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Application of PCR Technologies to Humans, Animals, Plants and Pathogens from Central Africa

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1. Introduction

The Central African region, also called Atlantic Equatorial Africa, harbors one of the biggest worldwide biodiversity. It is true for human, with a great diversity of ethnic groups, but also for animals, plants, and microorganisms including pathogen species. Although this region is lagging behind in various domains, few research centers and laboratories have been able to develop sophisticated research work for diagnostics, fundamental research, and operational research, using polymerase chain reaction (PCR) techniques. This present paper intends to give an overview of the use of PCR technology in Central Africa and its various applications in the field of genetics, phylogeography, ecology, botany, and infectious diseases, which may have a broad impact on interspecies relationships, diagnostics of diseases, environment and biodiversity.

We will successively describe the main research findings in humans, animals, plants and pathogens from Central Africa, and show how the PCR has allowed scientists from this region to contribute significantly to generalized knowledge in these fields. Then, we'll discuss opportunities and challenges in conducting such kind of research in these particular limited-resources settings before concluding this chapter.

2. Humans

Since the nineties, the extensive use of molecular techniques has contributed to deepen the knowledge on human genetics. In most studies related to Central Africa, such

methodologies have often been used in the context of immunogenetics or genetic epidemiology of infectious diseases. The host genetic background is as important as immunity in the individual fight against infections. These studies were a fabulous opportunity to investigate the richness and extreme diversity of the genetic polymorphisms that characterize populations from Central Africa.

2.1 HLA characterization

The major histocompatibility complex (MHC) is one of the most polymorphic genetic systems of many species, including human leukocyte antigen (HLA) in humans. The class I and class II MHC genes encode cell-surface heterodimers that play an important role in antigen presentation, tolerance, and self/non-self recognition. The HLA molecules bind intracellularly processed antigenic peptides, forming complexes that are the ligands of the antigen receptors of T lymphocytes. In addition, the class I and class II histocompatibility antigens play an important role in allogeneic transplantation. Matching for the alleles at the class I and class II MHC loci impacts the outcome of both solid-organ and hematopoietic stem cell allogeneic transplants.

The HLA class II typing of 167 unrelated Gabonese individuals living in the village of Dienga, located in the South-East of Gabon (province of the Haut-Ogooué) was assessed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) [2]. All individuals belonged to the Banzabi ethnic group, which represents the second most important population grouping in Gabon after the Fang, with 55,000 to 60,000 individuals living in an area of 32,000 km^2. At the date of realization, in 1996, restriction endonuclease mapping of the PCR products provided profiles that allowed identification of 135 major alleles or groups of alleles among the 184 known DRB1 alleles [3]. Similarly, 9, 24 and 53 major alleles or groups of alleles were recognizable out of a total of 19, 35 and 83 DQA1, DQB1 and DPB1 alleles respectively, so far reported in the literature. For each locus, the PCR-RFLP identified alleles include all major alleles, while unidentifiable alleles were corresponding to rare and newly described alleles. The most frequent alleles at each locus were DRB1*1501–3 (0.31), DQA1*0102 (0.50), DQB1*0602 (0.42) and DPB1*0402 (0.29). The estimation of the haplotype frequencies as well as the observation of the segregation of several haplotypes using additional HLA typing of relatives, revealed that the three-locus haplotype DRB1*1501–3-DQA1*0102-DQB1*0602 was found at the highest frequency (0.31) among these individuals. This haplotype is not typically African and has already been described in Caucasians, but its presence at high frequency is exclusive to populations originating from Central Africa, and can thus be designated as a particular genetic marker of these populations. On the other hand, the absence in the Gabonese Banzabi group of DRB1*04 and the concomitant predominance at equal prevalence rates of DRB1*02 and DRB1*05, conforms to the other sub-Saharan population groups which have already been typed for their DR1-DR10 allospecificities [4]. Similarly, the predominant alleles observed at the DQA1, DQB1 and DPB1 loci studied have already been described in other sub-Saharan populations [5]. As an example, the determination of DRB1-DQA1-DQB1 haplotype frequencies for 230 Gabonese individuals belonging to tribes as different as Fang, Kele, Myene, Punu, Sira and Tsogo, revealed, as for the Banzabi group, the highest frequency (0.24) for the DRB1*15/16-DQA1*0102-DQB1*0602 haplotype [6]. The same predominant haplotype was observed with a high frequency of 0.27 among 126 healthy individuals in Cameroon, by means of a determination by high-resolution PCR using sequence-specific oligonucleotide probes (PCR-SSOP) and/or DNA sequencing [7].

Few studies investigated the extensive allelic diversity in the class I loci (to date, more than 250 HLA-A, 500 HLA-B, and 120 HLA-C alleles) by means of molecular methods among populations of Central Africa [5]. In populations as geographically close as Cameroonians (Yaoundé) [8] and Gabonese (Dienga, South-East of Gabon) [9], the two most frequently detected HLA-A and HLA-B allele families diverged, illustrating the patchwork representation of the different genetic backgrounds (Cameroon: HLA-A*23, A*29, HLA-B*53 and B*58; Gabon: HLA-A*19, A*10, HLA-B*17 and B*70). In Cameroon, where populations are very heterogeneous in their origin, culture and language, the most frequently encountered HLA-A, HLA-B and HLA-C alleles differed in four ethnic groups distributed from the north to the south of the country, reflecting the complex migrations and admixtures that occurred in this area located in the borders of Central and west Africa, before that populations settled [10].

2.2 Red blood cell polymorphisms

Red blood cell polymorphisms are frequently found in areas where malaria is currently or was historically endemic. This observation led to the idea that some of these polymorphisms might provide a relative advantage for survival [11]. The best-characterized polymorphism in this context is the sickle cell trait (HbAS), comprising heterozygous carriage of hemoglobin (Hb) S, which results from a valine substitution for glutamic acid at position 6 of the hemoglobin β chain. HbAS provides carriers with a high degree of protection against severe *Plasmodium falciparum* malaria during early life, which explains the relatively high penetrance of this mutation— in some areas reaching 30%—in sub-Saharan African communities exposed to high rates of infection with *P. falciparum* [12]. The mutation in the homozygous state (HbSS) leads to the disease referred to as "sickle cell anemia," a life-threatening condition that usually results in early death [13, 14]. HbAS in such populations thus exemplifies a balanced polymorphism that confers a selective advantage to the heterozygote [15]. Molecular determination of the HbS carriage is assessed by PCR-RFLP, where a 369-bp segment of the codon 6 in the beta-globine gene, encompassing the A>T substitution, is amplified, before being digested with the restriction endonuclease *DdeI*.

In sub-Saharan populations, the ABO blood group distribution is in large part dominated by the O blood group, with prevalence rates of at least 50%. Strong hypotheses favor a selection pressure exerted by the plasmodial parasite on its host cell, and include i) the worldwide distribution of the ABO blood groups with a type O predominance in malarious regions of the world [16], ii) the fact that *Plasmodium falciparum* has substantially affected the human genome and was present when the ABO polymorphisms arose [17], iii) the associations of ABO blood groups and clinical outcome of malaria with the observation of a degree of protection conferred by blood group O against severe courses of the disease [18] and iv) the potential role that erythrocyte surface antigens may play in cytoadhesion of infected erythrocytes to micro vessel endothelia and in parasite invasion [19]. No molecular method is used for the determination of ABO blood groups, as hematological methods (Beth-Vincent and Simonin techniques) are both simple and robust.

G6PD is a cytoplasmic enzyme allowing cells to withstand oxidant stress. It is encoded by one of the most polymorphic genes in humans, located on the X chromosome. In Africa, G6PD is represented by three major variants, G6PD B (normal), G6PD A (90% enzyme activity) and G6PD A- (12% enzyme activity) [20]. The location of the G6PD gene on the X chromosome and the subsequent variable X-chromosome inactivation implies that the expression of G6PD

deficiency differs markedly among heterozygous females and therefore that these females do not constitute a homogeneous group [21]. PCR-RFLP is used for the molecular determination of the predominant G6PD A- variant in sub-Saharan Africa: mutation 376 A>G responsible for the G6PD A electrophoretic mobility and mutation 202 G>A responsible for the A- deficiency, are determined by PCR amplification of exons 5 and 4 respectively, followed by restriction enzyme analysis, using *FokI* (376 A>G mutation) and *NlaIII* (202 G>A mutation). However, the 376 A>G mutation may also be associated with other deleterious mutations such as 542 A>T (G6PD Santamaria), 680 G>T or 968 T>C, revealed after electrophoretic migration of digested amplified products with *BspEI*, *BstNI* and *NciI* respectively.

Table 1 presents data obtained among healthy individuals in order to avoid distribution bias due to selection of genetic traits by secularly settled diseases such as malaria. No HbSS individual was recorded in the studies gathered in this Table, because of an age range beyond the life expectancy of most HbSS patients in developing countries. Since the G6PD A and B variants have almost the same enzyme activity, the patients were stratified into groups with normal (female BB, AB, AA and male B and A genotypes), heterozygous (female A-B and A-A genotypes) and homo-/hemi-zygous (female A-A- and male A- genotypes) state, corresponding to decreasing levels of G6PD enzymatic activity. Some research teams have extensively studied erythrocyte polymorphisms in relation to malaria morbidity, among children hospitalized at the Albert Schweitzer Hospital from Lambaréné, in the Moyen Ogooué province of Gabon. As these genetic traits strongly influence the distribution of the clinical pattern of malaria, their frequency distribution is not representative of the whole population, and therefore they could not be reported in Table 1.

Erythrocyte polymorphisms	**Prevalence rate (%)**		
	Gabon (Dienga)	**Cameroun (Ebolowa)**	**Republic of Congo (Brazzaville)**
ABO blood groups:	N = 279 [22] [23]	N = 1,007 [24]	N = 13,045 [27]
Group O	54	51	53
Group A	27	24	22
Group B	17	19	21
Group AB	2	6	4
HbS genotypes:	N = 279 [22] [23]	N = 240 [25]	N = 868 [28]
Hb AA	77	81	80
Hb AS	23	19	20
Hb SS	0	0	0
G6PD state:	N = 271 M & F [22] [23]	N = 561 M [26]	N = 398 M & F [29]
- Normal (genotypes BB, AB, AA, B & A)	78	93	68
- Heterozygous (genotypes A-B & A-A)	13	0	21
- Homo-/hemi-zygous (genotypes A-A- & A-)	9	7	11

M: males; F: females.

Table 1. Erythrocyte polymorphisms among healthy individuals from Central Africa

Other erythrocyte polymorphisms characterize the sub-Saharan populations, including Central Africans. It is the case of the alpha-thalassemia, which consists in the deletion of 1, 2, 3 or the 4 genes encoding the alpha chain of the globin. Several forms of alpha-thalassemia are distributed worldwide, and the form encountered in sub-Saharan Africa resides in a gene deletion of 3.7 kb ($-\alpha^{3.7}$ type), which generates the formation of a functional hybrid gene. A PCR amplification strategy using three primers allows to determine the normal ($\alpha\alpha/\alpha\alpha$), heterozygous ($-\alpha^{3.7}/\alpha\alpha$) and homozygous ($-\alpha^{3.7}/-\alpha^{3.7}$) state as well as the - -/$-\alpha^{3.7}$ form (H haemoglobin) [30]. The prevalence of α^{+}-thalassemia in Africa ranges from 5 to 50%, according to a gradient from North Africa to equatorial Africa and from South Africa to equatorial Africa: so, the highest prevalence rates are reached in the Central African Republic [31] and in a Bantu population from the republic of Congo [32]. Different erythrocyte polymorphisms may coexist in the same individual, as the results of advantageous interactions. Namely, a beneficial effect of α^{+}-thalassemia on the hematological characteristics of sickle-cell anemia patients has been found, in accordance with the observation in HbAS individuals of decreasing values of HbS quantification accompanying decreasing numbers of α-globin genes (from 4 to 2) [32].

2.3 Innate immunity

For the needs of malaria-linked studies, polymorphisms of some products of the inflammatory response have been investigated among populations from Central African countries.

Mannose binding lectin (MBL) is a member of the collectin family of proteins, which are components of the innate immune system, acting therefore against multiple pathogenic organisms. MBL is thought to be more effective at an early age, before effective acquired immune responses have developed, and low plasma concentrations of non-functional MBL have been attributed to mutations in the first exon of the MBL gene: $MBL_{IVS\text{-}I\text{-}5}$ G>A, MBL_{54} G>A and MBL_{57} G>A. PCR-RFLP determination may be performed, using *NlaIII (*codon 52), *BanI* (codon 54) and *MboII* (codon 57) endonucleases. At least one MBP gene mutation was present in 34% of a Gabonese population sample (Banzabi), with an overall gene frequency of 0.03, 0.02 and 0.18 mutations at codons 52, 54 and 57, respectively [22, 25]. There are other published MBL2 genotyping techniques, based on sequence-specific PCR, denaturing gradient gel electrophoresis of PCR-amplified fragments, real-time PCR with the hybridization of sequence-specific probes and sequence-based typing. A new strategy that combines sequence-specific PCR and sequence-based typing (Haplotype Specific Sequencing or HSS) was recently improved and allowed identification of 14 MBL allele-specific fragments (located in the promoter and exon 1) among Gabonese individuals [33].

Inducible nitric oxide synthase 2 (NOS2) is the critical enzyme involved in the synthesis of nitric oxide, a short-lived molecule with diverse functions including antimalarial activity, that can also cause damage to the host cell. The most investigated polymorphism is located in the promoter region of NOS2, and concerns the point mutation $NOS2_{-954}$ G>C, which is associated with an increased production of NOS2. By the means of a PCR amplification followed by enzymatic digestion with *BsaI*, this point mutation was found in 18% of Gabonese individuals from the Banzabi ethnic group, mainly in the heterozygous state [22, 25]. A similar high prevalence was found in another Gabonese population group, recruited in Lambaréné [34].

Tumor necrosis factor α (TNF-α) is a proinflammatory cytokine that provides rapid host defense against infection but is detrimental or fatal in excess. The main studied

polymorphisms are located in the promoter region of the gene and are TNFα$_{-308}$ G>A and TNFα$_{-238}$ G>A base substitutions. These two polymorphisms have not been related to any variation in cytokine production, but may serve as markers for a functional polymorphism elsewhere in the TNF-α gene. Indeed, the TNFα$_{376}$ A allele (G>A substitution), which is frequently found in linkage disequilibrium with TNFα$_{-238}$ A allele, is related to enhanced secretion of TNF and might be responsible for increased antigen- or T-cell mediated B-cell stimulation and proliferation [35]. Molecular determination is assessed by PCR-RFLP using *NcoI* (-308), *AlwI* (-238) and *FokI* (376) restriction endonucleases. Prevalence rates of 22% (TNFα$_{-308}$ A allele) and 17% (TNFα$_{-238}$ A allele) were found in a Gabonese population (Banzabi), mainly in the heterozygous state [22, 25].

Haptoglobin (Hp) is an acute-phase protein that binds irreversibly to hemoglobin (Hb), enabling its safe and rapid clearance. Therefore, Hp has an important protective role in hemolytic disease because it greatly reduces the oxidative and peroxidative potential of free Hb. Haptoglobin exists in three phenotypic forms: Hp1-1, 2-1, and 2-2, which are encoded by two co-dominant alleles, Hp^1 and Hp^2. A fourth phenotype HpO, referred to as hypo- or an-haptoglobinaemia has been reported to be the predominant phenotype in West Africa. Functional differences between the different Hp phenotypes have been reported, the ability to bind Hb being in the order of 1-1 > 2-1 > 2-2. The gene frequencies of different Hp phenotypes show marked geographical differences as well as large variations among different ethnic groups. Hp genotypes determined by PCR in 511 Gabonese children from the village of Bakoumba (South-East of Gabon), distributed into 36.5%, 47.6% and 15.9% for Hp1-1, Hp2-1 and Hp2-2 respectively [36]. In South-West Cameroon, the genotype distribution among 98 pregnant women was 53% for Hp1-1, 22% for Hp2-1 and 25% for Hp2-2 [37].

2.4 Polymorphism of the cytochrome P450 superfamily

The DNA samples of the Gabonese individuals from the Banzabi ethnic group already described [2] entered a dataset of DNA samples from European (French Caucasians), African (Senegalese), South American (Peruvians) and North African (Tunisians) populations, in order to evaluate the inter-ethnic variations in the genetic polymorphism of several components of the cytochrome P450 superfamily (CYP) which gathers a large and diverse group of enzymes (Table 2). The function of most CYP enzymes is to catalyze the oxidation of organic substances. Their substrates include metabolic intermediates such as lipids and steroidal hormones, as well as xenobiotic substances such as drugs and other toxic chemicals. The investigation of the variable number of tandem repeat (VNTR) polymorphism of the human prostacyclin synthase gene (CYP8A1) revealed a particular distribution of the nine characterized alleles in the Gabonese population group, which may be associated with a more frequent and severe form of hypertension found in some Black populations [38]. The frequencies of three single nucleotide polymorphisms occurring in the CYP2A13 were determined by PCR-single strand conformational polymorphism (PCR-SSCP) (578C>T (Arg^{101}Stop)) and PCR-RFLP (3375C>T (Arg^{257}Cys) and 720C>G (3'-untranslated region)) and were respectively 0, 15.3 and 20.8 among the Gabonese group, differing from those of other groups under comparison: these marked inter-ethnic variations in an enzyme involved in the metabolism of compounds provided by the use of tobacco, have consequences on the susceptibility to lung cancer [39]. More precisely, it appears that black populations could present a higher deficit in CYP2A13 activity compared with other population groups, compatible with a reduced risk for smoking-related lung adenocarcinoma. In the same way, a frameshift mutation, responsible for the

synthesis of a truncated protein of the CYP2F1, which activity in lung tissue is linked to carcinogenic effects, was mostly represented in the Gabonese population sample [40]. The genetic polymorphism of the CYP3A5 enzyme, implicated in the metabolism of chemotherapeutic agents but also toxins, was analyzed using a PCR-SSCP strategy, leading to the observation of great inter-ethnic differences in the distribution of a maximum of 17 alleles, some of them being linked to the synthesis of a non functional enzyme. According to the determination of the CYP3A5 predicted phenotype, Gabonese individuals were the most numerous (90.0%) to express a complete and functional CYP3A5 protein compared to French Caucasians (10.4%) and Tunisians (30.0%) [41]. The CYP4A11 enzyme is involved in the regulation of the blood pressure in the kidney, and an 8590T>C mutation has been associated to an increased prevalence of hypertension. Using PCR-SSCP and nucleotide sequence analysis, the frequency of this mutation was found lower in Gabonese compared to other investigated African population groups (Tunisians, Senegalese) [42]. Lastly, 3 single nucleotide polymorphisms (SNPs) affecting the human type II inosine monophosphate dehydrogenase (IMPDH2) gene have been determined by PCR-SSCP. This enzyme participates in the metabolism of purines and constitutes a target for antiviral drugs. It resulted that African

P450 Tissue location	Clinical implication	Gene polymorphism	DNA samples origin (n)	Reference
CYP8A1 Ovary, heart, skeletal muscle, lung and prostate	Pathogenesis of vascular diseases	9 VNTRs in the 5'-proximal regulatory region of the *CYP8A1* gene	European (78 French Caucasians); African (50 Gabonese and 50 Tunisians)	[38]
CYP2A13 Lung tissue	Susceptibility of tobacco-related tumorigenesis	3 SNPs: 578C>T (exon 2), 3375C>T (exon 5) and 720C>G (3'UTR)	European (52 French Caucasians); African (36 Gabonese and 48 Tunisians)	[39]
CYP3A5 Liver	Metabolism of chemotherapeutic agents and toxins	17 SNPs on the 13 exons of the *CYP3A5* gene	European (51 French Caucasians); African (36 Gabonese and 36 Tunisians)	[41]
CYP2F1 Lung tissue	Metabolism of pneumotoxicants with carcinogenic effects	Frameshift mutation in *CYP2F1* exon 2 (c.14_15insC)	European (90 French Caucasians); African (32 Gabonese, 37 Tunisians and 75 Senegalese)	[40]
CYP4A11 Liver and kidney	Regulation of blood pressure in the kidney	1 SNP on *CYP4A22*-exon 11: 8590T>C	European (99 French Caucasians); African (36 Gabonese, 53 Tunisians and 50 Senegalese); South American (60 Peruvians)	[42]

VNTR: variable number of tandem repeats; SNP: single nucleotide polymorphism; 3'UTR: 3' untranslated region

Table 2. Genetic polymorphisms in enzymes of the cytochrome P450 superfamily (CYP), in diverse populations including Gabonese

population groups (Tunisians, Gabonese, and Senegalese) presented a higher IMPDH2 activity than Caucasians, with implications for the dose requirement of IMPDH2 inhibitors administered to patients [43].

This compilation of genetic data on populations from Central Africa is far from being exhaustive. As an example, the genetic polymorphism of Toll-Like Receptors (TLR) is to date extensively explored in order to deepen the understanding of the first steps of the immune recognition. Also, cytokines that regulate adaptive immune responses (humoral immunity and cell-mediated immunity) may present inter-individual genetic variations such as it is the case for IL-2, IL-4, IL-5, IFN-gamma, TGF-beta, LT-alpha or IL-13. Finally, increasing information is generated every day thanks to equipments (such as real-time PCR systems or DNA sequencers) that allow handling simultaneously a great number of biological samples. Altogether, this review of genetic data gathered during the last twenty years among Central African populations, illustrates in which point Africa, which is thought to be the homeland of all modern humans, is the most genetically diverse region of the world.

3. Animals

Methods used to infer the respective role of historical, environmental and evolutionary processes on animal distribution are related to the molecular ecology field and, as such, very similar to those employed to study plant dynamic (see section 4.). For animal, sequence of genes of mitochondrial DNA (mtDNA) such as cytochrome b or control region genes are largely used in phylogenetic and phylogeographic studies. The evolutionary pace of mitochondrial genomes being relatively fast, mtDNA sequences can also be used in population genetics study even if nuclear markers (microsatellites, SNP, etc.) provide a higher level of information.

3.1 Species identification from fecal pellets

The inability to correctly identify species and determine their proportional abundance in the wild is of real conservation concern, not only for species management but also in the regulation of illegal trade. However, estimating species abundance using classical ecological methods based on direct observation is very challenging in Central Africa. Indirect methods based on animal tracks, especially fecal pellets have been proposed; however pellets of parapatric related species are sometimes very similar and difficult to use to reliably differentiate species in the field. To address this problem, a PCR-based method has been proposed to differentiate Central African artiodactyls species and especially duikers (*Cephalophus*) from their fecal pellets [44]. In this purpose, a mtDNA sequence database was compiled from all forest *Cephalophus* species and other similarly sized, sympatric *Tragelaphus, Neotragus* and *Hyemoschus* species. The tree-based approach proposed by the authors is reliable to recover most species identity from Central African duikers.

3.2 Rivers are playing a major role in genetic differentiation for large primates in central Africa

For both Gorillas (*Gorilla gorilla*; [45, 46]) and Mandrills (*Mandrillus sphinx*; [47]) phylogeographic studies based on mtDNA (for both species) and microsatellite (only for Gorilla) markers have shown that rivers hamper gene flow among populations and have a major role in partitioning the species diversity. For Mandrills, the Ogooué river (Gabon)

separates populations in Cameroon and northern Gabon from those in southern Gabon [47]. For Gorilla, rivers are more permeable and allow limited admixture among populations separated by waterways [45]. Anthony et al. also showed that like for plant species (see section 4) past vicariance events and Pleistocene refugia played an important role in shaping genetic diversity of current Gorilla populations [45].

3.3 Central African elephants: Forest or savannah elephants?

Despite their morphology typical from forest elephants, a genetic study based on mtDNA [48] shows that Central African elephants are sharing their history with both forest and savannah elephants from Western Africa. It also gives evidence that Central African forest populations show lower genetic diversity than those in savannahs, and infers a recent population expansion. These results do not support the separation of African elephants into two evolutionary lineages (forest and savannah). The demographic history of African elephants seems more complex, with a combination of multiple refugial mitochondrial lineages and recurrent hybridization among them rendering a simple forest/savannah elephant split inapplicable to modern African elephant populations.

4. Plants

4.1 Methods and approaches

This paragraph is giving on overview of approaches and methods related to the molecular ecology field and used to study natural or human-induced dynamic of plant species in Central Africa. Acknowledging the past history of the Central African forest domain is crucial for our understanding of spatial and temporal evolution of species living throughout the region.

Historical processes responsible for the contemporary distributions of individuals can be studied within the field of historical biogeography or phylogeography. For phylogeographic studies the distribution of genetic lineages within or among closely related species is considered throughout the geographical space and current patterns are interpreted in light of past vicariance events, population bottleneck, survival in glacial refugia and/or colonization routes [49, 50, 51]. This approach can be combined with landscape genetic methods to respectively infer impact of historical and environmental processes on the distribution of the genetic diversity. Landscape genetic methods allow to correlate the distribution of the genetic diversity with environmental parameters and to reveal, for example, the impact of topographic features on gene flow or the role of soil heterogeneity in structuring the genetic diversity [52]. At finer scales, classical population genetic approaches address the role of additional evolutionary forces (drift, dispersal, mutation, mating system, etc.) in shaping current patterns. All these genetic-based approaches belong to the molecular ecology field and are combined to address questions linked to the natural species dynamic or more importantly, questions linked to the survival of threatened species facing forest fragmentation, logging activities, etc.

All these approaches primary necessitate analyses of the genetic diversity at individual level. In this purpose, various techniques based on PCR are used. Different genetic markers can be chosen based on their respective evolutionary properties. For analyses of large-scale patterns, sequences of cytoplasmic DNA (ctDNA) like chloroplastic DNA (cpDNA) for plants are chosen. Cytoplamic DNA are haploid, non-recombining (or recombination events are rare) and generally characterized by uniparental inheritance (chloroplasts are generally

maternally inherited for angiosperm, paternally for gymnosperm plant species). These markers allow inference in genealogical histories of individuals, populations and/or species. It is however highly recommended to combine cytoplasmic with nuclear markers for intraspecific phylogeographic studies because of the uniparental inheritance of ctDNA. It is especially true for species with sex-biased dispersal capacities. For instance, cpDNA would show a very strong spatial structure for tree with heavy barochore (dispersed by gravity) seeds whereas nuclear genes dispersed by both seed and anemophilous (transported by wind) pollen, would not reveal any spatial structure. Therefore, sequences from nuclear genes could provide valuable information in phylogeographic assessments. They are nonetheless more complicated to analyze because of i) the difficulty to isolate haplotype from diploid organisms, ii) intragenic recombination and iii) the relatively slow pace of sequence evolution at most nuclear loci. Other nuclear PCR-based genetic markers such as microsatellites, AFLP (Amplified Fragment Length Polymorphism), RAPD (Random Amplification of Polymorphic DNA) or SNPs (described in section 2.4) are also used for phylogeographic studies, most of them being particularly valuable for population genetic studies.

4.2 Importance of the past climatic changes in shaping pattern of genetic diversity in Central Africa

The Lower Guinea forest domain (the Atlantic coastal forest distributed from Nigeria to Congo) has undergone major distribution range shifts during the Quaternary, but few studies have investigated their impact on the genetic diversity of plant species. Several phylogeographic studies using either cpDNA polymorphism [52, 53, 54, 55, 56, 57] and/or nuclear markers such as RAPD [58] and microsatellite markers [53, 59, 60] have recently been published, considering Central African trees as model species, to give insight into the historical biogeography of the region. For most of the studied species, the genetic diversity is very spatially structured throughout the species distribution giving strong phylogeographic signals. These results show that the Central African rainforest domain was very fragmented during the cool and dry periods from the Last Glacial Maximum period at the end of the Pleistocene (20000-13000 years before present) and more recently during the Little Ice Age (about 4000-2500 years before present). During these periods, most tree species and probably forest species in general, only survived in a reduced number of isolated populations in areas where environmental conditions remained suitable. The question is now to test for the presence of forest refugia in Central Africa, in other words: did forest-species all survived in the same areas? In this case, effort for the conservation of these areas must be treated with the highest priority as refugia may play a major role in the survival of forest-species, while climate is changing, probably in buffering effect of the fluctuations. First results show that some refugia were shared among several tree species with one main refugium in the North and one in the South of the thermal equator (e.g. *Milicia excelsa* in [53], *Erythrophleum suaveolens* in [55], *Irvingia gabonensis* in [56], *Distemonanthus benthamianus* in [60]. Other species managed to survive in additional areas with at least four remaining populations for *Aucoumea klaineana* in Gabon [59]. More species covering all functional groups (pioneer, understorey, long-lived, etc.) must be studied to be able to infer general trends to allow predictions about impact of the Global Climate Change on species distribution.

4.3 Importance of species life history traits in the maintenance of genetic diversity

At finer scale, microsatellite loci were used to infer species dispersal ability of threatened tree species. *Baillonella toxisperma* Pierre Sapotaceae is a very low-density tree. The species is

insect-pollinated and its seeds are dispersed by animals, including elephants. Using spatial genetic structure analyses, Ndiade-Bourobou et al. were able to demonstrate that dispersal distances were uncommonly high and able to connect trees present in very low density throughout forest [61]. This process allows the maintenance of high genetic diversity in reducing inbreeding effect and assures as such the survival of the species. This equilibrium is very vulnerable as both tree and animal-vectors densities have dramatically dropped due to additional effects of logging, hunting and poaching activities. For *Aucoumea klaineanea* Pierre Burseraceae, a highly logged tree species in Gabon, Born et al. show that dispersal distance is very limited and that founder effects associated with colonization processes are avoided by the homogeneity in reproductive success in adults [62]. Their results also suggest [63] that reduced density of trees and/or forest opening is balanced by higher gene dispersal distances. This result is linked with dispersal syndromes of the species that locally contribute to the maintenance of the genetic diversity.

5. Pathogens

A lot of diseases of animal origin and their rapid spread and possible transmission to humans (HIV/AIDS, Ebola, Avian Influenza, etc.) can pose a threat to human health. Tools have evolved from simple serological screenings to specific amplification using conventional or Real Time PCR methods, hence allowing more suitable diagnostic methods for early stage detection, identification and characterization of emerging or re-emerging pathogens. We'll successively take examples of pathogens infecting i) humans (parasites, viruses, bacteria, in section 5.1), non-human primates and other animals (section 5.2), and finally pathogens of plants (section 5.3).

5.1 Pathogens in humans

5.1.1 Parasites

Health in Central Africa is triggered by malaria, the most studied human parasite. Malaria transmission remains holoendemic in Central Africa in spite of decades of efforts in implementation/operational research. Other parasitic diseases are of utmost importance in term of public health, as human African trypanosomiasis (or sleeping sickness), filariasis, intestinal parasites, schistosomiasis, toxoplasmosis and amibiasis; however, they are all considered as neglected diseases. The PCR techniques contribute to the diagnostic of these infections. These techniques also improve our understanding of the physiopathology of these diseases through basic research. PCR indubitably helps to diagnose more efficiently and to find new therapeutic strategies.

5.1.1.1 PCR and diagnostic for human parasites in Central Africa

The Table 3 shows a few examples of PCR-based diagnostics for human parasites, although these techniques are not the gold standard for diagnosis of human parasites. The high cost of the PCR-based techniques is mainly mentioned as inconvenient. New diagnostic techniques should be implemented once it's demonstrated that the balance cost/benefit is lower than 1. First, the technique must be feasible in routine laboratories in terms of equipment and training of local agents. Secondly, the new technique has to offer a benefit in terms of clinical treatment of the patients. This clinical benefit may result in a better specificity and sensitivity, and in a reduced time to diagnosis. The improvement of sensitivity allows the detection of sub-microscopic infections, as detailed in the chapter of this book titled "Submicroscopic infections of human parasitic diseases" by Touré-Ndouo.

The main advantages of diagnosis by PCR for human parasites from Central Africa are both the higher specificity and the small amounts of blood or tissue required. The specificity of DNA sequences offers a simple tool to distinguish species. As an example, the species spectrum of intestinal parasites involved in hospitalized AIDS patients was determined in the Democratic Republic of the Congo [64]. Opportunistic infections were detected by PCR, as *Cryptosporidium* sp., *Enterocytozoon bieneusi*, *Isospora belli* and *Encephalitozoon intestinalis*. The other intestinal parasites detected by PCR were *Entamoeba histolytica*, *Entamoeba dispar*, *Ascoris lumbricoides*, *Giardia intestinalis*, hookworm, *Trichiuris trichiura*, *Enterobius vermicularis*, and *Schistosoma mansoni*. Furthermore, the PCR-based diagnostic is quite more sensitive than microscopic examination, which is sometimes not sufficient to differentiate various parasite species. This is clearly the case for filariasis [65] and schistosomiasis [66]. In human sleeping sickness, PCR on blood allows avoiding painful lumbar punctures and was proposed as a less-invasive alternative to replace the cerebrospinal fluid examination. However, in this case, PCR is a good tool for primodiagnostic but cannot be used for post-treatment follow-up. Indeed, the high sensitivity of PCR leads to detection of persisting DNA in blood of patients even after successful treatment [67].

	Se.*	**Spe.***	**Advantage**	**Inconvenient**	**Ref. technique**	**Reference**
Plasmodium spp (qPCR)§	99.40%	90.90%	Limit of detection greatly reduced	High cost	Microscopy examination of thick and thin blood smears	[70]
T. brucei gambiense in blood by PCR	88.40%	99.20%	Non invasive	Not suitable for follow-up	Microscopic analysis of the CSF	[67]
L. loa, *M. perstans* and *W. bancrofti* by nested PCR	100%$	100%$	High se. and spe. for 3 filariosis co-endemic	Cost	Knott's concentration and microscopic examination	[65]
S. mansoni in fecal samples by qPCR	86.50%	100%	High spe. to distinguish species	High cost; Not intended for routine diagnostic	Microscopic examination of Kato	[66]
S. haematobium in fecal samples by qPCR	82.80%	100%			Microscopic examination of filtrated urine samples	

*Se. sensitivity, Spe. Specificity, CSF cerebrospinal fluid
§ qPCR, quantitative polymerase chain reaction
$ 30% of samples not done by PCR

Table 3. Efficiency and characteristics of PCR-based diagnostic in several endemic human parasitosis that are prevalent in Central Africa

Malaria constitutes one of the major public health problems in Central Africa. As *Plasmodium falciparum* infection is deadly when untreated in children and pregnant women, its diagnostic has to be accurate and fast. At hospital level, where many malaria diagnostics are performed a

day, cost/benefit may be convincing and PCR-based diagnostic may be implemented. However, the benefits linked to PCR-based diagnosis for malaria are the identification of the different *Plasmodium* species and a lower detection limit. This is not necessarily clinically relevant. In addition, the existence of alternative diagnostic techniques as rapid diagnostic tests (RDTs) based on immunochromatographic assays to detect specific *Plasmodium* antigens that are recommended by the WHO, increases the cost/benefit ratio for PCR [68, 69].

Finally, PCR-based diagnosis is a very good tool for epidemiological survey. It still needs improvement in terms of cost, feasibility and quickness to deserve an implementation in Central African routine laboratories.

5.1.1.2 PCR and research on human parasites in Central Africa

As malaria is the most prevalent infection in Central Africa with the higher mortality incidence, this part will focus on malaria. The aim of this part is to point out the central role of PCR techniques in malaria research performed in Central Africa, without providing an exhausting list of its applications. The Figure 1 summarizes the research applications in the malaria field related to PCR-based techniques.

Fundamental research

The link between fundamental and operational research is tight, particularly for pathologies like malaria that need field studies to confirm hypotheses. Molecular epidemiology for malaria parasite is an example of this tight link. The study of SNPs related to drug resistance in *P. falciparum* on a genome-wide scale in a diversity of strains from Africa provides information on the frequency of the studied SNPs. If drug resistance requires several SNPs and those naturally occurring SNPs are rare in most genes, it may last years for the parasite to acquire a drug resistant phenotype. So, it is important to know whether *P. falciparum* genome presents low or high level of SNPs in endemic areas. However, the generation of new *P. falciparum* variants encoding for different levels of SNPs can result of tandem repeats of similar sequences (called RATs) that could undergo slip-strand mispairing. Replication slippage or deletion mechanisms lead to the apparition or lost of different RATs. Interestingly, the high frequency of RATs close to drug resistance or immune response target sequences can result in a fast increase of important SNPs (reviewed in [71]).

The development of new diagnostics for malaria is also dependant of PCR-based techniques. The first RDTs for malaria were supplied more than 15 years ago. Some of them are based on immunochromatographic detection of *P. falciparum* histidine-rich protein 2 (PfHRP2), using monoclonal antibodies. PfHRP2 is an abundant circulating protein easily detectable in the blood of patients. However, some studies reported variable test performances. In that way, complementary studies were necessary to compare the PfHRP2 sequences from several parasite strains and the potential consequences on the performance of PfHR2-based RDTs. The genetic diversity of the *pfhrp2* gene was studied in isolates originating from 19 countries including Central African countries and the relationship between the *pfhrp2* diversities and the sensitivities of PfHRP2-based RDTs was assessed [72]. The results indicated that 2 types of repeats in the DNA sequence of PfHRP2 were predictive of RDT detection sensitivity with 87.5% accuracy. These results pointed out the importance of the genetic background of the parasites and their diversity in the different geographic endemic areas.

Parasite antigen diversity studies at the molecular level are also performed for vaccine research. *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) is a major vaccine target as

evidence supports the central role of PfEMP1 in the development of a protective acquired immunity in children and pregnant women living in high level endemic areas. However, PfEMP1 undergoes a serious problem. PfEMP1 is highly polymorphic and encoded by a gene family of 50-60 *var* genes. To identify specific *var* genes or domain structuring these genes and related to protective immunity, many molecular studies were done and are still currently performed, all based on the basic molecular technique, PCR. In pregnancy-associated malaria, some studies showed that the *var* gene expressed called *var2csa* is relatively conserved. A comparative study showed that Duffy binding–like domains from placental parasites from Gabon and Cameroon shared 85%–99% amino-acid identities, confirming the conserved nature of placental variants [73]. This demonstration of sequence conservation in PfEMP1 DNA and its implication in the binding to chondroitin sulfate A (CSA) and to the pathology was clearly relevant to vaccine development for pregnancy-associated malaria. Today, it is largely recognized that the parasite ligand mediating CSA binding and causing malaria in pregnancy is VAR2CSA, a member of PfEMP1 family, and that it is a promising target for vaccine design. Recent researches focus on the molecular variability of *var2csa* in field isolates and on the immune response induced by different domains of the protein. Vaccine research largely depends on immunological studies, as this is clearly the case with the example of PfEMP1. However, PCR is not the favorite technique for such studies unlike flow cytometry or Enzyme Linked Immunosorbent Assay (ELISA). For immunological topics related to malaria, PCR is mainly used in studies on human genetic markers linked to malaria protection (see section 2 of this chapter).

Operational research

The evaluation of the therapeutic and control strategies implemented to fight against malaria constitutes operational research. First, PCR has become an essential technique for the evaluation of antimalarial treatment efficiency. Historically, *in vivo* resistance of *P. falciparum* to antimalarial drugs was classified into three grades, RI (low), RII (intermediate), and RIII (high) [74]. Since 2002, therapeutic failures are divided in early and late treatment failures (ETF, LTF), and LTF includes late clinical failures and late parasitological failures [75]. Both classifications are based on follow-up studies of parasitemia in patients treated with antimalarial treatments. Usually, follow-ups last 28 days, but are now extended to 42 days with the use of artemisinin-based treatment combinations (ACT) [75]. The classification relies on the reappearance or not of parasites during the follow-up. In highly endemic areas for malaria, the reappearance of parasites may be linked to the persistence of the initial infection, or to a new infection that occurred during the follow-up (the incubation time for *P. falciparum* is 7 to 10 days). A first study was performed in Central Africa in Gabon to demonstrate the great advantage of PCR to distinguish recrudescent *P. falciparum* clones from new ones, in studies of antimalarial treatment efficacy [76]. The technique involves amplification by PCR of regions of 3 highly polymorphic parasite genes, merozoïte surface protein-1 (*msp-1*), *msp-2* and glutamate-rich protein (*glurp*). Through this study, the authors showed that 39% of RI resistant cases were in fact due to new infections. Today, PCR genotyping is systematically included in treatment efficacy studies [75].

The implementation of therapeutic strategies for malaria in a specific area has an impact on the deployment of parasite resistance to the drug used. It is of high importance to study the development of parasite resistance in malaria endemic areas, in order to suggest new policies once treatments become inefficient. PCR is definitely the basic tool to perform such studies once molecular mechanisms of resistance have been demonstrated through more

fundamental research. Sulfadoxine-pyrimethamine (SP) treatment has been used for a long time as second-line treatment for uncomplicated malaria in case of chloroquine treatment failure. The parasite mechanisms of resistance to SP have been well described and result in SNPs located on *Pfdhfr* and *Pfdhps* genes that appear in a few years following the implementation of such molecules. PCR followed by sequencing is the usual technique to study the rate of these mutations. In Gabon, Congo and Cameroon, the rate of *Pfdhfr* and *Pfdhps* mutations has been followed for years and constituted serious arguments to search other alternative treatments to chloroquine [77, 78, 79]. Since the era of ACT has begun, research teams based in Central Africa also use PCR-based techniques to follow the emergence of molecular markers related to the resistance to artemisinin-based molecules [80, 81].

Malaria prevention is also carried out through the use of insecticide treated materials or indoor residual spraying in Central Africa. This strategy has some implications on the spread of pyrethroid resistance in *Anopheles gambiae* and this has become a major concern in Africa. A PCR-RFLP assay was developed in Cameroon to follow two SNPs in the gene encoding subunit 2 of the sodium channel, also called the knockdown (*kdr*) mutations [82]. Since that time, studies to follow the situation of insecticide resistance are performed. In Gabon, both *kdr-e* and *kdr-w* alleles were shown to be present at high frequency in the *Anopheles gambiae* population. Of course, these results have implications for the effectiveness of the current vector control programmes that are based on pyrethroid-impregnated bed nets [83].

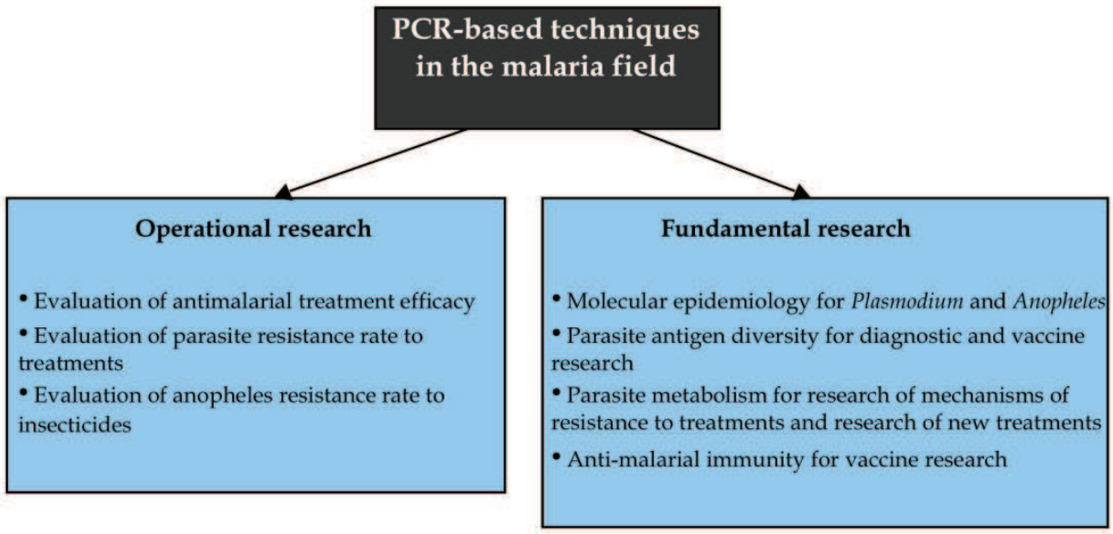

Fig. 1. The use of PCR-based techniques in the malaria field for operational and fundamental research

5.1.2 Viruses

This part will describe how the PCR-based techniques have been applied to many viruses infecting humans living in Central Africa, such as Human Immunodeficiency Virus (HIV), Human T cell Leukemia Virus (HTLV), Influenza virus, Hepatitis virus, and Ebola virus, for their origin, circulation, diversity, diagnosis, surveillance, and/or monitoring. Table 4 gives

several examples of pathogens infecting humans in Central Africa, which have benefited from PCR technologies, with a particular emphasis on viruses.

5.1.2.1 Human Immunodeficiency Virus (HIV)

Central Africa has been described as the "epicenter of the HIV pandemic"[84]. Scores of articles have used PCR methods to report findings related to the viral diversity of HIV in this region, emergence of new strains [85] and recombinant forms [86], emergence of resistance to antiretroviral drugs [87], and challenges encountered for the genotyping tests because of the broad diversity of HIV strains [88]. In this section we'll explain the usefulness of PCR in i) the identification of various HIV strains found in Central Africa, ii) the early diagnosis of HIV, especially in exposed infants, iii) the management of infected patients, iv) implementation research and finally, we'll underline the need of an African AIDS vaccine.

PCR has help in the discovery and description of the virus

Since the discovery of HIV in the early 80s by Montagnier and Gallo, many strains, types, subtypes, circulating recombinant forms (CRFs) and unique recombinant forms (URFs) have been described and characterized in patients from the Central African region. The discovery of new HIV variants occurred by atypical serological reaction, and confirmation was obtained by simple PCR, nested PCR, heteroduplex mobility assay (HMA) (see Box 1) or sequencing. Particularly, full-length genomes sequencing has been instrumental in the characterization of new HIV CRFs, such as HIV-1 CRF 25_cpx [89] and CRF 22_01A1 [86, 90] in Cameroon. Obviously, the characterization of all these variants has an impact on HIV diagnosis, treatment and vaccine development, especially for the HIV-infected individuals leaving in Central Africa. The genetic diversity of HIV-1 group M in the republic of Congo was described and documented [91]. This was achieved using specific PCR coupled to HMA techniques of the *env* and *gag* genes (see Box 1). In Equatorial Guinea, Hunt et al. described the variability of HIV-1 group O, while Peeters et al. performed a wider study of HIV-1 group O distribution in Africa [92, 93, 94]. Although ELISA was mainly used in this latter study, indeterminate cases were solved using PCR. In Gabon, a great quantity of HIV strains collected from 1986 to 1994 was characterized by molecular biology techniques (PCR, sequencing); then phylogenetic trees were constructed [95]. A high prevalence of HIV-1 recombinant forms has been reported in Gabon [96]. In Cameroon, many studies have been carried out on genotyping subtypes of HIV-1 [86, 97, 98, 99]. Recently, new HIV-1 groups named group N and group P have been identified from Cameroonian patients [100, 101, 102, 103].

PCR is used routinely for the diagnosis of HIV

Despite antibody testing being commonly used in HIV RDTs, this methodology is not suitable in children born of HIV seropositive mothers during the first 15 to 18 months of life. The reason is that maternal antibodies transferred to the infant during pregnancy or breastfeeding persist up to 18 months and could give false positive results. Therefore, detection of proviral DNA by PCR is recommended for the early diagnosis in HIV-exposed infants. Detection of HIV proviral DNA is performed using the Roche Amplicor HIV-1 DNA commercial test, which is so far considered as the gold standard. This test reveals an HIV-1 infection within neonates and infants from 6 weeks of life and beyond. This test targets the *gag* gene during amplification where a fragment of 120bp is amplified and then, detection is based on ELISA. The kit is stored at 4°C and was especially designed for HIV-1 group M. Blood samples are collected as Dried Blood Spots (DBS), which have already been used for nationwide HIV prevalence survey in Africa [104]. More than 305,000 children in 34

countries worldwide have been offered early infant diagnosis (EID) and antiretroviral treatment thanks to the Clinton HIV/AIDS initiative (CHAI) and UNICEF, both managing the funds from UNITAID. The Amplicor HIV-1 DNA commercial test is currently used in several laboratories throughout Africa, and Cameroon is probably the leading country in Central Africa with a well-developed national EID programme, implemented by the Ministry of Public Health in the 10 regions of the country since 2007 [105].

PCR allows the management of HIV infection

Two main tests employing PCR techniques are useful for the biological follow-up of HIV-1-infected individuals i) the viral load (VL), which uses RNA PCR and ii) the resistance testing, which consists in amplification of specific viral fragments and sequencing. Viral load is mostly useful to follow the progression of the disease and for therapeutic monitoring as well. According to the commercial kits that are currently available, products of amplification can either be detected at the end of the reaction or while they accumulate in a real time manner. The lack of a commercially available viral load assay for HIV-2 is a concern for the proper management of patients infected with HIV-2 strains [106]. The resistance testing is actually an HIV-1 genotyping assay where the protease and the reverse transcriptase conserved regions of the *pol* gene are amplified and sequenced, as described by Fokam et al. [107]. Only two commercial tests approved by the Food and Drug Administration are currently available, and have been used widely to follow-up patients under antiretroviral treatment [108, 109, 110] and to report drug resistance mutations in HIV-1 reverse transcriptase or protease [109, 111, 112]. However, such commercial kits are very expensive for resource-limited countries like those of Central Africa and also their performance is questionable because of the great diversity of strains found in that region. For these reasons, an in-house genotyping assay has been developed in Cameroon recently and it is considered as more performant and cost effective than commercial kits [107].

The heteroduplex mobility analysis (HMA) is a molecular biology technique based on PCR amplification then followed by polyacrylamide gel electrophoresis analysis. This method has been first used for the subtype determination of HIV-1 group M envelope sequences, but has been recently developed for *gag* gene sequences.

Principle of the HMA test:
Heteroduplexes are formed with uncharacterized DNA fragments and known DNA sequences (as reference) included in the kit. Importantly, *env* gene fragments of uncharacterized DNA fragments are amplified by nested PCR whereas the reference sequences are obtained by direct amplification of plasmids from the kit.
Mobility of such heteroduplexes is analyzed on polyacrylamide gels. The closest is the unknown DNA sequence with the reference sequence; the fastest is the mobility of the heteroduplex on the polyacrylamide gel.

The HMA technique has been used to characterize HIV strains from Cameroon [1].

Box 1. Heteroduplex Mobility Analysis

The use of PCR in implementation research

Implementation research is essential for the control of infectious diseases of poverty [113]. Although PCR technologies are sophisticated and require a certain level of technical

expertise and facilities that are usually not available and not affordable in poor-resources settings, implementation research studies can help to find alternative solutions. For example, the fact that DBS can replace blood samples advantageously has been instrumental in increasing access to HIV diagnosis in exposed infants living in remote settings, through the implementation and scale-up of the EID program [105]. Equally, DBS can improve the biological follow-up of HIV-1-infected individuals, both for the VL quantification and the resistance testing. Indeed, DBS, which can be collected on sites, transported and tested after a long-term storage, are suitable for the differed quantification of HIV-1 RNA, thus allowing people living with HIV/AIDS in rural areas to have access to this sophisticated test [114]. On another hand, implementation of resistance testing on DBS is in progress in Africa [115, 116] and will soon benefit HIV-1-infected patients living far from urban areas in Central Africa [108]. While waiting for the development of point of care assays, DBS appear to be a good alternative for the monitoring of HIV-1-infected people in remote settings (reviewed in [117]). However, the transport of samples and the return of results remains challenging, and need additional implementation research.

Back to the sites

Central Africa could be the ideal place where an AIDS vaccine could be designed, because of the great diversity of strains that are found in this region. However, when the scientific community is reflecting on how simian immunodeficiency virus infections hosted by African nonhuman primates could help in designing an AIDS vaccine for example, Central African scientists are absent [118]. This situation should change and African institutions, supported by their government, should advocate strongly for and invest in an African AIDS vaccine. To this end, the African AIDS Vaccine Partnership (AAVP) intends to promote cutting-edge research for the development of an African HIV vaccine [119]. In addition, the European Developing Clinical Trial Partnership (EDCTP) is supporting several African institutions from Gabon, Congo and Cameroon to build capacity for the conduct of future HIV/AIDS clinical trials [120] and is advocating for support from governments.

5.1.2.2 Human T cell Lymphotropic Virus (HTLV)

Central Africa is one of the few regions of the world where HTLV type 1 (HTLV-1) is highly endemic, as reviewed by Gessain & Mahieux [121]. Sequencing of HTLV-1 focuses on the gene *env* and the long terminal repeat fragments [122]. Molecular studies have demonstrated that the several molecular subtypes (genotypes) are related to the geographical origin and not to the disease. For example, while the subtype A is considered as cosmopolitan, the subtype B is mainly found in Central Africa (Democratic Republic of Congo, Gabon, and Cameroon). The subtype D has also been described in individuals from Cameroon, Gabon, Central African Republic, but less frequently than the subtype B, and more specifically in Pygmies. New subtypes (E and F) would be equally present in this region [121]. Interestingly, the first complete nucleotide sequence of HTLV type 2 (HTLV-2) has been obtained in a 44-year-old male living in a rural area of Gabon, by using nested PCR [123]. However, HTLV-2 does not seem to be as prevalent as HTLV-1 in this region since in a recent epidemiological survey performed on 907 pregnant women, only one case of HTLV-2 was reported [122]. In Cameroon however, HTLV-2 seroprevalence was 2.5% in Bakola Pygmies, but no HTLV-2 infection was found in Bantus [124]. HTLV type 3 (HTLV-3) and HTLV type 4 (HTLV-4) have been recently identified in primate hunters in Central Africa. Real-time PCR quantitative assays have been developed in the USA and allow detecting as

few as 10 copies of HTLV-3 or HTLV-4 sequences of the gene *pol* in a small amount of DNA from human peripheral blood lymphocytes [125]. However, a new method using a single tube, multiplex, real time PCR has been developed at the Centre International de Recherches Médicales de Franceville (CIRMF), Gabon, which allows detecting HTLV-1, HTLV-2 and HTLV-3 simultaneously [126]. This new PCR-based technique could be of valuable use for epidemiological studies in countries where those viruses are prevalent.

5.1.2.3 Influenza virus

Despite influenza surveillance was increasing worldwide, developing countries in general and Central Africa in particular paid very little attention to the 2009 pandemic. Very recently however, samples from patients living with influenza-like illness in Yaounde, Cameroon were analyzed with various techniques including real time reverse transcription-polymerase chain reaction (RT-PCR) thus allowing the detection and subtyping of influenza A (H1N1 and H3N2) and B viruses from these patients [127]. Because of the H1N1 influenza A pandemic, Cameroon entered in a global surveillance network and received a laboratory equipped with a robust PCR platform for diagnosing influenza viruses in remote settings [128].

5.1.2.4 Hepatitis viruses

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are endemic in the Central African region. Since the last two decades, the use of PCR techniques and phylogenetic analysis has led to characterize the genotype distribution of HCV in this area. The RNA is amplified by RT-PCR and nested PCR and the primers commonly used are specific to the 5'UTR and NS5B regions. In Cameroon, genotypes 1 and 4 are the most prevalent, but highly heterogeneous, with 5 subtypes 1, 4 subtypes 4 and unclassified subtypes, while the genotype 2 prevalence is low, with homogeneous sequences [129, 130]. Further work has help to understand the history of the HCV epidemic in Cameroon, where mass therapeutic or vaccine campaigns would have contributed to the spread of this infection during the colonial era [131]. In Gabon and Central African Republic, the predominance of the heterogeneous genotype 4 has been reported [132, 133, 134]. Equally, few HBV genotype studies have been conducted Central Africa. Makuwa et al. reported the identification of HBV-A3 in rural Gabon [135], while this genotype is co-circulating with HBV-E among Pygmies in Cameroon [136]. More recently, a pilot study was conducted in the village of Dienga, Gabon (previously described in section 2.1) with the aim of looking at potential interactions between HBV, HCV and *P. falciparum* infections, which are all very prevalent in this region [137]. In this study, HCV chronic carrier were identified by ELISA and by qualitative RT-PCR amplification of the 5' non coding region, and *P. falciparum* infection were assessed by microscopic examination and in case of negative result, by PCR targeting the gene encoding *P. falciparum* SSUrRNA, previously described by Snounou et al. [138]. Interestingly, these results showed that HCV infection may lead to slower emergence of *P. falciparum* in blood [137]. Other studies have demonstrated the usefulness of the PCR as a tool for the description of the molecular diversity of other less known/marginal viruses in this region, such as hepatitis delta virus in Cameroon [139] and in Gabon [140], or hepatitis GB-C/HG virus and TT virus in Gabon [141].

5.1.2.5 Ebola virus

Since the first declaration of deaths due to Ebola virus in Zaïre in 1976, the Central African region has been particularly affected by repeated Ebola outbreaks, which affected

populations from Gabon and Republic of Congo in addition to the Democratic Republic of Congo. However, publications on the detection of Ebola virus in humans by molecular studies such as RT-PCR are scarce. The first reason is that infected patients have been reluctant to any type of invasive sampling method. The second is that for cultural reasons, families strongly refuse that researchers collect postmortem skin biopsies [142]. By analyzing few serum samples and less invasive specimens such as oral fluid samples, Formenty et al. could detect Ebola virus by RT-PCR and compare the two types of specimens [142]. This RT-PCR method has been developed, implemented and evaluated for diagnostics purposes at the CIRMF in Gabon, where a tremendous work is being done in the field of Ebola and other hemorrhagic fevers [143]. It is clear that the RT-PCR is the most appropriate tool not only to diagnose the infection in patients at a very early stage, but also to follow-up recovering patients [144]. Of note, studies were more easily carried out in animals, where important findings using PCR technologies were reported (see section 5.2).

In conclusion to this section on viruses, it is important to mention that new random priming methods adapted from the sequence independent single primer amplification (SISPA) technology are now available, and could be used to sequence whole genomes of all sorts of (known or unknown) RNA and DNA viruses [145]. This methodology, together with molecular clock analyses are needed to better understand the origin, circulation and diversity of all the viruses present in Central African populations.

5.1.3 Bacteria

In a review on the molecular epidemiology of bacterial infections in sub-Saharan infections, almost no information is reported from Central Africa [156]. Recently, molecular epidemiology methods have been applied to the genetic typing of *Mycobacterium tuberculosis* complex strains, the etiologic agents of tuberculosis, whose incidence is increasing dramatically in sub-Saharan Africa [157]. In 1993, a novel typing method called spoligotyping has been described [158]. This PCR-based method uses the DNA polymorphism of *M. tuberculosis* complex strains to detect and differentiate clinical isolates simultaneously, and allows their genotypic classification [159]. Briefly, this method aims at analyzing the so called DVR regions, which is composed of direct repeat (DR) regions, in which variable repeat sequences are inserted [160]. Spoligotyping, which is frequently compared to the conventional and more powerful RFLP method, remains a useful tool for genotyping clinical isolates in various epidemiological settings. In Cameroon, Niobe-Eyangoh et al. have used spoligotyping for analysis of hundreds of *M. tuberculosis* complex isolates from patients living in the West region [155]. This technique, which is considered as rapid, simple, and cost-effective, has been found accurate and easy to implement in that country, where the distribution of *M. tuberculosis* complex strains remains however still poorly documented, as well as the rest of Central Africa (see Table 4).

5.2 Pathogens in animals

Non-human primates from Central Africa have been extensively studied because it has been found that they are naturally infected with viruses or parasites similar to those affecting humans. The fact that humans are living in permanent contact with wild animals through hunting and butchering can explain transmission of pathogens from animals to humans.

Pathogen-genotype	Group/ Subtype	Regions (specific group)	Technique	Zone of amplification
HIV-1	M/A,C, D, G, H, F, J, K, CRF01-AE	DRC	PCR & HMA	*env* V3-V5 region
	M/CRFs	Cameroon	Nested PCR	*gag*, *pol*, *env* genes
	M/CRFs	South Est Gabon	PCR	*pol* gene
	N	Cameroon	PCR	LTR-*gag*, *pol-vif*, *env* genes, entire genome
	O	Cameroon, Equatorial Guinea	PCR Nested PCR	LTR-*gag*, *pol-vif*, *env* genes, entire genome
	P	Cameroon	RT PCR	*pol* integrase and *env* fragments
HIV-2		Equatorial Guinea	nested PCR	*pol* gene
HTLV-1	A	Congo, DRC, Chad	nested PCR, PCR multiplex, real time PCR	gene *env* and LTR, gene *tax*
	B	DRC, Gabon, Cameroon, CAR		
	D	Cameroon, Gabon (Pygmies)		
	E	DRC		
	F	Gabon		
HTLV-2	Gab, B	Gabon Cameroon (Bakola Pygmies)	nested PCR, PCR, multiplex, real time PCR	entire proviral genome, gene *env* and LTR, gene *tax*, Long Terminal Repeats
HTLV-3		Gabon, Cameroon	multiplex, real time PCR, nested PCR	gene *tax* genes *tax* and *pol*

Pathogen-genotype	Group/ Subtype	Regions (specific group)	Technique	Zone of amplificatio
HTLV-4		South East Cameroon	nested PCR	gene *tax* genes *tax* and
Influenza A	H1N1 H3N2	Cameroon	RT PCR	HA NA and
Influenza B	B/Victoria/2/87 lineage and B/Yagamata/16/88 lineage			sequences
HCV-1	1a, 1b, 1c, 1e, 1h, 1l	Cameroon South-West CAR	RT PCR & nested PCR	NS5b gene NS5b and E2 reg 5'UTR regio
HCV-2	2f	Cameroon South-West CAR		
HCV-4	4e, 4f, 4k, 4c 4r, 4t, 4p, unclassified	Cameroon, South-West CAR, Gabon		
HBV	A3	Gabon, DRC, Cameroon (Pygmies)	Semi nested PCR	HBs (surface) g
	E	Cameroon (Pygmies)		
Ebola		DRC, Gabon, Congo	RT PCR	RNA polymer L and NP ge
Mycobacterium	*tuberculosis*	Cameroon	spoligotyping	DVR regio
Plasmodium	*falciparum*	Gabon	PCR	SSUrRNA ge

HIV: Human Immunodeficiency Virus, HTLV: Human T cell Leukemia Virus, HCV: Hepatitis Virus C, HBV: Hepatitis Virus B, LTR: Long Terminal Repeats, CAR: the Central African Republic, DRC: Democratic Republic of Congo

Table 4. Examples of pathogens infecting humans in Central Africa, which have benefited from PCR technologies

5.2.1 Pathogens in non-human primates

A substantial proportion of wild-living primates in Central Africa are naturally infected with Simian Immunodeficiency Viruses (SIVs) [161, 162, 163], Simian T-cell Lymphotropic Viruses (STLVs) [164, 165, 166, 167], Simian Foamy Viruses (SFV) [168] and also Hepatitis B Viruses (HBV) [169].

SIVs are lentiviruses that are found naturally in a great variety of nonhuman primates from Equatorial Africa, including but no restricted to chimpanzees (SIVcpz), mandrills, (SIVmnd-1 and SIVmnd-2), drills (SIVdrl), talapoin monkeys (SIVtal), sun tailed monkeys (SIVsun), African green monkeys (SIVagm), red-capped mangabeys (SIVrcm) (see [162, 163, 170] and [171] for review). The evolutionary origins of these related viruses have been studied by amplification of the *gag*, *pol*, and *env* genes, and by construction and analysis of evolutionary trees. Sequence analysis of the entire genome and phylogenetic analyses have led to the identification of distinct primate lentivirus lineages in which most of the SIV strains described so far can be classified (see [171] and Table 5). The example of SIVs illustrates how the PCR techniques have been instrumental in the characterization of new strains of pathogens in non-human primates of Central Africa. As previously mentioned for animals (see section 3) phylogeographic studies have been equally carried out for pathogens. In mandrills for example, the two types of viruses appear to be geographically distributed, since SIVmnd-1 was found in mandrills from central and southern Gabon whereas SIVmnd-2 was identified in northern and western Gabon, as well as in Cameroon [163].

Other examples of pathogens in non-human primates from Central Africa could have been used, like the STLVs (the simian counterpart of HTLVs), the SFVs and/or HBV, which similarly to SIVs have been described and characterized with molecular techniques including PCR. With no pretention of being exhaustive, the Table 5 summarizes several examples of pathogens found in animals from this region, with the technique used, the gene amplified, and appropriate references for more details. Of note, molecular techniques adapted to non-invasive fecal samples have been pivotal to identify simian viruses in quite a number of species, especially in case of wild living primates.

These findings from Central Africa on pathogens in non-human primates together with those reported in humans, give a more comprehensive picture of the relationship between simian viruses and their counterpart in humans.

Indeed, the use of PCR related technologies and the clustering of sequences has helped to understand that i) cross species transmission of viruses (from non-human primates to humans) occurred in Central Africa through highly exposed population such as hunters and people handling primates as bush meat [164] and ii) species barriers could be easier to cross over than geographic barriers [165]. Taken together, these observations reveal that the risk of emergence of new viral diseases in Central Africa is still latent.

Similarly, various species of *Plasmodium*, including *P. falciparum* have been found in great apes (chimpanzees and gorillas) in Central Africa [172, 173]. If blood samples are not suitable for systematical analyses in primates, especially in case of wild primates; the identification of *Plasmodium* by PCR has been facilitated by the use of fecal primate samples, which are also broadly collected for the identification of simian viruses (see above). The identification of new species of *Plasmodium*, such as *P. gaboni*, which infects chimpanzees and *P. GorA* and *P. GorB*, which infect gorillas, has help to obtain a more comprehensive view of the phylogenetic relationships among *Plasmodium* species [173].

Pathogen-genotype	Subtype/ lineage	Regions (animals)	Technique	Zone of amplifi
	gaboni	Gabon (chimpanzees)	PCR	complete mitocho genome (includin *Cox I* and *Cox III*
Plasmodium	*GorA* *GorB*	Gabon (wild chimpanzees, wild gorilla, captive wild-born gorilla)	Plasmodium-specific PCR assay	mitochondrial *cyto* gene
	falciparum	Gabon (wild chimpanzees, gorilla)		nuclear and mitoch genomes
SIV	SIVmnd-1 SIVmnd-2	Gabon (mandrills), Cameroon (mandrills)	PCR	entire genom
	SIVtal	Cameroon (talapoin monkeys)	PCR	entire genom
	SIVsun	Gabon (wild-caught sun tailed monkey)	PCR	entire genom
	SIVrcm	Gabon (red capped mangabeys); Nigeria/Cameroon border (red-capped mangabeys)	PCR	entire genom
	SIVcpz	Cameroon, Gabon, DRC (chimpanzees)	PCR	entire genom
STLV-1	D, F	Cameroon (agile mangabeys, mustached monkeys, talapoins, gorilla, mandrills, African green monkeys, agile mangabeys, and crested mona and greater spot-nosed monkeys); Gabon (mandrills)	Discriminatory PCR	LTR & *env* sequ
STLV-2		DRC (wild-living bonobos)	Generic PCR	*tax* gene
STLV-3		Cameroon (agile mangabeys)	Discriminatory PCR	LTR & *env* sequ

Pathogen-genotype	Subtype/ lineage	Regions (animals)	Technique	Zone o amplifica
SFV	SFVcpz	Gabon, Cameroon (chimpanzees); Cameroon, CAR, Gabon, Republic of Congo, DRC (wild chimpanzees); Gabon (wild and semi-free ranging captive mandrills)	nested PCR RT PCR	integrase ar region *gag*, and *pol*-IN
Ebola		Gabon (Fruit bats)	PCR	RNA polyn
Influenza	H5N1	Northern Cameroon (ducks)	PCR	NA seque

SIV: Simian Immunodeficiency Virus, STLV: Simian T cell Lymphotropic Virus, SFV: Simian Foamy Virus, LTR: Long Terminal Repeats, CAR: the Central African Republic, DRC: Democratic Republic of Congo

Table 5. Examples of pathogens infecting animals of Central Africa that have benefited from PCR technologies

By sequencing the complete mitochondrial gene or at least a part of the cytochrome b, and Bayesian or maximum-likelihood methods, phylogenetic analyses can be performed, hence allowing a better understanding of the origins and evolution of malaria parasites and possibly transmission between apes and humans [172].

5.2.2 Pathogens in other animal species

Apart from non-human primates, other animals from the Central African region have been studied for their possible implication in the life cycle of viruses causing hemorrhagic fever like Ebola or Marburg, which are both affecting great apes and humans. For example, sequences of Ebola were detected by PCR in small rodents and shrews, suggesting that common terrestrial small mammals living in peripheral forest areas may play a role in the life cycle of the Ebola virus [174]. More recently, Ebola and Marburg viruses were found in symptomless infected fruit bats in Central Africa, indicating that these animals could therefore act as the natural reservoir of these both viruses [153, 175].

In the context of outbreaks of highly pathogenic avian influenza, ducks from the far north region of Cameroon were found to host a highly pathogenic avian influenza subtype H5N1, whose sequence was closely related to H5N1 isolates reported in other African countries [176].

5.3 Pathogens in plants

For plant pathogen, PCR-based techniques are essentially used in two purposes: i) to identify pathogen species, comparing pathogen sequences to known pathogen sequence libraries or ii) to characterize pathogen colonization dynamic. One example of each application is summarized below.

5.3.1 Which fungi are attacking Central African *Terminalia* species?

Begoude et al. collected fungal inoculum on *Terminalia* in Cameroon to identify which pathogens are threatening these highly logged tree species. They compared DNA sequence for the ITS and tef 1-alpha gene regions to known pathogen libraries and showed that the majority of isolates are from the *Lasiodiplodia* genus [186].

5.3.2 The colonization dynamic of *Mycosphaerella fijiensis* in Cameroon

Dispersal processes of fungal plant pathogens can be inferred from analyses of spatial genetic structures resulting from recent range expansions. The fungus *Mycosphaerella fijiensis*, pathogenic on banana, is an example of a recent worldwide epidemic and is currently threatening Cameroonian banana plantations. Halkett et al. collected fungal isolates in Cameroon and analyzed them using 19 microsatellite markers. They demonstrated that large gene flows are linking populations even separated by long distances, through dense banana plantations, and so ensuring stable demographic regime and promoting efficient colonization dynamic of the fungus [187].

6. Opportunities and challenges

Some of the few research institutes and molecular biology laboratories that have been mainly involved in the findings reported above are the CIRMF (Franceville, Gabon), which

is equipped with BSL3 and BSL4 facility, and the CIRCB (Yaounde, Cameroon), among others. Despite the amount of work and publications that have been generated from the Central African region, institutions and scientists involved in molecular biology research in Central Africa are facing several problems including procurement, maintenance, human resources, capacity building and ethics–related issues.

Obtaining the valuable results depends on multiple factors including methodology of sampling, processing, storage and shipment of samples to laboratory with respect of maintain of the cold chain. As described above, problems related to sampling were well circumvented with animals. Indeed, by using shed hair or feces, which are non invasive methods of sampling, phylogenetic analyses have allowed a better understanding of the evolutionary history of gorillas [46] mandrills [47] or elephants [48]. Equally, a number of simian viruses have been characterized in fecal samples, which is more convenient, especially in case of wild-living primates. In these contexts, new reagents such as the RNA later® have been very helpful to stabilize and protect RNA in fresh collected specimens, hence allowing an extended period of storage before processing the samples. In humans, the collection of samples via DBS is simple, convenient, and cost effective. Transportation does not require any cold chain, and storage is easier than samples obtained from whole blood. In the field of HIV, DBS are advantageous for the biological follow-up of infected patients living in remote areas [117].

Another issue, which has to be taken into consideration, is related to the issue of the quality control and quality assurance, which need permanent improvement and capacity building efforts. Due to limited resources and equipment, and possibly because the culture of research is still dramatically lacking in most of sub-Saharan African countries [188], only few laboratories have obtained certification and the roadmap to accreditation is still far ahead. Therefore there is an urgent need that institutions from Central Africa participate more in laboratory accreditation programs, with the goal of seeking lab accreditation and excellence in general. For example, the World Health Organization (WHO)-AFRO and the Center for Disease Control Global AIDS program have launched recently an accreditation program for quality improvement of African laboratories for HIV monitoring. However, such programs will also improve the monitoring of HIV-TB coinfected patients, and by extension, the follow-up of patients suffering from other diseases, such as malaria or any neglected disease. Equally, support from the EDCTP is currently helping African institutions -grouped in regional Networks of Excellence- to conduct future clinical trials in the four regions of sub-Saharan Africa. To achieve this goal, a lot of efforts have been put into building capacity of young African scientists and laboratories, which have to meet international standards and respect good clinical and laboratory practices [120].

Studies reported here have been carried out mainly in the framework of collaborative research with institutions from the North. However, DNA samples are often kept abroad, with the partners, without any signed material transfer agreement. In some other cases, African scientists and institutions from the region are not associated to the work and/or publications. The researcher's community has to be aware of avoiding the "banking" of DNA from African populations outside from Africa, mutualising benefits with the concerned populations and scientific partners as well as respecting ethical issues, such as establishing a fair partnership between African scientists and scientist from the North. The lack of these aspects have been demonstrated in a recent bibliometric review on human genetic studies performed during the two last decades in Cameroon [189]. Recently, the

African Society of Human Genetics launched the Human Heredity and Health in Africa (H3Africa) initiative, with the support of the National Institutes of Health and the Wellcome Trust (see http://h3africa.org/). The aim of this initiative, which was first discussed at the Yaoundé meeting in March 2009, is to conduct genomics-based research projects in Africa in order to better understand health and diseases in various African populations and to identify possible populations that are at risk of developing a specific disease. To this end, various calls for proposals have been launched, in which African institutions will take the leading role. One of these calls is the H3 Africa biorepository grant, which will address the need of biobanking samples in Africa for Africa. This H3Africa programme gives a lot of hope that capacity building and ethics-related will be soon addressed in favor of African institutions and African scientists and other scientists living in Africa, and that partnerships will eventually result in true win-win collaborations.

7. Conclusion

The contribution of PCR technologies to humans, animals, plants and pathogens from Central Africa is considerable, hence allowing the discovery of new species of plants and pathogens in this region, particularly in Gabon (see http://www.cirmf.org/en/publications). The richness of animals, plants, and pathogens is unquestionable and the Central African region is notorious for its great biodiversity.

In this chapter, a great number of PCR-based techniques have been described, including but not limited to PCR-restriction fragment length polymorphism, PCR using sequence-specific oligonucleotide probes, combination of sequence-specific PCR and sequence-based typing also called Haplotype Specific Sequencing, PCR-single strand conformational polymorphism, reverse transcriptase PCR, sequence independent single primer amplification technology, nested and semi-nested PCR, quantitative PCR, real time PCR, PCR multiplex, Heteroduplex Mobility Analysis, and spoligotyping. Applied to humans, these techniques have contributed significantly to increase the knowledge on human genetics, through immunogenetics and genetics epidemiology of infectious diseases. Particularly, a great number of molecular studies describe the genetic polymorphism of the various populations and ethnic groups living in this region (section 2). Applied to wild animals and non-invasive samples such as shed hair or feces, PCR technologies have for example facilitated the identification of related species, which are not easy to differentiate just by direct observation as done by ecologists, by using mitochondrial DNA (section 3). Applied to plants, PCR-based methods have contributed to a better understanding of spatial and temporal evolution of species found in that region, including colonization routes, and tree densities than can be modified because of activities of humans in that region (section 4). Finally, application of PCR technologies has been reported for pathogens infecting humans, animals and plants (section 5). Parasites, viruses, and bacteria that are prevalent in humans, non-human primates and other animal species, and fungal plant pathogens have been discovered and characterized through PCR-based techniques.

The PCR-generated knowledge is benefiting to a broad range of disciplines, such as genetics, molecular ecology, phylogeography, botany, evolution, molecular epidemiology, and infectious diseases, amongst others.

Altogether, these finding have contributed to a better understanding of the relationship between humans from Central Africa and their environment (animals, plants and

pathogens), and particularly the inter relationship between species. Indubitably, this will be of help for a better management of resources at the global level. In addition, progresses have been made in fundamental research, operational research, and research applied to diagnostics and monitoring of infected individuals.

Challenges in conducting PCR-based research are procurement and storage of reagents and blood samples due to the cold chain, maintenance of equipment, as well as human resources, capacity-building and ethics-related issues. However, new initiatives such as those launched by the African Society of Human Genetics (H3 Africa), the AAVP (promoting an African AIDS Vaccine), and the EDCTP (supporting regional Networks of Excellence for the future conduct of clinical trials) are real opportunities for the scientific community that is working in Africa, to perform cutting-edge research where sophisticated molecular biology laboratories and bioinformatics platforms will be created/renovated and will complement each other.

In conclusion, despite a challenging research environment and though the paucity of facilities, scientists from Central Africa have brought a significant contribution to the scientific community, through PCR-related technologies. Collaborative research with northern partners has been fruitful and must be always conducted while keeping in mind a fair partnership and authorship. PCR-based research is increasing significantly in Central Africa and must be recognized at the level of the scientific community.

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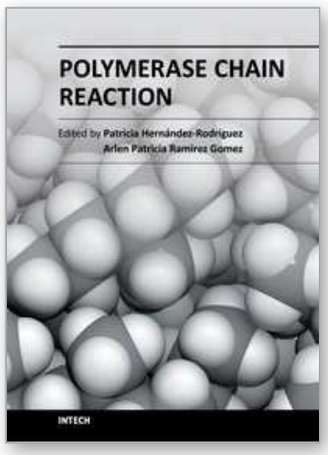

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