Evolving moldy murderers: How species in *Aspergillus* section *Fumigati* became pathogenic and identifying the genetic elements responsible

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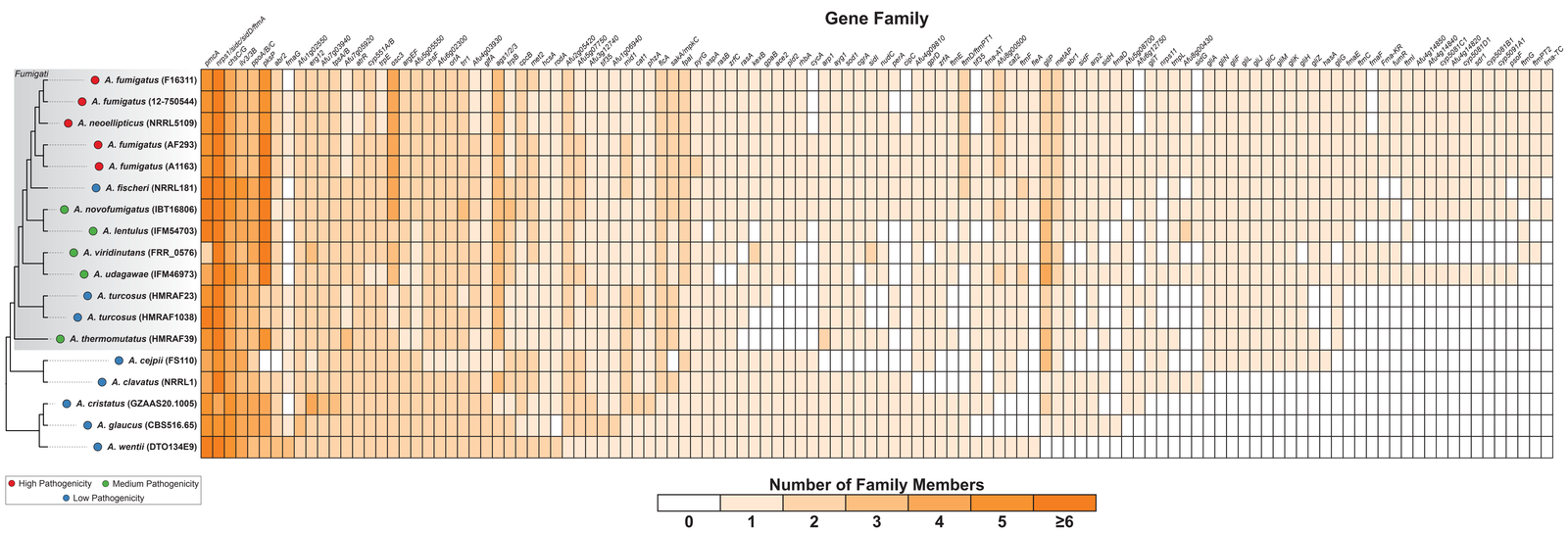
Abstract

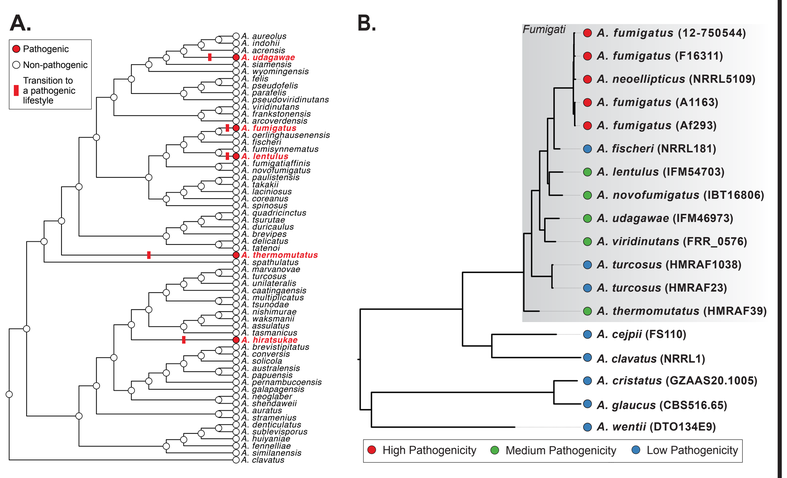
- Approximately 70% of all *Aspergillus* infections are caused by *A. fumigatus* whereas the remaining 30% stem from other species in the genus.
- Some pathogens are related to *A. fumigatus* and also belong in taxonomic section *Fumigati*; however, the majority of species in section *Fumigati* are not pathogenic.
- To study the evolution of pathogenicity in section *Fumigati* and identify targets for future therapeutics, we utilized comparative genomics, in vitro phenotyping, natural product chemistry, and an insect model of aspergillosis.
- Phylogenies of section *Fumigati* suggest that pathogenicity has independently evolved many times.
- *A. fischeri,* a non-pathogenic species very closely related to *A. fumigatus*, differs with regard to *A. fumigatus* for multiple disease-relevant traits.
- However, most of the *A. fumigatus* genes known to be associated with virulence are highly conserved in non-pathogens, and functional testing of multiple conserved virulence factors showed that they do not affect the pathogenic potential of *A. fischeri*.
- Many genes in pathogenic species exhibit higher rates of evolution than their homologs in lowly (or non-) pathogenic species.
- Our results establish a broad, comparative framework for explaining why *A. fumigatus* is such a potent killer and more generally, how major fungal pathogens evolve from historically innocuous organisms.

Most *A. fumigatus* genes known to be associated with virulence are highly conserved across species in section *Fumigati*, including in non-pathogens



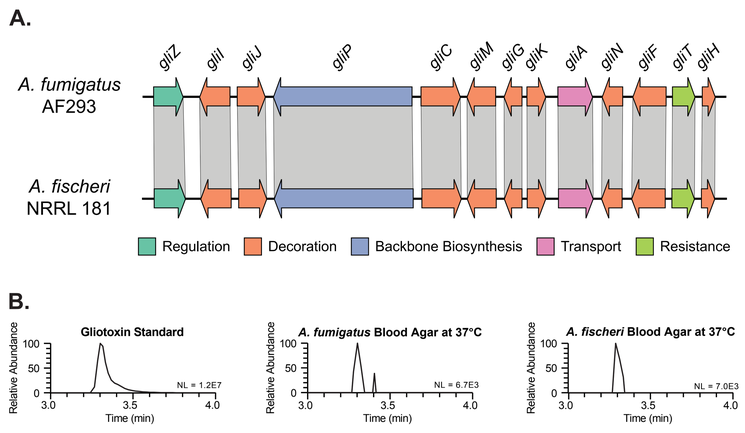
Heatmap of gene count in virulence-related gene families. Highly similar genes were placed in orthologous groups and were considered proxies for gene families. Virulence-related genes from *A. fumigatus* were acquired from the literature as well as the PHI-base database (4). Only those gene families that exhibited some change in gene family number across the species of interest (116/201) are shown. Note, there are no "*A. fumigatus*-Specific", "Section *Fumigati*-Specific", or "Pathogen-Specific" gene families. Left, cladogram of species based on their phylogenetic relationships.

Pathogenicity in section *Fumigati* has evolved multiple times independently



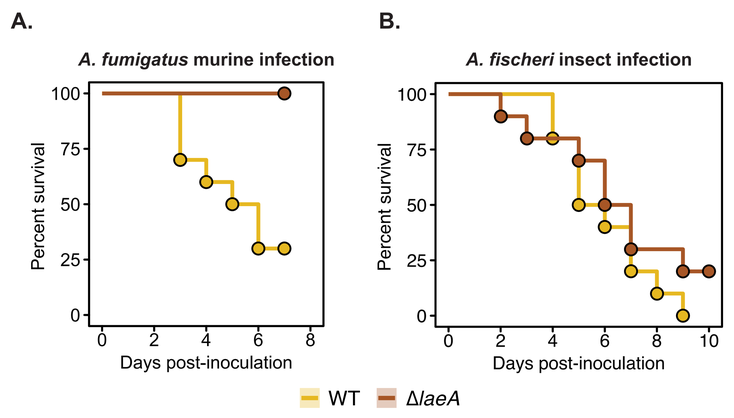
A. Reconstruction of the ability to cause human disease on a 4-gene phylogeny of section *Fumigati*. From (1) and (2). **B.** A 3,601-gene phylogeny of 5 outgroup species and all genomes available (as of April 27, 2019) from section *Fumigati* has multiple non-pathogenic strains interspersed with pathogens, suggesting that pathogenesis has evolved multiple times.

A. fischeri contains the gliotoxin biosynthetic gene cluster and produces gliotoxin in the same conditions as *A. fumigatus*



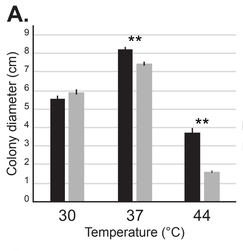
A. *A. fischeri* contains a biosynthetic gene cluster homologous to the one known to produce the toxin gliotoxin in *A. fumigatus*. **B.** Extracted chromatograms (using the protonated mass of gliotoxin) demonstrating the biosynthesis of gliotoxin in *A. fischeri* and *A. fumigatus*. The first panel shows the analysis of the gliotoxin standard for comparison. NL - normalization level (i.e. base peak intensity). From (5).

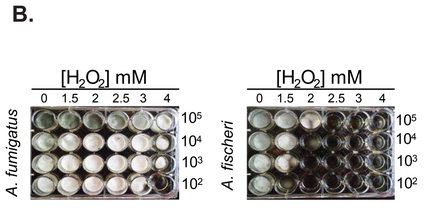
Deletion of a conserved virulence-related gene from *A. fumigatus* does not affect pathogenicity in *A. fischeri*



A. *laeA* is required for full virulence during *A. fumigatus* infection of immunocompromised mice. Redrawn from (6). **B.** Deletion of *laeA* in *A. fischeri* does not alter its virulence. Cumulative survival curves of moth (*Galleria mellonella*) larvae inoculated with asexual spores from either a Δ*laeA* mutant or wild-type (WT) *A. fischeri* NRRL 181 strain. Comparisons of moth cumulative survival when infected with either strain revealed no statistically significant differences (P-value = 0.30; log-rank test). For the inoculations, 10 moths were infected per group. From (5).

A. fischeri, a very close but non-pathogenic relative of *A. fumigatus*, exhibits divergent infection-relevant phenotypes



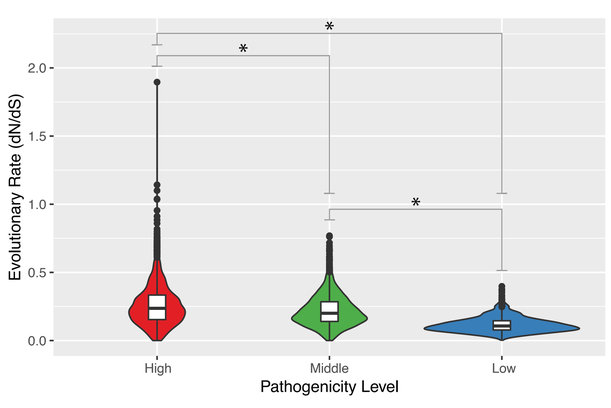


C.

Secondary Metabolite	Produced by	
	A. fumigatus	*A. fischeri*
Fumagillin	+	-
Pseurotin	+	-
Acetylaszonalenin	+	+
Fumitremorgin A	+	+
Fumitremorgin B	+	+
Verruculogen	+	+
C-11 epimer of verruculogen TR2	-	+
13-O-Fumitremorgin B	-	+
Sartorypyrone A	-	+
Sartorypyrone E	-	+
14-Epiaszonapyrone A	-	+
Aszonalenin	-	+

A. *A. fischeri* is less able to thrive at infection-relevant temperatures. Error bars indicate standard deviations between biological duplicates (**P-value < 0.005 in a paired, equal variance student t-test). **B.** *A. fumigatus* is more tolerant of a molecule used for immune defense (hydrogen peroxide) than *A. fischeri*. **C.** While *A. fischeri* can produce some of the same secondary metabolites as *A. fumigatus*, it also produces novel compounds. All data from (3).

Genes in more-pathogenic species exhibit faster rates of evolution than the same genes in less-pathogenic species.



A. Distribution of dN/dS values for 1,743 single-copy gene families in section *Fumigati* species. Only the 1,743 genes that rejected (p<0.01) the null hypothesis of a single dN/dS rate across the phylogeny are shown. *, adjusted P-value < 0.0001 for a Wilcoxon Signed-Rank Test.

References

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