Do all stoneflies nymphs have respiratory proteins? Further data on the presence of hemocyanin in the larval stages of plecoptera species

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Abstract

Contrary to what was assumed regarding the presence of respiratory proteins in insects, a functional hemocyanin was recently found in larvae and adults of the stoneflies species *Perla marginata*, whereas in the close species *Perla grandis*, hemocyanin functionality was deduced from sequence data. In order to verify if the presence of this ancient trait is widespread within the order and to investigate why stoneflies have maintained it, we have extended the search for hemocyanin to species of other Plecoptera families. In particular, we assessed the presence of hemocyanin in the larval stage of nine Plecoptera species, belonging to six of the seven families of the European stonefly-fauna, and analyzed its potential functionality as deduced by sequence data. We cloned and sequenced the corresponding cDNAs and studied their expression with RT-PCR technique. Moreover, we performed homology studies using the deduced amino acid sequences. On the basis of our analysis, we hypothesized a functional role of the hemocyanin only for two species: *Dinocras cephalotes* and *Isoperla grammica* (Perloidea). In all the investigated Nemouroidea and in *Siphonoperla torrentium* (Perloidea), this protein may have been lost. Larval size, life-cycle length, trophic role and environmental induction are discussed as possible explanations of these different physiological requirements.

Keywords: insects respiration, respiratory pigments, cDNA, oxygen.

Introduction

For long time it has been paradigmatically affirmed that, due to the presence of the tracheal system, insects do not have respiratory proteins, with the exception of several chironomid midges larvae, the aquatic Hemiptera genera *Buenoa* and *Notonecta* and the parasitic horse botfly genus *Gasterophilus*. More recently, a potential hemocyanin was characterized in the embryo hemolymph of the grasshopper *Schistocerca americana* (Sánchez *et al.*, 1998) and a respiratory hemoglobin was found in *Drosophila melanogaster* (Hankeln *et al.*, 2002); hemocyanin was found in larvae and adults of the stonefly *Perla marginata* (Hagner-Holler *et al.*, 2004) and this protein proved to be functional and to act as an oxygen carrier. A potentially functional hemocyanin has been found also in another stonefly species, *Perla grandis*, and its 3-D structure tentatively described and analyzed through a modelling approach (Fochetti *et al.*, 2006). Hankeln *et al.* (2006) cloned and studied the expression of a true hemoglobin gene in the honeybee *Apis mellifera* (Hymenoptera). They also stated that hemoglobins exist in other insect orders (Hemiptera, Coleoptera, Lepidoptera), suggesting that the corresponding genes belong to the standard set of an insect genome. Besides, Burmester & Hankeln (2007) reported the presence of hemocyanin in many insect orders, from Collembola to Blattodea, without further comments. All these data indicate that insect respiration is a more complex subject than previously thought.

Plecoptera is a very interesting and enigmatic group from the systematic and phylogenetic points of view. The order is considered a key group among the Insecta: many morphological characters of the Plecoptera are so primitive that the order has been hypothesized to be the sister-group of the rest of the Neoptera (Henning 1981; Kristensen 1991; Zwick, 2000; Beutel & Gorb, 2001). We have extended the search for hemocyanin to other species of different

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