Expression of the 70 kDa Heat shock protein family in Alpine freshwater chironomids (Diptera, Chironomidae) under natural conditions

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ABSTRACT

Chironomidae represent up to 100% of the fauna of Alpine streams. Because they survive stress conditions such as extremely low temperature (annual mean <4 °C), these animals represent a good organism model to analyze the relationship between adaptation to cold and expression of stress proteins such as the 70 kDa Heat shock protein family. Fourth instar larvae of ten species of cold-stenothermal chironomids (Pseudodiamesa branickii, Diamesa latitarsis, D. laticauda, D. cinerella, D. insignipes, D. zernyi, D. vaillanti, Orthocladius (O.) frigidus, O. (Euorthocladius) thienemanni and Paratrichocladius nivalis) were collected in a glacier-fed stream in NE Italy at two stations (1300 and 2600 m a.s.l.) and in two seasons (summer 2005 and spring 2006). Immunodetection and quantification of the relative levels of Hsp70 family were performed via Western blot analysis. Significantly different levels of Hsp70 were detected among species. The highest amounts were recorded in P. nivalis and D. insignipes, the lowest in P. branickii. Within the genus Diamesa, lower levels of Hsp70 were observed in the most cold-stenothermal species than in the less cold-stenothermal ones. These differences may be explained by different species autecology. The results provide information on biochemical strategies of alpine midges to face cold temperatures under natural conditions and new insights into their possible response to global warming.

Key words: cold-stenothermal species, Diamesinae, Orthocladiinae, glacial streams, stress proteins, Italian Alps

1. INTRODUCTION

In Alpine freshwaters, food-chains are simplified and few organisms are adapted to such environmental constraints (Irons et al. 1993). These habitats are colonised mainly by Chironomidae (Diptera) (Lods-Crozet et al. 2001) which possess adaptations to a variety of environmental rigors such as desiccation, anoxia, high or extremely low temperatures and freezing (Danks 1971; Lencioni 2004). Chironomids are the most widely distributed insect family in freshwaters, with about 3700 species widespread throughout all the zoogeographic regions (Ashe et al. 1987; Cranston 1995). In particular, in Alpine streams fed by ice- and snowmelt they account for the majority of the macroinvertebrate species, accounting for up to 100% of the fauna in the kryal (the first km downstream of the glacial snout) (Füreder 1999; Lencioni & Rossaro 2005). The kryal is characterised by extremely low temperatures (annual mean <4 °C), coupled with considerable seasonal and daily highly variability in channel stability, turbidity and discharge (Brittain & Milner 2001; Maioini & Lencioni 2001). For these reasons, chironomids are an appropriate taxa to study the adaptive strategies evolved to survive stresses such as low temperatures and temperature variations (Lencioni et al. 2008).

The involvement of heat shock proteins (Hsps) in resistance towards heat, but also cold and in a range of other stresses such as heavy metals, pesticides, desiccation, anoxia and diseases has been documented for many organisms, from bacteria to plants and animals (e.g., Lindquist 1986; Feder & Hoffmann 1999; Sørensen et al. 2003), including chironomids (e.g., Morcillo et al. 1997; Rinehart et al. 2006). However, there is no reference to cold stenothermal species such as Alpine chironomids. Hsps function as molecular chaperones and play a primary role in folding, assembly, intracellular localization, secretion, and degradation of other proteins.

In many organisms, Hsp of 70 kDa is considered the major Hsp family consisting of inducible (Hsp) and constitutive (heat shock cognate, Hsc) forms. The expression of both forms can be activated and/or increased in heat shock response (HSR) (Fader et al. 1994; Feder & Hoffmann 1999). Recently, the ecological importance of inducible Hsps was also demonstrated in recovery and survival of organisms under stressful conditions (Sørensen et al. 2003).

In a wide range of organisms, the expression of Hsps can be influenced by seasonal and altitudinal temperature variations, or by the different geographical areas in which the organisms occur (Fader et al. 1994; Hofmann & Somero 1995; Feder & Hoffmann 1999; Tomanek & Somero 1999). Because vital cellular processes may be susceptible to temperature, ectothermic organisms that live at thermal extremes have altered Hsp expression and function in order to facilitate protein folding (Hofmann 1999). Antarctic organisms represent a good