

Ageing and the evolution of female resistance to remating in seed beetles

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Female remating behaviour is a key mating system parameter that is predicted to evolve according to the net effect of remating on female fitness. In many taxa, females commonly resist male remating attempts because of the costs of mating. Here, we use replicated populations of the seed beetle *Acanthoscelides obtectus* selected for either early or late life reproduction and show that 'Early' and 'Late' females evolved different age-specific rates of remating. Early females were more likely to remate with control males as they aged, while Late females were more resistant to remating later in life. Thus, female remating rate decreases with age when direct selection on late-life fitness is operating and increases when such selection is relaxed. Our findings not only demonstrate that female resistance to remating can evolve rapidly, but also that such evolution is in accordance with the genetic interests of females.

Keywords: sexual conflict; senescence; mating frequency; experimental evolution; polyandry

1. INTRODUCTION

Despite its central importance, our understanding of the evolution of female remating rate is limited (Arnqvist & Nilsson 2000; Jennions & Petrie 2000). The sexes often have divergent interests in mating and remating rates can thus reflect the outcome of sexually antagonistic coevolution (Parker 1979; Holland & Rice 1999; Arnqvist & Rowe 2002; Chapman *et al.* 2003; Arnqvist & Rowe 2005). Female remating is a key mating system parameter that dictates the pattern of sexual competition and coevolutionary dynamics (see Shuster & Wade 2003). Therefore, the evolutionary causes and consequences of female remating rate are at the heart of sexual selection (Arnqvist & Nilsson 2000; Wiklund *et al.* 2001; Arnqvist *et al.* 2005; Maklakov *et al.* 2005a) and selection on female remating rate has profound effects on female longevity and reproductive success (Holland & Rice 1999; Martin & Hosken 2004; Martin *et al.* 2004). However, if female remating rate represents an adaptation subject to life history trade-offs, we would expect female resistance to remating to evolve under selection on female life histories.

Two studies have examined the effects of selection on female life histories on remating frequency, both using lines of fruit flies, *Drosophila melanogaster*, selected for reproduction early and late in life.

Pletcher *et al.* (1997) found no significant effect of selection for early (henceforth 'Early' lines) and late (henceforth 'Late' lines) age at reproduction on female mating probability, although they only assayed female mating rate early in life. In contrast, Sgro *et al.* (2000) found higher late-life mating frequencies for flies in Late lines and higher early-life mating frequencies for Early lines.

Here, we used replicated populations of the seed beetle *Acanthoscelides obtectus* (Bruchidae) selected for early and late age at reproduction to test for evolution of female remating rates. In bruchid beetles, remating carries direct costs (Das *et al.* 1980; Crudgington & Siva-Jothy 2000) and resisting courting males has immediate fitness effects (Arnqvist *et al.* 2005; Edvardsson & Tregenza 2005). We thus expect Early females to evolve higher rates of resistance (i.e. lower remating rates) early in life than at an older age, when there is no selection. In contrast, Late line females should remain resistant or may even increase resistance (i.e. lower remating rates) later in life.

2. MATERIAL AND METHODS

(a) Selection lines

The selection lines had been maintained under Late (late age of reproduction; $N=4$) or Early (early age at reproduction; $N=4$) selection regimes since 1989 (109 generations for L lines and 150 for E lines) under adult aphagy (see Tucic *et al.* 1996, 1998). We maintained the lines at 28 °C and 65% RH and beetles were reared on common beans, *Phaseolus vulgaris*. During selection, males and females in Early 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 Late 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; see 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 three generations (first block) and five generations (second block) prior to the start of the experiments.

(b) Experimental procedure

All females were virgin and 48 h old at the start of the experiments. Control virgin males from the base population were produced continuously during the experiment, and we only used virgin males that were 48–72 h old in all of our mating trials to control for confounding effects of male age and mating history. We placed each female with a randomly selected control male 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 per mating trial, males and females were separated immediately after they completed copulation. All virgin females were first mated once to a control male on day 2 after hatching. In our subsequent remating assays, each female was exposed to two randomly selected virgin control males during remating trials at days 3, 7 and 11 after hatching and we observed their behaviour for 120 min. Remating rate was measured by following groups of females through time, in two replicate blocks each containing one group per line (seven females per group in block 1 and eight in block 2). Females that remated during one trial were not exposed to males in subsequent trials. Our measure of age-specific remating rate was formed by dividing the number of females in a group that remated during a given trial with the total number of females in that group that were exposed to males in that trial. This proportion was then square-root arcsine transformed prior to statistical analyses.

(c) Data analysis

We analysed the effect of selection regime on female age-specific remating rate using a repeated measures analysis of variance, using SYSTAT 11, where each female group was considered an independent replicate (i.e. subject), treating selection as a between-subjects factor and female age and block as within-subjects factors. The distribution of pooled residuals from this model (see below) was not significantly different from normality (Shapiro–Wilk test; $p=0.588$). *Post hoc* tests were employed to test for differences

in remating rate over time within selection regimes. To compensate for multiple *post hoc* tests, we controlled for false discovery rate and consequently report adjusted *p*-values (i.e. *q*-values; Storey 2003).

3. RESULTS

In total, 47% of all females remated during our trials. There was no main effect of selection on female remating rate (see table 1). There was, however, a significant effect of female age on remating rate. As predicted, we also found a significant interaction between female age and selection, showing that age-specific remating rates differed in the two selection regimes. An assessment of the polynomial contrasts from our inferential model indicated that the significant interaction between female age and selection was mainly due to a difference in the linear effect (first-order polynomial: $F_{1,6}=5.12$) rather than in a non-linear trend (second-order polynomial $F_{1,6}=2.01$). This was supported by the *post hoc* tests (see table 2 and figure 1), that identified a significant increase in female remating rate with age in the Early lines and a significant, although less marked, decrease in remating rate with age in the Late lines.

4. DISCUSSION

Female age-specific remating rates have evolved in *A. obtectus* as a result of adaptation to different life-history conditions, such that remating rate increased with age in Early lines but not in Late lines. Females from Early lines have evolved under conditions where fitness is gained early in life while females from Late lines evolved under conditions where fitness is accrued late in life (Tucic *et al.* 1996, 1998). In essence, our results thus show that female resistance to remating decreases with age when selection late in life is absent and increases with age when selection late in life is intense. Our results differ from earlier work on *Drosophila*, where female remating rates were reported to decrease with age in Early lines relative to late lines (Sgro *et al.* 2000). Although, Sgro *et al.* (2000) ascribed their results to a more rapid somatic deterioration with age in Early females, we note that their study did not control for effects of male mating frequency. Male flies cohabiting with Early females mated at a higher rate early in life, which should reduce male persistence in courtship later in life. It is thus difficult to directly compare the results of the two studies.

Mating can reduce female lifespan via harassment by males, by increased exposure to predators and/or energetic costs (Arnqvist 1997), physical injuries (Crudginton & Siva-Jothy 2000; Blanckenhorn *et al.* 2002), or seminal compounds with toxic side-effects (Das *et al.* 1980; Chapman *et al.* 1995). However, mating can also prolong female lifespan by providing females with water and/or nutrients contained in male ejaculates (Maklakov *et al.* 2005b; reviewed in Eberhard 1996; Arnqvist & Nilsson 2000; Simmons 2001). The trade-off between the costs and benefits of remating, as well as that between the costs and benefits of resisting remating, can be complex and the relationship between female fitness and remating rate may often be nonlinear (e.g. Arnqvist & Nilsson

Table 1. Repeated measures analysis of variance of female remating rate.

source	d.f.	<i>F</i>	<i>p</i>
<i>between subjects</i>			
selection	1	1.544	0.26
error	6		
<i>within subjects</i>			
block	1	0.257	0.63
selection × block	1	0.166	0.698
error	6		
age	2	8.994	0.004
selection × age	2	4.046	0.045
block × age	2	2.94	0.092
selection × block × age	2	0.507	0.615
error	12		

Table 2. Effect sizes, 95% confidence intervals (CI) and *p*-values adjusted for false discovery rate (i.e. *q*-values) for *post hoc* tests of differences in female remating rate within selection regimes (see figure 1).

selection regime	level of comparison (days)	mean difference	95% CI (from-to)	adjusted <i>p</i> -value
Early	3–7	−0.521	−0.749 to −0.239	0.006
	3–11	−0.229	−1.435 to 0.976	0.372
	7–11	0.292	−0.857 to 1.44	0.306
Late	3–7	−1.12	−0.85 to 0.61	0.372
	3–11	0.219	−0.15 to 0.588	0.066
	7–11	0.339	−0.049 to 0.727	0.036

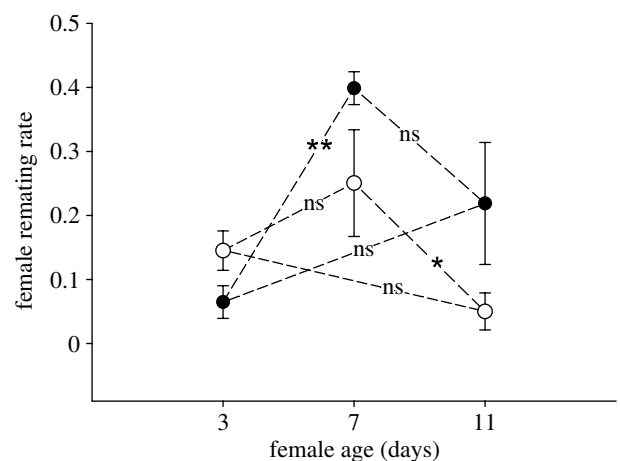


Figure 1. Changes in female remating rates with age in 'Early' lines (filled dots) and 'Late' lines (empty dots). Remating rates represent mean (\pm s.e.) averaged across the two blocks. Asterisks indicate differences in remating rates that are significantly different within selection regimes and 'ns' indicates non-significant differences (see table 2 for adjusted *p*-values and effect sizes). An overall statistical evaluation is presented in table 1.

2000; Arnqvist *et al.* 2005). Three facts collectively suggest that the costs of remating often outweigh any benefits in *A. obtectus*. First, females are very resistant to remating (Huignard 1974). In our study, females were constantly harassed by two virgin males at each exposure. Yet, only 47% of all females remated during the entire experiment. Females from the Early selection regime are likely to be effectively monandrous despite the presence of males. Second, there are direct costs of mating, caused by toxic seminal compounds (Das *et al.* 1980) and probably also by physical injury (Crudginton & Siva-Jothy 2000). Third, the pattern of evolution of age-specific remating rates documented here can be understood only if rematings carry net costs to females. Most importantly, females evolved increased resistance to remating with age when selected for long lifespan while resistance to remating decreased with age under relaxed selection for longevity.

In conclusion, we show that females that experience different life-history conditions evolve different age-specific remating rates and that this evolution is consistent with sexual conflict over remating. We clearly need more experimental studies across different taxa to determine the general pattern of evolutionary modulations of female remating behaviour as a result of changes in life-history conditions.

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