



# Subtelomeric Diversity as a Major Force in Evolution of *Aspergillus* Secondary Metabolism and Virulence Pathways

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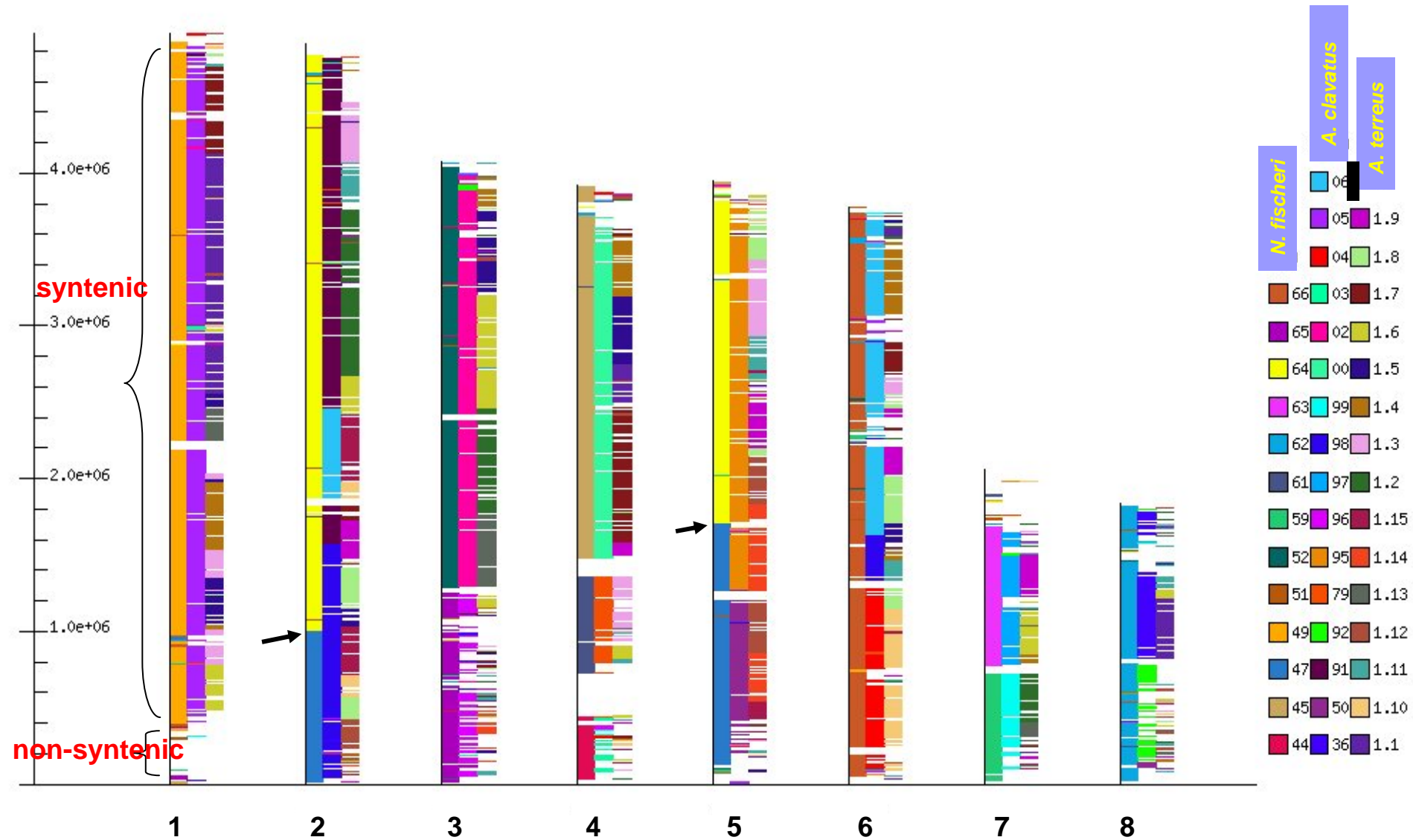
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**“We hold these truth to be self-evident..”**

- **That *A. fumigatus* and others evolved to be opportunistic pathogens of humans with compromised immune systems in the soil environment.**
- **That life in the soil as a grasseater (Latge) is no picnic.**  
*Adapted from Thomas Hobbes*
- **That secondary metabolites comprise a consequential component of their armamentarium for competitive advantage in the environment and for survival in the mammalian host.**

# *A. fumigatus* Whole Genome Alignment



# SM Biosynthesis Pathways (SMPs)

- “**Backbone**” enzymes (catalyze the first step):
  - Nonribosomal peptide synthases (NRPSs)
  - Polyketide synthases (PKSs)
  - Hybrid NRPS-PKS enzymes
  - Dimethylallyltryptophan synthase (DMATs)
  - **Terpene cyclases (TC)**
- “**Decorating**” enzymes (catalyze subsequent steps):
  - Cytochrome P450 oxydases
  - Methyltransferases
- **Transporters**
  - ABC-type
  - MSF-type
- **Transcriptional regulators**
  - Zn2C6-type
  - Global regulators (LaeA)

# The *A. fumigatus* gliotoxin cluster



- |  |                             |                      |
|--|-----------------------------|----------------------|
| ■ Peptide synthetase                     | ■ O-methyl transferase      | ■ Methyl transferase |
| ■ Thioredoxin reductase                  | ■ Glutathione S transferase | ■ Zinc Finger        |
| ■ Cytochrome P450 monooxygenase          | ■ Dipeptidase               | ■ Transporter        |
| ■ Aminocyclopropane carboxylate synthase |                             | ■ Other              |

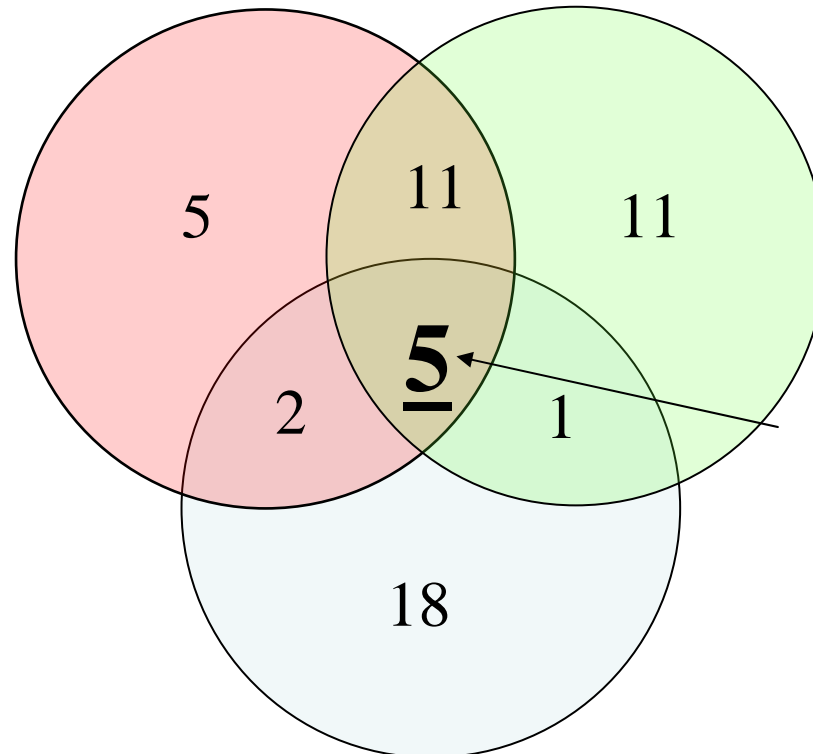
# Core and Species-specific Secondary Metabolite Biosynthesis Gene Clusters

*A. fumigatus* Af293

23 clusters

*N. fischeri*

29 clusters



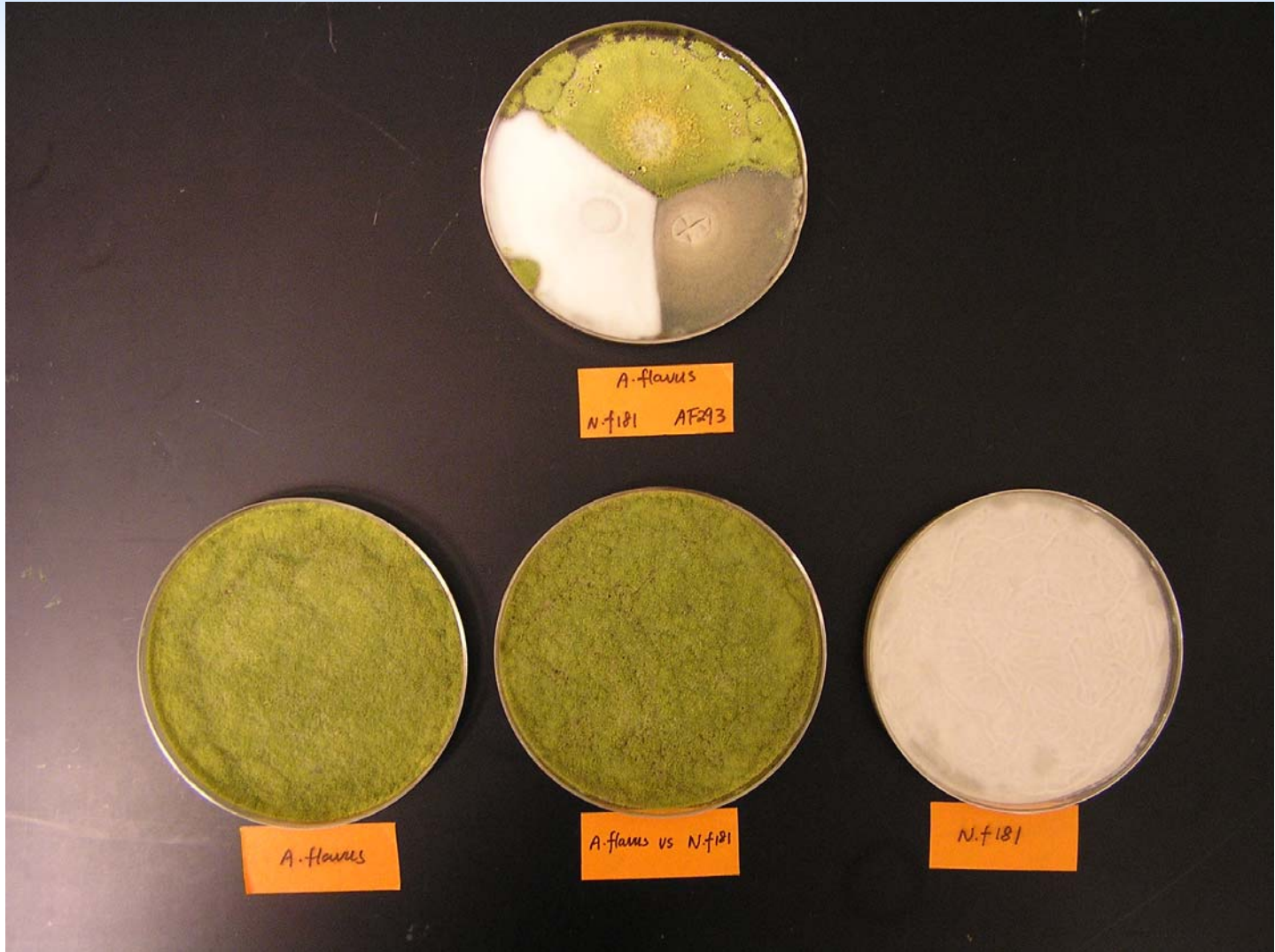
**Core clusters:**  
Pes1-containing  
spore pigment  
siderophore(s)  
## 5, 10

*A. clavatus*

26 clusters

# Af293 Clusters and Their Orthologs

Af293	CEA10	<i>N. fischeri</i>	<i>A.clavatus</i>	<i>A.terreus</i>	<i>A.oryzae</i>	<i>A.nidulans</i>
Pes1				*		
2						
pigment					*	*
fumigaclavine						
5						
6						
siderophore				*		
ETP toxin						
9						
10						
11						
12						
13						
14						
15						
16						
gliotoxin						
18						
19						
20						
fumitremorgin						
22						
pseurotin ?						



A. flavus  
N.f181 AF293

A. flavus

A. flavus vs N.f181

N.f181

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# HPLC Analysis of Extracts

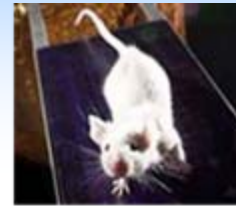
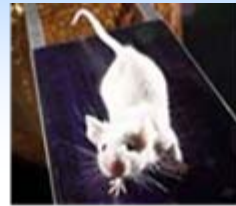
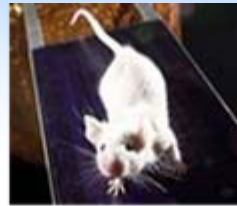
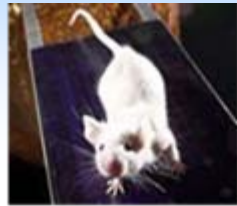
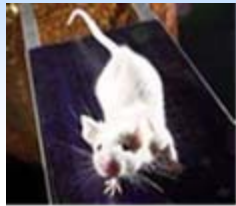
Jens Frisvad

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- *A. clavatus* at 30°C – Orlandin and Kotanin.
- *A. clavatus-A. fumigatus* at 30°C – Cytochalasin E and Cytocalasin K.
- *A. clavatus-B. thailandensis* at 30°C – Cytochalasin E, Cytocalasin K, Antafumicin, Antafumicin derivative, Unkown Indol.
  
- *A. flavus* at 30°C - 5 products
- *A. flavus-A. fumigatus* at 30°C – same 5 products plus Kojic Acid
  
- *A. fumigatus-A. flavus* at 37°C - Only epi detected
- *A. fumigatus* at 37°C - Nine products detected
- *A. flavus* at 37°C - Four products detected including two aflatoxins and epi

# *In Vivo* Expression Profiling

- Association with *in vitro* profiles
- Association with lineage specific genomic regions (A. fumigatus is environmentally adapted)



24 mice

24 mice

24 mice

24 mice

24 mice



Infect with  $10^8$   
*A. fumigatus* spores

Sacrifice 12 – 14 hours  
post infection

Bronchoalveolar lavage  
Snap freeze.

RNA extraction  
Quantification

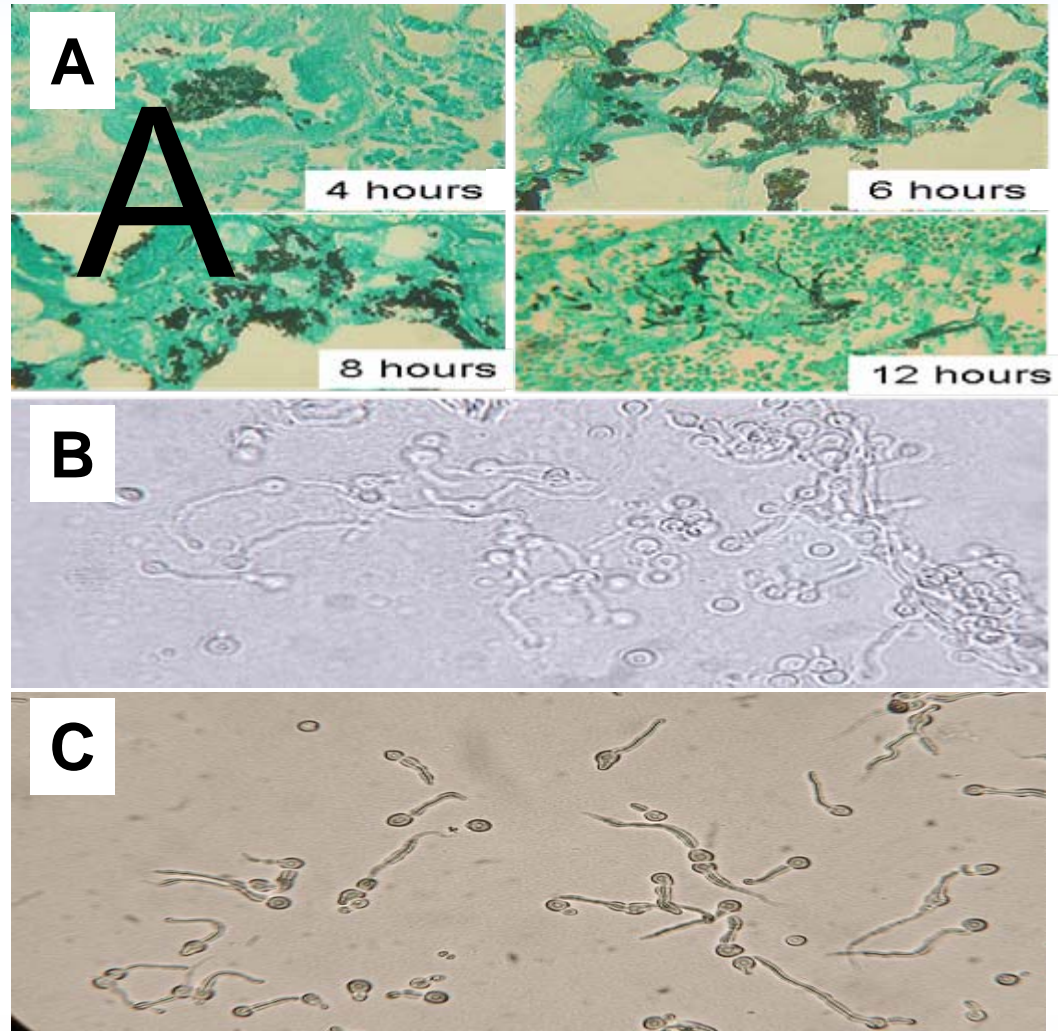
# RNA Amplification

- RNA yields:
  - Unamplified 108 – 800 ng Total RNA
  - Round 1 amplification: 3.7 – 19.3  $\mu\text{g}$
  - Round 2 amplification: 172.3 – 258.4  $\mu\text{g}$
- Amplification factor:  $4 \times 10^5$  –  $3 \times 10^8$

# Amplification Validation

- Three Hybridization Reactions
  - T0Tot vs T60Tot
  - T0aRNA1 vs T60aRNA1
  - T0aRNA2 vs T60aRNA2
- Treat pairs as replicates
  - Tot RNA – aRNA1 942 genes rejected (9.4%)
  - aRNA1 – aRNA2 14 genes rejected (0.14%)
  - TotRNA – aRNA2 1094 genes rejected (11%)

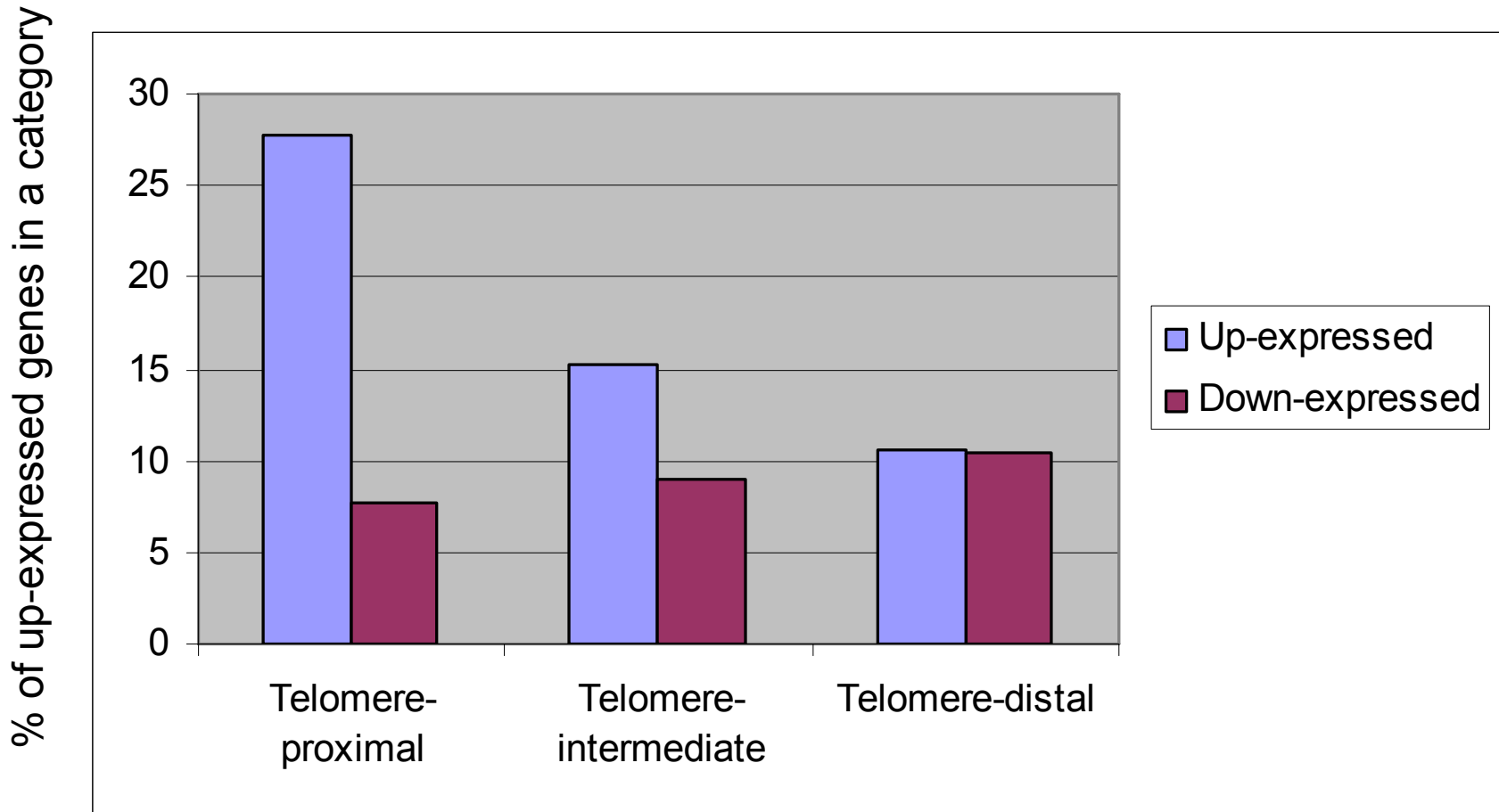
# Tissue and BALF Morphology



# Up-expressed Biological Processes

- Transport (amino acid, carbohydrate, endocytosis)
- Catabolism (amino acid, lipid, carbohydrate)
- Iron acquisition
- Transcriptional regulation
- Metabolism (C-N hydrolases, acyl-transferases, oxidoreductases)

# 30% of Subtelomeric Genes Are Up-expressed *In Vivo*



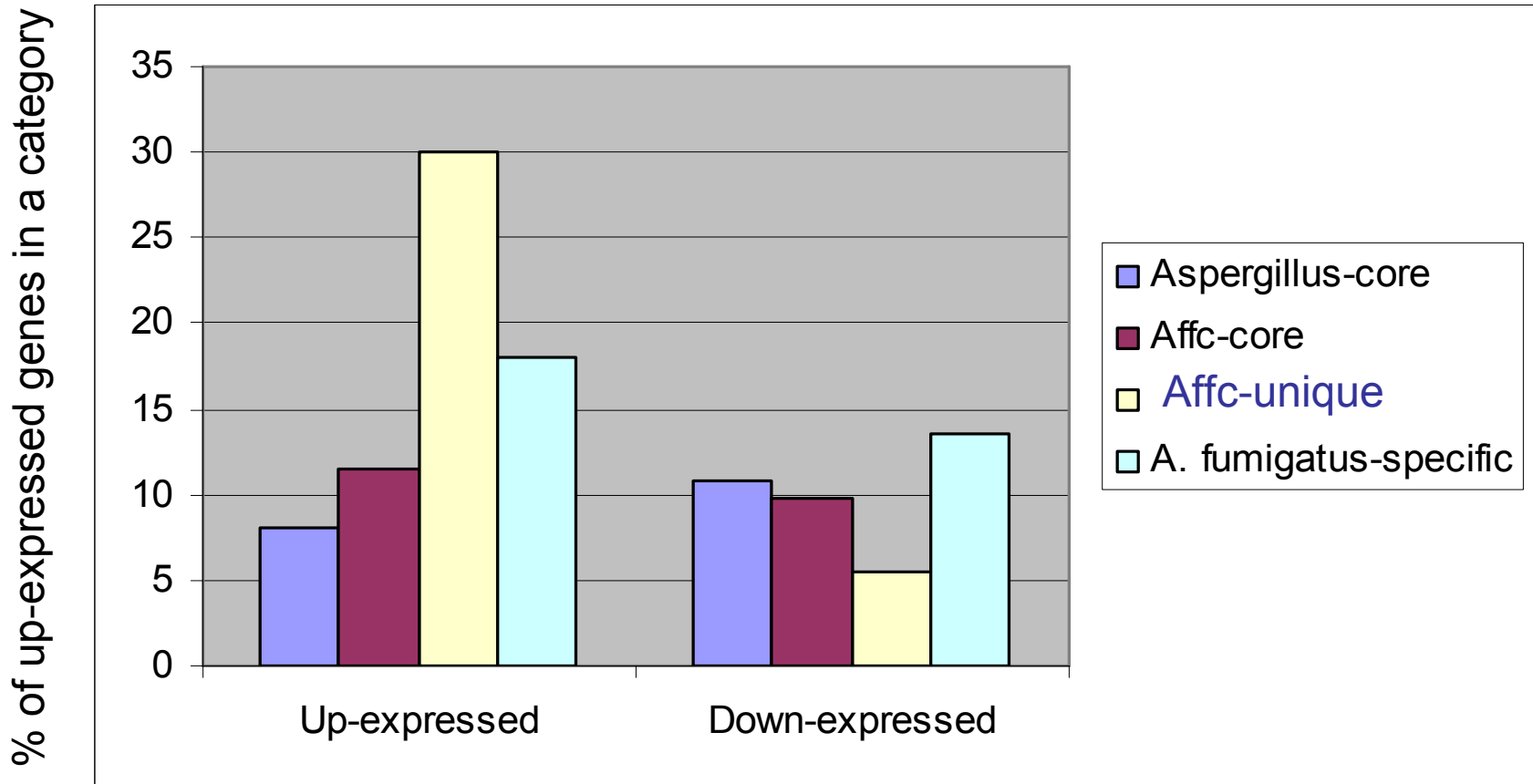
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Chromosomal coordinates

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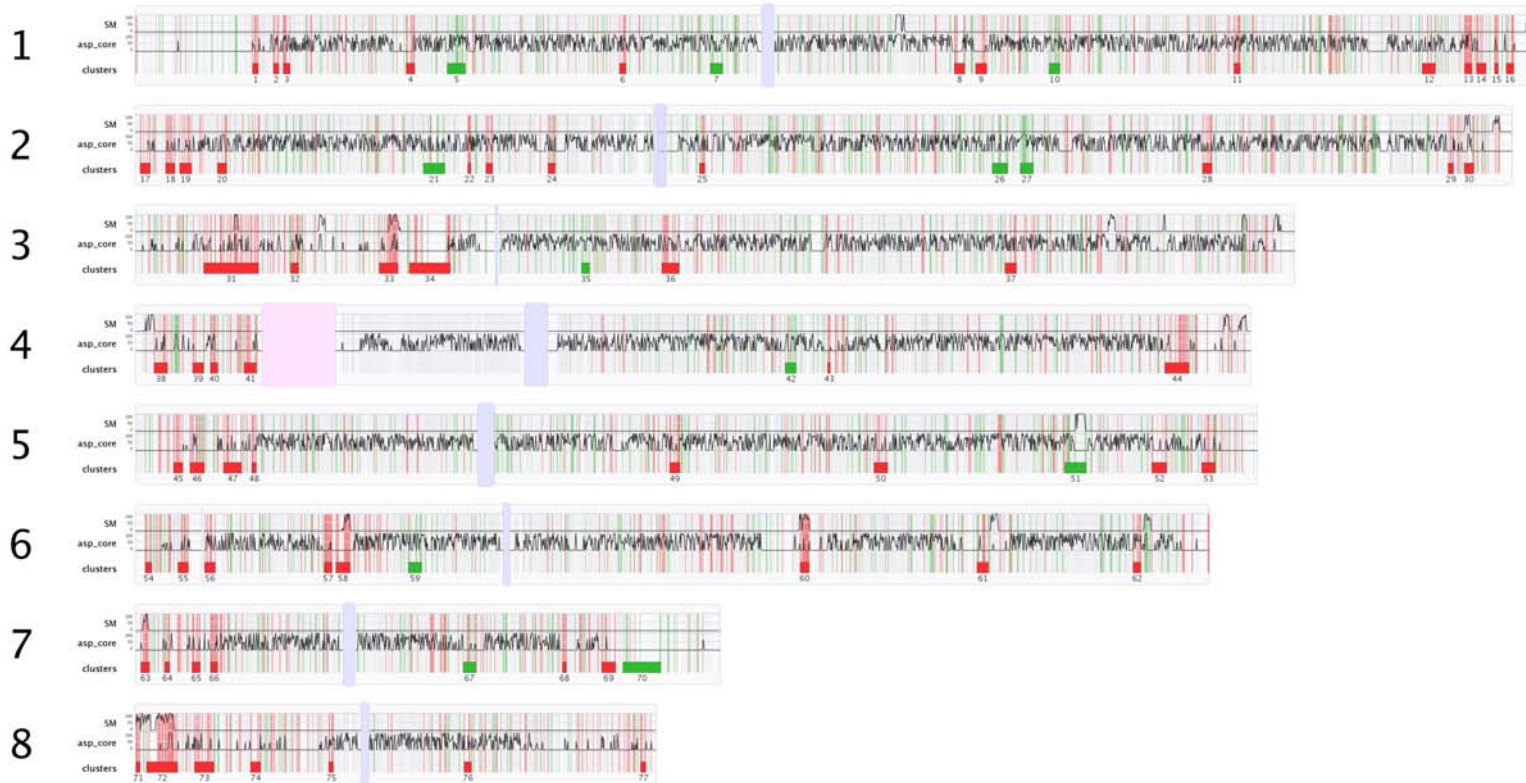
# 30% of Affc-Unique Genes Are Up-expressed *In Vivo*



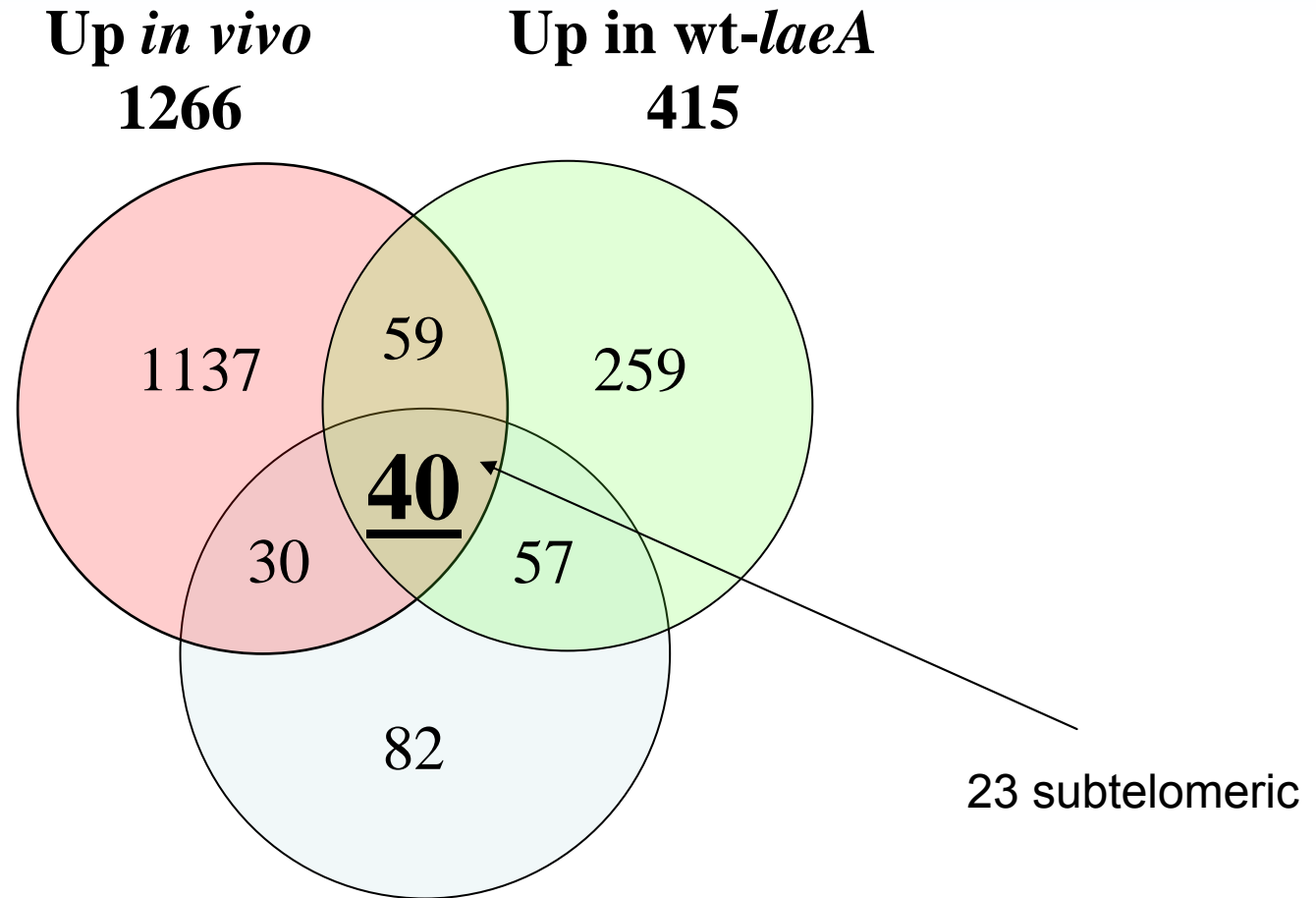
# *In Vivo* Up-expressed Genes Are Clustered on Chromosomes

- Most (51%) up-expressed genes are found in 68 contiguous gene clusters (5 - 30 genes)
- Including 7 secondary metabolite biosynthesis clusters (e.g. gliotoxin, siderophore) and 61 unknown metabolic clusters
- Almost 50% of the clusters are subtelomeric (within 300 Kb from telomeres)

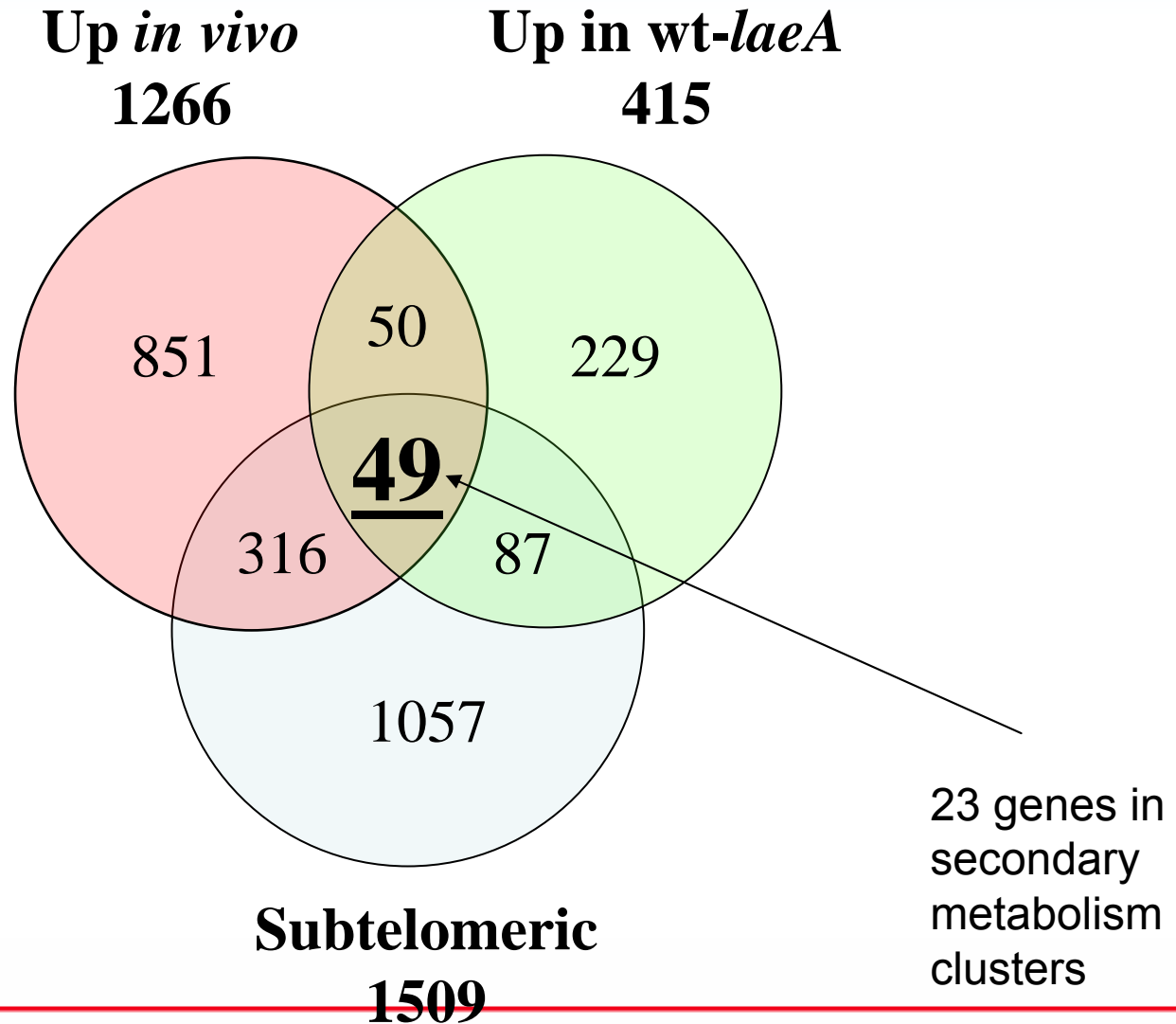
# A genome-wide transcriptional snapshot of *A. fumigatus* during initiation of murine infection



# Up-expressed *in vivo* and in wt *laeA*



# Up-expressed *in vivo* and in wt *laeA*



# Conclusions/Speculation

- RNA amplification allows for the microarray transcriptome analysis of trace amounts of RNA.
- Affc unique genes of the *A. fumigatus* genome appear to play a consequential role in mammalian host adaptation.
- Lineage specific clusters function as factories for new genes/roles.
- These genes evolved by selection in the environment and are secondarily adaptive for the host environment.

# The Secondary Metabolism Unique Regions Finder (SMURF) Web Site



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## SMURF - SECONDARY METABOLITE UNIQUE REGIONS FINDER

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NEW AND RETURNING USERS  
[CLICK HERE TO GET STARTED](#)

### About SMURF

Secondary Metabolite Unique Regions Finder is a web-based tool that finds secondary metabolite biosynthesis genes and pathways in fungal genomes. The predictions are based on [PFAM](#) and [TIGRFAM](#) domain content as well as on a gene's chromosomal position. [Precomputed results](#) for putative secondary metabolite biosynthesis clusters in sequenced fungal genomes are also available. The software is described in Nora Khaldi, Fayaz T. Seifuddin, Geoff Turner, Daniel Haft, Ken Wolfe, William C. Nierman, Natalie D. Fedorova, 2008 (in preparation).

### Sample Output

Example: *Aspergillus flavus*

[View backbone genes for \*Aspergillus flavus\*](#)

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