

## COMMENTARY

# From perplexing to predictive: are we ready to forecast insect disease susceptibility in a warming world?

Laura V. Ferguson<sup>1,\*</sup> and Shelley A. Adamo<sup>2</sup>

## ABSTRACT

Insects are critical to our ecosystems, but we do not fully understand their future in our warming world. Rising temperatures are affecting insect physiology in myriad ways, including changes to their immune systems and the ability to fight infection. Whether predicted changes in temperature will contribute to insect mortality or success, and the role of disease in their future survival, remains unclear. Although heat can enhance immunity by activating the integrated defense system (e.g. via the production of protective molecules such as heat-shock proteins) and accelerating enzyme activity, heat can also compromise the immune system through energetic–resource trade-offs and damage. The responses to heat are highly variable among species. The reasons for this variability are poorly known, and we are lagging in our understanding of how and why the immune system responds to changes in temperature. In this Commentary, we highlight the variation in insect immune responses to heat and the likely underlying mechanisms. We suggest that we are currently limited in our ability to predict the effects of rising temperatures on insect immunity and disease susceptibility, largely owing to incomplete information, coupled with a lack of tools for data integration. Moreover, existing data are concentrated on a relatively small number of insect Orders. We provide suggestions for a path towards making more accurate predictions, which will require studies with realistic temperature exposures and housing design, and a greater understanding of both the thermal biology of the immune system and connections between immunity and the physiological responses to heat.

**KEY WORDS:** Climate change, Heat, Immunity, Infection, Insect

## Introduction

Insects are critical components of terrestrial ecosystems and vectors of disease for humans and wildlife; thus, predicting how climate change will affect them is crucial to our ability to prepare for the future (Harvey et al., 2020, 2022). One key issue is how higher temperatures will affect their ability to withstand infection. Increasing temperatures will alter insect immunophysiology, thereby changing their ability to resist and tolerate pathogens (Laughton et al., 2017; St Leger, 2021). Climate-change-induced temperature increases may reduce insect immune function (e.g. owing to the negative effects of heat stress), leading to reduced population numbers, or may enhance insect immune systems (e.g. because of increased activity rates of immune enzymes), possibly boosting insect populations. Being able to predict which scenario

will occur is important: a reduction in the number of beneficial insects would be catastrophic (Sánchez-Bayo and Wyckhuys, 2019), and a waning of disease resistance in insect vectors could lead to increased disease transmission to humans (Murdock et al., 2012b). Conversely, increased disease resistance in agricultural pests could lead to biocontrol failures and loss of crops. Unfortunately, the complexities of immunophysiology make predictions about changing susceptibility to disease problematic.

In this Commentary, we review (1) how increased temperature affects immunophysiological networks, (2) where the chief difficulties lie for our ability to make predictions about changing disease susceptibility and (3) our suggestions for increasing our predictive power through careful experimental design. The majority of our examples draw from the insect groups that provide the most comprehensive overview of heat effects on immunity, mainly the lepidopteran pests. We examine the effects of chronic increases in average temperature and extreme weather events (i.e. heat waves), both of which are predicted to occur (more frequently) as a result of to climate change (IPCC, 2022). We note that the overall effects of climate change on disease in insects will hinge on several interacting variables (e.g. drought, nutrition) and their integrative impacts on host physiology (Laughton et al., 2017; St Leger, 2021); here, we highlight the roles of heat and immunity. Further, although the effects of climate change on pathogens will also be critically important in determining changes in disease susceptibility (St Leger, 2021) (Box 1), we focus on the insect side of the interaction.

## Insect immunophysiology and its implications for the effects of heat


Insects have dynamic immune systems, allowing them to combat pathogens and flourish in almost all ecological niches, including some that are replete with microbes (e.g. dung beetles). Insects have a combination of coordinated, thermally sensitive, cell-mediated and humoral immune responses (Buchon et al., 2014; Eleftherianos et al., 2021; St Leger, 2021) that can be both ever-present (i.e. constitutive) and/or upregulated (i.e. induced) in response to infection (Schmid-Hempel, 2005) (Box 2). The ability to coordinate and mobilize different immune components allows insects to reconfigure their immune responses and optimize immune function for different environmental conditions (Adamo, 2014, 2017a,b), including changes in temperature (St Leger, 2021).

The immune system is heavily regulated owing to its ability to cause self-damage and thus requires insects to walk a fine line between a robust defense and immunopathology (Lazzaro and Tate, 2022). For example, the phenoloxidase (PO) pathway, an important component of humoral immunity, produces reactive molecules (Eleftherianos et al., 2021; González-Santoyo and Córdoba-Aguilar, 2012) that attack pathogens but can also damage host tissues (Khan et al., 2017; Sadd and Siva-Jothy, 2006). Therefore, the PO pathway, and other immune responses, has mechanisms to constrain over-activity (e.g. serpins; González-Santoyo and

<sup>1</sup>Department of Biology, Acadia University, Wolfville, NS B4P 2R6, Canada.

<sup>2</sup>Department of Psychology and Neuroscience, Dalhousie University, Halifax, NS B3H 4R2, Canada.

\*Author for correspondence (laura.ferguson@acadiau.ca)

 L.V.F., 0000-0002-8366-8561; S.A.A., 0000-0002-5973-571X

### Box 1. Pathogens in a warming world

Pathogens are ectotherms that are sensitive to changing temperatures. For some, a warming world may shift them beyond the temperature of their optimal growth, allowing hosts to gain an upper hand, whereas others will benefit from more rapid growth (St Leger, 2021). Warming may also affect their off-host survival by crossing damaging thermal maxima and compromising their ability to infect a new host (Gehman et al., 2018). Because the outcome of infection will depend on matches and mismatches in the thermal performance of the host and the pathogen (Thomas and Blanford, 2003), warming has the potential to create new 'winners' and 'losers' in this match. Thus, the outcome of infection and disease depends on the interaction between hosts and symbionts, which are both sensitive to changing temperatures (St Leger, 2021). Similar to the hosts, we are only beginning to understand how heat will affect growth, persistence and virulence in pathogens. Nevertheless, we can employ similar tools and approaches to understanding the thermal biology of pathogens – for example, using thermal performance curves for pathogen performance traits (Molnár et al., 2017).

Pathogens may also contribute to the ability for hosts to withstand warming. Pathogens may alter the thermal maxima of their insect hosts (Hector et al., 2021); consequently, infection may compromise the ability to respond to heat stress and contribute to heat-induced mortality, or, conversely, help hosts survive extreme heat (Bates et al., 2011). Thus, microbial responses to heat will have effects on both infection-related mortality as well as the ability to tolerate abiotic stressors such as heat. Overall, although we focus on insect hosts in this Commentary, the thermal biology of pathogens and beneficial symbionts will be key in understanding insect responses to climate change (St Leger, 2021).

Córdoba-Aguilar, 2012). Insects also have molecules that can buffer self-damage (e.g. antioxidants) and are examples of infection tolerance (Ayres and Schneider, 2012). Infection tolerance allows a host to mitigate against pathogen-induced damage. These responses enhance survival without actively reducing pathogen numbers (Ayres and Schneider, 2012). Unfortunately, suppressing immune-generated damage (i.e. infection tolerance) can also reduce the effectiveness of resistance mechanisms (e.g. reactive molecules generated by PO; Clark et al., 2010). The need to balance resistance mechanisms (e.g. reactive molecules) with infection tolerance helps explain the complex regulatory loops that both activate and constrain immune activity (e.g. the PO pathway; González-Santoyo and Córdoba-Aguilar, 2012) and are integral to understanding shifts in immune function under changing environments.

The immune responses within cells (i.e. the pathways activated by cellular receptors for immune-stimulating molecules) are intertwined with other cellular pathways. For example, the intracellular signaling pathways responsible for inducing the production of antimicrobial peptides (AMPs) intersect with the insulin signalling pathway, coupling the immune response with cellular metabolism (Davoodi et al., 2019; Musselman et al., 2018). The immune system is also interconnected with other physiological networks (Fig. 2), e.g. the stress response (Adamo, 2014). These connections permit different defense systems (e.g. fight-or-flight and detoxification responses) to share molecular resources, allowing the immune system to function as part of a dynamic integrated defense system (IDS; Adamo, 2022). During an infection, these interconnections optimize the body for immune defense, increasing the chance of survival (Davoodi et al., 2019; Dolezal et al., 2019; Galenza and Foley, 2019) while minimizing energetic and resource costs (Adamo, 2022).

Heat is a stressor (King and MacRae, 2015) that activates the same hormonal stress response (i.e. octopamine, a key stress neurohormone; Davenport and Evans, 1984) that occurs during

### Box 2. Overview of insect immune systems

Insect immune responses are usually classified into two categories: cell-mediated and humoral (Beckage, 2011; Eleftherianos et al., 2021). Cell-mediated immunity is carried out by insect blood cells (haemocytes) (Eleftherianos et al., 2021). In response to pathogen-associated molecular motifs, haemocytes respond to invaders by engulfing (phagocytosis) or sequestering (nodulation, encapsulation) them (Eleftherianos et al., 2021). Haemocyte responsiveness can be altered by cytokines (i.e. immune signalling molecules) (Vanha-aho et al., 2016) and hormones (e.g. ecdysteroids) (Clark et al., 2005). In many insects, not all haemocytes are in circulation simultaneously (Strand, 2008). Therefore, although cell-mediated immunity is always present at a constitutive (i.e. baseline) level, when under pathogen attack, haemocyte numbers and their activity can be increased (i.e. an inducible response) (Eleftherianos et al., 2021). However, non-immune challenges (e.g. stressful stimuli; Mowlds and Kavanagh, 2008) can also result in changes in cell-mediated immunity as part of a stress response (Adamo, 2010).

Humoral immunity is composed of antimicrobial molecules, most of which are made by the fat body, an immune organ (Engström, 1999). One such molecule is phenoloxidase, an enzyme that results in the production of reactive molecules that can destroy pathogens (González-Santoyo and Córdoba-Aguilar, 2012). The enzyme is present in the haemolymph in an inactive form (prophenoloxidase). Because of the non-specific nature of reactive molecules, phenoloxidase activity can lead to substantial self-damage (Khan et al., 2017). During an immune challenge, receptors on fat body cells (e.g. IMD and Toll receptors) are activated, triggering complex intracellular signalling pathways, leading to the production of anti-microbial peptides (AMPs) (Buchon et al., 2014). These inducible molecules augment the constitutive cell-mediated and humoral responses and help to destroy any pathogens that survived the initial immune onslaught (Haine et al., 2008). The intracellular signalling pathways responsible for inducing AMP production intersect with other pathways (e.g. the insulin signalling pathway) (Davoodi et al., 2019; Musselman et al., 2018), coupling the immune response with other major physiological systems (Adamo, 2021, 2022) such as intermediate metabolism.

exposure to pathogens, poisons and predators (Adamo, 2022). Heat stress also activates important hub molecules (e.g. FoxO; Gruntenko et al., 2016) that are also activated by other stress responses, including the immune response (Adamo, 2022). Activated FoxO is linked to the production of heat-shock proteins (HSPs; Donovan and Marr, 2016) that both protect molecular molecules critical for cellular survival from heat-induced degradation (King and MacRae, 2015) and reduce immune-generated damage (Hector et al., 2020; Wojda, 2017), leading to better tolerance of and survival from infection (i.e. cross-tolerance; Sinclair et al., 2013). Such cross-tolerance between heat and immune stress responses helps explain why exposure to heat stress often leads to an enhancement of immune function and can increase survival after infection (Kryukov et al., 2020, 2018; Wojda, 2017). Moreover, hub molecules such as FoxO can have different alleles, resulting in variation in disease resistance (Wang et al., 2017). Similarly, they may also endow individuals with immune systems of varying sensitivity to temperature.

Although stress responses to any damaging stimulus might be expected to increase the production of broadly protective molecules, short-term heat (i.e. less than 24 h) can also upregulate the expression of immune genes (Wojda, 2017). Part of this upregulation may be due to FoxO activation, which typically leads to AMP production (Becker et al., 2010) and suggests the existence of cross-talk (Sinclair et al., 2013) between heat and the immune system. However, this increase in immune gene expression with heat is not ubiquitous. In some insects, heat has only a modest

effect on AMP gene expression (e.g. *Manduca sexta*; Alston et al., 2020), and in some transcriptomics studies, no increase in AMP gene expression has been found (e.g. *Bombyx mori*; Tang et al., 2016). The reasons for these species differences are unknown.

In addition to activating immune-enhancing stress responses, heat also increases the rate of enzyme activity (Chown and Nicolson, 2004) within the immune system (St Leger, 2021). These increases should boost immunocompetence in a warming world, at least until temperatures start to degrade the enzymes associated with the immune response. The benefit of this temperature effect is underscored by the ability of some insects to raise their body temperature by basking in sunlight, boosting their own immune responses (i.e. behavioural fever; Ouedraogo et al., 2003), resulting in increased survival after infection (Stahlschmidt and Adamo, 2013). Nevertheless, heat does not have a universally positive effect on the immune system.

### Why is heat not always immunoenhancing?

#### Negative effects of heat on immune function

Behavioural fever is not ubiquitous in insects, nor is it induced for every infection (Stahlschmidt and Adamo, 2013), perhaps because heat can also reduce immune function, even at temperatures below the temperatures needed to denature enzymes (Table 1, Fig. 1). Relatively modest heat shock (e.g. 30°C) can reduce immune gene expression in *B. mori* (Tang et al., 2016), and elevated temperatures reduce aphid resistance to parasitoid infection (Bensadia et al.,

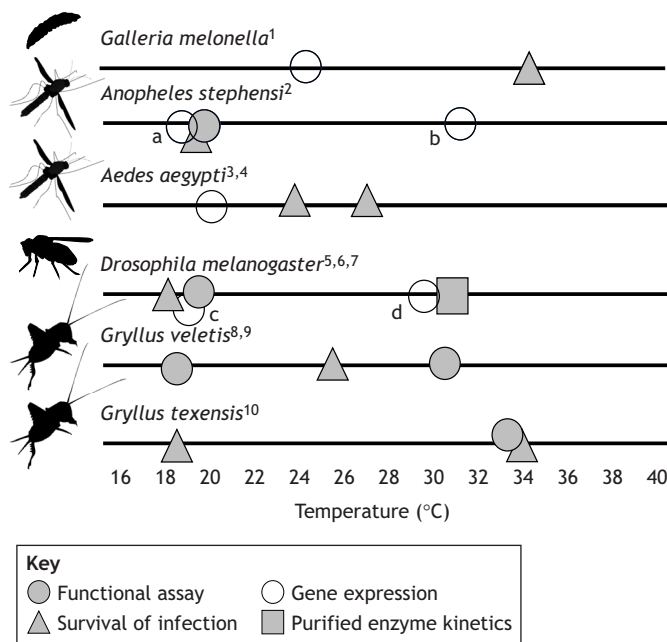
2006). In fact, some insects, such as *Drosophila melanogaster*, *Moniliformis moniliformis* and *Frankliniella occidentalis*, employ reverse behavioural fever (anapyrexia), preferentially occupying cooler habitats than their uninfected counterparts during infection, leading to improved survival (Hunt et al., 2016; Liu et al., 2019; Moore and Freehling, 2002). Further, species of *Drosophila* that are more susceptible to infection with a novel virus become increasingly susceptible with heat exposure, suggesting that heat can exacerbate susceptibility to emerging pathogens in some species (Roberts et al., 2018). Thus, the effects of heat on immunity and disease susceptibility are (perhaps unsurprisingly) variable, and therein lies the difficulty in our ability to predict the future.

Heat is sometimes immunocompromising because not all network interactions result in enhanced function (Adamo, 2017a, b). Under some conditions, resources are shifted away from the immune system to help fuel other responses, resulting in physiological trade-offs (Adamo, 2017a,b; Schwenke et al., 2016). For example, producing the molecules needed to tolerate heat (e.g. HSPs) can lead to decreased growth and reproduction, and may also limit resource availability for immune responses (González-Tokman et al., 2020). Moreover, because increased reproduction can decrease immunocompetence (McKean and Nunney, 2001), and increased temperatures increase reproductive rate in many insects (Adamo et al., 2012), resources diverted to egg production could decrease immunity under elevated temperatures. These types of trade-offs may be sex specific, which will be

**Table 1. Examples of variation in the response to different heat treatments among species.**

Immune measure	Type of heat exposure		
	Rearing/chronic temperature elevation	Acute (<24 h)	Heat wave (>24 h)
Circulating haemocytes	<i>Anticarsia gemmatilis</i> <sup>1</sup> <i>Lycaena tityrus</i> <sup>2</sup> <i>Pieris napi</i> <sup>3</sup> <i>Plodia interpunctella</i> <sup>4</sup>	<i>Galleria melonella</i> <sup>5</sup>	<i>Lobesia botrana</i> <sup>6</sup>
Phenoloxidase activity	<i>Ischnura elegans</i> <sup>7</sup> <i>Enallagma cyathigerum</i> <sup>7</sup> <i>Galleria melonella</i> <sup>9</sup> <i>Paropsis atomaria</i> <sup>10</sup> <i>Pieris napi</i> <sup>3</sup>	<i>Bicyclus anyana</i> <sup>8</sup>	<i>Lobesia botrana</i> <sup>6</sup>
Melanisation	<i>Ischnura elegans</i> <sup>7</sup> <i>Enallagma cyathigerum</i> <sup>7</sup> <i>Anticarsia gemmatilis</i> <sup>1</sup> <i>Paropsis atomaria</i> <sup>10</sup>		<i>Choristoneura fumiferana</i> <sup>11</sup> <i>Coenagrion puella</i> <sup>12</sup>
Encapsulation	<i>Anticarsia gemmatilis</i> <sup>1</sup>		<i>Choristoneura fumiferana</i> <sup>11</sup>
Lysozyme and/or AMP gene expression	<i>Pieris napi</i> <sup>3</sup> <i>Aedes aegypti</i> <sup>9</sup>	<i>Galleria melonella</i> <sup>13</sup> <i>Bombyx mori</i> <sup>14</sup> <i>Ostrinia furnicalis</i> <sup>15</sup> <i>Apis mellifera</i> <sup>16</sup> <i>Galleria melonella</i> <sup>17</sup> <i>Galleria melonella</i> <sup>13</sup> <i>Chilo suppressalis</i> <sup>19</sup> <i>Galleria melonella</i> <sup>13,17</sup> <i>Mamestra brassicae</i> <sup>21</sup>	<i>Aedes aegypti</i> <sup>18</sup>
Survival of fungal infection	<i>Melanoplus sanguinipes</i> <sup>20</sup> <i>Galleria melonella</i> <sup>9</sup>		
Survival/clearance of viral infection	<i>Anticarsia gemmatilis</i> <sup>1</sup> <i>Aedes aegypti</i> <sup>22</sup>		
Survival/clearance of bacterial infection	<i>Galleria melonella</i> <sup>23</sup>		<i>Gryllus texensis</i> (Gram +) <sup>24</sup> <i>Gryllus texensis</i> (Gram -) <sup>24</sup>
Survival of parasite infection	<i>Meccus pallidipennis</i> <sup>25</sup>		

Species in orange represent negative impacts on immune measures; species in blue represent positive impacts; species in black represent no change. Note: this is not an exhaustive list of immune responses to heat. Some insects, such as *G. mellonella*, appear to have heat resilient immune systems. However, the lack of data makes comparisons difficult. Data sources: <sup>1</sup>Silva and Elliot (2016); <sup>2</sup>Fischer et al. (2011); <sup>3</sup>Bauerfeind and Fischer (2014); <sup>4</sup>Triggs and Knell (2012); <sup>5</sup>Laughton et al. (2017), but see Triggs and Knell (2012); <sup>6</sup>Browne et al. (2014); <sup>7</sup>Illis et al. (2021); <sup>8</sup>Van Dievel et al. (2017); <sup>9</sup>Karl et al. (2011); <sup>10</sup>Kryukov et al. (2018); <sup>11</sup>Gherlenda et al. (2016); <sup>12</sup>Seehausen et al. (2018); <sup>13</sup>Tüzün and Stoks (2021); <sup>14</sup>Vertyporokh et al. (2015); <sup>15</sup>Guo et al. (2018); <sup>16</sup>Chen et al. (2019); <sup>17</sup>Li et al. (2022); <sup>18</sup>Wojda and Jakubowicz (2007); <sup>19</sup>Muturi et al. (2012); <sup>20</sup>Shamakhi et al. (2019); <sup>21</sup>Srygley and Jaronski (2022); <sup>22</sup>Richards et al. (2017); <sup>23</sup>Muturi et al. (2011); <sup>24</sup>Hurst et al. (2015); <sup>25</sup>Adamo and Lovett (2011); <sup>26</sup>Gonzalez-Rete et al. (2019).



**Fig. 1. The optimal temperature for different immune components and survival after infection differ both within individuals and between species.** Each marker along the line represents the thermal optimum for: a functional immune response, immune gene expression or survival after infection. The discrepancy between these thermal optima demonstrates that there is variation in the thermal performance of the immune system within a species, depending on which type of immune measurement we consider. Further, the optimum for a particular immune response does not necessarily correspond with the optimum for survival after infection – nor is the thermal optimum of survival the same when comparing across infections with different pathogens. Finally, there is variation among species in the thermal optima for the same types of immune measures. This variation complicates our ability to predict the outcome of infection and disease with warming. Letters denote similar measures (e.g. gene expression) with different thermal optima: a, *Defensin* 1; b, *Cecropin*; c, *Diptericin*; d, *Metchnikowin*. Data sources: <sup>1</sup>Kryukov et al. (2018); <sup>2</sup>Murdock et al. (2012a); <sup>3</sup>Tesla et al. (2018); <sup>4</sup>Ferreira et al. (2020); <sup>5</sup>Hunt et al. (2016); <sup>6</sup>Linder et al. (2008); <sup>7</sup>Fedorka et al. (2016); <sup>8</sup>Ferguson et al. (2016); <sup>9</sup>Ferguson and Sinclair (2020); <sup>10</sup>Adamo and Lovett (2011).

important to disentangle to understand broader impacts on population size. Whether physiological trade-offs (negative effects) or cross-talk/tolerance (positive effects) predominates will vary across species and relies on a variety of factors that we discuss throughout this section.

#### Balancing positive and negative effects of heat on immune function

Immune activity requires resources (Ardia et al., 2012); therefore, the impact of trade-offs resulting from heat exposure will depend on the nutritional state of the insect (Ponton et al., 2011), and on any co-occurring stressors that affect energy use and resources (St Leger, 2021; Van Dievel et al., 2017). For example, in crickets and damselflies, abundant food allows for both reproduction and immunity to increase with temperature (Adamo and Lovett, 2011; Van Dievel et al., 2017); however, with limited food, reproduction and survival decline at higher temperatures (Adamo et al., 2012), suggesting that physiological benefits of heat can be lost. Climate change is likely to reduce nutrient availability for many insects

(Harvey et al., 2020; St Leger, 2021), leading heat to have both direct and indirect negative effects on immunity. Heat can exacerbate the metabolic costs of immunity (Catalán et al., 2012), leading to depleted resources and compromised immunity. For example, nutrient stress compounds the negative effects of heat on immunity in the lepidopterans *Lobesia botrana* (Iltis et al., 2021) and *Bicyclus anynana* (Karl et al., 2011). Further, increasing temperature also increases energy use (Gonzalez-Tokman et al., 2020; Tüzün and Stoks, 2021) and decreases net energy gain (Huey and Kingsolver, 2019), leading to a direct resource-depleted limitation of the immune system. Overall, when heat is combined with other stressors, immune function is likely to suffer (Kaunisto et al., 2016; St Leger, 2021) through trade-offs within physiological networks (Adamo, 2017a,b).

Protective mechanisms initiated after heat stress (e.g. HSPs, antioxidants) also have the potential to inhibit the cytotoxic activity necessary for an effective immune response (e.g. Clark et al., 2010). For example, *Anopheles gambiae* mosquitoes that are more resistant to *Plasmodium* infection locally suppress antioxidant mechanisms and promote states of oxidative stress (Kumar et al., 2003; Molina-Cruz et al., 2008), suggesting that this activity is required for resistance. Alternatively, an upregulation of protective mechanisms after heat exposure (e.g. HSPs; Wojda, 2017) could also indicate a switch in immune strategy from an emphasis on resistance (i.e. reducing pathogen load) to one on infection tolerance (i.e. reducing damage associated with infection). This potential reconfiguration of the immune system could explain why decreases in PO activity are common after heat exposure (Table 1).

Heat may provide a hormetic effect wherein stress associated with one exposure is immuno-enhancing, but these effects are lost with increased intensity or duration. For example, acute exposure to 34°C increases immune activity in *Chilo suppressalis*, but chronic exposure does not (Shamakhi et al., 2019). Similarly, heat exposures of 36°C increase expression of immune genes such as *cecropin*, *defensin* and *transferrin* in *Ae. aegypti* larvae, but exposure of the same duration to 40°C does not (Muturi et al., 2012). Repeated or chronic heat stress is likely to be damaging, creating ‘wear and tear’ (Kingsolver et al., 2021; Marshall and Sinclair, 2015; Romero et al., 2009) that compromises the immune system and negates any benefits of acute exposure. Additionally, the continued over-expression of HSPs during prolonged heat exposure can lead to a reduction in growth and survival, suggesting that chronic activation carries a substantial penalty (Kingsolver and Woods, 2016), which may depress immunity. For example, rearing at high temperatures (i.e. a chronic exposure) compromises immunity in *Melanoplus sanguinipes* (Srygley and Jaronski, 2022) and *D. melanogaster* (Kutch et al., 2014). Further, the immune system may trade off with the heat shock response; in bees, heat exposure reduces immune gene expression and, conversely, wounding compromises the heat shock response (McKinstry et al., 2017). Thus, if heat is high enough to activate the heat shock response, this may compromise immunity in some insects. Overall, the intensity, duration and frequency of heat exposure is likely to determine the costs and benefits of heat to the immune system.

#### Pathogen-dependent immune responses in a warming world

Because the immune system is selected to respond to dynamic, thermally sensitive pathogens (Thomas and Blanford, 2003), the thermal performance of pathogens, or the environments in which infection is likely to occur, appear to influence immune system dynamics (Ferguson and Sinclair, 2020; Kirk et al., 2022). Even though key enzymes of the immune system, such as lysozyme (an

**Table 2. Considerations for designing experiments on the impact of heat on insect immunity**

Variable	Design parameters		Mechanisms underlying importance of design parameters
Heat	Duration	Acute (minutes–hours) versus chronic exposures (days–weeks)	Hormesis versus trade-offs; accrued stress versus recovery and repair; matches/mismatches in thermal performance of host and pathogen
	Intensity	Maximum temperature appropriate for predicted climate change maximums	
	Frequency	Realistic fluctuating temperatures	
	Timing	Repeated heat waves Heat experienced before, during or after infection	
Host	Life stage	Time of day	Thermal plasticity; matches/mismatches in thermal performance; impacts of infection on heat tolerance
		Juvenile versus adults	
	Nutrients	Sources of nutrition/amount of available food	Interactions between temperature and circadian rhythms
Pathogen	Physiological condition	Acclimated; aestivation/quiescence; growing; reproducing; presence of multiple stressors; age	Variation in network responses/thermal performance among life stages
	Behaviour	Relevant housing with refuges	Resource limitations on immunity and trade-offs; impacts of microbiomes on immunity
	Species	Ecologically relevant to host and geographic location	Trade-offs; cross-talk/cross-tolerance; immune plasticity
Experiment	Type	‘Omics’ versus functional assays versus survival; tissues of interest; multi-trait approach	Preferred temperatures that the insect will choose to occupy
	Temperature	Appropriate temperatures for enzyme assays, other functional measures	Thermal biology of pathogen; matches/mismatches in thermal performance

Careful consideration of each design parameter ensures that we capture the sources of variation in response to heat that arise through a variety of different physiological and behavioural mechanisms. If we can account for these sources of variation in our design, then we can create the most ecologically and physiologically relevant heat exposures to determine how warming will modify disease susceptibility in insects.

enzyme that cleaves peptidoglycan in bacterial cell walls) and PO are stable at a wide range of temperatures, and reach maximal activity between 30 and 45°C for insects from a broad range of species [e.g. *Heliothis virescens* (Lepidoptera), Lockey and Ourth, 1992; *Drosophila melanogaster* (Diptera), Asada and Sezaki, 1999; *Gryllus texensis* (Orthoptera), Adamo and Lovett, 2011], the optimal temperature for the organismal-level response is frequently much lower (Fig. 1). For example, the melanization response (i.e. the deposition of the pigment melanin on a pathogen) produced by PO activity in both *Anopheles stephensi* (Diptera) and *Gryllus veletis* (Orthoptera) has an optimal temperature close to 18°C and declines at temperatures above this point (Ferguson et al., 2016; Murdock et al., 2012a) (Fig. 1). *Anopheles stephensi* are nighttime feeders and are thus more likely to encounter blood-borne parasites in the cooler part of the day, which can fluctuate to temperatures near 18°C and promote parasite development (Suh et al., 2020). These nighttime temperatures are an intriguing match to the optimal activity of multiple immune metrics in *An. stephensi* (Murdock et al., 2012a), and suggest that perhaps selection has favoured increased immune activity at lower temperatures to optimize the ability to counter pathogen exposure during feeding. If the immune system is this highly tuned to pathogen thermal performance, then variations of a few degrees are likely to affect the outcome of infections (Suh et al., 2020; Thomas and Blanford, 2003).

This potential thermal specificity also suggests that the outcome of infection with warming will vary on a host species- and pathogen-specific basis (Sternberg and Thomas, 2014), and may be easier to predict in specialist host–parasite interactions (St Leger, 2021). Indeed, the effects of heat can be very pathogen specific, wherein heat can promote the survival of one pathogen while compromising survival of another (Adamo and Lovett, 2011; Mastore et al., 2019). The thermal performance of the multiple independent components of the immune system means that a performance curve of the immune system overall is the sum of these independent performance

curves. The ‘curve’ is likely to have local maxima and minima of resistance that shift as temperature increases, depending on how temperature affects individual immune components and their associated network interactions. As this ‘curve’ interacts with the thermal performance of pathogens, disease resistance to some pathogens may be enhanced with global warming (i.e. if the main immune component needed to vanquish the pathogen is enhanced by increased temperature), but not for others (Adamo and Lovett, 2011). Overall, hotter is not always better for the immune system or disease resistance, and thus warming will produce a suite of responses that the field must now disentangle.

**Immunity in a warming world: how do we move towards predictions?**

Despite the disparate responses to heat among immune measures and species (Table 1, Fig. 1), we suggest that we can gather the data we are lacking to predict patterns amongst the complexity and variation (Table 2). The ‘grunt’ work lies in collecting more of the right type of data (Gonzalez-Tokman et al., 2020; Harvey et al., 2020; St. Leger, 2021). However, we will also need to synthesize these data using multifactorial data analysis (e.g. discriminant analysis) and develop appropriate mathematical models. There are models for insect immune systems (Adamo and Spiteri, 2009; Ellner et al., 2021; Tate and Schulz, 2022) and for the effects of climate change on insect populations (Humphreys et al., 2022), but not on how climate change will affect insect immunity. In this section, we draw on approaches outlined by Gonzalez-Tokman et al. (2020) and Kaunisto et al. (2016) to discuss the data we need to collect to develop these mathematical models.

**Ecologically relevant thermal parameters**

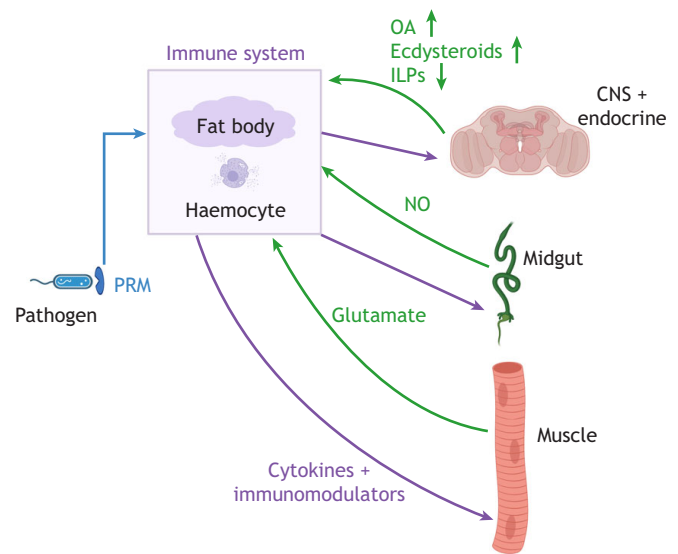
Future studies should use temperature protocols that do not just reflect the predicted increase in average temperatures but also incorporate real-world temperature fluctuations. Fluctuating

temperatures impact physiology, including immunity, in fundamentally different ways than constant temperatures (Colinet et al., 2015; Ferguson and Sinclair, 2020). Fluctuating temperatures can trigger a preparatory response to heat exposure (Sørensen et al., 2016), which could afford insects increased protection against infection as temperatures increase. For example, fluctuating temperatures improve the survival of *H. virescens* to fungal infection relative to constant temperatures (Ghazanfar et al., 2020). Fluctuating conditions may also allow for periods of recovery and repair (Colinet et al., 2015; Ma et al., 2021; Romero et al., 2009; Torson et al., 2015) that may decrease immunopathology and rescue compromised resistance against infection during heat exposure. In contrast, fluctuating temperatures may also cycle between the relative thermal optima/ advantage of a given host and pathogen, thereby compromising survival if the performance of the pathogen is favoured (Stoks et al., 2017). Because climate change is likely to exacerbate fluctuations in temperature, leading to higher extremes and greater variability (IPCC, 2022), it is important to determine whether fluctuating temperatures will help to mitigate, or will exacerbate, the negative impacts of heat exposure on the immune system.

Ecologically relevant temperature exposures are also critical prior to heat events. The ability to prepare for heat exposure through acclimation, hardening or developmental responses (Sgro et al., 2016) may modify how heat affects the immune system by altering the immune system's network configuration (e.g. balance of resistance versus infection-tolerance mechanisms). If insects can acclimate to elevated temperatures, this could allow insects to overcome trade-offs that compromise the immune system. Further, phenotypic plasticity may allow insects to keep pace with the thermal adaptations of their pathogens (e.g. if pathogens rapidly adapt growth rates and virulence to higher temperatures) (Gehman et al., 2018; Harvell et al., 2002). We suggest that we need to use realistic thermal parameters even prior to heat events (e.g. simulated heat waves) to determine the effect of changes in the duration, intensity and frequency of high temperatures on the immune system.

The duration of any benefits (or impairments) to the immune system after heat exposure is also unclear, although many appear to be transient. For example, exposure to heat (37°C) in *G. melonella* increases the expression of genes related to the immune system and improves subsequent survival after a fungal infection, but these benefits are lost 72 h after the heat wave (Browne et al., 2014). Similarly, increases in haemocytes (Box 2) immediately after an acute heat shock in *Mamestra brassicae* disappear 24 h post exposure (Richards et al., 2017). Thus, it will be important to use multiple time points to assess immune function both during and after heat exposure to determine whether these impacts have any bearing on survival, or whether acute exposures are inconsequential for subsequent infections.

One neglected issue in determining the effect of climate change on insect immunity is the capacity for behavioural thermoregulation in insects. Insect thermoregulation is a complex field of its own, with an individual's temperature preference varying depending on a variety of factors (Chown and Nicolson, 2004). Thermoregulation allows many species to find refuge against damaging temperatures (Gonzalez-Tokman et al., 2020), and thus the reported air temperatures may not be relevant for exploring immune function. However, thermoregulation itself may alter the exposure of insects to pathogens, for example by increasing their exposure to dark, moist spaces, leading to changes in the incidence of disease. Temperature may alter other insect behaviours, for example

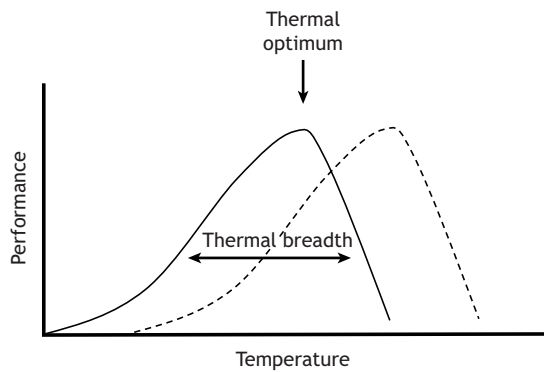


**Fig. 2. Immune systems are bidirectionally connected with other physiological systems, allowing the immune response to be context-dependent (e.g. influenced by nutritional status; Adamo et al., 2016; Dolezal et al., 2019; Zhao and Karpac, 2021).** Cytokines (purple arrows) induce the release of glutamate, NO and OA that in turn result in changes in immunometabolism and immune function (for further discussion, see Adamo, 2022; Eleftherianos et al., 2021; Yu et al., 2022). Selected references: ecdysteroids (Sun et al., 2016); OA (Adamo, 2010), ILPs (Sharma et al., 2019); NO (Das De et al., 2018). CNS, central nervous system; ILPs, insulin-like peptides; OA, octopamine; NO, nitric oxide; PRM, pathogen recognition molecule.

weakening behavioural defenses against parasitoids (Iltis et al., 2018), leading to changes in disease prevalence. Future studies should consider using housing that reflects the natural environment (including the availability of refuges) during temperature studies.

### Accounting for thermal performance of host and pathogen

Whether any influence of heat on the immune system leads to changes in the frequency or severity of infection will also depend heavily on the pathogen, and is likely linked to the matches or mismatches between host immune and pathogen thermal performance (Ferguson and Sinclair, 2020; Kirk et al., 2022; Le Lann et al., 2021; Moore et al., 2021; Thomas and Blanford, 2003) and any effects that heat may have on the pathogen itself. For example, survival of a fungal infection in crickets depends on whether the thermal environment during infection favours the performance of the host or the pathogen (Ferguson and Sinclair, 2020). We can create thermal performance curves (i.e. a graph of organism performance versus temperature that is a type of reaction norm; Fig. 3) describing the outcome of infection (i.e. survival across a suite of temperatures; Stoks et al., 2017), which is particularly useful for modeling disease for vectors, pests and species of conservation interest that may be targeted by individual, well-described pathogens and parasitoids. These curves allow us to determine the thermal optimum of responses, the thermal breadth, matches and mismatches with pathogens, and the potential for thermal plasticity in the immune system – all of which provide power for predicting the outcome of infection under different thermal regimes (Ferguson and Sinclair, 2020). However, this approach may not be tractable for predicting disease susceptibility in



**Fig. 3. Example of a thermal performance curve of the immune system.** Immune performance will decline on either side of a thermal optimum. Building thermal performance curves allows us to determine thermal optima and breadth of activity (solid line) as well as potential for plasticity (dashed line).

the general population of insects, in which we know very little of the diversity of pathogens that plague such a large variety of hosts, while the responses of their pathogens to climate change are also poorly understood (St Leger, 2021). Nevertheless, model systems and comparative approaches (Kaunisto et al., 2016; St Leger, 2021) should provide insight into potential patterns of infection across temperature. Finally, increases in understanding of within-host dynamics (Duneau and Ferdy, 2022) will help us determine whether there are critical temperature stages (e.g. within hours) in the course of infection that modify the survival of the host.

Because of the variation in the thermal performance of different immune components and the dynamic nature of the IDS, optimal immune activity of one component will not always translate into optimal survival across temperatures (Fig. 1). Measuring multiple immune components simultaneously is important for assessing immunocompetence (Adamo, 2004) and can allow us to differentiate a reduction in immune function from a change in immune system strategy (i.e. immune reconfiguration; Adamo, 2017a,b). Understanding these details allows us to predict how these changes in immunity will affect susceptibility to different types of pathogens (Adamo and Lovett, 2011; Laughton et al., 2017). Additionally, we need to ensure that we are measuring all immune activity at relevant temperatures. For example, PO assays examining the effect of heat on PO activity levels should be run at the ‘heat wave’ temperature. Because PO activity generally increases with increasing temperature (Adamo and Lovett, 2011; Ferguson et al., 2016), insects experiencing higher temperatures could maintain the same level of PO activity as baseline by reducing the amount of PO they produce. If PO activity is tested at room temperature, it might be erroneously concluded that heat reduces PO function.

### Disentangling physiological networks

Developing a realistic model of temperature effects will require a better understanding of the intra- and extra-cellular signaling systems activated by heat, and how they interact with different immune components at the molecular level. Transcriptomics, proteomics, metabolomics and the ability to integrate these large data sets will help us to disentangle physiological network responses, although we are still developing the ability to interpret the non-linear, dynamic, highly interconnected and redundant nature of these networks (Adamo, 2021). To facilitate predictions, molecular studies should be coupled with functional assays,

preferably assays measuring survival, of infection with ecologically relevant pathogens. This would allow multifactorial analyses such as discriminant function analysis or principal component analysis (i.e. statistical analyses that allow us to interpret the impacts of multiple, interacting factors) to provide some ability to interpret the changes in gene expression (including immune gene expression) with actual disease resistance. In terms of modelling, functional data are key, but understanding the underlying molecular mechanisms will allow modelling beyond a ‘black box’ approach, leading to more robust, and potentially more generalizable, predictions.

### Increasing taxonomic breadth and comparative approaches

Strong species differences are a hallmark of heat effects on immunity, even across insects in a similar climate zone (Table 1). Understanding why these differences exist remains a struggle. To uncover the underlying causes of this variation, we need to study the effects of heat on immunity and disease susceptibility in a broader range of taxa (Kaunisto et al., 2016; Ma et al., 2021; St Leger, 2021) as well as within species (e.g. local adaptation). Comparative studies will help us to determine whether there are larger patterns that can aid our ability to predict the future of disease in insects. For instance, are there geographic patterns based on the thermal evolution of the immune system (e.g. tropical versus temperate versus Arctic; Deutsch et al., 2008; Kankaanpää et al., 2020) that translate into patterns of disease resistance under climate warming? Geographic, microclimatic and phylogenetic patterns in the thermal performance of immunity and the balance between trade-offs and cross-tolerance/talk will be key to making broad-scale predictions. Further, common-garden experiments, especially those with population-specific pathogens, will provide insight into the costs and benefits of adaption to increasing heat for immunity (Tüzün and Stoks, 2021). Finally, populations that have been experimentally adapted to heat (e.g. *Drosophila*; Hsu et al., 2021) may be useful to compare with wild-type to determine how adaptation to heat will affect the immune system. It is also important to note that even within a species, the multifactorial correlations across different immune measures and functional assays can vary depending on the insect’s life stage (St Leger, 2021) and sex (Bauerfeind and Fischer, 2014). Nevertheless, these comparative approaches are required if we want to understand which species and populations will suffer or flourish in the face of infection in a warming world.

### Conclusions

Although insect immune function is only one aspect of insect disease susceptibility (Adamo, 2004; St Leger, 2021), it is an important one that is also intertwined with energy use and thermal tolerance (Hector et al., 2020, 2021). Further, the impacts of warming on disease susceptibility extend beyond insects, and the variation in responses to heat that we have outlined are relevant to all arthropods. We have shown that the effects of temperature are typically a complex blend of both negative and positive effects on the immune system, and differ across species (e.g. Table 1, Fig. 1). This complexity suggests that climate change will not have a generalizable effect on susceptibility to disease in all insects, even neglecting the effect of temperature on pathogens. Being able to make predictions about the effects of climate change, on even a small number of species, will require more appropriately designed studies, advanced data analysis and modelling. We need to make this effort – insects are crucial for healthy ecosystems, and they are also our competitors for food and vectors for disease. Our future depends on our ability to predict their future.

## Acknowledgements

We thank Dr David Duneau and two anonymous reviewers for their helpful comments that improved the paper.

## Competing interests

The authors declare no competing or financial interests.

## Funding

This work was supported by a National Sciences and Engineering Research Council grant to S.A.A. and a Killam Postdoctoral Fellowship to L.V.F.

## References

- Adamo, S. A. (2004). Estimating disease resistance in insects: phenoloxidase and lysozyme-like activity and disease resistance in the cricket *Gryllus texensis*. *J. Insect Physiol.* **50**, 209–216. doi:10.1016/j.jinsphys.2003.11.011
- Adamo, S. A. (2010). Why should an immune response activate the stress response? Insights from the insects (the cricket *Gryllus texensis*). *Brain Behav. Immun.* **24**, 194–200. doi:10.1016/j.bbi.2009.08.003
- Adamo, S. A. (2014). The effects of stress hormones on immune function may be vital for the adaptive reconfiguration of the immune system during fight-or-flight behavior. *Integr. Comp. Biol.* **54**, 419–426. doi:10.1093/icb/ctu005
- Adamo, S. A. (2017a). The stress response and immune system share, borrow, and reconfigure their physiological network elements: evidence from the insects. *Hormones Behav.* **88**, 25–30. doi:10.1016/j.yhbeh.2016.10.003
- Adamo, S. A. (2017b). Stress responses sculpt the insect immune system, optimizing defense in an ever-changing world. *Dev. Comp. Immunol.* **66**, 24–32. doi:10.1016/j.dci.2016.06.005
- Adamo, S. A. (2021). How insects protect themselves against combined starvation and pathogen challenges, and the implications for reductionism. *Comp. Biochem. Physiol. B* **255**, 110564. doi:10.1016/j.cbpb.2021.110564
- Adamo, S. A. (2022). The integrated defense system: optimizing defense against predators, pathogens, and poisons. *Int. Comp. Biol.* **62**, 1536–1546. doi:10.1093/icb/icac024
- Adamo, S. A. and Lovett, M. M. E. (2011). Some like it hot: the effects of climate change on reproduction, immune function and disease resistance in the cricket *Gryllus texensis*. *J. Exp. Biol.* **214**, 1997–2004. doi:10.1242/jeb.056531
- Adamo, S. A. and Spiteri, R. J. (2009). He's healthy, but will he survive the plague? Possible constraints on mate choice for disease resistance. *Animal Behav.* **77**, 67–78. doi:10.1016/j.anbehav.2008.09.011
- Adamo, S. A., Baker, J. L., Lovett, M. M. E. and Wilson, G. (2012). Climate change and temperate zone insects: the tyranny of thermodynamics meets the world of limited resources. *Environ. Entomol.* **41**, 1644–1652. doi:10.1603/EN11188
- Adamo, S. A., Davies, G., Easy, R., Kovalko, I. and Turnbull, K. F. (2016). Reconfiguration of the immune system network during food limitation in the caterpillar *Manduca sexta*. *J. Exp. Biol.* **219**, 706–718. doi:10.1242/jeb.132936
- Alston, M. A., Lee, J., Moore, M. E., Kingsolver, J. G. and Willett, C. S. (2020). The ghost of temperature past: interactive effects of previous and current thermal conditions on gene expression in *Manduca sexta*. *J. Exp. Biol.* **223**, jeb213975. doi:10.1242/jeb.213975
- Ardia, D. R., Gantz, J. E., B. C., Schneider, and Strebel, S. and Strebel, S. (2012). Costs of immunity in insects: an induced immune response increases metabolic rate and decreases antimicrobial activity. *Func. Ecol.* **26**, 732–739. doi:10.1111/j.1365-2435.2012.01989.x
- Asada, N. and Sezaki, H. (1999). Properties of phenoloxidases generated from prophenoloxidase with 2-propanol and the natural activator in *Drosophila melanogaster*. *Biochem. Genet.* **37**, 149–158. doi:10.1023/A:1018730404305
- Ayres, J. S. and Schneider, D. S. (2012). Tolerance of infections. *Ann. Rev. Immunol.* **30**, 271–294. doi:10.1146/annurev-immunol-020711-075030
- Bates, A. E., Leiterer, F., Wiedeback, M. L. and Poulin, R. (2011). Parasitized snails take the heat: a case of host manipulation? *Oecologia* **167**, 613–621. doi:10.1007/s00442-011-2014-0
- Bauerfeind, S. S. and Fischer, K. (2014). Integrating temperature and nutrition: environmental impacts on an insect immune system. *J. Insect Physiol.* **64**, 14–20. doi:10.1016/j.jinsphys.2014.03.003
- Beckage, N. E. (2011). *Insect Immunology*. Academic Press.
- Becker, T., Loch, G., Beyer, M., Zinke, I., Aschenbrenner, A. C., Carrera, P., Inhester, T., Schultze, J. L. and Hoch, M. (2010). FOXO-dependent regulation of innate immune homeostasis. *Nature* **463**, 369–373. doi:10.1038/nature08698
- Bensadia, F., Boudreault, S., Guay, J.-F., Michaud, D. and Cloutier, C. (2006). Aphid clonal resistance to a parasitoid fails under heat stress. *J. Insect Physiol.* **52**, 146–157. doi:10.1016/j.jinsphys.2005.09.011
- Browne, N., Surlis, C. and Kavanagh, K. (2014). Thermal and physical stresses induce a short-term immune priming effect in *Galleria mellonella* larvae. *J. Insect Physiol.* **63**, 21–26. doi:10.1016/j.jinsphys.2014.02.006
- Buchon, N., Silverman, N. and Cherry, S. (2014). Immunity in *Drosophila melanogaster* — from microbial recognition to whole-organism physiology. *Nat. Rev. Immunol.* **14**, 796–810. doi:10.1038/nri3763
- Catalán, T. P., Wozniak, A., Niemeyer, H. M., Kalergis, A. M. and Bozinovic, F. (2012). Interplay between thermal and immune ecology: effect of environmental temperature on insect immune response and energetic costs after an immune challenge. *J. Insect Physiol.* **58**, 310–317. doi:10.1016/j.jinsphys.2011.10.001
- Chen, K., Tang, T., Song, Q., Wang, Z., He, K., Liu, X., Song, J., Wang, L., Yang, Y. and Feng, C. (2019). Transcription analysis of the stress and immune response genes to temperature stress in *Ostrinia furnacalis*. *Front. Physiol.* **10**, 1289. doi:10.3389/fphys.2019.01289
- Chown, S. L. and Nicolson, S. W. (2004). *Insect Physiological Ecology*. New York: Oxford University Press.
- Clark, K. D., Kim, Y. and Strand, M. R. (2005). Plasmacyte sensitivity to plasmacyte spreading peptide (PSP) fluctuates with the larval molting cycle. *J. Insect Physiol.* **51**, 587–596. doi:10.1016/j.jinsphys.2005.03.002
- Clark, K. D., Lu, Z. and Strand, M. R. (2010). Regulation of melanization by glutathione in the moth *Pseudoplusia includens*. *Insect Biochem. Mol. Biol.* **40**, 460–467. doi:10.1016/j.ibmb.2010.04.005
- Colinet, H., Sinclair, B. J., Vernon, P. and Renault, D. (2015). Insects in fluctuating thermal environments. *Annu. Rev. Entomol.* **60**, 123–140. doi:10.1146/annurev-ento-010814-021017
- Das De, T., Sharma, P., Thomas, T., Singla, D., Tevatiya, S., Kumari, S., Chauhan, C., Rani, J., Srivastava, V., Kaur, R. et al. (2018). Interorgan molecular communication strategies of 'local' and 'systemic' innate immune responses in mosquito *Anopheles stephensi*. *Front. Immunol.* **9**, 148. doi:10.3389/fimmu.2018.00148
- Davenport, A. P., Evans, P. D. (1984). Stress-induced changes in the octopamine levels of insect haemolymph. *Insect Biochem. Mol. Biol.* **14**, 135–143. doi:10.1016/0020-1790(84)90021-0
- Davoodi, S., Galenza, A., Panteluk, A., Deshpande, R., Ferguson, M., Grewal, S. and Foley, E. (2019). The immune deficiency pathway regulates metabolic homeostasis in *Drosophila*. *J. Immunol.* **202**, 2747–2759. doi:10.4049/jimmunol.1801632
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C. and Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proc. Natl. Acad. Sci. USA* **105**, 6668–6672. doi:10.1073/pnas.0709472105
- Dolezal, T., Krejčová, G., Bajgar, A., Nedbalová, P. and Strasser, P. (2019). Molecular regulations of metabolism during immune response in insects. *Insect Biochem. Mol. Biol.* **109**, 31–42. doi:10.1016/j.ibmb.2019.04.005
- Donovan, M. R. and Marr, M. T., II. (2016). dFOXO activates large and small heat shock protein genes in response to oxidative stress to maintain proteostasis in *Drosophila*. *J. Biol. Chem.* **291**, 19042–19050. doi:10.1074/jbc.M116.723049
- Duneau, D. and Ferdy, J.-B. (2022). Pathogen within-host dynamics and disease outcome: what can we learn from insect studies? *Curr. Opin. Insect Sci.* **52**, 100925. doi:10.1016/j.cois.2022.100925
- Eleftherianos, I., Heryanto, C., Bassal, T., Zhang, W., Tettamanti, G. and Mohamed, A. (2021). Haemocyte-mediated immunity in insects: cells, processes and associated components in the fight against pathogens and parasites. *Immunology* **164**, 401–432. doi:10.1111/imm.13390
- Ellner, S. P., Buchon, N., Dörr, T. and Lazzaro, B. P. (2021). Host-pathogen immune feedbacks can explain widely divergent outcomes from similar infections. *Proc. R. Soc. B* **288**, 20210786. doi:10.1098/rspb.2021.0786
- Engström, Y. (1999). Induction and regulation of antimicrobial peptides in *Drosophila*. *Dev. Comp. Immunol.* **23**, 345–358. doi:10.1016/S0145-305X(99)00016-6
- Fedorka, K. M., Kutch, I. C., Collins, L. and Musto, E. (2016). Cold temperature preference in bacterially infected *Drosophila melanogaster* improves survival but is remarkably suboptimal. *J. Insect Physiol.* **93–94**, 36–41. doi:10.1016/j.jinsphys.2016.08.005
- Ferguson, L. V. and Sinclair, B. J. (2020). Thermal variability and plasticity drive the outcome of a host-pathogen interaction. *Am. Nat.* **195**, 603–615. doi:10.1086/707545
- Ferguson, L. V., Heinrichs, D. E. and Sinclair, B. J. (2016). Paradoxical acclimation responses in the thermal performance of insect immunity. *Oecologia* **181**, 77–85. doi:10.1007/s00442-015-3529-6
- Ferreira, P. G., Tesla, B., Horacio, E. C. A., Nahum, L. A., Brindley, M. A., De Oliveira Mendes, T. A. and Murdock, C. C. (2020). Temperature dramatically shapes mosquito gene expression with consequences for mosquito–Zika virus interactions. *Front. Microbiol.* **11**, 901. doi:10.3389/fmicb.2020.00901
- Fischer, K., Kolzow, N., Holtje, H. and Karl, I. (2011). Assay conditions in laboratory experiments: is the use of constant rather than fluctuating temperatures justified when investigating temperature-induced plasticity? *Oecologia* **166**, 23–33. doi:10.1007/s00442-011-1917-0
- Galenza, A. and Foley, E. (2019). Immunometabolism: insights from the *Drosophila* model. *Dev. Comp. Immunol.* **94**, 22–34. doi:10.1016/j.dci.2019.01.011
- Gehman, A. M., Hall, R. J. and Byers, J. E. (2018). Host and parasite thermal ecology jointly determine the effect of climate warming on epidemic dynamics. *Proc. Natl. Acad. Sci. USA* **115**, 744–749. doi:10.1073/pnas.1705067115
- Ghazanfar, M. U., Hagenbucher, S., Romeis, J., Grabenweger, G. and Meissle, M. (2020). Fluctuating temperatures influence the susceptibility of pest insects to biological control agents. *J. Pest. Sci.* **93**, 1007–1018. doi:10.1007/s10340-020-01215-9

- Gherlenda, A. N., Haigh, A. M., Moore, B. D., Johnson, S. N. and Riegler, M. (2016). Climate change, nutrition and immunity: effects of elevated CO<sub>2</sub> and temperature on the immune function of an insect herbivore. *J. Insect Physiol.* **85**, 57–64. doi:10.1016/j.jinsphys.2015.12.002
- Gonzalez-Rete, B., Salazar-Schettino, P. M., Bucio-Torres, M. I., Cordoba-Aguilar, A. and Cabrera-Bravo, M. (2019). Activity of the phenoloxidase system and survival of triatomines infected with different *Trypanosoma cruzi* strains under different temperatures: understanding Chagas disease in the face of climate change. *Parasit. Vectors* **12**, 219. doi:10.1186/s13071-019-3477-9
- González-Santoyo, I. and Córdoba-Aguilar, A. (2012). Phenoloxidase: a key component of the insect immune system. *Entomol. Exp. Appl.* **142**, 1–16. doi:10.1111/j.1570-7458.2011.01187.x
- Gonzalez-Tokman, D., Cordoba-Aguilar, A., Dattilo, W., Lira-Noriega, A., Sanchez-Guillen, R. A. and Villalobos, F. (2020). Insect responses to heat: physiological mechanisms, evolution and ecological implications in a warming world. *Biol. Rev. Camb. Philos. Soc.* **95**, 802–821. doi:10.1111/brv.12588
- Gruntenko, N. E., Adonyeva, N. V., Burdina, E. V., Karpova, E. K., Andreenkova, O. V., Gladkikh, D. V., Ilinsky, Y. Y. and Rauschenbach, I. Y. (2016). The impact of FOXO on dopamine and octopamine metabolism in *Drosophila* under normal and heat stress conditions. *Biol. Open* **5**, 1706–1711. doi:10.1242/bio.022038
- Guo, H., Huang, C., Jiang, L., Cheng, T., Feng, T. and Xia, Q. (2018). Transcriptome analysis of the response of silkworm to drastic changes in ambient temperature. *Appl. Microbiol. Biotechnol.* **102**, 10161–10170. doi:10.1007/s00253-018-9387-5
- Haine, E. R., Moret, Y., Siva-Jothy, M. T. and Rolff, J. (2008). Antimicrobial defense and persistent infection in insects. *Science* **322**, 1257–1259. doi:10.1126/science.1165265
- Harvell, C. D., Mitchell, C. E., Ward, J. R., Altizer, S., Dobson, A. P., Ostfeld, R. S. and Samuel, M. D. (2002). Climate warming and disease risks for terrestrial and marine biota. *Science* **296**, 2158–2162. doi:10.1126/science.1063699
- Harvey, J. A., Heinen, R., Gols, R. and Thakur, M. P. (2020). Climate change-mediated temperature extremes and insects: from outbreaks to breakdowns. *Glob. Change Biol.* **26**, 6685–6701. doi:10.1111/gcb.15377
- Harvey, J. A., Tougeron, K., Gols, R., Heinen, R., Abarca, M., Abram, P. K., Basset, Y., Berg, M., Boggs, C., Brodeur, J. et al. (2022). Scientists' warning on climate change and insects. *Ecol. Monogr.* **93**, e1553. doi:10.1002/ecm.1553
- Hector, T. E., Sgrò, C. M. and Hall, M. D. (2020). The influence of immune activation on thermal tolerance along a latitudinal cline. *J. Evol. Biol.* **33**, 1224–1234. doi:10.1111/jeb.13663
- Hector, T. E., Sgrò, C. M. and Hall, M. D. (2021). Thermal limits in the face of infectious disease: how important are pathogens? *Glob. Change Biol.* **27**, 4469–4480. doi:10.1111/gcb.15761
- Hsu, S.-K., Belmouaden, C., Nolte, V. and Schlötterer, C. (2021). Parallel gene expression evolution in natural and laboratory evolved populations. *Mol. Ecol.* **30**, 884–894. doi:10.1111/mec.15649
- Huey, R. B. and Kingsolver, J. G. (2019). Climate warming, resource availability, and the metabolic meltdown of ectotherms. *Am. Nat.* **194**, E140–E150. doi:10.1086/705679
- Humphreys, J. M., Srygley, R. B. and Branson, D. H. (2022). Geographic variation in migratory grasshopper recruitment under projected climate change. *Geographies* **2**, 12–30. doi:10.3390/geographies2010003
- Hunt, V. L., Zhong, W., McClure, C. D., Mlynski, D. T., Duxbury, E. M. L., Keith Charnley, A. and Priest, N. K. (2016). Cold-seeking behaviour mitigates reproductive losses from fungal infection in *Drosophila*. *J. Anim. Ecol.* **85**, 178–186. doi:10.1111/1365-2656.12438
- Hurst, M. R. H., Beattie, A. K., Jones, S. A., Hsu, P.-C., Calder, J. and Koten, C. V. (2015). Temperature-dependent *Galleria mellonella* mortality as a result of *Yersinia entomophaga* infection. *Appl. Environ. Microbiol.* **81**, 6404–6414. doi:10.1128/AEM.00790-15
- Ittis, C., Martel, G., Thiéry, D., Moreau, J. and Louâpre, P. (2018). When warmer means weaker: high temperatures reduce behavioural and immune defences of the larvae of a major grapevine pest. *J. Pest. Sci.* **91**, 1315–1326. doi:10.1007/s10340-018-0992-y
- Ittis, C., Louâpre, P., Vogelweith, F., Thiéry, D. and Moreau, J. (2021). How to stand the heat? Post-stress nutrition and developmental stage determine insect response to a heat wave. *J. Insect Physiol.* **131**, 104214. doi:10.1016/j.jinsphys.2021.104214
- IPCC (2022). Climate change 2022: impacts, adaptation, and vulnerability. In *Contribution of Working Group II to the Sixth Report of the Intergovernmental Panel on Climate Change* (ed. H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegria, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem and B. Rama). Cambridge University Press. doi:10.1017/9781009325844
- Kankaanpää, T., Vesterinen, E., Hardwick, B., Schmidt, N. M., Andersson, T., Aspholm, P. E., Barrio, I. C., Beckers, N., Bêty, J., Birkemoe, T. et al. (2020). Parasitoids indicate major climate-induced shifts in arctic communities. *Glob. Change Biol.* **26**, 6276–6295. doi:10.1111/gcb.15297
- Karl, I., Stoks, R., De Block, M., Janowitz, S. A. and Fischer, K. (2011). Temperature extremes and butterfly fitness: conflicting evidence from life history and immune function. *Glob. Change Biol.* **17**, 676–687. doi:10.1111/j.1365-2486.2010.02277.x
- Kaunisto, S., Ferguson, L. V. and Sinclair, B. J. (2016). Can we predict the effects of multiple stressors on insects in a changing climate? *Curr. Opin. Insect Sci.* **17**, 55–61. doi:10.1016/j.cois.2016.07.001
- Khan, I., Agashe, D. and Rolff, J. (2017). Early-life inflammation, immune response and ageing. *Proc. R. Soc. B* **284**, 20170125. doi:10.1098/rspb.2017.0125
- King, A. M. and Macrae, T. H. (2015). Insect heat shock proteins during stress and diapause. *Annu. Rev. Entomol.* **60**, 59–75. doi:10.1146/annurev-ento-011613-162107
- Kingsolver, J. G. and Woods, H. A. (2016). Beyond thermal performance curves: modeling time-dependent effects of thermal stress on ectotherm growth rates. *Am. Nat.* **187**, 283–294. doi:10.1086/684786
- Kingsolver, J. G., Moore, M. E., Augustine, K. E. and Hill, C. A. (2021). Responses of *Manduca sexta* larvae to heat waves. *J. Exp. Biol.* **224**, jeb236505. doi:10.1242/jeb.236505
- Kirk, D., O'Connor, M. I. and Mordecai, E. A. (2022). Scaling effects of temperature on parasitism from individuals to populations. *J. Anim. Ecol.* **91**, 2087–2102. doi:10.1111/1365-2656.13786
- Kryukov, V. Y., Yaroslavlseva, O. N., Whitten, M. M. A., Tyurin, M. V., Ficken, K. J., Greig, C., Melo, N. R., Glupov, V. V., Dubovskiy, I. M. and Butt, T. M. (2018). Fungal infection dynamics in response to temperature in the lepidopteran insect *Galleria mellonella*. *Insect Sci.* **25**, 454–466. doi:10.1111/1744-7917.12426
- Kryukov, V. Y., Kosman, E., Tomilova, O., Polenogova, O., Rotskaya, U., Tyurin, M., Alikina, T., Yaroslavlseva, O., Kabilov, M. and Glupov, V. (2020). Interplay between fungal infection and bacterial associates in the wax moth *Galleria mellonella* under different temperature conditions. *J. Fungi* **6**, 170. doi:10.3390/jof6030170
- Kumar, S., Christophides, G. K., Cantera, R., Charles, B., Han, Y. S., Meister, S., Dimopoulos, G., Kafatos, F. C. and Barillas-Mury, C. (2003). The role of reactive oxygen species on *Plasmodium* melanotic encapsulation in *Anopheles gambiae*. *Proc. Natl. Acad. Sci. USA* **100**, 14139–14144. doi:10.1073/pnas.2036262100
- Kutach, I. C., Sevçili, H., Wittman, T. and Fedorka, K. M. (2014). Thermoregulatory strategy may shape immune investment in *Drosophila melanogaster*. *J. Exp. Biol.* **217**, 3664–3669. doi:10.1242/jeb.106294
- Laughton, A. M., O'Connor, C. O. and Knell, R. J. (2017). Responses to a warming world: integrating life history, immune investment, and pathogen resistance in a model insect species. *Ecol. Evol.* **7**, 9699–9710. doi:10.1002/ece3.3506
- Lazzaro, B. P. and Tate, A. T. (2022). Balancing sensitivity, risk, and immunopathology in immune regulation. *Curr. Opin. Insect Sci.* **50**, 100874. doi:10.1016/j.cois.2022.100874
- Le Lann, C., Van Baaren, J. and Visser, B. (2021). Dealing with predictable and unpredictable temperatures in a climate change context: the case of parasitoids and their hosts. *J. Exp. Biol.* **224**, jeb238626. doi:10.1242/jeb.238626
- Li, X., Ma, W. and Jiang, Y. (2022). Heat stress affects the expression of antimicrobial peptide genes in adult honeybee (*Apis cerana* and *Apis mellifera*). *Int. J. Trop. Insect Sci.* **42**, 2465–2471. doi:10.1007/s42690-022-00774-x
- Linder, J. E., Owers, K. A. and Promislow, D. E. (2008). The effects of temperature on host-pathogen interactions in *D. melanogaster*: who benefits? *J. Insect Physiol.* **54**, 297–308. doi:10.1016/j.jinsphys.2007.10.001
- Liu, X., Reitz, S. R., Lei, Z. and Wang, H. (2019). Thermoregulatory response of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) to infection by *Beauveria bassiana*, and its effect on survivorship and reproductive success. *Sci. Rep.* **9**, 13625. doi:10.1038/s41598-019-49950-z
- Lockey, T. D. and Ourth, D. D. (1992). Isolation and characterization of hemolymph phenoloxidase from *Heliothis virescens* larvae. *Comp. Biochem. Physiol.* **102B**, 891–896.
- Ma, C.-S., Ma, G. and Pincebourde, S. (2021). Survive a warming climate: insect responses to extreme high temperatures. *Ann. Rev. Entomol.* **66**, 163–184. doi:10.1146/annurev-ento-041520-074454
- Marshall, K. E. and Sinclair, B. J. (2015). The relative importance of number, duration and intensity of cold stress events in determining survival and energetics of an overwintering insect. *Func. Ecol.* **29**, 357–366. doi:10.1111/1365-2435.12328
- Mastore, M., Quadroni, S., Toscano, A., Mottadelli, N. and Brivio, M. F. (2019). Susceptibility to entomopathogens and modulation of basal immunity in two insect models at different temperatures. *J. Therm. Biol.* **79**, 15–23. doi:10.1016/j.jtherbio.2018.11.006
- Mckean, K. A. and Nunney, L. (2001). Increased sexual activity reduces male immune function in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **98**, 7904–7909. doi:10.1073/pnas.131216398
- Mckinstry, M., Chung, C., Truong, H., Johnston, B. A. and Snow, J. W. (2017). The heat shock response and humoral immune response are mutually antagonistic in honey bees. *Sci. Rep.* **7**, 8850. doi:10.1038/s41598-017-09159-4
- Molina-Cruz, A., Dejong, R. J., Charles, B., Gupta, L., Kumar, S., Jaramillo-Gutierrez, G. and Barillas-Mury, C. (2008). Reactive oxygen species modulate *Anopheles gambiae* immunity against bacteria and *Plasmodium*. *J. Biol. Chem.* **283**, 3217–3223. doi:10.1074/jbc.M705873200

- Molnár, P. K., Sckrabulis, J. P., Altman, K. A. and Raffel, T. R. (2017). Thermal performance curves and the metabolic theory of ecology—a practical guide to models and experiments for parasitologists. *J. Parasitol.* **103**, 423–439. doi:10.1064/16-148
- Moore, J. and Freehling, M. (2002). Cockroach hosts in thermal gradients suppress parasite development. *Oecologia* **133**, 261–266. doi:10.1007/s00442-002-1030-5
- Moore, M. E., Hill, C. A. and Kingsolver, J. G. (2021). Differing thermal sensitivities in a host–parasitoid interaction: high, fluctuating developmental temperatures produce dead wasps and giant caterpillars. *Func. Ecol.* **35**, 675–685. doi:10.1111/1365-2435.13748
- Mowlds, P. and Kavanagh, K. (2008). Effect of pre-incubation temperature on susceptibility of *Galleria mellonella* larvae to infection by *Candida albicans*. *Mycopathologia* **165**, 5–12. doi:10.1007/s11046-007-9069-9
- Murdock, C. C., Paaijmans, K. P., Bell, A. S., King, J. G., Hillyer, J. F., Read, A. F. and Thomas, M. B. (2012a). Complex effects of temperature on mosquito immune function. *Proc. Biol. Sci.* **279**, 3357–3366. doi:10.1098/rspb.2012.0638
- Murdock, C. C., Paaijmans, K. P., Cox-Foster, D., Read, A. F. and Thomas, M. B. (2012b). Rethinking vector immunology: the role of environmental temperature in shaping resistance. *Nat. Rev. Microbiol.* **10**, 869–876. doi:10.1038/nrmicro2900
- Musselman, L. P., Fink, J. L., Grant, A. R., Gatto, J. A., Tuthill, B. F. and Baranski, T. J. (2018). A complex relationship between immunity and metabolism in *Drosophila* diet-induced insulin resistance. *Mol. Cell Biol.* **38**, e00259-17. doi:10.1128/MCB.00259-17
- Muturi, E. J., Kim, C. H., Alto, B. W., Berenbaum, M. R. and Schuler, M. A. (2011). Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. *Trop. Med. Int. Health* **16**, 955–964. doi:10.1111/j.1365-3156.2011.02796.x
- Muturi, E. J., Nyakeriga, A. and Blackshear, M. (2012). Temperature-mediated differential expression of immune and stress-related genes in *Aedes aegypti* larvae. *J. Am. Mosq. Control Assoc.* **28**, 79–83. doi:10.2987/11-6194R.1
- Ouedraogo, R. M., Cusson, M., Goettel, M. S. and Brodeur, J. (2003). Inhibition of fungal growth in thermoregulating locusts, *Locusta migratoria*, infected by the fungus *Metarhizium anisopliae* var. *acridum*. *J. Invertebr. Pathol.* **82**, 103–109. doi:10.1016/S0022-2011(02)00185-4
- Ponton, F., Wilson, K., Cotter, S. C., Raubenheimer, D. and Simpson, S. J. (2011). Nutritional immunology: a multi-dimensional approach. *PLoS Pathog.* **7**, e1002223. doi:10.1371/journal.ppat.1002223
- Richards, E. H., Dani, M. P., Lu, Y., Butt, T. and Weaver, R. J. (2017). Effect of stress on heat shock protein levels, immune response and survival to fungal infection of *Mamestra brassicae* larvae. *J. Insect Physiol.* **96**, 53–63. doi:10.1016/j.jinsphys.2016.10.013
- Roberts, K. E., Hadfield, J. D., Sharma, M. D. and Longdon, B. (2018). Changes in temperature alter the potential outcomes of virus host shifts. *PLoS Pathog.* **14**, e1007185. doi:10.1371/journal.ppat.1007185
- Romero, L. M., Dickens, M. J. and Cyr, N. E. (2009). The reactive scope model — a new model integrating homeostasis, allostasis, and stress. *Horm. Behav.* **55**, 375–389. doi:10.1016/j.yhbeh.2008.12.009
- Sadd, B. M. and Siva-Jothy, M. T. (2006). Self-harm caused by an insect's innate immunity. *Proc. Biol. Sci.* **273**, 2571–2574. doi:10.1098/rspb.2006.3574
- Sánchez-Bayo, F. and Wyckhuys, K. A. G. (2019). Worldwide decline of the entomofauna: a review of its drivers. *Biol. Conserv.* **232**, 8–27. doi:10.1016/j.biocon.2019.01.020
- Schmid-Hempel, P. (2005). Evolutionary ecology of insect immune defenses. *Ann. Rev. Entomol.* **50**, 529–551. doi:10.1146/annurev.ento.50.071803.130420
- Schwenke, R. A., Lazzaro, B. P. and Wolfner, M. F. (2016). Reproduction-immunity trade-offs in insects. *Annu. Rev. Entomol.* **61**, 239–256. doi:10.1146/annurev-ento-010715-023924
- Seehausen, M. L., Naumann, P. H., Beliveau, C., Martel, V. and Cusson, M. (2018). Impact of rearing temperature on encapsulation and the accumulation of transcripts putatively involved in capsule formation in a parasitized lepidopteran host. *J. Insect Physiol.* **107**, 244–249. doi:10.1016/j.jinsphys.2018.04.013
- Sgro, C. M., Terblanche, J. S. and Hoffmann, A. A. (2016). What can plasticity contribute to insect responses to climate change? *Annu. Rev. Entomol.* **61**, 433–451. doi:10.1146/annurev-ento-010715-023859
- Shamakhi, L., Zibae, A., Karimi-Malati, A. and Hoda, H. (2019). Effect of thermal stress on the immune responses of *Chilo suppressalis* walker (Lepidoptera: Crambidae) to *Beauveria bassiana*. *J. Therm. Biol.* **84**, 136–145. doi:10.1016/j.jtherbio.2019.07.006
- Sharma, A., Nuss, A. B. and Gulia-Nuss, M. (2019). Insulin-like peptide signaling in mosquitoes: the road behind and the road ahead. *Front. Endocrinol.* **10**, 166. doi:10.3389/fendo.2019.00166
- Silva, F. W. S. and Elliot, S. L. (2016). Temperature and population density: interactional effects of environmental factors on phenotypic plasticity, immune defenses, and disease resistance in an insect pest. *Ecol. Evol.* **6**, 3672–3683. doi:10.1002/ece3.2158
- Sinclair, B. J., Ferguson, L. V., Salehipour-Shirazi, G. and Macmillan, H. A. (2013). Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects. *Integr. Comp. Biol.* **53**, 545–556. doi:10.1093/icb/ict004
- Sørensen, J. G., Schou, M. F., Kristensen, T. N. and Loeschcke, V. (2016). Thermal fluctuations affect the transcriptome through mechanisms independent of average temperature. *Sci. Rep.* **6**, 30975. doi:10.1038/srep30975
- Srygley, R. B. and Jaronski, S. T. (2022). Increasing temperature reduces cuticular melanism and immunity to fungal infection in a migratory insect. *Ecol. Entomol.* **47**, 109–113. doi:10.1111/een.13088
- St Leger, R. J. (2021). Insects and their pathogens in a changing climate. *J. Invertebr. Pathol.* **184**, 107644. doi:10.1016/j.jip.2021.107644
- Stahlschmidt, Z. R. and Adamo, S. A. (2013). Context dependency and generality of fever in insects. *Naturwissenschaften* **100**, 691–696. doi:10.1007/s00114-013-1057-y
- Sternberg, E. D. and Thomas, M. B. (2014). Local adaptation to temperature and the implications for vector-borne diseases. *Trends Parasitol.* **30**, 115–122. doi:10.1016/j.pt.2013.12.010
- Stoks, R., Verheyen, J., Van Dievel, M. and Tüzün, N. (2017). Daily temperature variation and extreme high temperatures drive performance and biotic interactions in a warming world. *Curr. Opin. Insect Sci.* **23**, 35–42. doi:10.1016/j.cois.2017.06.008
- Strand, M. R. (2008). The insect cellular immune response. *Insect Sci.* **15**, 1–14. doi:10.1111/j.1744-7917.2008.00183.x
- Suh, E., Grossman, M. K., Waite, J. L., Dennington, N. L., Sherrard-Smith, E., Churcher, T. S. and Thomas, M. B. (2020). The influence of feeding behaviour and temperature on the capacity of mosquitoes to transmit malaria. *Nat. Ecol. Evol.* **4**, 940–951. doi:10.1038/s41559-020-1182-x
- Sun, W., Shen, Y.-H., Zhou, L.-X. and Zhang, Z. (2016). Ecdysone titer determined by 3DE-3 $\beta$ -reductase enhances the immune response in the silkworm. *J. Immunol.* **196**, 1646–1654. doi:10.4049/jimmunol.1500158
- Tang, X., Li, N., Wang, W., Yu, J., Xu, L. and Shen, Z. (2016). DGE analysis of changes in gene expression in response to temperature and deltamethrin stress in the silkworm (*Bombyx mori*). *J. Asia-Pac. Entomol.* **19**, 45–50. doi:10.1016/j.aspen.2015.11.001
- Tate, A. T. and Schulz, N. K. E. (2022). The within-host ecology of insects and their parasites: integrating experiments and mathematical models. *Curr. Opin. Insect Sci.* **49**, 37–41. doi:10.1016/j.cois.2021.11.001
- Tesla, B., Demakovskiy, L. R., Mordecai, E. A., Ryan, S. J., Bonds, M. H., Ngonghala, C. N., Brindley, M. A. and Murdock, C. C. (2018). Temperature drives Zika virus transmission: evidence from empirical and mathematical models. *Proc. Biol. Sci.* **285**, 20180795. doi:10.1098/rspb.2018.0795
- Thomas, M. B. and Blanford, S. (2003). Thermal biology in insect-parasite interactions. *Trends Ecol. Evol.* **18**, 344–350. doi:10.1016/S0169-5347(03)00069-7
- Torson, A. S., Yocum, G. D., Rinehart, J. P., Kemp, W. P. and Bowsher, J. H. (2015). Transcriptional responses to fluctuating thermal regimes underpinning differences in survival in the solitary bee *Megachile rotundata*. *J. Exp. Biol.* **218**, 1060–1068. doi:10.1242/jeb.113829
- Triggs, A. and Knell, R. J. (2012). Interactions between environmental variables determine immunity in the Indian meal moth *Plodia interpunctella*. *J. Anim. Ecol.* **81**, 386–394. doi:10.1111/j.1365-2656.2011.01920.x
- Tüzün, N. and Stoks, R. (2021). Lower bioenergetic costs but similar immune responsiveness under a heat wave in urban compared to rural damselflies. *Evol. Appl.* **14**, 24–35. doi:10.1111/eva.13041
- Van Dievel, M., Stoks, R. and Janssens, L. (2017). Beneficial effects of a heat wave: higher growth and immune components driven by a higher food intake. *J. Exp. Biol.* **220**, 3908–3915.
- Vanha-Aho, L.-M., Valanne, S. and Rämet, M. (2016). Cytokines in *Drosophila* immunity. *Immunol. Lett.* **170**, 42–51. doi:10.1016/j.imlet.2015.12.005
- Vertyporokh, L., Taszlow, P., Samorek-Pieróg, M. and Wojda, I. (2015). Short-term heat shock affects the course of immune response in *Galleria mellonella* naturally infected with the entomopathogenic fungus *Beauveria bassiana*. *J. Invertebr. Pathol.* **130**, 42–51. doi:10.1016/j.jip.2015.07.001
- Wojda, I. (2017). Temperature stress and insect immunity. *J. Therm. Biol.* **68**, 96–103. doi:10.1016/j.jtherbio.2016.12.002
- Wojda, I. and Jakubowicz, T. (2007). Humoral immune response upon mild heat-shock conditions in *Galleria mellonella* larvae. *J. Insect Physiol.* **53**, 1134–1144. doi:10.1016/j.jinsphys.2007.06.003
- Yu, S., Luo, F., Xu, Y., Zhang, Y. and Jin, L. H. (2022). *Drosophila* innate immunity involves multiple signaling pathways and coordinated communication between different tissues. *Front. Immunol.* **13**, 905370. doi:10.3389/fimmu.2022.905370
- Zhao, X. and Karpac, J. (2021). Glutamate metabolism directs energetic trade-offs to shape host-pathogen susceptibility in *Drosophila*. *Cell Metab.* **33**, 2428–2444.e8. doi:10.1016/j.cmet.2021.10.003