

GENDER DIFFERENCES IN LONGEVITY IN EARLY AND LATE REPRODUCED LINES OF THE SEED BEETLE

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Abstract - In most animals the average longevity of females is greater than that of males. Among the many explanations of the evolution of sex-specific mortality rates, we tested the hypothesis that sexual selection plays an important role in shaping the longevity and ageing of both sexes. The present study was performed using laboratory populations of *Acanthoscelides obtectus*, which were obtained either at an early (E) or late (L) age, where the inadvertently created conditions provided opportunities for the evolution of a monogamous and polygamous mating system, respectively. As predicted, under intense sexual selection (L populations) substantial sex differences in longevity were detected both in mated and virgin experimental beetles. On the other hand, monogamy, i.e. relaxation of sexual selection, in E populations resulted in elimination of the differences in longevity between mated females and males, and even led to postponed senescence of virgin males beyond the level exhibited by virgin females.

Key words: Sexual selection, sexual conflict, longevity, ageing, mortality rate

UDC 595.76:591.139:59

INTRODUCTION

Despite a long-standing interest in the evolution of gender differences in longevity, the reasons why in most animals the average life-span of females is greater than that of males is still a puzzle to evolutionary biologists (Fox et al., 2003). According to the traditional life-history theory, which predicts a trade-off between investment in reproduction and somatic maintenance (Stearns, 1992), natural selection should shape ageing and longevity so as to achieve an optimal balance between the reproductive rate and somatic deterioration (Partridge and Barton, 1993). Since males exhibit higher variance in reproductive success than females, "males are often selected to pursue a 'live fast, die young' reproductive strategy" (Bonduriansky et al., 2008, p. 443).

It has been argued that a balance between the costs and benefits of mating frequency (i.e. number of matings) may also play an important role in the evolution of ageing and longevity of both sexes (Promislow, 2003). Moreover, sexual selection via sexual conflict (Parker, 1979) can modify the sex-specific longevity and ageing rate since mating behavior that is optimal for one sex may be deleterious for the other (Promislow, 2003; Bonduriansky et al., 2008). For example, this hypothesis predicts that some of the sexual adaptations of males, such as the increased toxicity of seminal fluid in polygynous males that maximizes their sperm competitive ability (possibly by destroying or disabling the sperm of previous mates), should lead to the evolution of accelerated senescence in females. Indeed, in the laboratory evolution experiment with *Callosobruchus maculatus* Maklakov et al. (2007) have

shown decreased rates of mortality and elevated longevity in monogamous virgin females relative to virgin females evolved under enforced polyandry (similar results were obtained with females in other insect species; e.g. Holland and Rice, 1999; Martin and Hosken, 2003). On the other hand, although it is predicted that more sexually active males which have evolved under elevated intra-sexual competition should have shorter longevity and higher mortality rates, there were no differences in the longevity between *C. maculatus* males originating from different populations – with or without sexual selection (see also Maklakov and Fricke, 2009).

It is important to note that the role of sexual conflict in shaping longevity has been tested in very few species. Additionally, most of these studies explore the consequences of conflict from the female perspective. By focusing on male longevity, Hall et al. (2009) have revealed that nuptial feeding of females, via water and nutrients in ejaculate, could be an important mechanism of antagonistic interaction between females and males. Under some circumstances, such as scarce resources in the environment, the male reproductive investment in offspring, together with the associated costs of mating, can exceed the costs experienced by females. As pointed out by Hall et al. (2009), “the sex roles can reverse with females actually competing for access to males as potential food sources” (p. 873). In other words, changes in the nature of sexual conflict may shape the longevity and mortality rates of males in the same way as elevated sexual conflict does in females.

The general effects of sexual selection on the evolution of longevity and mortality rates can mediate, to some extent, the species-specific adaptations. For example, it seems that seed beetle (*Acanthoscelides obtectus*) females under starvation, benefit from nutrients and/or water received with ejaculates (Tucić et al. 1996; Maklakov et al. 2005; Šešlija et al., 2008). Since *A. obtectus* is a capital breeder (adults do not need water or food to reproduce successfully), this can be treated as

an important adaptation of the females (similar effects of male ejaculates on females has also been observed in other bruchids (Savalli and Fox 1999; Edvardsson, 2007)). Moreover, it is well known that in this species of seed beetle, males can reduce female longevity through seminal compounds with toxic side-effects (Das et al., 1980). Therefore, *A. obtectus* represents an appropriate experimental system for testing the effects of sexual conflict on the longevity and mortality rates from both the female and male perspectives.

The present study was performed using *A. obtectus* laboratory populations which markedly differ from each other with respect to average longevity and mortality rates. These populations were obtained from selection for reproduction either at an early (E) or late (L) age (Tucić et al., 1996, 1997). Females in the L treatment were selected for postponed senescence by allowing the adults to mate freely for a period of time before the eggs were collected, which resulted in a decreased resistance to remating and polyandrous mating behavior (a proxy for overall intensity of sexual selection). At the same time, L males evolved under elevated post-copulatory sperm competition and exhibited an increase in male courtship persistence and mating speed (Šešlija et al., 2009). On the other side, the E selection treatment, where only very young adults were allowed to reproduce, promoted the evolution of short-lived individuals and a monogamous mating system.

The primary goal of our study is to shed more light on the role of conflict between members of the same sex over the opportunity to mate in shaping sex-specific longevity and mortality rates. We compared the E and L populations with regard to the direction of sex differences in longevity and mortality rates in order to test Promislow’s (2003) hypothesis that sexual selection could lead to the evolution of sex differences in longevity. We predict a low level of differences in longevity between the sexes in the E populations because the males and females evolved under conditions that reduce the expression of sexually selected costly traits.

On the other hand, in the L selection treatment, which provides opportunities for the evolution of a polygamous mating system and intense sexual selection, we expect substantial sex differences in longevity.

MATERIALS AND METHODS

Life history and experimental conditions

The seed beetle, *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae: Bruchidae) is a cosmopolitan pest of stored legumes. The primary host of these weevils is the common bean, *Phaseolus vulgaris*. Populations of *A. obtectus* are most commonly detected in stores of dried legumes; their life cycle appears well-adapted for reproduction in a storage environment. This beetle is aphygous as an adult, and the amount of resources gained during larval development thus determine the resources available for allocation later in life. Larval development takes place in plant seeds and the adult emerges at the final molt. *A. obtectus* males are protandrous. Females do not remate until a few days after their initial mating (Huignard 1974). Since female resistance to remating is very high, some authors regard *A. obtectus* as being monoandrous (Jermy 1970; cited in Maklakov et al. 2007). *A. obtectus*, like a number of other insect species, exhibits a transfer of male secretions from the spermatophore to the female hemolymph (Das et al. 1980).

All experiments reported here were performed in a dark incubator at about 30°C and 70% humidity. All seeds were brought from one source and were frozen before their use in the experiments. No food or water was offered to experimental adults.

Experimental populations

The base population, from which all selection lines were originally derived, was obtained by mass-mating beetles from three different locations in the vicinity of Belgrade (Tucić et al. 1996). Experimental females and males originated from the lines of *A. obtectus* on which selection had been imposed for

early (E) and late (L) reproduction. Four replicates per selection treatment were maintained under adult aphygy.

Through the course of selection for early reproduction (E), about 300-500 females and males were kept together with beans and allowed to reproduce freely for 48 h after emergence; then the adults were removed. Since the females of this beetle species did not remate during this period, the E selection treatment inadvertently created conditions for the evolution of a monogamous mating system. Indeed, the rate of a female remating within the E treatment was very low, about 13% (Šešlija et al. 2009). This treatment gave rise to beetles with enhanced fitness during early life and a short life span. In the present study, we used E populations selected for 236 generations.

The L beetles used in this experiment were obtained from replicate populations selected for 167 generations. To establish each generation in the L selection treatment, over 1,000 females and males were kept together in about 10 separate vials (i.e., about 100 individuals per vial (diameter = 3 cm x height = 4 cm) thus, at a very high adult density) without beans and were allowed to mate from emergence until death. The beetles were introduced into bottles with about 100 bean seeds at day 10 and thus the eggs laid prior to this day did not contribute to the next generation (for details see Tucić et al. 1996). The adults of both sexes from the L treatment were bigger and lived longer than those from the E treatment. Because the females and males were kept together during their whole life, there was an opportunity for the females to remate and for the evolution of a polyandrous mating system. The estimated rates of female remating in the replicate populations within the L treatment varied from 59% to 66% (Šešlija et al. 2009).

Prior to the start of the present experiments, we kept all replicate populations at a population size of about 1,000 individuals under common housing regimes and completely relaxed selection for two generations.

Female and male longevities

Longevity assays were performed using mated and virgin females and males. For the study of longevity when females and males were mated, we paired about 30 newly emerged females and males from each of the four replicate populations within both selection regimes. The beetles were weighed and then placed in separate 35mm Petri dishes with a single bean seed. The beetle pairs spent their whole life together and the number of eggs laid and dead females and males were counted every day.

To consider virginity as a completely 'cost-free' state, we followed the procedure of other studies concerned with the mortality effects of mating and reproduction, and kept the newly emerged virgin females and males from both E and L populations isolated individually during their whole life in separate vials without bean seeds and any food or water supplies. The numbers of dead individuals were recorded daily.

Data analysis

To examine whether the body weight differed between females and males within selection treatments, we used mixed-effect nested ANOVAs (SAS Institute 2004), where the replicate populations (random factor) were nested within each sex (fixed factor). Because body size was positively correlated with longevity, the appropriate ANCOVA models were applied for the longevity data.

The longevity may differ due to differences in the baseline mortality rate and/or the exponential increase in mortality with age. For each group of experimental beetles we used WinModest software (Pletcher 1999) to describe the mortality function according to the Gompertz model. This mortality function has the form $u_x = ae^{bx}$, where u_x is the predicted instantaneous mortality rate at age x , a is the baseline or age-independent mortality rate, and b is the exponential increase in mortality with age. These parameters were estimated using the maximum likelihood estimation procedure of WinModest. To test

whether individual parameter estimates (a and b) differed significantly between the sexes, we used the log-likelihood-ratio test of WinModest.

RESULTS

As in all previous studies of *A. obtectus* populations, E females and males have a lower body weight than L beetles of both sexes (Fig. 1A). As expected for this beetle species, the females were heavier than the males ($F_{1,6} = 76.8$ for E treatment, $F_{1,6} = 32.9$ for L treatment, $P < 0.001$ for each). Interestingly, the body size differences between the genders were almost identical for both selection regimes; the females were about 20% heavier than the males.

Mean longevity also differed substantially between the populations; the L beetles lived longer than the E beetles, regardless of sex (Fig. 1 B and C). Since *A. obtectus* is a capital breeder (i.e. relying primarily on larval-derived resources which affect adult size) it could be expected that body weight explains much of the differences in longevity between the populations and females and males. Indeed, in both treatments larger individuals lived longer than smaller individuals, regardless of sex. Body weight explained a smaller percentage of the total variation in longevity in the E selection treatment ($R^2 = 0.04$, $P > 0.05$) than in the L treatment ($R^2 = 0.34$, $P < 0.01$). There was no evidence that the relationship between body weight and longevity differed between the sexes (no significant body weight \times sex interaction in an analysis of covariance), so we used ANOVA instead of ANCOVA in further analyses of gender differences in longevities.

In the L populations the females lived, on average, longer than the males ($F_{1,6} = 64.9$, $P < 0.001$ for mated beetles, $F_{1,6} = 6.9$, $P < 0.05$ for virgins; Fig. 1C). In contrast to the L populations, the average longevities of females and males in the E line were not different from each other for mated individuals ($F_{1,6} = 4.3$, $P > 0.05$), whereas virgin E males outlived females by about 8% ($F_{1,6} = 6.2$, $P < 0.05$; Fig. 1B).

Also, in the E populations virgin males lived, on average, about 3 days longer (or 29%) than mated

Figure 1.

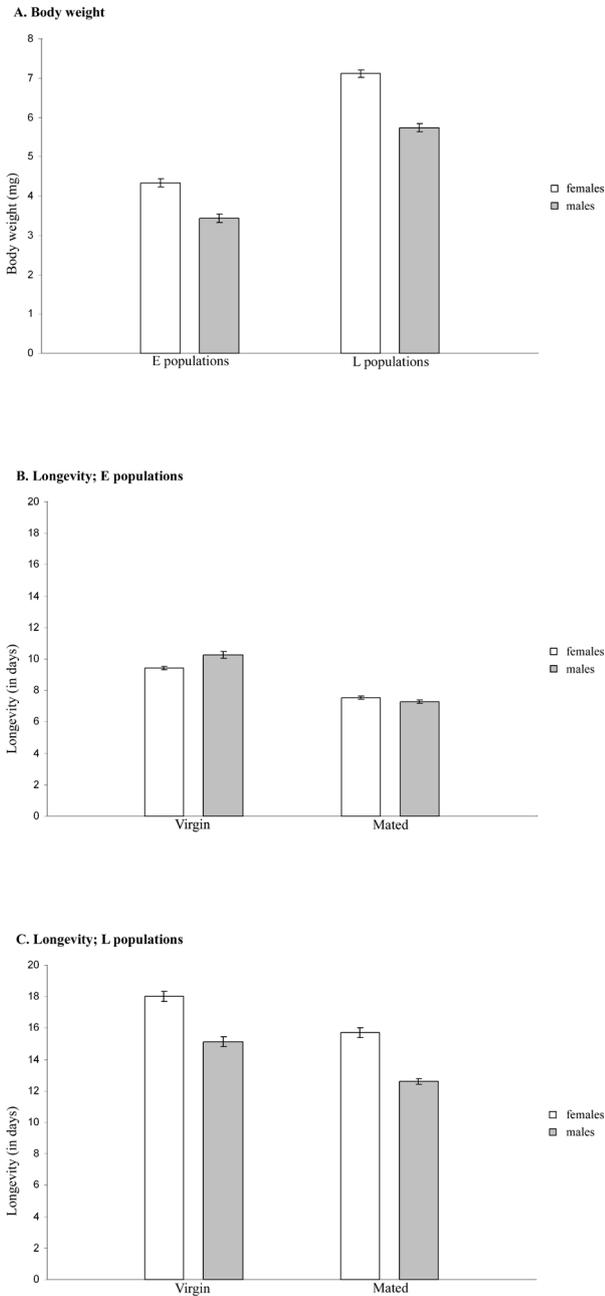


Fig. 1. Mean (\pm SE) body weight (A), longevity in the E populations (B) and in the L populations (C) of *Acanthoscelides obtectus* mated or virgin females and males.

Table 1. Parameter values for the Gompertz model of virgin and mated E and L females and males. a = the baseline mortality rate; b = the rate of senescence; MRDT (mortality rate doubling time) = $\ln 2/b$.

	Parameter estimates (95% confidence intervals)		MRDT (in days)
	a	b	
	E populations		
Virgin			
Females	0.83 (0.37, 1.86)	0.65 (0.57, 0.74)	1.06
Males	2.74 (1.54, 4.87)	0.451 (0.39, 0.51)	1.54
χ^2	16.62 (P=0.000)	5.84 (P=0.02)	
Mated			
Females	2.61 (1.31, 5.21)	0.67 (0.59, 0.77)	1.02
Males	0.72 (0.29, 1.81)	0.91 (0.79, 1.05)	0.76
χ^2	5.18 (P=0.02)	5.13 (P=0.02)	
L populations			
Virgin			
Females	2.22 (1.38, 3.57)	0.23 (0.21, 0.26)	2.97
Males	2.89 (1.69, 4.94)	0.27 (0.24, 0.30)	2.57
χ^2	2.92 (P=0.09)	0.53 (P=0.47)	
Mated			
Females	1.76 (0.92, 3.38)	0.29 (0.26, 0.34)	2.33
Males	0.27 (0.10, 0.70)	0.57 (0.49, 0.65)	1.22
χ^2	44.02 (P=0.000)	11.08 (P=0.0001)	

males ($F_{1,6} = 103.1$, $P < 0.001$), whereas in the L populations a similar trend was observed, even though the mated males lived only about 17% shorter than virgin ones ($F_{1,6} = 17.5$, $P < 0.01$).

In order to understand the observed longevity differences between females and males we compared the parameters of the Gompertz's model. The analysis of the initial or baseline mortality rate (a), and the rate of exponential increase in mortality rates with age or rate of ageing (b), revealed significant differences between both virgin and mated females and

males from the E population (Table 1). Interestingly, in both groups of E beetles, an inverse relationship was observed between the a and b parameters. Although virgin E males exhibited a significantly higher baseline mortality rate (a) than virgin E females ($\chi^2_{(1)} = 16.62$, $P < 0.001$), it seems that the lower exponential increase in mortality with age (b) produced the shift in longevity for males relative to females that can be seen in Fig. 1B.

The rate of exponential increase in mortality with age (b) showed higher values for L males compared with L females, but was significantly higher only for the mated beetles ($\chi^2_{(1)} = 11.1$, $P < 0.001$). Intriguingly, despite the fact that there were significant differences in the longevities of virgin L females and males, we did not find statistically significant differences between the a and b model parameters. However, both of these parameters have higher values in males than in females, indicating that their small but synergetic effects increase the longevity of females relative to males.

DISCUSSION

In the base population from which our lines were derived, the average longevities of mated females were about 25% higher than in males (Tucić et al., 1996). The results of the present study showed that this sexual difference in longevity persisted in the L populations, whereas in the E populations we did not detect differences in mortality patterns between mated females and males. Moreover, virgin E males lived about 8% longer than virgin females.

Gender differences in longevity within the E treatment

Since our E selection regime resulted in a marked relaxation of sexual selection compared with the ancestral conditions, as well as those prevailing in the L selection regime, we suggest that these circumstances may have led to the elimination of differences in longevity between mated females and males. If the consequence of monogamy in the E line was reduced sperm competition among males,

as suggested by Šešlija et al. (2009), this released both sexes from the adaptations used in sexual conflict. There are several costly traits that may have been reduced as a result of the removal of sexual antagonism. For example, as we pointed out earlier, sperm competition is thought to be responsible for the maintenance of substances in ejaculates that are unfavorable for females (Das et al., 1980), or for the evolution of male genitalia that harm females (Edvardsson and Tregenza, 2005). Thus, the removal of sperm competition may have promoted the evolution of mates that are more benign towards females. Also, under conditions that do not impose costly counter-adaptations, the males did not trade-off resources between traits associated with male-to-male competition and longevity.

Additionally, besides the possibility that in the E populations antagonistic coevolution between females and males, or inter-locus conflict (Arnqvist and Rowe, 2005) did not play an important role in shaping longevity, it seemed that intra-locus conflict was also relatively weak or absent in this line. Note that, while inter-locus sexual conflict involves different genes in each sex, in intra-locus conflict the same allelic variation has opposite effects on fitness when expressed in a male or a female. Although intra-locus sexual conflict has not been directly measured, we believe that the prevailing conditions in the E selection regime were inadequate for selecting a different optimal longevity in females and males – a characteristic that is thought to be an important determinant of intra-locus sexual conflict (see e.g. Zajitschek et al., 2007). If our interpretation is correct, the imposed selection for early reproduction should lead to accelerated senescence with similar intensity in both sexes. It is noteworthy that this conclusion is congruent with the results of divergent selection on male longevity in the black field cricket, *Teleogryllus commodus*, where only selection for a shorter male longevity yielded a strong correlated response in female longevity (Hunt et al., 2006). Therefore, we suggest that the absence of sexual difference in the mated beetles in the E populations is in broad agreement with the inadvertent removal of both inter-locus and intra-locus sexual conflict.

However, an important question remains - how can virgin E male senescence be postponed beyond the level exhibited by virgin E females, even though the males have a higher baseline mortality rate (*a*), which is related to the genetically determined vigor of the individuals? The lower exponential increase in mortality with age (*b*), which can be seen in the virgin males (Table 1) could be, at least in part, a consequence of the elevated activity of the E females relative to males (personal observation, BS). Additionally, it is possible that an elevated homosexual activity, which is more pronounced in the E females than males (Stojković et al., 2010), is positively correlated with the general activity of beetles. Therefore, in accordance with “rate of living theory” (Pearl, 1928), relatively active E females and “lazy” (Stojković et al., 2010) E males will produce the pattern of longevity between virgin females and males observed in these populations.

Gender differences in longevity within the L treatment

The elevated sexual conflict in the L selection regime provides a potential explanation for the sex differences in longevity observed in these populations. If males in the L populations are exposed to intense pre- and post-copulatory sexual selection, they need to trade-off resources between traits associated with male-to-male competition and longevity-increasing traits. Note that this longevity cost for males will be present regardless of their mating status; both virgin and copulated males produce various sperm and ejaculate substances. On the other hand, it is very plausible that evolved costly counter-adaptations in L females, which prevent the toxic effect of the proteinaceous accessory seminal substances of their mates (Das et al., 1980), are inducible, i.e. they are synthesized only if copulation has occurred. This could explain, at least in part, the observed sex differences in longevity within the L treatment.

The negative impact of copulation on male longevity has been recently well documented in different insect species (see Arnqvist and Rowe, 2005; Rankin and Kokko, 2007). Tucić et al. (1996) have

also shown that the copulatory activity of *A. obtectus* males substantially reduces their longevity. Interestingly, Šešlija et al. (2008) found that female longevity was not influenced by copulation with L males; virgin and mated females (when females were not allowed to lay eggs) did not differ from each other with respect to mortality patterns. This indicates that copulation *per se* does not reduce female longevity in the L line. This is in sharp contrast to one other seed beetle, *C. maculatus*, where virgin females, if evolved in populations with intense sexual selection, had a lower longevity than females from monogamous populations (Maklakov et al., 2007). Contrasting results with respect to copulation costs of females were also found in two related species of dung flies. In *Saltella sphondylli* copulation had no significant effect on female longevity (Martin and Hosken, 2003), whereas in *S. cynipsea* female longevity was dramatically reduced by copulation (Blanckenhorn et al., 2002). The observed intra-species variation in female longevity costs of copulation likely reflects a variation in the idiosyncratic adaptations which can modulate the costs and benefits of mating (Promislow, 2003; Maklakov et al., 2007). Although empirical data directly addressing this question are absent, it can be argued that sexual selection may even increase the rate of fixation of beneficial mutations leading to such adaptations.

We also note that, because females of the seed beetle have to contact host seeds for ovary maturation, the trait under selection in the L regime was the age at physiological maturity of females, whereas the reproductive activity schedules of the L males did not play an important role (Tucić et al. 1996). In this case, selection should lead to a different optimal longevity in females and males. We therefore suggest that intra-locus conflict might have been among the forces that produced a sex difference in longevity observed in the L populations.

Acknowledgments - We are grateful to Prof. N. Tucić for stimulating discussions about sexual conflict and longevity cost and for constructive suggestions for an early draft of the manuscript. This study was supported by the Ministry of Sci-

ence and Technological Development of Serbia, project No. 173007.

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