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# Plasticity and superplasticity in the acclimation potential of the Antarctic mite *Halozetes belgicae* (Michael)

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## Summary

**The plasticity of an organism's phenotype may vary spatially and temporally, and across levels of physiological organisation. Given the adaptive value of plasticity in heterogeneous environments, it might be expected that it will be expressed most in a phenotype's most significant adaptive suites; at high latitudes, one of these is low temperature adaptation. This study examines the phenotypic plasticity of cold acclimation in the Antarctic mite, *Halozetes belgicae* (Michael). Both plastic and 'superplastic' (extreme plasticity) acclimation responses were found. Plastic responses were evident in responses to laboratory acclimation and field acclimatisation. 'Superplasticity' was found in its ability to rapidly cold harden (RCH) at 0, –5 and –10°C. For example, after just 2 h of acclimation at 0°C, mites acclimated at 10°C shifted their supercooling points (SCPs) by approx. 15°C. In terms of the combined speed of induction and lowering of lethal temperature, this is the most potent RCH response yet reported for a terrestrial arthropod. RCH was also expressed in thermal activity thresholds. Mechanisms responsible for significant differences in recovery from chill torpor are unknown; however, analysis of gut nucleator abundance suggest that the dynamic management of supercooling potential is largely achieved behaviourally, *via* evacuation. Comparisons with the literature reveal that plasticity in this species varies latitudinally, as well as temporally. The high degree of plasticity identified here is coincident with *H. belgicae*'s occupation of the most exposed spatial niche available to Antarctic terrestrial arthropods.**

Key words: phenotypic plasticity, rapid cold hardening, maritime Antarctic, *Halozetes belgicae*, acclimation.

## Introduction

Phenotypic plasticity plays an important role in many organisms' responses to environmental variation (DeWitt and Scheiner, 2004). In variable environments, plasticity in biological traits may have considerable adaptive value, optimising fitness over widely ranging conditions. At high latitudes temperature is one of the primary environmental variables and adaptation to low temperatures – in particular, acclimation potential – is one of the primary derived features of terrestrial arthropods. This study examines the relationship between plasticity and acclimation potential in a common, but largely unexamined, species of maritime Antarctic mite.

Plasticity in the cold tolerance of polar terrestrial arthropods is expressed as the change from a non-cold-hardy to a cold-hardy phenotype. In the mites (Acari) and springtails (Collembola) that dominate the Antarctic terrestrial fauna, this switch is well known and described at seasonal temporal scales. Accomplished primarily by the management of supercooling capacity, it involves the evacuation of gut contents, accumulation of colligative antifreezes and concentration of body fluids with the onset of winter (Cannon and Block, 1988). In this sense, these animals all demonstrate a kind of bimodal plasticity moving physiologically between the two states with predictable seasonal thermal variation.

However, although these phenotypic changes are fixed components of their low temperature adaptation, to what extent do polar arthropods demonstrate plasticity at other scales of thermal variability? In other words, at what level of spatial or temporal resolution has plasticity been incorporated into their acclimation and acclimatisation responses?

Evidence accrued to date from a variety of polar contexts suggests that there is no common level of 'incorporation' (e.g. Klok and Chown, 2005; Worland and Convey, 2001; Sinclair et al., 2003; Sinclair and Chown, 2003; Lee et al., 2006b; Hawes et al., 2006a). Is this evidence for species-specific or context-specific variability? The expression of phenotype is a result of an interaction between genotype and environment and although some of the documented variance (or lack of it) in plasticity may be partitioned phylogenetically (e.g. Klok and Chown, 2003; Klok and Chown, 2005), there are nonetheless observable

correlations between levels of plasticity and exposure to thermal variability (e.g. Hawes et al., 2006a). However, the answer to the species-*versus*-context question can only be arrived at accretively by accumulating data on a range of species in a variety of environmental contexts. Here, we examine a species at one end of the variability gradient – extreme exposure.

*Halozetes belgicae* (Michael) is distributed throughout the sub-Antarctic and maritime Antarctic (Minto et al., 1991; Starý and Block, 1998). Known in Antarctica as the 'lichen-mite' (Bryant, 1945), it is a small oribatid (adult body length <700 μm) often found in association with the more common and well-studied oribatid, *Alaskozetes antarcticus* (Cannon and Schenker, 1985). On Signy Island (South Orkney Islands) it is described as being relatively scarce with a distribution restricted to coastal areas (Cannon and Schenker, 1985). Further south, on the Antarctic peninsula, at Marguerite Bay, it can be found in dense aggregations feeding near gull middens (T.C.H., personal observation), where it grazes the algal and fungal components of encrusting lichens. The extension of its geographical range to higher latitudes (and lower temperatures) is correlated with an expansion in niche from littoral to terrestrial environments (Marshall and Convey, 2004) that is associated with increased exposure to thermal variability. In fact, its preference for gull middens – characteristically on exposed rock surfaces with elevated aspects – means that it occupies the most thermally exposed sites of any terrestrial arthropod in maritime Antarctica. This study tests the hypothesis that the combined environmental context of low temperatures and greater thermal variability has led to a high degree of plasticity in the acclimation potential of this species.

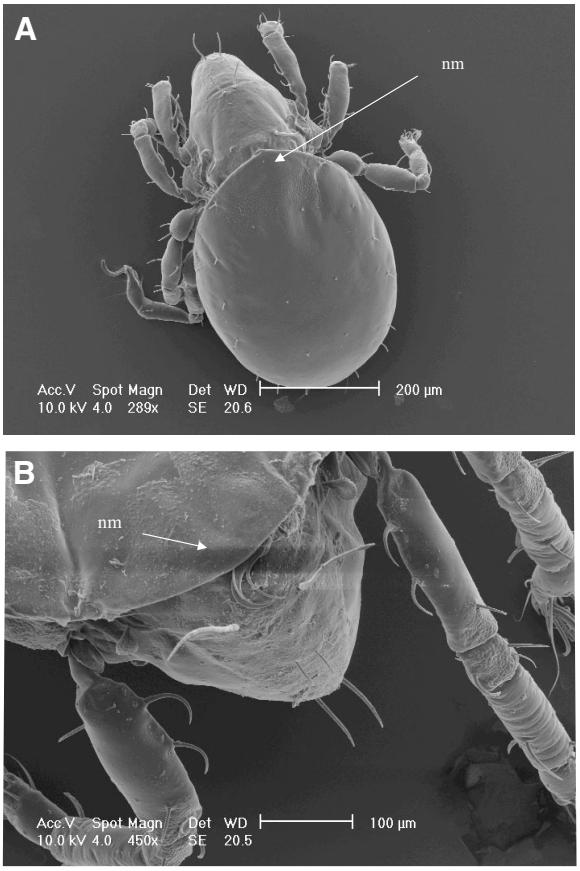


Fig. 1. Scanning electron micrograph of (A) *Halozetes belgicae* with (B) *Alaskozetes antarcticus* for comparison. nm, notogastral margin. Scale bars, 200 μm (A); 100 μm (B).

## Materials and methods

### *Sample collection*

Investigations were carried out from December 2004 to March 2005 at the British Antarctic Survey research station at Rothera Point, Adelaide Island, on the west coast of the Antarctic Peninsula (maritime Antarctic) (67°34′S, 66°08′W). Ancillary investigations – pre-freeze mortality, cold torpor, gut content analysis – were carried out in the UK at British Antarctic Survey laboratories (Cambridge) and the University of Birmingham. *Halozetes belgicae* (Michael) were collected from lichen-encrusted rocks at nearby Lagoon Island. Cultures were transported back to UK by ship in controlled temperature cabinets at 5°C (for approx. 4 months) and, upon arrival at Cambridge, kept at 5 and 10°C for approx. 1 month before experiments. Frequently found in association with *Alaskozetes antarcticus*, *H. belgicae* is distinguished from the former by its size (adults are approximately half the size of those of *A. antarcticus*), its colour (wine red), and by the possession of a strongly peaked anterior notogastral margin (Fig. 1) (Wallwork, 1967).

### *Culture methods*

Bulk cultures of feeding animals on lichen-encrusted rocks were established at 5 and 10°C. Control supercooling point (SCP) distributions for each acclimation treatment were determined after 1 week. To determine the responses of animals to temperature variation under field conditions, rocks with encrusted lichens and feeding mite aggregations were transplanted into an open container and established in an exposed site outside the laboratory. Field temperatures were monitored throughout with a Tinytag datalogger (Gemini Data Loggers, Gemini, Chichester, UK; logging interval=5 min; resolution=0.01°C) with the probe (unshielded) resting on the rock surface of one of the aggregations. For the field experiment on diurnal variation in cold hardiness, as sampling intervals were not uniform, habitat temperatures for each sample were determined as the mean temperature of the previous 4 h. Visual comparison with the complete temperature data set confirmed that the overall temperature trends were preserved.

### *Measurement of cold tolerance*

#### *Supercooling points*

SCPs were measured using differential scanning calorimetry (DSC) (Block, 1994). Standard SCP determinations followed previously published protocols (Worland and Convey, 2001). For temperature exposure treatments involving acclimation

prior to SCP determination, warming and cooling was carried out in an alcohol bath (Thermo Haake Phoenix P2 Circulator; Thermo Haake International, Karlsruhe, Germany) containing 95% ethanol solution. Animals ($N$=30) were placed in five replicate Eppendorf tubes, sat in boiling tubes within the bath, exposed to the set temperature for the chosen duration, then removed, weighed and placed directly into the DSC for SCP determination. The cooling and warming rate for acclimation experiments was 0.5°C min$^{-1}$. To ensure animals reached the desired temperature, a digital thermometer with sensor inserted into an Eppendorf tube, monitored the actual temperature of animals in the bath. As the experiments were directly aimed at identifying changes in the shape of SCP distributions, statistical comparisons of SCPs were carried out using the Kolmogorov–Smirnov test (which makes no assumptions about the shape of distributions). Common non-parametric statistics (e.g. Kruskal–Wallis) were employed to compare SCP distributions of the same shape.

*Chill torpor*

Torpor temperature of mites was measured by filming animals cooled on a cold stage. Ethanol (95% solution) was circulated around a metal cold stage using an alcohol bath (see above) to control temperature and cooling rate. Mites ($N$=7–15) were released into a small arena (diameter=5 mm) with metallic sides and an open bottom with a circle of filter paper sat in between the cold stage surface and the mites. To maintain relative humidity, moist filter paper covered the stage surface, but to prevent inoculation at sub-zero temperatures, the filter paper in the arena was kept dry (the lip on the open bottom of the arena prevented direct contact with the wet filter paper). A microscope cold-light was placed over the observation area to increase visibility. A JVC colour video camera (TK L148B) with macro lens (MLH, 10×) sat upon a tripod was placed over the arena and used to film the mites. Images were captured using Studio Capture (Version 1.5.3) software (www. studio86designs.freeserve.co.uk) with temperature overlap (simultaneously recording the temperature of a digital thermometer with thermocouple placed at the base of the cold stage). Video images were captured at a rate of 2 s frame$^{-1}$, at a resolution of 320×240 pixels. Films of cooled animals were saved and played back to identify individual torpor and recovery temperature for mites that survived cooling treatments. Torpor was defined as the complete cessation of movement (occasional twitching was ignored). Recovery temperature was identified as the temperature at which animals resumed locomotion. Individual animals were used for statistical analysis of torpor temperature (after Gibert and Huey, 2001; Gaston and Chown, 1999). Each experiment was repeated five times to provide a reliable sample size (not all animals survived, and a small number of animals had to be used for each treatment so that aggregational or clumping behaviour did not obscure response observations). Medians for chill coma and recovery were compared using the Mann–Whitney tests; mean survival with the $t$-test.

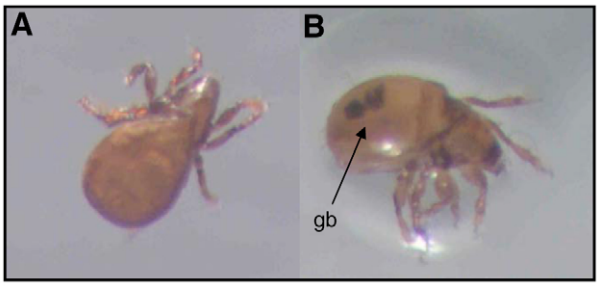


Fig. 2. *Halozetes belgicae* cleared in lactic acid without (A) and with (B) gut boli (gb).

*Experiments*

*Pre-freeze mortality*

To identify whether mites experience pre-freeze mortality, animals acclimated at 5°C (2+ weeks) in the UK were exposed to a modified DSC protocol with cooling and warming rates of 1°C min$^{-1}$. In the first treatment, 5 individual replicates ($N$=10) were cooled separately to –10°C, held there for 1 min, then rewarmed to 5°C. Sample pans were opened and living animals counted and removed. The number of SCPs measured by the DSC was then compared to the number of animals dead and alive (after 24 h). The experiment was then repeated with an additional five replicates cooled to –20°C.

*Effect of acclimation and acclimatisation*

SCPs were determined for mites acclimated at 5 and 10°C for 1 week on lichen-encrusted rocks. Season-scale acclimatisation in field-fresh animals was measured by snapshot sampling of SCPs on 5, 12 and 28 February. For each snapshot sample the mean weekly temperature (mean temperature of the sample day and previous 6 days) was calculated from mean hourly temperatures recorded at the nearby micro-met station on Anchorage Island (British Antarctic Survey, Biological Sciences Division). Diurnal

Table 1. *The relationship between mortality and crystallisation of body fluids in H. belgicae*

| | Replicate | $N_f$ | $N_d$ | Difference ($N_f$–$N_d$) |
|---|---|---|---|---|
| Cooled to –10°C | 1 | 2 | 2 | 0 |
| | 2 | 2 | 2 | 0 |
| | 3 | 2 | 2 | 0 |
| | 4 | 7 | 7 | 0 |
| | 5 | 3 | 3 | 0 |
| Cooled to –20°C | 1 | 8 | 8 | 0 |
| | 2 | 5 | 5 | 0 |
| | 3 | 7 | 7 | 0 |
| | 4 | 8 | 8 | 0 |
| | 5 | 10 | 10 | 0 |

Strategy classified as highly chill-tolerant. $N_f$, number frozen; $N_d$, number dead. $N$=10.

acclimatisation was measured by daily sampling (1–3 per day) of mite aggregations maintained outside the laboratory over 7–11 February.

*Effect of endogenous and exogenous factors on SCPs*

The SCPs of animals acclimated at each temperature were compared with the SCPs of starved and externally ice-inoculated mites. Starved animals were maintained at the acclimation temperature on moistened filter paper without access to food for 1 week. Inoculated animals were daubed with a layer of surface moisture using a paintbrush (size=00) immediately prior to SCP measurements.

*Rapid cold hardening*

*Cooling rate*

The effect of cooling rate on the SCPs of *H. belgicae* was determined by cooling animals from each acclimation temperature at 1, 0.5 and 0.1°C $min^{-1}$.

*Acclimation at 0, –5 and –10°C*

The effect of non-lethal low temperature exposure on the SCPs of animals was determined by cooling mites from each acclimation treatment to 0°C and holding them at this temperature for 2, 4, 6, 8 and 10 h, then determining their SCPs. This experiment was repeated with animals from both acclimation temperatures using sub-zero (–5, –10°C) temperatures for the induction of RCH. To prevent acclimation adjustments, animals were transferred directly to DSC sample pans after sub-zero temperature acclimation. Survival at these temperatures was therefore not monitored directly. Median SCPs therefore include animals that may have frozen during the acclimation process. As there is a good correspondence between 1st and 2nd SCPs of refrozen arthropods that are highly chill-tolerant (Hawes, 2007), these may be identified as SCPs above the acclimation temperature. These SCPs were included in the calculation of medians for each treatment: as (i) they reflect a component of the sample not rapidly cold hardening; (ii) their exclusion would artificially depress the calculated medians; and (iii) their inclusion ensures that calculations of change are conservative.

*Cooling to just below $CT_{min}$ (–12°C)*

To compare the effect of acclimation and RCH on low temperature activity thresholds, mites were cooled and filmed (as described above) to –12°C, which a pilot study indicated was just below their critical thermal minimum ($CT_{min}$). Torpor and recovery temperatures of all individuals that survived were determined by examination of the videos. Survival of each trial was assessed at the end of each experiment.

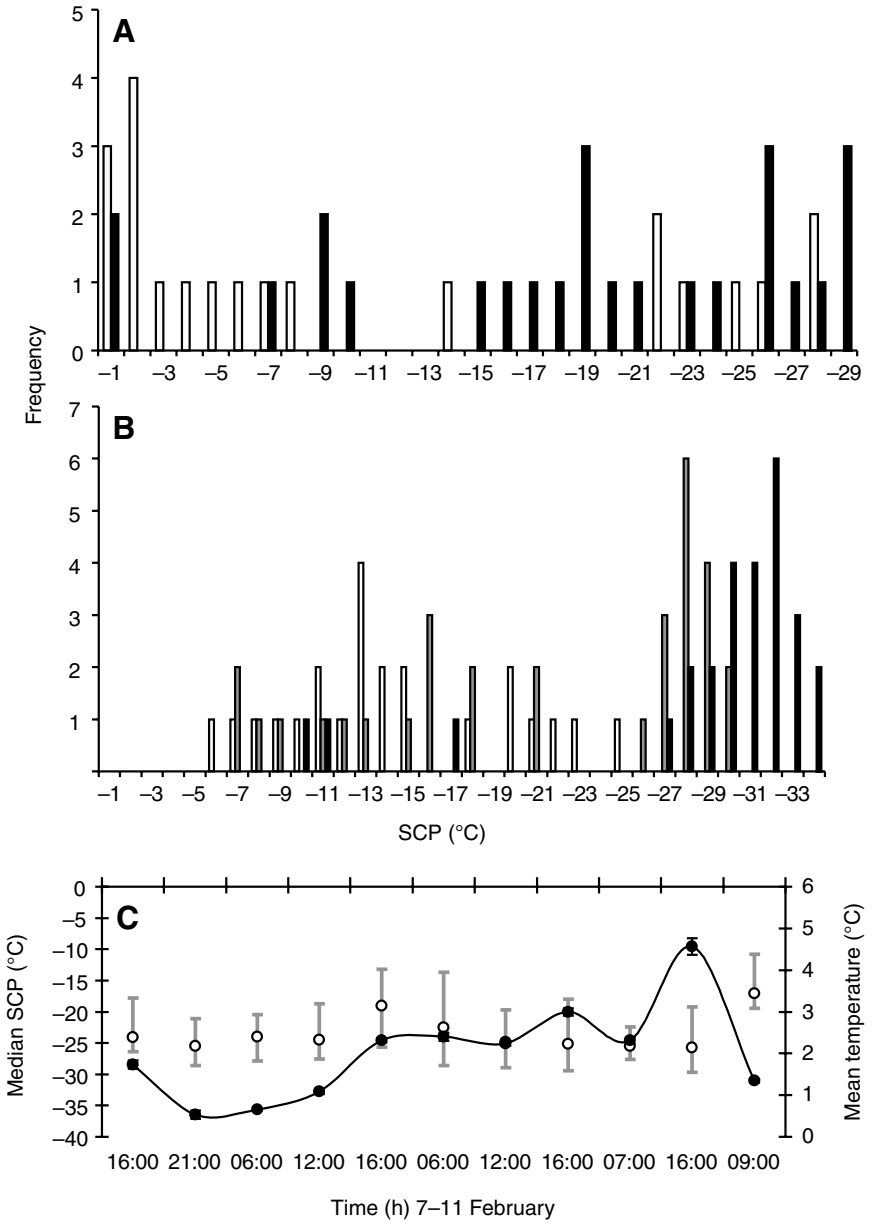


Fig. 3. Acclimation and acclimatisation in *Halozetes belgicae* at (A) weekly timescales; after 1 week acclimation at 10°C (white bars) and 5°C (black bars); (B) seasonal timescales – snapshot samples of field-fresh animals on the 5 February (white bars), 12 February (grey bars) and 28 February (black bars); (C) diurnal timescales: snapshot sampling of field-fresh animals over 7–11 February; filled circles, mean temperature of the rock surface for the 4 h prior to sampling; open circles, median SCP. Values are means ± s.e.m. (for *N* values, see text).

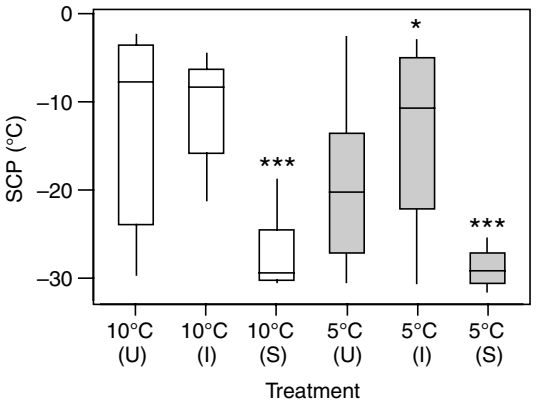


Fig. 4. Effects of starvation and ice inoculation on the SCPs of mites acclimated at 5°C (grey bars) and 10°C (white bars). U, untreated; I, inoculated; S, starved. *Significant different from 'U'; bars represent interquartile range; horizontal line through bars represents median; whiskers represent upper and lower limits of observations.

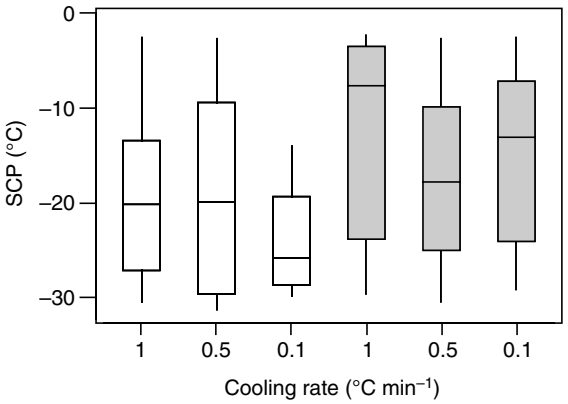


Fig. 5. Effect of cooling rate on the SCPs of mites acclimated at 5 (grey bars) and 10°C (white bars).

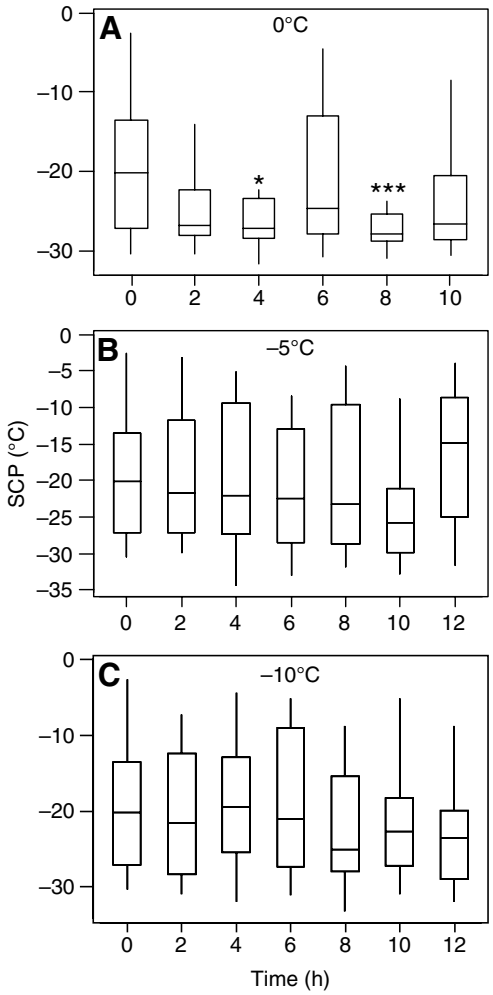


Fig. 6. RCH in 5°C acclimated mites then held for various times at 0°C (A), –5°C (B) and –10°C (C). *Significantly different ($P \geqslant 0.05$); ***highly significant different ($P \geqslant 0.001$).

*Return to acclimation temperature after 12 h RCH at 0°C*

The loss of cold hardiness after rapid cold hardening was investigated by exposing animals from each acclimation temperature to 0°C for 12 h, rewarming them to their acclimation temperature at 0.5°C $min^{-1}$, holding them there for 2, 4, 6, 8, 10 and 12 h and then determining their SCPs.

*Water contents*

Water content was determined gravimetrically. Animals were weighed on a Mettler-Toledo microbalance (UMXW d=0.1 μg) (Mettler-Toledo Ltd, Leicester, UK) prior to SCP determination, oven dried for 24 h at 60°C and then reweighed. Water content was calculated as the percentage difference between fresh and dry mass. Forty eight samples were examined, representing the range of SCPs in the experiments, and regression analysis used to identify any relationship between water content and SCP.

*Gut contents*

To identify the presence of gut boli in mites from *in situ* investigations, samples were preserved in industrial methylated spirit (IMS), transported back to the UK, cleared in 70% lactic acid and examined individually under a dissecting microscope (Fig. 2). Gut boli were scored as present or absent. A total of 75 treatment samples ($N$=20–30 for each) were examined. To determine whether gut contents were responsible for significant differences between acclimation treatments, a comparison was made between median SCPs of treatments and median SCPs of treatments adjusted for gut contents using a correction factor. Corrected SCPs were calculated as: median $SCP_{(N-z)}$, where $N$=sample size, and $z$=the number of animals with gut contents. (As gut boli can only decrease supercooling capacity, SCPs removed for calculation of adjusted SCPs were the highest SCPs.) For example, if five out of 30 animals had gut contents, the highest five SCPs were removed.

Table 2. *Significant* differences between untreated and rapidly cold hardened mites*

| Acclimation temperature | Treatment | Time at treatment temperature | *N* | $d_{max}$ | *P* |
|---|---|---|---|---|---|
| 10°C | RCH at 0°C | 2 | 21,21 | 0.429 | 0.042 |
| | | 4 | 21,27 | 0.582 | 0.001 |
| | | 6 | 21,29 | 0.550 | 0.001 |
| | | 8 | 21,32 | 0.619 | <0.001 |
| | | 10 | 21,30 | 0.619 | <0.001 |
| | RCH at –5°C | 2 | 21,29 | 0.481 | 0.007 |
| | | 4 | 21,28 | 0.548 | 0.001 |
| | | 6 | 21,30 | 0.552 | 0.001 |
| | | 8 | 21,28 | 0.393 | 0.049 |
| | | 10 | 21,24 | 0.536 | 0.003 |
| | | 12 | 21,28 | 0.464 | 0.011 |
| | RCH at –10°C | 2 | 21,28 | 0.440 | 0.019 |
| | | 4 | 21,31 | 0.490 | 0.005 |
| | | 8 | 21,21 | 0.476 | 0.017 |
| | | 10 | 21,26 | 0.485 | 0.008 |
| | | 12 | 21,29 | 0.468 | 0.010 |
| 5°C | RCH at 0°C | 4 | 25,26 | 0.408 | 0.029 |
| | | 8 | 25,24 | 0.472 | 0.009 |

*Kolmogorov-Smirnov two-sample test; $d_{max}$, maximum difference statistic.
RCH, rapid cold hardening.

## Results

### *Pre-freeze mortality*

Table 1 compares the number of 'frozen' and dead mites for each cooling treatment. There was no difference in any of the trials. In the maritime Antarctic, *Halozetes belgicae* may be described as highly chill-tolerant (*sensu* Bale, 1993; Bale, 1996).

### *Effect of acclimation and acclimatisation*

The SCP distributions of mites were significantly different ($N$=25,21; $d_{max}$ =0.499; $P$=0.007) after acclimation for 1 week at 5 and 10°C (Fig. 3A). Fig. 3B shows the changes in cold hardiness of field-fresh *H. belgicae* from mid to late summer. There were highly significant differences between the SCP distributions of mites sampled on the 5 and 12 February ($d_{max}$=0.516; $P$=0.002), 5 and 28 February ($d_{max}$=0.897; $P$<0.001) and 12 and 28 February ($d_{max}$=0.662; $P$<0.001). Mean (± s.e.m.) weekly temperatures for the week preceding these snapshot samples were: 3.39±0.27°C for 5 February; 2.60±0.20°C for 12 February; and 2.29±0.17°C for 28 February. [For a complete picture of temperature variation over the summer, see Hawes et al. (Hawes et al., 2006b).] Over a 5-day period, when environmental temperatures ranged between 0 and 5°C, field-fresh mites showed a significant difference between SCPs ($H$=28.52; d.f.=10; $P$=0.001 (adjusted for ties); Kruskall–Wallis test), but no direct relationship with temperature (Fig. 3C).

### *Effects of starvation and ice inoculation*

Fig. 4 shows the effect of starvation and ice inoculation on mites from both acclimation temperatures. Starvation reduced the SCPs of 10°C acclimated mites significantly ($d_{max}$=0.611; $P$=0.001), but as untreated mites had high SCPs, ice inoculation made no significant difference to SCPs. Mites from the 5°C treatment showed significant increases ($d_{max}$=0.593; $P$<0.001) and reductions ($d_{max}$=0.442; $P$=0.021), respectively, in cold hardiness, as a result of starvation and inoculation.

### *Rapid cold hardening*

Slower cooling rates did not result in significantly different SCPs in mites from either acclimation temperature (Fig. 5). Fig. 6 shows the effect of rapid cold hardening on mites acclimated at 5°C. Untreated mites already had low SCPs so little change was detected, except for significantly lower SCPs in mites acclimated at 0°C for 4 and 8 h (Table 2). Mites acclimated at 10°C showed evidence of significant rapid cold hardening at all three induction temperatures (Fig. 7, Table 2). Indeed, the only sample that did not show significant changes were the mites acclimated at –10°C for 6 h, and their change closely approached significance ($N$=21, 30; $d_{max}$=0.386; $P$=0.051).

There was no significant difference in chill coma temperature in untreated and rapidly cold hardened mites from either acclimation temperature (Fig. 8A), nor was there a difference between acclimation temperatures. Rapidly cold hardened mites acclimated at 10°C were found to have

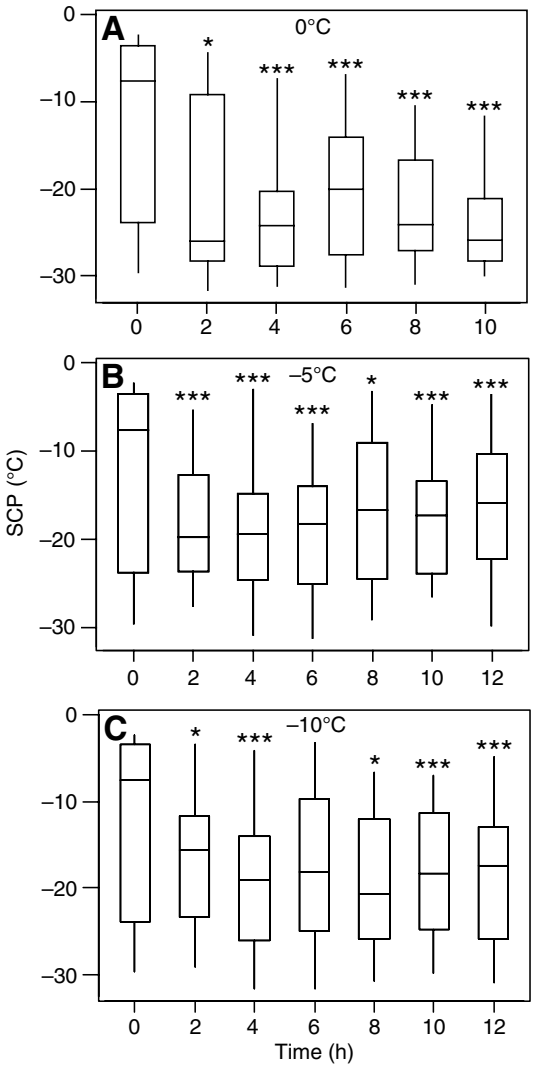


Fig. 7. RCH in 10°C acclimated mites then held for various times at 0°C (A), –5°C (B) and –10°C (C). *Significantly different ($P \geqslant 0.05$); ***highly significant different ($P \geqslant 0.001$).

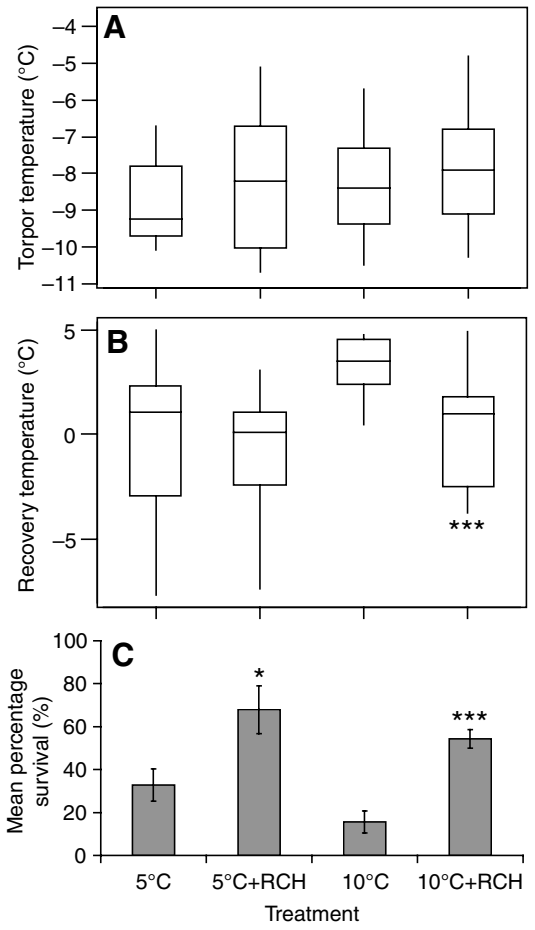


Fig. 8. Comparison of effect of cooling to –12°C on untreated and rapidly cold hardened (4 h at 0°C) mites from 5°C and 10°C. (A) Chill coma temperature; (B) recovery temperature; (C) survival. Mean values are ± 1 s.e.m. *Significantly different ($P \geqslant 0.05$); ***highly significant different ($P \geqslant 0.001$).

significantly lower recovery temperatures than untreated mites ($N$=19.26; $W$=577.5; $P$=0.0013) (Fig. 8B). Survival of the treatment was significantly greater in mites that underwent RCH from both 5 ($N$=5,4; d.f.=5; $T$=–2.61; $P$=0.048) and 10°C ($N$=6,6; d.f.=9; $T$=–5.72; $P$=0.0003; $T$-test) acclimation treatments (Fig. 8C).

Mites cold hardened for 12 h at 0°C and then returned to their acclimation temperatures for 2–12 h showed no significant loss of cold hardiness (Fig. 9).

### *Water contents*

The mean water content of samples examined was 70.1% (±1.3; $N$=48). There was no relationship between SCP and water content ($R^2$=0.0114; $F$=0.52; $P$=0.476).

### *Gut contents*

Overall, mites from all treatments had a high proportion of empty guts ($N$=75; mean=84.9±1.8%). Even mites acclimated at 10°C with high SCPs were found to have 61% empty guts. Nonetheless gut contents are likely to be responsible for the significantly higher SCPs in untreated mites. The adjusted median SCP of mites acclimated at 10°C (the control for RCH treatments) was –19.4°C *versus* an unadjusted median of –7.6°C. By contrast, for 10°C mites whose SCPs were affected exogenously by ice inoculation there was little difference between median SCP (–8.3°C) and adjusted median SCP (–8.9°C).

## Discussion

Phenotypic plasticity in the cold tolerance of *H. belgicae* is evident at multiple scales of temporal resolution. It adjusts its supercooling potential in response to seasonal acclimatisation, weekly acclimation, and hourly acclimation. RCH is expressed both in its supercooling thresholds and its low temperature thermal activity thresholds. The rate and extent of its acclimation potential at hourly scales is unmatched in any

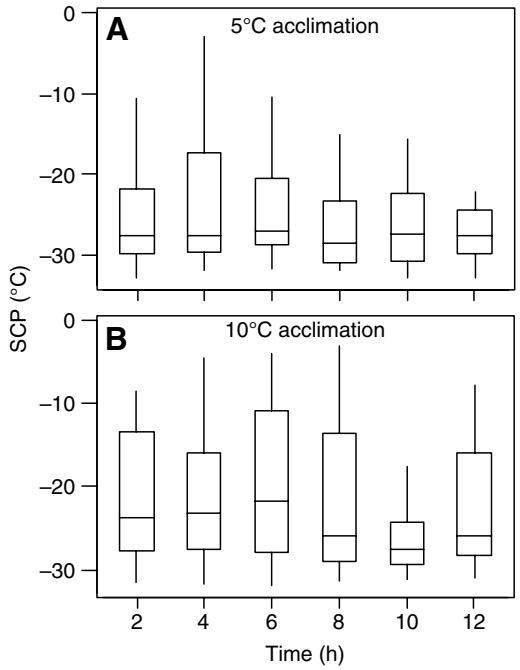


Fig. 9. Effect of return to acclimation temperature on the SCPs of acclimated at 5°C (A) and 10°C (B) mites rapidly cold hardened at 0°C for 12 h.

arthropod examined to date and may be designated as an example of extreme plasticity – or 'superplasticity'.

Comparisons with a sub-Antarctic phenotype (Table 3) add support to an adaptive interpretation of this physiological malleability: lower latitude mites demonstrate pre-freeze mortality and less pronounced acclimation potential compared to mites investigated in this study that occupy extremely exposed ecological niches.

Although survival at extended low temperature exposure was not examined here, Cannon and Schenker found 96.4% survival in field-fresh mites collected in April and held at –15°C for 14 days (Cannon and Schenker, 1985), suggesting that maritime Antarctic phenotypes also show little pre-freeze mortality over extended durations.

Susceptibility to inoculative freezing might be expected to counteract some of the advantages of *H. belgicae*'s plasticity in acclimation potential. However, the exposed and rocky habitats occupied by these mites are dry environments. Summer rain – in recent years a more frequent phenomenon in the maritime Antarctic – occurs only in tandem with warmer temperatures. Although snowfall during summer freeze–thaw cycles and winters offers a real risk of inoculation, contact with ice can be minimised behaviourally; when they are not grazing lichens, *H. belgicae* utilise the microtopography of the rock surface (grooves, micro-fissures) as a sheltering environment.

To date, the RCH response of polar soil arthropods is unique in its effect on supercooling capacity. Although there are undoubtedly other mechanisms involved in rapid cold hardening (e.g. Chen et al., 1987; Overgaard et al., 2005; Lee et al., 2006a) – particularly, in the context of *H. belgicae*, with regard to its effects on chill torpor – the parsimonious explanation for its dramatic effect on the supercooling potential of *H. belgicae* is gut-evacuation. By no means does this connote a lack of sophistication in the cold adaptation of these animals. Gut evacuation has long been known to play an important part in the management of supercooling potential in Antarctic terrestrial arthropods (e.g. Burn, 1981; Cannon and Block, 1988; Block, 1990). Indeed, these results only emphasise the extent to which this behaviour has been dynamically incorporated into their low temperature responses and the extent to which evacuation is an efficient means of ice nucleator management.

The existence of a 'rapid gut emptying response' was first proposed in the Antarctic collembolan, *Cryptopygus antarcticus* (Burn, 1981). Notwithstanding this, no significant evidence for manipulation of gut contents in this springtail was found when it was rapidly cold hardened (Worland and Convey, 2001). However, that study employed comparisons of faecal pellet production rather than gut contents, and comparisons using the former may be affected by the thermal energetics of polar soil arthropods. For example, maximum consumption rates (measured using faecal pellet production) of *C. antarcticus* occur at 5–10°C (Burn, 1984). Indeed, maximum faecal pellet production was found in springtails acclimated at 5°C rather than at lower temperatures (Worland and Convey, 2001). Recent experiments have shown that feeding is certainly involved to some extent in the reverse phase of the process (short term cold de-acclimation) in *C. antarcticus* (Worland et al., 2007), which might explain why mites in this study that were returned to their acclimation temperature in the absence of food did not lose their cold hardiness.

Table 3. *Comparison of acclimation potential in sub-Antarctic and maritime Antarctic phenotypes of H. belgicae*

| Site | Reference | Measure of central tendency | 5°C | 10°C | Pre-freeze mortality | Class of freeze avoidance |
|---|---|---|---|---|---|---|
| Marion Island | (Deere et al., 2006) | $LLT_{50}$ Dry | –5.6 | –7.0 | Yes | Moderately chill-tolerant |
| | | $LLT_{50}$ Wet | –4.6 | –4.7 | | |
| Maritime Antarctic | This study | Median SCP Dry | –20.2 | –7.6 | No | Highly chill-tolerant |
| | | Median SCP Wet | –10.7 | –8.3 | | |

SCP, supercooling point; $LLT_{50}$, low lethal temperature for 50% of sample population.

It is not often acknowledged that the sub-lethal experimental temperature (0°C) employed in laboratory inductions of RCH, for most species that have been examined (i.e. temperate species), is below or close to their critical thermal minimum for activity. Although experiments on Antarctic micro-arthropods (Worland and Convey, 2001; Lee et al., 2006b) (this study) and on the 'fine-tuning' of RCH in *Drosophila melanogaster* (Shreve et al., 2004) have demonstrated the induction of the response in animals not in coma; for many documented cases of RCH, it has been induced in animals effectively under chill coma, i.e. when metabolic activity is minimal.

In the present study, we found RCH was successfully induced above (0°C, –5°C), and at or just below (–10°C) the $CT_{min}$ of *H. belgicae*. Results for the latter were less significant, although not enough to definitively suggest a gradient in acclimation response. Extensive metabolic regulation (at least in terms of a system-level response) may not be necessary for improved performance in rapidly cold hardened insects; for example, at least some of the improvements can be attributed to responses at the cellular level (Yi and Lee, 2004). Nonetheless, one might anticipate a more comprehensive physiological response above the chill coma temperature when metabolic pathways (e.g. for the production of desaturase enzymes involved in membrane lipid modification) are more readily mobilised. In the context of this study, given the apparent importance of gut-evacuation to *H. belgicae*'s response, it would seem that the period of time these mites spend above their $CT_{min}$ would be particularly important.

In conclusion, *H. belgicae* would appear to exemplify the notion of adaptive plasticity in cold tolerance. Its seasonally scaled responses are adaptive and, indeed, fundamentally necessary for high latitude life. Its evidence of genotypic plasticity (latitudinal variation) and short-term 'superplasticity' are in agreement with environmental gradients in low temperature stress and variability.

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## References

**Bale, J. S.** (1993). Classes of insect cold hardiness. *Funct. Ecol.* **7**, 751-753.
**Bale, J. S.** (1996). Insect cold hardiness: a matter of life and death. *Eur. J. Entomol.* **93**, 369-382.
**Block, W.** (1994). Differential scanning calorimetry in ecophysiological research. *Acta Oecologea* **15**, 13-22.
**Bryant, H. M.** (1945). Biology at East Base, Palmer Peninsula, Antarctica. *Proc. Am. Philos. Soc.* **89**, 256-269.
**Burn, A. J.** (1981). Effects of temperature on the feeding activity of *Cryptopygus antarcticus*. *Comité National Français Des Recherches Antarctiques* **51**, 209-216.
**Burn, A. J.** (1984). Energy partitioning in the Antarctic collembolan *Cryptopygus antarcticus*. *Ecol. Entomol.* **9**, 11-21.
**Cannon, R. J. C. and Block, W.** (1988). Cold tolerance of microarthropods. *Biol. Rev. Camb. Philos. Soc.* **63**, 23-77.
**Cannon, R. J. C. and Schenker, R.** (1985). Cold tolerance of a cryptostigmatid mite at Signy Island, Maritime Antarctic. *Brit. Antarct. Surv. Bull.* **67**, 1-5.
**Chen, C. P., Denlinger, D. L. and Lee, R. E.** (1987). Cold-shock injury and rapid cold hardening in the flesh fly *Sarcophaga crassipalpis*. *Physiol. Zool.* **60**, 297-304.
**Deere, J. A., Sinclair, B. J., Marshall, D. J. and Chown, S. L.** (2006). Phenotypic plasticity of thermal tolerances in five oribatid mite species from sub-Antarctic Marion Island. *J. Insect Physiol.* **52**, 693-700.
**DeWitt, T. J. and Scheiner, S. M.** (2004). Phenotypic variation from single phenotypes: a primer. In *Phenotypic Plasticity Functional and Conceptual Approaches* (ed. T. J. DeWitt and S. M. Scheiner), pp. 1-9. Oxford: Oxford University Press.
**Gaston, K. J. and Chown, S. L.** (1999). Elevation and climatic tolerance: a test using dung beetles. *Oikos* **86**, 584-590.
**Gibert, P. and Huey, R. B.** (2001). Chill-coma temperature in *Drosophila*: effects of developmental temperature, latitude, and phylogeny. *Physiol. Biochem. Zool.* **74**, 429-434.
**Hawes, T. C.** (2006). Re-crystallisation temperature in an Antarctic springtail. *CryoLetters* In Press.
**Hawes, T. C., Couldridge, C. E., Bale, J. S., Worland, M. R. and Convey, P.** (2006a). Habitat temperature and the temporal scaling of cold hardening in the High Arctic collembolan, *Hypograstrura tullbergi* (Schäffer). *Ecol. Entomol.* **31**, 450-459.
**Hawes, T. C., Bale, J. S., Convey, P. and Worland, M. R.** (2006b). Ecologically realistic modalities in arthropod supercooling point distributions. *Eur. J. Entomol.* **103**, 717-723.
**Klok, C. J. and Chown, S. L.** (2003). Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biol. J. Linn. Soc. Lond.* **78**, 401-414.
**Klok, C. J. and Chown, S. L.** (2005). Inertia in physiological traits: *Embryonopsis halitcella* caterpillars (Yponomeutidae) across the Antarctic Polar Frontal Zone. *J. Insect Physiol.* **51**, 85-97.
**Lee, R. E., Damodaran, K., Yi, S.-X. and Lorigan, G. A.** (2006a). Rapid cold hardening increases membrane fluidity and cold tolerance of insect cells. *Cryobiology* **52**, 459-463.
**Lee, R. E., Elnitsky, M. A., Rinehart, J. P., Hayward, S. A., Sandro, L. H. and Denlinger, D. L.** (2006b). Rapid cold-hardening increases the freezing tolerance of the Antarctic midge *Belgica antarctica*. *J. Exp. Biol.* **209**, 399-406.
**Marshall, D. J. and Convey, P.** (2004). Latitudinal variation in habitat specificity of ameronothrid mites (Oribatida). *Exp. Appl. Acarol.* **34**, 21-34.
**Minto, L. B., Shepherd, G. J. and Usher, M. B.** (1991). The cryptostigmatid mite *Halozetes belgicae* (Michael) in the maritime Antarctic. *Antarct. Sci.* **3**, 53-59.
**Overgaard, J., Sorensen, J. G., Petersen, S. O., Loeschecke, V. and Holmstrup, M.** (2005). Changes in membrane lipid composition following rapid cold hardening in *Drosophila melanogaster*. *J. Insect Physiol.* **51**, 1173-1182.
**Shreve, S. M., Kelty, J. D. and Lee, R. E.** (2004). Preservation of reproductive behaviours during modest cooling: rapid cold-hardening fine-tunes organismal response. *J. Exp. Biol.* **207**, 1797-1802.
**Sinclair, B. J. and Chown, S. L.** (2003). Rapid responses to high temperature and dessication but not to low temperature in the freeze tolerant sub-Antarctic caterpillar *Pringleophaga marioni* (Lepidoptera, Tineidae). *J. Insect Physiol.* **49**, 45-52.
**Sinclair, B. J., Klok, C. J., Scott, M. B., Terblanche, J. S. and Chown, S. L.** (2003). Diurnal variation in supercooling points of three species of Collembola from Cape Hallet, Antarctica. *J. Insect Physiol.* **49**, 1049-1061.
**Starý, J. and Block, W.** (1998). Distribution and biogeography of oribatid mites (Acari: Oribatida) in Antarctica, the sub-Antarctic islands and nearby land areas. *J. Nat. Hist.* **32**, 861-894.
**Wallwork, J. A.** (1967). Cryptostigmata (Oribatid Mites). *Antarct. Res. Ser.* **10**, 105-122.
**Worland, M. R. and Convey, P.** (2001). Rapid cold hardening in Antarctic microarthropods. *Funct. Ecol.* **15**, 515-524.
**Worland, M. R., Hawes, T. C. and Bale, J. S.** (2007). Temporal resolution of cold acclimation and de-acclimation in the Antarctic collembolan, *Cryptopygus antarcticus* (Willem). *Physiol. Entomol.* In Press.
**Yi, S.-X. and Lee, R. E.** (2004). In vivo and in vitro rapid cold-hardening protects cells from cold shock injury in the flesh fly, *Sarcophaga crassipalpis*. *J. Comp. Physiol. B* **174**, 611-615.