Open access • Journal Article • DOI:10.1111/J.1365-3032.2005.00481.X

Rapid cold-hardening in a Karoo beetle, Afrinus sp. — Source link

Brent J. Sinclair, Steven L. Chown

Institutions: Stellenbosch University

Published on: 01 Mar 2006 - Physiological Entomology (Blackwell Science Ltd)

Topics: Cold hardening

Physiological Entomology (2006) **31**, 98–101 DOI: 10.1111/j.1365-3032.2005.00481.x

SHORT COMMUNICATION

Rapid cold-hardening in a Karoo beetle, *Afrinus* sp.

BRENT J. SINCLAIR and STEVEN L. CHOWN
Spatial, Physiological and Conservation Ecology Group, Department of Botany and Zoology, University of Stellenbosch, South Africa

Abstract. In the insect rapid cold-hardening response, survival at subzero temperatures is greatly improved by a brief pre-exposure at a milder temperature. It is predicted that insects with minimal cold tolerance capabilities living in variable environments should use rapid cold-hardening to survive sudden cold snaps. This is tested in *Afrinus* sp., a beetle that lives in an exposed habitat on rock outcrops in the Karoo Desert, South Africa, where microclimate temperatures drop infrequently to below freezing. *Afrinus* sp. shows a significant rapid cold-hardening response: survival of a 2-h exposure to −6.5 °C is much improved after pre-exposure to −2 °C, to 0 °C with a 2-h return to the rearing temperature, and to 40 °C, but not after pre-exposure to 0 °C. Little is known about the mechanism of the rapid cold-hardening response, although the data suggest that rapid cold-hardening may be mediated via several different mechanisms.

Key words. Cold tolerance, microclimate, Tenebrionidae, Pimeliinae.

Introduction

In general, insects survive subzero temperatures by either maintaining their body fluids in a liquid state at temperatures where they might otherwise be expected to freeze (freeze avoidance) or by withstanding the formation of internal ice (freeze tolerance) (Bale, 2002; Sinclair *et al*., 2003c). A global-scale analysis has suggested that the latter strategy is more common in temperate latitudes of the Southern Hemisphere than in the Northern, possibly because the unpredictable fluctuation of temperatures around freezing in the oceanic-influenced Southern Hemisphere favors a strategy that does not require prolonged seasonal build-up to become cold tolerant (Sinclair *et al*., 2003a). However, the majority of insect species show only opportunistic survival of subzero temperatures, and mortality in these species generally results from factors other than freezing (Bale, 1996; Chown & Nicolson, 2004). Furthermore, some groups (e.g. the Collembola; see Cannon & Block, 1988) may be phylogenetically constrained to freeze avoidance, and are thus unable to adopt freeze tolerance, regardless of its fitness benefits. In these circumstances, it has been hypothesized that the rapid cold-hardening response (for a comprehensive review, see Chown & Nicolson, 2004) may be of considerable ecological importance in allowing insects to respond to unexpected cold snaps (Lee *et al*., 1987).

Rapid cold-hardening comprises a phenomenon whereby the survival of an insect at a low temperature can be considerably increased by pre-exposure to a less severe low temperature, or other environmental stresses (Bale, 2002). For example, survival of a 2-h exposure at −10 °C by adult *Sarcophaga crassipalpis* is increased from 5 to 99% after a 2-h pre-exposure at 0 °C (Lee *et al*., 1987), and such an improvement in survival has now been recorded in a wide variety of species (Chown & Nicolson, 2004). Kelty & Lee (2001) have shown that rapid cold-hardening is induced in *Drosophila melanogaster* under a seminatural thermoperiod, suggesting that rapid cold-hardening is a readily-utilized mechanism for surviving short periods of subzero temperatures that may be experienced during daily temperature cycles in the field.

Thus, it is predicted that, in the variable environments of the Southern Hemisphere, freeze-intolerant insects, including those that survive low temperatures opportunistically, should utilize the rapid cold-hardening response to survive unpredictable cold snaps (Sinclair & Chown, 2005). In the present study, an initial test of this hypothesis is made using *Afrinus* sp. (Coleoptera: Tenebrionidae: Pimeliinae), a small (approximately 160 mg) beetle collected from beneath flat stones on the surfaces of rock outcrops in the Karoo desert of South Africa. *Afrinus* sp. only has a limited cold tolerance, with a mean temperature of crystallization of −6.8 °C, and some degree of prefreeze mortality (Sinclair & Chown, 2005).

Correspondence: Brent Sinclair, Department of Biological Sciences, University of Nevada, 4505 Maryland Parkway, Las Vegas, NV 89154-4004, U.S.A. Tel.: +1 702 895 5917; fax: +1 702 895 3956; e-mail: celatoblatta@yahoo.co.uk

A rapid cold-hardening response is shown in *Afrinus* sp., and this response is placed into the context of temperatures measured in a representative field microhabitat.

Materials and methods

Afrinus sp. were collected by hand from beneath small rocks on rock outcrops at Ezeljacht farm, near Sutherland, Northern Cape, South Africa (1550 m above sea level, 32.4105 °S 20.57747 °E). Beetles were returned to the laboratory in plastic containers and kept en masse at 22 °C under an LD 12 : 12 h photoperiod with lichens from the collection site for at least 7 days before use in rapid cold-hardening experiments. Moisture was provided daily in the form of a 1-cm diameter ball of damp cotton wool. Voucher specimens are lodged in the South African National Arthropod collection (accession number AcP 9494).

Rapid cold-hardening

To detect a rapid cold-hardening response, a discriminating temperature was determined at which survival was low, and survival at this temperature was then compared with that of individuals subjected to a mild pretreatment. To determine the discriminating temperature, a plunge protocol based on the method of Nunamaker (1993) was used, in which groups of five beetles ($n = 1$ group per temperature, except for −6.5 °C, where $n = 2$ groups) in 1.5-mL tubes were exposed to subzero temperatures for 2 h (−5, −5.5, −6, −6.5, −7 °C, Grant LTD 20 water bath, Grant Instruments, U.K). A test temperature of −6.5 °C was chosen because this temperature elicited approximately 20% survival (Fig. 1). Five groups of five beetles per treatment were placed in 1.5-mL tubes and pre-exposed for 2 h to 0, −2 and 40 °C, as well as to 0 °C with a 2-h recovery period at 22 °C ('0 gap'), and a control (22 °C). The gap treatment was used because stress protein responses to low temperatures generally take place after the exposure rather than during it (Denlinger & Lee, 1998). Survival was compared between each treatment and the control using a resampling technique employing the 'Resampling Stats for Excel 2.0' add-in (Resampling Stats, Inc., Arlington, Virginia). Pairwise comparisons between treatments and controls were made using multiple independent-sample *t*-tests with significance was determined by a table-wide resampling with replacement protocol (1000 iterations; Manly, 1997) followed by step-up false discovery rate correction (Garcia, 2004).

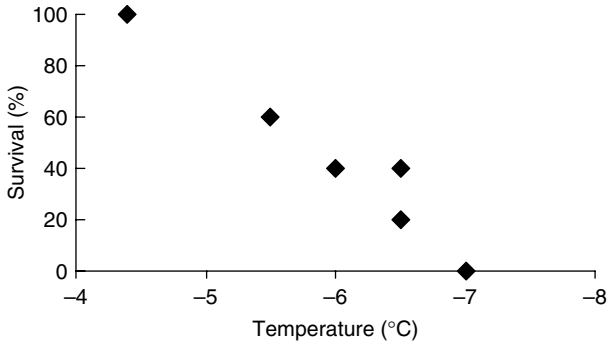

Fig. 1. Survival of adult *Afrinus* sp. after 2-h exposures to subzero temperatures (n = five individuals per point).

Microclimate monitoring

Microclimate temperatures were measured hourly using a thermochron iButton (± 0.5 °C) (Dallas Semiconductor, Dallas, Texas, U.S.A) placed beneath a large flat rock in the locality where *Afrinus* sp. were collected. Temperatures were recorded from 8 May 2003 to 19 July 2004.

Mean temperature and the number of events below the two low temperature treatments (0 and −2 °C) were calculated across the entire logging period, with the latter using macros modified from those described by Sinclair (2001). Further summary statistics (mean daily maximum and mean daily minimum) were calculated for two periods representating summer (December 2003 to March 2004) and winter (June to September 2003).

Results and discussion

Afrinus sp. displayed a significant rapid cold-hardening response to a pre-exposure temperature of −2 °C, to 0 °C with a 2-h recovery period, and to a 2-h high temperature (40 °C) exposure (Fig. 2). A 2-h exposure to 0 °C did not

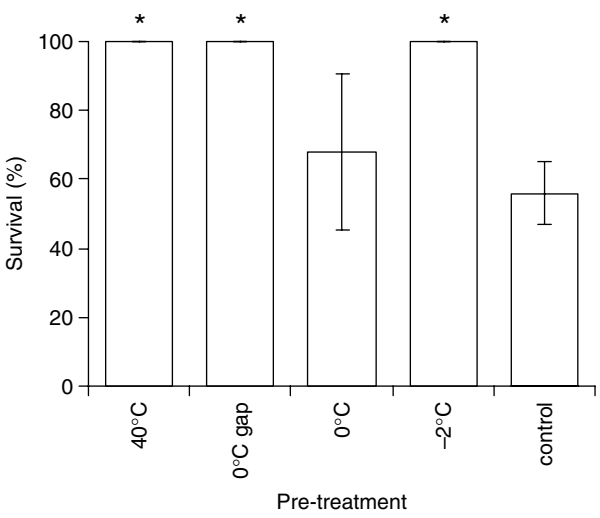

Fig. 2. Increase in survival of subzero temperature exposure after pretreatments in adult *Afrinus* sp., indicative of rapid cold-hardening. The asterisk indicates that survival for 2 h at −6.5 °C after 2-h pretreatments was significantly better than in control groups ($P < 0.01$); 0 °C gap refers to a 2-h treatment at 0 °C followed by 2 h at the acclimation temperature (22 °C). Although percentages are presented for ease of presentation, statistical comparisons were conducted on raw count data (see methods).

elicit significantly greater survival compared with the control group, although survival in the 0 °C group was more variable than in the controls (Fig. 2).

The use of rapid cold-hardening to survive fluctuating environmental temperatures in the field has been postulated for *Drosophila melanogaster* (Lee *et al*., 1987; Kelty & Lee, 1999, 2001), and demonstrated for Antarctic Collembola (Worland & Convey, 2001; Sinclair *et al*., 2003b). Field microclimate temperatures dropped below 0 °C on multiple occasions during the logging period, but only continued to decrease to below −2 °C on two occasions (Table 1). The minimum temperature recorded (−4 °C) is not expected to result in mortality of *Afrinus* sp., but mean minimum July air temperatures well below this value are common at nearby Sutherland (e.g. −5.8 °C in 2004), where absolute minimum temperatures below −11 °C (the record is −13.6 °C) are not uncommon (data provided by the South African Weather Services). In practice, the rock under which the logger was placed was two- to three-fold larger (both area and thickness) than the rocks commonly lifted to collect beetles and, accordingly, the temperatures presented are likely more buffered than the general habitat, and thus present a conservative estimate of microclimate temperatures. Therefore, it is probable that rapid cold-hardening is an important survival strategy in some microhabitats in at least some years and, occasionally, may be essential for survival in any habitat. It is such infrequent severe events that often determine the strategies of animals (Gaines & Denny, 1993; Grant & Grant, 1993).

Afrinus sp. displays rapid cold-hardening in response to low temperature pretreatments, as well as to high temperature pretreatment (as described in several other species) (Burton *et al*., 1988; Chen & Denlinger, 1992; Sinclair & Chown, 2003). The latter response is likely to be a consequence of the production of heat-shock proteins and other cellular protectants (Denlinger *et al*., 1991). The induction of rapid cold-hardening in response to the '0 gap' treatment is consistent with this hypothesis: heat-shock proteins are not produced during the cold shock response in several species but are produced during subsequent recovery (Denlinger & Lee, 1998; Chown & Nicolson, 2004), and may impart the increased cold tolerance observed in the present study. It is hypothesized that the increase in cold tolerance after heat shock in the caterpillars of the tineid moth, *Pringleophaga marioni*, might also be a consequence of the production of heat-shock proteins (Sinclair & Chown, 2003). However, a lack of rapid cold-hardening in the treatment at 0 °C (which has proven effective in most other studies; Chown & Nicolson, 2004) and the observation of rapid cold-hardening in beetles held at −2 °C suggests that rapid cold-hardening may not be entirely due to this induction, or that the rapid cold-hardening effect may be elicited via several alternative pathways. The microclimate temperature dropped below 0 °C many more times than it dropped below −2 °C (Table 1). Therefore, it is possible that natural selection has acted to reduce the rapid cold-hardening response to 0 °C in this species to minimize any costs associated with unnecessary cold-hardening. Generally, the mechanisms underlying the rapid cold-hardening response (and their associated fitness costs) are not well-understood (for reviews, see Denlinger & Lee, 1998; Chown & Nicolson, 2004). However, recent work (Michaud & Denlinger, 2004; Chown & Storey, 2005) has shown that there are significant differences in the roles of Hsp23 and Hsp70 during rapid cold-hardening in *Sarcophaga crassipalpis*, despite both being up-regulated during the process. Other cryoprotectants, such as polyhydric alcohols, are also up-regulated during rapid cold-hardening in some species, and their role cannot be ruled out in the *Afrinus* sp. Clearly, further studies are required before the mechanisms underlying rapid cold-hardening in this and other species are thoroughly understood.

In conclusion, *Afrinus* sp. shows a rapid cold-hardening response, and this may be sufficient to allow survival of unexpected cold snaps in the field. However, the geographical and phylogenetic distribution of the rapid cold-hardening response among insects has not been thoroughly explored (Chown & Nicolson, 2004), and it is not yet clear whether rapid cold-hardening is an ubiquitous aspect of insect physiology, or an adaptation to environments where cyclic temperatures prevail.

Table 1. Temperature summary for habitat typical of the localities where *Afrinus* sp. were collected at Sutherland.

Period	Temperature (°C)
Summary for entire period (8 May 2003 to 19 July 2004)	
Mean	13.7
No. events below 0 °C	21
No. events below −2 °C	2
Winter 2003	
Mean	7.4 ± 0.1
Mean ± SE daily maximum	14.8 ± 0.5
Mean ± SE daily minimum	3.2 ± 0.3
Absolute maximum	26.0
Absolute minimum	− 4.0
Summer 2003/2004	
Mean	20.5 ± 0.1
Mean ± SE daily maximum	29.3 ± 0.4
Mean ± SE daily minimum	13.1 ± 0.3
Absolute maximum	39.0
Absolute minimum	2.0

'Winter' measurements were taken from June to September; 'Summer' measurements were taken from December to March.

Acknowledgements

This work was supported by the National Geographic Society and the New Zealand Foundation for Research, Science and Technology. Beetles were collected under Northern Cape Nature Conservation Permit no. 058/2003. We are grateful to the Steenkamp family for access to land at Sutherland, to Riaan Stals for beetle identifications, and to Mhairi McFarlane for field assistance.

References

Bale, J.S. (1996) Insect cold hardiness: a matter of life and death. *European Journal of Entomology*, **93**, 369–382.

Bale, J.S. (2002) Insects and low temperatures: from molecular biology to distributions and abundance. *Philosophical Transactions of the Royal Society of London B*, **357**, 849–861.

Burton, V., Mitchell, H.K., Young, P. & Petersen, N.S. (1988) Heat-shock protection against cold stress of *Drosophila melanogaster*. *Molecular and Cellular Biology*, **8**, 3550–3552.

Cannon, R.J.C. & Block, W. (1988) Cold tolerance of microarthropods. *Biological Reviews*, **63**, 23–77.

Chen, C.P. & Denlinger, D.L. (1992) Reduction of cold injury in flies using an intermittent pulse of high-temperature. *Cryobiology*, **29**, 138–143.

Chown, S.L. & Nicolson, S.W. (2004) *Insect Physiological Ecology. Mechanisms and Patterns*. Oxford University Press, U.K.

Chown, S.L. & Storey, K.B. (2005) Linking molecular physiology to ecological realities. *Physiological and Biochemical Zoology*, in press.

Denlinger, D.L. & Lee, R.E. Jr (1998) Physiology of cold sensitivity. *Temperature Sensitivity in Insects and Application in Integrated Pest Management* (ed. by G. J. Hallam and D. L. Denlinger), pp. 55–95. Westview, Boulder, Colorado.

Denlinger, D.L., Joplin, K.H., Chen, C.-P. & Lee, R.E. Jr (1991) Cold shock and heat shock. *Insects at Low Temperature* (ed. by R.E. Lee Jr and D.L. Denlinger), pp. 131–148. Chapman & Hall, New York, New York.

Gaines, S.D. & Denny, M.W. (1993) The largest, smallest, highest, lowest, longest, and shortest: extremes in ecology. *Ecology*, **74**, 1677–1692.

Garcia, L.V. (2004) Escaping the Bonferroni iron claw in ecological studies. *Oikos*, **105**, 657–663.

Grant, B.R. & Grant, P.R. (1993) Evolution of Darwin's finches caused by a rare climatic event. *Proceedings of the Royal Society of London B*, **251**, 111–117.

Kelty, J.D. & Lee, R.E. Jr (1999) Induction of rapid cold hardening by ecologically relevant cooling rates in *Drosophila melanogaster*. *Journal of Insect Physiology*, **45**, 719–726.

Kelty, J.D. & Lee, R.E. Jr (2001) Rapid cold-hardening of *Drosophila melanogaster* (Diptera: Drosophilidae) during ecologically based thermoperiodic cycles. *Journal of Experimental Biology*, **204**, 1659–1666.

Lee, R.E. Jr, Chen, C.-P. & Denlinger, D.L. (1987) A rapid cold-hardening process in insects. *Science*, **238**, 1415–1417.

Manly, B.F.J. (1997) *Randomization, Bootstrap and Monte Carlo Methods in Biology*, 2nd edn. Chapman & Hall, U.K.

Michaud, M.R. & Denlinger, D.L. (2004) Molecular modalities of insect cold survival: current understanding and future trends. *Animals and Environment* (ed. by S. Morris and A. Vosloo), pp. 32–46. Elsevier, Amsterdam.

Nunamaker, R.A. (1993) Rapid Cold-Hardening in *Culicoides variipennis sonorensis* (Diptera, Ceratopogonidae). *Journal of Medical Entomology*, **30**, 913–917.

Sinclair, B.J. (2001) Biologically relevant environmental data: Macros to make the most of microclimate recordings. *Cryo-Letters*, **22**, 125–134.

Sinclair, B.J. & Chown, S.L. (2003) Rapid responses to high temperature and desiccation but not to low temperature in the freeze tolerant sub-Antarctic caterpillar *Pringleophaga marioni* (Lepidoptera, Tineidae). *Journal of Insect Physiology*, **49**, 45–52.

Sinclair, B.J. & Chown, S.L. (2005) Climatic variability and hemispheric differences in insect cold tolerance: support from southern Africa. *Functional Ecology*, **19**, 214–221.

Sinclair, B.J., Addo-Bediako, A. & Chown, S.L. (2003a) Climatic variability and the evolution of insect freeze tolerance. *Biological Reviews*, **78**, 181–195.

Sinclair, B.J., Klok, C.J. & Scott, M.B. *et al.* (2003b) Diurnal variation in supercooling points of three species of Collembola from Cape Hallett, Antarctica. *Journal of Insect Physiology*, **49**, 1049–1061.

Sinclair, B.J., Vernon, P., Klok, C.J. & Chown, S.L. (2003c) Insects at low temperatures: an ecological perspective. *Trends in Ecology and Evolution*, **18**, 257–262.

Worland, M.R. & Convey, P. (2001) Rapid cold hardening in Antarctic microarthropods. *Functional Ecology*, **15**, 515–524.

Accepted 6 July 2005
First published online 30 September 2005

