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USDA

Genotypic and Phenotypic Diversity Among a Familial Population of *Aspergillus flavus*

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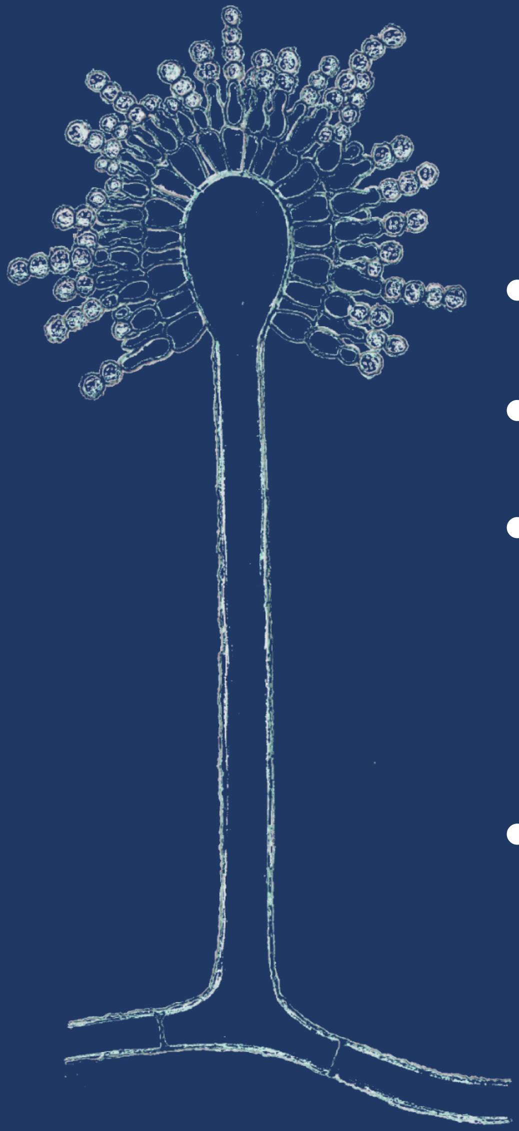
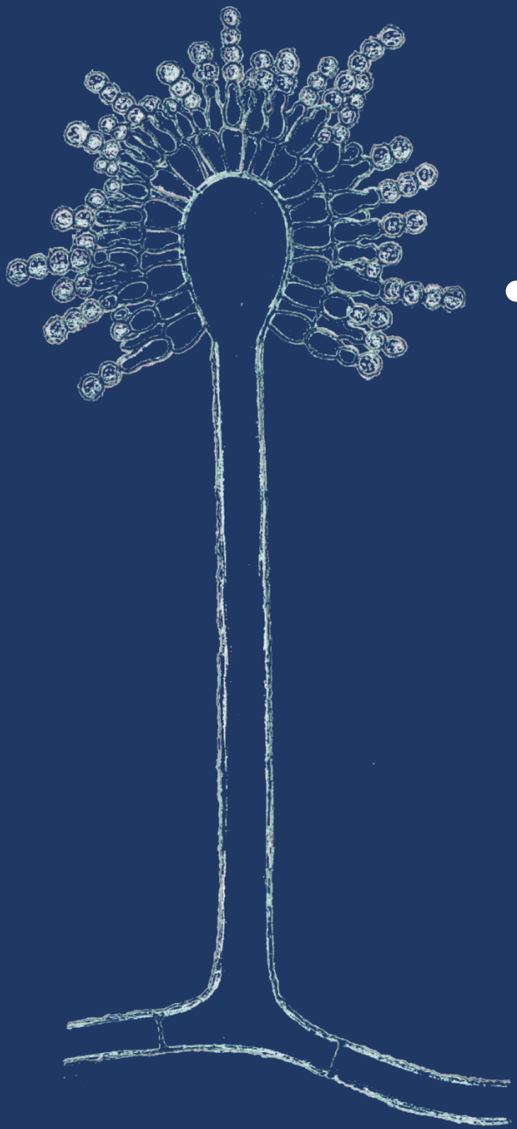


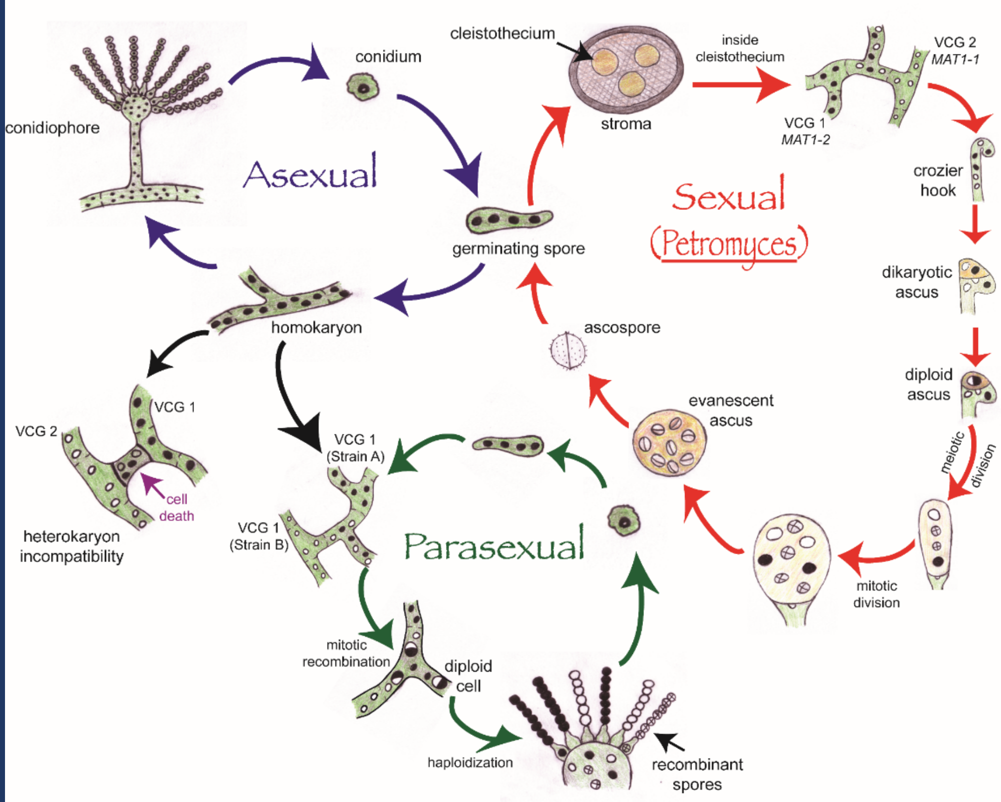
Aspergillus flavus is…

- A filamentous ascomycete fungus
- Ubiquitous in its existence
- A pathogen of plants, animals and humans
  - Mainly plants of agricultural importance
- A producer of potentially harmful mycotoxins
  - Most serious of its toxins is aflatoxin

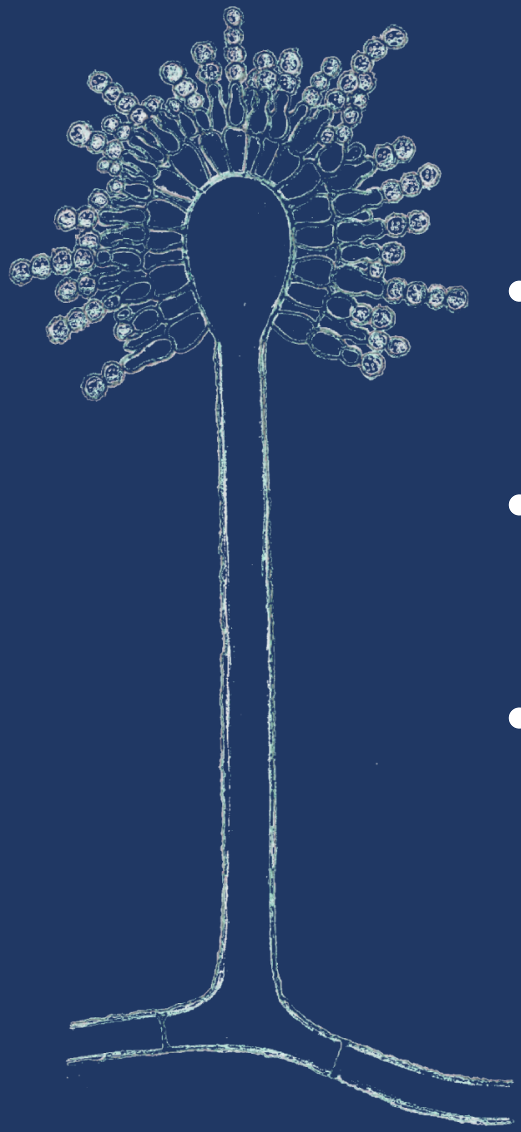
A. flavus has…



- Three modes of reproduction

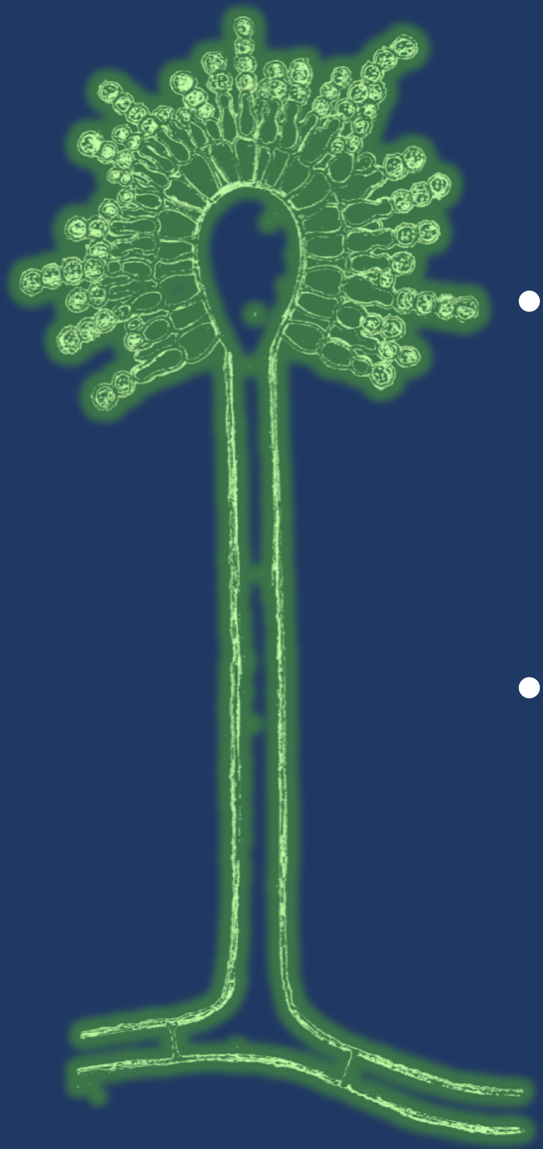


A. flavus undergoes recombination

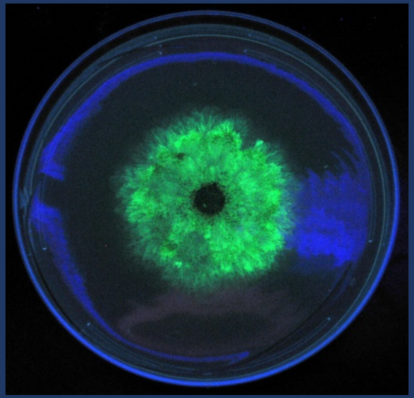


- An extensive history (Moore et al. 2009)
  - Evidence from the aflatoxin gene cluster
- Frequency is unknown
  - Detectable in a single generation?
- Extensiveness is unknown
  - Prevalence throughout the genome?

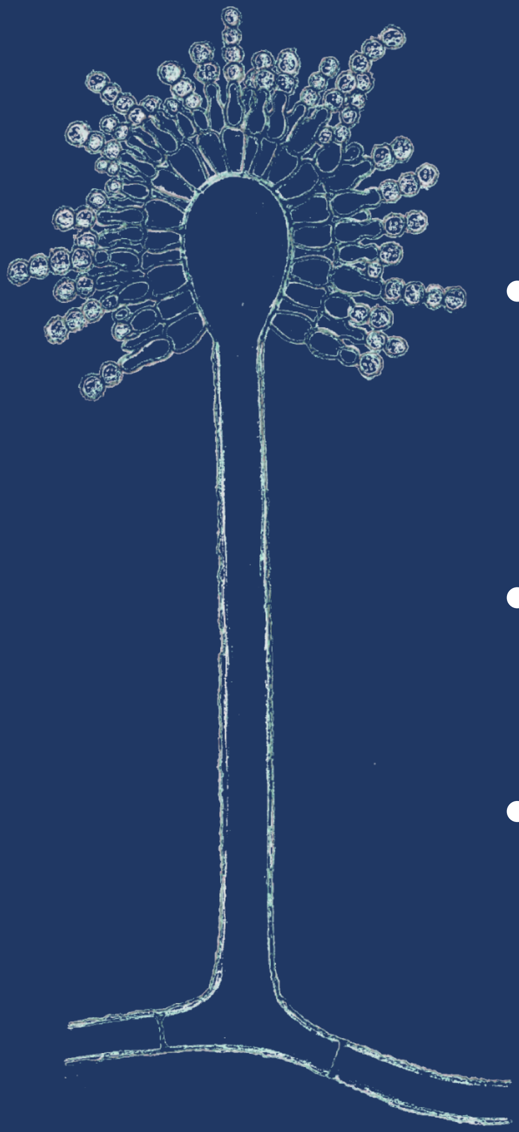
Mating Experiment

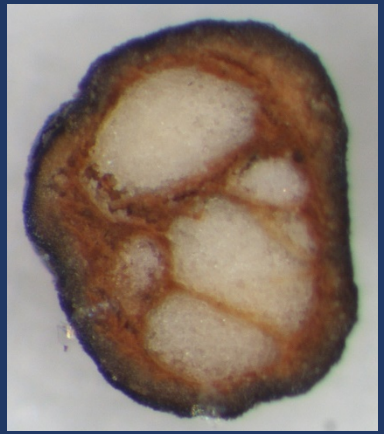


- *A. flavus* parental strains
  - *MAT1*-1 Parent = SRRC 1582 (AF+, CPA+, WT)
  - *MAT1*-2 Parent = K49G (AF-, CPA-, eGFP)
- Mixed spore suspensions on slants (Horn et al. 2009)
  - Mixed Grain Agar medium
  - Incubated 3 months in darkness at 30C

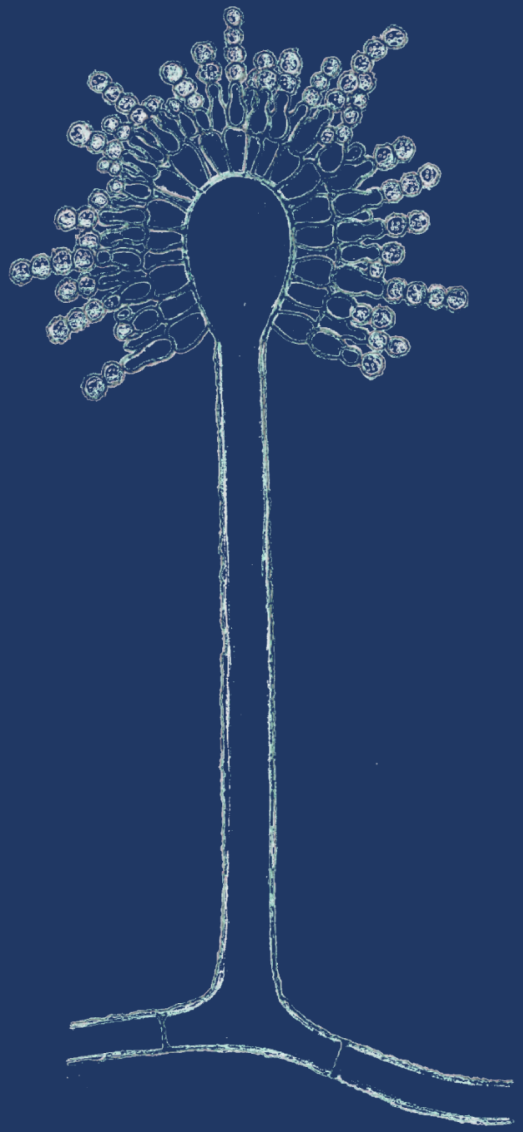


Selection of F1s

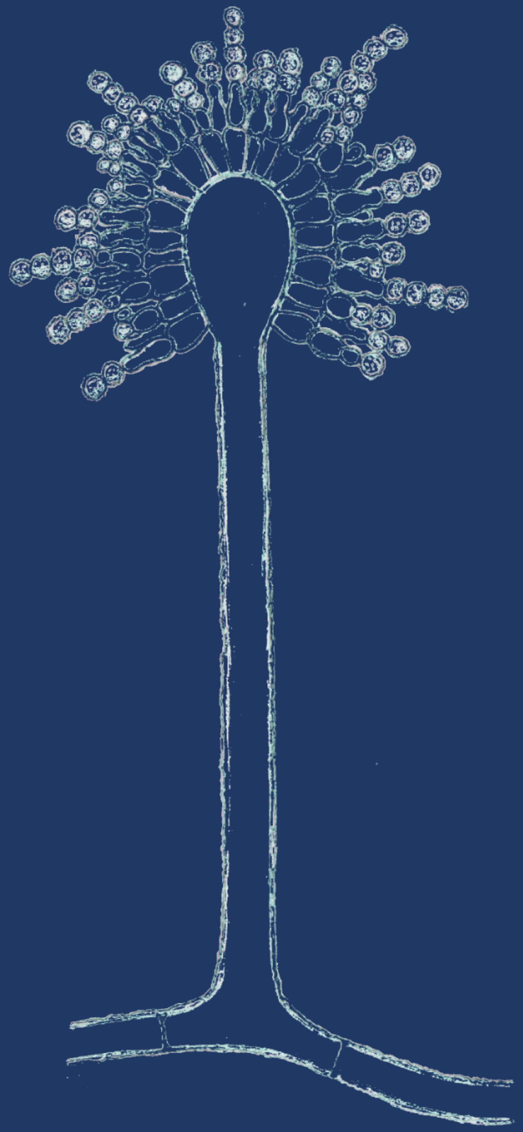




- Ascospores harvested and mixed
  - From different cleistothecia
  - From different stromata
- Suspension spread onto CZ plates
  - Incubated 24-48 hrs at 30C
- Hyphal-tipping for single-spore colonies on PDA
  - Five fluorescent progenies (O1-O5)
  - Five non-fluorescent progenies (O6-O10)



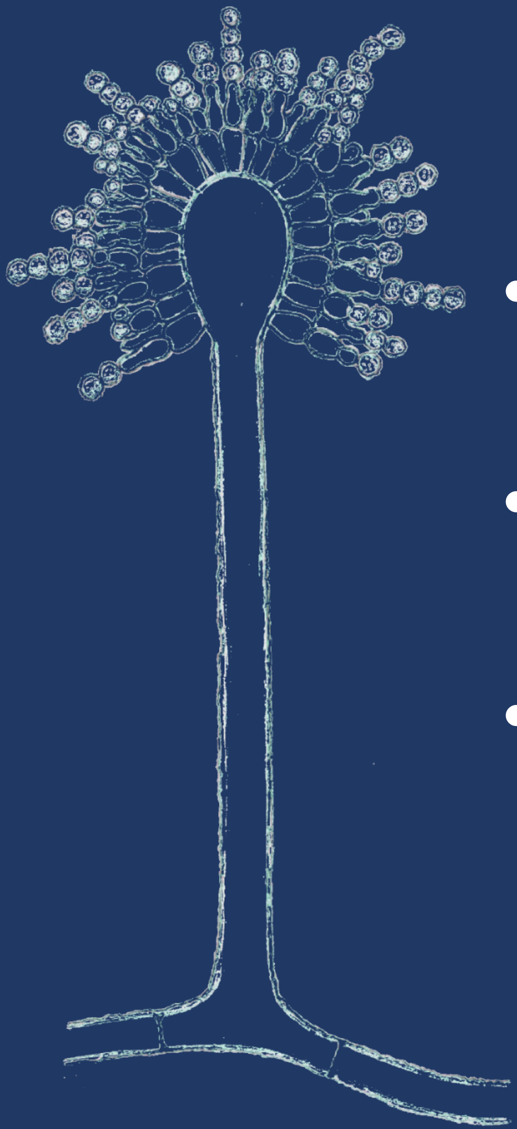
Preliminary Tests to Detect Evidence of Recombination



Are F1s clonal to either parent and/or siblings?

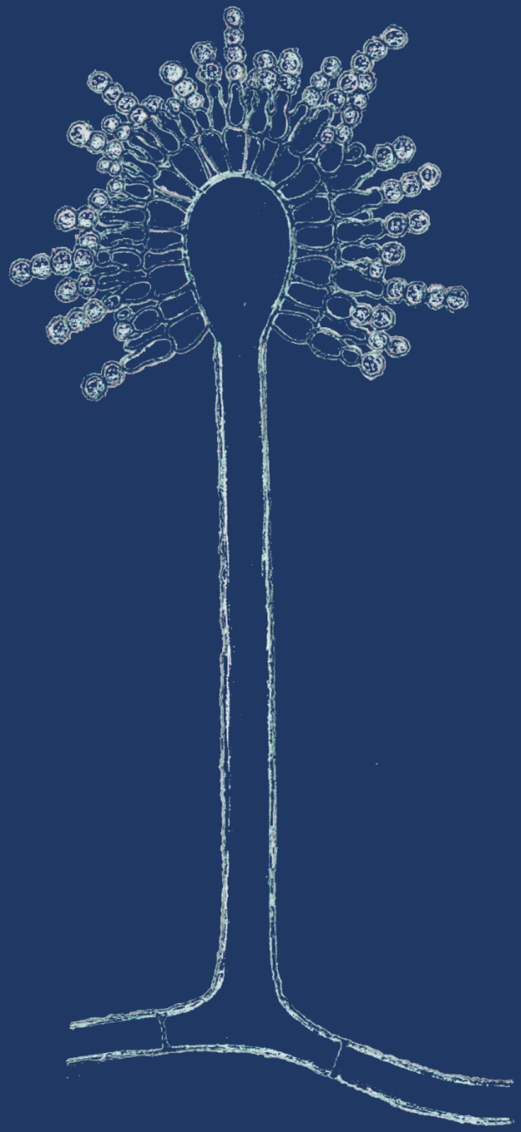
(Classical VCG Testing)

Findings: Classical VCG Testing



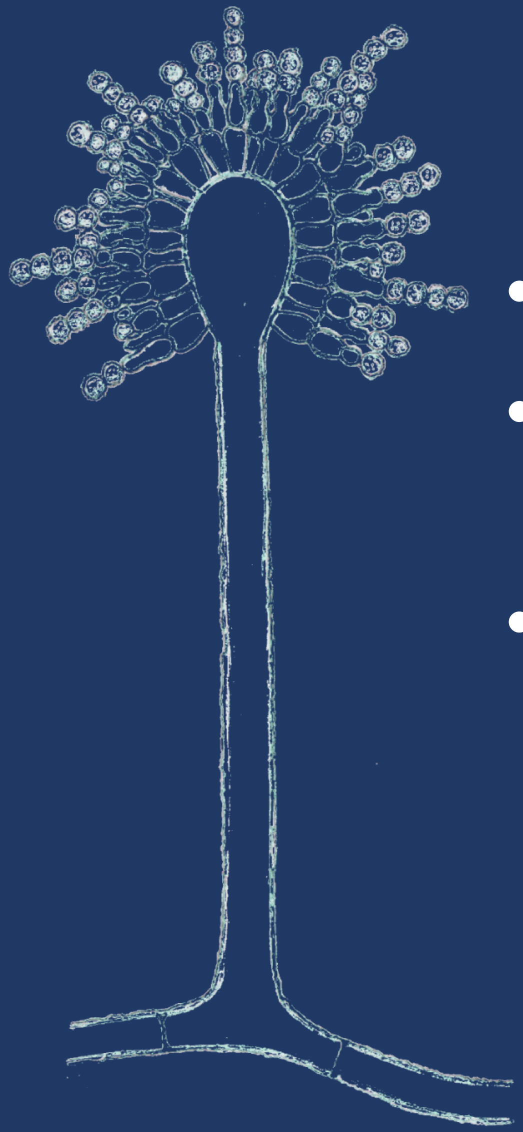
- Fluorescent F1s appear clonal to K49G parent 🟥
- Four of the F1s are unique VCGs
  - O7-O10
- Non-fluorescent F1s likely recombinant offspring 🟦

Strain	VCG
1582	A
K49G	B
O1	B
O2	B
O3	B
O4	B
O5	B
O6	B
O7	C
O8	D
O9	E
O10	F



What are the MAT genotypes for the 10 F1s?

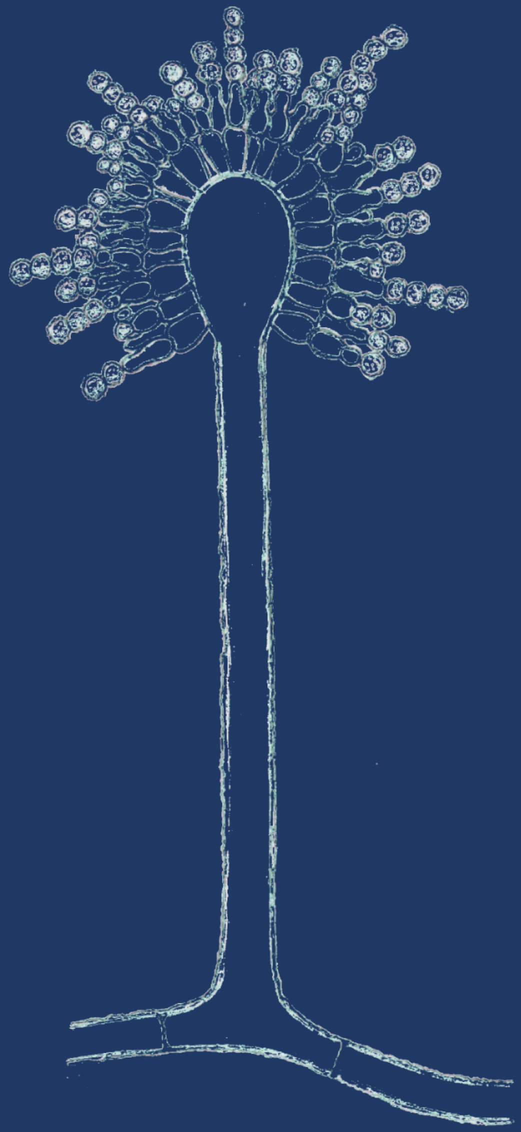
(Diagnostic PCR)



Findings: Diagnostic PCR

- All of the F1s were *MAT1*-2
- Fluorescent F1s appear clonal to K49G parent 🟥
- Non-fluorescent F1s likely recombinant offspring 🟦

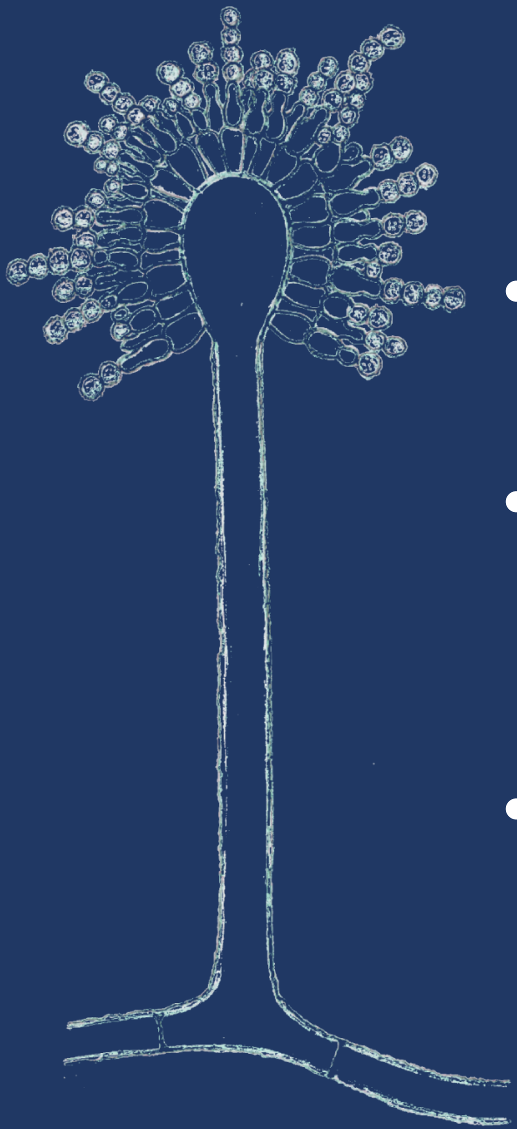
Strain	**MAT**
1582	1
K49G	2
O1	2
O2	2
O3	2
O4	2
O5	2
O6	2
O7	2
O8	2
O9	2
O10	2



Were there heritable chemotypes for the 10 F1s?

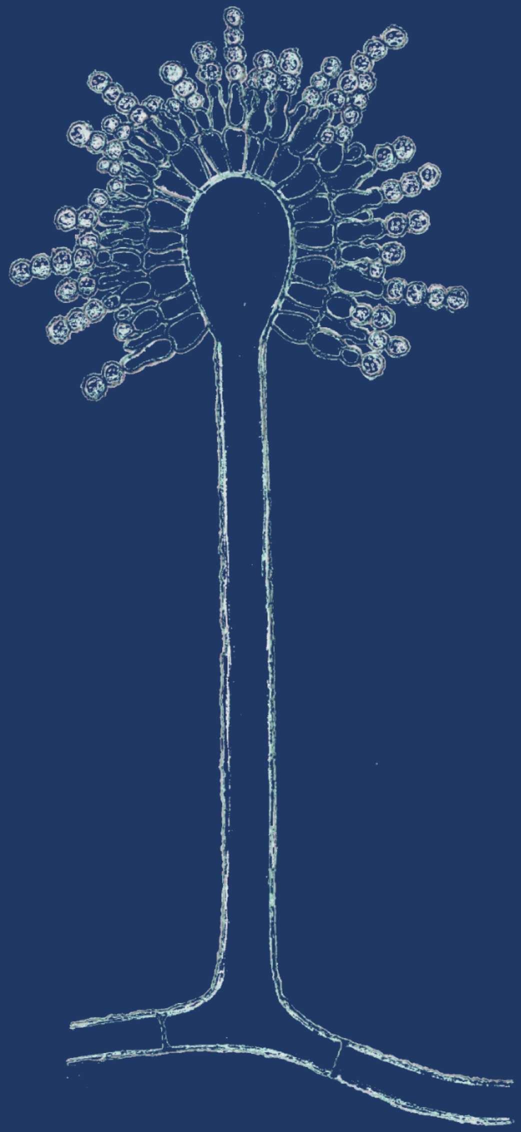
(UPLC Analysis)

Findings: UPLC Analysis

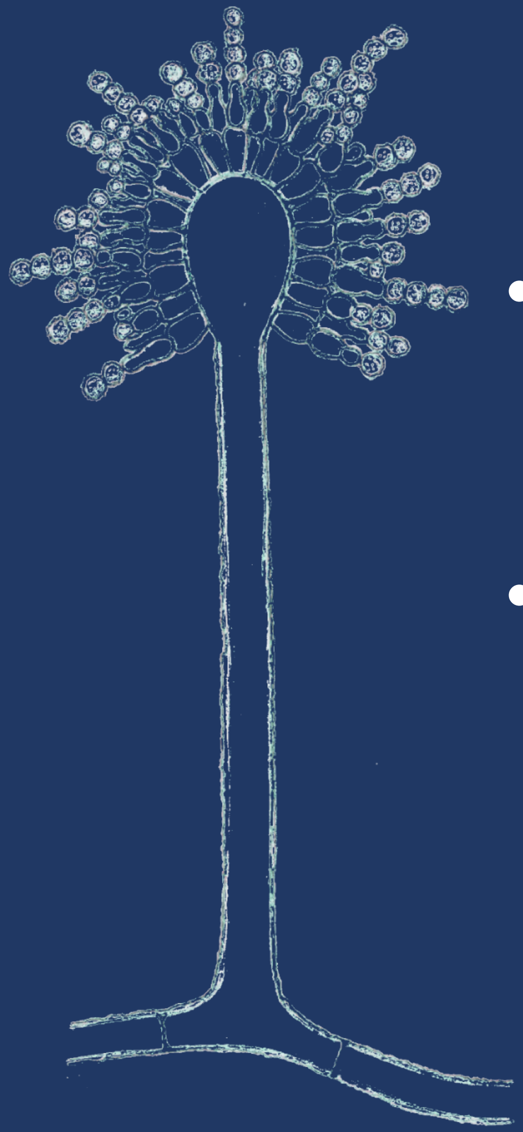


- Four fluorescent F1s appear clonal to K49G parent 🟥
- Two of the F1s were toxigenic
  - AF+/CPA-: O5
  - AF+/CPA+: O9
- Six of the F1s likely recombinant offspring 🟦

Strain	Tox
1582	AF+/CPA+
K49G	AF-/CPA-
O1	AF-/CPA-
O2	AF-/CPA-
O3	AF-/CPA-
O4	AF-/CPA-
O5	AF+/CPA-
O6	AF-/CPA-
O7	AF-/CPA-
O8	AF-/CPA-
O9	AF+/CPA+
O10	AF-/CPA-

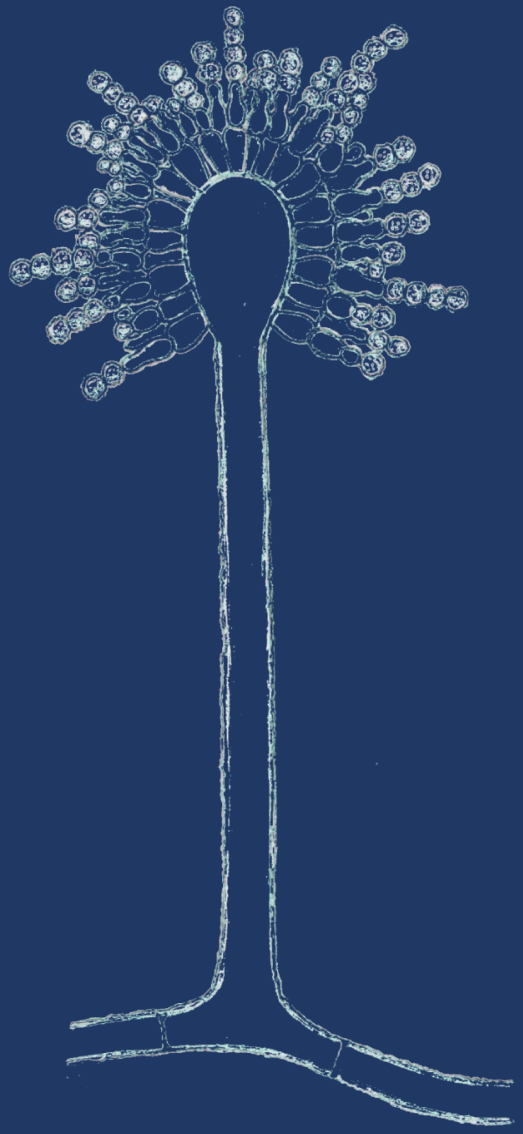


Investigating Recombination Via Crossover Events



Analysis: Recombination

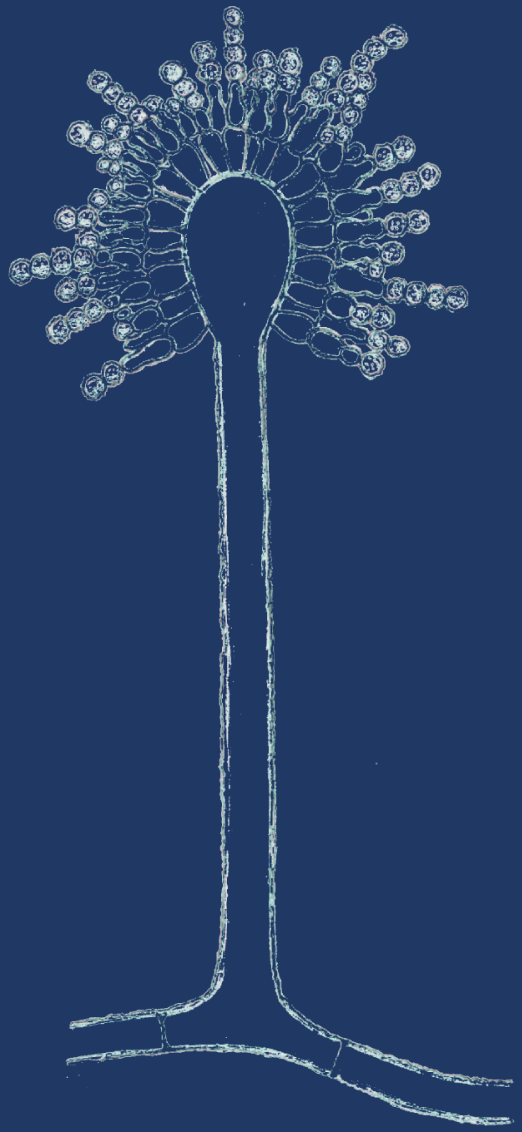
- Primer design, PCR amplification and sequencing of overlapping segments across the 75kb aflatoxin gene cluster (to start)
- Scan sequences for changes in SNP inheritance (i.e. crossover events)



Will I ever finish this recombination study???

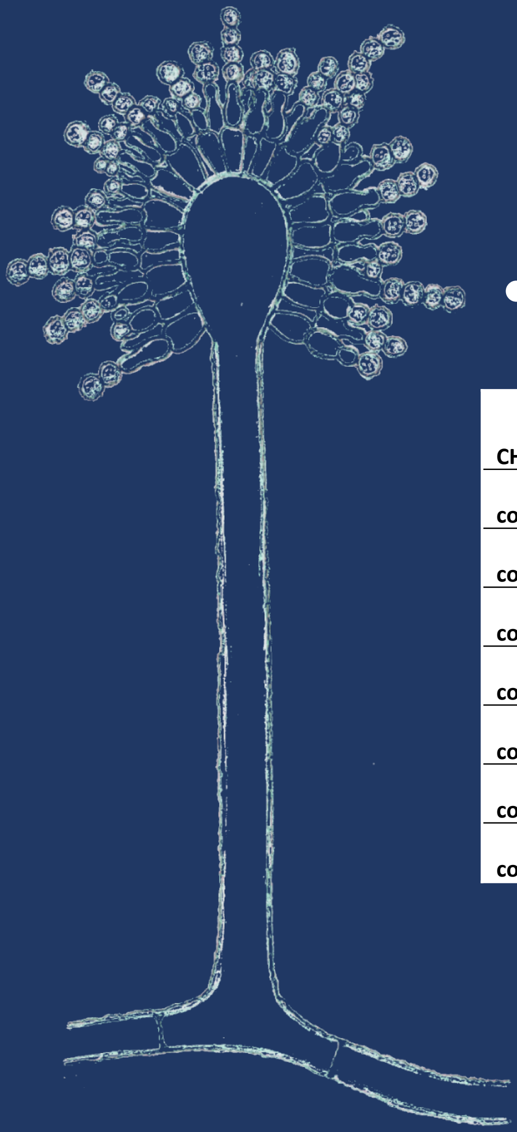
(Don’t Panic)

Whole-Genome Analyses for A Familial *A. flavus* Population



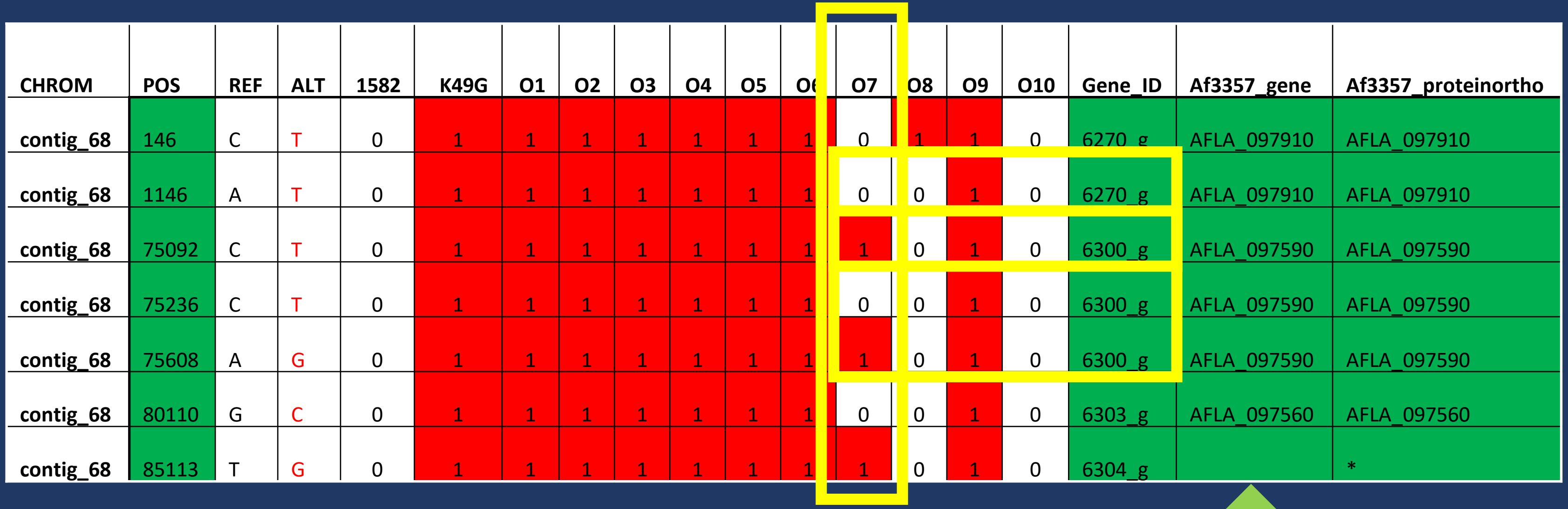
Is there genomic evidence of recombination?

(Variant Mapping)



Variant mapping shows crossover events

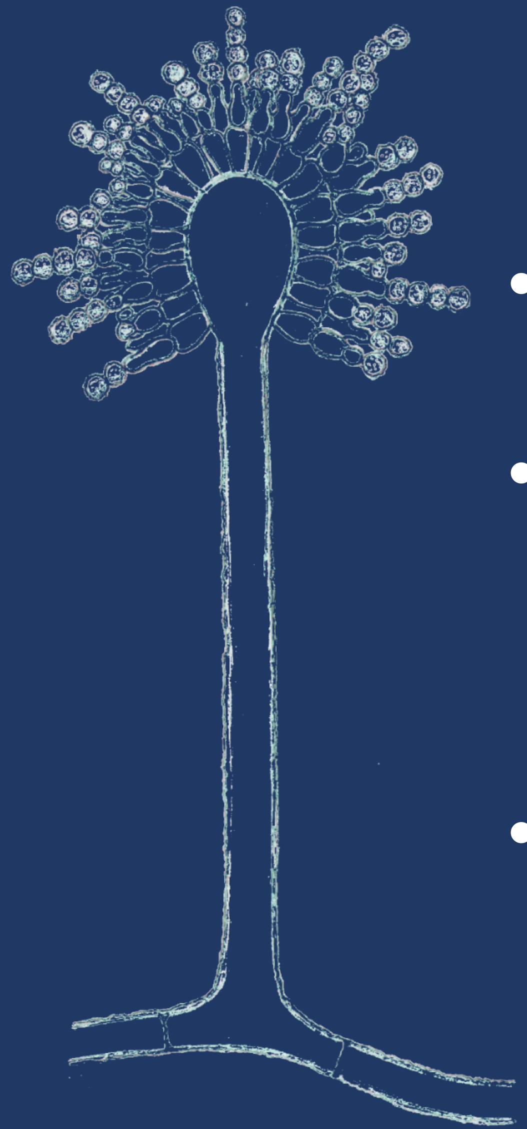
- Between genes and within genes



CHROM	POS	REF	ALT	1582	K49G	O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	Gene_ID	Af3357_gene	Af3357_proteinortho
contig_68	146	C	T	0	1	1	1	1	1	1	1	0	1	1	0	6270_g	AFLA_097910	AFLA_097910
contig_68	1146	A	T	0	1	1	1	1	1	1	1	0	0	1	0	6270_g	AFLA_097910	AFLA_097910
contig_68	75092	C	T	0	1	1	1	1	1	1	1	1	0	1	0	6300_g	AFLA_097590	AFLA_097590
contig_68	75236	C	T	0	1	1	1	1	1	1	1	0	0	1	0	6300_g	AFLA_097590	AFLA_097590
contig_68	75608	A	G	0	1	1	1	1	1	1	1	1	0	1	0	6300_g	AFLA_097590	AFLA_097590
contig_68	80110	G	C	0	1	1	1	1	1	1	1	0	0	1	0	6303_g	AFLA_097560	AFLA_097560
contig_68	85113	T	G	0	1	1	1	1	1	1	1	1	0	1	0	6304_g		*

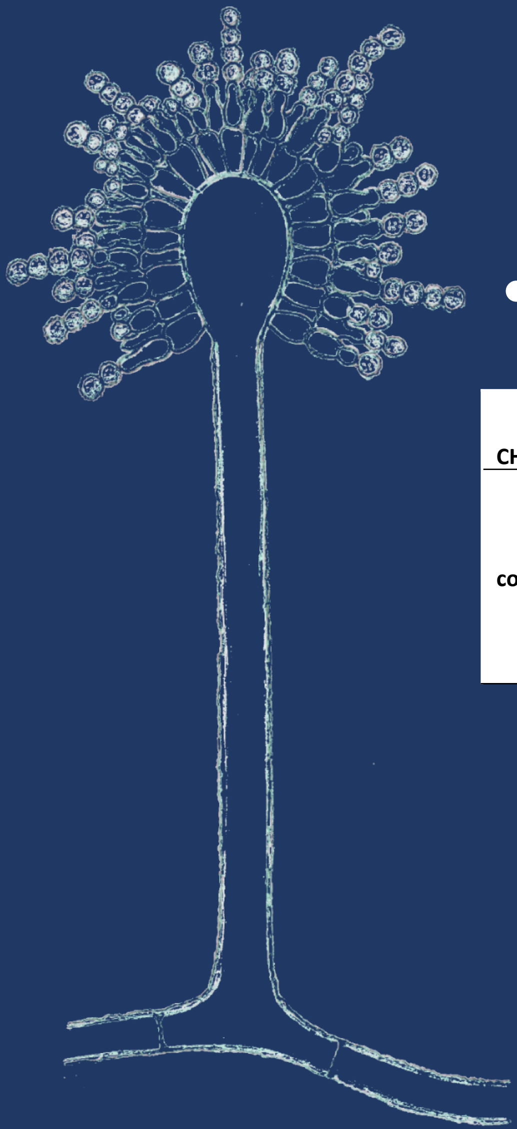
Hypothetical proteins
Chromosome 6

Variant mapping shows crossover events



- No fluorescent F1s exhibited obvious crossover events 🟥
- Four non-fluorescent F1s showed evidence of crossover events
  - O7-O10
- One non-fluorescent F1 exhibited no obvious crossover events 🟦

Strain	C.E.
1582	N/A
K49G	N/A
O1	0
O2	0
O3	0
O4	0
O5	0
O6	0
O7	29
O8	23
O9	26
O10	17

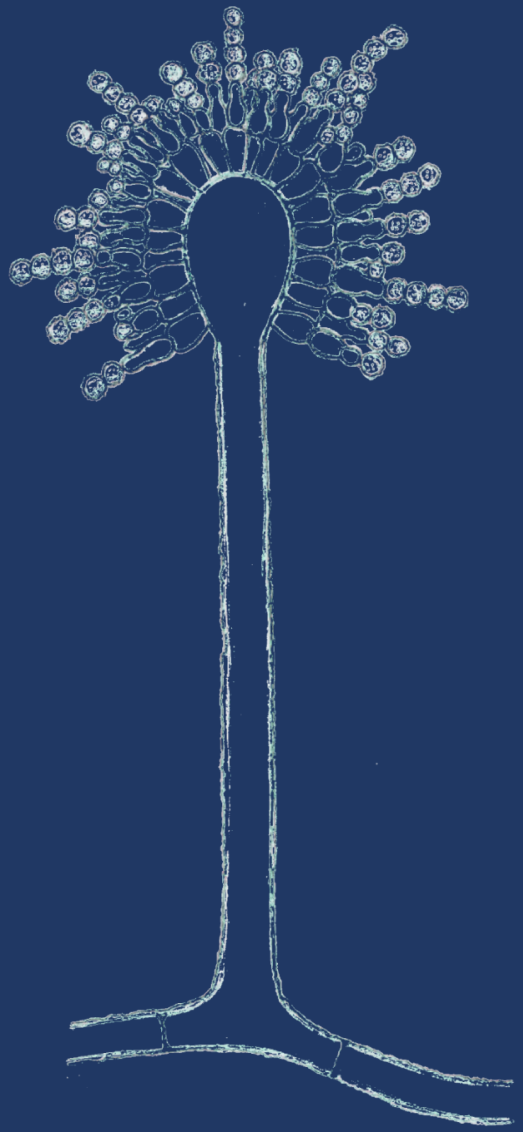


Variant mapping shows random mutations

- Rare events throughout the genome

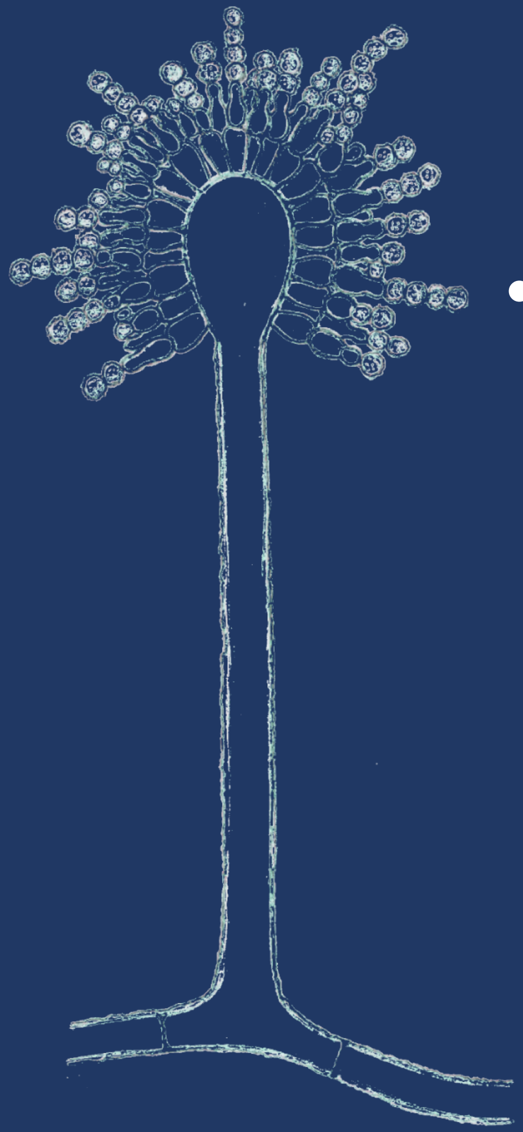
CHROM	POS	REF	ALT	1582	K49G	O1	O2	O3	O4	O5	O6	O7	O8	O9	O10	Gene_ID	Af3357_gene	Af3357_proteinortho
contig_106	59242	GTT CTT CTT CTT GTT CT	GT, CTTCTT CTTCTT GT, CTTCTT CTTCTT CTTCT	0	1	1	2	1	1	3	2	1	3	1	0	8264_g	AFLA_108120	AFLA_108120

2-5A-dependent ribonuclease, putative
Chromosome 3



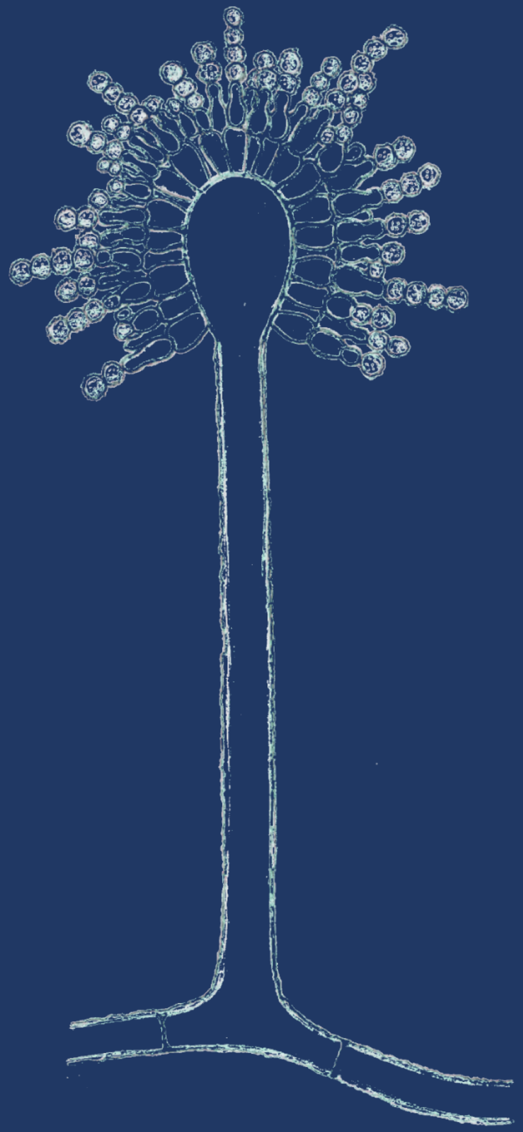
How many unique het genes = a new VCG?

(Variant Mapping)



Findings: het Loci

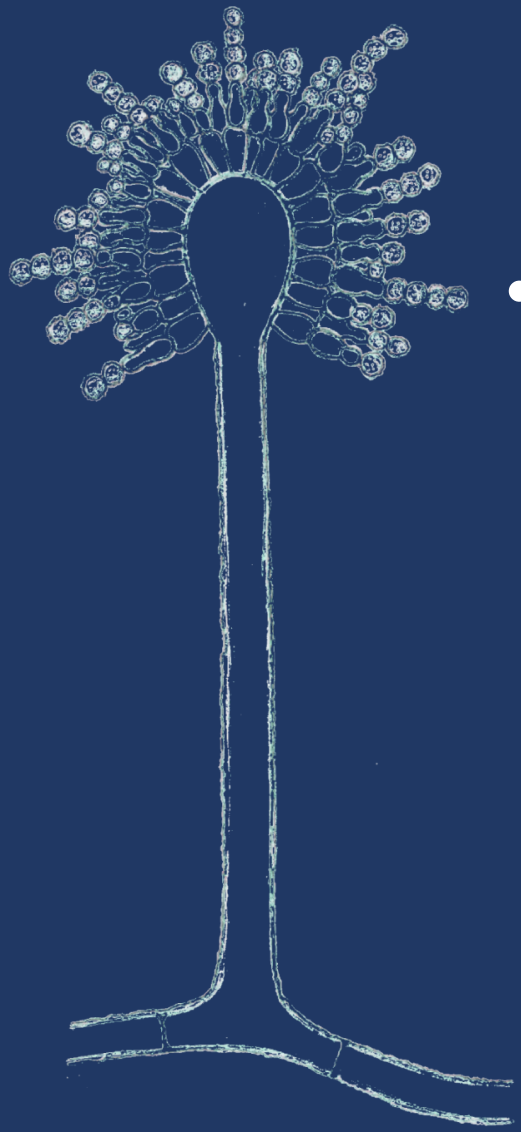
- May only take a single unique het locus to create heterokaryon incompatibility
  - 48 of 57 het loci had distinguishing variants
  - 42 of 48 loci had E-value < 1e-10
  - Numbers of het loci unique to each VCG:
    - A = 7; B = 1; C = 11; D = 7; E = 14; F = 2



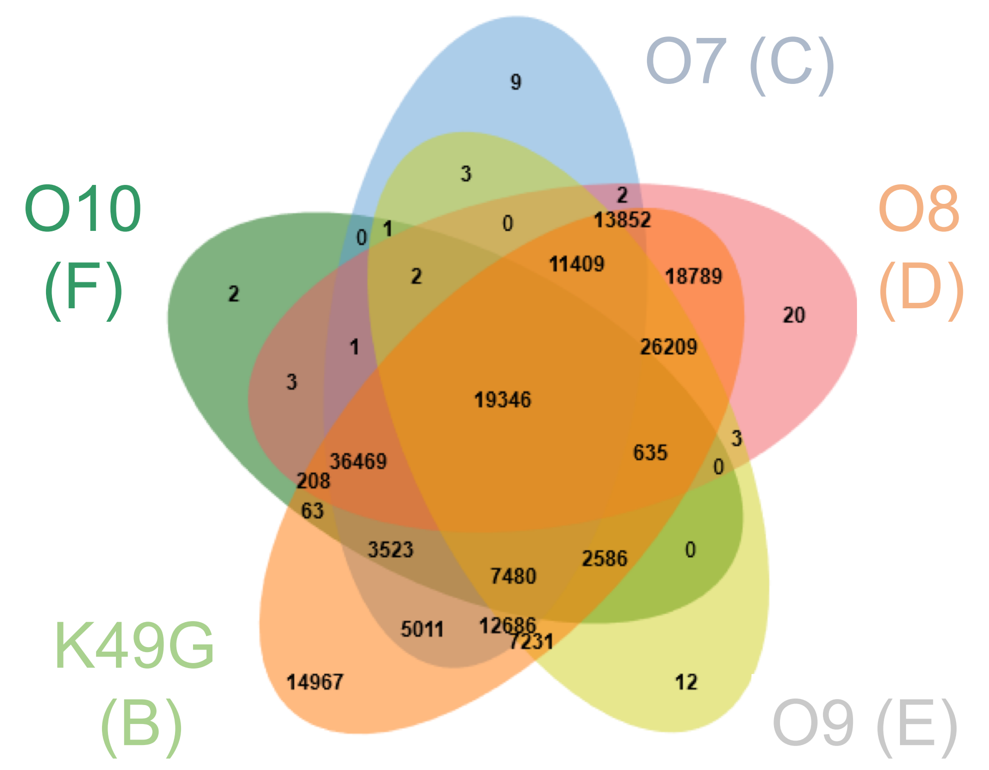
Are there any unique variants in each VCG?

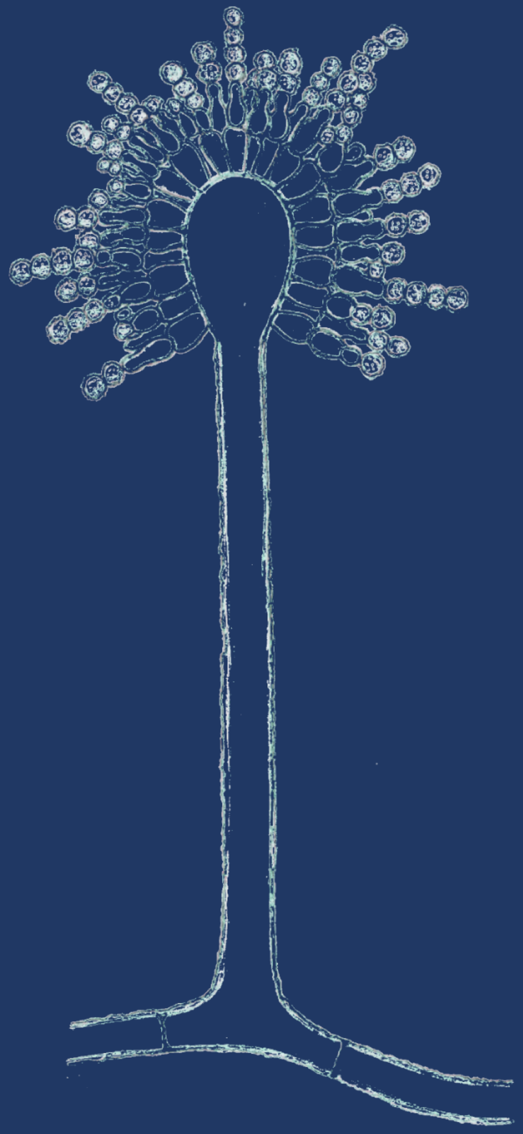
(Venn Analysis)

Venn Analysis of shared and unique variants



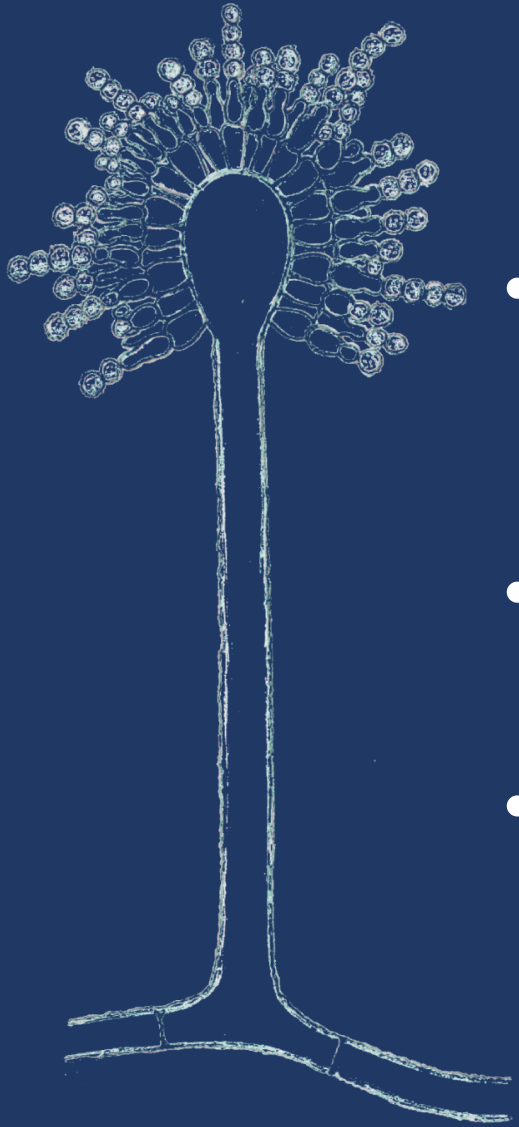
- Includes each unique VCG strain in relation to 1582 parent
  - O7-O10 very similar to aflatoxigenic parent





Do the fluorescent strains have the eGFP construct?

(Genome BLAST)

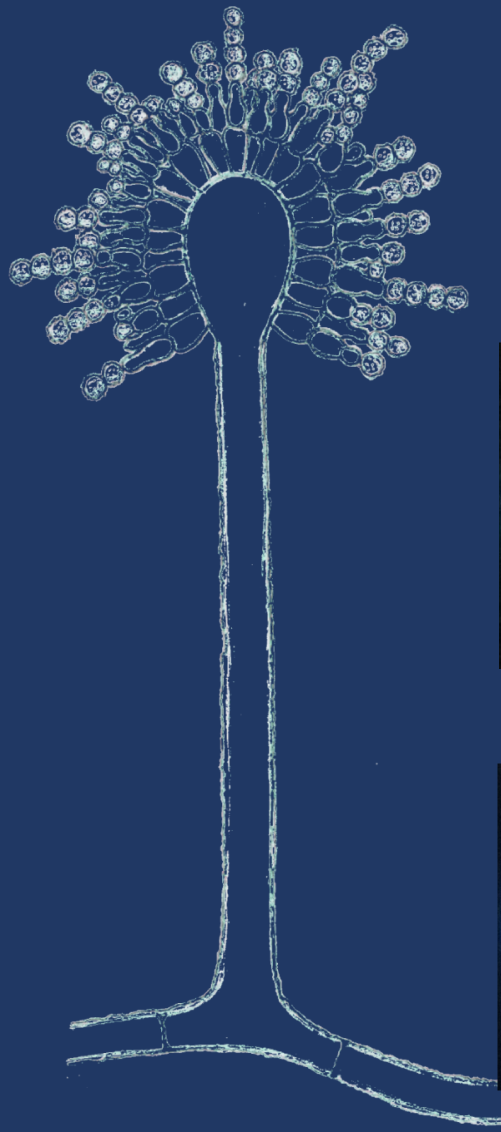


Findings: Genome BLAST

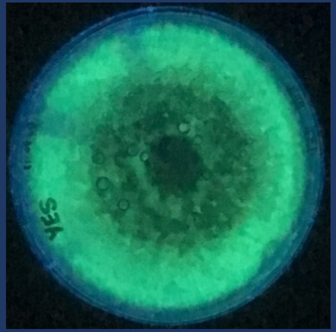
- Eight of the F1s contained the eGFP construct
  - O1-O6, O8, O9
- Two F1s exhibited highly mutated eGFP construct
- Only two of the F1s were truly non-fluorescent

Strain	**eGFP**
1582	0
K49-G	1
O1	1
O2	1
O3	1
O4	1
O5	1
O6	1
O7	0
O8	1 (50m)
O9	1 (79m)
O10	0

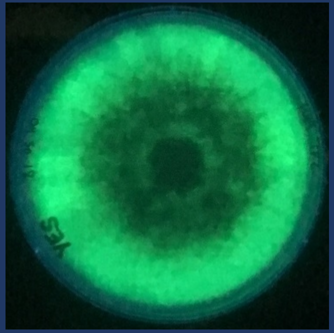
‘Fluorescence’ is in the eye of the beholder…



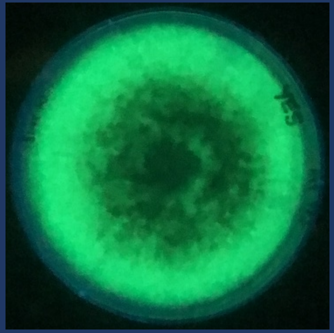
O1



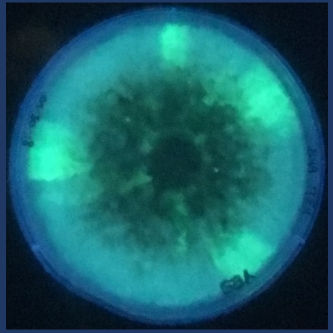
O2



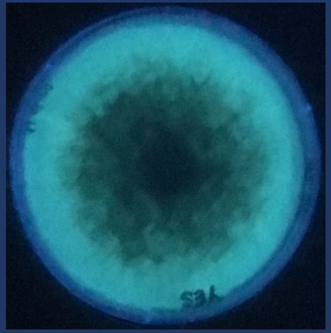
O3



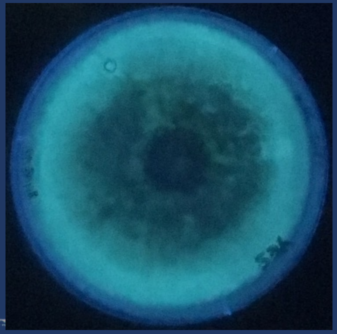
O4



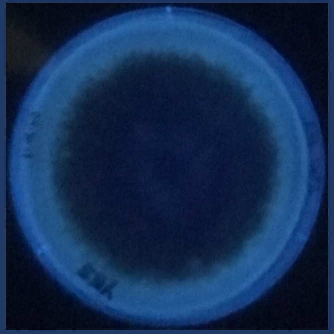
O5



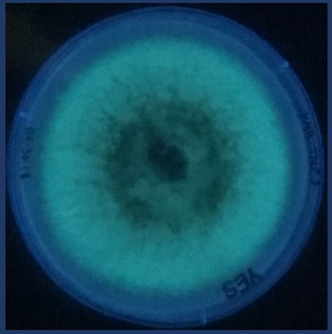
O6



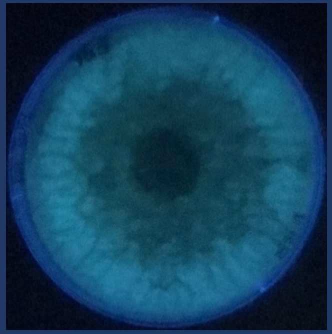
O7



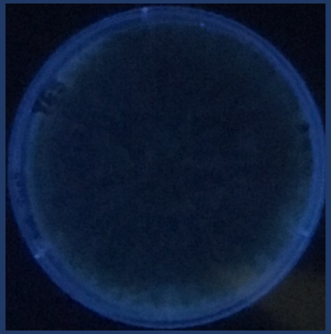
O8



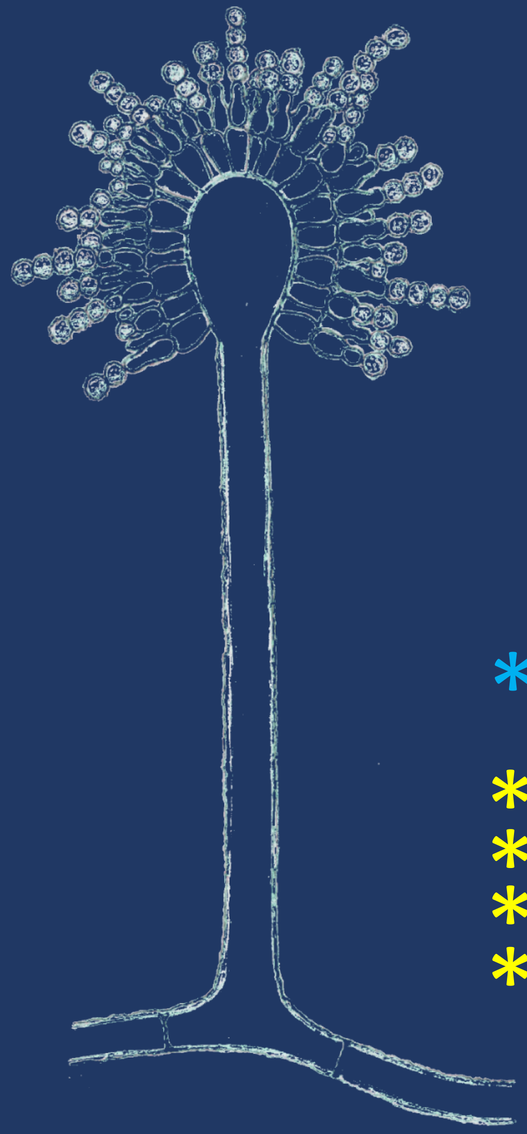
O9



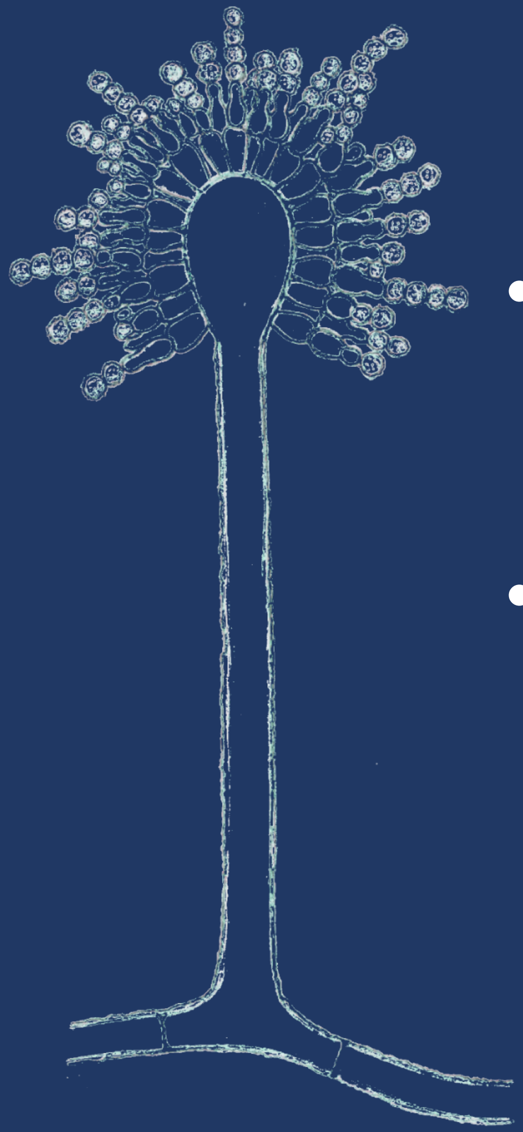
O10



Conclusions for Recombination



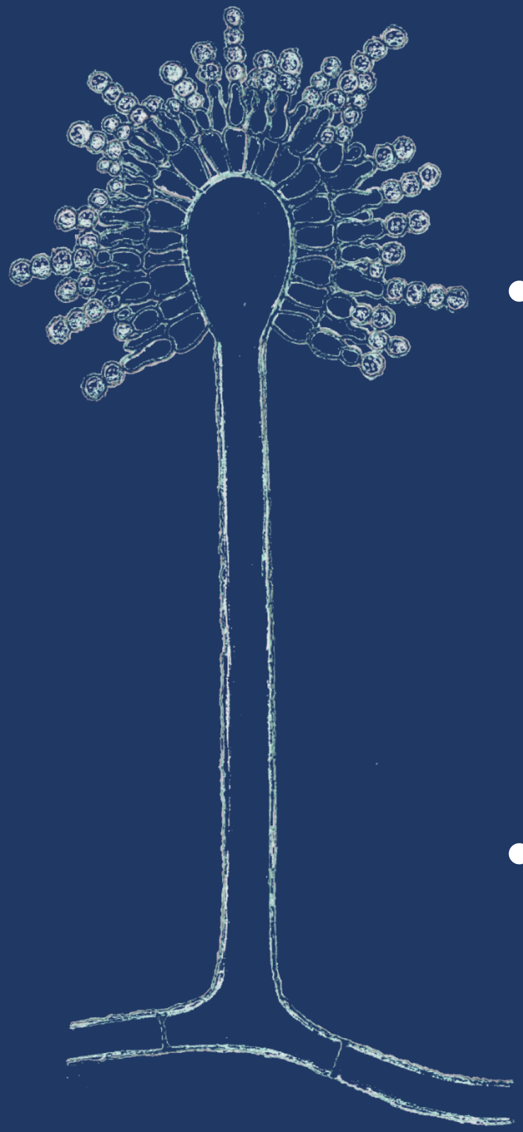
Strain	eGFP	VCG	MAT	Tox	C.E.
1582	0	A	1	AF+/CPA+	N/A
K49-G	1	B	2	AF-/CPA-	N/A
O1	1	B	2	AF-/CPA-	0
O2	1	B	2	AF-/CPA-	0
O3	1	B	2	AF-/CPA-	0
O4	1	B	2	AF-/CPA-	0
* O5	1	B	2	AF+/CPA-	0
O6	1	B	2	AF-/CPA-	0
* O8	1 (50m)	D	2	AF-/CPA-	29
* O9	1 (79m)	E	2	AF-/CPA-	23
* O7	0	C	2	AF+/CPA+	26
* O10	0	F	2	AF-/CPA-	17



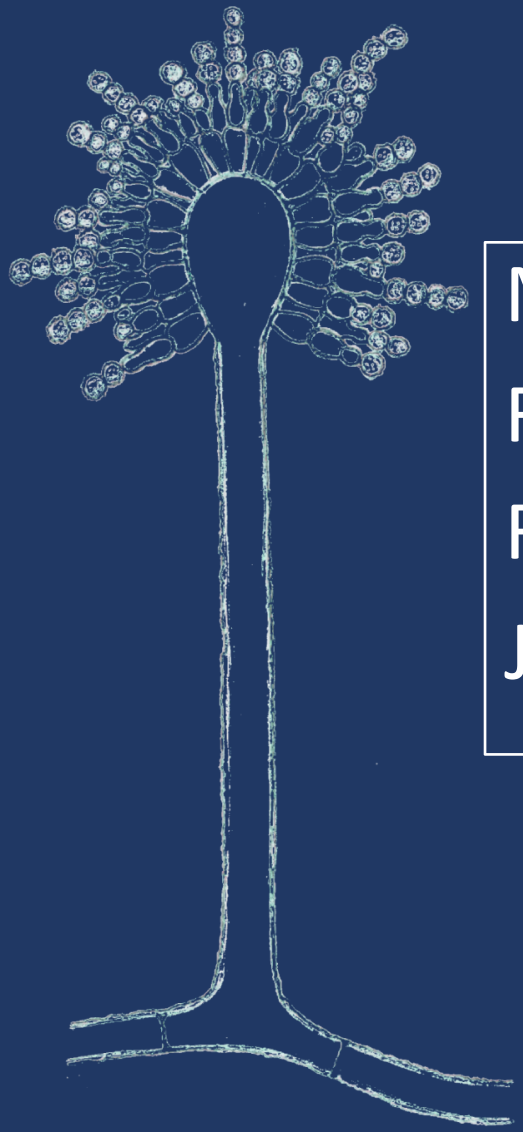
Future Analyses

- Generate metabolome data for the familial *A. flavus* population
    - Are there any obvious metabolite changes?
- Explore second generation (F2s) involving back-crosses to parents with F1s as well as crosses between F1s
    - Are F1s fertile, and will we further diversify the familial population?

Future Analyses: The Art of 'Re'



- Re-examination of progenies
  - O5 = Is its AF+/CPA- chemotype legitimate?
  - O6 = Why muted fluorescence when it has eGFP construct that appears complete and intact?
  - O8-O9 = What is responsible for their muted fluorescence?
- Repeat mating experiment and select more F1s



Thanks! Grazie! Merci! ¡Gracias! Xièxiè!Danke! Arigatō! Shukran!

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FFSR Unit @ SRRC

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José Di Mavungu (Ghent Univ.)

Mihi mai rā! Dhanyaavad! Obrigado! Blagodarnost'! Gamsa! Efcharistó!