Expansion of Adhesion Genes Drives Pathogenic Adaptation of Nematode-Trapping Fungi

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HIGHLIGHTS
Expansion of subtilisin, adhesion protein, and polygalacturonase gene families
Trap simplification during evolution of nematode-trapping fungi
Connection between trap simplification and expansion of adhesion genes

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Expansion of Adhesion Genes Drives Pathogenic Adaptation of Nematode-Trapping Fungi

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SUMMARY
Understanding how fungi interact with other organisms has significant medical, environmental, and agricultural implications. Nematode-trapping fungi (NTF) can switch to pathogens by producing various trapping devices to capture nematodes. Here we perform comparative genomic analysis of the NTF with four representative trapping devices. Phylogenomic reconstruction of these NTF suggested an evolutionary trend of trapping device simplification in morphology. Interestingly, trapping device simplification was accompanied by expansion of gene families encoding adhesion proteins and their increasing adhesiveness on trap surfaces. Gene expression analysis revealed a consistent up-regulation of the adhesion genes during their lifestyle transition from saprophytic to nematophagous stages. Our results suggest that the expansion of adhesion genes in NTF genomes and consequential increase in trap surface adhesiveness are likely the key drivers of fungal adaptation in trapping nematodes, providing new insights into understanding mechanisms underlying infection and adaptation of pathogenic fungi.

INTRODUCTION
Fungal infections have devastated agricultural crops and contributed to severe diseases in humans and animals (Fisher et al., 2012). Plant pathogenic fungi can destroy plant tissues and result in great crop losses. Animal fungal pathogens can cause systemic and opportunistic mycoses, with two ongoing epidemics that have caused millions of deaths of amphibians (O’Hanlon et al., 2018) and bats (Kramer et al., 2019). In humans, life-threatening fungal diseases have been among the most challenging medical problems. Indeed, fungal infestations continue to rise and pose a significant threat to all plants and animals, including humans. However, fungal infections are difficult to treat, partly because fungi are evolutionarily closely related to plants and animals and many of them are not obligate pathogens but arise through adaptations from pre-existing characteristics of non-parasitic lifestyles. The mechanisms underlying such adaptations are poorly understood.

Most fungal pathogens have two or more lifestyles, and they can switch to pathogenic mode under specific environmental cues (Gauthier, 2015). Therefore, understanding fungal lifestyle transition is of great significance to uncover the mechanisms underlying fungal pathogenesis. For instance, Magnaporthe oryzae can cause rice blast. Its pathogenesis starts from the attachment of conidia to plant surface and followed by conidia germination and penetration (Ebbole, 2007). M. oryzae has been a model for understanding plant-fungal interactions. The dimorphic fungus Candida albicans is a commensal of the mammalian mycoflora and the most common opportunistic pathogen of humans. Its ability to transition between yeast and hyphal form is essential for pathogenesis (Mayer et al., 2013). The insect pathogen Metarhizium anisopliae grows naturally in soils as a saprophyte and causes disease in various insects initiated by conidia germination after the adhesion of conidia to the host cuticle (Aw and Hue, 2017). However, most fungal lifestyle transitions are difficult to determine. Among the pathogenic fungi, the nematode-trapping fungi (NTF) are unique in that they have evolved specialized morphological adaptations to capture nematodes. The formation of trapping devices is the key indicator of their lifestyle transition from saprophytes to predators and thus makes them good models for studying the mechanisms of fungal pathogenesis and adaptation (Abad et al., 2008; Yang et al., 2011, 2012; Zhang and Hyde, 2014).
Four representative types of traps are known among the nematode-trapping fungi, including constricting ring (CR), adhesive network (AN), adhesive column (AC), and adhesive knob (AK). In CR, when triggered, the three curved ring cells swell rapidly inward and lasso the victim quickly using mechanical force (Figure 1A). Instead of mechanical forces, fungi with AN form interlocking loops by growing branching hyphae and fusing with the parent hyphae to develop adhesive networks to capture nematodes (Figure 1B). Fungi with the AC device form a string of cells with adhesive surfaces (Figure 1C). Lastly, AK is an erect stalk with an adhesive bulb at the end (Figure 1D).

Of the four types of nematode-trapping devices, two have had their representative genomes sequenced, including Arthrobotrys oligospora representing AN (Ji et al., 2019; Yang et al., 2011) and Monacrosporium haptotylum representing AK (Meerupati et al., 2013). Here we sequenced the genomes of three additional species, including Drechslerella brochopaga (representing CR), Dactylellina cionopagum (representing AC), and Dactylellina entomopaga (representing AK). In addition, a close relative to NTF but without a trapping device, Dactylella cylindrospora, was also sequenced for comparative analyses. The combined analysis showed that the expansion of genes encoding adhesion proteins (APs) among fungal genomes and their up-regulation during the fungal lifestyle change were responsible for the increasing adhesiveness on trap surface, driving the evolution of the nematode-trapping fungi and their adaptation in capturing nematodes using different trapping devices. The expansion of adhesion gene families has likely played a
very important roles during the evolution and adaptation of the predatory lifestyle by the NTF. They function in capturing nematodes, ensuring their lifestyle transition from saprophytes to predators and pathogens. Our results provide insights into the mechanisms underlying fungal pathogenesis and adaptation.

RESULTS
The four types of nematode traps have distinct features. Morphologically, CRs are most complicated (Xue-Mei Niu and Zhang, 2011; Yang et al., 2007). They immobilize nematodes actively and mechanically by rapid swelling of three highly ordered ring cells (Figure 1E). In contrast, ANs use complex three-dimensional adhesive nets to capture nematodes (Figure 1F), whereas ACs and AKs use two-dimensional adhesive columns and simple adhesive knobs, respectively (Figures 1G and 1H). Compared with the adhesive traps, one of the limiting factors for CRs is nematode size. Fungi with different-sized CRs were likely selected to capture nematodes of various sizes (Figure 1I). However, nematodes can escape CR even after entrapment (Figure 1J). Among the four types of traps, AK is the most efficient where a single knob is often sufficient to capture large nematodes (Figure 1H).

Genome Sequencing of Nematode-Trapping Fungi
The estimated genome sizes of the sequenced NTF ranged from 35 to 43 Mb (Table S1). The GC contents were about 44%–50% (Table S2). Their gene models, ranging from 9,924 to 10,716 (Table S3), were predicted by combining ab initio prediction and evidence-based searches. The average lengths of protein-coding genes were about 1.5 kb, and each gene contained averages of 2.8–3 exons, with 2,500–3,000 genes containing only one single exon each. About 60% of the inferred proteins had matches in public databases.

Phylogenomic Analysis Reveals Simplification of Trapping Devices
Previous research based on fossil records and phylogenetic analyses suggested potential evolutionary relationships among various nematode-trapping devices (Yang et al., 2007, 2012). To further study the pathogenic adaptation of the nematode-trapping fungi at the genome level, the phylogenomic relationships among the representative NTF were constructed using the maximum likelihood method based on 395 conserved genes coding for orthologous proteins. These 395 genes were conserved across all the 16 genomes used in phylogenomic analysis (Table S4), including the 5 representative NTF, the close relative D. cylindrospora, 7 other pathogenic fungi, and 3 saprophytes. These orthologous genes were mostly involved in house-keeping functions. Saccharomyces cerevisiae was used as the outgroup member. The bootstrap analysis based on 1,000 replications strongly supported the tree topology (Figure 2A). The phylogenetic tree showed that the NTF and their close relative Dactylella cylindrospora belonged to a monophyletic clade and suggested an unambiguous trend during the evolution of trapping devices. Specifically, the fungal nematode-trapping ability originated from a saprophytic ancestor of Orbiliomycetes. After D. cylindrospora split at around 339 Myr ago, the organisms with the active mechanical trap (CR) emerged first, followed by those with passive adhesive traps, including the fungi developing complex 3-D adhesive networks, adhesive columns, and adhesive knobs evolved in sequence.

The derived phylogenomic tree suggested that the nematode-trapping fungi underwent morphological simplification in trapping devices through evolution. However, the morphological simplification of traps has not weakened the nematode-capturing capacity. On the contrary, one single adhesive knob can immobilize nematodes of different sizes, whereas each constricting ring can only catch nematodes of a specific-size category (Figure 1H). Ultrastructural studies revealed that the adhesive trapping devices captured nematodes by means of an adhesive layer covering the trap surfaces (Belder et al., 1996; Tunlid et al., 1991). Specifically, the adhesion of nematodes to fungal traps as well as the nematode-trapping efficiency significantly decreased when the adhesive layer was denatured (Tunlid et al., 1991).

Ultrastructural Measurements Reveal Increase of Adhesiveness on Trap Surfaces
We measured the thickness of the adhesive layers on trap surfaces of the examined species. We found no adhesive layer on cells of CRs or on un-induced hyphae of all trapping devices (Figure 2B). On the contrary, different adhesive traps produced adhesive layers that differed greatly in their thickness. Specifically, a 0.2- to 0.4-μm-thick layer matrix was observed on the surface of ANs (Figure 2C), and about 1-2 μm for ACs (Figure 2D) and up to 20 μm for AKs (Figure 2E), respectively. Thus, the combined results based on ultrastructural observations and the phylogenomic tree suggested that increasing adhesiveness on trap surfaces was...
A key innovation allowing NTF to capture nematodes with a high efficiency even with morphologically simple trapping devices.

Comparative Genomic Analyses Reveal Expansion of Genes Encoding Adhesion Proteins

To further investigate the underlying genetic basis for pathogenic adaptation of the nematode-trapping fungi, genomic and comparative genomic analyses were performed. Genomic analysis showed that more than 40% of protein-coding genes belonged to multi-gene families (Table S5). The results revealed that selected groups of genes have significantly expanded in NTF genomes. Specifically, comparative analyses identified that 12 multi-gene families and 23 gene domains have significantly expanded (p < 0.05) in NTF genomes (Figures S1A and S1B). Interestingly, nine of the expanded multi-gene families lack significant matches in public databases, suggesting the existence of potentially novel genetic pathways underlying pathogenesis of NTF. Examples of the expanded gene families include lectins and proteins containing the yeast cell wall-integrity and stress-response component (WSC) domain and the Winged-helix (WH) domain. Lectins have been previously proposed to mediate the interactions between several parasitic fungi (including NTF) and their hosts (Rosen et al., 1996). Proteins containing the WSC domain serve as cell wall sensors (Dupres et al., 2009) and are involved in fungal adhesions (Linder and Gustafsson, 2008). Proteins containing the WH domain play very important roles during DNA binding of transcription factors (Lilley, 1995).

Previous studies have identified a diversity of genes and gene families related to fungal pathogenicity (Meerupati et al., 2013; Yang et al., 2011). Comparative analysis showed that some of them significantly expanded in NTF genomes (Figure S1C). For example, three gene families, including subtilisins, adhesion...
proteins, and polygalacturonases, showed continuous expansion during the evolution of progressively simplified trapping devices (Figure 3A). Subtilisins are known to play important roles in the pathogenicity of carnivorous fungi (Ahman et al., 2002). The polygalacturonases degrade lectin networks comprising cell walls and play an important role in fungal pathogenicity to plants (D’Ovidio et al., 2004). And, adhesion proteins help fungi adhere to a diversity of surfaces (Ebbole, 2007; Mayer et al., 2013).

Ultrastructural studies showed that the trapping devices acquired a progressively thicker adhesive layer on the surface of trap cells after they evolved the ability to trap nematodes by adhesion. In the NTF genomes, the genes encoding adhesion proteins were significantly expanded and the expansion paralleled the increasing adhesiveness of the trapping devices (Figures 3A and 3B). Specifically, there are 11 adhesion protein-encoding genes in D. brochopaga (CR), 16 in A. oligospora (AN), 21 in D. cionopagum (AC), and 31 in D. entomopaga (AK). There is a positive correlation between the number of adhesion proteins and the thickness of adhesive layer of corresponding trapping devices.

![Figure 3. Comparative Genomic Analyses](image)

(A) The gene members showed continuous expansion along the evolutionary history of nematode-trapping fungi. (B) Real-time PCR analysis showed the number of up-regulated genes encoding adhesion proteins (AP) was consistent with their distributions on genomes, i.e., the numbers increased in the order of CR-AN-AC-AK. There was no adhesion gene up-regulated in D. brochopaga (CR), whereas D. entomopaga (AK) had the most adhesion genes with up-regulated expression.

(C) The species-specific genes of the representative nematode-trapping fungi were found differently enriched in gene expansion. Blue circle represents “not significantly enriched,” yellow circle represents “significantly enriched,” and red circle represents “significantly enriched after Bonferroni correction”; circle size represents gene number.

(D) Nematode-trapping fungi (NTF) possess few highly similar genes, whereas the model fungus S. cerevisiae (without RIP) had many highly similar genes.

NTF, nematode-trapping fungi; CR, constricting ring; AN, adhesive network; AC, adhesive column; AK, adhesive knob; AP, adhesion protein. See also Figures S1 and S2, and Tables S2–S6.
**Genes Encoding Adhesion Proteins Show Consistent Up-Regulations**

To further investigate the relationship between adhesion proteins and trap induction of NTF, quantitative RT-PCR was performed to measure the gene expression levels of APs during the trap formation when induced by live nematodes. Gene expression analysis showed that the up-regulation of APs during trap induction was also consistent with both expansion of APs in genomes and the increasing adhesiveness of traps. Specifically, 7 genes encoding APs were significantly up-regulated (3 replicates, fold change >2) during induction of ANs and 10 were significantly up-regulated in ACs, whereas 23 were significantly up-regulated during induction of AKs (Figure 3B). In contrast, no APs were found up-regulated during the induction of CRs, which capture the nematodes by means of mechanical forces instead of adhesive layers. The positive correlation also exists between the thickness of adhesive layer and the numbers of up-regulated adhesion proteins during trap induction. Taken together, the nematode-trapping fungi using CRs have the least number of APs encoded in genome, whereas the fungi using AKs have most APs and the thickest adhesive layer on trap surface.

Our previous work showed that disruption of adhesion-related gene reduced the production of adhesive layer on trap surface of A. oligospora and significantly decreased its ability to capture nematodes (Liang et al., 2015). The results suggest that the significant expansion of APs along the NTF lineage is likely responsible for the gradual thickening of the adhesive layers. Given that capturing the nematodes with various traps is the most crucial step of infection by NTF, the number of APs and their regulation represent a key factor in shaping the evolution of various traps and achieving corresponding adaptation.

**Species-Specific Genes Emerged in Different Genomes**

Comparative analysis has also identified large numbers of species-specific genes among NTF genomes (Figure 2A). Interestingly, the species-specific genes were found enriched in many of the expanded pathogenicity-related gene families (Figure S2). Compared with the non-species-specific genes that are shared with non-NTF species, the species-specific genes possessed a few marked features. For example, the average length of proteins encoded by species-specific genes was 332 amino acids, much shorter than that of non-species-specific genes (509 amino acids). Second, a large proportion (72%) of the species-specific genes had no homologous matches in public databases. Third, there were fewer paralogs in species-specific genes than in other genes. Specifically, in non-species-specific genes, an average of 47% of the protein-encoding genes belonged to multi-gene families (Table S5), whereas in species-specific genes, the average number was 19%. Indeed, less than 3% of species-specific genes belonged to 12 large clusters consisting of more than 10 paralogs. The results on species-specific genes suggest that their expansions are likely related to gene diversification that contributes significantly to functional innovation of the nematode-trapping fungi.

Enrichment analysis showed that the species-specific genes among the NTF differed in gene expansion patterns. For example, all the species-specific genes of NTF are enriched in genes encoding subtilisin-like proteins, but D. brochopaga has much fewer coding genes than those developing adhesive trapping devices (Figure 3C). D. cionopagum, D. entomopaga, and M. haptotylum with morphologically simple adhesive traps are enriched in genes encoding subtilisins and peptidases, suggesting important roles of proteolysis in their infectious attack. In A. oligospora, the genes coding for GAS proteins are highly enriched. GAS proteins bind to the plasma membrane through a lipid anchor and are involved in cell wall synthesis and cell signaling (van Zanten et al., 2009; Verghese et al., 2006). In particular, M. haptotylum contains 19 genes with WSC domains, which is consistent with trap adhesion being very important for adhesive knobs.

**Repeat-Induced Point Mutations Involved in Gene Expansion**

Gene-family expansion can be caused by gene duplication and is recognized as one of the main mechanisms of adaptive innovation (Gladieux et al., 2014). However, the underlying mechanism for gene duplication and diversity is often unknown. Genomic analyses revealed that less than 2% of NTF genomes consisted of repetitive sequences (Table S2), even lower than that in the model filamentous fungus Neurospora crassa (Galagan et al., 2003; Selker, 1990) in which the repeat-induced point mutation (RIP) has been considered as responsible for its low percentage of repetitive sequences. RIP is a homology-based process that mutates repetitive sequences. Thus, RIP can significantly impact genome evolution by slowing the creation of new genes through genomic duplication (Galagan and Selker, 2004). Here, we calculated RIP indices to determine whether RIP contributed to the evolution of NTF genomes. By using the default settings (Hane and Oliver, 2008), positive RIP responses
were detected in various genomic regions (Table S6), including multi-gene families and repetitive sequences. In addition, consistent with the actions that RIP mutates duplicated sequences with greater than about 80% nucleotide similarity (Galagan et al., 2003), there are very few highly similar genes within the sequenced NTF genomes (Figure 3D). In particular, homologous genes with more than 70% similarity are almost absent from NTF genomes, whereas there are many such genes in S. cerevisiae. In addition, the RIP process requires the RID1 gene (Freitag et al., 2002). The homologous gene of RID1 has been found in all the NTF genomes. Furthermore, DNA methylation is usually associated with RIP (Singer et al., 1995). We performed a DNA methylation analysis of the whole genome of A. oligospora and identified a total of 737 5-mC positions, and positive RIP responses were detected in 70% of them. The results suggest that the RIP mechanism has had a profound impact on gene expansion in nematode-trapping fungi and contributes significantly to their pathogenic adaptation.

**DISCUSSION**

Lifestyle transition is a fundamental property of fungi in their responses to environmental changes. For nematode-trapping fungi, nitrogen deficiency is a common inducer for developing traps to capture nematodes (Nordbring-Hertz et al., 2006). Through evolution, various trapping devices likely emerged to deal with nitrogen deficiency. As the earliest emerged and morphologically the most complicated trapping device, CRs immobilize nematodes actively and mechanically by rapid swelling of three highly ordered ring cells. However, large nematodes cannot enter CRs and small nematodes can pass through the traps without triggering any response (Figure 1J). In contrast, the passive adhesive traps capture nematodes by means of adhesive layers on the surfaces of trap cells and can capture nematodes using a single adhesive knob (Figure 1H). Therefore, the simplification of traps has not reduced trapping efficiency but instead has likely provided competitive advantages. However, the simplification of trapping structures may have caused a reduction in the contact area between the fungi and nematodes, thus increasing the requirement for surface adhesiveness. The observed continuous expansion of adhesion proteins and the consequent thickening of adhesive layers are consistent with the above hypothesis to ensure capture efficiency with even simple trap structures (Figure 4). In addition, the continuous expansion of subtilisins and other related genes could enhance the ability of the fungi to penetrate and digest the nematodes after the nematodes are captured.

Gene duplication and divergence are important mechanisms for adaptive innovation (Gladieux et al., 2014; Hit-tinger and Carroll, 2007). Among NTF genomes, 44% of protein-coding genes belong to multi-gene families. Our analyses showed that trap evolution was associated with significant expansion of multiple gene families and many of them may function in pathogenicity of the nematode-trapping fungi. RIP plays important roles in gene duplication. Positive RIP responses have been detected in repetitive sequences, multi-gene families, species-specific genes, and DNA methylation positions in NTF genomes. In S. cerevisiae, more than 30% of gene pairs have more than 50% sequence identities, whereas in nematode-trapping fungi, the highly similar genes are almost completely absent (Figure 3D). Genomic rearrangement can also result in gene duplication. Syntenic analyses can be used for identification of genomic rearrangement. An example of genomic inversion among the sequenced NTF is shown in Figure S4. Genomic rearrangement could also facilitate the emergence of species-specific genes. Indeed, some of the species-specific genes are highly enriched in certain genomic regions. The low sequence similarity in gene families, large numbers of species-specific genes and few paralogs in species-specific genes suggest functional diversification following gene duplication. Other than the nematode-trapping fungi, other fungal pathogens (Table S5) also showed patterns of gene expansion and accumulation of species-specific genes. For example, search against PHI (pathogen-host interaction) gene database (Winnenburg et al., 2008) identified that the nematode-trapping fungi share more putative PHI genes with fungal pathogens (including animal and plant pathogens, Table S4) than with saprophytes (101 versus 19). Interestingly, they share more putative PHI genes with plant fungal pathogens (including M. oryzae) than with animal fungal pathogens (37 versus 23). The result is consistent with previous observations showing the putative PHI genes being enriched in plant-pathogen interaction and the important roles of poly-galacturonases in fungal pathogenicity to plants.

For most fungal pathogens, attachment to host tissues is among the most crucial stage for successful infection. These attachments are generally mediated by cell surface adhesion molecules (Aw and Hue, 2017; Ebbolé, 2007; Mayer et al., 2013). These molecules play critical roles in the establishment of fungal infections of plants, animals, and humans. In this study, we demonstrated that expansion of genes encoding adhesion proteins in nematode-trapping fungi was positively correlated with the thickening of adhesive layers on the surface of trapping devices during lifestyle transitions, which ensure high efficiency for capturing nematode preys using simplified trapping devices (Figure 4). Expansion of adhesion proteins is thus a key driver in the evolution of nematode-trapping fungi and their trapping devices. Our results suggest that adhesion
proteins play critical roles in the adaptive evolution and pathogenesis of nematode-trapping fungi. However, lifestyle transitions of nematode-trapping fungi involve multiple complex stages and adhesion is just the beginning of fungal predation on nematodes. The genomic resources generated here should help further studies on the genetic bases and molecular mechanisms underlying lifestyle transitions and pathogenesis of nematode-trapping fungi.

Limitations of the Study

Our analyses indicated a likely process for the evolution of nematode trapping devices and suggested the possible mechanisms underlying trapping device simplification while enhancing nematode trapping efficiencies. However, given the prevalence of RIP, how exactly the gene family expansion escaped RIP during evolution remains unknown. Similarly, previous studies have shown that disruption of adhesion-related gene reduced the adhesive layers of trap surface of A. oligospora, and how the adhesion genes interact to control the thickness of the adhesive layers among various NTF requires further investigation. In our investigations, we also identified the expansion of several other gene families such as subtilisins and polygalacturonases involved in the degradations of proteins and other macromolecules. Their roles in trapping device evolution and NTF adaptation, including how they interact with adhesion proteins to ensure capturing capacity, remain to be investigated.

METHODS

All methods can be found in the accompanying Transparent Methods supplemental file.

DATA AND CODE AVAILABILITY

All the data and methods necessary to reproduce this study are included in the manuscript and Supplemental Information. The genome projects have been deposited at GenBank under the BioProject
accession number PRJNA283584, PRJNA283942, PRJNA283944, and PRJNA283946. The gene annotation information has been included.

SUPPLEMENTAL INFORMATION
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AUTHOR CONTRIBUTIONS
K.-Q.Z and X.J. conceived the study and designed scientific objectives. K.-Q.Z. led the project. X.J. analyzed the data and prepared the manuscript. Z.Y. provided the materials and performed the experiments. J.Y. contributed to experiments. J.X. contributed to manuscript preparation. Y.Z., S.L., C.Z., J.L., and L.L. participated in discussions and provided suggestions.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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Supplemental Information

Expansion of Adhesion Genes
Drives Pathogenic Adaptation
of Nematode-Trapping Fungi

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Figure S1 Expanded gene families. Related to Figure 3. (A) Significantly expanded multigene families. (B) Significantly expanded gene domains. SSPs were not shown. (C) Comparison of pathogenicity-related gene families.
Figure S2 Enrichments of the species-specific genes in expanded gene families. Related to Figure 2 and 3. Top panel, from left to right are trapping-specific (D.cionopagum, D.entomopaga, M.haptotylum, A.oligospora and D.brochopaga), passively trapping-specific (D.cionopagum, D.entomopaga, M.haptotylum and A.oligospora ONLY), passively trapping-specific 2 (D.cionopagum, D.entomopaga and M.haptotylum) and passively trapping-specific 3 (D.entomopaga and M.haptotylum ONLY) genes; bottom panel, from left to right are D.cylindrospora-specific, D.brochopaga-specific, ao03-specific, D.cionopagum-specific, M.haptotylum and D.entomopaga-specific genes. Yellow box represents "significantly enriched"; red box represents "significantly enriched after Bonferroni correction". "SSP" was highly significantly enriched.
Figure S3 Enrichments of the species-specific genes in biological processes. Related to Figure 2. Top panel, from left to right are trapping-specific (D.cionopagum, D.entomopaga, M.haptotylum, A.oligospora and D.brochopaga), passively trapping-specific (D.cionopagum, D.entomopaga, M.haptotylum and A.oligospora ONLY), passively trapping-specific 2 (D.cionopagum, D.entomopaga and M.haptotylum) and passively trapping-specific 3 (D.entomopaga and M.haptotylum ONLY) genes; bottom panel, from left to right are D.cylindrospora-specific, D.brochopaga-specific, ao03-specific, D.cionopagum-specific, M.haptotylum and D.entomopaga-specific genes. Yellow box represents "significantly enriched"; red box represents "significantly enriched after Bonferroni correction".
Figure S4 An example of genome rearrangement. Related to Figure 4. On the top is the dot plot of the sequence matches between two scaffolds of *Arthrobotrys oligospora* and *Dactylella cylindrospora*; on the bottom is the detail of the sequence inversion.
Table S1 Statistics of genome assemblies. Related to Figure 1.

The four sequenced genomes have different numbers of assembly scaffolds. They also differ in genome size, N50/N90 length, minimum and maximum length of the scaffolds.

| Species                  | Total Num (#) | Total Length (bp) | N50 (bp) | N90 (bp) | Min Length (bp) | Max Length (bp) |
|--------------------------|---------------|-------------------|----------|----------|-----------------|---------------|
| *Dactylellina entomopaga* | 296           | 38,393,354        | 579,495  | 163,087  | 501             | 1,599,543      |
| *Dactylellina cionopagum*| 571           | 43,120,050        | 668,736  | 91,422   | 1,000           | 2,185,162      |
| *Drechslerella brochopaga*| 242           | 35,431,589        | 513,123  | 157,171  | 1,005           | 1,040,938      |
| *Dactylella cylindrospora*| 140           | 37,713,459        | 501,354  | 180,374  | 1,050           | 1,328,745      |

Table S2 Statistics of genome features. Related to Figure 1 and 3.

The GC contents of the four genomes range from 44% to 50%. More than 10,000 items have been annotated through Rfam database search. Low percentages of repetitive sequences were identified in these genomes, even lower than in *N. crassa* which use the RIP mechanism to reduce gene duplications. tRNAs have also been predicted as described in “Transparent Methods”.

| Species                  | Genome Size | GC% | Rfam Number (#) | GC% | Coverage% | Repeat GC% | tRNA Number (#) | GC% |
|--------------------------|-------------|-----|-----------------|-----|-----------|------------|-----------------|-----|
| *Dactylellina cionopagum*| 43,120,050  | 44.3| 11,284          | 43.4| 0.97      | 29.38      | 80              | 45.48%|
| *Dactylellina entomopaga*| 38,393,354  | 44.9| 11,130          | 43.3| 1.69      | 26.18      | 102             | 50.33%|
| *Drechslerella brochopaga*| 35,431,589  | 49.42| 10,234         | 46.75| 0.59     | 38.59      | 65              | 48.48%|
| *Dactylella cylindrospora*| 37,713,459  | 46.02| 10,785         | 44.2| 1.25     | 22.52      | 82              | 47.99%|
| *Neurospora crassa*      | 38,047,924  | 49.87| -              | -   | 2.57      | 29.59      | -               | -    |
## Table S3 Statistics of gene prediction. Related to Figure 2 and 3.

The statistics show the distributions of various sequence regions based on gene prediction results, including the intergenic regions, protein coding regions (combining exons and introns), exon and intron regions. The statistics include the coverage of these regions in the genome, the GC content of these sequence regions, the numbers and mean lengths of these sequences, the average numbers of exons and introns per gene and the numbers of genes having only one exon, for each of the genomes.

| Species                | S       | D       | D       | M       | A       | A       | A       | A       | C       | G. meae | V       | M       | C. glabrata | N. crassa |
|------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|------------|----------|
|                        | O. ae   | O. gum  | O. paga | O. lum  | O. ora  | O. brochs| O. tenua| O. humgal| O. nidulans| O. capsula| O. immula| O. dahliae| O. oryzae  | O. crassa |
| Intergenic Coverage%   | 27.81   | 60.96   | 57.69   | 46.84   | 55.53   | 56.37   | 56.83   | 46.40   | 40.69   | 50.05   | 49.98   | 44.23   | 50.22      | 52.95     | 43.27     | 55.69     |
| GC%                    | 34.57   | 41.45   | 42.22   | 44.41   | 41.68   | 46.54   | 43.72   | 43.70   | 46.83   | 39.15   | 41.81   | 44.31   | 50.24      | 47.54     | 50.26     | 45.89     |
| Coding Coverage%       | 72.19   | 39.04   | 42.31   | 53.16   | 44.47   | 43.63   | 43.17   | 53.51   | 59.31   | 49.95   | 50.02   | 55.77   | 49.78      | 47.05     | 56.73     | 44.31     |
| Number(#2)             | 5907    | 10716   | 10471   | 10959   | 10885   | 9924    | 10649   | 9630    | 9440    | 9254    | 9910    | 11628   | 10535      | 12336     | 15048     | 10082     |
| Mean(bp)               | 1503.56 | 1570.95 | 1551.46 | 1805.66 | 1636.96 | 1557.63 | 1528.98 | 1632.92 | 1872.83 | 1645.65 | 1494.13 | 1746.67 | 1601.77    | 1524.58   | 1791.66   | 1674.03   |
| Intergenic Lengths(bp) | 2.058   | 4.024   | 3.667   | 3.397   | 3.681   | 3.570   | 3.542   | 3.051   | 3.157   | 3.294   | 2.935   | 3.126   | 3.218      | 3.193     | 3.158     | 3.774     |
| Intron Coverage%       | 0.68    | 4.18    | 4.42    | 7.58    | 9.38    | 5.19    | 4.38    | 5.05    | 8.59    | 8.70    | 6.23    | 6.69    | 5.58       | 6.29      | 9.54      | 6.08      |
| Mean(bp)               | 313.231 | 90.029  | 91.0579 | 308.575 | 88.7623 | 92.5691 | 84.6262 | 79.5848 | 101.373 | 129.224 | 87.1301 | 94.4250 | 99.7683    | 119.804   | 142.054   | 135.474   |
| Intron Lengths(bp)     | 0.00    | 1.87    | 1.78    | 2.61    | 2.04    | 1.83    | 1.93    | 2.68    | 2.22    | 2.22    | 2.14    | 2.21    | 1.8        | 1.7       | 2.12      | 1.7       |
| Exon Coverage%         | 71.61   | 34.86   | 37.91   | 41.44   | 38.76   | 43.97   | 41.52   | 46.37   | 46.02   | 43.77   | 42.27   | 42.8    | 44.02      | 44.35     | 47.70     | 38.24     |
| Mean(bp)               | 1400.56 | 489.066 | 499.523 | 478.781 | 485.392 | 503.999 | 422.384 | 416.286 | 478.295 | 508.193 | 477.741 | 535.856 | 400.111    | 692.348   | 1523.18   | 1995      |

The statistics include the distributions of various sequence regions based on gene prediction results, including the intergenic regions, protein coding regions (combining exons and introns), exon and intron regions. The statistics include the coverage of these regions in the genome, the GC content of these sequence regions, the numbers and mean lengths of these sequences, the average numbers of exons and introns per gene and the numbers of genes having only one exon, for each of the genomes.
Table S4 List of species used for comparative analysis.
Related to Figure 2 and 3.

The 16 genomes listed in this table have been used for comparative genomics analysis and phylogenomics tree construction. These genomes represent fungi with a diversity of ecological roles, including nematode-trapping fungi, a close relative of NTF, other plant and animal fungal pathogens, and saprophytes.

| Abbreviation | Species               | Notes          |
|--------------|-----------------------|----------------|
| sc01         | Saccharomyces cerevisiae | Saprotroph   |
| dc01         | Dactylellina cianopagum | Trap: AC   |
| de02         | Dactylellina entomopaga | Trap: AK   |
| dh01         | Monacrosporum haptotylum | Trap: AK   |
| ao03         | Arthrobotrys oligospora | Trap: AN   |
| db02         | Drechslerella brochopaga | Trap: CR  |
| dt02         | Dactylella cylindrospora | No Trap  |
| af01         | Aspergillus fumigatus  | Animal pathogen |
| an01         | Aspergillus nidulans   | Saprotroph   |
| ac01         | Ajellomyces capsulatus | Animal pathogen |
| ci01         | Coccidioides immitis   | Animal pathogen |
| gr01         | Gibberella zeae        | Plant pathogen |
| vd01         | Verticillium dahliae    | Plant pathogen |
| mo01         | Magnaporthe oryzae     | Plant pathogen |
| cg01         | Chaetomium globosum    | Animal pathogen |
| nc01         | Neurospora crassa      | Saprotroph   |
Table S5 Statistics of multigene families. Related to Figure 3.

The gene members are grouped to multigene families based on their sequence similarities. The total gene numbers included in multigene families for each species are shown in this table. The total number of multigene families, as well as the average gene numbers per multigene family are also show below for each of the species.

| Species       | S. cerevisiae | D. cianopagum | D. entomopagum | M. haptotylum | A. oligospora | D. brochopagum | D. cylindrospora | N. capsulatus | A. fumigatus | A. nidulans | A. nidulans albo- | C. immitis | G. zeae | V. dahliae | M. oryzae | C. globosum | N. crassa |
|---------------|---------------|---------------|----------------|---------------|---------------|----------------|------------------|---------------|--------------|-------------|---------------------|-----------|--------|------------|------------|-------------|--------|
| Genes (#)     | 2762          | 5057          | 4470           | 5166          | 4839          | 3826           | 4233             | 4917          | 4924         | 3214        | 3939                 | 6143      | 5015   | 5708       | 4878       | 3468        |
| Ratio (%)     | 46.76         | 47.19         | 42.69          | 47.14         | 44.46         | 38.55          | 39.75            | 51.06         | 52.33        | 34.73       | 39.75                | 52.83     | 47.60  | 44.47      | 44.15      | 34.40       |
| Families (#)  | 752           | 1019          | 965            | 1020          | 988           | 850            | 918              | 877           | 731          | 879         | 1130                | 968       | 1132   | 888        | 793        |             |
| Perfamily (#) | 3.67          | 4.96          | 4.63           | 5.06          | 4.9           | 4.5            | 4.61             | 5.2           | 5.61         | 4.4         | 4.48                 | 5.44      | 5.18   | 5.04       | 5.49       | 4.37        |
Table S6 Statistics of detected RIP regions. Related to Figure 3.

Evidence for RIP was detected in various categories of DNA sequences, including the intron and exon regions, coding regions (combining exon and introns), the intergenic (noncoding) regions, the repetitive sequences, gene families, single exon genes and single introns, and the whole genome (with scaffolds). As described in Transparent Methods, the 200-bp window with 100-bp shift is used for RIP detection. When the criteria of RIP indices were met, the sequence fragment was labelled RIP positive (RIP prevalence=number of RIP-positive sequences/total number of sequences), and the 200-bp window-size sequence counts as RIP regions (length=total length of RIP regions/total length of sequences).

| Sequence type | RIP abundance (%) | Dactylellina clionopagum | Dactylellina entomopaga | Monacrosporiym haptotylum | Arthrobotrys oligospora | Drechslerella brochopaga | Dactylella cylindrospora | Neurospora crassa |
|---------------|-------------------|--------------------------|-------------------------|-------------------------|------------------------|-------------------------|------------------------|-----------------|
| single.intron | length            | 52.65                    | 50.34                   | 51.12                   | 53.24                  | 41.52                   | 50.67                  | 48.3            |
|               | prevalence        | 64.04                    | 62.41                   | 61.47                   | 66.05                  | 51.76                   | 62.54                  | 65.2            |
| noncoding     | length            | 60.96                    | 58.22                   | 58.07                   | 63.32                  | 53.41                   | 58.58                  | 61.84           |
|               | prevalence        | 98.69                    | 98.6                    | 97.3                    | 98.77                  | 97.43                   | 98.65                  | 98.84           |
| family.exon   | length            | 49.88                    | 49.34                   | 49.81                   | 49.94                  | 43.79                   | 48.16                  | 43.98           |
|               | prevalence        | 75.42                    | 75.57                   | 74.05                   | 74.94                  | 72.26                   | 75.09                  | 73.07           |
| genome        | length            | 59.45                    | 57.16                   | 57.41                   | 60.74                  | 52.18                   | 57.35                  | 56.5            |
|               | prevalence        | 100                      | 99.32                   | 100                     | 100                    | 100                     | 100                    | 100             |
| coding        | length            | 55.28                    | 53.93                   | 55.3                    | 55.81                  | 48.89                   | 53.77                  | 48.21           |
|               | prevalence        | 99.67                    | 99.49                   | 99.9                    | 99.77                  | 99.33                   | 99.62                  | 99.44           |
| single.exon   | length            | 50.04                    | 48.49                   | 49.18                   | 50.2                   | 43.88                   | 48.63                  | 43.25           |
|               | prevalence        | 77.68                    | 77.9                    | 75.45                   | 77.16                  | 75.14                   | 78.33                  | 65.85           |
| intron        | length            | 52.43                    | 49.75                   | 51.29                   | 53.64                  | 41.46                   | 51.43                  | 47.53           |
|               | prevalence        | 63.55                    | 61.61                   | 61.47                   | 66.22                  | 51.91                   | 62.95                  | 63.29           |
| family.coding| length            | 55.71                    | 54.94                   | 56.2                    | 56.46                  | 49.75                   | 54.46                  | 48.45           |
|               | prevalence        | 99.96                    | 99.82                   | 100                     | 99.94                  | 99.97                   | 99.98                  | 100             |
| exon          | length            | 49.97                    | 48.88                   | 49.49                   | 50.08                  | 43.84                   | 48.42                  | 43.54           |
|               | prevalence        | 76.51                    | 76.77                   | 74.71                   | 76.03                  | 73.81                   | 76.79                  | 68.59           |
| family.intron| length            | 52.23                    | 49.19                   | 51.44                   | 53.99                  | 41.4                    | 52.2                   | 46.15           |
|               | prevalence        | 63.13                    | 60.87                   | 61.47                   | 66.36                  | 52.06                   | 63.33                  | 60.35           |
| single.coding| length            | 54.85                    | 53.07                   | 54.42                   | 55.22                  | 48.25                   | 53.21                  | 48.06           |
| single.coding| prevalence        | 99.42                    | 99.25                   | 99.81                   | 99.64                  | 98.93                   | 99.39                  | 99.15           |
| repetitive    | length            | 21.23                    | 17.64                   | 38.88                   | 45.93                  | 27.01                   | 17.82                  | 34.84           |
|               | prevalence        | 12.83                    | 9.8                     | 19.02                   | 23.66                  | 17.13                   | 13.9                   | 16.68           |

Note: “coding” represents the whole gene encoding sequences, including exons and introns; The term “length” represents the length ratio of the detected RIP regions; The term “prevalence” represents the ratio of the sequence numbers with RIP regions detected.
Transparent Methods

Fungal strains

More than 90% of the carnivorous fungi belong to the Orbiliomycetes in the Ascomycota (Li et al., 2000; Yang et al., 2012), forming different types of trapping devices to capture and kill nematodes and other prey. In the present study, the genomes of the typical nematode-trapping fungi with different types of traps have been sequenced. *Dactylellina entomopaga* (CBS642.8), purchased from Centraalbureau voor Schimmelcultures (CBS), forms AK to capture the nematodes (Scholler et al., 1999). *Dactylellina cionopagum* (YMF1.00569, isolated from soil collected in Deqin County, Yunnan province) typically develops AC, consisting of one or more cells that may stick to the nematode cuticle and prevent it from escaping (Nordbring-Hertz et al., 1995; Yang and Liu, 2006). *Drechslerella brochopaga* (YMF1.01829, isolated from teleomorph collected in Jinggang mountain, Jiangxi province) captures nematodes with the aid of mechanical CR, consisting of three cells that will swell quickly when a nematode enters the ring (Nordbring-Hertz et al., 1995; Scholler et al., 1999). Besides, the genome of *Dactylella cylindrospora* (CBS325.70, purchased from CBS), a close relative to the nematode-trapping fungi without the capability of trapping nematodes, has also been sequenced for comparison.

The strains of *Dactylellina entomopaga* and *Dactylella cylindrospora* were purchased from Centraalbureau voor Schimmelcultures (CBS). The strains of *Dactylellina cionopagum* and *Drechslerella brochopaga* were isolated from soil of Yunnan Province and Jinggang Mountain, Jiangxi Province, China. All these strains were maintained on cornmeal agar (CMA).

Genome sequencing, assembly and analysis

Genomic DNA was extracted from the mycelia cultured on cellophane membranes on PDA medium using the modified CTAB method (Yu et al., 2007). 5μg of DNA was fragmented, end-repaired, A-tailed and ligated to Illumina paired-end adapters. The ligated fragments were then size-selected at 500 base pairs (bp) on agarose gels and amplified by LM-PCR to yield the short insert libraries, which were sequenced on the Illumina sequencing platform. A stringent filtering process has been performed on the WGS (Whole Genome Shotgun) reads. The additional bases (15-20bp) at the 5'-end were trimmed. The adapter bases (27bp) were trimmed. The duplicated reads were filtered out. The reads with 10 or more Ns and low-quality bases were filtered out.

Previously, the *A. oligospora* was sequenced by the ABI 3730 Sanger sequencing platform to a 2x coverage and the Roche 454 Genome Sequencer Titanium/FLX platforms to additional 34.6x coverage. The *M. haptotylum* was sequenced with 454 pyrosequencing technology using a titanium shotgun protocol (XLR70) at KTH Stockholm and paired end sequencing of 3 kb insert libraries at the DNA Sequencing
Facility at Lund University.

The WGS reads were assembled by SOAPdenovo(Li et al., 2010). The summaries of the resulting assemblies were listed in Table S1. The resulting assemblies had scaffold N50 of more than 500kb and were suitable for further analysis.

Repetitive sequences in the genome assemblies were identified by searching the Repbase database(Jurka et al., 2005) using RepeatMasker(Tarailo-Graovac and Chen, 2009) and by de novo repetitive sequence search using RepeatModeler (http://www-repeatmasker.org/RepeatModeler.html). tRNAs were predicted by tRNAscan-SE(Lowe and Eddy, 1997). Ribosomal RNAs were identified by RNAmmer(Lagesen et al., 2007). Other non-coding RNAs were predicted by searching the Rfam database(Gardner et al., 2009) using Infernal(Nawrocki et al., 2009). For faster detection, the sequences were first searched against Rfam database using FASTA(Lipman and Pearson, 1985), with a loose cutoff. The sequences having positive hits were then searched using the program Infernal. The statistics of some genome features were listed in Table S2. The descriptions of other species for comparison can be found below.

Gene prediction and annotation

Ab initio gene predictions were performed on the genome assemblies by Augustus(Stanke et al., 2006), GeneMark-ES(Borodovsky and Lomsadze, 2011), GlimmerHMM(Allen et al., 2006), and PASA(Haas et al., 2003) trained with the transcript sequences from this study (see below). The final sets of gene models were selected by EvidenceModeler(Haas et al., 2008), combing the ab initio gene predictions with the supports by alignments, including spliced alignments derived from non-redundant database(Pruitt et al., 2007) (NR) and other fungi using AAT(Huang et al., 1997), and GeneWise protein-homology-based gene predictions(Birney et al., 2004; Finn et al., 2010). The statistics of gene prediction results were listed in Table S3.

The predicted genes were annotated by BLAST(Altschul et al., 1990) searches against the public databases, including NR and UniProtKB/Swiss-Prot(Consortium, 2011). The function classifications were assigned with COGs(Tatusov et al., 1997) and KOGs(Tatusov et al., 2003). The protein signatures were identified using InterProScan(Zdobnov and Apweiler, 2001). Gene ontology(Ashburner et al., 2000) (GO) annotations and EC(Webb, 1992) (Enzyme Commission) numbers were analyzed by Blast2GO(Conesa et al., 2005). KO (KEGG Orthology) assignments and KEGG(Kanehisa et al., 2012) pathways were annotated using KAAS(Moriya et al., 2007) (KEGG Automatic Annotation Server). The two sequences with 30% identity over at least half of their entire sequence lengths and with e-value 1e-5 were taken as homologs.

Synteny analysis

Synteny mapping was performed using SyMAP software(Soderlund et al., 2011).
Repeat-induced point mutation (RIP) analysis

The RIP indices, TpA/ApT and (CpA+TpG)/(ApC+GpT), were calculated to detect RIP relics (Hane and Oliver, 2008; Margolin et al., 1998; Watters et al., 1999). The RIP indices were calculated for all types of sequences. Windows of 500-bp and 200-bp with 100-bp shifts were used for different types of sequences, such as the whole genomes, coding regions, non-coding regions, exons, introns, repetitive sequences, etc. The 200-bp window with 100-bp shifts was used for detecting RIP regions, complying with the criteria of $T_{pA/A_{pT}} \geq 0.89$ and $(C_{pA}+T_{pG})/(A_{pC}+G_{pT}) \leq 1.03$. Parts of the results were listed in Table S6.

Orthology and phylogenomic analysis

The predicted proteins of the three nematode-trapping fungi and one close fungus without traps were compared with the predicted proteins of the two nematode-trapping fungi, *Arthrobotrys oligospora* (Yang et al., 2011) and *Monacrosporium haptotylum* (Andersson et al., 2013; Meerupati et al., 2013), and the other 10 fungi (listed in Table S4). All proteins were searched against all other proteins in those genomes using BLASTP (Altschul et al., 1990). The matches with $E \leq 1e^{-5}$ and at least 30% sequence identity (Rost, 1999) over 60% of both protein lengths (Galagan et al., 2003) were taken as homologous sequences, from which the bidirectional best hits (BBHs) (Overbeek et al., 1999) were taken as orthologous sequences.

A total of 395 orthologous proteins shared by all the species, were obtained and concatenated to infer the phylogenomic relationships among these species using PHYLIP software (Felsenstein, 1989) with different methods, including Neighbor-joining (Saitou and Nei, 1987) (NJ), Maximum parsimony (Fitch, 1971) (MP) and maximum likelihood (Felsenstein, 1981) (ML). Divergence times were estimated and chronogram was constructed using PAML (Yang, 1997, 2007) based on the ML tree with three calibrations: root node (310-420 Mya), node A (100-420 Mya) and node B (290-420 Mya) (Cracraft and Donoghue, 2004; Prieto and Wedin, 2013; Sipiczki, 2000).

Multiple gene family analysis

Multigene families were constructed from the homologous sequences based on the rule of single linkage transitive closure (Galagan et al., 2003). The results were listed in Table S5.

Some multigene families were found to be significantly expanded in nematode-trapping fungi ($p<0.05$). The expansions were continuous along NTF lineage. Neutrality tests were performed by calculating Tajima's $D$ and Fu's $Fs$ using DnaSAM (Eckert et al., 2010), and by calculating Ka/Ks ratio using PAML (Yang, 1997, 2007).
Gene domain analysis

The gene domains (from InterProScan annotations) were compared among the nematode-trapping fungi and the other fungi. Some gene domains were found significantly expanded in the nematode-trapping fungi (p<0.05) and along the NTF lineage. The SSPs (Small Secreted Proteins) were retrieved as in previous studies(Andersson et al., 2013; Meerupati et al., 2013).

Pathogenicity-related gene families

A few gene families related to fungal pathogenicity were manually selected based on previous findings. The gene members of these families were expanded from the homologous sequences based on the rule of single linkage transitive closure. The seed genes for searches were retrieved from a previous study(Yang et al., 2011). The homologous sequences were then added to the gene families with a criterion of more than 30% sequence identity over at least half of the entire sequence lengths and an e-value 1e-5. The process continued until no new member could be added.

The pathogenicity-related genes were also predicted by homology search against the PHI gene database(Winnenburg et al., 2008), the Pathogen-Host Interaction database.

Comparative analyses

The species were divided into trapping-, pathogen- and saprotroph- species (see Table S4). Based on orthology analysis, the genes shared by all trapping-species in Arthrobotrys oligospora were classified into four categories: trapping-pathogen shared, trapping-saprotroph shared, trapping only and trapping-pathogen-saprotroph shared. The pathogen category can be further divided into plant and animal pathogen sub-categories (Table S4). The distribution analysis was also performed on the PHI putative genes shared by all trapping-species in Arthrobotrys oligospora.

Enrichment analysis

Functional analysis of genes remains a challenge for high-throughput data analysis. The gene-annotation enrichment analysis is one of the important strategies contributing to functional analysis of large gene sets(Huang da et al., 2009), helping the investigators identify biological processes. In the present study, the enrichment analysis was performed on different types of gene annotations for various gene lists, using the hypergeometric distribution(Rice, 2007) adjusted by the Bonferroni correction(Dunn, 1961).

Real-time PCR analysis

The total RNAs were reverse-transcribed and amplified by SYBR Premix Ex Taq II (TaKaRa) in a Roche LightCycle 480 II instrument (roche Diagnostics, Mannheim, Germany). Tublin mRNAs were amplified as an internal control for normalization of
each sample. The primer sequences were 5'-ACTGGGCGAAGGGTCATT-3' (F) and 5'-CCGAGGGAGTGGGTAATCT-3' (R). All samples were analyzed in triplicate using the 2-ΔΔCt method. For each gene, three replicates were performed.

**DNA methylation**

Genomic DNA were fragmented by sonication to 200-300bp with Covaris S220. The DNA fragments were treated twice with bisulfite using EZ DNA Methylation-Gold™ Kit followed by PCR amplification. The library preparations were sequenced on an Illumina Hiseq 2000/2500 platform. The adaptor sequences were removed and low quality reads were filtered out. The clean bisulfite-treated reads were mapped to the reference genome using Bismark software (version 0.12.5). The methylation state of all cytosine positions in the reads was inferred. Methylation level (ML) for each C site was defined as ML=reads(mC)/(reads(mC)+reads(C)). ML was further corrected with the bisulfite non-conversion rate according to previous studies (Lister et al. 2013).

**List of 395 conserved genes from the A. oligospora genome used for the phylogenetic analysis**

```
ao0307905 ao0307739 ao03010262 ao0309640 ao0307148 ao0306840 ao0301748 ao0309840 ao0306874 ao0301575 ao0303091 ao0305774
ao0304511 ao0307728 ao0308859 ao0306581 ao0303652 ao0307437 ao0306037 ao0302965 ao0301430 ao0305334 ao0304886
ao0304719 ao0301106 ao03010992 ao0306431 ao0303037 ao0307027 ao0305372 ao0300680 ao0308227 ao0305149
ao0304637 ao0303003 ao0304482 ao0302085 ao0304566 ao0305773 ao0307639 ao0308989 ao0310743 ao0305409
ao0302873 ao0303397 ao0301674 ao0304968 ao0301679 ao0308413 ao0304713 ao0305324 ao0305642 ao0301534 ao0303035
ao0304065 ao0304788 ao0309286 ao0308566 ao0302173 ao0307494 ao0308109 ao0304125
ao0304368 ao0306187 ao0308875 ao0305776 ao0307290 ao030189 ao0304472 ao0304720 ao0307623 ao0308203
ao0309459 ao0309656 ao0304433 ao030752 ao0308416 ao0309251 ao0306203 ao0309222 ao0302469
ao0309786 ao0306531 ao030242 ao0301403 ao0308855 ao0308201 ao0304748 ao0302896 ao0308602 ao0307372
ao0308816 ao0304843 ao0305667 ao0309924 ao0301067 ao0305362 ao0302313 ao0304787 ao0302333
ao0304573 ao0301115 ao0309266 ao03010619 ao0304237 ao0305364 ao0305768 ao0308649 ao0309066 ao0308117
ao0308793 ao0310566 ao0309767 ao0309695 ao03010070 ao0304773 ao0306146 ao0303164 ao0301243 ao0308354
ao0308775 ao0301893 ao0301332 ao0302912 ao0308564 ao0301210 ao0307444 ao0305898 ao0306771 ao0308862
ao0303122 ao0308018 ao03010272 ao03010648 ao0309802 ao0309287 ao0301124 ao0305253 ao0300432 ao0301356
ao0306470 ao0304751 ao0302077 ao03010036 ao0303061 ao0305455 ao0302563 ao0302879 ao0303915 ao0310297
ao0300330 ao030653 ao0303826 ao0307344 ao0305617 ao0303575 ao0309367 ao0304151 ao0308919 ao0306978
ao0301936 ao0307731 ao0303773 ao0303924 ao0301642 ao0307097 ao0308728 ao0309038 ao0309176 ao0307767
ao0306657 ao0300837 ao0302833 ao0308166 ao0302833 ao0305449 ao0302620 ao0302359 ao0305719 ao0308791
ao0307210 ao0309712 ao0309527 ao0304665 ao0301234 ao0307097 ao0301189 ao0310408 ao0305182 ao0305427
ao0304714 ao0306473 ao0303011 ao0310589 ao0306227 ao0303116 ao0304555 ao0309350 ao0305364 ao0307217
ao0302442 ao0304446 ao0309859 ao0302901 ao0304059 ao0305062 ao0301774 ao0306301 ao0303943 ao0309084
ao03082845 ao0306411 ao0304401 ao03010023 ao0307514 ao0308171 ao0301740 ao0302326 ao0308393 ao0303592
ao0301725 ao0302134 ao0309581 ao0304284 ao0310614 ao0307466 ao0306366 ao0300065 ao0303273 ao0302159
ao0306652 ao0306761 ao0306833 ao0301192 ao0304852 ao0303229 ao0304887 ao0304190 ao0301583 ao0303131
```
List of 12 multi-gene families

| Family | Genes |
|--------|-------|
| cluster28095 | ao0302270, ao0110186, ao0308574, ao0306182, ao0306626, de0203657, ao0307718, de0208402 |
| cluster35186 | ao0304953, ao0307789, ao0305571, de0204308, dc0110493, ao0301041, dc0102917, de0205490, dc0110258, dc0103145 |
| cluster24093 | de0209325, de0202642, ao0306609, ao0303315, de0205460, ao0302293, ao0306514, ao0304017, de0205490, ao010258, ao010345 |
| cluster28487 | de0200317, de0100356, de0109990, de0103419, de0103523, de0200836, de0206924, de0201097, de0206373 |
| cluster10205 | de0200841, de0202796, de0205706, ao0303163, de0202889, de0203567, de0201852, de0104250, de0209493, de0208838 |
| cluster34135 | de0208669, de0109390, de0102129, de0102636, de0207616, de0106603, de0108982, ao0303141, de0202231, de0208259 |
| cluster25335 | de0105024, ao0308326, de0201315, ao0308311, de0108173 |
| cluster1493 | de0104321, de0202710, de0104657, ao0310379, de0107675, de0206543, de0308787, de0105870, de0203055, ao0303884 |
| cluster234170 | de0110488, ao0310350, de0104744, de0103523, de0200836, de0206924, de0201097, de0206373 |
| cluster34170 | de0105374, de0208274, de0108761, de0104599, de0107392, de0107072, de0109525, ao0303153, ao0308280, de0103961, ao0105856 |
| cluster34170 | de0105374, de0208274, de0108761, de0104599, de0107392, de0107072, de0109525, ao0303153, ao0308280, de0103961, ao0105856 |
| cluster34170 | de0105374, de0208274, de0108761, de0104599, de0107392, de0107072, de0109525, ao0303153, ao0308280, de0103961, ao0105856 |
| cluster34170 | de0105374, de0208274, de0108761, de0104599, de0107392, de0107072, de0109525, ao0303153, ao0308280, de0103961, ao0105856 |

List of 12 multi-gene families
Three expanded gene families related to fungal pathogenicity

subtilisin: ao0300035 ao0300452 ao0300467 ao0302307 ao0302461 ao0302587 ao0302609 ao0302633 ao0302609 ao0302633
ao030389 ao0303234 ao0303945 ao0305325 ao0305499 ao0306396 ao0306894 ao0307129 ao0308714
ao0309515 ao0310012 ao031032 ao0310696 ao0310800 db0200043 db2000453 db0201690 db0201718 db0202051
db020236 db0202816 db0203791 db200475 db0204997 db0205442 db0207218 db0207758 db0207874
db0209344 dc0100038 dc0100192 dc0100271 dc0100580 dc0100612 dc0100622 dc0100882 dc0101290
dc0102117 dc0102262 dc0102521 dc0103275 dc0103451 dc0103515 dc0103547 dc0105317 dc0105686 dc0106062
dc0106211 dc0106564 dc0106635 dc0107453 dc0108336 dc0108541 dc0109248 dc0109186 dc0109917
dc0109442 dc0109828 dc0110014 dc0110051 dc020006 dc0200569 dc0201480 dc0201540 dc0201568
dc0201752 dc0202115 dc0202292 dc0202756 dc0202810 dc0202816 dc0203361 dc0203814 dc0204118
dc0204176 dc0204771 dc0204791 dc0204830 dc0205389 dc0205804 dc0205902 dc0206762 db0208442
dc0209475 db0100383 db0100643 db0100965 db0101067 db0101666 db0101728 db0102847 db0102888 db0103115
db0103608 db0103790 db0103812 db0104534 db0105368 db0105639 db0105698 db0106016 db0106556 db0106758
db0106821 db0106929 db0107210 db0107413 db0107949 db0108438 db0108637 db0108665 db0109417 db0109886
db0109983 db0109992 db0110364 db0110944
adhesion protein: ao0300459 ao0300868 ao0301050 ao0301050 ao0301866 ao0302277 ao0302326 ao0303120 ao0303366
ao0303621 ao0304739 ao0305758 ao0306189 ao0306454 ao0306918 ao0307325 ao0310129 db0200736 db0201336
db0201471 db0201630 db0202047 db0202371 db0203913 db0205306 db0205636 db0207722 db0208813 db0208022
dc0100304 dc0100635 dc0101196 dc0101751 dc0102872 dc0103959 dc0104531 dc0104969 dc0105388
dc0106447 dc0106512 dc0107116 dc0107123 dc0108370 dc0109991 dc0110176 dc0110587 dc0110709
dc0200373 dc0200602 dc0200969 dc0201109 dc0201619 dc0201670 dc0201933 dc0201979 dc0202066 dc0202354
dc0202519 dc0202652 dc0202757 dc0202773 dc0203299 dc0203387 dc0204934 dc0204943 dc0205671
dc0203877 dc0204684 dc0207272 dc0207433 dc0207734 dc0208209 dc0208770 dc0209131 dc0209499 dc0209652
dc0210005 db0100105 db0100288 db0100779 db0101133 db0101458 db0101787 db0101846 db0102038
db0102398 db0102431 db0103085 db0103263 db0103363 db0103517 db0103588 db0103629 db0103688
db0103770 db0104187 db0104386 db0104903 db0105179 db0105614 db0105914 db0106702 db0106902
db0106910 db0107110 db0107438 db0107476 db0107496 db0107540 db0108404 db0108482 db0108506
db0108560 db0108622 db0108828 db0109680 db0109735 db0110233 db0110318 db0110743 db0110842
poly-galacturonase: ao0301348 ao0303436 ao0303931 ao0304793 ao0305082 ao0305391 ao0306342 ao0308463
List of genes analyzed by RT-PCR for their expressions during trap formation

A. Oligospora: ao0305758 ao0301886 ao0306454 ao0302277 ao0306918 ao0303366 ao0303621 ao0300459 ao0301050 ao0300863 ao0306189 ao0304739 ao0303120 ao0301129 ao0302126 ao0307325

D. brochopaga: db0203913 db0200736 db0205306 db0208813 db0203271 db0201630 db0202047 db0205836 db0201471 db0207722

D. cionopagum: dc0102872 dc0107123 dc0105488 dc0100504 dc0101731 dc0104693 dc0110709 dc010655 dc0103959 dc0108370 dc0103926 dc0109605 dc0106532 dc0109911 dc0107116 dc0106447 dc01010176 dc0101996 dc01010587 dc0100282 dc0104531

D. entomopaga: de0203453 de0209499 de0203299 de0201619 de0207734 de0202066 de0207433 de0201109 de0208770 de0205671 de0205877 de0204934 de0204943 de0202773 de0202519 de0209131 de0203387 de0202757 de0201933 de0208209 de0201979 de0201670 de0202652 de0206652 de0206464 de0207272 de0202354 de0209602 de0200969 de0203373 de0210005
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