CHAPTER 2

The Source

So, HIV-1 originated in central Africa. But why, one may ask, central Africa? The answer, as we will see, is because this region is the habitat of the simian source of the virus. As an introduction to the wonderful world of chimpanzees, let us briefly review the career of the British primatologist Jane Goodall, some of whom's findings will illuminate the rest of our story.

A VIEW FROM GOMBE

Valerie Jane Morris-Goodall was born in London in 1934. Her father was a race car driver and military man, who was rarely at home and then asked for a divorce in 1950. Thus, Jane was raised by her mother 'Vanne', grandmother and aunt in a female-only household in the coastal town of Bournemouth. This was a significant advantage, since no one ever tried to tell her that there are certain things that women shouldn't do. Despite the war and her father's absence, she seems to have had quite a happy, if rather solitary, childhood. Like many children, she was interested in animals from an early age, but unlike most children, she decided and managed to devote her life to them.[1,2]

An average student with little interest in the usual academic subjects, and without any financial support at a time when higher education for girls was the exception rather than the rule, Jane went to secretarial college after secondary school. Her first few jobs included one at the London Zoo. In 1957 she had a stroke of luck when a childhood friend invited her to visit her in Kenya, which was still a British colony. Once

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there, after some other jobs, she had a second stroke of luck: she was hired as a secretary by Louis Leakey, an internationally renowned paleoanthropologist and director of a museum in Nairobi. Leakey and his second wife, Mary, had spent twenty-five years looking for fossils of early humans in the gorges of Olduvai in neighbouring Tanzania, and their discoveries would make them famous. Initially, the relationship between Mary and Jane was fraught; Louis had just ended an affair with his previous secretary, sending her off to study mountain gorillas in Uganda, close to the border with the Belgian Congo. He also had romance in mind when he hired Jane. He was to be disappointed in that respect, but was impressed by her patience and overriding interest in animals.

Louis Leakey was interested in apes and making connections and extrapolations between their behaviours and those of the first humans – their way of life, diet, and means of communication before language appeared. He didn't have much luck with gorillas as his former secretary only stayed in Uganda for a few months, and Dian Fossey, who he recruited many years later, came to a tragic end, assassinated in a Rwandan forest, a victim of her own abusive behaviour towards the local people. Leakey had more luck with chimpanzees and Jane Goodall. After identifying the 52 km^2 Gombe nature reserve on the banks of Lake Tanganyka as a study site, Leakey went looking for funding. Despite his reputation, his first grant applications were rejected, partly because his desire to send his secretary to work on the project seemed dubious from a scientific viewpoint.

He eventually succeeded in camouflaging this small detail, and secretly offered the position to another woman with a degree in anthropology, who turned it down. Initial support for the study came first from a small foundation, then the National Geographic Society, which had deep pockets thanks to its magazine sales. The Kigoma district commissioner refused to let a young white woman live alone in this isolated place, which was also potentially dangerous given the events unfolding in the Belgian Congo on the other side of the lake. The problem was solved by having Jane's mother accompany her for a few months, starting in July 1960. Vanne took care of the logistics and a small dispensary while Jane went into the forest to look for the chimpanzees, numbering about

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160 animals in all. At first they were difficult to find since the chimpanzees obviously didn't know they were in a nature reserve where, at least in principle, humans weren't allowed to shoot them.

Prior to that, most studies of chimpanzee behaviour had been on captive animals in zoos and research centres. Only one scientist had tried to observe them in their natural habitat but, unlike Jane, he didn't have the time or the patience and only caught furtive glimpses of his subjects. Goodall, on the other hand, was willing to spend months, and eventually years, in the forest, sometimes accompanied by a tracker but often alone, gradually getting closer and closer until her presence was considered normal. She came to recognise individual animals and gave them names. She called the most sociable one David Greybeard. All this time, Louis Leakey continued to seek funding for this observational study, which still continues today, almost five decades after his death.

Quite soon, Leakey decided that Jane should get a university degree to give more credence to her observations and make it easier to get grants. He must have had excellent contacts in British academia because he achieved the impossible: he managed to get Jane directly into a PhD programme at Cambridge University. Her PhD in ethology (the study of animal behaviour) would be her first university degree, which gave her the opportunity to spread her wings and become the principal investigator on the grant applications. The matter was resolved in three years, half of which was spent in the field collecting additional data.

The most interesting thing about Jane Goodall's early work is that she spent enough time observing chimpanzee communities in the Gombe Reserve to realise that, like humans, they had their own personalities. Some chimpanzees are gentle, others 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 don't. This marked individualism and their ability to laugh and even dance in the rain are perhaps what makes 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.

Over the years, Goodall collected a large number of observations on the sexual behaviour of chimpanzees. Most of this 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. No preliminaries, and as many as six different males may copulate with the same female in just ten minutes. Obviously this type of promiscuity favours the transmission of microbes present in their genital secretions. Some males will 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. However, this usually only lasts for a week or two, during which they copulate as often as five times a day. Then, back to the usual behaviour.

Jane Goodall documented for the first time that chimpanzees are able to develop and use tools, mostly sticks to procure food (for instance, to dig out ants or termites or to extract honey from hives). More germane to our story, as we shall see later, is that the Gombe study showed that, unlike gorillas, chimpanzees are omnivorous. In addition to fruits, leaves, insects, eggs and all kinds of plants, they sometimes hunt and eat vertebrates, including monkeys, antelopes and small warthogs. Primatologists even observed instances of cannibalism at Gombe, where a female and two of her offspring ate other baby chimpanzees, but this may have been aberrant or at least quite rare behaviour.

Like humans, chimpanzee communities are occasionally stricken by epidemics. In Gombe in 1966, during an outbreak in the region's human population, poliomyelitis caused four deaths and left some chimpanzees permanently paralysed. Respiratory infections (influenza and pneumonia in particular) followed, also with fatal consequences. This reflects not just the communal nature of life among the chimpanzees, who 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. This observation will be crucial for the rest of our story.

And like humans, chimpanzees sometimes engage in planned and organised violent behaviour. The Gombe team documented a war between two neighbouring communities which, after three years of attacks and killings, ended with the complete annihilation of the weaker troop. The myth of the 'noble savage' doesn't apply here.

In 1975, the kidnapping of four American student interns at Gombe by Congolese rebels led by Laurent-Desire Kabila, who was to bring down

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Mobutu more than two decades later, radically changed the structure of the research project. Goodall, who appeared to have little sympathy for the victims and their families, lost some of her gloss. Fortunately, the students were freed after a ransom was paid. This led to an Africanisation of the research at Gombe, which was a normal and desirable development in a newly independent country. While supervising the Gombe research work from afar, Jane Goodall became an activist who wanted to improve the living conditions of captive chimpanzees and to protect the species. She became part of the global environmental movement and would spend much of her time flying from place to place, speaking at fundraising meetings. She was now an icon.

Although the definitive biography of Jane Goodall is rather hagiographic, she clearly made a monumental contribution to knowledge about chimpanzees' behaviour and way of life, and showed patience, perseverance and courage (and perhaps some recklessness) in doing so, and this information will be very helpful in understanding early parts of the long journey of HIV. We will now review more systematically what is known about the distribution and behaviour of various species and subspecies of chimpanzees throughout tropical Africa.[1]

OUR CLOSEST RELATIVES

Chimpanzees are the closest relatives of humans, sharing 98–99% of their genome with us, and they are the most intelligent non-human animal. Chimpanzees and humans shared a common ancestor and diverged between four and six million years ago. In fact, chimpanzees are so close to humans that it was proposed to move them into the genus *Homo*, a somewhat perplexing idea. Much of our fascination with chimpanzees revolves around the question: what makes us human?

According to current taxonomy, there are two species: *Pan troglodytes*, the common chimpanzee, and *Pan paniscus*, the bonobo. Based on analyses of DNA, there are 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).

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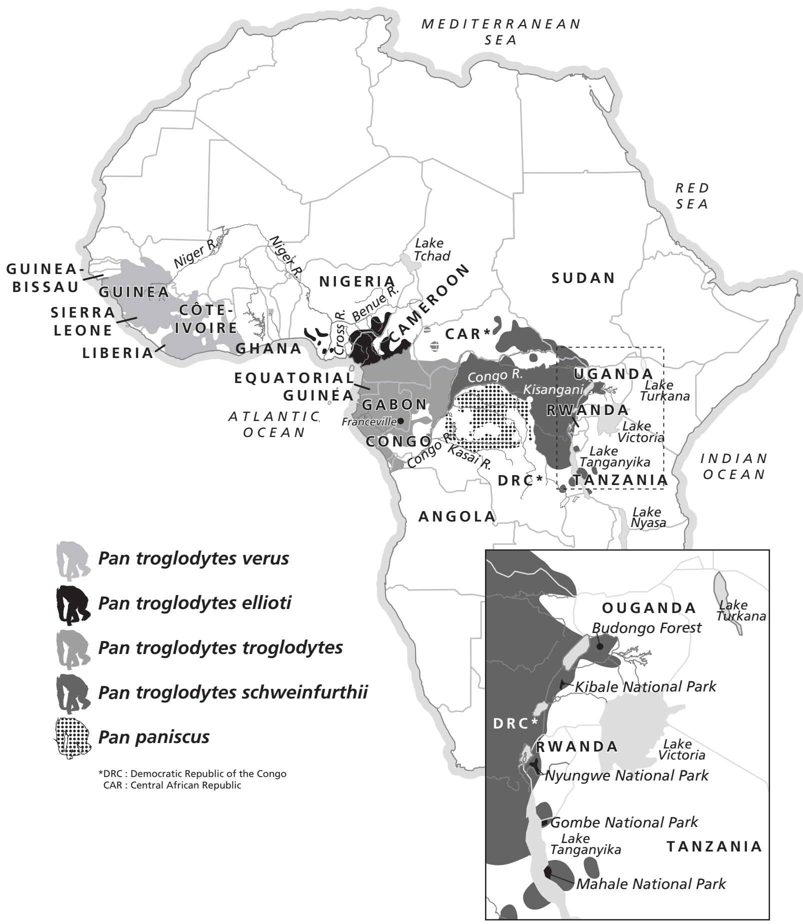
Chimpanzees are poor swimmers, so large rivers like the Cross, Sanaga, Ubangi and Congo became rather tight natural boundaries between the habitats of various species and subspecies, as can be seen in Map 2. *Pan troglodytes verus* (total population in 2016: 18,000–65,000 according to the International Union for Conservation of Nature (IUCN)) 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. *Pan troglodytes ellioti* (total population: fewer than 6,500) is found from east of the Cross to the Sanaga River in Cameroon, its southern boundary. *Pan troglodytes*



Map 2 Distribution of the four subspecies of *Pan troglodytes* and the *Pan paniscus* bonobo.

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schweinfurthii (total population: 181,000–256,000) inhabits mostly the DRC, east of the Ubangi and north of the Congo rivers, but its range extends into the Central African Republic, southern Sudan and eastwards to Uganda, Rwanda and Tanzania.[3]

Pan troglodytes troglodytes (total population: around 140,000) inhabits an area south of the Sanaga in Cameroon and extending eastward to the Ubangi 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 Congo-Brazzaville (40% of the total), Gabon (one-third) – where unfortunately they are rapidly declining – and Cameroon (one-fifth). Other countries have fewer than 2,000 each, with fewer than 200 in the DRC.[3]

Chimpanzee populations in the first half of the twentieth century were certainly higher than they are 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 were 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 to capture for medical experiments.[4–6]

The rest of this section focuses 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 15–160 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.

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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.[7–8]

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 territories. 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.

P.t. troglodytes chimps have low fertility: on average, 800 matings occur for each conception. During their reproductive years (age 14–40), 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. This demographic vulnerability explains why the IUCN consider chimpanzees to be a threatened species.[3,9]

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. Then, as shown by Goodall and others, chimpanzees are sexually promiscuous. 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.

ALL KINDS OF TREES

We will now examine how it gradually became clear that one subspecies of chimpanzee was the source of HIV-1. The focus in the next section is

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on molecular biology, but I decided not to skip over this part of the story, which explains the very beginning of the pandemic. Readers who find this topic daunting could go directly to the conclusion of this chapter.

Let's quickly review a science called phylogenetics. Phylogenetics uses nucleotide sequences (long lists of the letters A, C, G and T) to reconstruct the evolutionary history of various forms of life, including viruses. A 'phylogenetic tree' (as seen later in Figures 2.1 and 2.2) superficially resembles a genealogical tree. However, phylogenetic trees describe the relatedness between living organisms 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. In other words, we can identify brothers and cousins and establish, if applicable, the absence of family relationships between particular viruses.

Each division in the tree is the common ancestor of the organisms or isolates identified to its right. After branching, the organisms and their sequences evolve independently. The 'root' (at the extreme left) is the assumed common ancestor of all the organisms in the tree. To construct a phylogenetic tree, molecular biologists compare the differences in sequences of many isolates of putatively related organisms, 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 to either the initials of the patient, the name of the city or country where it was obtained, or whatever the researcher decides to call it (for instance, ZR59 for Zaire 1959 or DRC60 for Democratic Republic of the Congo 1960). 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 difference in their sequences, 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 difference. This is like brothers and sisters, born of the same mother and

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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. Or that two living organisms are not related in any way.

Phylogenetics, therefore, were used to look for animal viruses most closely related to human HIV – those with the most similar sequences – in order to determine the source of the pandemic. At this stage of the investigation, researchers were certain that HIV had appeared in humans sometime in the previous century, and that it must have come from an animal. Because of their genetic proximity to our own species and their obvious susceptibility to many of our pathogenic microbes, primates were the first suspects. Thus, the goal was to find the simian virus that was HIV's closest relative, its brother rather than its tenth cousin.

A BRAVE CONCLUSION

The first report of the isolation of a simian immunodeficiency virus (SIV) from a chimpanzee born in the wild came in 1989. This isolate, named $SIV_{cpz\text{-}gab1}$, was obtained from a chimpanzee kept at a research centre in Franceville in Gabon's equatorial forest, where fifty chimps were 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' (i.e. made to multiply) and then analysed. This chimpanzee, Amandine, came from an area near the border between Gabon and Cameroon, where she had been captured at six months of age after her mother had been slaughtered by a hunter. Amandine was four years old when the blood sample was obtained and seemed healthy despite presenting enlarged lymph nodes. Phylogenetic analyses suggested that $SIV_{cpz\text{-}gab1}$, Amandine's virus, was closer to HIV-1 than to HIV-2 and to SIVs from small monkeys. This was only a tentative start, but nonetheless a step in the right direction.[10–11]

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It was not possible to isolate the virus from the second seropositive chimp, a two-year-old animal shot by hunters that died of its wounds shortly after being brought to Franceville for care. He remained nameless. A few years later, thanks to technological advances, DNA amplification was used on this chimp's lymphocytes (which had been kept frozen) to sequence parts of the viral genome. This isolate became known as $SIV_{cpz\text{-}gab2.}$ It was close to $SIV_{cpz\text{-}gab1}$, as could have been expected, seeing that they both came from the same animal species living in the same area.[12]

In 1992, a third isolate ($SIV_{cpz\text{-}ant}$, this time Ant for Antwerp) 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. According to some – perhaps apocryphal – sources, he was presented to King Baudouin by Mobutu when the former visited Zaire in 1986, but the king did not want to keep the chimp at his chateau in Laeken, big as it was. Noah was two years old when he left his African birthplace. His isolate differed somewhat from HIV-1 and from the two previous SIV_{cpz} isolates.[13]

In 1999, a fourth isolate, $SIV_{cpz\text{-}US}$, was obtained from Marylin, caught in the wild and brought to the USA in 1963, aged four. Marilyn was probably Cameroonian, since she lived in a group of primates at an air force base in Alamogordo, New Mexico. A few years earlier, soldiers from the base had procured dozens of chimpanzees in Cameroon for NASA experiments designed to measure the tolerance of humanoids to extreme gravitational acceleration and explore the potentially harmful effects of exposure to weightlessness. Some unfortunate chimpanzees were even sacrificed to determine the acceleration, the number of Gs, that invariably cause death (237 G!). After a long training period, NASA blasted the first chimpanzee into space in January 1961 and a second 'chimponaut' a few months later. It was soon realised, however, that the astronauts tolerated these manoeuvres quite comfortably: the Soviets had just put Yuri Gagarin into orbit around the Earth without any apparent ill effects. The Alamogordo chimpanzees, now obsolete, were recycled into medical research. Marylin lived in a primate facility until she died in 1985 at the age of twenty-six, after delivering stillborn twins.[14–15]

In a survey of captive chimpanzees, Marilyn was the only one that was seropositive for HIV-1 antibodies. She had not been used in AIDS

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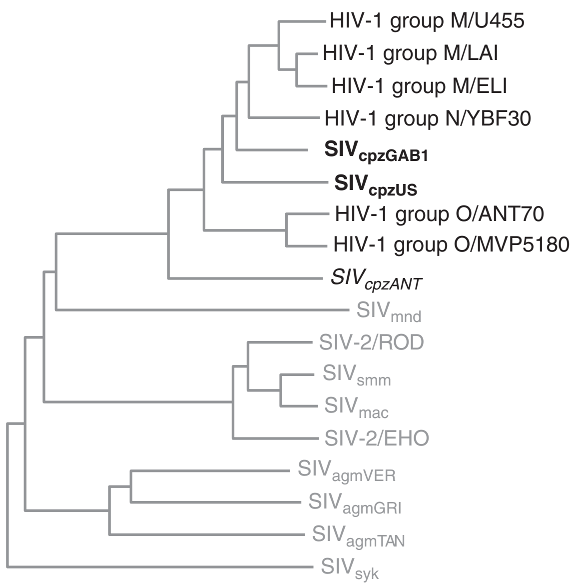
research, but had received human blood products between 1966 and 1969. During this early period, the blood products could not have contained HIV-1, so Marilyn had certainly acquired her SIV_{cpz} infection in Africa. SIV sequences were recovered from the spleen and lymph nodes procured at autopsy. Using DNA analyses, researchers identified the subspecies of chimpanzees from which this recent and the previous three isolates had been obtained.[16]

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 (from Gabon and probably, in Marilyn's case, from Cameroon) were *P.t. troglodytes*. As illustrated in Figure 2.1, 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 $SIV_{cpz\text{-}ant}$ differed and lay outside this cluster, as did HIV-2 and SIVs obtained from other non-human primates. To repeat the analogy used



2.1. Phylogenetic analysis showing the relationship between $SIV_{cpz\text{-}US}$ and $SIV_{cpz\text{-}gab1}$ and human isolates of HIV-1. The $SIV_{cpz\text{-}US}$ and $SIV_{cpz\text{-}gab1}$ isolates obtained from *P.t. troglodytes* chimpanzees (bold) 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.[17]

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earlier, HIV-1 from humans and SIV_{cpz} from *P.t. troglodytes* were similar, like brother and sister, while SIV_{cpz} from *P.t. schweinfurthii* was a third cousin. 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 evolution of SIV_{cpz} in chimpanzees resulting in *P.t. troglodytes* and *P.t. schweinfurthii* being infected with different lineages of SIV. Incidentally, note the power of these methods: this assertion concerning the origin of HIV was based on just four viral isolates, obtained from only four chimpanzees, but subsequently was proved right.[17]

Scientists could not rule-out 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 available: the one from Noah. 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*.

PRECIOUS FAECES

Since this initial work was conducted mostly with chimpanzees that had been in captivity for some time, there was some doubt about 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 solved, while in the second researchers had ventured down the wrong track.

Non-invasive technologies were developed to measure the presence of SIV 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. The ground underneath chimpanzee nests is apparently the best place to collect these precious stools. Urine samples proved inferior and were abandoned.

Among 100 wild *P.t. schweinfurthii* from Uganda and Tanzania, only one was infected with $SIV_{cpz\text{-}tan1}$ (tan for Tanzania). This isolate was

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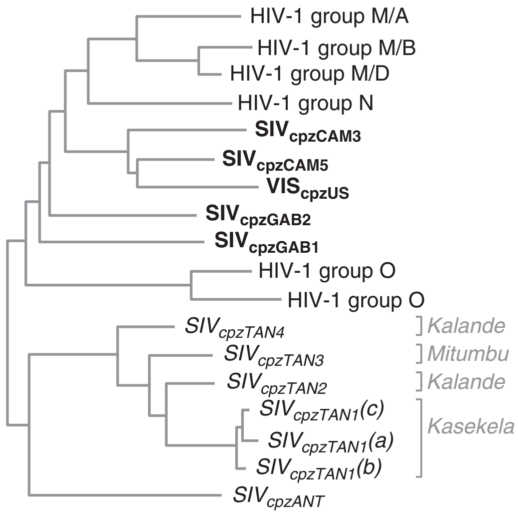

2.2. Phylogenetic analysis showing the relatively distant relationship between SIV_{cpz} isolates obtained from *P.t. schweinfurthii* chimpanzees and human isolates of HIV-1. SIV_{cpz} isolates obtained from *P.t. schweinfurthii* chimpanzees in Tanzania (italics) and $SIV_{cpz\text{-}ant}$ obtained from a *P.t. schweinfurthii* chimp in the DRC (italics) are 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).[19]

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 around 20%. These isolates clustered with $SIV_{cpz\text{-}ant}$, but diverged from the *P.t. troglodytes* isolates and from HIV-1 (Figure 2.2), confirming that *P.t. schweinfurthii* was not the source of HIV-1. In the DRC, an ambitious study involving forty sites showed that overall 15% of *P.t. schweinfurthii* were infected, with prevalences varying all the way from 0% to 100% between communities. For some unknown reason, this *P.t. schweinfurthii* SIV_{cpz} was not transmitted to humans – or, if it was, its subsequent propagation was ineffective and the virus disappeared from our species. Or perhaps it exists in humans but remained confined to a remote region of the DRC, where roads are impassable and seropositive patients never underwent sophisticated molecular biology tests. In fact, in these regions, the simian viruses were much better characterised than their human counterparts.[18–19]

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This heterogeneous distribution of SIV_{cpz} 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.

SIV is non-existent among captive *P.t. verus* (the western chimpanzee), about 1,500 of which were tested. 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. Presumably, some African rivers were easier to cross half a million years ago than they are today, allowing the two species and four subspecies of chimpanzees to evolve from a common ancestor, then populate different parts of the continent.[20]

Prevalence of SIV_{cpz} among wild populations of *P.t. troglodytes* was 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 molecularly fingerprint each and every individual ape who had defecated. After excluding degraded specimens and 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 in the highest it was 35%.[21]

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

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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, thanks to a multinational group led by molecular biologists Beatrice Hahn and Martine Peeters. An essential piece of the puzzle concerning the origins of HIV had just been decoded and put in the right place; it explains why in the previous chapter we were able to conclude that central Africa was the geographical birthplace of HIV-1. Quite simply because the animal source of human HIV-1 lived there.

Additional faecal samples from *P.t. troglodytes* were collected over the following years in Cameroon, where the prevalence of SIV_{cpz} infection is now estimated to be 6%. In Gabon, where overall prevalence is 26%, the same geographic variability was found. The virus is absent from the west and south of the country, but on the other side and especially in the north-east, going towards the border with Congo-Brazzaville, up to 55% of *P.t. troglodytes* are carriers. In the Central African Republic, as well as in the Mayombe region of the DRC, the small number of animals that could be tested were free of the virus.[22]

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 similarity 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. And, a crucial finding for our story, the SIV_{cpz} isolates from south-east Cameroon towards the border with Congo-Brazzaville and the Central African Republic were most closely related to what are presumed to be the ancestors of all HIV-1 group M strains, while those from south-central Cameroon were closer to HIV-1 group N.[23–25]

To support what we said at the end of the previous chapter, it is perfectly possible that 'patient zero', the first case that sparked the pandemic, was Cameroonian, even if the essence of the subsequent amplification of the virus occurred in another country, present-day DRC. In a later chapter, we will see how, even as far back as the days of German colonisation, trade from south-east Cameroon was conducted via the Congo River towards Léopoldville and Brazzaville. But since there were no isolates of SIV_{cpz} infecting *P.t. troglodytes* characterised from any country other than Cameroon and Gabon, it is not impossible that sequences still closer to HIV-1 could be found one day in one of these lands, as yet *terra incognita*, such as north of Congo-Brazzaville.

SIV infection was found among faeces from western gorillas (*Gorilla gorilla gorilla*), a virus that 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.[26–27]

Until proven otherwise, it is most likely that the modes of transmission of SIV_{cpz} between chimpanzees are the same as in humans: sex, from mother to child and possibly through blood–blood contacts. The efficiency of the transmission of SIV_{cpz} during sexual intercourse between chimpanzees appears to be similar to that measured with HIV-1 in humans, but the potential is magnified by the sexual promiscuity of these great apes. For instance, one adult male in Gombe is known to have mated since puberty with twenty-five 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 close-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.[28]

THE FOURTH APE

A weakness in the investigations of SIV among chimpanzees was the dearth of information about the fourth ape, *Pan paniscus*, the bonobo (the orangutan is the third one, found only in Asia and SIV-free). The bonobo used to be called the pygmy chimpanzee, but this was a misnomer since the difference in size compared to *Pan troglodytes* is trivial. In evolutionary terms, the bonobo diverged from *Pan troglodytes* a million years ago. It inhabits parts of the DRC south of the Congo but north of the Kasaï–Sankuru river system (Map 2) in the Congo central basin, which has small human populations but is linked by rivers to Kinshasa, the main market for its farming and fishing products. The bonobo's contacts with *P.t. schweifurthii*, which live north of the river, and with *P.t. troglodytes*, to its west, must be exceptional, since the Congo, the second greatest river in the world, is impassable by chimpanzees.[29]

Bonobos are less aggressive, 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; 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'). Highly dedicated primatologists described how 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. Some primatologists have even talked about 'pansexuality', so varied are their sexual contacts.[30]

Some form of courtship precedes about half of intercourses, but once they copulate fifteen seconds suffice. Intercourse is used to solve conflicts and to 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 a few bonobos living in zoos or primate centres in Europe and the USA 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 30,000–50,000 individuals scattered around a large area of the DRC. Their distribution is discontinuous and bonobos

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are well aware that their main predator is humans. Over the last few years, samples from around 200 wild-living bonobos, obtained from six sites in the DRC, have been tested and were all negative for SIV. Given the heterogeneity of 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.[31]

ORIGINS OF SIV IN CHIMPANZEES

Discussion of 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 SIV_{cpz} originated from the recombination of distinct SIVs infecting smaller monkeys, principally the SIV_{rcm} of red-capped mangabeys and a SIV that infects 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]

Since the geographic distribution of the species of small monkeys mentioned above overlaps with that of *P.t. troglodytes*, this viral recombination was certainly achieved among this subspecies and the transmission of the virus to *P.t. schweinfurthii* occurred at a later stage when, exceptionally, an infected chimpanzee managed to cross the Ubangi–Congo line. While the Congo is an absolute barrier, the Ubangi can be forded at the end of the dry season via strings of islands, especially near the city of Bangui. SIV_{cpz} appeared well after the differentiation between *P.t. troglodytes* and *P.t. schweinfurthii* about 90,000 years ago.[29]

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To conclude, there can no longer be any doubt that the common chimpanzee from central Africa, *Pan troglodytes troglodytes*, is the source of HIV-1. It is in the natural habitat of this primate that the first case of human infection must have occurred, the mythical ‘patient zero’ that triggered the pandemic. And it is in this region in the heart of Africa that the virus started its journey.

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