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Mitochondrial replacement and its effects on human health

Sir,

In a recent paper Dobler *et al.* (Dobler *et al.*, 2018) assess the likely consequences of mitochondrial replacement therapy using estimates of the effects of combining mitochondrial DNA with a novel nuclear background in animal and human studies. However, their conclusions are unsound since they do not follow elementary statistical principles.

What they fail to draw attention to, and properly model in their statistical analysis, is the high degree of statistical non-independence in their data. They have collected 1004 estimates of the effect of placing a mitochondrial DNA in a novel nuclear background. However, many of these estimates are from the same lines assayed for multiple traits. For example, 364 estimates come from the seed beetle, *Callosobruchus maculatus*, in which the same 25 lines, from the reciprocal crossing of only five lines, have been assayed for multiple traits, and 131 estimates come from the copepod *Tigriopus caifornicus*, where six crosses have been made between three lines. In some cases, the traits are very similar; 22 of their estimates come from a comparison of two mouse strains in which the mice were weighed every few days. They claim to account for the non-independence by adding publication ID into their model as a random factor, however this will fail to account for the non-independence for two reasons.

First, the random factor accounts for mean differences in effect size between publications, it does not take account of the fact that estimates of the variances in the model are incorrect. This is elementary statistics; you cannot test whether males and females differ in height by repeatedly measuring the height of a single male and female over and over again.

Second, including publication ID in an analysis does not take into account that multiple publications have used the same strains. In short, they have far less statistical power than they acknowledge.

Finally, they present a risk analysis of mitochondrial replacement therapy. They use estimates of how often significant negative effects are observed in animal studies to estimate what they expect in humans. However, again, the problems of non-independence are evident. A large proportion of the significant negative estimates (65 out of 136) come from a single study in which four traits were measured for three pairs of reciprocal crosses. The experiment in question was conducted by Ellison and Burton (Ellison and Burton, 2008) on *T. californicus*. Each pair of strains were crossed to form an F1; here the mtDNA finds itself in a nuclear genome which is 50% novel (i.e. from the paternal strain). They then crossed the F1s to yield F2s; the mtDNA is now in a variant of the F1 genome; on average 50% of the genome is paternal and 50% maternal. Finally, they backcrossed the F2s to the original parental strain such that on average 75% of the genome would be of one parental strain. They treat each of these as a statistically independent estimates of the effect of placing a mtDNA in a novel nuclear environment. This is inappropriate. A risk assessment conducted with this level of care could essentially come to any conclusion.

In summary, while the aims of the study of Dobler *et al.* (Dobler *et al.*, 2018) are laudable, they have not followed basic statistical principles and as such their analysis does not substantiate their claims. Other reviews of the issues and evidence surrounding mito-nuclear interactions and mitochondrial replacement therapy are available (Eyre-Walker, 2017).

Conflict of interest

Professor Adam Eyre-Walker declares a conflict of interest, since Dr. Ted Morrow works within the same research grouping at the University of Sussex.

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Reply: Mitochondrial replacement and its effects on human health: accounting for non-independence of data in meta-analyses

In a letter regarding our recent meta-analysis (Dobler *et al.*, 2018) showing pervasive health effects of mitochondrial replacement (MR), Eyre-Walker (2018) suggested that our treatment of non-independence of data results in our analysis being obsolete. Below we explain why we consider this statement is incorrect.

Non-independence of the data within a study has been correctly accounted for

The letter stated that the majority of the data points in our study originated from the same set of beetle or mouse strains, and effect size estimates are, therefore, inflated. We disagree; only three pairs of studies used the same strains—but these investigated different traits.

In his letter, Eyre-Walker suggests that entering study ID as a random factor does not account for the fact that multiple traits from the same study count as independent data points. Hence, he alleges that we used all 1004 effect size estimates as independent data points. This statement is incorrect. It is in fact the exact purpose of random effects in statistical modelling to account for multiple traits within a study to only produce one estimate per study. It is these estimates per study that are being compared across studies. This can be seen from the fact that our effective sample size has always been 44 cases/studies (each weighted by sample size of the respective study, by study ID and by phylogenetic relatedness).

Further, the letter suggests that, even if study ID was correctly employed, the model would be wrong because the variances in the model were incorrectly calculated. The inference, that our variances are erroneously calculated by a population-wide random factor approach, is incorrect. Every single effect size (Hedge's g) had its own variance calculation incorporated into the analysis. Clearly, variances for each effect-size estimate are accounted for in our meta-analysis. The statement 'that repeated measures of the same male and female are invalid to reveal overall sex differences' is true, but irrelevant to our study.

Correlations of traits within studies produce only small effects on effect size estimates

Recently, it has been pointed out that the use of random factors may not always sufficiently account for non-independence of the data if measurements of different traits within one study are correlated (Noble *et al.*, 2017). The consequences of this correlated error structure are assumed to be small, but we took the opportunity to examine its effect in a novel analysis adding 'reported trait' as a random factor to study ID. If our analysis was inflated because of the correlation of measurements of traits within a study, the difference between biomedical and biological studies should increase. However, the opposite is true and our original conclusion is strengthened. The marginally significantly higher effect size of biomedical (0.697, CI: 0.450;0.956)) compared to biological studies (0.462, CI: 0.287;0.688) in our original study now disappears. The new effect size estimates are 0.58 (CI: 0.49;0.70) for biomedical studies, and 0.53 (0.45;0.68) for biological studies. The difference of 0.05 (CI: -0.02;0.12) is not significant (autocorrelation of fixed factors: 0.08, of random factors: 0.15).

Risk analysis of MR is not unduly influenced by a single model species

As no risk analysis had been carried out before the clinical introduction of MR, we used our data set to calculate potential risks associated with MR. We used the epidemiologically ultraconservative approach that all effects observed between treatment and control group were only caused by a single offspring of a single maternal individual in the treatment group. The letter suggests that our risk analysis has been so poorly carried out that we could have come to any conclusion; that because most negative effects (65 out of 136 = 47.8%) were contributed by a single study organism, *Tigriopis californicus*, our results would be biased. However, Eyre-Walker (2018) failed to report that this species contributed an identical proportion of positive effects (30 out of 63 = 47.6%) and is, therefore, unlikely to produce a bias in our analysis.

Eyre-Walker further suggests that our analyses comparing differences between *Tigriopus* populations that differ on average in around 25%, or 50%, of the parental nuclear genome, are not appropriate to be treated as separate genomes. The authors of the original study treated these populations as independent units in their statistical analyses. Notwithstanding, we have presented all details of our risk analysis and readers are welcome to do their own calculations using one or other assumption. If our risk analysis lacks value, it is because we have been too conservative, not too careless, in our inferences.

Finally, in his letter, Eyre-Walker recommends his own analysis of MR effects (Eyre-Walker, 2017) instead of our systematic meta-analysis. We do not support this recommendation as his own study does not account for pseudoreplication or for phylogenetic dependence, it contains various traits whose relation to health are unclear, and it is based on a much smaller subset (23% of the data points and 45% of the studies) of data than that incorporated into our study, even though the study spanned the same period as our meta-analysis.

In conclusion, we have avoided pseudoreplication, by using state-of-the-art meta-analyses, based on Bayesian inference, with phylogenetic control, and utilise a multilevel modelling framework of analysis. As a consequence, we disagree with Eyre-Walkers suggestion that our analysis violated elementary statistical principles, and note that our analyses followed the current gold standard of meta-analyses in biology (e.g. Winternitz *et al.*, 2017; Noble *et al.* 2017, 2018). Moreover, addressing a recent concern on random factor use in meta-analyses (Noble *et al.*, 2017) allowed us to confirm that correlation of trait measurments within a study had no decisive impact on the results. We firmly refute Erye-Walker's criticisms of our study.

Conflict of interest

Dr Edward Morrow declares a conflict of interest, since Professor Eyre-Walker works within the same research grouping at the University of Sussex.

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