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Increased Respiration Rate as a Result of Adaptation to Copper in Confused Flour Beetle, *Tribolium confusum* Jacquelin du Val

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Abstract Multi-generation exposure of organisms to toxicants may lead to adaptations increasing their resistance to a chemical. However, the increased tolerance may have a negative effect on fitness in uncontaminated environments due to increased maintenance costs in adapted individuals. Herein we present results of a multi-generation experiment on the flour beetle, *Tribolium confusum*, showing that animals bred for ca. 10–13 generations in copper-contaminated medium had higher maintenance costs then their counterparts originating from uncontaminated medium. The results show that significant changes in energy budgets may occur even after relatively short selection in small laboratory cultures.

Keywords Metal pollution · Costs of tolerance

Chronic multi-generation exposure to environmental stressors, such as heavy metal contamination, often leads to the evolution of adaptation, demonstrated by increased fitness of organisms from adapted populations in comparison with non-adapted individuals in the presence of these stressors (Posthuma and Van Straalen 1993; Shirley and Sibly 1999). However, increased tolerance to environmental stressors may have a negative effect on fitness when the stressors are absent (Sibly and Calow 1989; Calow 1991; Van Straalen and Hoffmann 2000). There is evidence for the costs of metal resistance: plants from contaminated sites may exhibit reduced growth rate in unpolluted conditions (Wilson 1988), and metal-adapted animals are characterized by decreased fecundity, lifespan and size at maturity (Shirley and Sibly 1999; Xie and Klerks 2004).

Two mechanisms possibly leading to fitness costs of heavy metal resistance were suggested (Posthuma and Van Straalen 1993; Shirley and Sibly 1999). Firstly, metal-resistant genotypes may be less efficient in the uptake or utilization of essential metals, what can lead to micronutrient deficiency at unpolluted sites (Posthuma and Van Straalen 1993); data supporting this hypothesis were mostly collected in plants. Alternatively, detoxification processes – e.g. metallothionein synthesis – may use up a significant part of resources and energy, which thus become unavailable for fitness-related traits such as growth or reproduction (Sibly and Calow 1989). This hypothesis is widely regarded as true (Calow 1991; Łagisz et al. 2005), but is poorly supported by experimental data (Van Straalen and Hoffmann 2000).

Respiration rate measurements may give an insight into energy budgets of organisms under stress. Short-term exposure to stressors like heavy metals may result in increase in respiration rate (Conradi and Depledge 1998; Rowe et al. 2001), but a decrease in metabolism was also observed (Migula 1986; Laskowski et al. 1996). Similarly, data on respiration rate in animals collected from contaminated sites or taken from strains metal-selected for several generations seem to depend on the species and metal used: crayfish from coal ash-polluted site, or moth pupae from lines treated for 15 generation with cadmium had higher respiration rates than reference animals (Rowe et al. 2001; Kramarz and Kafel 2003), while a negative correlation was found between respiration rate of grasshoppers and zinc level in grass at their sites of origin (Zygmunt 2006). These studies, however, confounded the effect of possible adaptation to contamination with the toxic effect of contamination itself, as the studied organ-

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isms were taken directly from polluted environments. In one of a few studies attempting to separate out these effects, a positive relationship was found between metabolic rate of males of the carabid beetle *Pterostichus oblongopunctatus* from F1 generation fed uncontaminated food and zinc concentration at the sites their parents were collected from (Łagisz and Laskowski 2002). However, in organisms originating from natural populations factors other than the level of contamination – for example, a food limitation – are likely to influence the optimal level of metabolism (e.g., Comoglio et al. 2004, Simcic et al. 2005).

Here we report the differences in respiration rate of the confused flour beetle, *Tribolium confusum* (Jacquelin du Val 1863) (Coleoptera: Tenebrionidae) originating from laboratory lines kept for 15 months in copper-contaminated and uncontaminated media.

Materials and Methods

We used the beetles originating from lines kept for 62–67 weeks (approximately 10–13 generations) in 25 g of standard medium (Sokoloff 1974), either contaminated with 5 g Cu kg^{-1} or uncontaminated. Every 4 weeks half of the media was removed and replaced with fresh media of the same copper concentration, and beetles in the replaced media were placed back in their appropriate container. During selection, populations were maintained at carrying capacities of their habitats, with emigration enabled by a strip of paper stretching from the medium to outside the breeding container. The small breeding containers were kept in larger beakers where from the emigrants were removed periodically. Limited by the availability of individuals from copper-exposed lines, resulting from extremely high mortality, we had to use the beetles from both the first (F1) and the second (F2) generation after the transfer to uncontaminated, standard medium. *T. confusum* from F1 generation hatched from the eggs laid by females taken directly from selection lines (after 67 weeks of selection), and were raised in 500 ml containers half-filled with fresh, uncontaminated medium. Beetles from F2 generation were obtained from the eggs laid by F1 females living in uncontaminated medium, and originating from the selection lines after 62 weeks of selection. F2 beetles were raised singly in separate wells of cell culture plates half-filled with uncontaminated medium. Twenty-four hours before respiration measurements they were put into 500 ml containers half-filled with uncontaminated medium. At pupa stage the beetles were separated by sex. The beetles from both generations were used for respiration measurements approximately 10 days after reaching the adult stage.

Six hours before the start of the measurements, the beetles were placed in 50-ml glass bottles used as respiration chambers, ten individuals in each bottle. No food was given, but to reduce the effects of desiccation, in each bottle we placed 1-ml Eppendorf-type tube with pierced lid, half-filled with distilled water. Limited by the availability of individuals and number of measurement channels in the respirometer (30), four replicate respiration chambers were set up per generation per sex for beetles originating from uncontaminated medium, while for those originating from contaminated medium there were three replicate chambers per sex for F1 generation and two per sex for F2 generation. Beetles from each bottle were weighed (ten individuals together) to the nearest 0.1 mg (WPA 180/k microbalance, Radwag, Poland). The amounts of oxygen consumed were measured with 30-channel MicroOxymax respirometer (Columbus Instr., Ohio, USA). The measurements were taken every 24 hours for 6 days; during that time, the bottles were kept in darkness at 21.0 ± 0.5°C.

The effect of origin on the respiration rate was analyzed by comparing the regression lines of oxygen consumption (in μl O_2 mg^{-1} h^{-1}) versus day for the two origins. The analysis was done separately for males and females from F1 generation, F2 generation, and both generations together. All statistical analyses were performed with Statgraphics Plus v. 5.1.

Results and Discussion

We obtained complete 6-day respiration records for all chambers. There was no mortality during the experiment – all 260 beetles survived until the end of measurements.

For the comparison of oxygen consumption, we used the transformed data (square root of oxygen consumption versus logarithm of day, the transformation which highly improved fit to the linear model for both sexes – for males r^2 = 0.847 and 0.770, for females r^2 = 0.910 and 0.861 for transformed and non-transformed data, respectively). In both sexes, differently-raised beetles from F1 and F2 generations differed with their initial respiration rates (intercepts), as well as with the rates of respiration decrease over time (slopes) (Table 1). However, despite these differences, a consistent trend was found: beetles originating from copper-contaminated medium had higher initial respiration rate, which decreased more steeply with time (Fig. 1). The differences were significant or close to significance for both generations of the two sexes, separately or together (Table 1).

The higher initial metabolic rates in beetles originating from copper-exposed populations suggest considerable costs of running metal detoxification machinery, even in the absence of contamination. These added costs of resistance obviously reduce the amount of energy available for

Table 1 Intercepts and slopes (±SE) of sqrt(O_2 consumption in µl h^{-1} mg^{-1}) versus log(time in days) regression lines for copper-adapted and non-adapted flour beetles, with *p* values computed with comparison of regression lines procedure

	Intercepts			Slopes		
	Non-adapted	Copper-adapted	*p*	Non-adapted	Copper-adapted	*p*
Females F1	0.802 ± 0.008	0.824 ± 0.009	0.075	–0.122 ± 0.006	–0.151 ± 0.007	**0.003**
Females F2	0.889 ± 0.011	0.900 ± 0.011	**0.039**	–0.175 ± 0.009	–0.201 ± 0.009	0.064
Females F1+F2	0.843 ± 0.008	0.869 ± 0.009	**0.019**	–0.148 ± 0.007	–0.171 ± 0.008	**0.046**
Males F1	0.751 ± 0.006	0.782 ± 0.009	**0.015**	–0.112 ± 0.005	–0.129 ± 0.007	**0.038**
Males F2	0.771 ± 0.018	0.826 ± 0.011	0.138	–0.115 ± 0.014	–0.148 ± 0.009	0.131
Males F1+F2	0.761 ± 0.009	0.800 ± 0.008	**0.045**	–0.114 ± 0.008	–0.137 ± 0.007	**0.038**

For each sex, regression lines were calculated and compared either for F1 generation only, or for F2 generation only, or for F1 and F2 generations combined together. Differences significant at 0.05 level are typed boldface

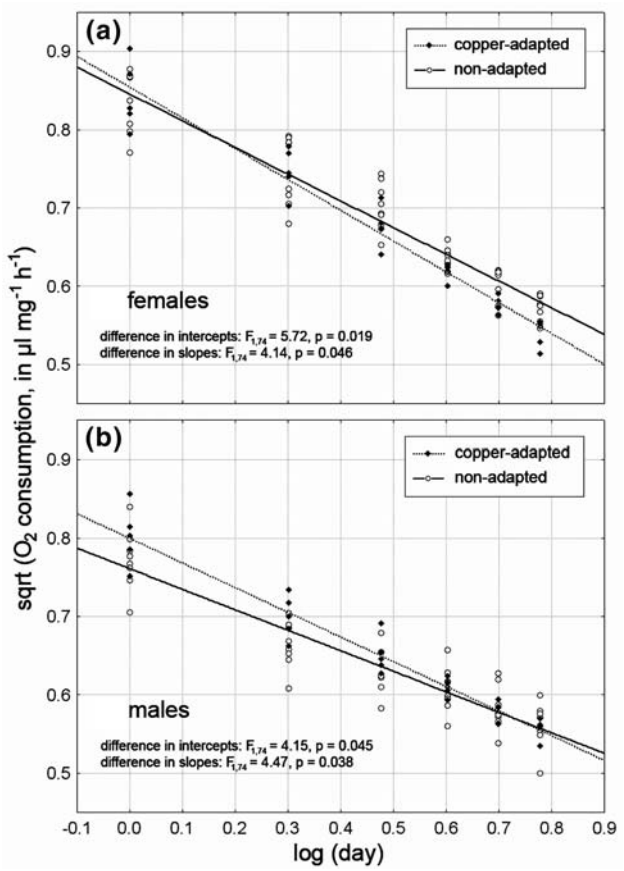

Fig. 1 Hourly oxygen consumption per mg of body mass during 6 days of starvation, computed for females (**a**, *above*) and males (**b**, *below*) from copper-adapted (*dotted lines*) or non-adapted (*solid lines*) lines. Regression lines were drawn for the two generations (F1 and F2) combined together

fitness-related traits. Indeed, in uncontaminated environment beetles originating from copper-contaminated medium had significantly lower fecundity and shorter median lifespan than control beetles, what translated into decreased population growth rate (Łukasik et al., unpublished data). The higher initial metabolic rate must lead to faster exhaustion of the reserves during starvation. This might be the reason why in our study the respiration rates decreased with time faster in resistant beetles. If this is the case, it may have negative consequences for adapted animals, making them more vulnerable to suboptimal environmental conditions, especially periods of food scarcity and stressors other than contamination (Stone et al. 2001).

Our data indicate on fitness costs of metal resistance in animals, which are associated with increased energy allocation into metal detoxification (Sibly and Calow 1989; Calow 1991; Van Straalen and Hoffmann 2000). It is one of few pieces of experimental evidence supporting this hypothesis. Obviously, other factors might as well be responsible for reduced performance of metal-adapted populations in uncontaminated environments, including micronutrient deficiency (Posthuma and Van Straalen 1993; Shirley and Sibly 1999) or negative genetic effects associated with selection at population sizes reduced by contamination (Łukasik et al., unpublished data). Nevertheless, our study confirms at least partly theoretical predictions about increased maintenance costs in animals adapted to contaminants. It also shows that even after relatively short selection in small laboratory cultures significant changes in energy budgets may occur.

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