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SEX DIFFERENCES, SEXUAL SELECTION, AND AGEING: AN EXPERIMENTAL EVOLUTION APPROACH

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Life-history (LH) theory predicts that selection will optimize the trade-off between reproduction and somatic maintenance. Reproductive ageing and finite life span are direct consequences of such optimization. Sexual selection and conflict profoundly affect the reproductive strategies of the sexes and thus can play an important role in the evolution of life span and ageing. In theory, sexual selection can favor the evolution of either faster or slower ageing, but the evidence is equivocal. We used a novel selection experiment to investigate the potential of sexual selection to influence the adaptive evolution of age-specific LH traits. We selected replicate populations of the seed beetle *Callosobruchus maculatus* for age at reproduction ("Young" and "Old") either with or without sexual selection. We found that LH selection resulted in the evolution of age-specific reproduction and mortality but these changes were largely unaffected by sexual selection. Sexual selection depressed net reproductive performance and failed to promote adaptation. Nonetheless, the evolution of several traits differed between males and females. These data challenge the importance of current sexual selection in promoting rapid adaptation to environmental change but support the hypothesis that sex differences in LH—a historical signature of sexual selection—are key in shaping trait responses to novel selection.

KEY WORDS: **Aging, actuarial, life-history, life span, reproductive, sex-specific selection**

Ageing is a nearly universal characteristic of living beings (reviewed in Hughes and Reynolds 2005; but see Sparkman et al. 2007), manifested as decreasing reproductive performance and increasing probability of death with age due to physiological deterioration (Rose 1991; Charlesworth 1994; Hughes and Reynolds 2005). The evolutionary theory of ageing rests on the fact that the probability of survival declines with age due to extrinsic mortality (Medawar 1952; Hamilton 1966; Rose 1991; Partridge and Barton 1993; Houle et al. 1994; Reynolds et al. 2007). This decline can result in accumulation of alleles with slightly deleterious effects that are most pronounced late in life (mutation accumulation theory, [Medawar 1952]) and/or alleles that increase fitness early in life at the cost of decreased fitness late in life (antagonistic pleiotropy theory, [Williams 1957]). Antagonistic pleiotropy reflects the optimization of the genetic trade-off between early- and late-life performance whereas mutation accumulation is essentially maladaptive (Partridge and Barton 1993). These theories are not mutually exclusive, however, and their relative importance remains unclear (Hamilton 1966; Rose 1991; Stearns 1992; Charlesworth 1994; Hughes and Reynolds 2005).

Sexual reproduction and anisogamy typically result in profound differences in life history (LH) between the sexes. Sexual selection and sexual conflict (Parker 1979; Andersson 1994; Arnqvist and Rowe 2005) alter the overall costs of reproduction and the benefits of early versus late reproduction for each sex, and can therefore affect the evolution of ageing by modifying sex-specific LH optima, and by shaping sex-specific mortality rates (Promislow 2003; Bonduriansky et al. 2008). This means that sexual coevolution, whether antagonistic or mutualistic, could contribute to the evolution of ageing via both

antagonistic pleiotropy and mutation accumulation. However, much controversy remains over the role of sexual selection, and the sexual conflict that inevitably results from sexual selection, in the evolution of ageing and life span.

Several theoretical ideas link sexual selection with ageing (reviewed in Bonduriansky et al. 2008). The potential for males to gain large, short-term fitness payoffs results in higher variance in mating success in males compared to females (Bateman 1948; Trivers 1972), and may select for male reproductive strategies that sacrifice longevity for mating opportunities (Vinogradov 1998). In support of this hypothesis, male crickets selected for shorter life span increase early reproductive performance (calling), whereas female longevity was reduced without affecting their reproductive performance (Hunt et al. 2006). The observation that males in most taxa are more likely to develop costly secondary sexual traits, and to engage in combat, has been put forward as a general explanation for sex differences in mortality rates (Trivers 1972; Promislow 1992; Liker and Szekely 2005; Clutton-Brock and Isvaram 2007). However, whether males' higher mortality rates necessarily select for faster ageing in males, as originally suggested by Williams (1957), is far from clear (Abrams 1993; Williams and Day 2003; Williams et al. 2006; Graves 2007, also see below). Sexual selection could also, in theory, decelerate ageing. If sexual selection results in increased male mating rate with age (Kokko 1997), this may lead to the evolution of slower ageing in males (Partridge and Barton 1996; Graves 2007).

Theoretically, sexual conflict (here, interlocus sexual conflict that modifies the direct costs of mating and reproduction) could promote either accelerated or decelerated ageing in both sexes. Sexual conflict can lead to increased rates of mortality (Svensson and Sheldon 1998), thereby reducing selection on late-life performance, which in turn could lead to the evolution of accelerated ageing (Promislow 2003). Again, however, increased mortality due to sexual conflict need not necessarily result in the evolution of accelerated ageing. Bonduriansky et al. (2008) suggested that this prediction can be reversed, provided that sexual conflict results in selection for somatic condition and vigor, analogous to the hypothesis that high predation rate may lead to the evolution of more robust organisms with slower ageing (Abrams 1993; Williams and Day 2003; Williams et al. 2006). Thus, the roles of sexual selection and sexual conflict in the evolution of ageing are likely to be complex. Empirical tests of these ideas are scarce, and published tests of how these processes may interact are, to the best of our knowledge, nonexistent.

The consequences of sexual selection for population mean fitness and adaptation to changed conditions are controversial as well. It has been suggested that sexual selection can enhance population mean fitness by facilitating the removal of deleterious mutations (Whitlock 2000; Agrawal 2001; Siller 2001), and accelerate the adaptation to new environments by hastening fixation of beneficial alleles (Whitlock 2000; Lorch et al. 2003). However, these hypotheses are opposed by sexual conflict theory, which suggests that sexual selection is likely to reduce population mean fitness and impede adaptation. Interlocus sexual conflict reflects direct harm that males impose on females (Parker 1979; Rice 1996; Arnqvist and Rowe 2005). Such harm should be opposed by natural selection when sexual selection is removed (Holland and Rice 1999; Martin and Hosken 2003; Martin et al. 2004; Crudgington et al. 2005; Tilszer et al. 2006), resulting in relatively higher fitness in monogamous populations (but see Crudgington et al. 2005; Simmons and Garcia-Gonzalez 2008). Populations evolving in the absence of sexual selection would, in theory, also experience weaker intralocus sexual conflict (Chippindale et al. 2001; Bonduriansky and Chenoweth 2009). Removal of the "sexual selection load" (Lande 1980; Houle and Kondrashov 2002) could therefore be expected to enhance the fitness of monogamous populations compared to polygamous populations. Moreover, sexually antagonistic coevolution could also impede adaptation to novel environmental conditions (Gavrilets et al. 2001; Kokko and Brooks 2003). Only three experimental evolution studies have addressed the role of sexual selection in adaptive evolution of LH traits in a changed environment, and the results are contrasting and support all possible outcomes: positive (Fricke and Arnqvist 2007), negative (Rundle et al. 2006), and null (Holland 2002) effects of sexual selection on the rate of adaptation.

Several experimental evolution studies have addressed the effects of sexual selection on the evolution of LH and ageing, but the results are inconsistent. A study of *Drosophila melanogaster*, where opportunity for sexual selection was manipulated for 10 generations by pairing female flies with either one or five different males, suggested that sexual selection increases adult viability of both sexes (Promislow et al. 1998). In this experiment, however, the flies were only allowed to mate once, thereby significantly reducing the opportunity for sexual conflict (prevalent in *D. melanogaster*, Rice et al. 2006), which could alter the effect of sexual selection on life span evolution (Promislow 2003). Studies in several taxa have indicated that increased sexual conflict reduces female life span (Holland and Rice 1999; Martin and Hosken 2003; Maklakov et al. 2007).

One problem that is shared by all of these studies is that they either manipulated LH evolution, which may inadvertently affect the mating system (MS), or manipulated MS, which may inadvertently affect LH evolution. This obviously complicates the interpretation of the results (Bonduriansky et al. 2008). For example, Maklakov et al. (2005) found that, in a bean weevil *Acanthoscelides obtectus*, females had increased background mortality but a decelerated rate of actuarial ageing (rate of increase in mortality with age) when mated to males from long-lived populations. These results could reflect increased sexual conflict in long-lived

lines, and males could therefore be caught in an evolutionary dilemma between the necessities of winning in sperm competition versus prolonging their mates' life span. Recently, Seslija et al. (2008, 2009) provided further discussion of the potential role of inadvertent manipulation of MS under different LH schedules for trait evolution in *A. obtectus*. The only way to avoid such problems and to directly test for the role of sexual selection in the evolution of age-specific traits is to experimentally impose different LH schedules while simultaneously manipulating the opportunity for sexual selection (Bonduriansky et al. 2008). In the present study, we adopted this approach by selecting replicate populations of the seed beetle *Callosobruchus maculatus*, for age at reproduction, while either allowing for sexual selection to operate under a natural level of polygamy or removing the opportunity for sexual selection by enforcing true random monogamy. This design allows us to investigate the roles of LH selection, sexual selection, and their interaction in the ongoing evolution of LH traits.

We predicted that LH selection for late reproduction (Old lines) would increase intrinsic life span and late-life reproductive performance of beetles compared to beetles selected for early reproduction (Young lines). Antagonistic pleiotropy theory (but not mutation accumulation theory) predicts that such an increase would be accompanied by a reduction in early life performance (Luckinbill et al. 1984; Rose 1984; Tucic et al. 1996; Partridge et al. 1999). Based on sexual conflict theory, we also predicted that experimental removal of sexual selection would enhance population mean fitness and hasten adaptation to a novel reproductive schedule (Rundle et al. 2006). Because males are expected to gain more by sacrificing life span for mating opportunities (Bonduriansky et al. 2008), we also predicted that male life span would respond more rapidly to selection on age at reproduction than female life span. *Callosobruchus maculatus* is a capital breeder and female fecundity is likely to correlate with virgin female life span regardless of LH selection because, in the absence of egg-laying substrate, female resources will be diverted to somatic maintenance. On the contrary, male mating success depends largely on male mate-searching activity, and males can mate multiply. Males are, therefore, more likely to gain fitness through strategies that increase mating opportunities at the cost of increased mortality risk.

To address these questions, we quantified life span and actuarial and reproductive ageing, as well as several correlated LH traits, in experimental Old and Young populations evolving with and without sexual selection. We found that LH selection resulted in rapid adaptive evolution of most traits measured. We also observed significant SEX $\times$ LH selection interactions, indicating that the sexes responded differently to selection on LH. However, sexual selection depressed population mean fitness, and had little effect on the rate of LH evolution.

Materials and Methods

STUDY ANIMALS

The seed beetle *C. maculatus* is an extensively studied model for evolutionary and LH studies (e.g., Fox 1993; Messina 1993; Tatar et al. 1993). *Callosobruchus maculatus* is a facultative capital breeder with a world-wide distribution, and one of the main pests of legume crops. The species' ability to reproduce successfully in the absence of food and water in the adult stage is an important component of its success in grain storages and scientific laboratories, where most of the present-day populations have evolved for many generations. Females lay eggs on the surface of dry seeds and larvae bore inside the seeds upon hatching where they forage, grow, and pupate to emerge after 3–4 weeks as fully functional adults. These newly emerging beetles can mate and reproduce almost immediately upon hatching (Fox et al. 2003). Males spend all of their lives pursuing females and deliver substantial ejaculates (up to 10% of their body weight in one ejaculate (Savalli and Fox 1998)). Although females reject most male mating attempts, they are polyandrous, can remate within 24 h (Fricke and Arnqvist 2007), and mate several times in their lives (Arnqvist et al. 2005).

We used a local Australian population of seed beetles *C. maculatus* as a source population for our experiments. This population was obtained from the Department of Primary Industries and Fisheries (DPIF), Queensland in 2006, where it was previously held since 2003. The source population (called QCMK1 by DPIF) originated from a total of 357 inoculated mung beans (*Vigna radiata*) collected in Kingaroy. The population was propagated with 250–300 beetles per generation placed on 450–500 g on fresh mung beans on four-weeks generation cycle under 30°C, 70% relative humidity (RH), and 12:12 light:dark (L:D) cycle at DPIF facilities. We obtained a sample of inoculated mung beans in September 2006. This sample produced $\sim$ 600 beetles and we propagated the population in our laboratory with $\sim$ 500 beetles per generation on 200 g of fresh organic mung beans that were frozen prior to use, for six months prior to the start of the selection experiment. The only difference in rearing conditions between the two locations was 14:10 L:D cycle imposed in our laboratory.

EXPERIMENTAL EVOLUTION

We started by creating two different LH regimes with eight replicate populations in each that we refer to as Young and Old lines. To avoid problems related to potential differences in larval competition between treatments, all lines were provided with a number of beans that exceeded the potential number of laid eggs (150 g of beans $\times$ $\sim$12 beans per g = $\sim$1800 beans per line per generation; please see Results for the estimates of egg-to-adult survival and offspring production per female), which, in combination with the propensity of these beetles to avoid laying eggs next to "occupied" beans ensured that only one egg was normally laid for each

bean. All lines shared the following procedure: at the beginning of each generation we isolated 192 beans (four 48-well plates) with a single hatched egg on each bean and collected virgin males and females over three days after the start of hatching. We used between 100 and 114 beetles to propagate each population (see below) but the surplus was needed to compensate for larvae that did not eclose and to reduce inadvertent selection for early maturation. The latter was achieved by randomly selecting among hatched beetles after three days from the start of hatching when most beetles have already eclosed, rather than collecting the first ones that emerged.

Young lines were propagated by placing the appropriate number of beetles on the fresh beans for 24 h after which all adults were discarded. Old lines were propagated by placing the appropriate number of beetles in fresh beans for 72 h, after which the adults were placed on a new sample of beans and all previously laid eggs were discarded.

Within each of the above LH regimes, we further manipulated the MS: half of the lines were propagated under sexual selection by keeping all beetles in the population together, which mimics the recent evolutionary history of this population ("Polygamy"), whereas in the other half sexual selection was removed by enforcing true random monogamy ("Monogamy") by randomly pairing virgin males and females for life. We used 100 beetles for "Monogamous" populations and 114 beetles for "Polygamous" populations to compensate for the anticipated reduction in effective population size (N_e) in sexually selected lines. This adjustment was based on the approximation that female beetles will remate twice prior to laying those of their eggs that will contribute to the next generation, and data on the variance in female fecundity and male reproductive success (Eady 1994; Fricke and Arnqvist 2007). Additionally, the size of our experimental populations suggests that they are unlikely to differ in the level of inbreeding over very short evolutionary scale such as 12–13 generations of selection (see also Rice and Holland 2005). We also note that we found no difference in body mass, development time or life span between different MS treatments, whereas larva-to-adult survival in all experimental populations was similar to or higher than that in the source population (see Results). Preliminary trials showed that $> 98\%$ of beetles survived 72 h. Therefore, we did not adjust our population sizes for LH regimes.

In summary, we used a 2×2 experimental design with a total of 16 experimental populations, which allowed us to investigate the interaction between LH and MS in trait evolution.

TRAIT ASSAYS

All assays were conducted simultaneously after 13 (Young) and 11 (Old) generations of selection followed by one generation of relaxed selection when 50 virgin males and 50 virgin females from each population were placed on 350 g of fresh mung beans (enough to ensure $\sim$1 egg per bean) for life and their offspring were used in subsequent experiments.

(1) Population hatching time and virgin individual life span: One-hundred ninety-two single beans with one egg each were isolated in four 48-well plates from each population. Upon hatching, the beetles were sexed and then checked daily for mortality. The dates of hatching and subsequent death were recorded.
(2) Body mass and virgin cohort life span: Hatching virgin beetles were isolated from another set of 48-well plates as in assay 1 over a 24-h period. The beetles were haphazardly assigned to single-sex cohorts of 20, with two replicates per population per sex in 65 mm aerated Petri dishes. Each replica was weighed using a semi-analytical balance and checked daily for mortality.
(3) Reproductive schedule and mated cohort life span: Hatching virgin beetles were isolated from another set of two 48-well plates as in assay 2. The beetles were haphazardly assigned to groups of three males and three females and five such replicates were created per population. These replicate groups were placed in 65 mm aerated Petri dishes with 12 g of fresh mung beans. After 24 h, the beetles were removed from the original dish and placed into a new dish with 12 g of beans for another 48 h. After that, the beetles were again replaced and put into yet another set of dishes with 10 g of beans until their death. The amount of beans used at each period of time was sufficient to allow beetles to lay only one egg per bean. This treatment allowed us to quantify offspring production during the first 24 h and after the first 72 h, which corresponds to the LH schedule imposed by two of our experimental evolution treatments (Young and Old). We also checked the dishes daily to record the time of death of all beetles. We allowed for 35 days after egg-laying prior to counting emerged offspring (assay 1 showed that the longest hatching time was 32 days, with a median of 25 days).
(4) Resistance to delayed reproduction: Hatching beetles were isolated as in assay 3 and haphazardly assigned to groups of 10 males and 10 females. Two such replicates were created for each population. The beetles were placed in 65 mm Petri dishes and left for 10 days; then 25 g of fresh mung beans were introduced. The offspring produced were counted as in assay 3. This experiment tested the resistance of beetles to long periods of absence of suitable laying material.

STATISTICAL RATIONALE

We used general linear mixed models to test for the effect of selection regimes on trait evolution. LH, MS, and SEX (where appropriate) were modeled as fixed factors, whereas experimental population (Population) was modeled as a random factor nested within LH and MS. The inclusion of SEX resulted in a split-plot design that we accounted for by including a SEX $\times$ Population [LH, MS] interaction. The significance of the random variance

components was estimated using log-likelihood ratio tests by comparing the models with and without a given factor. SEX × Population [LH, MS] interaction could not explain any additional variance in any model and we therefore do not include it in the outcome Tables. Because virgin beetles were weighed (in groups) prior to the experiment, we tested for the potential role of body mass by including it as a covariate in the model of virgin cohort life span. However, the effect of body mass was nonsignificant ($P = 0.6974$) and was subsequently removed from the model. Data on larva-to-adult survival were arcsine-square-root transformed. In all models, we confirmed the assumption of normality by testing model residuals using Shapiro-Wilks test (all $P > 0.05$). The data on offspring production at different ages (assay 3) were analyzed with multivariate analysis of variance (MANOVA).

We used a maximum-likelihood approach implemented in WinModest software (Pletcher 1999) to fit mortality data to the Gompertz family of nested models (Gompertz, Gompertz-Makeham, Logistic and Logistic-Makeham) (Promislow et al. 1999). The best fit was decided based on likelihood-ratio tests. When beetles were kept singly, the best fit was provided by a Logistic model, $\mu_x = \alpha\, e^{\beta x}/[1 + (\alpha\, s/\beta)\,(e^{\beta x} - 1)]$, where μx is the mortality hazard at age x, α is the baseline mortality rate, and β is the rate of increase in mortality with age (Rate-of-senescence), and s is the rate of deceleration in mortality late in life (Late-life-deceleration). When beetles were kept in same-sex virgin cohorts, the best fit was provided by a Gompertz model, $\mu_x = \alpha\, e^{\beta x}$. This means that there was no significant late-life deceleration of mortality in most virgin cohorts, such that the parameter s equaled zero and the Logistic model collapsed to Gompertz, reflecting the nested nature of these descriptive models. We derived the estimates of α, β, and s (where appropriate) and tested them for the effects of LH, MS, and SEX using the same statistical approach as for life span data. Because mortality rate is commonly log-normally distributed (Promislow et al. 1996), we ln-transformed α ($\ln \mu_x = \ln \alpha + \beta x$) prior to the analysis. Because $\ln \alpha$ is phenotypically and genetically correlated with β and s, we statistically controlled for the effects of $\ln \alpha$ when analyzing β, and controlled for $\ln \alpha$ and β when analyzing s (Miyo and Charlesworth 2004). We did not examine mortality rates in mated cohorts because the sample size would be too low for meaningful estimations (Pletcher 1999; Promislow et al. 1999).

Results

HATCHING TIME AND LARVA-TO-ADULT SURVIVAL

Young lines hatched faster than Old lines (mean ± SE [days]: Young: 24.13 ± 0.09; Old: 25.74 ± 0.09; $F = 103.51$, df = 1, 12, $P < 0.0001$), but there was no sex difference ($F = 1.37$, df = 1, 12, $P = 0.2643$) and no effect of MS ($F = 0.01$, df = 1, 12, $P = 0.9367$). None of the two- or three-way interactions were significant (all $P > 0.1$). Young lines had much higher larva-to-adult survival (97.5%) than Old lines (75.9%) ($F = 120.79$, df = 1, 12, $P < 0.0001$), but there was no effect of MS ($F = 0.02$, df = 1, 12, $P < 0.0001$) or LH–MS interaction. We note that larva-to-adult survival of Old lines was similar to that of the source population measured at the time of the experiment (72.9%).

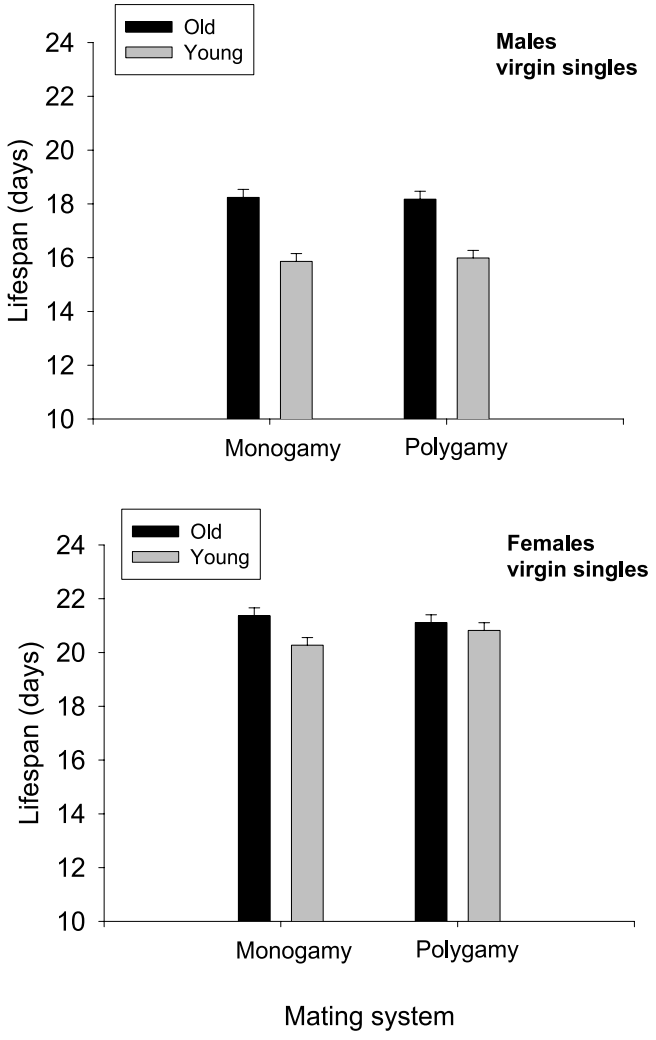

Figure 1. Effect of LH (Old vs. Young) and sexual (Monogamy vs. Polygamy) selection on life span (mean ± SE) in virgin males and females kept singly.

VIRGIN SINGLES: LIFE SPAN AND MORTALITY RATES

Virgin singly kept beetles from Old lines lived longer than their counterparts from Young lines (Fig. 1, Table 1). Females lived longer than males. There was a significant SEX × LH interaction because the difference between Old and Young males was more pronounced that the difference between Old and Young females (Fig. 1). That said, there was a significant difference between LH treatments in both sexes, as indicated by within-model Student's t contrasts at $\alpha = 0.05$.

Table 1. The effects of LH, MS, and SEX on mean life span of virgin singly kept beetles, virgin cohorts, and mated cohorts from 16 replicate populations nested within LH and MS. The results are shown as degrees of freedom (df), *F* values (differences in $-2\times LLR$ [ΔLLR] for random effects) and probabilities (*P*). See Figure 1 and 3 and text for the mean values±SE.

Statistic effects	df	$F/\Delta LLR$	*P*
Virgin singles			
LH	1, 12	32.31	0.0001
MS	1, 12	0.16	0.6963
LH × MS	1, 12	1.09	0.3166
SEX	1, 12	572.34	<0.0001
LH × SEX	1, 12	24.34	0.0003
MS × SEX	1, 12	0.32	0.5830
LH × MS × SEX	1, 12	1.01	0.3350
Population[LH, MS]	1	2.96	0.0853
Virgin cohorts			
LH	1, 12	3.79	0.0752
MS	1, 12	0.27	0.6109
LH × MS	1, 12	2.73	0.1241
SEX	1, 12	580.11	<0.0001
LH × SEX	1, 12	9.78	0.0087
MS × SEX	1, 12	2.11	0.1722
LH × MS × SEX	1, 12	3.03	0.1072
Population[LH, MS]	1	2.05	0.1522
Mated cohorts			
LH	1, 12	0.14	0.7149
MS	1, 12	1.54	0.2405
LH × MS	1, 12	0.25	0.6254
SEX	1, 12	43.31	<0.0001
LH × SEX	1, 12	8.63	0.0123
MS × SEX	1, 12	0.01	0.9149
LH × MS × SEX	1, 12	2.22	0.1619
Population[LH, MS]	1	9.44	0.0021

Table 2. The effects of LH, MS, and SEX on mean mortality rates, measured as Gompertz model parameters (baseline mortality [ln α], rate-of-senescence [β], and late-life deceleration [s]) of virgin singly kept beetles from 16 replicate populations nested within LH and MS. The results are shown as degrees of freedom (df), *F* values (differences in $-2\times LLR$ [ΔLLR] for random effects) and probabilities (*P*). See Figure 2 for age-specific mortality hazard curves.

Statistic effects	df	$F/\Delta LLR$	*P*
ln α			
LH	1, 12	0.11	0.7401
MS	1, 12	0.01	0.9275
LH × MS	1, 12	0.15	0.7052
SEX	1, 12	3.57	0.0831
LH × SEX	1, 12	3.49	0.0863
MS × SEX	1, 12	0.55	0.4727
LH × MS × SEX	1, 12	0.43	0.5232
Population[LH, MS]	1	1.06	0.3032
β			
LH	1, 11.89	18.85	<0.0001
MS	1, 11.89	0.03	0.8487
LH × MS	1, 11.9	0.37	0.56
SEX	1, 11.89	398.84	<0.0001
LH × SEX	1, 11.88	33.40	0.0001
MS × SEX	1, 11.3	0.01	0.9073
LH × MS × SEX	1, 11.28	0.01	0.9326
ln α	1, 15.15	789.35	<0.0001
Population[LH, MS]	1	−3.29	0.0697
s			
LH	1, 12.19	0.01	0.9359
MS	1, 10.5	0.17	0.6846
LH × MS	1, 10.58	0.99	0.3432
SEX	1, 17.09	6.89	0.0177
LH × SEX	1, 19.38	0.04	0.8367
MS × SEX	1, 11.57	0.11	0.7485
LH × MS × SEX	1, 11.52	0.04	0.8513
ln α	1, 14.95	2.38	0.1434
β	1, 13.74	0.02	0.8949
Population[LH, MS]	1	1.6	0.2059

There were no significant effects on baseline mortality rate (Table 2). However, males had higher rate-of-senescence than females, and Young males had higher rate-of-senescence (0.88 ± 0.02) than Old males (0.76 ± 0.02), whereas there was little difference between females from different LH treatments (Young: 0.65 ± 0.02; Old: 0.64 ± 0.02), resulting in a SEX × LH interaction (Table 2). Males had higher late-life deceleration in mortality than females ("Males": 1.44 ± 0.16; "Females": 0.64 ± 0.16; Table 2). Age-specific mortality curves based on pooled data for each sex and treatment are shown in Figure 2.

VIRGIN COHORTS: COHORT BODY MASS, LIFE SPAN, AND MORTALITY RATES

Females were heavier than males (least squares mean ± SE [gram]: females: 0.0817 ± 0.0006; males: 0.0569 ± 0.0006) and Old beetles were heavier than Young beetles (Young: 0.0671 ± 0.0006; Old: 0.0715 ± 0.0006), but there was no interaction between SEX and LH and effect of MS (Table 3). Females lived longer than males (Fig. 3, Table 1). There was a significant SEX × LH interaction because the difference between Old and Young males was obvious in both MS regimes whereas the difference between Old and Young females was reversed across MS regimes (Fig. 3, Table 1).

Females had much lower baseline mortality than males and there was a SEX × LH interaction because Old females had higher baseline mortality (ln α: least squares means ± SE: −11.24 ± 0.46) than Young females (−13.06 ± 0.46), whereas the opposite was true for males (Old: −6.07 ± 0.46; Young: −5.54 ± 0.46)

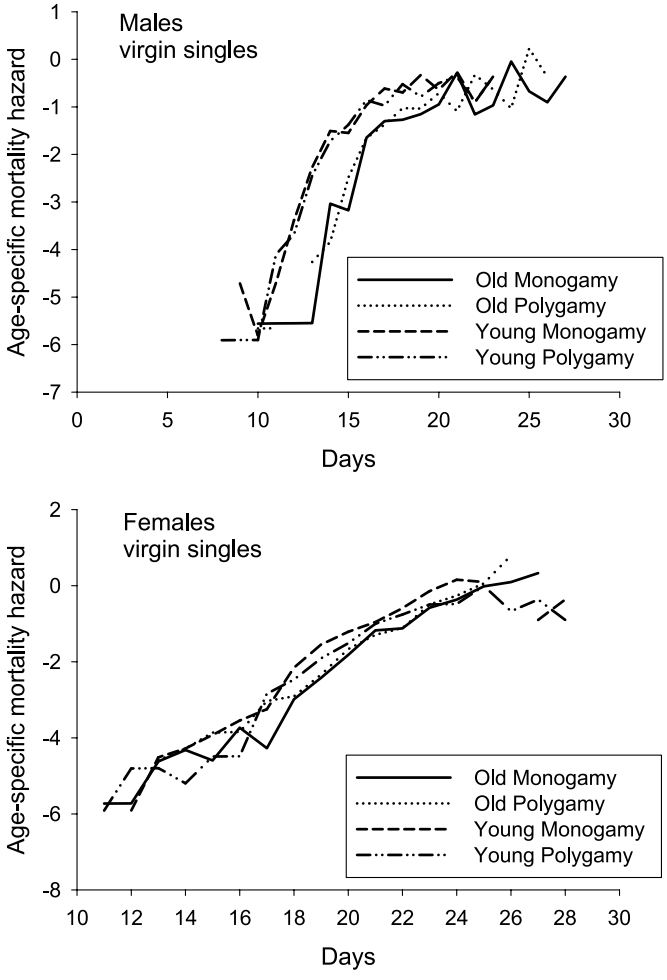

Figure 2. Effect of LH and sexual selection on age-specific mortality hazard (μ_x) in virgin males and females from four different selection regimes kept singly.

(Table 4). Females also had lower rate-of-senescence than males and there was a significant SEX × LH interaction because although Old males had lower rate-of-senescence (β: 0.58 ± 0.03) than Young males (0.67 ± 0.03), there was no such difference for females (Old: 0.40 ± 0.03; Young: 0.40 ± 0.04). Old beetles had lower rate-of-senescence under Monogamy (Old: 0.48 ± 0.02; Young 0.57 ± 0.02) but not under Polygamy (Old: 0.52 ± 0.02; Young 0.51 ± 0.02), resulting in a significant LH × MS interaction (Table 4).

MATED COHORTS: LIFE SPAN

Males outlived females in mated cohorts (mean life span ± SE: males: 8.03 ± 0.16) females: 7.28 ± 0.16; Table 1). Old females lived longer (least squares means ± SE: 7.50 ± 0.22) than Young females (7.05 ± 0.22), whereas the opposite was true for males (Old: 7.91 ± 0.2; Young: 8.15 ± 0.46), resulting in SEX × LH interaction (Table 1).

Table 3. The full factorial model of the effects of LH, MS, and SEX on mean wet body mass of virgin beetle cohorts from 16 replicate populations nested within the above fixed factors. The results are shown as degrees of freedom (df), *F* values (differences in $-2\times LLR$ [ΔLLR] for random effect) and probabilities (*P*). See text for the mean values±SE.

Statistic effects	df	$F/\Delta LLR$	P
LH	1, 12	23.11	0.0004
MS	1, 12	1.30	0.2763
LH × MS	1, 12	2.16	0.1673
SEX	1, 12	990.34	<0.0001
LH × SEX	1, 12	0.78	0.3948
MS × SEX	1, 12	0.66	0.4326
LH × MS × SEX	1, 12	0.27	0.6124
Population [LH, MS]	1	0.28	0.5967

AGE-SPECIFIC OFFSPRING PRODUCTION

Young populations produced more offspring than Old populations and Monogamy populations produced more offspring than Polygamy populations (Table 5, Fig. 5). However, Old populations produced more offspring than Young after three days of reproduction (Fig. 5A), resulting in significant age by LH interaction (Table 5).

RESISTANCE TO DELAYED REPRODUCTION

Monogamy populations (least squares mean ± SE [offspring per vial]: 155.12 ± 9.44) produced more offspring than Polygamy populations (118 ± 9.44) ($F = 7.73$, df = 1, 12, $P = 0.0167$), but there was no effect of LH ($F = 0.95$, df = 1, 12, $P = 0.3496$) and no significant interaction ($F = 1.77$, df = 1, 12, $P = 0.2086$).

Discussion

We found that LH selection affected most of the traits we measured, whereas the effect of sexual selection was modest. Sexual selection failed to affect most of the traits related to age-specific performance, depressed reproductive performance, and failed to promote adaptation. Notably, the sexes responded differently to selection on the LH.

RESPONSES TO LIFE-HISTORY SELECTION

Selection favoring offspring production either early or late in life resulted in direct evolutionary response in age-specific offspring production. Young lines produced more offspring than Old populations early in life, whereas the Old lines produced more offspring after three days of age. This result is consistent with the antagonistic pleiotropy theory of ageing, suggesting that an increase in late-life performance was accompanied by a reduction in early-performance on Old populations. This result is in line with some of the earlier studies in *D. melanogaster* (Luckinbill et al. 1984;

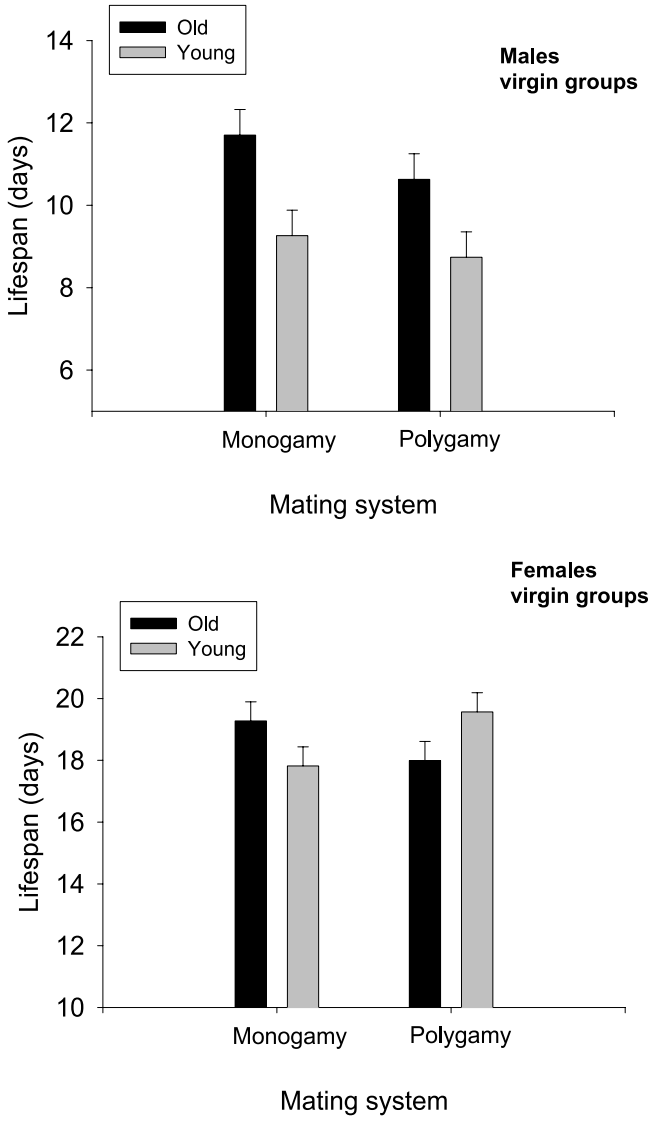

Figure 3. Effect of LH (Old vs. Young) and sexual (Monogamy vs. Polygamy) selection on life span (mean ± SE) in virgin males and females kept in single-sex groups.

Table 4. The effects of LH, MS, and SEX on mean mortality rates, measured as Gompertz model parameters (baseline mortality [ln α] and rate-of-senescence [β] of virgin cohorts of beetles from 16 replicate populations nested within the above fixed factors. The results are shown as degrees of freedom (df), *F* values (differences in $-2\times LLR$ [ΔLLR] for random effect), and probabilities (*P*). See Figure 4 for age-specific mortality hazard curves.

Statistic effects	df	$F/\Delta LLR$	*P*
ln α			
LH	1, 12	2.99	0.1093
MS	1, 12	2.00	0.1826
LH × MS	1, 12	1.46	0.2498
SEX	1, 12	141.95	<0.0001
LH × SEX	1, 12	4.93	0.0464
MS × SEX	1, 12	0.39	0.5428
LH × MS × SEX	1, 12	0.06	0.8162
Population[LH, MS]	1	1.49	0.2222
β			
LH	1, 12	5.12	0.0418
MS	1, 12	0.54	0.4758
LH × MS	1, 12	8.08	0.0147
SEX	1, 12	16.78	0.0006
LH × SEX	1, 12	4.97	0.0445
MS × SEX	1, 12	0.05	0.8221
LH × MS × SEX	1, 12	0.17	0.6845
ln α	1, 12	54.74	<0.0001
Population[LH, MS]		0.03	0.8625

Rose 1984) and *A. obtectus* (Tucic et al. 1996), but is contrary to another set of studies (Partridge and Fowler 1992; Roper et al. 1993; Leroi et al. 1994a,b). However, none of these studies explicitly controlled for larval densities, which could have led to differences in nutrition, and to direct selection for rapid preadult development (discussed by Partridge et al. [1999]). Controlling for larval density and inadvertent selection in *D. melanogaster* showed an increase in survival accompanied by a decrease in early fertility but, contrary to all previous studies, no increase in late fertility was detected in Old populations (Partridge et al. 1999). We controlled for larval density within selection lines and performance assays. We also took precautions to avoid selecting directly on preadult development time. Therefore, our results do suggest a genetic trade-off between early and late reproductive performance.

Young lines produced more offspring overall than Old populations, probably as a result of much higher larva-to-adult survival. Because Old lines had similar larval survival compared to the source population at the time of the experiment, we suggest that evolution of this trait occurred mostly in Young lines. Tentatively, it reflects a positive genetic correlation between investment in early reproduction and net reproductive success. Beetles from Old populations were slightly larger, took one day longer to emerge, lived longer, and had a lower rate of senescence as virgins than beetles from Young populations. These results also contrast with findings for *D. melanogaster*, where selection for age at reproduction produced no effect on body mass or preadult development time (Partridge et al. 1999). Unlike fruit flies, *C. maculatus* are capital breeders that rely on the resources acquired at the larval stage for their survival and reproduction. Old beetles would have to stay alive for four to six days, and maintain the capacity to mate and reproduce during the last three days of life, to contribute to the next generation. Such selection is expected to affect their body mass and, in the absence of egg-laying substrate (i.e., virgin assays), they would use these resources for body maintenance and senesce slower. However, the inclusion of body

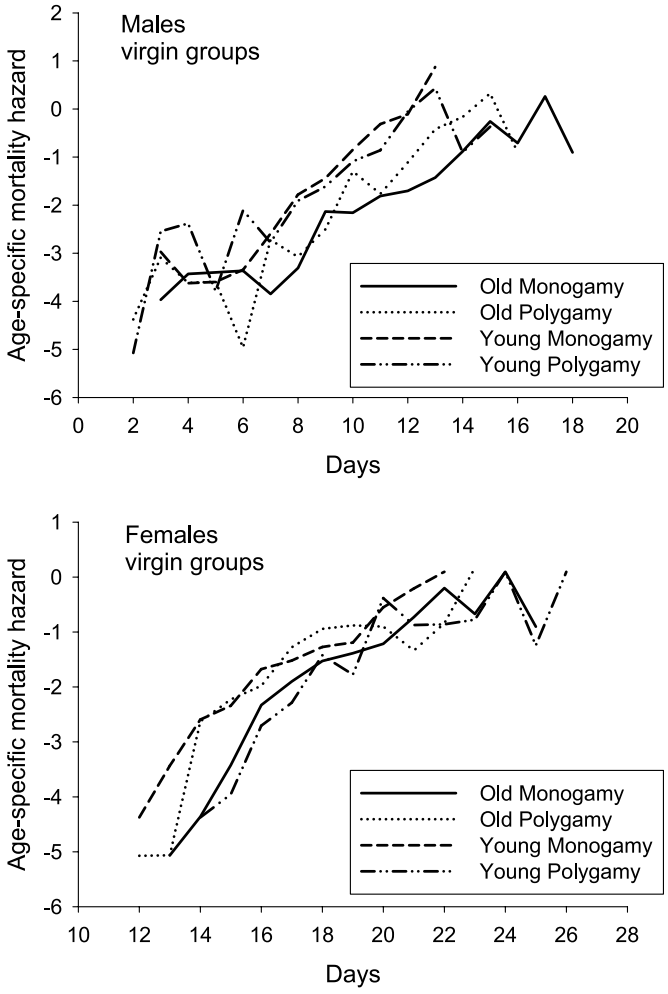

Figure 4. Effect of LH and sexual selection on age-specific mortality hazard (μ_x) in virgin males and females from four different selection regimes kept in single-sex groups.

mass as a covariate in the model did not affect the outcome, suggesting that other mechanisms (perhaps desiccation resistance or reduced metabolic rate) were responsible for the observed effect. Nevertheless, Old beetles were larger and selection for increased body mass could, in theory, select indirectly for longer development time. However, our measure of population eclosion time cannot be compared to development time in the previous studies, because our assays were not designed to test the actual time of larval development, and the observed differences in time to eclosion could as well result from delayed egg-laying by Old females. This question requires further investigation.

SEX DIFFERENCES IN LIFE SPAN

Sexual dimorphism in life span is ubiquitous. Several hypotheses that have been put forward as potential explanations can be divided into two main categories—asymmetric inheritance and sex-specific selection. The first class of these hypotheses is based on

Table 5. The full factorial MANOVA model of the effects of LH and MS on mean offspring from 16 replicate populations measure at day 1, 2–3, and >3 days after the start of reproduction. The results are shown as degrees of freedom (df), *F* values, and probabilities (*P*). Roy's Max Root for all within interactions: $F_{3,12}$=5.6, *P*=0.012. See Figure 5 for mean values±SE.

Statistic effects	df	*F*	*P*
Between-subjects			
LH	1, 12	4.88	0.0473
MS	1, 12	6.94	0.0218
LH × MS	1, 12	0.16	0.6888
Within-subjects			
Day	2.11	295.36	<0.0001
Day × LH	2, 11	7.58	0.0085
Day × MS	2, 11	1.06	0.3800
Day × LH × MS	2, 11	0.09	0.9127

differences in inheritance patterns between the sexes and stresses the importance of either heterogamety (the unguarded X hypothesis, Trivers 1972; Liker and Szekely 2005) or maternal transmission of the mitochondrial genome (Tower 2006). The second class of explanations depends on sex-specific selection on LHs (Trivers 1972; Bonduriansky et al. 2008). In *C. maculatus*, females are commonly known to outlive males (Fox et al. 2003, 2004; Maklakov et al. 2007). However, our study shows that this pattern is true only when virgin life span is considered, whereas males actually outlived females in mated cohorts. This suggests that the longer life span of virgin females results from a larger amount of resources accumulated during larval stage. This conclusion is supported by data from the source population suggesting that females do not live longer than males under natural reproductive conditions (Maklakov and Bonduriansky 2009). Why did virgin female life span respond less strongly to selection for age at reproduction (as suggested by consistent SEX × LH interactions) than virgin male life span? First, virgin female life span is likely to be directly related to female fecundity in a capital breeder such as *C. maculatus*, because resources accumulated at the larval stage can be allocated either for egg laying or for body maintenance. Selection on offspring production at a particular age class selects for the most fecund females, regardless of whether they are more fecund overall or only at the this particular age class. This could inadvertently select for larger, more fecund females that would also exhibit longer virgin life span. On the other hand, the relationship between life span and fitness is less obvious in males. Hunt et al. (2004) showed that male crickets fed good diets live shorter life spans than males fed poor diets (but see Maklakov et al. 2008; Zajitschek et al. 2009). Also, in *C. maculatus*, inbreeding reduces male fitness while increasing male life span (Bilde et al. 2009), or while having no effect on male life span (Fox et al. 2006), depending on inbreeding level. Thus, we have no a priori reason

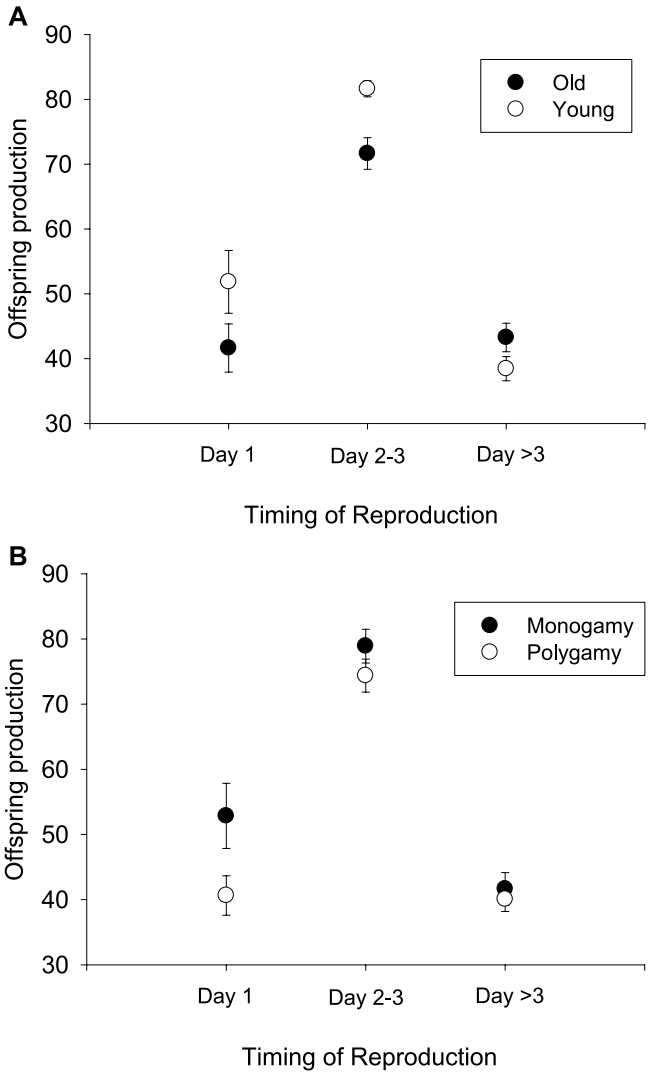

Figure 5. Effect of LH (Old vs. Young, [A]) and sexual (Monogamy vs. Polygamy, [B]) selection on offspring production (mean ± SE) in groups of three females and three males during first day (Day 1), second, and third day (Day 2–3) and rest of their life (Day >3).

to expect a strong positive relationship between male fitness and male life span in this species. We also note that the lack of a female response in virgin life span was mainly due to Polygamy females (Figs. 1B and 3B), which could result from sexually antagonistic coevolution in these populations (see below). Finally, the finding that males from Young populations outlived males from Old populations in mated cohorts may be explained by reduced density and lack of available mating partners in Young populations in which females died sooner and were probably less receptive late in life. In the absence of receptive females, male reproductive investment in Young populations could be lower than in Old populations, where females survived for longer and also reproduced better late in life.

SEXUAL SELECTION AND SEXUAL CONFLICT

Sexual selection can improve population fitness by removing deleterious mutations or increasing the spread and fixation rate of beneficial mutations (Proulx 1999; Whitlock 2000; Agrawal 2001; Siller 2001; Lorch et al. 2003). However, sexual conflict, an integral part of sexual reproduction except under random monogamy, can in theory reduce population fitness (Holland and Rice 1999; Kokko and Brooks 2003; reviewed in Arnqvist and Rowe 2005). Several studies in flies (Diptera) (Holland and Rice 1999; Martin et al. 2004; Wigby and Chapman 2004; Crudgington et al. 2005) and in the bulb mite *Rhizoglyphus robini* (Tilszer et al. 2006) suggest that polygamy plays a role in the evolution of either male-induced harm or female resistance to male-imposed costs or both, upholding some of the predictions of sexual conflict theory.

In *C. maculatus*, sexual selection has been shown to depress population fitness under stabilizing natural selection but to enhance population fitness under directional natural selection (Fricke and Arnqvist 2007). However, a recent study in dung beetles *Onthophagus taurus* provides the first clear example of population-level benefits of polygamy, which is in line with direct or indirect benefits models of sexual selection (Simmons and Garcia-Gonzalez 2008). This suggests that the net outcome of sexual coevolution under polygamy versus monogamy may depend on species-specific idiosyncrasies, such as the relative costs and benefits to females of traits that increase male mating success, but we need much more data from different taxa before any general patterns can emerge. *Callosobruchus maculatus* males transfer sizable ejaculates (up to 10% of their body weight in a single ejaculate), which have been hypothesized to benefit females directly (Fox 1993; Savalli and Fox 1998; Arnqvist et al. 2005; Edvardsson 2007). At the same time, mating is associated with costly harassment and genital damage to females (Crudgington and Siva-Jothy 2000; Ronn et al. 2006, 2007). It appears that there is a complex and nonlinear relationship between costs and benefits of multiple mating to females in *C. maculatus* (Arnqvist et al. 2005), making this a good candidate species in which to examine the relative costs and benefits of polygamy. In our study, enforced monogamy increased offspring production in two different assays, supporting the idea that sexual selection imposes a net cost. Our assays were not designed to test whether male harm decreased in Monogamy lines compared to Polygamy lines and whether the observed differences in offspring production between MS regimes were mainly due to male or female effects. These questions provide considerable potential for future study.

INTERACTION BETWEEN LH AND MS

Despite the observed effects of MS on population fitness in this study and the widespread effects of LH selection, there were almost no MS × LH interactions. The only exception was the rate of senescence in virgin cohorts, suggesting that the evolution of

reduced actuarial ageing in Old populations was hampered by sexual conflict in polygamous populations relative to monogamous populations (also compare with data for virgin cohort life span). Consequently, MS did not accelerate the rate of adaptation in age-specific reproductive performance and was even detrimental for this correlated trait.

Combined with negative net effects of Polygamy on population fitness, these results support the idea that potential benefits of sexual selection in this system were outweighed by the costs associated with sexually antagonistic coevolution. In this, our study supports the results of Holland (2002) and Rundle et al. (2006) that found no positive effect of sexual selection on the rate of adaptation to a new environment. However, this result also contrasts with a recent study in *C. maculatus*, which found that sexual selection accelerated adaptation to a novel food source (Fricke and Arnqvist 2007). In that study, Polygamy females were exposed sequentially to two males only for a total period of 24 h (Fricke and Arnqvist 2007). Because Polygamy females in our study would be constantly exposed to male mating attempts for life and such costs are substantial in seed beetles both in terms of longevity and egg laying (Ronn et al. 2006), we expect much stronger sexual conflict in our experiment. A recent study of the genetic architecture of fitness in *C. maculatus* suggested that a similar laboratory population harbored a sufficient amount of additive genetic variance for female fitness (CVA = 14%) for good-genes selection to operate (Bilde et al. 2008). Therefore, polygamous populations in Fricke and Arnqvist's (2007) study could potentially reap the indirect benefits of sexual selection while being relatively free from the costs of sexual conflict associated with male harassment. This implies that the relative importance of antagonistic versus good-genes coevolution between the sexes can depend on environmental conditions (here, social environment), providing a fruitful research venue for future studies of sexual selection.

CONCLUSIONS

We showed that LH selection resulted in rapid evolution of age-specific reproductive performance and mortality, supporting the antagonistic pleiotropy theory of ageing. In particular, we provide evidence for a genetic trade-off between early and late offspring production while controlling for larval density and inadvertent direct selection for faster development time. We also demonstrated that population fitness increased with the removal of sexual selection, as predicted by sexual conflict theory.

Although experimental populations responded rapidly to both LH and sexual selection, there was very little interaction between these two major evolutionary processes. Sexual selection certainly did not promote the evolution of adaptive age-specific performance and was probably weakly detrimental in the process of adaptation, which is in line with the bulk of the empirical data on adaptation in other LH traits (reviewed in Candolin and Heuschele 2008). This is particularly intriguing because two recent studies in the same species suggested that: (1) there is sufficient genetic variance for fitness for good-genes selection to operate (Bilde et al. 2008) and (2) sexual selection can, in principle, increase the rate of LH adaptation (Fricke and Arnqvist 2007). Taken together, these results imply that the relative importance of antagonistic versus mutualistic effects of sexual coevolution depend on the ambient or social environment, providing an exciting area for future research.

Although the effects of our sexual selection treatment on LH evolution were modest, the sexes responded differently to selection on LH. This implicates a key role of sex-specific reproductive strategies in the evolution of life span and ageing.

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