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Chill injury at alternating temperatures in *Orchesella cincta* (Collembola: Entomobryidae) and *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae)

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Key words. Cold hardiness, chill injury, low temperature, survival, mortality, Collembola, *Orchesella cincta*, Heteroptera, *Pyrrhocoris apterus*

Abstract. Survival and Lt_{50} after exposures at constant low temperature were compared to the values obtained at alternating temperatures in two active (summer acclimated) temperate terrestrial arthropods. The experimental regimes used interruptions – daily transfers from the lower temperature to various higher temperatures for two hours or to one high temperature for various durations. In both species the alternating conditions improved survival, implying reparation of the chill injury. In the collembolan *Orchesella cincta*, there was a maximum Lt_{50} when the higher exposure temperature was equal to the temperature of rearing (19°C). In the bug *Pyrrhocoris apterus*, Lt_{50} increased strongly with increasing higher temperature from 0 to 15°C, and was subsequently constant over the entire physiological range suitable for development (to 35°C). Exposure at 0°C was harmful if continuously applied, but survival increased, relative to a constant exposure at –5°C, if the temperature alternated between –5 and 0°C.

INTRODUCTION

Laboratory experiments investigating the cold hardiness of terrestrial arthropods are usually performed at constant low temperatures, although in nature the animals are exposed to cyclical temperature fluctuations. The importance of measuring survival in insects at temperatures fluctuating below and above zero, as happens in mild temperate winters, has been stressed by Bale (1989, 1991).

We can hypothesize several different effects on heterothermic animals that may be caused by the fluctuation or alternation between two temperatures. Let us set aside the freeze tolerant species in which the temperature fluctuations may cause freeze-thawing cycles. In freeze susceptible species, the lower temperature (above SCP) causes chill injury (Nedvěd et al., 1998), but may also serve as a signal for triggering increase in cold hardiness. The synthesis of stress proteins is induced by low temperature but occurs only after return to higher temperature (Joplin & Denlinger, 1990; Yocum et al., 1991). At the higher temperature some chill injury may be repaired (Chen & Denlinger, 1992; Nedvěd et al., 1998; Turnock & Bodnaryk, 1991). The higher temperature may allow physiological processes of cold hardening that is cued by the low temperature, but requires the higher temperature for effective expression. Finally, the higher temperature may deacclimate / activate an overwintering animal, which would subsequently suffer more injury at the lower temperature.

In the weevil *Rhynchaenus fagi*, survival i.e. lethal time was much longer at cycling temperatures (–15°C and +2°C cycling over 48 h) than at a constant low temperature (–15°C) (Coulson & Bale, 1996). In contrast, a slight negative effect of alternating temperatures (–5°C, 16 h / +10°C, 8 h) compared to constant –5°C was found in nymphalid butterfly *Inachis io*, and no difference was found in *Aglais urticae* (Pullin & Bale, 1989). In the collembolan *Orchesella cincta*, an increased duration of the exposure at the higher temperature (0.25 to 2 h per day) or higher temperatures (+5 to +19°C) of constant duration (2 h) had an increasingly positive effect on survival (Nedvěd et al., 1998).

Similar to the effect of alternating temperatures is the effect of post exposure conditions. It has been demonstrated that higher post exposure temperatures result in higher survival than lower ones. Turnock & Bodnaryk (1991) reported positive effects on survival of high (≥15°C) temperature exposure following cold stress (3 days at –15°C) in comparison to a lower (≤10°C) post-stress temperature in moth *Mamestra configurata*. When exposure at –15°C for 3 days was followed by exposure at 0°C for various lengths of time before incubation at 20°C, survival decreased linearly with the time spent at 0°C, although long exposure at 0°C only caused no mortality.

A method to quantify cold hardiness in freeze susceptible terrestrial arthropods recently proposed by Nedvěd (1998) is based on summation of degree days of the cold exposure. One of the two parameters calculated from the survival data, sum of injurious temperatures (SIT, DD or K.d), indicates the dose of injurious chilling, i.e. product of exposure duration and the temperature difference between the exposure temperature and the upper limit of the cold injury zone (ULCIZ, °C) that kills a half of a given sample. The model successfully fitted several sets of data on insect survival, from the literature and from original measurements. However, all the data were measurements at constant temperatures. This study examines the use of

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the degree day concept to quantify the amount of chill injury under regimes of two alternating temperatures in two summer-acclimated arthropods.

MATERIAL AND METHODS

The modelling was performed on survival data of the collembolan *Orchesella cincta* (L.) as published previously (see Nedvěd et al., 1998, and results hereafter for rearing and experimental conditions), and on new data of a pyrrhocorid bug.

Rearing and exposure conditions

The bug *Pyrrhocoris apterus* (L.) was reared under long day conditions (18L : 6D, 25°C). Larvae 3–4 days after ecdysis into the final (fifth) instar were used in experiments without any low temperature acclimation.

We measured the supercooling points of 16 individuals together in a single apparatus (Brunnhofer et al., 1991; Nedvěd et al., 1995) using cooling rate 10 K/h. Observation of survival after exposure at a series of constant low temperatures and various durations of exposure (Hanč, 1998) allowed the calculation of the upper limit of the cold injury zone (ULCIZ) and sum of injurious temperatures (SIT, according to Nedvěd, 1998).

Groups of 20 larvae per vial were transferred to a low temperature cabinet at a constant –5°C. The exposure was interrupted daily by transfer to a higher constant temperature. In the first experiment, we used a 2 h exposure at 0, 3, 10, 15, 25, and 35°C. In the second experiment, we used 25°C as the higher constant temperature, and various exposure duration: 0.5, 1, 2, 3, and 4 h.

One group per treatment was removed at daily intervals, and survival assessed as the number of walking larvae after an hour of recover at 25°C.

Calculations of the lethal time

We used the logistic equation to describe the sigmoid curve of the decrease of survival proportion (S) as a function of time (t):

$$S = \frac{e^{a+b \cdot t}}{1 + e^{a+b \cdot t}}$$

The parameters a and b characterize the shape of the curve, with the ratio $-a/b$ equal to the lethal time for 50% of the sample (Lt_{50}). The exposure time in all treatments was recalculated to include only the time spent at the lower temperature (–5°C), e.g. exposure periods with 2 h interruptions were 22, 44, 66 h, etc.

RESULTS

Cold hardiness parameters

The fifth instar larvae of *P. apterus*, from long day conditions, supercooled on average to –8°C. The upper limit of cold injury zone was calculated as +4.2°C, and the sum of injurious temperatures was 34 K.d (data after Hanč, 1998).

The supercooling point in adult *O. cincta*, from long day conditions ranged from –5 to –11°C (according to van der Woude, 1987). ULCIZ calculated according to survival data from Nedvěd et al. (1998) was +1.4°C, SIT about 14 K.d.

Improvement of survival in *P. apterus*

Continuous exposure at –5°C caused 50% mortality after 3.4 days. When repeated transfer to 25°C was applied, survival improved markedly, even when the duration of such an interruption was only 0.5 h. The longer the duration up to 4 h, the longer the resulting Lt_{50} (up to 9.6 days, Figs 1, 2).

Survival was also much improved by the repeated transfer to various temperatures for two hours, even when the temperature was relatively low. The higher the temperature, up to 35°C, the longer the resulting Lt_{50} (up to 8.7 days, Figs 3, 4).

Improvement of survival in *O. cincta*

In summer acclimated (19°C, 16L : 8D) adult *O. cincta*, the Lt_{50} was 3.1 days at a constant –3°C. Survival increased (up to 10 days) when the exposure was interrupted for 0.5, 1, 2, and 4 h at 19°C (Fig. 5), but remained unchanged (3.18 days) if the interruption lasted only 0.25 h. The longest interruption period (4 h) helped slightly less than the nearest shorter period (2 h).

The Lt_{50} was longer than in a constant low temperature when the exposure was interrupted for 2 h at the higher temperatures used (Fig. 6). The highest interruption temperature (30°C) was much less effective in increasing survival than the temperature at which the insects were reared.

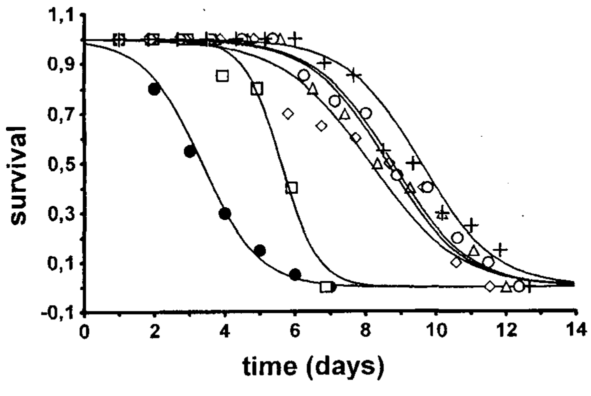

Fig. 1. Survival of *Pyrrhocoris apterus* at constant –5°C (●) and at alternating temperatures –5 / +25°C with various duration of the higher temperature: 0.5 h (□), 1 h (◇), 2 h (△), 3 h (○), and 4 h (+). The curves ordered respectively; plotted by logistic regression.

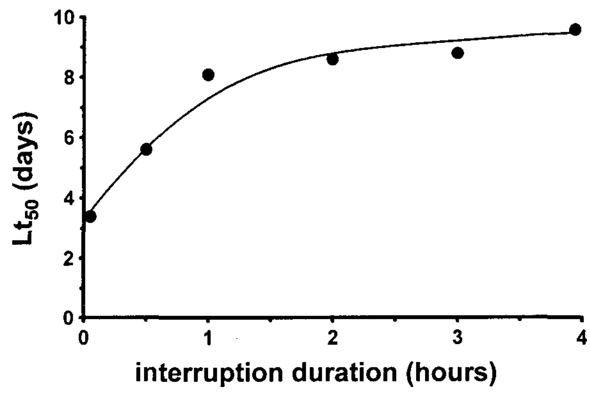

Fig. 2. Prolongation of Lt_{50} in *Pyrrhocoris apterus* by transfer from the low temperature (–5°C) to 25°C for various periods of time. The curve plotted by least squares smoothing.

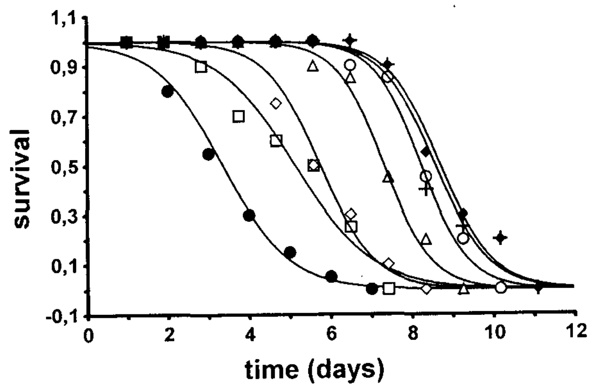

Fig. 3. Survival of *Pyrrhocoris apterus* at constant –5°C (●) and at alternating temperatures from –5 to 0°C (□), 3°C (◇), 10°C (△), 15°C (○), 25°C (+), and 35°C (◆). The curves ordered respectively; plotted by logistic regression.

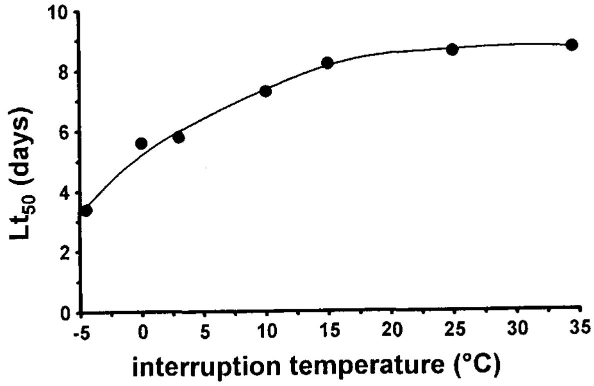

Fig. 4. Prolongation of Lt_{50} in *Pyrrhocoris apterus* by transfer from the low temperature exposure (–5°C) to various temperatures for 2 h. The curve plotted by least squares smoothing.

DISCUSSION

Direction of the effect of high temperature pulses

In both species, survival was higher (Lt_{50} longer) when the exposure at low temperature was interrupted every day for a short period by transfer to a higher temperature. All combinations of duration and temperature had a positive effect, thus agreeing with results on the weevil *Rhynchaenus fagi* (Coulson & Bale, 1996), but contrasting with the butterfly *Inachis io* (Pullin & Bale, 1989).

There is a great physiological difference between the arthropods investigated in this paper and the two studied earlier. Both the bug and the collembolan were taken from summer conditions – long day and high temperature – and were active, not cold acclimated. In such cases it is not surprising that high temperatures decreased mortality caused by chilling through reparation of the cold injury (Nedvěd et al., 1998; Turnock & Bodnaryk, 1991). The weevil and the butterfly were overwintering, cold acclimated. In such samples we may expect two opposite reactions. If the higher of the two alternating temperatures is not too high, just within the range of temperatures that the overwintering insect may encounter, repair of injury may occur and hence a reduction in mortality, as was the case in the weevil. If the higher temperature is too high, it may cause deacclimation as occurs in spring, and the subsequent survival at low temperature will decrease, as in the butterfly.

Effect of various pulse durations

The positive effect of alternating temperatures increased with the duration of the interruption of the cold exposure. However, beyond certain rather short periods, the improvement became constant, i.e. extension of the high temperature pulse produced no further increase in survival. Possibly, the switch from the increasing to the steady improvement (here about 1 h, see Fig. 2) might be shifted to longer durations if lower temperatures were used. In natural fluctuating conditions, the duration of the warm period during the day may last several hours, but the highest temperature of the day in the insect hibernacula may be lower than in our experiments when animals were not acclimated to cold.

Effect of various high temperatures

Although the effect of alternating temperatures was positive in both species, there was an interspecific difference (Figs 4, 6). In *O. cincta*, the effect was weak at lower temperatures and increased almost exponentially with increasing temperature. Very high temperature was either less effective in repair of the cold injury, or it repaired cold injury but also caused some heat injury.

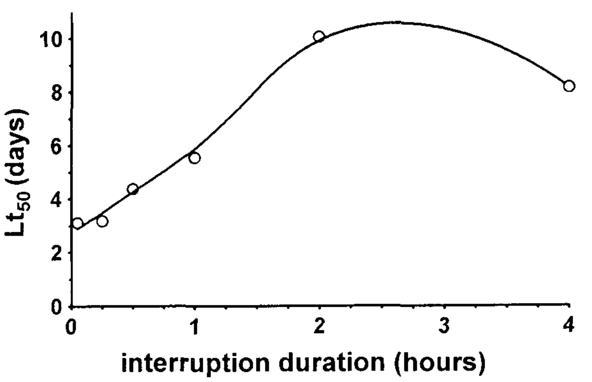

Fig. 5. Prolongation of Lt_{50} in *Orchesella cincta* by transfer from the low temperature exposure (–3°C) to 19°C for various periods of time. The curve plotted by least squares smoothing.

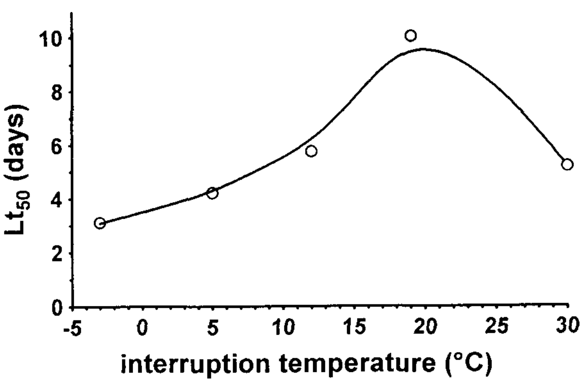

Fig. 6. Prolongation of Lt_{50} in *Orchesella cincta* by transfer from the low temperature exposure (–3°C) to various temperatures for 2 h. The curve plotted by least squares smoothing.

In *P. apterus*, the increase in survival was quite high even at low temperatures, it increased at moderate temperatures and was constant over the entire physiological range suitable for development (LDT 15°C – UDT 35°C, Nováková & Nedvěd, 1999). The higher lethal temperature (38°C) was not used in the experiments. Whether the switch from increasing to constant part of the curve on Fig. 4 would be also close to LDT if a shorter duration of the interruption were applied, or whether it would be at higher temperatures is unknown.

The lower of the two alternating temperatures in the experiments with *P. apterus* was very low, close to the SCP. At higher temperatures there would be less mortality as can be deduced from the known ULCIZ and SIT. In such conditions with less injury there would also be less possibility of repair of the injury during the high temperature interruption.

A different pattern of improvement of survival by a high temperature pulse was found in *Sarcophaga crassipalpis* (see Chen & Denlinger, 1992): interruption of cold exposure had a positive effect on survival of subsequent cold shock when the insects were exposed at +15°C. Neither lower (5, 10) nor higher (20, 25, 30°C) temperatures had the protective effect.

Helpful injurious temperatures

An interesting phenomenon becomes apparent when the improvement of survival by various high temperatures is considered in relation to the upper limit of the cold injury zone. The data on both species can be recalculated in such a way that only the duration of exposure at the lower of the two alternating temperatures is cumulated. Hence, if the higher of the two temperatures has no effect on the animals – neither injurious nor reparative – the resulting Lt_{50} should be the same as in the constant exposure. It seems to be true when the temperatures are close to ULCIZ (1°C) in *O. cincta*. However, temperatures well below the ULCIZ (4°C) in *P. apterus* had a clearly positive effect on survival. This phenomenon makes it impossible to summarize chill injury in this species in alternating temperatures according to a degree-day concept. The parameter SIT (Nedvěd, 1998) is valid only in experiments with constant temperatures.

However, we do not know, whether in the overwintering, cold-acclimated animals, the chill injury is simply cumulative or if there is a repair of injury similar to the situation in *P. apterus*. The present results might be usefully employed in designing regimes for the long-term cold storage of active insects for laboratory experiments or for the timed release of biological control agents.

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