the pattern of activity in reindeer. However, the daily pattern was modified when there was a distinct light/dark cycle, and reindeer in northern Norway, in particular, displayed a significant rhythm of exactly 24 hours throughout autumn, winter and spring (see supplementary information).

Free-living reindeer do not therefore show evidence of the classical prerequisite for circadian organization— persistence under constant conditions. Seasonal absence of rhythmicity in the circadian range has been recorded in the daily activity of the Svalbard ptarmigan (*Lagopus mutus hyperboreus*)⁵, and in circulating levels of the hormone melatonin in ptarmigan⁶ and in reindeer⁷, indicating that the expression of an internal clock is reduced in both Arctic species under constant light conditions. We therefore suggest that seasonal absence of circadian rhythmicity is a ubiquitous trait among resident polar vertebrates.

Reduced circadian organization may enhance animals' responsiveness and speed of phase adaptation to the light/dark cycle, as proposed for migrating birds⁸ and mammals emerging from hibernation⁹. And for herbivores in polar regions, there can be little selective advantage in maintaining strong internal clocks in an effectively non-rhythmic environment.

Bob E. H. van Oort*, Nicholas J. C. Tyler†, Menno P. Gerkema‡, Lars Folkow*,

Arnoldus Schytte Blix*, Karl-Arne Stokkan* *Department of Arctic Biology and Institute of Medical Biology, †Centre for Sámi Studies, University of Tromsø, 9037 Tromsø, Norway e-mail: karlarne@fagmed.uit.no ‡Department of Chronobiology, University of

Groningen, 9750 AA Haren, The Netherlands

- 1. Lowrey, P. L. & Takahashi, J. S. Annu. Rev. Genom. Hum. Genet. **4**, 407-441 (2004).
- Daan, S. & Aschoff, J. in *Circadian Clocks* (eds Takahashi, J. S. et al.) 7–43 (Plenum, New York, 2001).
- de Mairan, J. J. in Histoire de l'Académie Royale des Sciences 35–36 (Paris, 1729).
- 4. Gerkema, M. P. in *Biological Rhythms* (ed. Kumar, V.) 207-215 (Narosa, New Dehli, 2002).
- Stokkan, K. A., Mortensen, A. & Blix, A. S. Am. J. Physiol. 251, 264–267 (1986).
- Reierth, E., van't Hof, T. & Stokkan, K. A. J. Biol. Rhyth. 14, 314–319 (1999).
- Stokkan, K. A., Tyler, N. J. C. & Reiter, R. J. Can. J. Zool. 72, 904–909 (1994).
- Hau, M. & Gwinner, E. *Physiol. Behav.* 58, 89–95 (1995).
 Hut, R. A., Van der Zee, E. A., Jansen, K., Gerkema, M. P. &
- Daan, S. J. Comp. Physiol. B **172**, 59–70 (2002). Supplementary information accompanies this

communication on *Nature*'s website. **Competing financial interests:** declared none. **doi:**10.1038/4381095a

A direct test of $E = mc^2$

One of the most striking predictions of Einstein's special theory of relativity is also perhaps the best known formula in all of science: $E = mc^2$. If this equation were found to be even slightly incorrect, the impact would be enormous — given the degree to which special relativity is woven into the theoretical fabric of modern physics and into everyday applications such as global positioning systems. Here we test this mass–energy relationship directly by combining very accurate measurements of atomic-mass difference, Δm , and of γ -ray wavelengths to determine *E*, the nuclear binding energy, for isotopes of silicon and sulphur. Einstein's relationship is separately confirmed in two tests, which yield a combined result of $1 - \Delta mc^2/E = (-1.4 \pm 4.4) \times 10^{-7}$, indicating that it holds to a level of at least 0.00004%. To our knowledge, this is the most precise direct test of the famous equation yet described.

Our direct test is based on the prediction that when a nucleus captures a neutron and emits a γ -ray, the mass difference Δm between the initial (including unbound neutron) and final nuclear states, multiplied by c^2 (where *c* is the speed of light), should equal the energy of the emitted γ -ray(s), as determined from Planck's relation E = hf (where *h* is Planck's constant and *f* is frequency).

The total energy of the γ -rays emitted as

the daughter nucleus decays to the groundstate was determined by summing the individual γ -ray energies. These were obtained by wavelength measurement using crystal Bragg spectroscopy. The mass difference Δm is measured by simultaneous comparisons of the cyclotron frequencies (inversely proportional to the mass) of ions of the initial and final isotopes confined over a period of weeks in a Penning trap.

For an atom X with a mass number of A

Albert Einstein: father of the famous formula.

undergoing this nuclear reaction, the comparison is expressed in terms of measured quantities as

$$\Delta Mc^{2} = (M[^{A}X] - M[^{A+1}X] + M[D] - M[H])c^{2}$$

= 10³N_Ah(f_{A+1} - f_D) mol AMU kg⁻¹ (1)

where the Avogadro constant N_A relates the measured mass M[X] in unified atomic mass units (AMU) to its mass in kilograms m[X]. We made comparisons for $^{A+1}X = {}^{29}Si$ and $^{A+1}X = {}^{33}S$. The mass of the neutron M[n] is determined from the masses¹ of hydrogen M[H]and deuterium M[D] combined with f_D , the frequency of the γ -ray corresponding to the deuteron binding energy². The molar Planck constant is $N_Ah = 3.990312716(27) \times 10^{-10}$ J s mol⁻¹; numbers in parentheses indicate uncertainty on the last digits. This figure has been independently confirmed at about the 5×10^{-8} level by a range of experiments through its relationship with the fine-structure constant¹.

The γ -ray frequencies on the righthand side of equation (1) have been measured using the GAMS4 crystal-diffraction facility at the Laue-Langevin Institute in Grenoble³. The γ -rays emitted from sources located near the high-flux reactor core are diffracted by two nearly perfect, flat crystals whose lattice spacing, d, has been determined in metres⁴. The diffraction angles, θ , are measured with angle interferometers calibrated using a precision optical polygon (Fig. 1a). Wavelengths are determined from the Bragg equation $\lambda = 2d\sin\theta$ and were measured for the 3.5-MeV and 4.9-MeV transitions in ²⁹Si, for the 0.8-MeV, 2.4-MeV and 5.4-MeV transitions in ³³S, and for the 2.2-MeV transition in deuterium ²H (see supplementary information).

Because the diffraction angle for a 5-MeV γ -ray by a low-order silicon reflection is less than 0.1°, our binding-energy determinations were limited by our ability to measure the diffraction angles of the highest-energy γ -rays with fractional accuracy better than about





10⁻⁷. A slightly revised value of $f_{\rm D}$ from that given in ref. 2 is also reported here (*c* is exact): $f_{\rm Si} = c/(0.146318275(86) \times 10^{-12} \text{ m})$ $f_{\rm S} = c/(0.143472991(54) \times 10^{-12} \text{ m})$ $f_{\rm D} = c/(0.557341007(98) \times 10^{-12} \text{ m})$ These values of $f_{\rm Si}$ and $f_{\rm S}$ are, respectively, 25 and 50 times more precise than earlier values⁴.

The mass difference was determined by direct comparison of the cyclotron frequencies of two different ions simultaneously confined in a Penning trap⁵. This two-ion technique achieves mass comparisons with fractional accuracies below 10^{-11} by virtually eliminating the effect of many sources of noise such as magnetic field fluctuations. During measurements, the two ions are placed on a common circular orbit (magnetron mode), on opposite sides of the centre of the trap and separated by a distance of about 1 mm. Figure 1b shows that the measured cyclotron frequency ratio is almost completely independent of ionion separation, tightly constraining the size of the largest possible systematic errors⁵. The ion mass ratios reported here, namely $M[^{33}S^+]/M[^{32}SH^+] = 0.9997441643450(89)$ and $M[^{29}\text{Si}^+]/M[^{28}\text{SiH}^+] = 0.9997151241812(65),$ are respectively more than 700 and 100 times more precise than previously known⁶.

The total uncertainties include the uncorre-lated uncertainties of 4×10^{-12} each from ion-ion interactions, trap field imperfections, and statistics. The ratios also include corrections of $45(5) \times 10^{-12}$ and $7.3(2) \times 10^{-12}$ to account for polarization-induced shifts of the cyclotron frequencies⁷ (see methods in supplementary information). After accounting for the mass of the missing electron and the chemical binding energies^{8,9}, the ion mass ratios give the neutral mass differences: $M[^{32}S] + M[H]$ $-M[^{33}S] = 0.00843729682(30)$ AMU, and $M[^{28}\text{Si}] + M[\text{H}] - M[^{29}\text{Si}] = 0.00825690198(24)$ AMU. And by the addition of M[D] - 2M[H] =-0.00154828629(40) AMU (refs 1,6) to each one, we obtain the desired mass differences of equation (1), with a fractional accuracy of about 7×10^{-8} for both.

Comparing the measured energies and

Figure 1 | Typical data for testing $E = \Delta mc^2$. a, ³³S wavelength data and fitted curves. The energy of the emitted 5,421-keV y-rays is measured from the angular separation of the second-order Bragg peaks resulting from diffraction from a silicon crystal. **b**, Measured mass ratio $M[^{33}S^+]/M[^{32}SH^+]$ (which largely determines the mass change, Δm) as a function of the distance between the ions, $\rho_{\rm s}$, in the Penning trap. The lack of variation strongly suggests that the predicted uncertainties from ion-ion interactions and field inhomogeneities (turquoise and blue-hatched bands, respectively) are too conservative. The final mass ratio (solid line) is determined with fractional accuracy of 7 parts in 1012 (dashed lines). All error bars are one standard deviation.

masses, we report two independent tests of $E = mc^2$. The two comparisons find a measured fractional difference between E and Δmc^2 of 2.1(5.2) × 10⁻⁷ and -9.7(8.0) × 10⁻⁷ with sulphur and silicon isotopes, respectively, and a combined value of $-1.4(4.4) \times 10^{-7}$. The error on this comparison is currently dominated by the uncertainty on the γ -ray measurements. This result is 55 times more accurate than the previous best direct test of $E = mc^2$, which was performed by comparing the electron and positron masses to the energy released in their annihilation¹⁰. Simon Rainville*+, James K. Thompson*, Edmund G. Myerst, John M. Browns, Maynard S. Dewey||, Ernest G. Kessler Jr||, Richard D. Deslattes||, Hans G. Börner¶, Michael Jentschel¶, Paolo Mutti¶,

David E. Pritchard*

*Research Laboratory of Electronics,

MIT-Harvard Center for Ultracold Atoms, and Department of Physics, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

e-mail: rainville@alum.mit.edu †Present address: Département de Physique, Université Laval, Québec G1K 7P4, Canada ‡Department of Physics, Florida State University, Tallahassee, Florida 32306-4350, USA §The Physical and Theoretical Chemistry Laboratory, Department of Chemistry, South



Parks Road, Oxford OX1 3QZ, UK ||National Institute of Standards and Technology, Gaithersburg, Maryland 20899, USA

¶Institut Laue-Langevin, 38042 Grenoble Cedex, France

- 1. Mohr, P. J. & Taylor, B. N. Rev. Mod. Phys. 77, 1-107 (2005).
- Kessler, E. G. et al. Phys. Lett. A 255, 221-229 (1999).
 Kessler, E. G. et al. Nucl. Instrum. Meth. Phys. Res. A 457,
- 187-202 (2001).
- Dewey, M. S. et al. Preprint nucl-ex/0507011 at http://arxiv.org (2005).
- . Rainville, S., Thompson, J. K. & Pritchard, D. E. Science **303**, 334–338 (2004).
- . Audi, G., Wapstra, A. H. & Thibault, C. *Nucl. Phys. A* **729**, 337–676 (2003).
- Thompson, J. K., Rainville, S. & Pritchard, D. E. Nature 430, 58–61 (2004).
- NIST Chemistry WebBook, NIST Standard Reference Database Number 69 (eds Linstrom, P. J. & Mallard, W. G.) (National Institute of Standards and Technology, Gaithersburg, Marvland. 2003).
- 9. Chase, M. W. J. Phys. Chem. Ref. Data Monogr. 9, 1-1951 (1998).
- Greene, G. L., Dewey, M. S., Kessler, E. G. & Fischbach, E. Phys. Rev. D 44, R2216-R2219 (1991).

Supplementary information accompanies this communication on *Nature's* website. Competing financial interests: declared none. doi:10.1038/4381096a

Chirality in elephant pheromones

Musth in male elephants is an annual period of heightened sexual activity and aggression¹⁻³ that is linked to physical, sexual and social maturation. It is mediated by the release of chemical signals such as the pheromone frontalin, which exists in two chiral forms (molecular mirror images, or enantiomers). Here we show that enantiomers of frontalin are released by Asian elephants in a specific ratio that depends on the animal's age and stage of musth, and that different responses are elicited in male and female conspecifics when the ratio alters. This precise control of communication by molecular chirality offers insight into societal interactions in elephants, and may be useful in implementing new conservation protocols⁴.

Frontalin (1,5-dimethyl-6,8-dioxabicyclo [3.2.1]octane)^{1,2,5,6} is discharged during musth in male Asian elephants (*Elephas maximus*) from the temporal gland on the face. We analysed more than 100 secretion samples from six males and found that this pheromone was first detectable in the late teens, the quan-

tity secreted rising about 15-fold over a 25-year age span (Fig. 1a). Both enantiomeric forms (designated plus and minus; Fig. 1b, inset) were present and each was quantified (for methods, see supplementary information).

In young males, significantly more (+) than (-) frontalin was secreted, but concentrations of these became almost equal (1:1, a racemic mixture) as the elephant matured (Fig. 1b). Serial samples collected throughout the musth episodes of two young and two old males confirmed that this change in ratio was significantly associated with the elephants' age groups (results not shown).

Musth periods get longer as males age. The enantiomeric composition of the frontalin secreted becomes significantly more varied in