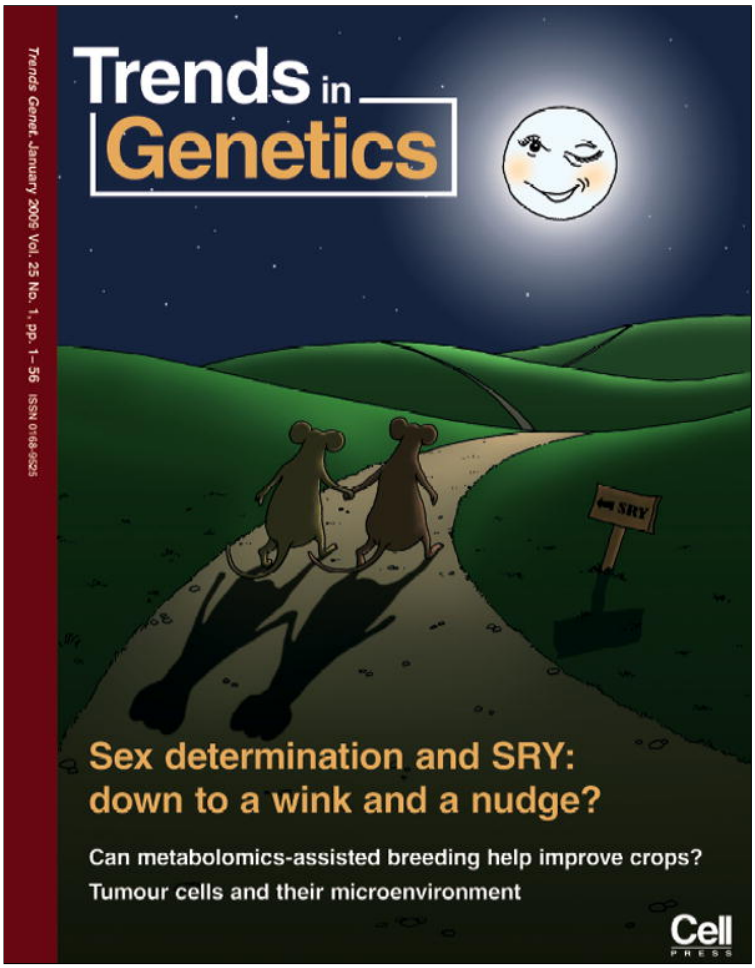

low estradiol doses indicates that their test system was insensitive to exogenous estrogens, calling into question their conclusions about BPA. From the standpoint of testing EDCs, this and other GLP studies have serious flaws; they are based on the assumption of a linear dose-response (an invalid assumption for hormone-like chemicals) and rely on analytical methods established in the 1950s without incorporation of new, more sensitive methodologies. For further discussion of the reasons why these GLP studies are incapable of detecting low dose effects of BPA, we refer the reader to a recent commentary [7].

Critical aspects of our findings have been replicated

Bell cites three papers by Eichenlaub-Ritter and colleagues as evidence that our original observations linking BPA and aneuploidy have not been replicated [8–10]. However, only one of these papers attempted to replicate our study [10]; the findings among the three studies differ and, importantly, all five studies published to date [2,8–11] have identified meiotic disturbances in response to BPA exposure. Differences in study design probably account for some discrepancies, but we believe that dietary phytoestrogens are an important confounder in these studies. The outcome of BPA-induced meiotic disturbances is, however, contentious. We have found that defects in chromosome alignment (congression failure) at metaphase I (MI) are correlated with aneuploid eggs [2], whereas Eichenlaub-Ritter argues that such oocytes arrest and cannot complete MI. [10] Although the finer details of oocyte cell-cycle control remain to be elucidated, the impact is essentially the same; failure to make an egg or making an aneuploid egg means failure to achieve a normal pregnancy.

BPA: the big picture

Importantly, Bell did not mention the other two vulnerable windows of oocyte development – meiotic entry and follicle formation – outlined in our review [1]. Both occur during fetal development, have the potential to compromise reproductive fitness and can be disrupted by estrogenic substances such as BPA. This underscores the findings from other low dose studies that fetal and neonatal exposures to BPA and other EDCs pose serious risks to human reproductive health [5]. Finally, two recent papers indicate that metabolic disturbances resulting from BPA exposure lead to adverse health outcomes, such as diabetes and heart disease in humans [12,13]. These papers, coupled with the literature on low dose effects in rodents, should cause regulatory agencies to reconsider their current view that low level BPA exposure poses little or no threat to human health.

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Letters Response

Molecular and morphological evolution in tuatara are decoupled

Sankar Subramanian[1,2], Jennifer M. Hay[3], Elmira Mohandesan[3], Craig D. Millar[2] and David M. Lambert[1]

[1] Griffith School of Environment, Griffith University, Nathan, QLD 4111, Australia
[2] Allan Wilson Centre for Molecular Ecology and Evolution, School of Biological Sciences, University of Auckland, Private 92019, Auckland, New Zealand
[3] Allan Wilson Centre for Molecular Ecology and Evolution, Institute of Molecular BioSciences, Massey University, Private Bag 102904 NSMC, Auckland, New Zealand

In the 1970s, Marie-Claire King and Allan Wilson [1] suggested that macromolecules and anatomical and behavioral features of organisms evolve at independent rates. Before then, the common view was that rates of molecular and morphological evolution are positively correlated. In accordance with this traditional view, Miller *et al.* [2]

Corresponding author: Lambert, D.M. (d.lambert@griffith.edu.au).

question our finding that tuatara (*Sphenodon*, Reptilia) exhibit a rapid rate of molecular evolution [3] because *Sphenodon* are a relict taxon with little skeletal change from Cretaceous relatives. Miller *et al.* [2] raise several points that we address here.

First, they argue that our conclusion is solely based on the point estimate of the tuatara molecular rate and ignores the associated 95% highest probability density (HPD) [2]. The point estimate we obtained for tuatara (1.56 substitutions per site per million years [$ss^{-1}My^{-1}$]; HPD 0.83–2.34) is the highest recorded for vertebrate animals. However, we did not ignore the HPD; we explicitly stated that the tuatara rate is not significantly different from the high rates recorded for penguins, aurochs and moa [3].

Miller *et al.* [2] also suggested that our estimate of the evolutionary rate of the hypervariable regions (HVRs) of the tuatara mitochondrial (mt) genome is 'implausibly high', compared with estimates from pedigree analyses and mutation accumulation lines. In fact, these methods result in rates as high as 2.5 and 8.9 $ss^{-1}My^{-1}$, respectively [4,5] and these are ~60% and sixfold greater than what we estimated for tuatara using time serial samples. Furthermore, Miller *et al.* [2] calculated a tuatara 'mutation rate' by simply dividing our evolutionary rate by a generation time estimate. Such a modified evolutionary rate is not comparable to an actual mutation rate obtained using pedigree methods or mutation accumulation lines because they estimate different parameters in different ways.

Miller *et al.* [2] also suggest that the high molecular rate recorded in our study [3] might be a result of what they argue is the low genetic variability (~2%) of our tuatara dataset. This claim is also incorrect. The nucleotide diversity of the nine other species compared in our study ranged from 0.2% to 6.2% (Figure 1a). Obviously, the diversity of our tuatara dataset is well within this range and the nucleotide diversity of only three species (horse, bison and brown bear) is significantly greater than that of tuatara ($P > 0.05$) (Figure 1a). Moreover, contrary to Miller *et al.*'s [2] hypothesis, the evolutionary rates for these three species with high diversity are significantly slower (<0.4 $ss^{-1}My^{-1}$) than that estimated for tuatara (1.56 $ss^{-1}My^{-1}$) [3]. Therefore, the claim that low nucleotide diversity will upwardly bias our Bayesian analysis of molecular rates is unfounded.

Although Miller *et al.* [2] suggest that there is no evolutionary signal in the dataset we presented, they nevertheless analyzed a subset of our original data and then claimed that their rate estimate is informative. By analyzing just the ancient samples, Miller *et al.* [2] estimated a much lower molecular rate (0.076 $ss^{-1}My^{-1}$; HPD 0.0016–0.32) for tuatara. This rate has very large confidence intervals close to zero and the mean is 47 times that of the lower HPD that actually indicates a lack of signal due to small sample size. Furthermore, they performed three randomization tests by interchanging the ages within the ancient samples. These tests resulted in rate estimates that are slightly less than but not significantly different from ours, which led them to conclude that the tuatara dataset does not contain sufficient information to estimate a valid rate of evolution. However, Miller *et al.* [2] did not include the modern samples in their randomization process. The latter constitute 55% (41 out of 74 sequences) of the dataset. Because their approach retained 55% of the signal, it is not surprising that the resulting rate estimates are not different from that estimated using the true ages. We repeated their analysis with a complete randomization protocol that randomized the ages of all sequences (ancient

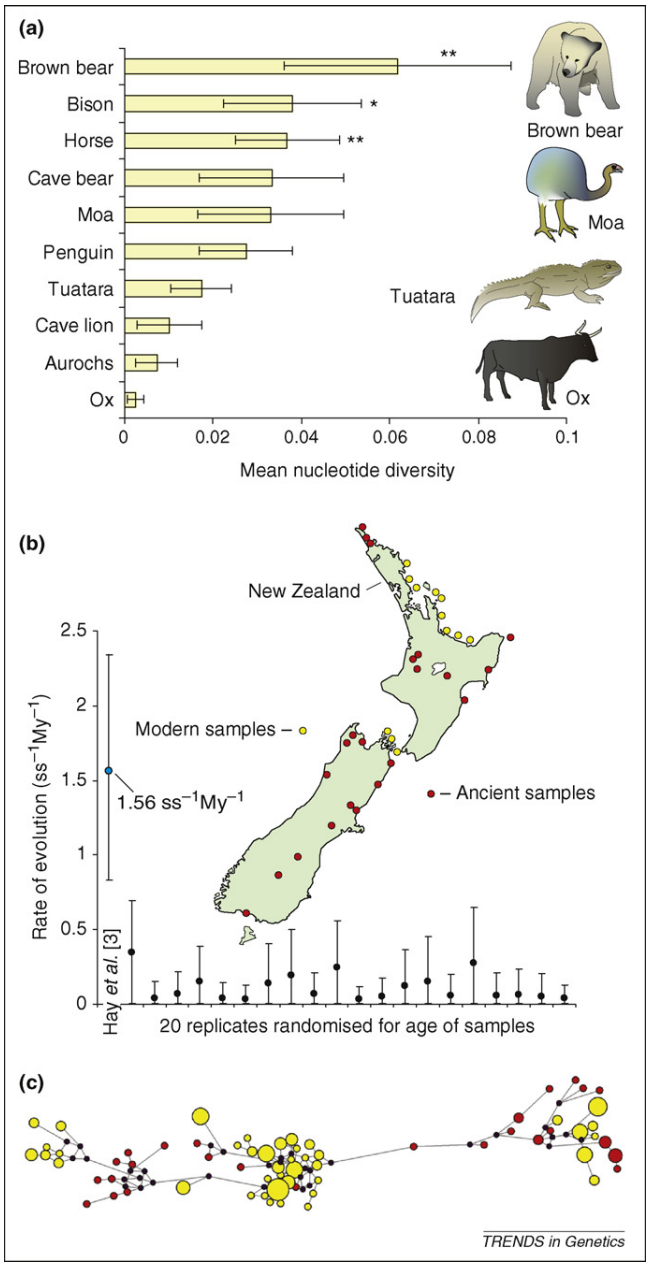

Figure 1. Nucleotide diversity, rates and genetic structure in tuatara. **(a)** Nucleotide diversities of bison, brown bear and horse are significantly higher than that of tuatara ($^{*}P < 0.05$ and $^{**}P < 0.01$). **(b)** Evolutionary rates of the mitochondrial HVR regions of tuatara estimated using serial samples. The original rate estimated by Hay *et al.* [3] is shown. The blue dot represents the point estimate and the 95% HPD intervals are shown. The results from 20 replicates, in which the ages of all samples were randomized, are indicated. Black dots represent the point estimates and the 95% HPD are also given. The geographic distributions of modern and ancient samples used in this study are shown. **(c)** Median joining network of tuatara haplotypes is shown. Ancient sequences are indicated in red, modern in yellow and hypothetical haplotypes in black. Branch lengths approximate the number of nucleotide differences between haplotypes and the sizes of circles are proportional to the number of samples.

and modern) and we then estimated the evolutionary rate using Bayesian evolutionary analysis sampling trees (BEAST; http://beast.bio.ed.ac.uk) [6]. The evolutionary rates for each of our 20 randomized datasets were much lower than the rate of 1.56 $ss^{-1}My^{-1}$ reported by us (Figure 1b). The results of our reanalysis are precisely what would be expected from randomizing the ages of all samples, when there is signal in the data.

We also conducted a simulation study to test if the results presented by Miller *et al.* [2] are derived from a methodological artifact as a result of ignoring the modern sequences (see supplementary material online for further details). When we randomized the dates of ancient simulated sequences only; we obtained similar results to those reported by Miller *et al.* [2] (Figure S1a). By contrast, randomizing the ages of modern and ancient simulated sequences (Figure S1b) gave similar results to those shown in Figure 1b, again indicating strong support for our original estimate [3].

Miller *et al.* [2] also suggested that tuatara populations are highly structured genetically and that this structure will have contributed to the rapid evolutionary rate we recorded. They claim that mainland (ancient) and offshore island (modern) populations of tuatara are genetically differentiated. However, their claim was based on patterns of geographic structure of tuatara from microsatellite DNA [7,8]. This inference is invalid because our study is about rates of evolution of mitochondrial DNA. Microsatellites typically show much more variation and reflect a different time and geographic scale to that of mitochondrial DNA [9]. Moreover, median joining network analyses using NETWORK 4.5 (http://www.fluxus-engineering.com) show that mtDNA sequences of tuatara from different island populations intermingle. Furthermore, our modern and ancient samples, although geographically distinct, are not genetically discrete and their mtDNA sequences also intermingle in a phylogenetic network (Figure 1c).

Finally, Miller *et al.* [2] noted that our Bayesian methods assume either a constant population size or exponential growth, whereas tuatara populations have declined substantially since humans arrived in New Zealand ~730 years ago [10]. However, all but three of the bones used in our original study [3] predate this decline. Therefore, the recent demographic history of tuatara cannot explain the high molecular rate we estimated.

It should be noted, however, that currently available methods for evolutionary rate estimation including BEAST [6] are based on simple models of evolution. Therefore, future rate estimates that take into account parameters such as DNA damage, migration, bottlenecks and population subdivision will influence the point rate estimates of all the vertebrates. However, the high relative rate estimate we reported for tuatara [3] is unlikely to change. Hence, despite its morphological stability, it is clear that tuatara evolve quickly at the level of neutral genetic variation, when compared with other vertebrates. This is not surprising because the biological processes underlying DNA sequence evolution and those that govern changes in morphology are very different. When it comes to tuatara, Allan Wilson and his co-workers would no doubt be pleased.

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Supplementary data

Supplementary data associated with this article can be found at doi:10.1016/j.tig.2008.11.001.

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