



## Genetic Basis and Function of Mating-Type Genes in *Aspergillus cristatus*

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### Abstract

*Aspergillus cristatus* is a homothallic fungus, the genome of which contains two mating-type (*MAT*) genes, *MAT1-1-1* and *MAT1-2-1*. Previous studies showed that the origin of *MAT* loci in this species differs from that of other fungi, and their functions have never been described. In this study, *MAT1-1-1* and *MAT1-2-1* were individually deleted by gene replacement, confirming that these genes regulate the sexual development of *Aspergillus cristatus*, with the two single-deletion mutants showing similar phenotypes. To explain this phenomenon, the evolutionary relationships of the core domains from high-mobility-group box (HMGB) domain, high-mobility-group (HMG) domain, and alpha 1 ( $\alpha 1$ ) domain were investigated, with the findings indicating that  $\alpha 1$  domain and HMG domain have a close evolutionary relationship. Transcriptomic analyses indicated that mating-type genes regulate the sexual development of *Aspergillus cristatus* by controlling the expression levels of pheromone precursor and receptor genes. This paper presents the first systemic investigation of the function of *MAT* genes from *Aspergillus cristatus*.

**Key words** – *Aspergillus cristatus* – mating-type gene – sexual development

### Introduction

Sexual development is a common, albeit not ubiquitous, mode of reproduction in fungi. In Ascomycota, sexual development involves cell fusion, nuclear fusion, and meiosis. These stages are orchestrated by the *MAT* locus, which encodes key transcription factor genes that govern identity and developmental fate (Lee et al. 2010, Dyer & O’Gorman 2012, Krijgsheld et al. 2013). Heterothallic fungi have two different *MAT* locus genes, one encoding a protein with a high-mobility-group (HMG) domain, called *MAT1-2-1*, and the other encoding a protein with an alpha box ( $\alpha 1$ ) domain, called *MAT1-1-1*. These two genes are assigned to different mating partners (Turgeon & Yoder 2000, Kück & Pöggeler 2009). In homothallic fungi, by contrast, the genome contains both *MAT1-1-1* and *MAT1-2-1* genes, so these species are self-fertile (Paoletti et al. 2007).

*Aspergillus cristatus* is a taxon that is dominant in the fermentation processes of Fuzhuan brick tea, and may produce abundant golden cleistothecia in later stages of the fermentation process (Tan et al. 2017). Among aspergilli, most species can reproduce exclusively asexually, but *A. cristatus* can undergo both sexual and asexual development under conditions of high and low

osmolarity; the genome of this species has also been sequenced, which has facilitated the study of *A. cristatus* (Ge et al. 2016, 2017). Genome analyses have shown that the *MAT* locus genes of *A. cristatus* are located in scaffolds 5 and 6, respectively. Evolutionary analyses have also revealed that the *MAT* loci of *A. cristatus* differ for those of other *Aspergillus* species (Ge et al. 2016). Moreover, the function of *MAT* genes from *A. cristatus* has never been reported.

In this study, we used gene replacement to assess the function of *MAT* genes in *A. cristatus*. According to the evolutionary relationship of the core domains from HMGB, HMG, and  $\alpha 1$ , we explained why single-deletion mutants of *MAT1-1-1* and *MAT1-2-1* have similar phenotypes. Moreover, we analyzed the regulatory mechanism by which *MAT1-1-1* and *MAT1-2-1* control sexual development via transcriptome sequencing of the wild-type strain and *MAT* gene mutants. This paper reports for the first time the function and evolutionary relationship of *MAT1-1-1* and *MAT1-2-1* in *A. cristatus*, and also provides some important results for understanding the regulatory mechanisms of *MAT* genes.

## Materials & Methods

### Strains, growth conditions, and morphological observation

*Aspergillus cristatus* E4 (CGMCC 7.193) was isolated from Fuzhuan brick tea manufactured by Yiyang Tea Factory Co. Ltd. (Yi Yang City, Hunan Province, China) and cultured on a cellulose membrane placed on MYA medium (malt extract, 20 g; yeast extract powder, 20 g; sucrose, 30 g; agar powder, 18 g; and water, 1000 mL) in the dark at 28 °C for 2 to 7 days. Mycelia were then fixed with 0.5% v/v glutaraldehyde in the dark at 4 °C for 12 h, washed three times with 0.1 mol/L PBS, fixed with 1% osmic acid for 2 h, washed three times with 0.1 mol/L PBS, dehydrated with 50%–99% thiobarbituric acid, freeze-dried under a vacuum, and coated with gold for morphological observation using a Hitachi S-3400N electron scanning microscope (Hitachi Corporation, Tokyo, Japan) (Ge et al. 2016). Moreover, mutant strains were cultured on MYA, low-osmolarity MYA (5% NaCl), and high-osmolarity MYA (17% NaCl), and the colony morphologies of wild-type and mutant strains were observed using an optical microscope (Olympus, Tokyo, Japan).

### Deletion of mating genes in *Aspergillus cristatus*

Two vectors for the deletion of *MAT1-1-1* (SI65\_05562) and *MAT1-2-1* (SI65\_06277) (Ge et al. 2016) were constructed, aiming at inserting the hygromycin-resistance cassette into the coding region of the genes to be deleted (Sugui et al. 2005, Mullins et al. 2001, Huang et al. 2015). In brief, the 5' and 3' flanking sequences of mating-type genes were amplified using genomic DNA as a template with the primer pairs *MAT1-1-1\_UL* and *MAT1-1-1\_UR* (resulting in a fragment size of 930 bp), *MAT1-1-1\_DL* and *MAT1-1-1\_DR* (fragment size of 713 bp), *MAT1-2-1\_UL* and *MAT1-2-1\_UR* (fragment size of 1121 bp), and *MAT1-2-1\_DL* and *MAT1-2-1\_DR* (fragment size of 834 bp) (Table 1). The products were digested with the restriction enzymes Xho I, BamH I, Spe I, and Xba I, respectively, and then inserted into the corresponding sites of the binary vector pDHt for fungal transformation (Mullins et al. 2001). The mutants were verified by Southern blotting with a probe (*Hyg-p*) and PCR with the primers *MAT1-1-1\_S* and *MAT1-2-1\_S* (Table 1).

**Table 1** Primers used for PCR amplification for mating-type genes deletion and detection

Primer names	Primer sequences	Products size (bp)
<i>MAT1-1-1_UL</i>	5'-CTCGAGCGACTTTCGCATCACTCC-3'	930
<i>MAT1-1-1_UR</i>	5'-GGATCCCCTACCCCATCACCCAT-3'	
<i>MAT1-1-1_DL</i>	5'-ACTAGTAAATGGGCTATCCTTGC-3'	713
<i>MAT1-1-1_DR</i>	5'-TCTAGAATGGCTTGTATCGTCCC-3'	
<i>MAT1-2-1_UL</i>	5'-CTCGAGTGGAGATAGAATCCGAAACG-3'	1121
<i>MAT1-2-1_UR</i>	5'-GGATCCACAGATACCGCAGAGCC-3'	

**Table 1** Continued.

Primer names	Primer sequences	Products size (bp)
MAT1-2-1_DL	5'-ACTAGTGCTTCATTTCGGTTCGTC-3'	834
MAT1-2-1_DR	5'-TCTAGAAGCCCAAGTCAATCTCG-3'	
MAT1-1-1_SL	5'-TCCGTCTGAATGAAGCC-3'	3158
MAT1-1-1_SR	5'-GGGCGGCATAGAAGTGT-3'	
MAT1-2-1_SL	5'-AAGGCTTTCTCGGTTTG-3'	2814
MAT1-2-1_SR	5'-ACCGTTCGTTAGTTGCT-3'	
Hyg-pL	5'-TTCGATGTAGGAGGGCGTGGAT-3'	626
Hyg-pR	5'-CGCGTCTGCTGCTCCATACAAG -3'	

### **MAT gene sequence acquisition and analysis**

We analyzed the promoters, terminators, and introns of *MAT* genes using Fgenesh<sup>+</sup> (Solovyev et al. 2006). The high-mobility-group box (HMGB), HMG, and alpha 1 ( $\alpha$ 1) amino acid sequences from aspergilli retrieved from AspGD (Aspergillus Genome Database) and NCBI (National Center for Biotechnology Information) are reported in Additional file 1. The core regions of these sequences were revealed using Kalign (Lassmann & Sonnhammer 2006) and datasets were analyzed with WebLogo (Crooks et al. 2004). Sequence alignments were generated with Clustal W2 (Larkin et al. 2007). The alignment results were used to construct a phylogenetic tree using maximum likelihood by MEGA 7.0 (Kumar et al. 2016). Moreover, secondary structures of *MAT* gene sequences were predicted by Jpred 4 (Drozdetskiy et al. 2015). Fold recognition and 3D structure prediction were performed with Phyre 2 (Kelley et al. 2015).

### **RNA extraction and transcriptome sequencing**

Mycelia of three different strains, namely, *A. cristatus* E4 (wild type), *MAT1-1-1* deletion strain ( $\Delta$ *MAT1-1-1\_2*,  $\Delta$ M1), and *MAT1-2-1* deletion strain ( $\Delta$ *MAT1-2-1\_3*,  $\Delta$ M2), were grown on MYA for 40 h (ascogonial coils begin to form; stage 1) and 48 h (cleistothecia can be observed and enumerated; stage 2). For the culture at each stage, three independent biological replicates were designed; thus, total RNA was extracted from a total of 18 samples using TRIzol reagent, in accordance with the manufacturer's instructions (Life Technologies Co., Ltd., Carlsbad, CA, USA). RNA was quantified using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Fisher Scientific Inc., Boston, MA, USA) based on the absorbance at 260 and 280 nm. A total of 1  $\mu$ g of RNA per sample was used as a template for the construction of cDNA libraries. The prepared libraries were sequenced on a BGISEQ-500 RNA-ref platform (BGI, Shenzhen, China), and 100-bp pair end (PE) reads were generated.

### **Identification and characterization of differentially expressed genes**

The high-quality clean reads were mapped to the genome and gene dataset of *A. cristatus* E4 by HISAT and Bowtie 2 (Kim et al. 2015), and then the expression levels of the transcripts were calculated by RSEM (Li & Dewey 2011). In this study, an absolute  $\log_2$  value (fold change)  $\geq 1$  and adjusted P value  $\leq 0.05$  were used as threshold values to screen for differentially expressed genes (DEGs). All data contributing to this study have been deposited with the NCBI under BioProject PRJNA431929. The RNA-seq expression dataset has been deposited at the NCBI's Sequence Read Archive under accession code SRP131629.

## **Results**

### **Analysis of MAT gene sequences**

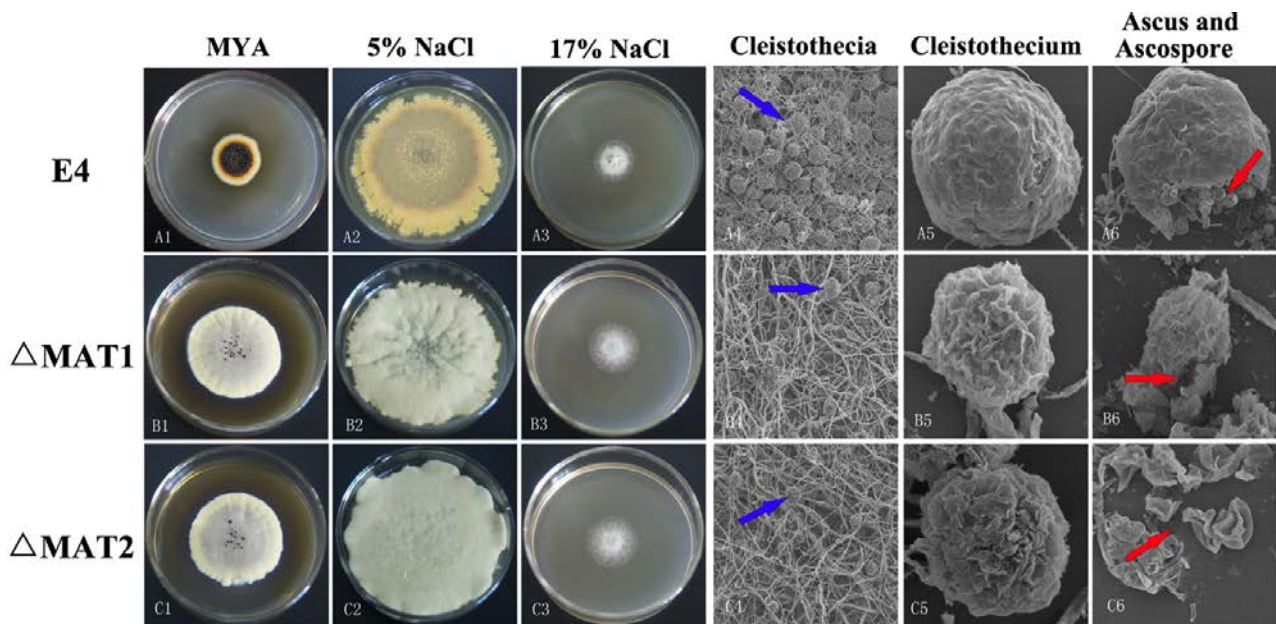
In this study, we used Fgenesh to analyze promoters, terminators, and introns of *MAT1-1-1* (SI65\_05562) and *MAT1-2-1* (SI65\_06277) from the *A. cristatus* genome (Ge et al. 2016, Solovyev et al. 2006). The results indicate that *MAT1-1-1* of *A. cristatus* is 1209 bases long and has one

intron of 62 bp, and encodes a protein with 384 amino acids. *MAT1-2-1* of *A. cristatus* is 1188 bases long, has two introns of 63 and 51 bp each, and encodes a protein with 357 amino acids.

### Functional characterization of mating-type genes

*MAT1-1-1* and *MAT1-2-1* of *A. cristatus* were individually deleted by gene replacement. A total of four *MAT1-1-1* and two *MAT1-2-1* deletion mutants were obtained, all of which showed defects in sexual development. One *MAT1-1-1* transformant ( $\Delta$ *MAT1-1-1\_2*,  $\Delta$ *MAT1*) and one *MAT1-2-1* transformant ( $\Delta$ *MAT1-2-1\_3*,  $\Delta$ *MAT2*) were chosen for further study. Subsequently, we observed the colony morphology of the wild-type strain and mutants. On MYA, the colonies of the mutant strains were lighter yellow and larger than for the control strain; moreover, the black exudate from the colony surface of wild-type strains was more extensive than that from the mutants (Fig. 1; A1, B1 and C1). On MYA with 5% NaCl, the colonies of the mutant strains and the wild-type strain were nearly the same size, and these cultures did not produce exudate; the color of the colonies of the mutant strains was lighter than that of the control strain (Fig. 1; A2, B2 and C2). The colony morphologies of the three strains were similar on MYA with 17% NaCl (Fig. 1; A3, B3 and C3).

The cleistothecium and ascospore morphology of the mutants and wild-type strains was observed by electron scanning microscopy. The results showed that two mutants produced fewer cleistothecia than the wild type under conditions of sexual development (Fig. 1; A4, B4 and C4). Moreover, the developed cleistothecia were immature and smaller than those in the wild-type strain (Fig. 1; A5, B5 and C5). Notably, no ascospores formed in the cleistothecia of the mutants (Fig. 1; A6, B6 and C6). This indicates that either of the *MAT* genes is essential for ascospore production and for the sexual development of *A. cristatus*.

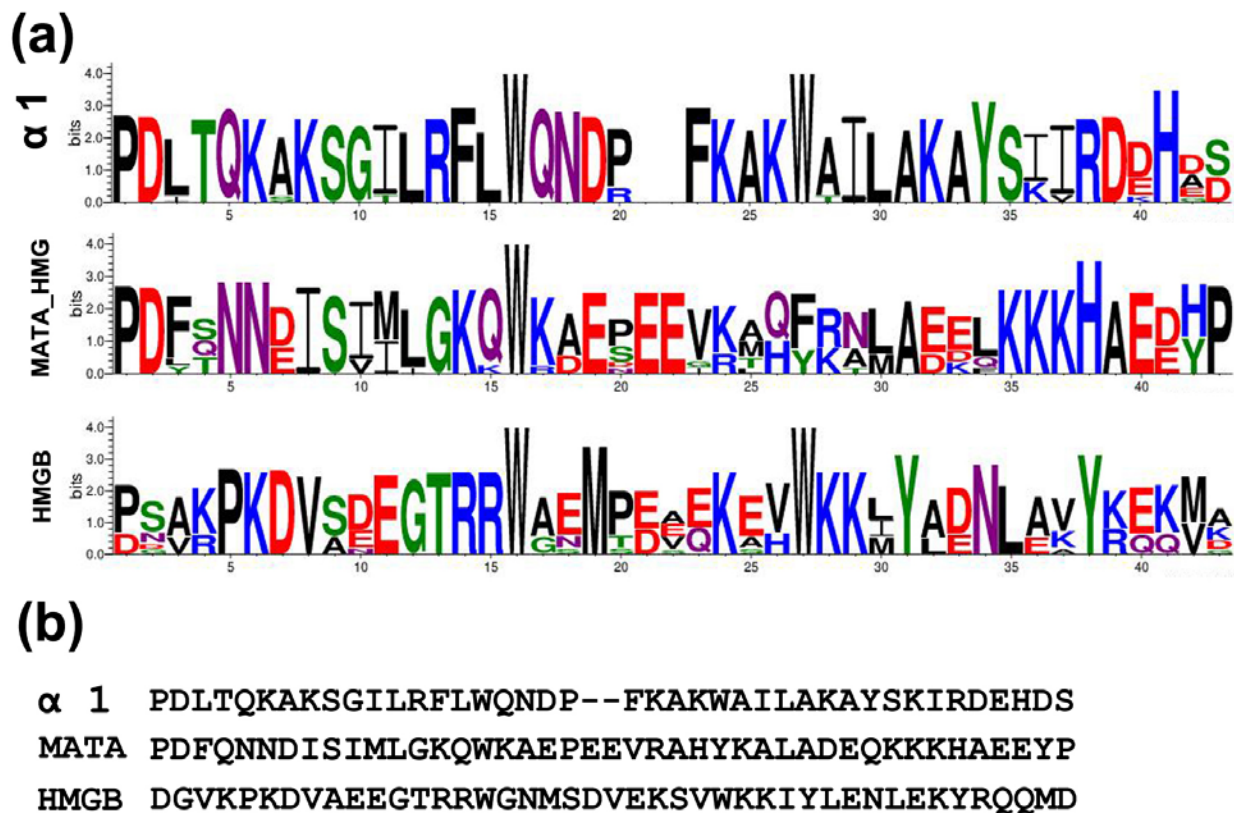


**Figure 1** – Phenotypic observation of the *MAT* mutants and wild-type strains under different growth conditions. A1–A6 show the results for wild-type strain *Aspergillus cristatus* E4. B1–B6 show the results for *MAT1-1-1* mutant. C1–C6 show the results for *MAT1-2-1* mutant. Blue arrows indicate cleistothecia on the mycelium, red arrows indicate the ascus and ascospore. In *Aspergillus cristatus*, E4 has ascus and ascospore, while *MAT1-1-1* and *MAT1-2-1* mutants have no ascus and ascospore.

Since the phenotypes of *MAT1-1-1* and *MAT1-2-1* mutants were similar, we speculated that the *MAT* genes from *A. cristatus* share similar functions. To confirm this, we performed an analysis of the evolution of the mating-type genes.

## Evolutionary analysis of mating-type genes

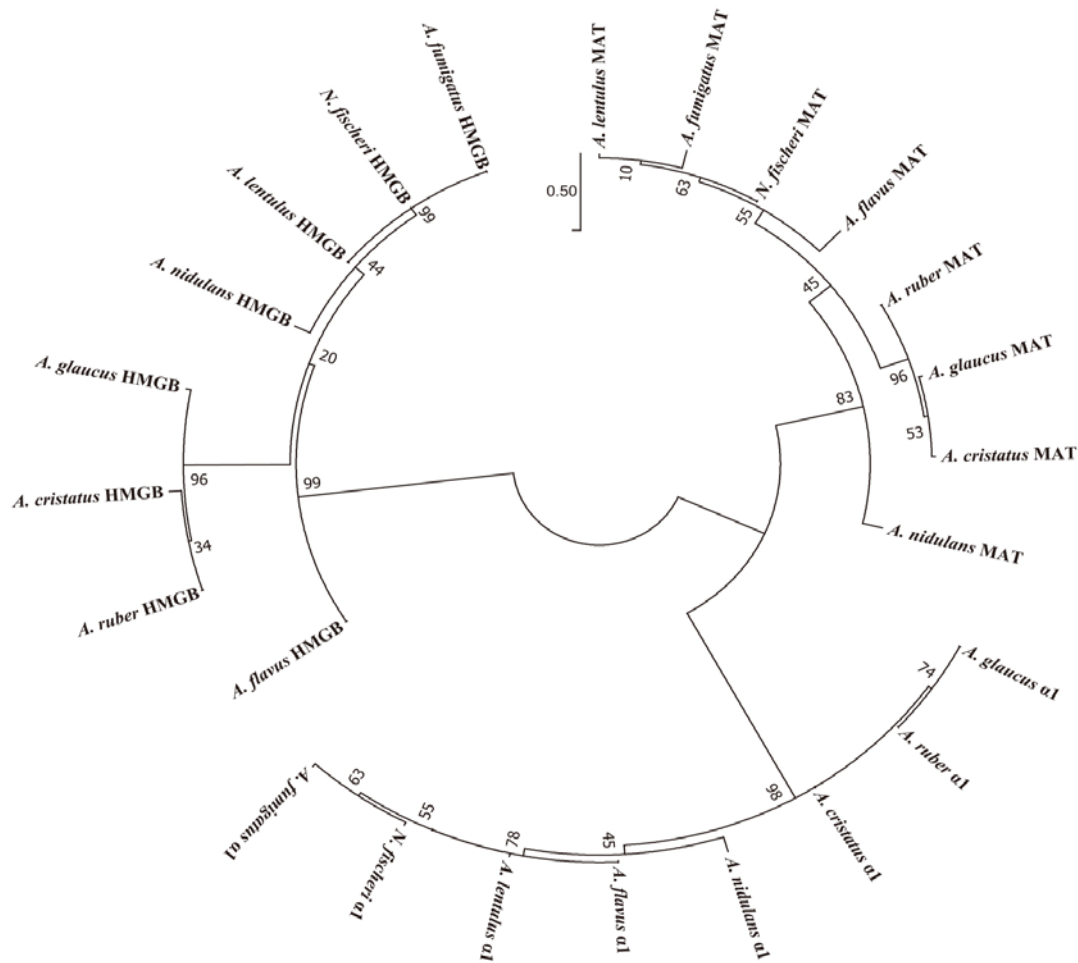
We selected protein sequences of aspergilli from AspGD and the NCBI database (Additional file 1) and divided them into three datasets, namely, HMGB domain,  $\alpha 1$  domain, and HMG domain datasets. Subsequently, we obtained the core domains of the three datasets using Kalign (Lassmann & Sonnhammer 2006); the core domain of  $\alpha 1$  contained 41 amino acids and the core domains of HMG and HMGB contained 43 amino acids. The correlation of these amino acid sequences was derived using Weblogo (Fig. 2a) (Crooks et al. 2004), and the results revealed higher similarity between HMG and  $\alpha 1$  domains than with the HMGB domain (Fig. 2b). Meanwhile, a maximum likelihood phylogram was constructed using the core domains of three datasets. The results showed that the sequences of the three domains were clustered into two clades. HMGB domain sequences were distributed in a monophyletic clade, while the HMG and  $\alpha 1$  domains were mostly distributed in another clade (Fig. 3). These results also indicated that HMG and  $\alpha 1$  domain sequences have closer affinity than the HMGB domain sequence.



**Figure 2** – Conserved sequence of HMGB, MAT\_HMG and  $\alpha 1$  domains. a Weblogo representation of a conserve sequence in  $\alpha 1$ , MATA\_HMG and HMGB. The X-axis represents amino acid position from the N to C terminal. The height of each letter within the stack reflects the relative frequency of the corresponding amino acid at the position, the overall height of each letter indicates the sequence conservation at the position (measured in bits). b Consensus core sequences of conserved amino acid residues.

The sequence conservation between HMG and  $\alpha 1$  domains suggested that they may have similar secondary and tertiary structures. As such, we first predicted the secondary structures of HMG and  $\alpha 1$  domains using Jpred 4 (Drozdetskiy et al. 2015). The results showed that the HMG and  $\alpha 1$  domains form three helices (Fig. 4a). Helix 1 of the  $\alpha 1$  domain is four residues shorter than that of the HMG domain, and helix 3 of the  $\alpha 1$  domain is three residues shorter than that of the HMG domain (Fig. 4b, c). Protein sequences used for secondary structure prediction were submitted to Phyre 2 for fold recognition (Kelley et al. 2015). As expected, the best matching templates for the  $\alpha 1$  domain of *A. cristatus* were known HMG-box template structures. The

likelihood of the homology was high (99%) and all tested mating-type proteins had the HMG-box family fold descriptor. Thus, the  $\alpha 1$  and HMG domains of *A. cristatus* have the same genealogy. According to these results, we speculated that *MAT1-1-1* (possessing an  $\alpha 1$  domain) and *MAT1-2-1* (possessing HMG domains) of *A. cristatus* have the same origin.

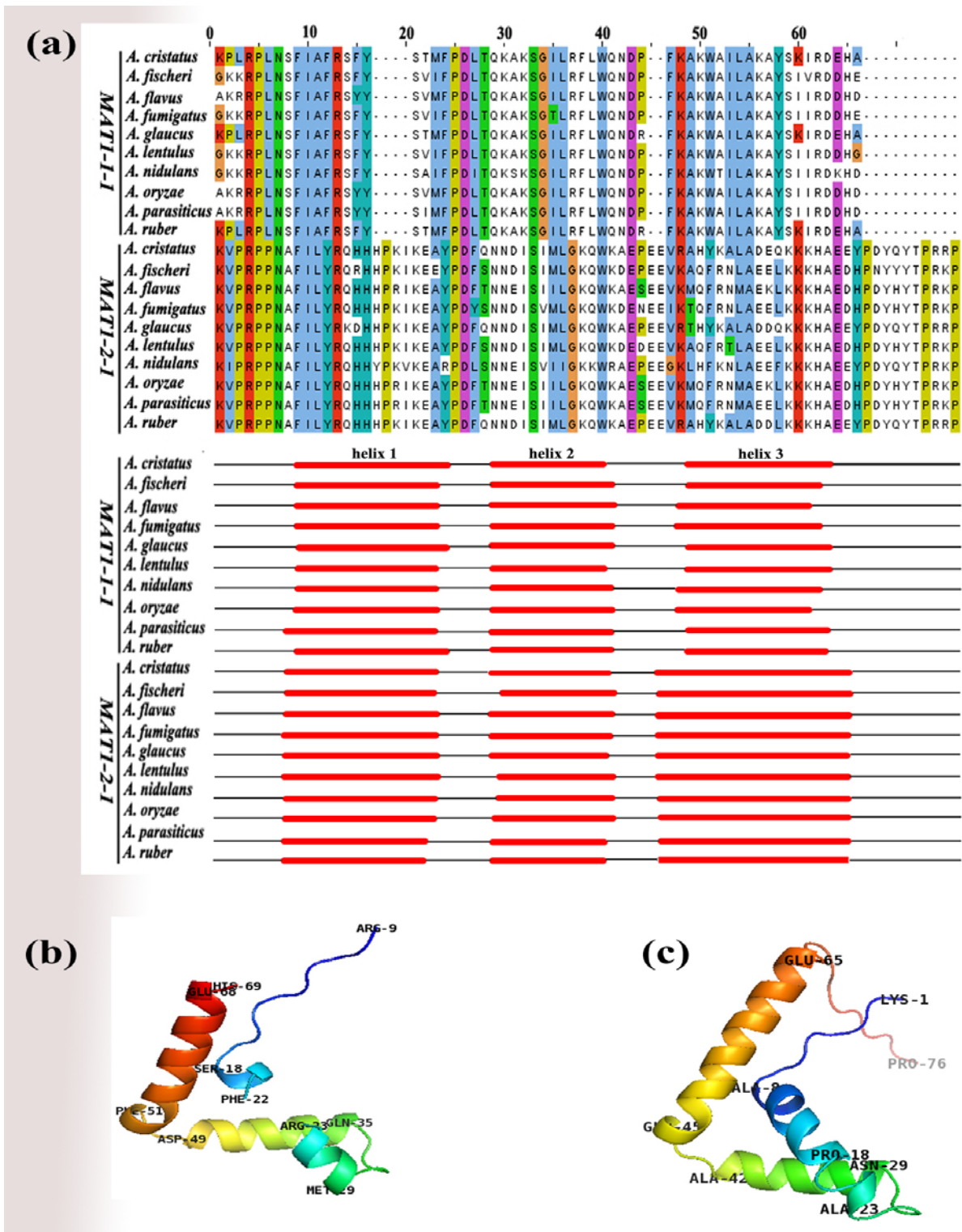


**Figure 3** – Unrooted phylogram for the HMGB, MAT\_HMG and the  $\alpha 1$  domain core amino acid sequences. Clustering of core amino acid sequences using maximum-likelihood from MEGA 7.0 (Kumar et al. 2016), 1000 bootstrap replicates were used. Three domains clustered in two clades, with HMGB domain sequences distributed in a monophyletic clade, and MAT\_HMG and  $\alpha 1$  domains mostly distributed in a clade. Abbreviations: HMGB, high mobility group box; MAT, MAT\_HMG;  $\alpha 1$ , alpha 1.

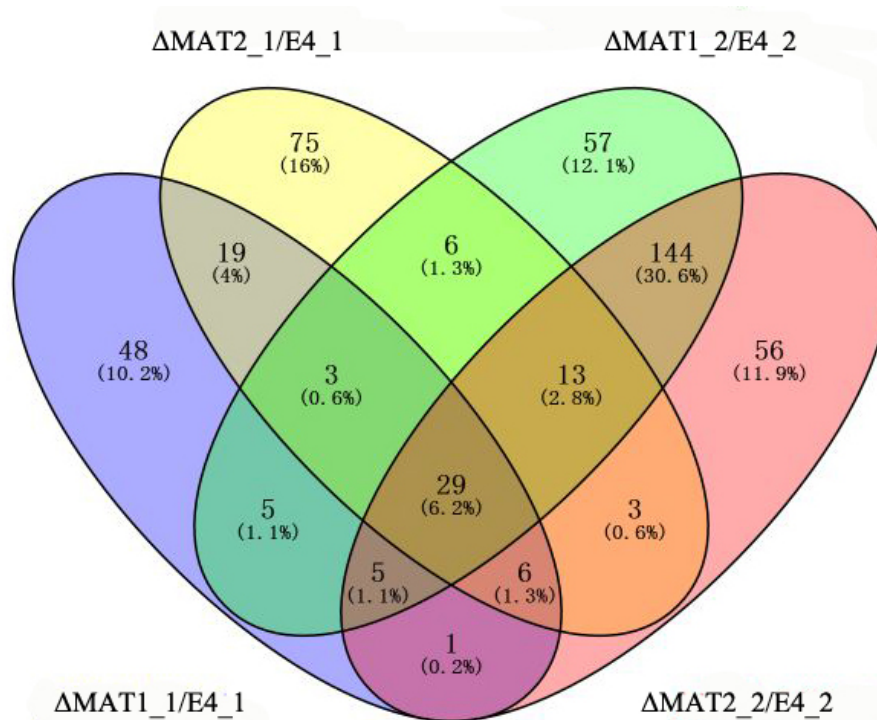
### Mechanism of regulation of sexual development by mating-type genes

This study identified 551 differentially expressed genes by comparing gene expression levels from the mutants and the wild-type strain, accounting for 5.44% of the total number of genes (Additional file 2). Of these, 116 and 262 genes were the differentially expressed genes from  $\Delta$ MAT1 at stage 1 and stage 2, respectively. 154 and 257 genes were the differentially expressed genes from  $\Delta$ MAT2 at stage 1 and stage 2, respectively (Fig. 5). Among these genes, 9 differentially expressed genes are associated with sexual reproduction of *A. cristatus* (Table 2; Fig. 6). The expression levels of *ppgA* and *preA* from  $\Delta$ MAT1 were down-regulated in both stages, while *preB*, *ste14*, *rce1*, and *fphA* of  $\Delta$ MAT1 were only down-regulated at stage 1, and *gprK*, *flbD*, and *flbA* were only down-regulated at stage 2. Meanwhile, *preB*, *ste14*, and *rce1* of  $\Delta$ MAT2 were down-regulated at both stages, while *gprK* and *flbD* of  $\Delta$ MAT2 were only down-regulated at stage 2, but *ppgA* of  $\Delta$ MAT2 was up-regulated at stage 2. Moreover, *flbD*, *ppgA*, *rce1*, *ste14*, and *gprK*

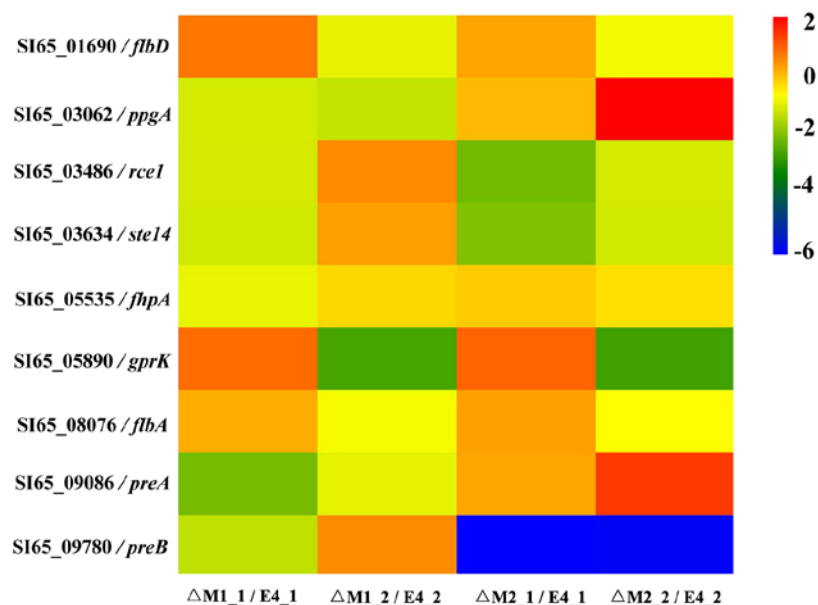
were regulated together by *MATI-1-1* and *MATI-2-1*, while other genes were only regulated by one of the *MAT* genes. These results suggest that *MATI-1-1* and *MATI-2-1* regulate the sexual development of *A. cristatus* by controlling genes for pheromone formation and signaling, and by controlling genes for cleistothecium development. The results also indicate that sporulation-related genes regulated by *MATI-1-1* differ from those regulated by *MATI-2-1*.



**Figure 4** – Predicted secondary and 3D structures of the  $\alpha 1$  and MAT\_HMG domains from *Aspergillus cristatus*. a Secondary structure of MAT\_HMG and the  $\alpha 1$  domain from other aspergilli. b *in silico* 3D structure model of the  $\alpha 1$  domain from *MATI-1-1* in *Aspergillus cristatus*. c *in silico* 3D structure of the MAT\_HMG domain from *MATI-2-1* in *Aspergillus cristatus*.



**Figure 5** Comparison of the differentially expressed genes from the wild strain and *MAT* genes mutants. In the figure,  $\Delta$ MAT1\_1/E4\_1 indicates the differentially expressed genes from the *MAT1-1-1* deletion strain relative to *Aspergillus cristatus* E4 at stage 1, while  $\Delta$ MAT1\_2/E4\_2 indicates that at stage 2. The results of  $\Delta$ MAT2\_1/E4\_1 and  $\Delta$ MAT2\_2/E4\_2 are similar to those described previously.



**Figure 6** Differentially expressed genes from *MAT1-1-1* and *MAT 1-2-1* mutants. In the figure, changes in background color from blue to red indicate changes in log<sub>2</sub> change fold values (−6, −4, −2, 0, and 2; upper right) of gene expression levels under conditions of 40 h and 48 h of growth on MYA. The codes on the right, such as SI65\_09086/*preA*, are gene IDs or names. Sample information is detailed beneath the images.  $\Delta$ MAT1\_1/E4\_1 indicates log<sub>2</sub> fold change of the gene expression level of the *MAT1-1-1* deletion strain relative to *Aspergillus cristatus* E4 at stage 1, while  $\Delta$ MAT1\_2/E4\_2 indicates that at stage 2. The results of  $\Delta$ MAT2\_1/E4\_1 and  $\Delta$ MAT2\_2/E4\_2 are similar to those described previously.

**Table 2** Differentially expressed genes associated with sexual reproduction of *Aspergillus cristatus* in *MAT1-1-1* and *MAT1-2-1* deletion-mutants.

Genes names	AspGD ID	<i>A. cristatus</i>	Function	Log2 Fold Change ( $\Delta$ <i>MAT1-1-1</i> _1 / E4-1)	Log2 Fold Change ( $\Delta$ <i>MAT1-1-1</i> _2 / E4-2)	Log2 Fold Change ( $\Delta$ <i>MAT1-2-1</i> _1/ E4-1)	Log2 Fold Change ( $\Delta$ <i>MAT1-2-1</i> _2/ E4-2)
<i>flbD</i>	AN0279	SI65_01690	Regulation of conidiophore formation	0.327948906	-1.17857348	-0.09443022	-1.03561797
<i>ppgA</i>	AN5791	SI65_03062	Pheromone precursor	-1.33098067	-1.48248346	-0.31501377	1.413918246
<i>rce1</i>	AN6528	SI65_03486	Role in pheromone precursor processing	-1.34618001	0.152754026	-2.26236605	-1.33090013
<i>ste14</i>	AN6162	SI65_03634	Role in pheromone precursor processing	-1.36356792	-0.05119989	-2.09475117	-1.35388858
<i>fhpA</i>	AN4521	SI65_05535	Regulation of sexual development	-1.13870613	-0.56537125	-0.44966608	-0.65871797
<i>gprK</i>	AN7795	SI65_05890	Putative heterotrimeric G-protein coupled receptor component	0.426691541	-2.63516061	0.492665098	-2.70359253
<i>flbA</i>	AN5893	SI65_08076	Inhibition of vegetative growth regulation Homothallic cleistothecia formation	-0.18121976	-1.00900537	-0.05402233	-0.92864768
<i>preA</i>	AN7743	SI65_09086	G-protein coupled pheromone receptor	-2.2166909	-1.17938831	-0.10736702	0.88184842
<i>preB</i>	AN2520	SI65_09780	G-protein coupled pheromone receptor	-1.5221798	0.123230336	-5.6924923	-5.61106

## Discussion

Mating-type genes are pivotal in the sexual development of fungi. In *Aspergillus nidulans*, it has previously been shown that deletion of either of the *MAT* genes resulted in the significant decreased production of cleistothecia, which are also small and entirely sterile (Paoletti et al. 2007). In this study, *MAT* gene mutants could not generate ascospores and failed to produce mature cleistothecia. These results are consistent with the reported function of *MAT* genes from *A. nidulans* (Paoletti et al. 2007). However, we also observed the interesting phenomenon that  $\Delta$ *MAT1* and  $\Delta$ *MAT2* deletion mutants of *A. cristatus* showed similar phenotypes. Thus, we further studied the mechanism behind this phenomenon by investigating the evolutionary relationship of mating-type genes.

In the literature on studies of *MAT* genes, there are reports about the evolutionary origin of HMG-domain proteins and  $\alpha$ 1-domain proteins (Idnurm et al. 2008). For example, Martin researched the evolutionary relationship of the HMG domain and  $\alpha$ 1 domain from representative species from major groups of Ascomycota and proposed that extant  $\alpha$ 1-box genes originated from an ancestral HMG gene (Martin et al. 2010). However, the genealogical analysis of *MAT1-1-1* and *MAT1-2-1* from *Ulocladium* indicated separate evolutionary events for the two *MAT* genes (Geng et al. 2014). The phylogenetic relationship of *MAT1-1-1* and *MAT1-2-1* from *A. cristatus* was analyzed using the conserved domain sequence of the corresponding

protein products. The results indicated that the  $\alpha 1$  domain from *MATI-1-1* and the HMG domain from *MATI-2-1* have a close relationship, and the two genes could have originated from a single ancestral HMG gene. These results are consistent with representative species of Pezizomycotina (Martin et al. 2010).

We detected the change of expression levels of sporulation-related genes from the mutants by transcriptomic sequencing. The results showed that there are nine differentially expressed genes putatively involved in sexual development. Among these genes, *flbD* and *fphA* are associated with the regulation of sexual development (Purschwitz et al. 2008, Krijgsheld et al. 2013, Arratia-Quijada et al. 2012), and the genes *ppgA*, *preA*, *preB*, *gprK*, *flbA*, *rce1*, and *ste14* are related to pheromone formation, transport, and sensing (Marr et al. 1990, Han et al. 2001, Pöggeler 2002, Seo et al. 2004, Yu 2006, Dyer & O’Gorman 2012). Moreover, the results from *Penicillium chrysogenum* showed that *MATI-1-1* is related to *ppg1* (*ppgA*, in aspergilli) and *pre1* (*preA*, in aspergilli) (Becker et al. 2015). In *A. cristatus*, *ppgA*, *preA*, and *preB* are downregulated in  $\Delta$ MAT1 and  $\Delta$ MAT2, indicating that mating-type genes are related to these genes. The results are consistent with previous reports on *A. nidulans* and *P. chrysogenum* (Paoletti et al. 2007, Becker et al. 2015).

In conclusion, the mating-type genes in *A. cristatus* are key for controlling sexual development. Mutants with the deletion of mating-type genes cannot form mature cleistothecia and ascospores. *MATI-1-1* and *MATI-2-1* in *A. cristatus* have a close evolutionary relationship. Notably, this paper reports for the first time the function of mating-type genes from *A. cristatus* and provides a valuable reference for research on the function of mating-type genes of fungi. However, the relationship between mating-type genes and other genes in *A. cristatus* remains to be clarified.

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