



Comparison of the two sequenced clinical isolates of *A. fumigatus*

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I N S T I T U T E





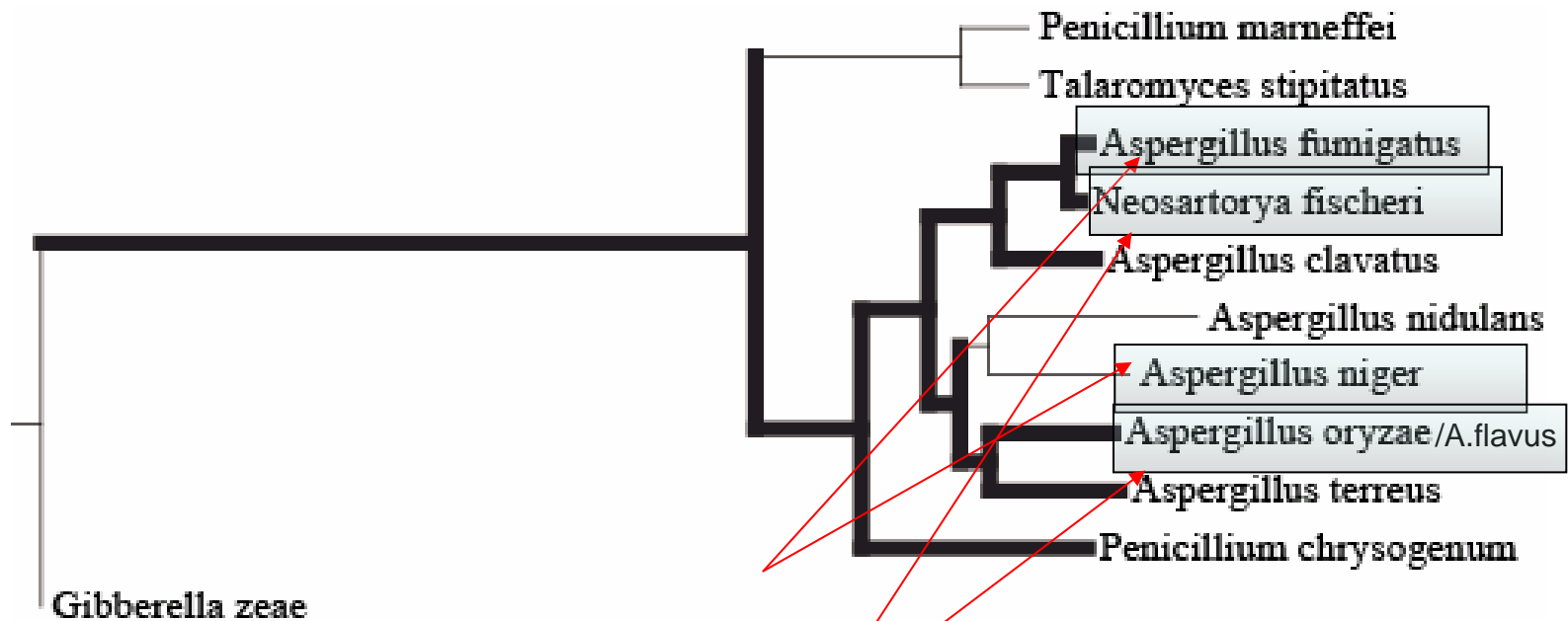
Outline

- Introduction: history of sequenced strains
- Continuous variation
- Discontinuous variation
- Vegetative incompatibility

Goal of Project

- examine the extent of diversity at the whole-genome level within *A. fumigatus* using the 2 sequenced strains (Af293 and CEA10)
- compare it to diversity within and between other species of the genus *Aspergillus* :
 - 2 sequenced *A. niger* strains
 - *A. flavus* and *A. oryzae*
 - *A. fumigatus* and *N. fischeri*
- Identify differential genetic traits associated with phenotypic differences in *A. fumigatus* isolates

Aspergillus/ Penicillium Tree



Species with 2 sequenced isolates

Pairs of closely related species

What is Molecular Diversity?

- DNA sequence variability
- Discontinuous variability:
 - Unique genes
 - Mating types
 - Vegetative compatibility groups
 - Copy number polymorphism
 - Sources: recombination, duplication, gene flow
- Continuous variability:
 - Nucleotide sequence divergence
 - Sources: mutation
 - Molecular markers: SNPs, restriction fragment length polymorphisms, SSRs (microsatellites)

Methods to Study Genetic Diversity

- Molecular typing: MLST (multilocus sequence typing)
- Microsatellites (tandem repeats 2-6 nt)
- High density SNP allele arrays
- Whole genome sequencing (WGS)
- Array comparative genomic hybridization (aCGH)



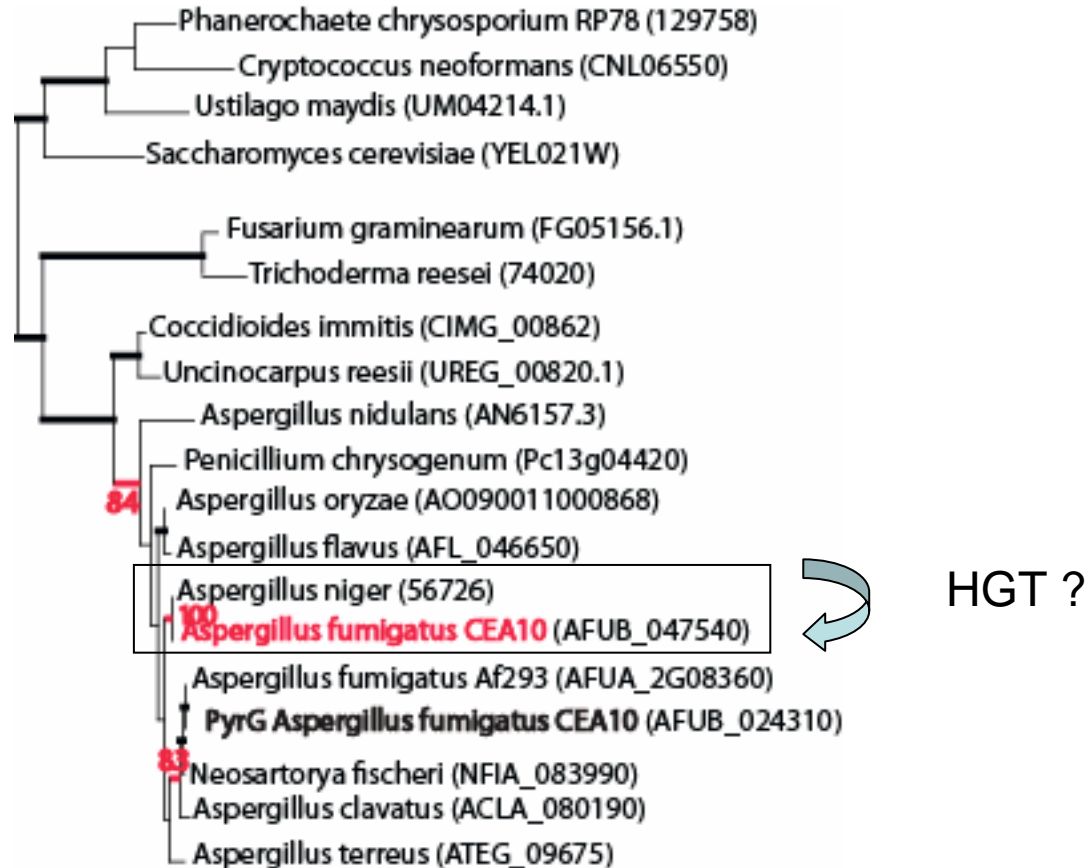
A. fumigatus Af293

- Isolated by David Denning in 1993 from a lung of a neutropenic patient who died from invasive aspergillosis
- Sequenced and annotated by TIGR/JCVI in 2005
- Used as a reference strain in CGH with other clinical isolates:
 - Af294 (France)
 - Af71 (USA)

A. fumigatus "CEA10"

- **CBS 14489/"Dal"/CEA10:** came from JP Latge's lab (from an IA patient, Mr. Dalage) renamed CEA10 by Christophe d'Enfert
- Given to the Elitra fungal team, which later moved to Merck
- Sequenced by Celera and annotated by TIGR/JCVI

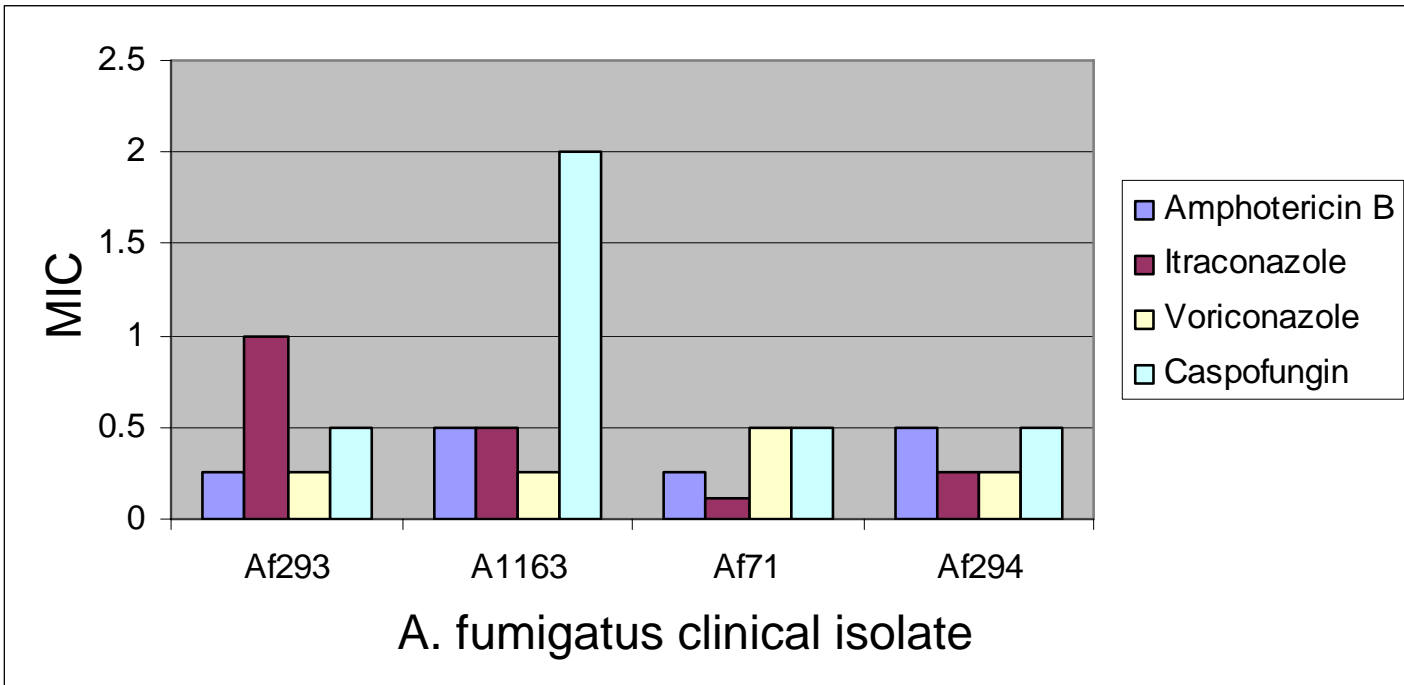
PyrG tree reveals the presence of *A. niger* *pyrG* in "CEA10"



“CEA10” = *A. fumigatus* A1163

- Horizontal gene transfer of *A. niger pyrG* into “CEA10”
- Or strain mix-up prior to sequencing ?
- “**CEA10**” is **FGSC A1163**: a derivative of *A. fumigatus* CEA17 (a uracil auxotroph of CEA10) converted to *pyrG+* via the ectopic insertion of *A. niger pyrG*

Preliminary Studies: Resistance to Antifungals





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Continuous Divergence: Intraspecific Sequence Identity

Species	<i>A.fumigatus</i> Af293/A1163	<i>A.niger</i> DSM/JGI	<i>A.flavus</i> / <i>A.oryzae</i>	<i>A.fumigatus</i> / <i>N.fischeri</i>
Genome vs. genome	99.80%	99.30%	99.50%	92.40%
CDS vs. genome	99.60%	99.10%	99.10%	94.30%
Protein vs. protein	99.50%	96.70%	98.00%	93.40%



The lowest level of sequence divergence

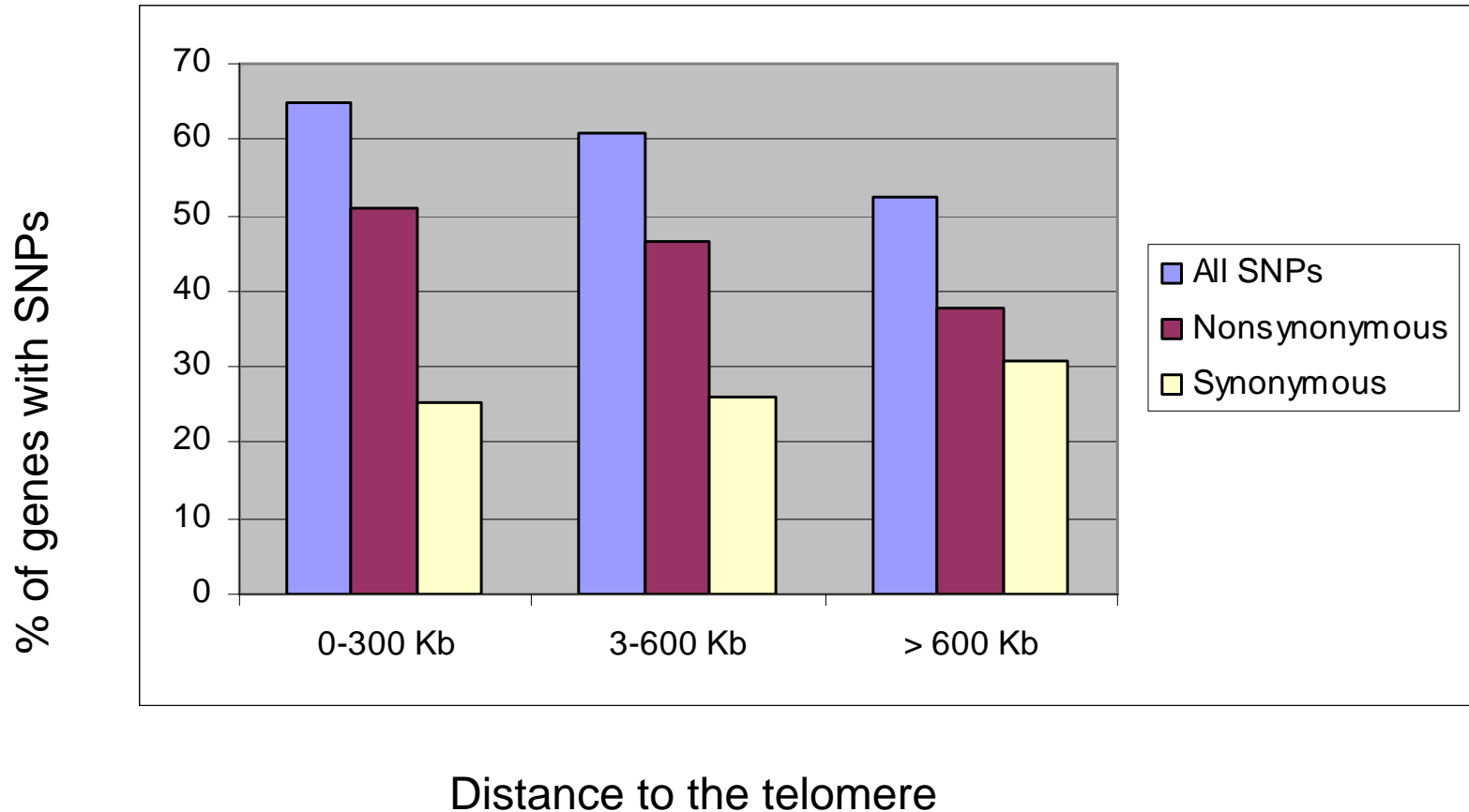
Continuous Divergence: Genes with SNPs

% CDS with SNPs	<i>A.fumigatus</i> Af293/A1163	<i>A.niger</i> DSM/JGI	<i>A.flavus</i> / <i>A.oryzae</i>	<i>A.fumigatus</i> / <i>N.fischeri</i>
nonsynononym.	41.27%	54.31%	61.45%	98.36%
only synonymous	28.81%	10.71%	15.72%	1.64%
total	57.86%	65.01%	77.18%	99.98%



The lowest level of sequence divergence

Genes with Nonsynonymous SNPs Have a Subtelomeric Bias

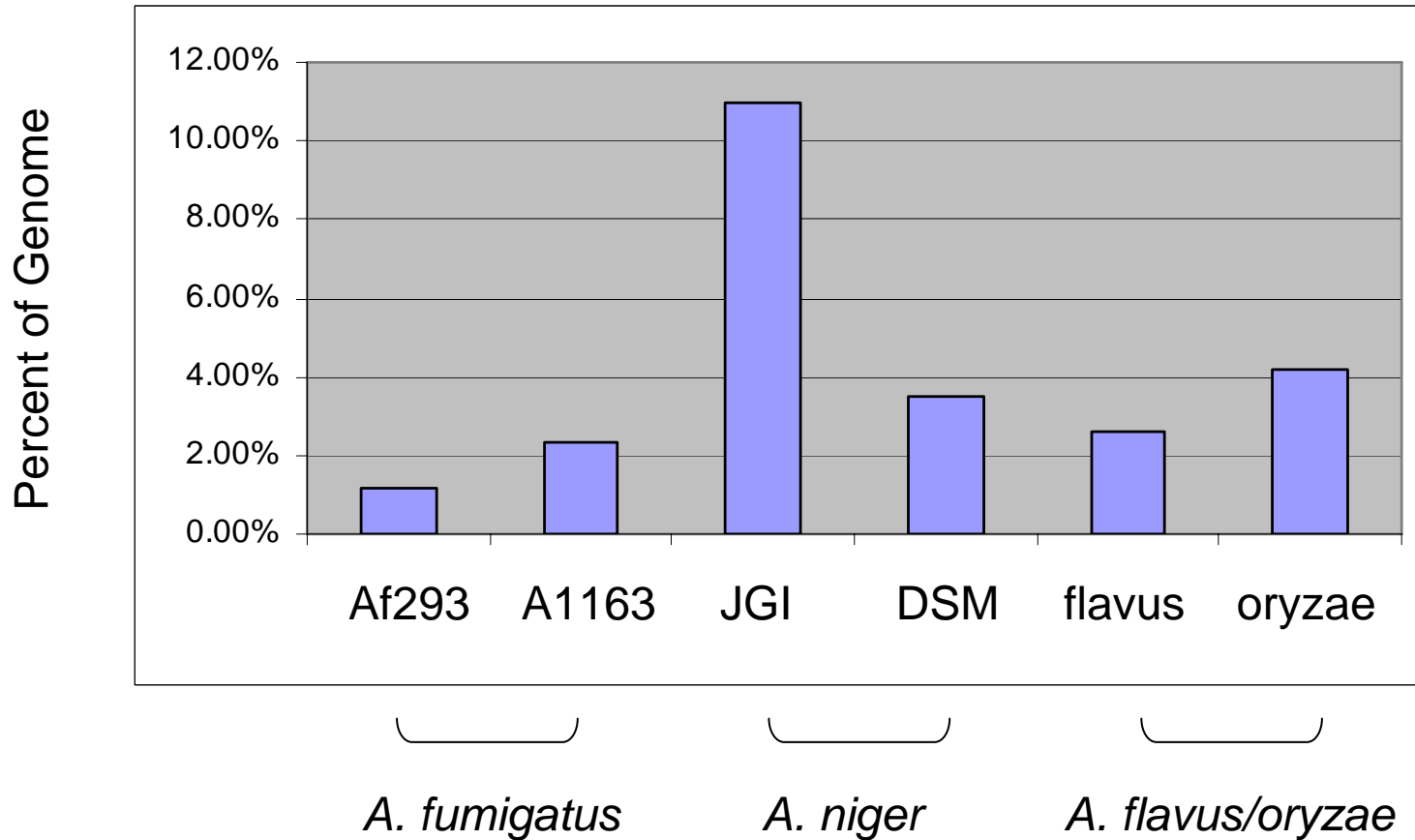




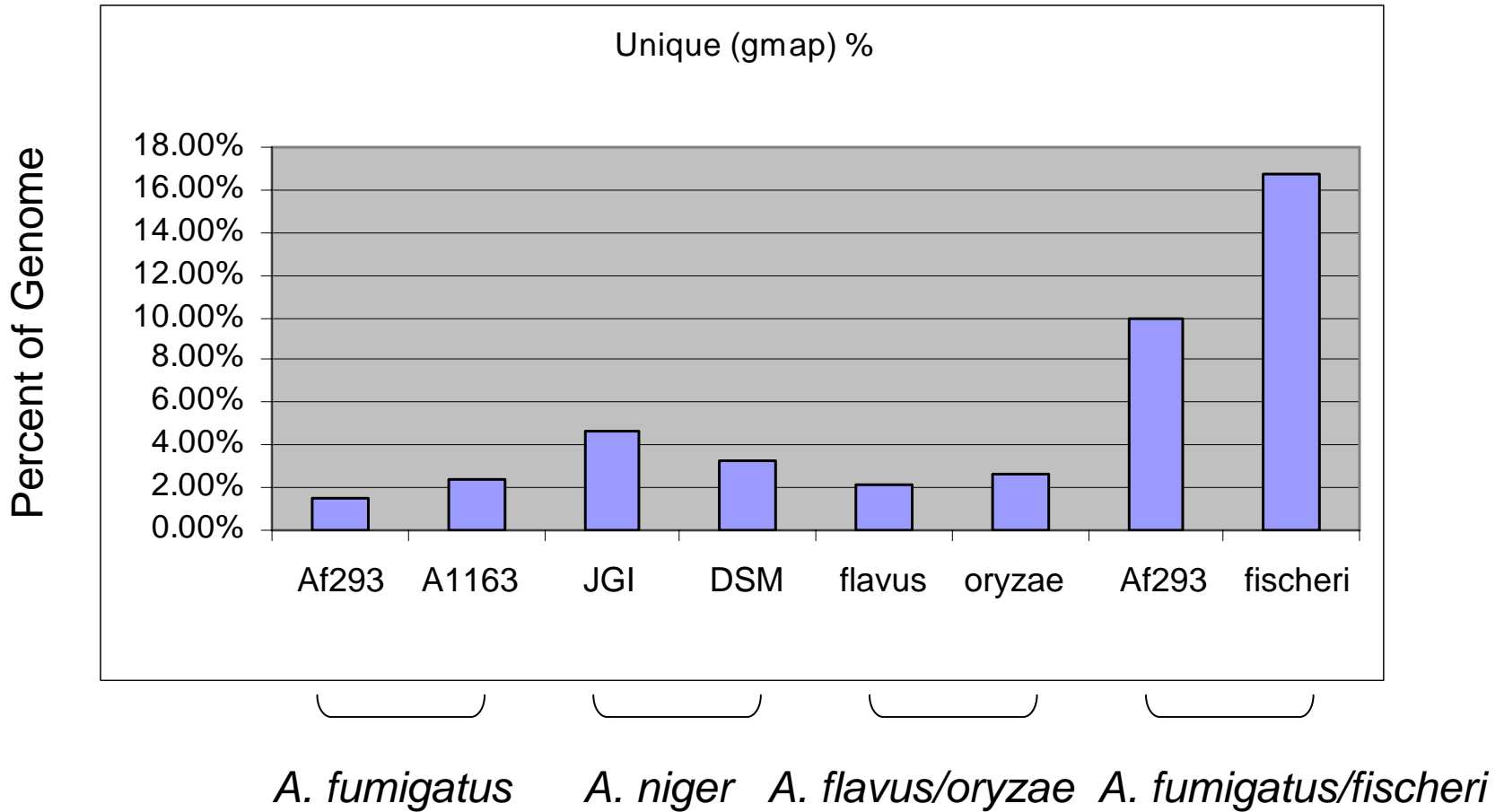
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Discontinuous Diversity: Isolate-specific Regions



Discontinuous Diversity: Isolate-specific Genes



Biological Roles of *A. fumigatus* Af293-specific Genes

- Unknown
- Metabolism
- Secondary metabolism (2 clusters)
- Transport



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Heterokaryon (Vegetative) Incompatibility

- Programmed cell death reaction in filamentous fungi
- Occurs during hyphal fusion between 2 genetically incompatible individuals from different VCGs
- Controlled by highly polymorphic *het* loci
- Characterized by trans-species polymorphism

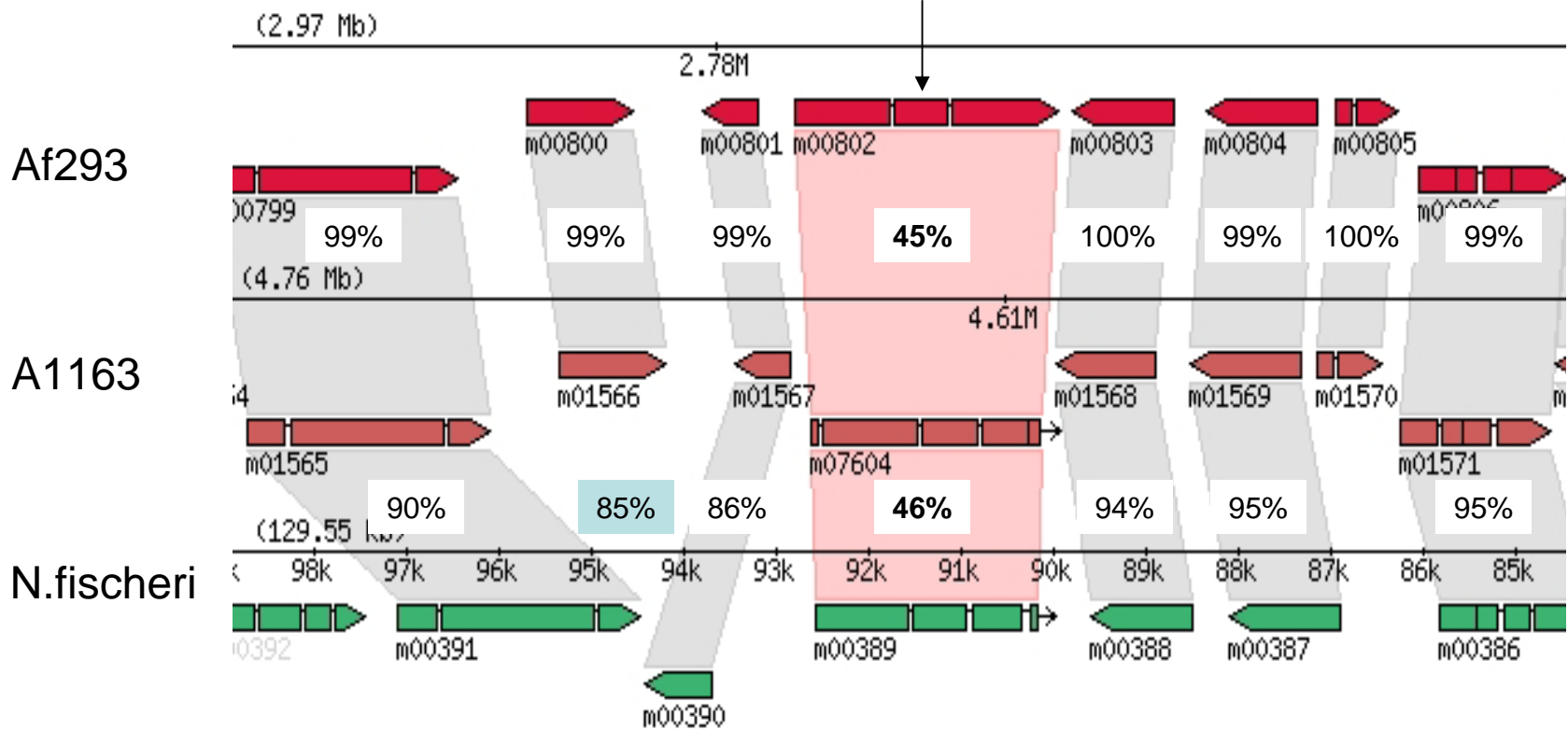
Highly Divergent Genes

Af293	Af294	Af71	A1163	Protein name	aCGH
AFUA_2G00910	SHARED	SHARED	DIVERGENT	Pfs, STAND and TPR domain protein	
AFUA_2G17420	ABSENT	SHARED	DIVERGENT	Pfs and STAND domain protein	
AFUA_7G08500	ABSENT	DIVERGENT	DIVERGENT	STAND and WD40 domain protein	
AFUA_6G07030	ABSENT	ABSENT	DIVERGENT	STAND and Ankyrin domain protein	
AFUA_6G07010	ABSENT	ABSENT	DIVERGENT	C6 transcription factor RosA	

AFUA_2G17420

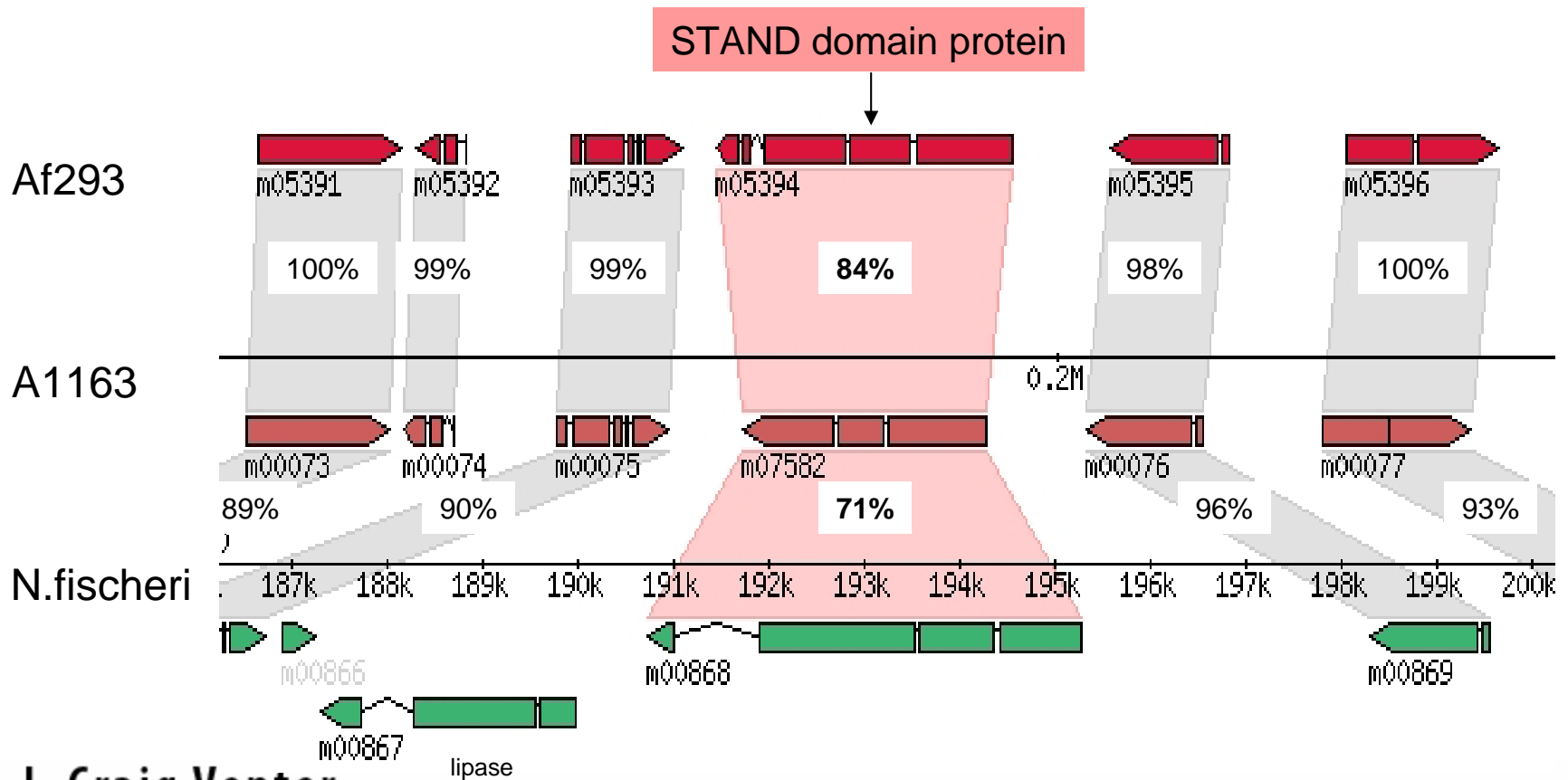
Genomic Neighborhood

STAND domain protein

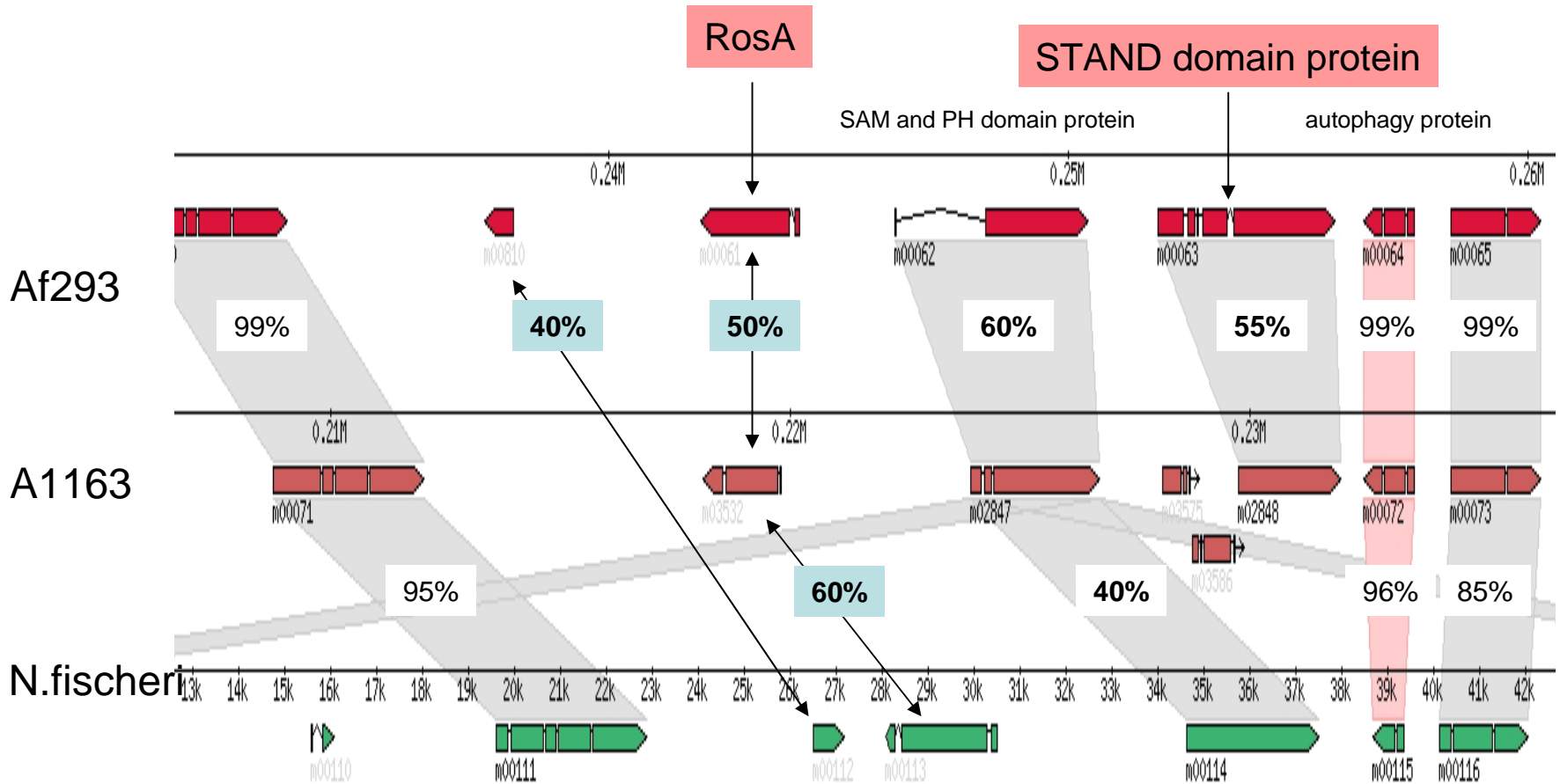


AFUA_2G00910

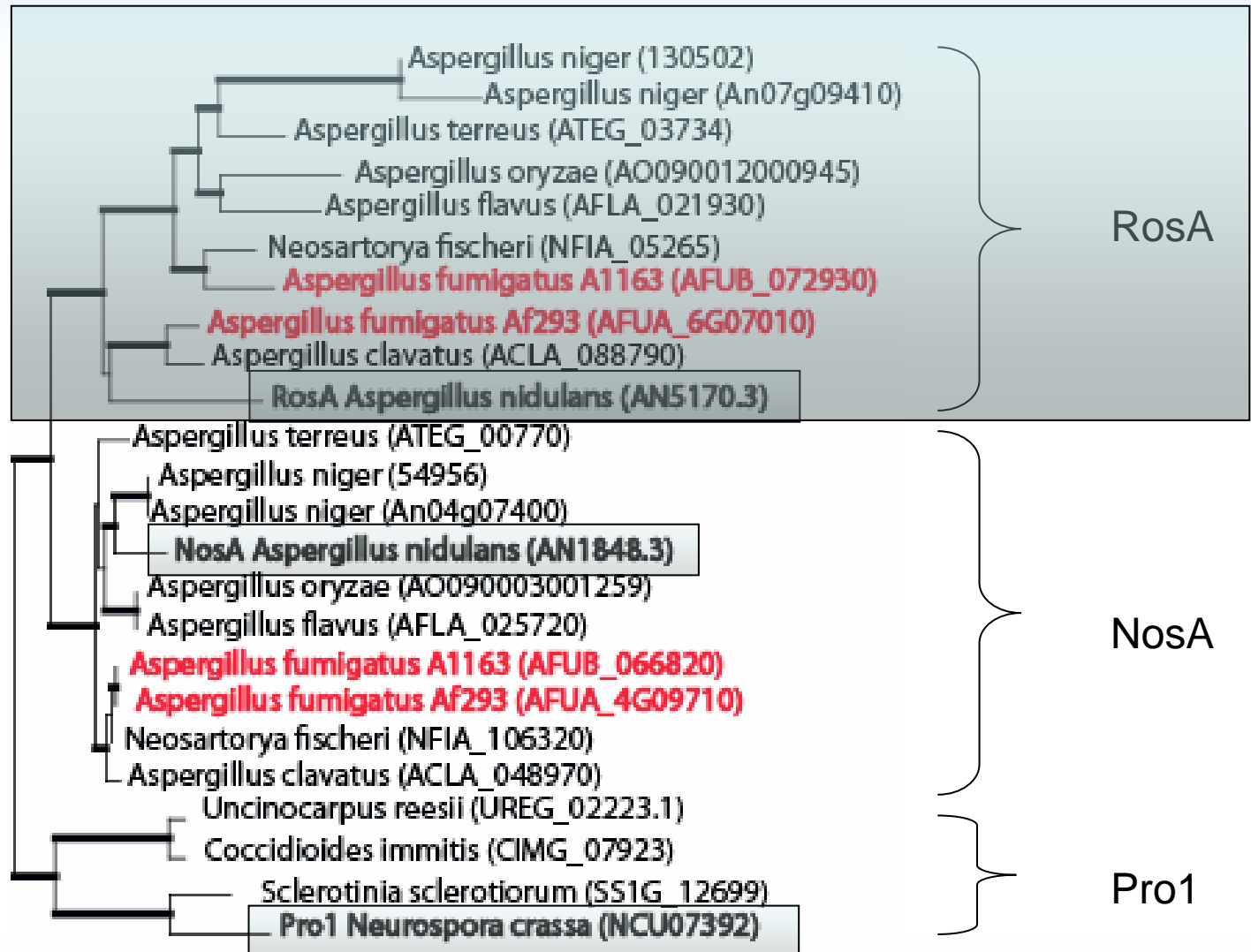
Genomic Neighborhood



AFUA_6G07030 and *rosA* Genomic Neighborhood



RosA/NosA Tree



Allelic Polymorphism among 22 *A. fumigatus* Isolates

Gene/ Isolate	AFUA_2G00910	AFUA_2G17420	RosA	Isolation site
WSA-419	1	1	1	Environmental, USA
WSA-446	1	1	1	Clinical, USA
WSA-621	1	1	1	Clinical, USA
WSA-622	1	2	1	Environmental, USA
Af293	2	2	1	Clinical, UK
A1163	1	1	2	Clinical, France
WSA-1195	1	1	2	Clinical, USA
WSA-270	1	1	2	Environmental, India
WSA-449	1	1	2	Clinical, USA
WSA-623	2	1	2	Clinical, USA
WSA-271	2	1	3	Environmental, USA

Different VCGs

The same VCG

Conclusions

- Isolate specific genes represent 2% of the *A. fumigatus* genome and ~60% genes contain SNPs
- Diversity within *A. fumigatus* (different VCGs) is similar to that within *A. niger* and *A. oryzae/flavus*
- Phenotypic differences can not be easily associated with either continuous or discontinuous divergence
- *A. fumigatus* has at least 3 *het* loci with 2 alleles
- CGH arrays must include the *A. fumigatus* “pan-genome” including all *het* alleles and unique genes

Acknowledgements



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NIAID

Thank you!

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