

## Comment

**Potential confounds to an assay of cross-generational fitness benefits of mating and male seminal fluid**

In Priest *et al.* (2008a), it was reported that lifetime reproductive success of female *Drosophila melanogaster* was increased by 28 per cent if their mothers had been housed under high remating conditions. As this gain could be due to mothers ‘trading up’ by remating with males with superior genes or via non-genetic parental effects, Priest *et al.*'s (2008b) cross-generational experiment was designed to differentiate between these two hypotheses. However, a review of this study leads us to question the validity of the results and the conclusions drawn from them.

In the assay, 4-day-old singly mated adult females were housed for 6 days, either in isolation from males (control) or in one of two male-exposure treatments where the males provided were sterile (i.e. lacked main-cell Acp<sub>s</sub> or were incapable of producing sperm). Over the last 2 days of this period, eggs produced were collected, counted and then permitted to develop for 12 days. At that time, adult daughters from all treatments were obtained and age-specific fertility (number of eclosed pupal cases produced) and longevity were measured. These data were used to calculate Charlesworth's (1994) index of fitness ( $r$ , intrinsic rate of increase). They found that early life fertility and fitness of daughters produced by mothers continuously housed with males were greater than those whose mothers were isolated from males. As the sterile males could not have passed on any ‘good genes’, non-genetic parental effects appeared to be responsible.

Our concerns about this study stem from the observation that maternal egg production differed significantly between treatments, with the mothers in male-exposed treatments laying approximately 40 per cent more eggs than mothers from the control group. This implies that there are substantial direct fitness benefits of polyandry in *D. melanogaster*, which conflicts with a number of studies (including Priest *et al.* 2008a), which demonstrate that prolonged exposure to males incurs large, direct, fitness costs to females (reviewed in Kujiper *et al.* 2006). Although the authors provide no explanation, we suspect that this abnormal result may be due to an unanticipated flaw in the experimental design relating to maternal housing conditions, which in turn casts doubts on the validity of the assays of daughter's fitness and fertility.

The accompanying reply can be viewed on page 28 or at <http://dx.doi.org/doi:10.1098/rsbl.2008.0434>.

It is conceivable that maternal egg production totals do not accurately reflect offspring production, if a large number of the eggs laid were unfertilized. In the 4–6 days that preceded the maternal egg-production monitoring phase of the assay, the amount of viable sperm in storage would have decreased naturally due to sperm death and use in fertilization. In the experimental treatments, where each female was assumed to have remated twice, additional sperm loss could arise via sperm dumping by the female and/or incapacitation by Acp<sub>s</sub>. The extent to which remating with sterile males leads to viable sperm loss is ambiguous. Snook & Hosken (2004) observed that following a single remating, roughly one-third of previously mated females possessed no sperm in storage, while higher rates of fertilized egg production subsequent to remating are reported by Prout & Clark (2000). Since Priest *et al.* (2008b) did not provide data on viable egg production rates, we can only speculate on the importance of sperm shortages.

Our concern regarding egg infertility in the maternal generation also has implications for the later measurement of daughter's fertility and fitness, as larval density can be an important source of variation in adult life-history parameters. If densities are exceedingly low, there will be insufficient larvae to effectively ‘work’ the media, while at higher densities, larvae experience greater competition for resources and increased exposure to waste products, both of which can influence adult fitness components (reviewed by Ashburner *et al.* 2005). In this assay, the extent to which larval densities (i.e. environmental conditions) differed between treatments is unknown, making it difficult to tell whether the differences in offspring fitness and fertility are due to larval environment or parental contribution. Ideally, if the authors had controlled larval density by adding fertilized eggs from another, marked population to the vials, this confound could have been avoided.

Even if there are no confounding effects of larval density on measurements of daughter's fitness and fertility, potential problems with the statistical analyses performed still exist. This is because one of the ANCOVA assumptions in Priest *et al.*'s (2008b) models is not met: the level of the covariate (number of eggs per vial) and treatment (maternal mating treatment) are highly dependent. This is problematic for the data's analysis and interpretation, as the effect of the covariate cannot be partitioned from the ‘independent’ treatment effect, leading to artificial ANCOVA results (Huitema 1980). It is thus unclear whether significant effects of maternal mating treatment on daughter's fitness or treatment  $\times$  age interaction in the analysis of age-specific fertility are spurious results.

Taken together, the concerns raised regarding the results, analysis and interpretation of the cross-generational assays described by Priest *et al.* (2008b) suggest that they should be treated with caution until further studies and re-analyses can be performed.

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- Ashburner, M., Golic, K. G. & Hawley, R. S. 2005 *Drosophila: a laboratory handbook*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Charlesworth, B. 1994 *Evolution in age-structured populations*. Cambridge, UK: Cambridge University Press.
- Huitema, B. E. 1980 *The analysis of covariance and alternatives*. New York, NY: Wiley.
- Kuijper, B., Stewart, A. D. & Rice, W. R. 2006 The cost of mating rises nonlinearly with copulation frequency in a laboratory population of *Drosophila melanogaster*. *J. Evol. Biol.* **19**, 1795–1802. (doi:10.1111/j.1420-9101.2006.01186.x)
- Priest, N. K., Galloway, L. F. & Roach, D. A. 2008a Mating frequency and inclusive fitness in *Drosophila melanogaster*. *Am. Nat.* **171**, 10–21. (doi:10.1086/523944)
- Priest, N. K., Roach, D. A. & Galloway, L. F. 2008b Cross-generational fitness benefits of mating and male seminal fluid. *Biol. Lett.* **4**, 6–8. (doi:10.1098/rsbl.2007.0473)
- Prout, T. & Clark, A. G. 2000 Seminal fluid causes temporarily reduced egg hatch in previously mated females. *Proc. R. Soc. B* **267**, 201–203. (doi:10.1098/rspb.2000.0988)
- Snook, R. R. & Hosken, D. J. 2004 Sperm death and dumping in *Drosophila*. *Nature* **428**, 939–941. (doi:10.1038/nature02455)