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Journal of Thermal Biology 30 (2005) 557–562

Journal of
THERMAL BIOLOGY

www.elsevier.com/locate/jtherbio

Acclimation, shock and hardening in the cold

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Received 25 May 2005; accepted 20 July 2005

Abstract

Recent articles by Bowler [2005. J. Therm. Biol. 30, 125–130] and Loeschcke and Sørensen [2005. J. Therm. Biol. 30, 255–257] have discussed the relationship between acclimation, hardening and heat shock in ectothermic animals, implying that analogous processes occur at low temperatures. We address this implication using the *Drosophila* literature. Cold tolerance in *Drosophila* has been measured in response to hardening (usually 1–3 h) or acclimation (usually days–weeks) using at least 27 different duration–intensity combinations. The metrics of response to these treatments include chill coma recovery or onset, survival and measures of reproductive success. However, it is unclear whether the mechanisms underlying the different metrics are the same, or whether the causes of injury are related over duration–intensity exposures ranging from a few minutes to hundreds of days. Furthermore, whilst there is ample evidence for acclimation and cold hardening in *Drosophila*, there is no clear evidence for a cold-shock response analogous to the well-characterised heat-shock response.

Keywords: *Drosophila*; Heat shock; Cold shock; Cold tolerance; Chill coma

1. Introduction

Bowler (2005) and Loeschcke and Sørensen (2005) have recently commented on the usage and definition of the terms 'acclimation', 'heat shock' and 'hardening' as they apply to (largely poikilothermic) animals. One of the key points of this debate is the question of whether these three processes (or the organism's responses to them, which often go by the same names) are in some sense different scales of the same thing. Loeschcke and Sørensen (2005) conclude that the three treatments (or responses) lie along a continuum defined by the temporal and thermal kinetics of the response, and that similar terms apply equally well to the cold end of the thermal scale. However, as we elaborate below, the effects of low and high temperatures on cells and organisms are qualitatively different, and these differences may be useful for informing the debate about whether acclimation, shock and hardening represent discrete categories or a continuum.

2. Treatments, responses and metrics

As with studies performed at high temperatures, some ambiguity exists as to whether acclimation can be both a response and a treatment (Loeschcke and Sørensen, 2005). At low temperatures, studies have tended to examine either acclimation (usually by way of longer-term exposure to slightly lower-than-rearing temperatures) or hardening (a shorter exposure to a much colder temperature—specifically rapid cold hardening, sensu (Lee et al., 1987). The responses to hardening and

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doi:10.1016/j.jtherbio.2005.07.002

acclimation are usually measured similarly—exposure to one or many episodes of cold varying into intensity and duration, followed by a measurement of some sort of organismal function (the metric).

The ways in which the response to low temperatures is determined are many, varied, and not easily comparable. Fig. 1 and Table 1 show 27 different duration vs. intensity treatments in studies of adult *Drosophila* spp. exposed to cold. These range from very long periods at relatively mild temperatures to acute exposures to much lower temperatures, yet most were referred to as measures of 'cold tolerance' by the authors. Historically, the insect cold tolerance literature focussed on low temperature damage associated with freezing (Salt, 1961), although this trend has recently given way to a more pluralistic view with the recognition that there are several forms of non-freezing injury as well (Bale, 2002).

Non-freezing injury has generally been divided into acute ('direct' or 'cold shock') injury caused by brief exposures of greater intensity and chronic ('indirect' or 'chilling') injury according to the period of time for which the insects were exposed. A third category (called 'latent chilling injury' by Turnock and Bodnaryk, 1991) refers to cold-induced damage that manifests later in development (for example as reduced fertility or developmental defect), but which has been only rarely investigated. Nedved (2000) proposed a system of classifying insect cold tolerance strategies based primarily upon the type of injury incurred. The points on Fig. 1 may be broadly (and arbitrarily) divided to reflect methods that are looking for responses that offer protection from acute and chronic cold injury. Latent chilling injury is distinguished by a change in the metric from survival to development or fecundity and could potentially be examined using either acute or chronic exposures.

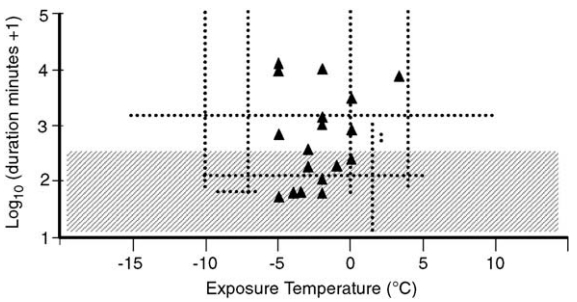

Fig. 1. Range of temperature–time exposures for 27 published methods of measuring cold tolerance in *Drosophila* species. Triangles represent single temperature–duration studies, vertical dotted lines indicate methods where duration was varied at a fixed temperature (to measure Lethal Time 50), horizontal lines where temperature was varied at a fixed duration (Lethal Temperature 50). Shaded box indicates all exposures of less than 6 h. Note logarithmic *Y* axis.

The mechanisms of acute and chronic cold injury are poorly understood, although it has been hypothesised that acute damage may be a consequence of immediate damage to cell membranes (Lee, 1991), while chronic damage may result from gradual equilibration of transmembrane ion gradients (Kostal et al., 2004). Thus, the literature examining responses to cold actually lies on two different trajectories (survival of acute or chronic cold injury) whose mechanistic link has not been established. By contrast, the effects of high temperatures at the molecular, cellular and organismal levels are reasonably well-established over a variety of durations (reviewed by Chown and Nicolson, 2004; Feder and Hofmann, 1999; Hochachka and Somero, 2002).

Not all metrics of acclimation or hardening responses are focussed on injury or survival. Although survival responses are easy to measure as a threshold trait, they are at the extreme end of a continuum of effects that may include a temporary or permanent loss of function, behaviour or fertility, all with attendant fitness consequences. For example, the temporal kinetics of chill coma induction/recovery has been a widely used metric of acclimation and hardening responses in adult *Drosophila*. This kind of assay has several advantages—it is non-lethal, shows variation that corresponds intuitively to geography, species distributions and acclimation, and allows selection both for and against the trait (David et al., 2003; David et al., 1998; Huey et al., 1992). As a direct measure of organismal function at low ambient temperatures, there are strong arguments to be made for chill coma temperature being an important and relevant metric (e.g. Gibert et al., 2001).

The relationship between low temperature survival and chill coma recovery has been demonstrated in interspecific studies showing covariance of the two traits among *Drosophila* species, suggesting that selection and acclimation on these traits may be related (Hori and Kimura, 1998). However, we invoke the age-old warning that 'correlation does not imply causation' when addressing these data: although a lack of correlation reasonably implies that the mechanisms behind two phenomena are different, it is far more of a leap to assert that a correlation is underpinned by a shared mechanistic basis. When acclimated to cold, an organism likely regulates a number of metabolic pathways. Because the organism is then shown to have better survival at low temperatures (or to regain activity faster, or lose fertility slower, or retain behaviours that would otherwise be lost) does not necessarily imply that each of the upregulated pathways observed is associated with all of the responses. Thus, not only may the measures of the responses of insects to acclimation be confounding two distinct types of injury, the metrics for the measure of 'tolerance' to cold or chilling, in *Drosophila* at least, may be confounding two or more different processes under a single name. An understanding of the causes of