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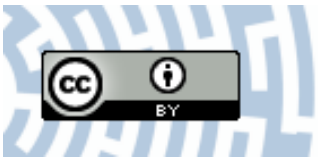

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The development al potential of the embryos of wolf spider *Xerolycosa nemoralis* from areas variously burdened with metals

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Abstract. The aim of this study was to assess and describe the developmental potential of the embryos of the wolf spider *X. nemoralis* collected from the reference site (Pilica) and a site heavily polluted with metals (Welnowiec) (southern Poland). For the analyses of the progress of development the embryos the computer microtomography was used. Adenylate concentration was measured using luminometric method. The macroscopic observations of the development of individual embryos revealed significant differences in the duration of embryogenesis (14,5 ±0.5 days in Pilica vs 16.5±0.53 in Welnowiec). The computer microtomography analyses enabled us to describe the geometry of the eggs inside the cocoon as well as to see the morphology of embryos inside the cocoon and to recognize selected developmental stages of the embryos. The concentration of cadmium in embryos in Welnowiec was significantly lower than in the embryos from the reference site, while the concentration of copper in the embryos from Pilica was significantly, 4 times lower than in Wełnowiec. The energy status indices (ATP concentration and ADP/ATP ratio) did not differ significantly in the embryos from the two sites.

Key words: heavy metals, spiders, embryos, computer microtomography, ATP, ADP/ATP

Introduction

The animals inhabiting polluted areas can be characterized by decreased tolerance to additional environment all stressors. This may result in the weakness of the population, reduction of its quantity and even in its extinction in case of dramatic change of the environmental conditions. Finally the species composition of the fauna can get changed since more sensitive species can be substituted by more resistant ones which are able to tolerate the pressure without any side effects in reproduction processes. The ecological assessment of the conditions of the arachnocenoses of degraded areas revealed that, usually, the quantity of spiders communities remains unchanged whereas their species composition changes significantly (Łuczak, 1984; Majkus, 1988, 2003).

Animals, including spiders, living in industrially polluted areas are forced to allocate an amount of energy to detoxification and defensive processes, often at the expenses of reproduction, development, growth and other life history parameters. Spiders can realize two opposite selection strategies enabling them to survive and maintain relatively stable populations in unfavourable habitats: the K- and r-strategies (Pianka, 1970). Web building spiders *Agelena labyrinthica* realize the r- strategy (numerous eggs but of low calorific value) while the wolf spiders *X. nemoralis* – the K-strategy: eggs are not very numerous but their energy contents is high and, consequently, hutching success is also relatively high under strong pollution conditions (personal, unpublished data). The aim of this study was to assess and describe the developmental potential of the embryos of the wolf spider *X. nemoralis* collected from two study sites, the reference site and a site heavily polluted with metals. This was to be achieved by an innovative method of computer microtomography, without the destruction of the cocoon and then verified by the observation and recording of the developmental stages of individual isolated eggs. Moreover, the obtained results will be referred to the energy status indices of the eggs laid by females collected from variously polluted areas.

Materials and Methods

Study sites were localized in Silesian voivodship, southern Poland. The reference site Pilica, is localized

over 30 km from the industrial district. As an abandoned rural area it is regarded as the reference, unpolluted site. The polluted area, the metallurgic waste heap is localized within The Upper Silesia Industrial District. The main pollutants there are waste originating from complex zinc and lead ore enrichment processes, comprising dolomites, clays and extremely toxic silts. The details concerning the quality and quantity of the pollutants and metal concentrations in the soil in the three sites are given in Babczyńska et al., 2011a, b.

Xerolycosa nemoralis (Westring, 1861) is an actively hunting spider inhabiting sunny sites, either open or covered with vegetation, between stones and dry leaves (Foelix, 1996). Sexually mature individuals appear between May and June. (Varol et al., 2006; http://srs.britishspiders.org.uk/portal.php/p/Summary/s/Xerolycosa+nemoralis). Adult fertilized females, were hand collected and immediately transported to the laboratory and placed into plastic containers with the layer of wet sand to assure a proper humidity (about 70%), covered with ventilation assuring covers and kept at the photoperiod L:D 14:10. The spiders under captivity were fed fruit flies *ad libitum*. As soon as the eggs were laid the egg cocoons were taken from the females and divided into four groups: cocoons scanned by computer microtomography without destroying them, the cocoons that have been opened and the eggs were incubated individually until the hatching of the youngsters, the eggs that were prepared to the cadmium and copper contents by AAS methods and the cocoons that were prepared for the measurements of ATP concentration and ADP/ATP ratio in order to assess possible apoptotic and/or necrotic changes of the embryos in the early stages of the development.

The values metal and ATP concentration as well as ADP/ATP ratio were analyzed statistically (Normality by the Kolmogorov-Smirnov test. Tukey test was used for post hoc one-way analysis of variance (ANOVA). Results with $p \leq 0.05$ were considered significant) using STATISTICA 8.0 package (StatSoft, Inc. (2007). STATISTICA (data analysis software system), version 8.0. www.statsoft.com.

Results

The concentrations of cadmium and copper in the embryos of *X. nemoralis* from Welnowiec and Olkusz are presented in Table 1.

Table 1. Concentration of cadmium and copper in the embryos of *X. nemoralis* from the polluted and the reference sites (mean ±SD; µg/g dry weight). a, b: different letters indicate statistically significant differences between sites (ANOVA, Tukey, $p \leq 0.05$).

metal	Pilica	Welnowiec
Cd	0.63±0.37a	0.06±0.01b
Cu	12.28±4.1a	48.31±12.69b

Table 2. ATP concentration [µmol g wet wt-1] and ADP/ATP ratio (mean ±SD) in the embryos of *X. nemoralis* from the polluted and the reference sites.

Welnowiec		Pilica	
ATP	ADP/ATP	ATP	ADP/ATP
6.6±3.2	0.1±0.05	13.2±7.6	0.06±0.05

The concentration of cadmium in embryos in Welnowiec was significantly lower than in the embryos from the reference site, while the concentration of copper in the embryos from Pilica was significantly, 4 times lower than in Wełnowiec. The energy status indices (ATP concentration and ADP/ATP ratio) did not differ significantly in the embryos from the two sites.

The computer microtomography analyses enabled us to describe the geometry of the eggs inside the cocoon as well as to see the morphology of embryos inside the cocoon and to recognize selected developmental stages of the embryos.

The macroscopic observations of the development of individual embryos revealed significant differences in the duration of embryogenesis (14,5±0.5 days in Pilica vs 16.5±0.53 in Welnowiec).

Discussion

The concentration of metals in the embryos confirm our previous results according to which the gonads, and consequently the eggs are protected against heavy metals which are deposed mainly in midgut glands (Wilczek and Babczyńska, 2000). Also, the relatively low concentrations of Cd and Cu did not change the levels of energy status indices (ATP concentrations and ADP/ATP ratio).

The concentration of ATP in the embryos from Welnowiec was higher than in the embryos from Pilica, although the differences between sites were not statistically significant. The relations between ATP concentration and ADP/ATP ratio may indicate intensive proliferation processes and explain the relatively high hatching success found for this spider species in polluted habitats (personal data, in preparation).

The description of developmental stages based on computer microtomography verified by macroscopic observation can confirm the developmental stages described by other authors for other spider species (Wolff C., Hilbrant M. 2011). It has been revealed, however, that the delay in the hatching was caused by of young spiders can be caused by the increase of the duration of developmental stages, as the result of increased the pollution by heavy metals in the environment (personal data, in preparation).

Conclusions

The study showed that the duration of the early stages of development and mortality eggs and embryos of *X. nemoralis* were site-dependent. Moreover, the results of this study may throw new light on the biology of spiders

and on the ability to use computer microtomography in embryological studies.

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