Response to Comment on "Global Genetic Change Tracks Global Climate Warming in Drosophila subobscura "

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Response to Comment on "Global Genetic Change Tracks Global Climate Warming in *Drosophila subobscura*"

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Rodríguez-Trelles and Rodríguez advocate standardizing old and new collections by climate rather than by calendar and also propose that some of our samples were biased by inappropriate timing. Their first suggestion applies to few species, and its implementation alters photoperiodic cues. Their second point is valid, but our conclusions are robust: Observed genetic changes reflect global warming, not sampling artifacts.

Rodríguez-Trelles and Rodríguez (*1*) assert that anyone attempting to evaluate genetic responses to climate warming should standardize sampling so that old and new samples are collected under equivalent climatic conditions. If, instead, old and new samples were standardized to the same month, then new ones would be collected under seasonally warmer conditions. If genetic markers shift seasonally, then observed between-sample differences might merely reflect seasonal rather than long-term changes. The authors raise an interesting caution that has been overlooked in many (*2*–*4*), but not all (*5*), previous studies. Nevertheless, we feel that they are overly cautious about existing and future studies of genetic responses to climate change.

First, their caution is relevant only to species having short generation times relative to season length (*6*) and to those having genetic markers that show marked seasonal cycles (*7*). Second, standardizing new samples to warmer but earlier dates will necessarily "destandardize" photoperiodic cues (*8*–*10*), which can have diverse phenotypic effects. Third, any genetic impact should be small. In Europe, spring/summer has advanced 2.5 days per decade (*11*). For samples separated by 25 years [median in our data in (*4*)], spring has advanced by only 6 to 7 days. Because few genetic samples of any species are separated by more than 25 years (*5*, *12*), seasonal advances are unlikely to alter genetic frequencies substantively in most species.

In their specific objection to the timing of our samples (*4*), Rodríguez-Trelles and Rodríguez (*1*) assert that inversion polymorphisms of *D. subobscura* undergo "pronounced seasonal cycles" and then argue that "it is conceivable" that our between-sample differences might be a sampling artifact. However, their statement that inversion polymorphisms cycle seasonally in *D. subobscura* is not universally accepted (*13*, *14*), and the example they cite (*15*) established cycles only for the O chromosome. To help resolve this controversy, we reanalyzed a large data set (*16*) for a Catalonian population of *D. subobscura*. For each of the 11 months, we computed a genome-wide PC1 score (*4*) and regressed it on month using a third-order polynomial weighted by sample size. PC1 shows temporal variation but no evidence of a sinusoidal trend (all $P > 0.24$). Thus, genome-wide seasonal cycles are undetectable at this site.

We did not include sampling dates in our study (*4*), so Rodríguez-Trelles and Rodríguez (*1*) relied on published dates (*17*) for the 13 European sites. They objected that four new samples were collected 1 to 2 months closer to summer than were the old ones. However, these selected sites are atypical of our samples: Three other new European samples were collected in a cooler month, all seven of our new North American sites were collected in a cooler month, and all six of our new South American samples were collected in a cooler or the same month. Thus, for most of our sites, seasonal shifts in inversion frequencies would only reduce the magnitude of change over time, not exaggerate it as suggested by Rodríguez-Trelles and Rodríguez (*1*).

If the four sites did bias the inversion data, then the difference in monthly ambient temperatures should be correlated with the difference in chromosome index (Ch_{PC1}) among sites. This correlation is significant (Spearman rho = 0.452, $P = 0.021$), and the correlation is eliminated when these four samples are deleted ($\rho = 0.0047$, $P = 0.834$). Thus, the four sites did induce bias. Is the bias sufficient to challenge our conclusions? To evaluate this, we deleted the four problematic samples and re-ran all analyses. The increase in the intercept from old to new samples remains significant for temperature ($F_{[1,21]} = 19.3$, $P = 0.00025$) and for chromosomes ($F_{[1,21]} = 37.6$, $P = 0.000004$). Moreover, chromosomes and temperature still shift in parallel (Rayleigh test: $\bar{r} = 0.60$, $P = 0.0002$). Therefore, our conclusions are robust.

We concur with Rodríguez-Trelles and Rodríguez (*1*) that the timing of collections should be considered carefully. However, their suggestion to standardize by climate rather than by calendar date will bias photoperiodic cues. Moreover, the observed growing-season shifts of a few days will generally be too small to have major genetic impact and thus bias results. Our new analyses reject their claim that inversions in this species show pronounced seasonal cycles. Finally, with regard to our own study, 4 of 26 samples are indeed problematic, but our original conclusions hold when those samples are excluded. We stand by our original conclusion that global genetic change in *D. subobscura* is tracking global warming and is not a sampling artifact.

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