

Variation in summer cold-hardiness of the Antarctic oribatid mite *Alaskozetes antarcticus* from contrasting habitats on King George Island

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Summary. The Antarctic oribatid mite *Alaskozetes antarcticus* was collected from several field habitats near Great Wall Station (62°13'S, 58°58'W) on King George Island during January and February 1990. The tritonymphs and adults were examined for their supercooling ability and survival at subzero temperatures in relation to inoculative freezing. The active tritonymphs and adults showed a wide range of supercooling points probably due to their polyphagous feeding activity and humid habitat conditions, with means ranging from -3.8° to -22.4° C. Detritivores were inferior to algivores in their supercooling ability. The former seemed to be transiently exposed to the hazard of freezing during the cool Antarctic summer. The resting (premolting) tritonymphs exhibited the lowest mean supercooling point of -28.3° C. Inoculative freezing reduced the survival of *A. antarcticus*. Its effect became conspicuous at temperatures below -20° C and was serious in the deeply supercooled individuals, such as resting tritonymphs and algivorous adults. During the active season, spontaneous freezing probably started from the gut contents seemed to be more fatal than inoculative freezing for this freeze intolerant species.

Introduction

Among the terrestrial arthropods living in the Antarctic, the oribatid mite *Alaskozetes antarcticus* is a very common and abundant species. It has been recorded from the continental and maritime Antarctic, especially from the Antarctic Peninsula and the adjacent islands (Wallwork 1967). Probably because of its abundance, this oribatid mite has been used as typical material for investigating the mechanism of cold-hardiness adopted by Antarctic terrestrial arthropods (eg. Block 1984).

Block et al. (1978) found that *A. antarcticus* is intolerant to freezing but possesses the ability to supercool to -30° C and the realization of this potential is influenced by starvation. Desiccation and cold-acclimation also act

to increase the supercooling ability with a concomitant accumulation of glycerol, while photoperiod has no effect (Young and Block 1980; Block and Sømme 1982). In addition to these experimental results, Lee and Baust (1981) observed that the mean supercooling points of this species in the field at Palmer Station, Anvers Island (64°46'S, 64°03'W) dropped from -11.0° to -29.2° C with a decline of microhabitat temperatures during late summer from February to the end of March: the supercooling points were substantially lower than the ambient temperatures. In the above studies, the interest was focused on the potential of cold-hardiness and its realization under experimental and natural conditions.

However, individual supercooling points of *A. antarcticus* collected in summer at various sites on Signy Island, maritime Antarctic (60°43'S, 45°36'W) largely fluctuated between the low (below -20° C) and high (0° to -20° C) groups of a bimodal distribution (Block and Sømme 1982; Cannon 1986a,b, 1987). The fluctuation is thought to reflect different diets and micro-climates (Cannon 1987). In the present study, we examined this assumption by comparing supercooling points of mites collected in summer from several contrasting habitats on King George Island (62°13'S, 58°58'W), in the maritime Antarctic. In addition, the effect of inoculative freezing on their survival, the possibility of which was once excluded (Cannon 1987), was re-examined over a wide range of subzero temperatures. Through these experiments we aimed to estimate the importance of dietary constituents in natural habitats to the cold tolerance of the active stages of this Antarctic oribatid mite.

Materials and methods

Materials

Tritonymphs and adults of *Alaskozetes antarcticus* (Cryptostigmata: Podacaridae) were collected from soil surfaces, beneath rocks (Fig. 1a), moss patches (Fig. 1b) and algal (*Prasiola crispa*) covered areas (Fig. 1c) near Great Wall Station (62°13'S, 58°58'W) on King

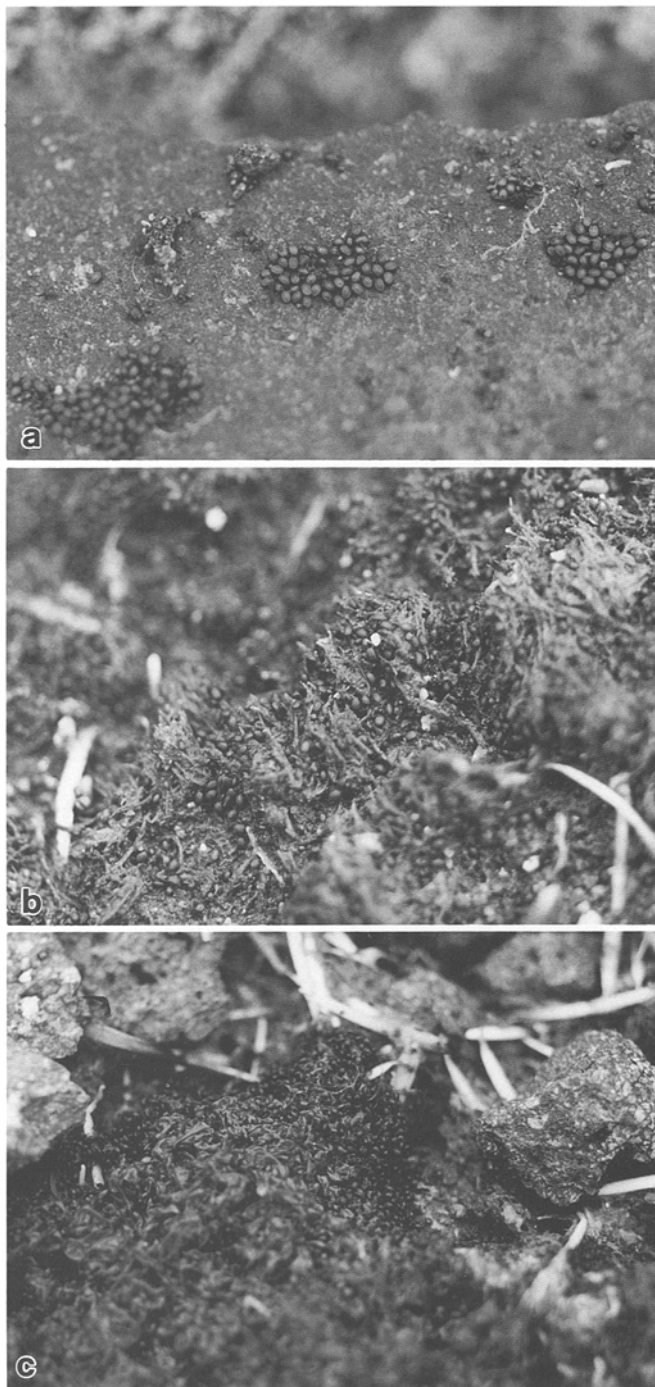


Fig. 1a-c. *Alaskozetes antarcticus* and its microhabitats on King George Island, 1990 summer. **a** clumps of resting nymphs and some wandering adults on the undersurface of a small rock; **b** mites inhabiting a moss patch fertilized by sea birds; **c** mites crowding on green foliose algae (*Prasiola crispa*)

George Island, South Shetland Islands during January and February 1990. These collection sites were nutrient enriched by sea birds with guano and cast feathers. The mites on soil surfaces and moss patches were distinguished into detritivores although their gut contents were not analyzed; those on green algae were termed algivores. Collected mites were confined to plastic bags or tubes with their habitat

materials and maintained outside (-1.5° to 7.5°C) under the floor of the station. They were used for experiments either on the day of collection or not later than four days after collection.

Supercooling points

Supercooling points were determined using conventional methods (Salt 1966). Three mites were attached to the fine tip (0.2 mm in diameter) of a copper-constantan thermocouple with a spot of silicone grease. To prevent desiccation during cooling, the tip of the thermocouple was enclosed in a glass vial (20 mm in diameter, 45 mm high) with a wet filter paper (20 mm in diameter, soaked with 0.1 ml distilled water) at the base, and suspended in an air-filled thermos flask. The flask was placed in a freezer (Ebara, ECS-190) at -50°C . At one time, 15 to 30 mites in separate vials were cooled to -30°C or below at rates between $0.06^{\circ}\text{C}/\text{min}$ (at -30°C) and $0.2^{\circ}\text{C}/\text{min}$ (at 0°C). The cooling curves were monitored by a portable 12-channel recorder (Yokogawa 3087). Each supercooling point was detected as the initiation of an exotherm due to ice formation within the animal's body.

Distributions of supercooling points displayed a variety of shapes with unimodal or bimodal peaks. The variations were compared statistically in terms of the high (supercooling points $> -20^{\circ}\text{C}$) and low (supercooling points $\leq -20^{\circ}\text{C}$) groups (Block and Sømme 1982; Cannon 1986a). The distribution of mites in the two supercooling-point groups was expressed as the ratio $R = \text{LG}/(\text{LG} + \text{HG})$, where LG and HG are the numbers of individuals in the low group and the high group, respectively. The mean supercooling point of the low group was expressed as \bar{x}_L ; the high group, \bar{x}_H ; the total, \bar{x}_T (all means were expressed with standard deviations). The difference between the means was statistically analyzed with *t*-test.

Survival tests

In each survival assay, 20 mites were placed on a wet (soaked with 0.1 ml distilled water) or dry filter paper (20 mm in diameter) in a glass vial (20 mm in diameter, 45 mm high). A copper-constantan thermocouple connected to a multi-channel recorder (Yokogawa 3087) was inserted into the vial to monitor temperature. As with the measurement for supercooling points, the vial was suspended in an air-filled thermos flask and cooled very slowly in a freezer (Ebara, ECS-190) to a subzero temperature between -5° and -30°C . The frozen (or supercooled) mites were rewarmed from the test temperatures without delay at a rate between 5° and $10^{\circ}\text{C}/\text{min}$. One day after rewarming to room temperature (about 18°C), the survival was judged by locomotive activity. The mites walking normally were classed as surviving. Mites which had lost their walking ability or only showing faint movements of the appendages were classed as dead.

Results

Supercooling points

A. antarcticus varied in frequency distribution of supercooling points according to the developmental stages, collection sites and habitat conditions, especially wetness. The resting (premolting) tritonymphs collected from beneath rocks showed the lowest mean supercooling point of $-28.3^{\circ} \pm 2.4^{\circ}\text{C}$ (Fig. 2a). The individual supercooling points ranging from -20.0° to -30.8°C wholly distributed in the low supercooling-point group ($R = 1$). By contrast, in the active tritonymphs on soil surfaces, a

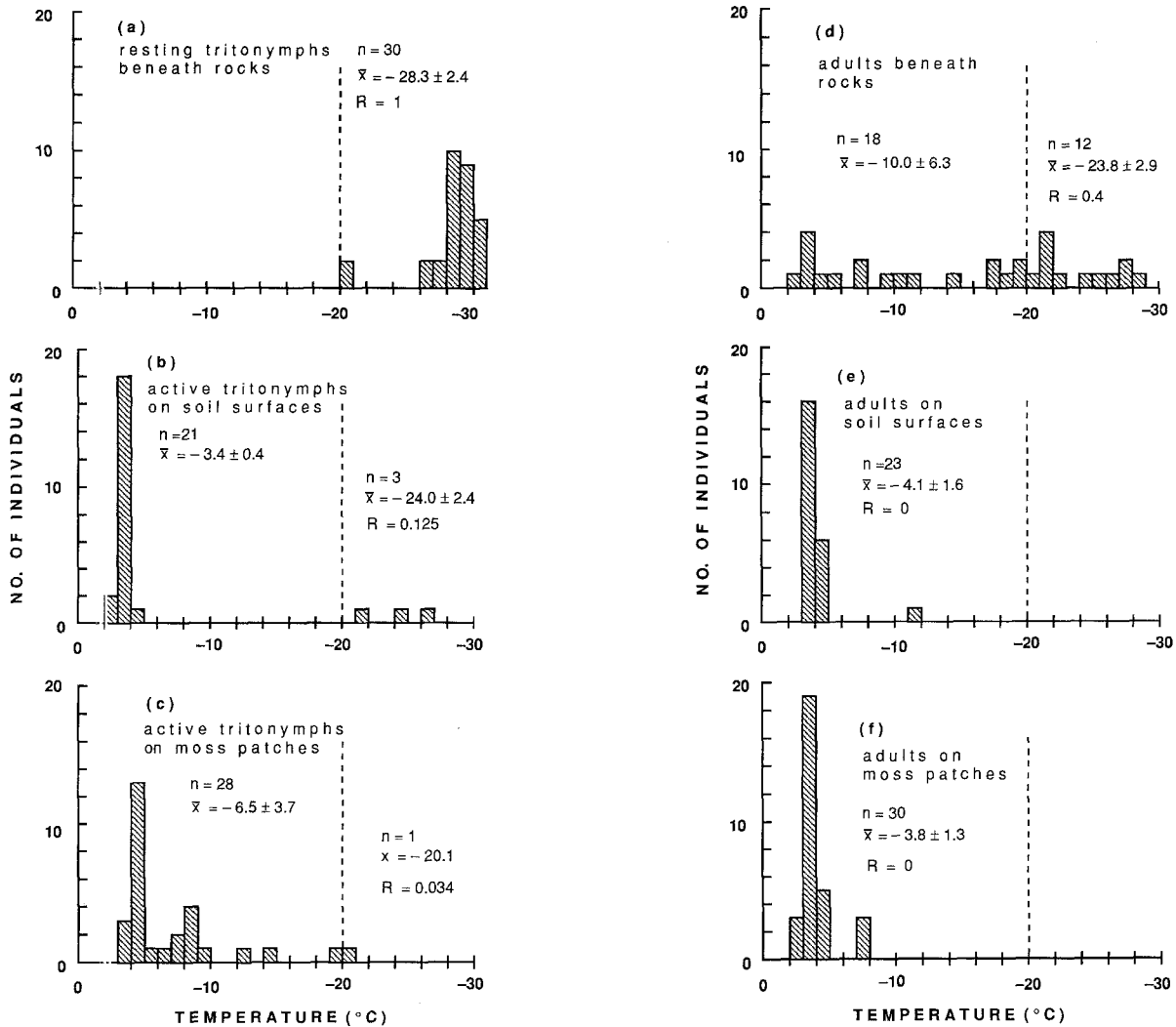


Fig. 2a-f. Frequency distributions of supercooling points of *Alaskozetes antarcticus* collected from three different sites. **a** resting tritonymphs from beneath rocks; **b** active tritonymphs from fertilized soil surfaces; **c** active tritonymphs from fertilized moss patches; **d** adults from beneath rocks; **e** adults from fertilized soil surfaces;

f adults from fertilized moss patches. Supercooling points are divided at -20°C into the low (*LG*) and high (*HG*) groups. $R = LG/(LG + HG)$. \bar{x} represents the mean and standard deviation of supercooling points in each divided group

majority of the supercooling points were distributed in the high supercooling-point group and concentrated around -3°C ($\bar{x}_H = -3.4 \pm 0.4$) [Fig. 2b] although three individuals supercooled below -21°C ($R = 0.125$). Supercooling points of active tritonymphs on moss patches were also distributed in the high supercooling-point group except one individual ($R = 0.034$) [Fig. 2c] and nearly half of them ranged above -5°C . However, the \bar{x}_H (-6.5 ± 3.7) was lower than that of the samples from soil surfaces and the difference between the two means was significant (t -test, $P < 0.001$).

Adult mites collected from beneath rocks, some of them freshly moulted, varied in their supercooling ability in contrast with the tritonymphs at the same site. Their individual supercooling points ranged between -2° and -29°C and showed neither the unimodal- nor the bimodal- distribution ($\bar{x}_T = -15.5 \pm 8.6$) [Fig. 2d]. Supercooling points of adults on soil surfaces and moss patches,

on the other hand, were entirely in the high supercooling-point group and concentrated around means of $-4.1^{\circ} \pm 1.6^{\circ}$ and $-3.8^{\circ} \pm 1.3^{\circ}\text{C}$, respectively (Fig. 2e, f). The difference between these means was not significant (t -test, $0.1 < P < 0.5$). However, there was a small but significant difference in the mean supercooling points between the active tritonymphs and adults at the same collection site: for the high group, $0.02 < P < 0.05$ in the samples from soil surfaces; $P < 0.001$ in those from moss patches.

Even for adult mites from the same collection site, the frequency distribution of supercooling points changed with the humidity of their habitat and/or dietary constituents. Many mites collected on a fine day (7 Feb. 1990) on semi-dried green foliose algae had an ability to supercool below -20°C ($\bar{x}_T = -22.4 \pm 5.9$, $R = 0.75$) [Fig. 3a]. In those on wet green algae in the same area after rainfall (9 Feb. 1990), the supercooling ability clearly shifted towards

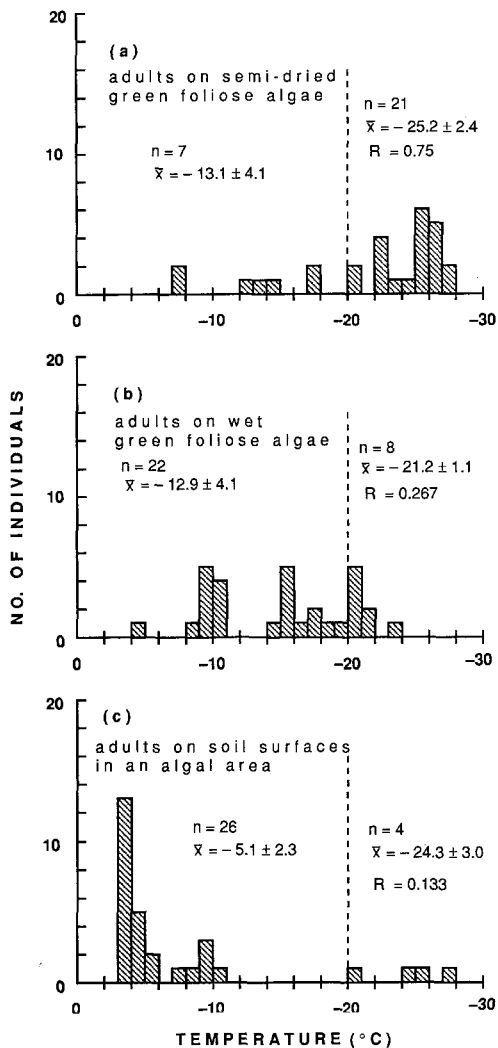


Fig. 3a-c. Frequency distributions of supercooling points of *Alaskozetes antarcticus* from an algal covered area. **a** adults from semi-dried green foliose algae; **b** adults from wet green algae after rainfall; **c** adults from soil surfaces around green algae

the high supercooling-point group ($\bar{x}_T = -15.1 \pm 5.1$, $R = 0.267$) [Fig. 3b]. Furthermore, the mites on the soil surface adjacent to the algal-covered area froze at relatively high subzero temperatures ($\bar{x}_H = -5.1 \pm 2.3$, $R = 0.133$) [Fig. 3c]. They seemed to be feeding on detritus instead of green algae.

Survival of subzero temperatures

An effect of inoculative freezing on the reduction in supercooling ability was detected in resting tritonymphs from beneath rocks and in the adults from semi-dried green foliose algae (Fig. 4a, c). Their mortalities in dry conditions at subzero temperatures increased along the cumulative frequency distribution curves of supercooling points, indicating that the mites survived at subzero

temperatures until they froze. On the other hand, mortalities in wet conditions at the subzero temperatures examined were almost always higher than the values expected from the cumulative frequency distribution curves in these samples (Fig. 4a, c). The increase in mortality became noticeable at temperatures below -20°C in both nymphal and adult mites. At -20°C , the mortalities of tritonymphs and adults probably due to inoculative freezing increased to 35 and 30% (whole mortality – cumulative frequency of supercooling points), respectively. The difference in mortality caused by inoculative freezing was not clear between the tritonymphs and adults because of the concomitant occurrence of spontaneous freezing.

No effect of inoculative freezing was observed in active tritonymphs from moss patches and in adults from soil surfaces in an algal area (Fig. 4b, d). Their mortalities in both dry and wet conditions increased in line with cumulative frequency distribution curves of their supercooling points. Because of the distribution of the supercooling points in the high group, the mites were killed by spontaneous freezing probably originated from the gut contents before they were exposed to inoculative freezing conditions.

Discussion

The present study demonstrated the extent of variation in supercooling ability in field-fresh samples of an Antarctic oribatid mite, *Alaskozetes antarcticus*, during the summer season at King George Island. Only the resting (pre-moulting) tritonymphs exhibited a constant ability to supercool to temperatures below -20°C . The active tritonymphs and adult mites, except those on semi-dried green foliose algae, readily froze at temperatures above -20°C . In particular, their supercooling points were concentrated in a narrow range of relatively high subzero temperatures between -2° and -5°C , when they were inhabiting soil surfaces fertilized by sea birds. Among the active mites, adults collected from beneath rocks showed various degrees of supercooling ranging from -2° to -29°C . Those on semi-dried green foliose algae, on the other hand, had relatively deeper supercooling ability, but their lower supercooling points shifted to the high supercooling-point group shortly after rainfall. Such rapid change in supercooling ability under natural conditions has not been observed before. Experimentally, Cannon et al. (1985) demonstrated a loss of supercooling ability in *Cryptopygus antarcticus* (Collembola) for short periods (3 and 7 days) in the presence of water.

Variation in supercooling ability seems to be caused by differences in the feeding activity, dietary constituents and dietary water content. Previous workers (Block et al. 1978; Young and Block 1980; Sømme 1982) have demonstrated that starved mites are superior in supercooling ability to fed individuals. In the present study, it was presumed that the resting tritonymphs attained a constant supercooling ability by cessation of feeding which is equivalent to starvation. On the contrary, active mites seem to reduce their supercooling ability with the resumption of feeding (beneath rocks) and with feeding on ice-nucleator-rich

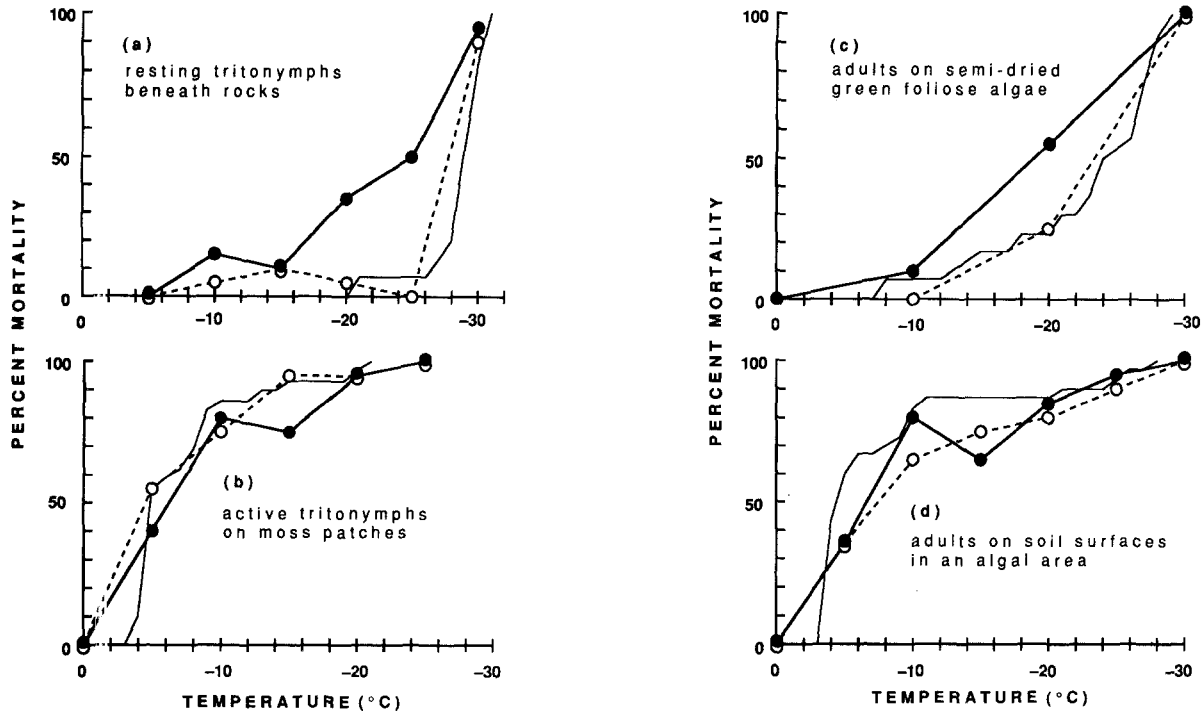


Fig. 4a-d. Mortalities of *Alaskozetes antarcticus* at subzero temperatures. a resting tritonymphs from beneath rocks; b active tritonymphs from fertilized moss patches; c adults from semi-dried green foliose algae; d adults from soil surface in an algal area. Solid circles

and solid lines represent the mortalities in wet experimental conditions; open circles and dotted lines, in dry experimental conditions. Fine solid lines represent the percent cumulative frequency distributions of supercooling points

habitats (fertilized soil surface and moss patches). Wynn-Williams (1985) states that the bacterium *Pseudomonas fluorescens* which occurs in soils on Signy Island has a potent ice-nucleating activity. The same bacterium is likely to be present in fertilized areas on King George Island and its presence and ingestion may reduce the supercooling ability of active mites. As demonstrated by Cannon (1986b), the uptake of water or a wet diet also seems to be related to the reduced ability of *A. antarcticus* to supercool. This is deduced from a difference in supercooling points between mites on semi-dried and on wet-green foliose algae. He suggested that the uptake of liquid reactivates organic nucleators present within the mites.

The present experiments demonstrate that inoculative freezing become effective in reducing the supercooling ability of *A. antarcticus* at temperatures below -20°C . Few mites may be exposed to the hazard of inoculative freezing during the maritime Antarctic summer. Thus the variation in supercooling ability produced by the feeding activity and habitat dietary conditions seems to be important to the summer cold-hardiness of this species. The daily minimum air temperatures at Great Wall Station during the study period occasionally dropped to subzero temperatures close to the supercooling points of mites on soil surfaces and moss patches. The detritivorous mites seemed to be exposed to the hazard of freezing. Block and Duman (1989) demonstrated the presence of haemolymph thermal hysteresis antifreeze proteins in *A. antarcticus* collected from Signy Island. The antifreeze proteins lowered the haemolymph freezing points from -1.37° to

-3.23°C in nymphs and from -1.08° to -2.28°C in adults. The presence of antifreeze proteins may play a key role in protecting detritivorous mites from lethal freezing during the cool Antarctic summer rather than during winter.

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