APPLICATIONS FOR MICROSATELLITE MARKERS IN THE MANAGEMENT AND CONSERVATION OF FOREST TREES,

illustration with: *Baillonella toxisperma and Milicia excelsa*

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FAO International technical conference of Agricultural Biotechnologie in Developing Countries (ABDC-10)
Guadalajara, 1 – 4 March 2010

Summarize

I- Forest risks linked to disturbances ?

II- Forest Management and Conservation challenges

III- Genetic of populations as a tool for forest Conservation and Management questions ?

IV- Application with two endangered tree species of the tropical rain forest

V- Futures

I- What are the main forest risks linked to disturbances ?

- Historical: Climate changes (Glacial and hot periods during pleistocene and Holocene)

⇒ **-Restricted and expansion phases of species area ranges**
-Bottleneck (reduction of gene diversity for many tree species)

- Contemporary : intensification of Human Activities (Agricultural, logging, urbanisation……), climate changes

⇒ **-Disturbances in tree populations dynamic (spatial population structure, changes in gene flow….)**
-Disturbances in landscape (forest fragmentation, reduction of tree populations density….)

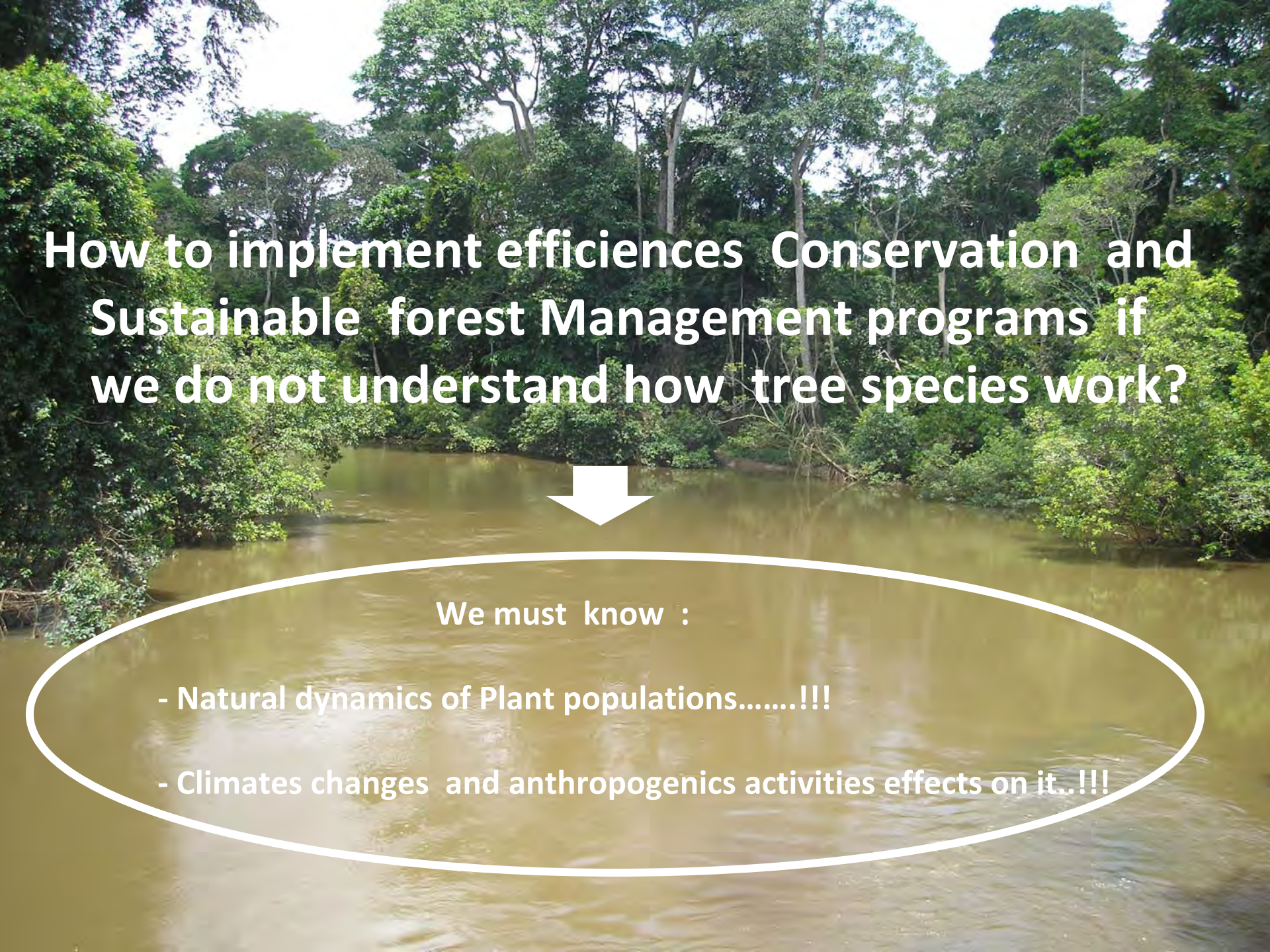
II- Forest Management and Conservation challenges

✓ Conservation and preservation of plants genetic diversity

- Reforestation programs
- Seeds bank collections (diversificated gene pools)
- Specific Protected Areas
- Preservation of endangered species

✓ Sustainable forest management

- Appropriated Minimum logging diameter (Adults tree)
- Respect of logging cycles for forest plots (20- 25 years)
- Certificated forest companies for logging (Economics statistics of logged wood, Forest plots logging planification ……)



How to implement efficiences Conservation and Sustainable forest Management programs if we do not understand how tree species work?

We must know :

- **Natural dynamics of Plant populations…….!!!**

- **Climates changes and anthropogenics activities effects on it..!!!**

III- Genetic of populations as a tool for forest Conservation and Management asks ?

What about?

- ✓ **Organisms are considered as genes and populations as gene pools.**

Main goals?

- ✓ **Study genes movement inside and among populations through space and time : Estimate genetic structure, gene flow and spatial patterns of gene movement in different landscape and geographical sites among life stages , between cohorts ….ect.**

- ✓ **Determinates ecologicals and biologicals traits that are linked to the genetic dynamic of plant populations .**

- ✓ **Issues and Recommandations for plants conservation and Forest Management**
- ✓ **Important insights to feed theoricals and biologicals models of simulations**

III-1.Microsatellites Markers : An usefull tool for assessing spatial and genetics patterns of tree populations

➟ What about?

- ✓ Sequences of tandem, tri, or tetra repetitions of nucleotids acids localised in all organisms DNA.
- ✓ Two microsats differ by their tall: polymorphism of fragment lenght
- ✓ Two most Used : Nuclear (biparental) and chloroplast (maternal) microsats

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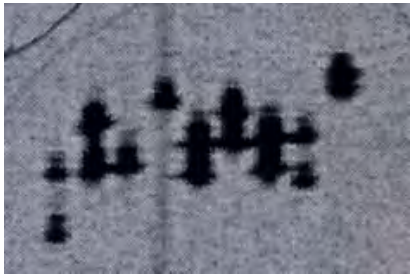


➟ Main Caracteristics ?

- ✓ High polymorphism : high power of segregation for individuals
- ✓ Good indicators of genetic diversity level
- ✓ Simple to use
- ✓ Even if……not always simple to implement robust specific microsatellite markers banks (with many highly polymorphic markers).

Usefull tools in most of Ecological and Biological studies……!!!!!

What do we know about Genetic Dynamics of very weak tree species of the tropical rain Forest?

B.toxisperma

M.excelsa

No genetic insights about..! Not enough..! nothing!!!!!!

How work tropical tree species with very weak density?

Asks:

Did these species present the same observed patterns of genetic diversity distribution as dense species?

1-What is the level of genetic diversity of a rare tropical tree specie?

2-What are the spatial patterns of genetic diversity for these kind of species?

- Spatial Genetic Structure (SGS)

- Gene flow estimates (seeds, pollen, global)

Application with *Baillonella toxisperma Pierre* (Moabi):
An endangered keystone specie of the tropical rain forest of congolese bassin (Central African Tree)

Baillonella Toxisperma , Sapotaceae

- Widespread ,Weak density : 5-6 isolated Adult /km^2

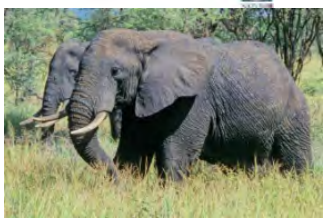
-One of the most large Guineo-Congolese bassin forest tree (Old forest): From South of Nigeria to Angola (Cabinda)

- Light demanding, Hermaphrodite, Entomophilous, Seeds Animal dispersed

- Regular fructification diameter = Minimum Logging Diameter (100 cm) …..!!!!!!

Fruits

Butter



Problem ???

Important very used tree!!!

✓All life stages predated,

✓Restricted regeneration,

✓slow growth,

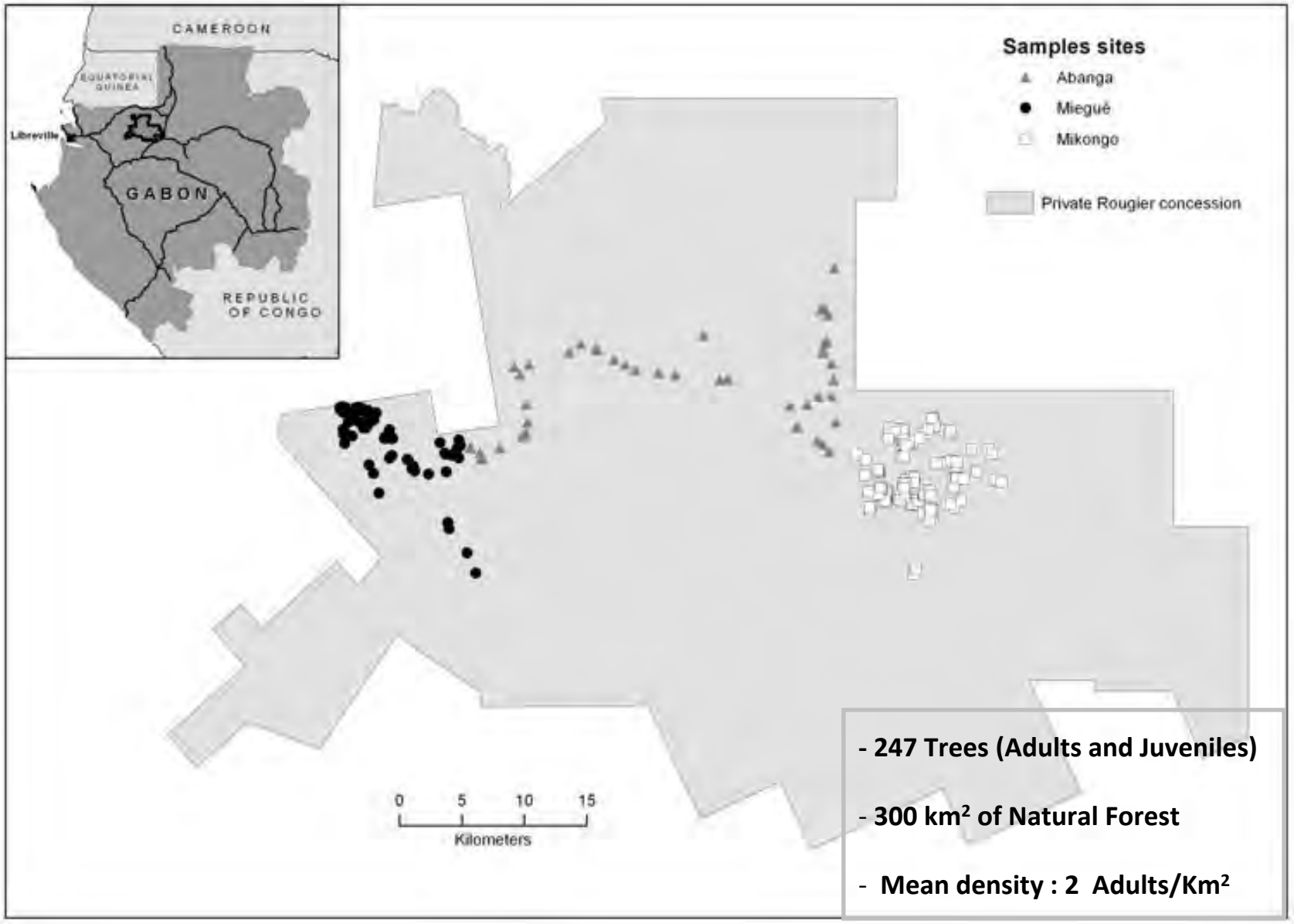
✓Logged when it begins to mate regulary (DBH=100cm)....!!!!

Seedlings

Isolated Adult tree

Joinery

Studied sites: Logging concession in Northwest of Gabon

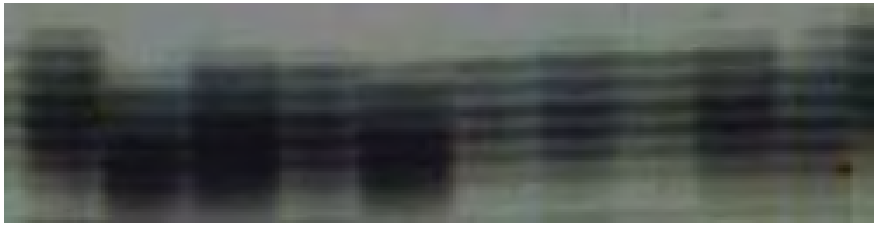


Materiels and Methods

-Molecular Biology Tools:

- 15 Nuclear microsatellites : na= 2-15
- 3 chloroplast microsatellites: na= 2-5

-Sequencing and genotyping



-Genetics of Populations softwares

✓ Spatial Autocorrelation methods

✓ Gene dispersal inference methods

1-What is the level of genetic diversity of *B.toxisperma*?

2-What are the spatial patterns of genetic diversity for these kind of species?

- Spatial Genetic Structure (SGS)
- Gene flow estimates (seeds, pollen, global)

Estimates of Genetic diversity parameters

Nuclear Microsatellites: He_{nuc} : 0.570, Similar to that of:

- ✓ The dense tropical tree specie *A.klaineana (Burseraceae)* (Born et al.2008) : He_{nuc}=0.38-0.55
- ✓ and one of the rare wind pollinated tropical tree *M.excelsa (Moraceae)*(Bizoux et al.2009): He_{nuc}=0.53-0.56

Chloroplast Microsatellites :He_{cp}: 0.761 and na_{cp}=12

- ✓ High haplotype diversity (maternal lineages) for a little scale compared to many others trees as:

- ✓ Vitellaria paradoxa (Sapotaceae) (Fontaine et al.2004) : He_{cp}: 0.71 and na_{cp}=7 in its all natural range (12 Countries).

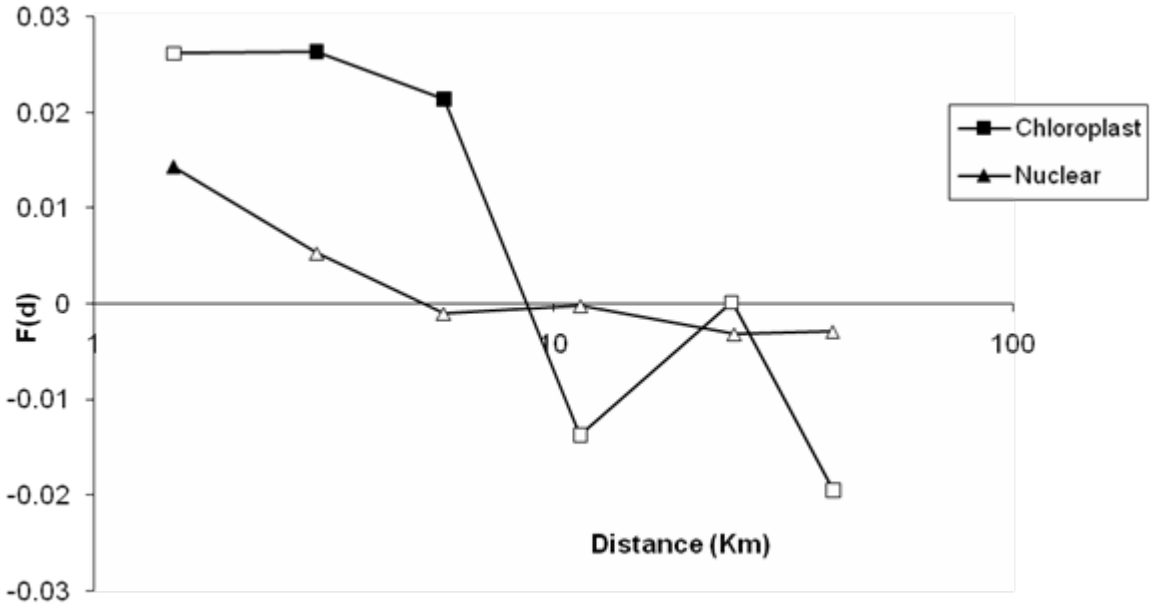
1-What is the level of genetic diversity of a rare tropical tree specie?

2-What are the spatial patterns of genetic diversity for these kind of species?

A- Spatial Genetic Structure (SGS) : How are genes organized in space?

B- Gene flow estimates (seeds, pollen, global)

Patterns of Spatial Genetic Structure (SGS)



Marker	b_{Ld}	S_p
Nuclear	-0.0032**	0.0032
Chloroplast	-0.0155**	0.0155

SGS: Kinship distance regression slope (b_{Ld}) show the Link between geographical distance and relatedness between pairwise individuals…..!!!!!!

✓**Observed SGS : show more genetic similarities between neighbors than distant individuals: Structured spatial organization of genes (individuals) inside population (not due to hazard)**

✓**Spatial extent (*Sp*) : SGS 5 times more stronger with chloroplast (haplotype) than nuclear, thus suggest restricted seeds dispersal compared to pollen.**

1-What is the level of genetic diversity of a rare tropical tree spec*y*?

2-What are the spatial patterns of genetic diversity for these kind of species?

A- Spatial Genetic Structure (SGS) : How are genes organized in space?

B- Gene flow estimates (seeds, pollen, global): How long *are B. toxisperma* genes dispersed

Inference of historical gene dispersals of pollen, seeds and global genes

According to two effective densities ($D_e/_4$;$D_e/_{10}$)

- ✓ Global gene flow : σ_g = 8.0 – 9.9km
- ✓ Seeds flow : σ_s = 4 - 6.3Km
- ✓ Pollen flow : σ_p = 9.8 – 10.8Km

•No common high gene dispersal events for an heavy diaspores tropical forest tree : probably due to efficient gene vectors

Guidelines for conservation and Forest Management

✓Increase the Minimum logging diameter (D>100cm)

- Permit to all maternal lineages to transfer their gene to many future generations

✓Seed collections even at a little spatial scale

-The weak observed SGS show that even at a fine scale we can collect much gene diversity for seeds banks with B. toxisperma

✓ Beside animals, Integration of forest inhabitants in Forest Management and Conservation programs

- The uncommon observed high seed flow highlight the strong contribution of elephant and Human in the expansion of *B.toxisperma* range. So besides to animals forest habitants have to be integrate in Conservation and Forest Management programs for this specie

Milicia excelsa (Moraceae)

Caracteristics:

-Low density : 10 Adults/km²

-Range : From Ivory Coast to Mozambic

-Dioecious, Light demanding

-Large tall , fleshy fruits

-Animal dispersed and one of the rare wind pollinated tropical tree

Problem ????

✓Intensively logged (one of the five) in Cameroon

✓Poor regeneration

Observation : *M.excelsa* stands declined in the last decades thus……. threatened specie

Spatial genetic structure in Milicia excelsa (BIZOUX, DAINOU et al.2009)

Experimentation:
Sites: 4 natural populations in Cameroon
Individuals : 287 Trees
Molecular Tools: 8 nuclear microsatellites

Results

Genetic diversity : Nuclear Microsatellites: He_{nuc}=0.38-0.55
Gene diversity similar to that of any tropical trees

✓**Spatial Genetic Structure (SGS): observed Spatial organization of gene**

✓**Intensity of SGS: Sp=0.006 , weak SGS compared to any others trees**

✓**Global gene flow (both seeds and pollen) : σg = 1.0 – 7.1km , long gene dispersal events probably due to pollen flow mediated by wind....!!!!**

■**Detection of logging effect on 2 populations presenting a high inbreeding coefficient (Fis>0): Mating between relatives probably as a consequence of pollen limitations due to Selective Logging....!!!!!**

Guidelines for conservation and Forest Management

Note that an increasing inbreeding depression in species reduces gene diversity and seedlings fitness ….!!!!, then…

- For the 2 populations presenting a high breeding coefficient :

✓ **Seed collection : collected seeds at least 10-20km from mothers tree (to avoid collecting related seeds or trees that represent a subset of genetic diversity…!!!)**

✓**Because of the observed extensive geneflow, no real risk of inbreeding effects,**
But…

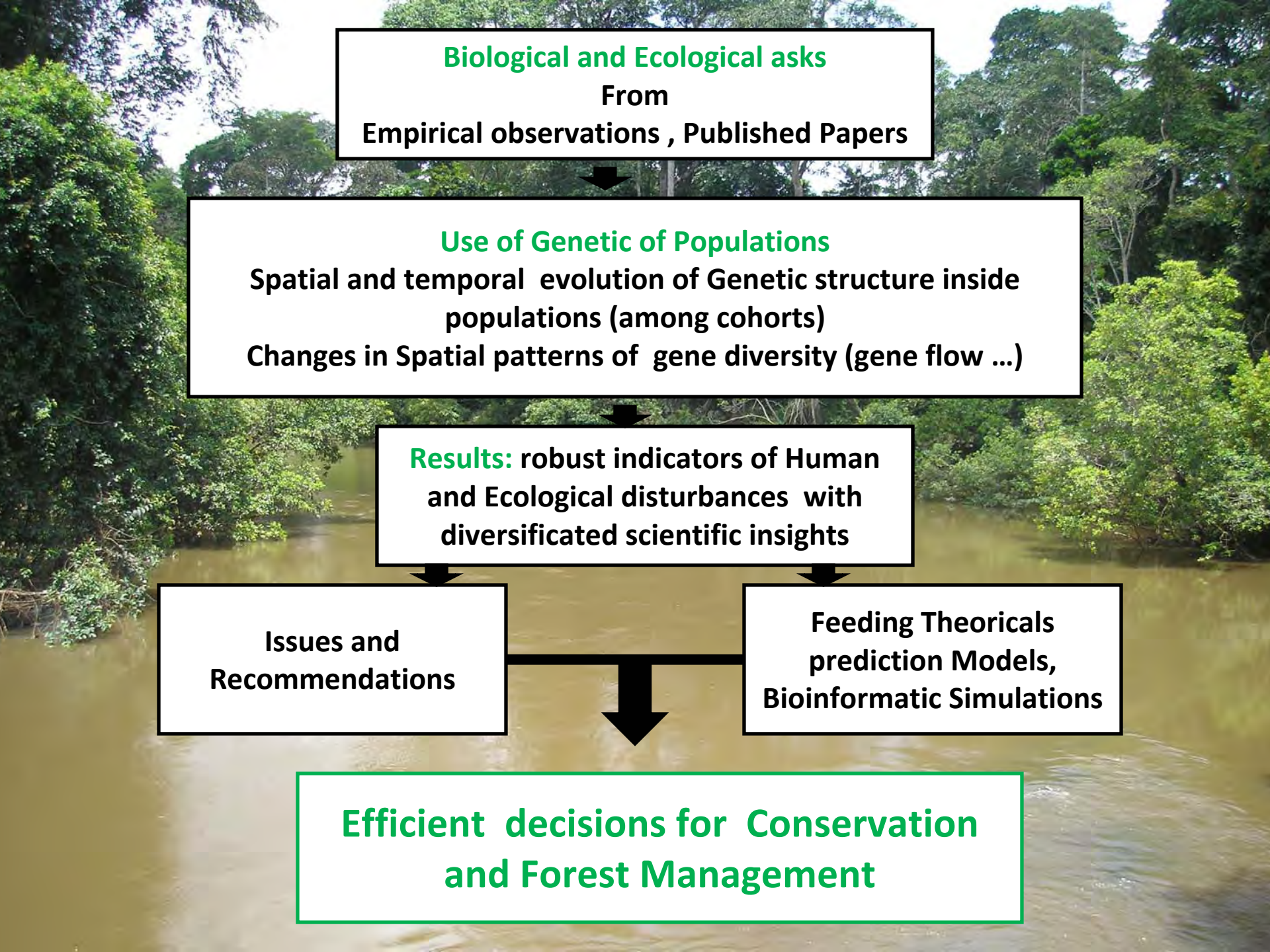
✓**Possibility to include reforestation programs with trees from different gene pools.**

•For the 2 other populations (not presenting a Fis>0) guidelines for seeds bank can be the same to that of B.toxisperma…..!!!!!

Futures for *B.toxisperma* Genetic studies

1- Spatial Genetic structures of *B.toxisperma* populations situated in the both sides of the Equator line (Gabon vs Cameroon) (Study in progress).

2- Impact of logging in spatial patterns of gene diversity with *B.toxisperma*: Parentage analysis, contemporary vs historical geneflow (Study in progress).


Biological and Ecological asks
From
Empirical observations , Published Papers
Use of Genetic of Populations
Spatial and temporal evolution of Genetic structure inside populations (among cohorts)
Changes in Spatial patterns of gene diversity (gene flow …)
Results: robust indicators of Human and Ecological disturbances with diversificated scientific insights
Issues and Recommendations
Feeding Theoricals prediction Models, Bioinformatic Simulations
Efficient decisions for Conservation and Forest Management

Thank for your attention!!!!