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Adaptive male effects on female ageing in seed beetles

Alexei A. Maklakov*, Natacha Kremer and Göran Arnqvist

Animal Ecology/Department of Ecology and Evolution, Uppsala University, Norbyvägen 18D, 752 36 Uppsala, Sweden

Selection can favour the evolution of a high reproductive rate early in life even when this results in a subsequent increase in the rate of mortality, because selection is relatively weak late in life. However, the optimal reproductive schedule of a female may be suboptimal to any one of her mates, and males may thus be selected to modulate female reproductive rate. Owing to such sexual conflict, coevolution between males and females may contribute to the evolution of senescence. By using replicated beetle populations selected for reproduction at an early or late age, we show that males evolve to affect senescence in females in a manner consistent with the genetic interests of males. 'Late' males evolved to decelerate senescence and increase the lifespan of control females, relative to 'early' males. Our findings demonstrate that adaptive evolution in one sex may involve its effects on senescence in the other, showing that the evolution of optimal life histories in one sex may be either facilitated or constrained by genes expressed in the other.

Keywords: male–female coevolution; senescence; sexually antagonistic coevolution; *Acanthoscelides obtectus*; life history

1. INTRODUCTION

Senescence can be defined as an age-specific increase in mortality rate and/or a corresponding decline in reproductive performance (Medawar 1952; Williams 1957; Rose 1991; Partridge & Gems 2002). Theory holds that senescence evolves as a result of an age-related decline in natural selection and studies of experimental evolution manipulating the temporal pattern of selection have largely confirmed this (Partridge & Mangel 1999). The cost of reproduction plays an important role and adaptive evolution of senescence involves organisms balancing reproduction early with mortality later in life (Sgro & Partridge 1999). However, because traits in one sex can affect both the economics and the timing of reproduction in the other (Sheldon 2000; Chapman 2001; Nilsson *et al.* 2002), it has been suggested that coevolution between genes with sex-limited expression may be involved in the evolution of senescence (Promislow & Pletcher 2002; Promislow 2003). This may be particularly true when interacting males and females have divergent genetic interests (Rice 1996; Arnqvist & Rowe 2002; Chapman *et al.* 2003; Arnqvist & Rowe 2005).

The suggestion that sexual coevolution is important for the evolution of senescence, however, rests on two specific yet unsupported postulates. First, evolutionary change in genes expressed in one sex should be capable of affecting senescence in the other sex. Second, evolution should be adaptive, such that selection in one sex for altering the rate of senescence among members of the other sex should result in changes in senescence in the latter sex that is adaptive for members of the former. In the current study, we provide direct experimental evidence for both of these suppositions.

While many types of trait in both sexes can affect the cost of reproduction for their mates, components in the male seminal fluid have recently been given a great deal of attention. In insects, males not only transfer nutrients and gonadotropic substances that influence the reproductive schedules of their mates, but also peptides and proteins that aid in sperm competition (Chapman 2001; Gillot 2003). The latter type of substances can be toxic to females (Chapman *et al.* 1995; Lung *et al.* 2002), presumably as a pleiotropic side-effect (Morrow *et al.* 2003), or as a result of a direct selection on males to affect female reproductive rate (Lessells 1999). Both of these types of substances can potentially influence age-specific mortality rates in females (Chapman *et al.* 1995). Earlier studies have shown that males evolve to decrease female mortality as a correlated response to selection for monandry (Holland & Rice 1999; Martin & Hosken 2003). This has been interpreted as the indirect result of evolution of reduced toxicity of the seminal fluid in the absence of sperm competition. In contrast to these studies, we test whether selection for a late age of reproduction in females, which directly favours males that delay senescence in their mates, results in the predicted evolutionary changes of the effects males have on female ageing.

We used replicated selection lines of the seed beetle *Acanthoscelides obtectus*. Males of this species are known to transfer different peptides and proteins to females at mating, and injection of purified seminal fluid substances into females have shown that one elevates egg production whereas one is toxic to females (Das *et al.* 1980). However, seminal fluid also contains substances with beneficial effects in females, since mated females live longer than virgins when not allowed to lay eggs (Tucic *et al.* 1996). The lines used here were selected for reproduction early (henceforth, 'E') or late ('L') in life for over 100 generations. In the E selection regime, offspring were recruited to the next generation during days 1–2 of adult life. In the L selection regime, recruitment occurred from day 10 until death. While males and females were kept

* Author for correspondence (alexei.maklakov@ebc.uu.se).

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together and mated from day 1 in both selection regimes, fitness was thus accrued during days 1–2 in the E lines and from day 10 until death in the L lines. Previous work based on these lines have shown that the L lines have evolved decreased rates of senescence, reduced early fecundity, increased body size, prolonged lifespan and elevated late life fecundity relative to the E lines (Tucic *et al.* 1996, 1998, 2004). In terms of selection among males on their effects on female mortality rates, these selection regimes differ dramatically. In contrast to E females, L females must survive until day 10 to produce any offspring at all. Therefore, we hypothesize that males that transfer seminal substances that decelerate senescence in their mates will be favoured in the L regime. Control females mated to males from the L regime should therefore show a lower rate of senescence compared to control females mated to males from the E regime.

2. MATERIAL AND METHODS

(a) *Selection regimes*

Selection lines were obtained from the laboratory of N. Tucic, University of Belgrade, where they had been maintained under the L ($N=4$) or the E ($N=4$) selection regimes since 1989 (109 generations of selection for L lines and 150 for E lines) under adult aphagy. All work reported here was performed at 28 °C and 65% relative humidity and beetles were reared on common beans, *Phaseolus vulgaris*. Beans were biodynamically grown and were frozen prior to use. During selection, males and females in the E lines were kept together with beans and allowed to mate and reproduce freely for 48 h after emergence after which adults were removed. Males and females in the L lines were kept together and allowed to mate from day 1, but eggs laid prior to day 10 did not contribute to the next generation (beans were introduced at day 10) (Tucic *et al.* 1996). We also used beetles from the base population from which all selection lines were originally derived. Base population beetles were allowed to reproduce during their entire lifespan. We kept all selection lines at a population size of approximately 1500 individuals under identical conditions and completely relaxed selection for five generations prior to the start of the experiments, to avoid any maternal effects that might occur. The lifespan of *A. obtectus* females ranges from 11 to 20 days (Tucic *et al.* 1996), depending on the availability of egg-laying substrate and raising conditions.

(b) *Experiments*

Virgin beetles for our experiments were generated in the following way. Beans showing the typical transparent 'windows' produced by pre-pupal larvae were placed individually in aerated plastic containers. The beans were inspected several times per day and any newly emerged adults were removed. Beetles were then immediately weighed and isolated individually. All beetles were 2 days old at the start of the experiments. We placed each male with a randomly selected control female from the base population in a 35 mm Petri dish for 30 min and observed whether mating occurred. To ensure that only one mating occurred, males and females were separated immediately after completed copulation. Pairs that did not mate during the trial were discarded (12% for E and 18% for L males overall; GLIM model (binomial errors using Williams's correction for overdispersion) test for difference between E and L lines, $\chi^2_1=1.4$, $p=0.237$) (Crawley 1993). We mated 20 males from each of the eight selection lines, as well as from the single base population, to one base population female each. This procedure was repeated three times and each time was treated as block in the analysis. In total, we successfully paired 60 males from each of the lines to base population females. Following these experimental matings, females were placed individually in 35 mm Petri dishes containing 10 fresh beans (replaced at days 2, 4 and 6) and were allowed to reproduce until death. Mortality was recorded daily. All eggs laid were counted in the first block only. We recorded (i) the time after egg laying that the first adult offspring emerged from each dish and (ii) the proportion of offspring emerged 34 days after egg laying, as alternative measures of offspring development time. Although we present only the data on the former here, separate analyses of the two measures yielded qualitatively identical results in terms of the effects of selection on offspring development time.

(c) *Data analysis*

We used WINMODEST (Pletcher 1999) to compare four different models for describing the temporal pattern of mortality (Gompertz, Gompertz–Makeham, logistic and logistic–Makeham) using log-likelihood tests. In most cases, the mortality function was best described by the Gompertz model (Gompertz 1825); $\mu_x=\alpha\, e^{\beta x}$, where μ_x is the predicted instantaneous mortality rate at age x, α is conventionally interpreted as the baseline mortality rate and β as the rate of increase in mortality with age (i.e. the rate of senescence). We then performed sensitivity analyses for all subsets of data, to test for robustness of estimated model parameters. The results of these analyses showed that maxima of the likelihood functions in all cases lay within a feasible range of parameter space (Pletcher 1999). The parameters α and β were estimated separately for each line and block and subsequently averaged across blocks prior to analysis to avoid pseudoreplication.

We used standard parametric statistics whenever the assumptions of such models were fulfilled. Data on baseline morality rate and the rate of increase in mortality rate were log-transformed, to normalize error distributions, prior to applying a multivariate general linear model (MANOVA) to compare patterns of mortality between selection regimes (SYSTAT 11). As we were unable to fully normalize the residual distribution in the model of female longevity, this model was evaluated by bootstrapping the residuals of the original model (ter Braak 1992; Manly 1997) (999 bootstrap replicates). We note that tests based on this bootstrap procedure were in no case conflicting with analogous conventional F-tests. The temporal pattern of egg production was analysed by first regressing the number of eggs laid on age for each individual female. The intercept and slope from these regressions were then collectively treated as response variables in a nested MANCOVA, with female mass as a covariate and line nested within selection regime. Variation in development time was analysed in a nested repeated-measures ANOVA, with female age as a within-subjects factor and selection regime, line nested within selection regime and block as between-subjects factors. Since the base population was not replicated, we were unable to test for differences between mates of each of the selection regimes and those of base population males. We note, however, that the observed average value for mates of base population males was intermediate to the mean values across replicates of the two selection regimes for most relevant parameters (Tucic *et al.* 1996).

3. RESULTS AND DISCUSSION

Male selection regime significantly affected mortality rates among control females (MANOVA of the two Gompertz parameters (Gompertz 1825), Wilks' $\lambda_{2,5}=20.73$, $p=0.004$). Univariate analyses of variance showed that this effect was due to a decelerated rate of senescence ($F_{1,6}=20.492$, $p=0.004$; figure 1*b*), but also to an elevated baseline mortality rate ($F_{1,6}=6.071$, $p=0.049$; figure 1*a*) in females mated to L males compared to those mated to E males (figure 2). Mortality decomposition analysis (Pletcher *et al.* 2000) suggests that baseline mortality rate accounted on average for 42%, while rate of senescence accounted on average for 58% of the differences in longevity observed between the E and the L populations.There was a negative phenotypic correlation across selection lines between male induction of female baseline mortality and female rate of senescence ($r=-0.783$, $p=0.021$), but, as predicted, females mated to L males lived longer on average than did females mated to E males (table 1). The average longevity of mates of base population males (18.5 days) was intermediate to the mean longevities observed among mates of E males (17.9) and those of L males (18.7).

Offspring development time was shorter among females mated to E males ($F_{1,12}=7.65$, $p=0.017$), no doubt a result of the paternal genetic contribution to offspring (Tucic *et al.* 2004). However, offspring development time also increased with maternal age ($F_{3,36}=10.46$, $p<0.001$) and, most importantly, did so at a faster rate among females mated to E males compared to L males (female age×selection regime; $F_{3,36}=5.07$, $p=0.005$; figure 3). This result shows that the rate at which the phenotypic quality of offspring deteriorates with female age, an important gauge of senescence (Kern *et al.* 2001), is to some extent attributable to their mates and so provides additional support for decelerated senescence among females mated to L males.

In general, our results provide evidence for adaptive evolution of the effect that males have on female senescence. As predicted, females enjoyed lower rates of senescence when mated to males that have been selected to lower the rates of senescence among their mates. Selection in both sexes should favour decelerated senescence in females in the L selection regime, so this effect is unlikely to be opposed by antagonistic adaptations in females. In accord with this, previous studies of these lines have shown that the rate of female senescence is indeed lower in L females (Tucic *et al.* 1996). In theory, males could achieve this effect by depressing early reproductive effort in their mates and/or by decreasing their cost of reproduction. The fact that we found no significant effect of male selection regime on the temporal pattern of egg production of their mates (Wilks' $\lambda_{2,150}=0.992$, $p=0.556$) shows that the effects seen were primarily mediated by a decreased net cost of reproduction. We suggest that males have achieved this by transferring a more beneficial ejaculate to females at mating. Since these beetles do not feed as adults, more nutrients and/or water in the seminal fluid could effectively lower the rate of senescence among females.

The effects seen in our experiment could, at least in part, represent a correlated response to other forms of adaptations in males. However, we believe that this is unlikely for the following reasons. In our selection lines,

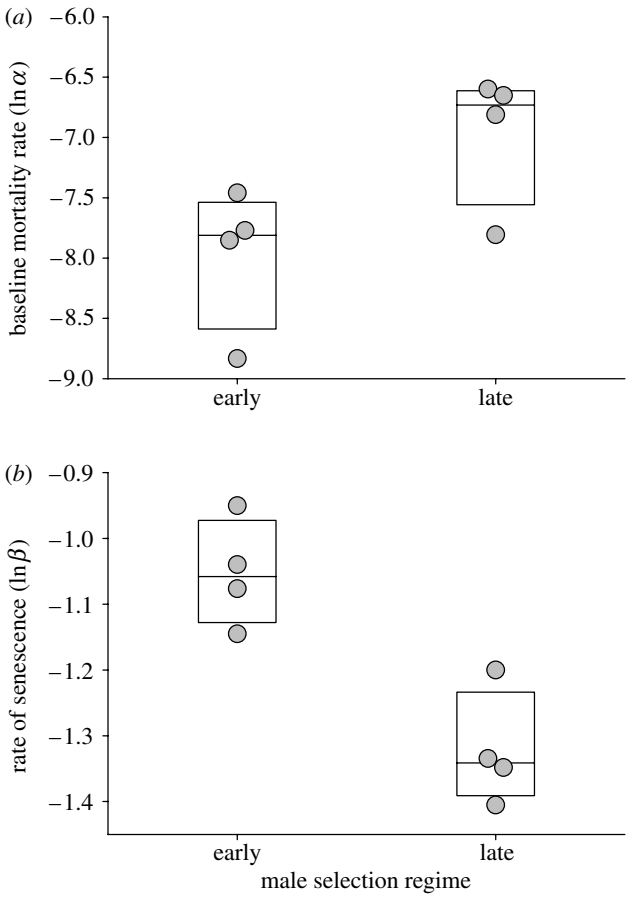

Figure 1. Male beetles evolved to affect mortality rates among their mates. Males from the 'late' selection regime induced (*a*) somewhat higher rates of baseline mortality but (*b*) decelerated rates of senescence in control females, compared to males from the 'early' regime. Figure shows box plots, where points represent mean Gompertz parameters for each replicated selection line across blocks as estimated by a maximum-likelihood procedure (Pletcher 1999).

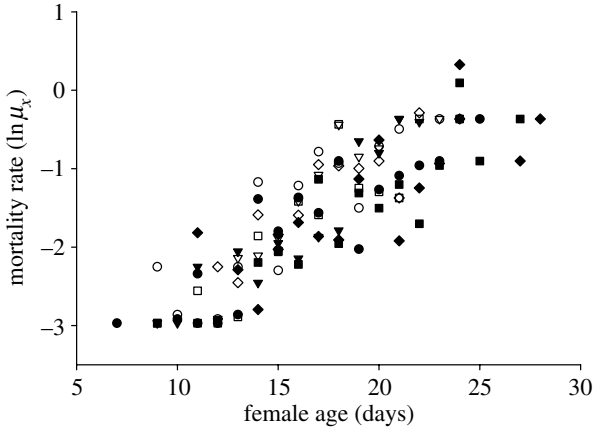

Figure 2. Mortality rates among control females mated to males from the eight selection lines, shown as averages per line across the three blocks. Mates of 'early' males (open symbols) exhibit accelerated ageing compared to mates of 'late' males (filled symbols). The data points represent raw data for mortality rate (μ_x).

there has been direct artificial selection for female age at reproduction (Tucic *et al.* 1996). This direct selection translates into two forms of indirect selection in males: (i) selection on the lifespan of males and (ii) selection on

Table 1. Nested ANOVA of female longevity (bootstrap significance tests).

	d.f.	F	p
male selection regime	1	5.282	0.026
block	2	39.162	0.001
female mass	1	20.788	0.001
male selection regime×block	2	0.347	0.714
line (male selection regime)	6	1.395	0.225
error	467		

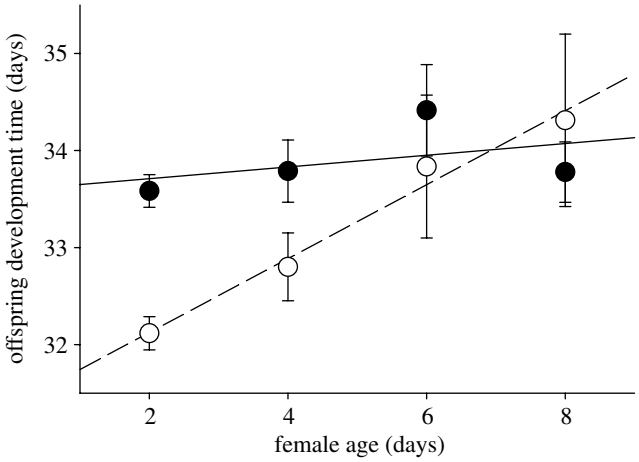

Figure 3. Senescence as manifested by an increase in offspring development time with female age. Again, senescence was decelerated in mates of males from the 'late' selection regime (filled symbols, solid line) compared to those of males from the 'early' selection regime (open symbols, dashed line). Symbols represent mean offspring age at first adult emergence (±s.e.) per line and block.

the lifespan of their mates. Because there is direct selection on female age at reproduction, however, male lifespan should be less closely related to male fitness than should the lifespan of their mates (e.g. males that are dead prior to day 10 can father many offspring in the L selection regime provided that their mates live past day 10). Thus, although our data do not allow us to determine the relative importance of these two forms of selection, we suggest that indirect selection in males generated via males' effects on female lifespan should be stronger. Female coevolutionary response to male adaptation is predicted to occur, but the direction of this response will depend on the interaction between sexual and natural selection and female resistance may exhibit complex age-dependent patterns (Pletcher *et al.* 1997; Sgro *et al.* 2000). Further theoretical and empirical work is needed to resolve this issue.

We note that females suffered a higher baseline mortality rate when mated to L males. Because L males have been selected to prolong female lifespan, this effect was unpredicted. One possible explanation for this result is that the female mating rate may differ in the two selection regimes. Because females do not remate until a few days after their initial mating (Huignard 1974), the E regime should be effectively monandrous while the L regime is polyandrous. Male adaptations that aid in sperm competition but are harmful to females may therefore have been lost in the E regime, where selection in one sex favours the same genes as does selection in the other sex and so there is no opportunity for sexual conflict (Holland & Rice 1999; Arnqvist *et al.* 2000), but may have been favoured in the L regime. Thus, it is possible that the higher baseline mortality rate experienced by control females mated to L males, compared to those mated to E males, reflects a relatively higher cost of mating with the former males. Because L males have then evolved under sperm competition, but E males have not, this is in line with other studies showing that male seminal fluid substances that are beneficial to males in terms of sperm competition may also be harmful to their mates (Chapman *et al.* 1995; Holland & Rice 1999; Martin *et al.* 2004).

Male beetles evolved to affect the mortality rate of their mates in a manner that is in line with the genetic interests of males, but not necessarily those of females. However, males appeared to be caught in an evolutionary dilemma. When females only reproduced late in life, males clearly evolved adaptations that decelerated ageing in their mates, but also evolved to increase female baseline mortality rate. The first and main of these effects is beneficial to individuals of both sexes, while the latter is likely to have sexually antagonistic fitness effects. Our results show that senescence in one sex can indeed be shaped by the adaptive evolution of sex-limited traits in the other, and so provide important evidence for a role of male–female coevolution in the evolution of ageing (Promislow & Pletcher 2002; Promislow 2003).

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