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(vervet), chimpanzee, Chinese and Indian rhesus or cynomolgus macaques successfully yielded product from both batches. Sequencing and phylogenetic analyses indicate that the cells used to prepare 19 CHAT 10A-11 were obtained from rhesus macaques, and those for CHAT 6039 were from cynomolgus macaques (Fig. 1).

Failure to detect HIV/SIV sequences or chimpanzee cellular components in two OPV CHAT stocks, together with the positive identification of macaque mitochondrial sequences, provides no support for the hypothesis that these materials were responsible for the entry of HIV into humans and the source of AIDS.

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1. Hoopes, E. *The River: A Journey to the Source of HIV and AIDS* (Penguin, London, 1999).
2. Garrett, A. J., Dunham, A. & Wood, D. *The Lancet* 342, 932–933 (1993).
3. Dickson, D. *Nature* 407, 117 (2000).
4. Hahn, B., Shaw, G., DeCock, K. & Sharp, P. *Science* 287, 607–614 (2000).
5. Berry, N. J. et al. *J. Hum. Virol.* 1, 457–468 (1998).
6. WHO Network for HIV Isolation and Characterisation *AIDS Res. Hum. Retrovir.* 10, 1327–1343 (1994).
7. Zhu, T. et al. *Nature* 391, 594–597 (1998).

Human immunodeficiency virus

Phylogeny and the origin of HIV-1

The origin of human immunodeficiency virus type 1 (HIV-1) is controversial. We show here that viruses obtained from the Democratic Republic of Congo in Africa have a quantitatively different phylogenetic tree structure from those sampled in other parts of the world. This indicates that the structure of HIV-1 phylogenies is the result of epidemiological processes acting within human populations alone, and is not due to multiple cross-species transmission initiated by oral polio vaccination.

According to the oral polio vaccination (OPV) hypothesis, the main (M) group of HIV-1 (the viruses responsible for the majority of global AIDS cases) emerged as a result of the vaccination of about one million people, who were largely living in the Congo from 1957–60, with an oral vaccine against polio virus that had allegedly been cultured in chimpanzee kidneys¹. This is claimed to have enabled the transfer to humans of chimpanzee simian immunodeficiency virus, the closest relative of HIV-1.

Conversely, phylogenetic analysis of HIV-1 sequences indicates that group M originated

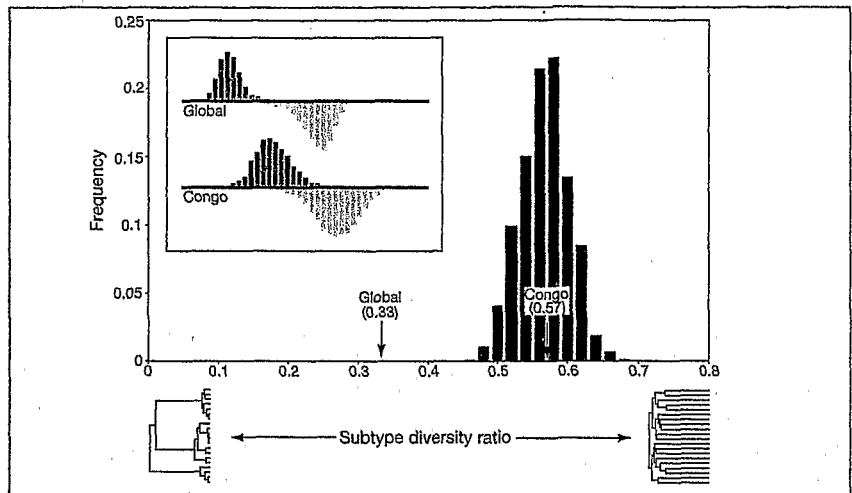


Figure 1 Maximum-likelihood phylogenies were estimated for three V3–V5 data sets: HIV-1 sequences from the Democratic Republic of Congo (423 base pairs), global isolates (426 base pairs), and Congo and global isolates combined (396 base pairs). Given a phylogeny with tips labelled according to subtype, the subtype diversity ratio (SDR) was calculated as the mean path length between tips of the same subtype divided by the mean path length between tips of different subtypes. For the phylogeny of the global isolates, 11 subtypes were allocated according to standard HIV-1 nomenclature⁷. For the Congo phylogeny, 11 subtypes were allocated so as to minimize the SDR score, using a heuristic optimization algorithm. This assignment is that which gives the maximum possible subtype structure for the Congo phylogeny. The global phylogeny gave an SDR of 0.33 and the Congo a value of 0.57. The analysis was repeated after removal of the Congo and global sequences previously identified as intersubtype recombinants^{4,5}. Our analysis will only be affected if recombination breakpoints fall within the V3–V5 region, so excluding recombinants changes the SDR only marginally (0.35 for the global phylogeny; 0.58 for the Congo). SDR values were similar when Congo isolates were assigned to different numbers of subtypes (for example, 0.59 and 0.55 in the case of 8 and 14 subtypes, respectively). To assess the significance of the difference between the global and Congo SDRs, we obtained a null distribution by simulating phylogenies under an exponential growth coalescent process inferred from *env* gene sequences of subtype A (ref. 6), which is common in Africa. The frequency distribution of minimum SDR values for these simulated phylogenies is shown in blue. Inset: normalized frequency distributions of intrasubtype path lengths (above the line) and intersubtype path lengths (below the line), plotted on the same horizontal scale (0.0–0.8 substitutions per site), for the global and Congo phylogenies. See supplementary information for details of trees and phylogenetic methods.

before the vaccination campaign², supporting a model of ‘natural transfer’ from chimpanzees to humans³. If this timescale is correct, then the OPV theory remains a viable hypothesis of HIV-1 origins only if the subtypes of group M differentiated in chimpanzees before their transmission to humans.

It has been suggested that the distinctive structure of the global group-M tree, which has been called a ‘starburst’ because of the apparently simultaneous appearance of viral subtypes, is consistent with the transfer of multiple viral lineages from chimpanzees to humans¹. To test this, we analysed partial *env* sequences (V3–V5) of 197 HIV-1 isolates sampled in 1997 from the Congo⁴, a likely location for the origin of HIV-1 group M under both hypotheses.

A phylogeny comprising the Congo data, plus 223 sequences representing the global diversity of HIV-1 (including all known subtypes), reveals comparable genetic diversity in the Congo strains to that among global strains, with many Congo lineages falling basal to the origin of each subtype as currently defined by the phylogeny of global strains⁵. We tested whether the structure of the Congo phylogeny differed from that of the global HIV-1 M group by comparing the subtype diversity ratio (SDR) of the two phylogenies (Fig. 1). This is defined as the ratio of the mean within-subtype pairwise distance to the

mean between-subtype pairwise distance.

Rather than assigning the Congo isolates to subtypes by their phylogenetic relationship to global strains, we used a heuristic algorithm to assign subtypes such that the subtype diversity ratio was minimized. The Congo and global phylogenies differ significantly in the SDR statistic, with the former showing no more subtype structure than phylogenetic trees simulated under a model of exponential population growth⁶ (Fig. 1; see supplementary information). This result is conservative because the minimum possible ratio value (representing maximum subtype structure) was used in the Congo analysis. Furthermore, although subtypes can be clearly identified in the distribution of pairwise distances for the global sequences (Fig. 1, inset), there is much less distinction between intra- and intersubtype comparisons for the Congo sequences. Hence, for any two randomly chosen Congo sequences, it is difficult to determine unambiguously whether they belong to the same or to different subtypes.

Our results indicate that the Congo and global phylogenies probably result from different epidemiological histories. As many Congo strains appear to be basal, we propose that each global subtype is the result of the chance exportation of some Congo strains to other geographical regions, thus producing an apparent starburst. Such founder effects have



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been proposed to explain the phylogenetically distinct subtypes B and E of HIV-1 group M (ref. 2). The observation that many Congo strains fall basal to the global subtypes also suggests that previous phylogenetic analysis has underestimated the number of lineages that pre-date 1957–60, and hence underestimated the minimum number of cross-species transmissions necessary to reconcile the OPV hypothesis with phylogenetic data.

In conclusion, the HIV-1 sequences from the Congo are evidence that the claim of the OPV theory¹ that it is “probably the only hypothesis of origin that can readily explain the starburst phenomenon” is incorrect. Our results give us no reason to doubt that the last common ancestor of HIV-1 group M was present in a human host.

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- Hooper, E. *The River: A Journey to the Source of HIV and AIDS* (Penguin, London, 1999).
- Korber, B. *et al. Science* 288, 1789–1796 (2000).
- Hahn, B., Shaw, G., de Cock, K. M. & Sharp, P. M. *Science* 287, 607–614 (2000).
- Vidal, N. *et al. J. Virol.* 74, 10498–10507 (2000).
- Los Alamos National Laboratory HIV Sequence Database, <http://hiv-web.lanl.gov>.
- Pybus, O. G., Rambaut, A. & Harvey, P. H. *Genetics* 155, 1429–1437 (2000).
- Robertson, D. L. *et al. Science* 288, 55–57 (2000).

Supplementary Information is available on Nature's website at www.nature.com or as paper copy from the London editorial office of Nature.

Chronobiology

Reversal of honeybee behavioural rhythms

Adult honeybees have sleep-like states^{1,2} and, like human infants³, bees develop their own endogenous circadian rhythms as they mature^{4,5}. But whereas disruption of our sleep cycles and synchronized internal rhythms may adversely affect our physiology and performance³, we show here that honeybees can revert to certain arrhythmic behaviours when necessary. To our knowledge, this chronobiological plasticity is the first example in any animal of a socially mediated reversal in activity rhythms.

Circadian rhythms in honeybees are an important component of the social behaviour development process that underlies the colony's division of labour. Larvae must be fed around the clock and are ‘nursed’ in the hive by young bees (5–15 days old) that work without any overt behavioural rhythms⁶. At about three weeks of age, however, a bee begins to forage outside the hive for pollen and nectar, an activity that calls for an inter-

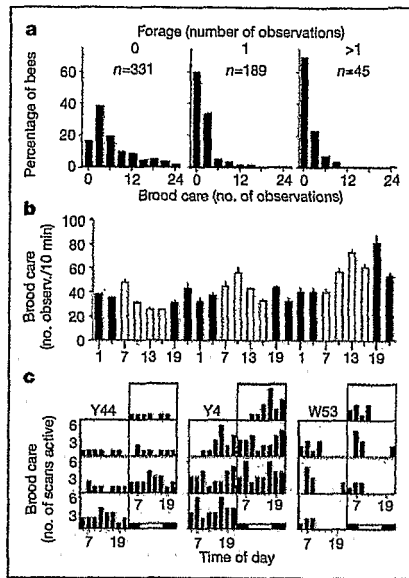


Figure 1 Reverted nurses care for brood with no diurnal rhythm. Brood care was observed under dim red light (invisible to bees) every 3 h for three days. Observations of brood care⁶: six 10-min visual scans of individually tagged bees in the vicinity of the brood. Foraging observations were made as described⁴. **a**, Reorganization of division of labour in reversion colonies: frequency distributions of brood care differed significantly (chi-square test, $P < 0.05$) for bees never observed foraging (0, left plot), observed foraging once (1, middle), or observed foraging more than once (> 1 , right). **b**, Colonial analysis. Mean (\pm s.e.) number of brood care events per observation period during the day (white bars) and night (black bars) ($n = 6$ scans per observation). To test comprehensively for diurnal rhythms, we pooled the data into two half-day categories and compared the amount of brood-care activity between them; this analysis was repeated for eight different half-day combinations. No behavioural rhythms were detected ($P > 0.05$, chi-square tests with Bonferroni correction). Results were similar for two other colonies (data not shown). Foragers and reverted nurses did not differ in age (29.9 ± 0.2 days, $n = 26$, and 29.8 ± 0.4 , $n = 8$, respectively; $P = 0.76$, unpaired *t*-test). **c**, Individual analyses. Number of scans with brood care (days double-plotted). Bars at the bottom right show the light–dark regime outside: black, night; white, day. Sixty-six reverted nurses were analysed individually. Y44: example of a bee active around the clock and showing no diurnal rhythm in brood care ($P > 0.05$; statistical analyses as above); this behaviour was seen in 80.3% of reverted nurses. Y4: example of a bee active around the clock and with a weak diurnal rhythm in brood care ($P > 0.05$); this behaviour was seen in 15.2% of reverted nurses. W53: one of only three bees (4.5%) showing clear diurnal rhythms ($P < 0.05$).

nal circadian clock for timing visits to flowers and for sun-compass navigation⁷.

Honeybees show great plasticity during their behavioural development, with their hive-to-field transition being accelerated, delayed, or even reversed in response to changing colony conditions⁸. We therefore investigated whether this plasticity extends to the bees' behavioural rhythms, focusing on the reversion from foraging to nursing as a particularly compelling challenge to the clock. This reversion occurs in response to a severe shortage of nurse bees and is associated with changes in exocrine, endocrine and neurochemical processes^{8,9}. Do foragers induced

to return to nursing also revert to an arrhythmic behavioural state?

We established three unrelated colonies, each composed initially of 2,000–2,500 foragers, their queen and young (sib) brood. Colonies composed only of foragers are known to induce behavioural reversion⁸, and indeed the division of labour was reorganized in these colonies: many bees continued to forage, participating in little or no nursing behaviour; some foragers reverted to nursing and stopped foraging completely, or almost completely (Fig. 1a).

As in typical colonies with young nurses⁶, brood care in our experimental colonies was performed around the clock, with no diurnal oscillations (Fig. 1b). The uninterrupted nursing occurred because individual bees had reverted to arrhythmic activity: analysis of individually tagged reverted nurses ($n = 66$) revealed that brood care was performed by arrhythmic bees nursing day and night, rather than by rhythmic bees working in shifts (Fig. 1c). We found that reversion also affected the activity–rest cycle: 21 reverted bees (31.8%) cared for the brood in seven or more consecutive observations for 21 hours or longer; foragers, in contrast, rest daily for periods of seven hours or more².

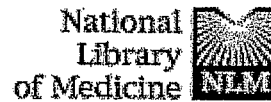
The underlying cellular and molecular basis of this striking natural behavioural plasticity is unknown. There may be task-dependent changes in a central clock mechanism, uncoupling of nursing activity from the clock, or an effect resulting from nursing behaviour that overrides the clock output. Comparing these possibilities should help to clarify the nature of the cellular and molecular bases of chronobiological plasticity.

Reverted nurses were able to rear the brood to maturity in all three colonies. Although we did not test other possible consequences of reversion, our findings may have wider implications, given the conservation of some molecular components of biological clocks¹⁰ and of sleep regulation^{11,12}.

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- Kaiser, W. & Steiner-Kaiser, J. *Nature* 301, 707–709 (1983).
- Kaiser, W. J. *Comp. Physiol.* 163, 565–584 (1988).
- Turek, F.W. & Zoc, P. C. *Regulation of Sleep and Circadian Rhythms* (Dekker, New York, 1999).
- Toma, D. P., Bloch, G., Moore, D. & Robinson, G.E. *Proc. Natl Acad. Sci. USA* 97, 6914–6919 (2000).
- Stussi, T. & Harmelin, M. L. C. *R. Acad. Sci. Helv. Seances. Acad. Sci. D* 262, 2066–2069 (1966).
- Moore, D., Angel, J. E., Cheeseman, I. M., Fahrbach, S. E. & Robinson, G. E. *Behav. Ecol. Sociobiol.* 43, 147–160 (1998).
- Von Firsch, K. *The Dance Language and Orientation of Bees* (Harvard University Press, Cambridge, 1967).
- Huang, Z. Y. & Robinson, G. E. *Behav. Ecol. Sociobiol.* 39, 147–158 (1996).
- Schulz, D. J. & Robinson, G. E. *J. Comp. Physiol. A* 184, 481–488 (1999).
- Dunlap, J. C. *Cell* 96, 271–290 (1999).
- Shaw, P. J., Cirelli, C., Greenspan, R. J. & Tononi, G. *Science* 287, 1834–1837 (2000).
- Hendricks, J. C. *et al. Neuron* 25, 129–138 (2000).



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Human immunodeficiency virus Phylogeny and the origin of HIV-1.

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