Causes of adaptive differences in age-dependent reproductive effort

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Abstract

Sexually selected ornaments are among the most spectacular traits in nature. Indeed, the extreme costs associated with producing sexual traits seem to play a crucial role in their evolution by enforcing honest levels of advertisement: only males with high levels of acquired resources (or high 'condition', as it is known in the literature) can afford to produce extravagant signals, a phenomenon which maintains signal reliability in a constant environment. In my thesis I examine many implications of this condition-dependent model of ornament and preference evolution for variation in age-dependent allocation to sexual signals and other life history traits.

In Chapter 1, I review theoretical implications of condition-dependent signalling for life history and sexual selection theory. I note that a universal cost of expenditure in sexual advertisement is metabolic in nature: metabolites used to fund ornament expression are by definition unavailable to other life history traits that compete for a limited resource pool. This universal constraint on expenditure does more than maintain honesty (as noted above), however: the reliance of sexual displays on high levels of nutrient acquisition may help maintain genetic variation in sexual signals

that would otherwise be eroded by strong mate choice, and without which the selective basis for good-genes choice would disappear. Three mechanisms in particular probably help to maintain genetic variation in acquisition. 1) Because acquiring resources and converting them efficiently to useful forms depends on the high function of many biochemical pathways, condition is undoubtedly highly polygenic, which slows the erosion of genetic variation under strong directional selection by females (especially in the presence of epistatic interactions). 2) The highly polygenic nature of condition also presents a large target for mutation, which continually restores variation at the loci under selection. 3) The many loci underlying condition may also be particularly sensitive to environmental heterogeneity in time or space. By favouring the most ornate males, females acquire high performing genes for their offspring, regardless of the precise allele combinations that have conferred the ability to acquire resources. Selection on specific alleles is liable to fluctuate over time or space whenever allelic performance is strongly context-specific. I close by noting the considerable challenges in advancing research on sexual selection and life history allocation, including the fact that two key processes central to life history (acquisition and allocation) are latent variables that interact in complex ways and are intrinsically difficult to measure empirically.

In the remainder of my thesis I conduct a series of experiments involving decorated crickets, *Gryllodes sigillatus*, which are useful models for studying life history because they enable precise measurement of male reproductive effort. Male *G. sigillatus* face important allocation decisions owing to the highly polyandrous nature of females,

and the substantial costs involved in signalling and mating.

Chapter 2 examines sex differences in age-dependent reproductive effort as a function of diet and development stage. I reared outbred crickets using four combinations of diet nutritional quality, and studied the effects of these combinations on male and female reproductive effort (calling effort in males and fecundity in females) and longevity. While I expected males to be more sensitive than females to variation in diet and developmental changes in its quality, I actually observed the opposite: males in all treatments increased calling effort over time, exhibiting consistently positive covariance between calling effort and longevity across treatments. By contrast, the relationships between female reproductive effort and longevity changed dramatically across treatments, and females who lived to intermediate ages had the highest fecundity. Although my results support sex-specific selection on life history allocation over time, a compelling additional explanation for my findings relates to the strategic role of calling for achieving male fitness. In the absence of positive feedback from potential mates, perhaps male allocation to sexual advertisement is careful and only increases gradually as a function of accumulating metabolic resources and increasing risk of intrinsic mortality.

Alleles underlying condition are expected to be particularly sensitive to environmental heterogeneity. While this sensitivity may help maintain additive variation in male quality (which is essential for the sustenance of adaptive good-genes mate choice, as noted in Chapter 1), too much environmental sensitivity could also under-

mine the signal value of the male trait. For example, if there are strong genotype-by-environment interactions (GEIs) for sexual advertisement, in a rapidly changing environment females risk favouring a male whose alleles are no longer best suited to current conditions. This problem is particularly pressing for animals like crickets where males exhibit a behaviourally plastic sexual display (such as calling), and so may dynamically adjust signalling effort over time. In Chapter 3, I used inbred lines of decorated crickets to quantify age and diet dependent genetic variation in male signalling. I demonstrate that while genetic correlations across diets were quite strong for morphological traits, correlations between measures of the male sexual trait rapidly approached zero as I increased the distance in time (i.e., across widely spaced ages) or diet (i.e., comparing more dissimilar dietary histories) between samples. While extrapolating from my laboratory experiments to nature is difficult, my findings nevertheless cast doubt on the value of behaviourally dynamic signals (such as cricket calls) for reliably indicating genetic quality in realistically complex environments.

In Chapter 4 I used physiological assays to evaluate factors affecting metabolite storage and use over time in decorated crickets. I manipulated the acquisition ability of all males using artificial diets that varied linearly in nutrient quality, and manipulated access to female mates over the course of the second week of adult life. By sacrificing crickets at key stages before and after manipulating the diet and social environment, I was able to estimate changes in stored metabolites, and relate these changes to calling effort and longevity. During the first week of adulthood (in the absence of females),

higher diet quality significantly increased calling effort and storage of lipid, glycogen, and carbohydrate (but not protein). The presence of females increased both the probability of calling and the amount of calling during the second week, whereas diet quality only improved calling effort. By the end of the second week, calling effort had decreased, even by high quality males in the presence of females, suggesting a depletion of resources. Furthermore, the loss of condition during week 2 covaried with calling effort during the previous week irrespective of diet. Males who started the second week in high condition lost more glycogen and carbohydrate than rivals; meanwhile, lipid accumulation covaried positively with calling effort during week 2. The contrasting patterns of storage and use for lipids compared to the 'quick-release' metabolites (glycogen and carbohydrates) affirms starkly distinct functions for the different storage components, and underlines the importance of specific physiological measures in life history research.

Finally, in the general discussion, I attempt to synthesise my thesis's contributions to the study of life history trade-offs involving behavioural sexual displays. I argue that my work may have strong implications for the general honesty of male advertisements, and invite further research focused on quantifying interactions between the genotype, age, and environment in natural systems. I also question the prevalence of adaptive age-dependent plasticity in sexual advertisement, arguing for the parsimony of a more mechanistic and non-adaptive explanation for variation among populations and taxa in age-dependent signalling: males vary in signalling effort primarily as a function of constraints on energy expenditure, rather than because they

are carefully saving resources for future use.

Declaration

I hereby declare that this thesis has been composed by myself and that it embodies the results of my own research. Where appropriate, I have acknowledged the nature and extent of work carried out in collaboration with others.

Thomas M. Houslay

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both of which I have been most grateful to receive. I should mention here that Luc and John's status in the field have afforded me support and advice from the best and brightest of those who have had the fortune to study the sex lives of crickets. Felix Zajitschek and Matt Hall deserve special mention, but my thanks also go out to Kevin Judge, Darryl Gwynne, Scott Sakaluk, Rob Brooks, Mike Kasumovic, Mike Ritchie, and Nathan Bailey.

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Introduction

Extravagant male ornamental traits include some of the most wonderful and outlandish sights that nature has to offer. Darwin's theory of sexual selection sought to explain the existence of such characters: although the development of these structures must be in some way 'injurious' to a male, the advantages afforded him through being favoured by choosy females must outweigh the disadvantages (Darwin, 1871). These traits evolve because females prefer to mate with ornamented males, which is easy to understand when the male trait indicates the quality or magnitude of a direct fecundity benefit a male provides to his mate (such as food, protection, or parental care) (Andersson, 1994; Kokko *et al.*, 2003). When males do not appear to offer their mates any direct benefits, an explanation for female choice is much more elusive (Kirkpatrick & Ryan, 1991).

One possible answer is that a male's trait indicates his underlying genetic quality, and that female choosiness secures the best genes for her offspring (Weatherhead & Robertson, 1979; Lande, 1981; Kirkpatrick & Barton, 1997; Houle & Kondrashov, 2002; Cameron *et al.*, 2003). However, strong female choice also poses a problem for

the persistence of genetic benefits: if a male sexual trait advertises his genetic quality, and females exhibit strong preferences for only the 'best' males, then female choice should rapidly erode the genetic variation between males that is associated with the display trait. Any benefits of choice therefore would be rapidly depleted, and soon outweighed by the costs of choosing (Kirkpatrick & Ryan, 1991).

Given this paradox, why does the natural world still exhibit such incredible variation in male display traits, and ample evidence of persistent female choice on the basis of male exaggerated characters (Andersson, 1994)? One potential solution is provided by the condition-dependence of male sexual traits and the 'genic capture' model. Male ornamental characters tend to covary with resource acquisition (a male's 'condition')(Andersson, 1994; Johnstone, 1995); genic capture is the process by which genetic variation for resource acquisition becomes associated with sexual trait expression (Rowe & Houle, 1996). Because resource acquisition ability represents the involvement of so many loci, genic capture can slow down the otherwise rapid depletion of additive genetic variation in the sexual trait by mate choice. The many loci associated with resource acquisition further provide a large target for mutations, which continually replenish additive variation removed by mate choice; furthermore, the polygenic nature of condition also renders it highly sensitive to environmental variation.

Condition-dependence means females can assess a male's overall condition via his sexual trait; given that a trait acts as a 'summary' of male resource acquisition ability,

female choice selects not on the trait itself, but rather on the currently high performance alleles underlying its expression (Rowe & Houle, 1996). The phenotypically plastic nature of condition-dependence means females favour the genes that code for highest performance in the environment in which the male trait developed, regardless of whether those alleles have historically been associated with high performance. If environments are heterogeneous or change over time, the selection imposed by mate choice also changes, which favours different alleles in different contexts. Error-prone female choice might represent a more evolutionarily stable strategy than perfect choice precisely because it maintains some proportion of poorly adapted alleles that females in future generations should select against (Holman & Kokko, 2014).

However, environmental heterogeneity and dispersal or temporal fluctuations in selection can lead to mate choice errors when a male's sexual signal does not reflect his condition accurately (Greenfield & Rodríguez, 2004; Bussière *et al.*, 2008b; Kokko & Heubel, 2008; Higginson & Reader, 2009; Holman & Kokko, 2014). When the relative performance of different genotypes depends on the environment in which they were expressed, genotype-by-environment interactions (GEIs) occur (Lynch & Walsh, 1998). Whether GEIs typically help or hinder effective mate choice remains an unresolved problem in sexual selection research (Greenfield & Rodríguez, 2004; Hunt *et al.*, 2004b; Ingleby *et al.*, 2010).

The central importance of sexual trait expression to any individual male's reproductive success, and thus to his fitness, begs the question of why individuals do not

'cheat' through disproportionate investment. Sexually selected traits are governed by the same constraints as all other traits, and are thus subject to trade-offs (Andersson, 1994). Trade-offs link traits together, and in so doing constrain their simultaneous evolution (Roff, 1992; Stearns, 1992; Roff & Fairbairn, 2007). A great contribution of life history theory to the study of sexual selection is the simple insight that all animals have a limited supply of resources from which to allocate to life history traits (Stearns, 1989). Given that resources are limited, all traits feature in a universal metabolic trade-off with other traits competing for the same resources, because investment of some proportion of the resource pool to any given trait necessarily decreases the availability of resources to all others (Roff, 1992; Stearns, 1992). Individuals that invest disproportionately in sexual traits should then suffer the consequences of having less to invest in other expensive life history traits. The existence of such trade-offs predicts negative correlations between each pair of life history traits; however, variation in acquisition can obviate the expected negative association between traits because some individuals have more to spend on both (Van Noordwijk & De Jong, 1986). Knowledge or manipulation of some feature of resource acquisition ability is therefore required in order to quantify how individuals allocate energy to competing life history traits.

In most species, measuring the reproductive effort of males presents a significant technical hurdle. Crickets have proven to be an ideal organism for this purpose: the main investment in reproductive effort by males is stridulation of their hardened forewings to produce a long-range calling song, which is energetically expensive (Ka-

vanagh, 1987; Hunt *et al.*, 2004a). Male calling can also attract acoustically orienting parasitoids (Cade, 1975) and predators (Walker, 1979). The amount of time spent calling (a male's 'calling effort') is directly related to the number of females he attracts, and is a strong predictor of mating success (Hunt *et al.*, 2004a; Bentsen *et al.*, 2006; Rodríguez-Muñoz *et al.*, 2010). Male reproductive investment can therefore be quantified accurately in the laboratory using a multi-channel event recorder that measures time spent calling (Bertram & Johnson, 1998).

A number of studies have tested the effects of variation in resource acquisition on male and female reproductive effort among several species of cricket. Because crickets can vary their calling with age, they are particularly useful for studying how life history trade-offs affect the ageing process. Resource based trade-offs between life history traits are of particular interest when males express their sexual trait on multiple occasions throughout life; in such cases, trade-offs occur not only between trait expression and survival, but also between current and future trait expression. Males might therefore be expected to trade off trait expression at some age in favour of greater investment in the trait at another (Roff, 1992; Stearns, 1992; Kokko, 1997, 1998). This problem is especially acute when the male trait is extremely phenotypically flexible, such as occurs in behavioural displays: if a male is free to alter his trait expression in dynamic fashion, to what extent does his phenotypic trait signal his underlying quality?

In my thesis, I use a combination of experimental manipulations, quantitative genet-

ics approaches and physiological assays to explore the complex interplay between an individual's acquisition of resources, the allocation of those resources to age-related sexual signalling, and the subsequent effects of these patterns on other life history traits. I will briefly outline each of the chapters below, but first describe in more detail the natural history of the study organism used for all of my empirical research.

Natural history of *Gryllodes sigillatus*

The study organism used throughout my thesis is the decorated cricket, *Gryllodes sigillatus* (F. Walker; Figure 1). This species is now widespread in tropical regions, having been most likely native to Asia (Walker, 2011). There is no overwintering stage, and generations are continuous. *G. sigillatus* often live in high density populations (Sakaluk, 1987); competition for mates is high and there is the opportunity for intense sexual selection. Males typically call to females from their burrows; calling intensity increases sharply after sunset, decreasing marginally thereafter until a rapid decrease near dawn (Sakaluk, 1987). Females travel much greater distances than do males, and often relocate to several different males during the course of the night (Sakaluk, 1987). Female *G. sigillatus* can incur costs of such activity: insectivorious Mediterranean house geckos, *Hemidactylus tursicus*, exhibit phonotaxis to male cricket calls and thus intercept (and consume) female crickets that are responding to males.

Figure 1: A male *Gryllodes sigillatus* attracts a female via stridulation of hardened forewings to produce a calling song.

Female *G. sigillatus* impose directional selection on male signalling (Ketola *et al.*, 2007), and appear to use a fixed internal threshold for determining male attractiveness (i.e., any male that passes a certain level of attractiveness will be mated)(Ivy & Sakaluk, 2007). Once a female mounts a male, he transfers a large spermatophore that attaches beneath the base of her ovipositor (Sakaluk, 1984, Figure 2). The spermatophore comprises a sperm ampulla, and a large gelatinous spermatophylax that contains no sperm (Sakaluk, 1984). Upon dismounting the male, the female removes and consumes the spermatophylax; the ampulla remains attached and transfers sperm to the

female's sperm storage organ. Once the female has consumed the spermatophylax, she removes the ampulla, so ending the process of sperm transfer. The spermatophylax takes an average of 40 mins to consume; larger spermatophores require more time for consumption, so increasing the period that the ampulla is attached to the female and the amount of sperm transferred (Sakaluk, 1984, 1985). Each spermatophore takes a substantial amount of time to construct (an average of 3.25 hours), although metabolic costs of their generation is uncertain (Will & Sakaluk, 1994; Sakaluk, 1985; Warwick *et al.*, 2009).

Figure 2: A female (above) *G. sigillatus* mounts a male (below). The gelatinous spermatophylax is clearly visible during copulation as the male transfers a spermatophore to his mate.

Female decorated crickets are highly polyandrous: the average mating frequency of females was 22 times over a 20-day period, while males averaged 10.9 matings in the same timeframe (Sakaluk, 1987). Multiple mating appears to improve female lifespan (Burpee & Sakaluk, 1993), and females that mate with several different males gain fitness benefits in terms of the proportion of offspring that survive to adulthood (Ivy & Sakaluk, 2005). The high remating rates of females decrease each individual male's fertilisation success (Sakaluk, 1986). The high remating rate of females and the costliness of mating for males (in terms of predation risk, the metabolic expense of spermatophore construction, and above all the time required to re-enter the mating pool) should all impose reasonably strong selection on males for careful investment in singing and mating activity. In my thesis, I first explore some of the theoretical issues covered in my thesis (Chapter 1), and then use decorated crickets as models to assess sex-specific investment in reproduction as a function of age (Chapter 2), genotype-by-environment interactions for age-dependent calling effort in males (Chapter 3), and metabolite acquisition and allocation over time as a function of diet and mate availability (Chapter 4).

1

Sexual Selection and Life History Allocation

Abstract

A comprehensive explanation for sexual trait diversity depends on integrating life history theory and sexual selection to compare costs and benefits of sexual traits. A universal cost for all sexual traits involves the resources required to create and maintain them, which are consequently unavailable to other life history characters. This resource trade-off typically causes covariance between an organism's resource budget and its level of sexual trait expression, which is known as condition-dependence. Condition-dependence has several implications. It may be particularly important for ornaments signalling genetic quality, by helping to maintain the genetic variation that favours mate choice. It may also reduce extinction risk in sexually selected populations. Further life history studies of sexually selected traits are required, mindful of the inherent difficulties in quantifying resource acquisition and allocation, not least of which is that these processes are not independent of one another.

1.1 Introduction

Sexual dimorphisms - those phenotypic differences between the sexes of a species - are among the most striking and fascinating traits in nature. Their sex-limited pattern of expression strongly suggests the operation of sexual selection, Darwin's (1871) proposed mechanism for the origin of secondary sexual characters. Much of sexual selection theory is focussed on estimating the fitness benefits for males of extraordinarily exaggerated sexual traits. Equally deserving of attention, and equally important for understanding diversity in sexual traits, are the potential fitness costs of investing in these traits. Undoubtedly, such costs play a role in the sex-limited expression that is the hallmark of sexually selected traits. Assessing variation in the relative importance of these costs is also essential for a convincing account of diversity in sexual traits, both within and among species.

To clarify the relative costs and benefits of investing in sexual traits, in this article we explore the intersection between sexual selection and life history theory: the biological discipline devoted to studying how individuals acquire and spend energy to increase fitness. We illustrate key concepts with a few examples from studies of insects; their diversity in size, morphology, behaviour, along with their relatively small size and short lifespans, make them ideal candidates for studying life history and sexual selection. However, the major themes of the article apply regardless of the taxon of interest.

We begin with some brief background information on life history theory, defining

terms and clarifying key concepts. We then explain the central role of life history trade-offs in the evolution of dimorphisms, and argue that sexual traits, especially those involved in mate choice, may be particularly prone to evolving a special relationship with an organism's resource budget. We explore some of the implications of this insight for research in sexual selection and some closely related disciplines. Finally, we close by discussing some of the main challenges to achieving further progress in the study of life history and sexual selection, and suggesting some directions that are likely to be fruitful in circumventing these.

1.2 A brief primer on life history trade-offs

Life history theory was developed to explain variation in traits associated with life tables (e.g., developmental rates, reproductive mode, offspring number, and offspring size), but it encompasses other traits - including secondary sex characters - that also covary closely with fitness. Theoretically, the best life history would feature very early and repeated reproduction, involving very many extremely large offspring, throughout an extremely long lifespan. Of course, an organism possessing all of these superfluous traits does not exist. Although each of these traits would clearly be favoured if it did not impose costs on others, in reality trait evolution is restricted by the existence of fitness trade-offs. The close associations of life history traits with fitness, and the persistent and important effect of trade-offs between them, are two key aspects that are crucial to understanding the evolution of sexually selected traits.

The study of trade-offs involves quantifying both costs and benefits of different levels of trait expression. These factors impose balancing or contrasting selection on these traits: although trait exaggeration is favoured in one context (by conferring fitness benefits, for example, through improved sexual attractiveness or increased competitive ability), it is disfavoured in another (for example, by increasing predation risk). The costs imposing the selection that opposes trait exaggeration can take several forms (see Box 1):

1. There can be direct negative consequences of expressing a trait (e.g., if the trait itself comes with survival or reproduction costs). In this case, we could formally describe the context-dependence of selection on the trait by examining differences in the covariance between the trait and fitness across different situations or life history stages.

2. The effect of the trait on one individual can differ from its effect in another (for example, if the expression of a trait has different effects on fitness for males and females, there can be contrasting selection across the sexes).

3. Even in the absence of direct costs to individuals, the exaggeration of costly traits can be constrained because resources used to express them are unavailable for other important life history functions that compete for the same resource pool.

Although there are many examples of the first two categories of costs, the third cat-

egory is especially interesting because it is universal: every expensive trait imposes an opportunity cost by depriving its bearer of the ability to invest more in other demanding life history characters. Whether greater expenditure on any individual trait is favoured in a particular situation depends on the relative marginal gains of investing a unit of resources in one trait as opposed to another. In the remainder of this article we focus on this particular kind of 'metabolic' trade-off, in which the chief cost is that which derives from the expenditure of limited resources on the exaggeration of an expensive trait.

Box 1 Three kinds of costs of increased trait expression.

- Male field crickets (Orthoptera: Gryllidae: *Teleogryllus oceanicus*) are targeted by females of the parasitic fly *Ormia ochracea* (Diptera: Tachinidae), which are attracted to cricket mating calls and deposit larvae that burrow into and consume their host (Cade, 1975).

- Although both male and female *Drosophila serrata* (Diptera: Drosophilidae) exhibit preference on the basis of the same signal trait (cuticular hydrocarbons, or CHCs), the form of sexual selection differs between the sexes (Chenoweth & Blows, 2005). While females exert a linear selection gradient on male CHC levels, the gradient for selection on female CHC levels is primarily nonlinear and convex. Effectively, alleles for particular levels of

CHCs have different fitness depending upon whether they are expressed in males or in females.

- In species with wing polymorphism, such as sand crickets *Gryllus firmus* (Orthoptera: Gryllidae), investment in costly flight muscles imposes a fecundity cost on females (Stirling *et al.*, 2001), and reduces calling effort (used for mate attraction) in males (Crnokrak & Roff, 2000). In both cases, the metabolic pathways that mediate these trade-offs have been quite well resolved.

1.2.1 Acquisition and allocation

It is useful to distinguish two fundamental processes at the heart of an individual's life history: its acquisition of resources, and its allocation of those resources to its many traits. Although these two concepts are centrally important, they are notoriously difficult to quantify precisely.

Acquisition (often called an organism's condition in the sexual selection literature) is an organism's ability to acquire and convert resources into metabolically useful forms; it represents the total budget for metabolic expenditure. Although this is an intuitive concept, it is empirically elusive because phenotypic traits available for assessment are all traits that have been constructed using resources (Tomkins *et al.*, 2004; Cotton *et al.*, 2004). Although many traits covary with the total budget, by definition

they also trade off metabolically with other important life history characters. This means that covariances between condition indices and life history traits need to be interpreted carefully.

Allocation refers to the pattern of investment of these resources in the production or maintenance of traits. Quantifying patterns of allocation is also a complex matter due to the sheer number of traits in which an individual may invest its acquired resources. In practice this can be simplified by considering a subset of traits and estimating the fraction of resources assigned to each, assuming expenditure can be estimated in a single currency. As an additional complication, the processes of acquisition and allocation are not independent of one another. Clearly, an individual's ability to acquire resources may depend on prior allocation to, for example, traits involved in gathering food. Such traits would, in turn, affect an individual's resource budget dramatically. Conversely, an allocation strategy may depend heavily on an organism's acquisition levels, because the fitness benefit provided by an additional unit of investment in a trait depends on that trait's current level of expression.

1.2.2 Detecting metabolic trade-offs

Superficially, the existence of metabolic tradeoffs predicts negative correlations between pairs of expensive life history traits. This is because individuals that overinvest in sexual traits should have less available for competing life history functions, such as immunity or survival, than rivals that invest more frugally in sexual characters.

In reality, even in the presence of a strong metabolic trade-off, the nature of the observed correlation between these characters need not be negative. The 'Y-model' (De Jong & Van Noordwijk, 1992) is an acquisition-allocation model that helps to explain why.

The Y-model illustrates how resources may be shared between two traits within an individual; whatever resources are used in expressing one of the traits is deducted from the total resource budget, leaving the residual fraction to be allocated to the second trait. If all members of a population have a fixed and equal level of resource acquisition, both the correlation and the covariance between the traits are negative as a consequence of the trade-off. However, variation between individuals in acquisition can cause the observed covariance between these traits to be positive (see Figure 1.1)(Van Noordwijk & De Jong, 1986). If, for example, most of the observed variation in trait expression is due to acquisition, and individuals vary little in allocation strategy, the phenotypic correlation between traits will be positive in spite of the fundamental trade-off between these traits (in fact, it is the means as well as the variances in acquisition and allocation that influence the nature of this correlation; see Roff & Fairbairn, 2007). The important implication of this insight is that phenotypic correlations are not necessarily good metrics for the strength or importance of a trade-off between traits.

In spite of the influence of the Y-model, the difficulties inherent in quantifying acquisition and allocation mean that it has rarely been robustly tested. However, there

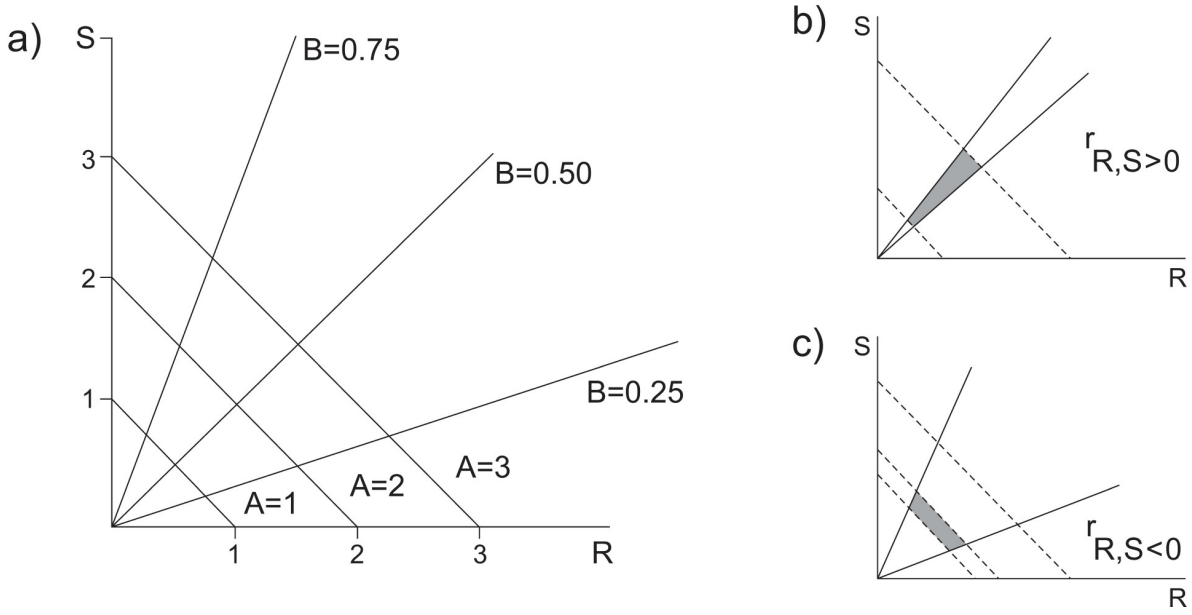

Figure 1.1: The Y-model: (a) The total energy, A, acquired by the individual, and the fraction, B, that it allocates to the life history trait R. The remaining fraction is allocated to the life history trait S. (b) Population wide variation in A is large and variation in B is small, leading to a positive phenotypic correlation between R and S (see hatched area). (c) The reverse case.

Figure reproduced from Van Noordwijk & De Jong (1986) with permission.

is strong support for its predictions from, for example, the well-studied trade-off between flight muscles and reproduction in the sand cricket Gryllus firmus, which has allowed explicit estimation of acquisition and allocation in units of energy (King *et al.*, 2011). Although these estimations were gained through the use of physiological methods, mechanistic analysis of life history tends not to use the Y-model, concentrating instead on the underlying pathways of physiological relationships and investigating the proximate mechanisms involved in resource allocation trade-offs (Leroi, 2001).

1.3 Secondary sexual characters

The theory of sexual selection is often broken into two main components: intrasexual competition between rivals for access to mates (often called male-male competition), and intersexual mate choice (often called female choice). These two forms of selection can favour the evolution of 'secondary sexual characters' in the form of armaments (that function to increase the competitiveness of an individual in intrasexual contests) and ornaments (that improve attractiveness to the opposite sex).

1.3.1 Life history theory and sexually selected weapons

Male competition for access to females has shaped the evolution of sex-dimorphic weaponry, both for fights against rivals and displays that intimidate potential opponents. Armaments occur in many species throughout the animal kingdom (reviewed in Emlen, 2008), and take many forms (Emlen *et al.*, 2005), yet are not typically targets for direct female choice (as Darwin, 1871, had suggested previously). Weapons may be used to dislodge rivals from mating sites, to pry them from females during copulations with rivals, or to trap females in mating burrows. Their importance in securing access to females raises the question of why individuals do not 'cheat' by investing in disproportionately large weapons.

Life history provides an obvious answer to this question: investment in one important trait means that resources are unavailable to other traits that also covary closely with fitness. Disproportionate investment in armaments therefore imposes direct metabolic costs to other functions. This is very similar to the phenomenon that maintains honesty in communicative signals (known as the handicap principle Zahavi, 1975). Briefly, signal honesty is maintained if a trait is costly, and if the marginal benefits of investing a trait depend on acquisition (e.g., if the cost of expressing a trait decrease with size, or conversely if the benefits increase with size).

1.3.2 Condition-dependence of ornaments

Unlike weapons, ornamental traits evolve not for the direct advantage they provide individuals in contests with rivals, but because the opposite sex prefers to mate with ornamented individuals. Why such preferences persist is a fascinating question; the benefits of mate choice vary greatly across species, and the relative importance of different models for the evolution of choice remains unclear. In many cases, ornaments appear to act as signals of the material benefits a male provides during courtship or mating. Males of the arctiid moth *Utetheisa ornatrix* (Lepidoptera: Arctiidae), for example, emit pheromones which females use in mate assessment (Dussourd *et al.*, 1991). These pheromones indicate the levels of protective alkaloids that a male will transfer to a female in his spermatophore, which she may then use to protect her eggs against predation.

In species where males do not provide any direct benefits, females nevertheless often choose males on the basis of ornaments. The most common explanation is that these ornaments reflect the male's genetic quality, which is the expected effect of a male's genetic contribution on the fitness of a female's offspring. Because ornaments are always signals communicating information across the sexes, the same mechanisms that prevent dishonesty in weapons and other signalling systems are important (see above).

In contrast to other kinds of signals, however, those that are favoured through strong mate choice for indirect genetic benefits face a special problem: maintaining genetic variation in ornament expression. This problem is known as the paradox of the lek, because it was originally articulated in trying to account for persistent strong choice in 'lekking' aggregations of birds in which females derive no apparent direct benefits, but still exercise strong choice. If females strongly prefer ornamented males advertising heritable differences, then this strong choice should eventually deplete the genetic variation between males that is associated with the ornamental trait. This in turn should undermine the benefits of choice, as these depend on genetic variation for the ornamental trait.

1.3.3 A life history solution to the lek paradox

One proposed mechanism for maintaining variation in ornamental traits is that they evolve a close association with resource acquisition, called condition-dependence

(Rowe & Houle, 1996). Higher quality males should acquire more resources, and should therefore be able to invest more in the ornament than lower quality rivals.

If sexually selected traits are condition-dependent, any mutation affecting a male's ability to acquire resources (including his ability to fight disease, catch prey, forage or metabolise nutrients effectively) would have some effect on ornaments. Consequently, to the extent that sexual traits are condition-dependent, they reflect the efficiency of virtually all of an organism's functions, and thereby the quality of virtually the entire genome. This forms the basis of the genic capture model: the involvement of so many loci (i.e., virtually all of them) helps preserve variation because it represents so many alleles in the first place (variation in which cannot easily be eroded even through strong choice), and because these loci provide a large target for mutations even at relatively low mutation rates (Rowe & Houle, 1996). Furthermore, the extremely polygenic nature of condition increases the possibility for epistatic interactions between loci and genotype-environment interactions that cause balancing selection, both processes that help sustain genetic variation in the face of strong selection via female choice.

1.4 Implications of condition-dependence

1.4.1 Allometry

Allometry is the study of relative growth patterns, and typically involves estimating a trait's level of exaggeration relative to another reference trait (Shingleton *et al.*, 2007). This relationship, when measured in individuals of the same population at a given developmental stage, is known as static allometry. If the ratio of exaggerated trait to reference trait remains constant, i.e., each unit increase in the reference trait returns a proportional increase in the target trait, the relationship is isometric. Positive allometry refers to disproportionately large increases in the exaggerated trait as the reference trait increases. Conversely, negative allometry indicates disproportionately small increases in focal trait relative to the reference trait.

Allometric relationships are clearly relevant to the study of sexual characters because conspicuous ornaments or armaments involve a redirection of resources towards the sexual trait and away from other aspects of the phenotype. Furthermore, the condition-dependent solution to the lek paradox explicitly invokes a correspondence between resource acquisition and trait expression that should be observable in allometric relationships, provided the reference trait covaries closely with resource acquisition (see Box 2). Much of the classic literature in sexual selection implies that sexually selected traits should exhibit positive allometry, because traits directly involved in sexual competition might be selected more strongly than body size itself. In fact, this expectation is not universally supported by theory or empirical reviews

(Bonduriansky & Day, 2003; Bonduriansky, 2007). Life history theory reveals why this prediction is perhaps too simplistic: it is not the intensity of selection on a trait alone that ought to affect the scaling relationship, but the change in intensity as a function of resource acquisition. If the benefits of investing in a sexual trait change with the level of resource acquisition, then the allometric relationship will deviate from isometry (to positive allometry if the marginal benefits of trait investment increase with size, and to negative allometry if they decrease). If the optimal allocation pattern does not depend on acquisition, then traits should exhibit isometry.

Box 2 Case study: armament evolution in dung beetles.

Beetles of the genus *Onthophagus* (Coleoptera: Scarabaeidae) suffer different functional costs of investment in exaggerated horns on the head or thorax (Emlen, 2001). These horns are used in competitive contests with rivals for access to females preparing brood balls from fresh dung. Large males possessing large horns are much more likely to win these contests, which confers clear benefits to males that assign resources to producing horns. In many *Onthophagus* species, only the largest 'major' males express horns, while smaller 'minor' males produce no discernible horns at all, and resemble females rather than producing proportionally smaller horns similar to those of major males. While major males fight rivals and guard their mates, minors attempt to 'sneak' copulations with females. The shift in allocation to horns closely matches the relative payoffs of

each mating strategy based on the acquisition level of the individual in question (Hunt & Simmons, 2001). It is also understandable in the context of life history theory, because horns are metabolically expensive, and trade-off with other morphological structures including eyes, wings and antennae (Emlen, 2001). This was illustrated by an ingenious experiment on *O. nigriventis* males that manipulated investment in horns by cauterising the cells associated with thoracic horn development at the larval stage (Simmons & Emlen, 2006). Cauterised males grew larger, but also developed disproportionately large testes, revealing the metabolic trade-off between horn expression and both testis size and body size (see Figure 1.2).

Onthophagus species with polymorphic males often exhibit other changes in the physiology and behaviour of the resource-poor minor males. Sperm competition theory predicts that investment in sperm should increase with the frequency of males employing sneak tactics, or the probability of a sneak mating occurring (Parker, 1990). Males with a lower mating probability should pursue a strategy that increases the likelihood of successful fertilisation. In *Onthophagus*, not only does testes size increase with the proportion of sneak males (and, extrapolating from this, the likelihood of sneak mating), but minor males invest more resources in testes than do major males (Simmons *et al.*, 2007).

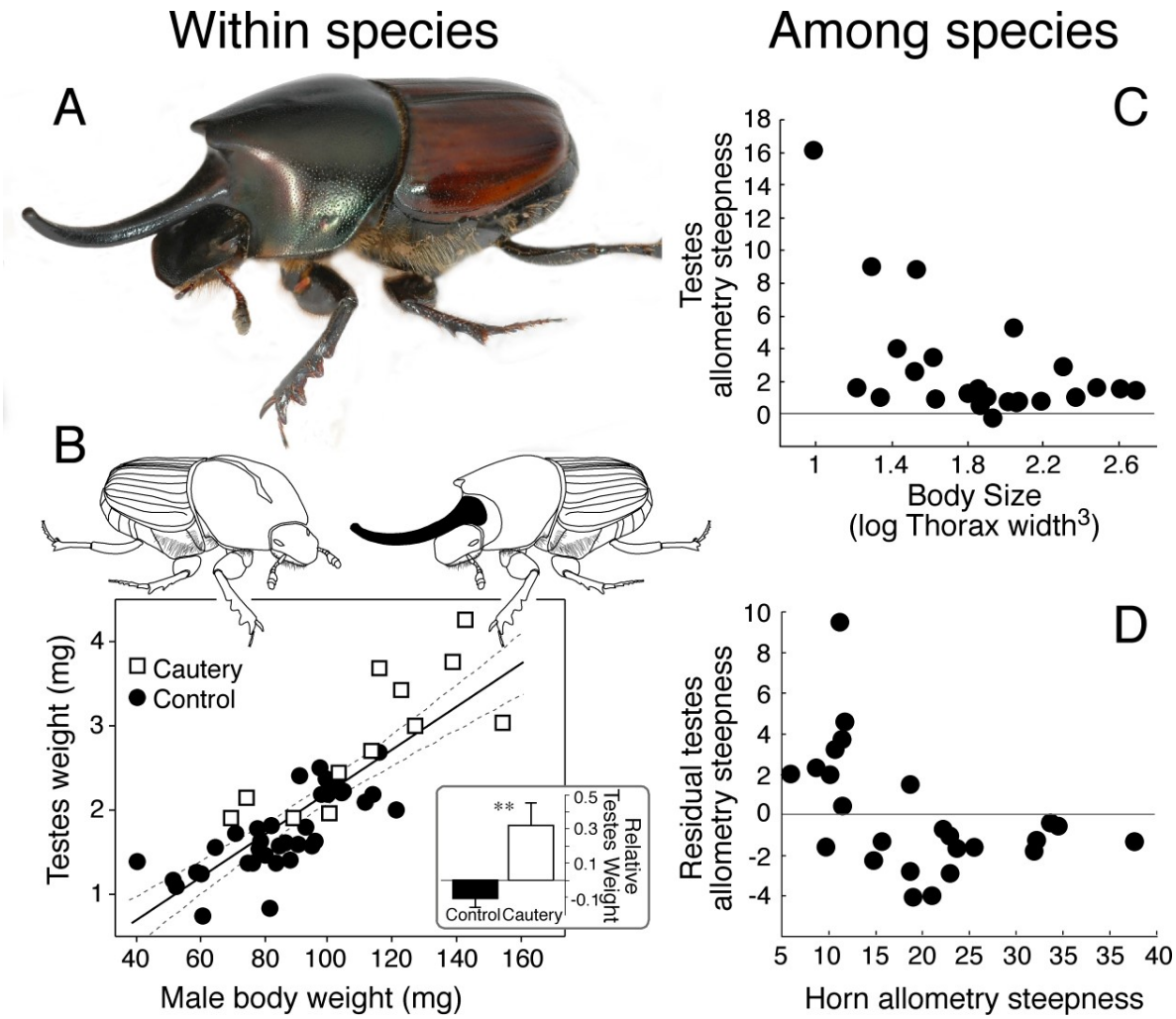

Figure 1.2: An allocation trade-off exists between weapons (thoracic horns) and testes in the beetle *Onthophagus nigriventis*. (A) Large males produce two horns on the thorax. (B) Cauterised males grew larger and invested disproportionately in testes compared to horned males. (Inset) Residual testes weight for cauterised and control males after controlling for body weight. (C,D) Analysis of 25 congeneric species shows that allometric slope steepness for log testes weight against log body weight declines as both body size (C) and horn allometry steepness (D) increases. These results indicate the decreasing marginal benefits of investment in testes with increasing horn and body size, presumably because larger males can better monopolize their mates and avoid sperm competition altogether.

Figure reproduced from Simmons & Emlen (2006) with permission.

1.4.2 Sexual selection and population fitness

In addition to the cost of sex itself, sexual selection was once thought to decrease the mean fitness of populations because it causes individuals to divert resources away from offspring production towards costly ornaments and armaments that contribute nothing to population growth, and in many cases are associated with direct fitness costs (see Box 1). However, if mutations have a more severe effect on male fitness because their deleterious effects disproportionately decrease performance in sexual competition, the resulting population-wide decrease in mutation load can theoretically overcome the cost of sex (Siller, 2001; Agrawal, 2001). The condition dependence of sexual traits can further benefit populations by increasing the adaptation rates of populations exposed to changing conditions (Lorch *et al.*, 2003). In fluctuating environmental conditions, genic capture effectively increases the intensity of environmental selection for resource acquisition through sexual selection on males. This has two advantages in the context of population fitness. First, if males do not contribute substantially to population growth, then strong selection on males can accelerate adaptation to new conditions without any substantial negative population consequences. Furthermore, condition-dependence allows remarkable flexibility in the trait combinations under selection: the alleles that covary most strongly with resource acquisition in the most recent generation are those favoured by sexual selection. Simple female preferences for exaggerated male traits can thereby select recently successful alleles even in environments that experience rapidly changing conditions. Whether this expectation helps explain the prevalence of sexual reproduction remains unclear.

1.4.3 Acquisition and allocation in sex-role reversed systems

While most mating systems feature intrasexual competition between males and intersexual selection via female choice, in some species females compete for male mates, and males exercise strong mate choice. Such sex-role reversed species present fascinating opportunities to study the evolution of secondary sex characters, because the nature of life history costs and benefits of sexually selected traits should vary considerably across the sexes. For one thing, whereas males often provide very little material benefits to females, females always provide energetically expensive commodities to males in the form of eggs. This means that issues relating to indirect genetic benefits are unlikely be as important in role-reversed species as they are for those with conventional sex roles. Furthermore, the role of females in egg production means that trade-offs between eggs and sexually selected traits may be more constraining in sex-role reversed species. In fact, the rarity of female ornaments (found in only a few of the many sex-role reversed animals known to science) may reflect the constraining role of trade-offs on ornament evolution in females (Fitzpatrick *et al.*, 1995). Not only does diverting resources from eggs to ornamentation potentially reduce female fecundity, it may also undercut direct benefits to choosy males, both by reducing the fecundity of their mates and increasing sperm competition if attractive females mate more often. The few species that do feature ornaments are intriguing subjects for study because they obviously represent exceptions to this rule (see Box 3). The exact causes allowing these exceptions remain unknown.

Box 3 Case study: deceptive female ornamentation in a sex-role reversed dance fly

In many dance flies (Diptera: Empididae), females receive a 'nuptial gift' in the form of a small prey item from males prior to mating, which they feed on during copulation. Males who offer larger gifts should be in greater demand by females, and thus can afford to be more exacting in their choice of partner. In some species such as *Rhamphomyia longicauda*, females have developed elaborate ornamentation, including inflatable abdominal sacs and pennate leg scales that appear to exaggerate body size during mating swarms, increasing their attractiveness (Funk & Tallamy, 2000). These ornaments have direct costs through increased predation (Gwynne *et al.*, 2007), but they may also have important, if difficult to estimate, metabolic costs. Although the population-wide covariance between ornamentation and fecundity is positive, the strength of this correlation is weaker in *R. longicauda* than in other species with conventional sex roles and no female ornamentation (see Figure 1.3)(Funk & Tallamy, 2000). One possible cause for the weaker covariance between the ornamentation and fecundity is the metabolic trade-off between ornaments and eggs. This trade-off nevertheless does not undermine mate choice in this system, perhaps because males cannot easily evaluate female size in the low-light swarming conditions during which pairing takes place.

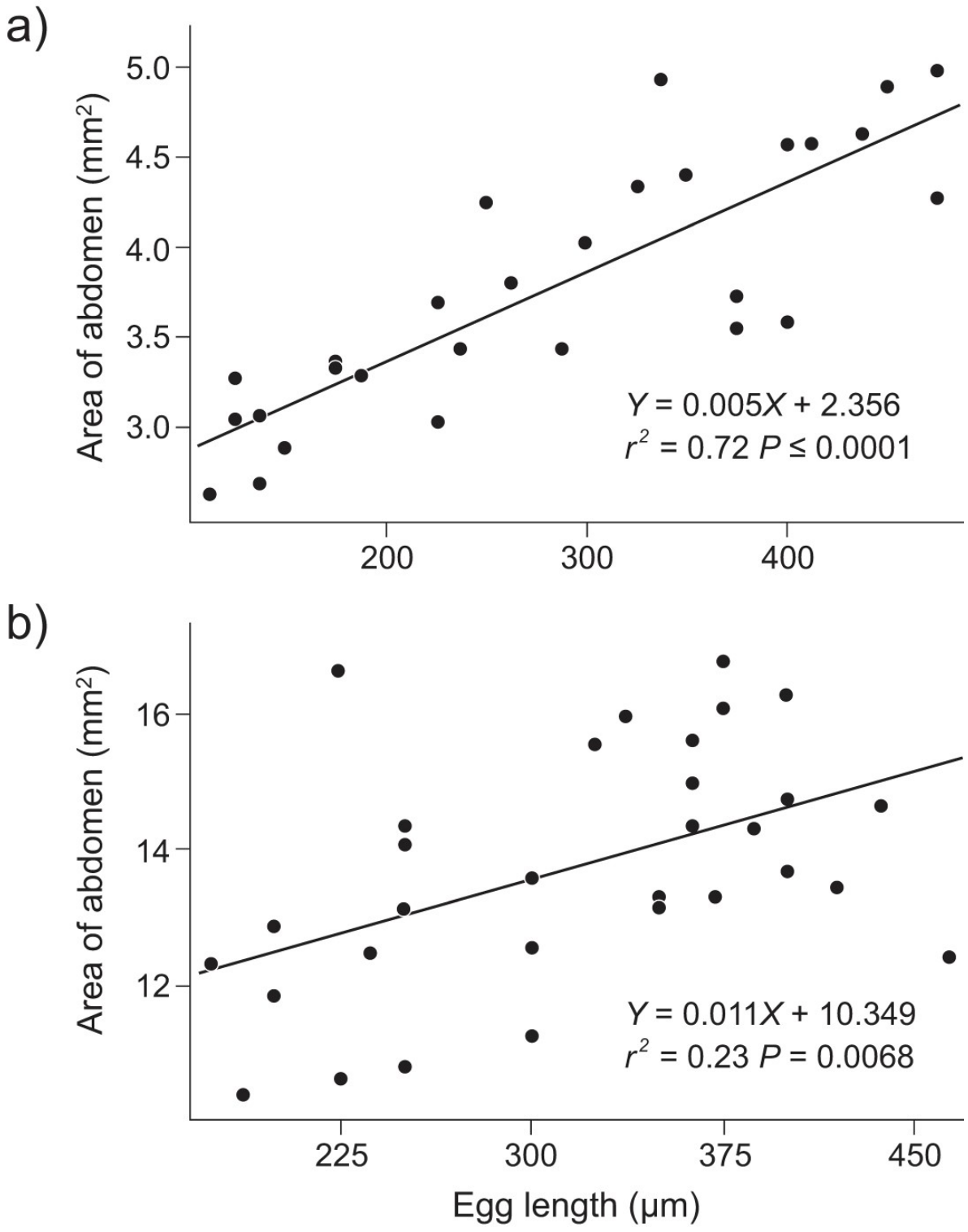

Figure 1.3: Male *Rhamphomyia* dance flies prefer females with larger, more mature eggs. The effect of egg length on abdominal area in females of (a) *R. sociabilis*, a species in which females cannot inflate their abdomens, reveals a strong positive relationship. In *R. longicauda* (b), abdominal area is a poorer indicator of egg length. Females of this species inflate their abdomens by swallowing air before entering the mating swarm to exaggerate their apparent size.

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1.5 Future challenges in the study of life history and sexual selection

Perhaps the most glaring challenge for this field is that the two concepts central to describing a life history, acquisition and allocation, cannot easily be measured. Because acquisition represents the resource budget before it is assigned to any traits, by definition any candidate index of acquisition will itself be a life history trait, and thus has the potential to trade off with other traits, including the sexual character of interest. In some cases it may nevertheless be possible to find indices that covary closely with total resource budget (e.g., the mass of pupae for insects such as lesser waxmoths, *Achroia grisella* (Lepidoptera: Pyralidae), which do not feed as adults). However, a more general solution to this problem is that any individual acquisition index should be regarded cautiously. Allocation presents its own barriers to assessment. It would be impossible to measure the allocation of energy to all characters and behaviours, and even were such a procedure attempted, comparing resource expenditure across traits is difficult because of differences in measurement units across traits. How can one compare gains in mass with gains in body size or longevity? Obviously, each of these measurements can help improve our understanding of selection on life history, but a comprehensive analysis of selection on life history will require a multivariate approach that simultaneously assesses interactions between traits. Such an approach is practically and analytically demanding, however, and much more work is needed to clarify effective methods for estimating multivariate interactions and comparing or combining different fitness episodes (see Hunt *et al.*, 2009). The integration of evo-

lutionary and molecular approaches may also yield dividends. While the study of the mechanisms underlying trade-offs has thus far concentrated on the relationship between reproduction and aging (Flatt & Schmidt, 2009), such an approach could also be used in the investigation of secondary sexual characters.

Perhaps an even more daunting challenge is that acquisition and allocation are not independent properties. The interdependence of resource budgets and allocation strategies is an extremely exciting, if somewhat intimidating avenue for future research. As individuals can effectively only express a single acquisition level and allocation strategy, the relationship between these two parameters is an emergent property of a genotype that has been reared in several different conditions, and can only be characterised for large groups of individuals. Selection on the interdependence of acquisition and allocation therefore depends on the cumulative effects of selection on individuals, each of which expresses only a single point on a reaction norm describing this interdependence. Studies of the evolution of phenotypic plasticity have begun to reveal the fascinating interplay between acquisition and sexual trait expression in several species, but many more examples are needed to clarify the important role of selection on life history in the tremendous diversity of sexual traits observed in nature.

2

Sex differences in the effects of juvenile and adult diet on age-dependent reproductive effort

Abstract

Sexual selection is predicted to cause differences between the sexes in optimal patterns of allocation of acquired resources. When individuals face allocation trade-offs between current and future reproductive effort, variation in resource acquisition might further result in sex differences in how investment changes with age, or in the sensitivity of the sexes to changes in resource availability over time. However, the nature and prevalence of sexual differences in age-dependent investment remain unclear . I manipulated resource acquisition at both juvenile and adult stages in male and female decorated crickets, *Gryllodes sigillatus*, and assessed whether this affected sex-specific patterns of resource allocation to age-dependent reproductive effort (energetically costly signalling in males, and fecundity in females) and longevity. Age-dependent reproductive effort in females was highly divergent across diet treatments, and appeared constrained by the gathering of resources and the time required to convert them to useful forms. Females of intermediate lifespan had the highest total fecundity, while males did not appear to prioritise reproductive effort over lifespan: long-lived males always signalled more than short-lived males. Contrary to predictions, patterns of male age-dependent reproductive effort did not differ greatly across diet treatments. Males in all treatments showed positive covariance between reproductive effort and lifespan, while diet treatment altered the relationship between these life history traits in females, reflecting differences between the sexes in

the potential benefits of allocation to longevity. My results are consistent with sex-specific selection pressures on patterns of allocation, but also suggest a less adaptive hypothesis: males may use feedback from the social environment to make allocation decisions, and the absence of such feedback means that resources are preferentially stored as energetic reserves. Patterns of calling effort could represent males sampling the environment, with the universal increase in calling effort with time being caused by gradual resource accumulation, heightened mortality risk over time, and a lack of feedback from available mates.

2.1 Introduction

An organism's total available resource budget for allocation to life history traits depends on its ability to acquire and convert resources to metabolically useful forms (its 'condition', *sensu* Rowe & Houle, 1996). Individuals invest resources in particular life history traits - such as current reproduction or somatic maintenance - to gain fitness benefits (De Jong & Van Noordwijk, 1992). However, an inherent cost of any such investment is the reduced availability of resources to other fitness-related traits, which results in trade-offs (Stearns, 1992). The allocation of resources to current and future reproduction is thought to be shaped by age-specific selection to optimise lifetime fitness, which is a function of early reproductive rate, the pattern of change in reproductive rate with age, and lifespan (Bonduriansky *et al.*, 2008).

Sexual selection is predicted to affect investment in life history traits for males, and consequently the optimal pattern of resource allocation to life history traits is likely to differ between the sexes (Promislow, 2003; Graves, 2007; Bonduriansky *et al.*, 2008). The limiting factors for achieving reproductive success are particularly sex-specific, with females typically constrained by the resources and time requirements of constructing eggs, while male mating success is mediated more by competition with other males for access to females (Bateman, 1948; Trivers, 1972; Andersson, 1994). Females should generally adopt fairly low-risk strategies of resource investment, and engage in less risky behaviour than males (Wedell *et al.*, 2006). In many species, fathers invest less in each offspring than do mothers, in part so that resources may be redirected towards acquiring additional mates (Trivers, 1972). Reproductive effort

is predicted to increase with age (because reproduction is increasingly the best option for resource investment when the risk of mortality from intrinsic sources grows, Kirkwood, 1977), and males may also sacrifice longevity in favour of increased allocation to current reproductive effort (Vinogradov, 1998). Theoretical models have demonstrated circumstances in which high-quality males invest disproportionately in costly sexual traits to the extent that they suffer lower survival rates than lower-quality males (Hansen & Price, 1995; Kokko, 1997; Höglund & Sheldon, 1998; Kokko, 1998; Kokko *et al.*, 2002).

In spite of the above-mentioned theory, a trade-off between reproductive effort and lifespan in males is not often discernible in empirical data: most studies report that preferred males also survive the longest (Jennions *et al.*, 2001) (but see also Hunt *et al.*, 2004a; Robinson *et al.*, 2006). One possible explanation is that differences in resource acquisition mask this trade-off within populations, because males with high levels of resource acquisition invest heavily in both reproduction and survival (Van Noordwijk & De Jong, 1986; Reznick *et al.*, 2000; Roff & Fairbairn, 2007). In addition to masking trade-offs, variation in acquisition within populations can also complicate life history studies by affecting the relative payoffs of different life history traits (Stearns, 1992). Because the marginal value of investment in a given trait is likely to change as a function of investment, the relationship between investment and marginal value also probably differs across traits.

Another reason that quantifying allocation to reproduction is complex is that prior

allocation (e.g., during the juvenile stage) to traits involved in gathering or processing food will surely affect an individual's current resource acquisition ability. Environmental effects encountered during juvenile development could, therefore, have long-term repercussions (Metcalfe & Monaghan, 2001). These may range from the 'silver spoon' effect, where individuals born into good environmental conditions always have fitness advantages as adults (Grafen, 1988), to 'environment-matching', where adaptive developmental plasticity shapes the phenotype according to current environmental conditions (Monaghan, 2008). For example, if the adult environment is characterised by poor nutritional quality, the best performing phenotype should be that which is produced by similar poor-quality conditions during the juvenile stage, and not that which is produced in high-quality juvenile conditions. However, because resource acquisition and allocation during development can affect adult acquisition and allocation, disentangling the effects of diet manipulations at specific life stages can be difficult (e.g. Scheuber *et al.*, 2003). Only by manipulating diet across life stages is it possible to examine the relationship between juvenile and adult acquisition and allocation explicitly, and assign the effects on investment in fitness-related traits to their sources precisely (Whattam & Bertram, 2011). As noted above, males and females optimise fitness in different ways, thus nutritional requirements are also likely to be sex-specific (Maklakov *et al.*, 2008). Applying juvenile and adult diet manipulations to both sexes can therefore help assess whether differences in sexual selection have led to divergence across the sexes in allocation strategies.

Crickets are ideal organisms for studying sex-specific patterns of survival and repro-

ductive investment, primarily because it is possible to measure investment in reproductive effort in both sexes in an age-dependent manner. Male crickets produce a long-range calling song through stridulation of their wings, which is energetically costly (Kavanagh, 1987; Hunt *et al.*, 2004a), and a male's 'calling effort' is a strong predictor of mating success (Hunt *et al.*, 2004a; Bentsen *et al.*, 2006; Rodríguez-Muñoz *et al.*, 2010). Female reproductive effort can be quantified by counting the number of eggs produced (Zajitschek *et al.*, 2007). The costs of reproductive effort are likely to differ between the sexes, and recent studies using several cricket species have shown that males and females exhibit dramatically different patterns of reproductive ageing (Maklakov *et al.*, 2009; Zajitschek *et al.*, 2009b; Archer *et al.*, 2012; Zajitschek *et al.*, 2012).

The relationship between diet , reproductive effort and ageing in crickets appears complex and depends on sex and context; for example, in the black field cricket, *Teleogryllus commodus*, males fed a nutrient-rich 'high-quality' diet (characterised by high relative protein content) produced higher levels of calling effort than those fed a 'low-quality' diet, but died at a younger age (Hunt *et al.*, 2004a). The reduced longevity of high-quality males was also associated with increased propensity to call in early adulthood, as well as increased nightly calling effort. A similar study on the fall field cricket, *Gryllus pennsylvanicus*, revealed very different patterns of life history allocation; males on high-quality diets survived longer and called more than those fed a low-quality diet (Judge *et al.*, 2008). Male *G. pennsylvanicus* fed a high-quality diet increased their nightly calling effort over time, although they suffered reproductive senescence in late life; this pattern of increasing calling effort until later

ages is widespread in studies of age-dependent calling in male crickets (Maklakov *et al.*, 2009; Zajitschek *et al.*, 2009b; Archer *et al.*, 2012). The nutrient requirements for optimising fitness in crickets also appear to be sex-specific, with increased carbohydrate intake leading to higher calling effort in males, while increased protein intake augmented egg production in females (Maklakov *et al.*, 2008). Unlike males, patterns of age-dependent reproductive effort in female crickets appear to be less dependent on diet: female fecundity typically peaks early in adulthood and then declines (Zajitschek *et al.*, 2009b; Archer *et al.*, 2012), and this pattern appears to hold irrespective of nutritional manipulations (Maklakov *et al.*, 2009; Zajitschek *et al.*, 2009b).

Zajitschek *et al.* (2009b) provided further evidence of sex-specificity in patterns of age-dependent reproductive investment and mortality in *T. commodus*; while male calling increased or reached a plateau with age, females exhibited reproductive senescence (a decline in fecundity with age). Zajitschek *et al.* (2009b) manipulated diet across both juvenile and adult stages, but found no effect of diet treatment upon male longevity, mortality rates, or age-dependent reproductive effort; furthermore, while calling effort increased with age, the rate of increase did not differ between treatment groups. Adult female longevity was actually decreased by the consumption of the nutrient-rich diet during adulthood, with no evidence of a reproduction-longevity trade-off. The lower lifespan of adult females on the nutrient-rich diet might instead be explained by negative effects of excess protein in this diet (Lee *et al.*, 2008; Maklakov *et al.*, 2008). Overconsumption of the nutrient-poor diet to reach a protein target might have increased intake of another nutrient with different survival effects: Maklakov

et al. (2008) showed that lifespan and reproductive effort are maximised by different regions of the nutritional landscape. Alternatively, females on the nutrient-poor diet may have diverted resources to extending longevity while accumulating protein required for making eggs.

Among male *T. commodus*, Zajitschek *et al.* (2009b) found a positive diet-independent relationship between total calling effort and lifespan. While females might invest preferentially in enhanced survival because of resource and time constraints on egg production, the opposite pattern could be expected among males (Kokko, 1998; Vinogradov, 1998): indeed, another study using animals descended from the same wild population of *T. commodus*, with qualitatively similar lifetime high- and low-quality diet treatments, found that distinct diet-dependent allocation strategies exist among male crickets (Hunt *et al.*, 2004a). A quantitative genetics study showed that longevity and both mean and total calling effort are positively genetically correlated in male *T. commodus* (Zajitschek *et al.*, 2007), yet selection experiments also found that antagonistic pleiotropy between calling effort and longevity can occur under some environmental conditions (Hunt *et al.*, 2006).

A strong positive genetic correlation between longevity and reproductive effort was also found in males of a related species, the decorated cricket *Gryllodes sigillatus* (Archer *et al.*, 2012). Archer *et al.* (2012) also provided evidence that sexual selection has played an important role in sexually dimorphic investment in reproductive effort across their lifetime. Further work is needed to investigate whether investment pat-

terns are subject to change depending on individual condition. Through measurement of how both male and female *G. sigillatus* allocate resources when acquisition is varied, I explore how selection has shaped optimal life history allocation strategies in both sexes. As inbred genetic lines of this species have been developed by Ivy *et al.* (2005), my study is partly motivated by the need for phenotypic data analogous to those of the studies mentioned above, but in a species that will later permit dissection of the genetic questions.

In this study, I investigate the effect of juvenile and adult diet on patterns of allocation to life history traits in both sexes of the decorated cricket *G. sigillatus*. I manipulate nutrient content to vary resource acquisition among males and females at both the juvenile and adult stages, and examine the consequences for allocation to development, reproductive effort and survival. I selected diets that contained high or low levels of a range of nutrients, particularly protein, and that were qualitatively similar to those used in previous life history studies with crickets (Hunt *et al.*, 2004a; Judge *et al.*, 2008; Zajitschek *et al.*, 2009b). For both sexes, I predicted that nutrient-rich diets at the juvenile stage would result in individuals developing to adulthood at larger size, and at a faster rate. However, I expected to see divergence between male and female patterns of investment in reproductive effort at adulthood, as male calling effort is probably under strong sexual selection while female reproduction should be shaped more by natural selection (Bonduriansky *et al.*, 2008). I predicted that females would invest in moderate strategies, with those on nutrient-rich adult diets allocating their greater resources evenly to increase both fecundity and longevity. Fecundity in

female crickets tends to peak at early ages (e.g. Maklakov *et al.*, 2009; Zajitschek *et al.*, 2009b); given that reproductive effort in females should be limited by protein acquisition and metabolism, we expected that females fed a nutrient-poor diet as adults might peak later than nutrient-rich diet females. All males were predicted to increase rates of calling effort with age; I expected that the gradient of increase in calling effort be steeper for males on the nutrient-rich adult diet, as the cost of increased investment in sexual traits should be lower in individuals in higher condition (Rowe & Houle, 1996). However, given that male reproductive success is limited by competition with other males for access to females, I also anticipated that nutrient-rich adult males might sacrifice longevity for increased early reproductive effort. I also tested for 'silver spoon' effects or 'environment-matching' by exploring whether individuals assigned to the nutrient-rich juvenile diet outperform those in the nutrient-poor group regardless of adult diet, or whether those raised on a nutrient-poor juvenile diet are superior when both groups are assigned to the nutrient-poor diet treatment as adults.

2.2 Methods

2.2.1 Cricket maintenance

Gryllodes sigillatus used in this study were descended from 500 adult crickets collected in Las Cruces, New Mexico in 2001, and used to initiate a laboratory culture maintained at a population size of approximately 5000 crickets and allowed to breed pan-

mictically (Ivy & Sakaluk, 2005). New genetic material was introduced periodically from cultures at other institutions. Crickets were housed in a 15-litre plastic container in an environmental chamber maintained at $32 \pm 1^{\circ}$C on a 14:10 hours light/dark cycle. Crickets were provided with a standard diet of ground dry cat food (Friskies Go-Cat Senior®, Purina, London, UK) and water in 60ml plastic test tubes plugged with cotton wool ad libitum, in addition to egg cartons for shelter. As soon as adults were detected, moistened cotton wool was provided in a petri dish (10 cm diameter) as an oviposition substrate. Each generation was maintained at a density of approximately 300 crickets per container.

2.2.2 Experimental design

Two different diet treatments were used to manipulate resource acquisition in experimental subjects. These diets were defined by high and low levels of a range of nutrients, and were selected on the basis of similar diets used in previous studies (Hunt *et al.*, 2004a; Judge *et al.*, 2008; Zajitschek *et al.*, 2009b). The high-nutrient, protein-rich diet (H) consisted of 100% fine-ground dry cat food (Purina, 32% protein), the standard diet on which our laboratory stocks are maintained. The low-nutrient, protein-poor diet (L) was a mixture comprising 50% fine-ground dry cat food and 50% fine-ground oatmeal (Aberfeldy Oatmeal, 11% protein).

I collected 360 cricket nymphs within 24 hours of hatching from eggs harvested from laboratory stock cultures. Nymphs were assigned randomly to either diet treatment

(N=180 for each juvenile diet treatment), and reared individually in clear plastic containers ($5 \times 5 \times 5$cm), which contained a piece of egg carton as shelter, a water bottle plugged with cotton wool, and the food treatment *ad libitum*. Containers were cleaned each week, and fresh water and food provided.

Individuals were checked for eclosion to adulthood daily from the fifth larval instar onward; on the day of eclosion, I measured body weight using a high precision electronic balance (Denver Instrument, model PI-225DA), and photographed individuals through a microscope (Motic, model SMZ-168 equipped with Moticam 2000). Measurements of pronotum length were obtained from these photographs using NIH ImageJ software (Schneider *et al.*, 2012). I calculated body condition at eclosion for each cricket using the scaled mass index (SMI) (Peig & Green, 2009, 2010):

$$SMI = M_i[L_0/L_i]^{b_{SMA}}$$

where M_i and L_i are the body mass and linear body measurement (pronotum length) of individual i respectively, L_0 is the population mean for L, and b_{SMA} is the scaling exponent estimated by the standardised major axis (SMA) regression of ln$M$ on ln$L$ (using R package lmodel2 (Legendre, 2013)). I calculated the mean and scaling exponent separately for males and females.

I assigned each newly-eclosed individual at random to an adult diet, resulting in 4 lifetime diet treatment groups: HH, HL, LH, LL. Individuals in treatment groups

HH and LL were maintained on the same diet at both juvenile and adult life stages; those in treatment groups HL and LH were switched to the alternate diet following eclosion to adulthood. Adults were maintained in the same manner as nymphs, and were checked daily for survival.

2.2.3 Male reproductive effort

I quantified male reproductive effort as his 'calling effort', the duration of time (in seconds) that each individual spent broadcasting his long-distance sexual advertisement call. I measured each male cricket overnight for 12 hours every seven days, beginning at seven days post-eclosion, until death. On the day of measurement, I replaced the lid of each male's container with a lid in which a microphone (C1163, Dick Smith Electronics) was mounted; I then placed each container into a hollowed-out cube of sound-proofing foam so as to minimise outside disturbance and ensure there was no crosstalk between containers. An electronic acoustic recording system (Bertram and Johnson 1998) sampled from the microphone of each individual cricket container 10 times per second to determine whether or not a male was calling.

2.2.4 Female reproductive effort

I quantified female reproductive effort as the number of eggs produced in a four-week period after mating. I gave each female the opportunity to mate seven days after eclosion, by housing her with a single random stock male overnight. Mated females were

provided with moist cheesecloth as a substrate for oviposition immediately after being separated from the male. I collected eggs and provided new cheesecloth every seven days, beginning at seven days after mating, for a total of four weeks.

2.2.5 Statistical analysis

I performed all statistical analyses using R 3.0.2 (R Core Team, 2013). For generalised linear models (GLMs) and generalised linear mixed models (GLMMs), categorical input variables such as sex, juvenile diet and adult diet were coded using binary dummy variables to aid interpretation of standardised coefficients (Gelman & Hill, 2007). Numeric input variables such as age or lifespan were standardised by centring (subtracting the mean) and scaling (dividing by 2 standard deviations), putting them on a common scale with each other and with the binary predictors, and aiding the interpretation of main effects (Gelman & Hill, 2007; Gelman, 2008; Schielzeth, 2010). Independence between linear and quadratic forms of numeric predictors (e.g., age and age^2, lifespan and lifespan^2) was achieved by standardising the input variable before squaring (Gelman & Hill, 2007).

Unless otherwise stated, model simplification was performed by dropping non-significant terms from the full model sequentially, and using an F test (lifespan) or chi-squared test (survival, lifetime reproductive effort) to compare the new model with the previous one. I retained more complex models whenever simplification resulted in a significant increase in model deviance.

Adult lifespan, survival, and lifetime reproductive effort

I used analyses of variance to test for differences in lifespan between the sexes and treatment groups. To explore differences in age-dependent mortality that might not affect average longevities, I also used Cox regression survival analysis to compare separate survival curves for different combinations of sex and treatment group.

For males, lifetime reproductive effort was the sum of all measurements of their nightly calling effort; for females, it was the total number of eggs laid within the 4-week period after mating. I estimated the effects of diet treatment on lifetime reproductive effort separately for each sex. Both male and female lifetime reproductive effort measurements produced over-dispersed count data, and so were fitted using separate generalised linear models with negative binomial error distributions in the R package MASS (Venables & Ripley, 2002). Juvenile and adult diet treatment and their interaction were included as fixed effects in each model; standardised lifespan was added as both a linear and quadratic term. The full models also included interactions between the lifespan covariates and each of the fixed effects (juvenile diet, adult diet, and their interaction).

Age-dependent male reproductive effort

Weekly male calling effort was over-dispersed and zero-inflated, and no data transformation adequately addressed these problems. For this analysis I fitted 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. The use of a ZAP model enabled me to ask two separate questions (Atkins *et al.*, 2013): which factors affect whether there is calling or no calling (i.e., zero or non-zero), and which affect the amount of calling when it occurs?

I used a generalised linear mixed model to specify the ZAP structure with a random effect that accounted for the repeated observations on individuals. I used Bayesian Markov chain Monte Carlo techniques and estimated the posterior mode and 95% credible intervals (CIs) for fixed effects and their interactions: the predictors were juvenile diet, adult diet, age at which the measurement was taken, and lifespan. Age and lifespan were included in both linear and quadratic forms. The full model included all possible interactions between predictor variables (excluding interactions between linear and quadratic forms of a single variable). A random effect of cricket ID was fitted to group measurements for each individual, with a variance term allowing the intercept and age-related slope to vary between individuals. I fitted an unstructured variance-covariance matrix to the Poisson model, allowing the random intercepts and slopes of this model to be correlated. There is no observed residual variance for binary data (the logit model), so this value was fixed at 1.

The analysis was carried out using the R package MCMCglmm (Hadfield, 2010) with 1,050,000 iterations, burn-in of 50,000, a sampling rate of 500, and parameter-expanded priors. I used the 'zapoisson' family for zero-altered Poisson; the Poisson part of the model as specified in MCMCglmm also has a vector of residuals that deals with over-dispersion in the data after accounting for fixed and random sources of variation (Hadfield, 2010). Fixed effect terms in MCMCglmm are considered significant if their 95% credible intervals do not straddle zero, so terms or interactions not meeting this criterion were removed if they were not involved in significant higher-order interactions, and if removal did not worsen the model fit according to DIC. Autocorrelation was low amongst consecutive thinned observations (<0.05), variance terms (<0.05), and fixed effects for both the Poisson (<0.05) and zero-altered (<0.13) parts of the model. I used Gelman-Rubin diagnostics to check that multiple runs of the model had converged upon the same posterior distribution (3 runs gave a multivariate potential scale reduction factor of 1.03)(Gelman & Rubin, 1992).

Age-dependent female reproductive effort

Female weekly egg count measurements were also over-dispersed relative to Poisson. I used the 'Poisson' family in the MCMCglmm R package, which handles over-dispersion (Hadfield, 2010), and a random effect of individual ID to group female measurements. As with the analysis of male age-dependent reproductive effort, I ran the model for 1,050,000 iterations with a burn-in of 50,000, a sampling rate of 500, and using parameter-expanded priors. Fixed effects terms that were not considered significant were removed if they were not involved in significant higher-order

interactions, and if removal did not worsen the model fit according to DIC. Autocorrelation was low amongst consecutive thinned observations (≤ 0.05), variance terms (≤ 0.05), and fixed effects (≤ 0.05). Gelman-Rubin diagnostics showed that multiple runs converged upon the same posterior distribution (multivariate potential scale reduction factor of 1.01).

Qualitatively similar results (not shown) were obtained using a generalised linear mixed model in the R package lme4 with Poisson family, random effect of cricket ID, and an additional over-dispersion term (Bates, 2010).

Visualisation of age-dependent reproductive effort

Graphic representations of the relationship between weekly reproductive effort measurements, diet treatment and lifespan were created by fitting non-parametric thin-plate spline contour plots separately for each sex, using the Tps function in the R package fields (Furrer *et al.*, 2009).

2.3 Results

Of the 320 nymphs raised individually from hatching, 205 eclosed to adulthood successfully: 99 from the high-nutrient (H) diet, and 106 from the low-nutrient (L) diet, and altogether comprising 100 males and 105 females. I found no significant effect of

diet treatment on survival to eclosion ($\chi^2_1 = 0.489, P = 0.485$; proportion surviving $\pm$ binomial S.E.: L = 0.590 $\pm$ 0.037, H = 0.550 $\pm$ 0.037). Of the 99 crickets surviving to eclosion on diet H, 57 were female, and 42 male; of the 106 on diet L, 48 were female and 58 male. I found no significant skew in the sex ratio of those surviving to eclosion overall or in each diet (Overall: $\chi^2_1 = 0.122, P = 0.727$; High-nutrient diet: $\chi^2_1 = 2.273, P = 0.132$; Low-nutrient diet: $\chi^2_1 = 0.943, P = 0.331$).

Male lifetime diet treatments had the following sample sizes: LL=27, LH=31, HL=24, HH=18. The asymmetry in treatment group size was due to individuals being assigned a new diet haphazardly upon reaching eclosion. A single male (diet HH) died prior to the first call effort recording, and another 3 (1 each of LL, LH, HH) did not call at all during measurement periods; these 4 males were therefore excluded from the analysis of reproductive effort, leaving a total of 96. Three females died prior to mating (one each of diets LH, HL, HH); the remaining 102 make up the following sample sizes: LL=22, LH=25, HL=33, HH=22. A further 15 females failed to lay any eggs throughout the 4-week period and were excluded from the analysis of reproductive effort, leaving a total of 87 remaining: LL=16, LH=23, HL=29, HH=19.

2.3.1 Juvenile development rate and adult morphology

Sex explained most of the variation in developmental rate and adult morphology (pronotum length and mass) at eclosion (Table 2.1), although there was also a marginally non-significant effect of diet treatment. Juvenile crickets reared on the high-nutrient

juvenile diet treatment group (H) had greater pronotum lengths and weighed more than those in the low-nutrient (L) group (Figure 2.1, Figure 2.2), although univariate ANOVAs showed that within-sex diet effects were only significant in males (Table 2.2). High-nutrient diet males had significantly larger scaled mass indices (SMI) at eclosion; they also developed to eclosion more rapidly, but not significantly so.

Table 2.1: MANOVA for development rate, weight and size at eclosion, including both sexes.

Parameter	Pillai's trace	$F_{3,201}$	P	
Sex	1.181	96.215	<0.001	***
Nymph diet	0.035	2.390	0.070	.
Sex × Nymph diet	0.003	0.228	0.877	

While there was no difference between males and females in the rate at which they developed to adulthood (development rate between-sex differences: low-nutrient $<0.001, F_{1,104} = 0.956, P = 0.331$; high-nutrient $<0.001, F_{1,97} = 0.007, P = 0.935$), females were larger and heavier than males at eclosion in both diet treatment groups (pronotum length between-sex differences: low-nutrient = 0.406mm, $F_{1,104} = 114$, $P<0.001$; high-nutrient = 0.369mm, $F_{1,97} = 66.18$, $P<0.001$; mass between-sex differences: low-nutrient = 0.049g, $F_{1,104} = 37.72$, $P<0.001$; high-nutrient = 0.041g, $F_{1,97} = 13.54$, $P<0.001$).

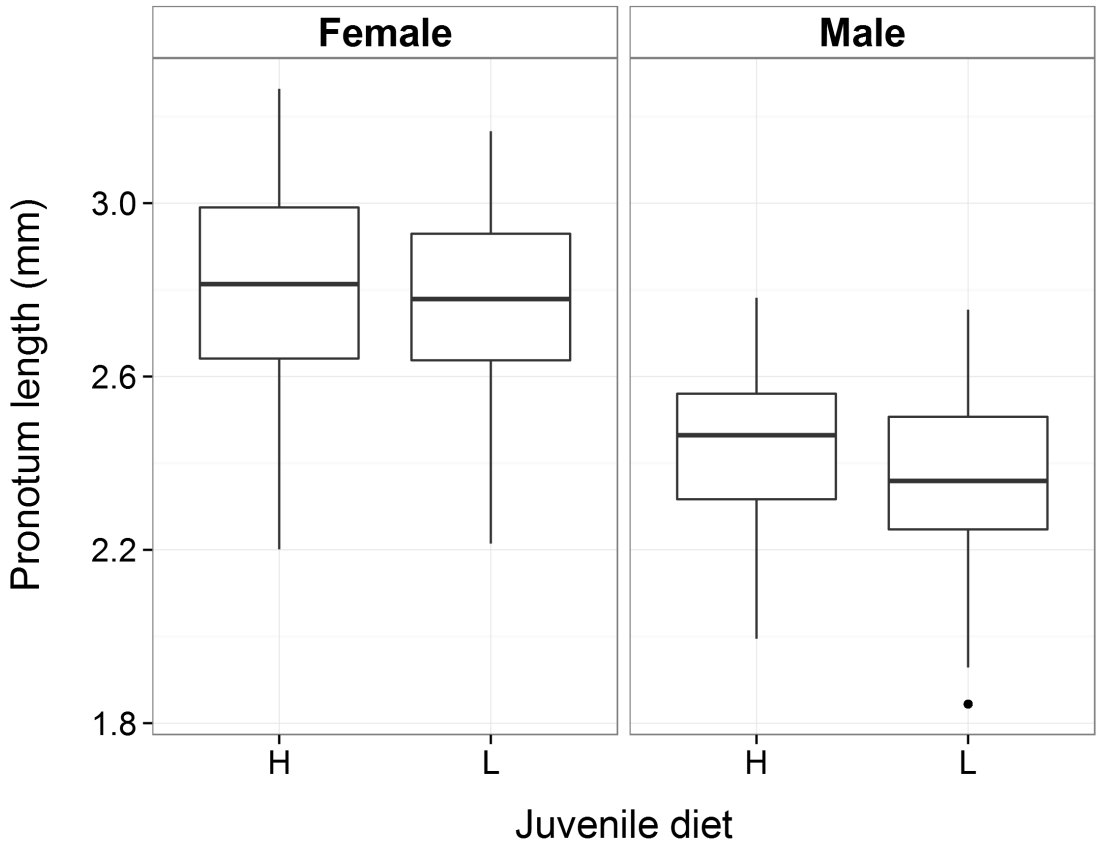

Figure 2.1: Boxplot showing median and first and third quantiles of pronotum length for females (left) and males (right) reared on high- (H) and low- (L) nutrient diets.

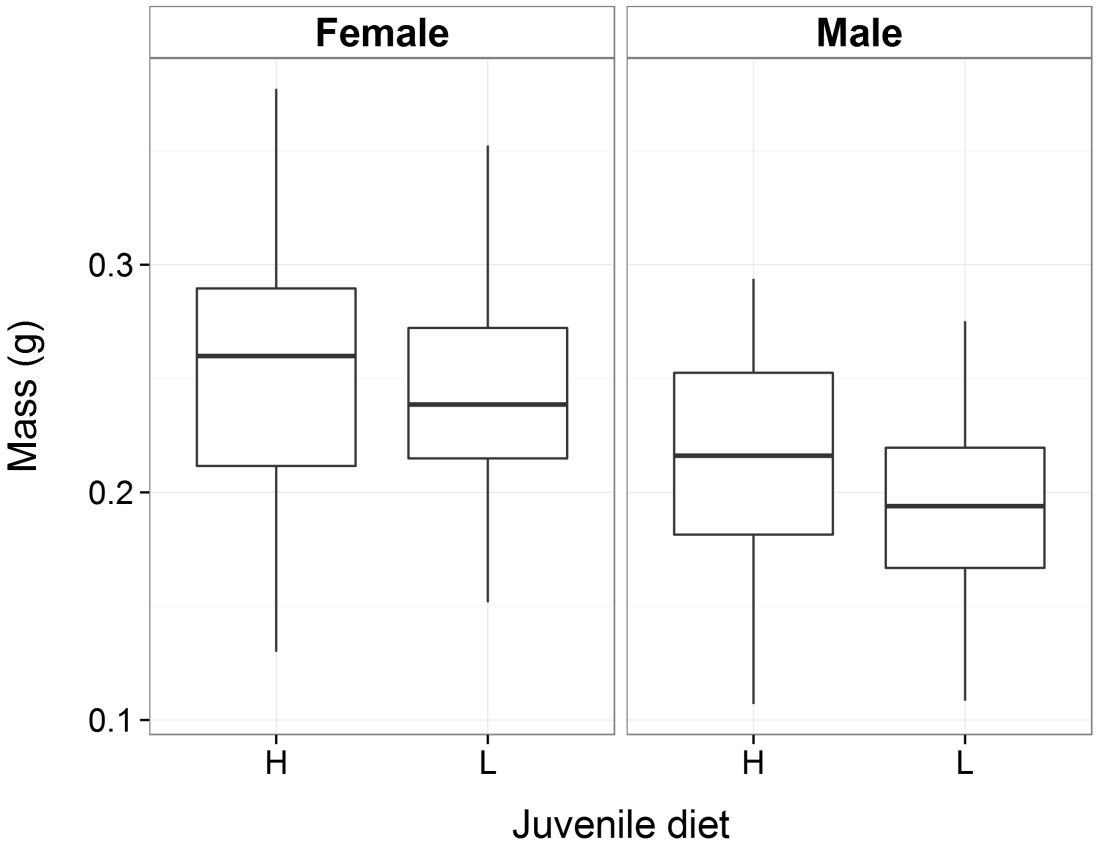

Figure 2.2: Boxplot showing median and first and third quantiles of mass for females (left) and males (right) reared on high- (H) and low- (L) nutrient diets.

Table 2.2: Univariate ANOVAs for development rate, weight and size at eclosion, separately for each sex. Parameter estimates are given as variable means ± standard errors of the means for both diet treatments at the nymph stage.

Males	Low-nutrient	High-nutrient	$F_{1,98}$	P	
Development rate (1/days)	0.020±0.001	0.021±0.001	3.060	0.083	.
Pronotum length (mm)	2.362±0.025	2.438±0.028	4.131	0.045	*
Weight (g)	0.192±0.005	0.212±0.007	6.142	0.015	*
Scaled mass index	0.194±0.010	0.234±0.013	6.023	0.016	*
Females	Low-nutrient	High-nutrient	$F_{1,103}$	P	
Development rate (1/days)	0.021±0.001	0.021±0.001	0.678	0.412	
Pronotum length (mm)	2.768±0.029	2.806±0.033	0.726	0.396	
Weight (g)	0.241±0.007	0.253±0.008	1.328	0.252	
Scaled mass index	0.246±0.013	0.274±0.016	1.856	0.176	

2.3.2 Adult longevity

Analysis of variance showed a marginally non-significant effect of sex on lifespan ($F_{1,203} = 3.077, P = 0.081$), with males tending to live longer than females (male lifespan: 46.79 ± 1.48 days, female lifespan: 43.08 ± 1.51 days). However, this was not consistent across all lifetime diet treatments: in the HL diet treatment group, females lived longer than males, although not significantly so ($F_{1,56} = 0.007, P = 0.326$; Figure 2.3). I also tested the effect of juvenile diet quality, adult diet quality, sex, and all interactions using a Cox regression survival analysis (Figure 2.4). None of these factors had significant effects on survival (Table 2.3), either before or after model simplification.

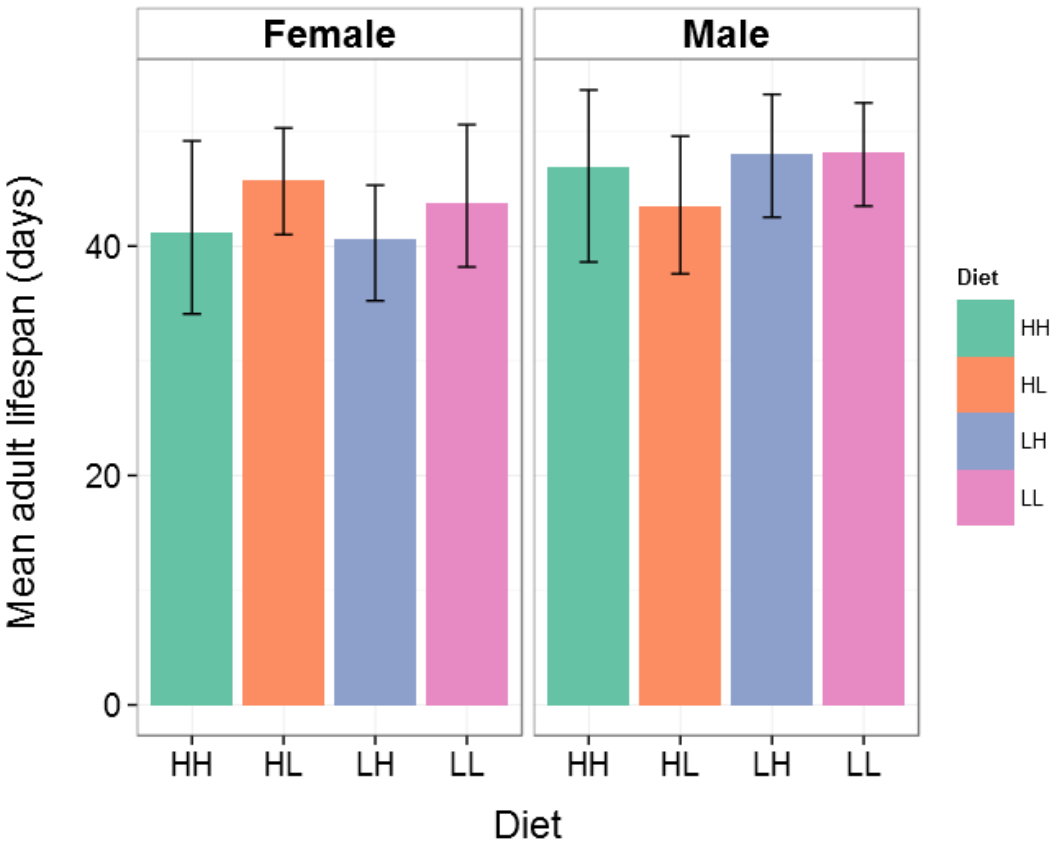

Figure 2.3: Adult lifespan in each of the four lifetime diet treatment combinations, separately for each sex. Values presented as the median $\pm$ 95% bootstrapped confidence interval of the median.

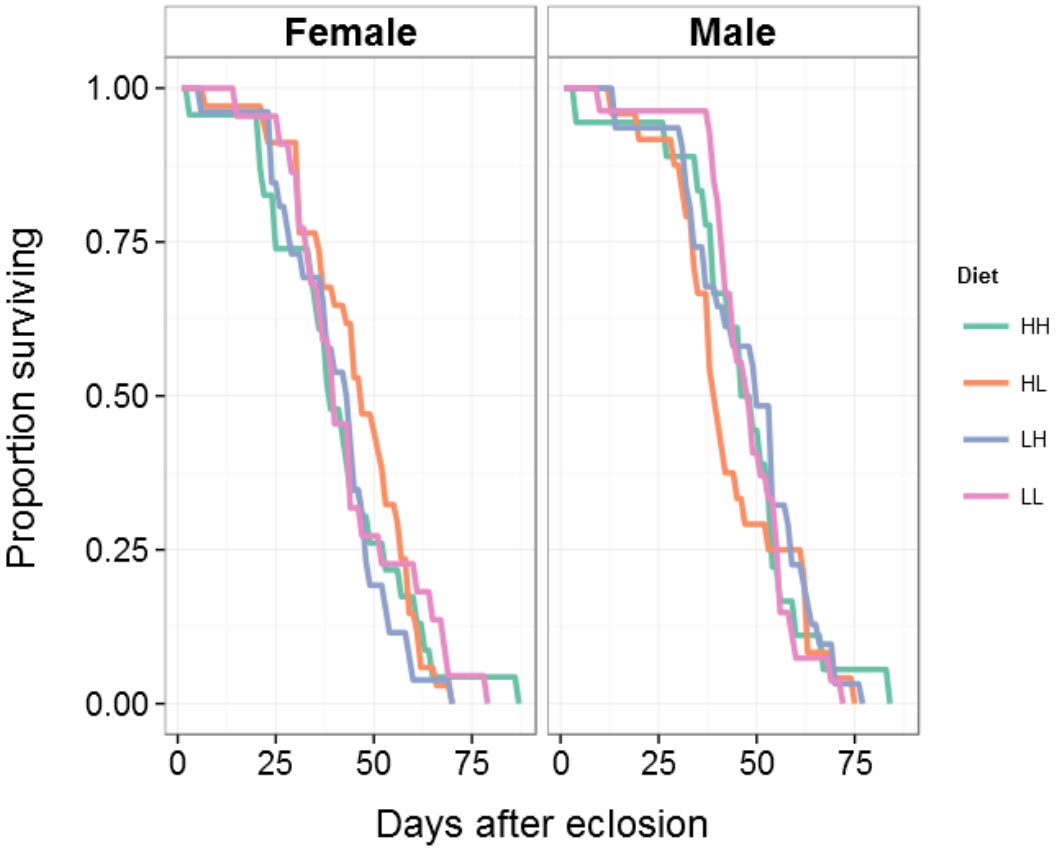

Figure 2.4: Post-eclosion survival curves for each lifetime diet treatment, separately for each sex.

Table 2.3: Cox regression survival analyses; complete full factorial model (i) and effects of nymph diet and adult diet in males and females (ii, iii).
*χ^2 for full model, Wald for separate variables.

		d.f.	χ^2/Wald*	P
(i)	Both sexes			
	Overall model	7	4.83	0.681
	Sex	1	-0.655	0.512
	Nymph diet	1	0.860	0.390
	Adult diet	1	-0.278	0.781
	Sex × Nymph diet	1	-0.791	0.429
	Sex × Adult diet	1	0.617	0.537
	Nymph diet × Adult diet	1	-0.580	0.562
	Sex × Nymph diet × Adult diet	1	0.323	0.747
(ii)	Males			
	Overall model	3	1.32	0.725
	Nymph diet	1	0.016	0.988
	Adult diet	1	0.886	0.376
	Nymph diet × Adult diet	1	-0.355	0.723
(iii)	Females			
	Overall model	3	0.49	0.921
	Nymph diet	1	0.263	0.793
	Adult diet	1	-0.370	0.711
	Nymph diet × Adult diet	1	-0.008	0.993

2.3.3 Lifetime reproductive effort

Total lifetime male calling effort was best described by a simplified model with a single significant term, a positive linear effect of lifespan (Table 2.4, Figure 2.5). The lack of significant interactions between lifespan and diet treatment indicates that longer-lived males called more during their lifetime, but that the relationship between lifes-

pan and total lifetime calling effort did not depend on diet. As males that live longer had more opportunities to call, I ran a model with mean calling effort (an individual's total calling effort divided by the number of measurements taken) as the response variable (and the same set of predictors) to investigate whether longer-lived males also called more per night on average. The final model suggested that mean calling effort was dependent on complex interactions between diet and the quadratic age term (Table 2.5). However, a single outlier - an HL male ranking in the top 15% of males for total calling effort but the lowest 5% for lifespan - influenced the final model heavily. After running the model again having removed the outlier and re-standardised lifespan, the final model was simpler, showing a positive linear relationship whereby longer-lived males called more on average per night (Table 2.5).

The final model for total female egg production included curvilinear effects of lifespan (indicated by significant positive linear and negative quadratic effects), along with (negative linear and positive quadratic) interactions between lifespan and high-quality adult diet (Table 2.4). In both low- and high-quality adult diets, females living to intermediate age had the highest levels of fecundity, but the curves were slightly different. However, the model was biased by an outlier, a single long-lived female on HH diet that was over 3 standard deviations from the mean, while all others were within 2 standard deviations. I removed this individual and standardised lifespan again before re-running model selection. The final model after removal of this outlier was slightly simpler (Table 2.4). The effect of lifespan was curvilinear, such that total fecundity increased with lifespan to intermediate values, but decreased at higher

Table 2.4: Generalised linear models for total lifetime reproductive effort, separately for each sex.

Term	df	z	Estimate $\pm$ SE	P	
Male					
(Intercept)	1,95	82.390	8.748 ± 0.106	<0.001	***
Lifespan	1,95	4.922	0.037 ± 0.008	<0.001	***
Female (all)					
(Intercept)	1,86	34.411	4.787 ± 0.139	<0.001	***
Lifespan	1,86	4.652	1.696 ± 0.365	<0.001	***
Lifespan^2	1,86	-4.739	-2.972 ± 0.627	<0.001	***
Adult diet (H): lifespan	1,86	-2.859	-1.338 ± 0.468	0.004	**
Adult diet (H): lifespan^2	1,86	3.184	2.025 ± 0.636	0.001	**
Female (outlier removed)					
(Intercept)	1,85	27.076	4.647 ± 0.172	<0.001	***
Adult diet (H)	1,85	2.175	0.471 ± 0.216	0.030	*
Lifespan	1,85	5.055	1.642 ± 0.325	<0.001	***
Lifespan^2	1,85	-6.123	-2.425 ± 0.396	<0.001	***
Adult diet (H): lifespan	1,85	-3.794	-1.749 ± 0.461	<0.001	***

values of lifespan (Figure 2.6). The linear effect of lifespan was also dependent upon adult diet, where total fecundity increased with lifespan at a lower rate for females on high-nutrient adult diet. The main effect of high-nutrient diet on fecundity was significantly positive. These effects are manifest in females fed a high-nutrient diet having peak total fecundity at earlier ages, and there being a fecundity benefit to long lifespan only in low-nutrient diet females.

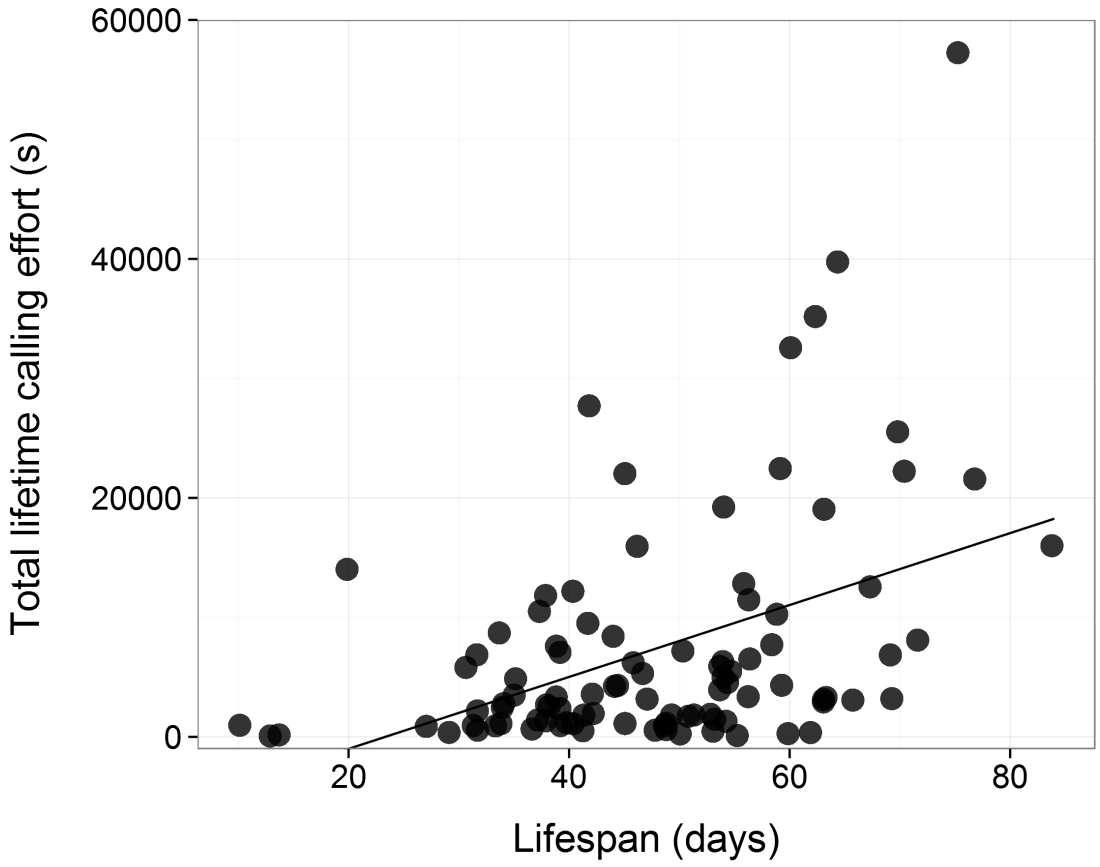

Figure 2.5: Total lifetime calling effort plotted against lifespan (all diets pooled).

2.3.4 Age-dependent reproductive effort

Male

The final model for age-dependent calling consisted of two parts, one of which (the zero-altered component) described aspects affecting the likelihood of calling (as opposed to remaining silent), and one of which (a Poisson model) described the intensity of calling among males that called. The whole model included juvenile and adult

Table 2.5: Generalised linear models for mean weekly calling effort, for all males and excluding single HL outlier.

Term	df	z	Estimate $\pm$ SE	P	
All					
(Intercept)	1,95	38.592	6.646 ± 0.172	<0.001	***
Adult diet (H)	1,95	1.702	0.430 ± 0.253	0.089	.
Lifespan	1,95	2.021	0.438 ± 0.217	0.043	*
Lifespan^2	1,95	2.885	1.150 ± 0.399	0.004	**
Adult diet (H): lifespan^2	1,95	-2.210	-1.321 ± 0.598	0.027	*
Outlier removed					
(Intercept)	1,94	67.449	6.924 ± 0.103	<0.001	***
Lifespan	1,94	2.853	0.598 ± 0.210	0.004	**

diet treatments and both linear and quadratic forms of the standardised age term, as well as all interactions between these; it also included the standardised lifespan term, and an interaction between age and lifespan (Table 2.6). By retaining lifespan in the model, a portion of change in performance can be described as within-individual ageing (van de Pol & Verhulst, 2006). Although there were several significant interactions (described further below) affecting both the probability of calling and the intensity of effort per night, the patterns of age-dependent calling effort were similar across diet treatments: calling increased with age, and longer-lived males called more at all ages (Figure 2.7).

In the zero-altered part of the model, a positive significant term indicates that a variable predicts reduced likelihood of zeroes, while a negative significant term indicates an excess of zeroes (zero-inflation) (Hadfield, 2010). The zero-altered part of

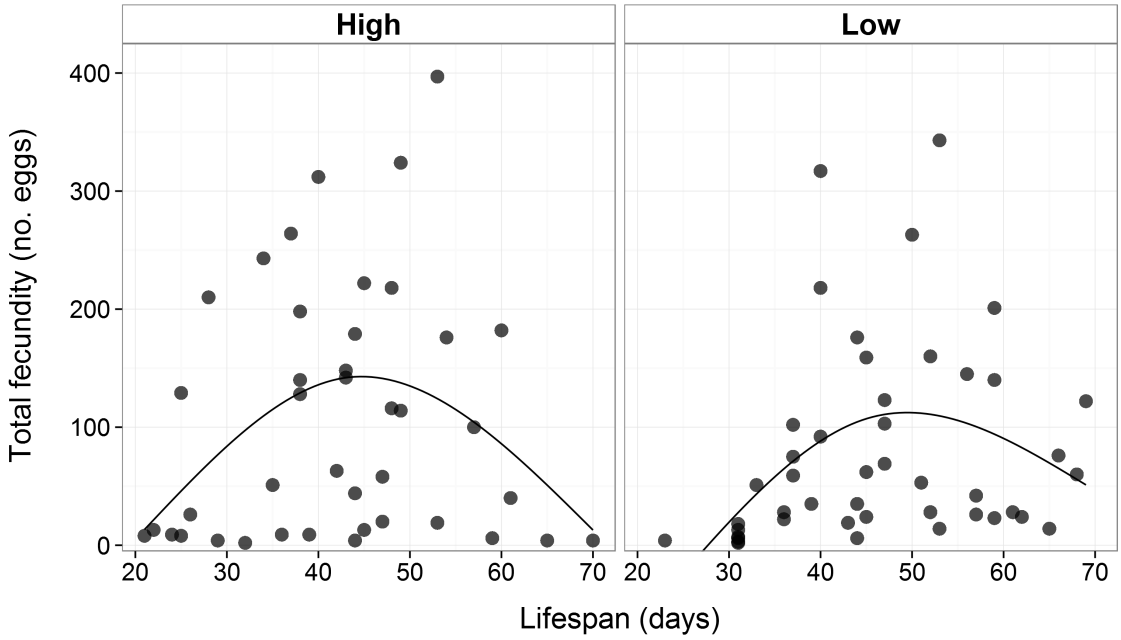

Figure 2.6: Total fecundity plotted against lifespan, separately for high- and low-quality adult diets. Lines show quadratic slopes for how total fecundity changes as a function of lifespan.

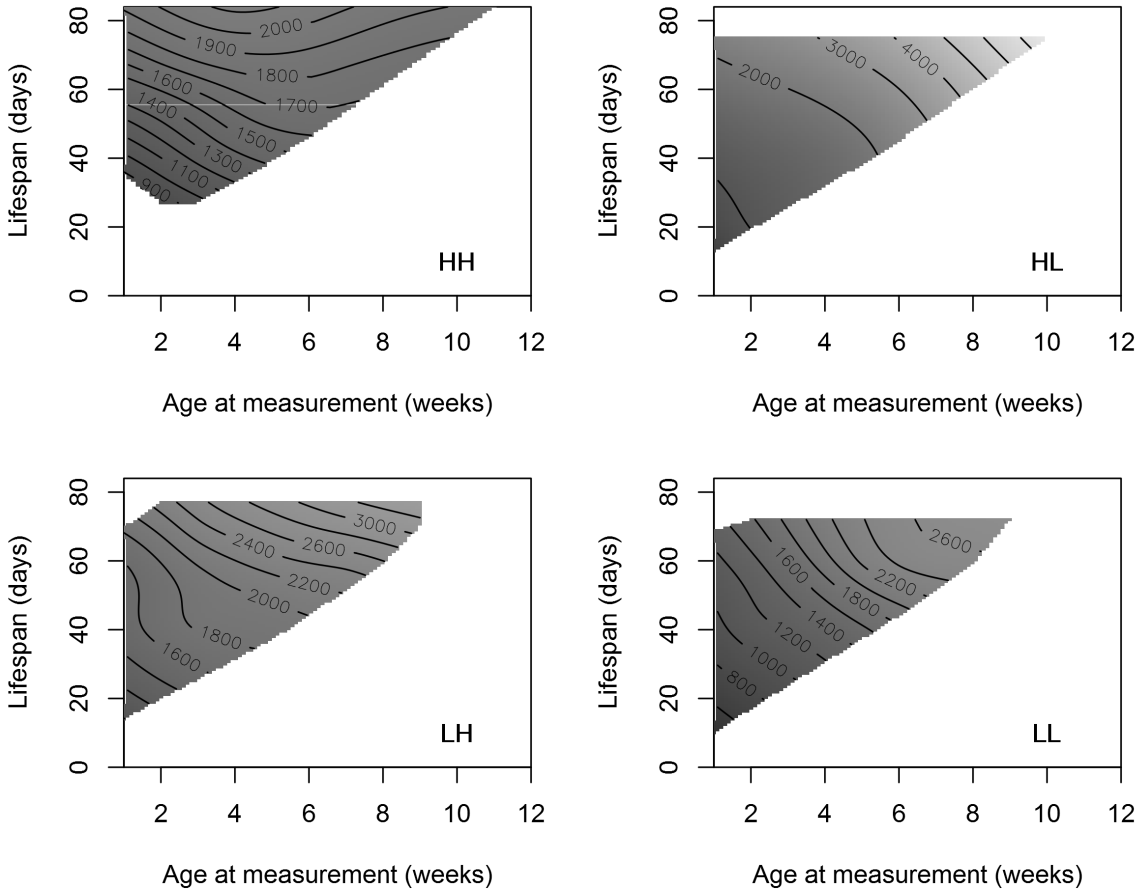

Figure 2.7: Age-dependent calling effort in relation to lifespan, excluding zero-calls and plotted separately for each diet treatment (HH, top left; HL, top right; LH, bottom left; LL, bottom right). Lighter shades represent higher values.

Table 2.6: MCMCglmm analysis of male weekly calling effort. The baseline category for diet treatment group is LL (*e.g.*, 'Juvenile diet (H)' indicates treatment group HL).

Fixed effect	Estimate	95% CI (lower, upper)	*P*	
Zero-altered				
(Intercept)	-6.214	(-6.850, -5.487)	<0.001	***
Juvenile diet (H)	-0.446	(-1.313, 0.730)	0.597	
Adult diet (H)	-0.079	(-0.888, 0.947)	0.996	
Juvenile diet (H): adult diet (H)	-0.167	(-1.764, 1.022)	0.606	
Age	-0.786	(-1.795, 0.056)	0.056	.
Age^2	-0.038	(-1.771, 1.917)	0.868	
Juvenile diet (H): age	1.739	(0.132, 2.949)	0.046	*
Juvenile diet (H): age^2	-2.652	(-4.350, 0.454)	0.111	
Adult diet (H): age	-0.673	(-1.881, 0.712)	0.306	
Adult diet (H): age^2	-0.319	(-2.741, 1.846)	0.669	
Juvenile diet (H): adult diet (H): age	-1.871	(-3.684, 0.428)	0.115	
Juvenile diet (H): adult diet (H): age^2	3.553	(0.158, 6.786)	0.040	*
Lifespan	-0.100	(-0.754, 0.608)	0.826	
Lifespan: age	0.731	(-0.546, 1.895)	0.313	
Poisson				
(Intercept)	6.428	(6.016, 6.873)	<0.001	***
Juvenile diet (H)	0.033	(-0.576, 0.671)	0.884	
Adult diet (H)	0.169	(-0.266, 0.866)	0.298	
Juvenile diet (H): adult diet (H)	0.090	(-0.855, 0.901)	0.892	
Age	0.378	(-0.242, 1.020)	0.200	
Age^2	-1.422	(-2.610, -0.006)	0.063	.
Juvenile diet (H): age	-0.400	(-1.452, 0.477)	0.336	
Juvenile diet (H): age^2	2.050	(0.121, 3.518)	0.025	*
Adult diet (H): age	0.380	(-0.512, 1.139)	0.484	
Adult Diet (H): age^2	-0.032	(-1.232, 1.895)	0.731	
Juvenile diet (H): adult diet (H): age	0.507	(-0.677, 2.095)	0.294	
Juvenile diet (H): adult diet (H): age^2	-2.793	(-4.809, -0.500)	0.040	*
Lifespan	0.330	(0.041, 0.773)	0.088	.
Lifespan: age	0.571	(-0.120, 1.438)	0.082	.

Variance component	Estimate	95% CI (lower, upper)
Zero-altered		
ID	0.607	(0.210, 1.053)
Poisson		
ID	0.320	(0.104, 0.577)
Age	0.103	(0, 0.400)
ID: age	-0.024	(-0.191, 0.149)
Residual	1.783	(1.486, 2.078)

the model showed that the age-related response of male call likelihood is dependent upon both juvenile diet and adult diet (Table 2.6). The significant interaction between diet treatment at both stages and the quadratic age term is manifest in the noticeable curvature of the response among HH males, where the likelihood of calling levels off after an initial decrease (Figure 2.8). The linear effect of age was also dependent upon juvenile diet, with the likelihood of recording a call increasing with age among males in diet group HL.

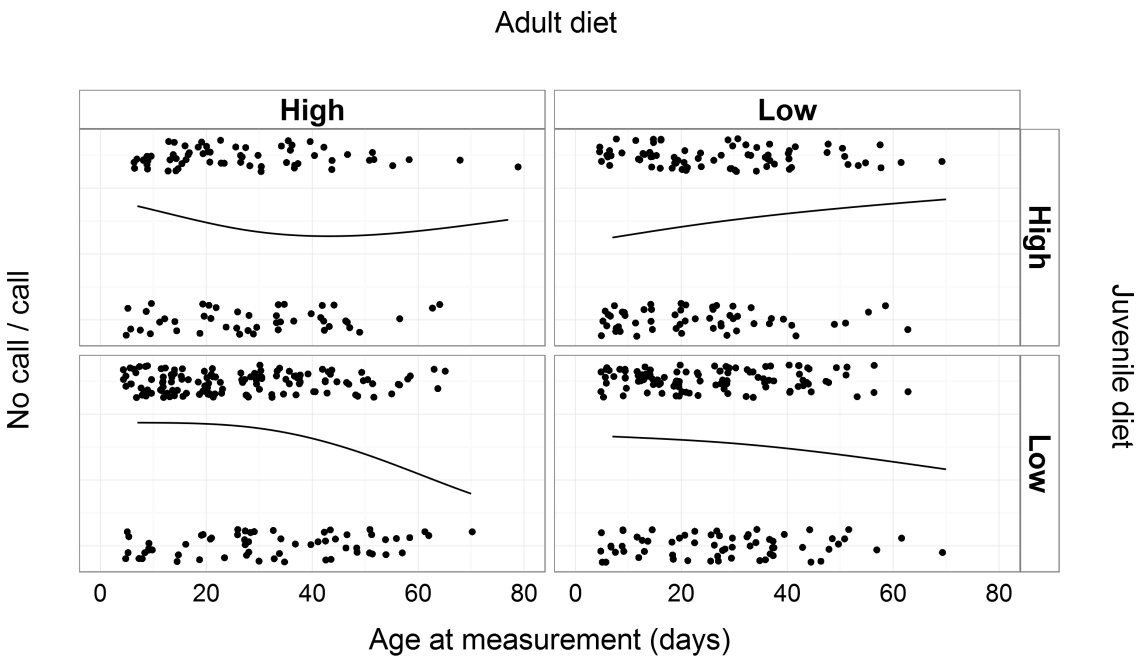

Figure 2.8: Age-dependent zero-call / call with fitted binomial GLM quadratic regression slope, separately for each diet treatment (HH, top left; HL, top right; LH, bottom left; LL, bottom right).

The Poisson part of the model predicts the amount of calling effort when males did

call. The effect of age on calling effort was dependent upon both juvenile and adult diet treatments: males that had access to the high diet treatment at juvenile and adult stages (HH) decreased calling effort at later ages (Table 2.6, Figure 2.7). Males fed the high diet treatment at only the juvenile stage (HL) increased calling effort sharply at later ages. The main effect of the quadratic age term was marginally non-significant and showed that male calling effort tended to decline at later ages. Lifespan and the interaction between lifespan and measurement age also had marginally non-significant effects, indicating that longer-lived males tended to call more, and that the effect of age was especially acute in longer-lived males.

Female

The final model for total female egg production indicated that the effect of lifespan depended on both juvenile diet and adult diet (Table 2.7). Because there were no 3-way interactions between juvenile diet, adult diet, and age, I pooled the adult diet treatments in Figure 2.9 (to illustrate the effects of juvenile diet) and the juvenile treatments in Figure 2.10 (to illustrate the effects of adult diet). Juveniles provided with a high-protein diet tended to show marked senescence, with their highest egg outputs early during life (Figure 2.9). Furthermore, in this treatment females who lived longest had the highest fecundity. In the low protein juvenile diet treatment, by contrast, fecundity during weeks 2-5 increased as a function of age and was negatively related to longevity. The effects of adult diet on age-specific fecundity were notably different (Figure 2.10). Females on high-protein diets tended to senesce (were most

fecund early in life), but longevity in this treatment covaried negatively with fecundity during weeks 2-5. In the low-protein diet treatment, fecundity did not seem to depend strongly on age, but was positively correlated with total lifespan.

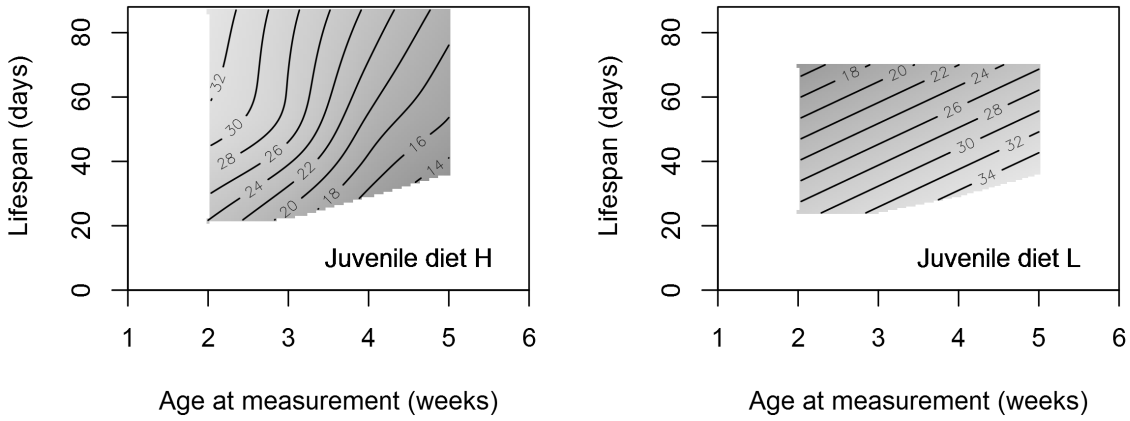

Figure 2.9: The effect of juvenile diet on age-dependent female reproductive effort (weekly egg counts) in relation to lifespan (left, high juvenile diet; right, low juvenile diet). Data are pooled across adult diets (i.e., high juvenile diet = HH + HL; low juvenile diet = LH + LL). Lighter shades represent higher values.

2.4 Discussion

Consuming a nutrient-rich juvenile diet resulted in larger and heavier adults of both sexes, as well as increasing the rate of development to adulthood, but without imposing differential mortality during this period. Both male and female crickets revealed distinct effects of diet quality on how they allocated resources to reproductive effort across their lifespan. Male crickets displayed a positive relationship between lifespan and calling effort, with longer-lived males calling more overall and on average

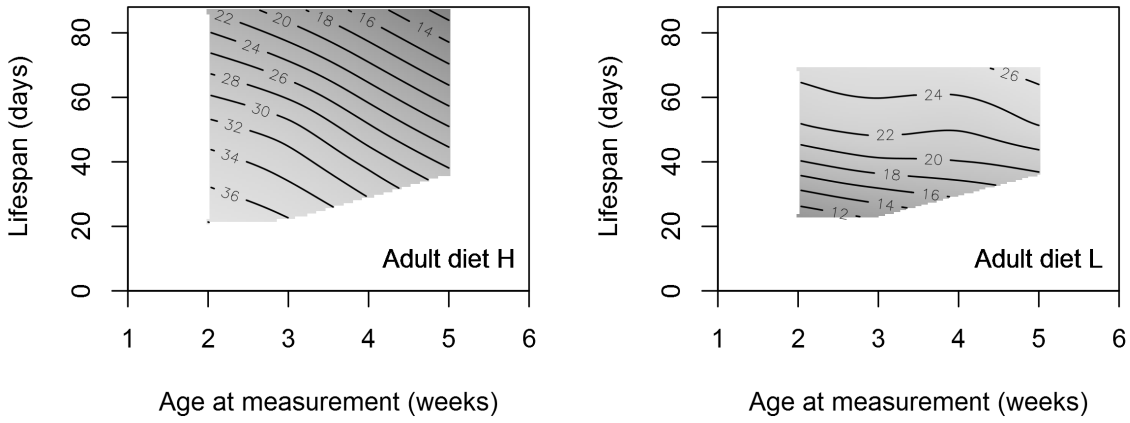

Figure 2.10: The effect of adult diet on age-dependent female reproductive effort (weekly egg counts) in relation to lifespan (left, high adult diet; right, low adult diet). Data are pooled across juvenile diets (i.e., high adult diet = HH + LH; low adult diet = HL + LL). Lighter shades represent higher values.

Table 2.7: MCMCglmm analysis of female weekly fecundity. The baseline category for diet treatment group is LL (*e.g.*, 'Juvenile diet (H)' indicates treatment group HL).

Fixed effect	Estimate	95% CI (lower, upper)	*P*	
(Intercept)	0.382	(-1.145, 1.956)	0.635	
Juvenile diet (H)	-1.044	(-3.036, 0.954)	0.289	
Adult diet (H)	0.150	(-1.985, 2.188)	0.909	
Juvenile diet (H): adult diet (H)	1.314	(-1.380, 4.409)	0.364	
Age	3.593	(1.479, 5.859)	0.001	***
Age^2	-1.859	(-6.666,3.164)	0.470	
Lifespan	0.566	(-1.467, 2.864)	0.618	
Juvenile diet (H): age	-3.288	(-5.802, -0.504)	0.016	*
Juvenile diet (H): age^2	3.577	(-2.525, 10.033)	0.268	
Juvenile diet (H): lifespan	0.240	(-2.529, 3.138)	0.855	
Adult diet (H): age	-2.952	(-5.758, -0.287)	0.030	*
Adult diet (H): age^2	1.392	(-4.969, 7.998)	0.673	
Adult diet (H): lifespan	-1.163	(-4.506, 1.823)	0.468	
Juvenile diet (H): adult diet (H): age	2.093	(-1.403, 6.071)	0.267	
Juvenile diet (H): adult diet (H): age^2	-5.507	(-14.870, 2.794)	0.208	
Juvenile diet (H): adult diet (H): lifespan	-0.386	(-4.620, 3.306)	0.847	
Variance component	**Estimate**	**95% CI (lower, upper)**		
ID	0.142	(0, 0.537)		
Residual	12.500	(9.316, 15.940)		

regardless of diet. I found a positive relationship between weekly calling effort measurements and lifespan across all diets, as well as between calling, lifespan and age, indicating that longer-lived males called more each night, and increased their calling with age at a higher rate than other males. Unlike males, in females I found differences across diets in both the presence and pattern of reproductive senescence, as well as in the covariance between longevity and fecundity. Whereas most other studies have shown diet-dependent plasticity in males, here I report intriguing changes in the age-dependent patterns of investment by females due to variation in resource acquisition.

2.4.1 Male life history strategies

Resource acquisition at both juvenile and adult stages affected the precise trajectories of age-dependent calling effort in males, yet the overall patterns among calling males were strikingly similar: calling effort increases with age, and longer-lived males call more. These results conform to the hypothesis that the marginal benefits of investment in reproductive effort (rather than survival) increase with age (Kirkwood, 1977), and other studies that indicate covariance between secondary sexual trait expression and longevity (Jennions *et al.*, 2001). However, the use of a zero-altered model also enabled me to use the same framework to assess the effects of these variables on not only the amount of calling effort when a male called, but also the likelihood of a male to call. I found that there were distinct responses to the same predictors: for example, males fed a low-quality diet as juveniles (LL and LH groups) increased calling effort

with age, but the likelihood of actually recording a call decreased dramatically. My results suggest that while overall patterns indicate age-related increases in calling effort, and that a positive correlation exists between lifespan and total calling effort, these mask more subtle differences between individuals as to how resources are invested in reproductive effort.

The use of a model to analyse the likelihood of calling and the amount of calling separately also serves to underline the integral difference between the sexes in how they accumulate fitness through reproductive effort. For females, the equation is generally straightforward: fecundity is amassed by the gathering of resources and the time required for converting these resources to useful forms. For males, resource acquisition is also a strong requirement for investment in energetically costly signalling, but allocation decisions are subject to greater uncertainty. Male reproductive success first requires that there be available females; optimal signalling is then dependent both on an individual's condition and the competition that he faces at any time (Houston & Mcnamara, 1987; Kokko, 1997; Lindström *et al.*, 2009). Males therefore need to sample the social environment far more than do females, which may select for phenotypic plasticity in male signalling decisions (Bretman *et al.*, 2011); my results show that males exhibit plasticity in both when and how much to call.

Given that male reproductive success is relative to the performance of rivals, why would high-quality males not sacrifice longevity for increased early reproductive effort (Kokko, 1998; Vinogradov, 1998)? Early signalling might be suppressed by high-

quality males as they have a good chance of outliving low-quality rivals, and high early investment might reduce future opportunities (Candolin, 2000; Proulx *et al.*, 2002; Lindström *et al.*, 2009). In addition to the effect of resource levels on the timing and quantity of signalling (Hunt *et al.*, 2004a; Judge *et al.*, 2008; Maklakov *et al.*, 2009; Zajitschek *et al.*, 2009b, 2012), plasticity in calling behaviour in crickets can be induced by manipulation of the social environment: perceived future competition (Kasumovic *et al.*, 2012b) and the presence of rivals (Callander *et al.*, 2013) have both been shown to affect how males invest in calling effort. In other species that are able to alter signalling behaviour dynamically, males have been shown to invest more based on female presence (Mappes *et al.*, 1996) or quality (Wong & Svensson, 2009). The importance of female cues on how male crickets allocate resources to signalling effort has thus far been largely neglected in the literature, and could yield greater insights into whether age-related increases in calling are due to strategic decisions or are governed more by residual reproductive value.

2.4.2 Honesty in signalling

HL males, despite being assigned to the nutrient-poor diet as adults, recorded the highest overall measurements of weekly calling effort. However, this was restricted to a few long-lived individuals at late measurement ages. Theory predicts that, when some proportion of condition can be transferred from one age to the next, low-quality males can signal 'dishonestly' by having a higher trait expression value at some late age than that of high-quality males (Kokko, 1997). Storing some proportion of ac-

quired resources as fat would be considered adaptive for males in most circumstances due to the energetic demands of calling, and male *T. commodus* accumulate more fat than do females over the first 14 days post-eclosion, regardless of diet (Maklakov *et al.*, 2008). In line with predictions from Kokko's model (Kokko, 1997), Hunt et al. showed that male *T. commodus* on a low-protein diet delayed the onset of calling compared to males fed a medium- or high-protein diet, gaining more weight during this period and then calling more at late ages than other males (Hunt *et al.*, 2004a). Because the proportion of these 'dishonest' males in the population should be small (in my study, HL males also had the steepest decline in survival, Figure 2.4), females selecting a male with a high trait value at any point will most likely obtain a high-quality male regardless of the apparent mismatch between calling effort and quality late in the season. Furthermore, the resource advantages of high-quality males mean that they should always be free to select the best allocation strategy (Kokko, 1997). Males fed a high-quality diet here showed a higher likelihood of recording a call at early ages (Figure 2.8), a strategy that would secure greater mating success according to the same model (Kokko, 1997).

2.4.3 Female overview

Although female crickets on the high-nutrient diet were larger and heavier than those on the low-nutrient diet, these differences were smaller than between-diet effects in males and were not statistically significant. I also found no effect of juvenile diet on lifespan or total reproductive effort. Female black field crickets (*T. commodus*) require

more protein (one of the main factors in distinguishing between my diet treatments) than do males in order to maximise reproductive effort (Maklakov *et al.*, 2008), thus low-quality diet females may have increased their feeding rate so as to compensate for the lower protein content of their diet. Adult diet did affect the parameters of the relationship between lifespan and total fecundity amongst females, but this did not change the overall shape: in line with predictions, the highest levels of fecundity were achieved by individuals of intermediate age. These patterns suggest that the best female allocation strategies are fairly moderate, with even investment between reproduction and survival being optimal due to the limiting factors of the nutrients and time required to construct eggs.

Although overall investment in survival and reproduction appeared very similar across diets, age-dependent fecundity in females differs markedly dependent upon both juvenile and diet. While the differences across diets in females were not predicted, it is possible to conceive of adaptive causes of such differences a posteriori. The following is speculation, and further testing of such hypotheses is needed. Females fed a high-quality diet at the juvenile stage display increased fecundity early in life and senesce rapidly; accumulated protein may have enabled these individuals to build more eggs early in adulthood, with metabolism then declining as a function of reproductive activity. Positive covariance between fecundity and lifespan is most likely due to individual differences in quality. Low-nutrient juvenile diet females should eclose to adulthood with less protein: more time at adulthood is required to accumulate the necessary resources, hence fecundity increases with age. The nega-

tive covariance between fecundity and lifespan might also be due to individual differences in quality; here, lower quality females may need to divert resources to extending survival so that they have more time to accumulate and convert resources to eggs.

Females that were fed high-nutrient adult diets show high initial fecundity, perhaps through access to greater amounts of protein at early adulthood. As with those fed a high-nutrient diet as juveniles, this group shows rapid reproductive senescence: again, this could be due to negative effects of reproductive activity. Such effects would also explain why those with high early fecundity also exhibit reduced survival. Fecundity in females fed a low-nutrient adult diet is reduced compared to other diets and shows little change with age, suggesting that females cannot accumulate enough protein to mature more eggs. Equal investment in both survival and reproductive effort would enable individuals to live longer so as to gather enough resources to build more eggs; the positive covariance between lifespan and fecundity could again be due to individual differences in quality, where females that have acquired more resources have more to allocate to both reproduction and longevity.

2.4.4 Sex differences may arise from sex-specific selection pressures

While the overall pattern among males is to increase calling with age, females exhibit reproductive senescence on some diet treatments. Male calling patterns are consistent with several previous studies of age-dependent reproductive effort in crickets (Judge *et al.*, 2008; Maklakov *et al.*, 2009; Zajitschek *et al.*, 2009b, 2012; Archer *et al.*,

2012). In females, I found that the effects of high-quality diet at juvenile and adult stages were similar, increasing early fecundity but with senescent effects on reproductive effort shortly thereafter. Such a pattern is also consistent with previous studies of age-dependent reproductive effort in crickets, where female fecundity peaked early and decreased thereafter (Maklakov *et al.*, 2009; Zajitschek *et al.*, 2009b; Archer *et al.*, 2012). The marked differences between male and female patterns of age-dependent reproductive effort suggest that optimal resource allocation is driven by sex-specific selection pressures. Furthermore, the relationship between lifespan and both total and age-dependent reproductive effort indicate differences between the sexes in the potential benefits of allocation to longevity. In males, regardless of diet, lifespan and total calling were positively correlated and the longest-lived males called the most at any given age. Peak calling effort also tended to occur beyond the average lifespan (median male lifespan: 46.5 ± 2.39 days). In contrast, total female fecundity was highest in those that lived to intermediate ages, and there was no consistent relationship between age-dependent reproductive effort and lifespan among females. Furthermore, fecundity among females fed a high-quality diet at either juvenile or adult stage peaked early, well before the average lifespan (median female lifespan: 44.0 ± 1.8 days).

Similar results were shown in the study by Maklakov *et al.* (2009), who used median and maximum predicted adult lifespan from a separate study of wild crickets (Zajitschek *et al.*, 2009a) to show that *T. commodus* female median lifespan occurred around peak fecundity, whereas male calling effort peaked beyond the maximum

predicted lifespan in the wild. My results, along with those of Maklakov *et al.* (2009), indicate some support for the hypothesis that sexual selection drives the evolution of increased male performance in later life (Graves, 2007). Another possibility supported by my results is that female choice for increased calling effort exerts directional selection on male condition, and thus might favour genes with positive pleiotropic effects on lifespan and whole-organism performance (Lailvaux & Irschick, 2006). A quantitative genetic approach using inbred lines of *G. sigillatus* held in a common environment has shown positive genetic correlations between lifespan and measures of calling effort at several ages (Archer *et al.*, 2012). Manipulating resource acquisition in genetically-related individuals (see Chapter 3) would help simultaneously assess genetic and environment-dependent male plasticity in allocation.

3

The effect of genotype, environment and age on the expression of a sexually-selected condition-dependent behavioural signal

Abstract

Despite the strong directional selection imposed by female choice, male sexual signals exhibit substantial variation. Genotype $\times$ environment interactions (GEIs) could help maintain variation in male trait expression, but present problems for the reliability of the trait in signalling male quality to choosy females. Signal reliability may also degrade for extremely plastic male traits such as behavioural displays, where trait expression can be altered in a dynamic fashion over time. In spite of the ubiquity of sexually selected behavioural display traits, little is known about how they are affected by the existence of GEIs. I used inbred lines of the decorated cricket, *Gryllodes sigillatus*, with dietary manipulations at the juvenile and adult stage to test how different genotypes respond to environmental variation. I measured morphological traits as male crickets matured to adulthood, and the sexually selected behavioural display trait (male calling effort) at weekly intervals thereafter. I then estimated interactions between genotype, age, and the environment in determining male sexual trait expression. Morphological traits exhibited plasticity due to diet, but males responded in a similar fashion across treatments. The expression of the trait was influenced strongly by genotype, age, diet, and higher order interactions between all three terms. Increasing the 'distance' between measurements (in terms of age, diet, or both) tended to sharply decrease the correlation between them. My results reveal added complications for the value of behaviourally plastic traits as reliable indicators

of male quality, and indicate the need for measurements of environmental and age-dependent variation of such traits in nature.

3.1 Introduction

In many species, females exhibit strong preferences for males displaying exaggerated traits, often when there are no observable direct benefits for doing so (Jennions & Petrie, 2000; Kotiaho *et al.*, 2008). Exhibiting preferences for certain males rather than mating randomly is probably costly (Pomiankowski, 1987); the evolution and persistence of female mate choice therefore requires that the potential benefits outweigh any costs. One commonly invoked adaptive explanation - that females choose males for the indirect genetic benefits that their offspring will receive - requires male traits be reliable indicators of 'genetic quality' (Weatherhead & Robertson, 1979; Lande, 1981; Kirkpatrick, 1982). However, because strong directional selection on a trait via mate choice should erode the additive genetic variation that provides any benefit, the prevalence and importance of female choice for genes remains controversial (Reynolds & Gross, 1990).

Despite this controversy, the presence of impressive diversity in sexual signals (many of which do not appear to indicate direct benefits such as food, protection, or parental care) invites explanations for how genetic variation is maintained for sexually-selected male traits (Andersson, 1994). The currently favoured mechanism involves condition-dependence, a form of phenotypic plasticity where expression of the signal trait covaries with the male's ability to acquire and allocate resources from the local environment (Andersson, 1994; Johnstone, 1995)(but see Cotton *et al.*, 2004). Exaggerated traits can therefore provide information to females about individual performance, even if the many alleles that confer high performance might change depending upon

environmental conditions. In addition to the large mutational target provided by the large number of loci contributing to condition, the maintenance of additive genetic variation via such 'genic capture' is probably further assisted by the fact that condition-dependent signals are highly sensitive to environmental variation (Rowe & Houle, 1996; Hunt *et al.*, 2004b; Tomkins *et al.*, 2004).

When the phenotypic expression of a genotype is altered by the environment, genotype-by-environment interactions (GEIs) are expected to occur: that is, the same genotype can express several phenotypes depending on environmental conditions, and genotypes differ in their responses to environmental variation (Via & Lande, 1985; West-Eberhard, 1989; Scheiner, 1993). Two features of GEIs are important in the context of sexual selection (Ingleby *et al.*, 2010). Firstly, the proportion of phenotypic variation that is genetically determined may change across environments and thus alter the benefits of exercising choice. Secondly, changes in the rank order of genotype performance across environments ('ecological crossover') mean the best male to sire offspring in one environment may not be the best in another.

The existence of GEIs can have contrasting effects on the evolution and persistence of female choice for indirect benefits. When the environment is spatially heterogeneous and migration is relatively low, GEIs enable local adaptation to occur as divergent selection favours different alleles in local environments (Via & Lande, 1985; Kawecki & Ebert, 2004; Ghalambor *et al.*, 2007; Blanquart *et al.*, 2012). Low migration rates would create a continual influx of new and often maladapted alleles, and may then favour

the evolution of female choice for males signalling high quality in the local environment (Day, 2000). If spatial variation in selection pressures acts alongside dispersal, or temporal variation combines with overlapping generations, GEIs can help to maintain additive genetic variation because an individual's signal trait may not reflect his current local environment accurately. In such cases, erosion of variation is slowed precisely because females will sometimes select the 'wrong' mate. Herein lies the problem: while GEIs can help maintain additive genetic variation, they also decrease the reliability of the male signal (Greenfield & Rodríguez, 2004; Kokko & Heubel, 2008; Higginson & Reader, 2009).

Signal reliability may also be compromised if individuals alter trait expression over the course of their lifespan. Although females can sometimes use changes in signal expression with age as a cue of genetic quality, the prevalence and importance of such age-based indicator mechanisms is unclear (Kokko, 1998; Brooks & Kemp, 2001). This is because signal expression requires the investment of acquired resources, so is dependent on individual life history strategies. Life histories can be determined by genetic variation for both resource acquisition and genetic variation in how acquired resources are allocated (Van Noordwijk & De Jong, 1986; Stearns *et al.*, 1991; Reznick *et al.*, 2000, Chapter 2)(but see also Robinson & Beckerman, 2013). Investment in early and late reproduction can also trade off against one another (Stearns, 1992). Life history trade-offs can cause weak or even negative covariance between longevity and fitness, enabling males to sacrifice future reproductive opportunities for increased early reproductive success (Kokko, 1998; Hunt *et al.*, 2004a; Charmantier *et al.*, 2006;

Robinson *et al.*, 2006). An ornament that reliably signals quality at one stage of an individual's lifetime may therefore not necessarily signal quality at all stages (Kokko, 1997; Proulx *et al.*, 2002).

GEIs in sexually-selected traits have been studied in many taxa, and they seem to be a common feature of male signals (Bussière *et al.*, 2008a; Ingleby *et al.*, 2010) . Long-term studies of within-individual variation in age-dependent sexual traits in wild populations have revealed a variety of patterns: ornamentation that increases with age and covaries with lifespan (Evans *et al.*, 2011), that changes predictably both between and within seasons (Griffith & Sheldon, 2001), or that is subject to complex environment- and context-dependent variation (Badyaev & Duckworth, 2003; Garant *et al.*, 2004). In one of the few studies of its kind, Miller & Brooks (2005) explored the effects of genotype, age and an experimental manipulation of the environment on sexual traits in 22 full-sibling families of male guppies (*Poecilia reticulata*) in the laboratory. Their results revealed age (but not treatment) effects in ornamentation, and treatment (but not age) effects in behaviour. Strong effects of family and a lack of significant family-by-environment, family-by-age or family-by-environment-by-age interactions indicated that neither age-dependent variation nor environmental variation should undermine male signal reliability in the population; however, the lack of these interactions also suggests no role for GEI in maintaining genetic variation in male signals in this system.

Behavioural signals and displays are especially flexible and their expression can be

changed rapidly, thus we might expect that such traits are more variable within individuals than are morphological characters (Falconer & Mackay, 1996). A signal's repeatability, i.e. the proportion of total variation that is reproducible among repeated measurements of the same subject or group (Lessells & Boag, 1987), indicates its level of consistency over time, and is often used to set an upper limit on trait heritability (Boake, 1989). A recent meta-analysis of repeatability in behavioural traits indicated a surprisingly high coefficient for repeatability of courtship behaviour (higher, in fact, than female preference behaviour repeatability)(Bell *et al.*, 2009). Despite the inherent differences between behavioural and morphological or physiological traits, plasticity in behaviour can be measured like any other phenotypic character (Sih *et al.*, 2004; Nussey *et al.*, 2007; Dingemanse *et al.*, 2010; Ghalambor *et al.*, 2010). While there is abundant evidence that behavioural traits are heritable and that sexually-selected behaviours are prevalent in nature, and despite substantial interest in how genetic variation is maintained in sexually-selected traits, there have been surprisingly few studies on the effects of GEI on behaviourally plastic male signals (Boake *et al.*, 2002; Ingleby *et al.*, 2010).

I conducted a longitudinal study of how male decorated crickets (*Gryllodes sigillatus*) invest in energetically expensive sexual signalling behaviour over their lifespan, using juvenile and adult diet treatments to vary the nutritional environment at these stages. By using inbred lines, I measured how different genotypes responded to environmental variation both during juvenile development and at adulthood. Male crickets (including *G. sigillatus*) signal to females via stridulation of their forewings,

a trait that is costly in terms of energy expenditure as well as in terms of increased predation or parasitisation risk . Among a number of cricket species, increased time spent calling (calling effort) covaries positively with the number of females responding to a calling male, an important factor in male reproductive success (Hunt *et al.*, 2004a; Bentsen *et al.*, 2006; Jacot *et al.*, 2008; Rodríguez-Muñoz *et al.*, 2010). First, I investigated whether male crickets exhibit plastic responses to environmental variation at the juvenile stage in terms of body size, mass, and development rate. After males reached adulthood, I measured male calling effort at weekly intervals to determine whether genotypes vary allocation of resources to this sexually-selected signal with age and across environments. I used a longitudinal analysis of these weekly calling effort measurements to estimate interactions between genotype, age, and environment in determining the expression of the male sexual trait.

3.2 Methods

3.2.1 Cricket maintenance and development of inbred lines

G. sigillatus used in this study were descended from 500 adult crickets collected in Las Cruces, New Mexico in 2001, and used to initiate a laboratory culture maintained at a population size of approximately 5000 crickets and allowed to breed panmictically. Nine inbred lines, denoted as A-I, were created by subjecting a random subset of crickets from this population to 23 generations of full-sib mating followed by 12 generations of panmixis within each line (Ivy *et al.*, 2005). This procedure was conducted

in the absence of any artificial selection, and effectively compartmentalises standing genetic variation in the starting population into discrete units that can be treated as distinct genotypes. Details of maintenance protocols can be found in Section 2.2.1.

3.2.2 Experimental design

I designed the experiment to evaluate the effects of age, nutritional environment (the quality of available resources), and genotype on male reproductive effort. Two different diet treatments were used to manipulate the quality of resources available to experimental subjects. These diets were characterised by high and low levels of a range of nutrients, and were selected on the basis of similar diets used to strong effect in previous studies (Hunt *et al.*, 2004a; Judge *et al.*, 2008; Zajitschek *et al.*, 2009b). The high-nutrient, protein-rich diet (H) consisted of 100% ground dry cat food (Purina, 32% protein), the standard diet on which our laboratory stocks are maintained. The low-nutrient, protein-poor diet (L) was a mixture comprising 50% ground dry cat food and 50% ground oatmeal (Aberfeldy Oatmeal, 13% protein)(proportions by dry weight).

I collected 160 cricket nymphs from each of the 9 inbred lines within 24 hours of hatching. Nymphs were assigned randomly to one of two diet treatments (N=80 for each diet treatment), and reared individually in clear plastic containers ($5 \times 5 \times 5$cm), which contained a shelter, a water bottle plugged with cotton wool, and the food treatment. Containers were cleaned each week, and fresh water and food provided.

Individuals were checked for eclosion to adulthood daily from the fifth larval instar onward; on the day of eclosion, I measured body weight using a high precision electronic balance (Denver Instrument, model PI-225DA), and photographed individuals through a microscope (Motic, model SMZ-168 equipped with Moticam 2000). Measurements of pronotum length were obtained from these photographs using NIH ImageJ software (Schneider *et al.*, 2012). Each individual was then randomly assigned an adult diet, resulting in 4 adult diet treatment groups: LL and HH, in which individuals were maintained on the same diet in juvenile and adult life stages, and LH and HL, in which individuals were switched to the alternate diet following eclosion. I maintained adults in the same manner as nymphs, except that they were provided with twice the volume of food . Adults were also checked daily for survival.

3.2.3 Male reproductive effort

Male reproductive effort was quantified as the time (in seconds) that each individual spent broadcasting his long-distance sexual advertisement call. I measured each male cricket overnight for 12 hours every seven days, beginning at seven days post-eclosion, until death. On the day of measurement, I replaced the lid of each male's container with a lid in which a microphone (C1163, Dick Smith Electronics) was mounted; each container was then placed into a hollowed-out cube of sound-proofing foam so as to minimise outside disturbance and ensure there was no crosstalk between containers. An electronic acoustic recording system (Bertram & Johnson, 1998)

sampled from the microphone of each individual cricket container 10 times per second to capture whether or not a male was calling.

3.2.4 Statistical analysis

All analyses were performed using R 3.0.2 (R Core Team, 2013). For linear regression models, categorical input variables such as juvenile diet and adult diet were coded using binary dummy variables to aid interpretation of standardised coefficients (Gelman & Hill, 2007). Numeric input variables such as age were standardised by centring (subtracting the mean) and scaling (dividing by 2 standard deviations), putting them on a common scale with each other and with the binary predictors (Gelman & Hill, 2007; Gelman, 2008; Schielzeth, 2010). Independence between linear and quadratic forms of numeric predictors (e.g., age and age^2) was achieved by standardising the input variable before squaring (Gelman & Hill, 2007).

I used a combination of cross-environment genetic correlation estimations and linear regression models to estimate the magnitude of genotype-by-environment interactions. A trait measured in two environments can be considered two separate traits for the purpose of calculating the cross-environment genetic correlation (Falconer & Mackay, 1996). If the cross-environment genetic correlation is high, performance in two different environments represents very nearly the same character, determined by very nearly the same set of alleles (Falconer & Mackay, 1996). Conversely, a low (or negative) correlation would mean that high performance requires a different set

of alleles in each environment. A cross-environment genetic correlation significantly different from 1 therefore indicates a genotype $\times$ environment interaction, in which the 'best' alleles in one environment are not necessarily the best in another. For labile traits, the genetic correlation can also be estimated across two age classes; a low genetic correlation would indicate genotype $\times$ age interaction, where the 'best' genotype at one age is not the best at another. This concept could be extended still further, to assess whether performance at some age in one environment predicts performance at another age in a different environment.

In this study, I generated estimates of genetic correlations across all combinations of environments and ages in which I measured male calling effort, many of which were based on relatively small sample sizes (due to differences in individual lifespan, and in whether a male called or not at each age). To avoid overinterpreting these low power analyses, I first used linear regression to determine the effects of genotype, diet and age on male calling effort. In linear regression models, genotype-by-environment interactions (GEIs) can be inferred from line $\times$ diet, line $\times$ age and line $\times$ diet $\times$ age interactions. These interactions should reduce the chances of observing significant line and diet effects, and thus correspond to reduced genetic correlations across environments (Falconer & Mackay, 1996; Lynch & Walsh, 1998) or ages.

To verify that linear regression and genetic correlation estimation return similar results, I used both methods for inferring GEI on measurements of juvenile development traits (pronotum size and body mass at eclosion, and the rate of development).

First, I used ANOVA to determine whether line × diet interaction exists for each of these traits. I then used multiple methods for estimating cross-environment genetic correlations to check whether they provide quantitatively similar results with my data. Astles *et al.* (2006) recommend the use of variance components when assessing the relative strength of correlations, but the use of family mean correlations is acceptable for simple tests of whether the correlation is significantly different from zero. I tested these methods (jackknifed family means, and variance components via least squares ANOVA and REML) alongside variance components estimated using Bayesian inference via MCMC (Hadfield, 2010). If the genetic correlation estimates for each trait are similar to linear regression results for juvenile traits, this supports the use of linear regression for estimating GEI in behavioural signalling.

Genotype and diet effects on juvenile development

To test for effects of diet and genotype on developmental traits during the juvenile stage, I used univariate ANOVAs for each of development rate to eclosion, mass at eclosion, and pronotum length at eclosion. I first estimated the cross-environment genetic correlation using the family means method; in the standard procedure, the estimate is the Pearson product-moment correlation between line means for each environment (Falconer & Mackay, 1996). This method is known to have a downward bias, so I used the jackknife technique described below to estimate correlations across environments, as simulations have shown that this provides a less biased estimate when the number of lines included in the analysis is small (<20)(Roff & Preziosi,

1994). Briefly, the genetic correlation between two traits is estimated using mean values for each line; a sequence of N (here, N = 8) pseudovalues is first calculated by dropping each line in turn, then estimating the Pearson product-moment correlation from the remaining values using the following formula:

$$S_{N,i} = Nr_N - (N-1)r_{N-1,i}$$

Where $S_{N,i}$ is the ith pseudovalue, r_N is the genetic correlation using all N lines, and $r_{N-1,i}$ is the genetic correlation estimated having dropped the ith line. The jackknife estimate of the genetic correlation is the mean of these pseudovalues, and an estimate of the standard error is given by:

$$SE = \sum_{i=1}^{i=N} (S_{N,i} - r_j)^2 / N(N-1)$$

I then estimated the genetic correlation across environments using variance components methods: least squares ANOVA, restricted error maximum likelihood (REML), and Bayesian inference. For least squares ANOVA, I used the following formula to calculate the genetic correlation:

$$r_g = V_F / \sqrt{(V_F V_{F\times E})}$$

V_F is the variance component due to overall family effects, and $V_{F\times E}$ is the variance component of the interaction between family and environment, calculated from a two-way ANOVA (Lynch & Walsh, 1998):

$$V_{F\times E} = MS_{F\times E} - MS_{Err}/n$$

where $MS_{F\times E}$ is the family-by-environment mean squares, MS_{Err} is the error term mean squares, and n is the approximation for the number of offspring per line per environment calculated using the general formula for n when group sizes are uneven (Sokal & Rohlf, 2012).

For the REML approach, I used the R package lme4 (Bates *et al.*, 2013) to fit a model to the data for both environments, with no constraints on the genetic variance across treatments. This model returns estimates of $Cov_{E1,E2}$, V_{E1} and V_{E2}, which I used to estimate the genetic correlation via the following equation:

$$r_g = Cov_{E1,E2}/\sqrt{(V_{E1}V_{E2})}$$

A similar technique was used to calculate the genetic correlation via Bayesian methods; I used the MCMCglmm R package (Hadfield, 2010) to fit an unstructured variance-covariance matrix to the data and create posterior distributions for the genetic variance in each diet treatment group, in addition to the covariance between them. I then applied the above formula to these distributions, and took the mode of the resultant distribution as the estimate of the genetic correlation across environments. The 95% highest posterior density interval of the distribution gives a 'credible interval' for the estimate, and indicates whether the estimate is significantly different from -1/0/1 when the interval does not include -1/0/1.

Genotype, treatment and age effects on adult survival and calling effort

I used Cox regression to test for the effects of juvenile diet, adult diet, genotype and all interactions on adult survival (i.e., after reaching eclosion). Weekly calling effort measurements were over-dispersed and had an excess of zeroes. I removed measurements of 0 seconds from the data set, restricting the analysis to only the variation in calling rather than presence or absence of a call. Measurements of calling effort were then log-transformed to conform to a Gaussian distribution. There were insufficient males calling from line A across the diet treatments, so all individuals from this line were excluded from the analysis of calling effort.

I investigated the causes of variation in weekly calling effort using a mixed-effects model in the R package lme4 (Bates *et al.*, 2013), which enabled me to group each male's measurements using an individual-level random effect. As male measurements were taken weekly, I first used the Akaike Information Criterion (AIC) to test whether random slopes for individual age provided a better fit to the data. Following Zuur *et al.* (2009), I used the full model (i.e., with all main effects and interactions of interest included in the fixed effects specification; see below) with REML estimation to test 3 different random effects structures: individual intercept only, individual intercept and linear slope for age, and individual intercept and both linear and quadratic slope for age. AIC showed that the best starting model allowed individuals to vary in both intercept and linear age-related slope (see Section 3.3).

My starting model for simplification thus contained all main effects and interactions of interest as fixed effects, and the random effects structure as specified above. Table 3.1 lists the main effects and interactions in the starting model, and how I have interpreted each such predictor of calling effort. I estimated this model using ML and performed model simplification by removing covariates from the model systematically, starting with the highest-order interactions, and using the likelihood ratio test to compare models. Finally, I refit the 'best' model in lme4 with REML estimation.

Table 3.1: Terms and interpretations of the model for genotype, treatment and age effects.

Term	Interpretation
Line	Genotypes differ in their levels of calling effort. A strong line effect indicates genetic variation for this trait.
Diet	Calling effort shows plasticity as a function of diet. I have pooled juvenile diet, adult diet and their interaction as sources of environmental variation, having previously shown that effects of diet on calling effort are not restricted solely to diet at a single life stage (see Chapter 2).

Age	Calling effort varies with an individual's age. I tested for both a constant amount of change with age (the linear age term) and a change in the linear amount of change with age (quadratic age term). Age-dependent changes in calling effort can reduce the strict correspondence between genotype and phenotype, especially if the population is age-structured (i.e., there are differences in the ages of individuals within the population), or if there is variation in the rate at which males reach sexual maturity (Kokko, 1997).
Line $\times$ Diet	Phenotype is influenced by diet, and genotypes do not all respond equivalently. If there is ecological crossover such that no single genotype is superior to all others in the environmentally relevant parameter space, spatial or temporal variation (combined with dispersal or overlapping generations respectively) can decrease the value of the signal as an indicator of potential indirect genetic benefits.

Line × Age	Genotypes differ in their age-related patterns of calling effort. This effect can also be considered a form of G×E, and similar considerations apply: if G×Age is strong enough that no single genotype is superior to all others at all ages, variation in the age structure of the population can decrease the value of the signal as an indicator of potential indirect genetic benefits.
Diet × Age	The response of calling effort to age depends on diet. As with environmental plasticity, the timing of migration across environments could decrease the correspondence between calling effort and potential indirect benefits.
Line × Diet × Age	Genotypes differ in their age-related patterns of calling effort, and these patterns themselves depend upon diet.

I also estimated genetic correlations and their 95% credible intervals for calling effort in a subset of the possible cross-diet and cross-age combinations (chosen because this subset is representative of high-power estimates across the many possible cross diet and age groups), using variance components from a Bayesian analysis implemented in MCMCglmm (as described in Section 3.2.4). Calling effort measurements of 0 seconds were removed from the data set, and individuals from lines A and C were excluded so as to ensure at least 2 individuals from each line, diet and age combination

recorded calls. For cross-diet correlations, I used measurements from weeks 2, 4 and 6 to show to what extent performance is correlated across different diet treatments, and whether this changes with age. Cross-age correlations are calculated separately for each diet treatment; I estimated the correlation between calling effort at week 1 and at weeks 2, 4, 6 to show how well performance at one age predicts performance at future ages.

3.3 Results

Of the 1440 nymphs raised individually from hatching, 676 eclosed to adulthood successfully: 352 from the high-nutrient diet (180 females, 172 males), and 324 from the low-nutrient diet (172 females, 152 males). The full breakdown of numbers surviving to adulthood by diet, line and sex is given in Table 3.2. Cox regression showed that juvenile diet had a significant effect on the rate of individuals surviving from hatching to eclosion, with those on the high-nutrient diet reaching adulthood more quickly ($\chi^2 = 10.956, d.f. = 1, P<0.001$; Figure 3.1). However, this was complicated by an interaction between juvenile diet and genetic line, such that some genotypes experienced especially longer development times on a low diet ($\chi^2 = 28.960, d.f. = 8, P<0.001$; Figure 3.2).

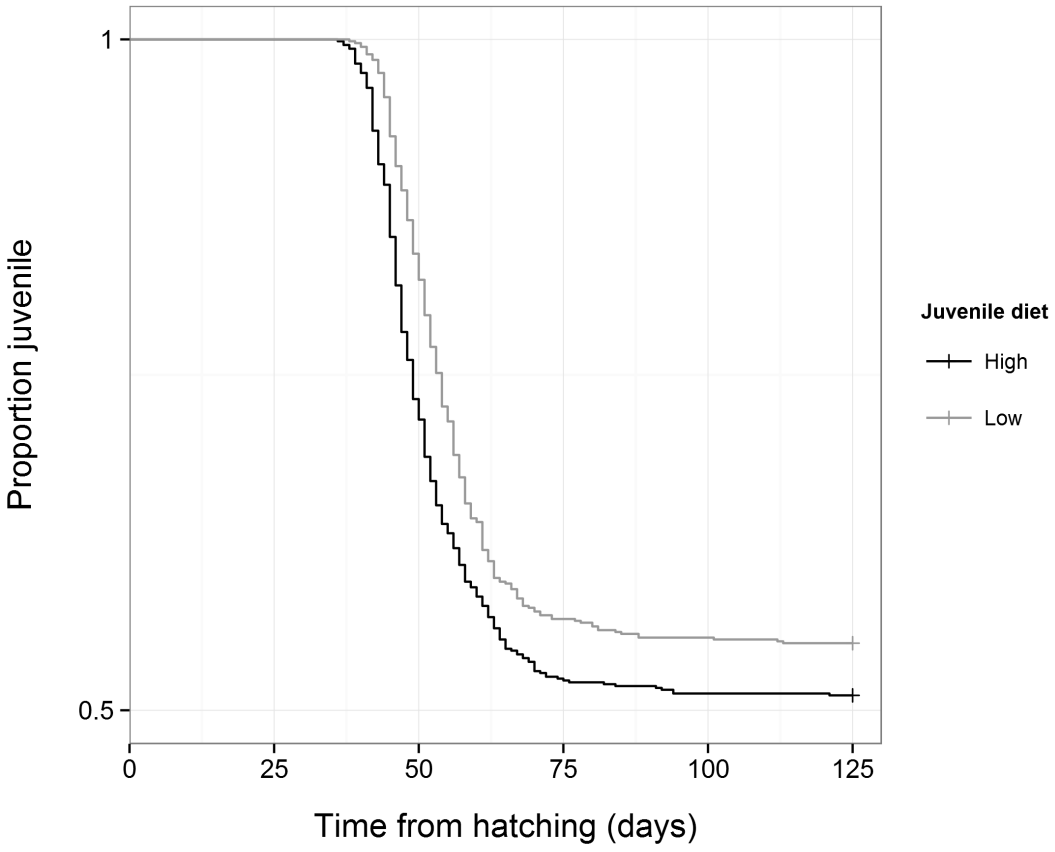

Figure 3.1: The effect of high (black line) and low (grey line) juvenile diet on the rate of development to adulthood. Measurements are pooled across all inbred lines, and are censored due to individuals that died before eclosing.

Table 3.2: Counts for survival to eclosion for each line, diet, and sex.

	High		Low	
	352		324	
	Female	Male	Female	Male
All	180	172	172	152
A	19	6	21	11
B	24	29	18	11
C	8	12	12	9
D	16	16	12	13
E	13	14	11	21
F	36	32	35	31
G	14	13	13	19
H	29	24	33	23
I	21	26	17	14

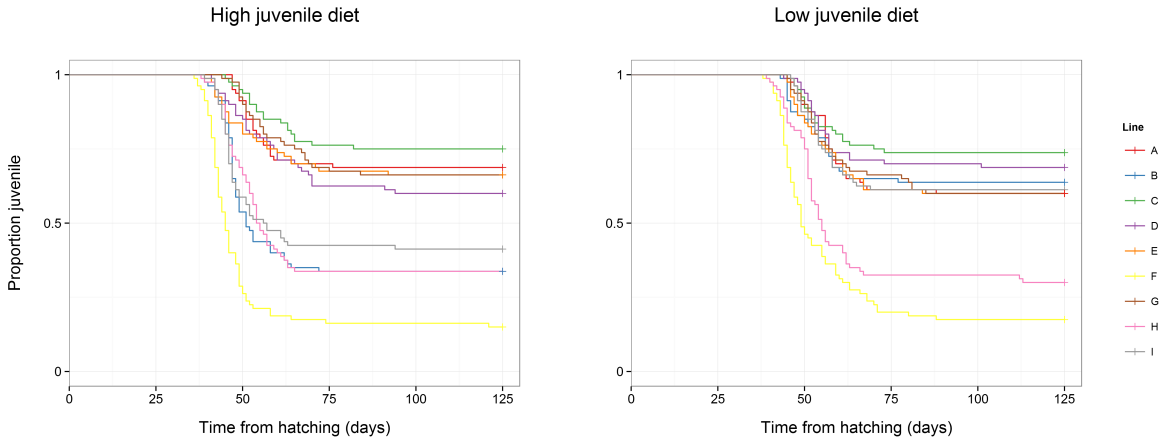

Figure 3.2: Line-specific development rates, plotted separately for each juvenile diet treatment. Measurements are censored due to individuals that died before eclosing.

3.3.1 Juvenile development

Multivariate ANOVA showed that the high quality juvenile diet typically increased development rate, pronotum length and body mass at eclosion (Table 3.3), but that there were clear differences between genotypes. Furthermore, the effect of diet depended on line. To determine which traits accounted for this multivariate effect, I conducted separate univariate ANOVAs for each trait (Table 3.4). The juvenile diet and line effects were sustained in the models for pronotum length and mass at eclosion, but the interaction effect was not statistically supported for these traits: Figure 3.3 shows clear differences between lines, and H diet individuals have greater body mass and pronotum length in each line. In contrast, for the rate of development to eclosion, we found that the effect of diet depended on line; Figure 3.3 shows clear differences between lines but an inconsistent effect of juvenile diet that depends on the genotype in question.

Table 3.3: MANOVA for development rate, mass and size at eclosion, including both sexes and all lines.

Parameter	Df	Pillai's trace	F	num Df	den Df	P	
Line	8	0.844	14.982	24	918	$<$0.001	***
Juvenile diet	1	0.132	15.385	3	304	$<$0.001	***
Juvenile diet $\times$ line	8	0.130	1.735	24	918	0.016	*

Estimates of the cross-environment genetic correlation for mass at eclosion and pronotum length for the two juvenile diet treatments were very close to 1 regardless of the estimation method (Table 3.5). The Bayesian 95% credible intervals do not overlap

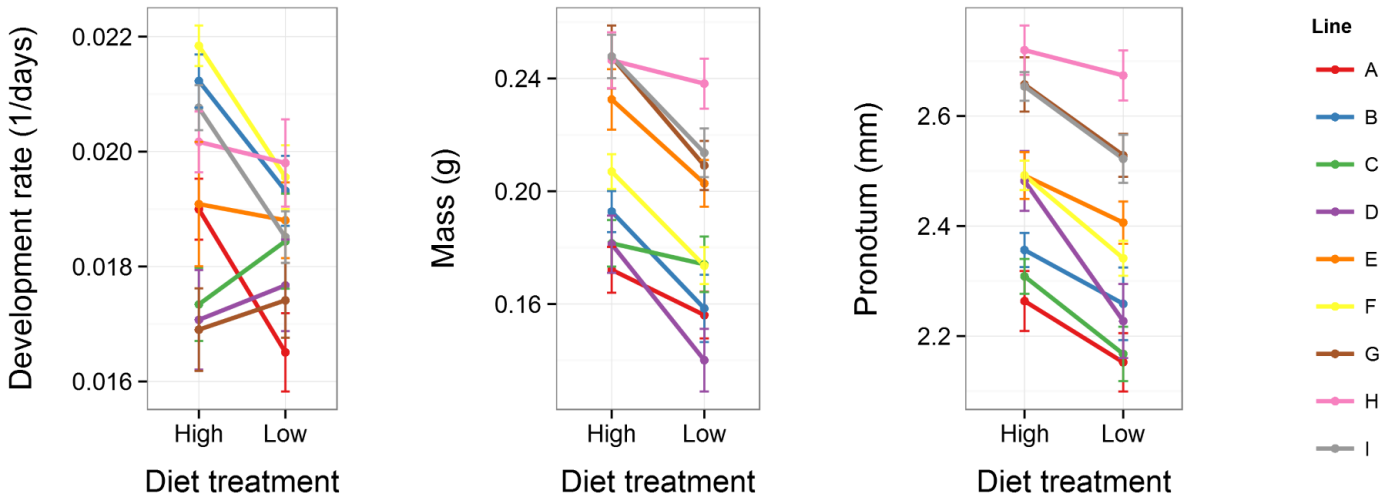

Figure 3.3: Reaction norms for traits measured at eclosion to adulthood in male crickets (development rate, mass, pronotum length; line means $\pm$ S.E.) across juvenile diet treatments.

Table 3.4: Univariate ANOVAs for development rate, mass and size at eclosion.

Trait	Parameter	df	F	P	
Development rate (1/days)	Juvenile diet	1	16.412	<0.001	***
	Line	8	9.474	<0.001	***
	Juvenile diet × Line	8	2.078	0.038	*
Mass (g)	Juvenile diet	1	36.480	<0.001	***
	Line	8	21.940	<0.001	***
	Juvenile diet × Line	8	0.899	0.517	
Pronotum length (mm)	Juvenile diet	1	33.41	<0.001	***
	Line	8	28.55	<0.001	***
	Juvenile diet × Line	8	0.880	0.534	

Table 3.5: Estimates of genetic correlations for developmental traits across juvenile diets, using multiple variance component methods. 95% confidence intervals for each correlation estimate are given in brackets where available.

Method	Development rate	Mass	Pronotum length
Family means	0.650 (0.104, 1)	0.919 (0.720, 1)	0.950 (0.822, 1)
Jackknifed family means	0.610 (0.524, 0.696)	0.903 (0.901, 0.905)	0.952 (0.950, 0.954)
LS ANOVA	0.774	1.010	1.009
Jackknifed LS ANOVA	0.768 (0.754, 0.782)	0.998 (0.994, 1)	1.001 (0.999, 1.002)
REML	1	1	1
Bayesian (MCMCglmm)	0.513 (-0.578, 0.979)	0.961 (0.448, 0.997)	0.976 (0.559, 1.000)

0, indicating that the estimate is significantly greater than 0. A genetic correlation close to 1 indicates that all genotypes react in the same way to a change in the environment. Reaction norms show mostly parallel effects of the diets among genotypes for mass and pronotum length, indicating no significant genotype-by-environment interaction (Figure 3.3).

The estimate for the genetic correlation across environments in development rate is lower than those for morphological characters in all methods except REML (where the model drives the correlation to -/+1 if the random effects have little explanatory power). The 95% credible intervals for Bayesian methods overlap 0 for development rate, indicating that the correlation is not significantly different from 0. The lower estimate of cross-environment genetic correlation for male development rate is consistent with the reaction norms that show changes in both the rank order of genotypes and the scale of variation across environments (Figure 3.3).

3.3.2 Adult survival and reproductive effort

Cox regression showed that lines differed significantly in survival rates after reaching adulthood (Table 3.6). I found a significant effect of juvenile diet; generally, crickets fed a low-quality juvenile diet tended to live longer (Figure 3.4). However, the effects of both juvenile and adult diet on survival were strongly genotype-dependent (Figure 3.5).

The best-fit random effects model for the effects of diet and genotype on male calling effort included a varying intercept and linear age slope for each individual (see Table 3.7). Removing higher order interactions from the starting model did not produce a better fit according to likelihood ratio testing, and diagnostic plots indicated that the full model with all main effects and interactions (see Table 3.1 for details of each) retained was a good fit to the data. Table 3.8 shows how the addition of each

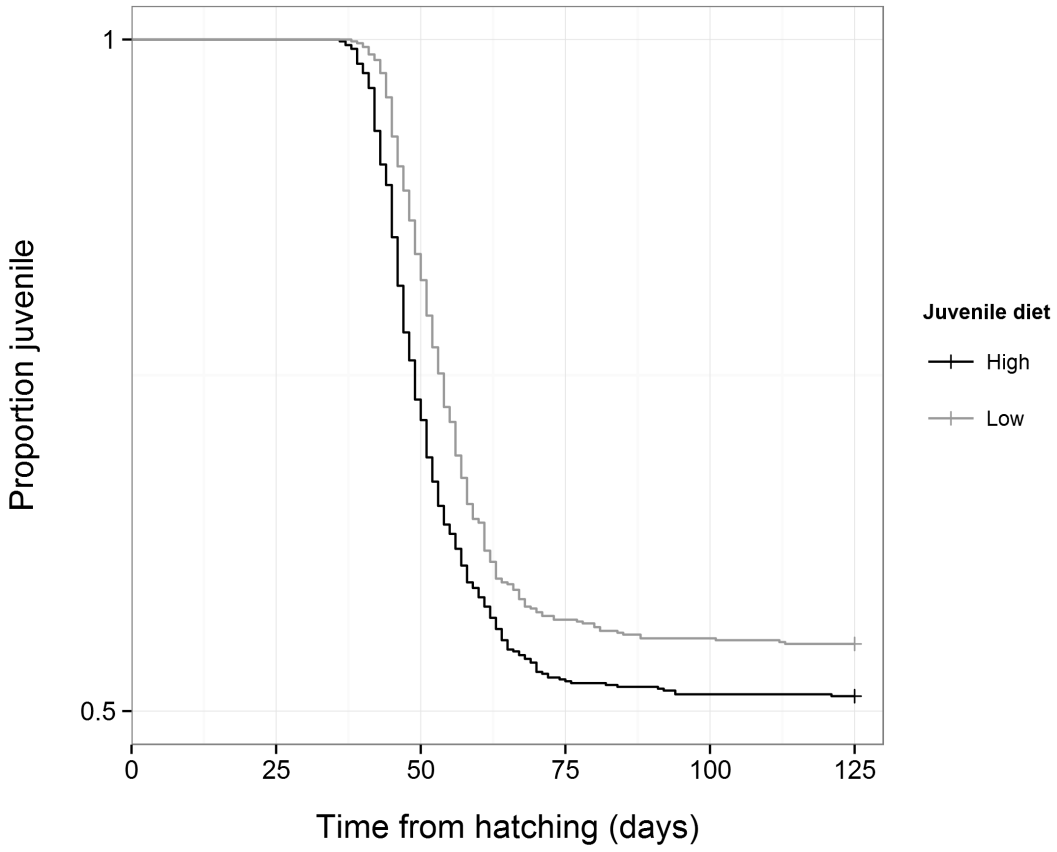

Figure 3.4: The effect of high (black line) and low (grey line) juvenile diet on survival rates post-eclosion. Measurements are pooled across adult diets and all inbred lines.

Table 3.6: Cox regression survival analysis for effects of juvenile diet, adult diet, genetic line and all interactions on adult survival post-eclosion.

	df	χ^2	P	
Juvenile diet	1	3.909	0.048	*
Adult diet	1	0.160	0.690	
Line	8	57.111	<0.001	***
Juvenile diet × Adult diet	1	0.008	0.930	
Juvenile diet × Line	8	11.357	0.182	
Adult diet × Line	8	5.374	0.717	
Juvenile diet × Adult diet × Line	8	16.064	0.041	*
Full model R^2 = 0.25; Wald = 110.2 on 35 df, P <0.001				

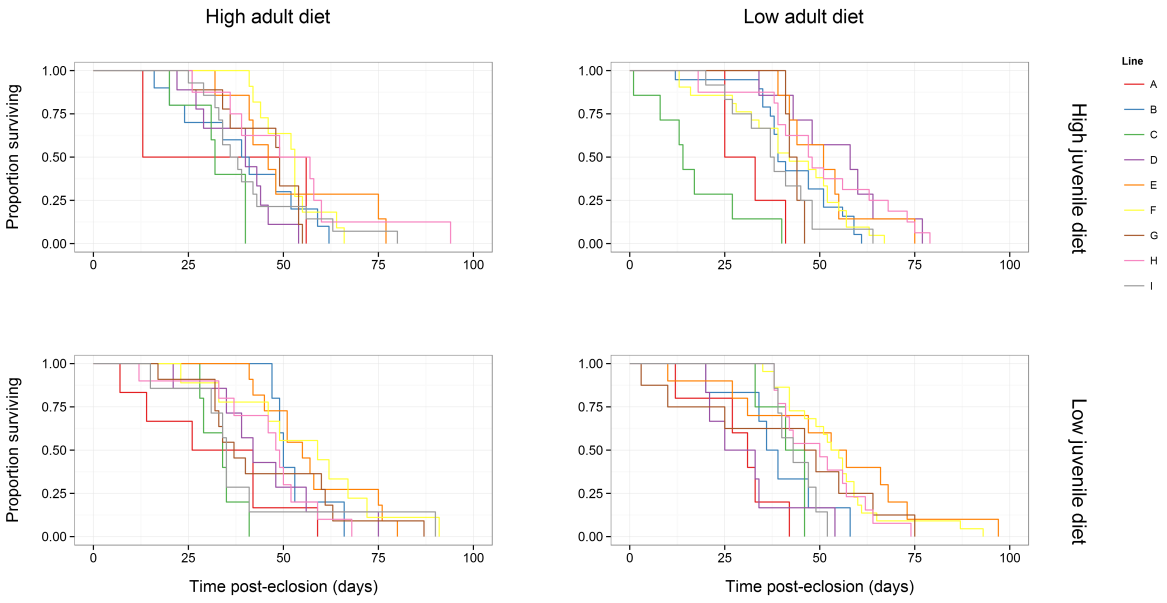

Figure 3.5: Line-specific survival rates, plotted separately for each combination of juvenile and adult diet.

predictor term affected the model deviance, AIC, and marginal R^2 (the variance explained by the fixed effects only Nakagawa & Schielzeth, 2013). Several predictors result in large jumps in each of these assessment statistics, including line, age, and their interaction term, as well as the three-way interaction between line, age and diet. Diet treatment only had explanatory power in the model when interacting with other predictor variables; visual inspection of the family and overall means across ages and diets shows that the overall mean nightly calling effort does not differ greatly across diet treatments (Figure 3.6).

Table 3.7: Selection of random effects specification for lme4 analysis of weekly calling effort (excluding Line A). Column 'df' gives the equivalent degrees of freedom for the fitted model.

Model	df	Random effects structure	AIC	ΔAIC
0	98	(1 | ID)	4307.6	N/A
1	100	(1 + age | ID)	4305.7	-1.9
2	103	(1 + age + age^2 | ID)	4310.4	4.6

Table 3.9 shows cross-environment genetic correlations for measurements at weeks 2, 4 and 6 post-eclosion. In most cases, the credible intervals overlap zero, indicating that the correlation is not significantly different from zero. The genetic correlation estimates in week 4 are high and positive, with smaller intervals than the other weeks shown; the correlation between diets LL and LH in particular is within a 95% credible interval that does not include zero ($r_{LL(4),LH(4)} = 0.89(0.04, 0.99)$). Reaction norms for this comparison show the mean values of calling effort for each line in diet treatments LL and LH at week 4; the slopes are close to parallel, indicating that per-

Table 3.8: Degrees of freedom, deviance, AIC and marginal R^2 changes as fixed effects are added to the base model of weekly calling effort.

Model	Added fixed effect	df	Deviance	ΔDeviance	AIC	ΔAIC	Marginal R^2
0	Intercept	5	4428.0	N/A	4438.0	N/A	0
1	Line	12	4352.0	-76.0	4376.0	-62.0	0.133
2	Diet	15	4356.8	4.9	4386.8	10.9	0.134
3	Age	16	4342.1	-14.7	4374.1	-12.7	0.157
4	Age^2	17	4333.1	-9.0	4367.1	-7.0	0.156
5	Diet × Age	20	4330.0	-3.2	4370.0	2.8	0.162
6	Diet × Age^2	23	4326.5	-3.5	4372.5	2.5	0.163
7	Line × Diet	44	4302.5	-24.0	4390.5	18.0	0.172
8	Line × Age	51	4282.6	-19.9	4384.6	-5.9	0.191
9	Line × Age^2	58	4267.0	-15.7	4383.0	-1.7	0.189
10	Line × Diet × Age	79	4196.8	-70.2	4354.8	-28.2	0.210
11	Line × Diet × Age^2	100	4105.7	-91.1	4305.7	-49.1	0.219

formance of a genotype at this age in one treatment strongly predicts performance at the same age in the other (Figure 3.7). In contrast, reaction norms for mean values of calling effort for each line in diets LL and HL in week 2 show both ecological crossover and a change in scale (Figure 3.8), reflected in a genetic correlation estimate close to zero and with very large credible intervals ($r_{LL(2),HL(2)} = -0.04(-0.87, 0.80)$).

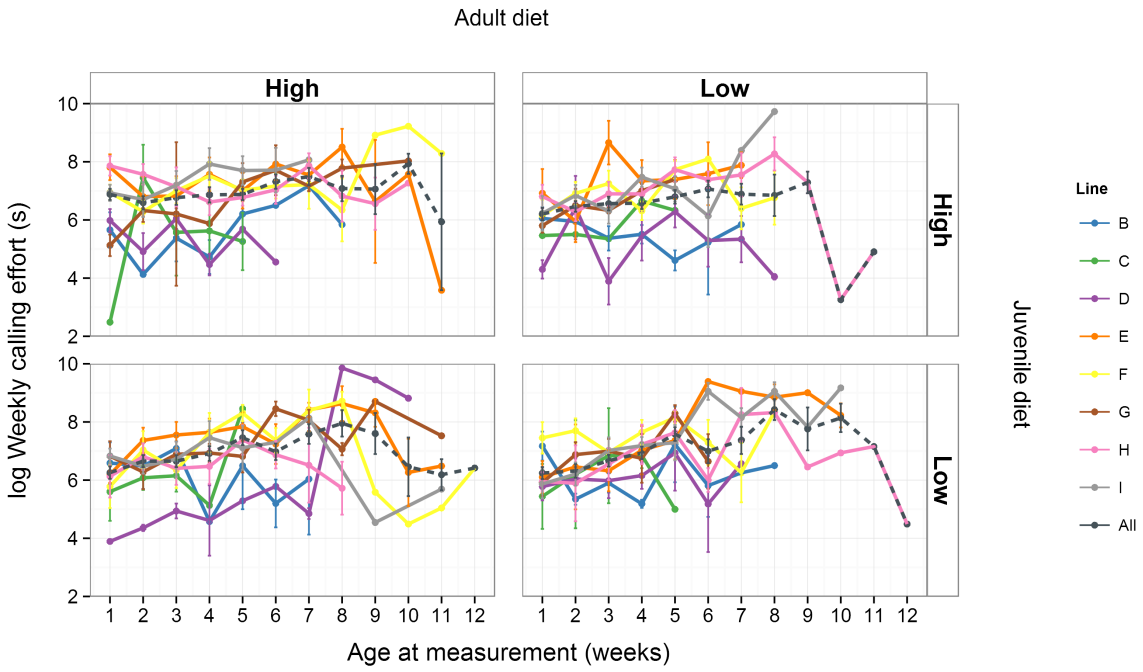

Figure 3.6: Mean log calling effort (±S.E.) over all weeks, separately for each line and diet treatment. The broken line indicates the overall mean in each treatment.

Table 3.9: Cross-environment genetic correlations for calling effort (measured in weeks 2, 4, 6). Values are posterior modes and 95% credible intervals from a Bayesian analysis using MCMCglmm.

	HH	HL	LH
Week 2			
HL	0.49 (-0.70, 0.88)		
LH	0.14 (-0.72, 0.91)	-0.31 (-0.86, 0.81)	
LL	0.17 (-0.79, 0.84)	-0.04 (-0.87, 0.80)	0.54 (-0.63, 0.94)
Week 4			
HL	0.81 (-0.24, 1.00)		
LH	0.90 (-0.20, 1.00)	0.85 (-0.08, 1.00)	
LL	0.91 (-0.21, 1.00)	0.91 (-0.04, 1.00)	0.89 (0.04, 0.99)
Week 6			
HL	0.57 (-0.60, 0.94)		
LH	0.26 (-0.63, 0.94)	0.75 (-0.44, 0.98)	
LL	0.39 (-0.57, 0.97)	0.58 (-0.58, 0.98)	0.44 (-0.63, 0.99)

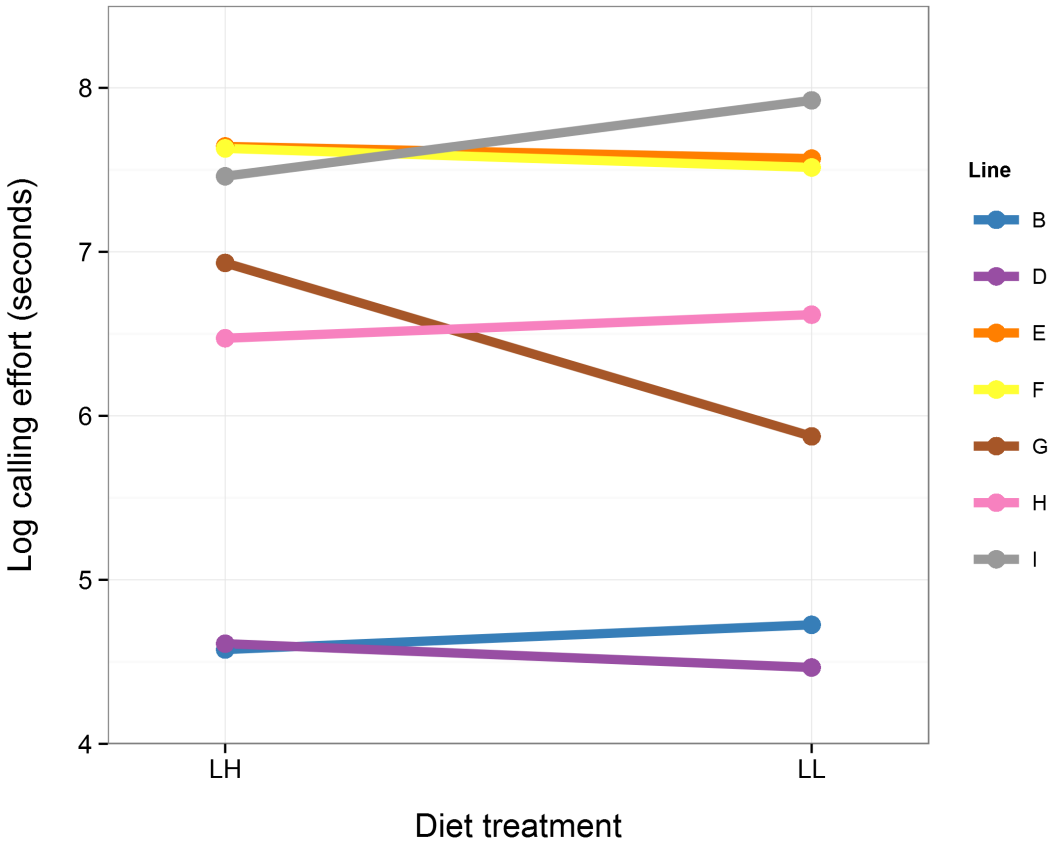

Figure 3.7: Reaction norms for log-transformed calling effort across diet treatments LH and LL at week 4. Points indicate the mean value for each genetic line in the different dietary environments.

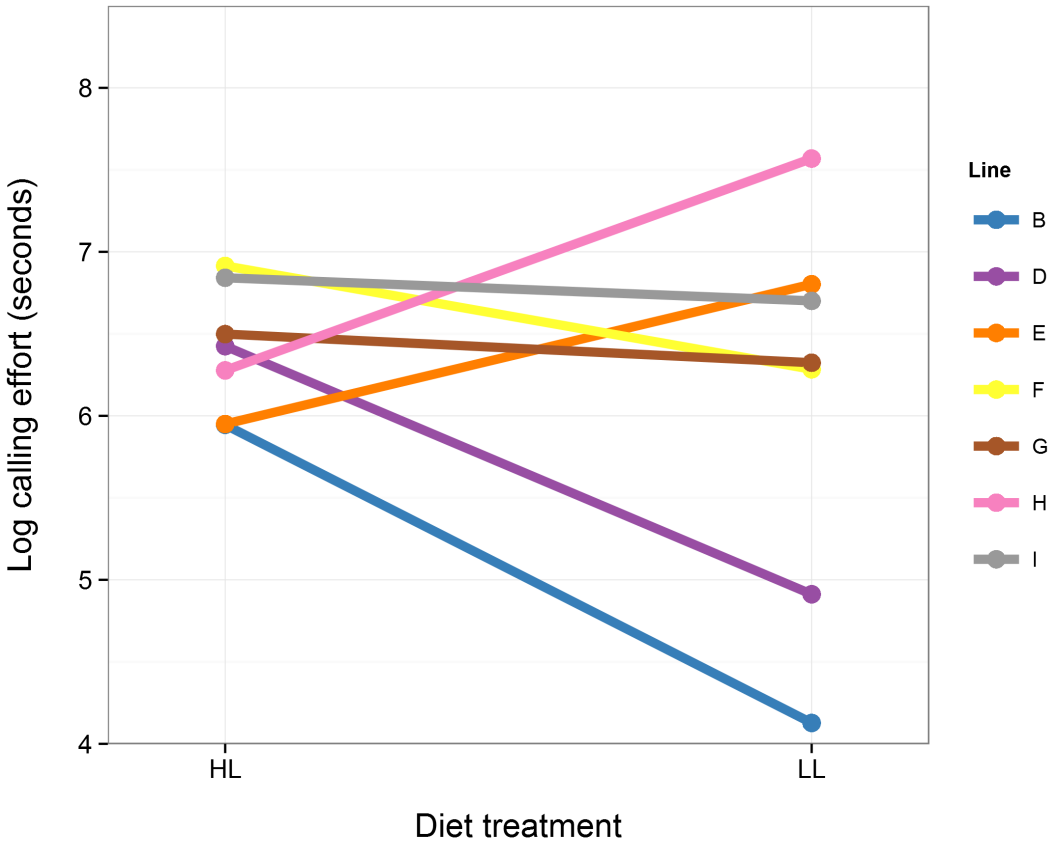

Figure 3.8: Reaction norms for log-transformed calling effort across diet treatments HL and LL at week 2. Points indicate the mean value for each genetic line in the different dietary environments.

A subset of cross-age genetic correlations (within diet treatments) shows that the correlation between calling effort at the first measurement and subsequent measurements tends to decay with time (Figure 3.9). This is most evident within diet treatment group LL: the correlation between ages 7 and 14 days post-eclosion is strongly positive with a credible interval that does not overlap zero ($r_{LL(1),LL(2)} = 0.64(0.07, 0.93)$), and reaction norms for mean values of calling effort across these ages shows largely parallel change (Figure 3.10). The correlation between ages 7 and 42 days in this group is closer to zero and with a wider interval ($r_{LL(1),LL(6)} = 0.02(-0.47, 0.70)$), and the reaction norms show greater crossover between performance at these two ages (Figure 3.11).

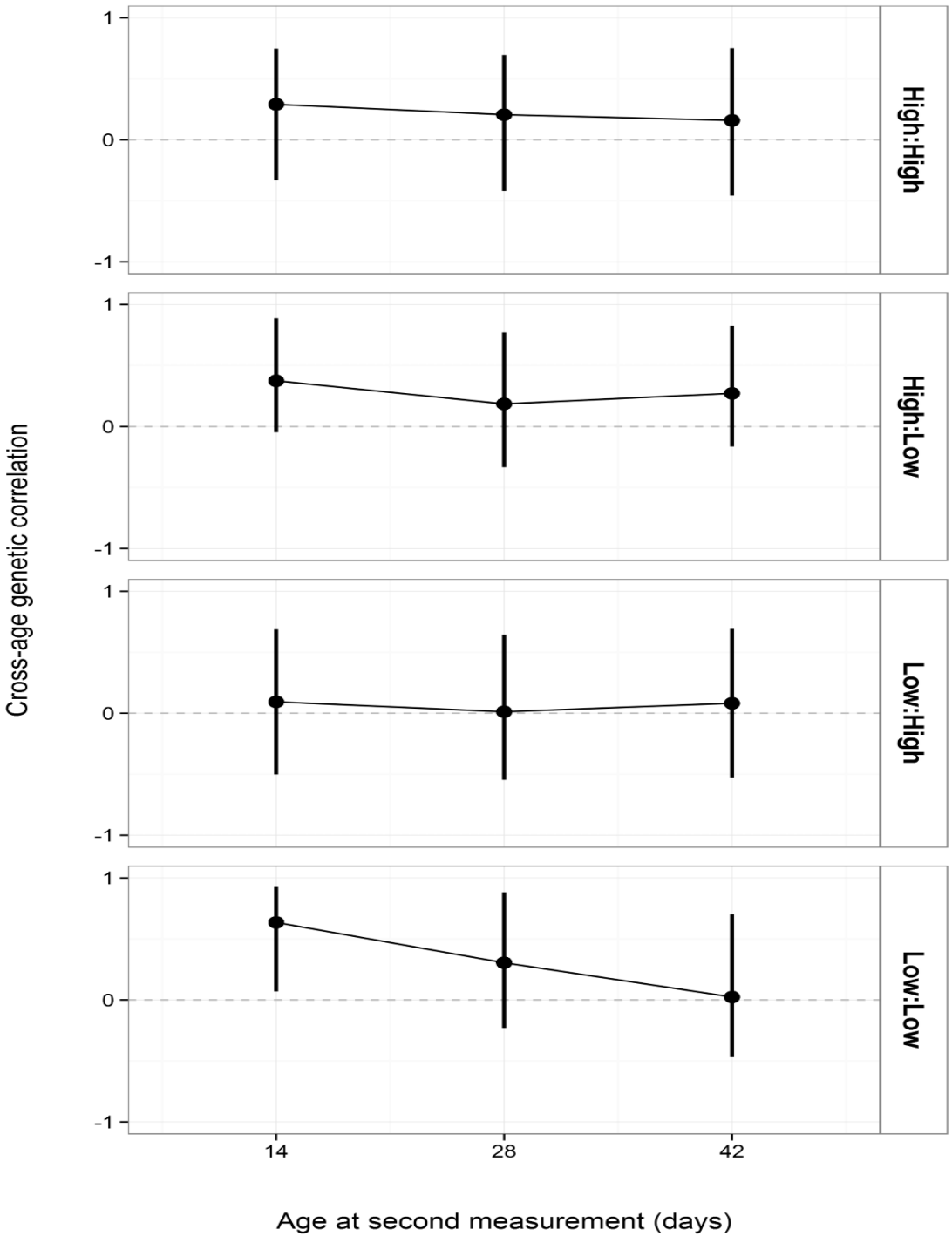

Figure 3.9: Estimates of cross-age genetic correlations between the first measurement (7 days post-eclosion) and measurements at ages 14, 28 and 42 days, separately for each diet treatment. Vertical bars indicate the 95% credible intervals for each estimate.

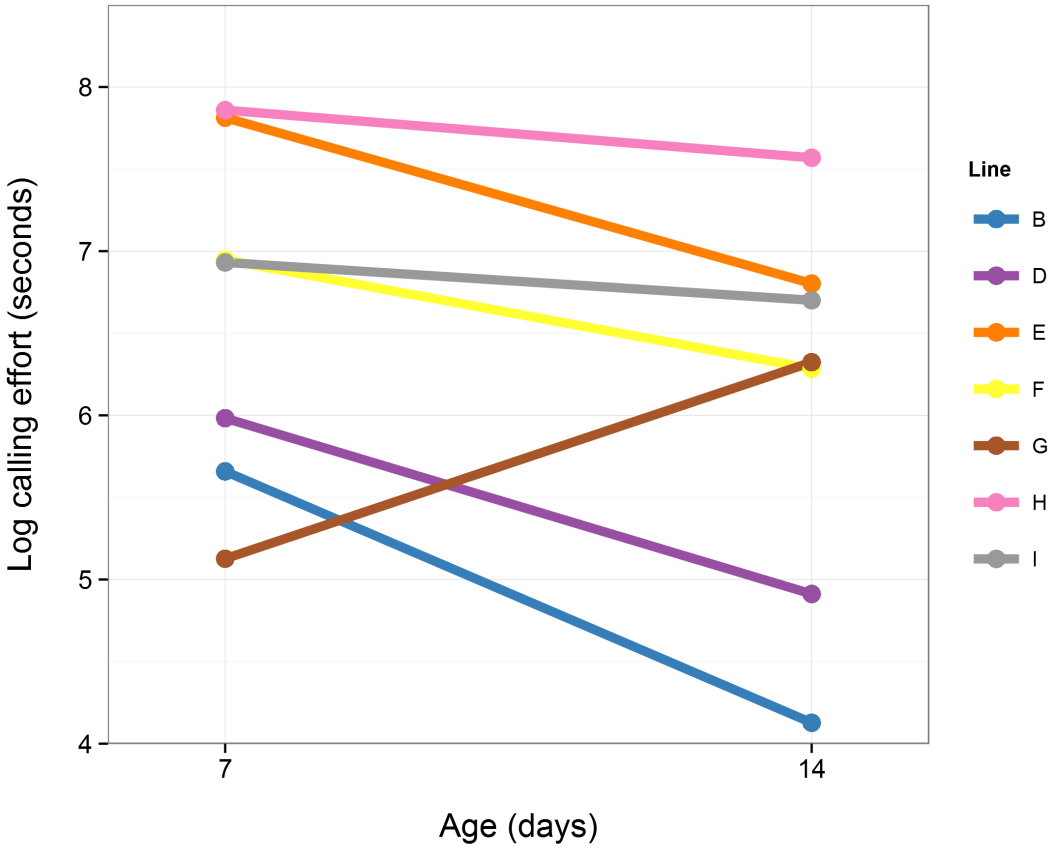

Figure 3.10: Reaction norms for log-transformed calling effort across ages 7 and 14 days post-eclosion for diet treatment group LL. Points indicate the mean value for each genetic line at these age groups.

3.4 Discussion

Diet-mediated phenotypic plasticity was evident from measurements taken at the end of the juvenile stage, with male crickets reared on a high-nutrient diet signifi-

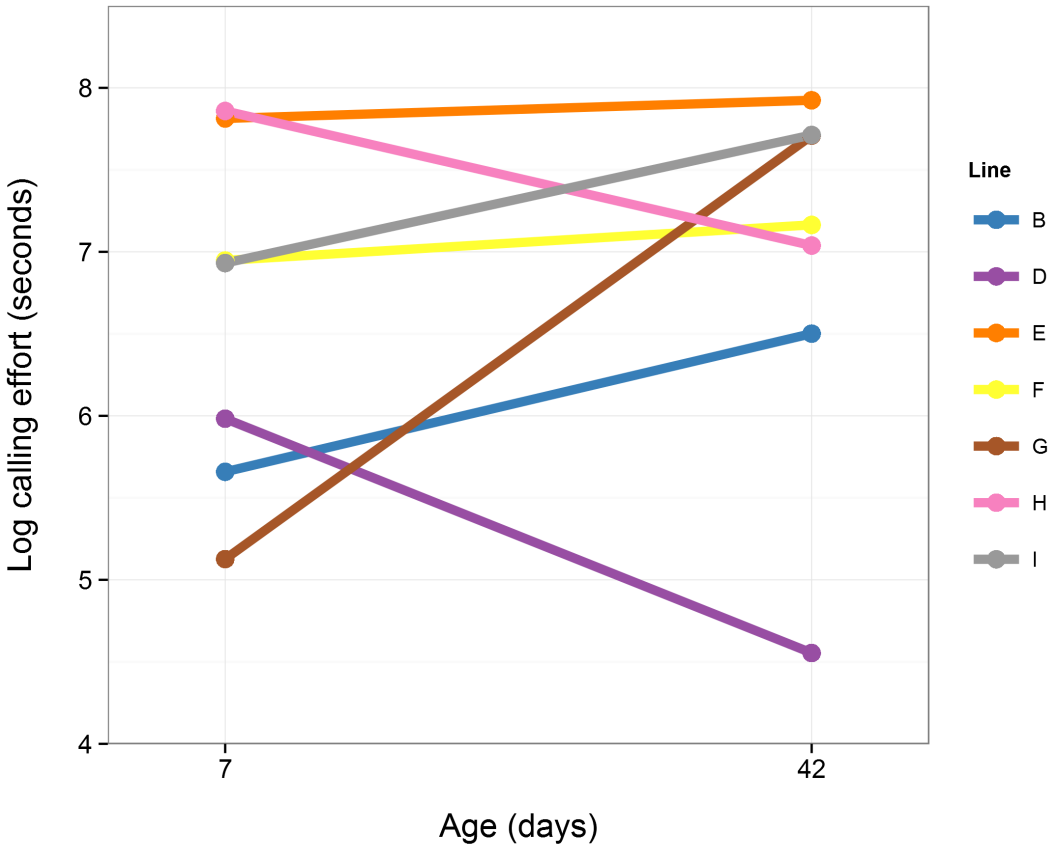

Figure 3.11: Reaction norms for log-transformed calling effort across ages 7 and 42 days post-eclosion for diet treatment group LL. Points indicate the mean value for each genetic line at these age groups.

cantly larger and heavier than those fed a low-nutrient diet. However, of the measurements available upon eclosion to adulthood, only the rate of development revealed strong differences in how genotypes responded to the diet. As predicted, this interaction resulted in a reduced genetic correlation across environments for development rate, while the cross-environment genetic correlation for pronotum length and mass were very close to 1.

The effect of genetic line on survival after reaching adulthood was also dependent on both juvenile and adult diet. In Chapter 2, I showed that dietary nutrition at both juvenile and adult stages affects age-related patterns of calling effort in adult *G. sigillatus*, and that a positive correlation exists between calling effort and longevity. However, measuring populations or groups can mask the underlying signalling dynamics occurring at the individual level (Lindström *et al.*, 2009). Here, the use of inbred lines allowed me to inspect how genetically identical individuals invest in sexual signalling over their lifetime, and when exposed to different nutritional environments. Calling effort was strongly influenced by the effects of and dependencies between genetic line, age, and diet treatment. The linear regression model of all calling measurements was supported by sample estimates of cross-environment and cross-age genetic correlations. The cross-environment genetic correlations varied over combinations of diet treatments, and the strength of these correlations also varied over time. Cross-age genetic correlations within diet treatments showed variation arising from the combinations of ages tested; diet treatment also affected how each genotype allocated to calling with age.

3.4.1 Phenotypic plasticity and genotype-by-age interactions

The phenotypic flexibility of behavioural signalling means that individuals have the ability to adjust their behaviour to act in an optimal fashion, within given constraints (in this case, the energetic requirements of calling)(Piersma & Drent, 2003). The strong effects of age on calling effort show that the intensity of signalling is liable to change over time, similar to patterns recorded in other cricket species (Judge *et al.*, 2008; Zajitschek *et al.*, 2009b). However, while an optimally behaving high-quality male might be expected to always signal more than a lower quality male (Kokko, 1997), such an assumption might not be realistic (McNamara & Houston, 2009). The genotype-by-age interactions for calling effort here clearly demonstrate that, in spite of general age-related trends, different genotypes resolve the trade-off between current and future signalling investment differently.

Kokko's (1997) model of evolutionary stable strategies in age-dependent signalling includes a scenario in which some proportion of condition can be stored from one time point to the next, meaning individuals can suppress signalling at some ages in favour of increased investment later. This can lead to lower quality males signalling more intensely at later ages than high quality males, and thus for females sometimes to make 'wrong' choices by preferring lower quality males. Honesty is still retained on average, however, because high quality males can maintain increased signalling over a greater period of time. The existence of genotype-by-age interactions in a

sexually-selected signal indicates that no single genotype produces the optimal phenotype across all ages, which could help to maintain additive genetic variation in natural populations.

3.4.2 Genotype-by-environment and genotype-by-environment-by-age interactions

Together, condition-dependent signals and genotype-by-environment interactions (GEIs) have often been invoked as a mechanism that helps explain costly female choice by maintaining additive genetic variation (Jia *et al.*, 2000; Hunt *et al.*, 2004b), but also blur the association between the male signal and the potential benefits to the female chooser (Greenfield & Rodríguez, 2004; Danielson-François *et al.*, 2006; Kokko & Heubel, 2008). Whether the balance of these effects favours the evolution of mate choice is not easily reconciled (Kokko *et al.*, 2007); the answer will depend both upon the amount of male mixing (e.g. due to migration from other environments) and the strength of selection (Kokko & Heubel, 2008; Holman & Kokko, 2014).

My results indicate that GEIs exist for male calling effort due to different dietary environments, and that the strength of the cross-environment genetic correlation depends not only upon which environments are selected, but also when the measurements take place. That GEIs are manifested as genotype-by-environment-by-age interactions is perhaps unsurprising: individuals resolve allocation trade-offs differently over time within a single environment, so the presence of GEI for condition is

liable to change how males signal at all ages. It is notable, however, that all cross-environment correlation estimates at week 4 are close to 1 (the 95% credible intervals almost all overlap 0, but also include 1), indicating that the ranked performance of each genotype is similar across all diet treatments at this stage (Table 3.9).

3.4.3 Female preferences for dynamic signals

Kokko & Heubel (2008) show that the relative strength of selection is important for the balance of positive and negative effects of variation-maintaining mechanisms. Low selection strength implies little gain for females from being choosy, while high selection strength implies that poorly performing males are excluded from reproducing at all (eroding genetic variation and therefore also reducing the benefits of choice) . In the black field cricket *Teleogryllus commodus*, field acoustic trials showed linear selection on the amount of time a call is broadcast: the number of responding females increases with call period, but some females still respond to shorter call time (Hunt *et al.*, 2004a). Female *G. sigillatus* are polyandrous (Sakaluk, 1987), and will mate up to five times per day (Burpee & Sakaluk, 1993). Females also have a fixed internal threshold for judging whether a male is attractive enough to mate with (Ivy & Sakaluk, 2007). So long as lower-quality males can invest enough in signalling to pass such a threshold, they may achieve some level of fitness and thus help sustain additive genetic variation in the population.

Plasticity in female preferences enables females to adjust their mate acceptance thresh-

old so as to discriminate against lower quality males and maximise indirect benefits (Cotton *et al.*, 2006). Evidence from related cricket species shows that the strength of female preferences may be altered by social experience (Bailey & Zuk, 2008, 2009; Bailey, 2011; Bailey & Macleod, 2013) and female condition (Hunt *et al.*, 2005). When there is variation in male quality, female *G. sigillatus* assess males based on phenotypic traits (Gershman & Sakaluk, 2010). They also gain genetic benefits through increased offspring survival from mating with multiple males (Ivy & Sakaluk, 2005), and are known to prefer novel males to those with which they have previously mated if male quality does not vary (Ivy *et al.*, 2005). Further research is required to determine whether females alter their preferences over the course of their lifespan, and how this affects male signalling dynamics.

3.4.4 Storage of condition, and optimal signalling

The optimal signalling level for a male should depend upon the signalling level of his competitors (Lindström *et al.*, 2009); crickets have been shown to alter their calling behaviour based both upon immediate (Callander *et al.*, 2013) and anticipatory (Bailey *et al.*, 2010; Kasumovic *et al.*, 2012b) assessment of the competitive environment. Given the resource costs of signalling, we should also expect that males might adjust effort based upon cues from receptive females (e.g. Rodríguez *et al.*, 2012). Crickets can store resources for future use in signalling effort, and adaptive signalling might entail opportunistic use of stored resources depending upon environmental and social cues. Trade-offs between current and future resource investment and the ability

to adjust signalling in a dynamic fashion lends an intriguing dimension to the results seen here and in other studies (Hunt *et al.*, 2004a; Judge *et al.*, 2008): are the patterns of male calling effort actually selected life history strategies? Or the optimal life history strategy when there is no feedback from the environment? If the latter, this might explain why solitary experimental males often do not call at all, and why effort tends to increase slowly with age (as the optimal investment is in reproductive effort as an individual ages, Kirkwood, 1977). More studies are required that not only manipulate condition, but also alter the current social environment to track how individuals optimise their signalling effort.

3.4.5 Concluding remarks

An increasing focus on the importance of within-individual variation in labile traits over recent years has been due largely to the maturing of long-term pedigree studies of wild populations, and the emergence of new statistical techniques (Nussey *et al.*, 2007; Brommer, 2013). Until relatively recently, individual plasticity was somewhat neglected in studies of variation in sexually-selected traits (Griffith & Sheldon, 2001). The dynamics of flexible behavioural signalling in shorter-lived animals provide similar avenues of study, but remain an underappreciated form of age-dependent plasticity.

4

Metabolic consequences of age-related allocation to a sexually selected behavioural display

Abstract

Allocating resources from a limited supply to one life history trait incurs a trade-off, as those resources are then unavailable for allocation to other traits. Male sexual signals are often condition-dependent, such that trait expression is closely related to the size of a male's resource pool. When a male expresses an energetically expensive trait repeatedly over his lifetime, the reliability of the signal as an indicator of his resource acquisition ability at any given time is suspect, because males can trade-off investment between current and future signalling effort. Identifying the relationships between trait investment and stored metabolites may help clarify whether males of differing resource levels vary in allocation strategy over time, and what maintains honesty in sexual trait expression.

Male decorated crickets, *Gryllodes sigillatus*, attract females using an energetically expensive behavioural display. I used a diet manipulation to study the effect of variation in resource acquisition ability on allocation to life history traits. I also manipulated the socio-sexual environment to induce greater signalling behaviour via the availability of females. Using physiological assays, I was able to determine the average energetic resource budgets of individuals at multiple time points. Increasing resource acquisition early in adulthood led males to call more, and also to accumulate their metabolic reserves at a higher rate. The availability of females increased both chorus participation (whether or not a male called in an evening) and the time

spent calling. High resource acquisition also increased how much a male called in the presence of females, although this effect weakened over time.

Males with lower resource budgets called less than males with high acquisition ability but still suffered metabolite storage loss and viability costs, suggesting that reduced signalling in these treatments was due to energetic constraints rather than an adaptive strategy to accumulate resources. 'Quick-release' metabolites (glycogen and sugars) were used up when females were available; lipid stores were better maintained in the presence of females, and greater lipid stores predicted increased longevity and future chorus participation. After the conclusion of the mate availability manipulation, males that had previous greater exposure to females showed decreased reproductive effort and reduced longevity, indicating lasting life history consequences of experimentally induced signalling effort.

4.1 Introduction

The fitness of an individual depends on its investment in life-history traits (Stearns, 1992). These traits are thought to be affected by trade-offs, caused when an increase in one life history trait that improves fitness is coupled to a decrease in another life history trait that reduces fitness (Stearns, 1989). One trade-off universal to all organisms occurs because individuals have a limited amount of resources to allocate to traits competing for a limited resource pool; the allocation of resources to one trait means that those resources are necessarily unavailable for allocation to another trait (Roff, 1992; Stearns, 1992).

An individual's ability to acquire resources, known as its 'condition', is often related strongly 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 resources and converting them to metabolically useful forms. If the fitness costs associated with increased investment in the sexual trait vary with underlying heritable quality, condition-dependent signal traits can be used as indicators of male quality by females (Nur & Hasson, 1984; Andersson, 1986; Grafen, 1990; Iwasa & Pomiankowski, 1994). The marginal costs of further investment in condition-dependent signal traits must therefore be lower for males in good condition than those in poor condition (Grafen, 1990; Rowe & Houle, 1996). These differential costs may be manifested through a reduction in future reproductive effort or survival.

Male resource allocation strategies are typically associated with high-risk tactics and

greater fitness variance in comparison to females (Bonduriansky *et al.*, 2008): males might therefore be expected to benefit by sacrificing longevity for increased early reproductive success (Vinogradov, 1998), causing negative covariance between early sexual activity and longevity. However, studies often show the opposite pattern, such that males that signal most intensely are also those that survive the longest (Jennions *et al.*, 2001). One possible explanation for this positive covariance is that high quality organisms have more resources to allocate to all aspects of their life history, which masks real life history trade-offs between expensive traits (Van Noordwijk & De Jong, 1986; De Jong & Van Noordwijk, 1992; Reznick *et al.*, 2000).

The problems inherent in assessing trade-offs in resource allocation to costly traits are particularly acute when an individual expresses an exaggerated sexual trait repeatedly over the course of its lifetime. When males can alter the strength of their condition-dependent signals in a dynamic fashion across ages, the value of the signals for indicating male quality is particularly suspect (Kokko, 1997). This is because when a male can adjust investment in a trait with age, he faces resource trade-offs between current and future expression as well as between the sexual trait and other components of life history: put simply, a male that invests his resources heavily at one stage has fewer resources to spend at the next stage, and vice versa (Badyaev & Qvarnström, 2002). To the extent that this creates variation between individual males in their patterns of age-dependent expression of sexual traits , it impedes the ability of females to use signal expression at a given moment as an index of a male's overall acquisition ability. In other words, the signalling level of a male at any given age in

its life is less likely to be an honest indicator of condition than would be true if this trait were less phenotypically plastic.

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 the metabolic costs associated with sexual trait expression are therefore crucial to determining how males vary in their age-dependent signalling, and to pinpoint the mechanism enforcing honesty in systems where females select males based upon relative trait exaggeration.

Assessing how life history trade-offs affect age-dependent 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 trade off with other life history characters (Hunt *et al.*, 2004b; Houslay & Bussière, 2012). One advocated workaround is to use residuals of mass over a fixed measure of body size as a proxy for condition, indicating an individual's available energy reserves (Peig & Green, 2009). However, simple body mass measurements may be inadequate: for example, energetically expensive trait expression (such as calling in crick-

ets) is fuelled by sugars that are underpinned by fat stores (Tomkins *et al.*, 2004), thus condition indices can only be used to compare individuals of different sizes in a reliable fashion if there is prior knowledge of how fat content is likely to scale with size (Kotiaho, 1999). Ideally, we need direct physiological studies (rather than genetic or phenotypic approaches) to study the mechanisms mediating metabolic storage and use, and their consequences for age-dependent variation in sexual trait expression (Zera & Harshman, 2001).

A primary model for studying investment in age-dependent sexual expression is acoustic signalling (Andersson, 1994), a trait which entails high energetic costs (Ophir *et al.*, 2010). In crickets, males produce a calling song through stridulation of their forewings; the amount of time a male spends calling (his 'calling effort') is a strong predictor of mating success in nature (Hunt *et al.*, 2004a; Bentsen *et al.*, 2006; Rodríguez-Muñoz *et al.*, 2010). Song production is highly energetically expensive [refs], and may increase mortality risk from both intrinsic (calling diverts energy from other functions, such as somatic maintenance Hunt *et al.*, 2004a) and extrinsic (male calls attract predators, Walker, 1979, or parasitoids, Cade, 1975) sources. Studies that have manipulated resource acquisition have shown that male condition strongly affects the timing and amount of calling effort (Judge *et al.*, 2008; Maklakov *et al.*, 2009; Zajitschek *et al.*, 2009b, 2012), and that high-quality males may invest so heavily in sexual displays that they suffer heightened mortality compared to lower-quality individuals (Hunt *et al.*, 2004a).

However, manipulating condition alone may not reveal differential costs of trait expression because males in good condition have more resources to allocate to all traits (Kotiaho, 2001). Testing for differential costs therefore requires manipulating both condition and trait investment, and then studying their effects on other life history traits (such as future reproductive effort or survival). Moreover, behavioural signals are highly phenotypically plastic, and thus enable males to react quickly to changes in the local environment so as to maximise fitness (Komers, 1997; Bretman *et al.*, 2011). Experimental manipulation of some aspect of the socio-sexual environment may therefore be used to induce investment in reproductive effort. Male crickets show plasticity in response to the social environment: juveniles reared in environments that indicate high competition for access to females invest more in reproductive tissues (Bailey *et al.*, 2010) and age-specific calling effort (Kasumovic *et al.*, 2012a). The presence of a rival male during adulthood can also cause plastic shifts in allocation to calling (Callander *et al.*, 2013). Manipulation of the socio-sexual environment can therefore be used not only to evaluate the metabolic and life history consequences of trait investment, but also to reveal whether plasticity in the male signal is due to strategic energy saving or the constraints of the resource pool. If the former, low levels of calling early in life among males with reduced resource acquisition ability should be associated with increased energy reserves and greater calling later in life (Kokko, 1997; Hunt *et al.*, 2004a). However, if there is no evidence of greater energy reserves for allocation at a later point, it may be assumed that low calling levels are due simply to a lack of energy for current allocation to reproductive effort.

In this study, I measure how allocation to sexual trait expression and resource storage changes as a function of both resource acquisition and the demands of allocation in male decorated crickets (*Gryllodes sigillatus*), which use an energetically expensive behavioural display to attract females. I measure the investment in reproductive effort through time spent signalling, and use physiological assays to estimate the energetic resource budgets of separate subsets of males before and after manipulating both resource acquisition and access to mates. I then assess the consequences of my manipulations of acquisition and allocation on longevity.

4.2 Methods

4.2.1 Cricket maintenance

Gryllodes sigillatus used in this study were descended from 500 adult crickets collected in Las Cruces, New Mexico in 2001, and used to initiate a laboratory culture maintained at a population size of approximately 5000 crickets and allowed to breed panmictically (Ivy & Sakaluk, 2005). Details of maintenance protocols can be found in Section 2.2.1.

4.2.2 Experimental protocol

I manipulated male cricket resource acquisition through diet treatment (7 levels), and resource allocation via mate availability (8 levels) (see further details of both treat-

ments below). I investigated variation in allocation to energy storage components by sacrificing subsets of the experimental population at set timepoints (Figure 4.1): immediately at eclosion; one week after eclosion (resource acquisition manipulation only); and two weeks after eclosion (both resource acquisition and allocation manipulations). Individuals were sacrificed by being placed in a $-80°$C freezer. Details of energy storage component estimation are given below. A final group were allowed to die naturally (to estimate investment in lifespan).

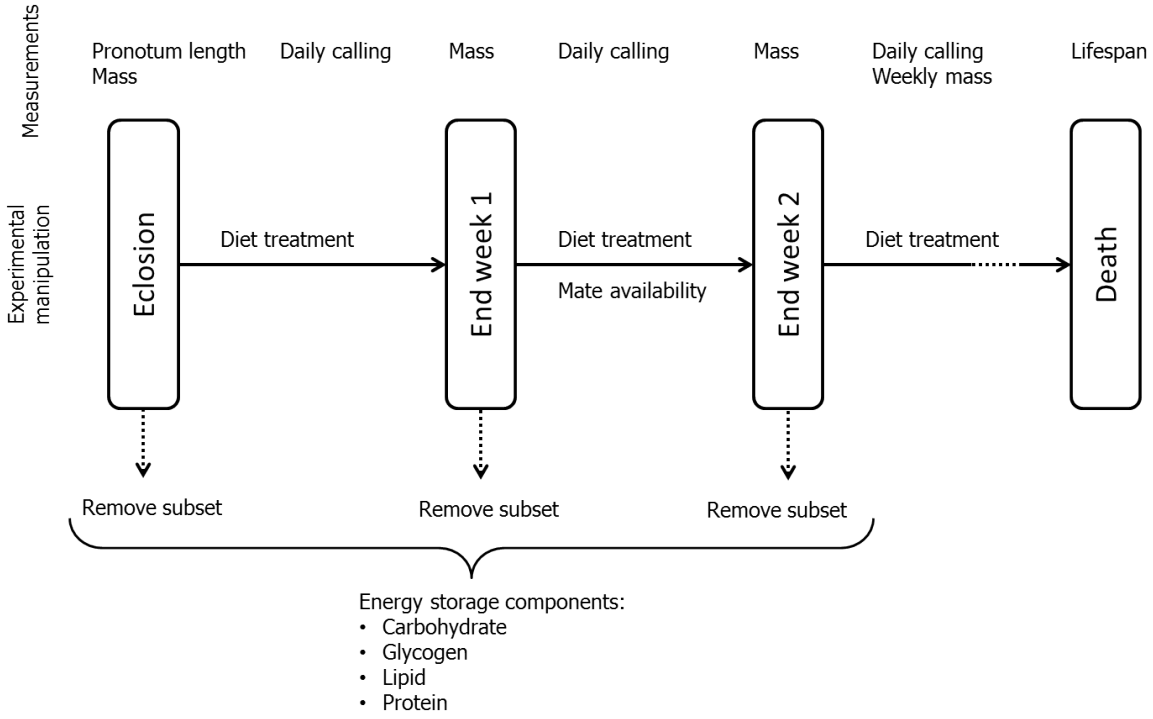

Figure 4.1: A schematic diagram of the experimental design used in this study, detailing experimental manipulations and the measurements taken at different stages.

I separated 630 male cricket nymphs from laboratory stock cultures during the fi-

nal larval instar. On the day of eclosion, I photographed individuals through a microscope (Motic, model SMZ-168 equipped with Moticam 2000) and obtained measurements of pronotum length from these photographs using NIH ImageJ software (Schneider *et al.*, 2012). I measured body weight using a high precision electronic balance (Denver Instrument, model PI-225DA) at eclosion and at weekly intervals thereafter. Upon eclosion, males were assigned randomly to one of four groups: those to be sacrificed immediately (N = 58), after one week (N = 56), after two weeks (N = 256), or those allowed to live out their natural lifespan (N = 260). Males that were not immediately sacrificed were transferred to individual clear plastic containers ($5 \times 5 \times 5$cm), which contained a piece of plastic mesh attached to the side as substrate, a water bottle plugged with cotton wool, and the food treatment. Containers were cleaned each week, and fresh water and food provided.

Male allocation to reproductive effort was quantified as 'calling effort', the duration of time (in seconds) that each individual spent broadcasting his long-distance sexual advertisement call. I measured each male cricket overnight from 2pm until 9am (dusk until afternoon of the following day on the reversed light cycle) every day from eclosion up to a maximum of 35 days. A microphone (C1163, Dick Smith Electronics) was mounted in the lid of each individual container; I then placed each container into a hollowed-out cube of sound-proofing foam so as to minimise outside disturbance and ensure there was no crosstalk between containers. A corner of the foam lid was removed so that crickets would be maintained on the set light cycle. An electronic acoustic recording system (Bertram and Johnson 1998) sampled from the microphone

of each individual cricket container 10 times per second to determine whether or not a male was calling.

Creation of artificial diets

I used seven artificial, dry, granular diets varying in the amount of total nutritional content to manipulate resource acquisition. All diets had a protein:carbohydrate ratio of 1:8, based on the effect of a high-carbohydrate diet in maximising calling effort and longevity in a related cricket species (Maklakov *et al.*, 2008). The diets ranged from 12% to 84% in total protein and carbohydrate content (referred to throughout as nutritional content): proteins consisted of a 3:1:1 mixture of casein, albumen and peptone, and digestible carbohydrates were a 1:1 mix of sucrose and dextrin. All diets contained Wesson's salts (2.5%), ascorbic acid (0.275%), cholesterol (0.55%) and vitamin mix (0.18%). After the appropriate dry weight of protein and carbohydrate had been added to the mixture, the remaining weight was made up with indigestible crystalline cellulose.

Female availability treatment

During the second week, male crickets were provided access to a different adult female cricket during the recording period each night for between 0 and 7 consecutive nights. As logistical constraints prevented me from providing virgin females of similar age each day, I instead controlled female 'experience' to be similar to the male with

which she would be placed: e.g., on the first day virgin females would be placed with virgin males, 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 placed with an experimental male, females would be housed together in single-sex groups, and provided with water, shelter, and ground cat food (the 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's rest from males. During the mate availability treatment week, the food was removed from each male's individual container during the call recording period (regardless of female presence). Males were given access to food while not being recorded (9am - 2pm).

4.2.3 Determination of metabolic storage components

The major components of energy storage were estimated using a modified version of the protocol of Foray *et al.* (2012). Cricket flight is fuelled by lipid breakdown (Zhao & Zera, 2002), and the existence 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 an individual can use to

fuel energetically expensive signalling. Together, these metabolites can be assumed to comprise an individual's energy budget. This method enables measurement of proteins, carbohydrates, glycogen and lipids in the same individual.

Each insect was placed into a 1.5 mL Eppendorf tube containing a stainless steel ball-bearing and 1 mL of lysis buffer (100 mM KH_2PO_4, 1mM dithiothreitol (DTT) and 1 mM ethylenedianimetetra-acetic acid (EDTA), pH 7.4) and was crushed by shaking the tube for 480s at 30 Hz using a Qiagen Tissue Lyser. Samples were then centrifuged at a low speed (3000 rpm) at 4°C to allow separation of insoluble debris. The soluble fraction of this was removed and used for further experiments.

Protein content

To determine protein quantity in each sample a Bradford assay was performed. 2.5 μL of the sample was transferred to a 96-well plate and combined with 200 μL Bradford reagent (Sigma-Aldrich). Absorbance was read on a Molecular Devices VersaMax microplate reader at 595 nm. A dilution-series of known concentrations of Bovine Serum Albumin (BSA) was used as a standard curve for absorbance versus known protein concentration.

Carbohydrate content

To dissolve all carbohydrates 180 μL of the original sample was combined with 20 μL of 20% sodium sulphate solution (Na_2SO_4). This solution was then mixed with 1500 μL of a 1:2 (v/v) chloroform-methanol solution. This led to solubilisation of total lipids and water-soluble carbohydrates. Samples were vortexed vigorously for 10 s and centrifuged for 5 mins at 10,000 rpm at 4°C. The supernatant was removed and used to determine soluble carbohydrate and lipid content while the pellet was retained for determination of glycogen content.

For determination of soluble carbohydrate an Anthrone assay was performed. Anthrone reagent was prepared by mixing 1.42 g/L of anthrone reagent (Sigma-Aldrich) with 70% sulphuric acid and protecting this solution from light. 150 μL of chloroform-methanol supernatant was transferred to a polypropylene 96-well plate and evaporated at room temperature for 30 min until a volume of approximately 10 μL remained. 240 μL anthrone reagent was then added and plates were incubated at room temperature for 15 min. The microplates were then heated at 70°C for 15 min in an incubator and absorbance was read at 625nm. A dilution-series of known concentrations of D-glucose was used as a standard curve for absorbance versus known carbohydrate concentration.

Glycogen content

To determine glycogen content the pellet fraction (collected following the chloroform-methanol reaction) was washed twice with 80% methanol, followed by vigorous vortexing and centrifugation at 16000 rpm for 5 min at 4°C. Supernatant was removed and 1 mL of anthrone reagent was added to the pellets and incubated at 70°C for 20 min. Samples were then cooled on ice and filtered through low-protein binding membrane 96-well plates (Durapore, 0.45 μM, polyvinylidene fluoride, Millipore). Absorbance at 625 nm was measured to determine glycogen content using a D-glucose standard curve as described above.

Lipid content

Lipid content was determined using a Vanillin assay. Vanillin reagent was prepared by mixing 1.2 g/L vanillin powder with 68% ortho-phosphoric acid and protected from light. 100 μL of supernatant (from the chloroform-methanol reaction) was added to a polypropylene 96-well plate and heated at 70°C for 30 min until the solvent had fully evaporated. 10 μL of 98% sulphuric acid was then added to each well and incubated at 70°C for 5 min, then cooled on ice. 190 μL of vanillin reagent was then added, incubated for 15 min and absorbance was measured at 525 nm. A dilution-series of known concentrations of triolein (Sigma-Aldrich) was used as a standard curve for absorbance versus known lipid concentration.

4.2.4 Estimating condition

I calculated body condition using the scaled mass index (SMI)(Peig & Green, 2009, 2010):

$$SMI = M_i[L_0/L_i]^{b_{SMA}}$$

where M_i and L_i are the body mass and linear body measurement (pronotum length) respectively of individual i, L_0 is the population mean for the linear body measurement, and b_{SMA} is the scaling exponent estimated by the standardised major axis (SMA) regression of ln$M$ on ln$L$ (using R package lmodel2, Legendre, 2013). I used all measurements of mass taken during the experiment to calculate the scaling exponent ($b_{SMA} = 2.563$), and L_0 was the mean pronotum length for all crickets used (mean pronotum length = 2.378mm). The scaled mass index for each cricket at any given timepoint was thus measured by substituting their fresh body mass M_i and pronotum length L_i into the equation $SMI = M_i[2.378/L_i]^{2.563}$. The use of a condition index enabled me to compare differences in mass over time having accounted for body size.

4.2.5 Statistical analysis

I performed all statistical analyses using R 3.0.2 (R Core Team, 2013). Diet treatment was centred, setting the 48% nutrition diet to 0. Other numeric input variables (such as SMI, when used as a predictor) were standardised by centring (subtracting the mean) and scaling (dividing by 2 standard deviations), putting them on a common

scale and aiding the interpretation of main effects (Gelman & Hill, 2007; Gelman, 2008; Schielzeth, 2010). Independence between linear and quadratic forms of numeric predictors (e.g., diet and diet^2, SMI and SMI^2) was achieved by centring or standardising the input variable before squaring (Gelman & Hill, 2007).

Unless otherwise stated, model simplification was performed by dropping non-significant terms from the full model sequentially, and using likelihood ratio tests to compare nested models. I retained more complex models whenever simplification resulted in a significant increase in model deviance.

Predictor variables in multiple regression models often included related measurements (e.g., body condition, change in body condition, and diet treatment in the analysis of energy storage components at the end of week 1). Before building the full models, I tested for collinearity of main effects by examining the variance inflation factors (VIFs) in models without interactions. If any covariates showed a VIF value greater than 2, I dropped the covariate with the highest VIF, recalculated VIFs, and repeated this process until all were below this threshold (Zuur *et al.*, 2010).

Zero-altered Poisson models for daily calling effort

Daily calling effort was over-dispersed and zero-inflated, and no data transformation addressed these issues adequately. For analyses featuring these data , I fitted 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. The use of a ZAP model enabled me to ask two separate question (Atkins *et al.*, 2013): which factors affect whether there is calling or no calling (i.e., non-zero or zero), and which affect the amount of calling when it occurs?

I used a generalised linear mixed model to specify the ZAP structure with a random effect of individual ID that accounted for the repeated observations on individuals. I used Bayesian Markov chain Monte Carlo techniques and estimated the posterior mode and 95% credible intervals (CIs) for predictor variables. The full model included all possible interactions between predictor variables (excluding interactions between linear and quadratic forms of a single variable). I fitted an unstructured variance-covariance matrix that allows covariance between the zero-altered and Poisson parts of the model. There is no observed residual variance for binary data (the logit model), so this value was fixed at 1. The analysis was carried out using the R package MCMCglmm (Hadfield, 2010) with 250,000 iterations, burn-in of 50,000, a sampling rate of 200, and parameter-expanded priors. I used the 'zapoisson' family for zero-altered Poisson; the Poisson part of the model as specified in MCMCglmm also has a vector of residuals that deals with over-dispersion in the data after accounting for fixed and random sources of variation (Hadfield, 2010). Fixed effect terms in MCMCglmm are considered significant if their 95% credible intervals do not straddle zero, so terms or interactions not meeting this criterion were removed if they were not involved in significant higher-order interactions, and if removal did not worsen the model fit according to DIC.

Metabolite content at eclosion

One individual was excluded from the analyses of metabolites at eclosion, as its standardised SMI value was over 2 standard deviations from the mean (all others were within ± 1.1 standard deviations). I used linear regression models to analyse the effect of body condition on protein, lipid and glycogen energy storage composition. Glycogen measurements were log-transformed to conform to a normal distribution. Carbohydrate measurements could not be transformed to a normal distribution, and were analysed using a generalised linear model with the negative binomial family.

Week 1

I analysed the effect of diet treatment and age on calling effort during the first week post-eclosion with a zero-altered Poisson (ZAP) model using MCMCglmm (see above). The full model included predictor variables of linear and quadratic terms for diet treatment and day post-eclosion (corresponding to the age of an individual), and all interactions (with the exception of that between the linear and quadratic forms of the same predictor). Individual cricket ID was included as a random effect to group the repeated measures.

I also used linear regression to test for effects of diet, condition at eclosion and change in eclosion on total calling effort over the first week (males that did not call over the

first seven days were excluded, and the data were log-transformed).

I used a multiple regression model to determine the causes of changes in body condition over the first week post-eclosion (SMI difference = SMI week 1 - SMI at eclosion). The full starting model included linear and quadratic terms for diet treatment and SMI at eclosion, and all interactions (with the exception of that between the linear and quadratic forms of the same predictor).

I also used multiple regression models to investigate the causes of variation in metabolites at the end of the first week. For each response variable (protein, lipid, glycogen and carbohydrate), I estimated the approximate changes in metabolic components over the first week post-eclosion by subtracting the mean content of each storage form at eclosion from every individual's measurements. I then tested for effects of body condition at eclosion, diet treatment, and their interaction. Glycogen measurements were log-transformed prior to analysis; carbohydrate measurements were analysed using a generalised linear model with the negative binomial family.

To study how diet mediated allocation to both calling effort and body condition, I used a linear regression model to test the effects of diet, calling effort (which was log-transformed before being standardised), and their interaction on the change in condition over the first week post-eclosion.

Week 2

I used a ZAP model in MCMCglmm (see above) to determine what affected the likelihood and amount of calling over the second week post-eclosion. The predictor variables were female presence (binary variable), linear and quadratic terms for diet treatment and day post-eclosion (corresponding to the age of an individual), body condition at the start of week 2, mate availability history, and up to three-way interactions between these (with the exception of those between linear and quadratic forms of the same predictor). The mate availability history variable was simply the number of females a male had already had access to prior to the current measurement. Individual cricket ID was included as a random effect so as to group the repeated measures.

I tested for effects of acquisition and allocation on the change in condition over week 2 using a multiple regression model, with predictors of total calling effort and diet treatment. I used a separate model to investigate the effects on the change in condition of my experimental manipulations in addition to condition at the start of the second week, and whether a male cannibalised a female during week 2. I used a generalised linear model with binomial family to test for effects of diet and mate availability manipulations on male survival during week 2.

Similarly to the analysis of Week 1 data, I estimated approximate changes in metabolite content over the week by subtracting the diet-specific mean content of each storage form at the end of week 1 from each individual's measurements. I then tested for effects of diet, mate availability treatment, condition at the beginning of week 2, and

interactions between them on each metabolite content change; log-transformed total calling effort was also included as a covariate.

Post-mate availability treatment

I tested for further consequences of my mate availability treatment using a multiple regression of lifespan on diet and mating treatments; I also used a negative binomial regression model to test for the number of days in the third week that a male produced a call.

To investigate how metabolite content at the end of the second week might affect future investment, I assigned each individual still alive beyond this point the mean values for metabolite content from the appropriate diet and mate availability treatment combination taken at the end of week 2. I used these values as predictors in a regression of lifespan, and a negative binomial regression model investigating how many days in the third week of adulthood a male called.

4.3 Results

As expected (because the treatments only began after eclosion), there were no significant differences between diet or mating treatment groups in pronotum length, body mass, or condition at eclosion (Table 4.1). Of the four types of metabolite measured, only lipids showed a significant association with body condition at eclosion: males

with a higher scaled mass index also had greater lipid content (Table 4.2, Figure 4.2).

Table 4.1: Differences among treatment groups (diet and mate availability manipulations) at eclosion.

	df	F	Estimate $\pm$ S.E.	P
Pronotum (mm)				
Diet	1,563	0.591	0.002 $\pm$ 0.003	0.443
Mating	1,507	0.165	-0.001 $\pm$ 0.003	0.685
Mass (g)				
Diet	1,570	0.633	0.0004 $\pm$ 0.0006	0.427
Mating	1,514	0.527	-0.0004 $\pm$ 0.0005	0.468
SMI				
Diet	1,563	0.788	0.001 $\pm$ 0.001	0.375
Mating	1,507	0.154	-0.0004 $\pm$ 0.0011	0.695

Table 4.2: Results from regressions of metabolites on condition at eclosion. **z-statistic for carbohydrate; t-statistic for all other components*.

	t/z^*	Estimate $\pm$ S.E.	P	
Protein				
(Intercept)	12.003	4.067 $\pm$ 0.339	$<$0.001	***
SMI	0.781	0.682 $\pm$ 0.873	0.438	
Lipid				
(Intercept)	24.000	5.616 $\pm$ 0.234	$<$0.001	***
SMI	2.127	1.323 $\pm$ 0.622	0.038	*
Glycogen				
(Intercept)	17.312	1.189 $\pm$ 0.069	$<$0.001	***
SMI	0.197	0.035 $\pm$ 0.178	0.845	
Carbohydrate				
(Intercept)	3.853	0.427 $\pm$ 0.111	$<$0.001	***
SMI	-0.163	-0.050 $\pm$ 0.305	0.871	

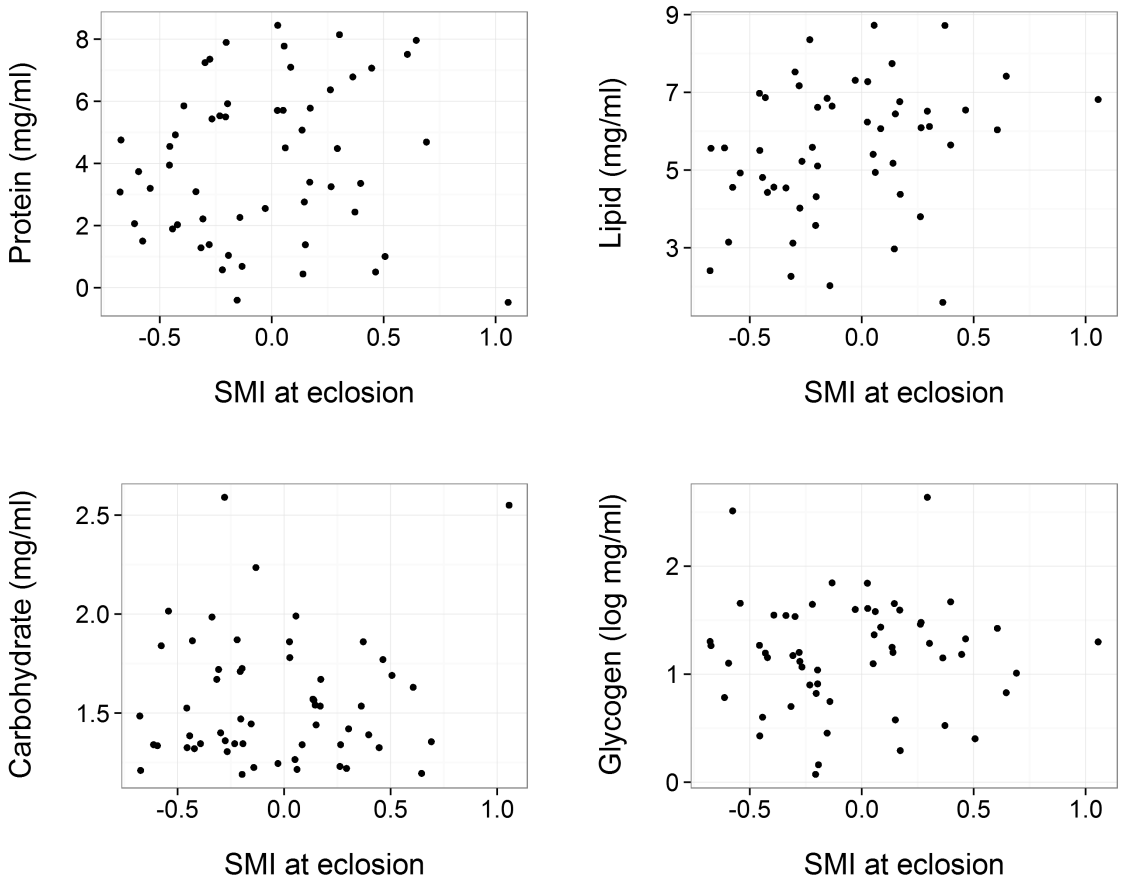

Figure 4.2: Metabolites at eclosion, plotted against standardised units of the scaled mass index (SMI) of body condition.

4.3.1 Manipulation of early adult nutrient acquisition (week 1)

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 zero-altered part of the ZAP model showed that the likelihood of calling increased significantly along with both nutritional diet content and age over the first week (Table 4.3). The age-related increase in likelihood of calling was less pronounced towards the end of the week. The Poisson part of the ZAP model showed how diet and age affect the amount of calling, given that calling took place. There was a significant two-way interaction between day and diet, indicating that calling effort increased over the course of the week especially when males were fed higher quality diets (Figure 4.3). Significant main effects showed that calling effort increased with improved diet, and that calling also increased with time (although a significant effect of the quadratic term showed that the increase in calling lessened over the course of the week). Total calling effort over the first week (the sum of each male's daily measurements in this period) increased with dietary nutrition content (diet parameter estimate $= 0.185 \pm 0.055$, $F_{1,333} = 11.33$, $P<0.001$).

I tested for effects on changes in body condition to investigate the overall phenotypic response to diet in relation to previous condition among all males. The change in body condition over the first week post-eclosion was best explained by a model that included a significant two-way interaction between diet treatment and SMI at eclosion (Table 4.4). Males with higher SMI at eclosion increased their body condition

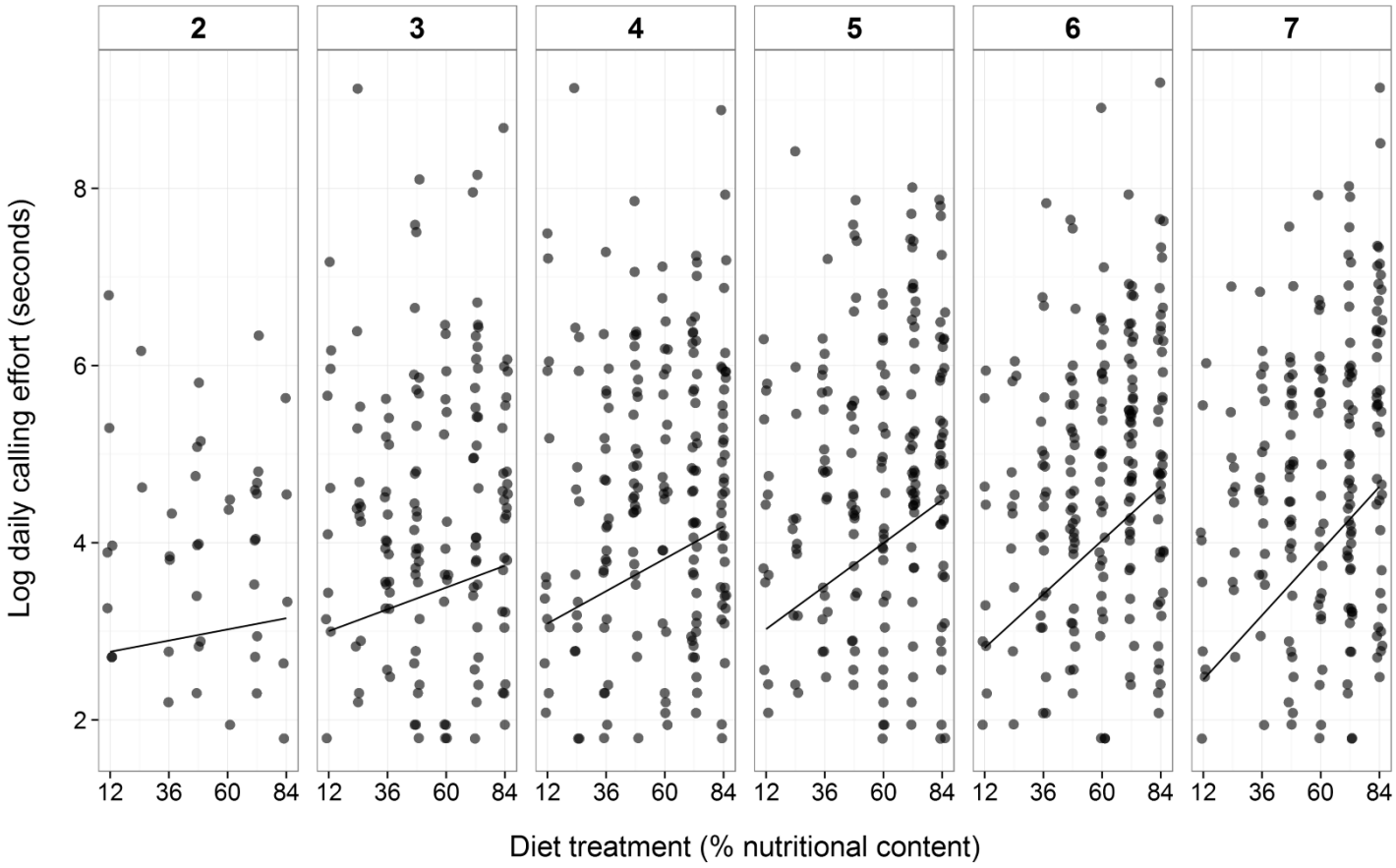

Figure 4.3: Log-transformed daily calling effort (in seconds, excluding zeroes) over the first week post-eclosion, plotted against diet treatment and separately for each day. Day 1 was excluded as no males called. Predicted slopes from the Poisson part of a MCMCglmm zero-altered Poisson model show how the relationship between diet and calling effort changes over the course of the week.

Table 4.3: MCMCglmm analysis of male nightly calling effort over the first week post-eclosion. Day 1 was excluded from the analysis as no males called.

Fixed effect	Estimate	95% CI (lower, upper)	*P*	
Zero-altered				
(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	
Poisson				
(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 component	**Estimate**	**95% CI (lower, upper)**		
Zero-altered				
ID	1.128	(1.009, 1.258)		
Poisson				
ID	1.559	(1.190, 1.979)		
Residual	5.000	(3.990, 6.086)		

more as dietary nutrition increased. The significant main effect of diet showed that change in body condition increased with improving nutritional content, although this increase levelled off at higher diets (Figure 4.5).

Testing for changes in metabolite content compared to estimates taken at eclosion then allowed me to investigate the underlying physiological response to my dietary manipulation. Protein content at the end of the first week post-eclosion was not sig-

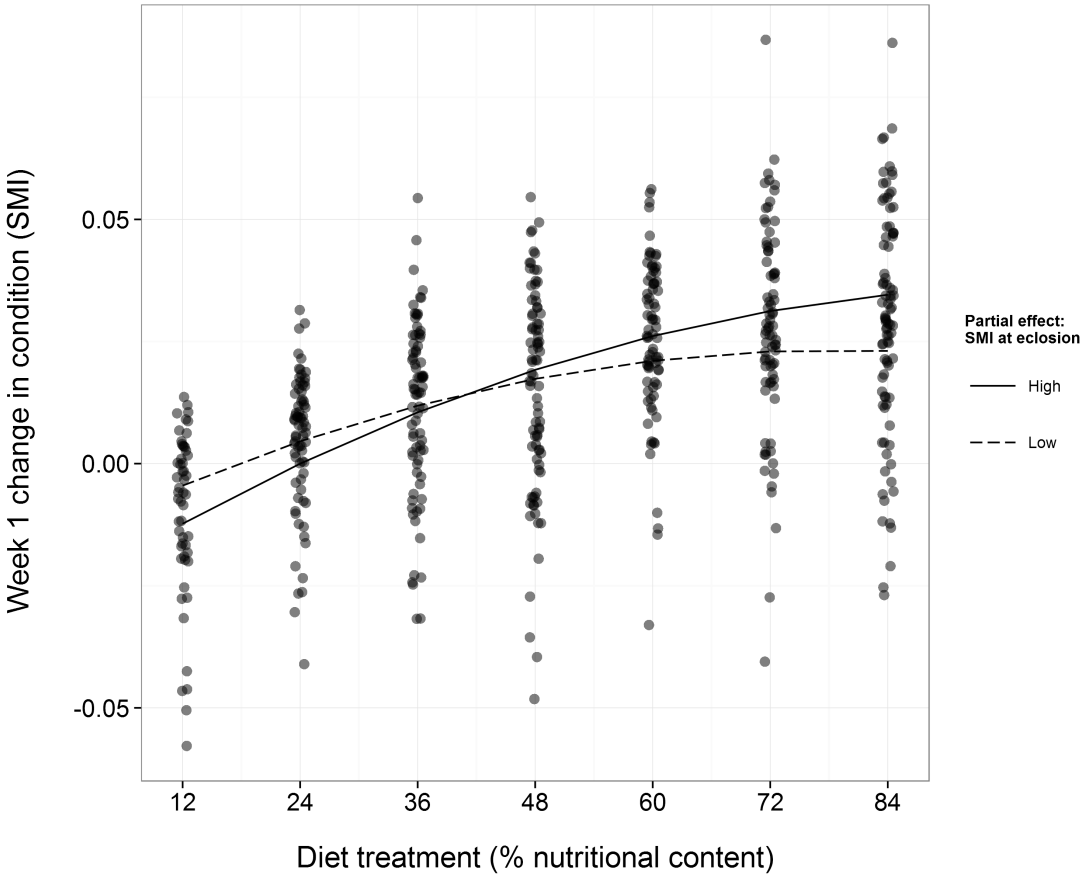

Figure 4.4: Predicted quadratic slopes from linear regression model showing the partial effects of high and low condition (SMI) at eclosion on the relationship between diet and the change in condition over week 1. Solid line represents high SMI at eclosion; dashed line low SMI at eclosion.

Table 4.4: Multiple regression showing how change in condition over the first week of adulthood (SMI week 1 - SMI at eclosion) is affected by diet treatment and initial condition.

	t	Estimate $\pm$ S.E.	P	
(Intercept)	14.961	0.018 $\pm$ 0.001	<0.001	***
Diet	14.432	0.006 $\pm$ 0.000	<0.001	***
SMI at eclosion	1.115	0.002 $\pm$ 0.002	0.265	
Diet^2	-3.732	-0.001 $\pm$ 0.000	<0.001	***
Diet $\times$ SMI at eclosion	3.812	0.003 $\pm$ 0.001	<0.001	***
Adjusted model $R^2 = 0.300$; $F_{4,534} = 58.75$, $P <0.001$				

nificantly different from measurements taken on the day of eclosion (Table 4.5, Figure 4.5). Increased dietary nutrition significantly increased carbohydrate, glycogen, and lipid content during week 1.

Table 4.5: Simplified models of predictors of change in energy storage components over week 1. The starting model included predictor variables of condition at eclosion, diet treatment, and their interactions.

	t	Estimate $\pm$ S.E.	P	
ΔProtein				
(Intercept)	0.060	0.022 $\pm$ 0.364	0.952	
ΔLipid				
(Intercept)	-3.409	-0.893 $\pm$ 0.262	0.001	**
Diet	2.617	0.363 $\pm$ 0.139	0.012	*
ΔGlycogen				
(Intercept)	28.390	1.613 $\pm$ 0.057	<0.001	***
Diet	3.739	0.110 $\pm$ 0.030	<0.001	***
ΔCarbohydrate				
(Intercept)	-8.324	-0.714 $\pm$ 0.086	<0.001	***
Diet	3.124	0.139 $\pm$ 0.045	0.003	**

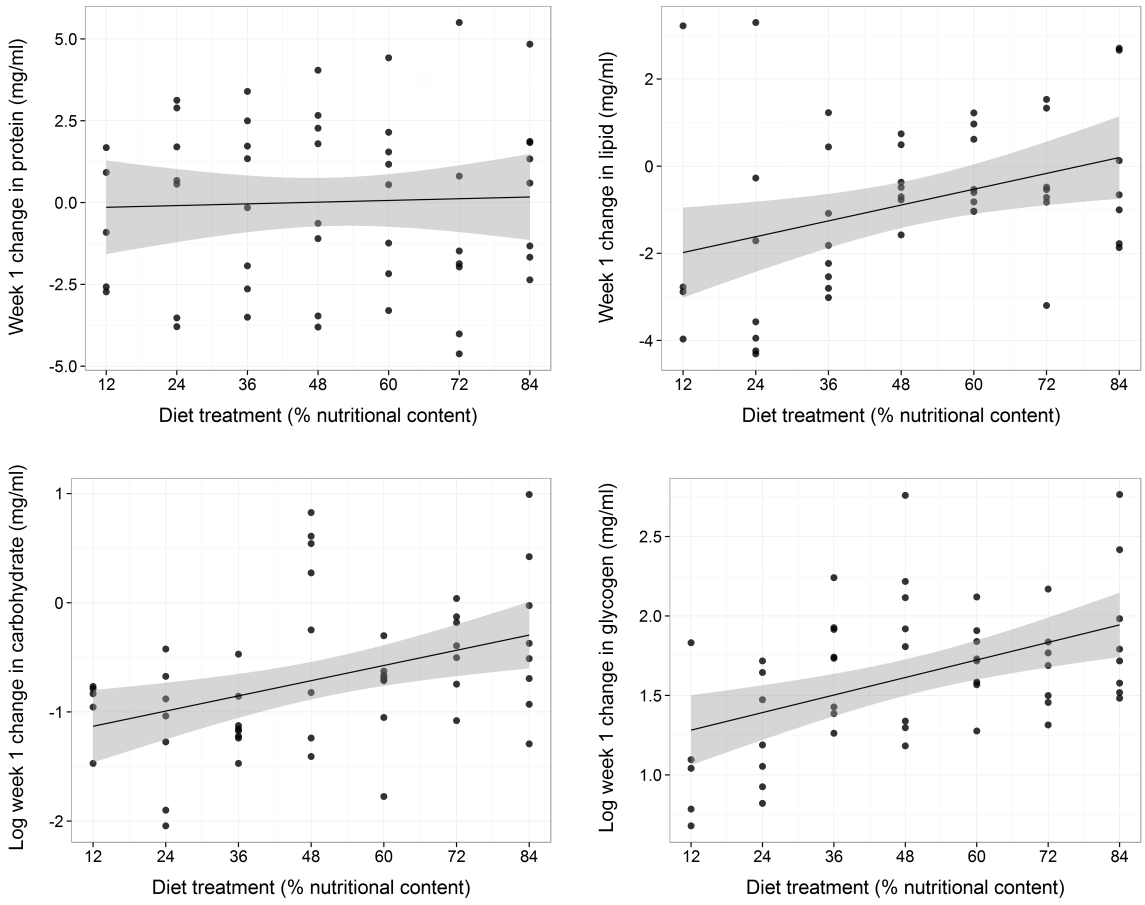

Figure 4.5: Change in metabolites over week 1, plotted against diet (with linear regression slope). Clockwise from top left: protein, lipid, glycogen, carbohydrate. Change in carbohydrate and glycogen content have been log-transformed.

The relationship between total calling effort and change in body condition over week 1 was strongly dependent upon diet (Table 4.6); increased dietary nutrition meant males that called more were better able to increase condition also (Figure 4.6). There was no significant relationship between calling effort and changes in metabolites over week 1.

Table 4.6: Parameter estimates for the model showing how change in condition over the first week of adulthood (SMI week 1 - SMI at eclosion) is affected by how much a male called over week 1 (log-transformed and standardised) and diet.

	t	Estimate $\pm$ S.E.	P	
(Intercept)	-2.318	-0.045 $\pm$ 0.019	0.021	*
Week 1 total calling effort	1.798	0.070 $\pm$ 0.039	0.073	.
Diet	12.869	0.128 $\pm$ 0.010	$<$0.001	***
Week 1 total calling effort $\times$ diet	2.877	0.056 $\pm$ 0.019	0.004	**
Adjusted model $R^2 = 0.279$; $F_{3,535} = 70.36$, $P <$0.001				

4.3.2 Manipulation of access to mates and of nutrient acquisition (week 2)

The zero-altered part of the mixed model analysis for daily calling effort during week 2 shows the effects of predictors on the likelihood of calling each day (Table 4.7). Males were significantly less likely to call as the week progressed, but this decrease in likelihood over time was mitigated by female presence (Female presence $\times$ day interaction term in Table 4.7; Figure 4.7). The main effect of female presence also significantly increased the likelihood of a male recording a call. Call likelihood was significantly reduced in males that had had greater access to females prior to the current measurement (Mate availability history term in Table 4.7).

The Poisson part of the model shows the effects of predictors on the amount of calling effort a male engaged in, given that a call was recorded (Table 4.7). Males called

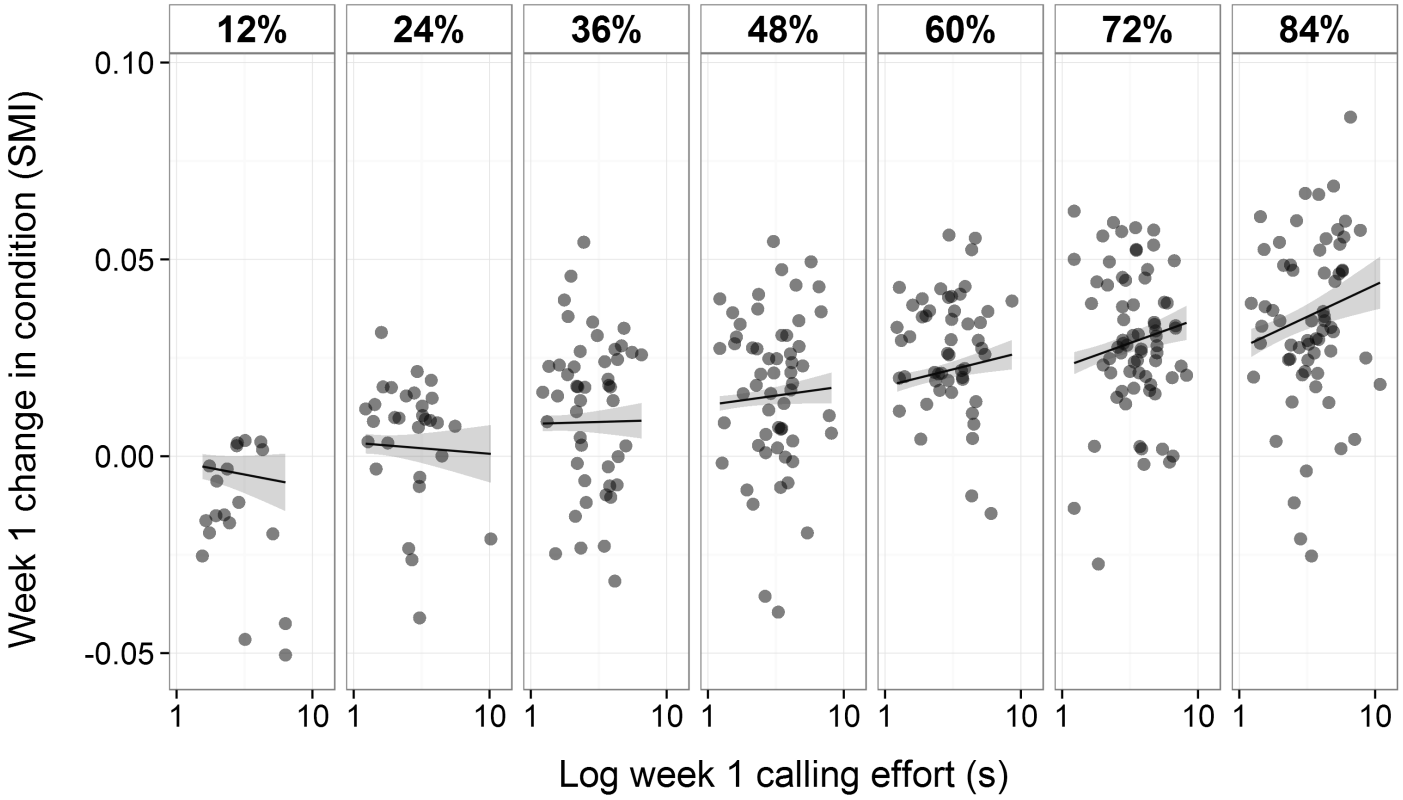

Figure 4.6: Predicted slopes from a linear model showing the relationship between total calling effort over week 1 (log-transformed, with zero-callers excluded) and the change in condition during this period, separately for each diet treatment.

Table 4.7: MCMCglmm analysis of male nightly calling effort over the second week post-eclosion. Males fed the 12% nutrition diet were excluded as they all died before the end of the week. Also excluded were males that cannibalised a female during the mate availability treatments.

Fixed effect	Estimate	95% CI (lower, upper)	*P*	
Zero-altered				
(Intercept)	-4.621	(-5.026, -4.195)	<0.001	***
Female presence	2.981	(2.523, 3.477)	<0.001	***
Diet	0.019	(-0.139, 0.173)	0.818	
Day	-0.120	(-0.237, -0.005)	0.036	*
Body condition (beginning week 2)	0.364	(0.010, 0.751)	0.062	.
Mate availability history	-0.640	(-0.865, -0.439)	<0.001	***
Female presence × day	0.776	(0.577, 0.993)	<0.001	***
Mate availability history × diet	0.053	(-0.025, 0.134)	0.188	
Mate availability history × day	0.012	(-0.038, 0.067)	0.600	
Mate availability history × diet × day	0.018	(-0.011, 0.045)	0.210	
Poisson				
(Intercept)	4.066	(3.758, 4.400)	<0.001	***
Female presence	0.736	(0.471, 0.961)	<0.001	***
Diet	0.348	(0.251, 0.461)	<0.001	***
Day	-0.195	(-0.265, -0.133)	<0.001	***
Body condition (beginning week 2)	0.825	(0.563, 1.153)	<0.001	***
Mate availability history	-0.075	(-0.203, 0.070)	0.296	
Female presence × day	-0.215	(-0.339, -0.094)	<0.001	***
Mate availability history × diet	0.023	(-0.014, 0.060)	0.204	
Mate availability history × day	0.053	(0.025, 0.076)	<0.001	***
Mate availability history × diet × day	-0.020	(-0.033, -0.007)	<0.001	***

Variance component	Estimate	95% CI (lower, upper)
Zero-altered		
ID	4.969	(3.844, 6.105)
Poisson		
ID	1.819	(1.557, 2.331)
Residual	1.273	(1.181, 1.391)

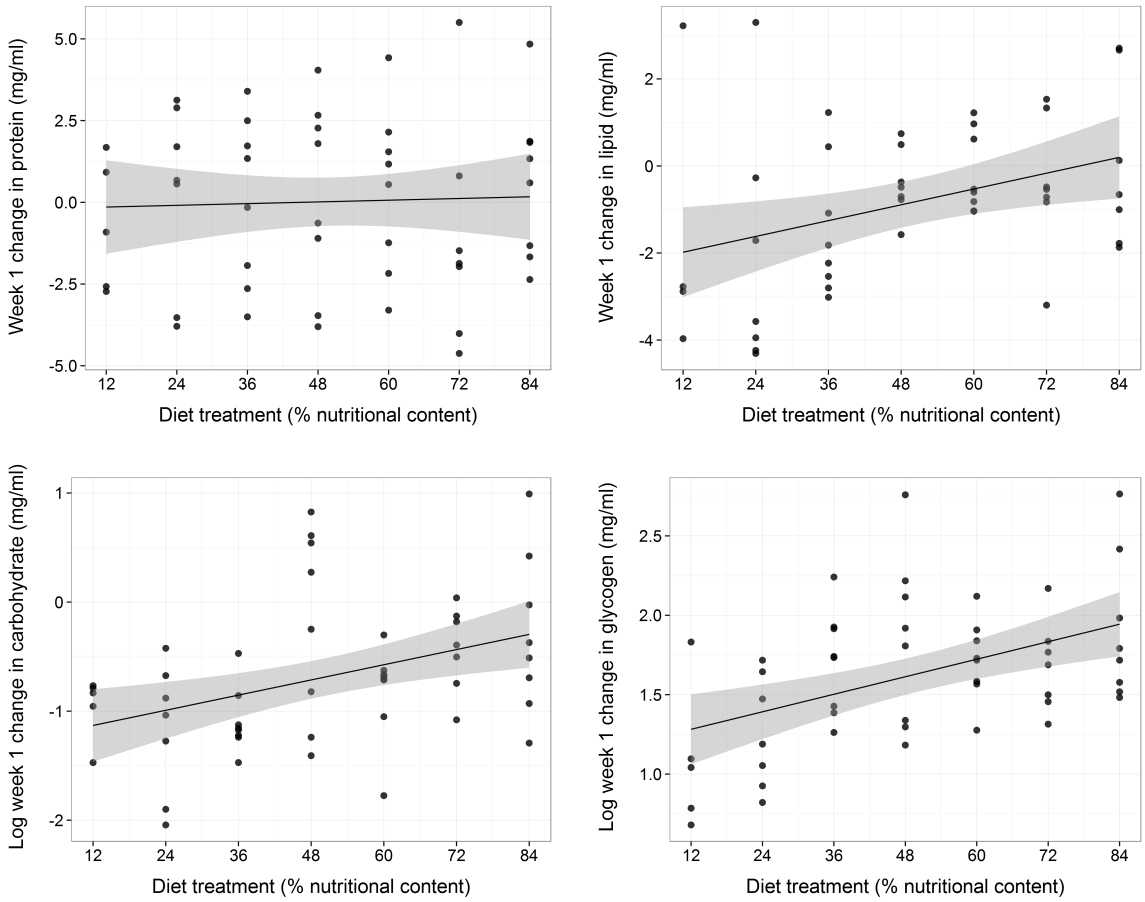

Figure 4.7: The effect of day of the week on the likelihood of male calling during week 2, plotted separately for whether a female was absent or present on the day of measurement. The line shows binomial regression, fitted with glm.

significantly more when a female was present, but the effect of female presence decreased over the course of the week (Figure 4.8). Dietary nutrition significantly increased calling effort, but this effect was reduced in males with greater access to females as the week went on (Mate availability history $\times$ diet $\times$ day interaction term in Table 4.7; Figure 4.9). Greater condition at the beginning of the week also significantly increased the amount of calling a male engaged in on any day.

Increased total calling effort during week 2 significantly decreased body condition over this period (Table 4.8, Figure 4.10). Diet treatment tended to increase condition, but this effect was not significant. Table 4.9 shows the minimum adequate model for a separate analysis of the effects of my experimental manipulations on the change in body condition over week 2. The effect of diet was dependent upon mate availability treatment; the main effect of diet was to increase condition with higher nutrition content, but increased mate availability led to decreased condition, particularly among males fed higher-quality diets (Figure 4.11). This effect could be due to the increased calling effort of males on higher diets and with greater access to females (Figure 4.9). Greater increase in condition over week 1 was associated with greater decrease in condition over week 2. Cannibalising a female cricket significantly increased condition among males; cannibalism was also significantly more likely to occur when males were exposed to females over a greater number of days ($z = -3.789, df = 1, P<0.001$). Re-analysing the data having excluded males that cannibalised females returned highly similar results for the remaining predictors (not shown).

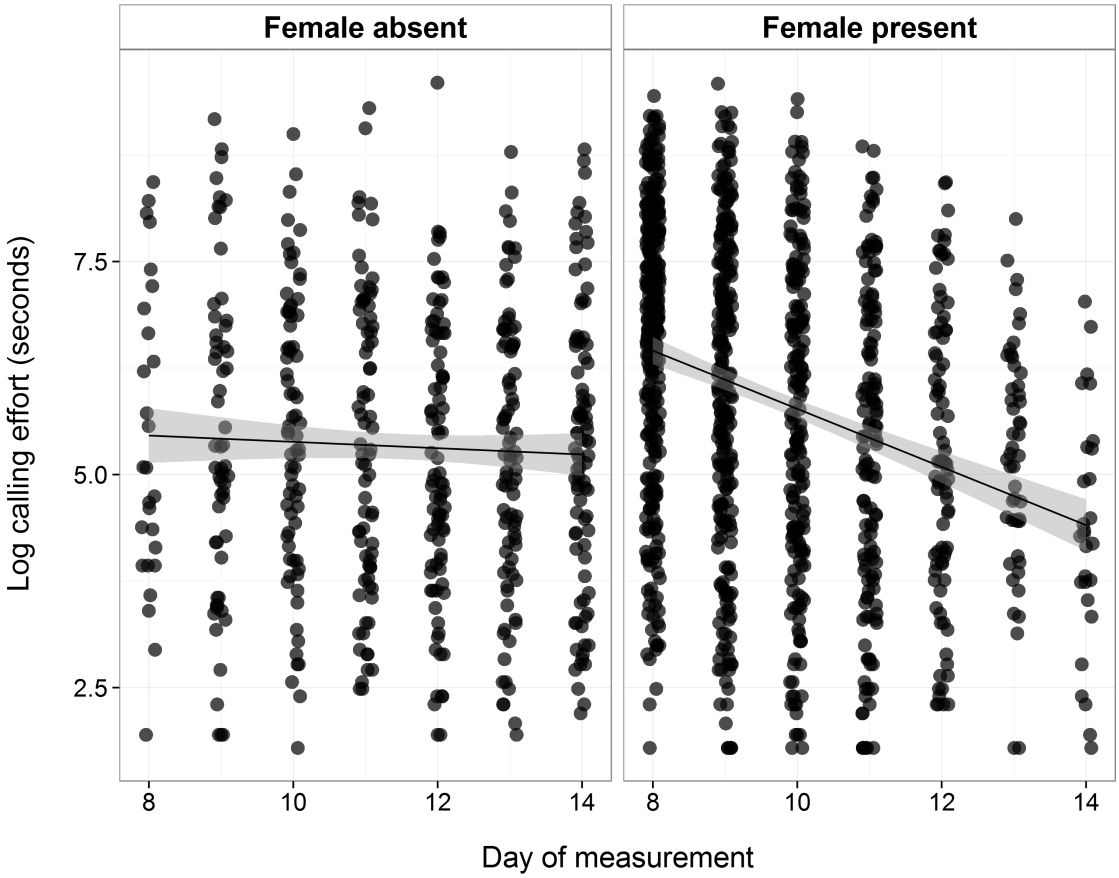

Figure 4.8: Log-transformed calling effort during week 2 post-eclosion, plotted separately for whether a female was present or absent during the measurement period. Zeroes and measurements from males fed 12% nutrition diet have been excluded. Lines show linear regression slopes with 95% confidence intervals.

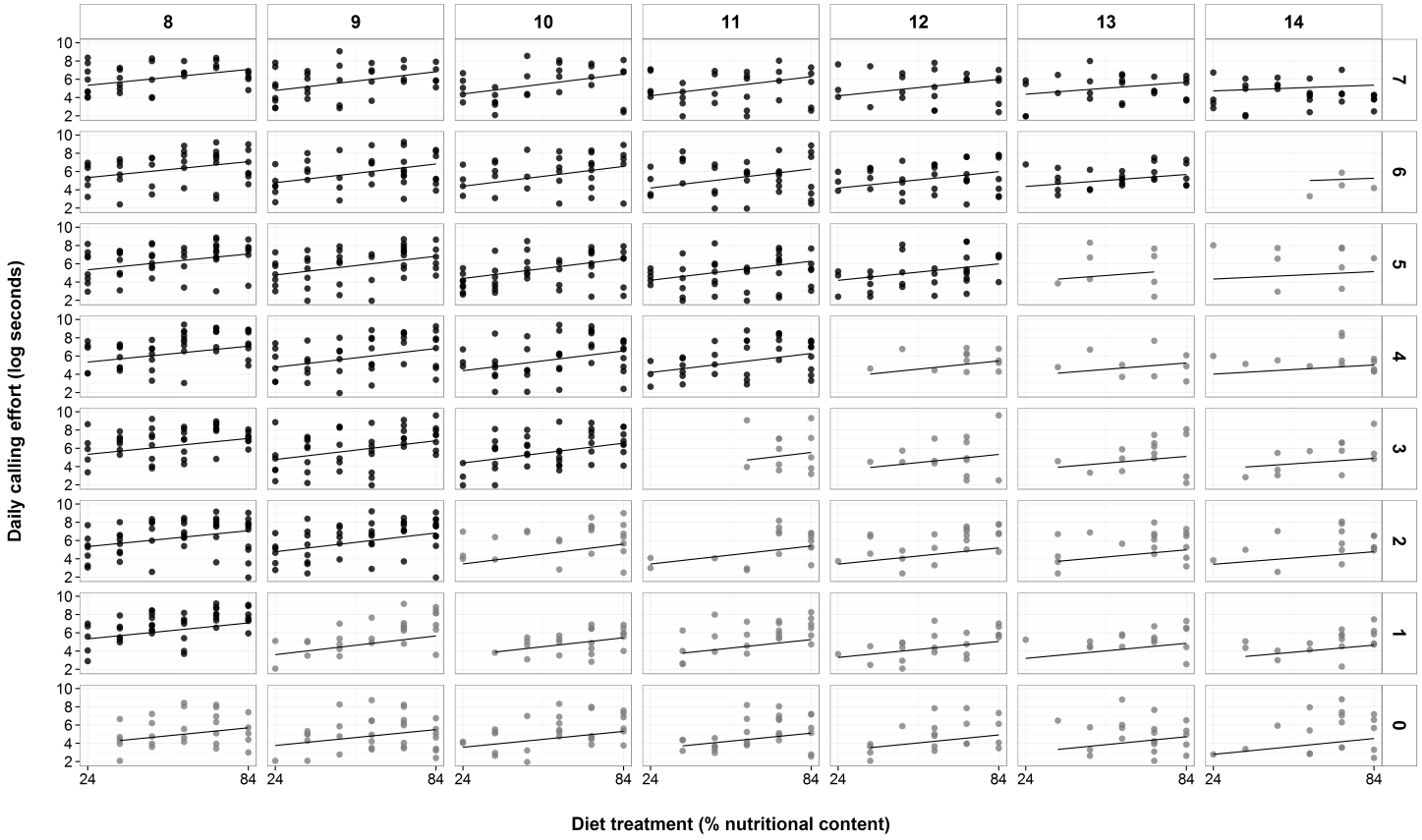

Figure 4.9: Small multiples show how the relationship between diet and daily calling effort changes as a function of day and mate availability treatment. Each individual panel plots the predicted regression slope from the Poisson part of a MCMCglmm zero-altered Poisson model of log-transformed daily calling effort against dietary nutrition (24-84%). Black circles represent measurements taken when females were present; grey circles when absent. Individual panels plot this relationship for each combination of day during week 2 (days 8-15 post-eclosion, overall horizontal axis) and mate availability treatment (0-7 days of consecutive female availability, overall vertical axis). Zero-call measurements have been excluded.

Table 4.8: Parameter estimates for the model showing how change in condition over the first week of adulthood (SMI week 2 - SMI week 1) is affected by how much a male called over week 2 (log-transformed) and diet. Males that did not call during week 2 were excluded from the analysis.

	t	Estimate $\pm$ S.E.	P	
(Intercept)	1.890	0.229 $\pm$ 0.121	0.060	.
Week 2 total calling effort	-2.782	-0.045 $\pm$ 0.016	0.006	**
Diet	1.819	0.030 $\pm$ 0.017	0.070	.
Adjusted model $R^2 = 0.021$; $F_{2,296} = 4.266$, $P = 0.015$				

Table 4.9: Multiple regression showing how change in condition over the second week of adulthood (SMI week 2 - SMI week 1) is affected by diet treatment, mate availability, initial condition, and whether cannibalism occurred.

	t	Estimate $\pm$ S.E.	P	
(Intercept)	-9.738	-0.014 $\pm$ 0.001	$<$0.001	***
Diet	5.740	0.005 $\pm$ 0.001	$<$0.001	***
Diet^2	-4.997	-0.0013 $\pm$ 0.0003	0.001	**
Mate availability	0.470	0.0015 $\pm$ 0.0003	0.638	
Week 1 SMI change	-6.922	-21.160 $\pm$ 3.056	$<$0.001	***
Cannibalised female	8.961	0.018 $\pm$ 0.002	$<$0.001	***
Diet $\times$ mate availability	-2.268	-0.0004 $\pm$ 0.0002	0.024	*
Adjusted model $R^2 = 0.333$; $F_{6,348} = 30.53$, $P <$0.001				

Mate availability significantly increased the likelihood of death during week 2 (Table 4.10, Figure 4.12). The chance of dying was significantly reduced by higher dietary nutrition, although this effect was curvilinear and levelled out at highest nutrition concentrations. Due to the high proportions of deaths in the two lowest diet treatments (12% and 24% nutrition: 100% and 60.5% mortality respectively during

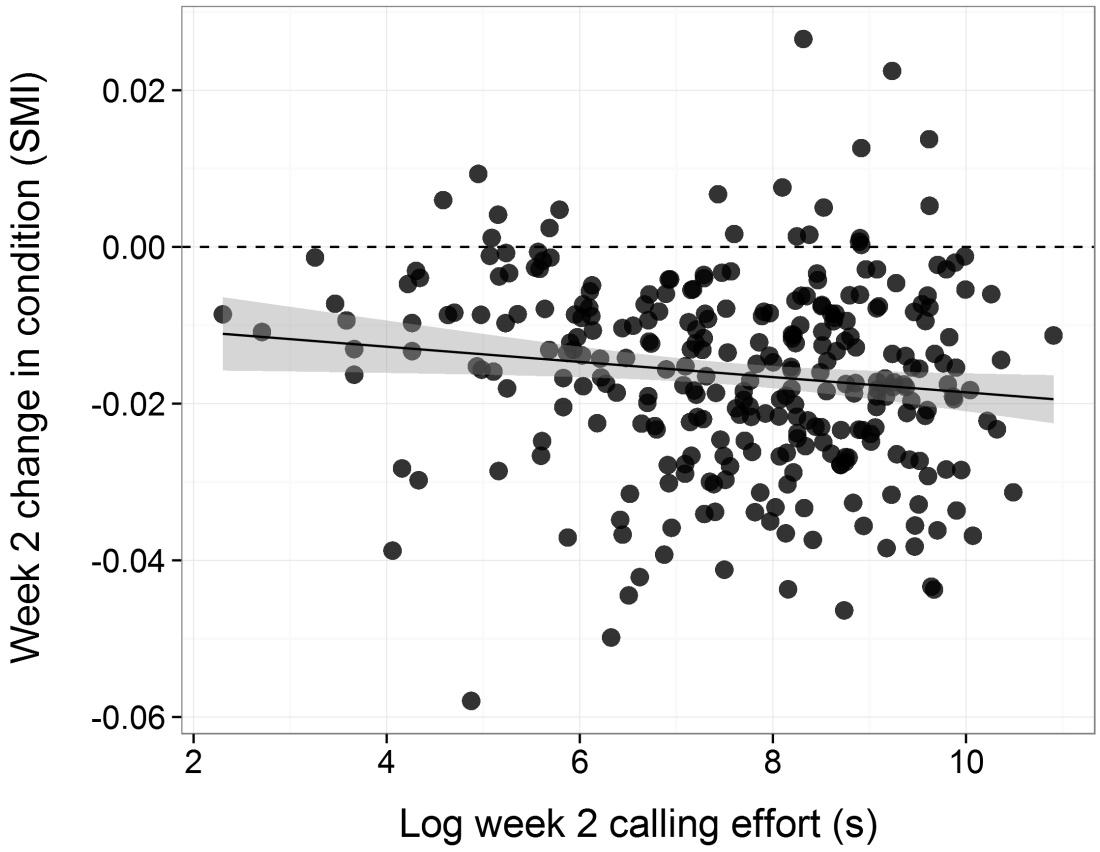

Figure 4.10: The relationship between total calling effort over week 2 (log-transformed, with zero-callers excluded) and the change in condition during this period. Measurements are pooled across all diets.

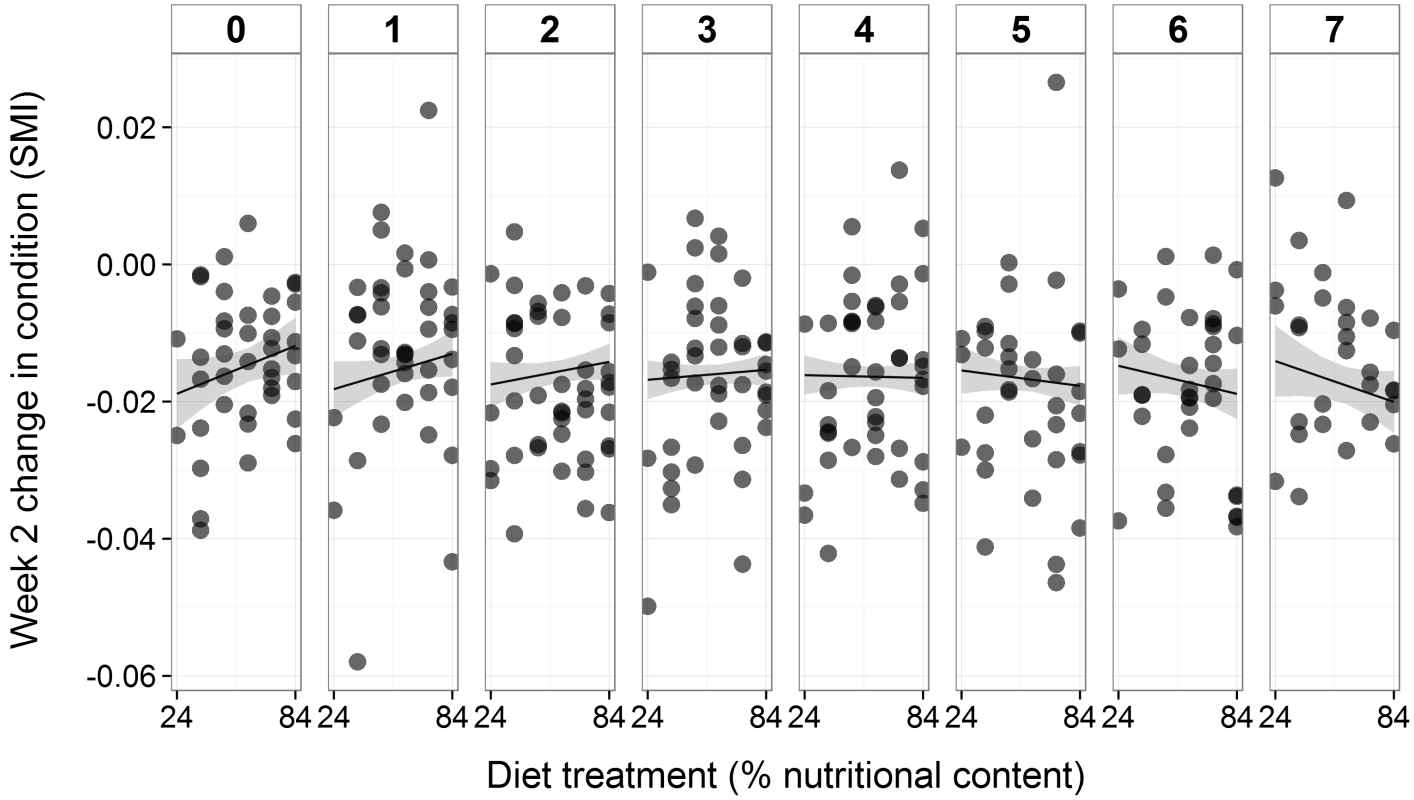

Figure 4.11: Predicted slopes from a linear regression model, showing the change in body condition over week 2 plotted against diet treatment, separately for each mate availability treatment. All males on the lowest nutrition (12%) diet died before the end of the week and so were excluded. Males that cannibalised females during the week have also been excluded.

week 2), I ran the analysis again excluding individuals from these diets (and with diet re-centred so that 60% nutrition was at zero). This analysis also showed that mate availability significantly increased the risk of dying, while diet treatment significantly reduced it.

Table 4.10: Generalised linear model with binomial family describing the influence of diet, mating treatment (plus interactions) on the likelihood of dying during the mating treatment week. Part (a) includes all data; (b) excludes the 12% and 24% diet treatments.

		z	Estimate $\pm$ S.E.	P	
(a)	(Intercept)	-8.198	-2.643 $\pm$ 0.322	$<$0.001	***
	Diet	-11.000	-0.819 $\pm$ 0.074	$<$0.001	***
	Diet^2	6.924	0.307 $\pm$ 0.044	$<$0.001	***
	Mate availability	2.074	0.121 $\pm$ 0.058	0.038	*
(b)	(Intercept)	-7.757	-2.892 $\pm$ 0.373	$<$0.001	***
	Diet	-1.987	-0.225 $\pm$ 0.113	0.047	*
	Mate availability	3.041	0.226 $\pm$ 0.074	0.002	**

There was no significant change in protein content from the expected diet-specific value over week 2 (Table 4.11). Males that called more in total over week 2 suffered the least reduction in lipid storage over this time (Figure 4.13). Body condition after week 1 was a significant predictor of change in glycogen and carbohydrate during week 2: males that had greater SMI at the beginning of week 2 lost more glycogen and carbohydrate over the week (Figure 4.14).

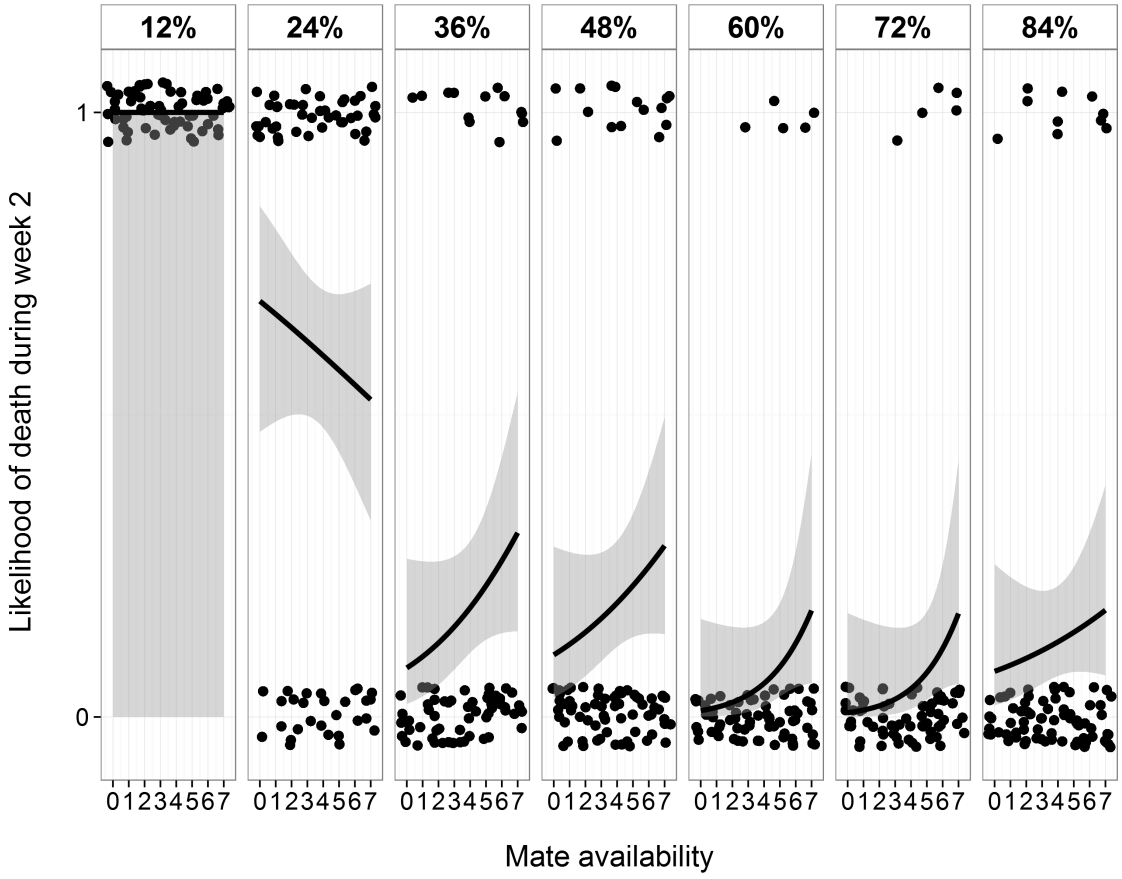

Figure 4.12: The likelihood of dying during the second week post-eclosion, plotted against mate availability treatment and separately for each diet. Lines indicate generalised linear model regression slopes with 95% confidence intervals.

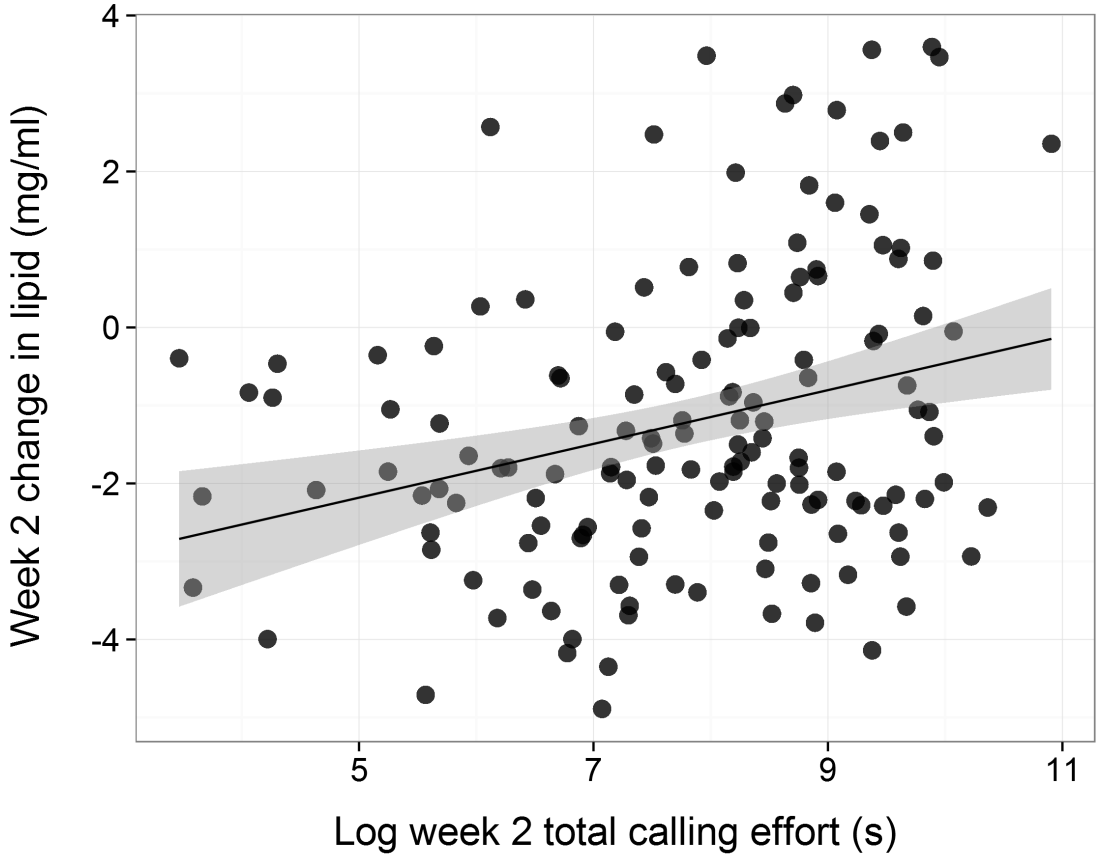

Figure 4.13: Change in lipid content over week 2, plotted against (log-transformed) total calling effort over the same period (with linear regression slope). Males that did not call during week 2 have been excluded.

Table 4.11: Simplified models of predictors of the change in metabolite content (mg/ml) over week 2. The 'change' for each response was calculated as the metabolite content at the end of week 2 minus the diet-specific mean for that metabolite at the end of week 1. The starting model included the following predictors: diet, mate availability treatment, condition at the beginning of week 2, up to third-order interactions between these variables, and log-transformed total calling effort over week 2 as a further covariate. Males that did not call during week 2 were excluded from the analysis; 2 males with body condition over 2.5 standard deviations from the mean were also excluded from the analyses of glycogen and carbohydrate.

	t	Estimate $\pm$ S.E.	P	
ΔProtein				
(Intercept)	0.586	0.122 ± 0.207	0.558	
ΔLipid				
(Intercept)	-5.159	-3.906 ± 0.757	<0.001	***
Week 2 total calling effort	3.630	0.345 ± 0.095	<0.001	***
ΔGlycogen (log-transformed)				
(Intercept)	40.578	1.682 ± 0.041	<0.001	***
SMI (end week 1)	-2.202	-0.198 ± 0.090	0.029	*
ΔCarbohydrate (log-transformed)				
(Intercept)	6.293	0.250 ± 0.040	<0.001	***
SMI (end week 1)	-3.044	-0.260 ± 0.086	0.003	**

4.3.3 Effects of prior exposure to available mates (week 3 onwards)

Mating treatment had a significant effect on lifespan after the end of the second week post-eclosion; males that had greater access to females during week 2 died sooner (Table 4.12, Figure 4.15). Lifespan was significantly increased by greater dietary nutrition, although this effect levelled out at higher quality diets. In the third week post-eclosion, the number of days in which a male recorded a call was significantly reduced by previous mate availability treatment and increased by diet (Table 4.13). The amount of calling effort a male recorded in total over week 3 was significantly

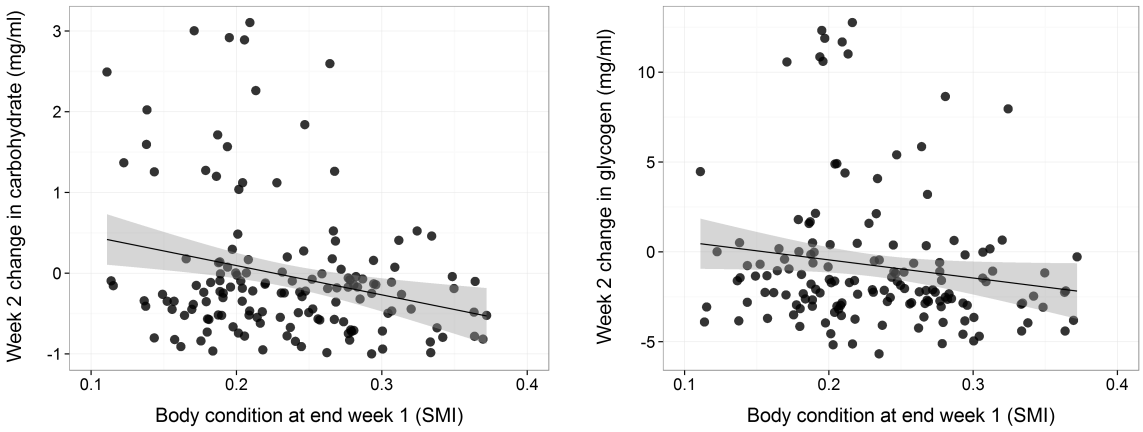

Figure 4.14: Change in carbohydrate (left) and glycogen (right) content over week 2, plotted against body condition at the beginning of the week (with linear regression slope).

increased by diet (parameter estimate $= 0.412 \pm 0.179$, $F_{1,59} = 5.32$, $P = 0.025$).

I used separate models to investigate whether the assumed metabolite content at the end of week 2 affected calling effort during week 3 and lifespan. There were no effects of protein, carbohydrate or glycogen content on calling effort or lifespan. Males with greater lipid content at the end of week 2 lived longer (parameter estimate = 5.588 ± 1.966, $F_{1,138} = 8.079$, $P = 0.005$; Figure 4.16), and called on more days during week 3 (parameter estimate $= 0.343$, $z_{1,138} = 2.947$, $P = 0.003$). Lipid content did not affect a male's total amount of calling effort in this period, however (parameter estimate $= 0.303 \pm 0.209$, $F_{1,59} = 2.10$, $P = 0.153$).

By contrast, the scaled mass index of condition at the end of week 2 was not a significant predictor of lifespan (parameter estimate $= 7.355 \pm 4.960$, $F_{1,140} = 2.199$, $P =$

0.140), likelihood of calling during week 3 (parameter estimate $= 0.463, z_{1,140} = 1,562, P = 0.118$), or the amount of calling during week 3 (parameter estimate $= 0.009 \pm 0.526, F_{1,59} < 0.001, P = 0.986$).

Table 4.12: Multiple regression showing how lifespan in those males surviving after the end of week 2 is affected by diet and mate availability treatments.

	t	Estimate $\pm$ S.E.	P	
(Intercept)	18.370	75.039 ± 4.085	<0.001	***
Diet	6.821	11.823 ± 1.734	<0.001	***
Diet^2	-2.868	-2.491 ± 0.869	0.005	**
Mate availability	-4.176	-3.913 ± 0.937	<0.001	***
Adjusted model $R^2 = 0.344$; $F_{3,143} = 26.55$, $P < 0.001$				

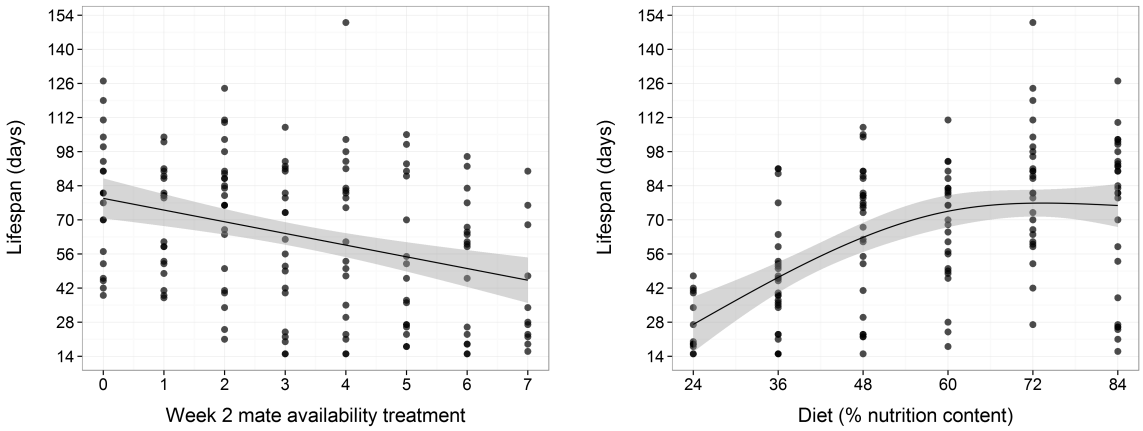

Figure 4.15: Effects of mate availability (left) and dietary nutrition (right) treatments on the lifespan of males that survived after the end of week 2.

Table 4.13: Negative binomial regression of the number of days during week 3 in which a male called on diet and week 2 mate availability treatment.

	z	Estimate $\pm$ S.E.	P	
(Intercept)	1.622	0.417 ± 0.251	0.097	.
Diet	4.200	0.388 ± 0.092	$<$0.001	***
Mate availability	-3.224	-0.211 ± 0.066	0.001	**

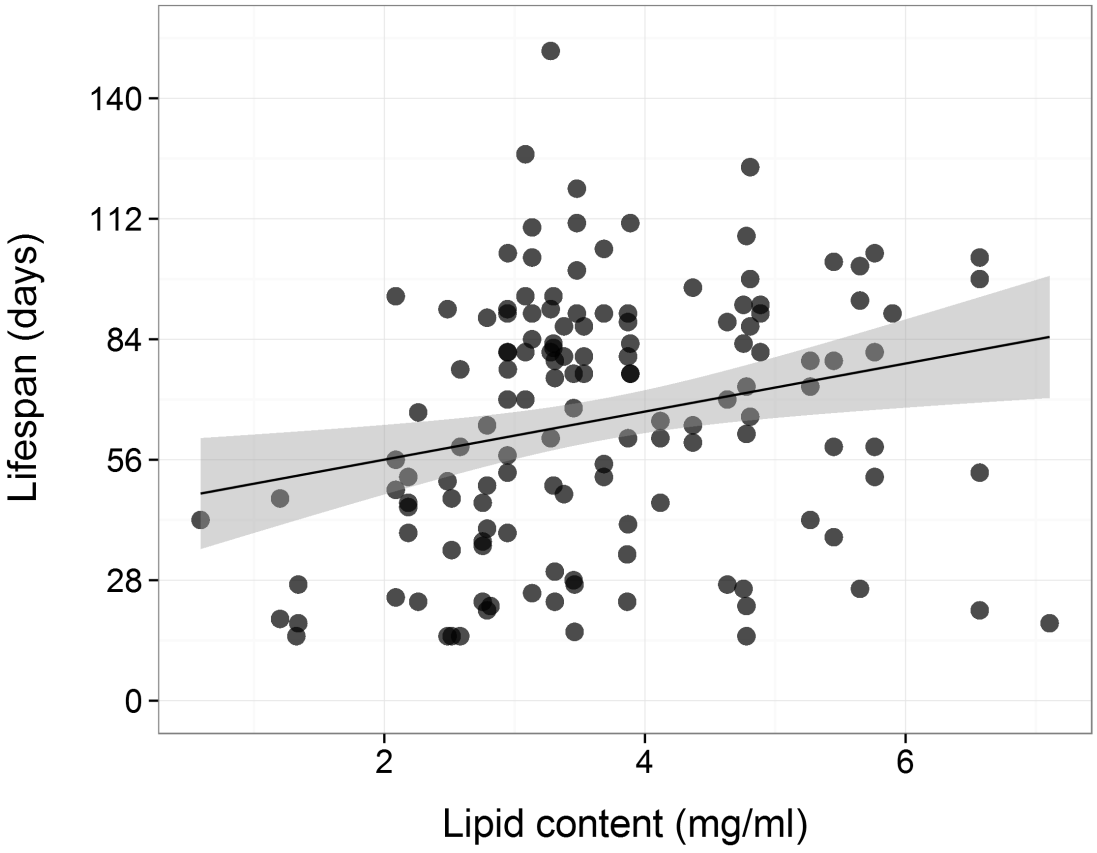

Figure 4.16: Effects of lipid content at the end of week 2 on lifespan of males surviving beyond this point.

4.4 Discussion

By manipulating dietary nutrition in male decorated crickets, I showed that greater resource acquisition led to an increase in both the likelihood and amount of signalling over the first week of adulthood, as well as to greater investment in energy stores (lipid, glycogen, and carbohydrate content) during this period. The relationship between investment in condition and calling effort was dependent on diet, switching from negative covariance when diet quality was low to positive when high.

During the second week, males responded strongly to the presence of a female by increasing both the likelihood of calling and time spent calling. The dietary manipulation did not affect the likelihood of whether a male signalled on any given day, but instead had a strong effect on the time spent calling. Calling effort was subject to complex interactions between resource acquisition and the availability of potential mates, with the positive effect of diet decreasing with greater exposure to females over the course of the week. The change in male condition during week 2 as a function of diet depended on access to females: greater female availability led to males with greater resource acquisition to lose more condition than those with poorer resource acquisition. Diminishing calling effort by the end of the week, even by high-quality males in the presence of females, suggests an exhaustion of available resources over this period. Indeed, the change in male condition over week 2 covaried negatively with total calling effort, and the likelihood of calling decreased as previous exposure to females increased - both results irrespective of diet. Greater exposure to potential mates also increased the risk of dying during week 2, although this was mitigated by diet qual-

ity. There were lasting consequences of my experimental manipulations: longevity following week 2 was increased by dietary nutritional quality, and decreased by exposure to females. However this reduced viability cannot be considered a true cost unless it is accompanied by a net decrease in fitness, because any decrease in lifespan may be offset in fitness terms by increased early reproductive success.

Loss of carbohydrate and glycogen content during the second week was greatest in those males that had been in best condition at the beginning of the week; meanwhile, males who called the most in total over week 2 also accumulated the most lipids in this period. Lipid content at the end of the second week was also associated with greater lifespan, and greater likelihood of calling during the following week. Variation in the conservation of different metabolites suggests lipids differ from carbohydrate and glycogen in usage and in importance for allocation to different life history traits.

4.4.1 Insights from physiological measures

Previous studies have mapped with great precision how nutrient acquisition affects male reproductive effort and lifespan in crickets (Maklakov *et al.*, 2008, 2009). Here, I examine the physiological storage compounds that may mediate functional mechanisms relating resource acquisition to allocation.

Increasing dietary nutrition after eclosion enabled male crickets to allocate resources

to storage in the forms of greater lipid, glycogen, and carbohydrate content. These appear to represent the major forms of energy stores; protein levels did not vary over the 3 time points that the metabolites were measured, indicating that males seem to maintain protein content rather than seek to increase it (while the nutrient ratio was heavily in favour of carbohydrate rather than protein, the variation in total nutrient content would enable individuals to differ in allocation if required). The increase in body condition over the first week was a significant predictor of the decrease in condition that males suffered over week 2, suggesting that resource acquisition early in the adult stage tended to be mobilized for fuelling calling effort. Body condition at the end of the first week also predicted the decrease in both glycogen and carbohydrate over week 2, both of which are quick-release forms of energy. By contrast, the change in lipid content over week 2 was positively correlated with total calling effort for this period. The absolute amount of acquired resources is known to affect trade-offs, the determination of which can be affected by priority rules (Zera & Harshman, 2001): the importance of lipids is demonstrated by the fact that lipid content at the end of week 2 predicts lifespan and the future likelihood of calling. Together, these results suggest that glycogen and carbohydrate are more freely expendable forms of energy, while lipids are more carefully conserved. If I had not measured glycogen and carbohydrate content, I might have mistakenly concluded that total calling effort in week two covaried negatively with the change in condition, but positively with the change in lipid content. This serves to underscore the importance of estimating multiple metabolic reserves.

4.4.2 Trade-offs or budget constraints

Early models concluded that reproductive effort should increase with age (Williams, 1966), yet recent studies employing game theoretical approaches and taking life history theory into account show that this is not necessarily the case (Kokko, 1997, 1998; Lindström *et al.*, 2009). Kokko (1998) illustrated 3 phenomena that can prevent positive correlations between life history traits: 1) trade-offs acting over lifetimes, such that fitness benefits early in life outweigh late life allocation (Hansen & Price, 1995); 2) increasing marginal gains in fecundity could mean that the optimal tactic for high-quality males is to decrease survival in favour of intense sexual advertisement; or, 3) allocation is not optimal. The last point is of particular interest: if individuals can vary trait expression in a plastic manner in heterogeneous environments, and trait expression is under selection, it seems paradoxical that adaptive plasticity for continuous traits is not the norm (Scheiner & Holt, 2012). However, the genetic and regulatory machinery required for controlling allocation across traits and over time is undoubtedly expensive to maintain, and may not confer a sufficiently high selection advantage over a simpler set of spending rules: expend resources on traits if you have them, and if not, focus on acquiring more resources instead. The differences we find in crickets that signal at high and low levels early in life may be primarily determined by budget constraints as opposed to careful anticipation of future opportunities.

Crickets are ideal for testing theories concerning age-related reproductive effort because it is possible to precisely quantify sexual advertisement effort by males. Several studies have manipulated resource acquisition to investigate its effects on life

history traits, with largely consistent results: males usually increase their calling effort with age, and longer-lived males call more (Judge *et al.*, 2008; Maklakov *et al.*, 2009; Zajitschek *et al.*, 2009b, 2012). One exception is the study of Hunt *et al.* (2004a), in which high-quality males called earlier and far more intensely than low-quality males, and also suffered survival costs such that high-quality males died earlier than low-quality males. This pattern of age-related allocation, in which the correlation between advertisement effort and survival is negative, has been shown to be adaptive for high-quality males in some cases (Kokko, 1997; Höglund & Sheldon, 1998; Kokko, 1998). In such situations, an evolutionarily stable strategy would suggest that low-quality males can benefit from suppressing signalling at early ages and investing in longevity, such that they benefit from increased advertisement later (Kokko, 1997). If this pattern of adaptive plasticity in investment to calling effort applied to *G. sigillatus*, optimally behaving males restricted to lower-nutrient diets in my study should have allocated resources preferentially to energy storage in lieu of early signalling.

Males fed lower nutrition diets did call less in my study, and the correlation between acquisition (the change in condition) and calling effort was negative during week 1 among males on lower-quality diets. However, although diet-restricted males were less likely to call over the first week, this effect was not evident during week 2. If low-quality males had continued to show a depressed propensity to call, and had increased condition 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, and

having less energy to allocate to signalling. The non-adaptive explanation is given further credence by dietary nutrition being a strong predictor of survival, with low-quality males suffering reduced longevity.

4.4.3 Response to female presence

Male crickets responded strongly to female presence in the second week, 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 focussed on adaptive plasticity for anticipating future conditions (Kasumovic & Brooks, 2011): manipulations of perceived density and future competition have shown adaptive effects on life history traits at adulthood (Bailey *et al.*, 2010; Kasumovic *et al.*, 2012b,a). Manipulations of the cricket social environment at adulthood have thus far been less common; Callander *et al.* (2013) used a manipulation of the social environment at adulthood by measuring the calling effort of male crickets who had been either kept isolated or housed with a rival male after reaching maturity. However, all males in this study were then isolated for the measurement period, which might explain the lack of behavioural plasticity across treatments (Callander *et al.*, 2013). The extreme phenotypic flexibility of behavioural traits means that individuals can react quickly in adjusting trait expression in response to social cues (Ghalambor *et al.*, 2010; Bretman *et al.*, 2011). Storage of resources in 'quick-release' form as sugars (carbohydrate and glycogen) provides male crickets the flexibility to signal more effectively in the presence of females, hence increased calling effort by males on higher-quality diets

during mate availability treatments.

An unresolved question from my study relates to the decrease in calling effort over the second week due to greater previous exposure to females. Decreases in condition and survival over this period make it likely that males are running low on resources; however, as noted earlier, lipid content covaried positively with total calling effort over week 2. Lipid metabolism is known to be important for a variety of functions in insects (Arrese & Soulages, 2010), and lipid content was here associated with greater longevity after the end of week 2. This result raises the question of why high-quality males do not sacrifice a greater proportion of this metabolite for increased signalling.

4.4.4 Big houses, big cars

Variation in condition 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 *et al.*, 2001). Figure 4.6 demonstrates clearly how such a correlation can arise when some males have acquired a greater amount of resources that they can then allocate to two competing traits. A negative correlation between two traits can be magnified substantially by reduced nutrient availability, as seen in the relationship between investment in condition and calling effort at low-nutrient diet treatments during week 1. Increased nutrient availability can mask the negative correlation between two traits because individuals have more resources to allocate to both (as seen in the positive relationship between the same traits when dietary nutri-

tion is high); here, the mean change in condition increased with diet and with total calling effort, indicating that individuals allocate more resources to both storage and current reproductive effort as acquisition levels increase. The biological implications of these results relate to how males are likely to sample the 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 increase in mean change in condition 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 quality males, as predicted by models of sexual selection under condition dependence (Grafen, 1990; Rowe & Houle, 1996). These findings mirror interspecific (Judge *et al.*, 2008) and intraspecific (Hunt *et al.*, 2004a) differences in the covariance between expensive traits under contrasting conditions, and invite more study in more taxa of the storage and use of metabolites over time.

General discussion

In this thesis, I have explored the relationship between an individual's resource pool and investment in age-related reproductive effort and survival. My first chapter set out how life history theory and sexual selection are intertwined, owing to the trade-offs that occur when individuals face allocation decisions about investing their finite resources in traits that affect fitness. I argued that the most glaring challenge to progress in this field was the inherent difficulty in measuring the elusive properties of acquisition and allocation, not least because these processes are not independent of one another.

In the subsequent chapters I used diet manipulations, quantitative genetic approaches and physiological techniques, to examine not only what maintains variation in a sexually selected display, but also to determine the potential life-long consequences of investment in mating traits that are so crucial to sexual selection theory (Zahavi, 1975; Andersson, 1982; Nur & Hasson, 1984; Andersson, 1986; Grafen, 1990; Johnstone, 1995; Rowe & Houle, 1996; Kotiaho *et al.*, 2001).

Chapter 2 showed that males and females differ substantially in how they allocate resources to important life history traits over time. I found that male age-dependent allocation to calling was quite consistent across diet treatments, while females showed substantial divergence across treatments in age-specific fecundity. Although the potential causes I outlined in Chapter 2 are somewhat speculative and require further investigation, females appear subject to greater time and nutrient-specific constraints than males in how they allocate to age-specific reproductive effort (see below). The positive correlations between reproductive effort and lifespan in males should not, perhaps, be surprising: within-treatment variation in male quality can cause some individuals to have more resources to spend on all fitness-enhancing traits (Van Noordwijk & De Jong, 1986; Reznick *et al.*, 2000). This begs the question of why positive correlations between reproductive effort and lifespan are not seen in females. One potential explanation stems from the work of Maklakov *et al.* (2008) using field crickets (*Teleogryllus commodus*), which showed that reproductive effort and longevity were maximised using similar nutritional profiles in males; by contrast, females maximised reproductive effort via protein intake and longevity via carbohydrates. Variation in male quality in my experiment might therefore be related to sheer metabolic efficiency, where the ability to acquire and convert resources to an all-purpose store enables positive correlations between life history traits. Females, meanwhile, are constrained by particular nutrient mixtures in how they maximise fitness, resulting in a necessary compromise between reproduction and longevity, which is ameliorated or deepened depending on the particular sequence and composition of diets through development.

The results from Chapter 2 suggested further questions that I attempted to address in the subsequent chapters. First, the similarity of male age-dependent signalling patterns across treatments required further investigation: if all males respond to an environmental manipulation in a similar fashion, what are the consequences for the maintenance of additive genetic variation when the signal trait is subject to directional selection through female choice? Resolving this question required a genetic approach, which I pursued in Chapter 3. Chapter 2 also highlighted the need to study resource allocation more directly, by manipulating acquisition and tracking metabolite storage and use over time, which I attempted in Chapter 4.

Genotype-by-environment interactions (GEIs) can help to maintain additive genetic variation under sexual selection when environmental conditions are variable (Kokko & Heubel, 2008; Higginson & Reader, 2009), because selection fluctuates over time and space as different combinations of alleles are favoured in different contexts. I assessed genetic variation and plasticity in how male *Gryllodes sigillatus* allocated their resources to life history traits in Chapter 3, using inbred genetic lines (Ivy *et al.*, 2005) and the same dietary manipulations as in Chapter 2. Recent interest in GEIs has been piqued by their potential to explain the persistence of female choice when there are no apparent direct benefits, and to help maintain additive genetic variation in sexual traits (Bussière *et al.*, 2008a; Ingleby *et al.*, 2010). However, this variance-maintaining mechanism may also exhibit what has memorably been termed a 'Jekyll & Hyde nature' (Holman & Kokko, 2014): GEIs acting alongside environmental heterogeneity

can cause problems for the value of the signal to the receiver (Greenfield & Rodríguez, 2004; Kokko & Heubel, 2008; Higginson & Reader, 2009). In such cases, any direct cost of choosing to females might override the potential benefits of choice (Kirkpatrick, 1982).

By testing for GEIs in a trait that is liable to change with age, I was able to investigate whether genotypes differed not only in their general ability to acquire resources from each environment, but also in how they resolved the trade-off between current and future reproduction. My results make for exciting, if perhaps slightly unsettling, reading: male signal trait expression was strongly affected by both age and the environment, such that the correlation between performance at one measure and another weakened as the 'distance' (in terms of both environment and age) grew. The implications of these findings for wild populations are uncertain, because my lab study by necessity focussed on a limited range of genotypes and environmental manipulations. Nevertheless, my results demonstrate the potential for substantial uncertainty when females choose males on the basis of behaviourally plastic signal traits. Clearly, we need estimates of age-dependent and environmentally determined variation in such traits in the wild to permit closer examination of the signal value of behavioural displays. Such wishes are not idle: Rodríguez-Muñoz *et al.* (2010) successfully used a combination of video monitoring and DNA profiling to investigate factors affecting reproductive success in a wild population of field crickets (*Gryllus campestris*). This kind of field study system has great potential for opening exciting new avenues for research on the real costs and benefits of investment in behaviourally plastic sexual

signals in wild populations.

Is there truth in advertising?

A central theme of my thesis has been whether honesty is maintained when males use energetically expensive displays to signal their quality to females. As noted in my first chapter, the existence of metabolic trade-offs provides a general answer to the question of why individuals do not 'cheat' through disproportionate investment in their sexual trait. However, when males are free to alter allocation to a repeatedly expressed trait, the correspondence between genotype (male quality) and phenotype (display) may be greatly impaired, leading to questions as to how females can make informed choices based on any single assessment of male display Kokko (1997). As detailed above, the existence of genotype-by-environment-by-age interactions casts serious doubt as to the reliability of male signals that are subject to trade-offs.

The optimal tactic for low-quality males might be to suppress early reproductive effort in favour of greater display intensity later (Roff, 1992; Kokko, 1997). For example, in Chapter 2, some males recorded very high calling effort measurements late in life despite being assigned to the low-nutrient diet treatment at adulthood. Males in this treatment were also less likely to call early in adulthood than those fed a high-quality diet. While these results correspond superficially to the pattern of dishonest signalling predicted above, simple resource acquisition manipulations allow only

measurement of each life history trait in isolation, rather than explicit determination of allocation trade-offs. One of the biggest constraints on studying life history allocation is our inability to precisely measure acquisition and allocation (as opposed to crude surrogates of these values), particularly because both processes encompass many characters, and affect virtually all aspects of a subject's biology. In my final chapter, I sought to address this shortfall by manipulating resource acquisition and sexual trait allocation while simultaneously assessing changes in metabolic reserves.

By using physiological assays to determine the average metabolic resource budgets of individuals at several time points, I found that males allocated resources to their energetic budget in the forms of longer-term storage (lipids) and quick-release energy (glycogen and carbohydrate). The presence of females caused increases in chorus participation and the intensity of calling, with males expending their glycogen and carbohydrate stores, presumably to fuel signalling effort. Males with higher quality food called more, but this effect weakened over time and with greater access to females. The negative covariance I report between total calling effort and the change in condition over time suggests that males exhausted their energetic budget in the presence of females. The reduction in calling effort over the week, even in the presence of females, could be due to an adaptive allocation decision to maintain energy reserves with an eye on future investment. An alternative, and more likely, explanation is that this reduction represents an inability to mobilise enough energy to match previous calling levels. That a male's current signalling effort is governed by his acquisition ability and previous expenditure suggests that metabolic trade-offs do pro-

vide a mechanism for maintaining honesty on average in male behavioural display (Kokko, 1997).

I also found that my manipulations of acquisition and allocation had long-term life history consequences, with longevity increased by diet quality but decreased by exposure to females. Notwithstanding the risks of extrapolating from laboratory studies, the lasting metabolic consequences of my allocation treatment highlight the potential for viability costs as a function of increased allocation to signalling behaviour. Before any bolder statements can be made, such results require estimations of natural variation in behaviour and reproductive success: decreased longevity cannot be considered a true cost if increased signalling results in greater fitness (Kotiaho *et al.*, 2001).

> *"You may be disappointed if you fail, but you are doomed if you don't try."*
>
> – Beverley Sills

The amount of time a male spends signalling to females is often the focus of studies of sexual selection, as it enables quantification of his reproductive effort as well as being a strong indicator of his reproductive success (Hunt *et al.*, 2004a; Bentsen *et al.*, 2006). However, I found that many males did not call at all during a given measurement period (a result common to studies of male cricket signalling behaviour, e.g., Hunt *et al.*, 2004a; Judge *et al.*, 2008). My statistical approach allowed me to analyse what factors affect the decision to call, in addition to the actual signalling level. The use of zero-altered models to ask these questions in a single framework has revealed subtleties

in the data that might otherwise have gone unnoticed. For example, in Chapter 4, the interaction between female presence and day is significantly positive for the decision to call, and negative for the amount of calling. Males were less likely to call as time went on, but more likely if a female was available; this could be due to decision rules whereby a male running low on reserves due to previous signalling effort should generally try to reduce calling in order to conserve resources, but the availability of a female makes the optimal behaviour that of a minimal amount of advertisement. The amount of calling effort was greater when a female was available, again perhaps because the situation makes advertisement optimal; the reduction of time spent calling in the presence of a female as the week went on is most likely due to decreased resource availability (particularly in terms of quick-release glycogen or carbohydrate). Distinguishing a male's effort from his binary decision to broadcast a call is therefore clarifying, but also raises far-reaching questions about the adaptive significance of plasticity in allocation, especially insofar as hypothesized adaptive plasticity is supported by experiments where males are isolated from a social environment featuring realistic numbers of choosy potential mates. Such studies are undoubtedly valid in their potential to address questions about the processes underlying metabolic tradeoffs between calling effort and other life history traits. Assessing the adaptive significance of behaviour would require housing crickets in conditions that more realistically mimic the natural situation. Although Chapter 4 does not mimic the natural situation, it does experimentally assess the plastic behavioural responses of males to females depending on their availability, further underlining the importance of the cricket social environment when considering calling effort over time.

Budget constraints or savings plans

In my thesis, I have been concerned with how animals spend and save resources; throughout, I have tried to remain focused on the process of acquisition and allocation, rather than straying into speculative adaptive explanations. Such is the danger when investigating phenotypically plastic traits, especially in the laboratory: while plasticity can often give the appearance of design, it is not necessarily adaptive (Piersma & Drent, 2003; Scheiner & Holt, 2012). Whether or not the age-dependent patterns I have reported have been strongly shaped by selection or not remains unclear.

Behavioural plasticity enables males to assess and respond to environmental conditions in a highly dynamic and flexible manner (Komers, 1997; Ghalambor *et al.*, 2010; Bretman *et al.*, 2011). What constitutes an adaptive plastic response? Ghalambor *et al.* (2007) define adaptive plasticity as a reaction norm that results in the production of a phenotype in the same direction as the optimal value that selection favours in the new environment. Bertram *et al.* (2013) provide evidence of adaptive phenotypic plasticity in the timing of calling by males of two wild-caught species of cricket (*Gryllus veletis* and *Gryllus pennsylvanicus*), where males aligned their signalling behaviour during those periods of the day when mating activity is typically at its highest. My results in Chapter 4, whereby males greatly increase both the positive decision to call as well

as the level of calling effort when females are present, almost certainly also represent adaptive plasticity in the sense that modulating advertisement to maximise its exposure to females is undoubtedly an economical use of metabolic resources.

A larger (and less well addressed by my thesis) question concerns whether the diet and age-dependent patterns of metabolic accumulation and expense are adaptive. Is it best to conserve resources when they are scarce, perhaps while waiting for rivals to have gone past their peak performance (Kokko, 1997, 1998; Hunt *et al.*, 2004a)? The notion that part of the age-dependent variation we see in cricket calls has been shaped by selection on life history is seductive (Bretman *et al.*, 2011; Bertram *et al.*, 2013). As noted above, a concern raised by my final chapter is whether the ability of crickets to modulate their behaviour in a dynamic fashion renders laboratory experiments on the adaptive significance of life history allocation patterns fruitless. In this vein, an alternative to adaptive explanations is simply that age-dependent patterns of calling effort need not be adaptive: the variation we see in age-dependent calling may be largely a consequence of current resource constraints, rather than selection on future prospects. The two alternatives above can be represented neatly by the ant and the grasshopper from Aesop's fable: in the summertime, when food is abundant, the grasshopper chirps and sings to his heart's content, with little care for the coming winter and its promised hardship. Meanwhile, the ant is thrifty and forward-looking, storing food away to be distributed in the cold of winter. To pose a question that would horrify any literal-minded taxonomist: are crickets ants, or are they grasshoppers? More pertinently, to what extent do my data tell us about the

prevalence of ant and grasshopper life history strategies among animals?

The answer to the first question is that crickets fall, as is so often the case, somewhere in between these two extremes. Perhaps a better or more familiar analogy for resource budgeting decisions than ants and grasshoppers is that of ready cash versus savings accounts. Is an individual's response to social conditions shaped primarily by future planning and the careful budgeting of resources, or limited simply by the amount of energy ready to spend at that time? To draw a direct comparison with my final chapter, glycogen and carbohydrate content appear to be ready cash, while lipids represent savings (to stretch the analogy to breaking point: inasmuch as lipids provide more energy than sugars on a gram-for-gram basis, savings can also be considered to accumulate interest, Campbell & Farrell, 2003). The standing phenotypic variation I observed in calling effort seemed to largely reflect resource limitation and so, combined with the exhaustion of glycogen and carbohydrate reserves over the second week of my experiment in Chapter 4, calls to mind energetic constraints as the dominant driver of behavioural variance. Nevertheless, there was substantial accumulation of lipids in some treatments, and a positive covariance between total calling effort and lipid content. Whether this kind of storage varies meaningfully in natural populations, and whether any such variation is the prime target of female good-genes choice is unclear.

The extent to which my data can generally inform about the prevalence of age-dependent adaptive plasticity in allocation across taxa likely depends on several aspects of ecol-

ogy and mating system. Decorated crickets show some peculiarities that made them good study subjects but may limit their relevance to a subset of the animal kingdom. Female *G. sigillatus* are highly polyandrous, mating frequently throughout life with many males, often more than once per night (Sakaluk *et al.*, 2002). While the spermatophylax transferred by males may not be as nutritious as those of bushcrickets (Will & Sakaluk, 1994; Warwick *et al.*, 2009), males nevertheless must invest substantially in each mating (including the average 3.25 hours required to rebuild a spermatophore after mating Sakaluk, 1985). Perhaps this type of mating system has selected for male allocation strategies that are more cautious and responsive to the social environment (Starrfelt & Kokko, 2012), and which require more careful allocation than the mating efforts of other taxa: given that behavioural signalling enables a dynamic response to changing conditions, ready cash should always be to hand in case a mating opportunity presents itself to a male decorated cricket. A sensible strategy is to first ensure that there is money in the bank to subsidise requirements in case of changing environmental conditions. As the moral goes: it is best to prepare for times of need.

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