Journal of Pest Science (2024) 97:1153–1169
https://doi.org/10.1007/s10340-023-01741-2

REVIEW

Insect resilience: unraveling responses and adaptations to cold temperatures

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Received: 2 August 2023 / Revised: 16 December 2023 / Accepted: 23 December 2023 / Published online: 22 February 2024

Abstract

Insects are vital arthropods that significantly impact various ecosystems. Their successful colonization of diverse habitats spanning from cold to warm environments is made possible by numerous adaptations shaped by environmental selection. This comprehensive review delves into the spectrum of physiological adaptations exhibited by insects to thrive in diverse environments, with a particular emphasis on the connection between these adaptations and the challenges posed by cold temperatures. Focusing on both long-term and short-term strategies, the review highlights the key protective mechanisms that insects employ to cope and thrive in cold temperatures. To withstand these constraints, insects have developed four main strategies: freeze tolerance, freeze avoidance, cryoprotective dehydration, and vitrification. These adaptive responses involve crucial physiological and biochemical changes that enable insects to withstand low temperatures. Specifically, insects exhibit cold tolerance through a range of molecular adaptive strategies, which encompass alterations in the expression of specific target genes, the synthesis of ice core formers, and the production of polyol cryoprotectants. Despite these remarkable results, there is still a lack of in-depth knowledge about the major factors contributing to successful overwintering of insects and their ability to withstand extremely low temperatures. To address these gaps, technological advances and genome sequencing of model organisms are critical to uncover the molecular mechanisms in insect responses to low temperatures. The knowledge gained from these advances provides valuable information about insect cold tolerance strategies and paves the way for a better understanding of their ecological importance and potential applications in conservation and ecological management.

Keywords Cold tolerance · Climate change · Protective mechanisms · Abiotic constraints · Environment · Physiological adaptations

Introduction

The most diverse and successful animal group on earth are insects, which have approximately one million known species (Stork 2018). Insects play crucial roles in nearly all ecosystems, including harsh and cold climates, deserts, and tropical forests (Harrison et al. 2012). Temperature is one of the most important abiotic factors that influence the life history of insects (Wang and Ma 2022; Ullah et al. 2022; Zhu et al. 2022). Insects can be found in different terrestrial environments despite variations in environmental temperatures (Sunday et al. 2011). The interaction of cold constraints and low tolerable temperatures might facilitate insect thermal tolerance and its association with biogeography (Kellermann et al. 2012; Alfaro-Tapia et al. 2022). Numerous studies have been conducted to learn more about the physiology that underpins insect cold tolerance variation (Teets and Denlinger 2013; Hayward et al. 2014). Insects exhibit a remarkable range of adaptations to changing environments. These adaptations have great diversity and complexity and can withstand seasonal adversity.

Adaptations of insects to cold climates can be divided into two groups, i.e., long-term and short-term adaptations. Over time, selection pressure is brought on by long-term adaptations. In cold-acclimated insects, better fitness is produced by selecting genotypes displaying phenotypic traits

Communicated by Bin Tang.

Z.L, N.D, and F.U designed the manuscript. F.U, A.A, and H.G prepared original draft. Z.L, F.U, M.H, A.A, H.G, B.G.G, L.C, R.R.R, A.G, and N.D critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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(physiological and behavioral). Short-term adaptations could be physiological and/or behavioral, e.g., if insects can detect cues about cold, they can hide in shelters to prevent death. Cold tolerance and tolerance of freezing stress are related concepts but have distinct differences regarding insect physiology and survival. Cold tolerance refers to the ability of insects to withstand and survive exposure to low temperatures. It encompasses various physiological, biochemical, and behavioral adaptations that allow insects to endure cold conditions. Tolerance of freezing stress, on the other hand, specifically refers to an insect's ability to survive the formation of ice crystals within its body during freezing temperatures. Insects that are freezing tolerant can withstand the physical damage caused by ice formation and recover upon thawing (Lee et al. 1987; Block 1990; Turnock and Fields 2005).

Currently, there are four recognized strategies for insects to survive cold temperatures: (1) freeze tolerance, (2) freeze avoidance, (3) cryoprotective dehydration, and (4) vitrification (Purać et al. 2016). Freeze tolerance is a survival strategy observed in insects that enables them to withstand internal ice formation. In freeze-tolerant insects, ice forms inside their bodies, which presents several challenges, such as cellular dehydration and mechanical damage. However, these insects have developed mechanisms to control the quality and quantity of ice, prevent or repair cellular and macromolecular damage, manage biochemical processes while frozen/thawing, and restore physiological processes post-thaw. The molecular basis and underlying mechanisms of freeze tolerance have been minimally explored, but it is believed to have evolved repeatedly in insects inhabiting environments with low temperatures and/or high risk of freezing (Toxopeus and Sinclair 2018). Certain insects, especially those in arctic environments, employ the freeze avoidance strategy to survive cold temperatures. Despite low temperatures, these insects keep their body fluids in a liquid state by producing ice-nucleating agents that foster the slow formation of ice crystals outside cells. This prevents intracellular ice formation and subsequent cellular damage. Some species of spiders and mites employ antifreeze proteins in their blood to prevent freezing altogether. This process, known as supercooling, allows them to remain in a liquid state until reaching the freezing point (Storey and Storey 2012). Some species host symbiotic bacteria and/or fungi in their guts that produce ice-nucleating agents, facilitating controlled freezing at relatively warm temperatures. The presence of these microbes in the gut may play a crucial role in supporting the widespread ability of alpine insects in New Zealand to tolerate freezing (Storey and Storey 2012; Morgan-Richards et al. 2023). Insects that utilize cryoprotective dehydration as a survival strategy reduce their metabolic activities and convert a significant portion of their body water into ice. This partial dehydration helps them survive freezing temperatures and harsh winter conditions. Cryoprotective dehydration allows insects to emerge early in spring when temperatures rise, offers protection against predators, and enables them to inhabit unique environments with freezing temperatures. This strategy has been observed in many cold-adapted invertebrates, particularly those in temperate and colder climates (Bale 1996; Storey and Storey 2012). Vitrification, the process by which a liquid loses its ability to flow and becomes brittle after cooling (Wasylyk et al. 1988), is a less common strategy observed in some insects. It's a critical physiological response in the cold tolerance of insects, particularly in freezing-tolerant species. This process involves the transformation of bodily fluids into a non-crystalline, glass-like state, preventing the formation of ice crystals that can be detrimental to cellular structures (Toxopeus and Sinclair 2018). Unlike freezing, vitrification avoids ice nucleation, reducing the risk of cellular damage during exposure to sub-zero temperatures. The vitrification process is linked to water, ice nucleators, and inoculation. These mechanisms remain vital components of insect cold survival, with water and ice nucleators influencing the efficacy of vitrification (Rozsypal 2015). Evidence of this strategy was found for the first time in a gall fly larva *Eurosta solidaginis* (Diptera: Tephritidae) in Texas (Wasylyk et al. 1988), and then on the beetle *Cucujus clavipes puniceus* (Coleoptera: Cucujidae) larvae in Alaska (Sformo et al. 2010). The mechanisms and prevalence of vitrification in insects are less studied than other strategies (Lee and Denlinger 1991; Clark and Worland 2008).

The physiological adaptations that allow insects to survive or avoid freezing include the production of antifreeze proteins, the removal of ice nucleators, extreme dehydration, and the accumulation of cryoprotectants (Duman 2001; Holmstrup et al. 2002; Sinclair et al. 2003). The adaptations and responses of insects and their underlying mechanisms have been extensively studied (Bale 2002; Danks 2005). The tolerance of freezing was quantified in several species of *Drosophila* as chill coma onset temperature (CT_{min}), chill coma recovery time (CCRT), and temperature that causes 50% mortality (LTe_{50}) (Andersen et al. 2015a). This review focuses on the physiological adaptations and important defense mechanisms used by insects to deal with cold constraints. We also discuss the newest technologies used in insect cold tolerance studies.

Phenotypic responses to cold constraints

Understanding the responses of insects to cold constraints necessitates exploration across various biological levels, encompassing the phenotype, tissue, and molecular domains. At the molecular level, insects exhibit intricate adaptations to cold stress. Notably, cold-induced stress

triggers molecular and cellular responses that are crucial for adaptation and acclimation. This involves changes in ion balance, membrane fluidity, and cellular homeostasis (Overgaard and MacMillan 2017; Enriquez and Colinet 2019; Overgaard et al. 2021).

At the molecular level, cold exposure induces fundamental changes in the insect's cellular and biochemical processes. This includes alterations in ion balance, as evidenced by the loss of K^+, Na^+, and H_2O equilibrium during chronic cold stress (Overgaard and MacMillan 2017). Molecular and cellular responses associated with cold adaptation involve significant adjustments to maintain homeostasis during cold exposure (MacMillan et al. 2015c). The impact of cold constraints at the tissue level becomes evident in physiological markers. Chronic exposure to low temperatures can lead to chilling injury, prolonged chill coma recovery, and disruptions in extracellular ion concentrations (MacMillan et al. 2015d). Physiological systems associated with muscle force, synaptic transmission, and epithelial barrier integrity are particularly sensitive to cold stress, manifesting at the tissue level (Tarapaki et al. 2021).

The phenotype level encapsulates observable outcomes of cold stress. Insects subjected to cold constraints display distinct phenotypic effects such as chill coma, chill injury, and mortality. These phenotypic responses are closely linked to the molecular and tissue-level changes, highlighting the interconnectedness of physiological processes in the cold-stressed insect (MacMillan et al. 2015d; Overgaard and MacMillan 2017). Understanding insect physiologies under cold constraints emphasizes the diverse and intricate mechanisms operating at molecular, tissue, and phenotype levels (MacMillan et al. 2015d; Enriquez and Colinet 2019; Tarapaki et al. 2021).

Chill susceptibility

Chill susceptibility alludes to the susceptibility of insects to physiological and cellular damage caused by exposure to low but non-freezing temperatures. The relevance of insect chill tolerance lies in its direct correlation with insect chill susceptibility, shaping the ability of insects to thrive in variable environmental conditions (Table 1). Chill tolerance refers to an organism's capacity to endure and function optimally at low temperatures, while susceptibility reflects the degree to which an insect is adversely affected by cold stress (Overgaard and MacMillan 2017). Chill susceptibility measures an insect's sensitivity to cold stress and its ability to withstand sublethal cold temperatures without suffering detrimental effects. In this sense, more chill-susceptible insects are more likely to experience physiological disruptions or even death when exposed to temperatures below their tolerance threshold (Colinet and Hoffmann 2012; Sørensen et al. 2013). When insects are exposed to cold temperatures, their main goal is to survive and resume their development, growth, and reproduction when the conditions become favorable. Under cold constraints, a fitness test is needed to accurately measure cold tolerance. These traits include population growth rates, sterility, fecundity, sperm count, mating, and courting success. These traits reveal adaptive variation among the species in different thermal environments (Shreve et al. 2004; Overgaard et al. 2007). In recent studies, the number of tolerance assays has been accelerated, and the critical thermal minimum (CT_{min}, steady fall of temperature at which insects suffer lack of neuromuscular activity) is used to assess the chill susceptibility or tolerance (Andersen et al. 2015a). Chill injury can also be measured using LT_{50} (50% of organisms tolerate a particular period of cold temperature or the time at which 50% of mortality occurs) (Sinclair et al. 2015). Insect chill tolerance is measured using CT_{min}, CCRT, damage, and survival. Importantly, these trait measures strongly correlate with species distribution (Kellermann et al. 2012; Overgaard et al. 2014).

Insect thermal ecology depends on the physiological adaptations influencing the variation in chill injury and chill coma phenotypes. Kostal et al. (2004) three acclimation groups [i.e. non-diapause (LD), diapause (SD) and diapause, cold-acclimated (SDA)] in adult firebugs, *Pyrrhocoris apterus*, exhibiting significant differences in chill tolerance levels. The investigation delved into the relationships between pre-freeze mortality, exposure duration to − 5 °C, recovery time post-chill coma, hemolymph volume, and fat body cell hydration in *P. apterus* adults from the three acclimation groups. The chill tolerance levels varied significantly among LD, SD, and SDA groups. LD adults showed 50% mortality after 7.6 days at − 5 °C, while SD adults required 35.6 days for the same mortality rate. SDA adults displayed no increase in mortality after 60 days of exposure. As exposure time to − 5 °C increased, the recovery time to resume locomotion post-chill coma linearly increased, with the shortest recovery time observed in the SDA group and the longest in the LD group. The mean hemolymph volume in the LD group rapidly decreased, reaching levels comparable to the SDA group after 8 days. Additionally, the SD group exhibited a slower loss of whole-body fat mass compared to the LD group during 35 days of exposure. Notably, the SDA group demonstrated a significant trend of fat body dehydration compared to the SD and LD groups.

Chill coma onset

Insect activity is impacted by temperature changes. If the temperature increases or decreases, the insect activity is affected, showing no response to external stimuli and

Table 1 Synthesis and differences among key concepts related to cold hardiness in insects

	Definition	Method	Measurement	Indicator
Chill susceptibility	Insect's vulnerability to the deleterious effects of exposure to cold temperatures. Is often associated with disruptions in ion and water homeostasis across membranes and epithelia, especially in chill-susceptible species	Assess the ability of insects to survive and function at low temperatures	Assessing the temperature at which an insect enters a state of chill coma onset. Evaluate behavioral and physiological changes, such as chill coma recovery time (CCRT) and the ability to maintain ion homeostasis at low temperatures	Higher chill susceptibility indicates greater sensitivity to cold stress and a lower tolerance to low temperatures
Chill Coma Onset	Point at which an insect enters a reversible comatose state when exposed to low temperatures. This state, known as chill coma, is characterized by a temporary loss of neuromuscular function due to decreased membrane potential caused by cooling	Determine the temperature at which insects enter chill coma	Use chill coma recovery time (CCRT), measuring the duration before an insect recovers normal functions after returning to room temperature	An earlier chill coma onset implies a lower threshold for neuromuscular dysfunction in response to cold exposure
Chill Injury	Physiological damage and adverse effects an insect incurs from exposure to low temperatures	Assess damage and adverse effects caused by exposure to low temperatures	Quantify physiological disruptions, including defects in the ability to fly, walk, or stand, and disruptions in ion and water homeostasis across membranes and epithelia	Increased chill injury indicates greater harm to physiological processes due to cold exposure

Based on Fitzgerald et al. 1996; Kostal et al. 2004; Overgaard et al. 2007; Terblanche et al. 2007; Colinet and Hoffmann 2012; Sørensen et al. 2013, MacMillan et al. 2015b; MacMillan et al. 2016b and Toxopeus & Sinclair 2018

eventually becoming neuromuscularly paralyzed (chill coma) (Terblanche et al. 2007). When the neuromuscular function is impaired and the insect is fully paralyzed, the chill coma onset temperature (CCO) is generally followed by a temperature that causes this stupor (MacMillan and Sinclair 2011). Many species recover from mild cold exposure and do not go into a chill coma (Terblanche et al. 2007). In a study with *Drosophila* insect species, it was found that variations in their ability to maintain ion and water balance at low temperatures explain the wide range of chilling tolerance, with chill-susceptible species losing ion selectivity and experiencing imbalanced ion concentrations, while chill-tolerant species maintain stability and can even recover from cold-induced comas (MacMillan et al. 2015a). In extremely cold conditions, insects go into a chill coma and prolong the time it takes to regain their fly efficiency until they adjust to warmer temperatures. Hence, another common and useful measure of chill tolerance is the chill coma recovery time (CCRT, the time for an insect to rise after exposure to cold) (Kostal et al. 2004). Insects get injured in an extremely cold environment, and ultimately death occurs. Neuromuscular phenotypes, such as gait defects, flying inability, or raising a leg, are all signs of sublethal injury (MacMillan et al. 2014).

Warm-adapted tsetse flies entered a chill coma at a temperature above 15 °C. In comparison, cold-adapted midges were observed in the Himalayas, where the environmental temperature decreases as low as − 16 °C (Terblanche et al. 2007). In chill coma onset temperature, interspecific variations have been observed in the species (Kellermann et al. 2012; Andersen et al. 2015a). In different insect species, cold exposure is linked to a significant depolarization of muscle resting membrane potential. The temperature at which an insect experiences a chill coma is often correlated with the threshold degree of muscle cell depolarization (Hosler et al. 2000). The extreme depolarized level linked with the beginning of a coma is often about − 40 mV membrane potential, considering the level of muscle stimulation (Andersen et al. 2015b). Due to insect membrane depolarization, the voltage-sensitive Ca^{2+} channels that activate the AP upstroke become disabled (Findsen et al. 2016).

Increased extracellular $[K^+]$ inhibits locust muscle force production in vitro at room temperature, resulting in membrane depolarization (Findsen et al. 2014). In *Drosophila* larvae, L-type Ca^{2+} channels responsible for AP are inactivated by low temperatures (Frolov and Singh 2013). According to previous studies, nervous and synaptic activity could be preserved at temperatures near or below the onset of coma (Findsen et al. 2016). In *Drosophila melanogaster* (Diptera: Drosophilidae), neuronal silence in the meta-thoracic ganglion and brain is linked to chill coma (Armstrong et al. 2012). Muscle contraction with differential expression of proteins may be involved in cold adaptation. During cold adaptation and repetitive thermal constraints, *Drosophila* changes the expression of contractile proteins (MacMillan et al. 2016a).

Chill injury

In chill-susceptible insects, prolonged cold exposure leads to cellular depolarization, unlike acute cold exposure. Chronic cold exposure generates and raises $[K^+]$ levels in the hemolymph. $[K^+]$ homeostasis is essential for maintaining a negative resting membrane potential (Vm) (Fitzgerald et al. 1996). Insects, like other animals, have high potassium permeability. Hence, the transmembrane distribution of $[K^+]$ accounts for the bulk of cellular diffusion potential (Dawson et al. 1989). The Cinereous Cockroach, *Nauphoeta cinerea* (Blattodea: Blaberidae), maintained constant concentrations of ions in their hemolymph and developed chilling injury that was significantly suppressed at 5 °C for 7 days (Koštál et al. 2006). During persistent cold exposure, the degree of chill injury suffered by insects is frequently associated with the loss of $[K^+]$ balance (MacMillan et al. 2015b, 2016b). Several studies also discovered that 100–200% rise in extracellular $[K^+]$ is commonly associated with the duration of cold exposure that causes 50% injury and/or mortality. While improving insect chill tolerance, cold acclimation, exposure to different temperatures, and cold adaptation to protect transmembrane $[K^+]$ dispersion (Koštál et al. 2007; Alvarado et al. 2015). The significance of elevated extracellular $[K^+]$ during chill injury was explored in vitro to examine the effects of hyperkalemia and cold on locust muscle cells. As a result, cell death may occur (MacMillan et al. 2015c). It was formerly considered that a breakdown of ion homeostasis caused cold-induced depolarization because ion balance is typically disturbed after persistent cold exposure. Several investigations have revealed that brief or mild cold exposures that elicit chill coma have little or no effect on ion homeostasis (Des Marteaux and Sinclair 2016). At low temperatures, cold-induced cell depolarization may cause the death of insect cells. The entrance of voltage-sensitive Ca^{2+} channels triggers an unregulated stimulation of Ca^{2+-} dependent lipases and proteases, resulting in cell death (Koštál et al. 2016; MacMillan et al. 2016b). Cold-induced cell death in the fruit fly, *D. melanogaster* flying muscles, is predominantly driven by apoptosis (Yi et al. 2007). Apoptosis is an early event in cell membrane depolarization produced by the $[K^+]$ gradient (Bortner et al. 2001). Inhibiting the activation of caspases, which ordinarily launch the apoptotic cascade, can also reduce the severity of chill injury (Yi and Lee 2011). If apoptosis is unavoidable, autophagy might help wounded cells recover from cold constraints (Gerken et al. 2015). Hence, inhibiting apoptotic

signaling pathways might have a key role in avoiding cell death during chilling.

Insect's physiology under cold constraints

Antifreeze proteins

Antifreeze proteins (AFPs) are essential for the survival of certain insect species in cold environments by preventing the formation of ice crystals and reducing freezing damage. Antifreeze proteins have been found in high concentrations in some freeze-resistant insects. They protect the insect from freezing by delaying the ice formation and reducing the freezing point of the hemolymph below the melting point (Wen et al. 2016; Duman and Newton 2020). Antifreeze proteins help freeze-resistant organisms overcome freezing in the winter. However, they can be found in species that survive in extremely cold environments (freeze-resistant species) and have significant antifreeze activity (Bale 2002). Furthermore, they protect freeze-tolerant species from recrystallization. By selecting AFP isoform combinations for ice-nucleating bacteria eradication, many polyols and glycerols assist them in preventing freezing. The antifreeze proteins have been observed in several insects, including the spruce budworm *Choristoneura fumiferana* (Lepidoptera: Tortricidae), the darkling beetle *Meracantha contracta* (Coleoptera: Tenebrionidae), snow scorpion flies, stone flies, milkwort bugs, budworm moths, wood cockroaches, Alaskan insects, and spiders (Tyshenko et al. 2005; Clark and Worland 2008). Antifreeze proteins (AFPs) have been derived from several parts of insects, including the heart, liver, and stomach (Chi Fai Cheung et al. 2017). Sequencing, cloning, and subsequent procedures of the insect antifreeze protein genes have been examined and produced in an *E. coli* transgenic system. In contrast, others have been produced in transgenic insect lines (Graham and Davies 2005). Neuroendocrine can control the regulation of genes; cfAFP337 from *C. fumiferana* and DAFP-1 from *Dendroides canadensis (Coleoptera:Pyrochroidae)* were first reported in *Drosophila* (Clark and Worland 2008). In transgenic flies, DAFP-1 could live longer at 0, while the transgenic line cfAFP337 showed a significant amount of thermal hysteresis (Clark and Worland 2008).

Cryoprotectants

Adaptive tactics present in an insect body are responsible for the survival of insects in severe conditions. Cryoprotectants in insects are crucial for antifreezing as they enable the organisms to survive extremely cold temperatures by preventing the formation of ice crystals within their cells, thereby preserving cellular integrity and metabolic function (Duman 2001). Cryoprotectants in the form of glycerol and sorbitol were reported for the first time from the *Bombyx* silkworm diapausing eggs (Chino 1957). The significance of cryoprotectants for the winter survival of insects was promptly acknowledged and subsequently explored by Wyatt (1961). In addition, a multi-cryoprotectants system in 1982 was identified based on the three components: trehalose, glycerol, and polyhydric alcohol (mannitol, sorbitol threitol) and low molecular weight sugars (Sømme 1982). To adapt to harsh conditions, insects accumulate trehalose molecules in their fluid bodies, which increase their resistance to freezing. Trehalose is found in most biological organisms and protects the cells and proteins against extreme cold injury (Gibney et al. 2015). Wen et al. (2016) determined the trehalose level in the hemolymph of *D. canadensis* larvae and reported that antifreeze proteins (AFPs) have a key role in inhibiting trehalose crystallization. The presence of trehalose also increases the antifreeze proteins in insects. AFPs attach to the particular surface of ice crystals in the hemolymph of cold-adapted animals to prevent the development of ice crystals (Davies 2014). Insect tolerance to cold is due to the production of molecular adaptive strategies like changes in expressions, ice-nucleator synthesis, and polyol cryoprotectants production (Sømme 1982; Pfister and Storey 2006; Clark and Worland 2008). In summer, during catabolism, the polyols are derived from accumulated glycogen storage (Worland et al. 2006). A signal transduction cascade system at different temperatures ranging from 5 to − 5 °C may activate the production of glycogen phosphorylase (Pfister and Storey 2002).

The cold reaction of protein phosphate 1 (PP1) and glycogen phosphorylase kinase (GPK) controls the glycogen phosphorylase, resulting in polyol synthesis in insects (Chowanski et al. 2015). Hormones and temperature stimulation also influence polyol synthesis due to the changes in the enzymatic apparatus involved in diapause and development (Clark and Worland 2008). Likewise, investigations have indicated changes in the activities of other enzymes in response to seasonal variations. For example, studies on the European corn borer [*Ostrinia nubilalis* (Lepidoptera: Crambidae)] revealed altered activities of enzymes like lactate dehydrogenase (Uzelac et al. 2020), while seasonal changes were implicated in the variations of phosphofructokinase in the goldenrod gall fly (*E. solidaginis*) as documented by Storey (1982). By freezing the bodily fluid, they develop very effective cryoprotectant systems, such as polyols and ice-nucleating proteins, that increase their freeze tolerance (Li 2016). In addition, it is also reported that polar species adopt several changes, like cryoprotective dehydration, in severe environmental conditions (Storey and Storey 2012). In difficult situations, cryopreservation tactics are effective for specific cells in their nature, like organs, tissues, red blood

cells, and conservation cells. *Gryllus veletis* (Orthoptera: Gryllidae) are freeze-tolerant by accumulating myo-inositol, proline, and trehalose in their hemolymph and body fats. These enzymes in crickets are important for survival at low temperatures for extended periods (Toxopeus et al. 2019). Similarly, for the survival of fat body cells in crickets, the exogenous myo-inositol and trehalose are essential.

Heat shock proteins

Heat shock proteins (HSPs) are induced when the insects are exposed to various constraints, including cold shock. Stressful conditions related to the environment are responsible for the synthesis of HSPs in insects (Overgaard and MacMillan 2017). These are the proteins related to stress and are only expressed in the reaction of external stress conditions like oxidation, starvation, chemicals, infection due to parasites, heavy metals, ultraviolet radiation, and extreme temperature exposure (Hao et al. 2007; Planelló et al. 2011; Liu et al. 2012; Sang et al. 2012; Wang et al. 2012; Zhang et al. 2015a, b; Hu et al. 2018). HSPs showed several biological functions in morphogenesis, early embryogenesis, and diapause (Hendrick and Hartl 1993). Depending on the classification, sequences' similarities, molecular masses, and function, the HSPs are divided into six families (Garrido et al. 2012). In insects, four major families are recognized with several co-chaperones, all sharing the common function of maintaining cell homeostasis through interaction with substrate proteins (King and MacRae 2015). The decreases in protein synthesis enhance the production of HSPs when insects are exposed to external environmental constraints such as crowding, cold, anoxia, and heat. Heat shock proteins are associated with co-chaperones often working in networks, e.g., J domain, also known as Hsp40 protein (confirming the homeostasis in intracellular protein) (Shorter 2011; Clare and Saibil 2013). They are also connected to the folding and localization of proteins, preventing protein aggregation (Hartl and Hayer-Hartl 2002).

In insects, *Hsp* mRNA or protein induction appears due to environmental constraints like cold shock and sublethal heat (Sørensen and Loeschcke 2007). The induction of heat shock proteins in the body of arthropods in response to cold shock has been widely studied (Chen et al. 2015). The transcript levels of *HSP70* and *HSP90* genes were altered in the orange wheat blossom midge ([*Sitodiplosis mosellana* (Diptera: Cecidomyiidae)]) larvae during heat and cold constraints (Chen et al. 2016). The HSP70 and HSP40 proteins were increased in freeze-tolerant gall fly (*E. solidaginis)* following exposure to − 16 °C for one day (Zhang et al. 2011). It was reported that no evidence was found in the *Drosophila* case. Rapid cold hardening (RCH) and cold restriction resistance did not affect the levels of expression of heat shock transcription factor (*HSF-1*), *HSP70*, and its regulatory genes (Nielsen et al. 2005). Due to exposure to cold constraints, a significant increase in *HIF-1* was observed in the larvae of the goldenrod gall fly, *E. solidaginis* (Morin et al. 2005). Cold shock as acute stress is unpredictable, while diapause as chronic constraints is extendable, and heat shock protein has the regulatory ability to overcome all these conditions (Rinehart et al. 2007). During the development and cold hardening processes, the expression levels of *HSPs* were increased in *Liromyza sativa* (Diptera: Agromyzidae) and *Bombyx (Lepidoptera: Bombycidae)* (Moribe et al. 2010). Besides, these also played a key role in diapause and thermal tolerance of several insects such as *Antheraea pernyi* (Lepidoptera: Saturniidae), *Calanus finmarchicus* (Calanoida: Calanidae), *Paratlanticus ussuriensis* (Orthoptera: Tettigoniidae), and *Apis cerana* (Hymenoptera: Apidae) (Aruda et al. 2011; Shim et al. 2012; Liu et al. 2013; Zhang et al. 2014). These findings demonstrated that HSPs can be activated in response to cold and other constraints, demonstrating the complexity of the regulatory processes controlling these genes. However, there remains a paucity of information on HSPs involved in cold constraint adaptation. In-depth research about HSPs might help us understand the underlying key mechanisms of insect cold temperature adaptations.

Ice nucleators

In insects, the function of the ice nucleator is to convert body fluid into ice, which leads to the development of ice crystals at a specific temperature (Salt 1961, 1966). Earlier research on bacterial nucleators showed that the constituents in the bacterial outer membrane, like lipids, proteins, and carbohydrates, are important in nucleation activity. Similarly, ice nucleators' activity was also observed in the extracellular fluid of freeze-tolerant invertebrates. Ice nucleators control the formation of ice into extracellular body fluids. In addition, removing these nucleators from the insect's body may increase the potential of the insects to become supercool (Sømme and Block 1982). Similarly, food particles and bacteria present in the gut of insects are considered prospective ice nucleators (Worland and Lukešová 2000). The impact of molting on Collembola ability to supercool has recently been investigated (Worland et al. 2006). During molting, SCP is depressed, and the midgut or its entire contents are shed in Collembola. Genes encoding for cuticle proteins are upregulated in animals with low SCPs, and interpretation revealed that the population with low SCP prefers to molt (Worland et al. 2006). Still, this topic is under consideration and needs further research to understand the in-depth knowledge about ice nucleators and their role in insect adaptation under cold constraints.

Membrane lipids

In non-freezing settings, cold damage occurs in the shift of cell membrane lipids from a liquid crystalline phase to a gel phase (Drobnis et al. 1993). It is not unexpected that changes in membrane phospholipid compositions are linked to insect's greater cold tolerance and diapause (Cossins 1994). Homeoviscous adaptation (HVA) changes cell membrane lipids in response to temperature variations. HVA has been examined in various insects at low temperatures, such as *E. solidaginis*, *P. apterus*, *Delia antiqua* (Diptera: Anthomyiidae), *Drosophila* species, *Chymomyza costata* (Diptera: Drosophilidae), and *Sarcophaga crassipalpis* (Diptera: Sarcophagidae) (Bennett et al. 1997; Ohtsu et al. 1998; Šlachta et al. 2002; Koštál et al. 2003; Kayukawa et al. 2007; Michaud and Denlinger 2007). Palmitoleic acid (C16:1) in phosphatidylethanolamine (PE) and oleic acid (C18:1) in phosphatidylcholine were considerably higher in the pupal stage of *D. antiqua* that leading to increased cold tolerance (Kayukawa et al. 2007; Carletto et al. 2009). When diapause rises from 30% of basal level, oleic acid contributes 58% of the total fatty acid pool in *S. crassipalpis*, indicating a significant re-modeling of membrane structure and fatty acid metabolism (Michaud and Denlinger 2007).

A9-acyl-CoA desaturase enzyme may express at low temperatures and increases the ratio of unsaturated to saturated fatty acids in cell membranes and is also produced by microorganisms, plants, fish, and insects (Tiku et al. 1996; Sakamoto and Bryant 1997; Vega et al. 2004; Kayukawa et al. 2007). The enzyme pattern has been identified in *D. melanogaster*, *B. mori*, *Trichoplusia ni* (Lepidoptera: Noctuidae), and *Musca domestica* (Diptera: Muscidae) (Liu et al. 1999; Yoshiga et al. 2000; Dallerac et al. 2000; Eigenheer et al. 2002). Due to their extensive tissue distribution, it is considered vital for homeostasis and development. It can also create sex-specific pheromones (Eigenheer et al. 2002) which are widely used for sustainable pest management (Alam et al. 2023). Kayukawa et al. (2007) discovered that cold constraint treatments boosted the expression of *D. antiqua* A9-acyl-CoA desaturase, accompanied by increased palmitoleic and oleic acid production.

Aquaporins

Aquaporins are channel proteins facilitating water transport across cell membranes, so they play a crucial role in excretion and osmoregulation. Aquaporins in insects, as in other organisms, have a crucial role in maintaining water homeostasis and facilitating water transport across membranes. They are integral for insect survival and adaptation to various environments (Soveral 2021). In insects, they are responsible for the ability of simple tubular epithelia, such as the Malpighian tubule, to create both hypo- and hyper-osmotic urine. Aquaporins determine the water transport capacity of a membrane, and their number and type within a membrane are crucial determinants of water movement. The presence of aquaporins in the tubules allows insects to regulate their internal osmotic balance effectively (Spring et al. 2009). They belong to the major intrinsic protein family and are divided into three subfamilies: orthodox or classical aquaporins, aquaglyceroporins, and S-aquaporins. Orthodox aquaporins are water selective, while aquaglyceroporins are permeable to glycerol and other small solutes in addition to water. S-aquaporins are a subfamily found only in animals and exhibit uncertain permeability. These subfamilies of aquaporins are present in various organisms, including insects (Madeira et al. 2016). Several aquaporins have been identified in insects, and their source, localization, and functional attributes have been studied (Spring et al. 2009).

Functional expression studies have revealed the specific roles of different aquaporins in water transport across the Malpighian tubules and their contribution to insect osmoregulation (Spring et al. 2009). Aquaporins played a crucial role in the cold tolerance of insects, facilitating water movement across cell membranes and maintaining cellular homeostasis in cold environments. Their importance in insect cold tolerance could be explained by their involvement in several key processes. Toxopeus and Sinclair (2018) discussed the mechanisms underlying insect freeze tolerance and highlighted the absence of a single molecule necessary or sufficient for cold tolerance. They suggested that freeze-tolerant insects control ice quality, repair cellular damage, manage biochemical processes, and restore physiological functions post-thaw, with aquaporins playing a role in all these processes. Cold temperatures can lead to dehydration due to increased evaporative water loss and reduced water intake. Aquaporins allow for the efficient movement of water across cell membranes, ensuring proper hydration and preventing excessive water loss (Zachariassen 1985; Toxopeus and Sinclair 2018). They also contribute to the prevention of ice formation and cellular damage. In freeze-tolerant insects, ice formation within cells can be detrimental. These proteins help control the quality and quantity of ice, preventing cellular dehydration and reducing the risk of mechanical damage (Zachariassen 1985; Toxopeus and Sinclair 2018). In addition, aquaporins play a role in maintaining biochemical processes during freezing and thawing. They help manage the movement of water and solutes, ensuring that essential metabolic reactions can continue even at low temperatures (Storey and Storey 2012; Toxopeus and Sinclair 2018).

In a study on *E. solidaginis* larvae, the role of aquaporins in desiccation and freeze tolerance was investigated. The presence of aquaporins was confirmed, and suggested that

their presence facilitates the redistribution of water and cryoprotective compounds between intra- and extra-cellular compartments, contributing to survival during freezing (Philip et al. 2008). Later, it was also found that changes in the abundance of aquaporin-like proteins correlated with the seasonal acquisition of freeze tolerance. Aquaporin proteins were upregulated as larvae became more tolerant to freezing, suggesting their involvement in permitting water redistribution and glycerol uptake during extracellular ice formation (Philip and Lee 2010). Another study on *E. solidaginis* characterized an aquaporin (EsAQP1) present in freeze-tolerant larvae. EsAQP1 exhibited high water permeability and was impermeable to glycerol and urea. The abundance of EsAQP1 increased during colder months, particularly in the brain, suggesting its potential role in protecting brain cells from water imbalance during freezing (Philip et al. 2011).

Genes linked with cold constraints

Understanding the genetic foundations of cold tolerance in insects is crucial for unraveling their adaptation to cold climates and predicting responses to changing environmental conditions. Recent progress in identifying genes linked to cold tolerance has provided insights into the molecular and physiological mechanisms that underlie insects' adaptability to low temperatures. This information can be categorized into two main groups: (1) studies identifying specific genes and (2) studies employing transcriptomics, metabolomics, and other omics sciences.

Studies of gene identification

Recent studies have pinpointed critical genes associated with cold constraints in insects. The muscle-LIM protein gene (*EsMlp*) and the *Drosophila* cold-acclimation gene (*Dca*) were identified as potential candidates through selection techniques and subtractive hybridization (Bilgen et al. 2001). The *Dca* gene, showing homology to the mammalian SMP-30, enhances cytosolic Ca^{2+} levels, influencing stress responses (Stronach et al. 1996; Goto 2000; Bilgen et al. 2001). The muscle-LIM protein gene (*EsMlp*) also facilitates muscle restructuring at sub-zero temperatures, playing a vital role in insect metamorphosis and development during the spring (Arber et al. 1994; Fujiwara and Denlinger 2007). De Jong and Saastamoinen (2018) found that the Glanville fritillary butterfly's cold tolerance was influenced by developmental and adult thermal conditions, implicating the flight gene.

Furthermore, the importance of the trehalose-6-phosphate synthase (*TPS*) and trehalase (*TRE*) genes in response to cold stress, related to the trehalose and glycogen production pathway, has been investigated (Wan et al. 2023). Recently, Zhang et al. (2023) showed that the *TPS* gene is linked with rapid cold hardening in *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae) by regulating trehalose metabolism. They reported that *TPS* regulates trehalose synthesis and accumulation *in L. oryzophilus* adults, ultimately improving insect survival under low temperature stress. The *TPS* gene is a fusion gene that encodes proteins containing TPP and TPS domains as well as exhibiting the activity of TPP and TPS enzymes (Jin et al. 2018). *TPS* genes have been identified in several insect species, including brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae) (Yang et al. 2017), *Heortia vitessoides* (Lepidoptera: Crambidae) (Chen et al. 2020), *M. domestica* (Zhang et al. 2019), and *Mythimna separate* (Lepidoptera: Noctuidae) (Yang et al. 2023). These genes play a crucial role in the insect's ability to respond to and tolerate cold conditions, adding another layer of complexity to the genetic basis of cold adaptation.

Omics sciences and the study of cold constraints.

Genomics and transcriptomics

Recent advances in genomics have shed light on the molecular mechanisms underlying insect cold stress adaptation. Genomic studies have revealed key genetic components and regulatory pathways involved in insect responses to low temperatures, providing valuable insights into their cold tolerance strategies. Fortunately, with the continuous reduction in costs associated with these technologies, the number of sequenced insect genomes is steadily increasing. Storey and Storey (2012) have reviewed the effects of "omics" studies in cold hardiness in insects. The focus of transcriptomics is gene expression, albeit there may be a large lag between gene and protein expression. Transcriptomics plays a crucial role in understanding cold tolerance in insects by providing insights into the underlying molecular mechanisms and identifying key genes and pathways involved in the response to cold stress. On cold acclimation by using suppression subtractive hybridization (SSH), first transcriptomic investigations were carried out in *D. melanogaster* (Goto 2000, 2001), demonstrated that by following cold adaptation to 15 °C, a gene similar to senescence marker protein 30 (*SMP30* or *Dca*) is upregulated. The initial microarray investigation on *D. melanogaster* cold tolerance was undertaken using commercially available 7000 sequence microarrays, followed by 12,000 sequence arrays (Qin et al. 2005).

Transcriptomic studies have identified numerous cold-regulated DEGs enriched in "metabolism" pathways, especially in fatty acid metabolism and biosynthesis (Zhang

et al. 2015a, b; Zhou et al. 2019; Wang et al. 2023). More specifically, some studies have been conducted on *Aphidius colemani* (Hymenoptera: Braconidae) and *Drosophila* species (Qin et al. 2005; Colinet et al. 2007; Sinclair et al. 2007). In addition to microarray and hybridization analysis, 37 transcripts out of 12,000 *Drosophila* clones exhibited upregulation in response to cold hardening. A majority of these transcripts encode membrane proteins and heat shock proteins. During this process, protein utility sequencing potentially yielded data indicating similarities in the prospective modification of oxidative pathways, leading from carbohydrates to fatty acids. It is plausible that hibernation and cold hardening share common mechanisms that give rise to similar modifications (Qin et al. 2005). These results showed the importance of technologies, such as genomics and transcriptomics, to compressively understand the possible mechanisms. During cold stress in *Drosophila*, an alternative approaches were employed to analyzing the transcription factors of five candidate genes (*desaturase 2*, *Hsp70a*, *Smp-30*, *Hsp23*, and *Frost*) and utilized *Drosophila* genome data (Goto 2000, 2001; Sinclair et al. 2007). The study focused on a few genes implicated in cold stress conditions. In the recovery phase, *Frost* and *Hsp70* genes were highly upregulated when exposed to cold stress conditions. The study made a precise distinction between the recovery phase and exposure. This factor was also reflected in the analysis of *A. colemani* proteomes under both constant cold temperature (CLT) and fluctuating temperature regime (FTR) treatments (Colinet et al. 2007). Shen et al. (2021) reported Treh1's association with freezing tolerance in *Bemisia tabaci* (Hemiptera: Aleyrodidae), suggesting a role in regulating geographical distribution. In *Dendroctonus valens* (Coleoptera: Curculionidae), an important invasive forest pest in China, Zhao et al. (2021) investigated the genes associated with cold tolerance and overwintering. By sequencing the transcriptomes of wintering and non-wintering adult and larval *D. valens*, they identified differentially expressed genes (DEGs) related to cold tolerance. The DEGs included genes involved in protein phosphatase, very long-chain fatty acid proteins, cytochrome P450, and putative leucine-rich repeat-containing proteins. Further investigations are required to elucidate the directly involved key genes and pathways and enhance our understanding of these phenomena. Utilizing multiple omics approaches enhances our understanding of insects' intricate molecular responses to low temperatures, shedding light on the broader spectrum of adaptations to cold stress conditions.

Proteomics

Although proteomics does not directly assess the activity of various proteins, this “omic” method alleviates the problem of post-transcriptional regulation by directly measuring the amount of functionally active molecules. The proteomic research on insect heat and acclimatory responses is quickly developing. Shotgun methods and 2-dimensional gel electrophoresis (2-DE) are two proteomic approaches (Bantscheff et al. 2012). Shotgun proteomic technologies, such as label-free, metabolic labeling, and isobaric chemical labeling mass spectrometry (MS), have fundamentally altered how biological systems are assessed (Bantscheff et al. 2012).

Increased proteomics studies have recently been conducted to identify insect diapauses' intrinsic processes. A study using 2DE-based proteomics investigated the effects of different temperatures and prolonged cold exposure on the parasitic wasp *A. colemani*. This study identified the specific proteins responsible for energy metabolism, as well as protein degradation and synthesis. In *D. melanogaster*, a freeze-intolerant species, proteomic approaches have explored rapid cold-hardening reactions (Overgaard et al. 2014). Despite substantial phenotypic implications, a 2D-DIGE proteome analysis showed a mild proteomic response in *D. melanogaster* adult species that were cold acclimated at 11 °C (Colinet et al. 2013). Bonnet et al. (2012) examined the proteomic profiles of over-wintering mountain pine beetle larvae from September and November to March and May. Due to glycerol accumulation and the activation of freeze-avoiding mechanisms, the larvae gradually gain cold constraints over the fall months. These results showed that the proteomics approach is one of the most advanced techniques for investigating the mechanisms involved in cold constraint adaptation.

Metabolomics

Metabolomics studies low molecular weight metabolites of numerous physiological circumstances and processes (Kopka 2006). Metabolomics focuses on alterations of transcription, translation, and protein activity. Metabolomics research relies on the identification of metabolites, which is commonly achieved by using nuclear magnetic resonance (NMR) methods, gas chromatography-mass spectrometry (GC–MS), and liquid chromatography-mass spectrometry (LC–MS), as well as strong multivariate statistical analysis. Individual metabolomics approaches are a strong and valuable analytical tool but have several drawbacks, such as a low detection limit and metabolite detection problems (Kopka 2006). Several notable metabolomics studies on cold constraints and hardiness recovery have been reviewed in arthropods, most of which used LC–MS and GC–MS methods (Storey and Storey 2012). Metabolites contain four main types: polyols, sugars, intermediate metabolites, and amino acids. Metabolomics observation of cold constraints in *Drosophila montana* (Diptera: Drosophilidae) and *D.*

melanogaster revealed an increased proline concentration rather than alanine (Pedersen et al. 2008). The metabolite is involved in the physiological response to abiotic constraints, such as sorbitol, which gives whiteflies resilience to high temperatures or glucose, protecting frogs from freezing damage. These reports showed that metabolomics is one of the most commonly used approaches to study the complex nature of how physiology is linked to external events and conditions (in our case, cold constraints).

Conclusion

Insects' ability to cope with cold conditions is a subject of study that has captured the attention of researchers across disciplines. Understanding the adaptations that enable insects to thrive in cold environments is crucial not only for basic ecological knowledge but also for practical applications in pest management and conservation efforts. There are four key themes of cold adaptations in insects: physiological strategies, metabolic adjustments, genetic and epigenetic regulation, and behavioral strategies. The first includes freeze tolerance, where insects can withstand the formation of ice crystals within their bodies, and freeze avoidance, where they actively prevent ice formation by supercooling. Additionally, cryoprotective dehydration and vitrification are strategies that involve the removal of water from cells to protect them from freezing. Secondly, many species undergo diapause, a state of dormancy that allows them to conserve energy and survive unfavorable conditions. Understanding the molecular and biochemical processes involved in metabolic adjustments is essential for comprehending their survival mechanisms. Third, cold adaptation in insects involves genetic responses to low temperatures. Unraveling the genetic basis of these adaptations is crucial for understanding the evolutionary history and potential for future adaptations. Epigenetic mechanisms may also play a role in regulating cold responses, and exploring these interactions holds promise for deeper insights. Finally, insects exhibit diverse behavioral adaptations to cope with low temperatures. Some species migrate to warmer regions during colder seasons, while others seek shelter or aggregate to conserve heat. Investigating the behavioral plasticity of insects in response to changing thermal conditions is an interesting area of research.

Cold adaptations in insects represent a rich and complex field of study, encompassing various physiological, genetic, and behavioral mechanisms. Future research should adopt interdisciplinary approaches and investigate the impact of changing environmental conditions to understand the mechanisms insects have to thrive in cold environments. Some interesting and pending research topics for understanding insect strategies for cold adaptation are: (1) the role of sugars in vitrification, by examining the biochemical pathways involved and their significance in cold tolerance; (2) collective behavior and thermoregulation by analyzing the factors influencing thermoregulatory behavior of social insects such as bees, ants, and termites, which use collective heat generation to maintain warmth in their nests during winter; (3) insect dormancy and metabolic suppression, exploring the physiological and molecular mechanisms underlying insect dormancy, a state of suspended animation during which insects minimize their metabolic activities to survive harsh winter conditions; (4) cellular responses to cold stress, including the activation of stress-related genes, protective mechanisms against freezing, and recovery processes after thawing; (5) biotechnological applications, exploring potential biotechnological applications of insect cold adaptation strategies, such as the development of new biomaterials or cryopreservation techniques inspired by insect antifreeze mechanisms; and (6) temperature sensing and signal transduction, investigating the sensory mechanisms through which insects detect temperature changes and how these signals are transduced to trigger cold adaptation responses at the molecular and physiological levels. A deeper understanding of how insects successfully adapt to cold environments will be gained by addressing these topics, contributing to our knowledge of their survival strategies and potential applications in diverse fields. This research can have significant implications in various fields, such as agriculture, pest control, and even biomedical research. By uncovering the mechanisms and processes involved in cold adaptation, scientists can develop innovative strategies to enhance crop production in cold climates, improve pest management techniques, and potentially discover new therapeutic approaches for human health conditions related to temperature sensitivity. Ultimately, this knowledge can pave the way for advancements in multiple industries and contribute to the overall understanding of biological adaptations to extreme environments.

Author contributions

ZL, ND, and FU designed the manuscript. FU, AA, and HG prepared original draft. ZL, FU, MH, AA, HG, BGG, LC, RRR, AG and ND critically revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgements We thank all other members of the Plant Quarantine and Invasion Biology Laboratory of China Agricultural University (CAUPQL) for valuable suggestions.

Funding This research was funded by the National Natural Science Foundation of China (32172394).

Declarations

Conflict of interest The authors declare no conflict of interest. ND serves as Editor-in-Chief of Journal of Pest Science and was not involved in the review process and decisions related to this manuscript.

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