

Diet-dependent female evolution influences male lifespan in a nuptial feeding insect

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Abstract

Theory predicts that lifespan will depend on the dietary intake of an individual, the allocation of resources towards reproduction and the costs imposed by the opposite sex. Although females typically bear the majority of the cost of offspring production, nuptial feeding invertebrates provide an ideal opportunity to examine the extent to which reproductive interactions through gift provisioning impose a cost on males. Here we use experimental evolution in an Australian ground cricket to assess how diet influences male lifespan and how the costs of mating evolve for males. Our findings show that males had significantly shorter lifespans in populations that adapted to a low-quality diet and that this divergence is driven by evolutionary change in how females interact with males over reproduction. This suggests that the extent of sexual conflict over nuptial feeding may be under-realized by focusing solely on the consequences of reproductive interactions from the female's perspective.

Introduction

Diet and reproduction are intimately linked with the evolution of aging and lifespan. The nutrient content of a diet can influence how individuals maximize lifespan and reproductive investment (Mair *et al.*, 2005; Piper *et al.*, 2005; Lee *et al.*, 2008; Maklakov *et al.*, 2008). Likewise, the allocation of dietary resources to either reproductive effort or longevity-enhancing pathways such as somatic maintenance (Kirkwood, 1977) can affect the trade-offs that are fundamental to life-history evolution (Roff, 1992; Stearns, 1992). Moreover, it has increasingly been recognized that both cooperative and antagonistic reproductive interactions between males and females at mating can also directly influence how long individuals live (Promislow, 2003; Maklakov *et al.*, 2005; Bonduriansky *et al.*, 2008). Such influences occur because of changes in the resource budgets of males and females that come about through mating (nutritional depletion or supplementation via the exchange of nutrients between mating partners, e.g. Gwynne, 1984; Bussière *et al.*, 2005), together with the direct costs of

mating or the manipulation of resource allocation that results from antagonistic reproductive interactions (inter-locus conflict, Arnqvist & Rowe, 2005). Despite considerable research effort directed at characterizing the effects of diet and reproductive investment on longevity (Hunt *et al.*, 2004; Mair *et al.*, 2005; Piper *et al.*, 2005; Lee *et al.*, 2008), few studies have addressed how diet quality and sexual behaviour interact to influence lifespan.

One potential source of diet-mediated changes in the costs of reproductive interactions consists of the nuptial feeding interactions of many invertebrates (Sakaluk *et al.*, 2006; Vahed, 2007; Wedell *et al.*, 2008). In these species, both males and females make significant reproductive contributions to the fitness of their offspring (Simmons & Gwynne, 1993), but the relative importance of male- and female-derived contributions to offspring can vary substantially, and can even change dramatically within species depending on the level of environmental resource availability. In some cases, when resources are scarce, male reproductive investment in offspring (Simmons, 1994), together with the associated costs of mating (Simmons & Kvarnemo, 2006), can exceed the investment of and/or the costs experienced by females. In such situations, the sex roles can reverse with females actually competing for access to males as potential food sources, and males exercising mate choice for high-quality partners (Gwynne & Simmons, 1990; Simmons, 1992;

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Cumming, 1994). However, although the nature of mate choice in these systems can be altered by environmental resource availability, the nature of sexual selection is not exactly the inverse of conventional systems because the balance of direct and indirect benefits available to choosy males is different from the typical situation for choosy females (Bonduriansky, 2001).

One of the most highly contested questions concerning sexual selection in nuptial feeding systems concerns whether or not nuptial feeding interactions are sites of antagonistic interactions between males and females. Most of the debate centres on whether nuptial gifts act to manipulate female current or future reproductive investment or if nuptial feeding imposes a net fitness cost on females (Sakaluk *et al.*, 2006; Vahed, 2007; Gwynne, 2008). Such arguments arise when considering mating systems like that of the decorated cricket, *Grylodes sigillatus*, in which the nuptial gifts that females receive contain very little nutritional benefit (Will & Sakaluk, 1994), but instead appear to contain substances that act to inhibit the sexual receptivity of the female (Sakaluk *et al.*, 2006). For the majority of nuptial feeding insects, however, negative effects of nuptial gifts are less likely as comparative and experimental evidence indicate that nuptial gifts typically provide direct fitness benefits to females (Gwynne, 2008). In part, the confusion regarding the role of sexual conflict over reproductive interactions in nuptial feeding insects occurs because the focus has centred on the costs and benefits received by females. Importantly, the extent to which providing the gift imposes a cost on males has received less attention.

Here, we present the results of a study in which we combined diet manipulation with experimental evolution in a nuptial feeding Australian ground cricket (*Pteronemobius* sp.) to understand how diet and the costs of mating interact to shape the evolution of male lifespan. In this species, the opportunity for females to exploit the nuptial gifts of males is clearly evident because males allow females to chew on a modified tibial spur and feed directly on their haemolymph. By exposing replicate populations to one of two artificial diets that vary in quality for seven generations, we have previously shown that *Pteronemobius* males from populations that have evolved on low-quality diet have a significantly reduced lifespan (Hall *et al.*, 2008a). We suggest three possible explanations as to why males from the low-quality diet populations had reduced lifespan. First, the differences may be a phenotypic response to the different carbohydrate and protein contents of the two diets, rather than the result of evolutionary change (Raubenheimer & Simpson, 1997; Raubenheimer *et al.*, 2005). Second, the males from the experimental populations of the two diet quality treatments may have diverged in how they allocate resources between reproductive investment and lifespan (Kirkwood, 1977; Hunt *et al.*, 2004). Third, our experimental diets may have altered the costs and benefits of donating and consuming

nuptial gifts for both mating partners, and changes in longevity come about in part because of the evolution of female exploitation of male-derived nuptial gift consumption. This third explanation is distinguished from the second because it presumes that any effect of experimental evolution relies not solely on allocation decisions by males, but rather on the interaction between males and females during mating.

To distinguish these competing explanations, we used a common garden approach in combination with mating trials to explore how dietary intake and the evolutionary histories of males and females contribute to male lifespan. Specifically, we raised individuals from each experimental population on a common diet (intermediate in quality to that of experimental evolution populations) and then mated the males to either a female from the same population or to a randomly selected control stock female (both of which were reared on the same, intermediate diet). This approach allows us to test three alternative predictions regarding the difference in male lifespan between high- and low-quality populations. First, if the divergence in male lifespan is due to differences in male dietary intake, then we would expect to find no significant difference in male lifespan between the treatments in any of the mating trials, as all individuals received the same diet. Second, if the divergence in male lifespan is primarily due to male evolution we would expect the differences in male lifespan to persist in both mating trials, irrespective of the identity of the female mating partner. Third, if the divergence in male lifespan is primarily due to female evolution, we would expect the differences in male lifespan to occur only when males are mated to females from their own population and disappear when mated to a random control stock female. Finally, having found support for this third alternative, we used model selection theory (Akaike, 1983; Burnham & Anderson, 2002) and response surface analysis (Draper & John, 1988) to examine how the costs of mating paid by males evolved under the high- or low-quality diets by relating mating effort, male condition and female condition to variation in male lifespan within each treatment. By dissecting how sex-specific evolutionary change influences male lifespan we will address two largely unresolved issues: how the costs of mating borne by males evolve in nuptial feeding insects and the potential role of inter-locus sexual conflict in shaping male lifespan.

Methods

Evolutionary history of the experimental populations

In April 2003, we established 10 experimental evolution populations of an undescribed Australian ground cricket from the genus *Pteronemobius* (Family: Gryllidae). These crickets originated from Waramanga (35°21'S, 149°03'E) in the Australian Capital Territory, Australia, and are

catalogued in the Australian National Insect Collection where voucher specimens were deposited in July 2006. The establishment and maintenance of the experimental populations have been described in detail previously in Hall *et al.* (2008a). Briefly, the experimental populations were assigned to one of two diet quality treatments (high or low) in which we manipulated the protein and carbohydrate content of the food received *ad libitum*, generating five replicate populations for each treatment. The 10 experimental populations were maintained with nonoverlapping generations under these conditions for seven generations and kept in a controlled temperature room throughout (28 °C, 14-h : 10-h light : dark cycle). The high-quality diet consisted of a blended dry weight mixture of 75% fish-rearing pellets (Nova Lo: 50% protein, Skretting Australia, Tas., Australia) to oatmeal (Quick Oats: 11% protein, Farmland, Vic., Australia), whereas the low-quality diet consisted of a blended mixture of 25% fish-rearing pellets to oatmeal. Similar diet manipulations have been used previously in *Pteronemobius* sp. (Hall *et al.*, 2008a) and another Australian Gryllid cricket, *Teleogryllus commodus* (Hunt *et al.*, 2004, 2005), where individuals raised initially on the high-quality diet tend to develop quicker and eclose heavier.

Experimental conditions

As part of a common garden experiment, we raised crickets from each experimental population from hatching on a single, intermediate diet (50% fish-rearing pellets to oatmeal) for a single generation. This ensured that any observed differences between the treatments in subsequent mating trials can be attributed to genetic divergence rather than a functional dietary response to variation in protein and carbohydrate content. In total, 200 nymphs from each of the eighth generation populations were housed individually on the day they hatched in small plastic containers ($8 \times 8 \times 5$ cm³), provided with vials of water stoppered with cotton wool, a piece of egg carton for shelter and fed *ad libitum* with the intermediate diet. Each day we checked for eclosions and weighed any new adults.

Mating trials

To assess if sex-specific evolutionary change influences male lifespan and the costs of mating, we mated males from each experimental population to two types of females: a female from the same experimental population and a control stock female. We also recorded the behaviour of the different mating pairs to assess how differences in the number or duration of copulations could contribute to the divergence in male lifespan between the high- and low-quality treatments. From the 200 nymphs of each experimental evolution population that were raised individually from hatching, we used the first 40 males and 20 females to eclose in the mating

trials. At the same time, newly eclosed females were collected from a large, well-mixed control stock that had been maintained on the same experimental diet (50% fish-rearing pellets to oatmeal) for the last eight generations. At the time of the behavioural trials, the males were randomly allocated to mate either with a female from the same experimental evolution population, or with a random control stock female. All crickets used in the behavioural trials were between 7- and 14-day-old post-adult eclosion.

Our experience is that these crickets are reluctant to mate in the presence of an observer. Consequently, to assess mating duration and frequency, each trial was monitored by filming the male and female mating pairs for 12 h, beginning at 18:00 hours, using a video camera equipped with infrared sensing and a built-in infrared source (model DCR-TRV340E; Sony Corporation, Tokyo, Japan). By attaching the camera to a computer running time-lapse computer recording software (RBCap, <http://www.rbartick.com/rbcap/>), we captured individual frames every 15 s rather than as a continuous recording. Each mating pair was placed in a small plastic container ($7 \times 7 \times 5$ cm³) with dampened paper towel lining the base, randomly arranged into four rows of three and placed under the tripod mounted camera. From the mating trial videos, we scored the number and duration of all mating events. In *Pteronemobius*, before a true copulation involving sperm transfer and nuptial feeding can occur, the female must first mount the male briefly in a mock copulation (Mays, 1971). Accordingly, we estimated a male's attractiveness as the inverse of the time taken from the start of the trial until the first mock copulation, as well as recording the number of true copulations and the average duration of the copulations. Consistent with previous studies on courtship-feeding ground crickets, we used the average copulation duration as an estimate of nuptial gift size (Fedorka & Mousseau, 2002a, b; Hall *et al.*, 2008a). Following the 12-h mating trial period, the males were returned to their individual containers and maintained in a controlled temperature room (28 °C, 14-h : 10-h light : dark cycle) where survival was monitored daily and the food and water replaced weekly.

We assessed the differences between the two diet quality treatments in mating behaviour and male lifespan with a mixed model ANOVA using restricted maximum likelihood for parameter estimation. In the analysis we treated diet treatment as a fixed factor and experimental population nested within treatment as a random factor. Before analysis, however, we transformed the inverse of latency to mock copulation (our measure of male attractiveness) using natural logarithms and the average copulation duration (our measure of nuptial gift size) using the square root transformation, as both were positively skewed. All analyses were conducted using SPSS (version 15; SPSS Inc., Chicago, IL, USA).

Model selection and the costs of mating

In order to test how mating effort, male condition and female condition predict male lifespan for the different diet quality treatments, we combined response surface analysis (Lande & Arnold, 1983; Draper & John, 1988) with model selection theory (Akaike, 1983; Burnham & Anderson, 2002; Johnson & Omland, 2004). Response surface analysis is a common approach for studies of evolution, whereby a second-order polynomial regression is used to characterize the relationship between multiple traits and an estimate of fitness. In evolutionary biology, previous uses include characterizing the nature of natural or sexual selection on a suite of traits (Blows & Brooks, 2003; Hall *et al.*, 2008b) or predicting what concentrations of nutrients in a diet maximize reproductive investment or longevity (Lee *et al.*, 2008; Maklakov *et al.*, 2008). The significance of such response surfaces are then tested using a sequential series of nested models using partial *F*-tests (Draper & John, 1988; Chenoweth & Blows, 2005). The use of model selection theory, however, allows for direct testing of different causal relationships (in this case the response surfaces) that are not preconditioned on a previous model (as per nested models).

We fitted a series of response surfaces to assess if the condition of the male, how much he invested in reproduction, the condition of his mating partner or any specific combinations of these factors best predicted male lifespan in each treatment. In this way a total of seven candidate response surfaces were evaluated by adding or removing groups of related variables from the models. We conducted the analysis using only the data collected when experimental population males were mated to experimental population females. The descriptive statistics for the variables used in the analysis are shown in Table 1. For both males and females we used development rate and mass at eclosion as our estimates of condition, whereas male mating effort was described by male attractiveness and nuptial gift investment (number of copulations by average copulation duration). Specifically, we fitted the seven candidate response surfaces separately for each treatment using a second-order polynomial regression model with experimental population as random factor, using restricted maximum likelihood for parameter estimation in *SPSS*. Before analysis, all traits were standardized to a mean of zero and a standard deviation of one.

For each candidate model, this routine generates Akaike information criteria (AIC) which describes how well the model describes the data (Akaike, 1983), with smaller values representing a better fit. The resulting AIC values were then used to rank the response surfaces evaluated, and from these ranked candidate models we inferred which combinations of mating effort, male condition or female condition best explain the observed patterns of male lifespan and hence the costs of mating in

Table 1 The descriptive statistics for mating effort, male condition and female condition used in the response surface analysis (mean $\pm$ SE) based on the mating trials between males and females from the same experimental populations.

Descriptive statistics	Abbreviation	High-quality treatment	Low-quality treatment
Mating effort			
Male attractiveness ($\log_e[s^{-1}]$)	MAT	-5.893 ± 0.194	-6.245 ± 0.190
Male gift investment ($\sqrt{s}$)	MGI	19.386 ± 2.054	19.417 ± 1.291
Male condition			
Male weight (mg)	MWT	12.933 ± 0.272	14.341 ± 0.253
Male development rate ($\text{day}^{-1} \times 10^3$)	MDR	33.165 ± 0.619	26.321 ± 0.163
Female condition			
Female weight (mg)	FWT	16.096 ± 0.272	17.880 ± 0.564
Female development rate ($\text{day}^{-1} \times 10^3$)	FDR	33.864 ± 0.837	27.266 ± 0.167

The mean values and standard errors are calculated based on the average value for each of the experimental evolution populations.

each of the two diet quality treatments. In general, only models that differ by two or more AIC units provide distinguishable levels of support (Burnham & Anderson, 2002). From the AIC values we also calculated the AIC weight for each model which provides a relative weight of evidence for given model in comparison with all other candidate models (Burnham & Anderson, 2002). Finally, we visualized the best fitting response surface for each treatment using a parametric contour plot implemented in *SIGMAPLOT* (version 10; Systat Software Inc., Chicago, IL, USA).

Results

Behavioural trials

Using two separate mating trials we assessed how male lifespan was influenced by the evolutionary history of both the male and his mating partner (Fig. 1). When males from each experimental population mated to a female from the same population, we found that the type of diet the population had previously evolved on for seven generations significantly influenced male longevity ($F_{1,8} = 12.6$, $P = 0.008$). Males from the high-quality diet populations lived on average 10 days longer than those males from the low-quality diet lines. This difference is consistent with that reported previously when individuals from the immediately previous generation were raised on the high- and low-quality diets [male lifespan (days $\pm$ SE): high quality = 46.19 ± 2.85 , low quality = 37.16 ± 2.53 , Hall *et al.*, 2008a]. There was no significant difference between the diet treatments in the attractiveness of the males [inverse of latency to mock copulation ($\log_e[s^{-1}] \pm$ SE): high quality = -5.893 ± 0.194 , low

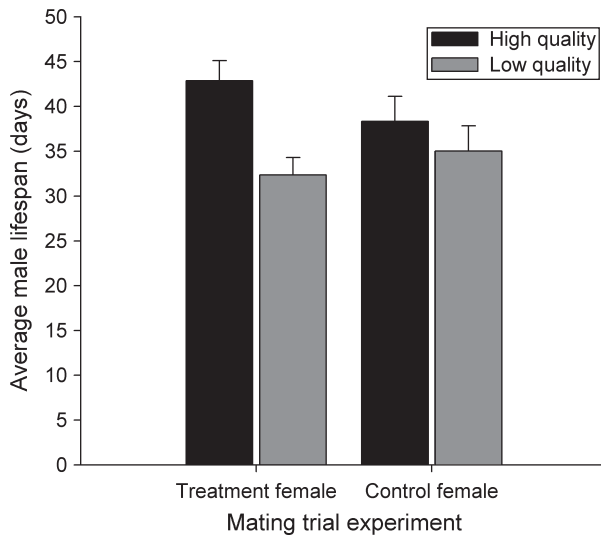


Fig. 1 The effect of partner identity on male lifespan for each of the diet quality treatments. The mean values and standard errors are calculated based on the average value for each of the experimental evolution populations.

quality = -6.245 ± 0.189 , $F_{1,8} = 1.6$, $P = 0.245$], the number of copulations obtained over 12 h (high quality = 1.890 ± 0.234 , low quality = 1.880 ± 0.136 , $F_{1,8} < 0.1$, $P = 0.971$) nor the average nuptial gift size (average copulation duration ($\sqrt{s} \pm SE$): high quality = 15.332 ± 2.054 , low quality = 15.527 ± 1.291 , $F_{1,8} < 0.1$, $P = 0.850$).

By contrast, when experimental population males were mated to random stock females, the diet quality treatment of the male had no significant effect on male lifespan ($F_{1,8} = 0.7$, $P = 0.427$). Once again, there was no significant difference between the diet treatments in the attractiveness of the males [inverse of latency to mock copulation ($\log_e[s^{-1}] \pm SE$): high quality = -6.809 ± 0.189 , low quality = -6.538 ± 0.329 , $F_{1,8} = 0.5$, $P = 0.498$], the number of copulations obtained (high quality = 1.690 ± 0.091 , low quality = 1.770 ± 0.106 , $F_{1,8} < 0.3$, $P = 0.583$) nor the average nuptial gift size [average copulation duration ($\sqrt{s} \pm SE$): high quality = 17.294 ± 0.204 , low quality = 16.630 ± 0.801 , $F_{1,8} < 0.5$, $P = 0.509$] when mated to a control stock female. An additional analysis that included treatment (high, low) and mating partner type (treatment, stock) as fixed factors confirmed that there is a significant interaction between the treatment of the male and his mating partner type ($F_{1,386} = 6.3$, $P = 0.013$) in determining male lifespan.

Response surfaces and model selection

The candidate response surfaces describing how male mating effort, male condition and female condition relate to male lifespan are shown in Table 2, whereas the

Table 2 The *a priori* set of seven candidate response surfaces that attempt to assess how male condition, female condition or mating effort best describe the variation in male lifespan within each diet quality treatment.

Candidate response surfaces	No. of parameters	AIC	ΔAIC	AIC weight
High-quality diet treatment				
ME	5	90.847	0.000	0.547
MC	5	91.930	1.083	0.318
FC	5	93.637	2.79	0.135
ME + FC	14	123.274	32.427	< 0.001
MC + FC	14	124.314	33.467	< 0.001
ME + MC	14	125.223	34.376	< 0.001
ME + MC + FC	27	207.337	207.337	< 0.001
Low-quality diet treatment				
FC	5	121.341	0.000	0.921
ME	5	127.341	6.223	0.041
MC	5	127.504	6.386	0.038
MC + FC	14	152.573	31.455	< 0.001
ME + FC	14	152.597	31.479	< 0.001
ME + MC	14	160.364	39.246	< 0.001
ME + MC + FC	27	218.375	97.257	< 0.001

In each treatment the models are ranked based on their AIC scores with a difference of two AIC units used to distinguish the levels of support, whereas the AIC weights indicate the probability that a given model is the best model. ME, mating effort; MC, male condition; FC, female condition.

parameter estimates of the three best fitting models per treatment are shown in Table 3. In total, we evaluated seven response surface models for each of the treatments. For the high-quality diet treatment the greatest support occurred for a response surface based on measures of mating effort, although a male condition response surface was only marginally less well supported. Visualization of the contour plot describing the mating effort surface (Fig. 2) reveals that male lifespan is predicted by specific combinations of male attractiveness and nuptial gift investment. For example, males that died sooner were either the attractive males with the largest gifts or unattractive males with small gifts. In terms of the male condition model, the parameter estimates (Table 3) indicate that male lifespan increases with the development rate of the male. In the low-quality treatment, however, the data clearly support only one model (Table 2). In this treatment the variation in male longevity is most strongly supported by the female condition response surface. The contour plot of the surface (Fig. 3) indicated that males who lived the longest were mated to females in the highest condition: those females that developed the fastest, and eclosed the heaviest.

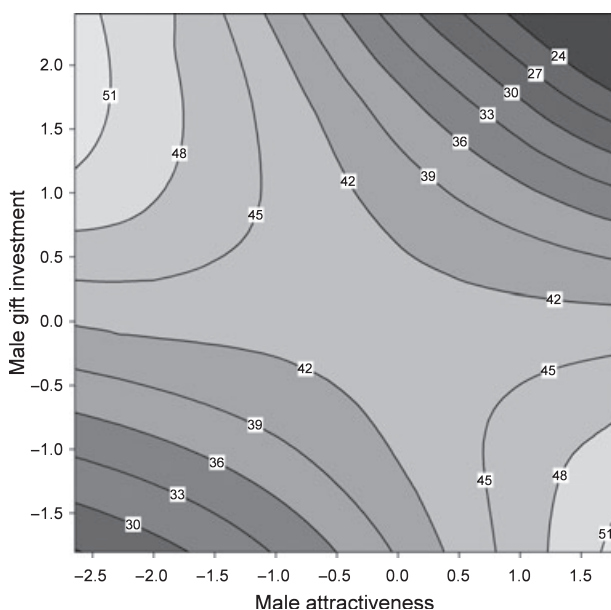
Discussion

We have shown that in an Australian ground cricket, *Pteronemobius* sp., male lifespan is influenced by diet through the different effects that high- and low-quality

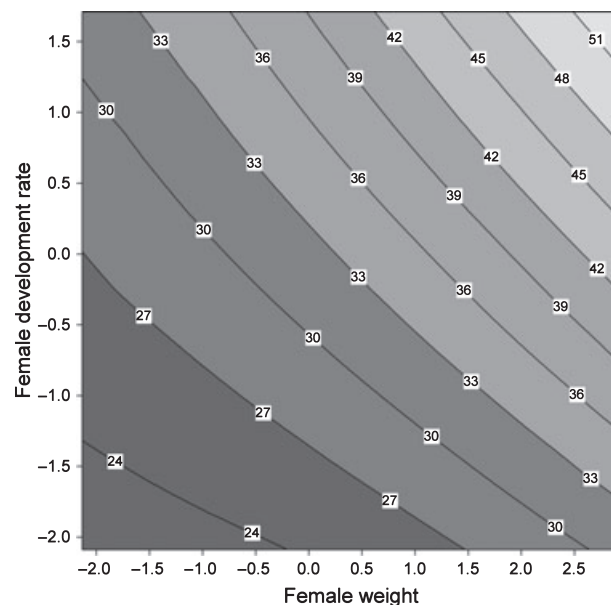
Table 3 The parameter estimates for the three simplest and best fitting response surface models for each treatment.

Candidate models	Response surface parameter estimates				
Mating effort	MAT	MGI	MAT × MGI	MAT × MAT	MGI × MGI
High-quality treatment	< 0.001	−0.019	−0.091*	< 0.001	−0.037
Low-quality treatment	−0.024	0.015	0.020	0.018	−0.010
Male condition	MWT	MDR	MWT × MDR	MWT × MWT	MDR × MDR
High-quality treatment	0.035	0.085**	0.021	0.006	0.019
Low-quality treatment	0.023	−0.045	0.044	0.020	−0.029
Female condition	FWT	FDR	FDR × FWT	FWT × FWT	FDR × FDR
High-quality treatment	0.054	0.044	−0.003	0.007	0.021
Low-quality treatment	0.095**	0.121**	0.025	0.009	0.002

The linear (β), quadratic (γ_{ii}) and correlational (γ_{ij}) coefficients were estimated using a single second-order polynomial regression (* $P < 0.01$, ** $P < 0.05$). Parameters include male attractiveness (MAT), male gift investment (MGI), male weight (MWT) and development rate (MDR) and female weight (FWT) and female development rate (FDR).

**Fig. 2** The parametric contour plot for the response surface predicting the relationship between male mating effort and male longevity in the high-quality diet treatment. For each contour line the predicted lifespan is indicated by the numbered label.

diets have on the evolution of nuptial feeding interactions between males and females. Male lifespan was significantly shorter in the populations that adapted to a

**Fig. 3** The parametric contour plot for the response surface predicting the relationship between female condition and male longevity in the low-quality diet treatment. For each contour line the predicted lifespan is indicated by the numbered label.

low-quality diet for seven generations, even though the replicate high- and low-quality diet populations did not differ in the number of copulations in our mating success assay, nuptial gift size or male attractiveness. Moreover, the differences in lifespan disappeared when males from each experimental population were mated to control stock females. The divergence in male lifespan appears to be driven by diet-mediated evolutionary change in how females interact with males over nuptial gifts. Our findings have important implications for how diet influences lifespan in nuptial feeding organisms like *Pteronemobius* sp., and more generally in how the costs of mating for males evolve and how antagonistic interactions between males and females can potentially shape male lifespan.

Across a wide range of species from classical model systems, such as *Caenorhabditis elegans* and *Drosophila melanogaster* to mammals, dietary intake has been shown to strongly influence lifespan and the costs of reproduction (Weindruch & Walford, 1988; Guarente & Kenyon, 2000). Until recently, the widely held view was that reducing the amount of calories consumed by an individual prolongs life (discussed in Masoro, 2006). The manipulation of dietary content, however, has revealed that the intake of specific nutrients may relate directly to longevity and the costs of reproduction (Mair *et al.*, 2005; Piper *et al.*, 2005). Using a state-space approach to nutritional research known as the geometric framework (Raubenheimer & Simpson, 1997; Simpson *et al.*, 2004), studies have shown that it is the precise ratio of

macronutrients such as protein and carbohydrates that contribute to lifespan and reproduction (Lee *et al.*, 2008; Maklakov *et al.*, 2008). Importantly, however, the effects of dietary restriction and nutrient intake are likely to be both species and sex specific (Magwere *et al.*, 2004). In the caterpillar *Spodoptera littoralis*, for example, excessive intake of carbohydrates results in increased mortality (Raubenheimer *et al.*, 2005), whereas in the field cricket, *T. commodus*, lifespan is maximized on a high-carbohydrate, low-protein diet (Maklakov *et al.*, 2008).

In *Pteronemobius* sp., we have previously shown that males from the low-quality populations that received the high carbohydrate, low protein diet over seven generations had significantly reduced lifespan (Hall *et al.*, 2008a). To examine whether or not lifespan has evolved, however, it is necessary to separate the immediate effect of macronutrient intake from the adaptive response that can occur to changes in the nutritional environment (Warbrick-Smith *et al.*, 2006). Importantly, in this study even when raised on the intermediate diet as part of a common garden experiment, the differences in male lifespan between populations adapted to the high- and low-quality diets still remained. The divergence in male lifespan, therefore, appears to have resulted from evolutionary change driven by difference in the nutritional environment, rather than the direct effects of specific macronutrient intake on how long males live.

Two lines of evidence, however, suggest the reduced lifespan of males from populations that have evolved under low-quality diets is not due to the direct evolution of male lifespan. First, in both experiments (here and in Hall *et al.*, 2008a) the males did not differ in how they invested in obtaining matings or providing nuptial gifts, as neither the number of copulations nor the average gift size differed between the two treatments. Second, the difference in male lifespan between the treatments occurs only when males are mated to females from their own experimental population. Together, these findings suggest that the divergence in male lifespan cannot be satisfactorily explained by genetic changes in the allocation of resources between reproduction and somatic maintenance (Kirkwood, 1977), nor in the variation in the quality of nuptial gift provision (Bussière *et al.*, 2005). We can also exclude the direct influence of maternal effects on male lifespan (Fox *et al.*, 2004) which our common garden design could not completely remove, given that the difference in male lifespan between the treatments disappears when males are mated to control females. Instead, our findings indicate that the divergence in male lifespan has occurred as a consequence of evolutionary change in how females interact with males over nuptial feeding. Given the nature of our experimental design, however, it is difficult to separate the potential co-evolution between females and males within each population from the direct influence of diet on female evolution without using population crosses during the mating trails.

Nevertheless, by relating variation in male lifespan to nuptial feeding and the condition of males and females, we examined how the costs of nuptial feeding to males evolved in each diet quality treatment. In the high-quality diet populations, we showed that the cost of mating for males was related to how the male invests in reproduction or the development of the male. By contrast, in the low-quality diet populations the best predictor of male lifespan was the condition of his mating partner, regardless of how much a male invested in copulation and nuptial gifts. In *Pteronemobius* sp., therefore, seven generations of evolving on a poor quality diet appears to have selected for manipulative females who impose greater costs on a male for a given time feeding directly on the haemolymph. Although previous studies have shown that the loss of haemolymph can significantly constrain the ability of males to obtain future matings (Sakaluk *et al.*, 2004), our results suggest that the loss of haemolymph may also be costly through the reduction in male lifespan. Importantly, however, the costs of providing a nuptial gift will also depend on the evolutionary history of the female. This suggests that not only is the way in which females extract costly nutrition from nuptial feeding males evolutionarily labile, but that it has considerable potential to cause male lifespan to diverge between populations by influencing how the costs of mating to males evolve and hence the nature of inter-locus sexual conflict over nuptial feeding.

Finally, by focusing on male lifespan, we have revealed that nuptial feeding is an important site of diet-mediated antagonistic interactions between males and females. Accordingly, we suggest that a change in the quality of the diet will have dramatic consequences for nuptial feeding mating systems not only in terms of the dynamics of sexual selection (Gwynne & Simmons, 1990; Simmons, 1992; Simmons & Kvarnemo, 2006; Hall *et al.*, 2008a), but also in how sexual conflict is potentially manifested in a mating system. In many species, however, the importance and extent of sexual conflict over nuptial feeding will be underestimated by focusing solely on the consequences of conflict between males and females from the female's perspective. More generally, our results reinforce predictions that changes in the nature of sexual selection, and hence sexual conflict, will significantly affect a species lifespan and rate of aging (Svensson & Sheldon, 1998; Promislow, 2003; Maklakov *et al.*, 2008). However, we suggest that in the same way elevated levels of sexual selection result in females experiencing reduced lifespan (Holland & Rice, 1999; Maklakov *et al.*, 2007), a reversal of sexual selection may consequently shape the aging and lifespan of males. Our findings highlight the potential for comparisons between sex role-reversed and conventional mating systems to examine how reproductive interactions between males and females can influence the evolution of lifespan, aging and the costs of mating.

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References

- Akaike, H. 1983. Information measures and model selection. *Bull. Int. Stat. Inst.* **44**: 277–291.
- Arnqvist, G. & Rowe, L. 2005. *Sexual Conflict*. Princeton University Press, Princeton, NJ.
- Blows, M.W. & Brooks, R. 2003. Measuring nonlinear selection. *Am. Nat.* **162**: 815–820.
- Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol. Rev.* **76**: 305–339.
- Bonduriansky, R., Maklakov, A., Zajitschek, F. & Brooks, R. 2008. Sexual selection, sexual conflict and the evolution of ageing and life span. *Funct. Ecol.* **22**: 443–453.
- Burnham, K.P. & Anderson, D.R. 2002. *Model Selection and Multi-model Inference*, 2nd edn. Springer, New York.
- Bussière, L.F., Basit, H.A. & Gwynne, D.T. 2005. Preferred males are not always good providers: female choice and male investment in tree crickets. *Behav. Ecol.* **16**: 223–231.
- Chenoweth, S.F. & Blows, M.W. 2005. Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. *Am. Nat.* **165**: 281–289.
- Cumming, J.M. 1994. Sexual selection and the evolution of dance fly mating systems (Diptera: Empididae; Empidinae). *Can. Entomol.* **126**: 907–920.
- Draper, N.R. & John, J.A. 1988. Response-surface designs for quantitative and qualitative variables. *Technometrics* **30**: 423–428.
- Fedorka, K.M. & Mousseau, T.A. 2002a. Nuptial gifts and the evolution of male body size. *Evolution* **56**: 590–596.
- Fedorka, K.M. & Mousseau, T.A. 2002b. Tibial spur feeding in ground crickets: larger males contribute larger gifts (Orthoptera: Gryllidae). *Fla. Entomol.* **85**: 317–323.
- Fox, C.W., Czesak, M.E. & Wallin, W.G. 2004. Complex genetic architecture of population differences in adult lifespan of a beetle: nonadditive inheritance, gender differences, body size and a large maternal effect. *J. Evol. Biol.* **17**: 1007–1017.
- Guarente, L. & Kenyon, C. 2000. Genetic pathways that regulate ageing in model organisms. *Nature* **408**: 255–262.
- Gwynne, D.T. 1984. Courtship feeding increases female reproductive success in bush-crickets. *Nature* **307**: 361–363.
- Gwynne, D.T. 2008. Sexual conflict over nuptial gifts in insects. *Annu. Rev. Entomol.* **53**: 83–101.
- Gwynne, D.T. & Simmons, L.W. 1990. Experimental reversal of courtship roles in an insect. *Nature* **346**: 172–174.
- Hall, M.D., Bussière, L.F. & Brooks, R. 2008a. The effect of diet quality and wing morph on male and female reproductive investment in a nuptial feeding ground cricket. *PLoS ONE* **3**: e3437.
- Hall, M.D., Bussière, L.F., Hunt, J. & Brooks, R. 2008b. Experimental evidence that sexual conflict influences the opportunity, form and intensity of sexual selection. *Evolution* **62**: 2305–2315.
- Holland, B. & Rice, W.R. 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proc. Natl. Acad. Sci. U S A* **96**: 5083–5088.
- Hunt, J., Brooks, R., Jennions, M.D., Smith, M.J., Bentsen, C.L. & Bussière, L.F. 2004. High-quality male field crickets invest heavily in sexual display but die young. *Nature* **432**: 1024–1027.
- Hunt, J., Brooks, R. & Jennions, M.D. 2005. Female mate choice as a condition-dependent life-history trait. *Am. Nat.* **166**: 79–92.
- Johnson, J.B. & Omland, K.S. 2004. Model selection in ecology and evolution. *Trends Ecol. Evol.* **19**: 101–108.
- Kirkwood, T.B.L. 1977. Evolution of aging. *Nature* **270**: 301–304.
- Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
- Lee, K.P., Simpson, S.J., Clissold, F.J., Brooks, R., Ballard, J.W.O., Taylor, P.W., Soran, N. & Raubenheimer, D. 2008. Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *Proc. Natl. Acad. Sci. U S A* **105**: 2498–2503.
- Magwere, T., Chapman, T. & Partridge, L. 2004. Sex differences in the effect of dietary restriction on life span and mortality rates in female and male *Drosophila melanogaster*. *J. Gerontol. A Biol. Sci. Med. Sci.* **59**: 3–9.
- Mair, W., Piper, M.D.W. & Partridge, L. 2005. Calories do not explain extension of life span by dietary restriction in *Drosophila*. *PLoS Biol.* **3**: 1305–1311.
- Maklakov, A.A., Kremer, N. & Arnqvist, G. 2005. Adaptive male effects on female ageing in seed beetles. *Proc. R. Soc. B* **272**: 2485–2489.
- Maklakov, A.A., Fricke, C. & Arnqvist, G. 2007. Sexual selection affects lifespan and aging in the seed beetle. *Aging Cell* **6**: 739–744.
- Maklakov, A.A., Simpson, S.J., Zajitschek, F., Hall, M.D., Dessman, J., Clissold, F.J., Raubenheimer, D., Bonduriansky, R. & Brooks, R. 2008. Sex-specific fitness effects of nutrient intake on reproduction and lifespan. *Curr. Biol.* **18**: 1062–1066.
- Masoro, E.J. 2006. Caloric restriction and aging: controversial issues. *J. Gerontol. A Biol. Sci. Med. Sci.* **61**: 14–19.
- Mays, D.L. 1971. Mating behavior of Nemobiine crickets: *Hygronemobius*, *Nemobius*, and *Pteronemobius* (Orthoptera: Gryllidae). *Fla. Entomol.* **54**: 113–126.
- Piper, M.D.W., Mair, W. & Partridge, L. 2005. Counting the calories: the role of specific nutrients in extension of life span by food restriction. *J. Gerontol. A Biol. Sci. Med. Sci.* **60**: 549–555.
- Promislow, D. 2003. Mate choice, sexual conflict, and evolution of senescence. *Behav. Genet.* **33**: 191–201.
- Raubenheimer, D. & Simpson, S.J. 1997. Integrative models of nutrient balancing: application to insects and vertebrates. *Nutr. Res. Rev.* **10**: 151–179.
- Raubenheimer, D., Lee, K.P. & Simpson, S.J. 2005. Does Bertrand's rule apply to macronutrients? *Proc. R. Soc. B* **272**: 2429–2434.
- Roff, D.A. 1992. *The Evolution of Life Histories*. Chapman & Hall, New York.
- Sakaluk, S.K., Campbell, M.T.H., Clark, A.P., Chadwick-Johnson, J. & Keorpes, P.A. 2004. Hemolymph loss during nuptial feeding constrains male mating success in sagebrush crickets. *Behav. Ecol.* **15**: 845–849.
- Sakaluk, S.K., Avery, R.L. & Weddle, C.B. 2006. Cryptic sexual conflict in gift-giving insects: chasing the chase-away. *Am. Nat.* **167**: 94–104.
- Simmons, L.W. 1992. Quantification of role reversal in relative parental investment in a bush cricket. *Nature* **358**: 61–63.

- Simmons, L.W. 1994. Reproductive energetics of the role reversing bush-cricket, *Kawanaphila nartee* (Orthoptera: Tettigoniidae: Zaprochilinae). *J. Evol. Biol.* **7**: 189–200.
- Simmons, L.W. & Gwynne, D.T. 1993. Reproductive investment in bushcrickets: the allocation of male and female nutrients to offspring. *Proc. R. Soc. B* **252**: 1–5.
- Simmons, L.W. & Kvarnemo, C. 2006. Costs of breeding and their effects on the direction of sexual selection. *Proc. R. Soc. B* **273**: 465–470.
- Simpson, S.J., Sibly, R.M., Lee, K.P., Behmer, S.T. & Raubenheimer, D. 2004. Optimal foraging when regulating intake of multiple nutrients. *Anim. Behav.* **68**: 1299–1311.
- Stearns, S.C. 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford.
- Svensson, E. & Sheldon, B.C. 1998. The social context of life history evolution. *Oikos* **83**: 466–477.
- Vahed, K. 2007. All that glitters is not gold: sensory bias, sexual conflict and nuptial feeding in insects and spiders. *Ethology* **113**: 105–127.
- Warbrick-Smith, J., Behmer, S.T., Lee, K.P., Raubenheimer, D. & Simpson, S.J. 2006. Evolving resistance to obesity in an insect. *Proc. Natl. Acad. Sci. USA* **103**: 14045–14049.
- Wedell, N., Tregenza, T. & Simmons, L.W. 2008. Nuptial gifts fail to resolve a sexual conflict in an insect. *BMC Evol. Biol.* **8**: 204.
- Weindruch, R. & Walford, R.D. 1988. *The Retardation of Aging and Disease by Dietary Restriction*. Charles C Thomas, Springfield, IL.
- Will, M.W. & Sakaluk, S.K. 1994. Courtship feeding in decorated crickets: is the spermatophylax a sham? *Anim. Behav.* **48**: 1309–1315.

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