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# From phenoloxidase to fecundity: food availability does not influence the costs of oxidative challenge in a wing-dimorphic cricket

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## Abstract

Stressed animals often struggle to maintain optimal investment into a number of fitness-related traits, which can result in some traits being more adversely affected than others. Variation in stress-related costs may also depend on the environment—costs can be facultative and only occur when resources are limited, or they may be obligate and occur regardless of resource availability. Dynamics of oxidative stress may be important in life-history evolution given their role in a range of biological processes—from reproduction to immunity to locomotion. Thus, we examined how resource (food) availability influences the costs of oxidative challenge to fitness-related traits spanning several levels of biological organization. We manipulated food availability and oxidative status in females of the wing-dimorphic sand field cricket (*Gryllus firmus*) during early adulthood. We then determined investment into several traits: reproduction (ovary mass), soma (body mass and flight musculature), and immune function (total phenoloxidase activity). Oxidative challenge (paraquat exposure) obligated costs to somatic tissue and a parameter of immune function regardless of food availability, but it did not affect reproduction. We show that the costs of oxidative challenge are trait-specific, but we did not detect a facultative (food-dependent) cost of oxidative challenge to any trait measured. Although the dynamics of oxidative stress are complex, our study is an important step toward a more complete understanding of the roles that resource availability and redox systems play in mediating life histories.

**Keywords** Antioxidant · Cricket · Flight · Life history · Immunity · Resource availability

## Introduction

Animals must balance investment into multiple fitness-related traits—from the growth and maintenance of somatic and reproductive tissues to components of physiological self-maintenance (e.g., antioxidant defenses and immune function) (Van Noordwijk and de Jong 1986; Reznick 1992; Zera and Harshman 2001; Cox et al. 2010; Stahlschmidt et al. 2013, 2015). However, this balancing act can become disrupted during stress (i.e., dysregulation can occur) when a given stressor has differential effects on important traits. For example, thermal stress can differentially influence immune parameters and disease resistance (Labaude et al. 2017), body mass and oxidative damage (Stahlschmidt et al. 2017), or fecundity and immune parameters (Leicht et al.

2017). Thus, understanding the costs of a stress response requires a thorough examination of stress-induced changes across scales of biological organization spanning from individual molecules to traits of morphology and life history.

Environmental conditions, such as food availability, may also influence the magnitude of costs induced by a stressor. Immune-challenged lizards reduce their investment into reproduction when food is limited, but the cost of this immune stressor is facultative: immune-challenged lizards with unlimited access to food are able to sustain reproductive investment (French et al. 2007). Food availability can similarly influence the reproduction- and survival-related costs of heat waves (Adamo et al. 2012). Yet, some stress-induced costs may be obligate and will occur regardless of food availability. For example, immune activation involves the production of reactive molecules that can damage enzymes, cell membranes, and tissues (Sadd and Siva-Jothy 2006; Molina-Cruz et al. 2008; Ayres and Schneider 2012), and the costs of an immune challenge to lifetime reproductive investment can be obligate (i.e., not facultative or food-dependent: Stahlschmidt et al. 2013). Therefore, both the trait(s) of interest

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(e.g., immune function vs. growth of somatic tissue) and resource availability must be considered when examining the costs of a stress response.

Oxidative stress occurs when an organism's antioxidant defenses are unable to neutralize damage to biomolecules caused by reactive oxygen species (ROS), which are by-products of oxidative phosphorylation in the mitochondria during normal metabolic processes (Costantini et al. 2010). Understanding the dynamics of costs due to oxidative stress is important, because oxidative stress may be a universal constraint in life-history evolution (Dowling and Simmons 2009; Costantini et al. 2010; Speakman and Garratt 2014). Oxidative stress is associated with a number of biological functions, including ornamentation, hibernation, immune function, digestion, locomotion, behavior, growth rate, and reproduction (Alonso-Alvarez et al. 2007; Costantini et al. 2008, 2010; van de Crommenacker et al. 2010; Herborn et al. 2011; Butler et al. 2016). Environmental oxidative stressors, such as heat waves and droughts, are expected to continue to exert strong effects on organisms due to ongoing global climate change (Desalvo et al. 2008; Portner 2010; Seebacher et al. 2010; Munne-Bosch et al. 2013; IPCC 2014; O'Neil et al. 2014; Zinta et al. 2014; Madeira et al. 2016; Ali et al. 2017; but see Enzor and Place 2014; Stahlschmidt et al. 2017). Food availability can influence the magnitude of oxidative stress (e.g., the amount of oxidative damage relative to the amount of antioxidant defenses: Fletcher et al. 2013; Tokolyi et al. 2014; Levin et al. 2017); yet, there is mixed support that the relationship between reproduction and oxidative stress is dependent on food availability (reviewed in Speakman and Garratt 2014).

Furthermore, life-history variation may influence costs associated with food limitation, oxidative challenge, or interactions thereof. For example, each discrete wing morph of *Gryllus* crickets adopts a different strategy to a flight-fecundity tradeoff. During early adulthood, long-winged (LW) females specialize in dispersal (invest in functional flight musculature) at a cost to reproduction, while short-winged (SW) females lack the ability to fly in return for greater reproductive investment (heavier ovaries) (Zera 2005). Investigations using both SW and LW crickets would allow for the examination of the effects of food availability and oxidative challenge in animals with different life-history allocation strategies. Flight muscle is rich in ROS-generating mitochondria; therefore, individuals investing into flight muscle may be more sensitive to (or tolerant of) oxidative challenge than those that do not invest into flight muscle.

We used the wing-dimorphic sand field cricket (*Gryllus firmus*) to examine how food availability influences the costs of oxidative challenge across a range of fitness-related traits. Our experimental design manipulated food availability (unlimited or limited access to food) and oxidative status (repeated injection of the oxidative stressor, paraquat, or a

sham injection) in both SW and LW female *G. firmus* during early adulthood (i.e., a  $2 \times 2 \times 2$  factorial design). We then determined investment into several traits: reproduction (ovary mass), soma (body mass and flight musculature), and immune function (total phenoloxidase activity). For each trait, we explicitly tested whether oxidative challenge (paraquat exposure) had one of the three effects:

1. no detectable cost—non-significant main and interactive effects of oxidative treatment
2. facultative cost—significant interactive effect of food and oxidative treatments, where oxidative challenge has a negative detectable effect only during food limitation
3. obligate cost—significant main effect of oxidative treatment, where a negative detectable effect of oxidative challenge occurs regardless of food availability

By determining the food-dependency of oxidative challenge costs to a range of fitness-related traits, we form a foundation for a further understanding of the physiological dynamics (e.g., food-dependent nodes and links in physiological networks) underpinning the role of oxidative stress in life-history evolution (Dowling and Simmons 2009; Costantini et al. 2010; Speakman and Garratt 2014; Salin et al. 2015).

## Methods

### Study animals

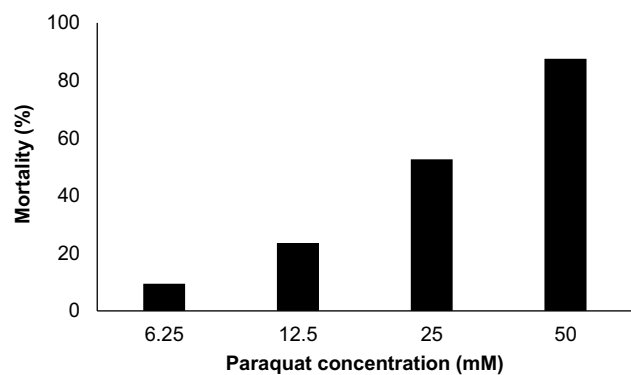
The wing-dimorphic *G. firmus* is endemic to the United States, ranging from Connecticut to Texas (Capinera et al. 2004). Crickets were acquired from three selected blocks that have been previously described (Zera 2005). Until adulthood, each block of crickets was group-reared in 75-l plastic bins with hundreds of hatchlings per bin and later dozens of larger nymphs per bin due to self-thinning. Bins were maintained at  $28 \pm 1$  °C and contained ad libitum access to water and commercial dry cat food until adulthood. At approx. 1 day of adulthood, *G. firmus* females used in the experiment ( $n = 240$ ) were removed from group housing and individually housed in translucent plastic containers (473 ml) with ad libitum access to water in a room maintaining a 16 h photoperiod and  $28 \pm 1$  °C.

### Experimental design

A  $2 \times 2 \times 2$  factorial design was used to manipulate food availability and oxidative status (i.e., oxidative challenge or the investment into antioxidant defenses) in newly emerged SW and LW adults over a 5 day period, as this is the period during which the flight-fecundity tradeoff is

most evident (Zera 2005; Zera and Larsen 2001). Each female's access to food (dry cat food) was manipulated. Half of the females had ad libitum access to food ('high food'). The remaining crickets had intermittent access ('low food'), where cat food was only available for 3 h on the 3rd day of the experiment, which results in *Gryllus* with similar levels of body fat as those found in the field (Adamo et al. 2012; Stahlschmidt et al. 2013, 2015). In addition, each female's oxidative status was manipulated on the 1st and 3rd days of the experiment: a systemic oxidative challenge (injection of an LD<sub>10</sub> dose of paraquat: see below), or no challenge [sham injection, whereby females were pierced in the abdomen with a sterile dissection pin of a similar diameter (0.5 mm) as the needle used to administer paraquat]. Although this 'no challenge' treatment did not control for the injection of saline fluid into the hemocoel, it was a control for wounding and handling stress. The sample sizes for each food-oxidative treatment combination were similar (low food + no challenge:  $n = 70$ ; high food + no challenge:  $n = 62$ ; low food + challenge:  $n = 54$ ; high food + challenge:  $n = 54$ ).

Paraquat (1,10-dimethyl-4,40-bipyridinium dichloride) is an herbicide that generates oxidative stress in animals (Ahmad 1995; Halliwell and Gutteridge 2007; Vecera et al. 2007; Rzezniczak et al. 2011). It facilitates the up-regulation of genes encoding antioxidants in many insect taxa (e.g., Order Diptera: Park et al. 2012; Zemanova et al. 2016; Order Hymenoptera: Choi et al. 2006; Koo et al. 2016; Order Lepidoptera: Kim et al. 2007; Tu et al. 2018), including *Gryllus* crickets (Order Orthoptera: ZRS unpublished). Thus, paraquat has been used to examine factors influencing oxidative stress resistance in several insect taxa, including female *Gryllus* crickets (Stahlschmidt et al. 2015; Adamo et al. 2016). It undergoes an in vivo, NADPH-dependent reduction resulting in a stable radical that generates reactive oxygen species, ROS (Bus and Gibson 1984). The LD<sub>10</sub> dose of paraquat for *G. firmus* of an average mass was estimated at 2  $\mu$ l of 6.25 mM paraquat in insect Ringer's solution (Fig. 1). As in previous work (Stahlschmidt et al. 2015), the same concentration (6.25 mM) was used for all injections, but the volume of injectate for each cricket was based on its body mass immediately prior to injection (BM; g), where volume of injectate ( $\mu$ l) =  $2.35 \cdot \text{BM}^{0.75}$ . The dose of paraquat injections ranged from 0.44 to 1.7  $\mu$ mol, depending on body mass. It is possible that paraquat exposure reduced food intake, which would functionally eliminate the 'high food' + 'challenge' treatment group combination. Although this was not tested for in *G. firmus*, the same protocol was used on females of the same age as those in our study in a related species (*Gryllus lineaticeps*), and results demonstrate that crickets in 'challenge' and 'no challenge' treatments consume food at similar rates (estimated marginal mean  $\pm$  s.e.m:  $15.5 \pm 2.0$  vs.  $17.8 \pm 2.0$  mg/day, respectively;



**Fig. 1** Mortality of *G. firmus* due to paraquat exposure ( $n = 136$ ). At 5 days of adulthood, each cricket was abdominally injected with one of the four concentrations of paraquat in insect Ringer's solution. Sham-injected crickets ( $n = 26$ ) exhibited no mortality. Crickets were maintained at  $28 \pm 1$  °C with ad libitum access to water and commercial dry cat food throughout adulthood—before and after injections. Survival was determined daily, and all mortality occurred 2–3 days after injections

$F_{1,37} = 2.7$ ,  $P = 0.11$ ) after accounting for initial body mass as a covariate ( $F_{1,37} = 32.3$ ,  $P < 0.001$ ).

At the end of the 5 day experiment, crickets were weighed to determine final body mass. Then, from a subset of crickets ( $n = 88$ ), 3  $\mu$ l of hemolymph was removed from the pronotal membrane and mixed with 14  $\mu$ l of distilled water (dH<sub>2</sub>O). After brief vortexing, each hemolymph–dH<sub>2</sub>O sample was stored at  $-80$  °C for assays of total protein and phenoloxidase (PO) activity (see below). For these assays, the sample sizes for each food-oxidative treatment combination were similar (low food + no challenge:  $n = 24$ ; high food + no challenge:  $n = 26$ ; low food + challenge:  $n = 19$ ; high food + challenge:  $n = 19$ ). Next, all crickets were euthanized by freezing at  $-20$  °C, and they were stored at  $-20$  °C until measurement at a later date. Crickets were then dissected, and flight musculature (dorso-longitudinal muscles [DLM]) was scored from 0 (DLM absent) to 1 (white, histolyzed, and non-functional DLM) to 2 (pink and functional DLM) as described in King et al. (2011). Each cricket's femur length was measured (a proxy for body size: Simmons 1986; Nguyen and Stahlschmidt 2019) using digital calipers, and its ovaries were removed to determine dry ovary mass (2 days of drying at 50 °C). Scoring wing musculature of *G. firmus* allowed for an estimate of investment into flight capacity while determining ovary dry mass provided an estimate of investment into reproduction (Roff and Fairbairn 1991; King et al. 2011).

### Total protein and phenoloxidase activity

Immunity in insects strongly relies on melanization, which is facilitated by the activity of phenoloxidase (PO; Siva-Jothy

et al. 2005; Kanost and Gorman 2008; Gonzalez-Santoyo and Cordoba-Aguilar 2012). Thus, in crickets and other insects, PO is typically correlated with or responds similar to treatments as pathogen clearance, disease resistance, and/or other immune parameters, such as encapsulation or lysozyme-like activity (Adamo 2004; Cerenius et al. 2008; Gershman 2008; Gershman et al. 2010; Srygley and Jaronski 2011; Srygley 2012). However, the insect immune system is complex (reviewed in Beckage 2008), and some results indicate an uncoupling of PO from disease resistance (e.g., Adamo 2004; Adamo and Lovett 2011). Therefore, our experimental design examines a single (albeit important) component of immunity (Siva-Jothy et al. 2005; Kanost and Gorman 2008; Gonzalez-Santoyo and Cordoba-Aguilar 2012), and thus, it can be used as a foundation for further study on the effects of food availability and oxidative challenge on immune function. Furthermore, total PO activity can covary with levels of total circulating protein (e.g., Stahlschmidt et al. 2015), and protein levels are also associated with disease resistance in *Gryllus* (Adamo 2004) presumably due to the presence of immune-related proteins other than PO (e.g., lysozymes). Therefore, total PO activity and protein content of hemolymph samples were measured (see below).

Hemolymph-dH<sub>2</sub>O samples were thawed on ice and 34 µl dH<sub>2</sub>O was added to each sample. From this solution, 20 µl was removed for Bradford assays of total circulating protein, and the remaining solution was used for total PO assays (all in triplicate; see below). The 20 µl sample was centrifuged at 10,000g for 5 min. Next, 6 µl of the supernatant was removed and added to 178 µl of Bradford reagent (Sigma-Aldrich) in a 96-well microtiter plate. After a 10 min incubation period at room temperature, the absorbance at 590 nm was measured using a spectrophotometer (BioTek Synergy H1). Absorbance values of samples were compared to those from a standard calibration curve that was calculated using bovine serum albumin (Sigma-Aldrich). Values of total protein content are reported as µg/µl hemolymph.

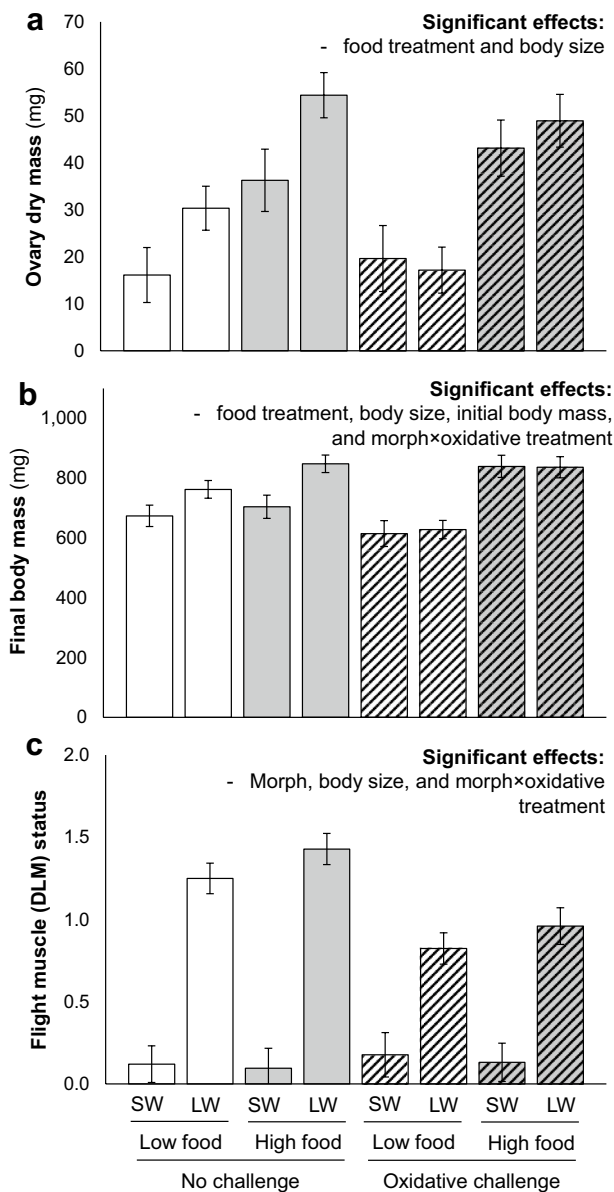
A kinetic spectrophotometric assay was used to measure the total PO activity of hemolymph (sensu Stahlschmidt et al. 2015). To the remaining hemolymph-dH<sub>2</sub>O solution for each sample, 30 µl of a solution containing bovine pancreas α-chymotrypsin (2 mg/ml PBS) was added. This mixture was incubated for 20 min at room temperature before being centrifuged for 5 min at 10,000g. Next, 15 µl of the supernatant was added to 180 µl of a solution containing l-DOPA (0.02 mol/l dH<sub>2</sub>O) in a 96-well microtiter plate. The change in absorbance was measured for 20 min at 490 nm using a spectrophotometer (BioTek Synergy H1). Absorbance values of samples were compared to those from a standard calibration curve that was calculated using tyrosinase (Sigma-Aldrich). Values of absolute total PO activity are reported as µg tyrosinase equivalents/µl hemolymph.

## Statistical analyses

Several linear models were performed in SPSS (v.22 IBM Corp., Armonk, NY), data were log-transformed when necessary, and two-tailed significance was determined at  $\alpha=0.05$ . To determine the effects on dry ovary mass, fixed effects included wing morphology (herein 'morph'), food, and oxidative treatments, and femur length was included as a covariate of body size. To determine the effects on final body mass, fixed effects included morph, food, and oxidative treatments, and initial body mass and femur length were included as covariates. To determine the effects on DLM and survival, ordinal and binary logistic generalized linear models were used, respectively. For these models, fixed effects included morph, food, and oxidative treatments, and femur length was included as a covariate of body size. To determine the effects on PO activity of hemolymph, fixed effects included morph, food, and oxidative treatments. Total protein content was also included as a covariate in the initial PO model. However, protein and PO were not significantly correlated ( $R=0.19$ ,  $P=0.094$ ), and protein content was removed from the final (reported) model, because this more compact model (i.e., the one in which protein levels were excluded) had a lower Akaike information criterion than the initial (full) model. Interactions between and among fixed effects were included for all models.

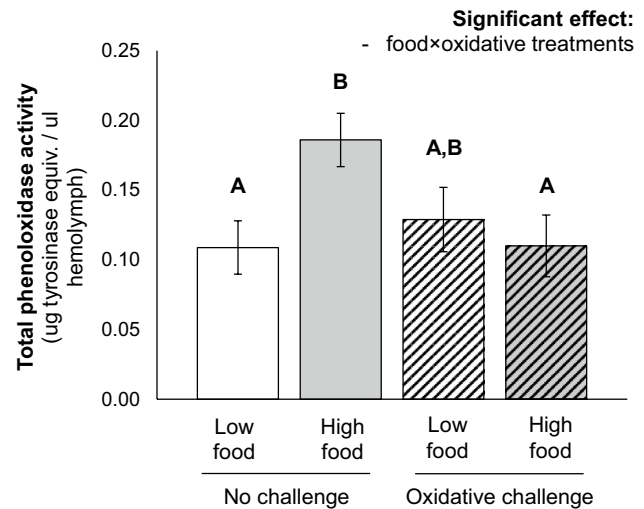
## Results

Overall, ovary mass was greater with higher food availability ( $F_{1,198}=51$ ,  $P<0.001$ ) and larger body size (femur length:  $F_{1,198}=11$ ,  $P=0.0012$ ), but it was not affected by any other fixed effects or interactions thereof (all  $P>0.090$ ) (Fig. 2a). Final (day 6) body mass was greater with higher food availability ( $F_{1,207}=100$ ,  $P<0.001$ ; Fig. 2b), body size (femur length:  $F_{1,207}=59$ ,  $P<0.001$ ), and initial (day 1) body mass (initial body mass positively covaried with final body mass:  $F_{1,207}=118$ ,  $P<0.001$ ). Final body mass was also influenced by an interaction between morph and oxidative status, where body mass in LW females was more sensitive to oxidative challenge ( $F_{1,207}=4.1$ ,  $P=0.044$ ) (Fig. 2b). Dorso-longitudinal (flight) musculature was greater in the LW morph ( $\chi^2=75$ ,  $df=1$ ,  $P<0.001$ ) and in larger females (femur length:  $\chi^2=15$ ,  $df=1$ ,  $P<0.001$ ) (Fig. 2c). There was also an interaction between morph and oxidative status on DLM, where flight musculature in LW females was more sensitive to oxidative challenge ( $\chi^2=4.8$ ,  $df=1$ ,  $P=0.028$ ), but there were not any other fixed effects or interactions thereof (all  $P>0.21$ ) (Fig. 2c). Total PO activity of hemolymph was influenced by an interaction between oxidative



**Fig. 2** Effects of oxidative treatment (sham injection [no challenge] or paraquat injection [challenge]), food treatment (intermittent access [low food] or *ad lib.* access [high food]), and wing morphology (short-winged [SW] or long-winged [LW]) on **a** ovary dry mass, **b** final body mass, and **c** flight muscle status (scored from 0 to 2; see text for details) in *G. firmus*. Values are displayed as mean  $\pm$  SEM

status and food availability ( $F_{1,72} = 5.3$ ,  $P = 0.025$ ), where the magnitude of PO was higher in high food, non-challenged females relative to those in high food, challenged and low food, non-challenged environments (Fig. 3). Yet, PO was not affected by any other fixed effects or interactions thereof (all  $P > 0.087$ ) (Fig. 3). Survival rate was high ( $> 90\%$ ) and unaffected by any covariates or fixed factors (or interactions thereof) (all  $P > 0.9$ ).



**Fig. 3** Effects of oxidative treatment (sham injection [no challenge] or paraquat injection [challenge]) and food treatment (intermittent access [low food] or *ad lib.* access [high food]) on total phenoloxidase activity of hemolymph in *G. firmus*. For clarity, wing morphology (short- or long-winged morph) is not shown, because it did not have a significant effect (see text and Table 1 for details). Pairwise comparisons using sequential Bonferroni corrections were used to determine differences across treatment combinations as denoted by letters. Values are displayed as mean  $\pm$  SEM

## Discussion

Empirical and meta-analytical evidence tends to indicate that key aspects of life history are linked to oxidative stress—yet, results are typically mixed, and the precise physiological mechanisms underlying links are contested (Dowling and Simmons 2009; Costantini et al. 2014; Speakman and Garratt 2014; Salin et al. 2015; Speakman et al. 2015; Blount et al. 2016). Equivocal results may stem from confounding aspects of experimental design, including uncontrolled (or unaccounted for) variables that obscure biologically relevant phenomena (Speakman and Garratt 2014). Thus, we focused on the role played by food availability, an important and widespread environmental factor, in oxidative challenge-related costs spanning life history and physiology. Our results indicate that the costs of paraquat exposure are trait-specific, ranging from no cost to an obligate (food-independent) cost (Figs. 1, 2; Table 1). Although we did not detect facultative (food-dependent) costs of oxidative challenge, the nutrient composition of food (e.g., Cotter et al. 2010) or the duration of food limitation (e.g., prolonged starvation rather than intermittent food availability) may play important roles in mediating stress-related tradeoffs. Clearly, the dynamics of oxidative challenge-related costs are complex and warrant further investigation into the “devil in the details” regarding underlying proximate mechanisms (*sensu* Harshman and Zera 2007).

**Table 1** Summary of results from linear mixed models ( $F$  or  $\chi^2$  values) for fixed effects (wing morphology, food treatment, oxidative treatment, and interactions thereof) on ovary mass, body mass, flight muscle (dorso-longitudinal muscle, DLM) status, total phenoloxidase (PO) activity, and total protein content of hemolymph

	Ovary mass	Final body mass	Flight muscle (DLM) status	Total PO activity	Total protein content
Morph	3.1	0.85	75**	0.23	0.24
Food treatment	51**	100**	0.021	1.9	6.2*
Oxidative treatment	0.59	0.74	1.6	1.8	0.046
Morph $\times$ food	0.45	1.5	1	1.4	1.9
Morph $\times$ oxidative	0.34	4.1*	4.8*	1.4	1.2
Food $\times$ oxidative	0.25	1.9	0.52	5.3*	< 0.001
Morph $\times$ food $\times$ oxidative	0.15	0.20	< 0.001	3.0	0.10

$F$  values (or  $\chi^2$  values: DLM) are displayed for each model. \* $P$  < 0.05, \*\* $P$  < 0.001, and values without asterisks were not significant. See text for full test results, including covariates

We detected obligate costs of oxidative challenge to investment into flight capacity (i.e., > 30% reduction in DLM status) and other somatic tissue in *G. firmus*. Only individuals investing heavily into flight musculature (i.e., LW crickets) were negatively affected by the oxidative challenge, and these costs to DLM and body mass occurred regardless of food availability (Fig. 2b, c). Intense exercise, such as flight, typically leads to oxidative stress across animal taxa (Powers and Jackson 2008; Fisher-Wellman and Bloomer 2009), even though antioxidants, such as glutathione (GSH), may be higher during flight (Magwere et al. 2006; Costantini et al. 2008; Jones et al. 2015; reviewed in Cooper-Mullin and McWilliams 2016). Glutathione is a key component of many animals' antioxidant defenses (Pompella et al. 2003). Although it was not measured in our study, the gene for glutathione-s-transferase (enzyme that facilitates the ability for GSH to bind to substrates) is expressed at a much higher rate in functional flight muscle relative to non-functional flight muscle in female *G. firmus* from the same selected blocks as our study (Vellichirammal et al. 2014). In addition to the oxidative stress-related costs of flight itself (Magwere et al. 2006; Costantini et al. 2008; Jones et al. 2015; reviewed in Cooper-Mullin and McWilliams 2016), aspects of flight metabolism may contribute to oxidative damage. In *G. firmus*, LW females accumulate higher levels of triglycerides (flight fuel) in their hemolymph than SW females (Zera 2005), and circulating triglycerides are associated with oxidative DNA damage in other taxa (Bukhari et al. 2015; Lohr et al. 2015). Thus, the growth and maintenance of flight musculature may generate significant amounts of ROS that require neutralization via up-regulated antioxidant defenses. Additional oxidative challenge (e.g., paraquat exposure in our study) may (1) directly reduce investment into tissue/physiological systems that generate high oxidative loads (e.g., flight muscle or immune resistance) and/or (2) lead to increased antioxidant defenses or oxidative stress repair mechanisms that allocate resources from some tissue/physiological systems (e.g., flight muscle or immune resistance). In sum, antioxidant defenses in flight-capable animals may

be constitutively high (i.e., they have a small physiological safety margin related to oxidative challenge), which may make these animals particularly susceptible to environmental oxidative stressors associated with ongoing global climate change (Desalvo et al. 2008; Portner 2010; Seebacher et al. 2010; Munne-Bosch et al. 2013; IPCC 2014; O'Neil et al. 2014; Zinta et al. 2014; Madeira et al. 2016; Ali et al. 2017; but see Enzor and Place 2014; Stahlschmidt et al. 2017).

Although there was an interactive effect of oxidative challenge and food treatments on total PO (Fig. 3), this interaction was more indicative of an obligate (rather than a facultative) cost of oxidative challenge to a parameter of immune function. That is, food limitation and oxidative challenge exhibited non-additive costs to PO. The magnitude of total PO activity was highest in females that had *ad lib.* access to food and were not exposed to oxidative challenge. Because neither food treatment nor oxidative treatment independently influenced total PO, the relative effects of each factor (i.e., food and oxidative treatment) are difficult to partition. However, one interpretation of this food  $\times$  oxidative interactive effect is that the benefit of high-food availability to immune function may have been eliminated when females were exposed to paraquat (Fig. 3). Although investigations into other tests of immunocompetence are warranted (e.g., disease resistance to a live pathogen), this suggests that a large investment into at least one metric of immune function only occurs if conditions are favorable. Investment into immune function entails energetic costs across taxa (reviewed in Lochmiller and Deerenberg 2000; Martin et al. 2008; Schwenke et al. 2016), and increased food abundance can eliminate potential immune-related costs to reproduction, a key life-history trait (Shoemaker et al. 2006; French et al. 2007; Xu et al. 2012; but see Stahlschmidt et al. 2013). Unlike previous work in *Gryllus* crickets (e.g., Stahlschmidt et al. 2015), we did not detect a correlation between protein and PO levels in hemolymph. Therefore, future work is required to better characterize the effects of oxidative challenge and food availability on non-PO proteins associated with immunocompetence (e.g., lysozymes), and whether



they protected individuals from other potential costs (e.g., oxidative challenge treatment had no effect on reproductive investment: Fig. 2a and discussed below). We also advocate for investigation into other drivers of immunological plasticity in response to oxidative challenge and food limitation, such as development (e.g., developmental plasticity: Muriillo-Rincon et al. 2017) and maternal effects (e.g., transgenerational priming: reviewed in Roth et al. 2018).

Food availability influenced ovary dry mass, but we detected no costs of oxidative challenge to investment into reproductive tissue (Fig. 2a) in support of our first (null) hypothesis. Much of the work on oxidative stress as a mediator of life histories has focused on its relationship to reproduction. The ‘oxidative stress life-history theory’ posits that reproduction obligates an increased production of ROS and that resource limitation prevents individuals from sufficient allocation toward protective mechanisms needed to offset increased ROS, which leads to oxidative stress during reproduction (Dowling and Simmons 2009; Costantini et al. 2010; Speakman and Garratt 2014). Our design examined whether an oxidative stressor entailed reproductive costs during this (potentially) physiologically vulnerable life-history stage. There are at least two explanations for our results that investment into reproduction (ovary mass) was not affected by oxidative challenge, while other traits were affected (Figs. 1, 2). First, reproductive tissue may be naturally buffered from oxidative challenge. However, previous work in insects indicates that chronic immune activation (which also induces oxidative damage: Sadd and Siva-Jothy 2006) via injection of an immunogen can influence reproductive output (Stahlschmidt et al. 2013; but see Shoemaker et al. 2006). Second, the maintenance of reproductive investment may be prioritized over soma and self-maintenance. Under stressful conditions, allocation to storage or maintenance can take precedence over allocation to reproduction in mammals, putatively to allow for reproduction in the future (Rogowitz 1996). Yet, reproduction is time-constrained in many insects, thereby incentivizing investment into early reproduction. For example, a *G. firmus* female in the field likely reproduces for only 1–2 weeks during her lifetime (Zera et al. 2007), and lab studies on *G. firmus* indicate that ovary mass increases tenfold during the time frame of our study (i.e., 5–7 days into adulthood: Mole and Zera 1994). We encourage continued comparative work on the manner in which oxidative stress affects key tissue types and drives allocation decisions.

Alternative to the explanations above, it is possible that paraquat exposure did not affect ovary mass in our study, because it did not induce oxidative challenge. For example, the half-lives of ROS (i.e., superoxide, hydrogen peroxide, and hydroxyl radicals) are short (< 1 s: reviewed in Forkink et al. 2010), meaning that paraquat exposure or paraquat-induced damage may have been minimal. However, our study included two exposures (injections of paraquat) for

each female in the paraquat treatment group. Furthermore, work in *G. firmus* and another *Gryllus* species demonstrates that at higher concentrations, a single paraquat exposure has longer lasting effects, because paraquat-induced mortality typically occurs 2–3 day post-exposure (Stahlschmidt et al. 2015; Fig. 1). However, the level of oxidative stress (e.g., lipid peroxidation levels: Guillet et al. 2000) induced by paraquat in our study was not measured, and the concentration of the paraquat dose used in our study may have been too low to initiate an oxidative stress response. For example, oxidative stress associated with stronger doses of paraquat reduces food intake in other insects (Ja et al. 2007), but the dosage of paraquat in our study likely did not affect food intake. That said, the dose of paraquat used in our study was an oxidative challenge as demonstrated by its costs to (i.e., independent or interactive effects on) all of the other traits measured. In addition to reducing final body mass (Fig. 2b), paraquat exposure had a greater effect on DLM in *G. firmus* that invest in DLM (i.e., LW individuals) than manipulations of food and water availability during adulthood (our study: Fig. 2c; ZRS unpublished), as well as manipulations of variation in food availability and temperature during development (Glass and Stahlschmidt 2019). The dose of paraquat used in our study also interacted with food availability to influence a component of self-maintenance (PO) in both wing morphs (Fig. 3). Nonetheless, validation of paraquat’s dose-dependent effect on oxidative stress levels is important (Koch and Hill 2017), and we also encourage the examination of other oxidative stressors (e.g., exposure to heat or heavy metals).

Dispersal is key to many life histories, because it allows individuals to move to higher quality environments (Roff 1994), but it can also come at a cost to other life-history traits (reviewed in Zera and Harshman 2001; Guerra 2011). Dispersal polymorphisms mediate these costs and benefits, and such polymorphisms are common among insects (reviewed in Zera and Denno 1997). Further work is required to understand how dispersal polymorphisms (e.g., wing dimorphisms) drive variation in circulating antioxidant defenses, and how these dynamics underlie tradeoffs between and among flight capacity, reproduction, and immune function. For example, GSH may mitigate flight-induced ROS in insects, and it neutralizes immune-induced ROS—yet, high levels of GSH prevent PO-mediated melanization, a key component of insect immunity (Clark et al. 2010; Gonzalez-Santoyo and Cordoba-Aguilar 2012; Vellichirammal et al. 2014). Therefore, a large investment into PO may obligate a concomitant large investment into GSH (Stahlschmidt et al. 2015). However, it is unclear how oxidative challenge, food availability, and oxidative challenge × food interactions influence this balancing act between GSH (not measured in our study) and PO in the context of dispersal and dispersal-related tradeoffs. Therefore, our study is an

important step toward a more complete understanding of the roles that resource (food) availability and redox systems play in mediating life histories.

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