Cold-tolerance and acclimation in the free-living nematode, *Panagrellus redivivus*

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SUMMARY

The supercooling points of *P. redivivus* adults, acclimated at 22°, are depressed by 2-3°, 3-5 hours after transfer to lower acclimation temperatures of 15°, 10° and 5°. At 15° supercooling points return to normal levels after 15 hours but at 10° and 5° the depression of supercooling points is more permanent. After 24 hours' exposure there is a significant negative correlation between supercooling points and acclimation temperature. The supercooling points of *P. redivivus* adults, acclimated at 10°, is elevated after transfer to 22°. These results indicate that *P. redivivus* adults are able to acclimate to changes in temperature in a way which affects their supercooling point. The possible rôle of cryoprotective substances in this response is discussed.

RÉSUMÉ

Tolérance et acclimatation au froid chez le nématode libre Panagrellus redivivus

Les points de surfusion des adultes de *Panagrellus redivivus* acclimatés à 22° sont abaissés de 2 à 3°, 3 à 5 heures après transfert aux températures d'acclimatation de 15°, 10° et 5°. A 15°, les points de surfusion reviennent à la normale après 15 heures, mais à 10° et 5° l'abaissement des points de surfusion devient définitif. Après une exposition de 24 heures, il y a forte corrélation négative entre les points de surfusion et la température d'acclimatation. Les points de surfusion des adultes de *P. redivivus* acclimatés à 10° sont élevés après le transfert à 22°. Ces résultats indiquent que les adultes de *P. redivivus* ont la capacité de s'acclimater aux variations de température par un mécanisme qui affecte leur point de surfusion. Le rôle possible de substances cryoprotectrices dans cette réaction est discuté.

A number of studies indicate that nematodes can survive sub-zero temperatures (Anderson, Wang & Levine, 1966; Balasingham, 1964; Vrain, 1978), but until recently the mechanisms involved were unknown. Arthropods which can survive sub-zero temperatures (cold-tolerant) can either withstand extracellular ice formation (freezing-tolerant) or avoid freezing by supercooling but die once the body fluids freeze (freezing-susceptible). An animal is said to supercool when it can maintain its body fluids in a liquid phase at temperatures below their melting point (Block, 1982). Most nematode species investigated are freezing-susceptible but can avoid freezing by supercooling. This mechanism has been demonstrated in *Trichostrongylus colubriformis* (J 3), *Ditylenchus dipsaci* (J 4), *P. redivivus* (adults), *Nematodirus battus* (J 3) and *Globodera rostochiensis* (J 2) (Ash & Atkinson, 1982; Wharton, Young & Barrett, 1984; Perry & Wharton, 1985). Supercooling points as low as — 28° have been recorded. *Aphelenchoides ritzemabosi* is the only species for which there is any evidence for freezing-resistance (Asahina, 1959).

Some arthropods can increase their cold-tolerance during winter by lowering their supercooling points (Sømme, 1982). Nematodes may also be able to do this. The ability of the infective juveniles of *Trichinella spiralis* to survive freezing at — 32° or — 45° is increased by preconditioning at — 15° (Smith, 1984). The mechanism of cold-tolerance in this species has yet to be demonstrated. Chilling lowers the supercooling points of *N. battus* eggs (Ash & Atkinson, 1982).

This paper investigates the ability of the adult stage of *P. redivivus* to acclimate to falling temperatures by lowering its supercooling point.

Materials and methods

P. redivivus were cultured on moist oats at 22°. For the acclimation studies cultures were transferred to 15°,

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10° or 5°. Acclimation temperatures were provided by cooled incubators, with an accuracy of ± 0.5°. At intervals after transfer, samples were removed and the nematodes cleaned and isolated by rapid migration through tissue paper. The supercooling points of more than 30 adults were measured. To investigate the effect of an increase in temperature on cold-acclimated nematodes a culture was acclimated at 10° for 48 hours and then transferred to 22°. Supercooling points were again measured at various intervals after transfer. The same nematode culture was used for each series of experiments.

Supercooling points were measured by direct observation using a thermoelectric cooling microscope stage. The design and operation of the stage is described in detail by Wharton and Rowland (1984). A drop of nematode suspension was transferred to a small square of cellulose acetate sheeting and the surface water removed with filter paper. A drop of liquid paraffin was placed on top of the sample to prevent water evaporation. The water content of nematodes will remain constant for several days in this medium (Perry, 1977). The specimen was placed on top of the thermocouple in the cooling chamber of the microscope stage and the chamber sealed and insulated. The stage was cooled rapidly to 0° and the control unit switched in to provide a controlled rate of cooling of 1°/min. Adult nematodes were observed as they were cooled from 0° to — 40° and the supercooling point recorded. The supercooling point was easily observed as a marked and sudden decrease in transparency as the nematode froze. Preliminary experiments had shown that clumps of nematodes had much higher supercooling points than individuals. Only the supercooling points of individual adults, not in contact with other nematodes, were noted.

Results

The supercooling points of *P. redivivus* adults acclimated at 22° and after transfer to acclimation temperatures of 15°, 10° and 5° is shown in Fig. 1. There is a marked depression of the supercooling points during the first 3-5 hours after transfer to the lower temperature. At 15° the supercooling points return to their original levels 15 hours after transfer, whereas at 10° and 5° the lowering of the supercooling point is more permanent.

The supercooling points of nematodes acclimated at 10° were elevated after transfer to 22° (Fig. 2). The supercooling points of nematodes after 24 hours exposure to an acclimation temperature is shown in Fig. 3. Supercooling points are negatively correlated with acclimation temperature ($r = -0.455$; $df = 137$; $p \leqslant 0.001$).

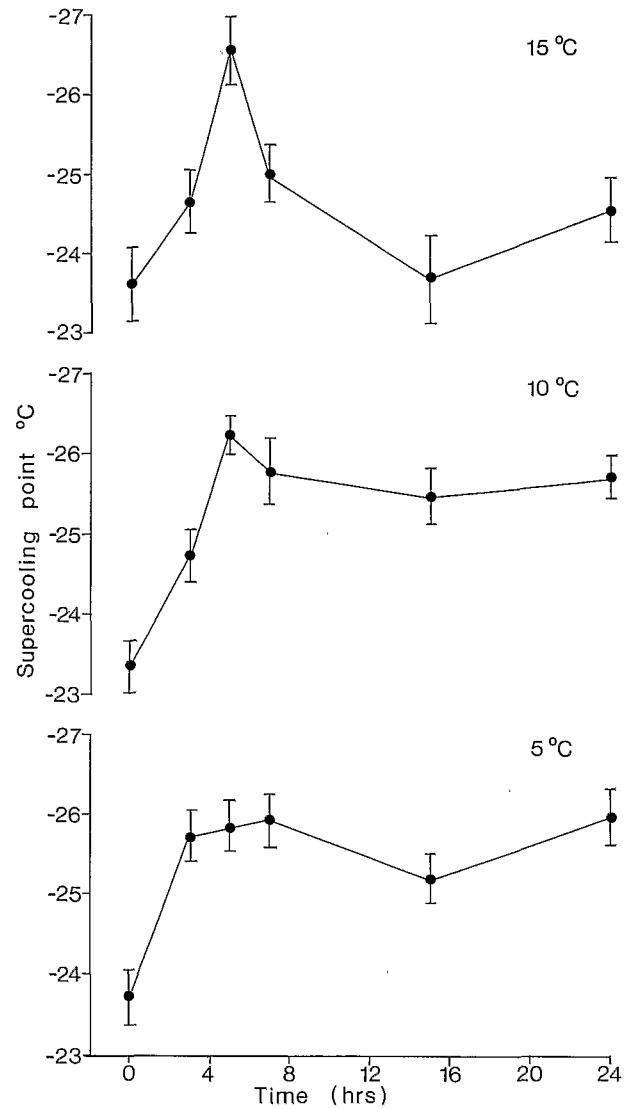

Fig. 1. The mean supercooling points of *P. redivivus* adults after transfer from an acclimation temperature of 22° to 15°, 10° and 5°. *(Vertical bars represent the standard error of the mean of more than 30 individual measurements).*

Discussion

After transfer from 22° to a lower acclimation temperature of 15°, 10° or 5° the adults of *P. redivivus* show a clear depression of their supercooling points in the order of 2-3°. After transfer to 15° supercooling points recover to their original levels after 15 hours. A decrease in temperature results in a lowering of the nematodes' supercooling points but this decrease is not sustained at 15°. After 24 hours' exposure there is a

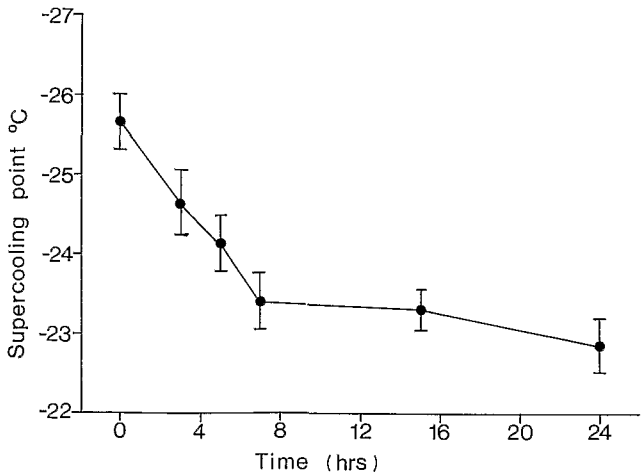

Fig. 2. The mean supercooling points of *P. redivivus* adults after transfer from an acclimation temperature of 10° to 22°. *(Vertical bars represent the standard error of the mean of more than 30 individual measurements).*

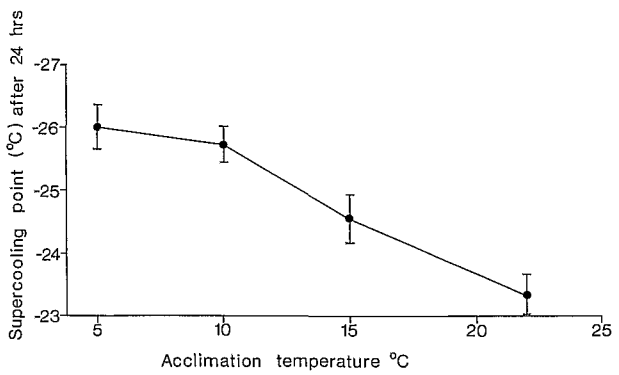

Fig. 3. The mean supercooling points of *P. redivivus* adults after 24 hours' exposure to different acclimation temperatures. *(Vertical bars represent the standard error of the mean of more than 30 individual measurements).*

significant correlation between supercooling points and acclimation temperature. The elevation of supercooling points after transfer from 10° to 22° indicates that the nematodes are not responding to a change in temperature, regardless of whether it is a decrease or an increase, and that the change in supercooling points observed are not due to some sort of ageing effect in the culture.

The depression of supercooling points in response to lowered acclimation temperatures in *P. redivivus* is comparable to that observed in some arthropods. Transfer of the antarctic terrestrial mite, *Alaskozetes antarcticus* from 0° to — 5° resulted in a 2-3° depression of supercooling points (Young & Block, 1980). Measurements of supercooling points have been made using a constant rate of cooling of 1°/min. A 2-3° depression of supercooling point under these conditions may reflect a greatly increased ability to survive sub-zero temperatures well above the measured supercooling point and close to those encountered in the field. The time of exposure is an important factor determining the temperature at which spontaneous freezing occurs (Sømme, 1982).

Most freezing-susceptible arthropods synthesise glycerol, or other polyhydric alcohols, as a cryoprotectant (Duman *et al.*, 1982). *P. redivivus* is known to synthesise glycerol as an end product of anaerobic catabolism (Butterworth & Barrett, 1985). Preliminary results, however, indicate no correlation between glycerol concentrations and supercooling points (Mabbett, Wharton & Butterworth, unpubl.). Glycerol concentrations may be too low to produce significant cryoprotective effects (Wharton, Young & Barrett, 1984). Nematodes are known to be able to synthesise other polyhydric alcohols, including sorbitol, ribotol and inositol (Barrett, 1981; Womersley, 1981). These are known to act or may be able to act as cryoprotective agents in arthropods (Duman *et al.*, 1982).

Trehalose, and other sugars, have also been suggested as cryoprotectants in arthropods (Duman *et al.*, 1982). During chilling, the trehalose concentration of the eggs of *N. battus* increases and the mean supercooling point decreases (Ash & Atkinson, 1982). Trehalose may, however, have other rôles during overwintering (Ash & Atkinson, 1983). The rôle of potential cryoprotective compounds in nematode cold-tolerance is unclear and merits further investigation.

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REFERENCES

ANDERSON, F. L., WANG, G. T. & LEVINE, N. D. (1966). Effect of temperature on survival of the free-living stages of *Trichostrongylus colubriformis*. *J. Parasitol.*, 52 : 713-721.

ASAHINA, E. (1959). Frost-resistance in a nematode, *Aphelenchus ritzema-bosi*. *Low Temp. Sci.*, B 17 : 51-62.

ASH, C. P. J. & ATKINSON, H. J. (1982). The possible rôle of trehalose in the survival of eggs of *Nematodirus battus* during dormancy. *Parasitology*, 85 : LV.

ASH, C. P. J. & ATKINSON, H. J. (1983). Evidence for a temperature-dependent conversion of lipid reserves to carbohydrate in quiescent eggs of the nematode, *Nematodirus battus*. *Comp. Biochem. Physiol.*, 76 B : 603-610.

BALASINGAM, E. (1964). Comparative studies on the effects of temperature on free-living stages of *Plococonus lotoris, Dochmoides stenocephala* and *Ancylostoma caninum*. *Can J. Zool.*, 42 : 907-918.

BARRETT, J. (1981). *Biochemistry of Parasitic Helminths.* London, Macmillans, x + 308 p.

BLOCK, W. (1982). Cold hardiness in invertebrate poikilotherms. *Comp. Biochem. Physiol.*, 73 A : 581-593.

BUTTERWORTH, P. E. & BARRETT, J. (1985). Anaerobic metabolism in the free-living nematode *Panagrellus redivivus*. *Physiol. Zool.*, 58 : 9-17.

DUMAN, J. G., HORWARTH, K. C., TOMCHANEY, A. & PATTERSON, J. C. (1982). Antifreeze agents of terrestrial arthropods. *Comp. Biochem. Physiol.*, 73 A : 545-555.

PERRY, R. N. (1977). The water dynamics of stages of *Ditylenchus dipsaci* and *D. myceliophagus* during desiccation and rehydration. *Parasitology*, 75 : 45-70.

PERRY, R. N. & WHARTON, D. A. (1985). Cold tolerance of hatched and unhatched second stage juveniles of the potato cyst-nematode *Globodera rostochiensis*. *Int. J. Parasitol.*, 15 : 441-445.

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SMITH, H. J. (1984). Preconditioning of *Trichinella spiralis nativa* larvae in musculature to low temperatures. *Vet. Parasitol.*, 17 : 85-90.

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

VRAIN, T. C. (1978). Influence of chilling and freezing temperatures on infectivity of *Meloidogyne incognita* and *M. hapla*. *J. Nematol.*, 10 : 177-180.

WHARTON, D. A. & ROWLAND J. J. (1984). A thermoelectric microscope stage for the measurement of the supercooling points of microscopic organisms. *J. Microsc.*, 134 : 299-305.

WHARTON, D. A., YOUNG, S. R. & BARRETT, J. (1984). Cold tolerance in nematodes. *J. Comp. Physiol.*, B 154 : 73-77.

WOMERSLEY, C. (1981). Biochemical and physiological aspects of anhydrobiosis. *Comp. Biochem. Physiol.*, 70 B : 669-678.

YOUNG, S. R. & BLOCK, W. (1980). Experimental studies on the cold-tolerance of *Alaskozetes antarcticus*. *J. Insect Physiol.*, 26 : 189-200.