

Discovery of a sexually reproducing population of the main fungal pathogen of rice, *Magnaporthe oryzae*, in Asia.

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Recombination greatly impacts the adaptive potential of populations. But determining if recombination occurs might be strenuous in fungi, where sex can be cryptic or facultative.

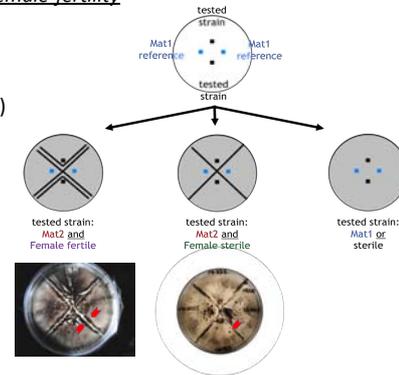
Magnaporthe oryzae is responsible for rice blast — the most damaging disease of the staple food crop of half the World's population. Although known as reproducing asexually, its sexual cycle is mastered *in vitro*. Recombination has been suspected¹, but never evidenced, in the Himalayan foothills, the centre of origin of the disease² and of domestication of Asian rice, *Oryza sativa*³.

→ Here we addressed the possibility that sexual reproduction still occurs in some populations of *M. oryzae* using biological, population genetics and simulation approaches.

1. *M. oryzae* sexual cycle requires opposite mating types (Mat) and one female-fertile strain

Figure 1. *In vitro* test of mating type and female-fertility. Example with a Mat1 reference strain

- Tested strain (Mat and fertility unknown) cultured with reference strains, until perithecia formation (3 weeks at 20°C).
- Reference strains :
 - 2 Mat1 and 2 Mat2
 - all female-fertile



2. Female-fertile strains are concentrated in South Asian populations

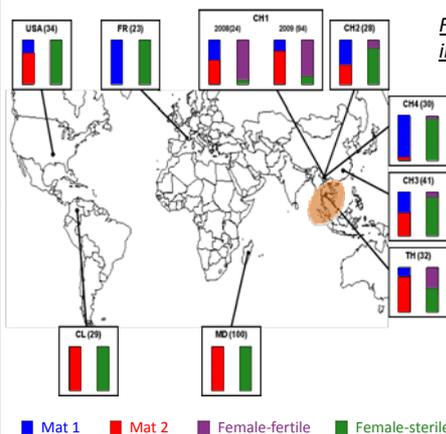


Figure 2. Mat and female-fertility in 9 populations

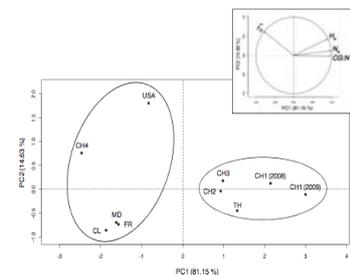
- Female-fertile strains were previously found exclusively in South Asia (not shown): this region is the candidate region for sexual reproduction.
- Mat and female-fertility test in 9 populations collected on cultivated rice (at least 20 individuals collected in the same field the same year) confirm that South Asian populations are the only candidates.

3. Linkage disequilibrium (LD) and clonal richness fit sexual reproduction in most South Asian populations

Table 1. Diversity and LD in the 9 populations

Populations	N	G	CG	CG:N	N _e	H _e	r _D
TH	27	20	18	66.7%	4.1	0.47	0.14
CH1-2008	24	21	18	75.0%	5.2	0.63	0.16
CH1-2009	83	76	63	75.9%	7.1	0.64	0.07
CH2	38	32	21	55.3%	3.8	0.50	0.21
CH3	23	14	14	60.9%	4.1	0.50	0.25
CH4	25	5	4	16.0%	1.5	0.08	0.62
CL	31	9	2	6.5%	1.5	0.06	NT
FR	23	12	4	17.4%	1.6	0.09	0.30
USA	37	20	6	16.2%	3.2	0.56	0.57
MD	95	18	10	10.5%	2.1	0.07	0.31

Figure 3. PCA of the 9 populations

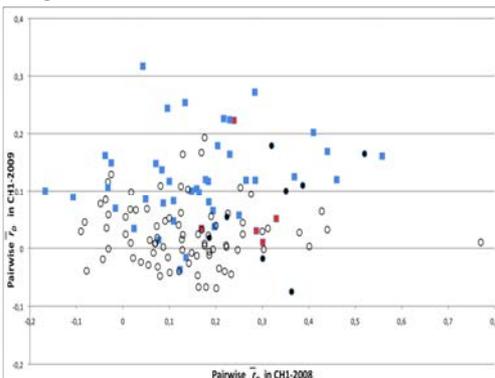


In South Asian populations (except CH4):

- Higher H_e (P=0.01) and N_e (P=6.10⁻³), as expected in the center of origin
- Lower r_D (P=0.03) and higher CG:N (P=2.10⁻⁵), as expected for sexual reproduction

4. One Chinese population (CH1) experienced sexual reproduction between two consecutive years

Figure 4. Pairwise LD in CH1 between 2008 and 2009



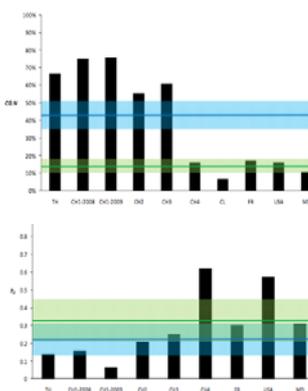
The CH1 population (China, Yunnan province), sampled in 2008 and 2009, showed:

- no correlation between pairwise LD values the two consecutive years (slope=0.05, P=0.28, R²=0.008)
- that pairs of loci remaining linked the 2 consecutive years are not over-represented (P=0.06)

Significantly linked pairs: ■ in 2008, not in 2009; ● in 2008 and 2009; ■ in 2009, not in 2008; ○ neither in 2008 nor in 2009

5. Simulations show that the levels of clonal richness and LD observed in the CH1 population cannot be reached without recombination

Figure 5. Data simulated for clonal populations with an island model



- Clonal populations were simulated with Quantinemo.

- The high CG:N and low r_D observed for South Asian populations (except CH4) could not be achieved by strictly clonal populations.

- Same results with 3 migration patterns: no migration, island model, stepping stone 2D.

Histograms: observed CG:N (up) and r_D (down). Lines and filled areas = mean and standard deviation for simulated data, (mutation rate μ=10⁻⁵, initial number of alleles Na=25, migration rate m=0.5, effective population size NS=1,000 (green) or 10,000 (blue)).

Using complementary biological and population genetics data, we demonstrate for the first time that

***Magnaporthe oryzae* populations pathogenic to rice reproduce sexually, at least locally, in their putative center of origin.**

This result is of main importance since it directly impacts the choice of control strategies against this major rice pathogen.