for mammal and bird species. It also suggests that z values may be taxon-specific when dealing with continental biotas.

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SIR — Heywood *et al.*[1] propose an explanation for the discrepancy between the high extinction rates predicted by theory and the low rates observed in reality. The admission that there is a discrepancy is something of a breakthrough. Theoretical predictions of extinction rates, derived from species–area relations, have previously been presented as if they were verified facts[2–4]. Indeed, the popular press, environmental organizations and many politicians have taken them to be such; witness the frequently cited 'fact' that 50,000 species a year are being driven to extinction, or a statement to the UN commission on sustainable development by US vice-president Al Gore that one-half of all species may be extinct within the lifetime of our grandchildren.

Heywood *et al.* seek to reconcile the discrepancy by arguing that there is a time-lag of some indeterminate length during which species that are 'committed' to extinction persist. An equally parsimonious explanation is that the predictions are simply wrong. In an earlier publication, two of the co-authors of ref. 1 argued that the species–area relation is a poor tool for making predictions: in a mainland situation, it is "nothing more than a self-evident fact: that as one enlarges an area, it comes to eventually encompass the geographical ranges of more species. The danger comes when this is extrapolated backwards, and it is assumed that by reducing the size of a forest, it will lose species according to the same gradient." Yet that is precisely what was done to obtain the oft-cited, very high extinction-rate figures. Wilson[2] uses the species–area relation $S=kA^z$, and, plugging in an estimate of the rate at which tropical forests are being cleared and using $z=0.15-0.35$, calculates that "if deforestation continues for thirty more years at the present rate, one-tenth to one-quarter of the rain-forest species will disappear".

As Heywood and Stuart[5] pointed out, there are many reasons why the species–area relation may be descriptive without being predictive, especially on a global basis: species are not distributed at random; conservation measures already protect many key habitats (including those critical to 95 per cent of Afrotropical avifauna, for example); some species may be able to adapt to secondary habitats. In an earlier review of the species–area relationship, Connor and McCoy[6] noted that the power relationship $S=kA^z$, first proposed in 1921, tends to exaggerate, producing impossibly high values of species when extrapolated to large areas. The authors' analysis of 100 sets of species–area data led to doubt that there is any biological significance to the relation whatsoever; they suggested it may be nothing more than a sampling phenomenon, a "correlation. . . without a functional relationship". Far from being proof that some consistent biological law was in operation, the fact that the values of z (determined by the slope of a best linear fit of $\log S$ versus $\log A$) typically fell in the range 0.2–0.4 was, they found, simply characteristic of any regression system with a high r-value and a large range in the independent variable relative to that of a monotonic dependent variable.

Heywood *et al.* take comfort from one quasi-empirical test of the theoretical extinction rates calculated from the species–area curve: A species-by-species analysis of threatened birds tallied 450 that appear doomed to extinction, a mere factor of 3 (as opposed to 100) off from the predicted value of 1,350. Yet Heywood and Stuart noted that hunting, collecting, disease and introduced species (as opposed to loss of habitat area, the presumed driving force in the theoretical predictions) have had "very major effects" on the decline of these particular species, most of which are not associated with tropical forests in any event. It is the loss of tropical forests that drives the very high global extinction-rate predictions, however. And even allowing for the operation of time lags, large losses in tropical forest area have occurred at least in one case without any comparable loss in species. The Atlantic coastal forests of Brazil, which have a higher species diversity than the now-threatened Amazon forests, were reduced by nearly 90 per cent, mostly in the nineteenth century; a recent survey by zoologists could not document a single extinction. On the contrary, several birds and six butterfly species believed 20 years ago to be extinct were rediscovered[7]. According to the species–area relationship, a loss of 50 per cent of species should have occurred. Given such discrepancies, one would appear to be justified in continuing to take the much-cited global extinction rates with a grain of salt.

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Self-assembly and protein stability

SIR — Shen *et al.* report[1] a dramatic increase (to 140 °C) in the thermal stability of bacteriorhodopsin in dry films of purple membrane. The potential high-temperature applications of these dry films were emphasized in an accompanying News and Views article[2]. In general, thermostabilization is often required (or desirable) for technological applications of proteins, and the results of Shen *et al.* suggest that removal of water may be a convenient way to achieve this goal.

In fact, protein thermostabilization on dehydration is not without precedents. In several cases, dry or lyophilized enzymes have been shown to be fairly resistant to thermal inactivation, even at temperatures above 100 °C; for instance, the half-lives for the irreversible thermal inactivation of dry trypsin and ribonuclease on heating *in vacuo* are about 2 h at 160 °C and about 10 min at 200 °C (calculated from ref. 3). Enhanced thermal stability is also observed when lyophilized enzymes are suspended in anhydrous organic solvents[4], and the possibility of high-temperature catalysis is one of the features of nonaqueous enzymology (note that, even under such anhydrous conditions, a certain number of essential water molecules must remain bound to the protein[4,5]).

Clearly, some applications of proteins may require a 'solid-like' support (such as a thin film); in these cases, immobilization of the protein in the suitable support (by, for instance, entrapment, covalent attachment, adsorption) followed by a controlled dehydration process appears to be an interesting possibility if thermostabilization is required.

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SAFINYA AND ROTHSCHILD REPLY — We agree that there are several previously reported examples in which dehydration or suspension in anhydrous organic solvents leads to enhanced protein stabilization against denaturation. However in our study we observed stabilization of the two-dimensional order of the protein lattice in dry bacteriorhodopsin films, together with stability against denaturation at high temperatures.

It is now well established that individual two-dimensional bilayers of hydrated purple membrane exhibit a lattice-melting transition around 70 °C (ref. 1 and refs 23, 24 therein), and subsequently undergo a broad denaturing transition (from the

disordered phase) around 90–100 °C. In contrast, our work indicates that the stacking of these sheets (higher order self-assembly achieved by water removal) results in: (1) the complete suppression of the melting transition with the purple-membrane lattice remaining ordered; and (2) the absence of protein denaturation up to 140 °C. We believe that the high temperature stability at 140 °C is related to the retention of the two-dimensional ordered lattice which under hydrated conditions melts away around 70 °C. In other words the inter-protein interactions coming from the two-dimensional ordered lattice appear to prevent the protein denaturation.

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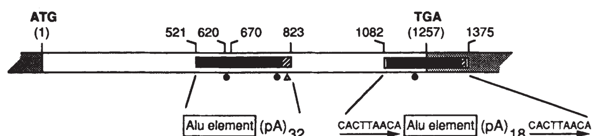
Alu sequences in RMSA-1 protein?

SIR — Yeo *et al.*[1] report a previously undescribed chromosomal protein that "regulates mitotic spindle assembly". The protein, termed RMSA-1, was identified as the antigen recognized by autoimmune serum from a patient with discoid lupus erythematosus. The antibodies in this serum bound to a protein of 47,000 (47K) on immunoblots of protein isolated from human, mouse, monkey and chicken cells. The serum was used to isolate a single human complementary DNA clone from a lambda zap cDNA expression library. The authors searched various sequence databases with the sequence of the putative RMSA-1 cDNA, but do not report the results of these searches in their paper.

We searched the RMSA-1 DNA and protein sequences against the US National Center for Biotechnology Information's non-redundant nucleotide and protein sequence databases using the BLAST network service. Surprisingly, we found that the 418-codon open reading frame reported in this paper contains two Alu sequences (see figure). The presence of these Alu elements is absolutely unambiguous, whether protein or DNA sequence is used as query in the database search. The first Alu element runs from codons 173 to 278 of the putative RMSA-1 open reading frame, whereas the second begins at codon 364 and extends 112 nucleotides past the translational stop codon. The two Alu elements align with more than 70 per cent nucleotide identity with putatively active Alu elements[2], both contain characteristic poly(A) tails, and the second Alu element is flanked by a typical target site duplication. Of particular note, all three consensus p34^{cdc2} phosphorylation sites identified by Yeo *et al.* are contained within these Alu regions. Also, the poly-L-lysine sequence reported by the authors as a potential nuclear localization signal is encoded in the poly(A) tail of the first Alu element, which would contribute significantly to a predicted p*I* of greater than 10, rather than a p*I* of 6.33, as stated by Yeo *et al.*

Several issues are raised in the light of our observations. The presence of two translated Alu elements (accounting for 34 per cent of the encoded protein) calls into question whether the cloned cDNA encodes the 47K protein recognized on immunoblots, or whether, for example, it represents the messenger RNA of an expressed pseudogene. In fact, given the co-migration of a crossreacting 47K species on immunoblots of proteins from humans, mice and chickens (and the fact that Alu elements are not present in the last two organisms), it seems unlikely that these Alu elements would be represented in the true 47K product. Furthermore, Yeo *et al.* used antisense RNA expression of the entire cloned cDNA (which includes both elements and a third Alu element downstream of the open reading frame) for functional studies. Given the very large number of Alu insertions within introns, untranslated regions and possibly, in rare cases, coding sequences of human genes[3,4], the results of such experiments are difficult to interpret. Of course, should it be the case that translated Alu elements exist in the functional 47K human protein, this would represent the first report of its kind and would have important evolutionary implications[4].



The RMSA-1 open reading frame and untranslated regions. The open reading frame is shown as a large white box; 5′ and 3′ untranslated regions are grey; Alu elements are represented by black boxes; poly(A) tails by hatched boxes and these are shown in more detail underneath. The white boxes flanking the second Alu element are 9-bp target-site duplications (inset). The filled circles and the triangle are the putative p34^{cdc2} kinase sites and nuclear localization signal, respectively.

In our opinion, this example supports the view that peer review of papers containing DNA or protein sequence data should involve direct evaluation by experts in sequence analysis. This would require direct access of sequence information at the time of review, together with the submitted manuscript, to allow searches to be repeated or performed by a sequence specialist before acceptance for publication.

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YEO *ET AL.* REPLY — We agree that Alu elements are present in the RMSA-1 cDNA sequence that we reported[1]. Tugendreich *et al.* suggest that one possible explanation is that we may have cloned an expressed pseudogene. However, we have reviewed our published and unpublished data and firmly believe this not to be the case. We are currently carrying out confirmatory, direct experiments to establish that the RMSA-1 coding sequence contains Alu elements.

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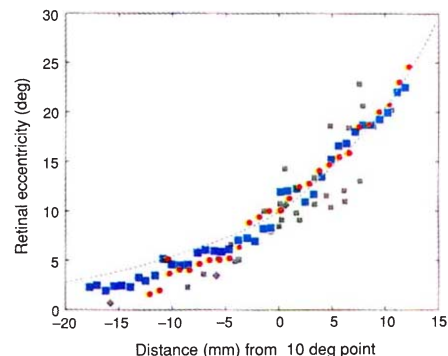
Erratum



IN the Scientific Correspondence by Stephen A. Engel *et al.* of 16 June ("fMRI of human visual cortex") the labelling of the graph axes were inadvertently omitted from Fig. 2. The correct figure is shown above.