vol. 168, no. 3 the american naturalist september 2006

E-Article

Artificial Selection on Male Longevity Influences Age-Dependent Reproductive Effort in the Black Field Cricket *Teleogryllus commodus*

John Hunt,[1,2,*] Michael D. Jennions,[3,†] Nicolle Spyrou,[1,‡] and Robert Brooks[1,§]

1. School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, New South Wales 2052, Australia;
2. Centre for Ecology and Conservation, University of Exeter in Cornwall, Penryn, Cornwall TR10 9EZ, United Kingdom;
3. School of Botany and Zoology, Australian National University, Canberra, Australian Capital Territory 0200, Australia

Submitted July 8, 2005; Accepted May 5, 2006;
Electronically published August 10, 2006

abstract: Although the trade-off between reproductive effort and longevity is central to both sexual selection and evolutionary theories of aging, there has been little synthesis between these fields. Here, we selected directly on adult longevity of male field crickets *Teleogryllus commodus* and measured the correlated responses of age-dependent male reproductive effort, female lifetime fecundity, and several other life-history traits. Male longevity responded significantly to five generations of divergent selection. Males from downward-selected lines commenced calling sooner and reached their peak calling effort at a younger age. They called more per night and, despite living less than half as long, called more overall than males selected for increased longevity. Females from the downward-selected lines lived significantly shorter lives than females from the upward-selected lines but still produced the same number of offspring. Nymph survival, development time, and body size and weight at eclosion did not show significant correlated response to selection on male longevity, despite evidence for substantial genetic variation in each of these traits. Collectively, our findings directly support the antagonistic pleiotropy model of aging and suggest an important role for sexual selection in the aging process.

* Corresponding author. Address for correspondence: Centre for Ecology and Conservation, University of Exeter in Cornwall, Penryn, Cornwall TR10 9EZ, United Kingdom; e-mail: j.hunt@exeter.ac.uk.

† E-mail: michael.jennions@anu.edu.au.

‡ E-mail: n.spyrou@unsw.edu.au.

§ E-mail: rob.brooks@unsw.edu.au.

Am. Nat. 2006. Vol. 168, pp. E72–E86. 0003-0147/2006/16803-41180$15.00.

Keywords: longevity, reproductive effort, senescence, sexual selection, life history, *Teleogryllus commodus*.

The trade-off between reproductive effort and longevity is fundamental to two vigorous fields of research that are both heavily grounded in life-history theory: sexual selection (Kokko 1997, 1998; Höglund and Sheldon 1998) and evolutionary theories of aging (Williams 1957; Hamilton 1966; Partridge and Barton 1993). Despite this fundamental connection, there has been surprisingly little integration of ideas between the two fields. Sexual selection researchers have mainly been concerned with the costs of male sexual signaling (reviewed by Kotiaho 2001) and whether preferred males have high breeding values for longevity (Moller and Alatalo 1999; Jennions et al. 2001). In contrast, research on the evolution of aging has focused mostly on female reproductive effort (Rose and Charlesworth 1981; Rose 1984; Partridge and Fowler 1992; Carey et al. 1998; Sgró and Partridge 1999), possibly due to the relative ease with which it can be measured in laboratory model organisms (counting eggs) compared with measuring lifetime investment by males in sexual signaling. Here, we report the results of a divergent selection experiment designed to test for a genetic association between male longevity and the timing and amount of calling effort in the Australian black field cricket *Teleogryllus commodus*. Calling effort is a strong predictor of male mating success in the field (Bentsen et al. 2006). Our results are therefore equally applicable to sexual selection theory and evolutionary theories of aging.

Sexual Selection and the Costs of Sexual Advertisement

Female mate choice imposes sexual selection on male sexual advertisement, resulting in its exaggeration over evolutionary time (Darwin 1871; Andersson 1994). The benefit to females of mate choice remains, however, the subject of considerable controversy, particularly when there are

no apparent direct benefits to being choosy (Andersson 1994; Kokko et al. 2003) and the only indirect benefits are that attractive males contribute superior genes to their offspring (Fisher 1930; Zahavi 1975; Grafen 1990; Andersson 1994; Eshel et al. 2000; Kokko et al. 2002, 2003).

It has been argued that one way for females to ensure they mate with males of superior genetic quality is to prefer older males that have demonstrated their superior viability (Trivers 1972; Manning 1985). This idea is intuitively appealing and consistent with evidence in many species that older males are more heavily ornamented, display more ardently, and attract more females than younger males (reviewed by Brooks and Kemp [2001]). Even though meta-analysis has shown that in many species, more heavily ornamented males tend to live longer (Jennions et al. 2004) and have offspring with greater juvenile survivorship (Møller and Alatalo 1999; but see Brooks 2000), this idea has been criticized because longevity is not necessarily positively correlated with lifetime fitness (Hansen and Price 1995; Brooks 2000; Kokko et al. 2002; Hunt et al. 2004*b*). One of the earliest applications of life-history theory to the relationship between male age and attractiveness (Hansen and Price 1995) pointed out that traits enhancing early- and late-life fitness components may be negatively genetically correlated, and natural selection may favor early-life fitness components more strongly than traits expressed later in life. Under such circumstances, old age may signal lower rather than higher total fitness (Hansen and Price 1995).

It is commonly accepted that honest advertisements of genetic benefits are costly (Zahavi 1975; Grafen 1990; Eshel et al. 2000; see review by Kotiaho [2001]). From a life-history perspective, sexual signaling is a form of reproductive effort, and any costs of signaling must be measured in the currency of residual reproductive value (Kokko and Lindström 1996; Kokko 1997, 1998). As residual reproductive value declines from the onset of maturity, it may often benefit males to increase their investment in sexual signaling with age (Kokko 1997), and this may reinforce the honesty of good-genes signaling (Kokko 1998; Proulx et al. 2002). Under these circumstances, late-life reproductive effort and longevity may be more important determinants of fitness than early-life signaling. It is important to stress, however, that females benefit from mating with males with high breeding values for total fitness (Hunt et al. 2004*b*). In some cases, such fitness is maximized by investing so heavily in sexual advertisement early in adulthood that the longevity of high quality males is less than that of lower quality males (Eshel et al. 2000; Kokko 2001; Kokko et al. 2002). Consequently, quantitative genetic studies examining the genetic basis of the relationship between longevity and age-dependent sexual signaling are crucial to understanding the adaptive value of male longevity.

Evolutionary Theories of Aging

Life-history theory predicts that extrinsic mortality rates affect the optimum trade-off between age-dependent reproductive effort and survival (Charlesworth 1994). High extrinsic mortality rates cause the strength of selection to decline rapidly after the onset of reproduction (Medawar 1946). This leads to selection for traits, such as greater reproductive effort early in adulthood, that increase fitness early in life even at the expense of a negative effect on fitness later in life due to accelerated somatic deterioration (i.e., senescence; Williams 1957; Hamilton 1966; Kirkwood 1977; Gasser et al. 2000). This is the antagonistic pleiotropy theory of senescence (Williams 1957), and it leads to a negative genetic correlation between traits that enhance fitness early and late in life. A second process that may also cause aging is the accumulation of deleterious mutations that only act late in life when selection is weak (Medawar 1946). The effects of these mutations will become more apparent in populations when extrinsic mortality is reduced (e.g., human cancers in westernized societies).

Most empirical evidence for a genetically mediated trade-off between age-dependent reproductive effort and senescence is based on studies of female reproductive effort (e.g., Rose and Charlesworth 1981; Partridge and Fowler 1992; Tucic et al. 1996; Müller et al. 1997; Carey et al. 1998). There is clear phenotypic evidence, however, that increased male sexual signaling is associated with accelerated aging (Partridge and Farquhar 1981; Cordts and Partridge 1996; Bonduriansky and Brassil 2002, 2005; Hunt et al. 2004*a*; Miller and Brooks 2005) and that male aging is accelerated when sexual selection is more intense (Carranza et al. 2004). Moreover, it is possible that the intensity of sexual selection may also play an important role in sexual differences in rates of aging. Sexual selection operating on one sex may influence aging in the opposite sex via pleiotropy (Svensson and Sheldon 1998; Promislow 2003) and thereby generate intralocus sexual conflict (sensu Rice and Chippindale 2001; Arnqvist and Rowe 2005) over the rate of aging.

To date, most studies of aging have selected for the age at which reproduction occurs rather than directly selecting on adult life span. This selection procedure cannot discriminate between selection for longevity and selection for age-dependent fecundity. For example, in a population selected to reproduce at an older age, there is selection for both increased longevity and increased fecundity at this age (Zwaan 1999). As such, changes in age-dependent fecundity schedules may be due to correlations between fe-

cundity at different ages that are independent of changes in longevity. The same would be true for age-dependent male investment in reproduction. Studies that directly select on longevity are therefore essential. Unfortunately, such studies are extremely rare, and the only one we are aware of is that of Zwaan et al. (1995*b*). There are no studies that have directly selected on male longevity to test whether there is antagonistic pleiotropy associated with age-dependent changes in male investment in reproduction. Such studies, especially if both the response of male and female longevity to selection were monitored, would clarify the role that sexual selection on males may play in creating difference in senescence between the sexes.

Teleogryllus commodus

In a recent diet manipulation experiment, we reported a phenotypic trade-off between male calling effort and adult longevity in the Australian black field cricket *Teleogryllus commodus* (Hunt et al. 2004*a*). Although both nymphs and adult females lived longer when reared on a high-protein rather than low-protein diet, adult males lived shorter lives. Reduced male longevity on the high-protein diet was probably due to the earlier onset of advertisement calling and a substantially greater time spent calling per night. It therefore appears that phenotypically increased reproductive effort early in adulthood is associated with more rapid senescence. However, the critical prediction that the same association is mediated by antagonistic pleiotropy as indicated by a genetic correlation between greater early reproductive effort and reduced longevity remains untested (Williams 1957).

Here, we imposed a regime of divergent selection on adult male longevity in *T. commodus*. We selected for increased or decreased male longevity and measured the direct response to selection as the divergence between four paired replicate lines. After five generations of selection, we measured age-dependent male calling effort to test for correlated responses in the timing of and nightly investment in sexual advertisement. We also assessed the potential for genetic conflict between the sexes by measuring the correlated responses of female longevity and lifetime fecundity to selection on male longevity. Finally, because the relationship between longevity and reproduction is shaped by trade-offs with other important fitness components (Roff 2002), we also tested for a genetic association between male longevity and four other important life-history traits, namely, nymph survival, development time, body size, and body weight.

Methods

Collection and Maintenance of Crickets

Approximately 200 adult female field crickets (*Teleogryllus commodus*) were collected from cattle pastures in Smith's Lake (32°22′S, 152°30′E), New South Wales, Australia, in March 2002. Gravid females were isolated in individual plastic containers (5 cm × 5 cm × 5 cm), provided with commercially produced cat food (Friskies Go-Cat Senior), water, and a petri dish containing moist cotton wool for oviposition. To reduce the likelihood that any environmental and/or maternal effects would influence the response to selection, crickets were reared in laboratory cultures for two generations before selection (Lynch and Walsh 1997). These cultures were maintained by rearing the offspring of 100 randomly mated females per generation in four stock culture containers (80 L) in a constant-temperature room at 28° ± 1°C with a 10D : 14L light regime. Each culture was provided with food and water ad lib., and egg cartons for shelter were cleaned weekly.

Selection Regime

We derived our starting populations from the third generation of laboratory reared crickets using the offspring of 200 randomly mated females that were haphazardly assigned to four stock culture containers (80 L). A total of 400 nymphs were established in each stock culture container. When these nymphs reached final instar, culture containers were checked daily and newly eclosed adults were removed. A total of 60 males from each culture container were placed into individually labeled plastic tubs (5 cm × 5 cm × 5 cm) and provided with food, water, and shelter (a piece of egg carton). Females were housed in a communal plastic tank (20 cm × 10 cm × 10 cm) according to their date of eclosion. On day 10 posteclosion, a randomly selected virgin female of the same age and from the same stock container as a male was placed in the male's tub for 3 days to mate (days 11–13). On day 14, the pair was separated and the female placed in her own plastic tub (5 cm × 5 cm × 5 cm) with food, water, and moist cotton wool and a Petri dish for oviposition. After 7 days (day 21) of oviposition, the egg pad was removed and placed in an individually labeled plastic tub (5 cm × 5 cm × 5 cm). Each week, nymphs were collected and established in communal plastic tanks (20 cm × 10 cm × 10 cm) according to their family and maintained until artificial selection was imposed. If both the male and his female partner were alive on day 21, the above protocol was repeated to ensure that females had sufficient sperm to continue producing offspring. Survival was monitored on a daily basis from day 0 (eclosion) onward for males and from day 10 onward for females.

When all 60 males had died, we imposed selection in opposing directions on male longevity ("divergent selection" sensu Falconer and Mackay 1996) to initiate a pair of selection lines. The minimum life span of selected males in the down lines was 10 days because no males mated before this age. In this and all subsequent generations, all 60 parental males per replicate (with one exception) died before any of their offspring eclosed. The offspring of the 50% of males with the highest longevity and the 50% of males with the lowest longevity were used to create the upward and downward lines, respectively. Under the assumptions of the infinitesimal model, this selected proportion ($p = 0.5$) theoretically maximizes the eventual selection limit by offering a compromise between the largest effective population size and the strongest intensity of selection (Latter and Robertson 1962; Hill 1970). To control for fecundity selection and differences in rearing density on the response to selection, an equal number of offspring from each selected male contributed to the next generation so that there were initially 400 offspring/replicate after the offspring of selected males were pooled for communal rearing. The number of selected males contributing to the next generation differed slightly across generations (from 16 to 22; fig. 1) because some of the 30 males died before mating or failed to produce sufficient nymphs. We ensured, however, that offspring from the same number of breeding pairs contributed to the upward- and downward-selected lines in each paired replicate to equalize any effects of inbreeding.

In each subsequent generation, we selected offspring sired by the top 50% of males in each of the four upward-selected replicate lines and the bottom 50% of males in each of the four downward-selected replicate lines. We always selected the oldest available nymphs (nymphs were housed in weekly batches) sired by each male to minimize maternal effects due to variation in the age of the female at the time of egg laying. On average, 75.5% of the nymphs produced per female came from eggs laid in the first week. Thus, most nymphs that contributed to the next generation were from eggs laid by young (day 14–21) females. For logistical reasons, our experiment did not include a set of unselected control lines. However, under a divergent selection regime, the downward-selected lines act as an internal control for the upward-selected lines and vice versa (Lynch and Walsh 1997). Moreover, common environmental effects are eliminated when practicing divergent selection, and more accurate estimates of realized h^2 are obtained because the sums of squares for the selection differential are increased (Hill 1972). Recently wild-caught crickets in a new laboratory environment may be subject to directional natural selection as they adapt to captivity, leading to temporal changes in the traits we measured. Such novel environments tend to promote positive fitness associations among traits (Hoffmann and Merilä 1999). Our finding that short-lived males called more (see "Results") suggests that laboratory adaptation is unlikely to confound our study. Furthermore, because of our paired design, the divergence in traits between the two artificially selected lines should still be largely independent of any adaptation to the laboratory rearing conditions.

We measured the response to selection each generation as the divergence between the upward- and downward-selected lines and the selection differential (S) was weighted ($S = [1/2]S_{\text{males}}$) because only male longevity was the target of selection in our experiment (Falconer and Mackay 1996). We calculated the realized heritability of male longevity using both the least squares regression of the cumulative response (R_c) on the cumulative selection differential (S_c) forced through the origin and the ratio estimator of R_c to S_c (Falconer and Mackay 1996). Except in instances where h^2 is very close to 0, such linear estimates are as accurate as maximum likelihood approaches (Hill 1972). Indeed, the ratio estimator is actually more robust when population sizes differ across generations (Hill 1972). As our selection experiment was replicated, we estimated the sampling variance of the average realized h^2 from the variance among replicate lines (Falconer and Mackay 1996).

After four generations of selection, we set up 70 males in individual containers as previously described to test for the effect of selection treatment on male longevity. We used the recently developed function "coxme" implemented in S-Plus 7.0 to fit a mixed-effects Cox model of survivorship (Therneau 2003). In our analysis we include replicate line as a random factor and selection treatment as a fixed factor. This analysis tested whether the survival curves differed between selection treatments. Survival curves may differ due to differences in the baseline rate of mortality or the senescent mortality. We therefore also explored these two aspects of survivorship using the freeware package WinModest (Pletcher 1999; available from http://www.hcoa.org/scott/softw-winmodest.asp). We fitted four models of age-dependent mortality (Gompertz, Gompertz-Makeham, logistic, and logistic-Makeham) for each replicate line. In most cases, the Gompertz model was the best model or did not differ significantly from the best-fitting model. We therefore estimated baseline and senescent mortality for each replicate line using this model. According to the Gompertz mortality model, the hazard at age x is given by

$$h(x) = \alpha e^{\beta x},$$

where α is the baseline mortality, and β is the senescent mortality component. This provided us with four estimates of α and β, respectively, per selection treatment. We com-

pared these parameters between selection treatments using two-sample *t*-tests). In all survival analyses, we excluded males that died on the day of eclosion.

Correlated Responses to Selection

After four generations of selection, we measured several traits in generation 5 to determine their correlated response to selection on male longevity. More specifically, we tested whether selection on male longevity was correlated with age-dependent male calling effort, female longevity and age-dependent fecundity, and a number of life-history traits (development time, body size and weight, nymph survival). Due to logistic and time constraints, it was not possible to relax selection for a few generations before making terminal measurements. The implications of this are considered further in the "Discussion."

Male Calling Effort

Total male call production is a good measure of male mating success because in both field (Bentsen et al. 2006) and laboratory studies (Hunt et al. 2005), females strongly prefer males with high call rates. To estimate male calling effort in generation 5, we established an extra 30 males in individual containers for each replicate line to monitor their calling effort. To control for the potentially confounding effect of a male's mating history on his calling effort, these males were maintained in an identical manner to the other 70 males in the replicate line but were not mated to a female. Furthermore, because mating may reduce male longevity, these 30 males were excluded from our terminal estimate of the response of male longevity to selection.

We monitored male calling effort using a custom-built recording apparatus that can measure 64 males/night (Hunt et al. 2004*a*). Each male is housed in an individual recording chamber (5 cm × 5 cm × 5 cm) with a condenser microphone (C1163, Dick Smith) mounted in the lid that is then acoustically isolated in a Styrofoam container (15 cm × 10 cm × 10 cm). Each microphone is connected to a data acquisition unit (DaqBook 120, IOTect, Cleveland; see Bertram and Johnson 1998 for a detailed description of a similar device) and a laptop. The data acquisition unit activates a single microphone at a time, which then relays the sound level to the PC board, where it is compared with the background noise. If the received signal is ≥10 dB louder, this is recorded as a call. The microphone is then deactivated and the next in the series activated. Each recording chamber was sampled 10 times/s. For the current analysis, we simply used the number of seconds of calling/night. Male calling was monitored from 1800 to 0900 hours in a constant-temperature room set to 28° ± 1°C.

Starting the day of eclosion, we measured the calling effort of each male ($n = 240$ males) every 2 nights for the first 10 days posteclosion and then every 3 nights thereafter until death. We increased sampling in the first 10 days to more accurately estimate when individuals commenced calling. We extracted the following five measures of calling effort for each male: the age at which he commenced calling (age at which calling commenced), the age at which he called the most (age with maximum calling effort), the number of seconds he called on that night (maximum calling effort), the mean number of seconds he called on all nights of sampling (mean calling effort), and the total number of seconds he called (total calling effort). Where necessary, we transformed these measures. We could not extract meaningful measures of the age at which calling commenced, the age with maximum calling activity, or the maximum calling activity for all males because several males did not call during their lifetime. Separate analyses were conducted for each of the five measures of calling activity. Analyses were run in S-Plus 7.0 using linear mixed models (LMM) using restricted maximum likelihood (REML) approximation with the identity of the replicate line as a random factor and selection treatment as a fixed factor. The significance of selection treatment was assessed using the conditional *t*-test of its parameter estimate. This is preferable to likelihood ratio tests of nested models with and without the fixed term of interest (Pinheiro and Bates 2000, pp. 87–92). We tested whether there was significant variation in calling among replicate lines within a treatment using a likelihood ratio test to compare the final model with and without the random factor term "replicate line." We examined the residuals of final models to ensure homoscedasticity and to ensure that they were normally distributed. Where necessary, dependent variables were transformed. We corrected for making multiple comparisons of call properties between the selection treatments using Bonferroni adjustments.

Female Longevity and Age-Dependent Fecundity

At generation 5, we measured the longevity of 70 females following the same protocol used in previous generations. We then compared female longevity between selection treatments using the same statistical analysis previously described for males. We excluded females that died on the day of eclosion because they generated an unusually high initial mortality in some lines. This was statistically conservative because this only occurred in down-line replicates. To estimate female lifetime offspring production, we established the egg pads of 30 randomly selected females per line in individual plastic containers (5 cm × 5

cm × 5 cm). Egg pads were checked daily, and the number of nymphs that emerged was counted. We monitored each female's egg pad(s) for 60 days after her death, because in an earlier study, there was an average of 53.7 ± 1.4 days between the first and last nymph hatching in a given batch of eggs (Jennions et al. 2004). We tested whether selection treatment affected females' lifetime nymph production using an LMM with the same analysis used for male calling. To translate nymph counts into fecundity requires the assumption that hatching success did not differ between selection treatments. We could not assume that the daily sequence of nymph emergence corresponds to the sequence of egg production because there is considerable within-female variation in the time to hatching (M. D. Jennions, unpublished data).

Life-History Traits

We estimated the correlated response of life-history traits to divergent selection on male longevity using a full-sib breeding design (Falconer and Mackay 1996). For each of the 30 pairs used to examine female lifetime offspring production in each replicate line, we attempted to set up 10 random offspring, each placed in an individual plastic container (5 cm × 5 cm × 5 cm; 30 full-sib families per replicate, maximum $n = 2{,}400$ offspring), and measured their development time, survival and body size, weight, and sex at eclosion. We monitored nymph survival on a weekly basis, when individual containers were cleaned and fresh food and water were provided to each nymph. On reaching the fourth instar, nymphs were checked daily, and newly eclosed adults were removed and their body size (pronotum width) and weight measured using a graticule in a binocular microscope (Leica MZ5) and an electronic balance (Mettler-Toledo 345G), respectively. Development time was calculated as the number of days between hatching and eclosion. All offspring were reared in a constant-temperature room set to 28° ± 1°C and a 14L : 10D light regime.

We used the function "coxme" implemented in S-Plus 7.0 to fit a mixed-effects Cox model (Therneau 2003) to test for the effects of selection treatment on nymph survival, with line and family nested within line as random factors. The number of days each nymph lived was entered for those that died as nymphs, and the age at eclosion was entered for those that survived to eclosion. Eclosing nymphs were treated as right-censored (i.e., still alive) cases.

To test for an effect of selection treatment and offspring sex on body mass, pronotum width, and development time, we conducted separate analyses for each of the three variables. Analyses were run in S-Plus 7.0 using LMMs, with the identity of the replicate line and family nested with line as random factors and selection treatment and sex as fixed factors. Model simplification proceeded from an initial maximal model containing the factors sex, selection treatment, and their interaction. We sequentially removed nonsignificant terms until the final minimal model only contained significant terms (Crawley 2002). The significance of fixed terms was determined by conditional *t*-tests of their parameter estimates (Pinheiro and Bates 2000, pp. 87–92). The *P* values associated with fixed terms that did not enter the final model were determined by adding them individually to the final model. There were no significant interactions between sex and selection treatment. We tested whether there was significant variation in calling among replicate lines within a treatment or families within replicate lines using likelihood ratio tests to compare the minimal model with and without the random factor of interest.

We estimated the heritability of nymph survival to eclosion using the method described by Falconer and Mackay (1996) for threshold traits (i.e., 0 = died, 1 = reached maturity). We used a randomization test to assess whether our observed heritability deviated significantly from 0 and a bootstrap method to estimate 95% confidence intervals (CIs). Both procedures were conducted using the Poptools version 2.5 (http://www.cse.csiro.au/poptools/) add-in for MS Excel. In the randomization test, we assigned individuals randomly (sampling with replacement) to families, and then we pseudoestimated the heritability. The proportion of pseudoestimates from 9,999 resample replicates that exceeded the actual heritability estimate is interpreted as the significance of the deviation of the actual heritability estimate from 0. For each bootstrap estimate, we excluded one individual per family and calculated a pseudoestimate of the heritability. The 95% CI is between the 25th smallest and 25th largest of the 1,000 pseudoestimates.

We estimated heritabilities of body size and weight at eclosion and development time separately for each of the eight selection lines using standard methods for full-sib breeding designs (Falconer and Mackay 1996). There were no treatment differences in these heritability estimates (paired-sample *t*-tests: all $t < 1.469$, $\mathrm{df} = 3$, $P > .238$), so we report the mean heritability and the standard error of that mean. Quantitatively similar estimates of heritability were obtained when extracting family and error variance components using LMMs with REML approximation.

Results

Direct Response to Selection

Male longevity showed a strong response to direct selection in both directions, as illustrated by the increasing divergence between upward- and downward-selected lines

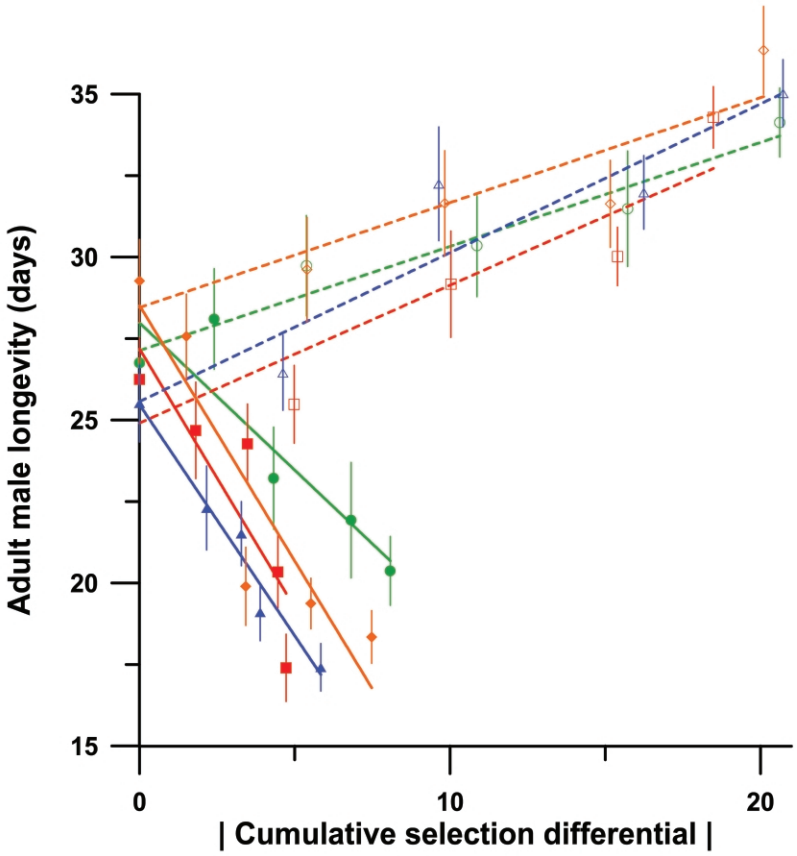

Figure 1: Direct response of male longevity to upward (*open symbols*, *dotted lines*) and downward (*solid symbols*, *solid lines*) selection on male longevity. Male longevity is plotted against the absolute cumulative selection differential. Fitted lines are regression slopes. Colors and symbol shapes represent paired replicate lines. The number of families contributing to each generation were equal in upward- and downward-selected lines: replicate 1 (18, 18, 18, 22), replicate 2 (19, 17, 19, 21), replicate 3 (19, 16, 20, 20), and replicate 4 (20, 16, 20, 20).

across generations (fig. 1) and the high realized heritabilities (table 1). There was, however, a large asymmetry in the response of male longevity to selection. The realized heritability of longevity in downward-selected lines was approximately four times greater than that of the upward-selection lines (table 1). After five generations of divergent selection on male longevity, males from the upward-selected lines lived significantly longer than males from the downward-selected lines (mixed-effects Cox model: treatment coefficient = −2.663 ± 0.202 SE, $Z = 13.18$, $P < .0001$; fig. 2*a*). Fitting a Gompertz model to the data for each replicate line showed that males from the upward-

Table 1: Realized heritability estimates for male longevity in the upward- and downward-selected lines and the divergence between these paired replicate lines

Replicate line	Upward selection		Downward selection		Divergence	
	OLS	Ratio	OLS	Ratio	OLS	Ratio
1	.34	.36	.71	.79	.45	.48
2	.33	.44	1.35	1.88	.56	.73
3	.45	.46	1.43	1.39	.68	.66
4	.27	.35	1.70	1.46	.65	.65
Mean ± SE	.34 ± .04	.40 ± .03	1.30 ± .21	1.39 ± .22	.59 ± .05	.63 ± .05

Note: OLS = ordinary least squares regression of cumulative response (R_c) on the cumulative selection differential (S_c) forced through the origin; Ratio = the ratio estimator of R_c to S_c (see Falconer and Mackay 1996).

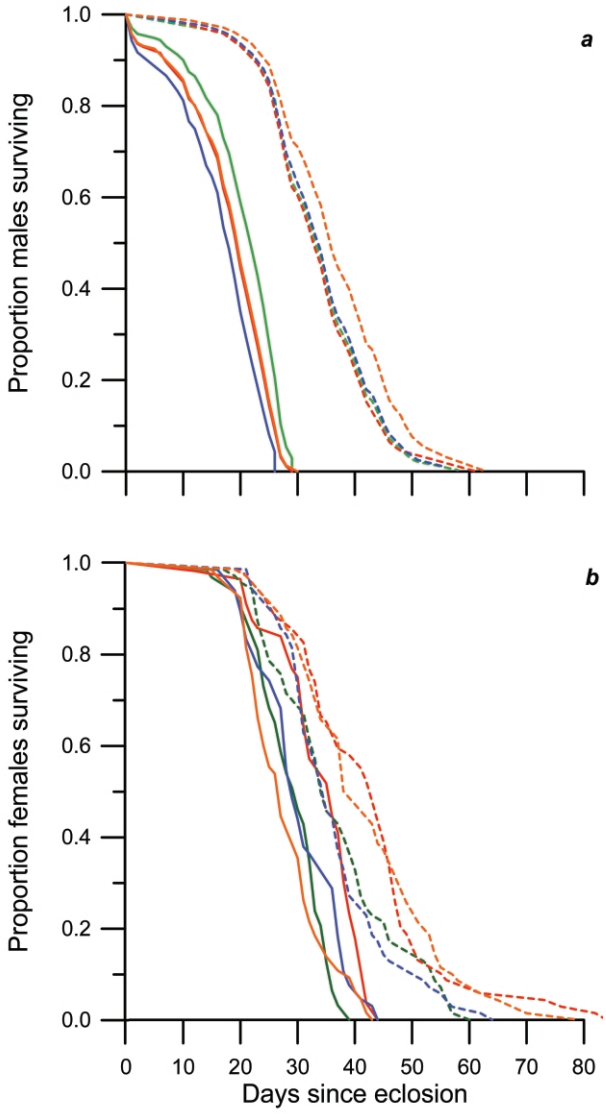

Figure 2: Cumulative survival curves of (*a*) adult males and (*b*) adult females in replicate selection lines for increased (*dotted lines*) and decreased (*solid lines*) adult male life span. Colors represent paired replicate lines that started at the same time.

selected line had a nearly significant increase in both baseline mortality (α; $t = 2.402$, df $= 6$, $P = .053$) and rate of senescence (β; $t = 6.163$, df $= 6$, $P = .001$; table 2).

Correlated Response in Male Calling Effort

Males selected for reduced longevity commenced calling sooner, reached their peak calling effort at a younger age, and called more per night than males selected for increased longevity (fig. 3; table 3). Despite living less than half as long as males selected for increased longevity, males from the reduced longevity lines actually called more in total (table 3).

Correlated Response in Female Longevity and Fecundity

Females from upward-selected lines lived significantly longer than those from the downward-selected lines (mixed-effects Cox model: treatment coefficient $= -1.142 \pm 0.212$ SE, $Z = 5.39$, $P < .0001$; fig. 2*b*). Fitting a Gompertz model to the data for each line showed that females from the upward-selected line had no significant increase in baseline mortality (α; $t_6 = 0.641$, $P = 0.55$) or senescence (β; $t_6 = 0.456$, $P = .67$; power to detect a medium effect sensu Cohen [1988] is <15% for $P = .05$ [two-tailed]; table 2). Based on the Cox regression, this correlated female response to selection on male life span suggests that there is the potential for intralocus genetic conflict between the sexes due to pleiotropic effects on female longevity of genes that reduce male life span. There were, however, no differences between selection treatments in the lifetime number of offspring produced per female (treatment: $F = .269$, df $= 1, 6$, $P = .623$; line within treatment: log-likelihood ratio $= 0.037$, df $= 1$, $P = .847$; up: 132.0 ± 10.8 SE, down: 124.3 ± 9.3 SE). Thus, unless there is a fitness cost to breeding sooner or over a shorter time frame, there was no detectable sexual conflict over longevity.

Correlated Response in Life-History Traits

In total, we established 1,473 nymphs from 150 families. Nymph survival did not significantly differ between the selection treatments (mixed-effects Cox model: treatment coefficient $= -0.127 \pm 0.881$, $Z = 0.79$, $P = .43$). There were no significant treatment effects on body size and weight at eclosion or development time (table 4), indicating a lack of correlated response in these life-history

Table 2: Mortality analyses fitting Gompertz models to each of the four replicate lines per selection treatment

Parameter	Up	Down	t, df $= 6$	P
Males:				
α	$.0017 \pm .00024$	$.0037 \pm .00081$	2.402	.053
β	$.1042 \pm .00655$	$.1755 \pm .00954$	6.163	**.001**
Females:				
α	$.0016 \pm .00056$	$.0022 \pm .00081$	.641	.545
β	$.1305 \pm .02814$	$.1115 \pm .03066$	.456	.665

Note: Separate models were fitted for males and females. The means and standard errors for the two parameter estimates α and β are presented (all $n = 4$). The t-tests compare these parameters between selection treatments. P values in bold remained significant ($P < .05$) after sequential Bonferroni correction.

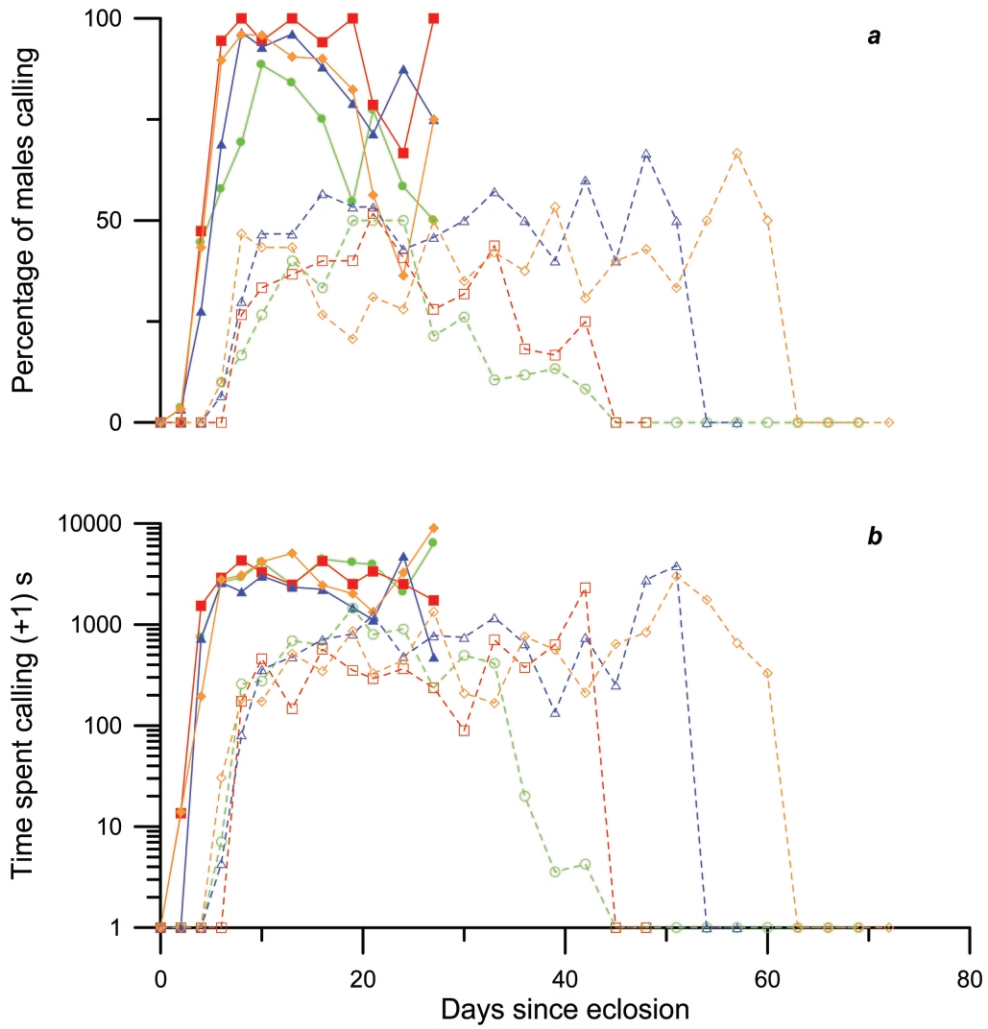

Figure 3: Age-dependent calling effort of males from lines selected for short (*solid symbols*, *solid lines*) and long (*open symbols*, *dashed lines*) adult male longevity. *a*, Proportion of males still alive that called each night. *b*, Mean number of seconds spent calling per male per night. Colors and symbol shapes represent paired replicate lines started at the same time.

traits to divergent selection on male longevity. There were, however, significant differences among lines within treatments and families within lines for all three life-history traits. Females had significantly smaller pronotum width, lower body mass, and shorter development time than males (table 4).

Analysis of nymph survival to eclosion as a threshold trait provided an estimated heritability of 0.463 with bootstrapped 95% CIs of 0.310 to 0.721. This estimated heritability was significantly >0 (randomization test $P < .001$). The REML estimation of variance components revealed significant amounts of heritable variation in development time ($h^2 = 0.466 \pm 0.076$, $P = .000$), pronotum width ($h^2 = 0.487 \pm 0.105$, $P = .001$), and weight at eclosion ($h^2 = 0.343 \pm 0.104$, $P = .006$).

Discussion

Direct Response to Selection on Male Longevity

The rapid direct response of adult male longevity to selection indicates that substantial additive genetic variation in male longevity is segregating within this cricket population. This finding is consistent with the results of selection directly on adult life span in *Drosophila melanogaster* (Zwaan et al. 1995*b*) and with significant reported heritabilities of longevity from laboratory breeding designs (e.g., dung flies; Mühlhäuser and Blanckenhorn 2004) and from pedigree analyses of free-living populations (e.g., red deer, Kruuk et al. 2000; humans, Pettay et al. 2005). Such evidence indicates that there is appreciable scope for evolution to shape rates of aging and longevity.

Table 3: Linear mixed model analysis of five correlated responses in male calling effort to divergent selection on male longevity

			Selection treatment		Line within treatment		Variance	
	Intercept	Treatment (up)	F, df = 1,6	P	χ^2, df = 1	P	Lines	Residual
Age at which calling commenced ($\log_{10}$[age])	.731 ± .016	.321 ± .023	187.53	**.000**	.00	.998	0	.0279
Age at maximum calling effort ($\log_{10}$[age])	1.030 ± .021	.254 ± .030	8.56	**.001**	.149	.700	.0003	.0362
Maximum calling effort ($\log_e$[s+1])	8.517 ± .224	−1.672 ± .319	27.49	**.002**	3.337	.068	.1221	2.0412
Mean calling effort ($\log_e$[s+1])	3.45 ± .174	−1.951 ± .247	62.23	**.000**	.207	.649	.0275	2.8421
Total calling effort ($\log_e$[s+1])	33.74 ± 2.19	−10.278 ± 3.102	10.98	**.016**	.00	.999	.000	577.2

Note: For model details, see text. The coefficient for the down selection treatment is set at 0. Thus, the estimated mean for down treatment = intercept; estimated mean for up treatment = intercept + up treatment estimate. The P values for the effect of selection treatment are based on conditional F-tests of parameter estimates. The P values for the effect of replicate lines are based on log-likelihood ratio tests. The P values in bold remained significant ($P < .05$) after sequential Bonferroni correction for multiple tests for the term in question.

Interestingly, selection for longer-lived males resulted in a response of approximately the same magnitude (R_c = 7.08 to 9.51 days) as selection for shorter-lived males (R_c = 6.38 to 10.92 days) despite the fact that approximately four times as much selection was applied when selecting upward on male longevity than when selecting downward. Thus, the realized heritability in our downward selection lines is about four times greater than in the upward-selected lines. Asymmetric responses are common in divergent selection experiments, and most such experiments show some degree of asymmetry (Frankham 1990). Indeed, asymmetry is expected if the trait under selection is a component of natural fitness, with selection toward increased fitness yielding a slower response than selection toward decreased fitness (Frankham 1990; Falconer and Mackay 1996). While there are a number of possible causes of asymmetric responses to selection (Falconer and Mackay 1996), the two most promising explanations for the asymmetric response we observed are inbreeding depression and genetic asymmetry.

Inbreeding depression frequently occurs in selection lines when the effective population size is small (Falconer and Mackay 1996). If the trait being selected is vulnerable to inbreeding depression, downward selection will be enhanced and upward selection opposed by inbreeding depression (Falconer and Mackay 1996). Our selection lines consisted of between 16 and 22 pairs of parents per generation, which is a reasonable population size for a short-term selection experiment. Even so, some inbreeding occurred during the course of our experiment, and *Teleogryllus commodus* is vulnerable to inbreeding depression in important fitness traits such as hatching success and juvenile survivorship (Jennions et al. 2004). The limited available empirical data suggest, however, that in *T. commodus* pedigree inbreeding has a statistically detectable effect on the longevity of adult females but not the longevity of adult males (J. Drayton and M. D. Jennions, unpublished data). In most species, however, inbreeding does appear to adversely affect male longevity (Charlesworth and Charlesworth 1987; Crnokrak and Roff 1999; de Rose and Roff 1999; van Oosterhout et al. 2000), so an effect of inbreeding depression on adult male survival may be partly responsible for the observed asymmetric response to selection.

Heritability is highest at so-called symmetric allele frequencies, that is, when alleles with strictly additive effects are at even frequencies, or when recessive alleles are at frequencies of approximately 0.75 (see fig. 8.1 in Falconer and Mackay 1996). Response to selection is most likely to be symmetric when the starting frequencies of additive alleles are close to the symmetric values. If selection in one direction (in this case toward shorter longevity) increases the frequencies of rare alleles, particularly recessive alleles at several loci, this will result in an increase in additive genetic variance until the symmetric frequencies are passed (Barton and Keightley 2002; Blows and Higgie 2003). No such increase in additive genetic variation will occur with selection in the opposite direction (for longer life span). It is conceivable that some of the initial genetic variation in male longevity was due to the effects of rare alleles, particularly deleterious recessives that persist at low frequencies in heterozygotes and are masked from selection. Indeed, theory predicts that a major source of genetic variation in male quality and condition-dependent traits, such as longevity and sexual signaling, will be deleterious mutations (Rowe and Houle 1996; Tomkins et al. 2004).

Table 4: Linear mixed model analysis of correlated responses in male and female size, weight, and hatching-to-adult development time

							Variances (× 100)		
	Intercept	Sex (male)	Selection[a]	Sex[b]	Line[c]	Family[d]	Lines	Family	Residual
Pronotum width (mm)	5.87 ± .06	.095 ± .023	.076 (.792)	16.691 (**<.001**)	114.9 (**<.001**)	127.5 (**<.001**)	2.564	4.311	12.907
Body weight (mg)	588.4 ± 15.5	15.9 ± 6.6	.004 (.954)	5.704 (**.017**)	88.43 (**<.001**)	82.43 (**<.001**)	162,750	257,590	1,068,240
Development time ($\log_{10}$[days])	1.744 ± .004	.011 ± .002	.06 (.814)	25.30 (**<.001**)	65.07 (**<.001**)	101.7 (**<.001**)	.0122	.0346	.1154

Note: The final models did not contain the selection treatment term (for details, see text). The coefficient for the effect of sex is set at 0 for females. Estimated mean for females = intercept; estimated mean for males = intercept + male estimate. The *P* values for the effect of sex are based on conditional *F*-tests of parameter estimates. The *P* values for the effect of replicate lines and families within lines are based on log-likelihood ratio tests. *P* values in bold remained significant ($P < .05$) after sequential Bonferroni correction for multiple tests for the term in question.

[a] F, df = 1, 6 (*P* value).

[b] F, df = 1, 936 (*P* value).

[c] χ^2, df = 1 (*P* value).

[d] χ^2, df = 1 (*P* value).

Such variation would be largely unavailable for selection on greater longevity, but selection for shorter adult life span should result in an increase in the frequencies of such alleles such that additive genetic variation would increase within these lines. This said, however, genetic asymmetry is not normally expected to generate an asymmetric response in the first few generations of selection because it requires substantive changes in allelic frequencies (Falconer and Mackay 1996).

Another explanation for the asymmetric response is that males favored by the environment used for selection display lower V_a. That is, the laboratory environment may reveal more additive genetic variation in traits that decrease longevity than those that increase it. More generally, there may be more ways to alter complex physiological and morphological traits adversely to decrease longevity than positively to increase it. Or, in genetic terms, there may be more loci that negatively affect longevity than those that positively affect it, creating a larger mutational target and greater V_a for reduced life span. Finally, the distribution of variance in genes affecting longevity may change with age. There may be many ways to for a cricket to die when it is young but far fewer if it survives past a certain point.

Correlated Response in Male Reproductive Effort

In an earlier dietary manipulation study, we found that male adult longevity was negatively associated with the timing and amount of male reproductive effort measured as advertisement calling (Hunt et al. 2004*a*). Call rate is a significant predictor of male mating success in both the laboratory and field (Hunt et al. 2005; Bentsen et al. 2006). The correlated response of both the timing and the amount of calling to selection on male longevity in the present study suggests that there is also a genetic basis to the tradeoff between male investment in sexual signals and longevity. The demonstrated negative genetic correlation therefore provides direct support for the antagonistic pleiotropy model of aging (Williams 1957; Hamilton 1966), in which genes that enhance fitness by increasing reproductive effort early in adulthood are negatively associated with longevity.

Previous studies of aging have focused almost exclusively on the effects of increased early-life reproductive effort by females (Zwaan 1999). Our findings put male reproductive effort in the form of calling effort on the same empirical footing as female reproductive effort as an agent of aging. They are consistent with the results of the only other study with direct selection on adult longevity, in which increased longevity in *D. melanogaster* was associated with lower female reproductive output at all ages (Zwaan et al. 1995*b*). Selecting directly on adult longevity requires considerable effort because selection can only be applied to a generation once most individuals in the preceding generation have died. A more commonly used tool to explore the effect of age-dependent selection is to artificially select on age at reproduction (e.g., Rose and Charlesworth 1981; Rose 1984; Partridge and Fowler 1992; Tucic et al. 1996). These studies have demonstrated a genetic association between female longevity and age-dependent fecundity in *D. melanogaster* (Rose and Charlesworth 1981; Rose 1984; Partridge and Fowler 1992), *Tribolium castaneum* (Mertz 1975), *Acanthoscedelis obtectus* (Tucic et al. 1996), and *Bactrocera cucurbitae* (Miyatake 1997). Whether the differences in the timing of reproductive effort in these studies is a correlated response to selection on longevity has been questioned, however, because it is the age dependence of reproductive output that is explicitly under selection, and this is a product of both survival past the age of selection and fecundity after this age is reached (Zwaan et al. 1995*b*). In our study, males from lines selected for longer lives started to call later in life and called less overall relative

to males from shorter-lived lines despite the fact that we selected on longevity rather than age-dependent reproductive effort. This result demonstrates that the pattern of reduced reproductive effort early in life for longer-lived animals predicted by the antagonistic pleiotropy theory of aging (Williams 1957; Kirkwood and Rose 1991) can be demonstrated by direct selection on longevity. More studies are needed, however, to confirm the generality of this widely cited but empirically undertested theory.

Our findings also have two important implications for sexual selection. First, the correlated response of male calling effort to selection on longevity demonstrates that calling effort has a genetic basis in *T. commodus*. Cade (1981) reached a similar conclusion by applying divergent selection on nightly calling effort in the field cricket *Gryllus integer*, obtaining realized heritability estimates of 0.50 and 0.53 in the high and low calling lines, respectively. Second, the genetic trade-off between male longevity and both the timing and the amount of calling provides new evidence for the costliness of sexual signaling. Calling significantly reduces male life span. This strong trade-off reinforces an important conclusion from the phenotypic correlation seen in our diet manipulation study (Hunt et al. 2004*a*): the fittest males in this population are unlikely to be those that live the longest. It also confirms the theoretical prediction that under strong sexual selection (documented in Hunt et al. 2004*a*; Bentsen et al. 2006), high-quality males may signal so intensely that they die sooner than lower-quality males (Eshel et al. 2000; Kokko 2001; Kokko et al. 2002).

It has been known for some time that signal expression changes with male age (reviewed by Brooks and Kemp 2001). Theory predicts that investment in sexual signaling should be age dependent (Hansen and Price 1995; Kokko and Lindström 1996; Kokko 1997, 1998; Beck and Powell 2000). Our findings show that monitoring age-dependent changes in sexual signaling is critical to the accurate assessment of fitness. When males commence calling, when they reach their peak calling effort, and how much they call throughout their lives are all likely to influence male longevity and be shaped by rates of extrinsic mortality. This makes it very difficult to use single samples of the intensity of plastic signals, such as calling effort, as an index of male fitness or genetic quality (Hunt et al. 2004*b*), even if they are made at a fixed age.

Correlated Response of Female Longevity and Fecundity

Female longevity and fecundity responded to selection on male longevity, although the responses were neither as stark nor as consistent as the responses of male longevity and calling effort. Clearly, longevity and age-dependent reproductive effort do not evolve independently in males and females due to genetic correlations between the sexes. The inability of male and female traits to evolve independently of one another is a potential source of intralocus sexual conflict, displacing the population from sex-specific optima (Chippindale et al. 2001; Rice and Chippindale 2001; Fedorka and Mousseau 2004). Our results suggest that age-dependent patterns of reproductive effort and rates of senescence are both likely to be subject to intralocus sexual conflict. Although others have noted that interlocus sexual conflict has the potential to cause senescence and generate sex-specific aging rates (Promislow 2003), we are unaware of any study that has provided evidence that intralocus conflict (sensu Arnqvist and Rowe 2005; Rice and Chippindale [2001] called this intersexual ontogenetic conflict) may influence the evolution of aging rates. This is a prediction that requires explicit testing.

Correlated Responses in Life-History Traits

Life-history theory predicts that patterns of survival and reproduction are shaped by trade-offs between fitness components (Roff 2002). However, while over two decades of laboratory selection experiments on aging-related traits in *D. melanogaster* have shown that the correlated responses of life-history traits are commonplace, these responses are highly variable across studies (Rose and Charlesworth 1981; Rose 1984; Partridge and Fowler 1992; Chippindale et al 1994). Part of this inconsistency is undoubtedly due to the various indirect procedures used to apply selection on rates of aging (Zwaan 1999). Some procedures may inadvertently place selection on juvenile stages, meaning that trade-offs between adult and juvenile life-history traits confound any observed responses to selection. For example, it is likely that many of the classic studies selecting for late-life fecundity in *D. melanogaster* (Rose 1984; Partridge and Fowler 1992) inadvertently selected for increased development time (Chippindale et al. 1994) and that this influenced the magnitude and direction of the correlated responses in larval survivorship and body size.

In our study we endeavored to ensure that the only trait that was directly selected was male life span and that terminal measures only reflected genetic changes between selection treatments. There are, however, some caveats that we need to note. First, for logistic reasons it was impossible to relax selection for two to three generations before making terminal measurements. There is, therefore, a possibility that maternal effects could influence the final measurements. For example, females may somehow differentially allocate resources to eggs sired by males that are longer lived. We are unaware of any studies that have shown such differential allocation based on male longevity,

and we therefore stand by our current interpretation of the selection experiment. Direct tests for differential allocation will be difficult, however, if male longevity is genetically correlated with other traits that are taken to be measures of differential allocation by females (e.g., nymph survival). Second, the nymphs that contributed to the next generation were not identical in their age. We did, however, minimize any differences in maternal age between selection treatments by preferentially using nymphs emerging from the first week's eggs. On average, across the replicate lines, 75.5% of the nymphs produced per female were from eggs laid in the first week. Thus, most nymphs that contributed to the next generation were from eggs laid by females 14–21 days posteclosion. Third, selected males could only be identified and their nymphs pooled once all males had died. To minimize the difference in the time of pooling between the paired upward- and downward-selected lines, we did not pool selected males' nymphs until all males had died. This means that there was no selection against early nymph mortality because sires contributed equally to their replicate line at the time of pooling. This relaxed selection was, however, equal between the upward- and downward-selected lines because the next generation was established at the same time for both selection treatments, and it should therefore not affect divergence between paired replicates.

Interestingly, we found that body size, body weight, nymph survival to eclosion, and development time did not show a correlated response to selection on adult male longevity despite the presence of substantial genetic variation in all of these life-history traits. In the only other study to select directly on adult longevity, Zwaan et al. (1995*b*) found that body weight at eclosion did not differ between *D. melanogaster* lines selected for long and short adult longevity and that neither differed from unselected controls. By contrast, lines selected for shorter adult longevity developed more slowly than those selected for longer adult longevity (Zwaan et al. 1995*b*). However, when the same authors selected directly on development time, there was no correlated response in adult longevity (Zwaan et al. 1995*a*).

These observed inconsistencies illustrate the need to complement laboratory selection experiments with observations made under natural or seminatural conditions on the relationship between senescence and life-history traits (Zwaan 1999). Tatar et al. (1997) demonstrated that populations of *Melanoplus* grasshoppers from high elevation have increased rates of senescence because more severe winters at high altitude result in stronger selection to shift reproductive schedules toward earlier breeding. Similarly, in a field population of translocated guppies (*Poecilia reticulata*), a change in the relative rate of extrinsic mortality on adults and juveniles due to predation was associated with an evolutionary shift toward late maturation, reduced reproductive effort, and the production of fewer, larger offspring per brood (Reznick et al. 1990; but see Reznick et al. 2004). Interestingly, however, in the laboratory, individuals from populations with higher extrinsic mortality rates do not have shorter life spans or cease reproducing sooner than those from populations with lower extrinsic mortality rates. The extent to which life span in the absence of extrinsic mortality is genetically correlated with major life-history variables is therefore an area where more research is required. We are currently undertaking a common garden experiment using six populations of *T. commodus* collected across southern Australia to examine large-scale genetic relationships between rates of senescence in males and females, age-dependent reproductive effort, and life-history traits. We anticipate that it will contribute to our understanding of the causes and evolutionary consequences of senescence in *T. commodus* populations.

Acknowledgments

We thank C. House, F. Zajitschek, and two anonymous reviewers for valuable comments and discussions that greatly improved this manuscript. We also thank A. Gasket and M. Smith for laboratory assistance in rearing and maintaining crickets in our selection lines. We thank S. Blomberg and F. Zajitschek for statistical advice. This research was supported by an Australian Research Council grant to J.H., R.B., and M.D.J., and J.H. was supported by a Natural Environment Research Council fellowship during the analysis and writing of this article.

Literature Cited

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, NJ.

Arnqvist, G., and L. Rowe. 2005. Sexual conflict. Princeton University Press, Princeton, NJ.

Barton, N., and P. D. Keightley. 2002. Understanding quantitative genetic variation. Nature Reviews Genetics 3:11–20.

Beck, C. W., and L. A. Powell. 2000. Evolution of female mate choice based on male age: are older males better mates? Evolutionary Ecology Research 2:107–118.

Bentsen, C. L., J. Hunt, M. D. Jennions, and R. Brooks. 2006. Complex multivariate sexual selection on male acoustic signaling in a wild population of *Teleogryllus commodus*. American Naturalist 167:E102–E116.

Bertram, S. M., and L. Johnson. 1998. An electronic technique for monitoring the temporal aspects of acoustic signals of captive organisms. Bioacoustics 9:107–118.

Blows, M. W., and M. Higgie. 2003. Genetic constraints on the evolution of mate recognition under natural selection. American Naturalist 161:240–253.

Bonduriansky, R., and C. E. Brassil. 2002. Rapid and costly ageing in wild male flies. Nature 420:377.

———. 2005. Reproductive ageing and sexual selection on male body size in a wild population of antler flies (*Protopiophila litigata*). Journal of Evolutionary Biology 18:1332–1340.

Brooks, R. 2000. Negative genetic correlation between male sexual attractiveness and survival. Nature 406:67–70.

Brooks, R., and D. J. Kemp. 2001. Can older males deliver the good genes? Trends in Ecology & Evolution 16:308–313.

Cade, W. H. 1981. Alternative male strategies: genetic differences in crickets. Science 212:563–564.

Carey, J. R., P. Liedo, H.-G. Müller, J.-L. Wang, and J. W. Vaupel. 1998. Dual modes of ageing in Mediterranean fruit fly females. Science 281:996–998.

Carranza, J., S. Alarcos, C. B. Sánchez-Prieto, J. Valencia, and C. Mateos. 2004. Disposable-soma senescence mediated by sexual selection in an ungulate. Nature 432:215–218.

Charlesworth, B. 1994. Evolution in age-structured populations. Cambridge University Press, London.

Charlesworth, B., and D. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237–268.

Chippindale, A. K, D. T. Hoang, P. M. Sevice, and M. R. Rose. 1994. The evolution of development in *Drosophila melanogaster* selected for postponed senescence. Evolution 48:1880–1899.

Chippindale, A. K., J. R. Gibson, and W. R. Rice. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. Proceedings of the National Academy of Sciences of the USA 98:1671–1675.

Cohen, J. 1988. Statistical power analysis for the behavioural sciences. 2nd ed. Erlbaum, New York.

Cordts, R., and L. Partridge. 1996. Courtship reduces longevity of male *Drosophila melanogaster*. Animal Behaviour 52:269–278.

Crawley, M. J. 2002. Statistical computing: an introduction to data analysis using S-Plus. Wiley, Chichester.

Crnokrak, P., and D. A. Roff. 1999. Inbreeding depression in the wild. Heredity 83:260–270.

Darwin, C. 1871. The descent of man and selection in relation to sex. J. Murray, London.

de Rose, M. A., and D. A. Roff. 1999. A comparison of inbreeding depression in life-history and morphological traits in animals. Evolution 53:1288–1292.

Eshel, I., I. Volovik, and E. Sansone. 2000. On Fisher-Zahavi's handicapped sexy son. Evolutionary Ecology Research 2:509–523.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longman, New York.

Fedorka, K. M., and T. A. Mousseau. 2004. Female mating bias results in conflicting sex-specific offspring fitness. Nature 429:65–67.

Fisher, R. A. 1930. The genetical theory of natural selection. Oxford University Press, Oxford.

Frankham, R. 1990. Are responses to artificial selection for reproductive fitness characters consistently asymmetrical? Genetical Research 56:35–42.

Gasser, M., M. Kaiser, D. Berrigan, and S. C. Stearns. 2000. Life-history correlates of evolution under high and low adult mortality. Evolution 54:1260–1272.

Grafen, A. 1990. Biological signals as handicaps. Journal of Theoretical Biology 144:517–546.

Hamilton, W. D. 1966. The moulding of senescence by natural selection. Journal of Theoretical Biology 12:12–45.

Hansen, T. F., and D. K. Price. 1995. Good genes and old age: do old mates provide superior genes? Journal of Evolutionary Biology 8:759–778.

Hill, W. G. 1970. Note: design of experiments to estimate heritability by regression of offspring on selected parents. Biometrics 26:566–571.

———. 1972. Estimation of realised heritabilities from selection experiments. I. Divergent selection. Biometrics 28:747–765.

Hoffmann, A. A., and J. Merilä. 1999. Heritable variation and evolution under favourable and unfavourable conditions. Trends in Ecology & Evolution 14:96–101.

Höglund, J., and B. C. Sheldon. 1998. The cost of reproduction and sexual selection. Oikos 83:478–483.

Hunt, J., R. Brooks, M. J. Smith, M. D. Jennions, C. L. Bentsen, and L. F. Bussière. 2004*a*. High-quality male field crickets invest heavily in sexual display but die young. Nature 432:1024–1027.

Hunt, J., L. F. Bussière, M. D. Jennions, and R. Brooks. 2004*b*. What is genetic quality? Trends in Ecology & Evolution 19:329–333.

Hunt, J., M.D. Jennions, and R. Brooks. 2005. Female mate choice as a condition-dependent life-history trait. American Naturalist 166:79–92.

Jennions, M. D., A. P. Møller, and M. Petrie. 2001. Sexually selected traits and adult survival: a meta-analysis. Quarterly Review of Biology 76:3–36.

Jennions, M. D., J. Hunt, R. Graham, and R. Brooks. 2004. No evidence for inbreeding avoidance through postcopulatory mechanisms in the black field cricket, *Teleogryllus commodus*. Evolution 58:2472–2477.

Kirkwood, T. B. L. 1977. Evolution of ageing. Nature 270:301–304.

Kirkwood, T. B. L., and M. R. Rose. 1991. Evolution of senescence: late survival sacrificed for reproduction. Philosophical Transactions of the Royal Society B: Biological Sciences 332:15–24.

Kokko, H. 1997. Evolutionarily stable strategies of age-dependent sexual advertisement. Behavioral Ecology and Sociobiology 41:99–107.

———. 1998. Good genes, old age and life-history trade-offs. Evolutionary Ecology 12:739–750.

———. 2001. Fisherian and "good genes" benefits of mate choice: how (not) to distinguish between them. Ecology Letters 4:322–326.

Kokko, H., and J. Lindström. 1996. Evolution of female preference for old mates. Proceedings of the Royal Society B: Biological Sciences 263:1533–1538.

Kokko, H., R. Brooks, J. M. McNamara, and A. I. Houston. 2002. The sexual selection continuum. Proceedings of the Royal Society B: Biological Sciences 269:1331–1340.

Kokko, H., R. Brooks, M. D. Jennions, and J. Morley. 2003. The evolution of mate choice and mating biases. Proceedings of the Royal Society B: Biological Sciences 270:653–664.

Kotiaho, J. S. 2001. Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. Biological Reviews of the Cambridge Philosophical Society 76:365–376.

Kruuk, L. E. B., T. H. Clutton-Brock, J. Slate, J. M. Pemberton, S. Brotherstone, and F. E. Guinness. 2000. Heritability of fitness in a wild mammal population. Proceedings of the National Academy of Sciences of the USA 97:698–703.

Latter, B. D. H., and A. Robertson. 1962. The effects of inbreeding and artificial selection on reproductive fitness. Genetical Research 3:110–138.

Lynch, M., and B. Walsh. 1997. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.

Manning, J. T. 1985. Choosy females and correlates of male age. Journal of Theoretical Biology 116:349–354.

Medawar, P. B. 1946. Old age and natural death. Modern Quarterly 1:56.

Mertz, D. B. 1975. Senescent decline in flour beetle strains selected for early adult fitness. Physiological Zoology 48:1–23.

Miller, L. K., and R. Brooks. 2005. The effects of genotype, age, and social environment on male ornamentation, mating behavior, and attractiveness. Evolution 59:2414–2425.

Miyatake, T. 1997. Genetic trade-off between early fecundity and longevity in *Bactrocera cucurbitae* (Diptera: Tephritidae). Heredity 78:93–100.

Møller, A. P., and R. V. Alatalo. 1999. Good-genes effects in sexual selection. Proceedings of the Royal Society B: Biological Sciences 266:85–91.

Mühlhäuser, C., and W. U. Blanckenhorn. 2004. The quantitative genetics of sexual selection in the dungfly *Sepsis cynipsea*. Behaviour 141:327–341.

Müller, H.-G., J.-L. Wang, W. B. Capra, P. Liedo, and J. R. Carey. 1997. Early mortality surge in protein-deprived females causes reversal of sex differential of life expectancy in Mediterranean fruit flies. Proceedings of the National Academy of Sciences of the USA 94:2762–2765.

Partridge, L., and N. H. Barton. 1993. Optimality, mutation and the evolution of ageing. Nature 362:305–311.

Partridge, L., and M. Farquhar. 1981. Sexual activity reduces lifespan of male fruitflies. Nature 294:580–582.

Partridge, L., and K. Fowler. 1992. Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Evolution 46:76–91.

Pettay, J. E., L. E. B. Kruuk, J. Jokela, and V. Lummaa. 2005. Heritability and genetic constraints of life-history trait evolution in preindustrial humans. Proceedings of the National Academy of Sciences of the USA 102:2838–2843.

Pinheiro, J. C., and D. M. Bates. 2000. Mixed-effects models in S and S-Plus. Springer, New York.

Pletcher, S. D. 1999. Model fitting and hypothesis testing for age-specific mortality data. Journal of Evolutionary Biology 12:314–328.

Promislow, D. 2003. Mate choice, sexual conflict, and the evolution of senescence. Behaviour Genetics 33:191–201.

Proulx, S., T. Day, and L. Rowe. 2002. Older males signal more reliably. Proceedings of the Royal Society B: Biological Sciences 269:2291–2299.

Reznick, D., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. Nature 346:357–359.

Reznick, D. N., M. J. Bryant, D. Roff, C. K. Ghalambor, and D. E. Ghalambor. 2004. Effect of extrinsic mortality on the evolution of senescence in guppies. Nature 431:1095–1099.

Rice, W. R., and A. K. Chippindale. 2001. Intersexual ontogenetic conflict. Journal of Evolutionary Biology 14:685–693.

Roff, D. A. 2002. Life history evolution. Sinauer, Sunderland, MA.

Rose, M. R. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. Evolution 38:1004–1010.

Rose, M. R., and B. Charlesworth. 1981. Genetics of life history in *Drosophila melanogaster*. II. Exploratory selection experiments. Genetics 97:196.

Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. Proceedings of the Royal Society B: Biological Sciences 263:1415–1421.

Sgró, C. M., and L. Partridge. 1999. A delayed wave of death from reproduction in *Drosophila*. Science 286:2521–2525.

Svensson, E., and B. C. Sheldon. 1998. The social context of life history evolution. Oikos 83:466–477.

Tatar, M., D. W. Gray, and J. R. Carey. 1997. Altitudinal variation for senescence in *Melanoplus* grasshoppers. Oecologia (Berlin) 111: 357–359.

Therneau, T. M. 2003. On mixed-effect Cox models, sparse matrices and modeling data from large pedigrees. http://mayoresearch.mayo.edu/mayo/research/biostat/upload/kinship.pdf.

Tomkins, J. L., J. Radwan, J. S. Kotiaho, and T. Tregenza. 2004. Genic capture and resolving the lek paradox. Trends in Ecology & Evolution 19:323–328.

Trivers, R. L. 1972. Parental investment and sexual selection. Pages 136–179 *in* B. Campbell, ed. Sexual selection and the descent of man 1871–1971. Heinemann, London.

Tucic, N., O. Stojkovic, I. Gliksman, D. Milanovic, and D. Šešlija. 1996. Laboratory evolution of life history traits in the bean weevil (*Acanthoscelides obtectus*): the effects of density-dependent and age-specific selection. Evolution 51:1894–1907.

van Oosterhout, C., W. G. Zijlstra, M. K. van Heuren, and P. M. Brakefield. 2000. Inbreeding depression and genetic load in a laboratory meta population of the butterfly *Bicyclus anynana*. Evolution 54:218–225.

Williams, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398–411.

Zahavi, A. 1975. Mate selection: a selection for a handicap. Journal of Theoretical Biology 53:205–214.

Zwaan, B. J. 1999. The evolutionary genetics of ageing and longevity. Heredity 82:589–597.

Zwaan, B. J., R. Bijlsma, and R. F. Hoekstra. 1995*a*. Artificial selection for development time in *Drosophila melanogaster* in relation to the evolution of aging: direct and correlated responses. Evolution 49: 635–648.

———. 1995*b*. Direct selection on life span in *Drosophila melanogaster*. Evolution 49:649–659.

Associate Editor: David Reznick
Editor: Michael C. Whitlock