Characterization of African Bush Mango trees with emphasis on the differences between sweet and bitter trees in the Dahomey Gap (West Africa)

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Contents

CHAPTER 1

General Introduction

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Background

Biological diversity plays a key role (nutritional, medical, socio-cultural, ecological, etc.) in the sustainability of human life and is therefore the most valuable resource on earth (SCBD, 2000; Jose, 2012). It includes the variability of organisms as well as the ecological systems that provide the environmental conditions necessary for their continuous regeneration (Probst and Crow, 1991; Swingland, 2001). Moreover, genetic variations as well as morphotypes resulting from the interaction with the environment are an integral part of biological diversity (Probst and Crow, 1991; SCBD, 2000; Sarkar, 2002). Since the 1992 Rio de Janeiro Earth Summit, the recognition of the fact that the knowledge present within human communities related to the management and use of biological resources is inextricably connected to biological diversity conservation has significantly increased (Swiderska, 2006).

Plants are a vital component of biological diversity and are central to human life, because they influence ecosystem resilience, soil stability and fertility as well as the agricultural systems on which human life depends (IPGRI, 1999; Eviner and Chapin, 2001). Among the large number of plant species valuable in human diets and other types of use, comparatively few contribute to the current exploitable agrobiodiversity (IPGRI, 1999). Agrobiodiversity includes the domesticated and cultivated plant species, and is defined by the Convention on Biological Diversity (http://www.cbd.int) as those plant species being manipulated during their evolutionary process in order to develop particular phenotypes that meet human needs. Among the limited set of domesticated / cultivated plant species, a comparatively low percentage have received adequate scientific attention with the intention to conserve their variability (Wood and Lenné, 1997). Many other valuable plants species are being collected from natural vegetations and integrated into the agricultural productive space by local communities. The vast majority of the useful plant species from a variety of ecosystems are exploited only locally and are known to the scientific community under various names among which "plant non timber forest products (NTFPs)" remains the most commonly used one.

Plant NTFPs in sub-Saharan Africa

Plant NTFPs refer to the products (apart from timber) collected from wild plant taxa occurring in natural forests (SCBD, 2001; van Andel, 2006). They can also be harvested from wild species preserved by local farmers on farms in various traditional agroforestry systems (Tabuna, 2000). The evaluation of the diversity of tropical plant species exploited as NTFPs already started during colonial times (see Reede tot Drakestein, 1678-1693; Rumphius, 1741-1750) and shows the very old symbiotic relationship between local communities and the environment (Irvine, 1952; Malaisse and Parent, 1985; Bergeret, 1986; Herzog, 1992; van Andel, 2000). This evaluation as well as the geographical areas covered are growing since the 1992 Rio de Janeiro Earth Summit which emphasizes the importance of the knowledge of local communities for the potential of biological resources (Hladik *et al*., 1996; Malaisse, 1997). The PROSEA and PROTA mega-programmes (http://proseanet.org/prosea/foundation.php, http://www.prota.org) are exemplary for such large-scale evaluations of botanical diversity resources in South-East Asia and Tropical Africa, respectively.

Because plant NTFPs are increasingly being used, in-depth studies on different aspects of their use, production and resilience in natural ecosystems and their economic importance at local, regional, national, and international levels have become a priority research topic over the last few decades (Ruiz Pérez and Arnold, 1995; Arnold and Ruiz Pérez, 2001; SCBD, 2001; Rai and Uhl, 2004; Ticktin, 2004; Adepoju and Salau, 2007). It has become clear that plant NTFPs play a significant role in the daily lives of millions of rural as well as urban populations around the world (Tabuna, 2000). Particularly, 80% of the rural populations in sub-Saharan Africa depend on plant NTFPs for their health needs (Kalaba *et al*., 2010). Their biochemical and nutritional evaluations (many are rich in proteins, lipid, carbohydrates, minerals and various vitamins and fibers (Augustino *et al*., 2011)) have demonstrated their potential to resolve the growing food, nutritional and health crises in Africa (Okolo *et al*., 1995; Chadare *et al*., 2010; Ahenkan and Boon, 2011; Sossa- Vihotogbé *et al*., 2012).

Two decades ago, an evaluation of the contribution of plant NTFPs to local populations' livelihood improvement was conducted throughout West and Central Africa by many organizations funded by the Consultative Group on International Agricultural Research (CGIAR). This evaluation intended to identify the key plant NTFP species on which research-development actions should be concentrated. It resulted in a great diversity of species including numerous multipurpose trees (Clark and Sunderland, 2004; Augustino *et al*., 2011). Even though there are many common types of use related to some species, geographically separated local communities not seldomly use them in different ways, in accordance with their culture, belief, food habits and traditional knowledge background accumulated through generations (Lesley and Brown, 2004; Sossa-Vihotogbé *et al.,* 2012). However, human migration and nutritional interactions among societies can gradually reshape food habits and modify knowledge regarding the utility of the local plant biodiversity (Turner and Turner, 2008). Therefore, many species may become widely used across their natural distribution area or even transported, marketed and eventually cultivated beyond that. African bush mango trees (ABMTs) are one of the most widely used and economically most important among the multipurpose trees species in West and Central Africa (Leakey *et al*., 2005).

ABMTs: revisiting the state of knowledge with emphasis on the Dahomey Gap

African bush mango trees are large, long-lived, evergreen tropical trees of 10 to 50 m high and are widely distributed throughout sub-Saharan Africa (Harris, 1996). Bush mangoes are their drupes, a mango-like fruit. Their flesh can be either pleasant and sweet or bitter and inedible, while the seed has various important uses as well. Throughout their entire distribution range, ABMTs are subjected to many types of use that differ among human communities. Lesley and Brown (2004) provided a detailed overview of the state of our knowledge on bush mangoes, and most importantly their seed, as major NTFPs in West and Central Africa. The flesh (mesocarp) of the sweet fruits is rich in Vitamin C (Fouda *et al*., unpublished data) and is consumed fresh. The seed provides one of the most important oily substances in West and Central Africa (Joseph, 1995; Ladipo, 1999; Oyen, 2007) and is reported to be valuable in food and cosmetic manufacturing (Lesley and Brown, 2004). Above all, the main value of the seed is its use as a sauce thickening agent, typical for the African diet (Lowe *et al*., 2000; Lesley and Brown, 2004; Leakey *et al*., 2005), causing the trade of the kernels, the hard endocarps containing the seeds, to reach all continents, following the migration of black African people (Tabuna, 2000). Their wood is fairly hard and is frequently used (Ayuk *et al.*, 1999; Dolor, 2011). Moreover, the leaf, bark and root of ABMTs know many medicinal uses, but these do not yet represent a significant economic value, although the analgesic effect of the bark was demonstrated (Okolo *et al.*, 1995). Economically, the seed is the most important NTFP that makes ABMTs the top priority food trees species worth of in-depth research programs in West and Central Africa (Franzel *et al.*, 1996). Ladipo (1999) reported that since 1975 the market for kernels was worth some US$ 50 million and this market has kept on growing. The economic potential related to kernel commercialization for a single ABMT was estimated at US$ 300 per year (National Research Council 2006). Recently, Nkwatoh *et al.* (2010) showed that the annual kernel production between 2002 and 2008 in a single Cameroonian natural reserve (the Ejagham Forest Reserve) and marketed toward Nigeria reached 155,000 tons, thus generating an annual revenue of US$ 675,000, clearly showing the market potential. Agbor (1994) already indicated that the production of ABMTs in natural areas was decreasing due to overexploitation and seedling mortality, which points to the urgent need for protection of natural stands because of the rapid increase in demand.

To boost the production of this NTFP and promote the establishment of economically profitable agrosystems based on ABMTs, the World Agroforestry Centre is leading a two decades-long research program on the morphological diversity of fruits and trees, cultivation, on-farm management, ideotype capture and fixation and shortening the time of first fruiting.

Today, ABMTs are exploited in at least twenty countries in sub-Saharan Africa: Senegal, Guinea (Conakry), Guinea-Bissau, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Equatorial Guinea, Gabon, Republic of the Congo, Democratic Republic of Congo, Angola, Uganda, South Sudan, Central African Republic, and Sao Tome and Principe (Harris, 1996; Lesley and Brown, 2004; Kengni *et al.*, 2011). While ABMTs are widely distributed, occurring naturally in the Upper and Lower Guinean forest blocks (Figure 1), and are used in various ways by local communities (Harris, 1996), research initiatives are still scarce. Only recently, an in-depth study (Sahoré *et al.*, 2012) indicated that the seeds of Ivory Coast provenances of sweet fruits are rich in fatty matter (64%), carbohydrates (24%), proteins (7%) and total mineral matter (6%). In Benin and Togo, only limited data is available on the use, occurrence and local commercialization of bush mangoes (Poidy, 2002; Tandjiékpon, 2001; Vodouhè, 2003; Vihotogbé *et al.*, 2007; Atato *et al.*, 2010).

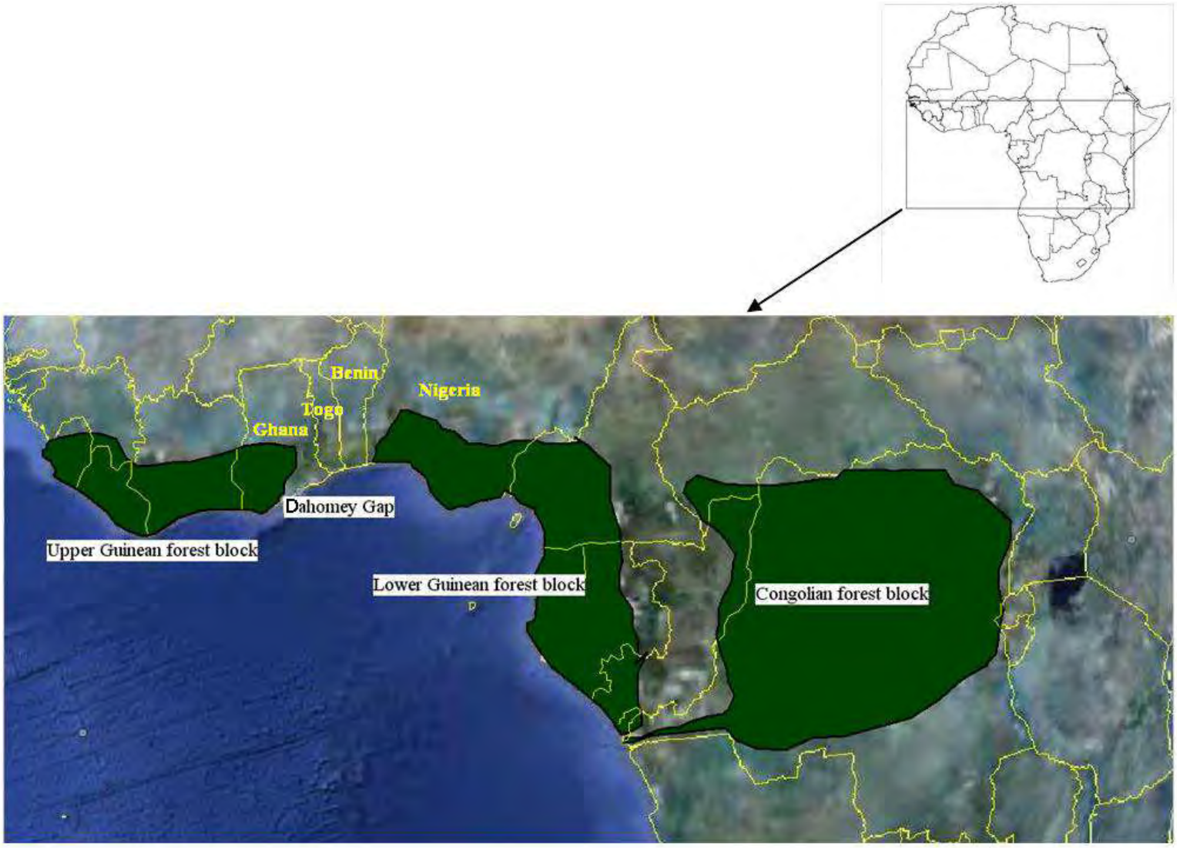

Figure 1: Location of Benin and Togo in the Dahomey Gap between the Upper and Lower Guinean forest blocks (adapted from satellite image downloaded from Google Earth, White, 1979 and Biodiversity Analysis and Technical Support for USAID, 2007).

Benin and Togo are located in a climatically particular eco-region called the Dahomey Gap (DG; see Figure 1) which is characterized by low annual rainfall (less than 1,200 mm) and high temperature (Maley, 1996; Salzmann and Hoelzmann, 2005). This eco-region extends from the plain of Accra (southern Ghana) to the area of Badagry (southwest Nigeria) and isolates the Upper Guinean forest block from the Lower Guinean one (White, 1979). The latter maintains its connection with the Central African Congolian forest block (Adebisi, 2007). The vegetation in the Dahomey Gap is mainly composed of a mosaic of savannah, with small extents of drier types of lowland rain-forests, and fallows and fields. Pollen records indicate that this gap, most probably, did not exist during the early and middle Holocene and that rainfall was sufficient to maintain a semi-deciduous/semi-evergreen forest (Salzmann and Hoelzmann, 2005). The latter authors also suggest that, before its establishment many pioneer tree taxa occurred in the Dahomey Gap. Even today, many forest tree species still occur in the Dahomey Gap but are critically threatened by the combined pressures of climate and human activities (Adomou, 2005). Among these forest tree species many produce important NTFPs that are locally consumed and regionally marketed throughout West Africa (Sokpon and Lejoly, 1996; Atato *et al*., 2010; Ekué *et al*., 2010). ABMTs belong to these important NTFPs that contribute to the nutrition, health and economy of communities in this eco-region (Tandjiékpon, 2003; Vodouhê, 2003; Vihotogbé *et al*., 2007; Atato *et al*., 2001).

The human population in the Dahomey Gap is multi-ethnic and communities are culturally linked (Assiwaju, 1979). Most of the local communities are rural and apart from agriculture, which is the most important source of their revenue and food, they also depend on plant NTFPs for their daily needs. Those NTFPs are found in human-made agro-systems as well as in the small-sized natural forests within the savannah dominated landscape.

Problem statement

ABMTs belong to the small family of Irvingiaceae which was taxonomically revised by Harris (1996). The family comprises three genera: *Desbordesia*, *Klainedoxa*, and *Irvingia* with 1, 2 and 7 species, respectively. Among the seven *Irvingia* species, six occur in West and Central Africa, while the seventh is native to South-East Asia (Harris, 1996). The term ABMTs refers to the two economically most important *Irvingia* species that occur in the humid lowland forests of West and Central Africa (Asaah *et al*., 2003), *I. gabonensis* (Aubry-LeComte ex O'Rorke) Baill. and *I. wombolu* Vermoesen. While the first generally has sweet mango like fruits the second has bitter ones and they co-occur in many eco-regions in their distribution range (Lesley and Brown, 2004). In the light of their domestication and cultivation potentials, the specific ecological characteristics of both taxa as well as the ecological variability present within each taxon are important research question to be addressed.

Morphologically, sweet and bitter ABMTs are very similar and difficult to discriminate in the field (Okafor, 1975; Harris, 1996). The mesocarp of *I. gabonensis* can be really sweet to slightly bitter and edible, while that of *I. wombolu* is totally inedible. This seems to be the only obvious distinctive character between the two taxa. Okafor (1975) stated that the taxonomic value of this character is low and presented sweet and bitter trees as varieties of a single species: *Irvingia gabonensis* var. *gabonensis* and *I. gabonensis* var. *excelsa* (Mildbr.) Okafor, respectively. In his taxonomic classification of the genus *Irvingia*, Harris (1996) acknowledged the overlap of many characters between both types. However, because in his opinion the mesocarp taste is correlated with other vegetative characters, which would thus be able to consistently distinguish bitter from sweet trees throughout their distribution range, he proposed to distinguish sweet and bitter trees at the species level (*I. gabonensis* and *I. wombolu*, respectively). Unfortunately, those other characters are often difficult to evaluate. Therefore, a thorough quantitative morphological as well as phenological evaluation of both taxa is needed in order to assess the key differences between them which will assist in the determination of the appropriate level of their taxonomic distinction.

Lowe *et al*. (2000) demonstrated significant genetic differences between sweet and bitter ABMTs. Although such a difference is generally indicative of a lack of exchange of genetic material, they did not present sound evidence of the absence of hybridization between the two types. Because certain weaknesses have been demonstrated in the methods they used, mostly the low reproducibility of the Random Amplified Polymorphism DNA technique, the taxonomic debate about ABMTs remains alive (National Research Council, 2006). As a result, both opinions (species-level and variety-level distinction) are found in recent studies (Dudu *et al.*, 1998; Nzekwe *et al.*, 2002; Nya *et al.*, 2006; Nya *et al.*, 2009; Dolor, 2011; Sahoré *et al.*, 2012). Therefore, the evaluation of genetic, but also of ecological, morphological, chemical or phenological differences between sweet and bitter ABMTs still are important research questions. It is particularly vital for any conservation and domestication program to insure the identity of the material being used, domesticated and conserved. For example, it is difficult to attribute the potential medicinal uses related to the biochemical properties of the bark and leaf of ABMTs (Okolo *et al.*, 1995; and Nosiri *et al.*, 2011) to either sweet or bitter trees (Tchoundjeu and Atangana, 2007). In addition, the kernels of bitter and sweet bush mangoes, the most important NTFP of ABMTs, are mixed for marketing, while it seems likely that differences in organoleptic properties, indicating different economic potential for sweet and bitter trees, are present (Ladipo, 1999). In the context of ABMTs domestication and conservation, the World Agroforestry Centre focuses on the germplasm from the Lower Guinean forest block, where sweet trees have been more intensively investigated. Broadening the geographical area for a comparative study to the Upper Guinean region seems logical, since a domestication and conservation program would want to use the maximum of ABMTs diversity (ecological, phenological, chemical and morphological as well as genetic). Capturing such diversity depends on well-balanced and sufficiently dense germplasm collecting activities covering the entire distribution range of ABMTs (see Ladipo *et al.*, 1996; Lowe *et al.*, 2000).

Most ABMTs material used for former cultivation and domestication efforts arose from Nigeria and Cameroon (Lowe *et al*., 2000; Ude *et al*., 2006; Lower *et al*., 2010). In the Dahomey Gap, ABMTs are distributed across different phytogeographical regions and in various agrosystems, but their truly wild occurrence is questionable. Thus, their geographical origin from outside that eco-region needs to be demonstrated (Kengni *et al*., 2011).

Objectives of this thesis

General objective

The overall objective of this research is to improve our knowledge on ABMTs, focussing on the possible differences between the sweet and bitter types, thus supporting the development of suitable strategies for their domestication, conservation and use within the Dahomey Gap (West Africa) and possibly beyond that region.

Specific objectives

This study aims to:

- Assess the ecological difference between sweet and bitter ABMTs in tropical Africa and discuss its taxonomic implication as well as the origin of the Dahomey Gap material.
- Quantify the phenological characteristics of both types of ABMTs and infer the potential drivers of this variation as well as its taxonomic implication.
- Investigate the distinction of sweet and bitter ABMTs in the field based on quantitative morphological data of leaves, bark and fruits.
- Revisit the pattern of genetic diversity and the phylogenetic relationships between and within sweet and bitter ABMT populations throughout tropical Africa.
- Relate traditional knowledge on AMBT usage to the decision of farmers to allocate productive space to bitter and sweet ABMTs in the Dahomey Gap.

Thesis outline

After this general introduction, which has briefly traced the knowledge and research issues related to ABMTs and their importance and presented the objectives of the thesis, this thesis comprises six additional chapters: five research papers and a general discussion. The second chapter gathers the indigenous knowledge related to the use of ABMTs in the Dahomey Gap, and uses the geographical location of the different levels of knowledge of local communities to postulate the origin of the Dahomey Gap material. In this chapter, the spatial abundance of ABMTs is modelled across the Dahomey Gap and the anthropogenic factors that influence their cultivation intensity as well as population survival are analysed. In the third chapter, presence data of sweet and bitter trees collected throughout the Dahomey Gap and in tropical Africa are used in ecological niche modeling analyses. The niche model of bitter trees is compared to that of the sweet while the model of cultivated material provides the potentially cultivatable area. Also, the origin of material cultivated in the Dahomey Gap is discussed. Phenological differences between bitter and sweet ABMTs and among populations in the Dahomey Gap are analysed in the fourth chapter. In addition, in this chapter we relate environmental factors to the reproductive success of each type of ABMT in different phytogeographical regions. In the fifth chapter, the possibility to differentiate sweet and bitter ABMTs in the field based on quantitative and qualitative morphological characters of leaves, bark, fruit and seeds, is investigated in populations across the Dahomey Gap. In the sixth chapter, the genetic diversity of and the relationships between sweet and bitter ABMT populations are assessed using Amplified Fragment Length Polymorphisms (AFLP) and chloroplast microsatellites. In the seventh chapter, the general discussion, the most important results of our attempts to discriminate bitter and sweet ABMTs are presented together with the implications these results have in terms of the domestication, cultivation and conservation of ABMTs as important food trees.

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Chapter 2

Ecological differences within African Bush Mango trees in tropical Africa with emphasis on the Dahomey Gap (West Africa)

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Abstract

Bush mangoes (Irvingiaceae) are priority food trees in humid areas of West and Central Africa. There are bitter and sweet fruited trees which are difficult to distinguish based on morphological characters. This has led to a taxonomic debate, hindered by the lack of quantitative comparative data. Furthermore, their occurrence in the Dahomey Gap, the dry savannah corridor between the two West African forest blocks, is unexplained. This study evaluates the ecological differences between bitter and sweet trees in tropical Africa and studies the possibility that the Dahomey Gap is part of the natural distribution range of bush mango trees. Occurrence data from the Dahomey Gap and elsewhere (in tropical Africa), were combined with climatic and soil data in Maxent to produce ecological niche models for bitter and sweet trees. Ecological niche identity tests were carried out in ENM-Tools. Wild sweet trees were predicted in the Guinean-Congolian phytogeographical region, while the predicted occurrence of bitter trees extended to the Guineo-Congolia/Sudania and Lake Victoria regions. This difference is significant, supporting the idea that bitter and sweet trees belong to two different species. We also conclude that bitter trees occur naturally only in a small part of the Dahomey Gap, the Volta forest region. Lastly, our results suggest that the natural distribution of sweet trees excludes the Dahomey Gap, where they occur only in cultivation. The history of their occurrence in this eco-region remains unclear.

Key-words: Benin, Climate, Ecological Niche Modeling, *Irvingia*, Maxent, Togo

Introduction

African bush mango trees (ABMTs) belong to the genus *Irvingia* within the family of Irvingiaceae. They are priority food tree species in the humid regions of West and Central Africa (Franzel *et al.*, 1996). They produce two economically important non-timber forest products (NTFPs) in sub-Saharan Africa (Tabuna, 2000): the sweet mesocarp, which is eaten fresh, and the seed used as a sauce thickening and flavouring agent (Lowe *et al.*, 2000; Tchoundjeu and Atangana, 2007).

Bush mangoes show a clear and steady difference in the mesocarp taste: bitter and totally inedible versus sweet or slightly bitter and edible. This is a key criterion that helps define bitter and sweet ABMTs throughout their distribution area. However, attempts to discriminate between bitter and sweet ABMTs using morphological characters are unsatisfactory (Okafor, 1975; Harris, 1996). Because sweet and bitter trees lack additional reliable discriminating characters, Okafor (1975) opted for a distinction only at the variety level within the species *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill.: *I. gabonensis* var. *gabonensis* and *I. gabonensis* var. *excels* (Mildbr.) Okafor, for sweet and bitter trees, respectively. However, in his recent taxonomic revision, Harris (1996) decided to recognize them at species level as *I. gabonensis* and *I. wombolu* Vermoesen, respectively, although he admitted a weak morphological distinction between them. Lowe *et al.* (2000) supported this decision using Random Amplified Polymorphic DNA (RAPD) molecular markers. But, because of some weaknesses related to these genetic markers, the taxonomic status of ABMTs remains controversial (National Research Council, 2006) and a random use of the variety as well as the species names abounds in literature. Finding a solution is hindered by poor sampling (Ladipo *et al.*, 1996; Lowe *et al.*, 2000) and the lack of centralizing the existing occurrences and the morphological and phenological data in quantitative and comparative studies over their entire distribution range.

Wild ABMTs occur in humid lowland forest areas in West and Central tropical Africa (Kang *et al.*, 1994; Harris, 1996; Van Dijk, 1997; Lowe *et al.*, 2010). Their distribution is centred in the Guineo-Congolian phytogeographical region, with some outliers to the Guinea-Congolian / Zambezian zone and the Guinea-Congolia / Soudania Transition zone (Fig. 2.1a, 2.1b; White, 1979). In West Africa, the Dahomey Gap refers to the region where a savannah corridor interrupts the rain forests of the Guineo-Congolian region (Maley, 1996). This particular eco-region, with higher temperatures and lower rainfall, extends from Accra, in south-eastern Ghana throughout Togo and Benin until Badagry, in south-western Nigeria and divides the Guineo-Congolian region into the Upper and Lower Guinean forest blocks (White, 1979; Maley, 1996; Sowunmi, 2007).

The increasing market demands of the hard bush mango endocarp (containing the valuable seed) causes an over-exploitation of trees in natural habitats and this results in poor regeneration in their postulated genetic diversity centres in Nigeria, Cameroon and Gabon (Agbor, 1994; Lowe *et al.,* 2000; Ude *et al.,* 2006; Lowe *et al.,* 2010). Therefore, cultivation initiatives are increasing throughout the ABMTs distribution range (Okafor and Fernandes, 1987; Shiembo *et al.,* 1996; Asaah *et al.,* 2003).

In general, cultivation broadens the geographic distribution of a species (Miller & Knouft, 2006) and accurate knowledge of cultivation success, as indicator of ecological adaptability, is required for an effective monitoring and conservation management (Shaffer *et al.,* 1998; Corsi *et al.,* 1999). Knowledge of the potential cultivatable area is also useful, for example in a national policy for agricultural development, to anticipate on the growing demands for many valuable NTFPs (Scheldeman *et al.,* 2007).

Ecological niche modelling combines the known occurrences of a species with corresponding environmental parameters in a geographic information system (GIS) to generate for any point within the geographic area under investigation the probability of the presence of this species (Phillips *et al.,* 2006; Pearson, 2007; McCormack *et al.,* 2011). Given their ability to identify areas where species should be able to successfully complete their life cycle, ecological niche models (ENMs) are powerful tools for agro-biodiversity conservation (Miller and Knouft, 2006; Sanchez *et al.,* 2010). Numerous algorithms are designed to refine species ecological niche modelling techniques. Among them, Maximum Entropy (Maxent, Phillips *et al.,* 2006) was identified as the more accurate even with a limited known occurrence dataset (Elith *et al.,* 2006; Pearson *et al.,* 2007; Wisz *et al.,* 2008; Leaché *et al.,* 2009). However, like any ecological modelling package, the authority of ENMs to provide quantitative ecological niche differences among closely related taxa is limited. ENM-Tools (Warren *et al.,* 2008) tries to overcome this weakness. It quantifies the ecological difference between two taxa by comparing estimated probabilities assigned to individual grid cells over the entire geographical space studied. This powerful package assists in the assessment of the ecological identity of a species (McCormack *et al.,* 2011; Wooten and Gibbs, 2011).

This study evaluates ecological differences between sweet and bitter trees in tropical Africa. We combined known occurrence data with environmental layers in Maxent (version 3.3.3k) addressing three main research questions:

- Are the ecological niches of bitter and sweet ABMTs different?
- Does the Dahomey Gap belong to the ecological niche of sweet and/or bitter ABMTs? In other words, do sweet and bitter ABMTs occur wild in the Dahomey Gap?
- What is the potential cultivatable area of sweet ABMTs in the Dahomey Gap?

Material and Methods

Presence data

A total of 3019 ABMTs occurrences from Central and West Africa were gathered from various sources including recent fieldwork (Table 2.1). Fieldwork was carried out only in the Dahomey Gap (Benin and Togo) in 2009. In this area, where human made agrosystems and relics of natural forests are the main vegetation, the occurrences were recorded throughout all areas where ABMTs grow in such a way that all phytogeographical regions (see Ern, 1979; Adomou, 2005) were covered. Using a Global Positioning System (GPS Garmin 60), each isolated tree scattered in the landscape was geo-referenced while in high density areas only one tree was sampled. The occurrence database was resampled to 5 arc-minute (10 x 10 km) to prevent overweighting. This resulted in 185 and 24 unique occurrences for sweet and bitter trees in the Dahomey Gap, respectively. We refer to these samples as Dahomey Gap bitter trees and Dahomey Gap sweet trees.

Outside the Dahomey Gap, the occurrences were obtained from the sources mentioned in Table 2.1. They are collections from wild populations occurring across the humid lowland forest areas on both sides of the Dahomey Gap. Herbarium specimens cited by Harris (1996) geo-referenced and the sample location maps of Lowe *et al.* (2000) were digitized and ABMTs' geographic positions were thus extracted. Data downloaded from the GBIF Data Portal (http://www.gbif.net) was checked and, if sufficiently trusted, added. Duplicate records were removed. The spatial distribution of the records range from between -13.93° to 33.63° longitude and -6.96° to 14.57° latitude.

Table 2.1: Sources and sample size for bitter and sweet African bush mango trees

Type ABMT	Geographic area	Source	Total sample size	Used sample size after duplication removed
Bitter	Dahomey Gap	Fieldwork (Vihotogbé R.)	213	24
	Tropical Africa	GBIF Data Portal	30	65
		Lowe et al. (2000)	28	
		Harris (1996)	21	
Sweet	Dahomey Gap	Fieldwork (Vihotogbé R.)	2565	185
	Tropical Africa	GBIF Data Portal (http://www.gbif.net)	86	121
		Lowe et al. (2000)	76	

Environmental parameters

Consistent conclusions can be drawn from species distribution models constructed with only climatic variables (see Scheldeman *et al.,* 2007). But, because climatic data have limited power to capture spatial micro-variations in a landscape and can display important spatial autocorrelations, additional environmental factors are often required to improve species distribution models. This is particularly important when assessing ecological differences between closely related species that geographically co-occur (see McCormack *et al.,* 2011). Moreover, the combination of soil characteristics and climatic variables play a key role in plant species distribution in sub-Saharan Africa (Linder *et al.,* 2005). Therefore, we use both climatic and soil data as ecological parameters to produce ecological niche models in tropical Africa and the Dahomey Gap. The nineteen 30 arc-seconds bioclimatic variables from the WorldClim database (http://www.worldclim.org/current) were tested for multicollinearity using Pearson's correlation ($r < 0.70$), and five independent variables were retained (Table 2.2). Soil data were collected from the ISRIC World Soil Information database (http://www.isric.org/). Soil data were tested for multicollinearity using Spearman rank correlation and twelve independent soil were retained (Table 2.2). The topsoil salinity, although shown complete independence against all other variables, was not retained because it holds very limited information within the ranges of ABMTs. In total, sixteen environmental layers were used to carry out the ecological niche modelling.

Table 2.2: Contribution of environmental factors to ecological niche models

Bioclimatic and soil variables	**Contribution to ecological niche models (%)**			
	Wild SBMTs	**DG SBMTs**	**Wild BBMTs**	**DG BBMTs**
Annual mean temperature	0.6	0.7	0.2	0.2
Isothermality (Bio_2/Bio_7)(* 100)	4.4	12	8.1	14.5
Minimum temperature of coldest month	12.6	48.6	2.5	2.9
Annual precipitation	50	15	17.2	1.8
Precipitation of coldest quarter	2.6	5.8	41.9	26
Available water storage capacity [class 1 (150mm/m) - 7 (0 mm/m)]	1.7	0.9	0.9	4.2
Drainage class [class 1 (very poor) – 7 (excessive)]	0.3	0.3	4.7	0.2
Reference depth of the soil unit	1.2	0.2	0	0
Topsoil calcium sulphate	0.2	0.5	2.9	3
Topsoil cation exchange capacity	0.2	0.3	0.6	1.2
Topsoil exchangeable sodium percentage	2.5	0.2	4	0.6
Topsoil gravel content	20.6	11.5	8	25.5
Topsoil organic carbon content	0.2	0.3	0.8	0.5
Topsoil pH [measured in a soil-water solution]	0.7	2.1	6.7	0
Topsoil silt fraction	0.9	0.9	1.2	19.2
Topsoil textural class [class 0 (none) – 3 (fine)]	1.1	0.8	0.3	0.3

DG = Dahomey Gap

Ecological niche modelling

We considered four occurrence sub-datasets: (i) wild bitter trees, (ii) wild sweet trees, (iii) bitter trees in the Dahomey Gap, and (iv) sweet trees in the Dahomey Gap. Assuming that occurrence records provide key information on the ecological conditions required for species occurrence and growth (Pearson 2007), we expected the modelled potential ranges to represent areas were the populations represented by each sub-dataset could potentially occur within the limits of the geographical area considered.

First, we combined each of those occurrence sub-datasets with the environmental layers in -2° to 15° Latitude and -15° to 35° Longitude in Maxent (using default parameters) to produce four ecological niche models. Second, using the same algorithm, the sweet trees occurrences from the Dahomey Gap were combined with the environmental layers in 5° to 13.5° Latitude and -1° to 5° Longitude to generate a fifth ecological niche model. The latter was used only to obtain the cultivatable area for sweet trees within that area. The projection of the obtained ecological niche models in the geographical space shows the potential distribution area of the corresponding species. Because in the Dahomey Gap only cultivated sweet trees were collected, this dataset was used to predict their potential cultivatable area.

The area under the curve (AUC) of the Receiver Operating Characteristic (ROC) was used to assess the accuracy of models created with the Maxent algorithm. This statistic describes the relationship between the percentage of correctly predicted occurrences against that of incorrectly predicted absences and ranges from 0.5 (random prediction) to 1 (high model consistency).

Ecological niche overlap and identity testing

This analysis aims to evaluate the difference in ecological niches between: (i) wild bitter and wild sweet trees, (ii) wild bitter trees from Tropical Africa and the Dahomey Gap bitter trees, and (iii) wild sweet trees from Tropical Africa and the Dahomey Gap sweet trees. The models produced by Maxent were used in ENM-Tools to calculate the Schoener’s *D* similarity index (see Warren *et al.,* 2008) measuring the niche-overlap between model pairs. This similarity index ranges from 0, for niches completely disjoint, to 1 for totally identical niches (Warren *et al.,* 2008).

In addition, using ENM-Tools occurrences from each model pair being compared were pooled and 100 pseudo-replicate random sets of occurrences were sampled from the pooled data. Original niches of the model pair were compared with those of the 100 random sets by means of the comparison of the observed values of the niche similarity (D) to the null distribution (see Warren *et al.*, 2008).

Results

Potential distribution areas of wild bitter and sweet trees

The potential distribution of wild occurrences of bitter and sweet trees was predicted in the area between -7° to 15° Latitude and -15° to 35° Longitude. The resulting ecological niche models were highly consistent: AUC = 0.940 and 0.964 for bitter and sweet trees, respectively.

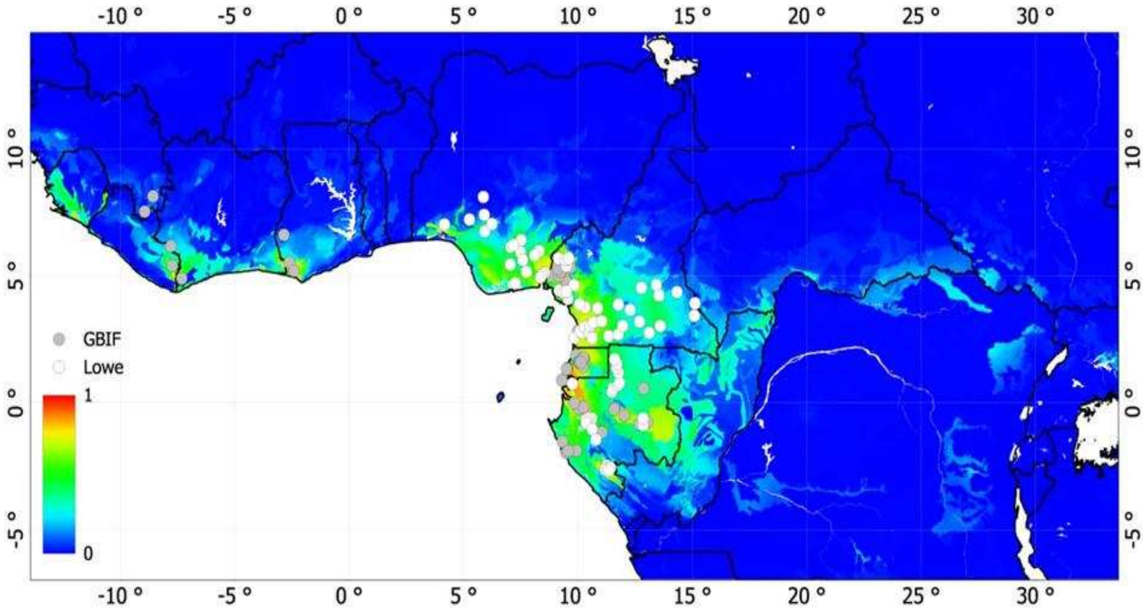

Figure 2.1a: Potential distribution of wild sweet ABMTs in tropical Africa

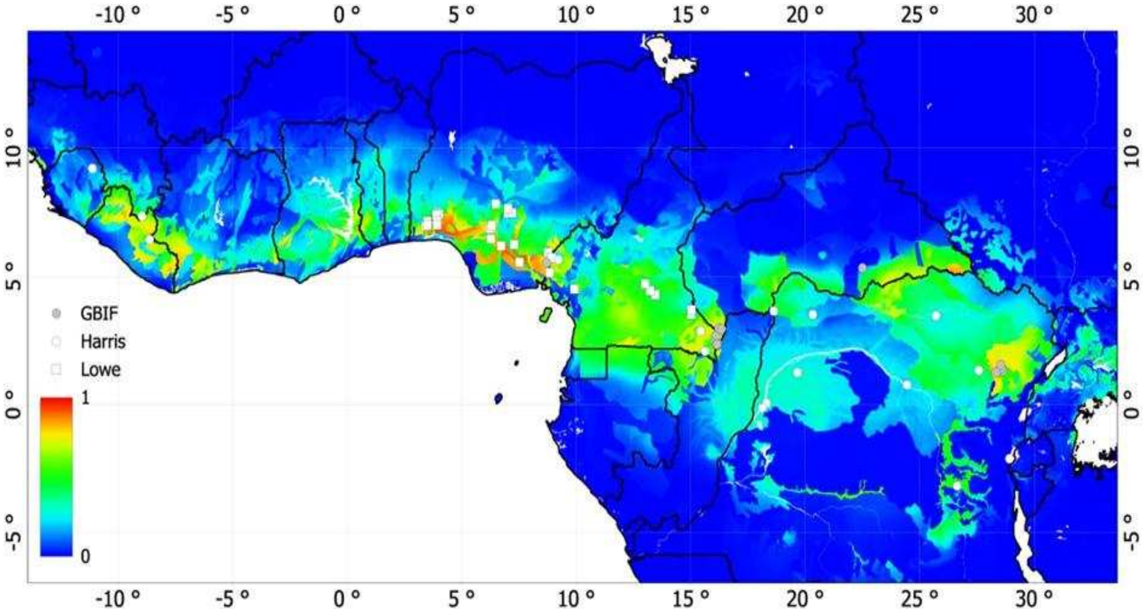

Figure 2.1b: Potential distribution of wild bitter ABMTs in tropical Africa

Wild sweet trees were predicted to occur in forest regions comparatively close to the coast with the highest distribution probabilities within -3° to 6° latitude and -12° to 14° longitude (Fig. 2.1a). Some high distribution probabilities were concentrated in the Upper Guinean forest block, but most are found in the Lower Guinean region (Nigeria to Gabon). In the Upper Guinean forest block their wild occurrence appears disjoint. The Dahomey Gap was excluded from the potential distribution range of wild sweet trees.

The potential distribution of the wild bitter trees, on the other hand, extends both to dryer regions and to other wetter regions. They were predicted to occur with high probabilities within -5° to 10° Latitude and -11° to 30° Longitude (Fig. 2.1b). Unlike wild sweet trees, high distribution probabilities were mapped with a more continuous pattern from Central and North Liberia to south-eastern Ivory Coast, away from the coast. Part of the Dahomey Gap (from south-western Togo to southern Ghana called the Volta forest region) is also included in the potential distribution area of wild bitter trees. The highest occurrence probabilities, with a more continuous pattern, are located in the Lower Guinean forest block: humid forests in southern Nigeria and Cameroon. The high occurrence probabilities extend to the northern edge of the Congolian forest block towards the Ituri region in the northeast of the Democratic Republic of Congo. Finally, wild sweet trees were predicted to occur throughout the Congo Basin but with low probabilities.

Ecological differences between wild bitter and sweet trees

Different environmental factors contribute at different levels to the ecological niche model of wild bitter and wild sweet trees (Table 2.2). The occurrence of wild bitter trees is most highly associated with the precipitation in the coldest quarter (42%) and annual precipitation (17.2%), while annual precipitation (50%), topsoil gravel content (20.6%), and minimum temperature in the coldest month (12.6%) govern the occurrence of wild sweet trees.

For wild bitter trees the optimum precipitation in the coldest quarter is around 500 mm while for wild sweet trees this niche parameter accounts for only 2.6% in the occurrence. Above this optimum, the occurrence probability of wild bitter trees steadily decreases (Fig. 2.2a). Wild bitter trees prefer an annual precipitation higher than 1500 mm/year, while the occurrence probability of wild sweet trees is steadily rising even above 2,500 mm/year. Wild sweet trees are more sensitive (negatively) to lower annual precipitations (< 2,500 mm) and their occurrence probability decreases more rapidly to 0 at 1,300 mm (Fig. 2.2b). With regard to the minimum temperature of the coldest month, wild sweet trees are sensitive to values lower than 17 °C, while wild bitter trees are not affected by this factor which accounts for only 2.5% in their niche model (Fig. 2.2c). Finally, the optimum topsoil gravel content is 0% for wild sweet trees displaying high negative sensitivity to high values, while this factor does not impact the occurrence probability of the wild bitter trees (Fig. 2.2d).

The Schoener's *D* statistic shows a degree of overlap of 0.5 between the ecological niches and the niche identity test indicates that this overlap is significantly less than that obtained when comparing the original models with those from randomization of the pooled dataset (Fig. 2.2e). Thus, the natural habitats populated by bitter and sweet trees are not identical.

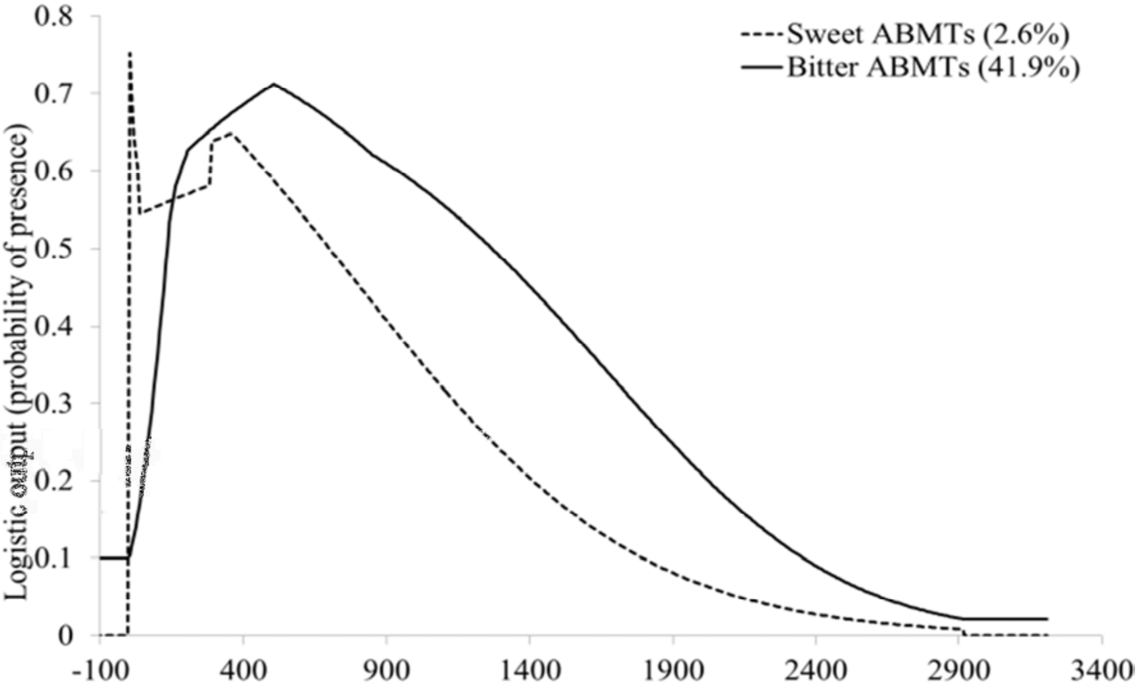

Figure 2.2a: Influence of precipitation of the coldest quarter (in mm)

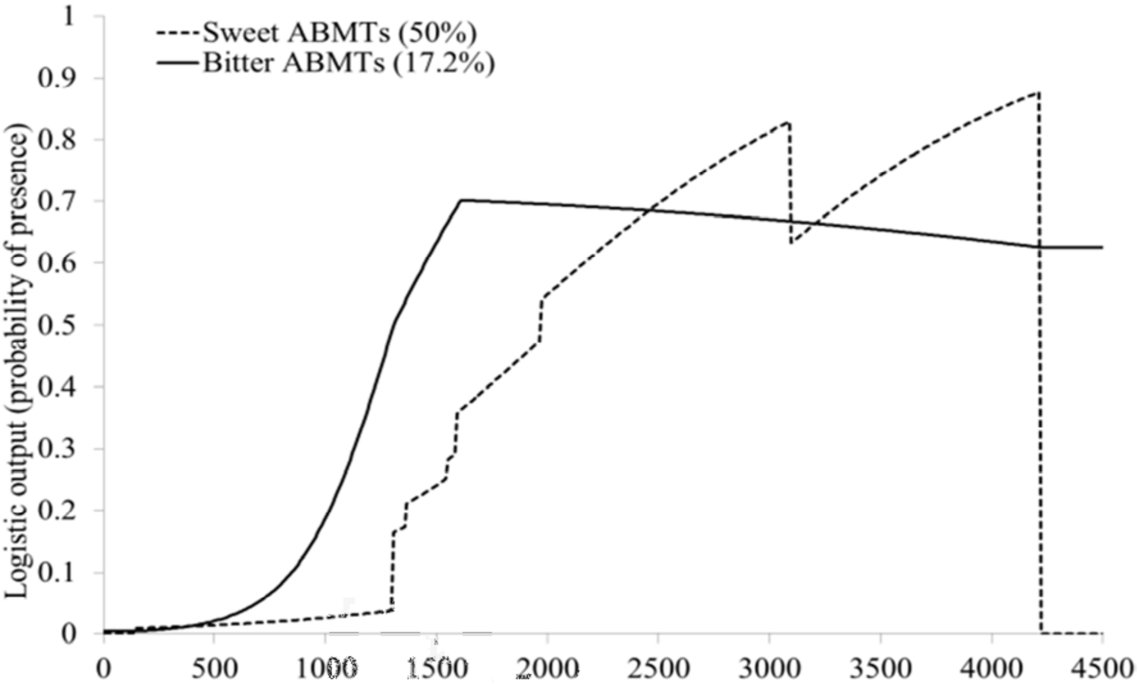

Figure 2.2b: Influence of annual precipitation (in mm)

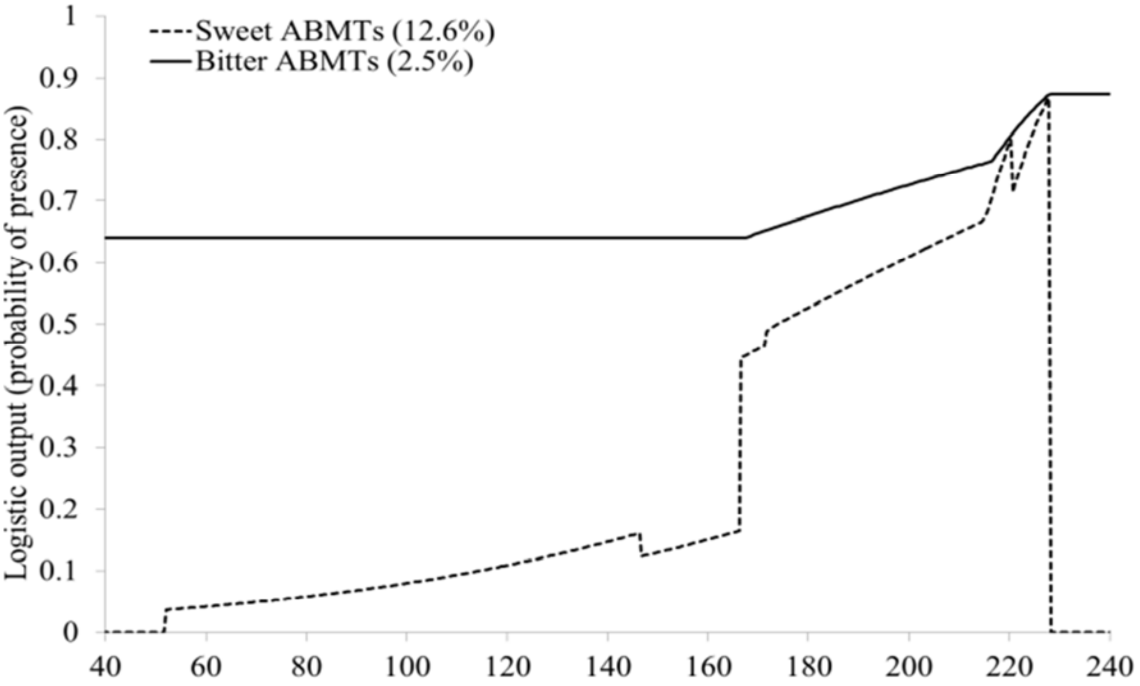

Figure 2.2c: Influence of minimum temperature of coldest month (0.1 degrees C)

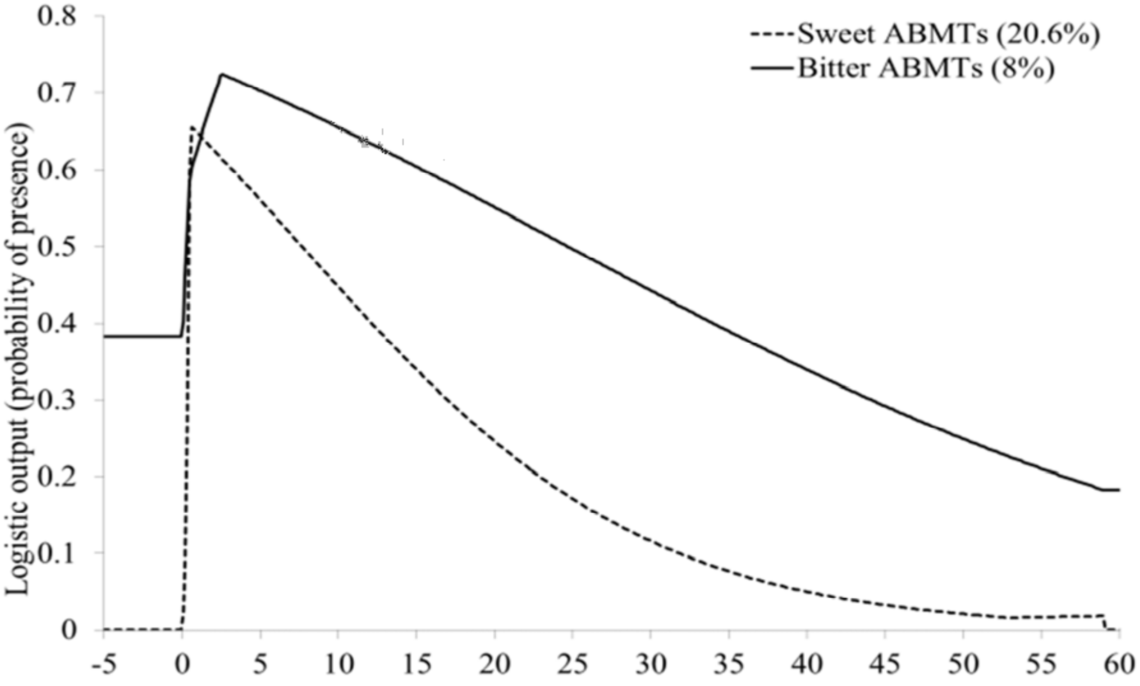

Figure 2.2d: Influence of topsoil gravel content (% volume)

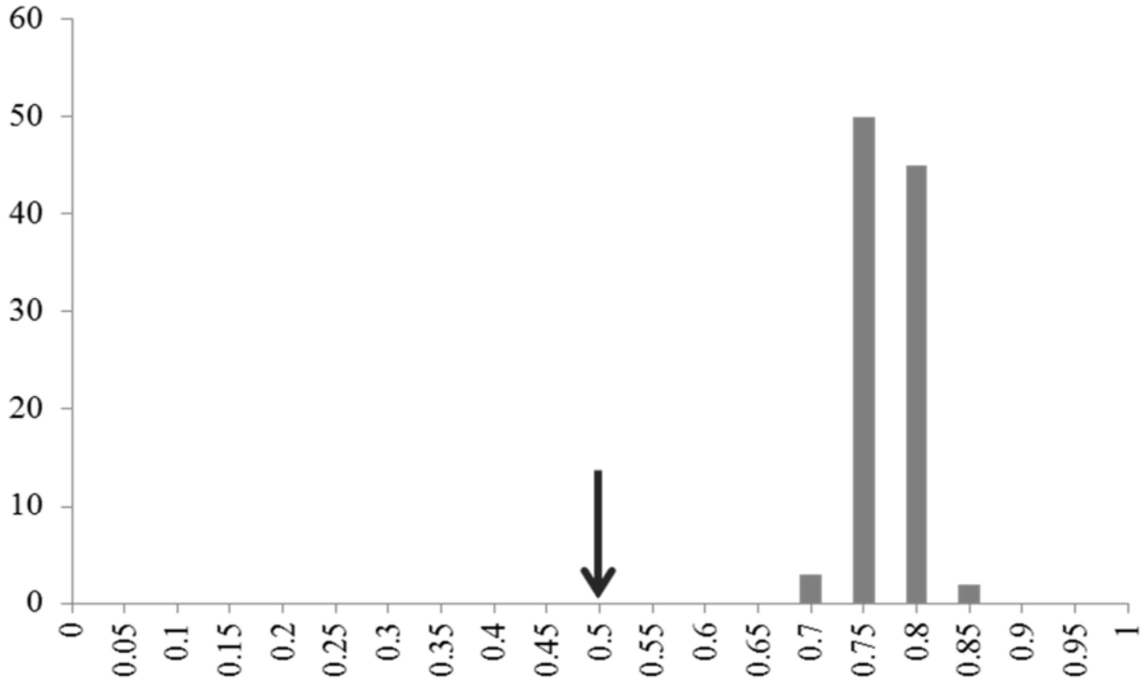

Figure 2.2e: Niche identity tests using Schoener's D: The similarity score of the models for the wild bitter and sweet trees is lower than expected by chance (arrow).

Differences between ABMT populations in the Dahomey Gap and wild populations

Within the Dahomey Gap, bitter trees occur only in the Volta forest region in Togo where they occur on farms, in forest gardens and in protected natural forests (Vihotogbé *et al.*, submitted-1). In this hilly area, they occur mostly in swampy places and along streams, but occasionally also on hill slopes. In contrast, sweet trees occur widespread in the Dahomey Gap from southern Togo to southern and central Benin. Sweet trees are found on farms and in home gardens, on plateau's, hill slopes as well as on hill summits and their distribution seems to follow that of the human settlements and agricultural productive areas. They are not found in flooded areas nor along streams. In the Volta forest region, bitter and sweet trees occasionally co-occur.

The potential distribution of the sweet tree populations in the Dahomey Gap is almost restricted to the Dahomey Gap (AUC = 0.987; Fig. 2.3a). The most important environmental factors contributing to this niche model are different from those of the wild sweet trees (table 2). For the Dahomey Gap sweet trees, minimum temperature of the coldest month is the most important factor (48.6%), while it was the third most important one in the niche model of the wild sweet trees (only 12.6%). However, both wild sweet trees and Dahomey Gap population models have a similar response with increasing probability of occurrence with increasing temperatures in the coldest month (Fig. 2.2c and 2.4a). Annual precipitation had the highest contribution to the distribution wild sweet trees (50%) while this factor only accounts for 15% in the potential distribution of the Dahomey Gap sweet trees. Finally, isothermality contributes to the potential distribution of the Dahomey Gap sweet trees (12%), while its contribution to that of wild sweet trees was very low (4%). In the Dahomey Gap, the occurrence of sweet trees is driven by monthly temperatures that change according to seasons in this region.

Little niche overlap (Schoener's *D* statistic = 0.13) was detected between the wild and Dahomey Gap sweet trees. The niche identity test indicated that this overlap was significantly less than expected based on the randomized draws from all pooled sweet tree occurrences (Fig. 2.4b). This implies that Dahomey Gap sweet trees are ecologically different from wild sweet trees.

The potential distribution of the Dahomey Gap bitter trees is restricted to the Volta forest region in south-western Togo (AUC = 0.998; Fig 3b). This distribution shows a high contribution of the same most important environmental factor controlling that of wild bitter trees: precipitation of the coldest quarter (26%, table 2). The response to this factor has the same optimum as well (500 mm: Fig. 2.1b & 2.4c). The occurrence of Dahomey Gap bitter trees is also associated with topsoil gravel content (25.5%) while this factor has low influence on the occurrence for the wild bitter trees collection (8%). Subsequently, topsoil silt fraction (19%) and isothermality (14.5%) are also influential factors for the Dahomey Gap bitter trees niche model (table 2).

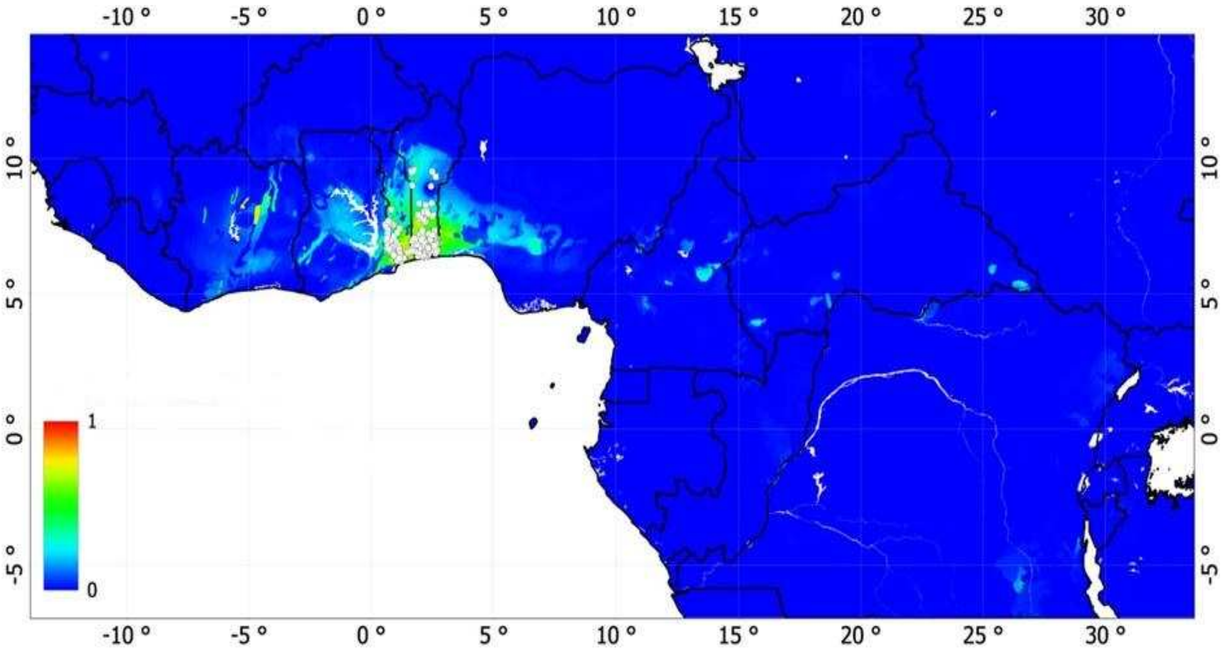

Figure 2.3a: Potential distribution of Dahomey Gap sweet ABMTs.

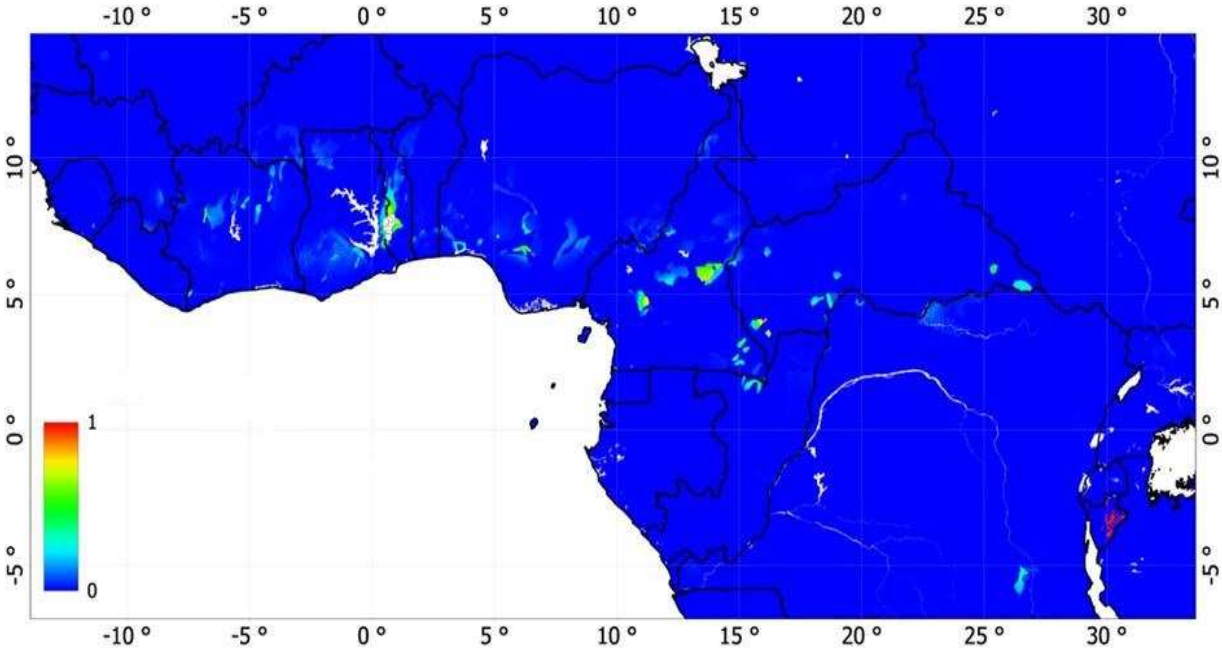

Figure 2.3b: Potential distribution of Dahomey Gap bitter ABMTs.

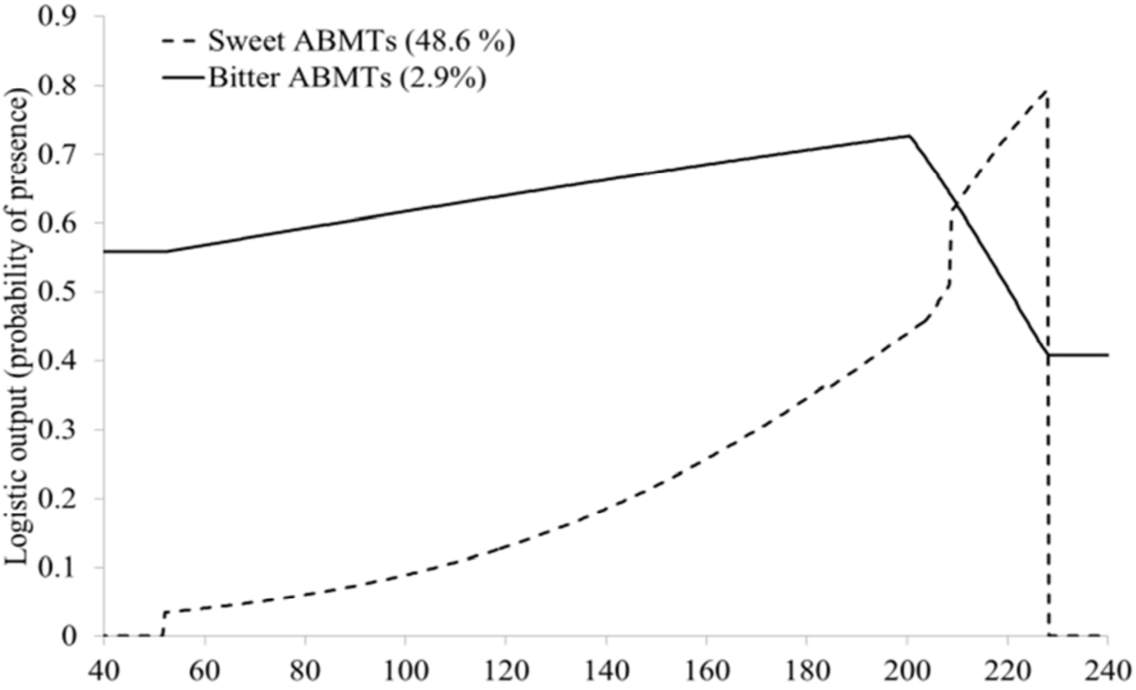

Figure 2.4a: Influence of minimum temperature of the coldest month to the distribution of Dahomey Gap SBMT and Dahomey Gap BBMT populations

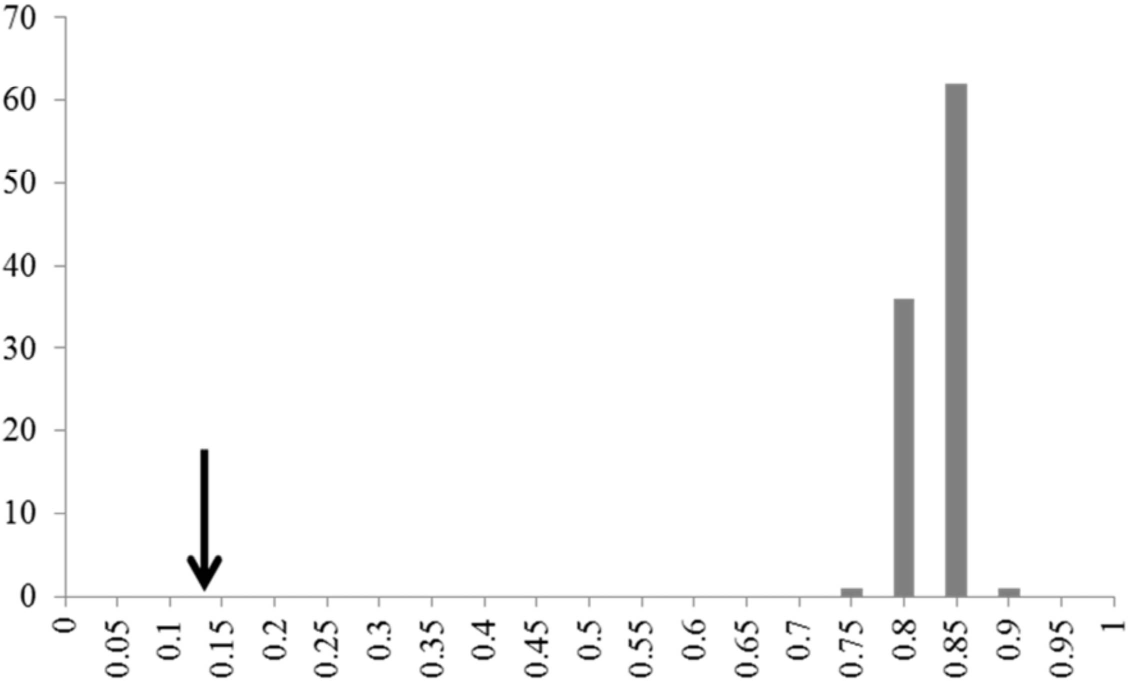

Figure 2.4b: Niche identity tests using Schoener's D: The similarity score of the models for the wild and Dahomey Gap SBMTs is lower than expected

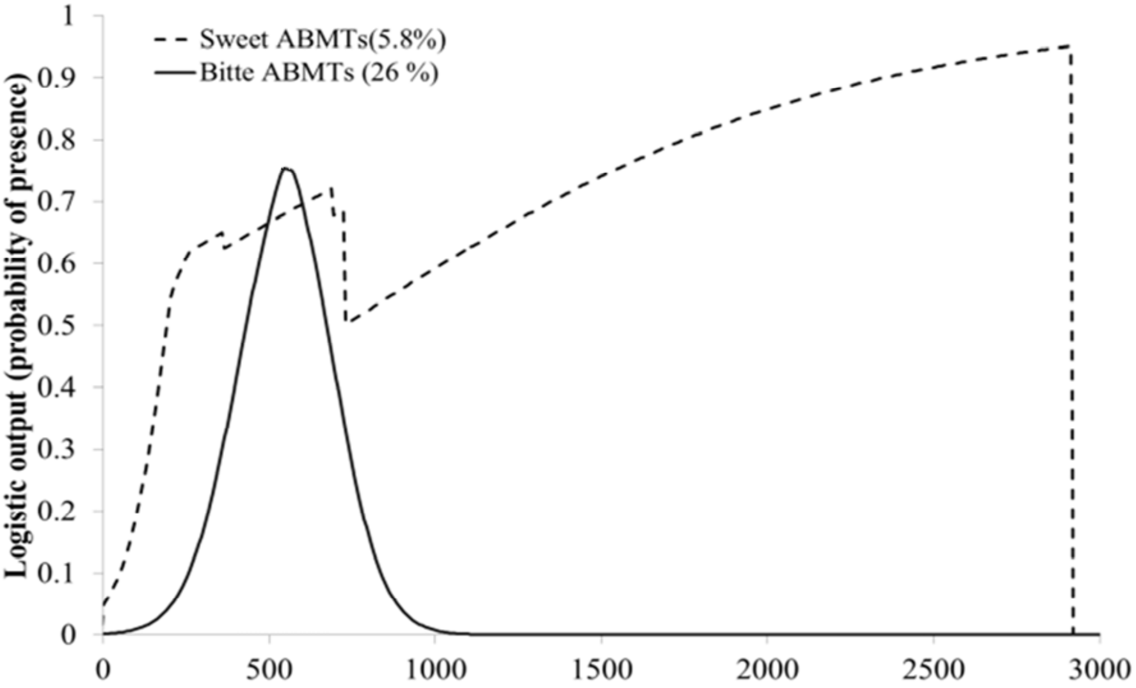

Figure 2.4c: Influence of annual precipitation of the coldest quarter on the distribution in Tropical Africa of Dahomey Gap BBMTs collection

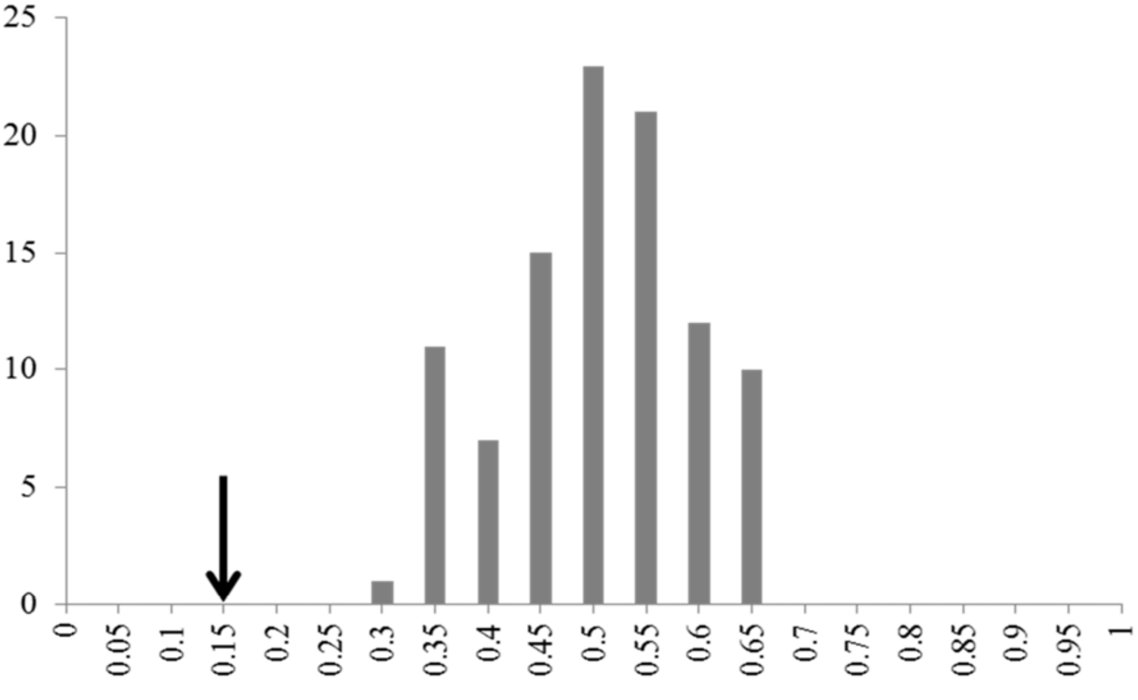

Figure 2.4d: Niche identity tests using Schoener's D: The similarity score of the models for the wild and Dahomey Gap BBMTs is lower than expected

The Schoener's *D* statistic indicates that there is only a slight niche overlap between the bitter trees in the Dahomey Gap and those in the wild throughout tropical Africa (0.15). The niche identity test indicates that this overlap is significantly less than expected by chance (Fig.

2.4d). The difference between observed and expected is much lower though than for the sweet trees. Therefore, the Volta forest region, in the Dahomey Gap, is an ecological area different from that of the wild bitter trees collection. This implies that Dahomey Gap bitter trees are ecologically different from wild bitter trees.

The ecological factors that govern potential distributions of bitter and sweet trees in the Dahomey Gap are different (Table 2.2). Where precipitation in the coldest quarter has a high influence on the occurrence of bitter trees (26%), it accounts for only 6% of the explanation of the potential distribution of sweet trees (Fig. 2.4c). Furthermore, the minimum temperature of the coldest month, detected as the most important factor influencing the distribution of sweet trees (48.6%), accounts for only 3% of the explanation of the potential distribution of bitter trees (Fig. 2.4a).

Finally, the potential cultivatable area for sweet trees in the Dahomey Gap is obtained, and indicates suitable habitat mainly in the southern part of the Dahomey Gap (Fig. 2.5; 6° to 8.5° latitude). Despite the occurrence of sweet trees in the central as well as the northern regions of Benin, habitat suitability for cultivation of sweet trees seems to be much lower in those regions (Fig. 2.5).

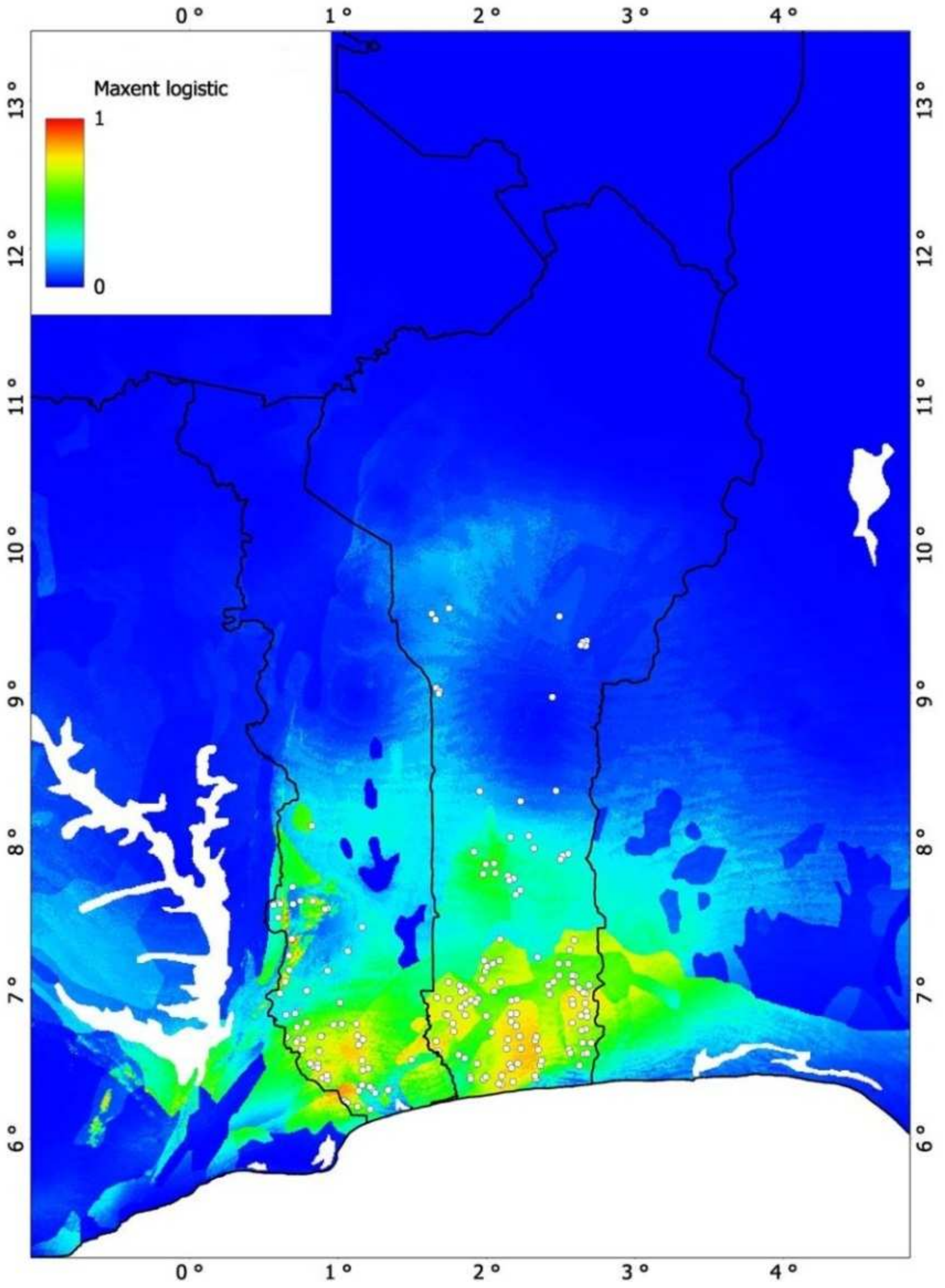

Figure 2.5: Potential cultivatable area of sweet trees in the Dahomey Gap: white dots are samples used in this study

Discussion

Ecological differences between wild bitter and sweet trees

The difficulty to distinguish between bitter and sweet trees based on morphological characters was the key argument of Okafor (1975) to opt for the taxonomic level of variety instead of species. In general, ABMTs occur in the wild on both sides of the Dahomey Gap from Senegal in the west to Sudan and Uganda in the east and Angola (Cabinda) in the south (Harris, 1996; Lesley and Brown, 2004). Our study demonstrates that within the distribution range, bitter and sweet trees populate significantly different ecological environments (Fig. 2.2e). The potential distribution of sweet trees is restricted to the wetter parts of the Guineo-Congolian phytochorion. This is consistent with findings of Kang *et al.* (1994), which indicated better growth and fruiting performances in Port Harcourt (south-eastern Nigeria, 2400 mm rainfall) than in Ibadan (south-western Nigeria, 1300 mm rainfall). In contrast, the extension of bitter trees to dryer climatic conditions (see Ndoye *et al.,* 1997) was also confirmed by our models (Fig. 2.1b) with optimum habitat suitability at relative low levels of precipitation in the coldest quarter. This is consistent with higher fruiting for bitter trees compared to sweet trees in the drier south-western Nigeria (Ibadan; Kang *et al.,* 1994). As such, the bitter trees display a higher ecological variability than the sweet trees (Fig. 2.1a & 2.1b). Therefore, soil total gravel content, determining soil structure and consequently its water holding capacity (Norman and Gary, 1990), has no limiting effect on the distribution range of bitter trees. The above can be regarded as support for the hypothesis that bitter and sweet trees should be viewed as taxonomically different species, *I. wombolu* and *I. gabonensis*, respectively (Harris, 1996). Our results suggest that such differences have a genetic basis and hence that the two taxa maintain their genetic identity and do not interbreed. The existence of a genetic difference is corroborated by the study of Lowe *et al.* (2000). Significant differences in phenology of bitter and sweet trees are also found by Vihotogbé *et al.* (submitted-2), further supporting the species level differentiation.

Occurrence and origin of ABMTs in the Dahomey Gap

This study shows that the Volta forest region, the wettest region in the Dahomey Gap, is an ecological area different from the ecological niche of wild bitter trees. In the Volta forest region, bitter trees are known as “the never cultivated ABMTs” or “wild only ABMTs”, while in the rest of the Dahomey Gap, local communities are not familiar to this taxon (Vihotogbé *et al.,* Submitted-1). Local communities in the Volta forest region confirmed that sweet trees have been introduced and are cultivated primarily for their sweet mesocarp, but the seed of any ABMTs has no place in their diet. Therefore, apart from a general use as timber, bitter trees have no direct value for these local communities: the mesocarp is inedible, and seed-based diets are still not admitted in the Volta region and even not totally accepted elsewhere in Togo. Also, local knowledge about social and medicinal use of ABMTs is very poor (Vihotogbé *et al.,* 2008). Bitter trees are sampled in the Volta forest region in natural but highly disturbed forest contiguous to farmlands and human settlements, and most importantly in primary agroforestry systems (forest gardens) and on farms. We postulate here that bitter ABMTs are native to the Volta forest region and are passing from a wild status within natural forest to a situation with scattered individuals preserved in agricultural productive spaces. Population viability might be threatened by the limited extent of this ecological region. When a tree species has no important socio-economic value, and in fact its wood presents the highest economic advantages for land owners, its distribution will potentially be reduced over time (Vihotogbé *et al.*, submitted-1). This is the case for bitter trees in the Volta forest region, since the expanding cultivation of coffee, cacao and bananas is strongly reducing the regeneration (Sodhi, 2007; Posner, 2008). The fact that the ecological niche models based on Dahomey Gap collections and those from elsewhere in Africa are less similar than expected by chance (Fig. 2.4d) might be explained by model over-fitting as a result of over-representation of collections in the Dahomey Gap related to collection efforts for this study.

Lowe *et al.*, (2000) postulated the presence of genetic diversity centres of bitter trees in southern Nigeria and south-eastern Cameroon. These coincide with our predicted highest occurrence probabilities (Fig. 2.1b). Additionally, our results also suggest high occurrence probabilities in the Upper Guinean forest (Harris, 1996). The Dahomey Gap isolates the Upper Guinean from the Lower Guinean forest block, and thus prevents the natural exchange of genetic material (Giresse, 2007). Therefore, the Lowe *et al.*, (2000) study should preferably be extended to include material from Upper Guinea to validate their conclusions for the entire distribution range of bitter trees.

The Dahomey Gap is an ecological zone where sweet trees occur, but outside their natural ecological niche (Fig. 2.4b). Even in forest relicts preserved throughout the Dahomey Gap, including the comparatively wetter Volta forest, no evidently wild sweet trees were recorded. Ethnobotanical data suggest the introduction of sweet trees in the Dahomey Gap from the Lower Guinean forest block through migration of the Yoruba people and their subsequent settlement in south-eastern Benin. From here, material spread to other parts of Benin and Togo (Vihotogbé *et al.*, submitted-1). This is consistent with the conclusion of Ude *et al.* (2006) who suggest that sweet trees dispersed from the Lower Guinean forests to drier ecological areas in the Soudania Transition zone. Therefore, we postulate that the occurrence of sweet trees in the Dahomey Gap is solely due to cultivation activities (Harris, 1996; Tchoundjeu and Atangana, 2007).

With respect to the presence and cultivation of sweet trees in the Dahomey Gap, a key question is: where does the genetic material comes from? In other words, is it true that sweet trees migrated from the Lower Guinean forest block? Firstly, old individual trees, particularly valuable food trees, are claimed to be planted by local communities in West Africa to prove land ownership (Neef, 2001). Secondly, in Benin, local communities in ancient forested areas consider old sweet trees as relicts of what has existed in the wild in the past before oil palm plantations were introduced. This latter argument is strengthened by an ancient taboo forbidding the cultivation of bush mango trees in Benin (Vihotogbé *et al.*, submitted-1), contradicting former widespread planting initiatives. Sayer (1992) postulated that in the Dahomey Gap the expected natural vegetation under the current climate would consist of semi-deciduous forest interspersed with tracts of denser vegetation types such as riverine forest as well as patches of swamp forest and lowland evergreen forest (preferred habitats of ABMTs; Lesley and Brown, 2004). Nagel *et al.* (2004) and Tossou *et al.* (2008) claimed that this implies that the present savannah vegetation was induced by humans. Salzmann and Hoelzmann (2005) show that before ca. 4500 yr BP the Dahomey Gap was covered with evergreen lowland rainforest containing typical Guineo-Congolian forest trees species. This past climatic phase plays an important role in explaining present-day tree species distributions (Linder *et al.*, 2005; Waltari *et al.*, 2007). Since the ecological niches obtained using the Maxent algorithm express the shared *current* environmental conditions between samples, they lack the possibility of tracing the influence of historical elements on the present-day distribution (Parmentier *et al.*, 2011). Therefore, hindcasting the potential distribution with past climatic data could be particularly useful in addressing this question to indicate habitat suitability in the past in order to infer the true origin of sweet trees being managed in the Dahomey Gap. Moreover, an effort to collect genetic material covering the entire actual distribution range is recommended to improve the conservation strategy for ABMTs.

Potential cultivatable area of sweet trees in the Dahomey Gap

Vodouhè (2003) demonstrated that the mesocarp quality of sweet trees plays an important role in the distribution in Benin. Moreover, in Togo, where sweet trees are also intensively cultivated, the seed-based diets are still considered to be foreign diets from Nigerian and Beninese people (Vihotogbé *et al.,* submitted-1). Therefore, especially the sweet mesocarp consumption has played a key role in the spread of cultivation initiatives. This led to an expansion of the cultivated area of sweet trees to new ecological zones that differ significantly from their natural ecological niche. The “easy to grow” nature of sweet trees is well illustrated by the non-significance of major soil fertility parameters (textural, organic carbon content, drainage, and water storage capacity) in the definition of the ecological niche. Trees are grown on a variety of soil types and the low rainfall in the Dahomey Gap appears to be sufficient to maintain an economically profitable yield (see Chinaka and Obiefuna, 1999).

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Chapter 3

Backgrounds of the domestication process of African bush mango trees (*Irvingiaceae*) in the Dahomey Gap (West Africa)

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Abstract

Bitter and sweet African bush mango trees (ABMTs) belong to the family Irvingiaceae and are valuable non-timber forest products in sub-Saharan Africa. They have not been studied well in the western part of their distribution range, and many aspects of their large-scale development remain unknown. In this study, we link the agroforestry status of ABMTs to differences in socio-cultural groups in order to identify the key factors influencing their abundance and survival in the Dahomey Gap.

First, we gathered the uses and local management strategies from the nine main socio-cultural areas in Benin and Togo. Second, occurrence data were obtained throughout the Dahomey Gap and imported into DIVA-GIS and MATLAB to calculate the spatial density pattern and analyse its structure. The variation of this pattern was analysed relative to three factors: the country, the phytogeographical zone and the dominant FAO soil category. Third, agroforestry system characteristics and farmers' social status relative to 841 trees were used in a multinomial logistic regression to identify anthropogenic factors driving the intensive cultivation of ABMTs. Finally, the impact of socio-cultural activities on extent and density of ABMT populations was analysed.

In the entire study zone, the sweet mesocarp is consumed and the valued seed of bush mangoes is commercialized. The application of seed-based diets and socio-therapeutic uses are common to communities in Benin. Sweet ABMTs are generally found either in home gardens or cultivation fields where they may occur at high densities (up to 1020 trees per 25 ha). Bitter trees, however, are confined to the Volta forest region in Togo and occur at low densities (< 462 trees per 25 ha) in the wild, sometimes in protected areas, in forest gardens as well as on fields. This indicates a clear difference in cultivation methods between bitter and sweet trees. Farmland status, farmer socio-cultural group and type of ABMTs determined the cultivation intensity. The fact that small farmlands are converted into sweet ABMT orchards indicates that farmers actively cultivate ABMTs in the Dahomey Gap. Diversity of indigenous knowledge, however, is not correlated to intensive cultivation nor to domestication efforts or local genetic conservation programs. Where slash and burn agriculture and intensive collection of fruits jeopardize bitter trees, traditional fishing systems (using twigs), a traditional selection strategy, and intensive land commercialization severely threaten sweet ABMT genetic resources.

Key words: Benin, conservation, ethnobotany, *Irvingia*, kriging, spatial distribution, Togo

Introduction

Non Timber Forest Products (NTFPs) are increasingly being studied due to their growing importance in enhancing livelihood. They play a key role in sustainable ecosystem management design (MMF and UNBC 2005; Delang, 2006) and are shaping land use systems in terms of their specific composition and spatial configuration. Millions of people have traditional knowledge on NTFPs and use them on a daily basis (Boateng *et al.*, 2007). The increasing demand for NTFPs is reflected in a growing commercial trade (Arnold and Ruiz Pérez, 2001) which is becoming an important economic incentive for local collectors. Consequently, natural ecosystems may well suffer from over-exploitation of their plant genetic resources (McLain and Jones, 2005), especially in areas where people are economically dependent on NTFPs (Ticktin, 2004). In the context of the rapid growth of the human population and a NTFP production limited by the natural ecosystem capacity, a sustainable harvest strategy reconciling plant genetic resources conservation and local community livelihood seems a utopia (Rai and Uhl, 2004). The development of traditional agroforestry systems, from forest gardens to orchards, indicates that local communities understand the need to carefully select useful plant species for cultivation and domestication (Wiersum, 2004). This partially decreases the dependence of communities on natural ecosystems although they still depend on natural forests for those NTFPs that cannot easily be cultivated such as primary rainforest species, wildlife, etc.

African bush mango trees (ABMTs: *Irvingia* spp.) belong to the small family of Irvingiaceae (Harris, 1996), of which seven species occur in West and Central Africa. Their fruits are economically one of the most important NTFPs in sub-Saharan Africa (Asaah *et al.*, 2003; Leakey *et al.*, 2003). They are among the priority food tree species abundant in traditional agroforestry systems and over the last decade have been subject to domestication trials by the World Agroforestry Centre (Franzel *et al.*, 1996). The mesocarp of the mango-like fruits is either bitter or sweet (Harris, 1996). Only the sweet mesocarp is edible, while the seeds (enclosed in the hard endocarp) of both bitter and sweet fruits are used as a sauce thickening agent (Leakey *et al.*, 2005, Tchoundjeu and Atangana, 2007). Bitter and sweet trees are taxonomically closely related and there is some debate about the correct level at which their differences should be valued (National Research Council, 2006). While Okafor (1975) presented the two types as varieties of the species *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill.: *I. gabonensis* var. *gabonensis* and *I. gabonensis* var. *excelsa*

(Mildbr.) Okafor, respectively), Haris (1996) argues they are best treated as different species (*I. gabonensis* and *I. wombolu* Vermoesen, respectively).

Because of the taxonomic uncertainties, it is difficult to assign many of the known uses to either the sweet or the bitter taxon (Oyen, 2007; Tchoundjeu and Atangana, 2007). In Central Africa, Lesley and Brown (2004) identified more than fifteen different indigenous uses related to ABMTs, but failed to distinguish between the therapeutic and biochemical properties of sweet and bitter trees.

Initially marketed for consumption by African migrants throughout the world (Tabuna, 2000), the ABMT fatty seed is nowadays increasingly becoming an important raw material for a growing number of food processing and cosmetic industries (Akubor, 1996; Oyen, 2007). Many CGIAR-funded organizations, among which the World Agroforestry Centre and Bioversity International, identified ABMTs as a priority food tree species for West and Central Africa. At the individual tree level, Vodouhê (2003) and Vihotogbé *et al.* (2008) studied the local economical profitability and the indigenous factors driving the eligibility of trees for conservation purposes in the Dahomey Gap, respectively. Throughout their entire distribution range, however, intensive cultivation systems of ABMTs remain rare (see Lowe *et al.,* 2006) and the basic drivers of and strategies for their domestication and intensive cultivation unstudied.

Traditional knowledge about food cultivation is often shared among a socio-cultural group as an integral part of their social heritage and natural resources management (Reyes-Garcia, 2001, De Caluwé *et al.,* 2009). Thus, assessing the relation between the main uses by a socio-cultural group and the abundance of ABMTs is important in order to evaluate their future potential as a crop for sub-Saharan Africa (Leakey *et al.,* 2005). Moreover, abundance, size-class distribution and threats are important structural parameters for the management of trees in traditional agroforestry systems (Gouwakinnou *et al.,* 2009) and as such they help detect local management strategies.

This study will focus on ABMT population structure in the Dahomey Gap (Benin and Togo). We want to quantify how traditional knowledge on AMBT usage and social status of farmers affect the allocation of productive space to bitter and sweet ABMTs. We aim at providing answers to three research questions:

(i) What is the spatial pattern of ABMT abundance in the Dahomey Gap?

(ii) Which anthropogenic factors are influencing this spatial pattern and hence the conservation of ABMT genetic resources in the Dahomey Gap?

(iii) Is there a difference in traditional knowledge on ABMT usage between the different socio-cultural areas in the Dahomey Gap, and how does this affect the local management strategies for ABMTs?

Material and Methods

Study area

The Dahomey Gap is the dry corridor dividing the West African rain forest block into the Upper and Lower Guinean phytochoria (Salzmann and Hoelzmann, 2005; White, 1979). This climatically dry corridor where savannah reaches the coast extends from Accra (southern Ghana) to Badagry in south-eastern Nigeria (Maley, 1996; Sowunmi, 2007). This study was carried out in Benin and Togo, representing the majority of the extent of this eco-region where small patches of natural forest and sacred forests are scattered in a wide grass or shrub savannah area. Large parts of this region have been transformed to cultivated fields (Kokou and Sokpon, 2006). The Dahomey Gap is located in a sub-humid Guinean climatic zone with a bimodal rainy season (April to June and September to November), mean annual rainfall of 900-1,200 mm and mean temperature of 25-29°C. This study covers all nine major socio-cultural areas: Aizo, Adja, Akposso, Ewe, Fon, Goun, Nago, Holli and Watchi (Heldmann, 2008; Atato *et al.,* 2010; Fig. 3.1).

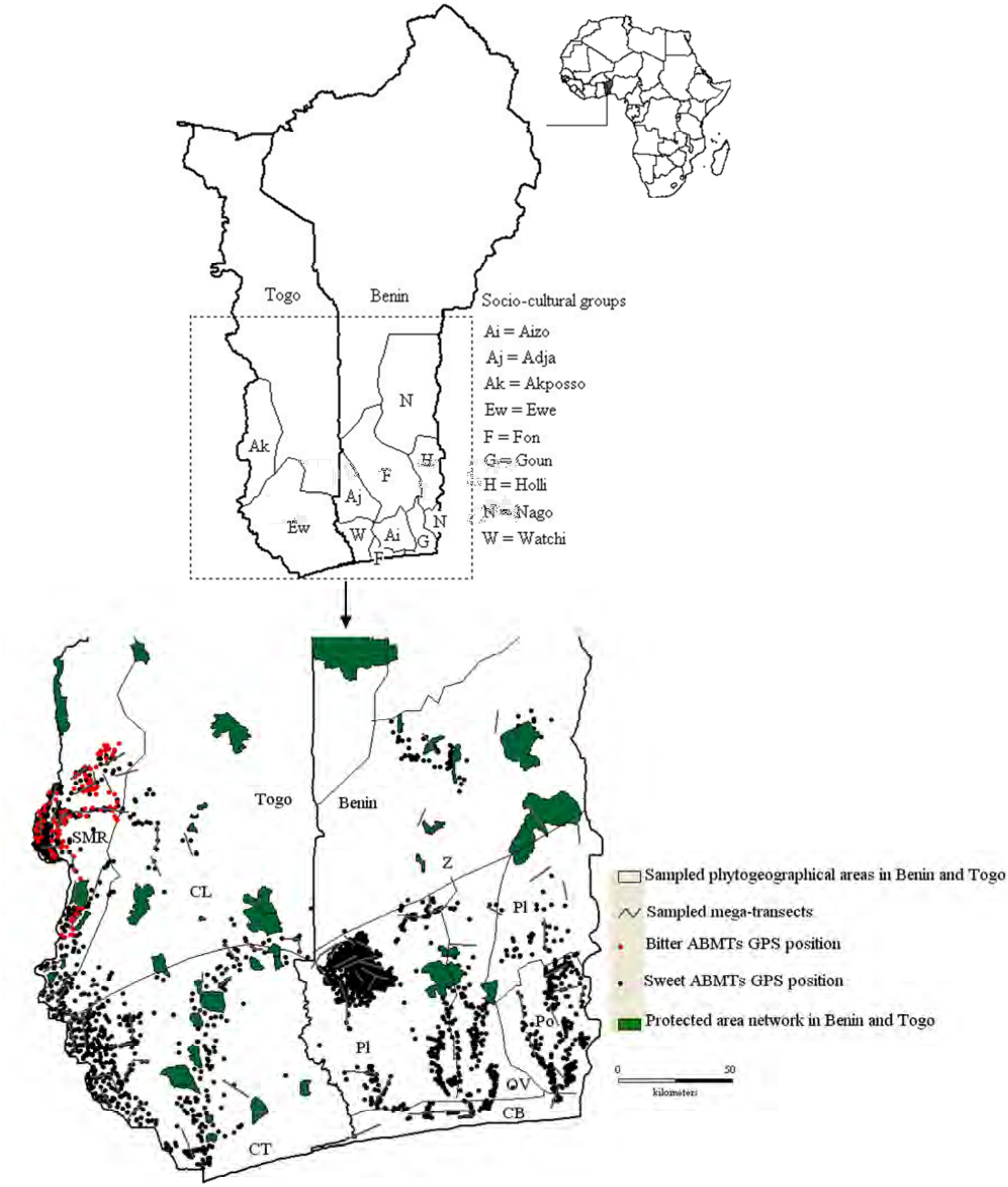

Figure 3.1: Sampled socio-cultural areas, mega-transect ABMTs records in different phytogeographical regions in Benin and Togo: Phytogeographical regions: Z = Zou; Pl = Plateau; OV = Oueme-Valley; PO = Pobè; CB = Coast-Benin; CL = Centre Lowlands; SMR = Volta zone = Southern Mountain Region; CT = Coast-Togo

Table 3.1: Sampled socio-cultural areas, main uses and anthropogenic activities impacting on ABMT populations.

Type of ABMTs	Geographic areas (see figure 1)	Socio-cultural groups	Profile of individuals	Most important economic product	Major socio-economic activities	Domestication process
Sweet trees	South West Benin	Adja	T_{35}:$O_{26}P_{9}$	Seed	Subsistence agriculture, Palm oil tree and food tree species	Only cultivated trees under a missal selection process importantly based on endocarp size regardless fruit's parasitism and the firmness and sweetness of the mesocarp.
	South Benin	Aizo	T_{32}:$O_{22}P_{10}$	Entire fruit (based on the mesocarp quality) and less importantly the seed	Subsistence agriculture plus most importantly traditional fishing using ABMTs branches for the fishing system construction	Preserved spontaneous trees obtained from human-thrown seeds. No action or interest for traditional selection.
	South Benin	Fon	T_{31}:$O_{21}P_{10}$			
	Lower South East Benin	Goun	T_{29}:$O_{22}P_{7}$			
	Upper South East Benin	Holli	T_{37}:$O_{19}P_{18}$	Entire fruit (based on the mesocarp quality) and less importantly the seed	Subsistence agriculture and palm oil trees	Unclear domestication status (pretended wild + spontaneous + planted). Trees are preserved importantly based on fruits parasitism and the firmness and sweetness of the mesocarp regardless mesocarp size.
	Upper South East Benin	Nagot	T_{22}:$O_{18}P_{4}$			
	Lower South West Benin	Watchi	T_{25}:O13P_{12}	None	Traditional fishing using ABMTs branches for the fishing system construction	Unclear domestication status
	Upper South West Togo	Akposso	T_{43}:$O_{29}P_{14}$	Seed	Cash crops (cocoa, coffee, banana, avocado), food tree species and subsistence agriculture	Unclear domestication status
	South Togo	Ewe	T_{40}:$O_{29}P_{11}$	Seed	Subsistence agriculture and	Only cultivated trees without

Type of ABMTs	Geographic areas (see figure 1)	Socio-cultural groups	Profile of individuals	Most important economic product	Major socio-economic activities	Domestication process
					food tree species	selection process
Bitter trees	Upper South West Togo*	Akposso		Seed, wood	Cash crops (cocoa, coffee, banana, avocado), food tree species and subsistence agriculture	Only wild trees preserved in traditional agroforestry systems
	Upper South West Togo	Akposso		Seed, wood	Protected areas	Only wild trees still far from cultivation initiative taking exploited by local communities and wood industries.

T = total surveyed, P = number of professional users (traditional practitioners, traditional religion priests), O= number of ordinary people.
Subscript numbers refer to the total number of individuals surveyed in each group

Sampling design and data collecting

The ethnobotanical database gathered in 2007 by Vihotogbé *et al.* (2008) in six major socio-cultural areas in Benin (Aizo, Adja, Fon, Goun, Nago, Holli) was supplemented with an ethnobotanical survey carried out in the three other major socio-cultural areas in the Dahomey Gap: Watchi in Benin and Ewe and Akposso in Togo. Moreover, since the 2007 database included relatively few professional users of biodiversity (i.e. traditional healers, religious specialists or vendors of medicinal plants), additional surveys were performed to specifically target such individuals in the six socio-cultural areas. In the three new areas, we aimed for a good mix of professional and non-professional users from the start.

Using a structured questionnaire, every individual was asked about his/her knowledge on different uses of any part of ABMTs (roots, stem, bark, leaves,) as well as his/her acceptance of the consumption of sweet bush mango and that of seed-based diets. Together with the 2007 database, data from a total of 294 respondents were analysed to apprehend the traditional knowledge and the current importance of ABMTs in the Dahomey Gap. Additionally, using direct on-farm observations and open survey techniques, data on the main traditional management systems were recorded.

Given the large size of the geographical area involved, a field research unit area was designed as a modified mega-transect, a technique used by Assogbadjo (2006) to quantify the occurrence of Baobab trees *(Adansonia digitata* L., Bombacaceae). Each mega-transect comprises a 10 km long line, along which observations were made in a zone of 250 m both left and right and hence covers a surface of 5 km^2. In total, 59 such mega-transects were sampled, three to seven in each socio-cultural area (Fig. 3.1).

When scattered at low density in the landscape, all individual adult and young bitter and sweet trees in a mega-transect were geo-referenced. In case an area with a high density of ABMTs was encountered, their number was estimated as follows. The trees bordering such high density areas were all accurately geo-referenced supplemented with all trees within two randomly defined 100 x 50 m sub-plots. Then, the total number of ABMTs present in the high density area was estimated from these data. Although not exhaustively, trees encountered between sampled mega-transects were also recorded and areas of ABMTs absence along and between mega-transects were marked as zero density points.

The influence of farmers on the spatial distribution pattern and conservation of genetic resources was assessed by analysing data from 841 adult trees from different agroforestry systems and corresponding to 841 farmers met in the field. For all 841 trees / farmers, the following ten parameters were recorded: (i) taste of mesocarp, (ii) nationality of farmer, (iii) socio-cultural group of farmer, (iv) farmer's respect of taboos regarding ABMT cultivation, (v) reason why the farmer cultivated or preserved ABMTs, (vi) local marketing system, (vii) local land use system, (viii) local land tenure combined with local judgment of farmland size, (xi) agroforestry system applied, and (x) the farmer's social-cultural status.

To investigate the structure and disturbance of *Irvingia* populations, six populations with enough adult trees (at least 30) were selected that had a unique combination of a particular agroforestry system, socio-cultural group, and a particular reason for ABMTs management (Table 3.1; Fig. 3.1). In low density areas, population data was obtained using two randomly selected 10 x 0.5 km mega-transects, while in high density areas, it was obtained from the two 100 x 50 m sub-plots used to estimate the tree density in four mega-transects (Fig. 3.1). Diameter at breast height (dbh) was measured for all adult trees (dbh ≥ 7 cm) of sampled *Irvingia* populations and all detected saplings and seedlings (dbh < 7 cm) were counted. In addition, these areas were visited throughout a year, specifically during fruit harvesting and land preparation, in order to identify signs of destructive management strategies and of any other human induced impact rooted in the main economic activities specific to the socio-cultural groups: pruning for different purposes, juvenile clearance and damage caused by harvesting techniques of bush mangoes or other NTFPs. Information on how people protect or remove/damage seedlings and saplings was also obtained through interviews.

Data analysis

Local use of ABMTs in the Dahomey Gap

Interviews led to a total of 23 different uses of ABMTs (Table 3.2b). For each respondent, each type of use was recorded as known / present (1) or unknown / absent (0). Thus, we assessed the level of ethnobotanical knowledge (Mn_{id}) for the i^{th} individual by:

$$Mn_{id} = \frac{Ni}{Nt}\ x\ 100\ \% \qquad (1)$$

Where Ni = number of uses known by the i^{th} individual and Nt = total number of uses recorded.

We distinguished two groups of *Irvingia* users: professional ones (traditional practitioners, traditional religion priests or vendors of medicinal plants) and non-professional ones (all other respondents).

Power transformation for percentage data (Box and Cox, 1964) was applied to Mn_{id} in order to normalize the data and stabilize their variance. Using Statistica version 6 (StatSoft, 2001), a two way Analysis Of Variance (ANOVA-2) was performed on the transformed Mn_{id} values in order to identify factors influencing the level of knowledge on ABMT uses. In this model, the two fixed factors are “socio-cultural group” and “user group”. The main effects of these factors as well as that of their interaction were assessed in this analysis.

In order to detect if respondents could be categorized geographically and to compare the two user groups, a Principal Component Analysis (PCA) was performed on a 0/1 matrix of all known uses per respondent and one column for each geographically defined socio-cultural region. Principal component scores from the first three axes of the PCA were visualized in two-dimensional plots. Since factors presenting no variability of responses introduce zero variation in the PCA, uses known to all respondents were deleted from the matrix.

Table 3.2a: Result of Analysis of Variance of level of ethnobotanical knowledge.

Source of variation	**Degree of freedom**	**Mean Square**	**F statistics**	**P value**
Socio-cultural group	8	0.75	185.45	< 0.0001
Socio-professional group	1	0.45	112.09	< 0.0001
Socio-cultural group * Socio-professional group	8	0.14	35.39	< 0.0001

Table 3.2b: First two principal components from the PCA on the ethnobotanical variables

Ethnobotanical variables	**First axis (48.56 %)**	**Second axis (10.95 %)**	**Third axis (8.49 %)**
1- First energetic use: ABMTs' branches + wood for domestic energy	-0.18	-0.27	0.90
2- Second energetic use: Bush mangoes' wooden for domestic energy	-0.18	-0.27	0.90
3- First socio-cultural use: dried branches to serve Holli + Nagot native fetish	-0.82	0.15	-0.06
4- Second socio-cultural use: Mature wood (trunk): incarnation of died twins	-0.78	0.31	0.09
5- Third socio-cultural use: dried mature wood for fetish drum making	-0.80	-0.26	-0.09
6- Economic uses: Leaf + fruit to accelerate other fruits ripening	-0.54	-0.76	-0.12
7- First therapeutic use: immature fruit accelerates the digestion of nitrogenous foods (mainly beans)	-0.24	0.10	0.17
8- Second therapeutic use: mature fruits as laxative	-0.64	-0.05	-0.24
9- Third therapeutic use: seed-based sauce against ulcers	-0.78	-0.30	0.05
10- Fourth therapeutic use: decoction of fresh leaves against malaria	-0.81	-0.26	-0.06
11- Fifth therapeutic use: decoction of fresh leaves to reinforce bladder excitation during the night.	-0.84	0.32	0.03
12- Sixth therapeutic use: fresh leaves against specific child diseases	-0.77	-0.43	-0.06
13- Seventh therapeutic use: bark in female gynaecology	-0.89	0.18	-0.07
14- Eighth therapeutic use: root decoction against impotence	-0.65	0.23	0.18
15- Ninth therapeutic use: bark decoction against haemorrhoid	-0.72	0.26	0.10
16- Tenth therapeutic use: seed oil against skin disease	-0.85	0.20	-0.06
17- Eleventh therapeutic use: small branches chewed against bad breath	-0.67	0.22	0.12
18- Twelfth therapeutic use: bark to treat wounds	-0.84	0.22	0.04
19- Thirteenth therapeutic use: bark decotion to balance human body temperature	-0.58	-0.25	-0.19
20- Fourteenth therapeutic use: bark to reinforcing babies' fontanel	-0.83	0.28	0.05
21- Fifteenth therapeutic use: bark to treat mycosis	-0.49	-0.19	-0.28
22- Sixteenth therapeutic use: leaf decoction as analgesic	-0.54	-0.76	-0.12
23- Seventeenth therapeutic use: bark decoction against dyspnoea	-0.84	0.32	0.03

Occurrence and density data reconstruction

Within each mega-transect, the border trees of each high density area were projected in DIVA-GIS (Hijmans *et al.*, 2001) and its extent was approximated by the derived convex polygon. The total number of ABMTs *(NT_j)* in each high density area was estimated by:

$$NT_j = N_s * S_{tj} \, / \, 10{,}000 \qquad (2)$$

Where N_s the total number of trees in the two sub-sampled areas of 100 x 50 m^2 and S_{tj} = the extent of the high density area in m^2.

Using DIVA-GIS, the NT_j trees were artificially generated in a random spatial pattern in the j^{th} area to complete the direct observation database (Fig. 3.1). Then, within each mega-transect, the number of trees that occurred within each 0.5 x 0.5 km^2, was obtained and geo-referenced in the centre of that unit area. Outside of the mega-transects, data on isolated individuals and small tree groups scattered in low density areas was entered as well with their GPS position. Zero density points were also added with their GPS position. The latter information was transferred to a 0.5 x 0.5 km^2 grid to thus complete the geo-referenced density database.

Spatial analysis of ABMT abundance in the Dahomey Gap

First, to assess which environmental variables influence the density, the layer of density points was superimposed with those of the countries (Benin and Togo), the FAO soil type (http://www.fao.org/geonetwork/srv/en/main.home#soils) and the phytogeographical zones in Benin (Adomou, 2006) and Togo (Ern, 1979). Using DIVA-GIS, the corresponding values were extracted into a database.

Bitter trees have a limited distribution being restricted to the Volta forest region (the Southern Mountains phytogeographical region in Togo). The density information turned out to have too few data points for a reliable statistical analysis. This is why, in this study, the analysis of their abundance and spatial structure will remain descriptive only.

For sweet trees zero density values were replaced by 0.1 and a natural logarithm transformation was applied. Using SAS 9.2 (SAS Institute Inc., 1999) an Analysis Of Variance (ANOVA) was carried out to assess the effect per variable.

Spatial variability of sweet ABMT density was measured using a geo-statistical approach (Rossi *et al.,* 1992; Christakos *et al.,* 2002). In this analysis, we ignored all short-term dynamic aspects that might change the spatial pattern, such as abundant regeneration in the rainy season, planting or selective elimination of trees, etc. Therefore, the density pattern was considered as a finite stock of trees expressing the environmental and conservational conditions in the geographic space. As such, we assume it to be a Gaussian random field at the second order stationarity (Goovaerts, 1997). This implies that the mean density is constant over the geographic space and the covariance between density values at position *x* and those at position $x + h$ exists and depends only on *h* not on *x*. In this study, we preferred the semivariogram function, which is simpler and more robust than the covariance (Baillargeon, 2005) to measure the patchiness or the spatial pattern of ABMTs' density across the Dahomey Gap. The Bayesian Maximum Entropy Library (BMELib: Christakos *et al.,* 2002) compiles consistent functions written in the Matrix Laboratory (Matlab) language to catch spatial structure by a geostatistical analysis. Using BMELib in MATLAB version R2011a (MathWorks Inc., Natick, MA, USA), the density semivariogram function was calculated as follows:

$$V(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [d(x_i) - d(x_{i+h})]^2 \text{ (Goovaert 1997)} \qquad (3)$$

With $V(h)$ = density semivariogram estimator; $d(x_i) - d(x_{i+h})$ = difference of mean of densities separated by a lag distance of (h) and $N(h)$ = number of data pairs in this lag distance. Omnidirectional as well as directional (North-South, West-East, 30° and 60°) semivariograms were calculated to check significant directional variations in ABMTs spatial structure. Only the omnidirectional semivariogram was modelled using the "eye fitting technique" (see Rossiter, 2007) and its parameters (nugget effect, sill and rang) were used to characterize the spatial structure of ABMT-based agroforestry systems in the Dahomey Gap.

Kriging is the more reliable estimation technique integrating semivariogram parameters and information regarding observed surrounding data points (mean and distance) and minimizing the prediction variance (Goovaert, 1997; Kumar and Remadevi, 2006). It promotes spatial visibility of information contained in the semivariogram. In non-sampled areas random points were generated in 0.5 km x 0.5 km grids cells. Using the BMELib in Matlab and based on the model of the obtained omnidirectional semivariogram, the density at non-sampled points within the Dahomey Gap was estimated by means of the ordinary kriging method (Goovaerts, 1997; Christakos *et al.,* 2002):

$$d_e(x_0) = \sum_{i=1}^{N(x_0)} w_i d(x_i) \qquad (4)$$

Where $d_e(x_o)$ is the estimated density value at location x_0, $\sum_{k=1}^{n(i)} w_k(i) = 1$ = the sum of the weights (w_i) of $N(x_0)$ surrounding points with $d(x_i)$ values.

Analysis of the anthropogenic drivers of ABMT spatial density patterns

Using Statistica version 6 (StatSoft, 2001), a Multinomial Logistic Regression Analysis (Hosmer and Lemeshow 1989) was carried out on the eight parameters influencing the conservation and cultivation of ABMTs. The Global Null Hypothesis (GNH: BETA=0) and the Type 3 Effects Analysis were used to validate the accountability of the CMLRA and identify the most causative parameters, respectively. The Maximum Likelihood Estimate and the Odds Ratio Estimate help to identify parameters that hinder or induce the conservation or cultivation and give a comparative basis of the influences related to different levels of the causative parameters.

Variation of ABMT population structure

For each population, the size distribution (DBH) was obtained and the coefficient of skewness (ℓ) was calculated to characterize its structure:

$$\ell = \frac{1}{n}\sum_{\alpha=1}^{n}\left(z(\alpha) - m\right)^3 / \sigma^3 \text{ (Goovaerts, 1997)} \qquad (3)$$

Where n = total number of individuals within the population, $z(\alpha)$ = dbh of the α^{th} individual within the population, σ = standard deviation of dbh values, m = mean of the dbh in the population. For each population, the percentage of trees affected by each type of physical damage was calculated for each diameter size-class: 0-7, 7-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, and > 100 cm. A two-way ANOVA was run in SAS 9.2 (SAS

Institute Inc., 1999) to assess differences in the level of damage per population. A Tukey test was used to distinguish populations based on their threat level.

Results

Traditional use and management of ABMTs

Sweet ABMTs were recorded in all socio-cultural areas while the bitter ones grow only in the Akposso area in the Volta forest region in Togo (Fig. 3.1).

In all areas the mesocarp of the sweet bush mango and the seed of both sweet and bitter bush mangoes are marketed and the seeds consumed. Table 3.2b provides the twenty-three other uses (socio-economic, medicinal and energetic, see also Vihotogbé *et al*. 2008) that were identified. In comparison to the 2007 study, only one additional use (fruits and / or leaves used for accelerating ripening of other fruits, mostly *Musa* spp. but less often also *Persea americana*, *Ananas comosus*, *Chrysophyllum albidum*), was found.

The ANOVA-2 results are presented in Table 3.2a. They show that the level of ethnobotanical knowledge (Mn_{id}) depends on both the socio-cultural group and the socio-professional group ($p < 0.001$). Also, the level of knowledge was significantly different between the two socio-professional groups ($p < 0.001$). Figure 3.2a shows that in the Adja, Holli and Nagot areas, the professional users detained higher local knowledge on *Irvingia* species, while in the other areas no clear difference was showed between these two groups, the professional and ordinary users showed no clear difference. However, the professional users in the Holli and Nagot areas (mean knowledge = 78% and 65%, respectively) have the most extensive ethnobotanical knowledge of *Irvingia* species in the Dahomey Gap. The Akposso area was that with the least indigenous knowledge (mean knowledge = 1%). Thus, the level of indigenous knowledge (Mn_{id}) appears to be higher in Benin than in Togo and is mostly concentrated in the southeast (Holli and Nagot; Fig. 3.2a).

The PCA shows that the first three axes together account for 68% of the variation within the ethnobotanical data (Table 3.2b). The first axis (48.04%) negatively correlates with all three socio-cultural uses plus fourteen medicinal uses and the second (10.95%) negatively correlates mainly with only one medicinal use, while the third axis (8.15%) represents intensive use of ABMTs as a source of domestic energy (fuel). The first axis (Fig. 3.2b) shows, again, that the Holli plus Nagot people in south-eastern Benin (and most importantly their professional users) had a higher indigenous knowledge than all other communities. The third axis shows that apart from the Akposso and few Nagot people, wood and seed are intensively used as fuel everywhere in the Dahomey Gap.

In Togo, the seed is mostly sold to Beninese and Nigerians while small amounts are locally used to thicken vegetable sauces based on *Corchorus olitorius* (Tiliaceae) and *Abelmoschus esculentus* (Malvaceae). The seeds are still considered to be an essential part of the diet of the Fon, Goun and Nagot people in Benin and the Nigerian Ibo’s. Apart from some Togolese Ewe groups, the high thickening property and brown color of this sauce makes it 'heavy' and not appreciated by Togolese.

From the interviews it turned out that, traditionally, there is a taboo on planting ABMTs among all ethnic groups studied in Benin, since the trees may expose neighbouring households to witchcraft, bad health and even death. However, only few farmers understand and confess that this taboo is rooted in a desire not to accept young owners of economically important trees. But his taboo is being broken and ABMTs are being integrated in intensive cultivation systems through the selection of mother trees and seeds for planting. The definition of mother tree depends on the targeted *Irvingia* products. Trees producing very sweet and 'pasty' fruits are prioritized in areas of fruit commercialization. In contrast, sweet fruits with large seeds (mean size ≥ 52 x 37 x 23 mm) are valued in areas where their usage is dominant. Transplantation of seedlings encountered under superior sweet mother trees is common. However, in some areas, seeds are used to establish orchards and agroforestry parks in taungya agroforestry systems.

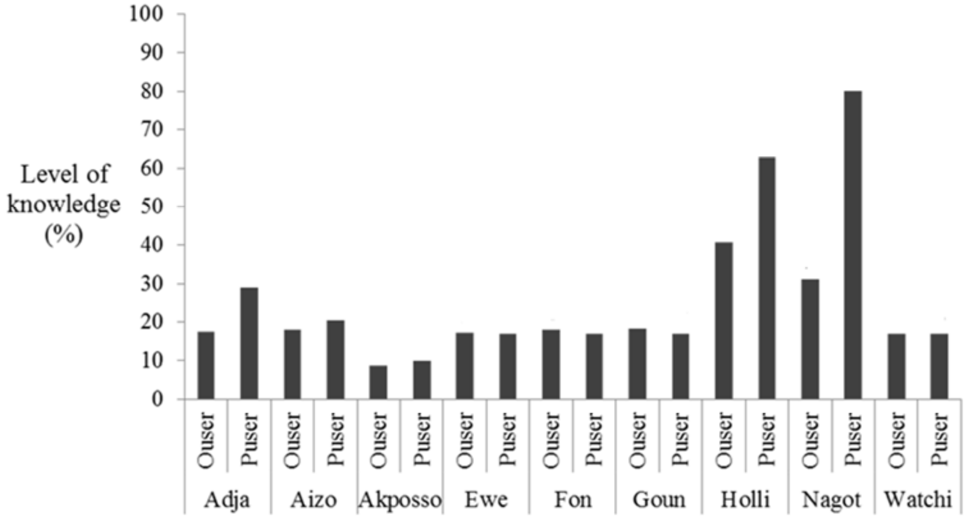

Figure 3.2a: Result of the Analysis of Variance: comparaison of socio-cultural groups, socio-professional groups and their co-influence

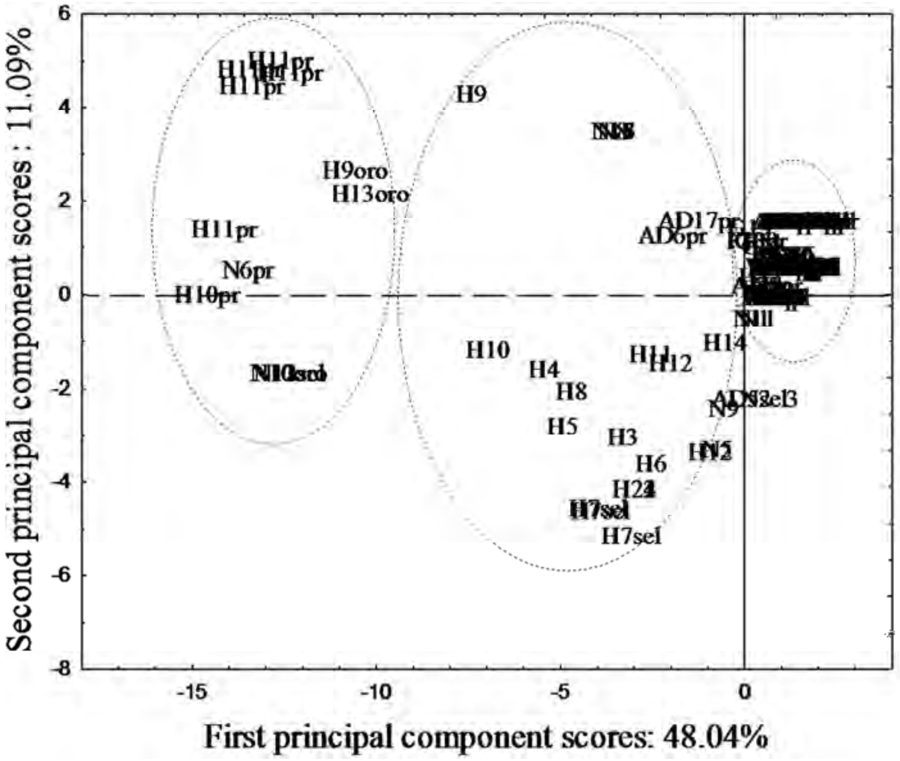

Figure 3.2b$_1$

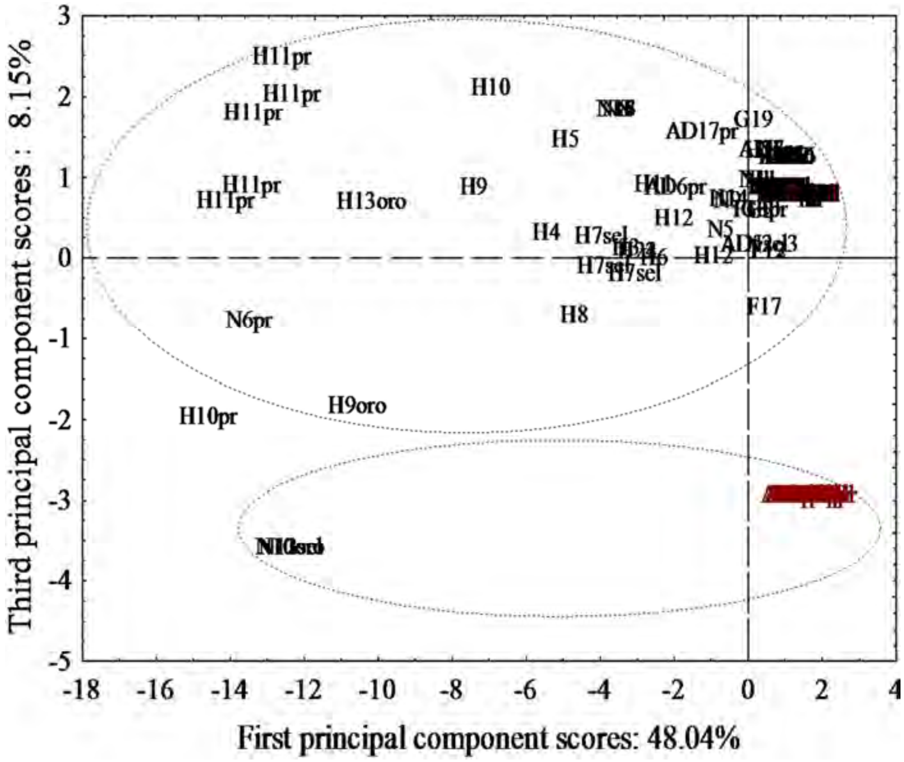

Figure 3.2b$_2$

Figure 3.2b: PCA Analysis of the 1 / 0 matrix of the ethnobotanical knowledge of the nine socio-cultural groups. AD = Adja, Ai = Aizo, Akp = Akposso, E = Ewe, F = Fon, G = Goun, H = Holli, N = Nagot, W = Watchi; pr = traditional healer, sel = merchant of medicinal plants, oro = head of ‘Oro’; rel = traditional religions keeper

In the Akposso area in the Volta forest region of Togo, trees with bitter fruits are called 'the never planted ABMTs'. Wild individuals are preserved on lands being transformed to cacao, coffee and banana fields or in forest gardens. Akposso communities reported that marketing of the seed started 20 years ago with the settlement of Ibo communities and is still considered as a marginal, female activity. However, men prefer the timber of bitter trees as the most economically valuable timber after *Milicia excelsa* (Moraceae).

Spatial structure of ABMT abundance

Density of sweet ABMTs ranges between 0 and 1020 trees / 25 ha (= 0.5 x 0.5 km). Low densities are common while high densities are spatially limited. The overall mean of sweet trees density is 35 (SD = 113) trees / 25 ha. Low densities (ranging from 0 to 462 trees / 25 ha) have also been recorded for bitter trees in the Southern Mountain phytogeographical region in Togo, with an overall mean of 55 (SD = 99.8) trees / 25 ha. In this phytogeographical region where bitter and sweet ABMTs co-occur, bitter trees are found in swampy areas, along streams and on hill slopes, while sweet trees occur everywhere even at the top of hills, but not in swamps or along streams, following human settlements.

The results of the ANOVA indicate that sweet tree density does not vary significantly at the country level ($P = 0.0994$). It is, however, significantly correlated with the FAO soil types and phytogeographical zones ($P < 0.001$ for both factors). The higher densities (mean = 90 and maximum = 1020 trees / 25 ha) are found on lateritic soils (Nd9 and Ne6-2b types) and on the complex ferric shallow soil types (I-Lf-Rd type). High densities occur in the phytogeographical regions of Plateau (in Benin), Central Lowlands and Southern Mountains (in Togo). More specifically, the high densities are located around Kpalimè in Togo and in the Adja and Sakété-Pobè areas in Benin.

The regionalized density data are presented in Figure 3.3a. An isotropic semivariogram model was observed. Only the West-East semivariogram displayed slightly different levels of spatial continuity while the others are all very similar or identical (Fig. 3.3b). However, the overall trend in all semivariograms is identical. The model fitted to the omnidirectional semivariogram was composed of a nugget effect (semivariance = 1), followed by a spherical pattern (semivariance = 2, range = 5 km) and an exponential pattern (semivariance = 1.25, range = 22 km). The highest semivariance value (4.5) in ABMT density is observed after 22 km. This means that the Dahomey Gap comprises patches of ABMT-based agroforestry with uniform densities in an average radius of 22 km. After this, range densities become significantly different in all directions (Fig. 3.3.b) and so, high density areas are spatially limited. Even though low density values are common, their level still significantly fluctuates, confirming the patchy spatial pattern for sweet trees over the Dahomey Gap (Fig. 3.3c).

Potential drivers of the spatial pattern

In the Dahomey Gap, we distinguish three agroforestry systems for *Irvingia* spp.: (i) orchards, (ii) agroforestry parks, and (iii) forest gardens. Orchards are managed through the taungya agroforestry system, and tree density varies between 256 and 400 per ha. The density of 400 trees per hectare ha was encountered in short-term and extensively managed taungya systems with many spontaneous occurrences, while that of 256 trees per hectare is common in intensively managed permanent taungya systems that combine trees and subsistence crops. Agroforestry parks come second in terms of density of ABMTs as priority crop. Most frequently, such parks are owned by farmers belonging to different families inheriting large and continuous farmlands. Each farmer marks his trees with a different colour of cloth to avoid exploitation by others. This confidence-based system relates to the exploitation of the ABMT seed, while the mesocarp consumption remains free (especially in southern Benin). Densities are low (161 ± 82 adult trees per ha) in those parks but more than 400 trees per ha can be detected in areas of natural regenerations. In forest gardens, lower densities are found than in the first two systems.

The results of the logistic regression used to investigate the causal factors behind the density patterns observed, presented a Pr > Chi-Sq < 0.001 for the GNH-Beta = 0 test, indicating that some parameters are indeed involved in this decision-making. Farmland status, ethnic group and the type of ABMT were identified as significant ones (Table 3.3a: Type 3 Effect Analysis). Having the lowest coefficient, an unclear inheritance status of the farmland apparently highly weakens the desire to develop an orchard (Table 3.3b: Maximum Likelihood Estimation). Considering farmland tenure, it appears that especially private, poor and small farmlands are being converted into ABMT orchards (Odd Ratio Estimation Point = 422 in land tenure options: Table 3.3b). On the other hand, farmlands with an uncertain inheritance status apparently give no confidence to farmers to engage in intensification of ABMT cultivation (Odd Ratio Estimation Point = 0.005; Table 3.3b). Regarding socio-cultural groups, significant initiatives for orchards development have been undertaken only by the Adja people.

Table 3.3: Logistic Regression Analysis of factors driving the desire of local farmers to intensify ABMTs cultivation.

Table 3.3a	**Type 3 Analysis of Effects**		
Effect	DF	Wald Chi-Sq	Pr > Chi-Sq
Farmland status	4	0.8297	< 0.0001
Socio-cultural group	8	2.4673	< 0.0001
ABMT Type	1	5.4446	< 0.0196

Table 3.3b		**Analysis of Maximum Likelihood Estimates**					**Odds Ratio**	
Parameters		**DF**	**Estimated coefficient**	**Standard Error**	**Wald Chi-Square**	**Pr > Chi- Square**	**Reference factor**	**Point Estimates**
ABMT type	*Edible*	*1*	*1.3779*	*0.5905*	*5.4446*	*0.0196***	*Vs. Inedible*	*15.734*
Farmland tenure	*LSH*	*1*	*1.1202*	*9.5985*	*0.0136*	0.9071^{NS}	*Vs. SPR*	*22.*
	LP	*1*	*3.2262*	*9.5636*	*0.1138*	0.7359^{NS}		*181*
	UHS	*1*	*-7.1923*	*47.7358*	*0.0227*	*0.0282***		*0.005*
	SPP	*1*	*4.0722*	*9.5669*	*0.1812*	*0.0104****		*422*
Ethnic group	*Adja*	*1*	*11.5780*	*34.8987*	*0.1101*	*0.0041****	*Vs. Watchi*	*> 999.9*
	Akposso	*1*	*6.7783*	*34.9013*	*0.0377*	0.8460^{NS}		*> 999.9*
	Aïzo	*1*	*3.2863*	*34.9096*	*0.0089*	0.9250^{NS}		*1*
	Ewe	*1*	*-8.4624*	*324.500*	*0.0007*	0.9792^{NS}		*2.33*
	Fon	*1*	*-9.3084*	*64.2539*	*0.0210*	0.8848^{NS}		*1*
	Goun	*1*	*-8.4392*	*54.7494*	*0.0238*	0.8775^{NS}		*2.385*
	Holli	*1*	*8.4553*	*34.9011*	*0.0587*	0.8086^{NS}		*> 999.9*
	Nagot	*1*	*7.1952*	*34.9018*	*0.0425*	0.8367^{NS}		*> 999.9*

LSH = lend farmland with agreement of sharing the harvest; LP= large and private farmland; UHS = farmland with unclear inheritance status; SPP = small and private farmland with poor soil; SPR = small and private farmland with rich soil; FTS = food tree species; ** = highly significant factor in the desire for intensive cultivation; *** = very highly significant factor in the desire for intensive cultivation; ^{NS} = factor with no significant effect in the determination of the motivation for intensive cultivation.

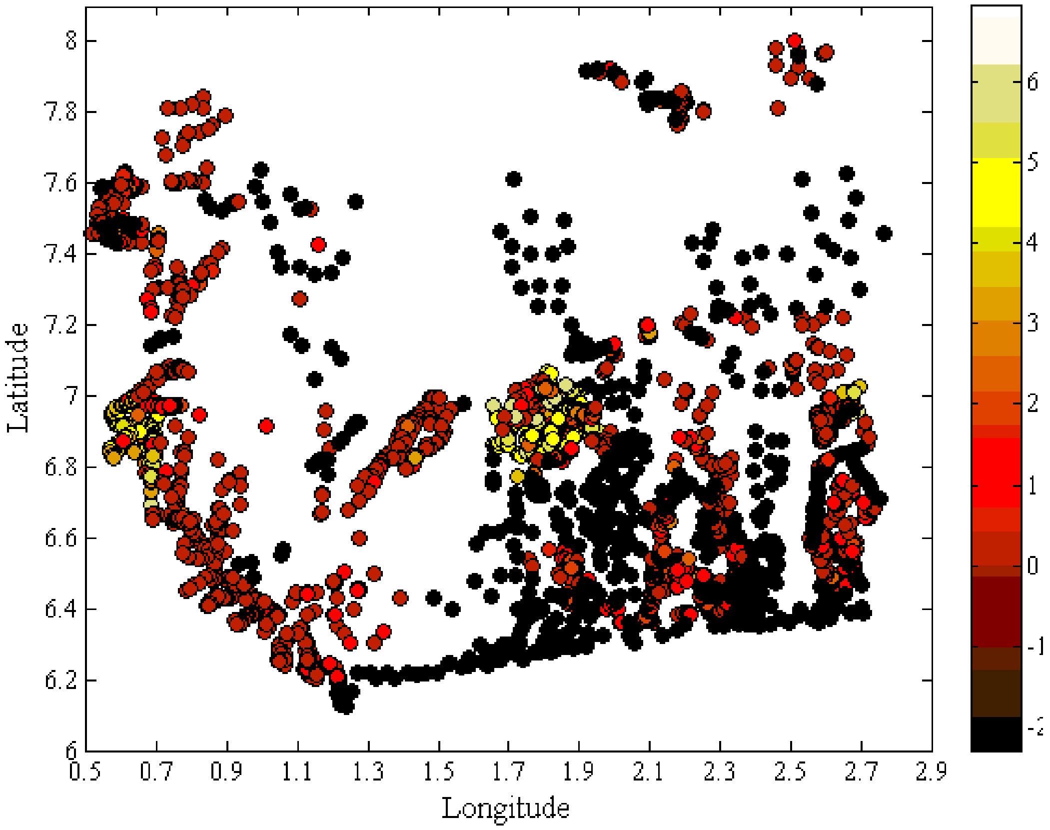

Figure 3.3a: Location of log transform values of sweet ABMTs density within 0.5 km x 0.5 km

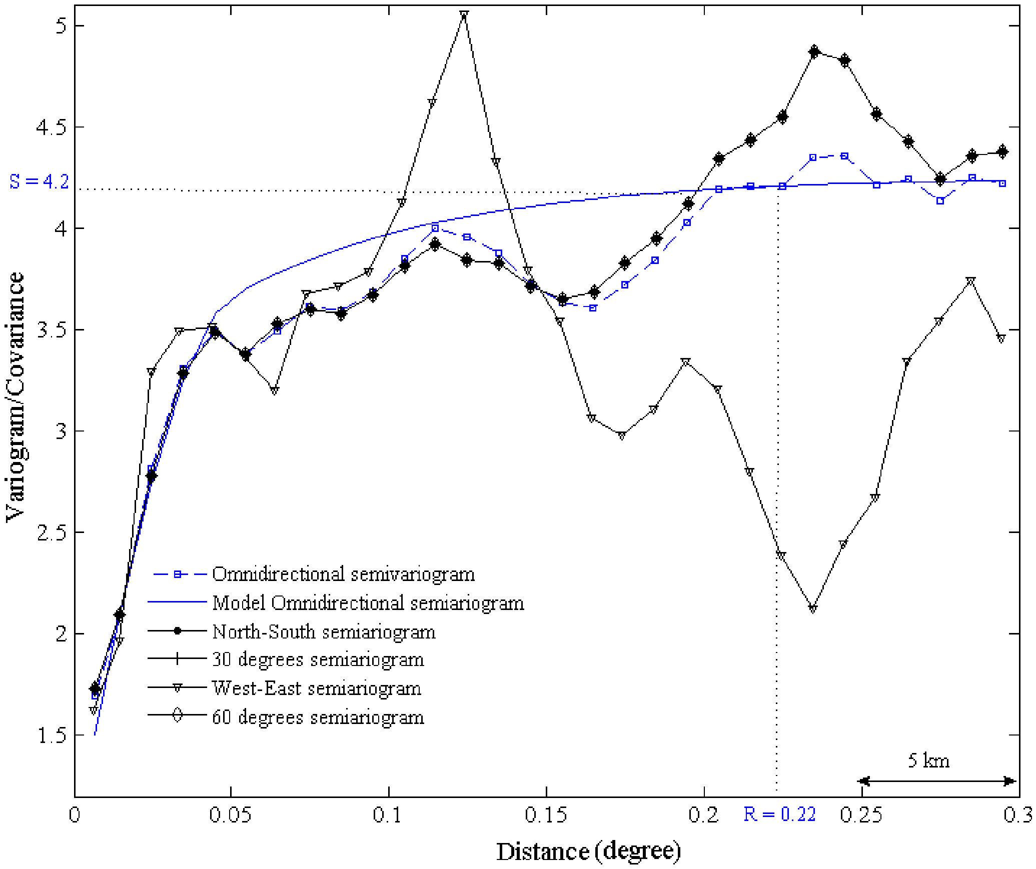

Figure 3.3b: Semivariogram expressing spatial structure of sweet ABMTs density

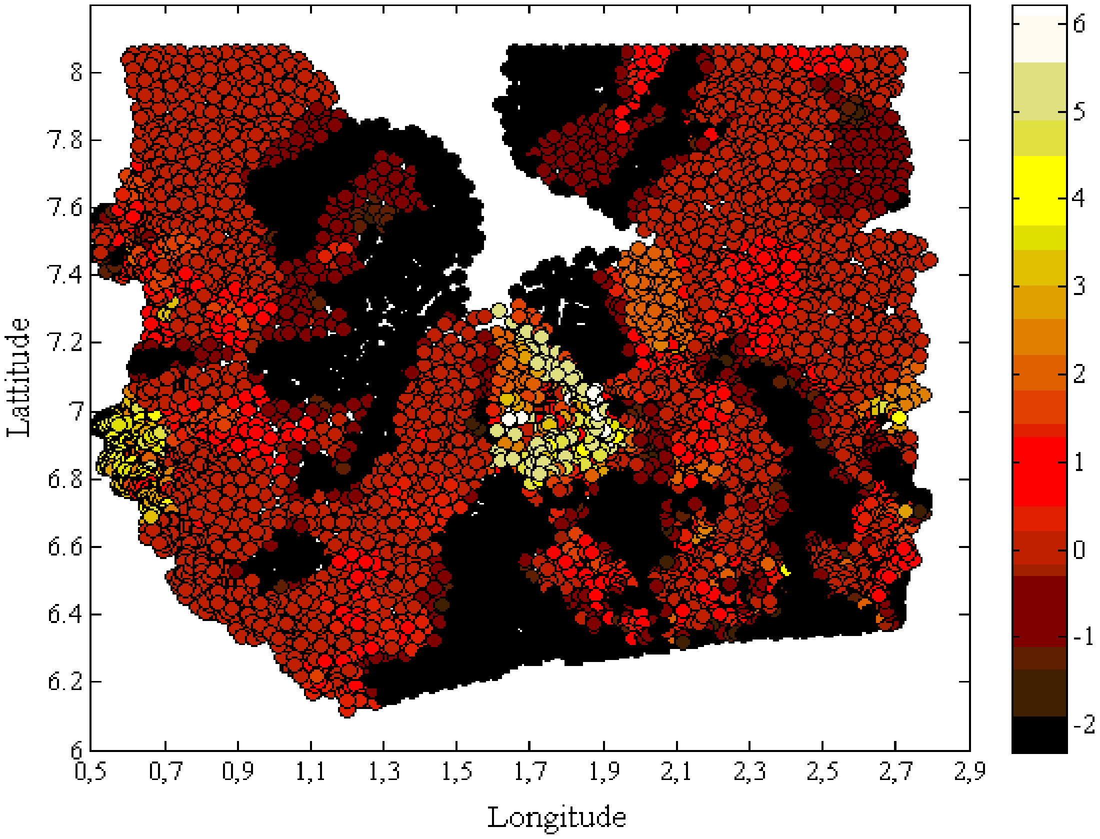

Figure 3.3c: Kriging map of sweet ABMTs density across the Dahomey Gap.
The colour bar indicates the natural logarithm of the density gradient: darker colours indicate lower density (black colour for zero density) and lighter ones higher densities (white colour for the highest values)

The Akposso people mostly use agroforestry parklands and forest gardens to manage bitter trees in the wild and do not establish any *Irvingia* orchards. Sweet trees are 16 times more frequently cultivated in orchards than bitter trees. Local marketing of the seed, the specific reason for bush mango cultivation, and existing taboos remain key factors determining the desire to intensify cultivation. However, their influence is still limited compared to the other significant parameters.

Differences in population structure and threats

In the Akposso area (Bena and Kounionhou), natural forest and forest gardens are mostly populated by large trees (skewness ≥ 1.5; Fig. 3.4a, b). In forest gardens, with a mean dbh of adult trees of 80 cm and where the largest trees are found, spontaneous regeneration is still abundant (32%). In contrast, regeneration in natural forest, where adult trees are on average slightly smaller than in forest gardens (mean dbh = 70 cm), is only 2%. In the Aizo area, the population is also composed of large trees (skewness ≈ 1; Fig. 3.4e). Here, two sub-populations can be recognized: spontaneous old populations (mean dbh = 70 cm), being replaced by cultivated young ones with a mean dbh of 20 cm. In this area, natural regeneration is very low (3%). A similar replacement initiative appears in the Holli and Nagot areas of Pobè, where the separation of the two sub-populations (mean dbh = 20 cm and 80 cm; Fig. 3.4f) in quasi equal frequency tends to be even clearer (skewness = 0.33). Planting was more intensive in the Adja area (Lalo-Klouekanmey) where young trees dominated the population (skewness = - 0.18; mean dbh = 30 cm) with high regeneration figures and an absence of large trees (> 80cm dbh; Fig. 3.4c). Similarly, the planted population in the Ewe area (Kpalimè) shows abundant regeneration and less large trees (mean dbh = 30 cm; Fig. 3.4d).

Apart from the intensive collecting of fruits (and seed), five other practices negatively affect the increase in population density and surface occupied by *Irvingia*. Some trees show the simultaneous occurrence of flowers, and fruits and the harvesting of unripe fruits, by shaking the branches or using long sticks or even a machete, causes profound damage: flower abortion, debarking, and reduction of the crown size. In some areas, saplings and seedlings are cleared for field establishment or maintenance. During other NTFP collecting in natural areas saplings may be systematically cleared. *Irvingia* trunks are sometimes severely debarked for medicinal purposes. Finally, the traditional fishing system uses *Irvingia* twigs for the construction of an ‘Akaja’, a structure that provides a breeding ground for fish. The resulting severe pruning is a major cause of population decrease and of low productivity.

Figure 3.4: ABMTs population structure and treat levels in different socio-cultural areas: a = wild bitter trees in protected area in the Volta region, b = wild bitter trees in traditional agroforestry systems in the Volta region (Kounionhou), c = cultivated sweet trees in southwestern Benin (Lalo-kouekamey), d = planted sweet trees in the lower Volta region (Kpalimè), e = sweet trees in old forest and current traditional fishing area in Benin (Calavi), f = sweet trees in old forest region in south east Benin (Pobè).

Skewness = 1.5

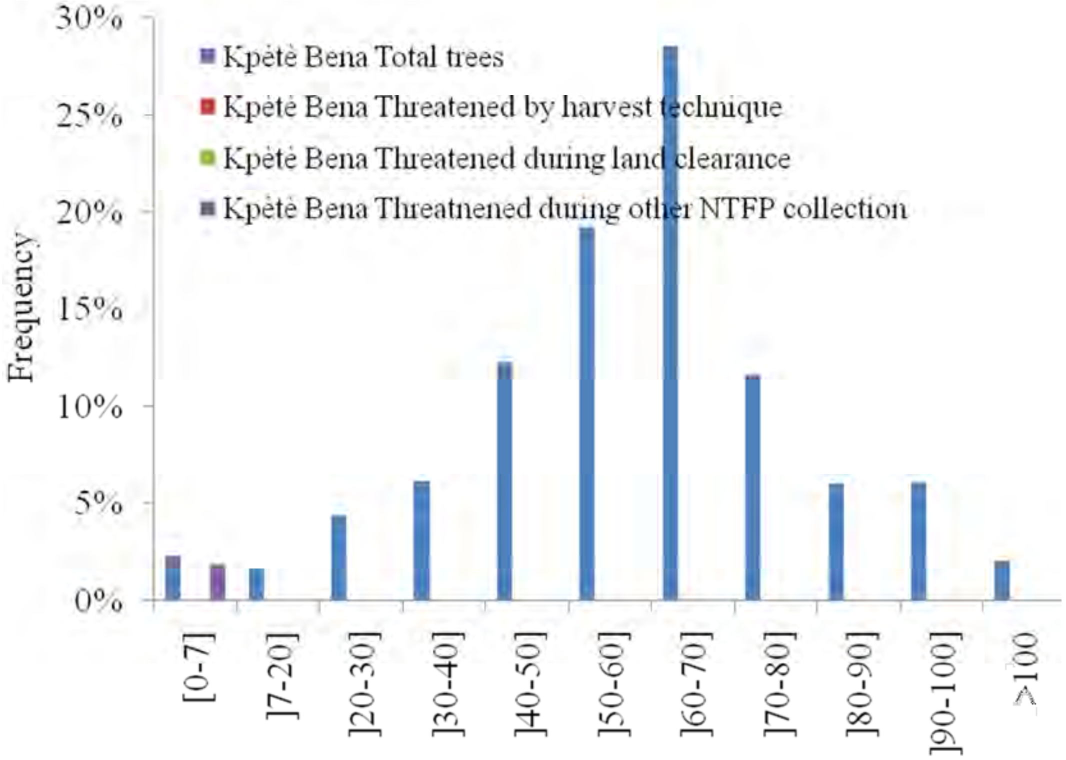

Figure 3.4a

Skewness = 1.63

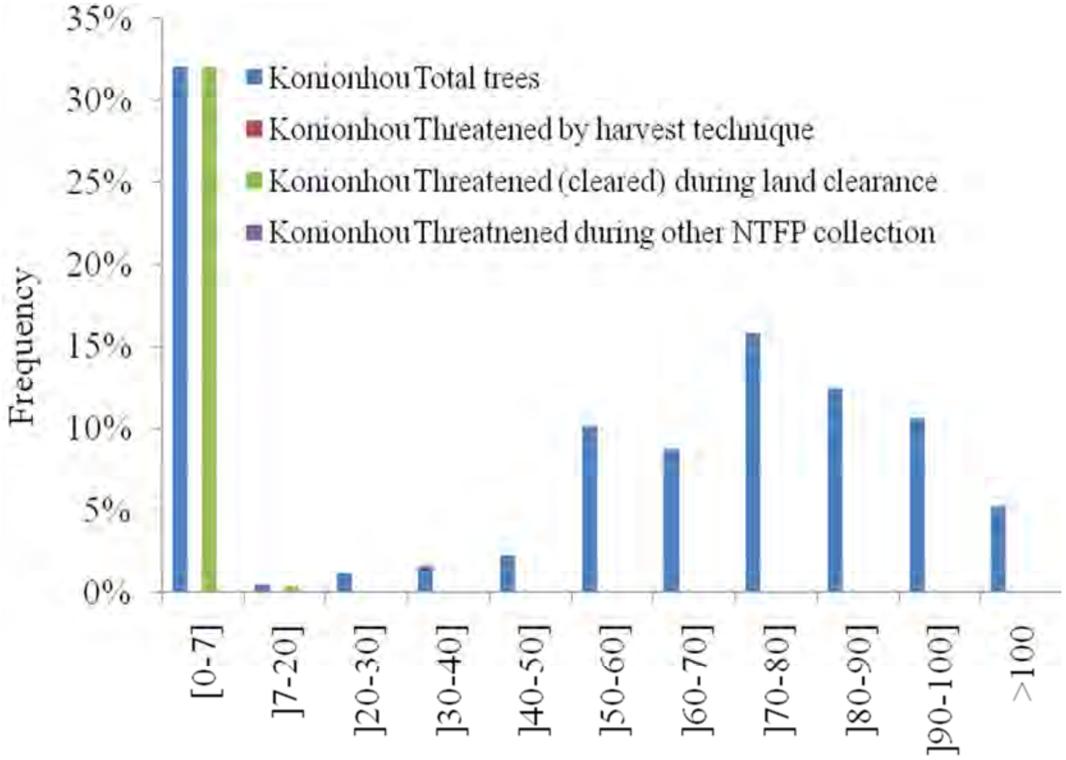

Figure 3.4b

Skewness = - 0.18

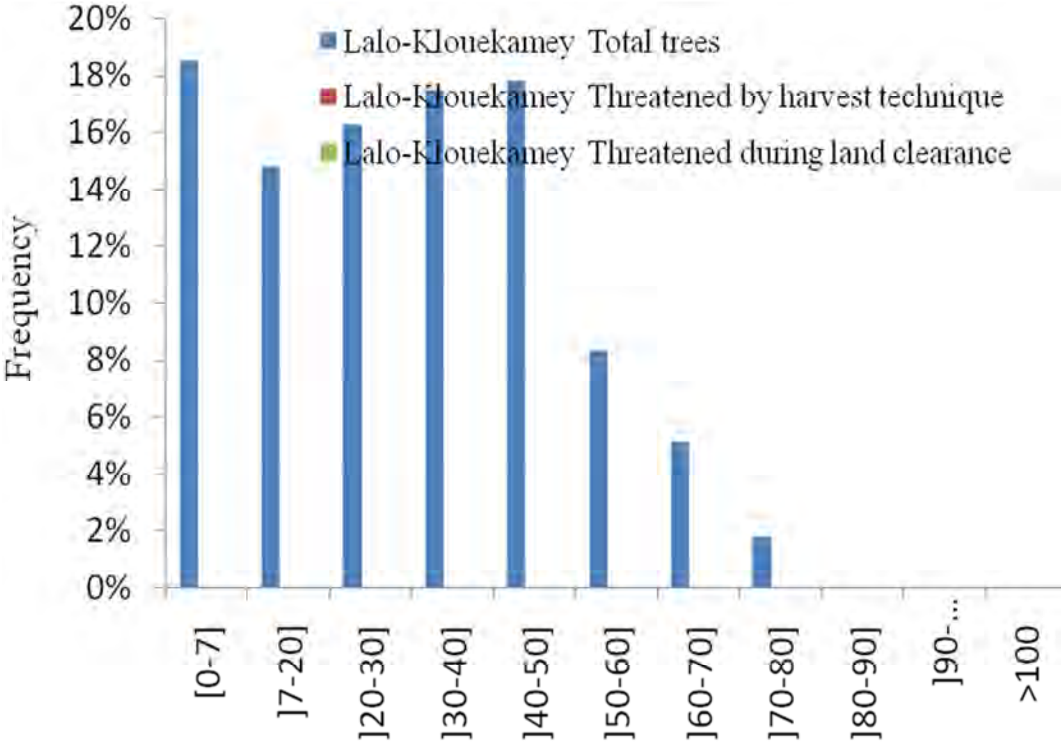

Figure 3.4c

Skewness = 0.35

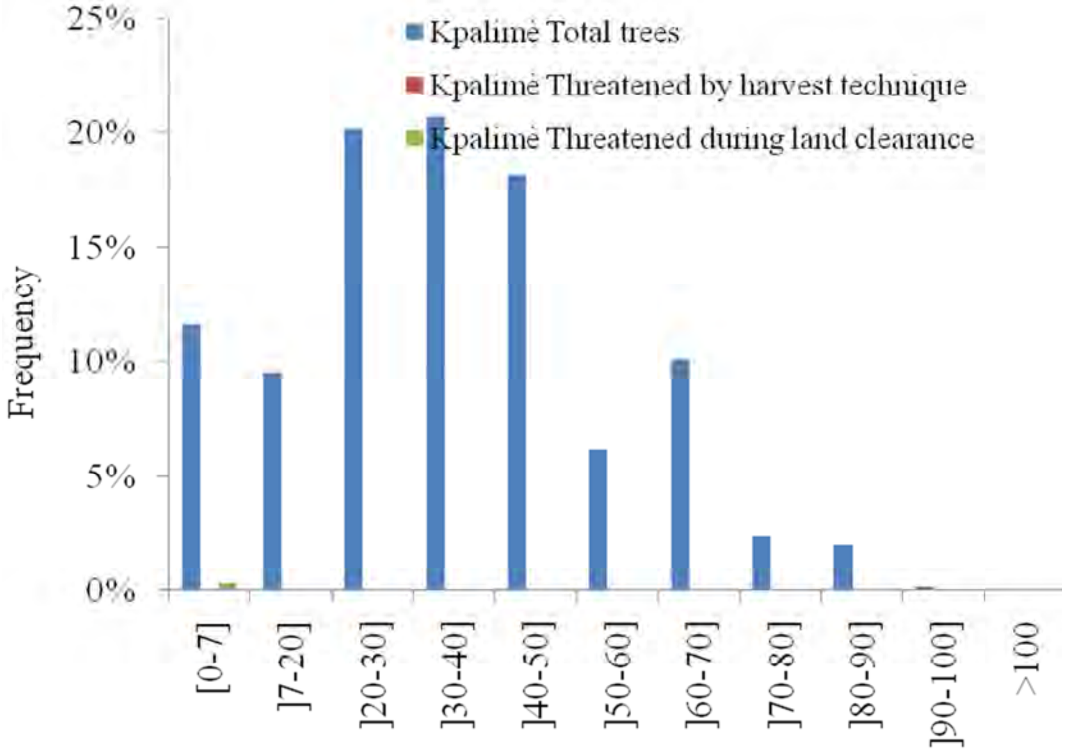

Figure 3.4d

Skewness = 0.926

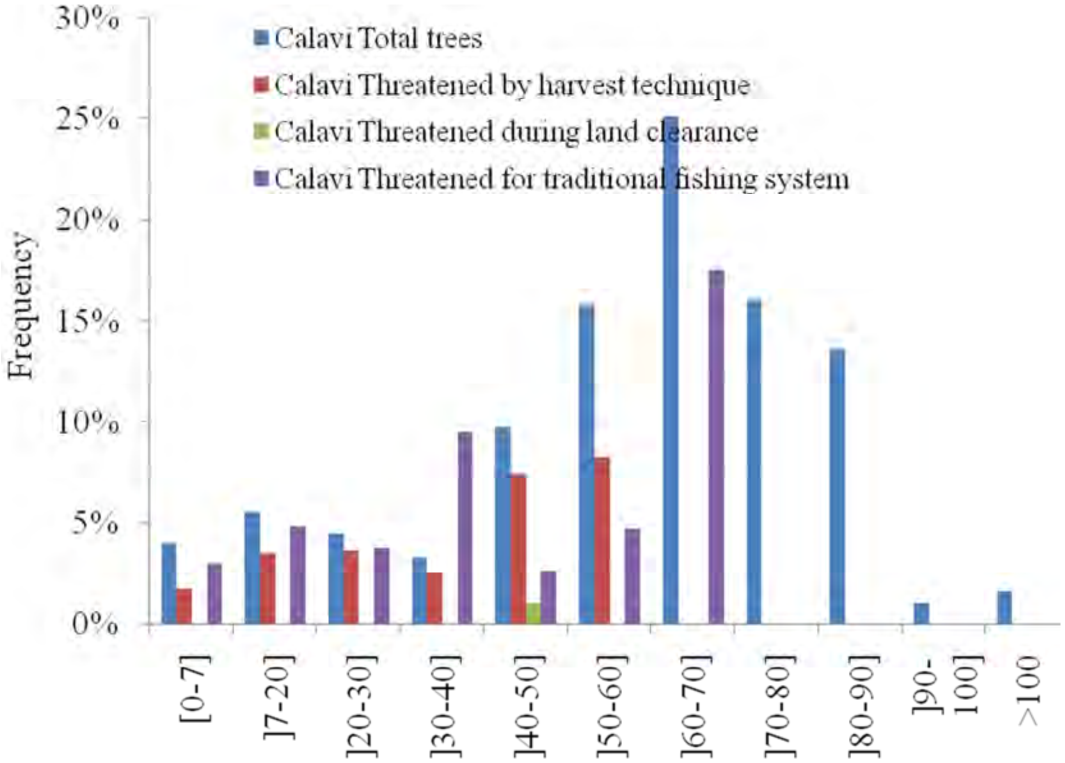

Figure 3.4e

Skewness = 0.33

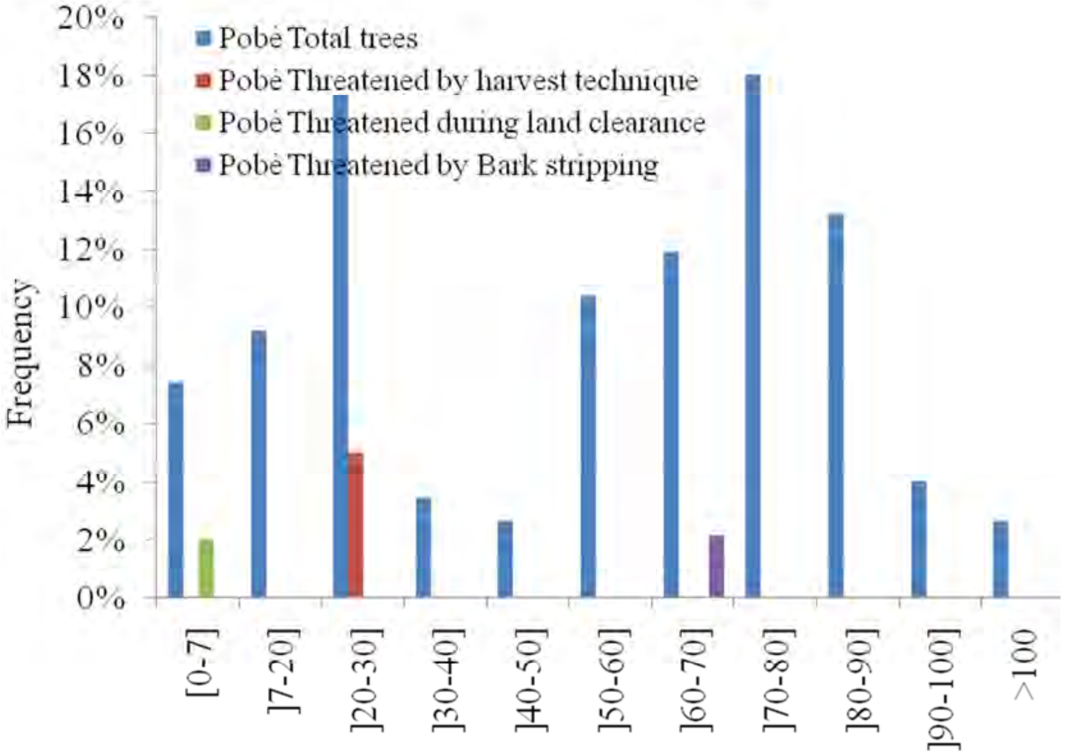

Figure 3.4f

Considering the combined threats, the Tukey test indicates there are three levels of population threats (Table 3.4). The frequency of threatened trees depends on the socio-cultural location of the population (P = 0.008), but all of the six identified practices equally contribute to tree damaging (P = 0.2563). Trees in the most intensive fishing area (Aizo, Calavi) are the most highly threatened. Fig. 3.4e shows that in this population, apart from the intensive collecting of fruits, all top three threat practices (branch pruning, juvenile clearance and damage caused by harvesting techniques) occur and may affect up to 17% of the adult trees of a given size class. Bitter trees in forest gardens of the Akposso area (Kounionhou) and sweet trees in the Holli and Nagot areas (Pobè) represent the second most disrupted populations with an important damage rate due to juvenile clearance (2-32%) during land preparation and inadequate harvesting techniques (Fig. 3.4b, f). In contrast, bitter trees in the protected area of the Akposso area (the disturbed forest land Kpètè-Bèna) and sweet trees managed by the Ewe community (Kpalimè) as well as most of these trees in the Adja area (Lalo-Klouekanmey) hardly show any damage (only 0–1.8% of threatened trees; Fig. 3.4a, d, c).

Table 3.4: Tukey test on population disturbance and categorization.

Tukey Grouping		**Disturbance levels generated by SAS**	**Population**	**Corresponding socio-cultural areas**
	A	0.013402	Calavi	Aïzo + Fon
B	A	0.005869	Kounionhou	Akposso
B	A	0.001927	Pobè	Holli + Nagot
B		0.000236	Kpètè Bena	Akposso
B		0.000060	Kpalimè	Eve (settled inside other socio-cultural groups dominated by Akposso)
B		0.000000	Lalo-KLouekamey	Adja

Populations with different letters are significantly different in terms of their actual disturbance level

Discussion

Usage heterogeneity and implications for ABMT occurrence

The diverse uses and economic contribution to livelihood are leading motives to cultivate *Irvingia* trees (Asaah *et al.*, 2003). In the 250 km wide area where ABMTs occur in Benin and Togo, most of the local communities are socially linked and have no migration limits (see

Assiwaju, 1979). Only the consumption of the sweet mesocarp and use of the seeds of bush mangoes are known to 100% of the local communities. Thus, the variation of the local socio-cultural knowledge showing a gradient with the lowest level in Togo (see Fig. 3.2a and Lesley and Brown, 2004, who reported a high diversity of use in Cameroon and Nigeria) indicates that ABMT conservation or cultivation did not initially occur because of the appreciation of their socio-cultural properties but rather for mesocarp and seed consumption and commercialization (Tchoundjeu and Atangana, 2007). In the particular case of the Dahomey Gap, this implies that people initially had no specific ethnobotanical knowledge on ABMTs and have obtained such via their contacts with the Yoruba people from Nigeria. This knowledge then spread further through population migrations. In this normally slow process of learning (see Turner and Turner, 2008) food properties may be accepted faster than any other types of use. Therefore, in the Dahomey Gap, ABMT cultivation is justified by the consumption of the sweet mesocarp plus that of the seed, and the economic value of these two NTFPs is leading the domestication process in their entire distribution range (Leakey and Tchoundjeu, 2001; Vodouhè, 2003; Atato *et al.,* 2010).

Given the fact that most of the communities in the Dahomey Gap are socio-culturally linked and have experienced long periods of migration (De Medeiros, 1984; Asiwaju, 1979), their diets regarding bush mangoes may not significantly vary if there is no difference in the history of the occurrence of ABMTs in their environment. Thus, the question is whether in the Dahomey Gap sweet ABMTs did occur naturally, or were introduced and are surviving only in cultivation. The fact that the origin of seed-based diets is situated in the Ibo and Yoruba areas (in Nigeria) supplemented by the decrease of ethnobotanical knowledge in a western direction, strengthens the hypothesis that sweet trees spread by population migration from the Lower Guinean forest block in Nigeria west over the Dahomey Gap (Lowe *et al.,* 2000). The change in behaviour caused by breaking taboos around ABMT cultivation and the recent start of seed consumption in Togo, further confirms this theory.

The purposes of the development of priority food trees species are clearly identified by local communities. There is a need to adopt different strategies in ABMT germplasm collecting and propagation. However, other activities, like control of the reproductive biology, tree improvement and breeding (Leakey *et al.,* 2005), are not significantly being taken into account. Therefore, like in many other valuable food tree species, the domestication of the sweet trees is mainly depending in initiatives of individual farmers and market opportunities (Leakey e*t al.,* 2005). In this process, a difference exists in the management of ABMT orchards depending on the local target. Where fruits are marketed for mesocarp consumption, less well-tasting trees or those having a fibrous mesocarp as well as infested trees are systematically eradicated (Vodouhe, 2003). Where the seeds are the commercial item, trees with small nuts (regardless of the mesocarp taste and nutrition quality) are eliminated. This inevitably leads to a loss of characteristics that might be valuable for the success of future agroforestry and plant breeding programs (Wood and Lenné, 1997). The very low tree densities in certain areas which potentially seem suitable to grow ABMTs may highlight not only an ethnic food preference or a lack of ethnobotanical or cultivation experience, but also regional differences in local market value of ABMTs or even NTFPs in general.

When a plant genetic resource presents no direct opportunity locally (food, medicinal or social), the good 'extractivism approach' proposed by Almeida (1996) as the best strategy of exploiting NTFPs without damaging the species’ life cycle guarantees no ecological balance (Rai and Uhl, 2004). Thus, the intensive exploitation of the *Irvingia* seeds from wild populations in the Volta forest region and its valuable timber are key factors jeopardizing especially the bitter tasting *I. wombolu* by narrowing its potential area of occurrence over time.

Variation in Irvingia *conservation and cultivation*

In the Volta region, bitter trees, *I. wombolu*, were found only as wild elements in forest gardens and in natural forest stands, while sweet trees, *I. gabonensis*, were found almost exclusively in cultivation. This difference in cultivation status is primarily caused by a preference of the local communities for a sweet mesocarp (Vodouhè, 2003). Bitter trees are therefore only preserved *in situ* in the Volta forest region. However, bitter trees belong to the most extensively exploited NTFPs in the Volta forest region and therefore this population may well be at severe risk of genetic erosion or even extinction.

The semivariogram characterizing the spatial pattern of ABMT density (Fig. 3.3b) shows a nugget effect with spherical and exponential characteristics within a short range of 22 km. This result implies that even at the sampled unit area level (25 ha), variation still exists within areas of uniform density level at different rates in a unique direction. However, the isotropic characteristic of the spatial structure implies that this unsteady pattern is repeated in all direction across the study areas where very local initiatives (within a radius of 22 km) of ABMT cultivation exist. Therefore, such initiatives are mainly taken by small-scale farmers who are more involved in indigenous tree species cultivation to enhance the sustainability of their poorer productive space (see Leakey and Tchoundjeu, 2001).

Both bitter and sweet trees occur in an overall low density pattern across their distribution range (see Ewane, *et al.*, 2009). The higher densities of sweet trees found in some areas agree with Leakey (2010) who reports that sweet bush mangoes are mostly exploited in traditional agroforestry systems. When plant species are not valued in the local diets or therapies there is no interest to conserve or cultivate them. This justifies the low density of bitter trees in the Volta region. In general, this may lead to rapid phenotypic as well as genetic erosion in a context of increasing land use change and biodiversity loss (Sodhi, 2007). In the Dahomey Gap, where natural forest is still steadily declining, wild bitter trees are under all kinds of threat (intensive collecting of seeds, specifically sought target of wood, eradication of juveniles in land conversion for agriculture, etc.). The low density of ABMTs found in natural stands in Nigeria, Cameroon and Gabon, related to high mortality of saplings and fruit collecting (Agbor, 1994; Van Dijk, 1999; Zapfack and Ngobo Nkongo, 2002), is also recorded in the natural reserves and forest gardens in the Dahomey Gap.

Impact of indigenous knowledge, socio-economic and ecological environments

Our results show that, in the Dahomey Gap, the products of *I. gabonensis* are generally not collected from ‘wild’ (spontaneously growing) trees (Lowe *et al.,* 2000). Because of their socio-economic value sweet ABMTs are as a rule integrated in intensive production systems by local farmers. This involves the manipulation, cultivation, and management of germplasm for a variety of products of the same species established in various systems (see Wiersum, 1996). Since such systems on average need a large surface, the priority crop status given to ABMTs works counterproductive to solve the crucial problem of farmland availability in the Dahomey Gap raised by Floquet and Mongbo (1998). Sweet *Irvingia* trees are the most intensively cultivated trees in the Dahomey Gap. The fact that mainly small farms are being converted to ABMT orchards indicates that the current cultivation process is mainly led by poor farmers (Leakey *et al.,* 2005). Unfortunately, the future improvement of ABMTs thus depends on the selection of genetic material made today by those small farmers. So, these currently unguided selection strategies may lead to a loss of genetic material, with an undeniable negative impact on their potential as a crop.

The cultivation of ABMTs in more organized systems is related to the presence of the Ibo people who locally organize the collection of the seed. Because the Ibo settlement occurred later than the local initiatives for intensive cultivation, the influence of socio-cultural groups in the cultivation efforts appears justified. This difference in time might be rooted in the variation of ethnic knowledge and taboos as well as the ability and willingness of certain local ethnic groups to break ancient taboos. However, the relationship ethnic group – indigenous knowledge – conservation remains strongly influenced by local economic opportunities. The seed and mesocarp value equally influence the desire to intensify the bush mango cultivation. Thus, not only the economical priority of the seed (Tchoundjeu and Atangana, 2007), but also the market for the entire fruits (sweet mesocarp) is an important stimulus for farmers' cultivation efforts. Moreover, the insignificant influence of the type of local market and the taboos on the desire to intensify the cultivation is indicative of a low production being offered to a very broad and diversified market. This has already led to the erosion of taboos and a high price of the fruit and mesocarp in the Dahomey Gap. However, the areas of intensive organization remain those where the seed represents the most important economical product, even regardless of the nature of the local collectors involved in the marketing. It appears that the presence of Ibo communities as local collectors stimulates a higher economic ambition. This is brought about by strict planting based on a traditional selection process for large size endocarp/seed germplasm.

Areas with high sapling densities indicate the core regions where intensive cultivation is practiced and the very low damage to trees confirms again the priority crop status of sweet *Irvingia* in these areas. From there, the intensive cultivation process is spreading to reach regions where the old populations are being progressively replaced by a better stock. In a continuously changing environment where local species have no direct interest of local communities, where slash-and-burn agriculture prevails, where planting of exotic tree species increases and where there is a demand for high quality wood, the survival of any food tree species in forest gardens or agroforestry systems is jeopardized. Most importantly, the free access to bitter bush mangoes in natural areas causes competition between collectors, which strongly limits the natural regeneration (Arnold Ruiz Pérez, 2001; Rai and Uhl, 2004). Intensive logging of bitter trees by local communities progressively extending the area of cash crop production is leading to a significant decrease of their density even in the protected area of Bena. Effective control is hindered by a lack of funding and personnel, inactivity of most of the environmental conservation institutions and social and political instability in Togo

(Posner 2008). Most importantly, the taxonomic integrity of bitter trees is not locally recognized and for any research institution in Togo they are still being considered as *I. gabonensis* (see Atato *et al.*, 2010). This remains a key issue to be addressed seeing the current confusing taxonomical trends and debates on ABMTs (Okafor, 1975 and all recent work in Nigeria: Omokaro *et al.*, 1999; Nya *et al.*, 2000; Nzekwe *et al.*, 2004; Nya *et al.*, 2006; Olawode, 2010; Harris, 1996 and publications by the World Agroforestry Centre). It clearly may harm the protection of bitter ABMTs against ecologically destructive factors and has a negative influence on its genetic conservation (Freese, 1998). Because of that, more thorough investigations on all levels of the assessment of the taxonomic integrity of ABMTs (ecology, reproduction biology, morphology, and genetics) are worthwhile to perform. In the Dahomey Gap, ABMTs experience an uncertain conservation situation and, like many other tree species, are suffering from a lack of wise and efficient usage and conservation strategies. Modern land use planning policies are generally based on space-consuming cash crops that are well adapted to the Guinean climatic zone but which may totally destroy a fragile area with a pattern of small forest patches in need of protective actions.

Conclusions

This study demonstrates the multiple potentials of ABMTs and the geographic pattern of indigenous knowledge and use stimulates questions about the geographic origin of sweet trees' genetic material in the Dahomey Gap. The increasing cultivation initiatives, rooted in the high economic potentials, are broadening ABMTs geographic distribution over exhausted soils in the Dahomey Gap. More organized and intensive cultivation systems are being driven by the Ibo communities that are also involved in the local commercialization systems. As this is a possible crop for future development, further research needs to be performed regarding ABMTs' potential cultivatable areas and productivity in different ecological areas. This is important, not only in the Dahomey Gap, but throughout their entire distribution range. This supposes a full morphological characterization, the definition of the bush mango ideotypes with respect to the type of product targeted, and the capture, fixation and development of desirable genetic material. Therefore, the material of the World Agroforestry Centre domestication program, not only from the Dahomey Gap but from the whole of the western part of the ABMT distribution range, is vital to ensure its wider impact in sub-Saharan Africa.

Regarding any food trees species, but ABMTs is particular; there are two important challenges for scientists and development policy makers regarding the active role of local communities in the domestication process. For scientist, considering the rapid fragmentation of natural habitats in the tropics, at least the distribution of wild populations of NTFP species needs to be known. Then, for policy makers, especially better insights in the potential cultivatable area of such species would be very useful to assist decisions on land-use planning, while a good knowledge of the genetic variability is necessary for the *in situ* and *ex situ* conservation of germplasm.

Acknowledgements

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Chapter 4

Phenological diversity and distinction within African Bush Mango trees (*Irvingia*: Irvingiaceae) in the Dahomey Gap (West Africa)

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Abstract

African Bush Mango trees are priority food trees in Sub-Saharan Africa. The occurrence of bitter and sweet fruited trees is still the subject of taxonomic debate which hinders their effective use and conservation. This study investigates differences in phenological behaviour between bitter and sweet fruited populations and their taxonomic implications. Monthly phenological data of seven populations in Benin and Togo are used to assess phenological diversity within bush mango trees, to discriminate bitter and sweet trees and to investigate their responses to environmental factors. The Shannon-Weaver phenological diversity index is used to describe variability of phenological states among populations and between bitter and sweet trees. A Canonical discriminant analysis is performed to identify the phenological states differentiating bitter and sweet trees and a principal component analysis supplemented with a cluster analysis is used to group individual trees. Finally, analyses of variance are carried out on the reproductive phenological states against the month (time), FAO soil type, type of bush mango tree, and the climatic zone to evaluate the reproductive responses of the taxa. Phenological diversity varies significantly among populations. The very low phenological diversity of bitter trees can be explained by their limited distribution range in the study area. Phenological states consistently discriminate bitter and sweet trees. The two types also display significantly different reproductive responses to the investigated environmental factors except soils, supporting the idea that they represent two different species. A small overlap in flowering time offers a possibility for hybridization between bitter and sweet trees, but fruiting subsequent to this overlap only occurred in bitter trees.

Key words: Adaptation, ecology, domestication, *Irvingia*, phenological states.

Introduction

African Bush Mango Trees (ABMTs) produce an economically emerging non-timber forest product (NTFP) that reaches the regional and international markets of sub-Saharan-Africa (Tabuna, 2000). This NTFP, the seed of their mango-like fruit, is an important part of the diet of sub-Saharan African communities (Ekpe *et al.,* 2007). It is marketed within its hard endocarp, has high oil content and is increasingly used in pharmaceutical and cosmetic manufacturing (Ogunsina *et al.*, 2008). The mesocarp of the fruit is either bitter and inedible or sweet and highly appreciated and forms the main character that discriminates between two types of ABMTs. Tree-to-tree variation in morphology greatly overlaps between bitter and sweet fruited trees and makes it difficult to distinguish between them without having tasted the fruit (Ofafor, 1975; Harris, 1996; Ladipo *et al*., 1996; Leakey and Tchoundjeu, 2001). Recently, Harris (1996) and Lowe *et al*. (2000) considered sweet and bitter trees to be distinct at species level: *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. and *I. wombolu* Vermoesen, respectively. However, the variety level claimed by Okafor (1975), *I. gabonensis* var. *gabonensis* and *I. gabonensis* var. *Excels* (Mildbr) Okafor, continues to be used in literature as well (see Nzekwe *et al*., 2002; Nya *et al*., 2006; Ekpe *et al*., 2009). ABMTs are phenologically highly diverse and this study aims at investigating its potential to discriminate between the bitter and sweet trees.

Regardless the taste of the mesocarp, the seed of both types is the most important non-timber forest product (NTFP) for local communities in the humid forest areas of West and Central Africa (Tchoundjeu *et al*., 2002). That of bitter fruits has greater economic value because of its higher food technological properties (Ladipo *et al*., 1996). Hence, in-depth observations are needed to clarify the taxonomic status of ABMTs and to design a better cultivation and conservation strategy.

ABMTs have a large distribution from Senegal to Angola, up to West Sudan (Lesley and Brown, 2004; Orwa *et al*., 2009; Nkwatoh *et al.*, 2010; Dolor, 2011). Naturally, they supposedly occur at 200–500 m altitude, under mean annual temperatures of 25–32°C and 1,500–3,000 mm of annual rainfall (Kengni *et al*., 2011). But they also abundantly occur at low altitudes in forest areas in the coastal regions of West and Central Africa (see Lesley and Brown 2004). Their diversity centres are located in the Lower Guinean and Congolian forest blocks (Lowe *et al.*, 2000; Ude *et al*., 2006). Currently, agroforestry systems of ABMTs are spreading across West and Central Africa due to their growing economic value (Shiembo *et* *al.,* 1996; Nkwatoh *et al.,* 2010). ABMTs are also found in the particular eco-region called the Dahomey Gap which is a dry corridor between the two West African forest blocks (Maley, 1996). Only few investigations compared bitter with sweet ABMTs in their entire distribution range and comparative quantitative phenological data are particularly lacking. Moreover, West Africa, which is the second important part of their distribution range, has been poorly prospected.

Phenology is demonstrated to be highly environmentally sensitive (Rumland and Vulie, 2005), and therefore it is a useful tool to assess responses of plants to global climate change as well as to periodic variations of climatic factors (temperature, rainfall, solar radiation) in specific climatic zones (Chmielewski and Rötzer, 2001; Crepinsek and Kajfez-Bogataj, 2006; Nord and Lynch, 2009). Moreover, phenological variations could reveal particularities of different taxa in their physiological responses to soil variability as well as the availability of nutrients and their uptake by plants (Nord and Lynch, 2009). Phenological characteristics can, therefore, be used to support taxonomic decisions and, because of its close links to crucial cultivation activities like planting, maintenance and harvesting, also be of great importance in predicting economic potential of emerging new crops like ABMTs in different eco-regions.

The aim of this study was to provide comparative quantitative data on phenological response and to infer potential drivers of this variation as well as its taxonomic implications within ABMTs. Data of a thirteen-month phenological survey were used to:

(i) evaluate the phenological variation and the diversity among and between bitter and sweet ABMTs across different climatic zones in the Dahomey Gap,
(ii) determine discriminating phenological properties between bitter and sweet ABMTs,
(iii) assess the response of ABMTs in their reproductive phase to environmental factors in the Dahomey Gap.

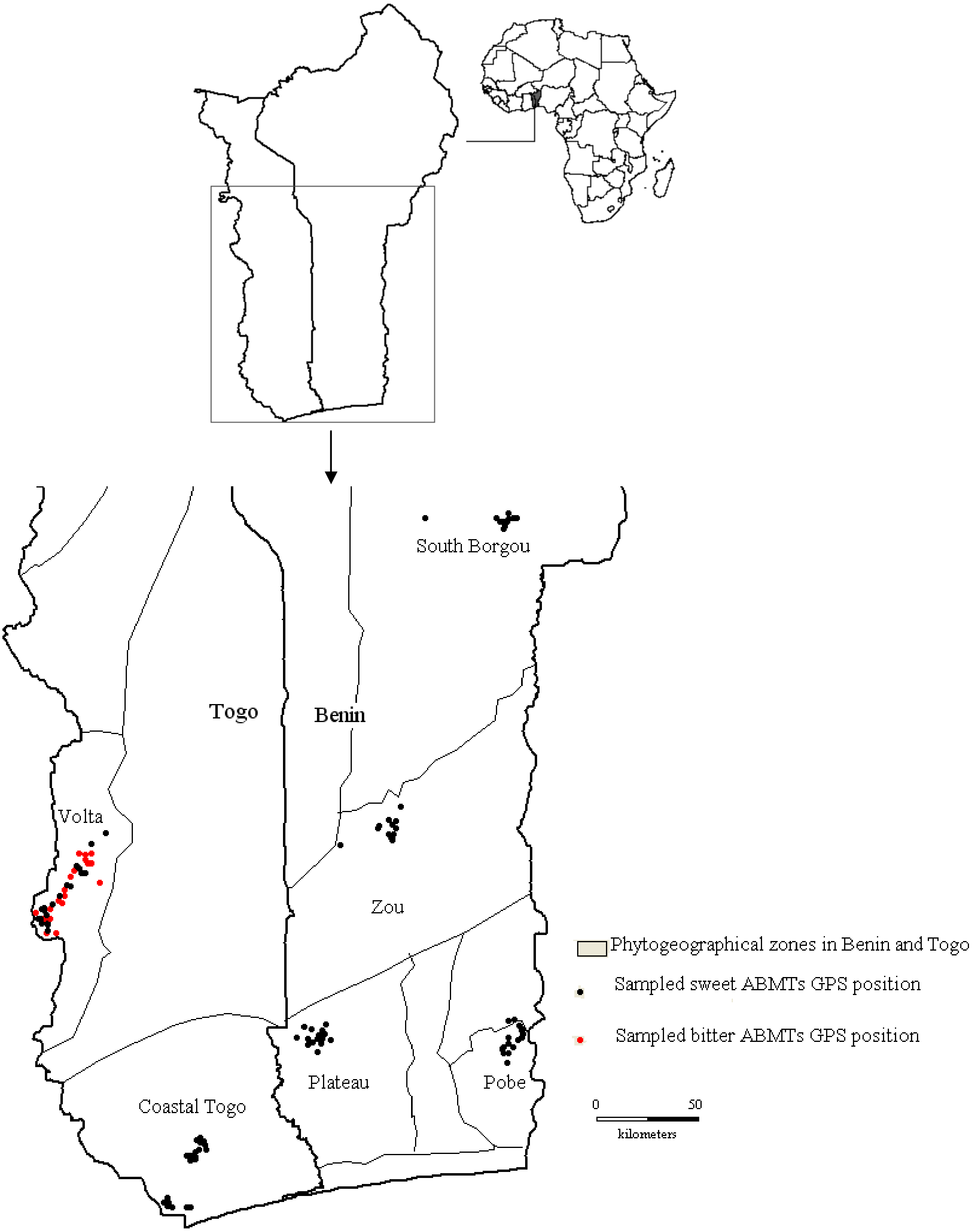


Figure 4.1: Geographic and phytogeographical locations of sampled populations.

Materials and methods

Study area

This study was carried out in six phytogeographical regions: Pobè, Plateau, Zou and South-Borgou in Benin (Adomou *et al.*, 2006), and the Coastal region and Southern Mountain forest region, also called Volta forest region, in Togo (Fig. 4.1). The southern parts of these two countries are located in the Dahomey Gap which splits the West African forest block into the Upper and Lower Guinean forest regions (Maley, 1996; Giresse, 2008). It consists of savannah stands with small-size forest relics and forms an obstacle for natural species exchange between the two forest blocks (Booth, 1957). It is a climatically induced anomaly dating from the Holocene (Maley, 1996) characterized by higher temperatures and lower rainfall. Despite these climatic parameters, numerous humid forest species still occur within this eco-region (Salzmann and Hoelzmann, 2005). Among them ABMTs, which occur in various land use systems and are increasingly cultivated to prevent soil degradation and enhance livelihood.

Sampling

Considering the type of bush mango tree, the local domestication strategy, and the phytogeographical region, seven populations of ABMTs were selected (Table 4.1). In each population a random sampling technique was applied and the number of trees was chosen in such a way that they could be surveyed in 1 to 2 days. Only trees that had already fruited at least once were selected. Within each population, eleven to thirty-eight mature trees separated by a distance of 100 m to 24 km were selected and geo-referenced, totalling to 173 individuals.

Monthly phenological surveys were carried out from October 2010 through October 2011, to describe three phenological phases: vegetative, flowering, and fruiting. Twelve phenological events describing the monthly changes that occur in the crown of each tree were defined. For the vegetative phase: (1) mature leaves, (2) leaves starting to drop, (3) leaves all dropped and (4) young leaves developing. For the flowering phase: (5) flower buds, (6) open flowers (ready for pollination), and (7) aborted flowers. For the fruiting phase: (8) immature fruits, (9) immature fruits aborted, (10) mature but unripe fruits, (11) mature unripe fruits aborted, and (12) mature and ripe fruits. Mature but unripe fruit was defined as a separate event, because it represents an important phase when looking at the effect of parasites, mostly in areas where the mesocarp remains the most important NTFP (Vihotogbé *et al.,* 2007) and might help design an improved pest management program. For each sampled tree and in each month, we observed and estimated the level at which each phenological event occurred in the volume of the crown: we referred to this as the monthly level of the related phenological event in this study. Five levels were defined and used to provide each tree with a monthly phenological score regarding each phenological event: 0 when the event did not occur in the crown; 1 when it occurred in 0-25% of the volume of the crown; 2 in 25-50%; 3 in 50-75%; and 4 in 75-100% (Fig. 4.2a, b, c). Thus, the raw phenological database consists of 173 rows (individuals) and 156 columns (13 months × 12 phenological events).

Table 4.1: Population characteristics.

Country	Phytogeographical region	Population name with reference to sampling and type of ABMTs	Climatic characteristic	Type and domestication state of the sampled population	Number of Sampled trees
Benin	Plateau	Couffo	Climate: Sub-humid Guinean; Rain fall: 900-1100 mm in bimodal regime (April-June and from September-November); Temperature: 25°C-29°C; Hygrometry: 76%-97%.	Only cultivated sweet trees under traditional selection process mainly for seed commercialization.	30
	Pobè	Pobè	Climate: Sub-humid Guinean; Rain fall: 1200-1300 mm in bimodal regime (April-June and from September-November) Temperature: 25°C-29°C; Hygrometry: 76%-97%	Mixture of pretended wild + spontaneous + cultivated sweet trees (with no selection process) for both entire fruit and mesocarp commercialization	38
	Zou	Dassa	Climate: Soudano-Guinean; Rain fall: 1100-1200 mm in unimodal regime (May-October); Temperature: 25°C-29°C; Hygrometry 31% to 98%.	Spontaneous and cultivated sweet trees (with no selection process) for household consumption and entire fruit commercialization	11
	Southern-Borgou	Parakou	Climate: Soudanian; Rain fall: lower than 900 mm in unimodal regime (June-November); Temperature: 24°C-31°C. Hygrometry: 18%-99%	Only cultivated sweet trees (with no selection process) for mesocarp and seed consumption and limited commercialization	13

Country	Phytogeographical region	Population name with reference to sampling and type of ABMTs	Climatic characteristic	Type and domestication state of the sampled population	Number of Sampled trees
Togo	Coastal region (Region V)	Lomé	Climate: Sub-humid Guinean with major rainfall deficit: 800 mm in bimodal regime (April-June and from September-November).	Only cultivated sweet trees (with no selection process) for mesocarp consumption and seed commercialization	32
	Southern Mountain or Volta Forest Region (Region IV)	Badou	Climate: Guinean; Rain fall: 1,168 - 2,103 mm in unimodal regime (Mars- October). Temperature: 21°C -32°C	Spontaneous and cultivated sweet trees (with no selection process) for entire fruit and seed commercialization.	15
				Only wild bitter trees still far from cultivation initiative and exploited by local communities and wood industries	32

Figure 4.2: Examples of levels attributed to phenological events. a = levels of mature leaves; b = levels of open flowers ready for pollination; c = levels of mature unripe fruits.

Data analysis

Using the raw phenological data for each phenological phase and for each month, a combination of the levels of the monthly phenological events (scores) was made to give a unique qualitative indicator that defines the phenological states for each sampled tree (see Goulart *et al.*, 2005). For example, a tree with ‘0004’ as vegetative state in December 2010 was characterized as that with a crown: (i) was completely devoid of mature leaves (0 %), (ii) was devoid of yellowing leaves ready to fall (0 %), (iii) has no freshly dropped leaves under it (0 %), and (iv) was completely filled with freshly renewed leaves (100 %) in December 2010. A tree coded ‘044’ for its flowering state in January 2011 had a crown: (i) with no flower buds, (ii) completely filled with open flowers able to be pollinated (100 %), but (iii) 100 % of these flowers aborted in this same month. Likewise, a fruiting state of ‘00012’ in October 2010 indicated a tree with: (i) no immature fruit in its crown (0%), (ii) no immature fruits being aborted (0 %), (iii) no mature and unripe fruit (0 %); (iv) few mature and unripe fruits aborted (25 %), and about 50 % of it volume filled of mature and ripe fruits.

For each month all possible phenological states were considered as separate columns in the data matrix. Each tree was then identified with 1 (presence) for its state, and 0 (absence) for all other states, resulting in a phenological presence/absence data matrix. This matrix was then used to calculate the monthly Shannon-Weaver phenological diversity index (Goulart *et al*., 2005) for each population and phenological phase:

$$H(ijk)H_{(ijk)} = \sum[p_{(ijk)\alpha} * \ln p_{(ijk)\alpha}] \tag{1}$$

H_{ijk} is the diversity index for the i^{th} population in the j^{th} phenological phase and in the k^{th} month and $p_{(ijk)}$ is the number of trees in the i^{th} population in the j^{th} phenological phase in the k^{th} month that presented the same α^{th} phenological state divided by the total number of individuals in that population.

The value of this index ranges from zero for a population in which all individuals present the same phenological state for a given phenological phase in a given month to high values (close to 1) for more phenologically diverse populations. This index was used to compare the seven populations and the two types (bitter and sweet) of ABMTs.

All the monthly phenological diversity values (H_{ijk}) per phenological phase were recorded. Using this H_{ijk} database and considering the phenological phases as replications, a one way analysis of variance (ANOVA-1) with repeated measurements was carried out in Statistica Version 6 against the population in order to evaluate differences in the overall phonological diversity. Moreover, individual trees that belong to either type of ABMTs (sweet versus bitter) were grouped and the monthly phenological diversity for each phenological phase was also calculated.

The raw phenological data matrix was checked and monthly phenological events that presented zero variance were removed. The correlation matrix of the remaining events was computed and multicollinearity was eliminated considering a cut-off value of 70%. A final phenological data matrix was created containing 117 monthly phenological events describing the 173 trees.

To analyse phenological differences between bitter and sweet ABMTs, the final phenological data matrix was used in a multivariate analysis. A Canonical Discriminant Analysis (CDA) was performed on the monthly levels of phenological events against the type of ABMTs using SAS. Prior probability proportional to sample size was assigned and posterior probabilities with the classification of individuals as being either bitter or sweet were computed. In order to allow the comparison under an uniform ecological condition, bitter and sweet ABMTs from the Volta region were used to calibrate the model which was used to classify the rest of individuals from the other populations, and the classification probability was also computed. An ANOVA-1 was run in SAS on the discriminating factors (monthly phenological events) against the type of ABMTs to obtain their levels of variation.

Phenological similarity among individuals is considered to be indicative of synchrony in life cycle events (Goulart *et al.*, 2005) and might help detect the spatial partitioning of the phenological diversity. To group individuals based on their phenological similarity, multivariate analyses were used. The application of two consecutive multivariate analyses was revealed useful to improve the classification of individuals (Mohammadi and Prasanna, 2003; Bidogeza *et al.,* 2009). First, a Principal Component Analysis (PCA) was carried out on the monthly levels of the 117 independent monthly phenological events using PAST (Hammer *et al.*, 2001). Second, the monthly phenological events correlated (using a cut-off of 70%) with the first six axes were used in a cluster analysis based on the Bray-Curtis similarity index (Dbc_{jk}; see Bloom, 1981 for the choice of this index):

$$(Dbc_{jk}) = \frac{\sum_{k=1}^{n} |x_{ik} - x_{jk}|}{\sum_{k=1}^{n} (x_{ik} - x_{jk})} \tag{2}$$

With i and j^n being two random individual trees characterized by a set of n monthly phenological events, k being a random phenological state, and with x_{ik} and x_{jk} being the levels for individuals i and j at the k^{th} monthly phonological event, respectively. Based on this index among individual ABMTs, a dendrogram was produced to visualize the similarities.

Reproductive response of ABMTs to environmental variations in the Dahomey Gap

To assess the impact of environmental variation on the reproductive response of ABMTs in the Dahomey Gap, four phenological events related to their reproductive success were studied in more detail. These are: level of flowers able to receive pollen, level of flower abortion, total level of fruit abortion and level of mature and ripe fruits. The total level of fruit abortion was obtained by adding the level of immature fruit abortion to that of the mature unripe fruit abortion. The GPS position of the trees was used to get the corresponding FAO soil type (http://www.fao.org/geonetwork). Using SAS, four ANOVA's (generalized linear model) were carried out on the level of these reproductive success variables against the type of ABMTs, phytogeographical region, soil type and season (month). The additive effects of those variables and the joint effects of type, phytogeographical region and soil type with season were assessed.

Results

Phenological variation and diversity within ABMTs

The ANOVA-1 with repeated measurements indicates significant differences in the overall phenological diversity among populations (P = 0.028). The population of Lomé followed by that of Pobè have the highest diversity index, while those of Dassa, Couffo and Parakou are phenologically the least diversified ones (Fig. 4.3a).

The bitter trees show slightly higher phenological diversity in November and December for the flowering phase and in January for the fruiting phase (Fig. 4.3c). Apart from those periods, a higher Shannon-Weaver diversity index was calculated for sweet ABMTs for all three phenological phases and in each month (Fig. 4.3b, c, d). For both types the vegetative diversity was generally higher in the most severe dry season from November through March than in the rainy season and in the short dry season from April through October (Fig. 4.3b). For the bitter trees, little variation of the diversity was found throughout the year for the flowering phase (1.5 to 2) as well as for the fruiting phase (1.2 to 2), while the sweet trees presented high variation of diversity ranging from 1.3 to 6 and 1.2 to 9 for flowering and fruiting phases, respectively (Fig. 4.3c, d).

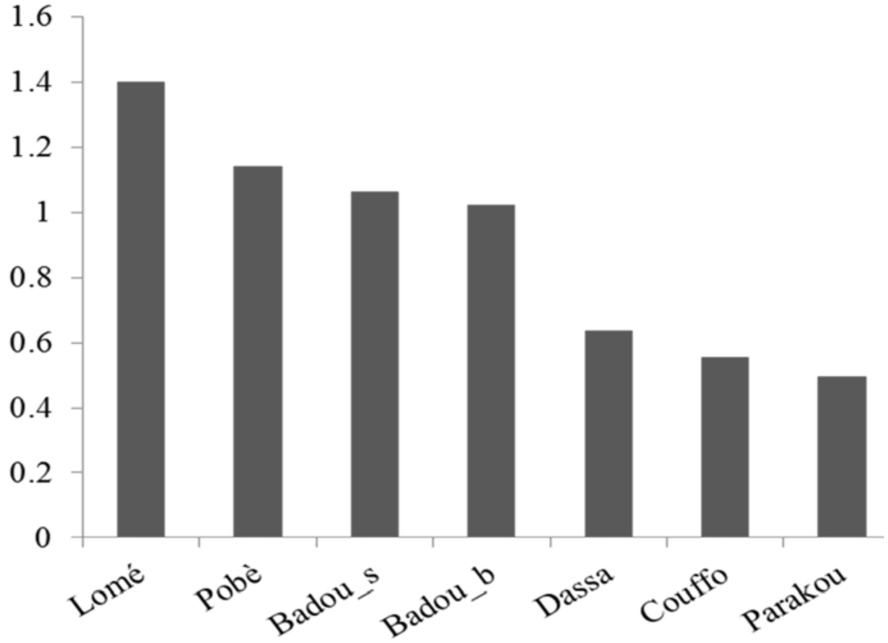


Figure 4.3a

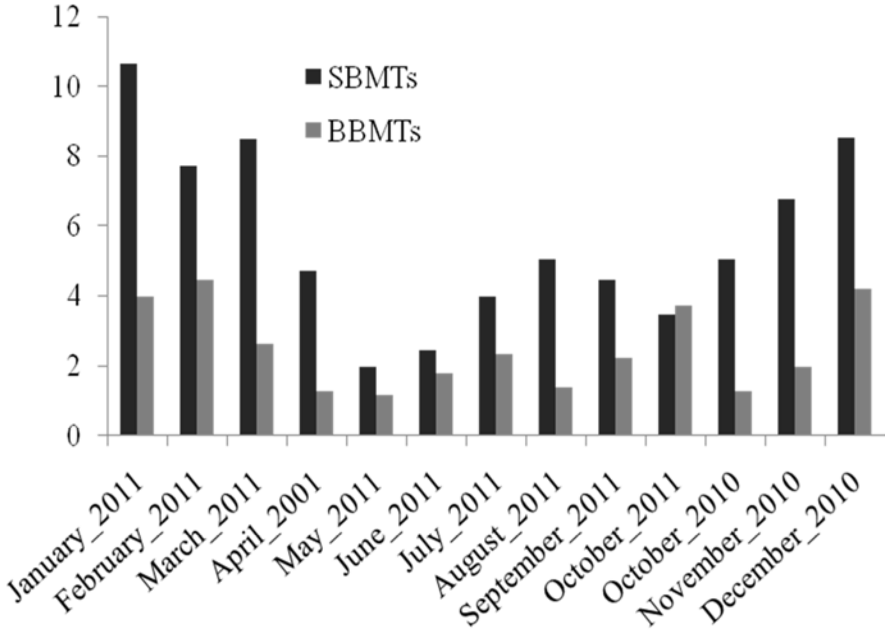


Figure 4.3b

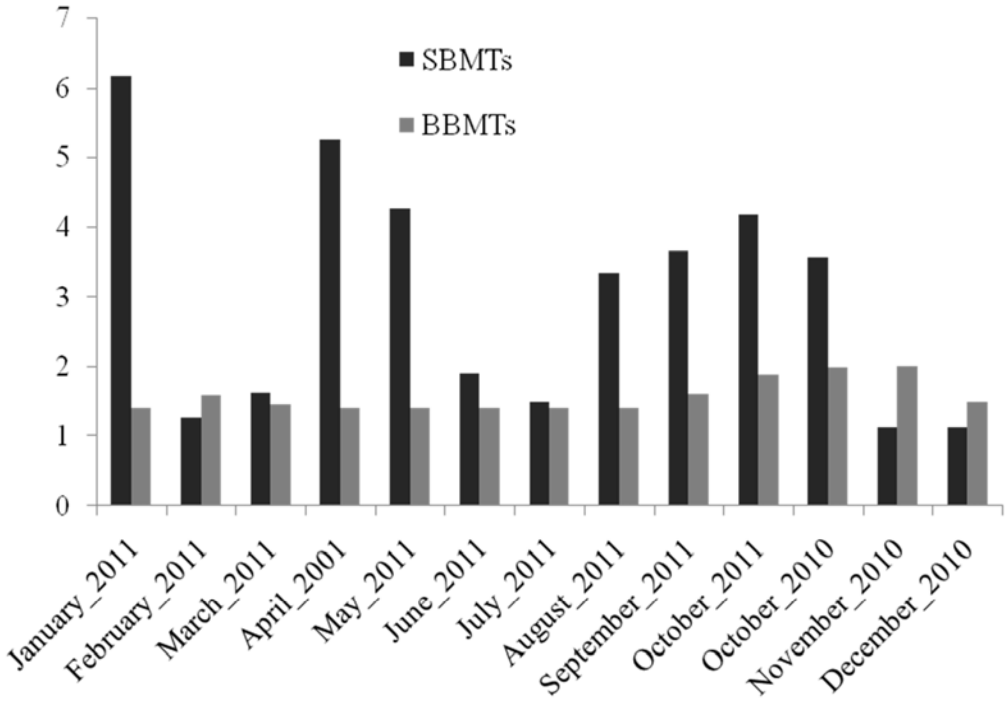


Figure 4.3c

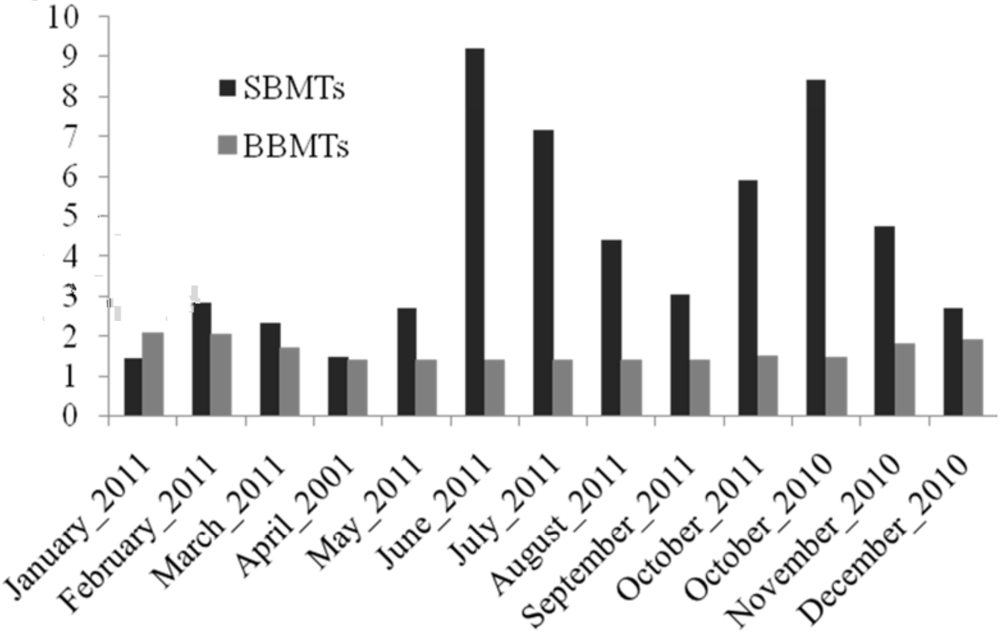
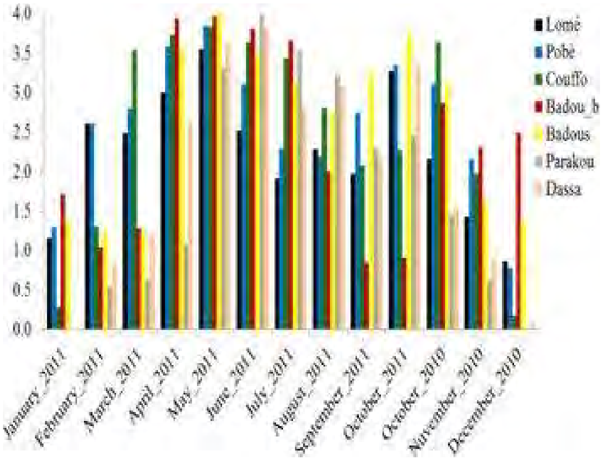


Figure 4.3d

Figure 4.3: Shannon diversity among populations and between bitter and sweet trees. Badou_s = sweet trees from Badou in the Volta region; Badou_b = Bitter trees from Badou in the Volta region; SBMTs = sweet bush mango trees; BBMTs = bitter bush mango trees: a = Global phenological diversity among populations; b = Vegetative diversity between bitter and sweet trees; c = Flowering diversity between bitter and sweet trees; d = Fruiting diversity between bitter and sweet trees

Phenological discrimination of bitter and sweet ABMTs

The vegetative phase does not distinguish among populations or between tree types (Fig. 4.4a-d). The reproductive phases (flowering and fruiting), on the other hand, show distinctive differences (Fig. 4.4e-l). For example, three main flowering periods were observed: December-January, March-May and August-November (Fig. 4.4e, f). While sweet trees flowered at all of these periods, bitter trees flowered only in September-October (Fig. 4.4e). Consequently, sweet trees bear immature fruits throughout the year with three peak periods in February-March, May-June and especially in September-November (Fig. 4.4h). For bitter trees, this period is mostly limited to November-December (Fig. 4.4h). Bush mango fruits are fully grown and ripe in three main periods: January-April, June-September and October-December (Fig. 4.4l). While mature and ripe sweet fruits are available in all of these three periods, the bitter ones are available only in the first period. It is clear that these three phenological characteristics are highly correlated, which is why subsequent analyses were performed on each of the states separately.



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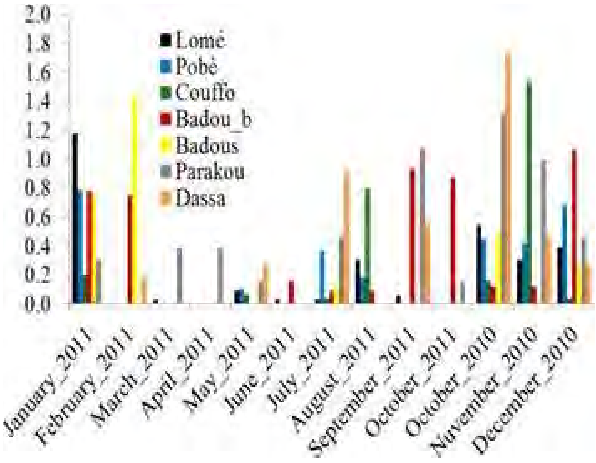

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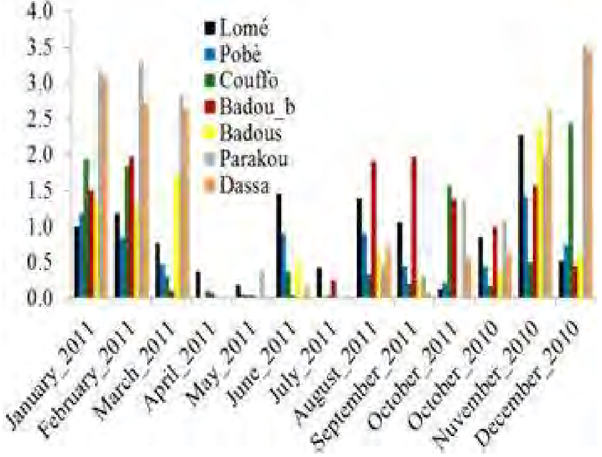

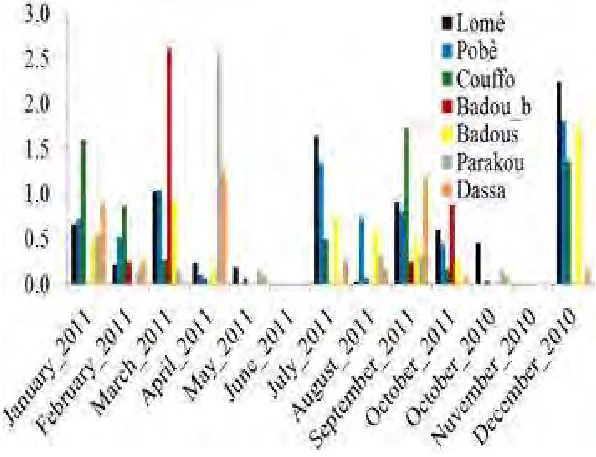

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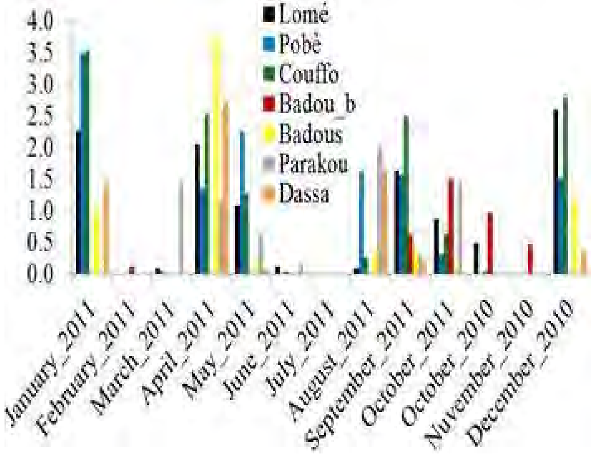

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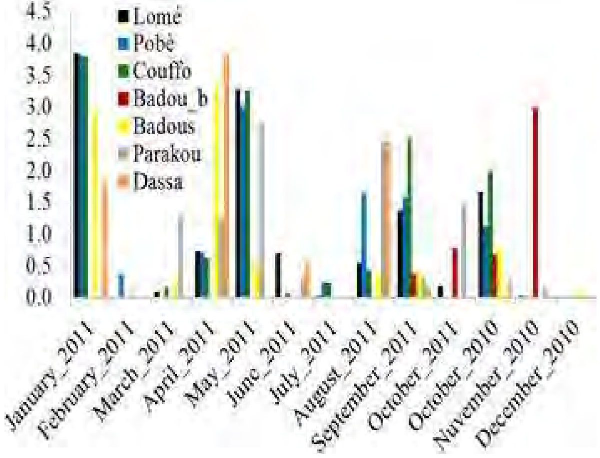

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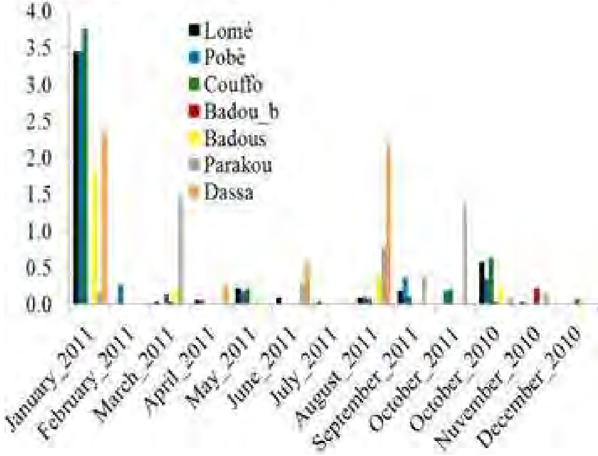

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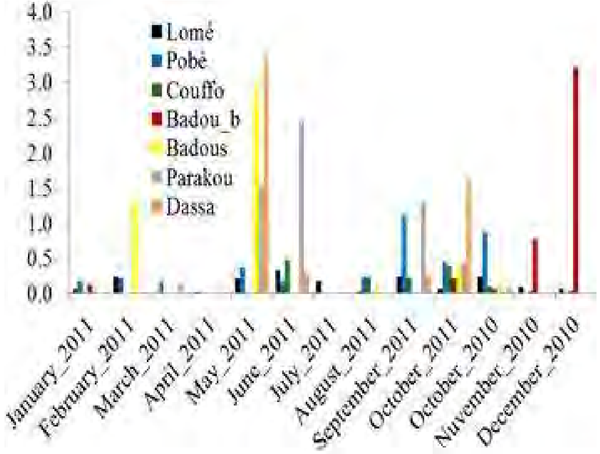


Figure 4.4g

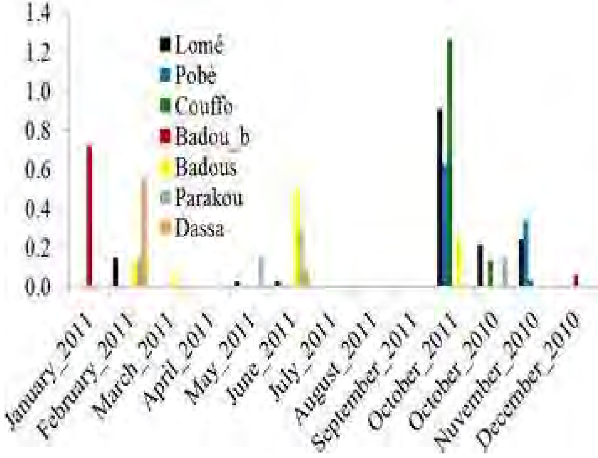


Figure 4.4h

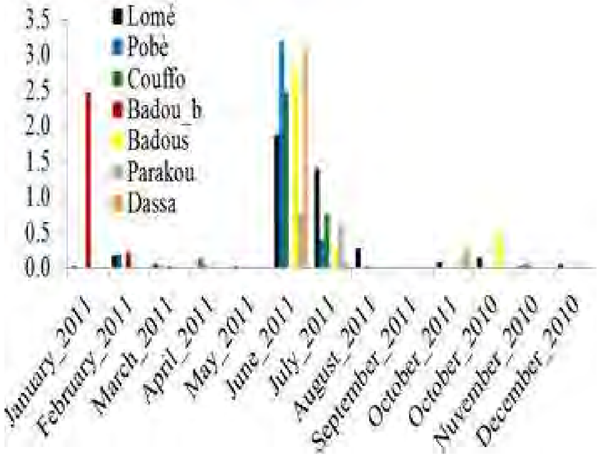
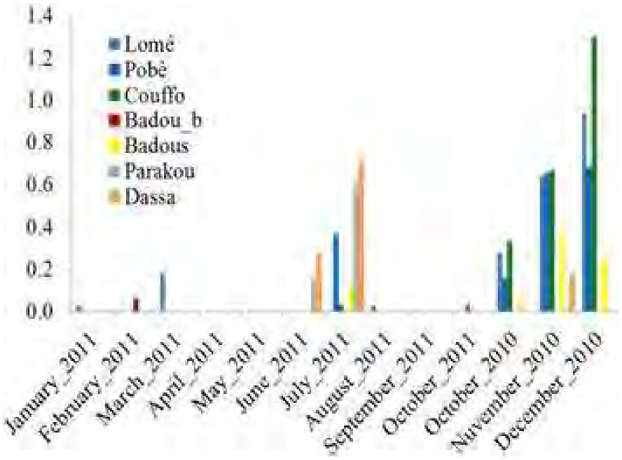


Figure 4.4i

Figure 4.4j



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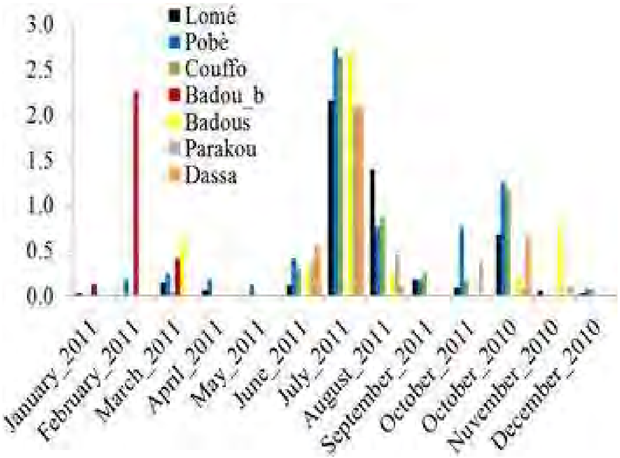


Figure 4.4l

Figure 4.4: Variations of the mean of the monthly level of the phenological events: (**a**) mature leaves, (**b**) leaves starting to drop, (**c**) leaves completely dropped (**d**) leaves freshly renewed, (**e**) flower buds, (**f**) open flowers, (**g**) aborted flowers, (**h**) immature fruits, (**i**) immature fruits aborted, (**j**) mature but unripe fruits, (**k**) aborted mature unripe fruits, and (**l**) mature ripe fruits.

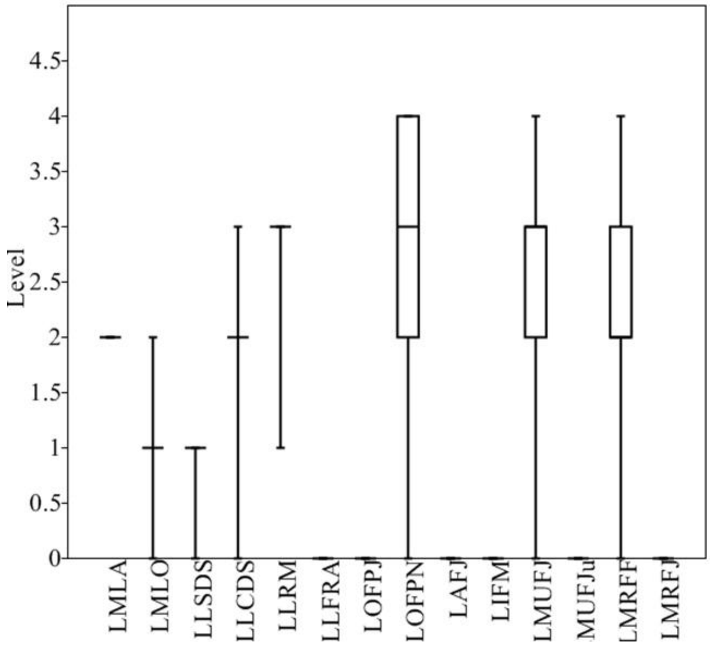


Figure 4.5a

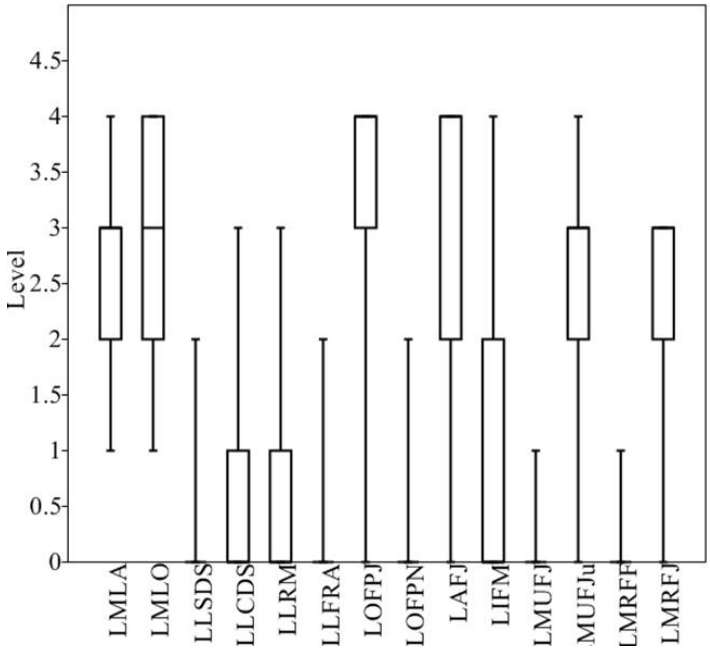


Figure 4.5b

Figure 4.5: Variation of the levels of discriminating monthly events: a = bitter trees, b = sweet trees: MLA=Level of mature leaves in August, LMLO=Level of mature leaves in October 2011, LLFRA=Level of leaf renewal in August, LOFPJ= Level of open flowers ready to be pollinated in January, LAFJ=Level of aborted flowers in January, LIFM=Level of immature fruits in May, LMUFJu=Level of mature unripe fruits in June, LMRFJ=Level of mature ripe fruits in July, LLSDS=Level of leaves starting to drop in September, LLCDS=Levels of leaves completely dropped in September, LLRM=Level of leaves renewed in March, LOFPN=Level of open flowers ready to be pollinated in November, LMUFJ=Level of mature unripe fruit in January, LMRFF=Level of mature ripe fruit in February.

The CDA to distinguish bitter and sweet ABMTs indicates that 52 of the 117 (that is 44%) monthly phenological events have significant discriminating power, when considered in isolation ($P < 0.001$). Together, their discriminant power is also consistent ($P < 0.001$). The total canonical structure of bitter and sweet ABMTs from the Volta region indicates the presence of fourteen significant discriminating monthly phenological events (absolute value of correlation ≥ 70%): level of mature leaves in August and in October 2011, leaves starting to drop in September, leaves completely dropped in September, leaves freshly renewed in March and in August, open flowers in January, and in November, aborted flowers in January, immature fruits in May, mature unripe fruits in January and in June, and mature ripe fruits in February and in July (Table 4.2a). Based on these discriminating monthly phenological events, all bitter and sweet ABMTs were consistently classified (100% with classification probability equalling to 1) in their respective group during the model calibration as well as during the classification of the samples of sweet trees from other populations. Results of the ANOVA-1 indicate that significant differences exist between bitter and sweet ABMTs regarding the discriminating monthly phenological events ($P < 0.001$). For example, bitter ABMTs (i) renewed leaves more intensively in March: level of leaves renewed= 2.6 (SD = 0.21) against 0.7 (SD = 0.11) for sweet trees; (ii) flowered more intensively in November: level of open flowers ready to be pollinated = 4 (SD = 0.5) against zero merely value for sweet trees and (iii) presented completely mature and ripe fruits in February: level of mature and ripe fruits = 2.2 (SD = 0.45) against almost zero values for sweet ABMTs. In the case of sweet ABMTs, their high flowering in January: level of open flowers ready to be pollinated = 4.4 (SD = 0.2) was followed by abundant abortion in the same month: level of flower abortion = 2.8 (SD = 0.16), and their two fruiting times in November and July distinguished them from bitter ABMTs (Fig. 4.5a-b).

The result of the PCA analysis shows that the first six PCA axes account for 74.51% of the phenological variation within the dataset. In total, 40 monthly phenological events are correlated with the first six PCA axes (Table 4.2b). The dendrogram obtained from the cluster analysis using those 40 monthly phenological events based on the Bray-Curtis similarity index separates all bitter ABMTs from the sweet ones at 0.24 similarity (Fig. 4.6). Three sub-groups can be distinguished within the group of sweet ABMTs: the first (G_1) clusters the population of Parakou (in the Southern-Borgou region) and is completely separated from other sweet trees at 0.44 similarity, the second (G_2) clusters trees of three populations: Pobè (Pobè region), Lomé (Coastal Togo region) and Couffo (Plateau region). In addition to those three populations, a few sweet trees from the Volta and Zou regions fall in this group. The last sub-group is separated from the second at 0.42 similarity and includes the other trees from the Volta and Zou regions (G_3; Fig. 4.6).

Table 4.2a: Results of the Discriminate Canonical Analysis.

Phenological event	Canonical Variable (CAN1)	Class Mean on Canonical Variable (CAN1)	Type of ABMTs Characterized
Level of mature leaves in August	0.82	4 * 106	Sweet ABMT
Level of mature leaves in October 2011	0.96		
Level of leaves freshly renewed in August	0.74		
Level of open flowers ready to be pollinated in January	0.89		
Level of aborted flowers in January	0.75		
Level of immature fruits in May	0.81		
Level of mature unripe fruits in June	0.92		
Level of mature ripe fruits in July	0.94		
Level of leaves starting to drop in September	-0.91	-2 * 106	Bitter ABMTs
Levels of leaves completely dropped in September	-0.89		
Level of leaves renewed in March	-0.74		
Level of open flowers ready to be pollinated in November	-0.82		
Level of mature unripe fruits in January	-0.77		
Level of mature ripe fruits in February	-0.81		

Table 4.2b: Results of the PCA analysis on the phenological states

Phenological state	**PCA 1 (28.86)**	**PCA 2 (12.94)**	**PCA 4 (11.14)**	**PCA 4 (8.47)**	**PCA 5 (7.75)**	**PCA 6 (4.45)**
Mature leaves in March 2011	-0.62	0.12	0.07	0.16	0.64	-0.06
Mature leaves in August 2011	-0.06	0.65	-0.54	-0.10	-0.01	-0.14
Mature leaves in October 2011	-0.06	0.15	0.40	0.04	0.69	-0.16
Mature leaves in December 2011	0.67	-0.21	0.41	0.18	0.14	-0.06
Start of leaf dropping in January 2011	-0.19	-0.62	0.14	0.14	-0.44	0.04
Start of leaf dropping in September 2011	0.65	0.01	-0.44	-0.18	-0.04	0.41
Start of leaf dropping in October 2011	0.74	-0.44	-0.08	0.01	0.26	0.02
Crown leafless in April 2011	-0.04	0.14	-0.11	0.77	-0.11	-0.14
Crown leafless in September 2011	0.46	-0.71	-0.04	0.26	0.01	0.06
Crown leafless in December 2011	-0.09	0.60	-0.62	-0.28	0.12	-0.02
Leaf renewal in March 2011	0.41	-0.71	0.24	-0.05	-0.01	0.10
Leaf renewal in May 2011	-0.04	0.24	-0.09	0.66	-0.17	-0.08
Leaf renewal in August 2011	0.05	0.40	0.71	-0.01	-0.02	0.09
Flower initiation in January 2011	-0.74	-0.04	0.26	-0.21	0.24	0.26
Flower initiation in September 2011	-0.61	-0.44	-0.22	-0.15	0.29	0.07
Flower initiation in December 2011	-0.71	-0.44	-0.09	0.05	-0.14	0.10
Mature flowers in January 2011	-0.82	0.04	0.42	0.18	0.06	0.00
Mature flowers in February 2011	0.06	0.44	0.70	-0.05	0.20	0.28
Mature flowers in May 2011	-0.69	-0.10	0.00	0.18	-0.16	0.45
Mature flowers in September 2011	-0.67	-0.40	-0.24	-0.18	0.40	0.06
Flower abortion in January 2011	-0.89	-0.06	0.10	0.02	0.06	0.06
Mature flowers in October 2011	-0.68	-0.50	-0.06	-0.19	-0.06	0.14
Immature fruit in October 2010	-0.04	0.44	-0.25	-0.22	0.12	-0.74
Mature unripe fruits in January 2011	0.69	-0.44	-0.05	0.01	0.27	-0.01
Mature unripe fruits in June 2011	-0.67	0.14	0.40	-0.44	-0.14	-0.15
Mature unripe fruits in August 2011	-0.02	0.12	-0.09	0.69	-0.16	-0.02
Aborting mature unripe fruits in November 2010	-0.64	-0.28	0.09	0.09	-0.09	0.10
Aborting mature unripe fruits in December 2010	-0.71	-0.47	-0.08	-0.21	-0.04	0.18
Mature and ripe fruits in February	0.71	-0.49	0.08	-0.01	0.41	0.05
Mature and ripe fruits in July	-0.74	0.20	0.19	-0.40	-0.25	0.05

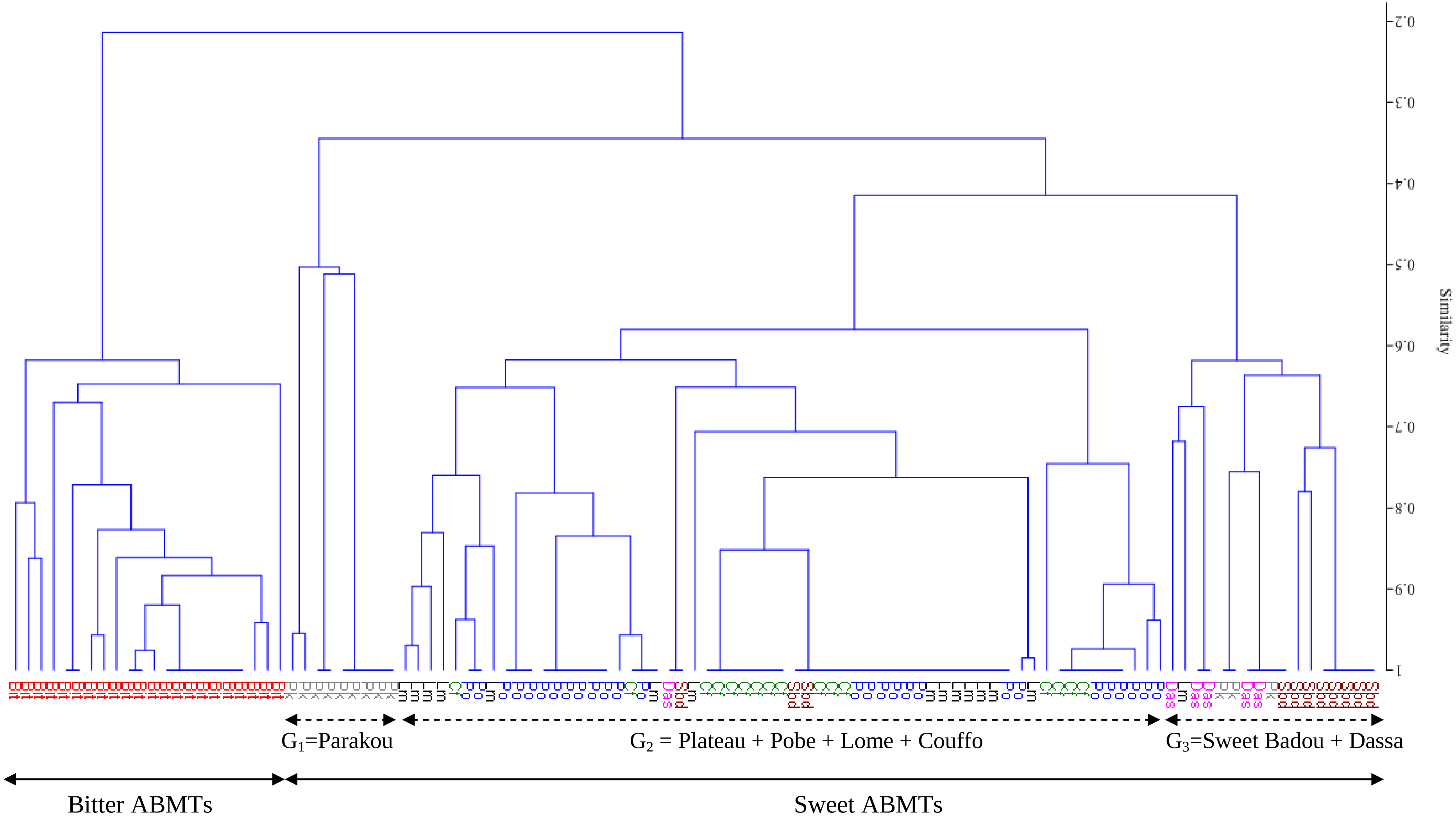


Figure 4.6: Classification of ABMTs based on the Bray-Curtis similarity index

Influence of environmental factors on ABMTs reproductive success

The results of the ANOVA show that the monthly variation of the flowering level of ABMTs significantly depends on the type of ABMTs and varies throughout the year ($P < 0.001$). Sweet ABMTs have the highest monthly flowering level in three peak times (January, May and September), while bitter trees flower only once (around November Fig. 4.7a). Although not during in the peak of their flowering, the two types of ABMTs co-flower in October (Fig. 4.7a). Flower abortion turns out to be higher for sweet ABMTs where it occurs throughout the year with its peak in January ($P< 0.001$; Fig. 4.7b). Significant differences exist between bitter and sweet ABMTs regarding the level and period of total fruit abortion ($P< 0.001$; Fig. 4.7c). Sweet trees abort repeatedly with their highest level in September-December, while bitter trees abort fruits once and intensely in January. Bitter and sweet ABMTs also show significant differences regarding the period of availability of mature and ripe fruits ($P< 0.001$; Fig. 4.7d). Mature and ripe fruits were recorded twice for sweet trees (July and October), while bitter trees have only one completely distinct fruiting period in February.

Significant differences exist among phytogeographical regions regarding the monthly variation of the flowering level ($P< 0.001$; Fig. 4.7e), and flower abortion level ($P< 0.001$; Fig. 4.7f).The observed flowering pattern in Fig. 4.7a was confirmed for the sweet tree populations of Pobè, Coastal Togo and Plateau as well as for the bitter trees in the Volta region. The January flowering was medium in the sweet tree populations of Zou and Volta and completely absent in South Borgou (see Fig. 4.7e). Apart from the January flower abortion, populations of Zou and South Borgou aborted flowers more repeatedly and at higher levels than in other phytogeographical regions (Fig. 4.7f). The phytogeographical regions also significantly influence the monthly level of mature and ripe fruits ($P< 0.001$; Fig. 4.7g). Apart from the Volta region, where mature and ripe fruits were found in three peak periods because of the presence of both bitter and sweet trees, the bimodal fruiting pattern observed for sweet trees (Fig. 4.7d) was observed in all regions. However, the populations of Pobè and Plateau have the highest mature and ripe fruit levels in the Dahomey Gap.

Finally, the monthly level of mature and ripe fruits significantly depended on the soil type ($P< 0.001$; Fig. 4.7h).

Figure 4.7: Variation in reproductive phenological events with type of ABMT and environmental factors. Fig. 4.7a: Difference in temporal variation of flowering level throughout the year between bitter and sweet ABMTs. Fig. 4.7b: Difference in temporal variation of flower abortion level throughout the year between bitter and sweet ABMTs. Fig. 4.7c: Difference in temporal variation of total fruit abortion level throughout the year between bitter and sweet ABMTs. Fig. 4.7d: Difference in temporal variation of mature ripe fruit level throughout the year between bitter and sweet ABMTs. Fig. 4.7e: Difference in temporal variation of flowering level throughout the year between phytogeographical regions. Fig. 4.7f: Difference in temporal variation of flowering abortion level throughout the year between phytogeographical regions. Fig. 4.7g: Difference in temporal variation of mature ripe fruit level throughout the year between phytogeographical regions. Fig. 4.7h: Difference in temporal variation of mature ripe fruit level throughout the year between soil types.

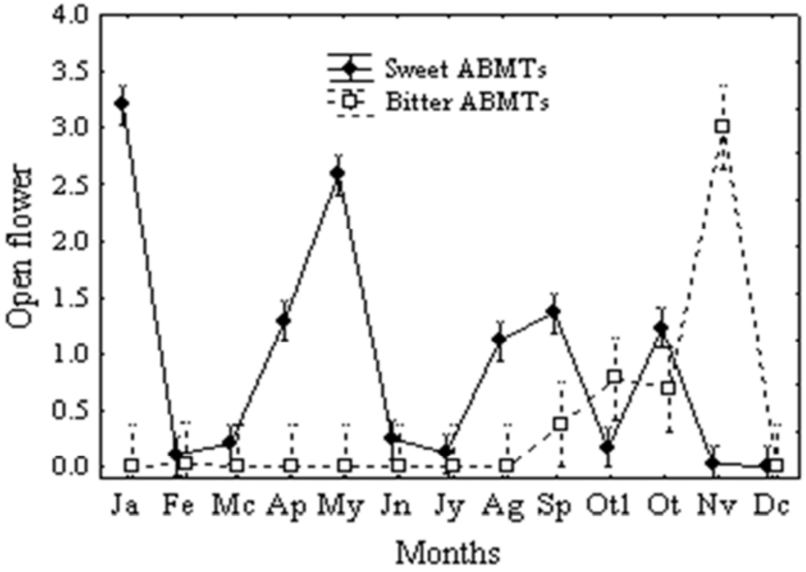


Figure 4.7a

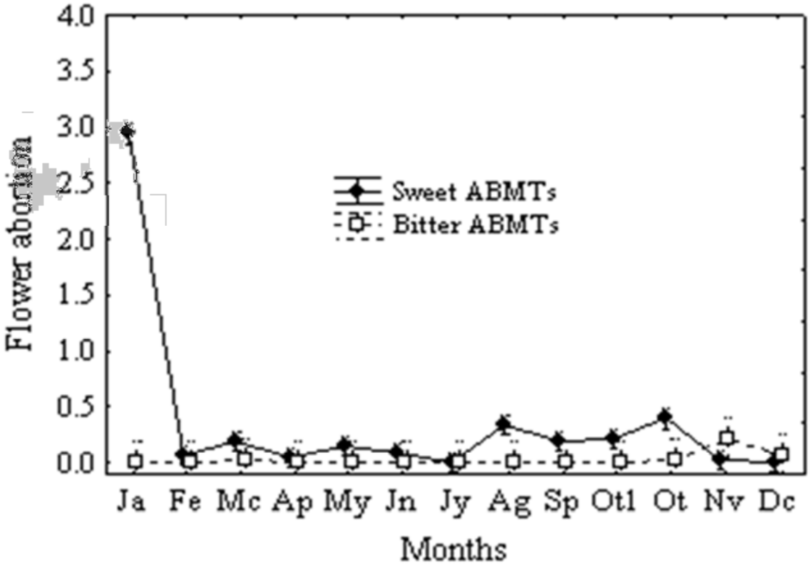
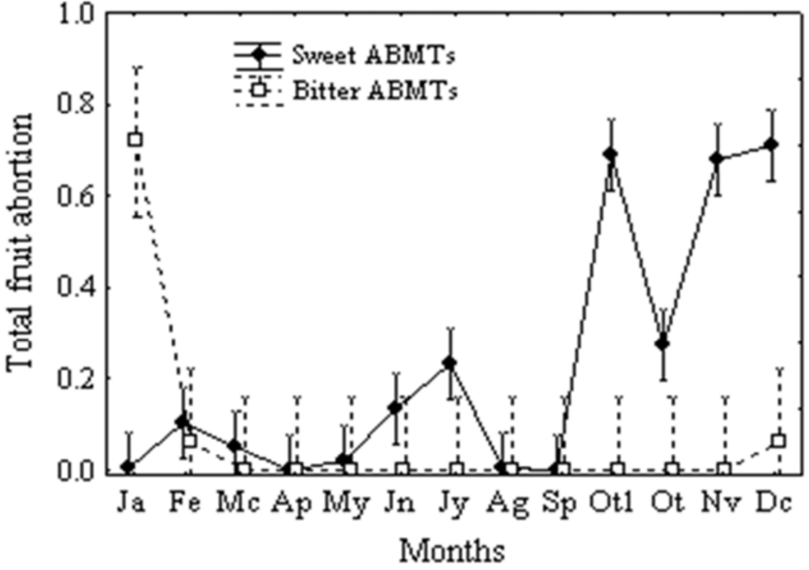


Figure 4.7b



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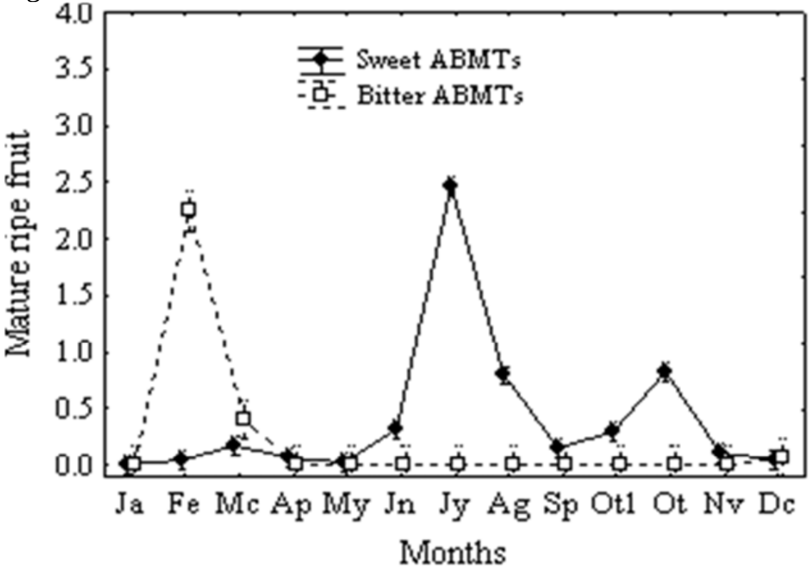

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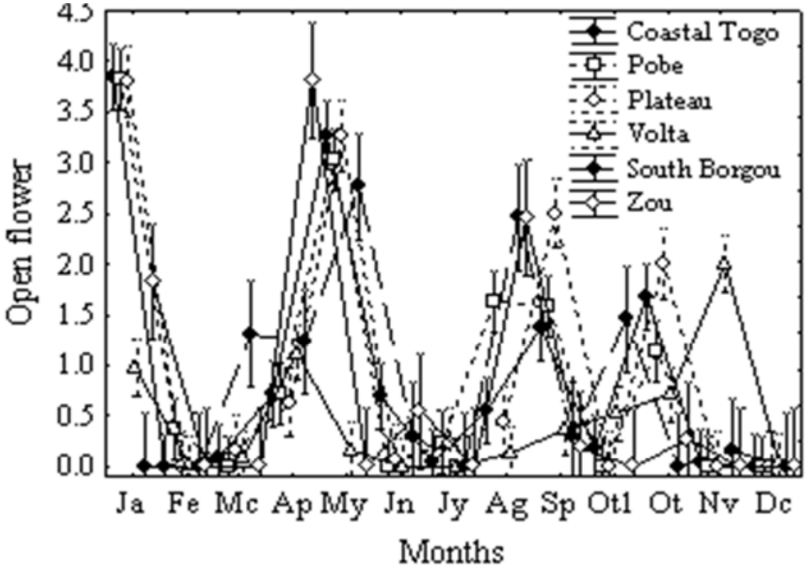

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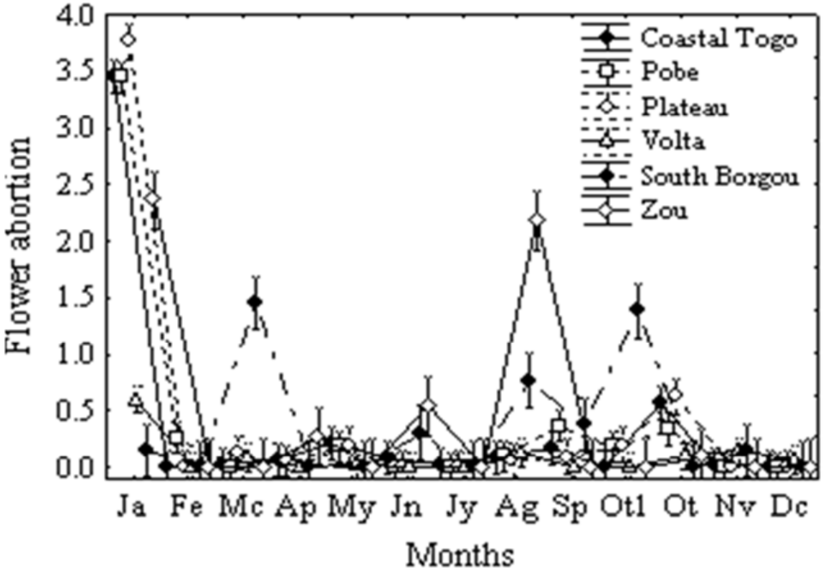

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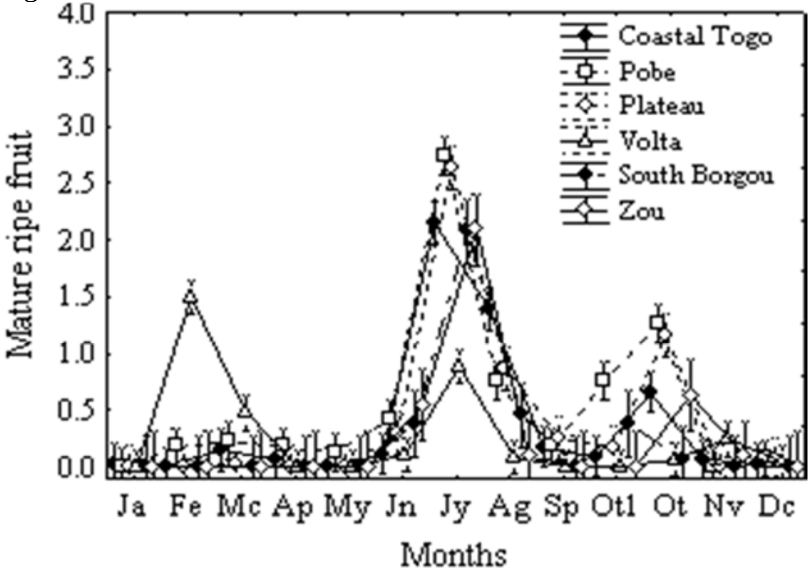

Figure 4.7g

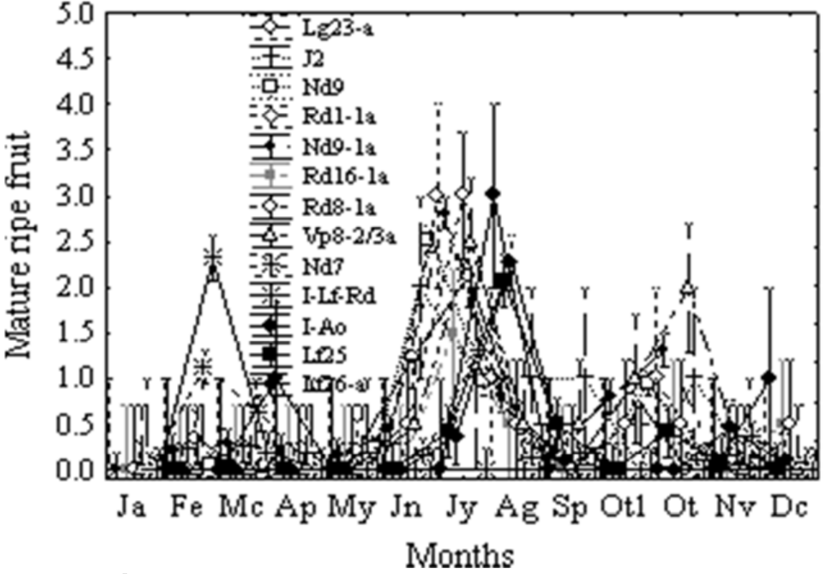

Figure 4.7h

Discussion

Phenological patterns and diversity

This study shows that in the Dahomey Gap the general phenological pattern of ABMT populations is confusing because of repetitive overlap in phenological events, mostly in the vegetative phase. However, phenological events (mostly in the reproductive phases) are generally more restricted in time for bitter trees while they stretch throughout the year for sweet trees. Lesley and Brown (2004) presented two flowering periods and repetitive fruiting events as a general phenological variation for both bitter and sweet trees. Harris (1996) showed variation in flowering and fruiting times in Central Africa (Cameroon and Gabon) and our results in the Dahomey Gap confirm the restricted reproductive phases detected by Harris (1996) and the results of Nzekwe *et al*. (2008) for bitter ABMTs in both the Guinean and Congolian African forest blocs. In Central Africa, Ladipo *et al*. (1996) reported natural bi-modal reproductive phases (as a general flowering and fruiting pattern) for sweet ABMTs, while Harris (1996) and Leakey and Simons (1998) presented the uni-modal flowering and fructification as the normal life cycle of ABMTs in the same eco-region, and attributed the bi-modal reproductive phases to the impact of domestication. Our data agree with this suggestion, because the highly domesticated sweet trees in the Dahomey Gap display an array from bi- to tri-modal and even higher flowering frequencies, but never a uni-modal one.

Considering the entire distribution range of ABMTs, evidence of bi-modal phenological patterns (see Newstrom *et al*., 1994) exists within natural as well as cultivated populations that show potential for selection and the establishment of more productive agroforestry systems in West and Central Africa. High rates of habitat degradation might play an important role in narrowing the phenological phases resulting in progressive irregularity of phenological events (see Herrerías-Diego *et al*., 2006). The extension of this bi-modal pattern for sweet trees to a much drier region like the Dahomey Gap (and even in some drier regions with precipitations less than 900 mm/year like in the North of Benin) is indicative of their wide ecological amplitude. In addition to their occurrence in contrasting ecological regions, the sudden occurrence of reproductive phases after unexpected short term changes within the climate regimes indicates the rapid adaptive response of sweet ABMTs to environmental change. This may well explain their abundance in agroforestry systems all over West and Central Africa (Shiembo *et al*., 1996).

The general phenological diversity is significantly different among populations. The sweet tree populations of Lomé, Pobè, and Couffo are all located in the Guinean climatic zone. Since plant phenological variation is partitioned by climate (Chuine, 2010), populations in the same climatic zone are expected to show identical phenological pattern. The differences in the phenological diversity within ABMTs populations in the Guinean climatic zone might be related to a difference in local domestication strategies (see Hodghin *et al.,* 1995) as well as to other local environmental factors, suspected here to be soil types. Populations of entirely spontaneous sweet trees (natural regeneration after mesocarp consumption by humans, without any management initiative) have a higher phenological diversity than the cultivated ones under the traditional mass selection process for seed commercialization. Since trees that show the same character (large seed) are being preserved and propagated, this directional selection strategy is narrowing the phenological diversity by synchronizing the reproductive phases (flowering and fruiting) and, thus, concentrating the harvest into two restricted times (see Ladizinsky, 1998, Leakey et *al.,* 2005; Munguia-Roas *et al.,* 2011). This is important for small farmers because it allows to easily detect the economic profitability of a crop and to better use the profit for livelihood enhancement. A lower phenological diversity was observed for all sweet tree populations located in hilly or drier areas with a uni-modal rainy season. Therefore, altitude as well as the climate regime partition ABMTs' phenological diversity across the Dahomey Gap (see Chuine, 2010; Munguia-Roas *et al.,* 2011). This low phenological diversity in high altitude and uni-modal climatic areas is not profitable in ABMTs cultivation because it negatively affects the fruiting success and reduces the productivity. Consequently, the climate regime and the altitude act like a domestication process, in shaping the tree species life cycle, which we postulate here as an adaptation strategy (Crepinsek and Kajfez-Bogataj, 2006).

Despite the fact that only wild bitter ABMTs were observed, they show low phenological diversity and more importantly a-synchronized reproductive phases compared to sweet trees. We postulate that, on the one hand this is related to their restricted distribution range devoid of ecological variability to which phenology might be linked (see Chuine, 2010). On the other hand, however, the repetitive reproductive events (flowering and fruiting) shown by the sweet trees in the same area (Fig. 4.4e-l) suggest low natural variation within bitter trees (see Doi *et al.*, 2010; Munguia-Roas *et al.,* 2011) and hence the existence of a genetic and taxonomic distinction between bitter and sweet ABMTs (Harris, 1996; Lowe *et al.,* 2000).

Phenological differences within ABMTs: implications for species distinction

Significantly discriminating monthly phenological events separate bitter and sweet ABMTs and justify the low similarity (similarity = 0.24; see Figs. 4.7) between the two types. Reproductive phases, especially the length of the flowering period and the period in which the flowers are receptive, are crucial for the possibility of hybridization (Doi *et al*., 2010). Although there are consistent differences regarding the period when flowers are receptive between bitter and sweet ABMTs, the extended flowering time of sweet ABMTs partly overlaps with the limited one of bitter ABMTs, suggesting the possibility of hybridization in their area of co-occurrence in October (Fig. 4.4f).When comparing our observations of October 2010 and October 2011, we conclude that the overlap of the flowering times of the two types of ABMTs is not the same every year (see Fig. 4.4f). Hence the possibility of hybridization probably fluctuates annually. However, the absence of fruits in December and January (Fig. 4.4h, j, l) in the sweet ABMTs population in the co-occurrence area (Volta region) and the steadiness in bush mango taste suggest no or unsuccessful hybridization through pollen transfer from bitter to sweet ABMTs. Therefore, we postulated that bitter and sweet ABMTs are better be considered as two different taxa. It is unknown whether successful hybridization happens from pollen transfer from sweet to bitter ABMTs. The compatibility between bitter and sweet ABMTs in their reproductive phases through different climatic zones in the entire distribution range would therefore be an interesting research topic.

The extended phenological pattern of sweet ABMTs allows the availability of fruits throughout the year with the highest levels in the rainy seasons. In contrast, in the Volta region, bitter ABMTs fruit once a year at the beginning of the rainy season (February) coinciding with the midst of the severest dry season in the rest of the Dahomey Gap. This implies that colonization of bitter trees from Volta to the rest of the Dahomey Gap is impossible, unless the distribution is promoted by human activities. Bitter ABMTs grow well in regions climatically similar to the Dahomey Gap like Ibadan in Nigeria (see Kang *et al*., 1994 and World Agroforestry field gene bank). Thus, the restriction of their distribution range in the Dahomey Gap is mostly related to their reproductive time (Chuine, 2010) and the lack of a human role in seed dispersal.

Impact of environmental factors on ABMTs occurrence and production

The timing and levels of phenological events of ABMTs in the Dahomey Gap varies throughout the year with important differences among phytogeographical regions and between bitter and sweet trees. This consistent difference explains the differences in the actual spatial distribution pattern in the Dahomey Gap (see Chuine, 2010; Doi *et al*., 2010). The wide distribution range of sweet trees is partitioned mostly by climatic factors (Crepinsek and Kajfez-Bogataj, 2006) causing differences among populations. Therefore, variation in reproductive success might be considered as an adaptation in response to differences in climate. High altitude plus high temperature with or without low rainfall reduce the repetitive reproduction of sweet ABMTs. Therefore, the highest potential of bush mango production is located in the Guinean climatic zone, a lowland area climatically very close to the postulated natural distribution ranges of ABMTs (see Kang *et al*., 1994; Van Dijk, 1999; Vihotogbé *et al.*, submitted-2).The extension of a reproductive phase or the development of a repetitive one are interesting key selection factors to increase a species' annual yield (Thurling, 1991). Therefore, the repetitive reproduction of sweet ABMTs gives them a higher economic value and determines their priority over bitter trees which justifies their abundance in advanced agroforestry systems (Okafor and Fernandes, 1987; Vihotogbé *et al.*, submitted-1). This economical advantage is hindered by the high level of flower and fruit abortion. Flower abortion in sweet ABMTs is mostly due to low rainfall and high temperatures occurring in all phytogeographical regions in January and which is more severe in the Northern populations. However, fruit abortion is also induced by pest infestation. In areas where the seeds are the most valuable product of ABMTs, infested trees are still important when the seed can be harvested from fruits before their abortion or even from unripe ones. In the situation that the entire fruit is the most important NTFP, infestation affects the quality of the mesocarp, lowering the value of the tree. This leads to the suppression of many infested sweet trees by local farmers and probably to a decrease of susceptible tree types.

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Chapter 5

Morphological Characterization of African Bush Mango Trees (*Irvingia* species) in the Dahomey Gap (West Africa)

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Abstract

This study investigates the morphological characteristics of bitter and sweet African bush mango trees (*Irvingia* species). African bush mangoes have been rated as the highest priority multi-purpose trees in need of improvement research in West and Central Africa. This study was carried out in the Dahomey Gap which is the West African savannah woodland area separating the Upper and the Lower Guinean rain forest blocks. We studied 128 trees from six populations and characterized their bark, fruits, mesocarp and seeds to assess the morphological differences among populations in the field. First, characteristics that might allow the distinction of bitter and sweet trees were analysed in a binary logistic regression. Second, a Principal Component Analysis was performed on fruit, mesocarp and seed measurements to define groups. The significance of factors that defined these groups was assessed in a Multivariate Analysis of Variance and a pairwise comparison of populations was performed using the Scheffe test. Lastly, characteristics were used in a cluster analysis (UPGMA). None of the variables: type of bark, mature fruit exocarp colour, fruit roughness and fresh mesocarp colour, were able to consistently distinguish bitter from sweet trees in the field. The analysis of the measurements of fruits, seeds and mesocarps demonstrated that bitter fruits have the heaviest seeds and this consistently distinguishes them from sweet fruits. However, the measurements of the fruit, mesocarp and seed did not have a joint effect in grouping sweet fruited populations. This indicates high diversity and potential for selection across all phytogeographical regions. The sweet trees of Couffo and those of Dassa are clearly different from all other populations. This can be attributed to traditional domestication and climate, respectively. The large fruits and the heavy seeds of the cultivated populations are evidence of successful on-going domestication and selection of sweet trees in the Dahomey Gap.

Key words: Dahomey Gap, domestication, Irvingiaceae, tree-to-tree variation, selection, species concept.

Introduction

African Bush Mango trees (ABMTs) belong to the small family of Irvingiaceae and are widely distributed in sub-Saharan Africa (Harris, 1996; Asaah *et al.,* 2003; Lesley and Brown, 2004). Significant phenological, morphological, and genetic variation was detected among populations across West and Central Africa and turned out to be correlated to geographical distance (Leakey *et al.,* 2000; Lowe *et al.,* 2000; Tairu *et al.,* 2000; Atangana *et al.,* 2001; Atangana *et al.,* 2002; Anegbeh *et al.,* 2003; Leakey *et al.*, 2003; Leakey *et al*., 2004; Leakey *et al.,* 2005; Ude *et al.,* 2006). However, quantitative morphological data relative to this variation have only been obtained from Central African populations. Sampling over the entire range is in turn a prerequisite for their effective conservation and for the development of an all-inclusive domestication program (Ladipo *et al.,* 1996; Lowe *et al.,* 2000; Ekue *et al.,* 2010).

Bush mango fruits taste either sweet or bitter and the corresponding trees have been treated as two different species: *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. and *I. wombolu* Vermoesen, respectively (Harris 1996; Lowe *et al.,* 2000). However, based on the minimal morphological differences, Okafor (1975) claimed that the taxa were best considered as two varieties (*I. gabonensis* var. *gabonensis* and *I. gabonensis* var. *excelsa* (Mildbr.) Okafor) and these infraspecific taxon names are also widely used. This has raised a controversial taxonomic situation causing some confusion in literature. Solving it requires a multi-disciplinary approach.

Sweet fruited trees are the most intensively studied ABMTs (Atangana *et al.,* 2001; Atangana *et al.,* 2002; Anegbeh *et al.,* 2003; Leakey *et al.,* 2003; Leakey *et al.,* 2004; Leakey *et al.,* 2005; Ude *et al.,* 2006 and other works of the World Agroforestry Centre and Centre for International Forestry Research) and apart from the comparative botanical data of Harris (1996), there is no quantitative assessment of the morphological variation of bitter and sweet ABMTs. Moreover, since bush mangoes are expected to generate high economic value in the near future based on at least two marketable products, the entire fruits and the endocarp containing the seed, important characteristics such as variation in fruit shape, mature fruit colour, and consumer preferences are particularly important (Ladipo *et al.,* 1996; Rashidi and Seyif, 2007). These are morphological features that vary within and between bitter and sweet trees and which are vital to define the nature of the material to be domesticated.

Fruit shape might particularly influence the choice of consumers (Luckwill, 1959; Rashidi and Seyif, 2007). Moreover, it will influence fruit size and weight which varies with environmental factors (Sinnott, 1932). Therefore, fruit shape is an important economical parameter and its diversity might be a valuable selection criterion in the domestication process. It can also be used to verify the taxonomic identity of the material being domesticated since it can be used in species delimitation (Saheed and Illoh, 2011). The diversity of bush mango fruit shape through different ecological zones and a comparison of bitter and sweet fruited trees in the Dahomey Gap as well as in their entire distribution range will provide important data to be related to the potential production of ABMT mesocarp and seed.

This work is part of a broader project intending to revisit differences within and between bitter and sweet ABMTs using ecological, phenological, morphological and genetic approaches. It focuses on the situation in a particular eco-region named the Dahomey Gap which is characterized by the virtual absence of lowland rainforest resources due to higher temperatures and less rainfall than is found in regions to the west and east. By tracing morphological features that will allow a distinction between and among bitter and sweet ABMTs we seek answers to two main research questions:

(i) Are bitter and sweet fruited ABMTs distinguishable in the field by features that can be easily observed?

(ii) Can the morphological variation of bush mangoes discriminate populations and types of ABMTs?

Materials and Methods

Sampling

The sampling design in this study follows, with small modifications that used by Vihotogbé *et al.* (Submitted-2) to evaluate phenological diversity within ABMTs. Six southern ABMTs populations (Pobè, Couffo, Dassa, Lomé and SVolta for sweet trees and Bvolta for bitter trees) in five different phytogeographical regions in Benin and Togo were investigated. In these populations, the sampled trees are a sub-set of those used for the phenological study. A sixth phytogeographical region, the Ouémé valley, was sampled in the surroundings of Abomey Calavi bringing the number of populations to seven.

In 2011, characteristics of a total of 128 fruit bearing trees (7 to 30 per population) were scored. In order to avoid incorporating variation due to differences in tree maturity, only adult trees that had been producing fruits for at least 5 years were selected. They were inspected for diseases to make sure that only totally healthy trees, without any kind of parasitism or human induced damage that might alter the morphological characteristics of trees and fruits, were selected. Fruit collecting was performed in February, June and July, the peak fruiting periods across the Dahomey Gap. Trees where visited early in the morning to collect mature fruits that had freely fallen down during the night. In total, 2,523 fruits (10 to 32 per tree) were collected and used in this study.

Morphological data

Each individual bitter and sweet fruited tree was described with the following six variables: (i) the type of bark, (ii) exocarp colour of the mature fruit, (iii) exocarp roughness of the mature fruit, (iv) mesocarp colour of the fresh fruit, (v) presence / absence of brown pigments in the fresh mesocarp, and (vi) the mesocarp tanning after 30 minutes. The type of bark was identified based on two main factors: its colour and roughness (Fig. 5.5.1). The colour of the exocarp and of the fresh mesocarp was scored using the Royal Horticultural Society Colour Chart (http://www.rhs.org.uk/Plants/RHS-Publications/RHS-colour-charts). The roughness of the fruit refers to the presence or absence of small pits in the fruit skin.

The seven detected bark types were arbitrarily coded from 1 to 7. Fruit skin roughness was coded as follows: smooth = 0, rough only on the side of the largest width = 1; uniformly but slightly rough = 2; uniformly medium rough = 3 and very rough = 4. The combination of standard numbers letters of the colour chart was used to identify the mature fruit exocarp and fresh mesocarp colour which were also arbitrarily coded: green group_135B = 1, green group_136B = 2, green group_139A = 3, yellow green group 144A = 4, yellow green group_146A = 5, yellow green group_146B = 6, yellow green group_152A = 7, yellow green group_152B = 8, yellow green group_152C = 9, yellow group_13A = 10, and yellow orange group_22A = 11 for exocarp colour and orange group_25A = 1, orange group_25B = 2, yellow orange_11A = 3, yellow group_11B = 4, yellow group_12A = 5, yellow group_13A = 6, yellow group_13B = 7, yellow group_13C = 8, yellow orange_20A = 9, yellow orange_21A = 10, and yellow orange_23A = 11 for mesocarp colour. For each individual tree, the occurrence of brown pigments in the fresh mesocarp and mesocarp tanning was reported as either 1 (for presence) or 0 (for absence).

Figure 5.5a: Arbitrary codes of bark types. The same letter implies the same type at different degrees of maturity: a: grey-orange colour smooth at immature stage and rough with splits and scales when mature; b: grey-green colour with little fine scales (only when mature) and without splits even when mature; c: grey colour with lateral crease but without splits and scales even when mature (rare bark type); d: grey-brown colour with earlier development of splits and scales (splits are particularly deep at the insertion point of branches on the trunk; type mostly found on low-branching trees in Couffo); e: grey colour with very fine scales and without splits; f: intense grey colour with abundant scales; g: grey-black colour with large and coarse scales.

The data collecting method proposed by Leakey *et al.* (2000) and used by Atangana *et al.* (2001) and Anegbeh *et al.* (2003) was used. Thus, for each fruit, the following ten variables were measured: (vii) fruit weight, (viii) fruit total length, (ix) fruit largest width, (x) fruit smallest width, (xi) mesocarp depth on the side of the seeds largest width, (xii) mesocarp depth on the side of the seeds smallest width, (xiii) seed weight, (xiv) endocarp length, (xv) endocarp width, and (xvi) endocarp thickness. The fruit and seed weight was measured using a Soehnle laboratory electronic portable scale (Min: 1g; Max: 2000g). Fruit length and diameter sand seed width and thickness were measured with an electronic digital calliper (0.01mm resolution) that also served to measure the mesocarp depths. The total length and both widths of individual fruits were converted into two fruit shape indices, the roundness ratio (RR) and ellipsoid ratio (ER) (Luckwill, 1959; Rashidi and Seyif, 2007):

$$RR = {}^{TL}\!/_{\sqrt{Ld\ x\ Sd}} \tag{1}$$

and

$$ER = {}^{Ld}\!/_{Sd} \tag{2}$$

TL = the total length of the fruit, *Ld* and *Sd* = its largest and smallest diameters, respectively.

For each sampled tree, we determined the mean RR (M_{RR}) and mean ER (M_{ER}). These values were used to assess the fruit shape as follows: oblate fruit ($0.86 < RR \leq 0.9$), spheroid to prolate spheroid fruit ($0.9 < RR \leq 1.14$) and oblong fruits ($RR \geq 1.15$); round fruits ($1 \leq ER < 1.1$) or elliptical fruits ($1.1 \leq ER \geq 1.2$).

Data analysis

The type of bark, fruit exocarp and mesocarp colour, exocarp roughness, the occurrence of brown pigments in the fresh mesocarp and its tanning were analysed in a binary logistic regression against the type of ABMT using SAS Version 9.2 (SAS Institute Inc. 2003). We used the Global Null Hypothesis Test and the Type 3 Effects Analysis to validate the model and identify factors that allow distinguishing bitter and sweet fruited ABMTs.

A principal component analysis (PCA) was performed on fruit shape indices, fruit total length and largest width, fruit and seed weight, mesocarp depths, and endocarp length, width and thickness, using PAST (Hammer *et al.*, 2001).

To identify significant factors responsible for the detected pattern, a multivariate analysis of variance (MANOVA) was carried out on the standardized values of these factors (fruit shape indices, fruit total length and largest width, fruit and seed weight, mesocarp depth, and endocarp length, width and thickness) against the populations. For each individual fruit, the mesocarp weight was calculated by deducting the seed weight from the fruit weight. The mean mesocarp weight was computed at individual tree level and used in the MANOVA. The Scheffe test was performed for the pairwise mean comparison and population grouping based on the significance of the mean of each factor. In order to determine which factor specifically distinguished bitter tree populations from the sweet tree ones, a second MANOVA analysis was performed on the same 10 factors against the type of ABMT.

Finally, the significant morphological factors that distinguished populations and types of ABMTs were used in a cluster analysis using the Euclidian distance among sampled trees.

Results

Field identification of bitter and sweet ABMTs

The logistic regression analysis indicates that none of the six considered factors (type of bark, fruit exocarp colour, fruit skin roughness, fresh mesocarp colour, occurrence of brown pigment in the fresh mesocarp, and mesocarp tanning after 30 min) are capable to consistently distinguish bitter from sweet ABMTs (P > 0.05 Table 5.1).

Table 5.1: Results of the logistic regression to test the possibility to identify bitter and sweet trees in the field.

Testing Global Null Hypothesis: BETA = 0			
Test	**Chi-Square**	**Degree Of Freedom**	**P > Chi-Square**
Likelihood Ratio	101.78	31.00	< 0.0001
Score	121.53	31.00	< 0.0001
Wald	0.17	31.00	1.00
Type 3 Analysis of Effects			
Effect	**DF**	**Wald Chi-Square**	**P > Chi-Square**
Bark type	6.00	0.02	1.00
Exocarp colour	10.00	0.08	1.00
Fruit skin roughness	4.00	0.00	1.00
Mesocarp colour	9.00	0.02	1.00
Presence of brown pigments in the mesocarp	1.00	0.00	0.91
Mesocarp tanning	1.00	0.00	0.90

Diversity of fruit shapes and quantitative variation in fruit, mesocarp and seed

In general, the RR values indicate that a spheroid to prolate spheroid fruit shape is common in bush mangoes in the Dahomey Gap (84.5%). Oblong and oblate shapes are less represented (14 and 1%, respectively). The ER values show that 85.27% of ABMTs produce fruits with a circular cross-section, while 14.7% produce fruits with elliptical outlines. The heaviest fruits (fruit weight > 400 g) are found in the population of Couffo and the heaviest seeds (seed weight > 40 g) are recorded in the bitter tree population. The population of Dassa produces the smallest fruits and seeds in the Dahomey Gap (Table 5.2a).

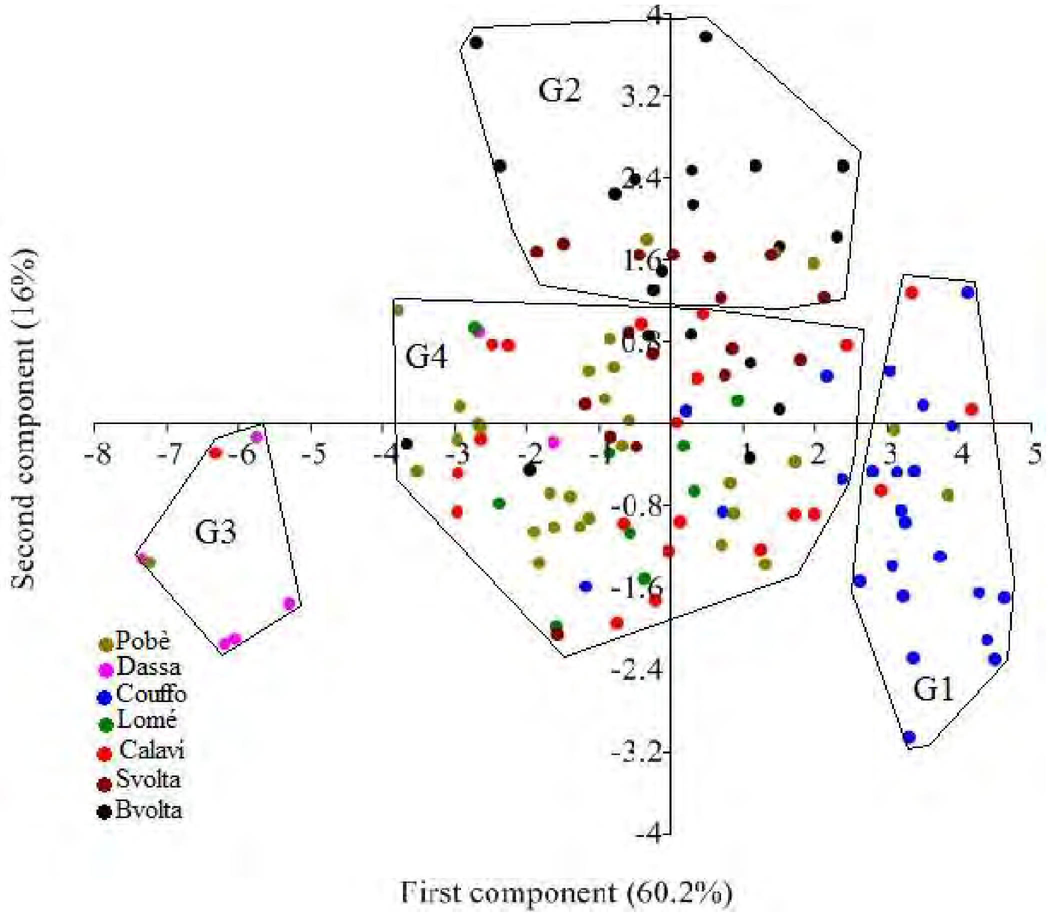


Figure 5.5b Result of the Principal Component Analysis: identification of groups within bush mangoes based on quantitative morphological characters of fruits and seeds.

The first two axes of the PCA explained 76.2% of the total variation. The first axis (60.2%) is mostly influenced by variables measured on the entire fruit, the mesocarp, and the seed, while the second (16%) is influenced by the roundness ratio (Table 5.2b). Four groups of trees were identified (Fig. 5.5b): (i) G1 comprises the majority of sweet ABMTs from the Couffo region with few individuals from Calavi and Pobè, (ii) G2 is composed of the majority of bitter trees with few individuals from Pobè and Volta region, (iii) G3is mostly composed of sweet ABMTs from Dassa, and (iv) G4 is a mixture of the majority of individuals from Pobè, Lomé, Calavi and the Volta region, but with a few individuals from the first three groups.

The results of the MANOVA against the populations indicate that there is no significant difference linked to fruit shape among populations ($P > 0.05$ for roundness and ellipsoid ratios; Table 5.2c). But all the other evaluated characters (fruit total length and largest width; fruit, mesocarp and seed weight; mesocarp depths; and seed length, width, and thickness) show significant differences among populations (Wilks' Lambda = 0.128; $F = 5.91$; $p < 0.001$; Table 5.2c).The population of Dassa has the lowest values for most of these factors (Fig. 5.3a-j). The heaviest, biggest and fleshiest fruits are found in the population of Couffo (Fig. 5.3a-f).For most of the evaluated factors, the population of Calavi presents the highest variation (Fig. 5.3).The Scheffe test indicates that the significant morphological factors related to the measurements on fruits, mesocarp and seeds do not define groups based on the pairwise difference of their means for populations (Table 5.2d). It also reveals that for most of the characters, the populations of Couffo and Dassa show significant differences both between them and from all others (Table 5.2d). The population of Dassa is different for most of the characters related to the entire fruit, the mesocarp and seed (Table 5.2d; Fig. 5.3) and that of Couffo is different from most other populations based on the fruits and mesocarp measurements (Table 5.2d; Fig. 5.3a-f). The Scheffe test for population grouping also indicates that the only factor that significantly distinguishes the bitter tree population from most of the sweet ones was the seed weight (Table 5.2d; Fig. 5.2 and 5.3j), but this character did not significantly separate the bitter tree population from the sweet ones in the Couffo and Volta regions. The largest and heaviest seeds are produced in both the bitter trees population and in that of the sweet trees in Couffo. The variation of most characters is higher in the population of Calavi. The results of the MANOVA against the type of ABMT indicates that the seed weight, width and thickness and mesocarp depths and weight are significantly different between the bitter and sweet ABMTs (Table 5.2c). Heavy, large and thick seeds are characteristic for bitter fruits, while deep and abundant mesocarp is in general characteristic of sweet fruits.

The cluster analysis shows a clear separation of the Couffo population from all other populations. Even though three other main groups appear, they do not express a complete distinction among the other populations of sweet trees and between bitter and sweet trees (Fig. 5.4).

Table 5.2a: Overall variation of bush mango fruit, mesocarp and seed characteristics.

Population	Morphological character	Mean	Std Dev	Minimum	Maximum
Bvolta	*Fruit weight (g)*	*117.6*	*39.7*	*38.0*	*252.0*
	Mesocarp depth (Ld) (cm)	1.2	0.3	0.4	2.2
	Mesocarp depth (Sd) (cm)	1.6	0.3	0.4	2.6
	Seed weight (g)	*20.1*	*5.2*	*5.0*	*41.0*
	Endocarp length (cm)	4.1	0.5	2.3	5.6
	Endocarp width (cm)	3.5	0.4	2.1	4.8
	Endocarp thickness (cm)	2.2	0.3	1.1	3.3
Calavi	*Fruit weight (g)*	*132.0*	*60.7*	*29.0*	*359.0*
	Mesocarp depth (Ld) (cm)	1.7	0.4	0.4	2.7
	Mesocarp depth (Sd) (cm)	1.8	0.5	0.6	3.0
	Seed weight (g)	*14.4*	*5.2*	*4.0*	*29.0*
	Endocarp length (cm)	3.9	0.7	1.4	5.8
	Endocarp width (cm)	3.0	0.4	1.9	4.9
	Endocarp thickness (cm)	2.0	0.3	1.0	2.9
Couffo	*Fruit weight (g)*	*213.7*	*53.6*	*78.0*	*426.0*
	Mesocarp depth (Ld) (cm)	2.2	0.4	0.5	3.8
	Mesocarp depth (Sd) (cm)	2.4	0.4	1.6	3.8
	Seed weight (g)	*18.1*	*4.4*	*6.0*	*30.0*
	Endocarp length (cm)	4.3	0.5	3.0	5.7
	Endocarp width (cm)	3.3	0.4	2.2	5.3
	Endocarp thickness (cm)	2.2	0.3	1.2	3.4
Dassa	*Fruit weight (g)*	*57.4*	*30.6*	*19.0*	*137.0*
	Mesocarp depth (Ld) (cm)	1.0	0.4	0.4	2.2
	Mesocarp depth (Sd) (cm)	1.2	0.3	0.6	1.9
	Seed weight (g)	*6.6*	*4.5*	*0.5*	*15.0*
	Endocarp length (cm)	2.6	0.6	1.6	3.9
	Endocarp width (cm)	2.0	0.6	1.0	3.2
	Endocarp thickness (cm)	1.3	0.3	0.8	1.8
Lomé	*Fruit weight (g)*	*114.5*	*32.8*	*56.0*	*200.0*
	Mesocarp depth (Ld) (cm)	1.8	0.3	1.2	2.7
	Mesocarp depth (Sd) (cm)	1.6	0.3	0.9	2.4
	Seed weight (g)	*11.8*	*3.3*	*6.0*	*22.0*
	Endocarp length (cm)	3.8	0.4	2.6	5.1
	Endocarp width (cm)	2.7	0.4	1.9	3.6
	Endocarp thickness (cm)	2.0	0.3	1.4	4.3
Pobè	*Fruit weight (g)*	*112.6*	*44.8*	*25.0*	*288.0*
	Mesocarp depth (Ld) (cm)	1.5	0.4	0.4	3.4
	Mesocarp depth (Sd) (cm)	1.7	0.4	0.6	3.4
	Seed weight (g)	*13.3*	*4.6*	*3.0*	*29.0*
	Endocarp length (cm)	3.7	0.6	1.9	6.7
	Endocarp width (cm)	2.9	0.4	1.2	5.5
	Endocarp thickness (cm)	2.0	0.4	1.0	3.5
Svolta	*Fruit weight (g)*	*120.5*	*33.3*	*11.0*	*252.0*
	Mesocarp depth (Ld) (cm)	1.5	0.3	0.7	2.3
	Mesocarp depth (Sd) (cm)	1.7	0.3	1.1	2.8
	Seed weight (g)	16.0	4.2	3.6	32.0
	Endocarp length (cm)	4.1	0.5	1.4	6.7
	Endocarp width (cm)	3.2	0.4	1.8	5.5
	Endocarp thickness (cm)	2.1	0.3	1.5	4.0

Ld = largest diameter, Sd = smallest diameter

Table 5.2b: Principal component loads on first and second axis from PCA of bush mangoes characteristics.

Morphological characters	PC_1 (60.2%)	PC_2 (16%)
Mean ellipsoid ratio	-0.14	0.42
Mean roundness ratio	-0.03	0.57
Mean endocarp thickness	0.72	0.34
Mean endocarp width	0.79	0.45
Mean endocarp length	0.87	0.38
Mean seed weight	0.80	0.50
Mean mesocarp depth (Ld)	0.88	-0.39
Mean mesocarp depth (Sd)	0.79	-0.52
Mean fruit weight	0.93	-0.27
Mean fruit length	0.95	-0.03
Mean Fruit width (Ld)	0.96	-0.23

Table 5.2c: Results of the multivariate ANOVAs.

Source of variation	**Morphological characters evaluated within populations**	**Type III Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F value**	**P value**
Between Populations	Mean of ellipsoid ratio	0.005	6	0.001	0.612	0.720
	Mean of roundness ratio	0.06	6	0.01	1.918	0.083
	Mean of endocarp thickness	*6.719*	*6*	*1.12*	*5.231*	*< 0.001*
	Mean of endocarp width	*13.72*	*6*	*2.28*	*10.142*	*< 0.001*
	Mean of endocarp length	*14.18*	*6*	*2.36*	*7.687*	*< 0.001*
	Mean of seed weight	*1339*	*6*	*233.3*	*12.22*	*< 0.001*
	Mean of mesocarp depth (Ld)	*16.288*	*6*	*2.71*	*17.504*	*< 0.001*
	Mean of mesocarp depth (Sd)	*15.525*	*6*	*2.58*	*15.74*	*< 0.001*
	Mean of mesocarp weight	*209275*	*6*	*34879*	*24.3*	*< 0.001*
	Mean of fruit weight	*221745*	*6*	*3695*	*21.808*	*< 0.001*
	Mean of fruit total length	*55.4*	*6*	*9.29*	*17*	*< 0.001*
	Mean of fruit largest width	*63*	*6*	*10.51*	*22.26*	*< 0.001*
Between types of ABMTs	Mean of ellipsoid ratio	0.1	1	0.1	1.9	0.15
	Mean of roundness ratio	0.003	1	0.003	0.22	0.63
	Mean of endocarp thickness	*0.75*	*1*	*0.75*	*7.46*	*0.007*
	Mean of endocarp width	*3.4*	*1*	*3.4*	*17.42*	*< 0.001*
	Mean of endocarp length	*0.75*	*1*	*0.75*	*2*	*0.16*
	Mean of seed weight	*507.15*	1	*507.15*	*21.04*	*< 0.001*
	Mean of mesocarp depth (Ld)	*3.92*	*1*	*3.92*	*23.67*	*< 0.001*
	Mean of mesocarp depth (Sd)	*1.13*	*1*	*1.13*	*5.7*	*0.019*
	Mean of mesocarp weight	*13633.1*	1	*13633*	*4.68*	*0.032*
	Mean of fruit weight	8881	1	8881	2.69	0.14
	Mean of fruit total length	1.14	1	1.14	1.21	0.27
	Mean of fruit largest width	2.35	1	2.35	2052	0.115

Table 5.2d: Results of the Scheffe test subsequent to the MANOVA analysis: comparison and grouping of populations based on the differences of means of the morphological factors; Ld = largest diameter, Sd = smallest diameter.

Significant morphological characters	Population (I)	Population (J)	Difference (I-J)	P Value
Mean endocarp thickness	Dassa	Bvolta	-0.85	0.011
		Couffo	-0.8	0.016
		Svolta	-0.76	0.042
Mean endocarp width	Dassa	Bvolta	-1.32	< 0.001
		Couffo	-1.27	< 0.001
		Pobè	-0.86	0.007
		Svolta	-1.25	< 0.001
		Calavi	0.95	0.003
Mean endocarp length	Lomé	Bvolta	-0.67	0.057
	Dassa	Bvolta	-1.16	0.002
		Calavi	-1.09	0.004
		Couffo	-1.47	< 0.001
		Lome	-1.27	0.004
		Pobè	-0.98	0.01
		Svolta	-1.42	< 0.001
Mean seed weight	Bvolta	Calavi	5.31	0.02
		Dassa	12.92	< 0.001
		Lome	7.94	0.003
		Pobè	6.6	< 0.001
	Dassa	Calavi	-7.62	0.015
		Svolta	-8.89	0.003
	Couffo	Dassa	10.82	< 0.001
		Pobè	4.5	0.026
Mean mesocarp depth (Ld)	Dassa	Calavi	-0.66	0.025
	Couffo	Bvolta	0.91	< 0.001
		Calavi	0.58	0.001
		Dassa	1.24	<0.001
		Lome	0.84	<0.001
		Pobè	0.76	<0.001
		Svolta	0.9	<0.001
Mean mesocarp depth (Sd)	Calavi	Couffo	-0.52	0.007
		Dassa	0.81	0.003
	Couffo	Bvolta	0.91	<0.001
		Dassa	1.33	<0.001
		Pobè	0.69	<0.001
		Svolta	0.76	<0.001
		Bvolta	0.91	<0.001
Mean mesocarp depth (Sd)	Dassa	Lome	-0.79	0.025
		Pobè	-0.63	0.035
Mean fruit weight	Dassa	Calavi	-74.19	0.013
	Couffo	Bvolta	98.14	<0.001
		Calavi	78.1	<0.001
		Dassa	152.29	<0.001
		Lome	96.66	<0.001
		Pobè	100.97	<0.001
		Svolta	93.3	<0.001
Mean fruit length	Couffo	Svolta	1.09	<0.001
		Calavi	1.13	<0.001
		Bvolta	1.36	<0.001

Significant morphological characters	**Population (I)**	**Population (J)**	**Difference (I-J)**	**P Value**
		Lomé	1.46	<0.001
		Pobè	1.46	<0.001
		Dassa	2.78	<0.001
	Dassa	Svolta	-1.68	<0.001
		Calavi	-1.65	<0.001
		Pobè	-1.31	<0.001
Mean fruit smallest diameter	Couffo	Calavi	1.22	<0.001
		Lomé	1.38	<0.001
		Svolta	1.44	<0.001
		Pobè	1.51	<0.001
		Bvolta	1.56	<0.001
	Dassa	Couffo	-2.77	<0.001
		Calavi	-1.55	<0.001
		Lomé	-1.38	<0.001
		Svolta	-1.32	<0.001
		Pobè	-1.25	<0.001
		Bvolta	-1.20	<0.001

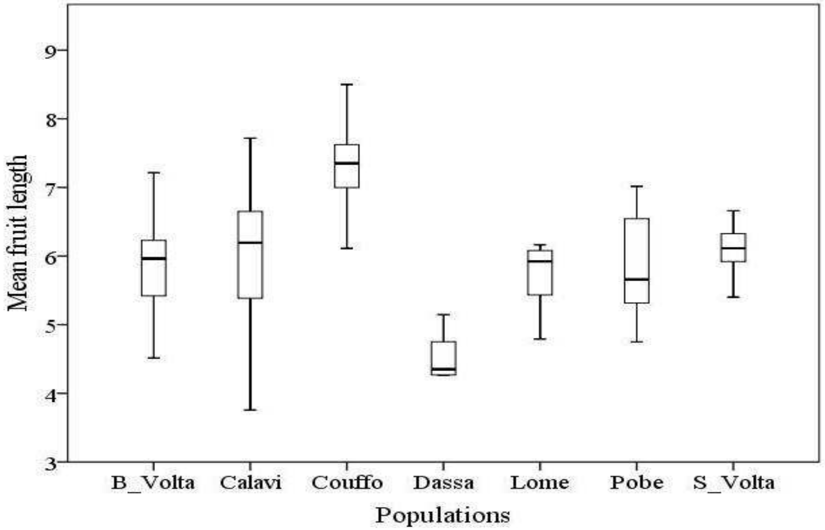


Figure 5.3a

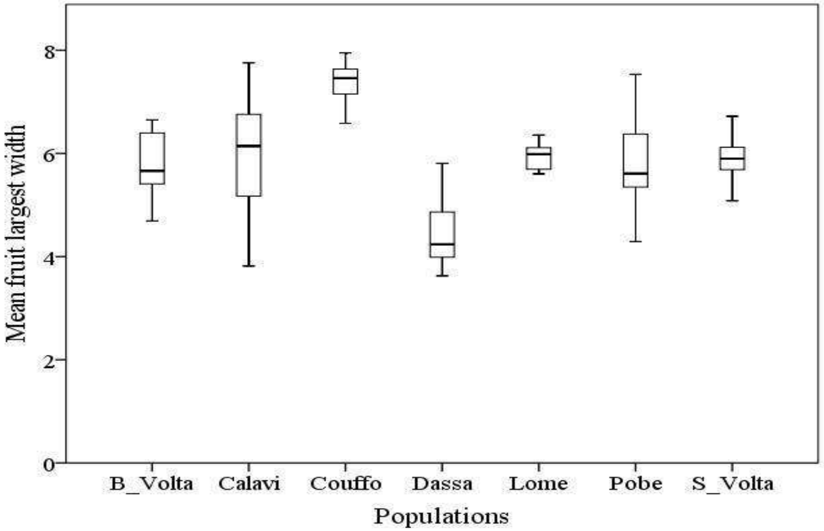


Figure 5.3b

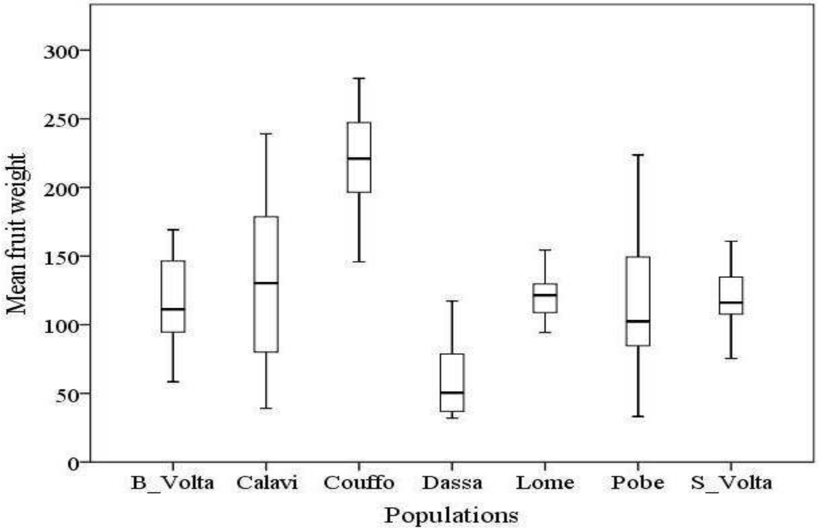


Figure 5.3c

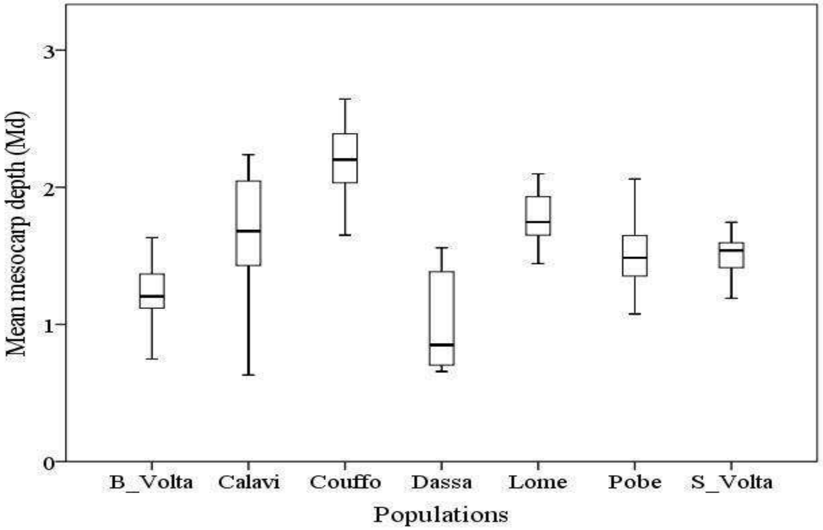
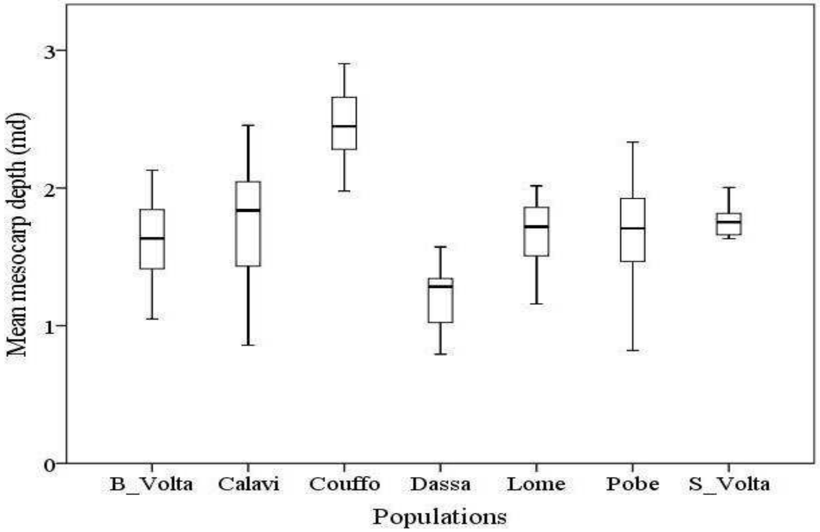


Figure 5.3d



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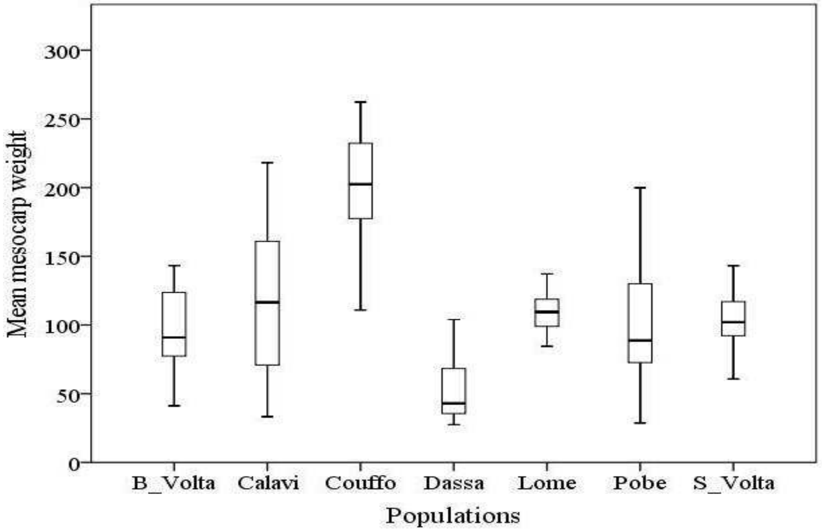

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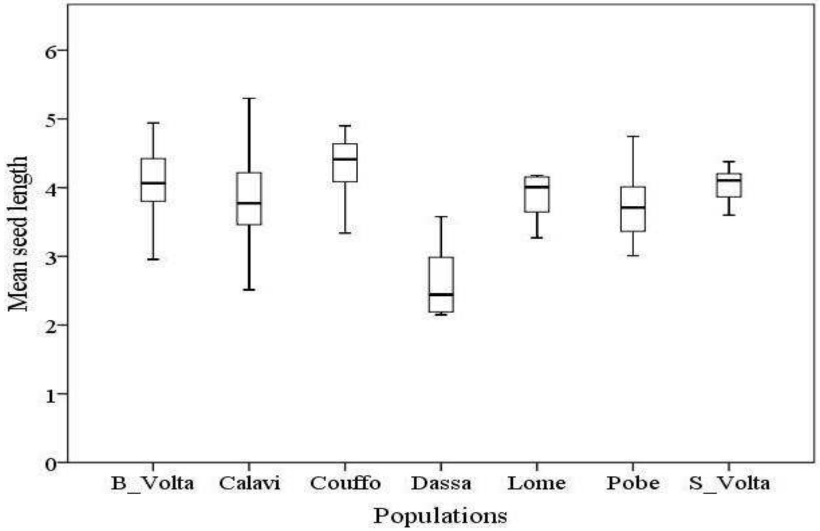

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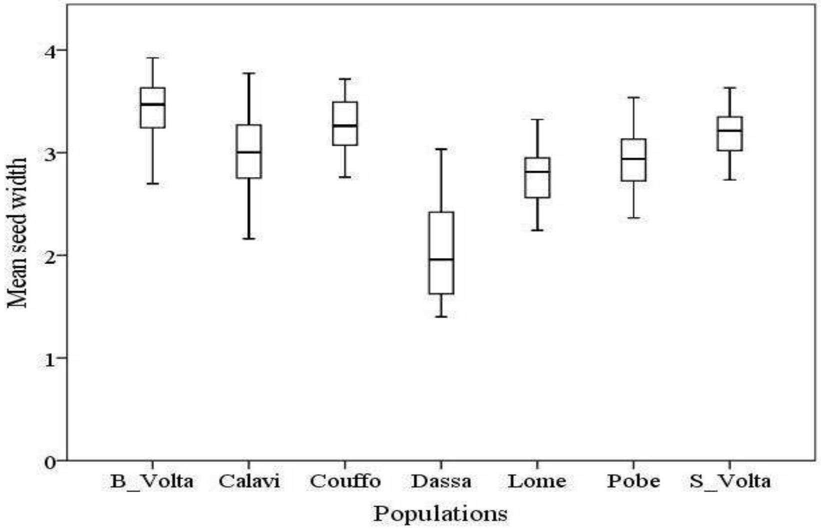

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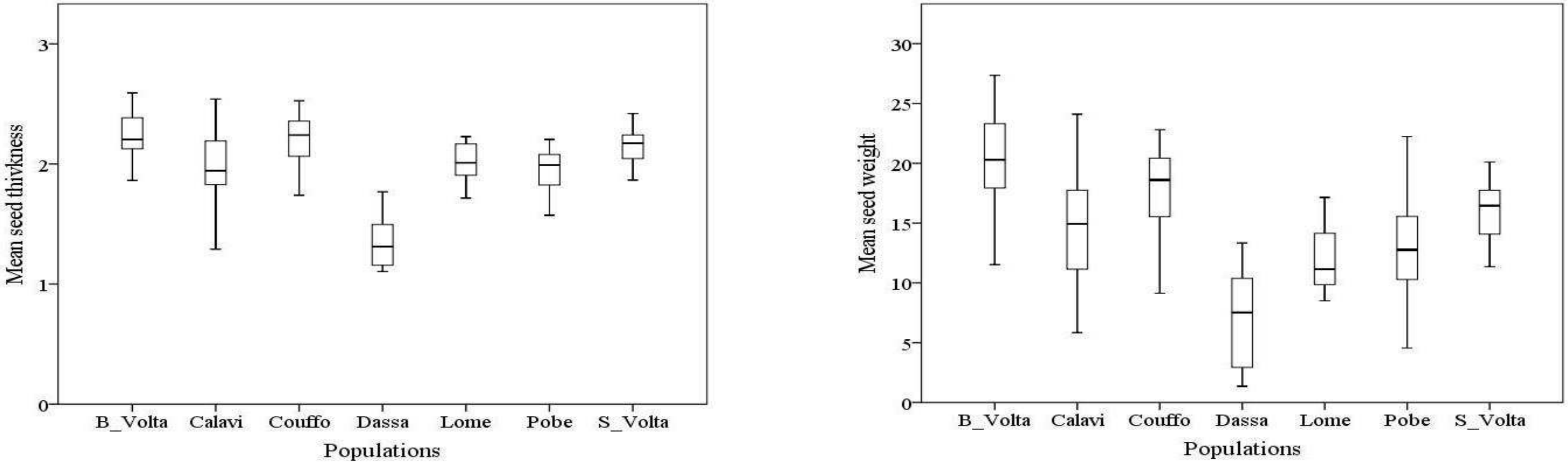

Figure 5.3i **Figure 5.3j**

Figure 5.3: Results of the MANOVA of fruit, mesocarp, seed and endocarp characteristics against populations.

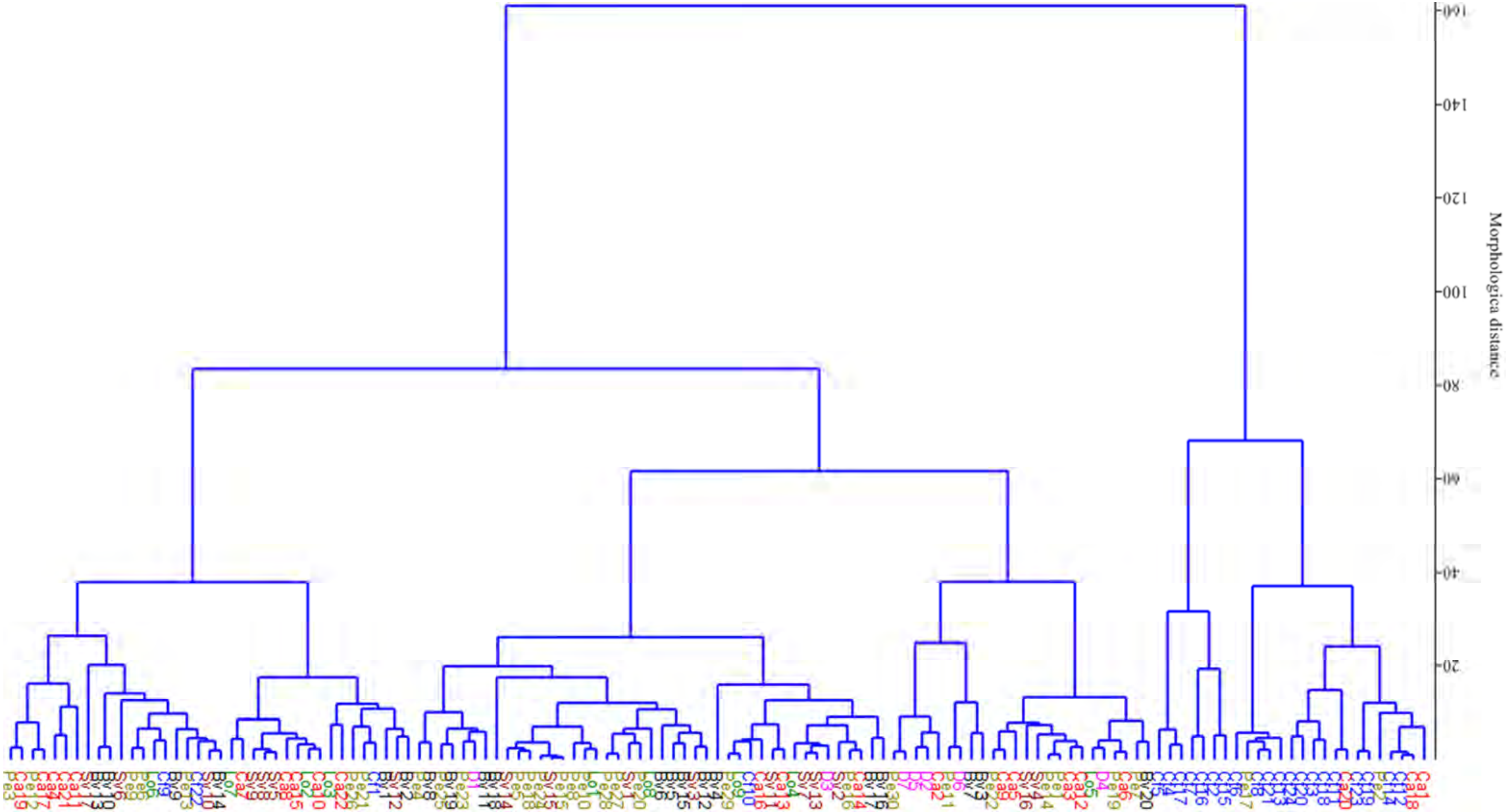

Figure 5.4: Result of the cluster analysis on the fruit, mesocarp, seed and endocarp characteristics (the colours indicate populations as presented in figure 2).

Discussion

Field identification of ABMTs

The type of bark, the roughness and colour of the mature fruit exocarp, colour of mature mesocarp, the brown pigments detected in the fresh mesocarp and its tanning all lack the power to consistently separate bitter and sweet trees. Even though we acknowledge that the colour of the fresh mesocarp seems to differentiate both taxa (Harris, 1996), the appreciation of this difference is difficult and subjective. The problematic distinction between bitter and sweet ABMTs was already extensively reported by Okafor (1975) and Harris (1996). In the present study, we attribute this difficulty to the large morphological variation within both bitter and sweet ABMTs. For instance, the yellow colour of the mesocarp is consistent within bitter fruits but also widely occurs within sweet fruits, which show different degrees of orange (from yellow-orange to orange). Moreover, even though brown pigments are thought to be typical for bitter fruits, this character is not always present within this taxon. This is important for the discussion about the taxonomic level on which the distinction between the two types should be recognized (Landolt, 1977; Leakey and Page, 2006). Even when considered as varieties, bitter and sweet ABMTs should exhibit at least one distinctive and obvious morphological character apart from the taste of their mesocarp. Unfortunately this remains an issue for which our data do not provide a convincing answer. Moreover, since we have only selected fully mature and healthy trees, the distinction in the field based on our morphological characters alone may well be even be more difficult than our results show.

The limited number of visible phenotypical characters and the limited number of populations evaluated for bitter trees may also explain our unsatisfactory results. Therefore, broadening the study area and taking more characters into account should be the next challenge to morphologically delimitate bitter and sweet trees. This is important for designing a better domestication program and an efficient use of ABMTs, since the morphological species concept is vital in plant breeding, selection and cultivation (Pickersgill, 2009).

Local communities instantly differentiate these taxa and it is possible to learn to quickly recognize them in the field. A higher juice seep characterized all bitter fruits leaving fibers difficult to remove from the seed during the removal of mesocarp for seed characterization. Some sweet bush mangoes also display this characteristic, but the removal of the mesocarp from sweet fruits always remains easier. The mesocarp of fruit ideotypes locally called “doughy bush mangoes”(viz. bush mangoes having a mesocarp with low water content, also called “the best fruit ideotype”) seeped no juice and is more easily detachable from the seed even when fruits from “doughy” mangoes have freely dropped. Only three mother trees of this kind (representing 2.7% of the sampled sweet trees) were identified. They show mature mesocarp that is deep orange and are highly desired to enrich agroforestry systems for entire fruits commercialization instead of the endocarp. Local communities stated that this ideotype was more common in the Dahomey Gap and was even the most shared germplasm for ABMTs domestication. Its decline started with the change over time in the type of mesocarp initially produced by desired mother trees. When this change happens, sweet ABMTs are systematically eliminated in areas where the type of mesocarp remains the most valuable NTFP. This explains the continuously low density of ABMTs in this area (see Vihotogbé *et al.,* Submitted-1). Many other characters (e.g. leaf shape and its development, the tree’s branching pattern, crown shape, petal color) seem worthy of a more detailed qualitative and quantitative evaluation. For example, tree-to-tree variation exists in leaf shape and our pilot observations show a higher diversity for bitter trees (Fig. 5.5a). Sweet trees display a higher diversity for seed shape (Fig. 5.5b) and for the arrangement of branches: their direction, the inner versus outer position of secondary branches, and the level of insertion of the first large branches. This determines a variety of crown shapes (see Ladipo *et al.*, 1996) that we did not address in this study. The colour of the petals could also be a valuable discriminant factor between bitter and sweet trees. While yellowish petals seem to be common for bitter trees, our preliminary observations showed a dominance of white petals in sweet trees which can, however, also have yellowish ones. If those characteristics appear to be consistently linked to the type of ABMT (bitter or sweet) and can be observed before the first fructification, this could considerably speed up the domestication process. Local communities also reported that fruit type may not be constant and thus can change from the best type to a completely undesirable one on a single tree. This needs further investigation and will lead to a great diversity of fruit types in a small geographical range. In this situation, even though desired traits are captured and disseminated through vegetative techniques (Tchoundjeu *et al.*, 2010), there is a risk of losing these again over time. Therefore, a permanent verification of the defined ideotype will be necessary to fix, keep and propagated desired characters durably (see Leakey and Page, 2006).

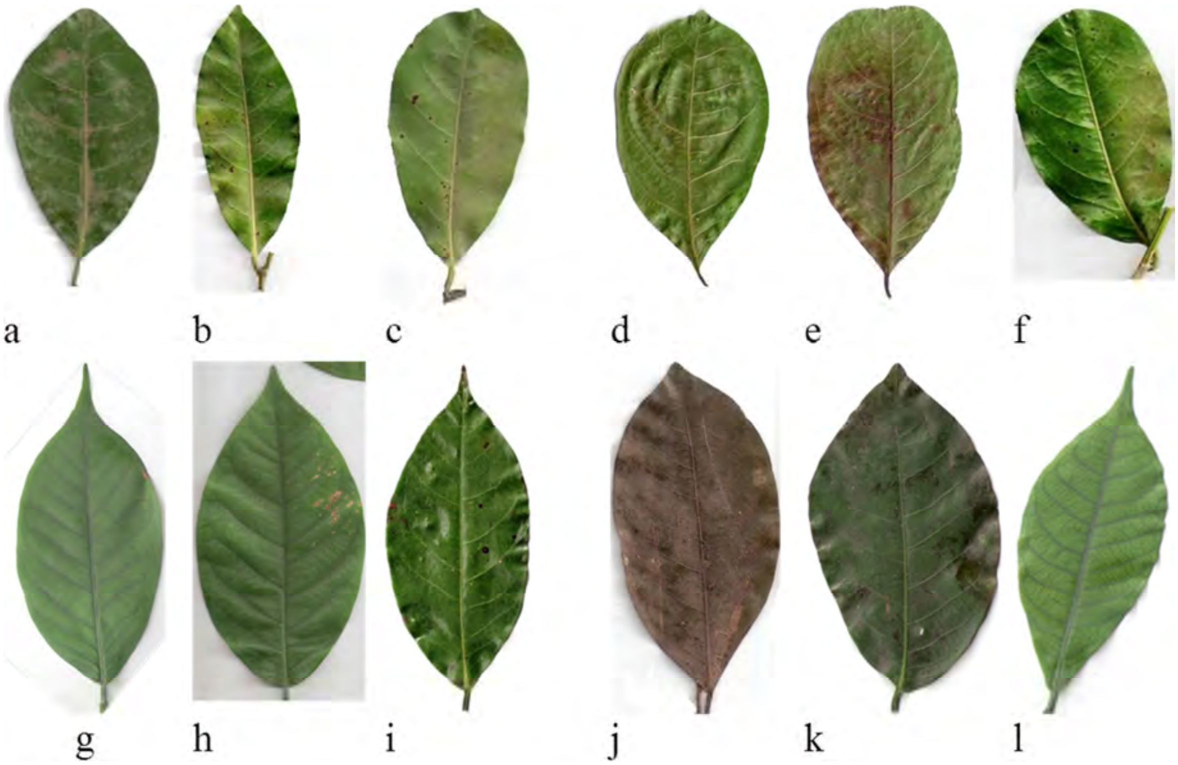

Figure 5.5a: Pilot records of leaf shape diversity in ABMTs. a – f: observed leaf shapes in bitter trees, g and h = leaf shape of juvenile bitter trees, i – k = observed leaf shapes in sweet trees, l = leaf shape of juvenile sweet trees.

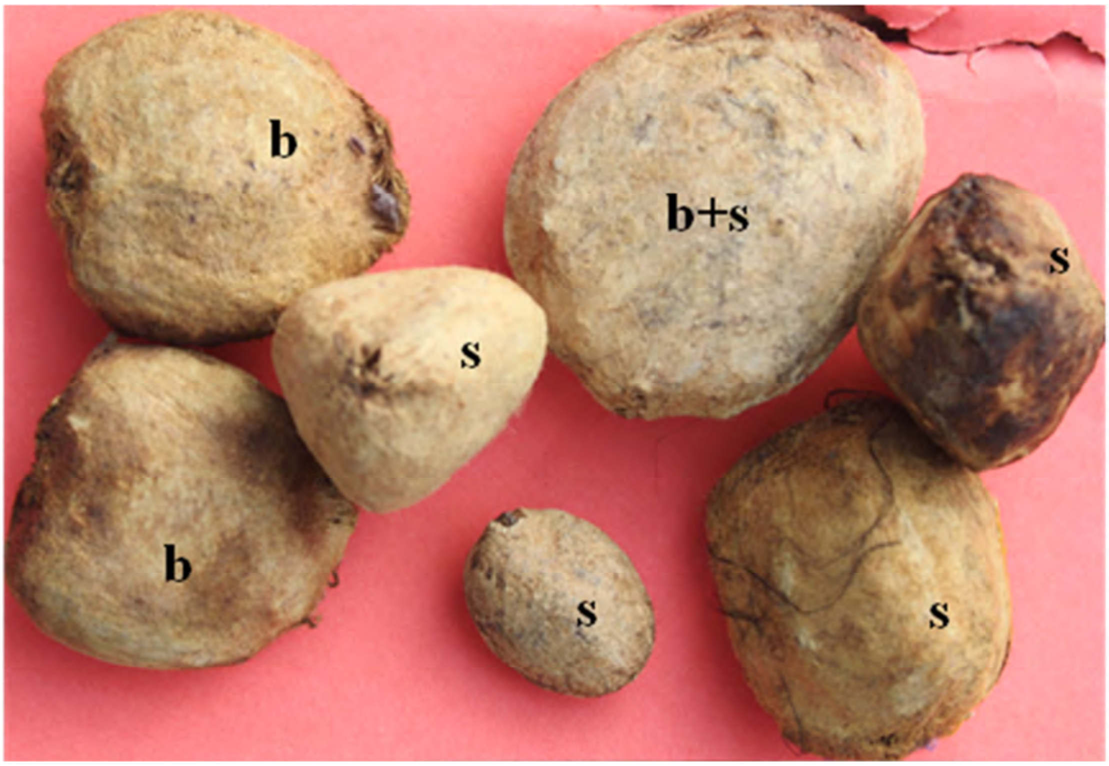

Figure 5.5b: Pilot records of seed shape diversity of bush mangoes. b = shape exclusively observed in bitter fruits, b + s = shape common to bitter and sweet fruits, s = shape exclusively observed in sweet fruits.

Variation of quantitative traits

Most fruits are spheroid to slightly prolate spheroid, in agreement with the results of Ladipo *et al.* (1996), and fruit shape does not significantly discriminate bitter and sweet trees.

Measurements related to the size of fruits and seeds and the abundance of mesocarp tissue distinguish the sweet tree population of Couffo from all other populations (bitter and sweet). This study presents the first quantitative morphological data on bitter ABMTs and indicates that, in the Dahomey Gap, their fruit and endocarp weigh 38 to 250g and 5 to 40g, respectively. In the Dahomey Gap, fruit and endocarp weights of sweet bush mangoes range between 11 to > 425 g and 0.5 to > 40 g, respectively. The ranges for sweet trees are wider than those detected in Cameroon and Nigeria (Leakey *et al.,* 2000; Atangana *et al.,* 2001; Anegbeh *et al.,* 2003). This suggests that in the Dahomey Gap the traditional domestication / selection process is already advanced, while in Central Africa the exploitation of bush mangoes and their seed relies mostly on wild populations (see Ladipo *et al.,* 1996; Lowe *et al.,* 2000; Dolor, 2011). Moreover, this high variability of sweet trees throughout contrasting ecological circumstances suggests a high ecological adaptability. Therefore, the morphological differentiation among sweet tree populations in the Dahomey Gap is determined by small farmer-lead domestication and a mass selection process (see Vihotogbé *et al.,* Submitted-1). In areas where bush mangoes are considered as cash crop and are traditionally managed, traditional selection is narrowing the morphological diversity toward heavy and big fruit ideotypes (see Cornelius, 1994; Van Hintum, 1995; Casas *et al.,* 1999) for the purpose of bush mango’s endocarp commercialization. This market-lead process is guided by the desire to limit the morphological diversity to ideotypes that guarantee high yield of the endocarp. The increase of fruit weight through mass selection is associated with that of the seeds. Consequently, local farmers might be leading successful traditional selection for the increase of endocarp weight due to unavoidable association among quantitative characters (Brown, 2010) of the endocarp. This points at a high economic interest and successful small farmer-lead domestication process taking place in the Dahomey Gap (Leakey *et al.,* 2004; Pickersgill, 2009). Despite the fact that reasons for domestication are clearly identified by local farmers disseminating the materials, the scientific basis and the more technical aspects that speed up a domestication strategy are still lacking (Simons and Leakey, 2004). Thus, the on-going mass selection is based on seed distribution by farmers and the appreciation of fruit ideotypes after the first fructification (normally after 6 to 8 years). This clearly shows the limit of traditional practices in speeding up domestication and selection processes for any tree genetic resource with a high morphological or genetic diversity. Seeing the important nutritional potential of the sweet mesocarp (Fouda *et al.*, Unpublished data; Lesley and Brown, 2004), the material in the Dahomey Gap is clearly interesting for domestication purposes aiming at an increase of both the mesocarp and the endocarp yield. This makes these sweet trees highly valuable since ABMTs have been rated as highest priority multi-purpose trees in need of improvement research in West and Central Africa In food-deficiency areas like Sub-Saharan Africa an efficient use of any valuable plant species is a challenge. This challenge is expressed clearly in the elite trees definition, prior to the Word Agroforestry Centre's on-going domestication program (Leakey and Tchoundjeu, 2001).

The difficulty to differentiate between sweet tree populations due to morphological overlap (Fig. 5.4) might be rooted in the continuous circulation of genetic material among ecological zones in the Dahomey Gap, and the continuous transfer of new genetic material via seeds between the Dahomey Gap and the neighbouring countries (Casas *et al.*, 1999). Therefore, we postulate that high morphological and genetic diversity within ABMTs is created and maintained in the traditional agroforestry systems throughout the Dahomey Gap (Casas *et al.*, 2005; Jose, 2012) and a valuable potential exists for any local as well as regional domestication program for sweet tree material. This is of particular advantage in a global climate change condition since this material is already adapted to low rainfalls conditions. While the different populations investigated belong to different phytogeographical zones, the fact that the characters do not act in concert to segregate populations indicates that the potential for selection of various desired characters is not geographically restricted, but widely distributed over the Dahomey Gap. This means that any fruit ideotype can be found in any region. This supports results of the World Agroforestry Centre identifying large and heavy sweet bush mangoes in drier region outside of the Dahomey Gap, and a variety of bush mango morphotypes that still remain to be carefully documented. The potential for selection is higher in the eastern part of the Guinean climatic zone of the Dahomey Gap, since individuals from this area are more spread over all the clusters defined (population of Pobè; Fig. 5.4). This is consistent with Pickersgill (2009) who reported that differences exist in geographical space regarding the distribution of the variation in plant species.

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Chapter 6

Genetic diversity and difference within and between bitter and sweet African bush mango trees (*Irvingia* spp., Irvingiaceae) in West and Central Africa

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Abstract

The domestication of the economically most important food tree species used in sub-Sahara African communities should be improved to enhance their production within agroforestry systems and combat the food deficit crisis in this region. African bush mango trees (*Irvingia* species) are top priority among the species that are preserved and integrated in agroforestry systems in the humid regions of tropical Africa. The taxonomic debate related to the species or varietal status of the bitter and sweet fruited African bush mango trees hinders their domestication process and rational use. AFLPs and cpSSRs were used in this study to assess the genetic diversity of African bush mango trees and to test the distinction between bitter and sweet fruited trees across Togo, Benin, Nigeria and Cameroon. Low genetic diversity was found for the isolated population of bitter trees occurring in south-western Togo due to the higher fragmentation of the small sized forest ecosystem in which they occur and the continuous reduction of the population size. The higher polymorphism and genetic diversity of the sweet tree populations in Benin and Togo indicate the effect of domestication of material with different geographical origin due to frequent long distance transfer of genetic material. When used separately, the AFLPs and cpSSRs failed to consistently discriminate populations and tree type. But the combined dataset from both markers generally differentiates geographically recognizable groups, and bitter from sweet trees. The suitability of AFLPs and cpSSRs to test our hypotheses within *Irvingia* is discussed.

Key words: AFLP, cpSSR, Benin, Togo, Dahomey Gap, *Irvingia*, taxonomy, domestication.

Introduction

In Africa, tropical forests host many multipurpose food tree species which are valuable in the light of the crucial food deficit in sub-Saharan Africa (Hladik *et al.*, 1996; Malaisse, 1997; FAO, 2008, Augustino *et al.*, 2011). While this food deficit is increasing forest ecosystems are being cleared at an alarming rate (Laurance, 1999; Archard *et al.*, 2002). The further domestication of the most important food tree species, widely used by local communities in their daily diets, seems a logical policy. This will strengthen traditional regional strategies for agrobiodiversity maintenance and, in general, enhance the global production of agroforestry systems.

The International Centre for Research in Agroforestry (ICRAF, now called World Agroforestry Centre) has become a leading institution in traditional food tree species domestication in West and Central Africa. Among the numerous species with nutritional importance or local communities, African bush mango trees (ABMTs) are economically the most important ones and are systematically preserved and integrated in various traditional agroforestry systems in humid sub-Saharan Africa (Okafor and Fernandes, 1987; Franzel *et al.*, 1996; Tabuna, 2000; Okunomo and Egho, 2010).

ABMTs are widely distributed and taxonomically confusing taxa within the family of Irvingiaceae within which a bitter fruited and sweet fruited form can be distinguished. Apart from the bitterness versus sweetness of the mesocarp, there is only limited morphological delimitation between the two types (Harris, 1996). On the other hand, ABMTs exhibit a high morphological and phenological diversity that is valuable for domestication and selection programs (see Harris, 1996; Atangana *et al.*, 2002) but there is no clear relation between biochemical properties and the type of ABMT (Tchoundjeu and Atangana, 2007). This overlap of morphological, phenological and biochemical properties generated a debate related to the correct taxonomy of ABMTs. Okafor (1975) proposed the variety level for sweet and bitter ABMTs, respectively *Irvingia gabonensis* (Aubry-LeComte ex O'Rorke) Baill. var. *gabonensis* and *I. gabonensis* var. *Excels* (Mildbr.) Okafor. But, based on a thorough taxonomic revision (Harris 1996) and a Random Amplified Polymorphic DNA (RAPD) analysis (Lowe *et al.*, 2000), a distinction at species level was suggested: *Irvingia gabonensis* and *I. Wombolu* Vermoesen, for sweet and bitter trees, respectively. There is a lack of support for the latter solution due to the weaknesses of the RAPD analysis, mostly their low reproducibility. Moreover, there is a lack of a sound quantitative morphological comparison between bitter and sweet ABMTs. Still, in order to ensure proper in- and ex-situ conservation of the genetically diverse material, and to support its development and genetic improvement (Grace *et al.,* 2008), a clear situation regarding the taxonomic identity of the material is essential. Furthermore, the geographic origin of populations present in the Dahomey Gap, the wide savannah area separating the West African forest into the Upper and Lower Guinean forest blocks, remains an important topic.

This study intends to revisit the pattern of genetic diversity of ABMTs in answering two main questions:

(i) What are the patterns of genetic diversity and differentiation within and between bitter and sweet ABMTs, and what do they tell us about the geographical origin of the Dahomey Gap material?

(ii) Are our markers (AFLPs and cpSSRs) suitable to consistently discriminate populations and types of ABMTs?

Materials and Methods

Sampling

Sweet and bitter ABMTs were sampled throughout the Dahomey Gap (Benin and Togo) and in Cameroon. Material was also sampled from four gene banks: IITA (International Institute for tropical Agriculture) and NAGRAB (National Centre for Genetic Resources and Biotechnology) in Ibadan (Nigeria) and Kolbison (Yaoundé) and Mbalmayo established by the World Agroforestry Centre in Cameroon (Table 1). For each sampled tree, young leaves were collected and stored in silica gel. DNA was extracted from each sample following the protocol described in Fulton *et al.* (1995) and an AFLP analysis was first carried out on 33 samples (10 bitter and 23 sweet trees). Moreover, an independent cpSSR analysis was carried out with 47 samples (14 bitter and 33 sweet trees), including more individuals from the Dahomey Gap and the regions postulated as ABMTs genetic diversity centres by Lowe *et al.* (2000) and Ude *et al.* (2006) in the Lower Guinean forest and the Congolian forest blocks. Thus, in total, 59 accessions (39 in the Dahomey Gap and 20 from Nigeria and Cameroon) were used in this study with 21 samples common to both analyses (Table 1).

Table 1: ABMT accessions and types of analysis applied

Accessions	Type of ABMT	Provenance: site of collection and country	Population	Types of analysis applied
P2	Sweet	Pobè, South Benin	SDG	AFLP
POB21	Sweet	Pobè, South Benin	SDG	AFLP
IP2	Sweet	Pobè, South Benin	SDG	AFLP
Ip4	Sweet	Pobè, South Benin	SDG	AFLP
Coco6	Sweet	Calavi, South Benin	SDG	AFLP
NPA4	Sweet	Parakou, North Benin	SDG	AFLP
NPA6	Sweet	Parakou, North Benin	SDG	AFLP
MBM1	Sweet	Sangmelima, South Cameroon	STA	AFLP
IW3BAD5	Bitter	Badou, Southwest Togo	BDG	AFLP
FNGB	Bitter	NAGRAB/ICRAF Gene bank, Nigeria	BTA	AFLP
DNGB	Bitter	NAGRAB/ICRAF Gene bank, Nigeria	BTA	AFLP
IWSAK1	Bitter	Centre Cameroon, ICRAF Kolbison Gene Bank, Cameroon	BTA	AFLP
POB20	Sweet	Pobè, South Benin	SDG	AFLP + SSR
Coco1	Sweet	Calavi, South Benin	SDG	AFLP + SSR
Coco5	Sweet	Calavi, South Benin	SDG	AFLP + SSR
NPA7	Sweet	Parakou, North Benin	SDG	AFLP + SSR
NPA9	Sweet	Parakou, North Benin	SDG	AFLP + SSR
TG1	Sweet	Atakpamè, Centre Togo	SDG	AFLP + SSR
TG18	Sweet	Lomé, South Togo	SDG	AFLP + SSR
BAD1	Sweet	Badou, Southwest Togo	SDG	AFLP + SSR
WAMP2	Sweet	Badou, Southwest Togo	SDG	AFLP + SSR
IGIBDGB2	Sweet	IITA Gene bank, Nigeria	STA	AFLP + SSR
IGIBDGB1	Sweet	IITA Gene bank, Nigeria	STA	AFLP + SSR
Limb	Sweet	Limbé, Southwest Cameroon	STA	AFLP + SSR
Limbe6	Sweet	Limbé, Southwest Cameroon	STA	AFLP + SSR
IGGBWAC	Sweet	ICRAF Kolbison Gene Bank, Yaoundé	STA	AFLP + SSR
NGMK1	Sweet	Sangmelima, South Cameroon	STA	AFLP + SSR
NMKIW02	Bitter	Mamfé, South Cameroon, ICRAF Mbalmayo Gene bank, Cameroon	BTA	AFLP + SSR
BSIW07	Bitter	Mamfé, South Cameroon, ICRAF Mbalmayo, Gene bank, Cameroon	BTA	AFLP + SSR
IWSAK2	Bitter	Centre Cameroon, ICRAF Kolbison Gene Bank, Cameroon	BTA	AFLP + SSR
CENRAD	Bitter	CENRAD Gene bank, Nigeria	BTA	AFLP + SSR
BAD4kiw	Bitter	Badou, Southwest Togo	BDG	AFLP + SSR
TGIW2	Bitter	Badou, Southwest Togo	BDG	AFLP + SSR
P2	Sweet	Pobè, South Benin	SDG	SSR
POB21	Sweet	Pobè, South Benin	SDG	SSR
CALI	Sweet	Calavi, South Benin	SDG	SSR
TORI13J	Sweet	Calavi, South Benin	SDG	SSR
TORI25	Sweet	Calavi, South Benin	SDG	SSR
Djot6	Sweet	Couffo, South Benin	SDG	SSR
LALO1G	Sweet	Couffo, South Benin	SDG	SSR
VODassa	Sweet	Dassa Centre Benin	SDG	SSR
Djoug	Sweet	Djougou, North Benin	SDG	SSR
Peninsou	Sweet	Peninsoulou, North Benin	SDG	SSR
Lom1	Sweet	Lomé, South Togo	SDG	SSR
L2	Sweet	Lomé, South Togo	SDG	SSR
TG4	Sweet	Lomé, South Togo	SDG	SSR

Accessions	Type of ABMT	Provenance: site of collection and country	Population	Types of analysis applied
TG12	Sweet	Lomé, South Togo	SDG	SSR
Atak	Sweet	Atakpamè, Centre Togo	SDG	SSR
BAD5	Sweet	Badou, Southwest Togo	SDG	SSR
IGGBWACII	Sweet	Centre Cameroon, ICRAF Kolbison Gene Bank, Cameroon	STA	SSR
MBUM	Sweet	Sangmelima, South Cameroon	STA	SSR
KGH1	Bitter	Kougnonhou, Southwest Togo	BDG	SSR
KGH2	Bitter	Kougnonhou, Southwest Togo	BDG	SSR
KGH3	Bitter	Kougnonhou, Southwest Togo	BDG	SSR
KGH4	Bitter	Kougnonhou, Southwest Togo	BDG	SSR
NKIW19	Bitter	Mamfé, South Cameroon, Mbalmayo Gene bank, Cameroon	BTA	SSR
BSIW324	Bitter	Mamfé, South Cameroon, Mbalmayo Gene bank, Cameroon	BTA	SSR
T2BSIW16	Bitter	Mamfé, South Cameroon, Mbalmayo Gene bank, Cameroon	BTA	SSR
IWSAK3	Bitter	Centre Cameroon, ICRAF Kolbison Gene Bank, Cameroon	BTA	SSR

SDG = sweet ABMTs from the Dahomey Gap, BDG = bitter ABMTs from the Dahomey Gap, STA = sweet ABMTs from Tropical Africa, BTA = bitter ABMTs from Tropical Africa.

Genetic diversity

The large number of bands generated and the high reproducibility of amplified fragment length polymorphisms (AFLPs) make them a marker widely used in genetic analysis (Berchowitz *et al.,* 2001; Assogbadjo *et al.,* 2010) and one that has advantages over RAPDs (Powell *et al.,* 1996). Microsatellites (or simple sequence repeats-SSRs) and especially chloroplast microsatellites (cpSSRs), have the power to reveal genetic diversity as well as phylogenetic relationships and hybridization between plant species (Wills *et al.,* 2005; Panwar *et al.,* 2010). We therefore decided to use both AFLPs and cpSSRs for our analyses.

AFLP data

The AFLP analysis was carried out at the Biosystematics Group, Wageningen University, the Netherlands, and followed the procedure of Vos *et al.* (1995) with minor modifications. Three primer combinations that had previously been demonstrated to successfully amplify sweet tree material (Ude *et al.,* 2006) were used to analyze all 33 samples. These are: E38M59 (Eco ACT / Mse CTA), E40M62 (Eco AGC / Mse CTT), and E33M48 (Eco AAG / Mse CAC).

PCR reactions were performed using a MJ PTC200 thermocycler. Prior to the selective amplification, the EcoR1 primer was fluorescently labelled with IRD700. AFLP fragments were separated on a LYCOR 4300 (Westburg, the Netherlands), and the resulting profiles were scored using the Quantar software (Key Gene Products, Wageningen, the Netherlands 2000) to produce a presence / absence data matrix.

cpSSR data

The cpSSR analysis was carried out in the Laboratory of Genetics and Biotechnology of the University of Abomey Calavi, Benin. Eighteen chloroplast SSR primers were tested on independent samples (bitter and sweet tree accessions). These are: CCMP 2, NTCP 8, NTCP 9, NTCP 30, NTCP 37, NTCP 39, NTCP 40, NTCP 5, NTCP 16, NTCP 19, NTCP 25, NTCP 26, NTCP 27, NTCP 29, NTCP 32, NTCP 33, NTCP 34 and NTCP 38. Of those 18 primers, seven (CCMP 2, NTCP 8, NTCP 9, NTCP 30, NTCP 37, NTCP 39 and NTCP 40) that amplified the chloroplast DNA of ABMT material were retained and applied to the complete set of 47 samples. PCR reaction was performed using a Peltier-Effect Cycling PTC 100 thermocycler programmed for an initial denaturation step at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s per cycle, a step at the annealing temperature for 1 min, a step at 72 °C for 1 min, and a final extension step at 72 °C for 5 min. Migration of the PCR products was visualized with denaturing polyacrylamide gel (5%) electrophoresis and then revealed with silver nitrate in accordance with Chair et al. (2005). The electrophoresis bands were scored to generate a presence / absence data matrix.

Data analysis

Genetic diversity and structure

Three datasets were considered in this study: the AFLP set, the cpSSRs set and the one containing the accessions that showed a result for both AFLP and cpSSR. An analysis of the genetic diversity and population structure based on allele frequency using AFLP-SURV version 1.0 (Vekemans, 2002) was performed on each dataset while the type of ABMT (sweet versus bitter) was considered as well as the geographical origin of the sample. Four geographic ‘populations’ were defined: (i) bitter trees from the Dahomey Gap (= population BDG), (ii) bitter trees from tropical Africa (= population BTA), (iii) sweet trees from the

Dahomey Gap (= population SDG) and (iv) sweet trees from tropical Africa (= population STA; Table 1). For each dataset we computed the mean Nei genetic diversity (Nei, 1973) per population, the global genetic differentiation (F_{st} statistics) and the pairwise genetic distance among populations and between sweet and bitter trees. Assuming no genetic structure among populations under a Hardy-Weinberg equilibrium (Vekemans, 2002), the significance of the genetic differentiation was assessed by comparing the observed F_{st} with the distribution of obtained F_{st} using 100 random individual permutations.

Identification of populations and distinction between sweet and bitter ABMTs

To assess the effectiveness of the genetic markers used in the discrimination of the four geographically recognized populations as well as the two types of ABMT, a cluster analysis was carried out on each dataset and a dendrogram was produced using the UPGMA method based on Jaccard similarity index (Jaccard, 1908) in Past (Hammer *et al.*, 2001):

$$I_j = {}^{a}\!/_{(a+b+c)} \qquad (1)$$

Where for a random pair of individuals, a = number of totally loci scored present for the two individuals, b = number of loci scored present exclusively present for only one individual and c = number of loci exclusively present for only the second individual.

When classifying individuals using a Principal Component Analysis (PCA), it can happen that the obtained grouping pattern is unclear due to the abundance of factors of low contribution to an existing pattern in the dataset. Therefore, the PCA axes that explain a high percentage of the total variance within the dataset or the factors correlated with those axes could be used in a subsequent multivariate analysis to get a better signal from the dataset (see Mohammadi and Prasanna, 2003; Bidogeza *et al.*, 2009). Because the separate use of the AFLPs and cpSSRs data generated confusing patterns, only the combined AFLPs + cpSSRs data were used in the rest of the analysis. First, all the alleles with zero variability in the AFLPs + cpSSRs data were removed. A Principal Coordinate analysis (PCO) was performed to obtain the characteristics of the main groups yielded by the cluster analysis. A Principal Component Analysis (PCA) was performed on the combined dataset and the axes that accumulated at least 70% of the total variation were retained. The loci that were highly correlated (at least 70%) with those axis were used to produce a Neighbor Joining (NJ) tree using a Kulczynski similarity index (Kulczynski, 1927). Like the Jaccard index, the Kulczynski similarity index is one of the most consistent similarity index used in systematics (Boyce and Ellison, 2001) and is calculated as follows:

$$I_K = 0.5 * (\frac{a}{a+b} + \frac{a}{a+c}) \tag{2}$$

Where I_k is the Kulczynski index, a and b are the same notation as for the Jaccard index.

Table 2a: Results of the genetic diversity analysis with AFLP-SURV (abbreviations see Table 1); highest figures in bold

	Population level				**Type level**	
	BTA	**BDG**	**SDG**	**STA**	**bitter**	**sweet**
	AFLPs					
Segregating fragments (%)	96.5				97.2	
Polymorphism (%)	22	66.7	**85.1**	60.3	66.7	**76.6**
Nei's genetic diversity	0.091	0.263	0.304	0.234	0.221	**0.264**
	cpSSRs					
Segregating fragments (%)	100				100	
Polymorphism (%)	55	70	65	**90**	60	**80**
Nei's genetic diversity	0.202	0.273	**0.289**	0.235	0.240	**0.278**
	AFLPs + cpSSRs					
Segregating fragments (%)	83.9				85.1	
Polymorphism (%)	24.8	50.9	**70.8**	63.4	50.9	**67.1**
Nei's genetic diversity	0.105	0.232	**0.251**	0.211	0.210	**0.245**

Table 2b: Pairwise F_{st} statistics among populations (abbreviations see Table 1); highest figures in bold.

	AFLP			
	BTA	**BDG**	**SDG**	**STA**
BTA	0.0000			
BDG	0.1490	0.0000		
SDG	0.0731	**0.2407**	0.0000	
STA	0.0000	0.1587	0.0789	0.0000
	cpSSRs			
BTA	0.0000			
BDG	0.0772	0.0000		
SDG	0.0000	0.1244	0.0000	
STA	0.0437	**0.3268**	0.0411	0.0000
	AFLPs + cpSSRs			
BTA	0.0000			
BDG	0.2511	0.0000		
SDG	0.1320	0.1618	0.0000	
STA	0.1646	**0.2916**	0.0960	0.0000

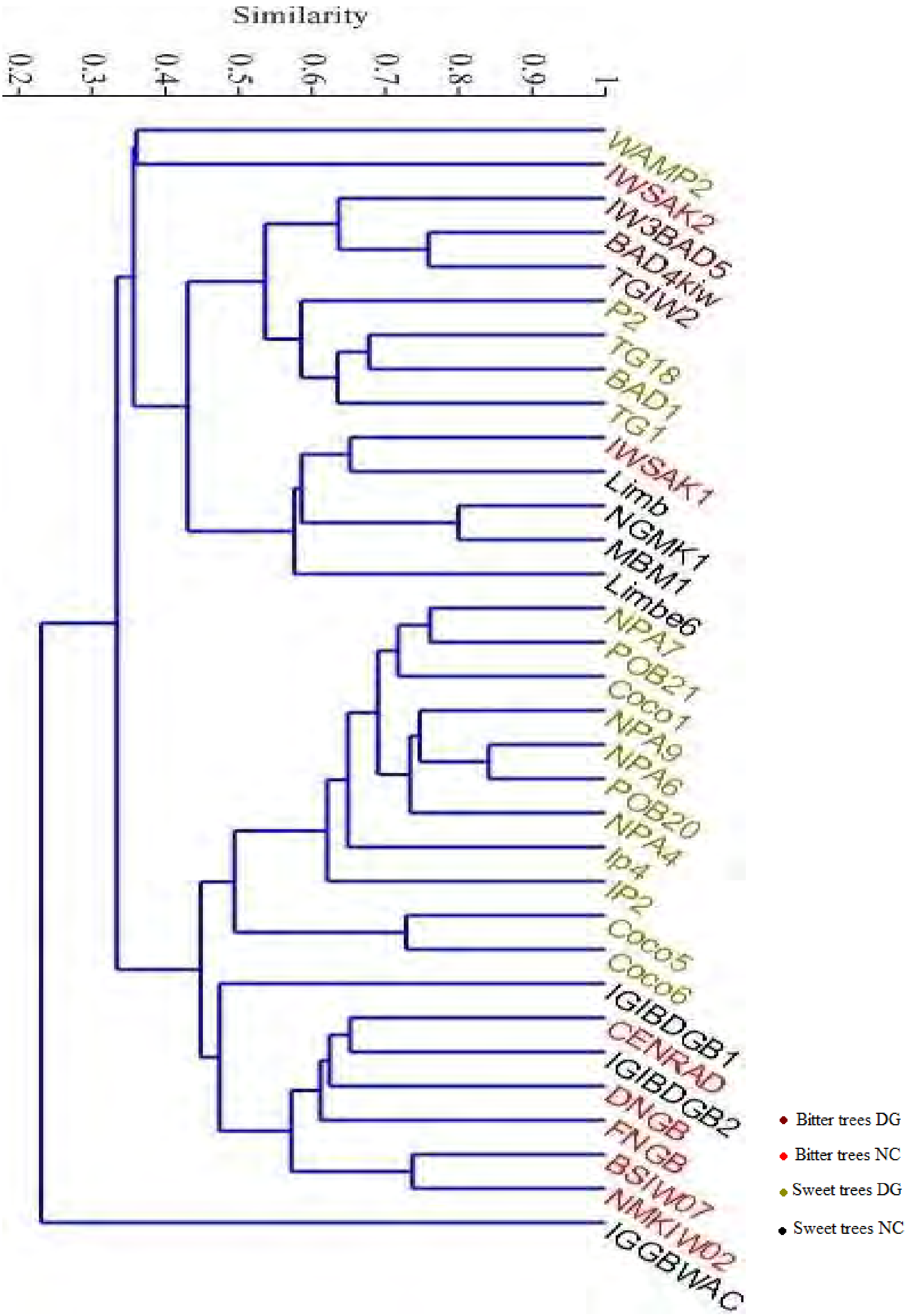

Figure 1a: UPGMA dendrogram for AFLP data of the 33 accessions based on Jaccard's similarity index

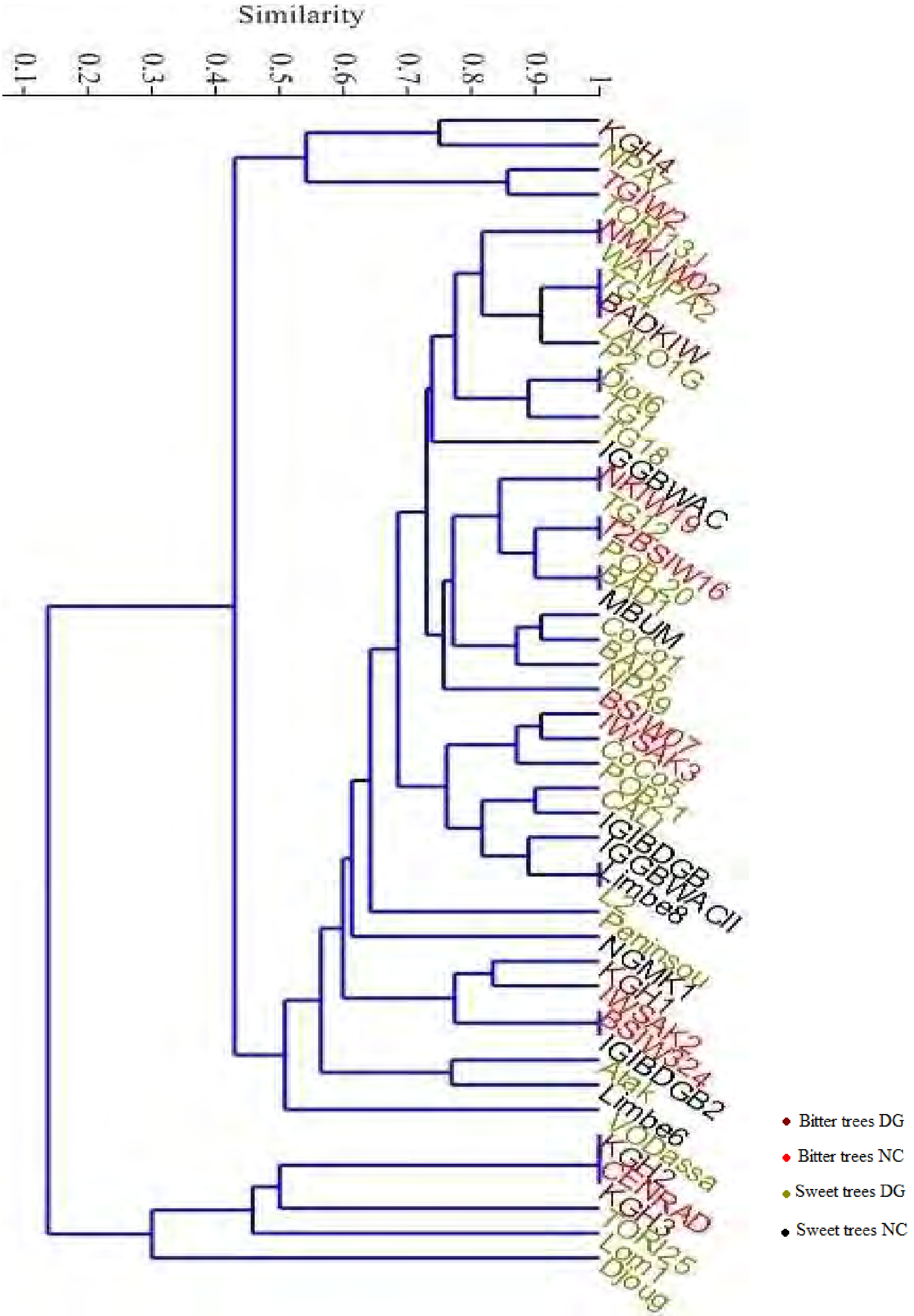

Figure 1b: UPGMA dendrogram for cpSSR data of the 47 accessions based on Jaccard's similarity index

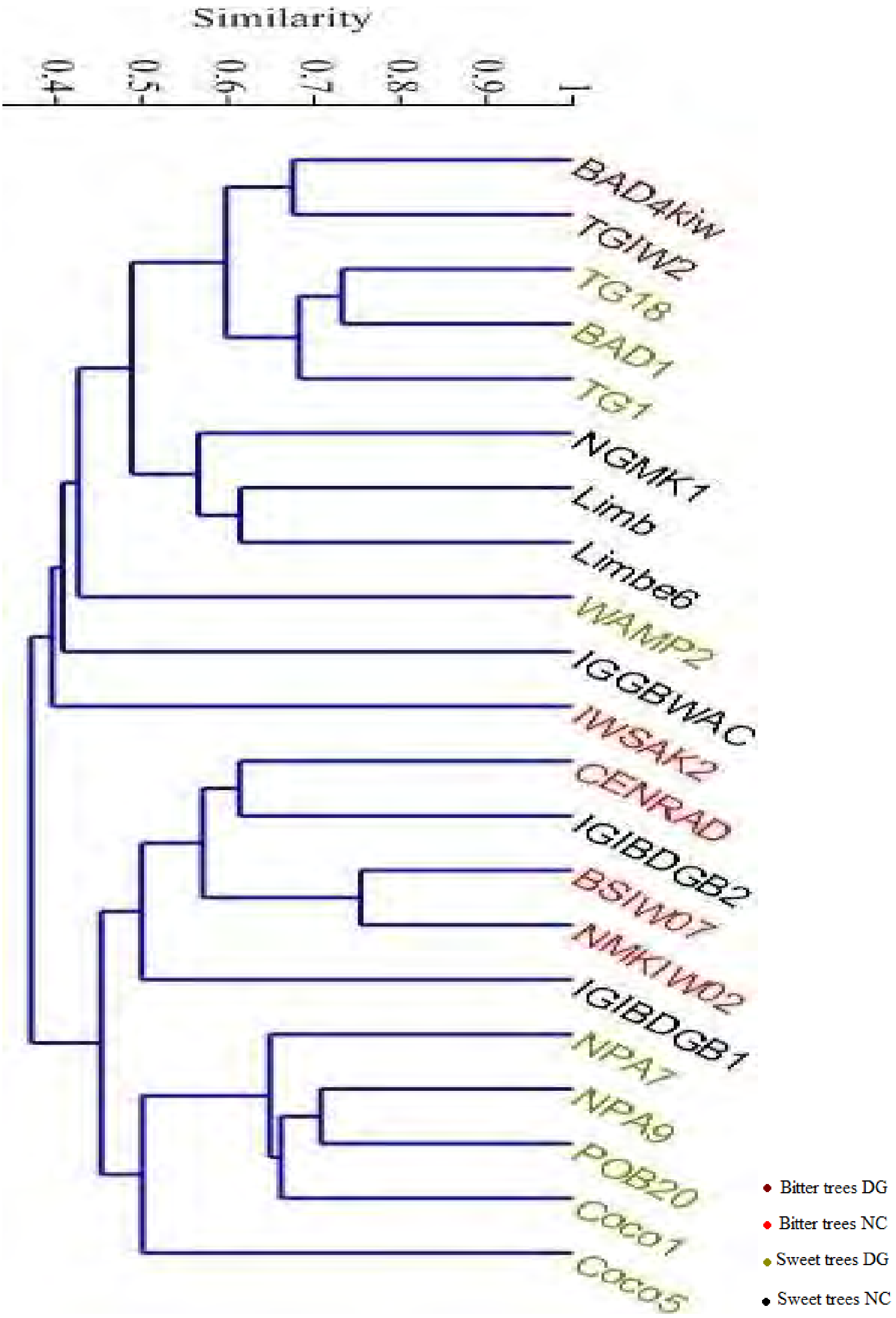

Figure 1c: UPGMA dendrogram for AFLP + cpSSR data of the 21 accessions based on Jaccard's similarity index

Results

For the AFLPs analysis a total of 141 polymorphic alleles were scored for all the 33 individuals. The cpSSR analysis yielded 20 polymorphic alleles (1 to 6 per locus). Thus, a total of 161 polymorphic alleles were available for the set of 21 samples with both AFLP and cpSSR results.

Genetic diversity and differentiation of ABMTs

The AFLP analysis indicates that the mean number of fragments scored as present for an individual tree was 47. The number of segregating fragments in the dataset is high (96.5 %). The polymorphism is highest within the sweet tree Dahomey Gap population (SDG) and lowest in the Dahomey Gap bitter trees population. The same tendency is observed regarding the within population Nei genetic diversity. The SDG population presents the significantly highest genetic diversity, while the BDG one presents the lowest diversity (Table 2a). The test for genetic differentiation among populations indicates a global F_{st} value of 0.108 (P = 0.024). The pairwise F_{st} values among populations is higher between bitter and sweet tree populations within the Dahomey Gap and no genetic difference is found between bitter and sweet tree populations outside of the Dahomey Gap (Table 2b). Sweet trees show a higher polymorphism and Nei genetic diversity than bitter trees (Table 2a). However, the difference based on this AFLP data is not significant (global F_{st} = 0.034; P =0.0639). The F_{st} between bitter and sweet tree populations is very low (0.011).

The cpSSR results show a mean number of fragments scored as present at individual tree level of 8 and all the 20 scored loci have segregation power. The polymorphism is highest in the sweet tropical African (STA) population and lowest for the BDG trees. The SDG population and the BTA one present the highest genetic diversity, while the BDG population presents the lowest diversity again (Table 2a). No clearly significant genetic differentiation is found among populations (global F_{st} = 0.105; P = 0.048 $\cong$ 0.05) even though the highest pairwise F_{st} is found between BDG and STA populations (Table 2b). Considering bitter versus sweet trees, the genetic diversity is highest in the sweet trees (Table 2a). However, based on the cpSSRs there is no significant genetic differentiation amongst the two types (F_{st} = 0.0537; P = 0.077), and the F_{st} between bitter and sweet tree populations is low (0.0540).

The combined AFLPs+ cpSSRs data also indicates a high number of alleles with segregating power (83.9%), with 24 alleles presenting no variability. The within population proportion of polymorphism and the Nei genetic diversity show the same tendency as the separate AFLPs and cpSSRs results: the highest value is calculated for the SDG population and the lowest in the BDG population (Table 2a). In this case, a significant genetic differentiation was detected among populations (global $F_{st} = 0.0176$; P = 0.016). The highest pairwise F_{st} is found between the BDG population and the STA one, while the lowest distance is present between the SDG and STA ones (Table 2b). Considering sweet and bitter trees, 85.1% of the combined AFLP and SSR alleles have segregation power. The proportion of polymorphic loci is higher within sweet trees than within bitter trees (Table 2b). The Nei genetic diversity is 0.2453 and 0.21 for sweet and bitter trees, respectively. The genetic differentiation between sweet and bitter trees is low ($F_{st} = 0.0335$) and not significant (global $F_{st} = 0.0333$; P = 0.064).

Cluster analysis

The dendrogram based on the AFLP results (Figure 1a) shows no clear pattern among populations and no clear distinction between bitter and sweet trees. The majority of individuals from each considered population are spread across many clusters. However, apart from few accessions, there is a tendency for the Togo (bitter and sweet) and Benin (sweet) materials to cluster together (Fig. 1a; Table 1).

The dendrogram based on the cpSSR results (Fig. 1b; Table 1) shows an even less clear pattern with higher similarity among individuals and completely fails to discriminate between the two types of ABMTs or geographically defined populations.

The dendrogram resulting from the cluster analysis of the combined APLP + cpSSR data (Fig. 1c; Table 1) is more discriminative than those obtained from the separate AFLP and cpSSR datasets. Apart from one accession from Togo (WAMP2), four geographically distinct groups can be distinguished from the lower to upper position: (i) all sweet trees from Benin, (ii) sweet and bitter trees from the Lower Guinean forest bloc (southern Nigeria and Mamfé region in South-west Cameroon), (iii) sweet and bitter trees from Central and South Cameroon, and (iv) bitter and sweet trees from Togo.

The first two axes of the PCO on the combined dataset with 137 alleles (Figure 2a; Table 1) accounts for 62.5%of the variance (46.13% and 16.38 % for coordinate 1 and 2, respectively). The PCO tends to separate the bitter from sweet ABMTs, with the two sweet trees from Nigeria (IGIBGB1 and IGIBGB2) falling within the bitter tree group. Within each of the two groups, the populations are not clearly distinguishable apart from the sweet trees from Benin that are clearly separated (as in Figure 1a and 1c).

Forty-eight alleles are highly correlated (at least 70%) with the first 8 PCA axes which account for 72.8% of the total variation. The clustering obtained with NJ based on the Kulczynski similarity index of these alleles (Figure 2b) confirms the pattern in the PCA. The inability of the combined AFLP + cpSSR data to accurately separate populations and the clustering of the sweet trees from Nigeria within the bitter tree cluster was also confirmed.

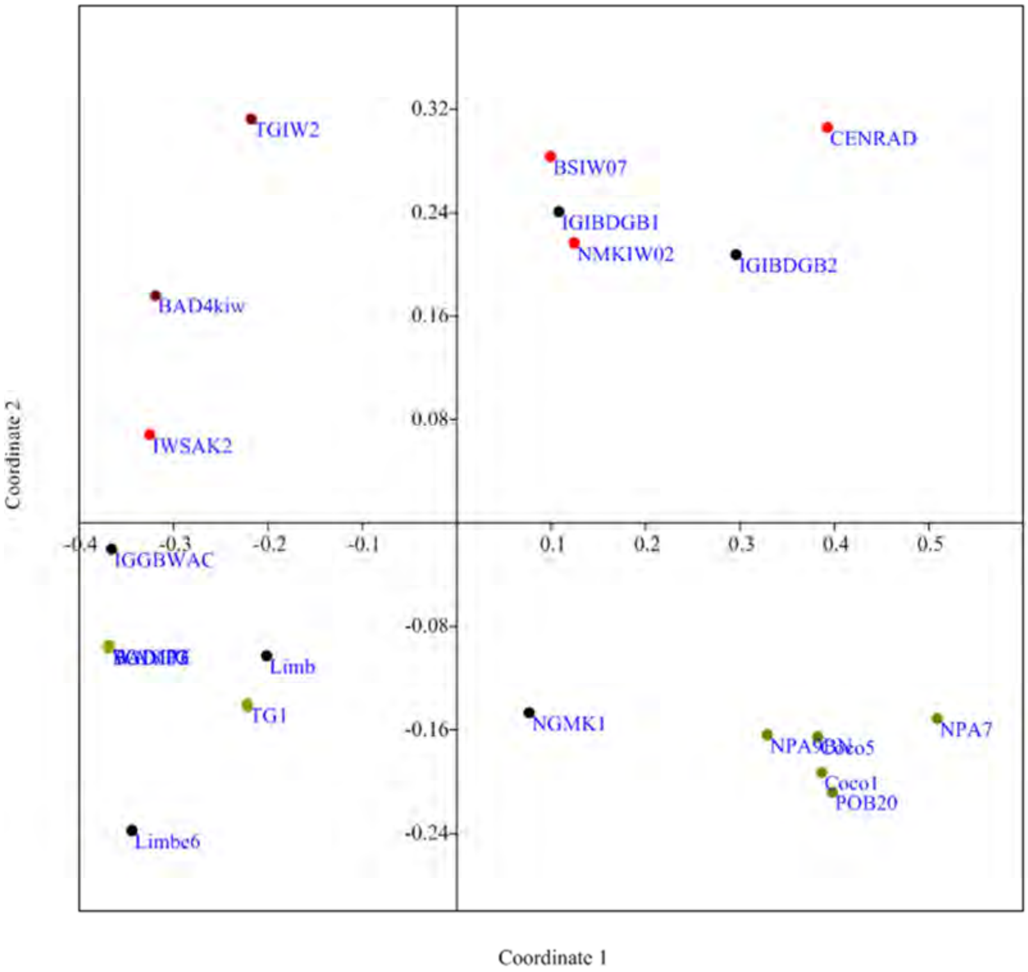

Figure 2a: Plot of first two principal coordinates based on Jaccard's similarity index with the 48 AFLPs+ cpSSRs for the 21 common accessions. Legend (color) is the same as in Figure 1

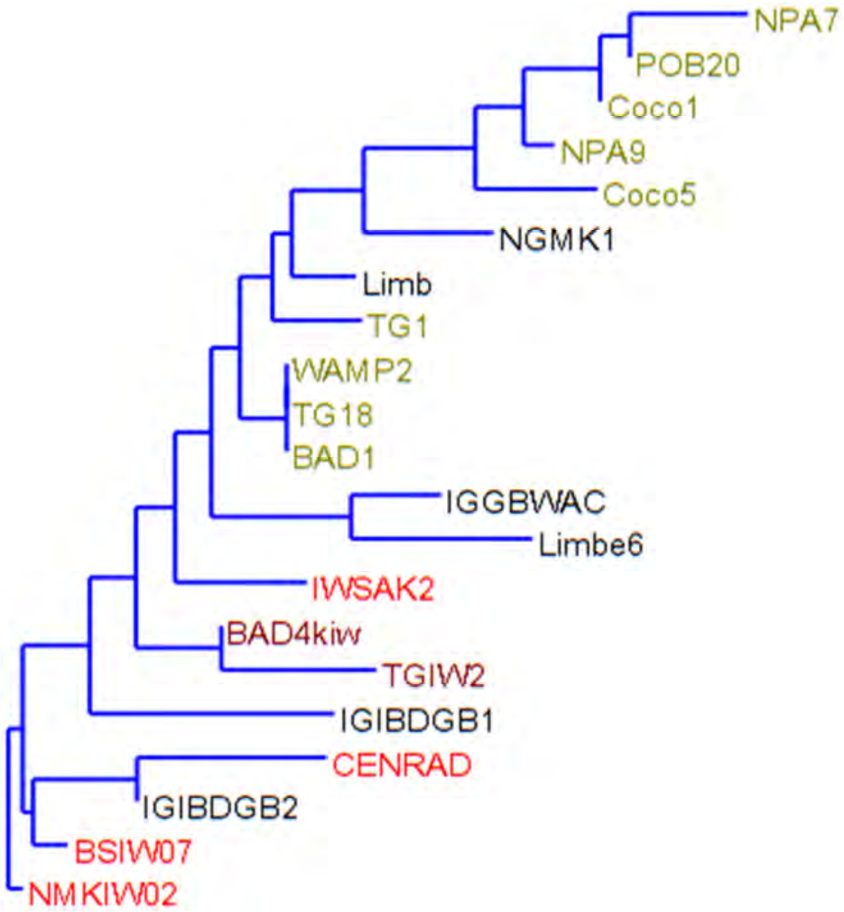

Figure 2b: Kulczynski similarity-based Neighbor Joining tree of the 48 AFLPs + cpSSRs loci. Legend (color) is the same as in Figure 1.

Discussion

ABMTs genetic diversity: failure of AFLPs and cpSSRs or influence of domestication?

For all three dataset considered, the lowest polymorphism and genetic diversity is found in the BDG population, while the highest values for these parameters are calculated for the cultivated SDG trees. These results are not consistent with those of Lowe *et al.* (2000) and Ude *et al.* (2006) who found a higher genetic diversity in Cameroon and Nigeria. Environmental transformation through logging, extension of agricultural productive space through yearly bush fires, and urbanisation are the main causes of biodiversity lost in tropical Africa (FRIG, 2003; Sodhi, 2007; Jose, 2012). Together with fragmentation and decrease of population size, these factors play a key role in shaping the climatic characteristics of the Volta forest region, a unique ecosystem in which bitter trees are found in the wild in the

Dahomey Gap (Vihotogbé *et al.,* submitted-1). In addition to these factors, the economic potential of bush mangoes' seed turns out to be one of the most important threats to ABMTs, since the market of this non timber forest product relies mostly on natural populations (Agbor, 1994; Lowe *et al.,* 2000). The population size of wild bitter ABMTs is decreasing in the Volta forest region as is the case in their entire distribution range due to a lack of sufficient natural regeneration (Agbor, 1994; Zapfack and Ngobo-Nkongo, 2002; Vihotogbé *et al.,* submitted-1). Consequently, this reduction of their ecological variability might be narrowing their morphological and genetic diversity. The domestication and cultivation of sweet trees, in various climatic zones in the Dahomey Gap, seem to preserve or increase the existing diversity (see Casas *et al.,* 2005; Jose, 2012). Although the provenance of sweet ABMTs in the Dahomey Gap is unknown (Harris, 1996; Asaah *et al.,* 2003; Lesley and Brown, 2004; Ude *et al.,* 2006; Vihotogbé *et al.,*submitted-1), their higher diversity in this eco-region may well be due to the fact that the ongoing traditional domestication process in this region includes material from geographically different origins: the Upper and Lower Guinean forest blocks as well as the Congolian forest region. We conclude that this is a consequence of the random genetic material transfer between and within local communities, not only for ABMTs but related to any economically important food tree species in agroforestry systems (Jose, 2012).

In general, for the three datasets no significant genetic differentiation is found between bitter and sweet ABMTs. Similarly, no genetic differentiation among populations was observed with the AFLP dataset. Genetic differentiation was detected within the cpSSR and AFLP +cpSSR datasets but their patterns are not the same. The dominance of cultivated material in our samples (collected in the field as well as in gene banks) might result in an expression of artificially generated variation.

Comparatively few sharp bands could be scored with the AFLP and the cpSSR products. This weakness also appeared in the AFLP study of Ude *et al.* (2006) and forced these authors to the use of no less than 12 pairs of primers. Our similar unclear results on the genetic diversity among populations and most importantly between bitter and sweet trees pose the important question concerning the suitability of the genetic markers used in our study. Thus, including wild material from every eco-region in the entire distribution range of ABMTs and analyse those in a sound genetic analysis will be of great importance in evaluating their genetic diversity, the influence of domestication, and their genetic adaptability.

Suitability of the markers

Apart from the sweet tree population from Benin, which forms the most consistently distinct cluster throughout our analyses, none of the methods used in this study clearly separate the geographically distinct populations (Figures 1a-2b). The PCA and NJ (Figure 2a and 2b)do show a distinction between the two types, with the exception of the sweet tree samples from Nigeria which cluster with the bitter trees (Figure 2a and 2b). This was also observed in the study of Lowe *et al.* (2000), but was explained in terms of inaccuracy of sampling. In our study, the fact that neither the PCA plots nor the NJ dendrogram are able to discriminate either geographical populations or fruit types implies that the markers used to achieve this goal are probably unsuitable. This idea is corroborated by the fact that we know that in the area where bitter and sweet ABMTs co-occur (in the Volta forest region) successful gene flow between bitter and sweet trees is hardly to be expected for phenological reasons: (i) very short co-flowering time, (ii) flowers abortion on all sweet trees after this co-flowering time and (iii) consistent overall difference in phenology between both types (Vihotogbé *et al.*, submitted-2). Further support comes from the presence of ecological differences between both types (see Lesley and Brown, 2004; Vihotogbé *et al.*, submitted-1). Therefore, the inability of the AFLP and cpSSR data to distinguish the two types might be attributed to the high level of sweet ABMTs diversity (Kelleher *et al.*, 2005). So, in conclusion, we attribute the failure of AFLPs, cpSSRs and AFLPs + cpSSRs to distinguish populations to the effect of domestication and large scale transfer of genetic material via seeds of the economically and nutritionally appreciated ABMT morphotypes.

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Chapter 7

General discussion and conclusions

Introduction

African bush mango trees (ABMTs) are important multi-purpose trees, belonging to two different taxonomic entities: one having fruits with a generally sweet and edible mesocarp and one with inedible bitter fruits. This thesis analyses the differences within and between sweet and bitter ABMTs, with an emphasis on the Dahomey Gap region, in order to decide upon their taxonomic status. Ecological, phenological, morphological and genetic approaches have been used to evaluate these differences. In this chapter, we also relate the traditional knowledge of local communities concerning bush mango morphotypes to the ecological, phenological, morphological and genetic data to draw an all-inclusive conclusion on the species distinction within ABMTs, their domestication and ecological adaptation and conservation, with emphasis on the Dahomey Gap. Possibilities for future research to further improve our knowledge related to some of the specific objectives addressed in the General Introduction are also discussed.

Key results concerning the differences between sweet and bitter ABMTs

A large-scale ethnobotanical inventory showed that, in terms of their value, local communities in the Dahomey Gap use bitter ABMTs only as timber, while sweet trees have a variety of other uses (nutritional, medicinal, socio-cultural, energetic). Even though the seed of bitter trees tends to be superior in quality to that of the sweet ones, it is not consumed by the indigenous communities in the Volta Region, the only region where bitter trees are found. They do not find any direct advantage for bitter trees cultivation. Therefore, the domestication of superior seed quality trees relies on wild material of bitter trees, while cultivation of sweet trees is much more common and highy stimulated by the additional advantage of consumption of the sweet mesocarp.

We demonstrated the existence of ecological differences between sweet and bitter ABMTs and that in the Dahomey Gap bitter trees are the only type of ABMTs occurring in the wild. The increase of the economic value of the seeds is causing the expansion of the area of cultivation of sweet trees and a restriction of the distribution area of bitter trees in the Dahomey Gap.

Based on provenances from the Dahomey Gap, important differences in phenological features, especially in the reproductive stages, were found. While bitter trees produce fruits once a year, sweet trees can have two or sometimes even three reproductive cycles per year.

Our morphological data failed to consistently discriminate between sweet and bitter trees and the quantitative measurements of fruits, mesocarp and seeds showed the absence of a joint effect to discriminate between populations. However, the data do show a clear effect of domestication.

Unexpectedly, the genetic diversity was higher in the cultivated sweet tree populations from the Dahomey Gap than in other cultivated as well as wild populations. However, AFLPs and cpSSRs failed to consistently separate the two types of ABMTs nor could they distinguish the various geographical populations.

Traditional taxonomy in the Dahomey Gap

In their symbiotic relationship with natural ecosystems, human communities have often developed traditional taxonomic systems based on plant traits (Hays, 1983). Traditional taxonomic systems have even assisted in relating the variability of the morphological characteristics of Baobab trees (*Adansonia digitata* L., Bombacaceae) to their differences in biochemical content and in the use of their non-timber forest products (Assogbadjo *et al.*, 2008). Similarly, such systems might allow the distinction of various types of ABMTs, based on the differences in their non-timber forest products. In the Nago socio-cultural area located in the centre of Benin, where no bitter trees are present, the sweet bush mango is locally called "Oro-N’Bèdjè" (Kengni *et al.*, 2010). This local name literally means “the bush mango with sweet / edible mesocarp”. This would suggest that local communities have experienced both sweet and bitter bush mangoes. However, the name "Oro-Koro", used by the Yoruba tribe to identify bitter trees in Nigeria, where both bitter and sweet trees occur in the wild (see Lesley and Brown, 2004), does not appear in the traditional taxonomic system in Benin and

Togo. The Nigerian origin of the Yoruba-Nagot-Holli tribes in Benin (Parrinder, 1947; Igue and Olabiyi, 1973) together with the comparatively low level of their ethnobotanical knowledge on ABMTs indicates that bitter trees were confined to the Volta region in Togo or became extinct in the Dahomey Gap before human settlement. At least, this extinction would have taken place before the migration of the Yoruba tribes into this eco-region. Furthermore, the local names "Oro-N’Bèdjè" and "Oro-Koro" are part of the taxonomic system of the Nigerian Yoruba migrants, and it seems likely that the use of "Oro-Koro" has eroded from their language when in Benin and Togo since they did not encounter bitter trees. However, since the migration of Nigerian Yoruba people to Benin and Togo is fairly recent (see Parrinder, 1947), it might also be that wild bitter trees, with their superior seed quality, did not occur in the Yoruba lands in Nigeria, because if this had been the case, they would most likely have propagated them along their migration routes. Finally, if bitter ABMTs did occur outside of the Volta forest region, they would most likely have been subjected to traditional domestication practices, and at least, they would have been mentioned by local communities during the ethnobotanical surveys. Moreover, the organoleptic superiority of their seeds would have been recognized locally and increased their economic value and this would have led to cultivation initiatives with an occurrence area wider than the very limited natural one of the Volta forest region in the Dahomey Gap.

The route along which ABMT material reached the Dahomey Gap remains a central issue. This is equally true for many other economically important food trees species in that area like the white star apple tree (*Chrysophyllum albidum*, Sapotaceae) and the akee tree (*Blighia sapida*, Sapindaceae). In this study, we postulate that ABMTs in the Dahomey Gap comprise a mixture of sweet trees from the Upper and Lower Guinean forest blocks and bitter trees native to this area. However, evidence that native sweet trees have not existed in the Dahomey Gap (Harris, 1996) needs to be demonstrated.

Even though the accuracy of traditional taxonomy could be questioned, it can help to get an overview of the diversity within a plant species (Sambatti *et al.*, 2001). To our knowledge, the traditional taxonomy of ABMTs has never been studied, but judging from the local names in the Dahomey Gap the taxonomical distinction of sweet and bitter trees remains a confusing issue. However, the names "never planted ABMT" and "wild ABMT", used in the Volta forest region to indicate bitter trees, do indicate that the local communities recognize their taxonomic distinction.

Cultivation of ABMTs and natural resources conservation

The limited surface of forest lands in the Dahomey Gap, combined with the predominance of traditional agricultural practices (slash and burn technique) inevitably exposes this eco-region to a high rate of soil degradation, and therefore to a fast soil carbon content exhaustion (Lal, 2004). The most important consequence of this exposure is the low subsistence crop yield, causing recurrent food crises in the region. The intensive cultivation of sweet ABMTs (and the selection of economically profitable tree ideotypes) acts as a restorative land use strategy for soil conservation that can be recommended to restore soil carbon content, reduce soil degradation and enhance arable soil water holding capacity. Therefore, ABMT cultivation has the potential to increase the productivity of traditional agroforestry systems (also through carbon sequestration, for example see Li and Feng, 2002). This productivity is rooted in the commercialization of the fruits and seeds of bush mangoes and the successful local initiatives to apply intercropping with water demanding crops (such as the vegetables *Capsicum frutescens, Solanum macrocarpon* and *Colocasia esculenta*) below ABMTs (Fig. 7.1 and 7.2). The development of such intercropping systems based on ecologically adapted fruit tree species makes it possible to: (i) use soils sustainably by increasing their resilience, (ii) enhance the livelihood and nutrition of communities, (iii) limit the use of chemical fertilizers and, therefore, reduce atmospheric and soil pollution. Because sweet trees present a direct benefit for local communities through the consumption of the seeds and the commercialization of the entire fruits, their domestication process has probably been faster than that of bitter trees. This has resulted in the collecting and domestication of more sweet tree ideotypes, increasing their genetic diversity in the Dahomey Gap.

Testing the taxonomic distinction within ABMTs

Local communities clearly distinguish bitter and sweet ABMTs and, as these have different economic and market aspects, this leads to a difference in their domestication process. Unfortunately, not all our analyses revealed a clear difference between sweet and bitter ABMTs. Where the ecological characteristics (chapter 2) are significantly different, the genetic analysis (chapter 6) gives no implications for a taxonomic distinction. This could be due to the markers used (AFLP and cpSSRs). For example, AFLPs failed to separate morphologically different species of oak (*Quercus petraea* and *Q. robur*, Fagaceae) and Kelleher *et al.* (2005) attributed this failure to a higher differentiation within populations than between species. This shows how closely related species might sometimes be difficult to separate, even with modern genetic methods (John *et al.,* 2005). Other studies on important African fruit trees, such as Assogbadjo *et al.* (2006, 2008) on Baobab, did find significant genetic differences among geographically isolated and morphologically distinct populations, while that of Ewédjè *et al.* (2012) on Butter tree (*Pentadesma butyracea*, Clusiaceae) detected that morphological traits consistently separated genetically distinct populations. The domestication process of these species still relies on wild trees preserved in traditional agroforestry systems in the Dahomey Gap. The absence of this link between ABMTs genetic, morphological and phenological data might be explained by a higher degree of manipulation of genetic material and hence a higher degree of hybridization amongst economically and nutritionally valuable trees over their entire distribution. Another explanation could be related to the fact that there is no fixed ABMT ideotype and that farmers rely on seed to enrich agroforestry systems. Petit and Excoffier (2009) pointed out that because cpDNA is only dispersed via seed, it is more frequently introgressed than nuclear material and, therefore, of less taxonomic value for species distinction. In addition, a high level of cross pollination may explain the failure of AFLPs and cpSSRs to assess the taxon delimitation within ABMTs.

Finally, our results suggest that an efficient conservation program of ABMTs is needed in order to plan an effective domestication program. Such a conservation program should encompass material of both sweet and bitter trees from a diverse set of ecological regions in order to ensure the capture of a maximum of variation.

Conclusions regarding ABMT diversity and taxonomic identity

The different analyses undertaken throughout this thesis intended to reveal the diversity patterns within ABMTs and establish the taxonomic characters valuable to distinguish sweet and bitter trees as well as assess the taxonomic level most suitable for the situation. Based on the different results obtained, we draw the following conclusions:

1- Ecological differences exist between wild sweet and bitter trees with bitter trees extending to drier areas. A seemingly conflicting situation is observed in the particular situation of the Dahomey Gap where sweet trees grow in much drier ecological zones than could be expected from their natural occurrence. Since, however, in the Dahomey Gap sweet trees do not occur wild, we assume this to be due to the domestication and cultivation efforts and so accept the presence of an ecological difference between sweet and bitter ABMTs.

2- Sweet and bitter trees show clear phenological differences even in areas where they co-occur. However, because all observations related to sweet trees were performed on cultivated material, we cannot rule out the possibility that this difference was in fact introduced during the domestication process.

3- Based on the characters we investigated, a morphological differentiation between sweet and bitter trees could not be demonstrated. During our fieldwork, we did, however, observe several potentially distinctive features in other characters (f.e. crown shape, branching pattern, leaf shape, petal colour) which should be the subject of additional study.

4- Our genetic markers failed to demonstrate a genetic differentiation into different taxa. However, we argue that the markers used are not the most suitable ones in the case of ABMTs, and suggest a more extensive genetic analysis of the material.

5- We judge the clear ecological and phenological differences between sweet and bitter trees we observed, along with the fruit taste and the morphological distinction made by Harris (1996), as sufficient to consider these taxa as taxonomically distinct. We expect the outcome of additional morphological and genetic studies to further support these observations, which is why we advise to recognize the two ABMT taxa at the species level.

Suggestions for future research

In chapter 3, the geostatistic approach (the variogram and kriging) used to estimate the spatial structure and population density across the Dahomey Gap was consistent in all directions, but we did not incorporate soil and climate micro-variation data in our model (as in chapter 2) in order to better approach the real situation. Even though our later knowledge on the ecological requirements of ABMTs indicated that soil characteristics play only a minor role in their potential distribution, differences in population density across the Dahomey Gap give rise to the question of their behaviour on different soil types. Because the density at a given location is the result of a combination of environmental factors and anthropogenic ones, both are important to strengthen the validity of our model. Therefore, applying a co-kriging method based on the correlation between the environmental plus anthropogenic data and the density values might be useful to further refine the results.

The morphological data used to assess the differences between bitter and sweet ABMTs did not include the diversity detected in complementary studies. Notably, features of the tree shape, leaf shape and flower colour seem promising (Chapter 5). Therefore, an additional morphological study should be undertaken using material from the entire distribution range and, since taxon distinction is expected to be difficult, the data analysed in a full scale statistical analysis.

Our study has used more cultivated / human influenced material than wild provenances of ABMTs. Thus, the patterns of variation present in ABMT material that has potentially been domesticated to adapt to new ecological environments outside of their natural niche, could well mask natural variations valuable for taxon distinction. Therefore, a new in-depth study using only wild provenances originating throughout the natural distribution of both taxa still seems a worthwhile undertaking.

Finally, in order to capture the maximum variation within ABMTs and design better strategies for the in- and ex-situ conservation as well as domestication of ABMTs, we propose to conduct the following additional studies:

1- Determine the economically profitable spatial arrangement of various agroforestry systems, including intercropping with various species, and model the productivity (fruit and seed) of both sweet and bitter trees under different agro-ecological conditions.
2- Specifically, domesticated material from the Dahomey Gap may well reveal the drought resistance of some ABMT ideotypes which are valuable to face future more severe climatic conditions or may lead to a further expansion of the cultivatable area of this valuable multi-purpose tree.
3- Collect and fully document the diversity of ABMTs and bush mango ideotypes and conduct a consumption preference test in order to classify those ideotypes.
4- Investigate the differences in biochemical and nutritional characteristics against the ABMT ideotypes in order to classify those ideotypes economically.
5- Investigate the capacity of ABMTs for soil carbon sequestration and thus their ability for climate change mitigation, or even whether ABMT plantations can be regarded as a form of carbon storage and thus through international incentives contribute to a higher net income of rural populations.

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Summary

African bush mango trees (ABMTs) are economically the most important species within the family of Irvingiaceae. They are priority trees producing non-timber forest products (NTFPs) and widely distributed in the humid lowland forests of West and Central Africa. To boost their production and develop them towards a major crop for rural communities in Africa, a domestication program was initiated in the 2000s which is being coordinated by the World Agroforestry Centre. ABMTs belong to two taxa, one with sweet and one with bitter fruits which are morphologically difficult to distinguish. The fresh mesocarp of the sweet bush mangoes are consumed, while the seed of both bitter and sweet fruits are an important component of the African diet. The high oil content of this seed further increases their potential use.

Apart from the overlap of their morphological characters, the ecological and phenological distinction between sweet and bitter ABMTs is unclear due to: (i) the lack of comparative quantitative data and (ii) the lack of centralizing the existing country-level databases. Therefore, their taxonomic status is still not clear. Do they represent distinct species or varieties or are they mere forms within the same gene pool? It is also unclear whether the occurrence of ABMTs in traditional agroforestry systems in the Dahomey Gap, the dry savannah corridor between the Upper and Lower Guinean rain forest blocks, forms part of the natural distribution or not . Moreover, genetic studies addressing ABMTs diversity have been geographically restricted, and conclusions regarding the taxonomic status of sweet and bitter trees were not unanimous.

This study was conducted in a perspective of developing suitable strategies for the conservation and use of ABMTs, mostly in the Dahomey Gap. First, differences in ethnobotanical knowledge of the major socio-cultural groups in the Dahomey Gap were linked to the agroforestry status of ABMTs. This was used to explain the characteristics of the spatial pattern of ABMTs abundance and the anthropogenic factors that govern this spatial structure as well as population survival in the Dahomey Gap. Second, occurrence data of wild and cultivated ABMTs were used in a species distribution modelling algorithm to calculate the niche space and potential distribution of bitter and sweet trees in Tropical Africa. The differences in the obtained distribution patterns were compared using ENM-Tools. Third, detailed monthly phenological data and morphological characteristics (qualitative as well as

quantitative measurements on the leaves, bark, fruits, and seeds) were used to analyse the diversity of ABMTs and to discover differences between them in order to be able to identify bitter and sweet trees in the field. Finally, the molecular markers AFLP and cpSSR were used in order to map the genetic diversity of ABMTs and to discriminate sweet and bitter trees across Togo, Benin, Nigeria and Cameroon.

The consumption of mesocarp and seed of bush mangoes is widely known throughout the Dahomey Gap. The level of knowledge within local communities of other types of uses (medical, social-cultural) is generally poor and decreases towards the western part of this region. This suggests that ABMTs (mostly the sweet trees) were introduced in this eco-region through the migration of human populations from the Lower Guinean forest block (Southeast Nigeria) to the West. In the Dahomey Gap, bitter trees are confined to the Volta forest region, a small-sized ecological area in south-western Togo. While low densities (< 462 trees per 25 ha) were recorded for wild bitter trees occurring in the Volta forest region, higher densities (up to 1020 trees per 25 ha) were found for sweet trees in human made agro-systems. This implies a clear difference in cultivation methods between bitter and sweet ABMTs. The intensive cultivation of ABMTs in the Dahomey Gap is influenced by farmland status, farmer’s socio-cultural group and type of ABMT. Small and exhausted farmlands are converted into sweet ABMT orchards indicating that their development is a small-scale process lead by individual farmers. Slash and burn agriculture and intensive collection of fruits for seed commercialization jeopardize bitter trees, while traditional fishing systems (using twigs), traditional mass selection strategy, and intensive land commercialization severely threaten sweet trees genetic resources.

Using species distribution modelling, the potential distribution of wild sweet trees was predicted in the wetter zones of the Guinean-Congolian phytogeographical region, while that of bitter trees extended to drier zones in the Guineo-Congolia/Sudania and Lake Victoria regions. This difference is significant, supporting the idea that bitter and sweet trees belong to two different species. In the Dahomey Gap, bitter trees occur in the wild in the wettest ecological region of the Volta forest region which is a very small part of the Dahomey Gap. This region is ecologically particular among the ecosystems in which wild bitter trees generally occur. We also conclude that in the Dahomey Gap sweet trees occur only in cultivation.

Within the Dahomey Gap, clear phenological differences exist between sweet and bitter ABMTs, mostly in their reproduction phases. Moreover, their reproductive success significantly depends on the type of ABMT, soil, climate and season and we conclude there is a low probability of hybridization between sweet and bitter trees in the area where they co-occur.

The qualitative morphological characters, the type of bark, colour of the mature fruit exocarp and mesocarp, and fruit roughness, do not consistently discriminate bitter and sweet trees in the field. We strongly recommend broadening the geographic area of this study by increasing more bitter trees as well as the wild samples of both taxa to validate this conclusion. The bitter trees in the Volta forest region produce the heaviest seeds and this consistently distinguishes them from all the sweet trees sampled in the Dahomey Gap. However, a combination of quantitative morphological characters (from fruits, mesocarp, and seeds) failed to discriminate populations. On the other hand this indicates the presence of a high diversity and thus high potential for selection across all phytogeographical regions. However, domestication and climate appear to be playing a key role in the morphological differentiation of Dahomey Gap populations, and evidence of success in the traditional domestication and selection of sweet trees is proven.

Low genetic diversity was found for the bitter trees occurring in the Volta forest region in the Dahomey Gap due to the high fragmentation of the small-sized forest ecosystem in which they occur and the continuous reduction of the population size. The higher polymorphism and genetic diversity observed in the sweet tree population in Benin and Togo indicate the effect of domestication of material from different geographical origins as well as a frequent long distance transfer of genetic material. When used separately, the AFLP and cpSSR data failed to consistently discriminate geographical populations and bitter from sweet trees. But a combined dataset of both markers tends to differentiate such populations as well as tree types. Our results also provide evidence that the suitability of AFLPs and cpSSRs to assess genetic diversity patterns in *Irvingia* material needs to be thoroughly reassessed.

Finally, although admitting that a broader study remains necessary, based on the presence of a consistent gap between both taxa regarding their reproductive periods, their different ecology and, of course, the consistent difference in taste of the fruit, we advise to treat the sweet and bitter ABMTs as two taxonomically different entities at species level: *Irvingia gabonensis* (Aubry-LeComte ex O'Rorke) Baill. and *I. wombolu* Vermoesen, respectively.

Samenvatting

Afrikaanse bush mango bomen (ABMTs) zijn economisch gezien de meest belangrijke soorten binnen de Irvingiaceae familie. Zij krijgen prioriteit als bomen die niet-hout bosproducten (NTFPs) leveren en zijn wijd verbreid in de vochtige laagland regenbossen van West en Centraal Afrika. Om hun productie te stimuleren en hen te ontwikkelen tot een belangrijk gewas voor de Afrikaanse plattelandsbevolking, werd in de jaren 2000 een domesticatieprogramma gestart, gecoördineerd door het World Agroforestry Centre. ABMTs behoren tot twee taxa, één met zoete en één met bittere vruchten, die morfologisch lastig van elkaar zijn te onderscheiden. Het mesocarp van zoete bush mango’s wordt vers geconsumeerd, terwijl het zaad van zowel bittere als zoete vruchten een belangrijke component is van het Afrikaanse dieet. De hoge olieconcentratie van dit zaad verhoogd haar potentieel gebruik.

Los van de overlap in hun morfologische kenmerken, is een ecologisch en fenologisch onderscheid tussen zoete en bittere ABMTs onduidelijk vanwege: (i) het ontbreken van vergelijkende kwantitatieve gegevens en (ii) het gebrek aan centralisatie van nationale databases. Hierdoor is hun taxonomische status nog steeds onduidelijk. Vertegenwoordigen zij verschillende soorten of variëteiten, of zijn het slechts vormen binnen dezelfde genenpool? Het is ook onduidelijk of het voorkomen van ABMTs in traditionele agroforestry systemen in de Dahomey Gap, de droge savanne-corridor tussen de Upper en Lower Guinean regenbossen, onderdeel is van hun natuurlijke verspreiding of niet. Bovendien zijn genetische studies naar de diversiteit van ABMTs geografisch beperkt en hun conclusies ten aanzien van de taxonomische status van zoete en bittere bomen niet eensluidend.

De huidige studie werd uitgevoerd binnen een kader van de ontwikkeling van passende strategieën voor de bescherming en het gebruik van ABMTs, voornamelijk in de Dahomey Gap. Allereerst werden verschillen in ethnobotanische kennis van de belangrijkste sociaal-culturele groepen in de Dahomey Gap gekoppeld aan de status van het op ABMTs gebaseerde agroforestry systeem. Dit werd gebruikt om de kenmerken van het ruimtelijke patroon van ABMT algemeenheid en menselijke factoren die deze ruimtelijk structuur beïnvloeden te verklaren, evenals het voortbestaan van populaties in de Dahomey Gap. Ten tweede werden gegevens over de aanwezigheid van wilde en gecultiveerde ABMTs gebruikt in een algoritme om de verspreiding van soorten te modelleren om zo de niche omvang en potentiele verspreiding van bittere en zoete bomen in tropisch Afrika te berekenen. De verschillen in de verkregen verspreidingspatronen werden vergeleken met behulp van ENM-Tools. Vervolgens werden gedetailleerde maandelijkse fenologische gegevens en morfologische kenmerken (zowel kwalitatieve als kwantitatieve metingen aan bladeren, bast, vruchten en zaden) gebruikt om de diversiteit van ABMTs te analyseren en verschillen tussen bittere en zoete bomen te ontdekken teneinde hen in het veld te kunnen identificeren. Tot slot werden de moleculaire merkers AFLP en cpSSR gebruikt om de genetische diversiteit van ABMTs uit Togo, Benin, Nigeria en Kameroen in kaart te brengen en zoete en bittere bomen te onderscheiden.

Kennis over de consumptie van bush mango mesocarp en zaad is wijd verbreid in de gehele Dahomey Gap. Het niveau van de kennis binnen lokale gemeenschappen over ander type gebruik (medisch, sociaal-cultureel) is over het algemeen laag en neemt af naar het westelijk deel van deze regio. Dit suggereert dat ABMTs (voornamelijk de zoete bomen) in deze eco-regio werden geïntroduceerd via de migratie van bevolking uit het Lower Guinea regenbos (zuidoostelijk Nigeria) naar het Westen. In de Dahomey Gap zijn bittere bomen beperkt tot de Volta bos-regio, een klein ecologisch gebied in zuidwestelijk Togo. Terwijl voor bittere bomen in deze regio lage dichtheden werden genoteerd (< 462 bomen per 25 ha), werden er hogere dichtheden waargenomen (tot 1020 bomen per 25 ha) voor zoete bomen in door mensen aangelegde agro-systemen. Dit impliceert een duidelijk verschil in cultiveringsmethodes tussen bittere en zoete ABMTs. De intensieve verbouwing van ABMTs in de Dahomey Gap wordt beïnvloed door de status van de landbouwgrond, de sociaal-culturele groep waartoe de boer behoort en het type ABMT. Kleine en uitgeputte gronden worden omgezet in zoete ABMT boomgaarden, wat aanduidt dat hun ontwikkeling een kleinschalig proces is, geleid door individuele boeren. Slash en burn landbouw en het intensieve verzamelen van vruchten voor commerciële zaadhandel brengen bittere bomen in gevaar, terwijl traditionele vistechnieken (gebruik van twijgen), de traditionele selectie-strategie en intensieve commercialisatie van land een ernstige bedreiging vormen voor genetische bronnen van zoete bomen.

Met behulp van het modelleren van arealen werd voorspelt dat de potentiele verspreiding van wilde zoete bomen in de nattere zones van de Guineo-Congolese fytogeografische regio ligt, terwijl die van bittere bomen zich uitstrekt tot de drogere zones van de Guineo-Congolese/Sudanese en Victoria Meer regio’s. Dit verschil is significant, wat het idee dat bittere en zoete bomen tot twee verschillende soorten behoren ondersteunt. In de

Dahomey Gap komen wilde bittere bomen voor in het ecologisch natste deel van de Volta bosregio, wat een zeer klein deel is binnen de Dahomey Gap. Deze regio is ecologisch afwijkend van de ecosystemen waarin bittere bomen doorgaans voorkomen. Ook concluderen we dat in de Dahomey Gap zoete bomen slechts in cultuur voorkomen.

Binnen de Dahomey Gap komen duidelijke fenologische verschillen voor tussen zoete en bittere ABMTs, voornamelijk in hun reproductieve fases. Bovendien hangt hun reproductieve succes significant af van het type ABMT, bodem, klimaat en seizoen, en we concluderen dat er een lage kans op hybridisatie is tussen zoete en bittere bomen in het gebied waar ze samen voorkomen.

In het veld onderscheiden de kwalitatieve morfologische kenmerken bast type, kleur van exocarp en mesocarp van rijpe vrucht en vrucht ruwheid niet consistent bittere van zoete bomen. We raden sterk aan het geografische gebied van deze studie uit te breiden met meer bittere bomen alsmede wilde bomen van beide taxa om deze conclusie te valideren. De zwaarste zaden worden door bittere bomen in de Volta regio geproduceerd en dit onderscheid hen consistent van alle zoete bomen in de Dahomey Gap. Echter, een combinatie van kwantitatieve kenmerken (van vruchten, mesocarp en zaden) slaagde er niet in populaties van elkaar te onderscheiden. Aan de andere kant wijst dit op de aanwezigheid van een hoge diversiteit en dus hoog potentieel voor selectie in alle fytogeografische regio’s. Echter, domesticatie en klimaat lijken een sleutelrol te spelen bij de morfologische differentiatie in de Dahomey Gap populaties en er is een bewezen succes van de traditionele domesticatie en selectie van zoete bomen.

Een lage genetische diversiteit werd aangetroffen bij bittere bomen in de Volta bosregio binnen de Dahomey Gap als gevolg van de hoge fragmentatie van het kleinschalige bos-ecosysteem waarin ze voorkomen en de gestage reductie van populatiegrootte. Het binnen populaties van zoete bomen in Benin en Togo waargenomen hogere polymorfisme en de hogere genetische diversiteit duiden op het effect van domesticatie van materiaal met verschillende geografische oorsprong alsmede een regelmatige aanvoer over lange afstand van genetisch materiaal. Afzonderlijk lukte het niet met AFLP en cpSSR gegevens eenduidig populaties met bittere bomen te onderscheiden van die met zoete. Maar, een dataset met de gecombineerde gegevens van beide merkers heeft de neiging dergelijke populaties alsook boom types wél te herkennen. Onze resultaten tonen ook aan dat de bruikbaarheid van AFLPs en cpSSRs om genetische diversiteitspatronen binnen *Irvingia* materiaal te bepalen grondig moet worden herzien.

Uiteindelijk, hoewel toegegeven dat een bredere studie noodzakelijk blijft, gebaseerd op de aanwezigheid van een consistent verschil in reproductieve fases tussen beide taxa, hun verschillende ecologie en, natuurlijk, het consistente verschil in smaak van de vrucht, adviseren we om de zoete en bittere ABMTs als twee verschillende taxonomische eenheden op soortniveau te behandelen: respectievelijk *Irvingia gabonensis* (Aubry-LeComte ex O'Rorke) Baill. en *I. wombolu* Vermoesen.

Curriculum Vitae

Romaric Vihotogbé was born on December 12th, 1975 in Pobé (Plateau), Republic of Benin. In June 1996, he graduated from high school (Davié, Porto-Novo, Benin) and obtained his Baccalauréat degree (option: Biology). In 1996 – 2001, he completed an Agricultural Engineer degree at the Faculty of Agronomic Sciences (FSA), Department of Natural Resources Management (Option: Forestry) at the University Abomey-Calavi (UAC, Benin), and presented his Engineer thesis on "The diversity of food plant species in the forest region of Pobè, South-East Benin, with emphasis on African bush mango trees in traditional agroforestry systems". From 2002 to 2005, he joined the Centre for Integrated Eco-Development in the African giant snails breeding and mushroom domestication and cultivation program. Back at the Faculty of Agronomic Sciences in 2005, he joined the Laboratory of Applied Ecology as Research Assistant on food tree species. In that year, he was granted an MSc scholarship by the Catholic University of Louvain La Neuve, Belgium. He studied at the Faculty of Biological Agronomic and Environmental Engineering and in 2006 he produced his MSc thesis on the endogenous conservation of the African bush mango trees in traditional agroforestry systems in Benin. From 2006 to 2007, he was appointed as Scientific Assistant for the Sub-Saharan African Forest Genetic Resources Programme (SAFORGEN) of Bioversity International, at the West African Office in Cotonou, Benin. With SAFORGEN, he was involved in the development of research projects on sub-Sahara African priority food trees among which the African bush mangoes. In 2008, he obtained a Sandwich PhD Scholarship from Wageningen University and started on June 1st, 2008 at the Biosystematics Group within the Department of Plant Science, Wageningen University. His PhD research focussed on the diversity of African bush mango trees and differences between sweet and bitter trees in the Dahomey Gap. The results of these studies (2008-2012) are presented in this thesis.

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CHAPTER 1

General Introduction

Romaric Vihotogbé

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Background

Biological diversity plays a key role (nutritional, medical, socio-cultural, ecological, etc.) in the sustainability of human life and is therefore the most valuable resource on earth (SCBD, 2000; Jose, 2012). It includes the variability of organisms as well as the ecological systems that provide the environmental conditions necessary for their continuous regeneration (Probst and Crow, 1991; Swingland, 2001). Moreover, genetic variations as well as morphotypes resulting from the interaction with the environment are an integral part of biological diversity (Probst and Crow, 1991; SCBD, 2000; Sarkar, 2002). Since the 1992 Rio de Janeiro Earth Summit, the recognition of the fact that the knowledge present within human communities related to the management and use of biological resources is inextricably connected to biological diversity conservation has significantly increased (Swiderska, 2006).

Plants are a vital component of biological diversity and are central to human life, because they influence ecosystem resilience, soil stability and fertility as well as the agricultural systems on which human life depends (IPGRI, 1999; Eviner and Chapin, 2001). Among the large number of plant species valuable in human diets and other types of use, comparatively few contribute to the current exploitable agrobiodiversity (IPGRI, 1999). Agrobiodiversity includes the domesticated and cultivated plant species, and is defined by the Convention on Biological Diversity (http://www.cbd.int) as those plant species being manipulated during their evolutionary process in order to develop particular phenotypes that meet human needs. Among the limited set of domesticated / cultivated plant species, a comparatively low percentage have received adequate scientific attention with the intention to conserve their variability (Wood and Lenné, 1997). Many other valuable plants species are being collected from natural vegetations and integrated into the agricultural productive space by local communities. The vast majority of the useful plant species from a variety of ecosystems are exploited only locally and are known to the scientific community under various names among which “plant non timber forest products (NTFPs)” remains the most commonly used one.

Plant NTFPs in sub-Saharan Africa

Plant NTFPs refer to the products (apart from timber) collected from wild plant taxa occurring in natural forests (SCBD, 2001; van Andel, 2006). They can also be harvested from wild species preserved by local farmers on farms in various traditional agroforestry systems (Tabuna, 2000). The evaluation of the diversity of tropical plant species exploited as NTFPs already started during colonial times (see Reede tot Drakestein, 1678-1693; Rumphius, 1741-1750) and shows the very old symbiotic relationship between local communities and the environment (Irvine, 1952; Malaisse and Parent, 1985; Bergeret, 1986; Herzog, 1992; van Andel, 2000). This evaluation as well as the geographical areas covered are growing since the 1992 Rio de Janeiro Earth Summit which emphasizes the importance of the knowledge of local communities for the potential of biological resources (Hladik *et al*., 1996; Malaisse, 1997). The PROSEA and PROTA mega-programmes (http://proseanet.org/prosea/foundation.php, http://www.prota.org) are exemplary for such large-scale evaluations of botanical diversity resources in South-East Asia and Tropical Africa, respectively.

Because plant NTFPs are increasingly being used, in-depth studies on different aspects of their use, production and resilience in natural ecosystems and their economic importance at local, regional, national, and international levels have become a priority research topic over the last few decades (Ruiz Pérez and Arnold, 1995; Arnold and Ruiz Pérez, 2001; SCBD, 2001; Rai and Uhl, 2004; Ticktin, 2004; Adepoju and Salau, 2007). It has become clear that plant NTFPs play a significant role in the daily lives of millions of rural as well as urban populations around the world (Tabuna, 2000). Particularly, 80% of the rural populations in sub-Saharan Africa depend on plant NTFPs for their health needs (Kalaba *et al*., 2010). Their biochemical and nutritional evaluations (many are rich in proteins, lipid, carbohydrates, minerals and various vitamins and fibers (Augustino *et al*., 2011)) have demonstrated their potential to resolve the growing food, nutritional and health crises in Africa (Okolo *et al*., 1995; Chadare *et al*., 2010; Ahenkan and Boon, 2011; Sossa- Vihotogbé *et al*., 2012).

Two decades ago, an evaluation of the contribution of plant NTFPs to local populations' livelihood improvement was conducted throughout West and Central Africa by many organizations funded by the Consultative Group on International Agricultural Research (CGIAR). This evaluation intended to identify the key plant NTFP species on which research-development actions should be concentrated. It resulted in a great diversity of species including numerous multipurpose trees (Clark and Sunderland, 2004; Augustino *et al*., 2011). Even though there are many common types of use related to some species, geographically separated local communities not seldomly use them in different ways, in accordance with their culture, belief, food habits and traditional knowledge background accumulated through generations (Lesley and Brown, 2004; Sossa-Vihotogbé *et al.,* 2012). However, human migration and nutritional interactions among societies can gradually reshape food habits and modify knowledge regarding the utility of the local plant biodiversity (Turner and Turner, 2008). Therefore, many species may become widely used across their natural distribution area or even transported, marketed and eventually cultivated beyond that. African bush mango trees (ABMTs) are one of the most widely used and economically most important among the multipurpose trees species in West and Central Africa (Leakey *et al*., 2005).

ABMTs: revisiting the state of knowledge with emphasis on the Dahomey Gap

African bush mango trees are large, long-lived, evergreen tropical trees of 10 to 50 m high and are widely distributed throughout sub-Saharan Africa (Harris, 1996). Bush mangoes are their drupes, a mango-like fruit. Their flesh can be either pleasant and sweet or bitter and inedible, while the seed has various important uses as well. Throughout their entire distribution range, ABMTs are subjected to many types of use that differ among human communities. Lesley and Brown (2004) provided a detailed overview of the state of our knowledge on bush mangoes, and most importantly their seed, as major NTFPs in West and Central Africa. The flesh (mesocarp) of the sweet fruits is rich in Vitamin C (Fouda *et al*., unpublished data) and is consumed fresh. The seed provides one of the most important oily substances in West and Central Africa (Joseph, 1995; Ladipo, 1999; Oyen, 2007) and is reported to be valuable in food and cosmetic manufacturing (Lesley and Brown, 2004). Above all, the main value of the seed is its use as a sauce thickening agent, typical for the African diet (Lowe *et al*., 2000; Lesley and Brown, 2004; Leakey *et al*., 2005), causing the trade of the kernels, the hard endocarps containing the seeds, to reach all continents, following the migration of black African people (Tabuna, 2000). Their wood is fairly hard and is frequently used (Ayuk *et al*., 1999; Dolor, 2011). Moreover, the leaf, bark and root of ABMTs know many medicinal uses, but these do not yet represent a significant economic value, although the analgesic effect of the bark was demonstrated (Okolo *et al*., 1995). Economically, the seed is the most important NTFP that makes ABMTs the top priority food trees species worth of in-depth research programs in West and Central Africa (Franzel *et al*., 1996). Ladipo (1999) reported that since 1975 the market for kernels was worth some US$ 50 million and this market has kept on growing. The economic potential related to kernel commercialization for a single ABMT was estimated at US$ 300 per year (National Research Council 2006). Recently, Nkwatoh *et al*. (2010) showed that the annual kernel production between 2002 and 2008 in a single Cameroonian natural reserve (the Ejagham Forest Reserve) and marketed toward Nigeria reached 155,000 tons, thus generating an annual revenue of US$ 675,000, clearly showing the market potential. Agbor (1994) already indicated that the production of ABMTs in natural areas was decreasing due to overexploitation and seedling mortality, which points to the urgent need for protection of natural stands because of the rapid increase in demand.

To boost the production of this NTFP and promote the establishment of economically profitable agrosystems based on ABMTs, the World Agroforestry Centre is leading a two decades-long research program on the morphological diversity of fruits and trees, cultivation, on-farm management, ideotype capture and fixation and shortening the time of first fruiting.

Today, ABMTs are exploited in at least twenty countries in sub-Saharan Africa: Senegal, Guinea (Conakry), Guinea-Bissau, Sierra Leone, Liberia, Ivory Coast, Ghana, Togo, Benin, Nigeria, Cameroon, Equatorial Guinea, Gabon, Republic of the Congo, Democratic Republic of Congo, Angola, Uganda, South Sudan, Central African Republic, and Sao Tome and Principe (Harris, 1996; Lesley and Brown, 2004; Kengni *et al*., 2011). While ABMTs are widely distributed, occurring naturally in the Upper and Lower Guinean forest blocks (Figure 1), and are used in various ways by local communities (Harris, 1996), research initiatives are still scarce. Only recently, an in-depth study (Sahoré *et al*., 2012) indicated that the seeds of Ivory Coast provenances of sweet fruits are rich in fatty matter (64%), carbohydrates (24%), proteins (7%) and total mineral matter (6%). In Benin and Togo, only limited data is available on the use, occurrence and local commercialization of bush mangoes (Poidy, 2002; Tandjiékpon, 2001; Vodouhè, 2003; Vihotogbé *et al*., 2007; Atato *et al*., 2010).

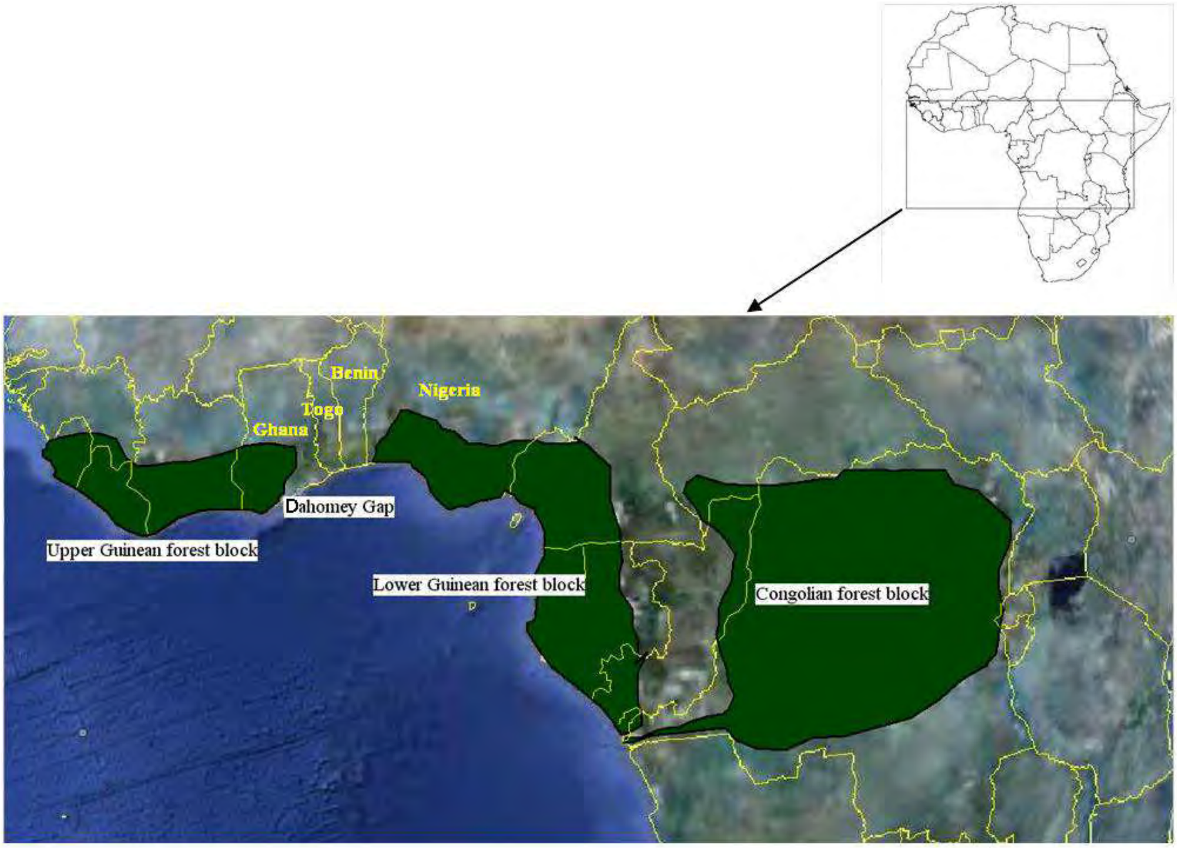

Figure 1: Location of Benin and Togo in the Dahomey Gap between the Upper and Lower Guinean forest blocks (adapted from satellite image downloaded from Google Earth, White, 1979 and Biodiversity Analysis and Technical Support for USAID, 2007).

Benin and Togo are located in a climatically particular eco-region called the Dahomey Gap (DG; see Figure 1) which is characterized by low annual rainfall (less than 1,200 mm) and high temperature (Maley, 1996; Salzmann and Hoelzmann, 2005). This eco-region extends from the plain of Accra (southern Ghana) to the area of Badagry (southwest Nigeria) and isolates the Upper Guinean forest block from the Lower Guinean one (White, 1979). The latter maintains its connection with the Central African Congolian forest block (Adebisi, 2007). The vegetation in the Dahomey Gap is mainly composed of a mosaic of savannah, with small extents of drier types of lowland rain-forests, and fallows and fields. Pollen records indicate that this gap, most probably, did not exist during the early and middle Holocene and that rainfall was sufficient to maintain a semi-deciduous/semi-evergreen forest (Salzmann and Hoelzmann, 2005). The latter authors also suggest that, before its establishment many pioneer tree taxa occurred in the Dahomey Gap. Even today, many forest tree species still occur in the Dahomey Gap but are critically threatened by the combined pressures of climate and human activities (Adomou, 2005). Among these forest tree species many produce important NTFPs that are locally consumed and regionally marketed throughout West Africa (Sokpon and Lejoly, 1996; Atato *et al*., 2010; Ekué *et al*., 2010). ABMTs belong to these important NTFPs that contribute to the nutrition, health and economy of communities in this eco-region (Tandjiékpon, 2003; Vodouhê, 2003; Vihotogbé *et al*., 2007; Atato *et al*., 2001).

The human population in the Dahomey Gap is multi-ethnic and communities are culturally linked (Assiwaju, 1979). Most of the local communities are rural and apart from agriculture, which is the most important source of their revenue and food, they also depend on plant NTFPs for their daily needs. Those NTFPs are found in human-made agro-systems as well as in the small-sized natural forests within the savannah dominated landscape.

Problem statement

ABMTs belong to the small family of Irvingiaceae which was taxonomically revised by Harris (1996). The family comprises three genera: *Desbordesia*, *Klainedoxa*, and *Irvingia* with 1, 2 and 7 species, respectively. Among the seven *Irvingia* species, six occur in West and Central Africa, while the seventh is native to South-East Asia (Harris, 1996). The term ABMTs refers to the two economically most important *Irvingia* species that occur in the humid lowland forests of West and Central Africa (Asaah *et al*., 2003), *I. gabonensis* (Aubry-LeComte ex O'Rorke) Baill. and *I. wombolu* Vermoesen. While the first generally has sweet mango like fruits the second has bitter ones and they co-occur in many eco-regions in their distribution range (Lesley and Brown, 2004). In the light of their domestication and cultivation potentials, the specific ecological characteristics of both taxa as well as the ecological variability present within each taxon are important research question to be addressed.

Morphologically, sweet and bitter ABMTs are very similar and difficult to discriminate in the field (Okafor, 1975; Harris, 1996). The mesocarp of *I. gabonensis* can be really sweet to slightly bitter and edible, while that of *I. wombolu* is totally inedible. This seems to be the only obvious distinctive character between the two taxa. Okafor (1975) stated that the taxonomic value of this character is low and presented sweet and bitter trees as varieties of a single species: *Irvingia gabonensis* var. *gabonensis* and *I. gabonensis* var. *excelsa* (Mildbr.) Okafor, respectively. In his taxonomic classification of the genus *Irvingia*, Harris (1996) acknowledged the overlap of many characters between both types. However, because in his opinion the mesocarp taste is correlated with other vegetative characters, which would thus be able to consistently distinguish bitter from sweet trees throughout their distribution range, he proposed to distinguish sweet and bitter trees at the species level (*I. gabonensis* and *I. wombolu*, respectively). Unfortunately, those other characters are often difficult to evaluate. Therefore, a thorough quantitative morphological as well as phenological evaluation of both taxa is needed in order to assess the key differences between them which will assist in the determination of the appropriate level of their taxonomic distinction.

Lowe *et al*. (2000) demonstrated significant genetic differences between sweet and bitter ABMTs. Although such a difference is generally indicative of a lack of exchange of genetic material, they did not present sound evidence of the absence of hybridization between the two types. Because certain weaknesses have been demonstrated in the methods they used, mostly the low reproducibility of the Random Amplified Polymorphism DNA technique, the taxonomic debate about ABMTs remains alive (National Research Council, 2006). As a result, both opinions (species-level and variety-level distinction) are found in recent studies (Dudu *et al.*, 1998; Nzekwe *et al.*, 2002; Nya *et al.*, 2006; Nya *et al.*, 2009; Dolor, 2011; Sahoré *et al.*, 2012). Therefore, the evaluation of genetic, but also of ecological, morphological, chemical or phenological differences between sweet and bitter ABMTs still are important research questions. It is particularly vital for any conservation and domestication program to insure the identity of the material being used, domesticated and conserved. For example, it is difficult to attribute the potential medicinal uses related to the biochemical properties of the bark and leaf of ABMTs (Okolo *et al*., 1995; and Nosiri *et al*., 2011) to either sweet or bitter trees (Tchoundjeu and Atangana, 2007). In addition, the kernels of bitter and sweet bush mangoes, the most important NTFP of ABMTs, are mixed for marketing, while it seems likely that differences in organoleptic properties, indicating different economic potential for sweet and bitter trees, are present (Ladipo, 1999). In the context of ABMTs domestication and conservation, the World Agroforestry Centre focuses on the germplasm from the Lower Guinean forest block, where sweet trees have been more intensively investigated. Broadening the geographical area for a comparative study to the Upper Guinean region seems logical, since a domestication and conservation program would want to use the maximum of ABMTs diversity (ecological, phenological, chemical and morphological as well as genetic). Capturing such diversity depends on well-balanced and sufficiently dense germplasm collecting activities covering the entire distribution range of ABMTs (see Ladipo *et al*., 1996; Lowe *et al*., 2000).

Most ABMTs material used for former cultivation and domestication efforts arose from Nigeria and Cameroon (Lowe *et al*., 2000; Ude *et al*., 2006; Lower *et al*., 2010). In the Dahomey Gap, ABMTs are distributed across different phytogeographical regions and in various agrosystems, but their truly wild occurrence is questionable. Thus, their geographical origin from outside that eco-region needs to be demonstrated (Kengni *et al*., 2011).

Objectives of this thesis

General objective

The overall objective of this research is to improve our knowledge on ABMTs, focussing on the possible differences between the sweet and bitter types, thus supporting the development of suitable strategies for their domestication, conservation and use within the Dahomey Gap (West Africa) and possibly beyond that region.

Specific objectives

This study aims to:

- Assess the ecological difference between sweet and bitter ABMTs in tropical Africa and discuss its taxonomic implication as well as the origin of the Dahomey Gap material.
- Quantify the phenological characteristics of both types of ABMTs and infer the potential drivers of this variation as well as its taxonomic implication.
- Investigate the distinction of sweet and bitter ABMTs in the field based on quantitative morphological data of leaves, bark and fruits.
- Revisit the pattern of genetic diversity and the phylogenetic relationships between and within sweet and bitter ABMT populations throughout tropical Africa.
- Relate traditional knowledge on AMBT usage to the decision of farmers to allocate productive space to bitter and sweet ABMTs in the Dahomey Gap.

Thesis outline

After this general introduction, which has briefly traced the knowledge and research issues related to ABMTs and their importance and presented the objectives of the thesis, this thesis comprises six additional chapters: five research papers and a general discussion. The second chapter gathers the indigenous knowledge related to the use of ABMTs in the Dahomey Gap, and uses the geographical location of the different levels of knowledge of local communities to postulate the origin of the Dahomey Gap material. In this chapter, the spatial abundance of ABMTs is modelled across the Dahomey Gap and the anthropogenic factors that influence their cultivation intensity as well as population survival are analysed. In the third chapter, presence data of sweet and bitter trees collected throughout the Dahomey Gap and in tropical Africa are used in ecological niche modeling analyses. The niche model of bitter trees is compared to that of the sweet while the model of cultivated material provides the potentially cultivatable area. Also, the origin of material cultivated in the Dahomey Gap is discussed. Phenological differences between bitter and sweet ABMTs and among populations in the Dahomey Gap are analysed in the fourth chapter. In addition, in this chapter we relate environmental factors to the reproductive success of each type of ABMT in different phytogeographical regions. In the fifth chapter, the possibility to differentiate sweet and bitter ABMTs in the field based on quantitative and qualitative morphological characters of leaves, bark, fruit and seeds, is investigated in populations across the Dahomey Gap. In the sixth chapter, the genetic diversity of and the relationships between sweet and bitter ABMT populations are assessed using Amplified Fragment Length Polymorphisms (AFLP) and chloroplast microsatellites. In the seventh chapter, the general discussion, the most important results of our attempts to discriminate bitter and sweet ABMTs are presented together with the implications these results have in terms of the domestication, cultivation and conservation of ABMTs as important food trees.

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Chapter 2

Ecological differences within African Bush Mango trees in tropical Africa with emphasis on the Dahomey Gap (West Africa)

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Abstract

Bush mangoes (Irvingiaceae) are priority food trees in humid areas of West and Central Africa. There are bitter and sweet fruited trees which are difficult to distinguish based on morphological characters. This has led to a taxonomic debate, hindered by the lack of quantitative comparative data. Furthermore, their occurrence in the Dahomey Gap, the dry savannah corridor between the two West African forest blocks, is unexplained. This study evaluates the ecological differences between bitter and sweet trees in tropical Africa and studies the possibility that the Dahomey Gap is part of the natural distribution range of bush mango trees. Occurrence data from the Dahomey Gap and elsewhere (in tropical Africa), were combined with climatic and soil data in Maxent to produce ecological niche models for bitter and sweet trees. Ecological niche identity tests were carried out in ENM-Tools. Wild sweet trees were predicted in the Guinean-Congolian phytogeographical region, while the predicted occurrence of bitter trees extended to the Guineo-Congolia/Sudania and Lake Victoria regions. This difference is significant, supporting the idea that bitter and sweet trees belong to two different species. We also conclude that bitter trees occur naturally only in a small part of the Dahomey Gap, the Volta forest region. Lastly, our results suggest that the natural distribution of sweet trees excludes the Dahomey Gap, where they occur only in cultivation. The history of their occurrence in this eco-region remains unclear.

Key-words: Benin, Climate, Ecological Niche Modeling, *Irvingia*, Maxent, Togo

Introduction

African bush mango trees (ABMTs) belong to the genus *Irvingia* within the family of Irvingiaceae. They are priority food tree species in the humid regions of West and Central Africa (Franzel *et al.*, 1996). They produce two economically important non-timber forest products (NTFPs) in sub-Saharan Africa (Tabuna, 2000): the sweet mesocarp, which is eaten fresh, and the seed used as a sauce thickening and flavouring agent (Lowe *et al.*, 2000; Tchoundjeu and Atangana, 2007).

Bush mangoes show a clear and steady difference in the mesocarp taste: bitter and totally inedible versus sweet or slightly bitter and edible. This is a key criterion that helps define bitter and sweet ABMTs throughout their distribution area. However, attempts to discriminate between bitter and sweet ABMTs using morphological characters are unsatisfactory (Okafor, 1975; Harris, 1996). Because sweet and bitter trees lack additional reliable discriminating characters, Okafor (1975) opted for a distinction only at the variety level within the species *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill.: *I. gabonensis* var. *gabonensis* and *I. gabonensis* var. *excels* (Mildbr.) Okafor, for sweet and bitter trees, respectively. However, in his recent taxonomic revision, Harris (1996) decided to recognize them at species level as *I. gabonensis* and *I. wombolu* Vermoesen, respectively, although he admitted a weak morphological distinction between them. Lowe *et al.* (2000) supported this decision using Random Amplified Polymorphic DNA (RAPD) molecular markers. But, because of some weaknesses related to these genetic markers, the taxonomic status of ABMTs remains controversial (National Research Council, 2006) and a random use of the variety as well as the species names abounds in literature. Finding a solution is hindered by poor sampling (Ladipo *et al.*, 1996; Lowe *et al.*, 2000) and the lack of centralizing the existing occurrences and the morphological and phenological data in quantitative and comparative studies over their entire distribution range.

Wild ABMTs occur in humid lowland forest areas in West and Central tropical Africa (Kang *et al.*, 1994; Harris, 1996; Van Dijk, 1997; Lowe *et al.*, 2010). Their distribution is centred in the Guineo-Congolian phytogeographical region, with some outliers to the Guinea-Congolian / Zambezian zone and the Guinea-Congolia / Soudania Transition zone (Fig. 2.1a, 2.1b; White, 1979). In West Africa, the Dahomey Gap refers to the region where a savannah corridor interrupts the rain forests of the Guineo-Congolian region (Maley, 1996). This particular eco-region, with higher temperatures and lower rainfall, extends from Accra, in south-eastern Ghana throughout Togo and Benin until Badagry, in south-western Nigeria and divides the Guineo-Congolian region into the Upper and Lower Guinean forest blocks (White, 1979; Maley, 1996; Sowunmi, 2007).

The increasing market demands of the hard bush mango endocarp (containing the valuable seed) causes an over-exploitation of trees in natural habitats and this results in poor regeneration in their postulated genetic diversity centres in Nigeria, Cameroon and Gabon (Agbor, 1994; Lowe *et al.,* 2000; Ude *et al.,* 2006; Lowe *et al.,* 2010). Therefore, cultivation initiatives are increasing throughout the ABMTs distribution range (Okafor and Fernandes, 1987; Shiembo *et al.,* 1996; Asaah *et al.,* 2003).

In general, cultivation broadens the geographic distribution of a species (Miller & Knouft, 2006) and accurate knowledge of cultivation success, as indicator of ecological adaptability, is required for an effective monitoring and conservation management (Shaffer *et al.,* 1998; Corsi *et al.,* 1999). Knowledge of the potential cultivatable area is also useful, for example in a national policy for agricultural development, to anticipate on the growing demands for many valuable NTFPs (Scheldeman *et al.,* 2007).

Ecological niche modelling combines the known occurrences of a species with corresponding environmental parameters in a geographic information system (GIS) to generate for any point within the geographic area under investigation the probability of the presence of this species (Phillips *et al.,* 2006; Pearson, 2007; McCormack *et al.,* 2011). Given their ability to identify areas where species should be able to successfully complete their life cycle, ecological niche models (ENMs) are powerful tools for agro-biodiversity conservation (Miller and Knouft, 2006; Sanchez *et al.,* 2010). Numerous algorithms are designed to refine species ecological niche modelling techniques. Among them, Maximum Entropy (Maxent, Phillips *et al.,* 2006) was identified as the more accurate even with a limited known occurrence dataset (Elith *et al.,* 2006; Pearson *et al.,* 2007; Wisz *et al.,* 2008; Leaché *et al.,* 2009). However, like any ecological modelling package, the authority of ENMs to provide quantitative ecological niche differences among closely related taxa is limited. ENM-Tools (Warren *et al.,* 2008) tries to overcome this weakness. It quantifies the ecological difference between two taxa by comparing estimated probabilities assigned to individual grid cells over the entire geographical space studied. This powerful package assists in the assessment of the ecological identity of a species (McCormack *et al.,* 2011; Wooten and Gibbs, 2011).

This study evaluates ecological differences between sweet and bitter trees in tropical Africa. We combined known occurrence data with environmental layers in Maxent (version 3.3.3k) addressing three main research questions:

- Are the ecological niches of bitter and sweet ABMTs different?
- Does the Dahomey Gap belong to the ecological niche of sweet and/or bitter ABMTs? In other words, do sweet and bitter ABMTs occur wild in the Dahomey Gap?
- What is the potential cultivatable area of sweet ABMTs in the Dahomey Gap?

Material and Methods

Presence data

A total of 3019 ABMTs occurrences from Central and West Africa were gathered from various sources including recent fieldwork (Table 2.1). Fieldwork was carried out only in the Dahomey Gap (Benin and Togo) in 2009. In this area, where human made agrosystems and relics of natural forests are the main vegetation, the occurrences were recorded throughout all areas where ABMTs grow in such a way that all phytogeographical regions (see Ern, 1979; Adomou, 2005) were covered. Using a Global Positioning System (GPS Garmin 60), each isolated tree scattered in the landscape was geo-referenced while in high density areas only one tree was sampled. The occurrence database was resampled to 5 arc-minute (10 x 10 km) to prevent overweighting. This resulted in 185 and 24 unique occurrences for sweet and bitter trees in the Dahomey Gap, respectively. We refer to these samples as Dahomey Gap bitter trees and Dahomey Gap sweet trees.

Outside the Dahomey Gap, the occurrences were obtained from the sources mentioned in Table 2.1. They are collections from wild populations occurring across the humid lowland forest areas on both sides of the Dahomey Gap. Herbarium specimens cited by Harris (1996) geo-referenced and the sample location maps of Lowe *et al.* (2000) were digitized and ABMTs' geographic positions were thus extracted. Data downloaded from the GBIF Data Portal (http://www.gbif.net) was checked and, if sufficiently trusted, added. Duplicate records were removed. The spatial distribution of the records range from between -13.93° to 33.63° longitude and -6.96° to 14.57° latitude.

Table 2.1: Sources and sample size for bitter and sweet African bush mango trees

Type ABMT	Geographic area	Source	Total sample size	Used sample size after duplication removed
Bitter	Dahomey Gap	Fieldwork (Vihotogbé R.)	213	24
	Tropical Africa	GBIF Data Portal	30	65
		Lowe et al. (2000)	28	
		Harris (1996)	21	
Sweet	Dahomey Gap	Fieldwork (Vihotogbé R.)	2565	185
	Tropical Africa	GBIF Data Portal (http://www.gbif.net)	86	121
		Lowe et al. (2000)	76	

Environmental parameters

Consistent conclusions can be drawn from species distribution models constructed with only climatic variables (see Scheldeman *et al.,* 2007). But, because climatic data have limited power to capture spatial micro-variations in a landscape and can display important spatial autocorrelations, additional environmental factors are often required to improve species distribution models. This is particularly important when assessing ecological differences between closely related species that geographically co-occur (see McCormack *et al.,* 2011). Moreover, the combination of soil characteristics and climatic variables play a key role in plant species distribution in sub-Saharan Africa (Linder *et al.,* 2005). Therefore, we use both climatic and soil data as ecological parameters to produce ecological niche models in tropical Africa and the Dahomey Gap. The nineteen 30 arc-seconds bioclimatic variables from the WorldClim database (http://www.worldclim.org/current) were tested for multicollinearity using Pearson's correlation (r < 0.70), and five independent variables were retained (Table 2.2). Soil data were collected from the ISRIC World Soil Information database (http://www.isric.org/). Soil data were tested for multicollinearity using Spearman rank correlation and twelve independent soil were retained (Table 2.2). The topsoil salinity, although shown complete independence against all other variables, was not retained because it holds very limited information within the ranges of ABMTs. In total, sixteen environmental layers were used to carry out the ecological niche modelling.

Table 2.2: Contribution of environmental factors to ecological niche models

Bioclimatic and soil variables	Contribution to ecological niche models (%)			
	Wild SBMTs	**DG SBMTs**	**Wild BBMTs**	**DG BBMTs**
Annual mean temperature	0.6	0.7	0.2	0.2
Isothermality (Bio_2/Bio_7)(* 100)	4.4	12	8.1	14.5
Minimum temperature of coldest month	12.6	48.6	2.5	2.9
Annual precipitation	50	15	17.2	1.8
Precipitation of coldest quarter	2.6	5.8	41.9	26
Available water storage capacity [class 1 (150mm/m) - 7 (0 mm/m)]	1.7	0.9	0.9	4.2
Drainage class [class 1 (very poor) – 7 (excessive)]	0.3	0.3	4.7	0.2
Reference depth of the soil unit	1.2	0.2	0	0
Topsoil calcium sulphate	0.2	0.5	2.9	3
Topsoil cation exchange capacity	0.2	0.3	0.6	1.2
Topsoil exchangeable sodium percentage	2.5	0.2	4	0.6
Topsoil gravel content	20.6	11.5	8	25.5
Topsoil organic carbon content	0.2	0.3	0.8	0.5
Topsoil pH [measured in a soil-water solution]	0.7	2.1	6.7	0
Topsoil silt fraction	0.9	0.9	1.2	19.2
Topsoil textural class [class 0 (none) – 3 (fine)]	1.1	0.8	0.3	0.3

DG = Dahomey Gap

Ecological niche modelling

We considered four occurrence sub-datasets: (i) wild bitter trees, (ii) wild sweet trees, (iii) bitter trees in the Dahomey Gap, and (iv) sweet trees in the Dahomey Gap. Assuming that occurrence records provide key information on the ecological conditions required for species occurrence and growth (Pearson 2007), we expected the modelled potential ranges to represent areas were the populations represented by each sub-dataset could potentially occur within the limits of the geographical area considered.

First, we combined each of those occurrence sub-datasets with the environmental layers in -2° to 15° Latitude and -15° to 35° Longitude in Maxent (using default parameters) to produce four ecological niche models. Second, using the same algorithm, the sweet trees occurrences from the Dahomey Gap were combined with the environmental layers in 5° to 13.5° Latitude and -1° to 5° Longitude to generate a fifth ecological niche model. The latter was used only to obtain the cultivatable area for sweet trees within that area. The projection of the obtained ecological niche models in the geographical space shows the potential distribution area of the corresponding species. Because in the Dahomey Gap only cultivated sweet trees were collected, this dataset was used to predict their potential cultivatable area.

The area under the curve (AUC) of the Receiver Operating Characteristic (ROC) was used to assess the accuracy of models created with the Maxent algorithm. This statistic describes the relationship between the percentage of correctly predicted occurrences against that of incorrectly predicted absences and ranges from 0.5 (random prediction) to 1 (high model consistency).

Ecological niche overlap and identity testing

This analysis aims to evaluate the difference in ecological niches between: (i) wild bitter and wild sweet trees, (ii) wild bitter trees from Tropical Africa and the Dahomey Gap bitter trees, and (iii) wild sweet trees from Tropical Africa and the Dahomey Gap sweet trees. The models produced by Maxent were used in ENM-Tools to calculate the Schoener's *D* similarity index (see Warren *et al.,* 2008) measuring the niche-overlap between model pairs. This similarity index ranges from 0, for niches completely disjoint, to 1 for totally identical niches (Warren *et al.,* 2008).

In addition, using ENM-Tools occurrences from each model pair being compared were pooled and 100 pseudo-replicate random sets of occurrences were sampled from the pooled data. Original niches of the model pair were compared with those of the 100 random sets by means of the comparison of the observed values of the niche similarity (D) to the null distribution (see Warren *et al.*, 2008).

Results

Potential distribution areas of wild bitter and sweet trees

The potential distribution of wild occurrences of bitter and sweet trees was predicted in the area between -7° to 15° Latitude and -15° to 35° Longitude. The resulting ecological niche models were highly consistent: AUC = 0.940 and 0.964 for bitter and sweet trees, respectively.

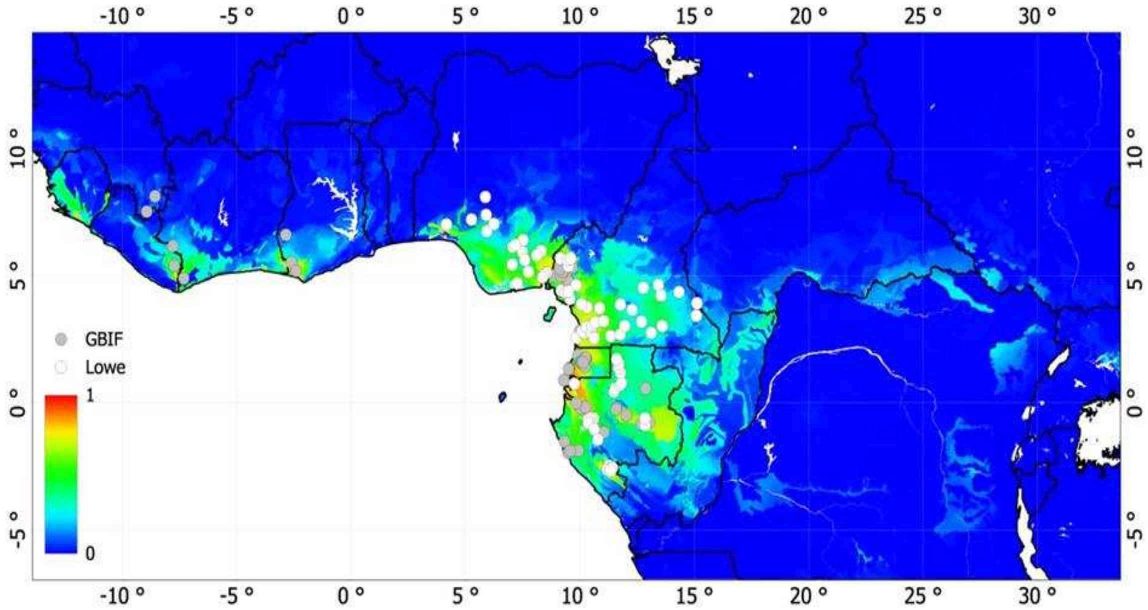

Figure 2.1a: Potential distribution of wild sweet ABMTs in tropical Africa

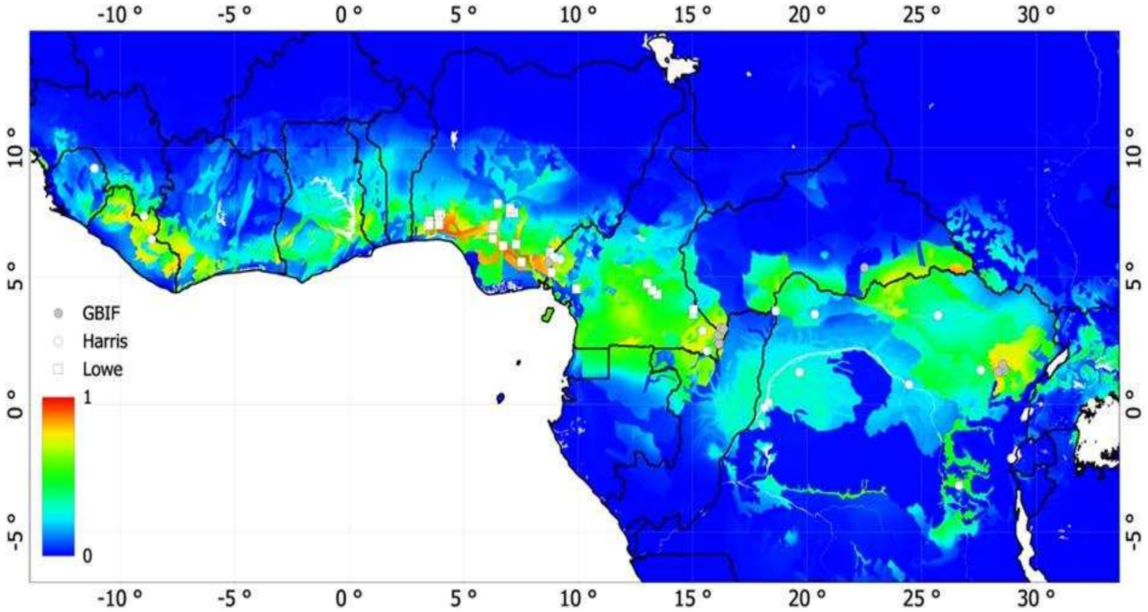

Figure 2.1b: Potential distribution of wild bitter ABMTs in tropical Africa

Wild sweet trees were predicted to occur in forest regions comparatively close to the coast with the highest distribution probabilities within -3° to 6° latitude and -12° to 14° longitude (Fig. 2.1a). Some high distribution probabilities were concentrated in the Upper Guinean forest block, but most are found in the Lower Guinean region (Nigeria to Gabon). In the Upper Guinean forest block their wild occurrence appears disjoint. The Dahomey Gap was excluded from the potential distribution range of wild sweet trees.

The potential distribution of the wild bitter trees, on the other hand, extends both to dryer regions and to other wetter regions. They were predicted to occur with high probabilities within -5° to 10° Latitude and -11° to 30° Longitude (Fig. 2.1b). Unlike wild sweet trees, high distribution probabilities were mapped with a more continuous pattern from Central and North Liberia to south-eastern Ivory Coast, away from the coast. Part of the Dahomey Gap (from south-western Togo to southern Ghana called the Volta forest region) is also included in the potential distribution area of wild bitter trees. The highest occurrence probabilities, with a more continuous pattern, are located in the Lower Guinean forest block: humid forests in southern Nigeria and Cameroon. The high occurrence probabilities extend to the northern edge of the Congolian forest block towards the Ituri region in the northeast of the Democratic Republic of Congo. Finally, wild sweet trees were predicted to occur throughout the Congo Basin but with low probabilities.

Ecological differences between wild bitter and sweet trees

Different environmental factors contribute at different levels to the ecological niche model of wild bitter and wild sweet trees (Table 2.2). The occurrence of wild bitter trees is most highly associated with the precipitation in the coldest quarter (42%) and annual precipitation (17.2%), while annual precipitation (50%), topsoil gravel content (20.6%), and minimum temperature in the coldest month (12.6%) govern the occurrence of wild sweet trees.

For wild bitter trees the optimum precipitation in the coldest quarter is around 500 mm while for wild sweet trees this niche parameter accounts for only 2.6% in the occurrence. Above this optimum, the occurrence probability of wild bitter trees steadily decreases (Fig. 2.2a). Wild bitter trees prefer an annual precipitation higher than 1500 mm/year, while the occurrence probability of wild sweet trees is steadily rising even above 2,500 mm/year. Wild sweet trees are more sensitive (negatively) to lower annual precipitations (< 2,500 mm) and their occurrence probability decreases more rapidly to 0 at 1,300 mm (Fig. 2.2b). With regard to the minimum temperature of the coldest month, wild sweet trees are sensitive to values lower than 17 °C, while wild bitter trees are not affected by this factor which accounts for only 2.5% in their niche model (Fig. 2.2c). Finally, the optimum topsoil gravel content is 0% for wild sweet trees displaying high negative sensitivity to high values, while this factor does not impact the occurrence probability of the wild bitter trees (Fig. 2.2d).

The Schoener's *D* statistic shows a degree of overlap of 0.5 between the ecological niches and the niche identity test indicates that this overlap is significantly less than that obtained when comparing the original models with those from randomization of the pooled dataset (Fig. 2.2e). Thus, the natural habitats populated by bitter and sweet trees are not identical.

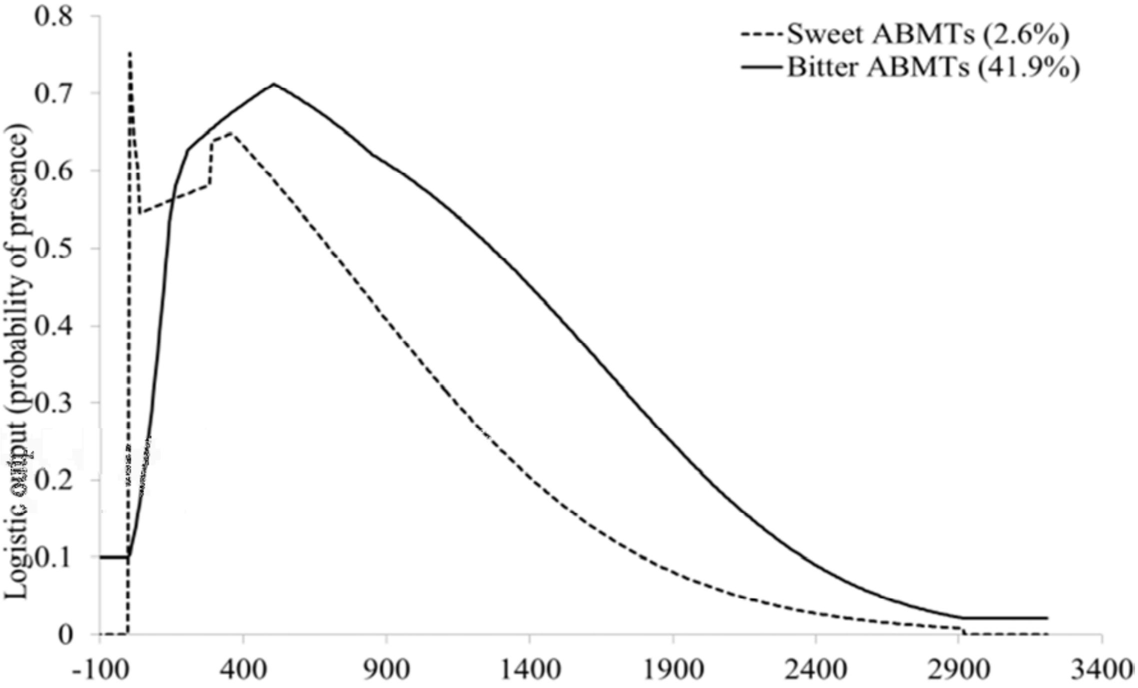

Figure 2.2a: Influence of precipitation of the coldest quarter (in mm)

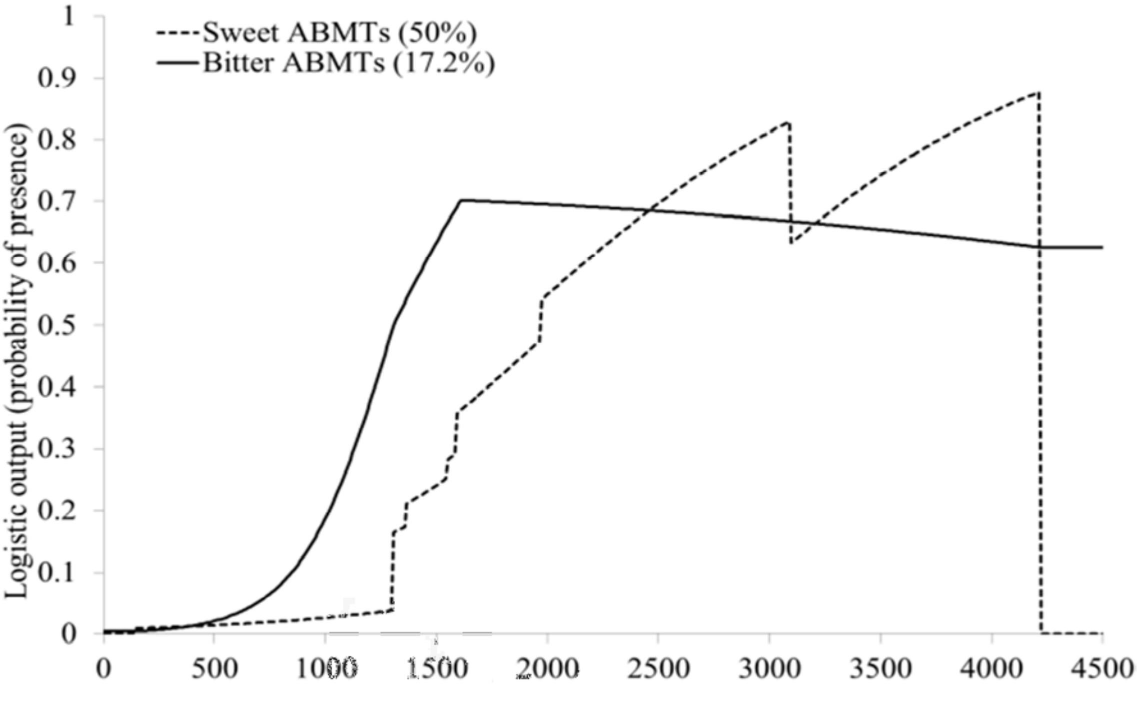

Figure 2.2b: Influence of annual precipitation (in mm)

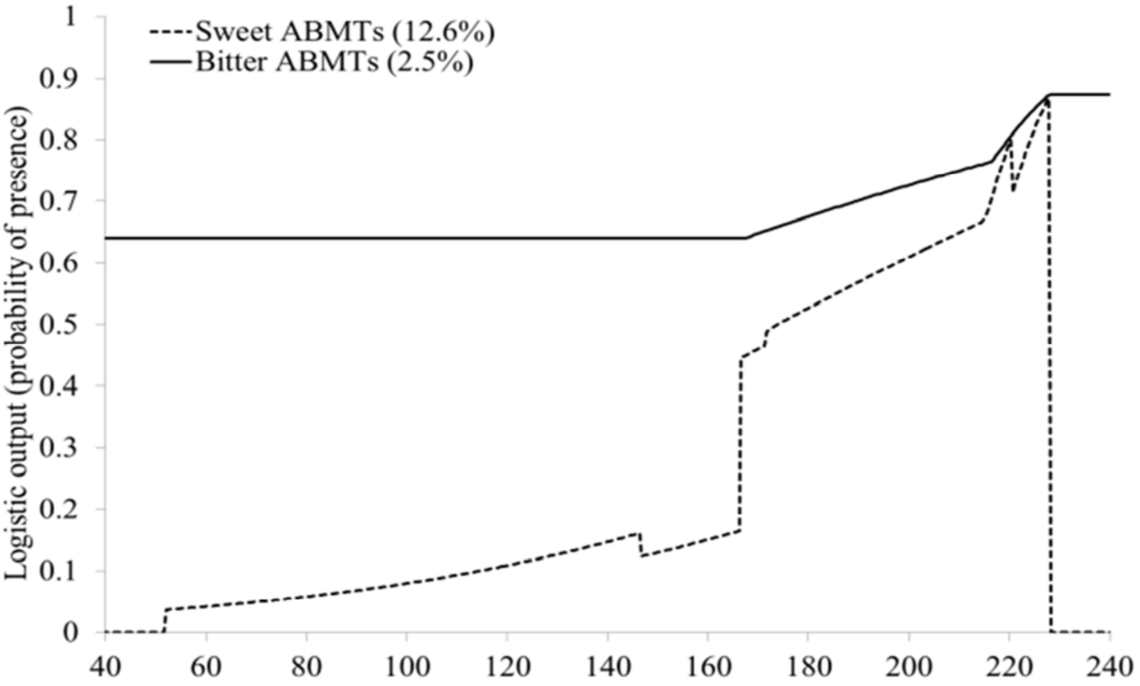

Figure 2.2c: Influence of minimum temperature of coldest month (0.1 degrees C)

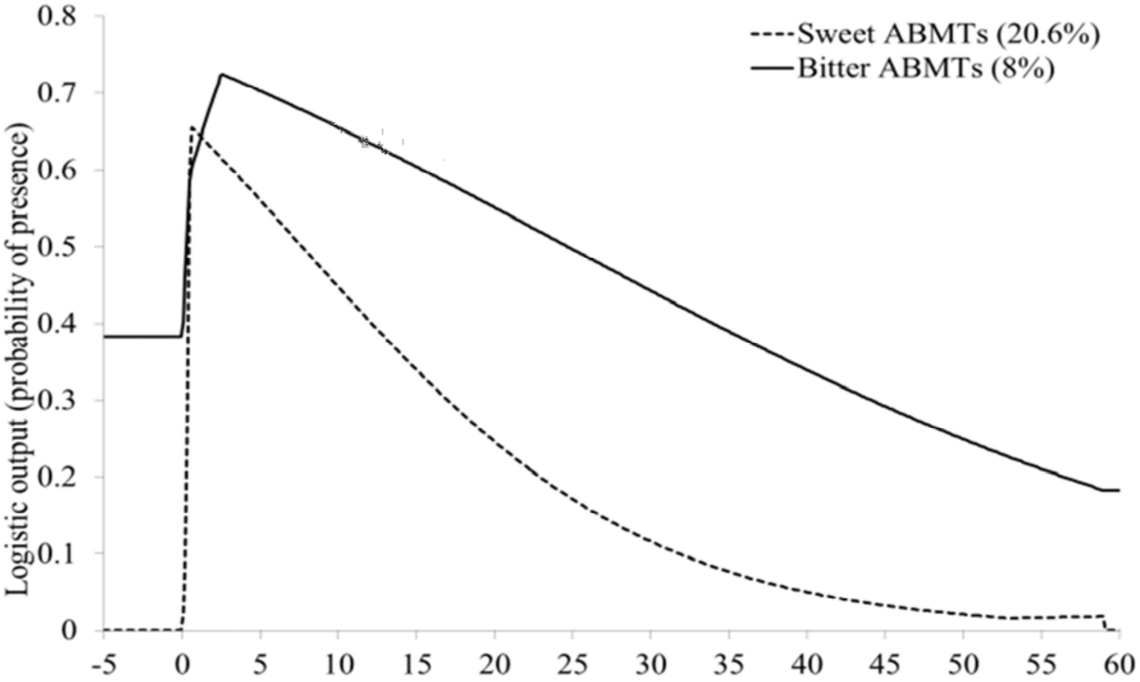

Figure 2.2d: Influence of topsoil gravel content (% volume)

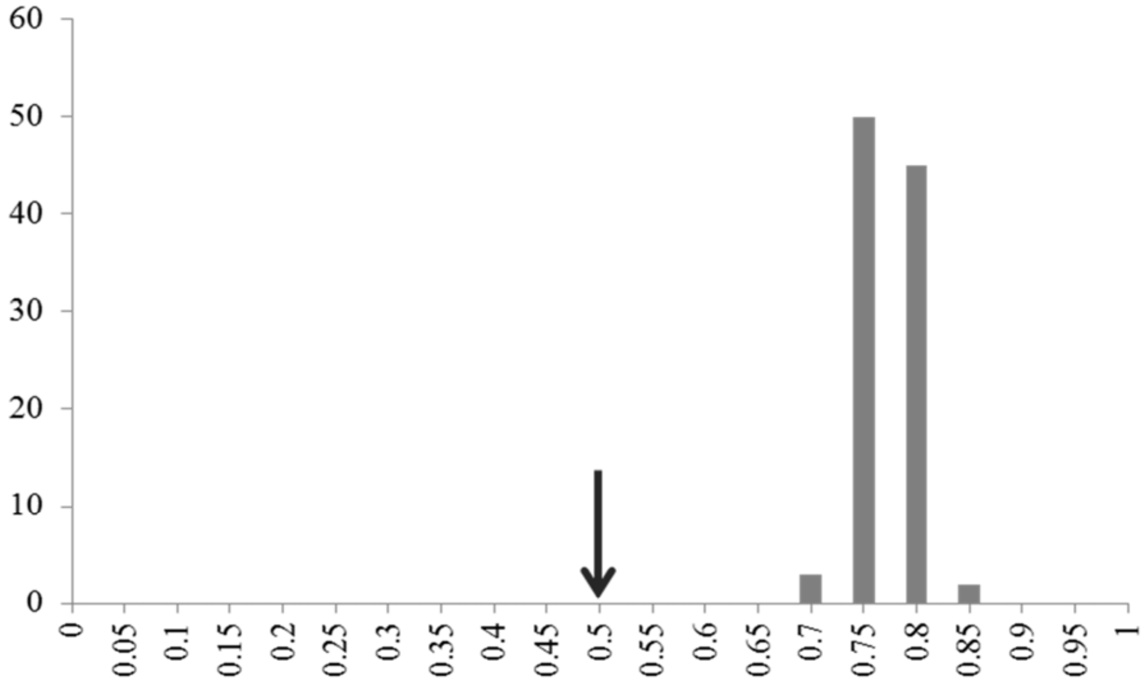

Figure 2.2e: Niche identity tests using Schoener's D: The similarity score of the models for the wild bitter and sweet trees is lower than expected by chance (arrow).

Differences between ABMT populations in the Dahomey Gap and wild populations

Within the Dahomey Gap, bitter trees occur only in the Volta forest region in Togo where they occur on farms, in forest gardens and in protected natural forests (Vihotogbé *et al.*, submitted-1). In this hilly area, they occur mostly in swampy places and along streams, but occasionally also on hill slopes. In contrast, sweet trees occur widespread in the Dahomey Gap from southern Togo to southern and central Benin. Sweet trees are found on farms and in home gardens, on plateau's, hill slopes as well as on hill summits and their distribution seems to follow that of the human settlements and agricultural productive areas. They are not found in flooded areas nor along streams. In the Volta forest region, bitter and sweet trees occasionally co-occur.

The potential distribution of the sweet tree populations in the Dahomey Gap is almost restricted to the Dahomey Gap (AUC = 0.987; Fig. 2.3a). The most important environmental factors contributing to this niche model are different from those of the wild sweet trees (table 2). For the Dahomey Gap sweet trees, minimum temperature of the coldest month is the most important factor (48.6%), while it was the third most important one in the niche model of the wild sweet trees (only 12.6%). However, both wild sweet trees and Dahomey Gap population models have a similar response with increasing probability of occurrence with increasing temperatures in the coldest month (Fig. 2.2c and 2.4a). Annual precipitation had the highest contribution to the distribution wild sweet trees (50%) while this factor only accounts for 15% in the potential distribution of the Dahomey Gap sweet trees. Finally, isothermality contributes to the potential distribution of the Dahomey Gap sweet trees (12%), while its contribution to that of wild sweet trees was very low (4%). In the Dahomey Gap, the occurrence of sweet trees is driven by monthly temperatures that change according to seasons in this region.

Little niche overlap (Schoener’s *D* statistic = 0.13) was detected between the wild and Dahomey Gap sweet trees. The niche identity test indicated that this overlap was significantly less than expected based on the randomized draws from all pooled sweet tree occurrences (Fig. 2.4b). This implies that Dahomey Gap sweet trees are ecologically different from wild sweet trees.

The potential distribution of the Dahomey Gap bitter trees is restricted to the Volta forest region in south-western Togo (AUC = 0.998; Fig 3b). This distribution shows a high contribution of the same most important environmental factor controlling that of wild bitter trees: precipitation of the coldest quarter (26%, table 2). The response to this factor has the same optimum as well (500 mm: Fig. 2.1b & 2.4c). The occurrence of Dahomey Gap bitter trees is also associated with topsoil gravel content (25.5%) while this factor has low influence on the occurrence for the wild bitter trees collection (8%). Subsequently, topsoil silt fraction (19%) and isothermality (14.5%) are also influential factors for the Dahomey Gap bitter trees niche model (table 2).

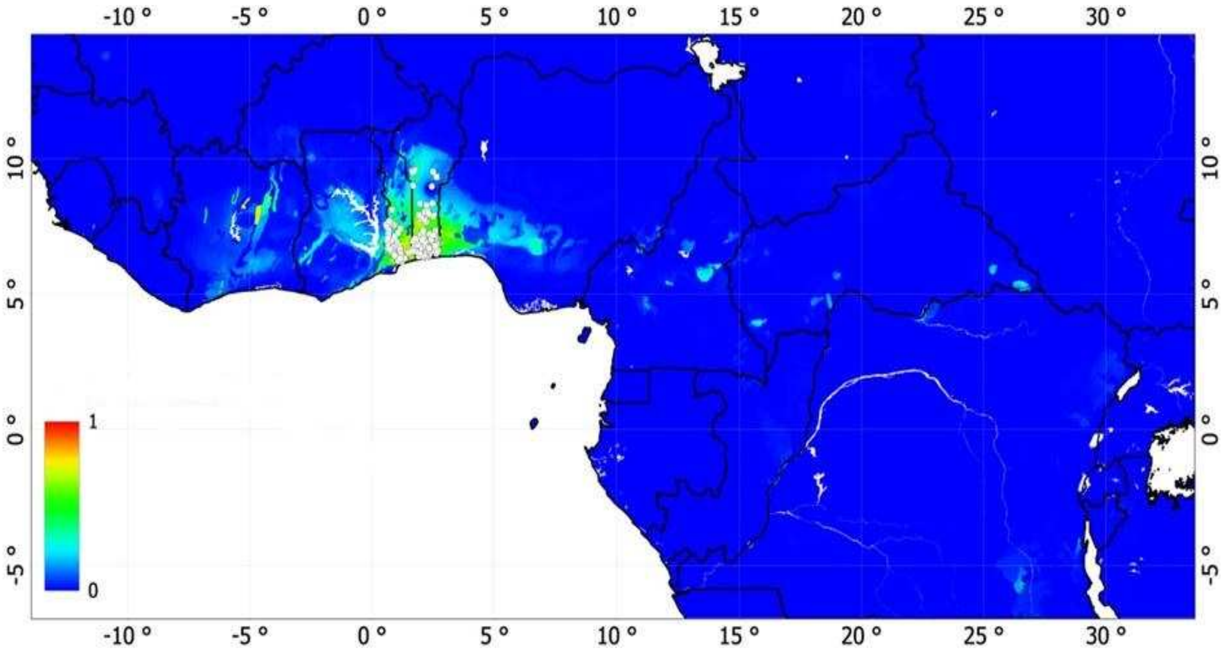

Figure 2.3a: Potential distribution of Dahomey Gap sweet ABMTs.

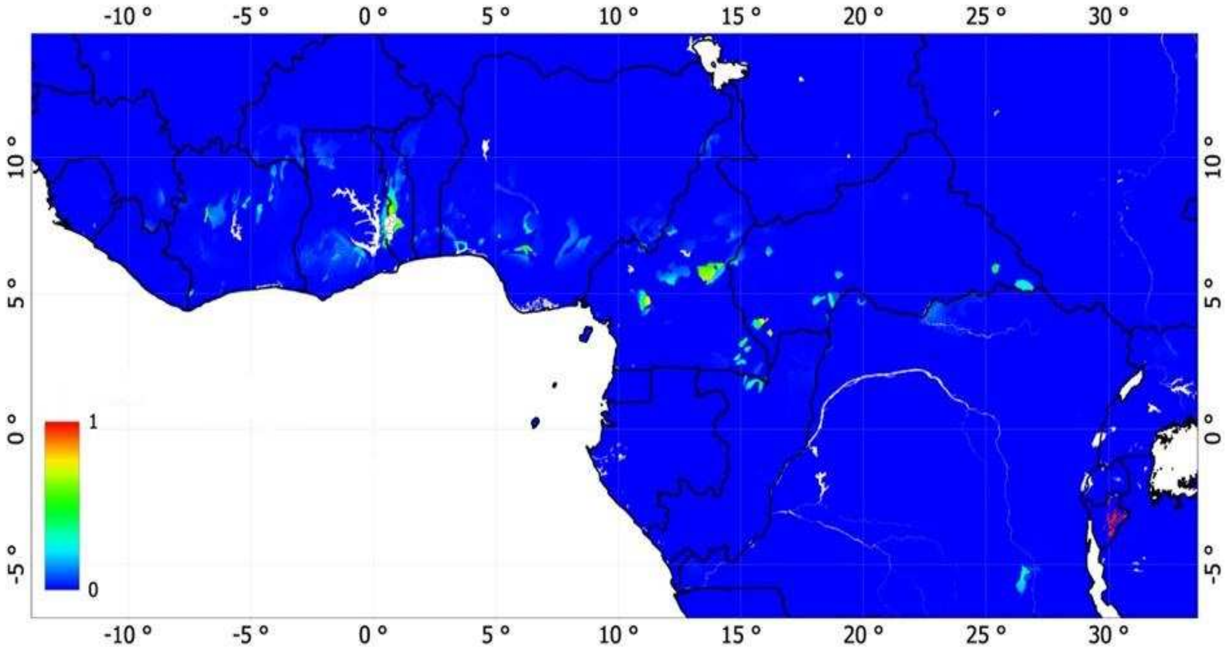

Figure 2.3b: Potential distribution of Dahomey Gap bitter ABMTs.

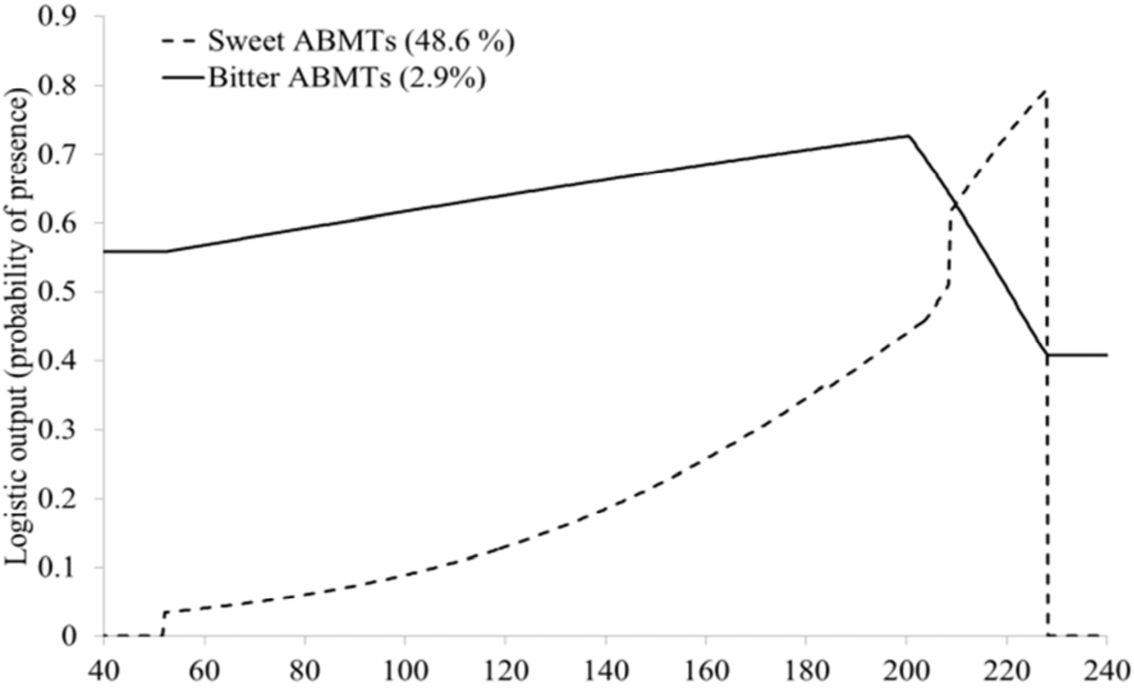

Figure 2.4a: Influence of minimum temperature of the coldest month to the distribution of Dahomey Gap SBMT and Dahomey Gap BBMT populations

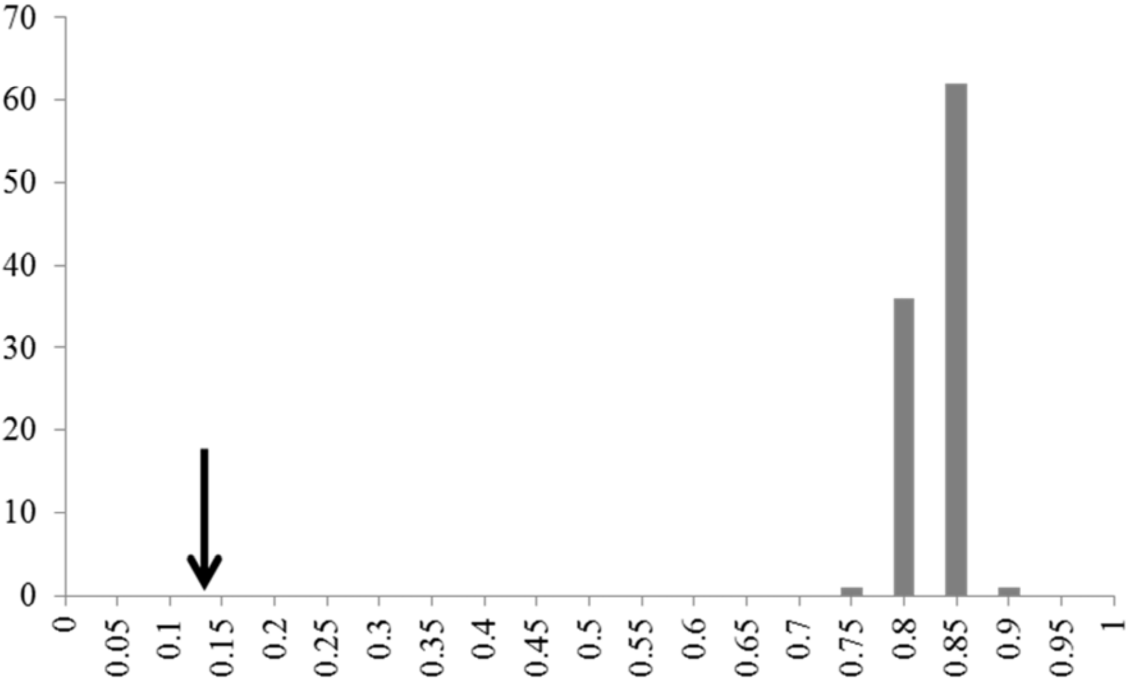

Figure 2.4b: Niche identity tests using Schoener’s D: The similarity score of the models for the wild and Dahomey Gap SBMTs is lower than expected

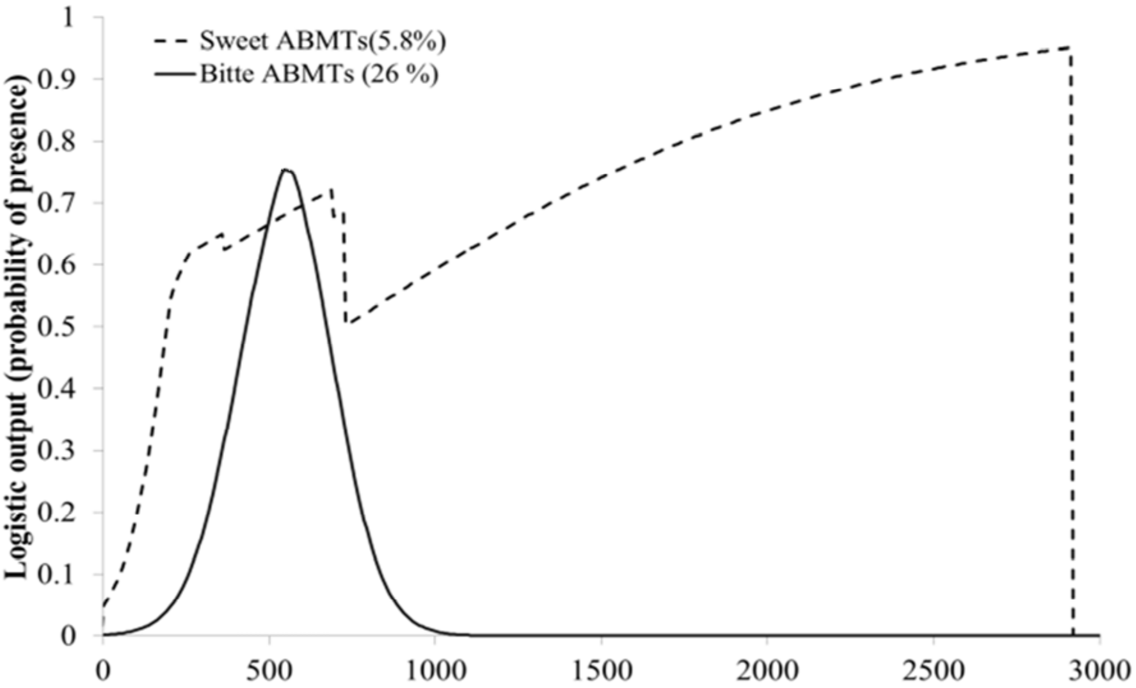

Figure 2.4c: Influence of annual precipitation of the coldest quarter on the distribution in Tropical Africa of Dahomey Gap BBMTs collection

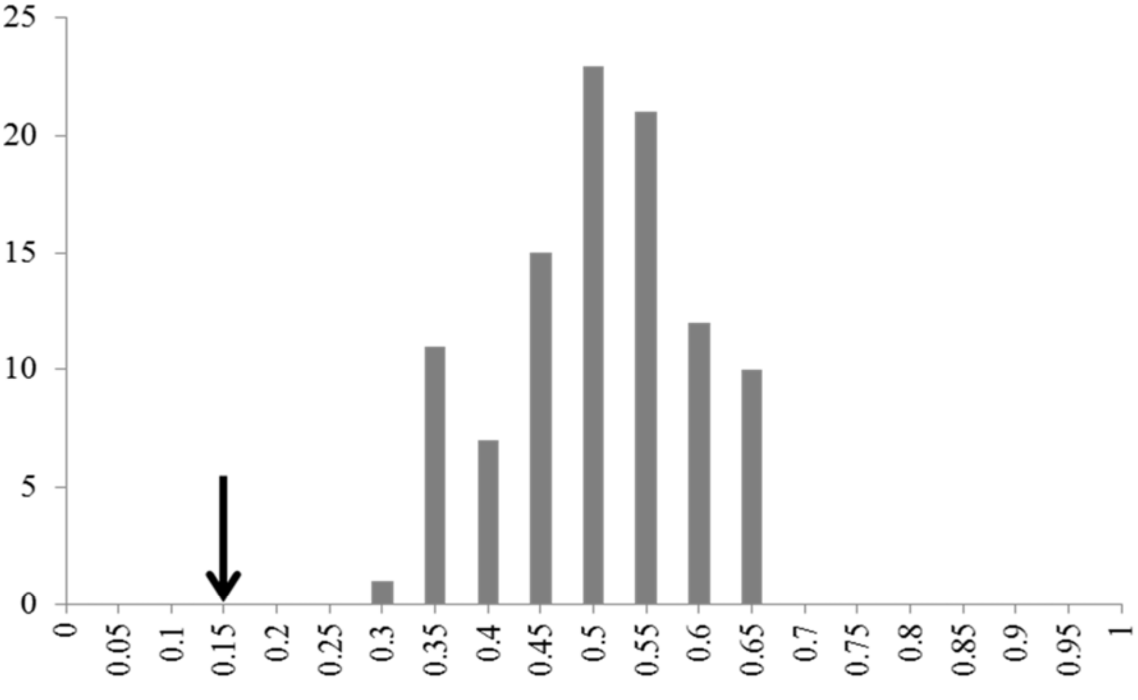

Figure 2.4d: Niche identity tests using Schoener's D: The similarity score of the models for the wild and Dahomey Gap BBMTs is lower than expected

The Schoener's *D* statistic indicates that there is only a slight niche overlap between the bitter trees in the Dahomey Gap and those in the wild throughout tropical Africa (0.15). The niche identity test indicates that this overlap is significantly less than expected by chance (Fig.

2.4d). The difference between observed and expected is much lower though than for the sweet trees. Therefore, the Volta forest region, in the Dahomey Gap, is an ecological area different from that of the wild bitter trees collection. This implies that Dahomey Gap bitter trees are ecologically different from wild bitter trees.

The ecological factors that govern potential distributions of bitter and sweet trees in the Dahomey Gap are different (Table 2.2). Where precipitation in the coldest quarter has a high influence on the occurrence of bitter trees (26%), it accounts for only 6% of the explanation of the potential distribution of sweet trees (Fig. 2.4c). Furthermore, the minimum temperature of the coldest month, detected as the most important factor influencing the distribution of sweet trees (48.6%), accounts for only 3% of the explanation of the potential distribution of bitter trees (Fig. 2.4a).

Finally, the potential cultivatable area for sweet trees in the Dahomey Gap is obtained, and indicates suitable habitat mainly in the southern part of the Dahomey Gap (Fig. 2.5; 6° to 8.5° latitude). Despite the occurrence of sweet trees in the central as well as the northern regions of Benin, habitat suitability for cultivation of sweet trees seems to be much lower in those regions (Fig. 2.5).

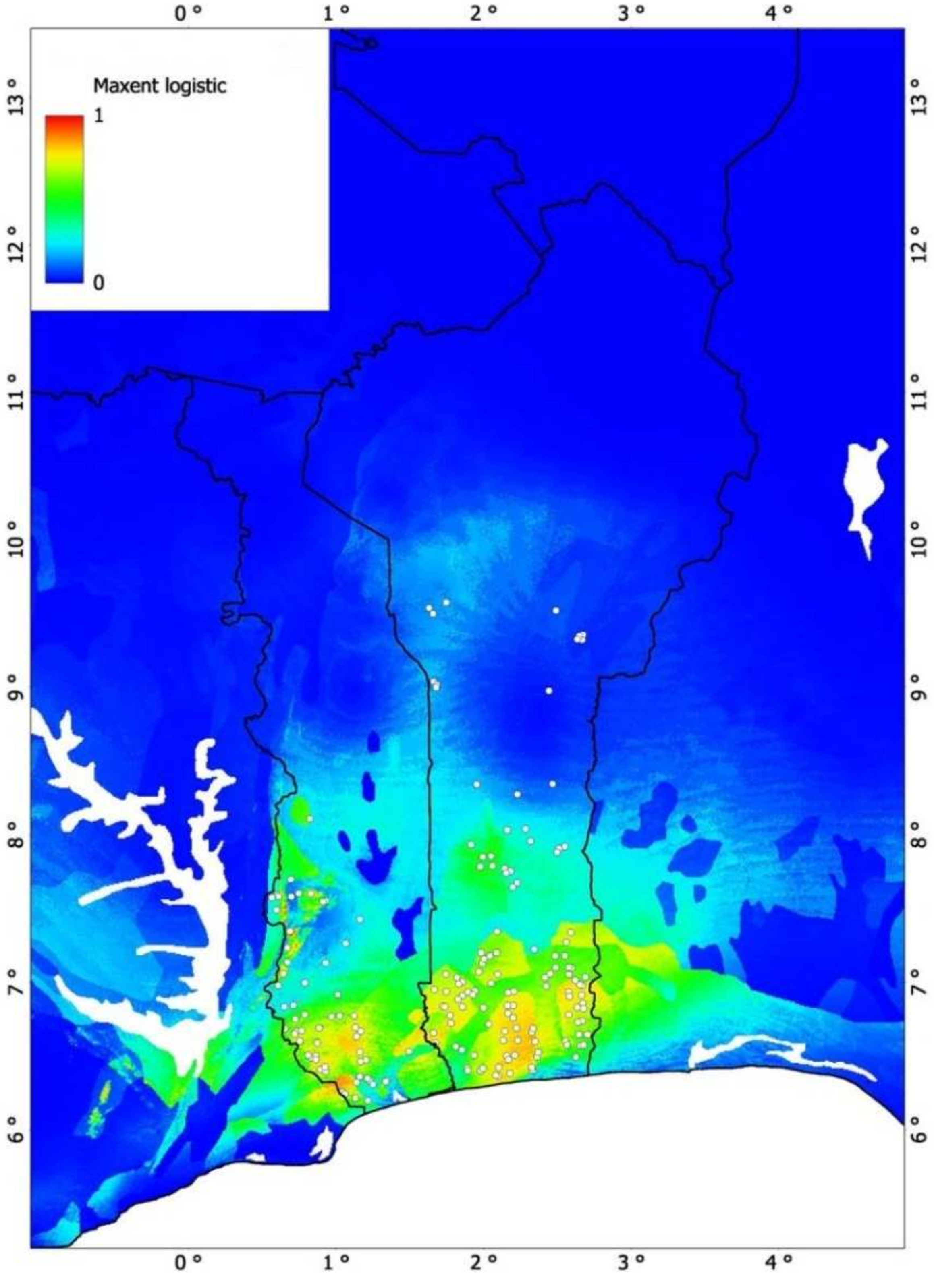

Figure 2.5: Potential cultivatable area of sweet trees in the Dahomey Gap: white dots are samples used in this study

Discussion

Ecological differences between wild bitter and sweet trees

The difficulty to distinguish between bitter and sweet trees based on morphological characters was the key argument of Okafor (1975) to opt for the taxonomic level of variety instead of species. In general, ABMTs occur in the wild on both sides of the Dahomey Gap from Senegal in the west to Sudan and Uganda in the east and Angola (Cabinda) in the south (Harris, 1996; Lesley and Brown, 2004). Our study demonstrates that within the distribution range, bitter and sweet trees populate significantly different ecological environments (Fig. 2.2e). The potential distribution of sweet trees is restricted to the wetter parts of the Guineo-Congolian phytochorion. This is consistent with findings of Kang *et al.* (1994), which indicated better growth and fruiting performances in Port Harcourt (south-eastern Nigeria, 2400 mm rainfall) than in Ibadan (south-western Nigeria, 1300 mm rainfall). In contrast, the extension of bitter trees to dryer climatic conditions (see Ndoye *et al.,* 1997) was also confirmed by our models (Fig. 2.1b) with optimum habitat suitability at relative low levels of precipitation in the coldest quarter. This is consistent with higher fruiting for bitter trees compared to sweet trees in the drier south-western Nigeria (Ibadan; Kang *et al.,* 1994). As such, the bitter trees display a higher ecological variability than the sweet trees (Fig. 2.1a & 2.1b). Therefore, soil total gravel content, determining soil structure and consequently its water holding capacity (Norman and Gary, 1990), has no limiting effect on the distribution range of bitter trees. The above can be regarded as support for the hypothesis that bitter and sweet trees should be viewed as taxonomically different species, *I. wombolu* and *I. gabonensis*, respectively (Harris, 1996). Our results suggest that such differences have a genetic basis and hence that the two taxa maintain their genetic identity and do not interbreed. The existence of a genetic difference is corroborated by the study of Lowe *et al.* (2000). Significant differences in phenology of bitter and sweet trees are also found by Vihotogbé *et al.* (submitted-2), further supporting the species level differentiation.

Occurrence and origin of ABMTs in the Dahomey Gap

This study shows that the Volta forest region, the wettest region in the Dahomey Gap, is an ecological area different from the ecological niche of wild bitter trees. In the Volta forest region, bitter trees are known as “the never cultivated ABMTs” or “wild only ABMTs”, while in the rest of the Dahomey Gap, local communities are not familiar to this taxon (Vihotogbé *et al.,* Submitted-1). Local communities in the Volta forest region confirmed that sweet trees have been introduced and are cultivated primarily for their sweet mesocarp, but the seed of any ABMTs has no place in their diet. Therefore, apart from a general use as timber, bitter trees have no direct value for these local communities: the mesocarp is inedible, and seed-based diets are still not admitted in the Volta region and even not totally accepted elsewhere in Togo. Also, local knowledge about social and medicinal use of ABMTs is very poor (Vihotogbé *et al.,* 2008). Bitter trees are sampled in the Volta forest region in natural but highly disturbed forest contiguous to farmlands and human settlements, and most importantly in primary agroforestry systems (forest gardens) and on farms. We postulate here that bitter ABMTs are native to the Volta forest region and are passing from a wild status within natural forest to a situation with scattered individuals preserved in agricultural productive spaces. Population viability might be threatened by the limited extent of this ecological region. When a tree species has no important socio-economic value, and in fact its wood presents the highest economic advantages for land owners, its distribution will potentially be reduced over time (Vihotogbé *et al.*, submitted-1). This is the case for bitter trees in the Volta forest region, since the expanding cultivation of coffee, cacao and bananas is strongly reducing the regeneration (Sodhi, 2007; Posner, 2008). The fact that the ecological niche models based on Dahomey Gap collections and those from elsewhere in Africa are less similar than expected by chance (Fig. 2.4d) might be explained by model over-fitting as a result of over-representation of collections in the Dahomey Gap related to collection efforts for this study.

Lowe *et al.*, (2000) postulated the presence of genetic diversity centres of bitter trees in southern Nigeria and south-eastern Cameroon. These coincide with our predicted highest occurrence probabilities (Fig. 2.1b). Additionally, our results also suggest high occurrence probabilities in the Upper Guinean forest (Harris, 1996). The Dahomey Gap isolates the Upper Guinean from the Lower Guinean forest block, and thus prevents the natural exchange of genetic material (Giresse, 2007). Therefore, the Lowe *et al.*, (2000) study should preferably be extended to include material from Upper Guinea to validate their conclusions for the entire distribution range of bitter trees.

The Dahomey Gap is an ecological zone where sweet trees occur, but outside their natural ecological niche (Fig. 2.4b). Even in forest relicts preserved throughout the Dahomey Gap, including the comparatively wetter Volta forest, no evidently wild sweet trees were recorded. Ethnobotanical data suggest the introduction of sweet trees in the Dahomey Gap from the Lower Guinean forest block through migration of the Yoruba people and their subsequent settlement in south-eastern Benin. From here, material spread to other parts of Benin and Togo (Vihotogbé *et al.*, submitted-1). This is consistent with the conclusion of Ude *et al.* (2006) who suggest that sweet trees dispersed from the Lower Guinean forests to drier ecological areas in the Soudania Transition zone. Therefore, we postulate that the occurrence of sweet trees in the Dahomey Gap is solely due to cultivation activities (Harris, 1996; Tchoundjeu and Atangana, 2007).

With respect to the presence and cultivation of sweet trees in the Dahomey Gap, a key question is: where does the genetic material comes from? In other words, is it true that sweet trees migrated from the Lower Guinean forest block? Firstly, old individual trees, particularly valuable food trees, are claimed to be planted by local communities in West Africa to prove land ownership (Neef, 2001). Secondly, in Benin, local communities in ancient forested areas consider old sweet trees as relicts of what has existed in the wild in the past before oil palm plantations were introduced. This latter argument is strengthened by an ancient taboo forbidding the cultivation of bush mango trees in Benin (Vihotogbé *et al.*, submitted-1), contradicting former widespread planting initiatives. Sayer (1992) postulated that in the Dahomey Gap the expected natural vegetation under the current climate would consist of semi-deciduous forest interspersed with tracts of denser vegetation types such as riverine forest as well as patches of swamp forest and lowland evergreen forest (preferred habitats of ABMTs; Lesley and Brown, 2004). Nagel *et al.* (2004) and Tossou *et al.* (2008) claimed that this implies that the present savannah vegetation was induced by humans. Salzmann and Hoelzmann (2005) show that before ca. 4500 yr BP the Dahomey Gap was covered with evergreen lowland rainforest containing typical Guineo-Congolian forest trees species. This past climatic phase plays an important role in explaining present-day tree species distributions (Linder *et al.*, 2005; Waltari *et al.*, 2007). Since the ecological niches obtained using the Maxent algorithm express the shared *current* environmental conditions between samples, they lack the possibility of tracing the influence of historical elements on the present-day distribution (Parmentier *et al.*, 2011). Therefore, hindcasting the potential distribution with past climatic data could be particularly useful in addressing this question to indicate habitat suitability in the past in order to infer the true origin of sweet trees being managed in the Dahomey Gap. Moreover, an effort to collect genetic material covering the entire actual distribution range is recommended to improve the conservation strategy for ABMTs.

Potential cultivatable area of sweet trees in the Dahomey Gap

Vodouhè (2003) demonstrated that the mesocarp quality of sweet trees plays an important role in the distribution in Benin. Moreover, in Togo, where sweet trees are also intensively cultivated, the seed-based diets are still considered to be foreign diets from Nigerian and Beninese people (Vihotogbé *et al.,* submitted-1). Therefore, especially the sweet mesocarp consumption has played a key role in the spread of cultivation initiatives. This led to an expansion of the cultivated area of sweet trees to new ecological zones that differ significantly from their natural ecological niche. The “easy to grow” nature of sweet trees is well illustrated by the non-significance of major soil fertility parameters (textural, organic carbon content, drainage, and water storage capacity) in the definition of the ecological niche. Trees are grown on a variety of soil types and the low rainfall in the Dahomey Gap appears to be sufficient to maintain an economically profitable yield (see Chinaka and Obiefuna, 1999).

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Chapter 3

Backgrounds of the domestication process of African bush mango trees (*Irvingiaceae*) in the Dahomey Gap (West Africa)

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Abstract

Bitter and sweet African bush mango trees (ABMTs) belong to the family Irvingiaceae and are valuable non-timber forest products in sub-Saharan Africa. They have not been studied well in the western part of their distribution range, and many aspects of their large-scale development remain unknown. In this study, we link the agroforestry status of ABMTs to differences in socio-cultural groups in order to identify the key factors influencing their abundance and survival in the Dahomey Gap.

First, we gathered the uses and local management strategies from the nine main socio-cultural areas in Benin and Togo. Second, occurrence data were obtained throughout the Dahomey Gap and imported into DIVA-GIS and MATLAB to calculate the spatial density pattern and analyse its structure. The variation of this pattern was analysed relative to three factors: the country, the phytogeographical zone and the dominant FAO soil category. Third, agroforestry system characteristics and farmers' social status relative to 841 trees were used in a multinomial logistic regression to identify anthropogenic factors driving the intensive cultivation of ABMTs. Finally, the impact of socio-cultural activities on extent and density of ABMT populations was analysed.

In the entire study zone, the sweet mesocarp is consumed and the valued seed of bush mangoes is commercialized. The application of seed-based diets and socio-therapeutic uses are common to communities in Benin. Sweet ABMTs are generally found either in home gardens or cultivation fields where they may occur at high densities (up to 1020 trees per 25 ha). Bitter trees, however, are confined to the Volta forest region in Togo and occur at low densities (< 462 trees per 25 ha) in the wild, sometimes in protected areas, in forest gardens as well as on fields. This indicates a clear difference in cultivation methods between bitter and sweet trees. Farmland status, farmer socio-cultural group and type of ABMTs determined the cultivation intensity. The fact that small farmlands are converted into sweet ABMT orchards indicates that farmers actively cultivate ABMTs in the Dahomey Gap. Diversity of indigenous knowledge, however, is not correlated to intensive cultivation nor to domestication efforts or local genetic conservation programs. Where slash and burn agriculture and intensive collection of fruits jeopardize bitter trees, traditional fishing systems (using twigs), a traditional selection strategy, and intensive land commercialization severely threaten sweet ABMT genetic resources.

Key words: Benin, conservation, ethnobotany, *Irvingia*, kriging, spatial distribution, Togo

Introduction

Non Timber Forest Products (NTFPs) are increasingly being studied due to their growing importance in enhancing livelihood. They play a key role in sustainable ecosystem management design (MMF and UNBC 2005; Delang, 2006) and are shaping land use systems in terms of their specific composition and spatial configuration. Millions of people have traditional knowledge on NTFPs and use them on a daily basis (Boateng *et al.*, 2007). The increasing demand for NTFPs is reflected in a growing commercial trade (Arnold and Ruiz Pérez, 2001) which is becoming an important economic incentive for local collectors. Consequently, natural ecosystems may well suffer from over-exploitation of their plant genetic resources (McLain and Jones, 2005), especially in areas where people are economically dependent on NTFPs (Ticktin, 2004). In the context of the rapid growth of the human population and a NTFP production limited by the natural ecosystem capacity, a sustainable harvest strategy reconciling plant genetic resources conservation and local community livelihood seems a utopia (Rai and Uhl, 2004). The development of traditional agroforestry systems, from forest gardens to orchards, indicates that local communities understand the need to carefully select useful plant species for cultivation and domestication (Wiersum, 2004). This partially decreases the dependence of communities on natural ecosystems although they still depend on natural forests for those NTFPs that cannot easily be cultivated such as primary rainforest species, wildlife, etc.

African bush mango trees (ABMTs: *Irvingia* spp.) belong to the small family of Irvingiaceae (Harris, 1996), of which seven species occur in West and Central Africa. Their fruits are economically one of the most important NTFPs in sub-Saharan Africa (Asaah *et al.*, 2003; Leakey *et al.*, 2003). They are among the priority food tree species abundant in traditional agroforestry systems and over the last decade have been subject to domestication trials by the World Agroforestry Centre (Franzel *et al.*, 1996). The mesocarp of the mango-like fruits is either bitter or sweet (Harris, 1996). Only the sweet mesocarp is edible, while the seeds (enclosed in the hard endocarp) of both bitter and sweet fruits are used as a sauce thickening agent (Leakey *et al.*, 2005, Tchoundjeu and Atangana, 2007). Bitter and sweet trees are taxonomically closely related and there is some debate about the correct level at which their differences should be valued (National Research Council, 2006). While Okafor (1975) presented the two types as varieties of the species *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill.: *I. gabonensis* var. *gabonensis* and *I. gabonensis* var. *excelsa*

(Mildbr.) Okafor, respectively), Haris (1996) argues they are best treated as different species (*I. gabonensis* and *I. wombolu* Vermoesen, respectively).

Because of the taxonomic uncertainties, it is difficult to assign many of the known uses to either the sweet or the bitter taxon (Oyen, 2007; Tchoundjeu and Atangana, 2007). In Central Africa, Lesley and Brown (2004) identified more than fifteen different indigenous uses related to ABMTs, but failed to distinguish between the therapeutic and biochemical properties of sweet and bitter trees.

Initially marketed for consumption by African migrants throughout the world (Tabuna, 2000), the ABMT fatty seed is nowadays increasingly becoming an important raw material for a growing number of food processing and cosmetic industries (Akubor, 1996; Oyen, 2007). Many CGIAR-funded organizations, among which the World Agroforestry Centre and Bioversity International, identified ABMTs as a priority food tree species for West and Central Africa. At the individual tree level, Vodouhê (2003) and Vihotogbé *et al.* (2008) studied the local economical profitability and the indigenous factors driving the eligibility of trees for conservation purposes in the Dahomey Gap, respectively. Throughout their entire distribution range, however, intensive cultivation systems of ABMTs remain rare (see Lowe *et al.*, 2006) and the basic drivers of and strategies for their domestication and intensive cultivation unstudied.

Traditional knowledge about food cultivation is often shared among a socio-cultural group as an integral part of their social heritage and natural resources management (Reyes-Garcia, 2001, De Caluwé *et al.*, 2009). Thus, assessing the relation between the main uses by a socio-cultural group and the abundance of ABMTs is important in order to evaluate their future potential as a crop for sub-Saharan Africa (Leakey *et al.*, 2005). Moreover, abundance, size-class distribution and threats are important structural parameters for the management of trees in traditional agroforestry systems (Gouwakinnou *et al.*, 2009) and as such they help detect local management strategies.

This study will focus on ABMT population structure in the Dahomey Gap (Benin and Togo). We want to quantify how traditional knowledge on AMBT usage and social status of farmers affect the allocation of productive space to bitter and sweet ABMTs. We aim at providing answers to three research questions:

(i) What is the spatial pattern of ABMT abundance in the Dahomey Gap?

(ii) Which anthropogenic factors are influencing this spatial pattern and hence the conservation of ABMT genetic resources in the Dahomey Gap?

(iii) Is there a difference in traditional knowledge on ABMT usage between the different socio-cultural areas in the Dahomey Gap, and how does this affect the local management strategies for ABMTs?

Material and Methods

Study area

The Dahomey Gap is the dry corridor dividing the West African rain forest block into the Upper and Lower Guinean phytochoria (Salzmann and Hoelzmann, 2005; White, 1979). This climatically dry corridor where savannah reaches the coast extends from Accra (southern Ghana) to Badagry in south-eastern Nigeria (Maley, 1996; Sowunmi, 2007). This study was carried out in Benin and Togo, representing the majority of the extent of this eco-region where small patches of natural forest and sacred forests are scattered in a wide grass or shrub savannah area. Large parts of this region have been transformed to cultivated fields (Kokou and Sokpon, 2006). The Dahomey Gap is located in a sub-humid Guinean climatic zone with a bimodal rainy season (April to June and September to November), mean annual rainfall of 900-1,200 mm and mean temperature of 25-29°C. This study covers all nine major socio-cultural areas: Aizo, Adja, Akposso, Ewe, Fon, Goun, Nago, Holli and Watchi (Heldmann, 2008; Atato *et al.,* 2010; Fig. 3.1).

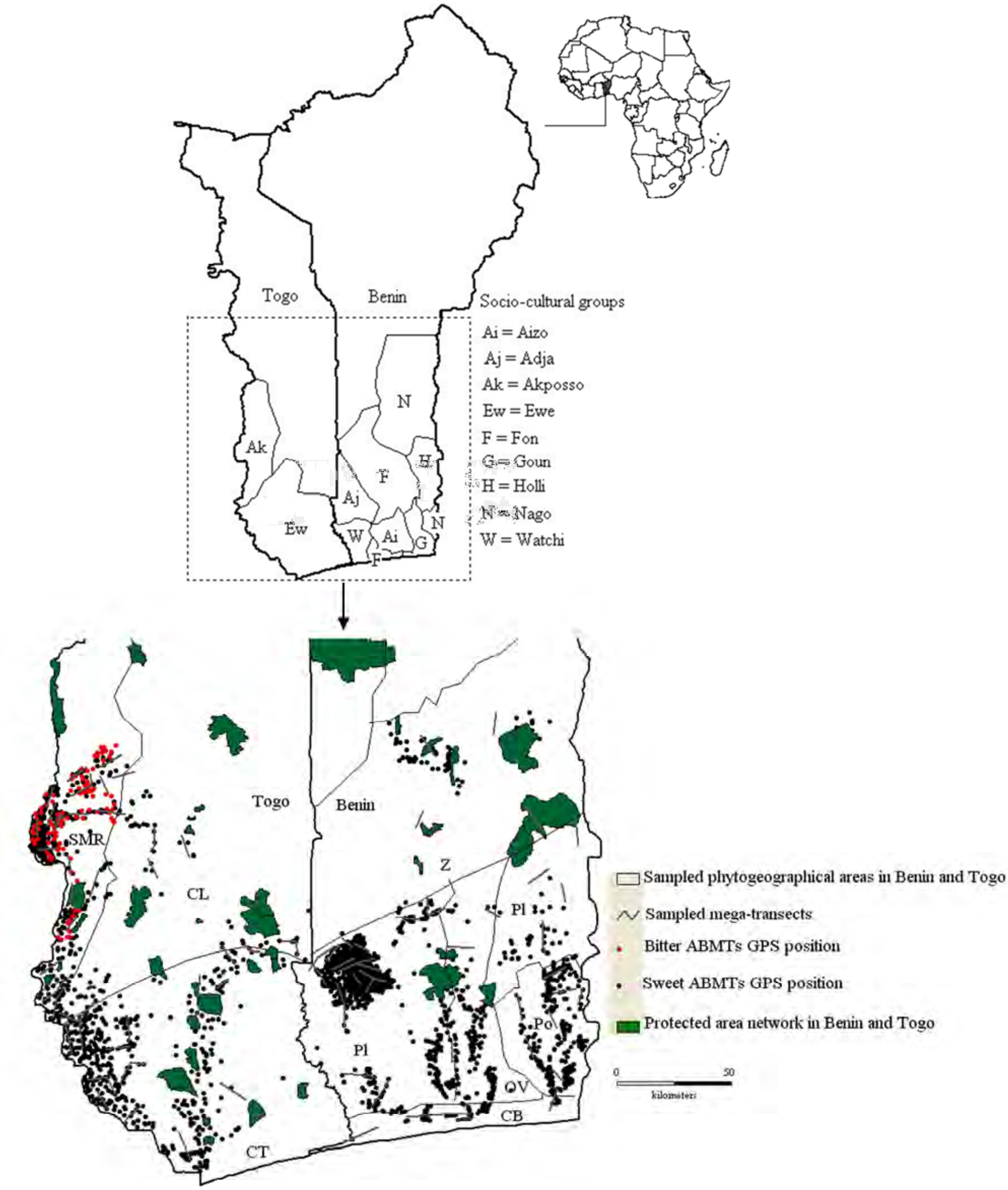

Figure 3.1: Sampled socio-cultural areas, mega-transect ABMTs records in different phytogeographical regions in Benin and Togo: Phytogeographical regions: Z = Zou; Pl = Plateau; OV = Oueme-Valley; PO = Pobè; CB = Coast-Benin; CL = Centre Lowlands; SMR = Volta zone = Southern Mountain Region; CT = Coast-Togo

Table 3.1: Sampled socio-cultural areas, main uses and anthropogenic activities impacting on ABMT populations.

Type of ABMTs	Geographic areas (see figure 1)	Socio-cultural groups	Profile of individuals	Most important economic product	Major socio-economic activities	Domestication process
Sweet trees	South West Benin	Adja	T_{35}:$O_{26}P_{9}$	Seed	Subsistence agriculture, Palm oil tree and food tree species	Only cultivated trees under a missal selection process importantly based on endocarp size regardless fruit's parasitism and the firmness and sweetness of the mesocarp.
	South Benin	Aizo	T_{32}:$O_{22}P_{10}$	Entire fruit (based on the mesocarp quality) and less importantly the seed	Subsistence agriculture plus most importantly traditional fishing using ABMTs branches for the fishing system construction	Preserved spontaneous trees obtained from human-thrown seeds. No action or interest for traditional selection.
	South Benin	Fon	T_{31}:$O_{21}P_{10}$			
	Lower South East Benin	Goun	T_{29}:$O_{22}P_{7}$			
	Upper South East Benin	Holli	T_{37}:$O_{19}P_{18}$	Entire fruit (based on the mesocarp quality) and less importantly the seed	Subsistence agriculture and palm oil trees	Unclear domestication status (pretended wild + spontaneous + planted). Trees are preserved importantly based on fruits parasitism and the firmness and sweetness of the mesocarp regardless mesocarp size.
	Upper South East Benin	Nagot	T_{22}:$O_{18}P_{4}$			
	Lower South West Benin	Watchi	T_{25}:O13P_{12}	None	Traditional fishing using ABMTs branches for the fishing system construction	Unclear domestication status
	Upper South West Togo	Akposso	T_{43}:$O_{29}P_{14}$	Seed	Cash crops (cocoa, coffee, banana, avocado), food tree species and subsistence agriculture	Unclear domestication status
	South Togo	Ewe	T_{40}:$O_{29}P_{11}$	Seed	Subsistence agriculture and	Only cultivated trees without

Type of ABMTs	Geographic areas (see figure 1)	Socio-cultural groups	Profile of individuals	Most important economic product	Major socio-economic activities	Domestication process
					food tree species	selection process
Bitter trees	Upper South West Togo*	Akposso		Seed, wood	Cash crops (cocoa, coffee, banana, avocado), food tree species and subsistence agriculture	Only wild trees preserved in traditional agroforestry systems
	Upper South West Togo	Akposso		Seed, wood	Protected areas	Only wild trees still far from cultivation initiative taking exploited by local communities and wood industries.

T = total surveyed, P = number of professional users (traditional practitioners, traditional religion priests), O= number of ordinary people.
Subscript numbers refer to the total number of individuals surveyed in each group

Sampling design and data collecting

The ethnobotanical database gathered in 2007 by Vihotogbé *et al.* (2008) in six major socio-cultural areas in Benin (Aizo, Adja, Fon, Goun, Nago, Holli) was supplemented with an ethnobotanical survey carried out in the three other major socio-cultural areas in the Dahomey Gap: Watchi in Benin and Ewe and Akposso in Togo. Moreover, since the 2007 database included relatively few professional users of biodiversity (i.e. traditional healers, religious specialists or vendors of medicinal plants), additional surveys were performed to specifically target such individuals in the six socio-cultural areas. In the three new areas, we aimed for a good mix of professional and non-professional users from the start.

Using a structured questionnaire, every individual was asked about his/her knowledge on different uses of any part of ABMTs (roots, stem, bark, leaves,) as well as his/her acceptance of the consumption of sweet bush mango and that of seed-based diets. Together with the 2007 database, data from a total of 294 respondents were analysed to apprehend the traditional knowledge and the current importance of ABMTs in the Dahomey Gap. Additionally, using direct on-farm observations and open survey techniques, data on the main traditional management systems were recorded.

Given the large size of the geographical area involved, a field research unit area was designed as a modified mega-transect, a technique used by Assogbadjo (2006) to quantify the occurrence of Baobab trees *(Adansonia digitata* L., Bombacaceae). Each mega-transect comprises a 10 km long line, along which observations were made in a zone of 250 m both left and right and hence covers a surface of 5 km^2. In total, 59 such mega-transects were sampled, three to seven in each socio-cultural area (Fig. 3.1).

When scattered at low density in the landscape, all individual adult and young bitter and sweet trees in a mega-transect were geo-referenced. In case an area with a high density of ABMTs was encountered, their number was estimated as follows. The trees bordering such high density areas were all accurately geo-referenced supplemented with all trees within two randomly defined 100 x 50 m sub-plots. Then, the total number of ABMTs present in the high density area was estimated from these data. Although not exhaustively, trees encountered between sampled mega-transects were also recorded and areas of ABMTs absence along and between mega-transects were marked as zero density points.

The influence of farmers on the spatial distribution pattern and conservation of genetic resources was assessed by analysing data from 841 adult trees from different agroforestry systems and corresponding to 841 farmers met in the field. For all 841 trees / farmers, the following ten parameters were recorded: (i) taste of mesocarp, (ii) nationality of farmer, (iii) socio-cultural group of farmer, (iv) farmer's respect of taboos regarding ABMT cultivation, (v) reason why the farmer cultivated or preserved ABMTs, (vi) local marketing system, (vii) local land use system, (viii) local land tenure combined with local judgment of farmland size, (xi) agroforestry system applied, and (x) the farmer's social-cultural status.

To investigate the structure and disturbance of *Irvingia* populations, six populations with enough adult trees (at least 30) were selected that had a unique combination of a particular agroforestry system, socio-cultural group, and a particular reason for ABMTs management (Table 3.1; Fig. 3.1). In low density areas, population data was obtained using two randomly selected 10 x 0.5 km mega-transects, while in high density areas, it was obtained from the two 100 x 50 m sub-plots used to estimate the tree density in four mega-transects (Fig. 3.1). Diameter at breast height (dbh) was measured for all adult trees (dbh ≥ 7 cm) of sampled *Irvingia* populations and all detected saplings and seedlings (dbh < 7 cm) were counted. In addition, these areas were visited throughout a year, specifically during fruit harvesting and land preparation, in order to identify signs of destructive management strategies and of any other human induced impact rooted in the main economic activities specific to the socio-cultural groups: pruning for different purposes, juvenile clearance and damage caused by harvesting techniques of bush mangoes or other NTFPs. Information on how people protect or remove/damage seedlings and saplings was also obtained through interviews.

Data analysis

Local use of ABMTs in the Dahomey Gap

Interviews led to a total of 23 different uses of ABMTs (Table 3.2b). For each respondent, each type of use was recorded as known / present (1) or unknown / absent (0). Thus, we assessed the level of ethnobotanical knowledge (Mn_{id}) for the i^{th} individual by:

$$Mn_{id} = \frac{Ni}{Nt} \, x \, 100\ \% \qquad (1)$$

Where Ni = number of uses known by the i^{th} individual and Nt = total number of uses recorded.

We distinguished two groups of *Irvingia* users: professional ones (traditional practitioners, traditional religion priests or vendors of medicinal plants) and non-professional ones (all other respondents).

Power transformation for percentage data (Box and Cox, 1964) was applied to Mn_{id} in order to normalize the data and stabilize their variance. Using Statistica version 6 (StatSoft, 2001), a two way Analysis Of Variance (ANOVA-2) was performed on the transformed Mn_{id} values in order to identify factors influencing the level of knowledge on ABMT uses. In this model, the two fixed factors are “socio-cultural group” and “user group”. The main effects of these factors as well as that of their interaction were assessed in this analysis.

In order to detect if respondents could be categorized geographically and to compare the two user groups, a Principal Component Analysis (PCA) was performed on a 0/1 matrix of all known uses per respondent and one column for each geographically defined socio-cultural region. Principal component scores from the first three axes of the PCA were visualized in two-dimensional plots. Since factors presenting no variability of responses introduce zero variation in the PCA, uses known to all respondents were deleted from the matrix.

Table 3.2a: Result of Analysis of Variance of level of ethnobotanical knowledge.

Source of variation	**Degree of freedom**	**Mean Square**	**F statistics**	**P value**
Socio-cultural group	8	0.75	185.45	< 0.0001
Socio-professional group	1	0.45	112.09	< 0.0001
Socio-cultural group * Socio-professional group	8	0.14	35.39	< 0.0001

Table 3.2b: First two principal components from the PCA on the ethnobotanical variables

Ethnobotanical variables	**First axis (48.56 %)**	**Second axis (10.95 %)**	**Third axis (8.49 %)**
1- First energetic use: ABMTs' branches + wood for domestic energy	-0.18	-0.27	0.90
2- Second energetic use: Bush mangoes' wooden for domestic energy	-0.18	-0.27	0.90
3- First socio-cultural use: dried branches to serve Holli + Nagot native fetish	-0.82	0.15	-0.06
4- Second socio-cultural use: Mature wood (trunk): incarnation of died twins	-0.78	0.31	0.09
5- Third socio-cultural use: dried mature wood for fetish drum making	-0.80	-0.26	-0.09
6- Economic uses: Leaf + fruit to accelerate other fruits ripening	-0.54	-0.76	-0.12
7- First therapeutic use: immature fruit accelerates the digestion of nitrogenous foods (mainly beans)	-0.24	0.10	0.17
8- Second therapeutic use: mature fruits as laxative	-0.64	-0.05	-0.24
9- Third therapeutic use: seed-based sauce against ulcers	-0.78	-0.30	0.05
10- Fourth therapeutic use: decoction of fresh leaves against malaria	-0.81	-0.26	-0.06
11- Fifth therapeutic use: decoction of fresh leaves to reinforce bladder excitation during the night.	-0.84	0.32	0.03
12- Sixth therapeutic use: fresh leaves against specific child diseases	-0.77	-0.43	-0.06
13- Seventh therapeutic use: bark in female gynaecology	-0.89	0.18	-0.07
14- Eighth therapeutic use: root decoction against impotence	-0.65	0.23	0.18
15- Ninth therapeutic use: bark decoction against haemorrhoid	-0.72	0.26	0.10
16- Tenth therapeutic use: seed oil against skin disease	-0.85	0.20	-0.06
17- Eleventh therapeutic use: small branches chewed against bad breath	-0.67	0.22	0.12
18- Twelfth therapeutic use: bark to treat wounds	-0.84	0.22	0.04
19- Thirteenth therapeutic use: bark decotion to balance human body temperature	-0.58	-0.25	-0.19
20- Fourteenth therapeutic use: bark to reinforcing babies' fontanel	-0.83	0.28	0.05
21- Fifteenth therapeutic use: bark to treat mycosis	-0.49	-0.19	-0.28
22- Sixteenth therapeutic use: leaf decoction as analgesic	-0.54	-0.76	-0.12
23- Seventeenth therapeutic use: bark decoction against dyspnoea	-0.84	0.32	0.03

Occurrence and density data reconstruction

Within each mega-transect, the border trees of each high density area were projected in DIVA-GIS (Hijmans *et al.*, 2001) and its extent was approximated by the derived convex polygon. The total number of ABMTs (NT_j) in each high density area was estimated by:

$$NT_j = N_s * S_{tj} / 10{,}000 \qquad (2)$$

Where N_s the total number of trees in the two sub-sampled areas of 100 x 50 m^2 and S_{tj} = the extent of the high density area in m^2.

Using DIVA-GIS, the NT_j trees were artificially generated in a random spatial pattern in the j^{th} area to complete the direct observation database (Fig. 3.1). Then, within each mega-transect, the number of trees that occurred within each 0.5 x 0.5 km^2, was obtained and geo-referenced in the centre of that unit area. Outside of the mega-transects, data on isolated individuals and small tree groups scattered in low density areas was entered as well with their GPS position. Zero density points were also added with their GPS position. The latter information was transferred to a 0.5 x 0.5 km^2 grid to thus complete the geo-referenced density database.

Spatial analysis of ABMT abundance in the Dahomey Gap

First, to assess which environmental variables influence the density, the layer of density points was superimposed with those of the countries (Benin and Togo), the FAO soil type (http://www.fao.org/geonetwork/srv/en/main.home#soils) and the phytogeographical zones in Benin (Adomou, 2006) and Togo (Ern, 1979). Using DIVA-GIS, the corresponding values were extracted into a database.

Bitter trees have a limited distribution being restricted to the Volta forest region (the Southern Mountains phytogeographical region in Togo). The density information turned out to have too few data points for a reliable statistical analysis. This is why, in this study, the analysis of their abundance and spatial structure will remain descriptive only.

For sweet trees zero density values were replaced by 0.1 and a natural logarithm transformation was applied. Using SAS 9.2 (SAS Institute Inc., 1999) an Analysis Of Variance (ANOVA) was carried out to assess the effect per variable.

Spatial variability of sweet ABMT density was measured using a geo-statistical approach (Rossi *et al.,* 1992; Christakos *et al.,* 2002). In this analysis, we ignored all short-term dynamic aspects that might change the spatial pattern, such as abundant regeneration in the rainy season, planting or selective elimination of trees, etc. Therefore, the density pattern was considered as a finite stock of trees expressing the environmental and conservational conditions in the geographic space. As such, we assume it to be a Gaussian random field at the second order stationarity (Goovaerts, 1997). This implies that the mean density is constant over the geographic space and the covariance between density values at position *x* and those at position $x + h$ exists and depends only on *h* not on *x*. In this study, we preferred the semivariogram function, which is simpler and more robust than the covariance (Baillargeon, 2005) to measure the patchiness or the spatial pattern of ABMTs' density across the Dahomey Gap. The Bayesian Maximum Entropy Library (BMELib: Christakos *et al.,* 2002) compiles consistent functions written in the Matrix Laboratory (Matlab) language to catch spatial structure by a geostatistical analysis. Using BMELib in MATLAB version R2011a (MathWorks Inc., Natick, MA, USA), the density semivariogram function was calculated as follows:

$$V(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [d(x_i) - d(x_{i+h})]^2 \text{ (Goovaert 1997)} \qquad (3)$$

With $V(h)$ = density semivariogram estimator; $d(x_i) - d(x_{i+h})$ = difference of mean of densities separated by a lag distance of *(h)* and $N(h)$ = number of data pairs in this lag distance. Omnidirectional as well as directional (North-South, West-East, 30° and 60°) semivariograms were calculated to check significant directional variations in ABMTs spatial structure. Only the omnidirectional semivariogram was modelled using the "eye fitting technique" (see Rossiter, 2007) and its parameters (nugget effect, sill and rang) were used to characterize the spatial structure of ABMT-based agroforestry systems in the Dahomey Gap.

Kriging is the more reliable estimation technique integrating semivariogram parameters and information regarding observed surrounding data points (mean and distance) and minimizing the prediction variance (Goovaert, 1997; Kumar and Remadevi, 2006). It promotes spatial visibility of information contained in the semivariogram. In non-sampled areas random points were generated in 0.5 km x 0.5 km grids cells. Using the BMELib in Matlab and based on the model of the obtained omnidirectional semivariogram, the density at non-sampled points within the Dahomey Gap was estimated by means of the ordinary kriging method (Goovaerts, 1997; Christakos *et al.,* 2002):

$$d_e(x_0) = \sum_{i=1}^{N(x_0)} w_i d(x_i) \tag{4}$$

Where $d_e(x_o)$ is the estimated density value at location x_0, $\sum_{k=1}^{n(i)} w_k(i) = 1$ = the sum of the weights (w_i) of $N(x_0)$ surrounding points with $d(x_i)$ values.

Analysis of the anthropogenic drivers of ABMT spatial density patterns

Using Statistica version 6 (StatSoft, 2001), a Multinomial Logistic Regression Analysis (Hosmer and Lemeshow 1989) was carried out on the eight parameters influencing the conservation and cultivation of ABMTs. The Global Null Hypothesis (GNH: BETA=0) and the Type 3 Effects Analysis were used to validate the accountability of the CMLRA and identify the most causative parameters, respectively. The Maximum Likelihood Estimate and the Odds Ratio Estimate help to identify parameters that hinder or induce the conservation or cultivation and give a comparative basis of the influences related to different levels of the causative parameters.

Variation of ABMT population structure

For each population, the size distribution (DBH) was obtained and the coefficient of skewness (ℓ) was calculated to characterize its structure:

$$\ell = \frac{1}{n}\sum_{\alpha=1}^{n}\left(z(\alpha) - m\right)^3 / \sigma^3 \text{ (Goovaerts, 1997)} \tag{3}$$

Where n = total number of individuals within the population, $z(\alpha)$ = dbh of the α^{th} individual within the population, σ = standard deviation of dbh values, m = mean of the dbh in the population. For each population, the percentage of trees affected by each type of physical damage was calculated for each diameter size-class: 0-7, 7-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, and > 100 cm. A two-way ANOVA was run in SAS 9.2 (SAS

Institute Inc., 1999) to assess differences in the level of damage per population. A Tukey test was used to distinguish populations based on their threat level.

Results

Traditional use and management of ABMTs

Sweet ABMTs were recorded in all socio-cultural areas while the bitter ones grow only in the Akposso area in the Volta forest region in Togo (Fig. 3.1).

In all areas the mesocarp of the sweet bush mango and the seed of both sweet and bitter bush mangoes are marketed and the seeds consumed. Table 3.2b provides the twenty-three other uses (socio-economic, medicinal and energetic, see also Vihotogbé *et al.* 2008) that were identified. In comparison to the 2007 study, only one additional use (fruits and / or leaves used for accelerating ripening of other fruits, mostly *Musa* spp. but less often also *Persea americana*, *Ananas comosus*, *Chrysophyllum albidum*), was found.

The ANOVA-2 results are presented in Table 3.2a. They show that the level of ethnobotanical knowledge (Mn_{id}) depends on both the socio-cultural group and the socio-professional group ($p < 0.001$). Also, the level of knowledge was significantly different between the two socio-professional groups ($p < 0.001$). Figure 3.2a shows that in the Adja, Holli and Nagot areas, the professional users detained higher local knowledge on *Irvingia* species, while in the other areas no clear difference was showed between these two groups, the professional and ordinary users showed no clear difference. However, the professional users in the Holli and Nagot areas (mean knowledge = 78% and 65%, respectively) have the most extensive ethnobotanical knowledge of *Irvingia* species in the Dahomey Gap. The Akposso area was that with the least indigenous knowledge (mean knowledge = 1%). Thus, the level of indigenous knowledge (Mn_{id}) appears to be higher in Benin than in Togo and is mostly concentrated in the southeast (Holli and Nagot; Fig. 3.2a).

The PCA shows that the first three axes together account for 68% of the variation within the ethnobotanical data (Table 3.2b). The first axis (48.04%) negatively correlates with all three socio-cultural uses plus fourteen medicinal uses and the second (10.95%) negatively correlates mainly with only one medicinal use, while the third axis (8.15%) represents intensive use of ABMTs as a source of domestic energy (fuel). The first axis (Fig. 3.2b) shows, again, that the Holli plus Nagot people in south-eastern Benin (and most importantly their professional users) had a higher indigenous knowledge than all other communities. The third axis shows that apart from the Akposso and few Nagot people, wood and seed are intensively used as fuel everywhere in the Dahomey Gap.

In Togo, the seed is mostly sold to Beninese and Nigerians while small amounts are locally used to thicken vegetable sauces based on *Corchorus olitorius* (Tiliaceae) and *Abelmoschus esculentus* (Malvaceae). The seeds are still considered to be an essential part of the diet of the Fon, Goun and Nagot people in Benin and the Nigerian Ibo’s. Apart from some Togolese Ewe groups, the high thickening property and brown color of this sauce makes it 'heavy' and not appreciated by Togolese.

From the interviews it turned out that, traditionally, there is a taboo on planting ABMTs among all ethnic groups studied in Benin, since the trees may expose neighbouring households to witchcraft, bad health and even death. However, only few farmers understand and confess that this taboo is rooted in a desire not to accept young owners of economically important trees. But his taboo is being broken and ABMTs are being integrated in intensive cultivation systems through the selection of mother trees and seeds for planting. The definition of mother tree depends on the targeted *Irvingia* products. Trees producing very sweet and 'pasty' fruits are prioritized in areas of fruit commercialization. In contrast, sweet fruits with large seeds (mean size ≥ 52 x 37 x 23 mm) are valued in areas where their usage is dominant. Transplantation of seedlings encountered under superior sweet mother trees is common. However, in some areas, seeds are used to establish orchards and agroforestry parks in taungya agroforestry systems.

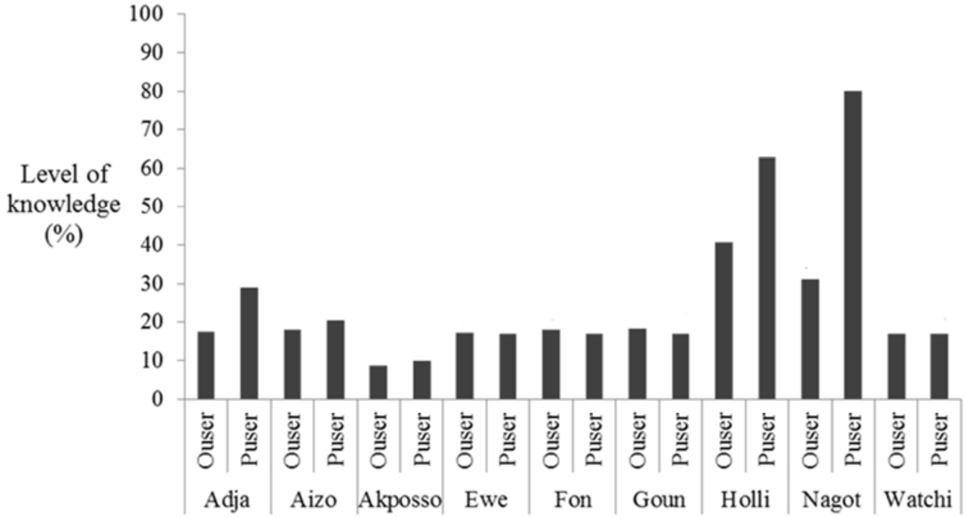

Figure 3.2a: Result of the Analysis of Variance: comparaison of socio-cultural groups, socio-professional groups and their co-influence

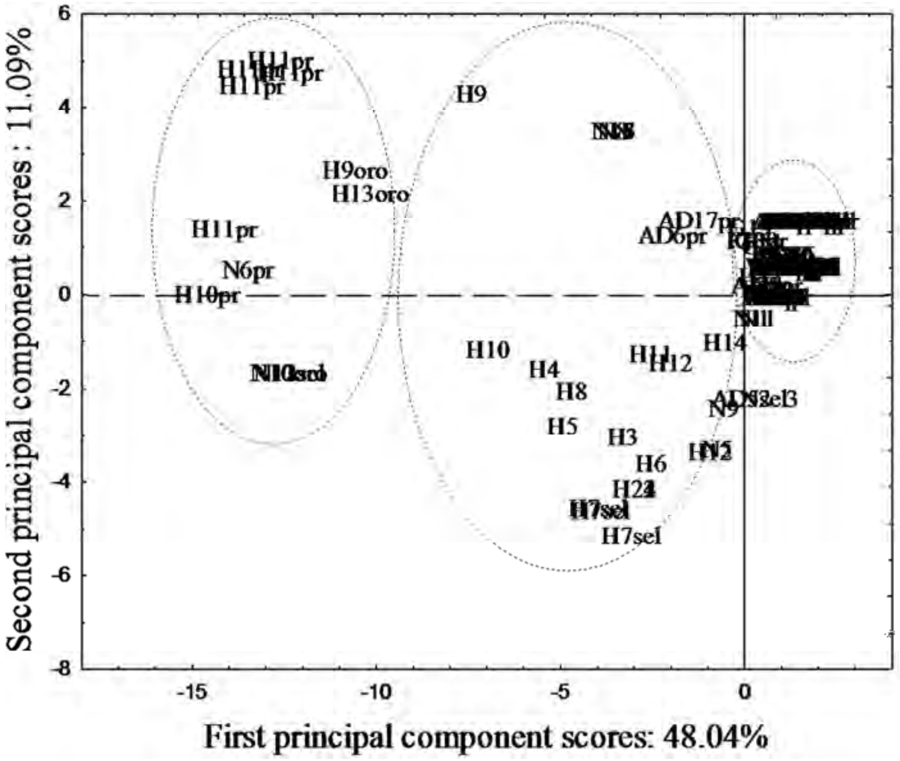

Figure 3.2b$_1$

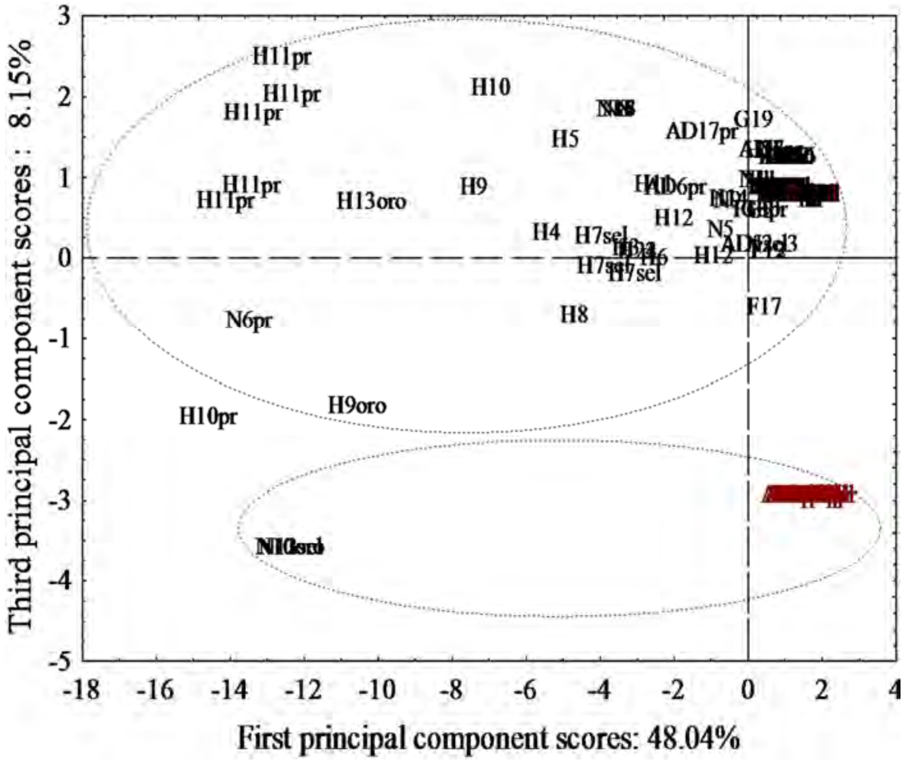

Figure 3.2b$_2$

Figure 3.2b: PCA Analysis of the 1 / 0 matrix of the ethnobotanical knowledge of the nine socio-cultural groups. AD = Adja, Ai = Aizo, Akp = Akposso, E = Ewe, F = Fon, G = Goun, H = Holli, N = Nagot, W = Watchi; pr = traditional healer, sel = merchant of medicinal plants, oro = head of 'Oro'; rel = traditional religions keeper

In the Akposso area in the Volta forest region of Togo, trees with bitter fruits are called 'the never planted ABMTs'. Wild individuals are preserved on lands being transformed to cacao, coffee and banana fields or in forest gardens. Akposso communities reported that marketing of the seed started 20 years ago with the settlement of Ibo communities and is still considered as a marginal, female activity. However, men prefer the timber of bitter trees as the most economically valuable timber after *Milicia excelsa* (Moraceae).

Spatial structure of ABMT abundance

Density of sweet ABMTs ranges between 0 and 1020 trees / 25 ha (= 0.5 x 0.5 km). Low densities are common while high densities are spatially limited. The overall mean of sweet trees density is 35 (SD = 113) trees / 25 ha. Low densities (ranging from 0 to 462 trees / 25 ha) have also been recorded for bitter trees in the Southern Mountain phytogeographical region in Togo, with an overall mean of 55 (SD = 99.8) trees / 25 ha. In this phytogeographical region where bitter and sweet ABMTs co-occur, bitter trees are found in swampy areas, along streams and on hill slopes, while sweet trees occur everywhere even at the top of hills, but not in swamps or along streams, following human settlements.

The results of the ANOVA indicate that sweet tree density does not vary significantly at the country level ($P = 0.0994$). It is, however, significantly correlated with the FAO soil types and phytogeographical zones ($P < 0.001$ for both factors). The higher densities (mean = 90 and maximum = 1020 trees / 25 ha) are found on lateritic soils (Nd9 and Ne6-2b types) and on the complex ferric shallow soil types (I-Lf-Rd type). High densities occur in the phytogeographical regions of Plateau (in Benin), Central Lowlands and Southern Mountains (in Togo). More specifically, the high densities are located around Kpalimè in Togo and in the Adja and Sakété-Pobè areas in Benin.

The regionalized density data are presented in Figure 3.3a. An isotropic semivariogram model was observed. Only the West-East semivariogram displayed slightly different levels of spatial continuity while the others are all very similar or identical (Fig. 3.3b). However, the overall trend in all semivariograms is identical. The model fitted to the omnidirectional semivariogram was composed of a nugget effect (semivariance = 1), followed by a spherical pattern (semivariance = 2, range = 5 km) and an exponential pattern (semivariance = 1.25, range = 22 km). The highest semivariance value (4.5) in ABMT density is observed after 22 km. This means that the Dahomey Gap comprises patches of ABMT-based agroforestry with uniform densities in an average radius of 22 km. After this, range densities become significantly different in all directions (Fig. 3.3.b) and so, high density areas are spatially limited. Even though low density values are common, their level still significantly fluctuates, confirming the patchy spatial pattern for sweet trees over the Dahomey Gap (Fig. 3.3c).

Potential drivers of the spatial pattern

In the Dahomey Gap, we distinguish three agroforestry systems for *Irvingia* spp.: (i) orchards, (ii) agroforestry parks, and (iii) forest gardens. Orchards are managed through the taungya agroforestry system, and tree density varies between 256 and 400 per ha. The density of 400 trees per hectare ha was encountered in short-term and extensively managed taungya systems with many spontaneous occurrences, while that of 256 trees per hectare is common in intensively managed permanent taungya systems that combine trees and subsistence crops. Agroforestry parks come second in terms of density of ABMTs as priority crop. Most frequently, such parks are owned by farmers belonging to different families inheriting large and continuous farmlands. Each farmer marks his trees with a different colour of cloth to avoid exploitation by others. This confidence-based system relates to the exploitation of the ABMT seed, while the mesocarp consumption remains free (especially in southern Benin). Densities are low (161 ± 82 adult trees per ha) in those parks but more than 400 trees per ha can be detected in areas of natural regenerations. In forest gardens, lower densities are found than in the first two systems.

The results of the logistic regression used to investigate the causal factors behind the density patterns observed, presented a Pr > Chi-Sq < 0.001 for the GNH-Beta = 0 test, indicating that some parameters are indeed involved in this decision-making. Farmland status, ethnic group and the type of ABMT were identified as significant ones (Table 3.3a: Type 3 Effect Analysis). Having the lowest coefficient, an unclear inheritance status of the farmland apparently highly weakens the desire to develop an orchard (Table 3.3b: Maximum Likelihood Estimation). Considering farmland tenure, it appears that especially private, poor and small farmlands are being converted into ABMT orchards (Odd Ratio Estimation Point = 422 in land tenure options: Table 3.3b). On the other hand, farmlands with an uncertain inheritance status apparently give no confidence to farmers to engage in intensification of ABMT cultivation (Odd Ratio Estimation Point = 0.005; Table 3.3b). Regarding socio-cultural groups, significant initiatives for orchards development have been undertaken only by the Adja people.

Table 3.3: Logistic Regression Analysis of factors driving the desire of local farmers to intensify ABMTs cultivation.

Table 3.3a	**Type 3 Analysis of Effects**		
Effect	DF	Wald Chi-Sq	Pr > Chi-Sq
Farmland status	4	0.8297	< 0.0001
Socio-cultural group	8	2.4673	< 0.0001
ABMT Type	1	5.4446	< 0.0196

Table 3.3b		**Analysis of Maximum Likelihood Estimates**					**Odds Ratio**	
Parameters		**DF**	**Estimated coefficient**	**Standard Error**	**Wald Chi-Square**	**Pr > Chi- Square**	**Reference factor**	**Point Estimates**
ABMT type	*Edible*	*1*	*1.3779*	*0.5905*	*5.4446*	*0.0196***	*Vs. Inedible*	*15.734*
Farmland tenure	*LSH*	*1*	*1.1202*	*9.5985*	*0.0136*	*0.9071*^{NS}	*Vs. SPR*	*22.*
	LP	*1*	*3.2262*	*9.5636*	*0.1138*	*0.7359*^{NS}		*181*
	UHS	*1*	*-7.1923*	*47.7358*	*0.0227*	*0.0282***		*0.005*
	SPP	*1*	*4.0722*	*9.5669*	*0.1812*	*0.0104****		*422*
Ethnic group	*Adja*	*1*	*11.5780*	*34.8987*	*0.1101*	*0.0041****	*Vs. Watchi*	*> 999.9*
	Akposso	*1*	*6.7783*	*34.9013*	*0.0377*	*0.8460*^{NS}		*> 999.9*
	Aïzo	*1*	*3.2863*	*34.9096*	*0.0089*	*0.9250*^{NS}		*1*
	Ewe	*1*	*-8.4624*	*324.500*	*0.0007*	*0.9792*^{NS}		*2.33*
	Fon	*1*	*-9.3084*	*64.2539*	*0.0210*	*0.8848*^{NS}		*1*
	Goun	*1*	*-8.4392*	*54.7494*	*0.0238*	*0.8775*^{NS}		*2.385*
	Holli	*1*	*8.4553*	*34.9011*	*0.0587*	*0.8086*^{NS}		*> 999.9*
	Nagot	*1*	*7.1952*	*34.9018*	*0.0425*	*0.8367*^{NS}		*> 999.9*

LSH = lend farmland with agreement of sharing the harvest; LP= large and private farmland; UHS = farmland with unclear inheritance status; SPP = small and private farmland with poor soil; SPR = small and private farmland with rich soil; FTS = food tree species; ** = highly significant factor in the desire for intensive cultivation; *** = very highly significant factor in the desire for intensive cultivation; ^{NS} = factor with no significant effect in the determination of the motivation for intensive cultivation.

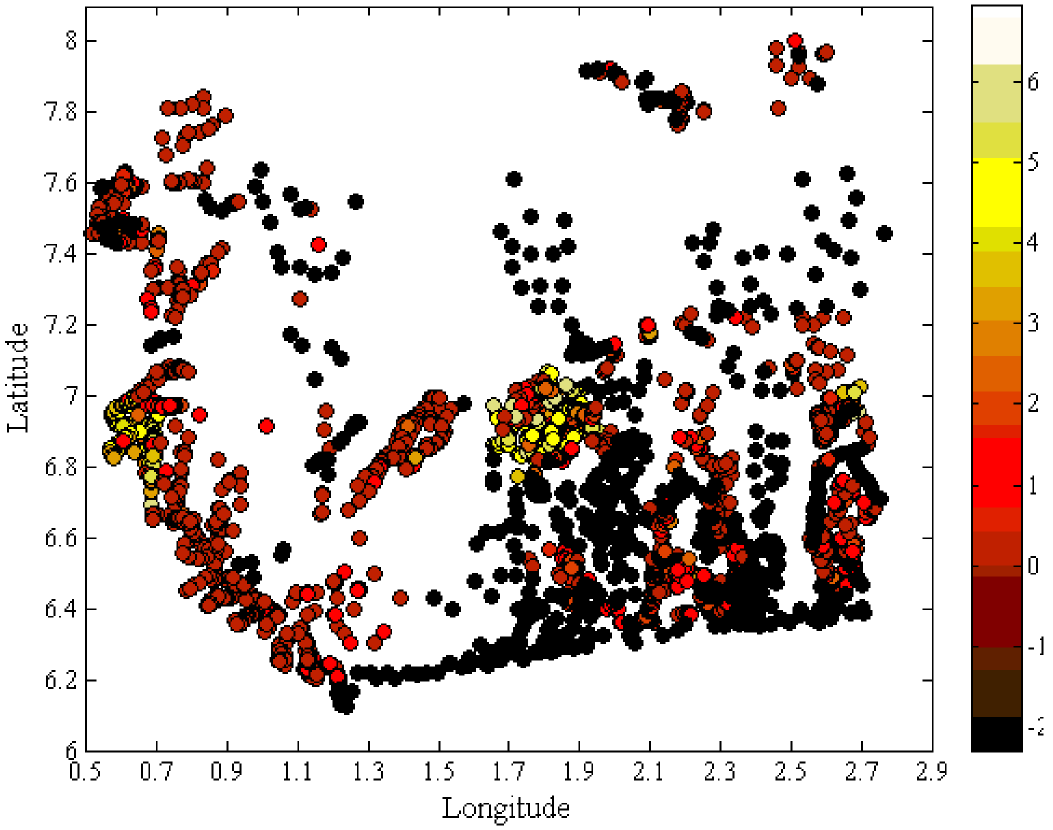

Figure 3.3a: Location of log transform values of sweet ABMTs density within 0.5 km x 0.5 km

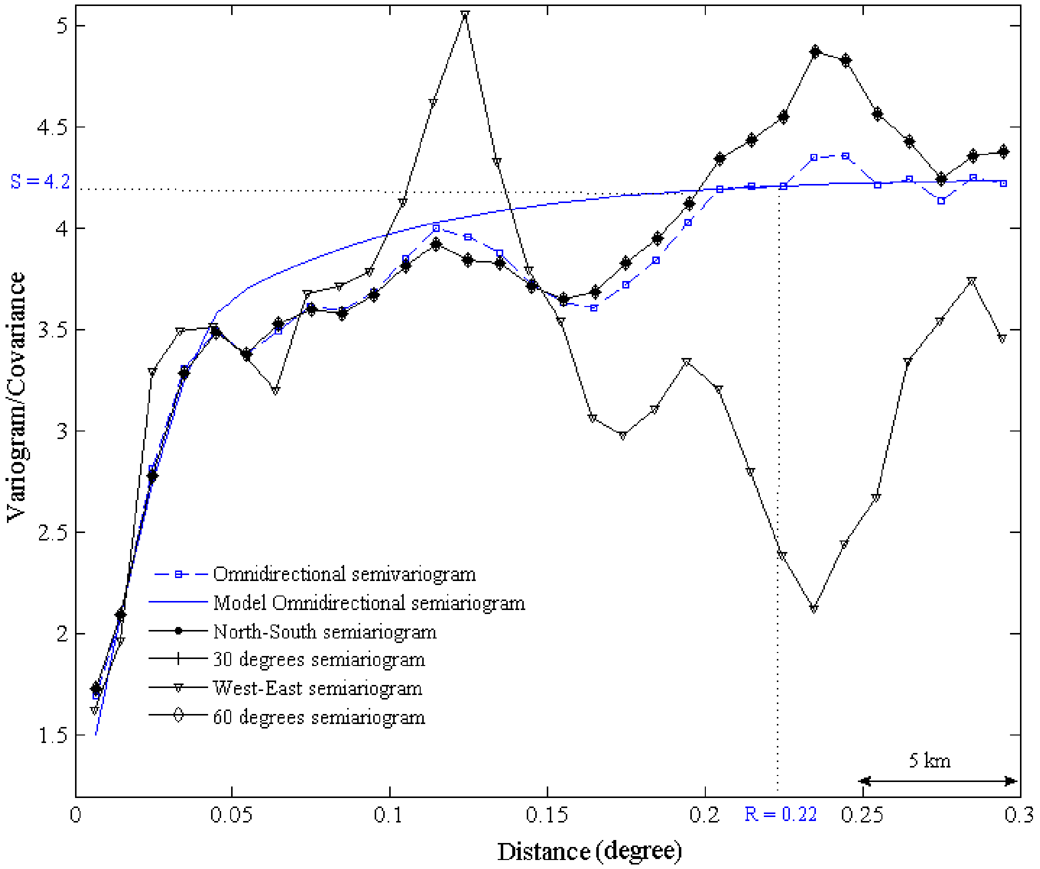

Figure 3.3b: Semivariogram expressing spatial structure of sweet ABMTs density

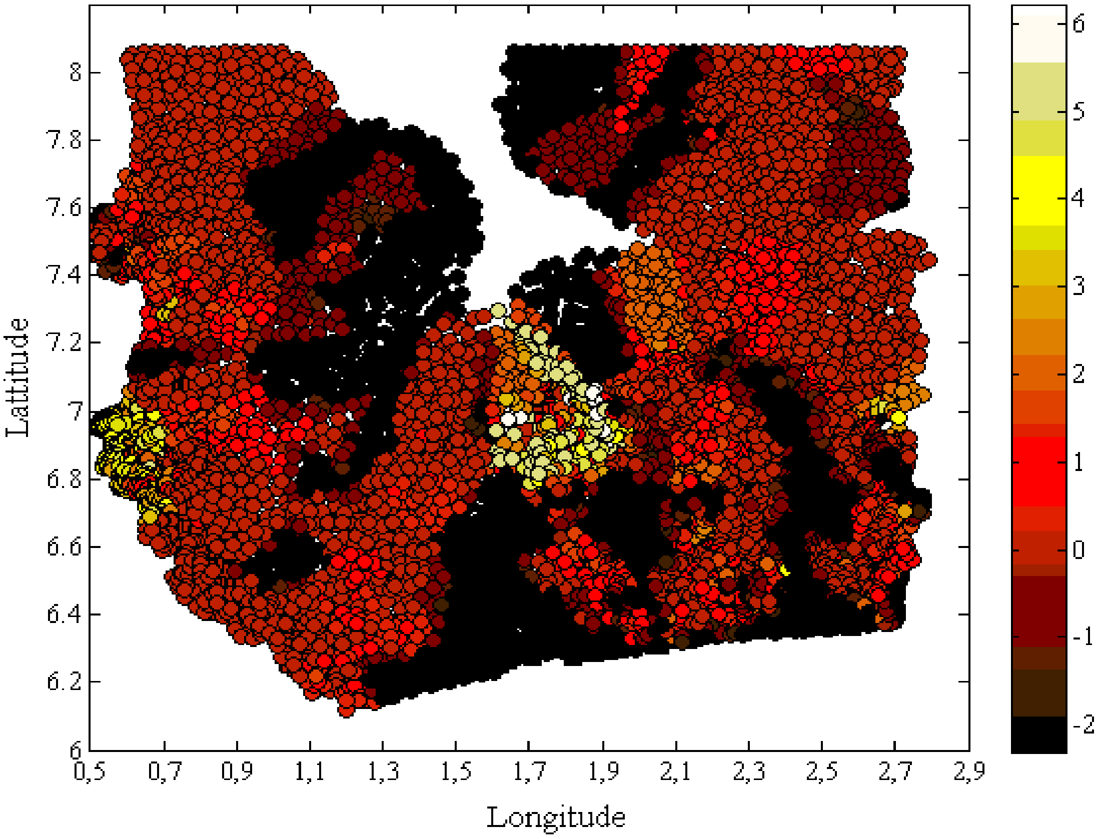

Figure 3.3c: Kriging map of sweet ABMTs density across the Dahomey Gap.
The colour bar indicates the natural logarithm of the density gradient: darker colours indicate lower density (black colour for zero density) and lighter ones higher densities (white colour for the highest values)

The Akposso people mostly use agroforestry parklands and forest gardens to manage bitter trees in the wild and do not establish any *Irvingia* orchards. Sweet trees are 16 times more frequently cultivated in orchards than bitter trees. Local marketing of the seed, the specific reason for bush mango cultivation, and existing taboos remain key factors determining the desire to intensify cultivation. However, their influence is still limited compared to the other significant parameters.

Differences in population structure and threats

In the Akposso area (Bena and Kounionhou), natural forest and forest gardens are mostly populated by large trees (skewness ≥ 1.5; Fig. 3.4a, b). In forest gardens, with a mean dbh of adult trees of 80 cm and where the largest trees are found, spontaneous regeneration is still abundant (32%). In contrast, regeneration in natural forest, where adult trees are on average slightly smaller than in forest gardens (mean dbh = 70 cm), is only 2%. In the Aizo area, the population is also composed of large trees (skewness ≈ 1; Fig. 3.4e). Here, two sub-populations can be recognized: spontaneous old populations (mean dbh = 70 cm), being replaced by cultivated young ones with a mean dbh of 20 cm. In this area, natural regeneration is very low (3%). A similar replacement initiative appears in the Holli and Nagot areas of Pobè, where the separation of the two sub-populations (mean dbh = 20 cm and 80 cm; Fig. 3.4f) in quasi equal frequency tends to be even clearer (skewness = 0.33). Planting was more intensive in the Adja area (Lalo-Klouekanmey) where young trees dominated the population (skewness = - 0.18; mean dbh = 30 cm) with high regeneration figures and an absence of large trees (> 80cm dbh; Fig. 3.4c). Similarly, the planted population in the Ewe area (Kpalimè) shows abundant regeneration and less large trees (mean dbh = 30 cm; Fig. 3.4d).

Apart from the intensive collecting of fruits (and seed), five other practices negatively affect the increase in population density and surface occupied by *Irvingia*. Some trees show the simultaneous occurrence of flowers, and fruits and the harvesting of unripe fruits, by shaking the branches or using long sticks or even a machete, causes profound damage: flower abortion, debarking, and reduction of the crown size. In some areas, saplings and seedlings are cleared for field establishment or maintenance. During other NTFP collecting in natural areas saplings may be systematically cleared. *Irvingia* trunks are sometimes severely debarked for medicinal purposes. Finally, the traditional fishing system uses *Irvingia* twigs for the construction of an ‘Akaja’, a structure that provides a breeding ground for fish. The resulting severe pruning is a major cause of population decrease and of low productivity.

Figure 3.4: ABMTs population structure and treat levels in different socio-cultural areas: a = wild bitter trees in protected area in the Volta region, b = wild bitter trees in traditional agroforestry systems in the Volta region (Kounionhou), c = cultivated sweet trees in southwestern Benin (Lalo-kouekamey), d = planted sweet trees in the lower Volta region (Kpalimè), e = sweet trees in old forest and current traditional fishing area in Benin (Calavi), f = sweet trees in old forest region in south east Benin (Pobè).

Skewness = 1.5

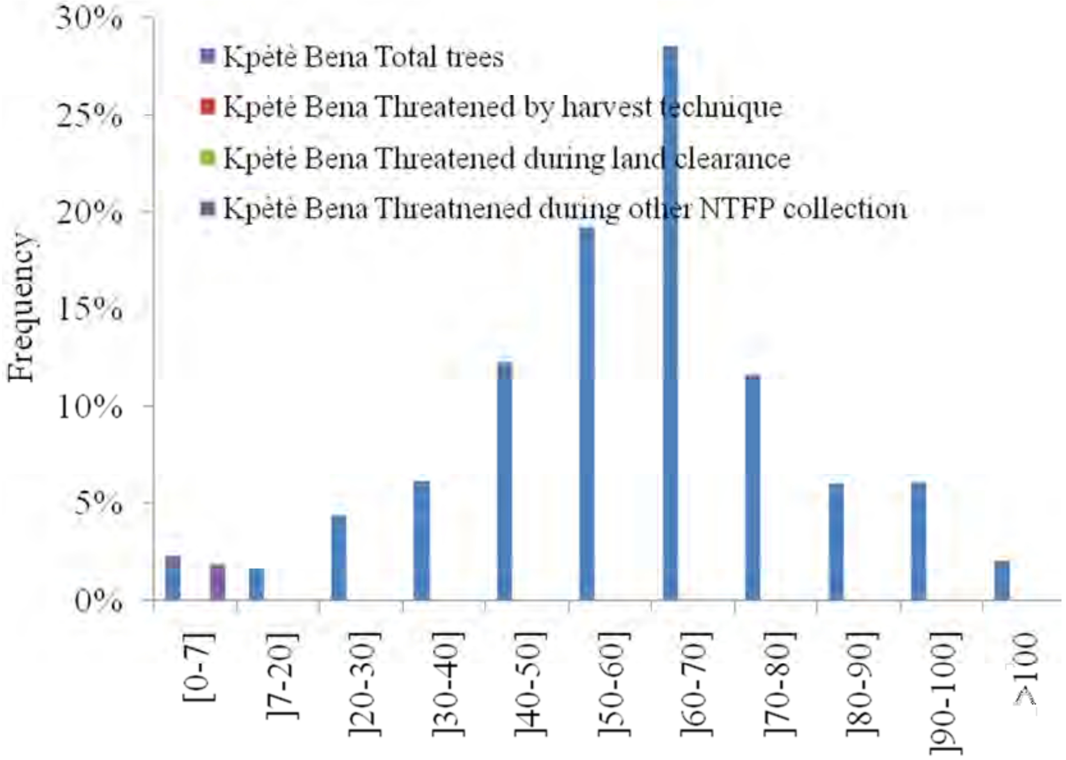

Figure 3.4a

Skewness = 1.63

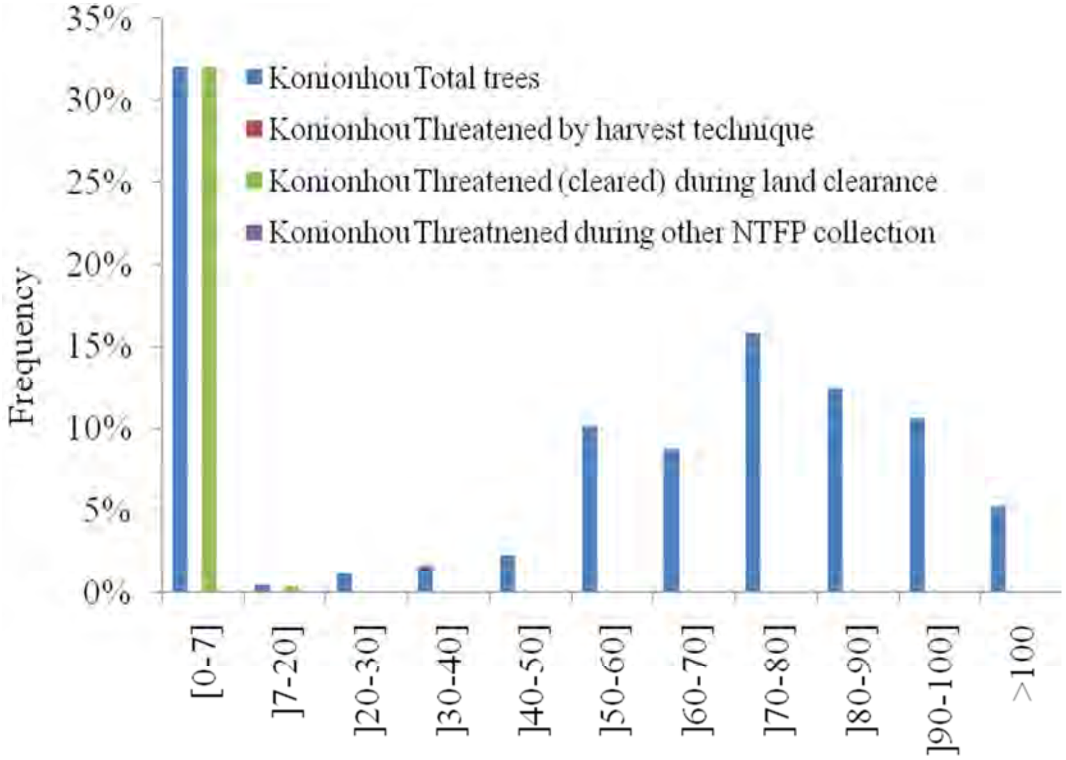

Figure 3.4b

Skewness = - 0.18

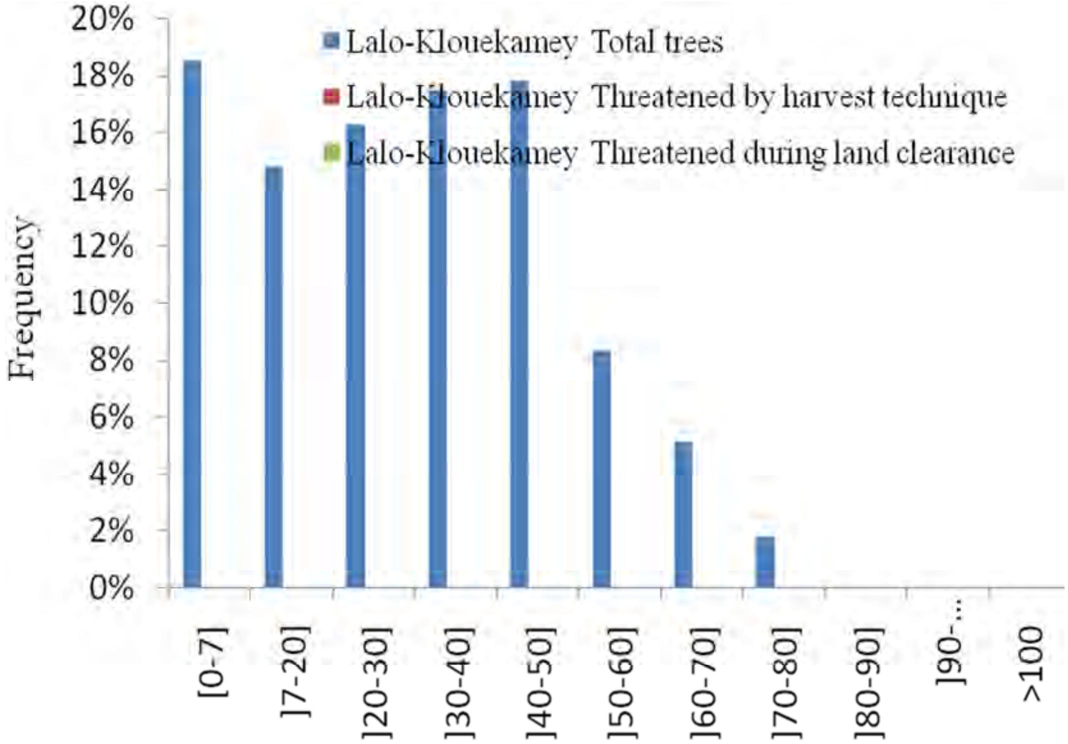

Figure 3.4c

Skewness = 0.35

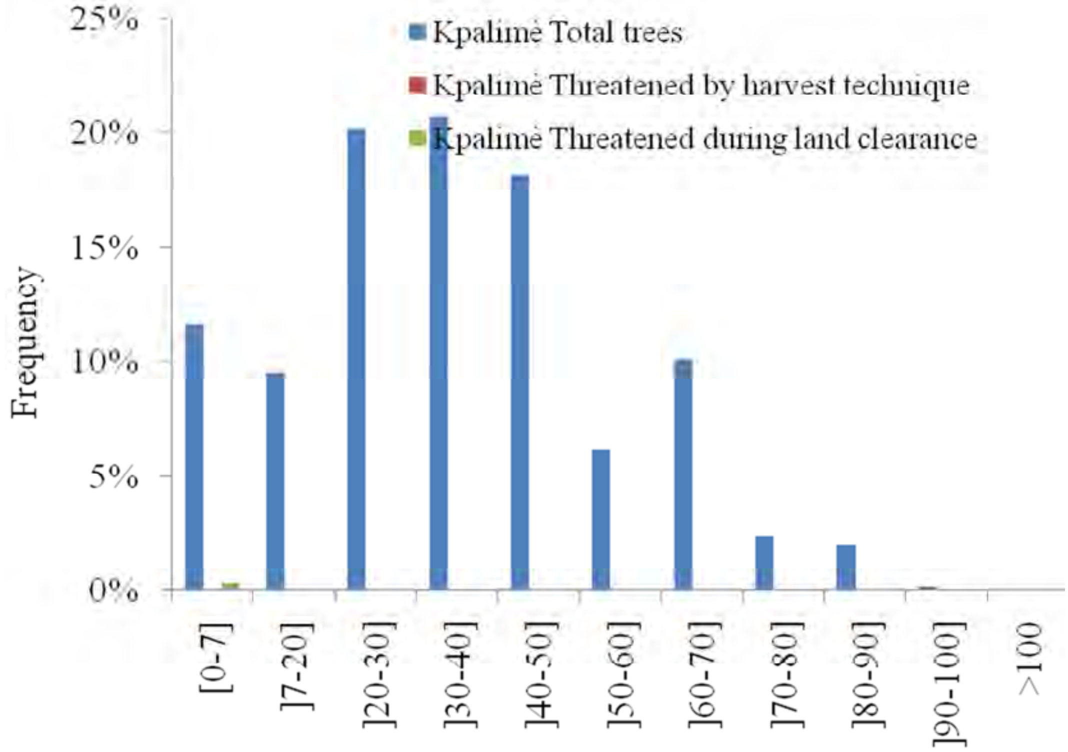

Figure 3.4d

Skewness = 0.926

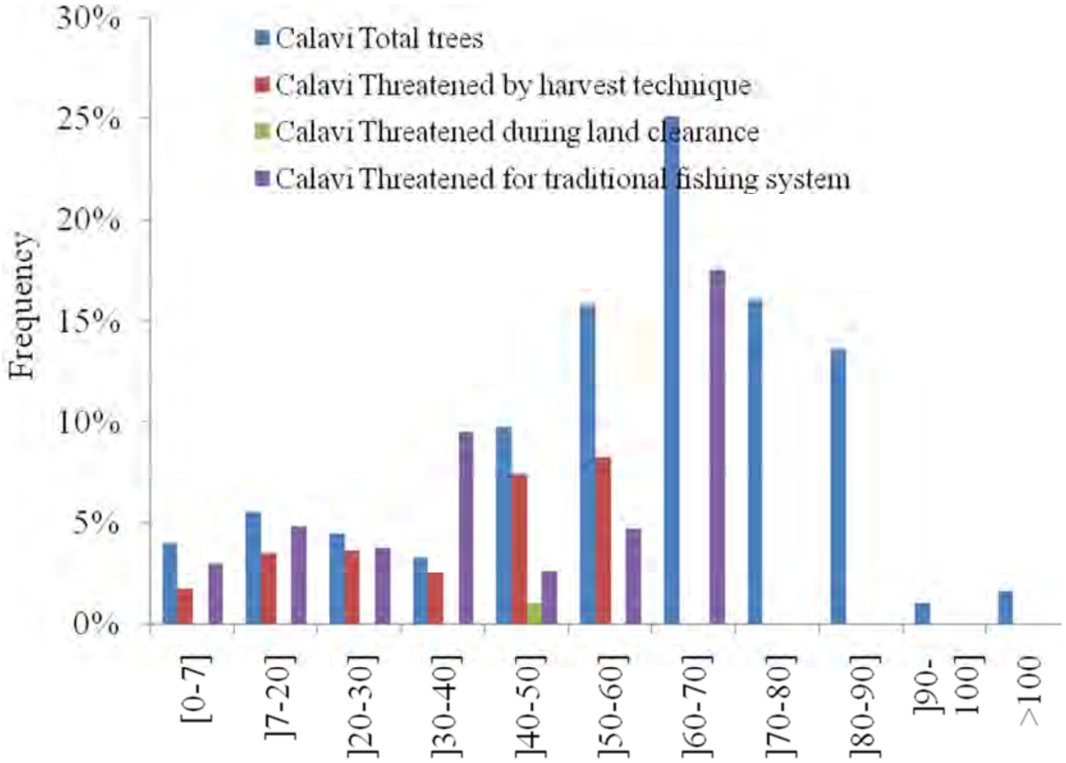

Figure 3.4e

Skewness = 0.33

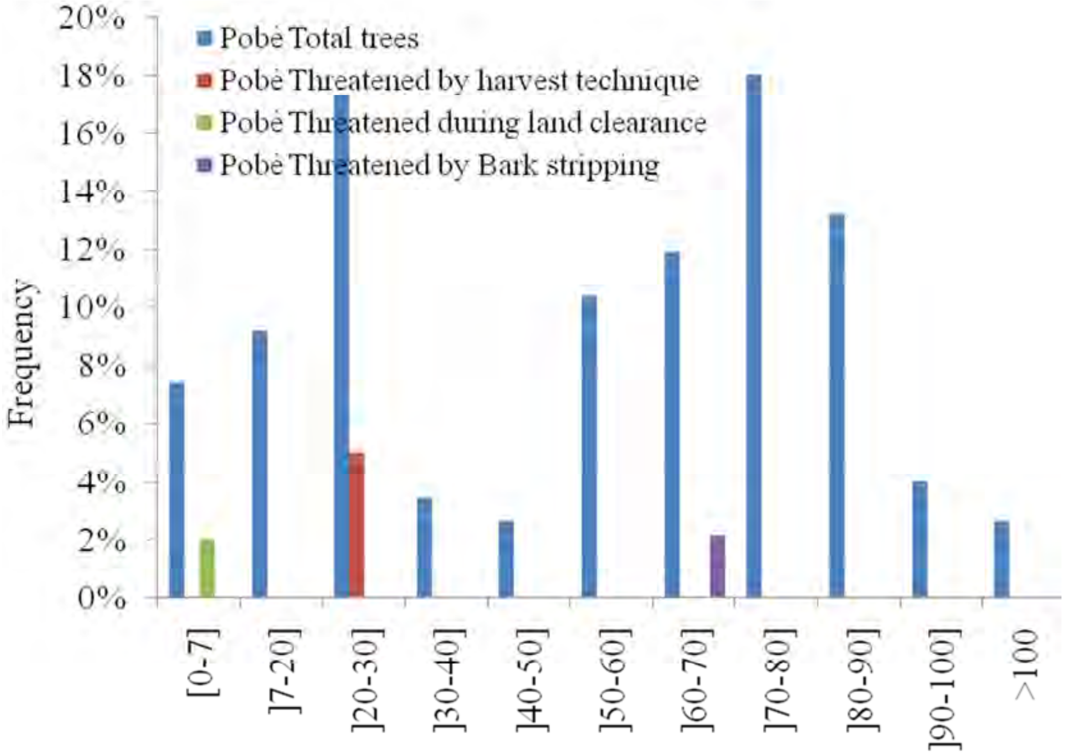

Figure 3.4f

Considering the combined threats, the Tukey test indicates there are three levels of population threats (Table 3.4). The frequency of threatened trees depends on the socio-cultural location of the population (P = 0.008), but all of the six identified practices equally contribute to tree damaging (P = 0.2563). Trees in the most intensive fishing area (Aizo, Calavi) are the most highly threatened. Fig. 3.4e shows that in this population, apart from the intensive collecting of fruits, all top three threat practices (branch pruning, juvenile clearance and damage caused by harvesting techniques) occur and may affect up to 17% of the adult trees of a given size class. Bitter trees in forest gardens of the Akposso area (Kounionhou) and sweet trees in the Holli and Nagot areas (Pobè) represent the second most disrupted populations with an important damage rate due to juvenile clearance (2-32%) during land preparation and inadequate harvesting techniques (Fig. 3.4b, f). In contrast, bitter trees in the protected area of the Akposso area (the disturbed forest land Kpètè-Bèna) and sweet trees managed by the Ewe community (Kpalimè) as well as most of these trees in the Adja area (Lalo-Klouekanmey) hardly show any damage (only 0–1.8% of threatened trees; Fig. 3.4a, d, c).

Table 3.4: Tukey test on population disturbance and categorization.

Tukey Grouping		Disturbance levels generated by SAS	Population	Corresponding socio-cultural areas
	A	0.013402	Calavi	Aïzo + Fon
B	A	0.005869	Kounionhou	Akposso
B	A	0.001927	Pobè	Holli + Nagot
B		0.000236	Kpètè Bena	Akposso
B		0.000060	Kpalimè	Eve (settled inside other socio-cultural groups dominated by Akposso)
B		0.000000	Lalo-KLouekamey	Adja

Populations with different letters are significantly different in terms of their actual disturbance level

Discussion

Usage heterogeneity and implications for ABMT occurrence

The diverse uses and economic contribution to livelihood are leading motives to cultivate *Irvingia* trees (Asaah *et al.*, 2003). In the 250 km wide area where ABMTs occur in Benin and Togo, most of the local communities are socially linked and have no migration limits (see

Assiwaju, 1979). Only the consumption of the sweet mesocarp and use of the seeds of bush mangoes are known to 100% of the local communities. Thus, the variation of the local socio-cultural knowledge showing a gradient with the lowest level in Togo (see Fig. 3.2a and Lesley and Brown, 2004, who reported a high diversity of use in Cameroon and Nigeria) indicates that ABMT conservation or cultivation did not initially occur because of the appreciation of their socio-cultural properties but rather for mesocarp and seed consumption and commercialization (Tchoundjeu and Atangana, 2007). In the particular case of the Dahomey Gap, this implies that people initially had no specific ethnobotanical knowledge on ABMTs and have obtained such via their contacts with the Yoruba people from Nigeria. This knowledge then spread further through population migrations. In this normally slow process of learning (see Turner and Turner, 2008) food properties may be accepted faster than any other types of use. Therefore, in the Dahomey Gap, ABMT cultivation is justified by the consumption of the sweet mesocarp plus that of the seed, and the economic value of these two NTFPs is leading the domestication process in their entire distribution range (Leakey and Tchoundjeu, 2001; Vodouhè, 2003; Atato *et al.,* 2010).

Given the fact that most of the communities in the Dahomey Gap are socio-culturally linked and have experienced long periods of migration (De Medeiros, 1984; Asiwaju, 1979), their diets regarding bush mangoes may not significantly vary if there is no difference in the history of the occurrence of ABMTs in their environment. Thus, the question is whether in the Dahomey Gap sweet ABMTs did occur naturally, or were introduced and are surviving only in cultivation. The fact that the origin of seed-based diets is situated in the Ibo and Yoruba areas (in Nigeria) supplemented by the decrease of ethnobotanical knowledge in a western direction, strengthens the hypothesis that sweet trees spread by population migration from the Lower Guinean forest block in Nigeria west over the Dahomey Gap (Lowe *et al.*, 2000). The change in behaviour caused by breaking taboos around ABMT cultivation and the recent start of seed consumption in Togo, further confirms this theory.

The purposes of the development of priority food trees species are clearly identified by local communities. There is a need to adopt different strategies in ABMT germplasm collecting and propagation. However, other activities, like control of the reproductive biology, tree improvement and breeding (Leakey *et al.*, 2005), are not significantly being taken into account. Therefore, like in many other valuable food tree species, the domestication of the sweet trees is mainly depending in initiatives of individual farmers and market opportunities (Leakey e*t al.*, 2005). In this process, a difference exists in the management of ABMT orchards depending on the local target. Where fruits are marketed for mesocarp consumption, less well-tasting trees or those having a fibrous mesocarp as well as infested trees are systematically eradicated (Vodouhe, 2003). Where the seeds are the commercial item, trees with small nuts (regardless of the mesocarp taste and nutrition quality) are eliminated. This inevitably leads to a loss of characteristics that might be valuable for the success of future agroforestry and plant breeding programs (Wood and Lenné, 1997). The very low tree densities in certain areas which potentially seem suitable to grow ABMTs may highlight not only an ethnic food preference or a lack of ethnobotanical or cultivation experience, but also regional differences in local market value of ABMTs or even NTFPs in general.

When a plant genetic resource presents no direct opportunity locally (food, medicinal or social), the good 'extractivism approach' proposed by Almeida (1996) as the best strategy of exploiting NTFPs without damaging the species’ life cycle guarantees no ecological balance (Rai and Uhl, 2004). Thus, the intensive exploitation of the *Irvingia* seeds from wild populations in the Volta forest region and its valuable timber are key factors jeopardizing especially the bitter tasting *I. wombolu* by narrowing its potential area of occurrence over time.

Variation in Irvingia *conservation and cultivation*

In the Volta region, bitter trees, *I. wombolu*, were found only as wild elements in forest gardens and in natural forest stands, while sweet trees, *I. gabonensis*, were found almost exclusively in cultivation. This difference in cultivation status is primarily caused by a preference of the local communities for a sweet mesocarp (Vodouhè, 2003). Bitter trees are therefore only preserved *in situ* in the Volta forest region. However, bitter trees belong to the most extensively exploited NTFPs in the Volta forest region and therefore this population may well be at severe risk of genetic erosion or even extinction.

The semivariogram characterizing the spatial pattern of ABMT density (Fig. 3.3b) shows a nugget effect with spherical and exponential characteristics within a short range of 22 km. This result implies that even at the sampled unit area level (25 ha), variation still exists within areas of uniform density level at different rates in a unique direction. However, the isotropic characteristic of the spatial structure implies that this unsteady pattern is repeated in all direction across the study areas where very local initiatives (within a radius of 22 km) of ABMT cultivation exist. Therefore, such initiatives are mainly taken by small-scale farmers who are more involved in indigenous tree species cultivation to enhance the sustainability of their poorer productive space (see Leakey and Tchoundjeu, 2001).

Both bitter and sweet trees occur in an overall low density pattern across their distribution range (see Ewane, *et al.*, 2009). The higher densities of sweet trees found in some areas agree with Leakey (2010) who reports that sweet bush mangoes are mostly exploited in traditional agroforestry systems. When plant species are not valued in the local diets or therapies there is no interest to conserve or cultivate them. This justifies the low density of bitter trees in the Volta region. In general, this may lead to rapid phenotypic as well as genetic erosion in a context of increasing land use change and biodiversity loss (Sodhi, 2007). In the Dahomey Gap, where natural forest is still steadily declining, wild bitter trees are under all kinds of threat (intensive collecting of seeds, specifically sought target of wood, eradication of juveniles in land conversion for agriculture, etc.). The low density of ABMTs found in natural stands in Nigeria, Cameroon and Gabon, related to high mortality of saplings and fruit collecting (Agbor, 1994; Van Dijk, 1999; Zapfack and Ngobo Nkongo, 2002), is also recorded in the natural reserves and forest gardens in the Dahomey Gap.

Impact of indigenous knowledge, socio-economic and ecological environments

Our results show that, in the Dahomey Gap, the products of *I. gabonensis* are generally not collected from ‘wild’ (spontaneously growing) trees (Lowe *et al.*, 2000). Because of their socio-economic value sweet ABMTs are as a rule integrated in intensive production systems by local farmers. This involves the manipulation, cultivation, and management of germplasm for a variety of products of the same species established in various systems (see Wiersum, 1996). Since such systems on average need a large surface, the priority crop status given to ABMTs works counterproductive to solve the crucial problem of farmland availability in the Dahomey Gap raised by Floquet and Mongbo (1998). Sweet *Irvingia* trees are the most intensively cultivated trees in the Dahomey Gap. The fact that mainly small farms are being converted to ABMT orchards indicates that the current cultivation process is mainly led by poor farmers (Leakey *et al.*, 2005). Unfortunately, the future improvement of ABMTs thus depends on the selection of genetic material made today by those small farmers. So, these currently unguided selection strategies may lead to a loss of genetic material, with an undeniable negative impact on their potential as a crop.

The cultivation of ABMTs in more organized systems is related to the presence of the Ibo people who locally organize the collection of the seed. Because the Ibo settlement occurred later than the local initiatives for intensive cultivation, the influence of socio-cultural groups in the cultivation efforts appears justified. This difference in time might be rooted in the variation of ethnic knowledge and taboos as well as the ability and willingness of certain local ethnic groups to break ancient taboos. However, the relationship ethnic group – indigenous knowledge – conservation remains strongly influenced by local economic opportunities. The seed and mesocarp value equally influence the desire to intensify the bush mango cultivation. Thus, not only the economical priority of the seed (Tchoundjeu and Atangana, 2007), but also the market for the entire fruits (sweet mesocarp) is an important stimulus for farmers' cultivation efforts. Moreover, the insignificant influence of the type of local market and the taboos on the desire to intensify the cultivation is indicative of a low production being offered to a very broad and diversified market. This has already led to the erosion of taboos and a high price of the fruit and mesocarp in the Dahomey Gap. However, the areas of intensive organization remain those where the seed represents the most important economical product, even regardless of the nature of the local collectors involved in the marketing. It appears that the presence of Ibo communities as local collectors stimulates a higher economic ambition. This is brought about by strict planting based on a traditional selection process for large size endocarp/seed germplasm.

Areas with high sapling densities indicate the core regions where intensive cultivation is practiced and the very low damage to trees confirms again the priority crop status of sweet *Irvingia* in these areas. From there, the intensive cultivation process is spreading to reach regions where the old populations are being progressively replaced by a better stock. In a continuously changing environment where local species have no direct interest of local communities, where slash-and-burn agriculture prevails, where planting of exotic tree species increases and where there is a demand for high quality wood, the survival of any food tree species in forest gardens or agroforestry systems is jeopardized. Most importantly, the free access to bitter bush mangoes in natural areas causes competition between collectors, which strongly limits the natural regeneration (Arnold Ruiz Pérez, 2001; Rai and Uhl, 2004). Intensive logging of bitter trees by local communities progressively extending the area of cash crop production is leading to a significant decrease of their density even in the protected area of Bena. Effective control is hindered by a lack of funding and personnel, inactivity of most of the environmental conservation institutions and social and political instability in Togo

(Posner 2008). Most importantly, the taxonomic integrity of bitter trees is not locally recognized and for any research institution in Togo they are still being considered as *I. gabonensis* (see Atato *et al.,* 2010). This remains a key issue to be addressed seeing the current confusing taxonomical trends and debates on ABMTs (Okafor, 1975 and all recent work in Nigeria: Omokaro *et al.,* 1999; Nya *et al.,* 2000; Nzekwe *et al.,* 2004; Nya *et al.,* 2006; Olawode, 2010; Harris, 1996 and publications by the World Agroforestry Centre). It clearly may harm the protection of bitter ABMTs against ecologically destructive factors and has a negative influence on its genetic conservation (Freese, 1998). Because of that, more thorough investigations on all levels of the assessment of the taxonomic integrity of ABMTs (ecology, reproduction biology, morphology, and genetics) are worthwhile to perform. In the Dahomey Gap, ABMTs experience an uncertain conservation situation and, like many other tree species, are suffering from a lack of wise and efficient usage and conservation strategies. Modern land use planning policies are generally based on space-consuming cash crops that are well adapted to the Guinean climatic zone but which may totally destroy a fragile area with a pattern of small forest patches in need of protective actions.

Conclusions

This study demonstrates the multiple potentials of ABMTs and the geographic pattern of indigenous knowledge and use stimulates questions about the geographic origin of sweet trees' genetic material in the Dahomey Gap. The increasing cultivation initiatives, rooted in the high economic potentials, are broadening ABMTs geographic distribution over exhausted soils in the Dahomey Gap. More organized and intensive cultivation systems are being driven by the Ibo communities that are also involved in the local commercialization systems. As this is a possible crop for future development, further research needs to be performed regarding ABMTs' potential cultivatable areas and productivity in different ecological areas. This is important, not only in the Dahomey Gap, but throughout their entire distribution range. This supposes a full morphological characterization, the definition of the bush mango ideotypes with respect to the type of product targeted, and the capture, fixation and development of desirable genetic material. Therefore, the material of the World Agroforestry Centre domestication program, not only from the Dahomey Gap but from the whole of the western part of the ABMT distribution range, is vital to ensure its wider impact in sub-Saharan Africa.

Regarding any food trees species, but ABMTs is particular; there are two important challenges for scientists and development policy makers regarding the active role of local communities in the domestication process. For scientist, considering the rapid fragmentation of natural habitats in the tropics, at least the distribution of wild populations of NTFP species needs to be known. Then, for policy makers, especially better insights in the potential cultivatable area of such species would be very useful to assist decisions on land-use planning, while a good knowledge of the genetic variability is necessary for the *in situ* and *ex situ* conservation of germplasm.

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Chapter 4

Phenological diversity and distinction within African Bush Mango trees (*Irvingia*: Irvingiaceae) in the Dahomey Gap (West Africa)

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Abstract

African Bush Mango trees are priority food trees in Sub-Saharan Africa. The occurrence of bitter and sweet fruited trees is still the subject of taxonomic debate which hinders their effective use and conservation. This study investigates differences in phenological behaviour between bitter and sweet fruited populations and their taxonomic implications. Monthly phenological data of seven populations in Benin and Togo are used to assess phenological diversity within bush mango trees, to discriminate bitter and sweet trees and to investigate their responses to environmental factors. The Shannon-Weaver phenological diversity index is used to describe variability of phenological states among populations and between bitter and sweet trees. A Canonical discriminant analysis is performed to identify the phenological states differentiating bitter and sweet trees and a principal component analysis supplemented with a cluster analysis is used to group individual trees. Finally, analyses of variance are carried out on the reproductive phenological states against the month (time), FAO soil type, type of bush mango tree, and the climatic zone to evaluate the reproductive responses of the taxa. Phenological diversity varies significantly among populations. The very low phenological diversity of bitter trees can be explained by their limited distribution range in the study area. Phenological states consistently discriminate bitter and sweet trees. The two types also display significantly different reproductive responses to the investigated environmental factors except soils, supporting the idea that they represent two different species. A small overlap in flowering time offers a possibility for hybridization between bitter and sweet trees, but fruiting subsequent to this overlap only occurred in bitter trees.

Key words: Adaptation, ecology, domestication, *Irvingia*, phenological states.

Introduction

African Bush Mango Trees (ABMTs) produce an economically emerging non-timber forest product (NTFP) that reaches the regional and international markets of sub-Saharan-Africa (Tabuna, 2000). This NTFP, the seed of their mango-like fruit, is an important part of the diet of sub-Saharan African communities (Ekpe *et al.,* 2007). It is marketed within its hard endocarp, has high oil content and is increasingly used in pharmaceutical and cosmetic manufacturing (Ogunsina *et al.*, 2008). The mesocarp of the fruit is either bitter and inedible or sweet and highly appreciated and forms the main character that discriminates between two types of ABMTs. Tree-to-tree variation in morphology greatly overlaps between bitter and sweet fruited trees and makes it difficult to distinguish between them without having tasted the fruit (Ofafor, 1975; Harris, 1996; Ladipo *et al*., 1996; Leakey and Tchoundjeu, 2001). Recently, Harris (1996) and Lowe *et al*. (2000) considered sweet and bitter trees to be distinct at species level: *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. and *I. wombolu* Vermoesen, respectively. However, the variety level claimed by Okafor (1975), *I. gabonensis* var. *gabonensis* and *I. gabonensis* var. *Excels* (Mildbr) Okafor, continues to be used in literature as well (see Nzekwe *et al*., 2002; Nya *et al*., 2006; Ekpe *et al*., 2009). ABMTs are phenologically highly diverse and this study aims at investigating its potential to discriminate between the bitter and sweet trees.

Regardless the taste of the mesocarp, the seed of both types is the most important non-timber forest product (NTFP) for local communities in the humid forest areas of West and Central Africa (Tchoundjeu *et al*., 2002). That of bitter fruits has greater economic value because of its higher food technological properties (Ladipo *et al*., 1996). Hence, in-depth observations are needed to clarify the taxonomic status of ABMTs and to design a better cultivation and conservation strategy.

ABMTs have a large distribution from Senegal to Angola, up to West Sudan (Lesley and Brown, 2004; Orwa *et al*., 2009; Nkwatoh *et al.*, 2010; Dolor, 2011). Naturally, they supposedly occur at 200–500 m altitude, under mean annual temperatures of 25–32°C and 1,500–3,000 mm of annual rainfall (Kengni *et al*., 2011). But they also abundantly occur at low altitudes in forest areas in the coastal regions of West and Central Africa (see Lesley and Brown 2004). Their diversity centres are located in the Lower Guinean and Congolian forest blocks (Lowe *et al.*, 2000; Ude *et al*., 2006). Currently, agroforestry systems of ABMTs are spreading across West and Central Africa due to their growing economic value (Shiembo *et* *al.,* 1996; Nkwatoh *et al.,* 2010). ABMTs are also found in the particular eco-region called the Dahomey Gap which is a dry corridor between the two West African forest blocks (Maley, 1996). Only few investigations compared bitter with sweet ABMTs in their entire distribution range and comparative quantitative phenological data are particularly lacking. Moreover, West Africa, which is the second important part of their distribution range, has been poorly prospected.

Phenology is demonstrated to be highly environmentally sensitive (Rumland and Vulie, 2005), and therefore it is a useful tool to assess responses of plants to global climate change as well as to periodic variations of climatic factors (temperature, rainfall, solar radiation) in specific climatic zones (Chmielewski and Rötzer, 2001; Crepinsek and Kajfez-Bogataj, 2006; Nord and Lynch, 2009). Moreover, phenological variations could reveal particularities of different taxa in their physiological responses to soil variability as well as the availability of nutrients and their uptake by plants (Nord and Lynch, 2009). Phenological characteristics can, therefore, be used to support taxonomic decisions and, because of its close links to crucial cultivation activities like planting, maintenance and harvesting, also be of great importance in predicting economic potential of emerging new crops like ABMTs in different eco-regions.

The aim of this study was to provide comparative quantitative data on phenological response and to infer potential drivers of this variation as well as its taxonomic implications within ABMTs. Data of a thirteen-month phenological survey were used to:

(i) evaluate the phenological variation and the diversity among and between bitter and sweet ABMTs across different climatic zones in the Dahomey Gap,

(ii) determine discriminating phenological properties between bitter and sweet ABMTs,

(iii) assess the response of ABMTs in their reproductive phase to environmental factors in the Dahomey Gap.

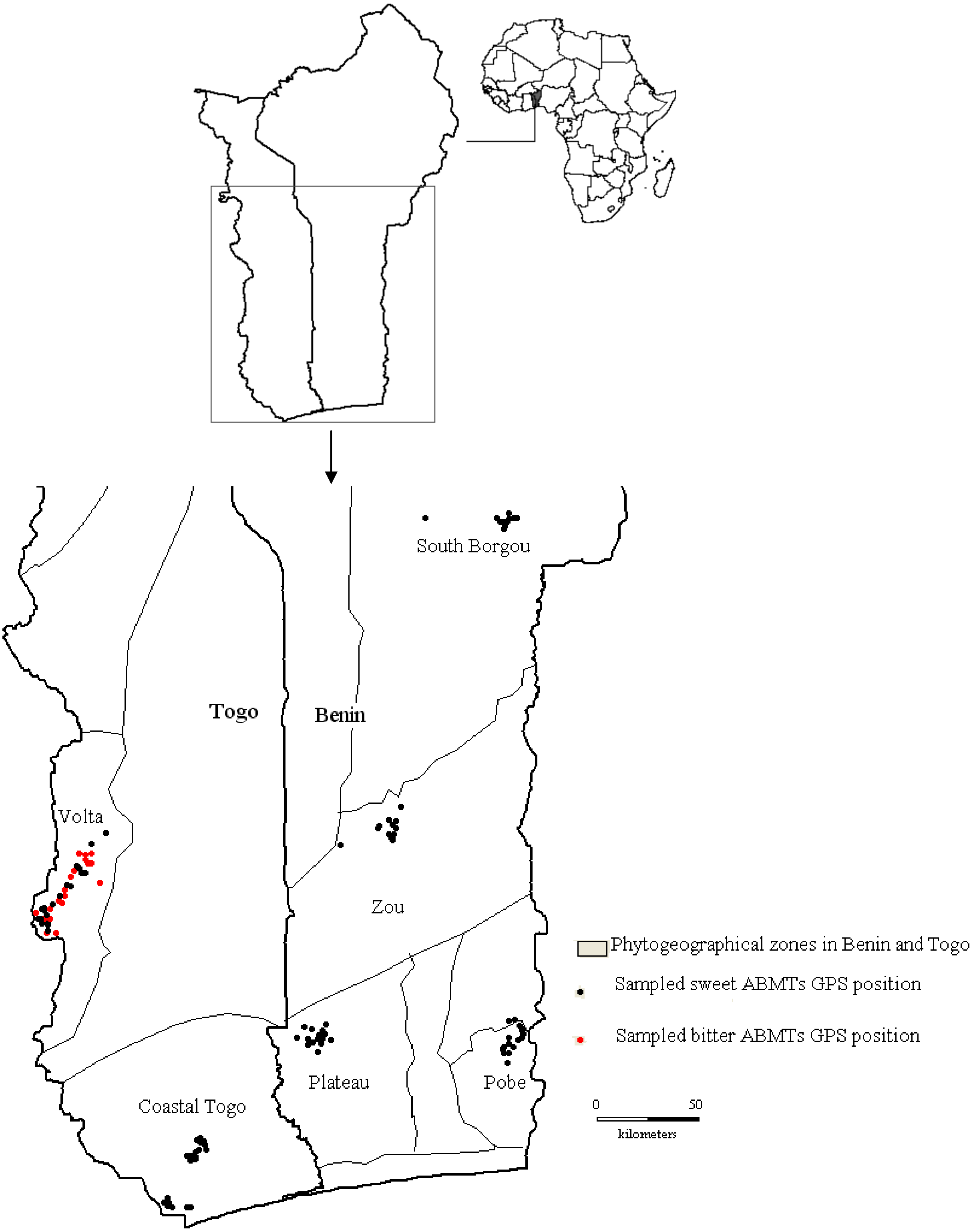


Figure 4.1: Geographic and phytogeographical locations of sampled populations.

Materials and methods

Study area

This study was carried out in six phytogeographical regions: Pobè, Plateau, Zou and South-Borgou in Benin (Adomou *et al.*, 2006), and the Coastal region and Southern Mountain forest region, also called Volta forest region, in Togo (Fig. 4.1). The southern parts of these two countries are located in the Dahomey Gap which splits the West African forest block into the Upper and Lower Guinean forest regions (Maley, 1996; Giresse, 2008). It consists of savannah stands with small-size forest relics and forms an obstacle for natural species exchange between the two forest blocks (Booth, 1957). It is a climatically induced anomaly dating from the Holocene (Maley, 1996) characterized by higher temperatures and lower rainfall. Despite these climatic parameters, numerous humid forest species still occur within this eco-region (Salzmann and Hoelzmann, 2005). Among them ABMTs, which occur in various land use systems and are increasingly cultivated to prevent soil degradation and enhance livelihood.

Sampling

Considering the type of bush mango tree, the local domestication strategy, and the phytogeographical region, seven populations of ABMTs were selected (Table 4.1). In each population a random sampling technique was applied and the number of trees was chosen in such a way that they could be surveyed in 1 to 2 days. Only trees that had already fruited at least once were selected. Within each population, eleven to thirty-eight mature trees separated by a distance of 100 m to 24 km were selected and geo-referenced, totalling to 173 individuals.

Monthly phenological surveys were carried out from October 2010 through October 2011, to describe three phenological phases: vegetative, flowering, and fruiting. Twelve phenological events describing the monthly changes that occur in the crown of each tree were defined. For the vegetative phase: (1) mature leaves, (2) leaves starting to drop, (3) leaves all dropped and (4) young leaves developing. For the flowering phase: (5) flower buds, (6) open flowers (ready for pollination), and (7) aborted flowers. For the fruiting phase: (8) immature fruits, (9) immature fruits aborted, (10) mature but unripe fruits, (11) mature unripe fruits aborted, and (12) mature and ripe fruits. Mature but unripe fruit was defined as a separate event, because it represents an important phase when looking at the effect of parasites, mostly in areas where the mesocarp remains the most important NTFP (Vihotogbé *et al.,* 2007) and might help design an improved pest management program. For each sampled tree and in each month, we observed and estimated the level at which each phenological event occurred in the volume of the crown: we referred to this as the monthly level of the related phenological event in this study. Five levels were defined and used to provide each tree with a monthly phenological score regarding each phenological event: 0 when the event did not occur in the crown; 1 when it occurred in 0-25% of the volume of the crown; 2 in 25-50%; 3 in 50-75%; and 4 in 75-100% (Fig. 4.2a, b, c). Thus, the raw phenological database consists of 173 rows (individuals) and 156 columns (13 months × 12 phenological events).

Table 4.1: Population characteristics.

Country	Phytogeographical region	Population name with reference to sampling and type of ABMTs	Climatic characteristic	Type and domestication state of the sampled population	Number of Sampled trees
Benin	Plateau	Couffo	Climate: Sub-humid Guinean; Rain fall: 900-1100 mm in bimodal regime (April-June and from September-November); Temperature: 25°C-29°C; Hygrometry: 76%-97%.	Only cultivated sweet trees under traditional selection process mainly for seed commercialization.	30
	Pobè	Pobè	Climate: Sub-humid Guinean; Rain fall: 1200-1300 mm in bimodal regime (April-June and from September-November) Temperature: 25°C-29°C; Hygrometry: 76%-97%	Mixture of pretended wild + spontaneous + cultivated sweet trees (with no selection process) for both entire fruit and mesocarp commercialization	38
	Zou	Dassa	Climate: Soudano-Guinean; Rain fall: 1100-1200 mm in unimodal regime (May-October); Temperature: 25°C-29°C; Hygrometry 31% to 98%.	Spontaneous and cultivated sweet trees (with no selection process) for household consumption and entire fruit commercialization	11
	Southern-Borgou	Parakou	Climate: Soudanian; Rain fall: lower than 900 mm in unimodal regime (June-November); Temperature: 24°C-31°C. Hygrometry: 18%-99%	Only cultivated sweet trees (with no selection process) for mesocarp and seed consumption and limited commercialization	13

Country	**Phytogeographical region**	**Population name with reference to sampling and type of ABMTs**	**Climatic characteristic**	**Type and domestication state of the sampled population**	**Number of Sampled trees**
Togo	Coastal region (Region V)	Lomé	Climate: Sub-humid Guinean with major rainfall deficit: 800 mm in bimodal regime (April-June and from September-November).	Only cultivated sweet trees (with no selection process) for mesocarp consumption and seed commercialization	32
	Southern Mountain or Volta Forest Region (Region IV)	Badou	Climate: Guinean; Rain fall: 1,168 - 2,103 mm in unimodal regime (Mars- October). Temperature: 21°C -32°C	Spontaneous and cultivated sweet trees (with no selection process) for entire fruit and seed commercialization.	15
				Only wild bitter trees still far from cultivation initiative and exploited by local communities and wood industries	32

Figure 4.2: Examples of levels attributed to phenological events. a = levels of mature leaves; b = levels of open flowers ready for pollination; c = levels of mature unripe fruits.

Data analysis

Using the raw phenological data for each phenological phase and for each month, a combination of the levels of the monthly phenological events (scores) was made to give a unique qualitative indicator that defines the phenological states for each sampled tree (see Goulart *et al.*, 2005). For example, a tree with ‘0004’ as vegetative state in December 2010 was characterized as that with a crown: (i) was completely devoid of mature leaves (0 %), (ii) was devoid of yellowing leaves ready to fall (0 %), (iii) has no freshly dropped leaves under it (0 %), and (iv) was completely filled with freshly renewed leaves (100 %) in December 2010. A tree coded ‘044’ for its flowering state in January 2011 had a crown: (i) with no flower buds, (ii) completely filled with open flowers able to be pollinated (100 %), but (iii) 100 % of these flowers aborted in this same month. Likewise, a fruiting state of ‘00012’ in October 2010 indicated a tree with: (i) no immature fruit in its crown (0%), (ii) no immature fruits being aborted (0 %), (iii) no mature and unripe fruit (0 %); (iv) few mature and unripe fruits aborted (25 %), and about 50 % of it volume filled of mature and ripe fruits.

For each month all possible phenological states were considered as separate columns in the data matrix. Each tree was then identified with 1 (presence) for its state, and 0 (absence) for all other states, resulting in a phenological presence/absence data matrix. This matrix was then used to calculate the monthly Shannon-Weaver phenological diversity index (Goulart *et al*., 2005) for each population and phenological phase:

$$H(ijk)H_{(ijk)} = \sum[p_{(ijk)\alpha} * \ln p_{(ijk)\alpha}] \tag{1}$$

H_{ijk} is the diversity index for the i^{th} population in the j^{th} phenological phase and in the k^{th} month and $p_{(ijk)}$ is the number of trees in the i^{th} population in the j^{th} phenological phase in the k^{th} month that presented the same α^{th} phenological state divided by the total number of individuals in that population.

The value of this index ranges from zero for a population in which all individuals present the same phenological state for a given phenological phase in a given month to high values (close to 1) for more phenologically diverse populations. This index was used to compare the seven populations and the two types (bitter and sweet) of ABMTs.

All the monthly phenological diversity values (H_{ijk}) per phenological phase were recorded. Using this H_{ijk} database and considering the phenological phases as replications, a one way analysis of variance (ANOVA-1) with repeated measurements was carried out in Statistica Version 6 against the population in order to evaluate differences in the overall phonological diversity. Moreover, individual trees that belong to either type of ABMTs (sweet versus bitter) were grouped and the monthly phenological diversity for each phenological phase was also calculated.

The raw phenological data matrix was checked and monthly phenological events that presented zero variance were removed. The correlation matrix of the remaining events was computed and multicollinearity was eliminated considering a cut-off value of 70%. A final phenological data matrix was created containing 117 monthly phenological events describing the 173 trees.

To analyse phenological differences between bitter and sweet ABMTs, the final phenological data matrix was used in a multivariate analysis. A Canonical Discriminant Analysis (CDA) was performed on the monthly levels of phenological events against the type of ABMTs using SAS. Prior probability proportional to sample size was assigned and posterior probabilities with the classification of individuals as being either bitter or sweet were computed. In order to allow the comparison under an uniform ecological condition, bitter and sweet ABMTs from the Volta region were used to calibrate the model which was used to classify the rest of individuals from the other populations, and the classification probability was also computed. An ANOVA-1 was run in SAS on the discriminating factors (monthly phenological events) against the type of ABMTs to obtain their levels of variation.

Phenological similarity among individuals is considered to be indicative of synchrony in life cycle events (Goulart *et al*., 2005) and might help detect the spatial partitioning of the phenological diversity. To group individuals based on their phenological similarity, multivariate analyses were used. The application of two consecutive multivariate analyses was revealed useful to improve the classification of individuals (Mohammadi and Prasanna, 2003; Bidogeza *et al.*, 2009). First, a Principal Component Analysis (PCA) was carried out on the monthly levels of the 117 independent monthly phenological events using PAST (Hammer *et al*., 2001). Second, the monthly phenological events correlated (using a cut-off of 70%) with the first six axes were used in a cluster analysis based on the Bray-Curtis similarity index (Dbc_{jk}; see Bloom, 1981 for the choice of this index):

$$(Dbc_{jk}) = \frac{\sum_{k=1}^{n} |x_{ik} - x_{jk}|}{\sum_{k=1}^{n} (x_{ik} - x_{jk})} \qquad (2)$$

With i and j^n being two random individual trees characterized by a set of n monthly phenological events, k being a random phenological state, and with x_{ik} and x_{jk} being the levels for individuals i and j at the k^{th} monthly phonological event, respectively. Based on this index among individual ABMTs, a dendrogram was produced to visualize the similarities.

Reproductive response of ABMTs to environmental variations in the Dahomey Gap

To assess the impact of environmental variation on the reproductive response of ABMTs in the Dahomey Gap, four phenological events related to their reproductive success were studied in more detail. These are: level of flowers able to receive pollen, level of flower abortion, total level of fruit abortion and level of mature and ripe fruits. The total level of fruit abortion was obtained by adding the level of immature fruit abortion to that of the mature unripe fruit abortion. The GPS position of the trees was used to get the corresponding FAO soil type (http://www.fao.org/geonetwork). Using SAS, four ANOVA’s (generalized linear model) were carried out on the level of these reproductive success variables against the type of ABMTs, phytogeographical region, soil type and season (month). The additive effects of those variables and the joint effects of type, phytogeographical region and soil type with season were assessed.

Results

Phenological variation and diversity within ABMTs

The ANOVA-1 with repeated measurements indicates significant differences in the overall phenological diversity among populations (P = 0.028). The population of Lomé followed by that of Pobè have the highest diversity index, while those of Dassa, Couffo and Parakou are phenologically the least diversified ones (Fig. 4.3a).

The bitter trees show slightly higher phenological diversity in November and December for the flowering phase and in January for the fruiting phase (Fig. 4.3c). Apart from those periods, a higher Shannon-Weaver diversity index was calculated for sweet ABMTs for all three phenological phases and in each month (Fig. 4.3b, c, d). For both types the vegetative diversity was generally higher in the most severe dry season from November through March than in the rainy season and in the short dry season from April through October (Fig. 4.3b). For the bitter trees, little variation of the diversity was found throughout the year for the flowering phase (1.5 to 2) as well as for the fruiting phase (1.2 to 2), while the sweet trees presented high variation of diversity ranging from 1.3 to 6 and 1.2 to 9 for flowering and fruiting phases, respectively (Fig. 4.3c, d).

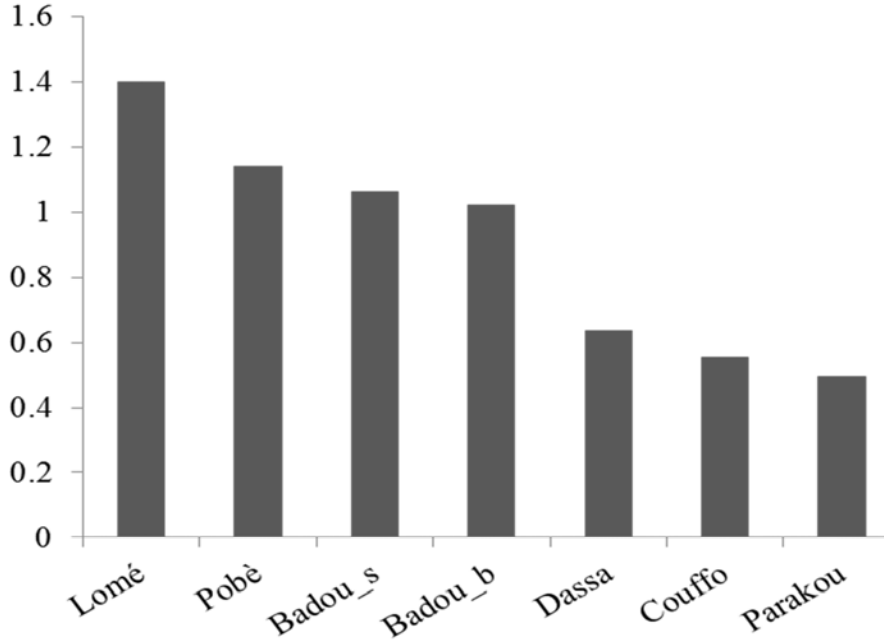


Figure 4.3a

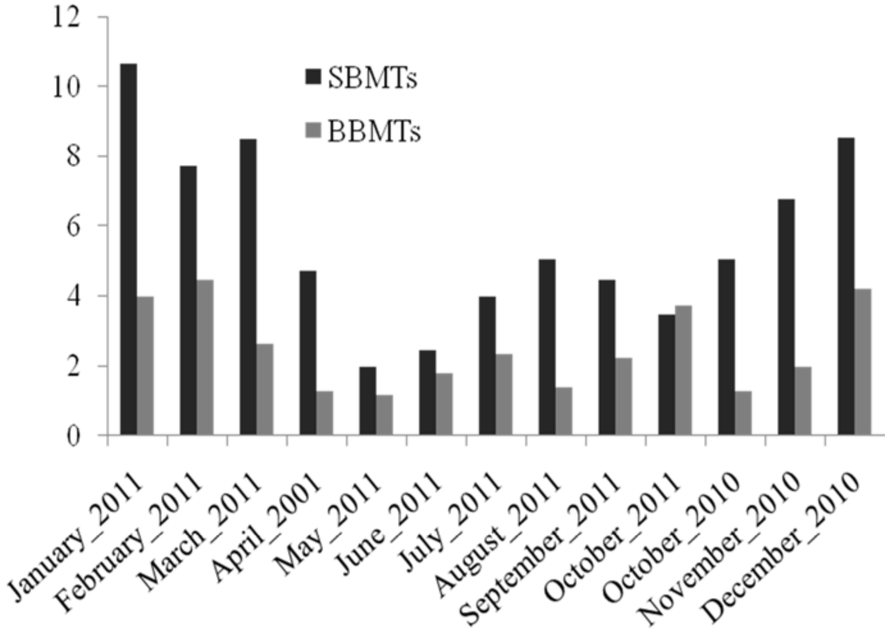


Figure 4.3b

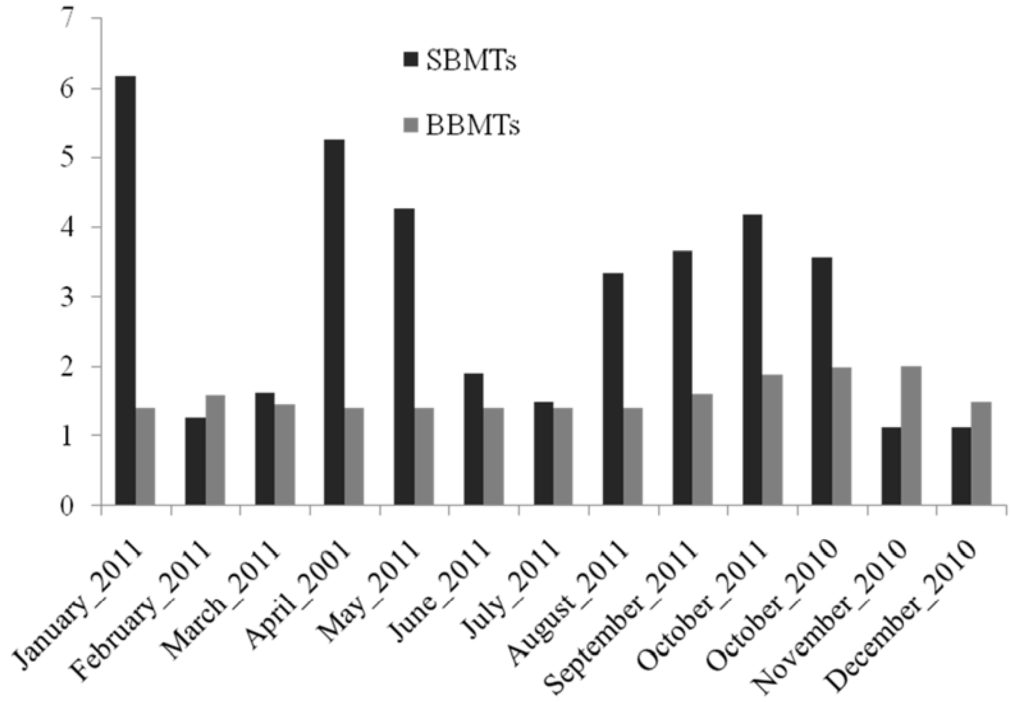


Figure 4.3c

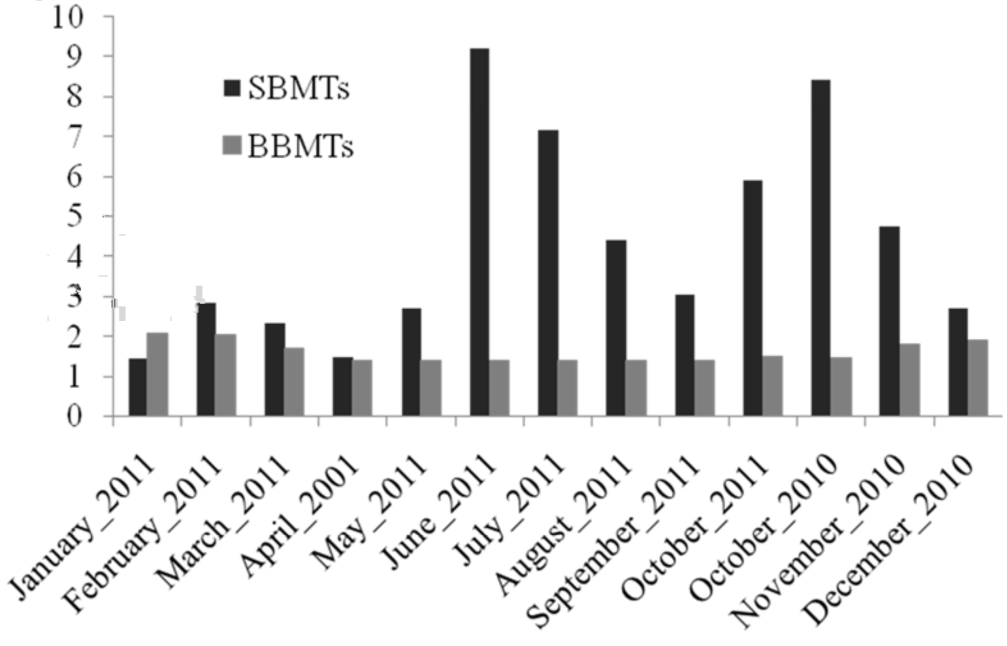


Figure 4.3d

Figure 4.3: Shannon diversity among populations and between bitter and sweet trees. Badou_s = sweet trees from Badou in the Volta region; Badou_b = Bitter trees from Badou in the Volta region; SBMTs = sweet bush mango trees; BBMTs = bitter bush mango trees: a = Global phenological diversity among populations; b = Vegetative diversity between bitter and sweet trees; c = Flowering diversity between bitter and sweet trees; d = Fruiting diversity between bitter and sweet trees

Phenological discrimination of bitter and sweet ABMTs

The vegetative phase does not distinguish among populations or between tree types (Fig. 4.4a-d). The reproductive phases (flowering and fruiting), on the other hand, show distinctive differences (Fig. 4.4e-l). For example, three main flowering periods were observed: December-January, March-May and August-November (Fig. 4.4e, f). While sweet trees flowered at all of these periods, bitter trees flowered only in September-October (Fig. 4.4e). Consequently, sweet trees bear immature fruits throughout the year with three peak periods in February-March, May-June and especially in September-November (Fig. 4.4h). For bitter trees, this period is mostly limited to November-December (Fig. 4.4h). Bush mango fruits are fully grown and ripe in three main periods: January-April, June-September and October-December (Fig. 4.4l). While mature and ripe sweet fruits are available in all of these three periods, the bitter ones are available only in the first period. It is clear that these three phenological characteristics are highly correlated, which is why subsequent analyses were performed on each of the states separately.

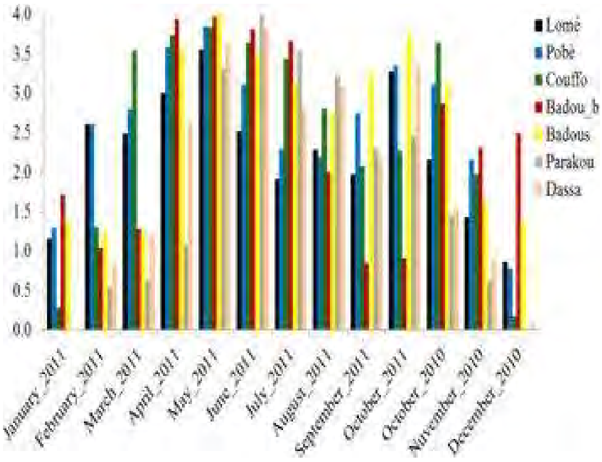
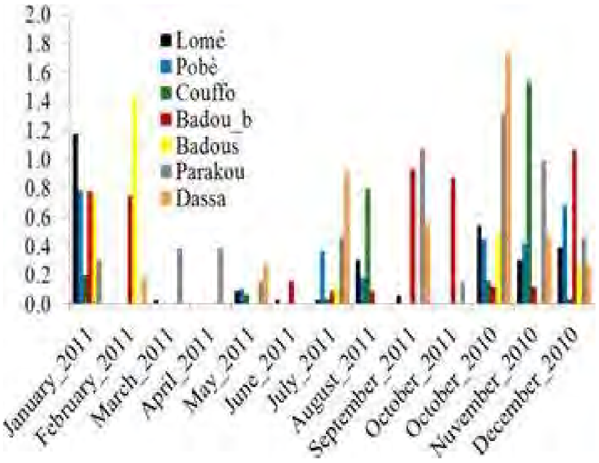


Figure 4.4a:



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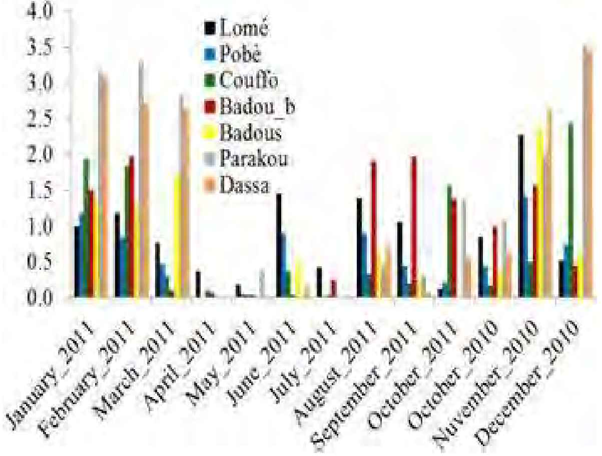


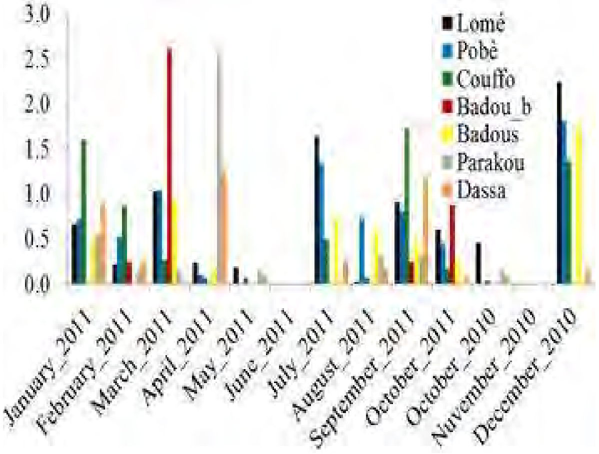
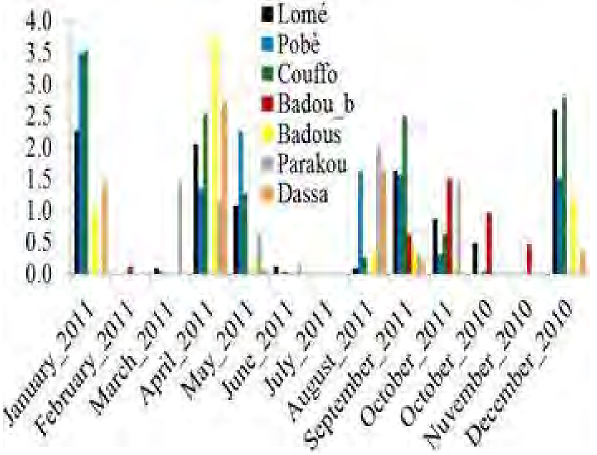


Figure 4.4c:

Figure 4.4d:



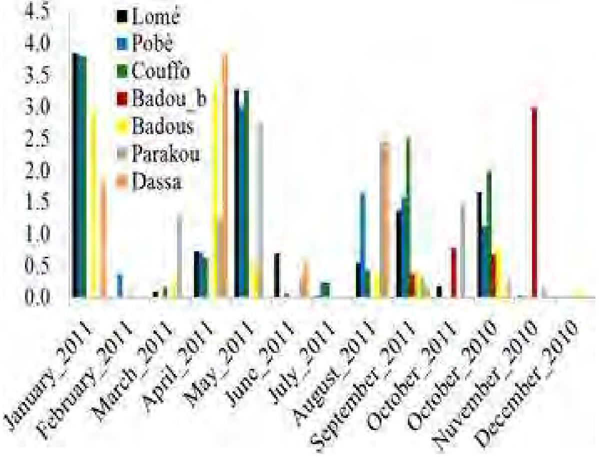
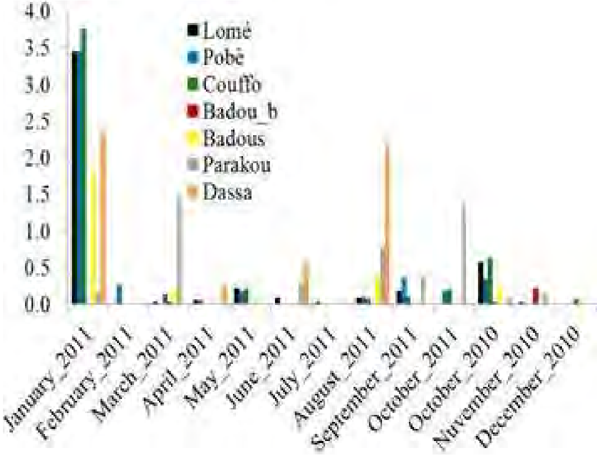


Figure 4.4e

Figure 4.4f



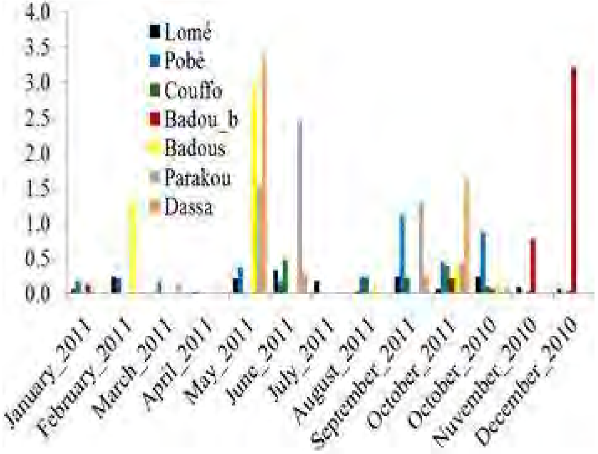
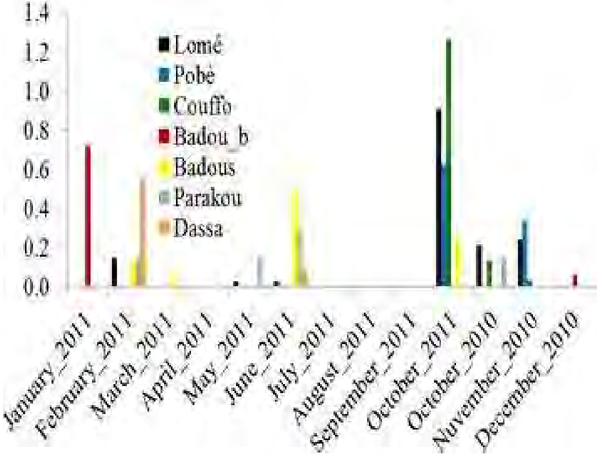


Figure 4.4g

Figure 4.4h



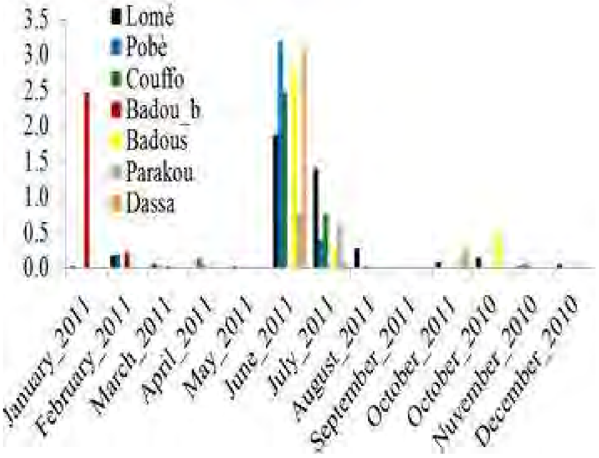


Figure 4.4i

Figure 4.4j

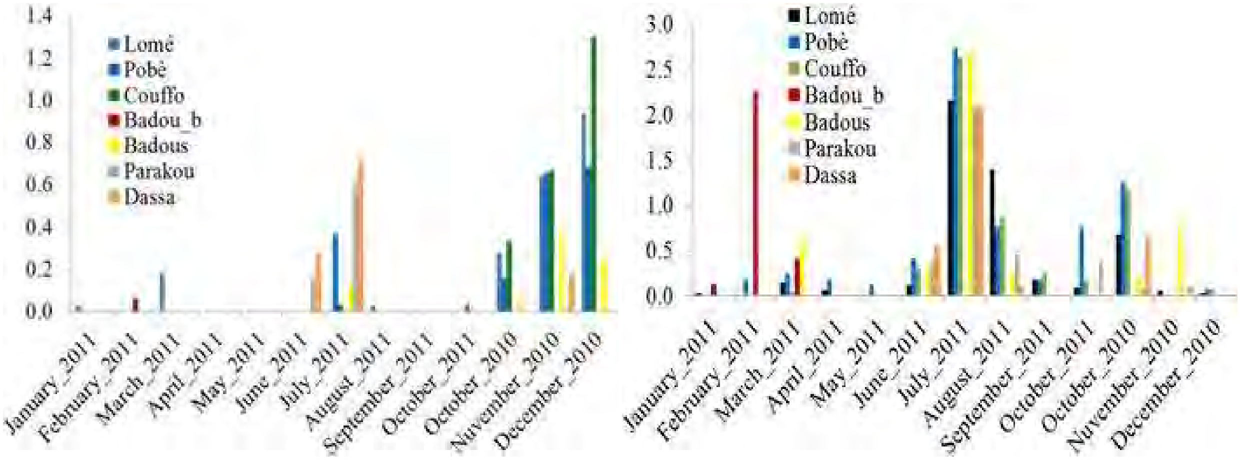


Figure 4.4k **Figure 4.4l**

Figure 4.4: Variations of the mean of the monthly level of the phenological events: (**a**) mature leaves, (**b**) leaves starting to drop, (**c**) leaves completely dropped (**d**) leaves freshly renewed, (**e**) flower buds, (**f**) open flowers, (**g**) aborted flowers, (**h**) immature fruits, (**i**) immature fruits aborted, (**j**) mature but unripe fruits, (**k**) aborted mature unripe fruits, and (**l**) mature ripe fruits.

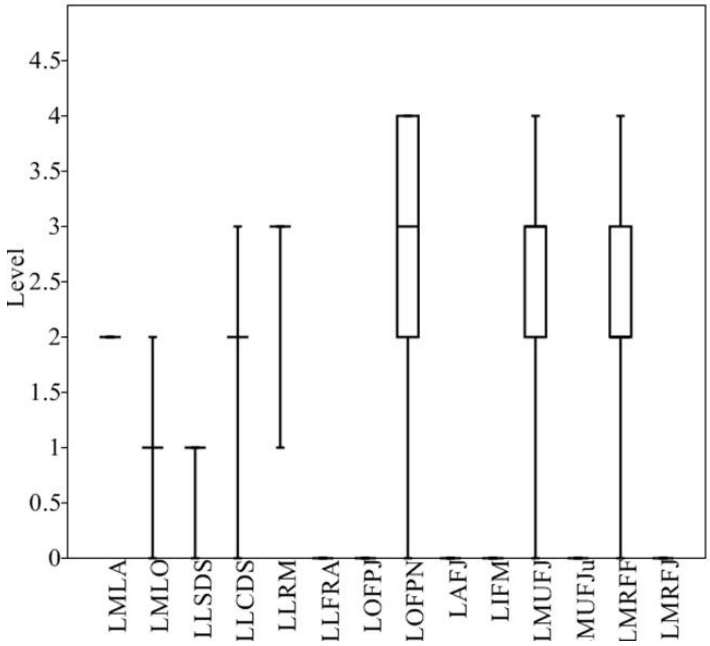


Figure 4.5a

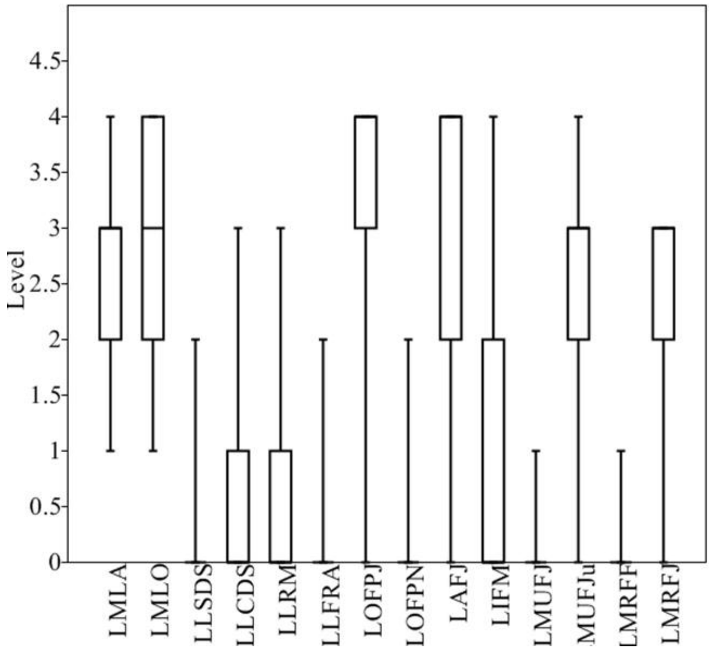


Figure 4.5b

Figure 4.5: Variation of the levels of discriminating monthly events: a = bitter trees, b = sweet trees: MLA=Level of mature leaves in August, LMLO=Level of mature leaves in October 2011, LLFRA=Level of leaf renewal in August, LOFPJ= Level of open flowers ready to be pollinated in January, LAFJ=Level of aborted flowers in January, LIFM=Level of immature fruits in May, LMUFJu=Level of mature unripe fruits in June, LMRFJ=Level of mature ripe fruits in July, LLSDS=Level of leaves starting to drop in September, LLCDS=Levels of leaves completely dropped in September, LLRM=Level of leaves renewed in March, LOFPN=Level of open flowers ready to be pollinated in November, LMUFJ=Level of mature unripe fruit in January, LMRFF=Level of mature ripe fruit in February.

The CDA to distinguish bitter and sweet ABMTs indicates that 52 of the 117 (that is 44%) monthly phenological events have significant discriminating power, when considered in isolation ($P < 0.001$). Together, their discriminant power is also consistent ($P < 0.001$). The total canonical structure of bitter and sweet ABMTs from the Volta region indicates the presence of fourteen significant discriminating monthly phenological events (absolute value of correlation ≥ 70%): level of mature leaves in August and in October 2011, leaves starting to drop in September, leaves completely dropped in September, leaves freshly renewed in March and in August, open flowers in January, and in November, aborted flowers in January, immature fruits in May, mature unripe fruits in January and in June, and mature ripe fruits in February and in July (Table 4.2a). Based on these discriminating monthly phenological events, all bitter and sweet ABMTs were consistently classified (100% with classification probability equalling to 1) in their respective group during the model calibration as well as during the classification of the samples of sweet trees from other populations. Results of the ANOVA-1 indicate that significant differences exist between bitter and sweet ABMTs regarding the discriminating monthly phenological events ($P < 0.001$). For example, bitter ABMTs (i) renewed leaves more intensively in March: level of leaves renewed= 2.6 (SD = 0.21) against 0.7 (SD = 0.11) for sweet trees; (ii) flowered more intensively in November: level of open flowers ready to be pollinated = 4 (SD = 0.5) against zero merely value for sweet trees and (iii) presented completely mature and ripe fruits in February: level of mature and ripe fruits = 2.2 (SD = 0.45) against almost zero values for sweet ABMTs. In the case of sweet ABMTs, their high flowering in January: level of open flowers ready to be pollinated = 4.4 (SD = 0.2) was followed by abundant abortion in the same month: level of flower abortion = 2.8 (SD = 0.16), and their two fruiting times in November and July distinguished them from bitter ABMTs (Fig. 4.5a-b).

The result of the PCA analysis shows that the first six PCA axes account for 74.51% of the phenological variation within the dataset. In total, 40 monthly phenological events are correlated with the first six PCA axes (Table 4.2b). The dendrogram obtained from the cluster analysis using those 40 monthly phenological events based on the Bray-Curtis similarity index separates all bitter ABMTs from the sweet ones at 0.24 similarity (Fig. 4.6). Three sub-groups can be distinguished within the group of sweet ABMTs: the first (G_1) clusters the population of Parakou (in the Southern-Borgou region) and is completely separated from other sweet trees at 0.44 similarity, the second (G_2) clusters trees of three populations: Pobè (Pobè region), Lomé (Coastal Togo region) and Couffo (Plateau region). In addition to those three populations, a few sweet trees from the Volta and Zou regions fall in this group. The last sub-group is separated from the second at 0.42 similarity and includes the other trees from the Volta and Zou regions (G_3; Fig. 4.6).

Table 4.2a: Results of the Discriminate Canonical Analysis.

Phenological event	**Canonical Variable (CAN1)**	**Class Mean on Canonical Variable (CAN1)**	**Type of ABMTs Characterized**
Level of mature leaves in August	0.82	4 * 106	Sweet ABMT
Level of mature leaves in October 2011	0.96		
Level of leaves freshly renewed in August	0.74		
Level of open flowers ready to be pollinated in January	0.89		
Level of aborted flowers in January	0.75		
Level of immature fruits in May	0.81		
Level of mature unripe fruits in June	0.92		
Level of mature ripe fruits in July	0.94		
Level of leaves starting to drop in September	-0.91	-2 * 106	Bitter ABMTs
Levels of leaves completely dropped in September	-0.89		
Level of leaves renewed in March	-0.74		
Level of open flowers ready to be pollinated in November	-0.82		
Level of mature unripe fruits in January	-0.77		
Level of mature ripe fruits in February	-0.81		

Table 4.2b: Results of the PCA analysis on the phenological states

Phenological state	**PCA 1 (28.86)**	**PCA 2 (12.94)**	**PCA 4 (11.14)**	**PCA 4 (8.47)**	**PCA 5 (7.75)**	**PCA 6 (4.45)**
Mature leaves in March 2011	-0.62	0.12	0.07	0.16	0.64	-0.06
Mature leaves in August 2011	-0.06	0.65	-0.54	-0.10	-0.01	-0.14
Mature leaves in October 2011	-0.06	0.15	0.40	0.04	0.69	-0.16
Mature leaves in December 2011	0.67	-0.21	0.41	0.18	0.14	-0.06
Start of leaf dropping in January 2011	-0.19	-0.62	0.14	0.14	-0.44	0.04
Start of leaf dropping in September 2011	0.65	0.01	-0.44	-0.18	-0.04	0.41
Start of leaf dropping in October 2011	0.74	-0.44	-0.08	0.01	0.26	0.02
Crown leafless in April 2011	-0.04	0.14	-0.11	0.77	-0.11	-0.14
Crown leafless in September 2011	0.46	-0.71	-0.04	0.26	0.01	0.06
Crown leafless in December 2011	-0.09	0.60	-0.62	-0.28	0.12	-0.02
Leaf renewal in March 2011	0.41	-0.71	0.24	-0.05	-0.01	0.10
Leaf renewal in May 2011	-0.04	0.24	-0.09	0.66	-0.17	-0.08
Leaf renewal in August 2011	0.05	0.40	0.71	-0.01	-0.02	0.09
Flower initiation in January 2011	-0.74	-0.04	0.26	-0.21	0.24	0.26
Flower initiation in September 2011	-0.61	-0.44	-0.22	-0.15	0.29	0.07
Flower initiation in December 2011	-0.71	-0.44	-0.09	0.05	-0.14	0.10
Mature flowers in January 2011	-0.82	0.04	0.42	0.18	0.06	0.00
Mature flowers in February 2011	0.06	0.44	0.70	-0.05	0.20	0.28
Mature flowers in May 2011	-0.69	-0.10	0.00	0.18	-0.16	0.45
Mature flowers in September 2011	-0.67	-0.40	-0.24	-0.18	0.40	0.06
Flower abortion in January 2011	-0.89	-0.06	0.10	0.02	0.06	0.06
Mature flowers in October 2011	-0.68	-0.50	-0.06	-0.19	-0.06	0.14
Immature fruit in October 2010	-0.04	0.44	-0.25	-0.22	0.12	-0.74
Mature unripe fruits in January 2011	0.69	-0.44	-0.05	0.01	0.27	-0.01
Mature unripe fruits in June 2011	-0.67	0.14	0.40	-0.44	-0.14	-0.15
Mature unripe fruits in August 2011	-0.02	0.12	-0.09	0.69	-0.16	-0.02
Aborting mature unripe fruits in November 2010	-0.64	-0.28	0.09	0.09	-0.09	0.10
Aborting mature unripe fruits in December 2010	-0.71	-0.47	-0.08	-0.21	-0.04	0.18
Mature and ripe fruits in February	0.71	-0.49	0.08	-0.01	0.41	0.05
Mature and ripe fruits in July	-0.74	0.20	0.19	-0.40	-0.25	0.05

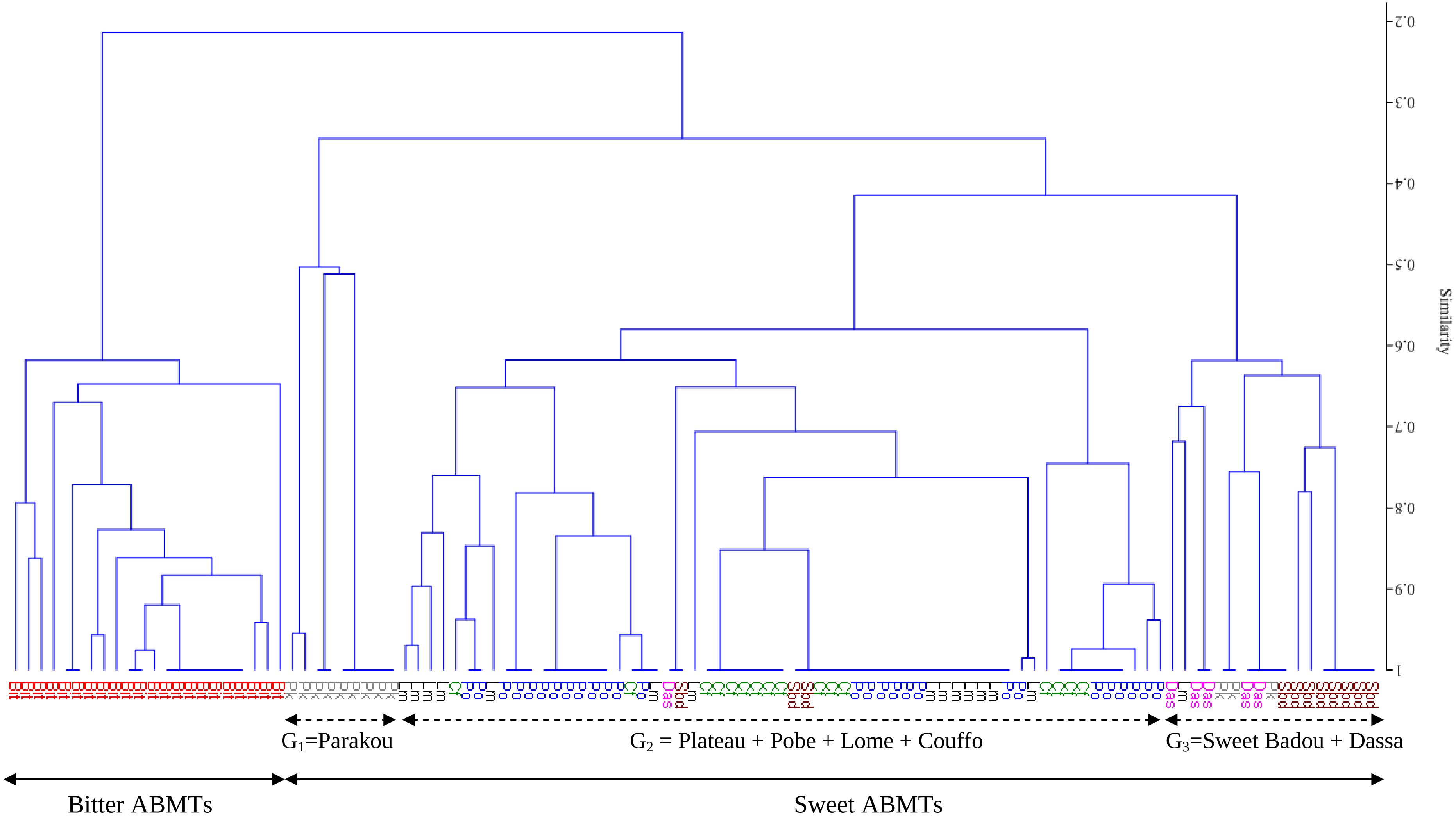


Figure 4.6: Classification of ABMTs based on the Bray-Curtis similarity index

Influence of environmental factors on ABMTs reproductive success

The results of the ANOVA show that the monthly variation of the flowering level of ABMTs significantly depends on the type of ABMTs and varies throughout the year ($P < 0.001$). Sweet ABMTs have the highest monthly flowering level in three peak times (January, May and September), while bitter trees flower only once (around November Fig. 4.7a). Although not during in the peak of their flowering, the two types of ABMTs co-flower in October (Fig. 4.7a). Flower abortion turns out to be higher for sweet ABMTs where it occurs throughout the year with its peak in January ($P< 0.001$; Fig. 4.7b). Significant differences exist between bitter and sweet ABMTs regarding the level and period of total fruit abortion ($P< 0.001$; Fig. 4.7c). Sweet trees abort repeatedly with their highest level in September-December, while bitter trees abort fruits once and intensely in January. Bitter and sweet ABMTs also show significant differences regarding the period of availability of mature and ripe fruits ($P< 0.001$; Fig. 4.7d). Mature and ripe fruits were recorded twice for sweet trees (July and October), while bitter trees have only one completely distinct fruiting period in February.

Significant differences exist among phytogeographical regions regarding the monthly variation of the flowering level ($P< 0.001$; Fig. 4.7e), and flower abortion level ($P< 0.001$; Fig. 4.7f).The observed flowering pattern in Fig. 4.7a was confirmed for the sweet tree populations of Pobè, Coastal Togo and Plateau as well as for the bitter trees in the Volta region. The January flowering was medium in the sweet tree populations of Zou and Volta and completely absent in South Borgou (see Fig. 4.7e). Apart from the January flower abortion, populations of Zou and South Borgou aborted flowers more repeatedly and at higher levels than in other phytogeographical regions (Fig. 4.7f). The phytogeographical regions also significantly influence the monthly level of mature and ripe fruits ($P< 0.001$; Fig. 4.7g). Apart from the Volta region, where mature and ripe fruits were found in three peak periods because of the presence of both bitter and sweet trees, the bimodal fruiting pattern observed for sweet trees (Fig. 4.7d) was observed in all regions. However, the populations of Pobè and Plateau have the highest mature and ripe fruit levels in the Dahomey Gap.

Finally, the monthly level of mature and ripe fruits significantly depended on the soil type ($P< 0.001$; Fig. 4.7h).

Figure 4.7: Variation in reproductive phenological events with type of ABMT and environmental factors. Fig. 4.7a: Difference in temporal variation of flowering level throughout the year between bitter and sweet ABMTs. Fig. 4.7b: Difference in temporal variation of flower abortion level throughout the year between bitter and sweet ABMTs. Fig. 4.7c: Difference in temporal variation of total fruit abortion level throughout the year between bitter and sweet ABMTs. Fig. 4.7d: Difference in temporal variation of mature ripe fruit level throughout the year between bitter and sweet ABMTs. Fig. 4.7e: Difference in temporal variation of flowering level throughout the year between phytogeographical regions. Fig. 4.7f: Difference in temporal variation of flowering abortion level throughout the year between phytogeographical regions. Fig. 4.7g: Difference in temporal variation of mature ripe fruit level throughout the year between phytogeographical regions. Fig. 4.7h: Difference in temporal variation of mature ripe fruit level throughout the year between soil types.

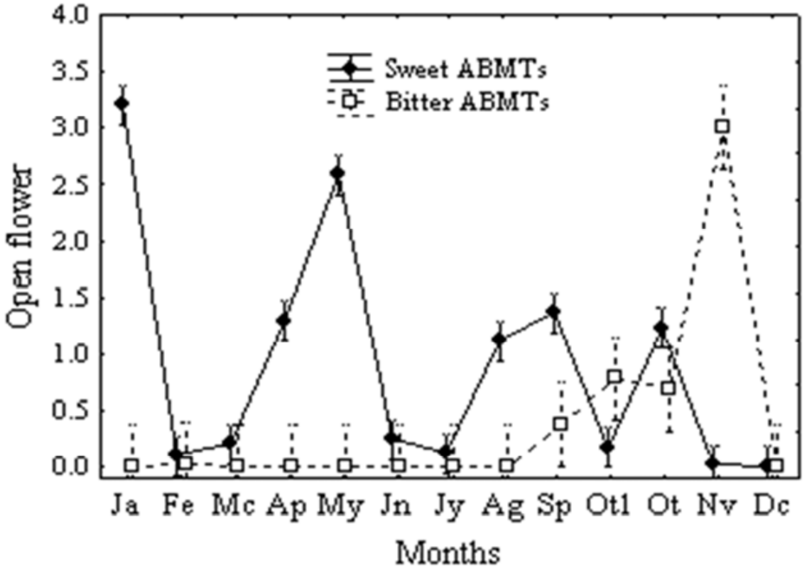


Figure 4.7a

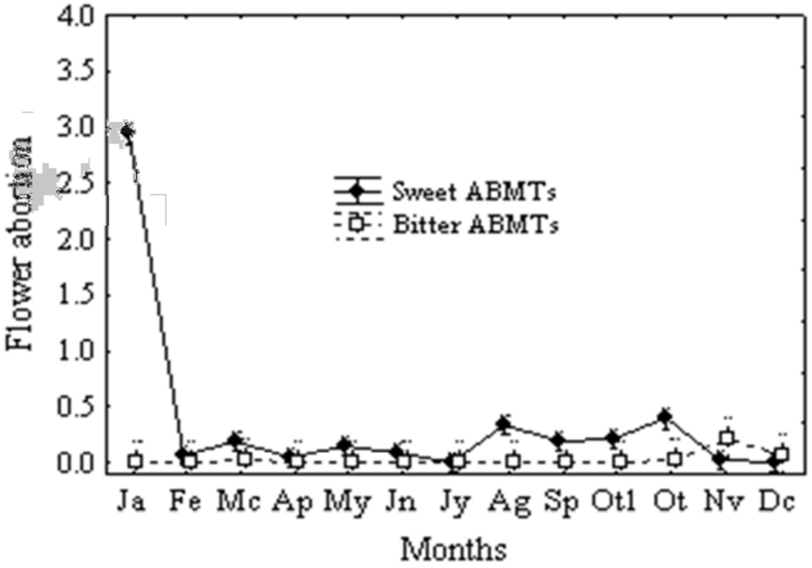


Figure 4.7b

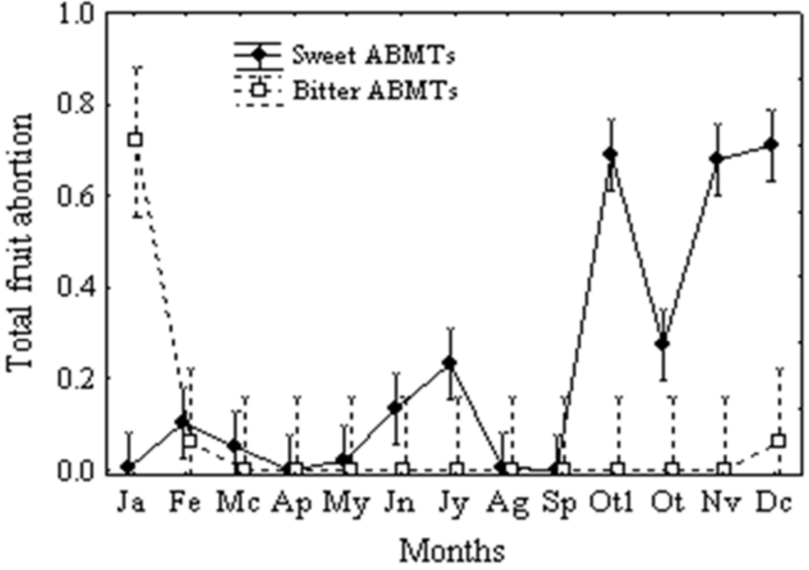


Figure 4.7c

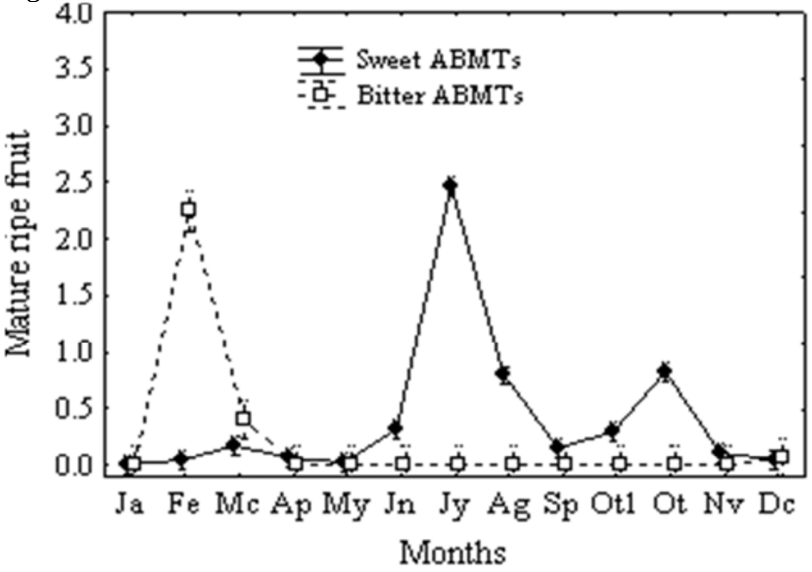


Figure 4.7d

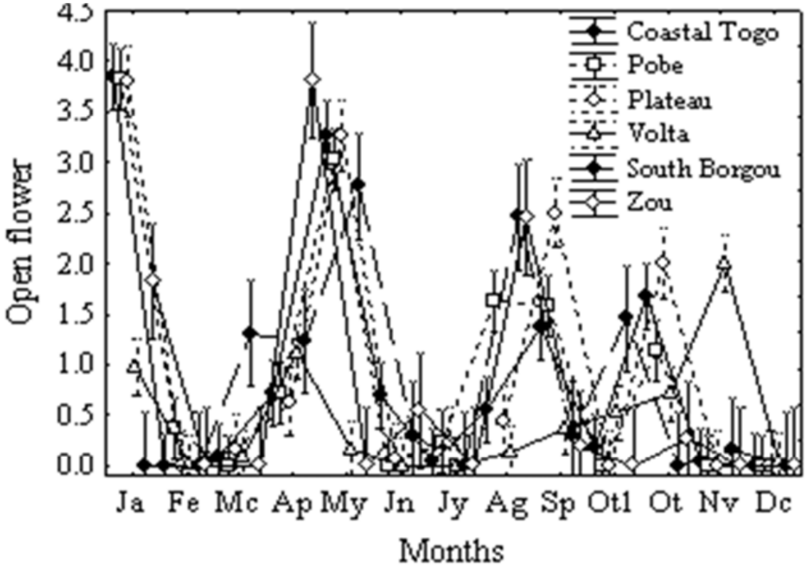


Figure 4.7e

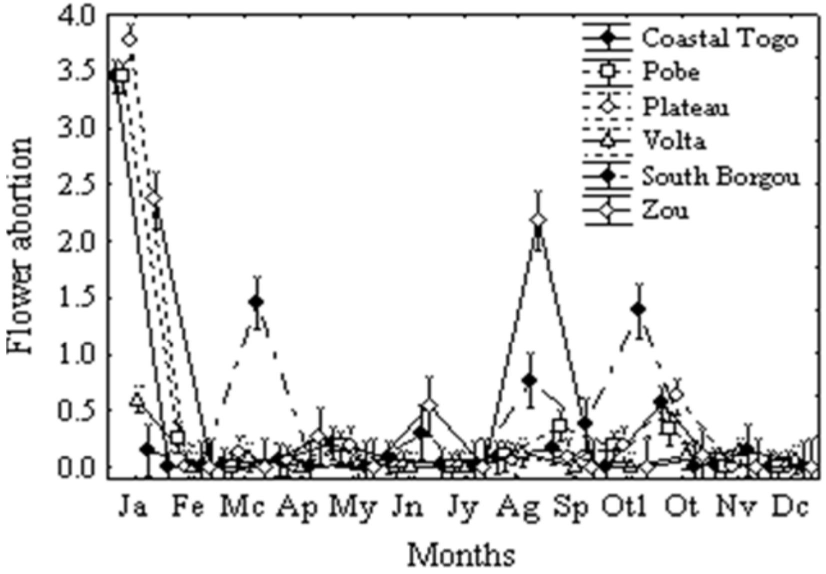


Figure 4.7f

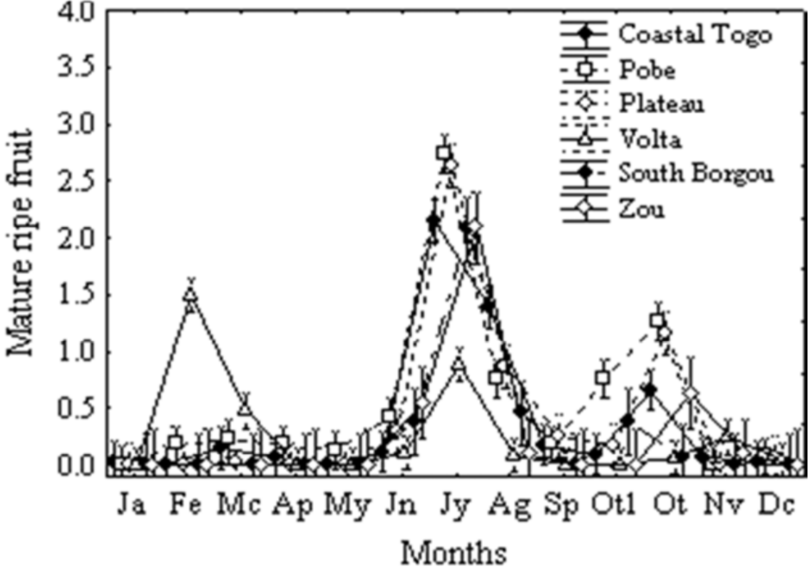


Figure 4.7g

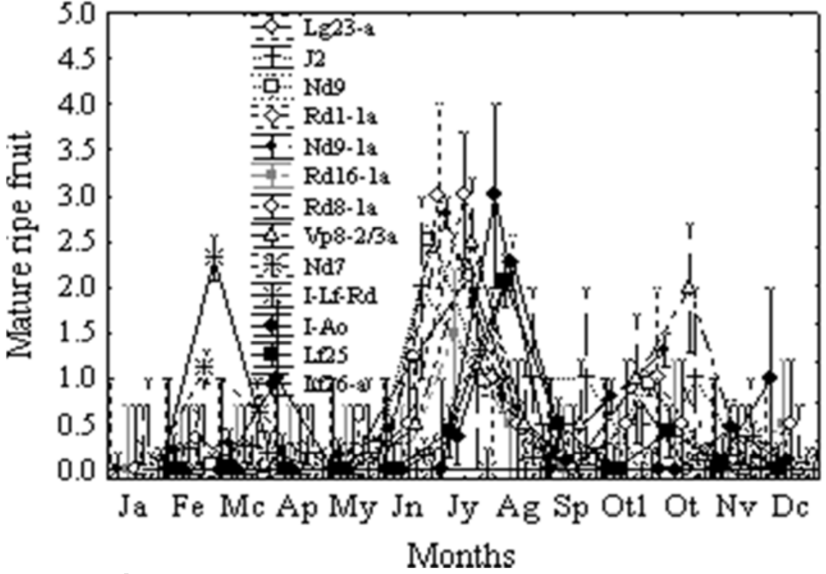


Figure 4.7h

Discussion

Phenological patterns and diversity

This study shows that in the Dahomey Gap the general phenological pattern of ABMT populations is confusing because of repetitive overlap in phenological events, mostly in the vegetative phase. However, phenological events (mostly in the reproductive phases) are generally more restricted in time for bitter trees while they stretch throughout the year for sweet trees. Lesley and Brown (2004) presented two flowering periods and repetitive fruiting events as a general phenological variation for both bitter and sweet trees. Harris (1996) showed variation in flowering and fruiting times in Central Africa (Cameroon and Gabon) and our results in the Dahomey Gap confirm the restricted reproductive phases detected by Harris (1996) and the results of Nzekwe *et al*. (2008) for bitter ABMTs in both the Guinean and Congolian African forest blocs. In Central Africa, Ladipo *et al*. (1996) reported natural bi-modal reproductive phases (as a general flowering and fruiting pattern) for sweet ABMTs, while Harris (1996) and Leakey and Simons (1998) presented the uni-modal flowering and fructification as the normal life cycle of ABMTs in the same eco-region, and attributed the bi-modal reproductive phases to the impact of domestication. Our data agree with this suggestion, because the highly domesticated sweet trees in the Dahomey Gap display an array from bi- to tri-modal and even higher flowering frequencies, but never a uni-modal one.

Considering the entire distribution range of ABMTs, evidence of bi-modal phenological patterns (see Newstrom *et al*., 1994) exists within natural as well as cultivated populations that show potential for selection and the establishment of more productive agroforestry systems in West and Central Africa. High rates of habitat degradation might play an important role in narrowing the phenological phases resulting in progressive irregularity of phenological events (see Herrerías-Diego *et al*., 2006). The extension of this bi-modal pattern for sweet trees to a much drier region like the Dahomey Gap (and even in some drier regions with precipitations less than 900 mm/year like in the North of Benin) is indicative of their wide ecological amplitude. In addition to their occurrence in contrasting ecological regions, the sudden occurrence of reproductive phases after unexpected short term changes within the climate regimes indicates the rapid adaptive response of sweet ABMTs to environmental change. This may well explain their abundance in agroforestry systems all over West and Central Africa (Shiembo *et al*., 1996).

The general phenological diversity is significantly different among populations. The sweet tree populations of Lomé, Pobè, and Couffo are all located in the Guinean climatic zone. Since plant phenological variation is partitioned by climate (Chuine, 2010), populations in the same climatic zone are expected to show identical phenological pattern. The differences in the phenological diversity within ABMTs populations in the Guinean climatic zone might be related to a difference in local domestication strategies (see Hodghin *et al.*, 1995) as well as to other local environmental factors, suspected here to be soil types. Populations of entirely spontaneous sweet trees (natural regeneration after mesocarp consumption by humans, without any management initiative) have a higher phenological diversity than the cultivated ones under the traditional mass selection process for seed commercialization. Since trees that show the same character (large seed) are being preserved and propagated, this directional selection strategy is narrowing the phenological diversity by synchronizing the reproductive phases (flowering and fruiting) and, thus, concentrating the harvest into two restricted times (see Ladizinsky, 1998, Leakey et *al.*, 2005; Munguia-Roas *et al.*, 2011). This is important for small farmers because it allows to easily detect the economic profitability of a crop and to better use the profit for livelihood enhancement. A lower phenological diversity was observed for all sweet tree populations located in hilly or drier areas with a uni-modal rainy season. Therefore, altitude as well as the climate regime partition ABMTs' phenological diversity across the Dahomey Gap (see Chuine, 2010; Munguia-Roas *et al.*, 2011). This low phenological diversity in high altitude and uni-modal climatic areas is not profitable in ABMTs cultivation because it negatively affects the fruiting success and reduces the productivity. Consequently, the climate regime and the altitude act like a domestication process, in shaping the tree species life cycle, which we postulate here as an adaptation strategy (Crepinsek and Kajfez-Bogataj, 2006).

Despite the fact that only wild bitter ABMTs were observed, they show low phenological diversity and more importantly a-synchronized reproductive phases compared to sweet trees. We postulate that, on the one hand this is related to their restricted distribution range devoid of ecological variability to which phenology might be linked (see Chuine, 2010). On the other hand, however, the repetitive reproductive events (flowering and fruiting) shown by the sweet trees in the same area (Fig. 4.4e-l) suggest low natural variation within bitter trees (see Doi *et al.*, 2010; Munguia-Roas *et al.*, 2011) and hence the existence of a genetic and taxonomic distinction between bitter and sweet ABMTs (Harris, 1996; Lowe *et al.*, 2000).

Phenological differences within ABMTs: implications for species distinction

Significantly discriminating monthly phenological events separate bitter and sweet ABMTs and justify the low similarity (similarity = 0.24; see Figs. 4.7) between the two types. Reproductive phases, especially the length of the flowering period and the period in which the flowers are receptive, are crucial for the possibility of hybridization (Doi *et al.*, 2010). Although there are consistent differences regarding the period when flowers are receptive between bitter and sweet ABMTs, the extended flowering time of sweet ABMTs partly overlaps with the limited one of bitter ABMTs, suggesting the possibility of hybridization in their area of co-occurrence in October (Fig. 4.4f).When comparing our observations of October 2010 and October 2011, we conclude that the overlap of the flowering times of the two types of ABMTs is not the same every year (see Fig. 4.4f). Hence the possibility of hybridization probably fluctuates annually. However, the absence of fruits in December and January (Fig. 4.4h, j, l) in the sweet ABMTs population in the co-occurrence area (Volta region) and the steadiness in bush mango taste suggest no or unsuccessful hybridization through pollen transfer from bitter to sweet ABMTs. Therefore, we postulated that bitter and sweet ABMTs are better be considered as two different taxa. It is unknown whether successful hybridization happens from pollen transfer from sweet to bitter ABMTs. The compatibility between bitter and sweet ABMTs in their reproductive phases through different climatic zones in the entire distribution range would therefore be an interesting research topic.

The extended phenological pattern of sweet ABMTs allows the availability of fruits throughout the year with the highest levels in the rainy seasons. In contrast, in the Volta region, bitter ABMTs fruit once a year at the beginning of the rainy season (February) coinciding with the midst of the severest dry season in the rest of the Dahomey Gap. This implies that colonization of bitter trees from Volta to the rest of the Dahomey Gap is impossible, unless the distribution is promoted by human activities. Bitter ABMTs grow well in regions climatically similar to the Dahomey Gap like Ibadan in Nigeria (see Kang *et al.*, 1994 and World Agroforestry field gene bank). Thus, the restriction of their distribution range in the Dahomey Gap is mostly related to their reproductive time (Chuine, 2010) and the lack of a human role in seed dispersal.

Impact of environmental factors on ABMTs occurrence and production

The timing and levels of phenological events of ABMTs in the Dahomey Gap varies throughout the year with important differences among phytogeographical regions and between bitter and sweet trees. This consistent difference explains the differences in the actual spatial distribution pattern in the Dahomey Gap (see Chuine, 2010; Doi *et al*., 2010). The wide distribution range of sweet trees is partitioned mostly by climatic factors (Crepinsek and Kajfez-Bogataj, 2006) causing differences among populations. Therefore, variation in reproductive success might be considered as an adaptation in response to differences in climate. High altitude plus high temperature with or without low rainfall reduce the repetitive reproduction of sweet ABMTs. Therefore, the highest potential of bush mango production is located in the Guinean climatic zone, a lowland area climatically very close to the postulated natural distribution ranges of ABMTs (see Kang *et al*., 1994; Van Dijk, 1999; Vihotogbé *et al.*, submitted-2).The extension of a reproductive phase or the development of a repetitive one are interesting key selection factors to increase a species' annual yield (Thurling, 1991). Therefore, the repetitive reproduction of sweet ABMTs gives them a higher economic value and determines their priority over bitter trees which justifies their abundance in advanced agroforestry systems (Okafor and Fernandes, 1987; Vihotogbé *et al.*, submitted-1). This economical advantage is hindered by the high level of flower and fruit abortion. Flower abortion in sweet ABMTs is mostly due to low rainfall and high temperatures occurring in all phytogeographical regions in January and which is more severe in the Northern populations. However, fruit abortion is also induced by pest infestation. In areas where the seeds are the most valuable product of ABMTs, infested trees are still important when the seed can be harvested from fruits before their abortion or even from unripe ones. In the situation that the entire fruit is the most important NTFP, infestation affects the quality of the mesocarp, lowering the value of the tree. This leads to the suppression of many infested sweet trees by local farmers and probably to a decrease of susceptible tree types.

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Chapter 5

Morphological Characterization of African Bush Mango Trees (*Irvingia* species) in the Dahomey Gap (West Africa)

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Abstract

This study investigates the morphological characteristics of bitter and sweet African bush mango trees (*Irvingia* species). African bush mangoes have been rated as the highest priority multi-purpose trees in need of improvement research in West and Central Africa. This study was carried out in the Dahomey Gap which is the West African savannah woodland area separating the Upper and the Lower Guinean rain forest blocks. We studied 128 trees from six populations and characterized their bark, fruits, mesocarp and seeds to assess the morphological differences among populations in the field. First, characteristics that might allow the distinction of bitter and sweet trees were analysed in a binary logistic regression. Second, a Principal Component Analysis was performed on fruit, mesocarp and seed measurements to define groups. The significance of factors that defined these groups was assessed in a Multivariate Analysis of Variance and a pairwise comparison of populations was performed using the Scheffe test. Lastly, characteristics were used in a cluster analysis (UPGMA). None of the variables: type of bark, mature fruit exocarp colour, fruit roughness and fresh mesocarp colour, were able to consistently distinguish bitter from sweet trees in the field. The analysis of the measurements of fruits, seeds and mesocarps demonstrated that bitter fruits have the heaviest seeds and this consistently distinguishes them from sweet fruits. However, the measurements of the fruit, mesocarp and seed did not have a joint effect in grouping sweet fruited populations. This indicates high diversity and potential for selection across all phytogeographical regions. The sweet trees of Couffo and those of Dassa are clearly different from all other populations. This can be attributed to traditional domestication and climate, respectively. The large fruits and the heavy seeds of the cultivated populations are evidence of successful on-going domestication and selection of sweet trees in the Dahomey Gap.

Key words: Dahomey Gap, domestication, Irvingiaceae, tree-to-tree variation, selection, species concept.

Introduction

African Bush Mango trees (ABMTs) belong to the small family of Irvingiaceae and are widely distributed in sub-Saharan Africa (Harris, 1996; Asaah *et al.,* 2003; Lesley and Brown, 2004). Significant phenological, morphological, and genetic variation was detected among populations across West and Central Africa and turned out to be correlated to geographical distance (Leakey *et al.,* 2000; Lowe *et al.,* 2000; Tairu *et al.,* 2000; Atangana *et al.,* 2001; Atangana *et al.,* 2002; Anegbeh *et al.,* 2003; Leakey *et al.*, 2003; Leakey *et al*., 2004; Leakey *et al.,* 2005; Ude *et al.,* 2006). However, quantitative morphological data relative to this variation have only been obtained from Central African populations. Sampling over the entire range is in turn a prerequisite for their effective conservation and for the development of an all-inclusive domestication program (Ladipo *et al.,* 1996; Lowe *et al.,* 2000; Ekue *et al.,* 2010).

Bush mango fruits taste either sweet or bitter and the corresponding trees have been treated as two different species: *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. and *I. wombolu* Vermoesen, respectively (Harris 1996; Lowe *et al.,* 2000). However, based on the minimal morphological differences, Okafor (1975) claimed that the taxa were best considered as two varieties (*I. gabonensis* var. *gabonensis* and *I. gabonensis* var. *excelsa* (Mildbr.) Okafor) and these infraspecific taxon names are also widely used. This has raised a controversial taxonomic situation causing some confusion in literature. Solving it requires a multi-disciplinary approach.

Sweet fruited trees are the most intensively studied ABMTs (Atangana *et al.,* 2001; Atangana *et al.,* 2002; Anegbeh *et al.,* 2003; Leakey *et al.,* 2003; Leakey *et al.,* 2004; Leakey *et al.,* 2005; Ude *et al.,* 2006 and other works of the World Agroforestry Centre and Centre for International Forestry Research) and apart from the comparative botanical data of Harris (1996), there is no quantitative assessment of the morphological variation of bitter and sweet ABMTs. Moreover, since bush mangoes are expected to generate high economic value in the near future based on at least two marketable products, the entire fruits and the endocarp containing the seed, important characteristics such as variation in fruit shape, mature fruit colour, and consumer preferences are particularly important (Ladipo *et al.,* 1996; Rashidi and Seyif, 2007). These are morphological features that vary within and between bitter and sweet trees and which are vital to define the nature of the material to be domesticated.

Fruit shape might particularly influence the choice of consumers (Luckwill, 1959; Rashidi and Seyif, 2007). Moreover, it will influence fruit size and weight which varies with environmental factors (Sinnott, 1932). Therefore, fruit shape is an important economical parameter and its diversity might be a valuable selection criterion in the domestication process. It can also be used to verify the taxonomic identity of the material being domesticated since it can be used in species delimitation (Saheed and Illoh, 2011). The diversity of bush mango fruit shape through different ecological zones and a comparison of bitter and sweet fruited trees in the Dahomey Gap as well as in their entire distribution range will provide important data to be related to the potential production of ABMT mesocarp and seed.

This work is part of a broader project intending to revisit differences within and between bitter and sweet ABMTs using ecological, phenological, morphological and genetic approaches. It focuses on the situation in a particular eco-region named the Dahomey Gap which is characterized by the virtual absence of lowland rainforest resources due to higher temperatures and less rainfall than is found in regions to the west and east. By tracing morphological features that will allow a distinction between and among bitter and sweet ABMTs we seek answers to two main research questions:

(i) Are bitter and sweet fruited ABMTs distinguishable in the field by features that can be easily observed?

(ii) Can the morphological variation of bush mangoes discriminate populations and types of ABMTs?

Materials and Methods

Sampling

The sampling design in this study follows, with small modifications that used by Vihotogbé *et al.* (Submitted-2) to evaluate phenological diversity within ABMTs. Six southern ABMTs populations (Pobè, Couffo, Dassa, Lomé and SVolta for sweet trees and Bvolta for bitter trees) in five different phytogeographical regions in Benin and Togo were investigated. In these populations, the sampled trees are a sub-set of those used for the phenological study. A sixth phytogeographical region, the Ouémé valley, was sampled in the surroundings of Abomey Calavi bringing the number of populations to seven.

In 2011, characteristics of a total of 128 fruit bearing trees (7 to 30 per population) were scored. In order to avoid incorporating variation due to differences in tree maturity, only adult trees that had been producing fruits for at least 5 years were selected. They were inspected for diseases to make sure that only totally healthy trees, without any kind of parasitism or human induced damage that might alter the morphological characteristics of trees and fruits, were selected. Fruit collecting was performed in February, June and July, the peak fruiting periods across the Dahomey Gap. Trees where visited early in the morning to collect mature fruits that had freely fallen down during the night. In total, 2,523 fruits (10 to 32 per tree) were collected and used in this study.

Morphological data

Each individual bitter and sweet fruited tree was described with the following six variables: (i) the type of bark, (ii) exocarp colour of the mature fruit, (iii) exocarp roughness of the mature fruit, (iv) mesocarp colour of the fresh fruit, (v) presence / absence of brown pigments in the fresh mesocarp, and (vi) the mesocarp tanning after 30 minutes. The type of bark was identified based on two main factors: its colour and roughness (Fig. 5.5.1). The colour of the exocarp and of the fresh mesocarp was scored using the Royal Horticultural Society Colour Chart (http://www.rhs.org.uk/Plants/RHS-Publications/RHS-colour-charts). The roughness of the fruit refers to the presence or absence of small pits in the fruit skin.

The seven detected bark types were arbitrarily coded from 1 to 7. Fruit skin roughness was coded as follows: smooth = 0, rough only on the side of the largest width = 1; uniformly but slightly rough = 2; uniformly medium rough = 3 and very rough = 4. The combination of standard numbers letters of the colour chart was used to identify the mature fruit exocarp and fresh mesocarp colour which were also arbitrarily coded: green group_135B = 1, green group_136B = 2, green group_139A = 3, yellow green group 144A = 4, yellow green group_146A = 5, yellow green group_146B = 6, yellow green group_152A = 7, yellow green group_152B = 8, yellow green group_152C = 9, yellow group_13A = 10, and yellow orange group_22A = 11 for exocarp colour and orange group_25A = 1, orange group_25B = 2, yellow orange_11A = 3, yellow group_11B = 4, yellow group_12A = 5, yellow group_13A = 6, yellow group_13B = 7, yellow group_13C = 8, yellow orange_20A = 9, yellow orange_21A = 10, and yellow orange_23A = 11 for mesocarp colour. For each individual tree, the occurrence of brown pigments in the fresh mesocarp and mesocarp tanning was reported as either 1 (for presence) or 0 (for absence).

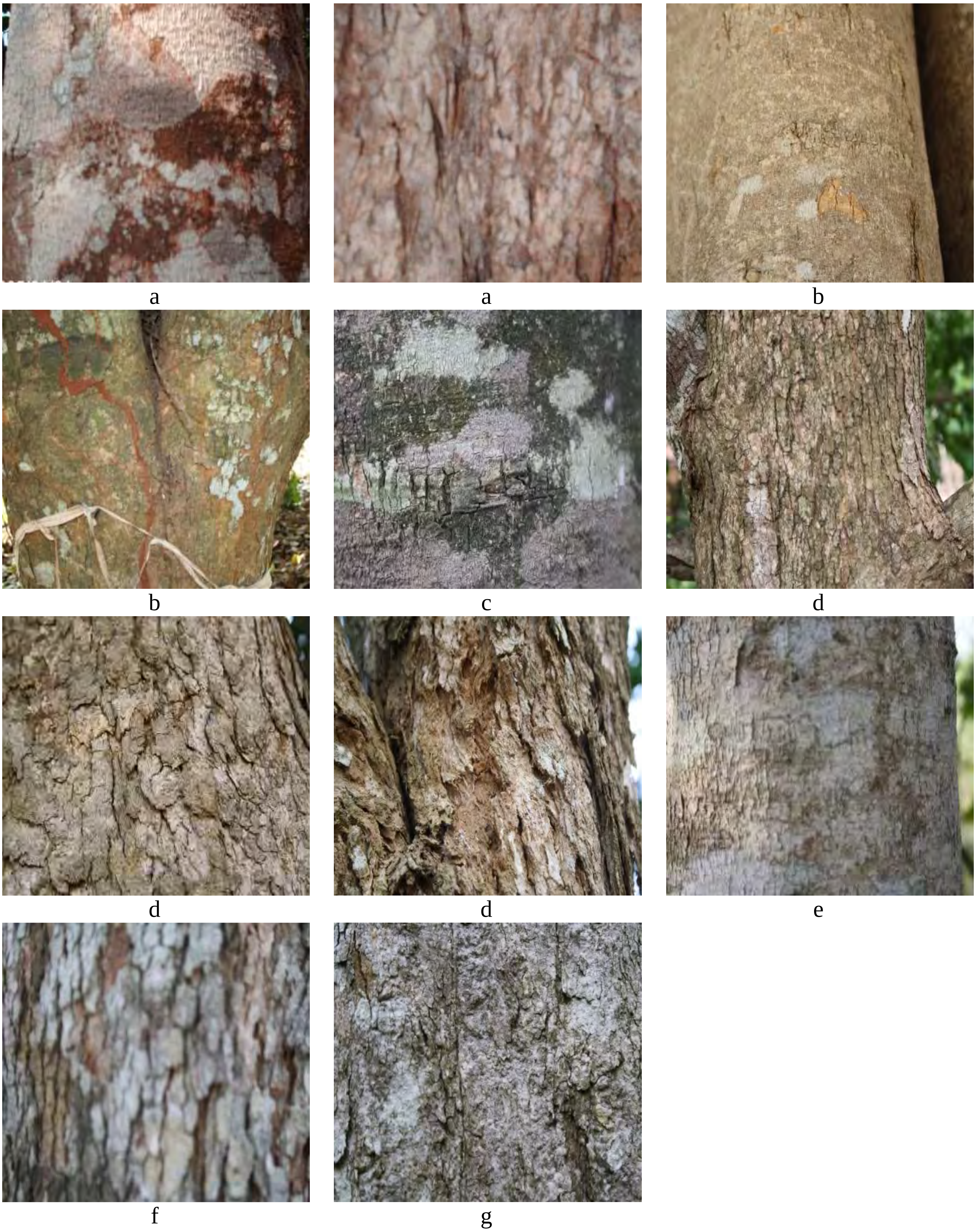


Figure 5.5a: Arbitrary codes of bark types. The same letter implies the same type at different degrees of maturity: a: grey-orange colour smooth at immature stage and rough with splits and scales when mature; b: grey-green colour with little fine scales (only when mature) and without splits even when mature; c: grey colour with lateral crease but without splits and scales even when mature (rare bark type); d: grey-brown colour with earlier development of splits and scales (splits are particularly deep at the insertion point of branches on the trunk; type mostly found on low-branching trees in Couffo); e: grey colour with very fine scales and without splits; f: intense grey colour with abundant scales; g: grey-black colour with large and coarse scales.

The data collecting method proposed by Leakey *et al.* (2000) and used by Atangana *et al.* (2001) and Anegbeh *et al.* (2003) was used. Thus, for each fruit, the following ten variables were measured: (vii) fruit weight, (viii) fruit total length, (ix) fruit largest width, (x) fruit smallest width, (xi) mesocarp depth on the side of the seeds largest width, (xii) mesocarp depth on the side of the seeds smallest width, (xiii) seed weight, (xiv) endocarp length, (xv) endocarp width, and (xvi) endocarp thickness. The fruit and seed weight was measured using a Soehnle laboratory electronic portable scale (Min: 1g; Max: 2000g). Fruit length and diameter sand seed width and thickness were measured with an electronic digital calliper (0.01mm resolution) that also served to measure the mesocarp depths. The total length and both widths of individual fruits were converted into two fruit shape indices, the roundness ratio (RR) and ellipsoid ratio (ER) (Luckwill, 1959; Rashidi and Seyif, 2007):

$$RR = {}^{TL}\!/_{\sqrt{Ld\ x\ Sd}} \qquad (1)$$

and

$$ER = {}^{Ld}\!/_{Sd} \qquad (2)$$

TL = the total length of the fruit, *Ld* and *Sd* = its largest and smallest diameters, respectively.

For each sampled tree, we determined the mean RR (M_{RR}) and mean ER (M_{ER}). These values were used to assess the fruit shape as follows: oblate fruit ($0.86 < RR \leq 0.9$), spheroid to prolate spheroid fruit ($0.9 < RR \leq 1.14$) and oblong fruits ($RR \geq 1.15$); round fruits ($1 \leq ER < 1.1$) or elliptical fruits ($1.1 \leq ER \geq 1.2$).

Data analysis

The type of bark, fruit exocarp and mesocarp colour, exocarp roughness, the occurrence of brown pigments in the fresh mesocarp and its tanning were analysed in a binary logistic regression against the type of ABMT using SAS Version 9.2 (SAS Institute Inc. 2003). We used the Global Null Hypothesis Test and the Type 3 Effects Analysis to validate the model and identify factors that allow distinguishing bitter and sweet fruited ABMTs.

A principal component analysis (PCA) was performed on fruit shape indices, fruit total length and largest width, fruit and seed weight, mesocarp depths, and endocarp length, width and thickness, using PAST (Hammer *et al.*, 2001).

To identify significant factors responsible for the detected pattern, a multivariate analysis of variance (MANOVA) was carried out on the standardized values of these factors (fruit shape indices, fruit total length and largest width, fruit and seed weight, mesocarp depth, and endocarp length, width and thickness) against the populations. For each individual fruit, the mesocarp weight was calculated by deducting the seed weight from the fruit weight. The mean mesocarp weight was computed at individual tree level and used in the MANOVA. The Scheffe test was performed for the pairwise mean comparison and population grouping based on the significance of the mean of each factor. In order to determine which factor specifically distinguished bitter tree populations from the sweet tree ones, a second MANOVA analysis was performed on the same 10 factors against the type of ABMT.

Finally, the significant morphological factors that distinguished populations and types of ABMTs were used in a cluster analysis using the Euclidian distance among sampled trees.

Results

Field identification of bitter and sweet ABMTs

The logistic regression analysis indicates that none of the six considered factors (type of bark, fruit exocarp colour, fruit skin roughness, fresh mesocarp colour, occurrence of brown pigment in the fresh mesocarp, and mesocarp tanning after 30 min) are capable to consistently distinguish bitter from sweet ABMTs (P > 0.05 Table 5.1).

Table 5.1: Results of the logistic regression to test the possibility to identify bitter and sweet trees in the field.

Testing Global Null Hypothesis: BETA = 0			
Test	**Chi-Square**	**Degree Of Freedom**	**P > Chi-Square**
Likelihood Ratio	101.78	31.00	< 0.0001
Score	121.53	31.00	< 0.0001
Wald	0.17	31.00	1.00
Type 3 Analysis of Effects			
Effect	**DF**	**Wald Chi-Square**	**P > Chi-Square**
Bark type	6.00	0.02	1.00
Exocarp colour	10.00	0.08	1.00
Fruit skin roughness	4.00	0.00	1.00
Mesocarp colour	9.00	0.02	1.00
Presence of brown pigments in the mesocarp	1.00	0.00	0.91
Mesocarp tanning	1.00	0.00	0.90

Diversity of fruit shapes and quantitative variation in fruit, mesocarp and seed

In general, the RR values indicate that a spheroid to prolate spheroid fruit shape is common in bush mangoes in the Dahomey Gap (84.5%). Oblong and oblate shapes are less represented (14 and 1%, respectively). The ER values show that 85.27% of ABMTs produce fruits with a circular cross-section, while 14.7% produce fruits with elliptical outlines. The heaviest fruits (fruit weight > 400 g) are found in the population of Couffo and the heaviest seeds (seed weight > 40 g) are recorded in the bitter tree population. The population of Dassa produces the smallest fruits and seeds in the Dahomey Gap (Table 5.2a).

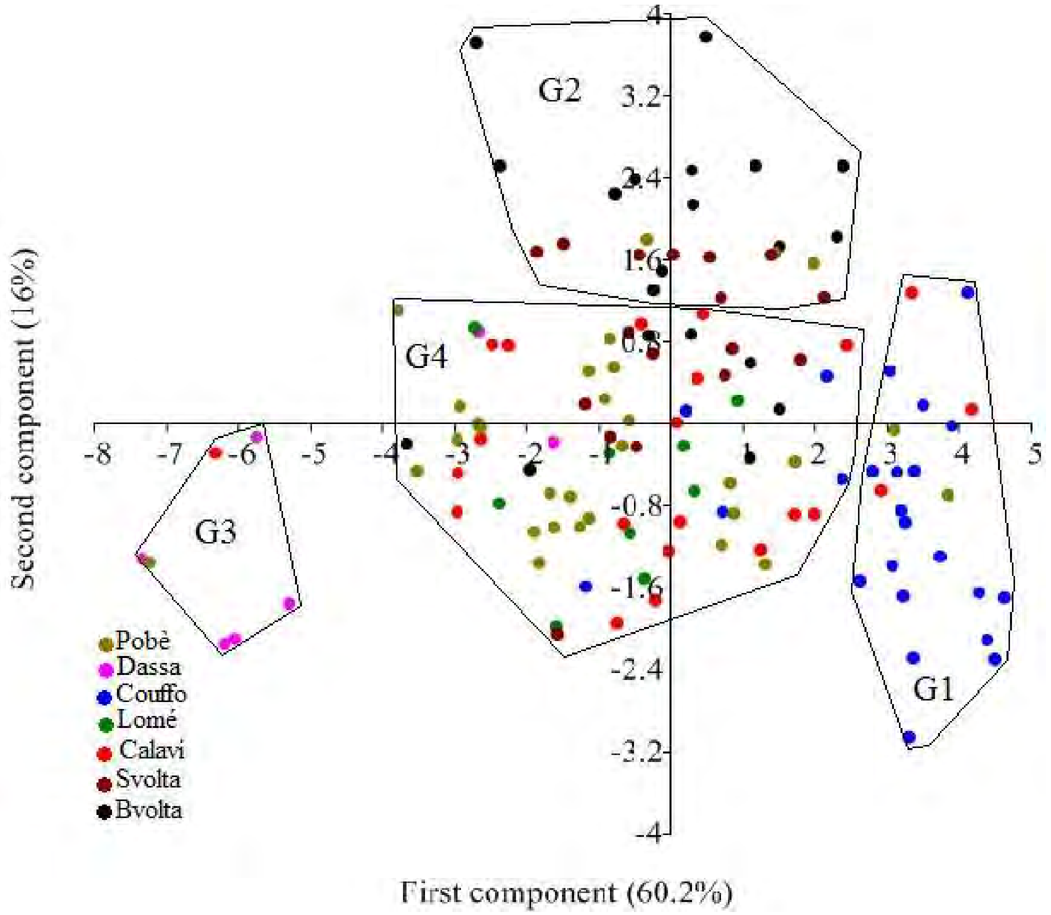


Figure 5.5b Result of the Principal Component Analysis: identification of groups within bush mangoes based on quantitative morphological characters of fruits and seeds.

The first two axes of the PCA explained 76.2% of the total variation. The first axis (60.2%) is mostly influenced by variables measured on the entire fruit, the mesocarp, and the seed, while the second (16%) is influenced by the roundness ratio (Table 5.2b). Four groups of trees were identified (Fig. 5.5b): (i) G1 comprises the majority of sweet ABMTs from the Couffo region with few individuals from Calavi and Pobè, (ii) G2 is composed of the majority of bitter trees with few individuals from Pobè and Volta region, (iii) G3is mostly composed of sweet ABMTs from Dassa, and (iv) G4 is a mixture of the majority of individuals from Pobè, Lomé, Calavi and the Volta region, but with a few individuals from the first three groups.

The results of the MANOVA against the populations indicate that there is no significant difference linked to fruit shape among populations ($P > 0.05$ for roundness and ellipsoid ratios; Table 5.2c). But all the other evaluated characters (fruit total length and largest width; fruit, mesocarp and seed weight; mesocarp depths; and seed length, width, and thickness) show significant differences among populations (Wilks' Lambda = 0.128; F = 5.91; $p < 0.001$; Table 5.2c).The population of Dassa has the lowest values for most of these factors (Fig. 5.3a-j). The heaviest, biggest and fleshiest fruits are found in the population of Couffo (Fig. 5.3a-f).For most of the evaluated factors, the population of Calavi presents the highest variation (Fig. 5.3).The Scheffe test indicates that the significant morphological factors related to the measurements on fruits, mesocarp and seeds do not define groups based on the pairwise difference of their means for populations (Table 5.2d). It also reveals that for most of the characters, the populations of Couffo and Dassa show significant differences both between them and from all others (Table 5.2d). The population of Dassa is different for most of the characters related to the entire fruit, the mesocarp and seed (Table 5.2d; Fig. 5.3) and that of Couffo is different from most other populations based on the fruits and mesocarp measurements (Table 5.2d; Fig. 5.3a-f). The Scheffe test for population grouping also indicates that the only factor that significantly distinguishes the bitter tree population from most of the sweet ones was the seed weight (Table 5.2d; Fig. 5.2 and 5.3j), but this character did not significantly separate the bitter tree population from the sweet ones in the Couffo and Volta regions. The largest and heaviest seeds are produced in both the bitter trees population and in that of the sweet trees in Couffo. The variation of most characters is higher in the population of Calavi. The results of the MANOVA against the type of ABMT indicates that the seed weight, width and thickness and mesocarp depths and weight are significantly different between the bitter and sweet ABMTs (Table 5.2c). Heavy, large and thick seeds are characteristic for bitter fruits, while deep and abundant mesocarp is in general characteristic of sweet fruits.

The cluster analysis shows a clear separation of the Couffo population from all other populations. Even though three other main groups appear, they do not express a complete distinction among the other populations of sweet trees and between bitter and sweet trees (Fig. 5.4).

Table 5.2a: Overall variation of bush mango fruit, mesocarp and seed characteristics.

Population	Morphological character	Mean	Std Dev	Minimum	Maximum
Bvolta	*Fruit weight (g)*	*117.6*	*39.7*	*38.0*	*252.0*
	Mesocarp depth (Ld) (cm)	1.2	0.3	0.4	2.2
	Mesocarp depth (Sd) (cm)	1.6	0.3	0.4	2.6
	Seed weight (g)	*20.1*	*5.2*	*5.0*	*41.0*
	Endocarp length (cm)	4.1	0.5	2.3	5.6
	Endocarp width (cm)	3.5	0.4	2.1	4.8
	Endocarp thickness (cm)	2.2	0.3	1.1	3.3
Calavi	*Fruit weight (g)*	*132.0*	*60.7*	*29.0*	*359.0*
	Mesocarp depth (Ld) (cm)	1.7	0.4	0.4	2.7
	Mesocarp depth (Sd) (cm)	1.8	0.5	0.6	3.0
	Seed weight (g)	*14.4*	*5.2*	*4.0*	*29.0*
	Endocarp length (cm)	3.9	0.7	1.4	5.8
	Endocarp width (cm)	3.0	0.4	1.9	4.9
	Endocarp thickness (cm)	2.0	0.3	1.0	2.9
Couffo	*Fruit weight (g)*	*213.7*	*53.6*	*78.0*	*426.0*
	Mesocarp depth (Ld) (cm)	2.2	0.4	0.5	3.8
	Mesocarp depth (Sd) (cm)	2.4	0.4	1.6	3.8
	Seed weight (g)	*18.1*	*4.4*	*6.0*	*30.0*
	Endocarp length (cm)	4.3	0.5	3.0	5.7
	Endocarp width (cm)	3.3	0.4	2.2	5.3
	Endocarp thickness (cm)	2.2	0.3	1.2	3.4
Dassa	*Fruit weight (g)*	*57.4*	*30.6*	*19.0*	*137.0*
	Mesocarp depth (Ld) (cm)	1.0	0.4	0.4	2.2
	Mesocarp depth (Sd) (cm)	1.2	0.3	0.6	1.9
	Seed weight (g)	*6.6*	*4.5*	*0.5*	*15.0*
	Endocarp length (cm)	2.6	0.6	1.6	3.9
	Endocarp width (cm)	2.0	0.6	1.0	3.2
	Endocarp thickness (cm)	1.3	0.3	0.8	1.8
Lomé	*Fruit weight (g)*	*114.5*	*32.8*	*56.0*	*200.0*
	Mesocarp depth (Ld) (cm)	1.8	0.3	1.2	2.7
	Mesocarp depth (Sd) (cm)	1.6	0.3	0.9	2.4
	Seed weight (g)	*11.8*	*3.3*	*6.0*	*22.0*
	Endocarp length (cm)	3.8	0.4	2.6	5.1
	Endocarp width (cm)	2.7	0.4	1.9	3.6
	Endocarp thickness (cm)	2.0	0.3	1.4	4.3
Pobè	*Fruit weight (g)*	*112.6*	*44.8*	*25.0*	*288.0*
	Mesocarp depth (Ld) (cm)	1.5	0.4	0.4	3.4
	Mesocarp depth (Sd) (cm)	1.7	0.4	0.6	3.4
	Seed weight (g)	*13.3*	*4.6*	*3.0*	*29.0*
	Endocarp length (cm)	3.7	0.6	1.9	6.7
	Endocarp width (cm)	2.9	0.4	1.2	5.5
	Endocarp thickness (cm)	2.0	0.4	1.0	3.5
Svolta	*Fruit weight (g)*	*120.5*	*33.3*	*11.0*	*252.0*
	Mesocarp depth (Ld) (cm)	1.5	0.3	0.7	2.3
	Mesocarp depth (Sd) (cm)	1.7	0.3	1.1	2.8
	Seed weight (g)	16.0	4.2	3.6	32.0
	Endocarp length (cm)	4.1	0.5	1.4	6.7
	Endocarp width (cm)	3.2	0.4	1.8	5.5
	Endocarp thickness (cm)	2.1	0.3	1.5	4.0

Ld = largest diameter, Sd = smallest diameter

Table 5.2b: Principal component loads on first and second axis from PCA of bush mangoes characteristics.

Morphological characters	PC$_1$ (60.2%)	PC$_2$ (16%)
Mean ellipsoid ratio	-0.14	0.42
Mean roundness ratio	-0.03	0.57
Mean endocarp thickness	0.72	0.34
Mean endocarp width	0.79	0.45
Mean endocarp length	0.87	0.38
Mean seed weight	0.80	0.50
Mean mesocarp depth (Ld)	0.88	-0.39
Mean mesocarp depth (Sd)	0.79	-0.52
Mean fruit weight	0.93	-0.27
Mean fruit length	0.95	-0.03
Mean Fruit width (Ld)	0.96	-0.23

Table 5.2c: Results of the multivariate ANOVAs.

Source of variation	**Morphological characters evaluated within populations**	**Type III Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F value**	**P value**
Between Populations	Mean of ellipsoid ratio	0.005	6	0.001	0.612	0.720
	Mean of roundness ratio	0.06	6	0.01	1.918	0.083
	Mean of endocarp thickness	*6.719*	*6*	*1.12*	*5.231*	*< 0.001*
	Mean of endocarp width	*13.72*	*6*	*2.28*	*10.142*	*< 0.001*
	Mean of endocarp length	*14.18*	*6*	*2.36*	*7.687*	*< 0.001*
	Mean of seed weight	*1339*	*6*	*233.3*	*12.22*	*< 0.001*
	Mean of mesocarp depth (Ld)	*16.288*	*6*	*2.71*	*17.504*	*< 0.001*
	Mean of mesocarp depth (Sd)	*15.525*	*6*	*2.58*	*15.74*	*< 0.001*
	Mean of mesocarp weight	*209275*	*6*	*34879*	*24.3*	*< 0.001*
	Mean of fruit weight	*221745*	*6*	*3695*	*21.808*	*< 0.001*
	Mean of fruit total length	*55.4*	*6*	*9.29*	*17*	*< 0.001*
	Mean of fruit largest width	*63*	*6*	*10.51*	*22.26*	*< 0.001*
Between types of ABMTs	Mean of ellipsoid ratio	0.1	1	0.1	1.9	0.15
	Mean of roundness ratio	0.003	1	0.003	0.22	0.63
	Mean of endocarp thickness	*0.75*	*1*	*0.75*	*7.46*	*0.007*
	Mean of endocarp width	*3.4*	*1*	*3.4*	*17.42*	*< 0.001*
	Mean of endocarp length	*0.75*	*1*	*0.75*	*2*	*0.16*
	Mean of seed weight	*507.15*	*1*	*507.15*	*21.04*	*< 0.001*
	Mean of mesocarp depth (Ld)	*3.92*	*1*	*3.92*	*23.67*	*< 0.001*
	Mean of mesocarp depth (Sd)	*1.13*	*1*	*1.13*	*5.7*	*0.019*
	Mean of mesocarp weight	*13633.1*	1	*13633*	*4.68*	*0.032*
	Mean of fruit weight	8881	1	8881	2.69	0.14
	Mean of fruit total length	1.14	1	1.14	1.21	0.27
	Mean of fruit largest width	2.35	1	2.35	2052	0.115

Table 5.2d: Results of the Scheffe test subsequent to the MANOVA analysis: comparison and grouping of populations based on the differences of means of the morphological factors; Ld = largest diameter, Sd = smallest diameter.

Significant morphological characters	Population (I)	Population (J)	Difference (I-J)	P Value
Mean endocarp thickness	Dassa	Bvolta	-0.85	0.011
		Couffo	-0.8	0.016
		Svolta	-0.76	0.042
Mean endocarp width	Dassa	Bvolta	-1.32	< 0.001
		Couffo	-1.27	< 0.001
		Pobè	-0.86	0.007
		Svolta	-1.25	< 0.001
		Calavi	0.95	0.003
Mean endocarp length	Lomé	Bvolta	-0.67	0.057
	Dassa	Bvolta	-1.16	0.002
		Calavi	-1.09	0.004
		Couffo	-1.47	< 0.001
		Lome	-1.27	0.004
		Pobè	-0.98	0.01
		Svolta	-1.42	< 0.001
Mean seed weight	Bvolta	Calavi	5.31	0.02
		Dassa	12.92	< 0.001
		Lome	7.94	0.003
		Pobè	6.6	< 0.001
	Dassa	Calavi	-7.62	0.015
		Svolta	-8.89	0.003
	Couffo	Dassa	10.82	< 0.001
		Pobè	4.5	0.026
Mean mesocarp depth (Ld)	Dassa	Calavi	-0.66	0.025
	Couffo	Bvolta	0.91	< 0.001
		Calavi	0.58	0.001
		Dassa	1.24	<0.001
		Lome	0.84	<0.001
		Pobè	0.76	<0.001
		Svolta	0.9	<0.001
Mean mesocarp depth (Sd)	Calavi	Couffo	-0.52	0.007
		Dassa	0.81	0.003
	Couffo	Bvolta	0.91	<0.001
		Dassa	1.33	<0.001
		Pobè	0.69	<0.001
		Svolta	0.76	<0.001
		Bvolta	0.91	<0.001
Mean mesocarp depth (Sd)	Dassa	Lome	-0.79	0.025
		Pobè	-0.63	0.035
Mean fruit weight	Dassa	Calavi	-74.19	0.013
	Couffo	Bvolta	98.14	<0.001
		Calavi	78.1	<0.001
		Dassa	152.29	<0.001
		Lome	96.66	<0.001
		Pobè	100.97	<0.001
		Svolta	93.3	<0.001
Mean fruit length	Couffo	Svolta	1.09	<0.001
		Calavi	1.13	<0.001
		Bvolta	1.36	<0.001

Significant morphological characters	**Population (I)**	**Population (J)**	**Difference (I-J)**	**P Value**
		Lomé	1.46	<0.001
		Pobè	1.46	<0.001
		Dassa	2.78	<0.001
	Dassa	Svolta	-1.68	<0.001
		Calavi	-1.65	<0.001
		Pobè	-1.31	<0.001
Mean fruit smallest diameter	Couffo	Calavi	1.22	<0.001
		Lomé	1.38	<0.001
		Svolta	1.44	<0.001
		Pobè	1.51	<0.001
		Bvolta	1.56	<0.001
	Dassa	Couffo	-2.77	<0.001
		Calavi	-1.55	<0.001
		Lomé	-1.38	<0.001
		Svolta	-1.32	<0.001
		Pobè	-1.25	<0.001
		Bvolta	-1.20	<0.001

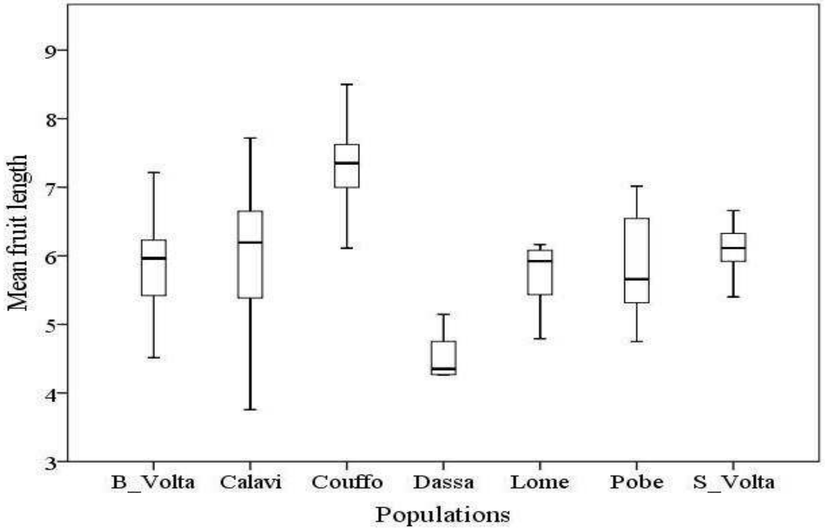


Figure 5.3a

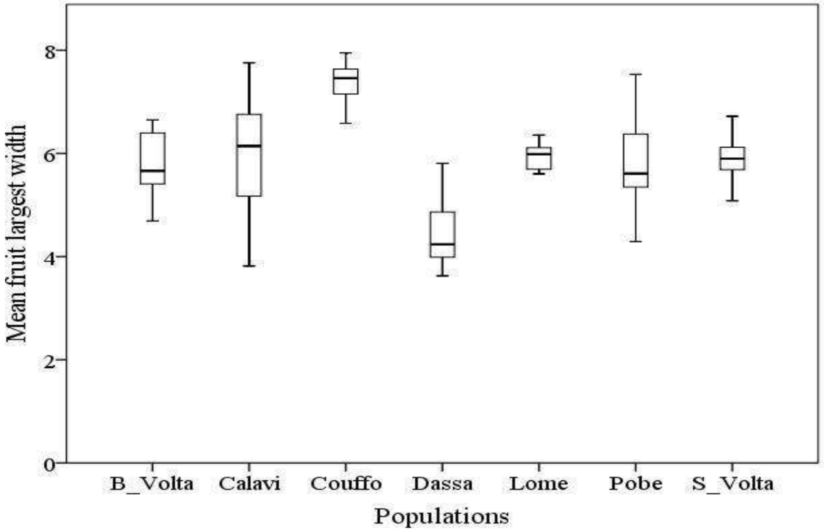
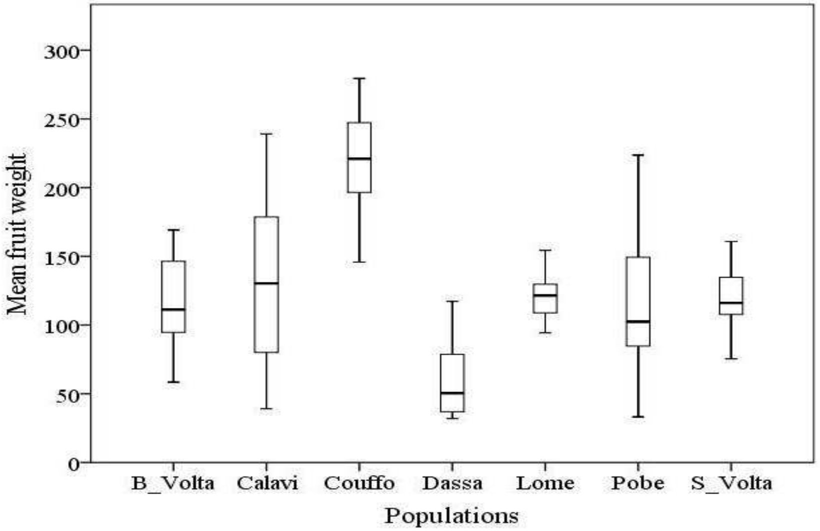


Figure 5.3b



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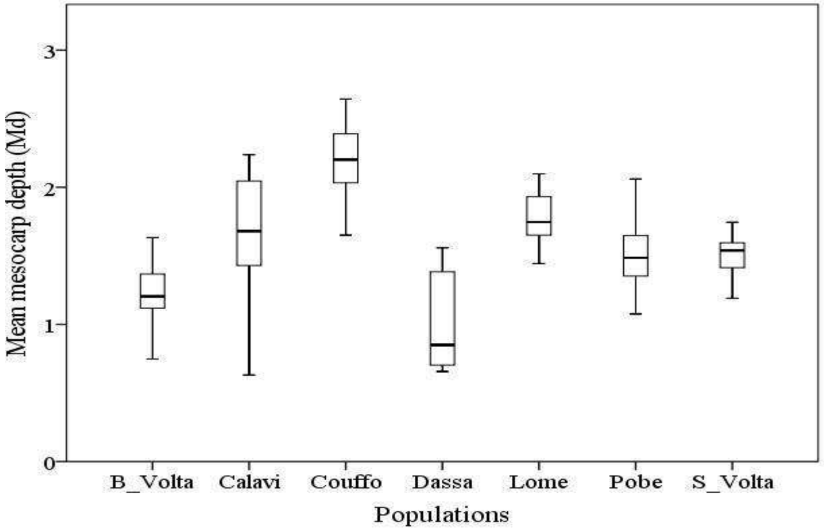

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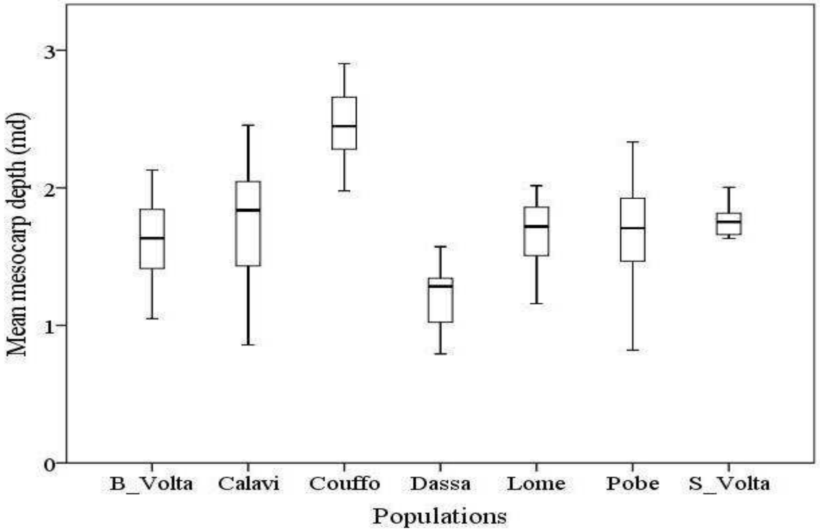

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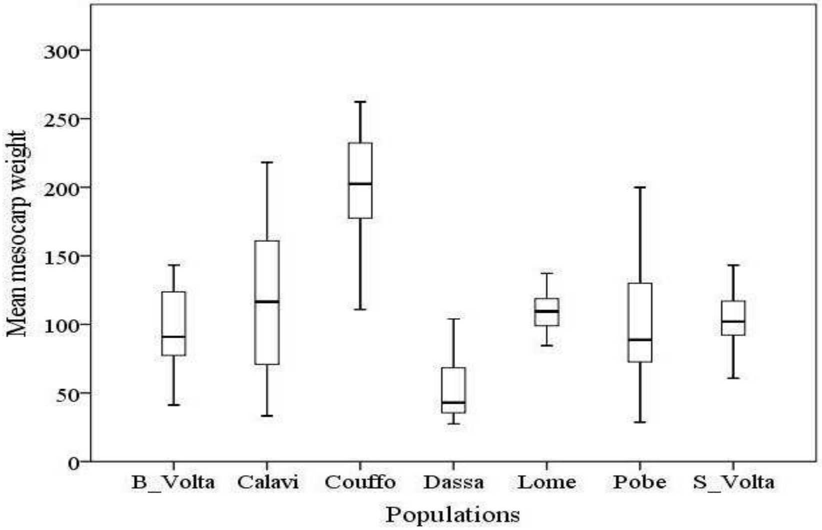

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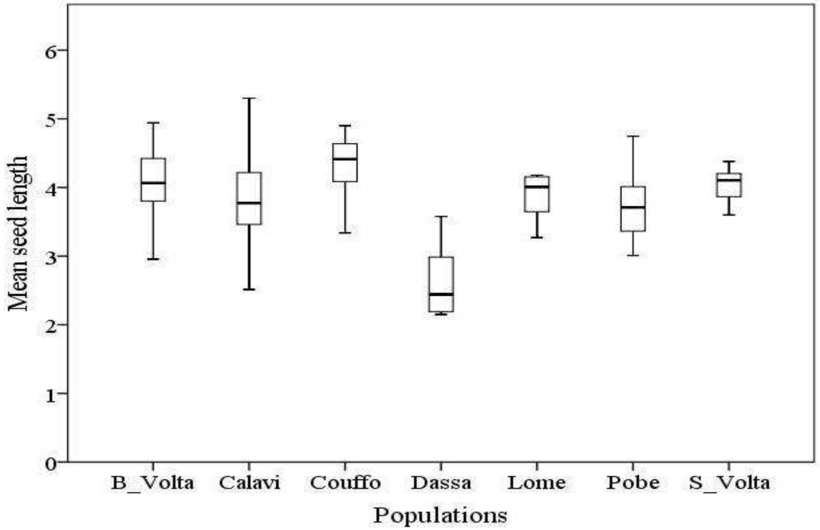

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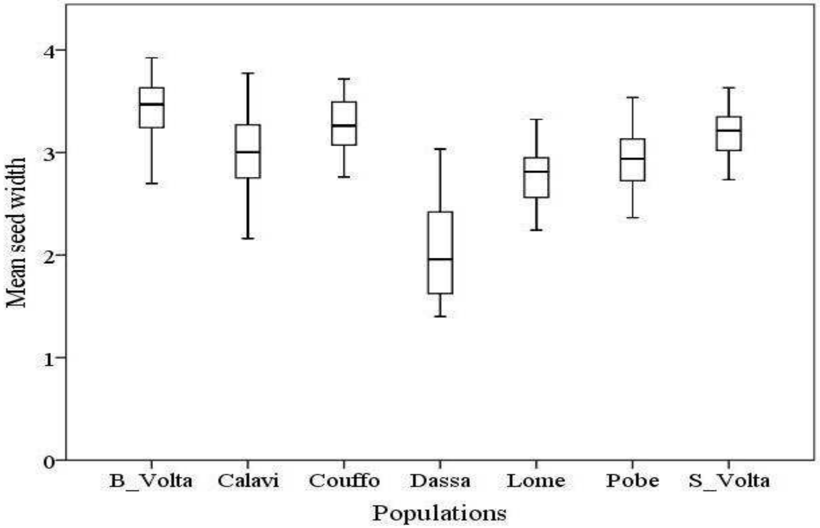

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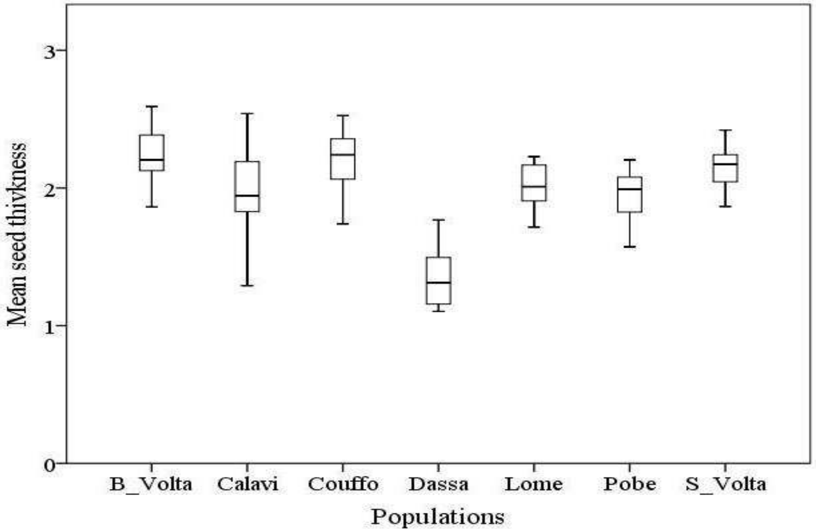

Figure 5.3i

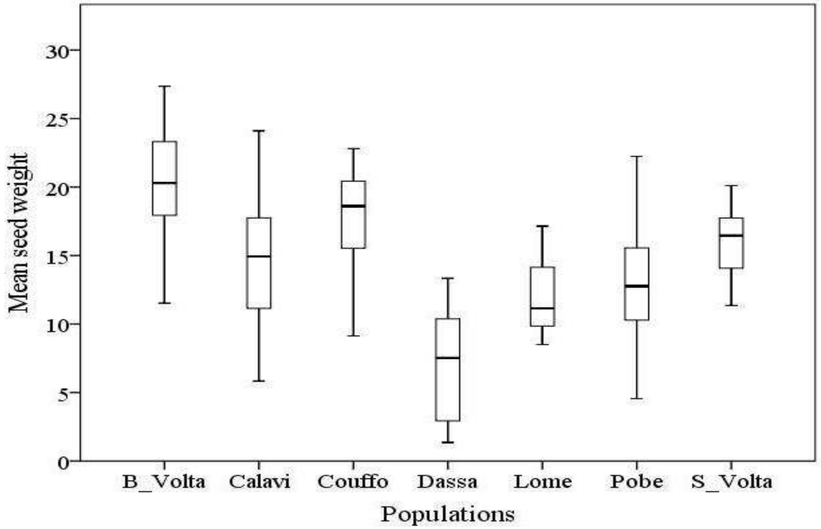

Figure 5.3j

Figure 5.3: Results of the MANOVA of fruit, mesocarp, seed and endocarp characteristics against populations.

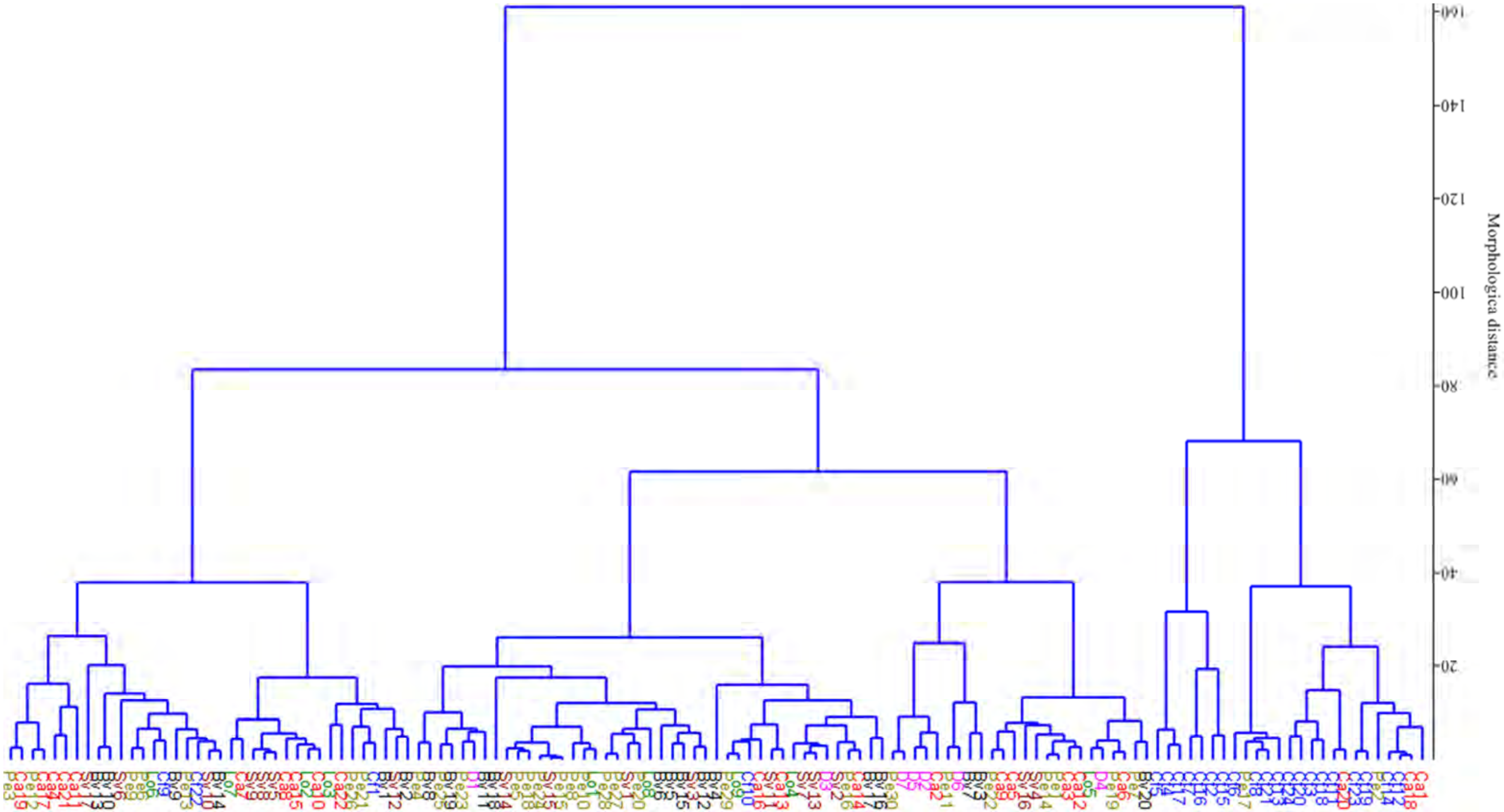

Figure 5.4: Result of the cluster analysis on the fruit, mesocarp, seed and endocarp characteristics (the colours indicate populations as presented in figure 2).

Discussion

Field identification of ABMTs

The type of bark, the roughness and colour of the mature fruit exocarp, colour of mature mesocarp, the brown pigments detected in the fresh mesocarp and its tanning all lack the power to consistently separate bitter and sweet trees. Even though we acknowledge that the colour of the fresh mesocarp seems to differentiate both taxa (Harris, 1996), the appreciation of this difference is difficult and subjective. The problematic distinction between bitter and sweet ABMTs was already extensively reported by Okafor (1975) and Harris (1996). In the present study, we attribute this difficulty to the large morphological variation within both bitter and sweet ABMTs. For instance, the yellow colour of the mesocarp is consistent within bitter fruits but also widely occurs within sweet fruits, which show different degrees of orange (from yellow-orange to orange). Moreover, even though brown pigments are thought to be typical for bitter fruits, this character is not always present within this taxon. This is important for the discussion about the taxonomic level on which the distinction between the two types should be recognized (Landolt, 1977; Leakey and Page, 2006). Even when considered as varieties, bitter and sweet ABMTs should exhibit at least one distinctive and obvious morphological character apart from the taste of their mesocarp. Unfortunately this remains an issue for which our data do not provide a convincing answer. Moreover, since we have only selected fully mature and healthy trees, the distinction in the field based on our morphological characters alone may well be even be more difficult than our results show.

The limited number of visible phenotypical characters and the limited number of populations evaluated for bitter trees may also explain our unsatisfactory results. Therefore, broadening the study area and taking more characters into account should be the next challenge to morphologically delimitate bitter and sweet trees. This is important for designing a better domestication program and an efficient use of ABMTs, since the morphological species concept is vital in plant breeding, selection and cultivation (Pickersgill, 2009).

Local communities instantly differentiate these taxa and it is possible to learn to quickly recognize them in the field. A higher juice seep characterized all bitter fruits leaving fibers difficult to remove from the seed during the removal of mesocarp for seed characterization. Some sweet bush mangoes also display this characteristic, but the removal of the mesocarp from sweet fruits always remains easier. The mesocarp of fruit ideotypes locally called “doughy bush mangoes”(viz. bush mangoes having a mesocarp with low water content, also called “the best fruit ideotype”) seeped no juice and is more easily detachable from the seed even when fruits from “doughy” mangoes have freely dropped. Only three mother trees of this kind (representing 2.7% of the sampled sweet trees) were identified. They show mature mesocarp that is deep orange and are highly desired to enrich agroforestry systems for entire fruits commercialization instead of the endocarp. Local communities stated that this ideotype was more common in the Dahomey Gap and was even the most shared germplasm for ABMTs domestication. Its decline started with the change over time in the type of mesocarp initially produced by desired mother trees. When this change happens, sweet ABMTs are systematically eliminated in areas where the type of mesocarp remains the most valuable NTFP. This explains the continuously low density of ABMTs in this area (see Vihotogbé *et al.,* Submitted-1). Many other characters (e.g. leaf shape and its development, the tree’s branching pattern, crown shape, petal color) seem worthy of a more detailed qualitative and quantitative evaluation. For example, tree-to-tree variation exists in leaf shape and our pilot observations show a higher diversity for bitter trees (Fig. 5.5a). Sweet trees display a higher diversity for seed shape (Fig. 5.5b) and for the arrangement of branches: their direction, the inner versus outer position of secondary branches, and the level of insertion of the first large branches. This determines a variety of crown shapes (see Ladipo *et al.*, 1996) that we did not address in this study. The colour of the petals could also be a valuable discriminant factor between bitter and sweet trees. While yellowish petals seem to be common for bitter trees, our preliminary observations showed a dominance of white petals in sweet trees which can, however, also have yellowish ones. If those characteristics appear to be consistently linked to the type of ABMT (bitter or sweet) and can be observed before the first fructification, this could considerably speed up the domestication process. Local communities also reported that fruit type may not be constant and thus can change from the best type to a completely undesirable one on a single tree. This needs further investigation and will lead to a great diversity of fruit types in a small geographical range. In this situation, even though desired traits are captured and disseminated through vegetative techniques (Tchoundjeu *et al.*, 2010), there is a risk of losing these again over time. Therefore, a permanent verification of the defined ideotype will be necessary to fix, keep and propagated desired characters durably (see Leakey and Page, 2006).

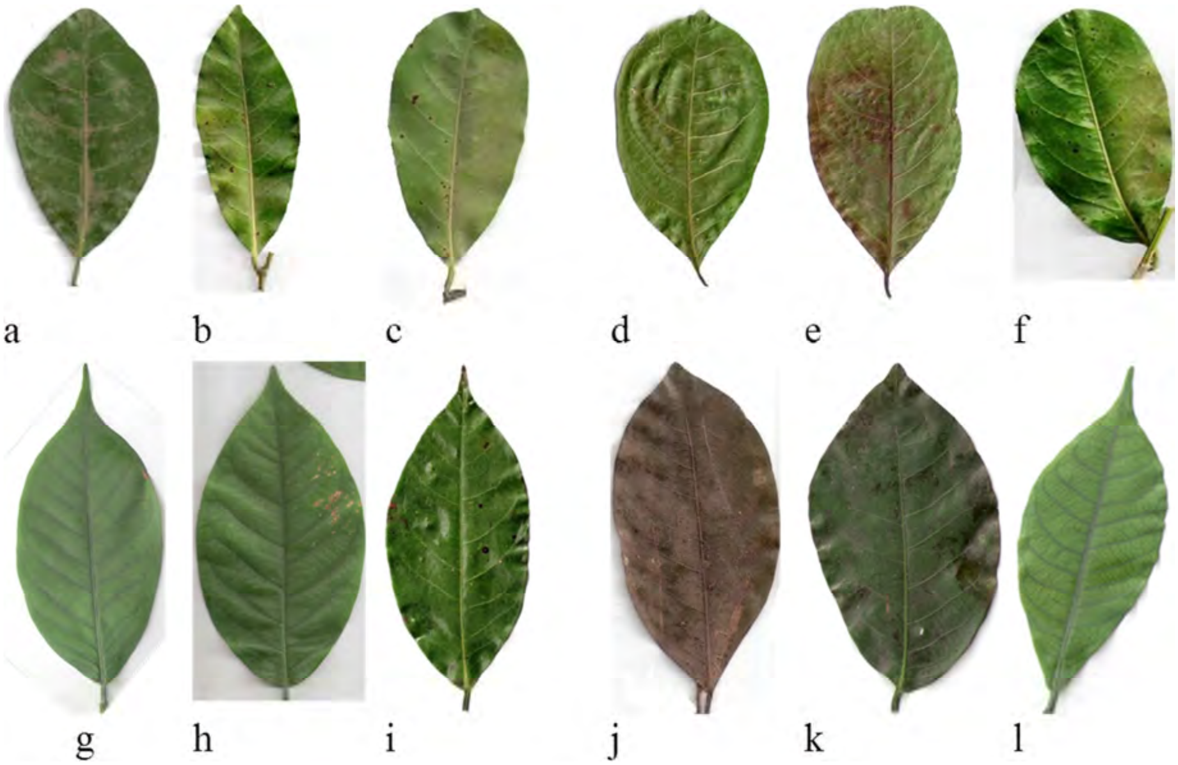

Figure 5.5a: Pilot records of leaf shape diversity in ABMTs. a – f: observed leaf shapes in bitter trees, g and h = leaf shape of juvenile bitter trees, i – k = observed leaf shapes in sweet trees, l = leaf shape of juvenile sweet trees.

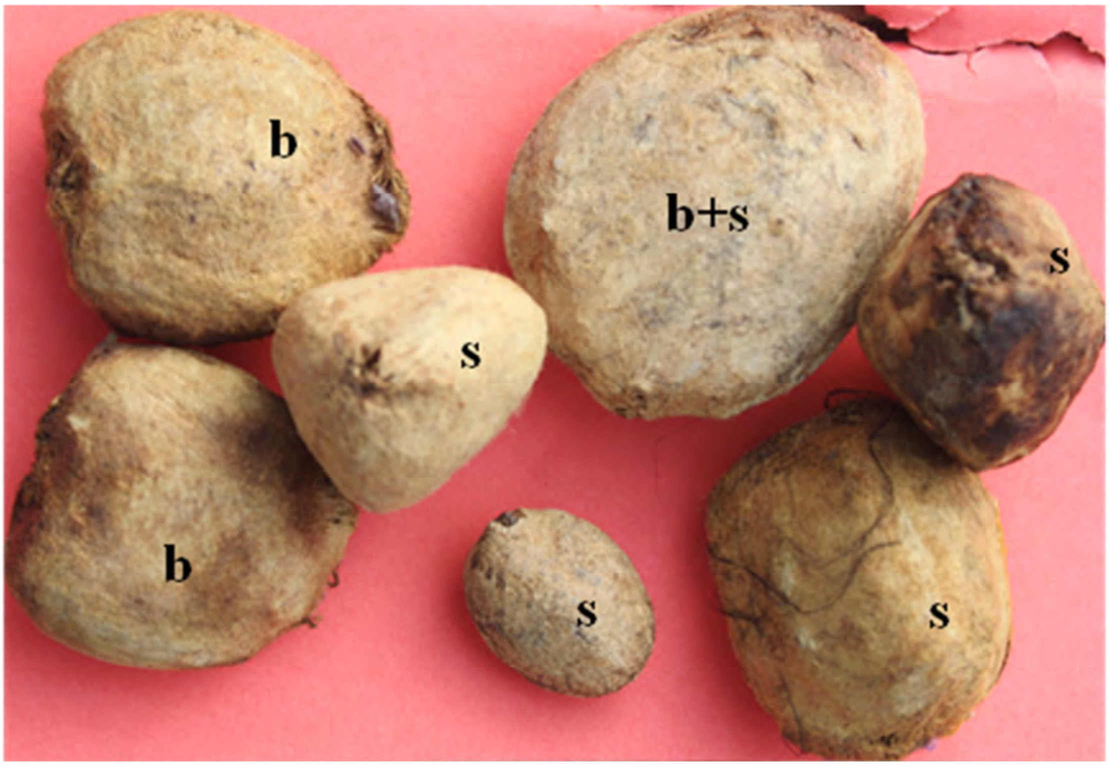

Figure 5.5b: Pilot records of seed shape diversity of bush mangoes. b = shape exclusively observed in bitter fruits, b + s = shape common to bitter and sweet fruits, s = shape exclusively observed in sweet fruits.

Variation of quantitative traits

Most fruits are spheroid to slightly prolate spheroid, in agreement with the results of Ladipo *et al.* (1996), and fruit shape does not significantly discriminate bitter and sweet trees.

Measurements related to the size of fruits and seeds and the abundance of mesocarp tissue distinguish the sweet tree population of Couffo from all other populations (bitter and sweet). This study presents the first quantitative morphological data on bitter ABMTs and indicates that, in the Dahomey Gap, their fruit and endocarp weigh 38 to 250g and 5 to 40g, respectively. In the Dahomey Gap, fruit and endocarp weights of sweet bush mangoes range between 11 to > 425 g and 0.5 to > 40 g, respectively. The ranges for sweet trees are wider than those detected in Cameroon and Nigeria (Leakey *et al.,* 2000; Atangana *et al.,* 2001; Anegbeh *et al.,* 2003). This suggests that in the Dahomey Gap the traditional domestication / selection process is already advanced, while in Central Africa the exploitation of bush mangoes and their seed relies mostly on wild populations (see Ladipo *et al.,* 1996; Lowe *et al.,* 2000; Dolor, 2011). Moreover, this high variability of sweet trees throughout contrasting ecological circumstances suggests a high ecological adaptability. Therefore, the morphological differentiation among sweet tree populations in the Dahomey Gap is determined by small farmer-lead domestication and a mass selection process (see Vihotogbé *et al.,* Submitted-1). In areas where bush mangoes are considered as cash crop and are traditionally managed, traditional selection is narrowing the morphological diversity toward heavy and big fruit ideotypes (see Cornelius, 1994; Van Hintum, 1995; Casas *et al.,* 1999) for the purpose of bush mango’s endocarp commercialization. This market-lead process is guided by the desire to limit the morphological diversity to ideotypes that guarantee high yield of the endocarp. The increase of fruit weight through mass selection is associated with that of the seeds. Consequently, local farmers might be leading successful traditional selection for the increase of endocarp weight due to unavoidable association among quantitative characters (Brown, 2010) of the endocarp. This points at a high economic interest and successful small farmer-lead domestication process taking place in the Dahomey Gap (Leakey *et al.,* 2004; Pickersgill, 2009). Despite the fact that reasons for domestication are clearly identified by local farmers disseminating the materials, the scientific basis and the more technical aspects that speed up a domestication strategy are still lacking (Simons and Leakey, 2004). Thus, the on-going mass selection is based on seed distribution by farmers and the appreciation of fruit ideotypes after the first fructification (normally after 6 to 8 years). This clearly shows the limit of traditional practices in speeding up domestication and selection processes for any tree genetic resource with a high morphological or genetic diversity. Seeing the important nutritional potential of the sweet mesocarp (Fouda *et al.*, Unpublished data; Lesley and Brown, 2004), the material in the Dahomey Gap is clearly interesting for domestication purposes aiming at an increase of both the mesocarp and the endocarp yield. This makes these sweet trees highly valuable since ABMTs have been rated as highest priority multi-purpose trees in need of improvement research in West and Central Africa In food-deficiency areas like Sub-Saharan Africa an efficient use of any valuable plant species is a challenge. This challenge is expressed clearly in the elite trees definition, prior to the Word Agroforestry Centre’s on-going domestication program (Leakey and Tchoundjeu, 2001).

The difficulty to differentiate between sweet tree populations due to morphological overlap (Fig. 5.4) might be rooted in the continuous circulation of genetic material among ecological zones in the Dahomey Gap, and the continuous transfer of new genetic material via seeds between the Dahomey Gap and the neighbouring countries (Casas *et al.*, 1999). Therefore, we postulate that high morphological and genetic diversity within ABMTs is created and maintained in the traditional agroforestry systems throughout the Dahomey Gap (Casas *et al.*, 2005; Jose, 2012) and a valuable potential exists for any local as well as regional domestication program for sweet tree material. This is of particular advantage in a global climate change condition since this material is already adapted to low rainfalls conditions. While the different populations investigated belong to different phytogeographical zones, the fact that the characters do not act in concert to segregate populations indicates that the potential for selection of various desired characters is not geographically restricted, but widely distributed over the Dahomey Gap. This means that any fruit ideotype can be found in any region. This supports results of the World Agroforestry Centre identifying large and heavy sweet bush mangoes in drier region outside of the Dahomey Gap, and a variety of bush mango morphotypes that still remain to be carefully documented. The potential for selection is higher in the eastern part of the Guinean climatic zone of the Dahomey Gap, since individuals from this area are more spread over all the clusters defined (population of Pobè; Fig. 5.4). This is consistent with Pickersgill (2009) who reported that differences exist in geographical space regarding the distribution of the variation in plant species.

Acknowledgements

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Chapter 6

Genetic diversity and difference within and between bitter and sweet African bush mango trees (*Irvingia* spp., Irvingiaceae) in West and Central Africa

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(Submitted to *African Journal of Biotechnology*)

Abstract

The domestication of the economically most important food tree species used in sub-Sahara African communities should be improved to enhance their production within agroforestry systems and combat the food deficit crisis in this region. African bush mango trees (*Irvingia* species) are top priority among the species that are preserved and integrated in agroforestry systems in the humid regions of tropical Africa. The taxonomic debate related to the species or varietal status of the bitter and sweet fruited African bush mango trees hinders their domestication process and rational use. AFLPs and cpSSRs were used in this study to assess the genetic diversity of African bush mango trees and to test the distinction between bitter and sweet fruited trees across Togo, Benin, Nigeria and Cameroon. Low genetic diversity was found for the isolated population of bitter trees occurring in south-western Togo due to the higher fragmentation of the small sized forest ecosystem in which they occur and the continuous reduction of the population size. The higher polymorphism and genetic diversity of the sweet tree populations in Benin and Togo indicate the effect of domestication of material with different geographical origin due to frequent long distance transfer of genetic material. When used separately, the AFLPs and cpSSRs failed to consistently discriminate populations and tree type. But the combined dataset from both markers generally differentiates geographically recognizable groups, and bitter from sweet trees. The suitability of AFLPs and cpSSRs to test our hypotheses within *Irvingia* is discussed.

Key words: AFLP, cpSSR, Benin, Togo, Dahomey Gap, *Irvingia*, taxonomy, domestication.

Introduction

In Africa, tropical forests host many multipurpose food tree species which are valuable in the light of the crucial food deficit in sub-Saharan Africa (Hladik *et al.*, 1996; Malaisse, 1997; FAO, 2008, Augustino *et al.*, 2011). While this food deficit is increasing forest ecosystems are being cleared at an alarming rate (Laurance, 1999; Archard *et al.*, 2002). The further domestication of the most important food tree species, widely used by local communities in their daily diets, seems a logical policy. This will strengthen traditional regional strategies for agrobiodiversity maintenance and, in general, enhance the global production of agroforestry systems.

The International Centre for Research in Agroforestry (ICRAF, now called World Agroforestry Centre) has become a leading institution in traditional food tree species domestication in West and Central Africa. Among the numerous species with nutritional importance or local communities, African bush mango trees (ABMTs) are economically the most important ones and are systematically preserved and integrated in various traditional agroforestry systems in humid sub-Saharan Africa (Okafor and Fernandes, 1987; Franzel *et al.*, 1996; Tabuna, 2000; Okunomo and Egho, 2010).

ABMTs are widely distributed and taxonomically confusing taxa within the family of Irvingiaceae within which a bitter fruited and sweet fruited form can be distinguished. Apart from the bitterness versus sweetness of the mesocarp, there is only limited morphological delimitation between the two types (Harris, 1996). On the other hand, ABMTs exhibit a high morphological and phenological diversity that is valuable for domestication and selection programs (see Harris, 1996; Atangana *et al.*, 2002) but there is no clear relation between biochemical properties and the type of ABMT (Tchoundjeu and Atangana, 2007). This overlap of morphological, phenological and biochemical properties generated a debate related to the correct taxonomy of ABMTs. Okafor (1975) proposed the variety level for sweet and bitter ABMTs, respectively *Irvingia gabonensis* (Aubry-LeComte ex O'Rorke) Baill. var. *gabonensis* and *I. gabonensis* var. *Excels* (Mildbr.) Okafor. But, based on a thorough taxonomic revision (Harris 1996) and a Random Amplified Polymorphic DNA (RAPD) analysis (Lowe *et al.*, 2000), a distinction at species level was suggested: *Irvingia gabonensis* and *I. Wombolu* Vermoesen, for sweet and bitter trees, respectively. There is a lack of support for the latter solution due to the weaknesses of the RAPD analysis, mostly their low reproducibility. Moreover, there is a lack of a sound quantitative morphological comparison between bitter and sweet ABMTs. Still, in order to ensure proper in- and ex-situ conservation of the genetically diverse material, and to support its development and genetic improvement (Grace *et al.,* 2008), a clear situation regarding the taxonomic identity of the material is essential. Furthermore, the geographic origin of populations present in the Dahomey Gap, the wide savannah area separating the West African forest into the Upper and Lower Guinean forest blocks, remains an important topic.

This study intends to revisit the pattern of genetic diversity of ABMTs in answering two main questions:

(i) What are the patterns of genetic diversity and differentiation within and between bitter and sweet ABMTs, and what do they tell us about the geographical origin of the Dahomey Gap material?
(ii) Are our markers (AFLPs and cpSSRs) suitable to consistently discriminate populations and types of ABMTs?

Materials and Methods

Sampling

Sweet and bitter ABMTs were sampled throughout the Dahomey Gap (Benin and Togo) and in Cameroon. Material was also sampled from four gene banks: IITA (International Institute for tropical Agriculture) and NAGRAB (National Centre for Genetic Resources and Biotechnology) in Ibadan (Nigeria) and Kolbison (Yaoundé) and Mbalmayo established by the World Agroforestry Centre in Cameroon (Table 1). For each sampled tree, young leaves were collected and stored in silica gel. DNA was extracted from each sample following the protocol described in Fulton *et al.* (1995) and an AFLP analysis was first carried out on 33 samples (10 bitter and 23 sweet trees). Moreover, an independent cpSSR analysis was carried out with 47 samples (14 bitter and 33 sweet trees), including more individuals from the Dahomey Gap and the regions postulated as ABMTs genetic diversity centres by Lowe *et al.* (2000) and Ude *et al.* (2006) in the Lower Guinean forest and the Congolian forest blocks. Thus, in total, 59 accessions (39 in the Dahomey Gap and 20 from Nigeria and Cameroon) were used in this study with 21 samples common to both analyses (Table 1).

Table 1: ABMT accessions and types of analysis applied

Accessions	Type of ABMT	Provenance: site of collection and country	Population	Types of analysis applied
P2	Sweet	Pobè, South Benin	SDG	AFLP
POB21	Sweet	Pobè, South Benin	SDG	AFLP
IP2	Sweet	Pobè, South Benin	SDG	AFLP
Ip4	Sweet	Pobè, South Benin	SDG	AFLP
Coco6	Sweet	Calavi, South Benin	SDG	AFLP
NPA4	Sweet	Parakou, North Benin	SDG	AFLP
NPA6	Sweet	Parakou, North Benin	SDG	AFLP
MBM1	Sweet	Sangmelima, South Cameroon	STA	AFLP
IW3BAD5	Bitter	Badou, Southwest Togo	BDG	AFLP
FNGB	Bitter	NAGRAB/ICRAF Gene bank, Nigeria	BTA	AFLP
DNGB	Bitter	NAGRAB/ICRAF Gene bank, Nigeria	BTA	AFLP
IWSAK1	Bitter	Centre Cameroon, ICRAF Kolbison Gene Bank, Cameroon	BTA	AFLP
POB20	Sweet	Pobè, South Benin	SDG	AFLP + SSR
Coco1	Sweet	Calavi, South Benin	SDG	AFLP + SSR
Coco5	Sweet	Calavi, South Benin	SDG	AFLP + SSR
NPA7	Sweet	Parakou, North Benin	SDG	AFLP + SSR
NPA9	Sweet	Parakou, North Benin	SDG	AFLP + SSR
TG1	Sweet	Atakpamè, Centre Togo	SDG	AFLP + SSR
TG18	Sweet	Lomé, South Togo	SDG	AFLP + SSR
BAD1	Sweet	Badou, Southwest Togo	SDG	AFLP + SSR
WAMP2	Sweet	Badou, Southwest Togo	SDG	AFLP + SSR
IGIBDGB2	Sweet	IITA Gene bank, Nigeria	STA	AFLP + SSR
IGIBDGB1	Sweet	IITA Gene bank, Nigeria	STA	AFLP + SSR
Limb	Sweet	Limbé, Southwest Cameroon	STA	AFLP + SSR
Limbe6	Sweet	Limbé, Southwest Cameroon	STA	AFLP + SSR
IGGBWAC	Sweet	ICRAF Kolbison Gene Bank, Yaoundé	STA	AFLP + SSR
NGMK1	Sweet	Sangmelima, South Cameroon	STA	AFLP + SSR
NMKIW02	Bitter	Mamfé, South Cameroon, ICRAF Mbalmayo Gene bank, Cameroon	BTA	AFLP + SSR
BSIW07	Bitter	Mamfé, South Cameroon, ICRAF Mbalmayo, Gene bank, Cameroon	BTA	AFLP + SSR
IWSAK2	Bitter	Centre Cameroon, ICRAF Kolbison Gene Bank, Cameroon	BTA	AFLP + SSR
CENRAD	Bitter	CENRAD Gene bank, Nigeria	BTA	AFLP + SSR
BAD4kiw	Bitter	Badou, Southwest Togo	BDG	AFLP + SSR
TGIW2	Bitter	Badou, Southwest Togo	BDG	AFLP + SSR
P2	Sweet	Pobè, South Benin	SDG	SSR
POB21	Sweet	Pobè, South Benin	SDG	SSR
CALI	Sweet	Calavi, South Benin	SDG	SSR
TORI13J	Sweet	Calavi, South Benin	SDG	SSR
TORI25	Sweet	Calavi, South Benin	SDG	SSR
Djot6	Sweet	Couffo, South Benin	SDG	SSR
LALO1G	Sweet	Couffo, South Benin	SDG	SSR
VODassa	Sweet	Dassa Centre Benin	SDG	SSR
Djoug	Sweet	Djougou, North Benin	SDG	SSR
Peninsou	Sweet	Peninsoulou, North Benin	SDG	SSR
Lom1	Sweet	Lomé, South Togo	SDG	SSR
L2	Sweet	Lomé, South Togo	SDG	SSR
TG4	Sweet	Lomé, South Togo	SDG	SSR

Accessions	Type of ABMT	Provenance: site of collection and country	Population	Types of analysis applied
TG12	Sweet	Lomé, South Togo	SDG	SSR
Atak	Sweet	Atakpamè, Centre Togo	SDG	SSR
BAD5	Sweet	Badou, Southwest Togo	SDG	SSR
IGGBWACII	Sweet	Centre Cameroon, ICRAF Kolbison Gene Bank, Cameroon	STA	SSR
MBUM	Sweet	Sangmelima, South Cameroon	STA	SSR
KGH1	Bitter	Kougnonhou, Southwest Togo	BDG	SSR
KGH2	Bitter	Kougnonhou, Southwest Togo	BDG	SSR
KGH3	Bitter	Kougnonhou, Southwest Togo	BDG	SSR
KGH4	Bitter	Kougnonhou, Southwest Togo	BDG	SSR
NKIW19	Bitter	Mamfé, South Cameroon, Mbalmayo Gene bank, Cameroon	BTA	SSR
BSIW324	Bitter	Mamfé, South Cameroon, Mbalmayo Gene bank, Cameroon	BTA	SSR
T2BSIW16	Bitter	Mamfé, South Cameroon, Mbalmayo Gene bank, Cameroon	BTA	SSR
IWSAK3	Bitter	Centre Cameroon, ICRAF Kolbison Gene Bank, Cameroon	BTA	SSR

SDG = sweet ABMTs from the Dahomey Gap, BDG = bitter ABMTs from the Dahomey Gap, STA = sweet ABMTs from Tropical Africa, BTA = bitter ABMTs from Tropical Africa.

Genetic diversity

The large number of bands generated and the high reproducibility of amplified fragment length polymorphisms (AFLPs) make them a marker widely used in genetic analysis (Berchowitz *et al.,* 2001; Assogbadjo *et al.,* 2010) and one that has advantages over RAPDs (Powell *et al.,* 1996). Microsatellites (or simple sequence repeats-SSRs) and especially chloroplast microsatellites (cpSSRs), have the power to reveal genetic diversity as well as phylogenetic relationships and hybridization between plant species (Wills *et al.,* 2005; Panwar *et al.,* 2010). We therefore decided to use both AFLPs and cpSSRs for our analyses.

AFLP data

The AFLP analysis was carried out at the Biosystematics Group, Wageningen University, the Netherlands, and followed the procedure of Vos *et al.* (1995) with minor modifications. Three primer combinations that had previously been demonstrated to successfully amplify sweet tree material (Ude *et al.,* 2006) were used to analyze all 33 samples. These are: E38M59 (Eco ACT / Mse CTA), E40M62 (Eco AGC / Mse CTT), and E33M48 (Eco AAG / Mse CAC).

PCR reactions were performed using a MJ PTC200 thermocycler. Prior to the selective amplification, the EcoR1 primer was fluorescently labelled with IRD700. AFLP fragments were separated on a LYCOR 4300 (Westburg, the Netherlands), and the resulting profiles were scored using the Quantar software (Key Gene Products, Wageningen, the Netherlands 2000) to produce a presence / absence data matrix.

cpSSR data

The cpSSR analysis was carried out in the Laboratory of Genetics and Biotechnology of the University of Abomey Calavi, Benin. Eighteen chloroplast SSR primers were tested on independent samples (bitter and sweet tree accessions). These are: CCMP 2, NTCP 8, NTCP 9, NTCP 30, NTCP 37, NTCP 39, NTCP 40, NTCP 5, NTCP 16, NTCP 19, NTCP 25, NTCP 26, NTCP 27, NTCP 29, NTCP 32, NTCP 33, NTCP 34 and NTCP 38. Of those 18 primers, seven (CCMP 2, NTCP 8, NTCP 9, NTCP 30, NTCP 37, NTCP 39 and NTCP 40) that amplified the chloroplast DNA of ABMT material were retained and applied to the complete set of 47 samples. PCR reaction was performed using a Peltier-Effect Cycling PTC 100 thermocycler programmed for an initial denaturation step at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s per cycle, a step at the annealing temperature for 1 min, a step at 72 °C for 1 min, and a final extension step at 72 °C for 5 min. Migration of the PCR products was visualized with denaturing polyacrylamide gel (5%) electrophoresis and then revealed with silver nitrate in accordance with Chair et al. (2005). The electrophoresis bands were scored to generate a presence / absence data matrix.

Data analysis

Genetic diversity and structure

Three datasets were considered in this study: the AFLP set, the cpSSRs set and the one containing the accessions that showed a result for both AFLP and cpSSR. An analysis of the genetic diversity and population structure based on allele frequency using AFLP-SURV version 1.0 (Vekemans, 2002) was performed on each dataset while the type of ABMT (sweet versus bitter) was considered as well as the geographical origin of the sample. Four geographic ‘populations’ were defined: (i) bitter trees from the Dahomey Gap (= population BDG), (ii) bitter trees from tropical Africa (= population BTA), (iii) sweet trees from the

Dahomey Gap (= population SDG) and (iv) sweet trees from tropical Africa (= population STA; Table 1). For each dataset we computed the mean Nei genetic diversity (Nei, 1973) per population, the global genetic differentiation (F_{st} statistics) and the pairwise genetic distance among populations and between sweet and bitter trees. Assuming no genetic structure among populations under a Hardy-Weinberg equilibrium (Vekemans, 2002), the significance of the genetic differentiation was assessed by comparing the observed F_{st} with the distribution of obtained F_{st} using 100 random individual permutations.

Identification of populations and distinction between sweet and bitter ABMTs

To assess the effectiveness of the genetic markers used in the discrimination of the four geographically recognized populations as well as the two types of ABMT, a cluster analysis was carried out on each dataset and a dendrogram was produced using the UPGMA method based on Jaccard similarity index (Jaccard, 1908) in Past (Hammer *et al.*, 2001):

$$I_j = {}^{a}/_{(a+b+c)} \tag{1}$$

Where for a random pair of individuals, a = number of totally loci scored present for the two individuals, b = number of loci scored present exclusively present for only one individual and c = number of loci exclusively present for only the second individual.

When classifying individuals using a Principal Component Analysis (PCA), it can happen that the obtained grouping pattern is unclear due to the abundance of factors of low contribution to an existing pattern in the dataset. Therefore, the PCA axes that explain a high percentage of the total variance within the dataset or the factors correlated with those axes could be used in a subsequent multivariate analysis to get a better signal from the dataset (see Mohammadi and Prasanna, 2003; Bidogeza *et al.*, 2009). Because the separate use of the AFLPs and cpSSRs data generated confusing patterns, only the combined AFLPs + cpSSRs data were used in the rest of the analysis. First, all the alleles with zero variability in the AFLPs + cpSSRs data were removed. A Principal Coordinate analysis (PCO) was performed to obtain the characteristics of the main groups yielded by the cluster analysis. A Principal Component Analysis (PCA) was performed on the combined dataset and the axes that accumulated at least 70% of the total variation were retained. The loci that were highly correlated (at least 70%) with those axis were used to produce a Neighbor Joining (NJ) tree using a Kulczynski similarity index (Kulczynski, 1927). Like the Jaccard index, the Kulczynski similarity index is one of the most consistent similarity index used in systematics (Boyce and Ellison, 2001) and is calculated as follows:

$$I_K = 0.5 * \left(\frac{a}{a+b} + \frac{a}{a+c}\right) \quad (2)$$

Where I_k is the Kulczynski index, a and b are the same notation as for the Jaccard index.

Table 2a: Results of the genetic diversity analysis with AFLP-SURV (abbreviations see Table 1); highest figures in bold

	Population level				**Type level**	
	BTA	**BDG**	**SDG**	**STA**	**bitter**	**sweet**
AFLPs						
Segregating fragments (%)	96.5				97.2	
Polymorphism (%)	22	66.7	**85.1**	60.3	66.7	**76.6**
Nei's genetic diversity	0.091	0.263	0.304	0.234	0.221	**0.264**
cpSSRs						
Segregating fragments (%)	100				100	
Polymorphism (%)	55	70	65	**90**	60	**80**
Nei's genetic diversity	0.202	0.273	**0.289**	0.235	0.240	**0.278**
AFLPs + cpSSRs						
Segregating fragments (%)	83.9				85.1	
Polymorphism (%)	24.8	50.9	**70.8**	63.4	50.9	**67.1**
Nei's genetic diversity	0.105	0.232	**0.251**	0.211	0.210	**0.245**

Table 2b: Pairwise F_{st} statistics among populations (abbreviations see Table 1); highest figures in bold.

	BTA	**BDG**	**SDG**	**STA**
AFLP				
BTA	0.0000			
BDG	0.1490	0.0000		
SDG	0.0731	**0.2407**	0.0000	
STA	0.0000	0.1587	0.0789	0.0000
cpSSRs				
BTA	0.0000			
BDG	0.0772	0.0000		
SDG	0.0000	0.1244	0.0000	
STA	0.0437	**0.3268**	0.0411	0.0000
AFLPs + cpSSRs				
BTA	0.0000			
BDG	0.2511	0.0000		
SDG	0.1320	0.1618	0.0000	
STA	0.1646	**0.2916**	0.0960	0.0000

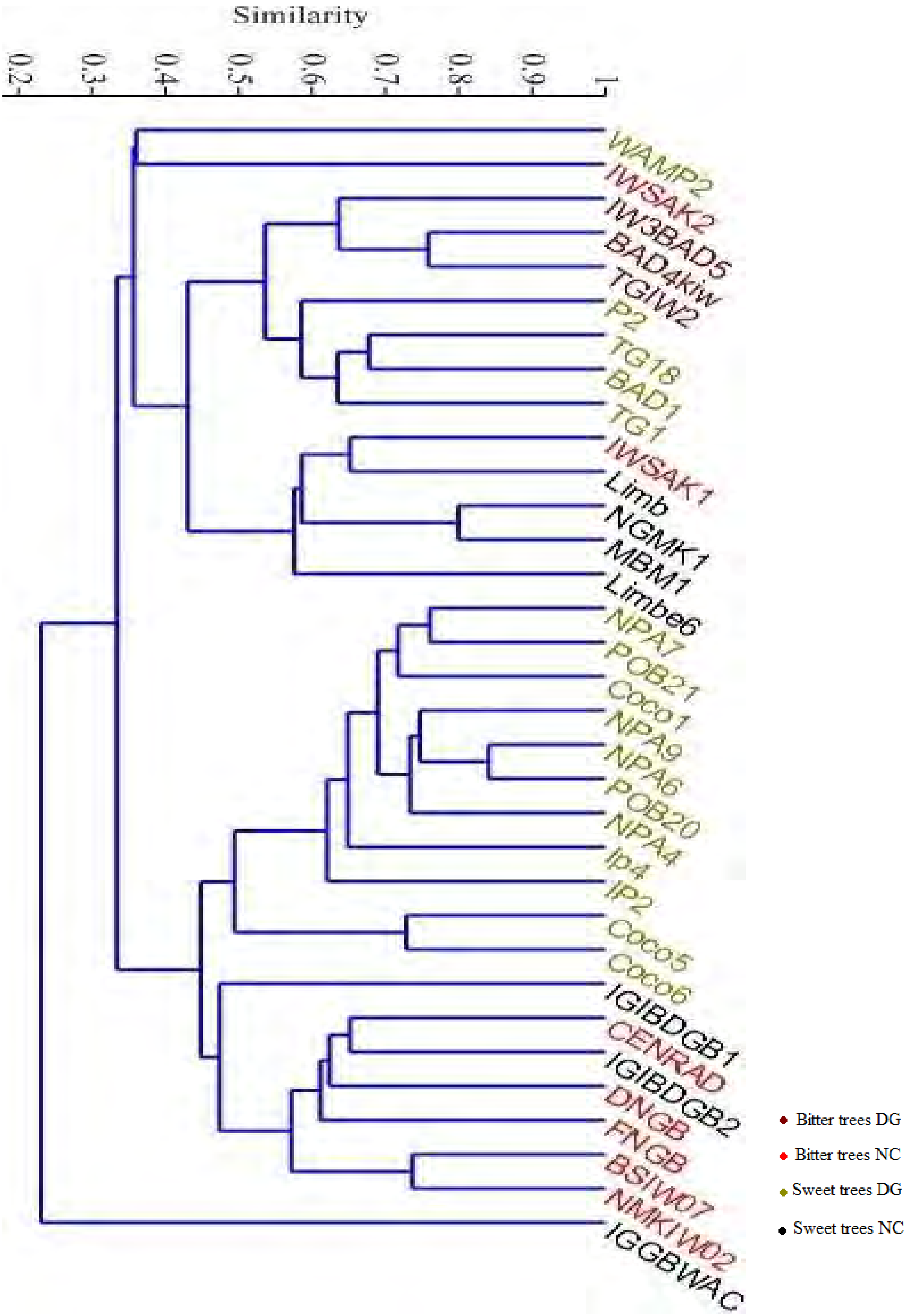

Figure 1a: UPGMA dendrogram for AFLP data of the 33 accessions based on Jaccard's similarity index

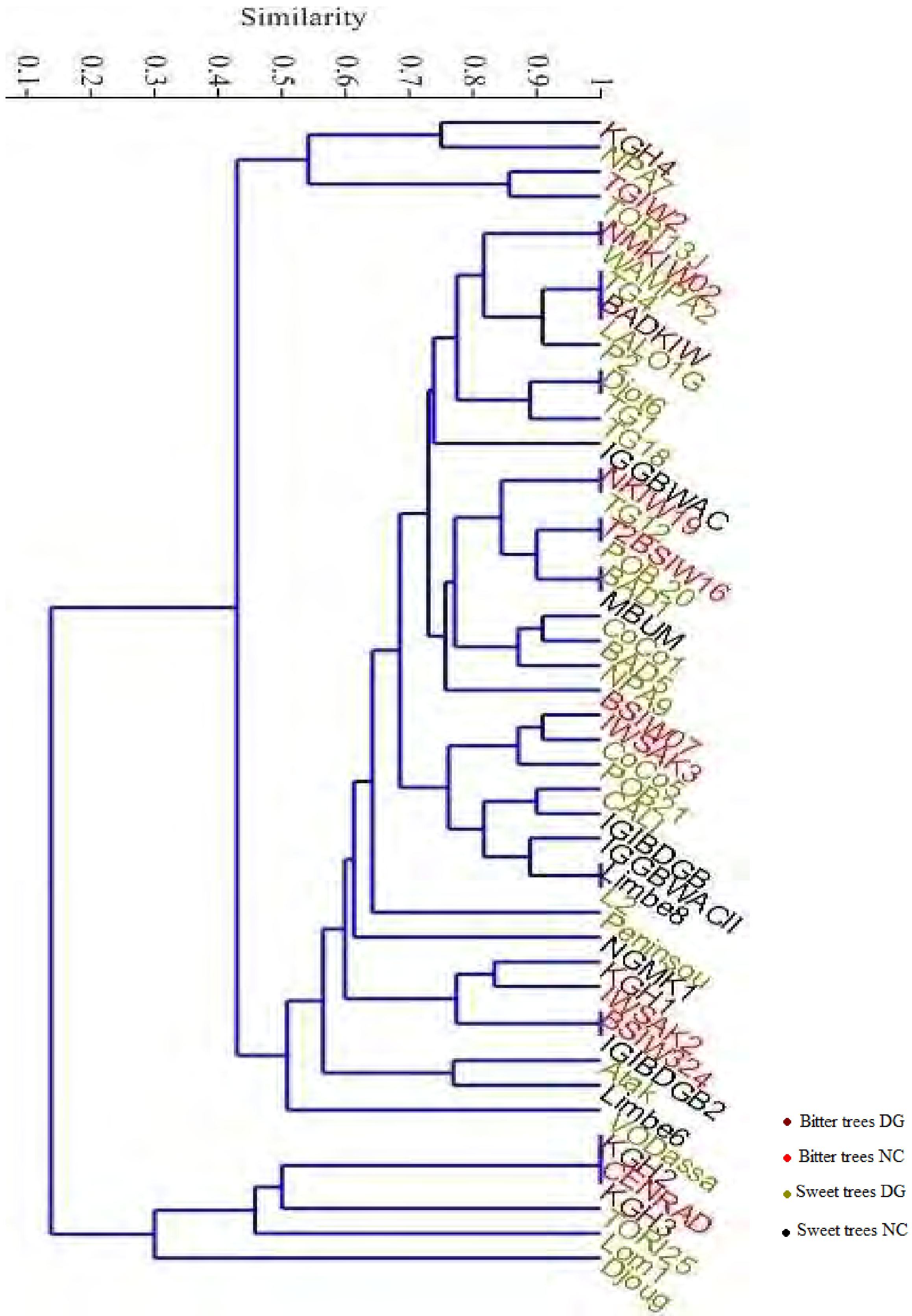

Figure 1b: UPGMA dendrogram for cpSSR data of the 47 accessions based on Jaccard's similarity index

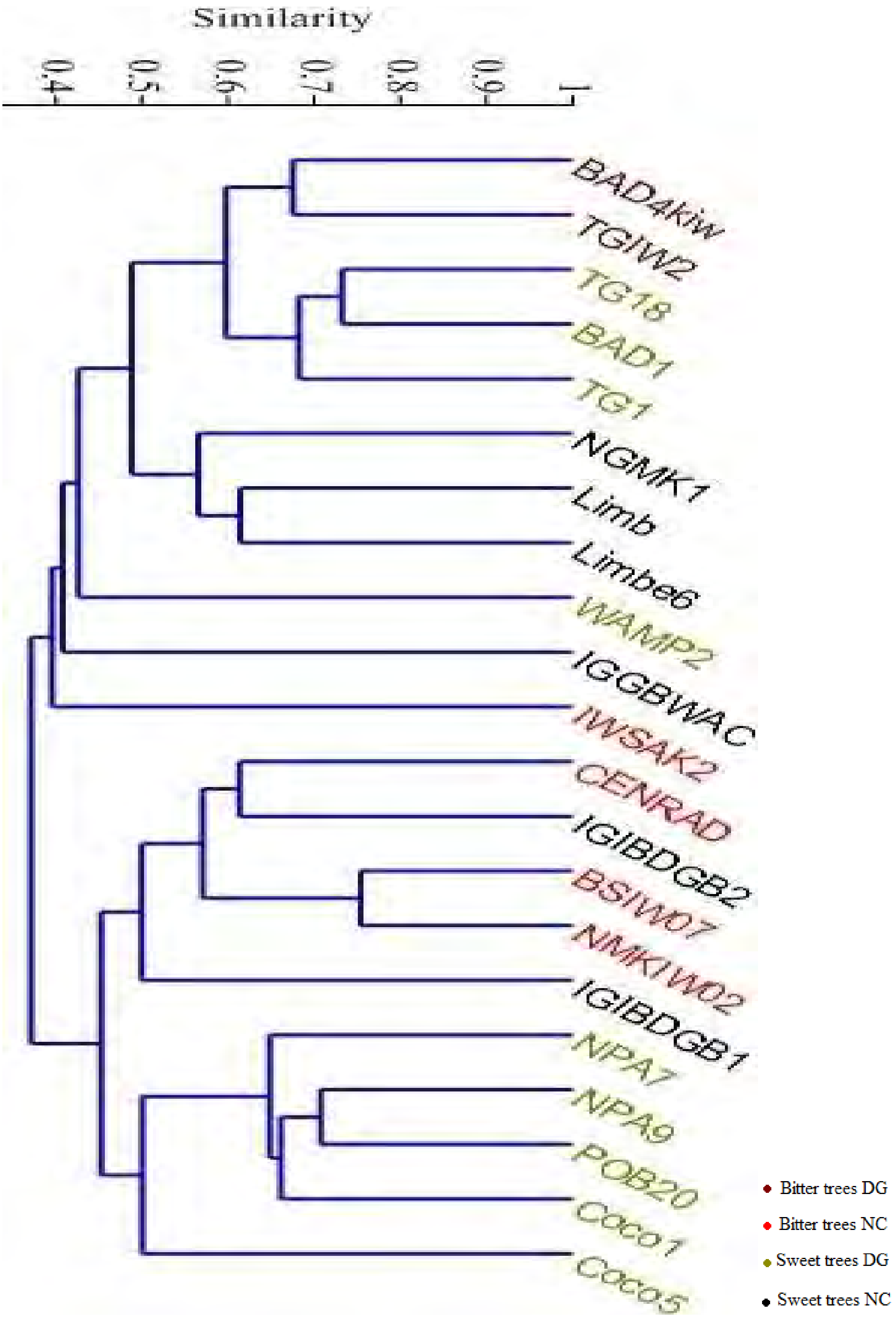

Figure 1c: UPGMA dendrogram for AFLP + cpSSR data of the 21 accessions based on Jaccard’s similarity index

Results

For the AFLPs analysis a total of 141 polymorphic alleles were scored for all the 33 individuals. The cpSSR analysis yielded 20 polymorphic alleles (1 to 6 per locus). Thus, a total of 161 polymorphic alleles were available for the set of 21 samples with both AFLP and cpSSR results.

Genetic diversity and differentiation of ABMTs

The AFLP analysis indicates that the mean number of fragments scored as present for an individual tree was 47. The number of segregating fragments in the dataset is high (96.5 %). The polymorphism is highest within the sweet tree Dahomey Gap population (SDG) and lowest in the Dahomey Gap bitter trees population. The same tendency is observed regarding the within population Nei genetic diversity. The SDG population presents the significantly highest genetic diversity, while the BDG one presents the lowest diversity (Table 2a). The test for genetic differentiation among populations indicates a global F_{st} value of 0.108 (P = 0.024). The pairwise F_{st} values among populations is higher between bitter and sweet tree populations within the Dahomey Gap and no genetic difference is found between bitter and sweet tree populations outside of the Dahomey Gap (Table 2b). Sweet trees show a higher polymorphism and Nei genetic diversity than bitter trees (Table 2a). However, the difference based on this AFLP data is not significant (global F_{st} = 0.034; P =0.0639). The F_{st} between bitter and sweet tree populations is very low (0.011).

The cpSSR results show a mean number of fragments scored as present at individual tree level of 8 and all the 20 scored loci have segregation power. The polymorphism is highest in the sweet tropical African (STA) population and lowest for the BDG trees. The SDG population and the BTA one present the highest genetic diversity, while the BDG population presents the lowest diversity again (Table 2a). No clearly significant genetic differentiation is found among populations (global F_{st} = 0.105; P = 0.048 $\cong$ 0.05) even though the highest pairwise F_{st} is found between BDG and STA populations (Table 2b). Considering bitter versus sweet trees, the genetic diversity is highest in the sweet trees (Table 2a). However, based on the cpSSRs there is no significant genetic differentiation amongst the two types (F_{st} = 0.0537; P = 0.077), and the F_{st} between bitter and sweet tree populations is low (0.0540).

The combined AFLPs+ cpSSRs data also indicates a high number of alleles with segregating power (83.9%), with 24 alleles presenting no variability. The within population proportion of polymorphism and the Nei genetic diversity show the same tendency as the separate AFLPs and cpSSRs results: the highest value is calculated for the SDG population and the lowest in the BDG population (Table 2a). In this case, a significant genetic differentiation was detected among populations (global F_{st} = 0.0176; P = 0.016). The highest pairwise F_{st} is found between the BDG population and the STA one, while the lowest distance is present between the SDG and STA ones (Table 2b). Considering sweet and bitter trees, 85.1% of the combined AFLP and SSR alleles have segregation power. The proportion of polymorphic loci is higher within sweet trees than within bitter trees (Table 2b). The Nei genetic diversity is 0.2453 and 0.21 for sweet and bitter trees, respectively. The genetic differentiation between sweet and bitter trees is low (F_{st} = 0.0335) and not significant (global F_{st} = 0.0333; P = 0.064).

Cluster analysis

The dendrogram based on the AFLP results (Figure 1a) shows no clear pattern among populations and no clear distinction between bitter and sweet trees. The majority of individuals from each considered population are spread across many clusters. However, apart from few accessions, there is a tendency for the Togo (bitter and sweet) and Benin (sweet) materials to cluster together (Fig. 1a; Table 1).

The dendrogram based on the cpSSR results (Fig. 1b; Table 1) shows an even less clear pattern with higher similarity among individuals and completely fails to discriminate between the two types of ABMTs or geographically defined populations.

The dendrogram resulting from the cluster analysis of the combined APLP + cpSSR data (Fig. 1c; Table 1) is more discriminative than those obtained from the separate AFLP and cpSSR datasets. Apart from one accession from Togo (WAMP2), four geographically distinct groups can be distinguished from the lower to upper position: (i) all sweet trees from Benin, (ii) sweet and bitter trees from the Lower Guinean forest bloc (southern Nigeria and Mamfé region in South-west Cameroon), (iii) sweet and bitter trees from Central and South Cameroon, and (iv) bitter and sweet trees from Togo.

The first two axes of the PCO on the combined dataset with 137 alleles (Figure 2a; Table 1) accounts for 62.5%of the variance (46.13% and 16.38 % for coordinate 1 and 2, respectively). The PCO tends to separate the bitter from sweet ABMTs, with the two sweet trees from Nigeria (IGIBGB1 and IGIBGB2) falling within the bitter tree group. Within each of the two groups, the populations are not clearly distinguishable apart from the sweet trees from Benin that are clearly separated (as in Figure 1a and 1c).

Forty-eight alleles are highly correlated (at least 70%) with the first 8 PCA axes which account for 72.8% of the total variation. The clustering obtained with NJ based on the Kulczynski similarity index of these alleles (Figure 2b) confirms the pattern in the PCA. The inability of the combined AFLP + cpSSR data to accurately separate populations and the clustering of the sweet trees from Nigeria within the bitter tree cluster was also confirmed.

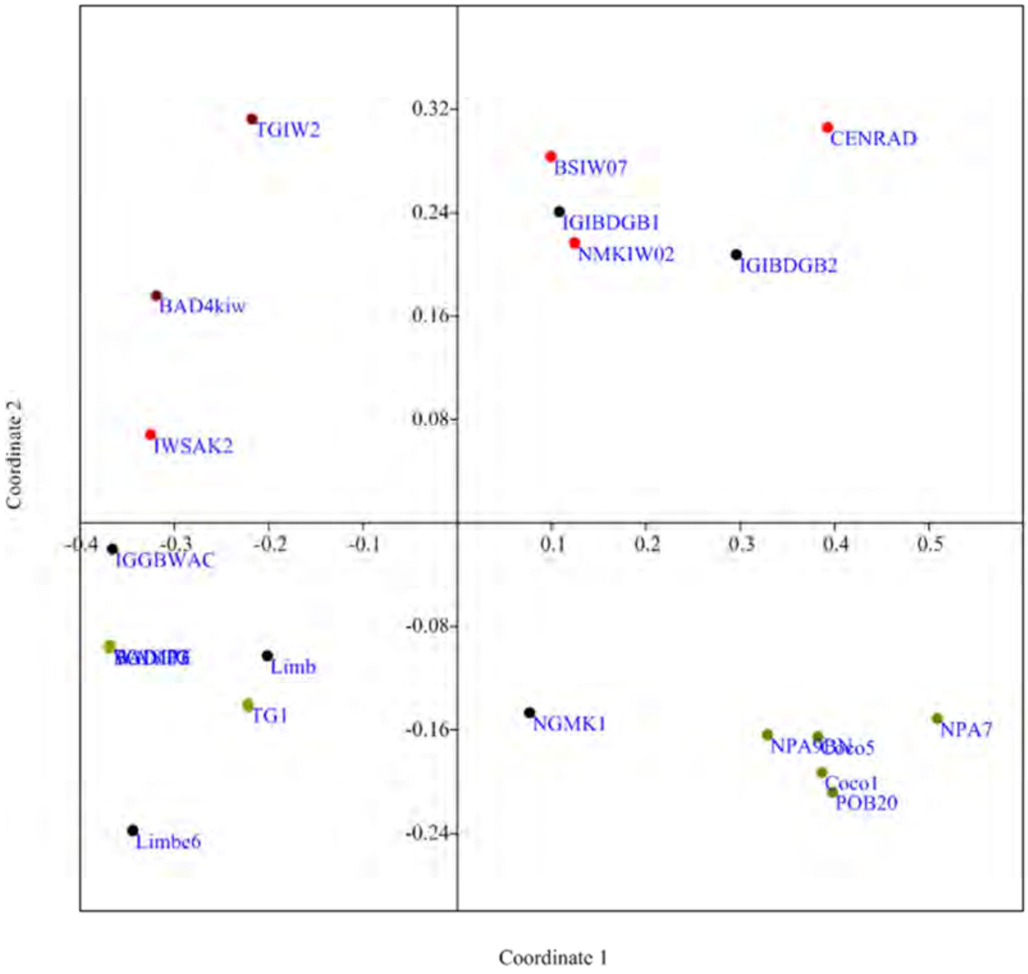

Figure 2a: Plot of first two principal coordinates based on Jaccard’s similarity index with the 48 AFLPs+ cpSSRs for the 21 common accessions. Legend (color) is the same as in Figure 1

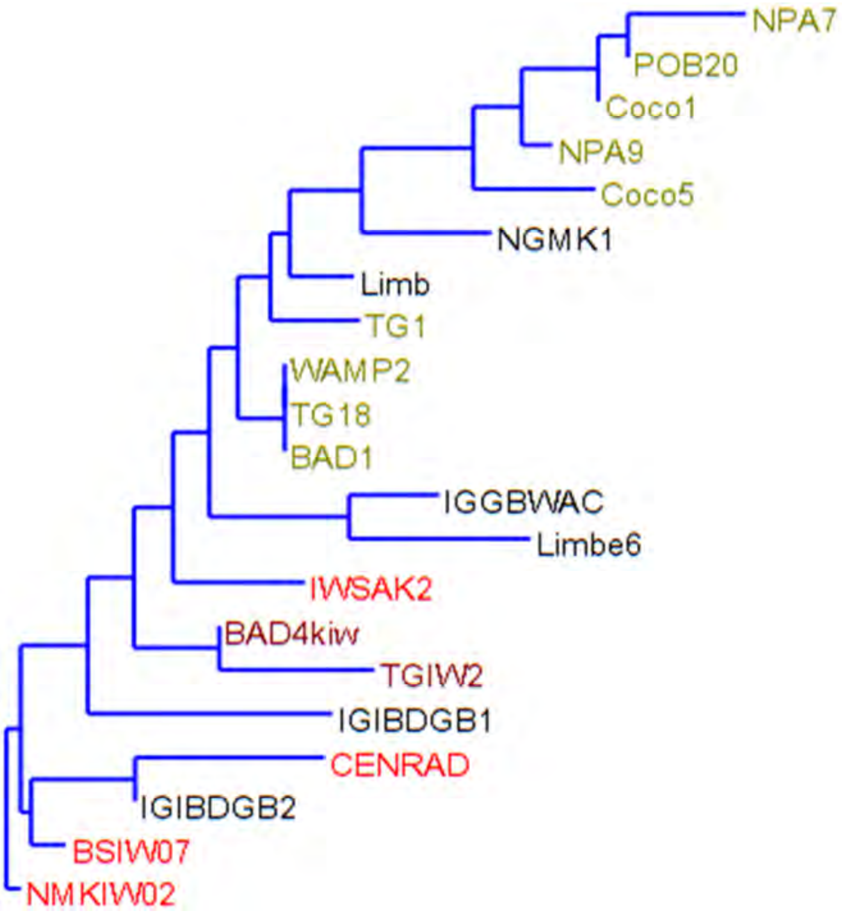

Figure 2b: Kulczynski similarity-based Neighbor Joining tree of the 48 AFLPs + cpSSRs loci. Legend (color) is the same as in Figure 1.

Discussion

ABMTs genetic diversity: failure of AFLPs and cpSSRs or influence of domestication?

For all three dataset considered, the lowest polymorphism and genetic diversity is found in the BDG population, while the highest values for these parameters are calculated for the cultivated SDG trees. These results are not consistent with those of Lowe *et al.* (2000) and Ude *et al.* (2006) who found a higher genetic diversity in Cameroon and Nigeria. Environmental transformation through logging, extension of agricultural productive space through yearly bush fires, and urbanisation are the main causes of biodiversity lost in tropical Africa (FRIG, 2003; Sodhi, 2007; Jose, 2012). Together with fragmentation and decrease of population size, these factors play a key role in shaping the climatic characteristics of the Volta forest region, a unique ecosystem in which bitter trees are found in the wild in the

Dahomey Gap (Vihotogbé *et al.,* submitted-1). In addition to these factors, the economic potential of bush mangoes' seed turns out to be one of the most important threats to ABMTs, since the market of this non timber forest product relies mostly on natural populations (Agbor, 1994; Lowe *et al.,* 2000). The population size of wild bitter ABMTs is decreasing in the Volta forest region as is the case in their entire distribution range due to a lack of sufficient natural regeneration (Agbor, 1994; Zapfack and Ngobo-Nkongo, 2002; Vihotogbé *et al.,* submitted-1). Consequently, this reduction of their ecological variability might be narrowing their morphological and genetic diversity. The domestication and cultivation of sweet trees, in various climatic zones in the Dahomey Gap, seem to preserve or increase the existing diversity (see Casas *et al.,* 2005; Jose, 2012). Although the provenance of sweet ABMTs in the Dahomey Gap is unknown (Harris, 1996; Asaah *et al.,* 2003; Lesley and Brown, 2004; Ude *et al.,* 2006; Vihotogbé *et al.,*submitted-1), their higher diversity in this eco-region may well be due to the fact that the ongoing traditional domestication process in this region includes material from geographically different origins: the Upper and Lower Guinean forest blocks as well as the Congolian forest region. We conclude that this is a consequence of the random genetic material transfer between and within local communities, not only for ABMTs but related to any economically important food tree species in agroforestry systems (Jose, 2012).

In general, for the three datasets no significant genetic differentiation is found between bitter and sweet ABMTs. Similarly, no genetic differentiation among populations was observed with the AFLP dataset. Genetic differentiation was detected within the cpSSR and AFLP +cpSSR datasets but their patterns are not the same. The dominance of cultivated material in our samples (collected in the field as well as in gene banks) might result in an expression of artificially generated variation.

Comparatively few sharp bands could be scored with the AFLP and the cpSSR products. This weakness also appeared in the AFLP study of Ude *et al.* (2006) and forced these authors to the use of no less than 12 pairs of primers. Our similar unclear results on the genetic diversity among populations and most importantly between bitter and sweet trees pose the important question concerning the suitability of the genetic markers used in our study. Thus, including wild material from every eco-region in the entire distribution range of ABMTs and analyse those in a sound genetic analysis will be of great importance in evaluating their genetic diversity, the influence of domestication, and their genetic adaptability.

Suitability of the markers

Apart from the sweet tree population from Benin, which forms the most consistently distinct cluster throughout our analyses, none of the methods used in this study clearly separate the geographically distinct populations (Figures 1a-2b). The PCA and NJ (Figure 2a and 2b)do show a distinction between the two types, with the exception of the sweet tree samples from Nigeria which cluster with the bitter trees (Figure 2a and 2b). This was also observed in the study of Lowe *et al.* (2000), but was explained in terms of inaccuracy of sampling. In our study, the fact that neither the PCA plots nor the NJ dendrogram are able to discriminate either geographical populations or fruit types implies that the markers used to achieve this goal are probably unsuitable. This idea is corroborated by the fact that we know that in the area where bitter and sweet ABMTs co-occur (in the Volta forest region) successful gene flow between bitter and sweet trees is hardly to be expected for phenological reasons: (i) very short co-flowering time, (ii) flowers abortion on all sweet trees after this co-flowering time and (iii) consistent overall difference in phenology between both types (Vihotogbé *et al.*, submitted-2). Further support comes from the presence of ecological differences between both types (see Lesley and Brown, 2004; Vihotogbé *et al.*, submitted-1). Therefore, the inability of the AFLP and cpSSR data to distinguish the two types might be attributed to the high level of sweet ABMTs diversity (Kelleher *et al.*, 2005). So, in conclusion, we attribute the failure of AFLPs, cpSSRs and AFLPs + cpSSRs to distinguish populations to the effect of domestication and large scale transfer of genetic material via seeds of the economically and nutritionally appreciated ABMT morphotypes.

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Chapter 7

General discussion and conclusions

Introduction

African bush mango trees (ABMTs) are important multi-purpose trees, belonging to two different taxonomic entities: one having fruits with a generally sweet and edible mesocarp and one with inedible bitter fruits. This thesis analyses the differences within and between sweet and bitter ABMTs, with an emphasis on the Dahomey Gap region, in order to decide upon their taxonomic status. Ecological, phenological, morphological and genetic approaches have been used to evaluate these differences. In this chapter, we also relate the traditional knowledge of local communities concerning bush mango morphotypes to the ecological, phenological, morphological and genetic data to draw an all-inclusive conclusion on the species distinction within ABMTs, their domestication and ecological adaptation and conservation, with emphasis on the Dahomey Gap. Possibilities for future research to further improve our knowledge related to some of the specific objectives addressed in the General Introduction are also discussed.

Key results concerning the differences between sweet and bitter ABMTs

A large-scale ethnobotanical inventory showed that, in terms of their value, local communities in the Dahomey Gap use bitter ABMTs only as timber, while sweet trees have a variety of other uses (nutritional, medicinal, socio-cultural, energetic). Even though the seed of bitter trees tends to be superior in quality to that of the sweet ones, it is not consumed by the indigenous communities in the Volta Region, the only region where bitter trees are found. They do not find any direct advantage for bitter trees cultivation. Therefore, the domestication of superior seed quality trees relies on wild material of bitter trees, while cultivation of sweet trees is much more common and highy stimulated by the additional advantage of consumption of the sweet mesocarp.

We demonstrated the existence of ecological differences between sweet and bitter ABMTs and that in the Dahomey Gap bitter trees are the only type of ABMTs occurring in the wild. The increase of the economic value of the seeds is causing the expansion of the area of cultivation of sweet trees and a restriction of the distribution area of bitter trees in the Dahomey Gap.

Based on provenances from the Dahomey Gap, important differences in phenological features, especially in the reproductive stages, were found. While bitter trees produce fruits once a year, sweet trees can have two or sometimes even three reproductive cycles per year.

Our morphological data failed to consistently discriminate between sweet and bitter trees and the quantitative measurements of fruits, mesocarp and seeds showed the absence of a joint effect to discriminate between populations. However, the data do show a clear effect of domestication.

Unexpectedly, the genetic diversity was higher in the cultivated sweet tree populations from the Dahomey Gap than in other cultivated as well as wild populations. However, AFLPs and cpSSRs failed to consistently separate the two types of ABMTs nor could they distinguish the various geographical populations.

Traditional taxonomy in the Dahomey Gap

In their symbiotic relationship with natural ecosystems, human communities have often developed traditional taxonomic systems based on plant traits (Hays, 1983). Traditional taxonomic systems have even assisted in relating the variability of the morphological characteristics of Baobab trees (*Adansonia digitata* L., Bombacaceae) to their differences in biochemical content and in the use of their non-timber forest products (Assogbadjo *et al.*, 2008). Similarly, such systems might allow the distinction of various types of ABMTs, based on the differences in their non-timber forest products. In the Nago socio-cultural area located in the centre of Benin, where no bitter trees are present, the sweet bush mango is locally called "Oro-N’Bèdjè" (Kengni *et al.*, 2010). This local name literally means “the bush mango with sweet / edible mesocarp”. This would suggest that local communities have experienced both sweet and bitter bush mangoes. However, the name "Oro-Koro", used by the Yoruba tribe to identify bitter trees in Nigeria, where both bitter and sweet trees occur in the wild (see Lesley and Brown, 2004), does not appear in the traditional taxonomic system in Benin and

Togo. The Nigerian origin of the Yoruba-Nagot-Holli tribes in Benin (Parrinder, 1947; Igue and Olabiyi, 1973) together with the comparatively low level of their ethnobotanical knowledge on ABMTs indicates that bitter trees were confined to the Volta region in Togo or became extinct in the Dahomey Gap before human settlement. At least, this extinction would have taken place before the migration of the Yoruba tribes into this eco-region. Furthermore, the local names "Oro-N’Bèdjè" and "Oro-Koro" are part of the taxonomic system of the Nigerian Yoruba migrants, and it seems likely that the use of "Oro-Koro" has eroded from their language when in Benin and Togo since they did not encounter bitter trees. However, since the migration of Nigerian Yoruba people to Benin and Togo is fairly recent (see Parrinder, 1947), it might also be that wild bitter trees, with their superior seed quality, did not occur in the Yoruba lands in Nigeria, because if this had been the case, they would most likely have propagated them along their migration routes. Finally, if bitter ABMTs did occur outside of the Volta forest region, they would most likely have been subjected to traditional domestication practices, and at least, they would have been mentioned by local communities during the ethnobotanical surveys. Moreover, the organoleptic superiority of their seeds would have been recognized locally and increased their economic value and this would have led to cultivation initiatives with an occurrence area wider than the very limited natural one of the Volta forest region in the Dahomey Gap.

The route along which ABMT material reached the Dahomey Gap remains a central issue. This is equally true for many other economically important food trees species in that area like the white star apple tree (*Chrysophyllum albidum*, Sapotaceae) and the akee tree (*Blighia sapida*, Sapindaceae). In this study, we postulate that ABMTs in the Dahomey Gap comprise a mixture of sweet trees from the Upper and Lower Guinean forest blocks and bitter trees native to this area. However, evidence that native sweet trees have not existed in the Dahomey Gap (Harris, 1996) needs to be demonstrated.

Even though the accuracy of traditional taxonomy could be questioned, it can help to get an overview of the diversity within a plant species (Sambatti *et al.,* 2001). To our knowledge, the traditional taxonomy of ABMTs has never been studied, but judging from the local names in the Dahomey Gap the taxonomical distinction of sweet and bitter trees remains a confusing issue. However, the names "never planted ABMT" and "wild ABMT", used in the Volta forest region to indicate bitter trees, do indicate that the local communities recognize their taxonomic distinction.

Cultivation of ABMTs and natural resources conservation

The limited surface of forest lands in the Dahomey Gap, combined with the predominance of traditional agricultural practices (slash and burn technique) inevitably exposes this eco-region to a high rate of soil degradation, and therefore to a fast soil carbon content exhaustion (Lal, 2004). The most important consequence of this exposure is the low subsistence crop yield, causing recurrent food crises in the region. The intensive cultivation of sweet ABMTs (and the selection of economically profitable tree ideotypes) acts as a restorative land use strategy for soil conservation that can be recommended to restore soil carbon content, reduce soil degradation and enhance arable soil water holding capacity. Therefore, ABMT cultivation has the potential to increase the productivity of traditional agroforestry systems (also through carbon sequestration, for example see Li and Feng, 2002). This productivity is rooted in the commercialization of the fruits and seeds of bush mangoes and the successful local initiatives to apply intercropping with water demanding crops (such as the vegetables *Capsicum frutescens, Solanum macrocarpon* and *Colocasia esculenta*) below ABMTs (Fig. 7.1 and 7.2). The development of such intercropping systems based on ecologically adapted fruit tree species makes it possible to: (i) use soils sustainably by increasing their resilience, (ii) enhance the livelihood and nutrition of communities, (iii) limit the use of chemical fertilizers and, therefore, reduce atmospheric and soil pollution. Because sweet trees present a direct benefit for local communities through the consumption of the seeds and the commercialization of the entire fruits, their domestication process has probably been faster than that of bitter trees. This has resulted in the collecting and domestication of more sweet tree ideotypes, increasing their genetic diversity in the Dahomey Gap.

Testing the taxonomic distinction within ABMTs

Local communities clearly distinguish bitter and sweet ABMTs and, as these have different economic and market aspects, this leads to a difference in their domestication process. Unfortunately, not all our analyses revealed a clear difference between sweet and bitter ABMTs. Where the ecological characteristics (chapter 2) are significantly different, the genetic analysis (chapter 6) gives no implications for a taxonomic distinction. This could be due to the markers used (AFLP and cpSSRs). For example, AFLPs failed to separate morphologically different species of oak (*Quercus petraea* and *Q. robur*, Fagaceae) and Kelleher *et al.* (2005) attributed this failure to a higher differentiation within populations than between species. This shows how closely related species might sometimes be difficult to separate, even with modern genetic methods (John *et al.,* 2005). Other studies on important African fruit trees, such as Assogbadjo *et al.* (2006, 2008) on Baobab, did find significant genetic differences among geographically isolated and morphologically distinct populations, while that of Ewédjè *et al.* (2012) on Butter tree (*Pentadesma butyracea*, Clusiaceae) detected that morphological traits consistently separated genetically distinct populations. The domestication process of these species still relies on wild trees preserved in traditional agroforestry systems in the Dahomey Gap. The absence of this link between ABMTs genetic, morphological and phenological data might be explained by a higher degree of manipulation of genetic material and hence a higher degree of hybridization amongst economically and nutritionally valuable trees over their entire distribution. Another explanation could be related to the fact that there is no fixed ABMT ideotype and that farmers rely on seed to enrich agroforestry systems. Petit and Excoffier (2009) pointed out that because cpDNA is only dispersed via seed, it is more frequently introgressed than nuclear material and, therefore, of less taxonomic value for species distinction. In addition, a high level of cross pollination may explain the failure of AFLPs and cpSSRs to assess the taxon delimitation within ABMTs.

Finally, our results suggest that an efficient conservation program of ABMTs is needed in order to plan an effective domestication program. Such a conservation program should encompass material of both sweet and bitter trees from a diverse set of ecological regions in order to ensure the capture of a maximum of variation.

Conclusions regarding ABMT diversity and taxonomic identity

The different analyses undertaken throughout this thesis intended to reveal the diversity patterns within ABMTs and establish the taxonomic characters valuable to distinguish sweet and bitter trees as well as assess the taxonomic level most suitable for the situation. Based on the different results obtained, we draw the following conclusions:

1- Ecological differences exist between wild sweet and bitter trees with bitter trees extending to drier areas. A seemingly conflicting situation is observed in the particular situation of the Dahomey Gap where sweet trees grow in much drier ecological zones than could be expected from their natural occurrence. Since, however, in the Dahomey Gap sweet trees do not occur wild, we assume this to be due to the domestication and cultivation efforts and so accept the presence of an ecological difference between sweet and bitter ABMTs.

2- Sweet and bitter trees show clear phenological differences even in areas where they co-occur. However, because all observations related to sweet trees were performed on cultivated material, we cannot rule out the possibility that this difference was in fact introduced during the domestication process.

3- Based on the characters we investigated, a morphological differentiation between sweet and bitter trees could not be demonstrated. During our fieldwork, we did, however, observe several potentially distinctive features in other characters (f.e. crown shape, branching pattern, leaf shape, petal colour) which should be the subject of additional study.

4- Our genetic markers failed to demonstrate a genetic differentiation into different taxa. However, we argue that the markers used are not the most suitable ones in the case of ABMTs, and suggest a more extensive genetic analysis of the material.

5- We judge the clear ecological and phenological differences between sweet and bitter trees we observed, along with the fruit taste and the morphological distinction made by Harris (1996), as sufficient to consider these taxa as taxonomically distinct. We expect the outcome of additional morphological and genetic studies to further support these observations, which is why we advise to recognize the two ABMT taxa at the species level.

Suggestions for future research

In chapter 3, the geostatistic approach (the variogram and kriging) used to estimate the spatial structure and population density across the Dahomey Gap was consistent in all directions, but we did not incorporate soil and climate micro-variation data in our model (as in chapter 2) in order to better approach the real situation. Even though our later knowledge on the ecological requirements of ABMTs indicated that soil characteristics play only a minor role in their potential distribution, differences in population density across the Dahomey Gap give rise to the question of their behaviour on different soil types. Because the density at a given location is the result of a combination of environmental factors and anthropogenic ones, both are important to strengthen the validity of our model. Therefore, applying a co-kriging method based on the correlation between the environmental plus anthropogenic data and the density values might be useful to further refine the results.

The morphological data used to assess the differences between bitter and sweet ABMTs did not include the diversity detected in complementary studies. Notably, features of the tree shape, leaf shape and flower colour seem promising (Chapter 5). Therefore, an additional morphological study should be undertaken using material from the entire distribution range and, since taxon distinction is expected to be difficult, the data analysed in a full scale statistical analysis.

Our study has used more cultivated / human influenced material than wild provenances of ABMTs. Thus, the patterns of variation present in ABMT material that has potentially been domesticated to adapt to new ecological environments outside of their natural niche, could well mask natural variations valuable for taxon distinction. Therefore, a new in-depth study using only wild provenances originating throughout the natural distribution of both taxa still seems a worthwhile undertaking.

Finally, in order to capture the maximum variation within ABMTs and design better strategies for the in- and ex-situ conservation as well as domestication of ABMTs, we propose to conduct the following additional studies:

1- Determine the economically profitable spatial arrangement of various agroforestry systems, including intercropping with various species, and model the productivity (fruit and seed) of both sweet and bitter trees under different agro-ecological conditions.
2- Specifically, domesticated material from the Dahomey Gap may well reveal the drought resistance of some ABMT ideotypes which are valuable to face future more severe climatic conditions or may lead to a further expansion of the cultivatable area of this valuable multi-purpose tree.
3- Collect and fully document the diversity of ABMTs and bush mango ideotypes and conduct a consumption preference test in order to classify those ideotypes.
4- Investigate the differences in biochemical and nutritional characteristics against the ABMT ideotypes in order to classify those ideotypes economically.
5- Investigate the capacity of ABMTs for soil carbon sequestration and thus their ability for climate change mitigation, or even whether ABMT plantations can be regarded as a form of carbon storage and thus through international incentives contribute to a higher net income of rural populations.

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Summary

African bush mango trees (ABMTs) are economically the most important species within the family of Irvingiaceae. They are priority trees producing non-timber forest products (NTFPs) and widely distributed in the humid lowland forests of West and Central Africa. To boost their production and develop them towards a major crop for rural communities in Africa, a domestication program was initiated in the 2000s which is being coordinated by the World Agroforestry Centre. ABMTs belong to two taxa, one with sweet and one with bitter fruits which are morphologically difficult to distinguish. The fresh mesocarp of the sweet bush mangoes are consumed, while the seed of both bitter and sweet fruits are an important component of the African diet. The high oil content of this seed further increases their potential use.

Apart from the overlap of their morphological characters, the ecological and phenological distinction between sweet and bitter ABMTs is unclear due to: (i) the lack of comparative quantitative data and (ii) the lack of centralizing the existing country-level databases. Therefore, their taxonomic status is still not clear. Do they represent distinct species or varieties or are they mere forms within the same gene pool? It is also unclear whether the occurrence of ABMTs in traditional agroforestry systems in the Dahomey Gap, the dry savannah corridor between the Upper and Lower Guinean rain forest blocks, forms part of the natural distribution or not . Moreover, genetic studies addressing ABMTs diversity have been geographically restricted, and conclusions regarding the taxonomic status of sweet and bitter trees were not unanimous.

This study was conducted in a perspective of developing suitable strategies for the conservation and use of ABMTs, mostly in the Dahomey Gap. First, differences in ethnobotanical knowledge of the major socio-cultural groups in the Dahomey Gap were linked to the agroforestry status of ABMTs. This was used to explain the characteristics of the spatial pattern of ABMTs abundance and the anthropogenic factors that govern this spatial structure as well as population survival in the Dahomey Gap. Second, occurrence data of wild and cultivated ABMTs were used in a species distribution modelling algorithm to calculate the niche space and potential distribution of bitter and sweet trees in Tropical Africa. The differences in the obtained distribution patterns were compared using ENM-Tools. Third, detailed monthly phenological data and morphological characteristics (qualitative as well as

quantitative measurements on the leaves, bark, fruits, and seeds) were used to analyse the diversity of ABMTs and to discover differences between them in order to be able to identify bitter and sweet trees in the field. Finally, the molecular markers AFLP and cpSSR were used in order to map the genetic diversity of ABMTs and to discriminate sweet and bitter trees across Togo, Benin, Nigeria and Cameroon.

The consumption of mesocarp and seed of bush mangoes is widely known throughout the Dahomey Gap. The level of knowledge within local communities of other types of uses (medical, social-cultural) is generally poor and decreases towards the western part of this region. This suggests that ABMTs (mostly the sweet trees) were introduced in this eco-region through the migration of human populations from the Lower Guinean forest block (Southeast Nigeria) to the West. In the Dahomey Gap, bitter trees are confined to the Volta forest region, a small-sized ecological area in south-western Togo. While low densities (< 462 trees per 25 ha) were recorded for wild bitter trees occurring in the Volta forest region, higher densities (up to 1020 trees per 25 ha) were found for sweet trees in human made agro-systems. This implies a clear difference in cultivation methods between bitter and sweet ABMTs. The intensive cultivation of ABMTs in the Dahomey Gap is influenced by farmland status, farmer’s socio-cultural group and type of ABMT. Small and exhausted farmlands are converted into sweet ABMT orchards indicating that their development is a small-scale process lead by individual farmers. Slash and burn agriculture and intensive collection of fruits for seed commercialization jeopardize bitter trees, while traditional fishing systems (using twigs), traditional mass selection strategy, and intensive land commercialization severely threaten sweet trees genetic resources.

Using species distribution modelling, the potential distribution of wild sweet trees was predicted in the wetter zones of the Guinean-Congolian phytogeographical region, while that of bitter trees extended to drier zones in the Guineo-Congolia/Sudania and Lake Victoria regions. This difference is significant, supporting the idea that bitter and sweet trees belong to two different species. In the Dahomey Gap, bitter trees occur in the wild in the wettest ecological region of the Volta forest region which is a very small part of the Dahomey Gap. This region is ecologically particular among the ecosystems in which wild bitter trees generally occur. We also conclude that in the Dahomey Gap sweet trees occur only in cultivation.

Within the Dahomey Gap, clear phenological differences exist between sweet and bitter ABMTs, mostly in their reproduction phases. Moreover, their reproductive success significantly depends on the type of ABMT, soil, climate and season and we conclude there is a low probability of hybridization between sweet and bitter trees in the area where they co-occur.

The qualitative morphological characters, the type of bark, colour of the mature fruit exocarp and mesocarp, and fruit roughness, do not consistently discriminate bitter and sweet trees in the field. We strongly recommend broadening the geographic area of this study by increasing more bitter trees as well as the wild samples of both taxa to validate this conclusion. The bitter trees in the Volta forest region produce the heaviest seeds and this consistently distinguishes them from all the sweet trees sampled in the Dahomey Gap. However, a combination of quantitative morphological characters (from fruits, mesocarp, and seeds) failed to discriminate populations. On the other hand this indicates the presence of a high diversity and thus high potential for selection across all phytogeographical regions. However, domestication and climate appear to be playing a key role in the morphological differentiation of Dahomey Gap populations, and evidence of success in the traditional domestication and selection of sweet trees is proven.

Low genetic diversity was found for the bitter trees occurring in the Volta forest region in the Dahomey Gap due to the high fragmentation of the small-sized forest ecosystem in which they occur and the continuous reduction of the population size. The higher polymorphism and genetic diversity observed in the sweet tree population in Benin and Togo indicate the effect of domestication of material from different geographical origins as well as a frequent long distance transfer of genetic material. When used separately, the AFLP and cpSSR data failed to consistently discriminate geographical populations and bitter from sweet trees. But a combined dataset of both markers tends to differentiate such populations as well as tree types. Our results also provide evidence that the suitability of AFLPs and cpSSRs to assess genetic diversity patterns in *Irvingia* material needs to be thoroughly reassessed.

Finally, although admitting that a broader study remains necessary, based on the presence of a consistent gap between both taxa regarding their reproductive periods, their different ecology and, of course, the consistent difference in taste of the fruit, we advise to treat the sweet and bitter ABMTs as two taxonomically different entities at species level: *Irvingia gabonensis* (Aubry-LeComte ex O'Rorke) Baill. and *I. wombolu* Vermoesen, respectively.

Samenvatting

Afrikaanse bush mango bomen (ABMTs) zijn economisch gezien de meest belangrijke soorten binnen de Irvingiaceae familie. Zij krijgen prioriteit als bomen die niet-hout bosproducten (NTFPs) leveren en zijn wijd verbreid in de vochtige laagland regenbossen van West en Centraal Afrika. Om hun productie te stimuleren en hen te ontwikkelen tot een belangrijk gewas voor de Afrikaanse plattelandsbevolking, werd in de jaren 2000 een domesticatieprogramma gestart, gecoördineerd door het World Agroforestry Centre. ABMTs behoren tot twee taxa, één met zoete en één met bittere vruchten, die morfologisch lastig van elkaar zijn te onderscheiden. Het mesocarp van zoete bush mango's wordt vers geconsumeerd, terwijl het zaad van zowel bittere als zoete vruchten een belangrijke component is van het Afrikaanse dieet. De hoge olieconcentratie van dit zaad verhoogd haar potentieel gebruik.

Los van de overlap in hun morfologische kenmerken, is een ecologisch en fenologisch onderscheid tussen zoete en bittere ABMTs onduidelijk vanwege: (i) het ontbreken van vergelijkende kwantitatieve gegevens en (ii) het gebrek aan centralisatie van nationale databases. Hierdoor is hun taxonomische status nog steeds onduidelijk. Vertegenwoordigen zij verschillende soorten of variëteiten, of zijn het slechts vormen binnen dezelfde genenpool? Het is ook onduidelijk of het voorkomen van ABMTs in traditionele agroforestry systemen in de Dahomey Gap, de droge savanne-corridor tussen de Upper en Lower Guinean regenbossen, onderdeel is van hun natuurlijke verspreiding of niet. Bovendien zijn genetische studies naar de diversiteit van ABMTs geografisch beperkt en hun conclusies ten aanzien van de taxonomische status van zoete en bittere bomen niet eensluidend.

De huidige studie werd uitgevoerd binnen een kader van de ontwikkeling van passende strategieën voor de bescherming en het gebruik van ABMTs, voornamelijk in de Dahomey Gap. Allereerst werden verschillen in ethnobotanische kennis van de belangrijkste sociaal-culturele groepen in de Dahomey Gap gekoppeld aan de status van het op ABMTs gebaseerde agroforestry systeem. Dit werd gebruikt om de kenmerken van het ruimtelijke patroon van ABMT algemeenheid en menselijke factoren die deze ruimtelijk structuur beïnvloeden te verklaren, evenals het voortbestaan van populaties in de Dahomey Gap. Ten tweede werden gegevens over de aanwezigheid van wilde en gecultiveerde ABMTs gebruikt in een algoritme om de verspreiding van soorten te modelleren om zo de niche omvang en

potentiele verspreiding van bittere en zoete bomen in tropisch Afrika te berekenen. De verschillen in de verkregen verspreidingspatronen werden vergeleken met behulp van ENM-Tools. Vervolgens werden gedetailleerde maandelijkse fenologische gegevens en morfologische kenmerken (zowel kwalitatieve als kwantitatieve metingen aan bladeren, bast, vruchten en zaden) gebruikt om de diversiteit van ABMTs te analyseren en verschillen tussen bittere en zoete bomen te ontdekken teneinde hen in het veld te kunnen identificeren. Tot slot werden de moleculaire merkers AFLP en cpSSR gebruikt om de genetische diversiteit van ABMTs uit Togo, Benin, Nigeria en Kameroen in kaart te brengen en zoete en bittere bomen te onderscheiden.

Kennis over de consumptie van bush mango mesocarp en zaad is wijd verbreid in de gehele Dahomey Gap. Het niveau van de kennis binnen lokale gemeenschappen over ander type gebruik (medisch, sociaal-cultureel) is over het algemeen laag en neemt af naar het westelijk deel van deze regio. Dit suggereert dat ABMTs (voornamelijk de zoete bomen) in deze eco-regio werden geïntroduceerd via de migratie van bevolking uit het Lower Guinea regenbos (zuidoostelijk Nigeria) naar het Westen. In de Dahomey Gap zijn bittere bomen beperkt tot de Volta bos-regio, een klein ecologisch gebied in zuidwestelijk Togo. Terwijl voor bittere bomen in deze regio lage dichtheden werden genoteerd (< 462 bomen per 25 ha), werden er hogere dichtheden waargenomen (tot 1020 bomen per 25 ha) voor zoete bomen in door mensen aangelegde agro-systemen. Dit impliceert een duidelijk verschil in cultiveringsmethodes tussen bittere en zoete ABMTs. De intensieve verbouwing van ABMTs in de Dahomey Gap wordt beïnvloed door de status van de landbouwgrond, de sociaal-culturele groep waartoe de boer behoort en het type ABMT. Kleine en uitgeputte gronden worden omgezet in zoete ABMT boomgaarden, wat aanduidt dat hun ontwikkeling een kleinschalig proces is, geleid door individuele boeren. Slash en burn landbouw en het intensieve verzamelen van vruchten voor commerciële zaadhandel brengen bittere bomen in gevaar, terwijl traditionele vistechnieken (gebruik van twijgen), de traditionele selectie-strategie en intensieve commercialisatie van land een ernstige bedreiging vormen voor genetische bronnen van zoete bomen.

Met behulp van het modelleren van arealen werd voorspelt dat de potentiele verspreiding van wilde zoete bomen in de nattere zones van de Guineo-Congolese fytogeografische regio ligt, terwijl die van bittere bomen zich uitstrekt tot de drogere zones van de Guineo-Congolese/Sudanese en Victoria Meer regio’s. Dit verschil is significant, wat het idee dat bittere en zoete bomen tot twee verschillende soorten behoren ondersteunt. In de

Dahomey Gap komen wilde bittere bomen voor in het ecologisch natste deel van de Volta bosregio, wat een zeer klein deel is binnen de Dahomey Gap. Deze regio is ecologisch afwijkend van de ecosystemen waarin bittere bomen doorgaans voorkomen. Ook concluderen we dat in de Dahomey Gap zoete bomen slechts in cultuur voorkomen.

Binnen de Dahomey Gap komen duidelijke fenologische verschillen voor tussen zoete en bittere ABMTs, voornamelijk in hun reproductieve fases. Bovendien hangt hun reproductieve succes significant af van het type ABMT, bodem, klimaat en seizoen, en we concluderen dat er een lage kans op hybridisatie is tussen zoete en bittere bomen in het gebied waar ze samen voorkomen.

In het veld onderscheiden de kwalitatieve morfologische kenmerken bast type, kleur van exocarp en mesocarp van rijpe vrucht en vrucht ruwheid niet consistent bittere van zoete bomen. We raden sterk aan het geografische gebied van deze studie uit te breiden met meer bittere bomen alsmede wilde bomen van beide taxa om deze conclusie te valideren. De zwaarste zaden worden door bittere bomen in de Volta regio geproduceerd en dit onderscheid hen consistent van alle zoete bomen in de Dahomey Gap. Echter, een combinatie van kwantitatieve kenmerken (van vruchten, mesocarp en zaden) slaagde er niet in populaties van elkaar te onderscheiden. Aan de andere kant wijst dit op de aanwezigheid van een hoge diversiteit en dus hoog potentieel voor selectie in alle fytogeografische regio’s. Echter, domesticatie en klimaat lijken een sleutelrol te spelen bij de morfologische differentiatie in de Dahomey Gap populaties en er is een bewezen succes van de traditionele domesticatie en selectie van zoete bomen.

Een lage genetische diversiteit werd aangetroffen bij bittere bomen in de Volta bosregio binnen de Dahomey Gap als gevolg van de hoge fragmentatie van het kleinschalige bos-ecosysteem waarin ze voorkomen en de gestage reductie van populatiegrootte. Het binnen populaties van zoete bomen in Benin en Togo waargenomen hogere polymorfisme en de hogere genetische diversiteit duiden op het effect van domesticatie van materiaal met verschillende geografische oorsprong alsmede een regelmatige aanvoer over lange afstand van genetisch materiaal. Afzonderlijk lukte het niet met AFLP en cpSSR gegevens eenduidig populaties met bittere bomen te onderscheiden van die met zoete. Maar, een dataset met de gecombineerde gegevens van beide merkers heeft de neiging dergelijke populaties alsook boom types wél te herkennen. Onze resultaten tonen ook aan dat de bruikbaarheid van AFLPs en cpSSRs om genetische diversiteitspatronen binnen *Irvingia* materiaal te bepalen grondig moet worden herzien.

Uiteindelijk, hoewel toegegeven dat een bredere studie noodzakelijk blijft, gebaseerd op de aanwezigheid van een consistent verschil in reproductieve fases tussen beide taxa, hun verschillende ecologie en, natuurlijk, het consistente verschil in smaak van de vrucht, adviseren we om de zoete en bittere ABMTs als twee verschillende taxonomische eenheden op soortniveau te behandelen: respectievelijk *Irvingia gabonensis* (Aubry-LeComte ex O'Rorke) Baill. en *I. wombolu* Vermoesen.

Curriculum Vitae

Romaric Vihotogbé was born on December 12th, 1975 in Pobé (Plateau), Republic of Benin. In June 1996, he graduated from high school (Davié, Porto-Novo, Benin) and obtained his Baccalauréat degree (option: Biology). In 1996 – 2001, he completed an Agricultural Engineer degree at the Faculty of Agronomic Sciences (FSA), Department of Natural Resources Management (Option: Forestry) at the University Abomey-Calavi (UAC, Benin), and presented his Engineer thesis on "The diversity of food plant species in the forest region of Pobè, South-East Benin, with emphasis on African bush mango trees in traditional agroforestry systems". From 2002 to 2005, he joined the Centre for Integrated Eco-Development in the African giant snails breeding and mushroom domestication and cultivation program. Back at the Faculty of Agronomic Sciences in 2005, he joined the Laboratory of Applied Ecology as Research Assistant on food tree species. In that year, he was granted an MSc scholarship by the Catholic University of Louvain La Neuve, Belgium. He studied at the Faculty of Biological Agronomic and Environmental Engineering and in 2006 he produced his MSc thesis on the endogenous conservation of the African bush mango trees in traditional agroforestry systems in Benin. From 2006 to 2007, he was appointed as Scientific Assistant for the Sub-Saharan African Forest Genetic Resources Programme (SAFORGEN) of Bioversity International, at the West African Office in Cotonou, Benin. With SAFORGEN, he was involved in the development of research projects on sub-Sahara African priority food trees among which the African bush mangoes. In 2008, he obtained a Sandwich PhD Scholarship from Wageningen University and started on June 1st, 2008 at the Biosystematics Group within the Department of Plant Science, Wageningen University. His PhD research focussed on the diversity of African bush mango trees and differences between sweet and bitter trees in the Dahomey Gap. The results of these studies (2008-2012) are presented in this thesis.

Publications

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