

*The 7th International Mycosphaerella and Stagonospora Symposium
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LANDSCAPE GENETICS AND *Gene flow in the banana pathogenic fungus
Mycosphaerella fijiensis*

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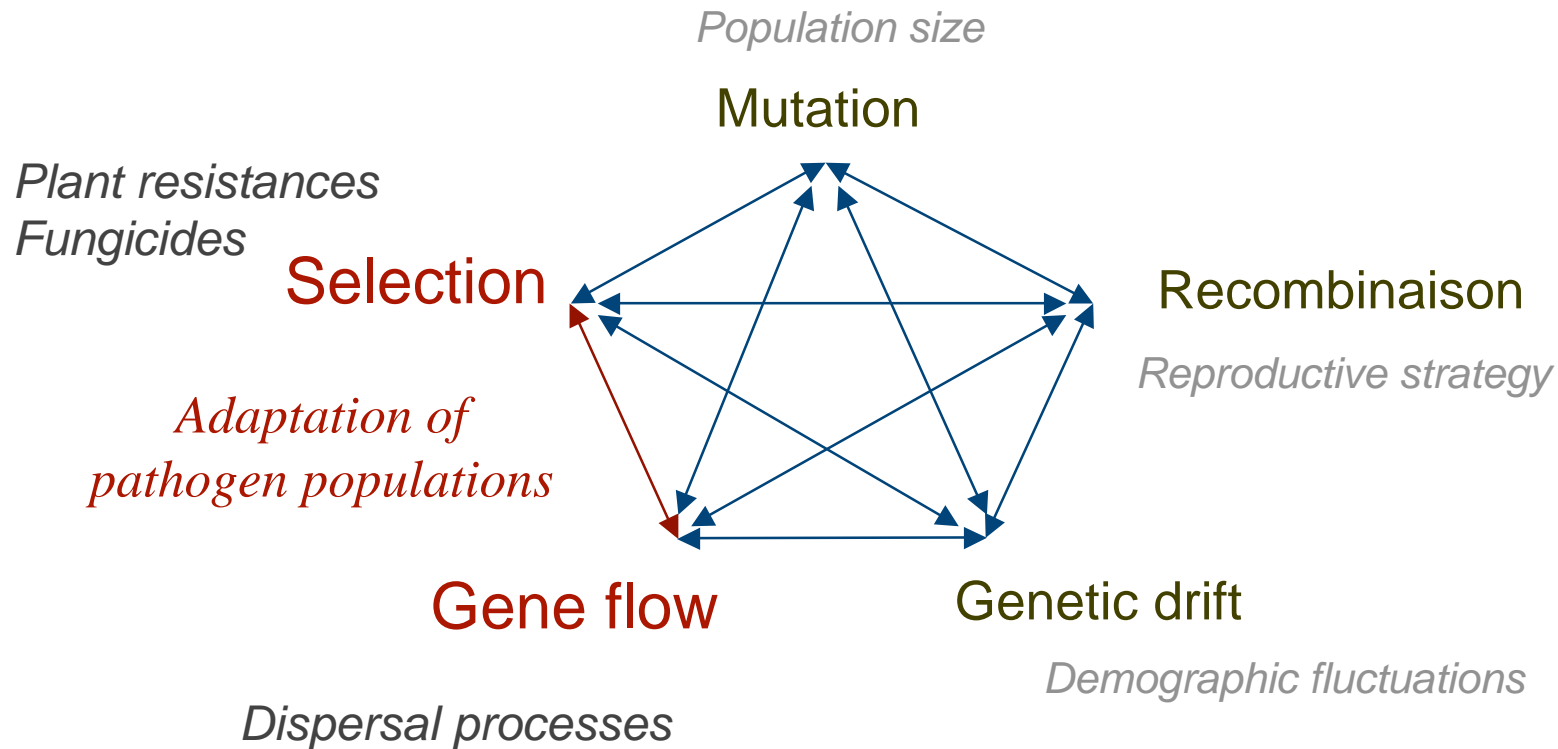
*UMR BGPI : "Biology and genetics of plant / pathogen interactions"
Campus International de Baillarguet - 34 398 MONTPELLIER – France*



UMR - BGPI
Biologie et Génétique
des Interactions Plante-Parasite

Context of this study or why study gene flow ?

→ Efficient and durable strategies of disease management should be defined in time and space taking into account epidemiology and evolutionary potential of pathogens



Objective: To infer gene flow and dispersal processes in
Mycosphaerella fijiensis

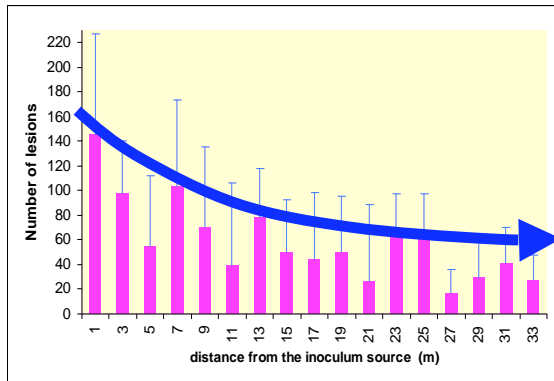
How to measure parameters related to gene flow ?

Exemple with two class of Methods

Direct methods

- Disease gradient analysis
- Average dispersal distance

Source
of inoculum 



Problems :

- Experiments costly and hard to realize
(Ex : problems with source identification)

Indirect methods

- Population genetics
- Neutral molecular markers
- Genetic differentiation between populations analysis

Q_s : Probability of identity
between 2 genes sampled in the
same habitat

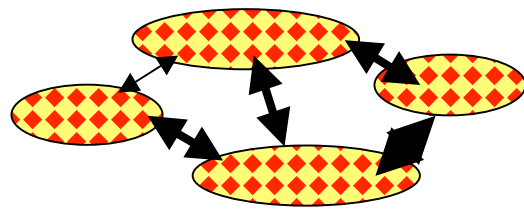
$$F_{ST} = \frac{Q_s - Q_T}{1 - Q_T}$$

Q_t : Probability of identity
between 2 genes sampled in the
whole habitats

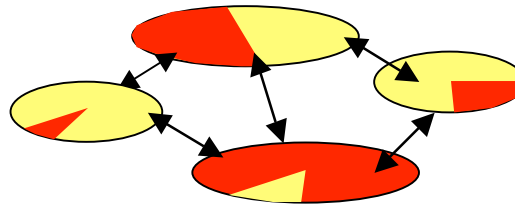
How to measure parameters related to gene flow ?

Back to the relation between F_{ST} & dispersal ability

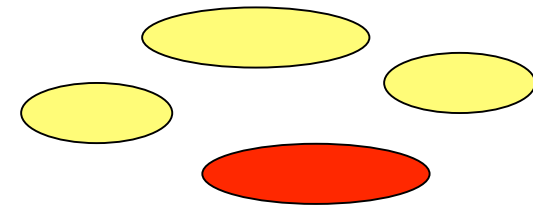
A high migration (rate or distance) will homogenize allelic frequencies between populations and will lead to a decrease of the F_{ST} parameter.



High Gene flow
 F_{ST} weak



Weak Gene flow
 F_{ST} moderate

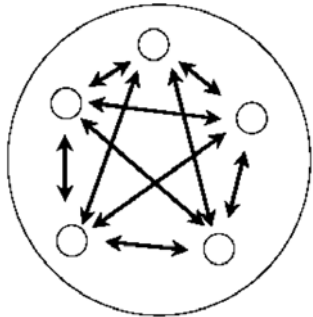


No Gene flow
 F_{ST} high

- **Objective** : Use F_{ST} (easy to measure) in order to estimate parameters related to dispersal (rate, average distance...) (difficult to measure)
- **Method** : Use a model to explicitly link F_{ST} and dispersal → dispersal parameters inference

How to measure parameters related to gene flow ?

Exemple with two models



Wright's Island model

- Population size : constant and identical
- Mutation – genetic drift equilibrium
- Equal contribution to the migrants pool

Migration rate / generation :

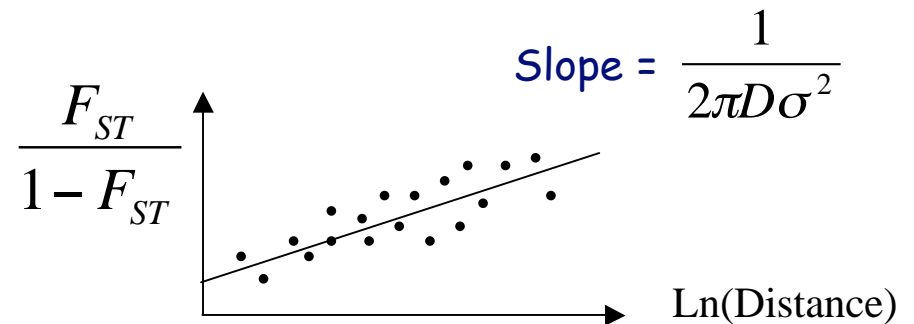
$$F_{ST} \approx \frac{1}{1 + 4Nm}$$

NOT REALISTIC

└── Migration rate
└── Effective size

Isolation by distance Model (IBD)

- Because of the limited migration in space, the probability of identity between genes is higher at short distance than at long distance.



- σ^2 = average squared axial parent-offspring distance

D = effective density of adults

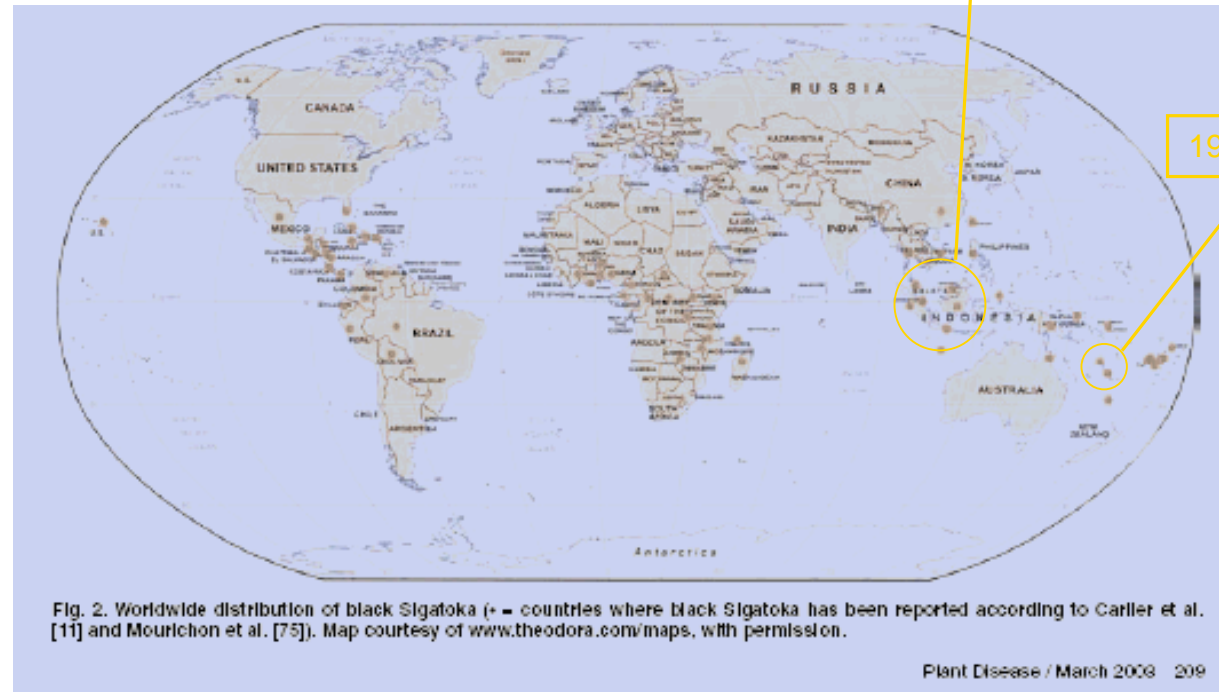
The patho-system *Mycosphaerella fijiensis* - Banana plant : A good model to study gene flow in pathogenic fungus populations



- Aerial Ascomycete, Haploid, Heterothallic
- Black leaf streak of Banana



South east of Asia :
Center of Origin



→ Recent worldwide expansion (1970') : replacement of *M.muscolola* (yellow Sigatoka)

The patho-system *Mycosphaerella fijiensis* - Banana plant : A good model to study gene flow in pathogenic fungus populations



- Aerial Ascomycete, Haploid, Heterothallic
- Black leaf streak of Banana
- 3 dispersal modes :



- Infected plant material transport
- Conidia dispersion (asexual reproduction)
- Ascospores dispersion (sexual reproduction)

- Panmictic populations
- Relative demographic stability

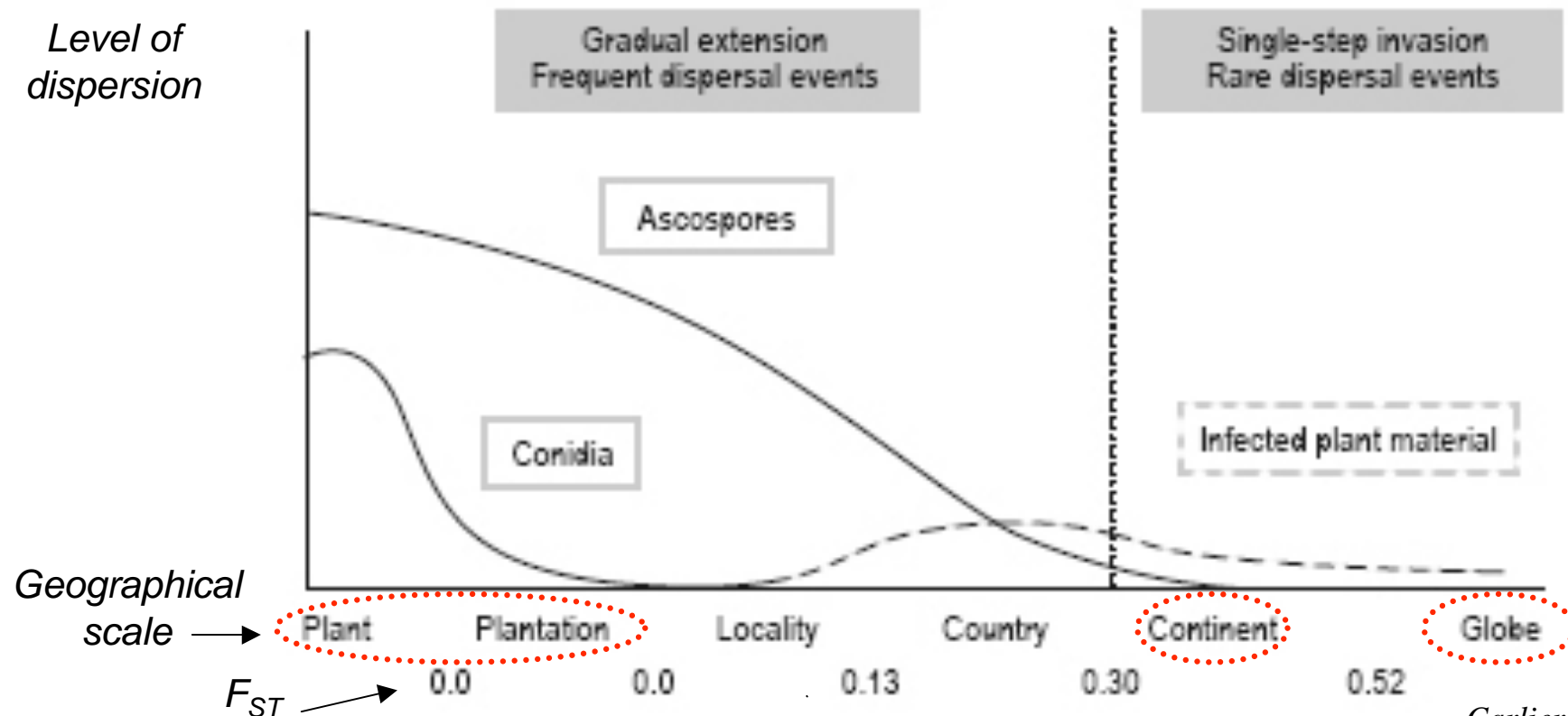
} Application of
classical
population
genetics methods

Previous studies related to the populations genetic structure of *Mycosphaerella fijiensis*

- Different scales & disease dissemination modes :

→ High level of genetic diversity

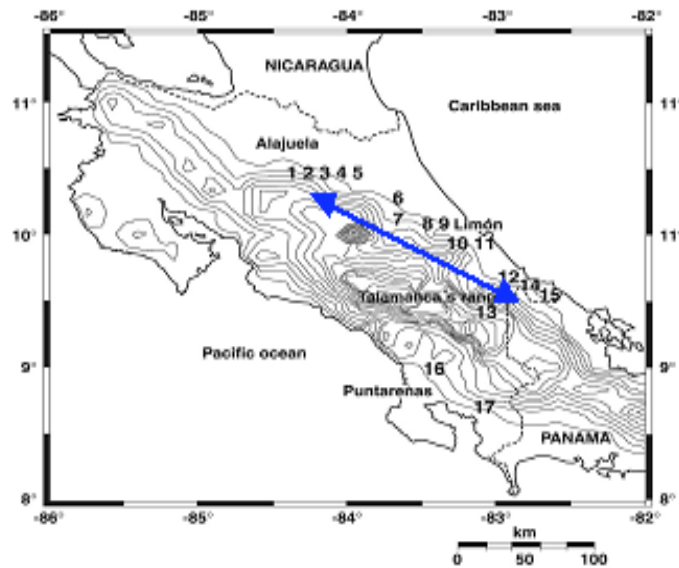
→ Relative importance of the dispersal modes of *M.fijiensis* as a function of geographical scale



Carlier, 2003

Previous studies related to the populations genetic structure of *Mycosphaerella fijiensis*

- Within a production area (few 100 Km long)

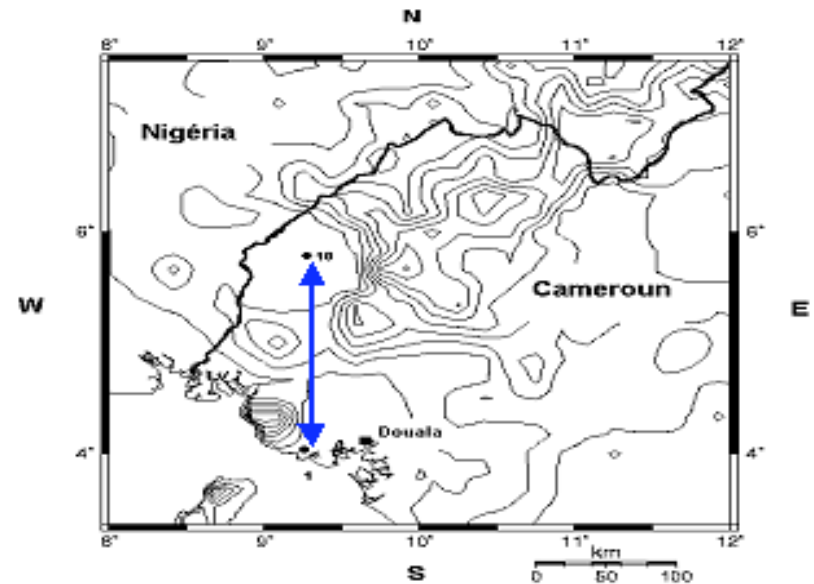


Costa Rica

Transect of 297 km

15 sites (26.4 km)

347 isolats / 7 PCR-RFLP and 9 microsatellites markers



Cameroon

Transect of 270 km

10 sites (26.8 km)

287 isolats / 15 microsatellites markers

Previous studies related to the populations genetic structure of *Mycosphaerella fijiensis*

- Within a production area (few 100 Km long)

Basic genetic analysis	Cameroon	Costa Rica
Gene diversity, H_E	0.31 to 0.46	0.41 to 0.59
Genotypic diversity, D_G	~ 1.00	~ 1.00
Linkage disequilibrium, r_D	< 0.02, NS	< 0.04 NS
Differentiation between populations, F_{st}	0.014 to 0.26	0.012 to 0.17

Previous studies related to the populations genetic structure of *Mycosphaerella fijiensis*



- Within a production area (few 100 Km long)

→ Differentiation between populations:

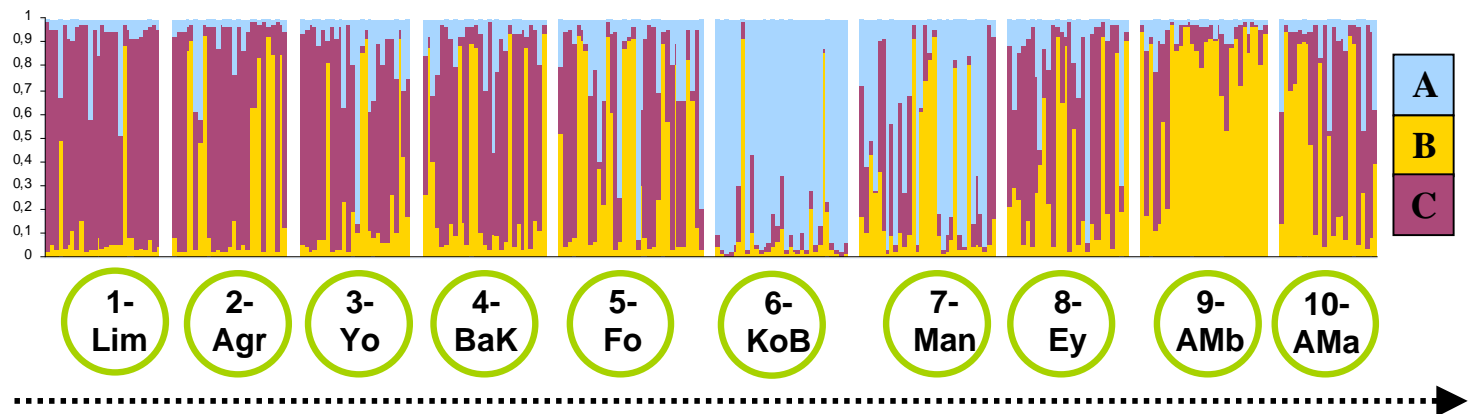
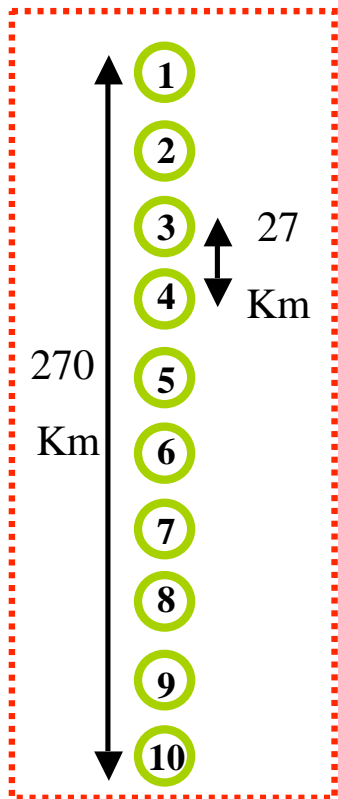
Costa Rica: $F_{st} = 0.012$ to 0.17 (Costa Rica)

Cameroon: $F_{st} = 0.014$ to 0.26 (Cameroon)

→ Clustering analysis (STRUCTURE, v2.2 — Pritchard et al., 2000)

Costa Rica: no structure detected

Cameroon: number of cluster $K = 3$



Previous studies related to the populations genetic structure of *Mycosphaerella fijiensis*



- Within a production area (few 100 Km long)

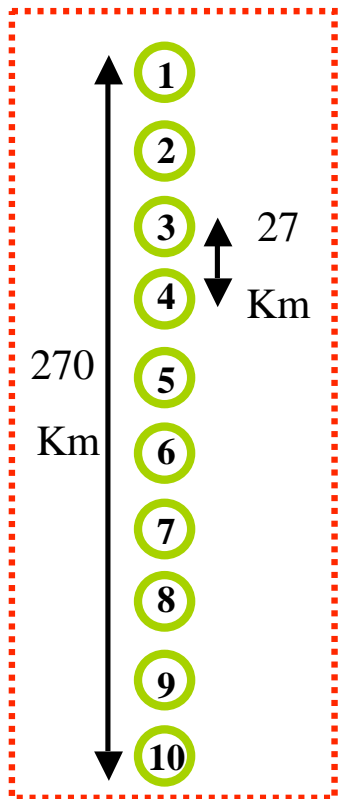
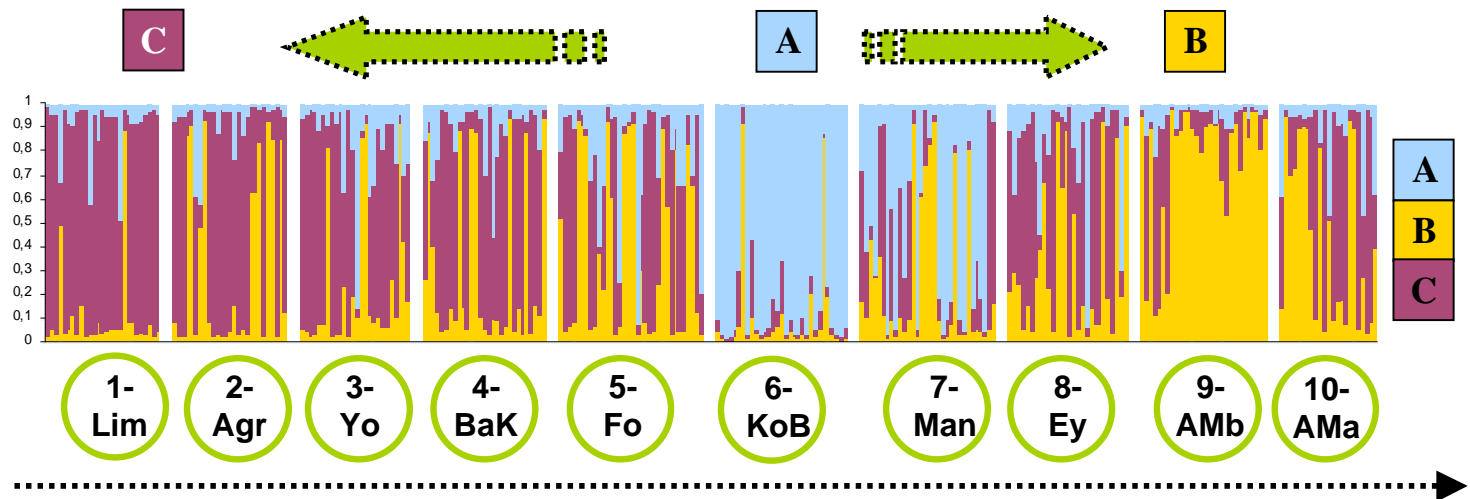
→ Differentiation between populations:

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→ Clustering analysis

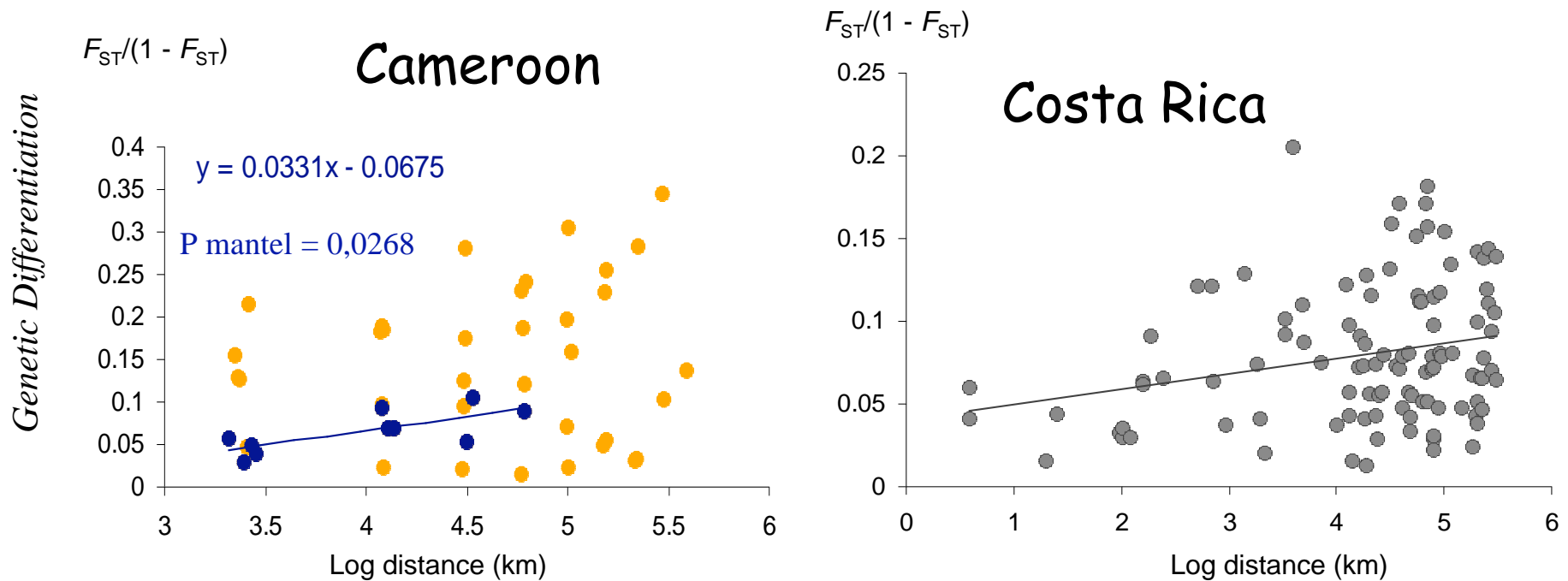
Cameroon: number of cluster $K = 3$



Previous studies related to the populations genetic structure of *Mycosphaerella fijiensis*

- Within a production area (few 100 Km long)

Isolation by distance analysis with discrete populations



- Whole transect : IBD not significant
- Sites 1 → 5 : IBD significant

- Whole transect : IBD significant



Direct method : $d \lll 1 \text{ Km}$

Previous studies related to the populations genetic structure
of *Mycosphaerella fijiensis*

- Within a production area (few 100 Km long)

Conclusions of this first study

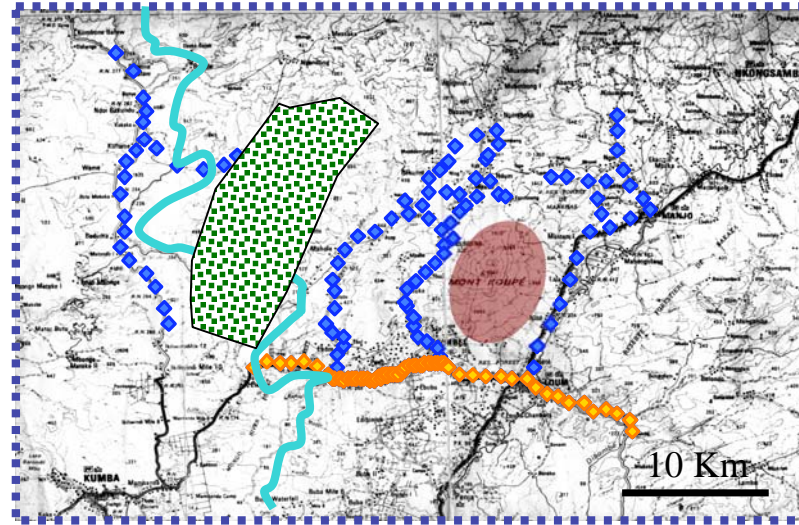
- IBD Analysis on a distance too long in regard with dispersal capacity of the pathogen (geographical scale considered still too big)
 - Effects of barriers
 - Effects of demographic events
- Unrealistic genetic model (discrete populations)

Population analysis in *M. fijiensis* at a local scale (50x50 Km)

New 2D & 3D Sampling (Cameroon)



IBD in a continuous population analysis



Landscape genetics analysis

Estimation of dispersal parameters

To delimit pathogen populations & to detect barriers to gene flow

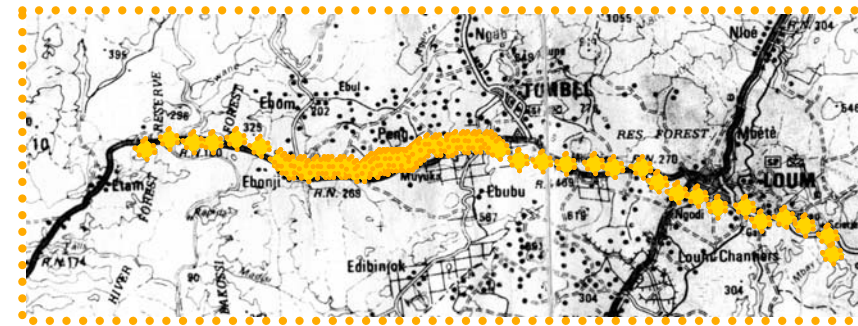
Not presented today...New analysis still under construction

Population analysis in *M. fijiensis* at a local scale (50x50 Km)

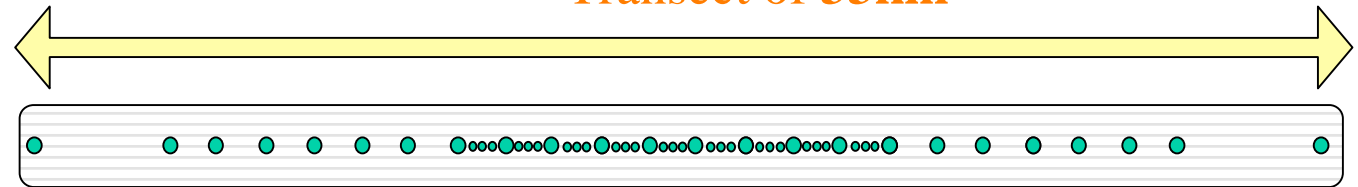
IBD in a continuous population analysis



- **Dispersal parameters estimation along a continuous population**



Transect of 33km



- 90 sites of sampling : 1Km, 250 m, 50 m
- 2-6 isolates / sites genotyped with 20 microsatellites markers
- Continuous population IBD method (haploids) (*Rousset, unpublished*)

Population analysis in *M. fijiensis* at a local scale (50x50 Km)

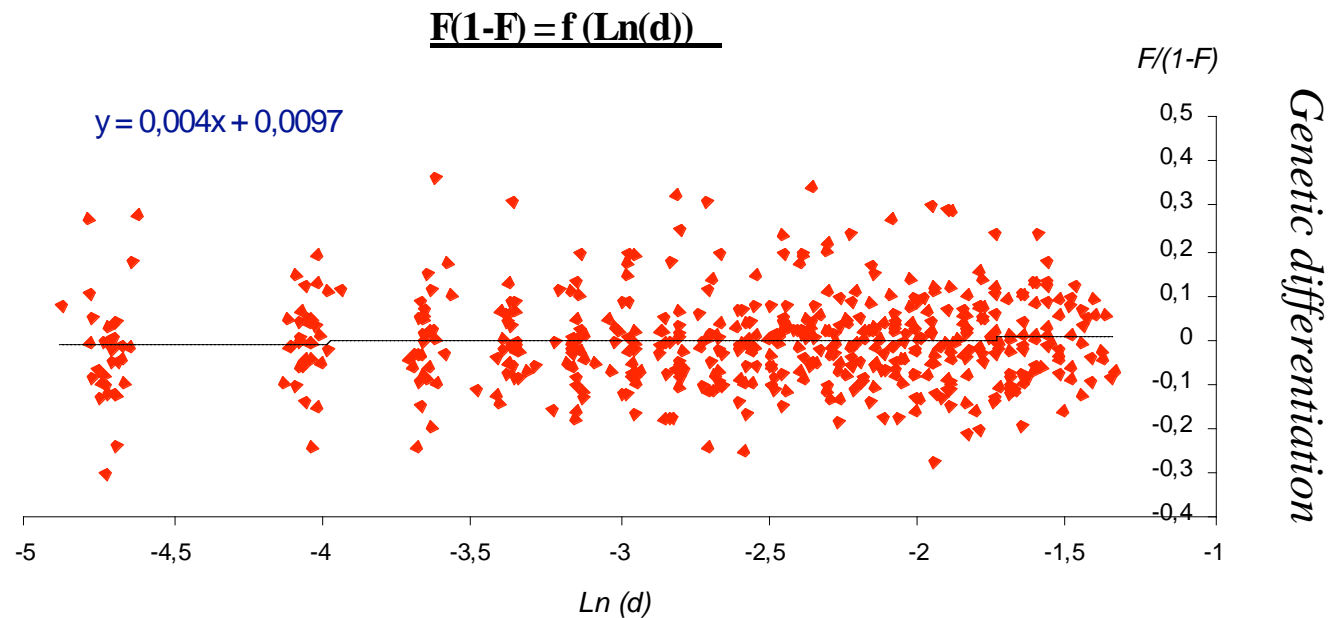
IBD in a continuous population analysis

- No IBD signal detected along the transect

$$b \approx \frac{1}{2 \pi D \sigma^2}$$

σ^2 = average squared axial parent-offspring distance

D = Density



Population analysis in *M. fijiensis* at a local scale (50x50 Km)

IBD in a continuous population analysis

How to explain an absence of IBD signal ? :

- High level of migration
- Lack of statistical power
- Method of IBD inappropriate to pathogenic fungus populations specificity

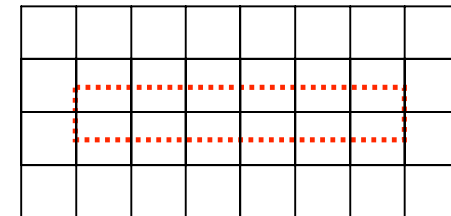
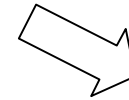
In particular : High effective size

Population analysis in *M. fijiensis* at a local scale (50x50 Km)

IBD in a continuous population analysis

• Lack of **statistical power** ?

→ Individual-based simulation model, spatially-explicit, based on the coalescence theory (*Leblois et al. 2003*)



→ Simulation of our sampling
(transect 33 km, X,Y,Z)

- Reproduction stage
- Migration stage
- Selection stage

⇒ Statistical power related to our sampling :

slope of 10^{-3} statistically detectable

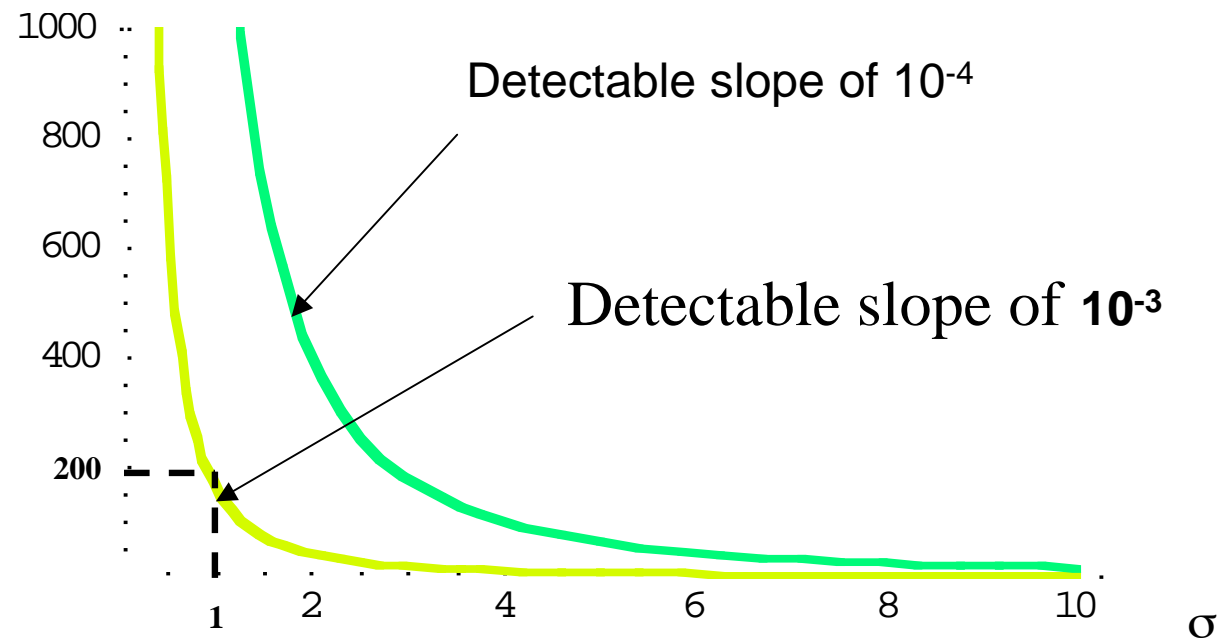
Population analysis in *M. fijiensis* at a local scale (50x50 Km)

IBD in a continuous population analysis

- **High effective size**

$$\text{Slope} = \frac{1}{2\pi N\sigma^2}$$

Effective size



Conclusions and prospects...

- IBD model might not be adapted to described dispersal processes and gene flow in *M. fijiensis*

- Test new methods to infer parameters related to dispersal processes (migration-selection models, estimation of dispersal curve, new direct methods ?...)

- Realize the spatialized genetic clustering analyse with a landscape genetics method (No *a priori* definition of populations)

- Understand the genetic structure of *M.fijiensis* through an agricol landscape

- Detect and define some eventual barriers to gene flow