REVIEW

Eur. J. Entomol. **96**: 1–10, 1999
ISSN 1210-5759

The physiology of cold hardiness in terrestrial arthropods*

Lauritz SØMME

University of Oslo, Department of Biology, P.O. Box 1050 Blindern, N-0316 Oslo, Norway; e-mail: l.s.somme@bio.uio.no

Key words. Terrestrial arthropods, cold hardiness, ice nucleating agents, cryoprotectant substances, thermal hysteresis proteins, desiccation, anaerobiosis

Abstract. Insects and other terrestrial arthropods are widely distributed in temperate and polar regions and overwinter in a variety of habitats. Some species are exposed to very low ambient temperatures, while others are protected by plant litter and snow. As may be expected from the enormous diversity of terrestrial arthropods, many different overwintering strategies have evolved. Time is an important factor. Temperate and polar species are able to survive extended periods at freezing temperatures, while summer adapted species and tropical species may be killed by short periods even above the freezing point.

Some insects survive extracellular ice formation, while most species, as well as all spiders, mites and springtails are freeze intolerant and depend on supercooling to survive. Both the degree of freeze tolerance and supercooling increase by the accumulation of low molecular weight cryoprotectant substances, e.g. glycerol. Thermal hysteresis proteins (antifreeze proteins) stabilise the supercooled state of insects and may prevent the inoculation of ice from outside through the cuticle. Recently, the amino acid sequences of these proteins have been revealed. Due to potent ice nucleating agents in the haemolymph most freeze tolerant insects freeze at relatively high temperatures, thus preventing harmful effects of intracellular freezing.

Due to the low water vapour pressure in frozen environments, supercooled terrestrial arthropods are at a risk of desiccation. Glycerol and other low molecular weight substances may protect against dehydration as well as against cold. In the arctic springtail *Onychiurus arcticus*, freezing is avoided due to dehydration in equilibrium with the ambient freezing temperature. In some frozen habitats terrestrial arthropods are enclosed by ice and survive an oxygen deficiency by anaerobic metabolism.

Suggestions for further research include investigating the nature of freeze tolerance, the physiology of prolonged exposures to cold, and the relation between desiccation, anaerobiosis and cold hardiness.

INTRODUCTION

Insects and other terrestrial arthropods are widely distributed in temperate and polar regions and overwinter in a variety of habitats. Many species overwinter in the ground well protected from extreme low air temperatures by plant litter and a cover of snow. Others are exposed to very low ambient temperatures under the bark or on branches of trees or on windswept mountain ridges. As may be expected from the enormous diversity of terrestrial arthropods, many different systems of cold hardiness have evolved.

The purpose of the present review is to discuss the different ways in which insects and other terrestrial arthropods are adapted to low temperatures. Various physiological mechanisms makes it possible to survive the harsh winter conditions of temperate and polar areas. When discussing this topic, some of the older literature should be kept in mind. Presently, modern researchers are able to explain cold hardiness by the molecular structure of certain proteins (Duman et al., 1998), but to reach this stage has taken almost one hundred years since the earliest ecological studies (Bachmetjew, 1901). The main advances that have been made during this period are discussed in the following paragraphs.

The first modern approach to studies on insects cold hardiness was conducted by Bachmetjew (1901), who introduced the use of thermocouples for measuring the freezing temperature of supercooled insects. The early literature on insect cold hardiness has been reviewed by Payne (1927) and Salt (1936). At present, the literature on cold hardiness is almost overwhelming, but different aspects of cold hardiness have been discussed in a number of review articles (e.g. Merivee, 1978; Sømme, 1982, 1999; Baust & Rojas, 1985; Zachariassen, 1985; Cannon & Block, 1988; Storey & Storey, 1988; Ushatinskaya, 1990; Duman et al., 1991a,b; Lee, 1991; Lee et al., 1996; Block, 1996; Danks, 1996) as well as in recent books (Lee & Denlinger, 1991; Leather et al., 1993; Sømme, 1995).

With regard to cold hardiness, it is practical to divide insects and other terrestrial arthropods in two main categories – *freeze tolerant* and *freeze intolerant* (Fig. 1). Both terms are relative, reflecting complicated mechanisms with great variation between species and seasonal variations within species. Freeze tolerance does not mean that an insect can tolerate any temperature in the frozen state, and its lower temperature limit must be defined by experimental procedures. Similarly, freeze intolerance, or freeze avoidance, does not mean that an insects can tolerate any temperature as long as it does not freeze. To avoid freezing at temperatures below the freezing point of their body fluids, freeze intolerant species depend on supercooling, as will be discussed in detail below.

---

* This paper was originally presented as an invited Plenary Lecture during the 6th European Congress of Entomology held in České Budějovice, Czech Republic, August 1998.

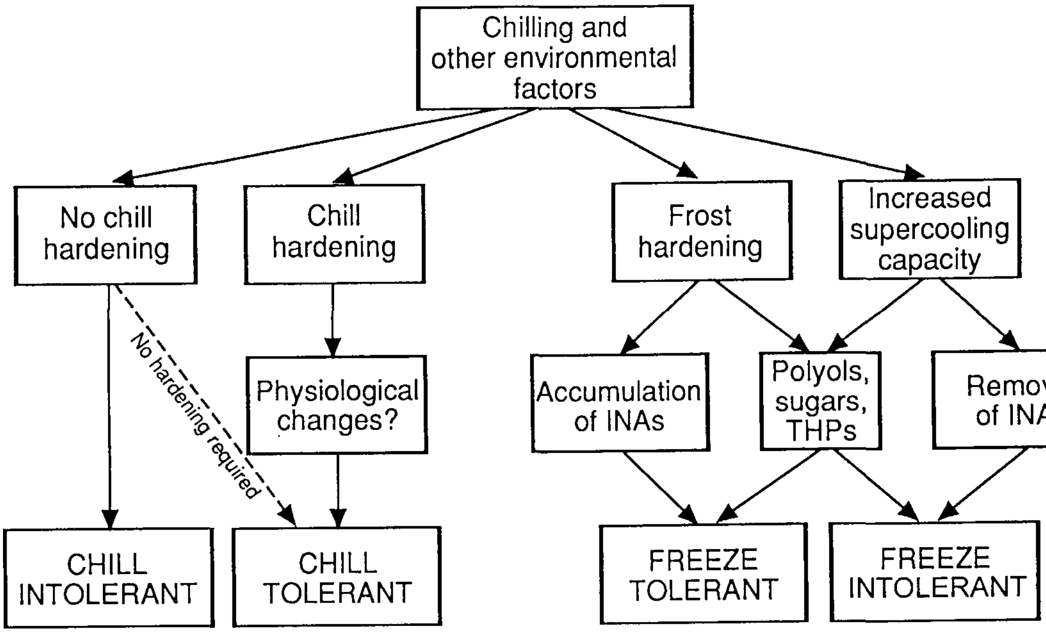

Fig. 1. Chart of processes leading to different categories of cold hardiness in terrestrial arthropods. Chill tolerance includes survival both above and below the freezing point of the body fluids in both freeze tolerant and freeze intolerant species.

PROLONGED EXPOSURE AT LOW TEMPERATURES

Neither the lower lethal temperature of freeze tolerant insects nor the capacity for supercooling should be considered as the only way to measure the cold hardiness of a given species. In addition, the ability to survive prolonged periods of exposure to cold must be evaluated at temperatures both above and below the freezing point of the insect's body fluids.

The early authors in the field were aware of the importance of the time of exposure to cold stress. Payne (1927), distinguished between the "quantity factor" which she defined as the ability to withstand relatively mild low temperatures, and the "intensity factor" which is the ability to withstand extremes of low temperatures. Similarly, Salt (1936) pointed out that many insects are killed at temperatures above their supercooling points; the lower the temperature the shorter the exposure necessary to kill them.

According to Salt (1969), and later pointed out by Bale (1993), some terrestrial arthropods are *chill intolerant* (Fig. 1) and will suffer from lethal injuries even at temperatures above 0°C. Other species are *chill tolerant*, or become chill tolerant during a hardening process, which enables them to survive temperatures both above and below the freezing point of their body fluids. Consequently, both freeze tolerant and freeze intolerant species are also adapted to avoid the risk of chilling.

For all categories of cold hardiness, pre-exposure history is crucial. Low temperature, short photoperiods and lowered quality of nutrition initiate cold hardening processes in the autumn. As reviewed by Denlinger (1991), there is a clear relationship between cold hardiness and diapause in some species, although generalisations cannot be made. Saunders & Hayward (1998) recently showed that survival during cold exposure in two northern European strains of the blow fly *Calliphora vicina* was greater in diapause-destined larvae than nondiapause-destined ones. According to Hodková & Hodek (1994, 1997), the supercooling capacity of the heteropteran bug *Pyrrhocoris apterus* (which is found on the cover of *European Journal of Entomology*) is increased by short photoperiods and low temperature, provided the bugs are in diapause. Extirpation of the retrocerebral complex from non-diapausing bugs enhances supercooling, suggesting that cold hardiness may be regulated by endocrinological processes.

Winter adapted temperate and polar species are apparently able to survive several months at sub-zero temperatures. In contrast, summer adapted temperate species and tropical species may be killed by short periods of chilling below or above the freezing point of their body fluids. Mortality during exposure to low temperatures is caused by the disruption of metabolic regulation which may result in lethal imbalances (Storey & Storey, 1988), but little is known about the physiological processes that enable winter adapted insects to avoid such damage. Another question that needs more attention is whether further development is affected by exposure to cold (Baust & Rojas, 1985). Although the overwintering stage may survive prolonged exposure to severe cold, subsequent stages may be harmed, and reproduction reduced.

The literature with regard to winter mortality in pest insects has been reviewed by Bale (1991) and Sømme (1999), but more comprehensive investigations are needed. Studies of this kind are quite time consuming since batches of insects must be tested at several low temperatures and for increasing periods of time. High numbers of experimental animals and adequate replicates must be provided for statistical analysis. Due to great seasonal variation, the experiments must be repeated several

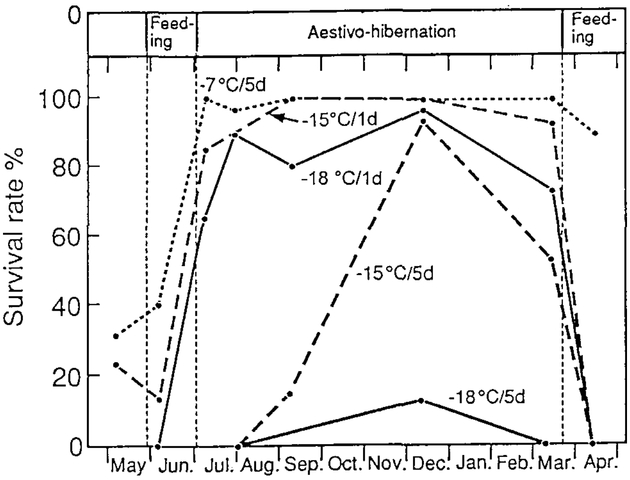

Fig. 2. The effect of feeding and aestivo-hibernation on seasonal changes in tolerance to cold in the apple blossom weevil *Anthonomus pomorum*. Redrawn from Košťál & Šimek (1996).

times during the winter. Following exposure at low temperatures, the insects should be reared for a considerable time to test their ability for further development and reproduction.

An example that clearly illustrates the seasonal changes in chill tolerance has been published by Košťál & Šimek (1996). Adults of the apple blossom weevil *Anthonomus pomorum* actively feed in May and June (Fig. 2), but enter a state of aestivo-hibernation in July. At this time the survival rates of weevils exposed to –7, –15 and –18°C in the laboratory were greatly increased. Highest tolerance to –15 and –18°C was observed in December, but survival rates decreased sharply when the weevils became active and resumed feeding in the spring. The results suggest that the ability to survive prolonged chilling develops as a gradual process, reaching its highest level at the coldest part of the winter.

Although data on winter mortality may not be known, the presence of a species in areas of severe climatic conditions is actually the best evidence of its ability to survive prolonged exposures at low temperatures. As an example, Lozina-Lozinskii (1974) reported that during the winter of 1941–1942 air temperatures in the Soviet Tatar Republic remained below –40°C for long periods, below –45°C for weeks, and even dropped to –55°C for one day. These conditions were endured by supercooled larvae of the bark beetle *Scolytus multistriatus* and frozen caterpillars of the cabbage moth *Mamestra* sp. hibernating above the snow cover.

SUPERCOOLING IN WATER AND TERRESTRIAL ARTHROPODS

When the temperature of pure water is lowered, freezing will, contrary to what may be expected, typically not take place at 0°C. Normally, water will supercool to some temperature below its melting point. If supercooled water is seeded by an ice crystal, freezing occurs spontaneously, and the temperature increases to the melting point of 0°C. Small volumes of highly purified water, e.g. droplets suspended in mineral oil, may supercool close to –40°C (MacKenzie, 1977), which is the temperature of “homogeneous nucleation”. In theory, initiation of freezing requires a nucleus about which water molecules can aggregate until a critical size is reached (Franks, 1985; Lee, 1991). The nucleus of crystallisation may be groups of water molecules themselves that by chance are arranged into the structure of ice. In nature, contact with foreign substances, like mineral particles, increases the chances of ice crystal formation, resulting in “heterogeneous nucleation”. In many biological systems, heterogeneous nucleation is caused by molecules acting as ice nucleating agents (see below).

The majority of insects and all spiders, mites and springtails are killed by freezing. In this respect they belong to the freeze intolerant category. For such species the avoidance of freezing is essential, and this is achieved by their greater or lesser ability to supercool. In the same way as highly purified water will supercool to temperatures far below the melting point, insects may be considered as containers in which the conditions for supercooling are exceptionally favourable (Sømme, 1982).

The avoidance of freezing in insects and other terrestrial arthropods is not unlimited, and the capacity of supercooling in a given species normally correspond to the lower winter temperatures of its overwintering habitat. The limit of supercooling in terrestrial arthropods is conveniently measured as the supercooling point (SCP), which also represents the lower limit of survival in freeze intolerant species. The SCP is the temperature at which spontaneous freezing occurs during gradual cooling, e.g. 1°C min^{-1} in the laboratory. When a supercooled insect freezes, the released heat of fusion causes the temperature to rise. Just as heat is required to melt ice, heat is released when ice is formed. Theoretically, the temperature inside the insect will rise almost to the melting point of its body fluid (Zachariassen, 1991).

REGULATION OF SUPERCOOLING IN TERRESTRIAL ARTHROPODS

Ice nucleating agents (INAs)

Several factors influence the degree of supercooling in terrestrial arthropods. To reduce the risk of heterogeneous nucleation, removal of ice nucleating agents (INAs) is of vital importance. INAs may be present in food particles and grains of mineral dust in the gut. In many species the cessation of feeding and clearing of the gut are important preparations for overwintering. Certain biological molecules that act as INAs have special functions in freeze tolerant insects (see below), but have to be removed or biochemically masked in freeze intolerant species.

Experiment on the antarctic springtail *Cryptopygus antarcticus* is an example of the effect of gut contents on supercooling (Sømme & Block, 1982). In specimens collected during the austral summer, almost all individuals had SCPs above –15°C, with a mean of –7.2°C. When another batch of springtails were starved for 6 days at 5°C a bimodal distribution appeared. In this case, half of the specimens had SCPs below –15°C, with a mean of –24.8°C. When starved specimens subsequently were fed a coarse homogenate of moss turf, the SCP returned to the high group level, strongly suggesting the presence of INAs in the food. In specimens fed on a purified unicellular green algae, however, the low group of SCPs were maintained. Green algae is an important food for antarctic springtails, that may experience freezing temperatures even in the middle of the summer. In this situation a diet free of INAs makes it less risky to feed.

Ice nucleating bacteria

Although there is good experimental evidence that heterogeneous nucleation begins in the gut of many insects, the precise nature of the INAs is usually not known. As reviewed by Lee et al. (1995), it has recently been discovered that nucleation in the gut is caused by ice nucleating bacteria. Due to the structure of special proteins on their surface, these rod shaped bacteria cause nucleation at temperatures as high as –1 or –2°C. Ice nucleating bacteria are commonly found on the surface of plants, and it is suspected that their presence causes substantial frost damage to agricultural crops.

Ice nucleating bacteria are consumed by phytophagous insects, and have been reported to constitute part of the normal gut flora (Lee et al., 1995). It appears that they are responsible for reduced supercooling capacity in a number of species. As demonstrated by Olsen & Duman (1997a) in larvae of the pyrochroid beetle *Dendroides canadensis*, the nucleating effect of these bacteria in the gut fluid is counteracted by masking them with certain proteins.

Ice nucleating bacteria have the potential to act as biological agents if they can reduce the supercooling capacity of overwintering insects (Lee et al., 1995). Thus, the mean SCP of diapausing adults of the Colorado potato beetle *Leptinotarsa decemlineata* rose from –7 to –3°C after exposure to various concentrations of the ice nucleating bacteria *Pseudomonas syringae* in the soil, resulting in increased rates of freezing at subzero temperatures.

Cryoprotectant substances

Seasonal changes in the supercooling capacity of terrestrial arthropods are also related to changes in the concentration of cryoprotectant substances in the haemolymph. Early studies of cold hardiness concentrated on low molecular weight substances (Sømme, 1982), while later it was realised that supercooling is affected by large protein molecules as well (Zachariassen, 1985; Duman et al., 1991a,b).

Glycerol is the most common low molecular weight cryoprotectant substance, but other polyols or sugars may have similar effects. These substances are often present in combination. As pointed out by Ring (1980), high concentrations of a single substance may be toxic, whereas this effect is avoided in a multicomponent system. In overwintering insects, the haemolymph may contain 1 or up to 5 Mol of glycerol. One Mol depresses the melting point of the haemolymph by 1.86°C, but the SCP is depressed about twice as much as the melting point (Zachariassen, 1985).

Seasonal changes in the SCPs and glycerol concentration in larvae of the pine shoot tortricid *Petrova resinella* is an example that has been frequently cited. According to Hansen (1973), glycerol is accumulated in the autumn, and reaches concentrations close to 30% of body fresh weight in the middle of the winter, concurrent with SCPs below –45°C. In the spring glycerol is lost and the supercooling capacity is greatly reduced.

Thermal hysteresis proteins

During the last twenty years it has been realised that so-called antifreeze proteins, or thermal hysteresis proteins (THPs) play an important role in the cold hardiness of terrestrial arthropods in addition to low molecular weight cryprotectant substances (Zachariassen, 1985; Duman, 1991a, b). THPs have an unusual characteristic in that they depress the freezing point (FP) of the haemolymph, but not the melting point (MP). The difference between MP and FP is termed thermal hysteresis. Apparently, the antifreeze proteins adhere to the surface of seedling ice crystals, thus preventing further growth which would have resulted in freezing.

THPs in hibernating insects was initially described in the tenebrionid beetle *Meracantha contracta* (Duman 1977), and have later been found in a number of other species. As shown in the pyrochroid beetle *Dendroides canadensis*, there are large seasonal fluctuations in THP concentration (Olsen & Duman, 1997b). Highest haemolymph concentrations are found in the middle of the winter, while thermal hysteresis disappears in the summer.

Antifreeze proteins have several functions. The lowering of the FP by 5–6°C in itself gives protection against freezing by a similar temperature interval. Furthermore, seasonal lowering of the supercooling points in many insects is also associated with increased THP concentrations. Even more importantly, THPs may actually stabilise the supercooled state by inhibiting the growth of seedling ice crystals (Husby & Zachariassen, 1980).

When insects are surrounded by ice in their overwintering habitat, there is a possibility that the ice may invade through the cuticle. In the bark beetle *Ips acuminatus* the risk of inoculative freezing is reduced by THPs (Gehrken, 1992). Recently, Olsen et al. (1998) have shown that THPs are present in the epidermal cells underneath the cuticle, thus neutralising any penetrating ice crystals.

The seasonal production of THP in the fat body of *Dendroides canadensis* is induced by juvenile hormone, and is cued by low temperature and short photoperiods (Duman et al., 1991b). Horwath et al. (1996) have shown that THPs in the mealworm *Tenebrio molitor* are produced in the fat body cells during the summer, and released into the haemolymph when triggered by stimulating photoperiods.

The structure and amino acid sequence have been identified in THPs from three insect species. According to Duman et al. (1998), larvae of *Dendroides canadensis* contain several THPs with sizes of 7.5–9.5 kDa. Their ability to form hydrogen bonds to ice can be explained by

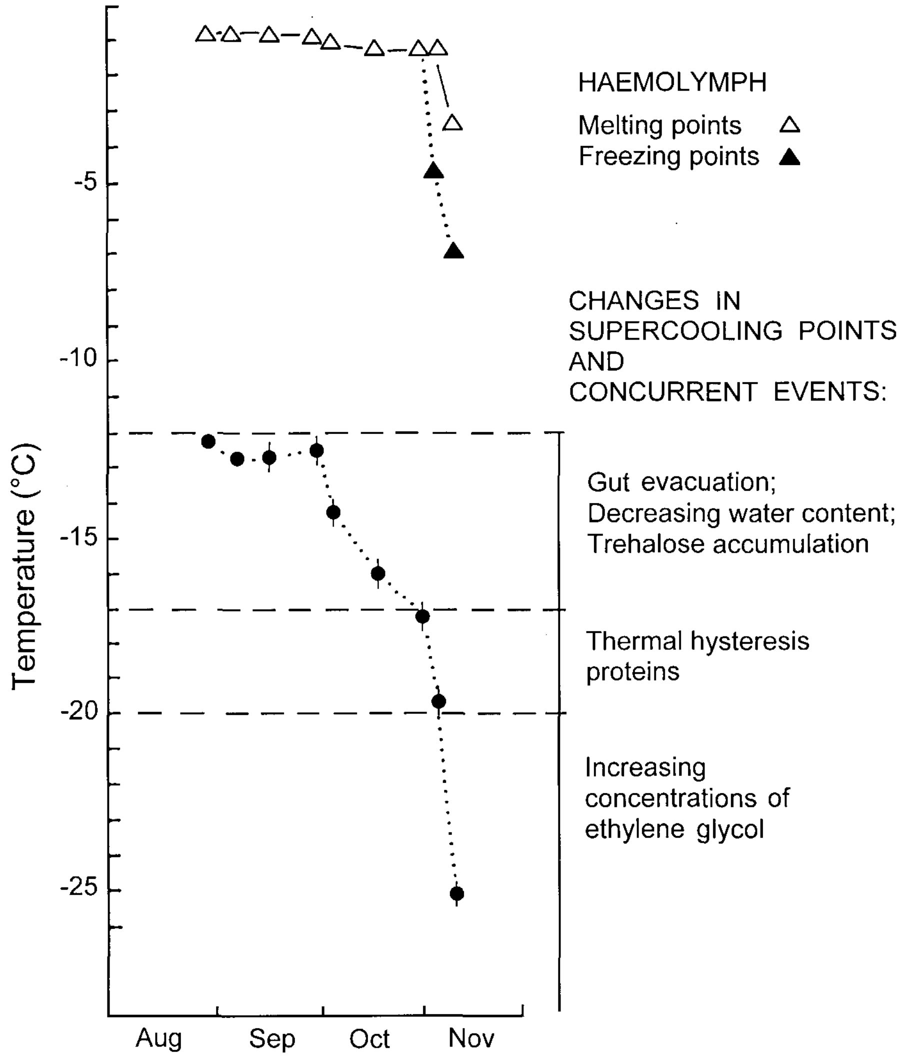

Fig. 3. Cold hardening in the bark beetle *Ips acuminatus* during the autumn. Haemolymph melting and freezing points (with thermal hysteresis in November) are shown in the upper part of the diagram; supercooling points and associated events in the lower part. Redrawn from Gehrken (1995).

the presence of repeated disulphide bridges in the protein sequences that function to present hydrophilic residues in the correct position. Furthermore, it has been shown that the haemolymph of *D. canadensis* contains proteins and low molecular weight substances that enhance the activity of the antifreeze proteins themselves. These latest results are remarkable by the fact that the nature of insect cold hardiness can be explained at the molecular level.

Multifactorial systems

There are few studies that show how different factors affect supercooling in the same species, but the bark beetle *Ips acuminatus* offers an example (Gehrken, 1995). Lowering of the SCPs in the autumn is initiated by the evacuation of the gut content (Fig. 3), and a decrease in water content accompanied by the accumulation of the disaccharide trehalose. Another drop in SCPs from –17°C to –20°C is closely correlated with an increased level of thermal hysteresis in the haemolymph, while further lowering of the SCPs is caused by increasing concentrations of ethylene glycol. In mid-winter the concentration of this substance reaches a level of 2.7 Mol.

FREEZE TOLERANCE

While most terrestrial arthropods are freeze intolerant, there are also many insects that can survive freezing. Although there are exceptions, freezing is considered lethal if it takes place within the cells. Freeze tolerant insects, however, are able to survive the formation of extracellular ice. Ice formation in the haemolymph results in an osmotic gradient causing water loss and shrinkage of the cells. For this reason the degree of freeze tolerance is partly a function of the tolerance of the cells to desiccation.

The freeze tolerance of insects is limited both by temperature and time. An example is provided by the alpine carabid beetle *Pelophila borealis* that is rapidly killed at –10°C, while some specimens may survive for more than 40 days at –5°C (Sømme, 1982). At –3°C most beetles survive for several months. The latter temperature is close

TABLE 1. Selected examples of freeze tolerant insects from different orders and geographical locations.

Species	Stage	Location	Mean SCP (°C)	Lowest temperature tolerance (°C)	Reference
Orthoptera					
Hemideina maori	Adult	New Zealand	–4	–9	Ramløv et al., 1995
Coleoptera					
Upis ceramboides	Adult	Alaska	–6	–60	Miller, 1982
Pterostichus brevicornis	Adult	Alaska	–11	–70	Miller, 1982
Pytho americanus	Larva	Rocky Mt	–5	–44	Ring, 1982
Pytho deplenatus	Larva	Rocky Mt	–54	–55	Ring, 1982
Cetonia aurata	Larva	France	–7	–16	Vernon et al., 1996
Lepidoptera					
Arctica caja	Larva	Estonia	–9	–78	Merivee, 1978
Gynaephora groenlandica	Larva	Ellesmere	–7	–70	Kukal, 1991
Diptera					
Belgica antarctica	Larva	Antarctica	–6	–15	Baust & Edwards, 1979
Hymenoptera					
Trichiocampus populi	Pupae	Hokkaido	–9	–70	Tanno, 1967
Bracon cephi	Larva	Alberta	–47	–47	Salt, 1959

to the temperature at the overwintering site of this species, under a deep cover of snow.

The nature of freezing injuries in insects is poorly understood. This is a field where more research is needed. Knowledge on how some insects survive freezing may be of importance for the cryopreservation of human organs.

Different degrees of freeze tolerance are found in different insects (Table 1). Some species, like the beetles *Pterostichus brevicornis* and *Upis ceramboides* from Alaska (Miller, 1982) as well as the arctic woolybear caterpillar *Gynaephora groenlandica* (Kukal, 1991), can tolerate temperatures down to –60 or –70°C. In contrast, the lowest temperature tolerated in the frozen state is only –9°C by the weeta *Hemideina mori* (Ramløv et al., 1992) and –5°C by the carabid beetle *Pelophila borealis* (Sømme, 1982)

A striking characteristic of many freeze tolerant insects is their poor capacity for supercooling. Mean SCPs are often in the range of –6 to –10°C, but there are also exceptions. The high SCPs are caused by the presence of ice nucleating agents (INAs) in the haemolymph (Zachariassen, 1985; Duman et al., 1991a, b). In freeze tolerant insects INAs may be maintained in both the gut and tissues, in contrast to the situation in freeze intolerant species, where they have to be eliminated in preparation for overwintering.

Haemolymph INAs are proteins or lipoproteins with high nucleating effects. Their function is apparently to inhibit lethal intracellular freezing. The risk of intracellular freezing, e.g. in the large fat body cells, increases with the degree of supercooling. Due to INAs, extracellular freezing in the haemolymph takes place at higher temperatures.

The degree of freeze tolerance in most species shows great seasonal fluctuations. The chrysomelid beetle *Phyllodecta laticollis* from alpine regions in Norway (van der Laak, 1982) has SCPs around –6°C all the year around (Fig. 4). The lower lethal temperature, however, decreases from –10°C in September to –40°C in February, following closely the ambient temperatures at the overwintering site.

As in freeze intolerant insects, accumulation of low molecular weight substances increase their cold hardiness. In freeze tolerant species glycerol and other polyols depress the lower lethal temperature. In beetles it appears that similar concentrations of polyols have greater effect on the cold hardiness of freeze tolerant than freeze intolerant species (Zachariassen, 1985). When the lower lethal temperatures of *Phyllodecta laticollis* or the pythid beetle *Pytho depressus* are plotted against the concentration of solutes in their haemolymph, a steep regression line appears. In comparison, similar concentrations of solutes have less effect on the SCPs of freeze intolerant beetles. Consequently, as suggested by Zachariassen (1985), freeze tolerance is the most successful strategy of cold hardiness in areas with extremely low temperatures. In fact, several species from the interior of Alaska and Arctic Canada are tolerant to freezing.

COLD HARDINESS AND OTHER ASPECTS OF LIFE HISTORY

The cold hardiness of terrestrial arthropods represents in fact only a part of their overwintering strategies. For a better understanding of overwintering, other aspects of life history must be considered (Danks, 1996). In species that overwinter in a specific stage, strict synchronisation of the life cycle with the season is required. In addition to phylogeny, this also involves adaptations for feeding, energy storage and metabolism. Other important aspects include behaviour to find the appropriate site of overwintering, adaptations to the relevant moisture conditions and the avoidance of predators.

As mentioned earlier, seasonal synchronisation is often associated with diapause which in some species is closely related to cold hardiness. Pullin (1996) even suggested that cold hardiness initially may have evolved in diapaus-

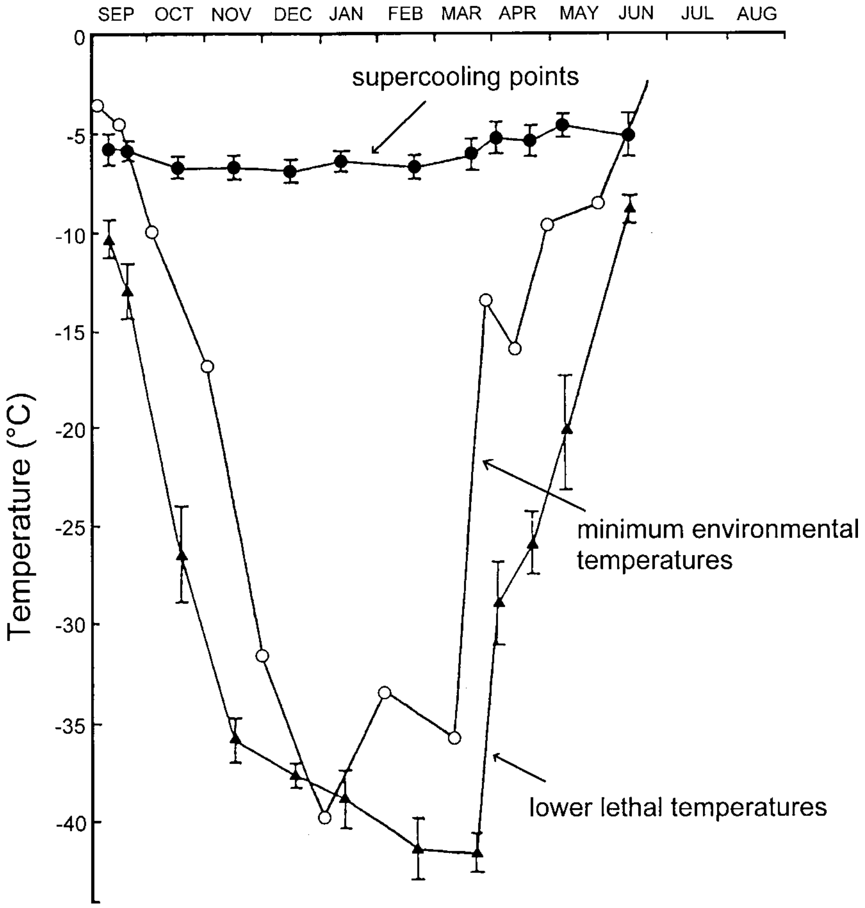

Fig. 4. Seasonal changes in supercooling points, and low temperature tolerance limit of *Phyllodecta laticollis*, compared with minimum environmental temperatures at the site of collection. Modified from van der Laak (1982).

ing insects. At an early stage in the evolution of insects, metabolic suppression during diapause may have resulted in low concentrations of carbohydrates. Enhanced accumulation of carbohydrates may have occurred in response to drought stress in tropical regions. In species pre-adapted like this, selection for cold hardiness may have been rapidly accomplished when invading colder regions.

In arctic areas, life cycles of several years duration may account for the low diversity of terrestrial arthropods, requiring the development of cold hardiness in several stages (Sømme, 1995). Extreme low temperature and prolonged winter duration offer other obstacles. As an adaptation to avoid extreme conditions, a large proportion of arctic species are restricted to moist and aquatic habitats.

More studies are required for the development of a holistic view of overwintering strategies (Danks, 1996). As will be discussed in the following paragraphs, physical factors other than temperature also have to be overcome.

COLD HARDINESS AND DESICCATION

The water vapour pressure of air above ice is lower than that above water. For this reason, unfrozen insects and other invertebrates are at a risk to loose water in frozen surroundings. Water losses may be reduced or prevented by low permeability of the cuticle, and terrestrial arthropods certainly show great variation in this respect (Hadley, 1994). Furthermore, high concentrations of solutes will lower the water vapour pressure of the haemolymph, and consequently reduce water losses. For this reason Ring & Danks (1994) have suggested that glycerol and other low molecular weight substances do not only protect against cold, but against desiccation as well.

The water content of freeze intolerant insects is commonly reduced in their overwintering stages (Sømme, 1982; Ring & Danks, 1994), and in this way the concentration of solutes is increased. Evidence for the relation between polyols and desiccation is provided by the bark beetle *Ips acuminatus*, which do not synthesise ethylene glycol until their water content has fallen below 52% of fresh weight (Gehrken, 1984). Similarly, glycerol synthetization in the antarctic mite *Alaskozetes antarcticus* is triggered by low relative humidity (Cannon & Block, 1988).

The presence of glycerol in aestivating pupae of the winter moth *Operophtera brumata* suggests that this substance protects against desiccation rather than cold (Ring & Danks, 1994). This relationship, however, was not found by Košťál et al. (1998) in aestivating prepupae of the Mediterranean tiger moth *Cymbalophora pudica*, while Klok & Chown (1998) suggested that the low SCPs of the sub-antarctic moth *Embryonopsis halticella* may have evolved as a consequence of pronounced desiccation resistance. Apparently, this question needs further investigation.

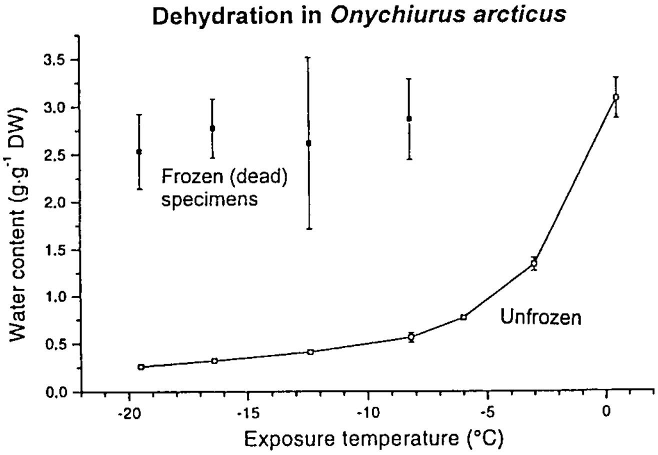

Fig. 5. Decrease in water content in unfrozen specimens of the arctic springtail *Onychiurus arcticus* kept over ice at decreasing temperatures. Specimens frozen spontaneously during exposure over ice maintained higher levels of water content. Redrawn from Holmstrup & Sømme (1998).

A PROTECTIVE DEHYDRATION STRATEGY

From earthworm cocoons and enchytraeids it is known that some invertebrates loose most of their water content in frozen soil, and in this way they avoid lethal freezing. Recently, Holmstrup & Sømme (1998) demonstrated a similar "protective dehydration strategy" in the arctic collembolan *Onychiurus arcticus* (Fig. 5). This may be considered as a previously unrecognised kind of cold hardiness, where the animals literally have to dry out to tolerate freezing temperatures.

O. arcticus is widely distributed in the Arctic. In bird-cliffs at Spitsbergen, Svalbard this species can tolerate temperatures below –20°C during the winter. Unlike other polar and alpine collembolan, *O. arcticus* does not respond to the onset of winter by increased supercooling capacity. Regardless of acclimation temperature, supercooling points remain at approximately –7°C, and all specimens are killed by freezing. This makes it difficult to understand how the collembolans are able to survive in their natural habitat.

When specimens of *O. arcticus* were placed in small containers over ice, their water content fell gradually until an equilibrium with the experimental temperature was reached (Fig. 5). From an initial value of 3.0 g.g^{-1} dry weight, the water content decreased to 1.5 g.g^{-1} at –3°C and 0.25 g.g^{-1} at –19.5°C. Following slow warming to above-zero temperatures, 80 to 90% survival was recorded in all groups. In this way *O. arcticus* avoids freezing by dehydration and will tolerate the low temperatures of its natural habitats.

ANAEROBIOSIS

Winter conditions do not only include cold and drought, but some terrestrial arthropods experience lack of oxygen as well (Sømme 1989, 1995). This was originally described for the carabid beetle *Pelophila borealis* that lives in alpine moors in Norway. During the winter the moist ground is frozen, but due to a deep layer of snow, temperatures rarely fall below –2°C. In the laboratory, the beetles survived for five months without oxygen at 0°C.

Similarly, the ground of windswept mountain ridges is solidly frozen during the winter. To collect soil samples with Collembola and mites, an axe is the best tool. An oxygen deficiency is likely to arise for animals enclosed in ice. Although their respiration is low at low temperatures, a change to anaerobic metabolism takes place.

In the laboratory some specimens of the collembolan *Tetracanthella wahlgreni* and of the oribatid mites *Carabodes labyrinthicus* survived for more than 90 d in an atmosphere of N_2 at 0°C. In both species lactate was accumulated as a result of anaerobic metabolism, although other end products may also have been produced.

Apparently, anaerobiosis is an important winter strategy in alpine and polar terrestrial arthropods. Soil animals from a variety of habitats may be enclosed in ice and experience an oxygen deficiency when water saturated soil freezes. More studies on anaerobiosis in terrestrial arthropods, in particular in relation to cold tolerance and desiccation, would be of great interest.

CONCLUSIONS

Insects and other terrestrial arthropods are found in some of the coldest polar and temperate environments. They overwinter in a variety of habitats, and different physiological mechanisms of cold hardiness have evolved.

While some species are freeze tolerant, most terrestrial arthropods depend on supercooling to survive low temperatures. Cold hardy species are able to survive low temperatures for prolonged periods of time. The degree of supercooling is regulated by the removal of ice nucleating agents from the body, by the accumulation of low molecular weight cryoprotectant substances and by thermal hysteresis proteins. Freeze tolerance also is enhanced by low molecular weight cryoprotectant substances and is often associated with the presence of ice nucleating agents in the haemolymph.

Increased haemolymph osmolality also protects against desiccation in a frozen environment. Some species must tolerate an oxygen deficiency during enclosure in ice. Other aspects of the entire life history of terrestrial arthropods will be affected by their overwintering strategies.

Suggestions for further research include investigating the nature of freeze tolerance, the physiology of prolonged exposure to cold and the relationships between desiccation, anaerobiosis and cold hardiness.

REFERENCES

Bachmetjew P. 1901: *Experimentelle entomologische Studien vom physikalisch-chemischen Standpunkt aus. Band 1.* Wilhelm Engelmann, Leipzig.

Bale J.S. 1991: Implication of cold hardiness in pest management. In Lee R.E. Jr. & Denlinger D.L. (eds): *Insects at Low Temperature*. Chapman and Hall, New York, pp. 461–498.

Bale J.S. 1993: Classes of insect cold hardiness. *Funct. Ecol.* 7: 751–753.

Baust J.G. & Edwards J. 1979: Mechanisms of freeze tolerance in an antarctic midge (Belgica antarctica). *Physiol. Entomol.* **4**: 1–5.

Baust J.G. & Rojas R.R. 1985: Insect cold hardiness: facts and fancy. *J. Insect Physiol.* **31**: 755–759.

Block W. 1996: Cold or drought – the lesser of two evils for terrestrial arthropods. *Eur. J. Entomol.* **93**: 325–339.

Cannon R.J.C. & Block W. 1988: Cold tolerance of microarthropods. *Biol. Rev.* **63**: 23–77.

Danks H.V. 1996: The wider integration of studies on insects cold-hardiness. *Eur. J. Entomol.* **93**: 383–403.

Denlinger D.L. 1991: Relationship between cold hardiness and diapause. In Lee R.E. & Denlinger D.L. (eds): *Insects at Low Temperature.* Chapman and Hall, New York, pp. 174–198.

Duman J.G. 1977: The role of macromolecular antifreeze in the darkling beetle Meracantha contracta. *J. Comp. Physiol.* **115**: 53–59.

Duman J.G., Wu D.W., Xu L., Tursman D. & Olsen T.M. 1991a: Adaptations of insects to subzero temperatures. *Q. Rev. Biol.* **66**: 387–410

Duman J.G., Xu L., Neven L.G., Tursman D. & Wu D.W. 1991b: Hemolymph proteins involved in insect subzero temperature tolerance: ice nucleators and antifreeze proteins. In Lee R.E. Jr. & Denlinger D.A. (eds): *Insects at Low Temperature.* Chapman and Hall, New York, pp. 94–127.

Duman J.G., Li N., Verleye D., Goetz F.W., Wu D.W., Andorfer C.A., Benjamin T. & Parmele D.C. 1998: Molecular characterization and sequencing of antifreeze proteins from larvae of the beetle Dendroides canadensis. *J. Comp. Physiol. (B)* **168**: 225–232.

Franks F. 1985: *Biophysics and Biochemistry at Low Temperatures.* Cambridge University Press, Cambridge, 210 pp.

Gehrken U. 1984: Winter survival of an adult bark beetle Ips acuminatus Gyll. *J. Insect Physiol.* **30**: 421–429.

Gehrken U. 1992: Inoculative freezing in the adult beetles Ips acuminatus and Rhagium inquisitor. *J. Insect Physiol.* **38**: 519–524.

Gehrken U. 1995: Correlative influence of gut appearance, water content and thermal hysteresis on whole body supercooling point of adult bark beetles, Ips acuminatus Gyll. *Comp. Biochem. Physiol. (A)* **112**: 207–214.

Hadley N.F. 1994: *Water Relations of Terrestrial Arthropods.* Academic Press, San Diego, 356 pp.

Hansen T. 1973: Variation in glycerol content in relation to cold-hardiness in the larvae of Petrova resinella L. (Lep., Tortricidae). *Eesti NSV Tead. Akad. Toim. Biol.* **22**: 105–112.

Hodková M. & Hodek I. 1994: Control of diapause and supercooling by the retrocerebral complex in Pyrrhocoris apterus. *Entomol. Exp. Appl.* **70**: 237–245.

Hodková M. & Hodek I. 1997: Temperature regulation of supercooling and gut nucleation in relation to diapause of Pyrrhocoris apterus (L.) (Heteroptera). *Cryobiology* **34**: 70–79.

Holmstrup M. & Sømme L. 1998: Dehydration and cold hardiness in the arctic collembolan Onychiurus arcticus Tullberg 1876. *J. Comp. Physiol. (B)* **168**: 197–203.

Horwath K.L., Easton C.M., Poggioli G.J. Jr., Myers K. & Schnorr I.L. 1996: Tracking the profile of a specific antifreeze protein and its contribution to the thermal hysteresis activity in cold hardy insects. *Eur. J. Entomol.* **93**: 419–433.

Husby J.A. & Zachariassen K.E. 1980: Antifreeze agents in the body fluid of winter active insects and spiders. *Experientia* **36**: 963–964.

Klok C.J. & Chown S.L. 1998: Interaction between desiccation resistance, host-plant contact and thermal biology of a leaf-dwelling sub-antarctic caterpillar, Embryonopsis halticella (Lepidoptera: Yponomeutidae). *J. Insect Physiol.* **44**: 615–628.

Košťál V. & Šimek P. 1996: Biochemistry and physiology of aestivo-hibernation in the apple blossom weevil, Anthonomus pomorum (Coleoptera: Curculionidae). *J. Insect Physiol.* **42**: 727–733.

Košťál V., Šula J. & Šimek P. 1998: Physiology of drought tolerance and cold hardiness of the Mediterranean tiger moth Cymbalophora pudica during summer diapause. *J. Insect Physiol.* **44**: 165–173.

Kukal O. 1991: Behavioral and physiological adaptations to cold in a freeze-tolerant arctic insects. In Lee R.E. Jr. & Denlinger D.L. (eds): *Insects at Low Temperature.* Chapman and Hall, New York, pp. 276–300.

Leather S.R., Walters K.F.A. & Bale J.S. 1993: *The Ecology of Insect Overwintering.* Cambridge University Press, Cambridge, 155 pp.

Lee R.E. Jr. 1991: Principles of insect low temperature tolerance. In Lee R.E. Jr. & Denlinger D.L. (eds): *Insects at Low Temperature.* Chapman and Hall, New York, pp. 17–46.

Lee R.E. Jr. & Denlinger D.L. (eds) 1991: *Insects at Low Temperature.* Chapman and Hall, New York, 513 pp.

Lee R.E. Jr., Lee M.R. & Strong-Gunderson J.M. 1995: Biological control of insects pests using ice-nucleating microorganisms. In Lee R.E. Jr., Warren G.J. & Gusta L.V. (eds): *Biological Ice Nucleation and its Application.* APS Press, St. Paul, pp. 257–269.

Lee R.E. Jr., Costanzo J.P. & Mugnano J.A. 1996: Regulation of supercooling and ice nucleation in insects. *Eur. J. Entomol.* **93**: 404–418.

Lozina-Lozinskii L.K. 1974. *Studies in Cryobiology.* John Wiley, New York, 259 pp.

MacKenzie A.P. 1977: Non-equilibrium freezing behaviour of aqueous systems. *Phil. Trans. R. Soc. London (B)* **278**: 167–189.

Merivee E. 1978: *Cold-Hardiness in Insects.* Acad. Sci. Estonian SSR, Tallin, 188 pp. (in Estonian, English abstr.).

Miller K. 1982: Cold-hardiness strategies in some adult and immature insects overwintering in interior Alaska. *Comp. Biochem. Physiol. (A)* **73**: 595–604.

Olsen T.M. & Duman J.G. 1997a: Maintenance of the supercooled state in the gut fluid of overwintering pyrochroid beetle larvae, Dendroides canadensis: role of ice nucleators and antifreeze proteins. *J. Comp. Physiol. (B)* **167**: 114–122.

Olsen T.M. & Duman J.G. 1997b: Maintenance of the supercooled state in overwintering pyrochroid beetle larvae, Dendroides canadensis: role of hemolymph ice nucleators and antifreeze proteins. *J. Comp. Physiol. (B)* **167**: 105–113.

Olsen T.M., Sass S.J., Li N. & Duman J.G. 1998: Factors contributing to season increases in inoculative freezing resistance in overwintering fire-colored beetle larvae, Dendroides canadensis (Pyrochroidae). *J. Exp. Biol.* **201**: 1585–1594.

Payne M.M. 1927: Freezing and survival of insects at low temperatures. *Q. Rev. Biol.* **1**: 272–280.

Pullin A.S. 1996: Physiological relationship between insect diapause and cold tolerance: Coevolution or coincidence? *Eur. J. Entomol.* **93**: 121–129.

Ramløv H., Bedford J. & Leader J. 1992: Freezing tolerance of the New Zealand alpine weta, Hemideina maori Hutton (Orthoptera; Stenopelmatidae). *J. Therm. Biol.* **17**: 51–54.

Ring R.A. 1980: Insects and their cells. In Smith A.M.J. & Farrant J. (eds): *Low Temperature Preservation in Medicine and Biology.* Pitman Medical, London, pp. 187–217.

Ring R.A. 1982: Freezing tolerant insects with low supercooling points. *Comp. Biochem. Physiol. (A)* **73**: 605–612.

RING R.A. & DANKS H.V. 1994: Desiccation and cryoprotection: overlapping adaptations. *Cryo-Letters* **15**: 181–190.

SALT R.W. 1936: Studies on the freezing process in insects. *Tech. Bull. Minn. Agric. Exp. Stn.* **116**: 1–41.

SALT R.W. 1959: Role of glycerol in the cold-hardening of Bracon cephi (Gahan). *Can. J. Zool.* **37**: 59–69.

SALT R.W. 1969: The survival of insects at low temperatures. *Symp. Soc. Exp. Biol.* **23**: 331–350.

SAUNDERS D.S. & HAYWARD S.A.L. 1998: Geographical and diapause-related cold tolerance in the blow fly Calliphora vicina. *J. Insect Physiol.* **44**: 541–551.

SØMME L. 1982: Supercooling and winter survival in terrestrial arthropods. *Comp. Biochem. Physiol. (A)* **73**: 519–543.

SØMME L. 1989: Adaptations of terrestrial arthropods to the alpine environment. *Biol. Rev.* **64**: 367–407.

SØMME L. 1995: *Invertebrates in Hot and Cold Arid Environments*. Springer, Berlin, 275 pp.

SØMME L. 1999: Cold adaptation in terrestrial invertebrates. In Margesin R. & Schinner F. (eds.): *Cold-Adapted Organisms – Fundamentals and Application*. Springer, Heidelberg, (in press).

SØMME L. & BLOCK W. 1982: Cold hardiness of Collembola at Signy Island, Maritime Antarctic. *Oikos* **38**: 168–176.

STOREY K.B. & STOREY J.M. 1988: Freeze tolerance in animals. *Physiol. Rev.* **68**: 27–84.

TANNO K. 1967: Freezing injury in fat body cells of the poplar sawfly. In Asahina E. (ed.): *Cellular Injury and Resistance in Freezing Organisms.* Institute of Low Temperature Science, Sapporo, pp. 245–257.

USHATINSKAYA R.S. 1990: *Latent Life and Anabiosis.* Nauka, Moscow, 182 pp. (in Russian).

VAN DER LAAK S. 1982: Physiological adaptations to low temperatures in freezing-tolerant Phyllodecta laticollis beetles. *Comp. Biochem. Physiol. (A)* **73**: 613–620.

VERNON P., VANNIER G. & LUCE J.M. 1996: Découverte de larves tolérantes et intolérantes à la congélation dans une même guilde de Cétoines (Coléoptères) en Forêt de Fontainbleau. *Écologie* **27**: 131–142.

ZACHARIASSEN K.E. 1985: Physiology of cold tolerance in insects. *Physiol. Rev.* **65**: 799–832.

ZACHARIASSEN K.E. 1991: The water relations of overwintering insects. In Lee R.E. Jr. & Denlinger D.L. (eds): *Insects at Low Temperature.* Chapman and Hall, New York, pp. 47–63.

Received September 17, 1998; accepted December 3, 1998