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Author(s): Pascal Gagneux, M. Katherine Gonder, Tony L. Goldberg and Phillip A. Morin

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Gene flow in wild chimpanzee populations: what genetic data tell us about chimpanzee movement over space and time

Pascal Gagneux¹*, M. Katherine Gonder², Tony L. Goldberg³ and Phillip A. Morin⁴

¹University of California San Diego, Glycobiology Research and Training Center, Cellular and Molecular Medicine-East, Room 1087, 9500 Gilman Drive, La Jolla, CA 92093-0687, USA

²Section on Genomic Structure and Function, Laboratory of Molecular and Cellular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Building 8, Room 205, 8 Center Drive, MSC 0830 Bethesda, MD 20892, USA

³University of Illinois College of Veterinary Medicine Department of Pathobiology, 2001 South Lincoln Avenue, Urbana, IL 61820, USA

⁴Laboratory for Conservation Genetics, Max Planck Institute for Evolutionary Anthropology, Inselstrasse 22, D-04103 Leipzig, Germany

The isolation of phylogenetically distinct primate immunodeficiency viruses from at least seven wild-born, captive chimpanzees indicates that viruses closely related to HIV-1 may be endemic in some wild chimpanzee populations. The search for the chimpanzee population or populations harbouring these viruses is therefore on. This paper attempts to answer the question of whether or not such populations of chimpanzees are likely to exist at all, and, if so, where they are likely to be found. We summarize what is known about gene flow in wild populations of chimpanzees, both between major phylogeographical subdivisions of the species, and within these subdivisions. Our analysis indicates that hitherto undocumented reproductively isolated chimpanzee populations may in fact exist. This conclusion is based on the observation that, despite limited geographical sampling and limited numbers of genetic loci, conventional notions of the nature and extent of chimpanzee gene flow have recently been substantially revised. Molecular genetic studies using mitochondrial DNA sequences and hypervariable nuclear microsatellite markers have indicated the existence of heretofore undocumented barriers to chimpanzee gene flow. These studies have identified at least one population of chimpanzees genetically distinct enough to be classified into a new subspecies (*Pan troglodytes vellerosus*). At the same time, they have called into question the long-accepted genetic distinction between eastern chimpanzees (*Pan troglodytes schweinfurthii*) and western equatorial chimpanzees (*Pan troglodytes troglodytes*). The same studies have further indicated that gene flow between local populations is more extensive than was previously thought, and follows patterns sometimes inconsistent with those documented through direct behavioural observation. Given the apparently incomplete nature of the current understanding of chimpanzee gene flow in equatorial Africa, it seems reasonable to speculate that a chimpanzee population or populations may exist which both harbour the putative HIV-1 ancestor, and which have remained reproductively isolated from other chimpanzee populations over the time-scale relevant to the evolution of the SIVcpz–HIV-1 complex of viruses. Continued extensive sampling of wild chimpanzee populations, both for their genes and their viruses, should be performed quickly considering the high probability of extinction that many wild chimpanzee populations face today. The history of human–chimpanzee contacts is discussed.

Keywords: chimpanzee; *Pan troglodytes*; HIV; SIV; phylogeography

1. INTRODUCTION

The isolation of HIV-like viruses (SIVcpz) from seven wild-born captive chimpanzees has prompted the search for the ancestor of HIV-1 in what is now considered to be the natural host of such an ancestral virus, the common chimpanzee *Pan troglodytes* (Gao *et al.* 1999; Corbet *et al.* 2000). Some of these SIV-positive animals were tested very soon after their capture in the wild. Unfortunately, all these animals were tested away from their capture sites, which remain unknown in most cases, and most were tested long after their dates of capture. Only one chimpanzee (from East Africa) has so far been docu-

mented to harbour SIVcpz in the wild (Sharp *et al.*, this issue). The precise locations of the natural population(s) that may harbour such a virus therefore remain unknown. The fact that SIVcpz isolates have been obtained that are phylogenetically distinct from any known forms of HIV-1 (Huet *et al.* 1990; Janssens *et al.* 1994; Van den Haesevelde *et al.* 1996; Gao *et al.* 1999; Corbet *et al.* 2000), combined with evidence for an innate resistance of chimpanzees to developing AIDS (Novembre *et al.* 1997), strongly suggests the existence of endemic SIVcpz viruses in wild chimpanzee populations.

The data we present illustrate that there are subpopulations of chimpanzees that have been isolated from each other for long periods of time (in the order of hundreds of thousands of years). Chimpanzee SIVs could therefore

*Author for correspondence (gagneux@biomail.ucsd.edu).

exist in some but not other populations. Alternatively chimpanzee SIVs could exist as widely different subtypes due to the prolonged isolation. Here we discuss the existing population genetic data with regard to movements of chimpanzees, both between major geographical regions and between local populations within these regions.

2. CHIMPANZEE TAXONOMY

Pan troglodytes, or common chimpanzees, are the most widely distributed of the African great apes. Until well into the 20th century, common chimpanzees lived in the forested or wooded tropical regions of Africa, ranging from Senegal and Gambia in the west to Uganda and Tanzania in the east. Their geographical range is limited by the presence of trees, for their nightly nests, and access to water (Kortlandt 1983). In the last five decades, their numbers have shrunk dramatically and they have been exterminated from at least six West African countries (Teleki 1989).

Attempts at classifying chimpanzees have a long and complicated history (Hill 1969; Reynolds & Luscombe 1971; reviewed in Gonder 2000). In the past, chimpanzees have variously been assigned to as many as 12 genera and many more species and subspecies (Hill 1969). The staggering morphological variation observed even within a single community of these animals has caused much confusion for taxonomists. Early European and American explorers would often claim the discovery of new species after capturing or killing unusual looking 'specimens'. Polymorphisms and ontogenetic changes in pigmentation of skin and fur continue to confuse observers of wild chimpanzees. Attempts to find distinguishing morphological characteristics for the subspecies commonly recognized until the mid 1990s have been only partially successful (Shea & Coolidge 1988; Groves *et al.* 1993). Bonobos, also called 'pygmy chimpanzees', were only described as a separate species in the 20th century (Coolidge 1933). The ranges of bonobos and chimpanzees do not overlap—bonobos live only south of the Congo River and they exhibit many clearly distinct, behavioural, morphological and genetic traits.

3. MOLECULAR TAXONOMY

With the advent of modern molecular biological techniques, the intraspecific diversity of chimpanzees has been further documented at the genetic level. Non-invasive genotyping has allowed the genetic characterization of hundreds of wild animals belonging to natural populations from known geographical locations (figure 1). With the exception of chimpanzees in the easternmost part of the species' range, all populations studied to date exhibit high levels of diversity, surpassing that found in the global human population (Gagneux *et al.* 1999b). Initial studies of mitochondrial DNA (mtDNA) haplotypes seemed to be consistent with the classification of chimpanzees into three subspecies: *Pan troglodytes verus* in West Africa (Upper Guinea), *Pan troglodytes troglodytes* in western equatorial Africa, and *Pan troglodytes schweinfurthii* in East Africa (Morin *et al.* 1994). Recent investigations involving more extensive and intensive geographical

sampling have, however, significantly complicated the initial picture of chimpanzee systematics that earlier molecular studies afforded (Gonder *et al.* 1997; Gonder 2000).

The current volatile state of chimpanzee molecular taxonomy is largely due to the fact that studies to date have relied heavily on only a handful of genetic loci. Different parts of the genome are useful for reconstructing evolutionary events on different time-scales. Nuclear genes with low mutation rates have long coalescence times (millions of years) and haploid genes, such as rapidly evolving parts of the mitochondrial control region, have much shorter coalescence times (tens to hundreds of thousands of years). More recent events in the species' evolution may thus only be reflected in the more rapidly evolving parts of the genome, especially those with smaller effective population sizes such as mtDNA. Hypervariable markers in the nuclear DNA, such as microsatellite (MSAT) loci, are best used for pedigree analyses and for the study of allele frequency variation between local populations.

Unfortunately, reliance on non-invasive samples has tended to limit the genetic markers that chimpanzee molecular taxonomists use. This is due to the minute amounts and often poor quality of DNA retrievable from such samples. Such limitations have made studies of nuclear DNA, which exists in low copy numbers per cell, difficult. Studies that have successfully employed nuclear DNA have been limited to very short genomic segments amplified by the polymerase chain reaction (PCR) and have had to take special precautions against 'allelic dropout', which would tend to bias results (Gagneux *et al.* 1997a). For these reasons, mtDNA has become the molecule of choice for chimpanzee molecular systematics. It is present in hundreds to thousands of copies in follicular cells at the end of shed hair and it can also be extracted much more easily from faeces, urine and chewed fruit pulp than nuclear DNA (Higuchi *et al.* 1988; Morin *et al.* 1992; Gagneux *et al.* 1997a; Constable *et al.* 1995; Vigilant 1999).

4. MOVEMENTS BETWEEN GEOGRAPHICAL REGIONS

By 1994, Morin and colleagues had already documented near-identical mtDNA haplotypes in East African chimpanzees living over 600 km apart, western equatorial African chimpanzees living over 400 km apart, and West Africa chimpanzees living over 900 km apart (Morin *et al.* 1994). Goldberg & Ruvolo (1997a) confirmed this finding in East Africa when analysing mtDNA control region sequence from 19 sampling sites for which they documented semi-restricted gene flow, low overall variability and clinal variation, but no geographical divisions (Goldberg & Ruvolo 1997a). In chimpanzees from upper Guinea, Gagneux *et al.* (1999b) documented sharing of mitochondrial DNA from Mali to south-eastern Côte d'Ivoire, spanning a distance of 1000 km. Within chimpanzees from upper Guinea, no geographical structure of genetic variation was apparent, except for one savannah population in north-eastern Côte d'Ivoire. That savannah population showed lower mtDNA diversity and significant subdivision from other populations in West Africa. Importantly, none of these

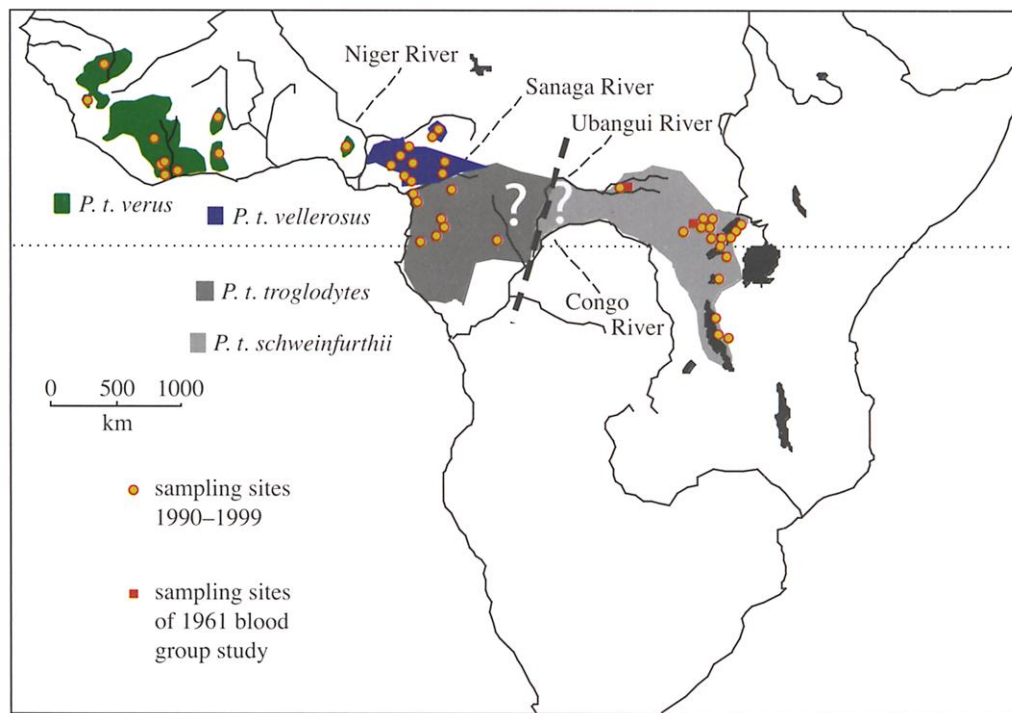


Figure 1. Ranges of the different chimpanzee subspecies across tropical Africa. Open circles indicate sampling sites, where samples of mtDNA have been collected between 1990 and 1999. Red squares indicate two sampling sites for a chimpanzee blood group study published in 1961. Dotted line and question marks indicate area for which samples are lacking and where a taxonomic break may or may not occur.

three studies found any haplotypes that were shared across the geographical regions of the three traditional subspecies, as proposed by Hill (1969).

More recently, Gonder sampled natural populations in Nigeria and Cameroon (Gonder *et al.* 1997; Gonder 2000). Surprisingly, chimpanzees from western Nigeria and north-eastern Cameroon had mitochondrial haplotypes uniquely different from any known populations. Their uniqueness indicates that these populations have been isolated for significant amounts of time.

Gonder's findings confirm the hypothesis that large rivers can form barriers for chimpanzee gene flow (Schwarz 1934). The Niger River in Nigeria and the Sanaga River in Cameroon have been identified as taxonomic barriers by Gonder (Gonder 2000). The Sanaga River has probably acted as a barrier for longer, but curiously this barrier is apparently not complete. How permeable the Niger was as a barrier remains unknown, due to the near extinction of chimpanzee populations to the west of the Niger. The only two available samples from Ise Forest Reserve in Nigeria have mtDNA haplotypes that cluster with the upper Guinean *P. t. verus* chimpanzees.

Analysis of the combined, large ($n=340$ haplotypes) mtDNA control region data set from these studies reveals that, unlike their upper Guinean and Nigerian counterparts, neither western equatorial nor East African chimpanzees form monophyletic clusters with respect to each other. The majority of *P. t. troglodytes* sequences clusters into two well supported branches, but a few others fall in-between the large number of East African sequences. The same result is obtained if only shorter sequences without

any insertions or deletions or missing base pairs are used for the analysis (figures 2 and 3).

Because the phylogenetic division between the eastern and western equatorial African chimpanzee subspecies remains uncertain, three avenues of research need to be pursued to resolve their true taxonomic relationship. First, better sampling across the putative subspecies boundary is needed to investigate levels of gene flow. Second, longer and higher-quality mtDNA sequences need to be characterized from those sampled animals to obtain a sufficient number of phylogenetically informative sites for the resolution of population-level events from the past 100 000–300 000 years. Unpublished data from 1.2 kb including the whole mitochondrial cytochrome *b* gene (P. A. Morin, unpublished data) continue to support subspecies distinctions between western equatorial and eastern populations, but more samples are still required to resolve the question. Finally, several independently assorting nuclear loci should be examined.

The proposed barrier between *P. t. troglodytes* and *P. t. schweinfurthii* is the Ubangui River (Schwarz 1934). As stated above, no-one has yet sampled chimpanzee populations on either side of this river. Haplotypes from animals from the Bondo region, northern Democratic Republic of Congo, sequenced for this study (accession nos. AF361230–AF361236) fall near the branch point from which several clades of *P. t. schweinfurthii* radiate (figure 2; P. Gagneux, unpublished data). Furthermore, the existence of four animals, two in Cameroon (accession nos. AF361237 and AF361238) and two in Gabon, with haplotypes clustering in-between those found in Tanzania and Uganda (figure 2) (Gonder 2000; Deinard & Kidd

2000), suggests that the distinction between a western equatorial and eastern subspecies may not be warranted. The clustering together of haplotypes from chimpanzees from different classical 'subspecies' ranges is rare, but its existence would indicate that the dispersal capacity of these animals may have been underestimated or, alternatively, that the role of barriers such as rivers has been overestimated. An alternative explanation is that the time-scale of the separation of *P. t. troglodytes* from *P. t. schweinfurthii* lies at or near the limit of the ability of mtDNA to resolve. Future studies using loci that evolve at rates intermediate between microsatellites and the mitochondrial control region DNA may someday validate the taxonomic separation of these two subspecies.

Two recent studies on genetic variability of captive chimpanzees have revealed little or incomplete differentiation between any of the chimpanzee subspecies from analysis of nuclear DNA sequence data. The 10 kb genomic sequence of X-chromosome non-coding sequence studied by Kaessmann *et al.* (1999) had an estimated coalescence time of 2.1 million years (Myr) for all chimpanzees, which is greater than the subspecies divergence estimates for all of the chimpanzee subspecies based on mtDNA. This may indicate that the slow mutation rate and presence of ancestral polymorphism make *Xq13* a poor marker for chimpanzee evolutionary history within the last 2 Myr, despite the large amount of polymorphism evident in chimpanzees as a whole. Deinard & Kidd's (2000) study of six nuclear loci revealed only partial resolution of each of the three classical subspecies. Sample size is also a factor in the resolution of trees based on nuclear DNA, as both studies were dependent on captive chimpanzee samples of known or inferred (from mtDNA haplotypes) origin, and were very limited in samples from *P. t. schweinfurthii* ($n=1-3$).

Nuclear DNA studies of wild chimpanzee populations have so far involved genotyping at several microsatellite loci. These are tandemly repeated di- tri- or tetranucleotide repeat loci in non-coding regions of the genome at which humans and chimpanzees exhibit high degrees of polymorphism (many alleles) and high heterozygosity (over 50%) (Tautz 1993). Only two studies have involved multiple populations in the same geographical region (Gagneux 1998; Gonder 2000). In upper Guinean *P. t. verus* chimpanzees, the degree of population subdivision between three distant populations in Mali, Sierra Leone and Côte d'Ivoire was minimal. Populations living in south-western Mali were not very distinct from forest populations living over 500 km to the south-east in Côte d'Ivoire, as is evident from the very low values of population subdivision (Gagneux 1998).

So far only Gonder *et al.* (Gonder *et al.* 1997; Gonder 2000) have sampled wild populations around a taxonomic break. The presence of a few mitochondrial haplotypes that clearly cluster with the samples from the other side of the Sanaga River indicates that small amounts of gene flow occur even across this putative phylogeographical barrier. Gonder's microsatellite data suggest that the effective number of migrants per generation across the Sanaga River is high (approximately 11). Gonder's microsatellite data for chimpanzee populations on both sides of the Sanaga River gave weaker subdivision signals and indicated more admixture than the mtDNA haplo-

type data. Thus it appears that even though nuclear microsatellite loci have a mutation rate about an order of magnitude higher than the most rapidly evolving piece of the mtDNA genome, the smaller effective population size of mtDNA leads to more rapid lineage sorting during periods of geographical isolation. Alternatively, the rapid rate of evolution at microsatellite loci may have begun to saturate these sites with mutations, leading to homoplasy and poor phylogenetic resolution.

In summary, movements of chimpanzees between upper Guinean regions in West Africa and regions to the east of the Niger River have not occurred for hundreds of thousands of years. There appears to have been some recent movement across the Sanaga River, but at relatively low frequencies. Whether barriers exist between western equatorial Africa and eastern Congo, Tanzania and Uganda, and what the locations of these barriers are, remains unknown due to the lack of data from the relevant regions (around the Sanga and Ubangui Rivers).

Five out of the seven chimpanzees positive for SIVcpz identified so far have mtDNA haplotypes that cluster phylogenetically with those characteristic of western equatorial Africa (Huet *et al.* 1990; Janssens *et al.* 1994; Van den Haesevelde *et al.* 1996; Gao *et al.* 1999; Corbet *et al.* 2000). The other two SIVcpz positive chimpanzees (Huet *et al.* 1990; Corbet *et al.* 2000) cannot be excluded from having originated in western equatorial Africa because (i) chimpanzees from western equatorial and eastern Africa may not form part of two different monophyletic groups, and (ii) there is some evidence of limited gene flow between *P. t. vellerosus* and *P. t. troglodytes* in the vicinity of the Sanaga River in central Cameroon.

5. MOVEMENT WITHIN GEOGRAPHICAL REGIONS

Field studies of wild chimpanzees date back to the 1960s, when Jane Goodall and Toshisada Nishida began habituating wild chimpanzee communities at two different sites on the shores of Lake Tanganyika in Tanzania (Goodall 1968; Nishida 1968). Since then, chimpanzees have been habituated to the presence of human observers at a handful of other sites across Africa.

All chimpanzees studied so far live in stable social units called communities and show a 'fission-fusion' type of social organization (reviewed in Goodall 1986). Communities typically comprise 20-100 individuals and defend 7-30 km² territories. Community members separate into smaller foraging parties, which rejoin and change size and composition several times in the course of each day. Most group members tend to sleep in close proximity in nests that are made at a different location every night. Males generally remain in their native social group, while females transfer to new social groups when they reach sexual maturity at *ca.* 14 years. The degree to which females disperse varies greatly between study sites. The distance to which females disperse is unknown (Goodall 1986; Nishida 1990; Wrangham *et al.* 1996; Boesch & Boesch-Achermann 2000). There is only one report of dispersal by males (Sugiyama *et al.* 1993) and only anecdotal evidence of migration by entire social groups.

Recent observation of 'rogue males' (R. W. Wrangham, personal communication) opens the possibility that males, too, move between groups, in spite of the fact

that this has only been observed a single time. In the study community at Bossou Guinea a single male temporarily joined the study group (he was in fact the only male in the group; Sugiyama *et al.* 1993). To what extent the absence of other mature males from that particular group facilitated his immigration remains an open question.

Fusion of social groups has been observed, in the form of 'takeovers' by the males of one group of the females of another group. Such a fusion involved the systematic killing of the males belonging to the group that was taken over (Goodall 1986). Chimpanzees are territorial in the sense that members of a social group cooperate in defending the territory against neighbouring groups. At some sites, females seem to hold individual territories within the larger territories defended by their male social partners (Wrangham & Smuts 1980). Antagonistic encounters with neighbours are frequent and males seem responsible for most of the fighting, which often results in serious injury (Wrangham 1999; Boesch & Boesch-Achermann 2000).

Patterns of gene flow have also been investigated using molecular genetic data from natural communities. Analyses of mtDNA from populations within the ranges of the four currently acknowledged subspecies have indicated, as discussed previously, that haplotypes are shared between local populations separated by very large geographical distances. This observation implies that within-subspecies gene flow may be extensive. Within the *P. t. schweinfurthii* range, for example, Goldberg (Goldberg & Ruvoilo 1997a) has documented that, on average, between three and four migrants are exchanged between populations per generation. This implies that gene flow on the 'mitochondrial time-scale' has indeed been extensive, and that the entire subspecies, at least within the geographical range sampled, is effectively panmictic. Whether gene flow in the more recent past has followed a similar pattern awaits data from more-quickly-evolving loci.

Nuclear data have been used to investigate chimpanzee social structure, which reflects gene flow between neighbouring communities. Chimpanzee communities are defined by a core of cooperative adult males, thought to be closely related. Kin selection has been used to explain this cooperation in an evolutionary context. A study of kinship in the Gombe community documented a slightly higher relatedness among group males than among group females, confirming female-biased dispersal (Morin *et al.* 1994). Gagneux *et al.*'s 1999a study of genetic structure in the West African Tai Forest study community, however, found no higher relatedness among males than among females. This discrepancy could result from real population differences, if the pattern documented at Tai turns out to be generalizable to other communities in the *P. t. verus* range (Gagneux *et al.* 1997b, 1999a).

Gene flow between neighbouring social groups may therefore consist not only of female dispersal but also of male dispersal. Males may physically move, or may sire offspring in other groups. Contrary to traditional views of chimpanzee social systems, therefore, the social and the reproductive units may not be identical (Morin 1993).

6. DISCUSSION

Investigations into the movements of chimpanzees within and between major biogeographical regions in equatorial Africa should be considered preliminary. Most conclusions to date are based on short and sometimes incomplete mtDNA sequences. The combined evidence does suggest that at least three, and possibly more, geographically and genetically isolated subpopulations of chimpanzees do exist in equatorial Africa. The presence of hitherto undocumented genetically distinct chimpanzee populations within these larger taxonomic groupings is entirely plausible. If geographical barriers have arisen to isolate such populations within the last several hundreds or thousands of years, these populations will remain effectively invisible until sampling becomes more extensive or other more-quickly-evolving genetic loci are examined.

Preliminary data do, however, suggest that such undiscovered reproductively isolated chimpanzee populations may exist. André *et al.* (1961) studied blood group antigens in 132 captive chimpanzees of known geographical provenance (see figure 1) in what was at the time the Belgian Congo (currently the Democratic Republic of Congo). They documented differences in A/O antigen frequencies for populations in northern Belgian Congo (around Ango and Bondo), and the presence of Rh antigens restricted to a population from Mambasa, near Ituri (eastern Belgian Congo). However, mtDNA haplotypes from the Bondo area and from the Ituri Forest do not form separate clades in phylogenetic reconstructions (Goldberg & Ruvoilo 1997b; K. Amman and P. Gagneux, unpublished data). Investigation of the Ango, Bondo and Mambasa populations using hypervariable nuclear markers is clearly warranted.

Even if reproductively isolated chimpanzee populations are someday discovered within the ranges of the currently acknowledged subspecies, it is unclear whether their presence will in fact be relevant to the evolution of SIVcpz and HIV-1. The notion of a co-speciation between SIVs and their chimpanzee host subspecies has been proposed (Gao *et al.* 1999; Hahn *et al.* 2000). Given the uncertain origin of the host of the more divergent SIVcpzANT (Van den Haesevelde *et al.* 1996) and the uncertain status of the eastern subspecies, there are little data to date to support or reject this hypothesis. If genetically distinct SIVcpz variants have indeed coevolved with their respective chimpanzee populations, this then suggests that the natural transmission of SIV across taxonomic breaks and biogeographical barriers is in fact a rare event. To understand the mechanisms by which SIVcpz may have been transmitted across a species barrier to humans, it may be necessary to examine the recent behavioural and cultural practices that bring wild chimpanzees into contact with humans.

Historical records on the nature and frequency of contact between humans and chimpanzees are poor or non-existent. However, given the long history of human and chimpanzee habitation of equatorial Africa, it should be assumed that contacts between the two species have always been varied and frequent. It is also likely that the advent of agriculture has increased the degree of contact between humans and chimpanzees. Crop-raiding by

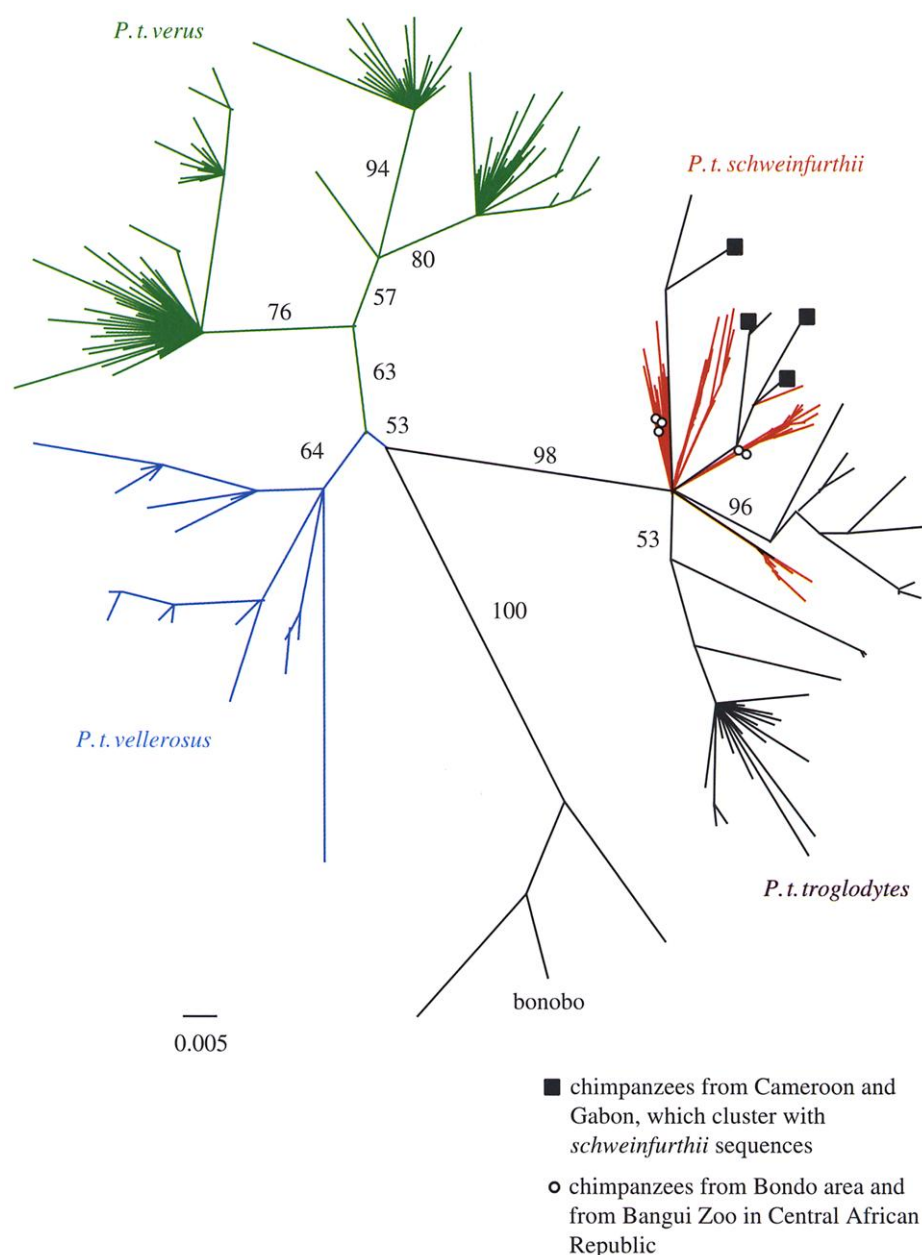


Figure 2. Neighbour-joining tree based on Kimura 2 parameter distances for 340 haplotypes of chimpanzee mitochondrial control region, hypervariable region I (415 bp). Sites with missing data were ignored in pairwise comparisons. Numbers indicate bootstrap values for 500 replications. While West African *P. t. verus* and Nigerian–western Cameroon *P. t. vellerosus* sequences each form a well supported clade, East African *P. t. schweinfurthii* and Central African *P. t. troglodytes* haplotypes do not fall into two different monophyletic groups. East African chimpanzee haplotypes (in dark grey) fall in-between Central African chimpanzee haplotypes (in black). Four sequences indicated by black boxes were found in animals living in Cameroon and Gabon. Sequences from the Bondo area in northern Republic of Congo are indicated by open circles (accession nos. AF361230–AF361238). Tree constructed with PAUP beta 4 version (Swofford 1998).

chimpanzees, for example, cannot be a new phenomenon, and would have led to regular interactions (mostly unfriendly) between humans and chimpanzees.

Historical accounts from the 16th and 17th centuries by Portuguese priests visiting West Africa mention typical chimpanzee behaviours such as nut cracking (palm nut) and the raiding of honeybee nests for honey (Hair 1984; Sept & Brooks 1994). Interestingly, these same accounts also mention the keeping of chimpanzees as pets by the local population. Cacao farming was introduced in Africa in the 1800s and would have created ample oppor-

tunity for contacts due to crop raiding by chimpanzees. Other opportunities include mango trees in close proximity to human settlements in south-western Mali that are visited by chimpanzees (Moore 1985), sugar cane fields in Gabon (S. Lahm, personal communication) and banana plantations in the eastern Republic of Congo (U. Rahm, personal communication).

The list in table 1 details the kinds of direct contacts that are likely to be occurring presently between humans and chimpanzees. It is by no means inclusive, but suggests modes of contact that have almost surely increased

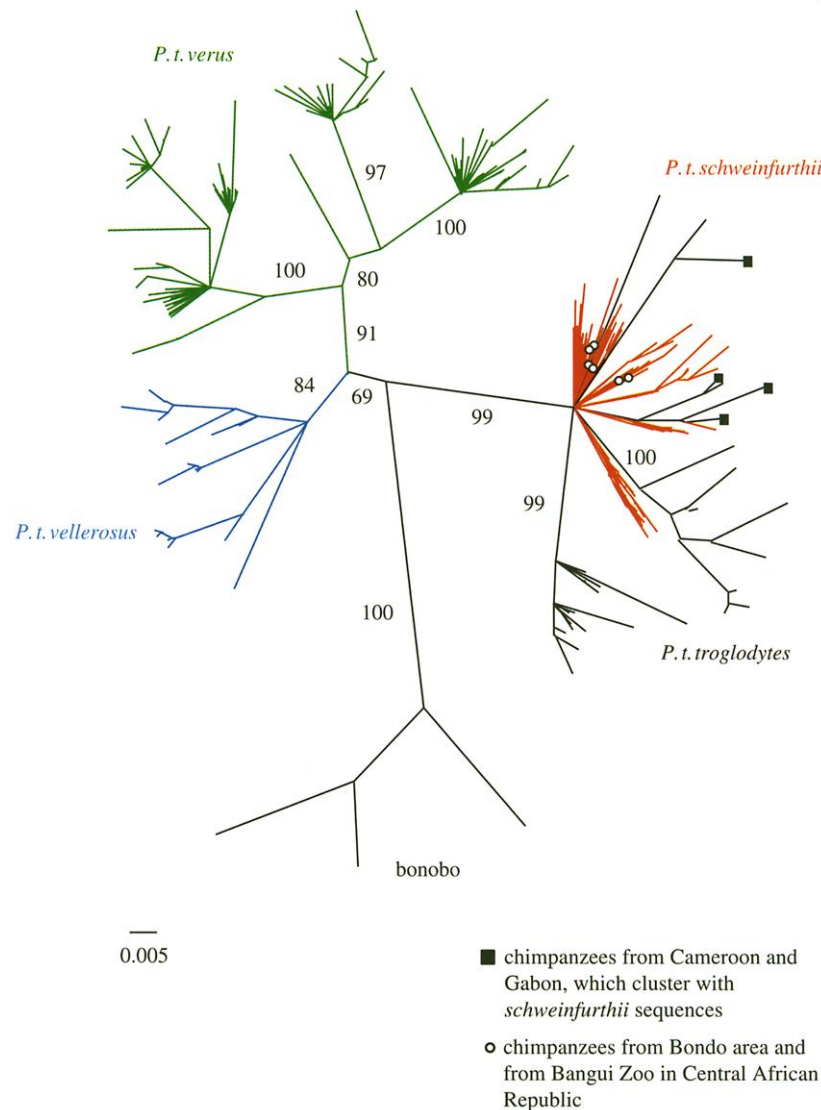


Figure 3. Neighbour-joining tree based on Tamura-Nei distances using a reduced set of 308 haplotypes (303 bp) with no insertions or deletions or missing bases. Numbers indicate bootstrap values for 1000 replications. The topology is very similar to the phylogram in figure 2. There is no support for monophyly of *P. t. troglodytes* or *P. t. schweinfurthii* subspecies.

interspecific contact rates within the last century. These are the sorts of contacts that would, therefore, be particularly relevant to the zoonotic or anthroponotic transmission of a virus such as SIVcpz-HIV-1. All these contacts drastically increased during the 20th century.

Chimpanzees must have been hunted before the advent of firearms because these animals would frequently have been both direct competitors and large prey. Chimpanzees are easy to find, although hunting them without guns is difficult. Traditional hunting in the forest was done with traps, nets, bow and arrows, and crossbows. Much of it would have resulted in capture rates proportional to the frequency of wild animals. Apes being rare, chimpanzees would not have fallen prey to forest people very regularly, but they would have been slaughtered and consumed if captured. Thus, opportunities for cross-species infection with a lentivirus from chimpanzees certainly existed in equatorial Africa for millennia. The pygmy hunting method using collapsible nets, later adapted by some European researchers, is undoubtedly an old invention. In 1963, 1964 and 1966 chimpanzees were

captured with the use of such nets on at least four occasions for a study of hepatitis B prevalence in eastern Congo (Rahm 1967).

Chimpanzee pets are often a by-product of hunting for meat, when the larger adult animals are slaughtered but the adorable infants are kept as pets. The Portuguese sources mentioned above are the earliest mention of pet chimpanzees in sub-Saharan Africa (17th century), but the practice has undoubtedly been in existence since much earlier.

The large number of chimpanzees in Europe and North America are all descendants of animals captured in Africa or actual wild-born 'founders' of the captive population. Records of these founder animals are mostly non-existent. The demand for chimpanzees in North America and Europe certainly created a large market for these animals during most of the 20th century. The increase in exposure to chimpanzees and their pathogens coming from their use in research or zoos was addressed early on by Hillis (1961) and later by Giunta & Groppa (1987) and Sandstrom *et al.* (2000). Because of the unavailability

Table 1. *Kinds of direct contact likely between humans and chimpanzees*

hunting and butchering of chimpanzees, including transport and handling during retail and cooking
keeping of chimpanzees as pets
antagonistic interactions with wild chimpanzees that raid crops or directly attack
capture of chimpanzees for international trade
biomedical research in Africa and abroad
zoos
entertainment industry
field research on chimpanzees
tourism involving close encounters between humans and wild chimpanzees

of data on chimpanzees captured and exported from Africa, it is impossible to tell the numbers, destinations and the uses made of these animals.

Assumptions about the geographical (or 'subspecies') origin of captive populations based on the site of purchase can be very misleading. The 35 founder animals of the Rijswijk chimpanzee colony were all assumed to be upper Guinean because they had been purchased from a dealer in Sierra Leone. However, based on their mtDNA genotypes, it is now clear that there were two *P. t. troglodytes* founders among the 35 founders in that population, bought in Sierra Leone, but captured somewhere in western equatorial Africa (R. Bontrop, personal communication). There is a Liberian *P. t. verus* chimpanzee living in a sanctuary in Zambia, and several *P. t. verus* chimpanzees and their descendents living on Rubondo Island, Lake Victoria, Tanzania (Hannah & McGrew 1991).

The prevalence of chimpanzee SIVs, as well as modes of transmission, remains unknown. We do not know whether transmission is: sexual; through fighting (as is the case in mandrills); perinatal; via coprophagy between infant and mother; or even via licking of wounds of other group members. This, and the preliminary nature of data on population movements, do not allow conclusions about the history of chimpanzee SIV in natural chimpanzee host populations. The almost complete lack of records on capture, transport, use and possibly release of chimpanzees by humans adds further uncertainty. The lack of SIVcpz in large numbers of upper Guinean chimpanzees, which could be tested because they have provided the bulk of animals used for research in Europe and North America, makes the presence of SIVcpz in upper Guinea unlikely. The limited amount of surveying carried out on wild populations from other regions does not allow us to determine which populations of chimpanzees are 'free' of SIVcpz infection.

Because historical records are incomplete and sometimes unreliable, we believe that the question of the geographical origin of SIVcpz and its purported evolution into HIV-1 must ultimately be answered through extensive sampling of wild chimpanzee populations. Such populations should be sampled both for the presence of SIVcpz and for the presence of genetic markers indicative of population isolation. Specific attention should be directed to whether a reproductively isolated subpopulation of chimpanzees exists that also harbours a variant of

SIVcpz directly ancestral to HIV-1 group M. The existence of such a population would be powerful evidence that the modern HIV-1 pandemic began with the zoonotic transmission of a specific virus from a specific population of chimpanzees to a specific population of humans. The appropriate directed historical investigations could then follow.

We do not, however, expect that the current existence of such a population of chimpanzees would be easy to document. If the overall prevalence of SIVcpz in wild populations of chimpanzees is low, as data suggest (Santiago *et al.* 2000), then large numbers of animals will have to be sampled in order to detect the virus at all. The logistics of such sampling are daunting given the political and social instability of the countries in which the likeliest chimpanzee populations are located. More worrisome is the fact that chimpanzee populations are disappearing at an alarming rate, due to such forces as deforestation and hunting (Teleki 1989). It is entirely possible that the 'critical' chimpanzee populations no longer exist.

Nevertheless, in the interests of documenting the remarkable biological variety inherent in our closest living relatives, it is imperative that studies of chimpanzees in the wild continue. Technologies for analysing non-invasive genetic samples will surely improve, including non-invasive methods for identifying and characterizing SIVcpz. Whether the most relevant populations of chimpanzees will survive to be studied remains to be seen.

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