SUPPORTING INFORMATION

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Maternal sexual interactions affect offspring survival and ageing

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SUPPORTING METHODS

Statistical analyses

We used multilevel models that provide a powerful and effective means of accounting for the hierarchical, nested structure of the data (Kreft & de Leeuw 1998; Gelman & Hill 2007), and that thus eliminate the issues of pseudoreplication that researchers historically experienced when dealing with hierarchically-structured datasets (Schank & Koehnle 2009; Lazic 2010). The parameter estimates for fixed effects in the longevity models are maximum likelihood estimates. Nonetheless, to conform to the historical paradigm for inferences based on orthogonal balanced designs, we report denominator degrees of freedom estimated using Satterthwaite's approximation in the *lmerTest* package for fixed effects (Kuznetsova, Brockhoff & Christensen 2013).

To estimate ageing rates, we explored which of four different models best described the temporal pattern of mortality in each cohort (Gompertz, Gompertz-Makeham, logistic and logistic-Makeham), using log-likelihood tests. The Gompertz model describes an exponential increase in mortality with age (Pletcher 1999), denoted $\mu_x = \alpha\, e^{\beta x}$, where μ_x is the predicted instantaneous mortality rate at age x, α is the Gompertz intercept (also called age-independent mortality rate, or frailty), and β is the rate of increase in mortality with age (also called age-dependent mortality rate, or the rate of senescence of the population) (Finch 1990). Logistic

models account for a deceleration in the rate of mortality with age, and when deceleration equals zero, logistic models are reduced to Gompertz. Finally, Makeham models include a constant that accounts for age-independent mortality (Pletcher 1999).

We fitted Gompertz models, for both sexes in our study, which were consistently the best fit to the data, as confirmed via sensitivity analyses that tested for the robustness of our parameter estimates (per cohort) using a maximum likelihood estimation procedure in *Winmodest*. The Gompertz parameters for frailty and the rate of senescence were estimated separately for each cohort.

SUPPORTING DISCUSSION

In our manuscript, we present results that indicate that heightened exposure, by females, to male sexual activity invoke trans-generational effects on offspring survival and ageing rates, and suggest that these effects are therefore putative trans-generational costs associated with multiple mating in females. We then contend that future studies that seek to fully appraise the evolutionary economics of multiple mating in females should take a broader scope than previous studies that have typically focused only on comparing the direct costs of multiple mating (to the mothers) to the indirect benefits in the offspring generation. Specifically, we suggest that such studies should routinely screen for the existence of trans-generational costs. The question must be asked – have we constructed a 'straw man' in making this argument? The answer would be *yes* under a scenario in which the costs and benefits would affect the same traits (since then researchers would already be inadvertently measuring all cost and benefit components). But this scenario is not the general case. Direct (Arnqvist & Nilsson 2000) and trans-generational benefits (Head *et al.* 2005; Priest, Galloway & Roach 2008; Priest, Roach & Galloway 2008; Garcia-Gonzalez & Simmons 2010) typically affect traits

linked to reproductive success [direct benefits on female fecundity, and trans-generational benefits via increases in offspring viability, competitive ability, attractiveness, or fecundity]. In contrast, the costs are typically manifested on traits linked to other components of organismal life-history. In species that form pair bonds, these costs might for instance come as reductions in paternal care by the social mate in instances where females mate with extra-pair males (Arnqvist & Kirkpatrick 2005). But, at least in the insects, the costs are most typically manifested as reductions on survival of the test subjects [direct costs] (Fowler & Partridge 1989; Chapman *et al.* 1995; Rice 1996; Crudgington & Siva-Jothy 2000; Garcia-Gonzalez & Simmons 2010; Gay *et al.* 2010), which as we have now identified, can even extend to effects on offspring survival [trans-generational costs]. Thus, studies seeking to fully appraise the costs and benefits of heightened sexual activity must balance these four components, and this will involve measuring either lifetime reproductive success (encompassing reproductive output across all life stages), or alternatively overlaying data on reproductive and mortality trajectories, in a multi-generational context (i.e., both mothers and offspring) – a feat that has rarely been achieved (Priest, Galloway & Roach 2008).

While our study has identified negative trans-generational effects on the offspring phenotype, the underlying mechanisms driving these results remain elusive. Our results do, however, suggest that it is not direct selection *per se* for particular paternal genotypes, brought about by polyandry, which contributed to divergence in the mortality trajectories of the offspring across the maternal sexual treatment. This is so because the greatest difference in offspring trajectories occurred between treatment levels in which the mothers mated only once (i.e. the *baseline* and *pre-copulatory* only levels). Thus, the reported effects are almost certainly attributable to variance in maternal effects induced by the levels of sexual interactions (harassment in this case) across the maternal sexual treatment. We note that our treatment did not result in differential (or indeed any) mortality on the mothers so the effects

reported here cannot be ascribed to direct selection on females leading to differential underrepresentation of maternal genotypes across treatments. It is possible that differences in the condition of mothers could have imposed selection for particular offspring genotypes during juvenile development, across the levels of the sexual mating treatment. For instance, offspring carrying alleles encoding larger body size, that might subsequently be associated with longer life, might have been less likely to survive to adult eclosion when mothers were subject to higher levels of harassment and thus were driven to poorer condition (in the *pre-copulatory* and *pre- plus post-copulatory* levels). Yet, there was no significant signature of differential mortality of focal juvenile flies across the treatment, with egg-to-adult viability of the focal juvenile flies (i.e. prior to their eclosion) unanimously high (> 90%). Alternatively, the differences in offspring ageing profiles across the treatment might reflect maternally-induced condition-dependent plasticity in the expression of offspring life-histories, or be epigenetically determined. Experimentally distinguishing among these possibilities to identify the precise mechanisms involved would provide a worthy avenue for further research.

Although we have documented trans-generational costs of sexual interactions on offspring survival and ageing, further studies will be required to test for generality of these effects across taxa and to home in on the true magnitude, hence importance, of these effects in natural populations. While our experiment provides proof-of-principle, we note that the focal offspring were raised and measured under benign laboratory conditions (controlled densities, one standardized mating per fly, ample live yeast on which to feed, constant temperature). We believe that it is therefore possible that the detected effects might have been amplified if measured under typical competitive conditions experienced by natural populations. Also, we note the possibility that the effects that we have identified on actuarial patterns of senescence might correlate with patterns of reproductive senescence (decline in

fertility and fecundity with age) throughout the life of the focal offspring. If this is the case, then the trans-generational effects we reported here might reflect much larger, currently cryptic, costs. Answers to these questions will require further research under a range of different environmental conditions, and involve measurement of reproductive senescence over the life of the focal flies. Although the collection of such data would represent a large undertaking, on the basis of our findings such an effort would be worthwhile in enabling an integrative assessment of the cost-benefit balance of sexual interactions.

Finally, we attend to the ongoing debate over the value that laboratory-based studies can add, when it comes to making general conclusions about evolutionary processes in natural populations. We note that prior to the experiment commencing, the population that we used had been reared in the laboratory for around two years, on a discrete generation, 14 day life cycle, whereby every new generation is propagated by adult females that are four days post-eclosion. This means that the population had been under an early-life selection regime for around 50 generations, and was laboratory-adapted. This approach has many advantages for studies that seek to make evolutionary inferences (Rice *et al.* 2005; Rice *et al.* 2006), and it results in experiments in which the focal flies are assayed in the environments in which they have evolved over long periods (in our case > 50 generations), rather than in a novel environment as happens when organisms are brought in from the field and assayed within one or a few generations of their arrival in the laboratory. However, the discrete generation protocol under which this population is maintained would in theory facilitate the accumulation of mutations that do not express their negative effects until after four days of adult life. Theoretically, this might result in a general acceleration of ageing, and shorter life spans, within the studied population relative to the ancestral natural population, as predicted by the classic evolutionary theory of ageing (Medawar 1952; Williams 1957; Stearns *et al.* 2000; Dowling 2012). Furthermore, alleles that might be maladaptive in natural populations,

might be swept to fixation in the laboratory population if they confer a benefit to early-life fitness (Dowling *et al.* 2009). However, we do not envisage these processes would bias the reported effects across the treatment levels in relation to those that would be expected, under the same experimental conditions, in the ancestral population. Clearly, the flies are still capable of reproducing for several weeks (and will therefore experience a reproductive senescence), and the population-specific exponential increase in mortality rate (i.e. onset of senescence) does not commence until around 30 days of adulthood. Admittedly, genetic architectures underlying certain phenotypes will undoubtedly change as organisms evolve to a new (laboratory) environment, and this might in certain instances affect the capacity of researchers to generalize their laboratory-produced results back to the wild populations from which their study organisms originally derived. In this particular case, however, we explore the effects of an 'ecological treatment' on trans-generational phenotypic expression, and it seems less likely that differences, between the laboratory and wild-evolved populations, in the fine-scale genetic architecture underpinning ageing and longevity would tangibly affect the response to this treatment. Nonetheless, our goal is not to make broad-scale inferences about how parental sexual interactions affect offspring phenotypes in the wild, or across taxa. Our goal is simply to provide a proof-of-principle for a scarcely considered concept.

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