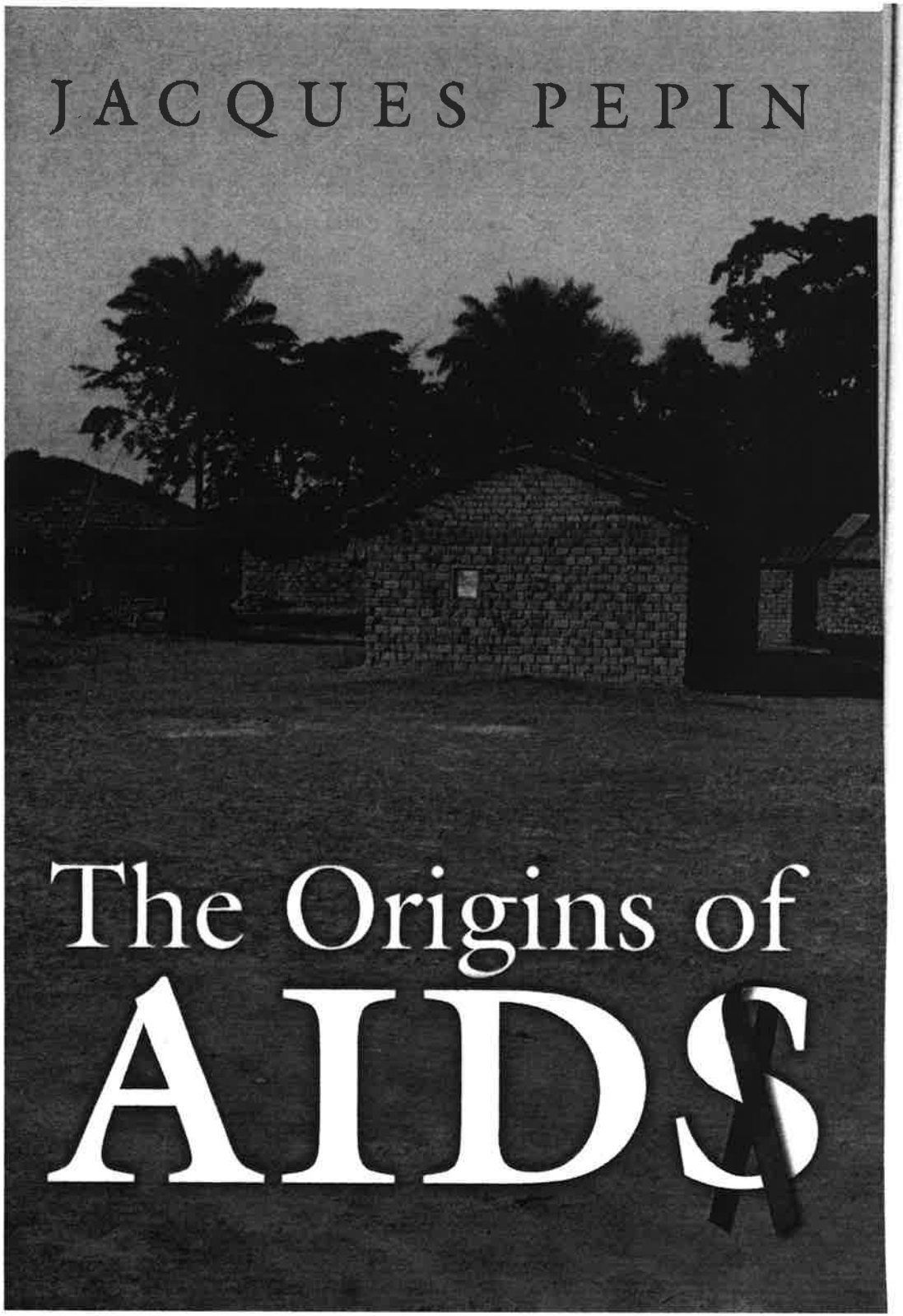

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1 Out of Africa

Ex Africa semper aliquid novi

Out of Africa, there is always something new, wrote historian Pliny the Elder more than 2,000 years ago. He was quite right. As early as 1984, just three years after the first description of the new disease, it was suspected that HIV, its recently discovered aetiological agent (then known as human T-cell lymphotropic virus (HTLV)-III in the US, LAV (lymphadenopathy-associated virus) in Europe), originated in central Africa. This was mainly because the first studies in Africa, conducted in Zaire and Rwanda, showed that AIDS was common in Kinshasa and Kigali, where nearly 90% of sex workers were infected. These field studies were prompted by the observation that of the first few hundred cases of AIDS diagnosed in Europe, about half occurred among patients coming from central Africa, mostly from Zaire. Over the following years, the epidemiology of HIV-1 infection in Kinshasa would be described in great detail by a group of American, Belgian and Congolese researchers known as Projet Sida, based at Hôpital Mama Yemo (Mama Yemo was dictator Mobutu's mother, a former sex worker, and she suffered the same fate as the Belgian colonists after her son was overthrown: this institution is now called Hôpital Général de Kinshasa). Projet Sida came to an abrupt end in 1991, when the whole of Kinshasa was looted by the city's poor people. During the same period and until the 1994 genocide, similar epidemiological work was conducted in Kigali, 1,500 kilometres east of Kinshasa.[1–3]

In retrospect, this early vision of central Africa as the source of HIV-1 was rather naive. Researchers assumed that since this was at the time the region with the highest prevalence (i.e. the proportion of the population that is infected) among groups representative of the general adult population, the virus must have originated there. There were at least two problems with this assumption.

First, there was an obvious bias, as little information on HIV prevalence was available from other parts of the continent, especially East and

southern Africa. Belgian researchers, the most prominent being Peter Piot, from the Institute of Tropical Medicine in Antwerp (who would later become the founding executive director of UNAIDS, the UN programme specifically dedicated to the control of HIV/AIDS), had naturally initiated HIV research in the former Belgian colonies where their institutions had maintained networks and contacts over the preceding decades. Much to their credit, Zaire and Rwanda were open to AIDS research from the start, but this was not the case in other countries such as Burundi and some English-speaking countries of East Africa, where there was a strong temptation to keep AIDS under wraps: if we ignore it, perhaps it will go away.

Second, the relationship between HIV prevalence and duration of the epidemic is not straightforward: it all depends on the annual incidence (the proportion of previously uninfected individuals who acquire HIV each year). We now know that in Kinshasa the HIV incidence among the general adult population was probably never higher than 1% per year. However, in some countries of southern Africa, annual incidence reached the extraordinary level of 5% in the 1990s (one seronegative adult out of twenty got newly infected with HIV each year). A prevalence of 10% could reflect an annual incidence of 1% continuing for more than ten years, or an incidence of 5% over just a couple of years. However, even if these assumptions about a central African origin of HIV/AIDS were naive, eventually they proved to be correct, showing that in the scientific domain intuition can sometimes be trusted!

Archival samples

Additional support for a central African origin of HIV-1 came from the testing of archival samples of blood. In the mid- and late 1980s, to understand the dynamics of HIV in the recent past, researchers tried to locate collections of sera obtained earlier for other purposes and kept frozen. Scientists tend to clean out their freezers once in a while to make room for new samples, or their samples are destroyed when they retire or move on to other positions. However, sometimes samples are forgotten for a long time or deliberately conserved. In Kinshasa, among mothers attending a well-baby clinic in the Lemba district, HIV-1 prevalence was 0.25% in 1970 (n=805) and 3.0% in 1980 (n=498). In the remote Catholic mission of Yambuku and surrounding communities of the Equateur province of Zaire, 0.8% of 659 samples collected in 1976

during an investigation of an epidemic of Ebola fever were found to be HIV-1 seropositive when tested ten years later. This proved that the virus had existed in this part of the world for some time, but not necessarily that it originated there; testing of archived samples of serum from American gay men who participated in epidemiological studies of hepatitis B also retrospectively documented cases of HIV-1 in the late 1970s, and even earlier for drug addicts.[4–8]

The Yambuku epidemic of Ebola fever which had prompted the collection of these samples had largely been 'iatrogenic' (healthcare related). In this small rural hospital, syringes and needles were scarce and constantly re-used, fuelling transmission of the blood-borne Ebola virus between patients attending the hospital for other reasons (malaria, gonorrhoea, etc.). The nuns issued only five syringes to the nurses each morning, which were then used and re-used on the 300 patients attending each day. Three-fourths of the first 100 cases of Ebola in Yambuku were infected through injections received at the hospital. The epidemic came to an end after the hospital was closed following the death of several nurses and nuns, infected by their patients. Clearly, noble intentions for providing health care to the underprivileged could have disastrous consequences when the risk of transmission of blood-borne viruses was not appreciated. This unfortunate situation was not new or specific to the Yambuku hospital, and had already had infinitely more dramatic consequences, although this was not known at the time, than these few hundred deaths from Ebola fever. It was decided to call this new disease after a nearby river rather than after the Yambuku mission, to avoid further stigmatisation after all it had gone through. The contrast between the two diseases is an excellent illustration of the genius of HIV. People infected with the Ebola virus quickly fall ill and die. This causes a spectacular epidemic, which triggers a massive (and always successful) reaction to control it. People infected with HIV, on the other hand, can live and quietly pass on the virus for ten years or more, and it will take even longer before physicians can recognise the emergence of this new disease, because symptomatic cases are not clustered within a short period of time.[9,10]

Elsewhere in Africa, no trace of HIV was found before the 1980s, which increasingly pointed to a central African origin of this 'new' virus. In West Africa, out of more than 6,000 samples obtained in Nigeria, Liberia, Ivory Coast, Togo, Senegal, Sierra Leone, Mali, Niger and Ghana in the 1960s and 1970s, not a single case of HIV-1 infection

was found. A few cases of HIV-2 infection were documented, however. Among 789 samples obtained in Senegal in 1981, one was positive but it is unclear whether this corresponded to HIV-2 or HIV-1.[11–16]

Meanwhile in East and southern Africa, in samples obtained from low-risk groups between 1959 and 1981, HIV was not found in Mozambique, Zimbabwe, Zambia, Uganda, Tanzania and northern Kenya, nor in mine workers in South Africa (who originated from Mozambique, Malawi, Lesotho, Botswana, Angola, Swaziland and South Africa itself). The earliest evidence of HIV in East Africa comes from Nairobi in 1980–1 where 1% of patients with STDs and 5% of sex workers were HIV-1-infected. Just three years later, 82% of Nairobi sex workers were HIV-1-infected. This exponential transmission among prostitutes is central to the story and will be examined later.[14,17–19]

Documentation of early cases of full-blown AIDS was also achieved retrospectively. First, let me say that no conclusions can be drawn from isolated cases of apparently immunocompetent patients found to have had, many years ago, a diagnosis of a condition now frequently associated with AIDS such as *Pneumocystis* pneumonia, if this is not substantiated by a specimen positive for HIV in the patient or his/her spouse. This is because there are rare non-infectious diseases of the immune system which lead to very low counts of CD4 lymphocytes (the cells which are the main target for destruction by HIV), and subsequently to any of a long list of opportunistic infections. Short of an archived specimen positive for HIV, the clustering of cases, geographically or temporally, or within a couple, is more suggestive of AIDS but never conclusive.[20–21]

Valuable journalistic information about some documented early cases can be found in *The river* as well as in *And the band played on*. The most interesting is that of a Norwegian family (father, mother and nine-year-old daughter), who all tragically died in 1976 from AIDS caused by HIV-1 group O, and whose sera were found to be HIV-positive when tested twelve years later. The child was born in 1967, which implies that the mother was already HIV-infected by then. The father had been a sailor, visiting a number of ports in Africa in the early 1960s, where he developed STDs, presumably after contacts with prostitutes. He probably acquired HIV-1 group O in Nigeria or Cameroon, where his boat stopped for a few days in 1961–2. A Danish surgeon died of AIDS in 1977, after working at the Abumonbazi rural hospital in Zaire in 1972–5 and in Kinshasa in 1975–7, following an earlier stint in

the same country around 1964. An eight-year-old Zairean child, infected perinatally in 1974–5, died in Sweden in 1982, and AIDS was serologically proven later on. A very unfortunate Canadian pilot involved in a plane crash in 1976 in northern Zaire, where he had surgery and received a blood transfusion, died of AIDS in 1980; his serum was later found to be HIV-1-positive (transfusion-acquired HIV infection progresses rapidly to AIDS, because of the huge quantity of viruses present in the blood bag). Former physicians at the university hospital in Kinshasa reported seven cases of AIDS diagnosed retrospectively, five of them confirmed serologically, which had been acquired sexually in the DRC (or Burundi in one case) in the late 1960s or the 1970s, mostly among Belgian nationals. Then in 1979, cases of AIDS started trickling down among the small proportion of Zaireans rich enough to seek health care in Belgium.[22–30]

We do not know whether other researchers tested ancient samples from other parts of Africa without reporting their findings. Studies with negative results tend not to be published in scientific journals, a phenomenon known as 'publication bias'. Thus although sketchy, testing of archival samples suggested that HIV-1 was present in the 1960s and 1970s, albeit at a low prevalence, in several locations in central Africa but not in West or East Africa.

The next step came from the documentation of the earliest case of HIV-1 infection in a sample obtained in the Belgian Congo around 1959, during the course of a study on genetic diseases of red blood cells. Of 672 samples, collected in Léopoldville and other locations, and miraculously kept (probably forgotten) in a freezer, one was found twenty-six years later to contain antibodies against HIV-1. Apparently, the HIV-1-positive specimen came from a male adult recruited in Léopoldville. HIV genetic material was amplified from this sample, and analyses confirmed that this was indeed the oldest HIV-1 isolate ever documented. It was named ZR59.[31–33]

It took more than twenty years for another ancient specimen containing HIV-1 to be located. Finding old tissue blocks collected between 1958 and 1960 and kept at the pathology department of the University of Kinshasa, scientists discovered HIV-1 sequences in a lymph node biopsy obtained in 1960 from an adult woman. It was given the name DRC60. Twenty-six other specimens (lymph nodes, livers and placentas) did not contain HIV. DRC60 and ZR59 differed by about 12%. It was calculated that DRC60 and ZR59 shared a common ancestor

around 1921, as we will discuss later. Although the exact time of its introduction into human populations remains debated, there is no doubt that HIV-1 was present in Léopoldville by 1959–60.[34]

Viral diversity

Now we will examine how the genetic diversity of HIV-1 in different parts of the world helped scientists trace back the origins of the virus. But first, we need to review quickly what 'sequencing' is all about. Sequencing is the identification in their proper order of the series of 'nucleotides' that constitute a gene. There are four types of nucleotides: adenine (A), thymine (T), guanine (G) and cytosine (C). The genome of any living organism is a long list of these four letters. When scientists compare viruses, the similarity between sequences is called 'homology', and non-similarity 'divergence'. If 90% of the nucleotide sequences between two isolates are the same, they have 90% homology or 10% divergence. This degree of divergence is used to decide whether two isolates constitute subtypes of one viral species, or two distinct species. For instance, sequences of HIV-1 and HIV-2 differ by more than 50%.

Based on such analyses, HIV-1 is now divided into four 'groups': group M (main), which is responsible for the current pandemic and causes more than 99% of all HIV-1 infections in the world, group O (outlier), group N (non-M non-O) and group P, which did not spread outside central Africa, for reasons still unclear.

HIV-1 group M is further subdivided into nine 'subtypes': A, B, C, D, F, G, H, J and K (the alphabet is not respected because subtypes E and I were found not to be real subtypes and have been renamed). HIV-1 often makes mistakes when replicating, a phenomenon compounded by the high level of viral production throughout the long natural history of the infection. Up to ten billion copies of the virus are produced every day, and the potential for errors in replication is commensurate. Over time, the accumulation of these errors leads to viral diversity. When around 20% of the nucleotide sequences of the initial virus have undergone replication errors, the result will be a new subtype, as defined arbitrarily by scientific consensus.[35–36]

High-risk individuals (especially in Africa) can get infected with a first subtype, and later with a second subtype, which can recombine into 'circulating recombinant forms' (CRF): part of their genome is derived from the first subtype, part from the second. Recombinants can be

2 The source

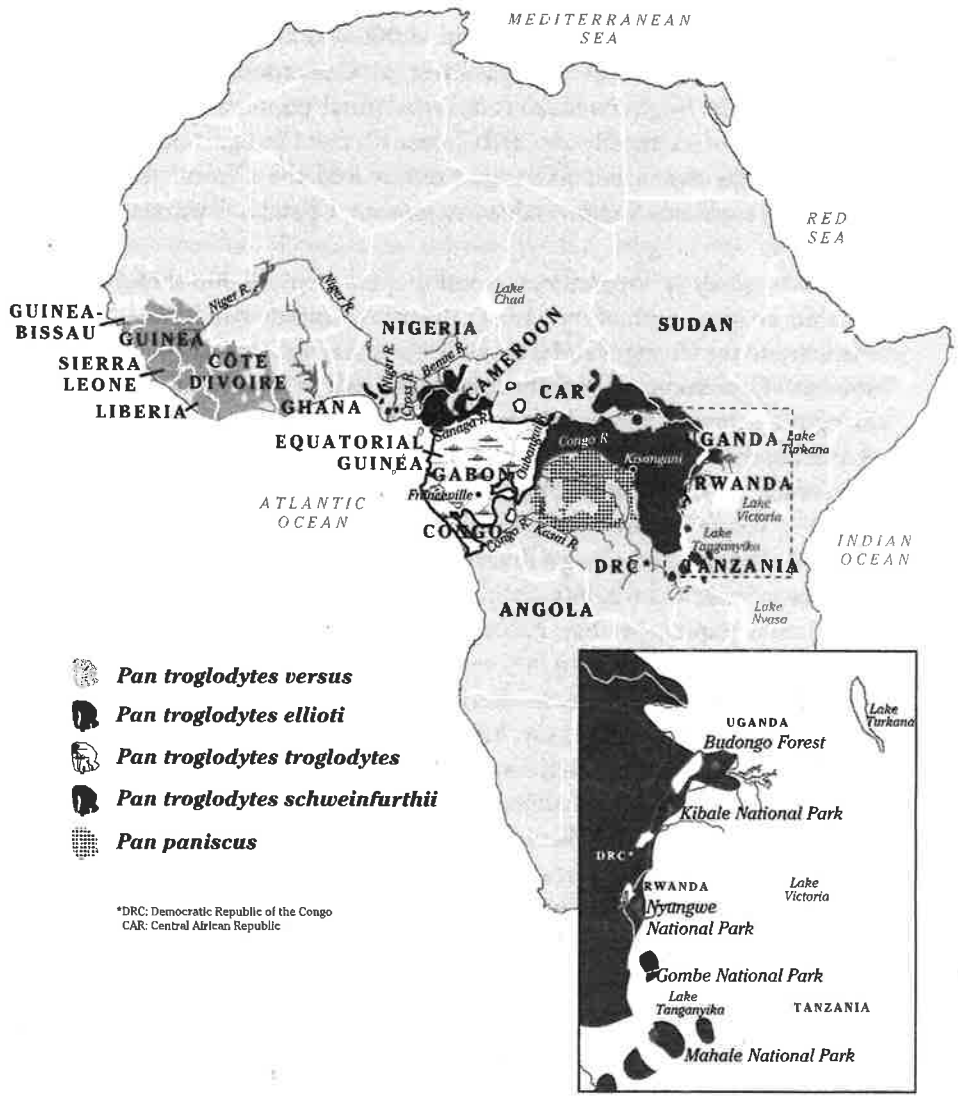
So, HIV-1 originated from central Africa. But then, one may ask, why central Africa? The answer, as we will see, is because this region corresponds to the habitat of the simian source of the virus.

Our closest relatives

Chimpanzees are the closest relatives of humans, sharing between 98 and 99% of their genome with us, and are considered the most intelligent non-human animal. Chimpanzees and humans shared a common ancestor and are thought to have diverged between four and six million years ago. In fact, chimpanzees are so close to humans that it was recently proposed to move them into the genus *Homo*. Long-term studies in the Gombe reserve of Tanzania revealed that, like humans, chimpanzees have their own personalities. Some are gentle, others are more aggressive. Some have a good relationship with their parents or other members of the troop while others are loners. Some have a strong maternal instinct, others do not. This marked individualisation and their ability to laugh are what make chimpanzees most like humans. Rather than reacting predictably and instinctively to a given situation, chimpanzees show intelligence and spirit and experience all kinds of emotions.[1–3]

According to current taxonomy, there are two species: *Pan troglodytes*, the common chimpanzee, and *Pan paniscus*, the bonobo. Based on analyses of mitochondrial DNA (DNA that comes solely from the mother), there are now four subspecies of *Pan troglodytes*: *Pan troglodytes verus* (western chimpanzee), *Pan troglodytes ellioti* (Nigerian chimpanzee, until recently *P.t. vellerosus*), *Pan troglodytes schweinfurthii* (eastern chimpanzee) and *Pan troglodytes troglodytes* (central chimpanzee) (Map 3).[4]

Chimpanzees are poor swimmers, so that large rivers like the Cross, Sanaga, Ubangui and Congo became natural boundaries between the



Map 3 Distribution of the four subspecies of *Pan troglodytes* and the *Pan paniscus* bonobo.

habitat of various species and subspecies. *Pan troglodytes verus* (total population in 2004: between 21,300 and 55,600, according to the International Union for Conservation of Nature) inhabits West Africa, from southern Senegal to the west bank of the Cross River in Nigeria;

most of its population is now found in Guinea and Ivory Coast. *Pan troglodytes ellioti* (total population: 5,000–8,000) is found from east of the Cross to the Sanaga River in Cameroon, its southern boundary. *Pan troglodytes schweinfurthii* (total population: 76,400–119,600) inhabits mostly the DRC, east of the Ubangui and north of the Congo rivers, but its range extends into the Central African Republic, southern Sudan and eastwards to Uganda, Rwanda and Tanzania.[5]

Pan troglodytes troglodytes (total population: 70,000–116,500) inhabits an area south of the Sanaga River in Cameroon and extending eastward to the Ubangui and Congo rivers, spread over seven countries: southern Cameroon, Gabon, the continental part of Equatorial Guinea, Congo-Brazzaville, a small area in the south-west of the Central African Republic, the Cabinda enclave of Angola and the adjacent Mayombe area of the DRC. The largest populations are found in Gabon (27,000–64,000), where unfortunately they are rapidly declining, Cameroon (31,000–39,000) and Congo-Brazzaville (about 10,000). Other countries have fewer than 2,000 each, with probably less than 200 in the DRC. It is estimated that *P.t. troglodytes* and *P.t. schweinfurthii* diverged approximately 440,000 years ago.[5,6]

Chimpanzee populations in the first half of the twentieth century were certainly higher than now, because there had been relatively little opportunity for human activities to disrupt the natural equilibrium of the species. Human populations were much smaller than today, with fewer hunters and fewer clients willing to purchase bush meat. As an educated guess, some experts suggested that, combining all subspecies, there was around one million chimps in 1960. The subsequent decline was particularly severe for *P.t. verus*, and is generally attributed to the destruction of its habitat by increasing human populations who farmed or logged and hunted for bush meat, to diseases like Ebola fever, and captures for medical experiments.[6–10]

The rest of this section will focus on the central *P.t. troglodytes* chimpanzee, but the morphologic, demographic and behavioural differences between the four subspecies of *Pan troglodytes* are minor, at least for the non-expert. *P.t. troglodytes* chimps have a life expectancy of 40–60 years. An adult male weighs 40–70 kg, a female 30–50 kg. They live in rather loose communities ('troops') of 15 to 160 individuals, with a dominant male leader. When they reach sexual maturity, males generally remain in the community into which they were born, while females often join other

troops. This intuitive exogamy maintains the genetic diversity of the subspecies and avoids the potentially devastating effects of inbreeding.

Chimpanzees are largely diurnal. To sleep at night, each individual builds a nest in a tree, complete with a pillow, 9–12 metres above the ground, which is normally used only once. For this reason, scientists have used nests to estimate chimpanzee populations, based on counts by surveyors who walk on line transects through forested areas as a sampling method. Population density of *P.t. troglodytes* is generally between 0.1 and 0.3 km^2. Most communities live in forested areas, and a minority in savannahs.

Chimpanzees are intensely territorial and most troops spend their entire lives within a 20–50 km^2 area. Adult males are aggressive, and spend much of their time patrolling their small territory. Males of one troop can form raiding parties to attack lone males (or couples) from other troops. *P.t. troglodytes* chimpanzees usually have a hostile and violent attitude towards members of other communities. Among their *P.t. schweinfurthii* counterparts in Tanzania, primatologists documented a war between two neighbouring communities which, after three years of attacks and killings, ended with the complete annihilation of the weaker troop.[1,11–13]

P.t. troglodytes chimpanzees are able to develop and use tools, mostly sticks to procure food (for instance, to dig out ants or termites or extract honey from hives). Unlike gorillas, chimpanzees are omnivorous, with a highly diversified diet consisting mostly of fruits, leaves, seeds, plants, insects and eggs, but they occasionally eat vertebrates, including monkeys, antelopes and warthogs.

An infant chimp spends the first five years of its life completely dependent on its mother. Like humans, they become progressively autonomous during adolescence, reaching sexual maturity at age 12–13. Chimpanzees are promiscuous, and most of their sexual activity takes place when the adult female is in heat and her vulva swells, which attracts the males, who copulate with her quickly, one after the other. As many as six different males may copulate with the same female in just ten minutes. Some males establish an exclusive relationship with a female of their choice, presumably for reproductive purposes, and take her on a 'honeymoon' far from the other chimpanzees. This usually only lasts for a week or two during which they copulate as often as five times a day. So while their behaviour limits the transmission of pathogens between troops, sexually transmitted infectious agents will easily

disseminate within a given troop once they have been successfully introduced.

P.t. troglodytes chimps have low fertility: on average, 800 matings occur for each conception. During their reproductive years (from age 14 to 40), females give birth to a mean of 4.4 babies, half of which die before reaching maturity. Each female has a lifetime reproductive success of only 2.3. A small increase in mortality, due to hunting or diseases, is sufficient to reduce this number to less than two and for the population to contract.[14,15]

Like humans, chimpanzee communities are occasionally stricken by epidemics. In Gombe, during an outbreak in the region's human population, poliomyelitis caused four deaths and left some chimpanzees permanently paralysed. Respiratory infections followed, also with fatal consequences. This reflects not just the communal nature of life among the chimpanzees, which have frequent and close contacts with other members of their troop, but also their biological similarity to humans, whose microbes can be transmitted to chimpanzees and vice versa.[1]

All kinds of trees

We will now examine how it gradually became clear that one subspecies of chimpanzees was the source of HIV-1. But first, let us review quickly a science called phylogenetics. Phylogenetics uses nucleotide sequences to reconstruct the evolutionary history of various forms of life, including microbial pathogens. A 'phylogenetic tree' superficially resembles a genealogical tree. However, phylogenetic trees describe the relatedness between living organisms (and their classification) rather than ancestry. They measure the genetic distance between organisms, and identify the nearest relatives. Because ancestors are not available to be tested, ancestry is assumed rather than proven. Each division in the tree is called a 'node', the common ancestor of the organisms or the isolates identified to its right. After such branching, the organisms and their sequences evolve independently. The 'root' (at the extreme left) is the assumed common ancestor of all organisms in the tree. To construct a phylogenetic tree, molecular biologists compare the differences in nucleotide sequences of many isolates of putatively related organisms. This exercise is repeated for various genes; if the findings are the same for two or

three genes, scientists are confident that they have produced the right phylogenetic tree.

An 'isolate' corresponds to a given pathogen obtained from one specific patient or animal at a specific point in time. If substantial laboratory work is done on any isolate, it will be given a name corresponding either to the initials of the patient, the name of the city or country where it was obtained or whatever the researcher decides to call it. Like children's names, these names serve only one purpose, to distinguish isolates from each other.

For two isolates belonging to the same species, a greater degree of divergence, corresponding to a larger cumulative number of errors in replication, indicates that their common ancestor was further back in time compared to isolates with a lesser degree of divergence. This is like brothers and sisters, born of the same mother and father, being more similar to each other than distant cousins who only share, say, great-grandparents. In practice, phylogenetic trees tell us that certain viruses are closely related and have a relatively recent common ancestor (these are said to 'cluster'), like brothers or first cousins, while for other viruses the relationship is similar to that of tenth cousins, whose common ancestors lived many generations ago.

The first report of the isolation of a simian immunodeficiency virus (SIV) from a chimpanzee born in the wild came in 1989. This isolate, given the name $SIV_{cpz\text{-}gab1}$, was obtained from a chimpanzee kept at the primate centre of Franceville, Gabon, where fifty chimps had been tested with assays used for the detection of anti-HIV antibodies in humans. Only two carried such antibodies; from one of them, the virus could be grown in cell culture, and its proteins were analysed. This chimpanzee, captured at six months of age, was four years old when the blood sample was obtained and seemed healthy despite presenting enlarged lymph nodes. Based on the crude methods available at the time, this SIV isolate was described as related although not identical to HIV-1. Phylogenetic analyses suggested that $SIV_{cpz\text{-}gab1}$ was closer to HIV-1 than to HIV-2 and to SIVs from African green monkeys, mandrills and other monkeys.[16–17]

It was not possible to isolate the virus from the second seropositive chimp, a two-year-old animal shot by hunters and that died of its wounds shortly after being brought to Franceville for care. A few years later, thanks to technological advances, nucleic acid amplification was used on this chimp's lymphocytes (which had been kept

frozen), in order to sequence parts of the viral genome. This isolate became known as $SIV_{cpz\text{-}gab2}$. It was phylogenetically close to $SIV_{cpz\text{-}gab1}$. In 1992, a third isolate ($SIV_{cpz\text{-}ant}$) was obtained from Noah, a five-year-old chimpanzee captured in the wild and impounded by customs officers in Brussels upon illegal arrival from Zaire. His isolate was somewhat divergent from HIV-1 and from the two previous SIV_{cpz} isolates.[18–20]

In 1999, a fourth isolate, $SIV_{cpz\text{-}US}$, was obtained from Marilyn, caught in the wild in an unknown African country and imported into the US as an infant in 1963. Marilyn was used as a breeding female in a primate facility until she died in 1985 at the age of twenty-six, after delivering still-born twins. During a survey of captive chimpanzees, Marilyn was the only one that was seropositive for HIV-1 antibodies. She had not been used in AIDS research, but had received human blood products between 1966 and 1969. During this early period, it is very unlikely that the blood products contained HIV-1, so there was a good chance that Marilyn had acquired her SIV_{cpz} infection in Africa. SIV sequences were amplified from the spleen and lymph node tissues procured at autopsy. Using mitochondrial DNA analyses, researchers identified the subspecies of chimpanzees from which this recent and the previous three isolates had been obtained.[21–22]

As could have been expected from the geographic distribution of *Pan troglodytes* subspecies, Noah (from Zaire) was a *P.t. schweinfurthii* while the other three, including Marilyn, were *P.t. troglodytes*. As illustrated in Figure 1, phylogenetic analyses revealed that the three SIV isolates obtained from *P.t. troglodytes* were similar to each other, and similar to HIV-1 strains from humans, while Noah's $SIV_{cpz\text{-}ant}$ diverged from these and lay outside this cluster, as did HIV-2 and SIVs obtained from other non-human primates.

Thus, naturally occurring SIV_{cpz} strains fell into two related but highly divergent, chimpanzee subspecies-specific, lineages: one for *P.t. troglodytes* and another for *P.t. schweinfurthii*. It was bravely concluded that *P.t. troglodytes* was the primary source of HIV-1 group M and its natural reservoir, and that there had been host-dependent evolution of SIV_{cpz} in chimpanzees resulting in *P.t. troglodytes* and *P.t. schweinfurthii* being infected with different lineages of SIV. Scientists could not rule out the possibility that other chimpanzee subspecies, especially *P.t. schweinfurthii*, could have transmitted their viruses to humans. This prudence was justified because a single isolate of SIV_{cpz} from *P.t. schweinfurthii* was

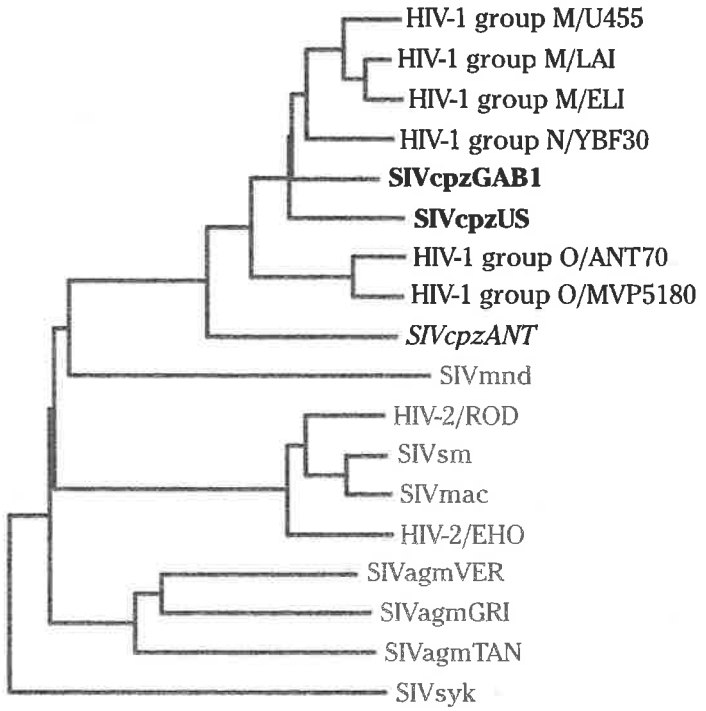


Figure 1 Phylogenetic analysis showing the relationship between $SIV_{cpz\text{-}US}$ and $SIV_{cpz\text{-}gab1}$ obtained from *P.t. troglodytes* chimpanzees (bold) and isolates from humans infected with HIV-1 (group M, group N, group O). The SIV_{cpz} isolates obtained from *P.t. troglodytes* cluster within the HIV-1 isolates, while $SIV_{cpz\text{-}ant}$ obtained from a *P.t. schweinfurthii* chimpanzee (italics) lies outside. Other SIV isolates obtained from monkeys and human isolates of HIV-2 lie further away. Adapted from Gao.[21]

available. It was possible that in the future other isolates of SIV_{cpz}, more similar to the human isolates of HIV-1, might be found in *P.t. schweinfurthii*. Additional isolates of SIV_{cpz} were later obtained from captive *P.t. troglodytes* in Cameroon, some of which were similar to those human HIV-1 isolates from the same country, reinforcing the view that HIV-1 originated in chimpanzees.[21,23]

Since this initial work was conducted mostly with chimpanzees which had been in captivity for some time, it was questionable whether the apes had acquired their SIV_{cpz} naturally in the wild or artificially in their cages where they had been in contact with other primates. In the first

case, the puzzle was close to being solved while, in the second, researchers had ventured down the wrong track. Non-invasive technologies were then developed to measure the presence of SIV antibodies and nucleic acids among chimpanzees living in the wild using urine and faecal samples, since obtaining blood samples was neither feasible nor ethically acceptable (some animals may have been hurt or killed in the process). We can but admire the motivation and expertise of these researchers and especially their trackers, roaming through the forest looking for chimpanzee urine or stools, which they had to distinguish from those of other animals. Urine samples proved inferior to faeces and were abandoned.

Among 100 wild *P.t. schweinfurthii* from Uganda and Tanzania, only one was infected with $SIV_{cpz\text{-}tan1}$. This isolate was similar to the previous $SIV_{cpz\text{-}ant}$ isolate from Noah, the Zairean *P.t. schweinfurthii*. More isolates were later found among *P.t. schweinfurthii* chimps in Gombe, where SIV_{cpz} prevalence was estimated to be around 20%. Phylogenetic analyses showed that these isolates clustered with $SIV_{cpz\text{-}ant}$ and diverged from the *P.t. troglodytes* isolates and from HIV-1 (Figure 2), confirming that *P.t. schweinfurthii* was not the source of HIV-1. Testing of additional *P.t. schweinfurthii* chimps from the Budongo forest of Uganda, the Mahale park in Tanzania and the Nyungwe reserve in Rwanda (Map 3) failed to identify a single animal infected with SIV_{cpz}. This heterogeneous distribution of SIV_{cpz}, which has recently been mirrored in a study of *P.t. schweinfurthii* in the DRC, probably reflects the community structures of chimpanzee populations and their behaviour: they have few contacts with chimpanzees belonging to other communities, except during territorial fights or when adolescent females migrate to other troops. But once SIV_{cpz} is successfully introduced into a community, there seems to be substantial transmission between its members, sexually or otherwise.[24–28]

SIV is non-existent among captive *P.t. verus* (the western chimpanzee), about 1,500 of which were tested and found to be uninfected. Surveys of wild *P.t. verus* and *P.t. ellioti* also failed to find a single case of SIV_{cpz} infection. Why is SIV_{cpz} absent within these two subspecies? Presumably, because SIVs were introduced into *P.t. troglodytes* and *P.t. schweinfurthii* only after these subspecies had diverged from *P.t. verus* and *P.t. ellioti* half a million years ago. Such a scenario would imply that there has been little contact between the subspecies ever since, which is possible since the large rivers of Africa constitute watertight barriers.[29]

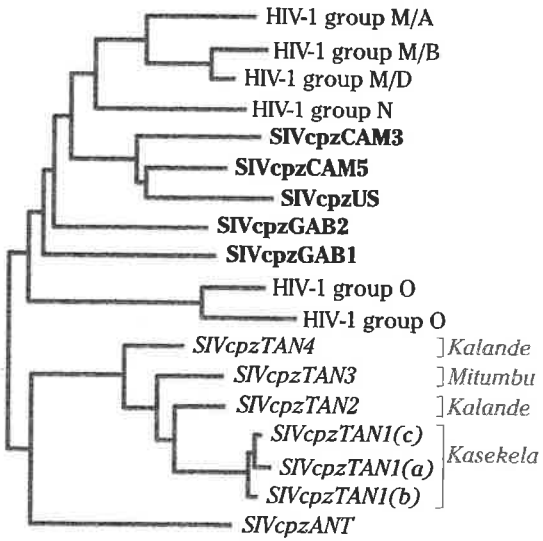


Figure 2 Phylogenetic analysis showing the relatively distant relationship between SIV_{cpz} isolates obtained in Tanzania from *P.t. schweinfurthii* chimpanzees (italics) and $SIV_{cpz\text{-}ant}$ obtained from a *P.t. schweinfurthii* chimp from the DRC (italics), clearly separated from the HIV-1 group M isolates. The latter are close to SIV_{cpz} isolates obtained from *P.t. troglodytes* (bold). HIV-1 group O lies outside the other HIV-1 isolates (in contrast to HIV-1 group N, which lies inside).

Adapted from Santiago.[26]

Prevalence of SIV_{cpz} among wild populations of *P.t. troglodytes* was then measured in an extraordinary study performed in ten forest sites throughout southern Cameroon. To make sure that the faeces originated from *P.t. troglodytes* and to avoid counting stools from any individual chimp more than once, the researchers amplified a number of host DNA sequences for species, gender and individual identification. In other words, they used the chimpanzee cells present in stools to fingerprint molecularly each and every individual ape who had defecated. After excluding degraded specimens, those that contained gorilla (the trackers' noses may not always be perfect!) or *P.t. ellioti* DNA, specimens were available from 106 individual *P.t. troglodytes* chimpanzees. Sixteen were infected with SIV_{cpz}. Again, there was a lot of variation in SIV_{cpz} prevalence between the study sites: in four of them not a single infection was found; in three sites prevalence was over 20% and the highest was 35%.[25]

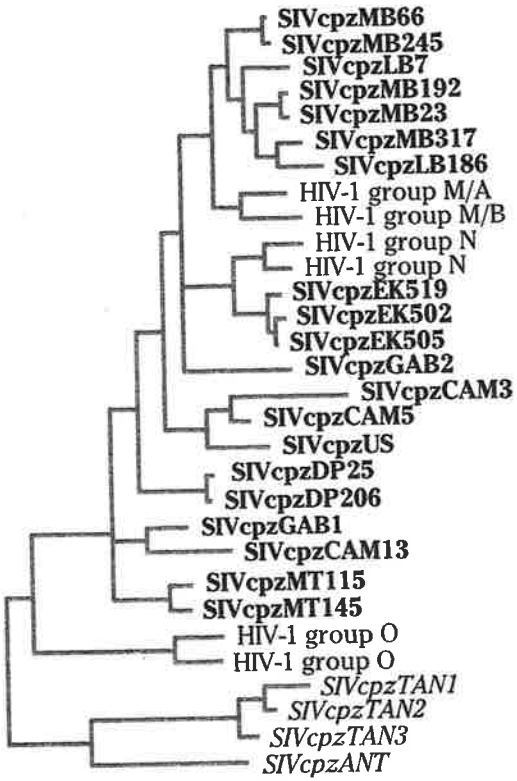


Figure 3 Phylogenetic analysis showing the relationship between SIV_{cpz} from *P.t. troglodytes* chimpanzees in Cameroon or Gabon (bold) and isolates from humans infected with HIV-1 group M, HIV-1 group N and HIV-1 group O. SIV_{cpz} isolates obtained from *P.t. troglodytes* cluster with the HIV-1 group M and N isolates, while HIV-1 group O remains an outlier. SIV_{cpz} obtained from *P.t. schweinfurthii* chimpanzees in Tanzania or DRC (italics) lie further away.

Adapted from Keele.[25]

Phylogenetic analyses (Figure 3) showed that all sixteen new SIV_{cpz} isolates were closely related to SIV_{cpz} isolates from captive *P.t. troglodytes* chimps and to HIV-1 groups M and N, but not to HIV-1 group O (always the outlier) or SIV_{cpz} obtained from *P.t. schweinfurthii*. This phylogenetic proximity confirmed – now irrefutably – that the SIV_{cpz} of *P.t. troglodytes* of central Africa was indeed the source of HIV-1 group M. Game over for this part of the story.

Chimpanzee populations separated by long distances or natural barriers like rivers harboured distinct lineages while adjacent troops harboured viruses closely related to each other. More detailed analyses of the genome showed strong clustering of human HIV-1 groups M and N viruses with the SIV_{cpz} lineages obtained from some specific *P.t. troglodytes* troops in southern Cameroon. In other words, in these rural communities, the local strains of HIV-1 infecting humans genetically resembled the local strains of SIV_{cpz} from the chimpanzees living close by. The SIV_{cpz} isolates from south-east Cameroon, towards the border with Congo-Brazzaville and the Central African Republic, were most closely related to HIV-1 group M, while those from south-central Cameroon were closer to HIV-1 group N.[30]

Additional faecal samples from *P.t. troglodytes* were collected over the following years, mostly in Cameroon, where the prevalence of SIV_{cpz} infection is now estimated to be 5.9%, a figure that I will use for calculations in forthcoming chapters. In the Central African Republic, no SIV_{cpz} infection was found but fewer than fifty specimens have been tested.[31]

SIV infection was found among faeces from western gorillas (*Gorilla gorilla gorilla*); a virus which was called SIV_{gor}. SIV_{gor} is very similar to HIV-1 group O, rather than to group M. Thus gorillas are not the source of the HIV-1 group M pandemic. Without getting into the details, chimpanzees may be the source of HIV-1 group O as well, which they transmitted to humans and to gorillas independently, or to gorillas first, which then infected some humans.[31–33]

Until proven otherwise, it is most likely that the modes of transmission of SIV_{cpz} between chimpanzees are the same as in humans: sexual intercourse, from mother to child and possibly through blood–blood contacts. There is much sexual promiscuity in chimpanzees. For instance, one adult male in Gombe is known to have mated since puberty at least 333 times with 25 different females, and of course only a very small proportion of all matings can be observed. A female called Flo was once observed to copulate fifty times within a twenty-four-hour period. The substantial genital swelling of females during oestrus may facilitate transmission of viruses by making the mucosa more fragile. Most of this sexual activity takes place within the closely knit community. A study of paternity among chimpanzee communities showed that only 7% of offspring had a father from outside the troop. Transmission

between troops could occur via out-migration of adolescent females, or during fights between males when blood-borne viruses could be exchanged.[3,11,12]

The fourth ape

A weakness in the investigations of SIV among chimpanzees is the dearth of virological information about the fourth ape, the *Pan paniscus* bonobo. Previously called the pygmy chimpanzee, this was a misnomer since the difference in size compared to *Pan troglodytes* is minor. It inhabits parts of the DRC south of the Congo but north of the Kasaï–Sankuru river system, in the Congo central basin which has low human populations but is linked by rivers to Léopoldville–Kinshasa, the main market for its farming and fishing products.

Bonobos are less aggressive and more mutually tolerant than *P.t. troglodytes*, and males and females have similar social ranks (some primatologists even describe an unusual situation of female dominance). Bonobos are not territorial so that males do not stalk or attack males from other troops and interactions with other communities are generally peaceful. They have a particularly intense, peculiar – and dare I say – quasi-human sexual activity: they do it for fun rather than just for reproductive purposes, and they have sex mostly in what biologists call a 'ventral–ventral mount' (the 'missionary position'). Among other practices that have been described by highly dedicated primatologists, they practise mutual genital–genital rubbing, genital massages, mouth kisses and even oral sex. Another unique feature of bonobos is their bisexuality, seen in both males and females.[34–36]

About half of intercourses are preceded by some form of courtship, but once they copulate fifteen seconds suffice. Intercourse is used to solve conflicts and maintain social interactions, and female bonobos are known to accept sex in exchange for food, a process quite similar to some human behaviour that we shall describe later. The period of sexual receptivity of female bonobos is twice as long as for *Pan troglodytes* and bonobos are more likely to have promiscuous matings outside their own group. In principle, these factors could facilitate the sexual transmission of viruses.

Until recently only thirty-two bonobos, all but four living in zoos or primate centres in Europe and the US, had been tested for SIV_{cpz} infection and none was infected. The main problem in studying

bonobos in the wild is that they are close to extinction, with between 10,000 and 20,000 individuals scattered around a large area of the DRC. Their distribution is discontinuous and bonobos are well aware that their main predator is humans. Just last year, samples from around sixty wild-living bonobos, obtained from two sites in the DRC, have finally been tested and were all negative for SIV. Given the heterogeneity in the distribution of SIV among *Pan troglodytes*, one would like a larger number of *Pan paniscus* troops to be tested, but in the meantime it is fair to say that there is no evidence that this primate played a role in the emergence of HIV-1.[28,37–38]

Origins of SIV in chimpanzees

What was the source of SIV_{cpz} infection in chimpanzees lies outside the scope of this book, which is to understand the early twentieth-century events that led to the current HIV-1 pandemic. To finish the story quickly, I will just add that, as reviewed elsewhere, SIV_{cpz} probably originated from the recombination of distinct SIVs infecting smaller monkeys, principally the SIV_{rcm} of red-capped mangabeys and a SIV which seems to infect greater spot-nosed monkeys, moustached guenons and mona monkeys. The most likely opportunity for such a recombination occurred when chimpanzees hunted and ate smaller monkeys. Perhaps the two SIVs that gave rise to SIV_{cpz} were transmitted independently to different chimpanzees and spread for some time before an ape became infected with both, allowing recombination to occur. Alternatively, one of the SIVs could have established itself within the chimpanzee population, the recombination occurring when one of the chimps infected with the original SIV acquired a second SIV from a small monkey, again via predation.[25–27,39]

3 The timing

Having identified the source of HIV-1, the next question is: *when* did the virus manage to cross species from chimps to humans? It has often been said that AIDS was a new disease on the African continent. Apart from the published cases mentioned in Chapter 1, clinicians working in central Africa, for instance Dr Bila Kapita, chief of internal medicine at Hôpital Mama Yemo in Kinshasa, reported that, at least since the mid-1970s, they started seeing cases that in retrospect were very likely to have been AIDS. This would be consistent with some degree of dissemination of the virus during the mid-1960s, given the average ten-year interval between infection and symptomatic disease. But could the disease have been present even earlier?[1,2]

Bush medicine

In most district or regional hospitals of countries inhabited by *P.t. troglodytes*, the diagnostic facilities during the colonial era (and even now) were so minimal that it would have been difficult, even for astute and experienced clinicians, to recognise the emergence of a new disease characterised by intermittent fevers and profound wasting. Most such institutions did not have any kind of half-decent microbiology laboratory. No cultures were done, either for common bacterial pathogens or the agent of tuberculosis, and diagnoses were based on stains made directly on the specimens, or solely on the combination of symptoms and signs found during the clinical examination. Fifty years later, I found the same situation at the Nioki hospital in Zaire: nothing had changed. This approach was relatively effective for diagnosing parasitic diseases (malaria, sleeping sickness, filariasis, intestinal parasites) but very insensitive for most bacterial diseases. Little radiological investigation was available either; only in the best hospitals was it possible to get something as elementary as a chest x-ray. The first x-ray machine in Brazzaville was installed in 1931, two years before one became available in Léopoldville.

Thus a patient with fever, chronic diarrhoea and wasting might initially have been administered an antibiotic active against, say, typhoid fever. An old antibiotic, chloramphenicol, used to be popular for this indication. If this did not work, then extra-pulmonary tuberculosis would be suspected and the patient started on antituberculosis drugs (only after 1950 because, prior to that, there was no drug treatment for tuberculosis). Several weeks would be required to determine whether the patient improved on this second empirical medication. Some responded, and probably indeed suffered from occult tuberculosis. Others did not and would slowly die, often at home after it had become clear that the hospital could not provide a solution, and the families did not want to waste all their meagre resources on unsuccessful therapeutic trials. The doctors would presume that these patients died from some form of cancer, the diagnosis of which was well beyond the scope of bush hospitals. A final diagnosis would never be made, as doctors had too many other things to worry about to try to determine the actual cause of a particular death by performing an autopsy. The capacity to recognise an emerging disease was minimal, for the simple reason that there was a long list of serious diseases, already recognised in every medical textbook of the time, that these hospitals could not diagnose.

In the capitals, diagnostic facilities were better but still far from the European standards of the time, even in the clinics whose main (or only) role was to provide care for Europeans. These hospitals had a few specialists, mostly surgeons who could carry out biopsies if some form of cancer was suspected. The histopathological slides would be sent to a collaborating hospital in Europe, and the results would come back months later. One such surgeon who worked in Brazzaville thought he had perhaps recognised a new disease, as we will see now.

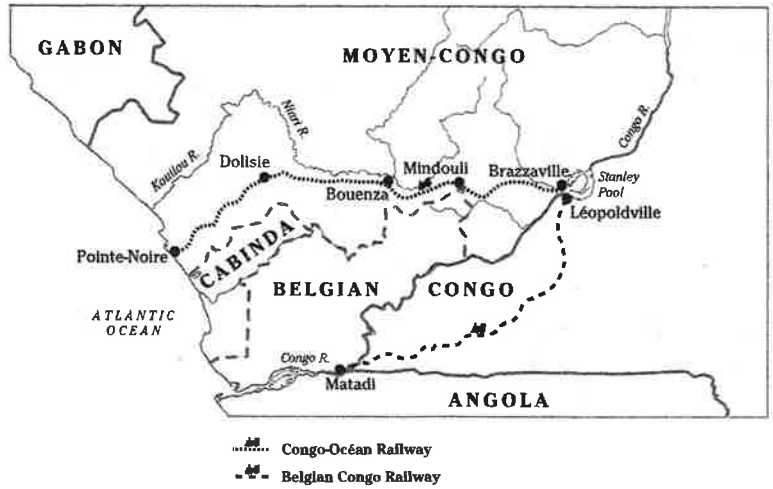
A colonial tragedy

Léon Pales was not an ordinary colonial doctor. He graduated from Bordeaux in 1929, aged twenty-four. During his medical studies, to earn some money Pales worked as an anatomical assistant at the medical school, helping with autopsies and the dissection of cadavers for medical students learning anatomy, an experience that would later prove very useful. While the usual MD thesis at the time consisted of a 60–80 pages literature review of some narrow medical topic, his was

429 pages long and addressed a very unusual field, palaeopathology: the study of diseases of prehistoric humans through examination of their bones. It would remain the standard French-language textbook for three decades. One of its main themes was that the study of ancient diseases could provide knowledge useful in understanding modern health problems. After the tropical medicine course in Marseilles, Pales was posted to Moyen-Congo (1931–3) and Tchad (1934–7). Back in France, he worked in Marseilles, taught anatomy and ethno-anthropology at the École du Pharo, and directed a field surgical unit during the invasion of France in 1940. Made a prisoner, he was repatriated to France the following year. He became assistant director of the Musée de l'Homme in Paris but does not seem to have been involved in the resistance movement organised around this institution. After WWII, the rest of his career (in France, and a few years in West Africa) would be devoted to palaeopathology, his first love, to anthropology and nutrition.[3,4]

During his two-year term in Brazzaville, Pales' career intersected with a colonial tragedy for the sake of 'economic development': the building of a railway between Brazzaville and Pointe-Noire, the Chemin de Fer Congo–Océan (CFCO), whose main purpose was to avoid depending on the Belgian railway. In a region with little infrastructure, a second railway was built only 100 kilometres from the Matadi–Léopoldville line, at the same time as the latter was expanded (Map 4). Started in 1921, the 511-kilometre railway would not be completed until 1934. Ninety-two bridges or viaducts had to be erected, as well as twelve tunnels, with the longest stretching over 1.5 km. During construction, the regions immediately west of Brazzaville and east of Pointe-Noire presented no major logistical problem; food could easily be delivered and the sick evacuated. In the middle, however, the 100-kilometre stretch in the Mayombe, a dense and hilly equatorial rain forest, became a nightmare. The Mayombe was thinly populated and the workforce had to be imported, creating a huge melting pot of all AEF ethnic groups, forced to live in squalid conditions highly propitious for the spread of microbial agents, perhaps including HIV-1.[5]

Initially in Moyen-Congo, and later in Oubangui-Chari and Tchad, 127,250 adult men were conscripted to work on the CFCO. Paid 1.5 francs per day, less than 1% of what their French foreman received, they worked ten hours a day, six days a week. Daily rations of food were inadequate, and the workers often received less than they were



Map 4 Itinerary of the Brazzaville–Pointe-Noire and Léopoldville–Matadi railways.

supposed to. They were housed in mud-brick buildings, where 50–60 men slept in the same room. As rumours spread concerning the fate of CFCO workers, it became increasingly difficult for the local chiefs to recruit their target numbers, for many fled to safer areas. The colonial authorities lowered the age limit, increased the duration of forced labour and coerced some unfortunate men in returning up to five times. Some workers absconded, usually in groups, but escape was harder to envision for men from Oubangui-Chari and Tchad. How could they possibly get back to their villages, a thousand kilometres away, without a penny in their pockets or any understanding of the local languages?[6,7]

Slave owners had an obvious interest in keeping their slaves alive: it was expensive to replace those who died. The situation was different with the CFCO. By contract, the AEF government had to supply the Société de Construction des Batignolles with 8,000 workers year round. Their recruitment, transportation, lodging and feeding was the responsibility of the state. As soon as a worker died, the state had to provide another and pay a penalty to the company if the minimum number of workers was not available. This was an early example of a public–private partnership in which the private company got excellent terms.

Grossly underpaid, underfed, overworked and housed in appalling conditions, between 15,000 and 23,000 workers died in the process, ten

times the death toll of the Léopoldville–Matadi railway thirty years earlier. The most murderous section, and the most difficult from the engineers' point of view, was the Mayombe. On top of the work accidents, epidemics broke out in the workers' camps. Mortality among the Mayombe workers was a staggering 496 per 1,000 men-years in 1926 (in other words, half would be dead within a year), 454 in 1927 and 384 in 1928. It declined to 173 per 1,000 men-years in 1929, when sanitary conditions improved after this scandal was revealed in France by writer André Gide and journalist Albert Londres. In absolute terms, the peak mortality occurred in 1927, when 2,892 workers died: eight per day. Mortality was highest among those recruited in Tchad.[6–9]

Inspection missions were sent by the French government to investigate whether the newspaper reports were true, and to come up with solutions. Two military doctors, General Lasnet and Lieutenant-Colonel Ferris, led these inspections. Ferris described the pathetic conditions of the primitive hospitals set up near the building sites, where huts erected for twelve patients could house thirty, causing transmission of pathogens between patients. Someone admitted for pneumonia ended up with dysentery a few days later, or vice versa. The main causes of mortality were: dysentery (bloody diarrhoea), caused by *Shigella dysenteriae*, endemic in the Mayombe; pneumonia, caused by a bacterium known as the pneumococcus; beriberi, a vitamin B1 deficiency which causes heart failure; other ill-defined febrile illnesses; and what the doctors called 'physiological misery', with some features (apathy, nostalgia) suggestive of major depression.[6,10]

The scandal in France and the inspection visits forced the AEF government to improve the workers' sanitary conditions. Governor Raphael Antonetti knew that he was in trouble and spent months writing detailed replies to the inspectors' reports. Instructions about how to take proper care of the workers were issued. Wages were increased, and some women were allowed into the workers' camps. Naturally, prostitution quickly developed, and STDs, hitherto inexistent, appeared among the workers. Prostitutes were noted to collect 'their fees on paydays amidst long palavers'.[9]

Instructive autopsies

When Léon Pales arrived in Brazzaville in 1931 as the colony's surgeon and obstetrician, the CFCO workers' health situation had already

improved. Surgical facilities in Brazzaville were limited, so Pales had a lot of free time to do what he had learned in Bordeaux and which nobody in AEF had done before: autopsies. He had access to the Institut Pasteur laboratory, where bacteriological cultures were available (for instance, to look for pathogens causing diarrhoea, such as *Shigella* and *Salmonella*) and where guinea pigs could be inoculated to look for the aetiological agent of tuberculosis. The Pasteur laboratory was even able to characterise pneumococci (the main agent of pneumonia) into serotypes.

Pales eventually published a few scientific papers on this necropsic work. First, he reported the findings from eighty-five patients who had died from pneumococcal infections, sixty-four of whom were CFCO workers. The pneumococcus was grown from the blood cultures, the cerebrospinal fluid, pleural fluid, pericardial fluid or other specimens obtained either pre-mortem or during autopsy. Pales described the autopsy findings, from the adrenals to the brain, which often revealed disseminated pneumococcal infections. This did not imply that the patients were immunologically impaired, but reflected the absence of an effective treatment which allowed this virulent pathogen to spread throughout the body. It certainly demonstrated Pales' unique competence and motivation in performing detailed autopsies and his access to the only laboratory in AEF where bacteriological cultures could be performed.[11–12]

He subsequently published a paper on tuberculosis in AEF, and more detailed information is available from a thesis written by medical student Jean Auclert in Marseilles using material provided by Pales. Pales described a new condition that he called Cachexie du Mayombe. Cachexia means profound weight loss. Adult male patients with Cachexie du Mayombe weighed as little as 30–5 kg, and were described as 'an assembly of bones held together by skin . . . whose only sign of life lay in their gaze'. They had a normal appetite and experienced no vomiting but suffered from chronic non-bloody diarrhoea. However, repeated examination of their stools failed to reveal a parasitic agent, and stool cultures performed at the Institut Pasteur were negative for the enteric pathogens known at the time, especially the *Shigella dysenteriae* which had killed many of the CFCO workers.[13–14]

Pales autopsied fifty such patients who, by his definition of the syndrome, had worked on the Mayombe part of the railway and sought care in Brazzaville after being declared unfit for service due to poor

health. In thirteen autopsies, he found confirmation of a tuberculosis that had been diagnosed pre-mortem, in seven others he found occult tuberculosis undiagnosed pre-mortem (tuberculosis of the intestine or the intra-abdominal lymph nodes), in four he found other diseases which killed the patient, but in twenty-six autopsies he did not find any macroscopically obvious medical condition explaining the profound wasting. He did note, however, that many of these patients had cerebral atrophy, very unusual for young adults, and that they also had generalised lymphadenopathy, including large mesenteric (around the small bowel) lymph nodes, which failed to reveal the tuberculosis bacillus via staining and/or guinea pig inoculation. We do not know the actual incidence of the Cachexie du Mayombe, but Pales presumably autopsied only a small fraction of cases, as many must have died elsewhere than at the Brazzaville hospital.

This new syndrome was certainly suggestive of AIDS. We can be pretty sure that these twenty-six patients did not have disseminated tuberculosis or cancer, which should have been easy to recognise during the autopsy. Severe malnutrition was also unlikely, because the patients' condition should have improved when properly fed in Brazzaville. The concentration of cases among patients who had worked in a well-defined area suggests a transmissible agent. Brain atrophy is common in patients with AIDS, and leads to a complication called AIDS dementia. Generalised lymphadenopathy is a hallmark of HIV infection, caused either by the virus itself or a variety of opportunistic infections which supervene. Such findings, as well as their chronic diarrhoea, would not have been noted had the patients died of major depression or some other severe psychological disturbance related to the hardship they had to endure.

One could speculate that the extremely high male/female ratio in the Mayombe work camps (ten men for each woman) and the intense prostitution that ensued would have facilitated the transmission of HIV-1, possibly from a single worker who had been infected with SIV_{cpz}. The time between the workers being sent to the CFCO camps and the development of their disease was not indicated in Auclert's thesis, but was probably less than the ten years we usually see today between getting HIV-1 and the first symptoms of AIDS. This does not exclude anything: for complex virological reasons, it is possible that this incubation period was actually shorter soon after the virus was introduced into human populations. And even nowadays, some unfortunate patients develop AIDS within two years after their infection.[9]

Unless the original tissue blocks or some of the slides prepared from the biopsies performed during these autopsies could be miraculously located, this will remain a hypothesis. I contacted the Pales family, the Institut Pasteur, the Musée de l'Homme and Le Pharo, and no such material seems to have survived over the past seven decades. Unfortunately, there is no longer an Institut Pasteur in Brazzaville, and whatever archives may have existed seem to have been destroyed during the long periods of civil strife this country went through. So we will never know for sure. But the point that can be made from this story is that the supposed absence of a clinical condition recognised by early twentieth-century doctors cannot be used as a strong argument for dating the emergence of HIV.

Molecular clocks

We have just seen the limitations of what can be extrapolated from reports or publications by colonial-era clinicians. Fortunately, molecular biology offers some help with dating. For readers who are unfamiliar with molecular biology, this is the last section where we will need to talk about these particular concepts.

First we will review what scientists call 'molecular clocks'. Their principle is simple, and is based on an assumption that the rate of genetic change is fairly constant over time. From a known mean rate of substitutions (which correspond to replication errors: mutations, deletions, etc.) within each gene, it is possible to estimate the chronology of the evolution of the organism. In its simplest form, if we assume a rate of evolution of, say, 0.002 substitutions per nucleotide per year at some given part of a gene and find, say, a 10% (0.1) difference in sequences between two isolates, we can back-calculate that they shared a common ancestor fifty years earlier, after which they diverged, each undergoing its own series of genetic changes. Of course, if the isolates were not obtained at roughly the same time, this will be taken into account.[15]

Viral recombination between two different subtypes of HIV-1 is the most difficult obstacle for molecular clocks. For instance, CRF02_AG isolates have part of their genome that originated from a subtype A, and part from a subtype G. It is not always clear which is which in the specific parts of the genome whose substitutions will be used to calculate molecular clocks. It is then impossible to evaluate how long the process

of evolution has been going on, because the starting point is unclear. The counter-strategy is simple: all inter-subtype recombinant viruses must be excluded. This works well as long as the recombinants are identified, which is not always easy. Other challenges associated with molecular clocks are reviewed elsewhere.[15]

In a landmark paper that tried to address these limitations through complex analytical strategies, scientists at the Los Alamos National Laboratory estimated that the most likely date for the common ancestor of all HIV group M isolates (that is, all subtypes and recombinants within group M) was 1931. This common ancestor corresponds to the root in phylogenetic trees, the point after which all divergence occurred. As in opinion polls, they calculated a 'confidence interval', which in this case was pretty wide, from 1915 to 1941. In other words, there were nineteen chances out of twenty that the true date of the common ancestor was somewhere within this range.[16]

To verify the validity of this dating, they back-calculated the date of the oldest HIV-1 sequences (ZR59) obtained in Léopoldville in 1959, as well as the date of isolates from Thailand, a country where HIV-1 was introduced in 1986–7 according to extensive epidemiological studies. They dated the Léopoldville sequence between 1957 and 1960, while for Thailand the common ancestor was calculated as having existed in 1986. So in both cases their dating was similar to what could be inferred from historical information, which suggested that their 1931 dating for the common ancestor of HIV-1 group M was relatively accurate.

However, the researchers could not directly address the question of when the cross-species transmission, from chimpanzee to man, had occurred. If the 1931 common ancestor was a human virus, the cross-species event would have occurred in the preceding decade (otherwise the patient would not have survived until 1931). But could the 1931 common ancestor still have been a simian virus at the time? This latter scenario was thought to be highly unlikely since it would have required multiple and close to simultaneous cross-species transmissions, all after 1931, that were all epidemiologically successful. On the contrary, analyses suggested that each group of HIV-1 (M, N, O and now P) represented a distinct cross-species transmission event, and that for pandemic HIV-1 group M the most likely number of such events was one. In other words, the 1931 ancestor indeed lay within a human host – and from this single individual, the true 'patient zero', more than sixty million people across the world were subsequently infected![17–20]

Then, from a database of HIV-1 isolates from the DRC, and using a sophisticated mathematical approach, the population dynamics of HIV-1 in that country were reconstructed by estimating, year after year, the number of infected individuals. This showed a very slow growth between 1930 and 1940, with an exponential growth later on. Prior to 1930, the number of HIV-1-infected individuals in the Belgian Congo was estimated at somewhere between 0 and 100.[17]

For a long time, ZR59 was the only ancient specimen of HIV-1. The recent discovery of DRC60, obtained from a lymph node biopsy performed in Léopoldville in 1960, provided additional information. ZR59 and DRC60 diverged by 12%, and were clearly phylogenetically distinct: ZR59 is an ancestor of subtype D while DRC60 is related to subtype A. This implies that HIV-1 group M began to diversify into human populations and entered Léopoldville a few decades before 1960. The inclusion of DRC60 changed the measure of the most recent common ancestor of HIV-1 group M, which was now dated at 1921 (confidence interval: between 1908 and 1933). Other models yielded slightly different dates. Reconstruction of the HIV-1 dynamics in the Belgian Congo was again compatible with a total of fewer than 100 HIV-1-infected individuals for a long time, then a very slow increase in the number of infected individuals until the mid-1950s, when exponential growth supervened.[21]

This gives us an appreciation of the lack of certainty in such measures. The addition of a single isolate, DRC60, changed the estimates by ten years, and the confidence interval remained wide. In practice, the common ancestor of all the subtypes of HIV-1 group M probably existed sometime in the first three decades of the twentieth century. To keep things simple, I will use '1921' from now on, but this should be viewed as indicating a period rather than a specific year.

For how long has SIV_{cpz} been present in chimpanzee populations? The same methods were used, based on sequences in the Los Alamos database. The results varied according to which gene was used for the molecular clocks and lacked precision. By and large, they suggested that the emergence of SIV_{cpz} in chimpanzees preceded its cross-species transmission to humans by something in the order of a few hundred years, not thousands or tens of thousands. This is probably why SIV_{cpz} is not found in *P.t. verus* and *P.t. ellioti* populations: the virus appeared long after these subspecies diverged from *P.t. troglodytes*.[22]

To summarise, there is compelling evidence that the common ancestor of HIV-1 existed in a human being sometime in the first three decades of the twentieth century, and that the whole group M pandemic was started by a single cross-species transmission. Now we will try to understand how the virus crossed species, what happened after 1921, and how HIV-1 eventually expanded into a global pandemic.

4 *The cut hunter*

The next question to be addressed is: how did the virus cross species to infect humans? How did the simian immunodeficiency virus of *P.t. troglodytes* chimps become the human immunodeficiency virus type 1? Again, science started out with an intuition: this must have occurred through the handling of chimpanzee meat by hunters, or their wives who would cut up the animals before cooking them. We will now examine whether this theory remains plausible after reviewing the various pieces of evidence accumulated over the past decade.

Hunters and their prey

Hunters and/or cooks can acquire infectious agents from their prey, including primates. For instance, Herpes B virus is a rare but highly lethal infection of individuals who handle monkeys, and especially laboratory technicians working with rhesus and cynomolgus macaques. Monkeypox is a smallpox-like but benign viral infection associated with exposure to monkeys. Highly lethal Ebola and Marburg haemorrhagic fevers have been reported in veterinarians and villagers who handled the carcasses of apes that had died in the wild from these infections. Recently, a retrovirus called simian foamy virus (SFV) has been associated with human exposure to monkeys and apes, and its sequencing allows the identification of the exact simian source. American veterinarians and animal caretakers working in primate centres or zoos were found to be infected with SFV acquired from chimpanzees. Fortunately, this virus does not seem to be pathogenic for humans, and no person-to-person transmission has ever been documented.[1–3]

Because of their intelligence, agility and aggressiveness, chimpanzees have no predators in the forest apart from leopards and, of course, humans. However, they are by no means easy prey for hunters, and rarely a specific target unless the apes had the bad idea of destroying the

crops in plantations near the villages. Although they might occasionally fall into wire snares, lianas knots, nets or pits of all kinds set up for other game animals, chimpanzees can escape from many such traps. Pygmies hunted year round with bows, crossbows and assegais. Targeting chimps would have been a dangerous undertaking, but the fact that pygmies could hunt elephants without firearms (albeit at considerable risk) was testament to their skills. For reasons still unclear, HIV-1 has remained remarkably rare among pygmies, and when present seems to have been acquired through their contacts with Bantus rather than from apes. Bantus hunted mostly during the dry season when the forest was easier to penetrate and when they had less farm work to do and needed additional sources of food until the next crop. If the hunt was successful, carcasses of great apes were generally first cut up in the forest to make them easier to carry, and then cut into smaller pieces in the village before being sold and eaten.[4–8]

It is difficult to hunt chimpanzees without firearms, and the firearms with small pellets generally available in the bush are not powerful enough to kill an ape. French colonisers had to deal with a number of armed rebellions from populations who opposed their rule, especially in the south-west of Oubangui-Chari and adjacent areas of the Moyen-Congo. Therefore, regulations made it difficult for the natives legally to procure powerful weapons. In France's annual reports to the League of Nations concerning Cameroun Français, the exact number of firearms and bullets imported into the country was spelled out (for instance, in 1922, 789 shotguns, 41 revolvers and 6,740 kg of ammunition). Africans could, however, own locally made piston firearms for small game hunting.[6,9,10]

In the Belgian Congo, regulations were looser. In 1927 an astounding 122,804 firearms permits were issued. This number gradually increased to 245,644 (a quarter of a million!) by 1945. The situation was made worse by a regulation forcing employers to provide their workers with meat at least once a week: hunting was much cheaper than farming. By 1925, Professor Leplae of Université Catholique de Louvain, an expert who also worked for the Ministry of Colonies, was complaining that many species of game animals were being butchered at such a rate that extinction would soon ensue. He estimated that 25,000 elephants were killed each year in the Belgian Congo, often with automatic rifles. Thirty years later, game animals of all kinds were indeed nearly extinct, and this was attributed to a combination of factors: the regulations

concerning meat for workers, the development of large cities which created lucrative markets for hunting products, the widespread availability of firearms and all kinds of materials that could be used for trapping, the bad example set by some European hunters and the disappearance of customary hunting regulations through which an equilibrium was maintained between human populations and game animals in the pre-colonial era.[11–13]

In edicts in April 1901 and December 1912, the Belgian Congo's governor prohibited the hunting of a number of animals, including chimpanzees, by both natives and Europeans. The ban was upheld in subsequent amendments of the law in 1934 and 1937, which divided game animals into four categories: gorillas belonged to category I and could not legally be hunted apart from what was required by scientific institutions, while chimpanzees, listed in category II, could be hunted by those who could afford to buy the appropriate, more expensive, permit. A tax also had to be paid for each animal killed: 1,500 francs ($30) for a *P.t. schweinfurthii*, and 3,000 francs for a *Pan paniscus*. That was far more expensive than the 50 francs charged for a monkey but a lot less than the 25,000 francs to be paid for a white rhinoceros. In practice, only expatriates could afford to pay such a high tax for killing a chimp. However, the extent to which the regulation could be enforced in such a huge territory was an entirely different matter.[14]

In French colonies of Africa, decrees issued in April 1930 and November 1947 prohibited the hunting of some species including chimpanzees. Sanctions for offenders included fines from 50 to 2,000 francs, confiscation of firearms or jail terms from six days to six months. The French colonial administrations did not have either the human resources or the will to enforce such regulations in remote, self-subsistent, communities but these decrees made it more difficult for hunters to sell chimpanzee meat openly in the markets of small provincial towns or in the workers' camps of private companies established in rural areas for logging or agriculture.[15]

Furthermore, in several ethnic groups of central Africa there were traditional cultural taboos against the consumption of chimpanzee meat because of their similarity to humans. For instance, among the Bayombe of the DRC and the Bakota of Gabon, eating apes is culturally prohibited for fear that women will give birth to apes, or to children with a simian face. In the Equateur region of the Belgian Congo, the bonobo was considered a human which had been transformed in the distant past.[6,16–18]

Thus, apart from pygmies, who lived in the forests, hunted daily and were not too concerned about government regulations (but were limited by their lack of firearms), the above factors tended to limit the number of natives who might potentially handle chimpanzee carcasses and acquire SIV_{cpz} from scratches or cuts on their hands. This may have changed in recent decades, as human populations increased and moved deeper into forested areas, using the dirt roads built by logging companies.

Quantifying the exposure

Now we will try to estimate the number of individuals who could have been occupationally infected with SIV_{cpz} in the 1920s. We will need to review findings from several studies which we will then assemble.

A few years ago, researchers visited remote villages in the Cameroonian rain forest and identified people who reported having had contacts (bites, scratches, wounds or other injuries) with animals at any time in their lives. This mostly involved a contact with small monkeys or non-primate animals, from rats to leopards and elephants. Twenty-nine individuals reported contact with gorillas or chimpanzees, up to fifty-three years earlier, and some had the scars to prove it. Antibodies against SFV, an innocuous retrovirus highly prevalent among apes and monkeys, were more common among villagers exposed to apes than those exposed to monkeys, presumably because the wounds, bites or scratches inflicted by the former were more severe.[1]

The exposure to apes was better quantified in seventeen other Cameroonian villages, where almost 4,000 adults were interviewed. A large majority of the exposures to primates involved monkeys rather than apes. Hunting was limited to men, 10% and 12% of which reported having hunted gorillas and chimpanzees respectively. Similar proportions of men and women reported having butchered these apes at least once in their lives. However, when asked about direct contact with primate blood or saliva (scratches, bites or other injuries) during hunting and butchering, only four men reported such exposures to chimpanzees, and seven to gorillas. No woman did so. In other words, only 0.1% of adults reported having had at least one direct contact with chimpanzee blood or body fluids, and 0.2% with gorillas.[19]

This enables us to estimate roughly the number of individuals living in the relevant parts of central Africa around 1921 who might have had

at least one contact with SIV_{cpz}-containing chimpanzee blood at some point in their lives. Let us assume that no exposure occurred among children under sixteen, that only exposure to chimpanzees was relevant (the prevalence of SIV_{gor} infection in gorillas is lower and its relevance to human transmission unclear) and that the frequency of lifetime exposure to chimpanzee blood among inhabitants of areas populated by *P.t. troglodytes* was the same in 1921 as in the recent past.

Around 1930, when reliable censuses became available, about 2.3 million persons lived in the areas inhabited by *P.t. troglodytes*: 900,000 in Cameroon south of the Sanaga River, 387,000 in Gabon, 664,000 throughout Moyen-Congo, 120,000 in continental Equatorial Guinea, 130,000 in the south-west of the Central African Republic, and at most 100,000 in the Cabinda enclave and the adjacent Mayombe area of the Belgian Congo. At the time, the natural growth of central African populations each year (the difference between the birth and death rates) was 0.6%, so we can estimate that the relevant populations in 1921 were around 2,177,000. Of all inhabitants, 62% were aged sixteen years or over (the population was older than today because of the high child mortality). If we multiply 2,177,000 by 62% by 0.1%, it can be estimated that 1,350 adults living in 1921 had been exposed to chimpanzee blood at least once in their lives.

Of course there are many sources of error: inaccuracies in the censuses, lower exposure to chimps in the 5% of the population that was truly urban, no exposure in parts of Moyen-Congo where there were no *P.t. troglodytes*, exposures among adolescents, recall biases in the estimate of the proportion exposed to chimpanzee blood, etc. However, this provides us with an order of magnitude of the number of individuals potentially exposed to SIV_{cpz}. This number must now be multiplied by the percentage of chimpanzees infected with SIV_{cpz} and by the probability of transmission during each exposure, if the source chimpanzee was indeed infected with SIV_{cpz}.

We will assume that the SIV_{cpz} prevalence in 1921 among *P.t. troglodytes* communities of central Africa was similar to what it is today. In Cameroon, 5.9% of wild *P.t. troglodytes* are infected with SIV_{cpz}. Thus, of the 1,350 adults exposed to chimpanzee blood, about 80 were exposed to a SIV_{cpz}-infected ape. Then how many of these 80 individuals did acquire SIV_{cpz}? This depends on the probability of transmission during each exposure.[20]

This probability must have varied according to the degree of viraemia, i.e. the quantity of SIV_{cpz} present in the ape's blood. The higher the

viraemia, the more infectious a given amount of blood was. In humans, the degree of HIV-1 viraemia increases markedly as the disease progresses towards AIDS. Although it was initially assumed that SIV_{cpz} is not pathogenic to chimpanzees, there is now much evidence supporting the opposite view. Experimental HIV-1 infection of chimpanzees can lead to rapid loss of their CD4 lymphocytes and full-blown AIDS with opportunistic infections. More recently, it was proven that SIV_{cpz} was pathogenic in wild *P.t. schweinfurthii*. In the Gombe reserve, ninety-four chimpanzees habituated to human contact were followed for nine years. Analyses of their stools showed which chimpanzees were infected with SIV_{cpz}. Over this period, the mortality rate in the seventeen chimpanzees infected with SIV_{cpz} was ten times higher than that among the non-infected animals. Although based on a limited number of observations, this relative mortality was similar to what happens in humans infected with HIV-1. An autopsy, done on three of the infected chimpanzees, revealed a reduction in the number of their CD4 lymphocytes. In one case, less than three years had elapsed between the infection with SIV_{cpz} and death. This suggested that SIV_{cpz} infection in chimpanzees could lead to immunodeficiency and high viraemia, which would make these animals more infectious, not just to other chimps, but to humans as well. It remains unknown whether SIV_{cpz} is as pathogenic in *P.t. troglodytes* as in their cousin *P.t. schweinfurthii*.[21–28]

So how much virus is there in their blood? In an early study using insensitive methods, our friend Noah, naturally infected with $SIV_{cpz\text{-}ant}$, was found to be viremic at a low level. Using the blood of Noah injected IV, a second chimp, Ch-Ni, was experimentally infected with $SIV_{cpz\text{-}ant}$. Very much like humans who develop acute HIV infection, Ch-Ni developed very high viraemia (5×10^6 viral copies/ml), which declined markedly in the ensuing months, eventually reaching levels similar to those of Noah. A few years later, using modern viral quantification methods, Noah was shown to have persistent and rather high viraemia (10^5 copies/ml). Ch-Ni maintained stable but lower viraemia (10^4 copies/ml). In humans, such levels are found at an advanced stage of HIV-1 infection, with a diagnosis of AIDS. Higher HIV-1 levels (10^5–10^7 copies/ml) are seen for a few weeks during acute infection and much later as a pre-terminal condition in untreated patients or in those whose virus has become resistant to all available therapies. To summarise, we can assume that in chimpanzees the degree of viraemia, and thus the potential for blood-borne transmission, is similar to that in humans.[23,29–31]

To estimate the frequency of SIV_{cpz} transmission when someone is exposed to chimpanzee blood, we can extrapolate from studies among healthcare workers exposed to blood from an HIV-1-infected patient, which correspond fairly well to what we are interested in here: the risk of transmission after a single accidental exposure through the skin. Since the mid-1990s, healthcare workers exposed to HIV-1 have been given preventive antiretroviral drugs, which decrease the probability of transmission by 80%. Before such prophylactic methods became widely used, the overall risk of transmission was 0.3% per exposure, but was modulated by factors reflecting the quantity of viruses inoculated during the injury. Transmission was much less common (0.03%) for exposures that involved only non-penetrating contact (say, a splash) between blood and mucous membranes (the mouth or eyes) or non-intact skin. The risk when blood came into contact with intact skin was near zero. Conversely, with deeper injuries, or accidents during which a larger quantity of blood was inoculated, the risk of transmission could be as high as 25%, especially when the incident involved a patient with terminal illness.[32,33]

When a hunter or cook was exposed to chimpanzee blood containing SIV_{cpz} in the bush of central Africa, some exposures would have been similar to those of healthcare workers, while others were far worse, for example a deep injury with a knife used for butchering or a traumatic wound inflicted by a struggling chimpanzee. Overall, the risk of transmission was probably higher than among healthcare workers sustaining needle pricks, because the injuries were more severe. As an educated guess, let us say that it was between 1 and 3%, that is, up to ten times more frequent than in occupationally exposed healthcare workers.

We had calculated that eighty adults living in central Africa in 1921 had been exposed to blood containing SIV_{cpz} while handling chimpanzee carcasses or hunting. With 1% transmission, the result is one human infected from chimps, and two or three if the risk of transmission per exposure was closer to 3%. Obviously, there are several sources of errors so these estimates cannot be taken at face value, but the bottom line is that the number of persons infected naturally with SIV_{cpz} around 1921 must have been small, and almost certainly less than ten.

We know that over several decades a cross-species transmission occurred at least four times for HIV-1, i.e. once for each of groups M, N, O and P, each of which is thought to reflect distinct cross-species transmission events rather than evolution within humans. It occurred at

least eight times for each of the different groups of SIV_{smm}, which became HIV-2, as we will see later. Thus, the epidemic of HIV-1 group M was triggered not because a lot of humans were infected directly from chimpanzees but because a rare case of infection managed to spread and multiply, something which all the others that preceded it had not managed to do. In the following chapters, we will try to understand why this time the subsequent human-to-human transmission was so effective. But apart from the occupational infections of hunters or cooks, could other modes of transmission have been responsible for the first case(s) of SIV_{cpz} transmission from chimpanzee to man? We will now review three such hypotheses.

The river

Oral poliovirus vaccines (OPV) distributed in the 1950s and early 1960s were massively contaminated with a simian virus (simian vacuolating virus 40), which originated from the macaque cells used to grow the vaccinal virus. Fortunately, this virus was not pathogenic for humans. In an article published in *Rolling Stone* in 1992, journalist Tom Curtis proposed the theory that HIV-1 came from the contamination of OPV with SIV from African green monkeys. Of course, it was later shown that African green monkeys were not the source of HIV-1, as their own SIVs were too different from HIV-1. Then in 1999, Edward Hooper published a book entitled *The river. A journey back to the source of HIV and AIDS*, which focused on the theory that chimpanzee cells had been used to produce an experimental oral polio vaccine called CHAT, developed by Hilary Koprowski at the Wistar Institute in Philadelphia. Koprowski had collaborated with Belgian scientists from the Stanleyville public health laboratory in the Belgian Congo. Clinical trials of the CHAT vaccine were conducted between 1957 and 1960 in the vicinity of Stanleyville, in the capital Léopoldville, as well as in the Ruzizi valley of Ruanda–Urundi. Eventually, the Koprowski vaccine proved inferior to the other OPV developed by his competitor Albert Sabin, and CHAT was never commercialised.[34]

The Stanleyville laboratory set up a colony of chimpanzees (presumably, all *P.t. schweinfurthii* or *Pan paniscus*) nearby, at a place called Camp Lindi, primarily to verify experimentally not just the efficacy but also the neurological virulence of the CHAT vaccine. Experimental oral polio vaccines were based on live viruses which had been empirically

attenuated through repeated passages over various cell cultures, and scientists were worried that these viruses could eventually regain their original virulence, in which case the vaccine would cause the disease rather than prevent it. Camp Lindi chimps were given the CHAT vaccine, then the wild polio virus, and monitored clinically for neurological deficits. Others were sacrificed for their spinal cord to be examined after intraspinal injection of the CHAT virus. In addition, about thirty chimpanzees were used for hepatitis research, in an attempt to identify the agents of viral hepatitis through inoculation of stool suspensions from human patients.

The main theory in *The river* was that the Stanleyville laboratory had supplied the Wistar Institute with chimpanzee tissues obtained from sacrificed animals, which had then been used in Philadelphia to produce the CHAT vaccine so that SIV_{cpz} had been introduced into some vaccine lots, which were then sent back to the Congo where their oral administration in the late 1950s started the pandemic. A number of elements described in the book itself made this theory implausible, most importantly the fact that Koprowski had conducted very large trials of the same CHAT vaccine in his native Poland, where HIV-1 did not emerge. It would have been an extraordinary coincidence for SIV_{cpz}-contaminated vaccines to be re-exported from Philadelphia only to the very country where, allegedly, the SIV_{cpz}-infected chimpanzee tissues had been obtained. Then Hooper changed his hypothesis somewhat, and suggested that the Stanleyville laboratory had produced batches of the vaccine locally, using cells from locally procured chimpanzees, which led to contamination with SIV_{cpz} in Stanleyville rather than in Philadelphia.

Many circumstantial elements argued against either version of the hypothesis. There is no documentary evidence that chimpanzee cells were ever used, anywhere in the world, to produce OPV. Scientists had easy access to small monkeys of the *Macaca* genus, which were abundant in Asia, cheaper and easier to handle than chimpanzees, raised fewer ethical issues and worked well in cell culture systems. In 1955, up to 200,000 rhesus monkeys were imported into the US for medical research. Furthermore, as noted by the late Dr Paul Osterrieth, a scientist who worked at the Stanleyville laboratory for a few years, it was technically impossible for this rather basic facility to produce any kind of novel viral vaccine. The laboratory just did not have the human and material resources for such an endeavour. The annual reports of the

Stanleyville laboratory for the crucial years never mentioned any local production of OPV, something that, had they achieved it, the laboratory workers would certainly have been proud of. Neither did the reports of the colony's health system mention any local production of OPV – while the same reports provided much information about other vaccines produced by the network of public health laboratories of the Belgian Congo. Hooper seems to have confused *conditionnement*, which meant local dilution of concentrated frozen vaccine stock or its distribution from a large container to smaller containers, with local production or amplification of the vaccine strain.[35–37]

Nevertheless, to test this hypothesis once and for all, old vials containing the CHAT vaccine, which had been kept frozen for decades, were located. Some came from the Wistar Institute, where CHAT had been developed and produced, and conspiracy theorists could argue that this institution had a vested interest in supplying vials which they already knew were not contaminated with HIV-1. But other CHAT vials were fished out of freezers at the CDC in Atlanta and at Britain's National Institute for Biological Standards and Control (NIBSC). Some vials contained the very batches (10A-11 and 13) that had been used in the Belgian colonies. Samples were tested by several institutions, including the NIBSC, the Institut Pasteur, the Max Planck Institute, the Karolinska Institute, the New York University School of Medicine and Roche Molecular Systems. All reached the same conclusions: there was no HIV or SIV nucleic acids in these vials; there was no chimpanzee DNA, only DNA from rhesus (*Macaca mulatta*) or cynomolgus (*Macaca fascicularis*) monkeys and, in one batch which had been grown on human diploid cells, *Homo sapiens* DNA; and there was poliovirus, which meant that there had not been extensive degradation of viral nucleic acids over the very long storage period.[38–40]

Furthermore, 131 faecal samples were collected from chimps in the forested areas around Kisangani (ex-Stanleyville), where the laboratory and chimpanzee colony had been located: only one was SIV-positive. This virus, obtained from a *P.t. schweinfurthii*, was called $SIV_{cpz\text{-}DRC1}$. In phylogenetic trees, it was close to the *P.t. schweinfurthii* viruses obtained from Uganda and Tanzania and with Noah's $SIV_{cpz\text{-}ant}$, and clearly distinct from all *P.t. troglodytes* SIVs.[41]

Therefore, there is no evidence that chimpanzee cells were used in the Stanleyville laboratory to prepare batches of OPV vaccines, or that any OPV was produced there. And even if chimpanzee cells had been used in Stanleyville, they could not have contained the *P.t. troglodytes* SIV_{cpz} that triggered the pandemic. There is no evidence either of any retrovirus having been present in the old batches of CHAT available for testing. Furthermore, even when taking into account the margin of error on these estimates, SIV_{cpz} emerged in human populations at least twenty-five years before the CHAT trials. This theory can be firmly rejected.

Bold experiments

Because of their similarity to humans, chimpanzees have been used for almost a century as animal models of many infectious diseases, to prove that a putative pathogen is the cause of a given disease, to evaluate new vaccines, etc. In the course of these experiments, chimpanzees have been injected with various amounts of human blood or other types of human specimens, containing a wide diversity of infectious agents: viruses (HIV-1 of course, poliomyelitis virus, hepatitis B virus (HBV), hepatitis C virus (HCV) and yellow fever virus), prions (proteins causing kuru, Creutzfeldt-Jakob disease and scrapie), bacteria (the aetiological agents of tuberculosis, leprosy, gonorrhoea and trachoma) and parasites (causing malaria and the Guinea worm). There was even a chimpanzee model of alcoholism and addiction to narcotics!

However, it is rather extraordinary that, on several occasions, the reverse was done: the IV injections of chimpanzee blood in humans. The first such experiment was performed in Sierra Leone by Donald Blacklock and Saul Adler, who injected two Europeans (presumably, themselves) subcutaneously (SC) and intravenously with small quantities of blood from a chimpanzee infected with malaria parasites. They did not develop malaria. We do not know what happened next but they certainly got worried when the donor chimpanzee died a few days later, with the autopsy showing a disseminated *Strongyloides* infection, with this intestinal worm being present in the chimp's bloodstream! Shortly thereafter, at the Institut Pasteur in Paris, a man was injected IV with 40 cc of chimpanzee blood as part of a comparative study of blood groups of chimpanzees and humans. The volunteer apparently tolerated the procedure well. With great foresight, the Pasteur scientist noted that such experiments should be avoided in the future, 'to keep off potentially transmitting to humans hitherto unknown infectious pathogens of chimpanzees'![42–45]

This advice seems to have fallen on deaf ears. Renowned parasitologist Jérôme Rodhain, director of the Tropical Medicine Institute in Brussels and then in Antwerp, studied whether malaria parasites of primates were transmissible to man. As a secondary interest, or perhaps as a moral justification, he also investigated whether fever induced by malaria could have a beneficial effect on patients with late-stage syphilis. Such work would be unthinkable today but in those days there were no ethics committees and each scientist could decide whether an experiment was morally acceptable. Similar investigations were conducted in other parts of the world, especially India, with inoculation to humans of blood obtained from Asian apes and monkeys.[46]

Rodhain carried out a series of experiments in which chimpanzee blood (5–10 cc) was injected IV in humans, most of them patients with syphilitic dementia. The chimpanzees (Thomas, Suzanne, Simone ...) originated from the Belgian Congo; although Rodhain alluded to them as *P.t. verus*, in retrospect they were probably *P.t. schweinfurthii*. Between 1938 and 1940, Rodhain injected chimpanzee blood to twenty-six patients. He did prove that some of these malaria parasites were infectious to humans, and that the parasite named by others *Plasmodium rodhaini*, in his honour, was in fact *Plasmodium malariae*. We should give him a lot of credit for being humble since he himself killed the chance of his name going down in history as the name of a species. Rodhain conducted similar experimentations with other species of malaria parasites, most notably *Plasmodium reichenowi*, a parasite of chimpanzees and gorillas. In 1954–5, he injected chimpanzee blood in four more psychiatric patients, and managed to transmit *Plasmodium schwetzi*.[47–52]

These experiments were done in hospitals in Antwerp and clearly could not have led to the emergence of HIV-1 in Africa. Furthermore, as the apes originated from the Belgian Congo, they were all presumably *Pan troglodytes schweinfurthii* (Rodhain would not have confused *P.t. verus* with *Pan paniscus*, the other ape potentially available from his Belgian collaborators). It seems unlikely that other scientists carried out similar experiments for malaria research, since Rodhain would have mentioned this in the detailed reviews of the subject that he wrote in the discussion section of his own papers. Of course, one could hypothesise that similar experiments were carried out in the nascent research institutions in Africa and the results never disseminated, but this would be pure speculation. There is no evidence of similar experiments being

conducted in the Léopoldville research laboratory, where Rodhain had worked during the early part of his career and which he continued to visit intermittently from his academic posts in Belgium.

At the Institut Pasteur in Paris, Auguste Pettit worked for sixteen years on developing a therapeutic serum containing high titers of antibodies against the poliomyelitis virus, to be given to patients with this disease. As it was difficult to obtain large enough quantities of serum from human cases recuperating from polio, he produced animal sera, which were then used on humans. Antipoliomyelitis serum was prepared from horses, monkeys and chimpanzees, and then administered to at least eighty patients. Two chimpanzees were used for this purpose; the first one was bled to death and the second was bled repeatedly over a two-and-a-half-year period. They were *P.t. verus* chimps from Guinea, and the patients were in France not Africa. So once again, this could not have led to the emergence of HIV-1.[53]

Eternal youth

Among French surgeons of the early twentieth century, Serge Voronoff had a most unusual résumé. Born in Russia, he emigrated to France as a teenager, changed his name to conceal his Judaism, got a medical degree in Paris in 1894, trained as a surgeon and worked in Egypt for fourteen years for the Khedive, the local monarch. There, according to his biographer, he was struck by the short life span of eunuchs among the Khedive's servants. His return to France in 1910 coincided with the emergence of a new speciality, endocrinology: the science of hormones. Hormones are secreted by some gland, transported via the bloodstream and have their main effect on distant organs. At the time, there was no hormone replacement therapy, neither for hypothyroidism, nor for diabetes, pituitary insufficiency and so on.[54]

Voronoff was given a chair of experimental surgery at the Collège de France in Paris, something to be taken with a pinch of salt since the Collège did not have a medical school. He did not choose the easiest route for treating patients with endocrine dysfunction. While the obvious solution (soon to be available) was to find animal sources of the defective hormone, concentrate it and administer the product to patients orally or by injections, Voronoff attempted to tackle their problems by transplanting animal organs. He trained for a few months

in the US under Alexis Carrel, soon to be awarded a Nobel for his pioneering work on organ transplants and vascular anastomoses.

Upon his return to Paris, Voronoff undertook experimental organ transplantations in animals, first within a given species and then between different species. In 1913, he performed his first xenotransplant (xeno- means foreign, in this case a foreign species), implanting a chimpanzee thyroid in a young man with congenital hypothyroidism. According to his surgeon, the patient improved. He performed a few more thyroid transplants, but this came to a stop when thyroid hormones were synthesised. During WWI, Voronoff grafted animal bones (in at least one case, from a chimpanzee) in soldiers with post-traumatic bone defects, but it was soon shown that in many cases bone could be obtained from the patient himself, at some other site.

Perhaps drawing on his earlier observations of eunuchs, Voronoff became convinced that the male hormones were necessary not only for sexual activity and phenotypes, but also for preserving various functions of the body, and especially the brain. Ageing was seen as a consequence of a degeneration of the testes. In his laboratory, he attempted to transplant testes from young animals to their older counterparts. Eventually, in 1920 this culminated in his first interventions on two humans, one of whom received the right and the other the left testes of the same baboon. These did not work well, and Voronoff attributed the failure to the use of organs from a species too different from humans. He decided to use chimpanzees instead.

Over the next two years, Voronoff performed twelve testicular transplants, from chimpanzee to man. It was not possible at the time to perform microvascular surgery, and Voronoff thought he could avoid the necrosis of the transplanted organ by actually grafting thin slices, with the assumption that small new blood vessels would form to re-vascularise the organ. That was very optimistic. Furthermore, nobody at the time had any understanding of the immunological rejection of a transplanted organ, presumably very severe when that organ was not of human origin. To maximise the use of the precious organs, each patient received half a testicle; from one chimpanzee donor four transplantations could be conducted. Finding chimpanzees was difficult. A Catholic order sent him a few dozen from Guinea. Through the Ministry of Colonies, he tried to arrange the shipment of chimps from Gabon but apparently none survived the journey. French newspapers reported that entrepreneurs in the Congo had sensed the potential for a quick profit so

that the price of a chimpanzee had increased ten-fold on the local market, but it is unclear whether any of these animals made it to the old continent. To maintain his supply, Voronoff visited Guinea, Senegal and Mali in the mid-1920s.[55–57]

Like many experimental surgeons, Voronoff claimed that his procedure was successful in more than half his patients. It is hard to say whether this claim was the result of unwarranted enthusiasm, the lack of objective measures, the placebo effect of surgery, data manipulation for commercial reasons or perhaps even a genuine effect. Despite the necrosis and immunological rejection, it is possible that for a few weeks, as the transplanted organ was disposed of by the recipient body's defence mechanisms, there was some absorption of the huge quantities of hormones present in the graft, some of which were not too dissimilar from the human ones.

Even if Voronoff might have had sincere scientific aims initially, he quickly realised the immense commercial potential of the procedure. Here was a renowned surgeon, holding a chair at the Collège de France, who had invented a procedure that could act not only as a surgical Viagra but prolong life and enhance quality of life for decades. Many rich and old men were willing to pay a fortune for a shot at eternal youth. Voronoff even travelled to India in 1929 to perform a testicular transplant on a maharajah. Although some of his fortune may have been inherited from his second wife, Voronoff certainly made a lot of money out of this surgical adventure, allowing him to spend the last three decades of his life in a fancy villa on the Italian Riviera, where he had set up a chimpanzee breeding colony, probably as a public relations ploy.

Such a lucrative business interested colleagues in France and overseas, and Voronoff's biographer estimated that about 2,000 testicular transplants were performed in Paris, Bordeaux, Nice, Lille, Alger, London, Rome, Turin, Milan, Genoa, Vienna, Madrid, Lisbon, Porto, Berlin, Alexandria, Constantinople, Chicago, New York, San Francisco, Buenos Aires, Valparaiso, Rio and even in Hanoi. Voronoff himself claimed to have performed 475 transplants. Eventually, the procedure was completely discredited for a number of reasons, including the fact that Voronoff had started performing ovarian transplants (inserting a chimpanzee ovary into a woman and vice versa), which raised extremely serious ethical concerns (could one of these females become pregnant with a half-chimp, half-human baby?). Voronoff became the butt of

popular humour in France and abroad. He died in 1951, aged eighty-five. We do not know whether he believed in the procedure strongly enough to have it performed on him, but he did transplant one of his older brothers, who came all the way from Russia for the surgery.[54;57]

So to get back to the question, could some of these chimpanzee organs have contained SIV_{cpz}, which may have infected the recipient, starting a chain of transmission? That seems unlikely for a number of reasons. First, all of the procedures seem to have been performed in countries unrelated to the emergence of HIV-1. Second, most of the recipients were elderly men, who were unlikely to have infected many sexual partners, even if re-invigorated by the procedure. Third, at least according to what was documented, Voronoff's chimps came from West Africa, thus were *P.t. verus*, a subspecies not infected with SIV_{cpz}. Fourth, it appears that in some of these procedures, the profit-seeking surgeons used testes from monkeys rather than apes due to a shortage of the latter. Therefore, this bizarre scenario can also be rejected.

In summary, there is now a reasonable body of evidence suggesting that the initial intuition of early researchers, the cut hunter theory, was correct and there is no alternative hypothesis that can be supported after a careful examination of the facts. Such occasional cases of cross-species transmission must have been occurring for hundreds of years, as humans and *P.t. troglodytes* chimpanzees have coexisted in the forests of central Africa for many generations. For a long time these ancient cases of SIV_{cpz} becoming HIV-1 did not manage to disseminate successfully. What happened in the early twentieth century for HIV-1 to spread successfully into human populations, while HIV-2 managed the same feat, albeit on a much more limited scale, in West Africa? We will start by briefly reviewing in the next chapter the history of European colonialism in central Africa, and how it created conditions propitious to the emergence of HIV.

5 Societies in transition

This background chapter aims to describe the settings in which the rest of the story took place. Africans understandably resent and reject as arrogant, or at least Eurocentric, historical accounts of their continent which consider the European penetration as the starting point and describe this process as discovery rather than what it really was: a military conquest for the purpose of economic exploitation. However, since the events relevant to the emergence of HIV-1 occurred during the colonial occupation of central Africa, and were facilitated by the profound social and economic changes brought about by colonisation, especially around the pool on the Congo River, we will focus on this period, but after a short detour which will enable us to examine how history confirms the molecular clocks of Chapter 3.

The slave trade and the exportation of infectious diseases to the Americas

The arrival of the Bantus in central Africa is, on the scale of human history, relatively recent, having occurred about 2,000 years ago, when migrants from around Lake Tchad managed to dominate the truly indigenous pygmy populations and for the first time introduced various forms of agriculture. In some areas, organisation was limited to small tribes that occupied geographically limited territories. Elsewhere, kingdoms were established, such as the Kongo kingdom, a loose confederation of tribes which corresponded to parts of current day Congo-Brazzaville, DRC, Angola and Gabon. These societies were not technologically advanced, which made it easy for Europeans to conquer the heartland of Africa once they found solutions to the health problems (mostly malaria) that decimated their early soldiers and settlers, many of whom died within two years of their arrival. But central African people had strong values, beliefs and traditions centred on the extended family, the clan. And there was