

Life History and Immune Challenge Influence Metabolic Plasticity to Food Availability and Acclimation Temperature

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ABSTRACT

Animals vary in their rates of energy expenditure for self-maintenance (standard metabolic rate [SMR]). Yet we still lack a thorough understanding of the determinants of SMR, potentially because of complex interactions among environmental, life-history, and physiological factors. Thus, we used a factorial design in female sand field crickets (*Gryllus firmus*) to investigate the independent and interactive effects of food availability (unlimited or limited access), acclimation temperature (control or simulated heat wave), life-history strategy (flight-capable or flight-incapable wing morphology), and immune status (control or chronic immune activation) on SMR (CO₂ production rate) measured at 28°C. Both environmental factors independently affected SMR where heat wave and food limitation reduced SMR. Furthermore, wing morphology and immune status mediated the plasticity of SMR to food and temperature. For example, the hypermetabolic effect of food availability was greater in flight-capable crickets and reduced in immune-challenged crickets. Therefore, although SMR was directly affected by food availability and acclimation temperature, interactive effects on SMR were more common, meaning several factors (e.g., life history and immune status) influenced metabolic plasticity to food and temperature. We encourage continued use of factorial experiments to reveal interaction dynamics, which are critical to understanding emergent physiological processes.

Keywords: cricket, factorial, *Gryllus*, heat wave, interactive effects, metabolic rate.

Introduction

Standard metabolic rate (SMR) is the energy cost of self-maintenance and thus is critical to understanding animal energetics (Glazier

2005; Lighton 2008; Burton et al. 2011). SMR has been linked to a range of biological processes—from rates of reproduction and aging to population growth and molecular evolution (sensu “metabolic theory of ecology”; reviewed in Brown et al. 2004; Glazier 2015). Although metabolic rate has been invoked as a universal “pacemaker” that drives important biological processes, metabolic rate may also be sensitive to (and thus driven by) a range of intrinsic and extrinsic factors (reviewed in Glazier 2015; Schulte 2015; Lefevre 2016). For example, locomotor activity and temperature strongly influence metabolic expenditure across animal taxa (Weibel et al. 2004; Glazier 2005, 2015; Angilletta 2009; Schulte 2015). Despite a rich history of investigations into metabolic rate within and among species (Glazier 2005; Konarzewski and Książek 2013), we still lack a comprehensive understanding of the determinants of metabolic rate (Burton et al. 2011; Glazier 2015).

Spatiotemporal variation in food availability affects many aspects of animal ecology (reviewed in Simpson and Raubenheimer 2012), and the composition of food can alter SMR (Cruz-Neto and Bozinovic 2004; Bozinovic et al. 2007; Konarzewski and Książek 2013; Auer et al. 2015; Clark et al. 2016). Likewise, environmental temperature influences a range of biological processes, including metabolic rate (reviewed in Angilletta 2009; Schulte 2015). For example, acclimation to warmer temperatures results in reduced SMR when controlling for test temperature for many organisms, including plants, fungi, invertebrates, and vertebrates (discussed in Stahlschmidt et al. 2015b), presumably as a means of conserving energy given the positive effect of temperature on metabolic rate (reviewed in Angilletta 2009). Food availability and temperature can vary simultaneously in nature (Mattson 1980; Stamp 1993) and interact to influence SMR in some taxa (snakes; Stahlschmidt et al. 2015b). Therefore, metabolic responses need to be characterized across equivalently complex contexts that take such co-varying environmental variables into consideration.

Metabolic rate is also sensitive to important physiological processes. For example, immune function is critical to host fitness (reviewed in Graham et al. 2011), and the acute metabolic costs of an immune challenge in vertebrates is well established (Kluger 1991; Lochmiller and Deerenberg 2000; Klasing 2004; Martin et al. 2008). Insects generally exhibit responses to immune challenge similar to those of vertebrates (Freitak et al. 2003; Ardia et al. 2012). Yet the detection of behavioral fever in insects is dependent on several factors (e.g., type of immunogen and the magnitude or duration of fever response; reviewed in Stahlschmidt and Adamo 2013), and immune-induced metabolic costs in insects may be similarly context dependent. An animal’s metabolic rate may also be linked to its life-history strategy (Crnokrak and Roff 2002; Książek et al. 2004; Bronikowski and Vleck 2010; but see Djawdan et al. 1996, 1997; Clark et al. 2016)—therefore, it is important to

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examine the nature of any potential interactions among life-history strategy, immune status, and environmental characteristics on metabolic rate.

Wing-dimorphic *Gryllus* cricket species may offer useful insight into the role of life history in explaining variation in SMR because they exhibit two discrete life-history strategies. A wing dimorphism influenced by both genetic and environmental factors mediates a flight-fecundity trade-off in *Gryllus* during early adulthood—long-winged (LW) females invest in flight muscle (dorsolongitudinal muscle [DLM] and dorsoventral muscle) at a cost to ovary mass, while short-winged (SW) females do not invest in DLM but exhibit heavier ovaries than LW females (Roff et al. 1984; Zera and Mole 1994; Zera et al. 1997; Zera 2005). Previous work indicates that LW female *Gryllus* tend to exhibit greater SMR than SW females (Zera and Mole 1994; Zera et al. 1997; Nespolo et al. 2008; but see Clark et al. 2016). Wing morphology can also influence immunocompetence in *Gryllus* (Park and Stanley 2015; Kirschman et al. 2017, 2019; but see Rantala and Roff 2006), but morph-specific effects of immune challenge on SMR in *Gryllus* are unknown. Likewise, little is known about interactive effects of wing morphology (life-history strategy) and other factors on SMR in *Gryllus* (but see Clark et al. 2016).

Important physiological processes are often influenced by a number of interactive variables. For example, variation in global gross primary production (i.e., carbon uptake by leaves) is driven by covarying environmental factors (temperature, vapor pressure deficit, and elevation; Wang et al. 2017). Likewise, metabolic rate may vary as a result of temperature-food or immune-life history interactions (see above). Furthermore, SMR may be influenced by more complex interactions among these four factors (i.e., three- or four-way interactions) that may exhibit additive or nonadditive effects (e.g., synergistic or antagonistic effects; reviewed in Todgham et al. 2013; Kaunisto et al. 2016). Therefore, we used a factorial experimental design to examine the independent and interactive effects of life-history strategy, acclimation temperature, food availability, and immune status on SMR in female sand field crickets (*Gryllus firmus*). We used our design specifically to test two hypotheses related to interactive effects on metabolic rate. First, we tested the hypothesis that hypometabolism due to heat acclimation is sensitive to other environmental factors (e.g., food availability) and/or wing morphology. Second, we tested the hypothesis that the metabolic response to chronic immune activation is also context dependent (e.g., food availability and immune challenge may interact to influence metabolic rate). Perhaps most importantly, our study will provide new insight into how SMR is affected by the complex interplay among life-history strategy, complex environments, and physiology.

Methods

Study Species

The sand field cricket (*Gryllus firmus*) is native to the southeastern United States and found most abundantly in Florida (Capinera et al. 2004). Crickets used in this study were acquired from six selected, nearly true-breeding blocks of SW and LW crickets (i.e., three SW blocks and three LW blocks) that have been previously

described (Zera 2005). Female *G. firmus* were reared at $28^{\circ} \pm 1^{\circ}\text{C}$ under a 16L:8D cycle with ad lib. access to water and commercial dry cat food.

Experimental Design

To investigate the plasticity of SMR in *G. firmus* ($n = 201$), a factorial design across two levels for each of three treatments (temperature, food availability, and immune status; described below) was used on SW and LW females ($n = 97$ and $n = 104$, respectively) during early adulthood (i.e., a $2 \times 2 \times 2 \times 2$ factorial design, including wing morphology; table 1). Newly molted adults (≤ 1 d after final ecdysis) were individually housed in small translucent deli cups (473 mL) containing water (water-filled shell vials plugged with cotton) and shelter (overturned 30-mL opaque containers with access holes) in incubators (I-36, Percival Scientific, Perry, IA) maintaining a 16-h photoperiod. They were randomly assigned to combinations of treatments described below.

Temperature treatment. Approximately half of the *G. firmus* experienced a control temperature treatment (sinusoidal daily temperature cycle of 20.5° – 32°C , which is the average daily temperature range during the May–September breeding season in Gainesville, FL; National Weather Service). The remaining crickets experienced a heat wave treatment (sinusoidal daily temperature cycle of 24° – 37°C , which was the temperature range during a heat wave in Gainesville, FL, during late July 2010; National Weather Service).

Food availability treatment. Crickets experienced one of two food treatment levels—high food availability (ad lib. access to food [commercial dry cat food]) or low food availability (access to food for 3 h on the third day of the study, as in Stahlschmidt et al. 2013; Stahlschmidt and Adamo 2015). The low-food treatment was ecologically relevant because of crickets' intermittent feeding habits (Gangwere 1961), and it results in individuals with levels of body fat similar to those found in the field for other *Gryllus* species (Adamo et al. 2012). After controlling for starting body mass, final body mass was 20% greater in high-food crickets relative to low-food crickets in our study (estimated marginal mean \pm SEM; control: 858 ± 6 vs. 710 ± 6 mg). Furthermore, prolonged exposure to intermittent food availability reduces survival, growth and developmental rates, and investment in reproductive tissue in *G. firmus* (Glass and Stahlschmidt 2019). Thus, the food availability treatments used in our study likely strongly influenced important biological processes.

Immune status treatment. On the first, third, and fifth days of the experiment, *G. firmus* experienced one of two immune treatments. Approximately half of the crickets experienced the challenge treatment (abdominal injection of $75 \mu\text{g}$ of lipopolysaccharide [LPS; Sigma Aldrich] in $2 \mu\text{L}$ of insect Ringer's solution) to chronically induce a systemic immune response. Other studies with crickets have demonstrated that immune activation due to exposure to LPS derived from *Serratia marcescens* can result in somatic and reproductive costs (Adamo 1999; Jacot et al. 2004, 2005; Leman et al. 2009; Drayton et al. 2013; McNamara et al. 2014; Charles and Killian 2015; but see Shoemaker and Adamo 2007). *Serratia marcescens* is distributed worldwide, and it has

Table 1: Factorial experimental design with 16 treatment group combinations where temperature treatment (control or simulated heat wave), food availability (high or low availability), and immune status (nonchallenged or chronic lipopolysaccharide challenged) were manipulated during early adulthood in short- and long-winged female *Gryllus firmus* ($n = 198$)

Wing morphology	Temperature	Food availability	Immune status (n)
Short winged	Control temperature	High food	No challenge (14) Challenge (8)
		Low food	No challenge (12) Challenge (12)
	Heat wave	High food	No challenge (11) Challenge (8)
		Low food	No challenge (14) Challenge (17)
Long winged	Control temperature	High food	No challenge (10) Challenge (9)
		Low food	No challenge (10) Challenge (14)
	Heat wave	High food	No challenge (17) Challenge (15)
		Low food	No challenge (15) Challenge (12)

been found in the bodies of orthopterans in the field (Steinhaus 1959). Similarly, the LPS used in our study was derived from *S. marcescens*, and the dosage (75 μg of LPS dissolved in 2 μL of insect Ringer's solution) was within the range of these studies (10–100 μg of LPS per cricket). Wounding associated with a sham injection activates the insect immune response (Gillespie and Khachatourians 1992; Wigby et al. 2008; Ardia et al. 2012). Thus, the remaining crickets experienced a no-challenge treatment, where they were simply handled similarly to challenge treatment crickets to control for the stress of repeated restraint without inducing an immune response.

The experiment ended after 6 d, which approximates the apex of the flight-fecundity trade-off (i.e., when the maximal differences in ovary and flight muscle masses between the two wing morphs occur; Zera and Larsen 2001). On the sixth (final) day of the experiment, crickets were weighed and then underwent flow-through respirometry trials at 28°C to determine whole-animal metabolic rate from 10:00 a.m. to 12:00 p.m. (see below). After which, each cricket was euthanized and stored at -20°C until it was dissected to determine the presence of DLM. Dissections indicated that >75% of LW individuals contained DLM, while DLM was completely absent in 99% of the SW individuals.

Respirometry Trials

Each cricket was placed in a small glass metabolic chamber (59 mL) in an incubator (I-30, Percival Scientific, Perry, IA) maintaining a constant 28°C. To reduce movement or activity during the trials, crickets were acclimated for ≥ 90 min in a darkened incubator before measurement. Other work on orthopterans demonstrates that carbon dioxide production rate (\dot{V}_{CO_2}) increases rapidly after feeding begins but returns to prefeeding levels within 10 min after

feeding ends (Gouveia et al. 2000). Therefore, the acclimation period in our study aimed to mitigate the potential confounding effect of feeding on metabolic rate.

Seven metabolic chambers at a time were connected to a multiplexer (MUX, Sable Systems, Las Vegas, NV). An eighth port on the multiplexer allowed for baseline measurements of influent air throughout the 86-min sampling period, which included four baseline measurements and seven respiratory measurements. Influent air was generated by a diaphragm pump (PP2, Sable Systems, Las Vegas, NV), and flow rates were regulated by needle valves and monitored by mass flow meters (FB8, Sable Systems, Las Vegas, NV). The multiplexer passed one airstream sequentially through each chamber for 10 min for respiratory measurements (90 mL min^{-1} per chamber), while the other airstream provided continuous air to crickets during nonmeasurement periods (~ 30 mL min^{-1} per chamber). Influent air was stripped of H_2O and CO_2 by flowing it through columns of CaSO_4 and soda lime, respectively. Sample effluent air was first passed through a water-vapor meter (FMS, Sable Systems, Las Vegas, NV), after which water was removed via CaSO_4 . Air was then passed through a CO_2 analyzer and then through an O_2 analyzer (FMS, Sable Systems, Las Vegas, NV). All hardware (i.e., FMS and FB8) interfaced with a software (ExpeData, Sable Systems, Las Vegas, NV) that recorded data each second to a computer. Each cricket's steady state \dot{V}_{CO_2} and oxygen consumption rate (\dot{V}_{O_2}) was determined from the average of the most level 5-min sequence (i.e., 300 samples) of data (Lighton 2008).

Oxygen consumption rate is directly proportional to metabolic rate, but O_2 analyzers are typically less sensitive than CO_2 analyzers (Harrison et al. 2012). For example, our O_2 analyzer was less reliably able to distinguish O_2 signal from background variation at the flow rate used in our study relative to our CO_2 analyzer,

which is not uncommon (Lighton 2008; Clark et al. 2016). Therefore, \dot{V}_{CO_2} was measured as an indirect estimate of SMR (Nespolo et al. 2005; Lighton 2008), which strongly correlated with \dot{V}_{O_2} in our study ($R = 0.75, P < 0.001$). The relationship between \dot{V}_{CO_2} and metabolic rate varies as a result of the composition (i.e., mixture of protein, carbohydrate, or lipid) and amount of fuel being used (Sinclair et al. 2011; McCue et al. 2015; Clark et al. 2016). Food limitation may shift crickets' oxidation of carbohydrate, lipid, and protein (Sinclair et al. 2011), thereby influencing crickets' respiratory quotient (RQ; $\dot{V}_{CO_2}/\dot{V}_{O_2}$), which could deflate \dot{V}_{CO_2} in low-food crickets. Therefore, a linear mixed model analysis was performed on RQ data to test the sensitivity of RQ to food treatment, with the caveat that measurements of \dot{V}_{O_2} were less reliable than those of \dot{V}_{CO_2} even though \dot{V}_{CO_2} was highly correlated with \dot{V}_{O_2} (see above). Wing morphology, temperature, immune status, and block were included as random effects, and food availability treatment was included as a main effect. Food treatment had no significant effect on RQ ($F_{1,195} = 0.003, P = 0.96$; fig. A1) and, presumably, fuel use.

Statistical Analyses

Data were tested for normality, and outlier data from three individuals were omitted to achieve a data set with normally distributed residuals. Data were analyzed using SPSS (ver. 25, IBM, Armonk, NY), and two-tailed significance was determined at $\alpha = 0.05$. To examine the independent and interactive effects of treatments (temperature, food availability, and immune status) and wing morphology (herein, "morph"), a linear mixed model analysis was performed on \dot{V}_{CO_2} . Treatments and morph were included as main effects, block was included as a random effect, and body mass was included as a covariate to control for body size. Nonsignificant interactions were removed from the model if doing so did not increase the Akaike information criterion. The final most parsimonious model is reported (table 2). Values are

Table 2: Effects of wing morphology (morph; short or long winged), temperature treatment (control or simulated heat wave), food availability (high or low availability), and immune status (nonchallenged or chronic lipopolysaccharide challenged) on CO_2 production rate at 28°C in female *Gryllus firmus* ($n = 198$)

	df	F	P
Morph	1, 183	3.1	.081
Temperature	1, 183	12.3	.001
Food availability	1, 183	77.0	<.001
Immune status	1, 183	.2	.651
Morph × food	1, 183	6.4	.012
Food × immune	1, 183	22.8	<.001
Morph × temperature × immune	1, 183	3.9	.010
Temperature × food × immune	1, 183	3.4	.035
Body mass	1, 183	179.3	<.001

Note. Nonsignificant interactions were removed from the initial (full) model if doing so did not increase the Akaike information criterion. The final most parsimonious model is reported. See figure 1 and text for further details.

displayed as estimated marginal means (95% confidence intervals) because body mass was included as a covariate—that is, reported values account for variation in body mass at the time of respirometry trials.

Results

Metabolic rate at 28°C was influenced by the main effects of temperature treatment (estimated marginal mean \pm SEM; control: 0.75 ± 0.02 mL h⁻¹; heat wave: 0.67 ± 0.02 mL h⁻¹), food availability (high food: 0.81 ± 0.02 mL h⁻¹; low food 0.60 ± 0.02 mL h⁻¹), and body mass where \dot{V}_{CO_2} was greater in heavier crickets (table 2; fig. 1). Yet it was not influenced by morph or immune status alone (table 2; fig. 1). Food availability interacted with morph to influence \dot{V}_{CO_2} where hypermetabolism associated with high food availability was greater in LW crickets (food × morph; table 2; figs. 1, A2). Food availability also interacted with immune status to influence \dot{V}_{CO_2} (food × immune) where immune challenge reduced the effect of food availability on \dot{V}_{CO_2} (table 2; figs. 1, A3). Morph, temperature treatment, and immune status interacted with one another to influence metabolic rate (morph × temperature × immune; table 2; figs. 1, A4). Relative to no challenge, immune challenge increased \dot{V}_{CO_2} for SW crickets in the control temperature treatment but decreased it for LW crickets (morph × temperature × immune; table 2; figs. 1, A4). There was an interactive effect of temperature treatment, food availability, and immune status on \dot{V}_{CO_2} where benign environmental conditions (high food availability, control temperature, and no immune challenge) increased \dot{V}_{CO_2} (temperature × food × immune; table 2; figs. 1, A5).

Discussion

Single-factor studies on metabolic rate are critical to understanding the determinants of metabolic plasticity because they are direct and convey the importance of a given factor while controlling for or eliminating confounding variables (e.g., manipulating temperature while controlling for immune status or vice versa). Yet many factors covary in nature and may interact to influence animal physiology (Mattson 1980; Stamp 1993; Todgham and Stillman 2013; Stahlschmidt et al. 2015b). Therefore, our study integrated the effects of complex environments (food availability and temperature treatments), life history (wing dimorphism mediating a flight-fecundity trade-off), and physiology (response to chronic immune challenge) on metabolic rate. Although SMR varied as a result of the individual effects of both environmental factors (food and temperature), it was not affected by morph or immune status alone (table 2; fig. 1). However, both morph and immune status interacted with several other factors to influence metabolic rate (table 2; figs. 1, A2–A5). Together, these results demonstrate the need to incorporate biotic and abiotic factors when investigating plasticity in SMR. For example, our results indicate that examining only one morph or only one temperature treatment would significantly underestimate the metabolic responses of *Gryllus firmus* to immune challenge for an entire population (i.e., both SW and LW individuals) experiencing variable temperature (morph × temperature × immune; table 1; figs. 1, A4).

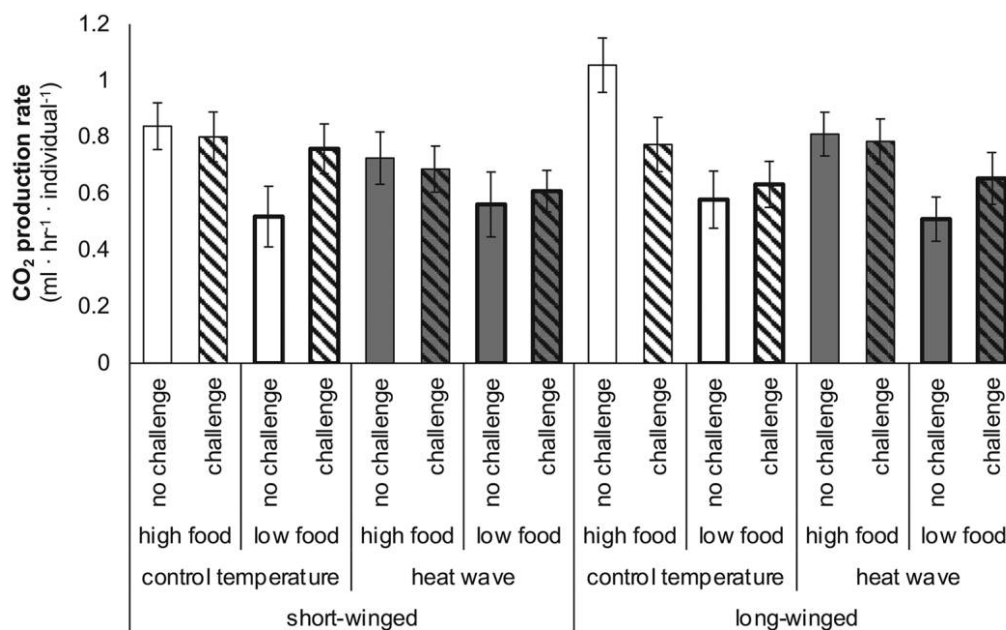


Figure 1. Effects of wing morphology (short or long winged), temperature treatment (control or simulated heat wave), food availability (high or low availability), and immune status (nonchallenged or chronic lipopolysaccharide challenged) on CO₂ production rate at 28°C in female *Gryllus firmus* ($n = 198$). Values are displayed as estimated marginal means (95% confidence intervals) because body mass was included as a covariate. See table 2 and text for further details.

Although immune status did not independently affect SMR, the metabolic response to chronic immune activation varied as a result of other factors, which supports our second hypothesis (metabolic response to chronic immune activation is context dependent). Immune status was involved in three significant interactions that affected SMR (table 2; figs. 1, A3–A5). Specifically, the effect of food availability on SMR was affected by immune status (food \times immune interaction), particularly in crickets from the control temperature treatment (temperature \times food \times immune interaction; table 2; figs. 1, A3–A5). Across animal taxa, immune activation generally leads to an acute increase in energy expenditure (reviewed in Kluger 1991; Lochmiller and Deerenberg 2000; Freitag et al. 2003; Klasing 2004; Martin et al. 2008; Ardia et al. 2012), but we found only partial support for this in *G. firmus*. For example, SW females exhibited increased SMR due to chronic immune challenge in the control temperature treatment (estimated marginal mean \pm SEM: 0.78 ± 0.03 vs. 0.68 ± 0.03 mL h⁻¹) but not in the heat wave treatment (table 2; figs. 1, A4). We further found that immune challenge actually decreased SMR by ~16% in LW crickets in the control temperature treatment (morph \times temperature \times immune; table 2; figs. 1, A4). A similar hypometabolic response to immune activation has been shown in other taxa (wound healing reduces SMR in lizards; Smith et al. 2017), possibly due to a sickness syndrome characterized by lethargy and anorexia. Animals vary in thermal biology and life history, and our results demonstrate that the metabolic response to immune challenge is modulated by a complex interplay between wing morphology and acclimation temperature. Related to this, mammals and birds typically increase metabolic rate during an immune challenge via a fever

response, but behavioral fever in reptiles and insects is highly dependent on context, such as sex or the timing, dose, and type of immune challenge (reviewed in Kluger 1986, 1991; Stahlschmidt and Adamo 2013; Todd et al. 2016; Ryan et al. 2018). Thus, it is important to integrate context (i.e., variation in biotic or abiotic factors) when examining the physiological responses to immune activation.

There is some evidence that life history can directly influence metabolic rate (Crnokrak and Roff 2002; Książek et al. 2004; Bronikowski and Vleck 2010; but see Djawdan et al. 1996, 1997; Clark et al. 2016). For example, across a range of temperatures, an ecotype of garter snake with a life history on the fast end of a pace-of-life continuum (i.e., fast growth and short life span) has a greater mass-specific SMR than an ecotype on the slower end of this continuum (Bronikowski and Vleck 2010). Yet SMR was not independently affected by wing morphology, which mediates a trade-off between investment in flight capacity and fecundity, and this result agrees with recent work in *G. firmus* (table 2; fig. 1; Clark et al. 2016). Rather, we found that life-history strategy (wing morphology) influenced metabolic plasticity in response to environmental variation and immune challenge (table 2; figs. 1, A2, A4). Wing morphology mediated the effect of food availability on SMR—the positive effect of food on SMR (i.e., high food availability increased \dot{V}_{CO_2}) was greater in the LW morph. Thus, SMR in LW individuals was more sensitive to food availability, and recent work indicates that non-SMR traits (i.e., flight capacity, body mass, and mortality) in LW *G. firmus* are more sensitive to other factors (e.g., water limitation and oxidative stress) relative to SW *G. firmus* (Z. R. Stahlschmidt, unpublished data; Stahlschmidt et al. 2019). Furthermore, the SMR for the two wing morphs

responded differently to immune challenge in the control temperature treatment—SMR increased in SWs and decreased in LWs (table 2; figs. 1, A4). It is possible that flightless SWs have a greater ability to upregulate some aspects of an energetically expensive immune response (Kirschman et al. 2017) because, unlike LW *Gryllus*, they are not forced to allocate resources between immunity and flight capacity (Adamo et al. 2008). Therefore, our results demonstrate that life history can influence metabolic rate through indirect or interactive means.

Crickets experiencing a simulated heat wave exhibited ~11% lower SMR than control-temperature crickets when measured at a test temperature of 28°C (table 2; fig. 1), potentially to save energy given the positive effect of temperature on energy expenditure (Angilletta 2009). The hypometabolic response to warmer acclimation temperatures may be a ubiquitous feature of thermal plasticity because it occurs across taxa—from plants and fungi (Malcolm et al. 2008; reviewed in Slot et al. 2015) to invertebrates (Powell and Watts 2006; this study) and vertebrates (Donelson et al. 2011; Seebacher and Grigaltchik 2014; Stahlschmidt et al. 2015b). In support of our first hypothesis (hypometabolism due to heat acclimation is sensitive to other environmental factors), thermal plasticity in metabolic rate was dependent on another environmental factor in our study. Specifically, the positive effect of the food availability treatment on SMR (i.e., high food availability increased \dot{V}_{CO_2}) was greater for nonchallenged crickets acclimated to the control temperature treatment (table 2; figs. 1, A5). That is, benign conditions (i.e., control temperature, high food availability, and no immune challenge) generally promoted metabolic activity. Given the natural covariation of temperature and food availability (Mattson 1980; Stamp 1993), it is important to continue to investigate both the independent and the interactive effects of these environmental factors on important physiological traits, such as metabolic rate.

When controlling for body mass, metabolic rate was ~35% lower in *G. firmus* that were food limited relative to those with food provided ad lib. during adulthood (table 2; fig. 1). Decreased food availability reduces nutrient acquisition, which reduces the amount of resources available for allocation to energetically expensive processes. For example, decreased food availability reduces investment in reproduction, flight capacity, and immune function in *Gryllus* crickets (King et al. 2011; Stahlschmidt et al. 2013, 2015a; Stahlschmidt and Adamo 2015). Yet there is mixed support for a hypometabolic response to food limitation in other animals, including insects (Hulbert et al. 2004; Nespolo et al. 2005; Roark and Bjorndal 2009; Sinclair et al. 2011; Speakman and Mitchell 2011; Niitepold et al. 2014; Liang et al. 2015; Lebeau et al. 2016; Smith et al. 2017; Redman et al. 2018; Zhang et al. 2018). Discrepancies in results across studies may be due to variation in the degree (or duration) of food restriction, phylogeny, or

measurements of metabolic rate (Sinclair et al. 2011; Speakman and Mitchell 2011; Redman et al. 2018). Our results further suggest that abiotic and biotic factors may modulate the effects of food availability on SMR—for example, hypometabolism associated with food limitation was blunted in crickets that were short winged or immune challenged (table 2; figs. 1, A2, A3).

In summary, we used a factorial approach to examine the independent and interactive effects of life-history strategy, environmental variables, and immune status on a key feature of animal energetics (i.e., SMR; Glazier 2005; Burton et al. 2011). Our results indicate that SMR was directly affected by food availability and acclimation temperature in *G. firmus* (table 2; fig. 1). Food availability had a stronger effect on SMR than heat wave (i.e., 35% vs. 11% change in SMR, respectively)—therefore, it is important to consider an experiment's feeding regimen because food availability may mask the effects of temperature on SMR if it is not properly accounted for. Interactive effects on SMR were more common than individual or independent effects in our study (table 2; fig. 1). Several factors (e.g., life history and immune status) influenced metabolic plasticity to food and temperature (table 2; fig. 1), so we encourage others to test whether these types of interactive effects on SMR are widespread and generally apply to other animal systems. Thus, factorial experimental designs are critical to identifying interactive (e.g., synergistic or antagonistic) effects on important physiological processes (Todgham and Stillman 2013). However, the interpretation of such complex dynamics requires sophisticated analytical methods. For example, network analyses have been used to quantify the role of diffuse indirect effects in shaping ecological communities (Fath and Patten 1999; Fath 2004). They have also shown how networks of genes respond to genetic and environmental shifts and how these interactions influence disease-associated traits, thereby demonstrating that common diseases are emergent properties of networks (Chen et al. 2008). Clearly, the dynamics of metabolic rate are complex (Brown et al. 2004; Glazier 2005, 2015; Burton et al. 2011; Redman et al. 2018; our study)—therefore, we encourage future research that integrates factorial designs with network analysis approaches to better understand the determinants of SMR.

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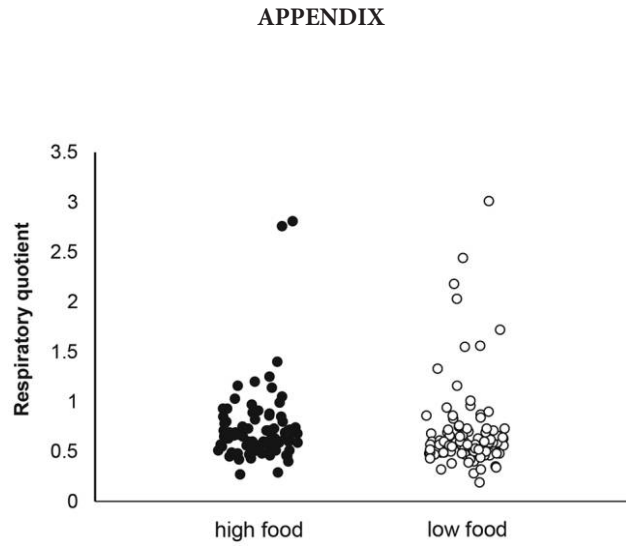


Figure A1. Effect of food availability (high or low availability) on respiratory quotient (RQ; $\dot{V}_{CO_2}/\dot{V}_{O_2}$) at 28°C in female *Gryllus firmus* ($n = 198$). To account for wing morphology, temperature, immune status, and block, these four factors were included as random effects when analyzing for the effect of food availability on RQ. There are seeming outliers (e.g., RQ values >1), and this is likely due to the limitations of O_2 analyzers (Harrison et al. 2012). Yet these data points were included because the data set exhibited normally distributed residuals, and only the more reliable \dot{V}_{CO_2} (not the \dot{V}_{O_2}) were used for subsequent analyses.

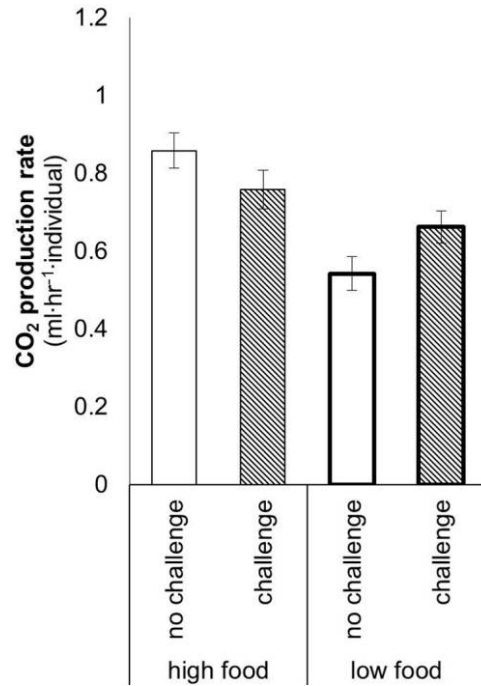


Figure A3. Effects of food availability (high or low availability) and immune status (nonchallenged or chronic lipopolysaccharide challenged) on CO_2 production rate at 28°C in female *Gryllus firmus* ($n = 198$). Values are displayed as estimated marginal means (95% confidence intervals) because body mass was included as a covariate.

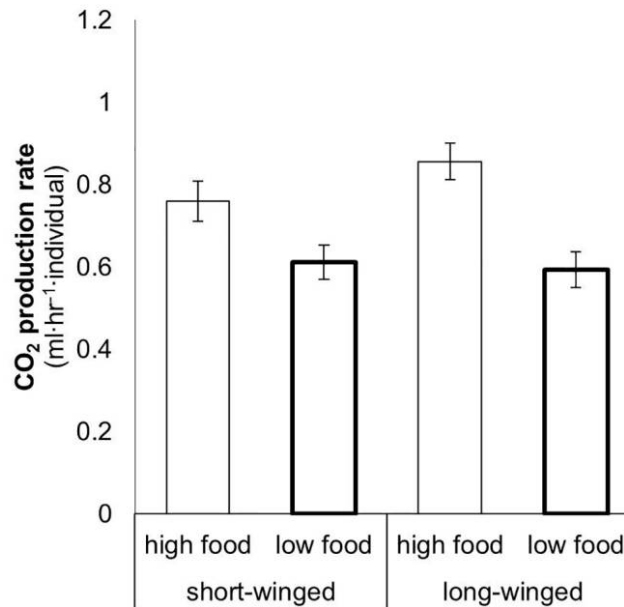


Figure A2. Effects of wing morphology (short or long winged) and food availability (high or low availability) on CO_2 production rate at 28°C in female *Gryllus firmus* ($n = 198$). Values are displayed as estimated marginal means (95% confidence intervals) because body mass was included as a covariate.

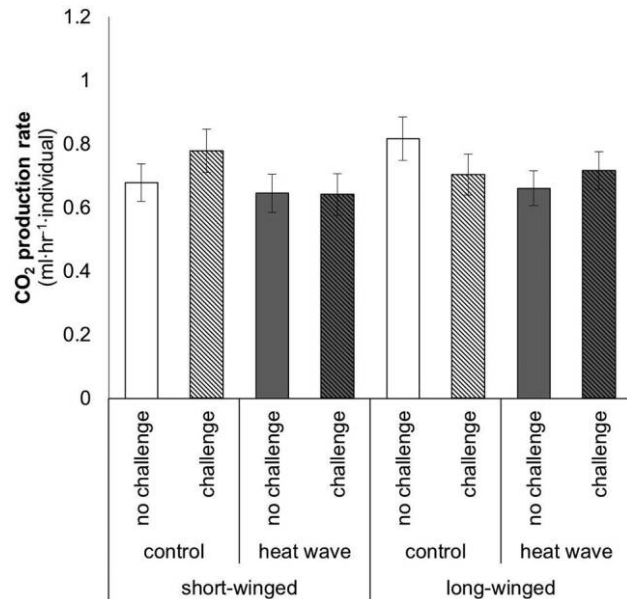


Figure A4. Effects of wing morphology (short or long winged), temperature treatment (control or simulated heat wave), and immune status (nonchallenged or chronic lipopolysaccharide challenged) on CO_2 production rate at 28°C in female *Gryllus firmus* ($n = 198$). Values are displayed as estimated marginal means (95% confidence intervals) because body mass was included as a covariate.

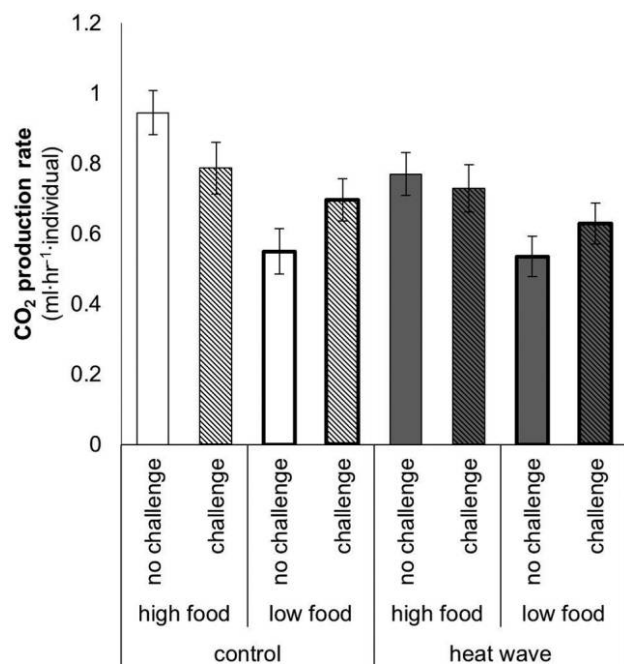


Figure A5. Effects of temperature treatment (control or simulated heat wave), food availability (high or low availability), and immune status (nonchallenged or chronic lipopolysaccharide challenged) on CO₂ production rate at 28°C in female *Gryllus firmus* ($n = 198$). Values are displayed as estimated marginal means (95% confidence intervals) because body mass was included as a covariate.

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