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Live-cell imaging of conidial fusion in the bean pathogen, *Colletotrichum lindemuthianum*

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Fusion of conidia and conidial germlings by means of conidial anastomosis tubes (CATs) is a common phenomenon in filamentous fungi, including many plant pathogens. It has a number of different roles, and has been speculated to facilitate parasexual recombination and horizontal gene transfer between species. The bean pathogen *Colletotrichum lindemuthianum* naturally undergoes CAT fusion on the host surface and within asexual fruiting bodies in anthracnose lesions on its host. It has not been previously possible to analyze the whole process of CAT fusion in this or any other pathogen using live-cell imaging techniques. Here we report the development of a robust protocol for doing this with *C. lindemuthianum in vitro.* The percentage of conidial germination and CAT fusion was found to be dependent on culture age, media and the fungal strain used. Increased CAT fusion was correlated with reduced germ tube formation. We show time-lapse imaging of the whole process of CAT fusion in *C. lindemuthianum* for the first time and monitored nuclear migration through fused CATs using nuclei labelled with GFP. CAT fusion in this pathogen was found to exhibit significant differences to that in the model system *Neurospora crassa*. In contrast to *N. crassa*, CAT fusion in *C. lindemuthianum* is inhibited by nutrients (it only occurs in water) and the process takes considerably longer.

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Mating type loci of *Botrytis cinerea*

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Botrytis cinerea is a heterothallic ascomycete with two mating types, MAT1-1 and MAT1-2. *B. cinerea MAT* loci have novel features. Fragments of the *MAT1-2-1* and *MAT1-1-1* genes were detected bordering idiomorphs of the MAT1-1 and MAT1-2 isolates, respectively. Both of these fragments encode truncated, non-functional proteins. *B. cinerea* has probably evolved from a homothallic ancestor containing complete *MAT1-1-1* and *MAT1-2-1* genes at the same locus, with MAT1-1 and MAT1-2 arising from the loss of HMG and alpha-domain sequences, leaving the disabled gene fragments seen in current *MAT* loci. Two ORFs, designated *MAT1-1*-5 and *MAT1-2-3*, have not previously been reported from other fungi. Homologs of *MAT1-1-5* are present in other leotiomycetes, whereas the *MAT1-2-3* gene is exclusively present within the genus *Botrytis.* Knockout mutants in *MAT1-1-5* are sterile, due to the inability of the dikaryon to develop a cap structure.

B. cinerea is unusual in that some isolates are capable of 'dual mating'. This refers to the observation that most isolates act in a standard heterothallic fashion (MAT1-1 or MAT-1-2), but some isolates can mate with both MAT1-1 ánd MAT1-2 isolates. Some dual mater isolates can self-fertilize and are truly homothallic. The *MAT* locus of five homothallic *B. cinerea* isolates was analysed. Four of those contain a MAT1-2 locus, without any sequence of the MAT1-1 locus being detected. Remarkably, one homothallic isolate contains a MAT1-1 locus, without any sequence of the MAT1-2 locus being detected. We conclude that dual mating and homothallism is controlled by sequences outside the *MAT* locus.