

Cadmium tolerance in a soil arthropod

a model of real-time microevolution

Insects are suitable models for the study of microevolution in real time. Springtails (Collembola) are common inhabitants of organic soils. When the soil is polluted by heavy metals, some springtails are still able to survive due to genetic adaptation. We have shown that the production of a metal-binding protein, metallothionein, is increased in strains of *Orchesella cincta* cultured from sites with heavy metal contamination. A higher production of metal-binding protein allows more metal to be retained in the gut cells and consequently more metal to be excreted when the gut epithelium is regenerated at moult. DNA analysis has shown that the promoter of the metallothionein gene shows a considerable degree of polymorphism in springtail populations while some promoter alleles confer increased expression of the gene. In populations exposed to high metal concentrations the frequency of these alleles is increased compared to reference populations, which is most likely due to a selective advantage of high expresser phenotypes in polluted ecosystems. The data illustrate a new mechanism of microevolution, selection on transcriptional regulation, rather than on gene structure.

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Introduction

Like all animals, insects require heavy metals in their metabolism, but suffer from metal toxicity when they are exposed to levels above their capacity for detoxification (box 1). Since life began, organisms have used heavy metals to perform certain functions in their metabolism. Some authors even argue that heavy metals played a crucial role in the origin of life itself (Russell & Hall 1997). Anyway, life has been very selective in the choice of metals for biological functions, because there is no correlation whatsoever between the abundance of metals in the earth's crust and their abundance in biological tissues (Ernst & Joesse 1983). Also insects have adopted heavy metals as essential elements in their metabolism. The role of copper is well-known because of its posi-

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tion in the oxygen transporting protein haemocyanin, but in general our knowledge of insect mineral nutrition is scanty compared to that of vertebrates and plants.

Notwithstanding the long history of interactions between organisms and heavy metals, adaptation processes can still be observed in present ecosystems. This is due to the fact that in a relatively short timespan, human activities have led to a large-scale redistribution of heavy metals over the planet. This redistribution basically takes the form of depletion in a few places (ore deposits) and enrichments in many other places (around mines and smelters, in industrial areas and around urban settlements). In this way, ecosystems are loaded with metal levels that were not there before. The question addressed in this paper is whether exposures associated with these increased metal loadings of the environment lead to new adaptations and what would be the mechanisms by which animals can develop such adaptations.

Adaptation to heavy metals can be considered an example of 'evolution through pollution'. In fact, one of the finest examples of evolution in action is provided by the adaptation of plants to metal-containing soils in areas that are naturally enriched with heavy metals. These examples have found their way to textbooks and are cited as evidence that evolution is still going on around us (Futuyma 1998). The vegetation of metal-enriched soils includes metal-tolerant varieties of species that are often rare in other places and species that are only known from metal-enriched sites.

In insects, the issue of 'evolution through pollution' is mostly known from pesticide resistance (Oppenoorth 1987). Metal tolerance is not often studied, partly because many insects are not exposed to high concentrations of heavy metals, at least not during their adult life. The story is different for insects that live in close contact with soils or sediments

and their associated dead organic matter, because these environmental compartments are sinks for many pollutants and heavy metals tend to accumulate there. Consequently, tolerance development to heavy metals in insects is best documented in soil- and sediment-living species. In this paper we document recent progress in the study of a soil-living collembolan and its adaptation to cadmium.

Adaptation to metals

Since the 1970s, many authors have reported cases of adaptation to metal pollution in field populations of animals (Joosse & Buker 1979, Van Straalen *et al.* 1987, Hopkin 1989, Posthuma *et al.* 1992, Baird & Barata 1999, Stürzenbaum *et al.* 2001). The term 'adaptation' is used in variable senses (see box 2). The aquatic studies published in the 1970s and most of the 1980s were summarized by Klerks & Weis (1987). Their review showed that metal tolerance had been reported more often in algae and invertebrates than in vertebrates (fish and amphibians). The authors also stated that if the published literature accurately represents the situation in polluted areas, it must be concluded that most, but not all, populations can develop tolerance. However, they warned against relaxing water quality criteria on this basis, because many populations failed to survive in polluted environments. How the tolerances were acquired, by physiological or genetic adaptation, was unclear in most of the studies. Posthuma & Van Straalen (1993) conducted a review with a scope similar to Klerks & Weis, but aimed to cover the terrestrial environment. These authors concluded that there was good evidence for metal tolerance in seven species of terrestrial invertebrate.

Box 1. What is a heavy metal?

All elements which have a density greater than 5 g/cm³ fall in the category 'heavy metals'. Heavy metals are a normal part of nature: they cycle through all compartments including living biomass, although their concentrations are usually very low (hence 'trace metals'). Some metals perform specific functions in the metabolism, for example:

copper is an essential constituent of the oxygen-transporting haemolymph protein of arthropods and molluscs, haemocyanin,

zinc is a crucial element of enzymes such as carbonic anhydrase and many DNA-binding peptides which regulate the expression of genes ('zinc fingers'),

iron is an essential part of cytochromes such as cytochrome P450, a family of enzymes associated with the metabolism of steroid hormones and biotransformation of plant secondary compounds.

Some metals such as lead and cadmium have no known biological function, but it cannot be excluded that they may have a function in very low concentrations. For example, recently a biological role was suggested for cadmium in a specific form of carbonic anhydrase in a marine diatom.

All heavy metals are toxic at certain doses. Usually it is only the free metal ion (e.g. Cd²⁺) that is the toxic agent. Consequently, toxicity of a heavy metal polluted soil depends not only on the total metal concentration but also on factors that determine the equilibrium between free metal and unavailable metal (oxides, carbonates, organic complexes, etcetera).

Evidence for metal tolerance is suggested for at least eighteen species, most of them invertebrates, but including two fishes (table 1). Only in a few cases it was actually proven that the adaptation was due to genetic change. The review (table 1) reinforces the previous conclusion that many animals can develop tolerance to metals, that invertebrates seem to be more prone to develop tolerance than vertebrates, and that some animals cannot develop tolerance at all. The strongest evidence for tolerance comes from studies at grossly polluted sites where selection is expected to be particularly strong, e.g. ore mines and point sources of industrial emission. It is interesting to note that no cases of tolerance (at least not in animals) are reported from some common cases of environmental pollution, e.g. lead in roadside verges and zinc below overhead electric power cables. Also, there is no evidence for copper tolerance in earthworms from vineyard soils.

Whereas table 1 illustrates cases of metal tolerance in field populations, the phenomenon of tolerance is also known from laboratory test species. Extensive work has been done on the variation between clones in parthenogenetic organisms, such as *Daphnia magna* Straus for the aquatic environment and *Folsomia candida* (Willem) for soil. A study by Baird & Barata (1999) has shown that substantial variability in the susceptibility to cadmium is present between clones of *Daphnia*. This has important implications for the use of these cladocerans as standard test animals in ecotoxicology, because the same level of water pollution may be evaluated differently depending on the clone used. The issue of interclonal differences was also investigated by Crommentuijn *et al.* (1995) in four strains of the parthenogenetic collembolan *F. candida*, exposed to three toxicants, including cadmium. Differences between clones amounted to a factor 4.

The evidence reviewed here (see table 1) suggests that adaptation to metals accompanied by genetic change is not an uncommon phenomenon in the wild. The following sections deal with one of the mechanisms underlying adaptation, induction of the metal-binding protein, metallothionein.

Metallothionein

Since its discovery in 1957, metallothionein (MT) has been subject to extensive research in a wide variety of organisms. The protein was called metallothionein because of its very high metal and sulphur content. The high sulphur content is due to an exceptional number of cysteine residues which results in a high affinity for heavy metals such as cadmium and copper. The cysteines are usually arranged in a Cys-XaaCys (Xaa being any amino acid) configuration. Metallothionein lacks aromatic amino acids. Notwithstanding these properties shared by all metallothioneins, the size of protein and amino acid composition can vary enormously between MTs of different organisms or even between isoforms within an organism.

Studies on vertebrates have shown that each molecule of MT has two metal-thiolate clusters. One cluster binds four cadmium atoms using eleven cysteines, the other binding three cadmium with nine cysteines. The two clusters were later identified as two separate protein domains, the C-terminal -domain (4-metal cluster) and the N-terminal -domain (3-metal cluster). The clusters are connected to each other by means of a short linker sequence, which does not contain cysteines.

Table 1. List of species for which metal tolerance in field populations has been suggested. The overview is limited to studies published after 1990. References are available from the authors.

Lijst van soorten waarvoor gesuggereerd is dat metaaltolerantie bij veldpopulaties optreedt. Het overzicht is beperkt tot onderzoek dat gepubliceerd werd na 1990. Referenties zijn verkrijgbaar bij de auteurs.

| species | metal | type of evidence |
|---|------------|--|
| <i>Drosophila melanogaster</i> and other <i>Drosophila</i> (Diptera) | Cd, Cu | duplication of MT gene confers resistance; frequency of duplication greater in industrial areas |
| <i>Asellus aquaticus</i> Linnaeus (Isopoda), <i>Gammarus pulex</i> (Linnaeus) (Amphipoda) | Zn | increased 24-h LC ₅₀ values in animals downstream a zinc put; altered life histories |
| <i>Orchesella cincta</i> (Collembola) | Cd | sustained growth in F1 population exposed to Cd; elevated Cd excretion through intestinal exfoliation |
| <i>Porcellio scaber</i> Latreille (Isopoda) | Cd | sustained growth of F1 under high Cd exposure; decreased growth in the absence of Cd |
| <i>Limnodrilus hoffmeisterii</i> Nicolet (Oligochaeta) | Cd, Co, Ni | sustained growth of F1 generation exposed to polluted sediment |
| <i>Orchesella bifasciata</i> , <i>Protaphorura armata</i> (Tullberg) (Collembola) | Cu, Zn | altered life-histories of F1 populations from contaminated site cultured in the absence of metal |
| <i>Gambusia holbrooki</i> Girard (Teleostei) | Hg | differential survival among Gpi-allozyme genotypes |
| <i>Cornu aspersum</i> (Muller) (Gastropoda) | Pb | higher deposition of Pb in shell and stronger shells in field populations from historically contaminated sites |
| <i>Chironomus riparius</i> Meigen (Diptera) | Cd | altered life-histories, increased Cd excretion; sustained growth under Cd exposure; Zn deficiency of F1 populations from contaminated site |
| <i>Fundulus heteroclitus</i> (Linnaeus) (Teleostei) | Hg | tolerance to methyl mercury in fish embryos raised from exposed populations; also tolerance to PCBs, dioxin and PAHs |
| <i>Polycelis tenuis</i> Ljima (Platyhelminthes) | Cd, Zn | higher LC ₅₀ and elimination rate constant in population from polluted site |
| <i>Lumbricus rubellus</i> Hoffmeister (Oligochaeta) | As | survival of worms in soils from mine site |
| <i>Cognettia sphagnetorum</i> (Vedovsky) (Oligochaeta) | Cu | worms from soil near smelter grow and survive better in polluted soil |
| <i>Biomphalaria glabrata</i> Say (Gastropoda) | Cd | decreased tolerance to Cd in <i>Schistosoma</i> -resistant strain of snail |
| <i>Pirata piraticus</i> (Clerck) (Araneae) | Pb | altered reproductive allocation in spiders from polluted sites |

Box 2. Adaptation: physiological and genetic

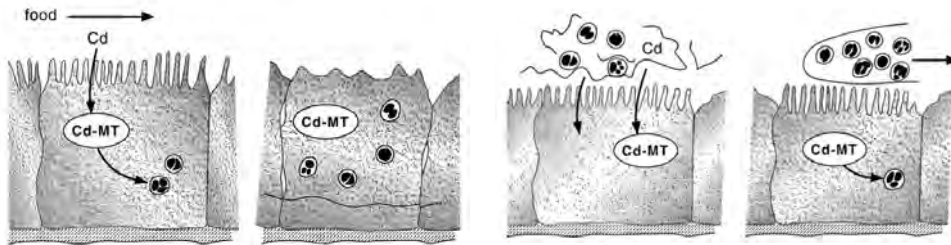
Adaptation has different meanings in the physiological and the evolutionary literature. We define *physiological adaptation* as the process by which an organism can cope with an environmental stress factor within its life time; physiological adaptation involves adjustments of the metabolic machinery in such a way that the consequences of the stress are minimized, at least temporarily. Physiological adaptations cannot be transmitted to the offspring via the DNA. However, sometimes the adaptation achieved by the mother can be partly transmitted by maternal effects, e.g. by adding proteins or mRNAs induced by exposure to the cytoplasm of the egg.

We define *genetic adaptation* as the process by which the genetic composition of a population changes under stress, in such a way that individuals with a (genetically determined) greater capacity to cope with the stress leave more offspring in the next generation. As a consequence, the average degree of tolerance to the stress factor increases in the next generation. Genetic adaptation is a form of microevolution.

Another issue of terminology is the concept of *genetic determination*, which applies equally to physiological and genetic adaptation. Physiological adaptation will often depend on enzymes, proteins or other structures whose production is encoded in the DNA of the organism. In that sense all physiological adaptations are genetically determined. In the case of genetic adaptation, there is variability between organisms in the efficiency or efficacy of these mechanisms, leading to changes in population composition when the stress factor is applied.

Like the vertebrates, all invertebrates investigated so far, except *Drosophila*, have metallothioneins with two metal-binding clusters. However, the amino acid sequence itself, which is mostly known from DNA sequences, varies enormously between species. Within the insects, metallothionein sequences are only available from *Drosophila* species and a collembolan, *Orchesella cincta* (Linnaeus). The MT of *O. cincta* is discussed in more detail below. In *D. melanogaster* Meigen two MT genes, named Mtn and Mto, were found initially (Lastowski-Perry *et al.* 1985, Mokdad *et al.* 1987). Recently, two other MTs were found in the *Drosophila*-genome, named MtnC and MtnD, while the earlier genes were re-named MtnA (= Mtn), and MtnB (= Mto) (Egli *et al.* 2003). All four *Drosophila*-metallothioneins seem to have one metal-binding cluster each. This was demonstrated for the MtnA-protein by Valls *et al.* (2000). Phylogenetic comparisons of the four single-domain MTs of *Drosophila* with the two-domain MTs of other invertebrates suggest that in the *Drosophila* lineage a reshuffling of the MT domains took place. The four *Drosophila*-MTs seem to derive from domain splits and duplications in an ancestral two-domain MT. Further sequencing of insect MTs is necessary to test this hypothesis.

There are a few studies on metal-binding proteins in insects providing evidence of the presence of metallothioneins, but these have not resulted in determination of the complete DNA or protein sequence. For example, Kasai *et al.* (1993) isolated two metal-binding proteins from the housefly, *Musca domestica* Linnaeus, and determined the amino acid composition of one of them. Similar studies have been done on cockroach *Blattella germanica* (Linnaeus), fleshfly *Sarcophaga peregrina* (Robineau-Desvoidy) and stonefly *Eusthenia spectabilis* (Gray). *Orchesella cincta* remains the only hexapod in which the MT-protein itself has been isolated and completely sequenced (see below).



1. metal enters gut epithelium
2. MT is induced
3. MT scavenges free metals
4. metal-MT is partly deposited in granules
5. feeding stops
6. gut epithelium degenerates
7. new cells are formed
8. cell contents of degenerated cells are resorbed
9. granules are concentrated in gut
10. gut pellet is excreted
11. feeding restarts

Figure 1. Model of the chain of events leading to cadmium excretion during the moulting cycle of *Orchesella cincta*, demonstrating the pathway of cadmium assimilated from the food and how this is partly removed from the animal by incorporation in metallothionein (MT) and deposition in lysosomal vesicles, followed by apoptosis of the gut epithelial cells during moulting.

Model van de keten van gebeurtenissen leidend tot cadmiumuitscheiding gedurende de vervellingscyclus van Orchesella cincta. Cadmium wordt opgenomen uit het voedsel, ingesloten in metallothioneïne (MT) en opgeslagen in blaasjes (lysosomale vesicula); verwijdering vindt plaats door apoptose (= geprogrammeerde celdood) van de darmepitheelcellen gedurende de vervelling.

Transcription of metallothionein genes can be induced by a wide variety of stimuli (e.g. hormones, growth factors, vitamins, cytotoxic agents) and stress in general. The most obvious and potent inducers are heavy-metal ions, especially those of cadmium, mercury and copper. Like for any gene, transcription of metallothionein can only take place if a complex of specific initiator proteins binds to the DNA-sequence in front of the gene. These proteins are called transcription factors and the region to which they bind is called the promoter. If free metal ions circulate in the cell, a transcription factor is activated, binds to the promoter of the MT-gene and stimulates transcription of the gene, which leads to a higher concentration of MT-protein, which can then bind the metal. This biochemical feedback loop ensures that the production of metallothionein is adjusted to the intracellular concentration of metal.

Cadmium tolerance in *Orchesella cincta*

We present an overview of recent research in the collembolan *Orchesella cincta* (box 3) as a case study of genetic metal adaptation, illustrating aspects of the mechanisms discussed above. Cadmium tolerance has been studied in *O. cincta* by comparing growth reduction upon exposure to cadmium, in the F1-generation of animals originating from populations in The Netherlands, Belgium and Germany (Posthuma 1990). In this study it was found that animals originating from heavy-metal polluted sites were less sensitive to cadmium than animals from relatively clean sites. Cadmium excretion efficiency was measured as the fraction of metal in the gut pellet compared to the total metal burden prior to the moult. This variable was considered as a metric character and analysed using quantitative genetic models. Significantly higher excretion efficiencies were found in animals (P0, F1, and F2) from polluted forest soils (Posthuma *et al.* 1992). Overall a negative correlation was apparent between growth reduction and cadmium excretion efficiency. Furthermore, it was shown that there existed additive genetic variation for cadmium ex-

cretion efficiency within a population originating from an unpolluted site; the heritability (h^2) of excretion efficiency was estimated as 33% using parent-offspring regression and 48% using a half-sib design (Posthuma *et al.* 1993). These data imply that there is significant genetic variation for cadmium tolerance in natural populations upon which selection may act.

In further studies attempts were made to elucidate the mechanism behind the genetically determined cadmium tolerance. Hensbergen *et al.* (1999, 2001) isolated a cadmium induced metallothionein from *O. cincta*, using protein separation methods and mass determinations. From the

amino acid sequence of the protein the gene was also identified. It turned out that only one copy of the gene is present in the *O. cincta* genome (Sterenberg & Roelofs 2003), both in reference populations and in tolerant populations. So the genetic adaptation of *O. cincta* to heavy metals is not achieved by gene duplication, as is the case in metal tolerance of *D. melanogaster* and many pesticide resistances.

Metallothionein is not the only cellular constituent that is involved in metal handling. There are numerous studies which show that metals can be sequestered within vesicles of the lysosomal system. When these vesicles contain a high amount of metals, they appear as dense granules in transmission electron microscopy. Different types of granular forms in terrestrial invertebrates have been described, depending on the specificity with which they bind certain metals. Granules of 'type B' are characterized by their high content of sulphur and sulphur-seeking metals such as cadmium, copper, mercury and silver. These granules seem to contain metallothionein or degradation products thereof (Hopkin 1989). Studies on *D. melanogaster* showed that cadmium is stored in granular structures in the anterior and posterior part of the midgut (Lauverjat *et al.* 1989). Such granules are present in tissues of many invertebrates (Brown 1982), including the gut of springtails (Dallai 1966, Humbert 1974). Stürzenbaum *et al.* (2001) showed that the cadmium- and sulphur-rich granules in cells lining the body cavity of earthworms are to be considered as concentrated deposits of metallothionein.

The relationship between metallothionein-bound cadmium and storage in lysosomes in *O. cincta* could explain several aspects of metal distribution and excretion within this species. Despite the fact that upon exposure approximately 90% of cadmium is present within the gut, only 40% is excreted at every moult by renewal of the gut epithelium and excretion of the old epithelium. This excreted epithelium is loaded with granular structures, probably containing metals in a form that prevents re-absorption when the old epithelium is digested. The remaining part of the cadmium load

Box 3. Springtails: the *Drosophilas* of the soil

Collembola (springtails) are hexapod soil-dwelling organisms and very abundant in forest soils with a fair cover of litter. The exact taxonomic position of the group of Collembola has come under debate recently. Nardi *et al.* (2003) developed a phylogeny on the basis of mitochondrial DNA sequences, showing that Collembola diverged earlier from the common ancestor of insects and crustaceans than the insects themselves. This would imply that the hexapod body plan has evolved twice. Indeed, Collembola have several typical characters such as the furca, the ventral tube, the post-antennal organ and the absence of malpighian tubules, which distinguish them from the true insects.



On the photograph an *Orchesella cincta* is shown, a common litter-dwelling representative of the family Entomobryidae. Three other *Orchesella* species, *O. villosa* (Geoffroy), *O. flavescens*, and *O. quinquefasciata* occur in The Netherlands, while the presence of *O. bifasciata*, which is known from North and Central Europe, is expected (Berg 2002). The single dark band on the abdomen is

diagnostic for *O. cincta*. All *Orchesella* species are hairy with white, yellow, brown and black colour patterns and surface-active habits. *Orchesella cincta* reaches a size of 3-5 mm. Populations in forest soil have an average annual density of some 2000 per m², with peaks up to several thousands in early autumn. There are two generations per year (Van Straalen 1985).

Collembola are numerous and easy to culture and manipulate in the laboratory. The parthenogenetic species *Folsomia candida* (Willem) is a major model for soil toxicity tests and is also proposed as a genomic model; DNA sequencing of Collembola is concentrated on this species. The research on heavy metal tolerance is however conducted with *O. cincta*, which is more easily found in the wild. Its sexual mode of reproduction allows crosses and pedigrees to be made for the study of heredity. Although springtails lack the possibilities for genetic transformation at the moment, they have many attractive features for ecological genetics and so are sometimes called the *Drosophilas* of the soil.

Springtails grow throughout their life without metamorphosis, which means that they moult regularly. As part of the moulting process the gut epithelial cells go into apoptosis (programmed cell death) and the whole epithelium is regenerated. After the exuvium is shed, the old gut epithelium is partly digested in the gut and then excreted via the anus to become visible as a yellow pellet of around 0.1 mm length on the substrate of a culture pot. Joosse & Buker (1979) were the first to suggest that exfoliation of the gut epithelium could play a role in metal tolerance of springtails. Van Straalen *et al.* (1987) started studies on the inheritance of metal excretion using micromethods for sample preparation and metal detection. These studies formed the basis for further molecular work, as described in this paper.

would be re-absorbed and incorporated in MT of the new cell. This scheme is illustrated in figure 1.

The research by Hensbergen *et al.* (1999, 2001) clearly showed that the MT of *O. cincta* plays a crucial role in physiological tolerance to cadmium. The question remained whether the genetic adaptation documented earlier could be traced back to some inherited modification of the metallothionein system. Molecular analysis showed that the cadmium-tolerant population over-expressed metallothionein two to three times in comparison to the reference, especially when the two groups of animals were exposed to cadmium (Sterenberg & Roelofs 2003). Sequencing the region upstream of the metallothionein gene revealed a lot of variation between individual springtails and it appeared that some promoter sequences are associated with high expression phenotypes while others are associated with low expressers (D. Roelofs, unpublished). The reason could be that some promoter sequences are more effective than others in binding metal-responsive transcription factor and thus can induce the gene to a higher degree.

Until recently population genetic analysis was focused mostly on structural variation in genes, e.g. variation in coding sequences leading to variation in run time of enzymes on electrophoresis gels (allozyme polymorphisms), or variation in non-coding regions such as microsatellites which can be used as markers of population structure. Only recently population genetics is expanding its analysis to genetic variation in transcriptional regulation (Wray *et al.* 2003). If such variation can be acted upon by natural selection in the wild

this would represent a new mechanism of microevolution.

The case of *O. cincta* is one of the best investigated examples of field-selected metal tolerance in animals. A comparable case is presented by *Drosophila*, where metal tolerance was shown to be associated with duplication of one of the metallothionein genes (Maroni *et al.* 1995), but it was never demonstrated that the MT duplication in *Drosophila* actually evolved in response to heavy metal exposure in the field (Lange *et al.* 1990).

Both in *O. cincta* and in *Drosophila*, research up to now has focused on the role of metallothionein, but the number of factors involved in the tolerance is unknown. Momose & Iwahashi (2001) applied a genomic approach to survey all the genes in the genome whose expression is regulated by cadmium. These authors were able to demonstrate that several genes associated with sulphate uptake, sulphate reduction and synthesis of cysteine were specifically upregulated by cadmium. Another study by Liao *et al.* (2002) documented the discovery of a novel cadmium-induced gene in the nematode *Caenorhabditis elegans* Maupas, that encodes a putative transmembrane protein associated with the lysosomal system. Both enzymes are associated with the sulphate pathway, and the metal transporters in lysosomal membranes could be the target for structural or regulatory mutations that could enhance the efficiency of the overall detoxification process. Therefore, the emphasis placed on metallothionein in metal tolerance research may prove to be flawed in the future. Our knowledge of the precise mechanism of adaptation to heavy metals still remains

fragmentary, even in well-investigated species. It is expected that the application of genomics approaches will further deepen our understanding and hopefully this will also improve the scientific basis for risk assessment.

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Samenvatting

Cadmiumtolerantie bij een bodemgeleedpotige: een model voor micro-evolutie in het heden

Evolutionaire processen op een tijdschaal van enkele jaren kunnen heel geschikt aan insecten onderzocht worden. De opkomst en teruggang van de zwarte variant van de berken-spanner onder invloed van luchtverontreiniging is een klassiek voorbeeld van milieu-geïnduceerde micro-evolutie dat in veel evolutiehandboeken besproken wordt. Ook de ontwikkeling van pesticidetolerantie behoort tot de beste voorbeelden van 'evolutie door pollutie'. In dit artikel geven we een overzicht van onderzoek aan een vergelijkbare casus, metaaltolerantie bij een springstaartsoort, *Orchesella cincta*. Bij deze soort is het genetische mechanisme diepgaand onderzocht en dit levert nieuwe inzichten in de manier waarop micro-evolutie kan plaatsvinden. Zware metalen zijn elementen die in metaalvorm een grotere dichtheid hebben dan 5 g/cm^3 . Veel zware metalen zijn nodig voor het metabolisme van insecten, bijvoorbeeld koper is een essentieel onderdeel van het zuurstofbindende bloedpigment haemocyanine en zink is aanwezig in allerlei enzymen en DNA-bindende peptiden. Bij hoge dosering zijn echter alle zware metalen toxisch, vooral in de vrije ionvorm. Bodemlevende insecten worden intensief aan zware metalen blootgesteld omdat metalen de neiging hebben te accumuleren in de bovenste bodemlagen. Het onderzoek heeft zich geconcentreerd op het metaal cadmium, dat niet essentieel is en al in lage concentraties toxisch. Het tolerantie-mechanisme van *Orchesella cincta* blijkt te bestaan uit binding van cadmium in een speciaal metaalbindend eiwit, metallothioneïne, dat vervolgens wordt vastgelegd in intracellulaire blaasjes (vesicula) van het lysosomale systeem in de darmepitheelcellen. Bij springstaarten wordt tijdens elke vervelling het gehele darmepitheel vernieuwd, waarbij de oude epitheelcellen gecontroleerd afsterven en verteerd worden in de darm, waarna de restanten op dezelfde manier als het meconium van holometabole insecten via de anus uitgescheiden worden. Dit mechanisme maakt het mogelijk dat bij de vervelling een groot deel van de metaalbelasting geëlimineerd kan worden. De uitscheiding van cadmium is des te hoger naarmate er vóór de vervelling meer cadmium in metallothioneïne-eiwit is vastgelegd. Nu blijkt dat tolerante dieren meer van het metaalbindende eiwit aanmaken bij blootstelling aan cadmium. DNA-onderzoek heeft laten zien dat er in een *Orchesella*-populatie een aanzienlijke mate van polymorfie is voor de promotersequentie van het metallothioneïne-gen, waarbij sommige allelen een verhoogde expressie veroorzaken en in een hogere frequentie voorkomen in populaties waar de bodem verrijkt is met zware metalen. Die verhoogde expressie blijft in stand als de dieren afkomstig van een vuil milieu meerdere generaties worden doorgekweekt in het laboratorium zonder blootstelling aan cadmium. Dit systeem, waarbij een milieufactor selecteert op de regulatie van een bepaald gen en niet op de structuur van het gen zelf, is een nieuw mechanisme voor in het heden waarneembare micro-evolutie.