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Evolution

Positive selection and rates of evolution in immunodeficiency viruses from humans and chimpanzees

(positive selection/relative rates of evolution/speciational evolution/immune surveillance/human immunodeficiency virus)

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ABSTRACT **Evolutionary theory predicts the recent spread of primate immunodeficiency viruses (PIVs) to new human populations to be accompanied by positive selection in response to new host environments and/or by random genetic drift. I assess evidence for positive selection in human and chimpanzee PIVs type 1 (PIV1s), using ratios of synonymous to nonsynonymous nucleotide change based on branch lengths and outgroup rooting. Ratios are smaller for PIV1s from humans than for PIV1 from a chimpanzee for the *pol*, *gag*, and *env* glycoprotein 120 (gp120) regions, indicating greater effects of positive selection in PIV1s from humans. Parsimony-based relative rate tests for amino acid changes showed significant differences between PIV1s from humans and chimpanzees in 18 of 48 pairwise comparisons, with all 18 showing faster rates of change in PIV1s from humans. This study indicates that in some instances, the recent evolution of human PIV1s follows a speciational pattern, in which increased diversification of taxa is correlated with greater amounts of character change appearing and being maintained through time. This extends the generality of the speciational pattern to a group of organisms (viruses) having the fastest known rates of anagenetic change for nucleotide characters and indicates that comprehensive understanding of PIV1 evolution requires consideration of both anagenetic change within viral lineages and the relative historical success of different viral clades. Phylogenetic analyses show that neither PIV1s infecting humans nor those infecting chimpanzees represent monophyletic groups and suggest multiple host-species shifts for PIV1s.**

Primate immunodeficiency viruses (PIVs) have been colonizing new human populations rapidly since the mid-1970s. Human PIV1s from Africa include the greatest known diversity of human PIV1 subtypes, representing the earliest divergent clades, and various subsets of these subtypes can now be found on all continents (1–3). Evolutionary theory predicts that this rapid geographic spread is likely to be accompanied by viral molecular sequence change that is either adaptive in response to new host environments or due to random genetic drift. Greater amounts of nonsynonymous (resulting in amino acid change) relative to synonymous (no amino acid change) nucleotide substitution characterize adaptive change and selection but not drift. Previous assessments of selection in PIV1s have focused on specific gene regions and have identified multiple, potentially overlapping, mechanisms, including selection for variants escaping the host's immune system surveillance (4–6), selection for macrophage or T-cell tropism (7, 8), selection for both convergence and diversification in the primary antigenic determinant (V3 loop) (7, 9), and selection for resistance to antiviral drugs such as 8-azidothymidine (AZT) (10). Theoretical studies have indicated the feasibility of selection favoring changes in replication rates, transmission rates, and levels of virulence in response to environmental variables such as changes in host density and behavior and the cost of resistance (11–13).

Multiple selective forces can operate concomitantly, and their effects on PIV1 sequence evolution may be mixed in varying degrees. Thus, no single selective mechanism is likely to explain patterns of change across diverse gene regions. In addition to positive selection operating on individuals within populations (microevolution), substantial evolutionary change may be due to differential success of clades in terms of speciation rate and longevity (macroevolution). A speciational or punctuational view of evolutionary tempo (14) posits that change accrues faster during periods of cladogenesis and potential adaptive radiation than is found afterwards. Here, I assess evidence for both positive selection and speciational evolution in PIV1s from humans and chimpanzee in comparisons of the PIV1 protein-coding genome overall and piecemeal. I provide a phylogenetic analysis of human and chimpanzee PIV1s to enable PIV1 comparisons based on branch lengths. Names for viral lineages follow a phylogenetic taxonomy based on common descent (15), with PIV1s including viruses previously called human immunodeficiency virus type 1 (HIV1s) and simian immunodeficiency viruses (SIVs) from chimpanzee (*Pan troglodytes*).

METHODS

I obtained PIV nucleotide sequences from the Human Retroviruses and AIDS data base [world wide web address: http://hiv-web.lanl.gov) and fit aligned amino acid sequences to corresponding nucleotide strings by using CLUSTAL V (16) and DNA STACKS (17). Human PIV1 sequences selected represent phylogenetically defined subtypes A, B, D, and O (ref. 18; Fig. 1), which includes all of the currently designated human PIV1 subtypes having entire genomes available for analysis. I conducted parsimony analysis for nine PIV1s and two PIV2s, using exhaustive branch and bound searches as implemented in PAUP version 3.1.1 (21). Gaps were treated as missing characters and regions of uncertain alignment with numerous gaps were omitted from analyses to reduce homoplasy. The data set includes 2789 base positions from the *pol* region, 891 from the *gag* region, and 1379 from the *env* region (5059 total). The third hypervariable region (V3) within *env* was omitted from analyses because of its hypervariability and uncertainties in alignment.

Relative proportions of synonymous and nonsynonymous change have been applied previously in assessment of potential selective effects in immunodeficiency viruses (6, 22, 23) and maintenance of polymorphism at major histocompatibility complex loci (24). Ratios of synonymous to nonsynonymous changes (S/N) also work to reduce potential biases imposed by any fluctuations across taxa in the mutation rate associated

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Abbreviations: PIV, primate immunodeficiency virus; S/N, ratio of synonymous/nonsynonymous changes; gp, glycoprotein; V3, third hypervariable region.

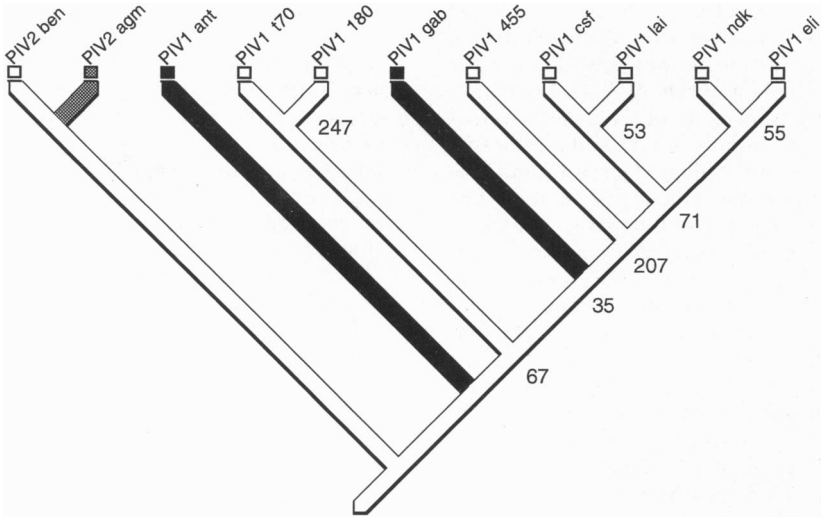

FIG. 1. Most parsimonious tree for nine PIV1s using two PIV2s as a combined outgroup based on 5059 nucleotide base positions total from the *pol, gag*, and *env* gene regions and three different weighting schemes (see text). With equal weighting for all characters, this tree entails 6720 steps and has a consistency index of 0.72. Numbers by branch nodes denote support indices (19) describing the difference in length between the most parsimonious tree and the shortest tree in which the particular clade is not present. Branch shading denotes viral host species and indicates the most parsimonious history of host-species shifts (black = *Pan troglodytes* [chimpanzee], white = *Homo sapiens*, gray = *Cercopithecus aethiops* [African green monkey]). Virus names are based on a phylogenetic taxonomy (15), with PIV1s including viruses previously called HIV1s and SIVs from chimpanzee, and PIV2s including former HIV2s and SIVs from African green monkeys. Virus abbreviations, host species (*Hs* = *Homo sapiens*), human PIV1 subtype (18), and data base accession numbers are: PIV1 455, *Hs*, subtype A, M62320; PIV1 csf, *Hs*, subtype B, M38429; PIV1 lai, *Hs*, subtype B, K02013; PIV1 eli, *Hs*, subtype D, K03454; PIV1 ndk, *Hs*, subtype D, M27323; PIV1 t70, *Hs*, subtype O, L20587, M31171; PIV1 180, *Hs*, subtype O, L20571; PIV1 gab, *P. troglodytes*, X52154; PIV1 ant, *P. troglodytes* (U42720; see ref. 20 for description); PIV2 ben, *Hs*, M30502; PIV2 agm, *Cercopithecus aethiops*, M30931.

with changing modes of viral transmission (endogenous or exogenous) and levels of virulence affecting viral replication rates. I use S/N ratios to test the hypothesis that PIV1s infecting humans have experienced greater selection pressure than PIV1s infecting chimpanzees. The power and novelty of this test derives from use of outgroups in calculating S/N ratios. Because the ingroup taxa are nearly equal in age relative to the outgroup, rate differences can be determined between them without knowledge of their absolute ages and without the assumption of equal time since divergence among lineages, which is required in genetic distance comparisons lacking reference to an outgroup. Among the study taxa, most human PIV1s were collected before the chimpanzee PIV1s, and potential bias due to slight age differences would favor slower rates in human PIV1s, opposite to the observed trend.

Both S/N ratios and relative rate tests are based on branch lengths from a series of phylogenetic trees with four taxa. These trees include all pairwise comparisons of the ingroup taxa (PIV1s) with two outgroup taxa and consider only the ingroup branch lengths following divergence from their most recent shared node. Relative rate tests use a binomial test for departure from the expectation of equal branch lengths for ingroup taxa if evolutionary rates are equal (25).

RESULTS

The same most parsimonious tree (Fig. 1) was found in three separate analyses for all gene regions combined: (*i*) equal weighting for all characters, (*ii*) use of first and second codon positions only, and (*iii*) giving an *a priori* weight of 0 to T ↔ C changes in all gene regions and a further weight of 0 for A ↔ T and A ↔ G changes in the *env* gene region (encoding gp120 and gp41) only. Unequal weighting schemes *ii* and *iii* are based on the faster rate of accumulation for substitutions at third codon positions, the generally faster rate of change in *env* compared with *pol* and *gag* (26), and plots (saturation curves) of numbers of substitutions for each of the six possible nucleotide pairs against percent divergence for all changes in pairwise comparisons of taxa. In these latter plots (not shown), substitution types given a weight of 0 in *iii* above appear most prone to saturation (and homoplasious similarity) on the basis of a reduction in the slope of their curves. Although analyses *ii* and *iii* involve less homoplasy, the homoplasy present in analysis *i* does not overwhelm the phylogenetically informative character changes. Congruent results from the three analyses increase confidence in the tree. One hundred bootstrap reanalyses using weighting scheme *iii* found all nodes in Fig. 1 to be supported 100% of the time. Neither PIV1s infecting humans nor PIV1s infecting chimpanzees represent monophyletic groups (Fig. 1).

P_S and P_N denote proportions of synonymous and nonsynonymous nucleotide differences per synonymous and nonsynonymous sites, respectively, and were calculated by using the program MEGA (27) to assess sequence saturation and to augment S/N ratios based on branch lengths. Mean P_S/P_N ratios for *pol, gag, env* gp41, and *env* gp120 were 7.28, 5.47, 3.40, and 2.81, respectively, for all pairwise comparisons of the five most closely related human PIV1s (455, lai, csf, ndk, eli). Negative (purifying) and positive selection are inversely related in that the former entails higher P_S/P_N ratios with most nonsynonymous changes selected against, and the latter entails lower ratios with more nonsynonymous changes allowed or selected for. Coffin (28) described retrovirus experiments showing selective effects for nearly all variants and argues, reasonably, that no retrovirus substitutions can be assumed to be entirely neutral. In this light, I interpret the P_S/P_N ratios as indicating that purifying selection is decreased and positive selection increased in considering the genes in the order listed above of decreasing P_S/P_N. This view does not rule out selective effects for synonymous changes (on features such as RNA structure), though it does suppose greater selective effects, on average, for amino acid substitutions.

S/N ratios, using values based on branch lengths and outgroup rooting, are smaller for PIV1s from humans than for a PIV1 from a chimpanzee for the *pol, gag*, and *env* gp120 regions (Fig. 2). This indicates greater amounts of nonsynonymous change relative to synonymous change and increased effects of natural selection in PIV1s from humans for these genes. S/N ratios are nearly the same for the *env* gp41 region,

indicating greater similarity in selection pressure. Multiple substitutions at numerous individual sites (saturation) can compromise sequence comparisons; however, this is not the case for the comparisons in Fig. 2 or for the P_S/P_N ratios above. Saturation can be inferred from plots of P_S and P_N versus percent sequence divergence for pairs of taxa and is indicated by a leveling of the P_S curve, with values staying around 0.60 to 0.70 despite increasing percent divergence for all four gene regions (Fig. 3). However, all pairwise comparisons used in Fig. 2 and the P_S/P_N ratios involve percent divergence values smaller than those falling under plateaus of the curves in Fig. 3. This is necessary to minimize confounding effects of homplasious similarity that can arise from saturation. Comparisons involving the earlier diverging chimpanzee sequence (PIV1 ant, Fig. 1) were not included in Fig. 2 or the P_S/P_N ratios because those percent divergence values do fall below the extended plateaus of the Fig. 3 curves. Fig. 3 indicates a faster rate of accumulation for synonymous changes and greater saturation for synonymous changes when considering older divergences, as expected. This also indicates greater constraints bearing on nonsynonymous than synonymous changes.

Relative rate comparisons for amino acid changes showed significant differences between PIV1s from humans and chimpanzees in 18 of the 48 pairwise comparisons (37.5%) with all 18 showing faster rates of change in PIV1s from humans (Table 1). These 18 significant differences are congruent with the S/N ratios and denote a tendency for accelerated rates of amino acid change in some PIV1s from humans. Significant rate differences were more common in the *env* gp120 (58.3%) and *pol* (66.7%) regions, compared with the *env* gp41 (16.7%) and *gag* (8.3%) regions. Plots of P_N (Fig. 3) suggest that the amino acid sequences involved in these comparisons are not saturated with change and thus are historically informative. Although 83.3% of all comparisons showed more amino acid character changes in PIV1s from humans compared with those from chimpanzees, 62% of the pairwise comparisons were not significantly different.

DISCUSSION

Selection Within Host Species. The S/N ratios indicate that natural selection has had greater effects on molecular sequence evolution in some PIV1s infecting humans than in related PIV1s from a chimpanzee (Fig. 2). This is found not just in the *env* gp120 region encoding surface antigens but also in the *gag* and, to a lesser degree, *pol* regions encoding (*i*) matrix, capsid, and nucleocapsid structural proteins and (*ii*) reverse transcriptase, endonuclease, and protease enzymes, respectively. When one considers the presence of this effect across different gene regions and recognizes individual virus particles, rather than particular genes or gene regions, as units of selection, the findings suggest that at least some PIV1 lineages are undergoing a degree of organismal adaptive radiation in humans as seen in other organisms either subject to changed environments or colonizing new environments (founder events). The recent history of PIV1s, spreading rapidly within and among human populations since the 1970s or earlier (3), is consistent with this view.

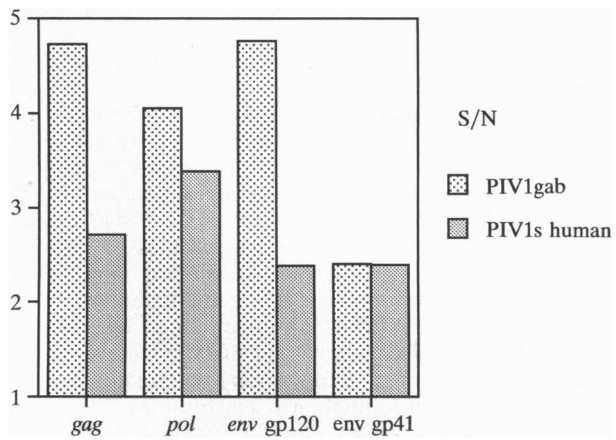

FIG. 2. S/N ratios for four genes in PIV1s from a chimpanzee (PIV1 gab) and averaged from five humans (PIV1s 455, lai, csf, eli, and ndk). Ratios are based on branch lengths from a series of four-taxon phylogenetic trees, each using PIV1s t70 and 180 as a combined outgroup. Smaller ratios indicate proportionally more nonsynonymous change and provide evidence of effects of natural selection.

Because each taxon is used repeatedly in calculation of S/N ratios and in calculation of relative rate tests, not all of the ratios or relative rate tests are independent from one another. This precludes significance tests for differences among S/N ratios in Fig. 2 and overinterpretation of the number of significant versus nonsignificant binomial tests in Table 1. Though I found evidence indicating the influence of natural selection across diverse gene regions for human PIV1s, the analyses do not suggest that selection explains all or even most human PIV1 diversification. Viral diversification does not depend on selection alone and is related to many other factors, including high mutation rates, high reproductive rates, variation in functional constraints, potential genetic drift, relative transmissibility, and differential extinction rates. In this context, ratios and relative rate tests in which some comparisons implicate natural selection while others do not is expected. Evidence of stronger positive selection is seen in studies focusing on the human PIV1 V3 region; however, even in this antigenic determinant region, varying degrees of susceptibility to natural selection among closely related viral lineages is seen (6).

The evolution of phylogenetically defined human PIV1 subtypes reflects coevolution between viruses and hosts, as viruses encounter variation among human individuals and populations in immune system capabilities, cultural factors influencing transmission (such as rates of sexual partner change), and occurrence of coinfecting pathogens that may influence PIV transmission and fitness. For example, increased PIV1 transmission rates have been found in individuals with other sexually transmitted diseases (29, 30). Distribution of other viruses may influence selective effects on PIV1s by means of "phenotypic mixing" in which PIV1 genomes are inadvertently placed within the envelope of other viruses, thereby acquiring different cellular host ranges. Viruses that are susceptible to such phenotypic mixing with PIV1s and that vary in frequency among human populations include PIV2, HTLV-I, vesicular stomatitis virus, and herpesviruses (reviewed in ref. 31). Relative rate tests showing faster amino acid evolution in PIV1s from humans compared with PIV1s from chimpanzees (Table 1) are consistent with the interpretation of greater selection pressure in human PIV1s as well.

I do not link the inferred increase in selection pressure to any specific mechanism for several reasons. There are numerous ecological variables that can influence coevolution between PIV1s and their hosts, and, when considering large sections of the genome, any selective effects stemming from those different variables are expected to be mixed. Further, specific selective forces may vary in impact over time as the host environment changes. Recombination events among PIV1s such as those identified by Robertson *et al.* (32) can provide additional variation for selection to act upon, and their potential effects will be embedded within results from virus sequence comparisons. Random sampling events in virus transmission and in the collection of virus sequences may also contribute to patterns of variation across taxa (33). However, random sampling events within PIV1 subtype populations, will yield less variability than that found in comparisons between different phylogenetically defined subtypes.

Difference in S/N ratios between PIV1s from humans and chimpanzees are greatest in *env* gp120, followed in descending

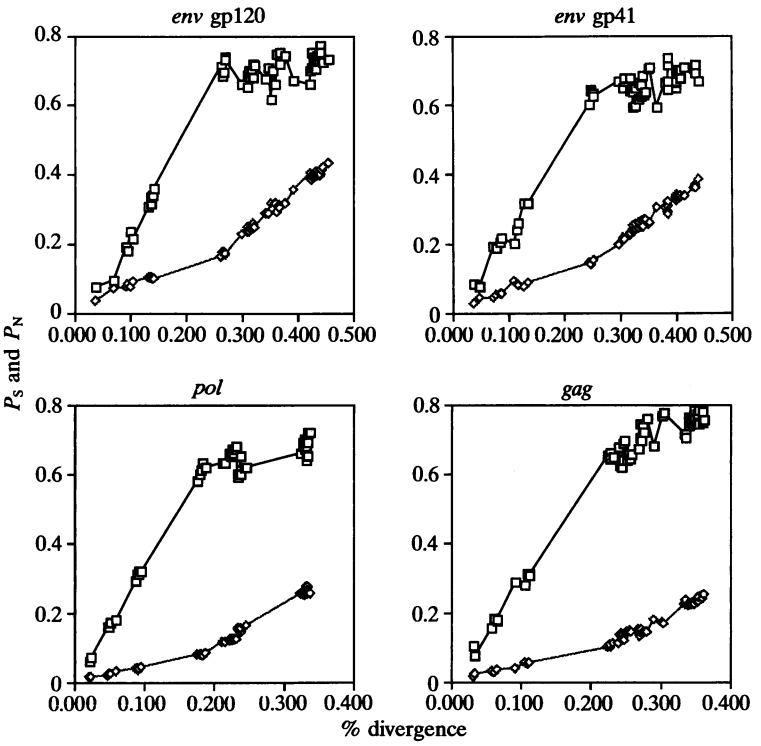

FIG. 3. Plots of P_S (□) and P_N (◇) versus percent divergence for all pairwise comparisons among 11 taxa (see Fig. 1 for taxa names). P_S and P_N denote proportions of synonymous and nonsynonymous nucleotide differences per synonymous and nonsynonymous sites, respectively (27).

order by *gag*, *pol*, and *env* gp41 (Fig. 2). The *env* gp120 ratios support previous hypotheses of selection for variants escaping immune surveillance, despite removal of primary epitope V3 sequences from the current analyses, as non-V3 *env* gp120 regions have been implicated in antigenic determination (see ref. 31). Similarly, the *gag* S/N ratios are also consistent with selection for escape mutants, as *gag* sequence variation has been implicated in loss of recognition of viral antigens by cytotoxic T lymphocytes (5). However, the number of base substitutions involved in immune surveillance escape is not well known and may be small. *pol* S/N ratios are also smaller in human PIV1s, though to a lesser degree. Greater positive selection on *pol* sequences could be related to several factors, including resistance to antiviral medications and coevolution for compatibility with the host cells' replication enzymes and processes. However, resistance to antiviral medication can entail only three or four nucleotide substitutions from "wild type" (10) and would have relatively little impact on S/N ratios. Nearly identical ratios for *env* gp41 are reflected in small relative rate test differences (Table 1) and do not suggest significant differences in selective pressure experienced by human and chimpanzee PIV1s.

Selection Between Host Species. Accelerated rates of amino acid evolution have been predicted for viral lineages colonizing new hosts in response to the new selective pressures imposed by the immune system and cell composition of a new host (34), and such an increased rate has been reported for influenza viruses (35, 36). This is an extension of the adaptive radiation phenomenon discussed above, put in the context of a shift or transmission between host species rather than within a host species. By this view, increased selective effects on human PIV1s might be seen as evidence for a PIV1 host shift from

Table 1. Phylogenetic relative rate tests for amino acids in pairwise comparisons of PIV1s from chimpanzees (chimp) (gab, ant) and humans

Chimp PIV1s	Human PIV1s						
	455	lai	csf	eli	ndk	t70	180
env gp120							
gab	+15/25	+12/27*	+13/30*	+16/24	+14/26*		
ant	+19/33*	+22/31	+21/30	+21/32	+20/34*	+15/42*	+22/42**
env gp41							
gab	+15/21	+14/15	+14/17	+14/18	+13/18		
ant	−20/16	+14/15	−21/16	−16/15	−18/17	+11/27**	+10/27**
gag							
gab	+15/22	+12/20	+13/19	+13/23	+13/25*		
ant	−22/18	−17/16	−19/18	−18/17	+15/16	+19/25	+21/27
pol							
gab	+34/54*	+32/49*	+35/48	+35/52*	+35/44		
ant	+52/78*	+50/75*	+52/77*	+49/77**	+49/73*	+63/78	+68/79

Numbers shown denote branch lengths for the taxon in the leftmost column/taxon in the top row, respectively; + or − denotes whether the PIV1 from humans showed more or fewer character changes, respectively. Combined outgroups used in calculating branch lengths were PIV1s t70 and 180 for comparisons involving PIV1 gab, and PIV2s agm and ben for comparisons involving PIV1 ant (see Fig. 1 for taxon abbreviations). *, $P < 0.05$; **, $P < 0.01$.

chimpanzees to humans. However, in this particular case that interpretation is not justified. Positive selection on human PIV1s has been demonstrated in sequential studies within host individuals and host populations (refs. 7 and 37; see ref. 8), and these selective effects will tend to overwrite any imposed earlier at the time of a host-species shift. Shpaer and Mullins (4) report a correlation between increased rates of nonsynonymous change and pathogenicity among PIVs, and the apparent greater virulence of PIV1s in humans than chimpanzees is consistent with the view of selection effects occurring within rather than between host species. However, apparent low virulence of PIV1s in chimpanzees is not well established. Thus, selection on PIV1s between and within host species can yield the same effect on rates of amino acid change and S/N ratios, and neither can be ruled out. Our poor understanding of the molecular-level features of PIVs limiting host specificity and previous observations (38) for other viruses that host specificity and pathogenicity may change with a small number of point mutations argue for a cautious approach.

The phylogenetic analysis (Fig. 1) suggests multiple independent colonizations (host-species shifts) by PIVs of humans, chimpanzees, or both. Although, our knowledge of the presence or absence of PIVs for primate taxa is limited, the current evidence on PIV phylogeny and PIV distribution among primates is consistent with humans being the ancestral host for PIVs currently found in chimpanzees and other primates species as well (15). This is based on (*i*) the use of parsimony to assess the fewest host-species shifts for PIVs and (*ii*) the fact that PIVs in humans show greater phylogenetic diversity than is currently found in viruses from any other infected primate species. To better estimate the sequence of host-species infections, more information is needed on the distribution of PIVs in other primates species (greater sampling of species and populations) and their pathogenicity.

Historical Maintenance of Diversity. Historical diversification of PIV1 taxa, stemming from founder events, barriers to dispersal, on-going nucleotide sequence change, and extinction, provides a mechanism for protecting change regardless of whether it is adaptive or not (see refs. 39 and 40). Relative rate tests for amino acid evolution indicate that the recent evolution of human PIV1s tends to follow a speciational pattern in which an apparent greater diversification of taxa is correlated with greater amounts of character change being maintained through time (Table 1). This extends the generality of the speciational, punctuated pattern to a group of organisms (viruses) having the fastest known rates of anagenetic change for nucleotide characters and indicates that relatively little character change being preserved in any particular clade cannot be attributed to low rates of anagenesis. Thus, comprehensive understanding of PIV1 (and other virus) evolution requires consideration of changes within viral lineages as well as the relative historical success of different higher level viral clades. Little work has been done on the latter, and we do not know whether differential historical success is due to particular attributes of entire clades being selected for or to randomly varying rates of branching and extinction.

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1. Louwagie, J., McCutchan, F., van der Groen, G., Peeters, M., Fransen, K., Piot, P., Gershy-Damet, G.-M., Roelants, G., van Heuverswyn, H., Eddy, G., Ungar, B. & Burke, D. (1992) *AIDS Res. Hum. Retroviruses* **8,** 1467–1469.
2. Louwagie, J., McCutchan, F., Peeters, M., Brennan, T. P., Sanders-Buell, E., Eddy, G., van der Groen, G., Fransen, K., Gershy-Damet, G.-M., Deleys, R. & Burke, D. (1993) *AIDS* **7,** 769–780.
3. Myers, G. (1994) *AIDS Res. Hum. Retroviruses* **10,** 1317–1324.
4. Shpaer, E. G. & Mullins, J. I. (1993) *J. Mol. Evol.* **37,** 57–65.
5. Phillips, R. E., Rowland Jones, S., Nixon, D. F., Gotch, F. M., Edwards, J. P., Ogunlesi, A. O., Elvin, J. G., Rothbard, J. A., Bangham, C. R., Rizza, C. R. & McMichael, A. J. (1991) *Nature (London)* **354,** 453–459.
6. Seibert, S. A., Howell, C. Y., Hughes, M. K. & Hughes, A. L. (1995) *Mol. Biol. Evol.* **12,** 803–813.
7. Holmes, E. C., Zhang, L. Q., Simmonds, P., Ludlam, C. A. & Leigh Brown, A. J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4835–4839.
8. Leigh Brown, A. J. & Holmes, E. C. (1994) *Annu. Rev. Ecol. Syst.* **25,** 127–165.
9. Holmes, E. C., Zhang, L. Q., Robertson, P., Cleland, A., Harvey, E., Simmonds, P. & Leigh Brown, A. J. (1995) *J. Infect. Dis.* **171,** 45–53.
10. Boucher, C. A., O'Sullivan, E., Mulder, J. W., Ramautarsing, C., Kellam, P., Darby, G., Lange, J. M., Goudsmit, J. & Larder, B. A. (1992) *J. Infect. Dis.* **165,** 105–110.
11. Levin, S. A. & Pimentel, D. (1981) *Am. Nat.* **117,** 308–315.
12. Anderson, R. M. & May, R. M. (1991) *Infectious Diseases of Humans: Dynamics and Control* (Oxford Univ. Press, New York).
13. Ewald, P. W. (1994) *The Evolution of Infectious Disease* (Oxford Univ. Press, New York).
14. Gould, S. J. & Eldredge, N. (1993) *Nature (London)* **366,** 223–227.
15. Mindell, D. P., Shultz, J. W. & Ewald, P. W. (1995) *Syst. Biol.* **44,** 77–92.
16. Higgins, D., Bleasby, A. J. & Fuchs, R. (1992) *Comput. Appl. Biosci.* **8,** 189–191.
17. Eernisse, D. J. (1992) *Comput. Appl. Biosci.* **8,** 177–184.
18. Myers, G., Korber, B., Wain-Hobson, S., Jeang, K.-T., Henderson, L. E. & Pavlakis, G. N., eds. (1994) *Human Retroviruses and AIDS: A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences* (Los Alamos National Laboratory, Los Alamos, NM).
19. Bremer, K. (1988) *Evolution* **42,** 795–803.
20. Peeters, M., Fransen, K., Delaporte, E., Vanden Haesevelde, M., Gershy-Damet, G.-M., Destens, L., van der Groen, G. & Piot, P. (1992) *AIDS* **6,** 447–451.
21. Swofford, D. L. (1993) PAUP (distributed by the Illinois Natural History Survey, Champaign), Version 3.1.1.
22. Burns, D. P. W. & Desrosiers, R. C. (1991) *J. Virol.* **65,** 1843–1854.
23. Simmonds, P., Balfe, P., Ludlam, C. A., Bishop, J. O. & Leigh Brown, A. J. (1990) *J. Virol.* **64,** 5840–5850.
24. Hughes, A. L. & Nei, M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 958–962.
25. Mindell, D. P. & Honeycutt, R. L. (1990) *Annu. Rev. Ecol. Syst.* **21,** 541–566.
26. McClure, M. A., Johnson, M. S., Feng, D.-F. & Doolittle, R. F. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2469–2473.
27. Kumar, S., Tamura, K. & Nei, M. (1993) MOLECULAR EVOLUTIONARY GENETICS ANALYSIS (The Pennsylvania State Univ., University Park), Version 1.01.
28. Coffin, J. M. (1992) *Curr. Top. Microbiol. Immunol.* **176,** 143–163.
29. Plummer, F. A., Simonsen, J. N., Cameron, D. W., Ndinya-Achola, J. O., Kreiss, J. K., Gakinya, M. N., Waiyaki, P., Cheang, M., Piot, P., Ronald, A. R. & Ngugi, E. N. (1991) *J. Infect. Dis.* **163,** 233–239.
30. Hunter, D. J. & Maggwa, B. N. (1994) *AIDS* **8,** 93–99.
31. Levy, J. A. (1994) *HIV and the Pathogenesis of AIDS* (Am. Soc. Microbiol. Press, Washington, DC).
32. Robertson, D. L., Sharp, P. M., McCutchan, F. E. & Hahn, B. H. (1995) *Nature (London)* **374,** 124–126.
33. Domingo, E. & Holland, J. J. (1994) in *The Evolutionary Biology of Viruses*, ed. Morse, S. S. (Raven, New York), pp. 161–184.
34. Steinhauer, D. A. & Holland, J. J. (1987) *Annu. Rev. Microbiol.* **41,** 409–433.
35. Gammelin, M., Altmüller, A., Reinhardt, U., Mandler, J., Harley, V. R., Hudson, P. J. & Fitch, W. M. (1990) *Mol. Biol. Evol.* **7,** 194–200.
36. Gorman, O. T., Bean, W. J., Kawaoka, T., Donatelli, I., Yuanji, G. & Webster, R. G. (1992) *J. Virol.* **65,** 3704–3714.
37. Cichutek, K., Norley, S., Linde, R., Kreuz, W., Gahr, M., Lower, J., von Wangenheim, G. & Kurth, R. (1991) *AIDS* **5,** 1185–1187.
38. Kawaoka, Y. & Webster, R. G. (1988) *Microb. Pathogen.* **5,** 311–318.
39. Futuyma, D. J. (1987) *Am. Nat.* **130,** 465–473.
40. Lenski, R. E. & Travisano, M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6808–6814.