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Sex-specific effects of natural and sexual selection on the evolution of life span and ageing in *Drosophila simulans*

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Summary

1. Variation in the strength of age-dependent natural selection shapes differences in ageing rates across species and populations. Likewise, sexual selection can promote divergent patterns of senescence across the sexes. However, the effects of these processes on the evolution of ageing have largely been considered independently, and interactions between them are poorly understood.

2. We use experimental evolution to investigate how natural and sexual selection affect life span and ageing in *Drosophila simulans*.

3. Replicate populations were evolved under lifetime monogamy (relaxed sexual selection) or lifetime polyandry (elevated sexual selection) and at one of two temperatures, 25 °C (relaxed natural selection) or 27 °C (enhanced natural selection), in a fully factorial design. We measured longevity in 150 individually housed flies taken from each of three replicate populations per selection regime.

4. We found that natural and sexual selection affected the evolution of life span via sex-specific effects on different ageing parameters (ageing rate vs. baseline mortality): natural selection reduced the rate of ageing in both sexes but increased male baseline mortality, while sexual selection elevated baseline mortality in both sexes but particularly in males.

5. This means that sexual and natural selection interacted to reduce male life span but acted on female life span by independently affecting particular ageing parameters. Sex-specific effects of sexual and natural selection may help explain the diverse patterns of ageing seen in nature but complicate predictions about how ageing and life span evolve across the sexes

Key-words: ageing rates, evolutionary response, experimental evolution, longevity, senescence, sexual conflict

Introduction

The strength of natural selection declines over an individual's lifetime (Hamilton 1966). As a result, selection to remove late-acting deleterious alleles from a population is weak. Thus, late-acting, harmful mutations accumulate in the gene pool (Mutation Accumulation – Medawar 1952) as do alleles that improve early fitness but have negative pleiotropic effects expressed later in life (Antagonistic Pleiotropy – Williams 1957). This age-dependent decline in natural selection may also favour intense investment in fitness early in life, rather than in long-term somatic maintenance (Disposable Soma – Kirkwood 1977). Evolutionary theory suggests that the accumulation of these costly, late-acting alleles and/or reduced investment in the soma over time causes ageing.

Evolutionary theories of ageing predict that the evolution of life span and ageing rates depend critically on

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variation in the strength of natural selection over time (Hughes 2010). Increasing the strength of natural selection late in life should promote the evolution of longer lives and slower ageing. In agreement with this prediction, *Drosophila melanogaster* selected for high late-life reproductive effort evolved significantly longer life spans (Partridge & Fowler 1992). Conversely, weakened natural selection over time, for example by increasing the risk of mortality in old animals, may favour the evolution of shorter lives and more rapid ageing (Stearns *et al.* 2000). This prediction becomes more complicated however, if high environmental mortality also selects for increased physiological condition and somatic maintenance, in which case the evolution of longer lives and slower ageing may be favoured (Williams & Day 2003). This appears to be the case in natural populations of guppies subjected to high rates of predation (Reznick *et al.* 2004) and laboratory populations of nematodes exposed to heat stress (Chen & Maklakov 2012), which evolve longer life spans despite higher mortality rates. In summary, if the strength of natural selection is elevated, leading to age- or condition-dependent increases in mortality risk, a diverse array of outcomes are possible with respect to the evolution of life span and ageing rates (Abrams 1993; Williams & Day 2003; Caswell 2007).

Sexual selection also plays an important, albeit less understood, role in the evolution of life span and ageing (Partridge & Barton 1996; Promislow 2003; Graves 2007; Bonduriansky *et al.* 2008). Sexual selection may drive sex differences in ageing and life span by favouring different reproductive schedules in males and females (Bonduriansky *et al.* 2008). For example, if sexual selection promotes high reproductive investment early in life in males relative to females (Kokko 1997, 1998), male life span is likely to decrease and their rates of ageing will increase (Promislow 1992). Alternatively, sexual selection could promote the evolution of longer male life span if reproductive effort increases with age (e.g. Botero *et al.* 2009), or if female choice selects for genes that have positive, pleiotropic effects on life span (Lailvaux & Irschick 2006). Crucially, if sexual selection promotes different life-history strategies across the sexes, it could cause divergence in the genetic interests of the sexes and sexual conflict could arise over ageing and life span (Maklakov & Lummaa 2013). Sexual conflict could generate antagonistic selection at a single locus expressed in both sexes (intralocus conflict) or across different loci (interlocus conflict) (Arnqvist & Rowe 2005), but in either case, life span could be reduced in one or both sexes (Bonduriansky *et al.* 2008). This means that although as a general rule elevating the intensity of sexual selection is expected to reduce selection for a long life in males relative to females, this is not the only possible outcome and it is hard to predict exactly how sexual selection will shape mortality trajectories in a population (Bonduriansky *et al.* 2008).

While we largely understand how natural and sexual selection can affect life span in the sexes, it is more challenging to predict how these processes jointly affect ageing. Changes in life span may occur due to variation in baseline mortality (i.e. population frailty) or ageing rate (Pletcher 1999), and we do not know whether natural and sexual selection affect one or both of these parameters independently or by acting together (Bonduriansky *et al.* 2008). Moreover, if these processes act together, do they influence the evolution of ageing parameters in the same or different directions and is this consistent across the sexes (Bonduriansky *et al.* 2008)? Generally, sexual selection is thought to oppose natural selection and favour traits that reduce population mean fitness (Kokko & Brooks 2003). However, it may also facilitate natural selection by enhancing condition and accelerating adaptation (Lorch *et al.* 2003). At present, it is unclear which of these scenarios is more applicable and hence, the joint impact of sexual and natural selection on ageing remains contentious (Bonduriansky *et al.* 2008).

Here, we examine how natural and sexual selection affect the evolution of life span and ageing parameters in male and female *Drosophila simulans* (Fig. 1). Using experimental evolution, we manipulated the intensity of natural selection (optimal temperature vs. mild thermal stress) and sexual selection (polyandry vs. monogamy) in replicate populations using a fully factorial design. This stressful temperature necessitates physiological changes to resist desiccation (Sharma, Hunt & Hosken 2012) and somatic damage (Landis, Shen & Tower 2012). After 45 generations of evolution, we assessed the life span of male and female flies in each of these populations to determine the effects of natural selection, sexual selection and their interaction on the evolution of life span and ageing in the sexes.

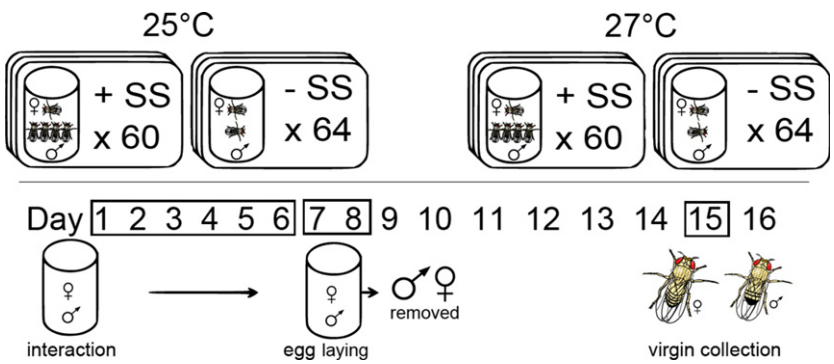

Fig. 1. A schematic of the protocol used to maintain selection lines used in our experiment. Flies of both sexes were housed for 6 days (1–6) in interaction vials before being transferred to egg-laying vials for a further 2 days (days 7 and 8). Adults were then discarded while eggs from egg-laying vials were allowed to develop. Virgin flies were collected from these vials on day 15 and used to start subsequent generations.

Materials and methods

DERIVATION AND MAINTENANCE OF FLY STOCKS

We used flies originating from 20 iso-female lines, collected from a wild population at Tuncurry, Eastern Australia in 2004. This stock population was reared on '*Drosophila* quick mix medium' (BLADES BIOLOGICAL, Kent, UK) and maintained at 25 °C under a 12:12 h light:dark cycle. Stock animals were kept in large population cages of 800–1000 flies, allowing overlapping generations and free mate choice for approximately 5 years prior to this study. Past work shows that these flies have substantial genetic variation for all phenotypic traits investigated to date (Taylor, Wedell & Hosken 2007; Hosken *et al*. 2008; Wright, Tregenza & Hosken 2008; Okada *et al*. 2011).

EXPERIMENTAL MANIPULATION OF NATURAL AND SEXUAL SELECTION

Replicate experimental populations of flies were established under relaxed (−) or elevated (+) natural selection (NS) and sexual selection (SS) in a fully factorial design to give four treatment combinations: +NS/+SS, −NS/+SS, +NS/−SS and −NS/−SS). A total of three replicate populations were established for each of these treatment combinations (n = 12 populations). The relaxed natural selection treatment corresponds to the standard rearing temperature of 25 °C (−NS), while for the elevated natural selection treatment the temperature was raised to 27 °C (+NS). This high temperature is very close to the *D. simulans* male sterility threshold (Chakir *et al*. 2002) and is also known to increase the risk of desiccation in both sexes (Sharma, Hunt & Hosken 2012). Therefore, this represents a stressful environment, which elevates natural selection relative to populations maintained at 25 °C. To relax the intensity of sexual selection, females were housed with only one male in monogamous pairs to remove the possibility of female mate choice (−SS). Conversely, to elevate the intensity of sexual selection, a single female was housed with four males (polyandry, +SS). A total of 60 females per population were used to propagate the elevated sexual selection treatment and 64 females for the relaxed sexual selection treatment. These different numbers of female were used to standardize effective population size (N_e) across treatments (as discussed in Sharma, Hunt & Hosken 2012).

Selection lines were maintained using the protocol outlined in Fig. 1. Replicate populations for each selection line were split between three incubators, such that there was a single population per selection regime in each incubator. These flies were housed for 6 days in 'interaction vials' and then transferred to 'laying vials' for 2 days. To reduce differential rates of development and mortality due to larval competition, food was always provided in excess (40 mL per vial). Adults were then discarded and virgin offspring collected and pooled by sex for each replicate population of each selection line. Individuals were selected at random from these pools to propagate the next generation (as discussed in Sharma, Hunt & Hosken 2012).

MEASURING LIFE SPAN AND SURVIVAL

After 45 generations of evolution, a total of 150 virgin flies of each sex were collected at random on their day of emergence from each of three replicate populations per selection regime. These flies were housed in individual vials (100 mL), provided with an excess of food (*Drosophila* quick mix medium) and maintained under a 12:12 h light:dark cycle at 26 °C. This temperature is intermediate between the two temperatures used in our natural selection regimes. This single assay temperature was chosen for logistical reasons and to ensure that no single population of flies were assayed at their ancestral population temperature, which has been shown to provide a survival advantage (e.g. Partridge *et al*. 1995). Survival of these flies was monitored daily when extra water was added to the food if it became too dry and flies transferred to a new feeding tube if any mould was observed. Flies were moved to a new vial of food weekly. In total, we measured the life span of 1800 male and 1800 female flies, although final sample sizes (a total of 3580 flies) vary slightly due to escape of some flies.

DATA ANALYSIS

We used a maximum likelihood approach implemented in the 'bbmle' (Bolker 2009) package of R (R core development team 2013) to compare five statistical different models that describe the demographic rate of change in mortality with age: Gompertz, Gompertz–Makeham, Logistic, Logistic–Makeham and Weibull. We compared these models separately for each sex of each replicate in our experiment. The best fitting model was taken as the one with the lowest Akaike Information Criterion (AIC). In the majority of cases (17 out of 24), the Weibull model provided the best fit to the data. When this was not the best fitting model, in all but one case (one +NS/+SS replicate for males), it was the second best fitting model. The equation describing the hazard function ($h(x)$) for the Weibull model is $h(x) = \lambda^{\beta}\beta x^{\beta-1}$ where x is time (or age), β is the shape parameter and λ is the scale parameter (Pletcher, Khazaeli & Curtsinger 2000). Biologically, β is interpreted as the 'rate of ageing', while $1/\lambda$ is interpreted as the 'baseline mortality' or 'frailty' of the population (Pletcher, Khazaeli & Curtsinger 2000). These are the definitions used in providing estimates of β and λ in our analysis.

To determine how life span responded to each form of selection in the sexes, we analysed our life span data using a two-factor ANOVA including natural selection, sexual selection and sex as explanatory variables. To determine how ageing parameters responded to each form of selection in the sexes, we analysed these two age-specific mortality parameters using MANOVA including natural selection, sexual selection and sex as explanatory variables. We adopted a MANOVA approach because age-specific mortality parameters (such as β and λ) are known to be phenotypically and genetically correlated, particularly in *Drosophila* (e.g. Pletcher 1999; Miyo & Charlesworth 2004). In both our ANOVA and MANOVA models, when there were significant interaction effects between natural and/or sexual selection and sex, we carried out sex-specific analyses to determine the underlying reason for this effect.

Results

LIFE SPAN

Sex interacted with both sexual and natural selection to affect the evolution of life span (Fig. 2, Table 1). To better understand these interactions, life span was then analysed separately by sex. Females evolving under elevated sexual selection died earlier than females evolving under relaxed sexual selection, but natural selection did not significantly influence female life span, either independently or via an interaction with sexual selection (Fig. 2b, Table 1).

In males, enhanced sexual and natural selection both reduced life span: males evolving under high temperatures (+NS) died almost 4 days before males evolving at optimal temperatures (−NS), while males evolving under enhanced sexual selection died 7 days before males under relaxed sexual selection. Additionally, there was a significant

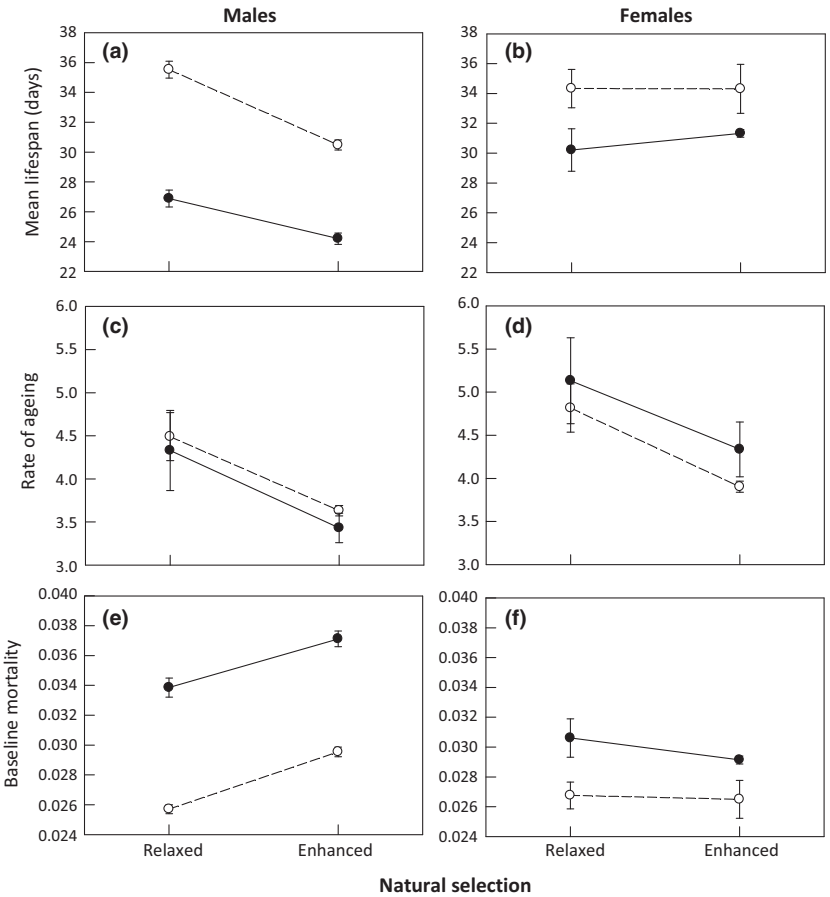

Fig. 2. The effect of natural and sexual selection on life span and ageing parameters in male and female *Drosophila simulans*. In each instance, open symbols with dashed lines represent populations evolving under relaxed sexual selection, whereas closed symbols with solid lines represent populations evolving under enhanced sexual selection. (a, c and e) Represent mean (±SE) life span, rate of ageing (β) and baseline mortality (1/λ) in males, while (b, d and f) represent these same measures in females.

Table 1. The effects of elevated or relaxed natural (NS) and sexual selection (SS) on life span in male and female *Drosophila simulans*. We started with an overall ANOVA model examining the effects of NS, SS and sex, as well as their interactions, on the evolution of life span. As NS and SS showed significant interactions with sex, we followed this overall model with a series of models examining the effects of NS, SS and their interaction within the sexes

Source	$F_{1,16}$	P
NS (A)	6·010	0·026
SS (B)	66·086	0·0001
Sex (C)	23·238	0·0001
A × B	1·671	0·214
A × C	10·633	0·005
B × C	8·306	0·011
A × B × C	0·202	0·659
	$F_{1,8}$	P
Females		
A	0·187	0·677
B	7·849	0·023
A × B	0·203	0·664
Males		
A	66·318	0·0001
B	246·428	0·0001
A × B	6·166	0·038

interaction between the two forms of selection affecting life span such that the negative effects of sexual selection on life span were less pronounced in flies also reared under elevated natural selection (Fig. 2a, Table 1). In other words, males evolving under relaxed natural selection and elevated sexual selection had mean life span reduced by almost 9 days, while in males evolved under elevated natural selection, increasing the intensity of sexual selection only reduced average life span by 6 days.

AGEING PARAMETERS

Patterns of mortality in our experimental populations were best described by the Weibull model (see Experimental Procedures). This model has two parameters, shape (β) and scale (λ) which equate to the rate of ageing and baseline mortality, respectively. An overall MANOVA analysis (including natural selection, sexual selection and sex as fixed effects) showed that the evolution of ageing parameters was significantly influenced by interactions between sex and natural selection ($F_{2,15} = 9{\cdot}741$, $P = 0{\cdot}002$) and between sex and sexual selection ($F_{2,15} = 8{\cdot}395$, $P = 0{\cdot}004$). To explore this interaction further, we therefore conducted sex-specific analyses.

Natural selection reduced the rate of ageing in males (Fig. 2c), while also increasing baseline mortality (Figs 2e and 3, Table 2). In females, only natural selection significantly reduced the rate of ageing (Figs 2d and 4; Table 2). Sexual selection elevated baseline mortality in both males and females (Table 2), but the magnitude of this effect was

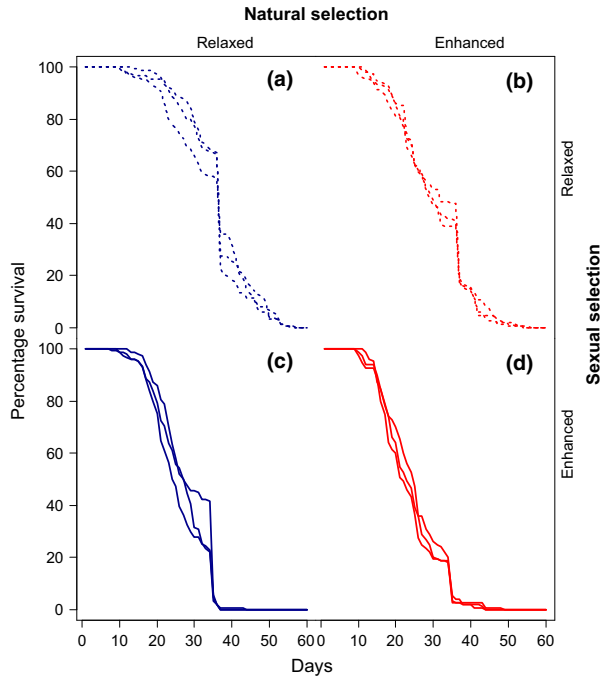

Fig. 3. Survival curves for males from each of the three replicate lines, evolved under relaxed sexual and natural selection (a), relaxed sexual and enhanced natural selection (b), enhanced sexual but relaxed natural selection (c) and enhanced sexual and natural selection (d).

far greater for males than females (Figs 2e,f, 3 and 4). Mortality curves for males (Fig. S1) and females (Fig. S2) in each of our replicate populations are provided in Appendix S1 in Supporting Information.

Discussion

Classical evolutionary theories have established a clear role for natural selection in the evolution of ageing (Medawar 1952; Williams 1957; Kirkwood 1977). More recently, it has been highlighted that sexual selection may also affect the evolution of senescence (Graves 2007; Bonduriansky *et al.* 2008). However, the effects of natural and sexual selection have largely been considered independently, and we now need to develop an understanding of how these processes operate together to influence the evolution of life span and ageing. Using experimental evolution, we found that both natural and sexual selection influence the evolution of ageing and life span in *Drosophila simulans*. However, these processes have independent effects on different ageing parameters (rate of ageing vs. baseline mortality) that differ in magnitude across the sexes. Our results may help explain why patterns of ageing in natural populations often fail to meet the predictions of theoretical models (Nussey *et al.* 2008) and illustrate the importance of adopting an integrated approach to gerontological research. Here, we discuss the mechanisms that are likely to underlie these patterns.

Under relaxed natural and sexual selection, males and females had very similar average life spans. However, each form of selection acted independently on different ageing parameters (i.e. baseline mortality or ageing rate) in the sexes to create pronounced sex differences in average life span. Increasing the strength of natural selection increased male baseline mortality, leading to a net reduction in male life span. This high baseline mortality meant that females evolving under increased natural selection lived longer than males also evolving under these conditions. However, enhanced natural selection simultaneously reduced rates of ageing in both males and females.

The reduced rate of ageing we observed in flies evolved under high temperatures suggests that evolution under heat stress selects against senescent decline, probably by promoting greater somatic maintenance. From a mechanistic perspective, there are several possible explanations for this result as many physiological responses to stress promote increased investment in the soma in *Drosophila* (Gems & Partridge 2008). For example, heat shock proteins, produced by many *Drosophila* species when exposed to high temperatures (Landis, Shen & Tower 2012), protect proteins from degradation (Tower 2011) and promote an increase in overall somatic maintenance, which may also extend life span (Sarup, Sørensen & Loeschcke 2013). This elevated somatic investment could also, in theory, help reduce ageing rates (Murphy *et al.* 2003). Stress responses, however, are costly (Rohmer *et al.* 2004): in *Drosophila,* surviving high temperatures requires specific nutrients (Sisodia & Singh 2012) and may even reduce immune function (Landis, Shen & Tower 2012). High temperatures also increase the costs of male reproduction. For example, evolving at higher temperatures is known to promote an increased investment in longer chained cuticular hydrocarbons (CHCs) to provide greater desiccation resistance, and this comes at the expense of investment in shorter chained CHCs that are more attractive to females in *D. simulans* (Sharma, Hunt & Hosken 2012). This means that males are likely to be required to invest more heavily in other courtship behaviours to obtain a mating. If the costs of surviving and reproducing during heat stress are high, low quality males could improve their fitness by investing more in early life reproductive effort, even if they died sooner, rather than saving resources to invest in surviving heat stress. This scenario could explain why male, but not female, flies evolve higher baseline mortality at high temperatures.

Sexual selection also had pronounced effects on the evolution of life span and ageing. Specifically, sexual selection reduced life span in both sexes but had the greatest effect on males. However, contrary to theoretical predictions (Promislow 2003), sexual selection reduced life span by elevating baseline mortality rather than accelerating the rate of ageing. A similar result in female seed beetles (Maklakov, Fricke & Arnqvist 2007) was attributed to the reliance of females on the male ejaculate to survive desiccation. In the absence of males and the vital water

Table 2. MANOVA examining the effects of elevated or relaxed natural and sexual selection on ageing rate and baseline mortality in male and female *Drosophila simulans*. We also present univariate GLMMs to determine how each of these response variables contributed to the overall multivariate effect

		MANOVA				
		Males			Females	
Source		Pillai's Trace	$F_{2,7}$	P	Pillai's Trace	$F_{2,7}$	P
Natural selection (A)		0·895	29·690	0·0001	0·605	5·372	0·039
Sexual selection (B)		0·985	231·211	0·0001	0·704	8·336	0·014
A × B		0·099	0·384	0·695	0·043	0·158	0·857
		Univariate tests					
			$F_{1,8}$	P		$F_{1,8}$	P
Natural selection (A)	Rate		9·480	0·015	Rate	6·771	0·032
	Baseline		57·931	0·0001	Baseline	0·720	0·421
Sexual selection (B)	Rate		0·402	0·544	Rate	1·306	0·286
	Baseline		283·973	0·0001	Baseline	10·124	0·013
A × B	Rate		0·005	0·947	Rate	0·031	0·864
	Baseline		0·383	0·553	Baseline	0·349	0·571

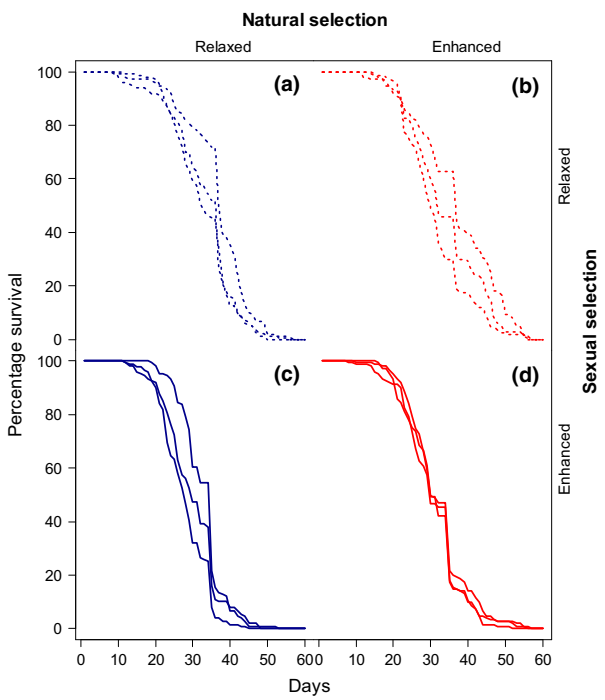

Fig. 4. Survival curves for females from each of the three replicate lines, evolved under relaxed sexual and natural selection (a), relaxed sexual and enhanced natural selection (b), enhanced sexual but relaxed natural selection (c) and enhanced sexual and natural selection (d).

source they provide, females evolving under intense sexual selection suffered higher baseline mortality (Maklakov, Fricke & Arnqvist 2007). Desiccation may also explain our results: males evolving under sexual selection invest in CHCs that attract females rather than those protecting against desiccation (Sharma, Hunt & Hosken 2012), which should increase their risk of dying due to water stress. Desiccation, however, is less likely to explain the elevated female mortality we see here because even under intense sexual selection, female CHC profiles remain relatively unchanged in response to sexual selection in *D. simulans* (Sharma, Hunt & Hosken 2012). Instead, sexual conflict may underpin the greater baseline mortality we see in females: female harassment under sexual selection (Taylor *et al.* 2008) may lead to maternal effects that negatively impact on offspring condition (Gasparini, Devigili & Pilastro 2012) and elevate baseline mortality in both sexes. Irrespective of the mechanism, sexual selection clearly reduced life span and increased population frailty. Thus, it is clear that 'good genes' sexual selection, which should improve condition and select for alleles that reduce mortality rates, played no apparent part in evolutionary responses. Instead, sexual selection appears to have favoured the evolution of traits that reduce population mean viability and survival.

Taken together, natural and sexual selection promoted the evolution of shorter lives in males via a conserved mechanism (greater population frailty), but in females, each form of selection acted antagonistically on life span via independent effects on specific ageing parameters. The magnitude of these effects differed however, and any modest positive effects of natural selection on the rate of ageing in females were outweighed by the negative effects of sexual selection on baseline mortality. Crucially, either form of selection principally affected one ageing parameter, with baseline mortality responding most to sexual selection, while only natural selection influenced ageing rate.

Natural and sexual selection did not interact to affect ageing parameters in either sex but did interact to affect male life span. Specifically, the costs of sexual selection for male life span were less pronounced under elevated natural

selection. Presumably this is because under high temperatures, natural selection selects against the exaggeration of traits that enhance male attractiveness but reduce male viability and robustness, reducing their net negative effect on mortality.

In conclusion, while we do not know the exact proximate mechanisms underpinning these results, both natural and sexual selection acted largely independently in *D. simulans* to drive the evolution of ageing and life span. Crucially, the magnitude and direction of these effects differs across the sexes. That different forms of selection act in a sex- and environment-specific manner could help explain why natural populations often fail to meet the predictions of theoretical models attempting to explain the variation in ageing rates seen in nature (Nussey *et al.* 2008), but help explain the variability in sex-specific mortality rates seen in different species (Fox, Dublin & Pollitt 2003) and in natural populations (Tafani *et al.* 2013). For example, we find that sex differences in life span are only absent in monogamous populations in benign environmental conditions, that is under relaxed natural and sexual selection. While these results illustrate the importance of disentangling the relative influence of natural and sexual selection on the evolution life span and ageing in the sexes, they also indicate the challenges associated with doing so. Future studies will benefit by examining traits other than age at death and attempting to understand the causes of mortality in the sexes (Bronikowski & Promislow 2005), and this is a direction we are currently taking to understand how and why the sexes respond differently to natural and sexual selection in our experiment. Furthermore, as life span and ageing are likely to share a common genetic basis in the sexes (Archer *et al.* 2012), it is possible that the sex differences we observe in these traits have evolved as a correlated response to selection (e.g. Rogell *et al.* 2014). We are currently also examining the genetic architecture of life span and ageing in the sexes of *D. simulans* in an attempt to better understand how sex differences in these traits evolve.

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Data accessibility

Data deposited in the Dryad Digital Repository: doi:10.5061/dryad.f70r3

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Supporting Information

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

Appendix S1. Mortality curves for female and male *Drosophila simulans* in our natural and sexual selection treatments, showing variation across replicate populations**.**

Fig. S1. Log mortality for females from each of the three replicate lines, evolved under relaxed sexual and natural selection (a), relaxed sexual and elevated natural selection (b), elevated sexual but relaxed natural selection (c) and elevated sexual and natural selection (d).

Fig. S2. Log mortality for males from each of the three replicate lines, evolved under relaxed sexual and natural selection (a), relaxed sexual and elevated natural selection (b), elevated sexual but relaxed natural selection (c) and elevated sexual and natural selection (d).