ed evidence that T-cell receptor rearrangement is exceedingly rare in B cells and at the same time reject the even more widely accepted evidence that T cells do not hypermutate their T-cell receptor loci.

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KELSOE *ET AL.* REPLY — Bachl and Wabl are mistaken in stating that we presented insufficient data for a proper analysis of our results. Mutations in Vα genes arise *in vivo* as a consequence of reaction in the germinal centre (GC) or *in vitro* during the PCR. Both processes are subject to Luria–Delbrück fluctuations and, in fact, the mutations observed in GCs fit a Luria–Delbrück distribution. However, the *in vivo* process is resistant to quantification since many factors are unkown (for example, replicative rates, independence of samples, effects of selection). In contrast, mutations introduced by the PCR can be rigorously analysed. Therefore, the null hypothesis that mutations in all samples were introduced *in vitro* can be tested.

We used a Monte Carlo algorithm to estimate the spectrum of PCR errors, including 'jackpot effects' due to early introduction of misincorporations, as follows. At each round of a simulated PCR, templates replicate with probability ε (0.8). If successful, each daughter independently incorporates a number of new mutations with parameter μ given by the product of the error rate of the *Pfu* polymerase and the length of the template sequence[1]; this is repeated for 80 cycles. This simulation was repeated 10^4 times to estimate the median, mean, 99% and 99.9% quantiles for frequencies of Vα11/Jα11 rearrangements containing 1, 2 or ⩾3 PCR errors. Predicted frequencies did not differ significantly from those observed in the B10 cell line (1 mutation observed versus 1.7 expected) and in periarteriolar T-cell sheath (PALS) cells (7 observed versus 4.2 expected). In contrast, Vα11 mutations in GC cells were significantly in excess of expected PCR mistakes; for example, the number of Vα exons with three mutations was about 3,500-fold above that expected. By chance, < 0.01% of sampled populations would contain triply mutated sequences at frequencies ⩾1/4,500; the frequency we observed for triple mutants in GCs was approximately 1/57.

Alternatively, problems of fluctuation may be avoided simply by scoring repeated mutations as single events. This analysis shows mutations in GCs to be significantly greater ($P = 0.03$; Fisher's exact test) than that found in the PALS.

Bachl and Wabl correctly note that mutated Vα rearrangements might originate in B lymphocytes. However, our present studies of single T cells dissected from GCs demonstrate Vα11 mutants in about 10% of productive rearrangements.

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Redshift and black-hole mass

SIR — The very interesting observations by M. Myoshi *et al.* (*Nature* **373**, 127–129; 1995) of water-vapour maser emission in the NGC 4258 disk offer an additional direct test for their model of a high-rotation velocity disk and allow an independent determination of the central black-hole mass. Indeed, a detectable amount of gravitational redshift should affect the observed line wavelengths.

As the distances of the maser emission regions from the centre of the black hole r are much larger than the black-hole Schwarzschild radius $r_S = 2GM/c^2 = 3.5\ 10^{-6}$ pc, the gravitational redshift is simply given by (assuming a spherical symmetry):

$$z(r) = \frac{r_S}{2r} = \frac{v_{circ}^2}{c^2}$$

where G is the gravitational constant, M the mass of the central object, v_{circ} the velocity of a particle in keplerian motion around it at radius r and c the velocity of light. The gravitational redshift expected for a central mass of $3.6 \times 10^7\ M_\odot$ at the inner edge of the disk is $cz(r = 0.13\ \text{pc}) = 4.0\ \text{km s}^{-1}$, whereas the one at the outer edge is $cz(r = 0.26\ \text{pc}) = 2.0\ \text{km s}^{-1}$, more than 10 times their velocity resolution, or about 4 and 2 times their quoted errors on the velocity determination for the high-velocity lines.

The high-rotation velocity lines offer an easy and safe way to test the disk model and to determine the black-hole mass. The reason is that they apparently arise very close to a line perpendicular to the line-of-sight. As a consequence, their velocities should systematically deviate from the values expected from keplerian motion, with residuals that are inversely proportional to the distances of the maser emission regions from the centre of the black hole. Further, the keplerian motion velocities have an antisymmetrical behaviour relative to the centre of the disk, whereas the gravitational redshifts have a symmetrical one. The low-velocity lines should also be affected by a gravitational redshift of 4.0 km s^{-1} as they are produced at the inner edge of the disk ($r = 0.13$ pc).

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Collision age

SIR — Beck *et al.*[1] have reported new biogeographical data, concluding that the collision of the Indian subcontinent with Asia was older than previously claimed. They suggested that the collision occurred between 66 and 55.5 Myr (million years) ago. However, palaeontological data constrain the latest possible date even more accurately. From the study of continental faunas and floras, it has been known for several years that terrestrial continuity between India and mainland Asia was already established by the time of the K/T boundary, 65 Myr ago, or probably slightly earlier[2,3]. More recent data again support this view[4,5].

Nevertheless, an Eocene collision, which was accepted for a long time, appears still to be admitted by various workers. The biostratigraphical result of Beck *et al.* is an additional argument which demonstrates that such an age can no be longer accepted.

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