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Interspecies Transmission of Simian Foamy Virus in a Natural Predator-Prey System▽

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Simian foamy viruses (SFV) are ancient retroviruses of primates and have coevolved with their host species for as many as 30 million years. Although humans are not naturally infected with foamy virus, infection is occasionally acquired through interspecies transmission from nonhuman primates. We show that interspecies transmissions occur in a natural hunter-prey system, i.e., between wild chimpanzees and colobus monkeys, both of which harbor their own species-specific strains of SFV. Chimpanzees infected with chimpanzee SFV strains were shown to be coinfected with SFV from colobus monkeys, indicating that apes are susceptible to SFV superinfection, including highly divergent strains from other primate species.

Simian foamy viruses (SFV) and their nonhuman primate hosts demonstrate coevolution (20). Despite the fact that SFV strains have been described for most Old World primate species, no human-specific foamy virus has yet been identified (16). However, zoonotic transmissions of SFV from various nonhuman primates to zookeepers and central African hunters or others having close contact with nonhuman primates are known to occur (5, 8–10, 21, 22). Such viruses have sequences that show a close relationship to SFV sequences from several nonhuman primate species (Fig. 1). Until now, it has been unclear whether such interspecies transmission can also take place when the hunter is naturally infected with its own species-specific SFV. Wild chimpanzees that regularly hunt and consume western red colobus monkeys (4) provide such a situation.

Wild chimpanzees (*Pan troglodytes verus*) and western red colobus monkeys (*Piliocolobus badius*) sharing a rainforest habitat, the Taï National Park, Côte d'Ivoire, were tested for SFV infection. The chimpanzees have been under human observation for more than 25 years and are known individually as a result of a project focusing on wild chimpanzee behavior. Tissue samples were obtained from 14 chimpanzees that had died of anthrax, respiratory disease, or other causes (12, 14, 15). Samples of blood, collected in EDTA, from nine red colobus monkeys were obtained under anesthesia, and organ samples were collected from the remains of a further two that had been killed and eaten by chimpanzees (15). All represented adult animals.

Using the SFV primers for the integrase gene (19), previous analyses revealed that 12 of the 14 chimpanzees harbored SFV strains (SFV_{cpz}) corresponding to strains described for the chimpanzee subspecies *Pan troglodytes verus* (details will be published elsewhere). Also 11 red colobus monkeys were tested positive for SFV using the same primers and standard conditions (96°C for 5 min; 40 cycles 96°C for 1 min, 56°C [first PCR] or 60°C [nested PCR] for 30 s, 72°C for 1 min; and a final elongation step at 72°C for 10 min). PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced directly in both directions without interim cloning.

Phylogenetic analysis using the neighbor joining method (BioEdit, PHYLIP 3.572 package) of these 389-bp sequences was performed (18). Bootstrap resampling with 1,000 replicates was employed to place approximate confidence limits on individual branches. The tree revealed a species-specific SFV lineage (SFV_{wrc}) unique to red colobus monkeys. Based on sequences derived from SFV_{wrc}, species-specific primers were designed for a first-round PCR (SFVwrc 1s, 5′ CATACAAT TACCACTCCAAGCCT; SFVwrc 2as, 5′ CAGACAAATCC AGTCATACCATC; 473 bp). Consecutively, two seminested PCRs combining primer SFVwrc 1s with SFVwrc 3s (5′ CTC AGTACTGGTGGCCAAATCTTAGA; 220 bp) and SFVwrc 2as with SFVwrc 4as (5′ CCAGTCATACCATCGACTACTA CAAGG; 423 bp) were performed. SFV sequences were amplified from spleen DNA of 2 of the 14 wild chimpanzees by using the SFV_{wrc}-specific primers. All PCR products were sequenced, aligned, and compared to the public database using BLAST NCBI (Table 1). High similarity of the sequences derived from the chimpanzees using the SFV_{wrc} primers and the colobus monkey sequences was seen (similarity of 98 to 99%). In contrast, the SFV chimpanzee sequences derived through PCRs using the generic SFV primers were only distantly related (78 to 81%), pointing to a double infection with two different SFV isolates.

Sequences were aligned using BioEdit, and phylogenetic analyses using the neighbor joining method as well as the

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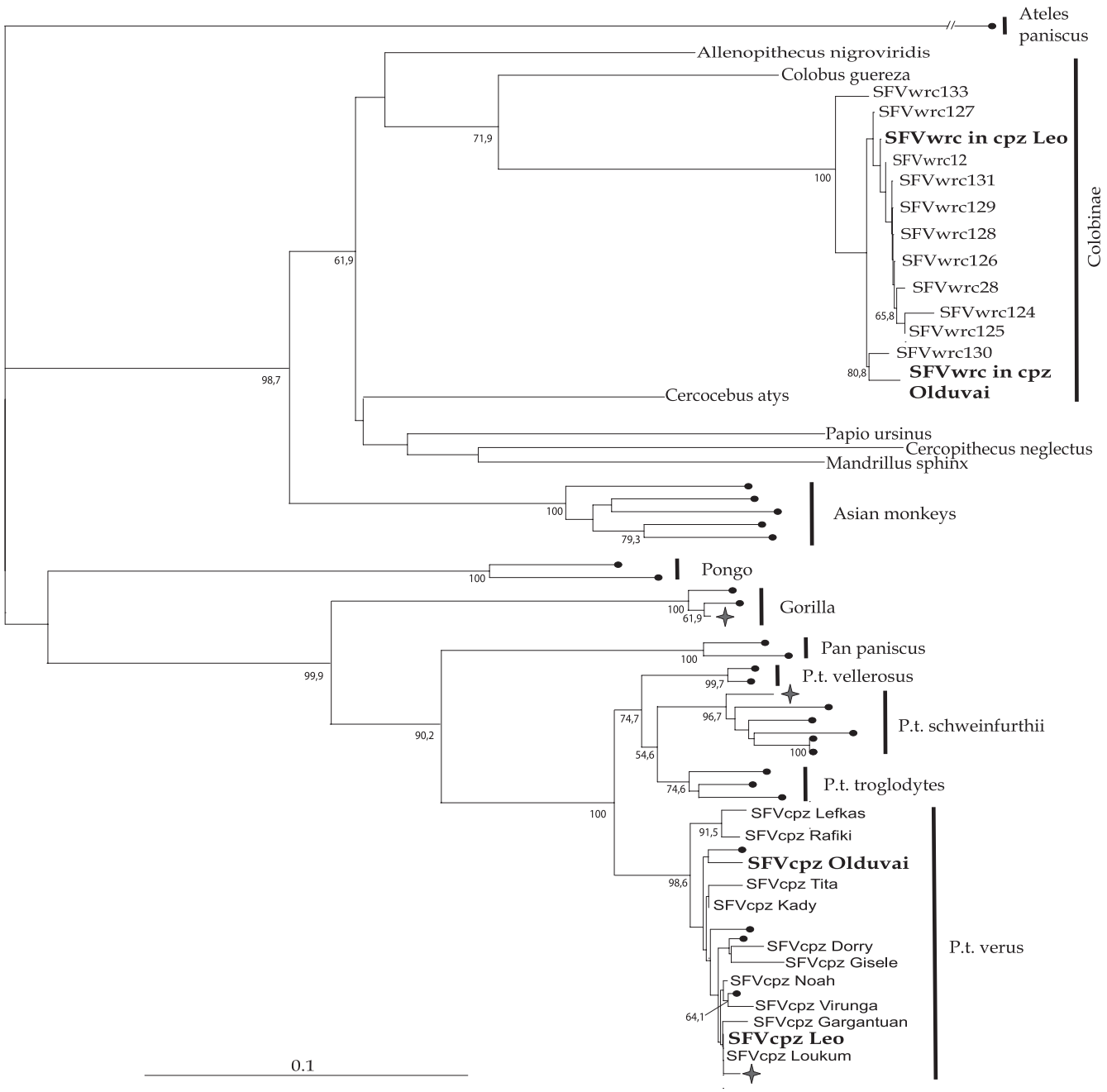

FIG. 1. Phylogenetic tree of 389-bp fragments of the SFV integrase-encoding regions of SFV_{cpz}, SFV_{wrc}, and other representative SFV strains. The tree was generated using the neighbor joining method (PHYLIP 3.572 package). Bootstrap resampling with 1,000 replicates was employed to place approximate confidence limits on individual branches. Percentages of bootstrap support for internal nodes are shown for values above 50. Dots represent SFV strains for the corresponding species, indicated by the vertical bar. Stars represent foamy viruses found in humans. For sequences generated in this study, exact names are given. Names of chimpanzees with double infection with SFV_{cpz} and SFV_{wrc} are in boldface and enlarged. An SFV spider monkey sequence (X83298) was used as an outgroup. Other sequences used were SFV sequences from African monkeys (*Allenopithecus nigroviridis*, AM492842; *Colobus guereza*, AY278791; *Cercocebus atys*, AF049079; *Papio ursinus*, AY686179; *Cercopithecus neglectus*, AY278782; *Mandrillus sphinx*, AY583781), Asian monkeys (*Macaca silenus*, AY686201; *Macaca cyclopis*, X83290; *Macaca tonkeana*, DQ354075 and DQ354089; *Rhesus* sp., X83292), and great apes (*Pongo* sp., AM492904 and AY686203; *Gorilla* sp., AY195688 and AY27879; *Pan paniscus*, AF049086 and AJ627550; *Pan troglodytes vellerosus*, AY639141 and AY639122; *Pan troglodytes schweinfurthii*, AY195675, AY195676, EU1027480, EU1027409, and EU1027490; *Pan troglodytes troglodytes*, AY639126, AY639123, and AY639128; *Pan troglodytes verus*, AY195685, X83297, X83296, AY195682, DQ142644, DQ142659 to 142666, EU248952 to EU248954) and foamy virus sequences from humans (AY195698, AY278776, and X83294).

maximum likelihood algorithms (PHYLIP 3.572 package; data not shown) revealed two well-separated clusters of colobus and chimpanzee SFV strains. Sequences obtained from chimpanzee samples using SFV_{wrc}-specific primers showed that 2 of the 14 chimpanzees already infected with SFV_{cpz} were simultaneously infected with SFV_{wrc} (Fig. 1). Two chimpanzees were SFV_{cpz} negative; however, these were infants, and it has been shown that seroconversion usually occurs when primates get older (3, 11). These two chimpanzees were also negative for SFV_{wrc}. It should be noted that chimpanzee and colobus sam-

TABLE 1. Chimpanzees from the Taï National Park (Côte d'Ivoire) analyzed for SFV_{cpz} and SFV_{wrc} infection

Chimpanzee name	Group/age (yr)/sex[a]	PCR result for[b]:		STLV result for LTR (8/14)[c]
		Int_all (12/14)	Int_wrc (2/14)	
Gisèle	N/~5/f	+	−	+
Gargantua	N/~10/m	+	−	+
Dorry	N/~10/f	+	−	+
Loukoum	N/~27/f	+	−	+
Lefkas	N/~8/m	+	−	−
Leonardo	N/~2/m	−	−	−
Leo	M/~29/m	+	+	+
Noah	M/~7/m	+	−	−
Kady	M/~30/f	+	−	+
Rafiki	S/~19/m	+	−	+
Tita	S/~20/f	+	−	−
Virunga	S/~25/f	+	−	+
Ophelia	S/~2/f	−	−	−
Olduvai	S/~8/m	+	+	−

[a] N, north group; M, middle group; S, south group (4, 13). f, female; m, male.
[b] +, positive result; −, negative result; Int_all, generic integrase primer used for the detection of SFV_{cpz}; Int_wrc, colobus-specific integrase primer. Fractions of positive results are in parentheses.
[c] STLV results are taken from reference 13. LTR, long terminal repeat. The fraction of positive results is in parentheses.

ples were collected at different times on different occasions and handled separately at all times and that extensive precautions were taken to avoid cross-contamination during DNA preparation and PCR analysis.

The two dually infected chimpanzees harbored unique SFV_{wrc} strains, indicating the introduction of SFV_{wrc} on independent occasions, rather than onward transmission of SFV_{wrc} among chimpanzees. Several studies have shown that exposure to saliva is a predominant route of SFV transmission (6, 7, 10, 21). It is thus likely that the two chimpanzees acquired SFV_{wrc} in the context of predation, as especially the males are frequent hunters and may be bitten by their prey. In addition, male chimpanzees consume significantly more monkey meat than females and chew entire bones, a habit that may cause lesions in the oral cavity (4). The two SFV_{wrc}-positive chimpanzees were both males, known to hunt frequently. We had previously demonstrated infection of these chimpanzees with another retrovirus, the simian T-cell leukemia virus type 1 (STLV-1), as a result of red colobus monkey consumption. However, in that study no preexisting infection with a chimpanzee-specific STLV was found (13). Noninvasive methods, such as those described by others (15a), will allow specific and more-extensive molecular epidemiological screenings of entire chimpanzee populations for SFV_{cpz} and for strains specific to other primate species. Others have, for example, shown the presence of one *Cercopithecus* SFV among 177 SFV-positive chimpanzee fecal samples (15a). However, generic integrase primers were used for this study. The use of species-specific foamy virus primers would most likely reveal a higher prevalence of SFV from other monkey species in the predator chimpanzee. Such screenings will provide further insight into interspecies SFV transmission and will help to identify risk factors associated with SFV transmission, such as hunting frequency and feeding strategies documented in the behavior studies. Our results demonstrate that, in addition to the presence of a species-specific SFV in a given host, transmission of other SFV from other species can occur in a natural primate predator-prey system, resulting in infections with divergent SFV isolates.

Moreover, prior infection with SFV_{cpz} does not seem to exclude infection with other SFV strains, a finding that may have important implications for foamy viruses as vaccine vectors (2). Coinfection has the potential to generate recombinants if the coinfecting viruses replicate in the same cell, possibly resulting in new pathological properties of viruses, as has been shown for simian immunodeficiency virus (1). Further studies are needed to specifically test for strain recombination and the existence of recombinant SFV lineages in wild chimpanzees. At this point, the phylogeny of the chimpanzee SFV_{cpz} strains described here clearly supports the cospeciation hypothesis suggested by others (20).

The high prevalence of SFV_{cpz} may be explained by intense social interactions, such as biting, whereas the low prevalence of SFV_{wrc} in the chimpanzees suggests that SFV_{wrc} is not or is rarely transmitted between the chimpanzees. This points to a dead-end infection of the superinfecting strain, as observed (albeit with a limited number of cases) in humans infected with SFV strains from nonhuman primates (5, 17, 21). Again, noninvasive methods will help address these issues, using wild chimpanzees as a model to investigate the fate of species-specific and new SFV in a host.

Nucleotide sequence accession numbers. New sequences of integrase-encoding regions of SFV strains were deposited in GenBank under accession numbers EU545383 to EU545395.

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