Population and Evolutionary Biology of Fungal Symbionts
Ascona
2007

Migration of the rice blast fungus Magnaporthe grisea

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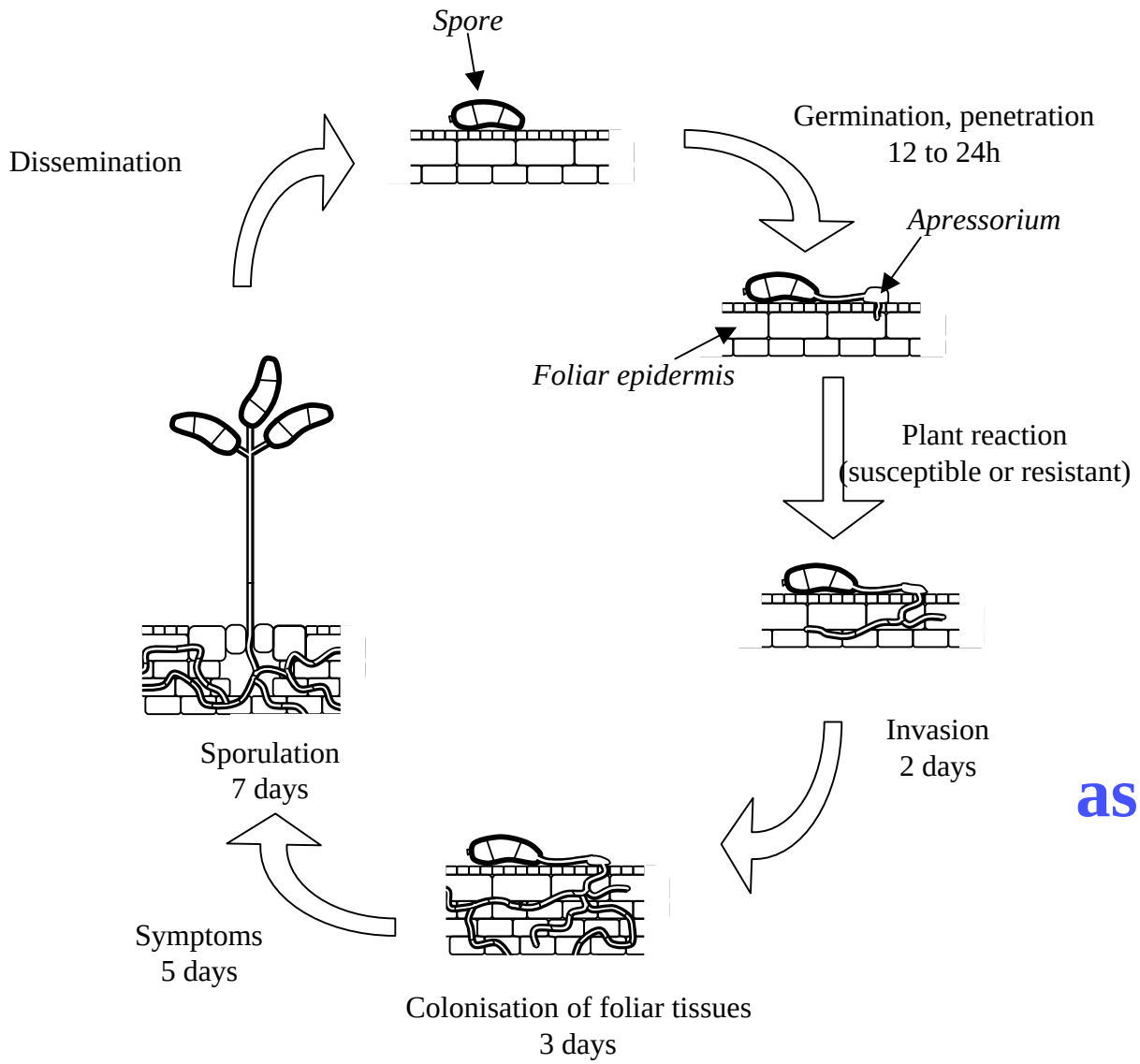
and soon **Elisabeth Fournier**

Introduction: Rice blast symptoms

Rice blast disease caused by the fungus *Magnaporthe oryzae*

Magnaporthe oryzae
haploid Ascomycete fungus

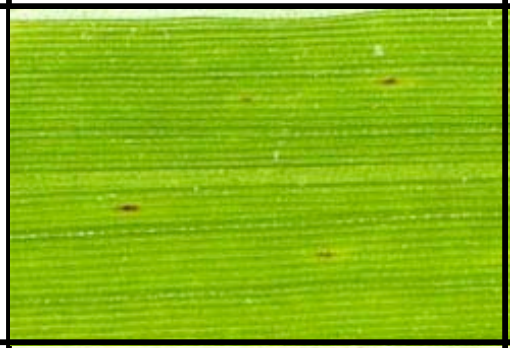
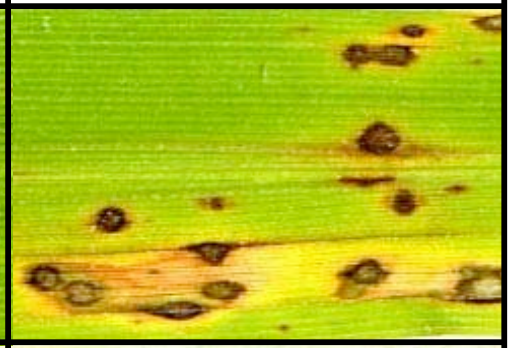
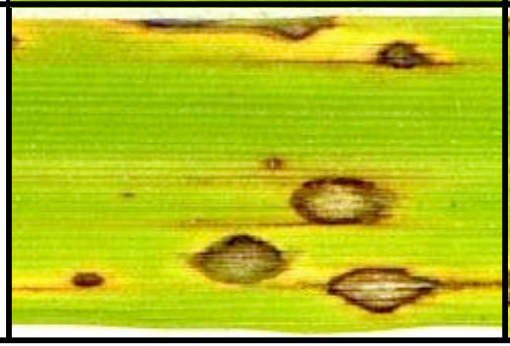
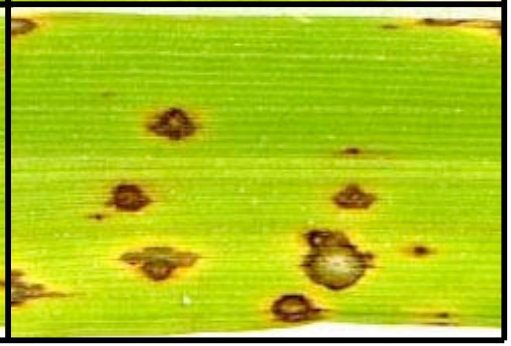


asexual reproduction

After Romain Berruyer, CIRAD

Rice -rice blast interactions follows the Gene for Gene theory

Plant

Pathogen	R/R or R/s	s/s
AVR		
vir		

Silué et al, 1992, Phytopathology

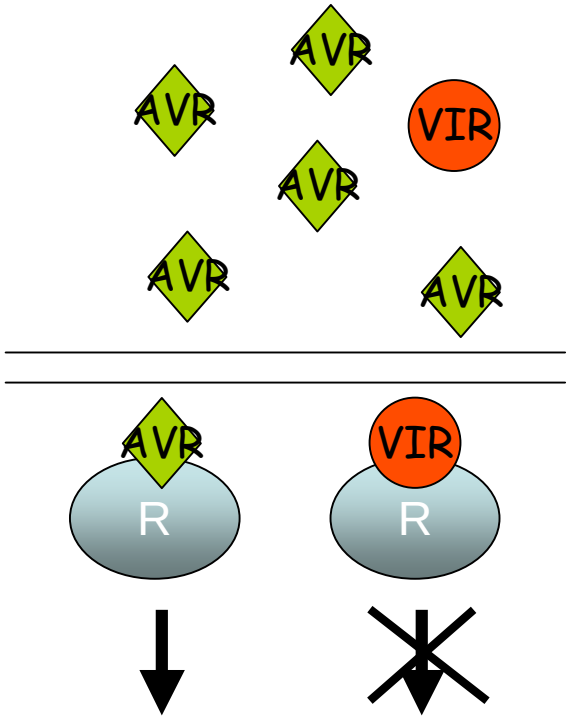
Resistance breakdown common and rapid

Varieties	69	70	71	72	73	74	75	76	77	78	79	80	81	82
IR8	O	X												
Cica4			O	X										
IR22			O	X										
Cica6						O	X							
Cica7								O		X				
Cica9								O	X					
Cica8										O		X		
Metica1													O	X

O release, X resistance breakdown

F. CORREA, CIAT, Colombia

Introduction: Population evolution



Understanding Resistance breakdown

Virulent strains emerge

Different mechanisms (lab studies)

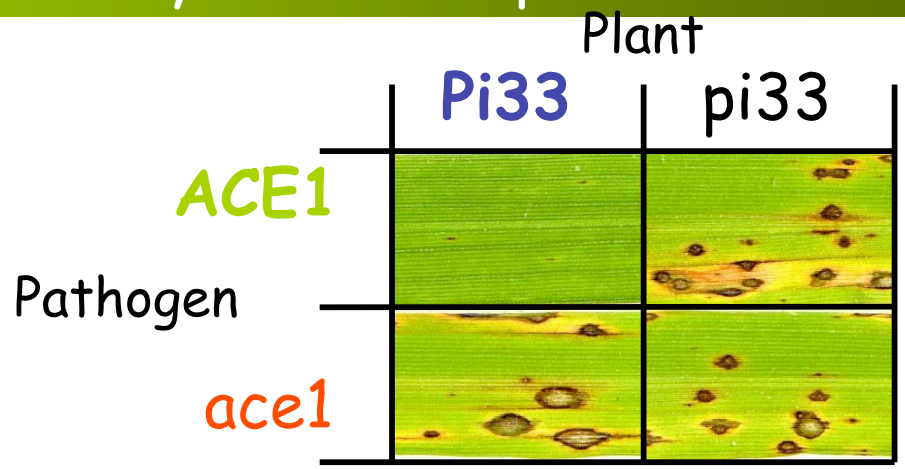
- Point mutation, insertion, deletion, recombination

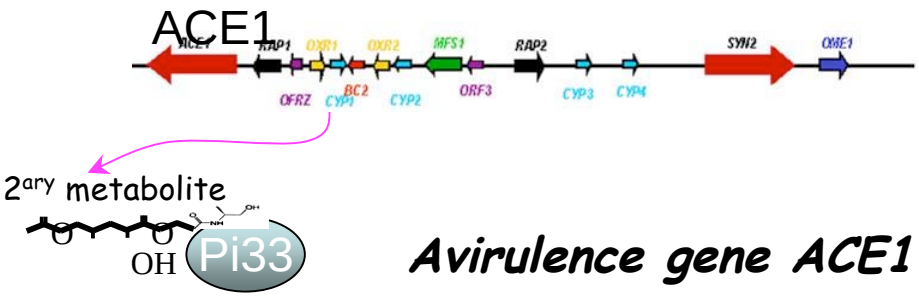
Few examples in natural populations

How virulent strains appear and spread in the field?

Identify and characterize alleles of avirulence genes in natural populations

ACE1/Pi33 : our model system for specific interactions





Avirulence gene ACE1 cloned

Functional studies

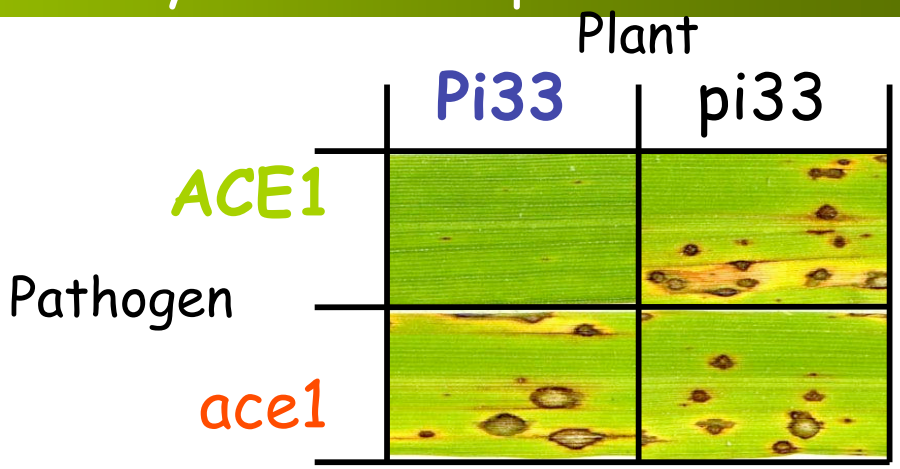
ACE1 **is specific of the infection**

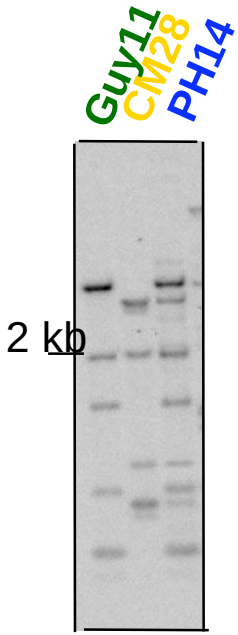
ACE1 **encodes a big, non secreted protein**

ACE1 **is a PKS/NRPS, 2ary metabolite biosynthesis enzyme**

Böhnert et al. Plant Cell 2004

ACE1/Pi33 : our model system for specific interactions





ACE1 probe

Avirulence gene ACE1 cloned

Population studies

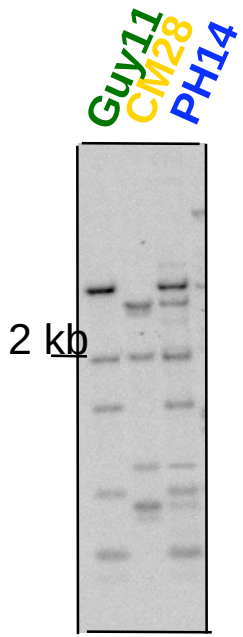
Identification and characterization of *ACE1* alleles in a worldwide collection (55 countries, 3,500 strains) by RFLP, then PCR

Fudal et al. in preparation

ACE1: rare emergence and long distance migration

Identification and characterization of *ACE1* alleles

3 virulent genotypes with 2 dominant
1 complex event (duplication/deletion)



ACE1 probe

ACE1: rare emergence and long distance migration

Identification and characterization of *ACE1 alleles*

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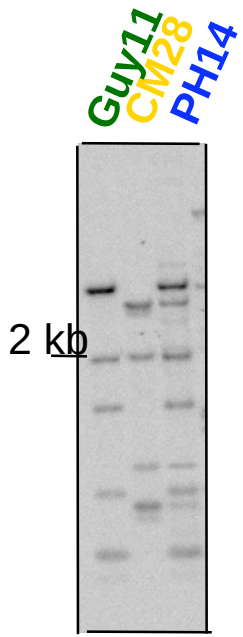
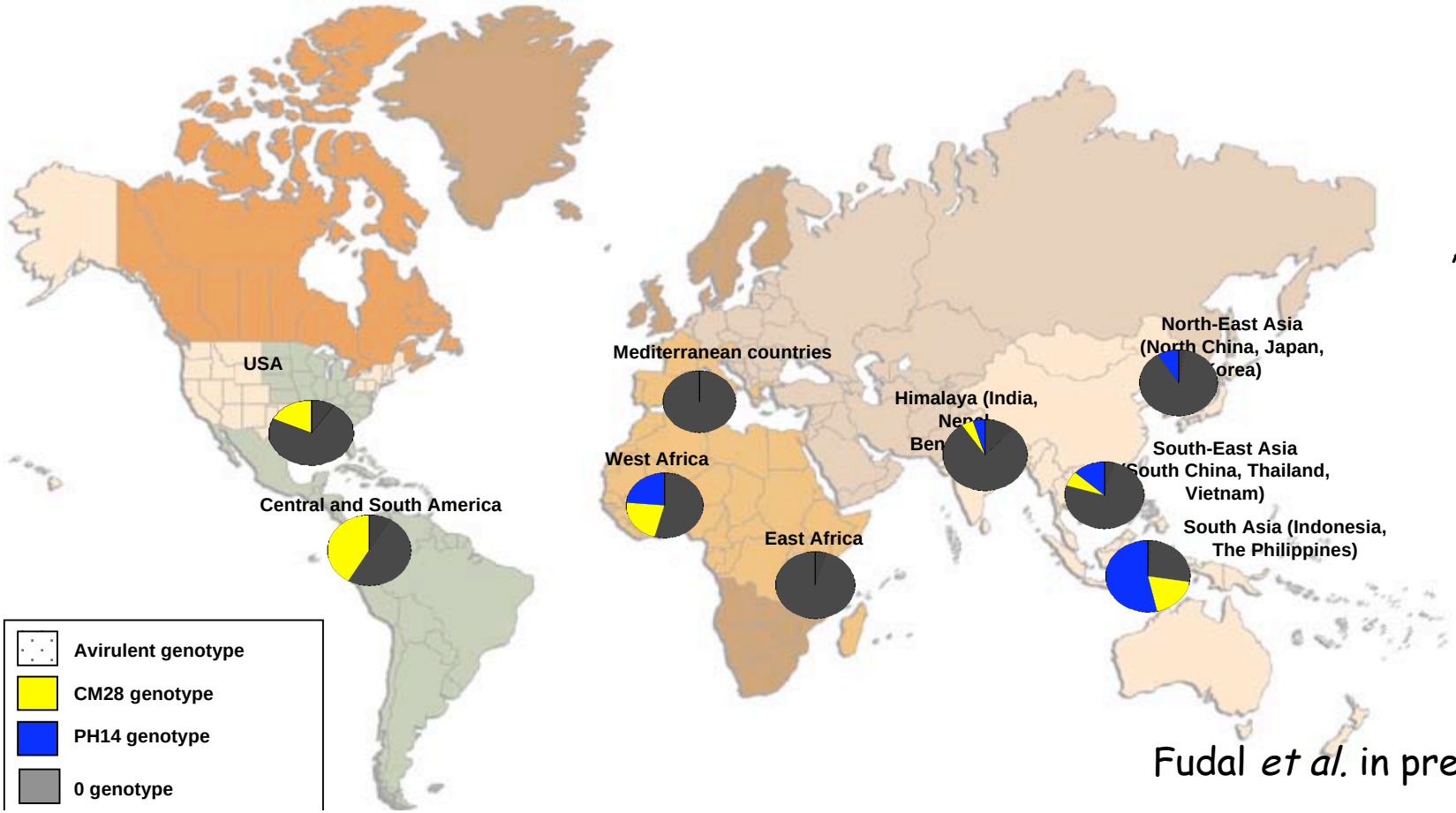
1 complex event (duplication/deletion)
1 unique apparition event
intercontinental distribution ← migration



ACE1 probe



Fudal *et al.* in preparation

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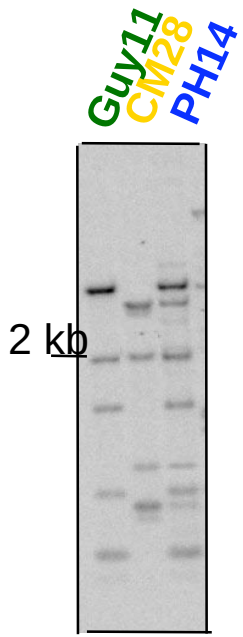


ACE1 probe

- **emergence of *ACE1* Vir. is rare in the field**
- **intercontinental migration of Vir. strains occured**

Fudal *et al.* in preparation

Couch *et al.* Genetics 2005

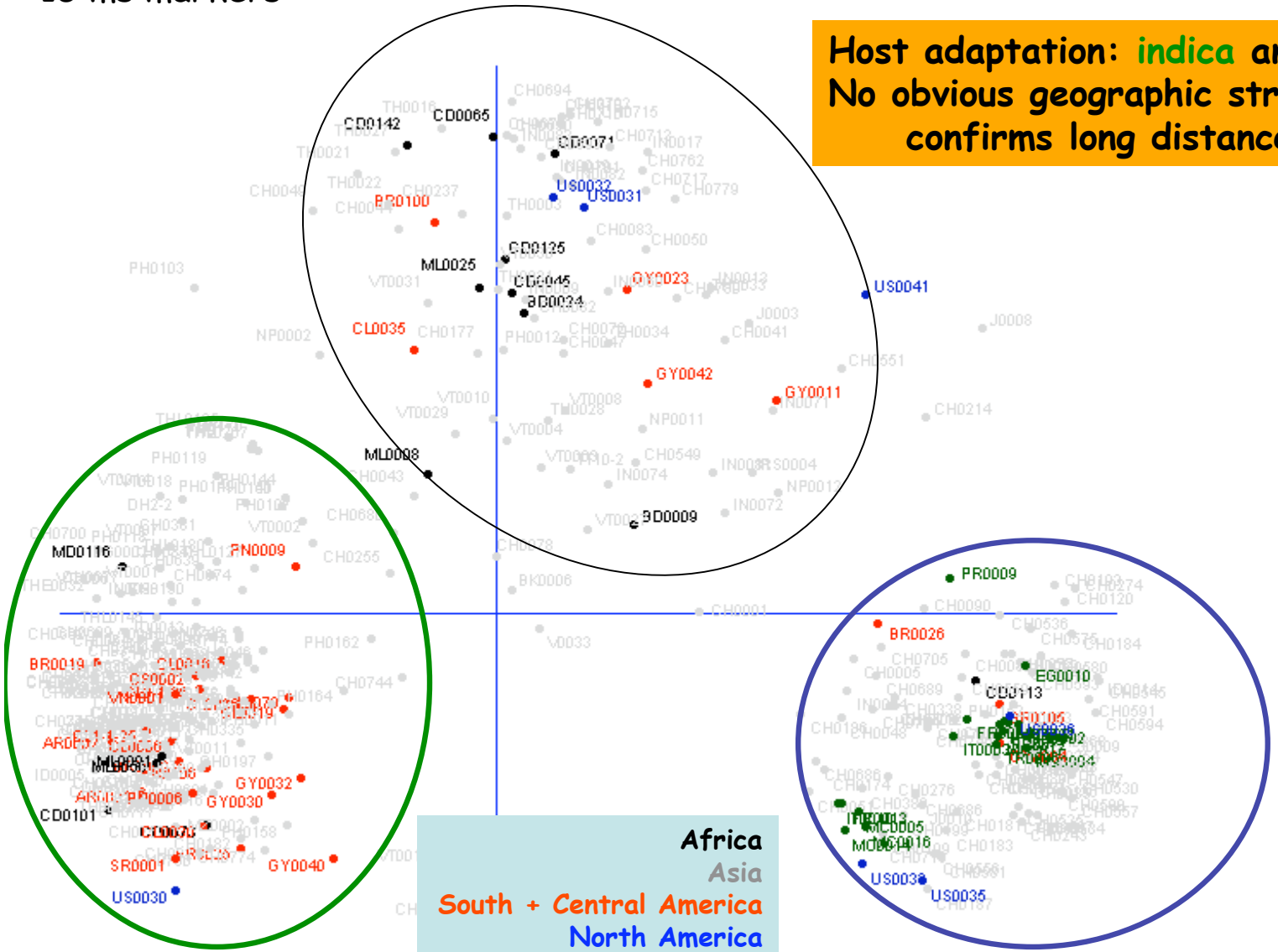
Fudal *et al.* Fungal Genetics and Biology 2005

World population structure confirms migration

World population structure preliminary study

500 isolates (reference isolates = maximum genetic diversity)
15 ms markers

Host adaptation: indica and japonica groups
No obvious geographic structure
confirms long distance migrations



Africa
Asia
South + Central America
North America
Mediterranean countries

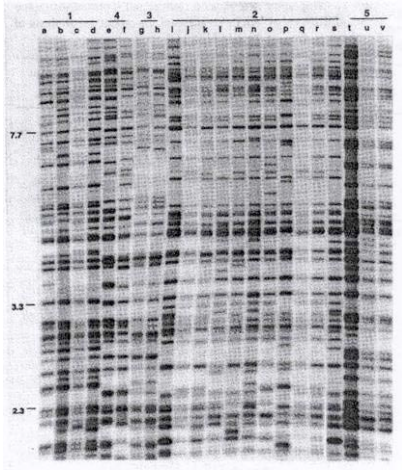
What is the quantitative importance of migration at different scales?

Pop. Genetics: measure migration = measure gene flow

Migration	High	0
Gene flow	High	0
Populations	identical	100% different
Differentiation	No	Yes
Fst value	0	1

Evaluating migration : development of new markers

MGR fingerprinting

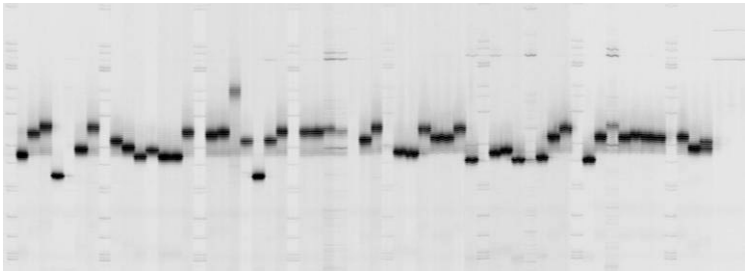


Appropriate genetic markers required
- identification of alleles for each locus
- no null allele

➜ Development of new markers for rice blast
Microsatellite markers commonly used for pop. genetics
Genome sequence available

➜ ca 1,500 microsatellite sequences

ms marker



- **300 primer pairs designed and tested**
- **130 microsatellite markers mapped**
- **18 markers chosen for pop.studies**

Kaye et al Fung. Genet. Biol. 2003
Kaye *et al.* in preparation

Adreit et al Mol. Ecol. Notes 2007

Differentiation at a local scale

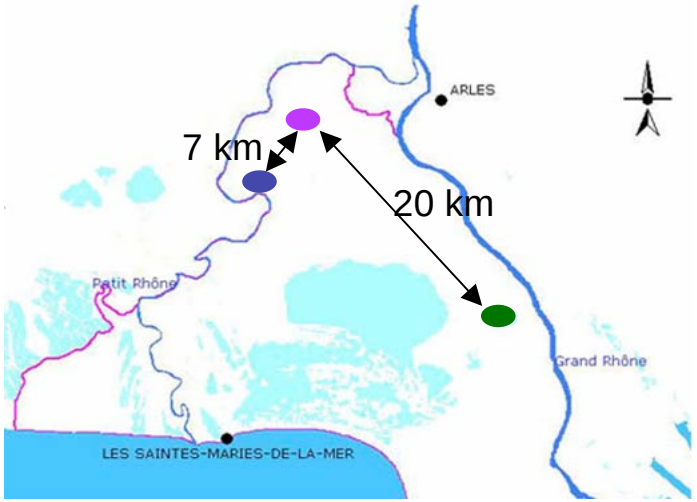
4 sites in Madagascar
Distance between sites: < 20 km

	L Mangalaza	L Manandona	U Belazao	U Andrano
L Mangalaza		0.01	0.04	0.30
L Manandona	*NS*		0.09	0.30
U Belazao	*S*	*S*		0.26
U Andrano	*S*	*S*	*S*	

NS / S = Fst value not significantly/significantly different of 0
L / U = Lowland / Upland agrosystems

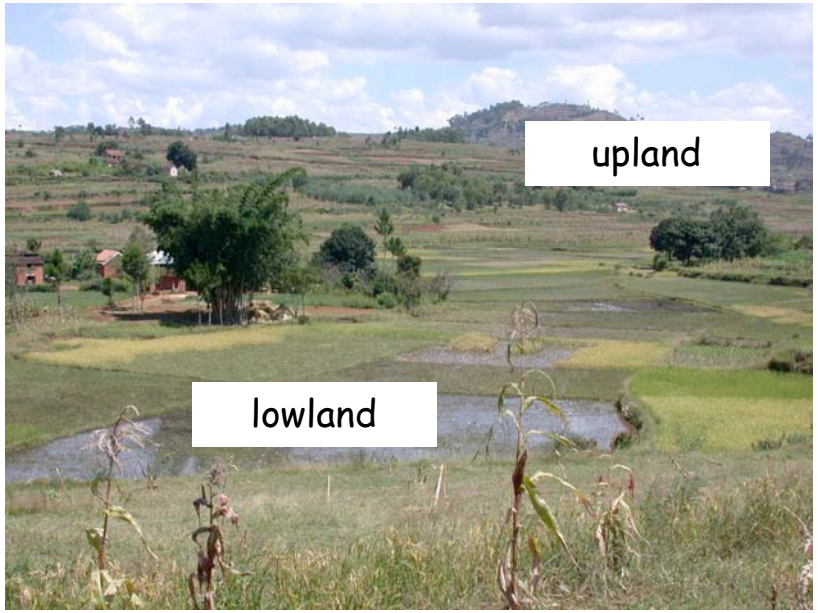
3 sites in France
Distance between sites: < 20 km

	Furane	Vigne	Gd Manusclat
Furane		0.590	0.094
Vigne	S		0.603
Gd Manusclat	NS	S	



Differenciation at local scale:
- evidence for short distance migration
- in agreement with short distance spore dispersal (1-5 m)

Evaluating migration : effect of selection?



Two agrosystems: lowland vs upland
One site in Madagascar

	2004	2005
Fst value	0.13	0.04
	S	S

	STRAINS																						
	From lowland											From upland											
CULTIVARS	MD398	MD802	MD803	MD804	MD1025	MD728	MD730	MD731	MD751	MD1030	MD1032	MD791	MD798	MD801	MD989	MD990	MD994	MD997	MD1004	MD1007	MD1010	MD1013	MD1016
Daniéla	R	.	.	S	R	R	R	R	MR	R	R	S	S	S	S	S	S	S	S	R	S	MR	MR
Fofifa133	R	R	MR	R	R	R	R	R	S	R	MR	S	S	S	S	S	S	S	S	S	S	S	S
Fofifa154	R	R	MR	R	R	R	R	R	S	R	MR	S	S	S	S	R	S	S	MR	S	MR	S	S
Fofifa152	R	R	R	R	R	R	R	R	S	R	MR	S	S	S	S	MR	S	S	S	S	S	S	S
Fofifa159	R	R	R	R	R	R	R	R	MR	R	R	S	R	S	S	R	S	S	MR	S	MR	R	R
Fofifa161	R	R	R	R	R	R	R	R	MR	R	R	R	R	S	S	R	S	S	S	S	S	MR	MR
Fofifa62	R	R	.	R	R	R	R	R	S	R	R	S	S	S	S	S	S	S	S	MR	R	S	MR
Fukunishiki	MR	MR	R	R	MR	R	R	MR	S	S	MR	S	S	S	S	S	S	S	S	S	S	S	S
K3	R	MR	MR	R	MR	MR	R	R	S	R	R	R	R	R	S	S	S	R	R	R	S	S	R
Nerica4	R	R	R	R	R	R	R	R	MR	R	MR	S	MR	S	S	MR	S	S	MR	MR	MR	MR	MR

Differentiation at local scale = limited migration + selection

Evaluating migration : Regional and intercontinental

Differentiation at different scales

Regional

2 sites in Indonesia (Indramayu and Sukabumi)
Distance between sites: 200 km

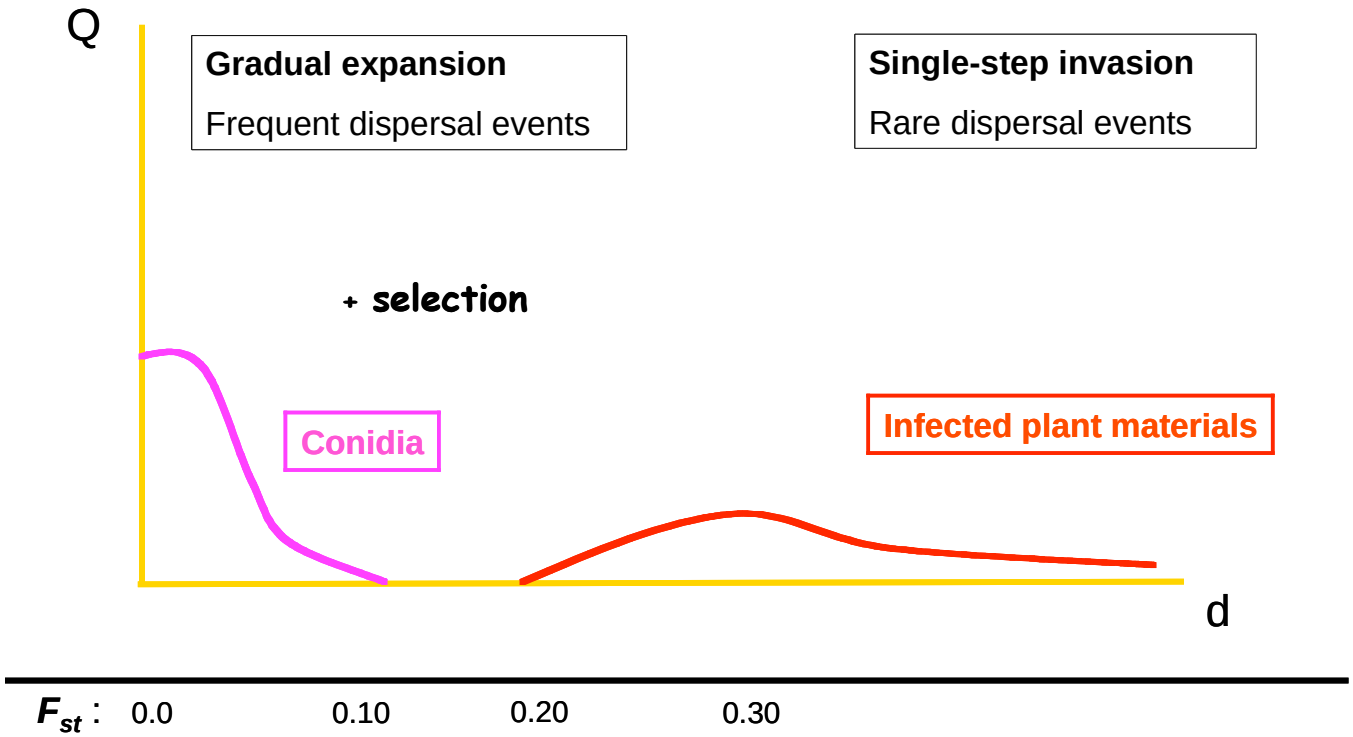
Fst = **0.19** *S*

Intercontinental

	Europe France + Spain	Asia Indonesia	Africa Madagascar
Europe		**0.45**	**0.60**
Asia	*S*		**0.41**
Africa	*S*	*S*	

Differentiation at regional and intercontinetal scales are also in agreement with limited migrations
Contradiction with ACE1 results ?

Summary



After Jean Carlier, CIRAD

Differentiation evaluations indicate limited migration (consistent with short distance movement of spores)

But :

- long distance migration is possible, probably by transportation of infected seeds
-in absence of recombination, selection by host resistance can also generate differentiation at local scale

Prospects

To confirm preliminary studies on differentiation and migration with more populations

To trace intercontinental migrations with “phylogenetic” markers

To measure migration at local scale by epidemiological studies in the field

To measure the relative importance of migration and selection on differentiation

In collaboration with:

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Dodelys Andriantsimialona — Fofifa, Antsirabe, Madagascar
Dwinita Utami — Icabiograd, Bogor, Indonesia
M. Santoso — Research Institute for Rice, Subang, Indonesia

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Jean Loup Notteghem, head of BGPI unit — SupAgro.M