Selection on age of female reproduction in the marula fruit fly, *Ceratitis cosyra* (Walker) (Diptera: Tephritidae), decreases total antioxidant capacity and lipid peroxidation

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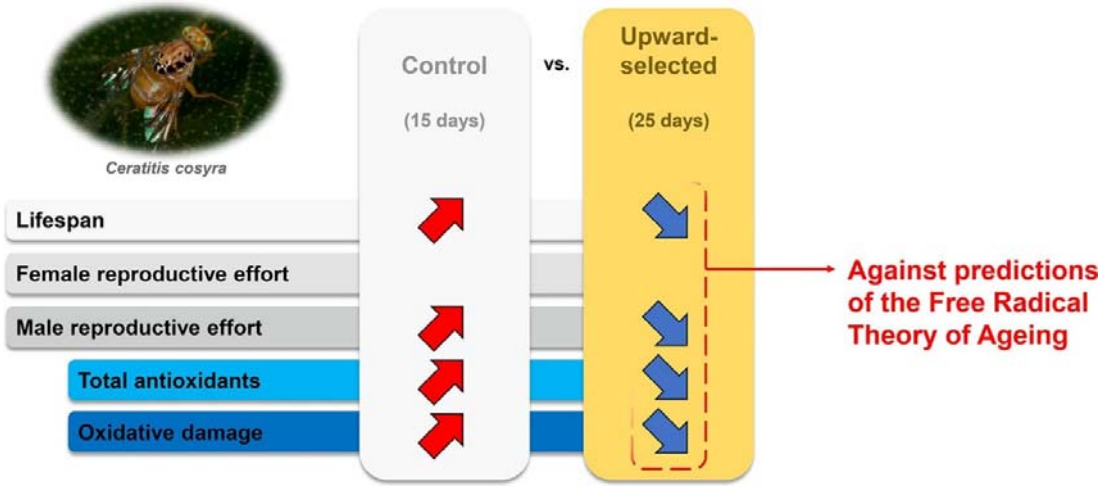
Highlights

- Oxidative stress may play a role in aging process and life-history trade-offs.
- Variation in lifespan and reproductive effort depends on life-history strategy.
- This study used experimental selection to induce a change in life-history strategy in fruit flies.
- Upward-selection on age of female reproduction reduced survival and delayed egg laying.
- Upward-selection was associated with lower lipid peroxidation and total antioxidant protection.

Abstract

The oxidative damage caused to cells by reactive oxygen species (ROS) is one of several factors implicated in causing ageing. Oxidative damage may also be a proximate cost of reproductive effort that mediates the trade-off often observed between reproduction and survival. However, how the balance between oxidative damage and antioxidant protection affects life-history strategies is not fully understood. To improve our understanding, we selected on female reproductive age in the marula fruit fly, *Ceratitis cosyra*, and quantified the impact of selection on female and male age-dependent mortality, female fecundity, male sperm transfer, calling and mating. Against expectations, upward-selected lines lived shorter lives and experienced some reductions in reproductive performance. Selection affected oxidative damage to lipids and total antioxidant protection, but not in the direction predicted; longer lives were associated with elevated oxidative damage, arguing against the idea that accumulated oxidative damage reduces lifespan. Greater reproductive effort was also associated with elevated oxidative damage, suggesting that oxidative damage may be a cost of reproduction, although one that did not affect survival. Our results add to a body of data showing that the relationship between lifespan, reproduction and oxidative damage is more complex than predicted by existing theories.

Graphical abstract



Keywords: tephritidae; oxidative stress; lifespan; reproduction; antioxidant

1. Introduction

Of the theories attempting to explain the mechanistic basis of ageing, the free radical theory of ageing (FRTA) (Harman, 1956) has received the most attention. Reactive oxygen species (ROS) are central to the FRTA. Oxidation-reduction reactions used by mitochondria to produce adenosine triphosphate for biochemical functions are one of the main sources of ROS formation (Selman et al., 2012). ROS are highly reactive and can cause cellular damage by oxidising cellular components such as proteins, lipids, or nucleic acids. To prevent damage accumulation, cells have antioxidant defences that render ROS inert, and can repair oxidative damage once it occurs. The FRTA predicts that when ROS levels exceed the antioxidant capacity of an organism, this leads to a state of oxidative stress and oxidative damage occurs. The accumulation of this damage causes cellular dysfunction and is thought to contribute towards ageing. However, several studies show that the relationship between oxidative damage and lifespan is oversimplified and the FRTA has fallen into disuse (Clancy and Birdsall, 2013; Pérez et al., 2009; Speakman and Selman, 2011; Stuart et al., 2014). Nevertheless, oxidative damage may still play a role in causing ageing (Hekimi et al., 2011; Kirkwood and Kowald, 2012).

More recently, ROS have been incorporated into an evolutionary framework, which suggests that ROS may still affect lifespan by mediating the life-history trade-off between reproduction and lifespan (Dowling and Simmons, 2009; Metcalfe and Alonso-Alvarez, 2010; Monaghan et al., 2009; Speakman et al., 2015). This could occur if reproduction increases ROS production, or if reproduction utilises resources that could otherwise be used to prevent or repair oxidative damage. Under either scenario, reproductive effort would increase oxidative damage accumulation, and potentially reduce lifespan. This idea has received some support from empirical studies (Archer et al., 2013; Blount et al., 2016; Costantini, 2008; Vágási et al., 2019). However, there is a lack of direct experimental work showing that laboratory selection on life-histories is accompanied by changes in oxidative damage and/or antioxidant protection in the direction predicted. In addition, the few studies that investigated the topic were performed on conventional model organisms such as *Drosophila melanogaster* (Arking et al., 2000; Harshman and Haberer, 2000).

Here, we investigated whether selection on the age of female reproduction affects sex-specific patterns of lifespan and reproduction. We then determined if those changes were associated with altered oxidative damage and antioxidant protection. If oxidative damage contributes to variation in lifespan, we predict that reduced oxidative damage is associated with longer lives. If oxidative damage is a proximate cost of reproduction, then greater reproductive investment should elevate oxidative damage. If reproduction reduces resources available to protect against oxidative damage, then greater reproductive effort should be associated with reduced antioxidant defences. We tested these predictions in the marula fruit fly, *Ceratitis cosyra* (Walker) (Diptera: Tephritidae), as an alternative to the conventional biological models (e.g. *D. melanogaster*), to broaden the diversity of species investigated

and because life-history traits and ageing patterns have been well studied in tephritids (Carey, 2011; Carey and Molleman, 2010; Carey et al., 2008; Chen et al., 2013; Fanson et al., 2012; Malod et al., 2017). We found that selection on female reproductive schedules was associated with altered life-history strategies. But against expectations, lines with longer lives and elevated reproductive investment were associated with both greater oxidative damage and total antioxidant capacity.

2. Materials and Methods

2.1 Fly husbandry

Infested mangoes from across Mpumalanga province, South Africa, were collected and pupae of *C. cosyra* retrieved. The flies emerging from these pupae were used to establish a culture that was maintained at ~ 23° C in a climate room with a 14:10 dark light photoperiod. To create optimal mating conditions, 1h of dawn and dusk was simulated by turning on 8 W fluorescent tubes (T4, Eurolux, Sandton, South Africa) placed above the fly culture 1 h before and after the main light of the room was switched on. The remaining room lights, comprising a combination of 20 W (G5, Eurolux, Sandton, South Africa) and 58 W (58W/840, Osram, Germany) fluorescent tubes were also turned on for the remainder of the light period. Adults were kept in groups of ca. 200 flies in 5 L plastic cages with unrestricted access to food (hydrolysed yeast and sugar in separate dishes) and water (water-soaked cotton wool). At 15 days after emergence, wild males from the culture were crossed with females from a laboratory culture provided by Citrus Research International (Nelspruit, South Africa). Flies for the selection regime were obtained by allowing laboratory females mated with wild males to lay eggs on a 125 mL plastic container (Plastilon, South Africa) covered with a layer of laboratory film (Parafilm M, Bemis, USA) pierced several times. Tissue paper soaked with 3 mL of guava juice (Hall's concentrate, Tiger Consumer Brands Limited, Bryanston, South Africa) was placed in the plastic container to encourage females to oviposit through the film. Eggs were then harvested and placed on 125 mL of a carrot-based diet (Citrus Research International, Nelspruit, South Africa) in a plastic container at an approximate rate of 2.5 eggs/mL of medium. The container was then placed in a 2 L plastic box with a layer of sand and a ventilated lid. After 15 days, during the pupal phase, the sand was sifted and the retrieved pupae placed in a Petri-dish (ø 65 mm) and transferred into a 5 L cage with unrestricted access to food and water for emerging adults.

2.2 Selection regime

Selection began three generations after laboratory females had been crossed with wild males and a strong culture had been established. We selected on the age of oviposition by only providing an oviposition substrate (a 125 mL plastic container with guava juice-soaked tissue paper) when flies were 15 days old (control, CT) or 25 days old (upward selected, US). In our laboratory, 15 days is the average

age when eggs are collected from this species and is also when oviposition peaked in an earlier study (Manrakhan and Lux, 2006). Upward selection at 25 days rather than an older age was to ensure that enough females would contribute to the subsequent generation due to a gradual decline in oviposition from three weeks of age (Manrakhan and Lux, 2006). This was to maintain populations of ca. 200 flies per replicate and avoid the risk of a population collapse. Selecting upwards on age of reproduction has been shown to extend lifespan in *Drosophila* (Harshman and Haberer, 2000; Rose, 1984), probably because only individuals that survive to reproduce late in life produce offspring. For each of the two selection lines (CT and US) we established five replicate populations. We maintained the selection regime for 20 generations. Lifespan and reproductive effort assays were performed for each line at generation 20 (G_{20}). Because flies were selected on age of oviposition, selection lines inevitably differed in their assay date.

2.3 Survival

Within 24 hours of emergence, 10 females and 10 males from each selection regime and replicate were transferred to individual containers. Each container comprised a 125 mL plastic cup with another cup nested inside with the base removed (for easy replacement of food and egg laying dishes; see below). The containers were covered with insect screen secured by two rubber bands. The flies were provided with filtered water through the insect screen with 200 µL pipette tips loosely capped at the wide end with putty-like pressure-sensitive adhesive (Prestik, Bostik, South Africa) to minimise evaporation. Sugar and hydrolysed yeast (Yeast Extract Powder, Biolab, Merck, Germany) were provided in the lids of two 2 mL microcentrifuge tubes. Mortality was recorded daily, and food and water were replaced when close to being depleted or if the sugar liquefied (due to its tendency to absorb water vapour). For each selection regime, lifespan data were obtained for 50 females and 50 males (n = 10 per sex, per replicate).

2.4 Female reproductive effort

Within a day of emergence, 50 females from each selection regime were placed into individual containers as described above (n = 10 per replicate, per selection regime). An artificial egg laying dish was added to each of the containers. This dish comprised a black screw-top lid (volume = 5 mL, diameter = 32 mm) containing a 1:10 orange essence-water solution (Robertsons, Johannesburg, South Africa) and covered with a double layer of laboratory film, pierced ten times with an entomological pin. The number off eggs laid by each female was counted every five days. The total number of eggs laid by females during their lifetime was calculated as the sum of all five-day oviposition intervals, and the average number of eggs per day as the total number of eggs divided by lifespan. The day of peak egg production was the day at the end of the five-day oviposition interval during which the maximum number of eggs were obtained from a female.

2.5 Male reproductive effort

For each selection regime, groups of 50 males were taken from each replicate shortly after emergence and kept in separate plastic cages to prevent mating. At ages 5, 15 and 25 days (t_5, t_{15} and t_{25}), focal males were paired with virgin females from an unselected laboratory culture one hour before the simulated sunset. This species only mate at dusk and new mating does not occur after darkness (personal observation). For each selection regime, age, and replicate, six pairs were assayed. The females used as mates were all between 10 and 20 days old to minimise the effect of female age on male reproductive measurements. The pairs were placed into cylindrical transparent plastic containers (height = 52 mm, diameter = 35 mm) for easy observation. All pairs were observed until all lights are turned off (two hours) to record if male calling and mating occurred. Due to the tendency of *C. cosyra* to mate for up to 12 hours (personal observation), flies were left to mate overnight.

The following morning, mated females were dissected under a stereo microscope to analyse sperm transfer, using methods described by Roets et al. (2018). A total of 110 females were dissected (CT: t_5 = 23, t_{15} = 17, t_{25} = 17; US: t_5 = 19, t_{15} = 13, t_{25} = 21). Spermathecae of females were removed and placed individually into 15 µL drops of water on microscope slides. Each spermatheca was then crushed with an entomological pin attached to a thin wooden dowel. The crushed spermatheca was then spread by vigorous stirring for 30 seconds before covering it with a 22 × 22 mm cover slip. The slides were left to dry for 2-3 days before gluing the corners of the coverslip to the slide using clear nail varnish.

The number of sperm transferred were estimated using a phase contrast microscope (BX43, Olympus Corporation, Japan) and methods described in Taylor *et al.* (2000). In summary, a matrix of 25 fields of view at 100 × magnification (17.36% of the coverslip area) were selected. The number of sperm counted in this area was multiplied by 5.76 to estimate the total number of sperm stored per spermatheca. If no sperm were found in the 25 fields checked, the whole slide was checked to confirm absence of sperm.

2.6 Lipid peroxidation and total antioxidant capacity

For each selection regime, 20 females and 20 males were collected at three different ages (0, 25 and 50 days). Flies were individually placed in 2 mL microcentrifuge tubes and frozen and stored at - 80º C. On the day of the assay, flies were removed from the freezer and weighed to determine wet mass. Flies were then placed in a 1.5 mL microcentrifuge tube with 1 mL of 5 mM phosphate-buffered saline and homogenised for 30 s using a homogeniser (T25 Ultra-Turrax, IKA, Germany). Malondialdehyde (MDA) concentration was measured using high-performance liquid chromatography according to Agarwal and Chase (2002). A standard curve was obtained using a serial dilution series of 1,1,3,3-tetraetoxypropane (Helfenstein et al., 2010; Mougeot et al., 2009). In order to determine total antioxidant capacity (TAC) for each individual, we used an antioxidant assay kit (Cayman, Cat no.

709001, USA) according to the manufacturer's recommendations. The TAC assay detects both non-enzymatic (e.g. ascorbic acid, α-tocopherol) and enzymatic antioxidants (e.g. superoxide dismutase, catalase) but cannot discriminate between them.

2.7 Data analyses

To determine the effect of selection and sex on survival, a Cox proportional hazards model was performed using the "coxme" function from the "survival" package (Therneau, 2015) in R (v 3.5.3, The R Foundation for Statistical Computing). Selection regime, sex and their interaction were fixed effects in the model, while replicate population was included as a random effect. We used backwards step-wise deletion of fixed effects to determine the minimal adequate model based on the lowest value for Akaike's information criterion. If a significant effect was detected, the model coefficient was used to determine the direction of the difference.

Generalised linear mixed effects models were used to analyse the other traits with Poisson (total eggs and sperm transfer), gamma (day of peak egg production, eggs per day, TAC and MDA) or binomial distributions (propensity of male calling or mating) with selection regime included as a fixed effect and replicate population included as a random effect. Other explanatory variables in each model are detailed below. Models were built using the function "glmer" from the "lme4" package (Bates et al., 2015). Where appropriate, we corrected for over-dispersion by adding an observation level random effect (Harrison, 2014). If a significant effect was detected, *post hoc* pairwise comparisons tests of the estimated marginal means were performed using the function "emmeans" (Russell, 2020).

Because zero values prevent use of the gamma family, if the number of eggs laid per day for a female was zero, this value was replaced with the smallest value of the dataset for this trait and divided by ten. To determine the effect of selection and age on the total number of sperm transferred to spermathecae, the number of sperm transferred per day, and the propensity of males to call and mate, selection regime and age, as well as their interaction were included as fixed effects.

If the value of the detected TAC for an individual was zero, it was replaced with the smallest TAC value in the dataset and divided by ten. For TAC and lipid peroxidation, the fixed effects added to the model were selection regime, age, and sex. Body mass was included as a covariate to account for variation in fly size. Interactions between all terms were included to the model, except for the covariate and random effect. If a significant effect of the covariate was detected, the coefficient and the significance of the slope were inspected. For graphical representation of TAC and lipid peroxidation, the means predicted by the models were plotted.

3. Results

3.1 Survival

Selection regime was the only variable retained in the minimal adequate model ($\chi 2 = 16.64$, df = 1, $p < 0.001$), with US flies having a significantly higher mortality risk (coefficient = 1.13, $p < 0.001$) than CT flies (Fig. 1). On average, US flies lived for 147.2 ± 6.85 days and CT flies for 210.0 ± 7.4 days (mean ± SE). The longer lifespan of CT was the consequence of an extended period when no mortality was recorded in either sex (females: 61 days; males: 73 days).

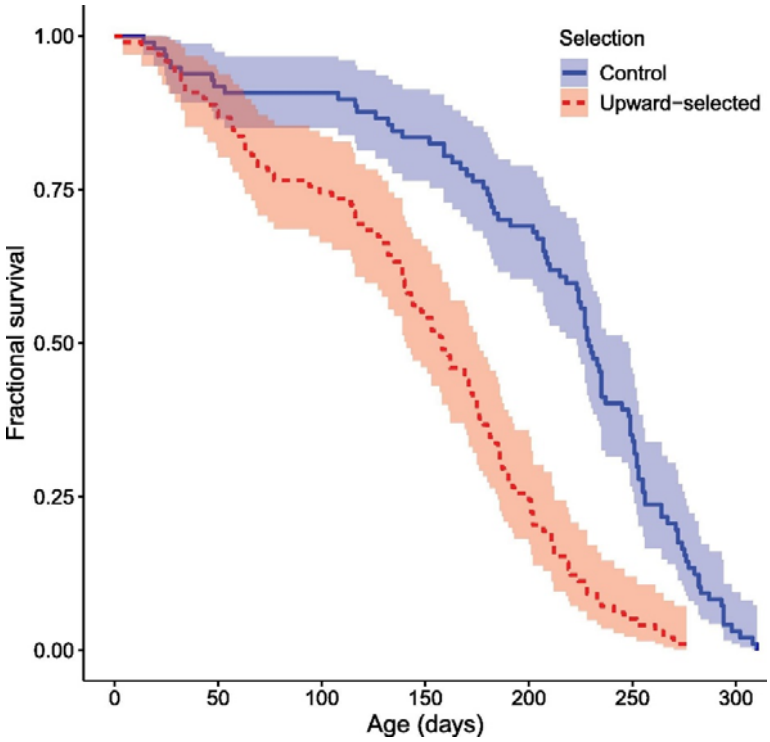


Figure 1. Survival curves of *C. cosyra* issued from control and upward-selected lines. Shading indicates 95 % interval. Upward-selection was performed by allowing females to oviposit only at 25 days after adult emergence, whereas eggs were collected from controls at 15 days. Each curve represents the sum of 50 virgin females and males kept individually in a container.

3.2 Female reproductive effort

We observed fewer eggs being laid by US females than CT females (Fig. 2a), but this difference was not statistically significant ($\chi 2 = 3.35$, df = 1, $p = 0.067$). The day of peak egg production was significantly later for US females ($\chi 2 = 8.46$, df = 1, $p = 0.004$) (Fig. 2b). The selection regime had a significant effect on the number of eggs laid per day ($\chi 2 = 8.11$, df = 1, $p = 0.004$), with females from US lines producing on average 0.42 ± 0.07 eggs per day in comparison with 0.96 ± 0.18 for CT line females.

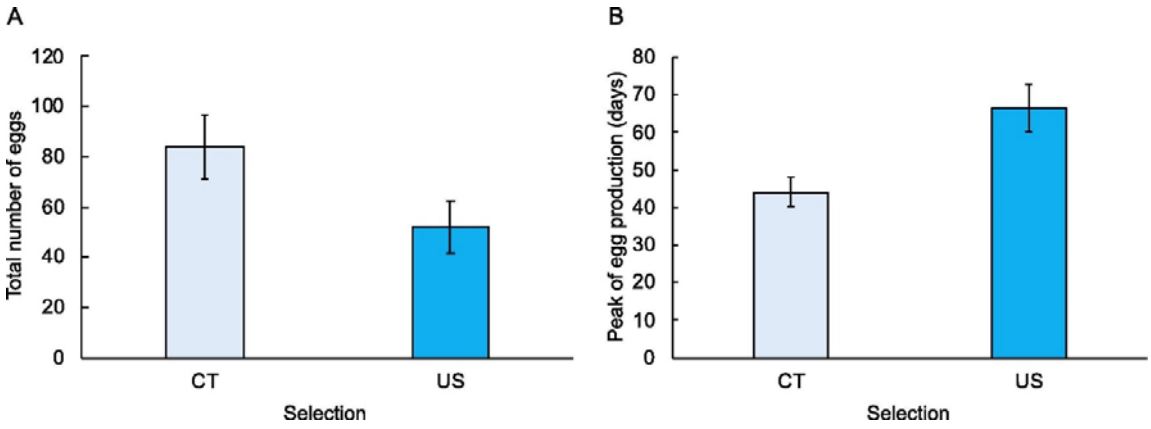


Figure 2. Reproductive effort of females from control (CT) and upward-selected (US) lines. The values displayed are the average total number of eggs (a) and average day of peak of egg production (b) of virgin females individually kept in a container. Each bar for panel a) represents 50 individuals, bars for panel b) represent 46 (CT) and 40 (US) individuals, respectively. Error bars indicate the standard error of the mean.

3.3 Male reproductive effort

All dissected females had sperm stored in their spermathecae. Both, selection regime and male age affected the number of sperm transferred, but there was no significant interaction between them ($\chi2$ = 0.76, df = 2, p = 0.683). Over the three tested ages, males from the CT lines consistently transferred more sperm (coefficient = 0.589, $p < 0.001$) than those from the US lines ($\chi2 = 4.72$, df = 1, p = 0.029) (Fig. 3). In both lines, sperm transfer changed with age ($\chi2 = 18.28$, df = 2, $p < 0.001$) and was lowest when males were 5 days old (5 vs 15 days: coefficient = - 0.581, p = 0.003; 5 vs 25 days: coefficient = - 0.847, $p < 0.001$), but there was no difference between sperm transferred at 15 and 25 days (coefficient = - 0.266, p = 0.301) (Fig. 3). The proportion of males calling or mating were not affected by the selection regime (Calling: $\chi2 = 1.16$, df = 1, p = 0.282; Mating: $\chi2 = 0.037$, df = 1 , p = 0.847) or male age (Calling: $\chi2 = 0.67$, df = 2, p = 0.716; Mating: $\chi2 = 3.44$, df = 2, p = 0.179). In addition, there was no significant interaction between the selection regime and male age (Calling: $\chi2 = 0.52$, df = 2, p = 0.769; Mating: $\chi2 = 4.22$, df = 2, p = 0.112). Across selection regimes and ages, 52% of the males exhibited courtship behaviour and 68% mated successfully.

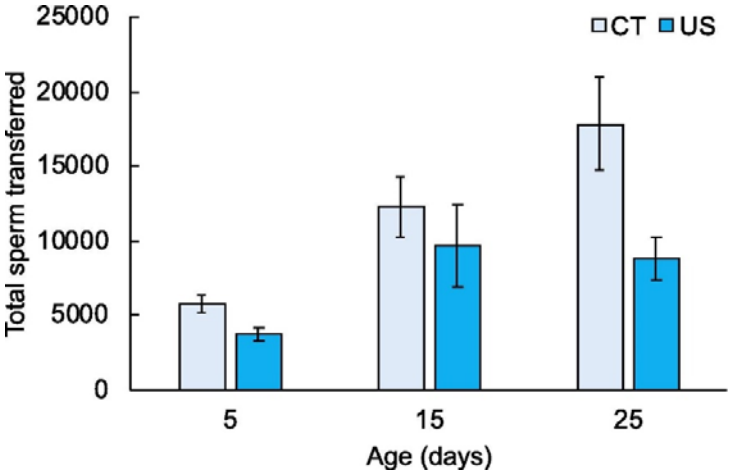


Figure 3. Reproductive effort of males from control (CT) and upward-selected (US) lines at three different ages. Bars show the average total number of sperm transferred by males to females' spermathecae. At ages 5, 15 and 25, males were individually paired with a virgin female of 10 to 20 days of age from an unselected laboratory culture. Each bar represents 13 to 23 individuals. Error bars indicate the standard error of the mean.

Table 1. Analysis of total antioxidant capacity (TAC) and lipid peroxidation using a generalised linear mixed model with gamma distribution.

Explanatory variables	Response traits	$\chi 2$	df	p
Selection	TAC	47.85	1	**< 0.001**
	Lipid peroxidation	35.66	1	**< 0.001**
Age	TAC	164.18	2	**< 0.001**
	Lipid peroxidation	49.23	2	**< 0.001**
Sex	TAC	0.25	1	0.615
	Lipid peroxidation	0.18	1	0.674
Body mass	TAC	0.01	1	0.905
	Lipid peroxidation	7.43	1	**0.006**
Selection x Age	TAC	89.78	2	**< 0.001**
	Lipid peroxidation	21.18	2	**< 0.001**
Selection x Sex	TAC	0.49	1	0.484
	Lipid peroxidation	0.17	1	0.677
Age x Sex	TAC	65.59	2	**< 0.001**
	Lipid peroxidation	11.39	2	**0.003**
Selection x Age x Sex	TAC	48.65	2	**< 0.001**
	Lipid peroxidation	7.37	2	**0.025**

3.4 Total antioxidant capacity and lipid peroxidation

There was a significant interaction between selection, age, and sex affecting TAC (Table 1). In newly emerged flies, US females and males had TAC that was 6-fold lower than CT flies of the same age, but by 50 days it was reversed with TAC being approximately double that of US flies (Table S1; Fig. 4).

At 25 days of age, US males had lower TAC than CT and US females. TAC of US females (Fig. 4) was similar at all ages (Table S1), while the TAC of the US males was the lowest at 25 days of age (Table S1; Fig. 4). In contrast, there was a significant decrease of TAC with age in both sexes of the CT lines (Table S1; Fig. 4).

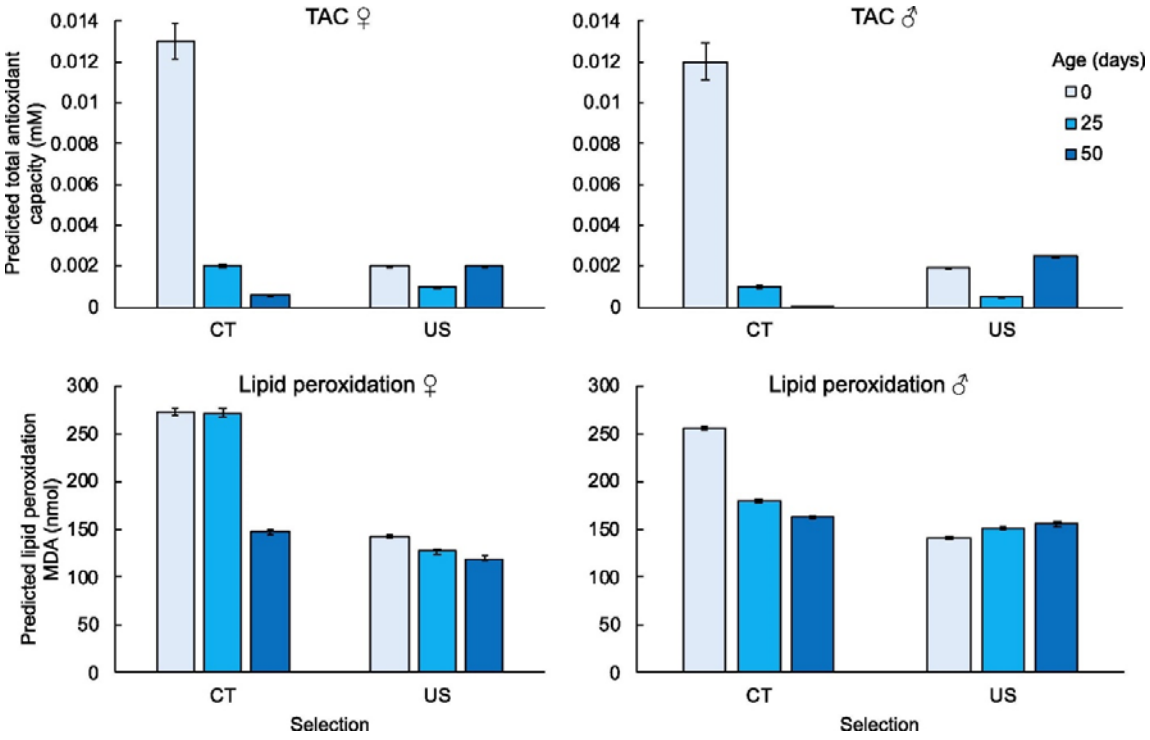


Figure 4. Total antioxidant capacity (TAC) and lipid peroxidation in females and males from control (CT) and upward-selected (US) lines at three different ages. Values displayed were predicted from the model used for statistical analyses where body mass was included as a covariate. Each bar represents 20 virgin individuals. Error bars indicate the standard error of the predicted means.

Lipid peroxidation was affected by an interaction between selection regime, age, and sex (Table 1), and there was a significant positive effect of body mass (Table 1; coefficient = 0.107, p = 0.006). Overall, oxidative damage was lower in US flies (Table S2; Fig. 4). Females from the US lines had significantly lower lipid peroxidation than those of the CT lines at 0 and 25 days, but not at 50 days where it was similar (Fig. 4). In males, oxidative damage to lipids was lower in US flies only on the day of emergence (Table S2) (Fig. 4). The changes in age-specific level of lipid peroxidation differed between selection regimes. In the US lines, oxidative damage to lipids remained constant, whereas in the CT lines damage decreased with age (Table S2; Fig. 4).

4. Discussion

Selecting upwards on age of female reproduction produced individuals with reduced survival and reproductive capacity, but a later peak in egg production. This latter observation is important as it reveals that selection regime was successful in altering how females schedule their reproductive effort. Fertility was negatively impacted in males, as they transferred less sperm to female's spermathecae. Male courtship and mating success, as well as female lifetime fecundity were the only traits that did not respond to the selection regime. In addition, lifespan was positively correlated with the number of sperm transferred and negatively correlated with the peak of egg production; showing that the value of these traits are a good indicator to determine if a fly belongs to the upward-selected or control lines (Appendix A, Fig. S1).

These results are surprising and contrast with existing literature on how selection on female reproductive scheduling should impact life-histories, where selection upwards on age of female reproduction extends lifespan. Here, we found the opposite; upwards selection on age of reproduction was associated with reduced survival. However, it is worth noting that the unselected control flies exhibited sixty to seventy days with zero mortality in either sex. This may be the main factor driving the survivorship difference between control and upward-selected flies. This pattern could reflect the study sample size, which is too small to allow accurate parameterisation of a survival curve using parametric survival models (Promislow et al., 1999; Shouman and Witten, 1995). However, forty individuals is sufficient to provide reasonable results using Cox models (the approach employed here) (Johnson et al., 1982). Some authors have suggested that it may be worth abandoning analyses of mortality rates and instead use summary statistics such as life-expectancy at birth, which should not depend heavily on sample size (Promislow et al., 1999). Furthermore, extended periods of high survivorship are not unusual and have been observed in various species (Cypser et al., 2006; Krainacker et al., 1987; Norry et al., 2006; Roets et al., 2018) as well as in earlier generations of our selected lines (unpublished data). For example, female Ceratitis capitata reared on plum experience negligible mortality for twenty days, which is approximately 40 % of the total lifetime of these flies (Krainacker et al., 1987). Therefore, while the period if zero mortality we observed is likely in part a statistical artefact, it seems reasonable to assume that mortality early in the life of our control group is low and that sizeable difference in longevity between control (147 days) and selected (210 days) flies is meaningful.

The reduced survival and sperm transfer in the upward-selected lines might reflect that in various species, including insects, older parental ages have less fit offspring due to deterioration of the parental germline with age (Monaghan and Metcalfe, 2019). Accordingly, selecting for older parents might result in reduced offspring fitness that accumulates over multiple generations. While we might expect

selection to counteract these effects (a parent that sires high-quality offspring late-in-life would have an important fitness advantage), this would only happen if there was a genotype in the starting population that could produce high quality offspring late in life. In addition, control flies experienced a no-mortality period of fifty days, which could be seen as the main factor driving the survivorship difference between control and upward-selected flies. Although we cannot say what caused these unusual results, selection did alter life-histories in such a way that we can make and test predictions about correlated changes in antioxidant protection and oxidative damage.

Selection lines varied in oxidative damage to lipids and TAC. Crucially, upward-selected flies began their adult life with lower total antioxidant protection, had the best protection at the oldest age, and suffered less from oxidative damage at all ages. Moreover, our lines did not show any age-associated changes in either trait. Low antioxidant protection and oxidative damage in upward-selected flies suggest that ROS production was decreased in these flies in comparison with control individuals. With upward-selected flies performing relatively poorly in comparison with control flies, it would be worthwhile studying bioenergetics efficiency of mitochondria as a potential mechanism for lower oxidative damage in upward-selected *C. cosyra* (López-Lluch et al., 2006). Control flies had higher lipid peroxidation and much better total antioxidant protection at emergence, but experienced age-associated declines in both traits over age, which occurred at a younger age in males than in females for oxidative damage. These observations contrast with studies in *D. melanogaster*, where selection for long lifespan (Arking et al., 2000) or late-life reproduction (Harshman and Haberer, 2000) led to extended lifespan, and increased antioxidant protection or oxidative stress resistance respectively. These results contrast with the FRTA because first, the selection regime associated with the highest survival experienced greater oxidative damage, and second, oxidative damage did not increase with age in either sex but actively declined over age in males. Our results are in line with the growing literature that undermine the FRTA and show no direct relationship between lifespan and oxidative damage (Speakman and Selman, 2011).

Higher total and daily fecundity, as well as earlier reproductive investment in males and females from control lines were associated with higher oxidative damage and greater antioxidant protection. For example, greater antioxidant protection is observed in response to conditions inducing higher ROS production (Towarnicki et al., 2020). This may suggest that here, reproduction is associated with greater ROS production, and in turn oxidative damage, and supports the suggestion that ROS production can be a cost of reproduction. However, there was no trade-off between survival and reproduction suggesting that while oxidative damage may be elevated following reproduction, it does not have costly survival impacts. Similar results were reported in *Teleogryllus commodus*, where reproduction was associated with greater oxidative damage in males, but not reduced lifespan (Archer et al., 2015).

In conclusion, selecting upwards on age of female reproduction in *C. cosyra* led to a decreased life expectancy, lower reproductive capacity in males and delayed reproductive investment in females. This was associated with lower oxidative damage to lipids and total antioxidant protection. Our results do not support the predictions of the FRTA as the highest survival was observed with flies sustaining the most oxidative damage but also experiencing the most important drop in total antioxidant protection as they aged. In addition, our observations support the idea that reproduction may be a source of oxidative damage but that this higher damage did not result in reduced lifespan.

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CRediT author statement

KM: Formal analysis, Data Curation, Writing – Original Draft, Validation, Visualization. **PDR:** Investigation, Data Curation, Writing Review & Editing. **CO:** Investigation, Data Curation, Writing – Review & Editing. **JDB:** Methodology, Resources, Writing – Review & Editing, Supervision. **CRA:** Conceptualization, Methodology, Formal analysis, Writing – Review & Editing, Validation, Supervision, Funding acquisition. **CWW:** Conceptualization, Methodology, Validation, Resources, Writing – Review & Editing, Supervision, Project Administration, Funding acquisition.

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Declaration of interests

The authors have no competing interests to declare.

Data availability

Data are available from the University of Pretoria online repository: https://doi.org/10.25403/UPresearchdata.12480569.v1

References

Agarwal, R., Chase, S.D., 2002. Rapid, fluorimetric–liquid chromatographic determination of malondialdehyde in biological samples. J. Chromatogr B 775, 121-126.

Archer, C.R., Hempenstall, S., Royle, N., Selman, C., Willis, S., Rapkin, J., Blount, J.D., Hunt, J., 2015. Testing the effects of DL-alpha-tocopherol supplementation on oxidative damage, total antioxidant protection and the sex-specific responses of reproductive effort and lifespan to dietary manipulation in Australian field crickets (*Teleogryllus commodus*). Antioxidants 4, 768-792.

Archer, C.R., Sakaluk, S.K., Selman, C., Royle, N., Hunt, J., 2013. Oxidative stress and the evolution of sex differences in life span and ageing in the decorated cricket, *Gryllodes sigillatus*. Evolution 67, 620-634.

Arking, R., Burde, V., Graves, K., Hari, R., Feldman, E., Zeevi, A., Soliman, S., Saraiya, A., Buck, S., Vettraino, J., Sathrasala, K., Wehr, N., Levine, R.L., 2000. Forward and reverse selection for longevity in *Drosophila* is characterized by alteration of antioxidant gene expression and oxidative damage patterns☆. Exp Gerontol 35, 167-185.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J Stat Softw 67, 48.

Blount, J.D., Vitikainen, E.I.K., Stott, I., Cant, M.A., 2016. Oxidative shielding and the cost of reproduction. Biol Rev 91, 483-497.

Carey, J.R., 2011. Biodemography of the Mediterranean fruit fly: Aging, longevity and adaptation in the wild. Exp. Gerontol. 46, 404-411.

Carey, J.R., Molleman, F., 2010. Reproductive aging in tephritid fruit flies. Ann N Y Acad Sci 1204, 139-148.

Carey, J.R., Papadopoulos, N.T., Müller, H.-G., Katsoyannos, B.I., Kouloussis, N.A., Wang, J.-L., Wachter, K., Yu, W., Liedo, P., 2008. Age structure changes and extraordinary lifespan in wild medfly populations. Aging Cell 7, 426-437.

Chen, E.H., Wei, D., Wei, D.D., Yuan, J.J., 2013. The effect of dietary restriction on longevity, fecundity, and antioxidant responses in the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). J. Insect Physiol. 59, 1008-1016.

Clancy, D., Birdsall, J., 2013. Flies, worms and the free radical theory of ageing. Ageing Res. Rev. 12, 404-412.

Costantini, D., 2008. Oxidative stress in ecology and evolution: lessons from avian studies. Ecol. Lett. 11, 1238-1251.

Cypser, J.R., Tedesco, P., Johnson, T.E., 2006. Hormesis and aging in *Caenorhabditis elegans*. Exp. Gerontol. 41, 935-939.

Dowling, D.K., Simmons, L., 2009. Reactive oxygen species as universal constraints in life-history evolution. ProC.R. Soc. Biol. Sci. Ser. B 276, 1737-1745.

Fanson, B.G., Fanson, K.V., Taylor, P.W., 2012. Cost of reproduction in the Queensland fruit fly: Y-model versus lethal protein hypothesis. Proc Biol Sci 279, 4893-4900.

Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298-300.

Harrison, X.A., 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. PeerJ 2, e616-e616.

Harshman, L.G., Haberer, B.A., 2000. Oxidative stress resistance: a robust correlated response to selection in extended longevity lines of *Drosophila melanogaster*? J Gerontol 55, B415-B417.

Hekimi, S., Lapointe, J., Wen, Y., 2011. Taking a "good" look at the free radicals in the aging process. Trends Cell Biol. 21, 569-576.

Helfenstein, F., Losdat, S., Møller, A.P., Blount, J.D., Richner, H., 2010. Sperm of colourful males are better protected against oxidative stress. Ecol. Lett. 13, 213-222.

Johnson, M.E., Tolley, H.D., Bryson, M.C., Goldman, A.S., 1982. Covariate analysis of survival data: a small-sample study of Cox's model. Biometrics 38, 685-698.

Kirkwood, T.B.L., Kowald, A., 2012. The free-radical theory of ageing - older, wiser and still alive: modelling positional effects of the primary targets of ROS reveals new support. Bioessays 34, 692-700.

Krainacker, D.A., Carey, J.R., Vargas, R.I., 1987. Effect of larval host on life history traits of the mediterranean fruit fly, *Ceratitis capitata*. Oecologia 73, 583-590.

López-Lluch, G., Hunt, N., Jones, B., Zhu, M., Jamieson, H., Hilmer, S., Cascajo, M.V., Allard, J., Ingram, D.K., Navas, P., de Cabo, R., 2006. Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. Proc Natl Acad Sci U S A 103, 1768-1773.

Malod, K., Archer, C.R., Hunt, J., Nicolson, S.W., Weldon, C.W., 2017. Effects of macronutrient intake on the lifespan and fecundity of the marula fruit fly, *Ceratitis cosyra* (Tephritidae): Extreme lifespan in a host specialist. Ecol. Evol. 7, 9808-9817.

Manrakhan, A., Lux, S.A., 2006. Contribution of natural food sources to reproductive behaviour, fecundity and longevity of *Ceratitis cosyra*, *C. fasciventris* and *C. capitata* (Diptera: Tephritidae). Bull. Entomol. Res. 96, 259-268.

Metcalfe, N.B., Alonso-Alvarez, C., 2010. Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death. Funct. Ecol. 24, 984-996.

Monaghan, P., Metcalfe, N.B., 2019. The deteriorating soma and the indispensable germline: gamete senescence and offspring fitness. Proc Biol Sci 286, 20192187.

Monaghan, P., Metcalfe, N.B., Torres, R., 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol. Lett. 12, 75-92.

Mougeot, F., Martínez-Padilla, J., Webster, L.M.I., Blount, J.D., Pérez-Rodríguez, L., Piertney, S.B., 2009. Honest sexual signalling mediated by parasite and testosterone effects on oxidative balance. Proc Biol Sci 276, 1093-1100.

Norry, F.M., Sambucetti, P., Scannapieco, A.C., Loeschcke, V., 2006. Altitudinal patterns for longevity, fecundity and senescence in *Drosophila buzzatii*. Genetica 128, 81-93.

Pérez, V.I., Bokov, A., Van Remmen, H., Mele, J., Ran, Q., Ikeno, Y., Richardson, A., 2009. Is the oxidative stress theory of aging dead? Biochim. Biophys. Acta. 1790, 1005-1014.

Promislow, D.E.L., Tatar, M., Pletcher, S., Carey, J.R., 1999. Below-threshold mortality: implications for studies in evolution, ecology and demography. J Evol Biol 12, 314-328.

Roets, P.D., Bosua, H., Archer, C.R., Weldon, C.W., 2018. Life history and demographic traits of the marula fruit fly, *Ceratitis cosyra* (Walker) (Diptera: Tephritidae): potential consequences of host specialisation. Physiol. Entomol. 43, 259-267.

Rose, M.R., 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution 38, 1004-1010.

Russell, L., 2020. emmeans: estimated marginal means, aka least-squares means. R package version 1.4.6.

Selman, C., Blount, J.D., Nussey, D.H., Speakman, J.R., 2012. Oxidative damage, ageing, and life-history evolution: where now? Trends Ecol. Evol. 27, 570-577.

Shouman, R., Witten, M., 1995. Survival estimates and sample size: what can we conclude? J Gerontol 50A, B177-B185.

Speakman, J.R., Blount, J.D., Bronikowski, A.M., Buffenstein, R., Isaksson, C., Kirkwood, T.B.L., Monaghan, P., Ozanne, S.E., Beaulieu, M., Briga, M., Carr, S.K., Christensen, L.L., Cocheme, H.M., Cram, D.L., Dantzer, B., Harper, J.M., Jurk, D., King, A., Noguera, J.C., Salin, K., Sild, E., Simons, M.J.P., Smith, S., Stier, A., Tobler, M., Vitikainen, E., Peaker, M., Selman, C., 2015. Oxidative stress and life histories: unresolved issues and current needs. Ecol. Evol. 5, 5745-5757.

Speakman, J.R., Selman, C., 2011. The free-radical damage theory: Accumulating evidence against a simple link of oxidative stress to ageing in lifespan. Bioessays 33, 255-259.

Stuart, J.A., Maddalena, L.A., Merilovich, M., Robb, E.L., 2014. A midlife crisis for the mitochondrial free radical theory of aging. Longev. Healthspan. 3, 4.

Taylor, P.W., Kaspi, R., Yuval, B., 2000. Copula duration and sperm storage in Mediterranean fruit flies from a wild population. Physiol. Entomol. 25, 94-99.

Therneau, T.M., 2015. A Package for Survival Analysis in S. version 2.38.

Towarnicki, S.G., Kok, L.M., Ballard, J.W.O., 2020. Yin and Yang of mitochondrial ROS in *Drosophila*. J Insect Physiol 122, 104022.

Vágási, C.I., Vincze, O., Pătraș, L., Osváth, G., Pénzes, J., Haussmann, M.F., Barta, Z., Pap, P.L., 2019. Longevity and life history coevolve with oxidative stress in birds. Funct. Ecol. 33, 152-161.