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Factors that influence freezing in the sub-Antarctic springtail *Tullbergia antarctica*

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Abstract

Effects of 12 biotic and abiotic factors on the freezing point of the sub-Antarctic springtail, *Tullbergia antarctica*, were investigated. Repeated cooling of individual springtails five times resulted in very similar freezing points suggesting that ice nucleation in this freeze-susceptible species is likely to be initiated by intrinsic factors rather than being a stochastic event. Mean supercooling point (SCP) was influenced by cooling protocol, showing a linear increase in mean SCP with cooling rates from 8 to 0.1 °C min^{-1}. However, the opposite effect (decreasing SCP) was seen with slower cooling. Slower rates may be ecologically realistic and allow time for appropriate physiological and biochemical changes. Feeding and food presence in the gut had no effect on SCP, and there was no correlation between the ice nucleating activity of bacteria isolated from the guts and the whole springtail SCP. Habitat altitude and diurnal light and temperature regimes also had no effect on SCP. There was no correlation between the cryoprotectant concentration of fresh animals and their SCP, but experimental desiccation resulted in increased osmolality and decreased SCP, although with considerable individual variation. The most significant influence on SCP was associated with ecdysis. As springtails cease feeding for a period either side of ecdysis, shedding the entire gut lining, moulting may be an efficient mechanism of clearing the gut of all ice nucleating material. This previously unrecognised relationship between ecdysis, cold tolerance and seasonal survival tactics may play an important role in over-winter survival of some arthropods.

Keywords: Cold tolerance; Cooling rate; Desiccation; Differential scanning calorimeter; Feeding; Freezing; Gut contents; Moulting; Rapid cold hardening; Starvation; Supercooling point

1. Introduction

The ability of insects to survive sub-zero temperatures is among the most researched of their remarkable adaptations enabling them to inhabit harsh environments. One of the most fundamental measurements used to assess cold tolerance is the temperature at which the insect's body fluids freeze, normally termed the supercooling point or SCP. This measurement, in conjunction with pre-freezing survival studies, allow the animal to be classified as either freeze avoiding or freeze tolerant (Block, 1991) or further subdivisions (Bale, 1993; Sinclair, 1999). Further detailed studies often reveal that the insect's cold-tolerance changes with season. Freeze avoiding insects normally have SCPs of between −5 and −10 °C whilst they are active and feeding during the summer. However, as winter approaches and environmental conditions become more severe, they may undergo a cold hardening process which typically results in a depression of the SCP to below −20 °C. With a few exceptions (Ring, 1982), freeze tolerant insects normally maintain a high freezing point to minimise the risk of tissue damage during the freezing process (Worland et al., 2004). This is achieved by retaining ice nucleating agents (INAs) within the body. INAs may be specialised proteins, or simply bacteria, which occur naturally in the gut flora. The SCP of a whole insect is determined by the probability of nucleation at a given temperature and is influenced by both time and

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temperature. It is also dependent on the nature and concentration of nucleating agents and the amount of liquid water in contact with them.

Freeze avoiding animals utilise three important mechanisms to reduce their SCP: (i) increase the solute concentration of body fluids, either by the synthesis of low molecular weight organic compounds (cryoprotectants) or by a reduction in water content, (ii) remove ice nucleators from the body which would otherwise catalyse the formation of ice, (iii) synthesise antifreeze compounds which delay the growth of ice crystals. Cold hardening may occur rapidly over a few hours (Worland and Convey, 2001) in some Antarctic species and may even change diurnally (Sinclair et al., 2003a). In species such as the Antarctic springtail *Cryptopygus antarcticus*, this cold hardening effect results in a biomodal distribution of their SCPs reflecting cold-hardened and non-hardened elements of the population (Block, 1990). The production of cryoprotectants such as glycerol have been shown to increase substantially in some species such as the flesh fly *Sarcophaga crassipalpis* (Lee et al., 1987), and it is conceivable that an insect may be able to quickly evacuate its gut. However, there is little evidence that either of these mechanisms is entirely responsible for rapid cold hardening. It is more likely that a suite of factors acting together determine the SCP.

In a preliminary study to examine the cold tolerance of the springtail *Tullbergia antarctica*, it was found that repeated freezing of the same individual produced remarkably similar SCPs (this paper). It can be argued that such measurements have little relevance to the survival of the springtail as, being freeze avoiding rather than freeze tolerant, it dies the first time it freezes. However, the fact that the freezing point of an individual remains similar during such repeated freeze thaw cycles indicates that freezing of the whole animal is not a stochastic event, but regulated by intrinsic factors. Prior to the initial freezing event, the animal's cold tolerance is determined by extrinsic factors, both biotic and abiotic.

The aims of this study were to assess the effects of several factors which are considered to influence the SCP of freeze avoiding insects and to determine the environmental conditions which trigger each response in a sub-Antarctic springtail.

Factors studied:

(i) Effect of repeated freezing.
(ii) Effect of experimental cooling rate.
(iii) Effect of habitat altitude (proxy for environment severity).
(iv) Rapid cold hardening.
(v) Effect of feeding.
(vi) Effect of gut contents.
(vii) Ice nucleating activity of gut contents.
(viii) Effect of moulting.
(ix) Effect of diurnal changes in light intensity (day length).
(x) Effect of diurnal changes in temperature.
(xi) Accumulation of cryoprotectants.
(xii) Effect of water content (desiccation).

These factors have all been implicated in the literature as having some effect on the cold tolerance of arthropods. Some, such as gut contents, are more controversial than others (Baust and Rojas, 1985). Although there have been many studies which have examined cold tolerance of arthropods in general, few studies have attempted to consider the effect of a wide range of factors on a single species.

2. Materials and methods

2.1. Study animal and collection site

T. antarctica (Lubbock) is a comparatively large white springtail weighing up to 0.75 mg and ca. 2 mm in body length. Its large size enabled cryoprotectant analyses to be made on samples of about 10 individuals (total of ca. 5 mg) and its semi-transparent cuticle allows examination of the gut contents of individuals without dissection. The lack of a furcula (springing organ) also facilitates handling live samples. The springtail is widely distributed in soil, moss and humus on Kerguelen and has been reported from Iles Crozet, (Dehaveng, 1981; Greenslade, 1986). With the exception of samples collected for the altitudinal study, springtails were extracted from leaf litter collected from around the base of the Kerguelen cabbage (*Pringlea antiscorbutica*) on the slopes of Mount Crozier (S 49.17°, E 70.02°) on Kerguelen.

The Kerguelen archipelago lies in the sub-Antarctic area of the Southern Indian Ocean on the Antarctic Convergence (Fig. 1) where upwelling cold water from the Antarctic mixes with the warmer waters of the Indian Ocean. This results in a cooler climate than would be expected for this latitude with an annual mean temperature of 4.6 °C (Météo-France, 1951–1999 records) and very strong winds of up to 200 kph. There are no distinct spring, or summer periods with monthly mean temperatures ranging between 2.1 and 7.7 °C (Frenot et al., 1998). The diversity of terrestrial arthropods is relatively poor but Dehaveng (1981) recorded 30 species of Collembola, 14 of which are endemic to the Kerguelen region.

Samples for this study were hand sorted using an aspirator from material returned to the laboratory and then stored until required in damp moss at 5 °C with a 12 h, low-level light regime. Specimens for the altitudinal study were collected by hand using an aspirator from the interface between moss and rocks along the edge of a