Article:
Bretman, A orcid.org/0000-0002-4421-3337 and Fricke, C (2019) Exposure to males, but not receipt of sex peptide, accelerates functional aging in female fruit flies. Functional Ecology, 33 (8). pp. 1459-1468. ISSN 0269-8463

https://doi.org/10.1111/1365-2435.13339

Revised manuscript April 2019

Exposure to males, but not receipt of sex peptide, accelerates functional aging in female fruit flies

Amanda Bretman[1] and Claudia Fricke[2]*

1. School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK

2. Institute for Evolution and Biodiversity, University of Muenster, Huefferstrasse 1, 48149 Muenster, Germany

*Corresponding author: Claudia Fricke Claudia.Fricke@uni-muenster.de

Phone: +49 (0)251 – 83 21042

Fax: +49(0)251 – 83 24668

ORCID Amanda Bretman 0000-0002-4421-3337

ORCID Claudia Fricke 0000-0002-0691-6779

Short title: Males affect female functional senescence

Key words: Senescence, sexual conflict, stress responses, mating costs

Abstract

1. Increased exposure to males can affect females negatively, reducing female lifespan and fitness. These costs could derive from increased mating rate and also harassment by males. Additionally, early investment in reproduction can increase the onset or rate of senescence in reproductive traits. Hence, there is a tight link between reproduction and aging.
2. Here, we assess how mating and encounter rate with males impacts declines in female functional traits that are not directly involved in reproduction. In *Drosophila melanogaster* fruit flies, exposure to males and mating reduces female lifespan through harassment and receipt of seminal proteins, including sex peptide. We manipulated the intensity of female exposure to males and regularly assessed female stress responses and recorded physiological traits over her lifetime.
3. Both mating itself and increased exposure to males accelerates declines in female climbing ability and starvation resistance. However, this is not related to changes in female body mass or fat storage. Moreover, these declines are not driven by the receipt of sex peptide.
4. Our results suggest some synchrony in senescence across traits in response to female exposure to males, however this is not universal, as we did not find this for physiological traits. Synchrony in senescence has been theorised but little supported in the literature. It is clear that aging is a multifaceted trait; to understand environmental impacts on aging rates we must measure more than lifespan, and indeed measure senescence in multiple traits. Specifically, our work shows that we must identify which female traits are sensitive to elevated mating activity to understand the impact of antagonistic interactions between the sexes on female aging patterns.

1 Introduction

Under the evolutionary theories of aging, a weakening of natural selection forces later in life results in the less effective purging of late-acting mutations. Here genes either accumulate mutations with age or these genes have pleiotropic effects, i.e. were selected for as they increase reproduction at younger ages even if they have deleterious effects at older ages (Williams 1957; Kirkwood & Rose 1991; Kirkwood 2005; Gaillard & Lemaître 2017). Apart from late acting genes with deleterious effects, classic theory suggests that aging results from a trade-off between resource allocation to reproduction rather than somatic maintenance. In this latter scenario, the resources invested into reproduction are not available for somatic maintenance (see Maklakov & Immler 2016). These early ideas have been refined and widely discussed. For example, it is predicted that there would be genetic correlations between early and late life fitness but these might not always be negative (e.g. Maklakov *et al.* 2015) and the unappreciated costs of germline maintenance challenge the fecundity-lifespan trade-off (Maklakov & Immler 2016). Elevated rates of reproduction have been shown to decrease lifespan in a range of species (Maynard Smith 1958; Partridge & Farquhar 1981; Tatar *et al.* 1993; Chapman *et al.* 1995; Helle & Lummaa 2013). Furthermore, the hypothesised trade-off between early and late life reproduction has been supported by various studies of wild vertebrates (Nussey *et al.* 2006; Reed *et al.* 2008; Bouwhuis *et al.* 2010), likewise the influence of early reproductive effort on late-life survival and late-life body condition (Beirne *et al.* 2015; Lemaître *et al.* 2015). Hence, elevated early reproductive effort not only shortens lifespan, but can also impact age-specific changes in reproductive traits (Nussey *et al.* 2006; Lemaitre & Gaillard 2017). In females this is variously measured through traits such as egg production, inter-birth interval and offspring weight and survival (Nussey *et al.* 2006; Reed *et al.* 2008; Hayward *et al.* 2013). However, what is rarely measured is the impact that mating has on senescence of functional traits not directly associated with reproduction.

Mating can affect female lifespan as part of the costs of mating inflicted by sexually antagonistic interactions (Chapman *et al.* 1995; Arnqvist & Nilsson 2000). In some species females are already affected after a single mating, as seen in the seed beetle *Acanthoscelides obtectus* (Maklakov *et al.* 2005) with males altering female aging rates to benefit their own genetic interests. In the fruit fly *Drosophila melanogaster*, a model in studies of aging, effects of mating on lifespan are well known, particularly in females (Flatt 2011). In *D. melanogaster* females, mating and increased egg production can increase susceptibility to oxidative

stress (Salmon *et al.* 2001; Rush *et al.* 2007), a much invoked driver of aging (Kregel & Zhang 2007). The sources of the effects of mating on female lifespan have been identified both as male harassment (Partridge & Fowler 1990) and the repeated receipt of seminal fluid transferred from males during mating (Fowler & Partridge 1989; Chapman *et al.* 1995), though even just the perception of male pheromones reduces female lifespan (Gendron *et al.* 2014). Constant exposure to males causes females to have a shorter total and reproductive lifespan (Edward *et al.* 2011) indicating that male exposure alters female life-history traits. Alterations of female physiology and behaviour are driven by components of the male seminal fluid. The best studied of these seminal proteins, sex peptide (SP), influences various female traits (Ram & Wolfner 2007). Some of these are directly related to reproductive effort, such as increasing egg production and reducing willingness to remate (Chapman *et al.* 2003), but SP also manipulates female nutritional decisions (Ribeiro & Dickson 2010), increases immune responses (Peng *et al.* 2005), increases activity and reduces sleep (Isaac *et al.* 2010). Female susceptibility to male-induced costs of mating is influenced by protein (likely crucial for egg production) available to them in their diet (Chapman & Partridge 1996; Fricke *et al.* 2010). Given this plethora of phenotypes, it is perhaps unsurprising that SP reduces female lifespan and so overall fitness, and is a much-cited example of sexual conflict (Wigby & Chapman 2005; Fricke *et al.* 2009; Smith *et al.* 2017).

Understanding functional senescence, the decline in physical functioning with age, can elucidate how different traits contribute to the gross aging phenotype and is obviously of great concern when assessing "health span". The move towards understanding health span requires knowledge of whether traits differ in the onset and rate of senescence and their responsiveness to factors that are known to alter senescence patterns (Promislow *et al.* 2006; Martin *et al.* 2007). Theory predicts that natural selection should act most strongly on those functions that impact the risk of death most strongly (reviewed by Gaillard & Lemaître 2017). Natural selection should then promote a stronger synchronicity in senescence patterns among traits (Williams 1957; Maynard Smith 1962). However, this is largely not borne out by empirical studies, which show asynchrony between traits in senescence in humans, laboratory and wild animals (Grotewiel *et al.* 2005; Walker & Herndon 2010; Nussey *et al.* 2013; Bansal *et al.* 2015; Hayward *et al.* 2015). There is therefore a need to uncover the environmental and genetic factors which contribute to this variation in senescence among traits (Nussey *et al.* 2013).

Functional senescence in *D. melanogaster* has been measured in a variety of traits including resistance to various stressors, climbing ability, immune and memory function, though the age of the onset of senescence in different traits ranges from ~10 to 100 days (reviewed by Grotewiel *et al.* 2005). Social environment can affect functional senescence as same-sex social contact versus isolation reduces the speed of decline in climbing ability in females (but not males) (Leech *et al.* 2017). Here we aimed to assess whether intensified mating interactions accelerate functional aging in females by measuring not just lifespan but also climbing ability and starvation resistance, plus potential underpinning physiological traits, i.e. fat content and body mass. If mating *per se* causes more rapid functional declines then we would expect virgins to decline more slowly, but females intermittently or constantly exposed to males to show similar patterns. However, if number of matings and/ or harassment by males plays a role in functional senescence, then females intermittently exposed to males will decline more slowly than those constantly held with males. If the receipt of SP is part of the underlying mechanism of reproduction-induced functional senescence, then females mated to males that do not produce SP should show slower declines than females receiving SP.

2 Material and Methods

2.1 Fly culturing

For these sets of assays Dahomey wild-type individuals were used. This strain was collected in the 1960s in Dahomey (now Benin) and has ever since been cultivated at 25°C and 60% RH on a 12:12 light: dark cycle in the laboratory in large, cage cultures in overlapping generations. All experiments were conducted under these standard conditions using vials containing 7 ml Sugar –Yeast (SY) medium (Bass *et al.* 2007)) with excess live yeast granules unless stated otherwise. We allowed the parental generation to lay eggs on agar-grape juice plates (50g agar, 600ml red grape juice, 42.5 ml Nipagin (10% w/v solution), 1.1 L water) supplemented with yeast paste. The following day first instar larvae were collected at 100 larvae per vial and ten days later virgin females and males were collected on ice. Adults were stored in same sex-groups of 20 per vial until used in the experiment when they were 4 days post-eclosion.

2.2 Sex-peptide knock out mutants

Mutant stocks were maintained as bottle cultures (70 ml of SY food in 1L bottles). We crossed virgin *Δ130*/*TM3,Sb,ry* females with SP^0/*TM3,Sb,ry* males. *Δ130*/SP^0 male offspring (SP^0) from this cross do not produce sex peptide (Liu & Kubli 2003). As a genotype matched control we crossed *Δ130*/*TM3,Sb,ry* females with SP^0,SP^+/*TM3,Sb,ry* males and the resulting *Δ130*/SP^0,SP^+ sons (SP^+) produce and transfer sex peptide at mating. The *Δ130*/*TM3,Sb,ry* stock was backcrossed for three generations into the Dahomey wild type genetic background and chromosomes 1, 2 and 4 of the other two stocks for four generations.

2.3 Experimental set-up

2.3.1 Male exposure treatment

Wild type females were assigned at random to one of three male exposure treatments. Females either encountered no males during their lifetime and remained virgin, were continuously held with males or experienced an intermittent exposure regime. In the intermittent exposure treatment females were held for three consecutive days with males and were held alone the remainder of the week. Once a week the batch of males used was discarded in both male exposure regimes and exchanged with a fresh batch of 4-5 day old males to account for age-related declines in male courtship and mating behaviour. All flies were moved to new vials with fresh media twice a week.

Against the backdrop of these three male exposure treatments we then performed three independent assays to test different functional aspects throughout female lifespan to measure a female's ability to maintain functional integrity while paying the cost of mating.

2.3.2 Negative geotaxis assay

Negative geotaxis or startle-induced climbing is a standard assay of locomotor senescence in flies (Jones & Grotewiel 2011). When tapped to the bottom of a cylinder, flies "escape" by climbing upwards, a response which becomes progressively slower with age (Arking & Wells 1990). In this assay, females were tested for

their ability to climb up 8 cm in an empty vial. Once a week females from the three exposure treatments were transferred individually into empty vials, allowed to adjust for 5 min and then the females were banged down to the bottom of the vial. Immediately afterwards females were observed and we recorded whether they a) tried to climb in the first place and b) the time it took them to cross the 8 cm line, up to a maximum of 180 seconds.

We started with 180 females and first tested them at 4 days post-eclosion and afterwards assigned them randomly to a male exposure treatment (n = 60 per treatment). Thus for the first measurement all females were virgins. For this assay females were either held in pairs with one male (continuous treatment and intermittent treatment during the male exposure time) or individually (virgin treatment and intermittent females during the no male time). We then tested each female once a week for her ability to perform this negative geotaxis task. We daily checked for female survival and recorded the day of death. When fewer than 20 females within one of the male exposure treatments remained alive we stopped assaying the females of that particular treatment but continued with the others.

2.3.3 Starvation resistance assay

We started with a total of 300 females per male exposure treatment and held females either in groups of ten (virgin treatment and intermittent exposure during the no male time) or in groups of 5 females and 5 males (continuous exposure and intermittent exposure when with males) per vial. During this time females were maintained on standard SY medium with live yeast grains added *ad libitum*. Once a week when females were transferred to new vials we made sure to reshuffle females groups to avoid common vial effects. We did not record female survival in the male exposure treatments.

For the starvation assay once a week a subgroup of 30 random females from each of the three male exposure treatments were put on agar-only food without any yeast added. Females were kept individually in these vials. We performed daily survival checks at roughly 24 hour intervals and recorded how long females survived to assay their starvation resistance. This sequence was repeated weekly until fewer than 30 females remained per treatment to perform this assay. Again, we performed the assay for the first time when females were 4 days post-eclosion and before assigning them to a male exposure treatment.

175

176 2.3.4 Triglyceride assay

177 Starvation resistance is directly linked to lipid reserves (Lee & Jang 2014), and triacylglyceride is the major
178 energy storage molecule in the fat body (Arrese & Soulages 2010). Hence, we here directly measured
179 triacyglyceride (TAG) content in females. For this we repeated the design of the starvation resistance assay
180 with one exception; instead of weekly starving females we snap froze 30 females in liquid nitrogen and
181 subsequently estimated the amount of triglycerides stored. We followed the protocol for the coupled
182 colorimetric assay for triglycerides as outlined by Tennessen et al. (2014). With this assay we compared the
183 stored triglycerides across ages and treatments. The triglycerides are macromolecules bound to proteins and
184 together form lipoproteins. We always pooled five females per sample and measured their wet weight (on a
185 Satorius MC 410S model at a resolution of 0.1mg) for an estimate of body mass before they were treated
186 according to protocol. We followed the protocol with a few minor exceptions, e.g. after the homogenisation
187 step (Step 3 in protocol by Tennessen et al. 2014) samples were centrifuged at 13 000 rpm at 4°C for 5 min.
188 After preparing the samples and adding the glycerol standard and for half of them the triglyceride reagent
189 (Sigma: T2449) we incubated tubes for 45 mins at 37°C (step 7 in protocol by Tennessen et al. 2014) before
190 measuring the colorimetric intensity of the sample at 540 nm in a Tecan Reader. Tennessen recommends
191 normalisation to an internal parameter to accurately reflect TAG levels across different conditions (here age)
192 and we here normalise to body mass as this has been done before (e.g. Hildebrandt *et al.* 2011) and allows
193 for comparisons across studies.

194

195 2.3.5 Sex-peptide treatment

196 To test potential mechanistic underpinnings of female functional aging responses to male exposure we
197 performed a further set of experiments to specifically test whether receipt of SP mediates responses in
198 females experiencing high costs of mating. Thus, we repeated the two stress response assays (negative
199 geotaxis and starvation resistance) exactly as described above (2.3.2 and 2.3.3) in terms of sample sizes,
200 starting age and sampling points. The only change implemented was that females were continuously exposed

to either males lacking SP (SP^0 treatment) or sex peptide transferring control males (SP^+ treatment) instead of wild type males.

2.4 Data analysis

Data were analysed using R v 3.3.1. For the male exposure experiment, we had two hierarchical questions, firstly what was the overall effect of male exposure (including mating), and then for those females that mated, what was the effect of different amounts of exposure to males. As such our approach was to analyse all three treatments first, and where an effect of treatment was found to then analyse the data without the virgin treatment. Survival data from females used in the negative geotaxis-climbing assay was analysed using Kaplan Meier log rank tests. Functional senescence data were analysed using GLMMs or GLMs as appropriate, using the package lme4 (using maximum likelihood rather than REML). Terms were subtracted from the maximal model by Analysis of Deviance (AOD) and by assessing the change in AIC (see Tables S1-4 in Supporting Information). Senescence in climbing ability was analysed as time to reach 8cm, with those individuals that tried but failed in the time allowed given a value of 180seconds. On only four occasions across the two experiments did the fly not try to climb (each in different treatments), and of those that tried only ~ 6% failed to reach 8cm within the time limit (53/913 trials in the male exposure experiment, 21/284 trials in the SP experiment). Climbing time was used as the response variable in a GLMM with male exposure treatment as a factor, age and lifespan (to account for selective disappearance e.g. (Hayward *et al.* 2015)) as covariates, and the random effect of fly identity to account for repeated measures. Age was fitted both as linear and quadratic functions in the models. A difference in senescence rate between our treatments will be indicated by a significant age x treatment interaction term. Response variables that required flies to be sacrificed (starvation resistance, body mass and TAG per mg) did not yield repeated measures or lifespan data, hence we used GLMs with age as a covariate and male exposure treatment as fixed factors. We initially assessed whether a linear or quadratic effect of female age within treatments was most appropriate, and where this was the case for at least one treatment, used the quadratic term in the full model (see Tables S1 and S3). For parameter estimates from the best supported model see Tables S2 and S4.

3 Results

3.1 Effect of exposure to males on lifespan and climbing ability

The amount of exposure to males significantly affected female survival (Kaplan Meier log rank test χ^2_2 = 121.639, $P < 0.001$) with virgin females surviving the longest (Fig 1A). Comparing only females that mated showed that females intermittently exposed to males survived longer than those continuously exposed (χ^2_1 = 18.141, $P < 0.001$; Fig 1A). We assessed the effect on climbing ability using time to reach 8cm. The maximal model contained the interaction between male exposure treatment, female age and lifespan (with fly identity included as a random factor). Removal of the 3-way interaction compared to a model with all pair-wise interactions, increased the AIC (see Table S1), and an Analysis of Deviance showed that a model without the three way interaction was significantly worse (χ^2_1 = 7.304, P = 0.007). This significant interaction remained when comparing only females that mated (χ^2_1 = 8.490, P = 0.004). This suggests that the way climbing ability is affected by female age differs between treatments, with females in the constant male exposure treatment becoming worse at the climbing task more quickly with the exception of the final assay (Fig 1B). Furthermore, this interaction is affected by female lifespan. To illustrate this we plotted lifespan and change in climbing time (day 32 – day 4 assay), noting that there is a negative relationship only in the constantly exposed treatment (Fig S1). This could indicate selective disappearance within the constantly exposed treatment, as those females that lived longer showed less of a decline in climbing ability with age. In sum, both mating *per se* and the amount of exposure to males affects female lifespan and locomotor senescence.

3.2 Effect of exposure to males on senescence of starvation resistance, body mass and body fat

We found that male exposure significantly affected senescence in female starvation resistance, (female age^2 x male exposure treatment: χ^2_1 = 11.995, P = 0.002, Table S1), likely caused by the virgin treatment showing an initial increase in starvation resistance before declining (Fig 1C). When the virgin treatment was removed an interaction between the linear effect of age and exposure treatment remained (female age x male exposure treatment: χ^2_1 = 7.038, P = 0.008), with females held intermittently with males surviving longer under starvation until day 32. This suggests that mating *per se* and the amount of contact with males affected age-

related decreases in starvation resistance. In order to assess whether this was due to differences in fat reserves between treatments we assayed female body mass and triacylglyceride content (TAGs). Whilst for body mass there was a significant interaction between female age and male exposure treatment ($F_{1, 84}$ = 5.670, P = 0.019), this pattern does not mirror that of the starvation assay as females held constantly with males were initially slightly heavier (Fig 1D) yet had lower starvation resistance (Fig 1C). Furthermore, accounting for body mass, there was no effect of female age or male exposure treatment on amount of TAGs, either as an interaction ($F_{1, 84}$ = 0.405, P = 0.526) or as main effects (treatment: $F_{1, 85}$ = 0.419, P = 0.519; age: $F_{1, 85}$ = 0.824, P = 0.367; Fig 1E).

3.3 Effect of receipt of sex peptide on lifespan and functional senescence

We then tested whether the results we had observed could be attributed to the receipt of sex peptide (SP). Female exposure to males that did or did not transfer SP had no effect on lifespan (Kaplan Meier log rank test: χ^2_1 = 0.496, P = 0.481; Fig 2A). Likewise, we found no effect on senescence in climbing ability. A model using all data (with fails fixed to 180s, the maximum observation period) showed no significant interactions (AOD model comparisons all P> 0.05, see Table S3), and no main effect of treatment (χ^2_1 = 0.018, P = 0.894) but a decline with age (χ^2_1 = 72.976, P < 0.0001) (Fig 2B). For starvation resistance, the interaction between female age and SP treatment was non-significant (χ^2_1 = 2.944, P = 0.088), and when this term was removed there was a significant effect of age (comparing models with age to that with age plus the quadratic term of female age χ^2_1 = 16.951, P < 0.0001) but not of treatment (χ^2_1 = 2.40, P = 0.123; Fig 2C). These results suggest that the effects of mating and male exposure on female functional aging are not driven by the receipt of SP.

4 Discussion

Whilst previous studies have reported that exposure to males affects female survival and reproductive senescence, our main findings show that this effect also applies to functional senescence in climbing ability and starvation resistance. Our data suggest that female lifespan and rates of functional senescence are altered

in response to both mating *per se* and amount of contact with males. However, these effects did not appear to be driven by differences in body mass or stored fats. Likewise, the patterns in functional senescence were not attributed to the receipt of sex peptide, as this did not cause any difference in the decline seen in either climbing ability or starvation resistance, or indeed in survival.

Largely there is consensus in the phenomenon that mating reduces lifespan in female *D. melanogaster* (reviewed by Flatt 2011), and this is confirmed in our data. Exposure to males seems to have an additive effect, in that females continuously exposed to males were more severely affected than those intermittently exposed, as in previous work (Chapman & Partridge 1996; Edward *et al.* 2011). Edward et al. (2011) used a similar experimental set-up to us and measured female offspring production. Continuous exposure lead to reduced reproductive lifespan with a strong correlation with lifetime reproductive success and females having a high reproductive output early in life (Edward *et al.* 2011). What we now show is that this pattern is reiterated in the senescence of two of the non-reproductive traits we measured. Indeed, for both traits, the constantly exposed females start to show a more obvious decline at the second assay at 11 days post eclosion, suggesting that there is some synchrony in senescence in these traits in response to exposure to males. It has been suggested that lifespan and health span are mechanistically connected (Rhodenizer *et al.* 2008) because longer-lived flies tend to have better climbing ability across ages (e.g. Gargano *et al.* 2005), though this is not always the case (Cook-Wiens & Grotewiel 2002). In addition, we found no corresponding senescence in female body mass or body fat here, thus highlighting that not all aspects of female physiology were equally affected by male exposure. Whilst it has been predicted that senescence should be observed as generalized deterioration rather than failure of single systems (Williams 1957) this is largely not borne out by empirical work (recently reviewed by Gaillard & Lemaître 2017). In general these opposing examples are either studies of wild populations that are subjected to multiple environmental drivers of aging (Massot *et al.* 2011; Hayward *et al.* 2015; Kervinen *et al.* 2015) or laboratory studies that do not impose any particular pressures (Herndon *et al.* 2002). By applying a specific environmental driver of aging in a controlled manner this may allow us to dissect which traits are predominantly affected and assess whether asynchronicity is a general aspect or instead explained by different sensitivities to multiple environmental drivers. It would be fruitful to establish whether this is generally observed when other known determinants of aging are manipulated.

Our finding that mating/ contact with males reduced starvation resistance is at odds with previous work. Multiple studies have found that mating increases female food intake and the size of the midgut, resulting in greater lipid storage and increasing starvation resistance (Rush *et al.* 2007; Jang & Lee 2015; Reiff *et al.* 2015). Both our measures of functional senescence rely on energy reserves, but we found no evidence that lipid content was increased by multiple mating. However, these previous studies were not designed to assess senescence patterns, hence usually only measure resistance once, relatively early in life. Additionally, females were exposed to males in a very limited way, perhaps a single mating or interactions were allowed for just a few days. Here instead we took a long-term approach were females mated repeatedly and were subject to male harassment, indicating that mating activity in the long-term results in the opposite pattern. Whilst receipt of seminal proteins might induce higher feeding rates in young females (also seen in our constantly exposed females who were heavier in the beginning before declining, see Fig. 1D), this might be countered by the harassment of females by males, therefore reducing feeding time and in the long-term energy stores. Also prior to being individualised for the starvation assay, individuals were held in groups of ten until they were chosen at the appropriate test age, potentially leading to competition over food particularly as also larvae were present in the treatments with mated females. Combined this might limit female access to resources and explain the discrepancy between studies. Additionally, it may be that there is selective disappearance of the lighter females (Nussey *et al.* 2008), but we cannot test for this directly in these destructive sampling assays (e.g. by adding individual lifespan into the model).

While in general body condition indices are used as a proxy for lipid content in animals, whether the two are tightly correlated is debated and further, whether either of these two measures influences fitness positively is not always clear cut (Wilder *et al.* 2016). At least in *D. melanogaster* dietary composition has a strong impact on fat deposition and fecundity as well as lifespan (Skorupa *et al.* 2008; Lee & Jang 2014). TAG levels are strongly dependent on the amount of carbohydrates in the diet (Skorupa *et al.* 2008). On a balanced diet the cellular composition of the female fat body is stable with age (Johnson & Butterworth 1985) or can show a slight decrease in TAG levels (Skorupa *et al.* 2008). Here we directly measured TAG levels in females as overall higher lipid reserve confers higher starvation resistant (Lee and Jang 2014) and there is a strong link between lipid storage and egg-production, as oocytes contain large amounts of lipids and are provisioned from the fat body (Arrese & Soulages 2010). Curiously, we found females continuously

exposed to males were least starvation resistant even though they had similar levels of triacylglycerides (TAG). This suggests male exposure (including mating rate) might alter female fat-metabolism. Mating status in redback spiders (*Latrodectus hasselti*) altered responses to food shortage, with mated females lowering their resting energetic rate in response to nutrient shortage, preserving energy, while virgin females maintained higher rates and had shorter lifespans (Stoltz *et al.* 2010). If similar metabolic mechanisms are at work in *Drosophila*, then we would expect male-exposed females to be more starvation resistant, but we found the opposite. Under starvation conditions female *Drosophila* first use up the non-lipid fraction of their bodies before switching to lipids (Lee and Jang 2014), but if anything continuously exposed females were slightly heavier. As we quantified the total amount of triglycerides, this did not allow us to distinguish whether it was dedicated to use in the ovaries and hence potentially not available for maintenance under starvation. This basic trade-off, where females under high male exposure invest more resources into egg production, might explain why these females were less resistant to starvation despite similar lipid energy reserves and this would be in line with the disposable soma theory (e.g. see Lemaitre et al. 2015). The physiological dynamics that underpin life-history trade-offs deserve further scrutiny.

That we could not attribute any of the senescence effects to the receipt of SP is curious, given the multitude of effects on female phenotypes that have previously been found (Chapman *et al.* 2003; Peng *et al.* 2005; Ram & Wolfner 2007; Isaac *et al.* 2010; Ribeiro & Dickson 2010) and the importance of SP in inducing female costs of mating (Wigby and Chapman 2005). For example, receipt of SP increases female activity (Isaac *et al.* 2010), but we did not see evidence of this in our climbing assay at any time point. It is possible that we did not measure these at old enough ages, though these measurements were within the timeframe for females constantly exposed to males to show a difference to virgins. At least for the induction of egg-laying by SP, females were only receptive when very young and this rapidly declined with age (Fricke *et al.* 2013), hence SP-induced female post-mating responses are female age-dependent and tend to diminish with age. It seems therefore that factors other than SP are more important in determining female aging phenotypes, either the other Sfps or the direct harassment by males (Partridge & Fowler 1990). While SP is one component implicated in the costs of matings, other Sfps are toxic (Lung *et al.* 2002; Mueller *et al.* 2007) and it is the entirety of the Sfps in the ejaculate that mediates the negative effects of multiple mating (Chapman *et al.* 1995). Mating (Zhou *et al.* 2014) and receipt of SP (Gioti *et al.* 2012) dramatically alter

female gene expression, including those implicated in lifespan such as the TOR pathway (Gioti *et al.* 2012). Simply hearing courtship song alters female expression of *Turandot* genes, a family of stress-response genes (Immonen & Ritchie 2012). Furthermore, ecdysone receptor as well as genes involved in germline maintenance and gustation/ odorant reception are candidates for female responses to continuous male exposure (Gerrard *et al.* 2013). These candidates represent potential hormonal and metabolic pathways that might influence resource allocation to the germ line. It would be interesting to test whether these pathways alter allocation to the germ line versus somatic maintenance and could potentially explain the heterogeneity in physiological versus functional senescence found here. For a general pattern to emerge though, a wider array of functional traits, representing a broader set of biological functions should be screened. This approach can be extended to include males, to test for sex-differences in functional aging to further our understanding how investment in mating activities alters senescence patterns. This could also reveal whether, in addition to being implicated in interlocus sexual conflict over mating rate, these traits might be targets for intralocus sexual conflict over aging profiles (Archer *et al.* 2018). Hence, there remains much work to be done on the molecular mechanisms underpinning how this environmental variable (mating activity) can alter senescence in multiple traits and how the different non-reproductive traits are integrated to contribute to observed aging phenotypes.

Conclusions

Overall we here showed that female costs of mating due to intensified male exposure leads to accelerated functional aging in female motor ability and resistance to starvation stress. This decline though was not underpinned by a matching decline in relevant physiological traits. Hence, while we found some synchronicity in aging phenotypes in response to mating activity across traits this was not universal. Understanding which traits contribute to the observed mating costs, are particularly affected by high mating effort and display high rates of functional aging should give valuable insights into aging patterns and integration of different traits to the aging phenotype.

Acknowledgements

We thank Dolors Amoros Moya, Sonja Schindler and Kristina Wensing for help with the assays, and Xavier Harrison for help with statistical analysis. We thank the Editor, Associate Editor and two reviewers for their helpful comments. We have no conflict of interest to declare. CF was supported by the DFG Emmy Noether Program (FR 2973/1-1).

Author Contributions

AB and CF conceived the idea and designed the experiment, CF conducted the experiment, AB analysed the data, both authors wrote the manuscript.

Data accessibility: Data are freely available from Dryad under doi:10.5061/dryad.9qb0dt6.

Literature cited

Archer CR, Recker M, Duffy E, Hosken DJ (2018). Intralocus sexual conflict can resolve the male-female health-survival paradox. *Nat. Commun.* **9**, 5048.

Arking R, Wells RA (1990). Genetic alteration of normal aging processes is responsible for extended longevity in *Drosophila*. *Developmental Genetics*. **11**, 141-148.

Arnqvist G, Nilsson T (2000). The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behaviour*. **60**, 145-164.

Arrese EL, Soulages JL (2010). Insect fat body: energy, metabolism, and regulation. *Annual Review of Entomology*. **55**, 207-225.

Bansal A, Zhu LHJ, Yen K, Tissenbaum HA (2015). Uncoupling lifespan and healthspan in *Caenorhabditis elegans* longevity mutants. *Proceedings of the National Academy of Sciences of the United States of America*. **112**, E277-E286.

Bass TM, Grandison RC, Wong R, Martinez P, Partridge L, Piper MDW (2007). Optimization of dietary restriction protocols in *Drosophila*. *Journals of Gerontology Series A-Biological Sciences and Medical Sciences*. **62**, 1071-1081.

Beirne C, Delahay R, Young A (2015). Sex differences in senescence: the role of intra-sexual competition in early adulthood. *Proceedings of the Royal Society B: Biological Sciences*. **282**, 20151086.

Bouwhuis S, Charmantier A, Verhulst S, Sheldon BC (2010). Individual variation in rates of senescence: natal origin effects and disposable soma in a wild bird population. *Journal of Animal Ecology*. **79**, 1251-1261.

Bretman A, Fricke C (2019) Data from: Exposure to males, but not receipt of sex peptide, accelerates functional aging in female fruit flies. Dryad Digital Repository: doi:10.5061/dryad.9qb0dt6.

Chapman T, Bangham J, Vinti G, Seifried B, Lung O, Wolfner MF, Smith HK, Partridge L (2003). The sex peptide of *Drosophila melanogaster*: Female post-mating responses analyzed by using RNA interference. *Proceedings of the National Academy of Sciences of the United States of America*. **100**, 9923-9928.

Chapman T, Liddle LF, Kalb JM, Wolfner MF, Partridge L (1995). Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature*. **373**, 241-244.

Chapman T, Partridge L (1996). Female fitness in *Drosophila melanogaster*: an interaction between the effect of nutrition and of encounter rate with males. *Proceedings of the Royal Society of London, Series B*. **263**, 755-759.
Cook-Wiens E, Grotewiel MS (2002). Dissociation between functional senescence and oxidative stress resistance in *Drosophila*. *Exp. Gerontol.* **37**, 1347-1357.
Edward DA, Fricke C, Gerrard DT, Chapman T (2011). Quantifying the life-history response to increased male exposure in female *Drosophila melanogaster*. *Evolution*. **65**, 564-573.
Flatt T (2011). Survival costs of reproduction in *Drosophila*. *Exp. Gerontol.* **46**, 369-375.
Fowler K, Partridge L (1989). A cost of mating in female fruitflies. *Nature*. **338**, 760-761.
Fricke C, Bretman A, Chapman T (2010). Female nutritional status determines the magnitude and sign of responses to a male ejaculate signal in *Drosophila melanogaster*. *Journal Of Evolutionary Biology*. **23**, 157-165.
Fricke C, Green D, Mills WE, Chapman T (2013). Age-dependent female responses to a male ejaculate signal alter demographic opportunities for selection. *Proceedings Of The Royal Society B-Biological Sciences*. **280**, 20130428.
Fricke C, Wigby S, Hobbs R, Chapman T (2009). The benefits of male ejaculate sex peptide transfer in *Drosophila melanogaster*. *Journal Of Evolutionary Biology*. **22**, 275-286.
Gaillard J-M, Lemaître J-F (2017). The Williams' legacy: A critical reappraisal of his nine predictions about the evolution of senescence. *Evolution*. **71**, 2768-2785.
Gargano JW, Martin I, Bhandari P, Grotewiel M (2005). Rapid iterative negative geotaxis: a new method for assessing age-related locomotor decline in *Drosophila*. *Exp. Gerontol.* **40**, 386-395.
Gendron CM, Kuo T-H, Harvanek ZM, Chung BY, Yew JY, Dierick HA, Pletcher SD (2014). *Drosophila* life span and physiology are modulated by sexual perception and reward. *Science*. **343**, 544-548.
Gerrard DT, Fricke C, Edward DA, Edwards DR, Chapman T (2013). Genome-wide responses of female fruit flies subjected to divergent mating regimes. *PLoS One*. **8**, e68136.
Gioti A, Wigby S, Wertheim B, Schuster E, Martinez P, Pennington CJ, Partridge L, Chapman T (2012). Sex peptide of *Drosophila melanogaster* males is a global regulator of reproductive processes in females. *Proceedings of the Royal Society B-Biological Sciences*. **279**, 4423-4432.
Grotewiel MS, Martin I, Bhandari P, Cook-Wiens E (2005). Functional senescence in *Drosophila melanogaster*. *Ageing Research Reviews*. **4**, 372-397.
Hayward AD, Moorad J, Regan CE, Berenos C, Pilkington JG, Pemberton JM, Nussey DH (2015). Asynchrony of senescence among phenotypic traits in a wild mammal population. *Exp. Gerontol.* **71**, 56-68.
Hayward AD, Wilson AJ, Pilkington JG, Clutton-Brock TH, Pemberton JM, Kruuk LEB (2013). Reproductive senescence in female Soay sheep: variation across traits and contributions of individual ageing and selective disappearance. *Functional Ecology*. **27**, 184-195.
Helle S, Lummaa V (2013). A trade-off between having many sons and shorter maternal post-reproductive survival in pre-industrial Finland. *Biology Letters*. **9**.
Herndon LA, Schmeissner PJ, Dudaronek JM, Brown PA, Listner KM, Sakano Y, Paupard MC, Hall DH, Driscoll M (2002). Stochastic and genetic factors influence tissue-specific decline in ageing *C. elegans*. *Nature*. **419**, 808-814.
Hildebrandt A, Bickmeyer I, Kuehnlein RP (2011). Reliable *Drosophila* body fat quantification by coupled colorimetric assay. *PLoS One*. **6**, e23796.
Immonen E, Ritchie MG (2012). The genomic response to courtship song stimulation in female *Drosophila melanogaster*. *Proceedings of the Royal Society B*. **279**, 1359-1365.
Isaac RE, Li C, Leedale AE, Shirras AD (2010). *Drosophila* male sex peotide inhibits siesta sleep and promotes locomotor activity in the post-mated female. *Proceedings of the Royal Society B: Biological Sciences*. **277**, 65-70.
Jang T, Lee KP (2015). The genetic basis for mating-induced sex differences in starvation resistance in *Drosophila melanogaster*. *Journal of Insect Physiology*. **82**, 56-65.
Johnson MB, Butterworth FM (1985). Maturation and aging of adult fat body and oenocytes in *Drosophila* as revealed by light microscopic morphometry. *Journal of Morphology*. **184**, 51-59.
Jones MA, Grotewiel M (2011). *Drosophila* as a model for age-related impairment and other behaviours. *Exp. Gerontol.* **46**, 320-325.

Kervinen M, Lebigre C, Alatalo R, Siitari H, Soulsbury CD (2015). Life-history differences in age-dependent expressions of multiple ornaments and behaviors in a lekking bird. *American Naturalist*. **185**, 13-27.
Kirkwood TBL (2005). Understanding the odd science of aging *Cell*. **120**, 437-447.
Kirkwood TBL, Rose MR (1991). Evolution of senescence- Late survival sacrificed for reproduction. *Philosophical Transactions Of The Royal Society B-Biological Sciences*. **332**, 15-24.
Kregel KC, Zhang HJ (2007). An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*. **292**, R18-R36.
Lee KP, Jang T (2014). Exploring the nutritional basis of starvation resistance in *Drosophila melanogaster*. *Functional Ecology*. **28**, 1144-1155.
Leech T, Sait SM, Bretman A (2017). Sex-specific effects of social isolation on ageing in *Drosophila melanogaster*. *Journal of Insect Physiology*. **102**, 12-17.
Lemaître J-F, Berger V, Bonenfant C, Douhard M, Gamelon M, Plard F, Gaillard J-M (2015). Early-late life trade-offs and the evolution of ageing in the wild. *Proceedings of the Royal Society B: Biological Sciences*. **282**, 20150209.
Lemaitre JF, Gaillard JM (2017). Reproductive senescence: new perspectives in the wild. *Biological Reviews*. **92**, 2182-2199.
Liu H, Kubli E (2003). Sex-peptide is the molecular basis of the sperm effect in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*. **100**, 9929-9933.
Lung O, Tram U, Finnerty CM, Eipper-Mains MA, Kalb JM, Wolfner MF (2002). The *Drosophila melanogaster* seminal fluid protein Acp62F is a protease inhibitor that is toxic upon ectopic expression. *Genetics*. **160**, 211-224.
Maklakov AA, Immler S (2016). The expensive germline and the evolution of ageing. *Current Biology*. **26**, R577-R586.
Maklakov AA, Kremer N, Arnqvist G (2005). Adaptive male effects on female ageing in seed beetles. *Proceedings Of The Royal Society B-Biological Sciences*. **272**, 2485-2489.
Maklakov AA, Rowe L, Friberg U (2015). Why organisms age: Evolution of senescence under positive pleiotropy? *Bioessays*. **37**, 802-807.
Martin GM, Bergman A, Barzilai N (2007). Genetic determinants of human health span and life span: Progress and new opportunities. *Plos Genetics*. **3**, 1121-1130.
Massot M, Clobert J, Montes-Poloni L, Haussy C, Cubo J, Meylan S (2011). An integrative study of ageing in a wild population of common lizards. *Functional Ecology*. **25**, 848-858.
Maynard Smith J (1958). The effects of temperature and of egg-laying on the longevity of *Drosophila subobscura*. *Journal of Experimental Biology*. **35**, 832-842.
Maynard Smith J (1962). Review lectures on senescence. I. The causes of ageing. *Proceedings of the Royal Society B: Biological Sciences*. **157**, 115-127.
Mueller JL, Page JL, Wolfner MF (2007). An ectopic expression screen reveals the protective and toxic effects of *Drosophila* seminal fluid proteins. *Genetics*. **175**, 777-783.
Nussey DH, Coulson T, Festa-Bianchet M, Gaillard JM (2008). Measuring senescence in wild animal populations: towards a longitudinal approach. *Functional Ecology*. **22**, 393-406.
Nussey DH, Froy H, Lemaitre J-F, Gaillard J-M, Austad SN (2013). Senescence in natural populations of animals: Widespread evidence and its implications for bio-gerontology. *Ageing Research Reviews*. **12**, 214-225.
Nussey DH, Kruuk LE, Donald A, Fowlie M, Clutton-Brock TH (2006). The rate of senescence in maternal performance increases with early-life fecundity in red deer. *Ecology Letters*. **9**, 1342-1350.
Partridge L, Farquhar M (1981). Sexual activity reduces lifespan of male fruitflies. *Nature*. **294**, 580-582.
Partridge L, Fowler K (1990). Non-mating costs of exposure to males in female *Drosophila melanogaster*. *Journal of Insect Physiology*. **36**, 419-426.
Peng J, Zipperlen P, Kubli E (2005). *Drosophila* sex-peptide stimulates female innate immune system after mating via the Toll and Imd pathways. *Current Biology*. **15**, 1690-1694.
Promislow DEL, Fedorka KM, Burger JMS (2006). Evolutionary biology of aging: future directions. In *Handbook of the biology of aging*. (EJ Masoro , SN Austad, eds). Burlington, MA: Academic Press.

Ram KR, Wolfner MF (2007). Seminal influences: *Drosophila* Acps and the molecular interplay between males and females during reproduction. *Integr. Comp. Biol.* **47**, 427-445.
Reed TE, Kruuk LE, Wanless S, Frederiksen M, Cunningham EJ, Harris MP (2008). Reproductive senescence in a long-lived seabird: rates of decline in late-life performance are associated with varying costs of early reproduction. *American Naturalist*. **171**, E89-e101.
Reiff T, Jacobson J, Cognigni P, Antonello Z, Ballesta E, Tan KJ, Yew JY, Dominguez M, Miguel-Aliaga I (2015). Endocrine remodelling of the adult intestine sustains reproduction in *Drosophila*. *eLife*. **4**, e06930.
Rhodenizer D, Martin I, Bhandari P, Pletcher SD, Grotewiel M (2008). Genetic and environmental factors impact age-related impairment of negative geotaxis in *Drosophila* by altering age-dependent climbing speed. *Exp. Gerontol.* **43**, 739-748.
Ribeiro C, Dickson BJ (2010). Sex peptide receptor and neuronal TOR/S6K signaling modulate nutrient balancing in *Drosophila*. *Current Biology*. **20**, 1000-1005.
Rush B, Sandver S, Bruer J, Roche R, Wells M, Giebultowicz J (2007). Mating increases starvation resistance and decreases oxidative stress resistance in *Drosophila melanogaster* females. *Aging Cell*. **6**, 723-726.
Salmon AB, Marx DB, Harshman LG (2001). A cost of reproduction in *Drosophila melanogaster*: Stress susceptibility. *Evolution*. **55**, 1600-1608.
Skorupa DA, Dervisefendic A, Zwiener J, Pletcher SD (2008). Dietary composition specifies consumption, obesity, and lifespan in *Drosophila melanogaster*. *Aging Cell*. **7**, 478-490.
Smith DT, Clarke NVE, Boone JM, Fricke C, Chapman T (2017). Sexual conflict over remating interval is modulated by the sex peptide pathway. *Proceedings of the Royal Society B-Biological Sciences*. **284**, 9.
Stoltz JA, Hanna R, Andrade MCB (2010). Longevity cost of remaining unmated under dietary restriction. *Functional Ecology*. **24**, 1270-1280.
Tatar M, Carey JR, Vaupel JW (1993). Long-term cost of reproduction with and without accelerated senescence in *Callosobruchus maculatus* - Analysis of age- specific mortality. *Evolution*. **47**, 1302-1312.
Walker LC, Herndon JG (2010). Mosaic aging. *Medical Hypotheses*. **74**, 1048-1051.
Wigby S, Chapman T (2005). Sex peptide causes mating costs in female *Drosophila melanogaster*. *Current Biology*. **15**, 316-321.
Wilder SM, Raubenheimer D, Simpson SJ (2016). Moving beyond body condition indices as an estimate of fitness in ecological and evolutionary studies. *Functional Ecology*. **30**, 108-115.
Williams GC (1957). Natural selection, and the evolution of senescence. *Evolution*. **11**, 398-411.
Zhou S, Mackay TFC, Anholt RRH (2014). Transcriptional and epigenetic responses to mating and aging in *Drosophila melanogaster*. *BMC Genomics*. **15**, 927.

Supporting Information

Additional supporting information may be found in the online version of this article

Appendix S1: Supplementary tables with supporting statistical information on model selection

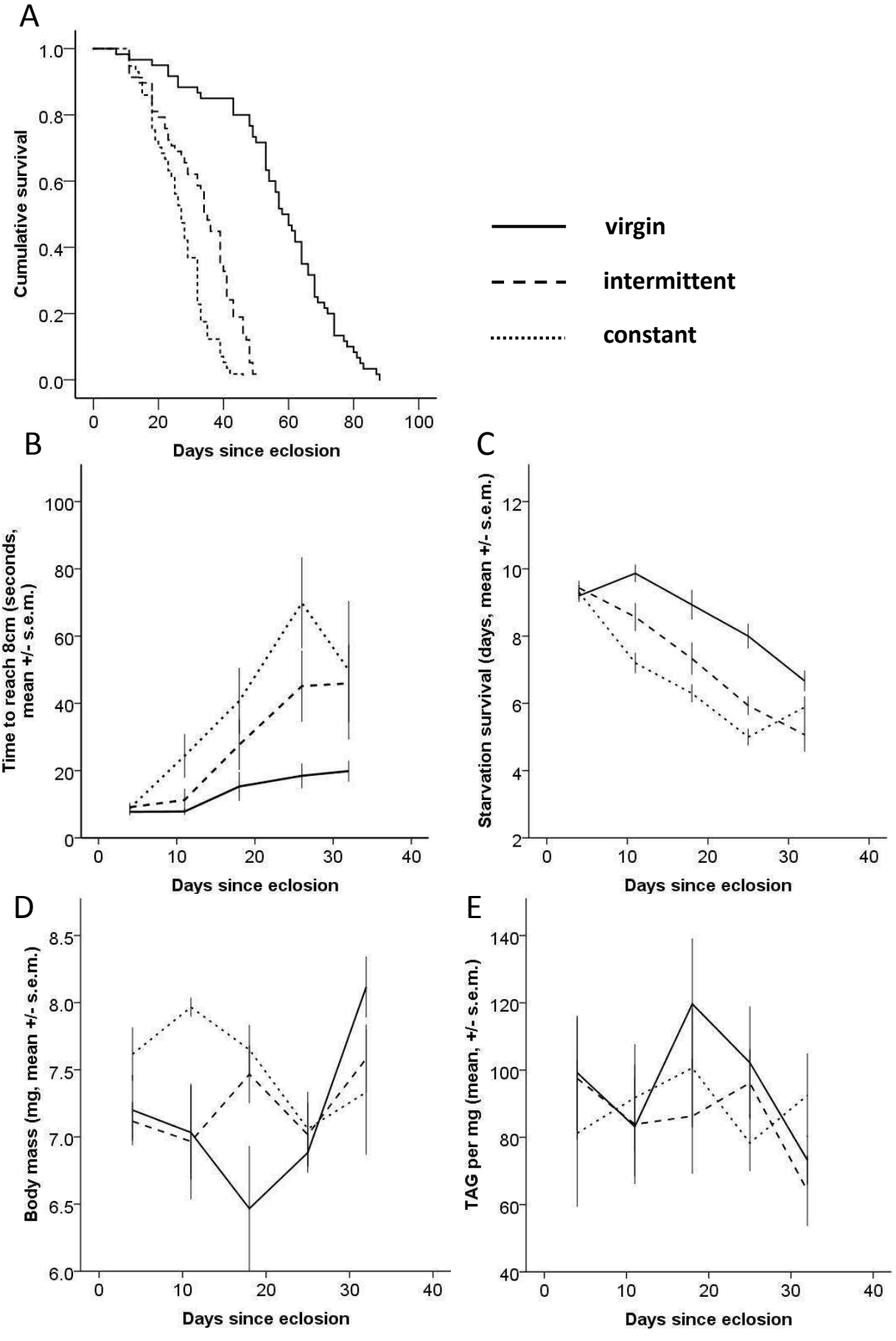
575 **Figure legends**

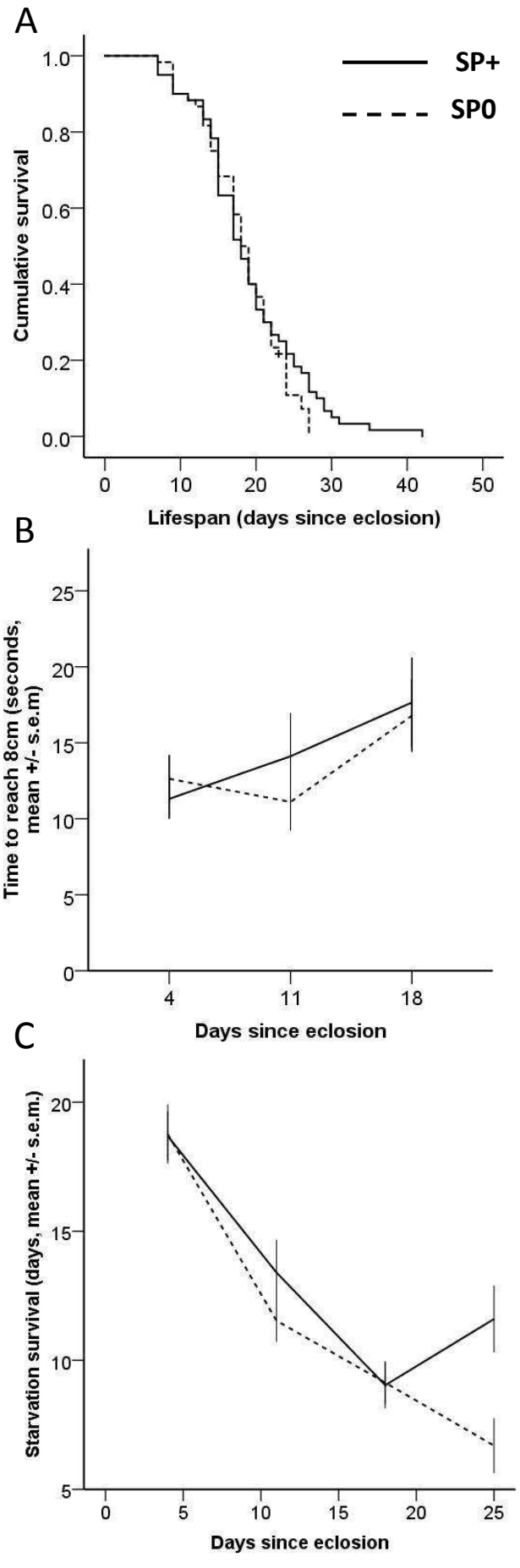
576 Figure 1 Adult lifespan and senescence of different physiological traits in females with varying exposure to
577 males. A) Lifespan of females kept singly as virgins (solid line), exposed to one male for 3 days per week
578 (dashed line), or constantly exposed to one male (dotted line). B) Climbing ability of these females was
579 assessed weekly and measured as the time taken to reach 8cm. C-E) In a further experiment, females were
580 kept in groups of ten and maintained as virgins (solid line), were exposed to males for 3 days per week
581 (dashed line), or constantly exposed to males (dotted line). C) For starvation resistance, on each test date 30
582 females were removed from each treatment and placed in vials containing only agar and checked daily for
583 death. For D) body mass and E) TAGs measurements a further 30 females were removed per assay per time
584 point and five pooled per sample.

585

586 Figure 2 The effect of receipt of sex peptide on female lifespan and physiological senescence. A) To measure
587 lifespan, females were kept in pairs with one male that either did (solid line) or did not (dashed line) produce
588 sex peptide. B) Climbing ability (the time taken to reach 8cm) of these females was assessed three times per
589 fly. C) In a further experiment, senescence of starvation resistance of females was measured. On each test
590 date 30 females were removed from each treatment and placed in vials containing only agar, and they were
591 checked daily for death.

592


A
Cumulative survival
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Days since eclosion
virgin
intermittent
constant
B
Time to reach 8cm (seconds, mean +/- s.e.m.)
100
80
60
40
20
0
0
10
20
30
40
Days since eclosion
C
Starvation survival (days, mean +/- s.e.m.)
12
10
8
6
4
2
0
10
20
30
40
Days since eclosion
D
Body mass (mg, mean +/- s.e.m.)
8.5
8.0
7.5
7.0
6.5
6.0
0
10
20
30
40
Days since eclosion
E
TAG per mg (mean, +/- s.e.m.)
140
120
100
80
60
40
0
10
20
30
40
Days since eclosion


A
SP+
SP0
Cumulative survival
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
Lifespan (days since eclosion)
B
Time to reach 8cm (seconds, mean +/- s.e.m)
25
20
15
10
5
0
4
11
18
Days since eclosion
C
Starvation survival (days, mean +/- s.e.m.)
20
15
10
5
0
5
10
15
20
25
Days since eclosion