

Food-limited mothers favour offspring quality over offspring number: a principal components approach

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Summary

1. Mothers are expected to balance the trade-off between the number and quality of offspring, and many theoretical studies describe how the maternal environment might influence the evolution of the number–quality trade-off.

2. However, few empirical studies attempt to test these theories (and their assumptions) by measuring the fitness consequences of variation in investment per offspring. Part of the problem is that measuring offspring fitness is difficult, which frequently leads experimenters to measure several proxies of offspring fitness in place of a comprehensive fitness assay. This strategy tends to result in multiple univariate analyses that involve different offspring fitness proxies, and these tests can have low power and may produce conflicting conclusions.

3. Here, we demonstrate the benefits of integrating maternal fecundity and proxies of offspring size and fitness into multivariate analyses to elucidate variation in reproductive allocation strategies. In a 2×2 factorial experiment, we manipulated the quality of maternal environment (food availability) throughout early and late adulthood (acute and chronic exposure to the maternal environment) in a field cricket.

4. We developed a multivariate index of reproductive allocation by incorporating maternal fecundity and the performance of offspring in low- and high-food environments into a principal components analysis. This index of reproductive allocation indicated that females decreased fecundity and increased offspring quality after chronic exposure to low-food environments, thereby providing evidence of adaptive plasticity in investment per offspring. In contrast, few treatment effects were observed using univariate analyses.

5. The present study demonstrates that multivariate analysis can increase our ability to assess the adaptive significance of reproductive strategies, particularly in situations when offspring size and fitness are difficult to measure with accuracy. Such an approach might ultimately help assess the adaptive significance of reproductive allocation across a wider range of taxa, thereby providing broader insight into the evolution of reproductive strategies.

Key-words: adaptive plasticity, cricket, environmental quality, *Gryllus texensis*, multivariate, optimal egg size, reproductive strategy, trade-off

Introduction

Parents must divide the energy available for reproduction between investment per offspring and fecundity (Lack 1947). Theory predicts that the optimal solution to the quality–number trade-off depends on environmental conditions, whereby parents who decrease fecundity and increase investment per offspring will have greater reproductive success when environmental quality is low (Smith &

Fretwell 1974; Brockelman 1975). Therefore, the decision to produce many poorly provisioned offspring, or a few well-provisioned offspring, can have a profound effect on parental reproductive success (Rollinson & Hutchings 2013a).

Natural environments are both spatially and temporally variable. If environmental variation is unpredictable, then theory suggests that a ‘conservative bet hedging’ reproductive strategy might evolve. In this case, parents increase investment per offspring leading to the consistent production of large offspring over space and time. Parents (or genotypes) adopting this strategy should exhibit a low

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variance in reproductive success and will attain a relatively high geometric mean fitness over time (McGinley, Temme & Geber 1987; Einum & Fleming 2004). On the other hand, when environmental variation is predictable, parents might use reliable cues to adjust the quality and number of offspring they produce to match the anticipated offspring environment (Fox, Thakar & Mousseau 1997; Allen, Buckley & Marshall 2008). Such 'adaptive egg size plasticity' essentially maximizes reproductive success of parents in both high-quality and low-quality environments (McGinley, Temme & Geber 1987).

These and other theories of egg size evolution have long been established (Smith & Fretwell 1974; Kaplan & Cooper 1984; McGinley, Temme & Geber 1987), and new theories continue to be formulated and reformulated (Hendry, Day & Cooper 2001; Hendry & Day 2003; Beck & Beck 2005; Olofsson, Ripa & Jonzén 2009; Marshall *et al.* 2010). Yet, an understanding of reproductive strategies is ultimately founded in empirical tests of theoretical models and the mechanisms of evolution they propose (Wiklund, Karlsson & Forsberg 1987; Sinervo *et al.* 1992; Rollinson, Edge & Brooks 2013). In fact, relatively few empirical studies endeavour to measure the fitness consequences of variation in investment per offspring (reviewed by Rollinson & Hutchings 2013b), and this paucity of studies impedes our ability to ask more general questions about the evolution of offspring investment. For example, what is the relative frequency of adaptive egg size plasticity (e.g. Fox, Thakar & Mousseau 1997) vs. conservative bet hedging (Einum & Fleming 2004) as adaptations to environmental variation in the wild? Clearly, the data are currently insufficient to address this question, and this is likely due to the difficulty associated with evaluating the adaptive significance of reproductive strategies.

Estimates of parental fecundity, investment per offspring and offspring fitness are required to evaluate the adaptive value of parental reproductive strategies. While estimating fecundity is generally straightforward, a sound estimate of offspring fitness requires that offspring survival be measured up to the time at which offspring mortality becomes random with respect to initial investment (Rollinson & Hutchings 2013b), which is not always feasible (e.g. Allen & Marshall 2013). For example, insects and fishes are incredibly speciose groups, and they feature many species that produce a very large number of very small eggs. For these groups, it can be difficult to measure with accuracy both investment per offspring and its fitness consequences, in which case several metrics of offspring viability, size and performance must serve as proxies for direct measures of investment per offspring and offspring fitness (e.g. Otto & Mackauer 1998; Geister *et al.* 2008; Boivin & Gauvin 2009). We suggest that applying multivariate analyses to this type of data might be a powerful way to examine patterns of reproductive allocation. Specifically, an 'index of reproductive allocation' could be developed, which would represent a major axis of phenotypic variation that is

correlated with parental fecundity, estimates of investment per offspring and correlates of offspring fitness (Wilson & Nussey 2009). The index could then be used to examine how reproductive patterns change across environments and to test the adaptive significance of reproductive allocation patterns. Combining maternal and offspring traits into a single metric is not at odds with theory (*sensu* Smith & Fretwell 1974) because investment per offspring can be both a maternal and an offspring phenotype (Wilson *et al.* 2005).

Previous research has demonstrated that multivariate analyses can reveal patterns of selection on investment per offspring that are not apparent in univariate analyses (Marshall & Monro 2013), but a multivariate approach has never been used to integrate measures of fecundity, offspring size and offspring fitness into a single metric. Thus, we used a multivariate approach to test adaptive plasticity in reproductive allocation (see McGinley, Temme & Geber 1987 for theoretical development) in the Texas field cricket (*Gryllus texensis*) under laboratory conditions. We manipulated the quality of maternal environment (food availability) throughout early and late adulthood (acute and chronic exposure to the maternal environment, e.g. egg production was examined during acute and chronic food limitation for some females) in a 2 × 2 factorial experiment. We then obtained estimates of maternal fecundity, as well as proxies of offspring size and performance in both low- and high-resource environments. In addition to univariate analyses, we used principal components analysis (PCA) to integrate measures of maternal fecundity with estimates of offspring performance into a single index of reproductive allocation. We then examined how this multivariate index varied with the quality of maternal environment over time to provide new insight into the dynamics of reproductive allocation.

Materials and methods

The *G. texensis* colony used herein has been described previously (Stahlschmidt *et al.* 2013). We isolated female crickets 11–13 days post-adult moult from group housing in the colony to ensure crickets selected for our study were mated, which typically occurs by 10 days post-adult moult (Solymar & Cade 1990). We randomly assigned crickets to different dietary treatments (described below), housed them individually in transparent plastic containers (18 × 14.5 × 9 cm) and supplied them with *ad libitum* access to water for the duration of the 10-day study.

We manipulated environmental quality and the duration of maternal exposure to environmental quality using a repeated measures approach ($n = 17$ –30 mothers per treatment group). To create 'high-food' environments, we provided crickets with *ad libitum* access to dry cat food. For 'low-food' environments, we provided crickets with food for only 3 h on the 5th day of the study, which results in *G. texensis* with similar levels of body fat as those found in the field (Adamo *et al.* 2012). To determine fecundity, we counted the number of eggs each female oviposited in a cotton-filled water bottle over multiday periods because *G. texensis* oviposits intermittently – thus, single day sampling procedures may over or underestimate fecundity. In fact, a strong proxy for lifetime fecundity in *G. texensis* is the mean oviposition rate during

the 4-day period between 12 days and 16 days post-adult moult ($R = 0.91$; Stahlschmidt *et al.* 2013). We counted the number of eggs each female oviposited early in the study (during the first 4 days) and late in the study (during last 4 days). Thus, early egg sampling reflected acute exposure to one of two environments ('high food' or *ad libitum* access to food vs. 'low food' or no access to food), while late egg sampling reflected chronic exposure to one of the two environments ('high food' or *ad libitum* access to food vs. 'low food' or reduced food availability). The end of the late sampling point approximates the end of crickets' life span in the field (Murray & Cade 1995). We then divided the number of eggs oviposited by four to determine oviposition rate during early and late sampling points.

We measured body mass and femur length (a proxy for body size) at the onset and conclusion of the study. Femur length does not change throughout adulthood – thus, it was used to control for body size-dependent effects on body mass (i.e. body condition). To determine body condition, we used the scaled mass index described in Eq. 2 in Peig & Green (2009), which uses a scaling exponent derived from the slope of the ln-transformed body size (femur length) vs. ln-transformed body mass regression of the population (i.e. all of the crickets in our study). Relative to other methods (e.g. calculation of residuals from an ordinary least squares regression of body mass against body length), this method has proven to be a more reliable indicator of the relative size of energy reserves in an array of animal taxa (Peig & Green 2009).

At each sampling point, we individually incubated 20 eggs from each female, and we estimated reproductive success as the product of oviposition rate and hatching success (Stahlschmidt *et al.* 2013). For half of each female's hatchlings, we determined the duration each hatchling could survive without food ('hatchling vigour'), which is a proxy for hatchling energy stores that is also positively correlated with hatchling body size (Stahlschmidt & Adamo 2013; Stahlschmidt *et al.* 2013). We housed the remaining hatchlings individually, supplied each of them with *ad libitum* food and water and determined 'moult success' (whether all of a given female's hatchlings survived and moulted into 2nd instar nymphs).

We used a multivariate statistical approach (PCA) on the entire data set to generate an index of reproductive allocation – that is, the tendency of a given female to allocate more to offspring quality relative to offspring quantity. Previous research has demonstrated that multivariate statistical methods can reveal patterns of correlational selection on investment per offspring and other life-history traits that are not apparent in univariate analyses (Marshall & Monro 2013). Yet, we note that our approach differs from multivariate analysis of selection (Phillips & Arnold 1989; Blows & Brooks 2003). Whereas multivariate selection analyses for offspring size would require mean-standardized parental fitness (e.g. parent or offspring fecundity) on the y-axis and an integrated offspring phenotype on the x-axis (e.g. Marshall & Monro 2013), the method developed herein integrates measures of fecundity, offspring size and offspring fitness into a single metric. In other words, the index simultaneously describes a reproductive strategy while approximating its fitness consequences for offspring and parents.

Principal components analysis uses orthogonal transformation to convert initial correlated variables into a smaller number of non-correlated variables called principal components (PCs), which can be used as dependent variables in subsequent analyses (e.g. linear mixed models). Based on several test statistics, our data set was appropriate for PCA. To begin, the initial variables (described below) were significantly correlated with one another – the sole exception was a lack of correlation between oviposition rate and hatchling vigour (Table 1). Further, the Bartlett's measure tests whether there is a significant pattern of correlations in a given data set. The Bartlett's measure for our data set was highly significant (<0.001), meaning a highly significant correlation pattern existed. At the other end of the spectrum, extreme multicollineari-

Table 1. Pearson product-moment correlation matrix of initial variables entered into principal components analysis

	Oviposition rate	Hatching success	Mean hatchling vigour
Oviposition rate			
Hatching success	−0.33**		
Mean hatchling vigour	−0.10	0.24**	
Moult success	−0.18*	0.21*	0.30**

PC1 reflects an index of reproductive allocation for adult *Gryllus texensis* where a higher PC1 reflects a lower oviposition rate coupled with higher offspring quality (increased hatching success, hatchling vigour and hatchling moult success). See text for specific details. *R*-values are displayed while * and ** denote one-tailed *P*-values ≤ 0.05 and ≤ 0.01 , respectively.

ty (very highly correlated variables) is undesirable because it may indicate that some initial variables are highly dependent upon one another. Our data set did not exhibit extreme multicollinearity because it had a relatively large determinant value (0.74). The Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy ranges from 0 (diffusion in the pattern of correlations where the sum of partial correlations is large relative to the sum of the correlations) to 1 (pattern of correlations is compact, meaning PCA should yield distinct, reliable PCs). The KMO value for our data set was >0.5 (specifically, 0.61), which is acceptable (Kaiser 1974). In sum, our data set satisfied the assumptions of having a significant and compact pattern of correlations.

The initial variables that are required for our method of reproductive allocation are provided in Table 2. In the present study, we included oviposition rate (eggs per day) as an estimate of parental fecundity (Stahlschmidt *et al.* 2013), hatchling vigour (day) as a proxy of investment per offspring and both hatching success (%) and moult success rate (%) as proxies of offspring fitness. Yet, other metrics of fecundity, offspring investment and offspring fitness can also be used (Table 2). One principal component extracted from our data had an eigenvalue >1 (specifically, 1.5), and it (PC1) accounted for 39% of the variation in the data (Table 3). PC1 loaded positively onto hatching success (0.74) and hatchling vigour (0.56), moult success (0.46) and negatively

Table 2. Adopting a principle components approach in an investigation of reproductive strategies requires that at least three metrics be incorporated into a principle components analysis. Metrics used in our study are italicized

Required traits	Examples
Fecundity	<i>Oviposition rate</i> , clutch or litter size, or lifetime number of eggs produced
Proxies of offspring fitness*	Fertilization success, <i>hatching success</i> , rate of deformities or survival to eclosion
Investment per offspring†	
Direct measures	Egg weight, egg volume or birth weight
Indirect measures (proxies)	Egg length or diameter, size at hatching or birth, or <i>starvation resistance</i>

*Estimates will generally be expressed as the proportion of offspring successfully fertilized, hatched, etc., in a group of offspring that arises from the same parent.

†Estimates will generally be expressed as the mean phenotype of a group of offspring (e.g. mean egg size) that arises from the same parent.

onto oviposition rate (-0.68). That is, higher values of PC1 reflected greater investment per offspring (hatchling vigour), greater offspring fitness (higher hatching success and hatchling moult success) and lower parental fecundity (lower oviposition rate).

To examine the relationship between the number and quality of offspring, we first performed PCA on variables of offspring quality (hatching success, hatchling vigour and moult success). As described above, this data set also met the criteria for PCA (e.g. sufficiently large determinant and KMO values). One principal component extracted from the data had an eigenvalue >1 (specifically, 1.4) that accounted for 50% of the variation in the data. This PC ('offspring quality index') loaded positively onto hatching success (0.65), hatchling vigour (0.74) and moult success (0.72). That is, higher values of offspring quality index reflected greater investment per offspring (hatchling vigour) and greater offspring fitness (increased hatching success and hatchling moult success). We then examined the relationship between the number of offspring (oviposition rate) and the quality of offspring (offspring quality index) using correlation analyses.

We performed all analyses with SPSS (version 21; IBM Corp., Armonk, NY, USA), and we determined two-tailed significance at $\alpha < 0.05$. All data met the assumptions of parametric statistics or were transformed as necessary. We used linear mixed models to determine the main and interactive effects of age and food availability on dependent variables (e.g. oviposition rate and PC1). We included individual as a random effect and included treatments (exposure duration and maternal environment) as fixed effects. For moult success, we used a binary logistic generalized linear model with maternal environment and exposure duration as fixed effects.

Results

Females with *ad libitum* access to food exhibited higher body condition during late adulthood than food-limited females (maternal environment \times duration of exposure: $F_{1,46} = 166$, $P < 0.001$) indicating that our manipulation of maternal environmental affected crickets' body reserves over time. Females in the high-food environment added body mass despite laying relatively more eggs and exhibiting higher estimated reproductive success over the course of the study (Fig. 1a,c). Further, oviposition rate was negatively correlated with index of offspring quality ($R = -0.28$, $P = 0.008$, Fig. 2).

Neither the duration of exposure nor maternal environment significantly influenced aspects of offspring quality in univariate analyses (Fig. 1b,d,e). However, when we used

our multivariate index of reproductive allocation (PC1) as the dependent variable, we found a significant interaction between maternal environment and the duration of exposure. Specifically, mothers decreased oviposition rate and increased offspring quality after chronic exposure to the low-food environment (Fig. 1f).

Discussion

Our study yielded two major findings. First, when *G. texensis* were removed from standard rearing conditions and placed in a chronically low-food environment, their reproductive strategy shifted from the production of 'many low-quality offspring' to the production of 'few high-quality offspring' (Fig. 1f) similar to other taxa (*Daphnia*: Gliwicz & Guisande 1992). Second this pattern was not detected using a standard univariate approach to data analysis. The pattern was only apparent after developing a multivariate metric that integrated maternal fecundity with proxies of investment per offspring and fitness correlates of offspring. While evolutionary studies have employed the use of multivariate statistics to examine patterns of selection (Phillips & Arnold 1989; Blows & Brooks 2003; Marshall & Monro 2013), ours is the first to point to the benefits of a simple PCA for ecological studies on the offspring quality–number trade-off.

We found that acute exposure to a low-food environment affected fecundity, but not aspects of offspring quality. In contrast, only chronic exposure resulted in the production of fewer, high-quality offspring (Fig. 1f). This delay may have been related to developmental mode. Like many animals, crickets are lecithotrophic and embryos rely solely on stored nutrients (yolk) for energy during development. In crickets, shifts in allocation (e.g. increasing mean investment to a set of developing follicles) are temporally constrained and will not appear until all the eggs formed previously have been oviposited (ca. 6 days in *G. texensis*: Shoemaker & Adamo 2007). On the other hand, matrotrophy allows mothers to provision their embryos continuously throughout embryonic development. In contrast to *G. texensis*, matrotrophic fish that are placed in low-resource environments immediately reduce allocation to offspring size, but exhibit a delay in a reduction in fecundity (Pollux & Reznick 2011). Together, these results suggest that reproductive mode may differentially delay adaptive shifts in offspring provisioning.

Unlike some other taxa (e.g. polychaetes: Bridges & Heppell 1996), *G. texensis* fed *ad libitum* exhibited a reduction in offspring quality with age (Fig. 1f) due to a decrease in offspring vigour coupled with a decrease in moult success rate (Fig. 1d,e). One explanation is that offspring quality is limited by some micronutrient (e.g. calcium or phosphorous) that is depleted over time, which occurs in other animals in captivity (Uni, Yadgary & Yair 2012) and in the field (Wilkin *et al.* 2009). Alternatively, senescence may obligate a reduction in offspring quality. Crickets fed *ad libitum* would be expected to have greater

Table 3. Principal components extracted from principal components analysis

Component	Eigenvalue	% of variance
1	1.5	39
2	0.9	24
3	0.8	20
4	0.7	17

The first component (PC1) explained 39% of the total variance, and it was the only PC with an eigenvalue >1 . It reflects an index of reproductive allocation for adult *Gryllus texensis* where a higher PC1 reflects a lower oviposition rate coupled with higher offspring quality (increased hatching success, hatchling vigour and hatchling moult success). See text for specific details.

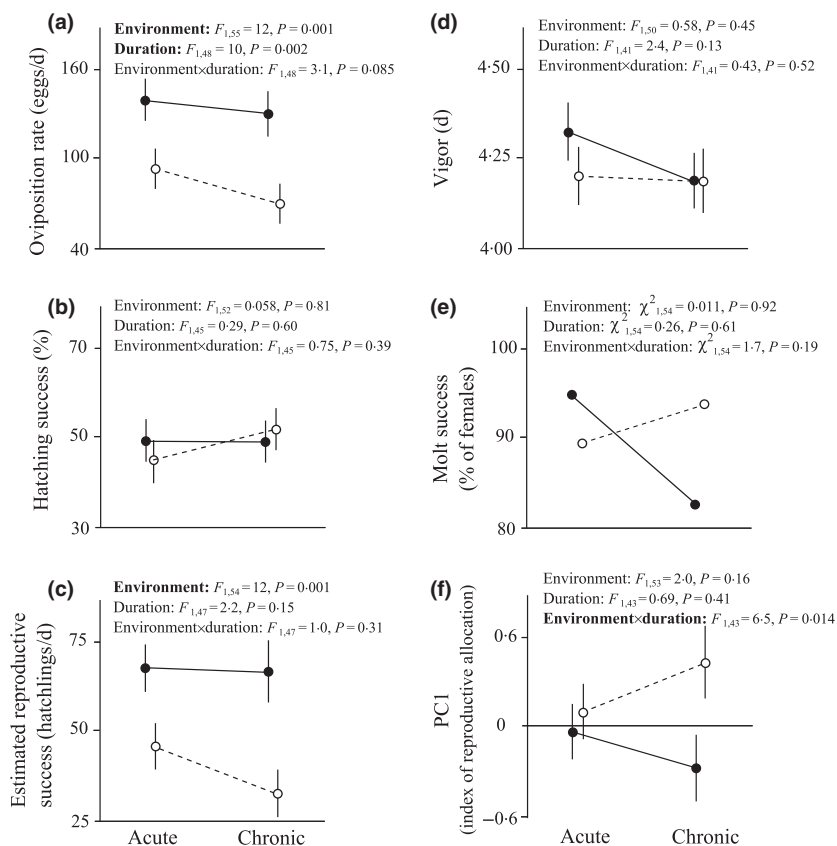


Fig. 1. Effects of the duration of exposure [acute (early adulthood only) and chronic (early and late adulthood)] to a food-limited environment (open symbols) or an *ad libitum* food environment (closed symbols) on (a) oviposition rate (a strong proxy for fecundity), (b) hatching success, (c) estimated reproductive success, (d) hatchling vigour, (e) molt success and (f) index of reproductive allocation (PC1) for adult *Gryllus texensis*. A higher PC1 reflects a lower oviposition rate coupled with higher offspring quality (increased hatching success, hatchling vigour and hatchling molt success). All values are presented as mean \pm SEM.

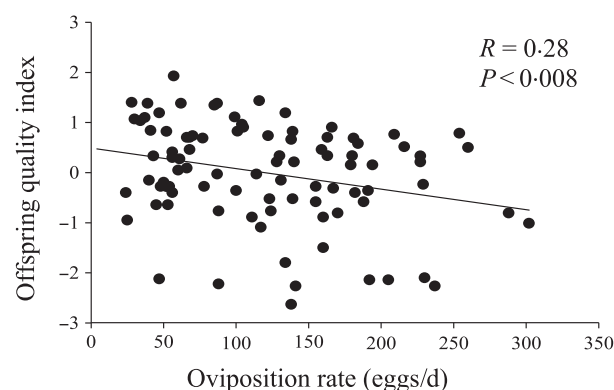


Fig. 2. Relationship between the number of offspring (oviposition rate, a strong proxy for fecundity) and the quality of offspring (principal component describing an index of offspring quality) in *Gryllus texensis*. This index was characterized by high hatching success, hatchling vigour and hatchling molt success. See text for specific details.

oxidative damage than those on a food-limited diet, suggesting that they will reach senescence faster (Lyn *et al.* 2011). Crickets of this species that are fed *ad libitum* exhibit signs of immunological senescence by the time they are of the age of our crickets during the last sampling point of our study (Adamo, Jensen & Younger 2001). Therefore, increased oxidative damage may reduce the efficiency of egg production in females fed *ad libitum* resulting in lower quality offspring over time. Clearly, future work is

required to better understand the mechanism(s) regulating shifts along the offspring quality–number axis.

Previous research on crickets has investigated the negative relationship between proxies of fecundity and offspring quality predicted by life-history theory (Carriere & Roff 1995; Simmons & García-González 2007). One of these studies reported a significant, although weak, negative relationship between proxies of fecundity and offspring quality ($P = 0.034$, $R = 0.41$; Simmons & García-González 2007). The other study did not detect a significant number–quality relationship within three cricket species, but they did find that egg weight (a proxy for egg quality) covaried with fecundity across these species ($P = 0.027$, R not reported). Likewise, the relationship between fecundity (oviposition rate) and offspring quality index in our study was highly significant ($P < 0.01$), but weak ($R = 0.28$; Fig. 2). Notably, these other studies reported significantly lower rates of hatching success (9–28%) than we found in *G. texensis* (ca. 50%; Fig. 1b). Discrepancies may not be due to reproductive strategy. Rather, they may be the result of differences in incubation methodologies because we used moist cotton as an egg-laying substrate, while previous studies used various depths of sand or soil (Carriere & Roff 1995; Simmons & García-González 2007). Indeed, hatching success in crickets is negatively affected by the depth of the substrate at which eggs are incubated and the moisture content of the substrate (Bradford, Guerette & Roff 1993; Carriere & Roff 1995). Thus, future efforts should continue to

examine the role of the incubation environment in reproductive strategies in this taxon (also see Stahlschmidt & Adamo 2013). Earlier studies also relied exclusively upon univariate approaches and less comprehensive metrics (e.g. hatching success as the only proxy for offspring fitness) to test for quality–number trade-offs, which may explain why a negative relationship between offspring number and quality was not detected within several cricket species (Carriere & Roff 1995).

A seasonal increase in offspring size has been observed in some Orthoptera (e.g. Landa 1992; Cherrill 2002). Landa (1992) describes this change in allocation as an adaptive response to an increasing threat of offspring starvation that occurs overwinter, although the fitness consequences of variation in investment per offspring were not measured in his study. Many North American field crickets, including *G. texensis*, also produce offspring that overwinter as juveniles (Alexander 1968; Walker 2011). The present study suggests that at least one species of Orthoptera is indeed able to respond to environmental cues and adjust investment patterns to increase fitness, which means adaptive explanations for offspring size plasticity may be well founded in this group. Mounting evidence suggests that adaptive egg size plasticity might be common in species where maternal and offspring environments are correlated (e.g. bryozoans: Allen, Buckley & Marshall 2008; fish: Leips *et al.* 2009). In fact, evidence of adaptive egg size plasticity has only recently been discovered in *Drosophila melanogaster* (Vijendravarma, Narasimha & Kawecki 2010), a well-studied species. However, while these studies do suggest adaptive plasticity might be relatively common, adaptive egg size plasticity can certainly not be taken as a foregone conclusion when maternal and offspring environments are correlated (e.g. Plaistow *et al.* 2007). Additionally, shifts in investment patterns may be accompanied by changes in oviposition behaviour (e.g. food-limited females may oviposit smaller batches of eggs or they may oviposit less frequently). As we outlined above, more research is needed to understand the adaptive significance of variation in reproductive allocation in the wild.

When parental fecundity, offspring size and offspring fitness were considered simultaneously, a pattern of reproductive investment that suggests adaptive plasticity became apparent, but this pattern was not apparent when traits were considered individually. The method suggested herein might therefore be useful in future studies of plasticity in reproductive strategy. Other multivariate statistical approaches [e.g. canonical correlation analyses, multivariate analyses of variance (MANOVA) or path analyses] may also yield insight into dynamics of reproductive strategy (e.g. Plaistow, Lapsley & Benton 2006). However, these approaches are more limited by sample size relative to PCA (Weinberg & Darlington 1976; Mitchell 1993; Hoyle 1995; Hair *et al.* 1998). Also, MANOVA does not quantify relationships between initial variables, such as fecundity and hatching success. Further, none of these alternative

multivariate approaches produce a variable of reproductive allocation (e.g. PC1 in our approach) that can be used in subsequent analyses to examine fixed or random effects. Running this variable through a linear mixed model also offers the advantage of robustness to missing values (e.g. PC1 from only one of two sampling points for some individuals).

Caution must nonetheless be used in applying a principle components approach to offspring quality–number data, and the following points must be stressed. (i) While there is a clear pattern that suggests adaptive plasticity in the present study, offspring and parental fitness were not directly measured. Without these direct estimates of fitness, the evidence of adaptive plasticity presented herein is not unequivocal. Thus, it should not be weighted as heavily as the evidence provided by studies in which estimates of fitness are more complete (e.g. Fox, Thakar & Mousseau 1997). Yet, a principle components approach may help provide reasonable tests of theory when fitness proxies of offspring and parents are used. (ii) In a similar vein, proxies of offspring fitness should be clearly related to offspring survival (see Wilson & Nussey 2009). For example, matrotrophic provisioning in the Pacific beetle cockroach (*Diploptera punctata*) influences the developmental rate of offspring where increased maternal nutrient investment per offspring favours fast-growing nymphs (Holbrook & Schal 2004). However, in the present study, we did not include developmental rate as a proxy of offspring fitness because the fitness consequences of this metric are not clear for *G. texensis*. If offspring fitness is indeed assessed by measuring survival at different developmental stages (e.g. in the present study and outlined in Table 2), then the major axis of variation extracted from the PCA will also approximate the fitness consequences for parents. This is important because parental fitness is at the crux of theories that describe the evolution of investment per offspring (Smith & Fretwell 1974). (iii) The major axis of phenotypic variation extracted from the principle components analysis should also explain a substantial proportion of the variation in the data. In the present study, PC1 explained 39% of the total variation. We believe that this result is biologically significant especially considering that trait values are difficult to measure with accuracy in small organisms. However, judgment must be used here because there is no consensus as to the minimum percentage of total variance that must be explained to reasonably conclude that the result is biologically meaningful.

The present study attempts to develop and apply a method that can be used to empirically test a burgeoning body of theory on the evolution of reproductive allocation strategies. If adopted widely, this approach could increase our ability to assess the adaptive significance of reproductive strategies – particularly, in situations when offspring size and fitness are difficult to measure with accuracy (Table 2). Ultimately, this will facilitate the comparison and communication of results, which will help provide a broader insight into the evolution of offspring size. We

encourage others to explore other multivariate approaches to analysing reproductive allocation strategies, such as canonical correlation analyses, MANOVA or path analyses.

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