

Assessment report on the PhD thesis by Sebastian Żmudzki: “Adaptations to elevated copper concentrations in invertebrates – multigeneration studies on *Tribolium castaneum*”, submitted to Jagiellonian University Krakow

The problem – its scientific relevance

This thesis addresses the question whether migration between subpopulations of the same species living in contaminated and uncontaminated soils influences the evolution of tolerance to pollution. The problem is relevant since metal soil pollution often knows a mosaic distribution with highly contaminated and less-contaminated patches and microsites. This may be the case even in forests exposed to large-scale aerial deposition of heavy metals since different microsites will accumulate metals to different degrees causing great heterogeneity of metal loading. It will certainly be the case at former mining sites, since eroding rocks, cinders and other mining remains are always distributed in a highly heterogeneous manner.

More generally, the problem is relevant in the context of evolution in a heterogeneous environment. When there is gene flow between subpopulations subjected to strong local selection for tolerance and subpopulations living in uncontaminated patches, what is the result for the (meta)population at large? I believe this is a still unresolved issue for evolutionary ecotoxicology, addressed only in some studies of pesticides.

Experimental approach

Candidate has chosen to address the question by means of experiments using the mealworm, *Tribolium castaneum* as a model. The beetles are cultured under different copper concentrations for many generations, with and without simulated migrations (transplantations) between the lines. The design of the experiments involved adequate replication. Due to the completely factorial set-up, the number of experimental groups was very large. The execution of the experiments must have been very laborious, however, the design was good.

Physiological variables

Analyses were done for reproduction and copper sensitivity, plus some physiological variables such as metallothionein expression, ATP, ADP, and respiration. Also, a study of copper kinetics was conducted, presenting a new model to analyse kinetics data showing an initial overshoot. The method for metallothionein analysis seems to me outdated. It does not measure a designated MT, but summarizes all proteins binding cadmium. At least one would have hoped for a validation of the method demonstrating that a significant part of the proteins analysed in this way are truly metallothioneins. Since the genome of *T. castaneum* is known to a large part, it would have been relatively easy to pick up an TcMT gene and measure its expression by qPCR. We cannot rely on the vertebrate MTs since literature data show an extremely rapid evolution of this protein, causing large sequence differences and molar MT/metal ratios even between the MTs of related species. Moreover, many invertebrates have a separate copper-binding MT (often not inducible, nor by copper nor by cadmium), and a cadmium-binding MT (induced by cadmium but not by

copper). So measurement of MT using cadmium saturation of unknown protein fractions might be wholly misleading. The measurements of ATP/ADP, as well as respiration, are adequate.

#### Genetic variation

Underlying the arguments is that strong directional selection using high copper exposure will exhaust genetic variation, which may be "rescued" by allowing migration between lines. The data indeed suggest this effect. It is a pity therefore that real measurements of genetic variation have not been done. If candidate could show that not only animal vigour but also neutral genetic variation (using, e.g. microsatellites) of a selection line is improved by migration between contaminated and non-contaminated lines, that would greatly reinforce the argument. This would also demonstrate that migrants actually interbred with resident populations.

#### Sex differences

In almost all variables there is a remarkable and significant influence of sex. This result is not discussed at all, although it is very apparent. Is it a question of body size, of activity or does it have a physiological basis? It would be very interesting to discuss this at more length.

#### Toxicokinetics

One of the most original parts of the thesis is the presentation of a new model for toxicokinetics, that may explain the sometimes large overshoot in the initial phase of the accumulation period. The model assumes physiological switches that change the kinetics parameters due to plastic adaptive mechanisms. For example, the fast initial uptake may lead to high intracellular copper concentrations, causing an active down-regulation of copper transporters. It would be interesting to back this up by molecular data on the activity of genes involved in copper uptake. The overshoot could maybe also be modelled by allowing for a second compartment with non-linear kinetics, rather than switches of parameters under the one-compartment model. However, the present analysis is very well elaborated, and the underlying data are very good and reliable.

#### Presentation

The thesis is written in remarkably good style, without major typos or errors in English grammar. The subdivision of each chapter is good. The presentation of figures and table is generally well done, however, sometimes I would have liked more explanation of the abbreviations used, which is however, compensated by providing a bookmark with the main terms.

#### Summary

This is a very good thesis, addressing a clearly defined problem, which is tested in a well-thought experimental design. The thesis clearly represents an original contribution to science. The chapters would make a fine contribution as a scientific article. I would like to recommend that if such a paper is produced from this thesis,

the various measurements which are now split over three chapters of the thesis are joined again in a single article. The toxicokinetics study may be a second paper. I think it should not be difficult to get this work published in journals of good international status (e.g. Environmental Pollution, Ecotoxicology).

In my opinion the thesis fulfils the requirements for a PhD thesis under Polish law, as communicated to me by the Institute of Environmental Sciences of Jagiellonian University Krakow.

A handwritten signature in black ink, appearing to be 'N. van Straalen', written in a cursive style.

Amsterdam 2 September 2015, Nico M. van Straalen, professor of Animal Ecology