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# Cold Tolerance of Insects and Other Arthropods [and Discussion]

W. Block, J. G. Baust, F. Franks, I. A. Johnston and J. Bale

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# Cold tolerance of insects and other arthropods

By W. Block

*British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, U.K.*

[Plate 1]

Arthropods, as poikilotherms, adapt to cold environments in a variety of ways that include extension of locomotory activity to low temperatures, enhancement of metabolic rate and maintenance of a positive energy balance whenever possible. The ecological implications for many such animals are extension of the life cycle and a requirement for an individual to overwinter several times. Prolonged sub-zero temperatures increase the risk of tissue freezing, and two main strategies have been evolved, first avoidance of freezing by supercooling, and secondly, tolerance of extracellular ice. In the first strategy, freezing is invariably lethal and extensive supercooling (to −30 °C and below) occurs through elimination or masking of potential ice nucleators in the body and accumulation of cryoprotective substances such as polyhydric alcohols and sugars. Such species are termed freezing intolerant. The second strategy, freezing tolerance, is uncommon in arthropods and other invertebrates, and usually occurs in a single life stage of a species. Freezing of liquid in the extracellular compartment is promoted by proteinaceous ice nucleators. Freezing is therefore protective, and the lethal temperature is well below the supercooling point in freezing tolerant individuals, whereas in most freezing intolerant species it is close to or at the supercooling point. Proteins also act as antifreezes in insects of both strategies, producing a thermal hysteresis by lowering the freezing point of haemolymph in a non-colligative fashion while not affecting the melting point temperature. Recent studies and developments in arthropod cold tolerance are discussed against this background, and a broader approach than hitherto is advocated, which integrates ecological information with physiological data.

## Introduction

Since the study by Reaumur (1734) showed that some insects survive winter in a frozen state, zoologists have been intrigued with the problems facing insects inhabiting cold environments. Insects are some of the largest metazoan animals to tolerate cold and to survive freezing. Investigation of the ecology and physiology of their cold tolerance is an important area of biological research, but it also has practical applications in understanding the overwinter survival of pest species in agriculture and forestry. In addition, the preservation of insect material is of fundamental interest in the field of cryobiology.

Low temperatures influence insects in a great variety of ways. It is important to distinguish between the effects of temperatures just above and around 0 °C (non-freezing) and sub-zero temperatures (potentially freezing), as their effects on poikilothermic animals may be very different. Many insects and other arthropods spend much of their lives in low temperature environments under conditions that would kill or prevent the normal activities of many temperate and tropical forms. Species living in polar and alpine regions have to withstand freezing conditions for a considerable part of the year and on several occasions during an

individual's life span. To merely survive in a cold environment is not sufficient, for insects have to grow, develop through various life stages and reproduce during often short summer periods. Low temperature effects, therefore, may be both deleterious and injurious in arthropods.

Two categories of cold injury can be defined for insects: chilling injury caused by the effects of low temperatures on their physiological processes, and freezing injury caused by physical damage due to ice crystals and its metabolic consequences (Storey & Storey 1988). Cold hardening is used as a general term covering adaptations both to sustain metabolism and homeostasis at low temperatures, and to preserve cell structure and function against the effects of internal ice.

This paper considers, first, the effects of non-freezing low temperatures around 0 °C, and secondly, those effects relating to sub-zero temperature conditions on insects and other arthropods. In preparing the material, it was evident that often, the insect is considered solely in relation to either freezing or low temperatures and not to both. Insufficient attention has been paid to environmental temperatures just above zero and to their effects on terrestrial arthropods. Such environmental conditions are those in which many cold-adapted species live for much of their active life, especially in polar regions. Therefore, many of the examples in this paper are for polar species with which I am most familiar. To further our understanding, it is important that a more integrated picture of the adaptations of insects to low temperatures be drawn, and one in which, for example, the mechanisms of cold-adapted metabolism and cold resistance are viewed against the biology and ecology of the species concerned. Only then will an accurate assessment of the interaction of insect and environment be achieved.

## Low temperature adaptations

The effects of low temperatures on insects and other terrestrial arthropods are many and varied. When ambient temperatures decline towards the cold end of the species' thermal range, individual overall metabolism is reduced, and locomotory activity is increasingly restricted until the onset of chill-coma when all movement ceases. Figure 1 shows the various stages in

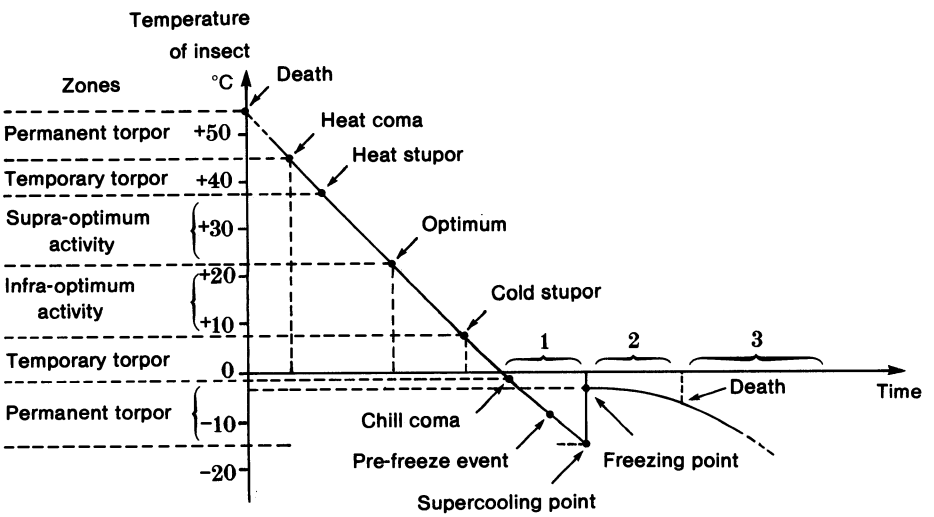


Figure 1. Diagram representing the thermobiology of an arthropod which is intolerant to freezing. 1, supercooling; 2, freezing; 3, solidification. (Modified from Vannier 1987.)

the thermobiology of an insect or arthropod that is intolerant to freezing. Before the onset of cold stupor, feeding may be limited and hence, the rate of energy assimilation will decline. Although growth rates may remain relatively high, if optimum environmental temperatures are of short duration, then extended development times result. Long life cycles are typical for many polar species. Successful terrestrial arthropods have a requirement to survive overwinter as a result of these ecological and physiological constraints, and overwintering may occur in any stage of the life cycle. The development of some form of cold hardening and resistance is a necessity. Adjustments to the physiological and biochemical machinery for overwintering in sub-zero temperatures result in metabolic costs to the individual. Finally, when optimal conditions return, the insect must undergo the processes of recovery and resumption of metabolic activity after cold exposure, before normal activity can take place. The interplay of these various effects with organism biology is a fascinating field of study.

Adaptations to low temperatures may involve morphological behavioural, ecological and physiological features (Sømme 1990). In insects morphological adaptations include reduction in body size, melanism, increased pubescence and wing reduction (brachyptery) or loss (aptery). Behavioural adaptations are avoidance of extreme environmental conditions by habitat selection, thermoregulation and activity patterns, whereas ecological adaptations include life cycle extension and univoltinism in special cases. Physiological adaptations are the elevation of metabolic rate, resistance to desiccation, cold tolerance and anaerobic metabolism in some species.

### (*a*) *Locomotion and activity*

Low temperatures directly restrict locomotory activities of insects and other arthropods, and this is demonstrated by the response of an Antarctic mite (*Alaskozetes antarcticus*) to environmental temperature (Block 1981). Over the range −4 to 24 °C, this species has a sigmoid activity curve with a peak between 16 and 24 °C. However, the optimum temperature for its activity, defined as that at which locomotory behaviour is most sensitive to thermal change, occurs between 12 and 16 °C. From an ecological and functional viewpoint, the crucial feature is that this mite is capable of locomotion at temperatures around 0 °C and for a few degrees below zero. Cold stupor temperatures (those which immobilize 50 % of the sample) for this species ranged from −5.0 to −6.6 °C and were not affected by low temperature acclimation (Young 1979*a*). Another Antarctic mite, *Nanorchestes antarcticus*, has been observed moving at temperatures as low as −11 °C (Rounsevell 1977).

Determinations of the lower temperature limit for activity in seven species of Antarctic micro-arthropods by observation of individual chill-coma levels (temperature at which all movement ceases) show a range from −3.0 to −11.0 °C (Block 1990). Experimental acclimation to constant low temperatures does not extend this range significantly downward, but even an extension of one or two degrees would be ecologically advantageous. The temperature at which locomotion restarts after chill-coma has been termed the reactivation temperature (Schenker 1984). Data for six species of micro-arthropods show that this is between one and two degrees higher than the individual chill-coma temperature, and that only rarely is the reactivation temperature positive.

Low temperature activity displayed by an individual insect in a given habitat will depend on a number of factors. Principally, locomotion depletes energy reserves and requires that the insect continues feeding, but in sub-zero conditions feeding will be suppressed. This has survival




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

value for such Antarctic species, which cannot survive freezing, by reducing the potential ice nucleators present in the gut compartment. In addition, suitable food resources may not be available during winter.

### (*b*) *Feeding and energetics*

In cold environments, insect feeding is regulated largely by temperature. In the springtail, *Cryptopygus antarcticus*, rates of feeding are high during summer in the Antarctic (Burn 1982), although with the onset of sub-zero soil temperatures an immediate decrease in the proportion of the population with gut contents occurs (figure 2). The virtual absence of feeding during winter is related to survival of sub-zero temperatures through enhancement of supercooling capacity. The rapid changes in the number of fed animals in field samples during spring freeze–thaw cycles suggest that such Collembola are capable of rapidly emptying their gut systems in response to lowered environmental temperatures, although their survival may be influenced by the cooling rate even within their ecological range. In energetic terms, a decline in temperature from 5 to 0 °C greatly reduces food consumption in both juvenile and mature individuals of *C. antarcticus* (Burn 1984) without similar effects on the rates of energy assimilation and respiration loss. In this species, the excess of energy assimilation over respiratory loss (that is, net positive energy balance) declines as the temperature decreases between 1.5 and 0 °C. Below this temperature, stored energy is utilized for metabolism. The models proposed by MacLean (1975) for the energy balance of cold adapted ectotherms do not entirely fit such observations, and Burn (1982) proposed that the critical relation is that of

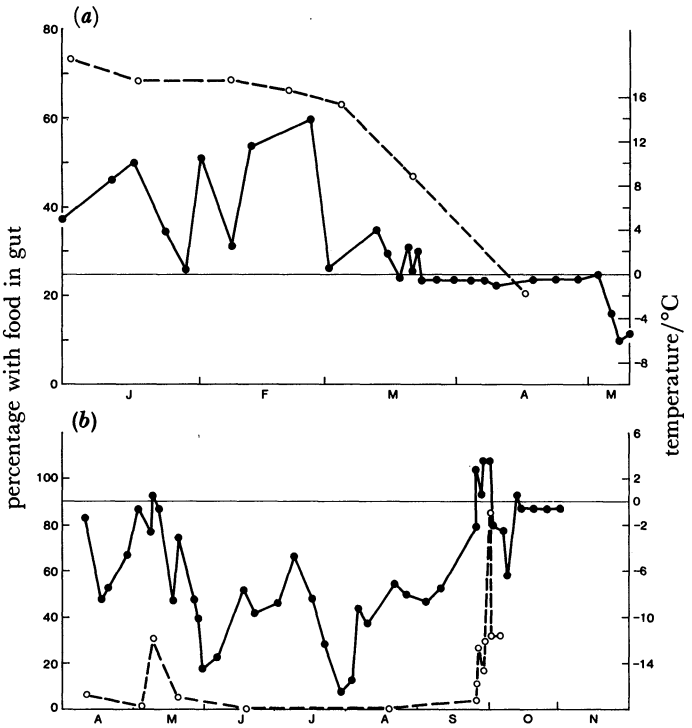


FIGURE 2. Influence of soil temperatures on feeding of the collembolan *Cryptopygus antarcticus* in the maritime Antarctic during (*a*) summer–autumn and (*b*) winter–spring. Changes of soil temperature (at 1.5 cm depth) (solid line) are plotted with proportions of animals with food in their gut (dashed line). (After Burn (1982).)

energy gain or loss at temperatures around 0 °C in species such as *C. antarcticus*. Thus, in utilizing the brief and intermittent thaw periods both during winter and in the austral spring and autumn, this springtail may sustain a negative energy balance during freezing periods. Therefore stored energy must be used to maintain metabolic processes. Above 0 °C, a large proportion (approximately 80 % in *A. antarcticus*) of assimilated energy is used in respiration by polar arthropods (Block 1977).

From calculations of the minimum energy required to support arthropod metabolism, knowing the individual respiratory rate and the calories lost per unit of oxygen consumed, it is concluded that in the polar environment energy is probably not a limiting factor. However, energy capture itself and its assimilation by the individual may be regulatory through the overall effects of temperature depressing locomotion, feeding and other activities at 0 °C. Most polar species probably utilize small enhancements of their mobility to significantly improve their foraging and feeding activities at such low temperatures, and thus gain a considerable advantage in biological terms.

### (*c*) *Respiration*

The relation of metabolic rate to temperature determined in the laboratory provides a comparative baseline of species' response to field temperature changes, and it has been investigated in several polar arthropods. Block (1977) derived a general curve for this relation from ten species of Antarctic land invertebrates over the temperature range of −4 to 22 °C. The overall $Q_{10}$ for the regression line is 3.04, which is slightly higher than most temperate species. Thus the metabolic response to rising temperature over this range is similar for the polar and temperate species studied, but the former have generally elevated levels. This was substantiated further by extending respiratory studies to include the adults and larvae of two species of phytophagous Coleoptera at South Georgia, in the sub-Antarctic, and comparing the results with alpine (Finse, Norway) and Arctic (Spitzbergen) beetles (Sømme *et al.* 1989). The single Arctic species showed the highest metabolic rates over the temperature range 5–20 °C.

For several Antarctic micro-arthropods there is a degree of cold adaptation in their rates of resting or standard metabolism, as determined by Cartesian Diver respirometry, when the data are compared with temperate forms (Block 1990). For an herbivorous mite, an elevation of metabolism of between 2–4 times was observed over their normal temperature range (figure 3), and between 3–4 times for predatory mites (Block & Young 1978). The Arrhenius equation can be used to relate insect metabolism to temperature, while allowing a derivation of the activation energy. In several polar insects, their activation energies occupy the lower part of the range found for all insects and support the suggestion (Young 1979*b*) that this is a feature of cold-adapted metabolism.

The evidence for metabolic cold adaptation in arthropods is of two seemingly irreconcilable kinds. First, the elevation of resting metabolism is required in species living in cold environments to prevent it from declining below the maintenance level, secondly, its elevation by such poikilotherms does not occur as it would divert energy from growth and reproduction and might be selectively disadvantageous. However, the energy intake for maintenance metabolism will be similar in both temperate and polar species, when compared at their normal environmental temperatures. The question of energy wastage would not arise, therefore, provided that energy intakes and metabolism are similar at their respective habitat temperatures. This conclusion depends on two assumptions: (*a*), that energy utilization is




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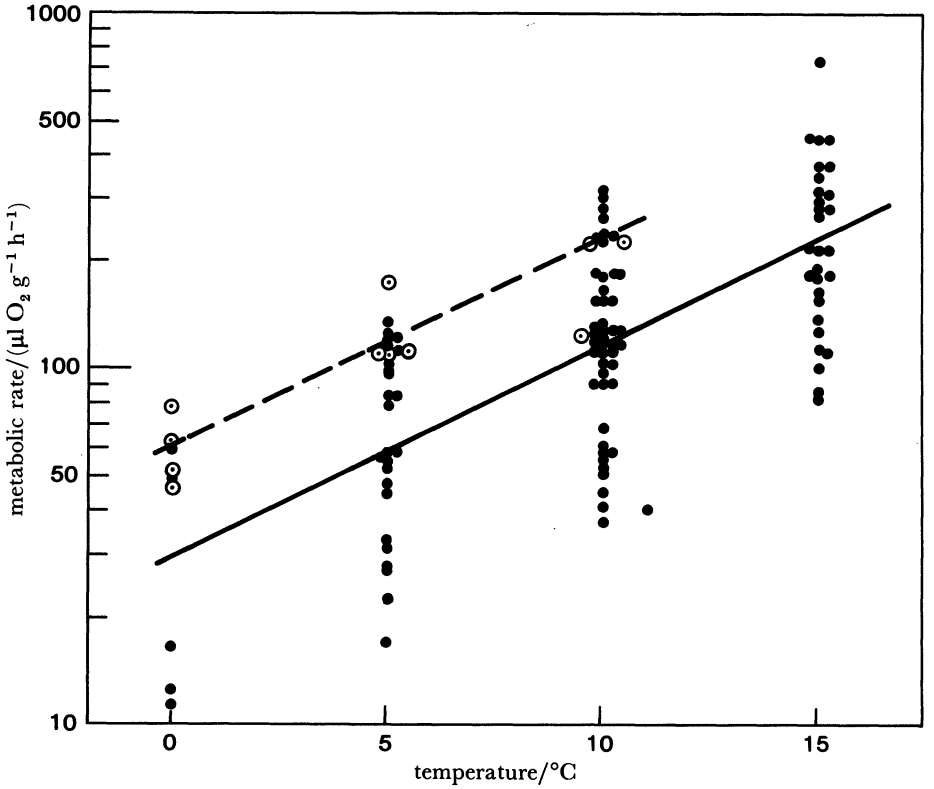


FIGURE 3. Relation of standard metabolic rate to temperature in all life stages of oribatid mites. Linear regressions have been fitted to data for *Alaskozetes antarcticus* (dashed line) from Signy Island, Antarctica, and to data for 36 temperate species (solid line). Regression equations of metabolic rate (MR, $\mu$l $O_2$ $g^{-1}$ $h^{-1}$) on temperature ($T$, °C) are for *A. antarcticus*: $\log_{10}$ MR $= 1.904 + 0.047\,T$ ($r = +0.859$), and for the temperate species: $\log_{10}$ MR $= 1.446 + 0.065\,T$ ($r = +0.711$). (From Block & Young 1978.)

minimal within the normal ecological range experienced by the individual, but is sufficient for maintenance of the animal while allowing as much as possible of the available energy to be used in growth and reproduction; (*b*), that the metabolic rate of the temperate species at polar temperatures is not sufficient for active life at these lower temperatures. From this assumption, it may be concluded that a minimum threshold of standard metabolism is necessary for maintenance of the arthropod in a physiological state where it can survive and remain active. More evidence is required from a wider range of species for an adequate resolution of this question. However, it is unlikely that all poikilotherms, or indeed all arthropods, will behave similarly, and it may be that diverse strategies have evolved in various cold environments to overcome the problems of energy balance at low temperatures.

### (*d*) *Growth, development and life cycles*

Data on growth rates of arthropods at low temperatures are sparse, but Burn (1981, 1984) has examined *C. antarcticus* in detail. Its moulting rate remains unchanged over 0–20 °C on a range of food materials, but maximum moulting frequency is attained at a lower temperature than in temperate isotomid Collembola. By using a relation between mandible size and body length, Burn (1981) showed that mature individuals (1040–1134 μm body length) either increased or decreased in size at subsequent moults. He suggested that degrowth, previously

demonstrated only in crustaceans, molluscs and turbellarians, may be of widespread occurrence in such species under cold conditions. By using knowledge of the soil microclimate experienced by populations of *C. antarcticus*, it was postulated that between two and four moult cycles could be completed in one polar summer, with individual longevity being 3–7 years. Thus overwintering occurs several times in the life cycle of such an insect; a similar situation seen in the terrestrial mites in the south polar region (Block 1980).

In some Antarctic oribatid mites, breeding is iteroparous and occurs throughout the austral summer with temperature controlling oviposition (Block 1980). As all post-embryonic stages overwinter, a mixed population of nymphal instars and adults occurs in the field, and from such a pool individuals mature to breed when environmental conditions permit. Individuals may live for 2–4 years. In the more diverse arctic insect fauna, a greater range of life cycle adaptations can be seen. Downes (1964) supposed that unpredictable weather might be exploited by opportunistic development, whereas in the high arctic, synchrony of growth and development with the seasonal cycle is common (Danks 1978). Nevertheless, extension of life cycle duration is observed in many arctic species of arthropods. Extreme examples of this are found in a chironomid living in tundra pools in northern Alaska, which has a seven-year life cycle (Butler 1982), and in the high arctic moth *Gynaephora groenlandica*, which requires up to 14 years to complete its life cycle (Kukal & Kevan 1987). By contrast, some alpine insects have evolved univoltine life cycles (a single generation per year), which may be considered as the most specialized (Sømme & Block 1990).

## Sub-zero temperature adaptations

As environmental temperatures approach 0 °C the need for survival becomes paramount in arthropods, and as temperatures decline below freezing, a capacity to avoid freezing or to survive physical damage due to ice formation in the body becomes crucial. Respiration continues below 0 °C and during the early phase of supercooling, and oxygen consumption has been measured down to −4 °C in antarctic mites (Young & Block 1980). Anoxic conditions may occur in the field, especially when ice forms, and survival under these conditions may be high for mites (Block & Sømme 1982) and other arthropods. Two main options for the survival of freezing temperatures have been exploited by insects and other arthropods (table 1). The first, and most common one, is the avoidance of freezing by supercooling (maintenance of the

Table 1. Scheme showing the main features of the cold tolerance strategies adopted by arthropods

| freezing intolerance | freezing tolerance |
|---|---|
| high supercooling capacity | poor supercooling capacity |
| supercooling point is usually the lower lethal temperature | supercooling point is not the lower lethal temperature |
| ice nucleating agents absent or masked | ice nucleating agents present and activated |
| polyols function as antifreezes | polyols function as cryoprotectants |
| thermal hysteresis proteins often present which may stabilize the supercooling point | thermal hysteresis proteins often present as antifreezes |
| ice formation is lethal | survive the formation of extracellular ice |

body fluids in the liquid phase below their normal freezing point). In this case, when freezing of the body tissues occurs, it is invariably lethal, and so various mechanisms are utilized by arthropods to enhance supercooling (see Block 1982; Sømme 1982). Such species are referred to as freezing intolerant or freezing susceptible. The second, and less frequent method, is that ice forms in the body tissues but is confined to the extracellular fluids. In this case, freezing at relatively high sub-zero temperatures is promoted by nucleators and supercooling is thereby reduced. Such species are termed freezing tolerant. For detailed reviews of the subject, see Zachariassen (1985); Bale (1987); Cannon & Block (1988) and Storey & Storey (1988). Comprehensive bibliographies are provided in Baust *et al.* (1982) and Lee *et al.* (1986).

In many biological systems and especially insects, avoidance of freezing by supercooling is the norm rather than tolerance of limited ice formation. This is the case in arthropods and other invertebrates that have been investigated (Block 1982). There are three principal physiological mechanisms underlying cold resistance in arthropods. First, ice nucleating agents (INAs) of various types promote nucleation in the fluid compartments and thereby terminate supercooling. Such agents may be any particle around which an ice embryo can form, including dust and bacteria, in addition to sub-microscopic proteins. INAs are present in both freezing tolerant and intolerant forms. Secondly, a thermal hysteresis may occur, where the freezing point of the haemolymph is depressed relative to the melting point temperature, thereby conferring protection during freezing conditions. This phenomenon is brought about by thermal hysteresis proteins (THPs) or antifreeze proteins, which act in a non-colligative manner, and are characteristic of hibernating freezing intolerant species. Thirdly, low molecular mass solutes (polyhydric alcohols and sugars) increase the cold hardiness of both freezing tolerant and freezing intolerant forms in a colligative (water-binding) manner. Such compounds may accumulate during preparation for winter, or they may occur year round.

The distribution of the two strategies of cold tolerance in arthropods is intriguing in that freezing tolerance is limited to representatives of 'higher' insect orders (for example, Coleoptera, Diptera and Hymenoptera), while freezing intolerance is of wide occurrence in both insects and arachnids (Cannon & Block 1988). A possible alternative strategy based on vitrification has been proposed by Wasylyk & Baust (1988), but this has only been observed in 'mock haemolymph' of the gallfly *Eurosta* between $-30$ and $-50$ °C.

### (*a*) *Supercooling*

The determination of the whole body freezing point temperature (supercooling point (SCP) of a single insect or arthropod, when cooled at a constant rate (usually 1 °C $min^{-1}$), is a widely used indicator of its cold hardiness (Salt 1961). Many factors influence the temperature at which the freezing process is initiated, including the duration of prior cold exposure, experimental cooling rate, ice nucleator content and polyols. Measured SCPs of freezing tolerant individuals are usually markedly higher than those for freezing intolerant individuals because of the higher INA activity in the former. It has been generally assumed that the measured SCP of a freezing intolerant species is also its lower lethal temperature, which is not always so (Bale 1987). Also, earlier studies have focussed on freezing as the most important lethal effect of sub-zero temperatures, but there are several others that affect the capacity of the supercooled insect to recover, survive and continue development. For example, Knight *et al.* (1986) reported considerable pre-freeze mortality in aphids occurring before the SCP. Studies of insect cold hardiness must be placed in an ecological context, to gain insight into the




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

processes controlled by low temperature that determine survival and mortality in the natural environment (Bale 1987).

Supercooling facilitated by biological processes is a major survival strategy in many terrestrial arthropods (see review by Sømme (1982)). In polar microarthropods, which are freezing intolerant, SCPs are the lower lethal temperatures of all the species studied to date (Cannon & Block 1988), and therefore are a good index of relative cold hardiness and potential survivability under the conditions of the experiment. Analyses of such SCP data have allowed: (*a*) tracking of seasonal changes in cold hardiness in relation to habitat microclimate, polyol and water contents, field survival, etc. and (*b*) comparison of the process of supercooling in living and physical systems.

Studies of seasonal fluctuations in SCPs of field populations of *A. antarcticus* and *C. antarcticus* at Signy Island, maritime Antarctic, show strong seasonal cycles in mean values ranging from −6 to −30 °C over six years (W. Block *et al.* unpublished data). The cycles were synchronized

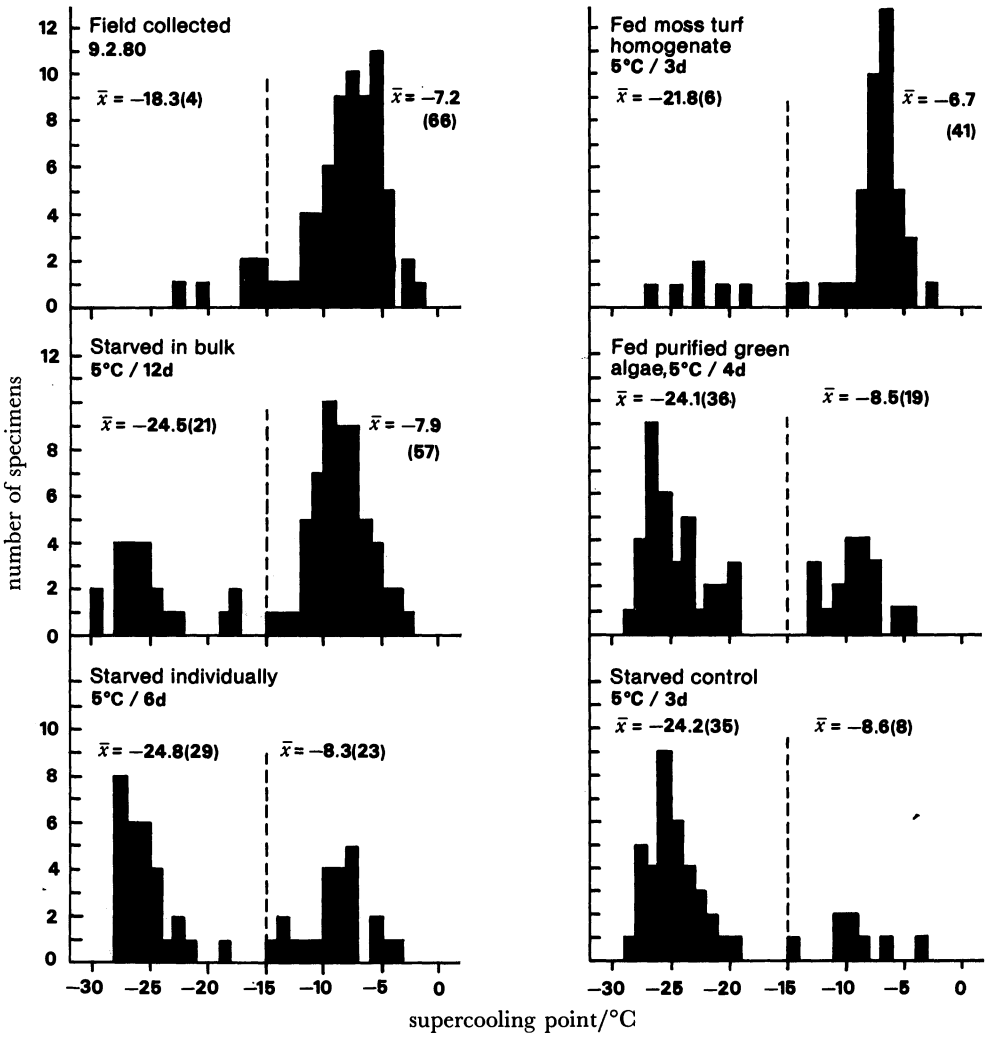


FIGURE 4. Supercooling point distributions for *Cryptopygus antarcticus* comparing field-fresh specimens with samples which were either starved or fed foods from their natural habitat at 5 °C. Mean high and low group supercooling points are shown together with the number of individuals per sample (after Sømme & Block 1982).

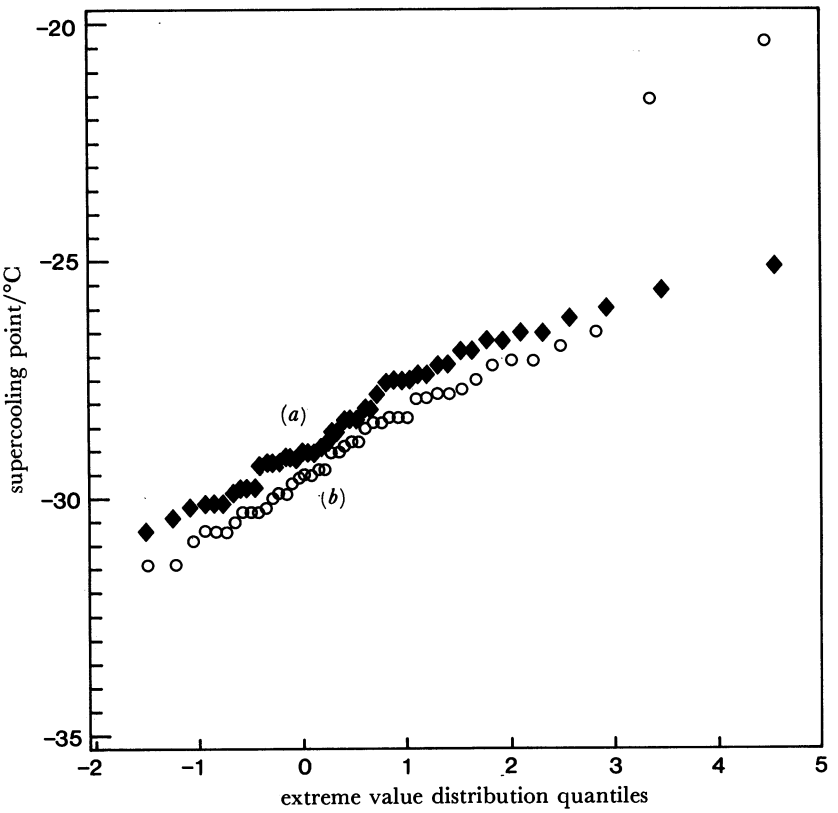


FIGURE 5. Quantile plots of extreme value distributions of two sets of supercooling point data for adult *Alaskozetes antarcticus* showing (*a*) an uncontaminated distribution similar to that for equivalent-sized water droplets, and (*b*) a distribution contaminated with two high-group data points. Only $< -20$ °C data are included.

with overall climate changes, which were reflected in the microclimatic conditions of their fellfield habitats. Concomitant seasonal changes in the levels of cryoprotectants were found in both species, with higher concentrations in winter than during summer. SCP frequency distributions of these species are often bimodal, having a 'high' group and a 'low' group with the division between $-15$ and $-20$ °C (figure 4). Attempts are being made to model the underlying mechanisms that may generate both uni- and bi-modal distributions, and to compare the arthropod distributions with those obtained for supercooled water droplets (Vali & Stansbury 1966). Developing Bigg's stochastic hypothesis that the probability of freezing increases exponentially with the decline in temperature (see Block 1983), Vali & Stansbury obtained the extreme value distribution to describe the variation in the temperature at which identical-sized water droplets freeze. The extreme value distribution clearly cannot mimic the bimodality of the arthropod system. However, the unimodal distributions, and particularly the 'low' group data, show a broad correspondence of behaviour with the water droplet model (figure 5). This supports the earlier suggestion by Block & Young (1979) that mites and water droplets of 1 $mm^3$ volume behave similarly when supercooled below $-20$ °C.

### (*b*) *Ice nucleators*

Potential ice nucleators in insects may be of external or internal origin. Mineral particles, microorganisms and ingested food materials are all important sources of externally derived

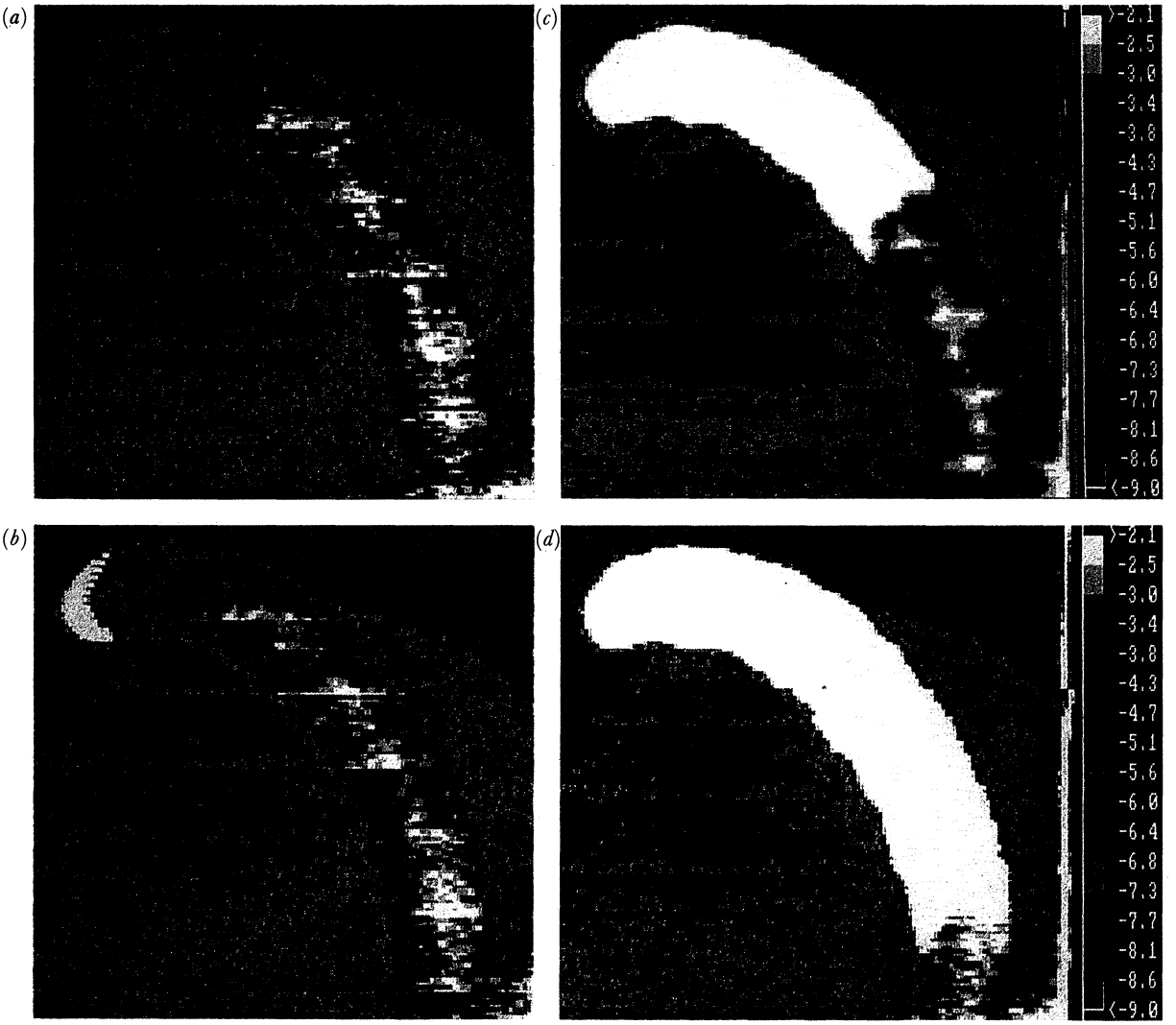


FIGURE 7. Thermographs showing the surface temperature patterns developed during freezing (*a–d*) of a larva of the tobacco hornworm (*Manduca sexta*) by using an infrared scanning system (Agema). The specimen was inoculated at the anterior end (upper left of the picture), and the freezing front can be followed as high temperatures are recorded because of the release of latent heat from the insect.


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

nucleators (Salt 1961), whereas internally derived nucleators are extracellular organic macromolecules (proteins, lipoproteins) and intracellular supramolecular structures (Zachariassen 1982). In freezing susceptible species that hibernate in a supercooled state, ice nucleators are either removed or masked, allowing an extension of supercooling to below −20 °C. Several species of freezing tolerant insects synthesize potent INAs that circulate in the haemolymph only in winter and ensure a protective extracellular freezing at relatively high sub-zero temperatures. Two haemolymph INAs have been characterized from freezing tolerant insects and Neven (1987) has shown that the apolipoprotein and phosphatidylinositol components are crucial for nucleator activity.

The idea that insects remove potential nucleators contained in food material in the gut system in preparation for winter (Salt 1953, 1961) has been challenged by Baust & Rojas (1985), and it is clear that this is not universally applicable. However, in several polar microarthropods the evidence suggests that clearing food materials from the gut correlates with increased supercooling capacity (figure 4) (Sømme & Block 1982), and that this process occurs in field populations (Burn 1982). It is not known whether this is an active process on the part of the animal or a response to declining environmental temperatures during autumn. However, Cannon (1986) simulated changes in supercooling point distributions of Antarctic mites by experimental treatments, which mimic those obtained from field samples at various seasons (figure 6).

In an attempt to locate the site of nucleation and to study the process of freezing in individual

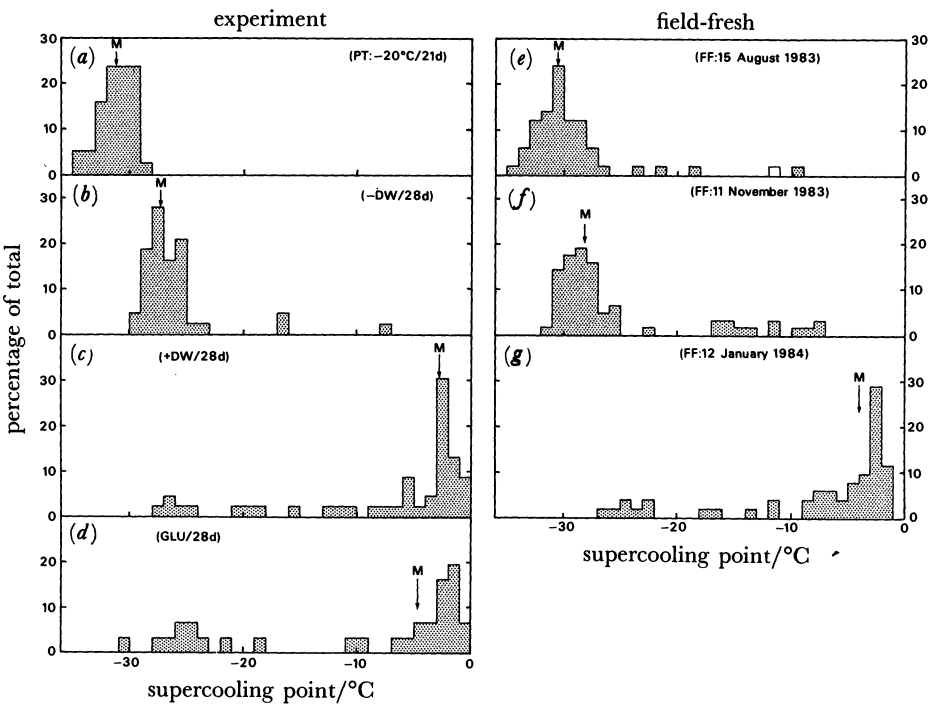


FIGURE 6. Supercooling point distributions for adult *Alaskozetes antarcticus* from a series of experimental treatments compared with field-fresh samples. (*a*) Pre-treatment (PT) sample; (*b*) without access to liquid water (−DW); (*c*) with access to double distilled water (+DW); (*d*) with access to 10% glucose solution (GLU); (*e*, *f*, *g*) field-fresh (FF) samples. In treatments (*b*), (*c*) and (*d*) the mites were maintained at 4 °C for 28 days in a saturated atmosphere (100% r.h.). M shows the position of the median supercooling point in each case. (Data from Cannon (1986)).

insects, recent studies have utilized infra-red thermography to measure temperatures at the surface of the insect's body when cooled at a constant rate in air (M. R. Worland & W. Block, unpublished data). The site of initial freezing (and hence nucleation) was detected by an observable rise in surface temperature, and its spread monitored with time. An infra-red detector and scanner (Thermovision 870, Agema) in conjunction with a Discon unit to convert the black and white thermal images to false colours and a video recording system was used to record and display the results. Analyses of the tapes were undertaken with a TIC-800 and CATS program. Six species of macro insects, including desert locust, cockroach, cranefly larvae and larvae and pupa of the tobacco hornworm (*Manduca sexta*) were examined (plate 1, figure 7). The results show that although the site of initial freezing varied within particular species, the abdomen was a common location. Total body freezing was rapid in small specimens, but the large lepidopterous larvae enabled rates of ice spread to be estimated. Such techniques allow a range of detailed freezing studies to be undertaken on large arthropods.

### (*c*) *Antifreezes*

Compounds that act as antifreezes in arthropods occur in both freezing tolerant and intolerant species, and are of two main types: low molecular mass compounds (polyols and sugars) and high molecular mass compounds (thermal hysteresis producing proteins or THPS).

Polyols and sugars function colligatively, and their accumulation causes a lowering of the whole body SCP of the arthropod. Concentrations of polyols are usually within the range 0.4–0.6 M (Cannon & Block 1988), but occasionally they may reach 4–5 M (Miller & Werner 1980). In terms of fresh weight, these amounts are normally 3–6%, and exceptionally 10–14%. In addition, polyols have important cryoprotective functions in stabilizing proteins and enzymes against cold denaturation, and promote desiccation resistance by increasing the proportion of unfrozen water. Polyols in solution have been found to depress the SCP by more than twice the corresponding freezing point depression of the haemolymph (Block & Young 1979). The compounds frequently identified from arthropods are glycerol, mannitol, sorbitol and trehalose, while ethylene glycol has been found to be important as an antifreeze in adults of the bark beetle *Ips acuminatus* (Gehrken 1984). Single and multicomponent polyol systems have been reported in various species of arthropods, the latter may prevent the build-up of high and potentially toxic levels of a single component. Two freezing tolerant species show a variety of cryoprotective compounds: larvae of the antarctic midge *Belgica antarctica* contain erythritol, glucose and trehalose, whereas those of the gallfly *Eurosta solidaginis* contain glycerol, sorbitol and trehalose. The primary environmental cue for antifreeze accumulation is thought to be temperature linked in most arthropods (Baust 1982), and this is also the case for ice nucleator activity (Zachariassen 1982).

Recent studies on the timecourse of ice accumulation in freezing tolerant insects by using differential scanning calorimetry and nuclear magnetic resonance techniques (Wasylyk & Baust 1988) have shown the formation of a partial glassy state in mixtures of cryoprotective solutions between −30 and −50 °C. This suggests that the cryoprotectant solution in *E. solidaginis* undergoes a glass transition at a relatively high temperature, which may be critical for its winter survival. Similar vitrification phenomena may be widespread in cold-adapted arthropods.

Antifreezes with high molecular masses are proteinaceous in character and are responsible for thermal hysteresis in several species of insects and other arthropods.




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

### (*d*) *Thermal hysteresis*

The phenomenon of several degrees difference in temperature between the melting and freezing points of isolated insect haemolymph was first thought to be part of the water conserving mechanism in *Tenebrio* larvae (Ramsay 1964). It was later recognized as an insect antifreeze mechanism in which the freezing point is depressed whereas the melting point temperature remains unaffected (Duman *et al.* 1982). The causative agents are macromolecular antifreezes circulating in the blood, which are similar to the antifreeze proteins and glycoproteins of polar marine fishes. Thermal hysteresis proteins in insects have more hydrophilic amino acids and lower alanine contents than those in fishes. They are found in both freezing tolerant and intolerant species, and the occurrence of thermal hysteresis has been reported in about 25 species of arthropods including spiders, a centipede and a mite (Block & Duman 1989). The phenomenon may be present for part or whole of the year in field populations. Where THPs are of seasonal occurrence, temperature and photoperiod are the most important environmental cues used to trigger their production and loss (Horwath & Duman 1983).

A study by Duman (1984) of the overwintering larvae of a cucujid beetle in northern Indiana showed more than three degrees of thermal hysteresis (table 2). However, comparison of two winter samples, in 1979 and 1983 showed that whereas most physiological cold hardiness parameters were the same, SCPs and hence the amount of undercooling (table 2) were markedly different. The individuals of the population in 1979 were freezing tolerant but freezing intolerant in 1983. INAs were not detected in the latter sample, but this was not thought to be the only critical factor in switching from freezing tolerance to susceptibility. A similar switch has been documented for larvae of the pyrochroid beetle *Dendroides canadensis* (Horwath & Duman 1984). Such results are intriguing and suggest that a more critical approach should be adopted in evaluating data for survival after exposure to freezing conditions, and for the effect of cooling rate on SCPs and thereby the assessment of freezing tolerance or intolerance in arthropods.

TABLE 2. COMPARISON OF SELECTED PHYSIOLOGICAL PARAMETERS OF COLD TOLERANCE IN TWO SAMPLES OF OVERWINTERING LARVAE OF THE BEETLE *CUCUJUS CLAVIPES*

(Data from Duman (1984). Values are mean ± standard deviation except for lower lethal temperatures and undercooling.)

| parameter | 2 Feb. 1979 | 16 Feb. 1983 |
|---|---|---|
| haemolymph melting point (°C) | −3.04 ± 0.89 | −2.58 ± 0.57 |
| haemolymph freezing point (°C) | −6.93 ± 1.18 | −5.71 ± 1.50 |
| thermal hysteresis (°C) | 3.89 ± 1.52 | 3.13 ± 1.12 |
| whole body supercooling point (°C) | −10.7 ± 1.7 | −30.1 ± 2.3 |
| undercooling (°C)† | 3.8 | 24.4 |
| lower lethal temperature (°C) | −26 | −30 |

† Haemolymph freezing point minus supercooling point.

Antifreeze proteins are thought to function in a non-colligative manner, and they do not generate the high osmotic pressures associated with other solutes such as polyols. Nor do they interfere with mainstream metabolic pathways. They are thought to function by adsorption-inhibition, binding onto embryo ice crystals and preventing their growth. In spring, they may




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

provide a source of amino acids for egg production, growth and moulting processes, which could be particularly important for arthropods in low temperature environments. Finally, it has been suggested that THPs function to stabilize supercooling in insects over their entire temperature range (Zachariassen & Husby 1982).

### (*e*) *Water*

The role of water is central to many aspects of arthropod cold tolerance, influencing as it does the activity of INAs, the antifreeze THPs and polyols, as well as being bound to macromolecules. Although no studies have linked seasonal fluctuations in body water content with cold tolerance parameters of any terrestrial arthropod, the effects of dehydration on micro-arthropods should not be ignored. The springtail *C. antarcticus* can sustain a reduction in total body water content from 60 to 40% of fresh weight (representing a fivefold increase in solute concentration asuming no osmoregulation). It also has a remarkable capacity to take in water when dehydrated with striking effects on its cold tolerance, as shown by its supercooling ability (figure 8) (Cannon *et al.* 1985). By comparison, the Antarctic mite *A. antarcticus* is very resistant to desiccation, but a 20% reduction in live weight (equivalent to a water content of less than 60% of fresh weight) by drying led to a significant accumulation of glycerol (Young & Block 1980). A possible explanation is that cell dehydration produces an increase in ionic concentration, which may inhibit a particular enzyme system and alter the carbon pathway.

Little is known of the distribution of water within the insect body and of its compartmentalization during supercooling and subsequent freezing. It is instructive to examine the relative quantities of body water that freeze when freezing tolerant and intolerant

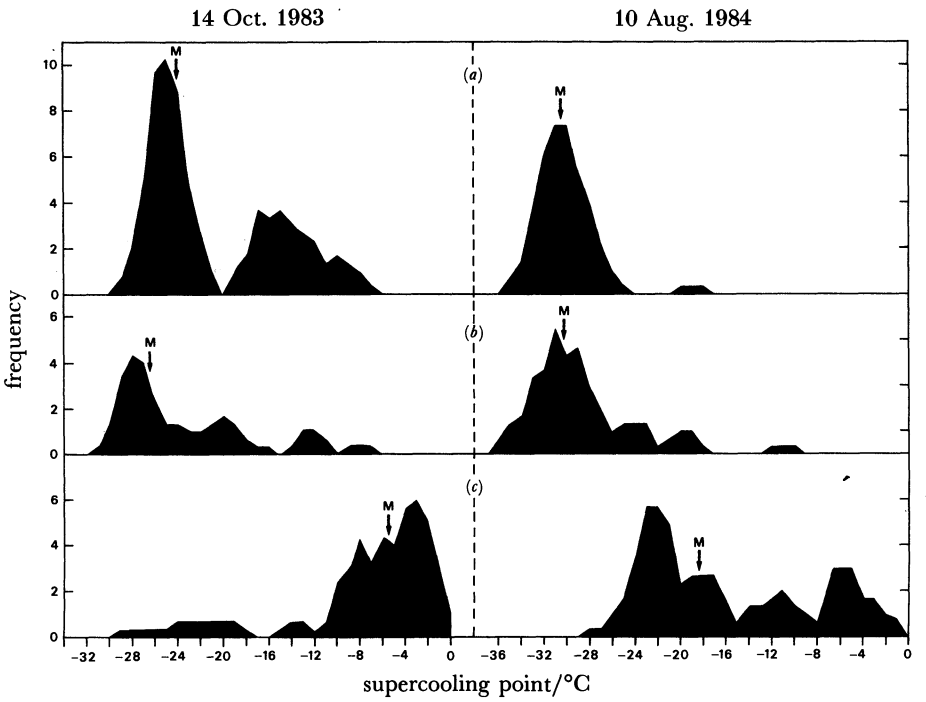


FIGURE 8. Effects of water uptake on the supercooling point distributions of two samples of *Cryptopygus antarcticus*: 14 October (early spring) and 10 August (winter). (*a*) Field-fresh; (*b*) dry; (*c*) insects allowed access to distilled water; M shows the position of the median supercooling point in each sample. (After Cannon *et al.* (1985).)

specimens are cooled under standard conditions. This has been undertaken for two Antarctic arthropods: the adult mite *A. antarcticus* (0.21 mg live weight, 66% water content) and the larva of a midge *Eretmoptera murphyi* (1.76 mg live weight, 74% water content) (W. Block & P. M. Harrisson, unpublished data). The former is intolerant, whereas the latter is tolerant to freezing (Block *et al.* 1980). A cooling rate of 1 °C $min^{-1}$ was used in a differential scanning calorimeter (DSC 700, Stanton Redcroft). Calculations based on the energy released during the freezing exotherms (figure 9) showed that almost 80% of the water froze in the mite, but only 57% froze in the midge, which is predicted on theoretical grounds. The use of calorimetric techniques to determine the amount of frozen water after supercooling in insects is a new approach. As Zachariassen (1988) stated 'The physiology of hibernating insects is to a great extent the physiology of body water at low temperatures.' He attempted to synthesize information on supercooling of both freezing tolerant and intolerant insects and to relate these data to haemolymph osmolality (figure 10), thereby highlighting the central role of water, ice nucleating agents and polyols such as glycerol in the ecophysiology of cold-adapted arthropods.

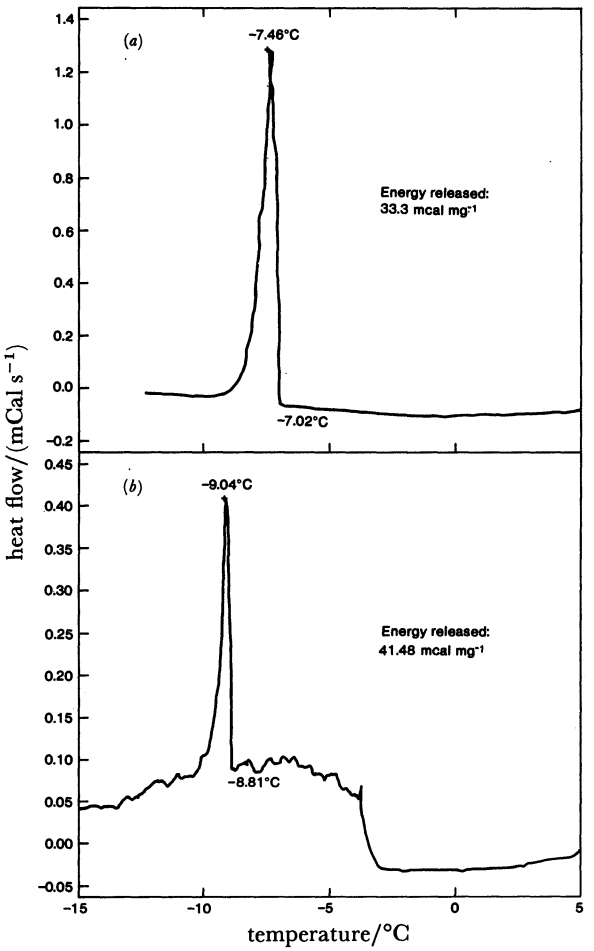


FIGURE 9. Exotherms produced during freezing of (*a*) a freeze tolerant insect (larva of the chironomid *Eretmoptera murphyi*), and (*b*) a freeze intolerant mite (adult *Alaskozetes antarcticus*), utilizing a low temperature differential scanning calorimeter (Stanton Redcroft).

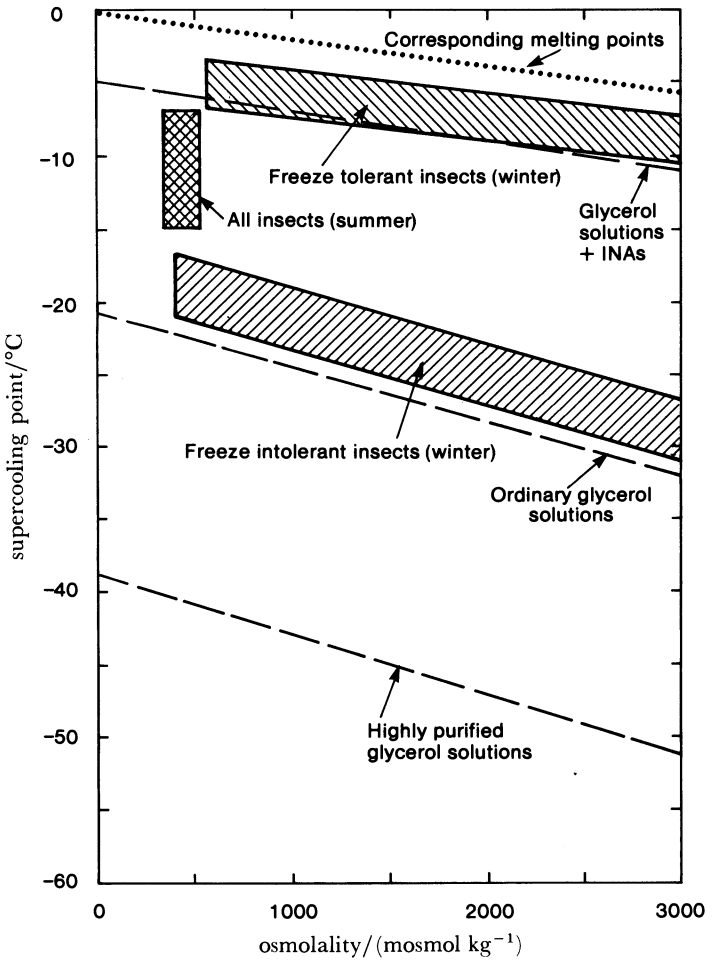


FIGURE 10. The relation between the supercooling points of whole insects and the osmolality of their body fluids compared with aqueous solutions of glycerol. Shaded areas show the positions of freeze tolerant and intolerant species in winter and summer. INAs show the presence of ice nucleating agents from the haemolymph of a freeze tolerant insect. (Modified from Zachariassen (1985)).

## CONCLUSIONS

A full understanding of the complexity of low temperature adaptations of insects and other terrestrial arthropods depends upon the integration of information from field and laboratory studies: integration of ecological data of the effects of low temperatures on locomotory activity, energetics, respiratory metabolism and development with physiological data for cold hardiness, including both freezing tolerant and intolerant species. In particular, the interplay of the three principal mechanisms underlying cold hardiness, supercooling, INAs, THPs and other antifreezes require investigation in appropriate species. The arthropod communities of polar and alpine habitats offer considerable scope in this respect (Sømme & Block 1990).

Data on cold hardiness require validation against the field situation so as to have ecological relevance, and few studies have attempted this in the past. Recent studies relating the cold hardiness of overwintering eggs of the autumn moth (a major defoliator of mountain birch) to topoclimate and outbreak damage in northern Fennoscandia by Tenow & Nilssen (1990) are an important contribution. However, the concerns of Baust & Rojas (1985) and Bale (1987)

are well founded and demand attention. In laboratory cold hardiness experiments, the rates of cooling and rewarming may be critical for arthropod survival, and hence the assessment of their freezing tolerance or intolerance. In this connection, we need to achieve a better understanding of cold survival, not just after the cooling or freezing experiment but in the short and long term. In the short term, the ability to recover from cold exposure and to resume normal activity is paramount, and the long-term requirement is for a capacity to develop, mature and complete the life cycle, preferably under field conditions. On present evidence, there is no typical cold-hardy insect or arthropod, and a generally applicable theory of cold hardiness must remain a future goal. In some respects, insect cryobiology has suffered because its protagonists have reached for generality prematurely.

Storey (1984) distinguished between adaptations for low temperature function and low temperature preservation in poikilothermic animals. Cold climate arthropods have evolved a diversity of both types of adaptations, and as yet, only a few have been studied. There has been much research concentrated on the effects of arthropods of a single process, freezing, albeit an important process in biological systems. The urgent requirement now is to place the results of supercooling and freezing studies *per se* in the wider context of both sub-zero and low temperature adaptational strategies found in arthropods.

I thank many of my Antarctic colleagues for helpful discussions and for collaboration in studies of arthropod cold tolerance, in particular Roger Worland, Ray Cannon, Paul Harrisson and Peter Rothery. The support of the British Antarctic Survey has been invaluable. I gratefully acknowledge the technical assistance provided by Agema Infrared Systems and Stanton Redcroft Limited for some of the experiments.

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Plate 1 was printed by George Over Limited, Rugby, U.K.

*Discussion*

J. G. Baust (*Center for Cryobiological Research, State University of New York, U.S.A.*). Dr Block's reference to Duman's description of the punctuate change in the overwintering strategy in an insect species from that of freezing tolerance to intolerance over one summer remains a fascinating incongruity, and causes me to raise the problem we face in understanding whether an insect is or is not freeze tolerant, as few studies of survival include cooling and warming rate optima. *Eurosta* is only freeze tolerant when exposed to certain optimal rates. Miller identified the critical nature of cooling rate optima in one insect where a rate of variation of 0.03 °C $min^{-1}$ made the difference between 100% and 0% survival. How then can we be confident that species described as intolerant are really not tolerant in nature and vice versa?

W. Block. Duman's data (see reference list) for the larvae of the beetles *Dendroides canadensis* and *Cucujus clavipes*, showing the probable switch of cold tolerance strategy is certainly intriguing, particularly as such events are uncommon. I agree wholeheartedly that more attention should be paid to evaluating the effects of cooling rate (and warming rate) on the long term survival of insects after freezing experiments. More importantly, we should experiment more with those cooling rates that are known to occur in the habitats in which the insect lives. At present, therefore, we can only describe a species as being tolerant or intolerant of freezing under the defined conditions of the laboratory experiment.

F. Franks (*Pafra Ltd, Cambridge, U.K.*). All cell types investigated in our laboratory show INA activity. The activity becomes effective at different temperatures. Thus, human red blood cells, and even ghosts, are able to catalyse ice nucleation only near −38 °C, whereas *Pseudomonas syringae* are more potent catalysts, becoming effective at −4 °C. INA is therefore a manifestation of cell catalytic efficiency.

W. Block. I agree with Dr Franks that ice nucleator activity may be a manifestation of cell catalytic efficiency in some biological systems.

I. A. Johnston (*Gatty Marine Laboratory, St Andrews, U.K.*). I was interested in Dr Block's figure of metabolic rate versus temperature for Antarctic mites and various temperate arthropods, which showed 2–4 times higher values in the former. Were these values for routine or resting metabolic rates? To what does Dr Block attribute higher rates in the Antarctic species?

W. Block. The metabolic rates described were derived from data for individual mite respiration measured while the animal was at rest. The elevated metabolic rates at low environmental temperatures can be attributed to translation of the metabolic rate–temperature




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

curve downwards to the low end of the temperature scale, showing that the Antarctic species is adapted to living in constantly colder conditions than the comparable temperate oribatid species.

F. Franks. I cannot believe that water content activates nucleators. The nucleation rate is a function of the volume of an aqueous compartment as well as of the temperature. This is true for homogeneous as well as for catalysed nucleation processes.

W. Block. My comments about water and ice nucleators were specifically related to the effects observed in dehydrated springtails collected from the field during the Antarctic winter, and exposed to a moist atmosphere and allowed to take in liquid water. Although their body water contents were low, it was not implied that it was water content *per se* that activated the nucleation process.

F. Franks. May I make a plea for the standardization of terminology and suggest that the term antifreeze protein which was coined by fish physiologists during the 1960s describes the phenomenon of nucleation inhibition much more clearly than thermal hysteresis protein (THP) which was used much later by insect physiologists to describe the same phenomenon.

J. Bale (*Department of Pure and Applied Biology, University of Leeds, U.K.*). I strongly endorse Dr Block's recommendations that laboratory ideas on insect cold tolerance should be validated by field studies; and that there should be a critical evaluation of the use of the supercooling point as an indicator of cold hardiness, but I would suggest another important requirement: the need for comparative studies across different climatic zones. The principles of insect cold hardiness have been derived from studies in a relatively small number of species in the harshest of climates, especially the polar regions. As we move from the poles through the sub-arctic to the temperate climate, the environmental stress of the winter season is less, and so we find that the so called 'general principles' are not so applicable: there are fewer if any freeze tolerant species and some insects die before they freeze. We have to consider the effects of this continuum of changing climate rather than specific, very cold zones.

W. Block. I agree that a more comparative approach to insect cold hardiness is necessary across wide climatic zones, especially to overwintering survival and lethal temperatures.

J. Bale. Professor Baust has drawn attention to the crucial importance of cooling (and warming) rates. Usually, in insect cold hardiness research we cool insects at 1 °C $min^{-1}$. Studies have shown that changes in cooling rate do not have a marked effect on the temperature at which an insect freezes, but this does not mean that differences in cooling rate have no effect on the ability of an insect to survive the freezing process. In a paper shortly to be published, it will be shown that cooling the freeze tolerant larvae of *Eurosta solidaginis* at 10, 5, and 0.1 °C $min^{-1}$ to −40 °C has a marked effect on the survival of the insect to the adult stage; 1 °C $min^{-1}$ is not the optimum rate of cooling, raising the idea that the rate at which an insect is cooled (frozen) will determine its tolerance or intolerance of freezing. In practice I think it will be easier to make a freeze tolerant insect intolerant at sub-optimal rates of cooling, rather than a freezing intolerant insect into a tolerant one, largely because unless the rates used are




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

very different, the temperature of ice formation in the freezing intolerant insect will be below −20 °C or even −30 °C and this, when freezing occurs, it will be very rapid. In freezing intolerant insects, freeze tolerance can only be conferred by artificial induction (seeding) of freezing above −5 °C.

W. Block. As stated in answer to Professor Baust's first question, cooling rates are one of the most important parameters in survival or non-survival of both freezing and potentially freezing temperatures in insects. It would be a most interesting experiment to attempt to change a freezing tolerant individual to a freezing intolerant one, and furthermore, to determine if similarly acclimatised individuals show such features in the next generation. It would probably be easier to switch from being freezing tolerant to intolerant rather than the reverse, as Dr Bale says.





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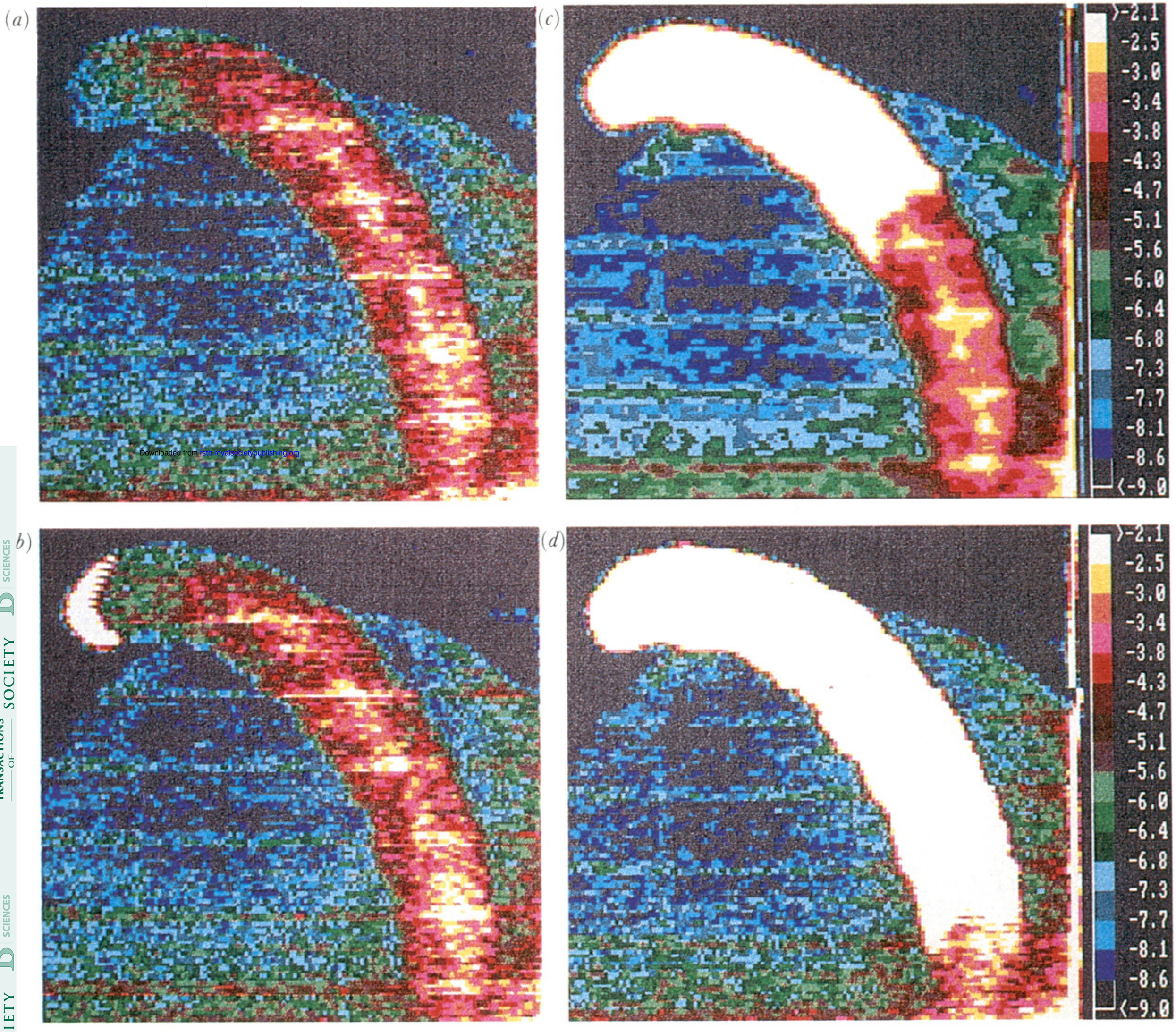


FIGURE 7. Thermographs showing the surface temperature patterns developed during freezing (*a–d*) of a larva of the tobacco hornworm (*Manduca sexta*) by using an infrared scanning system (Agema). The specimen was inoculated at the anterior end (upper left of the picture), and the freezing front can be followed as high temperatures are recorded because of the release of latent heat from the insect.