High-quality male field crickets invest heavily in sexual display but die young

John Hunt[1], Robert Brooks[1], Michael D. Jennions[2], Michael J. Smith[1]*, Caroline L. Bentsen[1] & Luc F. Bussière[1]

[1]*School of Biological, Earth and Environmental Sciences, The University of New South Wales, Sydney, New South Wales, 2052, Australia*
[2]*School of Botany and Zoology, Australian National University, Canberra, ACT, 0200, Australia*

* Present address: Arthur Rylah Institute for Environmental Research, 123 Brown Street, Heidelberg, Victoria, 3084, Australia

Only high-quality males can bear the costs of an extreme sexual display[1–4]. As a consequence, such males are not only more attractive, but they often live longer than average[5]. Recent theory predicts, however, that high-quality males should sometimes invest so heavily in sexual displays that they die sooner than lower quality males[2,6–9]. We manipulated the phenotypic quality of field crickets, *Teleogryllus commodus*, by altering the protein content of their diet. Here we show that nymphs and adult females reared on a high-protein diet lived longer than those on a low-protein diet. In contrast, adult males reared on a high-protein diet died sooner than those on low-protein diets because they invested more energy in calling during early adulthood. Our findings uphold the theoretical prediction that the relationship between longevity and sexual advertisement may be dynamic[2,3,6–8] (that is, either positive or negative), depending on local conditions[3,6] such as resource availability. Moreover, they caution the use of longevity as a proxy for fitness in sexual selection studies, and suggest avenues for future research on the relationship between sexual attractiveness and ageing.

The resources acquired by individual males (described as "condition" in ref. 4) may vary according to extrinsic ecological factors and genetic differences in acquisition and assimilation ability[4,10,11]. Phenotypically plastic allocation in response to accumulated resources is thought to be the basis for condition-dependent signals of male quality[3,4,8,11–13]. Male field crickets, for example, have been shown to decrease the time they spend calling with reduced food availability as adults[14,15]. However, adult life-history decisions often depend critically on the resources acquired during juvenile development[16].

Here, we manipulated the protein content of the diet fed to field crickets, *Teleogryllus commodus*, from hatching until death, to mimic consistent life-long differences in the ability of individuals to acquire resources. We then measured the time that males spent calling, and where possible the call structure of males, at five-day intervals. We also monitored the longevity, development time, size and weight at eclosion, as well as weight gain during the first ten days post-eclosion, of all individuals to test the prediction that males in good condition (that is, that have acquired more resources) may allocate so much energy to sexual advertisement that they die sooner than males in poorer condition.

Higher dietary protein was associated with greater nymph survival to eclosion (Fig. 1a), faster development and heavier weight at eclosion for both sexes, and with larger body size at eclosion for males (Table 1; for full details of these and all other analyses see Supplementary Information). It was also associated with greater longevity of adult females (Fig. 1b). In contrast, adult males reared on higher protein diets had shorter adult lives than those reared on a lower protein diet (Fig. 1c). This reduction in adult male longevity was associated with elevated calling effort early in life (10–20 days after eclosion) by males reared on a high-protein diet, both in terms of the proportion of males calling on a given night (Fig. 2a) and nightly calling effort (Fig. 2b). Despite dying earlier, males reared on the high-protein diet called more in their lifetime than those reared on the lower protein diets ($F_{2,198} = 47.8$, $P < 0.001$; high protein: 16,271 ± 1,173 s (mean ± s.e.m.); medium protein: 7,286 ± 781 s; low protein: 4,489 ± 599 s; Tukey's post-hoc comparison: high versus medium, and high versus low, $P < 0.001$; medium versus low, $P = 0.090$). Call structure did not, however, change significantly with either diet treatment or male age and there was no significant interaction between the two (multivariate analysis of variance (MANOVA); diet treatment, Pillai's trace = 0.096, $F_{10,128} = 0.772$, $P = 0.772$; male age, Pillai's trace = 0.126, $F_{5,63} = 1.816$, $P = 0.122$; interaction, Pillai's trace = 0.073, $F_{10,128} = 0.485$, $P = 0.897$). Given the effect of diet on male body size and the fact that adult size is generally correlated with call components (such as dominant frequency) in field crickets and other acoustic insects[17], why our diet treatment had no effect on call structure is the subject of current investigation in our laboratory.

Three lines of evidence suggest that the higher mortality rate of males reared on high-protein diets is due to elevated calling effort in early adulthood. First, within each treatment there was a negative relationship between early calling effort (days 10–20) and longevity (Fig. 3a). Second, males on high- and medium-protein diets tended to lose weight after the fourth day post-eclosion, whereas males on the low-protein diet continued to gain or maintain their body

Table 1 **Diet and sex affect development time, size and weight at eclosion and weight gain**

	MANOVA			
	Pillai's trace	*F*	d.f.	*P*
Diet	0.316	19.19	8, 818	<0.001
Sex	0.466	89.17	4, 408	<0.001
Diet × sex	0.098	5.29	8, 818	<0.001

	Univariate analyses				
	Diet means			ANOVA	
	High	Medium	Low	*F**	*P*
Males					
Development time (days)	96[a] (12)	98[a] (12)	105[b] (12)	10.64	< 0.001
Pronotum width (mm)	6.64[a] (0.20)	6.66[a] (0.17)	6.52[b] (0.23)	9.28	< 0.001
Weight at eclosion (mg)	737[a] (142)	722[ab] (122)	674[b] (125)	4.34	0.014
Weight gain, days 0–10 after eclosion (mg day^{-1})	0.5 (9.4)	2.1 (8.9)	4.6 (13.2)	2.68	0.071
Females					
Development time (days)	97[a] (12)	100[a] (11)	115[b] (17)	32.57	< 0.001
Pronotum width (mm)	6.82 (0.23)	6.91 (0.32)	6.85 (0.27)	1.95	0.145
Weight at eclosion (mg)	733[a] (98)	714[ab] (96)	682[b] (99)	4.78	0.009
Weight gain, days 0–10 after eclosion (mg day^{-1})	17.6[a] (15.6)	8.8[b] (15.6)	8.1[b] (15.6)	7.86	0.001

The effect of the interaction between sex and diet treatment occurs because males on high- and medium-protein diets gain weight more slowly than males reared on low-protein diets, whereas the opposite is true for females. Standard deviations are provided in parentheses. Letters in superscript indicate homogeneous subsets after Tukey's post-hoc tests. *$F_{2,200}$ (males); $F_{2,211}$ (females).

weight until day 15 (Fig. 2c), which coincided with when they started calling (Fig. 2a, b). Further support for an association between male weight loss and the onset of calling comes from the fact that in females, greater weight gain is associated with higher protein diets (Table 1). Third, males on high- and medium-protein diets lost a greater proportion of their body mass after each night's calling than males from the low-protein treatment (linear mixed model: treatment effect, $F_{2,169} = 73.18$, $P < 0.001$; least significant difference post-hoc comparisons, high versus medium, $P = 0.064$; high versus low, and medium versus low, $P < 0.001$). In the field, the negative relationship between early calling effort and longevity should, if anything, be stronger than in the laboratory because of the increased risks faced by calling males[18].

Within treatments, calling effort in early adulthood (10–20 days) was consistently negatively correlated with longevity (Fig. 3a). However, the relationships between late calling effort (25–30 days) and longevity were consistently positive (Fig. 3b). The shift from early to late calling across the treatments changes the relationship between mean calling effort per male per night and longevity from negative in the high- and medium-protein treatments to positive in the low-protein diet treatment (Fig. 3c). The mean condition of males in the population, therefore, not only determines how much a male invests in calling but also the nature of the

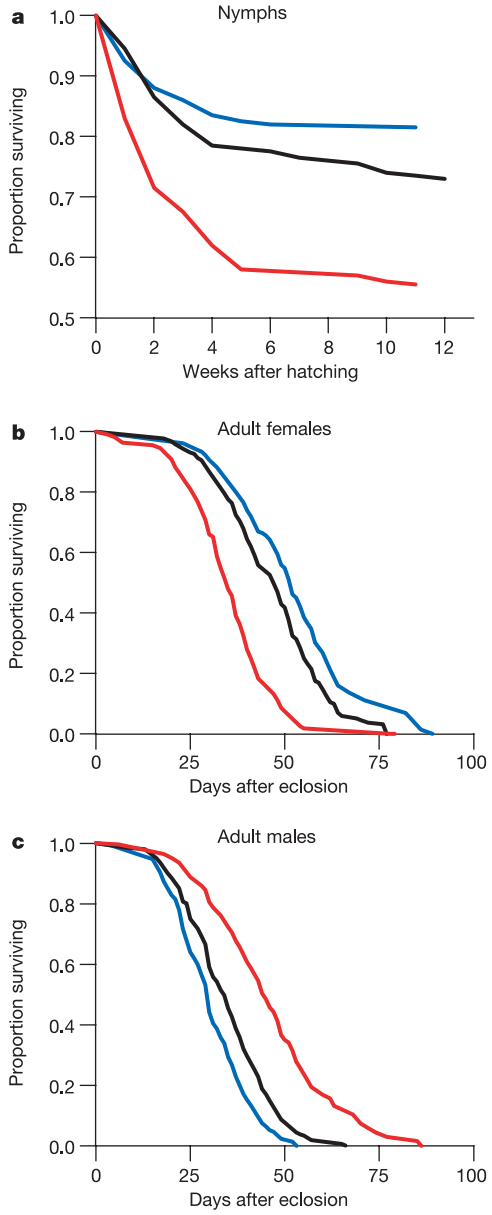

Figure 1 Nymph survival and adult longevity in the high- (blue line), medium- (black) and low-protein (red) diet treatments. **a**, Nymph survival to eclosion was highest in the high-protein treatment and lowest in the low-protein treatment (Cox regression, $\chi^2 = 29.31$, d.f. $= 2$, $P < 0.001$). **b**, Similarly, adult female longevity was highest in the high-protein treatment and lowest in the low-protein treatment, but **c**, the opposite was true in adult males (Cox regression, treatment $\chi^2 = 60.78$, d.f. $= 2$, $P < 0.001$; sex $\chi^2 = 31.06$, d.f. $= 1$, $P < 0.001$; treatment × sex $\chi^2 = 113.43$, d.f. $= 1$, $P < 0.001$).

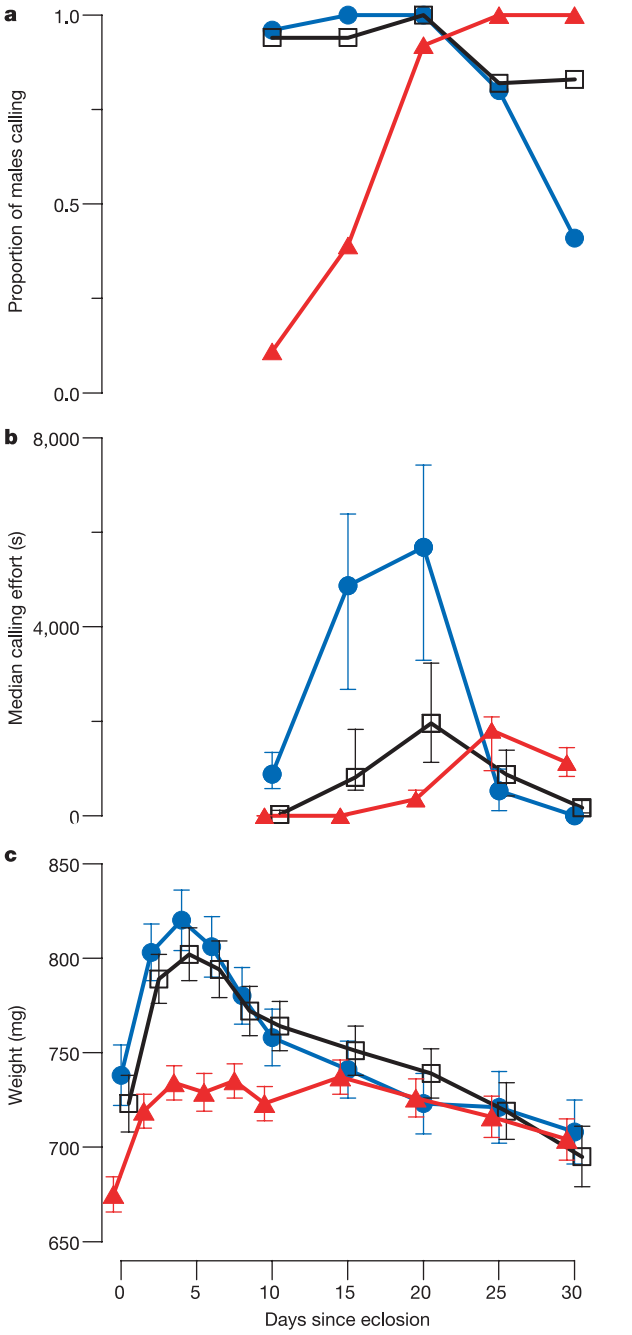

Figure 2 Reproductive effort and body weight of males in the high- (closed circles, blue line), medium- (open squares, black line) and low-protein (triangles, red line) diet treatments. **a**, Proportion of males calling. **b**, Median calling effort per night (with jackknifed 95% confidence intervals). **c**, Male weight change (±s.e.m.) in each treatment. There were significant differences among treatments in the quadratic and cubic coefficients describing these weight changes (treatment × (time)^2, $F_{2,1660} = 20.34$, $P < 0.001$; treatment × (time)^3, $F_{2,1660} = 12.43$, $P < 0.001$).

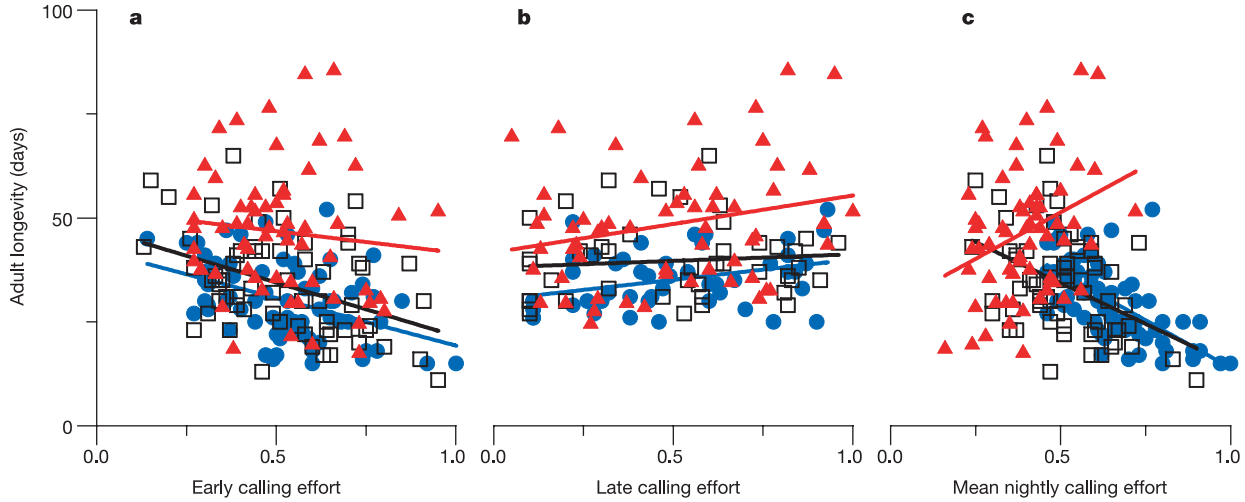

Figure 3 The relationships between longevity and early, late and mean nightly calling effort in the high- (closed circles, blue line), medium- (open squares, black line) and low-protein (triangles, red line) diet treatments (see Supplementary Information). **a**, Early calling effort: there were no treatment effects on the slope ($F_{2,192} = 0.86$, $P = 0.426$), but the common slope was significantly less than zero ($F_{1,197} = 24.38$, $P < 0.001$) and there were significant differences in elevation ($F_{2,197} = 34.09$, $P < 0.001$). **b**, Late calling effort: there were no treatment effects on the slope ($F_{2,145} = 1.70$, $P = 0.187$), but the common slope was significantly greater than zero ($F_{1,151} = 6.62$, $P = 0.011$) and there were significant differences in elevation ($F_{2,151} = 23.06$, $P < 0.001$). **c**, Mean nightly calling effort: slopes were significantly different from one another ($F_{2,195} = 20.06$, $P < 0.001$) due to differences between the low-protein treatment and the other two treatments.

relationship between reproductive effort and longevity within populations. Our current research is examining the relative contributions of phenotypic plasticity and selection on nymph survival within treatments to the overall relationship that exists between calling effort and longevity in *T. commodus*.

Abundant indirect and limited direct evidence has shown that sexual advertisement is costly[19]. Nonetheless, the fundamental tenet of handicap-signalling theory that high-quality males can better bear these costs than low-quality males[1–4], leads to the widespread expectation that quality, signalling and longevity will be positively correlated. This viewpoint is bolstered by the fact that studies reporting a positive relationship between sexual signalling and longevity are more commonly reported in the literature[5]. For example, in the wolf spider (*Hygrolycosa rubrofasciata*) male drumming is a costly sexual display that results in weight loss, increased intrinsic mortality risk[20] and higher chances of predation[21]. Despite this, within treatments, faster drumming males tend to survive longer than slower drummers[20]. This, like many similar studies[5], has been interpreted as showing that a costly sexual display is a signal of a viability benefit to offspring[20]. In contrast, the shorter lifespan of male crickets in our high-protein diet treatment provides the first direct confirmation of the theoretic prediction that males acquiring more resources may invest so heavily in sexual signals that they experience higher mortality than low condition males[2,6,7]. Unlike previous studies on the costs of sexual signalling[17], this relationship is not dependent on extrinsic sources of mortality (that is, predators or parasitoids[16]).

Dietary restriction increases lifespan in a range of organisms[22–25]. Caloric restriction is thought to retard ageing via a number of metabolic pathways[22,23]. The magnitude, but not the direction, of the effects of dietary restriction on longevity are often sex-specific[26,27] and this may, in part, be due to differences in the way that the sexes allocate resources to reproduction. For example, limiting the caloric content of the diet of female *Drosophila melanogaster*[24,27] and the protein content of the diet of female medflies (*Ceratitis capitata*)[25] results in reduced egg laying and concomitant increases in lifespan. However, dietary restriction also increases male longevity in these species, albeit to a lesser degree than female longevity[22–25], so that an overall reduction in metabolic rate can not be excluded as an alternative explanation for the increased longevity of individuals on a restricted diet. The opposing effects of diet on male and female *T. commodus* (Fig. 1b, c) suggest that the reduced longevity of males reared on a high-protein diet is not simply a metabolic by-product, but rather the direct result of increased reproductive effort in the form of calling as a sexual advertisement early in adulthood. More importantly, females from this population impose strong positive selection on calling effort in playback experiments in the wild (Fig. 4). Collectively, these findings suggest that phenotypic plasticity in the age-specific calling effort of males is an adaptive strategy to increase mating success.

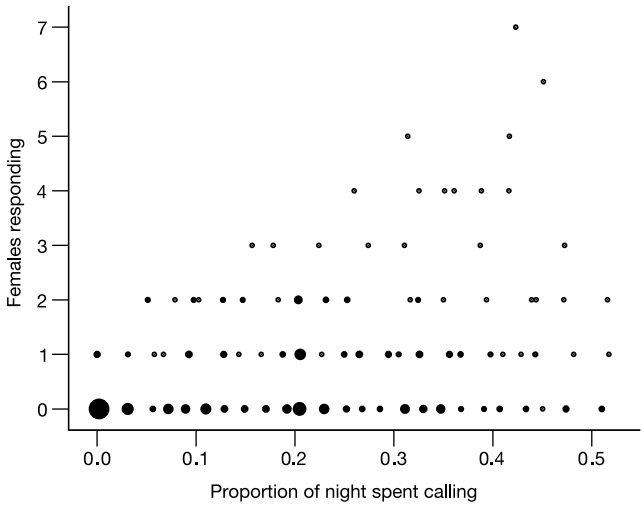

Figure 4 The number of females responding to a call in the field is positively correlated with the amount of time that the call is broadcast for. There was significant linear selection on the amount of time a call is broadcast in the field ($\beta = 0.355 \pm 0.062$ s.e, randomization test $P = 0.0001$). The largest circle represents 23 observations, the smallest represents 1 observation.

Our results demonstrate that whether the relationship between sexual advertisement and longevity is positive or negative depends critically on the mean condition of males in the population. Several authors have stressed the importance of understanding male sexual

advertisement as a form of reproductive effort within the framework of life-history theory[6,8,10,13,28], and of measuring such investment throughout a male's lifetime[28–30]. Our findings provide further evidence of the value of this approach. We demonstrate that longer-lived males are not always those in the best condition and therefore our findings support theoretical claims that longevity may not always be a reliable measure of male quality[6,9,10]. □

Methods

Laboratory feeding experiment

Animals for the laboratory feeding experiment were the F_3 descendants of 200 field-mated females collected at Smith's Lake, New South Wales, Australia, in March 2002. Cultures were provided with cat food (Friskies Go-Cat Senior) and water *ad libitum* and maintained by rearing the offspring of 100 randomly paired adults per generation in six large stock-culture containers (80 litre). Both the cultures and experimental animals were maintained in a room with a constant temperature of 28 ± 1 °C and a 10 h:14 h dark:light regime.

Experimental manipulation of condition

Manipulation of condition was achieved by feeding crickets on pellets comprising different mixtures of high-protein fish food (Pisces Enterprises, 45% protein) and oatmeal (Farmland, 12% protein). The high-, medium- and low-protein diet treatments consisted of 100% fish pellets or a dry weight mixture of 75% or 50% fish pellets with oatmeal. We created pellets by grinding fish food and oatmeal, adding water and drying the mixture in a custom-built plexiglass mould in an oven (60 °C for 12 h). Dry pellets weighed 0.121 g ± 0.002 s.e.

Experimental design

We collected 600 nymphs within 24 h of hatching and randomly assigned each to a low-, medium- or high-protein diet (200 nymphs per diet). Each cricket was housed in a separate individual plastic container (5 × 5 × 5 cm) for the duration of its life and provided with water, three diet pellets per week and a piece of egg carton for shelter. Food and water were replenished weekly and the container cleaned and nymph survival recorded. Fifth-instar nymphs were checked daily for eclosion. On the day of eclosion, and on the second, fourth, sixth, eighth and tenth day afterwards, each adult was weighed (to 0.00005 g) and pronotum width measured using an eyepiece graticule in a binocular microscope. We recorded the calling effort of living males at 10, 15, 20, 25 and 30 days after eclosion. Males were weighed the day before and the day after each calling effort measure. Food and water were replenished and the container cleaned the morning after. Females were maintained on an identical regime to males, apart from calling effort measures. Adult survival was monitored daily.

Calling effort

The calling effort of individual males was measured using an electronic monitoring device (see Supplementary Information) that monitored 64 males per night from 18:00 to 09:00 in a room set to a constant temperature of 28 ± 1 °C. Males, in their individual containers, were placed in separate styrofoam containers (15 × 10 × 10 cm), which were closed to keep males in acoustic isolation.

Field acoustic trials

We estimated the strength of sexual selection that females exert on male calling effort using playbacks of artificial calls conducted over 25 consecutive nights in the field at Smith's Lakes, New South Wales. We used SoundEdit (version 1) to construct 300 calls that varied randomly in dominant frequency, inter-call interval, number of pulses per chirp, inter-pulse interval and number of trills. We manipulated the amount of time that each call was broadcast for by randomly varying the number of times the call was repeated in the 5-min loop. Calls were broadcast from 13 sets of two speakers facing in opposite directions at 75 dB (65 cm from speakers) from 21:00 until 05:30. Females responding to a call were captured on a sticky trap (60 × 60 cm) surrounding the speakers.

Received 15 June; accepted 6 October 2004; doi:10.1038/nature03084.

1. Zahavi, A. Mate selection—a selection for a handicap. *J. Theor. Biol.* **53,** 205–214 (1975).
2. Grafen, A. Biological signals as handicaps. *J. Theor. Biol.* **144,** 517–546 (1990).
3. Nur, N. & Hasson, O. Phenotypic plasticity and the handicap principle. *J. Theor. Biol.* **110,** 275–297 (1984).
4. Rowe, L. & Houle, D. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* **263,** 1415–1421 (1996).
5. Jennions, M. D., Møller, A. P. & Petrie, M. Sexually selected traits and adult survival: a meta-analysis. *Q. Rev. Biol.* **76,** 3–36 (2001).
6. Kokko, H., Brooks, R., McNamara, J. M. & Houston, A. I. The sexual selection continuum. *Proc. R. Soc. Lond. B* **269,** 1331–1340 (2002).
7. Eshel, I., Volovik, I. & Sansone, E. On Fisher-Zahavi's handicapped sexy son. *Evol. Ecol. Res.* **2,** 509–523 (2000).
8. Höglund, J. & Sheldon, B. C. The cost of reproduction and sexual selection. *Oikos* **83,** 478–483 (1998).
9. Hansen, T. F. & Price, D. K. Good genes and old age: Do old mates provide superior genes? *J. Evol. Biol.* **8,** 759–778 (1995).
10. Hunt, J., Bussière, L. F., Jennions, M. D. & Brooks, R. What is genetic quality? *Trends Ecol. Evol.* **19,** 329–333 (2004).
11. Tomkins, J. L., Radwan, J., Kotiaho, J. S. & Tregenza, T. Genic capture and resolving the lek paradox. *Trends Ecol. Evol.* **19,** 323–328 (2004).
12. Kotiaho, J. S., Simmons, L. W. & Tomkins, J. L. Towards a resolution of the lek paradox. *Nature* **410,** 684–686 (2001).
13. Kokko, H. Good genes, old age and life-history trade-offs. *Evol. Ecol.* **12,** 739–750 (1998).
14. Wagner, W. E. J. & Hoback, W. W. Nutritional effects on male calling behaviour in the variable field cricket. *Anim. Behav.* **57,** 89–95 (1999).
15. Holzer, B., Jacot, A. & Brinkhof, M. W. G. Condition-dependent signaling affects male sexual attractiveness in field crickets, *Gryllus campestris*. *Behav. Ecol.* **14,** 353–359 (2003).
16. Roff, D. A. *Life History Evolution* (Sinauer Associates, Sunderland, Massachusetts, 2002).
17. Gerhardt, H. C. & Huber, F. *Acoustic Communication in Insects and Anurans* (Princeton Univ. Press, Princeton, 2002).
18. Zuk, M. & Kolluru, G. R. Exploitation of sexual signals by predators and parasitoids. *Q. Rev. Biol.* **73,** 415–438 (1998).
19. Kotiaho, J. S. Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. *Biol. Rev.* **76,** 365–376 (2001).
20. Kotiaho, J. S. Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait. *Behav. Ecol. Sociobiol.* **48,** 188–194 (2000).
21. Kotiaho, J., Alatalo, R. V., Mappes, J., Parri, S. & Rivero, A. Male mating success and risk of predation in a wolf spider: a balance between sexual and natural selection? *J. Anim. Ecol.* **67,** 287–291 (1998).
22. Lin, S.-J. *et al.* Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration. *Nature* **418,** 344–348 (2002).
23. Sohal, R. S. & Weindruch, R. Oxidative stress, caloric restriction, and aging. *Science* **273,** 59–63 (1996).
24. Chippindale, A. K., Leroi, A. M., Kim, S. B. & Rose, M. R. Phenotypic plasticity and selection in *Drosophila* life-history evolution. I. Nutrition and the cost of reproduction. *J. Evol. Biol.* **6,** 171–193 (1993).
25. Carey, J. R., Liedo, P., Müller, H.-G., Wang, J.-L. & Vaupel, J. W. Dual modes of ageing in Mediterranean fruit fly females. *Science* **281,** 996–998 (1998).
26. Müller, H.-G., Wang, J.-L., Capra, W. B., Liedo, P. & Carey, J. R. Early mortality surge in protein-deprived females causes reversal of sex differential of life expectancy in Mediterranean fruit flies. *Proc. Natl Acad. Sci. USA* **94,** 2762–2765 (1997).
27. Magwere, T., Chapman, T. & Partridge, L. Sex differences in the effect of dietary restriction on life span and mortality rates in female and male *Drosophila melanogaster*. *J. Gerontol. Biol. Sci.* **59A,** 3–9 (2004).
28. Gustafsson, L., Qvarnström, A. & Sheldon, B. C. Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. *Nature* **375,** 311–313 (1995).
29. Kokko, H. *et al.* Female choice selects for lifetime lekking performance in black grouse males. *Proc. R. Soc. Lond. B* **266,** 2109–2115 (1999).
30. Candolin, U. Changes in expression and honesty of sexual signalling over the reproductive lifetime of sticklebacks. *Proc. R. Soc. Lond. B* **267,** 2425–2430 (2000).

Supplementary Information accompanies the paper on **www.nature.com/nature**.

Acknowledgements We are grateful to S. Bertram for advice on the construction of the call-monitoring device. Thanks to J. Evans, S. Griffith, D. Gwynne, M. Head, T. Ivy, H. Kokko, J. Kotiaho and A. Lindholm for discussions and help in the field. J.H., R.B. and M.D.J. were funded by an ARC grant and L.F.B. by a NSERC Fellowship.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to J.H. (john.hunt@unsw.edu.au).

Foxa2 regulates lipid metabolism and ketogenesis in the liver during fasting and in diabetes

Christian Wolfrum[1], Esra Asilmaz[2], Edlira Luca[1], Jeffrey M. Friedman[2] & Markus Stoffel[1]

[1]*Laboratory of Metabolic Diseases, and* [2]*Laboratory of Molecular Genetics, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, New York 10021, USA*

The regulation of fat and glucose metabolism in the liver is controlled primarily by insulin and glucagon. Changes in the circulating concentrations of these hormones signal fed or starvation states and elicit counter-regulatory responses that maintain normoglycaemia. Here we show that in normal mice, plasma insulin inhibits the forkhead transcription factor Foxa2 by nuclear exclusion and that in the fasted (low insulin) state Foxa2 activates transcriptional programmes of lipid metabolism and ketogenesis. In insulin-resistant or hyperinsulinaemic mice,