UNIVERSITY OF ABOMEY-CALAVI

FACULTY OF AGRONOMIC SCIENCES

POST GRADUATE SCHOOL OF AGRONOMIC SCIENCES

SUSTAINABLE MANAGEMENT OF THE REMNANT IROKO POPULATIONS IN BENIN

Structural characterization, morphological and genetic variation, and conservation strategies

Christine A. I. Nougbodé OUINSAVI

Friday, 8th december 2006

UNIVERSITY OF ABOMEY-CALAVI

FACULTY OF AGRONOMIC SCIENCES

POST GRADUATE SCHOOL OF AGRONOMIC SCIENCES

SUSTAINABLE MANAGEMENT OF THE REMNANT IROKO POPULATIONS IN BENIN

Structural characterization, morphological and genetic variation, and conservation strategies

Thesis submitted to the Faculty of Agronomic Sciences, University of Abomey-Calavi, Republic of Benin

In fufillment of the requirement for the degree of Docteur ès Sciences Agronomiques
Defended on the 8th december 2006

By: Christine A. I. Nougbodé OUINSAVI

Promotor: Prof. Dr. Ir. Nestor SOKPON

Members of Jury

- Président : M. SANNI Ambaliou, Full Professor, FAST / UAC
- Promotor : M. SOKPON Nestor, Associate Professor, FA / UP
- Examiners:
  - M. SINSIN Brice, Full Professor, FSA / UAC
  - M. KHASA Damase, Full Professor, Université Laval, QUEBEC
  - M. AHANCHEDE Adam, Associate Professor, FSA / UAC
  - M. OFORI A. Daniel, Senior Researcher, FORIG, KUMASI

GESTION DURABLE DES POPULATIONS RELIQUES D'IROKO AU BENIN

Caractérisation structurale, variabilité morphologique et génétique, et strategies de conservation

Christine A. I. Nougbodé OUINSAVI

Thèse soumise et présentée publiquement pour l'obtention du grade de Docteur ès Sciences Agronomiques

Soutenue le 08 décembre 2006

Membres du Jury

- Président : M. SANNI Ambaliou, Professeur Titulaire, FAST / UAC
- Directeur de Thèse : M. SOKPON Nestor, Maître de Conférences, FA / UP
- Examinateurs:
  - M. SINSIN Brice, Professeur Titulaire, FSA / UAC
  - M. KHASA Damase, Professeur, CEF-Laval, Université Laval, QUEBEC
  - M. AHANCHEDE Adam, Maître de Conférences, FSA / UAC
  - M. OFORI A. Daniel, Maître de Recherches, FORIG, KUMASI

To my lovely parents, Delphine M. Boko and, Augustin Z. Ouinsavi

To all those who always work for my progress in Science and who believe that there is no Development without Sustainable Research

Table of contents

RÉSUMÉ

Titre de la these : Gestion durable des populations reliques d'iroko (*Milicia excelsa* welw. C.C. Berg) au Bénin: caractérisation structurale, morphologique et génétique et stratégies de conservation

Dans la présente thèse, nous avons étudié les caractéristiques structurales, la variabilité morphologique et génétique des populations reliques d'iroko (*Milicia excesla* welw. C.C. Berg) au Bénin et fait des propositions concrètes quant aux stratégies de conservation à adopter pour améliorer et renforcer les efforts de conservation déjà fournis par les populations locales.

Le **Chapitre 1** (Introduction générale) présente la justification de l'étude et la problématique abordée, les objectifs visés et la structuration de la thèse.

Le **Chapitre 2** présente les caractéristiques générales de *Milicia excelsa* et une revue de la literature sur l'espèce. En effet *Milicia excelsa* est une espèce decidue dont les individus sont très majestueux pouvant atteindre 30 à 50 m de haut et 1,70 à 2 m de diamètre avec une cime haute, large, très dense et en forme de parapluie, croissant au bout de quelques branches. C'est une espèce dioïque qui fleurit et fructifie chaque année mais rencontre d'importants problèmes sylviculturaux dus à l'attaque de Homoptera Psyllideae, *Phytolyma lata* qui détruit les plants d'iroko et rend les plantations non attractives. Les nombreuses études antérieures ont essayé sans succès total, de lutter contre cet ennemie naturel de diverses manières. Malgré les problèmes sylvicuturaux rencontrés par cette espèce, les arbres d'iroko restent encore soumis à la pression anthropic à travers leur exploitation et la fragmentation des ecosystèmes.

Le **Chapitre 3** discute de la fragmentation des écosystèmes et sa contribution dans l'isolement des populations d'arbres en mettant l'accent sur la fragmentation des écosystèmes dans le contexte béninois. Le processus d'apparition des populations d'arbres dispersés dans le paysage végétal, ainsi que les îlots de forêts est documenté pour mieux appréhender l'importance et le rôle de ces îlots de forêts et des arbres dispersés dans le maintien de la diversité biologique. Il se révèle que la fragmentation des habitats due au couloir sec dahoméen (Dahomey gap), et la pression anthropique ont

conduit à la destruction de la végétation et l'apparition de petits îlots de forêts (essentiellement des forêts sacrées) et de nombreuses populations d'arbres distribués à travers le paysage végétal au nombre desquels les arbres de *Milicia excelsa* dont la structure démographique, morphologique et génétique mérite d'être étudiée pour des fins de conservation de l'espèce.

Le **Chapitre 4** présente les caractéristiques structurales et la variabilité morphologique des populations de *Milicia excelsa* au Bénin étudiées par la méthode d'échantillonnage par transects et par des analyses multivariées à savoir l'analyse en composante principale, le cluster analysis, et l'analyse canonique discriminante. Quatre groupes de populations d'iroko morphologiquement differents ont été identifiés et se présentent comme suit :

- Groupe 1 composé des arbres d'iroko de la population de Niaouli qui possèdent les plus grandes feuilles et un ratio Dbh/Ht plus élevé.
- Groupe 2 constitué des arbres des populations de Aplahoué, Lokossa, et Save avec les feuilles de taille moyenne et un ratio Dbh/Ht moyen.
- Groupe 3 composé des arbres d'iroko des populations de Sakete, Bohicon et Bante présentant les plus petites feuilles et un ratio Dbh/Ht plus faible.
- Groupe 4 regroupant les arbres d'iroko des populations de Ketou, Tamarou, Bassila, Biro et Djougou avec des feuilles de taille moyenne et un ratio Dbh/Ht faible.

Par ailleurs, la discrimination des groupes montre que la variation des paramètres foliaires et la croissance des arbres sont fortement liées aux facteurs édaphiques tandis que la variation des paramètres mesurés sur les fleurs est liée à la pluviométrie. La caractérisation structurale montre une faible densité de population et de surface terrière pour toutes les populations. De plus la répartition par classe de diamètre des individus d'iroko présente une structure érractique avec des classes de diamètre vides bien que la plupart des courbes d'ajustement présentent une allure en cloche.

Le **Chapitre 5** présente la variabilité génétique de *Milicia excelsa*, sur la base des marqueurs RAPD (Random Amplified Polymorphic DNA). La variabilité révélée à partir de sept amorces est modérée de même que la valeur du coefficient de différentiation génétique entre populations (G_{ST} = 0.331); ce qui signifie que 33 % de la variation totale est inter- population. Le flux de gènes estimé est assez faible Nm = 1.01 indiquant un faible échange de graine et de pollen entre les populations. En outre trois barrières génétiques ont été distinguées divisant l'ensemble des populations en quatre

groupes séparés par trois principales zones de rupture génétique et un faible flux de gène probablement dû à un effet d'isolement par distance.

Dans le **Chapitre 6**, onze marqueurs microsatellites ont été mis au point pour *Milicia excelsa* en vue d'étudier avec plus de précision, la structure génétique et pour améliorer le plan de conservation de l'espèce. Une librairie génomique enrichie a été construite et a permis de séquencer 188 clones suivant les deux orientations. Au total 44 paires d'amorces oligonucléotidiques ont été synthétisées et testées au nombre desquelles les 11 paires amorces ayant présenté une bonne amplification et un polymorphisme élevé ont été évaluées à l'aide de l'ADN total génomique extrait aussi bien de *Milicia excelsa* que de l'espèce voisine, *M. regia*; Ce qui signifie que les amorces ainsi élaborées sont valables pour être utilisées dans l'étude de la génétique et de l'écologie d'autres espèces du genre *Milicia*.

Le **Chapitre 7** présente de façon beaucoup plus détaillée et plus précise la structure de la variabilité génétique de *Milicia excelsa* sur la base des marqueurs microsatellites. Les résultats de ce chapitre confirment l'existence d'une structuration génétique au sein des populations d'iroko au Bénin, précédemment révélée par les marqueurs RAPD. De même une différentiation génétique modérée mais statistiquement significative est observée entre les populations d'iroko. Toutes les populations présentent un déficit d'hétérozygotie pour le test d'équilibre de Hardy Weinberg avec des coefficients de consanguinité significativement positifs. Il existe également une corrélation significativement positive entre les distances génétiques et les distances géographiques ($r = 0.432$; $P = 0.007$, test de Mantel) indiquant que les populations sont différenciées sur la base d'un isolement par la distance. Le "Bayesian analysis of population structure" montre une division de la variabilité génétique en quatre révélant ainsi l'existence d'une hétérogénéité dans la structure génétique des populations d'iroko au Bénin. En somme ces divers résultats révèlent l'existence d'une structuration géographique de la variabilité génétique de *Milicia excelsa*.

Le **Chapitre 8**: présente l'importance socioéconomique et culturelle de l'iroko pour les populations béninoises et les stratégies traditionnelles de conservation de l'espèce. Il ressort de cette étude que l'iroko contribue au traitement de 45 maladies. Tous les organes de la plante sont utilisés à des degrés divers. L'iroko est aussi utilisé dans l'artisanat comme bois d'oeuvre en menuiserie et en sculpture et cette forme d'utilisation de l'arbre représente la plus grande menace de destruction pour l'espèce. Du fait de sa grande importance culturelle, l'arbre d'iroko est sacré et les individus

reliques sont donc protégés et conservés au champ, dans les forêts sacrées, dans les cimétières et au niveau des couvents fétiches, par les pratiques ethnobotaniques de conservation. Divers objets sont utilisés pour matérialiser les arbres d'iroko sacrés. Cependant ces pratiques de conservation ne protègent pas strictement les arbres d'iroko et méritent donc d'être renforcées par d'autres stratégies plus efficaces de conservation.

Dans le **Chapitre 9**, nous avons utilisé les informations obtenues au niveau des chapitres précédents pour proposer les stratégies de conservation les mieux adaptées à l'iroko au Bénin. La conservation i*n situ* est la plus indiquée pour cette espèce avec un effort complémentaire pour favoriser le flux de gènes entre les groupes de populations identifiés. Pour cela et étant donné que *Milicia excelsa* commence déjà par être intégré au système agroforestier au Bénin, la structure écologique de ces systèmes agroforestiers a été étudiée ainsi que les paramètres nécessaires à la réhabilitation de l'espèce au niveau des exploitations agricoles. Ainsi des densités optimales de conservation des arbres d'iroko ont été suggérées sur la base des caractéristiques morphologiques de l'arbre et de sa densité actuelle au niveau de différentes exploitations à travers le pays. Trois options ont été faites pour l'amélioration de la structure de l'iroko dans les exploitations agricoles qui serviront de couloir biologique de flux génétique interconnectant les populations rémanentes de cette espèce.

Le **Chapitre 10** présente une discussion générale des différents résultats. La fragmentation des habitats a eu une influence négative sur la structure démographique, morphologique et génétique de *Milicia excelsa.* Les groupes formés sur la base de la variabilité morphologique ne correspondent pas exactement à ceux génétiquement formés, ce qui signifie que la croissance aérienne de l'arbre et les dimensions foliaires sont beaucoup plus dues aux facteurs liés à l'environnement.

SUMMARY

In the current study we assessed structural characterization, morphological and genetic variation in the remnant iroko (*Milicia excesla* welw. C.C. Berg) populations in Benin and made some practical suggestions in order to improve in situ conservation of the species.

In **Chapter 1** (general introduction), we presented the study's background, objectives and the thesis structure.

Chapter 2 presented general characteristics of *Milicia excelsa* and an overview of previous studies which dealt with the species. In fact *Milicia excelsa* is a large deciduous tree up to 30-50 m height, with a diameter of 1.70 – 2 m and a high, umbrella-like crown, growing from a few thick branches. It is a dioeicious species that flowers and fruits every year but faces important silvicultural problems due to its attack by the Homoptera Psyllideae, *Phytolyma lata* which damage iroko trees and make plantations non attractive. Most of the previous studies attempted to control the pest in various ways without fully success. Despite the silvicultural problems faced by the species, iroko trees were still subjected to natural and human-caused pressure through tree logging and habitat fragmentation.

In **Chapter 3**, habitat fragmentation and its implications in tree populations' isolation were assessed with emphasis on fragmentation patterns and extent in Benin. The process of occurrence of tree populations and tree parklands as well as isolated small vegetation patches were reviewed to better emphasize their importance and their critical role in maintaining biological diversity. Apart from strong climate oscillation during the pleistocene which separated the West Africa guinean forest bloc into west and east blocs creating the Dahomey gap and consequently a forest-savannah mosaic vegetation type in Benin, human caused habitat fragmentation through continuous land clearing for agriculture, extensive forests exploitation and urbanization induced the occurrence of many isolated forest plots and trees species among which *Milicia excelsa* trees. As fragmentation was proved to have deleterous effects on genetic diversity within a species and its morphological structure, it was of interest to investigate the current demographic, morphological and genetic structure of *Milicia excelsa* in Benin before coming up with conservation strategies.

In **Chapter 4**, morphological variation and ecological structure of *Milicia excelsa* populations were assessed in Benin using transect sampling method and

multivariate analyses including principal component, cluster and canonical discriminant analysis. Four morphologically different groups of iroko populations were identified as followed:

- Group 1 composed of individuals from Niaouli region, which have the highest ratio dbh/TH and largest leaf sizes.
- Group 2 made of trees from Aplahoue, Lokossa, and Save regions with medium leaf size and dbh/TH.
- Group 3 composed of trees from Sakete, Bohicon and Bante regions showing the smallest leaf size and lowest dbh/TH.
- Group 4 gathering trees from Ketou, Tamarou, Bassila, Biro and Djougou regions with medium leaf size and low dbh/TH.

Moreover, discrimination of groups indicated that variation in total height growth and leaf size was highly correlated to edaphic factors while flowers size variation was best explained by annual rainfall. Ecological structure showed low stand density and hence low stand basal area for all of iroko populations. In addition, erratic diameter distribution was found for many populations with large gap in their diameter distribution, while most of them showed bell-shaped diameter distribution.

Chapter 5 revealed the level of genetic variation and pattern of genetic structure in populations of *Milicia excelsa*, based on Random Amplified Polymorphic DNA (RAPD) markers. Based on seven primers, genetic variation and genetic differentiation among populations were moderate (G_{ST} = 0.331) indicating that 33 % of the total genetic variation accounted for among-population differentiation. Estimated gene flow equaled to Nm = 1.01 showing limited seed and pollen dispersal among populations. In addition three genetic barriers were distinguished separating iroko populations into four groups by three main zones with sharp genetic change and lower gene flow, indicating effect of isolation by distance. Based on the various discussions on the accuracy of RAPD markers, genetic variation and population structure were reinvestigated using microsatellite markers.

In **Chapter 6**, eleven microsatellite primer pairs were developed for *Milicia excelsa* to accurately estimate the genetic diversity and population structure for improved conservation planning of the species. Enriched genomic DNA library was constructed from which 188 random clones were sequenced from both orientations. We designed and tested 44 oligonucleotide primer pairs of which the 11 primers that showed high amplification and high polymorphism were evaluated using genomic DNA

from both *Milicia excelsa* and *M. regia* indicating that the designed primers were useful for population and ecology genetic studies for other species of the genus *Milicia*

Chapter 7 presented a more detailed structure of genetic variation in *Milicia excelsa* using microsatellite markers. Results in this chapter confirmed the existence of genetic structure in *Milicia* species in Benin previously revealed by RAPD markers. Considerable genetic variability was detected for all populations at seven microsatellite loci. Moderate but statistically significant genetic differentiation was found among populations. All of the populations showed heterozygosity deficits in test of Hardy-Weinberg Equilibrium and significantly positive F_{IS} values due to inbreeding occurring in the species. Pairwise F_{ST} values were positively and significantly correlated with geographical distances (r = 0.432; P = 0.007, Mantel's test) indicating that populations are differentiated by 'isolation by distance'. Bayesian analysis of population structure showed clustering of the genetic variation into four groups revealing the existence of heterogeneity in population genetic structure. Altogether, these results indicated that genetic variation in *Milicia excelsa* is geographically structured.

Chapter 8 assessed socioeconomic and cultural importance of the species to Benin people and traditional strategies of conservation. It was found that *Milicia excesla* contribute to cure 45 diseases. All of the components of the tree are used in various proportions. Iroko tree is also highly exploited for timbers which are used in carpentry and joinery and this type of use represent the main threat on the species. Because of the high cultural value of the species, it is regarded as sacred and the remnant trees are protected on farm, in sacred groves, in cemeteries, and isolated sites of fetish worship by traditional ethnobotany practices, using various sacred objects to materialize the sacred trees. But these traditional strategies of conservation are not totally efficient and need to be reinforced.

In **Chapter 9** we used information gained from the above studies to suggest the most suitable conservation strategies for *Milicia excelsa* conservation in Benin. The four chapters presented above indicated that *Milicia excelsa* populations underwent an effect of isolation by distance and that *in situ* conservation is the most indicated for the species conservation with a need to favor gene flow among the groups of iroko populations. Therefore traditional agroforestry systems were assessed for their ecological structure and iroko status and management in farmlands. Then optimal iroko densities were suggested based on the morphological features (Crown diameter, height) and current stem number per hectare of the species in different regions across the country. Three

options were made to improve iroko structure in agroforestry systems which will serve as gene flow biological corridors for interconnecting these remnant populations.

Chapter 10 presented the general discussion of the different issues. Habitat fragmentation appeared to have influenced *Milicia excelsa* demographic, morphological and genetic structure. The groups formed on the basis of morphological variation did not correspond exactly to those depicted from genetic variation indicating that leaf size and the ratio dbh/TH variation in *Milicia excelsa* in Benin was highly influenced by environmental factors.

1

General introduction

1.1- Background

The world's tropical rain forests are the most important ecosystems in terms of biological diversity. They are by far richer in species diversity than any other biome but, they are changing due to rapid degradation and destruction (Myers, 1991; Grainger, 1993; FAO, 2000). Most studies estimate that about half of the earth's land area was covered by forests 8 000 years ago as opposed to 30 percent today (e.g. FAO, 2000; Ball, 2001). Based on comprehensive analysis of the latest inventory data available for each country, FAO (2000) estimated that the world's forests covered 3,869 million hectares in 2000, of which about 95 percent is natural forest and 5 percent forest plantations. The net change in forest area was estimated at –9.4 million hectares per year, representing the difference between a deforestation rate of 14.6 million hectares per year of natural forest, and an expansion of 5.2 million hectares per year (3.6 million hectares per year of natural forest regrowth and 1.6 million hectares per year of plantations by afforestation). In addition, 1.5 million hectares per year of natural forest were converted to forest plantations (FA0, 2001). It is noteworthy that most of the forest losses were in the tropics (Goldsmith, 1998).

Since habitat destruction and fragmentation are the major causes of species extinction (Ehrlich and Ehrlich, 1981; Lawton and May, 1995), this is the greatest conservation crisis. Indeed, increasing human populations and attendant land-use intensification (e.g., cultivation, grazing, urban development) resulted in the loss and subdivision of native habitats (Saunders *et al.*, 1991 Flather and Bevers, 2002), increasing species extinction rates (Pimm *et al.*, 1995), and lowered species diversity within managed ecosystems (Rapport *et al.*, 1985). Previous to that or concomitantly, several strong climate oscillations which occurred during the Pliocene affected vegetation shape and species distribution all over the world. Indeed, the forest-savannah mosaic vegetation type observed in the Dahomey gap was reported by several authors as the result of mid-Holocene marine transgression, followed by drier and wetter climatic conditions successively (Booth, 1958; Mayr and O'Hara, 1986; Dupont & Weinelt,

1996; Salzmann and Hoelzmann, 2005). This phenomenon could be referred to as natural habitat loss in terms of changes in landscape composition that might have caused a proportional loss of individuals from the landscape and, to natural habitat fragmentation in terms of additional effects resulting from the configuration of habitat (reduction of habitat patch size and isolation of patches, Andren, 1994; Wiegand *et al.*, 2005). Adaptation of a species to these variations may produce different morphological and physiological characteristics, resulting in the development of ecotypes. In addition, such fragmentation of natural plant communities can have deleterious effects on the genetic diversity within a species because there will be a decreasing in levels of gene flow, particularly over longer distances. The subsequent effects of genetic drift in small, isolated populations will lead to loss of diversity, leaving plants less able to adapt to changes in their environment and ultimately increasing the risk of extinction (Keller and Weller, 2002; Wilson and Provan, 2003). Indeed, many species faced extinction given the current rate of habitat loss and degradation (Ehrlich and Ehrlich, 1981; Tilman *et al*, 1994; Huxel and Hastings, 1999). Then, patterns of population genetic structure within plant species are shaped by the interaction of many factors some of which are intrinsic to the species life history (breeding system, modes of seed and pollen dispersal, life form) whereas others are perturbations induced by natural processes such as climatic changes (Heuertz *et al.*, 2004) and the multiple interactions between human and the complex functioning of forest ecosystem.

Iroko (*Milicia excelsa*) is a commercially important timber tree species formerly known by local people in Benin. Because of the highly attractive technological properties of its wood and its multipurpose uses, the species was subjected to intensive human pressure. According to Ouinsavi (2000), iroko lumbers and veneers became very scarce on wood market as well as in sawmills and carpentries where furniture made with iroko wood were more than two decades old. It is therefore very urgent to think of conservation and sustainable use of the species genetic resources.

Milicia excelsa species has been widely investigated through phytopathology research works (e.g., Cobbinah and Wagner, 1995; Nichols *et al.*, 1999; Bosu *et al.*, 2000; Ofori *et al.*, 2000) but studies dealing with genetic characterization are still scarce and result in *Milicia* species genetic variation assessment with emphasis on the geographical distribution of *Milicia* species in different ecological zones of Ghana, Ivory Coast and Sierra Leone (Ofori *et al.*, 2001).

According to Chevallier (1999), the main goal of forest genetic resources conservation is to preserve the various processes which maintain populations' genetic diversity. Meanwhile, sustainable management of renewable natural resources requires

analysis and master of the multiple interactions between human and the complex functioning of forest ecosystem. The first aspect to be addressed is the population structure of the remnant iroko trees in terms of ecological, morphological and genetic structure. Ecological structure assessment appeared to be necessary as habitat for plant species are naturally fragmented and most species occur in a number of discrete populations (Bonnin *et al*., 2002). The remnant iroko trees occur parsely distributed across natural landscape and continuous reduction of population size due to natural changes of climatic and soil conditions, human caused habitat fragmentation may disturb the genetic integrity of iroko population by affecting the size and degree of isolation of individual populations, thus contribution to genetic impoverishment and adaptive flexibility and consequently morphological structure of the populations.

Therefore, to come up with conservation strategies of *Milicia excelsa*, several research questions remained not yet addressed by previous research works among which the following questions: Did *Milicia excelsa* reamnant trees populations differ morphologically and genetically? How can these variations be assessed? For which reasons people preserve iroko trees and what are their strategies of conservation? How can *Milicia excelsa* genetic resources be efficiently conserved and managed in Benin?

1.2- Objectives

Global Objective

The global objective of this study is to assess possibilities of sustainable management of the remnant iroko populations in Benin and find out whether whether these isolated trees and populations differ morphologically and/or genetically from each other.

The specific Objectives are to:

1- Establish distribution map of remnant iroko populations in Benin and to characterize their structure.

2- Assess morphological variation in iroko populations in Benin.

3- Assess genetic variation and population structure in *Milicia excelsa*

4- Analyse socio – economic and cultural importance of iroko and find out the main reasons which undertake its preservation by farmers in Benin.

5- Explore the potentiality of agroforestry systems; improve on them and to use them as tools for an efficient *Milicia excelsa* genetic resources conservation.

1.3- Thesis Structure

The thesis is composed of 10 chapters among which 7 chapters (Chapters 4 to 10) presented different methods used for data collection and analysis, the obtained results, discussion and conclusions while the 3 other chapters dealt with the general introduction, the species characteristics, habitat fragmentation and consequences on tree populations' isolation.

So, I presented in a general introduction (Chapter 1) the loss of biological diversity of forest ecosystems due to rapid degradation and habitat destruction, and emphasized the priority need to assess the different processes that maintain *Milicia excelsa* genetic resources. To carry out a complete research work on *Milicia excelsa*, it was of interest to get some more detailed descriptive information on the species.

Chapter 2 presented the studied species through its botanical description, ecological features as well as silvicultural problems and previous investigations on pest and pathogen control.

In Chapter 3, I presented an overview of habitat fragmentation and its consequences on tree populations' isolation with a particular emphasis on its patterns and extent in Benin. It appeared that habitat fragmentation due to Dahomey gap and human activities has lead to the vegetation destruction and occurrences of small patches (mainly sacred groves) and many sparsely distributed tree populations among which *Milicia excelsa* which need to be investigated for its current demographic, morphological and genetic structure for conservation purposes.

In Chapter 4, I used trees inventory and measurement to characterize iroko population structure. Twelve iroko populations were drawn from geographical distibution. These populations were assessed for morphological variation.

In Chapter 5, I carried out molecular analysis to investigate *Milicia excelsa* genetic structure using RAPD markers.

As there were no existing microsatellite markers for *Milicia excelsa* in the international gene bank, I developped and optimized 11 microsatellite primers pairs for the genus *Milicia* (Chapter 6).

In Chapter 7, the developed microsatellite primers were used to assess more accurately *Milicia excelsa* population structure.

In Chapter 8 I highlighted the socioeconomic importance of the species showing utilization and traditional strategies of iroko conservation, management and use in Benin. It appeared that iroko trees are preserved on farms and in sacred places. However the species is still under threat because of its usefulness.

In **Chapter 9** we used information gained from the above studies to suggest the most suitable conservation strategies for *Milicia excelsa* conservation in Benin. I*n situ* conservation is the most indicated for the species conservation with a need to favor gene flow among the groups of iroko populations. Therefore traditional agroforestry systems were assessed for their ecological structure and iroko status and management in farmlands. Then suggestions were made to improve iroko structure in agroforestry systems which will serve as gene flow biological corridors for interconnecting these remnant populations.

Chapter 10 presented the general discussion of the different issues. Habitat fragmentation appeared to have influenced *Milicia excelsa* demographic, morphological and genetic structure. The groups formed on the basis of morphological variation did not correspond exactly to those depicted from genetic variation indicating that leaf size and the ratio dbh/TH variation in *Milicia excelsa* in Benin was highly influenced by environmental factors.

2

General characteristics of the species

2.1- Botanical and biological features of Milicia excelsa

Milicia excelsa (formerly *Chlorophora excelsa*) welw C.C. Berg. commercially known as iroko, is from Moraceae family, Urticaceae order, Tracheophyta phylum and planteae kingdom. It is a large deciduous tree up to 30-50 m height, with a diameter of 1.70 – 2 m (Fig. 2.1 *a*). Its bark is thick, pale, ash grey to nearly black, then brown, usually fairly rough and flaking off in small scales, but seldom fissured. The slash is thick, fibrous, cream coloured with brown spots, exuding white latex. Iroko has a lofty trunk, straight and cylindrical, up to 25 m or more to the 1st branches, usually with short, blunt buttresses. Iroko tree's crown is high, umbrella-like and growing from a few thick branches. Its thick branchlets, rather zigzag and angular are all more or less horizontal. Branches of female trees hang down but male individuals have upright branches.

Milicia excelsa leaves are simple and alternate and the stipules caduceus (Fig. 2.1 *b* and *c*). Leaves of seedlings, unlike those of the mature trees have serrated edges. Leaves in young trees are sandpapery and green above, paler and pubescent below while older leaves often become a bright yellow, serrulate at the margin, simple, alternate, 9 to 20 cm long and 5 to 10 cm wide, broadly elliptic or ovate, very shortly acuminate, usually unequally glabrous above and beneath except for minute hairs between the network of veins. About 15 pairs thick parallel, upcurving, pale-coloured lateral nerves, very prominent beneath and looped close to the margin. The ultimate veins are thick, forming a highly characteristic, more or less rectangular network on the under surface. The base is subcordate and the apex shortly acuminate with edge finely toothed. The stalk (2.5 to 6 cm), is stout and glabrous.

Milicia excelsa is a dioeicious species. According to Nyong'o *et al.* (1994), male and female trees have somewhat different appearances. Males tend to have a trunk which is longer and more slender and crowns which are smaller, narrower, with lighter colour foliage. Male and female flowers appear on separate trees and they are borne on single spikes in the axils of young leaves (Fig. 2.2 *a* and *b*). Male flowers are white in a

slender catkin with 6 to 8 mm large, closely crowded on pendulous, slender catkins (spikes) 15-20 cm long, dangling from twigs of the outer crown (Fig. 2.2 *c*). Female trees produce erect flower spikes about 5-6 cm long and 2 cm thick. Female flowers are greenish, in shorter and much fatter spikes, the styles of each flower project so that the inflorescence appears hairy (Fig. 2.2 *d*).

Fruit, 5 to 7.5 cm long with a diameter of 2 to 2.5 cm, are arranged along a longitudinal axis with 1 seed on each side, green, wrinkled, fleshy and resembling a fat green caterpillar; no change in the colour of the syncarp when mature, but the flesh between the actual fruit softens (Fig. 2.2 *e*). Seeds are hard, small and lie in the pulp (Fig. 2.2 *f*).

Flowering occured from January to April with a pic in February (Aubreville, 1959). Nyong'o *et al.* (1994) have reported that flowering begin in male trees, about three to four weeks before female flowers start flowering. According to these authors, male iroko trees appear to flower every year while some female trees may fail to flower some years. *Milicia excelsa* is an extremly allogamous species. Pollinisation is by wind, often during a period (December to January) when the trees have lost their leaves. Fruit become mature 5 to 6 weeks after fertilization and premature abscission of fruit is common (Keay, 1989). According to Nyong'o *et al.* (1994), the mean number of seed per strobilum is approximately 73, and the mean number of seed per kg of fruit is 404. Many of the fruits fall beneath parent trees while, others are dispersed by birds and especially by bats (Taylor, 1960).

The fruit bat *Eidolon helvum*, feeds on *Milicia* fruits and is considered as the principal agent of dispersal of this giant African tree both quantitatively and qualitatively. Taylor *et al.* (2000), through the most conservative estimate suggested that the Kumasi *Eidolon* colony disperses more than 140 million *Milicia* seeds in a single night, and that bat dispersal may increase seed survival and germination rates. These authors have also demonstrated that faecal iroko seeds have higher germination rate than rejecta seeds. This was explained by the fact that *Eidolon* feeds on *Milicia* fruit by sucking to select the more viable seeds, while immature, deformed or aborted seeds remain in the rejecta pellet hence, the low percent germination. A part from bat, it was noticed that several other agents are involved in the *Milicia* seeds dispersion among which, the red-crowned parrot which defecate seeds primarily in the seed-hostile environment below parent tree crowns, elephants and monkeys or duikers which probably kill and defecate many seeds in large clumps which predisposes them to seed predation, seedling competition, or fungal attack. It is noteworthy that these agents are poor dispersers compare to fruit bat.

a

b

c

Figure 2.1: *Milicia excelsa* tree. *(a, the whole tree; b, seedling; c, leaves)*

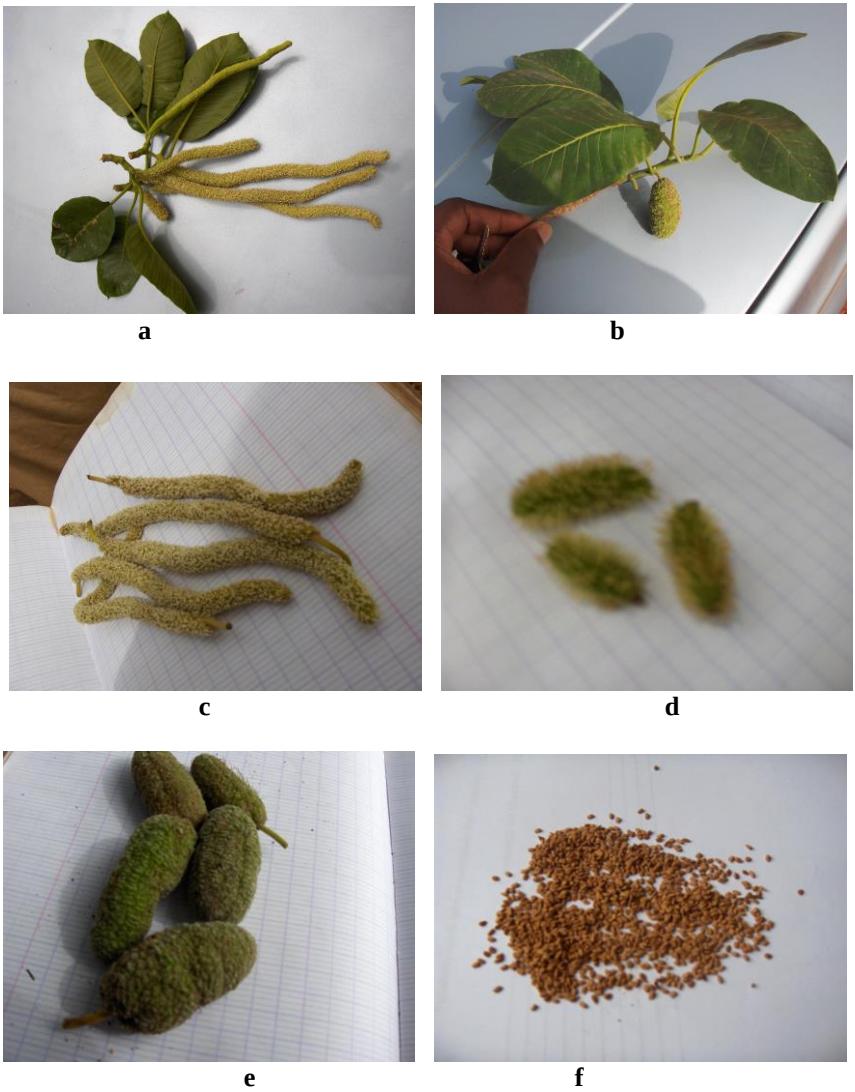

Figure 2.2: Different organs of iroko tree (*a. & b. Phyllotaxy, c. male flowers, d.female flowers, e. mature fruits, f. seeds*).

2.2- Ecological characteristics

Milicia excela (formerly *Chlorophora excelsa*) wew. C.C Berg (*Moraceae*) is a geographical widespread tree in western and Central Africa (fig 2.3). It occurs in Angola, Benin, Burundi, Burkina Faso, Central Africa Republic, Cameroon, Congo, Côte d'Ivoire, Ethiopia, Gabon, Equatorial Guinea. Ghana, Kenya, Malawi, Mozambique, Nigeria, Sao Tome & Principle, Sierra Leone, Sudan, Tanzania, Togo, Uganda, Zaire and Zimbabwe (Vroom, 2000).

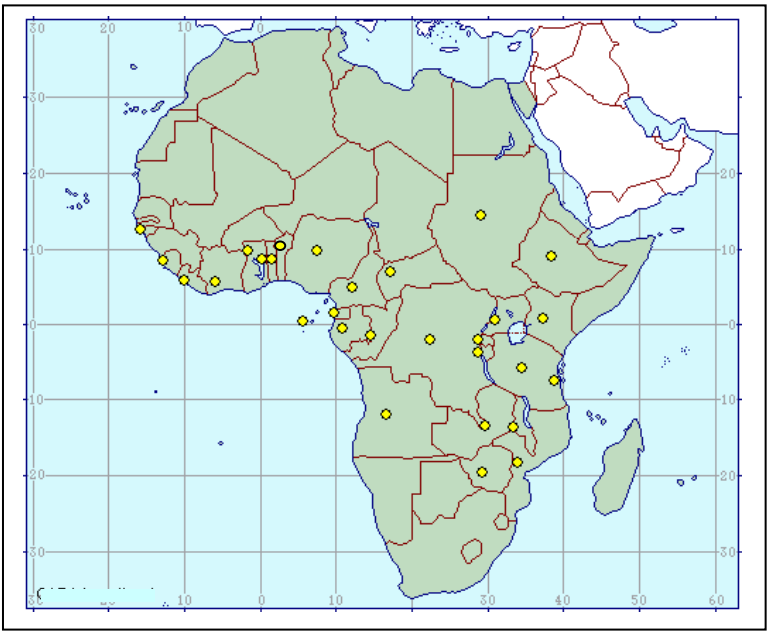

Fig 2.3: *Milicia excelsa* natural distibution (CAB International, 2004)

Milicia excelsa species is found in transitional vegetation between closed forest and savanna. It is often found in deciduous forests, semi deciduous forests, and evergreen forest and sometimes in savanna woodlands. Occasionally it is found in isolated relict forest up to about 1300 m from sea level. Most effective seed germination occurs in half – shade, the seedlings are most commonly found in medium size – light gaps and then become light dependant (Hawthorne, 1995). *Milicia excelsa* is a pioneer species which regenerates in disturbed, open areas and in logged forest (Hawthorne, 1995). In West

Africa this species is found in areas where rainfall is between 1,100 mm and 1,900 mm and the temperature is between 25˚C and 35˚C. In Kenya the species is found in relict moist forest and wooded grassland (Beentje, 1994) along the coast and in the Central New district and Nyanza province (Marshall and Jenkins, 1994). Especially, it has been found at an altitude of 4500 m on Mount Kilimanjaro in Tanzania, although it is usually found between sea level and 1,200 mm (FAO, 1986).

In germination tests of 19 tree species including pioneers, non-pioneer light demanders and non pioneers shade tolerant species (Swaine *et al.*, 1997), only three species among which *Milicia excelsa* showed the highest germination rate under light than under shade treatments. Agyeman (1994) demonstrated that the light compensation point of *Milicia* is somewhere between 2 and 6 % of full irradiance and its relative growth rate was highest at 42 % of full irradiance. Riddoch *et al.* (1991), measuring photosynthetic response of weeds, and pioneer and late successional trees, found that *Milicia excelsa* had similar photosynthetic rates (6 mol/m²/sec) to the weed *Chromolaena odorata* and to the pioneer tree species *Ceiba pentandra*. In contrast, they found that the late successional species had lower rates of dark respiration, lower mesophyll conductance, and lower light compensation points, compared with the weed and the pioneer species. It could be deduced that *Milicia* have a relatively high growth rate in open area confirming the statement of White (1968) stipulating that *Milicia* shows the highest growth rate in absence of light competition.

Milicia exclesa is a deciduous species which leaves completely fall every year during the period running from November to January and may extend to February.
Milicia excelsa prefers moist, well-drained soils and appears to be intolerant of impeded drainage (Taylor, 1960). The species is drought tolerant and fire-adapted and fungal pathogen resistant (Hawthorne, 1994; Swaine *et al.*, 1997).

2.3- Phytopathology and pest control on Milicia excelsa

Despite the high rate of seed production *Milicia excelsa* seedling are rare in forest with close canopy and readily appear in gap created in such a forest. Very little information is available on factors affecting germination of *Milicia excelsa* seeds. Fruit can be pulped in water and dried out, resulting in about 281,000 seeds per kg. Usually, seeds are immediately sown as viability of seeds is quickly lost (Taylor, 1960). However Taylor (1960) has reported that after 1 year at 0°C, a sample of iroko seeds retaind 71 % viability while another sample dropped from 48 % to 1 %. When it is sown immediately after extraction, iroko seeds can show an average germination rate up to 50 %.

According to Olatoye (1965), white light and gibberellic acid are known to promote germination in *Milicia* species.

Apart from the low regeneration rate observe for iroko, it is noteworthy the various difficulties of iroko plantations establishment. Previous attemps to establish plantations of stands of these species have been hampered by the gall forming Homoptera: Psyllideae *Phytolyma spp* which damage iroko trees and make plantations non attractive. Harris (1936), White (1966), Cobbinah (1988) and Wagner *et al.* (1991) have described the life history of *Phytolyma*. About 30 to 35 eggs are laids at a time in clusters by adult *Phytolyma*, on new buds and young leaves. After about 8 days of incubation, the first instar nymph hatches, and its probing stimulates parenchyma cells to devide fast to form gall tissue within 1 to 2 days. The first instar and subsequent instar stages feed within the gall which is many times larger than the insect within. Five nymphal instars have been identified over a developemental period of two to three weeks. Gall formation increases branching. The weight of the galls may bend over seedlings or shoots, particularly the heavily infested ones. Phytolyma attack is followed by dieback of foliage down to the woody tissue.

Reported the use of systemic pesticids in nusery at a dosage of 0.05 % of Fodiol (Parathion) applied for a period of four consecutive nights. Lowe (1963) gave details of a technics developed at Enugu station, which made mention of the application of fluoroacetamide at 1 % fornightly applied at a dosage of 425 g per ha. The results of these investigations indicated that in the experimental conditions, fluoroacetamide efficiently control gall formation but as systemic pesticides, the period of protection is shorter than expected. In addition, fluoroacetamide is toxid for human and its use is expensive

During the last twenty years, important research works were carried out in Ghana on the biology, population dynamic and control of the gall bug *Phytolyma lata*. In Ghana where coexist both *Milicia excelsa* and *M. regia*, three *Phytolyma* species were found (*P. lata, P. tuberculata* and *P. fusca*). In Sierre Leone where occurs *Milicia regia*, only *P. lata* is found. However, identification of partial genetic resistance to *Phytolyma lata* within the natural populations of *Milicia* (Cobbinah & Wagner, 1995) has stimulated the research for line resistant to Phytolyma for conservation and future genetic research. Bosu *et al.* (2000) have also investigated the feasibility of biological control of *P. lata* in Africa. Natural enemy studies indicated that most parasitoide recorded occur in the order Hymenoptera. Information gathered by these authors indicated parasitoids were less selective and were restricted, at least, to the family of Psyllidae, although their hosts were specific. So, parasitoid such as *Psyllaephagus phytolymae* (Encrytidae), *Aprostecetus phytolymae*, *A. roseveari*, *Tetrasitchus sp*, and

Tetrastichinae sp (all Eulophidae) were reared from nymphs. Four indigenous parasitoid species, *Psyllaephagus phytolymae*, *Aprostocetus salebrosus*, *A. roseveari* and *A. trichionotus* appear to be good biocontrol agent on *Phytolyma*. However it is not clearly known whether *Psyllaephagus phytolymae* could be manipulated to parasitize P. lata nymphs on seedling galls, where *Phytolyma* critically damage *Milicia*. If that is impossible, then it is recommended that *Aprostocetus salebrosus* be considered for release in nurseries and newly established plantations.

3

Habitat fragmentation and tree populations isolation: patterns and extent in Benin

Ouinsavi, C.; Sokpon, N.

Laboratoire d'Etudes et de Recherches Forestières, Faculté d'Agronomie, Université de Parakou, BP 123 Parakou, Rép. du Bénin ouinsch@yahoo.fr ; nsokpon@yahoo.fr

Abstract

Habitat fragmentation and its implications in tree populations' isolation were assessed with emphasis on fragmentation patterns and extent in Benin. Habitat fragmentation is known to be the major threat to ecosystems and species and appears to be the primary concern in conservation biology. The main causes of fragmentations are historical related to demography and space use, ecological and biological causes. Fragmentation causes are either natural or human - caused or both.

The strong climate oscillations that occurred during the Pleistocene and resulted in the location of Benin in the Dahomey Gap (savanna corridor between Guinean and Congolean rain forest) was the main natural fragmentation which shaped Benin vegetation. In addition to that, human-caused habitat fragmentation through continuous land clearing for agricultural purposes, extensive forests and trees exploitation, and urban areas expansion because of population increasing, have lead to the vegetation destruction and occurrences of small patches (mainly sacred groves) and many sparsely distributed tree populations among which *Milicia excelsa*. Those species are still under threat and need to be given priority for conservation actions.

Key words: Dahomey gap, vegetation destruction, isolated tree populations, Benin

3.1- Introduction

In the tropics, conservationists have focused their attention on the protection of natural forests and woodlands and, until recently, not given much attention to the widely dispersed forest patches and sparsely distributed trees throughout human-occupied landscape (Schellas and Greenberg, 1996; Attah-Krah, 2004). These patches and isolated trees are often critical component of the environment and their role in maintaining biological diversity is crucial (Sokpon *et al.*, 1998; Sokpon and Agbo, 1999; Sokpon and Ago, 2001; Kokou and Sokpon, 2006). For long-lived organisms such as trees, a better understanding of key factors determining the long-term conservation of these resources is vital and requires an interdisciplinary approach, integrating ecological, genetic and socioeconomic information (Atta-Krah, 2004).

Habitat fragmentation is a primary concern in conservation biology and needs to be focused on, for ecosystem and species diversity conservation. Fragmentation has been historically and recently evaluated for species and ecosystems through ecological studies and, by addressing its genetic consequences (Whitcomb *et al.*, 1981; Harris, 1984; Wilcove *et al.*, 1986; Santos *et al.*, 1999; 2002; White *et al.*, 1999; 2002; Young and Clarke, 2000; Bonnin *et al*, 2002; Lindenmayer & Franklin, 2002; Wilson & Provan, 2003; Mandelson *et al.*, 2004; Sumner *et al.*, 2004; Keller *et al.*, 2004; Tchir *et al.*, 2004; Wiegand *et al.*, 2005; Garcia *et al*, 2005). Some authors have suggested that small isolated populations are likely to be driven to extinction by demographic or environmental stochasticity before genetic factors can have an effect (Lande, 1988; Pimm *et al.*, 1988; 1989; Caughley, 1994; Wang *et al.*, 2005). Most contemporary researchers studying the phenomenon emphasized different causes of habitat fragmentation. Indeed natural disturbance process such as climate change, fire, wind storms and insect epidemics were always involved to alter the landscape, breaking up continuous stands of interior forest and creating edge habitat (Thomson *et al.*, 2003; Bonnin *et al.*, 2002; Franklin *et al.*, 2002; Pearson and Dawson, 2005). However Human - caused habitat fragmentation is also viewed as an important factor which destroys species, disrupts community interactions and interrupts evolutionary processes (Ehrlich and Ehrlich, 1981; Leakey and Lewin, 1995; Pimm *et al.*, 1998; Hanski, 1999; Keller, 2004). Fragmentation consequences induce increased effects on local populations dynamic (Dufrêne, 1995). Therefore spatial distribution of a species is a result of combined historical and anthropogenic factors as well as ecological and biological factors.

This paper aims to review existing literatures on habitat fragmentation and to assess the mechanism causing fragmentation in Benin context in order to better explain

the set of interactions between individual tree species and demographic, morphological and genetic processes in some tree populations.

3.2- Different causes of habitat fragmentation and their effects on ecosystems and species distribution

3.2.1- Definition of habitat fragmentation

Habitat fragmentation is defined as a disruption of once large continuous blocks of habitat into less continuous habitats (Hanski, 1999; Alan *et al.*, 2002), and implies occurrence of isolated individual trees and several patches, which differ by size and shape. The remaining habitat patches are separated by less suitable landscape elements such as agricultural areas, settlements and roads (Keller *et al.*, 2004; Solé *et al.*, 2004).

3.2.2- Different causes of habitat fragmentation

3.2.2.1- Historical causes of habitat fragmentation

Historical factors causing habitat fragmentation are those resulting from past event that influenced the previous generations and which determine the current distribution of the habitat. These include population's evolution factors and land use and settlement factors (demography).

The vegetation destruction as well as the wild fauna is continuously increasing. The phenomenon is strictly related to increase human population and consequently increase need of agricultural lands, timber and fuel woods, and all type of infrastructure. According to Pimm (1991) and Hanski (1999), when native vegetation is cleared (usually for agriculture or other kinds of intensive exploitation) habitat which were once continuous become divided into separate fragments. Solé *et al.* (2004) reported that rainforests, which comprise 6% of the world's land area and which contain at least 50% of the world's total species, are being cleared and fragmented at a rate far exceeding all other types of habitat. The case of Cascade Crest Forest at Washington (USA) is a typical example which could better illustrate the influence of historical factors on forest loss, habitat fragmentation and wildness (Thomson *et al.*, 2003). Indeed, for a century, forested lands in that ecosystem provided many of the resources to shelter, feed, and clothe indigenous cultures. As Washigton population has doubled over the next 50

years, and following the arrival of Euro-American settlers, the mountain slopes became the basis for fishing and timber economy and those industries persisted as part of the region's diverse economy. Then, about a century of logging has removed old-growth forests, caused fragmentation of wildlife habitat, and reduced the amount of viable habitat for threatened and endangered species, and increased stream sedimentation and damage to riparian areas.

3.2.2.2- Ecological and biological causes of habitat fragmentation

Biological causes of habitat fragmentation deal with factors which act through environment physical constraints, and the physiological limitations of individuals and their adaptation (speciation) while ecological causes include interspecific relations such as competition, predation or parasitism which can influence habitat distribution. Assessing ecological causes of fragmentation requires sufficient knowledge on species ecology (in terms of processes which influence species abundance, species richness, and fragmented landscape's diversity), on the biological conservation of those species (by developing the necessary strategies to maintain the focus species, to keep floristic and faunistic diversity and the different trophic interactions which undertake the key processing in the ecosystems), and a good understanding of the influence of landscape architecture on the population dynamic and interactions with natural enemies and control agents of individuals.

Climate type and climate changes determine soils and habitat structure. Predicted rapid climate change over the next century (Houghton *et al.,* 2001) poses a potenally severe threat to global biodiversity (Hannah *et al.*, 2002; Parmesan and Yohe, 2003; Thomas *et al.*, 2004; Pearson & Dawson, 2005). Thus species are expected to respond to the changing climate by migrating to track the environmental conditions to which they are adapted (Collingham & Huntley, 2000). According to Sala (2000), the ability of species to track future climates will be tested not only by the rate of change, but also by the loss and fragmentation of habitat that is characteristic of modern landscapes. Paleological evidence suggests that species have responded to past climate changes with rates of migration in the order of magnitude of kilometers per year (Davis, 1981; Huntley & Birks, 1983).

3.2.3- Effets of habitat fragmentation on ecosystems and species

One of the key issues in ecological genetics today is the effect of habitat fragmentation on the biodiversity of a range of ecosystems (Saunder *et al.*, 1991). Habitat fragmentation influences species diversity and ecosystems density and induces in a long term, demographic, morphological and genetic variability of populations because of the combined effect of population size reducing, station conditions changes and progressive isolation (Frankel & Soule, 1981). It was reported that populations of plants and animals occupying underglaciated regions have expanded their ranges following glacial retreat and the shift of climatic zone as consequently ranged expansions were predicted to result in reduced levels of genetic variation and genetic structure, as repeated long distance dispersal of individuals (founder events) imposed a series of bottlenecks on the colonizing genome (Hewitt & Ibrahim, 2001). Assessing the effect of habitat fragmentation on communities of mutualists (Amazonian ants and their host plants), Bruna *et al.* (2005) have found that twenty five years after fragment isolation, most species are rare and population sizes in fragments are extremely low indicating that environmental and demographic stochasticity could limit long-term population viability

3.2.4- Effect of habitat fragmentation on ecological structure of ecosystems and species

As ecological conditions change, sites may become unsuitable for a given species, as others may become suitable and be colonized. Many species are characterized by a balance between local population extinctions and establishment of new populations, e.g. they live in metapopulations (Hanski & Gilpin, 1997). Metapopulation viability is determined by a balance between opposite forces: migration and selection acting at local and regional scales a well as extinction and colonization (Bonnin *et al.*, 2002). Such balances are disrupted by human activities which often increase habitat fragmentation, reducing the size of suitable sites and increasing distances between them (Andrén, 1994; Young *et al.*, 1996).

Meanwhile, regeneration dynamic of species, which is intimately linked to the ecological structure of ecosystems or trees populations, appears to be highly influenced by seeds and pollen dispersion in fragmented habitats. Indeed, according to Clark *et al.* (1998), Cain *et al.* (2000; 2003), Pearson and Dawson (2005), there has been renewed interest in recent years, in the potential role of rare long distance dispersal events as drivers of rapid plant migrations. Mechanism by which seeds may be transported

unusually large distances are diverse and include the transportation of seeds in updrafts, dispersal by birds in nest material and movement of seeds whilst attached to the fur of mammals (Higgins *et al.*, 2003). Studies that attempted to predict migration rates driven by long-distance dispersal were severely limited by uncertainty as to the dispersal mechanism, difficulties of parameters estimation and more fundamentally, inherent stochasticity (Clark *et al.*, 2003). However, phenomenological modeling studies have greatly advanced the understanding of large-scale dispersal processes providing a foundation for addressing the potential effect of habitat fragmentation on the ability of the species to undertake large-scale migrations

3.2.5- Effect of habitat fragmentation on morphological and genetic structure of ecosystems and species

Until recently indicators of biodiversity have long been restricted to ecological parameters such as population dynamic and species richness. But recent advances in molecular genetic technology have opened new chapter in conservation efforts and results from molecular studies are becoming increasingly important in conservation and management of a wide range of rare or threatened species. Such techniques are of particular relevance to the analysis of plant populations, because plants vary widely in such factors as mode of reproduction (sexual versus asexual, selfing versus outcrossing), relative importance of pollen and seeds movement, and the role of dormancy in the re-establishment of populations (Young *et al.*, 1996).

Assessment of the evolution study of the genus *Dryas* in northern Atlantic region, in fragmented habitat into isolated sub-units, following the Pleistocene events, revealed great morphological variation in the circumpolar species *Dryas octopetala* L. (Hultén & Fries, 1986) and even the creation of new species from hibridazation of post-glacial migrants : *D. integrifolia* in north America, *D. octopetala* in Europe in the southerns part of the polar circle and *D. punstata* distributed in Siberia and Svalbard (Philipp & Siegismund, 2003). According to the distribution of *Dryas*, and the new living conditions, many morphological traits were developed which allow identification of three morphologically different populations in *Dryas octopetala.* The discriminant morphological characteristics developed mainly concerned leaves (leaf size, occurrence of glands on leaf upper face, etc.). Siegismund and Philipp (1999) combining genetic markers (isozymes) and morphological features « presence or absence of gland on leaf upper face », have shown that morphological variation did not match genetic variation in the species and have concluded the population identified morphologically were of

different species. Other studies were carried out on morphological variation in *Dryas octopetala* based on leaf size variation and it was shown that the observed variation was partially controled by genes.

According to Wilson & Provan (2003), fragmentation of natural plant commuties can have deleterious effects on the genetic diversity within a species because there will be a decrease in level of gene flow, particularly over longer distances. The sudsequent effects of genetic drift in small, isolated populations will lead to loss of diversity, leaving plants less able to adapt to changes in their environment and ultimately increasing the risk of extinction (Keller and Waller, 2002). Several studies revealed that populations from forests that had never been cleared or heavely logged exhibited significantly higher genetic diversity than those in disturbed forests (Wyatt, 1992; Lienert *et al.*, 2002; Wilson & Provan, 2003). They suggested that the reduction in genetic diversity could be due to a combination of founder effects and genetic drift, which were a consequence of habitat destruction that reduced population size and forced colonies to re-establish from a limited number of surviving sources.

The influence of fragmentation on genetic structure at the gametophytic stage has been addressed through mating system studies (e.g., Murawski *et al.*, 1994; James *et al.*, 1998) and through estimates of pollen dispersal (e.g., Nason *et al.* 1996; Aldrich *et al.*, 1998). These studies are sensitive to short-term disturbance in the pollination system, a critical portion of the life cycle. Hamrick *et al.* (1993) examined multiple stage classes of several species in continuous forest revealing that adult density and mode seed dispersal interact to determine levels of allozyme genetic structure in seedlings. Therefore low stand density and limited seeds dispersal imply strong structure in seedlings. Similarly, Alvarez-Buylla *et al.* (1996) reported that recolonisation of gap seems to influence allozyme variation in some gap-colonizing species. Deforestation and limited gene flow among populations induce strong genetic structure due to isolation. However gene flow among remnant forest populations could mitigate the effects of physical isolation which could be revealed by microsatellite analysis of pollen movement in fragmented populations (Chase *et al.*, 1996).

Results of Aldrich *et al.* (1998) provide compelling evidence that forest fragmentation may induce changes in the genetic structure of tropical tree populations. It is noteworthy that pre-existing genetic structure is shown to exist between collections of adults separated by about 1 km and this level of genetic differenciation among sites, and genetic similarity within sites appear to pass on to the saplings. According to Aldrich *et al.* (1998), it is more likely that a dramatic change in the breeding structure that may occur following fragmentation could result in difference between seedling cohorts and adults and sapling stages.

3.3- West African forest bloc fragmentation: the Dahomey gap

3.3.1- The Dahomey gap: Historical causes and influence on forests and species distribution

During the Pleistocene, strong climate oscillations affected dramatically the living world, resulting in the repetitive advance and retreat of the marine on the inland in tropical regions (Booth, 1958; Mayr and O'hara, 1986; Dupont & Weinelt, 1996; Zeitoun, 2000; Salzmann and Hoelzmann, 2005) and the polar ice sheet on the circumpolar land in temperate regions.

Many authors who have assessed vegetation history of the savanna corridor between Guinean and Congolean rain forest (named Dahomey Gap, Fig 3.1) during the last 150,000 years by inferences from fossil records (Booth, 1958; Mayr and O'Hara, 1986; Dupont & Weinelt, 1996; Salzmann and Hoelzmann, 2005), have demonstrated that the Dahomey Gap underwent several vegetational changes during that period. According to these authors, the western Guineo - Congolean part of the rain forest was connected to the main Congolean part during the early and middle Holocene. The Dahomey Gap might have been established from the mid-holocene marine transgression which caused a spread of mangrove forest along the inland lagoon, followed by a drier climatic condition which led to a rapid deterioration of the rain forest and subsequent spread of sudano-guinean savannas (Salzmann and Hoelzmann, 2005). Dahomey Gap is therefore characterized by a declined of annual precipitation from more than 2000 mm in the rain forest areas of Nigeria and Ivory Coast to about 1200-1000 mm in the savanna region (Dupont & Weinelt, 1996; Salzmann and Hoelzmann, 2005). In Benin this climate change is perceived through a rain fall gradient East-West (1300 mm at Pobe and 900 mm at Grand-Popo) and South-North (1300 mm at Cotonou, 1100 mm at Bohicon- Save and 900 - 1000 mm at Bembereke). The reduced precipitation rates of the Dahomey Gap are explained by the lessened convergence in combination with cooler surface waters of the seasonal coastal upwelling which occurs between July and October causing a short dry season in this region during the otherwise wettest monsoon months (Hayward and Oguntoyinbo, 1987; Salzmann and Hoelzmann, 2005). Later on, after the establishment of the Dahomey Gap, there was a return to wetter climatic conditions which resulted in a renewed spread of forest into savanna creating forest-savanna mosaic vegetation with a high number of pioneer tree taxa. The contemporary authors have all agreed with the explanation of the observed distributional patterns and areas of endemism of tropical forest trees. They stipulated that wide-ranging ancestral

taxa were isolated into forest refuges during glacial periods, and that, this isolation provided them opportunity to speciate. Mayr and O'hara (1986) have recognized five possible outcomes of the secondary contact derived from the return of more wetter climatic conditions, which are: *(i)* a smooth fusion if the populations had not diverged appreciably during the period of isolation, *(ii)* development of a conventional subspecies border if morphological (e.g., color) differences had evolved, but not isolating mechanisms or ecological differences, *(iii)* development of a hybrid zone along the line of contact, *(iv)* parapatry, with minimal overlap or hybridization, and *(v)* invasion (unidirectional or mutual) and overlap of the previously allopatric populations. The first three outcomes are possible when the speciation is not yet completed while the last two outcomes are envisageable if the speciation is largely or fully completed.

Benin vegetation was since then shaped as described by Adomou (2005) and three main phytochorological zones could be distinguished:

1- The Guineo-Congolean zone characterised by a bimodal regime of rainfall with two rainy seasons. It is composed of a mosaic of coatal forest and derived thickets, mangrove, swamp forest and semi-deciduous forests. In this zone, one can distinguish beach vegetation with abundance of *Remirea maritima* and *Ipomea pescaprae*, anthropic vegetation of mangrove swamp forests characterized by *Rhizophora racemosa, Avicennia germinans* and *Dalbergia ecastaphylum,* riparian forests, oil palm and coconut plantations, the humid semi-deciduous forest of Pobe characterized by *Dialium guineense* and *Triplochyton scleroxylon* plant community, *Strombosia glaucescens* and *Triplochiton scleroxylon* plant community, *Cleistopholis patens* and *Ficus mucuso* plant community (Sokpon and Lejoly, 1996); the humid semi-deciduous forest of Ke (Dangbo) composed of species such as *Cola gigantea, Cola cordifolia, Ceiba pentandra, Trilepisium madagascariense (= Bosqueia angolensis), Parkia bicolor* and *Celtis spp*. (Akoegninou, 1984); the humid semi-deciduous forest complex of Ichede - Toffo composed of species such as *Albizia adianthifolia, Albizia ferruginea, A. glaberrima, A. zygia, Antiaris toxicaria*, (Akoegninou, 1984); the humid semi-deciduous forest of Lama characterized by abundance of species such as *Dialium guineense, Diospyros mespiliformis, Ceiba pentandra, Drypetes floribunda, Memecylon afzelii, Celtis brownii* and *Mimusops andongensis*. These forest patches are separated by dry semi-deciduous forests characterized by *Anogeissus leiocarpa* plant communities, forest woodlands dominated *Isoberlinia spp.* plant communities, wooded savannas of *Daniellia oliveri, Parkia biglobosa* and *Vitellaria paradoxa* plant communities and shrub savannas ranging from Godomey, Kouhounou, to southern Ouidah, Come, Bopa, Pahou (with *Lophira lanceolata* et *Vitellaria paradoxa* plant communities).

2- The Guineo-Sudanian Transition zone considered by Aubreville (1937) as a wooded savanna region and by White (1983) as forest woodlands dominated by *Isoberlinia* species. The climate in this zone exhibit a tendency to a unimodal rainfall regime and the two rainfall peaks tend to merge into one peack indicating one rainy season. It is a mosaic of semi-deciduous forest (Bassila phytogeographical district), dry forests, woodlands, sometimes dry forests and wooded savannah crossed by gallery forests. The most abundant species found in the zone are *Isoberlinia doka, I. tomentosa*, *Monotes kerstingii, Uapaca togoensis Anogeissus leiocarpa, Antiaris toxicaria*, *Ceiba pentandra, Blighia sapida, Dialium guineense*, *Combretum fragrans, Entada africana*, *Maranthes polyandra, Pterocarpus erinaceus, Terminalia laxiflora*, and *Detarium microcarpum*.

3- The sudanian zone where rainfall regime is unimodal is composed of dry forests, woodlands, and savannah crossed by gallery forests. It is characterized by the predominance of species such as *Haematostaphis barteri, Lannea spp, Khaya senegalensis, Anogeissus leiocarpa, Tamarindus indica, Capparis spinosa, Ziziphus micronata, Combretum spp* and *Cissus quadrangularis*.

However the distribution of forests and tree species in Benin is also determined by other natural and physical factors such as Lama Depression and Atacora chain.

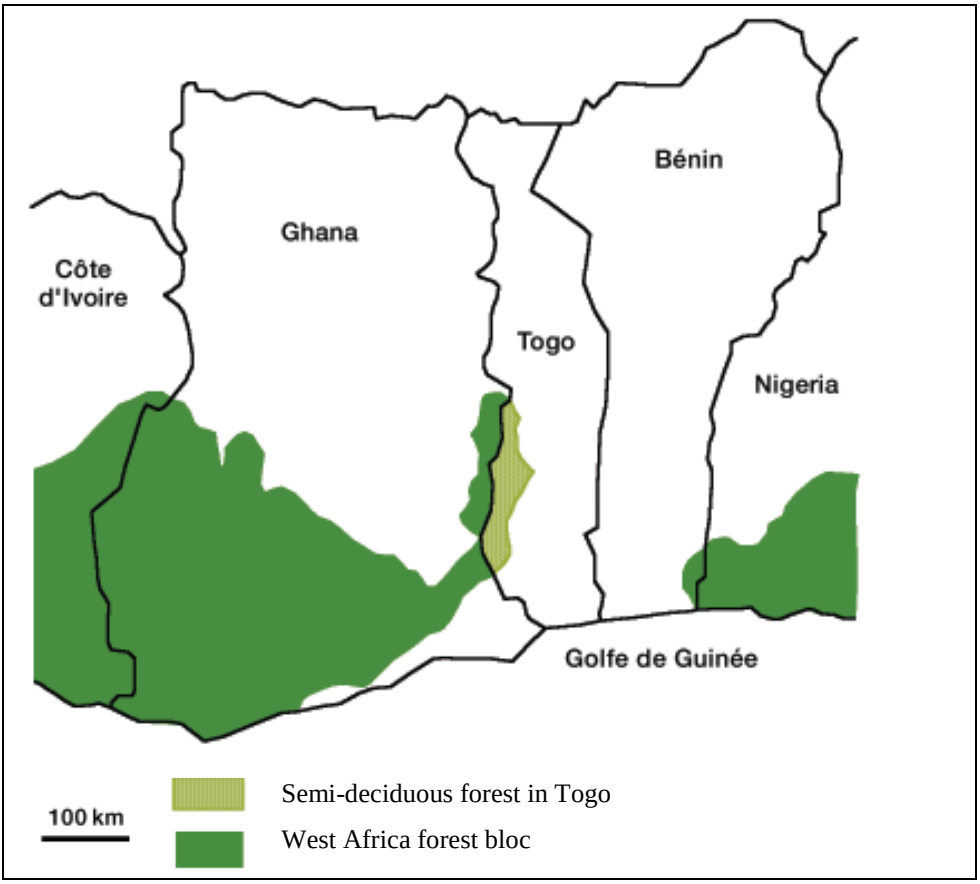

Fig 3.1. Benin and Togo location in the Dahomey gap, (Kokou and Sokpon, 2006)

3.3.2- Influence of the Lama depression on vegetation distribution in Benin

The combined effects of climate and soil types strongly determine the plant community species composition and structure. The Lama depression on one hand, and Atacora mountain which divided the Mekrou-Pendjari basin into two parts on another hand, have maintained local micro conditions which favored establishment of semi-deciduous forest and occurrence of rain forest trees species in the Sudanian region of Benin

Indeed, Lama depression shelters one of the last remnant forest reserves in the Dahomey Gap, the Lama humid semi-deciduous forest. Lama depression in Benin is a large and flat depression, located at about 100 km from Atlantic coast moving to the north which ranges from Togo to Nigeria and is mainly characterized by its soil type which is a typical vertisol with high clay content. The vertisols show strong swelling and shrinking during the year and broad cracks down to a depth of 60 cm and at the bottom of the cracks topsoil material (dark and rich in organic matter) can be found. They have parallelepiped aggregates with slickness sides in the subsoil. Classified as protected government forest reserve in 1946, Lama forest was a 16,250 hectares vegetation cover of which about 11,000 hectares were dense semi-deciduous forest. Lama forest has suffered from devastating exploitation by Holli people, a migrant population from Nigeria and leaving within the forest since 1950, and riparian population composed of Fon and Aïzo. The deforestation has led to the reducing forest area to about 1,900 hectare strictly protected as central core and surrounded by a buffer zone planted of exotic species such as *Tectona grandis, Gmelina arborea* and Australian acacias. The Central core of Lama forest constitutes a unique natural reserve growing on vertisol, with a particularly status of being the refuge of number of fauna and flora species of the Guinea-Congo biome. It is characterized by plant species such as *Triplochiton scleroxylon, Antiaris africana, Milicia excelsa, Afzelia africana, Ceiba pentandra, Diospyros mespiliformis* and *Dialium guineense*.

3.3.3- Influence of the Atacora chain on vegetation distribution in Benin

Despite its location in the sudanian zone, Bassila region shows a semi-deciduous forest floristically similar to those in the Guineo-Congolian zone. Bassila vegetation is characterised by plant species such as *Triplochiton scleroxylon, Celtis zenkeri, Holoptelea grandis, Cola gigantea, Trilepisium madagascariense, Rinorea dentata,*

Trichilia prieureana, *Albizia ferruginea*, *Rothmannia longiflora* and *Pierreodendron kerstingii* (Adomou, 2005), which are all semi-deciduous forest species. This particular vegetation type in Bassila region was explained by the existence in the past, of a bridge of rain forest connecting Bassila region to the Upper Guinea during the humid period of early Holocene to mid-Holocene (Salzmann and Hoelzmann, 2005; Adomou, 2005). However, such vegetation distribution and the occurrence of typical semi-deciduous forest dominated by Celtidaceae, Sterculiaceae and Moraceae in the region of Djougou, Northern west of Benin, were interpreted to derive from the presence of Atacora mountain which served as a refuge to many rain forest species such as *Milicia excelsa*, *Antiaris toxicaria*, *Pentadesma butyracea*, *Leucaniodiscus cupanioides*, *Milletia thonningii* and *Detarium senegalense* (Adomou, 2005).

3.4- Human – caused habitat fragmentation

3.4.1- Main causes of anthropogenic fragmentation in Benin

For several millennia, humans have attempted to domesticate tropical ecosystems and landscapes in order to channel a larger share of primary production toward their own consumption (Schroth *et al.*, 2004). However, the contribution of human action to the isolation of tree populations and its effect on the evolution of those populations are also important. Dupont & Weinelt (1996) reported that forest clearing by early humans and agriculture might have significantly contributed to the vegetation distribution patterns observed in the Dahomey Gap. This corroborated the hypothesis of Paradis (1975) that investigated isolated forest stands within the savanna of southern Benin and assumed that the present rainfall is still sufficient to allow the establishment of a dense semi-evergreen forest on a continuous stand, which therefore represents the natural vegetation of this region without anthropogenic influence. In Benin the main causes of human-caused ecosystems fragmentation are (*i*) land clearing for agricultural purposes combined with the improvement of plugging systems, (*ii*) extensive forests and trees exploitation, (*iii*) urban areas expansion because of population increasing.

Indeed as reported by Schroth *et al.* (2004), people initially exploited ecosystems in a subtle way by enriching forests close to campsites with useful plants species or by clearing small patches of forest or savanna with primitive tools and fire. But as human populations and their technological capabilities increased and markets for tropical, agricultural products developed, the impact of agriculture on tropical ecosystems and landscapes became more dramatic. Shifting cultivation and bush fallow were basically agricultural practices used by farmers in Benin and vegetation was covering the expenses. In addition, the development of cotton culture as cash crop has

induced farmlands increasing throughout the country especially in the central and northern regions where the remnant forest and savanna areas exist. Cotton culture has given raise to the introduction of some improved land cleaning systems such as the use of plough or tractors which required wide areas to be optimally useful to farmers. Furthermore, development of cashew as cash crop led to the conversion of important forest and savanna areas into mono specific cashew plantations with continuous need of new land clearing as cashew plantation immobilizes the land few years after its establishment.

Until past decade, forest reserves in Benin did not have management plan and any technical support for their sustainable use. Therefore selective logging led to scarcity of some tree species such as *Milicia excelsa, Khaya senegalensis, Afzelia africana* etc., in forests and savannas, as results of continuous exploitation of large, superior individuals, with no caution to their regeneration dynamic. Forests and species overexploitation was reinforced by the fact that the main household energy used in Benin is still fuelwood. Then most of the forests reserves (e.g. Bassila, forest reserve, Oueme-Boukou, Ouenou-Benou) were creamed of their most valuable, hardwood tree species for charcoal production and for firewood.

3.4.2- Sacred groves and phytodiversity conservation

Sacred forests in Benin have been reported for a very long time and were widely investigated for their conservation status, their structure and their contribution to biodiversity conservation (Sokpon *et al.*, 1998; Sokpon and Agbo, 1999; Sokpon and Ago, 2001; Kokou and Sokpon, 2006). They are numerous (a total number of 2,940 sacred groves in Benin, Table 3.1) and relatively small (69.4 % of sacred groves have an area lower than 1 ha), and they help to protect forest fauna and flora in the country (Kokou and Sokpon, 2006). Sacred groves are mostly relic forests affected by human pressure and they are conserved by sanctification (Chevalier, 1933; Aubreville, 1937; Coulibaly, 1978; Guinko, 1985).

Table 3.1: Total number, areas and size classification of sacred groves in Benin (Sokpon and Agbo, 1999)

Provinces	Areas			Total number		Total area	
	S≤1 ha	1<S<5 ha	S≥5 ha	N	%	S	%
Atacora	306	112	118	536	18.2	2,140	11.7
Borgou	223	136	127	486	16.5	2,083	11.3
Zou	581	146	85	812	27.6	12,552	68.4
Mono	125	11	3	139	4.7	91	0.5
Atlantique	404	54	16	474	16.1	412	2.2
Oueme	401	79	13	493	16.8	1,082	5.9
Total	2,040	538	362	2,940	100	18,360	100

N = total number and S = total area

3.4.3- Land clearing and the establishment of parklands and relict tree populations

Although large vegetation cover is destroyed by land clearing for agricultural purposes, many farmers maintained or actively included trees as part of their agricultural landscape. In the tropics, trees were essential components of the fallow vegetation on temporarily abandoned fields, and many trees were also retained based on some specific purposes on farm land where they did not interfere with the use of the land (McNeely and Schroth, 2006). These practices have developed parklands establishment. Parkland, defined as a regular, systematic and ordered presence of trees within fields (Sautter 1968 cited by Bagnoud *et al.*, 1995), is the result of a long evolutionary process during which an association between natural elements (trees, shrubs conserved, maintained and enhanced because of their utility) and crops, happens within a regularly exploited space (Kelly *et al.*, 2004). Generally, trees are preserved to provide shade, shelter, energy, food fodder, ecological services including soil fertility and microclimate improvement, medicine and many other goods and services that enabled the farmstead to prosper. In the central and northern Benin, the most abundant species preserved on farm are *Parkia biglobosa* and *Vitellaria paradoxa* and more recently, *Borassus aethiopum.* Evidence for anthropogenic selection of *Parkia biglobosa* and *Vitellaria paradoxa* was widely assessed by many scientists (e.g. Tomlinson *et al.*, 1995; Ouedraogo, 1995; Boffa, 1999; Lovett and Haq, 2000; Bayala *et al.*, 2002; Kelly *et al.*, 2004; Teklehaimanot, 2004) and development agencies in the last decade because, in countries where they occur, local people derive a lot of benefits from these trees mainly from their nonwood products. In

the southern Benin, the most abundant species preserved on farms is *Elaeis guineensis*. All over the whole country, other tree species (*Milicia excelsa*) which were not formerly known as agroforestry tree species are now becoming domesticated and preserved on farms because of their high utility but also because they have been almost exterminated from forests and savannas. As reported by Leakey *et al.* (1999), human pressure on forests and savannas, has resulted in increased establishment of trees on farms where farmers have secured land tenure or tree tenure.

Meanwhile it can be noticed that existing trees on farms are aged and regeneration is almost inexistent. Several factors could explain this situation among which one can distinguish harvesting form (farmers collect almost all of the fruits from trees every year especially in case of *Parkia biglobosa*, *Vitellaria paradoxa*, and hypocotyls in case of *Borassus aethiopum*), farm clearing by fire or ploughing, high seedling mortality, lack of knowledge on the species silviculture and as reported by Okullo *et al.* (2003), decreasing or suppression of fallow length due to growing human- or livestock-population pressures.

The landscape changing into a mosaic of parklands or remnant isolated trees surrounded by farms or unfavorable areas raises a crucial problem of management and maintenance of this fragmented ecosystems and trees populations. Indeed, these practices may lead to genetic diversity reduction through genetic bottlenecks and subsequently founder effect restricted gene flow and genetic drift may increase population genetic isolation and divergence. These genetic hazards, added to the possible enhancement of inbreeding may lead to fixation of deleterious alleles, endangering species persistence in fragments and jeopardizing their conservation (Gilpin and Soulé, 1986; Collevatti *et al.*, 2001). Evidence that fragmentation and isolated trees populations may induce changes in genetic structure, gene flow and mating patterns of tropical trees populations was found by several authors (e.g. Hall *et al.*, 1996; Nason and Hamrick, 1997; Aldrich and Hamrick, 1998; Aldrich *et al.*, 1998; Dayanandan, 1999; Collevatti *et al.*, 2001; Lemes *et al*., 2003). As it became clear that the Pleistocene and anthropogenic pressure produced drastic changes in the distribution in the tropics and sub-tropics as well as in the temperate regions, many authors argued that isolation by vegetational barriers, could have been as effective in tropics as isolation by vegetational and physical barriers had been in cooler regions. Therefore, there is a priority need to evaluate and mitigate the genetic effects of Human-caused fragmentation on tree species in general and on those which have a socio economic value for local people in particular.

4

Morphological variation and ecological structure of iroko (*Milicia excelsa* welw. C.C. berg) populations across different biogeographical zones in Benin

Ouinsavi, C.* and N. Sokpon

Laboratoire d'Études et de Recherches Forestières, Faculté d'Agronomie, Université de Parakou, BP 123 Parakou, République du Bénin; *Author for correspondence (E-mail: ouinsch@yahoo.fr; Phone : (229) 2361 2010, Fax: (229) 2361-0712

Submitted to Sciences & Nature

Abstract

The morphological variation and ecological structure of *Milicia excelsa* populations were assessed in Benin using transect sampling method and multivariate analyses including principal component, cluster and canonical discriminant analyses. The first principal components explained 91.23 % of total variation with leaf dimensions (length and width), height growth (*DBH/TH)* male flowers width and female flowers size (length and width) being the loading variables (eigenvectors equaled to 0.99, 0.89, 0.27, 0.98, 0.47and 0.26 respectively). Cluster analysis grouped individuals and populations into four clusters with greater accessions from similar adaptation zones being in the same cluster. Multivariate analysis of variance indicated for most of the traits, significant variation among populations (F =12.28, df =133, $P < 0.001$). Moreover, discrimination of groups indicated that variation in height growth and leaf size was highly related to edaphic factors while flowers size variation was related to annual rainfall. Ecological structure showed low stand density and hence low stand basal area for all of iroko populations. Moreover, erratic diameter distribution was found for many populations although most of them showed bell-shaped diameter distribution. The results of this study, raised urgent needs of genetic variation and population structure assessment in *Milicia excelsa* species.

Key words: Ecological structure; morphological variation; *Milicia excelsa*; environmental factors; Benin.

4.1- Introduction

Wide environmental and geographical variation often occurs within the natural range of plant species (Teklehaimanot *et al.*, 1998). Adaptation of a species to this variation may produce different morphological and physiological characteristics, resulting in the development of ecotypes. Widespread species are known to show marked among-population variation in morphological traits, which can be the result of genetic variation (Quiroga *et al.*, 2002). Furthermore, genotypic variation in the physiology and morphology has been reported for several tree species, which is often related to the plant habitat. For example, Abrams *et al.* (1990) and Kubiske and Abrams (1992) have reported that *Fraxinus pennisylvanica* and *Quercus rubra* both tree species widely distributed in North America, exhibited morphological and physiological variation related to their habitat conditions changes and consequently to development of abilities to drought tolerance. Morphological structure and spatial distribution of a species is an output of combined historical and anthropogenic factors as well as ecological and biological factors. According to Hamrick *et al.* (1992), Loveless (1992) and Chevallier (1999), these factors are important for quantifying genetic variation of a species. Master the bio-ecological parameters is also necessary for best sampling while carrying out genetic studies of the species.

Iroko tree (*Milicia excelsa* Welw. C.C. Berg.) is a hardwood tree of great socio-economical and cultural importance in Sub-Saharan Africa. It is a dioecious species which occurs in a wide range climatic and edaphic environment and adapts to various ecological conditions. In Benin, its natural range extends from the south to 10°30' N and from east to west of the country. The species has suffered from its habitat destruction and fragmentation and human pressure through intensive logging and land-use practices. A recent study carried out on timber wood exploitation in Benin (Sokpon and Ouinsavi, 2004) revealed almost inexistent lumber of iroko in sawmills and wood markets. The remnant iroko trees are sparsely distributed across the landscape, either on farms and public places (Ouinsavi *et al.*, 2005), or in sacred groves (Sokpon *et al.*, 1998; Sokpon and Agbo, 1999; Sokpon and Ago, 2001; Kokou and Sokpon, 2006), owing their existence to traditional ethnobotanic practices of conservation. As reported by Bruschi *et al.* (2003), such a situation might have disturbed the genetic integrity of iroko population by affecting the size and degree of isolation of individual populations,

thus contribution to genetic impoverishment and adaptive flexibility and consequently morphological structure of the populations.

This paper aims to assess ecological structure and morphological variation in *Milicia excelsa* populations in Benin.

4.2- Methods

4.2.1- Population samples

Given that the remnant *Milicia excelsa* trees are sparsely distributed across the landscape and relict forest patches, tree inventory was carried out using megatransect method. Ten 500 m width megatransects with variable length, the minimum length being 50 km were layed across different biogeographical zones within the species natural distribution area in order to cover as much as possible the species range in Benin. Along those transects, all iroko trees were inventoried and registered with the GPS (Global Positioning System) within 4 to 6 ha plots. A total of 1,028 iroko trees were measured.

4.2.2- Assessing structural characteristics of iroko populations

For each of the censured iroko tree, the diameter at the breast height (DBH), the total height (TH), bole height (BH) and crown diameter (CD) were systematically measured. DBH data were used to draw the diameter class distribution of the species for each population. Stand basal area (G) were calculated using the formula: $G = \pi D^2/4$ where d is the DBH. In addition trees number per hectare was estimated dividing the total number of iroko tree censured by the area concerned.

4.2.3- Morphometric analysis

Eight morphometric parameters were analyzed including bearing and architectural parameters (diameter at the breast height, total height, bole height, crown diameter) and descriptive parameters of the tree's organ (length and width of the limb, the fruit, and the petiole, Ayana and Bekele, 1999). The bearing and architectural parameters were measured on all of the censured trees while organ descriptive parameters were measured

on 10 leaves and 10 flowers randomly collected from adult trees. A total of 1,880 leaves, 1,340 male flowers and 1,360 female flowers were measured (Table 4.1). For leaves and flowers measurements, data were averaged over individual tree before undertaking the series of multivariate analyses using appropriate procedures.
Principal component analysis (PCA) was performed on the untransformed morphometric data using the correlation matrix. Two populations were clearly separated and the third group composed of individuals from the remaining populations, was subjected to a partial PCA using the same variables.

4.2.4- Assessing morphological variation of iroko populations and its relationship with environmental factors

To evaluate the importance of environmental factors in the morphological variation of iroko population, rainfall data were collected from ASECNA (Agence pour la Securité de la Navigation Aérienne) for the last thirty years and soil physical and chemical characteristics were collected from LSSE (Laboratoire des Sciences de sol et de l'Eau) for the study area. PCA was performed on environmental data including average rainfall (mm) and edaphic variables such as clay (%), silt (%), sand (%), Carbon (%), Nitrogen (%), C/N ratio, organic matter (MO %), Ca (me %) Mg (me %), K (me.%), Na (me %), total exchangeable bases (TEB me %), caption exchange capacity (CEC me %). Cluster analysis (CA) was performed to examine the morphological similarity, at individual level, between the twelve sampled populations. A total of 134 individuals which were measured all at once for DBH, total height, leaf dimensions and flower size, were clustered using PAST software version 1.43 (Hammer *et al.*, 2001). The measure of dissimilarity was Euclidian distance and clustering methods was Unweighted Pair-Group Method using Arithmetic Average (UPGMA). For all of the morphometric variables, multivariate analysis of variables (MANOVA) was used to test for significance of variation among populations and among groups of populations. Multiple regressions were carried out to correlate patterns of populations' differentiation to environmental variables. A canonical Analysis of Correspondence (CCA) was performed on the two sets of variables, the first set containing morphometric variables weighed in the principal components (PC) and the second set composed of the most PC weighed environmental variables. This is a direct gradient analysis which incorporates both ordination and multiple regression techniques to reveal the relationship between tables of multivariate data (Ter Braak, 1988; Mapaure, 2001). CCA ordination was done on standardized environmental variables. MANOVA and multiple regressions were

carried out using SPSS software. PCAs were performed using PAST software version1.43 (Hammer *et al.*, 2001) while CCA was performed using CANOCO for Windows 4.0 (Ter Braak, 1988).

4.3- Results

4.3.1- Principal component analysis and morphological variation among individuals

The recorded geographical coordinates were plotted onto Benin map and twelve *Milicia excelsa* populations (Aplahoue, Lokossa, Niaouli, Sakete, Bohicon, Save, Ketou, Tamarou, Biro, Bassila, Djougou, and Bante population) were inferred from geographical distribution of iroko trees on the map (Fig. 4.1, Table 4.1).

In principal component analysis, 91.23 % of morphological variation was explained by the first two principal axes (Table 4.2). The first axis, with eigenvalue of 9.55 explained 63.84 % of total variation and the second axis explained 27.8 % of variation with eigenvalue of 4.09. Morphological trait coefficient (i.e., eigenvectors) indicated that leaf dimensions (length and width) and the ratio *DBH/TH* were the loading variables in the first axis (0.99, 0.89 and 0.27 respectively) while male flowers width and female flowers size (length and width) were the loaded variables onto the second principal axis (0.98, 0.47, 0.26 respectively, Table 4.2). Along the principal component axis 1, most of individuals from Niaouli population occupied the right side whereas the mixed group drew aside to the centre and the left side (Fig. 4.2 a). This first axis differentiated populations with tallest and wider leaf trees. Along the principal axis 2, most of individuals from Save population occupied the middle lower part while the mixed populations were in the middle around the central point. Partial PCA dispatched the mixed populations into two groups (Fig. 4.2 b).

4.3.2- Patterns of morphological variation among iroko populations

Cluster analysis of iroko individuals revealed four clusters (Fig. 4.3). The first cluster contained most of individuals from Niaouli population. The three others cluster were rather mixed with cluster 2 grouping most of individuals from Save Aplahoue and Lokossa populations, cluster 3 mainly composed of individuals from populations Bohicon Bante and Sakete and cluster 4 encompassing individuals from Djougou,

Bassila, Tamarou, Ketou and Biro populations. Analysis of variance indicated significant morphological variation among groups of populations (F =12.28, df =133, P < 0.001). Population Niaouli which composed cluster 1 has the highest leaves size (mean *LL* =18.93, mean *LW* = 11.84) and showed highest height growth (average *DBH/TH* = 0.022, Table 5.4). Cluster 2 showed medium leaf size and height growth (mean *LL* ranged from 15.69 to 16.69, mean *LW* ranged from 9.64 to 10.41, and Average *DBH/TH* = 0.03). Cluster 3 has the smallest leaf size and lowest height growth (mean *LL* varied from 12 to 13.16, mean *LW* from 8.02 to 8.68 and mean *DBH/TH* varied from 0.064 to 0.085). Medium leaf size and low height growth were observed for populations in cluster 4 (Mean *LL* ranged from 13.04 to 14.9, average *LW* ranged from 7.35 to 7.50 and mean *DBH/TH* varied from 0.032 to 0.039) except Tamarou population which has small leaf size (*LL* = 10.37, *LW* = 6.76). Almost all group of iroko populations produced similar female flowers in terms of flower size except Populations Djougou, Tamarou and Bassila from cluster 4 which showed shortest female flowers (Table 4.4).

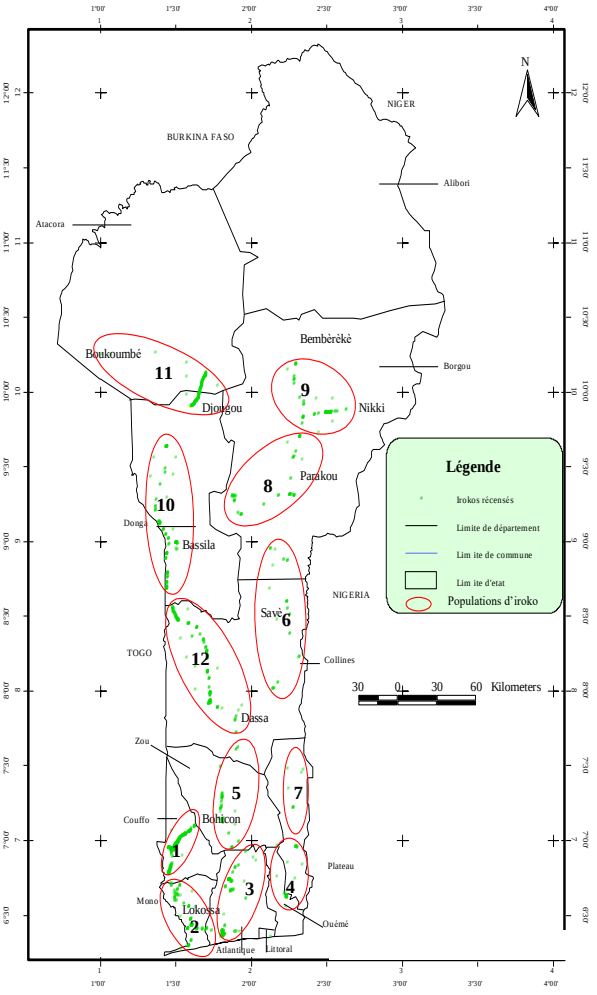

Fig. 4.1: *Milicia excelsa* populations and their geographical location in Benin. *Aplahoue (1), Lokossa (2), Niaouli (3), Sakete (4), Bohicon (5), Save (6), Ketou (7), Tamarou (8), Biro (9), Bassila (10), Djougou (11), Bante (12).*

Table 4.1: Sample size geographical locations and ecological characteristics of iroko populations sites

Populations	Geographical coordinates		Soil type	Average rainfall	Number of samples collected			
	Longitude (°E)	Latitude (°N)		(mm)	MF	FF	Leaves	Trees (DBH, H, CD)
Aplahoue	001°50’42.6”	07°05’06.4”	S1	1,200 – 1,300	160	140	300	143
Bante	001°58’06.6”	08°13’49.2”	S2	1,100 – 1,200	120	90	110	175
Basssila	001°39’16.9”	09°02’36.1”	S2	1,200 – 1,300	90	120	120	131
Biro	002°56’28.8”	09°56’11.2”	S2	900 – 1,100	130	110	120	60
Bohicon	002°04’49.6”	07°18’16.1”	S1	1,200 – 1,300	80	130	130	21
Djougou	001°26’04.8”	10°07’27.4”	S2	900 – 1,100	180	130	140	153
Ketou	002°35’45.2”	07°20’59.2”	S1 & S3	1,200 – 1,300	90	80	90	23
Lokossa	001°40’16.4”	06°45’26.1”	S1 & S3	1,200 – 1,300	140	150	270	144
Niaouli	002°08’08.1”	06°44’16.1”	S1 & S3	1,200 – 1,300	80	90	130	23
Sakete	002°35’34.8|”	06°37’05.2”	S1	1,200 – 1,300	120	110	220	122
Save	002°31’16.1”	08°04’27.9”	S2	1,100 – 1,200	60	90	90	16
Tamarou	002°39’38.4”	09°38’17.7”	S2	900 – 1,100	90	120	160	19
Total					1,340	1,360	1,880	1,028

Male flowers (*MF*), female flowers (*FF*), diameter at breast height (*DBH*), Height (*H*) crown diameter (*CD*). ferralitic soil (S1), tropical ferruginous (S2) and vertisols (S3)

Table 4.2: Results from principal component analysis (PCA) of morphometric traits

	Axis1		Axis2	
	Coefficient	Loading	Coefficient	Loading
DBH/TH	-0.0047	0.2704	-0.0043	-0.1628
DBH/BH	-0.0015	0.2206	0.0015	0.1508
DBH/CD	-0.0002	-0.0579	0.0001	0.0203
LL	0.8943	0.9900	0.1142	0.0828
LW	0.4346	0.8927	-0.0250	-0.0337
MFL	0.0006	0.0163	-0.0090	-0.1700
MFW	0.0776	0.1209	0.9683	0.9882
FFL	0.0709	0.2380	0.2165	0.4759
FFW	0.0196	0.1849	-0.0422	0.2608
eigenvalue	9.551		27.387	
%variance	63.841		4.097	

Diameter at breast height (*DBH*), total height (*TH*), bole height (*BH*)crown diameter (*CD*), leaf length (*LL*), leaf width (*LW*), male flowers length (*MFL*), male flower width (*MFW*), female flowers length (*FFL*), female flower width (*FFW*)

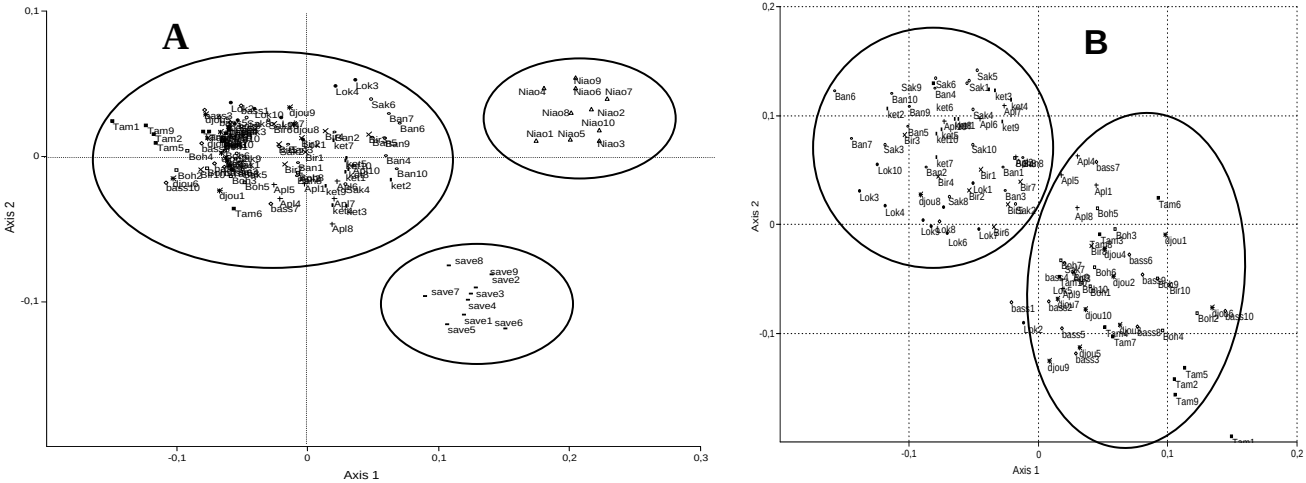

Fig. 4.2: Projection of individuals of *Milicia excelsa* in the space of the first and second principal components. *Sak = Sakete; Ket = Ketou; Tam = Tamarou; Bir = Biro; Djo = Djougou; Bas = Bassila; Ban = Bante; Boh = Bohicon; Nia = Niaouli; Apl = Aplahoue; Lok = Lokossa; Sav = Save*. *A* = first PCA, *B* = partial PCA

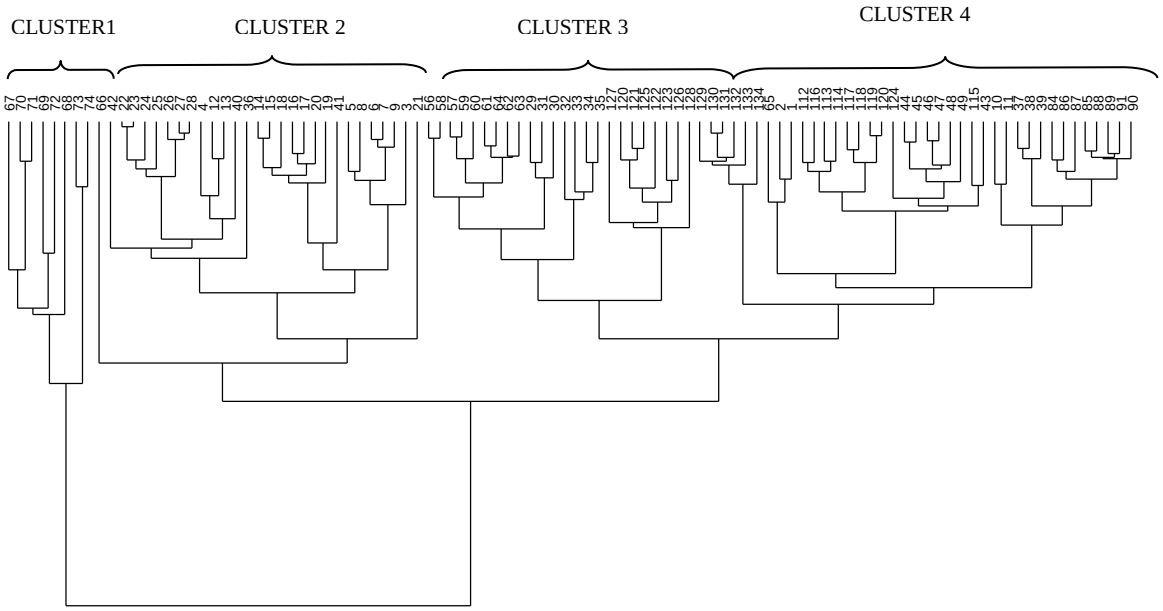

Fig. 4.3: UPGMA cluster of *Milicia excelsa* individuals from twelve populations. *1-11 (Aplahoue), 12-20 (Lokossa), 21-28 (Save), 29-37 (Sakete), 38-49 (Ketou), 50-62 (Bohicon), 63-80 (Niaouli), 81-92 (Tamarou), 93-102 (Biro), 103-118 (Bassila), 119-126 (Bante), 127-134 (Djougou)*

4.3.3- Influence of environmental factors on morphological variation in *Milicia excelsa*

Principal component analysis based on environmental variables revealed that the first two principal components accounted for 80.36 % of total variation (Table 4.3). The order of importance of the various parameters in the first principal component was high (*CEC*, 0.97; *Mg* and *Ca*, 0.96; Sand, -0.95; *Na*, 0.85; Clay, 0.80 and *N*, 0.59). This principal component, with eigenvalue of 6.05 explained 65.11 % of variation. Rainfall (-0.88) and Silt (0.59) were the variables loaded into the second component which explained 15.25 % of variation with eigenvalue of 1.41 (Table 4.3).

Table 4.3: Results from principal component analysis (PCA) of environmental data

	PC1		PC2	
	Coefficient	Loading	Coefficient	Loading
Clay	0.327	0.805	-.400	0.476
Silt	0.195	0.483	0.498	0.599
Sand	0.388	0.954	0.051	0.060
N	0.013	0.592	-0.015	-0.336
M.O	0.192	0.481	0.105	0.127
Ca	0.392	0.964	0.121	0.144
Mg	0.389	0.960	0.063	0.075
Na	-0.203	-0.847	-0.032	-0.064
Cat.	0.397	0.975	0.101	0.120
CEC	0.397	0.976	-0.028	-0.033
Rainfall	-0.082	-0.201	-0.740	0.881
Eigenvalue	6.048		1.417	
Variance (%)	65.112		15.252	

Multiple linear regressions of morphological traits on environmental factors indicated that height growth in *Milicia excelsa* was moderately related to silt content in the soil and rainfall (R^2 = 0.53 and R = 0.35 respectively, Table 4.5) and highly but negatively related to Na content in the soil (R^2 = 0.62, $P < 0.001$). Soil texture as clay, silt and sand amount in soil appeared to be important factors for explaining leaf size variation among iroko populations (R^2 = 0.49, R^2 = 0.52 and R^2 = 0.49, $P < 0.01$ respectively, Table 4.5). Leaf

length variation was dependent on soil chemical properties such as N (R^2 = 0.58, P < 0.001), Ca (R^2 = 0.54, P < 0.001), Mg (R^2 = 0.63, P < 0.001) Na (R^2 = 0.38, P < 0.01), Mo (R^2 = 0.38, P < 0.05) and CEC (R^2 = 0.64, P < 0.01). Rainfall has moderate but significant influence on flowers size variation (R^2 = 0.26, R^2 = 0.47, and R^2 = 0.22).

CCA combining ordination and multiples regression of morphological traits on environmental variables confirmed that rainfall and edaphic factors significantly affect morphological variation in iroko populations (r = 0.92 and r = 0.87, Table 4.6). Directions and influences of environmental factors (Fig. 4.4) clearly indicate that variation in the iroko Cluster 1 was explained by soil content of Na and rainfall, and to a lesser extend by the amount of clay, silt and N in soil. Morphological variation in cluster 2 (Save, Aplahoue and Lokossa) and cluster 3 (Bohicon, Bante and Sakete) was related to amount of Ca, Mg, clay in the soil and to rainfall and soil's caption exchangeable capacity. Sandy texture and amount of organic matter in soil are the main factors which explained morphological variation in iroko cluster 4 (Fig 4.4).

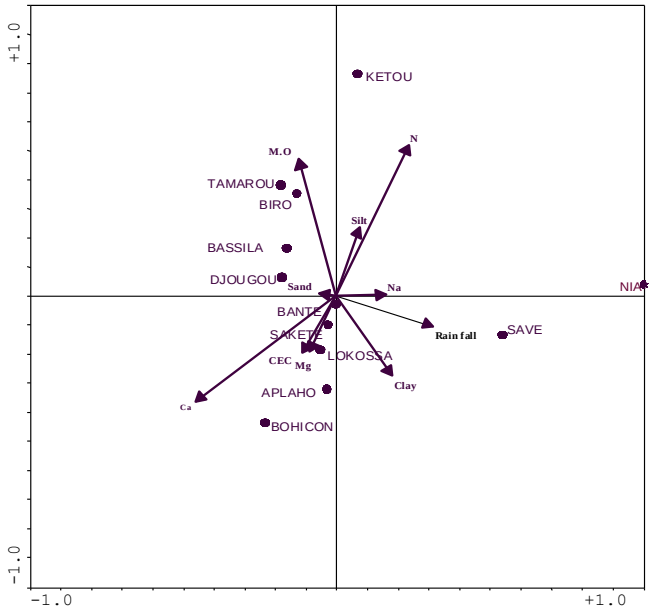

Fig. 4.4: CCA ordination diagram indicating the influence of rainfall and edaphic factors on the morphological distribution of iroko populations

4.3.4- Structural characteristics of *Milicia excelsa* populations

Milicia excelsa populations density varied from 1 to 9 stems / km² (Table 4.4). The highest average DBH values were recorded for Biro, Bohicon, Niaouli, Tamarou and Save populations. Stand basal area ranged from 0.19 to 2.05 m² / km². All of the iroko populations showed bell-shaped diameter class distribution except Bassila population (Fig 4.5). They typically have fewer number of stem in the smaller and larger diameter classes and more in the intermediate classes as observed for a well-thinned tree population structure. Among these populations four (Tamarou, Save, Ketou and Bohicon) showed an erratic structure with large gap in their class distribution. Population of Bassila follows an inverted J shape curve with large number of small trees and small number of large trees.

Table 4.4: Morphological variation of the twelve studied *Milicia excelsa* populations

Populations	DBH/TH	DBH/BH	DBH/CD	LL	LW	MFL	MFW	FFL	FFW	D (Ni/ km²)	G (m²/ km²)
Aplahoue	0.0296 b	0.0138 d	0.0677 f	15.943 d	9.755 d	8.844 a	0.5 a	4.555 b	1.8556 b	6.00	2.05
Bante	0.0636 d	0.0163 c	0.0476 c	13.16 b	8.68 c	9.511 a	0.5 a	4.733 b	2 b	1.00	0.19
Basssila	0.0399 c	0.0848 a	0.0323 a	14.46 c	7.44 b	11.5 c	0.65 c	6 c	2.36 c	1.00	0.70
Biro	0.0346 c	0.0892 a	0.0458 c	14.9 c	7.767 b	12.833 c	0.6 b	5.217 b	2.217 b	1.00	0.68
Bohicon	0.0661 d	0.0205 c	0.0667 f	12.377 b	8.022 c	9.6 b	0.7 c	4.789 b	1.933 b	2.00	0.35
Djougou	0.0348 c	0.0825 a	0.0406 b	14.043 c	7.957 b	8.886 a	0.533 b	3.057 b	1.686 a	1.00	0.46
Ketou	0.0320 c	0.0797 b	0.0512 d	13.043 c	7.357 b	9.071 a	0.514 b	4.871 b	2.071 b	1.00	0.24
Lokossa	0.0305 b	0.0147 d	0.0578 e	15.69 d	9.64 d	10.76 b	0.54 b	4.02 b	1.73 b	9.00	2.0
Niaouli	0.0222 a	0.0102 e	0.0516 d	18.933 e	11.844 e	14.2 d	0.66 b	5.98 b	1.8 b	2.00	1.25
Sakete	0.0854 e	0.0191 c	0.0635 e	12 b	8.2 c	12.5 c	0.56 b	5.357 b	2 b	2.00	0.89
Save	0.0292 b	0.0128 d	0.0457 c	16.867 d	10.417 d	12.667 c	0.578 b	3.833 b	2 b	1.00	0.57
Tamarou	0.0390 c	0.0902 a	0.0430 b	10.371 a	6.757 a	10.328 b	0.586 b	3.5 a	1.814 b	1.00	0.61

Diameter at breast height (*DBH*), total height (*TH*), bole height (*BH*)crown diameter (*CD*), leaf length (*LL*), leaf width (*LW*), male flowers length (*MFL*), male flower width (*MFW*), female flowers length (*FFL*), female flower width (*FFW*), stand basal area (*G*), population density *(D)*

Table 4.5: Coefficient of determination (R^2) of the linear regressions of morphometric traits on environmental variables.

	Clay	**Silt**	**Sand**	**N**	**M.O**	**Ca**	**Mg**	**Na**	**CEC**	**Rainfall**
DBH/TH	0,285	0,532**	0,053	0,244	0,250*	0,235	0,050	0,620***	0,159	0,350*
DBH/BH	0,219	0,092	0,148	0,379*	0,058	0,016	0,027	0,121	0,027	0,157
DBH/CD	0,209	0,369**	0,169	0,272*	0,188	0,033	0,002	0,426***	0,017	0,057
LL	0,494**	0,025	0,488**	0,579***	0,376*	0,545***	0,626***	0,386**	0,637***	0,089
LW	0,037	0,525**	0,080	0,287	0,053	0,036	0,099	0,005	0,068	0,062
MFL	0,137	0,190	0,252*	0,002	0,107	0,285*	0,201	0,192	0,263*	0,062
MFW	0,013	0,178	0,086	0,083	0,034	0,211*	0,188*	0,148	0,205*	0,262**
FFL	0,259*	0,030	0,131	0,028	0,193	0,004	0,036	0,065	0,015	0,473***
FFW	0,080	0,060	0,073	0,038	0,197	0,016	0,038	0,063	0,006	0,220*

Diameter at breast height (*DBH*), total height (*TH*), bole height (*BH*)crown diameter (*CD*), leaf length (*LL*), leaf width (*LW*), male flowers length (*MFL*), male flower width (*MFW*), female flowers length (*FFL*), female flower width (*FFW*). Significant level of the correlations are indicated by *($P < 0.05$), **($P < 0.01$) and ***($P < 0.001$).

Table 4.6: Relationship among morphological variation in iroko populations and environmental factors, derived from CCA

Axis	Eigenvalue	Population - environment correlations	Cumulative % variance of population-environment relations	Cumulative % variance of population data
1	0.059	0.92	78.9	62.6
2	0.015	0.87	99.1	78.7

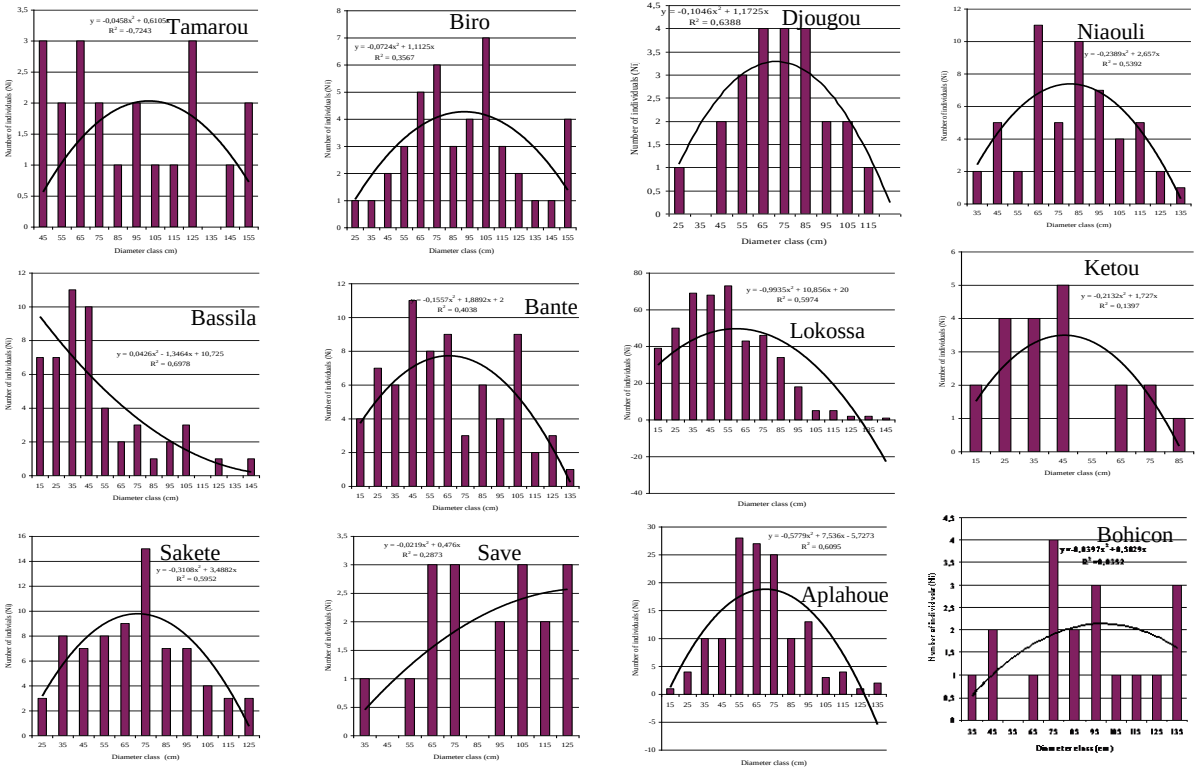

Fig. 4.5: Diameter class distribution of *Milicia excelsa* populations

4.4- Discussion

4.4.1- Morphological variation in *Milicia excelsa*

Our results suggested that morphological variation in the studied *Milicia excelsa* populations was strongly influenced by environmental factors. Several studies have indicated that morphological variation is apparently the result of an adaptive response to the environment; For example, variation of some traits is associated with a latitudinal and altitudinal range (Kleinschmit, 1993, Beaulieu *et al.*, 2004) or by contrasting climatic conditions (Bruschi *et al.*, 2003). Our results suggested that morphometric characters vary among populations without showing any geographic trend. The observed trend of morphological variation made mention of adaptation to the contrasting micro-edaphic conditions prevailing for these groups and this was supported by the significant correlation with soil physico-chemical characteristics. The greater discrimination power of adaptation micro edaphic conditions compared to the geographical regions of origin of accession in this study clearly indicated the greater importance of environmental factors (soil texture, soil chemical characteristics, and annual rainfall) than geographical location, in discriminating populations. This corroborated the results of Carter *et al.* (1987) who reported that water stress, together with soil nutritional deficiencies, have led to the development of adaptation aptitudes and hence morphological variation in trees populations. Similar results were found on other plant families (e.g. Casas *et al.*, 1999 on *Stenocereus stellatus* in Central Mexico, Bruschi *et al.*, 2003 on Italian populations of *Quercus petraea*).

The variation observed in *Milicia excelsa* morphological structure could indirectly reveal the consequences of natural habitat fragmentation and human pressure on ecosystems and tree populations. Indeed, most of individuals from cluster 1 in this study (Niaouli population) which showed the highest height growth and wider leaf size came from a region which harbors a typical humid semi-deciduous forest as a proof of its soil nutritional richness. In addition, majority of those sampled trees were from the protected site of a research centre, which has probably experienced various soil fertilizations. Similarly, populations from cluster 2 and 4 have most of their sampled trees being either the oldest one whose growth might have not suffered from recent human pressure on ecosystems (Save population), or dwelling sites that benefited from consequences of natural fragmentations such as *(i)* humid forest vegetation type and consequently soil type in Bassila and Djougou region due to Atacora mountain and the

phenomenon of dense forest species eradiation; *(ii)* eastern and western extension of Lama depression which maintained edaphic micro conditions in the surrounded areas in Aplahoue and Lokossa zones in the west and Ketou zone in the east. This explanation is congruent with our CCA results which indicated the amount of clay and silt, MO and CEC as the main explanatory variables of variation in these clusters. In this category, the inclusion of Tamarou and Biro populations harboring trees with moderate height growth and leaf size, could be explained by the extensive land use and intensive chemical fertilization of soils due to cotton culture in that region. To the contrary, the smallest leaf size and lowest height growth revealed by cluster 3 (Bohicon, Sakete and Bante populations) could result from intensive land use, lack of soil fertilization and absence of fallow. The flower size variation observed among populations with relation to annual rainfall made mention of development of adaptation abilities to climatic conditions, although it could be related to genetic variation in *Milicia excelsa* populations. Indeed, Camussi *et al.* (1985) have stated that human actions and natural selection factors, by affecting morphological traits related to adaptation of a population, could allow interference with adaptation due to genetic distances from quantitative traits.

4.4.2- *Milicia excelsa* populations' structure

The stand density calculated for *Milicia excelsa* populations was low but in the range with that reported by Peres and Baider (1997) on *Bertholletia excelsa* from line-transect censuses. The bell-shaped stem class distribution exhibited by iroko populations supported the species temperament as light demanding species are known to show such distribution. They are gap demanding for their regeneration and mortality is higher in earlier stage under closed forest canopy (Geldenhuys, 1992; Sokpon, 1995; Sokpon and Biaou, 2002). Combining the species temperament and seeds dispersal pattern of iroko which is a barochore (mature fruits are heavy and drop under the mature trees, Sokpon and Lejoly, 1994) and widely disseminated by bat (*Eidolon helvum*, Taylor et. al., 2000), iroko is expected to show large number of seedling under parent tree but it did not, although inventoried trees are mostly in open areas (in fallow lands, on farm, etc.) and seedling are protected by traditional ethnobotanic practices in Benin (Ouinsavi *et al.*, 2005). This adequate light level and abundant *Milicia* fruits, but lower than predicted regeneration under and around iroko trees suggest that some factor or combination of factors limits successful regeneration. Indeed, iroko female trees produce abundant fruit but the germination rate is low and decreases very quickly. In

addition the bulk part of fruit found beneath iroko tree is rejecta pellets. According to Taylor *et al.* (2000) the rejecta seeds have a very low percent germination because *Eidolon* feeds on *Milicia* fruit by sucking to select the more viable seeds, while immature, deformed or aborted seeds remain in the rejecta pellet. Thomas (1982) examining *Eidolon* excreta, found that its diet during migration was 88.8% *Milicia* fruits. Nichols *et al.* (2000) reported that fruit fall was heavy beneath female *Milicia*, but seedlings are found in small clumps, 150 m distanced away from supposed parent trees, which represent sites at which birds or especially bats defecated seeds.

The inverted J shaped curve shown in Bassila population class distribution can be explained by the heavily selective logging activity in that region which creamed valuable species in term of tree size.

Combining the ecological structure of the remnant iroko populations and the above mentioned morphological variation, it could be inferred that the current trend of those populations mirrored back the adaptation of *Milicia excelsa* to environmental changes due to its habitat destruction and fragmentation. However fragmentation might not induce only occurrence of micro-vegetational and edaphic conditions to which species has to adapt but also isolation of individual populations may have been causing decreasing of genetic variability and genetic drift progressively moving towards a discrete extinction of the species. Although morphological traits and ecological structure are known to represent only a small proportion of plants genome because there are influenced by environmental factors (Atta-Krah, 2004), morphological variation and spatial structure may have some genetic basis which could be useful for studies of the developmental mechanisms of plant populations (Casas, 1999; Klingenberg, 2002). Therefore the results of this study raised urgent needs of genetic variation and population structure assessment in *Milicia excelsa* species.

5

Spatial structure of genetic variation of *Milicia excelsa* populations in Benin revealed by random amplified polymorphic DNA markers

Christine Ouinsavi*, Nestor Sokpon

Laboratoire d'Études et de Recherches Forestières, Faculté d'Agronomie, Université de Parakou, BP 123 Parakou, République du Bénin;
*Author for correspondence :E-mail: christine.ouinsavi@sbf.ulaval.ca; ouinsch@yahoo.fr Phone : (229) 2361 2010, Fax: (229) 2361 0712

Abstract

The level of genetic variation and the pattern of genetic structure in twelve natural populations of *Milicia excelsa*, covering the species range in Benin, were assessed using Random Amplified Polymorphic DNA (RAPD) markers. Based on seven primers, a total number of 49 bands were recorded in 116 *Milicia excelsa* sample investigated, of which 44 bands (89.8 %) were polymorphic. Genetic variation was moderate (N_A = 1.933 ± 0.258, N_E = 1.416 ± 0.364, h = 0.252 ± 0.177 and H_S = 0.168 ± 0.012). Genetic differentiation among populations was moderate (G_{ST} = 0.331) indicating that 33 % of the total genetic variation accounted for among-population differentiation. Estimated gene flow equaled to Nm = 1.01 showing limited seed and pollen dispersal among populations. Cluster analysis dispatched populations into four groups based on genetic distances. In addition, Barrier analysis combining geographical coordinates and genetic differentiation among populations pairs, has distinguished three main zones with sharp genetic change and lower gene flow, likely due to an effect of isolation by distance. Based on these results, conservation actions could be planned to ensure the maintenance of appropriate levels of genetic variation in *Milicia excelsa* populations in Benin.

Keywords: genetic variation, population structure, *Milicia excelsa*, RAPD.

5.1- Introduction

Milicia excelsa Welw. C.C. Berg *(*Moraceae) commonly known as iroko is a dioecious species widely distributed across West Central and East Africa and occurs in a wide belt from Côte d'Ivoire to Tanzania. *M. excelsa* grows in transitional vegetation between closed forests and savanna (Howthorne, 1995). It is often found in gallery forest and can be found in deciduous, semi-deciduous or evergreen forest. Occasionally it is found in isolated relict forests from sea level to about 1300m. It is fairly abundant in the drier areas of semi-deciduous *Antiaris-Chlorophora* forest. In Republic of Benin, *M. excelsa* is distributed across the whole country area between latitude 6°30′N to 11°N.

From the early 1980s up to late 1990s, this species was the focus of an important transaction between Africa and Europe. Many African countries were exporters of iroko timber, especially Ghana (traded together with *M. regia*), Côte d'Ivoire, Cameroon, Congo and Gabon (WCMC, 1991). As a result of its over-exploitation, this species was classified on the IUCN red list as close to vulnerable (IUCN, 2006) and has been given conservation priority in many African countries. Genetic diversity assessment is necessary for *M. excelsa* genetic resources conservation. Therefore, attention needs to focus on the level of biological structure within the species.

Nowadays, a panel of molecular markers is available for genetic resources characterization, management and use. This wide array of DNA markers such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) has enabled researchers to investigate genetic diversity among various plant species across natural populations (Vanijajiva *et al*., 2005; Jeandroz *et al*., 2004; Jain *et al*., 2003; Archak *et al*., 2003, Lowe *et al*., 2000; Yeh *et al*., 1995).

Random Amplified Polymorphic DNA (RAPD), a technique generated by PCR using single decamer primers to amplify arbitrary fragments of DNA from priming sites throughout the entire genome (Gillies and Abbott, 1998), may provide a good basis for the estimation of intra – and inter- specific relationship especially in case there is lack of genetic information on a species. RAPD markers have been past and recently used for number of species in a wide variety of applications such as: assessment of genetic diversity within and between populations (Chalmers *et al*., 1992; Bhat and Jarret, 1995; Yeh *et al*., 1995; Changtragoon *et al*., 1997 ; Farooqui *et al*., 1998; Mamuris *et al*., 1999; Lowe *et al*. 2000; Jeandroz *et al*., 2004; Besse *et al*., 2004; Mehrnia *et al*., 2005), species and cultivars identification (Khasa et Dancik, 1996 ; Gillies *et al*., 1998; Schnell *et al*., 1999), marker linked to disease resistance and agronomic traits (Hayley *et al*., 1993; Yu and Paul, 1993), and establishment of species relationship in plant genera

(Thormann *et al*., 1994; Graham and McNicol, 1995; Comincini *et al*., 1996; Skepner and Krane, 1998; Ofori *et al*., 2001; Besse *et al*., 2004; Vanijajiva *et al*., 2005; Khanuja *et al*., 2005). Ofori *et al*. (2001) have used RAPD markers to dispatch the different species of *Milicia* which occur in Ghana, Côte d'Ivoire and Sierra Leone with relation to their geographical distribution in Ghana. But to date, there is no information on the extent and partitioning of genetic variation in *Milicia excelsa* in the whole Dahomean Gap.

In this study, we use Random Amplified Polymorphism DNA (RAPD) to provide insights into the genetic variability of *Milicia excelsa* and population structure in Benin.

5.2- Materials and methods

5.2.1- Plant material

Leaf samples from a total of 116 *Milicia excelsa* trees were collected in twelve natural populations representing the geographical distribution of the species in Benin (Table 5.1). Populations were geographically distant for more than 50 km and trees sampled within a population were distant from at least 100 m. The collected leaves were oven dried and used for DNA extraction.

5.2.2- DNA extraction and RAPD Amplification

Total genomic DNA was extracted from about 0.05 g of dried leaves weighed from each sample using the protocol provided in DNeasy Plant Mini Kit (QIAgen, UK, Ltd). DNA concentration was determined by electrophoresis on agarose - TAE gel stained with ethidium bromide (harry, 2001) by comparison with a 200 bp Hypperladder (Bioline, UK Ltd)..

Ten decamers primers (GEN 1-60, A-J) designed by GENOSYS (BioRad, UK Ltd) were screened and seven of them selected for clarity of the produced patterns were used for the amplification. Polymerase Chain Reaction (PCR) was carried out using a 96-well block thermocycler (PCR express, Hybaid, UK Ltd) in 20µl reaction volume containing 16 ng genomic DNA templates, 1x reaction buffer, 2.5mM $MgCl_2$, 2mM dNTPs, 1µM primer and 1 unit Taq polymerase. The PCR conditions were: initial denaturation at 94°C for 3 min followed by 36 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min and extension at 72°C for 2 min, with final extension at

72°C for 7 min. PCR products were held at 4 °C until they were analyzed. RAPD fragments were separated electrophoretically in 1.4 % TAE agarose gel stained with ethidium bromide at 0.5 μg/ml. Gel was run at a constant voltage 100 volts for 3 hours and image were analyzed using AlphaImager digital imaging and analysis system (Flowgen Bioscience, UK Ltd). Sizes were assigned to bands by comparison with a 200 bp Hypperladder (Bioline, UK Ltd).

5.2.3- Data analysis

Unequivocally scorable and consistently reproducible amplified DNA bands were transformed into binary character matrices as present (1) or absent (0) on the basis of size comparison with external standards (DNA Ladder Plus).

The following parameters were generated using the program POPGENE version 1.32 (Yeh *et al*., 1997) to describe intra- and inter-population genetic variation: Percentage polymorphism (*P*), heterozygosity (*h*), also referred to as genetic diversity, Shannon's information index (*I*), the observed number of alleles (N_A) and the effective number of alleles (N_E) (Lewontin, 1972; Nei, 1973, Yeh *et al*., 1997). Genetic divergence between populations were investigated using Nei's unbiased genetic distances (*GD*) and genetic identities (*GID*, Nei, 1978). These data were used to construct a phylogenetic dendrogram based on unweighted pair group method with arithmetic average (UPGMA, Nei, 1978) modified from NEIGHBOR procedure of PHYLIP Version 3.5. The genetic structure was investigated using Nei's gene diversity statistics, including the total genetic diversity (H_T), genetic diversity within populations (H_S), and the relative magnitude of genetic differentiation among populations ($G_{ST} = (H_T - H_S)/ H_T$) (Lewontin, 1972). An estimate of gene flow among populations (*Nm*) was computed using the formula [$Nm = (1 - G_{ST})/2\ G_{ST}$)] (McDermott and McDonald, 1993). A geographic matrix were generated by calculating geographic distances between populations based on their latitude and longitude, using the great-circle method (available at http://www2.nau.edu/~cvm/latlongdist.html).

The program BARRIER version 2.2 was used to highlight geographical areas with pronounced genetic barriers between populations (Manni, 2004) based on Monmonier algorithm (Monmonier, 1973). The method allows defining zones of maximum genetic change along a network of connecting populations (the 12 populations of *Milicia excelsa*). First, a Delaunay triangulation network (Brassel and Reif, 1979) was created to connect the adjacent populations by a set of triangles. Then a Voronoï tessellation, a polygonal neighborhood for each population that is constituted of those points, on the

plane, that are closer to such sample than to any other one (Voronoi, 1908), was superimposed on the corresponding Delaunay network. Finally the maximum difference algorithm of Monmonier was applied to identify zones of discontinuity. A barrier was initiated by tracing a line on the edge of Voronoï polygon that was associated with the highest distance between two neighbouring populations. The barrier was extended progressively to the adjacent edge that was associated to the highest distance until it reached the edge of the network or until it closed a circle around one or more populations. This new method uses pairwise measures of differentiation among populations with known locations to determine where breaks in gene flow might occur.

Table 5.1: Summary of Genetic Variation Statistics in twelve *Milicia excelsa* populations

Populations	Sample size	Geographical coordinates		N_A (± SD)	N_E (± SD)	h (± SD)	**I (± SD)**
		Longitude (°E)	Latitude (°N)				
Sakete	10	002°04'49.6"	07°18'16.1"	1.333 ± 0.488	1.146 ± 0.304	0.087 ± 1.163	0.137 ± 0.235
Ketou	06	002°35'45.2"	07°20'59.2"	1.267 ± 0.458	1.136 ± 0.293	0.081 ± 0.160	0.125 ± 0.234
Bohicon	10	002°39'38.4"	09°38'17.7"	1.267 ± 0.458	1.191 ± 0.358	0.107 ± 0.191	0.157 ± 0.275
Biro	10	0023°5'34.8|"	06°37'05.2"	1.333 ± 0.488	1.221 ± 0.361	0.127 ± 0.197	0.188 ± 0.285
Djougou	10	001°26'04.8"	10°07'27.4"	1.800 ± 0.414	1.504 ± 0.375	0.291 ± 0.190	0.433 ± 0.263
Bassila	10	001°39'16.9"	09°02'36.1"	1.666 ± 0.488	1.391 ± 0.411	0.222 ± 0.213	0.332 ± 0.297
Bante	10	001°58'06.6"	08°13'49.2"	1.733 ± 0.457	1.415 ± 0.405	0.239 ± 0.205	0.364 ± 0.283
Tamarou	10	002°56'28.8"	09°56'11.2"	1.600 ± 0.507	1.377 ± 0.362	0.224 ± 0.203	0.334 ± 0.294
Niaouli	10	002°08'08.1"	06°44'16.1"	1.600 ± 0.507	1.352 ± 0.397	0.203 ± 0.211	0.303 ± 0.298
Aplahoue	10	001°50'42.6"	07°05'06.4"	1.533 ± 0.516	1.297 ± 0.365	0.177 ± 0.199	0.268 ± 0.287
Lokossa	10	001°40'16.4"	06°45'26.1"	1.733 ± 0.457	1.258 ± 0.271	0.173 ± 0.158	0.283 ± 0.229
Save	10	002°31'16.1"	08°04'27.9"	1.400 ± 0.507	1.120 ± 0.234	0.081 ± 0.135	0.136 ± 0.204
Mean	116			1.522 ± 0.186	1.284 ± 0.119	0.167 ± 0.067	0.255 ± 0.099

N_A = Observed number of alleles; N_E = Effective number of alleles; h = Nei's (1973) gene diversity; I = Shannon's Information index

5.3- Results

5.3.1- RAPD variation and Genetic diversity within iroko populations

A total number of 49 scoreable bands were recorded over the 7 primers in the 116 *Milicia excelsa* tree samples investigated with a range from 3 bands (GEN 1- 60 B) to 9 bands (GEN 1-60 C) and an average of 7 bands per primer (Table 5.2). Most of the bands were polymorphic across populations with percentage polymorphic bands ranging from GEN 1-60 C (*P*= 66 %) to GEN 1-60 B and GEN 1-60 H (*P* = 100 %), and an average of 89.8 %.

The mean number of alleles varied from Ketou and Bohicon (N_A = 1.267 ± 0.458) to Djougou (N_A = 1.800 ± 0.414) with an average of N_A = 1.522 ± 0.186 while the mean effective number of alleles ranged from Save (N_E = 1.120 ± 0.234) to Djougou (N_E = 1.504 ± 0.375) with an average of N_E = 1.284 ± 0.119 (Table 5.1). Nei's gene diversity (*h*) varied from 0.081 ± 0.135 to 0.291 ± 0.190 with an average of 0.252 ± 0.177 and Shannon's information index ranged from 0.125 ± 0.234 to 0.433 ± 0.263 with an average of 0.167 ± 0.067. *h* and *I* showed a similar trend in all of the populations. Population Djougou showed the highest gene diversity while populations Save and Ketou have the lowest gene diversity.

Nei's estimate of gene diversity under Hardy-Weinberg disequilibrium revealed a total genetic diversity of H_T = 0.251 ± 0.031 and genetic diversity within populations equalled to H_S = 0.168 ± 0.012 (Table 5.3).

Table 5.2: Number of bands and percent polymorphism reveled by RAPD primers

Primers identification	Primer sequence	Number of bands	Number of polymorphic bands	Percent polymorphism
GEN 1-60 B	GTCCTACTCG	3	3	100 %
GEN 1-60 C	CTACACAGGC	9	6	66 %
GEN 1-60 D	GTCCTTAGCG	8	8	100 %
GEN 1-60 E	GTCCTGAACG	8	8	100 %
GEN 1-60 G	GAGTCAATCG	7	6	86 %
GEN 1-60 H	GTCCTCAGTG	6	6	100 %
GEN 1-60 J	GCAGACTGAG	8	7	87.5 %
Average		7	6.29	89.8 %

Table 5.3: Nei's (1973) genetic diversity statistics under the assumption of Hardy-Weinberg disequilibrium.

Parameter	Entire Data			
	H_T	H_S	G_{ST}	*Nm*
Hardy-Weinberg disequilibrium	0.251 ± 0.031	0.168 ±0.012	0.331	1.010

H_T = Total heterozygosity, H_S = within population heterosigosity, G_{ST} = genetic differentiation among populations, *Nm* = estimate of gene flow from G_{ST}, *Nm* = 0.5 (1 - G_{ST}) / G_{ST}

5.3.2- Population genetic structure

Genetic differentiation among populations estimated for multiple populations by G_{ST} was equaled G_{ST} = 0.331 (Table 5.3). Estimated value of gene flow obtained from the estimate of *GST* (Slatkin, 1989) was *Nm* = 1.01 migrants per generation between populations (Table 5.3), suggesting a restricted extent of gene flow among populations. The calculated genetic distance between populations pairs varied from *GD* = 0.007 to *GD* = 0.337. The highest values were recorded between populations Djougou, Bassila and Bante on one hand and populations Sakete, Ketou and Biro on another hand (Table 5.4).

UPGMA dendrogram constructed from modified NEIGHBOR procedure of PHYLIP, showed division of the populations into four clusters (Fig 5.1). This result revealed the existence of geographical structure of genetic variation of *Milicia excelsa* in Benin. Northern east populations clustered to form group 1, southern east populations including one population from the center (Bohicon) composed group 2, northern west populations grouped in group 3 and southern west populations clustered with one population from the center to form group 4. Two more broad groups can be distinguished at a less significant similarity level which are composed each of populations from east of the country, and western populations.

Monmonier's maximum difference algorithm identified three main genetic boundaries (Fig. 5.2), supporting the previous results. By definition, these boundaries correspond to zones of most abrupt genetic change in space and are identified in a descending order. The first genetic barrier (***a*** on Fig. 5.2) separated the two northern east populations. The second boundary (***b*** on Fig. 5.2) separated the two southern east populations from the other eight populations while the third genetic barrier (***c*** on the Fig

5.2) separated the three northern west populations from the rest. The average genetic diversity estimated for each of the four groups delineated by the Monmonier spatial analysis were 0.11 for northern east group, 0.14 for southern east group, 0.26 for northern west group and 0.20 for southern west and central group.

5.4- Discussion

Dominant markers have been widely used for genetic variability and cultivars identification in number of species (Aga *et al.*, 2005). They are reproached with having low reliability and repeatability. In this study, repetitiveness was ensured by repeating amplification at least twice and selecting the most reproducible bands for the analysis.
Based on this RAPD investigation, genetic variability in *Milicia excelsa* (N_A, N_E, and h) was moderate. Estimated within population genetic diversity (H_S) was lower than the values reported for the same species in moist forest (0.405) and dry forest populations in Ghana (Khanuja *et al.*, 2005). But this value is higher than those estimated for Ivory Coast (0.054) and Sierra Leone (0.122) populations (Khanuja *et al.*, 2005). The total genetic diversity for *Milicia excelsa* (H_T) is greater than that reported for animal-pollinated (0.167 – 0.182) plants and widespread (0.204) tropical tree species (Loveless & Hamrick, 1984, Hamrick & Godt, 1989, Loveless, 1992, Desphande *et al.*, 2001). Regarding the bioecological features and mating system of the species (widespread, majority outbreeding species), it is expected to show a higher genetic diversity than observed (Hamrick & Godt, 1992). This results raised a suspicion that there may be a reduction of heterozigosity probably due to inbreeding occurring in the species or other factors such as Walhund effect, genetic drift and the maintained of ancestral polymorphism which could lead to the same results.

The amount of genetic differentiation observed among populations was considerable (G_{ST} = 0.331) indicating that 33 % of the total genetic variation accounted for among population differentiation. Since the species of concern is a tropical cross pollinated species with bat and bird dispersal – seeds Taylor *et al.*, 2000) and some gravity-dispersed seed, this value seems to be large taking into account the general observation that woody, perennial and outbreeding species maintain most of their variation within populations (Hamrick & Godt, 1992). However the large G_{ST} value was supported by the relatively low gene flow between populations (Nm = 1.01). Theoretically, gene flow of more than four migrants per generation among populations is necessary to prevent genetic drift causing local genetic differentiation and therefore, population divergence (Slatkin, 1987). Similar result was obtained for other outbreeding animal-pollinated plant species (Desphande *et al.*, 2001).

Cluster analysis dispatched populations into four groups based on genetic distance among population pairs, separating northern east populations, northern west populations, southern east populations and, central and southern west populations thus suggesting a geographical heterogeneity in *Milicia excelsa* genetic structure. Other authors have investigated the spatial genetic structure in various tropical or temperate tree species and reported either a lack or a weak geographical heterogeneity which, they argued by the limited seed dispersal and extensive gene flow (Berg & Hamrick, 1994, Loiselle *et al*., 1995; Chung & Chung, 1999) or by extensive gene flow, wide seed dispersal, self incompatibility and dispersal agent (Waser, 1987; Dewey & Heywood, 1988; Doliguez & joly, 1997; Chung *et al.*, 2000). According to these authors, woody insect-pollinated species with seed widely and independently dispersed by birds, at most weak genetic structure will result. In the current study, Barrier analysis identifying three main zones with sharp genetic change and lower gene flow, clearly suggested an impact of limited seed and pollen dispersal and evidence effect of isolation by distance.

The information gained from this study is of tremendous use for planning conservation genetic actions to ensure the maintenance of appropriate levels of genetic variation in *Milicia exclesa* populations in Benin. However, further researches on the species, should address the level of inbreeding and distribution patterns of ancestral polymorphism in the species.

Table 5.4: Nei's Genetic Identity and Genetic distance among populations of *Milicia excelsa*.

Populations	Sakete	Ketou	Bohicon	Biro	Djougou	Bassila	Bante	Tamarou	Niaouli	Aplahoue	Lokossa	Save
Sakete	****	0.9495	0.9453	0.9085	0.8116	0.7950	0.8210	0.9193	0.8874	0.8910	0.8943	0.9119
Ketou	0.0518	****	0.9825	0.9598	0.7835	0.7338	0.7748	0.9058	0.8807	0.8522	0.8192	0.8182
Bohicon	0.0562	0.0177	****	0.9506	0.7572	0.7136	0.7536	0.8909	0.8579	0.8405	0.8069	0.8163
Biro	0.0960	0.0410	0.0506	****	0.8266	0.7871	0.8309	0.9572	0.9082	0.8776	0.8581	0.8636
Djougou	0.2087	0.2440	0.2781	0.1905	****	0.9709	0.9933	0.9075	0.9522	0.9233	0.9238	0.8826
Bassila	0.2295	0.3096	0.3375	0.2394	0.0296	****	0.9833	0.8939	0.9510	0.9381	0.9403	0.9113
Bante	0.1972	0.2552	0.2829	0.1852	0.0068	0.0168	****	0.9166	0.9583	0.9374	0.9485	0.9140
Tamarou	0.0842	0.0990	0.1155	0.0437	0.0971	0.1121	0.0871	****	0.9585	0.9486	0.9355	0.9465
Niaouli	0.1194	0.1270	0.1533	0.0963	0.0490	0.0502	0.0426	0.0424	****	0.9761	0.9508	0.9280
Aplahoue	0.1154	0.1599	0.1738	0.1306	0.0798	0.0639	0.0647	0.0527	0.0242	****	0.9686	0.9585
Lokossa	0.1117	0.1994	0.2146	0.1530	0.0793	0.0616	0.0529	0.0667	0.0505	0.0319	****	0.9790
Save	0.0922	0.2006	0.2030	0.1467	0.1249	0.0929	0.0900	0.0549	0.0747	0.0423	0.0212	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

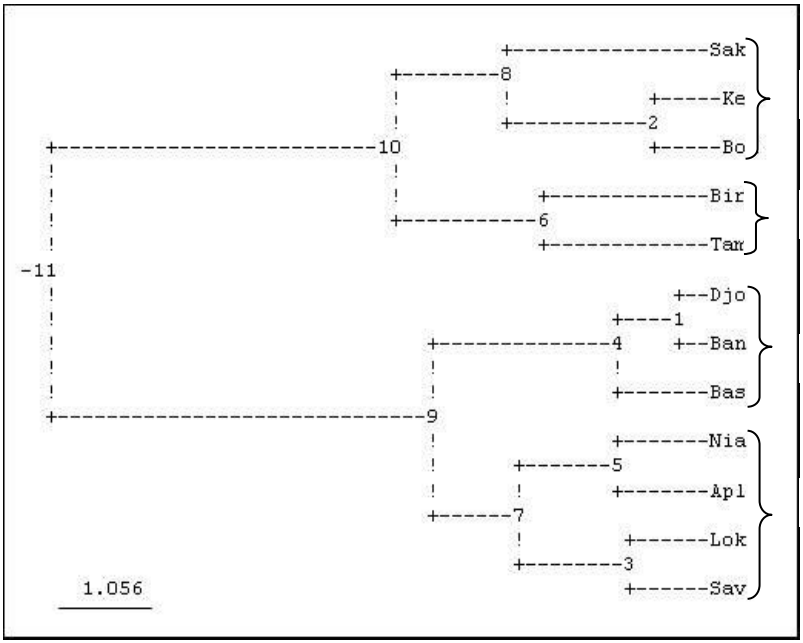

Fig. 5.1: UPGMA dendrogram of *Milicia excelsa* populations based on Nei's unbiased genetic distances among populations.

Sak = Sakete; Ket = Ketou; Tam = Tamarou; Bir = Biro; Djo = Djougou; Bas = Bassila; Ban = Bante; Boh = Bohicon; Nia = Niaouli; Apl = Aplahoue; Lok = Lokossa; Sav = Save

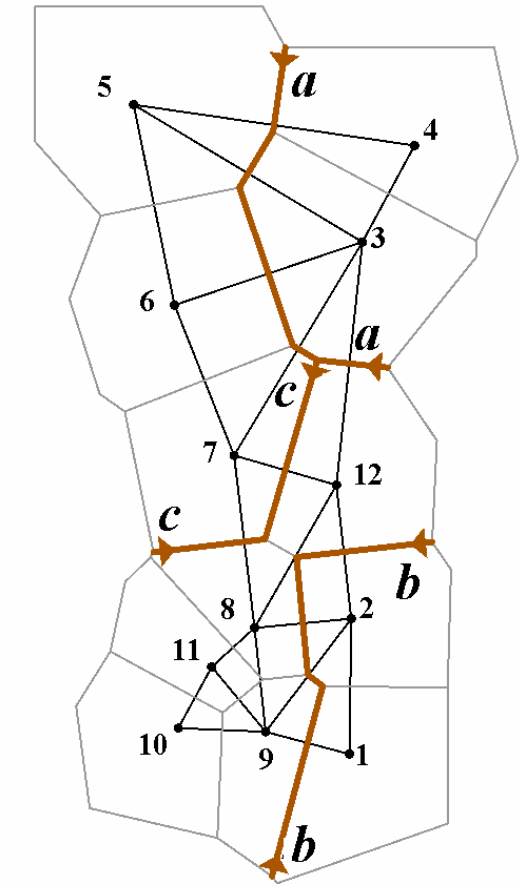

Fig. 5.2: Result of BARRIER analysis using pairwise G_{ST}
1 = Sakete; 2 = Ketou; 3 = Tamarou; 4 = Biro; 5 = Djougou; 6 = Bassila; 7 = Bante; 8 = Bohicon; 9 = Niaouli; 10 = Aplahoue; 11 = Lokossa; 12 = Save; a, b, and c are the genetic barriers; 1,2,…12 are the geographical locations of populations; Cluster 1= (3,4); Cluster 2 =(1,2); Cluster 3 = (5,6,7) and Cluster 4 = (8,9,10, 11, 12)

6

Novel microsatellite DNA markers for the threatened African endemic tree species, *Milicia excelsa* (Moraceae), and cross-species amplification in *Milicia regia*

Christine Ouinsavi[1,2*], Nestor Sokpon[1], Jean Bousquet[2], Craig H. Newton[3] and Damase P. Khasa[2]

[1]Laboratoire d'Études et de Recherches Forestières, Faculté d'Agronomie, Université de Parakou, BP 123 Parakou, République du Bénin
[2]Chaire de Recherche du Canada en Génomique Forestière et Environnementale and Centre de Recherche en Biologie Forestière, Université Laval, Sainte-Foy, Québec, Canada, G1K 7P4
[3]Vizon SciTecInc., 3650 Wesbrook Mall, Vancouver, BC, Canada, V6S 2L2

Molecular Ecology Note 6 (2006), 480 – 483

Abstract
Eleven microsatellite primer pairs were developed for the tropical African tree *Milicia excelsa.* Genomic DNA was enriched for dinucleotide (TC_n and TG_n) and tretranucleotide ($GATA_n$) and 188 random clones were sequenced from both orientations. We designed and tested 44 oligonucleotide primer pairs which were evaluated using genomic DNA from 30 *Milicia excelsa* mature trees collected from a natural population in Benin. Eleven of the 44 markers showed good amplification and were polymorphic. The number of putative alleles for polymorphic primer pairs varied from 3 to 7, with expected and observed heterozygosities ranging from 0.10 to 0.64 and from 0.10 to 0.80, respectively. All 11 loci amplified the related species *M. regia*, indicating that these primers will be useful for population and ecology genetic studies in other species of the genus *Milicia.*

6.1- Introduction

Milicia species *(M. excelsa* Welw. C.C. Berg and *M. regia A. Chev. C.C Berg*, Moraceae) commercially known as iroko, are African endemic species widely distributed across Africa. They occur in Angola, Benin, Burundi, Burkina Faso, Central African Republic, Cameroon, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Ethiopia, Gabon, Equatorial Guinea, Sao Tomé and Principe, Ghana, Kenya, Malawi, Mozambique, Nigeria, Sierra Leone, Sudan, Tanzania, Togo, Uganda, and Zimbabwe. Both species show a preference for flat and open areas in dry as well as moist and wet forest types (Hawthorne, 1995). They are both timber species in Sub-Saharan Africa and are very useful for people as they contribute to cure human diseases and pathologies in traditional medicine, to make furniture in carpentry and joinery, and to protect cultural values in local religions (Ouinsavi *et al.*, 2005). As a result of their over-exploitation, these species are classified on the IUCN red list as threatened with extinction (IUCN, 2004) and become the focus of conservation concern in many African countries. We developed microsatellite DNA markers for *Milicia excelsa* to investigate gene flow and population structure and examined the transferability of these markers for a congeneric species, *M. regia*.

6.2- Methodology

For the construction of enriched genomic libraries, DNA was extracted from a single individual of *Milicia excelsa* following the protocol of Dayanandan *et al.* (1997). We used the modified biotin-enrichment protocol described by Khasa *et al.* (2000). Briefly, 0.5 to 1.0 µg of genomic DNA were digested separately with *Hae III, Rsa I* and *Alu I* restriction enzymes (New England Biolabs, Beverly, Massachusets) in the presence of T4 DNA ligase (New England Biolabs), and M28 (5'-CTCTTGCTTGAATTCGGACTA-3') and M29 (5'-pTAGTCCGAATTCAAGCAAGAGCACA-3') polynucleotide linkers. A third restriction enzyme, *PsbAI* (New England Biolabs) was used to cleave any linker dimers produced by ligase. The adaptor modified genomic DNAs were then combined, denatured, and hybridized with biotinylated oligonucleotides AC_{12} and TC_{12} at 60°C and biotinylated $GATA_6$ at 50°C. These products were immobilized onto Dynabeads M-270 streptavidin magnetic beads (Dyna1) and washed extensively, first at the respective hybridisation temperature and then at room temperature. The enriched fractions were then amplified with primer M28 used as forward and reverse primer because the same

adaptor is on both ends of enriched DNA, digested with *EcoRI* as the adaptor contains an *EcoRI* site which helps to increase the efficiency of ligation by using a restriction endonuclease that creates 3' overhangs or 'sticky ends'. The products were then ligated into the plasmid vector pGEM3Z+ (Promega, Madison, USA). After transformation and selection on ampicillin agar plates, transformants were screened by colony hybridization with ^{32}P labelled $(AC/TC)_n$ or $GATA_n$, to identify microsatellite containing clones. Sequences of the positive colonies were determined using templates amplified with the forward and reverse M13 primers. Purified templates were then sequenced using the BigDye Terminator sequencing kit and an ABI 3600 automated sequencer (PE Applied Biosystems, Foster City, California).

7.3- Results

Of 188 sequenced clones, 81 revealed microsatellite loci and sufficient flanking regions for primer design. Primers to all 81 clones were designed using PRIMER 3 Output program (Rozen and Saletsky, 2000) and were synthesized by Operon (Qiagen-Operon, Alameda, California). Primers were first tested on genomic DNA from the original tree and the 44 primers that amplified clearly were then tested for their polymorphism on 30 individuals of *Milicia excelsa* randomly collected from natural habitat of the species in Benin (between 6°30' and 11° latitude N and 1° and 3° longitude E) and 10 individuals of *M. regia* collected in Ghana. Polymerase chain reaction (PCR) amplifications were carried out using a 96-wells thermal cycler PTC Peltier (MJ Research, Waltham, Massachusetts) and a 10-µL reaction volume, with 10 ng DNA, 1x PCR buffer (Invitrogen, Carlsbad, California), 3 mM $MgCl_2$ (Invitrogen), 10 ng BSA (LaRoche, Basel, Switzerland), 0.1 M Betaine (Sigma-Aldrich, St-Louis, Missouri), 2 nmol dNTPs (Invitrogen), 1µl (10µM) each of forward and reverse primers, 20 pmol Cy™5.5dCTP (Amersham-Bioscience, Buckinghamshire, UK) and 0.8U of Platinum *Taq* polymerase (Invitrogen). The PCR conditions were: initial denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30s and extension at 72°C for 3 min, with final extension at 72°C for 15 min. Unincorporated fluorescent dCTPs were removed from the PCR products by filtering them on 56 g/L Sephadex G-50 medium (Amersham Bioscience, Uppsala, Sweden) loaded on a multiscreen filter plate (Millipore, Bedford, Massachusetts) and 4 µl of stop buffer (Li-cor, Lincoln, Nebraska) were added to each purified product which was denatured at 94°C prior to use. PCR products were analysed on 6.5% denaturing polyacrylamide gels (Long Ranger, Biowhittaker Molecular Applications, Rockland, Maine) using an IR2DNA analyser (Li-cor). Allele sizes were determined using SAGA^{GT} v.2.0 software (Li-cor).

The number of alleles per locus, observed and expected heterozygosities as well as Wright's fixation index were estimated using the GDA program version 1.1 (Lewis & Zaykin, 2002). GDA was also used to test for conformance to Hardy-Weinberg expected genotypic proportions as well as linkage equilibrium.

Of the 44 tested primers, 11 loci amplified consistently and were polymorphic for both *M. excelsa* and *M. regia* (7 from di and 4 from tetra-nucleotides, Table 6.1). The total number of alleles varied from 3 to 7 (Table 6.1). Tetra-nucleotide polymorphic loci exhibited the smallest number of alleles. Observed (H_O) and expected (H_E) heterozygosities ranged from 0.10 to 0.80 (mean ± SE, 0.35 ± 0.08) and from 0.10 to 0.64 (mean ± SE, 0.44 ± 0.05), respectively (Table 6.1). Four loci among the eleven above mentioned (*Mex81, Mex137, Mex198, Mex243*) deviated significantly from Hardy-Weinberg expectations ($P < 0.05$). These departures may indicate population structure, as the 30 *M. excelsa* genotypes screened in this study are from diverse geographical origins, which may represent differentiated genetic pools. These loci show a significant disequilibrium in pairwise comparisons with estimated exact probabilities varying between 0.00 and 0.04.

One of the primer pairs (Mex163) amplified two different polymorphic loci (*Mex163a* and *Mex163b*) in *M. excelsa* and one polymorphic locus in *M. regia* (*Mex163a*). Amplification in the related species *Milicia regia* (Table 6.1) indicated a high level of cross-species transferability of the iroko microsatellites, which should facilitate the population genetic study of the diverse species of the genus *Milicia*. Such cross-species amplification at the genus level has also been observed in other tropical angiosperm genera (Dayanandan *et al.*, 1997; Stacy *et al.*, 2001; Lee *et al.*, 2004; Nettel *et al.*, 2005).

Table 6.1: Characteristics of microsatellite loci for *Milicia excelsa*.

Locus	Accession number	Repeat motif	Primer sequences (5'-3')	T_a (°C)	Size range (bp)	*Milicia excelsa* (*n* = 30)					*Milicia regia* (*n* = 10)	
						Nb of alleles	H_O	H_E	F	*H-WP-values*	Nb of alleles	H_O
Mex51	DQ153158	$(CA)_{14}$	F TGCCCGACCCAATATATTACA R ATTTGGCCGGAAGTCTAAGG	50	158 - 172	4	0.70	0.60	-0.18	0.39	3	0.00
Mex63	DQ153157	$(CA)_{24}(TA)_5$	F AAACTCCGCCTCACAAAAGA R ACGAGAGGGAAAATGGGAAA	50	232 - 242	7	0.45	0.46	0.02	0.11	5	0.33
Mex69	DQ153165	$(TG)_{17}$	F GCAAGCTCTGATGCTCACTG R AGGAGGCAAATTCAAAAGCA	50	180 - 188	4	0.50	0.61	0.19	0.16	3	0.14
Mex81	DQ153163	$(TG)_{19}$	F CCAAATCATTCTTCCGGCTA R ACAGTCCACCCATCGAAAAC	50	180 - 198	6	0.58	0.60	0.04*	0.01	3	0.89
Mex95	DQ153164	$(TC)_{15}$	F CTGAAAAAGTCCGGCTATGC R GGTTTCCAGATGCAGCAAAT	50	192-204	3	0.10	0.18	0.47	0.14	2	0.56
Mex137	DQ153162	$(TG)_{13}(GA)_9$	F ACTACCCCAACCCCAAGTTC R CCTGCACAAGATAGCAACGA	50	194 - 198	3	0.04	0.10	0.66*	0.02	4	0.70
Mex163a	DQ153160	$(CA)_{18}$	F TAGTTCCACATGGCTCATCG R AGCCACTCTCTGGTGCAGTT	50	207 - 217	4	0.80	0.64	-0.25	0.38	3	0.70
Mex163b	DQ153160	$(CA)_{18}$	F TAGTTCCACATGGCTCATCG R AGCCACTCTCTGGTGCAGTT	50	164 - 170	3	0.12	0.56	0.78*	0.00	-	-
Mex198	DQ153166	$(TCTA)_8$	F ACGCCATTGGAATGTAGACC R CTGTTGGAAAACATACTACAG	50	168 - 174	3	0.08	0.47	0.83*	0.00	2	0.60
Mex202	DQ153159	$(GATA)_9$	F ATTGCATGGGACACAAGACA R CATAGCTGCGGAGAAAGTCC	50	166 - 170	3	0.59	0.52	-0.12	0.17	3	0.78
Mex214	DQ153161	$(TCTA)_{11}$	F TTGAACTCTAGCAGCACGAAA R TCGGACTAACTCGAAAACGAA	50	147 - 163	3	0.17	0.16	-.0.6	1.00	2	0.11
Mex243	DQ153167	$(TCTA)_8$	F ACGCCATTGGGAATGTAGAC R TTGGAAAACATACTACAGCTT	50	168 - 178	3	0.12	0.43	0.74*	0.00	2	00
Mean ± SE						4 ± 0.4	0.35±0.08	0.44±0.05	0.21± 0.12		3± 0.28	0.44±0.1

Observed (H_O) and expected (H_E) heterozygosities, annealing temperature (T_a), sampling size (*n*), Wright's *F*

* Significant departure ($P < 0.05$) from Hardy-Weinberg equilibrium; -, no amplification.

7

Nuclear microsatellites reveal regional heterogeneity in population genetic structure of a threatened African species, *Milicia excelsa*

Christine Ouinsavi[1,2*], Nestor Sokpon[1], Damase P. Khasa[2]

[1]Laboratoire d'Études et de Recherches Forestières, Faculté d'Agronomie, Université de Parakou, BP 123 Parakou, République du Bénin
[2]Chaire de Recherche du Canada en Génomique Forestière et Environnementale and Centre de Recherche en Biologie Forestière, Université Laval, Sainte-Foy, Québec, Canada, G1K 7P4
Keywords: Microsatellites, *Milicia excelsa*, Population genetic structure, biological corridors
*Corresponding author: Christine Ouinsavi, Phone: (418) 656-2131 ext. 12587
Fax: (418) 656-7493, E-mail: ouinsch@yahoo.fr

Submitted to *Molecular Ecology*

Abstract

To accurately estimate the genetic diversity and population structure for improved conservation planning of *Milicia excelsa*, 212 individuals from twelve population samples covering the species' range in Benin were surveyed at seven specific microsatellite DNA loci. All loci were variable, with the mean number of alleles per locus ranging from 5.86 to 7.69. Considerable genetic variability was detected for all populations at the seven loci (Na = 4.60; H_E = 0.811). Moderate but statistically significant genetic differentiation was found among populations considering both F_{ST} (0.112) and R_{ST} (0.342). All of the populations showed heterozygosity deficits in test of Hardy-Weinberg Equilibrium and significantly positive F_{IS} values due to inbreeding occurring in the species. Pairwise F_{ST} values were positively and significantly correlated with geographical distances (r = 0.432; P = 0.007, Mantel's test) indicating that populations are differentiated by 'isolation by distance'. Bayesian analysis of population

structure showed division of the genetic variation into four clusters revealing the existence of heterogeneity in population genetic structure. Altogether, these results indicate that genetic variation in *Milicia excelsa* is geographically structured. Information gained from this study also emphasized the need for *in situ* conservation of the relict populations and establishment of gene flow corridors through agroforestry systems for interconnecting these remnant populations.

7.1- Introduction

Tropical trees suffered from several threats which have considerable long-term effects on the demographic and genetic structure of populations world-wide (Aldrich *et al*., 1998). Deforestation, habitat fragmentation and selective logging result in the loss of genetic diversity, the extinction of local populations, the reduction of population size and disruption of mutualisms within pollinators and seed-dispersal agents (Hall *et al*., 1996; Young *et al*., 1996; Aldrich *et al*., 1998; Dayanandan, 1999; Lemes, 2003). To evaluate and reduce the genetic effect of deforestation and logging, it has become a priority to obtain information on the natural levels and distribution of genetic variation in population of tropical trees. Investigations on population genetic diversity and structure of populations within a species may not only illustrate evolutionary histories, processes and mechanisms, but also provide useful information for the biological conservation of endangered species (Gao, 2004).

Earliest studies of tropical trees have used isozymes as the primary genetic markers and have shown that most of the species investigated are outcrossed, exhibit high levels of genetic diversity and gene flow, and carry much of the variation within rather than among populations (Loveless, 1992; Alvarez-Buylla and Garay, 1994; Hall *et al.*, 1994; Hamrick and Godt 1996). However more recent studies using microsatellites with strong discriminatory power, have reported high level of inbreeding, restricted gene flow and isolation by distance in some tropical trees due to fragmentation (Aldrich *et al*., 1998; White *et al*., 1999; Collevatti *et al.*, 2001; Lemes *et al*., 2003).

From the early 1980s up to late 1990s, *Milicia excelsa* (Moraceae) a large deciduous, dioeicious species up to 30-50 m height, with a diameter of 1.70 – 2 m, commonly called iroko, was the focus of an important trade between Africa and Europe. Indeed, many African countries were exporters of iroko timber, especially Ghana (traded together with *M. regia*), Côte d'Ivoire, Cameroon, Congo and Gabon (WCMC, 1991). In Benin, as the species ranks the first among valuable timber species, superior individuals were almost all logged from forests. Thus as a result of its over-exploitation,

the species was classified on the IUCN red list as close to vulnerable (IUCN, 2004) and become the focus of conservation concern in many African countries. Therefore, there is an urgent need for effective conservation and management of the remnant populations. For this purpose, estimates of population genetic parameters are essential. The variability observed at microsatellite loci provides estimates of inbreeding, heterozygosity, gene flow and outcrossing rate, all of which are important measures for assessing the conservation and management status of tropical trees under intense human pressure (Lemes *et al*., 2003).

In this work, we assessed the genetic diversity and population structure of the remnant natural populations of *Milicia excelsa* across its range in Benin using microsatellite markers recently developed for the species (Ouinsavi *et al*., 2006). The specific questions addressed in this study were: i) what is the amount of genetic diversity and differentiation harboured within-and among populations; ii) what are the implications for enhanced conservation of this threatened species.

7.2- Materials and methods

7.2.1- Plant material

Young leaves samples from a total of 212 *Milicia excelsa* mature trees were collected in twelve natural populations representing the geographical distribution of the species in Benin (see Table 7.1 for more details). Populations were geographically distant for more than 50 km and trees sampled within a population were distant from at least 100 m. Leaves were silicagel-dried and kept in freezer at –20ºC until DNA extraction.

7.2.2- DNA extraction and PCR protocols

Total genomic DNA was extracted from about 0.05 g of dried leaves using the DNeasy Plant Mini Kit (QIAgen, UK, Ltd) and following the protocol provided by the manufacturer. DNA concentration was determined by electrophoresis on agarose - TAE gel stained with ethidium bromide (Harry, 2001).

Molecular variation at 7 microsatellite loci was investigated using the following primers designed for *Milicia excelsa* (Ouinsavi *et al*., 2006): Mex163, Mex63, Mex137, Mex51, Mex202, Mex81, Mex95. Polymerase chain reaction (PCR) amplifications were carried out using a 96-wells thermal cycler PTC Peltier (MJ Research, Waltham, Massachusetts) and a 10-µL reaction volume, with 10 ng DNA, 1x

PCR buffer (Invitrogen, Carlsbad, California), 1.5 mM $MgCl_2$ (Invitrogen), 0.4 nmol dNTPs (Invitrogen), 2 nM each of forward and reverse primers and 0.6U of Platinum *Taq* polymerase (Invitrogen). 0.2nM of fluorescent M13 (5' CACGACGTTGTAAAACGAC 3') labeled with IRDye700, was added to the 5' end of forward primers. This technique is time consume less and cheaper than the use of fluorescent dCTP The PCR conditions were: initial denaturation at 94°C for 4 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30s and extension at 72°C for 3 min, with final extension at 72°C for 15 min. (Ouinsavi *et al*., 2006). PCR products were analyzed on 6.5% denaturing polyacrylamide gels (Long Ranger, Biowhittaker Molecular Applications, Rockland, Maine) using an IR2DNA analyzer (Li-cor). Allele sizes were determined using the SAGA^{GT} v.2.0 software (Li-cor).

7.2.3- Numerical analysis

7.2.3.1- Microsatellite diversity and population differentiation

Test for deviation from Hardy-weinberg equilibrium (HWE) at each locus and genotypic linkage disequilibrium among seven pairs of microsatellite loci in each population were performed using Fisher's exact tests with unbiased *P*-values available in the GDA program version1.1 (Lewis & Zaykin 2002). *P*-values were corrected for multiple comparisons by applying a sequential Bonferroni correction (Rice, 1989). Using the GDA program, genetic parameters for each population and overall loci were assessed by computing the average number of alleles per locus (*Na*) adjusted for the differences in sample size by resampling (El Mousadik and Petit, 1996), average percentage of polymorphic loci (*P*), observed heterozygosity (H_O), and heterozygosity expected under Hardy-Weinberg equilibrium (H_E). Nei's estimates of heterozygosity for all populations at each locus including the observed heterozygosity (H_O), the total genetic diversity pooled over all populations (H_T), the mean genetic diversity within populations (H_S) and the proportion of the total genetic diversity that occurs among populations due to different genotypes were calculated using FSTAT version 2.9.3.2 (Goudet, 2002), available online at http://www2.unil.ch/popgen/softwares/fstat.htm. For purpose of comparison, genetic differentiation was quantified using both F_{ST} statistics and differentiation based on allele size (R_{ST}). Weir and Cockerham (1984); Excoffier *et al*. (1992); and Weir (1996) estimators of F_{IT}, F_{ST}, F_{IS} and R_{ST} were estimated for each allele, locus and overall using FSTAT version 2.9.3.2 (Goudet, 2002). Jackknifing over

samples and loci and bootstrapping over loci were automatically performed for the statistics F_{IT}, F_{ST}, F_{IS}. Values of R_{ST} overall loci were estimated as weighted and unweighted R_{ST} (Rousset, 1996; Goodman, 1997).

7.2.3.2- Geographic structure

Geographical trends in the distribution of genetic diversity were investigated by three different methods. First, the null hypothesis of independence between pairwise F_{ST} and geographical distance was tested using Mantel test (Mantel, 1967) implemented in ARLEQUIN program version 3.1 (Excoffier *et al*., 2005). In addition, the Bayesian methods for the analysis of population genetic structure were used to estimate hidden population substructure by clustering sampled populations into panmictic groups by the Bayesian Analysis of Population Structure (program BAPS, Corander *et al.*, 2003, available online at http://www.rni.helsinki.fi/~mjs/). This does not require conditioning on known population structure. BAPS was run five times for 10^5 iterations after a burn-in period of 20 000, randomly mixing the order of populations in the input file. The resulting partitions were presented on a UPGMA dendrogram using Kullback - Leibler divergences among populations.

The model-based clustering algorithm was applied to identify clusters of genetically similar individuals and to test the proportion of genetic admixture among the clusters using STRUCTURE version 2.1 (Pritchard *et al*. 2003), available online at http://pritch.bsd.uchicago.edu/software/structure2_1.html. This was to assign individual multilocus genotypes probabilistically to a user defined number K of cluster or gene pools (Heuertz *et al*., 2004), achieving linkage equilibrium within cluster. We used an admixture model in which the fraction of ancestry from each cluster is estimated for each individual. A burn-in period of 1 000 000 iterations and 100 000 Markov Chain Monte Carlo (MCMC) repetitions were used and the program was run three times for each $K \in \{1,\ldots,12\}$ on the total dataset without any prior information on the origin of each sampled individuals and populations. Parameter of individual admixture alpha was chosen to be the same for all clusters and was given a uniform prior. The allele frequencies were kept independent among clusters to avoid overestimating the number of clusters (Falush *et al.*, 2003; Heuertz *et al*., 2004). Proportions of ancestry were averaged over individuals within each population sample and the corresponding pie charts were plotted onto the Benin map. The clusters are referred to as gene pools (Heuertz *et al.*, 2004).

7.3- Results

7.3.1- Within-and among population variation at the microsatellite level

Almost all loci met Hardy-Weinberg expectations except Mex81 in Bante population and Mex95 in Tamarou population which showed significant deviation from HWE equilibrium after Bonferroni corrections ($P < 0.05$). Exact test for genotypic linkage disequilibrium showed significant deviations for 7 out of 252 comparisons ($P < 0.05$), which may be due to chance alone and these deviations are all associated with Mex81 and Mex95 suggesting that this disequilibrium could largely be due to the Hardy-Weinberg disequilibrium in these two loci since pairwise measures include both within- and among-loci disequilibrium. A total number of 147 alleles were recorded over the 7 loci in the 212 individuals with a range from 13 alleles (Mex202) to 32 alleles (Mex63) and an overall average of 6.805 (Table 7.2). Most of the loci were variables across populations with mean observed heterozygosity ranging from Mex95 (H_O= 0.204) to Mex163 (H_O =0.765). Total gene diversity is high and similar for each locus with a range from Mex202 (H_T=0.866) to Mex63 (H_T =0.941); and gene diversity within populations ranging from Mex202 (H_S=0.743) to Mex81 (H_S=0.811).

Allelic richness in populations varies from 3.71 (Sakete) to 5.86 (Lokossa) with an average number of alleles per locus within populations (Na) of 4.60 (Table 1). The number of private alleles (Pa) across populations ranged from 1 to 5 with a total number of 29. The mean percentage of polymorphic loci within populations (P) was 100 % (0.99 criterions). At the population level, the observed heterozygosity ranged from H_O =0.48 (Niaouli and Aplahoue populations) to H_O =0.64 (Save population) with an average value of 0.54. The average gene diversity within population was H_E = 0.811 (a range from 0.732 to 0.855). Populations were on average inbred with a mean inbreeding coefficient of F_{IS} = 0.316, indicating a significant ($P < 0.05$) excess of homozygotes relative to Hardy-Weinberg expectations (Tables 7.1 and 7.2). Bante and Tamarou Populations showed significant pairwise disequilibrium between Mex81 and Mex51, and Mex95 and Mex51 respectively ($P < 0.05$).

Table 7.1: Geographical locations and genetic diversity parameters estimated in 12 *Milicia excelsa* populations

Populations	Longitude (°E)	Latitude (°N)	*n*	**Na**	**Pa**	*P(%)*	H_O	H_E	F_{IS}
Ketou	002°35'45.2"	07°20'59.2"	20	4.71	2	100	0.50	0.853	0.440
Aplahoue	001°50'42.6"	07°05'06.4"	21	4.29	2	100	0.48	0.783	0.332
Lokossa	001°40'16.4"	06°45'26.1"	20	5.86	5	100	0.57	0.838	0.320
Niaouli	002°08'08.1"	06°44'16.1"	15	4.00	2	100	0.48	0.826	0.432
Sakete	002°04'49.6"	07°18'16.1"	17	3.71	1	100	0.51	0.762	0.327
Biro	0023°5'34.8|"	06°37'05.2"	15	4.00	3	100	0.53	0.732	0.233
Bohicon	002°56'28.8"	09°56'11.2"	18	5.00	2	100	0.59	0.855	0.314
Bante	001°58'06.6"	08°13'49.2"	15	3.86	2	100	0.52	0.823	0.365
Bassila	001°39'16.9"	09°02'36.1"	17	4.43	2	100	0.63	0.806	0.211
Save	002°31'16.1"	08°04'27.9"	16	4.57	1	100	0.64	0.826	0.166
Tamarou	002°39'38.4"	09°38'17.7"	17	5.57	4	100	0.53	0.850	0.358
Djougou	001°26'04.8"	10°07'27.4"	21	5.14	3	100	0.53	0.780	0.296
Mean				4.60	29	100	0.54	0.811	0.316
Standard error			212				0.016	0.012	0.025

Sample size (*n*); mean number of alleles per locus (*Na*); total number of privates alleles (*Pa*); percentage of polymorphic loci (0.99 criterion) (*P*); Observed heterozygosity (H_O) and Expected heterozygosity (H_E); and inbreeding coefficient (F_{IS}). Put this formula in the text after citing Slatkin (1987)

Table 7.2: Estimates of single locus heterozygosity and genetic differentiation of the twelve *Milicia excelsa* populations over seven microsatellite loci

Locus	**A**	*Na*	***Pa(fq)***	H_O	H_S	H_T	G_{ST}	F_{IS} (‡‡)	F_{IT} (‡‡)	F_{ST} (‡‡)	R_{ST} (‡‡)
Mex163	26	7.50	5 (0.2)	0.765	0.873	0.929	0.061	0.121 (±0.06)***	0.180 (±0.06)***	0.067 (±0.01) ***	0.082***
Mex63	32	7.69	8 (0.6)	0.506	0.835	0.941	0.113	0.357 (±0.07)***	0.440 (±0.06)***	0.129 (±0.02) ***	0.692***
Mex51	16	6.65	1 (0.1)	0.554	0.770	0.900	0.144	0.277 (±0.04)***	0.388 (±0.03)***	0.154 (±0.02 ***	0.367***
Mex137	15	6.27	3 (0.1)	0.659	0.788	0.886	0.110	0.139 (±0.07)***	0.231 (±0.07)***	0.106 (±0.02) ***	0.413***
Mex202	13	5.86	4 (0.2)	0.645	0.743	0.866	0.143	0.144 (±0.08)***	0.286 (±0.09)***	0.165 (±0.06) ***	0.208***
Mex81	25	6.93	5 (0.4)	0.542	0.881	0.906	0.028	0.371 (±0.06)***	0.391 (±0.06)***	0.031 (±0.01) ***	0.077**
Mex95	20	6.74	3 (0.2)	0.204	0.784	0.906	0.135	0.739 (±0.05)***	0.774 (±0.05)***	0.134 (±0.04) ***	0.468***
Mean (±SE)	147	6.81	29	0.554 (±0.07)	0.811 (±0.02)	0.905 (±0.01)	0.104 (±0.02)	0.308 (±0.08)***	0.385 (±0.07)***	0.112 (±0.02) ***	0.342 (¶¶¶)

Total number of alleles per locus (*A*); mean number of alleles per locus (*Na*); total number (*Pa*) and frequency (*fq*) of privates alleles; observed heterozygosity (H_O); total gene diversity pooled over all population (H_T); mean genetic diversity within populations (H_S); proportion of the total genetic diversity that occurs among populations (G_{ST}). Weir and Cockerham (1984) estimates of F (F_{IT}), Theta (F_{ST}), f (F_{IS}) (± *SE*) and R_{ST} . Statistically significant levels of the tests are indicated by *($P < 0.05$), **($P < 0.01$) and ***($P < 0.001$).

7.3.2- Geographical patterns of population genetic structure

Genetic differentiation among populations estimated over loci by G_{ST} was moderate (G_{ST} = 0.104) but within the range of the estimated values using F_{ST} (0.112 ± 0.018). Mean values of R_{ST} and F_{ST} were 0.342 and 0.112 respectively suggesting that about 34% and 11% of the total variation resided among populations under stepwise-mutation model (SMM) and infinite allele model (IAM). Differentiation among populations based on the allele sizes, R_{ST} = 0.342 was significantly larger than differentiation based on allele identities (F_{ST} = 0.112 ± 0.018), indicating that stepwise-like mutations contributed to overall among population differentiation. Based on the private allele method Gene flow estimated for the studied populations from the formula [Nm = 0.25 (1 – F_{ST}) / F_{ST}] (Slatkin, 1987) equaled to Nm = 0.277 migrant per generation suggesting a restricted extent of gene flow among populations.

Correlation between geographical and genetic distances drawn from Mantel test was significantly positive (r = 0.432; P = 0.007) as shown by Fig. 7.1, indicating an evidence for isolation by distance, that geographically close populations tended to be genetically more similar.

BAPS analysis showed division of the populations into four clusters almost identical to the Neighbor-Joining dendrograms based on Nei's genetic distance (Fig 7.2). This result clearly showed the existence of heterogeneity in population genetic structure of *Milicia excelsa*. Southern east populations clustered to form group 1, southern west populations composed group 2, central and northern east populations grouped together except Biro which clustered with the first group. Population of Djougou remained alone in the fourth group. The model-based clustering method with STRUCTURE confirmed the heterogeneity in patterns of population genetic structure in *Milicia excelsa* in Benin although the inference of the number of gene pool K was not straightforward. Seeing that the log-likelihood values, *ln Pr X/K* increased with K values increasing, we choose the smallest value that captures the major structure in the data as suggested by Pritchard and Wen (2003). The value of K = 4 brought us close to the results from BAPS analysis moreover *ln PrX/K* values vary among different runs for the same K value when K > 4 (Fig. 7.3). The proportion of an individual genome from each population that contributed to each of the four clusters under a model with the highest probability is given by Fig 7.4. Gene pool 1 is most abundant across central region's populations; gene pool 2 is predominant in southern east populations while gene pool 4 mainly occurs in southern west populations. Gene pool 3 makes the bulk part of northern west population but is also remarkably represented in some populations from

the south. Biro population which, clusters with southern east populations, is geographically located in the northern east and genetically shares some common features (gene pool 1, gene pool 2 and gene pool 3) with central, southern east and north populations respectively.

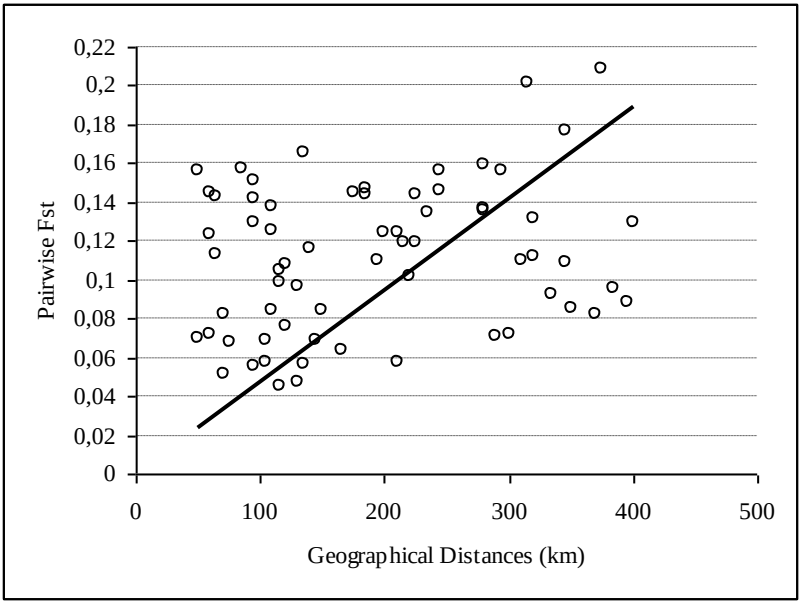

Fig 7.1 : Scatterplots of pairwise F_{ST} vs. geographical distance. Mantel test shows significant association (r = 0.432; P = 0.007)

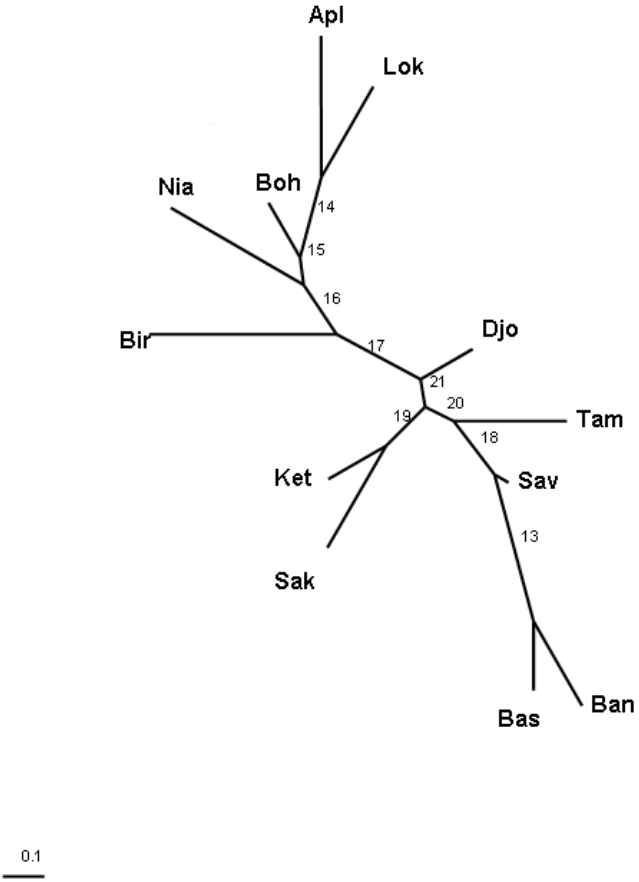

Figure 7.2: Neighbor-joining analysis of pairwise genetic distance between twelve *Milicia excelsa* populations. *Sak = Sakete; Ket = Ketou; Tam = Tamarou; Bir = Biro; Djo = Djougou; Bas = Bassila; Ban = Bante; Boh = Bohicon; Nia = Niaouli; Apl = Aplahoue; Lok = Lokossa; Sav = Save*. Numbers are boostrapping values

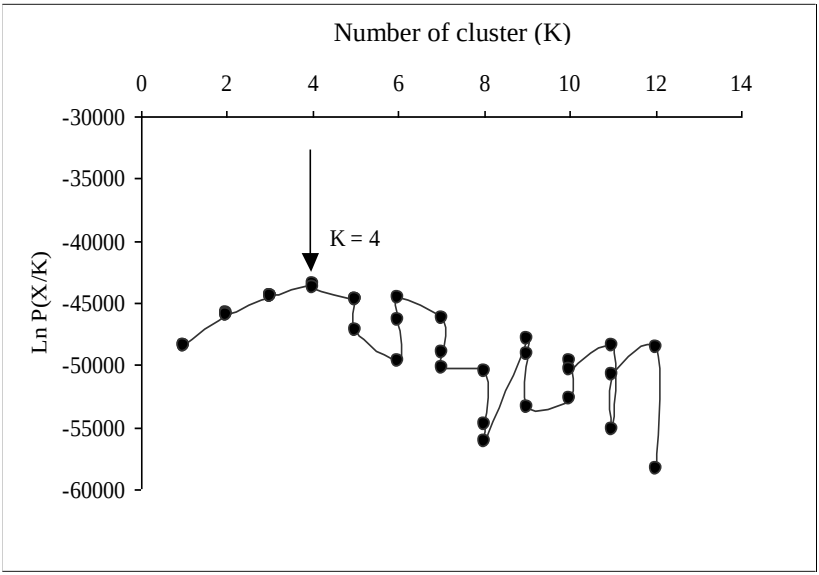

Figure 7.3: Estimated posterior probability of the number of cluster K for *Milicia excelsa* populations, with STRUCTURE program

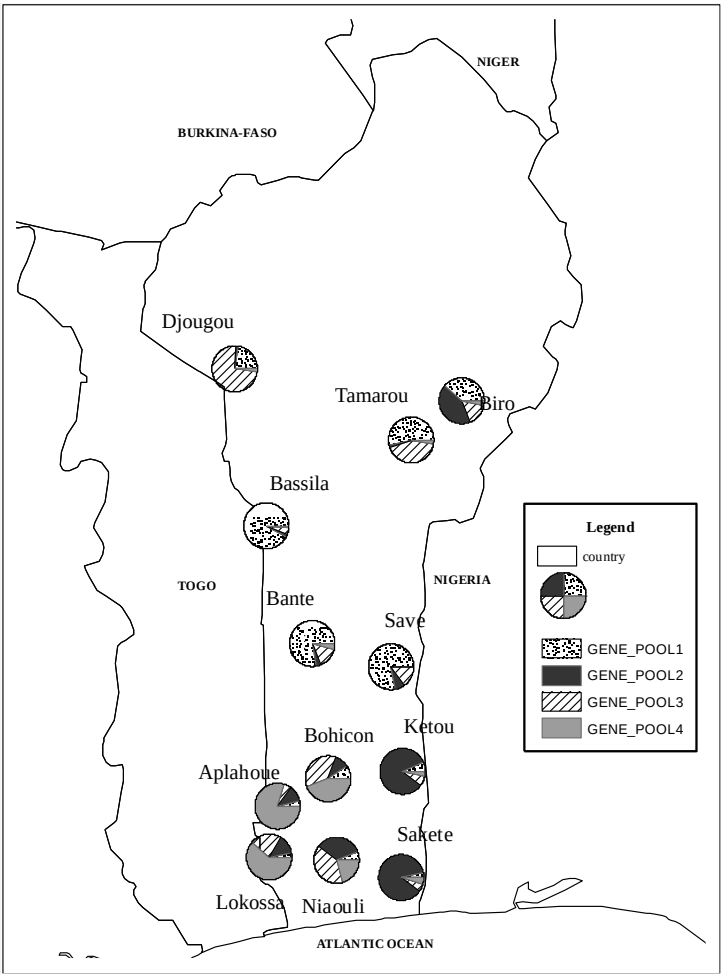

Figure 7.4: Proportions of ancestry of each population in each of the four gene pools defined with the model-based clustering method from Pritchard *et al.* (2000)

7.4- Discussion

7.4.1- Microsatellite diversity in *Milicia excelsa*

The overall pattern of genetic variation at microsatellite loci in *Milicia excelsa* revealed considerable genetic diversity (*Na* = 4.6, *P* = 100%, H_O = 0.54 and H_S = 0.81). The very high level of polymorphism detected could be due to high number of repeats (14 to 29 repeats) in the used loci (Ouinsavi *et al*., 2006) as SSRs with many repeats have been shown to be more polymorphic than ones with few repeats (Chase *et al.*, 1996; Van de Ven and McNicol, 1996; Dayanandan *et al*., 1999). Genetic diversities observed in *Milicia excelsa* (*Na*, H_O, H_E and H_S) are lower than the pattern found in other microsatellite studies of tropical tree species including *Symphonia globulifera* (Aldrich *et al*., 1998), *Carapa guianensis* (Dayanandan *et al*., 1999), *Swietenia humilis* (White *et al*., 1999), *Caryacar brasilense* (Covellati *et al*., 2001) and *Swietenia macrophylla* (Lemes *et al*., 2003) but higher than genetic diversity observed in *Vitellaria paradoxa* (Kelly *et al*., 2004).

7.4.2- Population structure and inbreeding level

Estimates of genetic differentiation among populations from microsatellites (F_{ST}= 0.112 ±0.018; G_{ST} = 0.104) indicated moderate but highly significant degree of differentiation ($P < 0.001$) among populations of *Milicia excelsa* in Benin. Since the species of concern is a tropical cross pollinated species with bat and bird dispersal–seeds (Taylor *et. al*., 2000) and some gravity-dispersed seed, this result may fit with the general observation that woody, perennial and outbreeding species maintain most of their variation within populations (Hamrick *et al.*, 1992; Hamrick and Godt, 1996). Other studies that have assessed genetic variation in natural populations of tropical tree species using microsatellites exhibited much lower levels of genetic differentiation than found here (0.031 for *Symphonia globulifera* in Southern Costa Rica, Aldrich *et al*., 1998; 0.041 for *Carapa guianensis* in Costa Rica, Dayanandan *et al*., 1999; 0.032 for *Swietenia humilis* in Honduras, White *et al.*, 1999; 0.097 for *Swietenia macrophylla* in the Brazilian Amazon, Lemes *et al*., 2003; and 0.026 for *Vitellaria paradoxa* in Mali, (Kelly *et al*., 2004). However, some authors have suggested that population differentiation is more accurately estimated by R_{ST}, because this measure better accounts for the high mutation rate of microsatellite markers while F_{ST} tends to underestimate

population differentiation (Slatkin, 1995; Hedrick, 1999; Collevatti *et al*., 2001; Lemes *et al*., 2003). On the other hand, different simulation studies (e.g. Di Rienzo *et al*., 1994; Estoup *et al*., 1995) have shown that most of the microsatellite markers formed by short or imperfect repeat motifs tend to fit more closely the IAM than the SMM.

Inbreeding coefficient (F_{IS}) indicated that alleles within populations were not united at random and that mating between close relatives may play an important role in determining the genetic structure of these populations. The estimated gene flow (Nm = 0.277) was also low reflecting a reduced gene flow among populations. The first possible explanation of such a situation is the occurrence of null alleles, which failed to amplify because of mutations in the flanking primer sequence (Callen *et al*., 1993; Lemes *et al*., 2003). But this seems to be an unlikely explanation because amplification failures were rare in this study. The second explanation which has been experienced in *Swietenia macrophylla*, a tropical outcrossing species is that, assortive mating caused by spatial clustering or coincidence in flowering time among related groups of trees, can lead to inbreeding and homozygote excess (Lemes, 2003). Such explanation seems to be more favorable in the case of *Milicia excelsa* in Benin. Indeed, *Milicia excelsa* is threatened throughout its range in Benin as a result of overexploitation and habitat destruction, which have clearly reduced local population size and led many populations to local extinction. The species' populations currently occupy fragmented habitats such as sacred groves which are small patches of the original vegetation protected by traditional ethnobotany or religious practices, and farmlands where trees are protected by farmers through traditional agroforestry systems (Ouinsavi *et al*., 2005). The spatial clustering of *M. excelsa* trees may have induced an increase local inbreeding level in progeny cohorts. Then the great majority of dispersal occurs between nearest-neighbour populations with only a few individuals moving longer distance. Other authors have also observed positive F_{IS} values and attributed it to populations' substructure and inbreeding (Ueno *et al*., 2002; Kelly *et al*., 2004).

7.4.3- Spatial genetic structure and conservation implications

Our data suggested that *M. excelsa* populations in Benin are differentiated by a process of 'isolation by distance'. This hypothesis is supported by a significant Mantel test between a matrix of F_{ST} values and a matrix of geographical distances. Spatial analysis by the model-based clustering method also indicated a geographical structure and clustered populations into four groups which correspond to southern west, southern east (including one population from the middle northern east), northern west, and

central populations. Several studies dealing with spatial genetic structure reported either a lack or a weak geographical heterogeneity in various tropical or temperate tree species which, they argued by the limited seed dispersal but extensive gene flow by pollen (e.g. Berg and Hamrick, 1994; Loiselle *et al*., 1995; Streiff *et al*., 1998; Chung *et al*., 1999) or by extensive gene flow by pollen, wide seed dispersal, self incompatibility and dispersal agent (Waser, 1987; Dewey and Heywood, 1988; Doligez and Joly, 1997; Chung *et al*., 2000). These authors made the general statement that for woody insect-pollinated species with seed widely and independently dispersed by birds, at most weak genetic structure will result. In the current study, the distribution of gene pool 2 and gene pool 4 in the south and the predominance of gene pool 1 in the center, suggested an impact of limited seed dispersal. Different factors could explain this genetic structure. For instance, fragmentation and overexploitation may be a valuable explanation of this situation. As the remaining *M. excelsa* trees in these regions are grouped into small patches in the built-up village areas (fetish worship sites, open places, near-by-villages farms, Ouinsavi *et al*., 2005), dispersal agent activities and hence seed dispersal may be reduced by human disturbance. Bats that are the main pollinators of this species may tend to forage inside patches or between nearest-neighbor clumps. Hence fragmentation may cause genetic isolation by keeping these mammals inside fragments (Kearns *et al*., 1998; Collevatti *et al*., 2001), restricting gene flow among populations.

In addition, fruiting and seed dissemination of *M. excelsa* occurs during the dry season and germination rate is low and decreases rapidly. Therefore bulk part of the seed may loose its viability before it is transported far away by water flow during the rainy season. The mixed composition observed in Niaouli population harbouring mainly gene pools 2, 3 and 4 could be understood by the fact that this population is composed in majority of planted trees of unknown seed origin. We suspect that seeds used to establish plantations may have come from genetically distinct localities.

The information gained on the levels and distribution of microsatellite variation in *Milicia excelsa* could be used to suggest appropriate management strategies. *M. excelsa* has long suffered from habitat degradation caused by selective logging and land use pressure which is now inducing inbreeding in most of the studied populations and may compromise the species genetic diversity by genetic drift. Traditional strategies of conservation were helpful to protect some mother trees and to assure populations renewal but these practices are not efficient for genetic resources conservation. The high level of genetic variation found within populations combined with the low density of adults trees indicate that *in situ* conservation strategies should be designed to preserve large areas to minimize the loss of diversity due to genetic drift. The isolation by

distance observed among populations suggests that *in situ* reserves should be distributed evenly across the species range in order to maximally conserve the regional diversity and integrity. Therefore we suggest that a key strategy for *Milicia excelsa* conservation should be the enhanced management of habitats to allow connections between populations. Agroforestry systems, if they are well managed, could contribute to maintain this connectivity across the landscape, hence acting as biological corridors between populations (Schroth *et al*., 2004). The species should be rehabilitated by transplanting seedlings into some preferential habitats such as Pobe, Sakete, Aplahoue and Bassila semi deciduous forests which should have high conservation priority as well as other protected reserves in savannah regions. These reserves could also serve as corridor among the existing patches. However, as mentioned by Storfer (1999) and Gao (2005), care should be taken to avoid mixing seeds or live plants collected from distinct populations to prevent changes in the genetic composition of the populations, producing a decreased fitness through outbreeding depression and disruption of locally adapted gene combinations.

8

Utilisation and traditional strategies of *in situ* conservation of iroko (*Milicia excelsa* welw. C.C berg) in Benin

C. Ouinsavi[a],*, N. Sokpon[a], O. Bada[b]

[a]Laboratoire d'Etudes et de Recherches Forestières, Faculté d'Agronomie, Université de Parakou, BP 123 Parakou, Benin
[b]Department of Forest Resources Management, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria

Forest Ecology and Management 207 (2005) 341 - 350

ABSTRACT

Socio – cultural surveys were carried out on the basis of a questionnaire administered on 346 respondents in order to investigate the cultural and ethnobotanic uses of *Milicia excelsa* in Vodun cultural area of Benin.

It was found that *Milicia excesla* contribute to cure 45 diseases. The different components of the tree used are leaves (30.3%), bark (25.8%), root (23.6%), latex (10.1%), flaking bark (6.7%), wood, lokotche and gum (1.1% each). Fruits or seeds are rarely used. Six different forms of utilization were recorded: soaking (46.3%), bark or leaves decoction (32.8%), herb tea (11.9%), powder (6.0%), leaves or bark grounded and rolled up into ball (1.5%), sacrifices (1.5%).

Iroko wood is also used in carpentry and joinery for construction purposes; furniture as well as for building boats / canoes.

Iroko tree is used as the conservatory of cultural values and incarnates many divinities which differ significantly from one province to another ($\chi^2 = 1830.27$; df = 25; $P < 0.01$ %). There is a significant difference between the provinces in respect of the

recognition of the species (χ^2 = 268.71; df = 17; p ≤ 0.01%) and the population awareness about iroko as a sacred tree also varied from one province to another (χ^2 = 308.66; df = 27; p ≤ 0.01 %).

Veneration of the tree is the main approach of its conservation by local people. *Milicia excelsa* is conserved on farm, in sacred groves, in public places and in cemeteries. The different sacred objects used to symbolise the divinities incarnated by iroko are: pottery (36.36 %), iron (11.11 %), calabash (4.04 %), candle (2.02 %), piece of cloth (18.18 %), sacrifice (13.13 %), piece of money (3.0 %), stone (2.05 %), glassware (broken bottle, 2.02 %), and convent (8.08 %). There is a highly significant difference between provinces as far as the sacred objects are concerned (χ^2 = 183.037; df = 19 and P < 0.001). The conservation purposes also vary significantly from one region to other (χ^2 = 8+94.47; df = 31; P < 0.01 %).

Key words: Strategies, conservation, cultural area, Vodun, Benin

RESUME

Des enquêtes sur les stratégies locales de conservation in situ de *Milicia excelsa* ont été réalisées sur la base de questionnaire administré à 346 personnes dans l'aire culturelle Vodun au Bénin.

Il ressort que l'iroko contribue au traitement de 45 maladies. Différents organes de la plante sont utilisés à savoir: les feuilles (30,3%), l'écorce (25,8%), les racines (23,6%), le latex (10,1%), les écailles (6,7%), le bois, lokotchê (une forme d'induration des minéraux accumulés dans le bois) et la colle (1,12%). Les fruits ou graines sont très rarement utilisés. Les différents modes d'utilisation de ces organes sont la macération (46,3%), la décoction de l'écorce ou des feuilles (32,8%), l'infusion et la trituration des feuilles (11,9%), la poudre (6%), la boule (1,5%), le sacrifice (1,5%).

L'iroko est aussi utilisé sur les plans artisanal (comme bois d'oeuvre en menuiserie et en sculpture) et culturel (comme conservatoire de la tradition).

La connaissance de cet arbre varie de façon hautement significative d'un département à un autre (λ^2 = 268,71; ddl = 17; P < 0,01%) de même que son titre d'arbre fétiche ou sacré ou pouvant recevoir des sacrifices (λ^2 = 308,66; ddl = 27 et P < 0,01%). L'arbre abrite plusieurs divinités qui diffèrent significativement d'un département à l'autre (λ^2 = 1830,27 ; ddl = 25 et P < 0,01%).

La sacralisation est le moyen fondamental de conservation de l'iroko par les populations locales. Les pieds d'iroko conservés se retrouvent au champ, dans les forêts sacrées, sur les places publiques, et dans certains cimetières. Les différents objets servant à matérialiser les divinités incarnées par l'iroko sont: les poteries (36,36 %), le fer (11,11 %), la calebasse (4,04 %), la bougie (2,02 %), des banderoles (18,18 %), la présence des sacrifices (13,13 %), les pièces de monnaie (3,0 %), la pierre (2,05 %), les verreries (2,02 %), la présence d'un couvent (8,08 %). Les objets utilisés varient de façon hautement significative d'un département à un autre (χ^2 = 183,04; df = 19 ; P < 0.001).Le degré de protection de *Milicia excelsa* varie de façon hautement significative d'un département à un autre dans l'aire culturelle *Vodun* (λ^2 = 258,14 ; ddl = 29 et P < 0,01). De la même manière les raisons de cette protection varient de façon hautement significative d'une région à une autre (λ^2 = 894,47 ; ddl = 31 ; et P < 0,01%).

Mots clés: Stratégies, conservation, aire culturelle, Vodun, Bénin

8.1- Introduction

Tropical forests are an important reservoir of biodiversity and, they play a fundamental role in giving satisfaction to many needs of the people. According to FAO (1999), the annual rate of forest destruction in the tropics was about 0.7% during the period 1990 to 1995.

Most of these forests disappear because of increasing human pressure and over – exploitation (Adebisi, 1999 quoting Lanly 1991; Faronsworth and Soejart, 1991). Consequently most of the identified important trees species (multipurpose trees, medicinal plants, hardwood) are threatened with extinction as a result of forest degradation.

In the Republic of Benin, tree species such as *Milicia excelsa, Khaya senegalensis, Afzelia africana,* and *Pterocarpus erinaceus,* are identified as threatened with extinction (Agbahungba *et al.,* 2001) because of uncontrolled forest destruction. It is therefore necessary that appropriate studies and programmes are put in place to enhance the conservation and sustainable use of forest genetic resources in Benin Republic.

Sustainable management of renewable natural resources requires analysis and master of the multiple interactions between human and the complex functioning of forest ecosystem. The enhancement of human capacity on forest genetic resources is

probably one of the most urgent challenges of conservation and sustainable management of forest resources in Africa.

The current study aims to assess socio – economic and cultural importance of iroko and find out the main reasons which undertake its conservation in cultural area of Vodun in Benin.

8.2- Methodology

Republic of Benin is located in West Africa between long 1° and 3° 40' E, and lat 06° 30' and 12° 30' N. It is bordered by Republic of Niger in the North, Burkina-Faso in the North - West, Togo in the West, Federal Republic of Nigeria in the East and the Atlantic Ocean in the South. It covers an area of 112622 km² and is divided into twelve departments which are: Atacora, Donga, Borgou, Alibori, Zou, Collines, Ouémé, Plateau, Littoral, Atlantique, Couffo and Mono. Cultural area of Vodun covers parts of the country, between long 1° and 3° 40' E, and lat 06° 30' and 9° N.

Adjanohoun *et al.* (1989) have distinguished in Benin four major types of vegetation landscape which are highlighted as follows:

- The coastal composed of beach vegetation and anthropogenic vegetation of mangrove swamp forests, riparian forests, oil palm and coconut plantations.;
- Guineo-Congolese zone characterized by relict of semi-deciduous forests, savannah and plantations
- the Guineo-Sudanean Transition zone with mosaic of forest woodlands, sometimes dry forests and wooded savannah crossed by gallery forests.
- The sudanean zone composed of shrub savannah, ticklish pseudo-steppe vegetation and gallery forests.

Apart from this natural vegetation, there are in Benin, some teak plantations (14.673,4 ha), and some *Khaya senegalensis* trial plantations, school plantations and at the border of roads (Toffo, Atchérigbé, Tanguiéta Kandi, Birni, Kouaba etc.).

According to INSAE (1994), the population of Benin Republic is about 5.6 million inhabitants with a growth rate of 3.2%. Population density varies from 16 inhabitants / km² in Borgou - Alibori province, to 322 inhabitants / km² in Atlantic - Littoral province with an average of 43 inhabitants / km² for the whole country.

Many ethnic groups are found in the study area. They include: Xueda, Xwla, Adja, Fon, Mahi, Gun - Ahizo, and Yoruba. According to Adam and Boko (1983); Dissou (1986) and Agbo and Sokpon (1998), there is a very significant similitude between ethnic groups in South and Central Benin. The main ethnic groups, which can be distinguished in this area, are: Fon, Adja and Yoruba.

More than 44% of the population practice traditional religions. They believe in many things such as stone, snake, and even some forest trees like *Milicia excelsa.* Apart from these groups who practice traditional religions, there are those who practice foreign religions such as Christianity (35.4%) and Islam (20.6%).

A survey was carried out on the basis of a questionnaire administered on 346 respondents in order to investigate the cultural and ethnobotanic uses of the species in the study area. 14 districts were chosen and in each district, a questionnaire was administered on each of the following 6 respondents: (i) the district head, (ii) President of Farmers Association, (iii) the forestry officer, (iv) chief of the traditional religion, (v) the Imam, and (vi) the Christian Priest. In addition, 2 villages were selected per district and in each village, the following respondents were interviewed: (i) the village head, (ii) President of Farmers Association, (iii) the Chief of the traditional religion, (iv) the President of tradipractitionners, (v) one representative of foreign religions, (vi) one representative of forest exploiters, (vii) the representative of carpenters, (viii) the forest officer in the village and (ix) two other people chosen at random from two different households. In some villages, the same person simultaneously occupied more than one of these positions, thus resulting in unequal sample sizes. A total number of 346 persons were interviewed.

Data collected from all sources were codified using numerical values and analysed with SPSS software using Chi-square test of independence.

8.3- Results

8.3.1- Socio-economics and cultural importance of the species

8.3.1.1- Medicinal uses of Milicia excelsa

8.3.1.1.1- Diseases cured with Iroko tree Components

Table 8.1 lists the diseases cured with *Milicia excelsa* components in southern and central Benin. The components are used solely or in combination with other materials in treating forty - five diseases. Four diseases were recorded in Atacora, only one each in Atlantique and Borgou, seven each in Mono and Couffo, two each in Oueme and Plateau and nine each in Zou and Collines provinces. The provinces of Zou and Collines are the most specialised in the magical use of *Milicia excelsa*.

Table 8.1: Diseases cured with *Milicia excelsa*

	Provinces					
Diseases	Atlan.	Ataco.	Borg.	Mono	Zou	Ouem.
Anti-witch-craft	+	+	+	+	+	+
Fortifiants	-	+	+	+	+	+
Fever	-	-	+	+	+	+
Body pains	-	+	-	+	+	+
Blurred vision	-	-	-	+	+	+
Miscarriage	-	-	-	+	+	+
Convulsion	+	-	+	+	+	-
Madness	-	-	+	+	+	-
Epilepsy	-	-	+	+	+	-
Malaria	+	+	-	+	+	-
Skin disease	-	+	-	+	+	-
Paralysis	-	-	-	+	+	-
Migrant	-	-	-	+	+	-
Menstrual disorder	-	-	-	+	+	-
Stomach ache	-	-	+	+	-	-
Antibiotic	-	-	-	-	+	+
Epidemics	-	+	-	-	-	-
impotency	-	+	-	-	-	-
Parasite of intestine	-	+	-	-	-	-
Catarrhs	-	+	-	-	-	-
Head (oka ori	+	-	-	-	+	-
Irritation	+	-	-	+	-	-
Whitlow	+	-	-	+	-	-
Compact of uterus	+	-	-	-	-	+
S.S. anaemia	+	-	-	-	-	-
Female sterility	-	-	+	-	-	-
Snake-bite	-	-	+	-	+	-
Hypertension	-	-	-	+	-	-
Inactive fœtus	-	-	-	+	-	-
Œdema	-	-	-	+	-	-
Dizziness	-	-	-	+	-	-
Yellow fever	-	-	-	+	-	-
Spleen disease	-	-	-	+	-	-
Bewitchment	-	-	-	+	-	-

Pile/haemorrhoid	-	-	-	-	-	+
Bleeding	-	-	-	-	-	+
Hernia	-	-	-	-	+	-
Hear ache	-	-	-	-	+	-
Anaemia	-	-	-	-	+	-
Jaundice	-	-	-	-	+	-
Shingles	-	-	-	-	+	-
Abortion	-	-	-	-	+	-
Dysentery	-	-	-	-	+	-
Sexually Transmissible Disease.	-	-	-	-	+	-
Dog-bite	-	-	-	-	+	-

(+) = *Milicia excelsa* is used to cure this disease in the province

(-) = *Milicia excelsa* is not used to cure this disease in the province.

Ataco = Atacora ; Atlan = Atlantique ; Borg = Borgou ; Ouem = Oueme.

8.3.1.1.2- Components of the tree used in traditional medicine

Figure 8.1 shows the frequency distribution of iroko tree components used in trado - medicine in southern and central Benin Republic. The leaves are the most used component whichever province or ethnic group is considered (30.3 %). The bark is used at a rate of 25.8 % and can sometimes replace the leaves. The root is used in a proportion of 23.6 %, the latex, 10.1 % and the flacking bark, 6.7 %. The wood, the lokotche and the gum are used in a proportion of 1.1 % each. The iroko fruits and seeds are rarely used to cure diseases.

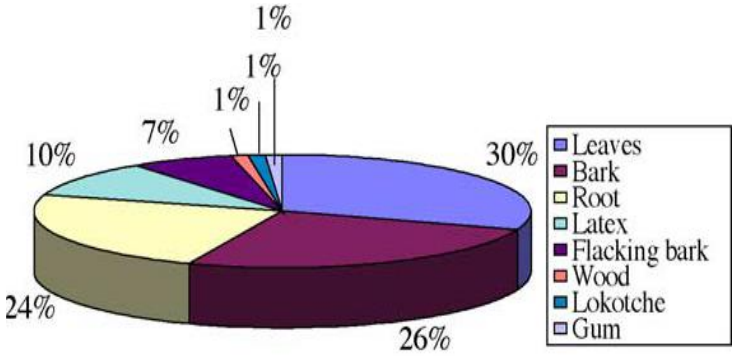

Fig. 8.1: Frequency distribution of iroko tree components used in trado - medicine

8.3.1.1.3- Forms of utilization

Fig. 8.2 shows different forms of utilization of *Milicia excelsa* in trado - medicine. Six different forms were recorded:
- Soaking (46.3 %): leaves and bark and root are soaked in water and used for the bath or to wash the face.
- The decoction (32.8 %): the decoction is drunk or used for bath according to the type of disease.
- Herb tea (11.9 %): this is also drunk, used for bath or for washing only the face.
- The powder (6 %): The flacking bark, the whole bark or the roots are dried, grounded or burnt. The powder is then mixed with water or maize pap and drunk. The powder is sometimes swallowed with alcohol or mixed with soap for the bath.
- In 1.5 % of cases, leaves and bark are grounded and rolled up into ball. Women use those balls to cure amenorrhoea.
Sacrifices (1.5 %): The root is used as component of sacrifices. This practice was recorded in Atacora and Mono-Couffo provinces.

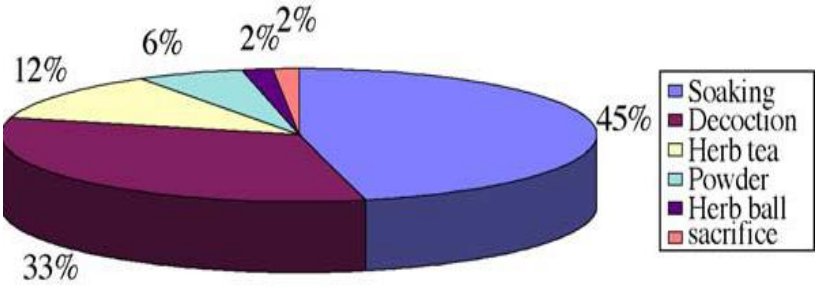

Fig. 8.2: Distribution of iroko form of utilization

8.3.1.2- Use of iroko timber

Milicia excelsa produces high quality wood which is used in carpentry and joinery for making tables, chairs, doors and windows. The species is also a very good wood used in sculpture for woodcarving in Zagnanado, Cove and Gbannanme regions. Iroko is used to make canoe in some lakeside villages. Its wood though durable is, however, so heavy

that when an accident occurs, it is difficult to save the victims by keeping the boat afloat. It is important to observe that *Milicia excelsa* is not used at all as fire wood in southern and central Benin.

8.3.1.3- Iroko tree as conservatory of cultural values

Milicia excelsa is considered by Benin local people as a very important tree which had mysterious powers that could protect or harm anyone. Many of the respondents associated the tree with witchcraft.

Iroko tree was known by 96 to 100 % of the respondents. Also, 80% regarded iroko tree as sacred with mystical powers. Nearly 9 % of the respondents knew the species just because of the good quality of its wood, while about 11 % had no knowledge of the species. There was a significant difference between the provinces in respect of the recognition of the species (χ^2 = 268.71; df = 17; $p \leq 0.01\%$). The population awareness about iroko as a sacred tree also varied from one province to another (χ^2 = 308.66; df = 27; $p \leq 0.01$ %). Nearly 55 % of the population believed and worshiped *Milicia excelsa*. The mode and intensity of devotion to the tree varied within the study area viz: 46 % in Atlantique province, 45.91 % in Zou and Collines, 53.06 % in Mono and Couffo, 57.69 % in southern Atacora, 29.16 % in southern Borgou and 30 % in Oueme and Plateau.

Consequent upon tribal wars in Benin, human migrations occurred during which some people carried their divinities to their new settlements in order to preserve them. Those divinities are then incorporated to various elements of the environment one of which is the iroko tree. Some villages were created around iroko tree after the settlement of populations. The tree is thus the previous object that generated the villages and it is called the Toxwyo of that village. All those villages which are created by a similar process are given the name of the iroko tree. As example, we have Vodun Loko in Lokossa district (lokossa means under iroko), Vodun Dan is the founder and the protector of Avegodo village in Aplahoue district, Ahozodjiloko is the tree which found Covedji village (Savalou district), Adanloko is the founder of Adjahouto (Allada district), Wassaloko (planted tree) is the founder of Adjara, Ahouanloko is the protector of Houedah community driven away by King Agadja in 1727, and iroko is the founder of Idiroko in Save district.

Milicia excelsa embodies many divinities which vary significantly from one province to another (χ^2 = 1830.27; df = 25 and $p \leq 0.01\%$). Among the respondents, 33 % did not know any divinity incarnated by iroko. For 10.4 %, iroko embodies only

"Vodun loko" with names as follows: Lokossou, Loko, Lokossi, Lokonon; 6.4 % know only about Vodun dan and toxwyo, 2.3 % for Hebiosso, 0.6 % for Sakpata and 47.4 % believe that iroko tree embodies many divinities.

8.3.2- Traditional strategies of *in situ* conservation of *Milicia excelsa*

8.3.2.1- Iroko tree conservation

People's reaction to the conservation of *Milicia excelsa* trees varies significantly between provinces ($\chi^2 = 258.14$; df = 29 and $p \leq 0.01\%$) and also between regions in the study area ($\chi^2 = 894.47$; df = 31; $p \leq 0.01\%$). For 64.7 % of those interviewed, iroko trees were conserved in order to protect the cultural practices, 15.9 % believed that the conservation was for commerce, while 16.2 % thought that the purposes are related to forestry legislation.

A sample of 803 iroko trees were recorded in the study area. Table 8.2 shows the distribution of these trees and the conservation level in each province. About 66.87 % of the iroko trees recorded were protected by people in Mono - Couffo provinces, 17.56 % in Plateau province, 7.58 % in Atlantique province, 3.37 % in Oueme province, 2.37 % in Zou and 2.21 % in Collines.

Table 8.2: Iroko tree conservation status per province

provinces	Number of iroko trees	Percentage (%)
Mono - Couffo	537	66.87
Plateau	141	17.56
Atlantique	61	7.58
Oueme	27	3.37
Zou	19	2.37
Collines	18	2.21
Total	803	100

8.3.2.2- Conservation strategies

Iroko tree conservation strategies could be summarized as follows:

(i)- On farm conservation: where iroko trees which grow naturally on the farm are protected by farmers who take care of them. Most of the time those trees are not sacred.

(ii)- Conservation in sacred groves: Here all the trees in the grove are protected including the iroko tree.

(iii)- Isolated sacred iroko trees: Some iroko trees are consecrated by traditional and religious authorities for various reasons even though they are not in a sacred grove. Because of the mystical status of the species, every iroko tree site is consecrated. Table 8.3 listed different iroko conservation sites according to the provinces.

Table 8.3: Different sites of iroko tree conservation

Provinces Sites	Plateau (%)	Oueme (%)	Atlantique (%)	Zou (%)	Collines (%)	Mono- Couffo (%)	Borgou (%)	Atacora- Donga (%)	Total
On farm	85,0	10,5	54,9	45,5	11,1	84,7	81,58	95,68	48,6
Sacred groves	5,8	21,1	2,0	0	0	0,4	2,64	0,71	4,8
Isolated sites of fetish worship	8,3	57,9	19,6	54,5	33,3	12,1	0	3,60	30,9
Public places	0,8	10,5	23,5	0	55,6	1,6	15,78	0	15,3
Cemeteries	0	0	0	0	0	1,2	0	0	0,2
	99,99	100,00	100,00	100,00	100,00	100,00	100,00	99,99	100,00

Apart from these strategies, people suggested other strategies for iroko tree conservation improvement. Those suggestions include (i) strengthen forestry legislation and continuous sensitization of the population (suggested by 49.1 %), (ii) iroko plantation establishment and protection against bush fire (37 %), and (iii) reward the

best iroko tree protectors (13.9 %). There is significant difference between the provinces as far as strategies suggested are concerned (χ^2 = 511.99; df = 27 and p ≤ 0.01%).

4.3.2.2.1- Conservation by sanctification approach

The sanctification of iroko trees is the main strategy used by local people for its conservation. By this practice, some trees are consecrated while others are not.
Fig. 8.3 shows the proportion of sacred and non sacred iroko trees in each province. In Zou - Collines provinces, there are more sacred iroko trees than non sacred ones. But in Borgou province there are very few sacred iroko trees compared to the non - sacred ones in that province.

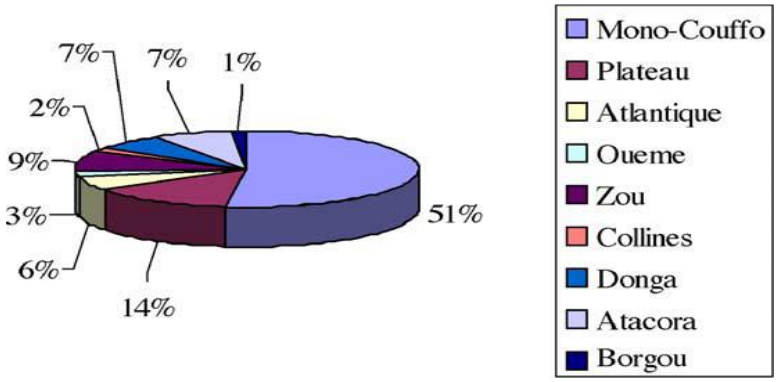

Figure 8.3: Different types of iroko per province

Among the iroko trees inventoried, 38.5 % were sacred. Table 8.4 shows the distribution of each category of iroko trees per province.

Table 8.4: Distribution of different categories of iroko tree per province

Provinces	Sacred Iroko (%)	Non sacred Iroko (%)
Mono	13	24
Atlantique	11	23,5
Zou	7	1,3
Atacora	3	4,5
Ouémé	4	5,5
Borgou	0,6	2,7
Total	38,5	61,5

In Mono – Couffo province, 13 % of the iroko trees were recorded in Mono-Couffo provinces, 11 % in Atlantique, 7 % in Zou, 4 % in Oueme, 3 % in Atacora, and 0.6 % in Borgou. Among the iroko trees which are not sacred but have sacrifices beneath, 24 % were recorded in Mono-Couffo provinces, 23.5 % in Atlantique, 4.5 % in Atacora, 2.7 in Borgou and 1.3 in Zou.

4.3.2.2.2- Nature of sacred objects

Table 4.5 lists different sacred objects used as symbol on iroko tree, according to the provinces. Ten sacred items were recorded in the whole study area (Fig. 8.4): Pottery (36.36 %), iron (11.11 %), calabash (4.04 %), candle (2.02 %), piece of cloth (18.18 %), sacrifice (13.13 %), piece of money (3.0 %), stone (2.05 %), glassware (broken bottle, 2.02 %), convent (fetish room, 8.08 %). There is a highly significant difference between provinces as far as sacred objects are concerned ($\chi^2 = 183.037$; df = 19 and $p \leq 0.001$). Tables 8.5 shows that pieces of cloth are used in all the provinces. Pottery is the most commonly used sacred item in Zou (66.66), Atlantique (75.0 %) and Mono (31.58) provinces while iron is predominant in Collines province (35.71 %). In Oueme province, the sacred status of iroko trees is often established by tying white cloth to the tree (28.57 %), or by the presence of a convent (fetish room) near from the trees (28.57 %). The most important sacred items used in Plateau province are calabash (36.37 %) and sacrifices under the trees (27.27 %). People from Plateau province are known to be the only ones who use calabash to establish the sacred status of iroko trees.

Table 8.5: Different items used in establishing sacred status of iroko trees

provinces Sacred objets	Plateau	Collines	Oueme	Mono-Couffo	Zou	Atlantique
Calabash	+	-	-	-	-	-
Pottery	-	+	+	+	+	+
Iron	+	+	-	+	-	-
Candle	+	-	+	-	-	-
piece of cloth	+	+	+	+	+	+
glassware	+	-	-	+	-	-
Sacrifice	+	+	+	+	+	-
Convent	-	-	+	+	+	+
Others (money, stone)	-	+	-	+	-	-

(+) = the sacred object was recorded in the province
(-) = the sacred object was not recorded in the province

8.3.2.2.3- Efficiency of traditional conservation strategies of iroko trees

In Benin Republic, because of the mystical considerations of iroko tree, its wood processing as timber wood or fire wood is strictly forbidden by traditional laws. For local uses, people are not allowed to collect iroko leaves from the tree. Leaves must be collected from the ground. Inspite of the proliferation of new foreign religions (especially Christianity and Islam), 45.4 % of the respondents still hallowed iroko tree, 54.6 % would still place sacrifices under the tree even if they did not hallow it. Therefore many iroko trees destroyed during the revolutionary period of witchcraft prevention in Benin Republic were re - planted and protected as the remaining iroko trees. Iroko stumps, seedlings and saplings are equally treated with respect.

Most of the protected iroko trees are in public places, near houses and in sites of fetish worship. These trees are protected by traditional authorities as well as religious authorities. Whoever wants to collect any component of the tree or do something around it, must obtain permission from the authorities in charge of the protected tree. Anyone who contravenes the conservation rules incurs several penalties which vary greatly between provinces ($\chi^2 = 205.55$; df = 27 and $p \leq 0.01$ %). In Zou - Collines provinces, the iroko tree logging is not common. In Atlantique and Mono provinces, the contravene is punished by the fetish responsible. The penalties ran from simple warning to the assassination of the offender according to the gravity of the fault.

It is important to note that the protection of iroko trees is not total. People are allowed to collect some components of the protected trees for medicinal uses or other purposes. The components often range from fruits to the logs. There are however, laid – down procedures which are strictly adhered to. These include: authorisation, offering, and initiation to traditional practices. Permission to collect some iroko components may be given to any people simply by offering a litre of the local brandy (sodabi) and kola nut (*Cola nitida*) to the chief priests or traditional chiefs.

People's perception of dead or felled iroko tree differs significantly from one province to another ($\chi^2 = 453.55$; df = 31 and $p \leq 0.01$ %). Among those interviewed, 63.9 % believed that dead or alive, the iroko tree was powerful. Others perform some rights to remove the power from an iroko tree in order to prune it or to log it. Such mystic power is restored to the tree stump after logging. About 88 % of iroko tree in Mono province were subjected to this practice, while in the Atlantique and Zou provinces, iroko stumps were venerated by 66.3 to 68 % of the respondents. There were fewer large – diameter iroko trees in Lokossa and Come districts of Mono province because of the practice of setting the powers of the tree aside in order to harvest its components or in deed to log the whole tree.

8.4- Discussion

8.4.1- Socio - economic importance of *Milicia excelsa* and pressure on the species

Milicia excelsa wood is appreciated by many wood users. Taylor (1960) and Nichols *et al*. (1998) emphasised the technical quality of this species. Iroko wood is hard and resistant to termite attack, which justifies its use in carpentry and joinery. According to WCMC (1991), the high quality timber of *Milicia excelsa* is used as a teak (*Tectona grandis*) substitute and is widely used for all kind of building and carpentry including domestic flooring, veneer and cabinetwork. It is used for building ships and barrels (WCMC, 1991). The species is also used externally because it has great resistance to bad weather.

In Cameroon, iroko wood is used in joinery as rapping fibres (Dounias, 1988; Seignobos, 1997). In southern Cameroon, *Milicia excelsa* is among the seventeen species essential for iron metallurgy because its wood is able to offer the properties needed for chemical and ox - redox reaction in the industry (Dounias, 1988).

In Benin Republic, *Milicia excelsa* has many medicinal uses. This corroborates the results of Adjanohoun *et al.* (1989) who identified five diseases cured with iroko leaves and bark. According to the authors, when *Milicia excelsa* leaves are burnt together with *Plumbago zeylanicum* leaves and added to black soap and the resulting compound is used to cure female sterility. *Milicia excelsa* is also used as a dye (FAO, African Regional Workshop, 1996).

It has been reported that *Milicia excelsa* bark, its ashes, the leaves, and the latex are all used in local medicine (Anon., 2001). The latex is used to reduce tumours and obstructions of the throat and for stomach problems. The bark is also used to treat coughs, dysentery, heart problems, and general tiredness.

Most of the time, *Milicia excelsa* is part of medico - magic prescriptions in Benin. Totin (1987) reported that witches always share all their secrets with iroko tree when they want to bewitch people. *Milicia excelsa* is also used in land improvement (Anon., 2001). It is often used as a shade tree and along streets as an ornamental. Its leaves are used as mulch and it is also nitrogen fixing (Anon., 2001).

Nowadays, *Milicia excelsa* is threatened with extinction in Benin Republic. This situation was emphasised by Agbahungba *et al.* (2001) who classified the species as an endangered species on which many conservation actions should be focused.

In Ghana (Hawthorne, 1995), this species is heavily exploited and its plantations tend to be unsuccessful.

In Zimbabwe, *Milicia excelsa* is threatened by habitat degradation but it is not exploited in that region (African Regional Workshop, 1996). It is found only in areas which are suffering from alluvial erosion.

According to the Benin National Department of Forest Resources reports (1959; 1960; 1965), the scarcity of the species on wood market in Benin started since 1965. *Milicia excelsa* is endangered in Benin Republic because of its over exploitation in the natural forests. There are however, other reasons such as difficulties of the species establishment in plantations because of attacks by *Phytolyma lata* (Wagner *et al.*, 1991), the very low natural regeneration rate owing to rapid loss of seed viability (Bada pers. comm.. 2002); and urbanization which induces the logging of the big iroko trees (Nichols *et al.*, 1998) which normally produce viable seeds.

A struggle against witchcraft by killing all big iroko trees was organised by the Government of Benin in 1972. This situation lead to the destruction of most of the big iroko trees and negatively affected seed production resulting in reduced regeneration of the species. As reported by Dave (2002), a serious threat to sacred trees and sacred groves is that the basis for there existence, the deeply rooted cultural and spiritual values of their sacred status are slowly eroding. The loss of cultural identity among the young generation is also an important threat for forest trees conservation (Gerden and Mtallo, 1990). Dave (2002 quoting Dorm-Adzobu *et al.*, 1991) describe the effect of the loss of cultural values as including the weakening of local traditional religious systems and community leaders loss of authority. These often happen when modern influences are introduced because the fetish priest loses his power over the people especially those removed from his daily influence. Then the religious rules are relaxed, the activities protecting sacred sites are preformed less frequently and less well, the forests are increasingly exposed to natural forces such as fires, and the forest resources are exploited. The grove's sanctity is violated, the forest becomes degraded and eventually some species disappear.

In Benin, *Milicia excelsa* density varies from 1 stem / ha in the dry semi deciduous forests to 7 stems / ha in gallery forests and its regeneration rate is also very low (less than 4 stems / ha). The situation is the same in Zimbabwe and in Mozambique where few regeneration seems to be observed in the open areas of cleared stands where large trees of *Milicia excelsa* are left standing (African Regional Workshop, 1996).

8.4.2- Socio - religious importance of *Milicia excelsa* and Traditional strategies of *in situ* conservation

In Benin, *Milicia excelsa* represent a reservoir of many traditional values. The results of this study confirm those of Agbahungba *et al.* (2001), Plya (1976), and Sokpon & Agbo (1999), who are reported that the species was sacred in Benin Republic irrespective of province or ethnic group. Indeed, many tree species are considered as sacred in Benin. According to Pazzi (1979), plants are considered as mystical objects which are employed in every ritual as liturgical plants, or as plant indicators of worship sites, or incarnating divinities. Then each divinity has its acceptable plant species which are distinct from those of others. According to Juhe – Beaulaton (1999), some plants are particularly appropriated to venerate some divinities.

The main strategy of *Milicia excelsa* conservation by local population is it deification. According to Sokpon & Agbo (1999), as iroko tree is sacred, its seedlings and saplings are protected by farmers wherever they grow. The remaining groves which are the relic forests affected by human pressure were conserved by sanctification (Chevalier, 1933; Aubreville, 1937; Coulibaly, 1978; Guinko, 1985). In general, when there is an iroko tree somewhere, the place is considered sacred (Juhe – Beaulaton & Roussel, 2002). According to Juhe – Beaulaton & Roussel (2002 quoting Michalon and Dury, 1998), the occurrence of trees from plant families such as Moraceae (*Milicia, Antiaris, Ficus*) and Bombacaceae (*Adansonia, Ceiba, Bombax*) is an act of God. As related by their history, sacred groves and sacred individual trees constitute the remaining old vegetation (Sokpon & Ago, 2001). Their religious status assures their conservation (Juhe-Beaulaton & Roussel, 1998).

In Togo, the sacred forest of Aneho-habitat seems to be the remaining littoral dense vegetation which, as reported by the traveller Zöller, existed in 1884 around Lome (Juhe-Beaulaton and Roussel, 1998). In Benin, the sacred status of some forests represents an important tool to maintain them until now (Sokpon and Agbo, 1999).

The role of religious and cultural beliefs in protecting trees is very important. Indeed according to Dave (2002 quoting Mathias-Mundy *et al.*, 1992), individual trees are often thought to possess ancestral or natural spirits, are at home to gods or are of special religious significance. According to Laird (1999), most of the sacred forests which are protected by traditional laws and have by no means a legal status have survived for many hundreds of years and today act as reservoirs of much local biodiversity.

8.5- Conclusion

The assessment of *Milicia excelsa* importance and local conservation shows that the species is used in carpentry and joinery for making furniture, in traditional medicine (to cure 45 diseases) and is an accepted reservoir of cultural values. It embodies many divinities which vary according to the provinces.

The fears of its mystical powers coupled with its veneration are the basis of its conservation. Iroko trees are conserved on farms, in sacred groves, in public places, and in cemeteries. The different sacred objects used to symbolize the sacred iroko trees include pottery, iron, calabash, candle, pieces of cloth, pieces of money, stone and glassware (broken bottle). The sacred status is not absolute as the mystical power could be removed temporary in order to harvest the tree or its components. Such powers are later restored to the stump of the tree.

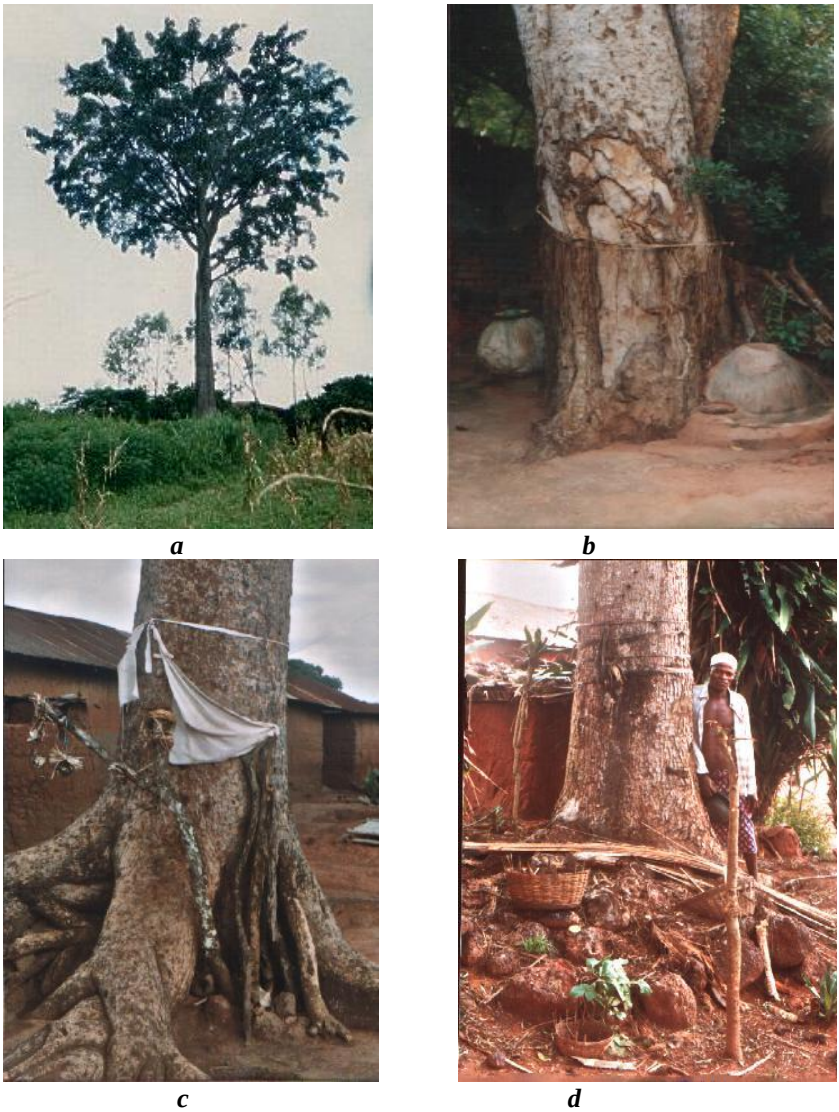

Fig. 8.4: Different items used to materialize sacred iroko trees. *a- Iroko tree preserved on farm; b- Sacred iroko tree materialized by potteries. Picture showing pressure on isolated iroko tree; c- Sacred iroko tree materialized by piece of cloth; d- Sacred iroko tree materialized by sacrifices.*

9

Traditional agroforestry systems as tools for *Milicia excelsa* welw. C.C. berg genetic resources conservation in Benin.

Christine Ouinsavi*, Nestor Sokpon

Laboratoire d'Études et de Recherches Forestières, Faculté d'Agronomie, Université de Parakou, BP 123 Parakou, République du Bénin;
**Author for correspondence (Email: ouinsch@yahoo.fr; christine.ouinsavi@sbf.ulaval.ca*
Phone: (229) 2361 2010, Fax: (229)2361 0712

Running title: Agroforestry systems for *Milicia excelsa* conservation in Benin
Key words: Agroforestry systems, conservation, *Milicia excelsa*, biological corridors.

In press in Agroforestry systems

Abstract

The potential contribution of agroforestry systems to the management and genetic resources conservation in iroko (*Milicia excelsa*), important and valuable timber tree species in sub-Saharan Africa, was addressed in this paper. The structure and dynamic of traditional agroforestry systems and the ecological structure of *Milicia excelsa* in farmlands, were studied through a survey carried out in 100 farmlands covering the natural range of iroko in Benin. Forty-five species belonging to 24 plant families were recorded in traditional agroforestry systems. Average tree density varied from 1 to 7 stems/km^2 with diversity index ranging from 2.6 to 2.9. *Milicia excelsa* sparsely occurs in agroforestry systems in all regions with density ranging from 1 to 4 stems/ha; stand basal area varying from 33.10^{-4} to 129.10^{-4} m²/ha, and almost inexistent seedling regeneration. However male and female trees seem evenly distributed on farmlands in all regions (F/M > 0). Iroko trees produced viable seeds with moderate germination rate and early growth (germination rate, *Gr* = 22 % and early growth is 7.29 cm after 3 months).

Suggestions were made regarding optimal iroko conservation densities in farmlands, according to farmers' socioeconomic conditions in different regions, in order to improve traditional agroforestry systems and their use as biological corridors in *Milicia excelsa* genetic resources conservation.

9.1- Introduction

In West African regions, it is common for farmers to retain useful trees when preparing a plot for cropping, thereby creating parklike landscape of scattered trees between crop field and rangelands (Boffa, 1999). These practice and other such as crop-fallow rotation, the use of timber trees in tree crop plantations, home gardens are various examples of the so-called agroforestry system. Agroforestry was recently defined by the Word Agroforestry Center (ICRAF, 2000) as a dynamic, ecologically based natural resource management practice that, through the integration of trees and other tall woody plants on farms and in the agricultural landscape, diversifies production for social, economic, and environmental benefits. In the last three decades, agroforestry has been widely promoted in the tropics as a natural resource management strategy that attempts to balance the goals of agricultural development with the conservation of soils, water, local and regional climate, and, more recently biodiversity (Schroth *et al*., 2004). Tropical agroforestry was also recently assessed in various ways such as the history and dynamic of the system (Kumar et Nair, 2004; Miller and Nair, 2006; Peyre *et al*., 2006), socioeconomic involvements (Montambault and Alavalapati, 2005), ecological interactions and management implications (Das and Chaturvedi, 2005; Lengkeek *et al*., 2005; Garcia-Barrios and Ong, 2004; Gaafar *et al*, 2006; Sharma and Ashwath, 2006), and genetic resources and biodiversity conservation (Izac and Sanchez, 2001; Schroth *et al*., 2004; Teklehaimanot, 2004; Atta-Krah *et al*., 2004; Simons and Leakey, 2004; McNeely, 2004; Acharya, 2006).

Milicia excelsa (commercially known as iroko) is a very important timber species in sub-Saharan Africa that is currently under threats because of its overexploitation. It is a large deciduous tree up to 30-50 m height, with a diameter of 1.70 – 2 m and a high, umbrella-like crown, growing from a few thick branches. The species was found to be sparsely distributed on farmlands which represented the main conservation sites for the remnant trees in Benin (Ouinsavi *et al*., 2005). A more recent study on the populations' genetic structure (Ouinsavi *et al*., 2006) revealed an effect of isolation by distance on relict populations of this species and suggested priority actions for increased gene flow by the creation of biological corridors between iroko populations in Benin.

In this study, we attempted to use agroforestry systems as tools for an efficient *Milicia excelsa* genetic resources conservation. Specifically we explored the potential of traditional agroforestry systems for biodiversity conservation. We improved it and made some suggestions about the optimum iroko trees densities to be maintained on

farmlands according to socio economic conditions and the relative importance of iroko in farmers' livelihood.

9.2- Methodology

9.2.1- The study area

The study was carried out in the Republic of Benin, located in West Africa between long 1° and 3°40' E and latitude 06°30' N and 12°30' N. It is bordered by Republic of Niger in the North, Burkina Faso in the Northwest, Togo in the West, Federal Republic of Nigeria in the East and the Atlantic Ocean in the South and covers an area of 112,622 km^2 (Fig.9.1).

Republic of Benin has a marginal ecosystem type because of its geographical situation in the Dahomean gap characterized by numerous physical constraints related to environment. Indeed, rainfall in Benin is low (900 - 1300 mm) comparing to its neighbor countries in the Guineo - Congolean zone such as Ghana, Côte d'Ivoire (average of 1500 mm), and Cameroon (1800 - 3000 mm).

Adjanohoun *et al*. (1989) distinguished in Benin four major types of vegetation landscape which were highlighted as follows:

- The Coastal and sub-coastal zone, which covered from the coast (6°20'N) up to 7°N, is composed of beach vegetation with abundance of *Remirea maritima* and *Ipomoea pescaprae*, anthropogenic vegetation of mangrove swamp forests characterized by *Rhizophora racemosa, Avicennia germinans* and *Dalbergia ecastaphylum*, riparian forests, oil palm and coconut plantations. This zone has a subequatorial climate type with two rainy seasons (from mid-March to mid-July and from mid-September to mid-November) which alternate with two dry seasons (from mid-July to mid-September and from mid-November to mid-March).
- The Guineo-Congolean zone composed of relict semi-deciduous forests characterized by species such as *Dialium guineense, Triplochyton scleroxylon, Strombosia glaucescens, Cleistopholis patens, Ficus mucuso, Cola cordifolia, Ceiba pentandra, Trilepisium madagascariense, Celtis spp, Albizia spp, Antiaris toxicaria, Diospyros mespiliformis, Drypetes floribunda, Memecylon afzelii, Celtis brownii, Mimusop andongensis, Daniellia oliveri, Parkia spp* and *Vitellaria paradoxa* (Sokpon, 1994; Sokpon and Lejoly, 1996), some savannahs and plantations.
- The Guineo-Sudanean Transition zone with mosaic of forest woodlands, sometimes dry forests and wooded savannah crossed by gallery forests. The most abundant species found in this zone are *Isoberlinia doka, I. tomentosa, Monotes kerstingii, Uapaca togoensis, Anogeissus leiocarpus, Antiaris toxicaria, Ceiba pentandra, Blighia sapida, Dialium guineense, Combretum fragrans, Entada africana, Maranthes polyandra, Pterocarpus erinaceus, Terminalia laxiflora*, and *Detarium microcarpum*.

These two zones are characterized by a Guinean climate type with two rainy seasons and two dry seasons distributed as above but the short rainy season of this region is sometimes absent. The total annual rainfall reaches 1,100 mm.
- The Sudanian zone composed of dry forests, savannah woodlands, shrub savannah and savannah grassland. The gallery forests is characterized by species such as *Haematostaphis barteri, Lannea spp, Khaya senegalensis, Anogeissus leiocarpa, Tamarindus indica, Capparis spinosa, Ziziphus mucronata, Combretum spp* and *Cissus quadrangularis*. This zone has a Sudanian climate type with one rainy season (from April to October) followed by one dry season (from November to March). The average rainfall varies from 1,100 mm in the southern part of this zone to 900 mm in the northern part.

According to FAO world classification of soils, the major type of soil distinguished in Benin are Leptosols in the coastal zone, Acrisols, Ferralsols or Lixisols and Vertisols in Guineo-Congolean zone, Luvisols, Lixisols, Alisols, Plinthosols and Arenosols in Guineo-Sudanean and Sudanean zones (FAO, 1999; Igué, 2000; Weller, 2002). Human pressure on land for agricultural purposes and forest exploitation is considerable. In southern Benin where population density varies from 200 to 450 inhabitants / km², average farmland area is 0.5 ha and fallow system is quasi absent. In the North where mean population density is 14 inhabitants / km², average farmland is 5 ha and fallow length is about five years.

Forest overexploitation has lead to the decreasing of some tree species density. So, species such as *Milicia excelsa, Khaya senegalensis, Afzelia africana, Pterocarpus erinaceus*, became scarce in their natural habitat (Agbahungba *et al.*, 2001). Human pressure on forest ecosystems also induced the occurrence of relicts forests which, are mainly sacred groves (Sokpon and Agbo, 1999) and isolated individual trees of some species such as *Milicia excelsa* trees which are protected by traditional ethnobotanic conservation practices (Ouinsavi *et al*., 2005).

9.2.2- Data collection and analysis

A semi-structured survey was carried out on rural households across the range of *Milicia excelsa* in Benin. Ten provinces over the twelve were investigated which are Oueme, Plateau in the Southeast, Mono, Couffo in the Southwest, Atlantique in the Southcentre, Zou, Colline in the Centre, Borgou in the Northeast, and Donga, Atacora in the Northwest. In each province, two villages were chosen at random, from which five households were selected to respond to the questionnaire, making sure that the selected households have their own farms. A total number of 100 households were interviewed. The survey was carried out using a questionnaire designed to assess the status of iroko trees on farms as well as different forms of on farm trees management. During the

survey, household heads were interviewed and direct observations were made on the field to record the distribution of annual crop compared to trees distribution on the farm, different treatments made to trees, the type of crop grown under trees and beyond the extent of the tree crown.

In addition, tree inventories were carried out on each farm and diameter at the breast height (Dbh, measured at 1.3 m above the ground), total height and crown diameter were measured for all woody perennials including iroko trees on the farms. It was noticed from farmers interview and visual observation whether the censured tree is a female or a male.

Natural regeneration of iroko (all iroko plant with Dbh < 10 cm) was planned to be counted on the farms but this activity failed to be achieved because regeneration were rare on most of the farms although iroko trees fruit every year. Therefore to test for seeds viability and their capacity to germinate during the rainy season, seeds were collected from twelve provenances in 2002 and were used for seeds germination and early growth trials. The provenance trials were carried out in the nursery in four different steps. In the first step, the freshly collected seeds were sown and the remaining seeds divided into two and kept, each at room temperature and in fridge at - 4°C. In the second step, the two sets of conserved seeds were sown separately after one year conservation in 2003. These two sets of conserved seeds were sown separately after two years conservation in 2004 (step 3) and after three years conservation in 2005 (step 4). The experimental design was a randomized bloc design with 3 repetitions. Each elementary plot (repetition) was sown of 100 seeds and watered every morning. Data collected concerned a daily counting of the number of germination. Seedlings from the first step's experiment were transplanted into polythene bags when they reached 4-leaves stage and their total height was fortnightly measured.

The primary data were used to describe traditional agroforestry systems in the range of *Milicia excelsa* distribution. From the inventory data, woody plant density and iroko density were calculated by dividing the total number of trees over the total number of hectare per region. Shannon diversity index (*H*) was calculated for each farm and averaged over provinces (Shannon and Weaver, 1949) as well as species richness (S) as:

$$H = -\sum_{i=1}^{k} Pi \, lnPi$$

, where P_i is the proportion of species *i* relative to the total number of species on the farm

and *S*, the total number of species at a site.

Variations in seeds germination and seedling early growth, among provenances were analyzed by Analysis of variance (ANOVA) using SPSS 9.1 software.

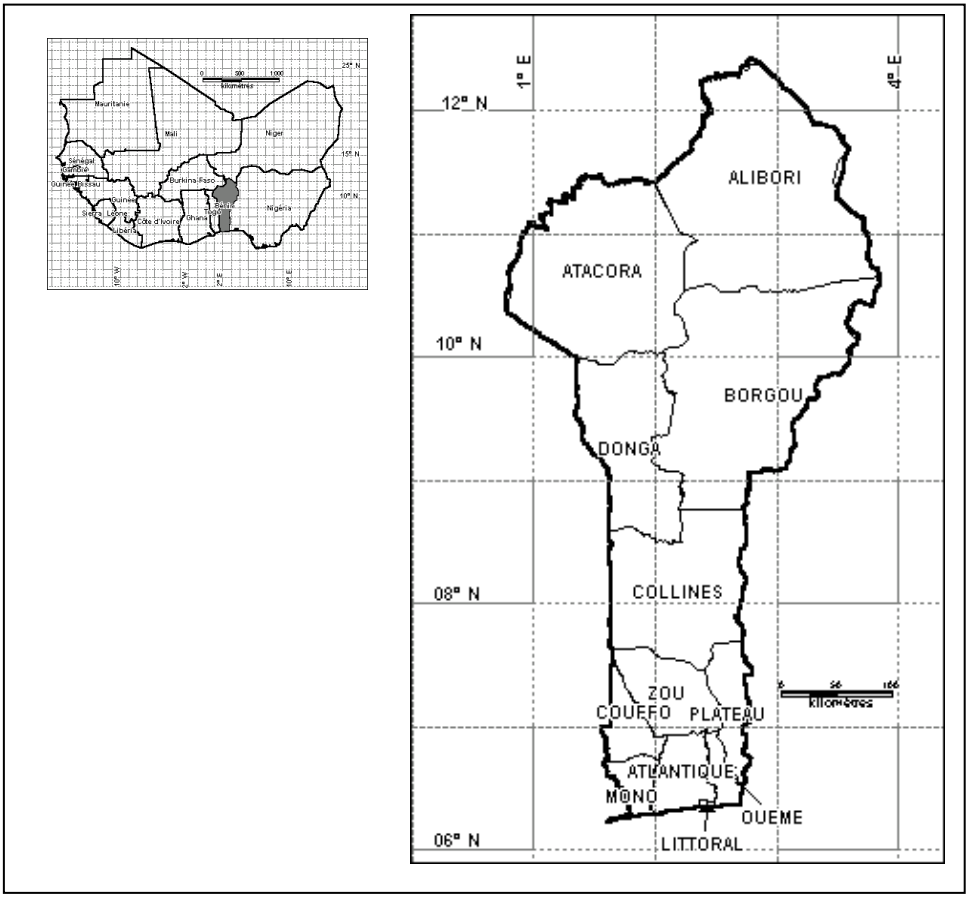

Fig 9.1: The study area

9.3- Results

9.3.1- Ecological structure of the woody component in traditional agroforestry systems

A total number of 24 plants families were recorded (Table 9.1). The major families were Fabaceae (with 5 species), Moraceae, Bombacaceae, Anacardiaceae (with 4 species), Rubiaceae, Sterculiaceae and Meliaceae (with 3 species). The average stand density of the woody component of farmlands was 6 ± 3.51 stems/ha in the South, 7 ± 3.62 stems/ha in the Central region and, mean densities were lower than 1 stem/ha (Table 9.2) in the Northwest. Traditional agroforestry practices differed according to different region in Benin. The protected species differed according to their utilities to farmers and their availability in the region. The reason why farmers protect woody plants on their farm varies significantly from a village to another in the South ($\chi^2 = 58$ d.f = 9 $p < 0.001$). Among the interviewed farmers in the south, 69.2 % protected trees for fire wood, 13.8 % for timber, 10.8 % for fruit sale, 3.1 % medicinal uses and 3.1 % for other uses of the wood (house building, carving, shade, etc.).

Species richness was S = 18 species with an average of 6 species per farm in the South, 33 species with an average of 4 species per farm in the Centre, and 15 species in the Northwest (Table 9.2). In the Northeast most of the investigated farms were more recently cleared and their structure could not be compared to the ones observed in the other regions. Shannon diversity index was slightly lower in the South (H = 2.59) than in the central region of the country (H = 2.94). The southern Benin (Mono, Couffo, Oueme, Plateau and Atlantique) is characterized by agroforestry system based on palm oil tree (*Elaeis guinensis*) in which palm oil trees regeneration is natural. The northern Benin (Borgou, Donga and Atacora) is characterized by predominance of shea butter tree (*Vitellaria paradoxa*) and locust bean tree (*Parkia biglobosa*) parklands, followed by some sparse trees of *Milicia excelsa*, *Borassus aethiopum*, *Adansonia digitata, Mangifera indica*. However a new form of land use is being developed now in that part of the country which is an agroforestry system based on cashew tree (*Anacardium occidentale*). In the Centre, *Mangifera indica*, *Citrus sinensis*, *Irvingia gabonensis* and *Elaeis guineensis*, are the most important trees on farmlands in the region close to the south (Bohicon, Setto) while the central north practices a system similar to the North's with shea butter tree and locust beans tree parklands and some cashew tree plantations.

Table 9.1: Botanical name, family and major uses of the woody component of agroforestry systems.

Botanical name	**Family**	**Region of occurrence**	**Major uses**
Anacardium occidentale	Anacardiaceae	N	Edible, cash crop, fire wood
Mangifera indica	Anacardiaceae	E	Edible, fire wood
Spondias mombin	Anacardiaceae	S	Edible, fire wood
Annona muricata	Anonaceae	E	Edible, fire wood
Annona senegalenis	Anonaceae	E	Edible, fire wood
Holarrhena floribunda	Apocynaceae	S	Medicinal, timber
Elaeis guineensis	Arecaceae	S	Edible, fire wood
Newbouldia laevis	Bignoniaceae	E	Medicinal, fire wood
Adansonia digitata	Bombacaceae	N	Edible
Bombax breviscuspe	Bombacaceae	N	Edible
Bombax costatum	Bombacaceae	N	Fire wood
Ceiba pentandra	Bombacaceae	S	Timber
Terminalia mentali	Combretaceae	N	Fire wood
Diospyros mespiliformis	Ebeneceae	E	Edible, timber
Acacia auriculiformis	Fabaceae	E	Fire wood, soil improvement
Albizia zygia	Fabaceae	S	Soil improvement, fire wood
Cassia siamea	Fabaceae	E	Medicinal, fire wood
Dialium guineense	Caesalpiniaceae	S	Edible fire wood
Gliricidia sepium	Fabaceae	E	Soil improvement
Prosopis africana	Fabaceae	E	Timber, medicinal
Irvingia gabonensis	Irvingiaceae	E	Edible
Persea gratissima	Lauraceae	E	Edible
Azadirachta indica	Meliaceae	E	Medicinal fire wood
Khaya senegalensis	Meliaceae	E	Medicinal, timber
Trichilia roka	Meliaceae	N	Fire wood
Parkia biglobosa	Mimosaceae	N	Edible, carving
Antiaris africana	Moraceae	E	Timber
Ficus capensis	Moraceae	E	Fire wood
Ficus elastica	Moraceae	E	Fire wood
Milicia excelsa	Moraceae	E	Timber, Medicinal, religious

Moringa oleifera	Moringaceae	E	Edible, fire wood
Eucalyptus camaldulensis	Myrtaceae	E	Medicinal, timber
Psidium goyava	Myrtaceae	E	Edible, medicinal
Morinda lucida	Rubiaceae	S	Medicinal, fire wood
Citrus sinensis	Rutaceae	E	Edible, medicinal
Xanthoxylum xanthoxyloîdes	Rutaceae	E	Fodder, fire wood
Blighia sapida	Sapindaceae	E	Edible, fire wood
Cola nitida	Sterculiaceae	E	Edible
Sterculia tragacantha	Sterculiaceae	E	Timber, fire wood
Triplochyton scleroxylon	Sterculiaceae	S	Timber, religious
Celtis zenkeri	Ulmaceae	S	Timber
Tectona grandis	Verbenaceae	E	Timber, fire wood
Sclerocarya birrea	Anacardiaceae	N	Edible fruit
Nauclea latifolia	Rubiaceae	E	Leaves for wrapping
Piliostigma thonningii	Caesalpiniaceae	E	Leaves for wrapping

N= North, S = south, E = everywhere (all regions)

Table 9.2: woody density and ecological diversity of agroforestry systems (n = 100 housholds)

Regions	Density	% planted trees	% non planted trees	S	H
South	6 ± 3.51	38.89	61.11	18	2.59
Center	7 ± 3.62	45.45	54.54	33	2.94
Northern west	1 ± 0.02	13.33	86.67	15	-
Mean	5	32.56	67.44	22.00	-

9.3.2- Ecological structure of iroko in farmlands

Milicia excelsa stand density varied across its range (*Ni* =1 stem/ km² in the North and the central region, *Ni* = 2 stems/ km² in the Northwest and *Ni* = 4 stems/ km² in the South (Table 9.3). Mean diameter and average stand basal area were respectively *Dbh* = 67.91 cm, *BA* = 129.10^{-4} m²/ha in the South; *Dbh* = 89.61 cm, *BA* = 62.10^{-4} m²/ha in Central region; *Dbh* = 91.63 cm, *BA*= 65.10^{-4} m²/ha in the Northern East and, *Dbh* = 64.32 cm, *BA* = 33.10^{-4} m²/ha in the Northwest. Fig. 9.2 shows stem class distribution of *Milicia excelsa* in farmlands from different regions. On Adja plateau in Southwest, iroko showed a bell shape distribution with dissymmetry on the left. This structure was almost similar to the expectations from tree populations' distribution under normal treatment. In the other regions, the low iroko density did not allow us to bring out its distribution.

In all regions few seedlings were recorded although the trees fruited annually. This might involve some management issues for efficient iroko conservation through agroforestry systems.

Table 9.3: Ecological and morphological characteristics of *Milicia excelsa* trees in agroforestry systems

Populations	Average DBH (cm)	Mean Stand basal area G (m²/ ha)	Density (Ni / km²)	Crown diameter (m)
South	67.91	0.0129	4	12.34
Centre	89.41	0.0062	1	16.51
Northern East	91.33	0.0065	1	14.32
Northern West	64.32	0.0033	2	14.15

9.3.3- Basic parameters for an efficient iroko on farm conservation

The most important condition to satisfy for the perenniality of a tree population is to assure that there are enough seedlings and growing conditions to compensate the mature population. This required an adequate number of male and female trees in the population, seeds viability and seedling growth.

In all of the investigated populations, there were both male and female iroko trees. The ratio, number of female trees over number of male trees (*F/M*) was lower than 1 for most of the provenances except two (Sakete and Tamarou) where $F/M \geq 2$. Provenance trials showed that germination starts 18 days after sowing in fresh iroko seeds. At that date, the highest number of seedlings rising was observed in Aplahoue provenance (10 %) and the lowest, in Sakete provenance (0 %) (Fig. 9.3). from the 28th day after sowing, all of the provenances reached their maximum germination rate (*Gr*) and three classes could be identified (fig.3): *(i)* provenances with $Gr \geq 18$ % (Save, Niaouli, Bassila and Aplahoue provenances), *(ii)* provenances with $12\ \% < Gr \leq 15\ \%$ (Tamarou and Biro provenances), and *(iii)* provenances with $Gr \leq 10$ % (Ketou, Bante, Djougou, Lokossa and Bohicon provenances). Altogether, the highest germination rate obtained for iroko was $Gr = 22$ % but this value decreased consistently according to the duration of conservation (average $Gr = 19\%$ after one year conservation at 4°C; $Gr = 5$ % after two years conservation at 4°C and $Gr = 2$ % after three years conservation at 4°C). Iroko seeds conserved at room temperature for one year do not germinate.

As far as the early growth of iroko seedling is concerned, significant differences were observed among provenances on the 75th day after transplantation ($F = 6.734$; $d.f = 11$ and $P < 0.001$) and on the 115th day after transplantation ($F = 6.033$; $d.f = 11$ and $P < 0.001$). On the 75th day after transplantation the highest growth was observed for the provenance of Save (3.71 cm) and the lowest in Lokossa provenance (2.90 cm). However there was a recovery in the growth speed and, at the 115th day after transplantation, provenances from Aplahoue, Bohicon and Save have the highest growth (7.29 cm).

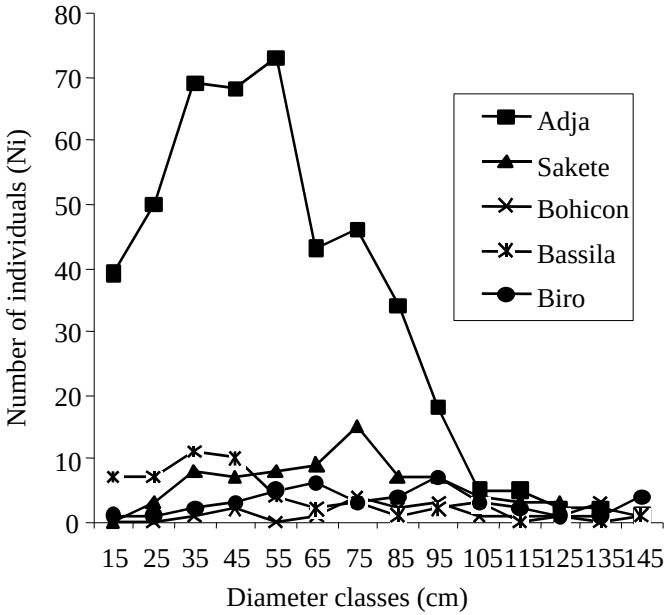

Fig. 9.2: *Milicia excelsa* diameter class distribution in different populations

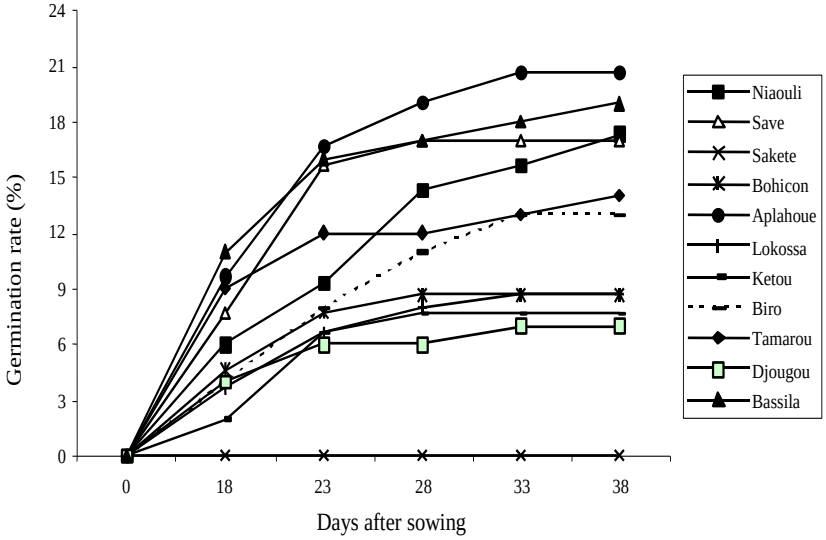

Fig 9.3: *Milicia excelsa* provenances test for germination

9.3.4- Farmer strategies of iroko trees management in agroforestry systems

Most of the crops produced in Benin such as maize, sorghum, rice, cotton, peanut beans, yam, cassava, and potatoes are light demanding species. Therefore, farmers are sometimes at a loss for allocating space to trees on their farm especially when they deal with a species that requires relatively large areas. The common management practice in agroforestry tree systems was tree pruning but this practice could have an impact on seed production. However, in Southwest of Benin, farmers developed another ingenious strategy for the shaded area exploitation using under iroko trees. They practiced market gardening which consists of growing shade tolerant crops such as tomato, pepper, and leaf vegetable plants under iroko trees and the light demanding crops are grown beyond the shade. Similar practices were noticed in the North with yam plants grown under the trees. This strategy has a triple benefit: it allowed farmers to fully use the space, to gain from iroko tree on their farm and dispense them from providing shade to the market garden plants.

9.4- Discussion

9.4.1- Ecological diversity of traditional agroforestry systems in Benin

In fragmented landscape, agroforestry could help to maintain a high level of biodiversity both within and outside protected areas (Schroth *et al*., 2004). Ecological diversity of traditional agroforestry systems in Benin was considerable although it was lower than what other authors recorded in agroforestry systems in Meru (54 species, Lengkeek *et al*., 2005); in Nepal (53 – 101 species from subsistence farming area, Acharya, 2006) and indigenous agroforestry systems in Amazonia (77 to 94 woody perennials tree species, Smith, 1996; Miller and Nair, 2006). The species diversity index calculated for agroforestry systems in this study was similar to what was reported for the subsistence farming systems in Nepal (2.91, Acharya, 2006). This value was lower than that of some home gardens in Sri Lanka (3.93, Sellathurai, 1997 quoted in Acharya, 2006) and in Bangladesh (3.24, Bashar 1999 quoted in Acharya, 2006). Many authors showed that traditional agroforestry practices contributed to the conservation of biodiversity through *in situ* conservation of tree species on farm, reduction of pressure on remnant forests, and the provision of suitable habitat for a number of plant and animal species on farmland (e.g. Atta-Krah *et al*., 2004; McNeely and Schroth, 2006; Acharya, 2006).

Tree density and type of tree species found on farms depended on local socio-economic and environmental factors. In the current study, tree density was low and the protected species differed according to their availability in the region and their usefulness to the farmer. Acharya (2006) reported that the number and types of tree species on farm in Nepal, depended on land holding size, livestock amount and the level of properties fragmentation.

9.4.2- *Milicia excelsa* ecological structure and conservation status in farmlands

On a broad point of view, *Milicia excelsa* appeared sparsely distributed in agroforestry systems in all regions in Benin (low density, 1 to 4 stems/ km²; low stand basal area, 33.10^{-4} - 129.10^{-4} m²/ha; and almost inexistent seedling regeneration). Although other recent studies of tree densities in Western and Central Kenya, Central Uganda and in Cameroon showed that 75 % of all tree species observed on farms were represented at a density of one or less individual per hectare (Atta-Krah *et al*., 2004), iroko density and structure in agroforestry systems need to be given a priority improvement. Indeed the species is currently under threats and a previous study (Ouinsavi *et al*., 2005) reported that most of the remnant iroko trees in Benin were conserved on farm. Agroforestry systems were therefore an appropriate issue to strengthen the species conservation. Our

suggestion of leaning traditional agroforestry management on *Milicia excelsa* density and ecological structure improvement could partially meet Hubbell (2001) and Lengkeek *et al*. (2004) suggestion related to agroforestry ecosystem diversification in terms of species evenness through substitution; i.e., fewer trees of a few major species and more trees of the rarer species. However care should be taken to avoid a system closed to mono specific one in order to assure an additional level of stability and resilience and minimize the chance of pest and disease outbreak. The suggested improvement should be seen as a tool for conserving the genetic viability of *Milicia excelsa* and contribute to both gene flow and the overall gene pool. It should contribute to conservation by providing habitat for pollinators and seed dispersers that facilitate gene flow in other tree species (Slocum and Horvitz, 2000) or by creating an environment that favors seedling regeneration (Boshier, 2004).

Our results showed that *Milicia excelsa* is evenly distributed across its range in terms of male and female trees; in addition the species fruits abundantly and produces viable seeds. However seedlings are almost inexistent on farms. Two reasons could explain this situation First, as iroko trees fruit sometimes in dry season and seeds are known to quickly loss their viability, it might not remain enough viable seeds to germinate during the rainy season. Secondly, farmers may destroy very young seedlings when there are plugging their farms as they do it every year and they are not awarded of the delicateness of iroko seeds viability and seedlings care. Based on these explanations, we suggest that farmers should allocate just a small plot of land in their farm, and establish iroko nursery from where they will pick seedlings for transplantation since they are all awarded of the species' scarcity.

9.4.3- Spatial distribution for iroko density optimization on farmlands

To help farmers to better implement the above suggested management techniques, we attempted to determine optimal iroko density for its conservation in the agroforestry systems. This was based on the total covering area of trees. The iroko density determined for farmlands in this study vary from 1 to 4 stems/ km². For the oil palm based agroforestry systems in the South, the National Center of Research on palm oil (SRPH) advices farmers to use a density of 80 trees/ha in association with crops. However, the currently enforced forest policy in Benin, based on the environmental conditions, suggests farmer to leave 50 trees/ha while clearing a new farm. Based on the average crown diameter of iroko tree in different region, the mean area covered by an iroko adult tree is 119.54 m² in the South, 213.97 m² in the Central region, 160.97 m² in the Northeast and 157.17 m² in the Northwest. When we report these values to iroko density in each region, the average area occupied by iroko trees on the farms is 478.16

m²/ha (4.78 %) in the South, 213.97 m²/ha (2.14 %) in the Central region, 160.97 m²/ha (1.61 %) in the Northeast and 157.17 m²/ha (1.57 %) in the Northwest. These proportions are too low to overcome the above mentioned requirement. Therefore we suggest that the woody perennial component of the agroforestry systems should be given the same importance as crops on the farm meaning that from one hectare plot, 5000 m² should be allocated to trees and 5000 m² to crops. Taking into account that *Milicia excelsa* is associated with a couple of other species depending on the regions, and considering our goal of laying the agroforestry system management on the improvement of iroko density and ecological structure, the following assumptions are made:

- 20 % of the 5000 m² allocated to trees per hectare is devoted to iroko (meaning 10 % of the total area)
- 25 % of the 5000 m² allocated to trees per hectare is devoted to iroko (meaning 12.5 % of the total area)
- 30 % of the 5000 m² allocated to trees per hectare is devoted to iroko (meaning 15 % of the total area)

On the basis of these assumptions, the optimal densities (Table 9.4) suggested for iroko trees in agroforestry systems are as followed: For the first option, farmers should use 8 trees/ha in the South, 5 trees/ha in the Central region and 6 trees/ha in the North. For the second option, they should use, a density of 10 stems/ha in the South, 6 stems/ha in Central region and 9 stem/ha in the North. When they choose the third option, they should use 12 stem/ha in the South, 7 stems/ha in Central region and 9 stems/ha in the North.

Altogether, farmer will choose one of these options according to their socio economic conditions and the importance of iroko in their livelihood.

Table 9.4: Optimal densities suggested for iroko trees in agroforestry systems

Regions	Optimal density suggested (Ni/ha)		
	Option 1 (20%)	Option 2 (25 %)	Option 3 (30 %)
South	8	10	12
Center	5	6	7
Northern east	6	8	9
Northern west	6	8	9
Average	6	8	9

10

General discussion and conclusions

10.1- Socio-economic and cultural importance of Milicia excelsa

Since they evolved, trees have had a great influence on the shaping of the ecology of our planet and in determining the present arrangements of life on earth. The role of trees in the evolution of mankind and the development of human cultures and communities is of particular importance for people. *Milicia excelsa* showed great usefulness in traditional medicine, in carpentry and joinery as well as religious practices. Almost all of the components of the tree are used to cure diseases and pathologies. Medicinal plants are known to occupy an important place in the healthcare systems of developing countries and their use became part of people traditional way of life (Keirungi & Fabricius, 2005). *Milicia excelsa* is also widely known for its mythical power which supported its importance in traditional religions. As reported by Kokou and Sokpon (2006), the species have long being considered as sacred and used to materialised very ancient sites dwelled by the ancestors spirits. Iroko tree was also regarded as sacred to symbolize the magnificence of the embodied person or family or spirit (Kokou and Sokpon, 2006). However, the first rank attached to this species in Benin took into consideration the strength of its healing properties and its sacred powers, but also its market price. Indeed, *Milicia excelsa* is widely used for all kinds of construction work and carpentry including domestic flooring, veneer and cabinetwork (WCMC, 1991). The timber is used for building ships and barrels. Iroko was a major commercial species in international trade. This economic importance of iroko is partially related to its natural resistance to decay-causing organisms, timber borers, termites and aquatic worms as well as its good working properties (Cobbinah *et al.*, 2000). These authors have reported that between 1985 and 1995, Iroko represented 12 and 18 % of the volume and value of timber export respectively. Tanzania and Uganda were in the past major sources of timber and some iroko wood is still exported from East Africa. In Kenya users of this species claimed that supplies were variable and unpredictable (Marshall & Jenkins, 1994). Many West African countries were the main exporters, especially Ghana (traded together with *M. regia*) and Côte d'Ivoire (WCMC, 1991). Alder (1989), comparing the current rate of iroko extraction in Ghana (172,983

cm^3/year and the annual growth rate (28,650 m^3/year), has predicted an extinction of the resource after ten years if nothing is done. In 1989, the United Kingdom imported 22 648m^3 of iroko wood of which, Côte d'Ivoire supplied 60% (WCMC, 1991). About 11,988m^3 of iroko wood were exported from Gabon in 1987, 8,236.664m^3 in 1994 and 12,823.169m^3 in 1995 (IUCN, 1990). Others countries such as Cameroon and Congo, were also exporter of iroko wood during that period either legally or illegally (Marshall & Jenkins, 1994).

More recent studies have emphasized other properties of iroko tree which reinforced its socio-economic importance and also highly supported the needs of the species conservation on farmlands. Indeed, Onuorah (2000) revealed the ability of heartwood extracts of *Milicia excelsa* to suppress attacks by two rotter (the brown rotter, *Lenzites tabea* and the white rotter, *Polyporous versicolor*). In addition, Cailleau *et al.* (2004), have made up the ability of iroko tree to accumulate mineral carbon as calcium carbonate ($CaCO_3$) in ferralitic soils, where $CaCO_3$ is not expected to precipitate and suggested the species conservation for maintenance of this mineral carbon sink.

10.2- Fragmentation and iroko populations' isolation in Benin

The results of habitat fragmentation for tree species are basically the reduced population sizes and greater physical isolation of populations (Hall *et al.*, 1996). The fragmented populations of *Milicia excelsa* were relatively small in a range of some few iroko remnant trees conserved on farms, in sacred groves and in public places by farmers or traditional religions, and mostly distant from each other. In natural forest reserves, iroko trees became nowadays scarce and isolation could be more easily assessed because the adult iroko trees can be seen clearly from long distances because of their large size and distinctive crown shape. This isolation patterns were also pronounced because of the few or lack of natural regeneration. Although the factors which support the low regeneration rate of this species are yet to be clearly elucidated, fragmentation could be seen as a favourite factor of such a situation in terms of low effective population size and greater spatial isolation of individuals which could consequently create an episode of low flowering intensity producing fewer seeds and no evidence for pollen immigration into the populations. Indeed, it occurred that some trees failed to flower or produce not viable flowers which abort after the rain, certainly because male trees where too distant from female trees and the flowers failed to be pollinated. This phenomenon was observed in populations such as Ketou and Save where individual trees were separated by long distance. Hall *et al.* (1996) have experienced similar impact of fragmentation on *Pithecellobium elegans* and reported the lack of complete

flowering synchrony in *P. elegans* and periods of low flowering frequency in large population with low effective size and greater spatial isolation of individuals.

10.3- Morphological variation and structural characterization of iroko populations

Despite the effort of some farmers to maintain *Milicia excelsa* in agricultural landscape, ecological structure and demographic assessment in the species showed low population density and erratic ecological distribution throughout the species range. This is in accordance with the statement of Powell *et al.* (1989), Gijsbers *et al.* (1994), Harvey & Harber (1999) that the diversity and density of isolated trees in fragmented landscape are still slowly eroding through a combination of tree harvesting and natural death. The diameter class distribution showed lack of regeneration in almost all of the iroko populations with few individuals in the small size classes suggesting a danger of physical extinction of the species. Harvey and Harber (1999) have explained such a situation by the fact that many of the primary forest tree species in fragmented landscape and pastures, that are relicts of the original forest do not regenerate in open habitats under management systems and they are not replaced after they die or are harvested. Therefore, as many of these species occur at low densities like *Milicia excelsa*, the elimination or natural death of even a few trees can result in the local loss of that species from the landscape (Guevara *et al.*, 1998; Harvey and Harber, 1999; Harvey *et al*, 2004). Although a strong family genetic structure could be observed in morphological mutant of some species in extremely dense populations (e.g., number of individuals per area, Mosseler, 1992), low stand density due to intense harvesting may result in increased inbreeding as a result of increased self-pollination and self-fertilization (Rajora and Mosseler, 2001). Therefore controlling density can affect relative level of inbreeding and outcrossing depending on spatial genetic structure within populations and reproductive biology of the species.

Morphological variation in *Milicia excelsa* populations revealed patterns of adaptation of the species to environmental variation. This result made agreement with the statement of Hamann *et al.* (2005) that populations of species which occur over wide ecological amplitude are physiologically and morphologically differentiated. Other authors have found similar results in various plant species (Bruschi *et al.*, 2003 in *Quercus petraea;* Fox and Brand, 1993; Byrne *et al.*, 2003 in *Santalum spicatum;* Serrato *et al.*, 2004 in *Ficus*) which they explained by the great heterogeneity of climate, physiography, soils and the geological history of study areas. Morphological variation was so pronounced that it has induced taxonomical uncertainties in *Ficus* species identification (Serrato *et al.*, 2004). The morphological variation in *Milicia*

excelsa reflected adaptation of the species to environmental condition but also may have some genetic basis. For example, variation in growth rate among provenances from higher rainfall area and those from arid area in western Australian sandalwood (*Santalum spicatum,* Brand *et al.*, 1990), have been proofed to indicate some genetic influence on growth characteristics as genetic differentiation of two lineages has been observed in cpDNA (Byrne *et al.*, 2003).

10.4- Genetic variation in iroko populations

Genetic variation in *Milicia excelsa* revealed by RAPD markers was moderate in the contrary to what should be expected based on the bioecological features and mating system of the species. In addition, genetic differentiation among populations was high and supported by a low gene flow value among population. Furthermore, cluster analysis distinguished four groups of iroko populations which were confirmed by Barrier analysis through notification of three genetic barriers which separated populations into four groups. Although patterns of differentiation of morphological traits did not coincide exactly with genetic differentiation of groups, RAPD results suggested geographical structure in *Milicia excelsa* genetic variation and raised a suspicion that there may be a reduction of heterozigosity probably due to inbreeding occurring in the species or other factors such as Walhund effect, genetic drift and the maintained of ancestral polymorphism which could lead to the same results. Several studies that have assessed genetic variation and population structure of tropical tree species in fragmented ecosystems, have reported various impact of fragmentation on genetic variation and spatial genetic structure of tree populations (Loiselle *et al*., 1995; Doligez and Joly, 1997; Aldrich *et al.*, 1998; Dayanandan *et al*., 1999; White *et al.*, 1999; Lemes *et al*., 2003; Kelly *et al*., 2004).

Despite the fact that RAPD was successful in revealing variation in *Milicia excelsa*, it was less pretentious to assess inbreeding level and mutation model in populations before giving a decision than to conclude management status and conservation strategies of the species on the only RAPD results. Indeed RAPDs are dominant markers which have important limitations in the assessment of population structure. They increase the error due to rare paternal alleles hence underestimating the within-population genetic diversity (Hallden *et al.*, 1996; Hansen *et al.*, 1998). In contrast, the variability observed at microsatellite loci was shown to provide estimates of inbreeding, heterozygosity, gene flow and outcrossing rate, all of which are important measures for assessing the conservation and management status of tropical trees under intense human pressure (Lemes *et al*., 2003). Furthermore, off all the marker systems,

microsatellites are considered highly efficient markers for population genetic studies because they are widely abundant, highly polymorphic, are usually inherited in a codominant manner and are randomly dispersed in the genome (Tautz, 1989). Microsatellite analysis confirmed almost all of the results obtained from RAPD analysis and gave more accurate estimates of genetic diversity and population structure for improved conservation planning of *Milicia excelsa.* Indeed, all of the populations showed heterozygosity deficits in test of Hardy-Weinberg Equilibrium and significantly positive F_{IS} values suggesting high inbreeding occurring in the species. Low gene flow and moderate genetic differentiation was observed among population under both stepwise-mutation model (SMM) and infinite allele model (IAM). Spatial structure of genetic diversity indicated evidence of an effect of 'isolation by distance' (IBD).
This heterogeneity observed in *Milicia excelsa* morphological and genetic structure, owed its origin to one more plausible event which is historical habitat fragmentation reinforced by continuous human pressure.

10.5- Pathways for Milicia excelsa genetic resources conservation in Benin

10.5.1- *In situ* conservation

In situ conservation refers to the protection of biological resources in their native environments and within naturally established and evolving populations (Rajora and Mosseler, 2001). Based on the ecological structure and genetic variation found for *Milicia excelsa* in our results, we suggested *in situ* conservation for this species. Millar *et al.* (1990), Finkeldey and Gregorius (1994) have already come to the conclusion that this dynamic approach of conservation is sometimes preferable to the more static *ex situ* conservation because it maintains ecological functions and processes, and associated organisms required to maintain both the target species and its genetic resources, and allow for continued evolution. This is more real in a country such as Benin which was naturally less favored in terms of vegetation cover because of natural habitat fragmentation resulting in its location within Dahomey gap. Knowing that majority of human population in Benin still depend on natural resources exploitation to overcome their daily needs and consequently legal settlements are often transgressed without strict penalty, *Milicia excelsa* resources conservation could not be established only on the regulations of national forest policy. Therefore our suggestion of improving on traditional agroforestry systems and using them as biological corridors (Schroth *et al.*, 2004) for *Milicia excelsa* conservation in Benin seems to be the most relevant strategy of *in situ* conservation of this species given that iroko tree is already integrated to

farmlands by farmers (Ouinsavi *et al.*, 2005). The additional effort needed is the rehabilitation of the species by transplanting seedlings into some preferential habitats such as Pobe, Sakete, Aplahoue and Bassila semi deciduous forests which should have high conservation priority as well as other protected reserves in savannah regions. This strategy is in accordance with Rajora and Mosseler's (2001) statement that a network of protected areas is a basic, but not in itself a sufficient requirement for adequate *in situ* protection of genetic resources. According to these authors, a protected areas network should be ecologically representative of the forest types present on the landscape and should relate to or be connected with the metapopulation dynamics of the species and habitat to be conserved.

10.5.2- *Ex situ* conservation

Ex situ conservation may be necessary where *in situ* conservation cannot be practiced or will not be sufficient to ensure adequate protection for genetic resources. Although *ex situ* conservation is said to be more adapted for conservation of species that are in decline (Rajora and Mosseler, 2001), its required paramount efforts for establishing protection by artificial or contrived means (Wang *et al*, 1993) and might not be easily implemented in developing countries. Furthermore, *ex situ* methods are found to be more limited in the range of genetic diversity that can be preserved (El-Kassaby, 1992). However, it will be prudent to apply a combination of *in situ* conservation and *ex situ* approaches for *Milicia excelsa* genetic resources conservation with emphasis on maintaining viable, healthy and genetically diverse naturally regenerating population *in situ*. Several authors have suggested various *ex situ* conservation strategies including germplasm banks and common garden archives, seed banks, tissue and cell cultures and cryopreservation, DNA banks and plantation establishment (Pence, 1992; Wang *et al.*, 1993, Boucaud *et al.*, 1994; Ryynanen, 1996; Beardmore and Vong, 1998; Rajora and Mosseler, 2001), for trees of commercial importance as well as trees at risk and trees of only ecological importance. For instance, *Milicia excelsa* seed could be collected from different *plus* trees in the four genetically different populations groups inferred from this study, to keep seed bank in Benin. But this required additional research on the suitable conservation temperature since our results indicated that the species still loose its seeds viability when it is conserved at – 4 ºC. Further researches also need to address disease and pest resistance of the species (basically, its attacks by *Phytolyma lata*) for efficient iroko plantations establishment.

References

Abrams M.D., Kubiske M.E., Steiner K.C. (1990) Drought adaptations and responses in five genotypes of *Fraxinus pennisylvanica* Marsh: Photosynthesis, water relations and leaf morphology. *Tree Physiol*. **6**, 305-315.

Acharya K.P. (2006). Linking trees on farms with biodiversity conservation in subsistence farming systems in Nepal. *Biodiversity and Conservation* **15**: 631-646.

Adam, K. S. & Boko, M., (1993). Le Bénin. Flamboyant/Edicef, 93 p.

Adjanohoun E., Adjakidje V., Ahyi M.R.A., Ake Assi L., Akoegninou A., D'almeida J., Akpovo F., Chadare M., Cusset G., Dramane K., Eyemi J., Gassita J.N., Gbaguidi N., Goudote E., Guinko S., Houngnon P., Issa Lokeita A., Kinifo H.V., Kone-Bamba D., Musampa Nseya A., Saadou M., Sodogandji Th., De Souza S., Tchabi A., Zinsou Dossa C. & Zohoun Th. 1989- Contribution aux études ethnobotaniques et floristiques en République du Bénin. Médecine traditionnelle et pharmacopée. ACCT, 895 p.

Adomou C.A. (2005)- Vegetation patterns and environmental gradient in Benin: Implications for biogeography and conservation. PHD Thesis, WU, 136 p.

Aga E, Bekele E., Bryngelsson T. (2005) Inter-simple sequence repeat (ISSR) variation in forest coffee trees (*Coffea arabica* L.) populations from Ethiopia, *Genetica* **124**, 213-221.

Agbahungba G., Sokpon N., Gaoué O.G. (2001). Situation des ressources génétiques forestières du Bénin. Atelier sous-régional FAO/IPGRI/ICRAF sur la conservation, la gestion, l'utilisation durable et la mise en valeur des ressources génétiques forestières de la zone sahélienne (Ouagadougou, 22-24 sept. 1998). Note thématique sur les ressources génétiques forestières. Document FGR/12F. Département des forêts, FAO, Rome, Italie.

Agbahungba, A. & Sokpon, N., (1998). Rapport national sur les ressources phytogénétiques forestières du Bénin. 30 p.

Agbo, V. & Sokpon, N. (1998). Forêts sacrées et patrimoine vital au Bénin. Rapport technique final. CRDI, Bénin, 32 p.

Agyeman V.K. (1994) Envronmental influences on tropical tree seedling growth, PHD dissertation, Universty of Aberden, 229 p.

Akoegninou, A (1984) : Contribution à l'étude des îlots de forêts denses humides semi-décidues en République Populaire du Bénin. Thèse de 3ème cycle, Univ. De Bordeaux III, 250p.

Franklin A.B., Noon B.R., George T.L. (2002) What is habitat fragmentation? *Studies in Avian Biology*, **25**, 20-29.

Aldrich PR, Hamrick JL, Chavarriaga P, Kochert G (1998) Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree *Symphonia globulifera*. *Molecular Ecology*, **7**, 933-944.

Alvarez-Buylla ER, chaos A, Pinero D, Garay AA (1996) Demographic genetics of a pioneer tropical tree species patch dynamics, seed dispersal, and seed banks. *Evolution*, **50**, 1155-1166

Alvarez-Buylla ER, Garay AA (1994) Population genetic structure of *Cecropia obtusifolia*, a tropical pioneer tree species. *Evolution*, **48**, 436-453.

Andrén H (1994). Effect of habitat fragmentation on birds and mammals in landscape with different proportion of suitable habitat- a review. *Oikos* **71**, 355-366.

Archak S., Gaikwad A.B., Gautam D., Rao E.V.V.B., Swamy K.R.M., Karihaloo J.L. (2003) DNA fingerprinting of Indian cashew (*Anacardium occidentale* L.) varieties using RAPD and ISSR techniques. *Euphytica*, **230**, 397-404.

Atta-Krah K., Kindt R., Skilton J.N., Amaral W. (2004). Managing biological and genetic diversity in tropical agroforestry. *Agroforestry Systems* **61**: 183-194.

Aubréville, A., (1937). Les forêts du Dahomey et du Togo. 1-113. In: Bulletin du Comité d'Etudes Historiques et Scientifiques de l'AOF, Tome XIX.

Aubreville, A. (1959). La flore forestière de la Côte d'Ivoire. 48–55. In: Publication n° 15 CTFT, Nogent-Sur-Marne, France.

Ayana A., Bekele E. (1999) Multivariate analysis of morphological variation in sorghum (Sorghum bicolor (L.) Moench) germplasm from Ethiopia and Eritrea. *Genetic Resources and Crop Evolution* **46**, 273-284.

Ball, J.B. (2001). Global forest resources; history and dynamics. In: Evans, J. (ed.). The forests handbook Vol. I. Oxford, Blackwell Science. 418 p.

Bayala J, Teklehaimanot Z, Ouedraogo SJ (2002) Millet production under pruned tree crowns in a parkland system in Burkina Faso. *Agroforestry systems* **54**, 203-214.

Beardmore T., Vong W. (1998) Role of the cotyledonary tissue in improving low and ultralow temperature tolerance of butternut (Juglans cinerea) embryonic axes. *Canadian Journal of Forest Research* **28**, 1-8.

Beaulieu J., Perron M., Bousquet J. (2004) Multivariate patterns of adaptive genetic variation and seed source transfer in *Picea mariana*. *Canadian Journal of Forest Research* **34**, 531-545.

Beentje H.J. (1994). Kenya trees, shrubs and lianas. National Museums of Kenya, Nairobi, Kenya

Berg R.R., Hamrick J.L. (1994) Spatial and genetic structure of two sand hills oaks: *Quercus laevis* and *Quercus margaretta* (Fagaceae), *American Journal of Botany*, **81**, 7-14.

Besse P., Da Silva D., Bory S., Grisoni M., Le Bellec F., Duval M-F (2004) RAPD genetic diversity in cultivated vanilla : *Vanilla planifolia*, and relationship with *V. tahitensis* and *V. pompona*, *Plant Science* **167**, 379 – 385.

Bhat K.V., Jarret R.L. (1995) Ramdom amplified polymorphic DNA and genetic diversity in India *Musa* germplasm, *Genet. Res. Crop. Evol.* **42**, 107 - 118

Boffa J.M. (1999). Agroforestry parklands in sub-Saharan Africa. Rome: Food and Agriculture Organization of the United Nations.

Bonnin I, Colas B, Bacles C, Holl A-C, Hendoux F, Destiné B, Viard F (2002) Population structure of an endangered species living in contrasted habitats: Parnassia palustris (Saxifragaceae). *Molecular Ecology* **11**, 979-990.

Boot AH (1958) The Niger, the Volta and Dahomey gap as geographic barriers. *Evolution* **12** (1), 48-62.

Boshier, D.H. (2004). Agroforestry systems: Improvement components in conserving the genetic viability of native tropical tree species? In Schroth G., Fonseca G.A.B., Harvey C.A., Gascon C., Vasconcelos H.L., Izac A-M.N. 2004. Agroforestry and biodiversity conservation in Tropical landscapes. Island Press, Washington. 290-313

Bosu P.P., Cobbinah J.R., Wagner M.R. (2000) Feasibility of biological control of *Phytolyma lata* in Africa. Proceeding of the International Workshop on Research advances in restoration of iroko as a commercial species in West Africa, FORIG Kumasi 15-16 Novemver, 2000, 77-90p.

Boucaud M.T., Brison M., Negrier P., Boucaud M.T. (1994) Cryopreservation of walnut somatic embryos. *Cryo-letters* **15**, 151-160

Brand J.E., Ryan P.C., Williams M.R. (1999) Establishment and growth of sandalwood (*Santalum spicatum*) in South-Western Australia: The northampton pilot trial. *Aust. For*. **62**,33-37

Brassel K.E, Reif D. (1979) A procedure to generate Thiessen polygons, *Geogr. Anal.*, **325**, 31-36.

Bruna EM, Vasconcelos HL, Heredia S (2005) The effect of habitat fragmentation on communities of mutualists: Amazonian ants and their host plants. *Biological conservation* **124**, 209-216.

Bruschi P., Vendramin G.G., Bussotti F. and Grossoni P. (2003) Morphological and molecular diversity among Italian populations of *Quercus petraea* (Fagaceae). *Annals of Botany* **91**, 707-716.

Buckland S. T., Anderson D. R., Burnham K. P., and Laake J. L. **(**1993). Distance sampling: estimating abundance of biological populations. Chapman and Hall, London.

Byrne M., MacDonald B., Broadhurst L., Brand J. (2003) Regional genetic differentiation in Western Australian sandalwood (*Santalum spicatum*) as revealed by nuclear RFLP analysis. *Theoretical and Applied Genetics* **107**, 1208-1214.

Cailleau G., Braissant O., Verrechia E.P. (2004) Biomineralization in plants as a long-term carbon sink. *Naturwissenschaften* **91**, 191-194.

Cain ML, Milligan BG, Strand AE (2000) Long-distance dispersal in plant populations. *American Journal of Botany* **87**, 1217-1222.

Cain ML, Nathan R, Levin S (2003) Long-distance dispersal. *Ecology* **84**, 1943-1944.

Callen DF, Thompson AD, Shen Y (1993) Incidence and origin of null alleles in the $(AC)_n$ microsatellite markers. *American Journal of Human Genetics*, **52**, 922-927

Camoussi A., Ottaviano E., Calinski T., Kaczmarek Z. (1985) Genetic distances based on quantitative traits. *Genetics* **111**, 945-962.

Carter S.P., Proctor J, Slingsby D.R. (1987) Soil and vegetation of the Keen of Hamar serpentine, Shetland. *Journal of Ecology* **75**, 325-333.

Casas A., Caballero J., Valiente-Banuet A., Soriano J.A., Davila P. (1999) Morphological variation and the process of domestication of *Stenocerus stellatus* (Cactaceae) in Central Mexico. *American Journal of Botany* **86** (4), 522-533.

Caughley G (1994) Directions in conservation biology. *J. Anim. Ecol*., **63**, 215-244

Chalmers K.J., Waugh R., Sprent J.I., Simons A.J., Powell W. (1992) Detection of genetic variation between and within populations of Gliricidia sepium and G. maculate using RAPD markers, *Heredity* **69**, 465 – 472.

Changtragoon S., Szmidt A.E., Wang X-R (1995) The use of molecular markers in the study of genetic diversity in ratta: preliminary results. In Ayad, W. G., T. Hodgkin, A. Jaradat and V. R. Rao, editors. Molecular genetic techniques for plant genetic resources. Report of an IPGRI workshop, 9 – 11 October, 1995, Rome, Italy. International plant genetic Resources Institute, Rome, Italy, pp. 39 – 43.

Chase M, Kesseli R, Bawa K (1996) Microsatellite markers for population and conservation genetics of tropical trees. *American Journal of Botany*, **83**, 51-57.

Chase MR, Moller C, Kesseli R, Bawa KS (1996) Distant gene flow in tropical trees. *Nature*, **383**, 398-399.

Chevalier, A. (1933). Les bois sacrés des Noirs, sanctuaires de la nature. P 37. In: Comptes rendus de la Société de biogéographie, Paris.

Chevallier M-H (1999)- La diversité génétique des arbres forestiers. in Ouédraogo, A. S. et J-M. Boffa, éditeurs. 1999. vers une approche régionale des ressources génétiques forestières en Afrique sub-saharienne. Actes du premier atelier régional de formation sur la conservation et l'utilisation durable des ressources génétiques forestières en Afrique de l'Ouest, Afrique Centrale et Madagascar, 16-27 mars 1998, Centre National de semences Forestières, Ouagadougou, Burkina Faso. Institut International des ressources phytogénétiques, Rome, Italie. 124- 127pp.

Chung MG, Chung JM, Chung MY, Epperson BK (2000) Spatial genetic distribution of allozyme polymorphisms following clonal and sexual reproduction in populations of *Rhus javanica* (Anacardiaceae). *Heredity*, **84**, 178-185.

Chung MG, Chung JM, Epperson BK (1999) Spatial genetic structure of allozyme polymorphisms within populations of *Rhus trichocarpa* (Anacardiaceae). *Silvae Genetica*, **48**, 223-227.

Clark JS, Fastie C, Hurtt G, Jackson ST, Johnson C, King GA, Lewis M, Lynch J, Pacala S, Prentice IC, Schupp EW, Webb III T, Wickoff P (1998) Reid's paradox of rapid plant migration: dispersal theory and interpretation of paleoecological records. *BioScience* **48**, 13-24

Cobbinah J.R. (1988) The biology, seasonal activity and control of Phytolyma lata. IUFRO Regional Workshop on pest and Diseases of forest plantations, June 5-10, 1988, Bangkok, Thailand

Cobbinah JR, Wagner MR (1995) Phenotypic variation in *Milicia excelsa* to attack by *Phytolyma lata* (Psyllidae). *Forest Ecology and Management*, **75**(1-3), 147-153

Collevatti RG, Grattapaglia D, Hay JD (2001) Population genetic structure of the endangered tropical tree species *Caryocar brasiliense*, based on variability at microsatellite loci. *Molecular Ecology*, **10** 349-356.

Collingham YC, Huntley B (2000) Impact of habitat fragmentation and patch size upon mogrationn rates. *Ecological applications* **10**, 131-144.

Comincini S., Sironi M., Bandi C., Giunta C., Rubini M., Fontana F (1996) RAPD analysis of systematic relationship among the *Cervidae, Heredity* **76**, 215 – 221.

Corander J, Waldmann P, Marttinen P, Sillanpää MJ (2004) BAPS 2: enhanced possibilities fort he analysis of genetic population structure. *Bioinformatics* **20** (15) 2363-2369.

Corander J, Waldmann P, Sillanpää MJ (2003) Bayesian analysis of genetic differentiation between populations. *Genetics*, **163**, 367-374.

Coulibaly, S. (1978). Le paysan Sénoufo. Abidjan, NEA, cartes, photos. 245 p.

CTFT (1973). Iroko 1-13 In : Revue Bois et Forêts des tropiques, n°148, Nogent -sur - marne, France.

Das D.K., Chaturvedi O.P. (2005). Structure and function of *Populus deltoides* agroforestry systems in eastern India: 2. Nutrient dynamics. *Agroforestry Systems* **65**: 223-230.

Davis MB (1981) Quaternary history and the stability of forest communities. In: West, DC., Shugart, HH, Bitkin, DB (Eds.), Forest succession: Concepts and application. Springer-Verlag, New-Yorg, pp. 132-153.

Dayanandan S, Bawa KS, Kesseli R (1997) Conservation of microsatellites among tropical trees (Leguminosae). *American Journal of Botany*, **84**, 1658 - 1663.

Dayanandan S, Dole J, Bawa K, Kesseli R (1999) Population structure delineated with microsatellite markers in fragmented populations of a tropical tree, *Carapa guianensis* (Meliaceae). *Molecular Ecology*, **8**, 1585-1592.

Deshpande A.U., Apte G.S., R.A. Bahulikar, Lagu M.D., Kulkarni B.G., Suresh H.S., Singh N.P., Rao M.K.V., Gupta V.S., Pant A., Ranjekar P.K. (2001), Genetic diversity across natural populations of three montane plant species from the Western Ghats, India revealed by intersimple sequence repeats, *Molecular Ecology*, 10, 2397-2408.

Dewey S.E., Heywood J.S. (1988) Spatial genetic structure in a population of *Psychotria nervosa* I. Distribution of genotypes, *Evolution*, **42**, 834-838.

Di Rienzo AD, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994) Mutational processes of simple-sequence repeat loci in human populations. *Proceeding of the National Academy of Sciences USA*, 9**1**, 3166-3170.

Doat, J. (1977). Pouvoir calorifique des bois tropicaux. Bois et Forêts des Tropiques **172**:33-55.

Doligez A, Joly H (1997) Genetic diversity and spatial structure within a natural stand of a tropical forest tree species, *Carapa procera* (Meliaceae), in French Guiana, Heredity, 79 (1997) 72-82.

Dounias E. (1993). Dynamique et gestion différentielles du système de production à dominante agricole des Mvae du Sud-Cameroun forestier. Thèse de Doctorat, Université des Sciences et Techniques du Languedoc, Montpellier.

Dounias E. (1988). Contribution à l'étude ethnoécologique *et al*imentaire des Koma gimbe, Monts Alantika, Nord-Cameroun. Mémoire de Maîtrise, ISTOM. Le Havre.

Dupont LM, Weinelt M (1996) Vegetation history of the savanna corridor between the Guinean and the Congolean rain forest during the last 150,000 years. *Veget. Hist. Archaeobot*. **5**, 273-292.

Ehrlich P.; Ehrlich, A. (1981)- Extinction. Oxford University Press, Oxford

El Mousadik A and Petit RJ, (1996). High level of genetic differentiation for allelic richness among populations of the argan tree [Argania spinosa (L.) Skeels] endemic to Morocco. *Theoretical and Applied Genetics*. **92**, 832-839

El-Kassaby Y.A. (1992) Domestication and genetic diversity: should we be concerned? Forest Chronicles 68, 687-700.

Elsevier Scienctific Publishing Company, Amsterdam, Netherlands. pp. 289-300.

Estoup A, Garnery L, Solignac M, Cornuet JM (1995) Microsatellite variation in honey bee (apis mellifera l.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. *Genetics*, **145**, 679-695.

Excoffier L, Laval G, and Schneider S (2005) ARLEQUIN ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online, **1**, 47- 50.

Excoffier L, Smouse P, Quattro, J.M (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479-491.

Falush D, Stephane M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567-1587.

FAO (2000). Global forest resources assessment main report. FAO Forestry Paper 140. 479p.

FAO (2001). State of the world's forests. FAO, Rome. 200 p.

FAO (1999). Situation des forêts du monde. Rome, Italie, 154 p.

FAO (1999b). Base de Référence Mondiale pour les Ressources en Sols. Rapport sur les ressources en sols du monde n°84, Rome, Italie.

FAO (1989) Some medicinal forest plants of Africa and Latin America. FAO Paper 67 pp. 252.

Farooqui N., Ranade S.A., Sane P.V. (1998) RAPD profile variation amongst provenances of neem. *Biochemistry and Molecular Biology International*, **45** (5), 931 – 939.

Finkeldey R., Gregorius H.-F (1994) Genetic resources: selection criteria and design. In Z.-S Kim and H.H. Hattemer (eds.), Conservation and manipulation of genetic resources in forestry, pp 322-347. Kwang Moon Kag, Seoul.

Flather CH, Bevers M (2002) Patchy reaction-diffusion and population abundance: The relative importance of habitat amount and arrangement. *American Naturalist* **159** (1) 40-56.

Fluzin P., (1983). Notions élémentaires de sidérurgie : 13-44. In : Echard, ed., Métallurgies africaines-nouvelles contributions, Mémoires de la Société des Africanistes 9, Paris.

Fox J.E.D, Brand J.E. (1993) Preliminary observations on ecotypic variation in *Santalum spicatum* L. Phenotypic variation. *Mulga Res. J*. **11**, 1-12

Frankel OH, Soule ME (1981) Conservation and evolution. Cambridge University Press, Cambridge.

Gaafar A.M., Salih A.A., Luukkanen O., El Fadl M.A., Kaarakka V. (2006). Improving the traditional Acacia Senegal-crop system in Sudan: the effect of tree density on water use, gum production and crop yields. *Agroforestry Systems*. **66**: 1-11.

Gao L-Z (2004) Population structure and conservation genetics of wild rice *Oryza rufipogon* (Poaceae): a region-wide perspective from microsatellite variation. *Molecular Ecology*,**13**, 1009-1024.

Gao L-Z (2005) Microsatellite variation within and among populations of *Oryza officinalis* (Poaceae), an endangered wild rice from China. *Molecular Ecology*, **14**, 4287-4297.

Garcia-Barrios L., Ong C.K. (2004). Ecological interactions, management lessons and design tools in tropical agroforestry systems. *Agroforestry Systems* **61**: 221-236.

Garcia D., Quevedo M., Obeso R.J., Abajo A. (2005) fragmentation patterns and protection of montane forest in the Cantabrian range (N.W Spain). *Forest Ecology and Management* **208**, 29-43.

Geldenhuys C.J. (1992)- The use of diameter distributions in sustained-Use management of forests: Examples from Southern Africa. In: Piearce, G.D., Gumbo, D.J.(Eds.), The Ecology and management of Indigenous forests in Southern Africa. Proceedings of an International Symposium, Victoria Falls, Zimbabwe, 27-29 July 1992. Zimbabwe Forestry Commission and SAREC, Harare, pp. 154-167.

Gijsbers HJM, Kessler JJ, Knevel MK (1994) Dynamics and natural regeneration of woody species in farmed parklands in the Sahel region (Province of Passore, Burkina Faso). *Forest Ecology and Management* **64**: 1-13.

Gillies C.M.A., Abbott J.R. (1998) Evaluation of random amplified polymorphic DNA for species identification and phylogenetic analysis in *Stylosanthes (*Fabaceae*),Pl. Syst. Evol.* 211, 201 – 216.

Gilpin ME, Soulé ME (1996) Minimum viable populations: process of specie extinction. In: Soulé ME (ed.), Conservation Biology, the science of scarcity and diversity p 19-34. Sinauer Associate, Sunderlands, MA.

Goldsmith, F.B. (ed.) (1998). Tropical rain forest: a wider perspective. Chapman and Hall, London. 416 p.

Goodman SJ (1997) Rst Calc: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and a determining their significance. *Molecular Ecology* **6**, 881-885.

Goudet J (2002) FSTAT version. 2.9.3.2: Department of Ecology Biology Building, University of Lausanne, Switzerland, available at http://www2.unil.ch/popgen/softwares/fstat.htm.

Graham J., McNicol R.J. (1995) An examination of the ability of RAPD markers to determine the relationship within and between *Rubus* species, *Theor. Appl. Genet.* **90**, 1128 – 1132.

Grainger, A. (1993). Controlling tropical deforestation. Earthscan Publication Ltd, London. 310 p.

Guevara S, Laborde J, Sanchez G (1998) Are isolated remnant trees in pastures a fragmented canopy? *Selbyana* **19**: 34-43.

Guinko, S. (1985). Contribution à l'étude de la végétation et de la flore du Burkina Faso. Les reliques boisées ou bois sacrés. 29-36. In : Bois et Forêts des Tropiques, 208.

Hall p, Chase MR, Bawa KS (1994) Low genetic variation but high population differentiation in a common tropical forest tree species. *Conservation Biology*, **8**, 471-482.

Hall P, Walker S, Bawa KS (1996) Effects of forest fragmentation on diversity and mating system of a tropical tree *Pithecellobium elegans*. *Conservation Biology*, **10**, 757-768.

Hallden C, Hansen M, Nilsson NO, Hjerdin A, Sall T (1996) Competition as a source of errors in RAPD analysis. *Theoretical and Applied Genetics* **93**, 1185-1192.

Hamann A., Smets P., Yanchuk A.D., Aitken S. N. (2005) An ecogeographic framework for *in situ* conservation of forest trees in British Columbia. *Canadian Journal of Forest Research* **35**, 2553-2561.

Hammer Ø., Harper, D.A.T., and P. D. Ryan (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* **4** (1): 9pp. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Hamrick J.L., Godt M.J.W. (1989), Allozyme diversity in plant species. In: Plant population Genetic, Breeding and Genetic Resources (eds Brown AHD, Clegg MT, Kahler AL, Weir BS), Chapman and Hall, New York, 1989, pp. 281-304.

Hamrick J.L.; Godt M.J.W.; Murawski M.D.A.; Loveless M.D. (1992)- Factors influencing levels of genetic diversity in woody plant species. *New Forests*. **6**: 95-124.

Hamrick JL, Godt MJW (1996) Effects of life history on genetic diversity in plant species. *Phylosophical transactions of the Royal Society of London Series B*, **351**, 1291-1298.

Hamrick JL, Murawski DA, Nason JD (1993) Influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. *Vegetation*, **107-108**, 281-297

Hannah L, Midgley GF, Millar D (2002) Climate change integrated conservation strategies. *Global Ecology and Biogeography* **11**, 485-495.

Hansen M, Hallden C, Sall T (1998) Error rates and polymorphism frequencies for three RAPD protocols, *Plant Mol. Biol. Rep*. **16**, 139-146.

Hanski I, Gilpin ME (1997) Metapopulation Biology: Ecology, Genetics and Evolution. Academic press, San Diego, CA.

Hanski, I. (1999)- Metapopulation Ecology. Oxford University Press, Oxford

Harris L.D. (1984) The fragmented forest. University of Chicago press, Chicago.

Harris WV (1936) Notes on two injurious psyllids and their control. *E. Afr. Agr. J*, **1**. 498-500.

Harry M (2001) Génétique moléculaire et évolutive. Collection Sciences fondamentales, Maloine, 326 p.

Harvey CA, Harber WA (1999). Remnant trees and the conservation of biodiversity in Costa Rican pastures. *Agroforestry Systems* **44**: 37-68.

Harvey CA, Tucker NIJ, Estrada A (2004) Live fences, isolated trees, and windbreaks: Tools for conserving biodiversity in fragmented tropical landscapes. In Schroth G., Fonseca G.A.B., Harvey C.A., Gascon C., Vasconcelos H.L., Izac A. –M.N. (eds.). Agroforestry and biodiversity conservation in Tropical landscapes. Island Press, Washington, 261-289 p.

Hawthorne WD (1995) Ecological profiles of Ghanaian forest trees. Oxford Forestry Institute:Oxford. pp.345.

Hayley S.D., Miklas P.N., Stavely J.R., Byrum J., Kelly J.D. (1993) Identification of RAPD markers linked to a major rust resistance gene block in common bean, *Theor. Appl. Genet.* **86**, 505 – 512).

Hedrick PW (1999) Highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313-318.

Heuertz M, Hausman J-F, Hardy OJ, Vendramin GG, Frascaria-Lacoste N, Vekemans X (2004) Nuclear microsatellites reveal contrasting patterns of genetic structure

between western and southern European populations of the common ash (*Fraxinus excelsior* L.). *Evolution* **58** (5), 976-988.

Higgins SI, Nathan R, Cain ML (2003) Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal. *Ecology* **84**, 1945-1956.

Hubbell S.P. (2001). The unified neutral theory of biodiversity and biogeography. Monographs in population biology 32, Princeton University Press.

Huntley B, Birks HJB (1983) An atlas of past and present pollen maps for Europe: 0-13,000 B.P. Cambridge University Press Cambridge.

Huxel GR, Hastings A (1999) Habitat loss, fragmentation and restoration. *Restoration Ecology* **7** (3), 309-315.

ICRAF (2000). Paths to prosperity through agroforestry. ICRAF's corporate strategy, 2001-2010. Nairobi: International Center for Research in Agroforestry.

Igué, M. (2000). The Use of a Soil and Terrain Database for Land Evaluation Procedures –Case Study of Central Benin -. Thèse PhD. University of Hohenheim Stuttgart Germany, 235p. ISSN 0942-0754.

INSAE (1994). Deuxième recensement général de la population et de l'habitation. Synthèse des résultats, 2 p.

INSAE (1999). Projection de la population de l'ensemble du Bénin de 1997 à 2032.Cotonou, 137 p.

IPGRI (1999). Vers une approche régionale des ressources génétiques forestières en Afrique sub-saharienne. Rome, Italie, VI-1.

IUCN (2004) IUCN Red List of Threatened Species. www.redlist.org , downloaded on 19 april 2005.

Izac A-M.N., Sanchez P.A. (2001). Towards a natural resource management paradigm for international agriculture: the example of agroforestry research. *Agricultural Systems* **69**: 5-25.

Jain N., Shasany A.K., Sundaresan V., Rajkumar S., Darokar M.P., Bagchi G.D., Gupta A.K., Kumar S., Khanuja S.P.S. (2003) Molecular diversity in *Phyllanthus amarus* assessed through RAPD analysis, *Curr. Sci.* **85**, 1454 – 1458.

Jeandroz S., Collignon A.M., Favre J.M. (2004) RAPD and mtDNA variation autochthonous and planted populations of *Picea abies* from the Voges mountains (France) in reference to other French populations, *Forest Ecology and Management* **197**, 225 – 229

Juhe-Beaulaton, D. & Roussel, B. (1998). A propos de l'historicité des forêts sacrées de l'ancienne Côte des Esclaves. 353-373. In : Plantes et paysages d'Afrique. Une histoire à explorer, Karthala-CRA, Paris.

Juhe-Beaulaton, D. (1999). Arbres et bois sacrés de l'ancienne Côte des Esclaves. Des repères historiques 101-118. In : Histoire d'Afrique. Les enjeux de mémoire, Karthala, Paris.

Kearns CA, Inouye DW, Waser NM (1998) Endangered mutualisms: The conservation of plant-pollinator interaction. *Annual review of Ecology and Systematics*, **29**, 83-112.

Keay, R.W.J. (1989). Trees of Nigeria. Clarendon Press. Oxford. pp. 287-304.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends Eco. Evol.* **17**, 230-241.

Keller, I.; Nentwig, W.; Largiadèr, C.R. (2004)- Recent habitat fragmentation due to roads can lead to significant genetic differenciation in an abundant flightless ground beetle. *Molecular ecology* **13**: 2983-2994

Kelly BA, Hardy OJ, Bouvet J-M (2004) Temporal and spatial genetic structure in *Vitellaria paradoxa* (shea tree) in agroforestry system in southern Mali. *Molecular Ecology*, **13**, 1231-1240.

Kennedy JO (1933) The iroko gall-maker. *Emp. For. J*. **12**, 37-57

Khanuja P.S.S., Shasany K.A., Pawar A., Lal R.K., Darokar M.P., Naqvi A.A., Rajkumar S., Sndaresan V., Lal N., Kumar S. (2005) Essential oil constituent and RAPD markers to establish species relationship in *Cymbopogon Spreng*. (Poaceae), *Biochemical Systematics and Ecology* **33**, 171 – 186.

Khasa D.P., Dancick B.P. (1996) Rapid identification of whithe-Engelmann spruce by RAPD, *Theor. Appl. Genet* **92**, 46 – 52.

Khasa DP, Jaramillo-Correa JP, Barry J, Bousquet (2006) Contrasting microsatellite variation between subalpine and western larch, two closely-related species with different distribution patterns. *Molecular Ecology* (in revision)

Khasa DP, Newton CH, Rahman MH, Jaquish B, Dancick BP (2000) Isolation, characterization, and inheritance of microsatellite loci in alpine larch and western larch. *Genome* **43**, 439-448.

Kleinschmit J. (1993) Intraspecific variation of growth and adaptive traits in European oak species. *Annales des Sciences Forestières* **50** (1), 166-185.

Klingenberg C.P. (2002) Morphometrics and the role of the phenotype in studies of the evolution of developmental mechanisms. *Gene* **287**, 3-10.

Kokou K., Sokpon N. (2006) Les forêts sacrées du couloir du Bénin. *Bois et Forêts des Tropiques* **288** (2) 15-23.

Kubiske M.E., Abrams M.D. (1992) Photosynthesis, water relations and leaf morphology of xeric versus mesic *Quercus rubra* ecotypes in central

Pennisylnania in relation to moisture stress. *Canadian Journal of Forest Research* **22**, 1402-1407.

Kumar B.M., Nair P.K.R. (2004). The enigma of tropical homegardens. *Agroforestry Systems* **61**: 135-152.

Lande R (1988) Genetics and demography in biological conservation. *Science*, **241**, 1455-1459.

Le Herisse, A. (1911). L'Ancien royaume du Dahomey. Larose, Paris. 384 p.

Leakey RRB, Wilson J, Deans JD (1999) Domestication of trees for agroforestry in drylands. *Annales of Arid Zone* **38**, 195-220.

Leakey, R., Lewin, R. (1995)- The sixth extinction. Dubbleday, New York

Lee SL, Tani K, Ng KKS, Tsumura Y (2004) Isolation and characterization of 20 microsatellite loci for an important tropical tree *Shorea leprosula* (Dipterocarpaceae) and their applicability to *S. parviflora*. *Molecular Ecology Notes* **4,** 222-225

Lemes MR, Gribel R, Proctor J, Grattapaglia D. (2003). Population genetic structure of mahogany (*Swietenia macrophylla* King, Meliaceae) across the Brazilian Amazon, based on variation at microsatellite loci: implications for conservation. *Molecular Ecology*, **12**, 2875-2883.

Lengkeek A.G., Kindt R., van der Maesen, L.J.G., Simons A.J., van Oijen D.C.C. (2005). Tree density and germplasm source in agroforestry ecosystems in Meru, Mount Kenya. *Genetic Resources and Crop Evolution* **52**: 709-721.

Lewis PO, Zaykin D (2002) *Genetic Data Analysis: Computer Program for the Analysis of Allelic Data, Version 1.1 (d16c).* Free program distributed by the authors over the Internet from http://lewis.eeb.uconn.edu/lewishome/software.html.

Lewontin R.C. (1972) The apportionment of human diversity, *Evol. Biol.* **6**, 381-398.

Lienert J, Fisher M, Schneller J, Diemer M (2002) Isozyme variability of the wetland specialist Swertia perennis (Gentianaceae) in relation to habitat size, isolation and plant fitness. *Am. J. Bot.* **89**, 801-811.

Lindenmayer D.B, Franklin J.F. (2002) Conserving forest biodiversity: A comprehensive Multiscaled approach. Island Press, Washington.

Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany*, **82**, 1420-1425.

Loveless M.D. (1992)- Isosyme variation in tropical trees: patterns of genetic organization. *New Forests* **6**: 67-94

Loveless M.D., Hamrick J.L. (1984) Ecological determinants of genetic structure in plant populations, *Annual Review of Ecology and Systematics*, **15**, 65-95.

Lovett PN, Haq N (2000) Evidence for anthropic selection of the sheanut tree (*Vitellaria paradoxa*). *Agroforestry Systems*, **48**, 273-288.

Lowe A.J., Gillies A.C.M., Wilson J., Dawson I.K. (2000) Conservation genetic of bush mango from central/west Africa: implications from random amplified polymorphic DNA analysis, *Molecular Ecology* 9, 831 – 841.

Mamuris Z, Stamatis C., Triantaphyllidis C. (1999) Intraspecific genetic variation of striped red mullet (*Mullus surmuletus* L.) in the Mediterranean Sea assessed by allozyme and random amplified polymorphic DNA, *Heredity* **83**, 30 – 38.

Mandelson TC, Siegel AM, Shaw KJ (2004) Testing geographicl pathways of speciation in a recent island radiation. *Molecular Ecology* **13**, 3787-3796.

Manni F., and Guérard E. (2004) BARRIER vs. 2.2. Manual of the user. Population genetics team, Museum of Mankind (Musée de l'Homme), Paris 2004, [Publication distributed by the authors].

Manni F., Guérard E., Heyer E. (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by "Monmonier's algorithm", *Human Biology*, **76**(2), 173-190.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Research. **27**: 209–220.

Mapaure I. (2001)- Small-scale variation in species composition of miombo woodland in sengwa, Zimbabwe: the influence of edaphic factors, fire and elephant herbivory. *Syst. Geogr. Pl.* **71:** 935-947

Marchais, C. des (1726). Journal du voyage de Guinée et Cayenne fait en 1724, 1725 et 1726.Bibliothèque nationale, Manuscrit, B. N., Paris. p 76.

Mayr E, O'Hara RJ (1986) The biogeographic evidence supporting the Pleistocene forest refuge hypothesis. *Evolution* **40** (1), 55-67.

McDermott J.M., McDonald B.A. (1993) Gene flow in plant pathosystems, *Annu. Rev. Phytopathol*. **31**, 353-373.

McNeely J.A. (2004). Nature vs. nurture: managing relationships between forests, agroforestry and wild biodiversity. *Agroforestry Systems* **61**: 155-165.

McNeely J.A., Schroth G. (2006). Agroforestry and biodiversity conservation - traditional practices, present dynamics, and lessons for the future. *Biodiversity and Conservation* **15**: 549-554.

Mehrnia M., Zarre S., Sokhan-Sanj A. (2005) Intra- and inter-specific relationship within the Astragalus microcephalus complex (Fabaceae) using RAPD, Biochemistry Systematics and Ecology **33**, 149 – 158

Millar C.I., Ledig F.T., Riggs L.A. (1990) Conservation of diversity in forested ecosystems. *Forest Ecology and Management* **35**, 1-4

Miller R.P., Nair P.K.R. (2006). Indigenous agroforestry systems in Amazonia : from prehistory to today. *Agroforestry Systems* **66**: 151-164.

Monmonier M. (1973) Maximum-difference barriers: an alternative numerical regionalization method, *Geogr. Anal*., **3** 245-61.

Montambault R.J., Alavalapati J.R.R. (2005). Socioeconomic research in agroforestry : a decade in review. *Agroforestry Systems* **65**: 151-161.

Mosseler A. (1992) Life history and genetic diversity in red pine: implication for gene conservation in forestry. *Forest Chronicles* **68**, 701-708.

Murawski DA, Gunatilleke IAU, Bawa KS (1994) The effect of selective logging on inbreeding in *Shorea megistophylla* (Dipterocarpaceae) from Sri Lanka. *Conservation Biology*, **8**, 997-1002.

Myers, N. (1991). Conversion rates in tropical moist forests. In: Golley, F.B. (ed.). Tropical rainforest ecosystems, structure and function. Ecosystems of the World 14A.

Nason JD, Herre EA, Hamrick JL (1996) Paternity analysis of the breeding structure of strangler fog population: Evidence for substantial long-distance wasp dispersal. *Journal of biogeography*, **23**, 501-512.

Nei M. (1973) Analysis of gene diversity in subdivided populations, *Proc. Natl. Acad. Sci.* **70**, 3321-3323.

Nei M. (1978) estimation of average heterozygosity and genetic distance from a small number of individuals, *Genetics* **89** 583-590.

Nettel A, Rafii F, Dodd RS (2005) Characterization of microsatellite markers for the mangrove tree *Avicennia germinans* L. (Avicenniacea). *Molecular Ecology Notes* **5,** 103-105

Newton AC, Allnutt TR, Gillies ACM, Lowe AJ, Ennos RA (1999) Molecular phylogeography, intraspecific variation and the conservation of tree species. *Trends in Ecology and Evolution*, **14**, 140-145.

Nichols J.D.; Wagner M.R.; Agyeman V.K.; Cobbinah, J. R. (2000)- patterns of occurrence of *Milicia* species in natural forest and its relationship to *Phytolyma lata*. Research advances in restoration of iroko as a commercial species in West Africa. International Workshop Proceedings, 15-16 November, 2000.

Nichols JD, Agyeman VK, Agurgo FB, Cobbinah JR, Wagner MR (1999) Patterns of seedling survival in the tropical African tree *Milicia excelsa. Journal of Tropical Ecology*, **15**, 451-461.

Nichols, J. D., Agurgo, F. B., Agyeman, V. K., Wagner, M. R. & Cobbinah, J. R., (1998). Distribution and abundance of *Milicia* species in Ghana. Ghana J. of forestry , Vol .6 ,1998, Ghana: 1-7

Nyong'o RN, Cobbinah JR, Appiah-Kwarteng J (1994) Flowering and fruiting patterns in *Milicia excelsa* and *Milicia* regia welw. *Ghana Journal of Forestry* **1**, 19-29

Ofori D.A., Cobbinah J.R., Swaine M.D., Price A.H., Leifert C., Adu Bredu, S. (2002) Screening for genetic resistance to Phytolyma lata in Milicia species (Odum/Iroko) using DNA fingerprinting and phenotypic characters. Proceeding of the International Workshop on Research advances in restoration of iroko as a commercial species in West Africa, FORIG Kumasi 15-16 Novemver, 2000, 47-53.

Ofori D.A., Swaine M.D., Leifert C., Cobbinah J.R., Price A.H. (2001) Population genetic structure of *Milicia* species characterized by using RAPD and nucleotide sequencing, *Genetic Resources and Crop Evolution* 48, 637-647

Okullo JB, Hall JB, Masters E (2003) Reproductive biology and breeding system of Vitellaria paradoxa. P 66-84. In Teklehaimanot, Z (ed.), Improved management of agroforestry parkland systems in sub-Saharan Africa. EU/INCO project Contract IC18-CT98-0261, Final Report, University of Wales Bangor, UK.

Olatoye ST (1965) the effect of light, pre-chilling and gibberellic acid on the germination of Chlorophora *excelsa* (Welw.) and Chlorophora regia A. Chev. *Jour. Agric. Soc. Of Nigeria* **2**(2) 71-74.

Ouedraogo AS (1995) Parkia biglobosa (Leguminosae) en Afrique de l'Ouest: Biosystematique et amelioration. PhD thesis. Institute for Forestry and Nature Research, Wageningen, 205p.

Ouinsavi C, Sokpon N, Bada SO (2005) Utilization and traditional strategies of *in situ* conservation of iroko (*Milicia excelsa* welw. C.c. berg) in Benin. *Forest Ecology and Management* **207**, 341-350

Ouinsavi C., Sokpon N., Khasa DP. (2006). Nuclear microsatellites reveal patterns of population genetic structure of a threatened African species, *Milicia excelsa*, *Molecular Ecology (in press)*

Ouinsavi, C. (2000). Contribution à la conservation *in situ* du *Khaya senegalensis* (Desr.) A. Juss : Importance socio-économique ; structure et dynamique des peuplements naturels ; productivité des plantations d'essai au Bénin. Thèse d'ingénieur FSA/UNB, 120 p.

Paradis G. (1975)Physionomie, composition floristique et dynamisme des formations végétales d'une partie de la basse vallée de l'Ouémé (Dahomey). *Ann. Univ. Abidjan*, sér.E, **8**, 281-315.

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37-42

Pazzi R. (1979). Eléments de cosmologie et d'anthropologie Evé, Adja, Gen, Fon. n° spécial. Annales de l'Université du Bénin, Togo, 41-55.

Pearson RG, Dawson TP (2005) Long-distance plant dispersal and habitat fragmentation: identifying conservation targets for spatial landscape planning under climate change. *Biological Conservation* **123**, 389-401

Pence V.C. (1992) Dessication and survival of *Aesculus*, *Castanea*, and *Quercus* embryo axes through cryopreservation. *Cryobiology* 29, 391-399.

Peres A.C., Baider C. (1997) Seed dispersal, spatial distribution and population structure of brazilnut trees (*Bertholletia excelsa*) in Southeastern Amazonia. *Journal of Tropical Ecology*, **13** (4), 595-616.

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conservation Biology*, **12**, 844-855.

Peyre A., Guidal A., Wiersum K.F., Bongers F. (2006). Dynamics of homegarden structure and function in Kerala, India. *Agroforestry Systems* **66**: 101-115.

Pimm SL, Gittleman JL, McCracken GF, Gilpin F (1989) Plausible alternatives to bottlenecks to explain reduced genetic diversity. *Trends Ecol. Evol*., **4**, 176-177.

Pimm SL, Jones HL, Diamond J (1988) On the risk of extinction. *Am. Nat*., **132**, 757-785.

Pimm SL, Russell GJ, Gittleman JL, Brooks TM (1995) The future of biodiversity. *Science* (Washigton DC) **269**, 347-350.

Pimm, S. L.; Rusell, G. J.; Gittleman, J. L.; Brooks, T. M. (1998)- The future of biodiversity. *Adv. Complex Sys*. **1**, 203

Pliya, J. (1976). L'arbre fétiche. 3è edition, Presbook Victoria, Cameroon. 90 p.

Powell G, Rappole JH, Sader JA (1989). Neotropical migrant landbirds use of lowland Atlantic habitats in Costa Rica: a test of remote sensing for identification of habitat. P. 287-298 in M Hagan, DW Johnston (eds.), *Ecology and Conservation of neotropical migrant landbirds*. Washington, DC: Smithsonian Institution Press.

Pritchard JK, and Wen W (2003) Documentation for structure software, version 2.1. Department of Human Genetics, University of Chicago. Available at http://pritch.bsd.uchicago.edu/software/structure2_1.html)

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945-959.

Quiroga M.P.; Premoli A.C.; Ezcurra C. (2002)- Morphological and isozyme variation in *Cerastium arvense* (Caryophyllaceae) in the southern Andes. *Can.J.Bot*. **80** 786-795.

Rajora O.P., Mosseler A. (2001) Challenges and opportunities for conservation of forest genetic resources. *Euphytica* **118**, 197-212.

Rapport DJ, Regier HA, Hutchinson TC (1985) Ecosystem behaviour under stress. *American Naturalist* **125**, 617-640.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*. **43**, 223-225

Rousset F (1996) Equilibrium Values of Measures of Population Subdivision For Stepwise Mutation Processes. *Genetics* **142**, 1357-1362.

Rozen S, Skaletsky H J (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365-386. Humana Press, Totowa, NJ.

Ryynanen L. (1996) Survival and regeneration of dormant silver birch buds stored at super-low temperature. *Canadian Journal of Forest Research* **26**, 617-623.

Sala OE, Chapin III FS, Armesto JJ, Berlow E, Bloomedfiled J, Dirzo R, Huber-Sandwald E, Huenneke LF, Jackson RB, Kinzizig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. *Science* **287**, 1770-1774.

Salzmann U, Hoelzmann P (2005) The Dahomey Gap: an abrupt climatically induced rain forest fragmentation in WEST Africa during the late Holocene. *The Holocene* **15** (2), 190-199.

Santos T., Telleria J.L., Virgos E. (1999) Dispersal of Spanish juniper Juniperus thurifera by birds and mammals in a fragmented landscape. *Ecography* **22**, 193-203.

Santos T., Telleria J.L., Carbonell R. (2002) Birds conservation in fragemented Mediterranean forests of Spain: Effects of geographical location, habitat and landscape degradation. *Biol. Conserv.* **105**, 113-125.

Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation: a review. Conservation biology.

Schellas J, Greenberg R (1996) Forest patches in tropical landscape. Island press, Washington, DC, 426 p.

Schnell R.J., Goenaga R., Olano C.T. (1999), Genetic similarities among cocoyam cultivars based on randomly amplified polymorphic DAN (RAPD) analysis, *Scientia Horticulturae* **80** (3-4), 267 – 276.

Schroth G., Fonseca G.A.B., Harvey C.A., Gascon C., Vasconcelos H.L., Izac A. –M.N. (2004). Agroforestry and biodiversity conservation in Tropical landscapes. Island Press, Washington, 523 p.

Seignobos, C. (1997). *Antiaris africana*, arbre relictuel de l'extrémité septentrionale des monts Mandara. 327-332. In : L'homme et le milieu végétal dans le bassin du

Lac Tchad. Séminaire du Réseau Méga-Tchad à Sèvres (France), ORSTOM, Paris.

Serrato A., Ibarra-Manriquez G., Oyama K. (2004) Biogeographie and conservation of the genus Ficus (Moraceae) in Mexico. *Journal of Biogeography* **31**, 475-485.

Service des Eaux, Forêts et Chasses (1959). Rapport annuel. 45 p.

Service des Eaux, Forêts et Chasses (1960). Rapport annuel. 39 p.

Service des Eaux, Forêts et Chasses (1965). Rapport annuel. 46 p.

Shannon C.E., Weaver W. (1949). The mathematical theory of communication. University of Illinois Press, Urbana, Illinois, USA.

Sharma A., Ashwath N. (2006). Land disposal of municipal effluents: importance of choosing agroforestry systems. *Desalination* **187**: 361-374.

Simons A.J., Leakey R.R.B. (2004). Tree domestication in tropical agroforestry. *Agroforestry Systems* **61**: 167-181.

Skepner A, Krane D.E (1998) RAPD reveals genetic similarity of *Acer saccharum* and *Acer nigrum*, *Heredity* 80, 422 – 428.

Slatkin M (1987). Gene flow and the geographic structure of natural populations. *Science*, **236**, 787-792.

Slatkin M., Barton N.H. (1989) A comparison of three indirect methods for estimating average levels of gene flow, *Evolution* **43,** 1349-1368.

Slocum M.G., Horvitz C.C. (2000). Seed arrival under different genera of trees in a neotropical pasture. *Plant Ecology* **149**: 51-62

Smith N.J.H. (1996). Home gardens as a springboard for agroforestry development in Amazonia. *Int. Tree Crops J.* **9**: 11-30.

Sokpon N. (1995) : Recherches écologiques sur la forêt dense semi-décidue de Pobè au Sud-Est du Bénin : Groupements végétaux, structure, régénération naturelle et chute de litière. Thèse de Doctorat, ULB, 350p.

Sokpon N. And Biaou S.H. (2002)- The use of diameter distribution in sustained-use management of remnant forests in Benin: case of Bassila forest reserve in North Benin. *Forest Ecology and Management* **161 :** 13-25

Sokpon N., Agbo, V. (1999). Sacred groves as tools for indigenous forest management in Benin. *Annales des Sciences Agronomiques du Bénin* **2** : 161-175.

Sokpon N., Ago E.E. (2001) Sacralisation et niveau de maturation des forêts denses semi-décidues du plateau Adja au Sud-Ouest du Bénin. *J. Rech. Sci. Univ. Lomé* (Togo) **5** (2), 319-331

Sokpon N., Ametepe A., Agbo V. (1998) Forêts sacrées et conservation de la biodiversité au Bénin. Cas du plateau Adja au Sud-Ouest du Bénin. *Annales des Sciences Agronomiques du Bénin* **1** : 47-64

Sokpon N., Lejoly J. (1996). Les plantes à fruits comestibles d'une forêt semi-caducifoliée: Pobè, au sud-est du Bénin. In: C.M. Hladik, A. Hladik, H. Pagezy, O.F. Linares and A. Froment (eds), L'alimentation en forêt tropicale: interactions bioculturelles et perspectives de développement. *Volume I*: Les ressources alimentaires: production et consommation, Paris, UNESCO, 115-124.

Sokpon N., Ouinsavi C. (2004): Gestion des plantation de *Khaya senegalensis* au Bénin. *Bois et Forêts des Tropiques* **279** (1): 37-46.

Sokpon, N. & Lejoly, J. (1994). Le groupement à *Triplochiton scleroxylon* et *Strombosia glaucescens* de la forêt naturelle semi-décidue de Pobè (Sud-Est, Bénin). In : J.H. SEYANI & A.C. CHIKUNI. Proc. XIIIth Plenary Meeting AETFAT, Malawi, 2 : 1453-1470.

Sokpon, N. (1994). Tenure Foncière et Propriété des Ligneux dans les Systèmes Agroforestiers traditionnels au Bénin. *Ann. Fac. Sc., Kisangani*, n° spéc., 115 – 122.

Solé RV, Alonso D, Saldana J (2004) Habitat fragmentation and biodiversity collapse in neutral communities. *Ecological complexity* **1**, 65-75.

Stacy EA, Dayanandan S, Dancik BP, Khasa DP (2001) Microsatellite DNA markers for the Sri Lankan rainforest tree species, *Shorea cordifolia* Dipterocarpaceae), and cross-species amplification in *S. megistophylla. Molecular Ecology Notes* **1**, 53-54.

Storfer A (1999) Gene flow and endangered species translocations: a topic revisited. *Biological Conservation*, **87**, 173-180

Streiff R, Labbe T, Bacilieri R, Steinkellner H, Glössl H, Kremer A (1998) Within-population genetic structure in *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. assessed with isozymes and microsatellites. *Molecular Ecology*, **7**, 317-328.

Sumner J, Jessop T, Paetkau D, Moritz C (2004) Limited effect of anthropologenic habitat fragmentation on molecular diversity in a rain forest skink, *Gnypetoscincus queenslandiae*. *Molecular Ecology* **13**, 259-269.

Swaine MD, Agyeman VK, Kyereh B, Orgle TK, Thompson J, Veenendaal EM (1997). Ecology of forest trees in Ghana. *ODA Forestry series No. 7*. London, 76 p.

Tautz D. (1989) Hypervariability of simple sequence as ageneral source for polymorphic DNA markers. *Nucleic acids research* **17**: 6463-6471

Taylor D.A.R., Kankam B.O., Wagner M.R. (2000), The role of the fruit bat, *Eidolon helvum*, in seed dispersal, survival, and germination in *Milicia excelsa*, a threatened west African hardwood. International Workshop Proceedings, 15-16 November, 2000, pp.29-39

Taylor, J. C., (1960) Synecology silviculture in Ghana. Thomas Nelson and Sons Ltd, 418 p.

Tchir TL, Johnson EA, Miyanishi K (2004) A model of fragmentation in the Canadian boreal forest. *Canadian Jouranal of Forest Research* **34**, 2248-2262.

Teklehaimanot Z., Lanek J., Tomlinson H.F. (1998) Provenance variation in morphology and leaflet anatomy of *Parkia biglobosa* and its relation to drought tolerance. *Trees* **13**, 96-102

Teklehaimanot, Z. (2004) Exploiting the potential of indigenous agroforestry trees: *Parkia biglobosa* and *Vitellaria paradoxa* in sub-Saharan Africa. *Agroforestry Systems* **61**: 207-220.

Ter Braak C.J.F. (1988)- CANOCO- an extension of DECORANA to analyze species-environment relationships. *Vegetation* **75**: 159-160

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, Ferreira de Siquira M, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Peterson AT, Phillips OL, Williams SE (2004) Extinction risk from climate change. *Nature* **427**, 145-148.

Thomas D.W. (1982)- The ecology of an African savanna fruit bat community: Resource partitioning and role in seed dispersal. PHD Thesis. University of Aberdeen, Scotland. 205 pp.

Thomson JL, Weller C, Severtsen B (2003) Cascade Crest Forests; Forest loss, habitat fragmentation and wildness. Landscape analysis of the central cascade in Washington state. The Wildness Society. 40p.

Thormann C.E., Ferreira M.E., Camargo M.E.A., Tivang J.G., Osborn T.C. (1994) Comparaison of RFLP and RAPD markers to estimating genetic relationship within and among crucuferous species, *Theor. Appl. Genet.* 88, 973 – 980.

Tilman D, May RM, Lehman CL, Novak MA (1994) Habitat destruction and extinction debt. *Nature* **371**, 65-66.

Tomlinson H, Teklehaimanot, Z, Traore A, Olapade EO (1995) Soil amelioration and root symbiosis of *Parkia biglobosa* (Jacq.) Benth. in West Africa. *Agroforestry Systems* **30**, 145-159.

Totin, A., (1987) Les multiples manifestations de la sorcellerie 1-15 In: Implication des guérisseurs traditionnels et des sages du village dans la formation de la jeunesse paysanne. Rapport de séminaire, INFOSEC, Bénin.

Ueno S, Tomaru N, Yoshimaru H, Manabe T, Yamamoto S (2002) Size class differences in genetic structure and individual distribution of *Camellia japonica* L. in a Japanese old-growth ever green forest. *Heredity*, **89**, 120-126.

Van de Ven WTG, McNicol RJ (1996) Microsatellites as DNA markers in Sitka spruce. *Theoretical and Applied Genetics*, **88**, 1-6.

Vanijajiva, O P. Sirirugsa, W. Suvachittanont, Confirmation of relationships among *Boesenbergia* (Zingiberaceae) and related genera by RAPD, Biochemical Systematics and Ecology 33 (2005) 159 - 170.

Voronoï M.G. (1908) Nouvelles applications des paramètres continus à la théorie des formes quadratiques, deuxième mémoire, recherche sur les parallélоedres primitifs, *Journal Reine Angew. Math.*, **134**, 198-207.

Vroom M. (2000) Evaluation of the iroko project in Côte d'Ivoire. Proceeding of the International Workshop on Research advances in restoration of iroko as a commercial species in West Africa, FORIG Kumasi 15-16 Novemver, 2000, 119-125p.

Wagner MR, Atuahene SKN, Cobbinah JR (1991) Forest entomology in west tropical Africa: Forest insect of Ghana. Kluwer Academic Publishers, Dordrecht, 210 pp.

Wagner, M. R., Atuahene, S. K. N. & Cobbinah, J. R. (1991). Forest entomology in west tropical Africa: forest insects of Ghana-Kluwer Academic Publishers, Dordrecht, 210 p.

Wang Y, Williams DA, Graines MS (2005) Evidence for a recent genetic bottleneck in the endangered Florida Keys silver rice rat (*Oryzomys argentatus*) revealed by microsatellite DNA analysis. *Conservation Genetics* **6**, 575-585.

Waser NM (1987) Spatial genetic heterogeneity in a population of the mountain perennial plant *Delphinium nelsonii. Heredity*, **58**, 249-256.

WCMC (1991) Provision of Data on Rare and Threatened Tropical Timber Species. 58p

Weir BS and Cockerham CC, (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**,1358-1370.

Weir BS (1996). Genetic data analysis II. Sinauer Publ., Sunderland, MA.

Weller, U. (2002). Land Evaluation and Land Use Planning for Southern Benin (West Africa). Ph. D. thesis, Helf 67. Hohenheimer Bodenkundliche Hefte 163p. Stuttgart Allemagne.

Whitcomb R.F., Robbins C.S., Lynch J.F., Whitcomb B.L., Klimkiewicz M.K., Bystrack D. (1981) Effects of forest fragmentation on avifauna of eastern deciduous forests. In: Burguess R.L., Sharpe D.M. (Eds), Forest Island dynamics in man-diminated epringer, New York, 125-205 pp.

White GM, Boshier DH, Powell W (1999) Genetic variation within a fragmented population of *Switietenia humilis* Zucc. *Molecular Ecology*, **11**, 1899-1910.

White MC (1968) Reaserch in Nigeria on the iroko gall bug (*Phytolyma* sp.). *Nigeria Forest Information Bulletin*. 47 pp.

White MG (1966) The problem of the Phytolyma gall bug in the establishment of Chlorophora. *Inst. Pap. Comm. For. Inst.* **37**, Oxford, London. 52 pp.

Wiegand T, Revilla E, Moloney KA (2005) Effect of habitat loss and fragmentation on population dynamics. *Conservation Biology* **19** (1), 108-121.

Wilcove D.S., McLellan C.H., Dobson A.P. (1986) Habitat fragmentation in the temperate zone. In Soulé M.E. (Ed), Conservation Biology: the Science of scarcity and diversity, the regents of the University of Michigan, Sinuauer Associates inc., Sunderland, Massachusetts, 237-256 pp.

Wilson PJ, Provan J (2003) Effect of habitat fragmentation on levels and patterns of genetic diversity in natural populations of the peat moss Polytrichum commune. *Proc. R. Soc. Lond* B **270**, 881-886.

Wyatt R (1992) Conservation of rare and endangered bryophyte: input from population genetics. *Biol. Conserv.* **59**, 99-107.

Yeh F.C, Yang R.C., Boyle T. (1997) POPGENE, version 1.32 ed. Software Microsofte Window-Based freeware for Population Genetic Analysis, University of Alberta, Edmonton, Alta, 1997

Yeh F.C., Chong D.K.X., Yang R.C. (1995) RAPD variation within and among natural populations of Trembling Aspen (*Populus tremuloides* Michx.) from Alberta. *Heredity* **86**, 454 – 460.

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution*, **11**, 413-418.

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. *Trends Ecol. Evol.* **11**, 413-418.

Young AC, Clark GM (2000) Genetics, demography and viability of fragmented population. Cambridge University Press, Cambridge.

Yu K., Pauls K.P. (1992) Optimisation of the PCR program for RAPD analysis, *Nucl. Acids Res.* **20**, 2606.

Zeitoun V (2000) Adéquation entre changements environnementaux et spéciations humaines au Plio-Pléistocène. *C.R. Acad. Sci. Paris, Sciences de la Terre et des planètes/ Earth and Planetary Sciences* **330**, 161-166.

Acknowledgements

I sincerely wish to express my special and heartfelt gratitude to my promotor, Prof Dr. Ir. Nestor Sokpon, Deputy Vice Chancelor of Scientific Research at University of Parakou. He directed me all along my university studies from undergraduate up to now. I owe him this enjoyable endhaviour of this programme.

I'm extremely grateful to Dr. Ofori A. Daniel, and the entire staff of Forestry Research Institute of Ghana (FORIG), especially the Director (Dr. J. R. Cobbinah) for providing me with laboratory facilities. I especially thank Dr. Ofori for his open mind, his helpful contribution and advice in Molecular Biology.

I'm also grateful to Prof. Damase Khasa, CEF, Laval University, Québec Canada for hosting part of the laboratory works. I sincerely thank him for his supportive kindness and guidance.

I would like to express my gratitude to Prof. Dr. Ir. Ahanchede Adam, Prof. Zoundjihekpon Jeanne, Dr. Ganglo Jean and Prof. Dr. Ir. Sinsin Brice for their critical comments and suggestions for the improvement of this thesis.

My special thank to André Gagné and Sauphie Senneville (CEF-Laval, Université Laval, QC) Dr Jean-Luc Jany (Department of Plant Science, University Cornell) for their technical assistance during the laboratory work.

This research was financially supported by the Agence Universitaire de la Francophonie (AUF) and Competitive funds of the Système National de la recherche Agricole (S.N.R.A – Benin)

I remain grateful to my colleagues in Laboratoire d'Etudes et de Recherches Forestières (L.E.R.F) and at the Faculty of agronomy University of Parakou, Ir. Biaou Honore and Ir. Latifou Idrissou, for their companionship.

My sincere gratitude to all of the staff of the Faculty of Agronomic sciences, University of Abomey-Calavi

Finally my special thanks to my lovely parents for their never-ending support, my sisters and brothers with particular thanks to Michel Sezonlin for his special assistance.

Curriculum vitae

Christine OUINSAVI was born on 24th July 1975 at Beterou, Parakou, Republic of Benin. She graduated for Bacalaureat D in 1994 in Benin and joined the Faculty of Agronomic Sciences, National University of Benin in October 1995. She obtained her BSc. in General Agronomy in July, 1999 and graduated for the degree of Agronomic Ingeneer (Option Natural Resources Management) in December, 2000. She started working in the Sylviculture and Forest Ecology Unit of the Laboratory of Applied Ecology (FSA/UNB) from January 2001 as Research Assistant. In July 2003 she graduated for MSc. degree in Silviculture and Forest Biology at University of Ibadan and registered for Ph.D programme at faculty of Agronomy, University of Abomey-calavi in October 2003. She was granted a fellowship in 2004 by Agence Universitaire de la Francophonie (AUF) to carry out part of her Ph.D programme at Centre d'Etude de la Forêt-Laval at Laval University, Québec, Canada. The same year, she was positionned at the Faculty of Agronomy, University of Parakou as Teaching and Research Assistant.

Her addresses in Benin are:

Postal: Faculty of Agronomy, University of Parakou, BP 123 Parakou, Benin

Email: ouinsch@yahoo.fr

List of publications

Ouinsavi C, Sokpon N, Bousquet J, Craig N, Khasa DP (2006) Novel microsatellite DNA markers for the threatened African endemic tree species, *Milicia excelsa* (Moraceae), and cross-species amplification in *Milicia regia*. *Molecular Ecology Notes* **6**, 480-483

Sokpon, N.; Biaou, S.H.; Ouinsavi, C; Hunhyet, O. (2006) - Gestion durable des forêts claires du Nord Bénin. *Bois et Forêts des Tropiques* **287** (1), 45-57

Ouinsavi C, Sokpon N, & Bada OS (2005) Utilization and traditional strategies of in situ conservation of iroko (*Milicia excelsa* Welw. C.C. Berg.) in Benin. *Forest Ecology and Management*, **207**, 341-350.

Sokpon, N. & Ouinsavi, C. 2004 - Gestion des plantations de *Khaya senegalensis* au Bénin. *Bois et Forêt des Tropiques*, **279** (1) pp : 37-46.

Sokpon, N. & Ouinsavi, C. (2002)- Utilisation de *Khaya senegalensis* en médecine traditionnelle au Bénin. *Rev. Méd. Pharm. Afric*. vol. **2**, 9-19

Ouinsavi C, Sokpon N, Khasa DP: Nuclear microsatellites reveal patterns of populations genetic structure of a threatened African species *Milicia excelsa*. *Molecular Ecology* (*submitted*)

Ouinsavi C, Sokpon, N.: Traditional agroforestry systems as tools for *Milicia excelsa* Welw. C.C Berg genetic resources conservation in Benin. *Agroforestry Systems* (*in press*)

Ouinsavi C, Sokpon, N.: Spatial structure of genetic variation of *Milicia excelsa* populations in Benin revealed by Random Amplified Polymorphic DNA markers. *Plant Sciences* (*submitted*)