	Zinc-binding
AFLA_036190	-1.10	*rap1*	
AFLA_089270	-1.11	*hacA*	bZIP
AFLA_083820	-1.13	Putative TF	C_6
AFLA_048650	-1.14	*steA*	Homeobox/Zinc-binding
AFLA_030580	-1.17	*pacC*	C_2H_2
AFLA_080270	-1.17	Putative TF	C_2H_2
AFLA_097380	-1.21	Putative TF	CP2
AFLA_020130	-1.21	*rgdA*	APSES
AFLA_018410	-1.21	Putative TF	C_2H_2
AFLA_129530	-1.22	Putative TF	Zinc-binding
AFLA_074060	-1.28	Putative TF	NF-X1
AFLA_051900	-1.35	*cnjB*	Zinc-binding
AFLA_067300	-1.37	Putative TF	Zinc-binding
AFLA_074200	-1.41	Putative TF	C_6
AFLA_135110	-1.44	Putative TF	HLH
AFLA_131640	-1.47	*devR/hpa3*	HLH
AFLA_070980	-1.48	Putative TF	C_6
AFLA_038860	-1.49	Putative TF	Zinc-binding
AFLA_058610	-1.54	*srbA*	HLH
AFLA_104780	-1.57	Putative TF	C_6
AFLA_127920	-1.57	*crzA*	C_2H_2
AFLA_002290	-1.58	*amdX*	C_2H_2
AFLA_084200	-1.59	Putative TF	C_6
AFLA_050970	-1.61	Putative TF	Zinc-binding
AFLA_020210	-1.68	*nsdD*	GATA
AFLA_109220	-1.73	Putative TF	C_6
AFLA_050250	-1.75	*cpcA*	bZIP
AFLA_099460	-1.76	*rfeG*	
AFLA_083100	-1.81	*zipA*	bZIP
AFLA_044680	-1.88	*ndtA*	NDT80_PhoG domain
AFLA_088390	-2.07	*egd1*	btf3-like
AFLA_035590	-2.12	Putative TF	C_6

AFLA_026250	-2.16	*rfeB*	Homeobox
AFLA_071330	-2.18	Putative TF	Zinc-binding
AFLA_110650	-2.23	*seb1*	C_2H_2
AFLA_091490	-2.27	*mtfA*	C_2H_2
AFLA_046990	-2.32	*stuA*	APSES
AFLA_064960	-2.36	Putative TF	Zinc-binding
AFLA_096370	-2.41	Putative TF	C_6
AFLA_008120	-2.47	Putative TF	Zinc-binding
AFLA_057080	-2.48	Putative TF	Zinc-binding
AFLA_067290	-2.60	Putative TF	Zinc-binding
AFLA_083560	-2.61	Putative TF	C_6
AFLA_119280	-2.63	Putative TF	Zinc-binding
AFLA_131330	-2.72	*nsdC*	C_2H_2
AFLA_087350	-2.86	*sltA*	C_2H_2
AFLA_017640	-2.87	*rpn4*	C_2H_2
AFLA_029620	-2.96	*abaA*	TEA/ATTS
AFLA_137320	-3.04	*flbC*	C_2H_2
AFLA_069460	-3.11	*egr2*	C_2H_2
AFLA_069100	-3.18	Putative TF	LIM/homeobox
AFLA_082850	-3.31	*brlA*	C_2H_2
AFLA_086110	-3.54	Putative TF	C_6
AFLA_057480	-4.22	Putative TF	Zinc-binding
AFLA_021240	-4.26	*glcD gamma*	HLH
AFLA_059960	-4.33	*aoiH*	C_6
AFLA_113790	-5.05	Putative TF	bZIP

CHAPTER 3

Comparative analyses of WetA-mediated gene regulatory networks in *Aspergillus*

The results described in this chapter are being prepared for publication

3-1 Abstract

Asexual development is widespread among the fungal class Eurotiomycetes (phylum Ascomycota) and is a key factor contributing the fecundity and fitness of Ascomycete fungi. These mitotically derived asexual spores are an efficient agent for genome protection, survival, propagation, and infecting hosts. Asexual sporulation (conidiation) is a highly successful and effective reproductive mechanism for *Aspergillus* fungi as vast numbers of asexual spores (conidia) can be produced from a single colony through repetitive cycles of mitosis. The *Aspergillus* conidiation is primarily governed by the highly conserved central regulatory circuit BrlA→AbaA→WetA, where WetA controls the proper formation, maturation, and viability of conidia and couples morphological differentiation and chemical development. While all *Aspergillus* species appear to require WetA function for sporulation, divergent gene regulatory networks (GRNs) governing distinct morphology of conidia and varying metabolic remodeling associated with conidiogenesis in each species are not known. To address this key research gap, we have carried out comparative expression analyses of conidia of wild type (WT) and *wetA* null mutant in *Aspergillus nidulans*, *Aspergillus flavus*, and *Aspergillus fumigatus*. Moreover, employing WetA-chromatin association in *A. nidulans* conidia, we prove that WetA is a DNA-binding protein that interacts with the WetA Response Element (WRE). Taken together, WetA is functionally conserved in regulation in *Aspergillus* cellular and chemical development while the Wet-mediated GRNs have been rewired during evolution. In summary, we have unveiled the evolutionary alterations of WetA-mediated GRNs that result in the changes in conidia development and metabolism in three *Aspergillus* species.

3-2 Introduction

Fungi are greatly important to humankind as pathogens, environmental recyclers, industrial producers, and agricultural aids. Filamentous fungi, particularly those in the species-rich class Eurotiomycetes (phylum Ascomycota), often reproduce asexually. Importantly, in some fungi, morphological development is coordinated with the production of secondary metabolites with toxic and antibiotic properties [1–3]. Asexual development (conidiation) in the fungal class Eurotiomycetes (phylum Ascomycota) results in the formation of mitotically derived conidiospores, or conidia. While all conidia represent non-motile asexual propagules, the morphology of the complex spore-producing structure conidiophore is distinct in various *Aspergillus* species [4]. As asexual sporulation is widespread among fungi, it represents a simple highly tractable system to understand how gene regulatory networks (GRNs) are re-wired to underlie the evolution of development and metabolic control in microbial eukaryotes.

Conidiation in *Aspergillus* begins with forming the specialized conidiogenous structure (conidiophore), and then the haploid conidia are formed by mitosis followed by repeated asymmetric division from the apex or side of the conidiophore. The conidia are isogenic to the haploid parent and serve in long-term viability and genome protection, which are capable of germinating and forming new colonies under appropriate conditions [5]. Conidiogenesis is a complex, precisely timed, and genetically programmed event involving specialized cellular differentiation, temporal and spatial regulation of gene expression, and cellular communications [4], which needs dedicate coordination of the gene regulatory networks (GRNs) to secure survival. The three distantly related species *Aspergillus nidulans*, *Aspergillus flavus*, and *Aspergillus fumigatus* whose genomes are more highly divergent than those of human-chicken-fish [6], form distinct conidiophores with varying sizes of conidia. The regulatory mechanisms of conidiation have been extensively studied in *A. nidulans* [7–23]. The regulatory genes can be divided into central regulators, upstream activators, negative regulators, light-dependent regulators, and the *velvet* regulators [24,25]. The central genetic regulatory circuit BrlA→AbaA→WetA is present in most *Aspergillus* and *Penicillium*, governing conidiation-specific GRNs and a sequentially dependent pathway of conidiation (Fig 3-1A) [15,24,26]. BrlA (bristle A) is a C_2H_2-zinc-finger type transcription factor (TF), which recognizes and interacts with the BrlA Response Elements (BREs; 5’-(C/A)(G/A)AGGG(G/A)-3’) (Fig 3-1B) [27,28]. The *brlA* gene is expressed in the early phase of conidiation and mediates vesicle formation and budding-like cell growth [19]. The *abaA* (abacus A) gene is activated by BrlA and regulates metulae and phialides formation. Similar to BrlA, AbaA is a TF, containing an ATTS/TEA DNA binding motif and a potential leucine zipper, that recognizes the AbaA Response Element (ARE: 5’-CATTCY-3’) (Fig 3-1B) [29].

WetA (wet-white A) is highly and broadly conserved in *Ascomycetes* [8–16,18–21,23,26,30]. The *wetA* gene is activated by AbaA at the late phase of conidiation and is essential for maturation of conidia [31]. WetA functions in the synthesis of crucial conidial wall component and makes the conidia impermeable and mature [13,14,30]. The deletion (Δ) of *wetA* results in various defects of conidia, including the formation of colorless conidia that undergo autolysis in *A. nidulans* [13–15,18–21,26], *A. fumigatus* [8,23], *A. oryzae* [11], and *A. flavus* [30]. The metabolism and expression control of several conidial components, including β-glucan and trehalose, are perturbed leading to reduced stress tolerance and viability of conidia in *A. nidulans*, *A. flavus*, and *A. fumigatus* [8,30].

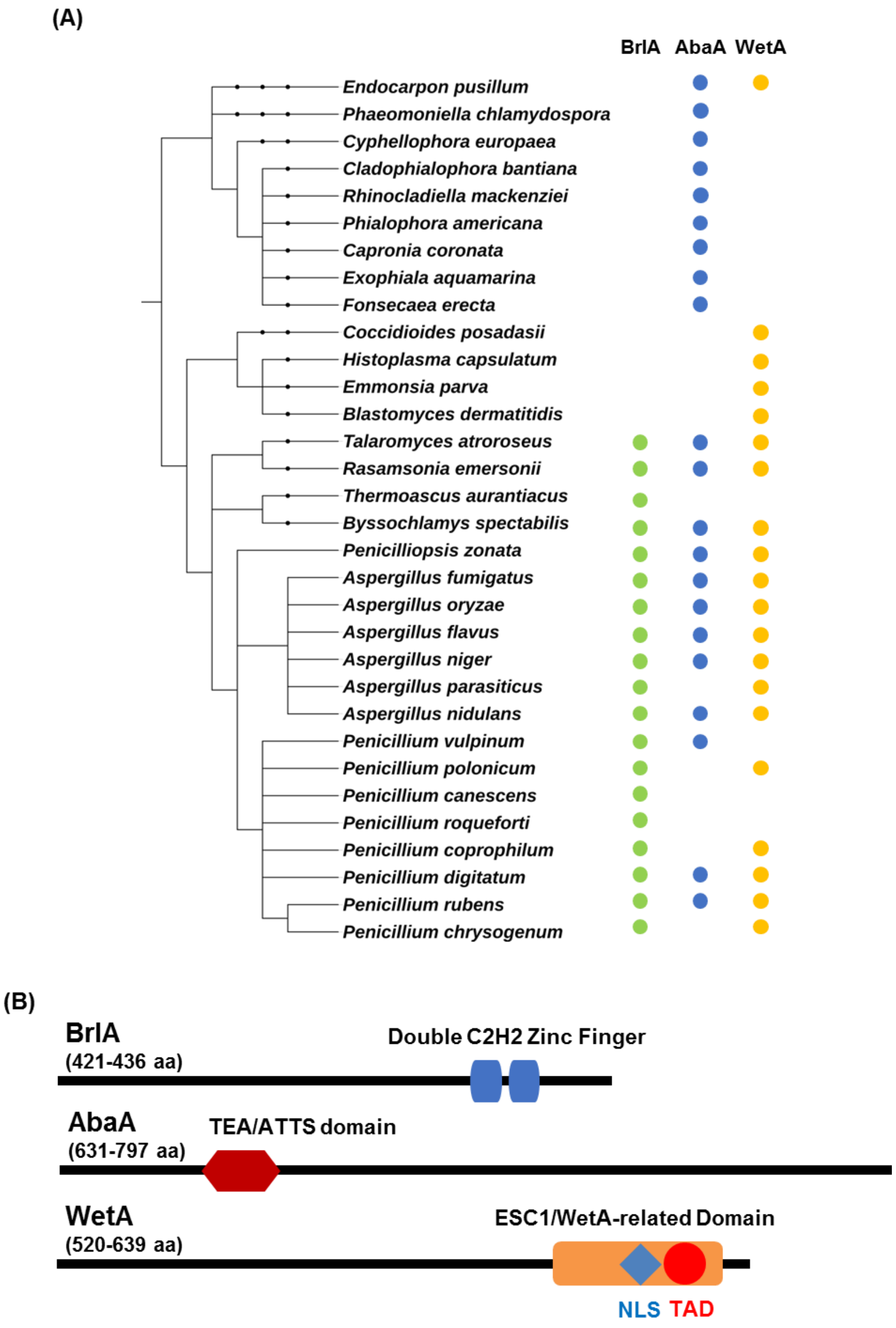


Fig 3-1 The central regulatory pathway is highly conserved in *Aspergillus* species

(A) The central regulators are highly conserved in *Aspergilli*. The phylogenetic tree was constructed based on NCBI taxonomy and visualized by iTOL [32]. Green, blue, and yellow circles represent that the species contains BrlA, AbaA, and WetA orthologs. The orthologs were identified by BlastP [33] using *A. nidulans* BrlA, AbaA, and WetA amino acid sequences as templates. (B) The predicted conserved central regulator protein architectures in *A. nidulans*, *A. fumigatus*, and *A. flavus*. The blue box and the brick hexagon represents C2H2 zinc finger domain and TEA/ATT domain in BrlA and AbaA, which were predicted in NCBI BlastP 2.6.0 [33]. The red circle and the red box represent the transcription activation domain (TAD) which was predicted by 9aaTAD using the “Less stringent Pattern” setting [34]. The blue diamond and the blue box represent the nuclear localization signal (NLS) predicted by NLStradamus using the 4 state HMM static model [35]. The orange rectangle and the orange box represent the ESC1/WetA-related domain (PTHR22934) predicted by the PANTHER classification system [36]. The consensus sequence and the consensus histogram are shown under the amino acid sequence multiple sequence alignment.

A. nidulans WetA is required for the activation of Class B genes, which are predicted to have spore-specific functions, and Class C and Class D genes, expected to have phialide-specific functions [13,15,37]. At least one Class B gene called *wA*, whose mRNA accumulates in phialide cells instead of in spores, is activated by WetA, indicating that WetA may regulate genes in these cells as well as in conidia [31]. Our recent *A. flavus* transcriptome analysis shows that WetA plays a broad regulatory role in conidia, and may serve as a key upstream regulator of multiple cellular and chemical developmental pathways [30]. Moreover, *A. nidulans* WetA, along with *A. fumigatus* WetA and *A. flavus* WetA, has a conserved ESC1/WetA-related domain (PTHR22934: SF29) with the putative DNA-binding ability originating near the C terminus (Fig 3-1B) [9,38], which also contains a predicted transcription activation domain (TAD) [34] and a nuclear localization signal (NLS) [35,39], suggesting that WetA is a potential TF (Fig 3-1B) [30]. Collectively, we propose that the evolutionarily conserved WetA plays a global regulatory role in bridging spore differentiation and survival in *A. nidulans*, *A. fumigatus*, and *A. flavus* [8,13–15,18–21,23,26,30]. Moreover, WetA functions differently in the early stage of conidiation and C2 layer condensation [8,30], suggesting that the WetA-mediated GRNs have been rewired during evolution.

In this report, we hypothesize that WetA is an evolutionarily conserved TF which directly binds to conserved and distinct targets in *A. nidulans*, *A. fumigatus*, and *A. flavus*, and results in respective WetA-mediated GRNs that cause distinct effects in cellular and chemical development. To test this, we have identified the WetA Response Elements (WRE) and elucidate WetA-mediated GRNs by employing ChIP-seq and RNA-seq. Furthermore, we interpret how WetA-mediated GRNs have been rewired during the evolution by the comparative transcriptome analysis in *A. nidulans*, *A. fumigatus*, and *A. flavus*. Our results shed the lights on the global regulatory roles of WetA in Aspergilli and the rewired WetA-mediated GRNs, which help us to understand the evolution of asexual development and metabolism in fungi.

3-3 Materials and methods

3-3-1 Strains, media, and culture conditions

Aspergillus strains used in this study are listed in Table S3-1.The fungal strains were grown on minimal medium (MM) with appropriate supplements as described previously [40,41] and incubated at 37°C (*A. nidulans* and *A. fumigatus*) or 30°C (*A. flavus*). To determine the number of conidia, WT and mutant strains were point-inoculated and grown on solid MM at 37°C (*A. nidulans* and *A. fumigatus*) or 30°C (*A. flavus*) for 2 days. The conidia were collected in ddH_2O from the entire colony and counted using a hemocytometer. For liquid submerged cultures, conidia of WT and mutant strains were inoculated in liquid MM and incubated at 37°C (*A. nidulans* and *A. fumigatus*) or 30°C (*A. flavus*), 220 rpm. Conidiation induction was performed as previously described [42]. *Escherichia coli* strains, DH5α and BL21 (DE3), were grown in Luria-Bertani medium with ampicillin (50 mg/ml) for plasmid amplification.

3-3-2 Generation of *wetA* deletion and complemented strains

In this study, we generated the deletion (Δ) and complement (C') strains of *AniwetA*. The oligonucleotides used in this study are listed in Table S3-1. Double-joint PCR was used to generate the deletion constructs of *AniwetA* [43]. Briefly, the deletion constructs containing *A. fumigatus pyrG* marker with 5' and 3' flanking regions of *AniwetA* were introduced into the recipient strains RJMP1.59 [44]. To generate complemented strains, a WT *AniwetA* gene region including its upstream 2 kb region was cloned to pHS13 [45], which contains ¾ *pyroA* [46], a 3xFLAG tag and the *trpC* terminator [47]. The resulting plasmid pMY1 was then introduced into the recipient Δ*AniwetA* strain TMY3, in which preferentially a single copy *AniwetA*$^+$ gets inserted into the *pyroA* locus complementing the *pyroA4* allele, and gives rise to TMY4. Multiple *AniwetA* deletion mutants were generated, which all behaved the same in every assay testing. We also generated three independent complemented strains (C'*AniwetA*), and they all behaved identically to one another as well. The Δ*AfuwetA* (TSGw4), Δ*AflwetA* (TMY1), and C'*AflwetA* (TMY2) strains were generated in previous studies [8,30].

3-3-3 Nucleic acid manipulation

To isolate genomic DNA, about 10^6 conidia of relevant strains were inoculated in 2 ml liquid MM and stationary cultures at 37°C (*A. nidulans* and *A. fumigatus*) or 30°C (*A. flavus*) for 2 days. The mycelial mat was collected, squeeze-dried, and genomic DNA was isolated as described [43]. Total RNA isolation for Northern blot analyses was performed as described [42,43,48]. For RNA-seq and ChIP-seq, 2-day-old conidia of WT and Δ*wetA* strains were harvested from solid MM.

3-3-4 Anti-WetA polyclonal antibody synthesis

Multiple alignment-based phylogenetic analysis using Clustal W/X [49] revealed that *Ani*WetA, *Afu*WetA, and *Afl*WetA contains a 14-aa-length conserved region near the C terminus (Fig 3-4A and Fig 3-4B). The 13-aa-peptide RKAGGDVEALEAV was selected, synthesized, and used for immunization in the rabbit and generation of an affinity-purified polyclonal antibody (GenScript Corp., Piscataway, NJ). The rabbit anti-WetA polyclonal antibodies were lyophilized in phosphate-buffered saline (pH 7.4) with 0.02% sodium azide as a preservative. Lyophilized antibodies were reconstituted with MilliQ water and aliquots were stored at -80°C until use.

3-3-5 RNA sequencing

Total RNA was extracted and submitted to ProteinCT Biotechnologies (Madison, WI) and the University of Wisconsin Biotechnology Center (Madison, WI) for library preparation and sequencing. A strand-specific library was prepared from total RNA using the Illumina TruSeq Strand-specific RNA sample preparation system. Briefly, mRNA was extracted from total RNA using poly-A selection, followed by RNA fragmentation. The strand-specific library was constructed by first-strand cDNA synthesis using random primers, sample cleanup, and second-strand synthesis using DNA Polymerase I and RNase H. A single 'A' base was added to the cDNA fragments followed by ligation of the adapters. Final cDNA library was achieved by further purification and enrichment with PCR, then quality checked using a Bioanalyzer 2100. The library was sequenced (PE100bp for *A. nidulans* and SE100bp for *A. fumigatus* and *A. flavus*) using the Illumina HiSeq2500, and over 10 million (*A. nidulans*), 9.1 million, and 19 million (*A. flavus*) high-quality reads per sample were achieved.

The quality of the raw sequence reads was verified using version 0.11.5 of FastQC [50]. The genome and gene annotations were downloaded from AspGD and NCBI and used for mapping. Mapping of the raw sequence reads to the genome was carried out with version 2.1.1 of Tophat2 [51], and the default options were used except for the maximum intron length was set to 4,000 bases (--max-intron-length 4000). Most (77-93%) of the reads from each of the samples mapped to the genome. The alignment BAM files were compared against the gene annotation GFF file, and raw counts for the number of reads mapping to each gene were generated using version 0.6.1 of HTSeq-count [52]. Approximately 70-80% of mappable reads from each of the samples could be assigned to genes. Differential expression analysis of the raw counts was carried out using version 1.14.1 of DESeq2 [53]. Genes were considered differentially expressed between the WT and Δ*wetA* conidia if their adjusted p-value was less than 0.05 and their $\log_2$ fold change was less than -1 or greater than 1.

3-3-6 Chromatin immunoprecipitation sequencing (ChIP-seq)

ChIP assays were performed using MAGnify ChIP assays (Invitrogen) according to the manufacturer's instructions. Briefly, 10^9 conidia were cross-linked with 1% formaldehyde, lysed and broken as described [54]. Cell lysates were sonicated to shear DNA to the 300-500 bp and were immunoprecipitated with the rabbit anti-WetA polyclonal antibody. Ten-percent of the supernatants were kept as input (input represents PCR amplification of the total sample). ChIP DNA samples were sent for ChIP-Seq service (ProteinCT, Madison, WI). Libraries were prepared using TruSeq ChIP Library Preparation Kit (Illumina, San Diego, CA), and sequenced on HiSeq2500 with single reads of 50 bp, about 8-30 M reads per sample. The raw sequence reads were aligned to *A. nidulans* genome (FGSC A4) using bowtie2 [55,56]. The Homer [57] program was used to create normalized genome coverage files and make peak calls. For each ChIP pair, the ChIP sample is compared to Input samples. Peaks were considered enriched between the ChIP samples and Input samples if their adjusted p-value was less than 0.001 and their $\log_2$ fold change was less than -1 or greater than 1. The peak corresponding DNA sequences were downloaded from AspGD for further analysis [58].

3-3-7 Motif discovery analyses

De novo motif finding program MEME-ChIP 4.11.3 [59] was used to predict WetA-binding motifs from the over-represented motifs in the 'peak sequences' with the settings: "meme-chip -oc . -index-name meme-chip.html -time 300 -fdesc description -order 1 -db db/JASPAR/JASPAR_CORE_2016_fungi.meme -meme-mod anr -meme-minw 4 -meme-maxw 50 -meme-nmotifs 10 -dreme-e 0.05 -centrimo-score 5.0 -centrimo-ethresh 10.0 sequences.fa" . The sequences of the identified 'peaks' were uploaded into meme. Possible functions associated with identified motifs were analyzed using Tomtom motif comparison tool against the JASPAR CORE (2016) fungi database [60]. The WetA-enriched regions were predicted and annotated by searching predicted WetA-binding-motif in the three *Aspergillus* genomes for the closest downstream genes (up to 2 kb) using FIMO [61] and FungiDB analysis tool [62].

3-3-8 Functional Enrichment Analysis

GO annotations were downloaded from the AmiGO 2 website (version 2.4) on February 8, 2017 [63], and terms enriched in either the WetA-activated or -repressed gene lists were detected using version 3.0.3 of the BiNGO application [64] for Cytoscape (version 3.4.0) [65]. Version 1-26-17 of the Gene Ontology (go.obo) [66] was used to establish GO term relationships. GO terms were considered enriched if their p-value, following the Benjamini-Hochberg correction as implemented in BiNGO, was less than 0.05.

3-4 Results

3-4-1 WetA is a potential transcription factor

The *A. nidulans wetA* (*AniwetA*), *A. fumigatus wetA* (*AfuwetA*), and *A. flavus wetA* (*AflwetA*) ORFs comprise 1,668 bp, 1,701 bp, and 1,692 bp with no introns and are predicted to encode 555, 566, and 563 amino-acid (aa) long proteins, respectively. BlastP analysis reveals that *A. nidulans* WetA (*Ani*WetA, XP_659541.1), *A. fumigatus* WetA (*Afu*WetA, XP_751508.1), and *A. flavus* WetA (*Afl*WetA, XP_002383329.1) amino acid sequences share about 60% aa identity with each other [33]. *Afl*WetA and is relatively more similar to *Afu*WetA compared to *Ani*WetA (Wu 2017). *Ani*WetA, *Afu*WetA, and *Afl*WetA proteins have a conserved 51-aa-length ESC1/WetA-related domain (PTHR22934: SF29) with the putative DNA-binding ability originating near the C terminus (Fig 3-1B) [9,38]. This highly conserved domain is further predicted by 9aaTAD and NLStradamus [34,35] to contain a 9-aa-length transcription activation domain (TAD) and a 16-aa-length nuclear localization signal (NLS), suggesting that WetA is a potential TF (Fig 3-1B).

3-4-2 Conserved and diverged roles of WetA in *Aspergilli*

A. flavus genome-wide expression analysis in conidia shows that WetA plays multiple roles in governing cellular and chemical development in *A. flavus* [30]. The deletion of *wetA* causes similar phenotypes in Δ*AniwetA*, Δ*AfuwetA*, and Δ*AflwetA* mutants, such as reduced hyphal branching rate, reduced trehalose and β-glucan amount in conidia, conidia autolysis, reduced stress tolerance, disrupted conidial pigmentation, etc [8,30], which suggests that WetA is functionally conserved in many developmental regulations. However, considering the different conidial morphology of *A. nidulans*, *A. fumigatus*, and *A. flavus*, and the different phenotypes of the Δ*AniwetA*, Δ*AniwetA*, and Δ*AniwetA* mutants (Table 3-1), we proposed that WetA acts differently in certain biological processes among the three *Aspergillus* species.

Table 3-1 Different phenotypes in three *Aspergillus* species

	A. nidulans	***A. fumigatus***	***A. flavus***
WT conidiophore head structure [67]	Biseriate	Uniseriate	Can be either uniseriate or biseriate
WT conidia size [68] **(Wu 2017)**	2.4 to 2.7 μm	1.3 to 1.8 μm	3.3 to 3.5μm
WT conidia color	Deep green	Grayish green	Yellowish green
Δ*wetA* conidial wall C2 layer thickness [68] **(Wu 2017)**	Thicker than WT conidia	Thicker than WT conidia	Thinner than WT conidia
Δ*wetA* conidiation timing [68] **(Wu 2017)**	Same with WT	Later than WT	Earlier than WT

To shed the light on the conserved and differential regulatory roles WetA appeared to play in the three *Aspergillus* species, we carried out genome-wide expression analyses in WT and mutant conidia using RNA-seq. The *A. flavus* RNA-seq data was generated in our previous study [30]. In short, Poly-A mRNA from four (*Ani*WT and Δ*AniwetA* strains) or three (*Afu*WT and Δ*AfuwetA* strains) technical replicates of 2-day-old conidia were purified and sequenced as described in the methods section.

Examination of global gene expression differences between the WT and mutant *wetA* indicate that WetA plays a broad regulatory role in conidia in all three *Aspergillus* species. Out of the 10,602 mapped *A. nidulans* genes, 5,706 (54% of the total) showed differential accumulation of mRNAs in the Δ*AniwetA* conidia in comparison to *Ani*WT conidia. Among 5,706 differentially expressed genes (DEGs), mRNA levels of 2,635 (25%) genes were lower (Down) in the Δ*AniwetA* conidia compared to *Ani*WT conidia, and those of 3,071 (29%) genes were higher (Up) in the Δ*AniwetA* conidia compared to *Ani*WT conidia (Fig 3-2). Out of the 9,908 mapped *A. fumigatus* genes, 5,730 (58% of the total) showed differential accumulation of mRNAs in the Δ*AfuwetA* conidia in comparison to *Afu*WT conidia. Among 5,730 differentially expressed genes (DEGs), mRNA levels of 2,734 (28%) genes were lower (Down) in the Δ*AfuwetA* conidia compared to *Afu*WT conidia, and those of 2,996 (30%) genes were higher (Up) in the Δ*AfuwetA* conidia compared to *Afu*WT conidia (Fig 3-2).

		A. nidulans	***A. fumigatus***	***A. flavus***
⬛	Unaffected genes	4,896 (46%)	4, 178 (42%)	7,730 (57%)
🟩	**DEG**	**5,706 (54%)**	**5,730 (58%)**	**5,755 (43%)**
🟨	Up-regulated in Δ*wetA*	3,071 (29%)	2,996 (30%)	2,899 (21%)
🟦	Down-regulated in Δ*wetA*	2,635 (25%)	2,734 (28%)	2,856 (21%)
	TOTAL	10,602	9,908	13,485

Fig 3-2 Summary of DEGs in the three *Aspergillus* Δ*wetA* conidia

The numbers of genes whose mRNA levels were similar (Unaffected, gray), or different between WT and Δ*wetA* conidia (DEG, green), with down (blue) and up (yellow) in the Δ*wetA* conidia compared to WT. A DEG is defined by having a > 2-fold change in mRNA levels between WT and Δ*wetA* conidia and an adjusted *p*-value of less than 0.05.

Functional category analysis was carried out by determining Gene Ontology (GO) terms that were enriched in DEGs. In *A. flavus*, the top enriched biological process GO categories are "single-organism metabolic process", "oxidation-reduction process", "transmembrane transport", "carbohydrate metabolic process", "cell wall organization or biogenesis", "polysaccharide metabolic process", and "secondary metabolic process", which are also highly enriched in *A. nidulans* and *A. fumigatus* (Table S3-2). Moreover, over 70% of all genes in the cellular component GO category, "fungal-type cell wall", is also highly enriched in all three species. These top enriched GO categories are consistent with the phenotypes in Δ*wetA* mutants, suggested that WetA plays key roles in carbohydrate metabolism, secondary metabolism, and conidial wall integrity. Of note is that the difference of the top enriched GO categories between the three species may result from the different degree of completion of the genome annotation. The top 100 DEGs with decreased/increased mRNA accumulation levels in the Δ*AniwetA* and Δ*AfuwetA* conidia are listed in Table S3-3, Table S3-4, Table S3-5, and Table S3-6, respectively.

To explore the conserved and differential regulatory roles of WetA, we checked the mRNA expression profiles of orthologs in the three *Aspergillus* genomes. We identified the orthologs in the three tested genomes using OrthoMCL database with the settings: "30% identity, 70% coverage, and an inflation value of 4" [69]. Total 17,537 orthogroups were identified, whereas 6,466 orthogroups contains orthologs in all three species (All_orthogroups), 546 orthogroups contains orthologs in *A. nidulans* and *A. fumigatus* (NU_orthogroups), 895 orthologroups contains orthologs in *A. nidulans* and *A. flavus* (NL_orthogroups), and 724 orthogroups contains orthologs in *A. fumigatus* and *A. flavus* (UL_orthogroups).

Among all orthogroups, 9,876 orthogroups (56% orthogroups) showed differential expression levels in Δ*wetA* conidia, where 4,561 orthogroups (46%) are species-specific. Total 5,315 All_orthogroups (82% of All_orthogroups), 436 NU_orthogroups (80% of NU_orthogroups), 724 NL_orthogroups (81% NL_orthogroups), and 583 UL_orthogroups (81% of UL_orthogroups) contain at least one ortholog differentially expressed in Δ*wetA* conidia (Table 3-2 and Table 3-3).

Table 3-2 Conserved and differential WetA-regulated All_orthogroups

	Expression pattern	Orthogroups number	Expression pattern	Orthogroups number	Total
NUL	↓↓↓	343	↑↑↑	419	762
NU↔L	↓↓↑	19	↑↑↓	51	70
NL↔U	↓↑↓	36	↑↓↑	56	92
N↔UL	↓↑↑	50	↑↓↓	42	92
N↔U	↓↑—	148	↑↓—	129	277
N↔L	↓—↑	53	↑—↓	50	103
U↔L	—↓↑	62	—↑↓	61	123
NU	↓↓—	297	↑↑—	265	562
NL	↓—↓	160	↑—↑	133	293
UL	—↓↓	175	—↑↑	179	353
N	↓——	513	↑——	292	805
U	—↓—	517	—↑—	458	975
L	——↓	177	——↑	168	345
Mixed					462
TOTAL					**5,315**

Note: Expression pattern: The mRNA expression levels in Δ*AniwetA*, Δ*AfuwetA,* and Δ*AflwetA* conidia. ↑: upregulated in Δ*wetA* conidia; ↓: downregulated in Δ*wetA* conidia; —: not regulated in Δ*wetA* conidia. *NUL*: all up-/down-regulated in Δ*wetA* conidia; *NU*↔*L*: up-/down-regulated in Δ*AniwetA* and Δ*AfuwetA* conidia, and down-/up-regulated in Δ*AflwetA* conidia; *NL*↔*U*: up-/down-regulated in Δ*AniwetA* and Δ*AflwetA* conidia, and down-/up-regulated in Δ*AfuwetA* conidia; *UL*↔*N*: up-/down-regulated in Δ*AfuwetA* and Δ*AflwetA* conidia, and down-/up-regulated in Δ*AniwetA* conidia; *NU*: all up-/down-regulated in Δ*AniwetA* and Δ*AfuwetA* conidia, but not regulated in Δ*AflwetA* conidia; *NL*: all up-/down-regulated in Δ*AniwetA* and Δ*AflwetA* conidia, but not regulated in Δ*AfuwetA* conidia; *UL*: all up-/down-regulated in Δ*AfuwetA* and Δ*AflwetA* conidia, but not regulated in Δ*AniwetA* conidia; *N*: only regulated in Δ*AniwetA* conidia; *U*: only regulated in Δ*AfywetA* conidia; *L*: only regulated in Δ*AflwetA* conidia. *Mixed*: WetA showed mixed regulatory effects on orthologs in Δ*wetA* conidia.

Table 3-3 Conserved and differential WetA-regulated N:NOrthogroups

NU_orthogroups		**NL_orthogroups**		**UL_orthogroups**	
NU expression pattern	Orthogroups #	NL expression pattern	Orthogroups #	UL expression pattern	Orthogroups #
↓—	45	↓—	97	↓—	85
↑—	55	↑—	142	↑—	91
—↓	68	—↓	85	—↓	57
—↑	63	—↑	67	—↑	62
↓↓	60	↓↓	67	↓↓	75
↓↑	26	↓↑	38	↓↑	35
↑↓	26	↑↓	60	↑↓	41
↑↑	66	↑↑	88	↑↑	82
Mixed	27	Mixed	80	Mixed	55
TOTAL	**436**	**TOTAL**	**724**	**TOTAL**	**583**

Among all All_orthogroups, 762 All_orthogroups (12% of All_orthogroups) are consistently up-/down-regulated in Δ*wetA* conidia (Table 3-2). The top enriched GO categories of these conserved WetA-regulated-All_orthogroups include "response to stimulus", "response to stress", "carbohydrate metabolic process", "sporulation", "filamentous growth", "cell wall organization or biogenesis", "pigment metabolic process", "negative regulation of sporulation", "glucan biosynthetic process", "trehalose metabolic process", and "cell wall chitin metabolic process", "cell tip growth" (Table S3-7). The functional analysis result shows that WetA is functionally conserved in stress response, pigmentation, cell wall organization, and cellular development, which is consistent with the observed phenotypes. However, 5,315 WetA-regulated-All_orthogroups (84% of All_orthogroups) were differentially regulated in different species, implies that WetA functionally diverges in the three species (Table 3-2). Among the three species, WetA shows more conserved regulatory effects on *A. flavus* orthologs and more diverted regulatory effects on *A. fumigatus* orthologs in All_orthogroups (Table 3-2).

Total 466 All_orthogroups (9% of All_orthogroups) contain more than one ortholog in single species (All_orthogroup$_{N:N:N}$). WetA plays mixed regulatory roles in almost all All_orthogroup$_{N:N:N}$, that is, some of the orthologs in the same All_orthogroup$_{N:N:N}$ were upregulated and some were downregulated in the same species. There were 127 All_orthogroup$_{N:N:N}$ (27% of All_orthogroup$_{N:N:N}$) shows consistent regulatory effects in single species (i.e. all orthologs in single species are all up-/down-regulated in Δ*wetA* conidia) (Table S3-8). Total 27 All_orthogroup$_{N:N:N}$ (6% of All_orthogroup$_{N:N:N}$) were all up-/down-regulated in all three species, including genes encode trehalose synthases (*tpsC* and *tpsD*), α-amylase (*amyA* and *amyB*), septation-required ser/thr protein kinase (*sepH*), and cell wall lysozyme (*gh25*). The other 100 All_orthogroup$_{N:N:N}$ shows differential regulatory patterns in the three species, including genes encoding putative transcription factors, putative major facilitator superfamily (MFS) transporters, kinases, GTPase, and carbohydrate metabolism-related proteins. Taken together, total 46% orthogroups were regulated by WetA in conidia, however, only 4% orthogroups were consistently all up-/down-regulated in all three species, which suggests that most of the WetA-mediated regulation has been genetically rewired during the revolution.

We further identified the species-specifically regulated All_orthogroups (genes show different WetA-regulated effect with the other two species) and performed function analysis against these genes (Table S3-9). Our results show that the WetA-mediated GRNs are rewired in the mitotic cell cycle, response to stimulus, DNA/RNA/protein metabolic process, nitrogen compound metabolic process, organic acid metabolic process, cytoskeleton organization, cell communication, aromatic compound metabolic process, regulation of signaling, cell communication, and another primary metabolic process.

Next, we checked the WetA-regulated NU_orthogroups, NL_orthogroups, and UL_orthogroups ($_{\mathrm{N:N}}$Orthogroups) (Table 3-3). Total 21~29% of the WetA-mediated regulation of $_{\mathrm{N:N}}$Orthogroups were consistently up-/down-regulated by WetA in both species. Functional analysis shows that the WetA-mediated GRNs has been rewired in the biological processes of the cell cycle, primary metabolism, secondary metabolism, transcription, and transmembrane transport (Table S3-10).

We further examined the DEGs in each species which has no orthologs in any other two species (N-/U-/L-specific DEGs). Functional analysis shows that N-specific DEGs are involved in “carbohydrate metabolic process”, “cell wall organization”, “response to stress”, “sporulation resulting in formation of a cellular spore”, “regulation of protein ubiquitination”, “response to drug”, “nucleotide-sugar transport”, “nucleotide transmembrane transport”, “protein sumoylation”, and “cellular calcium ion homeostasis”; U-specific DEGs are involved in “secondary metabolic process”, “alkaloid metabolic process”, “ergot alkaloid metabolic process”, “terpenoid indole alkaloid metabolic process”, “alkaloid biosynthetic process”, “epoxide metabolic process”, “fumagillin metabolic process”, and “plasma membrane selenite transport”; L-specific DEGs are involved in “oxidation-reduction process”, “obsolete electron transport”, “glycosaminoglycan metabolic process”, “peptidoglycan metabolic process”, and “transmembrane transport”.

Taken together, WetA exerts broad regulatory effects in conidia by controlling about half the *Aspergillus* transcriptomes. Although 56% orthogroups in the three *Aspergillus* species were regulated in Δ*wetA* conidia, most of their mRNA expression levels are differentially regulated in each species, suggesting that the WetA-mediated GRNs has been rewired during the revolution in *Aspergilli*.

3-4-3 Rewired WetA-mediated GRNs of asexual development, signal transduction, and conidial integrity in *Aspergilli*

To explore the conserved and differential molecular roles of WetA in conidiation in the three species, we checked mRNA levels of the interested genes related to asexual development (Fig 3-3 and Table S3-11).

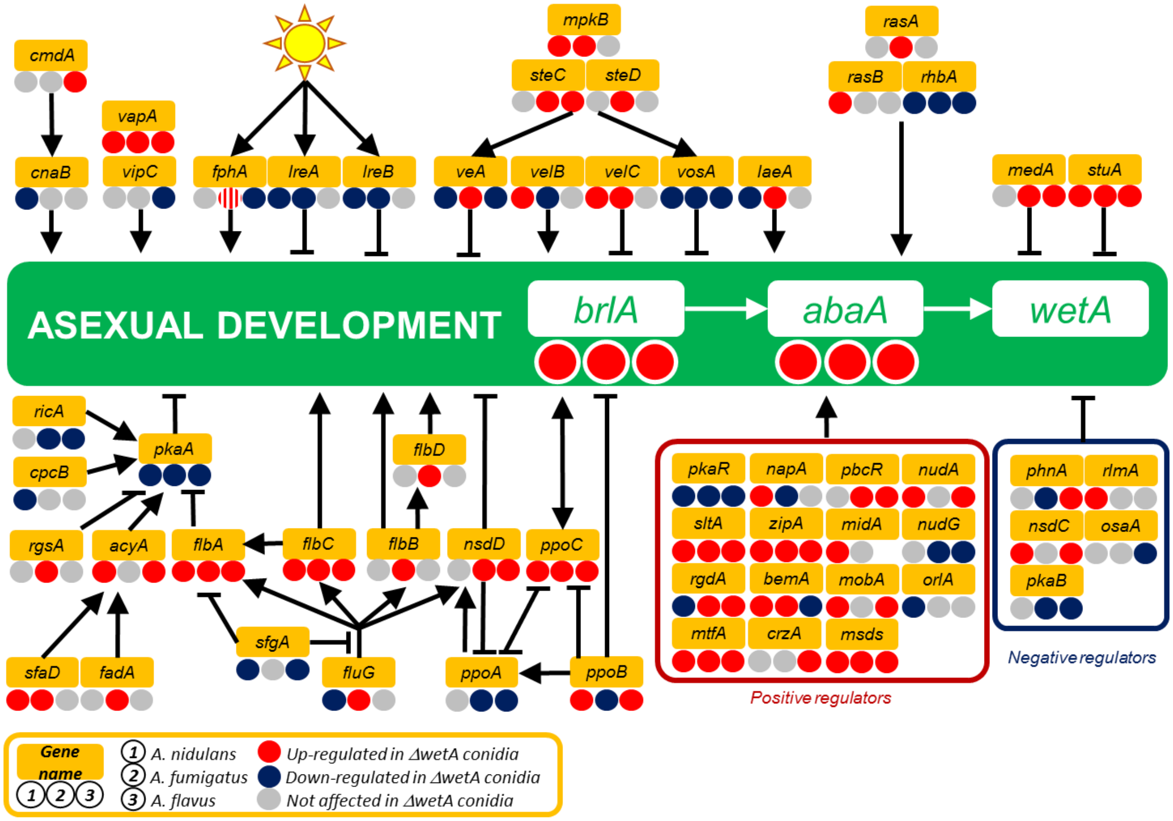


Fig 3-3 WetA-mediated regulation of asexual development in the three *Aspergillus* species

A Schematic diagram of the WetA-mediated regulatory model of asexual development. In this model, those genes with increased, decreased, and unaffected mRNA levels in the Δ*wetA* conidia are labeled with red (WetA-inhibited), blue (WetA-activated), and grey (not affected by WetA) circles and the WetA-regulatory effects in Δ*AniwetA*, Δ*AfuwetA*, and Δ*AflwetA* conidia are listed under the gene name from left to right, respectively.

In short, WetA negatively feedback regulates asexual develop by repressing its upstream regulator *brlA* in *Aspergillus* conidia. To achieve the conserved repression of *brlA* mRNA accumulation, WetA shows species-specific regulatory effects on *brlA* upstream regulatory networks. Take the *velvet* protein family (*veA*, *velB*, *velC*, and *vosA*) for example, although *vosA* mRNA levels were consistently decreased in the three Δ*wetA* conidia, the WetA effects on *veA*, *velB*, and *velC* are different in each species. Similarly, the light-dependent regulators were differentially regulated by WetA. The blue-light-dependent regulators, *lreA,* and *lreB* were unaffected in Δ*AflwetA* conidia but repressed in in Δ*AniwetA* and Δ*AfuwetA* conidia. Put together, the WetA-mediated feedback repression of asexual development is functionally conserved but its GRNs are rewired during evolution.

Our previous study shows that *Afl*WetA is involved in G-protein regulatory pathways. Here we examined whether WetA-mediated regulation of G-protein regulatory pathways is conserved in the other two *Aspergillus* species. The result shows that the mRNA levels of three G-protein receptors, *gprC*, *gprF*, *gprG*, and *nopA*, and the downstream regulators, *flbA* and *pkaA,* were consistently differentially regulated in Δ*wetA* conidia (down-, down-, up-, down-, up-, and down-regulated, respectively), while other members in the G-protein regulatory pathways were either not affected by WetA or showed species-specific regulatory patterns in Δ*wetA* conidia (Table 3-5). Since WetA plays a role in G-protein regulatory pathways, we proposed that WetA is involved in other signal transduction pathways as well. We examined the mRNA levels of kinases in Δ*wetA* conidia. The result shows that WetA differentially regulates about 100 kinase-encoded genes in Δ*wetA* conidia (Table S3-12). Most of the WetA-regulated kinase-encoded genes have one ortholog in each species and only 31 only exist in one species. Although WetA-mediated regulation targeted in a conserved group of kinase-encoded genes, but only 21 of them were consistently up- or down-regulated in all Δ*AniwetA*, Δ*AfuwetA*, and Δ*AflwetA* conidia.

Our previous study reports that the *Afl*WetA is involved in the regulation of TFs [30]. The comparative RNA-seq results show that about 150 putative TF-encoded genes in each species were differentially regulated in Δ*wetA* conidia (Table S3-13). There are 249 WetA-regulated TF-encoded genes have one ortholog in each species, and 41 are species-specific. Among the 153 conserved WetA-regulated putative TF-encoded genes, only 32 were consistently up- or down-regulated in all Δ*AniwetA*, Δ*AfuwetA*, and Δ*AflwetA* conidia.

Since our functional analysis data shows that WetA is involved in secondary metabolism in all three species, we checked the mRNA levels of the backbone genes in the secondary metabolite gene clusters (SMGs) in each species. The SMG backbone gene list was identified by Inglis *et al.* and our previous study [30,70]. Total 70, 37, and 74 SMG clusters were completely/partially showed altered mRNA expression levels in Δ*AniwetA*, Δ*AfuwetA*, and Δ*AflwetA*, respectively (Table 3-4). One of the SMG backbone gene *wA* is conserved in *Aspergilli* and encodes the conidial pigment. The previous study shows that *wA* is activated by WetA [13], which is consistent with the colorless conidia phenotype of the Δ*wetA* mutants. Although the *wA* mRNA level was decreased in Δ*AniwetA* and Δ*AflwetA* as expected, we found *wA* mRNA level was increased in Δ*AfuwetA*, suggesting that the conidial pigmentation regulatory pathway is more complex in *A. fumigatus*.

Table 3-5 G-protein pathway related DEGs in Δ*wetA* conidia in three *Aspergillus* species

GENE ID	***A. nidulans***		***A. fumigauts***		***A. flavus***	
		Log$_2$ Fold Change		**Log$_2$ Fold Change**		**Log$_2$ Fold Change**
fadA/gpaA	AN0651	-	Afu1g13140	3.82	AFLA_018340	-
flbA	AN5893	4.08	Afu2g11180	2.7	AFLA_134030	1.71
ganA	AN3090	-1.72	Afu3g12400	2.88	AFLA_079780	1.20
gprA	AN2520	-	Afu3g14330	1.58	AFLA_060740	2.03
gprB	AN7743	-3.67	Afu5g07880	-1.24	AFLA_061620	-
gprC	AN3765	1.85	Afu7g04800	4.4	AFLA_074150	2.04
gprD	AN3387	-1.72	Afu2g12640	4.12	AFLA_135680	-
gprE	AN9199	3.01	NA	NA	NA	NA
gprF	AN12206	-3.59	Afu5g04100	-2.79	AFLA_006880	-2.68
gprG	AN10166	2.71	Afu1g11900	3.86	AFLA_067770	3.09
gprH	AN8262	-	Afu5g04135	2.07	AFLA_006920	-3.09
gprK	AN7795	4.29	Afu4g01350	1.67	AFLA_009790	-1.41
gprM	AN6680	2.01	Afu7g05300	2.04	AFLA_075000	-
gprO	AN4932	-	Afu3g10570	1.32	AFLA_032130	-
gprR	NA	NA	NA	NA	AFLA_023070	-2.93
gprS	NA	NA	NA	NA	AFLA_006320	1.16
nopA	AN3361	-1.16	Afu7g01430	-3.51	AFLA_117970	-3.04
pdeA	AN0829	1.29	Afu1g14890	-	AFLA_084770	-1.39
pkaA	AN6305	-2.35	Afu2g12200	-3.46	AFLA_135040	-2.63
pkaB	AN4717	-	Afu5g08570	-1.16	AFLA_091910	-2.09

Note: “NA“: not exist in the genome. “-”: not differential expressed in Δ*wetA* conidia

Table 3-4 WetA-mediated SMG regulation

	A. nidulans	***A. fumigatus***	***A. flavus***
Total cluster number	70	37	74
Regulated cluster number	64 (91%)	35 (95%)	68 (92%)
100% regulated cluster number	5 (7%)	8 (22%)	8 (11%)
Cluster w/ all genes upregulated in DwetA conidia	3 (4%) Terriquinone cluster Emericellamide cluster *sidC* cluste	5 (14%) Conidial pigment cluster Fumigaclavine cluster Fumipyrrole cluster Fumiquinazoline cluster Pseurotin cluster	1 (1%) Cluster 71
Cluster w/ all downregulated in DwetA conidia	0 (0%)	2 (5%) Cluster 30* Cluster 36*	2 (3%) Cluster 23* Cluster 52*

Finally, we checked the expression levels of genes involved in conidia content and conidial wall integrity. All the DEGs associated with trehalose biosynthesis were downregulated in Δ*wetA* conidia in all three species, while *treA*, which is involved in trehalose degradation, was upregulated in Δ*AniwetA* and Δ*AflwetA* conidia but downregulated in Δ*AfuwetA* (Table S3-14). Loss of *wetA* resulted in increased mRNA levels of almost all DEGs involved in the biosynthesis of chitin and β-(1,3)-glucan, but had a mixed regulatory effect on the DEGs involved in the degradation process and the metabolic processes of α-(1,3)-glucan (Table S3-15, Table S3-16, and Table S3-17). Of note, all DEGs involved in the biosynthesis of melanin or hydrophobin DEGs were upregulated in Δ*AfuwetA* conidia (Table S3-18 and Table S3-19), which is not consistent with the phenotypes we observed.

In sum, WetA is a critical regulator of various signaling, developmental, and metabolic pathways in conidia. Even the WetA-mediated regulation results in similar phenotypes, the underneath GRNs has been rerouted in *Aspergilli*.

3-4-4 Identification of WetA response elements (WREs)

To understand the WetA regulatory mechanism in conidia, we carried out ChIP using customized Anti-WetA polyclonal antibody which specifically against the conserved 14-aa-length region (RKAGGDVEALEAVL) at the C' terminus of *Ani*WetA (Fig 3-4A and Fig 3-4B). ChIP-seq was performed to identify the *Ani*WetA response elements in conidia as described in methods. Total 2,210 peaks from three ChIP-seq replicates were reported with False Discovery Rate (FDR) less than 0.001 and the Fold Change (FC, sample tag counts divided by input tag counts) more than 2. The WetA-binding motif was predicted by MEME-ChIP [59]. The motif CCGCAWRCGGM was identified as the top candidate and exists in *AniwetA* upstream 2kb region (Fig 3-4C).

The potential *Ani*WetA binding regions were predicted by searching the predicted motif in the upstream 2kb regions of ORFs in *A. nidulans* genome using the FIMO [61] and annotated by FungiDB [62]. Total 2,437 genes were predicted to contain the WetA-binding motif within their upstream 2 kb regions. Function analysis of these potential *Ani*WetA targeted genes shows that the GO categories including "carbohydrate metabolic process", "transport", "DNA-mediated regulation of transcription", "chromatin modification", "cell cycle", "cell wall organization", "response to stress", "regulation of secondary metabolite biosynthesis", "conidium formation", and "pigment biosynthesis" (Table S3-20), which matches the observed phenotypes and transcriptome analysis results in Δ*AniwetA* conidia.

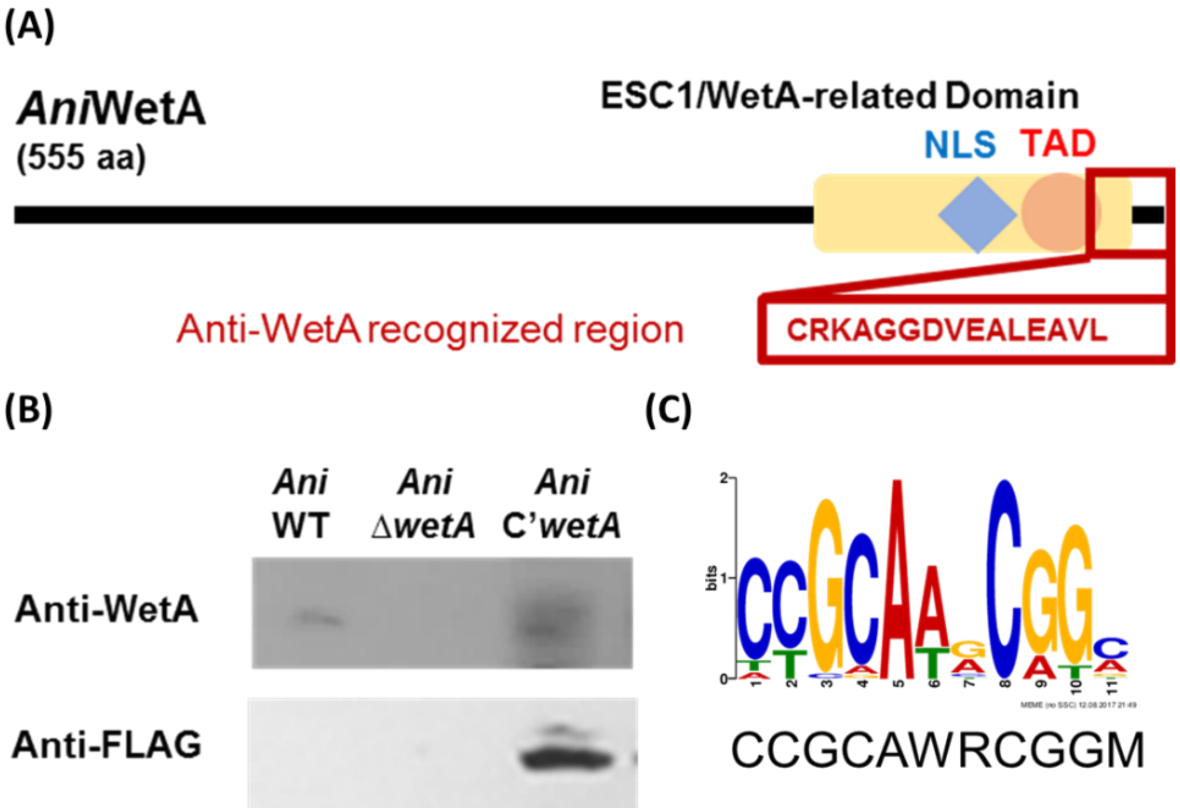
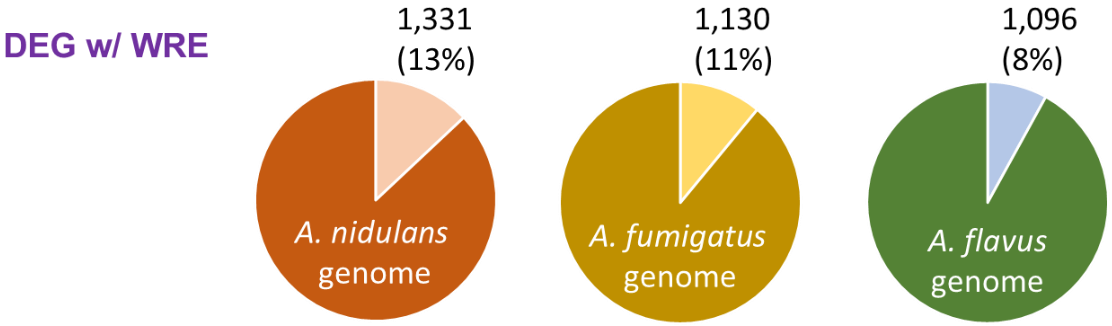


Fig 3-4 Identification of WetA binding motif

(A) The diagram of the recognized region of the customized anti-WetA polyclonal antibody. The anti-WetA recognized region overlaps with partial highly conserved Esc1/WetA-related domain near the WetA C' terminus. (B) Western blot analysis of the crude proteins extracted from *Ani*WT, Δ*AniwetA*, and C'*AniwetA* conidia using anti-WetA and anti-FLAG polyclonal antibodies. Only the C'*AniwetA* expresses WetA::3xFLAG and can be recognized by both anti-WetA and anti-FLAG antibodies. The result showed that the customized anti-WetA polyclonal antibody can specifically recognize conidial *Ani*WetA. (C) The predicted WetA response element (WRE).

To investigate the expression profile of *Ani*WetA target genes in conidia, data from the previous transcriptomic analysis was utilized. Total 1,331 *Ani*WetA-targeted genes were differentially regulated (Fig 3-5). The *Ani*WetA-targeted DEGs includes 3 G-protein-pathway associated genes, 17 conidial integrated associated genes, 21 putative kinase-encoded genes, 31 putative transcription factor encoded genes, 5 SMG backbone genes, and 17 asexual development associated genes (Table 3-5).

*Ani*WetA, *Afu*WetA, and *Afl*WetA show high similarity in their protein sequence and have conserved functions in conidia. We proposed that *Afu*WetA and *Afl*WetA may recognize similar DNA binding motif with *Ani*WetA. To test our hypothesis, we search the AniWetA binding motif in the *A. fumigatus* and *A. flavus*. Total 2,147 and 2,826 *Afu*WetA and *Afl*WetA targeted genes were identified, and 1,238 (58% of *Afu*WetA targeted genes, 22% of DEGs in Δ*AfuwetA* conidia) and 1,244 (44% of *Afu*WetA targeted genes, 22% of DEGs in Δ*AfuwetA* conidia) of them were differentially regulated in Δ*AfuwetA* and Δ*AflwetA* conidia, respectively.



	A. nidulans	*A. fumigatus*	*A. flavus*
Total Gene	**10,602**	**9,908**	**13,485**
DEG	5,706 (54%)	5,730 (58%)	5,755 (43%)
WRE	2,437 (23%)	1,859 (19%)	2,483 (18%)
DEG w/ WRE	1,331 (**13%**, 23%, 55%)	1,130 (**11%**, 20%, 61%)	1,096 (**8%**, 19%, 44%)

Fig 3-5 Summary of DEG, WRE, and DEG with WRE in *Aspergillus* Δ*wetA* conidia

The number and percentage of genes were differentially regulated in Δ*wetA* conidia (DEG), with down (blue), contains predicted WRE in the upstream 2kb regions (WRE), and DEG with WREs. The percentage is shown in black: the percentage of the total genome; The percentage is shown in red: the percentage of all DEGs. The percentage is shown in blue: the percentage of total genes with WRE.

Table 3-5 WetA targeted DEGs in Δ*AniwetA* conidia

G-protein pathway	*gprC, nopA, flbA*
Conidial integrity	*bglA, bglK, btgA, btgC, chsA, dewA, eglC, exgD, hpdA, orlA, treA,* AN0031, AN0499, AN1069, AN1837, AN5021, AN7869
Kinase	*aromA, atg1, clgA, ffkA, gin4, mst1, nimO, nimX, pho80, psk1, rio2, sD, sudD, tear, tinC,* AN0156, AN10551, AN1854, AN8886, AN9022, AN9500
TF	*creA, fhpA, flbC, napA, qutA, vosA, wetA,* AN0094, AN0388, AN0585, AN0817, AN0902, AN10491, AN10550, AN10600, AN10809, AN1500, AN2615, AN3391, AN3502, AN3769, AN4001, AN4133, AN4324, AN6790, AN7919, AN8111, AN8164, AN8355, AN8645, AN8949
SMG backbones	*apdA, micA, nisA,* AN0016, AN9129
Asexual development	*velB, mns1B, odeA, atg1, wetA, vosA, flbC, msd2, orlA, gprC, flbA, cnaB, chsA, dewA, esdC,* AN7683, AN4745

Although about 50% of the DEGs which are associated with 5,619 orthogroups contain WetA-binding motif in their upstream 2 kb regions in Δ*AniwetA*, Δ*AfuwetA*, and Δ*AflwetA* conidia, only 18 genes which contain WRE in the upstream 2kb regions were differentially regulated in all three species, including *vosA*, *wetA*, *flbC*, AN8121, AN11058, AN10265, AN9038, AN3706, AN5935, AN1528, AN1524, AN0315, AN5715, AN8763, AN8927, AN6048, AN3752, and AN2982 orthogroups (Table 3-6).

Table 3-6 Conserved and differential WetA-directly-regulated All_orthogroups

	Expression pattern	Orthogroups number	Expression pattern	Orthogroups number	Total
NUL	↓↓↓	13	↑↑↑	5	18
NU↔L	↓↓↑	1	↑↑↓	1	2
NL↔U	↓↑↓	4	↑↓↑	0	4
N↔UL	↓↑↑	0	↑↓↓	0	0
N↔U	↓↑—	5	↑↓—	2	7
N↔L	↓—↑	1	↑—↓	0	1
U↔L	—↓↑	1	—↑↓	1	2
NU	↓↓—	3	↑↑—	2	5
NL	↓—↓	4	↑—↑	0	4
UL	—↓↓	5	—↑↑	0	5
N	↓——	8	↑——	7	15
U	—↓—	10	—↑—	5	15
L	——↓	5	——↑	3	8
Mixed					3,033
TOTAL					3,119

Note: Expression pattern: The mRNA expression levels in Δ*AniwetA*, Δ*AfuwetA,* and Δ*AflwetA* conidia. ↑: upregulated in Δ*wetA* conidia; ↓: downregulated in Δ*wetA* conidia; —: not regulated in Δ*wetA* conidia. *NUL*: all up-/down-regulated in Δ*wetA* conidia; *NU↔L*: up-/down-regulated in Δ*AniwetA* and Δ*AfuwetA* conidia, and down-/up-regulated in Δ*AflwetA* conidia; *NL↔U*: up-/down-regulated in Δ*AniwetA* and Δ*AflwetA* conidia, and down-/up-regulated in Δ*AfuwetA* conidia; *UL↔N*: up-/down-regulated in Δ*AfuwetA* and Δ*AflwetA* conidia, and down-/up-regulated in Δ*AniwetA* conidia; *NU*: all up-/down-regulated in Δ*AniwetA* and Δ*AfuwetA* conidia, but not regulated in Δ*AflwetA* conidia; *NL*: all up-/down-regulated in Δ*AniwetA* and Δ*AflwetA* conidia, but not regulated in Δ*AfuwetA* conidia; *UL*: all up-/down-regulated in Δ*AfuwetA* and Δ*AflwetA* conidia, but not regulated in Δ*AniwetA* conidia; *N*: only regulated in Δ*AniwetA* conidia; *U*: only regulated in Δ*AfywetA* conidia; *L*: only regulated in Δ*AflwetA* conidia. *Mixed*: WetA showed mixed regulatory effects on orthologs in Δ*wetA* conidia.

Finally, we checked the expression levels of N-/U-/L-specific WetA targeted genes in Δ*wetA* conidia. Total 503, 327, 852 WetA targeted genes were differential regulated in Δ*AniwetA* (9% of *Ani*DEGs), Δ*AfuwetA* (6% of *Afu*DEGs), and Δ*AflwetA* (15% of *Afu*DEGs) conidia, respectively. Taken together, WetA regulates the *Aspergillus* conidial transcriptomes by both direct and indirect ways and controls species-specific GRNs to achieve conserved and diverged functions.

3-5 Discussion

While WetA is well known as the key regulator of many cellular and chemical development processes, including spore viability, wall integrity, and stress tolerance in Ascomycetes [7–23,30], the behind regulatory mechanism is still vague. In this study, we investigated the roles of the WetA-mediated GRNs in the model organism *A. nidulans*, the human pathogen *A. fumigatus*, and the aflatoxin producer *A. flavus*, and further identified the putative WetA binding motif in *A. nidulans*.

Previous studies suggest that the *Ani*WetA is required for activating a set of genes (Class B, Class C, and some Class D genes) whose products comprise or direct the assembly of the conidial wall layers and ensure proper cytoplasmic status [13,15,37]. We also reported that *Afl*WetA is involved in the regulation of the metabolic pathways of conidial contents and secondary metabolism which is possibly through controlling a group of transcription factors and signaling pathways [30]. Our RNA-seq results show that 54%, 58%, and 43% of *A. nidulans*, *A. fumigatus,* and *A. flavus* transcriptomes were differentially regulated in Δ*wetA* conidia respectively, suggest that the broad regulatory effect of WetA in *Aspergilli* conidia (Fig 3-2). Although the percentage of the *Afl*WetA-regulated genes of whole *A. flavus* genome is about 10~15% lower than the other two species, the total number of *Afl*WetA-regulated genes is almost the same with other two species (Fig 3-2). The *A. flavus* genome size (36.8 Mb) is significantly larger than the *A. nidulans* (30.1 Mb) and *A. fumigatus* (29.4 Mb) genomes and is highly similar to *A. oryzae* genome regarding both the genome size and the sequence similarity [71]. The previous comparative genomic study proposed that the smaller genome size of *A. nidulans* and *A. fumigatus* relative to *A. oryzae* is more likely due to the independent gene loss during evolution [72]. This hypothesis explains our findings here.

The WetA-mediated regulation is crucial for survival and therefore is conserved during evolution while about 3,000 genes were lost during evolution. Our orthologroup analysis shows that about among 9,876 WetA-regulated orthogroups, there are 5,315 WetA-regulated orthogroups (54%) exist in more than one *Aspergillus* species. Moreover, function analysis shows that WetA-regulated orthogroups shared in all three species are involved in stress response, sporulation, cell wall integrity, and carbohydrate metabolic process (Table S3-7), which match to the observed phenotypes in the three species. Taken together, our data support that the WetA-mediated regulation is functionally conserved in *Aspergilli*.

While *Ani*WetA, *Afu*WetA, and *Afu*WetA are functionally conserved in many aspects of development processes in conidia, the WetA regulatory roles are divergent in each species. Even WetA regulates a large number of common orthogroups in *Aspergilli*, only 8% WetA-regulated orthogroups were consistently all up-/ down-regulated in Δ*wetA* conidia in the three species (Table 3-2 and Table 3-3), suggests that even the WetA-mediated regulation is functionally conserved, the WetA-mediated GRNs are highly rewired. The *Aspergillus* asexual development serves as a good example to illustrate the divergent WetA-mediated GRNs of functionally conserved negative feedback regulation (Fig 3-3 and Table S3-11). Loss of *wetA* leads to increased level of the central regulator *brlA* in conidia and shutdown the asexual development. WetA-mediated repression of *brlA* is likely through controlling a wide spectrum of upstream regulators of *brlA* (Fig 3-3 and Table S3-11).

Although WetA shows broad regulatory effects in *Aspergillus* species, however, only several genes with WRE in their upstream regions were consistently down-/ up-regulated in Δ*wetA* conidia including *vosA* and *flbC* (Table 3-5), which suggests that these genes may play a crucial roles in survival and thus be conserved during the revolution. Both VosA and FlbC were reported as TFs in *A. nidulans* [73,74]. Loss of *vosA* causes some phenotypes like reduced trehalose amount common with loss of *wetA* [40], which suggests that the part of the WetA-mediated regulation may be conducted by regulating VosA. Previous studies show that *Ani*WetA contains *Ani*VosA binding motif in its upstream 2kb region [74] and was down-regulated in Δ*AnivosA* conidia (unpublished data), which indicates the cross feedback regulation of VosA and WetA. Moreover, when comparing VosA and WetA co-regulated genes in Δ*AniwetA* and Δ*AnivosA* conidia, about 95% WetA/VosA co-regulated genes were consistently up-/down-regulated (unpublished data). Taken together, it is possible that WetA/VosA mediated coregulation is through forming a complex to directly bind to downstream genes. *Ani*FlbC exists in the nuclei of hyphae and in developmental cells except conidia and serves a C_2H_2 TF regulating development in *A. nidulans* [73]. Overexpression of *AniflbC* leads to inhibition of hyphal growth, reduced conidiation, and activation of *AnibrlA*, *AniabaA*, and *AnivosA* [73], which is the same with the phenotypes of Δ*AniwetA* mutant. Although *Ani*FlbC is a TF and binds to *AnibrlA*, *AniabaA*, and *AnivosA* upstream regions, however, theres no *Ani*FlbC predicted binding site in the *AniwetA* upstream region [73]. Moreover, overexpression of *AniflbC* does not induce *AniwetA* mRNA expression [73]. When comparing the *AniflbC* and *AniwetA* expression patterns during development in WT, we can found that *AniflbC* decreases when *AniwetA* starts to express [73]. In sum, we propose that part of the WetA-mediated regulation is through directly binds to *flbC* upstream region and represses the *flbC* expression. Interestingly, VosA may also play a role in the WetA-mediated regulation of *flbC*, since *flbC* contains predicted *vosA* binding motif and is also upregulated in Δ*vosA* mutant (data not shown).

Our data shows that *Afl*WetA is more specified in the light-dependent regulation of asexual development. Both AniWetA and AfuWetA downregulates the blue light sensing regulators, *lreA,* and *lreB,* in Δ*AniwetA* and Δ*Afu*WetA conidia, but *Afl*WetA shows no effect on the blue light sensing regulators, *AfllreA* and *AfllreB,* mRNA levels (Fig 3-3 and Table S3-11). Instead, *Afl*WetA downregulates the red light sensing regulator *AflfphA* in Δ*AflwetA* conidia, while the expression mRNA levels of *AnifphA* is not affected and *AfufphA* ortholog is increased in Δ*AniwetA* and Δ*Afu*WetA conidia, respectively (Fig 3-3 and Table S3-11).

We further examine the WetA-mediated GRNs in other aspects based on the conserved functions characterized in previous studies. First, we checked the genes involved in conidial integrity. As we reported before, *Afl*WetA is involved in the regulation of metabolic processes of trehalose, chitin, β-(1,3)-glucan, α-(1,3)-glucan, melanin, and hydrophobin [30]. The mRNA levels of the genes associated with trehalose biosynthesis are all reduced in Δ*AniwetA*, Δ*Afu*WetA, and Δ*Afl*WetA conidia (Table S3-14). Similarly, the mRNA levels of almost all the genes associated with β-(1,3)-glucan biosynthesis increased in Δ*AniwetA*, Δ*Afu*WetA, and Δ*Afl*WetA conidia (Table S3-16). These results explain the dramatically reduced amount of trehalose and increased amount of β-(1,3)-glucan in Δ*wetA* conidia [8,30] and suggest the conserved WetA-mediated GRNs in trehalose and β-(1,3)-glucan biosynthesis. WetA's function is likely diverged in α-(1,3)-glucan metabolism. *Ani*WetA downregulates the α-(1,3)-glucan synthase *Aniags2* but upregulates all the genes associated with α-(1,3)-glucan synthase degradation Δ*AniwetA* conidia except AN1604 (Table S3-17). In contrast, *Afu*WetA upregulates all the α-(1,3)-glucan synthase *Afuags1*, *Afuags2*, and *Afuags3*, but has mixed effects on the genes associated with α-(1,3)-glucan synthase degradation in Δ*AfuwetA* conidia (Table S3-17). In Δ*AflwetA* conidia, *Afl*WetA shows mixed effects on both the genes associated with α-(1,3)-glucan biosynthesis and degradation (Table S3-17).

WetA is involved in the regulation of hydrophobin expression. All four reported hydrophobin encoded genes in *A. nidulans* were regulated in Δ*wetA* conidia, and only *AnidewA* was downregulated (Table S3-19). In *A. fumigatus*, all six hydrophobins encoded genes were upregulated in Δ*wetA* conidia (Table S3-19). In *A. flavus*, four of five reported hydrophobin encoded genes were down-regulated in Δ*wetA* conidia except for *AflrodA* (Table S3-19). Since the loss of *wetA* causes lower hydrophobicity of conidia, it is possible there are other unidentified hydrophobins were controlled by *Afu*WetA.

*Afu*WetA is more specialized in the regulation of melanin biosynthesis. The previous study shows that *wA*, which is the first regulator in the DHN-melanin synthesis pathway, is activated by WetA in *A. nidulans* conidia [13,75]. Our RNA-seq results show that both *AniwA* and *AflwA* were downregulated in Δ*AniwetA* and Δ*AflwetA* conidia (Table S3-18). Moreover, *Aflayg1*, the second gene in the DHN-melanin pathway [76], and *Aflbck1*, the MAP kinase of the brownish pigment pyomelanin biosynthesis pathway [77], are downregulated inΔ*AflwetA* conidia (Table S3-18). Surprisingly, although the Δ*AfuwetA* conidia are colorless, all the DEGs associated with both DHN-melanin and pyomelanin biosynthesis were upregulated in Δ*AflwetA* conidia (Table S3-18), suggests the melanin biosynthesis pathway may be more complicated in *A. fumigatus*.

We identified the putative *AniWetA* binding motif by performing ChIP-seq. The 11-aa-length predicted motif (CCGCAWRCGGM) is highly similar to the *S. cerevisiae* LEU3 motif. LEU3 is a C6 type transcription factor, which controls a group of leucine-specific genes [78]. About 23% genes in the *A. nidulans* contain the *Ani*WetA binding motif in their upstream 2 kb regions but only 55% of them are differentially regulated in Δ*AniwetA* conidia (Fig 3-5), suggests that the WetA-mediated regulation may have a temporal and spatial difference. Moreover, only 23% of the DEGs in Δ*AniwetA* conidia contain the *Ani*WetA binding motif (Fig 3-5), which suggests that *Ani*WetA may serve as a conserved regulatory hub which controls a group of regulators of various biological processes. *Ani*WetA directly targets and regulates genes associated with G-protein regulatory pathways, putative kinases, and putative TFs (Table 3-5). The RNA-seq results also show a lot more genes associated with G-protein regulatory pathways, putative kinases, and putative TFs were differentially regulated in Δ*wetA* conidia. Our data support that the WetA-mediated regulation is through the combination of hierarchical and cooperative modes to control its downstream genes. We also scanned the *A. fumigatus* and *A. flavus* genomes with *Ani*WetA binding motif, the result shows that 3,942 orthogroups containing the *Ani*WetA binding motif are differentially regulated in at least one species. WetA binding motif is somehow conserved in the three *Aspergillus* species and targets to similar set of genes to conserve the essential functions for survival.

In conclusion, we present a comprehensive comparative transcriptome study to illustrate the WetA-mediated regulation in *A. nidulans*, *A. fumigatus*, and *A. flavus* conidia. WetA is functionally conserved in the regulation of cellular and chemical development through directly and indirectly controlling the genetic rewired GRNs. Furthermore, we identified the *Ani*WetA binding motif which is likely also conserved in *A. fumigatus* and *A. flavus*.

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Table S3-1 *Aspergillus* strains and oligonucleotides used in this study.

STRAINS		
Name	GENOTYPE	SOURCE
NRRL3357	Wild-type	Fungal Genetic Stock Center
3357.5	*pyrG*$^{-}$	[79]
TMY1	Δ*AflwetA::AfupyrG*$^{+}$; *pyrG*$^{-}$	[30]
TMY2	Δ*AflwetA::AfupyrG*$^{+}$; *pyrG*$^{-}$*; wetA*	[30]
Af293	Wild-type	[80]
TSGw4	*AfupyrG1* Δ*AfuwetA*::*AnipyrG*$^{+}$	[8]
FGSC4	Wild-type	Fungal Genetic Stock Center
RJMP1.59	*pyrG89*; *pyroA4*	[44]
TMY3	*pyrG89*; *pyroA4*; Δ*AniwetA::AfupyrG*$^{+}$	This study
TMY4	*pyrG89*; *pyroA*::*AniwetA*(p)::*AniwetA*::FLAG$_{3X}$::*pyroA**; Δ*AniwetA::AfupyrG*$^{+}$	This study

*The 3/4 pyroA marker selects for the targeted integrationat the *pyroA* locus

OLIGONUCLEOTIDES		
Name	**SEQUENCE (5’→3’)**	**PURPOSE**
oMY-43	tagcgcattgttgcttaggg	5’ flanking of *AniwetA*
oMY-44	gccgttaccgacggatactc	3’ flanking of *AniwetA*
oMY-45	gtaatagactcagtggaccgggc	5’ nested of *AniwetA*
oMY-46	ctcctcctagaacccattatggc	3’ nested of *AniwetA*
oMY-47	gtgaagagcattgtttgaggcaggaagaggctgccagaagacctg	5' *AniwetA* with *AfupyrG* tail
oMY-48	agtgcctcctctcagacagaataggaggaagcttagatctgtggc	3' *AniwetA* with *AfupyrG* tail
oMY-25	gaccactcgttcaacaacgatg	5’ *AniwetA*
oMY-26	cgtactgcattaagtgcgg	3’ *AniwetA*
oMY-53	ccgaattcttgaagtattgattatgtaattatgc	5’ *AniwetA* with *Eco*RI
oMY-54	tagcggccgcgcagaggacagcctctaggg	3’ *AniwetA* with *Not*I

oJH-84	gctgaagtcatgatacaggccaaa	5’ *AfupyrG* marker
oJH-85	atcgtcgggaggtattgtcgtcac	3’ *AfupyrG* marker

Table S3-2 Top enriched GO categories of differential expressed genes in the Δ*wetA* conidia.

GO Category	# of Genes	% of Genes in Category
A. nidulans		
oxidoreductase activity	660	57.5
organonitrogen compound metabolic process	540	58.8
amide biosynthetic process	183	62.7
extracellular region	155	66.5
peptide biosynthetic process	153	64.8
nucleolus	141	70.1
secondary metabolite biosynthetic process	137	65.6
ribonucleoprotein complex biogenesis	135	69.9
ribosome	132	69.5
ribosome biogenesis	129	75.9
translation	129	65.2
ncRNA processing	122	65.2
structural constituent of ribosome	98	73.1
rRNA metabolic process	87	75
rRNA processing	87	75
preribosome	68	77.3
external encapsulating structure	66	73.3
cell wall	65	74.7
fungal-type cell wall	62	74.7
cell surface	60	78.9
ribosomal small subunit biogenesis	48	80
maturation of SSU-rRNA	43	84.3
cytosolic ribosome	43	86
ribosomal large subunit biogenesis	40	81.6
90S preribosome	40	83.3
maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	38	86.4
maturation of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	33	82.5
maturation of 5.8S rRNA	33	82.5
A. fumigatus		

material entity	3224	62.2
specimen	3219	62.2
oxidoreductase activity	577	64.3
oxidation-reduction process	463	64.8
carbohydrate metabolic process	316	67.4
secondary metabolic process	170	79.4
extracellular region	162	71.4
secondary metabolite biosynthetic process	121	79.1
fungal-type cell wall	62	78.5
cell wall macromolecule metabolic process	60	80
toxin metabolic process	53	82.8
toxin biosynthetic process	53	82.8
nucleoside triphosphate metabolic process	49	81.7
phenol-containing compound biosynthetic process	36	87.8
respiratory chain complex	26	96.3
respiratory chain	26	96.3
mitochondrial respiratory chain	25	96.2
A. flavus		
catalytic activity	1967	43.6
single-organism metabolic process	1028	43.4
oxidoreductase activity	703	49
metal ion binding	573	44.1
oxidation-reduction process	515	47.7
intrinsic component of membrane	434	46.5
integral component of membrane	429	46.3
transmembrane transport	379	46.4
obsolete electron transport	266	49.2
cofactor binding	247	48.8
carbohydrate metabolic process	226	47.4
cell periphery	217	50.7
coenzyme binding	188	48.6
plasma membrane	162	50.8
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	131	50.6

iron ion binding	128	51.8
monooxygenase activity	120	52.9
tetrapyrrole binding	112	49.8
heme binding	112	49.8
cytosol	110	55.3
cell wall organization or biogenesis	79	62.2
hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds	77	52.7
polysaccharide metabolic process	58	55.2
intrinsic component of plasma membrane	56	55.4
cytosolic part	52	59.8
cytosolic ribosome	50	61.7
external encapsulating structure	46	67.6
cell wall	43	70.5
carbohydrate catabolic process	42	58.3
pyridine nucleotide metabolic process	39	58.2
fungal-type cell wall	39	72.2
transferase activity, transferring nitrogenous groups	39	60
cell wall macromolecule metabolic process	30	81.1
aminoglycan metabolic process	28	65.1
cytosolic large ribosomal subunit	27	67.5
cell wall macromolecule catabolic process	12	85.7

Table S3-3 Top 100 genes showing decreased mRNA levels in the Δ*AniwetA* conidia.

GENE ID	Log_2 Fold Change	Description
AN6403	-11.38	Ortholog of Aspergillus clavatus NRRL 1 : ACLA_055850 and Aspergillus aculeatus ATCC16872 : Aacu16872_061969
AN10028	-11.36	Putative cytochrome P450
AN3783	-11.06	protein of unknown function
AN12038	-11.00	protein of unknown function
AN1616	-11.00	Ortholog of A. nidulans FGSC A4 : AN5887, AN4831, A. fumigatus Af293 : Afu2g11250, A. niger CBS 513.88 : An08g09700 and A. oryzae RIB40 : AO090026000525, AO090005000474, AO090113000193
AN1937	-10.99	Regulatory protein involved in conidial development
AN8335	-10.94	Ortholog of A. oryzae RIB40 : AO090038000479, AO090009000246, Aspergillus flavus NRRL 3357 : AFL2T_10422, Aspergillus versicolor : Aspve1_0046662 and Aspergillus sydowii : Aspsy1_0136121
AN3782	-10.90	Has domain(s) with predicted FAD binding, oleate hydratase activity and role in fatty acid metabolic process
AN10040	-10.80	Ortholog of A. fumigatus Af293 : Afu5g09180, A. niger CBS 513.88 : An07g03930, A. oryzae RIB40 : AO090020000514, Aspergillus wentii : Aspwe1_0035291 and Aspergillus terreus NIH2624 : ATET_06551
AN3966	-10.79	protein of unknown function
AN10041	-10.73	Ortholog of A. niger CBS 513.88 : An07g01780, Neosartorya fischeri NRRL 181 : NFIA_113800, Aspergillus wentii : Aspwe1_0281884 and Aspergillus versicolor : Aspve1_0082092
AN11624	-10.60	protein of unknown function
AN5371	-10.52	Ortholog of A. nidulans FGSC A4 : AN6727, AN0386, A. fumigatus Af293 : Afu1g01610, Afu4g01060, Afu6g03180, Afu7g05880 and A. niger CBS 513.88 : An04g08650, An07g02000
AN5325	-10.51	protein of unknown function
AN11574	-10.48	protein of unknown function
AN3079	-10.47	Secreted thaumatin-like protein

AN6963	-10.40	Ortholog(s) have flavin-linked sulfhydryl oxidase activity and role in oxidation-reduction process
AN3968	-10.37	protein of unknown function
AN3485	-10.13	Ortholog of Aspergillus acidus : Aspfo1_0060189
AN12115	-10.09	protein of unknown function
AN1605	-9.99	Ortholog of Aspergillus sydowii : Aspsy1_0679421
AN3243	-9.98	Ortholog of A. niger CBS 513.88 : An11g08150, Neosartorya fischeri NRRL 181 : NFIA_040880, Aspergillus wentii : Aspwe1_0036368 and Aspergillus versicolor : Aspve1_0033884
AN7278	-9.70	Putative glutamate decarboxylase with a predicted role in the 4-aminobutyrate (GABA) shunt
AN8382	-9.69	protein of unknown function
AN2838	-9.69	Ortholog of Aspergillus glaucus : Aspgl1_0163409, Neosartorya fischeri NRRL 181 : NFIA_097910, Aspergillus clavatus NRRL 1 : ACLA_058550 and Aspergillus zonatus : Aspzo1_0133076
AN11337	-9.60	Ortholog of A. niger CBS 513.88 : An18g01250, Aspergillus tubingensis : Asptu1_0114551, Aspergillus brasiliensis : Aspbr1_0322983 and Aspergillus kawachii : Aspka1_0173401
AN9194	-9.57	Transcript enriched in dormant conidia
AN10984	-9.51	protein of unknown function
AN8581	-9.50	Putative laccase related protein
AN8006	-9.48	Hydrophobin, protein of the conidium wall responsible for hydrophobicity of conidium surface
AN11917	-9.45	Ortholog(s) have fungal-type vacuole localization
AN5355	-9.37	Has domain(s) with predicted oxidoreductase activity, transferase activity, transferring acyl groups other than amino-acyl groups, zinc ion binding activity and role in oxidation-reduction process
AN3781	-9.34	Has domain(s) with predicted inorganic phosphate transmembrane transporter activity, role in phosphate ion transport and membrane localization
AN2611	-9.31	Has domain(s) with predicted role in isoprenoid biosynthetic process
AN3967	-9.25	protein of unknown function
AN2471	-9.22	Ortholog of A. niger CBS 513.88 : An02g07790, Neosartorya fischeri NRRL 181 : NFIA_065240, Aspergillus clavatus NRRL 1 : ACLA_039940, ACLA_078340 and Aspergillus niger ATCC

		1015 : 144091-mRNA
AN0396	-9.09	Has domain(s) with predicted protein dimerization activity
AN10494	-9.03	Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN7395	-8.96	Ortholog of A. nidulans FGSC A4 : AN5639, AN2587, AN9444, A. fumigatus Af293 : Afu5g00840, A. niger CBS 513.88 : An03g01000 and A. oryzae RIB40 : AO090102000018
AN10507	-8.90	Ortholog(s) have identical protein binding, mRNA binding, unfolded protein binding activity
AN10499	-8.86	Ortholog(s) have cytosol, nucleus localization
AN8921	-8.81	Has domain(s) with predicted 3-hydroxyacyl-CoA dehydrogenase activity, coenzyme binding, oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor activity
AN11477	-8.63	Ortholog of Aspergillus sydowii : Aspsy1_0137382 and Aspergillus aculeatus ATCC16872 : Aacu16872_040719
AN4177	-8.55	Has domain(s) with predicted catalytic activity, coenzyme binding activity and role in cellular metabolic process
AN5411	-8.54	Has domain(s) with predicted catalytic activity, metal ion binding, phosphoric diester hydrolase activity
AN3539	-8.45	Ortholog of A. nidulans FGSC A4 : AN3540, A. fumigatus Af293 : Afu8g00120, A. oryzae RIB40 : AO090038000188, AO090026000095 and Neosartorya fischeri NRRL 181 : NFIA_032270
AN0216	-8.40	Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN10233	-8.38	Has domain(s) with predicted NADP binding, coenzyme binding, oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor, phosphogluconate dehydrogenase (decarboxylating) activity
AN6460	-8.38	Ortholog of A. nidulans FGSC A4 : AN12398, A. oryzae RIB40 : AO090023000067, AO090010000683, Aspergillus wentii : Aspwe1_0075588 and Aspergillus versicolor : Aspve1_0086685
AN8607	-8.37	Ortholog of A. nidulans FGSC A4 : AN1754, A. fumigatus Af293 : Afu3g02050, A. niger CBS 513.88 : An11g06690, An18g01930 and A. oryzae RIB40 : AO090001000144
AN6457	-8.31	Has domain(s) with predicted peroxidase activity

AN1740	-8.29	protein of unknown function
AN7053	-8.29	Ortholog of Aspergillus versicolor : Aspve1_0054863
AN2720	-8.24	Has domain(s) with predicted catalytic activity
AN8553	-8.24	Putative catalase with a predicted role in gluconic acid and gluconate metabolism
AN1314	-8.15	Ortholog of A. niger CBS 513.88 : An11g08070, An09g03760, Neosartorya fischeri NRRL 181 : NFIA_030520, Aspergillus versicolor : Aspve1_0031176 and Aspergillus niger ATCC 1015 : 39297-mRNA, 43311-mRNA
AN1614	-8.12	Has domain(s) with predicted methyltransferase activity and role in metabolic process
AN5015	-8.11	Putative conidiation gene
AN5553	-8.04	Putative cytochrome P450
AN8640	-7.93	Ortholog of N. crassa conF, light-induced transcript expressed during conidiation in N. crassa
AN5089	-7.90	Ortholog of A. oryzae RIB40 : AO090103000089, Aspergillus flavus NRRL 3357 : AFL2T_12280, Aspergillus terreus NIH2624 : ATET_03032 and Aspergillus aculeatus ATCC16872 : Aacu16872_034601
AN0391	-7.89	Ortholog of A. nidulans FGSC A4 : AN7222, A. fumigatus Af293 : Afu1g01490, Afu2g01760, A. niger CBS 513.88 : An03g06670, An15g07300 and A. oryzae RIB40 : AO090010000045/aoiD, AO090102000259
AN1040	-7.81	Ortholog of A. oryzae RIB40 : AO090038000523, Aspergillus flavus NRRL 3357 : AFL2T_07855 and Aspergillus sydowii : Aspsy1_0160459, Aspsy1_0165518
AN7990	-7.74	Ortholog of A. niger CBS 513.88 : An11g06780, Aspergillus wentii : Aspwe1_0176973, Aspergillus sydowii : Aspsy1_0047960 and Aspergillus terreus NIH2624 : ATET_06661
AN6972	-7.73	protein of unknown function
AN11945	-7.68	Ortholog of A. oryzae RIB40 : AO090001000155
AN1603	-7.68	Ortholog of A. fumigatus Af293 : Afu8g05985, Neosartorya fischeri NRRL 181 : NFIA_098760, NFIA_099140, Aspergillus wentii : Aspwe1_0689083 and Aspergillus versicolor : Aspve1_0037404

AN3535	-7.67	Has domain(s) with predicted ATP binding activity
AN8398	-7.61	Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN3474	-7.59	Ortholog of A. oryzae RIB40 : AO090003001515, Aspergillus flavus NRRL 3357 : AFL2T_01577 and Aspergillus wentii : Aspwe1_0071034
AN5409	-7.51	Has domain(s) with predicted catalytic activity, coenzyme binding activity and role in cellular metabolic process
AN11122	-7.50	Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AN1604	-7.48	Putative alpha-1,3-glucanase
AN8776	-7.47	Ortholog of A. niger CBS 513.88 : An08g03560, A. oryzae RIB40 : AO090038000281, Aspergillus wentii : Aspwe1_0508022, Aspergillus sydowii : Aspsy1_0035009 and Aspergillus terreus NIH2624 : ATET_00334
AN2351	-7.45	Putative zinc-containing alcohol dehydrogenase
AN7988	-7.40	protein of unknown function
AN11533	-7.40	Ortholog of A. fumigatus Af293 : Afu8g06974, A. niger CBS 513.88 : An01g03010, Neosartorya fischeri NRRL 181 : NFIA_099710 and Aspergillus kawachii : Aspka1_0178966
AN6962	-7.39	Ortholog of the non-ribosomal peptide synthetase (NRPS) of A. fumigatus, nrps14/Afu8g00540
AN11916	-7.35	Has domain(s) with predicted ATP binding activity
AN8122	-7.34	Ortholog(s) have role in cellular response to drug, hexose transport, pathogenesis
AN5654	-7.33	protein of unknown function
AN0397	-7.32	protein of unknown function
AN11000	-7.30	Ortholog of Aspergillus flavus NRRL 3357 : AFL2T_11759, Neosartorya fischeri NRRL 181 : NFIA_094100, Aspergillus versicolor : Aspve1_0085617 and Aspergillus clavatus NRRL 1 : ACLA_044880
AN2472	-7.25	Has domain(s) with predicted catalytic activity
AN3484	-7.15	Ortholog of Aspergillus acidus : Aspfo1_0160947
AN5326	-7.14	protein of unknown function
AN8358	-7.08	Putative cytochrome P450
AN5938	-7.08	Ortholog(s) have cytosol, nucleus localization

AN0400	-7.04	Ortholog of A. fumigatus Af293 : Afu4g09610, A. niger CBS 513.88 : An04g07500, A. oryzae RIB40 : AO090003001281, Neosartorya fischeri NRRL 181 : NFIA_106400 and Aspergillus wentii : Aspwe1_0022929
AN4127	-7.04	Ortholog(s) have intracellular localization
AN3330	-7.00	protein of unknown function
AN2558	-7.00	Ortholog of A. fumigatus Af293 : Afu3g15050, A. niger CBS 513.88 : An14g03430, A. oryzae RIB40 : AO090701000428, Aspergillus wentii : Aspwe1_0175480 and Aspergillus sydowii : Aspsy1_0055978
AN7102	-6.96	Ortholog of A. fumigatus Af293 : Afu4g03830, A. niger CBS 513.88 : An14g01068, A. oryzae RIB40 : AO090011000343, Aspergillus wentii : Aspwe1_0030332 and Aspergillus sydowii : Aspsy1_0060599
AN4627	-6.96	protein of unknown function
AN11946	-6.93	protein of unknown function
AN6719	-6.92	Ortholog of A. fumigatus Af293 : Afu7g05770, A. oryzae RIB40 : AO090005000440, Aspergillus wentii : Aspwe1_0167865, Aspergillus sydowii : Aspsy1_0049271 and Aspergillus terreus NIH2624 : ATET_06356
AN2610	-6.89	Putative cytochrome P450
AN3537	-6.89	Has domain(s) with predicted O-methyltransferase activity
AN8774	-6.87	Transcript induced in response to calcium dichloride in a CrzA-dependent manner
AN5709	-6.86	Ortholog of A. fumigatus Af293 : Afu1g06670, A. niger CBS 513.88 : An18g05730, A. oryzae RIB40 : AO090001000467, Aspergillus wentii : Aspwe1_0038290 and Aspergillus sydowii : Aspsy1_0056984

Table S3-4 Top 100 genes showing increased mRNA levels in the Δ*AniwetA* conidia.

GENE ID	Log_2 Fold Change	Description
AN8656	9.32	Has domain(s) with predicted 2 iron, 2 sulfur cluster binding, iron ion binding, oxidoreductase activity, oxidoreductase activity and acting on paired donors, more
AN3331	9.27	Has domain(s) with predicted catalytic activity, metal ion binding, phosphoric diester hydrolase activity
AN6383	9.17	Ortholog of A. fumigatus Af293 : Afu5g00450, Afu6g10780, A. niger CBS 513.88 : An03g05860, A. oryzae RIB40 : AO090003001528, AO090023001010 and Aspergillus wentii : Aspwe1_0052273, Aspwe1_0067889
AN3882	9.01	Has domain(s) with predicted cysteine-type endopeptidase activity and role in proteolysis
AN0787	9.00	Putative mannosyl-oligosaccharide 1,2-alpha-mannosidase with a predicted role in mannose polymer metabolism
AN11510	8.94	Has domain(s) with predicted role in defense response
AN9273	8.93	Ortholog of Aspergillus flavus NRRL 3357 : AFL2T_00195, Aspergillus wentii : Aspwe1_0174848, Aspergillus versicolor : Aspve1_0083996 and Aspergillus sydowii : Aspsy1_0900274
AN6382	8.89	Has domain(s) with predicted phosphoric diester hydrolase activity and role in lipid metabolic process
AN5611	8.83	Putative carbonic anhydrase
AN3858	8.59	Possible pseudogene
AN3264	8.51	Putative xylose transporter
AN0638	8.47	Ortholog of A. nidulans FGSC A4 : AN6319, A. fumigatus Af293 : Afu1g16870, A. oryzae RIB40 : AO090005000988, Aspergillus wentii : Aspwe1_0038696 and Aspergillus sydowii : Aspsy1_0129541, Aspsy1_0995740
AN1618	8.41	protein of unknown function
AN0760	8.40	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN5505	8.32	Ortholog of Aspergillus versicolor : Aspve1_0052822 and Aspergillus sydowii : Aspsy1_0046382
AN4806	8.26	Ortholog of A. fumigatus Af293 : Afu3g07000, A. niger CBS

		513.88 : An02g13810, Aspergillus wentii : Aspwe1_0042056, Aspergillus sydowii : Aspsy1_0043180 and Aspergillus terreus NIH2624 : ATET_08722
AN2705	8.25	Ortholog(s) have catalytic activity and role in ascospore wall assembly
AN4871	8.23	Class V chitinase
AN7975	8.21	Ortholog of Aspergillus kawachii : Aspka1_0183274
AN10995	8.17	protein of unknown function
AN6472	8.17	Putative endo-mannanase GH76 family protein
AN6929	8.15	Putative alpha-L-rhamnosidase
AN3555	8.15	Small heat-shock protein
AN2530	8.12	Heat shock protein 30
AN0195	8.08	Ortholog of A. nidulans FGSC A4 : AN8020, A. fumigatus Af293 : Afu5g02380, A. niger CBS 513.88 : An03g04900, An02g10420 and A. oryzae RIB40 : AO090001000067, AO090102000350
AN6658	7.97	Has domain(s) with predicted oxidoreductase activity and role in oxidation-reduction process
AN4131	7.97	Has domain(s) with predicted solute:proton antiporter activity, role in cation transport, transmembrane transport and integral component of membrane localization
AN8977	7.94	Putative gluconolactonase with a predicted role in gluconic acid and gluconate metabolism
AN4004	7.84	Ortholog of A. nidulans FGSC A4 : AN8977/alcP, A. fumigatus Af293 : Afu1g08990, Afu1g11330 and A. niger CBS 513.88 : An05g02030, An10g00900, An16g06620
AN9324	7.81	Has domain(s) with predicted carbon-nitrogen ligase activity, with glutamine as amido-N-donor activity
AN0499	7.81	Has domain(s) with predicted chitin binding activity, role in chitin metabolic process and extracellular region localization
AN8322	7.76	Has domain(s) with predicted ADP binding, microtubule motor activity, nucleoside-triphosphatase activity, nucleotide binding activity and kinesin complex localization
AN9007	7.75	Putative cytochrome P450
AN3303	7.73	Ortholog of A. nidulans FGSC A4 : AN3346, AN3348, AN2044, AN8727 and A. fumigatus Af293 : Afu4g10080, Afu7g00420,

		Afu8g06100, Afu4g01242
AN5506	7.73	protein of unknown function
AN2895	7.73	Has domain(s) with predicted ADP binding activity
AN7670	7.66	Ortholog of A. fumigatus Af293 : Afu2g01430, Aspergillus wentii : Aspwe1_0114055, Aspergillus versicolor : Aspve1_0030702 and Aspergillus clavatus NRRL 1 : ACLA_093480
AN5407	7.61	Has domain(s) with predicted intracellular localization
AN10385	7.60	protein of unknown function
AN3304	7.59	Putative GABA transporter
AN1898	7.56	Ortholog(s) have role in melanin biosynthetic process from tyrosine, tyrosine catabolic process and cytoplasm localization
AN2921	7.56	Ortholog of A. nidulans FGSC A4 : AN4177, AN12488, A. fumigatus Af293 : Afu8g00600, A. niger CBS 513.88 : An03g00920, An02g01430, A. oryzae RIB40 : AO090010000696 and Aspergillus wentii : Aspwe1_0029035
AN7177	7.55	Ortholog of A. fumigatus Af293 : Afu4g03322, A. niger CBS 513.88 : An14g01990, A. oryzae RIB40 : AO090011000139, Aspergillus sydowii : Aspsy1_0052594 and Aspergillus terreus NIH2624 : ATET_02136
AN9137	7.52	Ortholog of A. fumigatus Af293 : Afu7g01695, Aspergillus clavatus NRRL 1 : ACLA_065860, Aspergillus zonatus : Aspzo1_0012532 and Aspergillus sydowii : Aspsy1_0093862
AN6579	7.51	Ortholog of A. fumigatus Af293 : Afu6g04470, A. niger CBS 513.88 : An15g01120, A. oryzae RIB40 : AO090701000109, Aspergillus wentii : Aspwe1_0179993 and Aspergillus terreus NIH2624 : ATET_07015
AN7716	7.48	Ortholog of A. fumigatus Af293 : Afu5g08180, A. oryzae RIB40 : AO090701000735, Aspergillus wentii : Aspwe1_0167587, Aspergillus sydowii : Aspsy1_0046259 and Aspergillus terreus NIH2624 : ATET_08308
AN6097	7.48	protein of unknown function
AN1197	7.44	Has domain(s) with predicted carbohydrate binding, catalytic activity and role in carbohydrate metabolic process
AN7200	7.40	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization

AN6088	7.39	Predicted metal ion transmembrane transporter
AN11851	7.39	protein of unknown function
AN10401	7.38	Ortholog of A. nidulans FGSC A4 : AN6780, A. fumigatus Af293 : Afu7g01210, Aspergillus sydowii : Aspsy1_0029236, Aspsy1_0043488 and Aspergillus terreus NIH2624 : ATET_08673
AN8981	7.33	Protein with homology to GPR1/FUN34/YaaH family members
AN6697	7.30	Putative Sun-family protein
AN10473	7.28	protein of unknown function
AN11641	7.26	Ortholog of Aspergillus versicolor : Aspve1_0117193
AN3325	7.25	protein of unknown function
AN7823	7.19	Putative peroxidase
AN6648	7.17	protein of unknown function
AN4109	7.17	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN7131	7.16	Putative cytochrome P450
AN6273	7.16	Ortholog(s) have intracellular localization
AN1646	7.14	Ortholog of Aspergillus versicolor : Aspve1_0064838 and Aspergillus sydowii : Aspsy1_0059989
AN12034	7.12	protein of unknown function
AN8617	7.10	protein of unknown function
AN9171	7.09	protein of unknown function
AN8083	7.06	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN8803	7.02	Hydrophobin
AN6960	7.00	Has domain(s) with predicted metal ion transmembrane transporter activity, role in metal ion transport, transmembrane transport and membrane localization
AN11193	6.99	Has domain(s) with predicted oxygen transporter activity
AN10493	6.98	Has domain(s) with predicted 2 iron, 2 sulfur cluster binding, oxidoreductase activity and role in oxidation-reduction process
AN4809	6.98	Putative glutaminase A with a predicted role in glutamate and glutamine metabolism
AN4374	6.97	Ortholog of Aspergillus glaucus : Aspgl1_0040120, Aspergillus versicolor : Aspve1_0054084 and Aspergillus sydowii : Aspsy1_0062289

AN11597	6.97	Ortholog of A. fumigatus Af293 : Afu5g03300, A. niger CBS 513.88 : An09g05625, Neosartorya fischeri NRRL 181 : NFIA_038810, Aspergillus wentii : Aspwe1_0114897 and Aspergillus versicolor : Aspve1_0203178
AN3053	6.96	Putative transglycosidase with a predicted role in glucan processing
AN5474	6.96	protein of unknown function
AN6940	6.95	Has domain(s) with predicted metal ion transmembrane transporter activity, role in metal ion transport, transmembrane transport and membrane localization
AN0788	6.95	Ortholog of A. niger CBS 513.88 : An09g02430, An03g05960, An09g00710, An09g00820, An09g01710 and A. oryzae RIB40 : AO090005000954, AO090003000250, AO090012000369, AO090010000570, AO090023000882, AO090120000022, AO090012000900
AN8979	6.94	Alcohol dehydrogenase with a role in two-carbon compound metabolism
AN4336	6.93	Putative L-arabinitol 4-dehydrogenase with a predicted role in L-arabinose/arabitol and D-xylose/D,L-xylulose/xylitol metabolism
AN0791	6.89	protein of unknown function
AN7892	6.89	Small heat-shock protein
AN8514	6.88	Asterriquinone prenyltransferase
AN8513	6.88	Putative single-module nonribosomal peptide synthetase (NRPS)
AN7858	6.87	Has domain(s) with predicted aspartic-type endopeptidase activity and role in proteolysis
AN9323	6.87	Ortholog of A. niger CBS 513.88 : An02g00370, An06g00670, A. oryzae RIB40 : AO090102000655, Aspergillus wentii : Aspwe1_0024055, Aspwe1_0181691 and Aspergillus versicolor : Aspve1_0090061, Aspve1_0142746
AN10482	6.86	Putative beta-glucosidase
AN2355	6.86	Has domain(s) with predicted role in response to stress and integral component of membrane localization
AN11966	6.85	protein of unknown function
AN6928	6.84	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization

AN5781	6.84	Putative 30 kilodalton heat shock protein
AN1897	6.83	Homogentisate 1,2-dioxygenase, enzyme in phenylalanine catabolism
AN1731	6.82	Putative proline dehydrogenase with a predicted role in proline metabolism
AN10238	6.81	Putative GNAT-type acetyltransferase
AN0374	6.80	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AN3265	6.80	Arrestin domains and PY motif-containing protein with homology to Saccharomyces cerevisiae Rod1p and Rog3p proteins
AN12440	6.79	Has domain(s) with predicted catalytic activity, phosphopantetheine binding, transferase activity and role in metabolic process
AN4268	6.77	Ortholog(s) have nitronate monooxygenase activity and role in denitrification pathway, detoxification of nitrogen compound
AN11923	6.77	protein of unknown function
AN12330	6.77	protein of unknown function

Table S3-5 Top 100 genes showing decreased mRNA levels in the Δ*AfuwetA* conidia.

GENE ID	Log_2 Fold Change	Description
AFU5G06830	-13.96	Has domain(s) with predicted RNA binding, ribonuclease III activity and role in RNA processing
AFU7G00260	-13.69	Has domain(s) with predicted intramolecular transferase activity and role in hopanoid biosynthetic process
AFU5G01260	-13.60	Ortholog of A. oryzae RIB40 : AO090138000080, Aspergillus flavus NRRL 3357 : AFL2T_08758, Neosartorya fischeri NRRL 181 : NFIA_040860 and Aspergillus fumigatus A1163 : AFUB_049760
AFU7G04920	-13.34	protein of unknown function
AFU4G13230	-12.90	Ortholog of A. nidulans WetA, a developmental regulatory protein involved in conidial development and activator of conidium-specific gene expression
AFU7G00270	-12.61	Ortholog(s) have role in austinol biosynthetic process, dehydroaustinol biosynthetic process
AFU2G14330	-12.44	Ortholog of Aspergillus fumigatus A1163 : AFUB_029950
AFU6G09570	-12.33	Ortholog of A. oryzae RIB40 : AO090005000031, Aspergillus glaucus : Aspgl1_0049355, Aspergillus flavus NRRL 3357 : AFL2T_00053 and Aspergillus wentii : Aspwe1_0032683
AFU7G08580	-12.23	Has domain(s) with predicted GTP binding, GTPase activity
AFU1G17060	-12.15	Ortholog(s) have nucleus localization
AFU6G03285	-12.12	protein of unknown function
AFU8G05770	-12.06	Ortholog of Aspergillus acidus : Aspfo1_0053584 and Aspergillus fumigatus A1163 : AFUB_081780
AFU8G01330	-12.04	Ortholog of Neosartorya fischeri NRRL 181 : NFIA_094800 and Aspergillus fumigatus A1163 : AFUB_085280
AFU3G03040	-11.93	Ortholog of A. oryzae RIB40 : AO090010000310, Aspergillus wentii : Aspwe1_0294100, Aspwe1_0531408, Aspergillus niger ATCC 1015 : 53802-mRNA and Aspergillus terreus NIH2624 : ATET_10090
AFU6G03210	-11.75	protein of unknown function
AFU4G00180	-11.69	Fatty acid 8,11-diol synthase
AFU1G01490	-11.59	Ortholog of A. nidulans FGSC A4 : AN0391, AN7222, A.

		fumigatus Af293 : Afu2g01760, A. niger CBS 513.88 : An03g06670, An15g07300 and A. oryzae RIB40 : AO090010000045/aoiD, AO090102000259
AFU4G00730	-11.52	Ortholog of A. nidulans FGSC A4 : AN8638/cetJ, A. fumigatus Af293 : Afu2g14320, Afu3g13130, Afu8g01530, A. niger CBS 513.88 : An12g10710, An01g01230 and A. oryzae RIB40 : AO090113000154
AFU3G13750	-11.52	Ortholog of A. oryzae RIB40 : AO090010000138, Neosartorya fischeri NRRL 181 : NFIA_063520, Aspergillus wentii : Aspwe1_0044901 and Aspergillus fumigatus A1163 : AFUB_035440
AFU8G05780	-11.51	Ortholog of Aspergillus fumigatus A1163 : AFUB_081770 and Aspergillus zonatus : Aspzo1_1313164
AFU4G00740	-11.47	Protein of unknown function identified by mass spectrometry, abundant in conidia
AFU4G14630	-11.42	Has domain(s) with predicted UDP-N-acetylmuramate dehydrogenase activity, flavin adenine dinucleotide binding activity and role in oxidation-reduction process
AFU3G03580	-11.35	Has domain(s) with predicted transferase activity, transferring acyl groups other than amino-acyl groups activity
AFU1G01175	-11.17	Has domain(s) with predicted role in cell redox homeostasis
AFU1G01500	-11.12	Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AFU3G00500	-11.08	Ortholog of A. nidulans FGSC A4 : AN2488, A. fumigatus Af293 : Afu4g03010, A. niger CBS 513.88 : An14g03080, A. oryzae RIB40 : AO090012000174 and Aspergillus wentii : Aspwe1_0176515, Aspwe1_0622364
AFU3G00605	-11.08	protein of unknown function
AFU7G04950	-10.88	Has domain(s) with predicted triglyceride lipase activity and role in lipid metabolic process
AFU5G09180	-10.87	Ortholog(s) have extracellular region localization
AFU8G06510	-10.86	Ortholog of A. oryzae RIB40 : AO090103000096, Aspergillus flavus NRRL 3357 : AFL2T_12273, Aspergillus niger ATCC 1015 : 127757-mRNA and Aspergillus terreus NIH2624 : ATET_04327
AFU7G04930	-10.85	Putative alkaline serine protease

AFU4G00450	-10.81	Ortholog of Aspergillus fumigatus A1163 : AFUB_100990
AFU6G00260	-10.80	Has domain(s) with predicted phosphatidylserine decarboxylase activity and role in phospholipid biosynthetic process
AFU5G00660	-10.64	Ortholog of Aspergillus fumigatus A1163 : AFUB_049130
AFU2G00440	-10.56	protein of unknown function
AFU6G11670	-10.46	Ortholog of A. nidulans FGSC A4 : AN12093, A. niger CBS 513.88 : An01g11550, Neosartorya fischeri NRRL 181 : NFIA_057330, Aspergillus wentii : Aspwe1_0039637 and Aspergillus clavatus NRRL 1 : ACLA_085310
AFU1G01170	-10.43	Ortholog of Aspergillus fumigatus A1163 : AFUB_079640
AFU7G08575	-10.37	Hsp70 family chaperone
AFU5G14800	-10.25	Lactate dehydrogenase
AFU1G01980	-10.22	Ortholog(s) have role in hyphal growth, response to cold, response to heat, response to oxidative stress, response to salt stress, sporocarp development involved in sexual reproduction
AFU5G01340	-10.21	Putative phospholipase B
AFU5G06910	-10.21	Has domain(s) with predicted carbon-sulfur lyase activity and role in metabolic process
AFU3G03570	-10.20	Has domain(s) with predicted FAD binding, oleate hydratase activity and role in fatty acid metabolic process
AFU1G17670	-10.08	Has domain(s) with predicted FAD binding, oxidoreductase activity and role in metabolic process
AFU7G05050	-10.05	Ortholog of A. niger CBS 513.88 : An07g06270, Aspergillus tubingensis : Asptu1_0057823, Aspergillus fumigatus A1163 : AFUB_090620 and Aspergillus terreus NIH2624 : ATET_00875
AFU7G08560	-9.99	Ortholog of A. oryzae RIB40 : AO090701000205, Aspergillus flavus NRRL 3357 : AFL2T_05851 and Aspergillus fumigatus A1163 : AFUB_097700
AFU1G17430	-9.97	Ortholog(s) have monophenol monooxygenase activity
AFU3G13755	-9.79	protein of unknown function
AFU8G04870	-9.75	Has domain(s) with predicted oxidoreductase activity
AFU8G01690	-9.73	Ortholog of A. oryzae RIB40 : AO090010000578 and Aspergillus fumigatus A1163 : AFUB_084900
AFU2G00470	-9.72	Has domain(s) with predicted RNA polymerase II

		transcription factor activity, sequence-specific DNA binding, zinc ion binding activity, role in regulation of transcription, DNA-templated and nucleus localization
AFU8G07260	-9.48	Has domain(s) with predicted transferase activity, transferring glycosyl groups activity and membrane localization
AFU5G10270	-9.39	Ortholog(s) have identical protein binding, mRNA binding, unfolded protein binding activity
AFU7G08570	-9.31	Ortholog of Neosartorya fischeri NRRL 181 : NFIA_032740, Aspergillus versicolor : Aspve1_0033447, Aspergillus niger ATCC 1015 : 45038-mRNA and Aspergillus sydowii : Aspsy1_0034803
AFU8G01640	-9.28	Protein similar to nonribosomal peptide synthases (NRPS-like), encoded in a predicted secondary metabolite gene cluster
AFU8G01110	-9.27	Ortholog of Neosartorya fischeri NRRL 181 : NFIA_094550 and Aspergillus fumigatus A1163 : AFUB_085490
AFU3G02780	-9.25	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AFU3G03560	-9.20	Putative coenzyme A disulfide reductase
AFU5G14810	-9.15	Putative pyruvate decarboxylase
AFU1G15610	-9.14	Putative zinc-binding dehydrogenase family oxidoreductase
AFU7G00700	-8.92	Ortholog(s) have oxidoreductase activity, acting on CH-OH group of donors activity and cytosol, nucleus localization
AFU4G08240	-8.88	Putative zinc-containing alcohol dehydrogenase
AFU1G16960	-8.82	Protein of unknown function identified by mass spectrometry
AFU4G00440	-8.72	Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AFU4G09255	-8.68	Has domain(s) with predicted phosphoric diester hydrolase activity and role in lipid metabolic process
AFU7G00805	-8.66	protein of unknown function
AFU1G16930	-8.65	Has domain(s) with predicted phosphatidylserine decarboxylase activity and role in phospholipid biosynthetic process
AFU1G04100	-8.65	Ortholog of A. nidulans FGSC A4 : AN8649, A. niger CBS 513.88 : An01g02930, A. oryzae RIB40 : AO090009000519, Aspergillus wentii : Aspwe1_0037325, Aspwe1_0104267 and Aspergillus sydowii : Aspsy1_0048337

AFU2G11610	-8.62	Has domain(s) with predicted catalytic activity, cation binding activity and role in carbohydrate metabolic process
AFU4G14500	-8.58	Putative questin oxygenase involved in trypacidin biosynthesis, member of the tpc secondary metabolite gene cluster
AFU3G02460	-8.56	Has domain(s) with predicted ATP binding, protein kinase activity, protein tyrosine kinase activity and role in protein phosphorylation
AFU6G11680	-8.54	Has domain(s) with predicted catalytic activity, magnesium ion binding, thiamine pyrophosphate binding activity
AFU6G09790	-8.50	Has domain(s) with predicted role in cell wall macromolecule catabolic process
AFU1G00300	-8.37	Ortholog of Aspergillus acidus : Aspfo1_0200728
AFU6G00460	-8.35	Ortholog of A. nidulans FGSC A4 : AN8144, AN1651, A. fumigatus Af293 : Afu5g14600, Afu6g13950, A. niger CBS 513.88 : An11g02490, An01g11420 and A. oryzae RIB40 : AO090001000384, AO090011000740
AFU7G06900	-8.35	Has domain(s) with predicted catalytic activity and role in metabolic process
AFU6G14010	-8.31	Ortholog of A. nidulans FGSC A4 : AN2445, A. fumigatus Af293 : Afu6g10580, A. niger CBS 513.88 : An11g00710, A. oryzae RIB40 : AO090023000216 and Aspergillus wentii : Aspwe1_0027041
AFU8G07265	-8.27	Has domain(s) with predicted polygalacturonase activity and role in carbohydrate metabolic process
AFU6G12210	-8.27	Putative endo-1,4-beta-xylanase
AFU3G00850	-8.26	Transcript up-regulated in conidia exposed to neutrophils
AFU5G14320	-8.25	Ortholog of A. nidulans FGSC A4 : AN2683, AN5069, A. fumigatus Af293 : Afu5g02860, A. niger CBS 513.88 : An09g04830, An14g05730 and A. oryzae RIB40 : AO090102000482, AO090001000197
AFU3G02050	-8.19	Ortholog of A. nidulans FGSC A4 : AN1754, AN8607, A. niger CBS 513.88 : An11g06690, An18g01930, A. oryzae RIB40 : AO090001000144 and Aspergillus wentii : Aspwe1_0028029, Aspwe1_0705658
AFU2G11620	-8.18	Has domain(s) with predicted catalytic activity, cation binding

		activity and role in carbohydrate metabolic process
AFU6G03200	-8.18	Has domain(s) with predicted transporter activity, role in transmembrane transport, transport and membrane localization
AFU7G06890	-8.10	Has domain(s) with predicted heme binding, peroxidase activity and role in oxidation-reduction process
AFU2G00190	-8.07	Ortholog of A. nidulans FGSC A4 : AN0020, A. niger CBS 513.88 : An15g07480, Aspergillus versicolor : Aspve1_0365574, Aspergillus niger ATCC 1015 : 40853-mRNA and Aspergillus zonatus : Aspzo1_0077270
AFU2G00460	-8.06	Has domain(s) with predicted oxidoreductase activity and role in metabolic process
AFU8G01340	-8.02	Major facilitator superfamily (MFS) sugar transporter
AFU8G06000	-7.99	Has domain(s) with predicted catalytic activity, oxidoreductase activity
AFU6G14370	-7.98	AraC-like ligand binding domain protein
AFU8G06020	-7.96	Glutamate decarboxylase
AFU5G06240	-7.95	Putative zinc-dependent alcohol dehydrogenase, involved in ethanol fermentation
AFU3G03460	-7.93	Has domain(s) with predicted hydrolase activity
AFU4G14620	-7.92	Has domain(s) with predicted zinc ion binding activity
AFU2G00200	-7.88	Ortholog(s) have catalase activity
AFU7G06820	-7.88	Has domain(s) with predicted role in cell adhesion
AFU1G13820	-7.85	Ortholog of Aspergillus glaucus : Aspgl1_0038114, Neosartorya fischeri NRRL 181 : NFIA_011700 and Aspergillus fumigatus A1163 : AFUB_013300
AFU4G03615	-7.82	Ortholog(s) have cytosol, nucleus localization
AFU6G12000	-7.81	protein of unknown function
AFU6G03220	-7.80	Ortholog of Aspergillus fumigatus A1163 : AFUB_095080

Table S3-6 Top 100 genes showing increased mRNA levels in the Δ*AfuwetA* conidia.

GENE ID	Log$_2$ Fold Change	Description
AFU8G00500	10.61	Putative acetate-CoA ligase
AFU8G00510	10.34	Predicted oxidoreductase
AFU4G14820	10.29	Putative transferase family protein with a predicted role in helvonic acid biosynthesis
AFU8G00940	10.24	Ortholog(s) have role in arginine transport, asperfuranone biosynthetic process and plasma membrane localization
AFU3G12910	10.01	Putative O-methyltransferase
AFU8G00550	9.99	Putative methyl transferase
AFU8G00580	9.92	Putative elongation factor ElfB
AFU6G00180	9.80	Fibrinogen-binding protein of unknown function
AFU7G05180	9.72	Afusin
AFU8G00570	9.54	Putative hydrolase
AFU8G00540	9.52	Non-ribosomal peptide synthetase (NRPS)
AFU8G00530	9.52	Putative alpha/beta hydrolase
AFU4G14770	9.43	Putative oxidosqualene:protostadienol cyclase (OSPC) which is involved in the biosynthesis of the antibiotic helvolic acid
AFU5G01990	9.35	BYS1 domain protein
AFU8G00520	9.31	Membrane-bound terpene cyclase
AFU8G06560	9.30	Has domain(s) with predicted role in transmembrane transport and integral component of membrane localization
AFU4G01450	9.25	Has domain(s) with predicted 3-deoxy-7-phosphoheptulonate synthase activity, catalytic activity and role in aromatic amino acid family biosynthetic process, biosynthetic process
AFU2G12680	9.12	Ortholog of A. nidulans FGSC A4 : AN7941, A. fumigatus Af293 : Afu7g00370, A. niger CBS 513.88 : An03g01770, Aspergillus sydowii : Aspsy1_0156290 and Aspergillus terreus NIH2624 : ATET_07370
AFU8G00430	8.93	Ortholog of A. nidulans AN1088
AFU4G14170	8.86	Ortholog of Aspergillus glaucus : Aspgl1_0034877, Neosartorya fischeri NRRL 181 : NFIA_102140, Aspergillus wentii : Aspwe1_0171319 and Aspergillus fumigatus A1163 : AFUB_071320

AFU1G00770	8.84	Ortholog of A. niger CBS 513.88 : An08g12010 and Aspergillus fumigatus A1163 : AFUB_075320, AFUB_096610
AFU3G03270	8.82	Has domain(s) with predicted catalytic activity and role in metabolic process
AFU8G00710	8.82	Has domain(s) with predicted role in defense response, negative regulation of growth
AFU7G00170	8.80	Dimethylallyl tryptophan synthase
AFU2G12630	8.67	Allergen Asp f 13
AFU4G01460	8.60	Ortholog of Neosartorya fischeri NRRL 181 : NFIA_043690, Aspergillus fumigatus A1163 : AFUB_101940 and Aspergillus clavatus NRRL 1 : ACLA_061720
AFU4G08870	8.56	Ortholog of A. nidulans FGSC A4 : AN9441, A. oryzae RIB40 : AO090023000672, AO090026000071, Neosartorya fischeri NRRL 181 : NFIA_107300 and Aspergillus wentii : Aspwe1_0064277
AFU5G13730	8.49	Ortholog of Aspergillus fumigatus A1163 : AFUB_061470
AFU8G00480	8.42	Putative iron-dependent oxygenase
AFU8G00930	8.41	Ortholog of A. fumigatus Af293 : Afu4g01290/csn, A. niger CBS 513.88 : An04g04530, A. oryzae RIB40 : AO090011000027, AO090020000697/csnA and Neosartorya fischeri NRRL 181 : NFIA_043990, NFIA_094360
AFU8G00560	8.22	Putative P450 monooxygenase
AFU4G14840	8.15	Putative transferase family protein with a predicted role in helvonic acid biosynthesis
AFU4G01270	8.05	Ortholog of A. nidulans FGSC A4 : AN3285, Neosartorya fischeri NRRL 181 : NFIA_044020, Aspergillus versicolor : Aspve1_0066429 and Aspergillus fumigatus A1163 : AFUB_101760
AFU3G01910	7.93	Putative cellobiohydrolase, a predicted secreted hydrolase with a fungal cellulose binding domain
AFU3G12920	7.80	Nonribosomal peptide synthase (NRPS)
AFU4G14780	7.79	Putative cytochrome P450 monooxygenase with a predicted role in helvonic acid biosynthesis
AFU5G01490	7.78	Putative hydrophobin
AFU8G06810	7.74	Ortholog of A. oryzae RIB40 : AO090003001576, Aspergillus glaucus : Aspgl1_0145643, Aspergillus flavus NRRL 3357 :

		AFL2T_01871 and Neosartorya fischeri NRRL 181 : NFIA_024040
AFU6G03450	7.74	Ortholog of A. oryzae RIB40 : AO090001000013/wykH, AO090102000466, Aspergillus flavus NRRL 3357 : AFL2T_07266, AFL2T_09860, Neosartorya fischeri NRRL 181 : NFIA_007610 and Aspergillus versicolor : Aspve1_0401363
AFU5G02330	7.70	Allergen Asp f 1
AFU6G09745	7.70	Ortholog of Aspergillus brasiliensis : Aspbr1_0199026, Neosartorya fischeri NRRL 181 : NFIA_055440, NFIA_112700, Aspergillus fumigatus A1163 : AFUB_075800 and Aspergillus clavatus NRRL 1 : ACLA_006680
AFU8G01760	7.70	protein of unknown function
AFU8G00440	7.68	Baeyer-Villiger monooxygenase (BVMO)
AFU3G12900	7.67	Putative transporter
AFU1G16190	7.60	Cell wall glucanase
AFU6G00270	7.51	protein of unknown function
AFU3G00870	7.49	Has domain(s) with predicted N-acetyltransferase activity
AFU8G00490	7.46	Putative polyketide synthase
AFU2G17160	7.45	Ortholog of A. fumigatus Af293 : Afu3g13630, Afu7g00570, A. niger CBS 513.88 : An15g01210, An09g06230, An09g04210, An01g07840 and Neosartorya fischeri NRRL 181 : NFIA_095980
AFU6G07820	7.43	Putative integral membrane protein
AFU4G01290	7.37	Glycosyl hydrolase family 75 chitosanase
AFU6G07000	7.33	Ortholog of A. nidulans FGSC A4 : AN5172, A. fumigatus Af293 : Afu4g09700, A. niger CBS 513.88 : An04g07430, A. oryzae RIB40 : AO090012000946, AO090003001270 and Aspergillus wentii : Aspwe1_0166832
AFU6G03440	7.31	fructosyl amino acid oxidase, putative
AFU4G01210	7.31	Ortholog of A. nidulans FGSC A4 : AN9206, AN1849, A. fumigatus Af293 : Afu5g02320, Afu5g09930, A. niger CBS 513.88 : An03g02680, An12g10080, An12g02060 and A. oryzae RIB40 : AO090001000129, AO090010000537
AFU4G00390	7.31	Has domain(s) with predicted catalytic activity, hydrolase activity, hydrolyzing O-glycosyl compounds activity and role in

		carbohydrate metabolic process
AFU8G02070	7.29	Ortholog of A. niger CBS 513.88 : An05g02310, A. oryzae RIB40 : AO090010000612, Neosartorya fischeri NRRL 181 : NFIA_095740 and Aspergillus clavatus NRRL 1 : ACLA_043710
AFU6G09680	7.28	Predicted O-methyltransferase, encoded in the putative gliotoxin biosynthetic gene cluster
AFU3G00880	7.27	Putative adhesin protein
AFU7G00970	7.24	Predicted repeat rich glycophosphatidylinositol (GPI)-anchored cell wall protein
AFU8G00380	7.24	Putative alpha/beta hydrolase
AFU6G03060	7.23	Putative major facilitator superfamily (MFS) monosaccharide transporter
AFU1G03210	7.20	Myb family transcription factor
AFU3G01270	7.19	Has domain(s) with predicted hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amides activity, role in ceramide metabolic process and integral component of membrane localization
AFU6G03490	7.19	phenol-2-monooxygenase, putative
AFU4G01200	7.17	Has domain(s) with predicted RNA binding, endoribonuclease activity, ribonuclease activity
AFU7G00370	7.11	Ortholog of A. nidulans FGSC A4 : AN7941, A. fumigatus Af293 : Afu2g12680, A. niger CBS 513.88 : An03g01770, Aspergillus sydowii : Aspsy1_0156290 and Aspergillus terreus NIH2624 : ATET_07370
AFU4G14830	7.10	Putative cytochrome P450 monooxygenase with a predicted role in helvonic acid biosynthesis
AFU7G01060	7.03	Ortholog of A. nidulans FGSC A4 : AN7836, A. oryzae RIB40 : AO090003000833, Neosartorya fischeri NRRL 181 : NFIA_113770 and Aspergillus terreus NIH2624 : ATET_02590
AFU4G14790	7.03	Putative cytochrome P450 monooxygenase with a predicted role in helvonic acid biosynthesis
AFU8G00360	7.03	Ortholog of Neosartorya fischeri NRRL 181 : NFIA_093900, Aspergillus versicolor : Aspve1_0045862, Aspergillus fumigatus A1163 : AFUB_086210 and Aspergillus sydowii : Aspsy1_0048601

AFU6G00690	7.02	protein of unknown function
AFU6G14480	6.97	Has domain(s) with predicted transferase activity, transferring glycosyl groups activity and role in protein glycosylation
AFU8G07090	6.95	Predicted adhesin-like protein, ortholog of A. nidulans AN3258
AFU3G10175	6.94	Ortholog of Neosartorya fischeri NRRL 181 : NFIA_067110 and Aspergillus fumigatus A1163 : AFUB_038990
AFU2G14520	6.92	Secreted hydrolase
AFU4G13610	6.91	Ortholog of Aspergillus fumigatus A1163 : AFUB_070530
AFU1G17180	6.90	Putative pyridine nucleotide-disulphide oxidoreductase
AFU6G03430	6.87	Putative C6 finger domain protein
AFU8G01770	6.85	Predicted adhesin-like protein
AFU4G13600	6.84	Has domain(s) with predicted nucleic acid binding, zinc ion binding activity
AFU3G12200	6.82	OPT family small oligopeptide transporter
AFU3G14665	6.81	Ortholog of Neosartorya fischeri NRRL 181 : NFIA_062590 and Aspergillus fumigatus A1163 : AFUB_034560
AFU2G03830	6.78	Allergen Asp f 4
AFU2G14480	6.78	Ortholog(s) have extracellular region localization
AFU8G06680	6.78	Ortholog of Aspergillus fumigatus A1163 : AFUB_081100 and Aspergillus terreus NIH2624 : ATET_08599
AFU5G03780	6.77	Putative L-PSP endoribonuclease family protein
AFU2G00640	6.76	Has domain(s) with predicted beta-N-acetylhexosaminidase activity, catalytic activity, hydrolase activity, hydrolyzing O-glycosyl compounds activity and role in carbohydrate metabolic process
AFU8G00910	6.76	Ortholog(s) have role in asperfuranone biosynthetic process
AFU3G14170	6.75	Putative high-affinity hexose transporter
AFU3G07580	6.73	Ortholog of Neosartorya fischeri NRRL 181 : NFIA_069600, Aspergillus wentii : Aspwe1_0176375, Aspergillus fumigatus A1163 : AFUB_041540 and Aspergillus clavatus NRRL 1 : ACLA_035370
AFU8G00800	6.72	Has domain(s) with predicted role in transmembrane transport and membrane localization
AFU3G01400	6.70	Ortholog(s) have role in drug transmembrane transport
AFU8G02090	6.70	Has domain(s) with predicted sugar:proton symporter activity, role in carbohydrate transport and Golgi membrane, integral

		component of membrane localization
AFU7G05450	6.69	Novel beta-1,3-glucan modifying enzyme involved in fungal morphogenesis
AFU1G01300	6.68	Has domain(s) with predicted poly(beta-D-mannuronate) lyase activity, role in alginic acid catabolic process and periplasmic space localization
AFU6G00290	6.67	Has domain(s) with predicted catalytic activity, pyridoxal phosphate binding activity and role in biosynthetic process
AFU8G00400	6.65	protein of unknown function
AFU8G05630	6.59	putative chitin synthase
AFU4G11480	6.58	Ortholog(s) have role in cellular potassium ion homeostasis
AFU3G01390	6.57	protein of unknown function

Table S3-7 Top enriched GO categories of orthologs in All_orthogroups consistently up-/down-regulated in Δ*wetA* condia

GO Category	# of Genes	% of Genes in Category
A. nidulans		
single-organism process	305	7.8
single-organism cellular process	240	8.1
single-organism metabolic process	189	8.2
response to stimulus	107	9.5
organonitrogen compound metabolic process	94	10.2
small molecule metabolic process	88	9
cellular response to stimulus	88	8.8
oxidation-reduction process	81	9.2
response to stress	72	10
carbohydrate metabolic process	68	13.4
developmental process	65	14.8
single-organism developmental process	62	14.9
phosphate-containing compound metabolic process	60	11.8
phosphorus metabolic process	60	11.4
response to chemical	59	10.3
reproduction	57	12.8
organonitrogen compound biosynthetic process	57	9.2
anatomical structure development	56	14.8
cellular response to chemical stimulus	53	10.6
cellular response to stress	53	9.1
cellular component biogenesis	52	9.7
anatomical structure morphogenesis	50	16
sporulation	49	16.8
anatomical structure formation involved in morphogenesis	49	16.4
multi-organism process	48	12.1
organophosphate metabolic process	46	14.4
carbohydrate derivative metabolic process	46	13.5
single-organism carbohydrate metabolic process	43	16.2
growth	43	11.2

cellular developmental process	42	17.4
reproductive process	42	13.3
filamentous growth	42	11.5
asexual reproduction	40	18.8
asexual sporulation	39	20.3
cell differentiation	39	17.6
sporulation resulting in formation of a cellular spore	37	17.8
cellular amide metabolic process	37	11.1
positive regulation of biological process	37	10.3
developmental process involved in reproduction	36	12.9
regulation of biological quality	33	11.6
nucleobase-containing small molecule metabolic process	32	13.6
amide biosynthetic process	31	10.6
cell wall organization or biogenesis	30	14.8
cellular carbohydrate metabolic process	30	14.5
response to abiotic stimulus	30	12.4
nucleotide metabolic process	29	16.7
nucleoside phosphate metabolic process	29	16.4
monocarboxylic acid metabolic process	29	12
multi-organism reproductive process	28	10.8
sexual reproduction	28	10.8
response to external stimulus	27	11.1
cofactor metabolic process	26	14.1
phosphorylation	26	12.3
peptide metabolic process	26	10.3
hyphal growth	25	13.8
peptide biosynthetic process	25	10.6
fungal-type cell wall organization or biogenesis	24	18.6
conidium formation	24	18.6
ribose phosphate metabolic process	23	18.5
response to oxidative stress	23	18.5
coenzyme metabolic process	23	15
single organism reproductive process	23	13.3
translation	23	11.6
carbohydrate catabolic process	22	13.3
external encapsulating structure organization	21	19.4

cell wall organization	21	19.4
regulation of developmental process	21	15.2
filamentous growth of a population of unicellular organisms	21	10.8
pyridine-containing compound metabolic process	20	27
regulation of anatomical structure morphogenesis	20	15.7
generation of precursor metabolites and energy	20	13
carbohydrate derivative biosynthetic process	20	12.1
asexual sporulation resulting in formation of a cellular spore	19	30.2
fungal-type cell wall organization	19	19.6
pathogenesis	19	15.6
organ development	19	12.1
reproductive structure development	19	12.1
system development	19	12.1
spore-bearing organ development	19	12.1
reproductive system development	19	12.1
multicellular organismal development	19	12
single-multicellular organism process	19	11.7
multicellular organismal process	19	11.7
nicotinamide nucleotide metabolic process	18	26.5
pyridine nucleotide metabolic process	18	26.5
oxidoreduction coenzyme metabolic process	18	22.5
nucleoside metabolic process	18	13
glycosyl compound metabolic process	18	12.7
small molecule catabolic process	18	11.8
carbohydrate biosynthetic process	17	23
monosaccharide metabolic process	17	18.1
cellular response to oxidative stress	17	16.8
purine-containing compound metabolic process	17	13.7
response to inorganic substance	17	12.1
conidiophore development	16	20.3
response to oxygen-containing compound	16	16.5
ribonucleoside metabolic process	16	15.7
ribonucleotide metabolic process	16	15.5
cellular response to oxygen-containing compound	15	17.9
ribonucleoside monophosphate metabolic process	15	15.3
nucleoside monophosphate metabolic process	15	15.2

purine ribonucleotide metabolic process	15	15
purine nucleotide metabolic process	15	14.6
cellular homeostasis	15	12
symbiosis, encompassing mutualism through parasitism	14	26.4
interspecies interaction between organisms	14	25.5
single-organism carbohydrate catabolic process	14	21.9
regulation of sporulation	14	19.2
purine ribonucleoside monophosphate metabolic process	14	14.9
purine nucleoside monophosphate metabolic process	14	14.9
purine nucleoside metabolic process	14	14.7
purine ribonucleoside metabolic process	14	14.7
cellular component morphogenesis	13	23.2
hexose metabolic process	13	20.3
cell wall biogenesis	13	19.7
response to osmotic stress	13	13.8
response to toxic substance	13	12.9
regulation of asexual sporulation	12	23.5
regulation of asexual reproduction	12	23.1
regulation of sporulation resulting in formation of a cellular spore	12	23.1
regulation of cell differentiation	12	22.2
positive regulation of developmental process	12	18.2
purine ribonucleoside triphosphate metabolic process	12	16.2
ribonucleoside triphosphate metabolic process	12	16.2
purine nucleoside triphosphate metabolic process	12	15.8
nucleoside triphosphate metabolic process	12	15.4
cell wall macromolecule metabolic process	12	13.3
biological adhesion	11	35.5
cellular carbohydrate biosynthetic process	11	21.6
regulation of response to stress	11	16.4
ATP metabolic process	11	15.1
organelle assembly	11	14.5
positive regulation of response to stimulus	10	14.7
glucose metabolic process	9	31
nucleoside diphosphate metabolic process	9	27.3
interaction with host	9	25.7

positive regulation of sporulation	9	25.7
pyruvate metabolic process	9	24.3
amino sugar metabolic process	9	19.1
cellular aldehyde metabolic process	9	18.8
fungal-type cell wall biogenesis	9	18.4
spore germination	9	18.4
pigment biosynthetic process	9	17.6
pigment metabolic process	9	16.1
positive regulation of cell differentiation	8	29.6
response to host defenses	8	29.6
positive regulation of sporulation resulting in formation of a cellular spore	8	29.6
response to host	8	29.6
response to other organism	8	27.6
ADP metabolic process	8	27.6
response to external biotic stimulus	8	27.6
response to defenses of other organism involved in symbiotic interaction	8	27.6
cellular component macromolecule biosynthetic process	8	26.7
ribonucleoside diphosphate metabolic process	8	26.7
purine ribonucleoside diphosphate metabolic process	8	26.7
cell wall macromolecule biosynthetic process	8	26.7
purine nucleoside diphosphate metabolic process	8	26.7
regulation of conidium formation	8	22.9
cellular polysaccharide biosynthetic process	8	21.6
response to salt stress	8	21.1
polysaccharide biosynthetic process	8	20.5
RNA export from nucleus	8	19
cell wall polysaccharide metabolic process	8	18.2
RNA transport	8	18.2
establishment of RNA localization	8	18.2
nucleic acid transport	8	17.8
glucosamine-containing compound metabolic process	8	17.8
aminoglycan metabolic process	8	17
RNA localization	8	16.7
tRNA modification	8	15.7

regulation of defense response	7	38.9
modulation by symbiont of host defense response	7	38.9
modulation by organism of defense response of other organism involved in symbiotic interaction	7	38.9
modification of morphology or physiology of other organism involved in symbiotic interaction	7	36.8
modification of morphology or physiology of other organism	7	36.8
modification by symbiont of host morphology or physiology	7	36.8
pentose-phosphate shunt	7	36.8
glyceraldehyde-3-phosphate metabolic process	7	36.8
glucose 6-phosphate metabolic process	7	33.3
positive regulation of asexual reproduction	7	30.4
spore wall biogenesis	7	30.4
NADP metabolic process	7	30.4
regulation of asexual sporulation resulting in formation of a cellular spore	7	30.4
nucleoside diphosphate phosphorylation	7	29.2
cell morphogenesis	7	25.9
nucleotide phosphorylation	7	25.9
response to reactive oxygen species	7	25
cellular component assembly involved in morphogenesis	7	23.3
positive regulation of reproductive process	7	21.2
vitamin metabolic process	7	18.4
cell growth	7	17.9
response to radiation	7	17.1
response to light stimulus	7	17.1
chitin metabolic process	7	16.7
positive regulation of asexual sporulation resulting in formation of a cellular spore	6	42.9
gluconeogenesis	6	31.6
aldonic acid metabolic process	6	30
cell wall polysaccharide biosynthetic process	6	28.6
hexose biosynthetic process	6	28.6
monosaccharide biosynthetic process	6	28.6
ATP generation from ADP	6	26.1
cell adhesion	6	26.1

glycolytic process	6	26.1
cellular response to reactive oxygen species	6	25
negative regulation of sporulation	6	22.2
negative regulation of developmental process	6	20
organic hydroxy compound catabolic process	6	20
establishment of nucleus localization	6	18.8
nucleus localization	6	18.8
single-species submerged biofilm formation	6	18.2
pentose metabolic process	6	18.2
single-species biofilm formation	6	18.2
lipid modification	6	17.6
rRNA transport	5	45.5
rRNA export from nucleus	5	45.5
induction by symbiont of host defense response	5	41.7
positive regulation by organism of defense response of other organism involved in symbiotic interaction	5	41.7
positive regulation by symbiont of host defense response	5	41.7
adhesion of symbiont to host	5	41.7
induction by organism of defense response of other organism involved in symbiotic interaction	5	41.7
positive regulation of defense response	5	41.7
glucan biosynthetic process	5	33.3
D-gluconate metabolic process	5	33.3
monosaccharide catabolic process	5	31.3
trehalose metabolic process	5	31.3
regulation of spore-bearing organ development	5	29.4
regulation of multicellular organismal development	5	29.4
regulation of multicellular organismal process	5	29.4
negative regulation of cell differentiation	5	27.8
negative regulation of sporulation resulting in formation of a cellular spore	5	27.8
cytoplasmic translation	5	25
beta-glucan metabolic process	5	25
spore wall assembly	5	23.8
negative regulation of meiotic cell cycle	5	22.7
cell wall assembly	5	22.7

alcohol catabolic process	5	22.7
primary alcohol metabolic process	5	22.7
NAD metabolic process	5	21.7
cell redox homeostasis	5	20.8
negative regulation of reproductive process	5	20
beta-glucan biosynthetic process	4	50
NADH metabolic process	4	44.4
regulation of conidiophore development	4	40
cell wall chitin metabolic process	4	36.4
septin ring organization	4	36.4
septin cytoskeleton organization	4	33.3
developmental growth involved in morphogenesis	4	33.3
positive regulation of conidium formation	4	33.3
negative regulation of sexual sporulation resulting in formation of a cellular spore	4	33.3
unidimensional cell growth	4	33.3
regulation of generation of precursor metabolites and energy	4	30.8
protein secretion	4	30.8
polyol catabolic process	4	30.8
developmental growth	4	26.7
cell-substrate adhesion	4	26.7
glucosamine-containing compound biosynthetic process	4	25
ascospore wall biogenesis	4	25
chitin biosynthetic process	4	25
aminoglycan biosynthetic process	4	25
amino sugar biosynthetic process	4	25
reactive oxygen species metabolic process	4	23.5
regulation of glucose metabolic process	4	23.5
cell wall beta-glucan metabolic process	3	75
pyrimidine nucleoside biosynthetic process	3	75
cell tip growth	3	75
pyrimidine ribonucleoside biosynthetic process	3	75
protein autophosphorylation	3	60
regulation of immune response	3	60
regulation of immune system process	3	60
positive regulation of conidiophore development	3	60

(1->3)-beta-D-glucan metabolic process	3	60
response to antibiotic	3	60
cell adhesion involved in single-species biofilm formation	3	50
mitochondrial electron transport, cytochrome c to oxygen	3	42.9
cell adhesion involved in biofilm formation	3	42.9
septin ring assembly	3	42.9
submerged biofilm formation	3	42.9
pyrimidine ribonucleoside metabolic process	3	37.5
development of symbiont involved in interaction with host	3	37.5
pyrimidine nucleoside metabolic process	3	37.5
development of symbiont in host	3	37.5
development involved in symbiotic interaction	3	37.5
positive regulation of spore-bearing organ development	3	33.3
positive regulation of multicellular organismal process	3	33.3
oligosaccharide biosynthetic process	3	33.3
cell wall chitin biosynthetic process	3	33.3
disaccharide biosynthetic process	3	33.3
trehalose biosynthetic process	3	33.3
response to cold	3	30
folic acid-containing compound metabolic process	3	30
modulation by symbiont of host cell-mediated immune response	2	100
fungal-type cell wall beta-glucan metabolic process	2	100
modulation by organism of cell-mediated immune response of other organism involved in symbiotic interaction	2	100
fungal-type cell wall beta-glucan biosynthetic process	2	100
endocytic recycling	2	100
age-dependent response to reactive oxygen species	2	66.7
response to superoxide	2	66.7
induction of conjugation upon nutrient starvation	2	66.7
aspartate transport	2	66.7
cellular age-dependent response to reactive oxygen species	2	66.7
negative regulation of cell development	2	66.7
siroheme metabolic process	2	66.7
siroheme biosynthetic process	2	66.7
xylulose 5-phosphate biosynthetic process	2	66.7
pyrimidine ribonucleotide biosynthetic process	2	66.7

regulation of ribosomal protein gene transcription from RNA polymerase II promoter	2	66.7
eisosome assembly	2	66.7
pentitol catabolic process	2	66.7
cellular response to oxygen radical	2	66.7
pentitol metabolic process	2	66.7
pentose-phosphate shunt, oxidative branch	2	66.7
cell wall (1->3)-beta-D-glucan metabolic process	2	66.7
induction of conjugation upon nitrogen starvation	2	66.7
cell wall beta-glucan biosynthetic process	2	66.7
cellular response to superoxide	2	66.7
cell wall (1->3)-beta-D-glucan biosynthetic process	2	66.7
xylulose 5-phosphate metabolic process	2	66.7
response to oxygen radical	2	66.7
A. fumigatus		
single-organism metabolic process	168	7.1
organonitrogen compound metabolic process	83	9.2
small molecule metabolic process	72	7.9
carbohydrate metabolic process	52	10.9
organonitrogen compound biosynthetic process	52	8.7
phosphorus metabolic process	47	9.7
phosphate-containing compound metabolic process	45	9.6
carbohydrate derivative metabolic process	38	11.9
organophosphate metabolic process	32	11.5
cellular amide metabolic process	30	9
cell wall organization or biogenesis	27	21.3
cell cycle	27	10.1
amide biosynthetic process	27	9.3
single-organism carbohydrate metabolic process	26	12.4
nucleobase-containing small molecule metabolic process	26	11.2
cell cycle process	25	10
multi-organism process	23	16.1
translation	23	9.5
nucleotide metabolic process	22	13.3
nucleoside phosphate metabolic process	22	12.6
cofactor metabolic process	21	12.1

phosphorylation	21	10.2
reproduction	20	12.9
cell wall organization	19	25.3
external encapsulating structure organization	19	25
polysaccharide metabolic process	19	18.1
ribose phosphate metabolic process	19	15.4
cellular carbohydrate metabolic process	18	17.3
coenzyme metabolic process	18	12.2
monocarboxylic acid metabolic process	18	10.3
generation of precursor metabolites and energy	16	13.3
developmental process	14	16.9
purine-containing compound metabolic process	14	11.6
glucan metabolic process	13	27.1
cellular aldehyde metabolic process	13	26.5
anatomical structure morphogenesis	13	22.4
cellular polysaccharide metabolic process	13	21.7
anatomical structure development	13	20
nicotinamide nucleotide metabolic process	13	19.7
pyridine nucleotide metabolic process	13	19.4
pyridine-containing compound metabolic process	13	17.8
oxidoreduction coenzyme metabolic process	13	16.7
single-organism developmental process	13	16.3
multi-organism reproductive process	13	14.1
sexual reproduction	13	14.1
reproductive process	13	12.7
ribonucleotide metabolic process	13	12.3
cellular glucan metabolic process	12	25.5
carbohydrate biosynthetic process	12	23.1
cellular developmental process	12	19.7
monosaccharide metabolic process	12	19
meiotic cell cycle process	12	17.4
meiotic cell cycle	12	17.4
purine ribonucleotide metabolic process	12	11.8
purine nucleotide metabolic process	12	11.5
hexose metabolic process	11	23.4
reproduction of a single-celled organism	11	16.2

glucose metabolic process	10	40
carbohydrate catabolic process	10	13.9
pathogenesis	9	25.7
cytokinesis	9	16.4
cell division	9	14.1
glucose 6-phosphate metabolic process	8	42.1
pyruvate metabolic process	8	23.5
anatomical structure formation involved in morphogenesis	8	22.9
sporulation	8	22.9
sporulation resulting in formation of a cellular spore	8	22.9
single-organism carbohydrate catabolic process	8	20
cell differentiation	8	20
amino sugar metabolic process	8	18.6
beta-glucan metabolic process	7	38.9
pentose-phosphate shunt	7	38.9
glyceraldehyde-3-phosphate metabolic process	7	38.9
NADP metabolic process	7	31.8
nucleoside diphosphate phosphorylation	7	29.2
nucleotide phosphorylation	7	28
nucleoside diphosphate metabolic process	7	23.3
ascospore formation	7	21.9
sexual sporulation	7	21.9
sexual sporulation resulting in formation of a cellular spore	7	21.9
cell development	7	21.9
nucleobase metabolic process	7	21.2
cytokinetic process	7	20.6
cytoskeleton-dependent cytokinesis	7	20.6
mitotic cytokinetic process	7	20.6
mitotic cytokinesis	7	20.6
reproductive process in single-celled organism	7	19.4
single organism reproductive process	7	17.5
aminoglycan metabolic process	7	16.3
developmental process involved in reproduction	7	14.9
(1->3)-beta-D-glucan metabolic process	6	46.2
gluconeogenesis	6	40
hexose biosynthetic process	6	37.5

monosaccharide biosynthetic process	6	37.5
glycolytic process	6	26.1
ATP generation from ADP	6	26.1
ADP metabolic process	6	23.1
purine ribonucleoside diphosphate metabolic process	6	23.1
purine nucleoside diphosphate metabolic process	6	23.1
ribonucleoside diphosphate metabolic process	6	23.1
cellular component morphogenesis	6	21.4
cytokinesis, site selection	6	18.8
mitotic cytokinesis, site selection	6	18.8
cellular bud site selection	6	18.8
chitin metabolic process	6	17.1
cell wall macromolecule metabolic process	6	16.2
glucosamine-containing compound metabolic process	6	15.4
response to oxidative stress	6	15.4
cellular lipid catabolic process	6	15.4
axial cellular bud site selection	5	55.6
cell morphogenesis	5	21.7
pyrimidine-containing compound biosynthetic process	5	19.2
cellular divalent inorganic cation homeostasis	4	33.3
divalent inorganic cation homeostasis	4	33.3
purine nucleobase metabolic process	4	22.2
superoxide metabolic process	3	42.9
reactive oxygen species metabolic process	3	37.5
calcium ion homeostasis	3	33.3
cellular calcium ion homeostasis	3	33.3
autophagic cell death	2	100
phosphatidylethanolamine metabolic process	2	66.7
leucine catabolic process	2	66.7
phosphatidylethanolamine biosynthetic process	2	66.7
branched-chain amino acid catabolic process	2	50
pyrimidine ribonucleotide biosynthetic process	2	50
pyrimidine ribonucleotide metabolic process	2	50
carbon utilization	2	50
pyrimidine ribonucleoside biosynthetic process	2	50
obsolete cytokinesis, completion of separation	2	50

A. flavus		
single-organism process	301	8.7
single-organism cellular process	232	8.6
single-organism metabolic process	179	9.1
response to stimulus	102	9.6
organonitrogen compound metabolic process	84	10.3
oxidation-reduction process	79	11.1
response to stress	68	9.9
developmental process	66	15.1
single-organism developmental process	63	15.1
reproduction	59	13.6
carbohydrate metabolic process	58	12.4
anatomical structure development	57	15.1
cellular component biogenesis	55	10.4
phosphate-containing compound metabolic process	54	11.7
phosphorus metabolic process	54	11.2
multi-organism process	53	13.4
anatomical structure morphogenesis	51	16.5
sporulation	49	17.3
anatomical structure formation involved in morphogenesis	49	16.7
reproductive process	44	13.9
cellular developmental process	43	17.9
growth	43	11.4
carbohydrate derivative metabolic process	42	14.5
filamentous growth	42	11.6
asexual reproduction	41	20.4
asexual sporulation	40	21.7
organophosphate metabolic process	40	14.4
cell differentiation	39	18
developmental process involved in reproduction	38	13.5
cellular component assembly	38	10.4
sporulation resulting in formation of a cellular spore	37	18
cell wall organization or biogenesis	34	18.7
regulation of biological quality	34	11.8
nucleobase-containing small molecule metabolic process	30	15.4
single-organism carbohydrate metabolic process	30	14.9

response to abiotic stimulus	30	13.7
multi-organism reproductive process	30	11.7
sexual reproduction	30	11.7
nucleotide metabolic process	28	18.5
nucleoside phosphate metabolic process	28	17.9
fungal-type cell wall organization or biogenesis	27	21.8
response to external stimulus	27	11.3
hyphal growth	26	14.3
single organism reproductive process	25	14.6
phosphorylation	25	12.9
conidium formation	24	19
cofactor metabolic process	24	14.3
cell wall organization	23	22.5
external encapsulating structure organization	23	22.5
ribose phosphate metabolic process	22	21.2
filamentous growth of a population of unicellular organisms	22	11.8
fungal-type cell wall organization	21	23.1
pathogenesis	21	16.8
coenzyme metabolic process	21	14.7
asexual sporulation resulting in formation of a cellular spore	20	33.3
carbohydrate catabolic process	20	13.2
organ development	20	12.2
system development	20	12.2
spore-bearing organ development	20	12.2
reproductive system development	20	12.2
reproductive structure development	20	12.2
multicellular organismal development	20	12.1
single-multicellular organism process	20	11.9
multicellular organismal process	20	11.9
pyridine-containing compound metabolic process	19	31.1
response to oxidative stress	19	16.4
regulation of anatomical structure morphogenesis	19	16
regulation of developmental process	19	14.7
sexual sporulation	19	12.6
sexual sporulation resulting in formation of a cellular spore	19	12.6
response to inorganic substance	18	12.3

pyridine nucleotide metabolic process	17	31.5
nicotinamide nucleotide metabolic process	17	31.5
oxidoreduction coenzyme metabolic process	17	26.2
conidiophore development	17	19.8
generation of precursor metabolites and energy	17	19.8
cell development	17	12.7
purine-containing compound metabolic process	16	15.8
nucleoside metabolic process	16	15.7
glycosyl compound metabolic process	16	15.1
cellular component morphogenesis	15	26.8
ribonucleotide metabolic process	15	17.9
ribonucleoside metabolic process	15	17.6
cell wall biogenesis	14	22.2
regulation of sporulation	14	20.9
ribonucleoside monophosphate metabolic process	14	18.2
nucleoside monophosphate metabolic process	14	17.7
purine ribonucleotide metabolic process	14	17.5
purine nucleotide metabolic process	14	17.3
single-organism carbohydrate catabolic process	13	23.6
carbohydrate biosynthetic process	13	20.3
purine nucleoside monophosphate metabolic process	13	17.6
purine ribonucleoside monophosphate metabolic process	13	17.6
cell wall macromolecule metabolic process	13	17.3
purine ribonucleoside metabolic process	13	17.1
purine nucleoside metabolic process	13	17.1
multi-organism cellular process	13	14.6
cellular response to oxidative stress	13	14.3
regulation of sporulation resulting in formation of a cellular spore	12	26.1
regulation of asexual sporulation	12	25.5
regulation of cell differentiation	12	25
regulation of asexual reproduction	12	24.5
symbiosis, encompassing mutualism through parasitism	12	23.1
interspecies interaction between organisms	12	22.2
response to osmotic stress	12	17.4
purine ribonucleoside triphosphate metabolic process	11	20.4

ribonucleoside triphosphate metabolic process	11	20
purine nucleoside triphosphate metabolic process	11	20
nucleoside triphosphate metabolic process	11	18.3
positive regulation of response to stimulus	11	16.4
regulation of response to stress	11	16.2
fungal-type cell wall biogenesis	10	21.3
ATP metabolic process	10	18.9
cellular carbohydrate biosynthetic process	10	18.5
response to oxygen-containing compound	10	17.9
cellular aldehyde metabolic process	9	29
biological adhesion	9	29
response to salt stress	9	26.5
cellular response to oxygen-containing compound	9	20.5
spore germination	9	19.6
cell wall polysaccharide metabolic process	9	18.8
monosaccharide metabolic process	9	17.6
spore wall biogenesis	8	40
nucleoside diphosphate metabolic process	8	40
pyruvate metabolic process	8	38.1
positive regulation of sporulation	8	28.6
cellular component assembly involved in morphogenesis	8	28.6
response to reactive oxygen species	8	28.6
cell wall macromolecule biosynthetic process	8	25.8
cellular component macromolecule biosynthetic process	8	25.8
regulation of conidium formation	8	23.5
interaction with host	8	23.5
cellular polysaccharide biosynthetic process	8	21.1
pigment biosynthetic process	8	20.5
polysaccharide biosynthetic process	8	20
RNA export from nucleus	8	19
RNA transport	8	18.2
establishment of RNA localization	8	18.2
pigment metabolic process	8	18.2
nucleic acid transport	8	17.8
RNA localization	8	17
tRNA modification	8	16.3

glyceraldehyde-3-phosphate metabolic process	7	43.8
pentose-phosphate shunt	7	43.8
ADP metabolic process	7	43.8
purine nucleoside diphosphate metabolic process	7	41.2
purine ribonucleoside diphosphate metabolic process	7	41.2
ribonucleoside diphosphate metabolic process	7	41.2
glucose 6-phosphate metabolic process	7	38.9
regulation of asexual sporulation resulting in formation of a cellular spore	7	36.8
NADP metabolic process	7	33.3
positive regulation of sporulation resulting in formation of a cellular spore	7	31.8
positive regulation of cell differentiation	7	30.4
response to host defenses	7	28
cellular response to reactive oxygen species	7	26.9
response to host	7	26.9
response to defenses of other organism involved in symbiotic interaction	7	25.9
cell morphogenesis	7	25
response to external biotic stimulus	7	25
response to other organism	7	25
establishment of nucleus localization	7	21.2
nucleus localization	7	21.2
biofilm formation	7	20
hexose metabolic process	7	17.5
nucleoside diphosphate phosphorylation	6	50
nucleotide phosphorylation	6	40
glucose metabolic process	6	37.5
regulation of defense response	6	35.3
positive regulation of asexual reproduction	6	35.3
modulation by symbiont of host defense response	6	35.3
modulation by organism of defense response of other organism involved in symbiotic interaction	6	35.3
spore wall assembly	6	33.3
cell wall assembly	6	31.6
modification by symbiont of host morphology or physiology	6	31.6

modification of morphology or physiology of other organism	6	30
modification of morphology or physiology of other organism involved in symbiotic interaction	6	30
cell wall polysaccharide biosynthetic process	6	27.3
cell adhesion	6	24
negative regulation of sporulation	6	21.4
negative regulation of developmental process	6	19.4
positive regulation of asexual sporulation resulting in formation of a cellular spore	5	50
rRNA export from nucleus	5	45.5
ATP generation from ADP	5	45.5
rRNA transport	5	45.5
glycolytic process	5	45.5
positive regulation by symbiont of host defense response	5	41.7
positive regulation of defense response	5	41.7
induction by organism of defense response of other organism involved in symbiotic interaction	5	41.7
induction by symbiont of host defense response	5	41.7
positive regulation by organism of defense response of other organism involved in symbiotic interaction	5	41.7
septin cytoskeleton organization	5	38.5
beta-glucan metabolic process	5	35.7
ascospore wall biogenesis	5	35.7
glucan biosynthetic process	5	33.3
monosaccharide catabolic process	5	33.3
response to hydrogen peroxide	5	26.3
negative regulation of sporulation resulting in formation of a cellular spore	5	26.3
negative regulation of cell differentiation	5	26.3
trehalose metabolic process	5	26.3
cytoplasmic translation	5	22.7
negative regulation of meiotic cell cycle	5	21.7
regulation of cytokinetic process	5	21.7
NAD metabolic process	5	21.7
beta-glucan biosynthetic process	4	50
NADH metabolic process	4	44.4

positive regulation of conidium formation	4	40
septin ring organization	4	36.4
protein secretion	4	36.4
cell wall chitin metabolic process	4	33.3
negative regulation of sexual sporulation resulting in formation of a cellular spore	4	33.3
unidimensional cell growth	4	30.8
microtubule polymerization	4	30.8
ascospore wall assembly	4	30.8
developmental growth involved in morphogenesis	4	30.8
fungal-type cell wall assembly	4	28.6
polyol catabolic process	4	28.6
cell-substrate adhesion	4	26.7
regulation of cell size	4	26.7
response to antibiotic	3	75
cell tip growth	3	75
cell wall beta-glucan metabolic process	3	75
pyrimidine ribonucleoside biosynthetic process	3	60
pyrimidine nucleoside biosynthetic process	3	60
(1->3)-beta-D-glucan metabolic process	3	60
cell adhesion involved in biofilm formation	3	50
submerged biofilm formation	3	50
regulation of actomyosin contractile ring contraction	3	50
gluconeogenesis	3	50
cell adhesion involved in single-species biofilm formation	3	50
septin ring assembly	3	42.9
mitochondrial electron transport, cytochrome c to oxygen	3	42.9
development of symbiont involved in interaction with host	3	42.9
development of symbiont in host	3	42.9
development involved in symbiotic interaction	3	42.9
hexose biosynthetic process	3	37.5
monosaccharide biosynthetic process	3	37.5
mitophagy	3	33.3
regulation of conidiophore development	3	33.3
mitochondrion disassembly	3	33.3
fungal-type cell wall beta-glucan biosynthetic process	2	100

fungal-type cell wall beta-glucan metabolic process	2	100
eisosome assembly	2	100
endocytic recycling	2	100
response to superoxide	2	66.7
aspartate transport	2	66.7
cellular response to superoxide	2	66.7
pentitol catabolic process	2	66.7
cell wall (1->3)-beta-D-glucan biosynthetic process	2	66.7
xylulose 5-phosphate metabolic process	2	66.7
glycolytic fermentation to ethanol	2	66.7
cellular response to oxygen radical	2	66.7
pentitol metabolic process	2	66.7
cellular age-dependent response to reactive oxygen species	2	66.7
hexose catabolic process to ethanol	2	66.7
regulation of ribosomal protein gene transcription from RNA polymerase II promoter	2	66.7
siroheme metabolic process	2	66.7
ethanol biosynthetic process involved in glucose fermentation to ethanol	2	66.7
cell wall beta-glucan biosynthetic process	2	66.7
negative regulation of cell development	2	66.7
xylulose 5-phosphate biosynthetic process	2	66.7
age-dependent response to reactive oxygen species	2	66.7
induction of conjugation upon nitrogen starvation	2	66.7
protein autophosphorylation	2	66.7
cell wall (1->3)-beta-D-glucan metabolic process	2	66.7
response to oxygen radical	2	66.7
pentose-phosphate shunt, oxidative branch	2	66.7
siroheme biosynthetic process	2	66.7
induction of conjugation upon nutrient starvation	2	66.7

Table S3-8 List of All_orthogroups$_{N:N:N}$ consistently regulated by WetA

Expression pattern in Δ*wetA* conidia (Ani/Afu/Afl)	*A. nidulans* Gene ID	*A. fumigatus* Gene ID	*A. flavus* Gene ID
↑— —	AN6673	Afu5g01190	AFLA_053690 AFLA_077650
	AN6338	Afu2g13630	AFLA_099700 AFLA_136830
	AN2598 AN7557	Afu1g17570	AFLA_024150 AFLA_089810
	AN3546	Afu4g14400 Afu6g09300	AFLA_040660 AFLA_040670
—↑—	AN8261	Afu5g04130	AFLA_006900 AFLA_035750
	AN11188	Afu4g00230	AFLA_010030 AFLA_063630
	AN4175	Afu6g07950	AFLA_036680 AFLA_126010
	AN7832	Afu5g00630	AFLA_037690 AFLA_092920
	AN7147	Afu4g03590	AFLA_040470 AFLA_121350
	AN5984	Afu2g10240	AFLA_044390 AFLA_090140
	AN11010	Afu5g07440	AFLA_061180 AFLA_061190
	AN1060	Afu1g12332	AFLA_067020 AFLA_067030
	AN4791	Afu3g06800	AFLA_100980 AFLA_100990
	AN10834 AN12335	Afu5g06500	AFLA_049020
	AN11089 AN1129	Afu1g11650	AFLA_068070
	AN0146 AN7806	Afu2g17560	AFLA_139300
	AN6385	Afu7g07030 Afu7g08290	AFLA_124690

	AN4135 AN6731	Afu7g05920	AFLA_004460 AFLA_076880
	AN1840 AN4353	Afu4g06380	AFLA_024060 AFLA_113580
	AN4082 AN4084	Afu1g05580 Afu1g05595	AFLA_050910
——↑	AN0970	Afu2g12500	AFLA_035810 AFLA_135320
	AN0119	Afu5g11790	AFLA_092780 AFLA_092790
	AN3341 AN5427	Afu8g02660	AFLA_057800
	AN5348 AN6432	Afu6g14160	AFLA_105410
	AN4818	Afu3g07130 Afu8g06140	AFLA_101910
↑↑—	AN5990	Afu2g10160	AFLA_001930 AFLA_044460
	AN7027	Afu4g04250	AFLA_009860 AFLA_114880
	AN4590	Afu2g02110	AFLA_043090 AFLA_097730
	AN3236	Afu6g01830	AFLA_060450 AFLA_120990
	AN3396 AN8513	Afu5g00150	AFLA_044200
	AN6477 AN8610	Afu1g01930	AFLA_108170
	AN8502	Afu3g01370 Afu5g13290	AFLA_054130
	AN2180	Afu2g08110 Afu2g15700	AFLA_015310 AFLA_023380
	AN5146	Afu1g07200	AFLA_022200 AFLA_063650 AFLA_079950
	AN3790 AN9042	Afu2g03980	AFLA_034920 AFLA_087590 AFLA_124510

	AN1950	Afu2g17650 Afu4g13340	AFLA_052220 AFLA_069580 AFLA_075860
—↑↑	AN11080	Afu2g18040	AFLA_083250 AFLA_139480
	AN3393 AN7962	Afu4g13750	AFLA_065450
	AN0773	Afu1g14340 Afu7g04970	AFLA_042050
↑↑↑	AN4385	Afu4g06750	AFLA_113190 AFLA_113200
	AN12126 AN9187	Afu4g09270	AFLA_003770
	AN1428	Afu1g00450 Afu8g04100	AFLA_012050
	AN1418	Afu1g00480 Afu8g04070	AFLA_012080
	AN9205	Afu5g00100 Afu7g06523	AFLA_038870
	AN11043	Afu3g01610 Afu4g09300	AFLA_110180
	AN9305	Afu3g14730 Afu7g00200	AFLA_117500
	AN1427	Afu1g00440 Afu8g04110 Afu8g06560	AFLA_012030
	AN2018 AN3402	Afu2g00710 Afu4g10130	AFLA_026140
	AN10062 AN5559	Afu4g00570 Afu4g11780	AFLA_027800
	AN6470 AN8969	Afu6g10130 Afu8g06980	AFLA_104880
↑↑↓	AN8019	Afu1g01020 Afu8g06400	AFLA_136760
	AN1620 AN2626	Afu8g02040	AFLA_120950 AFLA_137460
↑↓—	AN1821	Afu8g07040	AFLA_009480 AFLA_072930

	AN2175	Afu2g14840	AFLA_010110 AFLA_076820
	AN3863	Afu5g10040	AFLA_052940 AFLA_135160
	AN6802	Afu5g14315	AFLA_058300 AFLA_126460
	AN7201	Afu6g10250	AFLA_106870 AFLA_138570
	AN11209 AN8768	Afu7g00280	AFLA_138480
	AN1430	Afu1g00470 Afu8g04080	AFLA_012070
−↑↓	AN7083	Afu8g00380	AFLA_003940 AFLA_041480
	AN9168	Afu8g05710	AFLA_097330 AFLA_126340
↑↓↑	AN3349	Afu2g14430	AFLA_007470 AFLA_024080
	AN2530 AN3555 AN7892	Afu3g14540	AFLA_060260
↑↓↓	AN1519	Afu8g05280	AFLA_021300 AFLA_078690
	AN8102	Afu3g01220	AFLA_038650 AFLA_121260
	AN9189	Afu5g00110 Afu7g00260	AFLA_125760
↑−↑	AN3117	Afu3g12740	AFLA_020960 AFLA_053470
	AN6227	Afu2g13295	AFLA_089880 AFLA_136340
	AN0118	Afu5g11810	AFLA_092800 AFLA_092810
↓−−	AN0507	Afu6g11450	AFLA_039960 AFLA_125160
	AN1227	Afu1g10525	AFLA_069160 AFLA_069170
	AN8787	Afu5g09850	AFLA_098220

			AFLA_098230
	AN3851	Afu4g07880	AFLA_111860 AFLA_111870
	AN1807	Afu3g02100	AFLA_113330 AFLA_120230
	AN3975	Afu3g15150 Afu6g03300	AFLA_115820
—↓—	AN3368	Afu7g01320	AFLA_079930 AFLA_117830
	AN10954	Afu2g06050	AFLA_096560 AFLA_130180
	AN6820	Afu4g00660	AFLA_119980 AFLA_123400
	AN12097 AN6395	Afu1g17230	AFLA_014290
	AN0509 AN7613	Afu6g13720	AFLA_054470
	AN4532 AN8998	Afu2g02910	AFLA_133150
	AN7598	Afu2g15420 Afu6g11720	AFLA_038270
	AN3414	Afu1g15970 Afu8g01560	AFLA_077260
	AN5483	Afu1g00630 Afu6g13290 Afu7g08480	AFLA_033660
	AN3369 AN6832	Afu6g12130 Afu7g01310	AFLA_117820
——↓	AN8063	Afu5g01880	AFLA_024970 AFLA_101770
	AN2237	Afu5g07330	AFLA_056810 AFLA_107990
	AN10869 AN1789	Afu2g14650	AFLA_043510
↓↑—	AN8814	Afu5g09470	AFLA_035450 AFLA_098530
	AN4182	Afu6g07980	AFLA_036730 AFLA_125910

	AN3489	Afu5g07540	AFLA_059060 AFLA_104630
	AN1506	Afu8g05090	AFLA_078840 AFLA_127110
	AN6730 AN6932	Afu7g05910	AFLA_076870
	AN1429	Afu1g00460 Afu8g04090	AFLA_012060
—↓↑	AN4153	Afu6g09640	AFLA_064450 AFLA_108700
	AN5591	Afu4g11460 Afu4g11467	AFLA_027440
	AN9021	Afu1g16070 Afu5g14980	AFLA_040590
↓↑↑	AN2834	Afu2g17250	AFLA_002200 AFLA_096230
↓↑↓	AN1414	Afu1g00580 Afu8g04050	AFLA_012100
↓↓—	AN10626 AN1523	Afu8g05320	AFLA_078650
	AN1772	Afu6g00450 Afu6g09040	AFLA_128870
—↓↓	AN2674	Afu5g14270	AFLA_002100 AFLA_066060
	AN6608	Afu6g04190	AFLA_056000 AFLA_056010
	AN11701	Afu4g03550 Afu6g04370	AFLA_120360
	AN8467	Afu1g11050 Afu3g01230	AFLA_097900 AFLA_117440
↓↓↓	AN0391	Afu1g01490	AFLA_003760 AFLA_013730
	AN8621	Afu3g02780	AFLA_008410 AFLA_015590
	AN5563	Afu4g11730	AFLA_027730 AFLA_049340
	AN0895	Afu1g15610	AFLA_061270 AFLA_083890

	AN11094	Afu6g10120	AFLA_077990 AFLA_110070
	AN6349	Afu2g14180	AFLA_105670 AFLA_105680
	AN0963 AN10629	Afu3g01900	AFLA_097450
	AN10421 AN3523	Afu6g13470	AFLA_097700
	AN3243 AN8549	Afu5g01248	AFLA_138550
	AN8639	Afu4g03190 Afu5g14300	AFLA_002830
	AN2470	Afu2g00170 Afu5g03930	AFLA_006730
	AN8587	Afu1g00500 Afu8g06000	AFLA_028100
	AN4913	Afu3g00370 Afu3g10760	AFLA_019650 AFLA_031900
	AN7632	Afu2g01040	AFLA_058310 AFLA_059790 AFLA_072340
	AN11921	Afu8g07200	AFLA_062120 AFLA_072920 AFLA_120010
↓−↑	AN3539	Afu8g00120	AFLA_126540 AFLA_138450
	AN2463 AN3201	Afu5g14550	AFLA_016530
	AN9022	Afu1g16060 Afu5g14970	AFLA_040580
↓−↓	AN6451 AN7295	Afu2g16860	AFLA_002590
	AN2422 AN2785	Afu4g09860	AFLA_025860
	AN5500	Afu5g00820 Afu6g14570	AFLA_049540

Table S3-9 Top enriched GO categories of orthologs species-specifically regulated in Δ*wetA* conidia

GO Category	# of Genes	% of Genes in Category
A. nidulans		
cellular process	652	15.2
metabolic process	583	13.8
single-organism process	526	13.5
organic substance metabolic process	482	15.2
cellular metabolic process	459	16.1
primary metabolic process	449	15.8
single-organism cellular process	421	14.2
macromolecule metabolic process	319	17.1
nitrogen compound metabolic process	314	17.1
single-organism metabolic process	301	13
cellular macromolecule metabolic process	300	17.8
cellular nitrogen compound metabolic process	273	17.5
organic cyclic compound metabolic process	249	16
biological regulation	236	16
heterocycle metabolic process	232	16.5
cellular aromatic compound metabolic process	228	16.3
biosynthetic process	225	14.9
organic substance biosynthetic process	221	15.6
cellular component organization or biogenesis	217	19.4
regulation of biological process	215	15.8
cellular biosynthetic process	213	15.7
nucleobase-containing compound metabolic process	210	17.6
response to stimulus	193	17.1
regulation of cellular process	188	15
nucleic acid metabolic process	176	18.7
cellular response to stimulus	175	17.5
cellular component organization	174	17.8
gene expression	157	18.9
organonitrogen compound metabolic process	150	16.3
protein metabolic process	148	17
small molecule metabolic process	148	15.1
regulation of metabolic process	143	14.7
cellular nitrogen compound biosynthetic process	135	16.3

RNA metabolic process	132	19.3
cellular protein metabolic process	132	17
organelle organization	131	18.3
regulation of cellular metabolic process	127	14
regulation of macromolecule metabolic process	125	14.2
regulation of primary metabolic process	125	14.2
response to stress	124	17.2
macromolecule biosynthetic process	124	16.4
cellular macromolecule biosynthetic process	122	16.4
organonitrogen compound biosynthetic process	120	19.4
cellular component biogenesis	117	21.8
single-organism biosynthetic process	114	14.1
response to chemical	108	18.9
cellular response to chemical stimulus	99	19.8
cellular localization	93	16
cellular response to stress	91	15.7
oxoacid metabolic process	87	15.8
organic acid metabolic process	87	15.6
carboxylic acid metabolic process	85	15.7
organic substance catabolic process	84	14.6
macromolecular complex subunit organization	83	19.3
cell cycle	81	17.9
reproduction	80	18
cell cycle process	79	17.9
macromolecule modification	77	14.8
single-organism organelle organization	75	16.7
RNA processing	72	25.5
growth	72	18.7
developmental process	71	16.1
cellular catabolic process	69	15.4
ncRNA metabolic process	68	27.6
single-organism developmental process	68	16.3
multi-organism process	67	16.8
macromolecule localization	67	15.4
cellular component assembly	66	17.8
filamentous growth	65	17.8
cellular amide metabolic process	64	19.2
small molecule biosynthetic process	64	16.8
positive regulation of biological process	63	17.5

anatomical structure development	63	16.7
negative regulation of biological process	63	16.5
protein localization	60	16
amide biosynthetic process	59	20.2
cell communication	59	16.2
ribonucleoprotein complex biogenesis	56	29
peptide metabolic process	56	22.1
cellular macromolecule localization	56	16.1
regulation of biological quality	54	18.9
negative regulation of cellular process	54	16
cellular protein localization	54	15.7
ribosome biogenesis	53	31.2
ncRNA processing	53	28.3
protein complex subunit organization	53	19.8
DNA metabolic process	53	17.4
positive regulation of cellular process	53	17.2
peptide biosynthetic process	52	22
macromolecular complex assembly	52	18.9
cellular amino acid metabolic process	51	17.6
chromosome organization	50	19.9
anatomical structure morphogenesis	50	16
reproductive process	50	15.8
anatomical structure formation involved in morphogenesis	48	16.1
cellular macromolecular complex assembly	47	20.5
sporulation	46	15.8
translation	45	22.7
developmental process involved in reproduction	44	15.8
rRNA metabolic process	43	37.1
rRNA processing	43	37.1
multi-organism reproductive process	43	16.6
sexual reproduction	43	16.6
organic acid biosynthetic process	42	16.5
carboxylic acid biosynthetic process	42	16.5
regulation of cell cycle	42	15.9
proteolysis	41	16
regulation of transcription from RNA polymerase II promoter	39	21
vesicle-mediated transport	37	18
meiotic cell cycle	37	17.2
mitotic cell cycle process	36	17.6

mitotic cell cycle	36	17.5
meiotic cell cycle process	36	17.1
response to drug	36	17.1
cellular response to nutrient levels	35	17
cellular response to extracellular stimulus	35	16.9
cellular response to external stimulus	35	16.6
response to nutrient levels	35	16.4
response to extracellular stimulus	35	16.4
cellular response to DNA damage stimulus	34	16.6
response to organic substance	33	21.3
hyphal growth	33	18.2
cellular response to drug	33	18.1
alpha-amino acid metabolic process	33	17.1
DNA repair	32	18.8
cellular macromolecule catabolic process	32	17.5
regulation of response to stimulus	31	21.1
homeostatic process	31	18.5
protein complex assembly	31	17.9
protein complex biogenesis	31	17.8
response to inorganic substance	29	20.7
sulfur compound metabolic process	28	20.4
cellular amino acid biosynthetic process	28	19.2
cytoskeleton organization	28	18.7
cell division	27	22.1
cellular response to organic substance	27	21.8
chromatin organization	27	19.7
organelle fission	27	17.6
response to metal ion	26	21.8
regulation of growth	26	20.2
nuclear division	26	17.8
regulation of molecular function	25	18.8
response to toxic substance	24	23.8
cellular response to metal ion	24	22.6
cellular response to inorganic substance	24	22.4
tRNA metabolic process	24	21.2
cellular homeostasis	24	19.2
detoxification	23	26.4
chromosome segregation	23	21.9
detoxification of inorganic compound	22	26.8

stress response to cadmium ion	22	26.8
detoxification of cadmium ion	22	26.8
stress response to metal ion	22	26.8
response to cadmium ion	22	25.3
mRNA metabolic process	22	20.4
ribosomal large subunit biogenesis	21	42.9
cellular response to cadmium ion	21	25.6
sulfur compound biosynthetic process	21	23.3
nuclear chromosome segregation	21	21.6
aromatic compound catabolic process	21	21.4
alpha-amino acid biosynthetic process	21	20.4
organic cyclic compound catabolic process	21	18.8
regulation of cell communication	20	23.3
DNA replication	20	19.6
protein-DNA complex subunit organization	19	24.1
regulation of signal transduction	19	23.5
regulation of signaling	19	22.9
heterocycle catabolic process	19	21.1
cellular ion homeostasis	19	20.9
cellular nitrogen compound catabolic process	19	20.9
monosaccharide metabolic process	19	20.2
ion homeostasis	19	19.8
cellular chemical homeostasis	19	19.2
actin filament-based process	18	25.7
regulation of cellular component biogenesis	18	24.3
cytokinesis	18	21.7
regulation of gene expression, epigenetic	18	20.5
inorganic ion homeostasis	18	20.2
DNA conformation change	17	28.3
actin cytoskeleton organization	17	25
filamentous growth of a population of unicellular organisms in response to starvation	17	19.3
maturation of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	16	40
maturation of 5.8S rRNA	16	40
DNA-dependent DNA replication	16	26.2
sister chromatid segregation	16	20.5
negative regulation of transcription from RNA polymerase II promoter	15	27.3
nucleic acid phosphodiester bond hydrolysis	15	26.3

cytokinetic process	15	25.9
ribosomal small subunit biogenesis	15	25
protein folding	15	20.8
maturation of SSU-rRNA	14	27.5
protein-DNA complex assembly	14	26.4
nucleobase-containing compound catabolic process	14	24.6
ribonucleoprotein complex assembly	14	23
RNA splicing	14	22.6
ribonucleoprotein complex subunit organization	14	21.9
regulation of response to stress	14	20.9
positive regulation of response to stimulus	14	20.6
RNA modification	14	20.6
maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	13	29.5
RNA phosphodiester bond hydrolysis	13	27.7
RNA splicing, via transesterification reactions	13	25
cleavage involved in rRNA processing	12	34.3
nucleosome organization	12	26.1
cellular aldehyde metabolic process	12	25
DNA packaging	12	23.5
endocytosis	12	23.1
aspartate family amino acid metabolic process	12	22.6
ribosome assembly	11	29.7
sulfur amino acid metabolic process	11	28.2
chromatin assembly or disassembly	11	25.6
cell wall polysaccharide metabolic process	11	25
regulation of hydrolase activity	11	23.4
regulation of cellular ketone metabolic process	11	22.4
chromatin remodeling	11	22.4
tRNA aminoacylation for protein translation	10	28.6
chromatin assembly	10	27
RNA catabolic process	10	26.3
tRNA aminoacylation	10	26.3
amino acid activation	10	26.3
post-Golgi vesicle-mediated transport	10	25.6
regulation of cytoskeleton organization	10	25
cellular component disassembly	10	23.8
ribosomal large subunit assembly	9	40.9
DNA replication initiation	9	37.5

endonucleolytic cleavage involved in rRNA processing	9	36
sulfur amino acid biosynthetic process	9	36
endonucleolytic cleavage of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	9	36
methionine metabolic process	9	34.6
RNA phosphodiester bond hydrolysis, endonucleolytic	9	34.6
regulation of actin cytoskeleton organization	9	33.3
regulation of actin filament-based process	9	33.3
regulation of cellular component size	9	31
regulation of anatomical structure size	9	31
cortical actin cytoskeleton organization	9	31
cortical cytoskeleton organization	9	30
regulation of cellular response to stress	9	29
serine family amino acid metabolic process	9	26.5
water-soluble vitamin biosynthetic process	9	25
RNA 3'-end processing	9	25
aspartate family amino acid biosynthetic process	9	25
vitamin biosynthetic process	9	25
water-soluble vitamin metabolic process	9	24.3
maturation of LSU-rRNA	8	44.4
methionine biosynthetic process	8	36.4
ncRNA 3'-end processing	8	36.4
endonucleolytic cleavage in ITS1 to separate SSU-rRNA from 5.8S rRNA and LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	8	36.4
response to nitrogen compound	8	34.8
protein complex disassembly	8	33.3
macromolecular complex disassembly	8	28.6
mRNA catabolic process	8	27.6
nuclear-transcribed mRNA catabolic process	8	27.6
phytosteroid metabolic process	8	26.7
ergosterol metabolic process	8	26.7
cellular alcohol metabolic process	8	26.7
cellular response to pheromone	8	26.7
endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	7	50
rRNA 5'-end processing	7	46.7
RNA 5'-end processing	7	46.7
ncRNA 5'-end processing	7	46.7

endonucleolytic cleavage to generate mature 5'-end of SSU-rRNA from (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	7	46.7
establishment or maintenance of cytoskeleton polarity	7	43.8
maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	7	43.8
nucleosome assembly	7	36.8
translational initiation	7	35
hemicellulose metabolic process	7	33.3
ncRNA transcription	7	30.4
mitochondrial genome maintenance	7	29.2
sphingolipid metabolic process	7	29.2
rRNA modification	6	54.5
postreplication repair	6	40
snRNA metabolic process	6	40
cysteine metabolic process	6	37.5
cellular protein complex disassembly	6	37.5
cellular response to nitrogen compound	6	33.3
regulation of carbohydrate biosynthetic process	6	31.6
protein stabilization	5	83.3
rRNA catabolic process	5	71.4
DNA geometric change	5	62.5
DNA duplex unwinding	5	62.5
ncRNA catabolic process	5	62.5
regulation of transcription during mitosis	5	62.5
regulation of protein stability	5	62.5
cellular response to oxygen levels	5	50
response to oxygen levels	5	50
snoRNA processing	5	41.7
establishment or maintenance of actin cytoskeleton polarity	5	41.7
positive regulation of chromatin modification	5	41.7
response to organic cyclic compound	5	38.5
snoRNA metabolic process	5	38.5
regulation of actin filament polymerization	5	35.7
response to endogenous stimulus	5	35.7
DNA unwinding involved in DNA replication	4	100
translational termination	4	50
sulfate assimilation	4	50
'de novo' IMP biosynthetic process	4	50
snoRNA 3'-end processing	4	44.4

D-xylose metabolic process	4	44.4
IMP metabolic process	4	44.4
IMP biosynthetic process	4	44.4
phosphate ion transport	4	44.4
glycerol catabolic process	3	75
negative regulation of actin filament polymerization	3	75
negative regulation of protein polymerization	3	60
regulation of cellular response to oxidative stress	3	60
N-terminal protein amino acid acetylation	3	60
polyadenylation-dependent RNA catabolic process	3	60
divalent inorganic anion homeostasis	3	60
tRNA catabolic process	3	60
nuclear polyadenylation-dependent ncRNA catabolic process	3	60
nuclear polyadenylation-dependent tRNA catabolic process	3	60
nuclear ncRNA surveillance	3	60
chromatin remodeling at centromere	3	60
polyadenylation-dependent ncRNA catabolic process	3	60
cellular divalent inorganic anion homeostasis	3	60
regulation of response to oxidative stress	3	60
galactonate metabolic process	3	60
A. fumigatus		
cellular process	720	18.4
single-organism process	634	18.2
metabolic process	592	15.6
single-organism cellular process	515	19
organic substance metabolic process	481	16.5
cellular metabolic process	462	17.9
primary metabolic process	448	17.2
single-organism metabolic process	330	16.8
macromolecule metabolic process	328	18.6
cellular macromolecule metabolic process	315	19.7
nitrogen compound metabolic process	290	17.4
biological regulation	259	19
cellular nitrogen compound metabolic process	259	17.9
biosynthetic process	244	17.7
cellular component organization or biogenesis	241	21.8
organic cyclic compound metabolic process	238	17
organic substance biosynthetic process	236	18
regulation of biological process	235	18.8

cellular biosynthetic process	235	18.3
localization	234	18.6
heterocycle metabolic process	223	17.2
cellular aromatic compound metabolic process	220	17.4
cellular component organization	219	22.5
establishment of localization	213	18.6
transport	208	18.6
regulation of cellular process	207	18.2
response to stimulus	201	18.8
nucleobase-containing compound metabolic process	194	17.8
protein metabolic process	188	21.8
single-organism localization	188	18.2
single-organism transport	180	18.2
cellular response to stimulus	179	19.2
cellular protein metabolic process	178	23.1
organelle organization	177	24.6
nucleic acid metabolic process	162	18.4
regulation of metabolic process	150	17.1
macromolecule biosynthetic process	148	20.8
cellular nitrogen compound biosynthetic process	148	19.2
gene expression	148	18.7
cellular macromolecule biosynthetic process	147	20.8
organonitrogen compound metabolic process	143	17.6
cellular localization	138	23.8
response to stress	134	19.4
single-organism organelle organization	122	26.9
establishment of localization in cell	114	24.7
RNA metabolic process	114	17.6
organonitrogen compound biosynthetic process	113	19.4
cellular response to stress	111	20.7
cell cycle	109	24.2
macromolecule modification	109	21.3
cell cycle process	107	24.3
cellular component biogenesis	107	20.3
intracellular transport	103	24.7
macromolecule localization	100	23.7
protein modification process	99	22
cellular protein modification process	99	22
organic substance transport	96	21.3

protein localization	94	25.5
developmental process	94	21.6
growth	92	24.4
filamentous growth	91	25.2
single-organism developmental process	91	21.9
macromolecular complex subunit organization	90	21.3
reproduction	90	20.8
cellular protein localization	89	26.2
cellular macromolecule localization	89	26
single-organism intracellular transport	88	24.2
phosphorus metabolic process	84	17.4
anatomical structure development	83	22
oxoacid metabolic process	83	18
organic acid metabolic process	83	17.8
cellular component assembly	82	22.5
carboxylic acid metabolic process	81	17.9
phosphate-containing compound metabolic process	81	17.5
cytoplasmic transport	76	24.3
cell communication	76	21.5
negative regulation of biological process	75	20.1
nucleobase-containing compound biosynthetic process	72	17.9
cellular amide metabolic process	71	21.1
multi-organism process	71	18
establishment of protein localization	69	26.5
positive regulation of biological process	69	19.7
protein transport	68	27
amide biosynthetic process	68	22.7
anatomical structure morphogenesis	68	22
regulation of cell cycle	67	26.5
reproductive process	67	21.2
intracellular protein transport	64	27.8
negative regulation of cellular process	64	19.5
macromolecular complex assembly	63	23.1
anatomical structure formation involved in morphogenesis	63	21.4
peptide metabolic process	61	22.6
developmental process involved in reproduction	61	21.7
peptide biosynthetic process	60	23.8
DNA metabolic process	60	21.2
positive regulation of cellular process	60	19.8

regulation of cellular component organization	59	23.7
chromosome organization	58	22.9
vesicle-mediated transport	57	27.8
single-organism cellular localization	57	24.7
sporulation	57	20.1
regulation of biological quality	57	19.8
regulation of cell cycle process	54	28.1
translation	54	26.7
protein complex subunit organization	54	20.5
hyphal growth	53	29.1
cellular macromolecular complex assembly	53	23.7
cellular developmental process	52	21.7
macromolecule catabolic process	52	18.6
protein localization to organelle	51	28.3
sexual reproduction	51	19.9
multi-organism reproductive process	51	19.9
ion transport	51	18.8
meiotic cell cycle	50	22.7
regulation of mitotic cell cycle	49	30.4
meiotic cell cycle process	49	22.8
mitotic cell cycle	47	22.6
mitotic cell cycle process	47	22.6
cell differentiation	47	21.7
response to external stimulus	46	19.2
regulation of organelle organization	45	24.1
cellular amino acid metabolic process	45	19.1
organelle fission	44	28
response to nutrient levels	44	21.1
response to extracellular stimulus	44	21
cellular response to DNA damage stimulus	43	21.4
sporulation resulting in formation of a cellular spore	43	21
negative regulation of metabolic process	43	19.1
nuclear division	42	28
cellular macromolecule catabolic process	42	23.5
filamentous growth of a population of unicellular organisms	42	22.5
asexual reproduction	42	20.9
response to starvation	41	21.9
cellular response to nutrient levels	41	20.4
cellular response to extracellular stimulus	41	20.3

cellular response to external stimulus	41	20
protein targeting	40	27.4
regulation of cell division	39	28.3
establishment of protein localization to organelle	39	26.9
protein complex assembly	39	22.5
protein complex biogenesis	39	22.4
homeostatic process	39	21.9
cytoskeleton organization	38	26.6
mitochondrion organization	38	24.7
single organism signaling	38	22.2
signal transduction	38	22.2
signaling	38	22.2
cellular response to starvation	38	21.1
asexual sporulation	38	20.7
DNA repair	37	22.4
single-multicellular organism process	37	22
multicellular organismal process	37	22
single organism reproductive process	37	21.6
single-organism membrane organization	36	25.9
membrane organization	36	25.7
organ development	36	22
system development	36	22
spore-bearing organ development	36	22
reproductive system development	36	22
reproductive structure development	36	22
multicellular organismal development	36	21.8
reproduction of a single-celled organism	34	22.7
cation transport	34	21.5
negative regulation of cell cycle	33	26
carbohydrate derivative biosynthetic process	33	20.4
Golgi vesicle transport	32	32.7
chromosome segregation	32	31.1
regulation of nuclear division	32	29.4
regulation of molecular function	32	24.1
sexual sporulation resulting in formation of a cellular spore	32	21.2
sexual sporulation	32	21.2
regulation of growth	31	24.8
regulation of developmental process	31	24
regulation of response to stimulus	31	21.2

organophosphate biosynthetic process	31	20.4
vacuolar transport	30	36.6
peptidyl-amino acid modification	30	30.9
reproductive process in single-celled organism	30	22.1
protein catabolic process	30	20.4
establishment or maintenance of cell polarity	29	24
regulation of catalytic activity	29	23.8
cell development	29	21.6
cellular protein catabolic process	29	20.9
microtubule-based process	28	31.1
nuclear chromosome segregation	28	29.5
negative regulation of cell cycle process	28	26.9
regulation of anatomical structure morphogenesis	28	23.5
cellular protein complex assembly	28	23
proteolysis involved in cellular protein catabolic process	28	21.2
cellular homeostasis	28	20.7
mitotic nuclear division	27	30.7
chemical homeostasis	27	24.3
modification-dependent protein catabolic process	27	23.1
ubiquitin-dependent protein catabolic process	27	23.1
modification-dependent macromolecule catabolic process	27	22.3
ascospore formation	27	22.3
intracellular signal transduction	27	21.6
regulation of mitotic cell cycle phase transition	26	28.3
regulation of cell cycle phase transition	26	28
tRNA metabolic process	26	22.6
regulation of cell communication	25	27.2
sporocarp development	25	21.4
regulation of mitotic nuclear division	24	37.5
regulation of signaling	24	26.7
cellular chemical homeostasis	24	23.1
microtubule cytoskeleton organization	23	31.9
organelle assembly	23	30.3
sister chromatid segregation	23	29.9
regulation of signal transduction	23	26.4
ion homeostasis	23	23
positive regulation of cellular component organization	23	22.1
mitotic sister chromatid segregation	21	31.8
regulation of localization	21	31.3

cation homeostasis	21	23.1
cellular ion homeostasis	21	22.1
protein localization to vacuole	20	42.6
negative regulation of mitotic cell cycle	20	28.6
metal ion transport	20	25
heterocycle catabolic process	20	24.7
establishment of protein localization to vacuole	19	41.3
protein targeting to vacuole	19	41.3
cellular response to nitrogen starvation	19	28.8
cellular response to nitrogen levels	19	28.8
regulation of catabolic process	19	25
peptidyl-lysine modification	18	31.6
small GTPase mediated signal transduction	18	30.5
regulation of intracellular signal transduction	18	28.1
regulation of chromosome organization	18	26.1
ER to Golgi vesicle-mediated transport	17	36.2
vacuole organization	17	34
proteasomal protein catabolic process	17	25.8
proteasome-mediated ubiquitin-dependent protein catabolic process	17	25.8
meiotic nuclear division	17	25.4
tRNA processing	17	24.3
transcription from RNA polymerase II promoter	17	24.3
positive regulation of cell cycle process	16	35.6
positive regulation of cell cycle	16	34
covalent chromatin modification	16	25.8
phospholipid biosynthetic process	16	25.8
histone modification	16	25.8
actin cytoskeleton organization	16	25
actin filament-based process	16	24.2
regulation of sporulation	16	23.9
autophagy	15	41.7
regulation of transport	15	28.3
endosomal transport	15	25.9
positive regulation of mitotic cell cycle	14	50
regulation of hydrolase activity	14	30.4
protein-DNA complex assembly	14	28
negative regulation of mitotic cell cycle phase transition	14	26.4
negative regulation of cell cycle phase transition	14	25.9

protein acylation	13	34.2
establishment of cell polarity	13	27.7
regulation of protein catabolic process	13	26.5
intracellular protein transmembrane transport	13	26
protein transmembrane transport	13	26
cellular amine metabolic process	13	25.5
regulation of small GTPase mediated signal transduction	12	36.4
protein acetylation	12	35.3
growth of unicellular organism as a thread of attached cells	12	34.3
positive regulation of cell division	12	31.6
regulation of Ras protein signal transduction	11	42.3
peptidyl-lysine acetylation	11	37.9
internal protein amino acid acetylation	11	37.9
internal peptidyl-lysine acetylation	11	37.9
movement of cell or subcellular component	11	33.3
regulation of chromosome segregation	11	31.4
nucleus organization	11	30.6
regulation of cellular localization	11	29.7
nucleotide-excision repair	11	28.9
RNA catabolic process	11	28.9
single-organism membrane invagination	10	52.6
membrane invagination	10	52.6
lysosomal microautophagy	10	52.6
regulation of GTPase activity	10	33.3
syncytium formation by plasma membrane fusion	10	32.3
protein maturation	10	32.3
organelle fusion	10	32.3
syncytium formation	10	32.3
positive regulation of nuclear division	10	32.3
spindle organization	10	32.3
divalent inorganic cation transport	10	31.3
regulation of sister chromatid segregation	10	31.3
regulation of cytokinesis	10	31.3
nucleobase metabolic process	10	30.3
establishment of nucleus localization	10	30.3
nucleus localization	10	30.3
secretion by cell	10	29.4
secretion	10	29.4
regulation of conidium formation	10	29.4

negative regulation of cell cycle G2/M phase transition	10	28.6
negative regulation of G2/M transition of mitotic cell cycle	10	28.6
CVT pathway	9	64.3
sister chromatid cohesion	9	42.9
negative regulation of catabolic process	9	36
meiotic chromosome segregation	9	33.3
chromatin silencing at silent mating-type cassette	9	33.3
histone acetylation	9	33.3
positive regulation of cellular amide metabolic process	9	31
protein dephosphorylation	9	31
late endosome to vacuole transport	9	30
positive regulation of cell communication	9	30
regulation of mitotic sister chromatid segregation	9	30
positive regulation of mitotic nuclear division	8	61.5
piecemeal microautophagy of nucleus	8	57.1
nucleophagy	8	57.1
mitotic sister chromatid cohesion	8	50
regulation of barrier septum assembly	8	47.1
pseudohyphal growth	8	44.4
regulation of mitotic cytokinetic process	8	42.1
regulation of mitotic cytokinesis	8	42.1
regulation of vesicle-mediated transport	8	40
invasive growth in response to glucose limitation	8	40
regulation of cell septum assembly	8	40
signal transduction involved in conjugation with cellular fusion	8	38.1
invasive filamentous growth	8	36.4
regulation of cytokinetic process	8	34.8
regulation of DNA-dependent DNA replication	8	33.3
amide transport	8	33.3
chromosome condensation	7	46.7
organelle disassembly	7	41.2
negative regulation of intracellular signal transduction	7	41.2
macroautophagy	7	41.2
homologous chromosome segregation	7	41.2
carbon utilization	7	36.8
acetate catabolic process	6	75
mitochondrion disassembly	6	66.7
mitophagy	6	66.7
regulation of penicillin biosynthetic process	6	60

acetate metabolic process	6	50
positive regulation of cell cycle phase transition	6	46.2
positive regulation of mitotic cell cycle phase transition	6	46.2
metallo-sulfur cluster assembly	6	40
iron-sulfur cluster assembly	6	40
regulation of penicillin metabolic process	6	37.5
pre-replicative complex assembly involved in cell cycle DNA replication	5	55.6
pre-replicative complex assembly involved in nuclear cell cycle DNA replication	5	55.6
pre-replicative complex assembly	5	55.6
positive regulation of chromosome segregation	5	50
regulation of cell cycle G1/S phase transition	5	45.5
negative regulation of small GTPase mediated signal transduction	5	45.5
regulation of G1/S transition of mitotic cell cycle	5	45.5
regulation of septation initiation signaling	5	45.5
intra-Golgi vesicle-mediated transport	5	45.5
regulation of endocytosis	5	45.5
negative regulation of Ras protein signal transduction	5	45.5
post-translational protein modification	4	100
establishment of mitotic sister chromatid cohesion	4	80
establishment of sister chromatid cohesion	4	80
peptide pheromone maturation	4	80
traversing start control point of mitotic cell cycle	4	80
positive regulation of cell cycle G1/S phase transition	4	66.7
N-glycan processing	4	66.7
positive regulation of G1/S transition of mitotic cell cycle	4	66.7
attachment of spindle microtubules to kinetochore involved in homologous chromosome segregation	4	57.1
kinetochore organization	4	57.1
microtubule cytoskeleton organization involved in homologous chromosome segregation	4	57.1
vesicle localization	4	57.1
'de novo' pyrimidine nucleobase biosynthetic process	4	57.1
positive regulation of penicillin biosynthetic process	4	57.1
regulation of vacuole fusion, non-autophagic	4	57.1
attachment of spindle microtubules to kinetochore involved in meiotic chromosome segregation	4	57.1
meiotic gene conversion	4	57.1
regulation of sister chromatid cohesion	4	57.1

regulation of centromeric sister chromatid cohesion	3	100
C-terminal protein amino acid modification	3	100
DNA replication, removal of RNA primer	3	75
protein folding in endoplasmic reticulum	3	75
phosphatidylethanolamine biosynthetic process	3	75
regulation of iron ion transport	3	75
histone H3 deacetylation	3	75
pyrimidine dimer repair	3	75
telomere capping	3	75
A. flavus		
cellular process	461	10.6
organic substance metabolic process	353	10.9
primary metabolic process	333	11.3
cellular metabolic process	328	11.5
single-organism cellular process	300	11
macromolecule metabolic process	213	11.1
nitrogen compound metabolic process	210	11.5
cellular macromolecule metabolic process	197	11.8
cellular nitrogen compound metabolic process	173	11.3
biosynthetic process	163	11
organic substance biosynthetic process	160	11.4
organic cyclic compound metabolic process	159	10.9
cellular biosynthetic process	157	11.8
cellular aromatic compound metabolic process	153	11
heterocycle metabolic process	147	11.1
nucleobase-containing compound metabolic process	135	11.5
biological regulation	121	10.9
small molecule metabolic process	120	13.1
organonitrogen compound metabolic process	114	12.6
regulation of biological process	113	11.3
cellular component organization or biogenesis	110	12.6
protein metabolic process	110	11.9
regulation of cellular process	109	12
cellular nitrogen compound biosynthetic process	99	11.6
cellular protein metabolic process	96	12.8
cellular macromolecule biosynthetic process	93	11.5
macromolecule biosynthetic process	93	11.2
cellular component organization	91	12.5
response to stimulus	82	14

organonitrogen compound biosynthetic process	82	13.7
phosphorus metabolic process	79	16.2
phosphate-containing compound metabolic process	77	16.4
single-organism biosynthetic process	74	11.5
heterocycle biosynthetic process	73	11.6
organelle organization	70	13
aromatic compound biosynthetic process	70	12
macromolecule modification	63	13.8
oxoacid metabolic process	62	12.5
organic acid metabolic process	62	12.4
carboxylic acid metabolic process	61	12.4
nucleobase-containing compound biosynthetic process	61	12.2
cellular response to stimulus	59	16.5
catabolic process	58	12.3
protein modification process	57	14
cellular protein modification process	57	14
organic substance catabolic process	56	12.4
organophosphate metabolic process	52	18.7
lipid metabolic process	50	12.3
cellular catabolic process	49	13.8
carbohydrate derivative metabolic process	47	14.7
response to stress	47	14.2
single-organism organelle organization	43	14.2
cell cycle process	41	16.3
cell cycle	41	15.3
cellular amino acid metabolic process	41	13.9
cellular lipid metabolic process	39	14
nucleobase-containing small molecule metabolic process	35	15
organophosphate biosynthetic process	34	22.8
cell communication	34	17.4
nucleoside phosphate metabolic process	33	18.9
signal transduction	32	18.4
single organism signaling	32	18.4
signaling	32	18.4
macromolecule catabolic process	32	13.8
nucleotide metabolic process	31	18.7
phosphorylation	31	15
carbohydrate derivative biosynthetic process	30	18.5
cellular response to stress	29	16.8

alpha-amino acid metabolic process	29	14.9
DNA metabolic process	29	13.7
cellular macromolecule catabolic process	27	15.6
reproduction	24	15.5
mitotic cell cycle	23	17
mitotic cell cycle process	23	17
nucleoside phosphate biosynthetic process	22	25.6
ribose phosphate metabolic process	22	17.9
protein catabolic process	22	16.1
purine-containing compound metabolic process	21	17.4
nucleoside metabolic process	21	15.4
glycosyl compound metabolic process	21	15.3
nucleotide biosynthetic process	20	25.6
nucleoside monophosphate metabolic process	20	20.8
purine nucleotide metabolic process	20	19.2
ribonucleotide metabolic process	20	18.9
cytoskeleton organization	20	16.1
cellular response to DNA damage stimulus	20	14.2
ribonucleoside monophosphate metabolic process	19	20.2
ribonucleoside metabolic process	19	18.8
intracellular signal transduction	19	17.1
purine ribonucleoside monophosphate metabolic process	18	19.8
purine nucleoside monophosphate metabolic process	18	19.6
purine ribonucleoside metabolic process	18	19.1
purine nucleoside metabolic process	18	18.9
purine ribonucleotide metabolic process	18	17.6
small molecule catabolic process	18	15.4
cofactor biosynthetic process	17	16.5
cellular carbohydrate metabolic process	17	16.3
mitochondrion organization	16	26.7
nucleoside triphosphate metabolic process	16	21.3
phospholipid metabolic process	16	18.8
ribonucleoside triphosphate metabolic process	15	20.5
energy derivation by oxidation of organic compounds	15	16.1
regulation of cell cycle	15	16
organelle fission	15	16
organic acid catabolic process	15	15.3
carboxylic acid catabolic process	15	15.3
microtubule-based process	14	23

regulation of cellular protein metabolic process	14	20.3
ATP metabolic process	14	19.7
purine nucleoside triphosphate metabolic process	14	19.4
purine ribonucleoside triphosphate metabolic process	14	19.4
regulation of protein metabolic process	14	18.7
coenzyme biosynthetic process	14	17.5
DNA replication	14	16.7
microtubule cytoskeleton organization	13	25.5
nucleoside monophosphate biosynthetic process	13	25
ribonucleotide biosynthetic process	13	24.1
ribose phosphate biosynthetic process	13	24.1
cell division	13	20.3
regulation of catalytic activity	13	20
regulation of molecular function	13	20
nucleoside biosynthetic process	12	25
ribonucleoside biosynthetic process	12	25
glycosyl compound biosynthetic process	12	24.5
ribonucleoside monophosphate biosynthetic process	12	24
purine nucleotide biosynthetic process	12	23.5
purine-containing compound biosynthetic process	12	19.7
cellular respiration	12	17.9
membrane organization	12	16.7
purine ribonucleoside biosynthetic process	11	25.6
purine nucleoside biosynthetic process	11	25.6
purine ribonucleoside monophosphate biosynthetic process	11	23.4
purine nucleoside monophosphate biosynthetic process	11	22.9
purine ribonucleotide biosynthetic process	11	22
cytokinesis	11	20
glycerophospholipid metabolic process	11	20
glycerolipid metabolic process	11	19
regulation of phosphate metabolic process	10	28.6
regulation of phosphorus metabolic process	10	28.6
regulation of cell communication	10	27.8
regulation of signal transduction	10	27.8
regulation of signaling	10	27.8
regulation of response to stimulus	10	27
phospholipid biosynthetic process	10	19.2
peptidyl-amino acid modification	10	17.9
glycoprotein metabolic process	10	17.9

mitochondrial transport	9	30
regulation of phosphorylation	9	29
nucleoside triphosphate biosynthetic process	9	28.1
phosphatidylinositol metabolic process	9	25.7
DNA recombination	9	23.7
regulation of transferase activity	9	23.1
regulation of protein modification process	9	21.4
protein import	9	20.9
aspartate family amino acid metabolic process	9	19.6
protein targeting to mitochondrion	8	38.1
protein localization to mitochondrion	8	38.1
establishment of protein localization to mitochondrion	8	38.1
regulation of intracellular signal transduction	8	32
regulation of protein kinase activity	8	28.6
regulation of kinase activity	8	28.6
regulation of protein phosphorylation	8	26.7
ribonucleoside triphosphate biosynthetic process	8	25.8
electron transport chain	7	33.3
regulation of protein serine/threonine kinase activity	7	28
energy coupled proton transport, down electrochemical gradient	7	26.9
ATP synthesis coupled proton transport	7	26.9
hydrogen ion transmembrane transport	7	25
ATP biosynthetic process	7	24.1
purine nucleoside triphosphate biosynthetic process	7	23.3
purine ribonucleoside triphosphate biosynthetic process	7	23.3
hydrogen transport	7	21.2
positive regulation of metabolic process	7	21.2
proton transport	7	21.2
ATP synthesis coupled electron transport	6	37.5
oxidative phosphorylation	6	37.5
respiratory electron transport chain	6	33.3
regulation of small GTPase mediated signal transduction	6	31.6
intracellular protein transmembrane transport	6	30
protein transmembrane transport	6	30
regulation of cyclin-dependent protein kinase activity	6	26.1
regulation of cyclin-dependent protein serine/threonine kinase activity	6	26.1
liposaccharide metabolic process	6	25

branched-chain amino acid metabolic process	6	23.1
mitochondrial transmembrane transport	5	45.5
intracellular protein transmembrane import	5	35.7
double-strand break repair	5	27.8
positive regulation of catalytic activity	4	57.1
positive regulation of molecular function	4	57.1
regulation of Ras protein signal transduction	4	40
mitochondrial ATP synthesis coupled electron transport	4	30.8
lagging strand elongation	4	30.8
termination of RNA polymerase II transcription, exosome-dependent	3	60
inositol metabolic process	3	60
obsolete mutagenesis	3	60
positive regulation of cellular protein metabolic process	3	50
positive regulation of protein metabolic process	3	50
DNA integrity checkpoint	3	42.9
DNA damage checkpoint	3	42.9
Mo-molybdopterin cofactor biosynthetic process	3	42.9
molybdopterin cofactor biosynthetic process	3	42.9
molybdopterin cofactor metabolic process	3	42.9
positive regulation of phosphorus metabolic process	3	42.9
prosthetic group metabolic process	3	42.9
positive regulation of phosphate metabolic process	3	42.9
pyrimidine nucleotide biosynthetic process	3	42.9
Mo-molybdopterin cofactor metabolic process	3	42.9
termination of RNA polymerase II transcription	3	42.9
pyrimidine deoxyribonucleotide biosynthetic process	2	100
deoxyribonucleotide biosynthetic process	2	100
glycoprotein catabolic process	2	100
deoxyribose phosphate biosynthetic process	2	100
cortical protein anchoring	2	100
plasma membrane fusion	2	100
2'-deoxyribonucleotide biosynthetic process	2	100
positive regulation of hydrolase activity	2	100

Table S3-10 Conserved and divergent GO categories of WetA-regulated N:NOrthogroups

	WetA-mediated NU_orthogroups
Conserved GO categories	benzene-containing compound metabolic process
	cellular ketone metabolic process
	phenol-containing compound metabolic process
	organic hydroxy compound biosynthetic process
	coenzyme M metabolic process
	maturation of LSU-rRNA
	dUTP metabolic process
	RNA methylation
	extracellular polysaccharide metabolic process
	ferrous iron transport
	peptidyl-glutamine methylation
	cellobiose transport
	arsenite transport
	quinone metabolic process
	monocarboxylic acid biosynthetic process
Diverged GO categories	calcium ion import
	xyloglucan metabolic process
	replication fork protection
	triglyceride metabolic process
	neutral lipid metabolic process
	methylglyoxal metabolic process
	acylglycerol metabolic process
	negative regulation of DNA replication
	ribosomal small subunit assembly
	hemicellulose metabolic process
	alkaloid metabolic process
	DNA integration
	glucan metabolic process
	indole alkaloid metabolic process
	pyruvate metabolic process
	triglyceride metabolic process
	austinol metabolic process
	neutral lipid metabolic process
	dehydroaustinol biosynthetic process
	glycolytic process
	dehydroaustinol metabolic process
	ATP generation from ADP

Mixed GO categories	DNA-mediated transposition
	DNA integration
	RNA-dependent DNA replication
	DNA metabolic process
	DNA recombination
	DNA replication
	phosphorelay signal transduction system
	peptidyl-histidine autophosphorylation
	protein autophosphorylation
	macromolecule biosynthetic process
	nucleic acid metabolic process
	defense response
	WetA-mediated NU_orthogroups
Conserved GO categories	secondary metabolite biosynthetic process
	oxidation-reduction process
	peptidyl-lysine oxidation
	peptide cross-linking
	spore wall assembly
	phosphatidic acid metabolic process
	protein oxidation
	cell wall assembly
	mycotoxin metabolic process
	organic heteropentacyclic compound metabolic process
	cellular lactam metabolic process
	antibiotic metabolic process
	drug metabolic process
	obsolete electron transport
	amine metabolic process
	transition metal ion homeostasis
Diverged GO categories	oxidation-reduction process
	secondary metabolic process
	transmembrane transport
	DNA-templated transcription,
	RNA metabolic process
	macromolecule biosynthetic process
	nucleobase-containing compound metabolic process
	nitrogen compound metabolic process
	obsolete electron transport
	mycotoxin metabolic process

	organic heteropentacyclic compound metabolic process
	transmembrane transport
	heterocycle biosynthetic process
	tetraterpenoid metabolic process
	formaldehyde metabolic process
	organic cyclic compound biosynthetic process
Mixed GO categories	alcohol metabolic process
	aldonic acid metabolic process
	amino acid transmembrane transport
	asparagine metabolic process
	austinol metabolic process
	cell wall macromolecule catabolic process
	cellular ketone metabolic process
	cinnamic acid metabolic process
	dehydroaustinol metabolic process
	D-gluconate metabolic process
	establishment of localization
	glycosyl compound metabolic process
	ketone biosynthetic process
	localization
	mycotoxin metabolic process
	nucleobase transport
	nucleobase-containing small molecule metabolic process
	nucleoside metabolic process
	obsolete electron transport
	organic hydroxy compound metabolic process
	oxidation-reduction process
	phenylpropanoid metabolic process
	quinone metabolic process
	regulation of asperthecin biosynthetic process
	regulation of nitrogen utilization
	regulation of sterigmatocystin biosynthetic process
	secondary metabolic process
	single-organism localization
	transmembrane transport
	transport

WetA-mediated UL_orthogroups

Conserved GO categories	(1->6)-beta-D-glucan metabolic process
	amino acid activation for nonribosomal peptide biosynthetic process

	amino acid adenylylation by nonribosomal peptide synthase amino sugar catabolic process aminoglycan catabolic process anaerobic amino acid catabolic process anaerobic glutamate catabolic process arginine catabolic process beta-glucan metabolic process carbohydrate derivative catabolic process cell killing cell wall organization or biogenesis cell wall polysaccharide metabolic process cellular glucan metabolic process cellular polysaccharide metabolic process chitin catabolic process cytolysis cytolysis by symbiont of host cells cytolysis in other organism 'de novo' protein folding defense response deoxyribonucleotide metabolic process energy derivation by oxidation of organic compounds fermentation generation of precursor metabolites and energy glucan metabolic process glucosamine-containing compound catabolic process glutamine family amino acid catabolic process hemicellulose metabolic process high-affinity iron ion transmembrane transport hormone transport iron ion transmembrane transport mycotoxin metabolic process obsolete cytokinesis, completion of separation obsolete vitamin or cofactor transport organonitrogen compound catabolic process peptide pheromone export polysaccharide metabolic process positive regulation of ergosterol biosynthetic process positive regulation of ergosterol biosynthetic process by positive regulation of transcription from RNA polymerase II promoter positive regulation of lipid transport

	positive regulation of steroid biosynthetic process
	positive regulation of sterol transport
	regulation of ergosterol biosynthetic process by regulation of transcription from RNA polymerase II promoter
	regulation of hormone levels
	regulation of lipid transport
	regulation of sterol transport
	succinyl-CoA metabolic process
	xylan metabolic process
Diverged GO categories	amine biosynthetic process
	aromatic amino acid family biosynthetic process
	carbohydrate metabolic process
	cellular biogenic amine biosynthetic process
	cellular potassium ion transport
	copper ion transmembrane transport
	cytoplasmic sequestering of transcription factor
	galacturonan metabolic process
	hemicellulose catabolic process
	negative regulation of cellular protein localization
	negative regulation of intracellular transport
	pectin metabolic process
	phosphatidic acid metabolic process
	polyamine metabolic process
	polysaccharide metabolic process
	potassium ion transmembrane transport
	production of siRNA involved in RNA interference
	regulation of nitrogen utilization
	RNA interference
	secondary metabolic process
	xanthone-containing compound metabolic process
Mixed GO categories	(R)-carnitine transport
	allantoin transport
	amide transport
	amine metabolic process
	amine transport
	amino acid transmembrane transport
	amino acid transport
	amino-acid betaine transport
	anion transmembrane transport
	antibiotic metabolic process

beta-lactam antibiotic metabolic process
biological adhesion
capsule organization
carboxylic acid transport
carnitine transport
cation transport
cell adhesion
cellular amine metabolic process
cellular lactam metabolic process
cellular nitrogen compound catabolic process
choline transport
dipeptide transport
divalent metal ion transport
drug metabolic process
establishment of localization
ethanolamine transport
fatty acid derivative metabolic process
fumiquinazoline metabolic process
gliotoxin metabolic process
glycine betaine transport
heterocycle catabolic process
icosanoid metabolic process
inorganic ion transmembrane transport
ion transport
iron assimilation by reduction and transport
lipid catabolic process
localization
mycotoxin metabolic process
neutral amino acid transport
nitrogen compound transport
nonribosomal peptide biosynthetic process
nucleobase transport
oligopeptide transport
organic acid transport
organic cyclic compound catabolic process
organonitrogen compound catabolic process
oxidation-reduction process
peptide transport
polyketide metabolic process
proline transport

prostaglandin metabolic process
prostanoid metabolic process
quaternary ammonium group transport
response to antibiotic
response to chemical
response to copper ion
response to drug
response to toxic substance
response to transition metal nanoparticle
secondary metabolic process
single-organism catabolic process
single-organism localization
single-organism process
single-organism transport
toxin metabolic process
transition metal ion transport
transmembrane transport
zinc II ion transport

Table S3-11 DEGs related with asexual development in Δ*wetA* conidia

	A. nidulans		***A. fumigauts***		***A. flavus***	
	ID	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**
abaA	AN0422	4.96	Afu1g04830	3.72	AFLA_029620	2.96
acyA	AN2623	4.03	Afu3g12620		AFLA_070880	3.90
ams1	AN2936	1.56	Afu3g08200		AFLA_086900	1.02
areA	AN8667		Afu6g01970		AFLA_049870	-0.45
argB	AN4409		Afu4g07190		AFLA_112660	-1.53
atfA	AN2911		Afu3g11330		AFLA_031340	-0.64
atfB	AN6849	-1.79	Afu5g12960	1.53	AFLA_094010	-6.01
atg1	AN1632	1.42	Afu4g09050		AFLA_110620	1.39
atgH	AN5131	3.34	Afu1g07470	1.08	AFLA_022400	1.23
bem1	AN7030	1.16	Afu4g04120	2.68	AFLA_114720	-1.12
brlA	AN0973	6.65	Afu1g16590	1.93	AFLA_082850	3.31
cch1	AN1168		Afu1g11110		AFLA_068440	1.53
chsA	AN4566		Afu2g01870	2.50	AFLA_042780	1.95
chsB	AN7032	2.19	Afu4g04180	1.25	AFLA_114760	1.72
chsE	AN6318	4.97	Afu2g13440	3.56	AFLA_136030	3.37
chsF	AN1555	3.05	Afu8g05630	6.59	AFLA_078290	2.43
chsG	AN2523	2.55	Afu3g14420	4.00	AFLA_060590	2.59
cmdA	AN2047		Afu4g10050		AFLA_026020	1.59
cnaA	AN8820		Afu5g09360		AFLA_098750	0.49
cnaB	AN6566	-1.59	Afu6g04540		AFLA_055480	-0.06
cpcB	AN4163	-2.58	Afu4g13170		AFLA_051980	-0.91
crzA	AN5726		Afu1g06900		AFLA_127920	1.57
dewA	AN8006	-9.48	Afu8g07060	3.48	AFLA_060780	-4.32
esdC	AN9121	1.02	Afu7g01930	3.32	AFLA_071090	2.51
fadA	AN0651		Afu1g13140	3.82	AFLA_018340	0.25
fbx15	AN2505	1.25	Afu3g14150	1.93	AFLA_019100	1.74
figA	AN3036	4.02	Afu3g09060		AFLA_086010	2.40
flbA	AN5893	4.08	Afu2g11180	2.70	AFLA_134030	1.71
flbB	AN7542		Afu2g14680	1.17	AFLA_131490	0.95
flbC	AN2421	1.91	Afu2g13770	4.92	AFLA_137320	3.04

flbD	AN0279		Afu1g03210	7.20	AFLA_080170	0.43
flbE	AN0721		-		AFLA_017380	0.14
fluG	AN4819	-2.37	Afu3g07140	1.88	AFLA_039530	-2.59
fphA	AN9008		Afu4g02900	1.02	AFLA_065850	-1.09
ganB/gpaB	AN1016		Afu1g12930		AFLA_018540	0.63
gpaA	AN0651		Afu1g13140	3.82	AFLA_018340	0.25
gpgA	AN2742		Afu1g05210		AFLA_030020	-0.41
gprC	AN3765	1.85	Afu7g04800	4.40	AFLA_074150	2.04
gprD	AN3387	-1.72	Afu2g12640	4.12	AFLA_135680	0.49
hsp90	AN8269	3.67	Afu5g04170	-2.33	AFLA_006960	-0.70
ime2	AN6243	2.24	Afu2g13140	3.44	AFLA_136540	1.71
kex1	AN10184		Afu1g08940		AFLA_088670	-1.05
laeA	AN0807	-2.93	Afu1g14660	2.92	AFLA_033290	0.15
llmB/vipC	AN8945		Afu8g01930		AFLA_121330	-4.53
llmF	AN6749		Afu8g01930		AFLA_008970	2.89
lreA	AN3435	-3.04	Afu3g05780	-1.73	AFLA_103610	0.35
lreB	AN3607	-1.06	Afu4g12690	-3.43	AFLA_051690	-0.49
meaB	AN4900		Afu3g10930		AFLA_031790	0.36
medA	AN6230		Afu2g13260	5.50	AFLA_136410	2.81
midA	AN4897	3.21	Afu3g10960	3.26	AFLA_031700	2.89
mkkB	AN4189		Afu1g05800		AFLA_051240	0.58
mob1	AN6288	2.26	Afu2g12390		AFLA_135550	2.38
mpkB	AN3719	4.29	Afu6g12820	1.98	AFLA_034170	0.96
msdS	AN0787	9.00	Afu1g14560	4.99	AFLA_033400	4.17
msnA	AN1652	-1.66	Afu4g09080	3.52	AFLA_110650	2.23
mtfA	AN8741	1.51	Afu6g02690	3.71	AFLA_091490	2.27
napA	AN8863		Afu5g05540	1.50	AFLA_048050	0.43
nce102	AN7683	-2.44	Afu2g01590	-4.35	AFLA_062460	-5.13
nsdC	AN4263	1.60	Afu7g03910		AFLA_131330	2.72
nsdD	AN3152		Afu3g13870	2.78	AFLA_020210	1.68
nudA	AN0118	2.76	Afu5g11810		AFLA_092800	2.17
nudG	AN0420		Afu1g04850	-1.22	AFLA_029640	-1.26
odeA	AN1037	1.50	Afu1g12530	3.08	AFLA_066330	1.85
orlA	AN3441	-2.90	Afu3g05650		AFLA_103730	-0.59
osaA	AN6578		Afu6g04490		AFLA_055650	-1.50
pac2/osaB	AN3074		Afu3g09640	2.06	AFLA_085200	1.08

pbcR	AN1599		Afu7g01640		AFLA_028410	-1.02
pcl1	AN0453	5.52	Afu1g04750	4.14	AFLA_029150	2.40
phnA	AN0082		Afu5g12200	-1.39	AFLA_093230	1.66
pkaA	AN6305	-2.36	Afu2g12200	-3.46	AFLA_135040	-2.63
pkaB	AN4717		Afu5g08570	-1.16	AFLA_091910	-2.09
pkaR	AN4987	-1.69	Afu3g10000	-1.81	AFLA_032870	-1.36
pksP	AN8209	-4.97	Afu2g17600	1.22	AFLA_006170	-5.70
ppoA	AN1967		Afu4g10770	-2.51	AFLA_026790	-1.46
ppoB	AN6320	4.75	Afu4g00180	-11.69	AFLA_120760	-11.01
ppoC	AN5028	3.00	Afu3g12120	5.41	AFLA_030430	1.23
ppoD	-		-		AFLA_021100	3.74
prpA	AN3129	1.85	Afu5g07320	2.50	AFLA_034670	5.31
rasA	AN0182		Afu5g11230	2.06	AFLA_132380	0.21
rasB	AN5832	1.88	Afu2g07770		AFLA_047110	0.17
rgdA	AN4745	2.26	Afu3g06280		AFLA_102850	2.22
rgsA	AN5755		Afu6g06860	1.01	AFLA_037370	0.56
rhbA	AN8868	-1.30	Afu5g05480	-2.91	AFLA_048000	-1.15
rho1	AN5740	1.09	Afu6g06900		AFLA_037320	1.58
ricA	AN1661		Afu4g08820	-1.33	AFLA_110790	-1.07
rlmA	AN2984	1.73	Afu3g08520		AFLA_086590	0.34
rodA	AN8803	7.02	Afu5g09580		AFLA_098380	1.72
rodB	-		Afu1g17250	6.54	AFLA_014260	-2.00
sfaD	AN0081	1.59	Afu5g12210	2.31	AFLA_093240	0.47
sfgA	AN8129	-1.13	Afu5g02800		AFLA_005520	-2.82
sidB	AN8751	2.40	Afu6g02840	2.09	AFLA_091740	1.39
sltA	AN2919	3.33	Afu3g08010	3.36	AFLA_087350	2.86
somA	AN9087		Afu7g02260		AFLA_071410	0.05
srgA	AN6974		Afu4g04810		AFLA_115520	-0.44
ssc1	AN9467		Afu5g02560	1.16	AFLA_004760	1.40
steC	AN2269		Afu5g06420	2.37	AFLA_048880	1.48
steD	AN7252		Afu2g17130	2.55	AFLA_002340	-0.45
stuA	AN5836	4.03	Afu2g07900	5.61	AFLA_046990	2.32
swoM	AN6037	-1.22	Afu2g09790	-1.29	AFLA_044820	-1.43
tcpA	AN0641		Afu1g16840		AFLA_082510	-1.07
tmpA	AN0055	-2.08	Afu5g12520		AFLA_093580	-2.48
tpsA	AN5523	-1.62	Afu6g12950	-2.09	AFLA_034030	0.25

tpsB	AN5523	-1.62	Afu2g04010	-1.84	AFLA_087630	-2.65
tpsC	AN10533	-1.50	Afu7g03940	-2.63	AFLA_131370	-2.30
ugtA	AN3113		Afu3g12700	2.63	AFLA_020990	1.34
vapA	AN0186	2.98	Afu5g11190	5.93	AFLA_132340	1.48
veA	AN1052	-2.43	Afu1g12490	2.44	AFLA_066460	-1.90
velB	AN0363	1.07	Afu1g01970	-2.20	AFLA_081490	0.20
velC	AN2059	2.36	Afu4g09770	1.07	AFLA_025780	0.82
vosA	AN1959	-3.35	Afu4g10860	-3.28	AFLA_026900	-3.70
vosB	-		-		AFLA_074470	-6.25
wetA	AN1937	-10.99	Afu4g13230	-12.90	AFLA_052030	-10.74
wsc1	AN4674	5.45	Afu5g09020	3.89	AFLA_099380	3.60
wsc3	AN5660	3.81	Afu4g13670	5.57	AFLA_052510	3.01
zipA	AN11891	1.74	Afu1g16460	3.77	AFLA_083100	1.81

Table S3-12 Kinase encoded DEGs in Δ*wetA* conidia

	A. nidulans		*A. fumigauts*		*A. flavus*	
	ID	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**
dakA	**AN0034**	1.18	**AFU5g12690**	-	**AFLA_093790**	-3.14
atmA	**AN0038**	-1.33	**Afu5g12660**	1.09	**AFLA_093750**	-
pkcA	**AN0106**	1.49	**AFU5g11970**	-	**AFLA_092990**	-
rio2	**AN0124**	-2.13	**Afu5g11730**	-1.90		
	AN0156	-1.08	**AFU5g11450**	-	**AFLA_132700**	-
ireA	**AN0235**	3.74	**Afu1g01720**	2.32	**AFLA_081860**	-
	AN0259	-	**Afu1g03420**	1.64	**AFLA_080110**	-
	AN0699	-	**Afu1g13600**	-1.72	**AFLA_017810**	-
aromA	**AN0708**	-1.12	**AFU1g13740**	1.65	**AFLA_017680**	-
	AN0929	1.66	**AFU1g15930**	-	**AFLA_083430**	1.32
pbsA	**AN0931**	-1.67	**Afu1g15950**	-2.63	**AFLA_083380**	-
oca2	**AN10019**	1.08	**Afu5g11840**	-1.41	**AFLA_092880**	-1.06
srpkF	**AN10082**	2.77	**Afu6g02242**	2.18	**AFLA_090470**	-
	AN10156	2.34	**AFU1g11550**	2.41	**AFLA_068180**	3.36
null	**AN10188**	-2.09	**AFU1g08840**	-	**AFLA_088770**	-
srpkG	**AN10462**	1.35			**AFLA_090280**	-2.55
stk21	**AN10485**	-	**Afu6g08590**	1.68	**AFLA_128210**	-
prk1	**AN10515**	3.43	**Afu1g05930**	2.01	**AFLA_090810**	2.29
	AN10551	-2.81	**AFU4g07140**	-1.97	**AFLA_112710**	-1.10
	AN10646	-	**AFU1g07040**	-2.48	**AFLA_022040**	-1.09
	AN10682	-	**Afu6g12890**	-1.76	**AFLA_034120**	-
	AN10731	-1.35				
pclB	**AN10741**	1.61	**AFU2g07660**	2.33	**AFLA_047210**	-
pkpA	**AN10800**	1.66	**Afu2g13600**	-1.91	**AFLA_136730**	-
snf4	**AN10854**	-	**AFU5g12990**	-1.11	**AFLA_094030**	-1.08
srpkB	**AN10895**	-2.24	**Afu6g02590**	4.06	**AFLA_106860**	-
srpkE	**AN10937**	4.04	**Afu3g02460**	-8.56	**AFLA_009240**	-
	AN1096	-1.08	**Afu1g11950**	-1.77	**AFLA_067680**	-2.15
	AN11032	-	**AFU5g02220**	-	**AFLA_004050**	1.50
gin4	**AN11101**	3.95	**Afu6g02300**	2.79	**AFLA_091080**	2.64
kin1	**AN1171**	-	**Afu1g11080**	1.15	**AFLA_068480**	3.19

	AN1176	-1.11	**AFU1g10980**	-	**AFLA_068540**	-
	AN11803	-2.71	**AFU4g10840**	-	**AFLA_026880**	-2.24
	AN11814	-6.30			**AFLA_119130**	1.67
	AN4279	-	**Afu7g03750**	1.33		
	AN11932	3.11	**AFU7g01620**	-	**AFLA_070720**	1.66
sD	**AN1194**	-2.00	**AFU1g10820**	-	**AFLA_068790**	-
pgkA	**AN1246**	-2.45	**Afu1g10350**	-3.19	**AFLA_069370**	-2.11
srpkC	**AN1315**	2.18			**AFLA_072390**	-1.69
	AN1315	2.18			**AFLA_072390**	-1.69
	AN1370	-	**Afu1g09170**	3.58	**AFLA_088430**	-
plkA	**AN1560**	-	**AFU8g05680**	1.72	**AFLA_078250**	-
atg1	**AN1632**	1.42	**Afu4g09050**	-	**AFLA_110620**	1.39
	AN1668	-	**Afu4g08900**	-1.64	**AFLA_110530**	-
nimO	**AN1779**	-1.76	**Afu6g09180**	1.22	**AFLA_129020**	-
sln1	**AN1800**	-	**AFU2g00660**	-	**AFLA_039130**	1.15
	AN1854	1.47	**Afu4g09910**	2.05	**AFLA_025890**	-
phoB	**AN1867**	1.17				
	AN2211	-1.42	**AFU5g07030**	-	**AFLA_057380**	-
gcn2	**AN2246**	-1.05	**AFU5g06750**	-	**AFLA_056720**	-
ksp1	**AN2265**	1.35	**AFU5g06470**	-		
	AN2269	-	**Afu5g06420**	2.37	**AFLA_048880**	1.48
	AN2272	-	**Afu5g06390**	1.02	**AFLA_048840**	-
	AN2311	-	**AFU5g10680**	-	**AFLA_119540**	1.82
ffkC	**AN2373**	1.98				
	AN2412	-	**Afu2g13680**	3.37	**AFLA_136990**	1.89
ssn3	**AN2489**	-	**Afu3g13990**	1.14	**AFLA_019840**	-
	AN2513	-	**AFU3g14290**	-	**AFLA_098060**	-1.32
	AN2766	-	**Afu3g06080**	1.06	**AFLA_103280**	-
mps1	**AN2927**	3.58	**AFU3g08100**	3.54	**AFLA_087000**	-
isr1	**AN3001**	-2.33	**AFU3g08710**	-		
	AN3065	-	**Afu3g09550**	1.83	**AFLA_085320**	-
	AN3110	-	**Afu3g12670**	2.58	**AFLA_021030**	2.35
	AN3177	-	**Afu3g13270**	1.08	**AFLA_020560**	-
	AN3181	-3.01	**AFU3g13210**	-	**AFLA_020620**	1.18
	AN3214	-2.86	**Afu4g01020**	-1.58	**AFLA_024540**	-8.52
cdc7	**AN3450**	1.54	**AFU3g05540**	-	**AFLA_103840**	1.13

	AN3619	1.20	**Afu4g12680**	1.08	**AFLA_051700**	2.46
	AN3648	2.27	**Afu4g12160**	2.94	**AFLA_028230**	-
mpkB	**AN3719**	4.29	**Afu6g12820**	1.98	**AFLA_034170**	-
	AN3755	-	**Afu7g04640**	-1.19	**AFLA_073980**	-
erg12	**AN3869**	1.73	**Afu4g07780**	1.99	**AFLA_112040**	-
srrC	**AN4134**	3.46				
teaR	**AN4214**	1.88	**AFU1g06090**	2.05	**AFLA_050110**	1.79
ura6	**AN4258**	-	**Afu7g03990**	-1.37	**AFLA_131420**	-
stt4	**AN4278**	1.17	**Afu7g03760**	1.45	**AFLA_131170**	1.11
phoC	**AN4310**	-	**Afu4g06020**	-1.06	**AFLA_113980**	-
	AN4380	1.65	**Afu4g06690**	1.28	**AFLA_113250**	-
	AN4382	-2.43	**AFU4g06710**	-2.39	**AFLA_113230**	2.69
	AN4447	1.96	**Afu4g07400**	4.51	**AFLA_112430**	-
	AN4483	-	**Afu2g03490**	-1.68	**AFLA_092240**	-
psk1	**AN4536**	1.80	**Afu2g02850**	-1.70	**AFLA_133230**	2.95
ckiA	**AN4563**	1.55	**AFU2g02530**	-	**AFLA_133580**	-
vps34	**AN4709**	-1.08	**Afu5g08670**	1.08	**AFLA_099880**	-
pkaC2	**AN4717**	-	**Afu5g08570**	-1.16	**AFLA_091910**	-2.09
bck1	**AN4887**	-	**Afu3g11080**	1.63	**AFLA_031560**	-
	AN4914	-	**Afu3g10750**	-4.53	**AFLA_031910**	-3.50
ran1	**AN4935**	1.23	**AFU3g10530**	-	**AFLA_032170**	-1.54
	AN4957	1.39	**Afu3g10300**	-2.30	**AFLA_032510**	-1.06
	AN4984	-1.23	**AFU3g10040**	-	**AFLA_032830**	-
pkaR	**AN4987**	-1.69	**Afu3g10000**	-1.81	**AFLA_032870**	-1.36
	AN5122	-1.40	**AFU1g07530**	-	**AFLA_022470**	-
	AN5144	-	**AFU1g07220**	-	**AFLA_022220**	-1.35
pho80	**AN5156**	1.18	**AFU1g07070**	-	**AFLA_022060**	-
	AN5167	1.07	**Afu6g06990**	5.82	**AFLA_021950**	2.31
pkiA	**AN5210**	1.44	**AFU6g07430**	-	**AFLA_087900**	-
fos-1	**AN5296**	-	**Afu6g10240**	-1.32	**AFLA_106830**	-
chkA	**AN5494**	2.04	**Afu6g13160**	1.95	**AFLA_033770**	1.54
	AN5529	-	**Afu4g11890**	4.79	**AFLA_027910**	1.72
mpkA	**AN5666**	-1.15	**Afu4g13720**	1.48	**AFLA_052570**	-
mst1	**AN5674**	2.44	**Afu7g04330**	2.23	**AFLA_073630**	-
	AN5719	-2.16				
stk22	**AN5728**	-1.71	**AFU1g06920**	-		

	AN5757	-	**Afu6g06870**	1.66	**AFLA_037350**	1.55
ark1	**AN5815**	-	**AFU2g07550**	-	**AFLA_047300**	-1.22
	AN5817	-	**Afu2g07570**	-	**AFLA_047280**	-
ankA	**AN5822**	3.54	**AFU2g07690**	3.22	**AFLA_047180**	3.31
pdkA	**AN5843**	2.44	**AFU5g14790**	-7.47		
pkcB	**AN5973**	2.02	**Afu2g10620**	1.62	**AFLA_043950**	2.63
tor	**AN5982**	-	**Afu2g10270**	1.02	**AFLA_044350**	-
npkA	**AN6044**	-	**Afu2g09710**	-1.32	**AFLA_044920**	-
	AN6053	-	**AFU2g09570**	1.53	**AFLA_045240**	2.68
ffkG	**AN6192**	2.36	**Afu2g11730**	4.71	**AFLA_134590**	-
imeB	**AN6243**	2.24	**Afu2g13140**	3.44	**AFLA_136540**	1.71
mob1	**AN6288**	2.26	**AFU2g12390**	-	**AFLA_135550**	2.38
pkaA	**AN6305**	-2.36	**Afu2g12200**	-3.46	**AFLA_135040**	-2.63
pod6	**AN6339**	-1.94	**AFU2g13640**	-	**AFLA_136840**	-
	AN6347	-	**Afu2g14200**	2.58	**AFLA_105620**	-
sudD	**AN6363**	-2.49	**AFU2g14090**	-1.04	**AFLA_138010**	-
	AN6367	-	**Afu2g14040**	-1.49	**AFLA_137970**	-1.68
gsk3	**AN6508**	1.36	**Afu6g05120**	1.05	**AFLA_054840**	-
	AN6570	-	**Afu6g04500**	-1.20	**AFLA_055520**	-1.44
	AN6572	-2.19	**AFU6g04380**	-	**AFLA_055540**	-
ffkJ	**AN6758**	2.70				
	AN6824	-	**AFU5g12870**	-2.24	**AFLA_093870**	-2.75
	AN6943	3.48				
uvsb	**AN6975**	-	**AFU4g04760**	-	**AFLA_115480**	1.24
bem1	**AN7030**	1.16	**AFU4g04120**	2.68	**AFLA_114720**	-1.12
	AN7185	-	**Afu4g03140**	-1.21	**AFLA_039070**	-
	AN7321	-	**AFU2g16620**	-	**AFLA_002970**	-1.07
	AN7469	-	**AFU2g05820**	-	**AFLA_129990**	-1.62
	AN7502	-1.14	**AFU2g05430**	-	**AFLA_129590**	-
ppk33	**AN7537**	-	**AFU6g09240**	-	**AFLA_129090**	-
chkC	**AN7563**	5.65	**AFU2g14920**	4.04	**AFLA_016700**	1.55
snf1	**AN7695**	-2.05	**Afu2g01700**	-1.80	**AFLA_062250**	-
	AN7737	-	**AFU5g07950**	-	**AFLA_061710**	6.15
ku70	**AN7753**	-	**AFU5g07740**	-1.12	**AFLA_061470**	1.69
	AN7787	-				
	AN7945	-			**AFLA_104660**	-1.55

ffkA	**AN7986**	2.87				
	AN7995	-	**Afu5g02530**	-2.32	**AFLA_004730**	-1.10
	AN8213	-3.22	**AFU5g03460**	-1.15	**AFLA_006260**	-
swoH	**AN8216**	-2.09	**Afu5g03490**	-3.32	**AFLA_006300**	-1.35
	AN8751	2.40	**Afu6g02840**	2.09	**AFLA_091740**	1.39
iki3	**AN8790**	-	**AFU5g09840**	-1.29	**AFLA_098240**	-1.49
cmkC	**AN8827**	2.16	**AFU5g05980**	-	**AFLA_048460**	1.48
cla4	**AN8836**	2.24	**Afu5g05900**	4.30	**AFLA_048350**	1.07
	AN8837	2.16	**Afu5g05890**	-1.71	**AFLA_048340**	-
	AN8843	-2.74	**Afu5g05820**	-1.05	**AFLA_048290**	-
	AN8859	-1.69	**AFU5g05590**	-	**AFLA_048100**	-
ptkA	**AN8865**	-1.10			**AFLA_048030**	-
	AN9022	-1.74			**AFLA_040580**	1.03
	AN9024	2.30				
tinC	**AN9067**	1.21	**AFU7g02570**	2.68	**AFLA_071680**	-1.55
ffkK	**AN9302**	-				
panK	**AN9446**	-1.35	**AFU3g07180**	-	**AFLA_102040**	-1.51
	AN9461	-	**Afu5g03240**	-1.35	**AFLA_005960**	-
	AN9500	3.58	**AFU6g02020**	-	**AFLA_049940**	-
nimA	**AN9504**	-	**Afu6g02670**	-1.43	**AFLA_091450**	-
			Afu1g00530	-2.02	**AFLA_028130**	-2.85
			AFU1g00640	2.66	**AFLA_003780**	3.03
			Afu2g00670	-1.09		
tcsC			**Afu2g03560**	3.16		
			Afu4g04680	2.25		
			Afu4g14735	2.24		
			Afu4g14740	3.85		
			Afu5g03950	-		
			Afu5g06730	1.79	**AFLA_138880**	-
			Afu5g08480	-3.44	**AFLA_091820**	-
			Afu5g13420	3.67		
			Afu6g03252	3.48		
			AFU7g00530	1.36	**AFLA_005410**	-1.05
			Afu7g04550	-1.84	**AFLA_073810**	2.42
			Afu7g04735	1.50		
			Afu8g06150	-3.14		

			Afu8g06180	2.46		
					AFLA_008230	-2.78
					AFLA_009250	-3.25
					AFLA_010150	-1.50
					AFLA_040840	-2.10
					AFLA_043510	-2.09
cdc2l1					**AFLA_069750**	2.37
bos1					**AFLA_092160**	1.10
phoA	**AN8261**	-	**Afu5g04130**	1.05	**AFLA_006900**	-
Following genes are grouped by their orthogroup						
fphA	**AN9008**	-	**Afu4g02900**	1.02	**AFLA_065850**	-1.09
					AFLA_095440	5.45
					AFLA_101910	2.09
sepH	**AN4385**	2.06	**AFU4g06750**	2.38	**AFLA_113190**	1.19
					AFLA_113200	1.62
nimX	**AN4182**	-1.23	**Afu6g07980**	4.43	**AFLA_036730**	-
					AFLA_125910	-
	AN6985	2.31	**Afu4g04680**	2.25	**AFLA_097830**	3.44
	AN1568	-		-	**AFLA_115360**	-
	AN4479	2.42	**Afu2g03560**	3.16	**AFLA_092150**	1.28
					AFLA_092160	1.10
					AFLA_119810	-
	AN1568	-	**Afu4g04680**	2.25	**AFLA_097830**	3.44
	AN6985	2.31		-	**AFLA_115360**	-
	AN1017	-1.20	**Afu1g12940**	-1.92	**AFLA_099500**	-
	AN4668	-	**Afu5g09100**	-3.10		
	AN8886	-1.22	**AFU1g11630**	1.87	**AFLA_068090**	-
	AN1132	-	**AFU8g02700**	-	**AFLA_122560**	1.18
	AN5589	-1.54	**Afu4g11540**	-2.20	**AFLA_027530**	-
	AN3916	-	**Afu6g08470**	2.68	**AFLA_128100**	-

Table S3-13 Transcription factor encoded DEGs in Δ*wetA* conidia

	A. nidulans		***A. fumigauts***		***A. flavus***	
	ID	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**
sfgA	**AN8129**	-1.13	**Afu5g02800**	-	**AFLA_005520**	-2.82
	AN12489	3.57	**Afu2g16230**	4.00	**AFLA_003630**	1.67
mdpE	**AN0148**	-2.04	**Afu4g14540**	-1.33		-
	AN0333	-	**Afu1g02360**	1.22	**AFLA_081130**	-
rsrA	**AN0273**	1.38				
			Afu5g09170	-1.45	**AFLA_099570**	-2.18
	AN10809	1.58	**Afu2g14250**	-1.31	**AFLA_105360**	-
	AN5806	-	**Afu2g07460**	-3.33		
	AN11962	-	**Afu3g14600**	2.27	**AFLA_059510**	-
	AN7434	-1.09	**Afu2g06130**	1.84	**AFLA_013090**	-2.16
	AN7434	-1.09	**Afu2g05360**	2.77	**AFLA_013090**	-2.16
			Afu3g01155	-1.21		
	AN0585	1.11	**Afu6g11110**	4.36	**AFLA_109220**	1.73
cnjB	**AN4133**	-3.17	**Afu4g13060**	-	**AFLA_051900**	1.35
	AN0658	-1.02	**Afu1g13190**	-		
	AN0671	-1.48	**Afu1g13310**	-1.73	**AFLA_018110**	-1.18
			Afu3g14750	-1.86		
	AN0644	3.62	**Afu1g13050**	-	**AFLA_018410**	1.21
	AN0718	-	**Afu1g14460**	2.22	**AFLA_017290**	1.36
zfpB	**AN1251**	3.37	**Afu1g10230**	4.64	**AFLA_069460**	3.11
	AN5929	2.03	**Afu2g10770**	2.04	**AFLA_043760**	-
uaY	**AN0891**	-	**Afu1g15470**	1.62	**AFLA_083940**	-
	AN0902	5.76	**Afu1g15680**	4.41	**AFLA_083820**	1.13
			Afu1g16620	2.82	**AFLA_082910**	-
	AN4175	-	**Afu6g07950**	2.91	**AFLA_036680**	-
	AN1298	5.64	**Afu1g09670**	5.38	**AFLA_021240**	4.26
brlA	**AN0973**	6.65	**Afu1g16590**	1.93	**AFLA_082850**	3.31
	AN6295	1.65	**Afu2g12310**	3.56	**AFLA_135110**	1.44
	AN10059	4.56			**AFLA_081600**	-
	AN10083	1.69	**Afu6g03700**	-	**AFLA_108220**	-
rosA	**AN5170**	-	**Afu4g09710**	1.52		

	AN10120	-	**Afu1g14360**	1.63	**AFLA_042030**	-1.29
	AN10128	1.12	**Afu1g15370**	1.28	**AFLA_084200**	1.59
	AN10294	-	**Afu5g09890**	-	**AFLA_120290**	-1.80
zipC	**AN10378**	1.79	**Afu4g02940**	-1.64	**AFLA_066000**	-
	AN10384	-	**Afu5g14530**	-		
rfeG	**AN4680**	3.88	**Afu5g08990**	5.57	**AFLA_099460**	1.76
	AN10491	1.08	**Afu6g08550**	-	**AFLA_128160**	-
	AN3667	-	**Afu4g12410**	1.09	**AFLA_050300**	-3.01
galX	**AN10543**	-2.46	**Afu1g11000**	-	**AFLA_049270**	-1.42
	AN10548	-1.57	**Afu4g06420**	-2.04	**AFLA_113510**	-
	AN10600	2.73			**AFLA_000770**	-
	AN11165	1.29	**Afu7g01960**	1.79		
	AN11222	2.59	**Afu2g17880**	-	**AFLA_124220**	2.51
qutA	**AN1134**	-1.06	**Afu1g11620**	2.05		
wetA	**AN1937**	-10.99	**Afu4g13230**	-12.90	**AFLA_052030**	-10.74
zipA	**AN11891**	1.74	**Afu1g16460**	3.77	**AFLA_083100**	1.81
	AN1217	2.10	**Afu1g10580**	3.97	**AFLA_069100**	3.18
	AN3761	2.31	**Afu7g04710**	-	**AFLA_074060**	1.28
	AN3063	-	**Afu3g09380**	-2.13	**AFLA_085360**	-1.13
srbA	**AN7661**	1.81	**Afu2g01260**	1.17	**AFLA_058610**	1.54
zapA	**AN1265**	-1.71	**Afu1g10080**	-1.38	**AFLA_069700**	-
	AN1536	1.67	**Afu8g05460**	3.26	**AFLA_078500**	-
	AN8753	2.80	**Afu6g02850**	2.25	**AFLA_091710**	-1.14
	AN0568	-1.46	**Afu6g11230**	-1.53	**AFLA_109030**	-
	AN1569	-			**AFLA_097720**	-2.91
rfeB	**AN2009**	2.14	**Afu4g10220**	4.22	**AFLA_026250**	2.16
msnA	**AN1652**	-1.66	**Afu4g09080**	3.52	**AFLA_110650**	2.23
	AN1678	2.18			**AFLA_054310**	-3.24
	AN1736	-1.45	**Afu2g00880**	1.64	**AFLA_104780**	1.57
jlbA	**AN1812**	1.48				
	AN0817	-1.59	**Afu1g14750**	-1.97	**AFLA_033160**	-1.27
	AN1924	1.52	**Afu6g07780**	4.63	**AFLA_036460**	-
	AN8596	-2.00	**Afu1g17150**	-	**AFLA_120780**	-3.10
	AN2025	2.89				
dopA	**AN2094**	-1.03	**Afu2g05020**	-	**AFLA_035130**	-
	AN1212	-2.04	**Afu1g10660**	1.68	**AFLA_068970**	-

	AN2367	-			**AFLA_024570**	-1.01
flbC	**AN2421**	1.91	**Afu2g13770**	4.92	**AFLA_137320**	3.04
	AN2615	-1.82	**Afu7g00210**	-4.32		
metR	**AN4361**	-1.08	**Afu4g06530**	-2.94	**AFLA_113450**	-
hexA	**AN4695**	4.36	**Afu5g03920**	-1.83	**AFLA_099650**	-
pacC	**AN2855**	2.08	**Afu3g11970**	-	**AFLA_030580**	1.17
nscR			**Afu7g00130**	1.44		
sltA	**AN2919**	3.33	**Afu3g08010**	3.36	**AFLA_087350**	2.86
	AN7170	-	**Afu4g03460**	-2.31	**AFLA_040260**	2.00
rlmA	**AN2984**	1.73	**Afu3g08520**	-	**AFLA_086590**	-
mbf1	**AN2996**	2.67	**Afu3g08630**	-	**AFLA_086430**	-
abaA	**AN0422**	4.96	**Afu1g04830**	3.72	**AFLA_029620**	2.96
tbp	**AN4976**	-	**Afu3g10120**	-2.03	**AFLA_032740**	-
	AN3216	1.54				
oefC	**AN3075**	5.13	**Afu3g09670**	3.05	**AFLA_085170**	-
	AN0026	-	**Afu5g09990**	-3.30	**AFLA_138930**	-1.72
	AN3385	-			**AFLA_116880**	-
prtT			**Afu4g10120**	2.40	**AFLA_026130**	-
	AN0096	-	**Afu5g12060**	-	**AFLA_093070**	-2.15
	AN3433	-4.20	**Afu3g05760**	-4.30	**AFLA_103640**	-4.14
lreA	**AN3435**	-3.04	**Afu3g05780**	-1.73	**AFLA_103610**	-
	AN3501	-1.72	**Afu4g14590**	-	**AFLA_009580**	-2.63
	AN3502	-1.75	**Afu1g06540**	-	**AFLA_107030**	-1.06
	AN3506	-2.63			**AFLA_123840**	-2.87
lreB	**AN3607**	-1.06	**Afu4g12690**	-3.43	**AFLA_051690**	-
	AN7610	1.08	**Afu2g15620**	1.28	**AFLA_015390**	-
cpcA	**AN3675**	1.92	**Afu4g12470**	3.74	**AFLA_050250**	1.75
flbD	**AN0279**	-	**Afu1g03210**	7.20	**AFLA_080170**	-
vosA	**AN1959**	-3.35	**Afu4g10860**	-3.28	**AFLA_026900**	-3.70
	AN3769	1.90	**Afu7g04820**	-1.98	**AFLA_074200**	1.41
fhpA	**AN4521**	1.46			**AFLA_132980**	-
	AN0486	-	**Afu3g02070**	-	**AFLA_013890**	-1.17
facB	**AN0689**	-	**Afu1g13510**	-2.72	**AFLA_017900**	-
rpn4	**AN0709**	1.17	**Afu1g13750**	-1.15	**AFLA_017640**	2.87
	AN0094	-1.10	**Afu5g12080**	-	**AFLA_093110**	-
	AN10432	-			**AFLA_040220**	-2.62

	AN3290	5.63			**AFLA_101420**	-4.05
	AN4001	-1.65	**Afu1g04170**	-		
	AN10192	-	**Afu8g04540**	-	**AFLA_139560**	-3.60
sln1	**AN1028**	-	**Afu3g02700**	2.90	**AFLA_038210**	-2.39
	AN4185	-1.02	**Afu4g03430**	-2.82	**AFLA_040300**	-7.03
nsdC	**AN4263**	1.60	**Afu7g03910**	-	**AFLA_131330**	2.72
	AN4324	1.94	**Afu4g06170**	6.27	**AFLA_113790**	5.05
	AN10295	-	**Afu2g14350**	1.84	**AFLA_105310**	-
	AN10334	-	**Afu2g00360**	-1.09	**AFLA_083510**	-1.14
	AN4773	1.29				
	AN4821	-	**Afu8g01150**	-1.02	**AFLA_101970**	-
thiA	**AN10492**	-	**Afu6g08350**	1.34	**AFLA_037170**	-1.50
	AN7971	-1.09				
	AN11003	-	**Afu8g07360**	-	**AFLA_062330**	-1.93
	AN11073	-			**AFLA_136880**	-2.19
	AN4788	-1.17	**Afu3g06770**	-	**AFLA_100950**	-
	AN11793	-	**Afu8g07000**	-3.21	**AFLA_009490**	-1.01
fcr1	**AN4861**	1.23	**Afu3g07670**	-1.12	**AFLA_100080**	-
	AN4878	1.36	**Afu3g11170**	1.39	**AFLA_031450**	-
btf3	**AN12485**	-	**Afu1g09130**	1.47	**AFLA_088390**	2.07
metZ	**AN5218**	-1.16	**Afu6g07530**	-	**AFLA_087810**	-1.07
zfpA	**AN1500**	1.09	**Afu8g05010**	2.32	**AFLA_078920**	4.44
	AN1824	-	**Afu5g14390**	1.10	**AFLA_097680**	-1.26
	AN1906	-	**Afu6g07560**	1.80	**AFLA_036190**	1.10
aslA	**AN5583**	1.87	**Afu4g11480**	6.58	**AFLA_027460**	1.78
rfeF	**AN2012**	2.72	**Afu4g10200**	2.84		
htfA	**AN2020**	-	**Afu4g10110**	1.16	**AFLA_026100**	-
	AN5775	1.90	**Afu6g06530**	1.83	**AFLA_037760**	2.87
amdA	**AN2270**	-	**Afu5g06410**	1.14	**AFLA_048870**	-1.29
stuA	**AN5836**	4.03	**Afu2g07900**	5.61	**AFLA_046990**	2.32
	AN5849	5.89	**Afu2g08040**	5.90		
	AN5870	-1.05	**Afu2g11460**	-	**AFLA_134320**	1.07
steA	**AN2290**	-	**Afu5g06190**	3.22	**AFLA_048650**	1.14
	AN2667	-	**Afu8g01990**	2.14	**AFLA_097920**	-1.59
fkh1/2	**AN2854**	-	**Afu3g11960**	-1.64	**AFLA_030600**	-1.45
	AN2957	-2.16			**AFLA_017040**	-1.03

areB	**AN6221**	-1.69	**Afu2g13380**	-	**AFLA_136100**	-
	AN3024	-	**Afu3g08880**	-2.96	**AFLA_085880**	-4.19
nsdD	**AN3152**	-	**Afu3g13870**	2.78	**AFLA_020210**	1.68
	AN3154	-1.06	**Afu3g13920**	1.45	**AFLA_020130**	1.21
	AN3217	-	**Afu4g01010**	-	**AFLA_024580**	-1.19
	AN3224	-	**Afu4g00950**	-1.05	**AFLA_024470**	-
regA	**AN3391**	2.99	**Afu1g17640**	-3.64	**AFLA_073870**	-1.45
	AN6715	-1.75	**Afu7g05620**	-	**AFLA_076560**	-
	AN3900	-	**Afu5g06460**	-	**AFLA_048920**	-1.24
	AN6790	-1.15			**AFLA_013200**	1.02
	AN4013	-	**Afu1g04110**	-1.07	**AFLA_028760**	-1.30
amdR	**AN4035**	-	**Afu1g03860**	-	**AFLA_028560**	-1.06
	AN7061	-3.95				
ace2	**AN4873**	-	**Afu3g11250**	-1.85	**AFLA_031400**	-
	AN7073	5.16			**AFLA_059960**	4.33
	AN7343	3.41				
	AN5048	1.48	**Afu3g12160**	2.25	**AFLA_030390**	-
napA	**AN7513**	1.81	**Afu6g09930**	-1.10	**AFLA_129340**	-
	AN5405	-	**Afu6g13680**	2.03	**AFLA_008860**	-
devR	**AN7553**	1.77	**Afu2g14800**	3.25	**AFLA_131640**	1.47
aflR	**AN8645**	-1.94			**AFLA_139360**	-2.99
crz1	**AN5726**	-	**Afu1g06900**	-	**AFLA_127920**	1.57
anbH1	**AN7734**	3.52	**Afu5g08020**	-	**AFLA_061790**	1.92
dbaA	**AN7896**	3.80				
	AN7919	2.46	**Afu8g05840**	-2.39	**AFLA_024040**	-
	AN5924	-	**Afu2g10850**	-	**AFLA_043710**	-1.17
	AN8111	-1.55			**AFLA_058750**	-
	AN6396	-	**Afu1g17240**	-	**AFLA_014270**	-1.81
azf1	**AN6503**	-	**Afu6g05160**	2.32	**AFLA_054800**	-
	AN8164	1.53	**Afu5g02880**	2.23	**AFLA_005580**	1.20
	AN8177	-1.04	**Afu5g03030**	1.34	**AFLA_005740**	-
	AN6747	-	**Afu7g00652**	-6.11	**AFLA_096330**	-2.40
palcA	**AN8271**	-1.57	**Afu5g04190**	-	**AFLA_006980**	-
	AN8355	-3.25	**Afu6g00220**	-	**AFLA_050970**	1.61
apdR	**AN8414**	1.67				
	AN8506	-1.65			**AFLA_049680**	-

	AN8535	1.24	**Afu5g09740**	-	**AFLA_118300**	1.00
	AN6828	-	**Afu5g12900**	-2.05	**AFLA_093810**	-
	AN6846	-	**Afu5g12930**	-	**AFLA_093980**	-1.45
farA	**AN7050**	-	**Afu4g03960**	1.02	**AFLA_041540**	-
	AN8655	2.64	**Afu8g06460**	2.25	**AFLA_123500**	-1.19
mcmA	**AN8676**	1.08	**Afu6g02110**	-	**AFLA_090910**	-
mtfA	**AN8741**	1.51	**Afu6g02690**	3.71	**AFLA_091490**	2.27
flbB	**AN7542**	-	**Afu2g14680**	1.17	**AFLA_131490**	-
	AN7592	-	**Afu2g15340**	-2.26	**AFLA_015850**	-1.62
mcnB	**AN8858**	1.30	**Afu5g05600**	-	**AFLA_048110**	-
	AN8918	-3.38			**AFLA_129400**	-2.83
	AN8949	5.67				
alcR	**AN8978**	4.15	**Afu5g07510**	-	**AFLA_013240**	-
	AN9013	1.77	**Afu8g02280**	-2.48	**AFLA_132200**	-
hsf1	**AN8035**	-	**Afu5g01900**	-1.14	**AFLA_025030**	-
	AN9240	3.35				
	AN8894	-	**Afu8g02640**	3.76	**AFLA_122500**	-2.37
	AN9328	1.03				
hacA	**AN9397**	4.00	**Afu3g04070**	3.12	**AFLA_089270**	1.11
	AN9117	-	**Afu7g01890**	2.03	**AFLA_071050**	-
amdX	**AN9492**	1.77	**Afu2g17220**	-	**AFLA_002290**	1.58
atfB			**Afu5g12960**	1.53	**AFLA_094010**	-6.01
					AFLA_080270	1.17
					AFLA_096320	-2.84
			Afu7g04811	3.98	**AFLA_074180**	-1.49
			Afu3g00490	1.56	**AFLA_059360**	2.05
			Afu5g01460	-2.04	**AFLA_038900**	-3.28
			Afu1g04140	-1.01	**AFLA_028840**	1.05
			Afu1g03775	-	**AFLA_028410**	-1.02
aro90					**AFLA_015920**	-1.25
			Afu1g01240	1.24	**AFLA_124010**	-2.44
			Afu3g01510	-1.75	**AFLA_105530**	-2.27
			Afu1g15850	-1.93	**AFLA_083560**	2.61
			Afu4g03670	2.41	**AFLA_041330**	-2.22
			Afu3g03315	-3.05	**AFLA_119890**	-2.01
			Afu3g00690	4.82	**AFLA_034610**	-2.28

			Afu3g13600	-1.27	**AFLA_110020**	-1.57
			Afu7g06370	2.09	**AFLA_126910**	-2.72
					AFLA_033480	-2.26
					AFLA_036490	-1.36
					AFLA_086110	3.54
					AFLA_100300	-1.44
acu-15					**AFLA_134920**	-2.36
			Afu3g02750	-	**AFLA_064370**	-1.08
			Afu5g00950	-3.18	**AFLA_049410**	-4.68
			Afu2g04600	-	**AFLA_035590**	2.12
			Afu1g17350	-	**AFLA_097380**	1.21
acuK	**AN7468**	1.08	**Afu2g05830**	-		-
					AFLA_023420	1.01
			Afu7g06500	-	**AFLA_123770**	-1.81
nirA	**AN0098**	-1.21	**Afu5g12020**	-1.02	**AFLA_093040**	-
			Afu5g10250	-2.50	**AFLA_120090**	2.62
creA	**AN6195**	1.09	**Afu2g11780**	-	**AFLA_134680**	-
					AFLA_096370	2.41
hasA			**Afu3g12890**	4.91		
fumR			**Afu8g00420**	1.12		
			Afu2g01190	2.97		
	AN1962	-	**Afu4g10820**	-	**AFLA_026850**	-2.34
			Afu2g03050	-1.13		
			Afu3g03230	1.68		
			Afu5g02655	1.65		
			Afu7g08320	-4.53		
			Afu8g07180	-1.37		
atfC			**Afu1g17360**	-1.66		
fmpR			**Afu6g03430**	6.87	**AFLA_064330**	-
			Afu1g17460	-1.12		
			Afu3g02000	-1.32		
			Afu7g04340	2.21	**AFLA_073640**	-
			Afu1g02860	4.84		
gliZ			**Afu6g09630**	-2.12		
	AN11098	-	**Afu4g14712**	1.65	**AFLA_076320**	-1.18
nosA	**AN1848**	1.57	**Afu6g07010**	-1.06	**AFLA_025720**	-1.69

					AFLA_066630	-1.30
					AFLA_093080	-2.33
Following genes are grouped by their orthogroup						
	AN10906	-	**Afu5g01662**	-1.49	**AFLA_023260**	-1.88
	AN11169	-1.98	**Afu7g01820**	-1.88	**AFLA_070980**	1.48
	AN10579	-	**Afu8g06630**	-		
			Afu1g16115	-1.82		
	AN0388	2.27	**Afu1g01590**	-	**AFLA_025430**	-
	AN10550	-2.10				
xprG	**AN1414**	-1.18	**Afu1g00580**	3.58	**AFLA_012100**	-2.16
			Afu8g04050	2.16		-
	AN2422	-1.41	**Afu4g09860**	-	**AFLA_025860**	-1.65
	AN2785	-1.69				
farB2	**AN1425**	-	**Afu1g00410**	-	**AFLA_012010**	-1.49
farB1			**Afu8g04130**	1.75		

Table S3-14 DEGs related to trehalose metabolic process in Δ*wetA* conidia

	A. nidulans		***A. fumigauts***		***A. flavus***	
	ID	**Log_2 Fold Change**	**ID**	**Log_2 Fold Change**	**ID**	**Log_2 Fold Change**
Biosynthesis						
rfab	**AN10506**	-1.65	**Afu5g14780**	-7.43	**AFLA_008180**	-2.02
tppC	**AN10533**	-1.50			AFLA_087640	-1.09
orlA	**AN3441**	-2.90	**Afu3g05650**		**AFLA_103730**	-2.24
treB	**AN5635**		**Afu4g13530**	-2.02	**AFLA_052430**	-2.65
ccg-9	**AN5021**	-1.81	**Afu3g12100**	-2.58	**AFLA_030450**	-2.65
tpsA	**AN5523**	-1.62	**Afu6g12950**	-2.09	AFLA_087630	-3.85
tpsB			**Afu2g04010**	-1.84		
tpsC	**AN8639**	-2.01	**Afu4g03190**	-4.68	**AFLA_002830**	
tpsD		-1.65	**Afu5g14300**	-5.46		-
Degradation						
treA	**AN9340**	1.58	**Afu3g02280**	4.26	**AFLA_090490**	-2.85

Table S3-15 DEGs related to chitin metabolic process in Δ*wetA* conidia

	A. nidulans		***A. fumigauts***		***A. flavus***	
	ID	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**
Biosynthesis						
chsD	**AN1555**	3.05	**Afu8g05630**	6.59	**AFLA_078290**	2.43
pcmA	**AN4234**	1.88	**Afu1g06210**	4.45	**AFLA_127350**	
	AN8765	1.37	**Afu6g02940**		**AFLA_131730**	3.44
	AN5152	1.90	**Afu1g07110**	5.80	**AFLA_022110**	
csmB	**AN6317**	4.75	**Afu2g13430**	4.78	**AFLA_136040**	3.86
csmA	**AN6318**	4.97	**Afu2g13440**	3.56	**AFLA_136030**	3.37
ungA	**AN9094**	-1.14	**Afu7g02180**		**AFLA_071350**	2.29
	AN1069	2.02	**Afu1g12040**	1.20	**AFLA_067530**	
gnaA	**AN8706**	1.58	**Afu6g02460**	-1.23	**AFLA_091260**	
chs5	**AN8710**		**Afu6g02510**	3.72	**AFLA_091300**	2.30
chs3	**AN1554**		**Afu8g05620**	3.25	**AFLA_078300**	1.85
chsA	**AN7032**	2.19	**Afu2g01870**	2.50	**AFLA_042780**	1.95
chsB	**AN2523**	2.55	**Afu4g04180**	1.25	**AFLA_114760**	1.72
chsC			**Afu3g14420**	4.00	**AFLA_013690**	3.97
chsG					**AFLA_060590**	2.59
Degradation						
	AN10838					
	AN11233	4.82	**Afu3g07160**	4.05	**AFLA_102010**	4.03
nagA	**AN1502**	5.51	**Afu8g05020**	2.20	**AFLA_078900**	2.97
chiB	**AN4871**	8.23	**Afu3g11280**	1.90	**AFLA_031380**	5.80
	AN5454	4.17	**Afu8g01410**	4.11	**AFLA_104680**	6.80
chiA	**AN8241**	2.84				
	AN0299	1.44	**Afu1g02800**	1.00	**AFLA_080650**	
csnC	**AN11051**	4.59	**Afu3g14980**	2.88	**AFLA_089380**	1.18
			Afu4g01290	7.37	**AFLA_038420**	
chi4	**AN11059**	1.06	**Afu5g03530**	3.60	**AFLA_006340**	
			Afu5g03760	4.09		
			Afu5g03960	2.79		
			Afu5g06840	-3.45	**AFLA_013280**	-1.97
			Afu6g09780	-3.61		

	AN10309		**Afu6g10430**	-1.93	**AFLA_106680**	
			Afu7g05140	6.47	**AFLA_101800**	4.16
			Afu8g00700	3.24		
	AN7794	5.34				
	AN9390		**Afu6g09307**	3.24	**AFLA_028280**	
			Afu7g00670	-1.77		
cts2					**AFLA_033550**	2.30
					AFLA_040330	1.62
null	**AN9327**	2.34				
chiA1					**AFLA_006590**	2.02
	AN077		**Afu1g14430**	1.63	**AFLA_041970**	2.17
	AN9380				**AFLA_025900**	5.08
	AN1852	3.70	**Afu4g09940**			
	AN12279	2.58			**AFLA_107830**	2.03
			Afu6g00500	-3.21		
	AN0499	7.81				

Table S3-16 DEGs related to β-(1,3)-glucan metabolic process in Δ*wetA* conidia

	A. nidulans		*A. fumigauts*		*A. flavus*	
	ID	**Log_2 Fold Change**	**ID**	**Log_2 Fold Change**	**ID**	**Log_2 Fold Change**
Biosynthesis						
gelB	**AN0558**	2.92	**Afu6g11390**	3.04	**AFLA_108860**	1.92
btgA	**AN10150**	4.56	**Afu1g11460**	2.16	**AFLA_068300**	-2.36
btgD	**AN3727**	3.17	**Afu6g12380**	1.27	**AFLA_052780**	1.57
fksA	**AN3729**	5.81	**Afu6g12400**	4.57	**AFLA_052800**	1.92
gelA	**AN7657**	4.03	**Afu2g01170**	5.59	**AFLA_058480**	2.67
	AN8846	1.80	**Afu5g05770**	1.98	**AFLA_048250**	
sunA	**AN6697**	7.30	**Afu7g05450**	6.69	**AFLA_076430**	2.88
chs3	**AN1554**		**Afu8g05620**	3.25	**AFLA_078300**	1.85
			Afu3g13200	2.34	**AFLA_020630**	3.56
gelD	**AN11152**	2.54	**Afu2g05340**	4.85	**AFLA_052810**	2.72
gelC	**AN3730**	4.37	**Afu2g12850**		**AFLA_064920**	2.81
gelE	**AN7511**	-1.53	**Afu6g12410**	4.23	**AFLA_121370**	2.45
			Afu8g02130	3.26	**AFLA_129440**	3.02
Degradation						
					AFLA_023650	-4.21
	AN2217		**Afu5g07080**		**AFLA_057310**	1.03
	AN7915	-2.63			**AFLA_066750**	3.68
					AFLA_075690	-2.09
	AN2690	3.17	**Afu5g14030**	2.62	**AFLA_095890**	2.10
					AFLA_107790	2.09
			Afu4g03350	2.91	**AFLA_107800**	1.15
exg1	**AN4052**		**Afu1g03600**	-1.59	**AFLA_028260**	-3.06
	AN7869	2.22				
eglC	**AN7950**	-1.37				
engA	**AN0245**	3.43	**Afu1g05290**	1.35	**AFLA_029950**	5.25
engA	**AN0472**	4.55	**Afu1g04260**	2.06	**AFLA_028950**	3.61
	AN3883	4.68	**Afu5g02280**	4.85	**AFLA_004480**	1.17
bglN	**AN3949**	5.39				
btgC	**AN4700**	2.10	**Afu5g08780**	5.29	**AFLA_099780**	

	AN4825	3.01				
exgD	**AN7533**	3.24	**Afu6g09250**	1.08	**AFLA_129100**	1.37
btgE	**AN1551**	6.59	**Afu8g05610**	4.39	**AFLA_078320**	2.36
exg9	**AN4852**	3.61	**Afu2g00430**	-5.62	**AFLA_102640**	
			Afu3g07520	4.62		
bglJ	**AN7865**	6.69	**Afu5g07190**	2.43	**AFLA_057030**	
bglH	**AN3903**	4.46	**Afu3g00230**		**AFLA_000810**	
bglK	**AN2612**	-2.74	**Afu6g11910**		**AFLA_116080**	-1.63
bglB	**AN0712**	5.19	**Afu6g14490**	5.05		
			Afu7g00240	-3.34		
exgO	**AN0779**	6.26	**Afu1g14450**	2.58	**AFLA_041950**	2.77
	AN0550		**Afu6g11980**	-2.41		
exg6	**AN8480**	-2.45	**Afu6g13270**	3.53		
exg9	**AN4852**	3.61	**Afu2g00430**	-5.62	**AFLA_102640**	
			Afu3g07520	4.62		
eng7	**AN0031**	1.33	**Afu3g03080**	4.23		
	AN6620		**Afu6g14540**	2.83		
bglF	**AN10482**	6.86	**Afu1g05770**	4.20	**AFLA_023350**	-2.54
bglA	**AN4102**	2.27	**Afu1g17410**		**AFLA_014190**	
	AN5976		**Afu6g03570**		**AFLA_051140**	1.76
	AN6652		**Afu6g08700**	3.77	**AFLA_049420**	
bglL	**AN2828**	4.84	**Afu8g02100**		**AFLA_063040**	3.33
					AFLA_126780	-1.38
					AFLA_128480	4.27

Table S3-17 DEGs related to α-(1,3)-glucan metabolic process in Δ*wetA* conidia

	A. nidulans		***A. fumigauts***		***A. flavus***	
	ID	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**
Biosynthesis						
ags1	**AN5585**		**Afu3g00910**	5.31	**AFLA_023460**	-1.76
ags2	**AN3307**	-1.24	**Afu2g11270**	3.14	**AFLA_134100**	4.21
ags3			**Afu1g15440**	1.68	**AFLA_116810**	
Degradation						
agnD	**AN11064**	1.26	**Afu5g03940**	-2.89	**AFLA_006740**	-2.18
	AN1604	-7.48	**Afu8g06030**	-7.06	**AFLA_077910**	-1.86
			Afu7g08350	2.09	**AFLA_091790**	1.43
agnB	**AN3790**	5.34	**Afu2g03980**	1.71	**AFLA_034920**	
agnC	**AN9042**	4.03			**AFLA_087590**	
					AFLA_124510	
mutA	**AN7349**	3.80				

Table S3-18 DEGs related to melanin metabolic process in Δ*wetA* conidia

	A. nidulans		***A. fumigauts***		***A. flavus***	
	ID	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**
DHN-melanin biosynthesis						
wA	**AN8209**	-4.97	**Afu2g17600**	1.22	**AFLA_006170**	-5.70
ayg1	**AN9171**	7.09	**Afu2g17550**	2.15	**AFLA_075640**	-11.09
arp1	**AN10049**		**Afu2g17580**	3.52	**AFLA_016140**	1.75
arp2	**AN0146**		**Afu2g17560**	2.98		
abr1			**Afu2g17540**	2.35		
abr2	**AN6635**		**Afu2g17530**	1.70	**AFLA_045660**	
Pyomelanin biosynthesis						
bck1	**AN4887**		**Afu3g11080**	1.63	**AFLA_073870**	-1.45
hmgX	**AN1898**	7.56	**Afu2g04210**	3.50	**AFLA_036100**	
hppD	**AN1899**	6.48	**Afu2g04200**	2.75	**AFLA_036110**	
maiA	**AN1895**	6.39	**Afu2g04240**	2.90	**AFLA_036070**	1.13

Table S3-19 Hydrophobin-encoded DEGs in Δ*wetA* conidia

	A. nidulans		***A. fumigauts***		***A. flavus***	
	ID	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**	**ID**	**Log$_2$ Fold Change**
rodA	**AN8803**	7.02	**Afu5g09580**	3.61	**AFLA_098380**	1.72
	AN6401	1.16				
dewA	**AN8006**	-9.48			**AFLA_060780**	-4.32
	AN1837	5.78				
	AN0940					
			Afu8g07060	3.48		
			Afu2g14661	4.44		
*rodB**			**Afu1g17250**	6.54		
			Afu5g03280	3.98		
			Afu5g13480	1.05	**AFLA_094660**	
*rodB**					**AFLA_014260**	-2.00
					AFLA_063080	-10.01

* Afu1g17250 and AFLA_014260 are both annotated as *rodB*, but not in the same orthogroup.

Table S3-20 Top enriched GO categories of potential *Ani*WetA target genes

GO Category	# of Genes	% of Genes in Category
metabolic process	47	3%
carbohydrate metabolic process	34	12%
polysaccharide catabolic process	23	27%
transcription DNA-templated	20	6%
transport	20	5%
DNA-templated regulation of transcription	20	4%
cell wall organization	18	37%
protein transport	14	14%
cellulose catabolic process	9	29%
ribosome biogenesis	9	16%
proteolysis	9	6%
glucan catabolic process	8	40%
rRNA processing	8	15%
sterigmatocystin biosynthetic process	8	12%
pectin catabolic process	7	23%
cell cycle	7	17%
cellular response to osmotic stress	7	16%
conidium formation	7	9%
carbon utilization	6	27%
response to stress	6	11%
intracellular protein transport	6	9%
DNA repair	6	7%
cellulose metabolic process	5	56%
oxalate metabolic process	5	38%
positive regulation of sterigmatocystin biosynthetic process	5	22%
chromatin modification	5	19%
cellular amino acid biosynthetic process	5	13%
aromatic amino acid family biosynthetic process	5	12%
cellular response to DNA damage stimulus	5	9%
mannan catabolic process	4	40%
xylan catabolic process	4	18%
autophagy	4	17%

gluconeogenesis	4	17%
cleistothecium development	4	15%
mitotic nuclear division	4	14%
sporocarp development involved in sexual reproduction	4	13%
sexual sporulation resulting in formation of a cellular spore	4	12%
cell division	4	12%
acetate metabolic process	3	43%
mannose metabolic process	3	30%
glutamine family amino acid metabolic process	3	30%
glucan metabolic process	3	27%
acetate catabolic process	3	25%
formation of translation preinitiation complex	3	23%
positive regulation of transcription from RNA polymerase II promoter	3	21%
regulation of conidium formation	3	19%
regulation of translational initiation	3	19%
monodictyphenone biosynthetic process	3	14%
establishment of cell polarity	3	14%
glycerol metabolic process	3	13%
protein import into peroxisome matrix	2	67%
asexual spore wall assembly	2	50%
GDP-mannose biosynthetic process	2	50%
glycogen biosynthetic process	2	50%
iron ion homeostasis	2	50%
regulation of carbon utilization	2	50%
peptidyl-diphthamide biosynthetic process from peptidyl-histidine	2	40%
nucleosome assembly	2	40%
quinate metabolic process	2	33%
arginine biosynthetic process	2	33%
glutamine metabolic process	2	33%
ER to Golgi vesicle-mediated transport	2	29%
xyloglucan metabolic process	2	29%
glucose metabolic process	2	29%
positive regulation of penicillin metabolic process	2	29%
DNA-templated, negative regulation of transcription	2	29%

CHAPTER 4

Conclusions and Future Directions

4-1 Conclusion

The *Aspergillus* life cycle is complex, precisely timed, and genetically programmed to involve specialized cellular differentiation, temporal and spatial regulation of gene expression, and cellular communications [1]. Numerous genes are involved in regulating fungal development. In asexual development, the central regulators, BrlA, AbaA, and WetA, play crucial roles and define sequentially-dependent pathway of conidiation [2,3]. However, the extent to how WetA functions in conidia maturation remains unknown. Previous studies show that WetA activates a series of spore-specific genes [3]. Importantly, WetA contains a conserved DNA-binding domain with a nuclear localization signal (NLS) inside of the domain, suggesting that it is a potential transcription factor. Moreover, as AbaA exerts differential regulatory effects on the *velvet* regulators in different *Aspergillus* species [4,5,3], we proposed that the WetA-mediated genetic regulatory networks (GRNs), as well as AbaA-mediated GRNs, reroute during evolution. To solve the last puzzle of the WetA-mediated regulation in *Aspergilli*, we carried out systematic analyses of the WetA phenotypes, transcriptomics, and direct targets in three important *Aspergillus* species: *A. nidulans*, *A. fumigatus*, and *A. flavus*.

*Ani*WetA and *Afu*WetA have been characterized in previous studies [2–4,6–12]. We generated the Δ*AflwetA* mutant and characterized the functions of *Afl*WetA (Chapter 2). *Afl*WetA is a multi-functional regulator that couples spore differentiation and survival, and governs proper chemical development in *A. flavus*. WetA is functionally conserved and required in *A. flavus* for many aspects of its biology, including spore viability, wall integrity, and stress tolerance. However, loss of *AflwetA* causes earlier conidiation and hyper-condensation of the C2 layer of the conidia wall in Δ*AflwetA* conidia, suggesting the differential regulatory roles of *Afl*WetA in comparison to *Ani*WetA and *Afu*WetA. Moreover, we performed transcriptome analysis to elucidate the functions of *AflwetA* in conidia. The results show that *Afl*WetA contributes the regulation of conidial components metabolic processes and conidiation. Furthermore, *Afl*WetA regulates a variety of transcription factors and signaling pathways, indicates that the *Afl*WetA is an upstream regulator of multiple development processes.

We further carried out RNA-seq to illustrate the functions of *Ani*WetA and *Afu*WetA in conidia (Chapter 3). The results show that the *Aspergillus* WetA regulates more than half of the genome in conidia. The *Aspergillus* WetAs are functionally conserved in the feedback repression of conidiation, activation of trehalose biosynthesis, repression of β-(1,3)-glucan, and the response to environmental stimuli. More than 40% WetA-regulated genes have at least one orthologs in other two species, suggesting that the WetA-regulated targets are highly conserved in *Aspergilli*. However, the WetA-mediated regulatory effect on the conserved targets is divergent.

To verify whether WetA is a transcription factor, we carried out ChIP-seq in *A. nidulans*. The result shows that WetA can physically interact with its target's promoter region, including WetA itself. The WetA response elements (WREs) are widely presented in *A. nidulans* genome, as well as in *A. fumigatus* and *A. flavus* genomes. About half of the *Ani*WetA-targeted genes are regulated by WetA in conidia, suggesting that the WetA-mediated regulation may be temporal- and spacious-specific. Moreover, only ~20% WetA-regulated genes contain WRE in their promoter regions, indicating that WetA is a supreme upstream regulator, which indirectly regulates it targets by controlling their regulators.

To sum up, WetA is evolutionally and functionally conserved key transcription factor, which bridges cellular and chemical development processes. Furthermore, the WetA-mediated GRNs are re-wired to underlie the evolution of development and metabolic control in *Aspergillus* species.

4-2 Future direction

4-2-1 WetA-regulated targets in *Aspergilli*

Based on the transcriptome analyses, we're able to identify several interesting WetA-regulated targets for further study. For example, AFLA_063080 is a predicted hydrophobin encoded gene which is almost fully shut down in Δ*AflwetA* conidia, as well as the *AnidewA* in Δ*AniwetA* conidia. AFLA_063080 is a *A. flavus*-specific gene, which may play an important role in *A. flavus* conidia maturation. AN1298, Afu1g09670, and AFLA_021240 are orthologs which are predicted as the bHLH transcription factor with unknown function. Their expression levels are increased 20~50 times in Δ*wetA* conidia, suggesting that they may play an important role in WetA-mediated GRNs.

4-2-2 Analysis of WetA-mediated VosA and FlbC regulatory mechanism

Based on our RNA-seq and ChIP-seq results, WetA activates *vosA* and represses *flbC* by direct binding. We plan to create the *vosA* overexpression mutant and *flbC* deletion mutant on the *wetA* deletion background to verify whether some of the Δ*wetA* phenotypes can be rescued. However, the WetA-VosA regulatory mechanism may be more complicated since our data suggests that WetA and VosA can cross-activate each other by direct binding and may form a complex and co-regulate the *Aspergillus* genomes. To prove our hypothesis, the *in vitro* and/or *in vivo* protein-protein interaction assays are necessary. Moreover, the binding motifs' co-occurrence in *Aspergilli* and cross-comparison of RNA-seq results from Δ*AniwetA* and Δ*AnivosA* analysis are in progress.

4-2-3 Metabolome analysis of WetA-regulated metabolic processes

Function analysis of our RNA-seq results shows that WetA contributes to both primary and secondary metabolism. However, WetA usually shows a mix regulatory effects on different members in the same pathway. To know the net effect of WetA, a comprehensive metabolome analysis is necessary. Our preliminary data shows that WetA is involved in several amino acid metabolic pathways. However, the extraction method of the primary and secondary metabolites still needs to be optimized.

4-2-4 WetA-mediated regulation in different development stages

In this study, we focused on a single cell type, conidia. However, both the phenotype observation and functional analysis of RNA-seq data suggest that WetA also plays regulatory roles in different development stages. For example, it will be interesting to check the transcriptome and WetA-DNA interaction at early conidiation stage, which WetA begins to feedback repress *brlA* expression. Our result also shows that *Ani*WetA and *Afu*WetA repress the blue-light receptors, *lreA,* and *lreB*, while *Afl*WetA represses the red-light receptor *fphA*. Comparative transcriptome analysis of different light source during conidiation would elucidate the WetA-mediated light-dependent regulation of conidiation.

4-3 References

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