

Are all eggs created equal? Food availability and the fitness trade-off between reproduction and immunity

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Summary

1. Reproduction and immune function are critical processes, but organisms can rarely optimize both traits. Resultant reproduction–immunity trade-offs may be ‘facultative’, occurring only when resources are scarce, or they may be ‘obligate’, occurring regardless of resource availability.
2. Previous research has tested for the ‘facultative’ or ‘obligate’ nature of reproduction–immunity trade-offs by measuring resource allocation (e.g. follicle size). However, measuring resource allocation alone may be insufficient when gauging the fitness consequences of reproduction–immunity trade-offs because the number and quality of eggs or offspring trade off with one another.
3. We used the Texas field cricket (*Gryllus texensis*) to provide the most direct test to date of whether a fitness trade-off between these two traits is ‘facultative’ or ‘obligate’. We used a factorial design to manipulate food availability and immune status throughout adulthood. We then estimated lifetime fecundity, hatching success and their product (reproductive success), and we also measured several aspects of offspring quality (e.g. egg size and protein content, and hatchling size and energy stores).
4. A reproduction–immunity trade-off was ‘obligate’ in this species because immune challenge reduced reproductive success estimates regardless of food availability. Females with unlimited food were more fecund and produced more and larger hatchlings, but neither food availability nor immune status affected egg size, egg phenoloxidase activity, incubation duration, hatching success or hatchling energy stores. We observed a trade-off between offspring size and number – females favouring offspring size over fecundity produced fewer hatchlings, but their hatchlings were of higher quality (larger and more robust).
5. By demonstrating that not all eggs are created equal, we provide key insight into the role of reproductive allocation in the fitness trade-off between reproduction and immunity.

Key-words: ecological immunology, egg size, *Gryllus texensis*, phenoloxidase, reproductive allocation

Introduction

Reproduction and self-maintenance are critical processes that require an appreciable investment of resources (Angilletta & Sears 2000; Nilsson & Raberg 2001; Freitak *et al.* 2003; Martin, Scheuerlein & Wikelski 2003; Cox *et al.* 2010; Ardia *et al.* 2012). While allocation trade-offs between aspects of reproduction and self-maintenance (e.g. immune function) can occur (Adamo, Jensen & Younger 2001; French, Johnston & Moore 2007; Knowles, Nakagawa & Sheldon 2009; Cox *et al.* 2010), some research has shown that both reproduction and immune function can be maintained if individuals have *ad libitum* access to food

(Shoemaker, Parsons & Adamo 2006; French, Johnston & Moore 2007; Xu, Yang & Wang 2012). Therefore, reproduction–immunity trade-offs may be resource dependent or ‘facultative’. Trade-offs between reproduction and immune function may be also ‘obligate’. For example, vertebrate sex steroids can bind directly to immune cells and suppress immune function (Schuurs & Verheul 1990). If such a physiological link is critical for homeostasis, a trade-off between reproduction and immune function would occur regardless of the resources available. Another potential example is the activation of damaging free radicals during the invertebrate immune response (Sadd & Siva-Jothy 2006; Molina-Cruz *et al.* 2008). For example, the enzyme phenoloxidase is an important component of invertebrate immune systems (Gonzalez-Santoyo &

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Cordoba-Aguilar 2012), but it can also induce self-damage (Sadd & Siva-Jothy 2006) and may lead to reduced reproductive output regardless of food availability.

Although allocation trade-offs between reproduction and immune function have been demonstrated (Adamo, Jensen & Younger 2001; French, Johnston & Moore 2007; Knowles, Nakagawa & Sheldon 2009; Cox *et al.* 2010), fitness trade-offs between these two processes are less understood. For example, immune-challenged lizards reduce their allocation of resources to follicles during food limitation (French, Johnston & Moore 2007), which may entail fitness costs such as less viable eggs or offspring. Yet, this result represents a fitness trade-off between reproduction and immunity only if a reduction in follicle size decreases the reproductive success of the parent. While larger, more energy-rich eggs typically result in larger offspring (e.g. bryozoans: Marshall & Keough 2008; insects: Gillooly & Dodson 2000; fish: Einum & Fleming 2000; lizards: Sinervo *et al.* 1992; birds: Christians 2002), larger offspring do not necessarily increase the fitness of the parent (e.g. Fox, Thakar & Mousseau 1997). Furthermore, because investment per offspring and parental fecundity trade off (Lack 1947), parents must balance the fitness benefits accrued from increases in offspring size and viability against any potential fitness costs resulting from decreases in fecundity (Smith & Fretwell 1974). Therefore, measuring aspects of resource allocation alone (e.g. the size or number of eggs or follicles) may be insufficient when gauging the adaptive significance of a maternal response to environmental perturbation (e.g. shifts in immunogen exposure). Rather, a true assessment of a fitness trade-off between reproduction and immunity requires a comprehensive examination of offspring size–fitness relationships and the effect of immune challenge on lifetime reproductive success – that is, the number and quality of eggs and offspring produced over a female's lifetime.

We used factorial manipulations of food availability and immune status in adult female Texas field crickets (*Gryllus texensis*) to test two competing hypotheses. The 'facultative regulation hypothesis' predicts that immune-challenged females will exhibit reduced reproductive success during food limitation. However, when food is provided *ad libitum*, immune challenge will have no effect on reproductive success. On the other hand, the 'obligate regulation hypothesis' predicts that immune-challenged females will exhibit reduced reproductive success regardless of food availability. To estimate reproductive success, we estimated lifetime egg production and measured hatching success. In addition, we measured several aspects of egg quality, including egg size, egg protein content, egg PO activity, duration of egg development, hatchling size and hatchling energy stores. Using this design, we were also able to detect shifts in allocation strategies due to chronic food limitation and/or immune challenge. For example, stressed females (e.g. those that were immune challenged and/or food limited) may allocate more resources to fewer offspring – that is, these females may produce fewer eggs, but

their eggs may be larger or have higher protein content resulting in higher quality offspring. In sum, we clarify the independent and interactive roles of two widespread environmental factors (food availability and immunogen exposure) on offspring quantity and quality.

Materials and methods

STUDY ANIMALS

We used long-winged adult *G. texensis* that were part of a long-term colony, which has been described previously (Adamo & Lovett 2011). Prior to adulthood, we supplied crickets with food (dry cat food) and water *ad libitum* (water-filled bottles with loosely packed cotton) and housed crickets in the colony room maintained at 26 ± 1 °C and a 12:12 light/dark cycle. Within 1 day of their adult moult, we assigned crickets to different dietary treatments (described below), but we housed all crickets in the colony room and supplied them with *ad libitum* access to water for the duration of the study. All experiments were approved by the Animal Care Committee of Dalhousie University (#19-026) and are in accordance with the Canadian Council on Animal Care.

EXPERIMENTAL DESIGN

We used a 2×3 design to manipulate nutritional state and immune status throughout adulthood in a factorial fashion ($n = 48\text{--}51$ for each of six treatment groups). We isolated 24 cohorts of newly moulted (≤ 1 day postadult moult) females and housed them individually in transparent plastic containers ($18 \times 14.5 \times 9$ cm). We manipulated each female's access to food (dry cat food, a high-quality food source: Adamo *et al.* 2010): *ad libitum* access or intermittent access (cat food was available for 3 h every 3 day, which results in *G. texensis* with similar levels of body fat as those found in the field: Adamo *et al.* 2012).

Over the course of the experiment (the first 17 day of adulthood), we also manipulated females' immune status: a systemic immune challenge treatment (injection with heat-killed bacteria every 3 day), a wounding control treatment (pierced with sterile dissection pin every 3 day) or an unhandled control treatment. Similar to Shoemaker & Adamo (2007), we induced a chronic systemic immune challenge by injecting females with heat-killed (previously boiled) *Serratia marcescens*, a Gram-negative bacterium. *Serratia marcescens* is found world-wide in both soil and water and has been recovered from the bodies of orthopterans in the field (Steinhaus 1959). A concentration of 1×10^4 live cells per 2 μL culture media of *S. marcescens* is the LD₅₀ dosage for *G. texensis* (Adamo *et al.* 2010). Using this concentration of heat-killed *S. marcescens*, we chronically activated crickets' immune systems without pathogenesis. Specifically, we injected crickets abdominally using a 10- μL Hamilton syringe with heat-killed *S. marcescens* (Carolina Biological Supply Co., Burlington, NC, USA) every 3 day of adulthood beginning ≤ 1 day postadult moult. As a control for wounding and handling stress, we pierced females in the abdomen with a sterile dissection pin every 3 day of adulthood beginning ≤ 1 day postadult moult. Wounding activates a truncated version of the insect immune response (Gillespie & Khachatourians 1992; Wigby *et al.* 2008; Ardia *et al.* 2012). We did not handle females in the control treatment group except immediately before and after mating and during weighing.

On the evening of 11 day postadult moult, we mixed females with sexually mature males at a ratio of one female per 1–2 males to elicit overnight mating, and we excluded from analyses females that did not mate (mating status was determined by the presence

of sperm-filled spermatheca during a post-mortem dissection 17 day postadult moult).

BODY CONDITION AND OVIPOSITION RATE

To determine the effects of diet and immune status on body condition, we weighed each cricket to determine body mass (± 0.1 mg) at the onset of the study, immediately prior to mating, and at the end of the study (≤ 1 , 11 and 17 day postadult moult, respectively). At the end of the study, we also removed the rear right leg from each cricket to determine femur length (a proxy for body size: Simmons 1986) using vernier callipers (± 0.1 mm; #5921, Scienceware, Switzerland). We corrected for interindividual variation in body mass due to body size using body mass and femur length to determine body condition on ≤ 1 , 11 and 17 day postadult moult via the scaled mass index (Peig & Green 2009). To determine the effects of food availability and immune status on oviposition rate, we counted the number of eggs each female oviposited in its cotton-filled water bottle between 12 and 17 day postadult moult. This 5 day period of oviposition strongly correlates with lifetime oviposition (for details, see Appendix S1 in Supporting Information).

QUANTITY AND QUALITY OF OFFSPRING

In nine cohorts, we isolated 15 randomly chosen eggs from each female to determine the quality of reproductive effort (for details, see Appendix S2 in Supporting Information). Briefly, we digitally imaged freshly laid eggs to determine egg size (length and two-dimensional surface area), and we then individually incubated 12 of these eggs from each female to determine incubation duration (duration of egg development), hatching success and hatchling size (femur length). For each female, we estimated reproductive success (the product of oviposition rate and hatching success) and reproductive effort (the product of oviposition rate and egg size [surface area]). We also determined hatchling vigour, which was the duration each hatchling could survive without food (a proxy for hatchling energy stores). We stored the remaining three eggs from each female at -80 °C for subsequent analyses of protein content and PO activity. Phenoloxidase is an important enzyme involved in melanization, which is critical to exoskeleton stability and pathogen resistance (Andersen, Peter & Roepstorff 1996; Gonzalez-Santoyo & Cordoba-Aguilar 2012). For details, see Appendix S2.

STATISTICAL ANALYSES

We performed all analyses with SPSS (version 19, IBM Corp., Armonk, NY, USA), and we determined two-tailed significance at $\alpha < 0.05$. All data met the assumptions of parametric statistics, were transformed as necessary or were analysed using nonparametric tests. We used linear mixed models to determine the main and interactive effects of treatments, age and/or morphological traits on dependent variables (e.g. oviposition rate and hatching success). We included cohort as a random effect for all mixed models and individual as a random effect for a repeated measures model on body condition. We included treatments (food availability and immune status) as fixed effects, and we included maternal femur length as a covariate when it was significant.

To test relationships between variables of egg quality (e.g. total protein content or egg length) and characteristics of development (e.g. incubation duration) and hatchling phenotype (e.g. vigour or femur length), we used simple linear regression analyses on variables we expected to have causal relationships with one another (e.g. we expected individuals with relatively large eggs to also have larger hatchlings). We used correlation analyses on variables in which causal relationships were not known *a priori* (e.g. egg PO

activity and hatchling femur length). We used Spearman's rank correlation to examine correlations between variables with non-parametric data sets. Finally, we tested for a negative correlation between the size and number of offspring while holding reproductive effort constant. To avoid multicollinearity among predictor variables, we specified oviposition rate as the dependent variable and regressed it against hatchling femur length (an independent metric of offspring size), reproductive effort and cohort in a multiple linear regression analysis.

Results

Regardless of treatment, crickets increased body condition throughout adulthood (age: $F_{2,857} = 344$, $P < 0.001$). Food availability strongly influenced the rate at which body condition increased ($F_{2,857} = 249$, $P < 0.001$), but there was no effect of immune status on body condition ($F_{2,857} = 0.001$, $P = 0.99$). A food \times immune effect on body condition was significant ($F_{2,857} = 249$, $P < 0.001$) where females with *ad libitum* access to food increased body condition at a faster rate prior to mating relative to after mating. Food availability and immune status significantly affected oviposition rate (Fig. 1a; Table 1). Estimated reproductive success and effort were significantly affected by food availability, and

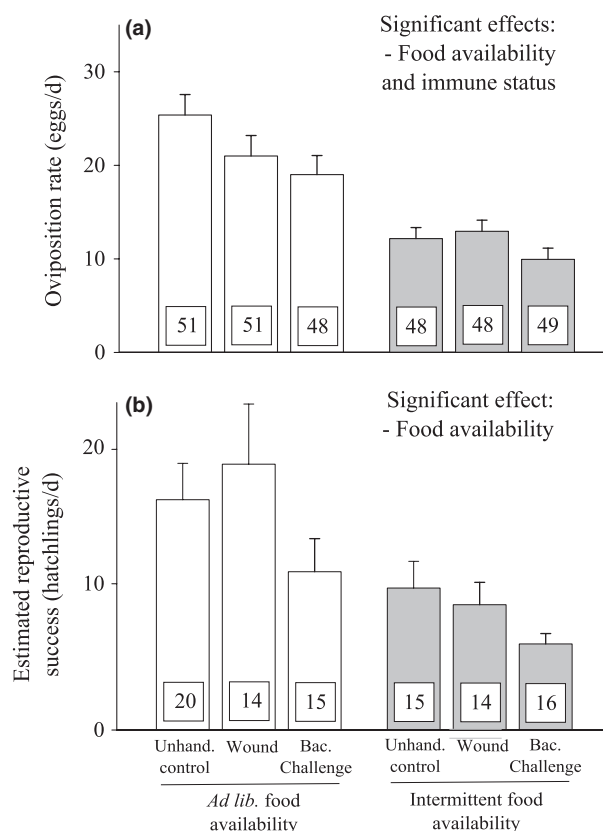


Fig. 1. Effects of treatment on fecundity. (a) Oviposition rate and (b) estimated reproductive success (the product of oviposition rate and hatching success) of *Gryllus texensis* that experienced varying access to food (*ad libitum* or intermittent) and were exposed to no immune challenge (unhandled control), a wound or a bacterial challenge. See text for details about dietary and immune challenge treatments. Sample sizes of treatment groups are represented on each bar. Values are displayed as mean \pm SEM.

Table 1. Summary results of reproductive effort for female *Gryllus texensis* that experienced varying access to food (*ad libitum* or intermittent) and were exposed to no immune challenge (unhandled control), a wound or a bacterial challenge. See text for details about dietary and immune challenge treatments and the variables described below

	df	F	P
Oviposition rate (eggs day ⁻¹)			
Food availability	1, 288	50.1	<0.001
Immune status	2, 288	3.2	0.043
Food × immune	2, 288	1.2	0.30
Estimated reproductive success (hatched eggs day ⁻¹)			
Food availability	1, 94	12.8	0.001
Immune status	2, 94	2.8	0.063
Food × immune	2, 94	0.52	0.60
Reproductive effort (mm ²)			
Food availability	1, 78	17.8	<0.001
Immune status	2, 78	2.6	0.080
Food × immune	2, 78	0.63	0.54
Egg length (mm)			
Food availability	1, 78	0.64	0.43
Immune status	2, 78	0.14	0.87
Food × immune	2, 78	0.061	0.94
Two-dimensional egg surface area (mm ²)			
Food availability	1, 78	2.61	0.11
Immune status	2, 78	0.29	0.75
Food × immune	2, 78	0.080	0.92
Egg protein content (µg egg ⁻¹)			
Food availability	1, 90	0.64	0.64
Immune status	2, 90	4.1	0.021
Food × immune	2, 90	0.43	0.65
Absolute phenoloxidase (PO) activity (µg tyrosinase equivalent egg ⁻¹)			
Food availability	1, 48	0.23	0.63
Immune status	2, 48	1.1	0.34
Food × immune	2, 48	0.26	0.78
Relative PO activity (µg tyrosinase equivalent µg protein ⁻¹ egg ⁻¹)			
Food availability	1, 93	0.32	0.58
Immune status	2, 93	0.21	0.81
Food × immune	2, 93	0.32	0.73
Incubation duration (day)			
Food availability	1, 92	0.32	0.58
Immune status	2, 92	0.21	0.81
Food × immune	2, 92	0.32	0.73
Hatching success (%)			
Food availability	1, 94	<0.001	0.99
Immune status	2, 94	0.65	0.53
Food × immune	2, 94	0.094	0.91
Hatching femur length (mm)			
Food availability	1, 86	4.1	0.045
Immune status	2, 86	1.2	0.30
Food × immune	2, 86	0.23	0.79
Hatching vigour (day)			
Food availability	1, 94	0.40	0.53
Immune status	2, 94	1.5	0.24
Food × immune	2, 94	0.36	0.70

there was a strong trend for immune-challenged females to exhibit lower estimated reproductive success and effort ($P = 0.06$; Fig. 1b; Table 1).

Immune status (but not food availability) significantly influenced egg protein content (Fig. 2a; Table 1). Food availability (but not immune status) significantly affected

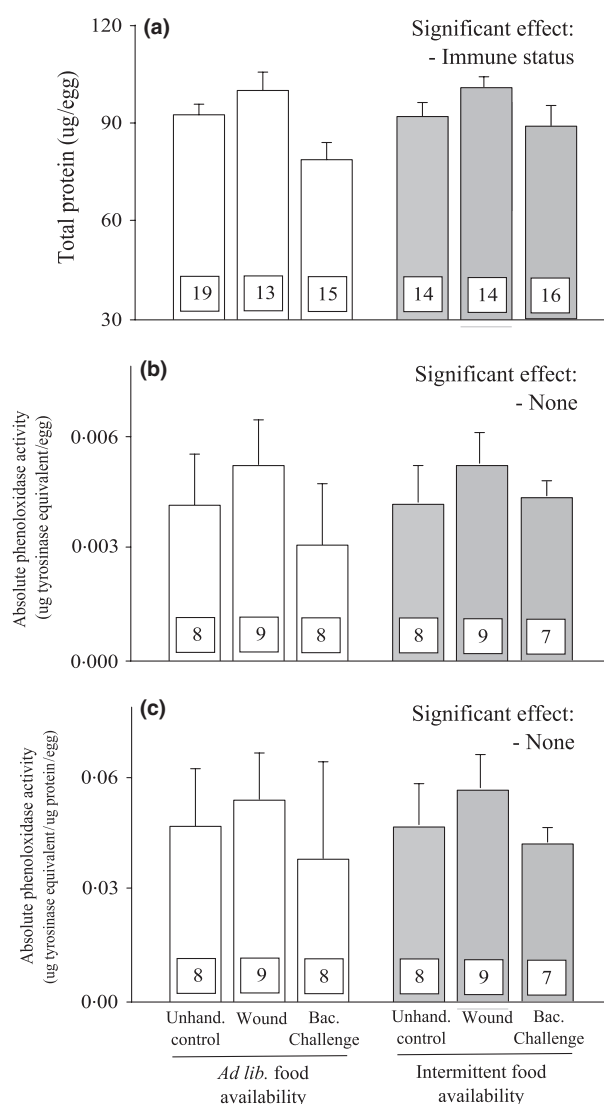


Fig. 2. Effects of treatment on eggs. (a) Total protein content, (b) absolute phenoloxidase (PO) activity and (c) relative (protein corrected) PO activity of eggs laid by *Gryllus texensis* that experienced varying access to food (*ad libitum* or intermittent) and were exposed to no immune challenge (unhandled control), a wound or a bacterial challenge. See text for details about dietary and immune challenge treatments. Sample sizes of treatment groups are represented on each bar. Values are displayed as mean \pm SEM.

mean hatchling size where females with *ad libitum* access to food produced hatchlings with larger femurs (Fig. 3; Table 1). There was no effect of food availability, immune status or a food \times immune interaction on egg length (mean \pm SEM: 2.433 ± 0.009 mm), two-dimensional surface area of eggs (1.076 ± 0.006 mm²), absolute PO activity (Fig. 2b), relative PO activity (Fig. 2c), incubation duration (16.5 ± 0.1 day), hatching success ($63 \pm 2\%$) or hatchling vigour (4.0 ± 0.1 day). However, there was a tendency for females with *ad libitum* access to food to lay larger eggs (surface area) than intermittently fed females (*ad libitum*: 1.085 ± 0.007 mm²; intermittent: 1.066 ± 0.0010 mm²) (Table 1).

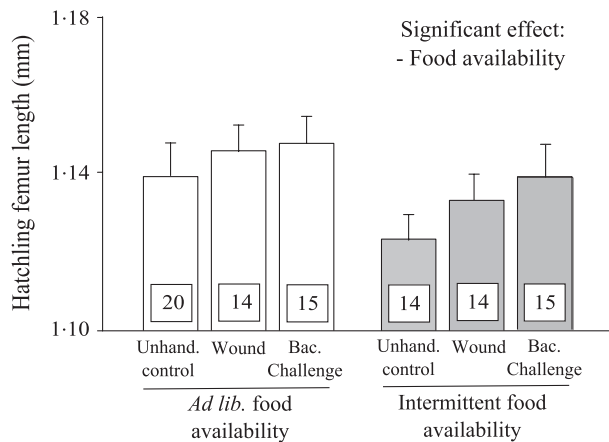


Fig. 3. Effects of treatment on mean femur length (a proxy for body size) of offspring from *Gryllus texensis* that experienced varying access to food (*ad libitum* or intermittent) and were exposed to no immune challenge (unhandled control), a wound or a bacterial challenge. See text for details about dietary and immune challenge treatments. Sample sizes of treatment groups are represented on each bar. Values are displayed as mean \pm SEM.

Egg length was significantly related to egg surface area ($n = 78$, $R = 0.82$, $P < 0.001$), and one or both of these metrics for egg size were positively related to several other aspects of offspring quality, including egg protein content (surface area: $F_{1,74} = 4.1$, $P = 0.046$, $R^2 = 0.053$), hatchling femur length (length: $F_{1,75} = 21$, $P < 0.001$, $R^2 = 0.22$; surface area: $F_{1,75} = 43$, $P < 0.001$, $R^2 = 0.37$), hatchling vigour (surface area: $F_{1,76} = 6.3$, $P = 0.015$, $R^2 = 0.077$) and the total duration of offspring life (sum of incubation duration and vigour: surface area: $F_{1,76} = 4.5$, $P = 0.038$, $R^2 = 0.056$). Females laying eggs with more protein also had larger hatchlings ($F_{1,88} = 4.7$, $P = 0.033$, $R^2 = 0.051$), but there were no significant relationships between egg PO activity and characteristics of development or hatchling phenotype. Hatchling femur length was positively related to hatchling vigour ($F_{1,88} = 6.6$, $P = 0.012$, $R^2 = 0.070$). After accounting for the effects of cohort and reproductive effort, hatchling femur length was strongly negatively related to oviposition rate (multiple regression model: $F_{3,72} = 6168$, $P < 0.001$, $R^2 = 0.99$; hatchling femur length: $t = -5.4$, $P < 0.001$).

Discussion

We provide the most direct test to date of the resource dependency of a fitness trade-off between immune function and reproduction. Although others have examined the role of resource availability in an allocation trade-off between these two traits (e.g. French, Johnston & Moore 2007), we examined this trade-off by estimating the number and quality of offspring produced over a female's entire lifetime. Regardless of food availability, crickets that were chronically immune challenged exhibited lower fecundity than those that were not challenged (Fig. 1) in support of the 'obligate regulation hypothesis'. Immune-challenged

females laid eggs at a relatively slow rate (Fig. 1a), and their eggs did not exhibit greater hatching success (Table 1). Thus, we detected a strong trend ($P = 0.06$, likely due to a smaller sample size) for immune-challenged females to exhibit relatively low estimated reproductive success independent of resource availability.

Obligate trade-offs between reproduction and immunity may be due to several proximate mechanisms, some of which could occur in both invertebrates and vertebrates. For example, the insect immune response relies heavily on non-specific free radicals to destroy pathogens (Molina-Cruz *et al.* 2008), but it can also result in oxidative damage to internal tissues (Sadd & Siva-Jothy 2006). Thus, oxidative damage due to immune activation may impair reproduction directly (e.g. damage to reproductive tissues) or indirectly (e.g. reduce the functionality of signalling molecules or enzymes involved in reproduction). The immune response also results in oxidative stress in vertebrates (Costantini & Moller 2009; Sorci & Faivre 2009), which suggests that oxidant-antioxidant mechanisms may underlie reproduction-immunity trade-offs across taxa (Harshman & Zera 2007). Therefore, future research should investigate the specific role of immune-induced oxidative damage in decreased reproduction.

Crickets given a chronic immune challenge starting in middle age do not show the decline in reproductive output (Shoemaker & Adamo 2007) that we find when the immune challenges begin in early adulthood. This result suggests that a developmental shift in immune function is required for the reproduction-immunity trade-off in this species to be obligatory. Early immune challenges are known to produce long-lasting effects in crickets. For example, a study on another field cricket (*Gryllus campestris*) found that immune challenge during the pre-adult stage induced a pronounced decline in investment in secondary sexual characteristics in males (Jacot *et al.* 2005). Thus, whether a trade-off is obligatory, facultative or occurs at all may also depend on the timing of immune challenge.

Although immune status affected reproduction, it did not influence body condition, and this is in agreement with previous work in *G. texensis* (Shoemaker & Adamo 2007). One explanation is that the decline in egg laying observed for immune-challenged females led to increased egg storage, resulting in no change in overall mass, despite a decrease in energy reserves and general condition. Alternatively, females may prioritize the maintenance of body condition over oviposition rate. In other words, females may divert resources from reproduction to immune activation, which is energetically costly in crickets and other insects (Freitag *et al.* 2003; Ardia *et al.* 2012). However, the role of food quality, as well as food quantity, in reproduction-immunity trade-offs requires more research (e.g. Cotter *et al.* 2011).

We found that immune challenge did more than reduce oviposition rate – it affected the protein content of eggs because females that were chronically wounded produced

relatively protein-rich eggs (Fig. 2a). This result may be due to physiological demands constraining adaptive allocation strategy. Wounding and melanization in the absence of a pathogen result in increased egg size or protein content in other orthopteran insects (Bascunan-Garcia, Lara & Cordoba-Aguilar 2010; Kelly 2011), suggesting that challenged females may be under added pressure to produce larger, higher quality eggs (egg protein content was positively correlated with hatchling size in our study). However, the physiological costs of responding to a bacterial challenge (e.g. protein used to sequester bacterial components and for melanization) were likely higher than the costs associated with wounding (melanization alone). Thus, females challenged with bacterial components and who also had limited access to food may have been unable to allocate more resources into eggs.

Yet, although females with *ad libitum* access to food should have been able to increase investment even with a more expensive immune response, they too exhibited this result (Fig. 2a). Rather, the two immune challenge treatments of our study may have sent very different messages to crickets, which resulted in different physiological responses that affected egg allocation decisions. The wounding challenge may signal a chronic risk of mechanical damage (e.g. predation risk), while the bacterial challenge may be perceived as chronic sickness (e.g. pathogen infection). Future work should investigate how these two seemingly similar immune challenge treatments affect individuals' physiology (e.g. oxidative stress and octopamine, a neurotransmitter involved in the insect stress response) and life history (e.g. developmental rate and longevity). Future research should also compare the effects of chronic *versus* acute immune challenge on cricket immune function and life history.

Food availability significantly affected oviposition rate, estimated reproductive success and hatchling size where females with *ad libitum* access to food produced more and larger hatchlings (Figs 1 and 3). There was also a nonsignificant trend for females with *ad libitum* access to food to produce larger eggs (Table 1), which was expected given the strong positive relationship between egg size and hatchling size. Classic egg size theory (*sensu* Smith & Fretwell 1974) predicts that increased reproductive effort should result in an increase in fecundity but not an increase in investment per offspring. The classic model assumes that the allocation of resources to the number and size of offspring comprises independent processes. Yet, there is evidence from a wide variety of taxa that investment per offspring and reproductive effort are positively correlated (Schwarzkopf, Blows & Caley 1999; Caley, Schwarzkopf & Shine 2001; Beck & Beck 2005).

In fact, when reproductive effort was held constant, we detected a negative phenotypic trade-off between fecundity and hatchling size. Because there was no relationship between egg size and hatching success, this trade-off suggests that females maximizing fecundity would have the greatest reproductive success. However, our measure of reproductive success incorporated only hatching success and fecundity,

and a reliable assessment of optimal reproductive strategies requires that offspring performance be monitored up to the time at which performance becomes random with respect to initial investment (Rollinson & Hutchings, 2013). There is a growing literature suggesting that effects of offspring size on fitness are generally delayed and are not detectable during the embryo or egg stage (Fox, Thakar & Mousseau 1997; Einum & Fleming 2002; Beckerman *et al.* 2006; Rombough 2006). Thus, our estimate of maternal reproductive success may not allow for an accurate or meaningful determination of the optimal reproductive strategy. Fortunately, we also measured several aspects of hatchling phenotype in our study. For example, we observed that larger eggs had more protein and resulted in larger hatchlings, which, in turn, survived longer without food. In the wild, the fitness of both offspring and parents may increase when offspring are hardier, especially if environmental conditions are unfavourable (e.g. Fox, Thakar & Mousseau 1997; Einum & Fleming 1999). To avoid incorrect conclusions about the fitness consequences of allocation trade-offs or environmental perturbation, biologists should continue to appreciate that not all eggs are created equal.

We explicitly tested the 'facultative regulation' and 'obligate regulation' hypotheses regarding a fitness trade-off between reproduction and immunity in a field cricket, and our results supported the 'obligate regulation hypothesis'. We demonstrate the fitness costs of chronic immune activation – *ad libitum* food availability cannot prevent a reduction in our estimates of reproductive success after chronic activation of the immune response. Future work should examine the temporal dynamics of reproduction–immunity trade-offs – that is, at what age do trade-offs switch from 'obligate' to 'facultative'? By demonstrating that not all eggs are created equal, we provide key insight into reproduction–immunity trade-offs and reproductive allocation in varying environments.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Estimating lifetime fecundity.

Appendix S2. Egg quality measurements.