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The environmental physiology of Antarctic terrestrial nematodes: a review

Accepted: 28 July 2003 / Published online: 28 August 2003
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Abstract The environmental physiology of terrestrial Antarctic nematodes is reviewed with an emphasis on their cold-tolerance strategies. These nematodes are living in one of the most extreme environments on Earth and face a variety of stresses, including low temperatures and desiccation. Their diversity is low and declines with latitude. They show resistance adaptation, surviving freezing and desiccation in a dormant state but reproducing when conditions are favourable. At high freezing rates in the surrounding medium the Antarctic nematode *Panagrolaimus davidi* freezes by inoculative freezing but can survive intracellular freezing. At slow freezing rates this nematode does not freeze but undergoes cryoprotective dehydration. Cold tolerance may be aided by rapid freezing, the production of trehalose and by an ice-active protein that inhibits recrystallisation. *P. davidi* relies on slow rates of water loss from its habitat, and can survive in a state of anhydrobiosis, perhaps aided by the ability to synthesise trehalose. *Teratocephalus tilbrookii* and *Ditylenchus parvevirens* are fast-dehydration strategists. Little is known of the osmoregulatory mechanisms of Antarctic nematodes. Freezing rates are likely to vary with water content in Antarctic soils. Saturated soils may produce slow freezing rates and favour cryoprotective dehydration. As the soil dries freezing rates may become faster, favouring freezing tolerance. When the soil dries completely the nematodes survive anhydrobiotically. Terrestrial Antarctic nematodes thus have a variety of strategies that ensure their survival in a harsh and variable environment. We need to more fully understand the conditions to which they are exposed in Antarctic soils and to apply more natural rates of freezing and desiccation to our studies.

Keywords Anhydrobiosis · Cold tolerance · Cryoprotective dehydration · Desiccation · Freezing · Osmoregulation

Introduction

Terrestrial Antarctic nematodes are part of a community of organisms that are living in one of the harshest environments on Earth. Less than 0.5% of continental Antarctica is ever free of snow and ice (Fox and Cooper 1994). Even rarer are the ice-free areas that receive sufficient meltwater in summer to support the growth of visible vegetation (moss, algae and lichens). Antarctic terrestrial communities consist of only a few species, making it feasible to attempt an overall understanding of their ecology (Block 1994). Since they are living at the limits of life on Earth, terrestrial Antarctic organisms may be particularly sensitive indicators of climate change. They are also good models for studying how life survives in extreme environments and they may allow us to discover improved methods of storing biological materials (Wharton 2002a).

There are a number of factors in terrestrial Antarctic environments that may be considered stressful (Convey 1996). Mean and extreme temperatures decrease with increasing latitude. This brings lower temperatures for growth and increased exposure to freezing. Daily temperature fluctuations in the microenvironments inhabited by terrestrial Antarctic organisms can be considerable (25–50 °C), depending on snow cover and the absorption of solar radiation, resulting in exposure to freeze/thaw cycles (Convey 1996). Since most water is locked up as snow and ice, the environment is generally extremely dry although water may be locally abundant, with the availability of water varying on a temporal and geographical scale. Light is limited on a seasonal, as well as a daily, basis from 24 h daylight in midsummer to 24 h darkness in midwinter. These factors combine to produce short growing seasons. Other stresses may

Communicated by: I. D. Hume

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include low nutrient availability, unstable substrates, ultraviolet radiation, osmotic stress and unpredictable environmental conditions (Convey 1996; Wharton 2002a).

Although conditions in terrestrial Antarctic habitats can generally be considered stressful the degree of stress will vary on both a continental and local scale. Two climatic regions are recognised. The maritime Antarctic comprises parts of the Antarctic Peninsula and nearby islands. This has a cold maritime climate with mean monthly air temperatures above 0 °C for several months during summer and rarely falling below -15 °C in winter (Block 1994). This encompasses a small proportion of the continent. The remaining areas are part of the continental Antarctic with mean monthly air temperatures rarely above 0 °C in summer and winter means as low as -25 °C even in coastal areas (Block 1994).

There are three main types of ice-free areas in the continental Antarctic (Walton 1984). There are nunataks, which are isolated mountain tops that project above the ice sheet, there are some large areas permanently free of snow and ice due to local climatic conditions (e.g. the Dry Valleys of Victoria Land), and there are coastal sites where the winter snow cover melts during summer to provide liquid water and ice-free ground. This review summarises our understanding of the environmental physiology of terrestrial Antarctic nematodes found in these areas.

The distribution and ecology of nematodes in terrestrial Antarctic ecosystems

The diversity of the terrestrial Antarctic nematode fauna is low. The most recent survey lists 29 species from the maritime Antarctic and 14 from the continental Antarctic (Andrássy 1998). Although this might seem to be a rather sparse fauna, nematodes are the most diverse and abundant invertebrates in these habitats. Nematode diversity declines with increasing latitude (Fig. 1), a pattern also found over a narrow range of latitudes within the maritime Antarctic (Maslen 1979). This may be related to increased abiotic stress with increasing latitude and a corresponding decrease in the biota.

Terrestrial Antarctic nematodes feed predominantly on bacteria, cyanobacteria and algae. Their habitats, however, often have very low microbial contents. Rates of decomposition are slow, due to low numbers of microbes, low temperatures and restricted water availability. There are a few fungivorous, plant-parasitic and predatory species (Andrássy 1998).

Some species of nematodes are associated with certain habitats. For example, *Panagrolaimus davidi* is associated with coastal sites (Wharton and Brown 1989) and in particular penguin colonies and ornithogenic soils (Porazinska et al. 2002; Sinclair 2001). Presumably debris from the penguin colonies boosts nutrient availability in these soils. *Scottinema lindsayae* is associated with dry and saline soils (Treonis et al. 1999) and

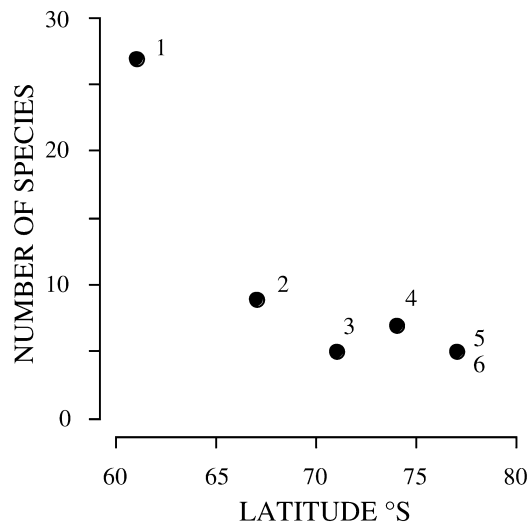


Fig. 1 The diversity of terrestrial Antarctic nematodes decreases with increasing latitude. Data from: 1 Signy Island (Maslen 1981), 2 Adelaide Island (Convey and Worland 2000; N.R. Maslen, personal communication), 3 Alexander Island (Convey and Wynn-Williams 2002), 4 Nunataks Dronning Maud Land (Sohlenius et al. 1996), 5 Ross Island (Sinclair 2001), 6 Dry Valleys (Wharton and Brown 1989)

P. magnivulvatus has been recorded only from nunataks (Sohlenius et al. 1996). Given the different nature of these habitats, we might expect these species to vary in their responses to environmental conditions.

Conditions in the maritime Antarctic support, in places, the growth of extensive moss turfs and carpets and even two species of higher plants (Block 1994). In contrast, visible vegetation in the continental Antarctic is extremely sparse. Nematodes inhabiting the latter are thus likely to be exposed to much harsher abiotic conditions.

Resistance and capacity adaptation in Antarctic terrestrial nematodes

Organisms exposed to extreme environmental conditions show two broad responses (Wharton 2002a, 2002b). Some are adapted to grow and reproduce under conditions that are constantly extreme (extremophiles, displaying capacity adaptation), whilst others survive extreme conditions in a dormant state, only growing and reproducing when conditions are favourable (cryptobiotics, displaying resistance adaptation).

There is evidence for capacity adaptation in some Antarctic nematodes. *Plecticus antarcticus* lays eggs and completes its life cycle at lower temperatures than nematodes from temperate regions (Caldwell 1981). *Scottinema lindsayae* has a higher fecundity, faster rates of juvenile development and better survival at 10 °C than at 15 °C (Overhoff et al. 1993). In contrast, *P. davidi* grows best at 25 °C (Brown 1993; Wharton 1997) and thus does not show capacity adaptation to cold. It is, however, resistant to low temperatures and desiccation.

The survival strategies of this and probably many other Antarctic nematodes are dominated by resistance adaptation.

Cold tolerance strategies of Antarctic terrestrial nematodes

Antarctic nematodes have to face the problems of exposure to very low temperatures and to freezing events. In the maritime Antarctic (Signy Island) the minimum soil surface temperature was -17.1°C and there were few freeze/thaw events (Davey et al. 1992). In the continental Antarctic (Cape Bird, Ross Island) the minimum soil surface temperature was -31.7°C and there were frequent freeze/thaw events during late spring and summer (Sjursen and Sinclair 2002; Wharton 1997). The moisture status of the soil varies on both a small geographical scale and with time (Campbell et al. 1997; Sinclair and Sjursen 2001a; Wharton 1997). This suggests that Antarctic nematodes face exposure to low temperatures under highly variable conditions.

Cold tolerance strategies have traditionally been divided into freezing tolerance and freeze avoidance (Lee 1991). Freeze avoiding animals maintain their body fluids as a liquid at temperatures below their melting point (they supercool) but die once freezing occurs (at the supercooling point). Freezing-tolerant animals can survive ice forming in their bodies and tend to show little supercooling ability. More recently a third cold tolerance mechanism (cryoprotective dehydration) has been described, where a soil organism surrounded by ice dehydrates rather than freezes (Holmstrup et al. 2002; Holmstrup and Westh 1994).

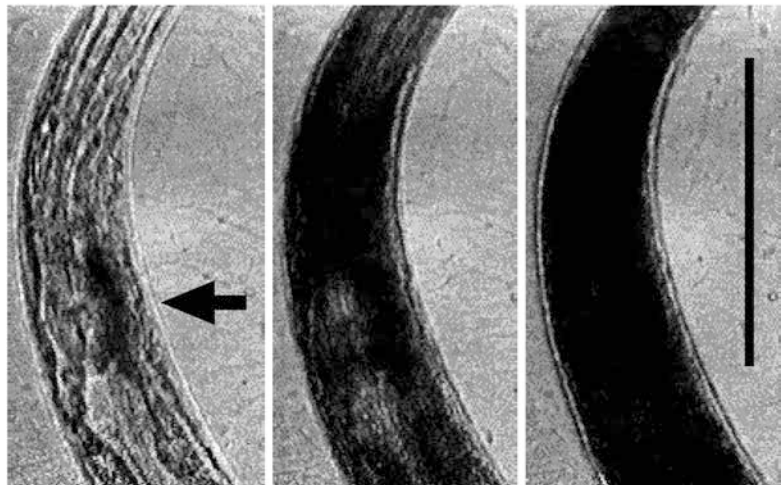
Our understanding of the cold tolerance strategies of Antarctic nematodes have undergone a number of significant shifts. At first the techniques used for studying the cold-tolerance mechanisms of arthropods were applied to nematodes (Pickup 1990a, 1990b, 1990c). This involved attaching nematodes to thermocouples to record freezing exotherms, after removing surface water

and coating with liquid paraffin to prevent desiccation. Under these conditions nematodes supercool and show mainly a freeze-avoiding strategy. Supercooling points vary seasonally and are also related to feeding activity. If the supercooling point was relatively high some nematodes survived, suggesting some freezing tolerance ability (Pickup 1990a, 1990b, 1990c).

Nematodes are essentially aquatic animals, requiring at least a film of water around soil particles for movement, growth and reproduction (Wharton 1986). They are thus much more likely to be exposed to inoculative freezing (ice from the surrounding water travelling through a body opening, or across the cuticle, to cause the freezing of the animal) than are the fully-terrestrial arthropods (Wharton 1995). Since nematodes are transparent, the formation of ice in their bodies can be observed on a cold microscope stage. *P. davidi*, isolated from Cape Bird Ross Island, has been the main model for these studies. When frozen in water, under conditions where there is a relatively rapid spread of ice through the sample, the ice enters the nematode's body via body openings—in particular the excretory pore (Wharton and Ferns 1995). The ice then spreads rapidly throughout the body and eventually all compartments freeze (Fig. 2). Freezing is not confined to the extracellular spaces and the nematode survives intracellular freezing (Wharton and Ferns 1995; Wharton et al. 2003). *P. davidi* is the only animal known to survive extensive intracellular freezing. It has survived exposure to -40°C (Wharton and Block 1997) and to -80°C (Wharton and Brown 1991) with high levels of survival. Freezing tolerance appears to be widespread amongst Antarctic nematodes (Convey and Worland 2000; Wharton and Block 1993). Freezing tolerance occurs in larval and adult nematodes but the eggs of *P. davidi* can survive by freeze avoidance, since the eggshell prevents inoculative freezing (Wharton 1994).

There is a clear relationship between the survival of *P. davidi* and the freezing rate of the sample, which in turn is dependant upon the sample volume and temperature (Wharton et al. 2002). Survival is favoured by

Fig. 2 Freezing of *P. davidi* starts near the posterior bulb of the oesophagus (arrow) and spreads throughout the body until all compartments, including intracellular compartments, freeze. From Wharton and Ferns (1995), reproduced with the permission of the Company of Biologists (scale bar = 100 μm)



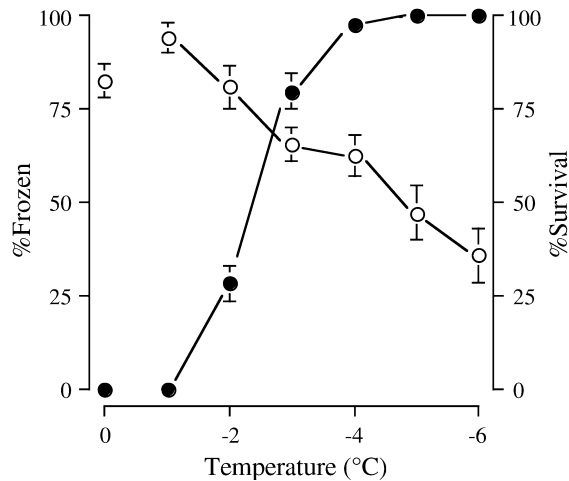


Fig. 3 The effect of temperature on the freezing (filled circles) and survival (open circles) of *P. davidi*. Samples were nucleated and held at the test temperature for 30 min. Samples at 0 °C were unfrozen controls. From Wharton et al. (2003), reproduced with the permission of the Company of Biologists

slow freezing rates. At fast freezing rates a proportion of nematodes appear to be physically damaged by the growth of ice crystals in the surrounding medium, with body contents protruding through breaks in the cuticle. Similar damage after freezing has been observed in other Antarctic nematodes (Convey and Worland 2000). Slow freezing rates (in samples nucleated at high subzero temperatures) tend to inhibit inoculative freezing and enhance survival (Fig. 3). In samples frozen at -1 °C no inoculative freezing occurred; instead the nematodes became visibly dehydrated (Wharton et al. 2003) and survived by cryoprotective dehydration (Holmstrup and Westh 1994). The dehydration is driven by the difference in vapour pressure between supercooled water and ice at the same temperature (Fig. 4). In samples frozen at lower temperatures, the nematodes freeze by inoculative freezing but a proportion survive (Fig. 3), confirming that they are freezing tolerant (Wharton et al. 2003). *P. davidi* thus appears to have a variety of strategies for surviving low temperatures. Using a freeze-substitution technique and transmission electron microscopy to visualise ice at an ultrastructural level it can be seen that increasing freezing rate produces a shift from cryoprotective dehydration to extracellular freezing to intracellular freezing (D.A. Wharton and Downes, unpublished results).

Cold tolerance mechanisms of Antarctic terrestrial nematodes

Studies on cold tolerance mechanisms have focused on *P. davidi*, given its ease of culture. After inoculative freezing of this nematode the freezing process can be completed extremely rapidly (0.2 s), facilitated by the small size of the animal (Wharton and Ferns 1995). This is much faster than in other freezing-tolerant animals

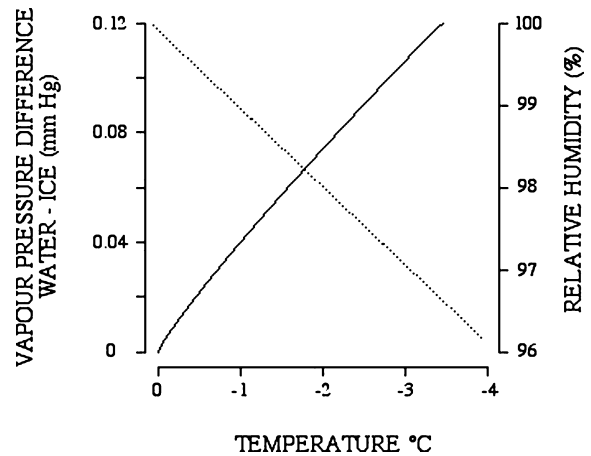


Fig. 4 The difference in the vapour pressure of supercooled water and ice at various temperatures (solid line) and the relative humidities generated (dotted line). Data from Weast (1989)

and may aid survival since it would prevent the osmotic stress that would result if different body compartments froze at different times. During freezing 82% of body water is converted into ice, about the limit of freezable water in biological systems (Wharton and Block 1997). Nematodes grown at 20 °C and then acclimated to lower temperatures show an increase in the production of the disaccharide trehalose and increased survival (Wharton et al. 2000). Trehalose is known to act as a cryoprotectant in some animals and may also provide protection against the effects of dehydration. A number of nematode species have been shown to synthesise trehalose in response to desiccation and/or low temperatures (Wharton 2002b). In some annelids and frogs cryoprotectants are synthesised during the freezing process itself, rather than as a response to low-temperature acclimation (Storey and Storey 1992; Holmstrup and Sjørnsen 2001). In nematodes the freezing process is probably too rapid to allow this to occur.

There also appears to be a protein component to the cold tolerance mechanisms of this nematode. Proteins that interact with ice (ice-active proteins, IAPs) can be divided into three classes. Ice-nucleating proteins (INPs) trigger ice formation, antifreeze proteins (AFPs) inhibit ice nucleation, whilst recrystallisation-inhibiting proteins (RIPs) have neither of these properties (or have them only weakly) but affect the stability of ice crystals after they have formed. INPs appear to be absent in *P. davidi* and this is to be expected in a freezing-tolerant animal that can rely on inoculative freezing (Wharton and Worland 1998). There is little or no thermal hysteresis activity (a difference between the melting and freezing point in the presence of an ice crystal), suggesting the absence of AFPs (D.A. Wharton and H. Ramløv, unpublished observations). In contrast, there is evidence that *P. davidi* produces a protein that inhibits the activity of organic ice nucleators, a property of AFPs (Olsen and Duman 1997a, 1997b; Wharton and Worland 1998). Antifreeze activity is not likely to be of

advantage to a freezing-tolerant organism but ice nucleation inhibition could assist cryoprotective dehydration by hindering inoculative freezing.

Recrystallisation occurs in ice held at a constant (or varying) subzero temperature. This involves a change in the size distribution of ice crystals as larger crystals grow at the expense of small crystals. This could be damaging to a frozen organism (Knight and Duman 1986; Knight et al. 1988, 1995). Recrystallisation inhibition may be the major function of antifreeze proteins in freezing-tolerant organisms, where antifreeze activity would not assist their survival (Duman 2001). *P. davidi* produces a protein (a RIP) that inhibits recrystallisation (Ramløv et al. 1996). The *P. davidi* RIP has now been isolated, purified and sequenced (G. Goodall, D. A. Wharton and C. J. Marshall, unpublished results). It has no sequence homology to any other IAP. It does, however, belong to a family of nematode-specific proteins of widespread distribution but with no assigned function. The role that this protein plays in freezing tolerance and/or cryoprotective dehydration has yet to be established.

Anhydrobiosis in Antarctic terrestrial nematodes

Although the absolute amount of freshwater in Antarctica is huge, most of it is unavailable to organisms—locked up as ice or attached to soil components (e.g. clay, organic matter). The availability of liquid water is thus a major determinant of the distribution of terrestrial Antarctic organisms and desiccation is a significant challenge to them (Kennedy 1993). The degree of desiccation stress varies in different sites. Some wet moss habitats in the maritime Antarctic are permanently saturated with water, whilst lichen-encrusted bryophyte communities are drier (Kennedy 1993). Communities supported by meltwater from snowbanks may become desiccated when the source of water becomes exhausted (Sinclair and Sjørnsen 2001a). The dry soil habitats of the Dry Valleys region support populations of nematodes, which must be able to survive desiccation (Treonis et al. 2000; Virginia and Wall 1999).

Some species of nematodes are capable of anhydrobiosis, losing all their body water and surviving in an ametabolic, dormant state (Wharton 2002b). Two broad groups of anhydrobiotic nematodes are recognised (Womersley 1987). Slow-dehydration strategists need to be dried slowly before they can survive exposure to extreme desiccation. They rely on their moss or soil habitat drying slowly to provide the slow rate of water loss necessary for anhydrobiotic survival. Fast-dehydration strategists themselves have the adaptations necessary to slow the rate of water loss and can survive immediate exposure to low relative humidity (Wharton 2002b).

P. davidi is a slow-dehydration strategist and only survives exposure to 0% relative humidity (RH) if it is first dried at 99% or 76% RH (Wharton and Barclay 1993). Drying on a model substrate (agar), that might more closely mimic the drying characteristics of its

environment, did not enhance its survival. Such a treatment is essential for some slow-dehydration strategists to survive desiccation even at 97% RH (Womersley and Ching 1989), suggesting that *P. davidi* can survive faster rates of water loss than these. Slow drying produces coiling, which may reduce the rate of water loss by reducing the surface area of cuticle directly exposed to air. The time taken for nematodes to recover upon rehydration is related to the severity of the stress experienced during desiccation (Wharton 2002b; Wharton and Barclay 1993). Trehalose is produced by *P. davidi* (Wharton et al. 2000) and is important for anhydrobiotic survival (Crowe et al. 1992). Antarctic nematodes are often found in a coiled state and this may indicate that they are in a state of anhydrobiosis (Treonis et al. 2000).

Pickup and Rothery (1991) compared the rates of water loss and anhydrobiotic survival of *Ditylenchus* sp. B (= *Ditylenchus parcevivens*, Andrassy 1998) and *Teratocephalus tilbrooki* at Signy Island. *D. parcevivens* inhabits erect branching thalli of a fructiose lichen (*Usnea* spp.) and hence might be expected to be exposed to high rates of water loss, whilst *T. tilbrooki* inhabits moss carpets (*Andreaea* spp.) that would produce slow rates of water loss when exposed to desiccation. Rates of water loss of *T. tilbrooki* at 60% RH/5 °C were high but nevertheless the nematodes could survive desiccation for many days. Survival of 0% RH was not reported for this nematode. *D. parcevivens* has much slower rates of water loss and could survive direct exposure to 0% RH (Pickup and Rothery 1991). It thus has the characteristics of a fast-dehydration strategist. The desiccation responses of *T. tilbrooki* are unusual. It does not have the characteristics of a slow-dehydration strategist, despite its habitat, since it will survive high rates of water loss. The only other nematode reported with these characteristics is a *Plectus* sp. isolated from moss growing on a flat roof (Hendriksen 1982).

Osmotic stress and Antarctic terrestrial nematodes

Some Antarctic soils are highly saline (Virginia and Wall 1999) and inputs of water, from precipitation or melting snow and ice, may subject nematodes to sudden variations in osmotic stress. Soil nematodes in general may have to tolerate three- to fivefold variations in external osmotic pressure as a result of rainy or drought conditions (Thompson and Geary 2002). Since nematodes are reliant upon a high internal turgor pressure within the pseudocoel for coordinated movement to occur (Wharton 1986), the ability to osmoregulate in the highly variable osmotic environment provided by Antarctic soils is likely to be of great importance. Osmoregulation in nematodes may involve the permeability of the cuticle and epidermis, the production of organic osmolytes (such as trehalose, glycerol, amino acids and urea) to balance hyperosmotic stress and the removal of excess water (via the excretory system or the intestine) during

hypoosmotic stress (Thompson and Geary 2002). None of these mechanisms have been investigated in Antarctic nematodes, although *P. davidi* is known to synthesise trehalose and glycerol (Wharton et al. 2000) and these could be acting as osmolytes.

P. davidi shrinks during exposure to single salt solutions ($0.1\text{--}0.4\text{ mol l}^{-1}$ NaCl). There is no recovery in length in hyperosmotic solutions, suggesting an inability to osmoregulate (Viglierchio 1974). Single salt solutions may not, however, give an accurate picture since other ions may be required for osmoregulation to occur (Wright and Newall 1976, 1980). *P. davidi* appears to be isotonic to $<0.05\text{ mol l}^{-1}$ NaCl (Viglierchio 1974). The survival of *P. davidi* declines with increasing osmolality. It can survive exposure to distilled water for at least 24 h (Wharton and To 1996), which may indicate an ability to osmoregulate in hypoosmotic solutions.

Interacting/changing stresses and Antarctic terrestrial nematodes

Although temperature, water and osmotic stress have been considered separately so far in this review they clearly have important interactions. When a solution starts to freeze the osmotic concentration of the unfrozen portion is raised as salts are excluded from the growing ice crystals (Shepard et al. 1976). This freeze concentration effect could dehydrate nematodes as a result of hyperosmotic stress. This may be sufficient to prevent the freezing of nematodes (Forge and Mac-Guidwin 1992). Inoculative freezing of *P. davidi* can still occur, however, in solutions of osmolalities up to $1130\text{ mosmol l}^{-1}$ and if salt concentrations are raised by a factor of 120 (Wharton and To 1996). Cryoprotective dehydration occurs in distilled water, in which a freeze concentration effect is not possible (Wharton et al. 2003) and may be the dominant effect during the freezing of soil water. There may, however, be an interaction between osmotic stress and cold tolerance. *P. davidi* survives a freezing stress better at intermediate, rather than high or low osmolalities (Wharton and To 1996). Water loss from soil through dehydration will also raise the osmotic concentration of soil water.

In *P. davidi* cryoprotective dehydration is favoured by high nucleation temperatures and slow cooling rates producing slow rates of freezing (Wharton et al. 2003). Cooling rates are likely to be slow in Antarctic soils. Sinclair and Sjørnsen (2001b) recorded maximum cooling rates of $0.021\text{ }^{\circ}\text{C min}^{-1}$ at Keble Valley, Cape Bird. In soil saturated with water the large number of nucleators present and slow cooling rates are likely to produce high nucleation temperatures and slow rates of freezing. This will favour cryoprotective dehydration. As the soil dries out, however, the nematodes will be in decreasing volumes of water and this will produce lower nucleation temperatures and faster freezing rates. Since the nematodes have only a restricted ability to resist inoculative freezing (Wharton et al. 2003) this will favour freezing

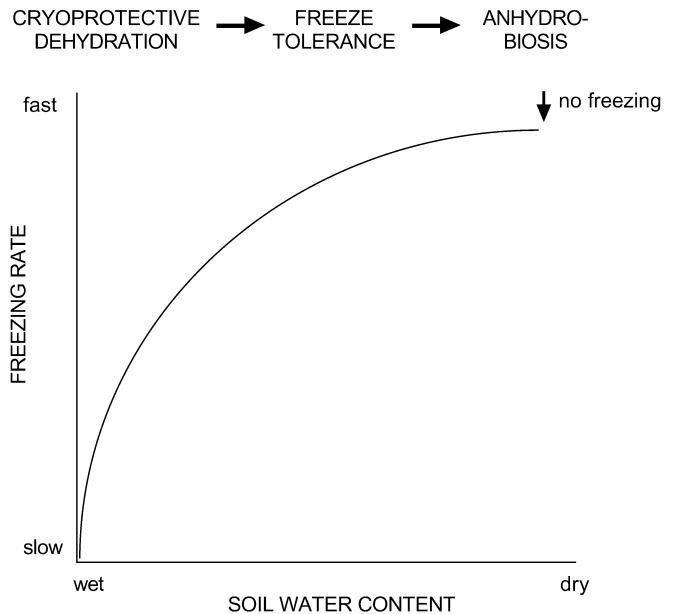


Fig. 5 The response of a soil nematode to freezing temperatures will depend upon the temperature of ice nucleation and the volume of soil water. If the water content is high the nucleation temperature that results in soil freezing will be high (given the large number of soil ice nucleators and the large volume of solution) and the freezing rate will be slow, favouring a cryoprotective dehydration mechanism. As the soil dries out the nematode will be in smaller volumes of fluid with lower nucleation temperatures. Freezing rates will thus be high, favouring inoculative freezing and freezing tolerance. As the soil continues to dry the nematode will become free of surface water, desiccate and survive in a state of anhydrobiosis. The form of the relationship between freezing rate and soil water content is hypothetical

tolerance. Eventually the soil will dry out completely and the nematodes survive anhydrobiotically (Wharton and Barclay 1993). There may thus be a shift from cryoprotective dehydration to freezing tolerance and then to anhydrobiosis as the soil loses water (Fig. 5).

Conclusions

Terrestrial Antarctic nematodes live in an extremely harsh and variable environment. Their repertoire of survival mechanisms allows them to persist in the face of extreme physical challenges but the relative importance of these (including freezing tolerance, cryoprotective dehydration, anhydrobiosis) is unclear. We need to apply techniques that can more directly assess the conditions that nematodes are exposed to in Antarctic soils. This may be difficult since nematodes have to be removed from their environment in order to study them, a process that changes their physiological state (Wharton 1995). More natural rates of change of temperature and water content need to be used in our experiments.

We have much to learn of the mechanisms underlying the survival mechanisms of nematodes. Such an understanding may have important implications for the

storage of biological materials and our interpretation of the ecology of terrestrial Antarctic organisms.

Acknowledgements I am grateful to Antarctica New Zealand who have supported my visits to Antarctica. Our current work on the ice active proteins of Antarctic nematodes is funded by the Marsden Fund of the Royal Society of New Zealand.

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