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**Sex-specific effects of social isolation on ageing in *Drosophila melanogaster***

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**Abstract**

Social environments can have a major impact on ageing profiles in many animals. However, such patterns in variation in ageing and their underlying mechanisms are not well understood, particularly because both social contact and isolation can be stressful. Here, we use *Drosophila melanogaster* fruitflies to examine sex-specific effects of social contact. We kept flies in isolation versus same-sex pairing throughout life, and measured actuarial (lifespan) and functional senescence (declines in climbing ability). To investigate underlying mechanisms, we determined whether an immune stress (wounding) interacted with effects of social contact, and assessed behaviours that could contribute to differences in ageing rates. Pairing reduced lifespan for both sexes, but the effect was greater for males. In contrast, for females pairing reduced the rate of decline in climbing ability, whereas for males, pairing caused more rapid declines with age. Wounding reduced lifespan for both sexes, but doubled the negative effect of pairing on male lifespan. We found no evidence that these effects are driven by behavioural interactions. These findings suggest that males and females are differentially sensitive to social contact, that environmental stressors can impact actuarial and functional senescence differently, and that these effects can interact with environmental stressors, such as immune challenges.

**Keywords:** functional senescence, lifespan, wounding, stress, social environment

## 1. Introduction

It is becoming increasingly clear that social environments can play a significant role in individual ageing rates in animals (Amdam, 2011; Holt-Lunstad et al., 2010; Pantell et al., 2013; Partridge and Prowse, 1997), regardless of the related costs of reproduction (Flatt, 2011). Often these studies only measure changes in lifespan, but functional senescence (decline in physical functions) may also be sensitive to social contact (Behrends et al., 2007). Whether social contact is beneficial or costly can depend on both frequency of contact and the identity of the interacting partners (Holt-Lunstad et al., 2010). What drives these patterns remains unclear. Social isolation may increase release of stress hormones and off-target inflammatory responses (Hawkley and Cacioppo, 2003), whereas social contact could provide beneficial environmental enrichment (Donlea et al., 2014), but is likely to increase competition for reproduction, food or territory, and exposure to communicable diseases (Krause and Ruxton, 2002). Moreover, social facilitation or ‘group effects’ have been observed whereby insects raised in groups rather than isolation develop faster and invest more in reproduction (Grassé, 1946; Lihoreau and Rivault, 2008; Schausberger et al., 2017; Uzsák and Schal, 2013), which may have carryover effects on adult lifespan (Lee et al., 2016).

Ageing may show sex-specific responses to social environments. Numerous studies have shown that females have reduced lifespans in male-biased populations, through male harassment and receipt of toxic seminal proteins (Chapman et al., 1995). Males can suffer both from contact with females, through elevated courtship activity (Cordts and Partridge, 1996), and also with other males, possibly through direct aggressive interactions though also through increasing investment in reproduction (Bretman et al., 2013). Such sex differences can be seen in wild populations, for example, population density affects senescence in male but not female red deer (Mysterud et al., 2001). It is therefore likely that what constitutes an adverse social environment, and hence the consequences for ageing phenotypes, is different for each sex, but the underlying mechanisms driving these differences are poorly understood.

Here we aim to investigate effects of social contact in both actuarial and functional senescence using *Drosophila melanogaster*. Social effects on longevity have previously been reported

in *D. melanogaster*, but these studies largely either used mixed-sex groups (hence incorporating the cost of mating) (Iliadi et al., 2009; Zajitschek et al., 2013) or measured one sex only (Bretman et al., 2013; Gendron et al., 2014; Ruan and Wu, 2008). There is some evidence that social contact also affects functional senescence in males in terms of locomotor activity (Ruan and Wu, 2008) and mating success in later life (Bretman et al., 2013). In this latter study, we attributed the effect on ageing partly to responses to sperm competition signalled by the presence of another male (Bretman et al., 2013). *D. melanogaster* lifespan in the wild has been estimated to be over 50 days (Robson et al., 2006), but assessing their natural social environments is extremely challenging and such data are scarce. We speculate that flies frequently spend time in crowded conditions around ephemeral food sources (Wertheim et al., 2006), or are solitary when moving between them, and are hence subject to a great deal of variation in social context. We suggest this underlies the behavioural plasticity that males of many species show in reproductive effort (Bretman et al., 2011a; Wedell et al., 2002), as without environmental variation, plasticity should not be maintained (Carroll and Corneli, 1995). Moreover, *D. melanogaster* show natural genetic variation in propensity to aggregate (Saltz, 2011), driving further variation in natural social environments and individual responses to them.

As this plastic investment in response to sperm competition is male-specific, and additionally, males are more aggressive towards each other than are females (Nilsen et al., 2004), we hypothesised that same-sex social contact would speed ageing in males but not females. To address this we measured the effect of social isolation versus pairing on both actuarial (lifespan) and functional ageing (decline in climbing ability) in both sexes. We also aimed to gain insights into the underlying mechanisms. Given the intimate link between social environments and immunity (Amdam, 2011; Pantell et al., 2013), we reasoned that if part of these effects are through increased risk of disease or resource allocation to immune function, these patterns would be exacerbated by an immune challenge. Injury is known to interact with ageing and stress resistance (Sepulveda et al., 2008) and wounding repair utilises many of the same molecular pathways as infection responses (Felix et al., 2012; Lemaitre and Hoffmann, 2007; Ramet et al., 2002). We therefore used wounding (amputation of a middle leg) as a general immune challenge, a methodology which has been previously used to

investigate stress and ageing in *D. melanogaster* (Carey et al., 2007; Sepulveda et al., 2008). We also assessed whether social contact affected ageing through behaviours such as aggression, increased activity or exclusion from the food.

## **2. Materials and Methods**

### **2.1 Fly stocks and maintenance**

*Drosophila melanogaster* (wild-type Dahomey strain) were maintained in mass population cages on standard sugar-yeast agar medium (Bass et al., 2007), at a constant 25°C, 50% humidity with 12:12 light:dark cycle. For experiments, larvae were raised at a density of 100 per vial. Upon eclosion, adult flies were sexed under ice anaesthesia put into groups of 10 and transferred to their experimental treatment the following day. To assess the effect of social contact on ageing we compared focal flies held as adults in isolation or paired with a non-focal partner of the same sex. This is sufficient social contact in males to elicit a sperm competition response, and increases in number or density of rivals does not increase this response (Bretman et al., 2010). As our main intention was to assess the effect of social contact *per se*, this design also minimises direct competition for food. Non-focal flies were identified using a small wing clip, carried out the day after eclosion under light CO<sub>2</sub> anaesthesia, as in our previous studies (Bretman et al., 2012; Bretman et al., 2013). Non-focal flies and food was changed weekly, hence non-focals were 2-8 days old throughout.

### **2.2 Measuring actuarial senescence (lifespan) in intact and wounded flies**

Virgin focal flies were maintained in isolation or same-sex pairs and their survival was checked daily until all the flies were dead. In addition, we tested how the patterns in lifespan of the focal flies differed if the fly was under immune stress, specifically amputation of a middle leg as used in previous studies (Carey et al., 2007; Sepulveda et al., 2008). Amputations were performed under CO<sub>2</sub> anaesthesia on the day after eclosion, with equal numbers having the left or right leg removed. We

therefore had eight groups; single or paired, uninjured or injured, male or female, (n = 50 per treatment group; Figure 1A).

### **2.3 Measuring functional senescence (climbing ability)**

Senescence in climbing ability was measured using a standard negative geotaxis assay (Cook-Wiens and Grotewiel, 2002). Male and female flies were kept singly or in same sex pairs as before, but as we wanted to measure climbing ability, all flies were uninjured. Once a week from 5 to 56 days post eclosion, we recorded whether focal flies reached a height of 10cm within 120 sec in an empty vial (i.e. without food, not used to house flies, and a unique vial for each focal fly). Non-focal flies and food were changed as before. Sample size started at n = 60 per treatment, but reduced as flies died, hence we stopped the experiment when only n~20 per treatment remained (isolated male = 19, paired male = 21, isolated female = 18, paired female = 21).

### **2.4 Measuring behaviour**

To evaluate the potential contribution of behavioural variation to the observed ageing patterns, flies were maintained as before; single or paired, uninjured or injured, male or female (n = 20 per treatment). We recorded whether the focal fly was inactive, walking, on the food and grooming. Paired flies were also scored for whether they were within a body length of the non-focal fly or being involved in an aggressive encounter. Observations were made at 9am, 12pm and 3pm on day 3, 5, 7, 10, 12 and 15 post-eclosion. During each observation period, the behaviour of each focal fly was recorded each minute for 10min.

### **2.5 Statistical analysis**

All data were analysed using R v 3.3.1, package lme4 (Bates et al., 2015) and graphs were made using SPSS v 20. As lifespan data violated the assumptions of a Cox regression, this was analysed using a

GLM with quasi Poisson errors (to account for over dispersion) with sex, injury and social environment as factors. Our general approach when using GLMs or GLMMs (for repeated measures where fly ID was used a random factor) with appropriate error structures was to simplify from the full model using Analysis of Deviance (AOD, using  $F$  or  $\chi^2$  tests as appropriate to the error structure) to test whether removing a term significantly affected the model's descriptive power. We analysed the proportion of flies in each trial successfully reaching 10cm in 120s using a GLM with quasi binomial errors, with sex, age and social environment as fixed factors. To analyse behavioural data, we used GLMMs with Poisson errors, with the number of observations of the behaviour of interest within the 10min scan period as the response, with sex, injury, and social treatment as fixed factors and fly ID, day and time of day as random factors. For behaviours that could only be expressed in pairs (aggression or sitting within a body length) the models were the same but without social treatment as a factor.

### 3. Results

#### 3.1 Lifespan when isolated or paired, uninjured or injured

The effect on lifespan of injury and social environment differed between the sexes, as there was a significant three way interaction between sex, injury and social environment (AOD  $F_{1,326} = 26.827$ ,  $P = 0.045$ ). To investigate this further, as females appeared to be living longer than males, we split the dataset by sex and analysed these data separately. For females, there was no significant interaction between social environment and injury (AOD  $F_{1,158} = 0.113$ ,  $P = 0.738$ ), but both injury (AOD  $F_{1,159} = 9.685$ ,  $P = 0.002$ ) and social environment (AOD  $F_{1,28} = 17.260$ ,  $P < 0.001$ ) reduced female lifespan (Figure 1B). For males, however, there was a significant interaction between injury and social environment (AOD  $F_{1,169} = 5.431$ ,  $P = 0.021$ ), as the reduction in lifespan due to injury was exacerbated for paired males (Figure 1C). Scaling by sex- and injury treatment-specific median

lifespan, pairing reduced female lifespan by ~12% whether injured or not, but reduced male lifespan by 20% if uninjured and ~38% if injured.

### 3.2 Senescence in climbing ability when isolated or paired

The proportion of flies completing the climbing task was determined by a significant three way interaction between sex, social environment and age (AOD  $F_{1,56} = 11.685$ ,  $P = 0.001$ ; Figure 1D).

To further investigate this, males and females were again analysed separately. For females, there was a significant interaction between age and social environment (AOD  $F_{1,28} = 17.313$ ,  $P < 0.001$ ); at ~35 days after eclosion isolated females become less successful than paired at climbing (Figure 1D). For males, the interaction was not significant (AOD  $F_{1,28} = 0.180$ ,  $P = 0.675$ ) but climbing ability declined with age (AOD  $F_{1,29} = 14.456$ ,  $P < 0.001$ ) and isolated males were more successful in completing the task (AOD  $F_{1,29} = 10.078$ ,  $P = 0.004$ ; Figure 1D).

### 3.3 Behaviour when isolated or paired, uninjured or injured

We found little evidence that behavioural patterns explained the observed differences in functional and actuarial senescence. Males were inactive more often than females (AOD  $\chi^2_1 = 21.246$ ,  $P < 0.001$ ; Figure 2A). Inactivity was also affected by a significant interaction between social environment and injury (AOD  $\chi^2_1 = 6.387$ ,  $P = 0.012$ ); injured flies were more often inactive if they were isolated, whereas there was little difference between isolated or paired uninjured flies (Figure 2B). Sex had no effect on the time spent walking (AOD  $\chi^2_1 = 1.370$ ,  $P = 0.242$ ). However, walking was affected by an interaction between social environment and injury (AOD  $\chi^2_1 = 6.386$ ,  $P = 0.011$ ); social environment had little effect in uninjured flies, but for injured flies, isolated flies walked more (Figure 2C). The effect of the social environment on the time spent on the food differed between sexes (AOD  $\chi^2_1 = 5.435$ ,  $P = 0.020$ ), with males spending more time on the food if paired (Figure 2D). Injured flies spent more time on the food (AOD  $\chi^2_1 = 11.337$ ,  $P = 0.0008$ ; Figure 2E). Injured flies groomed more (AOD  $\chi^2_1 = 5.110$ ,  $P = 0.024$ ; Figure 2F), but there was no effect of sex (AOD  $\chi^2_1 = 1.495$ ,  $P = 0.221$ ).

or social environment (AOD  $\chi^2_1 = 1.394$ ,  $P = 0.238$ ) on grooming. For paired flies, observations of flies within a body length was affected by an interaction between sex and injury (AOD  $\chi^2_1 = 11.335$ ,  $P = 0.0008$ ); there was little effect of injury on female proximity, but males were more often observed in close proximity if they were injured (Figure 2G). Aggression was very rarely observed between females, hence there was a strong main effect of sex on amount of aggression (AOD  $\chi^2_1 = 116.54$ ,  $P < 0.0001$ ; Figure 2H). Injured flies were less likely to be observed in aggressive encounters (AOD  $\chi^2_1 = 7.741$ ,  $P = 0.005$ ; Figure 2I).

#### 4. Discussion

As predicted, social contact had profound effects on actuarial senescence; pairing reduced lifespan, but this was more severe for males (~20% compared to 12% for females). Injury reduced lifespan for both sexes, almost doubling the effect of pairing on male lifespan. Social contact also affected functional senescence in a sex-specific manner, such that pairing for males, but isolation for females, caused more rapid declines in climbing ability. We found no evidence that these effects are driven directly by behaviour, as neither pairing nor wounding increased activity and flies were not excluded from the food. Whilst there was more aggression between males than between females, this was not more intense for wounded flies, so could not have driven the interactive effect of wounding and social environment on male lifespan. Wounded flies groomed more, but this was unrelated to sex or social environment.

Previous studies on the social effects on ageing in *D. melanogaster* have found reduced lifespan in group-housed flies, but sometimes only in males (Carazo et al., 2016) or in both sexes (Iliadi et al., 2009). A further study showed that social environments had an interactive relationship with food resources, as diet affected female lifespan regardless of social environment but dietary restriction reduced male lifespan only in mixed sex groups (Zajitschek et al., 2013). Males maintained continuously with other males had longer life spans than those kept with females (Cordts and Partridge, 1996), which was attributed to the cost of courtship, specifically mounting attempts (Partridge and Prowse, 1997). Males carrying a mutation in the gene *Sod* (a sulfoxide dismutase

involved in responses to oxidative stress) lived longer if housed with “helpers”, but only if those helpers were young wild-type males (Ruan and Wu, 2008). Taken together these findings suggest that the effect of social environments on lifespan are complex and dependent on the amount of contact, density and identity of the social partners.

Sex-specific ageing patterns are widely observed amongst animals (Austad and Fischer, 2016) and are predicted if one sex suffers from greater extrinsic mortality rates (Williams, 1957). In polygynous species this is most often males, likely because of the costs of mating behaviours and secondary sexual traits (Bonduriansky et al., 2008). This might contribute to the sex differences we found in ageing *per se* and the response to social contact. Many previous studies show that male *D. melanogaster* respond to the presence of rivals by increasing mating duration (Bretman et al., 2009; Bretman et al., 2011b; Bretman et al., 2012) and altering ejaculate content (Garbaczewska et al., 2013; Moatt et al., 2014; Wigby et al., 2009). These strategies appear to be costly, as starved males are unable to mount this response (Mason et al., 2016) and paired males die sooner regardless of whether they are actually able to mate (Bretman et al., 2013). It is possible, therefore, that anticipating sperm competition elicits a response that is costly even if the ejaculate is not used. Clearly this is a consideration only for males, but whilst females appear less sensitive, they still did respond to social contact, hence we investigated other potential contributing factors.

One direct consequence of social contact is enhanced competition for resources. The patterns in ageing we observed do not seem to be driven by flies being excluded from food. Injured flies and paired males were more often on the food, though we do not know if they were eating at different rates. Injured flies may simply be on the food more since it is more difficult to rest on the sides of the vial. Likewise, the amount of activity was reduced in shorter-lived paired and injured flies, so does not suggest they were spending more energy in general activity. We observed low levels of aggression in both sexes, but there was clearly more between males, as seen in previous studies (Nilsen et al., 2004). Between males, aggression declines quickly with increasing familiarity (Liu et al., 2011). Indeed, previous social experience reduces aggression, as previously isolated males (Wang et al., 2008) and females (Ueda and Kidokoro, 2002) are more aggressive than socially experienced counterparts. Our data show that injured males were less often involved in aggressive encounters,

though we cannot assess whether this is because they were less likely to initiate fights. Wounded males are outcompeted by unimpaired males in gaining matings (Sepulveda et al., 2008), so they may be perceived as less of a threat generally. So whilst differences in aggression might contribute to a reduction in male compared to female lifespan, it cannot explain the doubling of the reduction in lifespan for injured paired males found in our study. This is in line with our previous work in males (Bretman et al., 2013) and it seems unlikely then that aggression plays a major role in the mechanisms underlying the social effect on ageing.

We used injury as a simple immune challenge as both wounding and infection responses utilise many shared underlying immunity mechanisms. The effect of injury on lifespan in *D. melanogaster* is not straight forward, as previous studies have found an effect in males only (Sepulveda et al., 2008) or in both sexes, but a stronger effect in females (Carey et al., 2007). Other invertebrates also show a lifespan cost to wounding (Carey et al., 2009) and encapsulation (Armitage et al., 2003). The effects of removing a leg could include haemolymph loss and increased risk of infection, plus the cost of wound healing, all of which could be physiologically costly through, for example, increasing metabolic rate (Ardia et al., 2012). If the costs of wounding, or even prophylactic increases in immune gene regulation, interact with the cost of being paired in males, this could explain why the effects of injury and pairing in males interact. Indeed, a recent analysis of transcriptomic responses of males to rivals shows differential expression of immune related and adult lifespan determining genes (Mohorianu et al., 2017). The costs of injury do not seem to arise from behavioural differences. Wounded flies were less often involved in aggressive encounters and we found a reduction in activity by wounded flies, consistent with the suggestion that sick animals reduce activity to conserve energy (Hart, 1988; Sullivan et al., 2016). We found injured flies groomed more, in line with a previous finding that even decapitated *D. melanogaster* increased grooming if triggered by contact with *Escherichia coli* (Yanagawa et al., 2014). It is likely that grooming is beneficial to sick invertebrates by removing surface pathogens without increasing heat loss (Sullivan et al., 2016).

Our finding that lifespan and functional senescence show different patterns in response to social contact aligns with the idea that traits do not all necessarily show the same senescence patterns

(Grotewiel et al., 2005; Nussey et al., 2013). The basis of this variation in senescence among traits is not yet understood (Nussey et al., 2013). *D. melanogaster* shows senescence in a wide range of traits, with declines becoming apparent at different ages, though comparisons across multiple studies is not straight forward (reviewed by Grotewiel et al., 2005). It would therefore be beneficial to explore social effects on senescence in multiple traits to fully understand the consequences for later life.

Clearly we only tested a very simple social environment manipulation. We did not use mixed sex pairs in order to avoid costs of reproduction, but being virgin throughout life is probably unusual, particularly for females (Markow et al., 2012). In addition, non-focal partners were always less than 10 days old as in previous work (Ruan and Wu, 2008), but as generations overlap, the age of interacting individuals may alter the effect of social contact on ageing (Souza, 2011). Our behavioural observations were made in relatively young flies, and these interactions could change with age. However, the general pattern is that various behaviours and overall activity declines with age (Grotewiel et al., 2005), hence we likely measured the stage at which we would see the most variation in behaviour. Future work could build on our observations by altering the frequency of social interactions, the number of flies per group, age of interacting partners and by mating all individuals.

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**Competing interests:** We have no competing interests to declare.

**Author's Contributions:** AB, SMS and TL designed the experiments, AB and TL conducted work in the lab and carried out the statistical analysis. The manuscript was written by all authors.

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**Data accessibility:** Data will be archived Research Data Leeds open access repository upon acceptance.

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**Figure legends**

**Figure 1 A) Experimental design.** Females and males (black tipped abdomen) were maintained throughout adult life in isolation or same-sex pairs. Non-focal flies (grey) were identified with a small wing clip. Injured flies had their 3<sup>rd</sup> leg (half left, half right) removed under CO<sub>2</sub> anaesthesia. **Median lifespan** of **B)** females and **C)** males when maintained throughout life socially isolated or in same-sex pairs. Pairs consisted of a focal individual and a non-focal which was changed weekly. To increase stress through an immune challenge, half of the flies had their middle leg amputated the day after eclosion (“injured”). N = 50 for all groups. Whiskers represent maximum and minimum non-outlier values. Circles indicate outliers (Q1/Q3±1.5 x IQ range). **D) Senescence** of climbing ability (flies reaching 10cm in 120s) of isolated or paired males and females was measured weekly in a separate experiment. Initial n = 60.

**Figure 2 Behavioural observations** of males and females, isolated or in same-sex pairs, uninjured or injured (middle leg amputated). Pairs consisted of a focal individual and a non-focal which was changed weekly. Plots are to illustrate the GLMMs of the effect of the three factors on each behaviour. Amount of inactivity was determined by **A)** sex and **B)** an interaction between social treatment and injury; **C)** amount of walking by an interaction between social treatment and injury. Number of observations of the focal fly on the food was determined by an interaction between **D)** social treatment and sex and **E)** injury treatment. **F)** The number of observations of the focal fly grooming was determined by injury treatment. For paired flies only, **G)** the amount of time spent within a body length was determined by an interaction between sex and injury treatment; the number of aggressive encounters by **H)** sex and **I)** injury. Whiskers represent maximum and minimum non-outlier values. Circles indicate outliers (Q1/Q3±1.5 x IQ range).

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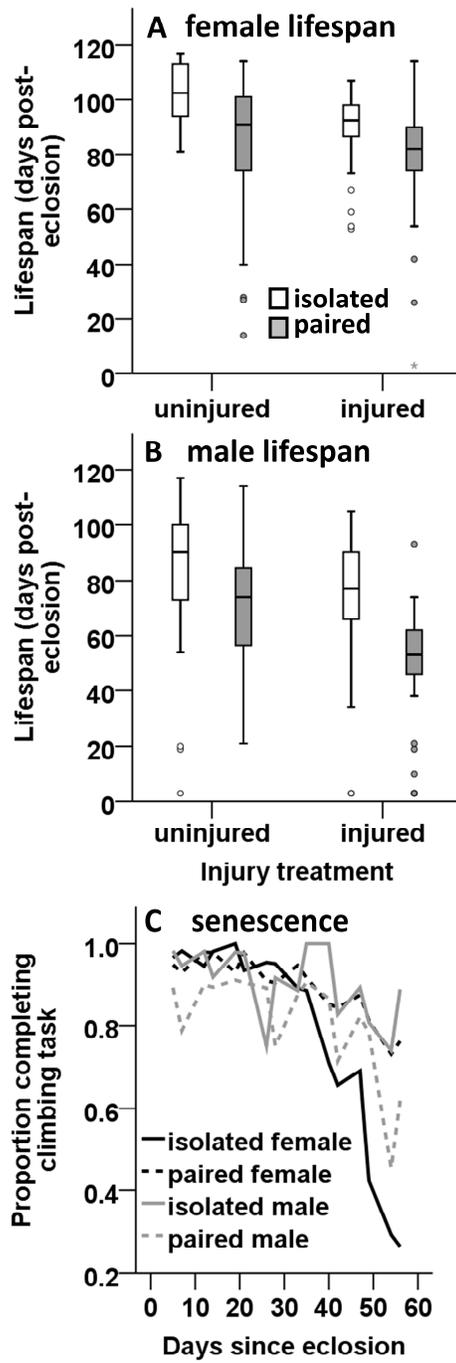
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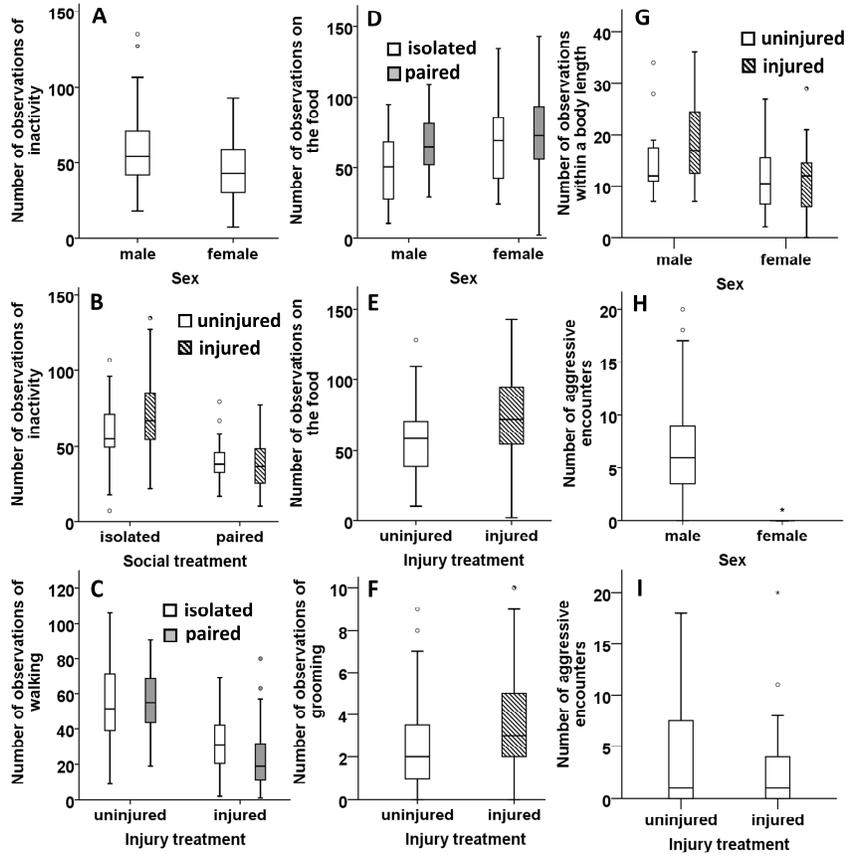
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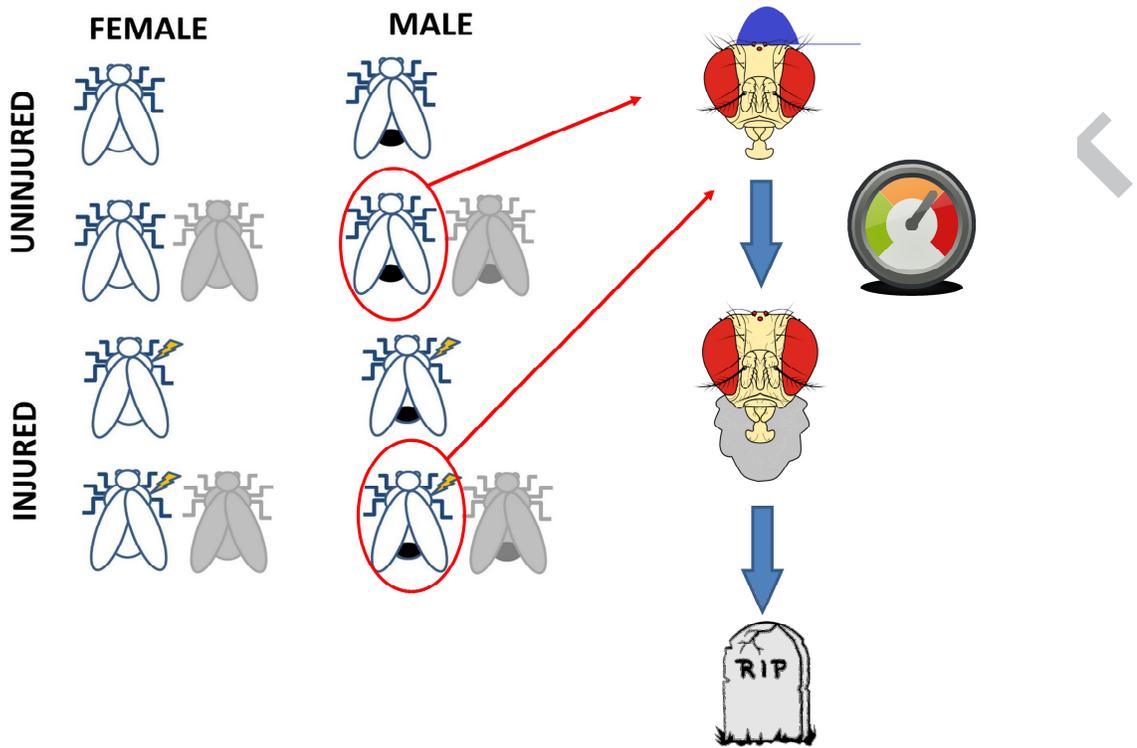
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**Sex-specific effects of social isolation on ageing in *Drosophila melanogaster***

## Highlights:

- Social contact reduces lifespan for both sexes, but is more severe for males.
- Climbing ability declines more quickly for paired males but isolated females.
- For males but not females, injury exacerbates the effect of pairing on lifespan.
- Behavioural observations do not explain these differences in ageing patterns.

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