



## SYMPOSIUM

### Ecological Immunology Mediated by Diet in Herbivorous Insects

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From the symposium “The Micro and Macro of Nutrient Effects in Animal Physiology and Ecology” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2014 at Austin, Texas.

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**Synopsis** A rapidly advancing area of ecological immunology concerns the effects of diet on animals' immunological responses to parasites and pathogens. Here, we focus on diet-mediated ecological immunology in herbivorous insects, in part because these organisms commonly experience nutritional limitations from their diets of plants. Nutritional immunology highlights nutrient-based trade-offs between immunological and other physiological processes as well as trade-offs among distinct immunological processes. This field reveals that nutrition influences the quality and quantity of immunological defense in herbivorous insects, and conversely that nutritional intake by herbivorous insects can be an adaptive response to the specific types of immune-challenge they face in the context of other physiological processes. Because the diets of herbivores challenge them physiologically with plants' secondary metabolites, another area of study analyzes constraints on immunological defense imposed by secondary metabolites of plants in the diets of herbivorous insects. Alternatively, some herbivores can use secondary metabolites as medicine against parasites or pathogens. Animal-medication theory makes an important contribution to ecological immunology by distinguishing prophylactic and therapeutic mechanisms of anti-parasite defense. Integrating ideas from animal-medication and nutritional immunology, we outline a conceptual framework in which the immunological role of the diet consists of mechanisms of prophylaxis, therapy, compensation, and combinations thereof. Then, we use this framework to organize findings from our own research on diet-mediated ecological immunology of woolly bear caterpillars. We show evidence that the woolly bear caterpillar, *Grammia incorrupta* (Hy. Edwards) (Lepidoptera, Erebidae, and Arctiinae), can employ both diet-mediated prophylaxis and therapy. First, increased consumption of carbohydrate-biased food prior to immune-challenge increased its melanization-response. Second, increased consumption of pyrrolizidine alkaloids (PAs) more than 24 h after parasitism by tachinid flies resulted in anti-parasite resistance. Caterpillars reduced feeding on protein-biased food within 24 h after immune-challenge, showing evidence of illness-induced anorexia. We synthesize our work to generate the hypothesis that a diet-mediated defense by the host against parasites acts as a temporally explicit, multi-stage process.

Ecological immunology applies evolutionary ecological theory to the analysis of immunological defense of organisms against their parasites and pathogens (e.g., Sheldon and Verhulst 1996; Rolff and Siva-Jothy 2003; Schmid-Hempel 2003; Wilson 2005). Traditionally, immunology has been studied almost exclusively in the domains of physiology, biochemistry, and molecular biology (Rolff and Siva-Jothy 2003), approaches that dominate the massive enterprise of biomedical research. Therefore, a great opportunity exists to leverage evolutionary ecological theory to gain new insight in a field that is already

richly informed by mechanistic study, albeit with a mammalian focus. However, the complexity of immunological components and pathways (e.g., Ponton et al. 2011, 2013) presents a practical challenge to the proper testing of theory (Schmid-Hempel 2003) and even to deciding what immunological parameters should be measured (Adamo 2004). In this article, we grapple with these opportunities and challenges in reference to ecological immunology as mediated by diet in herbivorous insects. We review how evolutionary ecological theory has been applied to the immunological role of diet in herbivorous insects.

Rather than being comprehensive in its coverage, our review focuses on key ideas and case studies, culminating in a review of our own work on the ecological immunology of woolly bear caterpillars. Based on our review, we develop a general conceptual framework for the immunological role of diet in herbivorous insects that integrates ideas from the related fields of nutritional immunology and animal-medication. In our study system, we use this framework to understand diet-mediated defense by hosts against parasites as a temporally explicit, multi-stage process.

### Review of the literature

A cornerstone concept of evolutionary ecology and ecological immunology is the resource-mediated trade-off among different vital functions (Stearns 1992; Moret and Schmid-Hempel 2000). Due to biophysical, genetic, and ecological constraints, organisms possess a finite pool of resources that must be allocated toward several distinct functions, such as maintenance, growth, reproduction, and defense. Although finite, this pool of resources is dynamic; an organism can alter its quantity, quality, and pattern of allocation, often in an adaptive manner. Feeding behavior and diet (resource acquisition) strongly determine the quantity and quality of the resource pool, with important consequences for the pattern of allocation (Cotter et al. 2011; Diamond and Kingsolver 2011). In theory, adaptive feeding behavior can ameliorate trade-offs in allocation through changes in dietary quantity or quality. For the immature stages of herbivorous insects, there has been a great emphasis on the trade-off between growth and immunological defense because of the physiological primacy of growth (Scriber and Slansky 1981; Simpson and Raubenheimer 2012) as well as the high risk of mortality due to parasitism and disease (Gross 1993; Godfray 1994; Cornell and Hawkins 1995) during this part of the life cycle.

Dietary nutrients are hypothesized to be central to this trade-off, a key concept in the field of nutritional immunology (Ponton et al. 2011, 2013). Many studies have specifically identified protein and amino acids as mediators of the trade-off between growth and immunological defense because they commonly act as growth-limiting nutrients for herbivorous insects (Scriber and Slansky 1981; Bernays and Chapman 1994; Schoonhoven et al. 2005). In addition, parasites and pathogens can induce immune-responses in insects that entail increases in hemocyte number, lysozyme-like activity, phenoloxidase (PO) activity, and the magnitude of encapsulation and melanization (Beckage 2008;

Carton et al. 2008; Strand 2008), all of which require substantial amino-acid investments for their structure and function. Some studies provide direct evidence that dietary protein limits immunological activity (Lee et al. 2006; Povey et al. 2009; Raubenheimer and Simpson 2009). However, the complexity of nutrient-mediated trade-offs is clearly exemplified by a comprehensive analysis of nutritional immunology of the caterpillar *Spodoptera littoralis* (Cotter et al. 2011). These researchers measured hemolymph protein, larval growth, and several immunological parameters across a range of diets that varied factorially in caloric density and nutritional composition (protein-to-carbohydrate ratio). Trade-offs between optimal growth and immunological responses were mediated by nutritional composition rather than by caloric density in the diet of immune-challenged caterpillars. Interestingly, the dietary nutritional composition that optimized the immunological response varied among different immunological parameters (especially PO activity versus lysozyme-like activity), providing a nutrient-mediated explanation for trade-offs between PO and lysozyme activity seen in other studies (Moret and Schmid-Hempel 2001; Cotter et al. 2004).

Immunological processes also can compete for the same physiological resources as processes such as digestion or excretion, thus causing other trade-offs (Smith and Holt 1996). Contrary to the notion that increased dietary intake should ameliorate allocation trade-offs, one putatively adaptive feeding-response to such trade-offs is decreased feeding during an infection. So-called illness-induced anorexia has been documented in many animal species, including herbivorous insects (Adamo 2006). Among various proposed hypotheses for illness-induced anorexia, the best supported is that it reduces physiological trade-offs between digestion and immunological function (Adamo et al. 2010). Immune-challenged, omnivorous crickets (*Gryllus texensis*) reduced their intake of high-lipid food, which they readily eat in the absence of immune-challenge. Furthermore, a high-lipid diet increased mortality of crickets experimentally infected with a bacterial pathogen (*Serratia marcescens*), but had no such effect on control crickets. These findings align with previous work showing that high levels of lipid in the cricket's hemolymph caused a decrease in the concentration of a lipid-transport protein (monomeric apolipoprotein III), and, in turn, reduced resistance to a bacterial pathogen (Adamo et al. 2008). Therefore, monomeric apolipoprotein III appears to mediate a physiological trade-off between lipid-digestion and immunological function, and

illness-induced anorexia toward high-lipid food ameliorates it. Evidence from the herbivorous caterpillar, *Manduca sexta*, also shows that dietary lipids reduce resistance against pathogens (Adamo et al. 2007).

The diets of herbivores challenge them physiologically, not only because of nutrient limitation, but also because of secondary metabolites of plants (Bernays and Chapman 1994; Schoonhoven et al. 2005; Forbey et al. 2009). Herbivorous insects, such as caterpillars, cope with these potential toxins through a variety of physiological processes, including excretion, maintenance of high pH in the gut, enzymatic detoxification, and sequestration (Nishida 2002; Desprès et al. 2007; Chapman et al. 2013). As these various physiological processes act against intoxication, they have the potential to compete for the same physiological resources as immunological processes, thus creating a resource-mediated trade-off (Schmid-Hempel 2003). Ingested secondary metabolites of plants also can compromise immunological function more directly through cytotoxicity and metabolic interference. Smilanich et al. (2009) showed that caterpillars of the buckeye butterfly (*Junonia coenia*) produce a weaker melanization-response to experimentally injected Sephadex beads when given a diet with high concentrations of iridoid glycosides (IGs). Path-analysis best supported a model in which dietary IG acts directly on melanization (Smilanich et al. 2009). This example is especially striking because buckeye caterpillars feed specifically on host-plants containing IG, and sequester IG for anti-predator defense (e.g., Dyer and Bowers 1996). Therefore, even herbivorous insects with physiological specialization toward specific plant toxins can be susceptible to their antagonistic effects on immunological function. As most herbivorous insects have specialized diets (Schoonhoven et al. 2005), such antagonism has the potential to be widespread. An interesting question for future research is whether the antagonism between ingested toxins and immunological function is more severe for host-specific than for grazing herbivores. The constraints in diet and physiology of host-specific herbivorous insects might limit their ability to ameliorate negative effects of toxins through behavioral plasticity in their choice of diet.

Medication-behavior by animals is one means by which behavioral plasticity in choice of diet can circumvent antagonisms and trade-offs. Whereas healthy animals might choose a diet that is optimal for growth and reproduction, animals infected with parasites or pathogens might choose an alternative diet that maximizes resistance or tolerance to parasites at the expense of optimal growth. Like the rest

of ecological immunology, the small, but growing, body of contemporary work on medication by animals analyzes the behavior of acquiring substances that prevent and treat illness from an evolutionary ecological perspective (Clayton and Wolfe 1993; Moore 2002). de Roode et al. (2013) argued that anti-parasite behavior, such as self-medication, should be regarded as a component of an animal's immune-system. Several other authors have reached similar conclusions through consideration of the preferential feeding by animals on pharmacologically active substances (pharmacophagy; Boppré 1984), hence the name "PharmEcology" for this branch of ecological immunology (Forbey et al. 2009; Raubenheimer and Simpson 2009).

### Integrating nutritional immunology and medication-behavior

Aside from expanding the behavioral dimension of ecological immunology, the field of animal-medication contributes a unique perspective. It importantly differentiates between prophylactic and therapeutic mechanisms of anti-parasite defense (e.g., Lozano 1998; Castella et al. 2008; Singer et al. 2009; de Roode et al. 2013) (Table 1). Prophylaxis refers to hosts' defenses that are in place prior to attack by the parasite. For example, workers of European wood ants (*Formica paralugubris*) routinely collect pine resin, which provides anti-pathogen resistance for the colony (Christe et al. 2003; Chapuisat et al. 2007; Castella et al. 2008). Based on evolutionary ecological theory of inducible defense (reviewed by Tollrian and Harvell [1999]), we propose that prophylaxis is expected when a defensive trait acts: (1) in response to an ever-present threat (constitutive prophylaxis) or (2) in response to a cue that reliably predicts an attack that can only be countered by deployment of a defensive trait prior to the onset of infection (induced prophylaxis). By contrast, therapeutic medication is an induced, adaptive behavioral response to infection, such that the host increases its likelihood of resistance against, or tolerance to, parasitism (Lozano 1998). From the perspective of evolutionary ecology, therapeutic medication is adaptive plasticity; it is expected when a defensive trait acts in response to a detectable threat and entails a fitness cost when not needed (Singer et al. 2009). The critical difference between prophylactic and therapeutic medication is not whether it is constitutive or induced, but rather, whether it occurs before or after the onset of infection. Induced prophylaxis is expected when environmental cues can accurately predict the risk of

**Table 1** Types of medication predicted by evolutionary ecological theory with regard to whether medicative behavior is plastic and when it occurs in relation to the onset of infection

Adaptive plasticity?	Timing with regard to onset of infection	
	Before	After
No.	Constitutive prophylaxis	In contrast to prophylaxis, therapy is induced, by definition
Behavior is constitutive: no fitness trade-offs exist between anti-parasite defense and other physiological functions	The likelihood of enemy attack is constantly high with no reliable, detectable cues that predict an attack	
Yes.	Induced prophylaxis	Therapeutic medication
Behavior is induced: fitness trade-offs exist between anti-parasite defense and other physiological functions	Reliable, detectable cues indicate the likelihood of attack; fitness benefit depends on medication prior to infection	Reliable, detectable cues indicate parasite infection and fitness benefit can occur from medication after the onset of infection

**Table 2** Conceptual framework for the immunological role of diet in herbivorous insects

Dietary parameter	Prophylaxis	Therapy	Compensation
Quantity of food	Intake of food providing nutritional status that circumvents trade-offs in allocation	Increased or decreased intake of food to aid the immune-response	Increased intake of food to restore homeostasis following therapy
Nutritive quality or quantity of food	Regulation of nutrient intake providing nutritional status that circumvents allocation trade-offs	Selective intake of nutrients that aid the immune-response	Selective intake of nutrients to offset depletion or bias from therapy
Medicinal quality or quantity of food	Intake of anti-parasite medicine prior to parasite attack	Intake of anti-parasite medicine after attack by parasites	Intake of medicine to heal damage from therapy

infection (Table 1). For example, according to the density-dependent prophylaxis hypothesis (Wilson and Reeson 1998), the risk of disease for an insect can be predicted by the density of conspecifics in a shared environment. Therefore, prophylaxis against parasites and pathogens will be induced at high densities of the host. In keeping with predictions, resistance to disease has been shown to increase in response to density in a number of herbivorous insects, including beetles (Barnes and Siva-Jothy 2000), locusts (Wilson et al. 2002), and caterpillars (Cotter et al. 2004). Although these effects are not mediated by changes in feeding behavior, they illustrate that the immune-system can be primed by environmental cues that anticipate infection.

Integrating ideas from animal-medication and nutritional immunology, we propose that the immunological role of the diet in herbivorous insects consists of mechanisms of prophylaxis, therapy, compensation, and combinations thereof. Hypothetically, each of these mechanisms can be achieved via alterations in the quantity of food ingested, the quality or quantity of the intake of specific nutrients, or the quality or quantity of the intake of specific medicines (Table 2). According to this framework, one mechanism of prophylaxis occurs when the amount of food

consumed prior to attack by parasites or pathogens changes the condition of the host in ways that circumvent or reduce trade-offs in allocation involving the immunological response. Following attack, increased or decreased (Adamo et al. 2010) consumption of food might act therapeutically to aid the immunological response. Following a therapeutic response, compensation refers to increased intake of food to restore physiological homeostasis that was disrupted by actions of the parasite, pathogen, or host. With regard to specific nutrients, prophylaxis would entail regulation of the intake of nutrients prior to attack by parasites, thereby causing a nutritional status that circumvents or reduces trade-offs in allocation (e.g., Fellous and Lazzaro 2010). Nutrient-mediated therapy refers to increases or decreases in the intake of specific nutrients that aid the immune-response after attack by parasites or pathogens (e.g., Lee et al. 2006; Povey et al. 2009). In this case, compensation is regulation of the intake of specific nutrients after therapy to restore nutritional status that was disrupted by actions of the parasite, pathogen, or host (e.g., Lee et al. 2006; Ponton et al. 2011). Finally, medicinal prophylaxis and therapy, respectively, refer to the intake of specific medicines before and after attack by parasites, as



described previously. Recent work also has identified several cases of transgenerational medication, in which an infected mother chooses a diet for her offspring that specifically increases anti-parasite pharmacological defense (e.g., Lefèvre et al. 2010; Kacsoh et al. 2013). Medicinal compensation could be used after therapy to heal damage from actions by parasites, pathogens, or hosts (e.g., Cornet et al. 2007; Babin et al. 2010). Some of these hypothetical mechanisms have empirical support from herbivorous insects, whereas others remain untested.

#### Case study: *Grammia incorrupta* caterpillars

We use the framework described in Table 2 to organize our current understanding of the immunological role of diet in grazing by caterpillars of the tiger moth, *G. incorrupta* (Hy. Edwards) (formerly *G. genitura* [Strecker]) (Lepidoptera, Erebidae, and Arctiinae), which inhabits grassland and savannah in southwestern United States (Singer 2008; Schmidt 2009). The life cycle of *G. incorrupta* lends itself to the study of herbivores' foraging behavior because selection of a host-plant occurs in the larval stage, rather than in the adult stage (Singer 2008). The late-instar caterpillars, the focus of our work, are individual grazers, locomoting, feeding, and switching among individual host-plants of many species over the course of minutes or hours (Singer et al. 2002), and show a feeding preference for forbs (Singer and Stireman 2001). Late-instar caterpillars also experience a variable risk of mortality from insect parasitoids (0–80%), with a median frequency of 8.5% (Singer and Stireman 2003). Of the 13 species of primary endoparasitoids recorded from *G. incorrupta*, two species of tachinid fly (*Carcelia reclinata* and *Exorista mella*) and one undescribed species of braconid wasp (*Cotesia* nr. *phobetrix*) comprise the majority of attacks (Stireman and Singer 2002). Each of these parasitoid species lays one or more eggs on, or in, host caterpillars, the larval parasitoids feed and develop inside the caterpillar, then emerge from, and kill, the host. Because previous studies showed that the diet of late-instar *G. incorrupta* caterpillars could modify anti-parasitoid resistance (Singer and Stireman 2003; Singer et al. 2004), our subsequent research sought to understand which dietary components contribute to anti-parasitoid resistance and how resistance operates.

The first portion of this research targeted the possible role of dietary PAs in therapeutic medication. Several physiological experiments demonstrated that the gustatory system of *G. incorrupta* caterpillars is specialized for PA consumption (Bernays et al. 2002),

and that these caterpillars sequester PA acquired from certain species of host-plant (Hartmann et al. 2004, 2005). Like other arctiines, *G. incorrupta* possesses a flavin-dependent monooxygenase enzyme that maintains sequestered PA in a non-toxic N-oxide form in the insect's hemolymph (Sehlmeyer et al. 2010), thereby avoiding autotoxicity. Complementary evidence from an ecological experiment suggested that dietary PA mediates anti-parasitoid resistance in *G. incorrupta* (Singer et al. 2004). The first evidence of therapeutic medication with dietary PA was seen in a comparison of the gustatory responses of parasitized versus nonparasitized caterpillars (Bernays and Singer 2005). Parasitized caterpillars exhibited a stronger gustatory response to PA than did nonparasitized caterpillars, but the status of parasitism did not change the gustatory response to sucrose, another stimulant for feeding. Follow-up experiments measuring caterpillars' feeding behavior confirmed this suggestive evidence (Singer et al. 2009; Smilanich et al. 2011). Singer et al. (2009) showed that parasitism by tachinids increased caterpillars' intake of PA-treated filter paper but not sucrose-treated filter paper, demonstrating that the increased feeding-response was specific to the putative medicine. This same study showed the therapeutic function of this medicine; dietary PA increased the survival of caterpillars parasitized by the tachinid fly, *E. mella*. However, dietary PA reduced the survival of nonparasitized caterpillars, indicating the cost of medication without a therapeutic need. Smilanich et al. (2011) examined the PA-medication response of *G. incorrupta* in early versus late temporal phases of parasitism by tachinids. PA-medication proved to be a delayed response to infection by tachinids, only becoming manifest approximately 48 h after infection. The latter finding suggests that PA-medication functions as a line of anti-parasitoid defense that is secondary to the physiological immunological response, which occurs within minutes or hours of infection (Beckage 2008).

The second portion of our ecological immunological research on *G. incorrupta* focused on the role of diet in the early phase of parasitism, concurrent with immunological recognition, encapsulation, and, ultimately, melanization of a foreign body. In part, this project tested whether certain dietary components might increase or perhaps decrease the melanization-response of *G. incorrupta*. In a series of experiments, we used injections of Sephadex beads as an artificial, standardized immune-challenge (Lavine and Beckage 1996). We first tested the feeding-response to PA in immune-challenged caterpillars

as well as the effect of dietary PA on the melanization-response. PA consumption by bead-injected caterpillars was reduced in the 24-h period after injection relative to PA consumption by sham-injected and non-injected controls (Smilanich et al. 2011). However, dietary PA had no measurable effect on the melanization-response of the immune-system (Smilanich et al. 2011), reinforcing our hypothesis that PA-medication is a secondary line of defense rather than a primary mechanism of anti-parasitoid defense that competes or interferes with the melanization-response.

Evidence from other systems prompted an examination of the role of dietary macronutrients in the immune-response of *G. incorrupta*. We reasoned that the findings by Lee et al. (2006), in which virus-challenged *S. littoralis* caterpillars showed protein-biased intake of macronutrients that increased several parameters of the immunological response and anti-virus resistance, forecasted a similar response by immune-challenged *G. incorrupta* caterpillars. Therefore, we compared the feeding-response with nutritionally defined foods of immune-challenged and control caterpillars, and we tested the effect of macronutrient content of the diet on the melanization-response. Prior to these experiments, we identified the feeding-intake-target of the dietary ratio of protein (P) to carbohydrate (C) in final instars of *G. incorrupta* caterpillars (Mason et al. 2014). According to the geometric framework of animal nutrition (Simpson and Raubenheimer 2012), the feeding-intake-target indicates the optimal ratio of dietary components (e.g., protein and carbohydrate) for animals' growth and development. Although *G. incorrupta* attained a maximum adult body mass over a range of protein-to-carbohydrate ratios (15%P:25%C, 20%P:20%C, 25%P:15%C, percentage of total diet by dry mass) in the larval diet, final instars self-selected a ratio near 25%P:15%C, which we considered the intake-target or optimal diet. In a no-choice assay of feeding, bead-injected *G. incorrupta* caterpillars showed reduced feeding-responses to the optimal diet (protein-biased) in the 24-h period after injection relative to sham-injected and non-injected controls (Mason et al. 2014). Interestingly, the immune-challenge treatment did not change the feeding-response to a carbohydrate-biased diet (15%P:25%C) (Mason et al. 2014), suggesting that illness-induced anorexia occurred only in response to protein-biased food. A feeding-choice assay reinforced this finding. Bead-injected caterpillars self-selected reduced food overall compared with control caterpillars in the 24-h period after injection, and the diet of bead-injected caterpillars shifted

disproportionately away from protein-biased food (Mason et al. 2014).

Like their feeding-responses, the immunological response of *G. incorrupta* differed from those of immune-challenged *S. littoralis* (Lee et al. 2006). The ratio of protein to carbohydrate in the diet of *G. incorrupta* had no effect on the melanization-response of the immune-system both in no-choice and feeding-choice experiments (Mason et al. 2014). Instead, the quantity of carbohydrate-biased food ingested by *G. incorrupta* caterpillars provided the only evidence of an immunological role of diet. In the no-choice experiment, the amount of carbohydrate-biased food consumed prior to immune-challenge was positively associated with the melanization-response (Mason et al. 2014). This result suggests that dietary carbohydrates might play a prophylactic role in immunological melanization. In the feeding-choice experiment, the amount of food consumed in the 24-h period after immune-challenge was positively associated with the melanization-response (Mason et al. 2014). This might indicate a therapeutic or compensatory role of total food-intake, although the caterpillars in this experiment showed evidence of illness-induced anorexia at the same time. Therefore, these seemingly contradictory pieces of evidence suggest an interaction between the quantity and nutritional quality of food-intake on immunological function following infection.

To summarize, we hypothesize that the diet-mediated ecological immunology of *G. incorrupta* caterpillars involves at least three mechanisms that putatively function as a multi-stage process (Fig. 1). First, there is evidence that increased quantity of carbohydrate-biased food has a positive prophylactic effect on the melanization-response. The underlying physiological mechanism is unclear, but might include enlarging the fat body, which is a site for production of molecules needed for PO and for melanization-activity (Beckage 2008). Second, in the

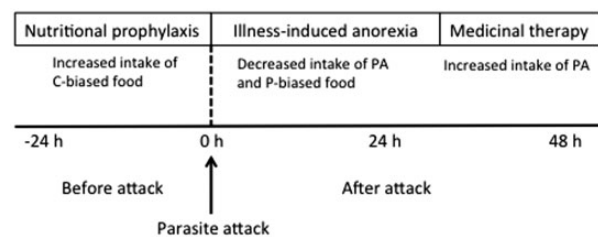


Fig. 1 Temporally explicit summary of the immunological role of diet in the caterpillar *G. incorrupta* as a multi-stage process that integrates components of the conceptual framework summarized in Table 2.

early phase of infection, caterpillars displayed anorexia both toward PA and toward food that is nutritionally optimal for growth. We hypothesize that illness-induced anorexia toward medicine and certain nutrient ratios is an initial therapeutic response to immune-challenge (Adamo 2006). The adaptive benefit of illness-induced anorexia is likely to be enhanced immunological function, although this remains mostly untested in this system because to date we have only measured melanization of beads. Third, substantial evidence supports the hypothesis that PA-medication is a subsequent therapeutic response to parasitism by tachinids. Presumably, medication-behavior is induced by physiological cues indicating the failure or insufficiency of the immunological response, but this hypothesis has not been tested directly. Finally, we found only weak evidence for the possibility of nutritional therapy or compensation. A stronger test of possible mechanisms of compensation will require experiments lasting over a longer time. It is notable that the largest increase in the intake of protein-biased food by virus-challenged *S. littoralis* caterpillars occurred 4–7 days post-infection (Lee et al. 2006, Fig. 4a), suggesting the possibility of nutritional compensation rather than therapy.

We conclude by reinforcing the proposal that the immunological response of herbivorous insects, and other animals, functions as a multi-stage process mediated by diet through a diversity of mechanisms (Schulenburg et al. 2009). Seen in this light, the current literature, including our own work, merely scratches the surface of diet-mediated ecological immunology. This field of study will benefit from further research that not only identifies the component mechanisms (e.g., Table 2), but also integrates them, including their possible combinatorial effects, using a systems approach (e.g., Fig. 1). Insect model systems, such as *Drosophila melanogaster*, have some great advantages for leading this charge (e.g., Lee et al. 2008; Kacsoh et al. 2013). Temporal activity patterns of genes with known immunological and dietary roles can be measured prior to, and over the course of, infection, similar to work already being carried out on a variety of insect species (e.g., Vogel et al. 2014). Ultimately, however, a strong theoretical understanding of ecological immunology must be supported by an empirical foundation of comparative biology. The study of diverse species possessing a variety of life-history traits and tested in a variety of environments offers the best opportunity to develop a theory of ecological immunology with broad predictive power.

## Acknowledgments

The authors thank Robin Warne and Dan Hahn for organizing the symposium that spawned this article. They also acknowledge research assistance from many undergraduates in the Singer laboratory and constructive comments by two anonymous reviewers and the editor that helped improve the article.

## Funding

Funding of this work came from Wesleyan University and National Science Foundation [grant 0744676, awarded to M.S.S.].

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