Progresses of Cryoecology and Immune Stress and Defense in Insect

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Abstract: The effect of temperature on physiology mediates many of the challenges that insects face under climate change. Insect immunity is thermally sensitive and, as such, environmental change is likely to have complex effects on survival, disease resistance and transmission. The effects of temperature on immunity will be particularly profound in winter because cold and overwintering are important triggers and regulators of insect immune activity. Low temperatures can both suppress and activate immune responses independent of pathogen, which suggests that temperature not only affects the rate of immune responses but also provides information that allow overwintering insects to balance investment in immunity and other physiological processes that underlie winter survival. Changing winter temperatures are now shifting insect immunity, as well as the demand for energy conservation and protection against parasites. Whether an insect can survive the winter will thus depend on whether new immune phenotype will shift to match the conditions of the new environment, or leave insects vulnerable to infection or energy depletion. This paper synthesize patterns of overwintering immunity in insects and examine how new winter conditions might affect insect immunity,.

Key words: Insect; Low Temperature; Overwintering Survival; Immunoecology; Immune Stress; Immune Defense

1. Introduction

In the context of increasingly frequent freezing rain and snow disasters and climate warming, ectotherms, including insects, face unprecedented challenges, including changes in host-pathogen interactions caused by changes in environmental temperature[1]. The functions and activities of immune cells and enzymes, the main components of the insect immune system, are particularly sensitive to temperature, so the insect immunity is affected by the environmental temperature[2], and therefore, air temperature changes may have complex effects on their survival, disease resistance and population dispersal. The effect of temperature on immunity is especially profound in winter, because cold and overwintering are one of the important triggers and regulators of the insect immune response[2]. Low temperatures both inhibit and activate pathogen-independent immune responses in insects, suggesting that temperature not only affects the rate of immune responses, but also provides signals for overwintering insects to balance the resource allocation of immune and other physiological processes, as energy conservation is essential for successful overwintering.

Ecoimmunology, an emerging discipline, studies the causes and outcomes of changing immune function in evolutionary, ecological and physiological contexts, and then predicting the impact of disease suppression and transmission on population dynamics in the context of climate change. As global temperatures change with climate, it is becoming increasingly important to understand the physiological adaptations of organisms to respond to environmental changes. In the context of climate change, the goals of ecological immunologists and biological thermodynamics are necessarily intertwined, as predicting biological fitness from thermodynamic metrics becomes increasingly complex[3]. Therefore, overlap between disciplines is at the heart of research predicting the uncertain effects of climate change on insects.

In temperate, polar and alpine regions, more than half of insect life is wintering or preparing for overwintering[4]. The overwintering period is accompanied by cold stress, and insects also face more biotic and abiotic stresses, such as starvation, drought, hypoxia, energy loss, parasites and the threat of pathogens and other[4]. Therefore, overwintering insects are a good model to understand the interaction between multiple biotic stresses and abiotic stresses. The behavior and adaptability of insects in the growing season tend to change significantly after the arrival of winter, improving by reducing metabolism and storing more cold-resistant substances[4]. In the northern temperate regions, many insects can overwinter through diapause. In addition, insects also need to activate commonly used low-temperature protection mechanisms, including antioxidant defense, reducing water loss rate, and producing heat shock proteins[5]. Research on the biological stress faced by overwintering insects shows that microorganisms have strong resistance to low temperature, which generally can only inhibit their growth and reproduction, and rarely kills them. Microorganisms such as bacteria and pathogens that are still active in cold winter make insects face severe survival challenges. The discovered cryogenic microorganisms include both eubacteria, cyanobacteria, yeast, fungi and algal[6]. They have the ability to adapt to low temperature and are widely distributed in all cold environments of the earth, including alpine areas, deep sea areas, north and southern poles, glaciers, freshwater lakes and soil[7]. For example, psychrophilic fungi (e. g. *Metarhizium* and *Beauveria*) are found in overwintering micro-habitats, and these fungi are associated with mortality of various insects in spring[8]. Thus, insects may be required to activate the immune responses during the winter months.

However, the impact and regulation of low temperature on immunity require coordination and balance with other physiological responses to low temperature, which play important roles in winter survival and reproductive adaptation during the subsequent growth season. Current relevant studies have only just begun to explore and understand this relationship between overwintering and immunity, and lack a framework to predict the impact of immunity on insect survival in cold regions.

This study investigated the effects of low temperature and overwintering on the thermal biology and immune competence of the insect immune system. Analysis of insect immunophenotyping during overwintering may represent a coordinated balance between energy saving and cold stress responses as well as pathogen stress. Furthermore, the possibility that climate change could alter immune phenotypes and host-pathogen interactions was explored.

2. Ecological Immunology in a Low-Temperature Environment: The Role of Immunity in the Overwintering Survival of Insects

2.1 Thermal Dependence of Insect Immunity

The insect immune system is mainly an innate immune response including various cellular immunity and humoral immunity. Cellular immunity is mediated by blood cells, which circulate through the hemolymph and are mainly responsible for phagocytosis of pathogens and parasites and for assembly cyst[9]. Humoral immunity acts by enzymes (e. g., lysozyme and phenoloxidase)[10] and the antimicrobial peptide[11], and involves the regulation of the Toll, IMD, JNK, and JAK-STAT signal transduction pathways. These independent components of the immune system are differentially altered by environmental stress, and the immune system has the ability to reconfigure itself, such as enhanced cellular immunity that can compensate for impaired humoral immunity and vice versa[12]. Because the immune system has this ability to reconfigure, it is important to measure multiple components of the immune system to determine immune changes due to temperature, covering constitutive or potential immunity, as well as inducible defense and resistance to infection[13].

Insect immune cells and enzymatic activities are particularly sensitive to temperature, so their immune function is directly dependent on the ambient temperature[14-16]. Although research on the thermodynamics of insect immune systems is limited, immune function typically follows a typical thermodynamic curve and can be performed over a wide temperature range[14-18]. In the thermodynamic curve, the immune response reaches the functional optimum as a curve with increasing temperature, after which the immune function will rapidly decline at higher temperatures[3]. However, the thermodynamics of the immune system varies between species to accommodate the specific habitat climate of this species. For example, individuals of *Drosophila melanogaster* from tropical Africa have weaker immune activity at low temperatures compared to individuals from temperate North America (who may be more adapted to cold)[19]. This suggests that the thermodynamics of the immune system is optimized for the environmental temperature of species survival and may also co-evolve with the thermodynamics of local pathogens.

Temperature may alter the optimal temperature for immune function, or alter the temperature range in which immune responses can occur. In insects, the optimal temperature for cell-mediated pathogen immune responses is generally below the optimal temperature for their corresponding physiological and biochemical responses. For example, phagocytosis and melanization in *Anopheles stephensi* and *Gryllus veletis* were strongest at 18°C[14,16]. In contrast, the expression and enzymatic activity of the gene encoding nitric oxide synthase (responsible for the production of the immune signal molecule nitric oxide) peaked at 30°C[16]. This suggests that different components of the immune system can be adapted to function at different temperatures to act against pathogens with different thermodynamics or, alternatively, remodeling of the immune system under different temperature conditions needs to be considered. Importantly, the difference in thermodynamics of the various components of the immune system[21], again indicating that the functional activity of multiple components of the immune system is required to obtain a comprehensive understanding of the performance of immunity under different temperature conditions.

Cold exposure to insects can alter the immune function of insects in many ways [22]. For example, repeated cold exposure stress leads to increased survival of fungal infection in *Drosophila melanogaster*[23] and *Pyrrharctia isabella*[24], which may be that cold damage (including damage produced during cold recovery) activates the immune system to play a repair role, resulting in insect defense against pathogens. In contrast, low temperature reduced the level of phagocytosis of the *Samia cynthia pryeri* pupa, indicating that cold stress suppresses immune function[25]. However, it is not clear whether cold stress is a byproduct of cold damage and stress response (adaptive), or a nonadaptive result of the cross-talk between stress response and immune response. Therefore, it is necessary to gain insight into the molecular mechanisms linking cold stress with immune responses to determine whether their interactions are of adaptive significance.

Immune function is also plastic under conditions of temperature changes. The researchers found that cold acclimation and accimatization can alter insect immunity in winter. Enhanced insect immune function in cold environments is generally considered as a preventive response to pathogen stress or tissue damage, or as a compensatory response to a coordinated balance between immune and other physiological activities[22]. For example, overwintering *Nicrophorus vespilloides* has a high humoral response, an increased percentage of phagocytic blood cells, and enhanced phenoloxidase activity[26]. Overwintering *Bombus lucorum* queen were also observed with higher levels of phenolic oxidase activity[27]. The overwintering *Aquarius najas* has enhanced cyst function and higher survival[28]. There are many other similar acclimation responses, and cold exposure and diapause can activate the immune system of many insects, even in the absence of pathogen induction[23-24, 29-31]. In contrast, reduced immune function may prevent or limit autoimmune damage during overwintering, or redistribute immune investment for energy conservation with limited resource and energy availability[20]. Energy saving is crucial for successful overwintering[32], insect immune response and cold stress response need to pay a high cost[32]. For example, in *Gryllus veletis*, cold acclimation increases cold tolerance, but low temperature reduces melanization and humoral antibacterial activity[14], overwintering *Apis mellifera* downregulates the gene that encoding the antimicrobial peptide[33], and *Hetaerina americana* reduces resistance to bacterial infection in winter[34]. This suggests that insects have a coordinated balance between immune defense, cold tolerance, and energy saving, which may affect the seasonal patterns of immune function.

2.2 Seasonal Immunity to Insect Overwintering

Overwintering insects consume a lot of energy to cope with multiple stress factors including cold, and winter dormancy must protect energy reserves[4]. Thus, insects can sacrifice immune defense functions to save energy and assign them to cryoprotection mechanisms. It is generally believed that low temperature in winter will inhibit immune function, and the rate of immune response is relatively slow. The thermodynamic curve of immune function illustrates this problem[17]. However, although the diversity and density of pathogens can be reduced in winter, the selective effect of low temperature in winter also enables more infectious and virulent pathogens to survive[33], so individual insects with higher immune activity can improve the overwintering survival rate of this species[28].

During overwintering, insects are subjected to a series of seasonal, comprehensive and species-specific stresses. In the face of multiple stress factors, insects must optimize energy allocation between immune defense function and other functions related to fitness to survive[35].

To balance the coordinated balance between immunity and multiple other physiological processes necessary for overwintering survival, insects can reconfigure the energy of the immune defense system to produce different seasonal changes in different immune defense responses. For example, in many vertebrate ectotherms, innate defense responses (e. g. phagocytosis) are maintained or enhanced in winter, while acquired defense responses (e. g. antibody production) are inhibited, suggesting that costly activity reduces due to limited energy or resources[36]. However, few studies have specifically analyzed the energy costs of comparing innate versus acquired immunity during the winter period. Insects lack adaptive immune responses, possess species-specific immune functions, and seasonal immunity to disease susceptibility, indicating that the insect immune system may also undergo reconfiguration during overwintering[35]. The seasonal reconfiguration of the insect immune system is not only a product of the coordinated balance of high-cost and low-cost immune responses, but also of winter resource constraints and in response to pathogen stress. Moreover, the combination of these winter stress factors will also vary according on the physiology of different insect species and their biotic and abiotic stresses in different overwintering microhabitats[35]. To predict how winter affects insect immune function and the role of immune system reconstitution in overwintering survival requires insight into the underlying causes of immune system reconfiguration and whether these causes are universal across populations.

After prolonged cold exposure to overwintering, spring onset can improve immune defense capabilities (possibly a preventive measure)[22,37].

The increase in temperature in spring often leads to an increase in pathogen biological stress on insects[38]. Therefore, the rise in temperature may be a "signal" for insects, indicating the need to enhance their immune defense, resulting in an increase in their preventive immunity. For example, frogs that cooled from 26℃ to 21℃ were more susceptible to fungal than those warming from 16℃ to 21℃[39] , indicating that the increased temperature did indeed affect the immune response of ectotherms. So, fluctuating temperatures can also activate insect immune responses, for example, fluctuating low temperatures increase the expression of transcripts associated with the immune response of *Megachile rotundata*[40]. In the context of climate change, it is not clear whether the average winter temperature rises and exceeds a certain threshold, or if the seasonal immunity of insects is independent of the absolute winter temperature (thermodynamic temperature).

The overwintering immunity of insects is relatively complex, and the immune phenotype in winter may be formed by the combination of various influencing factors (Figure 1). Since insects have programmed and preventive changes in immune function in winter, whether they are subject to pathogen stress, the cross-talk between stress responses in response to multiple overwintering stresses should be the underlying the seasonal changes in immune function[22]. For example, immune responses activated by cold damage, byproducts of stress reactions or other seasonal products (multiple seemingly unrelated phenotypic effects caused by the expression of a single gene) [41], these non-adaptive immune responses can provide favorable effects for overwintering insects to defend against pathogens. In addition, the process of pathogen infection itself can also alter the immune function of insects (e. g., nematodes inhibit the activity of phenol oxidasewhen infecting *Galleria mellonella*[42]). Meanwhile, due to the significant impact of environmental temperature on infection outcomes, such as affecting host resistance, host recovery, pathogen virulence, etc., the interaction between host and pathogen will change under different temperature environments[43]. However, although researchers hypothesize that these are influencing factors of seasonal immune changes, the roles and relative importance of each factor in shaping immune phenotypes remain to be explored.

Dashed lines indicate prophylactic changes in immunity driven by trade-offs/energy constraints and parasites as selective pressures. Solid lines indicate direct effects of a variable on immunity. During overwintering, extreme temperatures could damage immune cells and tissues. Furthermore, energy/resource use and the presence of parasites during winter will also directly impact immune function. Overall, the resulting immune phenotype created through these mechanistic interactions will drive winter survival.

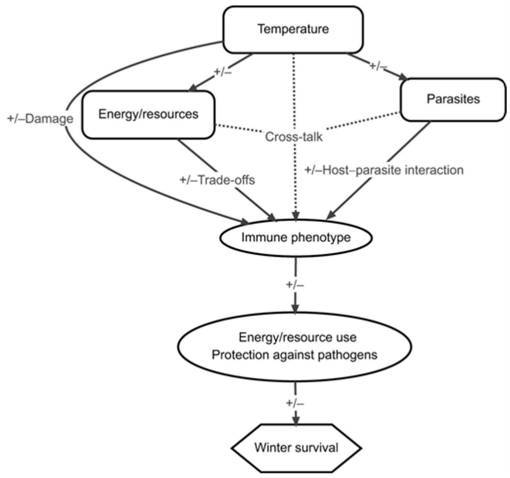

Figure 1. The Putative Mechanisms Underlying Seasonal Immune Phenotypes and the Interconnectedness of Each With Respect to Changes in Temperature

2.3 Impact of Winter Climate Change on Insect Immunity

Due to global warming, winter temperatures will become increasingly warmer, variable and unpredictable[4], which may alter the relative importance of immune-related winter survival influencing factors (i. e., energy saving and survival after infection) (Figure 1). In warmer or variable environments, the metabolic rates of ectotherms like insects may increase in[4], and winter climate change means that insects will face greater challenges to protect their limited energy storage. Also in such environments, infection and host-pathogen interactions can increase or decrease intensity[43]. Higher temperatures may increase the growth of native and new pathogens; instead, psychophilic microorganisms may no longer dominate[4]. Therefore, the success of overwintering will depend on whether the insect can produce new immunophenotypes under new winter conditions to achieve an appropriate balance between pathogen defense and energy conservation.

Currently, a model framework is lacking to describe how changing winter temperatures will affect immunity in overwintering insects. This is partly because experimental evidence suggests that the effects of temperature on immunity are species-specific and highly dependent on the type of environmental temperature experienced by the species. Higher mean air temperatures can increase the rate of the immune response in some insects, and fluctuating temperatures may have more complex effects on the immune response[44]. For example, exposure to fluctuating temperature conditions during overwintering increased immunity and disease resistance in *Pyrrharctia isabella*[24] and *Megachile rotundata*[29,40]. But *Hetaerina americana* were also observed to reduce their resistance to bacterial infection during the winter period[34] . Moreover, other studies have shown that seasonal immunity in some species may be regulated endogenously and is largely independent of temperature changes[45]. So far, it is not known why the changing overwintering temperature causes changes in the immune system, much less to predict the effect of variable temperature on insect immunity and survival fitness.

Each influence of seasonal immunity may

cause immune phenotype changes with climate (Table 1, Figure 1). Especially high temperatures and large fluctuations can provide new signals about environmental challenges, triggering physiological changes in insects to meet these new stress demands[44], thus forming new immunophenotypes. However, whether these signals are reliable and whether changes in immunity adapt to these new environments is unclear. If insects are subjected to increased biological stress in new environments with higher temperatures and greater fluctuations, then increasing energy investment in immunity may be beneficial(Table 1, Figure 2). But the increase of immune function also has such a possibility, similar to the in winter warmer weather, insects inappropriately reduced its cold tolerance function and improve the immune function to adapt to the environmental temperature "error", after temperature fluctuations into the low temperature state, insects but maintain the adjusted higher immune defense ability[46]. In such cases, if the insect immune function increases and the biological stress does not increase accordingly, it will only inappropriately increase the bodys energy and resource consumption, thus impairing other physiological processes necessary for winter survival. On the contrary, insects are susceptible to infection if pathogens stress increases and immune function is reduced or unchanged, or the organism chooses to save energy and allow energy diversion to other physiological functions (Table 1, Figure 2). In summary, the ability to predict insect overwintering survival is required to understand how each influencing factor contributes to seasonal immune changes.

Table 1. Potential Scenarios Driving Driving Changes in Immunity and Success Under Warmer and More Variable Winters

Mechanism	Condition	Scenario	Outcome for immune system	Match to new winter demands
Damage	Warmer winters	Less exposure to damaging temperatures	No damage to immune system	+ for energy savings + for protection against parasites
	Variable winters	More exposure to damaging temperatures (e.g. repeated cold)	Increased damage to immune system	– for energy savings – for protection against parasites
		Increased opportunity to repair damage	Increased strength of immune system	– for energy savings + for protection against parasites
Trade-offs		Increases in metabolic rate and energy use	Potential for resources to be unavailable for immunity	– for protection against parasites
Cross-talk		New signals	No change if endogenous regulation	+/– for protection against parasites (see Fig. 2)
			Changes to configuration of and investment in immune activity	+/– for energy savings (see Fig. 2)
Host–parasite interactions		Increased if permitting more parasite growth	Potential for changes to immune phenotype if new parasites can suppress the immune system	+ for energy savings – for protection against parasites
		Decreased if cold-active parasites inhibited	Less immune activation if infection decreases	+ for energy savings

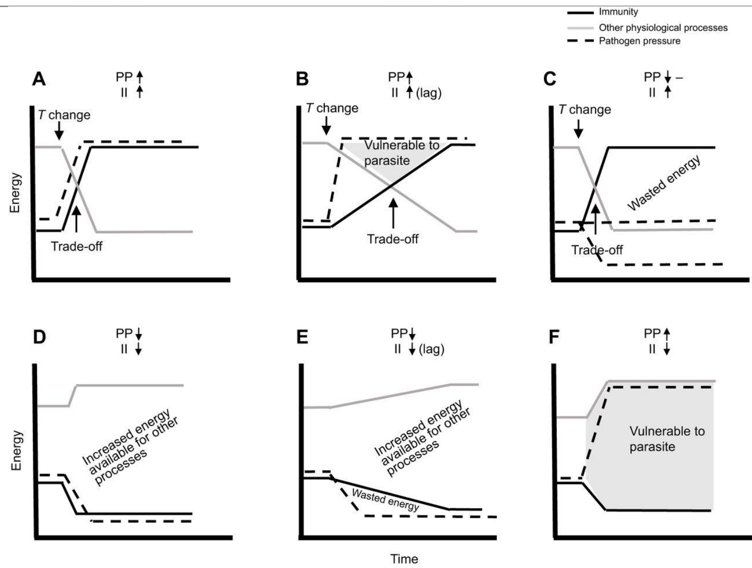

Figure 2. Potential Scenarios of Changes in Immune Investment under Temperature Changes and the Resultant Trade-Offs or Vulnerability to Pathogens

Dashed lines represent potential increases or decreases in pathogen pressure. *T*, temperature. (A) Immune investment (II) increases as pathogen pressure (PP) increases, providing protection against infection but trading-off energy/resources with other physiological processes. (B) As in A, but with a lag between the increase in pathogen pressure and immune investment, leaving a period of time in which insects are still vulnerable to infection. (C) Pathogen pressure decreases or remains unchanged but there is increased immune investment resulting from an unreliable cue in the environment, leading to wasted energy that cannot be used for other physiological processes. (D) Pathogen pressure decreases and immunity is suppressed, providing energy savings with no trade-off for pathogen protection. (E) As in D, but with a lag between the decrease in pathogen pressure and immune suppression (i.e. energy savings). (F) Pathogen pressure increases but immunity is suppressed as a result of an unreliable cue in the environment, leaving the insect vulnerable to infection.

2.4 The Prospects of Biological Thermodynamics and Ecological Immunology Research

All studies of climate change predict increased global temperature means as well as the magnitude of seasonal temperature changes. The nonlinear relationship between temperature and biological life activity implies that fluctuating temperature causes serious consequences that disrupt physiology, life history, and ecology. Currently, the combination of biological thermodynamics and ecoimmunology has delineated complex seasonal immune phenotypes and fluctuations in infection rates[20], which deserves further in-depth research, especially predicting how seasonal immunity responds to winter climate change. This aspect can be studied in two directions: (1) to understand the potential influencing factors of immune phenotype. In the conditions of climate change, the change direction of immune function will depend on the outcome of the seasonal immune change process (Table 1, Figure 2). Therefore, before starting to predict how winter changes will change immune phenotypes, it is important to understand the contribution of each influencing factor (e. g. interaction and injury) to seasonal immunity and whether these influencing factors will maintain or alter seasonal changes in immune function under conditions of winter climate change. (2) The thermodynamic curve of immune function was used to predict the seasonal changes. Thermodynamic curve[3] to explore the seasonal changes in immune function, will help to speculate the influencing factors behind these changes (pathogen stress and energy saving coordination balance) and its adaptive significance, and can predict the effect of winter climate change on infection rate, and predict the cost of immune function change (energy consumption) (figure 3). You can even map thermodynamic changes in various components of the immune system (cellular immune response and humoral immune response) to predict the adaptive significance of insect immune phenenotypes due to winter climate change (e. g., warmer, more variable climate).

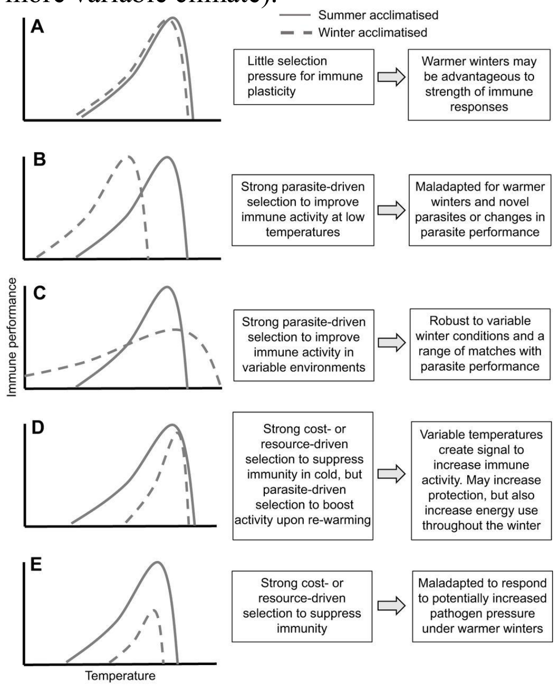

Figure 3. Inferring Adaptive Significance and Outcomes of Climate Change from Thermal Performance Curves of Immune Activity

The thermal plasticity of immune activity compared among seasons can help us to generate hypotheses about the selective pressures underlying these changes and their adaptive significance. From here, we can create predictions about the impact of changing winters on protection against parasites or the energetic consequences of changes in immune activity. (A) A lack of thermal plasticity of immunity. (B) A shift in immune activity that favour performance at low temperatures. (C) Increased breadth of immune thermal performance. (D) Narrowing of the breadth of immune thermal performance. (E) Overall decrease or suppression of immune activity.

Acknowledgments

This paper is supported by Topic: "Analysis and Application of Antibacterial Activity of Attacin", 2023 Young Talents Project of Tianshan Talents Training Program of Xinjiang Uygur Autonomous Region (2023TSYCQNTJ001645);The Young Science and Technology Talent Project of Changji Hui Autonomous Prefecture "Analysis and study of antibacterial activity of Attacin" (2023 QN 03);2024 Xinjiang Characteristic Pharmaceutical Resources Development and Application Innovation team of Xinjiang Agricultural Vocational and Technical College (XJNZYTD202401)

References

[1] Rohr JR, Palmer BD. Climate change, multiple stressors, and the decline of ectotherms [J]. Conservation Biology, 2013, 27 (4): 741-751.

[2] Sheldon BC, Verhulst S. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology [J]. Trends in ecology & evolution, 1996, 11 (8): 317-321.

[3] Sinclair BJ, Marshall KE, Sewell MA, *et al*. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? [J]. Ecology Letters, 2016, 19 (11): 1372-1385.

[4] Williams CM, Henry HAL, Sinclair BJ. Cold truths: how winter drives responses of terrestrial organisms to climate change [J]. Biological Reviews, 2015, 90 (1): 214-235.

[5] Storey KB, Storey JM. Insect cold hardiness: metabolic, gene, and protein adaptation [J]. Canadian Journal of Zoology, 2012, 90 (4): 456-475.

[6] Feller G. Molecular adaptations to cold in psychrophilic enzymes [J]. Cellular and Molecular Life Sciences, 2003, 60 (4): 648-662.

[7] Morita RY. Psychrophilic bacteria [J]. Bacteriological Reviews, 1975, 39 (2): 144-167.

[8] Bidochka MJ, Kasperski JE, Wild GAM. Occurrence of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* in soils from temperate and near-northern habitats [J]. Canadian Journal of Botany, 1998, 76 (7): 1198-1204.

[9] Lavine MD, Strand MR. Insect hemocytes and their role in immunity [J]. Insect biochemistry and molecular biology, 2002, 32 (10): 1295-1309.

[10] González-Santoyo I, Córdoba-Aguilar A. Phenoloxidase: a key component of the insect immune system [J]. Entomologia Experimentalis et Applicata, 2012, 142 (1): 1-16.

[11] Gillespie and JP, Kanost MR, Trenczek T. Biological mediators of insect immunity [J]. Annual review of entomology, 1997, 42 (1): 611-643.

[12] Adamo SA. The effects of stress hormones on immune function may be vital for the adaptive reconfiguration of the immune system during fight-or-flight behavior [J]. 2014, 54: 419-426.

[13] Fedorka KM, Linder JE, Winterhalter WE, *et al*. Post-mating disparity between potential and realized immune response in *Drosophila melanogaster* [J]. Proceedings of the Royal Society B: Biological Sciences, 2007, 274 (1614): 1211-1217.

[14] Ferguson LV, Heinrichs E, Sinclair BJ. Paradoxical acclimation responses in the thermal performance of insect immunity [J]. Oecologia, 2016, 181 (1): 77-85.

[15] Catalán TP, Wozniak A, Niemeyer HM, *et al*. Interplay between thermal and immune ecology: effect of environmental temperature on insect immune response and energetic costs after an immune challenge [J]. Journal of Insect Physiology, 2012, 58 (3): 310-317.

[16] Murdock CC, Paaijmans KP, Bell AS, et al. Complex effects of temperature on mosquito immune function [J]. Proceedings of the Royal Society B: Biological Sciences, 2012, 279 (1741): 3357-3366.

[17] Butler MW, Stahlschmidt ZR, Ardia DR, *et al*. Thermal sensitivity of immune function: evidence against a

generalist-specialist trade-off among endothermic and ectothermic vertebrates [J]. The American Naturalist, 2013, 181 (6): 761-774.

[18] Zimmerman LM, Carter AW, Bowden RM, *et al*. Immunocompetence in a long-lived ectothermic vertebrate is temperature dependent but shows no decline in older adults [J]. Functional ecology, 2017, 31 (7): 1383-1389.

[19] Lazzaro BP, Flores HA, Lorigan JG, *et al*. Genotype-by-environment interactions and adaptation to local temperature affect immunity and fecundity in *Drosophila melanogaster* [J]. PLoS pathogens, 2008, 4 (3): e1000025.

[20] Ferguson LV, Kortet R, Sinclair BJ. Eco-immunology in the cold: the role of immunity in shaping the overwintering survival of ectotherms [J]. Journal of Experimental Biology, 2018, 221 (13): jeb163873.

[21] Adamo SA. Estimating disease resistance in insects: phenoloxidase and lysozyme-like activity and disease resistance in the cricket *Gryllus texensis* [J]. Journal of insect physiology, 2004, 50 (2-3): 209-216.

[22] Sinclair BJ, Ferguson LV, Salehipour-shirazi G, *et al*. Cross-tolerance and cross-talk in the cold: relating low temperatures to desiccation and immune stress in insects [J]. Integrative and comparative biology, 2013, 53 (4): 545-556.

[23] Le Bourg É, Massou I, Gobert V. Cold stress increases resistance to fungal infection throughout life in *Drosophila melanogaster* [J]. Biogerontology, 2009, 10 (5): 613-625.

[24] Marshall KE, Sinclair BJ. The sub-lethal effects of repeated freezing in the woolly bear caterpillar *Pyrrharctia isabella* [J]. Journal of Experimental Biology, 2011, 214 (7): 1205-1212.

[25] Nakamura A, Miyado K, Takezawa Y, *et al*. Innate immune system still works at diapause, a physiological state of dormancy in insects [J]. Biochemical and biophysical research communications, 2011, 410 (2): 351-357.

[26] Urbański A, Czarniewska E, Baraniak E, *et al*. Impact of cold on the immune system of burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae) [J]. Insect Science, 2017, 24 (3): 443-454.

[27] Vesterlund SR, Lilley TM, van Ooik T, *et al*. The effect of overwintering temperature on the body energy reserves and phenoloxidase activity of bumblebee *Bombus lucorum* queens [J]. Insectes sociaux, 2014, 61 (3): 265-272.

[28] Krams I, Daukšte J, Kivleniece I, *et al*. Overwinter survival depends on immune defense and body length in male *Aquarius najas* water striders [J]. Entomologia experimentalis et applicata, 2011, 140 (1): 45-51.

[29] Xu J, James RR. Temperature stress affects the expression of immune response genes in the alfalfa leafcutting bee, *Megachile rotundata* [J]. Insect Molecular Biology, 2012, 21 (2): 269-280.

[30] Zhang J, Marshall KE, Westwood JT, *et al*. Divergent transcriptomic responses to repeated and single cold exposures in *Drosophila melanogaster* [J]. Journal of Experimental Biology, 2011, 214 (23): 4021-4029.

[31] Štětina T, Poupardin R, Moos M, *et al*. Larvae of *Drosophila melanogaster* exhibit transcriptional activation of immune response pathways and antimicrobial peptides during recovery from supercooling stress [J]. Insect biochemistry and molecular biology, 2019, 105: 60-68.

[32] Sinclair B J. Linking energetics and overwintering in temperate insects [J]. Journal of thermal biology, 2015, 54: 5-11.

[33] Steinmann N, Corona M, Neumann P, *et al*. Overwintering is associated with reduced expression of immune genes and higher susceptibility to virus infection in honey bees [J]. PloS One, 2015, 10 (6): e0129956.

[34] Córdoba-Aguilar A, Jiménez-Cortés JG, Lanz-Mendoza H. Seasonal variation in ornament expression, body size, energetic reserves, immune response, and survival in males of a territorial insect [J]. Ecological Entomology, 2009, 34 (2): 228-239.

[35] Ferguson LV, Sinclair BJ. Insect immunity varies idiosyncratically during overwintering [J]. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 2017, 327 (5):

222-234.

[36] Abram Q, Dixon B, Katzenback B. Impacts of low temperature on the teleost immune system [J]. Biology, 2017, 6 (4): 39.

[37] Salehipour-Shirazi G, Ferguson LV, Sinclair BJ. Does cold activate the *Drosophila melanogaster* immune system? [J]. Journal of insect physiology, 2017, 96: 29-34.

[38] Altizer S, Dobson A, Hosseini P, *et al*. Seasonality and the dynamics of infectious diseases [J]. Ecology letters, 2006, 9 (4): 467-484.

[39] Greenspan SE, Bower DS, Webb RJ, *et al*. White blood cell profiles in amphibians help to explain disease susceptibility following temperature shifts [J]. Developmental & Comparative Immunology, 2017, 77: 280-286.

[40] Torson AS, Yocum GD, Rinehart JP, *et al*. Transcriptional responses to fluctuating thermal regimes underpinning differences in survival in the solitary bee *Megachile rotundata* [J]. Journal of Experimental Biology, 2015, 218 (7): 1060-1068.

[41] Fedorka KM, Copeland EK, Winterhalter WE. Seasonality influences cuticle melanization and immune defense in a cricket: support for a temperature-dependent immune investment hypothesis in insects [J]. Journal of Experimental Biology, 2013, 216 (21): 4005-4010.

[42] Brivio MF, Pagani M, Restelli S. Immune suppression of *Galleria mellonella* (Insecta, Lepidoptera) humoral defenses induced by *Steinernema feltiae* (Nematoda, Rhabditida): involvement of the parasite cuticle [J]. Experimental parasitology, 2002, 101 (2-3): 149-156.

[43] Thomas MB, Blanford S. Thermal biology in insect-parasite interactions [J]. Trends in Ecology & Evolution, 2003, 18 (7): 344-350.

[44] Colinet H, Sinclair BJ, Vernon P, *et al*. Insects in fluctuating thermal environments [J]. Annual Review of Entomology, 2015, 60: 123-140.

[45] Sandmeier FC, Horn KR, Tracy CR. Temperature-independent, seasonal fluctuations in immune function of the *Mojave Desert Tortoise* (Gopherus agassizii) [J]. Canadian journal of zoology, 2016, 94 (8): 583-590.

[46] Sobek-Swant S, Crosthwaite JC, Lyons DB, *et al.* Could phenotypic plasticity limit an invasive species? Incomplete reversibility of mid-winter deacclimation in emerald ash borer [J]. Biological Invasions, 2012, 14 (1): 115-125.