JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12276

Maternal sexual interactions affect offspring survival and ageing

D. K. DOWLING*, B. R. WILLIAMS* & F. GARCIA-GONZALEZ†‡

*School of Biological Sciences, Monash University, Melbourne, Vic., Australia
†Doñana Biological Station, Spanish Research Council CSIC, Isla de la Cartuja, Seville, Spain
‡Centre for Evolutionary Biology, School of Animal Biology, The University of Western Australia, Nedlands, WA, Australia

Keywords:

ageing;
indirect benefits;
indirect costs;
life-history;
lifespan;
maternal effects;
polyandry;
sexual conflict;
sexual selection;
trans-generational.

Abstract

In many species, females exposed to increased sexual activity experience reductions in longevity. Here, in *Drosophila melanogaster*, we report an additional effect on females brought about by sexual interactions, an effect that spans generations. We subjected females to a sexual treatment consisting of different levels of sexual activity and then investigated patterns of mortality in their offspring. We found reduced probabilities of survival, increases in the rate of senescence and a pattern of reduced mean longevities, for offspring produced by mothers that experienced higher levels of sexual interaction. We contend that these effects constitute trans-generational costs of sexual conflict – the existence or implications of which have rarely been considered previously. Our results indicate that ongoing exposure by mothers to male precopulatory interactions is itself sufficient to drive trans-generational effects on offspring mortality. Thus, we show that increases in maternal sexual activity can produce trans-generational effects that permeate through to latter life stages in the offspring. This helps to elucidate the complex interplay between sex and ageing and provides new insights into the dynamics of adaptation under sexual selection.

Introduction

Much of evolutionary theory is grounded on the principle that investment in reproduction is costly. For example, the cost of reproduction is central to life-history theory because it drives the fundamental trade-off between an individual's investment into current reproduction at the expense of future reproductive potential and survival prospects (Williams, 1966; Reznick, 1985; Stearns, 1989). Reproductive costs have also been invoked in classic theories that seek to explain the evolution of ageing, such as the *Antagonistic Pleiotropy* and the *Disposable Soma* theories (Williams, 1957; Kirkwood & Rose, 1991).

It has become clear that the costs associated with sexual interactions extend far beyond those that are directly tied to reproductive investment. In several species, the act of mating alone can drive reductions in female longevity, when controlling for variance in reproductive rates. Arguably, the best examples come from the fruit fly *Drosophila melanogaster* and the seed beetle *Callosobruchus maculatus*. In *Drosophila melanogaster*, the receipt by females of seminal proteins that are present within the male ejaculate decreases their longevity, with those females receiving greater loads of accessory gland proteins living shorter lives (Chapman *et al.*, 1995). In fact, females experience decreases in survival simply by co-habiting with males in the absence of any mating, implying that male precopulatory behaviours are detrimental for female fitness (Partridge & Fowler, 1990). Experimental evolution studies have confirmed the sexually antagonistic nature of male adaptations in *D. melanogaster*. In one study, females were prevented from co-evolving with males by effectively holding the female phenotype static over numerous generations. Under such conditions, males rapidly adapted to the opposite sex, and a by-product of this male adaptation was a reduction in female survivorship (Rice, 1996), although this result was not replicated in a later study using descendants of the same base population (Jiang *et al.*, 2011). In a subsequent selection experiment, females mated to promiscuous

Correspondence: Damian K. Dowling, School of Biological Sciences, Building 18, Monash University, Clayton Campus, Clayton, Melbourne, 3800 Victoria, Australia.
Tel.: +61 3 99 020 479; fax: +61 3 99 055 613;
e-mail: damian.dowling@monash.edu

control males (sourced from populations where sexual selection was free to operate) suffered a longevity cost compared with females that mated to males sourced from populations evolving under conditions of enforced monogamy (Holland & Rice, 1999), demonstrating that sexual selection may select for male adaptations that harm females. Other studies in *D. melanogaster* have confirmed similar patterns, namely that co-habitation with males generally results in a reduction in female fecundity (Linder & Rice, 2005; Lew *et al.*, 2006). In *Callosobruchus maculatus*, multiply-mated females live shorter lives than singly mated (Crudgington & Siva-Jothy, 2000), and these reductions in longevity seem at least partly attributable to scar damage (Gay *et al.*, 2010) induced via male genital spines that puncture the connective tissue within the female reproductive tract (Rönn *et al.*, 2007).

The aforementioned examples form part of a portfolio of evidence, which contends that sexual selection can routinely favour the evolution of male traits that negatively impact on female life histories (Arnqvist & Rowe, 2005), including components of ageing (Promislow, 2003; Bonduriansky *et al.*, 2008). Of the many traits involved in sexual interactions, there are numerous examples of cases in which optimal expression of a trait in one sex takes the other sex away from its optimum (Arnqvist & Rowe, 2005). Indeed, the widespread prevalence of sex differences in reproductive tactics, parental investment and potential reproductive rates across the animal kingdom sets the stage for strong sex specific and sexually antagonistic selection on traits involved in sexual reproduction (Chapman *et al.*, 2003; Arnqvist & Rowe, 2005). As such, sexually antagonistic selection is expected to be a pervasive force in shaping evolutionary trajectories under sexual selection.

A longstanding conundrum for evolutionary biologists is an explanation for why the females of so many species mate multiply within a given reproductive bout (i.e. polyandrously). On the one hand, polyandry might evolve adaptively if females garner direct benefits (Arnqvist & Nilsson, 2000), or genetic (indirect) benefits for their offspring (Jennions & Petrie, 2000), from multiple mating. On the other, theory would suggest that polyandry could result entirely from sexual conflict over mating patterns (Holland & Rice, 1998; Gavrilets *et al.*, 2001) and thus be primarily associated with direct costs. Although the direct fitness benefits to females of polyandry seem common, at least in the insects when mating rates are restricted to intermediate levels, further increase in mating rates results in diminished returns on fecundity and longevity (Arnqvist & Nilsson, 2000). Furthermore, there are numerous documented cases of species for which there are no apparent direct benefits associated with multiple mating (Jennions & Petrie, 2000; Arnqvist & Kirkpatrick, 2005). As such, much attention has focused on whether the direct costs associated with multiple mating will typically be compensated by genetic benefits transmitted to the offspring (Kirkpatrick & Barton, 1997; Holland & Rice, 1998; Chapman *et al.*, 2003; Cordoba-Aguilar & Contreras-Garduno, 2003; Eberhard & Cordero, 2003; Kokko *et al.*, 2003; Arnqvist & Kirkpatrick, 2005, 2007; Griffith, 2007). Theory indicates that the direct costs will outweigh any accrued indirect genetic benefits (Cameron *et al.*, 2003), and this has been supported by some (Arnqvist & Nilsson, 2000; Arnqvist & Kirkpatrick, 2005; Orteiza *et al.*, 2005; Stewart *et al.*, 2005, 2008; Maklakov & Arnqvist, 2009; Brommer *et al.*, 2011), but not all (Head *et al.*, 2005; Garcia-Gonzalez & Simmons, 2010) empirical studies. Recent studies highlight further complexity to the debate because they show that multiple mating can result in trans-generational benefits to offspring that are generated by paternal effects or paternally induced maternal effects (García-González & Simmons, 2007; Priest *et al.*, 2008b), rather than via any indirect genetic (principally, good genes) process traditionally suggested to explain such patterns (Moller & Alatalo, 1999).

The debate to date has focussed on balancing the direct costs of polyandry to the potential trans-generational benefits that females might recoup. However, two recent studies provide some suggestion that the trans-generational effects associated with multiple mating might on occasion manifest themselves as costs rather than benefits. First, female guppies (*Poecilia reticulata*), exposed to higher levels of male sexual harassment, produced daughters with smaller bodies and sons with smaller gonopodia, and this resulted in these sons exhibiting lowered reproductive success (Gasparini *et al.*, 2012). Second, a recent study in *D. melanogaster* reported that females, subjected to frequent exposure to males, produced sons with increased fitness, but grandsons with decreased fitness (Brommer *et al.*, 2011). This would suggest that the benefits of multiple mating can be eroded via conflicting effects across generations.

Here, we contribute a novel insight into this field of research, by presenting results that indicate that the previously identified survival costs of heightened sexual interaction in female *D. melanogaster* (Chapman *et al.*, 1995; Rice, 1996; Holland & Rice, 1999) can be reinforced by trans-generational reductions in the survival, and accelerated ageing, of their offspring. These effects represent putative trans-generational costs to females of engagement in polyandry or exposure to heightened male sexual interaction under sexual selection. Furthermore, the trans-generational effects we uncover affect a fundamental pillar of organismal life history – offspring mortality trajectories. Although longevity, survival and ageing are traits that have been studied within a trans-generational framework in the context of maternal age effects (i.e. the Lansing effect; Lansing, 1947; Priest *et al.*, 2002; Fox *et al.*, 2003; Opit & Throne, 2007; Yilmaz *et al.*, 2008; Bouwhuis *et al.*, 2010), they have

generally not been examined in the context of research into the evolution of polyandry and dynamics of sexual selection (Priest *et al.*, 2008a). This probably accounts for why the effects we report here have previously remained elusive. We discuss how the existence of trans-generational costs stemming from maternal sexual interactions could have broad evolutionary implications and suggest that such costs should be incorporated into future 'cost-benefit' appraisals of the evolutionary economics of sexual selection, sexual conflict and the evolution of polyandry.

Materials and methods

Study population

The experiments were performed on an Australian population of *Drosophila melanogaster*. This population had been seeded by 60 wild-caught nonvirgin females, collected from three localities (20 females per site) within Coffs Harbour, New South Wales, Australia, in 2010. Ten sons and 10 daughters of each of these females were added to those of the other females to create a single mass-bred population. The population was thereafter propagated in the laboratory on a 14-day discrete generation life cycle, at 25 °C and a 12 : 12 light–dark cycle, across numerous 10-dram vials on a potato dextrose–yeast–agar medium. Each vial in the population is propagated by 20 four-day-old pairs over a 20-h egg-laying period, and all eclosing offspring are admixed with those of the other vials, each generation, prior to their sorting into new vials. Larval densities of each vial are trimmed to a moderate density of 150 eggs per vial. Previous research has shown that this population harbours an abundance of functional genetic variance for numerous life-history traits (Williams *et al.*, 2012).

Experimental design

The prospective mothers entering the experiment were collected as virgins within 7 h of eclosion and stored in groups of eight, with *ad libitum* access to live yeast. When the mothers were 4 days old, each group was subjected to a 2-h exposure to a group of twelve 4-day-old virgin males, during which time virtually all mothers would have mated once and only once [DKD, BRW; personal observation on this population, and general to other populations; see for example (Holland & Rice, 1999)]. The males were then removed from the mating vials, and each respective group of mothers subjected to a treatment, comprised of one of three mating levels (maternal sexual treatment), for the following 10 days.

Prospective mothers assigned to the first level received no further contact to males over the subsequent 10-day period. From here on, we refer to this level as the *baseline*, as all females in the experiment first experienced this 2-h exposure to males prior to their subsequent assignment to the other levels. Following this baseline level of sexual exposure, the eight mothers in each of the vials assigned to the second level of the treatment were immediately exposed to a new group of 12 virgin males, whose genitals had been previously cauterized using a fine tungsten wire probe connected to a 6 V, 1 A power source. This cauterization procedure had been extensively pilot-tested within our laboratory to ensure that it was completely effective in precluding copulation by the male subjects, without noticeably impairing their activity levels or desire to engage in precopulatory sexual behaviours. Thus, mothers assigned to this level remained mated only once, but were subjected to ongoing male precopulatory activity (harassment and courtship) over the 10-day period. From here on, we refer to this level as the *precopulatory only* level. Mothers assigned to the third level were immediately exposed, after the baseline level of sexual exposure, to a group of 12 virgin males, whose genitals were fully intact, over the 10-day period. Prospective mothers assigned to this level will have in general experienced effects of ongoing male precopulatory activity, several copulations and post-copulatory effects associated with the receipt of multiple ejaculates. From here on, we refer to this level as the *pre- plus post-copulatory* level. In total, 10 maternal vials (i.e. 10 vials, each containing a group of eight mothers) were allocated to each level of the treatment (thus, 30 maternal vials in total).

Every second day, each group of mothers was transferred to a fresh vial with *ad libitum* access to live yeast, and males that had died or escaped were replaced. On the fifth and tenth days of the treatment, we collected the eggs laid by each group of mothers over a 32-h laying period. Within each maternal age category (5 or 10 days of sexual treatment), these eggs were all of the same age (within 32 h) regardless of the maternal sexual treatment level from which they were sourced. This therefore precludes variance in egg-age effects from confounding our interpretations. The eggs collected from each 'maternal vial' were then placed in new vials at a maximum density of 25 eggs per vial. These vials, denoted 'juvenile vials', were the vials in which the focal offspring were reared, prior to their eclosion as adults and subsequent redistribution into vials of adults for the ageing assay described below. Given this cap on larval density in the juvenile vials at very low levels (less than twenty per cent of typical egg densities of 150 per vial, under which the population has evolved in the laboratory), and given that we scored the resultant egg-to-adult viability of these vials and can report that maternal clutch viabilities were unanimously high across the experiment (mean = 0.94 ± 0.005), there was therefore little scope for density-dependent effects to influence our interpretations. Nonetheless, as a further precaution in mitigating the potential for density-dependent effects at the larval stage to confound

our interpretations, we also included this parameter (egg-to-adult viability) as a covariate in our subsequent statistical analyses.

The focal sons and daughters of these mothers were then collected from the juvenile vials, as virgins, and stored by sex in 'assay vials', in cohorts that were capped at a maximum of 30 same-sex individuals (mean = 22.7 ± 0.57). Each cohort of focal flies, from a given assay vial, shared the same set of eight mothers that had been exposed to the maternal sexual treatment. When 5 days old, the focal flies entered the ageing assay, with the sexes assayed separately. At this point, each cohort of focal flies was exposed to an equal-sized group of 5-day-old virgin flies of the opposite sex, collected from Coffs Harbour population, and allowed to mate over a 2-h period during which time almost all flies would have mated one time only. We mated focal flies in this way to reconcile two observations. First, the natural physiological state in which a female fly would spend the majority of her life is most likely to be that of a mated rather than a virgin, given that females are sexually mature within 8 h of their eclosion to adulthood. Second, the transcriptomes of multiply-mated and once-mated females are known to exhibit very similar baseline gene expression profiles, but these baseline profiles differ substantially from those of virgin females (Innocenti & Morrow, 2009). At the end of the mating period, the mates of the focal flies were removed. From thereon, each cohort of focal flies was transferred to a fresh vial, with food and 0.001 g of live yeast, every second day, and the number of deaths recorded at the time of transfer. Each cohort was monitored until such time that all individuals within it had deceased. In total, 2106 offspring were followed through to death across the three levels of the treatment.

Statistical analyses

Using the data, we obtained measures of offspring longevity, ageing rates and survival. We conducted longevity and survival analyses on individual flies (n = 2106 flies, 93 assay vials, 30 maternal vials), using multilevel models (see Data S1). Multilevel linear models of offspring longevity were fitted using the *lmer* function in the *lme4* (Bates *et al.*, 2012) package in R 2.14.2 (R Development Core Team, 2012), with maternal sexual treatment (*baseline*; *precopulatory*; *pre- plus post-copulatory*), maternal age at oviposition (5 or 10 days), offspring sex (male; female) and their interactions as fixed effects. The egg-to-adult viability of the juvenile vials was included as a covariate (see Experimental design for exact rationale). Survival differences across the same fixed effect classes were analysed using mixed effects Cox proportional hazards regression in the *coxme* package of R (Therneau, 2012). In both the longevity and survival analyses, maternal vial replicate (there were 10 replicate vials per treatment level, thus 30 in total; denoted replicate ID in Table 1) and assay vial identity (offspring were scored in vials capped at 30 same-sex individuals, denoted Assay vial ID) were included in the model as random effects to account for the multiple levels of data.

As described above, during the ageing assay, cohorts of flies were monitored in replicated assay vials of on average 22.73 ± 0.57 individuals. We used Winmodest 1.0.2 (Pletcher, 1999) to estimate components of ageing using Gompertz models (see Data S1), where 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

Table 1 Effects of maternal sexual history on offspring longevity, survival and ageing. (a) General linear mixed model (GLMM) of offspring longevity, with maternal sexual treatment (treatment), maternal age at oviposition (maternal age) and offspring sex as fixed effects, the egg-to-adult viability associated with the rearing vial of the juvenile focal offspring (i.e. the vials that the offspring developed in) as a covariate, and assay vial ID (of adult offspring during the assay) and replicate ID (corresponding to maternal vial within maternal sexual treatment) as random effects (see Methods). b) Type III GLM of the Gompertz rate of senescence (β), with model effects as described above for *a)* and frailty (*ln* transformed) as a covariate. Cohorts here represent those of large membership (see Methods). c) Cox proportional hazards mixed model regression of survival. Standard deviation (SD) is reported for random effects. Integrated log-likelihood, χ^2 = 228.5 and 6 d.f; and penalized log-likelihood, χ^2 = 317.9 and 40.79 d.f. Further details about statistical analyses are reported in the Methods and Data S1 file.

(*a*, Offspring Longevity)	Df (num,den)	L-R χ^2	*P*
Maternal Treatment	2, 29.01	5.39	0.068
Maternal Age	1, 72.79	0.01	0.940
Offspring Sex	1, 75.51	84.69	< 0.001
Egg-to-adult viability	1, 110.22	0.60	0.439
	SD		
Assay vial ID	1.83		
Replicate ID	1.41		
Residual	12.81		

(*b*, Offspring Ageing)	Df	Type III SS	*F*	*P*
Maternal Treatment	2	0.009533	5.819	0.032
Offspring Sex	1	0.015017	18.33	0.004
ln(alpha)	1	0.090481	110.46	<0.001
Residual	7	0.005734		

(*c*, Offspring Survival)	Df	L-R χ^2	*P*
Maternal Treatment	2	8.53	0.014
Maternal Age	1	2.22	0.136
Offspring Sex	1	81.08	<0.001
Egg-to-adult viability	1	0.046	0.830
	SD		
Assay vial ID	0.172		
Replicate ID	0.044		

rate, or the rate of senescence of the population) (Finch, 1990). Such estimates are known to be highly sensitive to sample sizes (Pletcher, 1999). Given that the maternal age at oviposition generally had little effect on longevity or survival patterns in the other analyses (see Results), we chose to use this factor as a natural level of biological structure in which to assign individuals to cohorts of larger membership. To clarify, for each treatment level and sex, focal flies were produced that had been reared from eggs that were laid by mothers whom had been exposed to the maternal sexual treatment for either five or 10 days. Thus, for each combination of treatment and sex, focal flies produced by 5-day-old mothers were assigned to one cohort and those produced by 10-day-old mothers to a second cohort. The membership of each cohort shared environmental interdependencies, having been produced by mothers of the same age, and assayed at the same time. Thus, having collapsed the vials of each treatment by sex combination into these two cohorts, the mean number of flies per cohort was 176 ± 19.4. We note that the rate-of-senescence analyses based on cohort sizes represented at the level of assay vials (22.73 ± 0.57 flies per vial) nonetheless provided qualitatively identical results.

We fitted general linear models (GLMs) to the rate-of-senescence means, in R 2.14.2 (R Development Core Team, 2012), in which the maternal sexual treatment, offspring sex and their interactions were modelled as fixed factors. A negative association typically exists between the rate of senescence and frailty (Hughes, 1995), and this was the case in our data set. Hence, rate of senescence was modelled with frailty (*ln* transformed) as a covariate in the subsequent analyses. We included the number of deaths per cohort as a weight statement in the models.

In all of the above models, we omitted nonsignificant interactions (alpha criterion of 0.05) from the model one at a time, starting with second-order, then first-order interactions, then main effects. None of the interactions, in any model, were significant, and thus, here we report only the test statistics associated with the main effects. *F* tests were used to assess statistical significance for the GLMs of the Gompertz data, and log-likelihood ratio tests for the multilevel models of longevity and Cox models of survival. Tukey's tests were implemented using the *multcomp* package in R (Hothorn *et al.*, 2008).

Data are available for public access, at DataDryad.org (doi:10.5061/dryad.j3823).

Results

The sexual history experienced by mothers prior to and during the production of the eggs that yielded the assayed offspring subsequently affected patterns of survival and ageing in their offspring. In particular, mothers that mated once only in their lifetime, during a brief exposure to males, produced offspring that tended to live longer (Table 1a, $P = 0.068$, Fig. 1a), that aged slower (Table 1b, $P = 0.032$, Fig. 1b) and that exhibited lower risks of mortality at any given time point (Table 1c, $P = 0.014$, Fig. 1c), than mothers exposed to regimes conducive to incessant male coercion for matings.

Tukey–Kramer tests revealed that the sole significant contrast in each analysis was between offspring of females exposed to the *baseline* level of sexual activity and those exposed to *precopulatory only* male sexual activity (rate of senescence: $P = 0.027$, survival: $P < 0.01$, whereas the P value associated for this contrast in the longevity analysis was $P = 0.057$). Although females exposed to male *pre- plus post-copulatory* activity produced offspring exhibiting longevities, survival and rates of senescence that were intermediate between the *baseline* and *precopulatory only* treatment levels (Fig. 1a–c), these differences were not significant.

Comparison of the risk ratios generated by the proportional hazards analysis revealed that offspring produced by mothers exposed to *precopulatory only* sexual activity experienced a 27% increase in the immediate risk of death at any given time point, although offspring whose mothers experienced both *pre- plus post-copulatory* sexual activity exhibited a 15% increased risk of mortality, over offspring of *baseline* mothers (Fig. 1c).

We also found strong sexual dimorphism for longevity (LS means, males = 43.65 ± 0.60, females = 51.80 ± 0.47), the rate of senescence (LS means, males = 0.087 ± 0.002, females = 0.073 ± 0.002) and the risk of mortality (Table 1). We found no effect of maternal age, which encompasses the length of time mothers were exposed to the treatment, on longevity or survival in the offspring (Table 1).

Finally, we examined the effect of the maternal sexual treatment on the associated egg-to-adult viability of the juvenile vials, in which the focal offspring were reared. Egg-to-adult viability in these vials was high across all the maternal sexual treatment levels ($F_{2,47} = 2.43$, $P = 0.10$, *baseline*: 90.67% ± 1.89, *precopulatory only*: 93.8% ± 0.99, *pre- and post-copulatory*: 94.50% ± 0.85: Tukey's HSD tests indicate no significant differences between levels).

Discussion

Here, we show the effects of the maternal sexual history on offspring survival and components of ageing in *D. melanogaster*. We contend that this result could provide new insights in the ongoing debate amongst evolutionary ecologists into whether indirect benefits (in terms of increased offspring fitness) associated with heightened levels of sexual activity can compensate for the direct costs experienced by females. Although previous studies have identified positive trans-generational

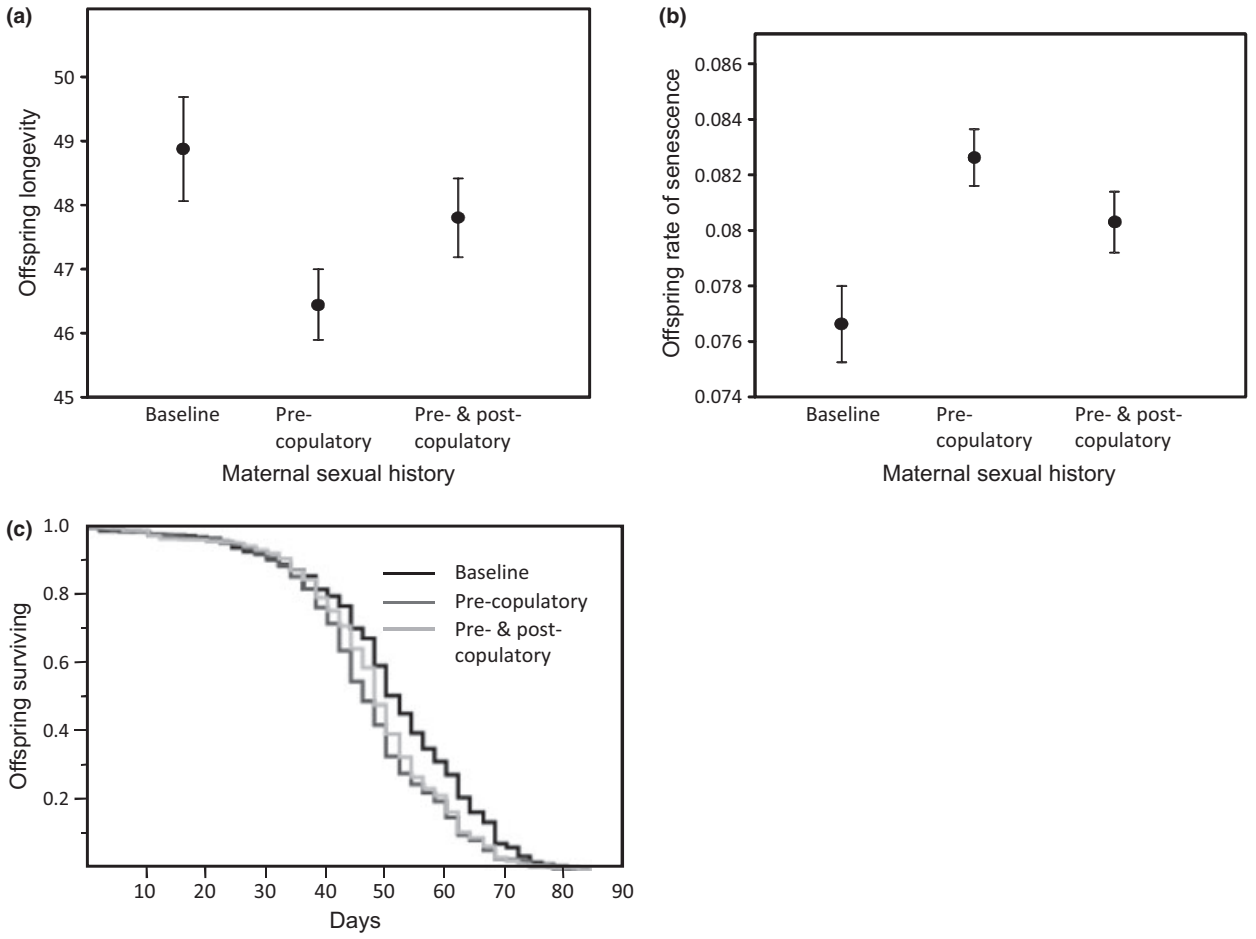

Fig. 1 (a) LS means of longevity (±1SE) in days, (b) LS means of rate-of-senescence and (c) survival curves for offspring produced by mothers that experienced differing sexual histories. (a) Longevities are LS means calculated from the level of the assay vial (n = 93). (b) Rates of senescence are LS means calculated from the large cohorts (membership determined by maternal age) assembled for the ageing analysis, as described in the Methods (n = 12). (c) Survival curves of each level of the treatment are indicated by lines of differing shades.

effects on components of offspring reproductive fitness, attributable to increases in maternal sexual interaction (Konior *et al.*, 2001; Head *et al.*, 2005; Fisher *et al.*, 2006; García-González & Simmons, 2007; Rundle *et al.*, 2007; Priest *et al.*, 2008a,b; Taylor *et al.*, 2008; Garcia-Gonzalez & Simmons, 2010; Firman & Simmons, 2012), or little or no such effects (Arnqvist & Kirkpatrick, 2005; Orteiza *et al.*, 2005; Stewart *et al.*, 2005, 2008; Maklakov & Arnqvist, 2009), little attention has focused on the possibility of effects to manifest as costs in the offspring generation (Brommer *et al.*, 2011; Gasparini *et al.*, 2012). Here, we have experimentally uncovered negative trans-generational effects on a set of core life-history traits (survival, ageing, longevity) that has been rarely studied in a trans-generational context and generally only in the framework of effects of maternal age on offspring (Lansing, 1947; Priest *et al.*, 2002; Fox *et al.*, 2003; Bouwhuis *et al.*, 2010). The effects we report could plausibly represent a hitherto hidden trans-generational cost (or indirect cost) of multiple mating, affecting a fundamental component of life history – mortality (Stearns, 1989). Thus, in brief, our results indicate that the previously identified direct survival costs to females, upon increased exposure to sexual interactions (Chapman *et al.*, 1995; Crudgington & Siva-Jothy, 2000), can be reinforced by survival costs in the following generation. This implies that a mother's sexual history can leave its footprint even on the latter life stages of her offspring.

An important question raised by our results is whether the negative trans-generational effects that we have identified on offspring survival and ageing parameters indeed reflect a trans-generational cost of heightened maternal sexual activity, and if so whether these costs are sizeable enough to warrant consideration in future appraisals of the economics of multiple

mating in females. Comparison of the hazard ratios for the significant comparisons suggests a large increase in the chance of mortality at any given time for offspring produced by *precopulatory only* mothers relative to the baseline and around a nine per cent decrease in longevity. Indeed, it is possible that the maternal sexual treatment induced a life-history trade-off in the offspring, resulting in increased early-life reproductive fitness at the expense of survival (Stearns, 1989), although we cannot determine whether the trade-off would be balanced in favour of *net* trans-generational benefits or costs, as we did not measure the reproductive success of the offspring in this study. The existence of early-life trans-generational fitness benefits in *D. melanogaster* have, however, been shown to be context dependent (Long *et al.*, 2010; Brommer *et al.*, 2011) and range from negligible (Orteiza *et al.*, 2005; Stewart *et al.*, 2008) to large (Priest *et al.*, 2008a). However, whether or not the trans-generational effects identified here actually represent *net* fitness costs of sexual interactions seems tangential to the issue of whether these effects should be termed trans-generational costs or not. If we take an evolutionary economic approach to the issue, then clearly there will be both estimable *benefits* and *costs* that result when females increase their exposure to sexual activity (i.e. under polyandry and increased sexual selection). These benefits and costs can manifest immediately on the subjects of the sexual interactions (hence be direct), or on the offspring generation (hence be trans-generational). Based on our results, we contend that it will be important for each of these four fitness components to be measured in future studies that seek to determine the net fitness consequences (hence adaptive value) of multiple mating by females. Historically, however, researchers have primarily focused on the issue of balancing the direct costs against indirect benefits (Head *et al.*, 2005; Stewart *et al.*, 2008; Garcia-Gonzalez & Simmons, 2010; Slatyer *et al.*, 2012). We address this issue in further length within the Data S1 file associated with this article.

Our data show that offspring produced by mothers exposed to increased male precopulatory sexual activity exhibited lower survival and faster ageing than offspring produced by mothers exposed to a baseline level of sexual interaction (one initial mating). Contrasts involving enhanced post-copulatory activity levels were not significant. Notably, this result indicates that female exposure to ongoing male precopulatory behaviours is, by itself, sufficient to drive trans-generational effects on components of offspring ageing and survival. Such precopulatory effects could arise from the complex male courtship ritual, which includes male orientation and courtship song, male foreleg tapping on females, incessant pursuit and circling of mating-resistant females, female genital licking and attempted mounting (Greenspan & Ferveur, 2000).

There was a subtle signal for offspring produced by mothers experiencing both pre- plus post-copulatory sexual interaction to exhibit intermediate ageing parameters to those of the other two treatment levels (although – as we have stated above – these contrasts were not statistically significant, and the existence of these effects therefore cannot be verified, and if present must be small). Nonetheless, the direction of the pattern is intriguing, given that it has previously been shown that the accessory cell proteins (*acps*), within the seminal fluid in *D. melanogaster*, exert negative effects on female survival (Chapman *et al.*, 1995) and are thus essentially toxic to female physiology (Wolfner, 2002; Wigby & Chapman, 2005). In the light of toxic effects of acps, it might have been reasonably expected that offspring produced by mothers experiencing both pre- plus post-copulatory male harassment would have exhibited lower survival and faster ageing than offspring produced by mothers experiencing only precopulatory male sexual activity. What might then explain this seemingly counter-intuitive result?

Although the receipt of multiple doses of seminal fluid might exert a direct negative effect on the survival of mothers (Chapman *et al.*, 1995), we suggest that the seminal fluid could possibly have an indirect nourishing, or hydrating effect, that positively augments survivorship in the offspring generation. Females are known to mate to hydrate in the seed beetle, *C. maculatus* (Edvardsson, 2007), but how plausible is it that the ejaculate contents could confer antagonistic effects across generations, harming mothers while benefitting offspring? Such antagonistic effects were recently documented by Brommer *et al.* (2011), who observed that heightened exposure to male sexual activity resulted in females producing sons of high fitness, but grandsons of low fitness (Brommer *et al.*, 2011). Our suggestion that the ejaculate can induce cross-generational opposing fitness effects is also consistent with the observation that multiple mating in *D. melanogaster* can result in the offspring exhibiting high early-life fitness in the absence of sexually selected 'good genes' processes (García-González & Simmons, 2007; Priest *et al.*, 2008b).

We also acknowledge the possibility of sexual selection to have influenced the *pre- plus post-copulatory* treatment level and contributed a compensatory 'rescue' effect amongst the offspring. This treatment level was the only level in which females received multiple ejaculates, thus enabling multiple paternities of each female's ova and invoking strong post-copulatory sexual selection on males. This could have resulted in selection for offspring genotypes conferring higher fitness and associated higher survival and slower ageing. If so, offspring of this treatment level would suffer the indirect costs associated with ongoing male precopulatory exposure by their mothers, but this effect could be moderated by the receipt of genetic benefits in the

offspring. However, we believe that there is little scope for this possibility, given that there was no differential mortality of focal juvenile flies across the treatment, and indeed, egg-to-adult viability of the focal juvenile flies (i.e. prior to their eclosion) was unanimously very high (>90%). Finally, we acknowledge that other less biologically interesting processes could result in a similar pattern in offspring survival and ageing, across the treatment. For instance, it is possible that females assigned to the *pre- plus post-copulatory* treatment level might well have experienced an overall lower level of male harassment than females assigned to the *precopulatory only* treatment level. This is because females of the former level would have routinely spent periods of time in a nonreceptive refractory period, following mating, whereas females of the *precopulatory only* level would have been receptive throughout the duration of the treatment. It has previously been observed that male courtship intensity towards nonreceptive females is lower than towards virgin females (McRobert *et al.*, 1997). However, it is probable that males in the *precopulatory only* level would have falsely identified the females as being 'multiply mated', as the cuticular hydrocarbon profiles of these females will have been influenced by repeated male contact and the transfer of male-specific pheromones to the females (Ejima *et al.*, 2007; Everaerts *et al.*, 2010). Finally, we note the possibility that males of the *precopulatory only* level may have generally increased their rate of post-copulatory courtship over the duration of the maternal sexual treatment, as a response to their sustained lack of copulatory success.

Regardless of the underlying finer mechanisms involved in driving the patterns across the maternal sexual treatment, clearly the salient conclusion to arise from our results is that male precopulatory activity alone was sufficient to drive the trans-generational effects on offspring life-history expression. Our experimental design enabled us to carefully control for all known potential confounding sources of environmental variance, such as egg-age effects, larval densities, variance in egg-to-adult viability, levels of sexual interaction in the offspring and variance in age in the focal offspring, parental and grandparental generations. Consequently, the trans-generational effects identified here can be traced directly to the sexual environments of the mothers. Thus, the sexual history of mothers can have effects on offspring survival and components of ageing. We contend that these effects represent putative costs that were hitherto unrealized. This suggests that the focus of the ongoing debate in sexual selection research, which is currently largely fixed on comparing direct costs to indirect benefits, should be broadened to include assessment of cryptic trans-generational costs. Doing so would require a shift in focus away from current practice of measuring only the early-life life-history components in the offspring.

Acknowledgments

The study was funded by a University of Western Australia Research Collaboration Award to FGG and DKD, a Monash University Research Fellowship to DKD and Discovery projects from the Australian Research Council to FGG and DKD. DKD and FGG were funded by Australian Research Council fellowships while writing the manuscript. FGG was also supported by the Spanish Ministry of Economy through the Ramon y Cajal program and grant (co-funded by the European Regional Development Fund CGL2012-34685). We thank Ian Stewart for designing the cautery used in the experiments.

References

Arnqvist, G. & Kirkpatrick, M. 2005. The evolution of infidelity in socially monogamous passerines: the strength of direct and indirect selection on extrapair copulation behavior in females. *Am. Nat.* **165**: S26–S37.

Arnqvist, G. & Kirkpatrick, M. 2007. The evolution of infidelity in socially monogamous passerines revisited: a reply to Griffith. *Am. Nat.* **169**: 282–283.

Arnqvist, G. & Nilsson, T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* **60**: 145–164.

Arnqvist, G. & Rowe, L. 2005. *Sexual Conflict*. Princeton University Press, Princeton.

Bates, D., Maechler, M. & Bolker, B. (2012) lme4: Linear mixed-effects models using S4 classes. R package version 0.999999-2. http://cran.r-project.org/web/packages/lme4/index.

Bonduriansky, R., Maklakov, A., Zajitschek, F. & Brooks, R. 2008. Sexual selection, sexual conflict and the evolution of ageing and life span. *Funct. Ecol.* **22**: 443–453.

Bouwhuis, S., Charmantier, A., Verhulst, S. & Sheldon, B.C. 2010. Trans-generational effects on ageing in a wild bird population. *J. Evol. Biol.* **23**: 636–642.

Brommer, J.E., Fricke, C., Edward, D.A. & Chapman, T. 2011. Interactions between genotype and sexual conflict environment influence transgenerational fitness in *Drosophila melanogaster*. *Evolution* **66**: 517–531.

Cameron, E., Day, T. & Rowe, L. 2003. Sexual conflict and indirect benefits. *J. Evol. Biol.* **16**: 1055–1060.

Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F. & Partridge, L. 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male Accessory gland products. *Nature* **373**: 241–244.

Chapman, T., Arnqvist, G., Bangham, J. & Rowe, L. 2003. Sexual conflict. *Trends Ecol. Evol.* **18**: 41–47.

Cordoba-Aguilar, A. & Contreras-Garduno, J. 2003. Sexual conflict. *Trends Ecol. Evol.* **18**: 439–440.

Crudgington, H.S. & Siva-Jothy, M.T. 2000. Genital damage, kicking and early death – the battle of the sexes takes a sinister turn in the bean weevil. *Nature* **407**: 855–856.

Eberhard, W.G. & Cordero, C. 2003. Sexual conflict and female choice. *Trends Ecol. Evol.* **18**: 438–439.

Edvardsson, M. 2007. Female *Callosobruchus maculatus* mate when they are thirsty: resource-rich ejaculates as mating effort in a beetle. *Anim. Behav.* **74**: 183–188.

Ejima, A., Smith, B.P.C., Lucas, C., Van Naters, W.V., Miller, C.J., Carlson, J.R. *et al.* 2007. Generalization of courtship learning in *Drosophila* is mediated by cis-vaccenyl acetate. *Curr. Biol.* **17**: 599–605.

Everaerts, C., Farine, J.P., Cobb, M. & Ferveur, J.F. 2010. *Drosophila* Cuticular Hydrocarbons Revisited: mating Status Alters Cuticular Profiles. *PLoS ONE* **5**: e9607.

Finch, C.E. 1990. *Longevity, Senescence, and The Genome*. University of Chicago Press, Chicago.

Firman, R.C. & Simmons, L.W. 2012. Male house mice evolving with post-copulatory sexual selection sire embryos with increased viability. *Ecol. Lett.* **15**: 42–46.

Fisher, D.O., Double, M.C., Blomberg, S.P., Jennions, M.D. & Cockburn, A. 2006. Post-mating sexual selection increases lifetime fitness of polyandrous females in the wild. *Nature* **444**: 89–92.

Fox, C.W., Bush, M.L. & Wallin, W.G. 2003. Maternal age affects offspring lifespan of the seed beetle, *Callosobruchus maculatus*. *Funct. Ecol.* **17**: 811–820.

Garcia-Gonzalez, F. & Simmons, L.W. 2010. Male-induced costs of mating for females compensated by offspring viability benefits in an insect. *J. Evol. Biol.* **23**: 2066–2075.

García-González, F. & Simmons, L.W. 2007. Paternal indirect genetic effects on offspring viability and the benefits of polyandry. *Curr. Biol.* **17**: 32–36.

Gasparini, C., Devigili, A. & Pilastro, A. 2012. Cross-generational effects of sexual harassment on female fitness in the guppy. *Evolution* **66**: 532–543.

Gavrilets, S., Arnqvist, G. & Friberg, U. 2001. The evolution of female mate choice by sexual conflict. *Proc. Biol. Sci.* **268**: 531–539.

Gay, L., Hosken, D.J., Eady, P., Vasudev, R. & Tregenza, T. 2010. The evolution of harm-effect of sexual conflicts and population size. *Evolution* **65**: 725–737.

Greenspan, R.J. & Ferveur, J.-F. 2000. Courtship in Drosophila. *Ann. Rev. Genet.* **34**: 205–232.

Griffith, S.C. 2007. The evolution of infidelity in socially monogamous passerines: neglected components of direct and indirect selection. *Am. Nat.* **169**: 274–281.

Head, M.L., Hunt, J., Jennions, M.D. & Brooks, R. 2005. The Indirect Benefits of Mating with Attractive Males Outweigh the Direct Costs. *PLoS Biol.* **3**: e33.

Holland, B. & Rice, W.R. 1998. Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution* **52**: 1–7.

Holland, B. & Rice, W.R. 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proc. Natl. Acad. Sci. USA* **96**: 5083–5088.

Hothorn, T., Bretz, F. & Westfall, P. 2008. Simultaneous inference in general parametric models. *Biometrical J.* **50**: 346–363.

Hughes, K.A. 1995. The Evolutionary Genetics of Male Life-History Characters in *Drosophila melanogaster*. *Evolution* **49**: 521–537.

Innocenti, P. & Morrow, E.H. 2009. Immunogenic males: a genome-wide analysis of reproduction and the cost of mating in *Drosophila melanogaster* females. *J. Evol. Biol.* **22**: 964–973.

Jennions, M.D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biol. Rev.* **75**: 21–64.

Jiang, P.P., Bedhomme, S., Prasad, N.G. & Chippindale, A. 2011. Sperm competition and mate harm unresponsive to male-limited selection in *Drosophila*: an evolving genetic architecture under domestication. *Evolution* **65**: 2448–2460.

Kirkpatrick, M. & Barton, N.H. 1997. The strength of indirect selection on female mating preferences. *Proc. Natl. Acad. Sci. USA* **94**: 1282–1286.

Kirkwood, T.B. & Rose, M.R. 1991. Evolution of senescence: late survival sacrificed for reproduction. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **332**: 15–24.

Kokko, H., Brooks, R., Jennions, M.D. & Morley, J. 2003. The evolution of mate choice and mating biases. *Proc. Biol. Sci.* **270**: 653–664.

Konior, M., Radwan, J. & Kolodziejczyk, M. 2001. Polyandry increases offspring fecundity in the bulb mite. *Evolution* **55**: 1893–1896.

Lansing, A.I. 1947. A transmissible, cumulative, and reversible factor in aging. *J. Gerontol.* **2**: 228–239.

Lew, T.A., Morrow, E.H. & Rice, W.R. 2006. Standing genetic variance for female resistance to harm from males and its relationship to intralocus sexual conflict. *Evolution* **60**: 97–105.

Linder, J.E. & Rice, W.R. 2005. Natural selection and genetic variation for female resistance to harm from males. *J. Evol. Biol.* **18**: 568–575.

Long, T.A.F., Pischedda, A. & Rice, W.R. 2010. Remating in *Drosophila melanogaster*: are indirect benefits condition dependent? *Evolution* **64**: 2767–2774.

Maklakov, A.A. & Arnqvist, G. 2009. Testing for Direct and Indirect Effects of Mate Choice by Manipulating Female Choosiness. *Curr. Biol.* **19**: 1903–1906.

McRobert, S.P., Adams, C.R., Wuttke, M., Frank, J. & Jackson, L.L. 1997. A comparison of female postcopulatory behavior in *Drosophila melanogaster* and *Drosophila biarmipes*. *J. Insect Behav.* **10**: 761–770.

Moller, A.P. & Alatalo, R.V. 1999. Good-genes effects in sexual selection. *Proc. Biol. Sci.* **266**: 85–91.

Opit, G.P. & Throne, J.E. 2007. Influence of maternal age on the fitness of progeny in the rice weevil, Sitophilus oryzae (Coleoptera: Curculionidae). *Environ. Entomol.* **36**: 83–89.

Orteiza, N., Linder, J.E. & Rice, W.R. 2005. Sexy sons from re-mating do not recoup the direct costs of harmful male interactions in the *Drosophila melanogaster* laboratory model system. *J. Evol. Biol.* **18**: 1315–1323.

Partridge, L. & Fowler, K. 1990. Non-mating costs of exposure to males in female *Drosophila melanogaster*. *J. Insect Physiol.* **36**: 419–425.

Pletcher, S.D. 1999. Model fitting and hypothesis testing for age-specific mortality data. *J. Evol. Biol.* **12**: 430–439.

Priest, N.K., Mackowiak, B. & Promislow, D.E.L. 2002. The role of parental age effects on the evolution of aging. *Evolution* **56**: 927–935.

Priest, N.K., Galloway, L.F. & Roach, D.A. 2008a. Mating frequency and inclusive fitness in *Drosophila melanogaster*. *Am. Nat.* **171**: 10–21.

Priest, N.K., Roach, D.A. & Galloway, L.F. 2008b. Cross-generational fitness benefits of mating and male seminal fluid. *Biol. Letters* **4**: 6–8.

Promislow, D. 2003. Mate choice, sexual conflict, and evolution of senescence. *Behav. Genet.* **33**: 191–201.

R Development Core Team 2012. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Reznick, D. 1985. Costs of reproduction – an evaluation of the empirical evidence. *Oikos* **44**: 257–267.

Rice, W.R. 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* **381**: 232–234.

Rönn, J., Katvala, M. & Arnqvist, G. 2007. Coevolution between harmful male genitalia and female resistance in seed beetles. *Proc. Natl. Acad. Sci. USA* **104**: 10921–10925.

Rundle, H., Odeen, A. & Mooers, A. 2007. An experimental test for indirect benefits in *Drosophila melanogaster*. *BMC Evol. Biol.* **7**: 36.

Slatyer, R.A., Mautz, B.S., Backwell, P.R.Y. & Jennions, M.D. 2012. Estimating genetic benefits of polyandry from experimental studies: a meta-analysis. *Biol. Rev.* **87**: 1–33.

Stearns, S.C. 1989. Trade-offs in life-history evolution. *Funct. Ecol.* **3**: 259–268.

Stewart, A.D., Morrow, E.H. & Rice, W.R. 2005. Assessing putative interlocus sexual conflict in *Drosophila melanogaster* using experimental evolution. *Proc. Biol. Sci.* **272**: 2029–2035.

Stewart, A.D., Hannes, A.M., Mirzatuny, A. & Rice, W.R. 2008. Sexual conflict is not counterbalanced by good genes in the laboratory *Drosophila melanogaster* model system. *J. Evol. Biol.* **21**: 1808–1813.

Taylor, M.L., Wigmore, C., Hodgson, D.J., Wedell, N. & Hosken, D.J. 2008. Multiple mating increases female fitness in *Drosophila simulans*. *Anim. Behav.* **76**: 963–970.

Therneau, T. 2012. Coxme: mixed effects Cox models. R package version 2.2–3. http://CRAN.R-project.org/package= coxme.

Wigby, S. & Chapman, T. 2005. Sex peptide causes mating costs in female Drosophila melanogaster. *Curr. Biol.* **15**: 316–321.

Williams, G.C. 1957. Pleiotropy, natural selection, and the evolution of senescence. *Evolution* **11**: 398–411.

Williams, G.C. 1966. Natural Selection, the Costs of Reproduction, and a Refinement of Lack's Principle. *Am. Nat.* **100**: 687–690.

Williams, B.R., Van Heerwaarden, B., Dowling, D.K. & Sgro, C.M. 2012. A multivariate test of evolutionary constraints for thermal tolerance in *Drosophila melanogaster*. *J. Evol. Biol.* **25**: 1415–1426.

Wolfner, M.F. 2002. The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. *Heredity* **88**: 85–93.

Yilmaz, M., Ozsoy, E.D. & Bozcuk, A.N. 2008. Maternal age effects on longevity in *Drosophila melanogaster* populations of different origin. *Biogerontology* **9**: 163–168.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1 Supporting methods, discussion and references.

Data deposited at Dryad: doi:10.5061/dryad.j3823

Received 21 June 2013; revised 3 September 2013; accepted 5 October 2013