Mating opportunities and energetic constraints drive variation in age-dependent sexual signalling

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Summary

1. When males repeatedly produce energetically expensive sexual signals, trade-offs between current and future investment can cause plasticity in age-dependent signalling. Such variation is often interpreted as alternate adaptive strategies: live fast and die young vs. slow and steady.
2. An alternative (yet rarely tested) explanation is that condition-dependent constraints on allocation cause variation in signalling with age ('late bloomers' do not have early investment options). Testing this hypothesis is challenging because resource acquisition and allocation are difficult to measure, and energetic reserves both affect and are affected by reproductive effort.
3. We simultaneously manipulated acquisition (through dietary nutrition) and access to potential mates (as a proxy for manipulating sexual trait allocation) in male decorated crickets (*Gryllodes sigillatus*), while measuring age- and signalling effort-mediated changes in energy storage components.
4. Increased diet quality caused increased signalling effort and energy storage, while access to females increased both the likelihood of and time spent signalling. Males with lower resource budgets signalled less, but still suffered energetic storage loss and viability costs.
5. Our results suggest that energetic constraints, rather than strategic resource accumulation, reduced signalling levels in males with lower resource acquisition ability. Our findings imply a non-adaptive explanation for age-dependent variation in sexual signalling, and an important role for energetic constraints in maintaining the honesty of costly behavioural displays.

Key-words: condition dependence, life history, phenotypic plasticity, sexual selection, trade-offs

Introduction

The fitness of an individual depends on its investment in life-history traits (Stearns 1992). The expression of these traits is thought to be affected by trade-offs, caused when a fitness-improving increase in one life-history trait is coupled to a fitness-reducing decrease in another (Stearns 1989). One trade-off universal to all organisms occurs because any individual has only a finite pool of resources, for which all of its traits are competing. Most studies on such trade-offs have focused on investment across traits at a single point in the life history, but allocation trade-offs can also occur between investments in the same trait at different ages. Although the concept of age-dependent changes in performance is prevalent in studies of senescence, it is often difficult to disentangle the effects of allocation from other potential causes of decreased performance (Monaghan *et al.* 2008; Nussey *et al.* 2008).

An individual's ability to acquire resources, known as its 'condition', is often strongly related to the expression of costly sexual traits in males (Rowe & Houle 1996). Condition is likely determined by a large number of loci because so many genes are involved in acquiring and metabolizing resources. If the ability to pay fitness costs associated with increased investment in a sexual trait (e.g. reduced future reproductive effort or survival) is heritable, females can use a male's condition-dependent signals to assess the quality of his genes (in terms of the probable condition of offspring; Nur & Hasson 1984; Andersson 1986; Grafen 1990; Iwasa & Pomiankowski 1994).

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This relationship between genetic quality and condition depends on trade-offs, with genetic correlations among fitness-related traits affected by relative levels of genetic variation in both the acquisition and allocation of resources (Van Noordwijk & De Jong 1986; Houle 1991; Hunt *et al.* 2004b). While theory suggests males should benefit by sacrificing investment in longevity in favour of increased early reproductive success (Vinogradov 1998; Bonduriansky *et al.* 2008), studies often show the opposite pattern such that males that signal most intensely are also those that survive the longest (Jennions, Møller & Petrie 2001). One possible explanation for this positive covariance is that high-condition individuals have more resources to allocate to all aspects of their life history, masking real life-history trade-offs between expensive traits (Van Noordwijk & De Jong 1986; De Jong & Van Noordwijk 1992; Reznick, Nunney & Tessier 2000; Hunt *et al.* 2004b).

Assessing how life-history trade-offs affect investment in sexual trait expression is a challenging task. The resource pool itself cannot be measured directly via phenotypic traits: many such traits may covary with an individual's resource budget, but must themselves have been constructed using resources and therefore will necessarily be traded against other life-history traits (Hunt *et al.* 2004b; Houslay & Bussière 2012). Residuals of body mass over a fixed measure of body size are commonly used as a proxy for condition (e.g. Peig & Green 2009), yet are unlikely to be consistently adequate (Barnett *et al.* 2015; Wilder, Raubenheimer & Simpson 2015): for example, energetically expensive trait expression (such as sexual display) is fuelled by sugars that are supplied by fat stores (Tomkins *et al.* 2004). Consequently, condition indices can only be used to reliably compare individuals of different sizes if we know how fat content scales with size (Kotiaho 1999). Ideally, direct physiological analyses (rather than genetic or phenotypic approaches) are needed to study the mechanisms mediating energy storage and use, and their consequences for variation in sexual trait expression (Zera & Harshman 2001).

The problems inherent in assessing resource trade-offs are compounded when an individual expresses a sexual trait repeatedly over its lifetime. Such individuals face trade-offs not only between the focal trait and other components of life history, but also between current and future expression of the sexual trait: a male that invests many resources when young has fewer resources to spend when old, and vice versa (Badyaev & Qvarnström 2002). To save resources for critical periods, males may elect not to signal at certain ages, or to delay maturation until later in life (Kokko 1997). These trade-offs create variation between individuals in patterns of age-dependent expression of sexual traits and impede females from using signal expression as an index of a male's overall acquisition ability. In other words, investment in sexual signalling at any given age is less likely to be an honest indicator of quality than if this trait remained constant across his lifetime (Kokko 1997).

The honesty of sexual traits has long been of interest to students of sexual selection (Zahavi 1975; Andersson 1982;

Nur & Hasson 1984; Grafen 1990; Johnstone 1995; Kokko 1997). Strict honesty in sexual trait expression may not always be fulfilled in a population (Candolin 1999; Hunt *et al.* 2004a), but the 'on-average' honesty enforced by life-history trade-offs maintains stability in systems where male advertisement is subject to within-individual variation over lifetimes (Kokko 1997). Identifying costs associated with sexual trait expression is therefore crucial to determining variation in age-dependent signalling and pinpointing mechanisms enforcing honesty in such systems.

A primary model for studying investment in age-dependent sexual expression is acoustic signalling (Andersson 1994). In crickets, males produce an advertisement call through stridulation of their forewings, and the amount of time a male spends calling ('calling effort') is a strong predictor of mating success in nature (Hunt *et al.* 2004a; Bentsen *et al.* 2006; Rodriguez-Munoz *et al.* 2010). Calling is highly energetically expensive (e.g. Kavanagh 1987; Hunt *et al.* 2004a; Ophir, Schrader & Gillooly 2010; Mowles 2014) and may increase mortality risk from both intrinsic (calling diverts energy from other functions, such as somatic maintenance, Hunt *et al.* 2004a) and extrinsic sources (male calls attract parasitoids, Cade 1975; or predators, Walker 1979). Among crickets, calling is likely fuelled by aerobic metabolism (Prestwich & Walker 1981; Bailey *et al.* 1993; Prestwich 1994; Hoback & Wagner 1997), with variation in calling effort linked to the ability to acquire and mobilize combinations of glycogen, carbohydrates and lipids (*Acheta domesticus,* Bertram *et al.* 2011; *Gryllus assimilis, Gryllus texensis,* Thomson, Darveau & Bertram 2014). Studies that manipulated resource acquisition have shown that male condition strongly affects the timing and magnitude of calling effort in several different cricket species (*Gryllus pennsylvanicus,* Judge, Ting & Gwynne 2008; *A. domesticus,* Bertram *et al.* 2009; *Teleogryllus commodus,* Maklakov *et al.* 2009; Zajitschek *et al.* 2009, 2012; *Gryllodes sigillatus,* Houslay *et al.* 2015) and also that high-condition males sometimes invest so heavily in sexual displays that they suffer heightened mortality compared to lower-condition individuals (*T. commodus,* Hunt *et al.* 2004a).

However, manipulating acquisition alone may not necessarily reveal differential costs of sexual trait expression, as males in good condition have more resources to allocate to all traits (Kotiaho 2001). Testing for differential costs requires manipulating both acquisition and trait investment and then studying their effects on other life-history traits (e.g. future reproductive effort or survival). Moreover, behavioural signals are highly phenotypically plastic, which may allow males to react quickly to changes in the local environment so as to maximize fitness (Komers 1997; Bretman, Gage & Chapman 2011). We do not know the importance of resource trade-offs relative to other factors in causing distinct age-specific investment strategies (live fast and die young vs. slow and steady). Male crickets respond plastically to the social environment: juveniles reared in environments indicating high competition for

females invest more in reproductive tissues (*T. oceanicus*, Bailey, Gray & Zuk 2010) and age-specific calling effort (*T. commodus*, Kasumovic, Hall & Brooks 2012). During adulthood, rival male presence can cause plastic shifts towards increased calling effort (*T. commodus*, Callander *et al.* 2013), while the introduction of females can affect call frequency (*Gryllus bimaculatus*, Simmons 1986) and timing (*G. sigillatus, Gryllus veletis,* Burpee & Sakaluk 1993). The simultaneous manipulation of diet and the socio-sexual environment can therefore be used to evaluate both the energetic and life-history consequences of trait investment and reveal whether plasticity in the male signal is due to the strategic saving of resources or resource constraints.

In this study, we measure changes in allocation to sexual signalling and resource storage as a function of both acquisition and the demands of prior signalling in male decorated crickets (*G. sigillatus*). We measure calling effort and use biochemical assays to estimate the energetic resource budgets of separate subsets of males before and after manipulating resource acquisition and access to potential mates. Furthermore, we assess the consequences of our experimental manipulations on longevity. Our measurements of male daily calling behaviour reflect two processes: a male's decision as to whether or not he calls and – given a positive decision – his calling effort. The presence of females should present universal incentive in the decision to call, enabling us to separate this choice from the more resource-dependent component (the nonzero amount of calling effort). By isolating components of male investment decisions in this way, we can disentangle variation due to constraints from variation due to adaptive planning. If future planning is the primary force shaping an individual's response to social conditions, low levels of calling early in life among males with reduced resource acquisition ability should also be associated with increased energy reserves and greater calling later in life (Kokko 1997; Hunt *et al.* 2004a). If, however, there is no evidence of greater energy reserves for allocation at a later point, it is likely that low calling levels are due simply to a lack of energy for current allocation to reproductive effort. In the latter case, we would also expect all males to respond to the availability of potential mates by investing their resources more heavily in calling effort, rather than saving for the future.

Materials and methods

CRICKET HUSBANDRY

We used *G. sigillatus* (Fig. 1) descended from a 2001 collection of 500 adult crickets in Las Cruces, New Mexico, which had been used to initiate a laboratory culture maintained at a population size of approximately 5000 crickets and allowed to breed panmictically (Ivy & Sakaluk 2005). Crickets were housed in 15-L plastic containers in an environmental chamber maintained at 32 ± 1 °C on a reversed 14:10-h light/dark cycle. We provided juvenile crickets with egg cartons for shelter, *ad libitum* water in cotton wool-plugged test tubes and a standard diet of ground cat food

Fig. 1. A male *Gryllodes sigillatus* calls to a female (photograph by T. Houslay).

(Friskies Go-Cat Senior®, Purina, London, UK). We provided moistened cotton wool pads to adults as an oviposition substrate. We maintained crickets at a density of approximately 300 individuals per container.

EXPERIMENTAL PROTOCOL

We manipulated male resource acquisition through diet treatment and also manipulated access to females (as a proxy for manipulating resource allocation). We investigated variation in allocation to four different energy storage components (carbohydrates, glycogen, lipids and proteins; details given below) by sacrificing subsets of the experimental population (by placing individuals in a −80 °C freezer) at three different ages: immediately at eclosion (baseline variation), one week post-eclosion (effect of resource acquisition manipulation) and two weeks post-eclosion (effects of both resource acquisition and female-access manipulations). A fourth group of males were allowed to die naturally, to estimate how acquisition and prior signalling investment affected life span.

We separated 630 male cricket nymphs from laboratory stock cultures during the final instar. Upon eclosion, we photographed individuals (Motic SMZ-168 with Moticam 2000) and measured pronotum length using NIH IMAGEJ v1.48 (Schneider, Rasband & Eliceiri 2012). We measured body weight using a high-precision electronic balance (Denver Instrument, model PI-225DA) at eclosion and weekly intervals thereafter. At eclosion, we assigned males randomly to one of four groups: those to be sacrificed immediately ($n = 58$), after one week ($n = 56$), after two weeks ($n = 256$) or allowed natural life span ($n = 260$). We transferred male crickets that were not immediately sacrificed to individual clear plastic containers (5 × 5×5 cm), each placing plastic mesh as substrate, a cotton wool-plugged water bottle and the food treatment. We cleaned containers and provided fresh food and water weekly.

We quantified allocation to the male sexual trait as 'calling effort', the duration (in seconds) that each individual spent broadcasting his sexual advertisement call. We measured each male overnight from 14.00 to 09.00 h (dusk until afternoon of the following day on the reversed light cycle; lights off 14.30 to 00.30 h, with 30-min rampdown) every day from the day after eclosion up to a maximum of 35 days. We mounted a microphone (C1163, Dick Smith Electronics) in the lid of each individual container, placing that container into a hollowed-out cube of soundproofing foam (15 × 15 × 15 cm) to minimize outside disturbance and prevent crosstalk between containers during recording. We removed a corner of the foam lid to maintain crickets on the set light cycle. An electronic acoustic recording system (Bertram & Johnson 1998) sampled from each microphone of each 10 times s^{-1} to determine whether or not a male was calling (see Hunt *et al.* 2004a).

CREATION OF ARTIFICIAL DIETS

We manipulated resource acquisition by creating seven artificial, dry, granular diets that varied in the amount of total nutritional

content (see Appendix S1, Supporting Information). All diets had a protein: carbohydrate ratio of 1:8, based on the effect of a high-carbohydrate diet in maximizing calling effort and longevity in this species (J. Hunt, unpublished data). Diets ranged from 12 to 84% (in 12% increments) in total protein and carbohydrate contents (referred to throughout as nutritional content).

FEMALE-ACCESS TREATMENT

During the second week post-eclosion, we allowed male crickets access to a single different adult female cricket during the recording period each night for between 0 and 7 consecutive nights (always beginning on the first day of the second week post-eclosion; that is, eight distinct treatment groups). As logistical constraints prevented us from providing virgin females of similar age each day, we instead controlled female 'experience' to be similar to the male with which she would be placed: on the first day, virgin males had access to virgin females; on the second day, a male would gain access to a female that had had a single night's access to males; and so forth. When not housed with an experimental male in his container, females were maintained in single-sex groups according to experience and provided with water, shelter and stock diet. Female experience was manipulated to the necessary level by housing them overnight with random stock males. Before being placed with an experimental male, females were also given at least a day without access to a male (considered sufficient as female *G. sigillatus* have a high remating rate, averaging 22 times over a 20-day period; Sakaluk 1987). During the week that access to females was manipulated, the food was removed from each male's container during the call recording period (regardless of female presence). Males were given access to food whenever calling effort was not being recorded (09.00 to 14.00 h).

During the female-access treatment period, we allowed paired crickets to mate freely. In many cricket species, males stop calling after mating until they have generated a new spermatophore and are ready to mate again (Huber 1962; Loher 1974; Cade & Wyatt 1984; Wagner 2005); this is also the case in *G. sigillatus* (T. Houslay, personal observation). Males transfer a two-part spermatophore, comprising the sperm ampulla and a spermatophylax 'nuptial gift' (Sakaluk 1984). While these take an average of 3·25 h to compose (Sakaluk 1985), they consist of 80–85% water and have little or no nutritional value (Will & Sakaluk 1994; Warwick *et al.* 2009), and as such, the metabolic costs of their generation are uncertain. We anticipate that one major effect of mating will be a reduction in calling, with the concomitant saving in stored resources that might otherwise be spent on calling activity. Our large sample size and use of continuous (rather than categorical) treatments enable us to capture diet- and calling-mediated changes in energy storage despite any additional effects of mating on males.

MEASURING ENERGY STORAGE

We used a modified version of Foray *et al.* (2012) to measure the major components of energy storage in each cricket: carbohydrates, glycogen, lipids and proteins (see Appendix S2). These can be assumed to comprise an individual's entire current energy budget. Cricket flight is fuelled by lipid breakdown (Zhao & Zera 2002), and the nature of the underlying pathways makes it likely that lipids are the major energy store for stridulatory calling effort. Their presence is likely to be greatest in males that have access to high-quality resources. Glycogen is the storage form of glucose and can be broken down in quick response to the need for energy in high-intensity activity (Campbell & Farrell 2003). High circulating glycogen levels may indicate males that are prepared to engage in sustained calling bouts, while carbohydrate content shows the existence of free sugars that can be used to fuel energetically expensive signalling. Protein contains the same energy content per gram as carbohydrates, but takes longer to break down and is not typically used as a short-term energy source. The relative value of these energy storage forms for sexual signalling is currently unclear; lipids provide more energy than sugars on a gram-for-gram basis (Campbell & Farrell 2003), yet mobilization of shorter-term energy forms is probably important for plastic responses to reproductive opportunities. If males were future planners, such that low-acquisition males restrict early signalling in favour of storage for use later in life, we would expect these males to show greater storage of lipids relative to glycogen and carbohydrate.

STATISTICAL ANALYSIS

We performed all statistical analyses using R 3.1.1 (R Core Team 2014). Independence between linear and quadratic forms of numeric predictors (e.g. diet and diet^2) was achieved by centring the input variable before squaring (Gelman & Hill 2007). Diet treatments were centred and scaled to single-unit deviations such that model coefficients show the effect on the response variable equivalent to one unit of change in diet treatment. In analyses of daily calling effort (discussed further below), male age post-eclosion was also centred but not scaled (coefficients show effect equivalent to 1 day's change). Continuous covariates (e.g. measurements of energy storage when used as predictors) were standardized by mean-centring and scaling (dividing by 1 standard deviation), putting them on a common scale and aiding interpretation of main effects (Gelman & Hill 2007; Schielzeth 2010). Unless otherwise stated, we performed model simplification by dropping non-significant higher-order terms (interactions and quadratic effects) from the full model sequentially and using likelihood ratio tests to compare nested models. We retained more complex models whenever simplification resulted in a significant increase in model deviance. We observed no cases in which non-significant main effects became significant during model simplification steps that did not involve the deletion of an interaction term including that particular main effect.

Daily calling decisions and effort

Daily calling effort data were overdispersed and zero-inflated; for analyses of these data, we used the R package MCMCGLMM (Hadfield 2010) to fit a zero-altered Poisson (ZAP) model: a two-part model that includes a logistic regression for the zeroes in the data and a Poisson regression for the zero-truncated counts (see Appendix S3). We accounted for repeated observations on individuals by specifying a random effect of cricket ID. The ZAP model enabled us to ask two distinct questions within a single statistical structure (Atkins *et al.* 2013): what factors influenced whether a male called or not (i.e. nonzero or zero), and, if he did call, what factors influenced the magnitude of calling (Houslay *et al.* 2015)?

For the first week post-eclosion, the full model included predictor variables of linear and quadratic terms for both diet treatment and days post-eclosion (corresponding to male age, modelled as a continuous variable), and all interactions (except those between the linear and quadratic forms of the same predictor, e.g. day × day^2, as such interactions are not interpretable).

For analysis of daily calling effort across the second week post-eclosion, female-access treatment could not be entered in the model as a single predictor because the differences among treatment groups only become apparent as the week progresses. Instead, we use both a binary predictor of 'female presence' (indicating whether a male had access to a female on the current day of observation) and a continuous 'prior female-access' variable (the number of days prior to the current one in which a male had

access to females). The full model for daily calling over the second week post-eclosion therefore included predictor variables of female presence (binary variable), linear and quadratic terms for both diet treatment and male age (in days post-eclosion), prior female access, and up to three-way interactions between these (except those featuring linear and quadratic forms of the same predictor).

Changes in body mass

We used multiple regression to assess the linear and quadratic effects of diet treatment on changes in individual body mass (using the scaled mass index, SMI; Peig & Green 2009) over the first week post-eclosion. SMI is a better index of the relative size of energy reserves (Peig & Green 2010), and a better correlate of standardized mass in crickets (*A. domesticus*, *G. texensis;* Kelly, Tawes & Worthington 2014), than other commonly used proxies.

To estimate the effects of both acquisition and allocation on the change in SMI (ΔSMI) over week 2, our predictors were diet treatment and log-transformed total calling effort. We used a separate model to investigate the effects on ΔSMI of diet treatment, female-access treatment and their interaction. Given that our individuals had already been subject to their diet treatments for the first week post-eclosion, we corrected ΔSMI over week 2 for the effect of regression to the mean, using the method outlined by Kelly & Price (2005).

Changes in energy storage

For those individuals sacrificed at eclosion, we used linear regression models to test whether carbohydrate, glycogen, lipid or protein contents predicted an individual's scaled body mass (SMI). We log-transformed SMI so that model residuals conformed to a normal distribution. We excluded one individual because its SMI was over four standard deviations from the mean (all others were within ± 2·2 standard deviations).

We also used linear regression models to investigate the causes of variation in energy storage components at the end of the first week post-eclosion. For each response variable (protein, lipid, glycogen and carbohydrate), we estimated approximate changes over this period by subtracting the mean concentration of each storage form at eclosion from every individual's measurements. We then tested for the effect of diet treatment on these changes. The values representing changes in carbohydrates, glycogen and lipids were log-transformed prior to analysis. We also tested whether the changes in each form of energy storage predicted changes in SMI.

We estimated approximate changes in energy storage components over the second week by subtracting the diet-specific mean content of each storage form at the end of week 1 from each individual's measurements. We then tested whether these estimated changes in energy storage were associated with individual changes in SMI. We also tested whether individual energy storage measurements at the end of week 2 predicted SMI at the same stage.

Results

As expected (because treatments began after males reached eclosion), there were no significant differences between diet or female-access treatment groups in pronotum length, raw body mass or scaled body mass (scaled mass index, SMI) at eclosion (all $P > 0.37$). Of the four energy storage forms measured, only lipids were a significant predictor of SMI at eclosion (lipid coefficient = 0.065 ± 0.030, $t_{1,50} = 2.19$, $P = 0.033$).

MANIPULATION OF RESOURCE ACQUISITION IN EARLY ADULTHOOD

The likelihood of calling and calling effort both increases with diet quality and age

The simplified ZAP model for days 2–7 post-eclosion included predictor variables of diet, linear and quadratic terms for the day of calling (i.e. the age of an individual post-eclosion), and an interaction between diet and the linear day term. The likelihood of calling increased significantly along with both nutritional diet content and age over the first week (Table 1, Fig. 2a). The age-related increase in the likelihood of calling was less pronounced towards the end of the week. When males did call, there was a significant two-way interaction between day and diet on the amount of calling effort: effort increased over the course of the week, especially when males were fed higher-quality diets (Table 1, Fig. 2b). The main effects of age and diet were to increase calling effort during this period, age acted in a nonlinear manner. This nonlinear effect of age can be seen in the change in the intercept (centred at 48% diet) across panels in Fig. 2b: the age-related change in the intercept does not increase at a constant rate, with the rate of increase dropping at higher values of male age.

Greater resource acquisition leads to increases in body mass and energy storage

The change in SMI among individuals increased with improving dietary nutrition, although this increase levelled off at higher diets (negative quadratic effect of diet; Table 2a). We estimated changes in energy storage content

Table 1. MCMCGLMM zero-altered Poisson (ZAP) analysis of male nightly calling effort over the first week post-eclosion. Day 1 was excluded from the analysis as no males called

Fixed effects	Estimate	95% CI (lower, upper)	pMCMC
Likelihood of calling			
(Intercept)	−5·636	(−5·926, −5·374)	<0·001
Diet	0·187	(0·081, 0·297)	<0·001
Day	0·420	(0·306, 0·540)	<0·001
Day^2	−0·147	(−0·198, −0·088)	<0·001
Diet × day	0·036	(−0·006, 0·082)	0·120
Calling effort			
(Intercept)	3·636	(3·391, 3·876)	<0·001
Diet	0·183	(0·094, 0·276)	<0·001
Day	0·191	(0·115, 0·265)	<0·001
Day^2	−0·074	(−0·108, −0·042)	<0·001
Diet × day	0·060	(0·033, 0·089)	<0·001

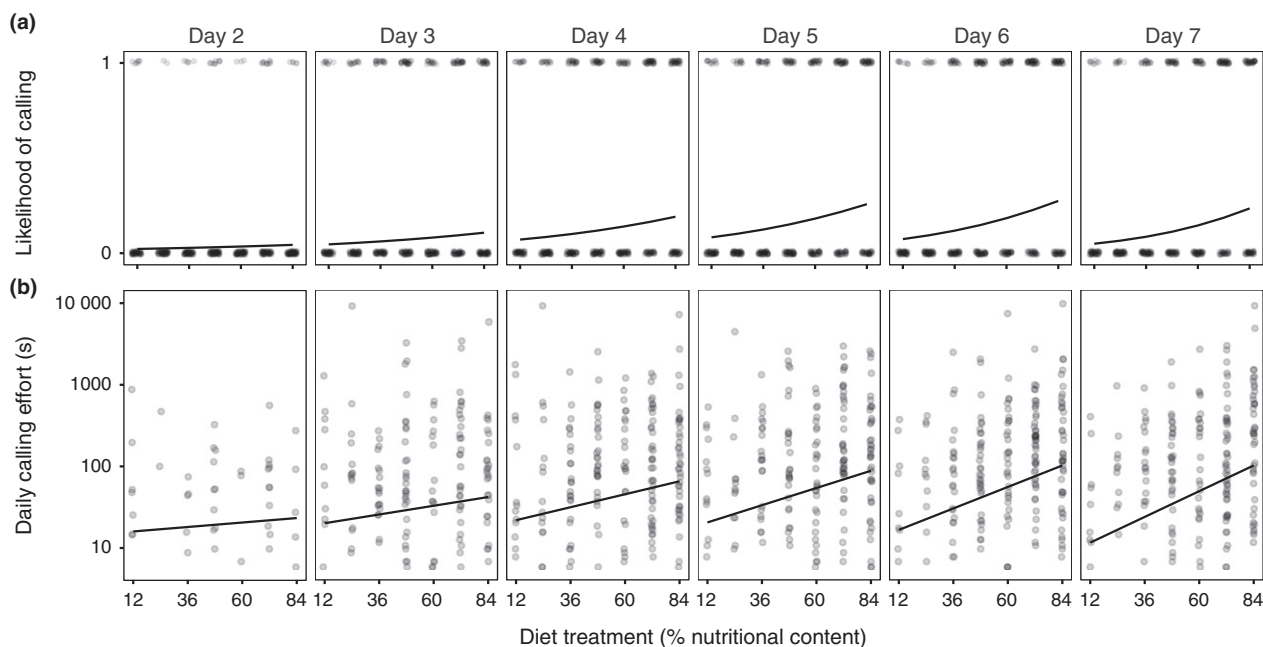| Variance components	Estimate	95% CI (lower, upper)	
Likelihood of calling			
ID	1·128	(1·009, 1·258)	
Calling effort			
ID	1·559	(1·190, 1·979)	
Residual	5·000	(3·990, 6·086)	

Fig. 2. The effects of diet treatment (*x*-axis) and day of measurement (equivalent to male age post-eclosion; modelled as a continuous variable and shown here as distinct panels) on male sexual advertisement during the first week post-eclosion. (a) The likelihood of male calling, with fitted regression line taken from coefficients from the zero-altered part of the daily calling analysis; (b) the amount of calling effort (plotted on log-scale *y*-axis), given a male called, with fitted regression line from the Poisson part of the daily calling analysis. The first day post-eclosion was excluded from the analysis as no males called.

Table 2. Linear regression models showing the effect of diet over the first week post-eclosion on (a) individual changes in body mass, as scaled mass index (SMI), and (b) approximated changes in four different energy reserve forms. Values representing changes in carbohydrate, glycogen and lipid contents were log-transformed prior to analysis

Response	Predictor	d.f.	t	Estimate $\pm$ SE	P
a) Δ SMI	Diet	1,536	13·97	0·0062 $\pm$ 0·0004	<0·001
	Diet^2	1,536	−3·62	−0·0009 $\pm$ 0·0002	<0·001
b) Δ Carbohydrate	Diet	1,43	3·39	0·050 $\pm$ 0·015	0·001
Δ Glycogen	Diet	1,47	3·90	0·135 $\pm$ 0·035	0·003
Δ Lipid	Diet	1,49	2·67	0·075 $\pm$ 0·028	0·010
Δ Protein	Diet	1,48	0·53	0·100 $\pm$ 0·189	0·597

by comparing individual measurements of each storage component to averages taken from the subset of crickets sacrificed at eclosion, enabling us to investigate the underlying physiological response to our dietary manipulation. Improving dietary nutrition significantly increased carbohydrate, glycogen and lipid reserves, but not protein (Table 2b). Separate models also showed that the estimated changes in lipid and glycogen were significant predictors of changes in SMI over the first week post-eclosion (carbohydrate = 0·015 $\pm$ 0·004, $t_{1,43} = 1·70$, $P = 0·096$; glycogen = 0·008 $\pm$ 0·003, $t_{1,47} = 2·30$, $P = 0·026$; lipid = 0·008 $\pm$ 0·003, $t_{1,46} = 2·34$, $P = 0·024$; protein < −0·001 $\pm$ 0·003, $t_{1,48} = -0·01$, $P = 0·992$).

Relationship between calling and change in body mass is dependent on resource acquisition

The significant interaction term (Table 3) indicated that the relationship between total calling effort and change in

SMI over week 1 was strongly dependent upon diet, switching from a negative to a positive relationship as diet improved (Fig. 3): increased dietary nutrition meant males that called more were also better able to increase body mass.

MANIPULATION OF RESOURCE ACQUISITION AND ACCESS TO POTENTIAL MATES

In the first part of our study, males on lower-quality diets called less often and less intensely than those on higher-quality diets (Fig. 2). While total calling effort and storage appeared to covary negatively among those low-acquisition males, those males did not invest more in energy reserves relative to higher-acquisition individuals (Fig. 3). To demonstrate 'future planning', low-acquisition males should remain less likely to call even in the presence of females, instead conserving energy reserves for greater calling later in life.

Table 3. Multiple regression showing how individual change in body condition over the first week of adulthood (SMI day 7 – SMI at eclosion) is affected by how much a male called over week 1 (log-transformed and standardized) and diet

	t	Estimate $\pm$ SE	P
(Intercept)	16·34	0·0143 $\pm$ 0·0009	<0·001
Log week 1 total calling effort	1·80	0·0016 $\pm$ 0·0009	0·073
Diet	12·87	0·0058 $\pm$ 0·0004	<0·001
Log week 1 total calling effort $\times$ diet	2·88	0·0013 $\pm$ 0·0004	0·004

Males are more likely to call when females are present

The simplified ZAP model for daily calling effort during the female-access treatment period included predictor variables of diet, female presence on the current day, the age of an individual at the time of measurement (in days post-eclosion) and prior female access (the number of days the male had access to a female prior to the current measurement). Multiple interactions were retained in the model, including a three-way interaction between prior female access, diet and day of measurement.

Female presence significantly increased the likelihood of calling (Table 4, Fig. 4a). Males were less likely to call as the week progressed, but this decrease was not observable when females were present ('Female presence $\times$ day' interaction in Table 4; Fig. 4a). Finally, call likelihood was significantly reduced in males that had greater access to females prior to the current measurement ('Prior female access', Table 4). Diet had no effect on the likelihood of calling: low-acquisition males were just as likely to call as high-acquisition males.

The positive effect of diet on calling effort is reduced by greater prior access to females

Calling effort increased significantly when a female was present, but the effect decreased over the week ('Female presence $\times$ day' interaction term in Table 4; Fig. 4b). Dietary nutrition also significantly increased calling effort (Fig. 5), but this effect was reduced in males with greater access to females as the week progressed ('Prior female access $\times$ diet $\times$ day' interaction in Table 4; Fig. 5). These effects are visualized in Fig. 5, which shows the relationship between diet treatment and calling effort for the first (left panels) and final (right panels) days of the female-access manipulation, for those given no female access (lower panels) or daily female access (upper panels).

Diet-related increase in body mass is reduced by access to females

Having excluded males that did not call or that cannibalized females during the female-access treatment period, we found that increased total calling effort during week 2 was associated with decreased SMI (log total calling = -0.0018 ± 0.0007, $t_{1,307} = -2.55$, $P = 0.011$). Diet treatment increased individual SMI, but this effect was reduced at higher diets (diet = 0.0021 ± 0.0006, $t_{1,307} = 3.51$, $P < 0.001$; diet^2 = -0.0018 ± 0.0003, $t_{1,307} = -3.76$, $P < 0.001$). Separately, we found no effect of diet on the frequency with which males cannibalized females during the female-access treatment period ($\chi_6^2 = 1.10$, $P = 0.98$).

Table 5 shows the minimum adequate model for a separate analysis of the effects of our experimental
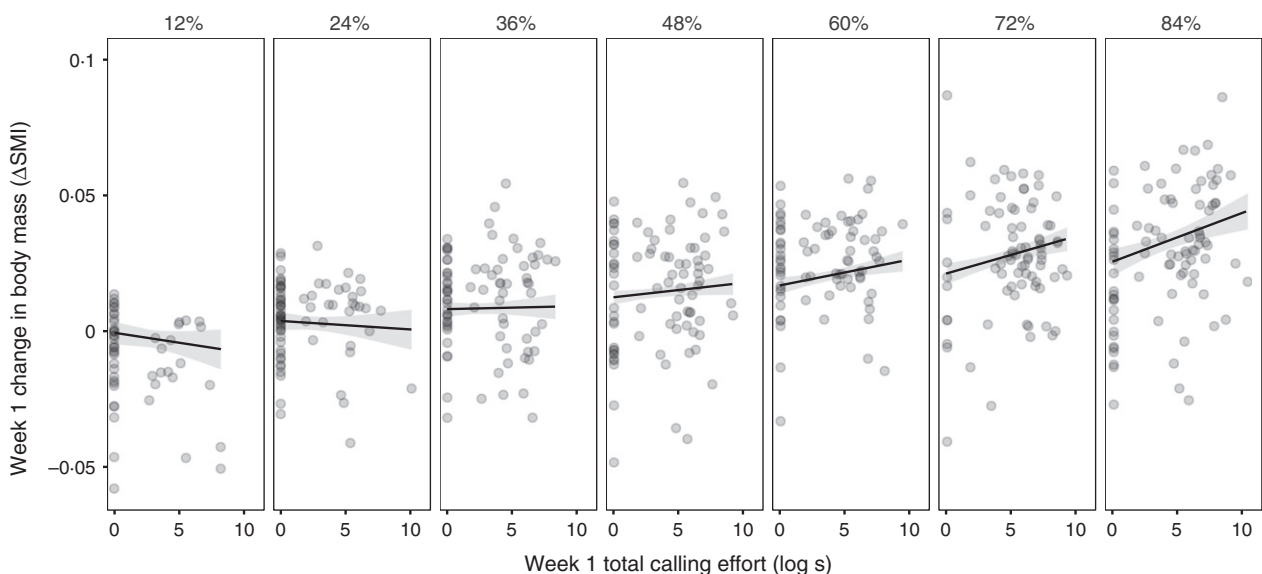

Fig. 3. The relationship between total calling effort over the first week post-eclosion (log-transformed) and the change in scaled mass index (SMI) during this period, plotted separately for each diet treatment (note that diet was modelled as a continuous predictor). Lines are predicted slopes from a linear regression model, and shaded areas represent 95% confidence intervals.

Table 4. MCMCGLMM zero-altered Poisson (ZAP) analysis of male nightly calling over the second week post-eclosion. Males fed the 12% nutrition diet were excluded as they all died prior to the end of the week. Also excluded were males that cannibalized a female during the female-access treatments

Fixed effects	Estimate	95% CI (lower, upper)	pMCMC
Likelihood of calling			
(Intercept)	−4·617	(−5·034, −4·179)	<0·001
Female presence	3·007	(2·491, 3·564)	<0·001
Diet	0·031	(−0·149, 0·196)	0·732
Day	−0·120	(−0·244, −0·005)	0·058
Prior female access	−0·647	(−0·871, −0·429)	<0·001
Female presence × day	0·779	(0·566, 1·001)	<0·001
Prior female access × diet	0·055	(−0·018, 0·144)	0·185
Prior female access × day	0·014	(−0·041, 0·067)	0·607
Prior female access × diet × day	0·017	(−0·010, 0·044)	0·223
Calling effort			
(Intercept)	4·065	(3·717, 4·381)	<0·001
Female presence	0·742	(0·498, 0·971)	<0·001
Diet	0·385	(0·281, 0·489)	<0·001
Day	−0·195	(−0·264, −0·130)	<0·001
Prior female access	−0·080	(−0·214, 0·058)	0·247
Female presence × day	−0·210	(−0·335, −0·082)	0·001
Prior female access × diet	0·024	(−0·012, 0·058)	0·191
Prior female access × day	0·054	(0·028, 0·079)	<0·001
Prior female access × diet × day	−0·020	(−0·032, −0·007)	<0·001

Variance components	Estimate	95% CI (lower, upper)
Likelihood of calling		
ID	5·365	(3·982, 6·876)
Calling effort		
ID	2·108	(1·694, 2·553)
Residual	1·283	(1·183, 1·394)

manipulations on the change in SMI over week 2 (again, excluding males that cannibalized females). The effect of diet depended on female-access treatment; in the absence of females, males on high-nutrition diets tended to gain more body mass during this period than those on low diets; however, high-nutrition males with consistent access to potential mates lost more mass ('Diet × Female-access' interaction term in Table 5; Fig. 6).

Changes in body mass are associated with changes in carbohydrate and glycogen reserves

We found that the estimated change in carbohydrate and glycogen reserves were significant predictors of changes in individual SMI over the second week post-eclosion (carbohydrate = 0.0045 ± 0.0008, $t_{1,143} = 5.52$, $P < 0.001$; glycogen = 0.0039 ± 0.0009, $t_{1,145} = 4.56$, $P < 0.001$;

lipid = -0.0006 ± 0.0009, $t_{1,142} = -0.72$, $P = 0.470$; protein = 0.0007 ± 0.0009, $t_{1,139} = 0.70$, $P = 0.486$).

We also tested whether any of our energy storage measurements correlated with individual SMI at the end of week 2; only lipid content significantly predicted SMI (lipid = 0.014 ± 0.005, $t_{1,142} = 2.96$, $P = 0.004$).

EFFECTS OF RESOURCE ACQUISITION AND ACCESS TO POTENTIAL MATES ON SURVIVAL

Due to the high proportions of deaths in the two lowest diet treatments (100% and 60·5%, respectively), we excluded individuals in those treatments from our analysis of the likelihood of dying during the female-access period. Greater female access significantly increased the risk of death during this period, while diet significantly reduced it (diet = -0.225 ± 0.113, $t_{1,377} = -1.99$, $P = 0.047$; female access = 0.226 ± 0.074, $t_{1,377} = 3.04$, $P = 0.002$; Fig. S2). A model retaining the excluded diet treatments gave qualitatively similar results, which are not presented here.

Our female-access treatment had a significant effect on life span after the conclusion of the female-access treatment period (i.e. after the end of the second week post-eclosion); males with greater access to females during week 2 had shorter life spans (female access = -3.913 ± 0.937 days, $t_{1,145} = -4.18$, $P = 0.001$; Fig. S3a). Life span was significantly increased by greater dietary nutrition, although this effect disappeared at higher-quality diets (diet = 11.823 ± 1.734 days, $t_{1,145} = 6.82$, $P < 0.001$; diet^2 = -2.491 ± 0.869, $t_{1,145} = -2.87$, $P = 0.005$; Fig. S3b).

Discussion

Male sexual signals are often condition-dependent, with trait expression closely associated with the size of the resource budget (e.g. Andersson 1994; Johnstone 1995). Few studies have simultaneously manipulated resource acquisition and access to reproductive opportunities while also examining the resultant patterns of energy storage and use (Kotiaho 2000). Recently, it has become clear that traits used to measure condition are themselves condition-dependent (and are built using the resources that make up condition), meaning that traits reflecting condition must inevitably trade-off with one another (Tomkins *et al.* 2004; Houslay & Bussière 2012). This fact prescribes careful interpretation of covariances between proxies for condition and other life-history traits and highlights the need for more direct measurements of energy reserves (Morehouse 2014; Wilder, Raubenheimer & Simpson 2015). Despite the inherent challenges, identifying relationships between trait investment and stored energy reserves can illuminate mechanisms leading to changes in resource storage and use, and therefore help clarify the adaptive significance of variation in resource allocation among males as well as the consequences of energetic trade-offs for honesty in sexual trait expression.
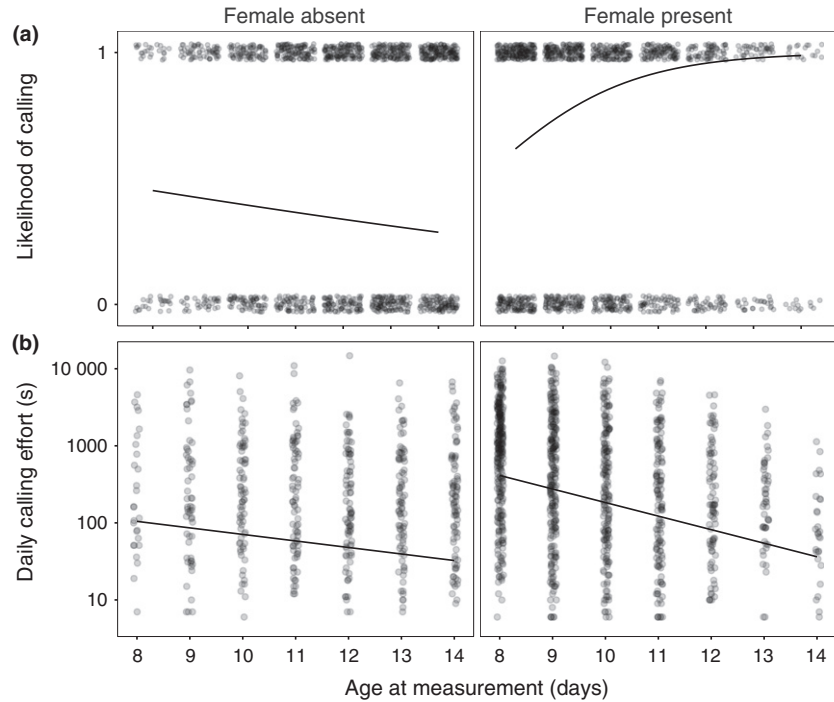
Fig. 4. The partial effect of day of measurement (equivalent to male age post-eclosion) on male sexual advertisement during week 2, plotted separately for whether a female was absent or present on the day of measurement. (a) The likelihood of male calling, with fitted regression line taken from coefficients from the zero-altered part of the daily calling analysis; (b) the amount of calling effort (plotted on log-scale *y*-axis), given a male called, with fitted regression line from the Poisson part of the daily calling analysis. Measurements from males fed 12% nutrition diet have been excluded.
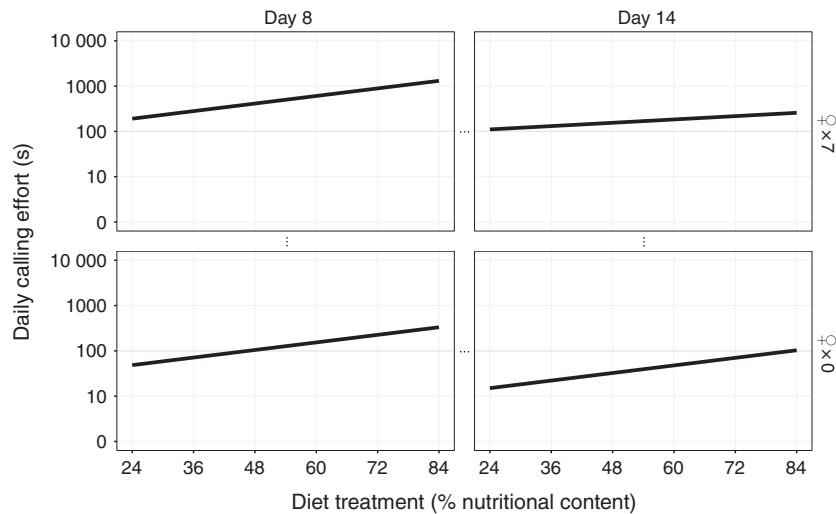
Fig. 5. 'Small multiples' show how the relationship between diet and daily calling effort changes as a function of day and female-access treatment. Each individual panel plots the predicted slope from the Poisson part of our MCMCGLMM ZAP model of daily calling effort against dietary nutrition (24–84%). Here, we present a subset of panels, showing how the effect of diet on calling effort (plotted on log-scale *y*-axis) changes from the first to last day of the female-access treatment period (male ages of 8 and 14 days post-eclosion, overall horizontal axis), among males that had either no access to females (lower panels) or daily access to females (upper panels). See text for model details; Fig. S1 presents the complete set of panels for all combinations of day and female availability treatment.

By manipulating dietary nutrition in male crickets, we showed that greater resource acquisition led to an increase in both the likelihood and amount of signalling in early adulthood (Table 1, Fig. 2), as well as greater investment in energy stores (lipid, glycogen and carbohydrate) during this period (Table 2). The relationship between allocation to scaled body mass (as scaled mass index, SMI) and calling effort depended on diet quality, switching from negative covariance when diet quality was low to positive covariance when high (Table 3, Fig. 3). While low-acquisition males

called less often, and less intensely, during this period than did higher-acquisition males, we found no evidence that such a suppression of signalling effort led to increased energy reserves. If low calling effort among low-acquisition males was due to energy constraints rather than future planning, we predicted that these males would respond to the presence of potential mates by investing more in calling instead of saving energy for the future.

Sexual trait expenditure was highly plastic depending on the availability of a potential mate: males responded

Table 5. Simplified multiple regression model showing factors affecting individual change in scaled body mass over the second week of adulthood (SMI day 14 – SMI day 7, corrected for the effect of regression to the mean). Males that cannibalized a female during this period were excluded from the analysis

	t	Estimate $\pm$ SE	P
(Intercept)	1·34	0·0019 $\pm$ 0·0014	0·182
Diet	3·5	0·0031 $\pm$ 0·0008	<0·001
Female access	0·09	0·0001 $\pm$ 0·0003	0·927
Diet^2	−3·72	−0·0011 $\pm$ 0·0003	<0·001
Diet $\times$ Female access	−2·16	−0·0004 $\pm$ 0·0002	0·032

strongly to the presence of a female by increasing both the likelihood of calling and time spent calling (Table 4, Fig. 4). During the female-access treatment period, diet did not affect the probability of signalling on any given day. It did, however, have a strong positive effect on the amount of time spent calling (Fig. 5), mediated by female access: the positive effect of diet on calling effort decreased with greater exposure to females over the course of the week (Fig. 5). The change in male scaled body mass (SMI) as a function of diet during this period was nonlinear and also depended on access to females (Table 5, Fig. 6). In the absence of females, males fed higher nutrition diets tended to increase or maintain body mass more than those on lower-nutrition diets. However, greater female access effectively reversed this relationship: males on high-

nutrition diets tended to lose more SMI than those on lower-nutrition diets when given daily access to females over the treatment period. Diminishing calling effort by the end of week 2, even by males on high-quality diets in the presence of females, suggests an exhaustion of available resources over this period (Fig. 5).

Indeed, the change in male body mass over week 2 covaried negatively with total calling effort, and the likelihood of calling decreased as previous exposure to females increased (Fig. 4a, Table 4) – both results irrespective of diet. Greater exposure to potential mates also increased the risk of dying during week 2, although this effect was mitigated by diet quality (Fig. S2). There were lasting consequences of our experimental manipulations: longevity following week 2 was increased by dietary nutritional quality and decreased by greater prior exposure to females (Fig. S3). However, this reduced viability cannot be considered a true cost unless it is accompanied by a net decrease in fitness, because any decrease in life span may be offset in fitness terms by increased early reproductive success (Kotiaho 2001; Hunt *et al.* 2004a,b).

FUTURE PLANNING OR BUDGET CONSTRAINTS?

Crickets are ideal for testing hypotheses concerning age-related reproductive effort because we can precisely quantify sexual advertisement effort by males. Several studies have manipulated resource acquisition to investigate its
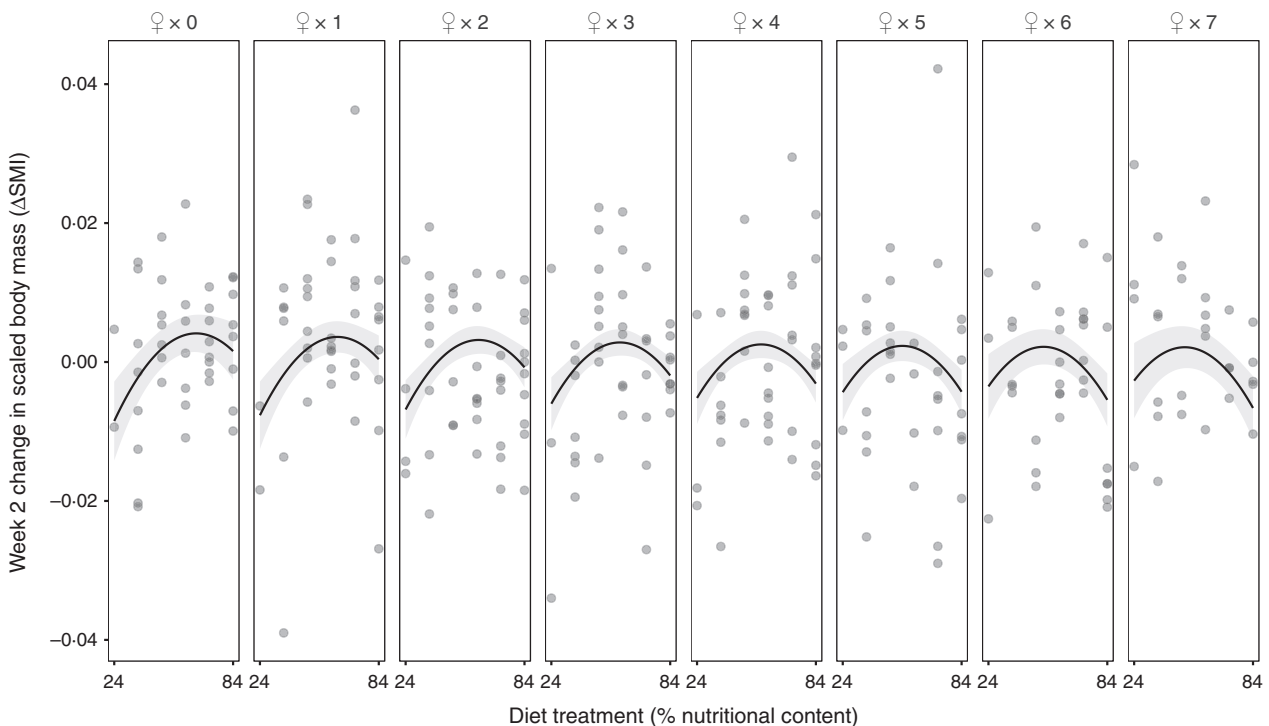

Fig. 6. Predicted quadratic slopes from a linear regression model show the effect of diet treatment on the change in individual scaled mass index (SMI) over week 2, separately for each female-access treatment. All males on the lowest nutrition (12%) diet died before the end of the week and were thus excluded; we also excluded males that cannibalized females. Shaded areas represent 95% confidence intervals. Values for the change in SMI have been corrected for the effect of regression to the mean.

effects on life-history traits, with largely consistent results: males fed higher-quality diets typically increase their calling effort with age, and longer-lived males call more (Judge, Ting & Gwynne 2008; Maklakov *et al.* 2008; Zajitschek *et al.* 2009, 2012; Houslay *et al.* 2015). One exception is the study of Hunt *et al.* (Hunt *et al.* 2004a), in which males fed a high-protein diet called earlier and far more intensely than those fed lower protein diets; high-protein diet males also suffered survival costs such that they died earlier than low-protein diet males. This pattern of age-related allocation may reflect status-dependent plasticity in allocation to reproduction and survival in some cases (Kokko 1997, 1998; Höglund & Sheldon 1998; Vinogradov 1998). In particular, low-condition males can benefit from suppressing signalling at early ages and investing in longevity and sexual advertisement later in life (Kokko 1997). If this adaptive plasticity in investment applied to *G. sigillatus*, males restricted to lower-nutrient diets should have allocated resources preferentially to energy storage instead of early signalling.

Males fed lower-nutrition diets in our study called less, and the correlation between allocation to storage (individual change in SMI) and calling effort was negative during week 1 among males on lower-quality diets (Table 2, Fig. 3). However, while diet-restricted males were less likely to call over the first week (Table 1, Fig. 2a), this effect was not evident during week 2 (Table 4). If males on low-quality diets had continued to show a depressed propensity to call and had increased body mass over this period, that would be more consistent with crickets suppressing advertisement in favour of increased future investment. Instead, it appears more likely that variation in calling effort was due simply to budget constraints: that is, having less energy to allocate to signalling. The non-adaptive explanation for this variation among males is given further credence by dietary nutrition being a strong predictor of survival, with males fed lower-nutrient diets suffering reduced longevity. The fact that a male's current signalling effort is primarily governed by his acquisition ability and previous expenditure also supports previous assertions that energetic trade-offs provide a mechanism for maintaining honesty on average in male behavioural display traits (Kokko 1997).

VARIATION IN USAGE AND IMPORTANCE OF ENERGY STORAGE FORMS

Increased resource acquisition from higher-nutrient diets enabled male crickets to allocate more resources to storage after eclosion in the forms of greater lipid, glycogen and carbohydrate contents (Table 2b). These appear to represent the major forms of energy stores; protein levels did not vary over the three time points that these stores were measured, indicating that males seem to maintain protein content rather than increase it (although the nutrient ratio in our diet treatment was heavily in favour of carbohydrate rather than protein, the variation in total nutrient content would enable individuals to differ in allocation if required). We hypothesized that 'future planning' would result in low-acquisition males prioritizing storage in the form of lipids; the positive effect of diet on this form indicates that this was not the case. Our results further corroborate findings in other cricket species, in which calling effort covaried with the ability to acquire, metabolize and mobilize these three storage forms (Bertram *et al.* 2011; Thomson, Darveau & Bertram 2014).

Males tended to lose scaled body mass (SMI) over week 2 (Table 5), and individual SMI change in this period was associated with estimated changes in glycogen and carbohydrate reserves – both of which are quick-release forms of energy. Absolute SMI at the end of that period was associated with current lipid reserves. Together, these results suggest that glycogen and carbohydrate are more freely expendable forms of energy, while lipids are more carefully conserved. Such variation in the conservation of storage forms indicates that lipids differ from carbohydrate and glycogen in usage and importance and highlights the importance of estimating multiple metabolic reserves when examining life-history trade-offs. The patterns of change in individual body mass and energy reserves over weeks one and two suggest that resource acquisition early in adulthood tended to be mobilized for fuelling calling effort as soon as the social circumstances favour it.

ACQUISITION CAN AFFECT COVARIANCES BETWEEN EXPENSIVE LIFE-HISTORY TRAITS

Variation in resource acquisition ability among individuals can lead to positive correlations between life-history traits, as predicted by Van Noordwijk & De Jong (1986) and often found in nature (Jennions, Møller & Petrie 2001). Figure 3 demonstrates clearly how such a correlation can arise when some males have acquired a greater amount of resources and can then allocate to two competing traits. Increased nutrient availability can mask any negative correlation between two traits because individuals have more resources to allocate to both, as seen in the increasingly positive relationship between calling effort and body mass when dietary nutrition increases. We found that the mean change in body mass also increased with diet and with total calling effort, indicating that individuals allocate more resources to both storage and current reproductive effort as acquisition increases. These results have implications for how males are likely to sample their social environment through calling effort: males probably call irregularly early in life, and the extent to which this activity affects the accumulation of resources depends on nutrient intake. The accumulation of body mass with increases in both diet and total calling effort may be due partly to those individuals on higher-quality diets having a greater resource intake, and also because of the smaller marginal costs of increased advertisement to higher condition males, as predicted by models of sexual selection under condition dependence

(Grafen 1990; Rowe & Houle 1996). These findings mirror interspecific (Judge, Ting & Gwynne 2008) and intraspecific (Hunt *et al*. 2004a) differences in the covariance between expensive traits under contrasting conditions, although it is not clear the extent to which our results are related to selection in the laboratory rather than in the wild. We hope to see more studies in more taxa that directly assess the storage and use of multiple energy forms over time – particularly in the face of varying resource acquisition regimes, and with varying reproductive opportunities.

SOCIALLY MEDIATED PLASTICITY IN REPRODUCTIVE EFFORT

Male decorated crickets responded strongly to female presence, with significant increases in both the likelihood and the time spent calling. To date, most work on male responses to the social environment in crickets have focused on adaptive plasticity for anticipating future conditions (Kasumovic & Brooks 2011): manipulations of juveniles' perceived density and future competition can affect adult life-history traits (*T. oceanicus*, Bailey, Gray & Zuk 2010; *T. commodus*, Kasumovic, Hall & Brooks 2012; Kasumovic *et al*. 2013). Manipulations of the social environment in adult crickets have thus far been less common. Callander *et al*. (2013) measured the calling effort of male Australian black field crickets (*T. commodus*) that were either kept isolated or housed with a rival male after reaching maturity. However, all males in that study were isolated for the measurement period itself, which might explain the lack of behavioural plasticity across treatments (Callander *et al*. 2013). The extreme phenotypic flexibility of behavioural traits means individuals can adjust trait expression rapidly in response to social cues (Ghalambor, Angeloni & Carroll 2010; Bretman, Gage & Chapman 2011); indeed, female presence has previously been shown to affect the frequency and timing of cricket calling (Simmons 1986; Burpee & Sakaluk 1993; although see Bertram, Orozco & Bellani 2004). Storage of resources in 'quick-release' form as sugars (carbohydrate and glycogen) may provide male crickets the flexibility to signal more effectively in the presence of females, explaining the increased calling effort by males on higher-quality diets during our female-access treatment period.

HOW STRONG IS THE EVIDENCE FOR ADAPTIVE PLASTICITY IN AGE-SPECIFIC REPRODUCTIVE EFFORT?

An unresolved question from our study relates to the decrease in calling effort over the second week due to greater previous exposure to females (Fig. 5). Early theoretical models concluded that reproductive investment should increase with age (Williams 1966), but recent studies using game theory and life-history theory paint a more complex picture (Kokko 1997, 1998; Lindström *et al*.

2009). Kokko (1998) illustrated three phenomena that can prevent positive correlations between life-history traits: (i) trade-offs acting over lifetimes, such that fitness benefits accrued early in life outweigh late life fitness (Hansen & Price 1995); (ii) increasing marginal gains in fecundity could favour decreased survival and intense sexual advertisement for high-quality males; and/or (iii) suboptimal allocation. The last point is of particular interest: if individuals can vary trait expression plastically in heterogeneous environments, and trait expression is under selection, shouldn't we generally expect adaptive plasticity for continuous traits (Scheiner & Holt 2012)? One explanation for suboptimal allocation is that the genetic and regulatory machinery required for controlling allocation across traits and over time is expensive to maintain and may not confer a sufficiently high selection advantage over a simpler set of rules: spend resources if you have them, and if not, focus on acquiring more resources instead. The differences we find in individuals that signal at high and low levels early in life may be primarily determined by budget constraints, as opposed to adaptive perception of future opportunities.

Data accessibility

Data deposited in the Dryad Digital Repository: http://doi.org/10.5061/dryad.tj693 (Houslay *et al*. 2016).

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Specifications for the creation of artificial diets.
Appendix S2. Details of methods used for estimating energy storage components.
Appendix S3. MCMCGLMM Zero-Altered Poisson (ZAP) model specification for the analysis of daily calling effort.
Fig. S1. The effect of diet on calling effort as a function of day and female-access treatment.
Fig. S2. The effects of diet and female access on the likelihood of death during female-access period.
Fig. S3. The effects of diet and female access on male lifespan.