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Evolution of male age-specific reproduction under differential risks and causes of death: males pay the cost of high female fitness

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Abstract

Classic theories of ageing evolution predict that increased extrinsic mortality due to an environmental hazard selects for increased early reproduction, rapid ageing and short intrinsic lifespan. Conversely, emerging theory maintains that when ageing increases susceptibility to an environmental hazard, increased mortality due to this hazard can select against ageing in physiological condition and prolong intrinsic lifespan. However, evolution of slow ageing under high-condition-dependent mortality is expected to result from reallocation of resources to different traits and such reallocation may be hampered by sex-specific trade-offs. Because same life-history trait values often have different fitness consequences in males and females, sexually antagonistic selection can preserve genetic variance for lifespan and ageing. We previously showed that increased condition-dependent mortality caused by heat shock leads to evolution of long-life, decelerated late-life mortality in both sexes and increased female fecundity in the nematode, *Caenorhabditis remanei*. Here, we used these cryopreserved lines to show that males evolving under heat shock suffered from reduced early-life and net reproduction, while mortality rate had no effect. Our results suggest that heat-shock resistance and associated long-life trade-off with male, but not female, reproduction and therefore sexually antagonistic selection contributes to maintenance of genetic variation for lifespan and fitness in this population.

Introduction

Ageing is often defined as the progressive physiological deterioration that increases the risk of death with advancing age (Finch, 1990; Hughes & Reynolds, 2005). Evolutionary theories of ageing rely on the fundamental idea that the strength of selection declines with age because few individuals can survive to old age and contribute to future generations in the face of extrinsic mortality hazards, such as predation, parasitism and harsh abiotic conditions (Medawar, 1952; Williams, 1957; Hamilton, 1966; Rose, 1991; Charlesworth, 1994). This decline in the strength of selection can result in two genetic processes that contribute to ageing: mutation accumulation (MA) (Medawar, 1952) and antagonistic pleiotropy (AP) (Williams, 1957). In the MA theory, ageing evolves because selection at old age is so weak that it cannot effectively weed out late-acting deleterious alleles (Medawar, 1952). On the other hand, the AP theory of ageing involves early- vs. late-life fitness trade-offs, where selection favours genes that increase fitness in early age even when the same set of genes are detrimental later in life (Williams, 1957). A mechanistic account of ageing under AP is disposable soma theory (DS), which addresses the trade-off between survival and reproduction, which has been observed in a wide range of species (Keller & Genoud, 1997; Carey, 2001; Von Wyschetzki *et al.*, 2015; also see Flatt, 2011, and the references therein). DS describes ageing as a consequence of optimal resource allocation. Because resource

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supply is often limited, and resources allocated for one function are no longer available for other functions, somatic maintenance in late-life can be killed for increased early-life reproduction (Kirkwood, 1977; Kirkwood & Holliday, 1979; Kirkwood & Rose, 1991). At the cellular level, autophagy may play an important role in the process of ageing and in mediating the trade-off between lifespan and reproduction (Vellai *et al.*, 2009; De Loof, 2011). The key prediction of the classic theories of ageing is that an increase in extrinsic (age independent) mortality increases 'selection shadow' in late life, leading to accelerated ageing and shorter lifespan (Medawar, 1952; Williams, 1957; Abrams, 1993; Williams *et al.*, 2006). Moreover, AP and DS theories predict that increased extrinsic mortality should select for increased investment in early reproduction.

However, the traditional framework for the evolution of ageing has been challenged by introducing the concept of condition–environment interactions (Abrams, 1993, 2004; Williams & Day, 2003; Williams *et al.*, 2006). Ageing can be viewed as progressive deterioration of an organism's condition. One obvious limitation of the conventional approach is that extrinsic mortality is assumed to be random. The emerging theory suggests that the trajectories of ageing evolution could be altered when extrinsic mortality is dependent on the individual's physiological condition, such that individuals in better condition are more likely to survive environmental mortality hazards, and as a consequence, high rate of extrinsic mortality should select for physiologically more robust individuals with prolonged intrinsic lifespan (Abrams, 2004; Williams *et al.*, 2006; Maklakov, 2013). Recently, we used an experimental evolution approach to test how increases in mortality rate affect the evolution of ageing under random vs. condition-dependent mortality (Chen & Maklakov, 2012). Condition dependence here means that mortality is nonrandom with respect to individual phenotype and results in selection for resistance against the environmental hazard that causes death. We thus explicitly predict that physiological condition is heritable and high-condition individuals are more likely to survive the environmental hazard. The results observed under random mortality agreed with the prediction of AP and DS theories that shorter intrinsic lifespan and increased early (and total) fecundity in females evolved under high rate of extrinsic mortality. By contrast, increase in nonrandom mortality under heat shock resulted in the evolution of physiologically robust phenotypes that not only showed increased resistance to heat stress (Chen & Maklakov, 2013), but also lived longer, had low mortality rate in late life and were more fecund (Chen & Maklakov, 2012; Chen *et al.*, 2013), supporting the general tenet that condition dependence of mortality plays an important role in moulding the evolution of life-history traits.

While our study focused on the role of condition dependence, it is important to emphasize that Medawar–Williams prediction has also been challenged by emphasizing that it holds only under certain assumptions about density dependence and age specificity of extrinsic mortality (Abrams, 1993; Caswell, 2007). It has long been noted that increased extrinsic mortality would have no effect under density-independent population growth, while under density dependence a range of possible outcomes exist depending on age specificity of density dependence on fertility and survival (Abrams, 1993). Our explorations into the role of condition-dependent mortality relied on the experimental design that imposed density dependence by restricting population growth rate, which is equivalent to assuming equal density effects on all fertilities, and such form of density dependence predicts that increased extrinsic mortality will result in the evolution of more rapid senescence when extrinsic mortality is random (Abrams, 1993; Kirkwood & Rose, 1991). Thus, our study is a test of refined predictions from the evolutionary theory of ageing (Abrams, 1993; Williams & Day, 2003).

Simultaneous increase in heat-shock resistance, lifespan and fecundity was unexpected and is not necessarily predicted by the condition–environment interactions model, which is based on the reallocation of resources to somatic maintenance under high-condition-dependent mortality (Williams & Day, 2003). Furthermore, it requires an explanation for why there is high standing genetic variation for fitness in the original population from which our experimental lines were derived, because the lines evolved under heat shock appear to have higher fitness at the first glance. Several explanations were discussed in the original paper (Chen & Maklakov, 2012), one of which was sexually antagonistic selection resulting in intralocus sexual conflict (IaSC) (Rice & Chippindale, 2001; Bonduriansky & Chenoweth, 2009) over lifespan and ageing (Bonduriansky *et al.*, 2008; Maklakov & Lummaa, 2013). IaSC over life-history traits arises because sexes often have different optima in lifespan and reproductive schedules due to anisogamy and sexual selection (Trivers, 1972, 1985; Maklakov & Lummaa, 2013) but share most of their genes (Rice, 1984; Rice & Chippindale, 2001; Griffin *et al.*, 2013; Connallon & Clark, 2014). Sexes often experience different levels of sexual competition – typically, males compete with each other for mating opportunities, whereas females invest heavily in exploiting resources for reproduction. For a female, fitness often can be increased only through gradual exploitation of resources, and increasing investment in resource acquisition does not automatically maximize her fitness owing to the time required to process the resources to produce offspring. On the other hand, for a male, not only fitness is increased with the number of matings (Bateman, 1948; Fritzsche & Arnqvist, 2013), but mating with high-quality females also brings a substantial

direct fitness benefit per investment (Adler & Bonduriansky, 2014). Consequently, (i) short-term fitness gain per investment is often greater for males than for females, and (ii) sexes have different optimal phenotypic values to maximize fitness.

Because the shared genes are expected to produce similar phenotypes with opposing fitness effects in the two sexes, males and females are often constrained from reaching their sex-specific optima in different traits (Bonduriansky & Chenoweth, 2009; Griffin *et al.*, 2013; Connallon & Clark, 2014), including lifespan (Lewis *et al.*, 2011; Berg & Maklakov, 2012; Berger *et al.*, 2014). Sexually antagonistic alleles can thus be maintained in the population because of their opposing fitness effects on the sexes – mutations that increase fitness in one sex but decrease fitness when expressed in the opposite sex will be maintained at intermediate frequencies (Bonduriansky & Chenoweth, 2009; Arnqvist, 2011). Recent empirical evidence suggests that IaSC over optimal resolution of lifespan-reproduction trade-off plays an important role in shaping sex-specific life histories (Lewis *et al.*, 2011; Berg & Maklakov, 2012; Berger *et al.*, 2014).

In this study, we investigated how mortality rate and mortality source affect the evolution of male reproductive ageing and the potential role of sexually antagonistic trade-offs in maintaining genetic variation for life-history traits under condition-dependent mortality. Specifically, we examined the evolution of male fitness and reproductive ageing using established experimental lines of *C. remanei* with different evolutionary history: high or low rates of extrinsic mortality imposed randomly or nonrandomly (by heat shock). We aimed to answer the following four questions: 1) Does increased rate of extrinsic mortality result in the evolution of increased early-life reproduction in males as predicted by DS theory?; 2) Does the source of extrinsic mortality affect the evolution of male reproductive performance?; 3) Is there a trade-off between heat-shock resistance and reproduction in males?; and 4) Can sexually antagonistic selection explain high genetic variation for female fitness found in the previous studies of these populations?

Materials and methods

Experimental lines

In this study, we used experimental lines that evolved from the strain SP8 of *C. remanei* (Chen & Maklakov, 2012). This wild-type strain, provided by N. Timmermeyer from the Department of Biology, University of Tuebingen, Germany, is a cross of three wild-type isolates (SB146, MY31 and PB206) and has been shown to harbour substantial standing genetic variation (Graustein *et al.*, 2002; Cutter *et al.*, 2006; Reynolds & Phillips, 2013). The experimental lines were derived by subjecting 16 replicated populations, each consisting of 25 pairs of males and females (except for the first generation in which populations consisted of 50 pairs of males and females), to experimental evolution under four life-history regimes (four populations per regime) by varying the rate [high (H) or low (L)] and source [random (R) or condition-dependent (C-d)] of extrinsic mortality (from here on abbreviated as 'HR', 'LR', 'HC-d' and 'LC-d', respectively; see Chen & Maklakov, 2012 for details). Extrinsic mortalities were applied to adult worms every 3 days by removing 28.6% or 85.5% of worms in L and H treatments in the populations with overlapping generations, respectively and, for example, in L treatments, a worm had a probability of ~0.54 to survive to the age of 13 days, whereas in H treatments, this probability was ~0.007 based on extrinsic mortalities alone. Condition-dependent mortality was imposed on resistance to thermal stress because 1) resistance to high ambient temperature is positively correlated with lifespan, stress resistance and immunity in *Caenorhabditis* species (Amrit *et al.*, 2010) and 2) heat-shock proteins (HSPs) that confer resistance to high temperature are molecular chaperones responsible for general defence against a wide range of stressors (Sorensen *et al.*, 2003). In both HC-d and LC-d treatments, individual worm's resistance to heat shock was determined by its ability for locomotion after heat shock, and only highly mobile individuals (i.e. Class A individual as defined by Herndon *et al.*, 2002) were considered resistant and were transferred to the next generation. Exactly the same criterion of selection was held across treatments, between sexes and throughout the experimental evolution. Thus, HR and LR treatments enable us to test the classic Medawar-Williams prediction that accelerated ageing evolves under increased extrinsic mortality. Simultaneously, HC-d and LC-d treatments directly test the role of condition dependence of extrinsic mortality on the evolution of ageing. For this reason, it was crucial that the same rates of mortality were maintained across R and C-d regimes. However, the exact number of worms that were removed following heat-shock differed in every population in each generation. Therefore, we first imposed different rates of condition-dependent mortality on HC-d and LC-d populations, and then imposed corresponding rates of random mortality on HR and LR populations. Each C-d population had a 'sister' R population in which exact same rate of mortality was imposed. For example, if 40 worms (say, 18 females and 22 males) were removed by heat-shock in HC-d1 population, we randomly removed 18 females and 22 males from HR1 population, and so on for every pair of 'sister' populations. By doing so, we ensured that C-d and R populations differed only in the source of mortality, but their mortality rates were exactly the same. Besides, upon each bout of imposed extrinsic mortality, both the population size and the sex ratio were restored to the original

set up (i.e. 25 pairs of males and females and 1:1 sex ratio) by adding in the appropriate number of worms using the offspring produced by the respective population (e.g. in the example above, 18 female offspring and 22 male offspring would be added to HC-d1 population). There was no difference in mortality rates between males and females (Table S2).

After 12 generations of selection, HR populations evolved shorter intrinsic lifespan than LR lines, whereas HC-d lines evolved longer intrinsic lifespan than LC-d populations resulting in mortality source × mortality rate interaction for this trait (Chen & Maklakov, 2012). Besides, C-d populations evolved stronger resistance to heat-shock than they had in the beginning of the experiment and HC-d populations evolved stronger resistance than LC-d populations (Chen & Maklakov, 2013). Female fecundity increased with the rate of extrinsic mortality but was unaffected by mortality source: HC-d and HR lines had similar fecundities, which were higher than those of LC-d and LR lines (see Fig. S2 in Chen & Maklakov, 2012).

By restricting population growth rate at zero, we imposed density dependence assuming that all fertilities are affected equally. Moreover, because our extrinsic mortality treatments were applied to adult worms only, it changed the population structure by increasing recruitment of young individuals in two high extrinsic mortality treatments (HR and HC-d). Essentially, our design modelled a population with stable population size where recruitment of young individuals is only possible following the death of an adult.

Male reproductive fitness assay

At the beginning of the assay, we isolated 10 focal males synchronized at the last larval stage from each of the 16 experimental populations. Age-specific fertility was measured every 3 days throughout male lifespan, from day one of adulthood (i.e. the next day of isolation) until death. Specifically, one focal male was paired with five virgin 'tester' females (approximately 2 days old) derived from SP8 populations for 3 h. After 3 h of mating, the focal male was removed, and the five females were transferred to a separate fresh plate and were left to lay eggs for 3 h. The number of eggs produced by the five females within this 3-h egg-laying period was used as an estimation of the focal male's age-specific fertility. Each of the focal males was paired with one mature SP8 'holding' female in-between the assays to reduce males' mate-search behaviour (males wander around in search of females when kept alone and may die when stranded on the walls of the Petri dish) and to standardize their mating status. These 'holding' females were replaced after each assay to avoid mixing with progeny nematodes. All assays were performed on 60-mm Petri dishes with standard medium and handling (Stiernagle, 2006).

Statistical analysis

We analysed fitness using an individual-based, rate-sensitive estimation of absolute fitness (λ_{ind}), which combines lifetime fertility and reproductive schedule (McGraw & Caswell, 1996; Brommer *et al.*, 2002; Metcalf & Pavard, 2007). Essentially, λ_{ind} is an analogous measurement of intrinsic population growth rate derived from Euler–Lotka equation using age-structured projection matrices: for individual i, fitness λ_{ind} is the largest root of $\Sigma(f_x)(l_x)(\lambda_{ind})^{-1} = 1$, where f_x is fertility at age x and l_x is survival at age x (either 1 or 0). λ_{ind} was calculated for each male and was fitted by a general linear mixed-effect model (GLMM) with restricted maximum-likelihood approach with mortality source (random or condition dependent), mortality rate (high or low) and the interaction fitted as fixed effects, and population included as a random effect (Table 1).

Age-specific fertility was fitted in a full GLMM using maximum-likelihood approach with mortality source (random or condition dependent), mortality rate (high or low), age (linear and quadratic, age and age^2), age at last reproduction (ALR) (van de Pol & Verhulst, 2006) and interactions fitted as fixed effects, and individual and population fitted as random effects. Age and ALR were grand-mean centred to facilitate interpretation of differences in age-specific fertility among treatments. Model selection was performed by both forward inclusion of variables from the null model and backward removal of insignificant effects or interactions from the full model using log-likelihood ratio test. Both model selection procedures returned the same model. This final model was refitted using restricted maximum likelihood (Table 2).

Early fertility (i.e. fertility of day one of adulthood) was fitted by a GLMM with restricted maximum-likelihood approach with mortality source (random or condition dependent), mortality rate (high or low) and the interaction fitted as fixed effects, and population included as a random effect (Table S1).

For all three models, significance of fixed terms was tested using Wald F-test with denominator degrees of freedom approximated by Kenward–Roger approach. All statistical analyses were performed using the R programming language (R 3.0.3; www.r-project.org) with

Table 1 The full general linear mixed-effects model of the effects of extrinsic mortality source (mortality source, random or condition dependent) and extrinsic mortality rate (mortality rate, high or low) on the evolution of male total fitness (λ_{ind}).

Fixed effect	*F* ratio	Prob > *F*
Mortality source	$F_{1,12} = 14.941$	0.002
Mortality rate	$F_{1,12} = 0.866$	0.370
Mortality source * mortality rate	$F_{1,12} = 0.580$	0.461

Table 2 The final general linear mixed-effects model of the effects of extrinsic mortality source (mortality source, random or condition dependent), extrinsic mortality rate (mortality rate, high or low), age (linear and quadratic, age and age^2) and age at last reproduction (ALR) on the evolution of male age-specific fertility.

Fixed effect	*F* ratio	Prob > *F*
Mortality source	$F_{1,11.54} = 6.337$	0.028
Mortality rate	$F_{1,11.42} = 0.874$	0.369
Mortality source * mortality rate	$F_{1,11.42} = 0.622$	0.446
Age	$F_{1,51.22} = 27.535$	<0.001
Age * Mortality source	$F_{1,39.73} = 6.780$	0.013
Age * Mortality rate	$F_{1,38.86} = 3.653$	0.063
Age^2	$F_{1,97.02} = 109.980$	<0.001
Age^2 * Mortality source	$F_{1,73.22} = 3.133$	0.081
Age^2 * Mortality rate	$F_{1,72.11} = 0.288$	0.593
ALR	$F_{1,180.32} = 38.717$	<0.001
ALR * age	$F_{1,442.20} = 35.3201$	<0.001
ALR * age^2	$F_{1,286.29} = 10.216$	0.002

nlme (Pinheiro *et al.*, 2015), lme4 (Bates *et al.*, 2014) and car (Fox & Weisberg, 2011) packages.

The evolution of intralocus sexual conflict over lifespan was illustrated by plotting reproductive performance (variance standardized within each sex) of males and females across control (i.e. R) and long-lived (i.e. C-d) lines (Fig. S1).

Results

Evolution under heat shock resulted in a reduction in male fitness, measured as λ_{ind} (Mortality Source: $F_{1,12} = 14.941$; $P = 0.002$; Table 1; Fig. 1). This reduction came mostly from reduced early fertility (Fig. 2), which is supported by significant interaction between Age and Mortality Source (Age * Mortality Source: $F_{1,39.73} = 6.780$; $P = 0.013$; Table 2). In general, male fertility increased in early life, with maximum fertility reached at day four, and decreased subsequently (Age: $F_{1,51.22} = 27.535$, $P < 0.001$; Age^2: $F_{1,97.02} = 109.980$, $P < 0.001$; Table 2; Fig. 2); however, C-d males showed significantly lower fertility than R males on day one (Mortality Source: $F_{1,11.90} = 23.307$, $P < 0.001$; Table S1; Fig. 2). There was no effect of mortality rate on the evolution of male fitness (mortality rate: $F_{1,12} = 0.866$; $P = 0.370$; Table 1) or reproductive ageing (mortality rate: $F_{1,11.42} = 0.874$; $P = 0.369$; Table 2), and no interaction between mortality rate and age (Age * Mortality Rate: $F_{1,38.86} = 3.653$; $P = 0.063$; Table 2).

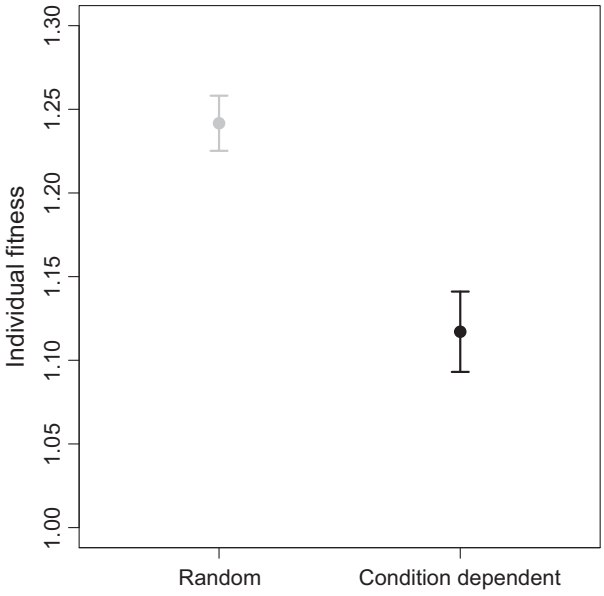

Fig. 1 Mean (±SE) individual fitness (λ_{ind}) of males evolved under condition dependent mortality (black) or random mortality (grey). Males evolved under condition dependent mortality had lower fitness.

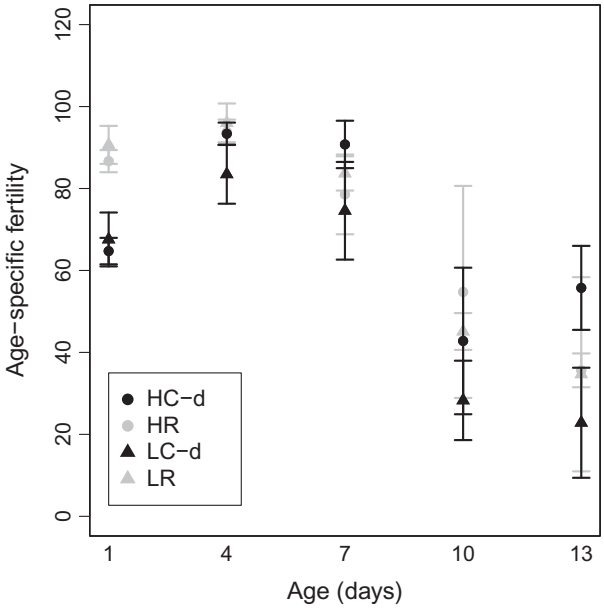

Fig. 2 Mean (±SE) age-specific fertility of males evolved under high (circles) or low rate (triangles) of mortality imposed by different mortality sources (condition dependent: black; random: grey). Males evolved under condition-dependent mortality had a reduced early-life fertility.

Discussion

Our results showed that male age-specific reproduction did not evolve under differential rates of extrinsic mortality. In particular, we did not find the evolution of increased reproduction under high mortality rate as predicted by AP theory. On the contrary, the source of extrinsic mortality had a strong impact on the evolution of male reproduction, especially on reproductive output in early ages. Nonrandom mortality by heat shock

resulted in reduction in early fertility and net individual fitness, suggesting that evolution of increased heat-shock resistance trade-offs with male fitness.

Life-history theory aims to understand how natural selection shapes the major events in the life of an organism so as to maximize its genetic representation in the future generations (Stearns, 1992; Roff, 2002). Life-history optimization, however, is constrained by the underlying genetic correlations, and major life-history traits are often negatively correlated with each other, such that increased performance in one trait trade-offs with reduced performance in another. Sexes often have different life histories, and the optimal resolution of key life-history trade-offs, such as the trade-off between lifespan and reproduction, can differ between males and females (Trivers, 1972; Bonduriansky *et al.*, 2008; Maklakov & Lummaa, 2013). However, sexually homologous traits are expected to show positive intersexual genetic correlations owing to the shared genetic machinery controlling their phenotypic expression (Lande, 1980; Rice, 1984; Chippindale *et al.*, 2001; Bonduriansky & Chenoweth, 2009; Connallon & Clark, 2014). As a result, sex-specific selection on lifespan can increase fitness in one sex but simultaneously pull the other sex away from its sex-specific optimum (Berg & Maklakov, 2012; Berger *et al.*, 2014), maintaining polymorphism at sexually antagonistic loci for lifespan (reviewed in Maklakov & Lummaa, 2013).

In our study system, we showed that evolution under condition-dependent mortality induced by heat shock resulted in increased lifespan and resistance to heat stress in both sexes (Chen & Maklakov, 2012, 2013). However, increased resistance to heat stress is associated with greater lifetime fecundity in females (Chen & Maklakov, 2012) but lower lifetime offspring production in males (Table 2 and Table S1; Fig. 1 and Fig. 2). Thus, the alleles favoured by heat-shock selection indeed confer high fitness when expressed in females; however, fixation of these alleles in the population is likely hampered because the same alleles, when expressed in males, carry a fitness cost (Fig. 2 and Fig. S1).

Condition-dependent mortality acts on one or a set of physiological traits and modifies the trajectory of lifespan evolution via the genetic correlation between the trait(s) in question and lifespan (Williams & Day, 2003). When this correlation is positive, increase in condition-dependent mortality can promote the evolution of increased lifespan, as we found in the earlier study (Chen & Maklakov, 2012). In nature, populations experience multiple sources of extrinsic mortality and it is likely that some sources of mortality result in selection for improved physiological condition and increased intrinsic lifespan, while other sources of mortality may be random with respect to organismal physiology and yet others may, in theory, accelerate ageing because resistance to a particular hazard can trade-off with intrinsic lifespan (Dowling, 2012). Importantly, different mortality sources can result in different outcomes in males and females because the correlations between resistance to extrinsic hazard, reproduction and intrinsic lifespan may be sex specific. Theory and empirical evidence suggest that suites of life-history traits with highly integrated genetic architecture (life-history syndromes) are reservoirs of sexually antagonistic genetic variation for fitness (Prasad *et al.*, 2007; Kwan *et al.*, 2008; Mank *et al.*, 2008; Bonduriansky & Chenoweth, 2009; Abbott, 2011; Bedhomme *et al.*, 2011; Delcourt *et al.*, 2012; Gosden *et al.*, 2012; Berger *et al.*, 2014). This study supports this premise and suggests that sexually antagonistic selection maintains genetic variation for fitness and ageing in *C. remanei* because of the sex-specific trade-offs between heat-shock resistance, lifespan and reproduction.

The evolutionary biology of ageing is moving beyond the traditional paradigm, both theoretically and empirically (Williams & Day, 2003; Williams *et al.*, 2006; Watchter *et al.*, 2013, 2014; Jones *et al.*, 2014; Maklakov *et al.*, 2015). Nevertheless, the fitness trade-offs associated with lifespan evolution are not fully understood (Gems & Partridge, 2013; Jones *et al.*, 2014). The classic theories of ageing, such as Antagonistic Pleiotropy (Williams, 1957) and disposable soma (Kirkwood, 1977), focused on trade-offs between early-life vs. late-life fitness components, particularly early reproduction vs. late survival. AP and DS predict that, as mortality rate increases, somatic maintenance should be killed for reproduction (Williams, 1957; Kirkwood, 1977; Kirkwood & Rose, 1991). On the other hand, condition–environment interactions model (Williams & Day, 2003; Williams *et al.*, 2006) predicts that an increase in environmental hazard that selects for physiologically robust genotypes can postpone the onset of ageing and sometimes result in the evolution of slower ageing and longer lifespan. However, this model does not necessarily predict a net increase in fitness; instead, an increase in physiological robustness may cause resources to be reallocated from reproduction to somatic maintenance. Here, we showed that sex-specific pleiotropy results in reduced reproductive performance in males in long-lived and stress-resistant males, despite increased reproductive performance in long-lived and stress-resistant females (Fig. S1). Moreover, our results strongly suggest that male reproductive performance trades-off directly with heat-shock resistance rather than with increased lifespan because even low level of heat-shock resistance that was not associated with increased lifespan resulted in reduction in male fertility (Table 1). These findings further highlight the key role of the source of extrinsic mortality in shaping not only the evolution of lifespan and ageing but also the evolution of sexual dimorphism in life history.

Life-history evolution in response to an increase in environmental hazard should rely heavily on the within- and between-sex genetic correlations between

lifespan, female fecundity, male fertility and the trait conferring resistance to this hazard. While sexually antagonistic selection is expected to play an important role in shaping sex-specific life histories and this study provides further evidence in this regard, empirical work suggests that there can be substantial sex-specific genetic variation for lifespan (Lehtovaara *et al.*, 2013). Therefore, it is likely that the effect of an environmental hazard on the evolution of sex-specific life histories will depend on the nature of this hazard. Is it affecting mortality of one sex or both sexes? Does resistance to hazard depend on sexually dimorphic (or sex limited) aspects of physiology or morphology? We recently showed that sex-specific condition-dependent mortality based on male performance during mate search can result in sex-specific evolution of lifespan and generate sexual dimorphism in this trait (Chen & Maklakov, 2014), while this study provides strong support for the role of intralocus sexual conflict when a different source of condition-dependent mortality (heat shock) affects both sexes. Little is known about specific genes involved mediating intralocus conflict, but genes involved in TGF-beta signalling pathway in nematodes are potential candidates, as mutations in these genes substantially impair male's reproductive performance but extend hermaphrodites' reproductive span, more than double in some cases (Savage-Dunn, 2005; Luo *et al.*, 2009, 2010; Luo & Murphy, 2011). Lifespan is a complex trait that is affected by both private (sex specific) and shared sets of alleles within each sex (e.g. Berger *et al.*, 2014) and, taken together, the recent findings suggest that different mortality sources may target both private and shared components of lifespan in males and females. Future studies should focus on testing the effects of different ecologically relevant sources of extrinsic mortality in both sexes on the evolution of sexual dimorphism in life history.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Figure S1 Intralocus sexual conflict for fitness across control (open circles) and long-lived (closed circles) lines.
Table S1 The full general linear mixed-effects model of the effects of extrinsic mortality source (Mortality Source, Random or Condition-dependent) and extrinsic mortality rate (Mortality Rate, High or Low) on early fertility (i.e. first day of adulthood).
Table S2 The mean survival after heat-shock and 95% confidence interval of males and females in HC-d and LC-d treatments.

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