

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 theory<sup>1</sup> 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.

Andrew Rambaut\*, David L. Robertson\*, Oliver G. Pybus\*, Martine Peeters†, Edward C. Holmes\*

\*Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

e-mail: edward.holmes@zoo.ox.ac.uk

†Laboratoire Retrovirus, IRD, BP 5045, 34032

Montpellier Cedex1, France

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

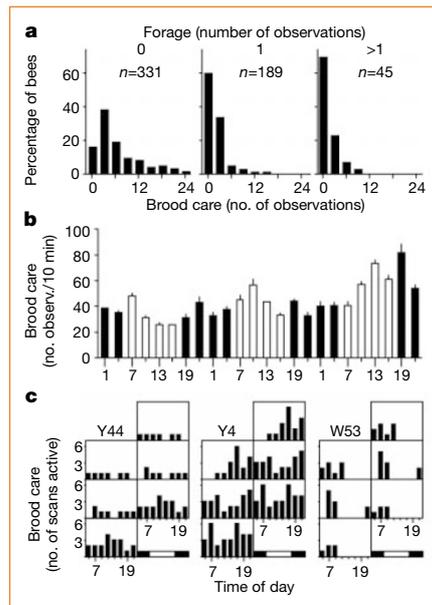
Supplementary information is available on Nature's website at [www.nature.com](http://www.nature.com) or as paper copy from the London editorial office of Nature.

Chronobiology

## Reversal of honeybee behavioural rhythms

Adult honeybees have sleep-like states<sup>1,2</sup> and, like human infants<sup>3</sup>, bees develop their own endogenous circadian rhythms as they mature<sup>4,5</sup>. But whereas disruption of our sleep cycles and synchronized internal rhythms may adversely affect our physiology and performance<sup>3</sup>, 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 rhythms<sup>6</sup>. 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<sup>7</sup>) every 3 h for three days. Observations of brood care<sup>6</sup>: six 10-min visual scans of individually tagged bees in the vicinity of the brood. Foraging observations were made as described<sup>6</sup>. **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 ( $29.9 \pm 0.2$  days,  $n = 26$ , and  $29.8 \pm 0.4$ ,  $n = 8$ , 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 navigation<sup>7</sup>.

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 conditions<sup>8</sup>. 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<sup>8,9</sup>. 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 reversion<sup>8</sup>, 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 nurses<sup>6</sup>, 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 more<sup>2</sup>.

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<sup>4</sup> 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 clocks<sup>10</sup> and of sleep regulation<sup>11,12</sup>.

Guy Bloch\*, Gene E. Robinson\*†

\*Department of Entomology, †Neuroscience Program, University of Illinois, 505 South Goodwin Avenue, Urbana, Illinois 61801, USA

e-mail: [guybloch@life.uiuc.edu](mailto:guybloch@life.uiuc.edu)

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