Lipid Metabolism in Response to Cold

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Abstract

Temperature directly shapes insect physiology and has a preponderant effect on life history traits. Winter conditions in temperate and polar regions are especially challenging for insects. Extremely low temperatures can indeed compromise insect survival by promoting freezing of body fluids, but mild cold temperatures above 0 °C (i.e. chilling) can also lead to complex and severe physiological dysregulations. Among physiological damages due to freezing and chilling, insect lipids are one of the primary targets. As low temperatures tend to rigidify phospholipid bilayers, membranes functions are compromised at cold. Lipid rigidification due to cold also decreases the accessibility of fat stores for metabolic enzymes, and therefore their availability for basal metabolism. These deleterious effects, combined with low food availability in winter, result in a substantial nutritional challenge for overwintering insects. Consequently, lipid modifications such as homeoviscous adaptation of cell membranes, fluidity maintenance of fat reserves, cuticular lipid accumulation, and production of antifreeze glyclolipids are essential components of the physiological response to cold stress. The aim of the present chapter is to present the physiological challenges caused by low temperatures, the lipid modifications linked with cold tolerance in insects, and the molecular regulation of lipid metabolism during cold exposure.

1. Introduction: Insects in the cold

Our planet shelters a wide diversity of ecosystems, in which living organisms face a large variety of ecological variables. Among abiotic factors, temperature has a preponderant effect on life by acting from the molecular scale (shaping protein conformation, directing chemical reactions rates, etc.; Hochachka and Somero, 1984) to the highest levels of biological organizations (shaping fundamental niches of species and population dynamics; Clark et al., 2003; Magnuson et al., 2015). Insects represent the most diverse and richest group of animals and are present in the vast majority of terrestrial ecosystems, and therefore face diverse climatic conditions. Most insects are poikilotherms, meaning that their body temperature is directly influenced by their surrounding environmental temperature (Potter et al., 2013). Their physiology and life history traits are therefore intimately linked to temperature. Consequently, insect species distribution is closely linked with the range of temperature they can tolerate, and in many cases distribution is most closely linked to the lowest temperatures they face in their habitat (Addo-Bediako et al., 2000; Bale, 2002; Kellermann et al., 2012; Kimura, 2004).

Under actual climatic conditions, winters in temperate zones are challenging for insects. Exposure to low temperatures can severely decrease fitness, and in extreme

Table 1: List of abbreviations

Term	Abbreviation
Adipokinetic hormone	AKH
AMP-activated protein kinase	AMPK
Critical minimal temperature	CTmin
Crystalline phase	Lα
Gel phase	Lβ
Glycerolphospholipid	GPL
Hexagonal phase	HII
Juvenile hormone	JH
Lysophospholipids	LPL
Phosphatidylcholine	PC
Phosphoethanolamine	PE
Polyunsaturated fatty acid	PUFA
Tryacilglycerid	TAG
Unsaturated fatty acid	UFA

conditions, low environmental temperatures can promote freezing of body fluids. Intracellular freezing is usually lethal in insects, but ice formation in the extracellular space can also directly cause cellular damage by physically disrupting membrane structure or tissue integrity. Extracellular freezing can also damage cells indirectly by increasing the concentration of osmolytes. This “freeze concentration” leads to an increase of osmotic pressure, resulting in fluid movement from the cell, and therefore cellular dehydration (for reviews on the physiology of freezing injuries see Rozsypal, 2022; Toxopeus and Sinclair, 2018). Historically, insect cold tolerance strategies are divided between “freeze intolerant” species that succumb to ice formation in their body and “freeze tolerant” insects that survive extracellular ice formation (Lee, 1989, 2010; Salt, 1961). Freeze intolerant species accumulate metabolites in winter such as antifreeze proteins, glycolipids and high concentration of low molecular weight cryoprotectant compounds. These metabolites decrease hemolymph melting point, which enter a supercooling state and therefore prevent ice formation (Lee, 2010). Oppositely, freeze tolerant insects usually initiate ice formation using ice-nucleating agents (proteins, crystalloid compounds or micro-organisms; Lee, 2010). Ice nucleating agents allow to control the formation of ice crystals, and therefore prevent their further propagation, and help to avoid intracellular freezing. However, numerous insect species suffer from cold damage well before freezing temperatures: for instance, the tsetse fly *Glossina pallidipes* shows signs of neuromuscular dysfunctions at quite high temperatures (21 °C; Terblanche et al., 2007). Thus, freeze intolerant species can be further divided into additional sub-categories: “freeze avoiding” species that can survive down to their supercooling point, “chill tolerant” species that succumb at temperatures below 0 °C but above the supercooling point, and “chill susceptible” insects that suffer cold injuries at mild cold temperatures, above 0 °C (Overgaard and MacMillan, 2017).

While freezing directly compromises cell and tissue integrity due to physical damage by ice crystals, chilling causes complex physiological perturbations that compromise homeostasis and survival (Colinet et al., 2012; Enriquez et al., 2018; Overgaard et al., 2007). Cold, by its thermodynamic effect, slows the rates of chemical reactions and can cause a depolarization of membrane potential, leading to neuromuscular dysfunction, also known as chill coma (Overgaard and MacMillan, 2017). Cold acts on the strength of

noncovalent interactions, therefore altering the structural integrity of macromolecules such as proteins (Todgham et al., 2007), nucleic acids (Lubawy et al., 2019) and cellular membranes (Hazel, 1989). When unfolded (denatured) proteins accumulate, it can form aggregates, which could be one of the causes of chronic chilling injuries (Rozsypal, 2022). These deleterious effects can compromise the function of essential proteins such as enzymes (Privalov, 1990) and cytoskeleton components (Cottam et al., 2006). As phospholipid bilayers, cell and organelle membranes are by nature sensitive to temperature, and their fluidity is dictated by temperature, with membranes being fluid at permissive temperature and rigidifying with decreasing temperature. The effects of low temperatures on membrane biophysical properties are presented in detail in part 2.a. Such deleterious effects on macromolecules can have aftereffects on metabolic pathways such as aerobic respiration pathways (Michaud and Denlinger, 2007), contributing to the global loss of homeostasis.

Beside temperature, a major limiting factor for insect overwintering survival is nutrient availability. Indeed, winter periods often correlates with food deprivation, and insects that overwinter in a quiescent or dormant state cannot feed, meaning that their survival relies on their own resources (Hahn and Denlinger, 2007; Sinclair, 2015), mainly triacylglycerides (TAGs) stored in lipid droplets. Lipid reserves not only serve during cold exposures, but also play major roles to meet energetic demands during warming waves, to resume activity (for insects overwintering in a motile life stage), or complete development (for insect overwintering at juvenile stages; Hahn and Denlinger, 2007; Sinclair, 2015). However, as for membranes lipids, low temperatures also affect biophysical properties of lipid droplets by decreasing their fluidity, compromising their accessibility for enzymatic hydrolysis and therefore their uptake for basal metabolism.

To face these physiological challenges, insects possess a vast arsenal of adaptations and plastic responses (acclimation, or diapause, which is presented in chapter 12) to counteract negative effects of cold temperatures. In addition to producing protective molecules such as antifreeze proteins, glycolipids and cryoprotectants, insects reshuffle membrane and lipid droplet composition in winter to increase their fluidity, a phenomenon known as homeoviscous adaptation (see part 3.a). Several exhaustive reviews are

available on the physiology of cold tolerance in insects (see: Hayward et al., 2014; MacMillan and Sinclair, 2011; Overgaard and MacMillan, 2017; Rozsypal, 2022), and the aim of the present chapter is therefore to highlight that among physiological damages due to low temperatures, and the corresponding underlying mechanisms to counteract these injuries, lipid metabolism plays a central role. Lipids are indeed among the primary targets of cold injuries and are consequently preponderant in the responses to cold temperatures. The current chapter will address how insect lipids are impacted by low temperatures and highlight the roles lipid plays in insect cold tolerance physiology.

2. Effects of cold temperatures on lipids and physiological consequences in insects

a. Biophysical properties of lipids at low temperature

Cell membranes are essential to organismal organization and physiology by providing cells and organelles a physical barrier that helps regulate water, solute and ion diffusion. Biological membranes consist of a glycerophospholipid (GPL) bilayer in which many molecules (mainly proteins) are embedded (Gennis, 1989). Membranes are highly dynamic structures from which many molecular pathways are initiated (Casares et al., 2019). GPLs are composed of a glycerol residue covalently linked to two non-polar (hydrophobic) fatty acids and one esterified phosphate group. This phosphate group is linked to a hydrophilic, polar headgroup which can vary in composition (Figure 1a). In insect cells, the main polar groups are choline, (forming phosphatidylcholines – PC) and ethanolamine (forming phosphoethanolamines – PE). GPLs have highly diverse structures, and aside from PC and PE other hydrophilic moieties are present in insects (but in lower abundance) such as phosphatidylglycerol, phosphatidylinositol or phosphatidylserine (Hammad et al., 2011; Jones et al., 1992). Fatty acid chains are also diverse as they can change in length and unsaturation ratio. Some GPLs also have one of the fatty acid chains cleaved, forming Lysophospholipids (LPLs; *e.g.* lysophosphatidylcholine; lysophosphatidylethanolamine).

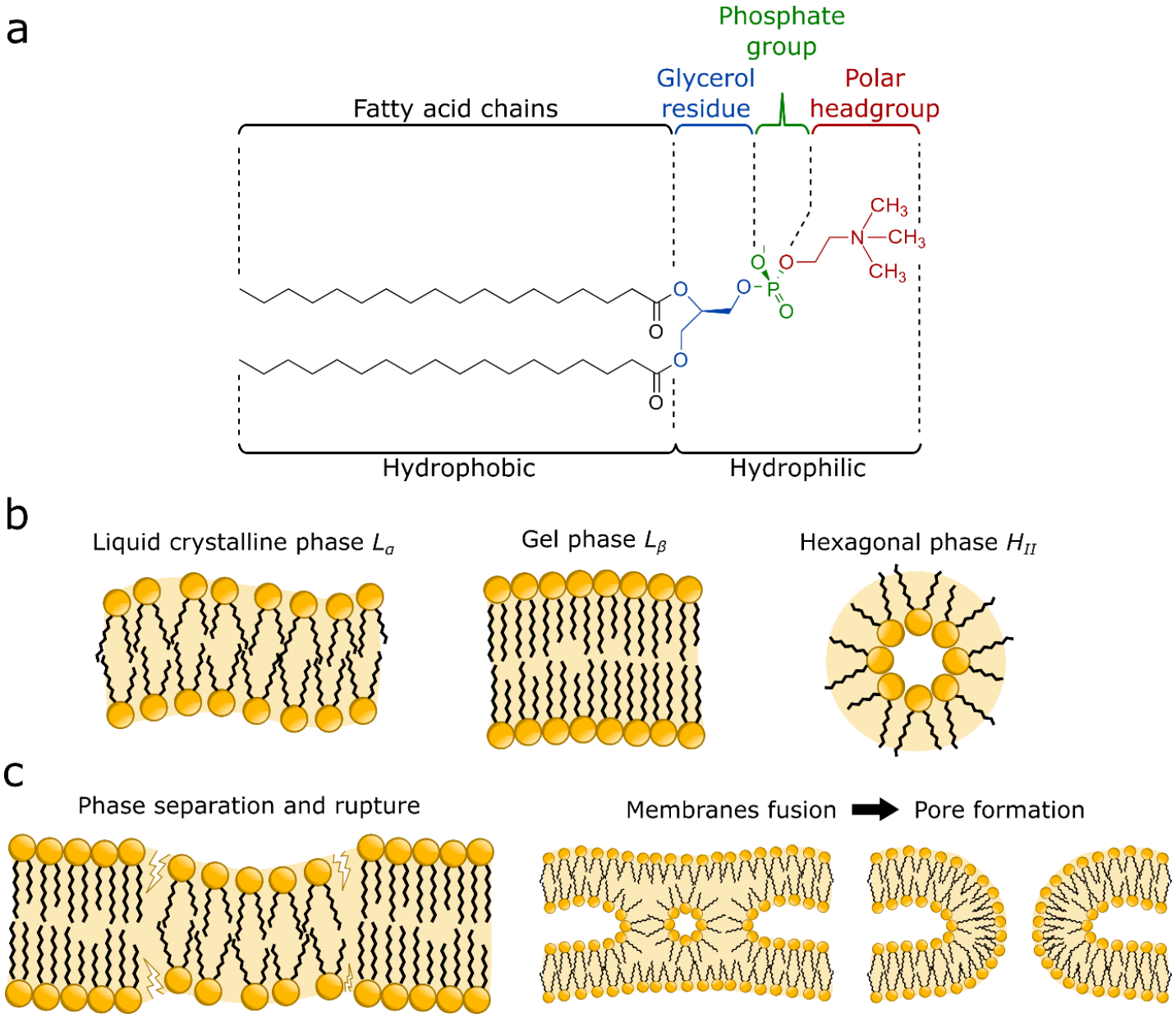

Figure 1: (a) Glycerophospholipid (GPL) organization. GPLs are composed of two non-polar (hydrophobic) fatty acids covalently linked to a glycerol residue which is esterified to a phosphate group. This phosphate group is linked to a hydrophilic, polar headgroup which can vary in composition (here a choline is represented).

(b) Alteration of biophysical properties of biological membranes by temperature. At permissive temperatures GPL bilayers show a disordered packing, giving the membrane a fluid state known as the liquid crystalline phase (L_α). At low temperatures, GPLs are more tightly organized, resulting in a rigid gel phase (L_β). Oppositely, high temperatures allow lipids to show a loose organization which can lead to a transition into a hexagonal phase (H_{II}). The H_{II} phase can also be promoted by conditions of low humidity.

(c) Alteration of membrane integrity due to low temperatures. Changes of phases within the bilayer can induce zones of phase-transition that can promote a lateral separation of the phospholipid bilayer at the interface between gel and fluid domains. The H_{II} phase can provoke membranes fusion, which initiate pore formation. These alterations can compromise the permeability of membranes and cause fluid and solutes to leak from the cells.

The formation of a GPL bilayer is mainly due to a hydrophobic effect: due to their hydrophobic nature, fatty acid chains are excluded from water and aggregate together within the bilayer structure, while the hydrophilic polar headgroups interact with water molecules and form the membrane surfaces (Gennis, 1989; Singer and Nicolson, 1972). Other noncovalent interactions act between GPL, such as van der Waals forces or hydrogen bonding, but these are relatively minor factors in comparison to hydrophobic forces (Gennis, 1989). These membrane bilayers are fluid structures, meaning that lipids and other molecules are moving within the bilayer. Lipids rotate around their own axis, diffuse laterally within their layer, and also move from one layer to another, a movement known as transbilayer flip-flop (Gennis, 1989). The fluidity of the membrane is essential for the functioning of enzymes and other proteins embedded in the bilayer. Because of their composition, membranes are highly thermosensitive, as their biophysical properties, defined by their viscosity (fluidity) and also their phase state, are directly dependent on temperature (Hazel, 1989).

At permissive temperatures, fatty acid carbon chains can show isomeric conformations such as gauche rotamers (i.e. a 60° rotation around a carbon-carbon single bond; Kučerka et al., 2011) that result in a disordered packing of GPLs, giving the bilayer a fluid state referred to as the liquid crystalline phase (L_α; Figure 1b). When temperature decreases, fatty acid chains tend to assume a trans conformation, which requires less energy, at the expense of rotamers forms. This conformational change leads to a more compact, tight organization of GPLs and therefore promotes a rigidification of the bilayer (Fernandez-Puente et al., 1994). TAGs contained in lipid droplets are similarly affected by low temperatures. GPL rigidification can lead to the transition to a rigid, highly ordered gel phase (L_β; Figure 1b; Hazel, 1995). Decreasing temperature also causes an increase in membrane thickness, due to the highly organized configuration of GPL within the bilayer (Kučerka et al., 2011; Rozsypal, 2022). Oppositely, at high temperatures, the molecular geometry of lipids can change for to “conical shape”, which in extreme cases can promote a phase transition to a highly disorganized inverted hexagonal phase (H_{II}; Figure 1b) which is incompatible with the integrity of the GPL bilayer (Hazel, 1995). H_{II} phase can in turn promote membranes fusion, which could initiate pore formation (Jahn et al., 2003), compromising the permeability of membranes and causing fluid and solutes

to leak from the cell (Figure 1c; Rozsypal, 2022). In addition to high temperature, transition to H_{II} phase can also be induced by low hydration of molecules, a condition which can be caused by ice formation in freeze tolerant insects for instance (Rozsypal, 2022).

b. Physiological consequences of lipids structures rigidification

These losses of membrane integrity can lead to cascading effects on insect homeostasis. In non-stressful conditions, hemolymph ionic concentration is regulated by secretion of K^+ rich fluids in Malpighian tubules and simultaneous Na^+ ions and water reabsorption in proctodeum (Des Marteaux and Sinclair, 2016; Harrison et al., 2012; Maddrell and O'Donnell, 1992). Membrane rigidification and GPL phase perturbation can greatly compromise the activity of transmembrane proteins such as ion channels or transporters (Cossins, 1994; Hazel, 1989; MacMillan and Sinclair, 2011), which will alter membrane ionic permeability. Furthermore, modification of membrane fluidity can cause non-uniform phase changes within the bilayer, resulting in phase-transition zones that can lead to a lateral separation of the GPL bilayer at the interface between gel and fluid domains, which can compromise membrane permeability to fluids and solutes (Figure 1c Clerc and Thompson, 1995). This lateral separation will further contribute to dysregulation of the balance between passive and active ionic transfer across the membrane, promoting a loss of ionic homeostasis (Koštál, 2010). In the cold, ionic imbalance leads to increased Na^+ concentration in the gut and other organ lumens, which increases osmotic pressure and causes water to leak into organs, and therefore a low hydric content in the hemolymph, resulting in hyperkalemia (Figure 2; Coello Alvarado et al., 2015; Des Marteaux and Sinclair, 2016; MacMillan et al., 2015). Loss of hydric and ionic homeostasis further exacerbates the depolarization of cell membrane caused by cold, which perturbs action potentials in muscular and nervous cells. Furthermore, membrane rigidification and depolarization result in the dysfunction of synaptic Ca^{2+} channels, which control neurotransmitter releases (Figure 2; Findsen et al., 2016). The combined effects of these dysregulations lead to a loss of neuromuscular system coordination, contributing to chill coma induction (Figure 2; MacMillan and Sinclair, 2011). Furthermore, membrane depolarization is a step of apoptosis initiation (Bortner et al., 2001). Loss of water and

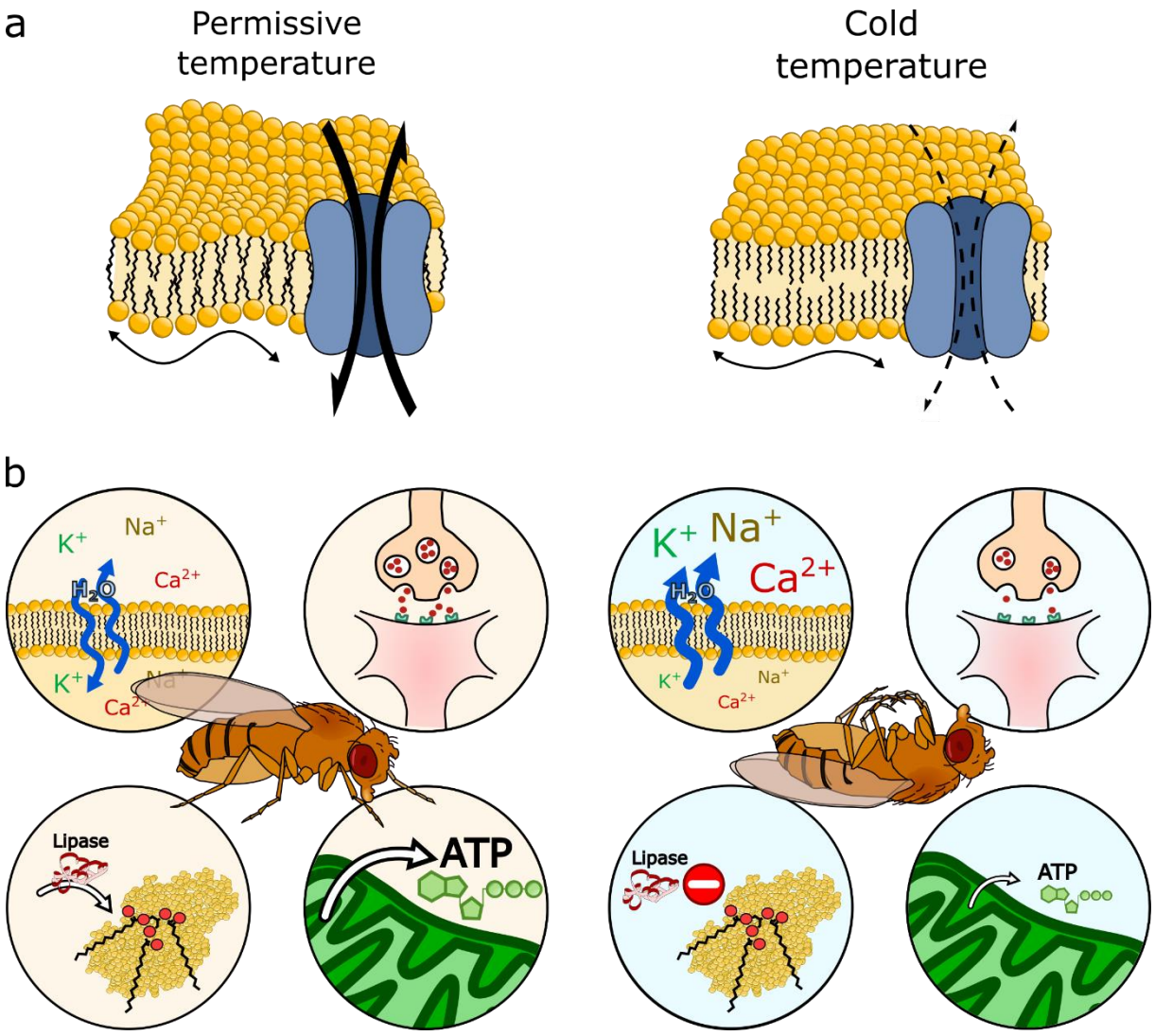

Figure 2: Summary of low temperature effects on lipids and their physiological consequences **(a)** Low temperatures promote the rigidification of membranes, impeding their biological functions, such as the activity of transmembrane proteins. The fluidity of stored lipids in lipid droplets (triacylglycerides, TAG) is also decreased in the cold. **(b)** These alterations lead to cascading effects such as dysregulation of the balance between active and passive ionic and water transfer across the membrane, which can cause a loss of water balance and ionic homeostasis. These physiological modifications, combined with membrane rigidification can lead to membrane depolarization, perturbing action potentials and release of neurotransmitters. Rigidification of mitochondria double membranes compromise their activity. Alteration of mitochondria function, together with the low accessibility of TAG by hydrolases due to lipid droplets rigidification, can lead to a deficit in ATP production. All these dysregulations lead to a global loss of homeostasis, which can result in neuromuscular dysfunction leading to chill coma or death.

ionic homeostasis can therefore promote death by apoptosis in insect cells subjected to cold stress (Yi et al., 2007).

Mitochondria activity is also compromised by low temperatures (Colinet et al., 2017; Kukal et al., 1989), probably due to rigidification of their double membranes. Alteration of mitochondria function, together with the low accessibility of TAG by hydrolases due to lipid droplets rigidification, can lead to a deficit in ATP production (Figure 2 Colinet, 2011; Coulson et al., 1992; Dollo et al., 2010), which can contribute to the disruption of metabolic homeostasis. Metabolism dysregulation also leads to an accumulation of deleterious molecules such as reactive oxygen species, creating an imbalance between reactive oxygen species production and elimination, leading to oxidative stress (Lopez-Martinez et al., 2008). Oxidative stress can further compromise mitochondria integrity and damage proteins, and can also degrade nucleic acids, by promoting mutation or deletion, which could lead to damage in tissues (Imlay, 2003).

3. Lipids modifications linked with insect cold tolerance

3.a - Homeoviscous adaptation

One of the most conserved physiological responses of ectotherms to low temperatures is homeoviscous adaptation of cell membranes (Hazel, 1995). The term homeoviscous adaptation incorporates all modifications of the GPL bilayer composition that adjust membrane fluidity and guarantee its function at different temperatures (Hazel, 1995; Sinensky, 1974). Such adjustments not only maintain membrane fluidity but also its phase, a phenomenon known as homeophasic adaptation (Hazel and Eugene Williams, 1990). Several changes in GPLs have been identified that impact membrane fluidity, including changes at the headgroup level, in the composition of fatty acid chains or in the nature of lipids embedded in the bilayer. These adjustments can be categorized as:

i. *Modifications of GPL headgroups*

ii. *Increased unsaturation (or polyunsaturation) of fatty acid carbon chains*

iii. *Shortening of fatty acid carbon chains length (increase of the C16/C18 ratio)*

iv. Increased proportion of LPL

v. Increased proportion of cholesterol in the cell membrane

i. Modifications of GPL headgroups

GPL polar headgroup modifications, typically an increase of PE proportion at the expense of PC, is a common adjustment of homeoviscous adaptation. PE and PC are the most abundant GPLs in insects. While PC is classically defined as having a cylindrical shape, which give bilayers a tight and compact organization (therefore poorly fluid), PEs have a conical shape that disorganizes GPLs and increase membrane fluidity (Figure 3). Downer and Kallapur (1981) demonstrated with wide angle X-ray diffraction that mitochondria acclimated to 31°C (which have a high PE/PC ratio) keep membranes in the fluid L_α state when exposed to 0°C, while mitochondria acclimated to 45°C (which have a low PE/PC ratio) have membranes that transition to the rigid L_β phase at 0°C. The ratio between PC and PE therefore greatly influences membrane fluidity, and for each range of temperature there is an optimal PE/PC ratio guaranteeing proper fluidity and functioning (Hazel, 1995). Consequently, increased PE/PC ratio in cell membranes is a typical modification linked with adaptation to cold, acclimation, diapause or winter season in insects (Colinet et al., 2016; Cooper et al., 2012; Cooper et al., 2014; Goto and Katagiri, 2011; Hodková et al., 1999; Koštál et al., 2011; Michaud and Denlinger, 2006; Overgaard et al., 2008; Tomčala et al., 2006; Trenti et al., 2022). However, some studies showed sex specific changes in the PE/PC ratio (in D. suzukii this ratio increase in acclimated females but decrease in acclimated males; Enriquez and Colinet, 2019a), or no modification following rapid cold hardening (i.e. a rapid acclimation process known to increase cold tolerance in insects; MacMillan et al., 2009). Oppositely, in the freeze tolerant larvae of the gall fly *Eurosta solidaginis* the proportion of PC increase at the expense of PE during winter (Pruitt and Lu, 2008). In that case, the ordering effect of PC in membrane bilayer could prevent the transition to the H_{II} phase that can occur due to dehydration of the cell cause by extracellular ice formation, which is consistent with homeophasic adaptation. In addition to PE, other GPLs can replace PC in the bilayer, including phosphatidylglycerol or phosphatidylserine which increase during cold acclimation in *D. suzukii* (Enriquez and Colinet, 2019a).

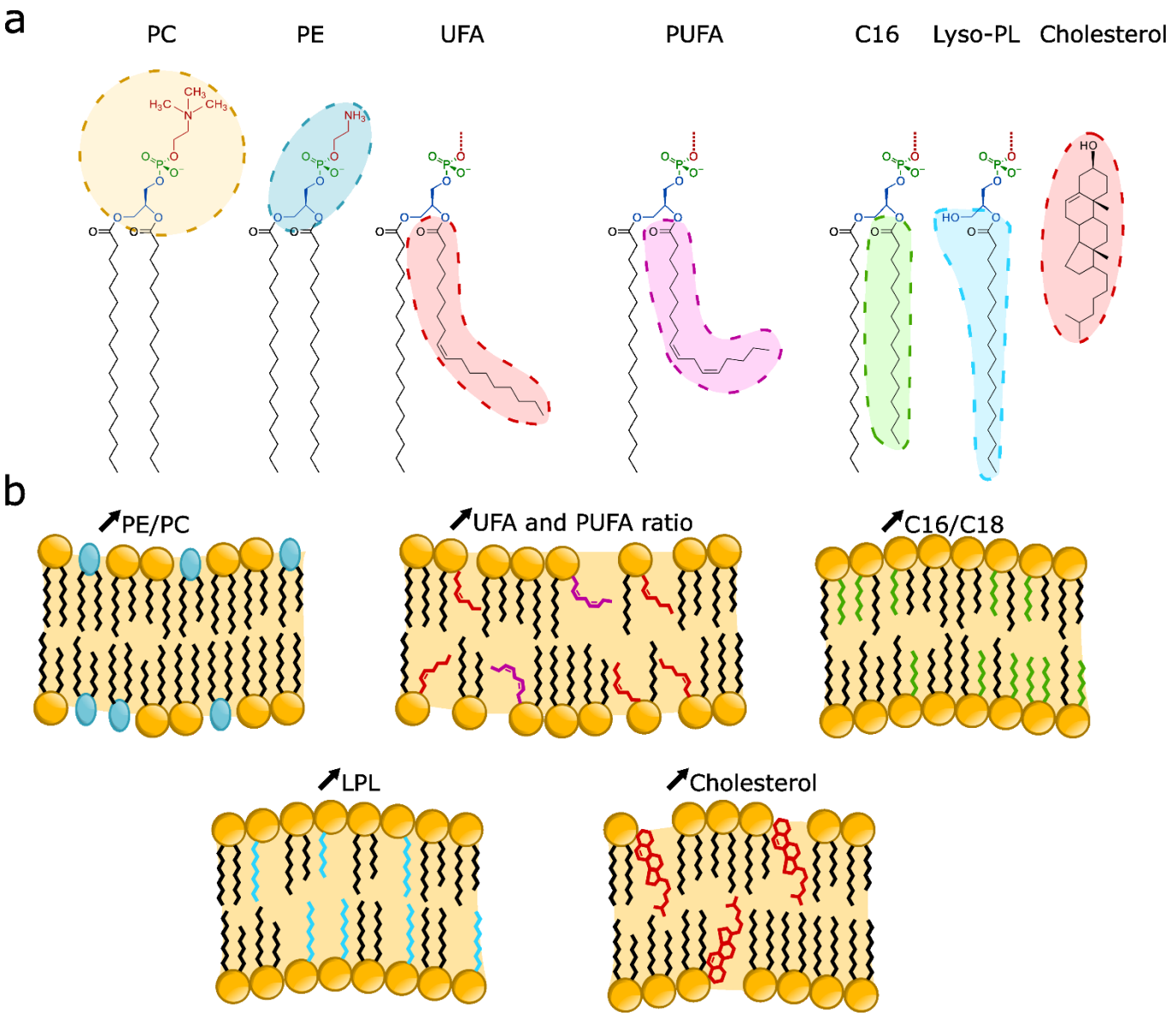

Figure 3: Modifications of lipid structures linked with homeoviscous adaptation of cell membranes. **(a)** overview of lipid structure modifications **(b)** effects on GPLs organization. PE have a conical shape, contrary to PC that show a cylindrical shape. Increased proportion of PE at the expense of PC increases space between GPLs, disordering the organization of GPLs bilayer, increasing membrane fluidity. Similarly, insertion of *cis* double bonds in fatty acids carbon chains disorders the organization of the bilayer. An increased proportion of C16 fatty acids in GPLs leads to modifications of non-covalent interactions between GPLs, which tend to increase the membrane fluidity. LPL have one of the cleaved fatty acid. This cleavage gives GPLs an “inverted cone” shape which participate to the disorganization of GPLs bilayer. Insertion of cholesterol molecules into the bilayer reduce mobility of the surroundings GPLs, ordering the membrane and decreasing its fluidity. This ordering effect could give cholesterol a role in maintaining membrane structure during freezing, preventing membrane phase separation and leakage caused by cold.

GPL: glycerophospholipid; PC: phosphocholine; PE: phosphoethanolamine; UFA: unsaturated fatty acid; PUFA: polyunsaturated fatty acid; LPL: Lyso-phospholipid.

ii. Increased unsaturation (or polyunsaturation) of fatty acid carbon chains

A major mechanism of homeoviscous adaptation in response to low temperatures is increased unsaturation (or polyunsaturation) of fatty acid carbon chains in GPL and TAG (Figure 3). Unsaturation of fatty acids is due to desaturase enzymes which insert a *cis* double bond, provoking a 30° curvature in the carbon chain. Unsaturated GPL have a largely conical shape and participate in disordering GPL organization, increasing the fluidity of membrane and TAG stored in lipid droplets. Brankatschk et al. (2018) indeed showed that *D. melanogaster* larvae with high level of unsaturated fatty acids were characterized by increased membrane fluidity at cold (determined from C-Laurdan emission measurements of liposomes prepared from lipid extracts of larvae). In other drosophilids, unsaturation of GPL fatty acids decreases the melting point temperature of membranes in cold acclimated flies, which could participate to decreasing their coma onset temperature (critical minimal temperature, CT_{min}; Slotsbo et al., 2016). Similarly, the transition temperature of TAG decreases in diapausing or cold adapted *Drosophila* flies (Ohtsu et al., 1993) and in *Cydia pomonella* moths collected in winter (Rozsypal et al., 2014). Increases in unsaturation or polyunsaturation of GPL and TAG are commonly linked with diapause, acclimation, acclimatization or adaptation to cold in insects and hexapods (Bashan and Cakmak, 2005; Bennett et al., 1997; Enriquez and Colinet, 2019a; Joanisse and Storey, 1996; Koštál and Šimek, 1998; Michaud and Denlinger, 2006; Ohtsu et al., 1993; Thiry and Hoffmann, 1986; Trenti et al., 2022; van Dooremalen and Ellers, 2010). However, in some insects, such as drosophilids, the effects of cold on fatty acid unsaturation is variable, as several studies reported no major changes in unsaturation of fatty acid (Colinet et al., 2016; MacMillan et al., 2009; Ohtsu et al., 1999; Overgaard et al., 2008), while others reported increase unsaturation and polyunsaturation in response to cold (Cooper et al., 2012; Cooper et al., 2014; Enriquez and Colinet, 2019a; Goto et al., 2010; Overgaard et al., 2005; Overgaard et al., 2006). In other taxa of insects, examples of fatty acid unsaturation absence due to low temperatures can also be found. For instance, in diapausing eggs of *Aedes albopictus* the unsaturation of GPLs and TAGs does not change in comparison with non-diapausing eggs (Batz and Armbruster, 2018), and similarly no changes are observed in cold acclimated *Sarcophaga similis* (Goto and Katagiri, 2011). No major changes in unsaturation are found in *Locusta migratoria*

following cold acclimation (Bayley et al., 2020; Gerber et al., 2021), nor in cold acclimated prepuae of the moth *Cymbalophora pudica* (Koštál and Šimek, 1998). Finally, during winter unsaturation of fatty acid can even decrease, for instance in thoracic muscles from *P. apterus* (Hodková et al., 1999).

iii. Shortening of fatty acid carbon chains length (increase of the C16/C18 ratio)

A widespread response to low temperatures is the shortening of fatty acid carbon chains length: the position and length of carbon chains influence hydrophobic effects between the two fatty acids of GPL. Consequently, shortening of carbon chains reduces hydrophobic effect and van der Waals forces between fatty acid chains (Kučerka et al., 2011) and therefore increases membrane fluidity (Figure 3). Increased cold tolerance due to fluctuating thermal regimes, adaptation to cold, diapause or acclimation is also often correlated with a shortening of fatty acid carbon chains length in insects, characterized in an increase of the C16/C18 ratio (Bahrndorff et al., 2007; Bashan et al., 2002; Colinet et al., 2016; Hodková et al., 1999; Ohtsu et al., 1999; Ohtsu et al., 1998; Overgaard et al., 2006; Thiry and Hoffmann, 1986; Trenti et al., 2022). However, fatty acid chain length does not change in cold acclimated *S. simili*s (Goto and Katagiri, 2011) nor differ between tropical and subpolar drosophilid species (Slotsbo et al., 2016), and the C16/C18 ratio even decreases in response to acclimation in *D. suzukii* (Enriquez and Colinet, 2019b).

iv. Increased proportion of LPL

In response to cold, the number of LPLs tends to increase in cell membranes (Figure 3). LPL are GPL in which one of the fatty acids has been cleaved by the action of a phospholipase A. This cleavage gives LPL an “inverted cone” shape, which disrupts the tight organization of GPL, increasing membrane fluidity. An increased proportion of LPLs within the GPL bilayer is known to play a role in the responses to low temperatures during thermal fluctuations in the model species *Drosophila melanogaster* (Colinet et al., 2016), during cold acclimation in *D. suzukii* (Enriquez and Colinet, 2019a) and during seasonal acclimatization in the bug *Pyrrhocoris apterus* (Koštál et al., 2013). Also, in *Ceratitis*

capitata flies, individuals showing the capacity to quickly recover after a chill coma are characterized by an increased proportion of LPLs (Pujol-Lereis et al., 2016).

v. Increased proportion of cholesterol in the cell membrane

After GPLs, cholesterol is the most abundant lipid within animal cell membranes. In contrast to GPL modifications presented previously, the presence of cholesterol is known to rigidify membrane bilayers. Cholesterol molecules reduces the rotamerisation capabilities of their surrounding GPLs, which tend to reduce their mobility and therefore the bilayer fluidity (Crockett, 1998; Yang et al., 2016). This rigidification capacities could give cholesterol a role in maintaining membrane fluidity during heat stress. Indeed, in mitochondria muscle cells from the desert locus *Schistocerca gregaria* acclimation at 45°C increase the proportions of cholesterol molecules embedded in the GPL bilayer, in comparison with mitochondria acclimated at 31°C, which increase the temperature of phase transition of mitochondria membranes (Downer and Kallapur, 1981; Kallapur et al., 1982). However, by interacting with fatty acyl chains of GPLs, cholesterol could also prevent membrane phase separation and leakage caused by low temperatures (Crockett, 1998; Mocé et al., 2010). In mammals, an abundance of cholesterol in spermatozoid membranes is indeed correlated to their resistance to cold shock and cryopreservation (Mocé et al., 2010). In insects yet, studies linking cholesterol to cold tolerance are scarce. Insects cannot synthetize cholesterol de novo, but Shreve et al. (2007) showed that *D. melanogaster* fed with medium supplemented in cholesterol resulted in an increased proportion of cholesterol in cell membranes, which in turn correlated with increased cold tolerance and cold acclimation capacity. However, in the gall fly *E. solidaginis* even if cholesterol levels increase in the hemolymph during winter acclimatization, the proportion of cholesterol embedded in membranes from Malpighian tubules does not increase (Yi and Lee, 2005). Similarly, in the woolly bear caterpillar *Pyrrhactia isabella* cholesterol levels remain stable during cold acclimation in tissues such as Malphighian tubules and cells from the midgut, but decrease both in the hemolymph, and in fat body cell membranes (Yi and Lee, 2016). As insect sterols originated from their diet, other sterols such as phytosterols can replace the role of cholesterol in insect membranes, as in *D.*

melanogaster where ergosterol is an abundant membrane sterol (Rietveld et al., 1999). In the firebug *P. apterus* winter is characterized by an increase concentration of phytosterols, but it is not clear which proportion of these sterols are incorporated in cell membranes, and what role they play in insect cold tolerance (Koštál et al., 2013). Thus, there is still a lack of knowledge on the function of cholesterol or other sterols in the responses to temperature changes in insects, which should be tackled in future studies.

An analysis of the body of literature that investigated lipid modifications linked with low temperatures reveals that homeoviscous adaptation plays a major role in insect overwintering, and that cold tolerance is linked with preservation of membrane fluidity, which helps to maintain insect cell viability at cold (Lee et al., 2006). However, some insect species show limited evidence of homeoviscous adaptation, like the locust *L. migratoria* (Bayley et al., 2020; Gerber et al., 2021). This lack of GPL modifications in response to cold can be due to the fact that this species overwinter as eggs and not at the adult stage (Wang and Kang, 2003), making GPL adjustments unnecessary for adults, which were the subject of these studies. Also, insects do not necessarily require all the adjustments previously presented to adjust membrane fluidity. This can be illustrated by taking insects from the genus *Drosophila* as an example. Indeed, in *D. melanogaster* low temperatures induces modifications of GPL headgroups and increase C16/C18 ratio but do not increase fatty acid unsaturation (Colinet et al., 2016). However, in *D. suzukii* cold similarly reshuffles GPL headgroups but decreases the C16/C18 ratio while increasing unsaturation of fatty acid chains (Enriquez and Colinet, 2019b). These discrepancies can be due to numerous different factors such as differences in experimental set ups or different range of temperatures used, but it is more likely that the precise adjustments used for homeoviscous adaptation vary across species. The several adjustments linked with homeoviscous adaptation could therefore be used alternatively in organisms to reach a similar outcome: guaranteeing membrane fluidity at cold temperatures.

3.b – Lipids as energetic resources during cold stress and acclimation

Overwintering is a challenging time for insects, as they are faced with the dual threats of abiotic environmental stress (e.g., low temperature, lack of water, limited

oxygen, etc.) and lack of food availability (Hahn and Denlinger, 2011; Overgaard and MacMillan, 2017). The inability of most insects to eat in the winter puts significant energy strain on them, and the role of lipid metabolism in coping with the energetic challenges of overwintering is covered in Chapter 12. Here, we will address the role of lipid metabolism in supporting the energetic costs of cold acclimation and recovery from cold stress. This chapter is not intended to be an exhaustive review but will instead focus on general principles and key examples.

At low temperatures, excitable membranes become depolarized, leading to a collapse of neuromuscular function and eventual loss of ion homeostasis (See section 2.b; Overgaard and MacMillan, 2017). This loss of ion homeostasis eventually contributes to cold injury by activating cell death programs. Thus, repairing cold injury requires restoring cellular osmotic gradients and reestablishing membrane polarity. The bulk movements of water and ions required to restore homeostasis are energetically costly, and indeed, recovery from cold stress is often accompanied by elevated metabolic rate relative to conditions before the cold stress (Lalouette et al., 2011; MacMillan et al., 2012). The timing of this "metabolic overshoot" corresponds with the time required to recover hemolymph Na^+ content (MacMillan et al., 2012), suggesting that the primary reason for this increased metabolic rate is to restore ion and water balance. However, it is worth noting that elevated metabolic rate during recovery from cold stress is not universal; in the freeze tolerant midge *Belgica antarctica,* in the absence of cold hardening, larvae have *reduced* metabolic rates during recovery (Teets et al., 2019), presumably due to impaired oxygen transport and/or mitochondrial function. Nonetheless, it is clear that cold stress is energetically costly, and in many cases lipid reserves fuel this increased demand for energy (see below).

In addition to restoring ion gradients, many of the molecular processes involved in resisting or repairing cold-induced damage to macromolecules are energetically costly. For example, heat shock proteins are molecular chaperones that are involved in numerous abiotic stress responses (even cold, despite their name), and recovery from cold stress elicits rapid upregulation of these genes (Joplin et al., 1990; Rinehart et al., 2007; Sinclair et al., 2007). In the case of heat stress, the energetic costs of activating the

heat shock response reduce energy availability for reproduction, and the number of heat stress events is proportional to declines in fecundity (Krebs and Loeschcke, 1994). While direct measurements have not been made for cold, it is well established that heat shock divert energy from essential functions like growth and reproduction, so expression after cold likely carries an energetic cost (Feder and Hofmann, 1999; Sørensen et al., 2003). Indeed, elevated expression of heat shock proteins sometimes corresponds with reduced lipid reserves (Teets et al., 2011), suggesting that lipid may be fueling energetically costly repair processes like heat shock protein activation. In some cases, heat shock proteins and other protective mechanisms are often upregulated in an anticipatory manor to increase resistance to cold injury (Teets and Denlinger, 2013). Beyond these canonical stress response pathways, cold acclimation often involves wholesale changes in transcript abundance; for example, cold acclimation in *D. melanogaster* results in nearly 1/3 of the transcriptome to be differentially regulated (MacMillan et al., 2016). These large-scale changes in gene expression likely carry energetic costs, although the extent to which lipid metabolism provides energy inputs for cold acclimation has not been quantified. However, it is important to note that cold acclimation can also cause compensatory changes in body composition. For example, *D. melanogaster* reared at 18°C have significantly higher lipid reserves than those reared at 30°C (Klepsatel et al., 2016), so they may be able to accommodate the energetic costs of cold acclimation.

The energetic costs of cold injury are often reflected by a depletion in lipid reserves during or after cold stress. In *D. melanogaster,* exposure to 0°C beyond 24 h rapidly depletes TAGs, such that less than half the initial reserves are present after 48 h of cold exposure (Chen and Walker, 1994). In the same species, cold exposure also causes TAG depletion during a recovery. Exposure to 0°C for 4 h, which is nonlethal, leads to ~25% reduction in TAG content, and these deficits are permanent and still apparent 10 days after cold exposure (Klepsatel et al., 2016). However, another study in *D. melanogaster* indicated that TAG depletion after cold exposure is only transient (Marshall and Sinclair, 2010), and it is unclear why these discrepancies exist. Outside of *D. melanogaster,* similar depletions in lipid reserves following cold stress have been observed in the parasitic wasps *Aphidius colemani* and *A. ervi* (Colinet et al., 2006; Ismail et al., 2010), as well the freeze-tolerant midge *B. antarctica* (Teets et al., 2011; Teets et al., 2019). Similarly, in the

freeze-tolerant fly *Chymomyza costata,* recovery from both chilling and freezing cause large increases in metabolic rate, and while lipid content was not directly measured, calculations indicate that as much as half of total lipid reserves could be depleted during recovery (Štětina et al., 2018). In freeze-tolerant larvae of *E. solidaginis,* repeated freeze-thaw cycles promote the conversion of long-chain TAGs to acetylated TAGs (see details below), which causes a net reduction in energy density and a concomitant decrease in spring reproductive output (Marshall and Sinclair, 2018). This depletion of long-chain TAGs results in decreased egg production in the spring (Marshall and Sinclair, 2018), indicating that lipid depletion in response to cold stress can have lasting fitness consequences. However, direct measures of fitness-related traits (e.g., behavior, reproductive output, etc.) are seldom incorporated into energetic studies of cold tolerance. Thus, additional work is needed to quantify the fitness effects of energy depletion caused by cold stress.

Several factors can influence the extent of lipid depletion in response to cold stress. First, and perhaps most intuitively, the severity and duration of cold stress influences rates of lipid usage. For example, in *B. antarctica,* lipid reserves decrease steadily as the total duration frozen increases, such that the amount of depletion after five cycles of 12 h freezing and 12 h thawing (i.e., 60 h total frozen) is the same as the amount of depletion observed after a continuous 60 h of freezing (Teets et al., 2011). In *E. solidaginis,* lipid metabolism is influenced by both the freezing temperature and the number of freezing events experienced, indicating that the severity of cold exposure and the number of threshold-crossing events can both influence lipid utilization (Marshall and Sinclair, 2018). Further, for freeze-tolerant insects, whether they are frozen or supercooled during a cold event can influence lipid metabolism. In *E. solidaginis,* metabolic rate is lower in frozen larvae than supercooled larvae at the same temperature (Irwin and Lee, 2002), presumably leading to less energy drain when larvae are frozen. Similarly, in the freeze-tolerant moth *Pyrrharctica isabella,* larvae that overwinter above the snow are more likely to freeze, and frozen larvae consumed less lipid, even when accounting for the temperature difference (Marshall and Sinclair, 2012). However, for *B. antarctica,* the opposite was observed, in which larvae that were inoculatively frozen had higher rates of lipid depletion than those that were supercooled at the same temperature

(Teets et al., 2011). Finally, cold acclimation can influence the energetic costs of cold exposure. In *B. antarctica,* 60 h of freezing at -5°C leads to ~20% depletion of lipid stores in summer-collected larvae (Teets et al., 2011), but winter acclimatized larvae experience no detectable lipid depletion after 2 weeks of freezing at -5°C. Thus, the precise consequences of freezing vs. supercooling on lipid metabolism can vary both across and within species, depending on the exact cold conditions and acclimation state.

While a reduction in lipid reserves is commonly observed following cold stress, this pattern is not a universal phenomenon. The stink bug *Halyomorpha halys* maintains consistent lipid reserves throughout winter, despite not feeding, although its protected overwintering habitats rarely experience subzero temperatures (Ciancio et al., 2021). Thus, this species may use habitat selection as a means to avoid energetic costs associated with low temperature stress. The tropic cockroach *Gromphadorhina coquereliana,* despite having a limited ability to survive low temperature, does not experience any lipid depletion in the fat body after repeated cold exposures (Chowanski et al., 2015). Thus, perhaps insects that seldom experience cold in their natural environments have not evolved energetically expensive repair processes that deplete lipid reserves. However, the moth *P. isabella* is also able to maintain lipid stores after repeated freeze-thaw cycles, despite experiencing considerable damage to tissues. Thus, lipid metabolism after cold stress varies across insect species, although differences in study designs and treatment conditions make it difficult to pinpoint the exact reasons for these discrepancies. One likelihood is that lipid metabolism during and after cold stress is related to life history, as outlined in Sinclair and Marshall (2018). For example, insects that are unable to feed and replenish lipid reserves after winter may preferentially use other energy sources so that they can reserve lipids for reproduction.

For insects that rely on lipids to fuel cold stress responses, conditions that deplete lipid reserves can affect the ability to survive low temperatures. While energy drain throughout the winter is a significant challenge for many insects (see Chapter 12), conditions that reduce lipid storage could leave less energy available to cope with cold stress (Sinclair, 2015). While the link between carbohydrate storage and cold tolerance is clear, given carbohydrates' role as cryoprotectant precursors (Storey, 1997), whether

lipid levels directly influence cold tolerance is less clear. In the case of *D. suzukii,* starvation prior to cold stress that reduces lipid reserves concurrently raises CT_{min} and lengthens chill coma recovery time but improves survival of acute cold shock (De Ro et al., 2021), indicating that distinct cold tolerance traits are differentially affected by energy depletion. These results are consistent with other work showing that dietary composition influences distinct cold tolerance traits in unpredictable ways, and that the precise effect of diet varies across genotypes (Littler et al., 2021). In antlions (Order: Neuroptera) and wormlions (Order: Diptera), starvation has no effect on cold tolerance, although individuals with higher lipid content have faster chill coma recovery time (Scharf et al., 2016). Thus, the relationship between lipid content and cold tolerance appears to be complex and requires further investigation across a wider range of species.

3.c - Other functions of lipids in relation to cold stress

In addition to their roles in homeoviscous adaptation and as energy sources for fueling cold stress responses, lipids play some other roles in cold stress. Most of these minor roles have not been studied extensively, so it is unclear how commonplace they are across species. One potential role for lipids is to serve as a precursor for cryoprotectant production. The glycerol motif of TAG is potent cryoprotectant, and indeed, glycerol is perhaps the most commonly used low molecular weight cryoprotectant among insects (Storey, 1997). Thus, liberation of glycerol by lipases could increase cryoprotectant titers in the bloodstream. However, insects predominantly use glycogen as a precursor for glycerol synthesis (Storey and Storey, 2012), and it doesn't appear that TAG plays a major role. Stoichiometrically, one glucose molecule can yield two glycerol molecules, and a single glycogen molecule can contain thousands of glucose subunits. Thus, glycogen is a much more efficient precursor for glycerol, as TAG (MW > ~500 g/mol, depending on fatty acid composition) can only produce a single glycerol molecule (MW = 92.1 g/mol). While lipids don't appear to be a major source of glycerol, there are other cases where they can be converted to cryoprotective molecules. For example, trehalose and proline, two potent cryoprotectants, can be derived from fatty acids (Arrese and

Soulages, 2010; McDougall and Steele, 1988), although the extent to which these processes operate in the context of cold tolerance is uncertain.

Another class of lipids that may be important for overwintering insects are cuticular lipids. The insect cuticle contains both structural lipids and a wax layer of free lipids that serves an important waterproofing function (Lockey, 1988). Cold stress is often accompanied by limited water availability, and when insects are in chill coma, they are at increased risk of desiccation due to an inability to drink water (which may be frozen anyway) and an inability to select humid microhabitats (Sinclair et al., 2013). Increased cuticular lipids are observed as part of the diapause program in several species (Ala-Honkola et al., 2020; Benoit and Denlinger, 2007; Urbanski et al., 2010), although it appears the primary benefit is to increase resistance to desiccation during dormancy. Similarly, in the high-elevation species *D. nepalensis,* cold acclimation increases cuticular hydrocarbon deposition (Parkash and Lambhod, 2021), although it is once again presumed that this response is to promote cross-tolerance to desiccation. Thus, in several species, cuticular lipids appear to be involved in the overwintering program, although it is unclear whether they play a role in cold tolerance.

Finally, some insects have evolved novel classes of lipids that play important roles in cold hardiness. In *E. solidaginis,* acetylated TAGs make up a staggering 36% of the total lipid pool in overwintering larvae (Marshall et al., 2014). Most animals rely exclusively on long-chain TAGs, and acetylated TAGs have only been observed in extremely trace amounts in animals. Larvae of *E. solidaginis* often overwinter above the snowpack in goldenrod stems, which provide little insulation, and are thus among the most freeze-tolerant insects that have been described (routinely surviving below -55°C; Lee et al., 1995). Thus, it is proposed that acetylated TAGs, which have lower viscosity than long-chain TAGs, may be more accessible for energy metabolism at extremely low temperature (Marshall et al., 2014). Furthermore, these lipids accumulate in droplets in the fat body, and the fat body of *E. solidaginis* is one of a few known insect tissues that can survival internal ice formation (Bennett and Lee, 1997), suggesting that these unique lipids may play a role in intracellular freezing tolerance. The freeze-tolerant beetle, *Upis ceramboides,* also has synthesizes a novel xylomannan glycolipid that consists of fatty

acid and saccharide components (although it is not clear if the lipid and saccharide are covalently linked), and this compound produces significant thermal hysteresis activity (Walters et al., 2009). This compound was the first non-protein thermal hysteresis agent to be isolated, and it has since been found in several other insect species, indicating that glycolipids may play a previously unappreciated role in regulating ice formation in insects (Walters et al., 2011).

4 - Molecular regulation of lipid metabolism during cold exposure

While the molecular regulation of metabolism in relation to diapause is well-described in some species (e.g., Hahn and Denlinger, 2011; Reynolds et al., 2012; Sinclair and Marshall, 2018), the regulation of lipid metabolism during cold acclimation and in response to stress has received less attention. Nonetheless, select studies have identified some of the key lipid metabolism enzymes and genes that directly respond to low temperature. Cold acclimation involves large-scale changes in both the transcriptome (MacMillan et al., 2016) and proteome (Colinet et al., 2013), and many of these changes prepare insects to cope with the physiological challenges of cold stress, including maintenance of ion homeostasis, preservation of protein structure and function, and synthesis of low molecular cryoprotectants to protect against cold injury. Indeed, some of the changes in lipid metabolism discussed above are supported by changes at the molecular level, and we will detail those changes in this section.

At the biochemical level, changes in the activities of select lipid metabolism enzymes accompany seasonal acquisition of cold tolerance. Two gall-forming insects, the moth *Epiblema scudderiana* and the fly *E. solidaginis,* have opposite changes in lipid metabolism, with the freeze-avoidance *E. scudderiana* depleting lipid reserves over the winter while the freeze-tolerant *E. solidaginis* preserves lipids (Joanisse and Storey, 1996). These changes are reflected by changes in enzyme activity in *E. solidaginis*, as enzymes involved in fat oxidation (hydroxyl-CoA dehydrogenase, carnitine-palmitoyl transferase, and acetoacetyl-CoA thiolase) all decrease in *E. solidaginis* over the winter to preserve lipid reserves. However, in *E. scudderiana,* enzyme activities are more variable and not as well-correlated with lipid reserves. Further, in *E. solidaginis,* cold

hardy larvae have higher activity of AMP-activated protein kinase (AMPK) (Rider et al., 2011), a signaling molecule that shifts metabolism from ATP consumption to ATP conservation during hypometabolic states. Activation of AMPK also suppresses lipid biosynthesis, and its activation in the winter ins consistent with reduced activity of enzymes associated with lipid biosynthesis (e.g., ATP -citrate lyase and malic enzyme) (Joanisse and Storey, 1996). AMPK is also phosphorylated in direct response to cold stress in the beetle *Tribolium castaneum* (Jiang et al., 2019), suggesting this enzyme may play a general role in regulating lipid metabolism at low temperatures.

At the gene level, several studies have shown that desaturases likely play an important role in mediating responses to cold stress (reviewed by Cossins et al., 2002). Desaturases catalyze the formation of double bonds in polyunsaturated fatty acids and thus play an important role in homeoviscous adaptation (discussed above). In the cricket *Acheta domesticus,* progressively decreasing temperatures lead to a 66% increase in Δ^{12} desaturase activity, the specific enzyme responsible for linoelic acid biosynthesis, and accordingly, increases in linoleic acid are sometimes observed in overwintering insects (Rozsypal et al., 2014). At the transcript level, desaturase genes are upregulated during cold acclimation in both the onion maggot *Delia antiqua* (Kayukawa et al., 2007) and *D. suzukii* (Enriquez and Colinet, 2019b). Also, in *D. melanogaster,* a desaturase gene is upregulated during recovery from cold stress (Zhang et al., 2011), suggesting desaturases play roles in both preparatory processes and in direct response to cold stress. While functional studies on desaturase enzymes in the cold are lacking in insects, in the worm *Caenorhabditis elegans* combined suppression of two desaturase genes reduces the degree of cold acclimation (Murray et al., 2007). However, the effect size of this manipulation is small, suggesting that other non-desaturase processes are responsible for the majority of protection at low temperature.

Select studies have also identified other players involved in lipid metabolism at low temperature. Adipokinetic hormone (AKH) is one of the primary neuroendocrine regulators of lipid metabolism in insects, and while a direct role for this hormone in cold stress has not been identified, genes encoding its receptor (*AkhR*) are activated by low temperatures. *AkhR* transcripts are upregulated in both *D. suzukii* after 9 d of cold

acclimation at 10°C (Enriquez and Colinet, 2019b) and after three days of decreased temperatures (from 34 to 25°C) in brood of *Apis melifera* (Ramirez et al., 2021), suggesting increased sensitivity to AKH may be required to maintain lipid metabolism at low temperatures. AKH is also involved in regulating supercooling capacity in some insects. In the beetle *Ceruchus piceus,* AKH signaling is shut down in the winter, and exogenous application reduces supercooling capacity by causing the mobilization of ice nucleating lipoproteins (Xu et al., 1990). In contrast, juvenile hormone (JH) causes a reduction in ice nucleating activity, indicating that the opposing actions of JH and AKH regulate circulation of ice nucleating lipoproteins. In the rice stem borer *Chilo suppressalis,* cold acclimation leads to upregulation of two transcripts encoding lipase genes (Ma et al., 2020), potentially to liberate glycerol for cryoprotection. In addition to these genes involved in preparatory cold acclimation processes, genes involved in glycerolipid metabolism are rapidly upregulated during recovery from cold shock in the flesh fly *Sarcophaga bullata* (Teets et al., 2012). Finally, during rapid cold hardening, a short-term cold acclimation response that does not require changes in gene expression, lipid storage droplet proteins are rapidly phosphorylated after 2 h of 0°C (Teets and Denlinger, 2016), which would promote lipolysis. However, the functional significance of this phosphorylation event is unclear. Together, the above studies suggest that lipid metabolism is regulated at several levels during cold stress, but work is needed in additional taxa to establish the generalizability of these results.

Conclusions and Perspectives

Lipid metabolism plays a central role for overwintering insects (Figure 4). In addition to its well-established role for long-term energy storage during diapause (See Chapter 12 and Hahn and Denlinger, 2011), lipid metabolism contributes in many other ways to survival at low temperature. Changes in cell membrane lipid composition facilitate homeoviscous adaptation, which permits membrane function and fluidity at low temperatures (Sinensky, 1974). Homeoviscous adaptation is commonly observed among overwintering insects, although the manner in which it is achieved can vary from species to species (i.e., changes in headgroups, fatty acid composition, and/or cholesterol

content), suggesting multiple evolutionary origins for homeoviscous adaptation. Lipids also play an important role in fueling energetically costly stress defense mechanisms in the cold, and indeed, the frequency and severity of cold stress during winter can have a significant impact on energy reserves available when the growing season resumes. Some especially cold-adapted species have also developed novel classes of lipids that function as antifreezes or that can be mobilized as energy sources during periods of freezing. Finally, the expansion of molecular research in recent years has supported an essential role for lipid metabolism in cold stress, as gene expression related to lipid metabolism is involved in both cold acclimation and recovery from cold stress. However, as with much of insect biology, diversity in study systems and methodology makes it challenging to draw general conclusions, so carefully designed, phylogenetically informed studies of lipid metabolism during cold stress are needed in future research.

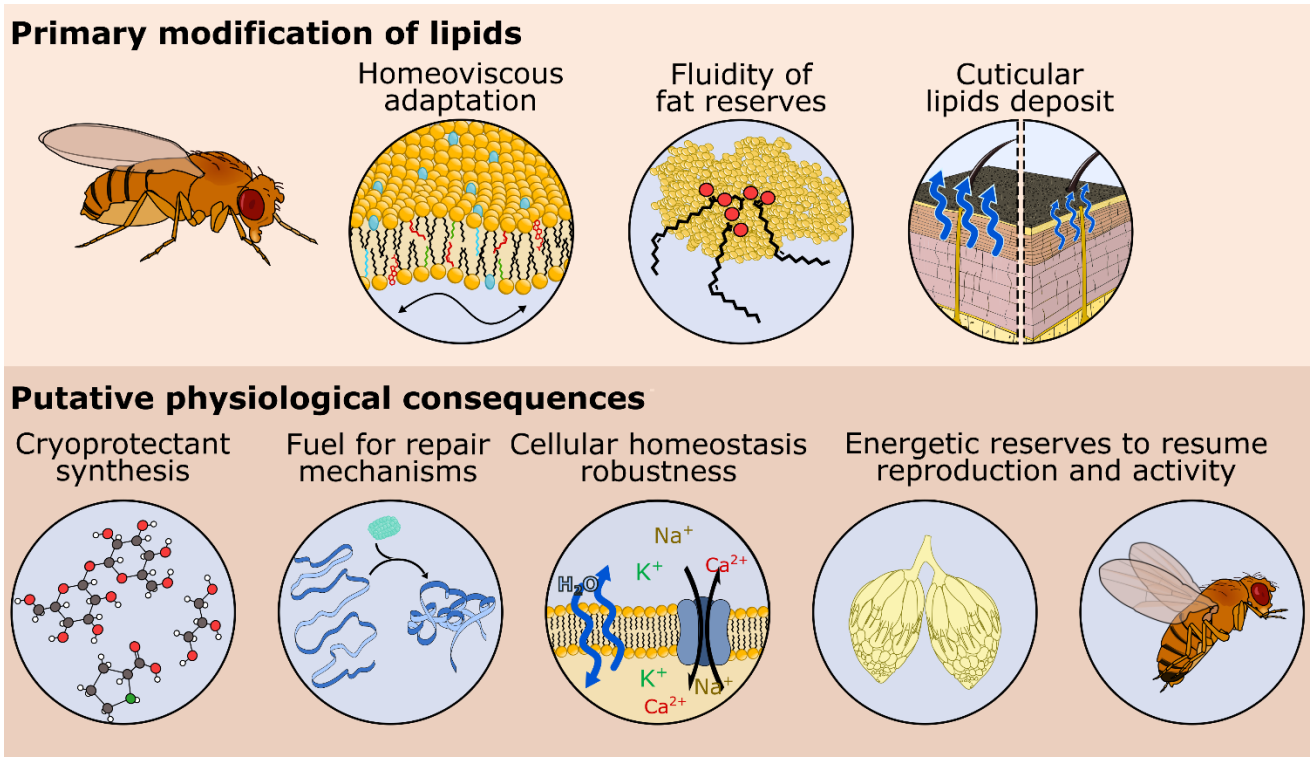

Figure 4: Modification of lipids during winter, and physiological repercussions. Maintenance of lipid structures fluidity allow membranes functioning (homeoviscous adaptation) and guarantees the availability of lipid reserves at cold temperatures. Increased cuticular lipid deposits could protect from dehydration during long term cold exposures. Lipid reserves can serve as precursors for synthesis of cryoprotectant molecules, such as saccharose, proline or glycol, and as fuel for the activation of the repairing machinery, such as production of heat shock proteins for instance. All these adjustments help to the maintenance of cellular homeostasis. Lipid reserves are also of major importance at the end of winter, as they will allow insects to resume their activity and reproduce.

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