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Sexual selection affects lifespan and aging in the seed beetle

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Summary

Sexual selection in general, and sexual conflict in particular, should affect the evolution of lifespan and aging. Using experimental evolution, we tested whether removal of sexual selection leads to the evolution of accelerated or decelerated senescence. We subjected replicated populations of the seed beetle *Callosobruchus maculatus* to either of two selection regimes for 35 generations. These regimes either allowed (polygamy) or removed the potential (monogamy) for sexual selection to operate. To test for the evolution of intrinsic differences between the two selection regimes, we assayed longevity in replicate cohorts of virgin females and males. Virgin females from populations evolving under sexual selection had reduced lifespan as predicted by the sexual conflict theory of aging. However, this reduction was due to increased baseline mortality rather than an increase in age-specific mortality rates with age. We discuss these findings in light of other data from this model system and suggest that system-specific idiosyncrasies may often modulate the general effects of male–female coevolution on the evolution of aging.

Key words: senescence; experimental evolution; longevity; coevolution; sexual conflict.

Introduction

Theory suggests that senescence evolves because the intensity of natural selection declines with age (Medawar, 1952; Williams, 1957; Partridge & Barton, 1993; Charlesworth, 1994; Hughes & Reynolds, 2005). This decline results in the accumulation of alleles with deleterious effects that are only expressed at late age (mutation accumulation; Medawar, 1952) and/or in the accumulation of alleles that enhance fitness in early life at the expense of fitness late in life (antagonistic pleitropy; Williams, 1957). Increased rates of extrinsic mortality should lead to accelerated rates of intrinsic mortality (i.e. senescence) under both of these scenarios (Partridge & Barton, 1993; Hughes & Reynolds, 2005), and this prediction has been supported experimentally (Stearns *et al*., 2000). However, it has also been suggested that a high extrinsic mortality rate can select for increased investment in somatic maintenance, which can in turn result in decelerated senescence (Abrams, 1993; Williams & Day, 2003; Williams *et al*., 2006). In support of this possibility, Reznick *et al*. (2004) found that higher extrinsic mortality mediated by predation in guppies *Poecilia* reticulata was associated with decelerated rates of intrinsic mortality.

Recent theory has also suggested that genetic conflicts between mates (i.e. sexual conflict; Arnqvist & Rowe, 2005) may affect the evolution of aging (Promislow & Pletcher, 2002; Promislow, 2003; Maklakov *et al*., 2005, 2006a). In theory, increased levels of sexual selection may either elevate or depress adult survival rates. Promislow *et al*. (1998) found that increased opportunity for sexual selection was genetically correlated with increased adult survivorship in fruit flies *Drosophila melanogaster*; but this study did not control for the potentially confounding effects of conflicting interests between males and females (Promislow, 2003). There are reasons to expect that sexual conflict *per se* should lead to the evolution of accelerated senescence in females: in face of an increase in female mortality caused by sexually antagonistic adaptations in males (Rice, 1996; Arnqvist & Rowe, 2005), females should accumulate alleles with deleterious late-life effects and hence age at a higher rate. Promislow (2003) used this logic to suggest that the sexual conflict theory of aging predicts that higher rates of sexual conflict lead to the evolution of higher rates of senescence. However, it is theoretically plausible that increased level of conflict will reduce age-related deterioration as a by-product of increased investment in somatic maintenance. Here we show that sexual conflict in beetles leads to reduced lifespan but does not cause a more rapid senescence in females.

We used an experimental evolution approach to test the sexual conflict theory of aging. We maintained seed beetles *Callosobruchus maculatus* under two selection regimes (monogamy and polygamy; four replicate populations of each) for 35 generations, after which selection was completely relaxed for two generations to avoid maternal effects. In monogamous populations, the opportunity for mate choice and sexual conflict was removed by mating females to one randomly chosen male each. In polygamous populations, both males and females could mate with two partners, thus allowing for mate choice and sexual conflict over reproduction to occur (Holland & Rice, 1999).

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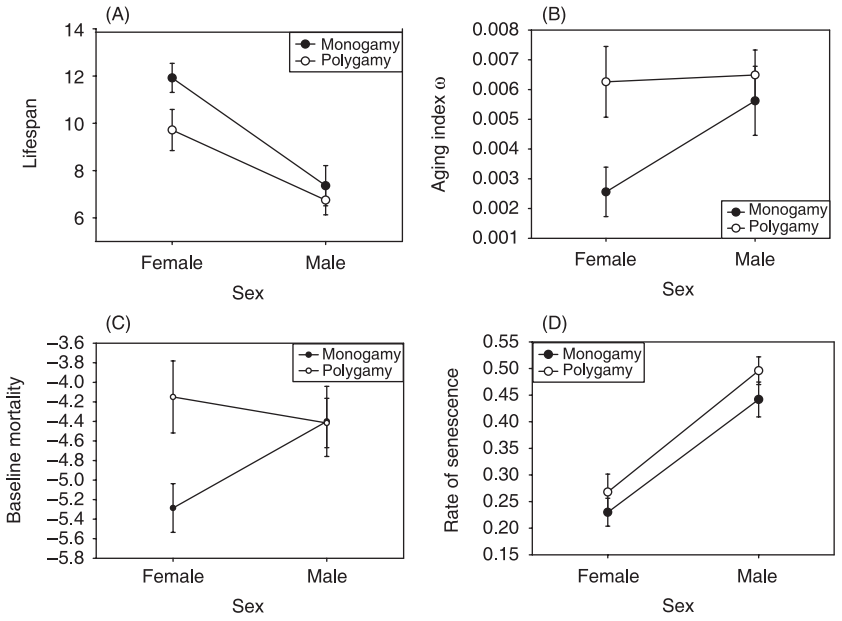

Fig. 1 The effect of experimentally enforced monogamy or polygamy on the evolution of longevity, Reznick's aging index (ω), baseline mortality (ln α) and rate of senescence (β). Females from monogamous populations (filled circles) lived longer (A) and aged slower (B) than females from polygamous populations (open circles) and this effect was mainly due to lower baseline mortality (C) rather than rate of senescence (D). See text and Table 1 for statistical analyses.

Table 1 The effects of selection regime, sex and their interaction on longevity, Reznick's aging index (ω), baseline mortality (Gompertz intercept, ln α) and rate of senescence (Gompertz slope, β). All models control for average body mass of beetles in each cohort and the model of rate of senescence additionally controls for baseline mortality (see text). Significant interactions are highlighted

Source/statistic	DF	DFDen	F ratio	*P* value
Longevity				
Selection	1	6.784	2.4373	0.1638
Sex	1	25.53	19.3190	0.0002
Selection–sex	**1**	**37.53**	**7.7811**	**0.0082**
Body mass	1	21.28	2.1229	0.1597
Reznick's ω				
Selection	1	6.522	4.0102	0.0883
Sex	1	16.19	1.7045	0.2100
Selection–sex	**1**	**38.1**	**5.8082**	**0.0209**
Body mass	1	10.61	14.1285	0.0034
Baseline mortality				
Selection	1	6.049	2.7593	0.1474
Sex	1	14.95	0.5904	0.4542
Selection–sex	**1**	**37.93**	**8.8789**	**0.0050**
Body mass	1	8.841	5.4076	0.0456
Rate of senescence				
Selection	1	7.03	1.4372	0.2694
Sex	1	36.86	57.3839	< 0.0001
Selection–sex	1	36.8	0.6284	0.4330
Body mass	1	34.9	0.0058	0.9399
Baseline mortality	1	37.65	152.0208	< 0.0001

Results

Females lived longer than males but there was no overall effect of selection on longevity (Fig. 1A, Table 1). However, the effect of selection interacted with sex, such that females from monogamous populations lived longer than females from polygamous populations ($P = 0.049$, Figs 1A and 2A) while there was no difference in lifespan between males from the two selection regimes ($P = 0.529$, Figs 1A and 2B).

To compare changes in mortality rates with age between females from monogamous and polygamous populations, we used the Gompertz model, $\mu_x = \alpha e^{\beta x}$, where μ_x is the mortality hazard at age x, α (Gompertz intercept) is the baseline mortality rate, and β (Gompertz slope) is the rate of increase in mortality with age, that is, the rate-of-senescence of the population in our analysis of age-dependent mortality (Promislow *et al*., 1999). We measured aging using two alternative methods. First, we analysed the effect of sex and selection on the rate of aging using the approach adopted by Reznick *et al*. (2004) (see Experimental procedures). This analysis revealed a significant sex–selection interaction for aging (Table 1, Fig. 1B), suggesting that females from polygamous populations aged faster than females from monogamous populations ($P = 0.017$) while there was no difference in rate of aging among males ($P = 0.509$). Second, we analysed different components of the Gompertz equation separately (Bronikowski & Promislow, 2005). Here, we found a significant sex by selection interaction for baseline mortality rate (Table 1, Fig. 1C), due to polygamous females showing a higher baseline mortality than monogamous females ($P = 0.015$), while there was no difference between polygamous and monogamous males ($P = 0.967$). The analysis of the rate of senescence showed that males generally senesce faster than females, while we found no effect of selection (Table 1, Fig 1D).

Discussion

Our experimental removal of reproductive competition among males and conflicts of interest between males and females triggered the evolution of decreased rates of mortality and elevated lifespan in monogamous females, presumably as a direct response to a release from a sexual selection load. These results

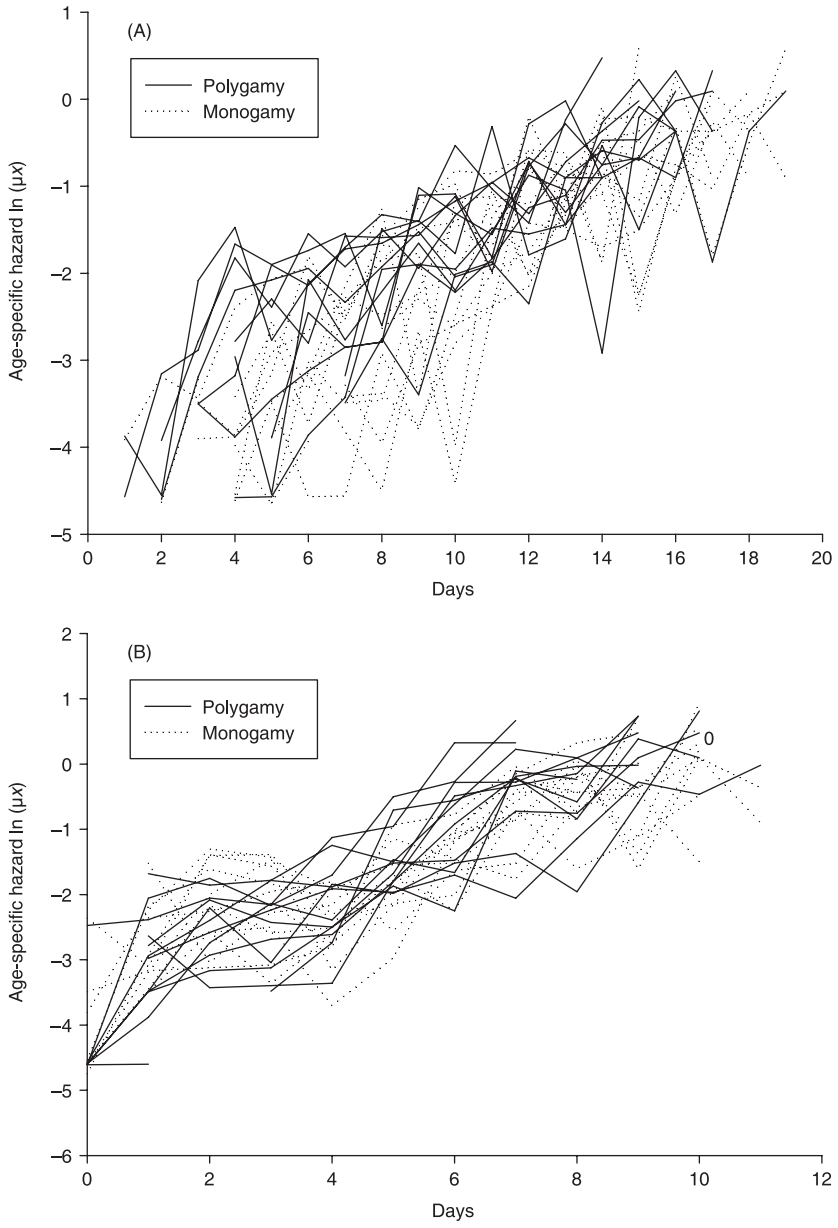

Fig. 2 Female and male *Callosobruchus maculatus* beetles evolving under different mating regimes show intrinsic differences in lifespan and mortality rate. This figure shows (A) female and (B) male age-specific hazard rate (ln μ_x) in replicate cohorts of beetles from populations selected under experimentally enforced monogamy or polygamy. Virgin females from monogamous populations (dotted lines) died less rapidly than did virgin females from polygamous populations (solid lines). There was no difference in hazard rates between cohorts of males from different selection regimes.

are in line with the hypothesis that sexual conflict is associated with the evolution of rapid aging (Promislow, 2003). However, our analysis of different components of mortality suggested that virgin females from polygamous lines did not exhibit significant acceleration of the rate of mortality with age. Rather, the difference in lifespan between selection lines was primarily caused by a higher baseline mortality rate. This finding is consistent with the general trend that differences in lifespan between populations are often attributable to differences in baseline mortality rather than to differences in the rate of senescence (Promislow *et al*., 1996; Pletcher *et al*., 2000; Bronikowski *et al*., 2002; Maklakov *et al*., 2006b; but see Fox *et al*., 2004a). For example, Bronikowski *et al*. (2002) attributed differences in α between different populations of baboons (*Papio hamadryas*) to environmental factors, such as diet, predation, stress and disease, as well as medical intervention in captive populations. In our study, we note that environmental factors cannot have a significant direct impact, so differences in aging in females must instead reflect intrinsic differences between different populations (Promislow *et al*., 1996; Pletcher *et al*., 2000; Maklakov *et al*., 2006b).

We suggest that the observed evolved differences in baseline mortality reflect the fact that seed beetles inhabit arid environments and that females have evolved an ability to utilize male ejaculates to their own needs. The male ejaculate constitutes about 6% of the male body weight, and ejaculates have a large influence on female life history as females benefit from water and/or nutrients in the ejaculate (Savalli & Fox, 1999; Edvardsson, 2007). Fricke & Arnqvist (2007) showed that females have evolved

a higher remating rate in the polygamous lines used here, such that polygamous females have evolved under a higher mating rate than females in monogamous populations. Polygamous females are thus expected to evolve to rely on male ejaculates to a higher extent, making them more susceptible to extrinsic mortality caused by desiccation in the absence of male ejaculate resources. In a more general sense, our results thus illustrate that the effects of male–female coevolution on the evolution of aging will to some extent depend on the system-specific idiosyncrasies that mediate these effects (Promislow, 2003).

Two distinct types of sexual conflict are recognized, depending on whether there is antagonistic selection on alleles in one locus (i.e. intralocus conflict) or in different loci (i.e. interlocus conflict) (Chippindale *et al*., 2001; Rice & Chippindale, 2001; Arnqvist & Rowe, 2005). Both types of conflict can contribute to the evolution of senescence (Promislow, 2003). Although we have so far discussed our data in terms of interlocus conflict, we note that the removal of sexual selection in monogamous populations will also reduce intralocus genetic conflicts between males and females. The fact that females from monogamous populations could thus be freed, at least in part, from the sexual selection load associated with intralocus conflicts (Fricke & Arnqvist, 2007) may certainly have contributed to the evolution of increased lifespan seen in monogamous females.

The sex differences in lifespan and mortality rates are generally consistent with previous studies in *C. maculatus*: males lived shorter and senesced faster than females (e.g. Fox *et al*., 2003, 2004a), although we did not find any effect of sex on baseline mortality rate. The genetic architecture underlying variation in lifespan is different for male and female beetles (Fox *et al*., 2004b, 2006), which is in line with the results of this study. While we did not find any effect of selection on male mortality, it is possible that males that evolved under sexual selection express pleiotropic traits that increase male mating success at the expense of survival. The chance to detect such effects was reduced in our study as we only tested virgin males. This subject requires further investigation.

In summary, we showed that the removal of sexual selection led to the evolution of increased lifespan in virgin females. Contrary to predictions derived from the interlocus sexual conflict theory of aging, however, the reduction in lifespan seen in polygamous females was apparently not due to the evolution of an accelerated rate of senescence. Further research should be aimed at elucidating the relative importance of inter- and intralocus genetic conflicts in the evolution of aging.

Experimental procedures

Selection lines and survival assay

We mass-mated three *C. maculatus* populations collected in large numbers at three adjacent locations in Nigeria and propagated this population with more than 300 individuals per generation for four generations prior to initiating the selection experiment. Selection populations were kept at 30 °C (± 1 °C) and 45% (± 10%) relative humidity and a 12:12 h light-dark cycle on black-eyed beans (*Vigna unguiculata*). Beetles used to propagate populations were collected as virgins during the first 72 h after the onset of hatching. We collected beans showing transparent 'windows' produced by prepupal larvae and placed them individually in aerated plastic chambers, to produce virgin adults. We used two selection regimes: monogamy (M; $n = 4$) and polygamy (P; $n = 4$) (see Fricke & Arnqvist, 2007). In M populations, we paired 50 virgin males individually with one virgin female each in each generation. In P populations, we paired 57 virgin males individually with one female each (see below), but females were allowed the opportunity to mate with two males consecutively. This was achieved by rotating males (within the set of 57 males per population) once among females 3 h after the introduction of the first male. Females were exposed to the males from the same generation. The overall exposure to males was identical in both treatments. We used unequal absolute population sizes in the two selection regimes (100 for monogamous populations and 114 for polygamous populations) in order to yield similar effective population sizes (Fricke & Arnqvist, 2007).

After 24 h, males were discarded and all females from a given population were transferred into a new glass vial provided with 120 g of beans and were allowed to lay eggs until death. As males were discarded after mating, there was no selection on male lifespan in this experiment. For our survival assays, we collected 300 virgin females and 300 virgin males from each of eight selection populations in the same manner as described above, following two generations of relaxed selection. We placed groups of 100 virgin females/males separately in 90×15 mm vials containing 10 g of beans as a substrate, and set up three replicates of such cohorts of each sex per each selection population, resulting in a total of 4800 beetles in 48 cohorts. The cohorts were weighed using Sartorius analytical balance and weight was used in our models to factor out the effect of average body mass of the cohort. Mortality was scored daily in the assay vials.

Data analysis

We used a mixed-model nested analysis of variance (ANOVA) to test for differences in lifespan between monogamy and polygamy selection regimes, with populations nested within selection regime. The lifespan of females was averaged for each cohort, such that each cohort provided only one data point in our statistical evaluation. We also retested the sex by selection interaction after removing the potential scale effect by dividing the mean values for each sex prior to the analysis. The sex–selection interaction remained significant ($P = 0.015$).

We used the maximum likelihood approach in WinModest (Pletcher, 1999) to compare four different models that all describe the demographic rate of change in mortality with age (Promislow *et al*., 1999): Gompertz, Gompertz-Makeham, Logistic and logistic-Makeham. Likelihood ratio tests were used to choose the model that best fit our data. The Gompertz model was the

best fit for males (70.8% of cohorts), while the Logistic model was the best fit for females (70.8%). Overall, the Gompertz model was the best for 50% of all cohorts when tested separately, while the logistic model provided best fit for 40% of cohorts. Further analysis of the maximization procedure revealed that (i) the logistic model fitted male cohorts poorly (in 25% of the cohorts, at least one of the parameters resided at the boundary of its feasible range and in two cohorts the iterative maximization procedure failed to converge), and (ii) the Gompertz model provided a good fit to data for all female cohorts (a maximum of the likelihood function was found in 100% of all female cohorts). We thus used the Gompertz model in all subsequent analyses of the effect of selection on age-specific mortality rates. We then extracted the maximum likelihood estimates of the two model parameters (baseline mortality, α; rate-of-senescence, β). Different ways of analysing these parameters can yield dissimilar conclusions (Bronikowski & Promislow, 2005). Here, we used the aging index ω^2 $\left(\omega = \sqrt{\alpha * \beta}\right)$ (Reznick *et al*., 2004) to test for the overall effect of selection regime on age-specific mortality rates, but also analysed the effect of selection on ln α and β separately (α was log transformed prior to the analysis because it is log normally distributed). As age-specific mortality rates are phenotypically and genetically correlated (Hughes, 1995; Miyo & Charlesworth, 2004; Maklakov *et al*., 2006b), we used nested analysis of covariance (ANCOVA) to test for the effect of selection on rate-of-senescence (β), while statistically controlling for baseline mortality.

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