Insect Pathogenic Fungi: Genomics, Molecular Interactions, and Genetic Improvements

Chengshu Wang* and Sibao Wang

Key Laboratory of Insect Developmental and Evolutionary Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China; email: cswang@sibs.ac.cn, sbwang@sibs.ac.cn

Annu. Rev. Entomol. 2017. 62:73–90

First published online as a Review in Advance on November 4, 2016

The *Annual Review of Entomology* is online at ento.annualreviews.org

This article's doi: 10.1146/annurev-ento-031616-035509

*Corresponding author

Keywords

convergent evolution, effector, entomopathogenic fungi, genetic engineering, host specificity, immune evasion

Abstract

Entomopathogenic fungi play a pivotal role in the regulation of insect populations in nature, and representative species have been developed as promising environmentally friendly mycoinsecticides. Recent advances in the genome biology of insect pathogenic fungi have revealed genomic features associated with fungal adaptation to insect hosts and different host ranges, as well as the evolutionary relationships between insect and noninsect pathogens. By using species in the *Beauveria* and *Metarhizium* genera as models, molecular biology studies have revealed the genes that function in fungus-insect interactions and thereby contribute to fungal virulence. Taken together with efforts toward genetic improvement of fungal virulence and stress resistance, knowledge of entomopathogenic fungi will potentiate cost-effective applications of mycoinsecticides for pest control in the field. Relative to our advanced insights into the mechanisms of fungal pathogenesis in plants and humans, future studies will be necessary to unravel the gene-for-gene relationships in fungus-insect interactive models.

INTRODUCTION

Gene-for-gene relationship: the inheritance of a parasite's ability to cause disease and a host's resistance is controlled by pairs of matching genes

Fungi are common pathogens of insects and therefore critical regulators of insect populations in nature. There are more than 1,000 species distributed in the phyla Entomophthoromycota, Blastocladiomycota, Microsporidia, Basidiomycota, and Ascomycota that are known to infect and kill insects (79). The phylum Entomophthoromycota is newly established from the former phylum Zygomycota and includes species largely pathogenic to insects (5). The mosquito pathogens *Coelomomyces* spp. were previously classified as chytrids and have now been reallocated to the recently established phylum Blastocladiomycota (62). Microsporidian parasites of insects are intracellular obligates, and those that are pathogens of mosquitos and locusts have been successfully used for the biological control of these insect pests (48). The genus *Septobasidium* of Basidiomycota contains more than 170 described species that are mutualistic/pathogenic to scale insects (33). Of these five phyla, ascomycete insect pathogens, mainly species in *Cordyceps* sensu lato, include more than 600 species that can infect not only insects but also spiders and mites (76). These pathogens are largely facultative, widely distributed, and better studied when compared with the insect parasites of other phyla. Unless otherwise indicated, this review focuses mainly on advances in the genomics and molecular biology of ascomycete entomopathogenic fungi.

Insect pathogenic fungi have evolved highly diversified lifestyles in nature (**Figure 1**). For example, the sexual stages of *Metarhizium* and *Beauveria* have been identified as *Metacordyceps* and *Cordyceps* species; however, their sexual stages rarely occur in the field and can hardly be induced in the lab (108). In contrast, *Cordyceps* species, such as *C. militaris*, can readily produce sexual fruiting bodies (**Figure 1*d***), which have been used for centuries in the traditional medicine of Asian countries (35, 107). Certain species of entomopathogenic fungi evolved specificity or different host ranges (5). Most interestingly, fungal infections by host-specific pathogens, such as *Ophiocordyceps unilateralis* and *Entomophaga grylli*, can alter host behavior, inducing the host to climb to an elevated position prior to its death to benefit the transmission of fungal spores (14, 69). Accumulating evidence has also demonstrated that ascomycete insect pathogens can function as plant endophytes, antagonists of plant pathogens, and plant growth-promoting agents (3). Unlike insect pathogenic bacteria and viruses that cause diseases after oral ingestion, fungi infect insects via direct penetration of the insect cuticle (**Figure 2**) and are therefore well suited for the development of contact mycoinsecticides to control not only chewing pests but also insects with sucking mouthparts, such as aphids and mosquitos (86).

In this review, we highlight recent advances in the genomics, molecular biology, and genetic engineering of entomopathogenic fungi. Future perspectives are also proposed for further investigating the gene-for-gene relationships in insect-fungus interactions.

COMPARATIVE GENOMICS OF FUNGAL ENTOMOPATHOGENICITY

To date, the genomes of 26 species of insect pathogens, which include 23 ascomycete species and 3 species of Entomophthoromycota, have been sequenced and/or published (**Table 1**). Most of them are *Metarhizium* species, which include 13 sequenced strains that belong to 9 species. In addition to understanding the fungal tree of life (30), obtaining genome information will advance our knowledge of the genomic traits that determine fungal entomopathogenicity and will further promote molecular biology studies of fungus-environment interactions that will benefit agriculture, the environment, and human health.

Insect Pathogen Evolution and Host Adaptation

Phylogenomic analysis of the first genome-sequenced pathogens—namely, *Metarhizium anisopliae* (with a broad host range; the sequenced strain was later reclassified as *M. robertsii*) and *M. acridum*

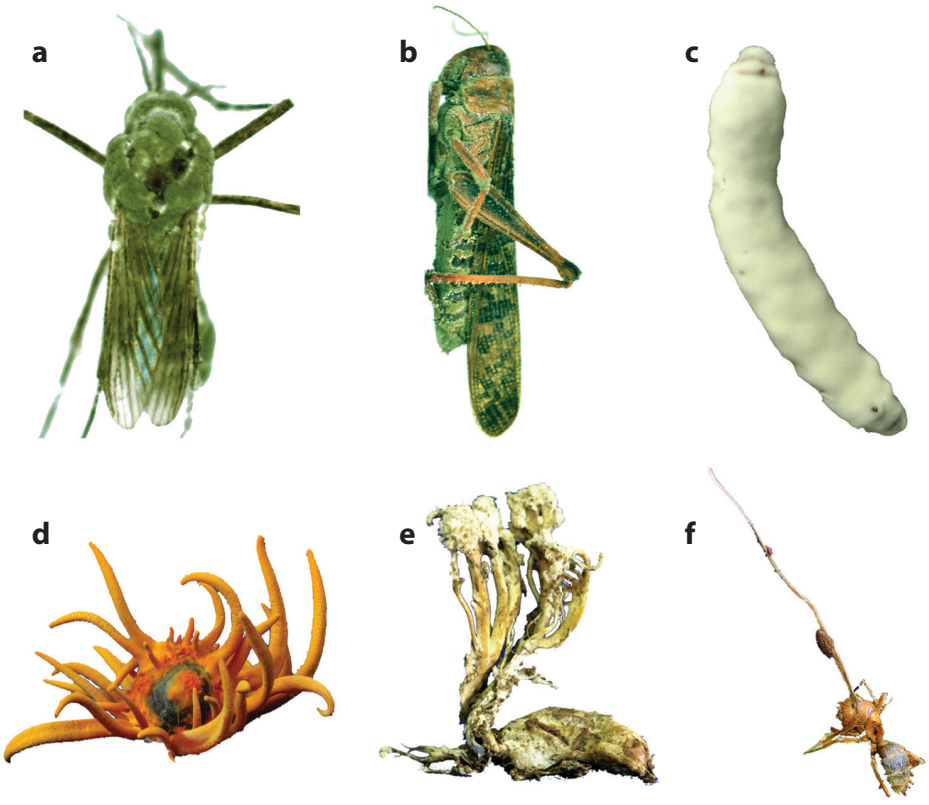

Figure 1

Insects killed and mycosed by different fungi. (*a*) Mosquito (*Aedes aegypti*) killed and mycosed by *Metarhizium robertsii*. (*b*) Locust (*Locusta migratoria*) killed and mycosed by *M. acridum*. (*c*) Silkworm (*Bombyx mori*) larva killed and mycosed by *Beauveria bassiana*. (*d*) Chinese tussah silkmoth (*Antheraea pernyi*) pupa colonized by *Cordyceps militaris* to form the sexual fruiting bodies. (*e*) Asexual synnemata of *Cordyceps cicadae* erupted from the head of a mycosed cicada. (*f*) Carpenter ant (*Camponotus* sp.) killed by a species of *Ophiocordyceps unilateralis* to form the sexual stroma. Figure courtesy of Daniel Winkler.

(acridid-specific)—indicated that the lineage diversified approximately 33–43 million years ago and is most closely related to plant endophytes (27). A further analysis of seven *Metarhizium* species with different host ranges indicated that specialists evolved first, followed by transitional species with intermediate host ranges and then the most recently diverged generalists (34). This speciation/evolution trajectory was paralleled by insect host speciation (34). In addition to the importance of the fossil record, the identification of extant transitional species greatly facilitates our understanding of fungal pathogen speciation and organism evolution.

A phylogenetic analysis of the currently sequenced ascomycete insect pathogens together with selected mycoparasites and plant and human pathogenic fungi revealed that fungal entomopathogenicity evolved multiple times (70) (**Figure 3**). Consistent with a previous taxonomic placement within the order Onygenales (5), the honey bee pathogen *Ascosphaera apis* was found to be a close relative of human pathogens, such as *Aspergillus fumigatus*, which causes aspergillosis. The insect pathogen *Sporothrix insectorum* is closely related to plant and human pathogens in the family Ophiostomatales. Otherwise, ascomycete insect pathogens have massively diversified in the

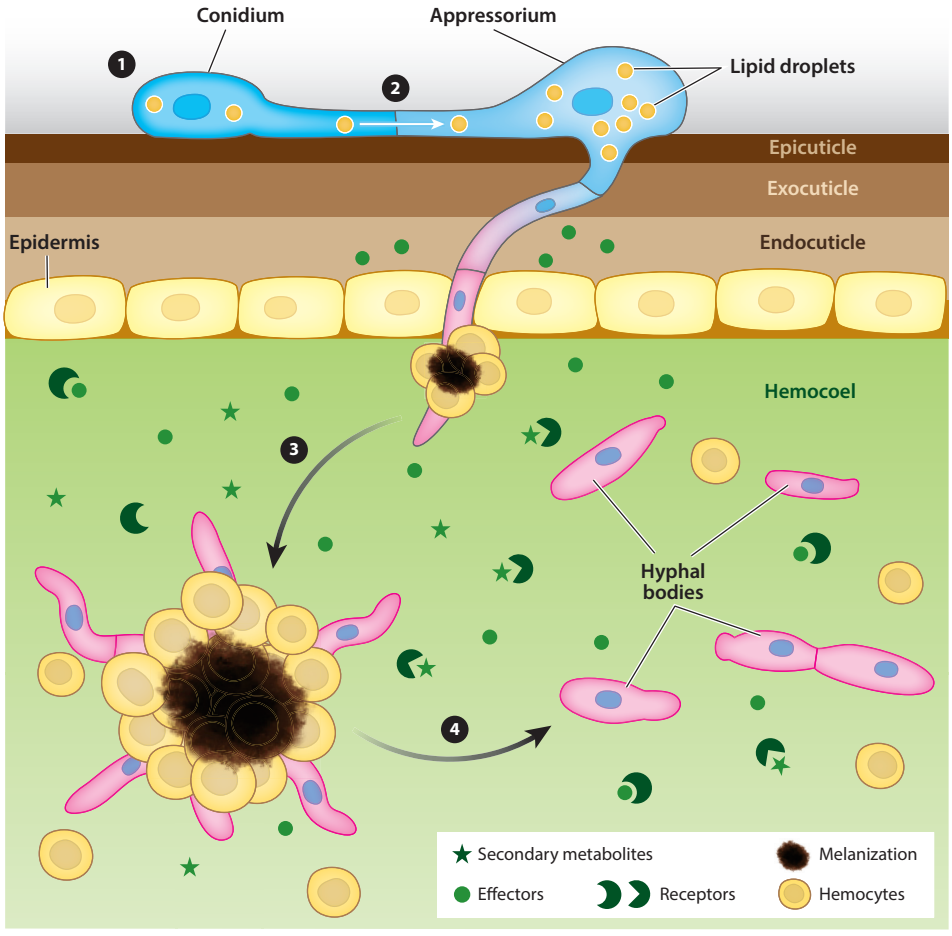

Figure 2

Schematic of the infection process of entomopathogenic fungi such as *Metarhizium robertsii*. (❶) A spore adheres to the insect cuticle, germinates, and forms the infection structure, the appressorium. (❷) In addition to the secretion of proteases and chitinases, lipid droplets are translocated from the mother conidium to the appressorium for hydrolysis, which generates a high concentration of glycerol to build up turgor pressure and breach the cuticle. (❸) During this process and after reaching the host hemocoel, the fungal cells modify their cell wall structures (represented in *pink*) in response to hemocyte recognition, encapsulation, and melanization. In addition, fungal cells secrete effector proteins and secondary metabolites to evade host immunity by counteracting host receptors (resistant proteins). (❹) To successfully colonize the hemocoel and kill the insect, escaped filamentous cells switch to a yeast-type propagation strategy to form hyphal bodies (also called blastospores) for quick growth and host nutrient deprivation.

order Hypocreales together with plant pathogens, endophytes, and mycoparasites (**Figure 3**). This evolutionary pattern is indicative of the frequent concurrence of cross-kingdom or cross-phylum host jumping during fungal pathogen speciation.

Two hypotheses have been proposed to explain the occurrence of cross-kingdom or cross-phylum host jumping, the host habitat hypothesis and the host relatedness hypothesis (5). The former suggests that new hosts were accidently acquired due to their proximity in the environment, and the latter argues that parasites are more likely to jump to new hosts that are closely

Table 1 Information on genome-sequenced entomopathogenic fungi

Species	Strain	Genome size (Mb)	Gene number	NCBI accession number	Reference(s)
Beauveria bassiana	ARSEF 2860	33.7	10,366	ADAH00000000	96
Beauveria bassiana	D1-5	34.7	11,861	ANFO01000000	NP
Cordyceps militaris	Cm 01	32.2	9,684	AEVU00000000	107
Hirsutella thompsonii	MTCC 3556	34.6	9,756	APKB00000000	1
Hirsutella thompsonii	MTCC 6686	34.7	10,259	APKU00000000	
Metarhizium acridum	CQMa 102	39.4	9,849	ADNI00000000	27, 34
Metarhizium album	ARSEF 1941	30.5	8,472	AZHE00000000	34
Metarhizium majus	ARSEF 297	42.1	11,535	AZNE00000000	
Metarhizium guizhouense	ARSEF 977	43.2	11,787	AZNH00000000	
Metarhizium brunneum	ARSEF 3297	37.1	10,689	AZNG00000000	
Metarhizium anisopliae	ARSEF 549	38.5	10,891	AZNF00000000	
Metarhizium anisopliae	BRIP 53293	38.6	11,415	APNB00000000	57
Metarhizium anisopliae	E6	38.4	10,817	JNNZ00000000	74
Metarhizium anisopliae	BRIP 53284	37.9	11,456	APNC00000000	NP
Metarhizium robertsii	ARSEF 23	41.7	11,689	ADNJ02000000	27, 34
Metarhizium robertsii	ARSEF 2575	39.7	12,384	JELW00000000	NP
Metarhizium frigidum	ARSEF 4124	50.03	13,547	PRJNA272623	NP
Metarhizium rileyi	RCEF 4871	32.0	8,764	AZHC00000000	70
Isaria fumosorosea	ARSEF 2679	33.5	10,060	AZHB00000000	
Aschersonia aleyrodis	RCEF 2490	30.9	8,461	AZGY00000000	
Lecanicillium lecanii	RCEF 1005	35.6	11,030	AZHF00000000	
Beauveria brongniartii	RCEF 3172	32.4	9,595	AZHA00000000	
Sporothrix insectorum	RCEF 264	34.7	9,496	AZHD00000000	
Ascosphaera apis	ARSEF 7405	20.4	6,442	AZGZ00000000	
Aschersonia badia	MTCC 10142	28.8	9,292	JMQE00000000	2
Tolypocladium inflatum	NRRL 8044	30.4	9,998	AOHE00000000	8
Ophiocordyceps sinensis	CO 18	120	6,972	ANOV00000000	35
Ophiocordyceps unilateralis	SC 16a	26.0	7,821	LAZP00000000	14
Ophiocordyceps polyrhachis-furcata	BCC 54312	43.0	6,799	LKCN00000000	94
Conidiobolus coronatus	NRRL 28638	39.9	10,635	PRJNA67455	10
Conidiobolus thromboides	FSU 785	24.4	8,867	PRJNA196084	NP
Basidiobolus meristosporus	CBS 931.73	88.7	16,111	PRJNA196075	NP

Abbreviations: Mb, megabases; NCBI, National Center for Biotechnology Information; NP, not published.

related to the original hosts (41, 55). The fossil record supports the idea that the incipient species of arthropod pathogens were concurrent with the emergence of angiosperms and the modern orders of insect herbivores around the Jurassic-Cretaceous boundary (77). Thus, the host habitat hypothesis better explains the dynamic evolution of host diversification and adaptation in the Hypocreales. In contrast, the close relationships between insect and human pathogens support the host relatedness hypothesis (**Figure 3**). For instance, fruit fly (*Drosophila melanogaster*) and wax moth (*Galleria mellonella*) larvae have frequently been used for the evaluation of the virulence potential of human pathogens (9).

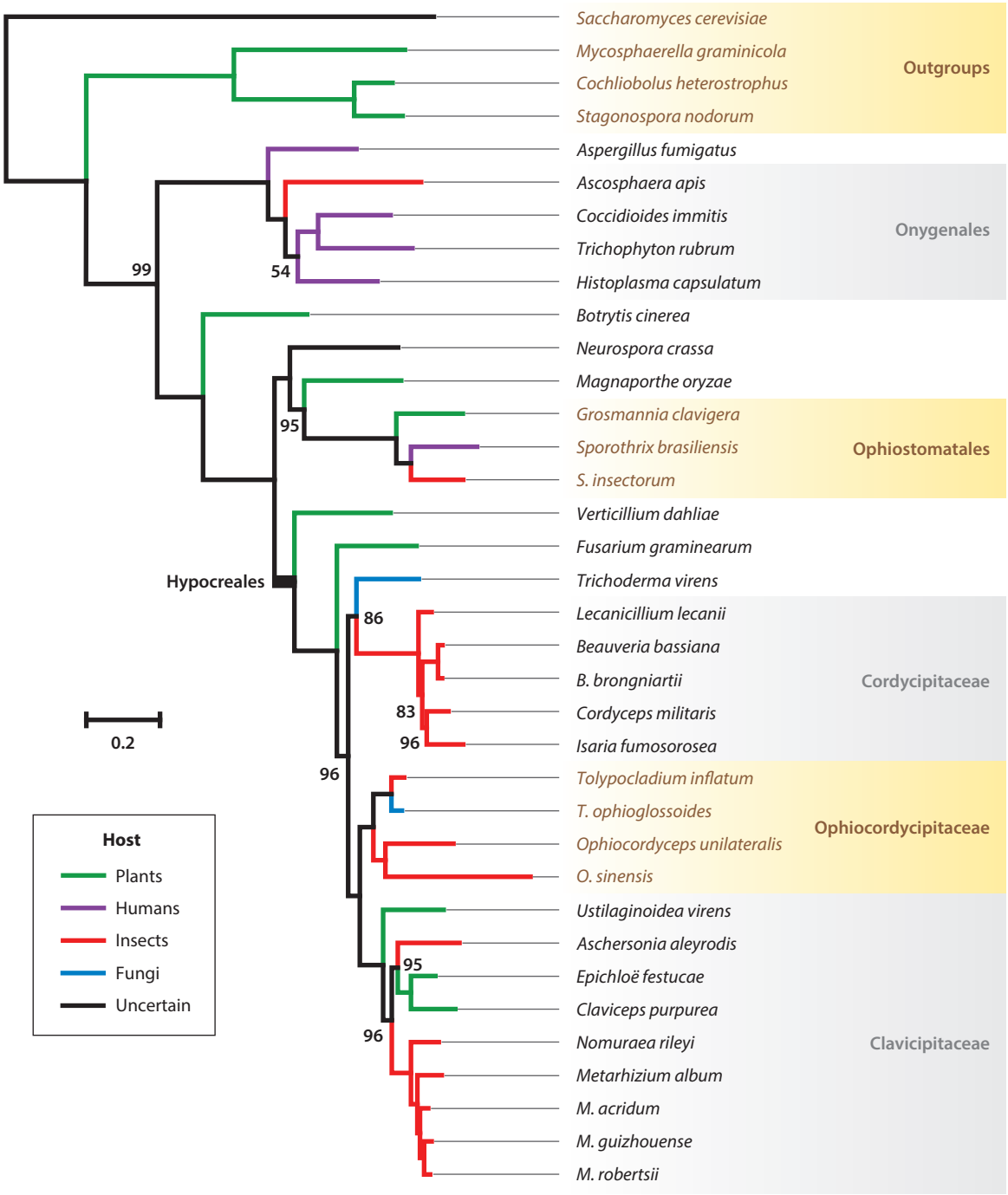

Figure 3

Phylogenetic relationships of ascomycete entomopathogenic fungi. The well-supported maximum likelihood tree (unlabeled branches have 100% bootstrap support) was generated using concatenated protein sequences with 37,272 amino acid positions. Branches are shown in different colors for fungal species associated with different hosts. For different lineages, frequent host switching/jumping is evident.

Genomic Determinants of Fungal Entomopathogenicity

Insect pathogenic fungi typically follow the sequential steps of infection/interaction to kill insects (**Figure 2**). Approximately 15% of the total proteins—that is, the putative determinants of fungal virulence—encoded in the genomes of insect pathogens are involved in fungus-insect interactions (27, 34, 96). To initiate the infection, G protein–coupled receptors (GPCRs) mediate the host recognition and activation of downstream pathways to regulate the differentiation of infection structures (27). Fungi encode an array of Pth11-like GPCRs with established roles in virulence (100). The gene family that encodes these receptors is well represented in the genomes of insect pathogenic fungi (34, 96, 107). However, these receptors remain "orphans" without identified ligands from insects.

Insect cuticles are rich in proteins and chitin, and the epicuticular wax layer is composed of an array of lipids and hydrocarbons (56). Thus, relative to noninsect pathogens, protein families such as proteases and chitinases are typically expanded in the genomes of insect pathogens to degrade insect cuticles (1, 70). This similarity is indicative of convergent evolution. Insect pathogens also encode different subfamilies of lipases, which degrade cuticular lipids and therefore play an important role in insect infection (13). Compared with plant pathogenic fungi, insect pathogens encode fewer carbohydrate-active enzymes, which include the families of glycoside hydrolase (GH), glycosyl transferase, polysaccharide lyases (PLs), and carbohydrate esterase, except for the GH18 family of chitinase (34, 70, 96). In particular, the PL family of pectinases and cutinases, the essential virulence factors in plant pathogens, is highly underrepresented in entomopathogenic fungi (70). Fungal cell walls are primarily composed of glucan polymers, chitin, mannans, and glycoproteins (6). The enzymes that contribute to fungal cell wall biosynthesis, remodeling, and protein glycosylation are connected to fungal developments and virulence (37, 63, 85).

Secreted proteins—in particular, the effectors—are key factors involved in pathogen-host interactions. The identification and functional elucidation of effectors in plant pathogens help establish the gene-for-gene relationships in pathogen-host interactions (75). Up to now, this hypothesis has not been as well determined in animal-pathogen interactions. Fungal effectors usually evolve quickly; that is, they are highly divergent or even originate at a species-specific level. However, common features have been found for these effector proteins, which are usually small (less than 300 amino acids in length) secreted cysteine-rich proteins (SSCPs) (75). On the basis of this algorithm, a plethora of SSCP-type effectors (more than 200 proteins on average) were identified in each genome of entomopathogenic fungi (70), suggesting the presence of a model of interaction similar to that of plant pathogens (**Figure 2**).

Hundreds of small molecules with insecticidal activity and probably other biological functions have been identified from entomopathogenic fungi, such as destruxins, beauvericin, oosporein, bassianolide, cordycepin, and beauverolides (54). Genome analyses of insect pathogenic fungi have unraveled the presence of dozens of secondary metabolic gene clusters, mainly nonribosome peptide synthetase (NRPS), polyketide synthase (PKS), NRPS-PKS hybrids, and terpene synthase, in each studied species. These gene clusters are highly associated with the fungal adaptation to different hosts (70). Indeed, the deletion of NRPS or PKS genes for cyclopeptide destruxin biosynthesis in *M. robertsii* and the production of cyclopeptides beauvericin and bassianolide and benzoquinone oosporein in *Beauveria bassiana* revealed that these genes are required for full fungal virulence (26, 80, 98, 99). *Metarhizium* produces both extracellular and intracellular cyclopeptide siderophores for iron sequestration and acquisition. Interestingly, the intracellular NRPS product ferricrocin but not the extracellular iron chelator metachelin is required for full virulence (29). Thus, small molecules produced by insect pathogenic fungi may also contribute to the fungal entomopathogenicity.

Convergent evolution: independent evolution of similar features in divergent species

SSCP: small secreted cysteine-rich protein

NRPS: nonribosomal peptide synthetase

PKS: polyketide synthase

Genomic Features Associated with Fungal Host Specificity

The mechanisms of host specificity are still poorly understood in pathogenic microbiology. In addition to enhancing the evolutionary understanding of fungal host specificity discussed above, the variations in genome size and gene-coding capacity of different entomopathogenic fungi (**Table 1**) may reflect features of fungal host specificity and/or pathogenic strategy. In particular, the honey bee pathogen *A. apis* and the caterpillar fungus *O. sinensis* encode a few thousand fewer proteins than do other pathogens. The adaptive genome features of these fungi are consistent with their specialized pathogenic lifestyle. The honey bee pathogen infects the host through ingestion (5), and the caterpillar fungus employs the stealth strategy of remaining latent for years before killing the insects (35). Likewise, the highly host-specific pathogens of the *O. unilateralis* species complex encode a number of proteins similar to the number encoded by the caterpillar fungus *O. sinensis* (**Table 1**). The association of genome size and coding capacity with host specificity is also evident in *Metarhizium* species. For instance, specialists, such as *M. album* and *M. acridum*, encode fewer proteins and therefore have reduced protein family sizes of secreted proteases, chitinases, SSCPs, GPCRs, and detoxifying enzymes than do generalists, such as *M. anisopliae* and *M. robertsii* (34). Generalists additionally acquired nonselective toxin gene clusters, which is a reasonable scenario for fungal adaptation to diverse host species. Similar features are found in plant pathogenic fungi, in which the genome and protein family sizes of parasites are larger than those of the free-living relatives in most if not all cases (65).

The driving forces that influence the evolution of fungal genome size have been associated mainly with the activity of transposable elements, events of horizontal gene transfer or horizontal chromosome transfer (HCT), genetic recombination, and mechanisms of repeat-induced point mutation (RIP) (65). An HCT event occurred in the plant pathogen *Fusarium oxysporum* and enabled the fungus to infect a wide range of hosts (53), whereas the loss of a dispensable chromosome in *Metarhizium* greatly reduced the fungal virulence against insects (84). RIPs occur during meiosis to mutate duplicated sequences as a genome defense mechanism in fungi (65). The estimation of RIP indices suggested that specialized *Metarhizium* species, *Ophiocordyceps* spp., and *C. militaris*, which have narrow host ranges, would employ the RIP strategy to prevent gene duplication. However, RIP did not occur in nonspecialist *Metarhizium* species and *B. bassiana*, which have expanded protein families (34, 96). In addition, the RIP discrepancy observed in different *Metarhizium* species implies that the specialists maintain sexual lives to enable RIPs to function. Therefore, sexuality is associated with narrow host ranges and asexuality with host generalization in entomopathogenic fungi. Intriguingly, the advantage of sexual reproduction confers faster generational variations in specialist species and therefore a greater possibility of infecting a wider range of hosts.

Intraspecific Genomic Variations

Screening of highly virulent strains has frequently been performed for the targeted control of insect pests (86), implying that in addition to the interspecific variations, intraspecific differences exist in insect pathogens. For example, the sequencing of two *Hirsutella thompsonii* strains revealed that one strain has 503 more genes than the other (1). Similarly, variations in genome size and coding capacity are also evident between two sequenced strains of *M. robertsii*, two strains of *B. bassiana*, and four strains of *M. anisopliae* (**Table 1**). In particular, a one-to-one orthology analysis of these two strains of *M. robertsii* showed that 601 unique genes are present in the genome of ARSEF 23 and 1,275 in that of ARSEF 2575. Most of these unique genes (379/601 in ARSEF 23; 832/1,275 in ARSEF 2575) have no conserved domain(s), including the putative SSCP-like effectors, suggesting that they quickly evolved after speciation. Consistent with those

results, these two strains of *M. robertsii* show varied abilities against ultraviolet (UV) irradiation and heat shock (66) and are genetically incompatible with each other (92).

It appears that the sequencing and resequencing of more strains will be required for population genomic studies to answer other questions as well. For example, it is still argued whether the genetic relatedness of different strains is highly associated with geographic locality or with insect host origin (17, 88). There are also concerns regarding the interference of local populations after the release of mycoinsecticides for pest control. Release and recovery studies of *B. bassiana* and *M. robertsii* revealed that parasexual recombinations could occur between the released and local strains (82, 83, 92). However, the long-term ecological effects on the possible displacement of local strains and the safety to nontargeted hosts have not been thoroughly evaluated.

Immune evasion: a strategy used by pathogens to escape host immunity and continue growing

MOLECULAR MECHANISMS OF FUNGAL PATHOGENESIS

Molecular biology studies were performed before the advent of insect fungal genomics and have since become of particular importance in understanding the mechanisms of molecular interactions between fungi and insects. By using *M. robertsii*, *M. acridum*, and *B. bassiana* as model species, different genes have been functionally characterized for their contributions to fungal infection processes, such as spore adhesion, infection structure differentiation, detoxification, insect hemocoel adaptation, nutrient deprivation, and immune evasion (**Figure 2**). The results of these studies shed light on the mechanisms of fungus-insect interactions.

Spore Adhesion and Host Recognition

First, fungal spores have to adhere to the host cuticle to initiate infection (**Figure 2**). Before the identification of adhesin proteins in *Metarhizium* (90), spore adhesion was considered to be passively mediated by fungal hydrophobins (104). *Metarhizium* species encode two divergent adhesins: MAD1 and MAD2. MAD1 mediates spore adhesion to the insect cuticle and thereby contributes to fungal infection and virulence, whereas MAD2 facilitates spore adhesion to plant surfaces to establish endophytic or plant root rhizosphere relationships (90). This characteristic family of proteins was later found to be widely present in different fungi, suggesting a common adhesive feature (47). The divergence of different adhesins in *Metarhizium* might suggest that active adhesion occurs after fungal recognition of the respective hosts. Thus, to establish a nutritional relationship with insects, entomopathogenic fungi must readily recognize their hosts. As in plant pathogens (100), it has been suggested that GPCRs mediate insect host recognition and the activation of downstream mitogen-activated protein kinase (MAPK) and protein kinase A (PKA) pathways, which trigger the formation of the infection structure (appressorium), the hallmark of host recognition (23, 27). Functional characterization of a GPCR in *B. bassiana* indicated that the receptor mediates nutrient sensing, stress response, and fungal development (102). In addition to GPCRs, a single copy of the transmembrane protein tetraspanin is present in the genomes of entomopathogenic fungi to mediate signal transduction in the early fungal infection process (50). Overall, further efforts are required to identify the host signals/ligands perceived by GPCRs and the downstream transcription factors (TFs) activated by the MAPK and PKA signal pathways.

Infection Structure Differentiation

After host recognition, successful differentiation and maturation of the appressoria are prerequisites for the fungus to penetrate the cuticle (**Figure 2**). Consistent with findings in plant pathogens, such as the rice blast fungus *Magnaporthe oryzae* (95), the MAPK pathway mediates appressorium

formation, whereas the PKA pathway primarily mediates appressorium maturation in entomopathogenic fungi (23, 38). For the infection structure to function, the buildup of turgor pressure is essential and is achieved by the accumulation of high concentrations of glycerol and glycogen in the appressoria (95). Glycogen metabolism has not been clearly understood in entomopathogenic fungi. However, in *Metarhizium*, lipid droplets (LDs) stored in conidia provide the source of triacylglycerol and are degraded and transformed into a high concentration of glycerol in the appressoria (28). The identification of an LD-specific and mammalian-like perilipin in *Metarhizium* revealed that the protein is critical for LD storage in conidia and therefore for the generation of turgor pressure in the appressoria (91). Furthermore, in *Beauveria*, an LD-surface caleosin-like protein participates in lipid storage and fungal infection (20). As in *M. oryzae* (95), the genes involved in cell autophagy or apoptosis are also required for fungal virulence because they intervene in LD accumulation and/or cell development in *M. robertsii* (11, 16, 67). However, unlike the observed phenotype of mother cell death and the requirement of cell wall melanin to generate turgor pressure in *Magnaporthe* (95), conidium death is not found in entomopathogenic fungi during early infection, and cell wall melanin is also not evident in connection with fungal virulence in *M. robertsii* (12).

Detoxification of Insect Cuticular Compounds

To penetrate the insect cuticle, entomopathogenic fungi have to detoxify cuticular antimicrobial/antifungal compounds, such as quinones, lipids, alkanes, and free fatty acids, that would inhibit spore germination and/or hyphal growth (60). For example, the red flour beetle (*Tribolium castaneum*) is resistant to fungal infection as it produces benzoquinone-containing secretions on the epicuticle, but the production of 1,4-benzoquinone oxidoreductase in *B. bassiana* can help detoxify these compounds and assist fungal infection, an outcome of the coevolutionary arms race between fungus and insect (59). In addition, the genomes of entomopathogenic fungi encode an array of cytochrome P450 (CYP), dehydrogenase, and lipase enzymes that are putatively involved in detoxification (61). Functional studies indicated that the deletions of a CYP52-encoding gene in *B. bassiana* and *M. robertsii* delayed spore germination and appressorium formation on the insect epicuticle and therefore impaired fungal virulence (46, 105). The treatment of *Metarhizium* spores with a lipase activity inhibitor disabled the fungal infection process (13), indicating the functional importance of lipases in the degradation/detoxification of cuticular lipids. The oxidative burst, a rapid and transient production of reactive oxygen species (ROS), is a well-known early defense strategy in plants in response to pathogen infections (42). Acute ROS production has not been established in insects as a mechanism to counteract pathogen infections. However, deletions of superoxide dismutase, catalase, and thioredoxin genes, which are involved in antioxidative stresses in *B. bassiana*, substantially impaired fungal virulence against insects (43, 93, 103). These results suggest that antioxidative responses are required for the progress of fungal infections.

Evasion of Host Immunity

To successfully colonize the insect hemocoel (**Figure 2**), fungal pathogens have to evade acute immune responses, such as hemocyte ingestion, encapsulation, melanization, and expression of antifungal peptides, that are activated in insects by the dual detection of cell wall structures (such as β-glucans and chitin) and fungal virulence factors (32). To counteract these defenses, fungal cells first upregulate the osmosensor and activate a downstream pathway to mediate adaptation to the osmotic pressure of the insect hemolymph (38, 81). Then the remodeling of cell walls coupled with camouflage of cell surfaces with a protective coat protein enables the fungal cells

to evade host recognition (**Figure 2**) and quickly escape hemocyte encapsulation (37, 85). Fungal proteases can trigger melanization of the insect hemolymph by activating prophenoloxidases (PPOs). The resulting melanin is toxic to both insect hosts and fungi (71). Thus, the expression of proteases will be switched off when the fungi grow in the insect hemolymph (87). In addition, the insecticidal secondary metabolites, such as destruxins produced by *Metarhizium* (80) and oosporein biosynthesized by *Beauveria*, can function as effectors to inhibit PPO activity and downregulate host expression of antifungal peptides (26). A recent metabolomics study revealed that fungal infection can also reduce the accumulation of immune-mediator eicosanoids in the insect hemolymph (97), a previously unsuspected immune evasion strategy that remains to be investigated.

Hyphal body: an irregularly shaped fragment formed by segmentation of a hypha or yeast-type propagation

Rapid proliferation through yeast-type budding to produce hyphal bodies is another strategy for fungal cells to quickly occupy the insect hemocoel (**Figure 2**). Fungal infections can cause an antifeedant effect in insects, and the fast propagation of fungal cells can facilitate the deprivation of nutrients to expedite the death of the insect (97). For example, the acid trehalase and phosphoketolase genes of *Metarhizium* are specifically upregulated in trehalose-rich insect hemolymph for degradation of this disaccharide, which contributes to fungal virulence (15, 39). The mechanism that controls dimorphic switching is still poorly understood in insect pathogens. Interestingly, deletion of the gene encoding the cell surface adhesin MAD1 in *Metarhizium* further impaired fungal filamentous fragmentation due to septin cytoskeletal dysfunction (90). An insect-like sterol carrier protein in *Metarhizium* was found to facilitate the formation of hyphal bodies when the fungus was growing in the insect hemocoel (106).

Regulation of the Fungal Infection Process

Transcriptome analyses indicated that gene expression is finely tuned in fungal pathogens in response to different infection steps (27, 96), that is, the functional outcomes of TFs. The downstream TFs of the MAPK and PKA pathways and their targeted genes activated in the infection process are largely unclear in entomopathogenic fungi. A few loss-of-function studies revealed that the deletion of a single TF can result in substantial phenotypic changes and virulence reduction in insect pathogens; for example, the ablation of the WetA developmental regulator, the VosA velvet protein, and the CreA carbon and nitrogen repressor homologous genes in *B. bassiana* impaired fungal conidiation, nutrient uptake, growth, pigmentation, sporulation, hyphal body formation, and virulence (44, 52). The deletion of the yeast stress-responsive activator Msn2 homologous gene *Bbmsn2* in *B. bassiana* revealed that Bbmsn2 is required for multistress responses and virulence due to the downregulation of proteases and lipases but deregulation of oosporein production (51).

During early infection, the ambient pH is a critical determinant for regulating the expression of cuticle-degrading enzymes (72). A functional study of a pH-responsive TF, PacC, in *M. robertsii* indicated that the gene is involved in the downregulation of chitinases but the upregulation of genes associated with cell wall remodeling and protein glycosylation (37). Likewise, the TFs containing the basic leucine zipper domain and the homolog of the response regulator in a two-component signaling pathway are also required for fungal development, stress response, and pathogenic processes in *Metarhizium* (36, 67). As indicated above, secondary metabolites produced by insect pathogens also contribute to fungal virulence (26, 80). Fungal secondary metabolisms are controlled either jointly or specifically by a pathway-specific regulator and global TF (40). The pathway-specific TF is functionally evident in the control of oosporein production (26). In contrast, the gene cluster involved in the destruxin biosynthesis does not contain a TF (80). Future studies are necessary not only for additional insights into the TFs involved in the direct or indirect control (e.g., the biosynthesis of insecticidal metabolites) of fungal virulence but also for

the establishment of epigenetic control of the fungal infection process, which has been indicated in other models of pathogen-host interactions (31).

GENETIC IMPROVEMENTS OF FUNGAL BIOCONTROL EFFICACY

In addition to a better understanding of their unique biology, the ultimate goal of studying entomopathogenic fungi is to promote the cost-effective applications of mycoinsecticides for the control of different insect pests (86). Barriers to the large-scale application of fungal biocontrol agents still exist due to their slow killing speed and environmental stability issues (56, 73). Apart from improving formulation techniques, genetic engineering efforts have been spent on improving the efficacy of mycoinsecticides—for example, through genetic modifications to increase fungal virulence against targeted pests and/or enhancing fungal environmental stabilities against stress factors, such as UV irradiation and high temperature.

Genetic Improvement of Fungal Virulence

The first trial of a genetic improvement in fungal virulence was performed by engineering *Metarhizium* to overexpress the gene encoding the endogenous cuticle-degrading protease Pr1, and the resulting transformant took 25% less time to kill insects (71). Engineering *B. bassiana* to constitutively express a chitinase gene also significantly increased the fungal performance in killing insects (19). Otherwise, exogenous genes encoding proteins toxic to insects were used to genetically improve fungal virulence. For example, an insect-selective scorpion neurotoxin peptide gene (*AaIT*) was synthesized and used to transform *M. anisopliae* for targeted expression in insect hemolymph. This transformation increased the fungal toxicity more than ninefold against mosquitos, tobacco hornworm (*Manduca sexta*), and coffee berry borer (*Hypothenemus hampei*) (58, 89). The combined use of *Pr1* and *AaIT* to genetically modify *B. bassiana* did not synergistically improve virulence, because the protease Pr1 degrades the toxic peptide AaIT (49). However, a synergistic effect was evident in *M. acridum* engineered with multiple neurotoxin genes (22). *M. anisopliae* was genetically engineered to combat malaria in mosquitos by expressing antimalarial and/or insecticidal genes (25). A similar experiment was successfully conducted with the integration into *B. bassiana* of an insecticidal protein from the insect pathogenic bacterium *Bacillus thuringiensis*. The recipient strain showed not only a significantly improved virulence but also the ability for per os infection of insects (64). The virulence of *B. bassiana* was increased through the expression of endogenous insect genes, such as those for a diuretic hormone, neuropeptide, and serpin, to disrupt or inhibit normal hormone levels, necrophoretic behavior, or phenoloxidase activation in insects (18, 21, 101).

Genetic Engineering to Improve Fungal Environmental Stability

To combat abiotic stress factors, the inclusion of UV protectants and heat stabilizers in the formulation of insect pathogens provides a way to increase fungal spore stability (4). Genetic engineering methods to improve fungal stability under field conditions have also been tried. For example, genetic modification of *B. bassiana* to overexpress a tyrosinase gene conferred on the fungus a significant increase in UV resistance (68). The integration of a PKS gene cluster for melanin biosynthesis from a plant pathogen into *M. anisopliae* enabled the fungus to resist UV irradiation and improved fungal virulence (78). The engineering of *Metarhizium* or *Beauveria* to overexpress a DNA repair photolyase also increased fungal resistance to solar radiation (24). Heat shock proteins (HSPs) are involved in multiple stress responses in different organisms. *M. robertsii*

was genetically transformed to overexpress an endogenous HSP-encoding gene (*Hsp25*), and the mutant not only survived extreme temperatures but also showed resistance to oxidative stress and osmotic agents (45). Thus, genetic engineering is an effective way to improve fungal environmental stability and therefore the efficacy of field applications. Depending on the regulations in different countries, developing these genetically modified strains will potentiate the cost-effective application of mycoinsecticides for the control of different insect pests or disease vectors.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Genomic analyses of entomopathogenic fungi have revealed the evolutionary and protein family features associated with fungal adaptation to insect hosts. Fungal entomopathogenicity evolved multiple times, and relative to noninsect pathogenic fungi, the formation of similar protein family size in divergent species suggests the occurrence of convergent evolution during the coevolutionary arms race between fungi and insects. Insect pathogenic fungi also evolved specificity for different ranges of host species. Species with narrow host ranges usually have reduced protein-coding capacities and protein family sizes. In addition, they frequently undergo sexual reproduction as compared with generalist species. Molecular biology studies have revealed the genes that function in fungal interactions with insect hosts at different infection stages. However, relative to our greater level of understanding regarding fungus-plant and fungus-human interaction mechanisms (7, 75), future efforts will be needed to investigate the function of effector-like proteins in fungus-insect interactions and to dissect the molecular mechanisms involved in regulating the cell dimorphic switch during fungal colonization of the insect hemocoel. The knowledge obtained will further improve the cost-effective application of mycoinsecticides in the field.

SUMMARY POINTS

1. Entomopathogenic fungi play a pivotal role in the regulation of insect populations in nature. The genomics and molecular biology of insect pathogens have been well developed in recent years.
2. Fungal entomopathogenicity evolved multiple times; convergent evolution led to the formation of similar protein families in different species for adapting to insect hosts.
3. The host specificity of insect pathogens is associated with fungal genome features and lifestyle. Species with narrow host ranges usually have reduced protein family sizes but keep the capacity for sexual reproduction as compared with generalists.
4. Similar to plant pathogens, entomopathogenic fungi encode an array of effector-like proteins in their genomes, suggesting the presence of analogous gene-for-gene relationships in fungus-insect interactions.
5. Different strategies are employed by insect pathogens to invade the host and evade its immune response, such as the production of secondary metabolites.
6. Genetic engineering provides useful strategies to either increase fungal virulence or enhance fungal resistance to different stress factors.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Yuzhen Lu, Kai Cen, and Shuai Zhan helped with the design and generation of the figures. The authors were supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB11030100, XDB11010500) and the National Nature Science Foundation of China (31225023, 31530001).

LITERATURE CITED

1. Agrawal Y, Khatri I, Subramanian S, Shenoy BD. 2015. Genome sequence, comparative analysis, and evolutionary insights into chitinases of entomopathogenic fungus *Hirsutella thompsonii*. *Genome Biol. Evol.* 7:916–30
2. Agrawal Y, Narwani T, Subramanian S. 2016. Genome sequence and comparative analysis of clavicipitaceous insect-pathogenic fungus *Aschersonia badia* with *Metarhizium* spp. *BMC Genom.* 17:367

3. Evidence of nutrient trading among insects, entomopathogenic fungi, and plants.

3. **Behie SW, Zelisko PM, Bidochka MJ. 2012. Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants. *Science* 336:1576–77**
4. Behle RW, Compton DL, Laszlo JA, Shapiro-Ilan DI. 2009. Evaluation of soyscreen in an oil-based formulation for UV protection of *Beauveria bassiana* conidia. *J. Econ. Entomol.* 102:1759–66
5. Boomsma JJ, Jensen AB, Meyling NV, Eilenberg J. 2014. Evolutionary interaction networks of insect pathogenic fungi. *Annu. Rev. Entomol.* 59:467–85
6. Bowman SM, Free SJ. 2006. The structure and synthesis of the fungal cell wall. *BioEssays* 28:799–808
7. Boyce KJ, Andrianopoulos A. 2015. Fungal dimorphism: The switch from hyphae to yeast is a specialized morphogenetic adaptation allowing colonization of a host. *FEMS Microbiol. Rev.* 39:797–811
8. Bushley KE, Raja R, Jaiswal P, Cumbie JS, Nonogaki M, et al. 2013. The genome of *Tolypocladium inflatum*: evolution, organization, and expression of the cyclosporin biosynthetic gene cluster. *PLOS Genet.* 9:e1003496
9. Chamilos G, Lionakis MS, Lewis RE, Kontoyiannis DP. 2007. Role of mini-host models in the study of medically important fungi. *Lancet Infect. Dis.* 7:42–55
10. Chang Y, Wang SS, Sekimoto S, Aerts AL, Choi C, et al. 2015. Phylogenomic analyses indicate that early fungi evolved digesting cell walls of algal ancestors of land plants. *Genome Biol. Evol.* 7:1590–601
11. Chen YX, Duan Z, Chen P, Shang YF, Wang CS. 2015. The Bax inhibitor MrBI-1 regulates heat tolerance, apoptotic-like cell death, and virulence in *Metarhizium robertsii*. *Sci. Rep.* 5:10625
12. Chen YX, Feng P, Shang YF, Xu YJ, Wang CS. 2015. Biosynthesis of non-melanin pigment by a divergent polyketide synthase in *Metarhizium robertsii*. *Fungal Genet. Biol.* 81:142–49
13. da Silva WOB, Santi L, Schrank A, Vainstein MH. 2010. *Metarhizium anisopliae* lipolytic activity plays a pivotal role in *Rhipicephalus* (*Boophilus*) *microplus* infection. *Fungal Biol.* 114:10–15
14. de Bekker C, Ohm RA, Loreto RG, Sebastian A, Albert I, et al. 2015. Gene expression during zombie ant biting behavior reflects the complexity underlying fungal parasitic behavioral manipulation. *BMC Genom.* 16:620
15. Duan Z, Shang Y, Gao Q, Zheng P, Wang C. 2009. A phosphoketolase Mpk1 of bacterial origin is adaptively required for full virulence in the insect-pathogenic fungus *Metarhizium anisopliae*. *Environ. Microbiol.* 11:2351–60
16. Duan ZB, Chen YX, Huang W, Shang YF, Chen PL, Wang CS. 2013. Linkage of autophagy to fungal development, lipid storage and virulence in *Metarhizium robertsii*. *Autophagy* 9:538–49
17. Enkerli J, Widmer F. 2010. Molecular ecology of fungal entomopathogens: molecular genetic tools and their applications in population and fate studies. *BioControl* 55:17–37

18. Virulence increase by transformation with endogenous insect genes.

18. **Fan Y, Borovsky D, Hawkings C, Ortiz-Urquiza A, Keyhani NO. 2012. Exploiting host molecules to augment mycoinsecticide virulence. *Nat. Biotechnol.* 30:35–37**
19. Fan Y, Fang W, Guo S, Pei X, Zhang Y, et al. 2007. Increased insect virulence in *Beauveria bassiana* strains overexpressing an engineered chitinase. *Appl. Environ. Microbiol.* 73:295–302
20. Fan Y, Ortiz-Urquiza A, Garrett T, Pei Y, Keyhani NO. 2015. Involvement of a caleosin in lipid storage, spore dispersal, and virulence in the entomopathogenic filamentous fungus, *Beauveria bassiana*. *Environ. Microbiol.* 17:4600–14

21. Fan Y, Pereira RM, Kilic E, Casella G, Keyhani NO. 2012. Pyrokinin β-neuropeptide affects necrophoretic behavior in fire ants (*S. invicta*), and expression of β-NP in a mycoinsecticide increases its virulence. *PLOS ONE* 7:e26924
22. Fang W, Lu HL, King GF, St. Leger RJ. 2014. Construction of a hypervirulent and specific mycoinsecticide for locust control. *Sci. Rep.* 4:7345
23. Fang W, Pava-Ripoll M, Wang S, St. Leger R. 2009. Protein kinase A regulates production of virulence determinants by the entomopathogenic fungus, *Metarhizium anisopliae*. *Fungal Genet. Biol.* 46:277–85
24. Fang W, St. Leger RJ. 2012. Enhanced UV resistance and improved killing of malaria mosquitoes by photolyase transgenic entomopathogenic fungi. *PLOS ONE* 7:e43069
25. Fang W, Vega-Rodriguez J, Ghosh AK, Jacobs-Lorena M, Kang A, St. Leger RJ. 2011. Development of transgenic fungi that kill human malaria parasites in mosquitoes. *Science* 331:1074–77
26. Feng P, Shang Y, Cen K, Wang C. 2015. Fungal biosynthesis of the bibenzoquinone oosporein to evade insect immunity. *PNAS* 112:11365–70
27. Gao Q, Jin K, Ying SH, Zhang Y, Xiao G, et al. 2011. Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum*. *PLOS Genet.* 7:e1001264
28. Gao Q, Shang YF, Huang W, Wang CS. 2013. Glycerol-3-phosphate acyltransferase contributes to triacylglycerol biosynthesis, lipid droplet formation, and host invasion in *Metarhizium robertsii*. *Appl. Environ. Microbiol.* 79:7646–53
29. Giuliano Garisto Donzelli B, Gibson DM, Krasnoff SB. 2015. Intracellular siderophore but not extracellular siderophore is required for full virulence in *Metarhizium robertsii*. *Fungal Genet. Biol.* 82:56–68
30. Gladieux P, Ropars J, Badouin H, Branca A, Aguileta G, et al. 2014. Fungal evolutionary genomics provides insight into the mechanisms of adaptive divergence in eukaryotes. *Mol. Ecol.* 23:753–73
31. Gomez-Diaz E, Jorda M, Peinado MA, Rivero A. 2012. Epigenetics of host-pathogen interactions: the road ahead and the road behind. *PLOS Pathog.* 8:e1003007
32. Gottar M, Gobert V, Matskevich A, Reichhart J, Wang C, et al. 2006. Dual detection of fungal infections in *Drosophila* via recognition of glucans and sensing of virulence factors. *Cell* 127:1425–37
33. Henk DA. 2005. New species of *Septobasidium* from southern Costa Rica and the southeastern United States. *Mycologia* 97:908–13
34. Hu X, Xiao GH, Zheng P, Shang YF, Su Y, et al. 2014. Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation. *PNAS* 111:16796–801
35. Hu X, Zhang YJ, Xiao GH, Zheng P, Xia YL, et al. 2013. Genome survey uncovers the secrets of sex and lifestyle in caterpillar fungus. *Chin. Sci. Bull.* 58:2846–54
36. Huang W, Shang YF, Chen PL, Cen K, Wang CS. 2015. Basic leucine zipper (bZIP) domain transcription factor MBZ1 regulates cell wall integrity, spore adherence, and virulence in *Metarhizium robertsii*. *J. Biol. Chem.* 290:8218–31
37. Huang W, Shang YF, Chen PL, Gao Q, Wang CS. 2015. MrpacC regulates sporulation, insect cuticle penetration and immune evasion in *Metarhizium robertsii*. *Environ. Microbiol.* 17:994–1008
38. Jin K, Ming Y, Xia YX. 2012. *MaHog1*, a Hog1-type mitogen-activated protein kinase gene, contributes to stress tolerance and virulence of the entomopathogenic fungus *Metarhizium acridum*. *Microbiology* 158:2987–96
39. Jin K, Peng G, Liu Y, Xia Y. 2015. The acid trehalase, ATM1, contributes to the in vivo growth and virulence of the entomopathogenic fungus, *Metarhizium acridum*. *Fungal Genet. Biol.* 77:61–67
40. Keller NP, Turner G, Bennett JW. 2005. Fungal secondary metabolism—from biochemistry to genomics. *Nat. Rev. Microbiol.* 3:937–47
41. Kepler RM, Sung GH, Harada Y, Tanaka K, Tanaka E, et al. 2012. Host jumping onto close relatives and across kingdoms by *Tyrannicordyceps* (Clavicipitaceae) gen. nov. and *Ustilaginoidea*_(Clavicipitaceae). *Am. J. Bot.* 99:552–61
42. Lamb C, Dixon RA. 1997. The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:251–75
43. Li F, Shi HQ, Ying SH, Feng MG. 2015. Distinct contributions of one Fe- and two Cu/Zn-cofactored superoxide dismutases to antioxidation, UV tolerance and virulence of *Beauveria bassiana*. *Fungal Genet. Biol.* 81:160–71

25. Genetically altered fungus to combat malaria in mosquitos.

27. First sequenced genomes of insect pathogenic fungi.

34. The finding of existing transitional species to bridge fungal speciation relationships.

44. Li F, Shi HQ, Ying SH, Feng MG. 2015. WetA and VosA are distinct regulators of conidiation capacity, conidial quality, and biological control potential of a fungal insect pathogen. *Appl. Microbiol. Biotechnol.* 99:10069–81
45. Liao X, Lu HL, Fang W, St. Leger RJ. 2014. Overexpression of a *Metarhizium robertsii* HSP25 gene increases thermotolerance and survival in soil. *Appl. Microbiol. Biotechnol.* 98:777–83
46. Lin LC, Fang WG, Liao XG, Wang FQ, Wei DZ, St. Leger RJ. 2011. The *MrCYP52* cytochrome P450 monoxygenase gene of *Metarhizium robertsii* is important for utilizing insect epicuticular hydrocarbons. *PLOS ONE* 6:e28984
47. Linder T, Gustafsson CM. 2008. Molecular phylogenetics of ascomycotal adhesins—a novel family of putative cell-surface adhesive proteins in fission yeasts. *Fungal Genet. Biol.* 45:485–97
48. Lomer CJ, Bateman RP, Johnson DL, Langewald J, Thomas M. 2001. Biological control of locusts and grasshoppers. *Annu. Rev. Entomol.* 46:667–702
49. Lu D, Pava-Ripoll M, Li Z, Wang C. 2008. Insecticidal evaluation of *Beauveria bassiana* engineered to express a scorpion neurotoxin and a cuticle degrading protease. *Appl. Microbiol. Biotechnol.* 81:515–22
50. Luo S, He M, Cao Y, Xia Y. 2013. The tetraspanin gene *MaPls1* contributes to virulence by affecting germination, appressorial function and enzymes for cuticle degradation in the entomopathogenic fungus, *Metarhizium acridum*. *Environ. Microbiol.* 15:2966–79
51. Luo Z, Li Y, Mousa J, Bruner S, Zhang Y, et al. 2015. *Bbmsn2* acts as a pH-dependent negative regulator of secondary metabolite production in the entomopathogenic fungus *Beauveria bassiana*. *Environ. Microbiol.* 17:1189–202
52. Luo Z, Qin Y, Pei Y, Keyhani NO. 2014. Ablation of the *creA* regulator results in amino acid toxicity, temperature sensitivity, pleiotropic effects on cellular development and loss of virulence in the filamentous fungus *Beauveria bassiana*. *Environ. Microbiol.* 16:1122–36
53. Ma LJ, van der Does HC, Borkovich KA, Coleman JJ, Daboussi MJ, et al. 2010. Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* 464:367–73
54. Molnar I, Gibson DM, Krasnoff SB. 2010. Secondary metabolites from entomopathogenic Hypocrealean fungi. *Nat. Prod. Rep.* 27:1241–75
55. Nikoh N, Fukatsu T. 2000. Interkingdom host jumping underground: phylogenetic analysis of entomoparasitic fungi of the genus *Cordyceps*. *Mol. Biol. Evol.* 17:629–38
56. Ortiz-Urquiza A, Luo ZB, Keyhani NO. 2015. Improving mycoinsecticides for insect biological control. *Appl. Microbiol. Biotechnol.* 99:1057–68
57. Pattemore JA, Hane JK, Williams AH, Wilson BA, Stodart BJ, Ash GJ. 2014. The genome sequence of the biocontrol fungus *Metarhizium anisopliae* and comparative genomics of *Metarhizium* species. *BMC Genom.* 15:660
58. Pava-Ripoll M, Posada F, Momen B, Wang C, St. Leger R. 2008. Increased pathogenicity against coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae) by *Metarhizium anisopliae* expressing the scorpion toxin (AaIT) gene. *J. Invertebr. Pathol.* 99:220–26

59. Molecular evidence of a coevolutionary arms race between a fungus and its host.

59. Pedrini N, Ortiz-Urquiza A, Huarte-Bonnet C, Fan Y, Juarez MP, Keyhani NO. 2015. Tenebrionid secretions and a fungal benzoquinone oxidoreductase form competing components of an arms race between a host and pathogen. *PNAS* 112:E3651–60

60. Pedrini N, Ortiz-Urquiza A, Huarte-Bonnet C, Zhang S, Keyhani NO. 2013. Targeting of insect epicuticular lipids by the entomopathogenic fungus *Beauveria bassiana*: hydrocarbon oxidation within the context of a host-pathogen interaction. *Front. Microbiol.* 4:24
61. Pedrini N, Zhang S, Juarez MP, Keyhani NO. 2010. Molecular characterization and expression analysis of a suite of cytochrome P450 enzymes implicated in insect hydrocarbon degradation in the entomopathogenic fungus *Beauveria bassiana*. *Microbiology* 156:2549–57
62. Porter TM, Martin W, James TY, Longcore JE, Gleason FH, et al. 2011. Molecular phylogeny of the Blastocladiomycota (Fungi) based on nuclear ribosomal DNA. *Fungal Biol.* 115:381–92
63. Qin Y, Ortiz-Urquiza A, Keyhani NO. 2014. A putative methyltransferase, *mtrA*, contributes to development, spore viability, protein secretion and virulence in the entomopathogenic fungus *Beauveria bassiana*. *Microbiology* 160:2526–37

64. Qin Y, Ying SH, Chen Y, Shen ZC, Feng MG. 2010. Integration of insecticidal protein Vip3Aa1 into *Beauveria bassiana* enhances fungal virulence to *Spodoptera litura* larvae by cuticle and *per os* infection. *Appl. Environ. Microbiol.* 76:4611–18
65. Raffaele S, Kamoun S. 2012. Genome evolution in filamentous plant pathogens: why bigger can be better. *Nat. Rev. Microbiol.* 10:417–30
66. Rangel DE, Butler MJ, Torabinejad J, Anderson AJ, Braga GU, et al. 2006. Mutants and isolates of *Metarhizium anisopliae* are diverse in their relationships between conidial pigmentation and stress tolerance. *J. Invertebr. Pathol.* 93:170–82
67. Shang Y, Chen P, Chen Y, Lu Y, Wang C. 2015. MrSkn7 controls sporulation, cell wall integrity, autolysis, and virulence in *Metarhizium robertsii*. *Eukaryot. Cell* 14:396–405
68. Shang Y, Duan Z, Huang W, Gao Q, Wang C. 2012. Improving UV resistance and virulence of *Beauveria bassiana* by genetic engineering with an exogenous tyrosinase gene. *J. Invertebr. Pathol.* 109:105–9
69. Shang Y, Feng P, Wang C. 2015. Fungi that infect insects: altering host behavior and beyond. *PLOS Pathog.* 11:e1005037
70. Shang Y, Xiao G, Zheng P, Cen K, Zhan S, Wang C. 2016. Divergent and convergent evolution of fungal pathogenicity. *Genome Biol. Evol.* 8:1374–87
71. St. Leger RJ, Joshi L, Bidochka MJ, Roberts DW. 1996. Construction of an improved mycoinsecticide overexpressing a toxic protease. *PNAS* 93:6349–54
72. St. Leger RJ, Joshi L, Roberts D. 1998. Ambient pH is a major determinant in the expression of cuticle-degrading enzymes and hydrophobin by *Metarhizium anisopliae*. *Appl. Environ. Microbiol.* 64:709–13
73. St. Leger RJ, Wang CS. 2010. Genetic engineering of fungal biocontrol agents to achieve greater efficacy against insect pests. *Appl. Microbiol. Biotechnol.* 85:901–7
74. Staats CC, Junges A, Guedes RL, Thompson CE, de Morais GL, et al. 2014. Comparative genome analysis of entomopathogenic fungi reveals a complex set of secreted proteins. *BMC Genom.* 15:822
75. Stergiopoulos I, de Wit PJGM. 2009. Fungal effector proteins. *Annu. Rev. Phytopathol.* 47:233–63
76. Sung GH, Hywel-Jones NL, Sung JM, Luangsa-Ard JJ, Shrestha B, Spatafora JW. 2007. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Stud. Mycol.* 57:5–59
77. Sung GH, Poinar GO, Spatafora JW. 2008. The oldest fossil evidence of animal parasitism by fungi supports a Cretaceous diversification of fungal-arthropod symbioses. *Mol. Phylogenet. Evol.* 49:495–502
78. Tseng MN, Chung PC, Tzean SS. 2011. Enhancing the stress tolerance and virulence of an entomopathogen by metabolic engineering of dihydroxynaphthalene melanin biosynthesis genes. *Appl. Environ. Microbiol.* 77:4508–19
79. Vega F, Meyling N, Luangsa-Ard J, Blackwell M. 2012. Fungal entomopathogens. In *Insect Pathology*, ed. F Vega, H Kaya, pp. 171–220. San Diego, CA: Academic
80. Wang B, Kang QJ, Lu YZ, Bai LQ, Wang CS. 2012. Unveiling the biosynthetic puzzle of destruxins in *Metarhizium* species. *PNAS* 109:1287–92
81. Wang C, Duan Z, St. Leger RJ. 2008. MOS1 osmosensor of *Metarhizium anisopliae* is required for adaptation to insect host hemolymph. *Eukaryot. Cell* 7:302–9
82. Wang C, Fan M, Li ZZ, Butt TM. 2004. Molecular monitoring and evaluation of the application of the insect-pathogenic fungus *Beauveria bassiana* in southeast China. *J. Appl. Microbiol.* 96:861–70
83. Wang C, Li ZZ, Butt TM. 2002. Molecular studies of co-formulated strains of the entomopathogenic fungus, *Beauveria bassiana*. *J. Invertebr. Pathol.* 80:29–34
84. Wang C, Skrobek A, Butt TM. 2003. Concurrence of losing a chromosome and the ability to produce destruxins in a mutant of *Metarhizium anisopliae*. *FEMS Microbiol. Lett.* 226:373–78
85. Wang C, St. Leger RJ. 2006. A collagenous protective coat enables *Metarhizium anisopliae* to evade insect immune responses. *PNAS* 103:6647–52
86. Wang CS, Feng MG. 2014. Advances in fundamental and applied studies in China of fungal biocontrol agents for use against arthropod pests. *Biol. Control* 68:129–35
87. Wang CS, Hu G, St. Leger RJ. 2005. Differential gene expression by *Metarhizium anisopliae* growing in root exudate and host (*Manduca sexta*) cuticle or hemolymph reveals mechanisms of physiological adaptation. *Fungal Genet. Biol.* 42:704–18
88. Wang CS, Shah FA, Patel N, Li ZZ, Butt TM. 2003. Molecular investigation on strain genetic relatedness and population structure of *Beauveria bassiana*. *Environ. Microbiol.* 5:908–15

71. First trial to genetically improve fungal virulence.

85. A strategy to evade insect immunity by molecular camouflaging of cell wall structure.

89. Genetic improvement of fungal virulence with a scorpion neurotoxin gene.

89. Wang CS, St. Leger RJ. 2007. A scorpion neurotoxin increases the potency of a fungal insecticide. *Nat. Biotechnol.* 25:1455–56

90. Wang CS, St. Leger RJ. 2007. The MAD1 adhesin of *Metarhizium anisopliae* links adhesion with blastospore production and virulence to insects, and the MAD2 adhesin enables attachment to plants. *Eukaryot. Cell* 6:808–16
91. Wang CS, St. Leger RJ. 2007. The *Metarhizium anisopliae* perilipin homolog MPL1 regulates lipid metabolism, appressorial turgor pressure, and virulence. *J. Biol. Chem.* 282:21110–15
92. Wang S, O'Brien TR, Pava-Ripoll M, St. Leger RJ. 2011. Local adaptation of an introduced transgenic insect fungal pathogen due to new beneficial mutations. *PNAS* 108:20449–54
93. Wang ZL, Zhang LB, Ying SH, Feng MG. 2013. Catalases play differentiated roles in the adaptation of a fungal entomopathogen to environmental stresses. *Environ. Microbiol.* 15:409–18
94. Wichadakul D, Kobmoo N, Ingsriswang S, Tangphatsornruang S, Chantasingh D, et al. 2015. Insights from the genome of *Ophiocordyceps polyrhachis-furcata* to pathogenicity and host specificity in insect fungi. *BMC Genom.* 16:881
95. Wilson RA, Talbot NJ. 2009. Under pressure: investigating the biology of plant infection by *Magnaporthe oryzae*. *Nat. Rev. Microbiol.* 7:185–95
96. Xiao G, Ying SH, Zheng P, Wang ZL, Zhang S, et al. 2012. Genomic perspectives on the evolution of fungal entomopathogenicity in *Beauveria bassiana*. *Sci. Rep.* 2:483
97. Xu Y-J, Luo F, Gao Q, Shang Y, Wang C. 2015. Metabolomics reveals insect metabolic responses associated with fungal infection. *Anal. Bioanal. Chem.* 407:4815–21
98. Xu Y, Orozco R, Kithsiri Wijeratne EM, Espinosa-Artiles P, Leslie Gunatilaka AA, et al. 2009. Biosynthesis of the cyclooligomer depsipeptide bassianolide, an insecticidal virulence factor of *Beauveria bassiana*. *Fungal Genet. Biol.* 46:353–64

99. First evidence of a small molecule contributing to fungal virulence.

99. Xu Y, Orozco R, Wijeratne EM, Leslie Gunatilaka AA, Stock SP, Molnar I. 2008. Biosynthesis of the cyclooligomer depsipeptide beauvericin, a virulence factor of the entomopathogenic fungus *Beauveria bassiana*. *Chem. Biol.* 15:898–907

100. Xue CY, Hsueh YP, Heitman J. 2008. Magnificent seven: roles of G protein-coupled receptors in extracellular sensing in fungi. *FEMS Microbiol. Rev.* 32:1010–32
101. Yang L, Keyhani NO, Tang G, Tian C, Lu R, et al. 2014. Expression of a Toll signaling regulator serpin in a mycoinsecticide for increased virulence. *Appl. Environ. Microbiol.* 80:4531–39
102. Ying SH, Feng MG, Keyhani NO. 2013. A carbon responsive G-protein coupled receptor modulates broad developmental and genetic networks in the entomopathogenic fungus, *Beauveria bassiana*. *Environ. Microbiol.* 15:2902–21
103. Zhang LB, Tang L, Ying SH, Feng MG. 2015. Subcellular localization of six thioredoxins and their antioxidant activity and contributions to biological control potential in *Beauveria bassiana*. *Fungal Genet. Biol.* 76:1–9
104. Zhang S, Xia YX, Kim B, Keyhani NO. 2011. Two hydrophobins are involved in fungal spore coat rodlet layer assembly and each play distinct roles in surface interactions, development and pathogenesis in the entomopathogenic fungus, *Beauveria bassiana*. *Mol. Microbiol.* 80:811–26
105. Zhang SZ, Widemann E, Bernard G, Lesot A, Pinot F, et al. 2012. CYP52X1, representing new cytochrome P450 subfamily, displays fatty acid hydroxylase activity and contributes to virulence and growth on insect cuticular substrates in entomopathogenic fungus *Beauveria bassiana*. *J. Biol. Chem.* 287:13477–86
106. Zhao H, Xu C, Lu HL, Chen X, St. Leger RJ, Fang W. 2014. Host-to-pathogen gene transfer facilitated infection of insects by a pathogenic fungus. *PLOS Pathog.* 10:e1004009
107. Zheng P, Xia YL, Xiao G, Xiong C, Hu X, et al. 2011. Genome sequence of the insect pathogenic fungus *Cordyceps militaris*, a valued traditional Chinese medicine. *Genome Biol.* 12:R116
108. Zheng P, Xia YL, Zhang SW, Wang CS. 2013. Genetics of *Cordyceps* and related fungi. *Appl. Microbiol. Biotechnol.* 97:2797–804