Appressorium
THE BREAKTHROUGH IN DIKARYA

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What’s an appressorium?

- **Emmett & Parbery, 1975**: “All structures adhering to host surfaces to achieve penetration, regardless of morphology”

![Diagram showing appressorium structures](image)

- **M. oryzae appressorium**
  - [Meng et al., 2009](#)

- **P. anserina appressorium**
  - [Brun et al., 2009](#)
Podospora’s appressorium

- *Podospora anserina* tagged with cytoplasmic GFP
What’s an appressorium?

- Emmett & Parbery, 1975: “All structures adhering to host surfaces to achieve penetration, regardless of morphology”

![Diagram of appressorium structures](image)

- The appressorium is not exclusive to pathogenic species!
- Homologous structures
- Is this structure widespread among saprotrophic *Eumycetes*?
What about other species?

- **38 Eumycetes species tested:**
  - **Ascomycota**
    - **Orbiliomycetes** (1)
    - **Pezizomycetes** (4)
    - **Eurotiomycetes** (6)
    - **Dothideomycetes** (2)
    - **Leotiomycetes** (1)
    - **Sordariomycetes** (16)
    - **Agaricomycetes** (5)
  - **Basidiomycota**
    - **Mucoromycota**
      - **Mucoromycetes** (3)
  - **Dikarya**

- **24/38 (63%) species tested develop an appressorium in our conditions**
- **Appressorium development is widespread among saprotrophic Dikarya**

Demoor et al., 2019
Some species of interest develop an appressorium

Trichoderma reesei
Hypocreales

Neurospora crassa
Sordariales

Sordaria macrospora
Sordariales

Demoor et al., 2019
A breakthrough in *Eumycetes*?

- The appressorium is an adhesion and penetration structure encountered in numerous fungal species: pathogenic/symbiotic/saprotrophic.

- The appressorium is an ancestral feature among *Dikarya*.

What about other *Eumycetes*?
- Could some *Mucoromycetes* actually develop an appressorium?
- Some *Glomeromycota* species can differentiate appressoria.
- Test other *Eumycetes* species.

When did the appressorium appear during the evolution?

What about the genetic program of appressorium development?
Podospora anserina: a genetic model

- Ascomycota
- Genome sequenced: 35 Mb, 7 chromosomes
- Non-pathogenic
- Easily cultured in the lab
- Fast growing: 7mm per day
- One-week sexual reproduction cycle
- Easy molecular genetics studies
How to study its genetic program?

**Aim:** Identify the genes involved in this mechanism

- Screening for suppressors restored for penetration
- No new gene identified → New strategy
How to study its genetic program?

Pils1, Nox2 and Mpk2

Appressorium differentiation

Appressorium differentiation

Ascospore germination

M. oryzae

P. anserina

P. anserina

➢ Share common regulating elements

Lambou et al., 2008
The AGADFUN project

- Ascospore Germination and Appressorium Development in FUNgi
- **Combined study:** Study germination mutants to identify genes involved in both ascospore germination and appressorium development

Screening for germination mutants (P. Silar)

- GUN1
- GUN2
- GUN3
- GUN4
- GUN5
- GUN6

- 6 Germination UNcontrolled (GUN) mutants sequenced; 1 candidate gene for each
- Unravel the regulation pathways of both processes
The $GUN2^1$ mutant

- $GUN2$: Transcription factor (Gal4 family)
- Never studied in filamentous fungi
- Involved in:
  - The control of germination: $\Delta GUN2$ germinates spontaneously
  - The appressorium formation: $\Delta GUN2$ has a delay in appressorium formation
- Transcriptomics analysis of $\Delta GUN2$ during germination: in progress
- Identification of new actors of both pathways
The \textit{GUN1}^{1} mutant

- Point mutation in \textit{Pa}_6\textunderscore1340 (\textit{GUN1}) which encodes a perox/mito Carnitin Acetyl-transferase (CAT)

- Identified in \textit{M. oryzae} as virulence factor (\textit{Pth2}; Bhambra et al., 2006)
  - Validates our approach

\textit{GUN1}=\textit{AcuJ}

\textit{A. nidulans}

Stemple \textit{et al.}, 2010
Study of the $\text{GUN1}^1$ mutant

- $\text{GUN1}$ is involved in:
  - The control of germination: $\Delta\text{GUN1}$ does not germinate
  - The setting up of appressorium: $\Delta\text{GUN1}$ has a delay of appressorium formation

- Tagging of the $\text{GUN1}$ protein:
The **GUN1** mutant

**GUN1::mCherry::AKI, mito-GFP**

**GUN1::mCherry::AKI, peroxi-GFP**
Study of the \textit{GUN1} mutant

- \textit{GUN1} is involved in:
  - The control of germination: \( \Delta \text{GUN1} \) does not germinate
  - The setting up of appressorium: \( \Delta \text{GUN1} \) has a delay of appressorium formation

- Tagging of the \textit{GUN1} protein:
  - Both peroxisomal and mitochondrial
  - Results for the mutant protein in progress: different localization?

- Epistasis studies place \textit{GUN1} upstream of Mpk2 and PIs1/Nox2 in the pathway

- Study of \textit{GUN1} mutant: Understand the role of the mitochondria and peroxisomes in ascospore germination and appressorium development
The *GUN* mutants

- Study both ascospore germination and appressorium development
- This strategy allowed to find a gene involved in pathogenicity
- Unravel their regulation pathways
- Candidate genes for other *GUN* mutants also connected to the acetate metabolism
- Promising strategy to find new actors of both processes
Take home message

1. The appressorium refers to all fungal mechanical penetration structures

2. It is widespread among Dikarya

3. *Podospora anserina* is a model species allowing us to find new regulators in these processes

4. Combined study of both ascospore germination and appressorium development

5. Find new actors of both pathways
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Thank you for your attention