

Insect Cold-Hardiness: Insights from the Arctic

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(Received 3 March 1994; accepted in revised form 19 September 1994)

ABSTRACT. Cold-hardiness and related adaptations of insects in the Arctic correspond to characteristic climatic constraints. Some species are long-lived and are cold-hardy in several stages. In the Arctic, diapause and cold-hardiness are less likely to be linked than in temperate regions, because life-cycle timing depends as much on the need to coincide development with the short summer as on the need to resist winter cold. Winter habitats of many species are exposed rather than sheltered from cold so that development in spring can start earlier. Several features of cold-hardiness in arctic species differ from the characteristics of temperate species: these include very cold-hardy insects with low supercooling points that are not freezing tolerant; freezing-tolerant species that supercool considerably rather than freezing at relatively high subfreezing temperatures; mitochondrial degradation linked with the accumulation of cryoprotectants; and the possibly limited occurrence of thermal hysteresis proteins in winter. Several interesting relationships between cold-hardiness and water have been observed, including different types of dehydration. Winter mortality in arctic insects appears to be relatively low. Adaptations to cold in summer include retention of cold-hardiness, even freezing tolerance; selection of warm sites; and behaviour such as basking that allows elevated body temperatures. Studies especially on the high-arctic moth *Gynaephora groenlandica* show that various factors including cold-hardiness and other summer and winter constraints dictate the structure of energy budgets and the timing of life cycles.

Future work should focus on the biological and climatic differences between arctic and other areas by addressing habitat conditions, life-cycle dynamics, and various aspects of cryoprotectant production at different times of year. Even in the Arctic cold-hardiness is complex and involves many simultaneous adaptations.

Key words: cold-hardiness, insects, supercooling, freezing-tolerance, cryoprotectants, metabolism, energy budgets, life cycles, habitat selection

RÉSUMÉ. L'endurance au froid et autres adaptations connexes des insectes de l'Arctique correspondent à des contraintes climatiques caractéristiques. Certaines espèces ont une grande longévité et sont résistantes au froid à plusieurs stades. Dans l'Arctique, il est moins probable que la diapause et l'endurance au froid soient reliées que dans les zones tempérées, parce que le cycle de vie dépend autant du besoin de faire coïncider la croissance avec le bref été que du besoin de résister au froid hivernal. Les habitats hivernaux de nombreuses espèces sont exposés au froid plutôt qu'abrités du froid de façon que la croissance débute plus tôt au printemps. Plusieurs caractéristiques de la résistance au froid chez des espèces arctiques diffèrent des caractéristiques des espèces tempérées: les premières comprennent des insectes très résistants au froid ayant des points de surfusion bas qui ne sont pas tolérants au gel; des espèces tolérantes au gel qui manifestent une surfusion considérable plutôt que de geler à des températures relativement hautes sous le point de congélation; la dégradation mitochondriale liée à l'accumulation de cryoprotecteurs; et l'occurrence peut-être limitée de protéines à hystérésis thermique en hiver. On a observé plusieurs rapports intéressants entre la résistance au froid et l'eau, y compris différents types de déshydratation. La mortalité hivernale chez les insectes de l'Arctique semble être relativement basse. Les adaptations au froid en été comprennent le maintien de la résistance au froid, et même de la tolérance au gel; le choix de sites tempérés et le comportement qui consiste à se chauffer au soleil, ce qui permet une élévation des températures du corps. Des études effectuées en particulier sur la noctuelle de l'Extrême-Arctique *Gynaephora groenlandica* montrent que divers facteurs y compris la résistance au froid et d'autres contraintes estivales et hivernales dictent la structure des bilans énergétiques et le rythme des cycles de vie.

De futurs travaux devraient porter sur les différences biologiques et climatiques entre des régions arctiques et d'autres zones en étudiant les conditions de l'habitat, la dynamique des cycles de vie et divers aspects de la production de cryoprotecteurs à différentes époques de l'année. Même dans l'Arctique, la résistance au froid est complexe et implique bien des adaptations simultanées.

Mots clés: résistance au froid, insectes, surfusion, tolérance au gel, cryoprotecteurs, métabolisme, bilans énergétiques, cycles de vie, sélection de l'habitat

Traduit pour la revue *Arctic* par Nésida Loyer.

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INTRODUCTION

Many insect species are able to survive temperatures well below freezing, and diverse aspects of insect cold-hardiness have been described (Lee and Denlinger, 1991). In the Arctic, cold is severe and conditions in many habitats approach the physiological limits for life. The region, therefore, is especially useful for studying cold-hardiness: adaptations to cold tend to be more extreme and more prevalent in arctic species than in their southern relatives. Arctic research also suggests the importance of considering events throughout the year, because in the high Arctic even summer is cold and short.

This paper reviews the cold-hardiness of insects in arctic regions, emphasizing key discoveries about cold-hardiness that were assisted by information from the Arctic. Valuable information has also come from studies in antarctic and alpine areas (e.g., Sømme, 1989; Block, 1990; Sømme and Block, 1991; and papers cited therein), but it is not emphasized here. On the basis of adaptations observed and the factors that appear to have influenced those adaptations, the paper points out further ways to explore cold-hardiness by using the Arctic as a laboratory. Such work is of potential interest to a range of arctic biologists. A glossary of common technical terms relevant to the field of insect cold-hardiness follows the acknowledgements.

Aspects of life cycles and their timing related to cold-hardiness are discussed first, followed by a review of adaptations to winter conditions on a range of scales from habitats to cells. Adaptations to summer conditions are then outlined. The energy required for cold-hardiness is considered, and a synopsis of techniques likely to be most useful or feasible in the Arctic is provided. A final section summarizes the key conclusions of this review, and highlights some aspects of insect cold-hardiness that can most profitably be explored further in arctic regions.

ASPECTS OF INSECT COLD-HARDINESS

Climatic Constraints

Many climatic features of arctic regions limit the ways in which insects can live there. Such features are exemplified by the measures of temperature and seasonality shown for a single high arctic site in Table 1. This site, Resolute, Cornwallis Island, Northwest Territories, represents the northwestern high Arctic, which is colder and cloudier in summer than the larger islands to the east, such as Ellesmere Island.

All species experience severe cold in winter (the mean February temperature of this site is below -33°C : Table 1). Species cannot escape by long-distance movement, because equable climates are very far away. Moreover, the presence of permafrost everywhere except in deep lakes and in the substrates of some running waters means that unfrozen habitats are not available to most species, and even individuals buried in substrates experience prolonged temperatures of about -20°C in the high Arctic (Danks, 1971a). Nevertheless, constant darkness in winter without solar heating or marked diel cycles means that temperatures, though severe,

TABLE 1. Selected climatic information for a sample site in the Canadian northwestern high Arctic: long-term mean values (chiefly, 1951–80) for Resolute, Cornwallis Island ($74^{\circ} 43' \text{N}$, $94^{\circ} 59' \text{W}$; 67 m elevation) (Danks, 1993).

Mean annual screen air temperature	-16.6°C
Mean February temperature (coldest month)	-33.2°C
Mean July temperature (warmest month)	4.1°C
Mean July maximum	6.8°C
Mean July minimum	1.4°C
Maximum temperature recorded	18.3°C (July)
Minimum temperature recorded	-52.2°C (Jan)
Mean cloud cover during June and July	75%
Mean seasonal melting degree days	248
Approximate date after which daily mean rises above 0°C	16 June
Approximate date on which daily mean falls below 0°C	30 August
Mean duration of melt period	74 days
Likelihood of frost during July	$>90\%$
Mean frost-free period (10–20 July)	9 days
Approximate duration of persistent snow cover	290 days
Annual rainfall (June, July, August)	52.7 mm
Annual snowfall (all months)	83.8 cm
Mean snow depth on ground at the end of January	10.2 cm

are somewhat less variable than in temperate regions.

The winter is very long. Species sealed in ice will not be exposed to the air again for many months, and might require the ability to respire anaerobically as temperatures rise before the ice melts. Summer is short and cool (e.g., mean air temperatures exceed 0°C for only 74 days; Table 1). The mean frost-free season is very short, only 9 days, so that cold-hardiness is required even during the warmest summer period. This short season starts slowly as habitats warm up. Wet habitats and low-lying areas where snow has accumulated through drifting in winter warm up especially slowly.

Many potential resources of food are in short supply in arctic regions (Danks, 1981), which makes insect development during the short summer season even more difficult. Constraints on development imposed by limited resources of food, heat and time are offset by the fact that the temperature of habitats, especially at the ground surface and in shallow waters where most insects live, can be greatly increased by solar heat. Therefore, the extent of summer cloud cover is very important. Cloud cover varies especially according to proximity to the sea, both on a local scale and according to the size of regional land masses, because the sea contributes moisture to the air when it is ice-free in summer. Arctic regions are very dry, and often have been referred to as polar desert. Only a few centimetres of rain fall each year in the high Arctic (Table 1). Such dryness may hinder insect development, and interacts with the effects of temperature.

Life Cycles

An insect's life cycle determines the type and number of stages that require cold-hardiness for winter, and for how many winters. In many species from temperate regions, life-cycle development lasts less than a year, and the species overwinters, typically in a single fixed stage, after completing several summer generations. Other species have one generation per year and overwinter in each generation. A few have still longer life cycles. In colder environments and at higher latitudes, more species

have prolonged life cycles (Sømme and Block, 1991; Danks, 1992).

In the Arctic, insect life cycles vary from one to many years (Table 2). For example, mosquitoes and bumble bees complete a generation each year. The high arctic lymantriid moth *Gynaephora groenlandica* regularly takes 14 years. Species that overwinter many times generally do so in successive larval stages (Table 2). For example, all instars except the first of *G. groenlandica* overwinter once or more (Kukal and Kevan, 1987). Each of these stages is freezing-tolerant. Moreover, in a few species with long-lived adults, both adults and larvae are very cold-hardy (Ring and Tesar, 1980 for the pythid beetle *Pytho americanus*).

TABLE 2. Duration of the life cycle and overwintering stages reported for some arctic insects.

Species	Life cycle (years)	Overwintering Stage(s)	Reference
Coleoptera			
<i>Isochnus arcticus</i> (Curculionidae)	1	adult	Downes, 1964 (as <i>Rhynchaenus</i> sp.)
Diptera			
<i>Pedicia hannai antennata</i> (Tipulidae)	4–5	larva I?, II, III, IV ¹	MacLean, 1973
<i>Aedes</i> spp. (Culicidae)	1	egg	Corbet & Danks, 1975
<i>Orthocladus lapponicus</i> (Chironomidae)	3	larva I, II, III	Welch, 1976
<i>Chironomus prior</i> (Chironomidae)	7	larva I, II, III, IV	Butler, 1982a, 1982b
<i>Chironomus tardus</i> (Chironomidae)	7	larva I, II, III, IV	Butler, 1982a, 1982b
Lepidoptera			
<i>Gynaephora groenlandica</i> (Lymantriidae)	14	larva II, III, IV, V and VI	Kukal & Kevan, 1987 Kevan & Kukal, 1993
Hymenoptera			
<i>Bombus</i> spp. (Apidae)	1	adult	Richards, 1973

¹ Roman numerals refer to larval instars.

Cold-hardiness and Diapause

Requirements for cold-hardiness vary seasonally, and the synthesis of cryoprotectants may be controlled by seasonal temperature, photoperiod or both (e.g., Baust, 1982; Horwath and Duman, 1983; Lee, 1991). Seasonal responses also adjust the timing of the active and inactive, usually sheltered, stages. Life cycles may include diapause, a programmed interruption in development controlled by token stimuli.

Conventional wisdom about the relationships between diapause and cold-hardiness in insects has been developed from experience with temperate species. Diapause suppresses oxidative metabolism (Sømme, 1967), and thus may enhance cryoprotectant synthesis. Cold-hardiness and diapause may or may not be linked to each other, although they usually develop at the same time; however, diapause has evolved many times independently (Danks, 1987a; Denlinger, 1991). Diapause serves a wider range of functions than cold-hardiness, especially with respect to timing of the life cycle, so that linkages between diapause and

cold-hardiness would not necessarily be expected (but see Pullin, 1994).

In the Arctic, active seasons are short and subfreezing temperatures may occur even during the summer, so that continuous or rapidly responding cold-hardiness may be required. Habitats are more limited and winter temperatures less variable than in temperate zones, reducing the range and fluctuation of conditions. The range of photoperiod and temperature cues available for the induction of cold-hardiness or of diapause is likewise more limited in arctic than in temperate regions. Farther south, photoperiod is the most informative indicator of conditions, but 24-hour daylight prevails in summer at the highest latitudes. There, temperature is more easily monitored, and more reliably indicates conditions, than photoperiod (Danks and Oliver, 1972b).

Data from the Arctic confirm that in species exposed at the soil surface or on vegetation, cold-hardiness tends to be retained all year, although not necessarily by the same mechanism all year. Freezing tolerance persists during summer in *G. groenlandica* (Kukal, 1991) and the blow fly *Protophormia terrae-novae* (Kukal, unpubl. data). Diapause occurs in at least some arctic species (e.g., Danks and Byers, 1972; Danks, 1981), but appears to be adaptive primarily in the context of very short summer seasons, rather than being closely linked with cold-hardiness as such. It prevents development from continuing dangerously late in the summer, even if it is relatively warm, so that individuals are in an appropriate stage for the onset of winter. In all high arctic chironomid midges, for example, only larvae that can pupate and emerge in spring without further feeding complete development in a given year. Any individual that must feed even a little in spring before it can pupate defers pupation and emergence until the next year. Some large larvae therefore remain in diapause for many weeks during summer when the ponds in which they live are still warm. Such a conservative mode of development reflects the fact that midges must emerge as early as possible in summer in order to reproduce before winter returns (Danks and Oliver, 1972a).

Winter Habitat Selection

Site selection is a well known component of winter survival in arthropods (Danks, 1991). In temperate regions, most species seek sheltered winter habitats in which changes and extremes of temperature are buffered. In the low Arctic, aquatic insects survive winter by moving away from a freezing front or by remaining in habitats which do not freeze. However, some chironomid, empidid and trichopteran larvae survive in frozen habitats (Winchester, 1984; Irons et al., 1993).

Information on the overwintering sites selected by most insects in the Arctic highlights the importance of timing the whole life cycle rather than simply avoiding winter cold. Many species overwinter in locations that are relatively exposed, which warm up and support activity early in the spring. Species overwintering there can begin development much earlier than species in low-lying sites where snow must first melt, and that may be flooded at the beginning of the growing season. Several species of caterpillars and beetles overwinter on exposed ridges (see references in Danks, 1981:278). The high Arctic mosqui-

toes *Aedes impiger* and *Aedes nigripes* deposit their freezing- and drying-resistant eggs only around pond margins on the warmest sites, which are the first to become free of snow in spring (Corbet and Danks, 1975). Because eggs are deposited only in such places, large numbers accumulate on these favourable sites. There, the eggs hatch as early as possible when they are flooded as the ponds fill in spring. Early hatch is important to allow time for individuals of these univoltine species to complete larval development, emerge and reproduce the same season.

Microhabitat Modification

A number of temperate species modify their winter habitats by means of earthen cells, cocoons, and other structures (Danks, 1991). The larvae of some species of chironomid midges build special winter cocoons. These cocoons differ in construction from the summer ones: less particulate material is included in winter cocoons, and they enclose the larvae much more tightly. Winter cocoons are believed to provide protection against mechanical damage by ice in frozen habitats (Danks, 1971b). In some species from temperate regions, winter cocoons are constructed only when habitat temperatures are just above freezing (Danks, 1971b; Danks and Jones, 1978). Most larvae that overwinter in frozen arctic ponds build winter cocoons.

Freezing and Supercooling

Most information about insect cold-hardiness has come from studies outside the Arctic. In temperate regions, insects survive the winter in a frozen state (freezing-tolerant species—see Glossary) or prevent freezing through depression of the supercooling point of their body fluids (freezing-intolerant species) (reviews by Salt, 1961; Zachariassen, 1985; Storey and Storey, 1988; Cannon and Block, 1988; Lee and Denlinger, 1991; Block et al., 1992). Insects normally achieve lower supercooling points by the synthesis of antifreeze or cryoprotectant compounds, together with removal of extra-cellular nucleating agents that might initiate the freezing process (see supercooling and nucleating agents in Glossary). Freezing by nucleation of unprotected individuals can be induced simply by ice on the cuticle (in damp sites and in frozen soil), by food in the insect gut, by soil particles on the cuticle (Sømme, 1982), or by ice-nucleating bacteria (Strong-Gunderson et al., 1989, 1990). Some freezing-tolerant insect species synthesize proteinaceous nucleating compounds which are found in the haemolymph or cell matrix (Duman, 1982; Zachariassen, 1982; Baust and Zachariassen, 1983; Duman et al., 1991). These compounds facilitate freezing at high subzero temperatures. Rapid and injurious freezing would otherwise take place after extensive supercooling. Freezing-tolerant insects also accumulate cryoprotectants, discussed in a separate section. Freezing tolerance is supposed to be likely in insects from regions where winters are especially cold, such as the Arctic (e.g., Downes, 1962), because supercooling is difficult to achieve at very low temperatures and for very long periods.

Studies in the Arctic have revealed interesting variations on the generalizations made in the preceding paragraph. Many arctic species indeed are freezing-tolerant, but some species,

including those with extremely low supercooling points (Table 3; Ring, 1982), survive unfrozen. The very low supercooling points, such as -60°C , are remarkable because the homogeneous nucleation temperature (and hence the spontaneous freezing or supercooling point) of small amounts of pure water is only -40°C . However, in at least some of the species with exceptionally low supercooling points, the true freezing point of the haemolymph is also very low, approaching -20°C (Miller, 1982).

TABLE 3. Supercooling points and cryoprotectants reported for some arctic and subarctic insects.

Species	Stage	Supercooling point ($^{\circ}\text{C}$)	Cryo-protectants detected	Reference
Freezing-Intolerant				
<i>Rhabdophaga</i> sp. (Diptera: Cecidomyiidae)	larva	-62	glycerol	Ring 1981, 1983
<i>Rhabdophaga strobilioides</i> (Diptera: Cecidomyiidae)	larva	-58	glycerol	Miller & Werner, 1987
<i>Mayetiola rigidae</i> (Diptera: Cecidomyiidae)	larva	-58	glycerol	Miller & Werner, 1987
<i>Euura</i> sp. (Hymenoptera: Tenthredinidae)	larva	-56	glycerol	Miller & Werner, 1987
<i>Coccinella quinque-notata</i> (Coleoptera: Coccinellidae)	adult	-24	none	Ring, 1981, 1983
<i>Papilio canadensis</i> (Lepidoptera: Papilionidae)	pupa	-27	ethylene glycol	Kukal et al., 1991
Freezing-Tolerant				
<i>Pytho americanus</i> ¹ (Coleoptera: Pythidae)	larva	-7	glycerol	Ring, 1981, 1983; Ring & Tesar, 1981
<i>Martyrhilda ciniflorella</i> (Lepidoptera: Oecophoridae)	adult	-23	glycerol	Miller, 1978, 1982
<i>Gynaephora groenlandica</i> (Lepidoptera: Lymantriidae)	larva	-8	glycerol, alanine, betaine	Kukal et al., 1988b; Kukal 1991
<i>Mycetophila</i> sp. (Diptera: Mycetophilidae)	adult	-33	glycerol	Miller, 1978, 1982
<i>Exechia</i> sp. (Diptera: Mycetophilidae)	adult	-33	glycerol	Miller, 1978, 1982
<i>Xylophagus</i> sp. (Diptera: Xylophagidae)	larva	-6	none	Ring, 1981, 1983
<i>Pontania</i> sp. (Hymenoptera: Tenthredinidae)	larva	-9	sorbitol	Ring, 1981, 1983
<i>Rogas</i> sp. (Hymenoptera: Braconidae)	adult	-30	glycerol?	Miller, 1978

¹ Pollock (1991) regards both *Pytho americanus* and *P. deplanatus* as synonyms of *Pytho planus*.

Some arctic insects survive very low temperatures without the expected cryoprotectants in their haemolymph. For example, larvae of the xylophagid fly *Xylophagus* sp., which supercool to -6°C (Table 3), are freezing tolerant to at least -43°C (Ring, 1981) but contain no glycerol or similar cryoprotectants.

Another unusual feature of cold-hardiness in some arctic insects, also known in a few temperate species, is that some freezing-tolerant species supercool to very low temperatures (see examples in Table 3), and lack the deliberate ice-nucleation at higher temperatures that is characteristic of most freezing-tolerant temperate species (Ring, 1982, 1983). In alpine regions, the freezing-tolerant beetle *Pytho deplanatus* supercools to -54°C (Ring 1983). Nucleators in some freezing-tolerant temperate insects are carried in the haemolymph (Duman et al., 1991),

but in larvae of *Gynaephora groenlandica* the supercooling point of haemolymph is -20°C (Kukal et al., 1988b), even though whole larvae supercool only to -8°C (Table 3). Likewise, the site of nucleation is not the haemolymph in some freezing-tolerant aquatic species. Inoculative freezing of arctic chironomid midge larvae occurs at relatively high subfreezing temperatures (e.g., -2°C to -3°C), although larvae not in contact with ice supercool to about -9°C (Danks, 1971b). Inoculative freezing of an arctic ichneumonid endoparasitoid by freezing of its sawfly larval host (Humble and Ring, 1985) has also been reported.

The fact that the cold-hardiness of arctic insects differs in some ways from generalizations derived chiefly from work on species from temperate regions suggests three possibilities: 1) conditions in the arctic are more severe, and require unusual forms of adaptation; 2) the severe arctic conditions intensify adaptations that also occur in other regions, making these adaptations easier to document in the Arctic; 3) the pattern of conditions in the Arctic permits or forces adaptations that are somewhat different from those in temperate regions. In winter, temperatures fluctuate less in many arctic habitats than farther south, because no solar energy warms the ground surface during the continual darkness of the arctic winter. In this context, note that some cryoprotective agents protect tissues against any temperature changes, especially during thawing (e.g., Duman et al. 1991), and not just against low temperatures.

Cryoprotectants

Several different cryoprotectants of low molecular weight are found commonly in insect species from temperate zones (reviews by Sømme, 1982; Storey and Storey, 1988; Lee, 1991). The most common of these compounds are polyhydroxy alcohols, such as glycerol, sorbitol, mannitol, threitol, and ethylene glycol, carbohydrates such as trehalose, glucose, and fructose, and amino acids such as alanine. Data for arctic insects (Table 3) suggest no dramatic differences from temperate-zone insects except for the apparent absence of cryoprotectants in some very cold-hardy species. As in temperate regions, glycerol is the most commonly detected cryoprotectant. For example, in *Gynaephora groenlandica* glycerol is easily detected in cold-acclimated (winter) larvae, but is absent from warm-acclimated larvae (Kukal et al., 1988b). The main cryoprotectant synthesized by overwintering pupae of the swallowtail butterfly *Papilio canadensis* in Alaska (ethylene glycol) is different from that of the closely related temperate species *P. glaucus* (alanine) (Kukal et al., 1991).

Thermal hysteresis proteins (THPs) are widespread, and have been documented in various ectothermic animals as well as in lower and higher plants (DeVries, 1983; Duman, 1992; Urrutia et al., 1992). They are synthesized by numerous species of overwintering insects, both freezing-tolerant and freezing-intolerant species (Duman 1982; Duman et al., 1991). THPs hinder ice-crystal growth, and inhibit the recrystallization of ice during freeze-thaw cycles. Freezing and thawing during winter are more common in temperate than in arctic regions, however. Preliminary evidence indicates that *Pytho americanus* and *P. deplanatus* show very limited thermal hysteresis with supercooling point depressions of 0.07°C and 0.11°C respectively.

Further, the degree of hysteresis does not change during brief (10-day) exposures to sub-zero temperatures (Ring, unpubl. data). However, thermal hysteresis proteins, in addition to polyols, have been found in antarctic mites (Block and Duman, 1989).

In some species of insects from temperate areas, temperature shocks result in the production of "stress proteins," which may contribute to cold-hardiness (Denlinger et al., 1992). Some of the stress proteins produced by "cold shock" appear to be identical to those triggered by "heat shock," which have been studied in much greater detail. The existence and potential role of stress proteins in arctic insects has not been studied.

Winter Metabolism

Cryoprotectants, such as glycerol, accumulate when oxidative metabolism is suppressed by diapause, or by anaerobic conditions (e.g., in organisms encased in ice, which essentially is impermeable to oxygen [Scholander et al., 1953b; Sømme, 1974]). Glycerol, alanine and betaine accumulate in the haemolymph of larvae of *Gynaephora groenlandica* in response to anaerobiosis. In *G. groenlandica*, larvae shelter near permafrost during July and August after a period of feeding in early June. Near permafrost the larval mitochondria degrade over a long period (Table 4), creating a bottleneck in oxidative metabolism (Kukal et al., 1989). Mitochondria are reconstituted rapidly after thawing. Similar mitochondrial degradation has been observed in frozen larvae of high arctic chironomid midges during winter (J.R. Byers, pers. comm. 1969). This striking response effectively suspends metabolism.

TABLE 4. Occurrence of mitochondria in tissues of *G. groenlandica* larvae after three months at different temperatures. N = 400–500 nuclei, 10 larvae. (after Kukal et al., 1989).

Acclimation temperature ($^{\circ}\text{C}$)	Mitochondria per nucleus	
	Brain	Fat body
15	100+	100+
5	1.43	1.18
-15	0.04	<0.01

Water Relationships

The ability to withstand low relative humidity has been considered a preadaptation to cold tolerance. Indeed, the most cold-tolerant organisms include tardigrades and brine shrimp, which lose nearly 100% of their body water in response to inclement conditions. During this cryptobiotic (anabiotic) phase they can survive temperatures near absolute zero (Crowe and Clegg, 1973, 1978; Yancey et al., 1982). The African chironomid *Polypedilum vanderplanki*, an inhabitant of temporary rock pools, also survives in a cryptobiotic state and then can withstand immersion in liquid nitrogen (Hinton, 1960). Most arctic regions are defined as desert or semi-desert because of the low precipitation, most of which falls as snow. Although many soil habitats are relatively moist, exposed microhabitats, well-drained ridges,

and rock fields are very dry after spring. Resistance to dehydration as well as to cold is also important in the Antarctic (e.g., Sømme and Block, 1991).

Desiccation resistance is also an important component of winter survival, especially in species from very cold, exposed habitats, which are exceptionally dry. The main adaptations associated with cold-hardiness—solute production, water content and habitat selection—also enhance desiccation resistance (Ring and Danks, 1994).

Resistance to desiccation and tolerance of body-water loss during winter differ among the various nematine sawflies attacking arctic willows (*Salix* spp.), and these differences appear to be correlated with the severity of conditions within overwintering habitats. Humble (1987) found that sawflies overwintering in exposed habitats are more resistant to desiccation and more tolerant of water loss than those overwintering beneath snow cover. Furthermore, water loss over time is reduced when winter temperatures are below the freezing points of sawfly prepupae. Endoparasitoids are more resistant to desiccative water loss than are their nematine sawfly hosts; and ectoparasitoids are more resistant to desiccation than endoparasitoids.

Work in the Arctic has already illustrated that water relationships during winter can be complex. Some species have much lower amounts of non-bound water during winter, for example, less than half of the summer level in *Rhabdophaga* sp. (Cecidomyiidae) (Ring, 1981). Larvae of *Rhabdophaga* overwinter in stem galls on willows and therefore are exposed to ambient air temperatures. The fact that they can supercool to -62°C indicates that much of their body water is physiologically bound and therefore unfreezable (see also Storey et al., 1981).

Many species show modest reductions in water content, such as 4–8%, that reflect physiological adjustments for winter, although these adjustments are not necessarily related to cold-hardiness (e.g., Danks, 1971b). Danks also noted that larvae of some species of chironomid midges frozen into the substrate of shallow arctic ponds dehydrate further, so that they appear wrinkled immediately after thawing. Presumably water is withdrawn from these individuals by osmosis just before they themselves freeze. At that time, the larvae are surrounded by a high concentration of salts left in the solution that remains after most of the pond water has already been frozen into surface ice. Such events might be expected to further enhance cold-hardiness. Few ponds from temperate regions freeze solidly in this way, because they are insulated by snow, temperatures are less severe, and permafrost is absent (Danks, 1971a).

Winter Mortality

Winter mortality in temperate regions is often high, and varies depending on the severity of a given winter and the nature of overwintering microhabitats (Danks, 1978, 1991). Arctic winters are long and severe, but two preliminary findings about mortality are of particular interest. First, natural winter mortality in the high Arctic can be low. Some arctic caterpillars survive repeated freezing and thawing (e.g., Johansen, 1910). Kukul and Kevan (1987) and Kevan and Kukul (1993) calculated that although mortality from parasitoids was about 70% for larvae of

Gynaephora groenlandica, winter mortality of larvae was very low, perhaps 13% over the whole of the 14-year generation time. Downes (1965) suggested that most arctic species are particularly well adapted to the severe conditions at high latitudes: the “A-strategists” of Greenslade (1983) adapted to more-or-less predictably adverse conditions, but with few competitors. Downes inferred that, in general, mortality must be low because the reproductive rate of most species is also low.

Second, mortality in arctic species, notwithstanding the remarkable cold-hardiness of some of them, appears to be temperature- and time-dependent and therefore depends partly on the variation or sequence of conditions in a given place. Exposures to both -4°C and -18°C for 12 weeks were injurious to frozen chironomid larvae collected in late winter from high arctic ponds (Danks, 1971b; Table 5). The alternative supposition that arctic species suffer little winter mortality once safely frozen to low temperatures (e.g., Downes, 1965) needs to be reexamined.

TABLE 5. Survival of mechanically undamaged larvae of some high arctic chironomids collected in frozen mud during late winter and subjected to different temperatures in constant-temperature rooms (data from Danks, 1971b).

Species	% Survival with treatment:		
	None	12 weeks at -4°C	12 weeks at -18°C
<i>Stictochironomus unguiculatus</i>	94	35	0
<i>Chironomus</i> sp. ¹	92	73	82
<i>Tanytarsus norvegicus</i>	95	85	13
<i>Procladius culiciformis</i>	96	68	38

¹ *Chironomus* sp. 2 of Danks, 1971b.

Cold Injury

Temperate insects can be harmed by cold temperatures in the absence of freezing, damage that can be termed “chilling injury” at temperatures above 0°C , and “cryoinjury” at temperatures below 0°C . Freezing-intolerant insects can suffer high mortality from such damage during prolonged supercooling, even though they do not freeze (Bale, 1987; Turnock et al., 1990). Many arctic insects are exposed to cool temperatures for most of their summer period of activity; all would be expected to withstand injury.

Summer Habitat Selection

Cold is the major abiotic deterrent to the growth and development of arctic insects. Most species assume the temperature of their microhabitats; in occupying favoured microhabitats they depend especially on heat provided by solar radiation (Danks, 1981). Sunshine warms shallow water (Oliver and Corbet, 1966), the surface of the ground (Powell, 1961), and certain plant parts (Warren Wilson, 1957; Kevan, 1975; Mølgaard, 1982; Danks, 1987b) by as much as $20\text{--}30^{\circ}\text{C}$ above the ambient temperature. Microhabitat temperatures depend also on substrate characteristics and wind. They decrease when insolation is

reduced during cloud cover and at the ends of the day when the sun is close to or below the horizon.

Most arctic insects cope with these low temperatures by seeking microhabitats that can absorb heat and that are buffered from the wind, such as depressions with thick, dark-coloured plant cover. Particularly suitable habitats tend to be widely dispersed. Adult mosquitoes and adult butterflies are confined to, or more common in, sheltered valleys (Mercanton, 1920; Kevan and Shorthouse, 1970). Many adults choose thermally favourable sites for their offspring. For example, mosquitoes deposit eggs near midday on the warmest, south-facing sides of ponds, in sites sheltered from the wind by sedges (Corbet and Danks, 1975). Blow flies deposit eggs on the southern exposure of carcasses (Nuorteva, 1966 for *Phormia*), and their larvae pupate at the insulated surface of the host rather than in sheltered sites (McAlpine, 1965).

Thermoregulation

Control of body temperature by basking is known in temperate insects, especially in larger species, and is very common in arctic insects. Basking species expose themselves and orientate preferentially towards the sun's rays. Basking by adult butterflies enables them to reach the temperature threshold required for flight (Kevan and Shorthouse, 1970). Other species bask to accelerate the development of their eggs, as in high arctic flies such as mosquitoes that sit at the centre of the parabolic corollas of heliotropic flowers (Hocking and Sharplin, 1965; Kevan, 1975, 1989). Complex basking behaviour accelerates larval development, as in the larvae of *Gynaephora groenlandica*, which can raise their body temperature by more than 20°C above ambient temperature during basking (Kevan et al., 1982; Kukul et al., 1988a). Larvae adjust their temperatures by orientation and movement (Kevan et al., 1982) and by the alternation of feeding and basking (Kukul et al., 1988a; Kukul and Dawson, 1989; Kukul, 1991, 1992). Cocoons are oriented to favour solar heating and minimize heat loss by wind during pupal development (Kevan et al., 1982). Anecdotal observations of basking have been made for many other kinds of arctic insects, including flies and bugs (Danks, 1981; Kevan, 1989).

Endothermic insects such as bumble bees generate heat by shivering their flight muscles, and consequently can forage at lower temperatures than ectothermic species (Heinrich, 1979, 1993). However, despite these adaptations, which enable the arctic species to forage when air temperatures are below freezing (Bruggemann, 1958), there are only two species of bumble bees in the high Arctic (Richards, 1973). Heinrich (1979) pointed out that endothermy has high energy demands; in arctic regions, both the length of summer and the availability of pollen and nectar are restricted.

Summer Metabolism

Some species from cold climates compensate for the effects of low temperature on development. Metabolism can take place at lower temperatures if the limits ("thresholds") for activity, growth or development are lower. Alternatively,

a relative increase in metabolic rate can occur at a given temperature, presumably through lowered activation energies of key enzymes (Hochachka and Somero, 1984).

Limits for activity and emergence are lower in some arctic insects than in their temperate relatives, including mosquito and chironomid larvae (e.g., Haufe and Burgess, 1956; Danks, 1981). Metabolic compensation or cold-adapted metabolism has been documented for several species of arctic and antarctic arthropods, including some springtails, mites, beetles and moth pupae (Scholander et al., 1953a; Sømme et al., 1989; Sømme and Block, 1991; Kukul et al., 1991).

There is also limited evidence of short-term acclimation in chironomid midge larvae from the Arctic, that is, the restoration of metabolic activity to higher levels within a few hours or days after lower temperatures have depressed it (Bierle, 1971; Butler et al., 1981). However, other species, even from the high Arctic, show no lowered thresholds, temperature compensation or acclimation (e.g., Richards, 1973; Procter, 1977; Kukul et al., 1989).

The fact that metabolic compensation is not universal in arctic species can be explained in two ways. First, many species live in habitats, such as shallow ponds and the surface soil, that are not especially cold in summer, and indeed some of these species are widely distributed outside the Arctic (Danks, 1981). Therefore, every arctic species does not require metabolic adaptations to survive there. Second, enhanced metabolism at low temperatures would not be advantageous if energy could not be assimilated at low temperatures as rapidly as it would have to be respired (MacLean, 1975). Such considerations lead to a wider view of the energetic requirements for cold-hardiness.

Seasonal Energy Budgets

All insects apportion energy according to their various life-cycle requirements. In the Arctic, the appropriate budgeting of energy is especially important. For example, the manufacture of winter cryoprotectants requires energy reserves in the form of glycogen and lipid. Therefore winter survival depends on how individuals balance their energy supplies obtained by feeding during the summer season against the amount and timing of their energy demands for activity, reproduction, storage and cryoprotection.

Factors influencing the energy budget of *Gynaephora groenlandica* are summarized in Table 6. Low summer temperatures can be offset by behavioural thermoregulation. However, growth rate diminishes when body temperatures approach 30°C while basking (Kukul et al., 1988a), because metabolic demands exceed the assimilation efficiency (Kukul and Dawson, 1989). Many factors interact in ensuring the build-up at the end of summer of the energy stores required for maintenance and cryoprotectant synthesis. Food quality of the primary food plant, the arctic willow (*Salix arctica*), declines as the season progresses (Kukul and Dawson, 1989), because the carbohydrate and nutrient content of the leaves and catkins diminishes while secondary metabolites accumulate. In some habitats, preferred food plants are scattered; yet these large larvae have to feed on more than one individual plant to complete development.

Several factors, including lower quality of food plants, increased abundance of parasitoids and increased cloud cover as

the summer progresses, favour activity early in the season (Table 6). Larvae of *Gynaephora groenlandica* cease feeding during mid-summer and move down into sheltered sites close to the permafrost, resulting in “voluntary hypothermia” and the conservation of energy (Kukal, 1990, 1992).

Such behaviour prolongs the life cycle of *Gynaephora groenlandica*, which lasts for 14 years (Table 2). Other factors likewise favour slow development (Table 6). For example, moults are energetically expensive and time-consuming, complicating any analysis of growth rate and assimilation efficiency. Moults are particularly sensitive to low temperatures and in some species can be the major limiting factor for development, as documented in the Alaskan geometrid moth *Epirrhita autumnata* (Ayres and MacLean, 1987).

Gynaephora groenlandica is adapted for early, relatively rapid feeding and growth in warm sites in spring, followed by withdrawal to cold sites and energy conservation. In species like this from regions where energy (in terms of food, heat, and other elements) is severely limited, thermal preferences and seasonal constraints, including extreme winter cold, appear to dictate the structure of energy budgets.

TECHNIQUES FOR FIELD STUDIES OF INSECTS IN THE ARCTIC

Many of the aspects of cold-hardiness just outlined can be studied especially profitably in the Arctic. However, conducting field research there is difficult, so we recommend applicable techniques. Some of the most useful equipment for studies of

cold-hardiness and allied topics is noted in Table 7. Most work in insect cold-hardiness cannot be conducted on material that has been transferred to “home” laboratories, because the physiological state of the insects changes during transport. For a few uses it is feasible to ship frozen material (e.g., organisms collected in frozen substrates) in heavily insulated boxes.

Major difficulties of arctic fieldwork are associated with transportation of equipment, suitable accommodation and laboratory facilities, and availability of electricity. Moreover, most data must be collected during the brief period of insect activity in summer. Shipping of equipment to the high Arctic is expensive, reducing the feasibility of studies that require heavy or bulky instruments. Complex instruments tend to require a lot of power and stable environmental conditions, which are difficult to attain without a permanent and preferably heated structure in which to house them. Power must be produced by generators, so frequent fuel replenishment and maintenance are required. Therefore, computers and accessory equipment should be driven by solar power whenever possible. Simple and portable devices are most useful. Careful initial planning and subsequent improvisation in the field are the keys to successful arctic fieldwork, because resupply is infrequent or absent.

CONCLUSIONS

A remarkable number and variety of insights about insect cold-hardiness have come from work in the Arctic, as summarized in Table 8. Most of the adaptations for cold-hardiness known in arctic insects are similar to those of species from

TABLE 6. Synopsis of some factors affecting the seasonal energy budget of the high arctic moth *Gynaephora groenlandica* (Lymantriidae).

Factor	Constraint	Effect	Sample Temporal Pattern	Presumed Adaptation
Temperature	Low summer temperature	Slows metabolism and assimilation	Cold conditions throughout summer	Basking, increased assimilation efficiency
	Cold and variable summer temperatures	Intermittent frost	Frosts at unpredictable intervals during summer	Constant cold-hardiness
	Cold winter	Energy requirements for cryoprotectants	Substantial energy investment required before late summer	Slow growth, conservative energy budget “voluntary hypothermia”
	Very low temperatures	Cold throughout winter	Extreme cold-hardiness	
Cloud cover	Little sunshine	Low effective temperature	Higher cloud, especially closer to the ocean after ice break-up in mid to late summer reduces solar warming	Early seasonal activity, slow growth
Food plants	Low quality	Reduced growth rate	Quality declines from spring through summer	Feed early in the season
	Low abundance	Difficult to move and find a new host plant	Rapid feeding by large caterpillars would quickly consume the individual plant currently fed upon	Slow growth, resist starvation
Natural enemies	High mortality	Decreases reproductive potential	Parasitoids more abundant later in summer	Activity early in season
Moulting	Moulting time (especially at low temperatures)	Slows development and uses energy	Throughout life cycle (6 instars)	Slow growth; overwintering possible in many instars

TABLE 7. Synopsis of some equipment useful for field study of insect cold-hardiness and related topics in the Arctic.

Equipment	Information obtained or procedure
Maximum-minimum thermometer	Extreme environmental temperatures (daily, monthly, and seasonal)
Thermocouple thermometer:	Immediate ambient, substrate, subsurface, and insect body temperatures (internal and surface)
	Microhabitat temperatures, aquatic and terrestrial; basking temperatures, flight threshold temperatures, temperatures under different behaviours, compartmentalization of body temperatures
– with a data logger	Long-term records of microclimatic and habitat temperatures
– with a portable -30° + freezer ¹	Supercooling temperatures of insects. Storage of samples for further analyses
Thermal gradient and thermocouples	Thermal preference and thermoregulatory behaviour
Environmental chambers ¹	Acclimation regimens
pH meter	Measurements of aquatic habitats
Emergence cages	Insect emergence in aquatic or terrestrial habitats
Collecting equipment:	Quantitative and/or qualitative data on insect taxonomy, phenology, physiology, and biochemistry. Laboratory studies using NMR, DSC, HPIC, GC/S. Samples prepared for TEM or SEM or cryostat.
– vials for hand and other collecting	
– insect nets (aerial and aquatic)	
– traps (malaise, pan, pitfall, intercept, baited, berlese funnels ¹)	
Microscope (¹ for higher power)	Examinations, identifications, dissections, sample preparations, behaviour
Vital dyes	Quantification of cell survivorship using microscopy
Stop watch, tape recorder, video camera	Behavioural and energy balance studies
Still camera	Documentation and identification
Outdoor cages	Life cycle data
Laboratory cages and incubators	Life cycle data
Transect and plot markers	Population information
Environment modification chambers	Simulation of environmental change; influence on insect life-cycles and feeding strategy
Thin-layer chromatography	Antifreezes/cryoprotectants synthesized by cold-hardy insects
Nanoliter osmometer ¹	Colligative depression of freezing point (indication of accumulation of antifreezes and cryoprotectants)
Micropipettes	Haemolymph samples sealed with mineral oil or heat
Computers and printers ¹	Data acquisition and data analyses

¹ generator or solar panels with batteries required.

temperate zones, as concluded for arctic adaptations more generally by Danks (1981). Nevertheless, interesting features that may be uniquely arctic, or just more easily visible there, have been reported in some species. These features include mitochondrial degradation, year-round retention of freezing-tolerance, extremely low supercooling points, and lack of the usual cryoprotectants. Other features reflect differences between arctic and temperate conditions, such as the greater stability of winter temperatures in the arctic, and the dryness of some arctic habitats.

Further arctic research on cold-hardiness should recognize these biological and climatic differences by addressing habitat conditions, life-cycle dynamics, and various aspects of cryoprotectant production at different times of year. Our sugges-

tions for such research are based on key features of cold-hardiness discovered or expected in arctic insects.

The incidence and control of diapause might be linked to the development of cold-hardiness to a lesser extent than in temperate regions, because arctic terrestrial species need cold-hardiness in summer as well as in winter, winter temperatures fluctuate less, and the short summer makes life-cycle timing especially critical. Diapause and cold-hardiness should therefore be examined together.

Seldom have conditions in insect habitats in the Arctic been assessed throughout the non-active season. Such assessments allow the actual requirements for cold-hardiness to be defined more closely, especially if direct estimates of winter mortality are made at the same time. Supercooling, and hence survival in

TABLE 8. Summary of some valuable insights obtained by the study of adaptations to cold in arctic insects.

Aspect	Sample finding from the Arctic
Life cycles	Many life cycles are very long, and successive stages are cold-hardy. Overwintering stage is usually the larva.
Cold-hardiness and diapause	Linkage between cold-hardiness and diapause may be less likely in the Arctic.
Winter habitat selection	Life cycle timing as well as winter severity is important.
Microhabitat modification	Cocoons and hibernacula have special roles in protection.
Freezing and supercooling	Supercooling can be maintained at very low temperatures and for very long periods. Cold-tolerant species can have low supercooling points.
Cryoprotectants	Freeze/thaw is limited in winter and so thermal hysteresis proteins are likely to be less favoured.
Winter metabolism	Mitochondrial degradation occurs.
Water relationships	There are several types of "dehydration."
Winter mortality	Winter mortality is relatively low.
Cold injury	Cold-hardiness is maintained in summer.
Summer habitat selection	Summer habitats often are very restricted and easier to observe.
Thermoregulation	Finely tuned behaviour such as basking is used (easier to observe where there is little vegetation).
Summer metabolism	Metabolic compensation for low temperatures does not necessarily occur in arctic species.
Seasonal energy budgets	Extreme constraints of temperature, food and time force conservation of energy (e.g., energy storage for winter is accomplished by curtailing summer activity).
Equipment for field studies	See Table 7.

freezing-intolerant species, are time- and temperature-dependent, and so is survival in at least some freezing-tolerant species (e.g., Table 5). Further information is required on patterns of mortality with time in both experimental and natural settings.

Winter habitat conditions can be modified by structures such as the distinctive winter cocoons, already described, made by the larvae of chironomid midges. In the Arctic, unlike in temperate zones where winter cocoons appear only sporadically, winter cocoons of many species could be collected readily from shallow fresh waters to clarify their possible roles in protecting larvae that overwinter in frozen substrates.

Arctic species are especially useful for studying the dynamics of cryoprotectant production and degradation over time. In particular, these processes can be followed in the larvae of long-lived species in successive seasons to assess differences in cold-hardiness and its basis from year to year or from stage to stage.

Mitochondrial degradation appears to favour the accumulation of cryoprotectants by blocking further metabolism of certain metabolic products, but it might play additional roles in freezing tolerance. Studies of the processes of mitochondrial degradation and reconstitution would also be of great interest.

Thermal hysteresis proteins should be looked for in arctic insects (and also in the plants on which they feed). If these proteins are most effective in protecting organisms against freeze-thaw cycles, as seems likely, they would not be expected in individuals during the relatively constant conditions of winter. In summer, however, the lowest temperatures are not far below 0°C, and individuals would normally be expected to avoid

repeated freezes and thaws by supercooling. Thermal hysteresis proteins could provide protection in spring and summer. It would be useful to know if any arctic species switch to THPs from cryoprotectants of low molecular weight (such as polyols, most common in winter; Table 3) or if they continue to use winter systems of protection against cold. Arctic species, even in summer, must also survive temperatures above but close to freezing, suggesting that they have developed efficient systems for the avoidance of chilling injury, about which we know much less than we do about freezing injury.

It would also be profitable to combine studies of cold-hardiness, especially freezing tolerance, with the study of desiccation resistance, because exposed arctic habitats are exceptionally dry in winter. Such investigations would lead to wider information about the roles of water in insect cold-hardiness.

On a broader scale, energetic constraints limit the adaptations that are feasible. Summer metabolism and its relationships with temperature, time (including energy budgets linked to the timing of diapause), and other constraints (such as food and natural enemies) should therefore be examined in a variety of arctic insects. Studies of the genetic control of these various aspects of cold-hardiness would be fruitful, because organisms from the Arctic are subjected to strong selection for cold-hardiness.

These findings for the Arctic confirm that insect cold-hardiness is complex, and is based on many interacting factors. Therefore, many aspects of cold-hardiness must be considered simultaneously. The physiology of cold-hardiness has preoccupied most students of the subject, and will continue to generate

useful findings. However, other more general aspects also contribute to cold-hardiness. For example, the controlled timing of the life-cycle ensures that insects are in an appropriate stage to survive winter cold, and the controlled budgeting of energy ensures that stores are sufficient for cryoprotectant production. Studying these interacting factors in arctic insects would be especially informative.

ACKNOWLEDGEMENTS

We thank Dr. Bill Block, British Antarctic Survey, Cambridge, England, Dr. Rick Lee, Miami University, Oxford, Ohio, and two anonymous reviewers for a variety of helpful comments on the manuscript.

GLOSSARY

Bound water: Water that is so closely associated with cellular or other components in an organism that it is not available to participate in any freezing processes.

Chilling injury: Injury caused by low temperatures above 0°C.

Cold-hardiness: Ability of organisms to survive at low temperatures.

Cryo-injury: Injury caused by low temperatures below 0°C in the absence of freezing.

Cryoprotectants: Substances manufactured by overwintering insects that protect tissues in various ways from the potentially injurious effects of low temperatures.

Cryptobiosis (also Anabiosis): Virtual suspension of metabolism, normally in a desiccated state, in some organisms, which then also are markedly freezing tolerant.

Cue (also Token stimulus): Environmental signal used, especially to control diapause, as an indicator of conditions, but one that does not act directly to inhibit development (as would low temperature, for example). In temperate regions the most common cue for diapause is photoperiod.

Diapause: Programmed suppression of morphological development not directly dependent on adverse conditions (see also **Cue**).

Ectothermy: Body temperature follows ambient temperature, and can be increased only by external sources of heat, such as sunshine.

Endothermy: Increase in body temperature through internal generation of metabolic heat. In insects, this heat is generated by flight muscles.

Freezing intolerance: Inability of organisms to tolerate the formation of ice in the body (see also **Freezing tolerance**).

Freezing point (True freezing point): The temperature at which, in the absence of supercooling, water or aqueous solutions would be expected to freeze. This temperature is the same as the melting point, the temperature at which the last ice crystal disappears from a frozen sample as it is thawed.

Freezing tolerance (also corrupted by many authors to the grammatically awkward “freeze tolerance”): Ability of organisms to tolerate the formation of ice (normally only extracellular ice) in the body.

Inoculative freezing: Freezing initiated by inoculating supercooled body fluids with ice nuclei, which triggers freezing, as by ice growth through insect cuticle.

Metabolic compensation: Adaptation whereby basal rates of metabolism are changed (compared to similar populations elsewhere) to compensate for lower or higher ambient temperatures in a particular region or habitat.

Nucleating agents (also Nucleators, Nuclei): Substances that assist the onset of freezing by acting as nuclei for the freezing process (see **Supercooling**). Most freezing-tolerant insects have such substances, which ensure that freezing takes place before the temperature gets so low that ice formation, once initiated, would be very rapid and so potentially injurious.

Polyols: Polyhydric alcohols, such as glycerol, a common class of cryoprotectant.

Recrystallization: Changes in the configuration of ice crystals, often injurious to organisms, that take place in frozen systems as the temperature changes. Such changes depend especially on the rate of thawing.

Stress proteins: Specific proteins synthesized by certain insects in response to heat shock (suddenly increased temperature) or cold shock (suddenly reduced temperature).

Supercooling: Phenomenon by which water and aqueous solutions remain unfrozen at temperatures below the true freezing point or melting point. This failure to freeze results from the fact that freezing is initiated only when sets of water molecules remain in the hexagonal configuration of the ice crystal for long enough periods; the period depends on the speed of water molecules (hence temperature), the volume of solution (hence size), and the presence of nuclei (see **Nucleating agents**).

Supercooling point (also Crystallization temperature): Temperature at which freezing occurs spontaneously in a supercooled liquid. The spontaneous freezing point or supercooling point of small volumes of pure water is -40°C.

Supercooling point depression: The difference between the supercooling point and the melting or true freezing point.

Thermal hysteresis protein (THP) (also Anti-freeze protein): Protein that lowers the temperature at which freezing takes place (the difference between this temperature and the true freezing point in the presence of THPs is “hysteresis”), apparently by inhibiting ice-crystal growth at the ice-water interface.

Token stimulus (see Cue)

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