Journal of Thermal Biology 32 (2007) 374–382

Journal of
THERMAL BIOLOGY

www.elsevier.com/locate/jtherbio

Does thermal-related plasticity in size and fat reserves influence supercooling abilities and cold-tolerance in *Aphidius colemani* (Hymenoptera: Aphidiinae) mummies?

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Received 13 February 2007; accepted 24 March 2007

Abstract

(1) The parasitoid *Aphidius colemani* was reared at 15 or 25 °C to induce variation in size and fat reserves; SCP and cold-tolerance were compared. Insects from both temperatures were also exposed to constant or fluctuating cold-exposure.

(2) The lower SCP in mummies reared at 25 °C may be partially explained by their smaller size, a negative relationship being observed between SCP and size.

(3) A bimodality was observed in SCP distributions, with two modes around −26 and −22 °C, likely because of presence/absence of gut content.

(4) The type of exposure had a striking impact, mortality being considerably lower under fluctuating regime.

(5) While energy storage is an important factor, vulnerability to chill-injury is supposed to be the primary factor regulating survival at low temperature.

Keywords: Parasitic wasp; Low temperature; Chill-injury; SCP; Fat reserves; Recovery

1. Introduction

Exposure to extreme temperatures, lethal or sub-lethal, is a major factor shaping life history traits in insect parasitoids (reviewed by Hance et al., 2007). Due to seasonal cycles, many insect species are frequently exposed to sub-optimal low temperatures in their natural environments. The underlying mechanisms allowing these organisms to increase their cold-hardiness have been extensively reviewed (e.g. Sinclair et al., 2003). In insect parasitoids, adaptations to low temperatures are similar to those of most ectotherms (Hance et al., 2007). Exposures to prolonged low temperature are known to have detrimental effects on the survival of parasitoids (Langer and Hance, 2000; Lysyk, 2004; Tezze and Botto, 2004; Levie et al., 2005; Colinet et al., 2006a, b). When the "dose" of cold-exposure (a combination of time of exposure and temperature) exceeds a specific threshold, chill-injuries accumulate, become progressively irreversible and eventually lethal (Bale, 1996, 2002; Kostal et al., 2006). In many insect species, the lethal cumulative injuries occur even at temperatures above 0 °C, but the main causes of death are still not well understood (Renault et al., 2002; Kostal et al., 2004, 2006). In studies devoted to the consequences of cold-exposure, the specific effects of starvation and cold are not easy to identify as they are acting together. Hence, consequences of cold-exposure may result either from cold effects or starvation, or a combination of both phenomena.

Several studies emphasized that exposing insects to fluctuating thermal regimes (FTR) (i.e. cold-exposure

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doi:10.1016/j.jtherbio.2007.03.005

interrupted by periodic short pulses at high temperature), *versus* constant low temperatures (CLT), increased significantly the survival (Chen and Denlinger, 1992; Nedvěd et al., 1998; Renault et al., 2004; Colinet et al., 2006a). FTR reduce the level of accumulated chill-injuries and thus the mortality, either because less chill-injuries accumulate at FTR, or because the effect of chilling is counteracted by the high temperature intervals (Hanč and Nedvěd, 1999; Renault et al., 2004; Colinet et al., 2006a, 2007b).

During low temperature exposure, insects fast and basal metabolism relies exclusively on body energy reserves (Pullin, 1987; Lavy et al., 1997). Energy is needed not only to survive to subzero temperatures, but also to survive low, but above zero temperatures (Lavy et al., 1997). Energy reserves, particularly from fat body, are consumed during starvation at low temperature exposure (David and Vannier, 1996; Renault et al., 2002, 2003). In a previous study, it has been shown that parasitoid mass loss increased with cold-exposure duration and was associated with a marked decrease in dry mass, due to lipid reserves depletion (Colinet et al., 2006b). Heavier insects with larger fat reserves should presumably have a significant advantage for survival at low temperature (Renault et al., 2003; Colinet et al., 2006b), an assumption that will be tested in this study.

There is strong evidence that an increase in lipid content underlies increased resistance to starvation, as indicated by numerous studies on *Drosophila* (reviewed by Hoffmann and Harshman, 1999). An increase in body mass has also been associated with increased starvation resistance (Chippindale et al., 1996; Harshman et al., 1999). The starvation-resistance hypothesis (SRH) states that because energy stores increase with size faster than metabolic rate, resistance to starvation should increase with body size (Cushman et al., 1993; Arnett and Gotelli, 2003).

The temperature-size rule states that ectotherms grow larger at lower temperature, and this rule is supported by numerous studies (reviewed by Atkinson, 1994; Atkinson and Sibly, 1997; Angilletta and Dunham, 2003). As a generally accepted guideline, decreased developmental temperature results in slower growth rate, longer development time, and larger adult size in insects (Sibly and Atkinson, 1994). Since, fat reserves are positively correlated with body mass (Ellers and van Alphen, 1997; Ellers et al., 1998; Strohm, 2000; Rivero and West, 2002; Colinet et al., 2007a), variations in developmental temperature is a convenient way to obtain bigger and fatter individuals (Colinet et al., 2007a).

Studies of insect cold-hardiness are generally achieved by measuring their capacity to survive at low temperatures for extended periods, and/or by measuring supercooling abilities. The SCP should represent the lower lethal temperature for freeze-intolerant insects, although death sometimes occurs at temperatures well above SCP. The real ecological value of the SCP thus remains ambiguous (Bale, 2002; Renault et al., 2002), but SCP can still be used as a convenient comparative index, especially since it depends on the organism's native characteristics, such as body composition and size (Renault et al., 2002).

In the present study we focused on the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiinae), which is commercially produced and distributed as an aphid biocontrol agent, targeting primarily *Myzus persicae* Sulzer (Homoptera: Aphididae) in glasshouses in many European countries. This parasitoid stops feeding at the end of third larval stage (Muratori et al., 2004), the larva then spins its cocoon inside the empty cuticle of the aphid, forms a mummy and pupates (Hagvar and Hofsvang, 1991). From that moment until emergence, it does not feed and all metabolic processes use energetic reserves accumulated during the larval stages.

Different developmental temperatures were used to induce a phenotypic variation in insect size. We then tested the hypothesis that body size may influence superccoling abilities and/or cold-tolerance via SRH. We also tested the crossed effect of the type of exposure (FTR *versus* CLT) on insect survival during a medium-term cold-exposure.

2. Materials and methods

2.1. Insect rearing

The green peach aphid, *M. persicae,* was used as host for the parasitoid rearing and laboratory cultures were established from individuals collected in fields during 2000 at Louvain-la-Neuve, Belgium (50.3°N Latitude). Aphids were reared in $0.3\,m^3$ cages on sweet pepper (*Capsicum annuum* L.) under $18\pm1\,°C$, $\pm60\%$ RH and LD 16:8 h. *A. colemani*, originally obtained from Viridaxis SA (Belgium), were subsequently reared in the laboratory under the same conditions.

2.2. Parasitoid development

To obtain standard mummies, batches of 50 standardized 3-day old aphids were offered to a mated female parasitoid for 4 h at 18 °C. Aphids were all synchronized at the same age in order to avoid host–age effects on parasitoid development (Colinet et al., 2005). The parasitoid females used were less than 48 h old, naïve, and mated. After parasitism, the females were removed and the aphids were immediately transferred to either 15 or 25 °C to continue development until mummification. Developmental temperature is known to induce phenotypic plasticity in body mass and fat reserves, wasps coming from a rearing at 15 °C being larger (size), heavier (dry mass) and fatter (fat content) than wasps developing at 25 °C (Colinet et al., 2007a).

2.3. Morphometric and gravimetric measures

For both developmental temperatures, one-day old mummies were subjected to individual mass measurements (Mettler-electrobalance Me22, sensitivity 1 μg) ($n = 84$).