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Reproduction and development of *Spodoptera exigua* from cadmium and control strains under differentiated cadmium stress

Anna Płachetka-Bożek*, Alina Kafel, Maria Augustyniak

Department of Animal Physiology and Ecotoxicology, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland

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ABSTRACT

The growth and development of living organisms is programmed in genes, but exogenous factors (e.g. cadmium) may modulate endogenous information. Heavy metals may disturb physiological functions and accumulate in the tissues. The insects under prolonged heavy metal stress show some modifications in their metabolism management.

The aim of this study was to compare the reproduction and development between individuals of *S. exigua* from the strain, exposed over 130 generations to sublethal concentration of cadmium (44 mg Cd/kg dry weight of larval diet), and the individuals from the control strain, both additionally exposed to different concentration of cadmium (22-704 mg Cd/kg dry weight of larval diet).

The exposure to various cadmium concentrations in the diet revealed survival difference between the cadmium and the control animals at the larvae stage. The differences between adults were not evident. The telomere length (responsible for the duration of a lifespan) in the cadmium strain was shorter in the females than in the males and the individuals from the control strain. *TERF1* gene expression (indirectly responsible for the telomere length) was higher in the individuals from the cadmium strain 24 hrs after eclosion.

The significant reduction in the larvae body mass was observed in both strains, when the metal concentration was equal to or higher than 264 mg/kg dry weight of larval diet. The EC50 values (defined as of body mass loss), calculated 48 hours after cadmium exposure of individuals from control and cadmium strains, were respectively 632 and 725 mg Cd/kg dry weight of diet.

However, some difference in reproduction (the total number of eggs laid and the oviposition time) between the strains appeared only in the groups fed on the uncontaminated diet. The control females laid almost two times more eggs than those from the cadmium strain, and the control ones had more than two times longer oviposition time than the females from the cadmium strain.

The fluctuation was also noted in the size of eggs and the hatching success on the following days when both strains were compared, while the hatching success was higher for the insects from the cadmium strain.

In conclusion, the insects from the cadmium strain are more resistant to cadmium contamination, as it is evidenced by the EC50 parameter. However, the females from the cadmium strain start laying eggs statistically later, have shorter telomeres and slightly reduced *TERF1* gene expression, but hutching success in the strain is significantly higher when compared with the control individuals.

1. Introduction

The morphogenesis, growth and development of organisms are controlled by a genetic program which is being constantly improved during the evolution, and is passed down from generation to generation. However, the endogenous information is modulated by external factors, and the responses of organisms to them, especially during the longer exposure, are fixed in the population (Jura, 1988). Prolonged exposure to anthropogenic factors may affect the selection process and permanently change the genetic program of development. Such phenomenon was presented in the case of different organisms including microbes (Azarbad et al., 2016), plants (Koźmińska et al., 2018), and also insects (Merritt and Bewick, 2017) being under stress of heavy metals. Metal-responsive genes were identified in different insect representatives in the field and laboratory studies. For example, differences in genes expression, as an effect of evolutionary adaptation to contaminants, was found in the case of sensitive and tolerant populations of springtails *Orchesella cincta* (Posthuma, 1990).

* Corresponding author.
E-mail address: anna.plachetka@us.edu.pl (A. Płachetka-Bożek).

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Heavy metals induce oxidative stress and can cause DNA damage both in plants and animals (Augustyniak et al., 2016, 2006; Babczynska et al., 2012; Huang et al., 2012; Kafel et al., 2012b; Lin et al., 2007; Suganya et al., 2016; Sun et al., 2016; Xie et al., 2014; Yang et al., 2016). Increased amounts of heavy metals present in contaminated environments may exert highly toxic effects. They could be biogenic metals like zinc, copper and iron, which are necessary to keep life functions of different organisms and non-biogenic ones, perceived as very toxic, like cadmium or lead (Straalen and Roelofs, 2005). Cadmium is present especially in anthropogenic degraded environments. The metal exhibits harmful effects on cells and may disturb their physiological functions (Cervera et al., 2004; Kafel et al., 2012b). It is particularly dangerous because of its rapid absorption by organisms, and the ease to accumulate in plant and animal tissues (Kaczyńska et al., 2015).

Specific mechanisms of defence responses to heavy metal stress, but also to reactive oxygen species (ROS), amount of which could be elevated after heavy metal exposure, are characteristic for organisms and can be clearly observed among herbivorous insects. Survival strategies resulting from adaptation to a specific plant diet can be universal, and can serve as the first line of defence against heavy metal contamination (Dallinger and Rainbow, 1993; Kafel et al., 2014; Konopka et al., 2013; Straalen and Roelofs, 2005). Removal of the toxic metals from sensitive sites in the cell and/or their accumulation to maintain cellular homeostasis through chelation, uptake with different intensity, and sequestration processes are some of the most important phenomena. The aforementioned mechanisms help minimize the damage resulting from exposure to nonessential metal ions like cadmium. The primary mechanism is usually the exclusion of the ions from active tissues, followed by the sequestration into non-active tissues (Bednarska and Świątek, 2016; Clemens, 2006; Song et al., 2017; Vijver et al., 2003). It should be noted here that cadmium can act as a pro-oxidant, and can generate ROS production in the tissues (Augustyniak and Migula, 2000; Kafel et al., 2012a, 2012b).

Insects are suitable models for microevolution studies, because many subsequent generations can be obtained in relatively short time, at a relatively low costs. Moreover, their widespread occurrence and their important role in ecosystems should be emphasised (Pölkki et al., 2012; Straalen and Roelofs, 2005). Subsequent generations of insects being under prolonged heavy metal stress revealed some modifications in their metabolism management, which can be involved in the process of adaptation to heavy metals. Presently, it is observed in ecosystems, which are overloaded with metals (Straalen and Roelofs, 2005). The presence of genetically based resistance was detected in the soil invertebrate species, and in *Drosophila melanogaster* (Spurgeon and Hopkin, 2000). The resistance may be connected with the induction, activation, and interaction of metallothioneins (Spurgeon and Hopkin, 2000; Straalen and Roelofs, 2005). The effect of heavy metals toxicity can also be observed at the physiological level, where it manifests, among others, through changes in the reproductive success. It is one of the most important parameter of organism fitness, perceived as an essential factor in species development characterisation (Sun et al., 2016).

The toxic effect of cadmium and its influence on insect physiology has been described in the literature. The reduction in the development, growth, reproduction and hatchability after cadmium intoxication has been described the most often (Augustyniak et al., 2008; Babczynska et al., 2012; Cervera et al., 2004; Kafel et al., 2012b; Yang et al., 2016). The investigation of cadmium influence on insects is complex, as their reproduction and development may dependent on the temperature, food quality and quantity, population density and female age (Augustyniak et al., 2008). Permanent contact with heavy metals can disturb the reproductive processes (Augustyniak et al., 2008; Xie et al., 2014) because energy resources can be shifted toward detoxification (Cresswell et al., 1992). Relatively new research on different species, such as *Oncopeltus fasciatus, Aphis medicaginis, Spodoptera litura* showed that heavy metal exposure may affect the reproduction both in females and males (Cervera et al., 2005; Shu et al., 2009; Sun et al., 2016; Xie et al., 2014). Some observations revealed that the organisms living in the environment contaminated with heavy metals are distinguished by an extended life cycle, what (in case of insects) is manifested mainly by longer duration of larval stages (Kafel et al., 2012b; Vedamanikam and Shazilli, 2008). But chronic exposure can reduce this effect (Kafel et al., 2012b). Both genetic and epigenetic mechanisms can be involved in the enhancement of tolerance of organisms to environmental pollutants. However, it seems obvious that time of exposure is crucial (Pölkki et al., 2012; Straalen and Roelofs, 2005).

Long-lasting exposure of animals to a particular stressor (such as cadmium) may lead to the selection of individuals which cope with other stressors better (Augustyniak et al., 2017; Kafel et al., 2012a, 2012b). For over ten years our team have been researching the mechanisms of *S. exigua* adaptation after long-term exposure to cadmium. One of the strains was constantly fed with cadmium (44 mg/kg of dry weight of food) and the other one was the control group. The details of the breeding conditions were described in Augustyniak et al. (2016). Our newest investigations showed that multigenerational contact with cadmium improved tolerance of the insect to survive under other stressor, e.g. pesticide - spinosad. We claimed that the insects from the cadmium strain that were poisoned with spinosad did not require higher energy consumption as it was in the control strain (Augustyniak et al., 2017).

The aim of this study was to check if there are some crucial differences in life cycle steps between individuals of *S. exigua* from control and cadmium strains (being under multigenerational selection to sublethal concentration of cadmium i.e. 44 mg Cd/kg dry weight of diet), and additionally exposed to food with other than 44 mg Cd/kg dry weight of diet. The reproduction and development parameters of *S. exigua* individuals from both strains was then discussed in the light of expression of vitellogenin (Vg), as well as mutation in Vg gene in the cadmium strain individuals that we found in our previous study. As the telomere length may be related to the length of life, especially in the heavy metal exposure context, and the telomere binding protein (*TERF1* - the protein which probably inhibits the action of telomerase) may be disrupted by heavy metals, in this study the telomere length and *TEFR1* gene expression analyses were also performed in the control and cadmium breeding strains.

2. Materials and methods

2.1. *Spodoptera exigua* as a model organism

S. exigua is a polyphagous pest, widely distributed in Europe, Africa, Australia, Asia and America. The insect inhabits and feeds on vegetables, field crops and flowering plants (Capinera, 2017; Steenwyk and Barnett, 1985; Takai and Wakamura, 1987). During our work with the strain we observed that the life cycle of *S. exigua* in good laboratory conditions varies between 28 and 39 days, a single female can produce 1000 eggs, oviposition lasts seven days, and then hatching of the offspring starts at 72 h (Płachetka-Bożek et al., 2018).

2.2. Insects and breeding conditions

Two strains (cadmium and control) of *Spodoptera exigua* were used in the experiments. The first one (cadmium) was fed a diet contaminated with cadmium (44 mg Cd/kg dry weight of larval diet) for more than 130 generations. The second one (control) was reared on a standard artificial diet for insects (Augustyniak et al., 2016). Individuals of both strains were kept in the following conditions: 25 ± 1 °C, a photoperiod 16 L: 8 D (light: dark) and RH 30 ± 5%.

In the experiment, individuals from the two breeding strains were additionally exposed for one generation to different concentrations of cadmium: 0, 22, 44, 88, 176, 264, 352, 528, 704 mg/kg of dry weight of